Title

Casein Kinase 1δ Splice Isoforms Differentially Regulate Kinase Activity Through Their Intrinsically Disordered C-terminus

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Casein Kinase 1δ splice isoforms differentially regulate kinase activity through their intrinsically disordered C-terminus

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Dedication

To my grandparents, Agnes and Anthony McArthur, who encouraged me to believe in myself and reach my fullest potential.

Abstract

Title: Casein Kinase 1δ splice isoforms differentially regulate kinase activity through their intrinsically disordered C-terminus

Author: Rachel L. Harold

Casein Kinase 1δ influences the timing of the mammalian clock by regulating PER2 stability through post-translational modifications. CK1δ regulates PER2 through a phosphoswitch mechanism, in which phosphorylation of the FASP (Familial Advanced Sleep Phase) region of PER2 stabilizes the protein to lengthen clock period, while phosphorylation of the degron region promotes its degradation to shorten clock period. This phosphoswitch is held in delicate balance to control proper timing of the clock; mutations in CK1δ and PER2 influence the selectivity of the kinase on this switch and result in sleep phase disorders. Two CK1δ splice variants, δ1 and δ2, that differ only in the last 15 residues, show different kinase activity towards the FASP region, which suggests that the extreme C-terminus of CK1δ is a regulator of this switch. Although autophosphorylation of the disordered C-terminus of CK1δ is known to inhibit its kinase activity, it is not clear how this might differ between the CK1δ splice variants. Using Hydrogen/Deuterium Exchange-Mass Spectrometry (HDX-MS), we have begun to map the differences in tail interaction with the catalytic kinase domain of CK1δ. Nuclear Magnetic Resonance (NMR) studies have also been used to measure autophosphorylation rates of the δ1 tail *in trans* and *in cis*, and to shed light

on potential differences in intramolecular interactions of the tail isoforms. Coupled with biochemical assays using full length kinase mutants, we demonstrate that anion binding sites on the kinase domain may influence the difference in activity between the splice isoforms. Inhibition assays with tail phosphopeptides and full-length tail mutants have also shown a difference in phosphorylation dependence between the splice variants. A deeper comparison of intermolecular interactions in CK1δ and the effects of phosphorylation in splice variants will shed light on how they exhibit differential activity on the PER2 phosphoswitch to control circadian timing.

Chapter 1

Biochemical mechanisms of period control within the mammalian circadian clock

Acknowledgements

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1.1 Abstract

Genetically encoded biological clocks are found broadly throughout life on Earth, where they generate circadian (about a day) rhythms that synchronize physiology and behavior with the daily light/dark cycle. Although the genetic networks that give rise to circadian timing are now fairly well established, our understanding of how the proteins that constitute the molecular ‘cogs’ of this biological clock regulate the intrinsic timing, or period, of circadian rhythms has lagged behind. New studies probing the biochemical and structural basis of clock protein function are beginning to reveal how assemblies of dedicated clock proteins form and evolve through posttranslational regulation to generate circadian rhythms. This review will highlight some recent advances providing important insight into the molecular mechanisms of period control in mammalian clocks with an emphasis on structural analyses related to CK1-dependent control of PER stability.

1.2 Introduction to the mammalian circadian clock

Circadian rhythms arise from genetically encoded molecular clocks that originate at the cellular level and operate with an intrinsic period of about a day (circa diem). The timekeeping encoded by these self-sustained biological clocks persists in constant darkness but responds acutely to changes in daily environmental cues, like light, to keep internal clocks aligned with the external environment [1]. Therefore, circadian rhythms are used to help organisms predict changes in their environment and temporally program regular changes in their behavior and physiology [2]. The circadian clock in mammals is driven by several interlocked transcription-translation feedback loops (TTFLs) [3]. The integration of these interlocked loops is a complicated process that is orchestrated by a core feedback loop in which the heterodimeric transcription factor complex, CLOCK:BMAL1, promotes the transcription of its own repressors, Cryptochrome and Period (CRY and PER) as well as other clock-controlled genes (Fig. 1.1A). Notably, there is some redundancy in this system as paralogs of both PER (PER1–3) and CRY (CRY1–2) proteins participate in the core TTFL. In general, these proteins accumulate in the cytoplasm, interact with one another, and recruit a kinase that is essential for the clock, Casein Kinase 1 δ/ε (CK1δ/ε), eventually making their way into the nucleus as a large complex to repress CLOCK:BMAL1 transcriptional activity [4, 5]. Despite this relatively simple model for the core circadian feedback loop, there is growing evidence that different repressor complexes that exist

throughout the evening may regulate CLOCK:BMAL1 in distinct ways [6, 7]. PER proteins are essential for the nucleation of large protein complexes that form early in the repressive phase [4] by acting as stoichiometrically-limiting factors that are temporally regulated through oscillations in expression [8, 9]. As a consequence, circadian rhythms can be disrupted by constitutively overexpressing PER proteins [8] or established de novo with tunable periods through inducible regulation of PER oscillations [10]. CK1δ/ε regulate PER abundance by controlling its degradation post-translationally [11-13]; accordingly, mutations in the kinases [14, 15] or their phosphorylation sites on PER2 [16, 17] can induce large (~4 h) changes in circadian period, firmly establishing this regulatory mechanism as a central regulator of the mammalian circadian clock. CRY proteins bind directly to CLOCK: BMAL1 [18] and mediate the interaction of PER-CK1δ/ε complexes with CLOCK:BMAL1 leading to phosphorylation of the transcription factor and its release from DNA [6, 19] as well as acting as direct repressors of CLOCK:BMAL1 activity by sequestering the transcriptional activation domain (TAD) of BMAL1 from coactivators like CBP/p300 [20, 21]. Much remains to be elucidated about the assembly and activity of these core clock proteins, but insight into the molecular basis of circadian period determination is growing thanks to the integration of genetic studies from model organisms and humans with biochemical, structural, and cell-based studies. High-resolution structures have now been determined for most of the globular

domains of core clock proteins and some of their complexes [22], with a growing appreciation for the important role that flexible linkers and intrinsically disordered regions play in tuning clock protein function and clock timing (Fig. 1.1B) [23]. We will focus here on recent advances in our understanding of the mechanisms of period control by some of the negative elements of the core feedback loop (Fig. 1.1C), highlighting the nanoscale structural and dynamic properties of clock proteins that influence their functional roles as repressors within the core TTFL. For a review of mutations in human CRY1 [24, 25] and CRY2 [26] that influence circadian timing, please refer to [27].

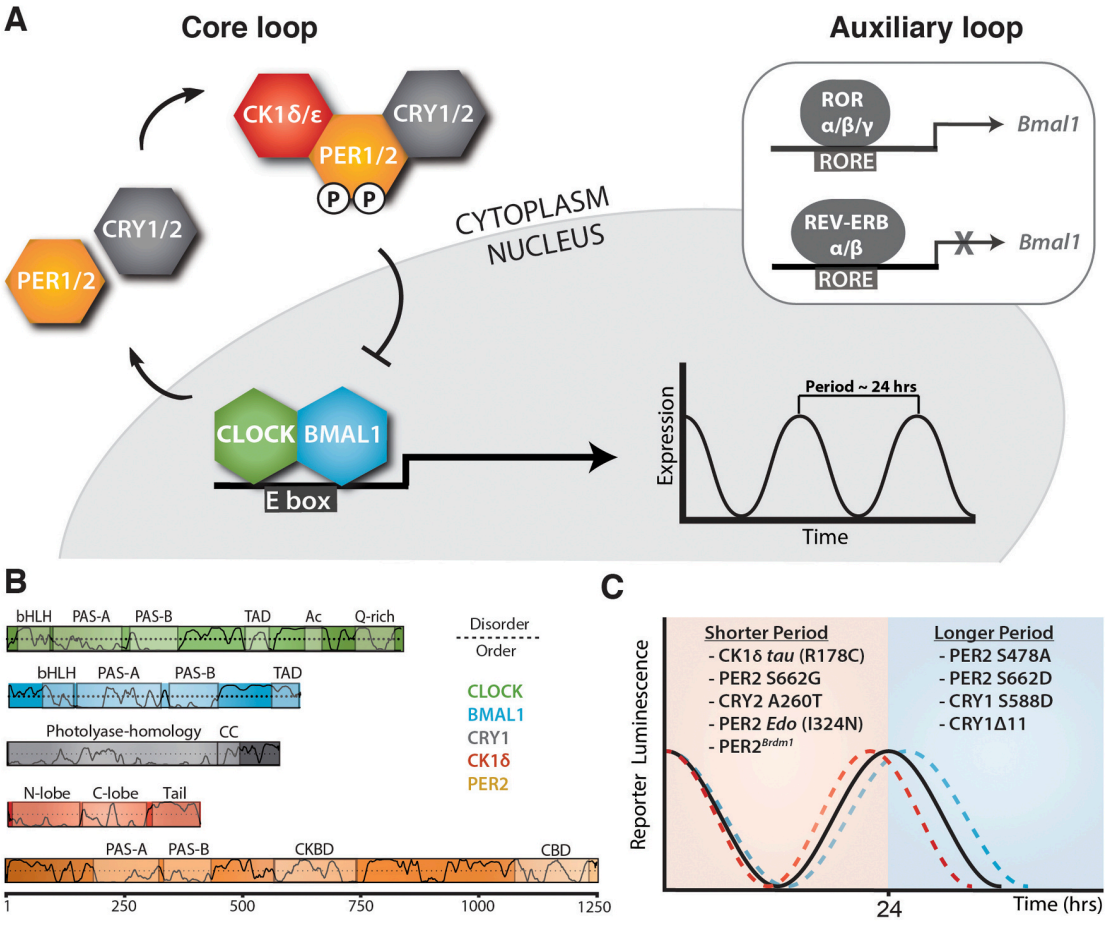

Fig. 1.1 Changes to the core molecular clock control mammalian circadian period. (A) Simplified schematic of the core mammalian feedback loop mediated by CLOCK:BMAL1 expression of CRY and PER genes with an auxiliary loop that consists of the retinoic acid receptor-related orphan receptor α (RORα) and the nuclear receptors REV-ERBα/β. (B) Functional domain architecture of core clock proteins with structured domains (boxes) and traces indicating the propensity for intrinsic disorder [28]: bHLH, basic helix-loop-helix; PAS, PER-ARNT-SIM; TAD, transactivation domain; Ac, acetyl-CoA binding, Q-rich, polyglutamine; CC, coiled-coil; CKBD, Casein Kinase 1-binding domain; and CBD, CRY-binding domain with residue numbering underneath. (C) Period effects from select mammalian clock alleles of core clock proteins.

1.3 Post-translational regulation of PER2 stability

Although transcriptional regulation by CLOCK:BMAL1 and downstream transcription factors is essential for generation of robust circadian rhythms, it is becoming increasingly clear that post-transcriptional and post-translational modifications of core clock components play an important role in both the generation of circadian rhythms and determination of its intrinsic period [29]. While many studies have identified roles for post-translational modification of CRY and PER proteins in clock timing, we will focus here on an in-depth analysis of the regulation of PER2 by CK1δ/ε, as it relies on an elaborate integration of post-translational modifications that ultimately determine the relative abundance of PER2 needed to maintain a ~24 h period [30].

1.3.1 Structural organization of PER2

PER1 and PER2 serve as interaction hubs for both CK1δ/ε and CRYs with dedicated binding sites that maintain stable complexes with these core clock proteins throughout most of the repressive phase of the clock each day [4, 5] (Fig. 1.2A), a feature that is notably absent in PER3 [31]. The flexibility of PER proteins likely contributes to their role as labile scaffolds for transcriptional regulators of the clock; PER1-3 are all predominantly intrinsically disordered, conferring a susceptibility to regulation by post-translational modifications and promiscuity for interaction partners [32-36] that is common among other intrinsically disordered proteins (IDPs) [37]. Two tandem PER-ARNT-SIM (PAS) domains present in the N-terminus of all three PER isoforms allow them to form homodimers and heterodimers (Fig. 1.2A) [38-40] mediated by the β-sheet surface of the PAS-B domain (Fig. 1.2B). Deletion of the core PAS-B motif in the Per2Brdm1 mutant leads to a loss of circadian rhythms, demonstrating that protein-protein interactions facilitated by this region are essential for clock function [41]. Notably, mutation of just a single residue, W419E, at the dimer interface in the mouse PER2 PAS-B domain potently disrupts formation of homodimers [38] and was recently shown to reduce phosphorylation by CK1δ [42]. Moreover, the PER2 earlydoors (Edo) mouse possesses a point mutation (I324N) in the interdomain linker connecting the PAS-A and B domains that reduces PER2 stability to shorten the circadian period [43]. Collectively, these findings

demonstrate that dimerization via the PER PAS domains is critical for protein stability and clock timing, although more work is needed to understand the exact role that PAS domains play in orchestrating clock protein complexes and PER turnover.

1.3.2 Control of PER2 degradation by phosphodegrons

The turnover of PER2 is primarily mediated by CK1δ/ε-dependent phosphodegrons to intimately link kinase activity with PER2 stability. One well-studied phosphodegron site of mouse PER2 (S478) is located immediately downstream of the PAS-B domain. Dimerization of the PER2 PAS-AB domains positions the phosphodegron site (S478) of each monomer to protrude from the same face of the PAS-AB domain homodimer (Fig. 1.2B) [38]. This phosphodegron largely conforms to the canonical β-TrCP recognition motif, DSGϕXS, where ϕ is a hydrophobic residue and the two conserved serines become phosphorylated to make the substrate competent for β-TrCP recognition (Fig. 1.2C) [44]. CK1δ/ε phosphorylation of S478 in mouse PER2, the first of the two serines in the motif, is required for interaction with the E3 ubiquitin ligases β-TrCP1/2, leading to ubiquitination of PER2 and its proteasomal degradation [12, 45, 46]. However, this PAS-B phosphodegron is unique to PER2; PER1 utilizes a different CK1δ/ε-dependent phosphodegron N-terminal to the tandem PAS domains [13]. This N-terminal phosphodegron is also conserved in PER2 [45] and may play an

auxiliary role in its turnover, as clock timing was only modestly impacted in the PER2 S478A transgenic mouse [47]. An interaction with the E3 ubiquitin ligase MDM2 also influences PER2 stability independently of CK1δ/ε activity [48], opening the door for a complex integration of signals to mediate PER2 degradation.

1.3.3 The Casein Kinase-Binding Domain stabilizes PER2 through a phosphoswitch

Although other kinases such as CK1α [49], CK2 [50], SIK3 [51] and Cdk5 [52] phosphorylate PER2, CK1δ/ε are the only kinases that stably associate with PER2 throughout the night, moving from the cytoplasm into the nucleus with the other core clock proteins [4, 5]. CK1δ and the related isoform CK1ε bind to the Casein Kinase-Binding Domain (CKBD) in PER2 via two conserved motifs that flank a serine-rich region [12, 31]. Notably, mutation of the first residue (S662G) in a series of five consecutive serines that are phosphorylated in human PER2 markedly decreases its stability and shortens circadian period by ~4 h to manifest as Familial Advanced Sleep Phase Syndrome (FASPS) [16, 17]. Because CK1δ/ε-dependent phosphorylation of PER2 in this region links circadian timekeeping to this human sleep disorder, the serine-rich cluster in the CKBD has been named the FASP region. Recent studies have begun to elucidate the molecular basis for CK1δ/ ε activity in the FASP region of PER2 to understand how it exerts such powerful control over

circadian period. Phosphorylation of the first serine in this cluster (S659 in mouse, S662 in humans) by CK1δ leads to the obligately sequential phosphorylation of downstream serines (Fig. 1.2A) [53]. Therefore, the human S662G FASPS allele eliminates the ability of CK1δ to prime its activity downstream, disrupting all phosphorylation in the FASP region. There is strong evidence that FASP phosphorylation plays a critical role in stabilizing PER2 protein [16], as the S662G mutation in human PER2 (or the analogous S659A mutation in mouse PER2) [54] leads to premature turnover of the protein and a dramatically shorter circadian period of ~20 h in a transgenic mouse model, while use of a phosphomimetic mutation (S662D) in human PER2 that presumably leads to constitutive priming of sequential FASP phosphorylation confers a long period of ~25 h in vivo [17]. Although it is not yet known how FASP phosphorylation contributes to regulation of PER2 stability, mutation of the priming serine that blocks phosphorylation of FASP downstream serines increases CK1δ/ε activity at the phosphodegron site S478 [55] to suggest that the phospho-FASP region could antagonize CK1δ/ε activity at the phosphodegron site S478. The opposing effects of FASP and phosphodegron phosphorylation likely involves cellular phosphatases like PP1 that contribute to CK1δ/ε-dependent regulation of circadian period through PER2 [56], although there could also be a direct mechanistic link between FASP phosphorylation and regulation of CK1δ/ε activity. In fact, the functional linkage of phosphorylation at the FASP region and phosphodegron

by CK1δ/ε has been described as a phosphoswitch that introduces a phase-specific delay to PER2 degradation necessary for proper circadian timekeeping (Fig. 1.2A) [57]. Interestingly, while introduction of the analogous priming site mutation in mouse PER1 (S714G) destabilized PER1 and led to a shorter circadian period, it also caused an advance in feeding rhythms not seen in the PER2 mutant [58], suggesting that further study of the regulation of PER turnover could help uncouple distinct functions of PER1 and PER2 in control of circadian period and clock outputs.

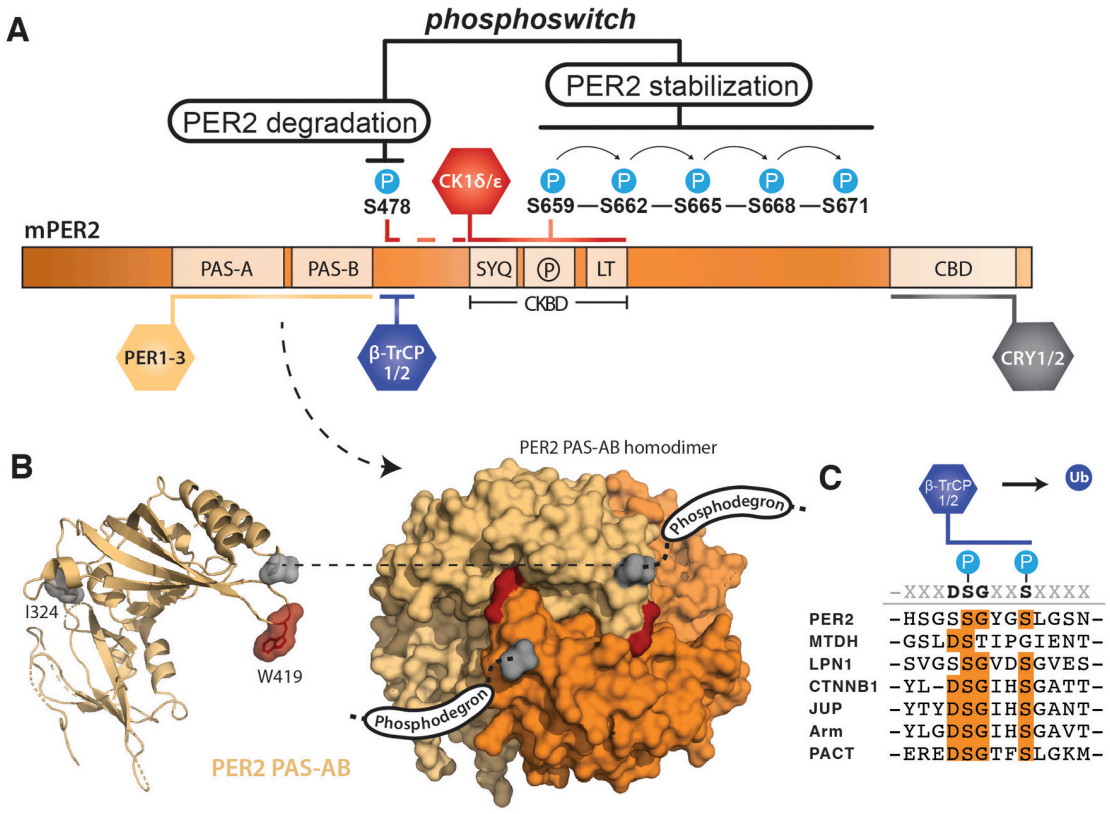

Fig. 1.2. PER2 stability is tightly regulated by post-translational modifications. (A) Domain map of mouse PER2 depicting clock protein binding sites and the CK1δ- dependent phosphoswitch model. CK1δ-dependent phosphorylation of the FASP region (P) within the

CKBD antagonizes activity at the upstream phosphodegron, which is used to recruit the E3 ubiquitin ligases, β-TrCP1/2, for subsequent proteasomal degradation of PER2. (B) Left, cartoon representation of the mouse PER2 PAS-AB domain monomer with the location of the *Edo* mutation (I324N) and Trp residue required for dimerization (W419) highlighted, PDB: 3GDI. Right, surface representation of the PER2 PAS-AB homodimer. The phosphodegron is located in a disordered region immediately downstream of the PAS-B domain; the C-terminal residue is depicted in surface mode (gray) to show how each respective phosphodegron is poised to protrude from the same face of the dimer. (C) Alignment of phosphodegrons within human proteins that are targeted by β-TrCP1/2.

1.3.4 Other post-translational modifications influence PER2 stability

PER proteins are also subjected to a number of other post-translational modifications aside from phosphorylation. PER2 is *O*-GlcNacylated within the FASP region [59], modifying the priming serine along with two sites downstream. Both *O*-GlcNAc transferase (OGT) and *O*-GlcNAcase (OGA), the enzymes responsible for adding or hydrolyzing *O*-GlcNAc, respectively, are expressed or activated in a circadian manner, and factors that increase *O*-GlcNacylation also lead to a concomitant decrease in FASP phosphorylation that reduces PER2 protein levels [59, 60]. These results support a model for competition between *O*-GlcNacylation and phosphorylation at this key regulatory region, suggesting a mechanism by which glucose metabolism could modulate the circadian clock by antagonizing phosphorylation of PER2 in the stabilizing FASP region to

represent a direct link between the circadian clock and metabolism as a "nutrition switch" [30, 59]. Acetylation also plays an important yet enigmatic role in PER2 regulation of the clock, first observed through manipulation of SIRT1, the nicotinamide adenine dinucleotide (NAD+)-dependent deacetylase. Although PER2 becomes acetylated as it accumulates throughout the repressive phase of the circadian clock [61], the identity of the acetyltransferase(s) that modify PER2 is currently not known, nor is it known where PER2 is acetylated throughout the protein. Nonetheless, loss of SIRT1 results in elevated levels of acetylated PER2 in mouse liver to attenuate the robustness of circadian rhythms, while overexpression of SIRT1 facilitates PER2 degradation [61, 62]. Because acetylation and ubiquitination both target lysine residues, it is possible that these modifications compete for the same residues to directly control PER2 stability. However, there is some evidence that regulation of PER2 by acetylation could be more complicated, as acetylation at K680 on mouse PER2, located downstream of the serine cluster in the FASP region, is hyperacetylated following inhibition of SIRT1 and leads to a decrease in FASP phosphorylation [63], suggesting that an interplay between acetylation and phosphorylation of the FASP region could also control CK1δ/ε activity on PER2 to regulate the balance of the phosphoswitch.

1.4 A central role for CK1 in eukaryotic circadian period determination

The timing of eukaryotic circadian rhythms from green algae to humans is heavily influenced by CK1δ/ε and its related orthologs [15, 64]. Like other Ser/Thr kinases, CK1δ/ε has a typical two-lobed structure (Fig. 1.3A), but little is known about the molecular mechanisms by which activity of the CK1 family is regulated. The activation loop is one key feature that distinguishes the CK1 family from other Ser/Thr kinases (Fig. 1.3B) [65]. Unlike many other kinases, the kinase domain of CK1 family members is not regulated by activation loop phosphorylation; therefore, they are considered to be constitutively active [66]. The CK1 family acts as phosphate-directed kinases that preferentially recognize a D/E/pSxxS consensus motif, where a phosphorylated serine or a similar negative charge within the substrate templates activity at a serine located 3 or 4 residues downstream (Fig. 1.3A) [67]. Interestingly, at least two functionally important CK1δ/ε-dependent phosphorylation sites on PER2, the phosphodegron and the FASP priming site, do not conform to this consensus motif and likely serve as slow, rate-limiting steps for PER2 regulation [53, 55, 68]. Therefore, understanding the molecular basis for kinase activity and substrate selectivity by CK1δ/ε has the potential to yield important insights into circadian timekeeping. In particular, a better understanding of the molecular mechanisms underpinning CK1δ/ε-dependent phosphorylation of PER will provide a framework for treating circadian disorders by targeting CK1δ/ε to modulate the clock [69].

1.4.1 Anion-dependent regulation of a structural switch in CK1δ

The kinase domain of CK1δ/ε contains several highly conserved anion binding sites located around the C-terminal lobe, including two that flank either side of the substrate binding cleft (Fig. 1.3A, B) [70-72]. We recently showed that these anion binding sites regulate the overall kinase activity of CK1δ/ε, as well as influence the substrate specificity of the kinase at both consensus and non-consensus sites [55]. The significance of these highly conserved anion binding sites was initially suggested by the discovery of the first period-altering allele in mammals, the CK1ε *tau* allele that causes a dramatically shortened circadian period of ~20 h [73]. The R178C substitution in the *tau* kinase was predicted to disrupt an anion-binding pocket near the substrate binding region to decrease CK1δ/ε activity [14]. While the *tau* mutant kinase did exhibit reduced activity on some generic kinase substrates (e.g., casein and phosvitin) as well as the FASP region of PER2, it led to a paradoxical gain of function at the PER2 phosphodegron that decreased stability of the protein [55, 74]. Crystal structures of the *tau* kinase domain recently revealed that disruption of the anion binding pocket at S1 in the mutant is linked to an allosteric structural switch in the activation loop that encodes a preference for the PER2 PAS-B phosphodegron site S478 [55]. Allostery is a common regulatory feature of protein kinases that allows for a switch-like, ultrasensitive regulation of their biological activity [75]. The

activation loop and flanking regions distinguish CK1 from all other Ser/Thr kinases (Fig. 1.3B) [76], containing residues involved in the coordination of anions at three conserved sites, S1-S3. Therefore, these sites likely play a role in the CK1 family-specific regulation of kinase activity, perhaps through binding of anionic, phosphorylated residues. Interestingly, the entire substrate binding cleft that allosterically links anion binding to substrate selectivity is 95% identical from humans to green algae [55], suggesting that the mechanisms discovered in mammalian CK1δ/ε may also regulate kinase activity and circadian period across other eukaryotic clocks.

1.4.2 Regulation of CK1δ/ε activity by its disordered C-terminal tail

The kinase activity of both CK1δ and CK1ε is inhibited by autophosphorylation of an intrinsically disordered inhibitory tail that follows the kinase domain to set these isoforms apart from other members of the CK1 family [77-80]. Because the full-length kinase autophosphorylates and slowly inactivates itself in vitro, most biochemical studies exploring the activity of CK1δ/ε on clock proteins utilize the truncated, constitutively active protein (CK1δ/ε ΔC) [53, 55, 71, 81], although new studies are finally beginning to explore the consequences of autophosphorylation in more detail [82, 83]. However, not much is known yet about how the phosphorylated tail interacts with the kinase domain to inhibit its activity; several autophosphorylation sites were previously identified on CK1ε at S323, T325, T334, T337, S368, S405,

S407 and S408 using limited proteolysis and phosphatase treatment [79] or through Ser/Thr to Ala substitutions in vitro [80], although it is currently not known which (if any) of these sites are important for kinase regulation of the clock. One potential interface has been mapped between the kinase domain and autoinhibitory tail through crosslinking and mass spectrometry to suggest that the tail might dock some phosphorylated Ser/Thr residues close to the anion binding sites near the active site [84]. This study also provided evidence that the tail may be able to regulate substrate binding, and therefore control specificity of the kinase, by comparing the activity of CK1α, a tailless kinase, with CK1ε on two substrates, PER2 and Disheveled [84]. Understanding the role of tail autophosphorylation and its regulation of kinase activity is sure to shed light on control of circadian rhythms by CK1δ/ε. Some sites within the C-terminal tail of CK1δ and/or CK1ε are known to be phosphorylated by other kinases, such as AMPK [85], PKA [86], Chk1 [87], PKCα [88], and cyclin-dependent kinases [89, 90]. PKA phosphorylates S370 in CK1δ to reduce its kinase activity; consistent with this, mutation of S370 to alanine increases CK1-dependent ectopic dorsal axis formation in *Xenopus laevis* [86]. Chk1 and PKCα also reduce CK1δ kinase activity through phosphorylation of overlapping sites at S328, T329, S331, S370, and T397 in the tail of rat CK1δ [87, 88]. Phosphorylation of CK1δ T347 influences its activity on PER2 in cells and was found to be phosphorylated by proline-directed cyclin-dependent kinases rather than autophosphorylation [89].

CDK2 was also found to reduce the activity of rat CK1δ in vitro through phosphorylation of additional sites at T329, S331, T344, S356, S361, and T397 [90]. Unlike the other kinases listed here, phosphorylation of S389 on CK1ε by AMPK increases the apparent kinase activity on the PER2 phosphodegron in cells; consequently, activation of AMPK with metformin increased the degradation of PER2 [85]. Therefore, the phosphorylation of CK1δ and/or CK1ε tails by these other kinases therefore has the potential to link its regulation of PER2 and the circadian clock to metabolism, DNA damage response, and the cell cycle. There is now strong evidence that the C-terminus of CK1δ plays a direct role in regulation of circadian period. Recently, tissue-specific methylation of CK1δ was shown to regulate alternative splicing of the kinase into two unique isoforms, δ1 and δ2, that differ only by the extreme C-terminal 15 residues (Fig. 1.3C) [91]. Remarkably, expression of the canonical δ1 isoform decreases PER2 half-life and circadian period, while the slightly shorter δ2 isoform increases PER2 half-life and circadian period [91]. Further biochemical studies revealed that these two variants exhibit differential activity on the stabilizing priming site of the PER2 FASP region—the δ1 isoform has a lower activity than δ2, which also closely resembles the C-terminus of the ε isoform [53]. These data suggest that a very short region at the C-terminal end of the tail could play a major role in regulation of CK1δ and the PER2 phosphoswitch to control circadian period. This is bolstered by the discovery of a missense mutation in

the same region of the CK1ε tail at S408N in humans that has been associated with protection from Delayed Sleep Phase Syndrome (DSPS) and Non-24 h Sleep-Wake Syndrome (N-24) [92]. Further studies will help to reveal biochemical mechanisms behind regulation of kinase activity and substrate selectivity by the C-terminal tail of CK1δ and CK1ε to determine how they play into regulation of circadian rhythms.

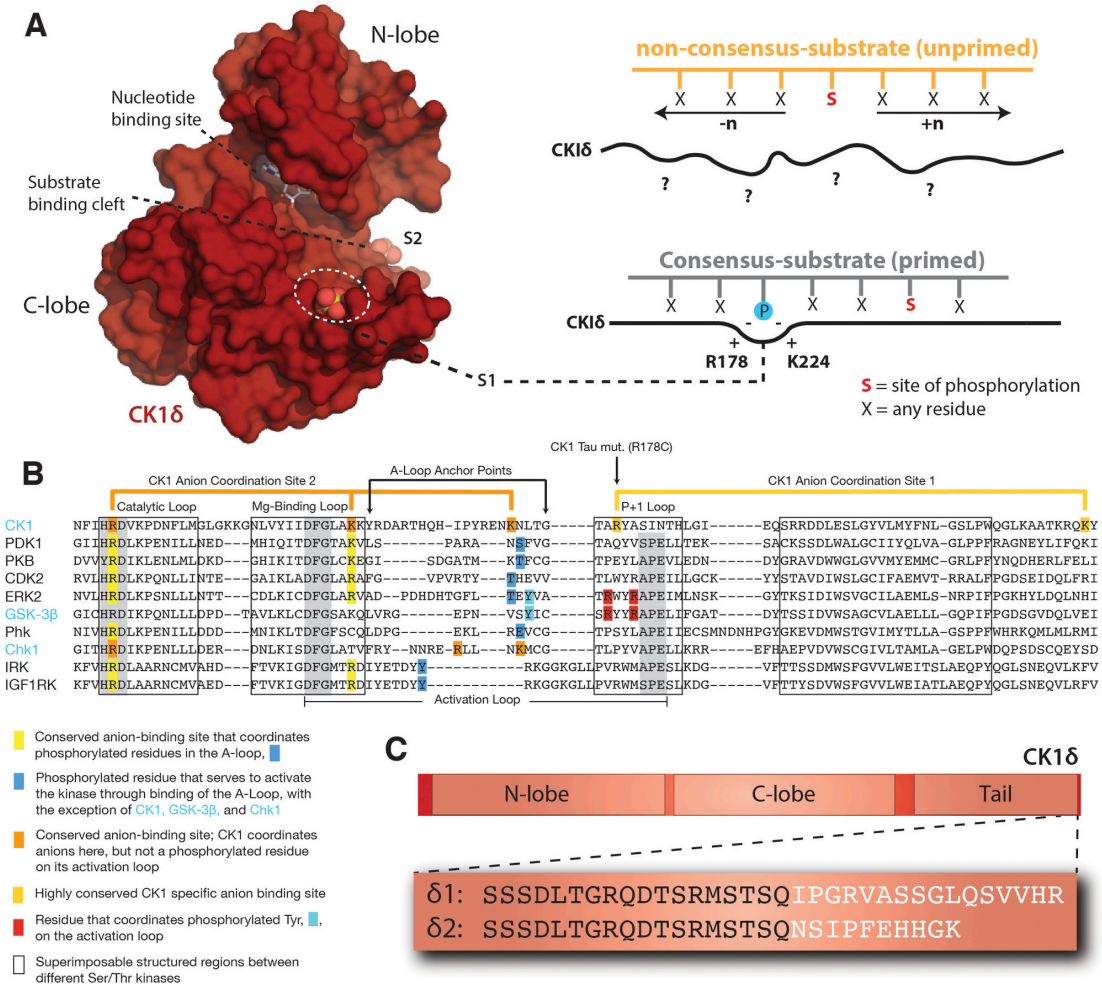

Fig. 1.3. Regulatory mechanisms control CK1δ _activity in the mammalian clock. (A) Left, Surface representation of the CK1δ _kinase domain, PDB: 5X17. The substrate binding cleft is flanked by two highly conserved anion binding sites, S1 and S2. Right, CK1 is

thought to use S1 to bind phosphorylated (or 'primed') substrates, leading to phosphorylation of the CK1 consensus motif, pSxxS. CK1 also exhibits non-consensus activity on unprimed sites. (B) Alignment of the activation loop and nearby regions in representative Ser/Thr kinases. CK1 lacks the conserved APE motif in the P + 1 loop involved in substrate recognition. (C) Differences between the alternatively spliced variants CK1δ1 and CK1δ2 at the C-terminus of the kinase.

1.5 Concluding remarks

While there is much more to be learned, data from human genetics and mammalian model systems are finally being integrated with biochemical and structural studies of the core clock components to provide clues to the molecular basis of the circadian clock. Recent advances in biophysical techniques, such as cryo-EM, will help to further enhance our understanding of how the molecular 'cogs' of the core clock come together to affect circadian period and influence human behavior and physiology.

Chapter 2

Isoform-specific C-terminal phosphorylation drives autoinhibition of Casein Kinase 1

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2.1 Abstract

Casein kinase 1 δ (CK1δ) controls numerous biological processes, including circadian rhythms and Wnt signaling. CK1δ is inhibited by autophosphorylation of its intrinsically disordered C-terminal tail, but it is not understood how this occurs. Two CK1 splice variants, δ1 and δ2, differ only in the last sixteen residues of the tail and have different activity *in vivo*, suggesting that the extreme C-terminus (XCT) of CK1 plays a role in autoinhibition. Using NMR and HDX-MS, we show that the δ1 XCT is preferentially phosphorylated by the kinase and the δ1 tail makes more extensive interactions across the kinase domain. Mutation of δ1-specific XCT phosphorylation sites disrupts its interaction with the kinase domain and

increases kinase activity *in vitro* and in cells. δ1 autoinhibition relies on conserved anion binding sites around the CK1 active site that are also used for feedback inhibition by phosphorylated substrates, demonstrating a common mode of product inhibition of CK1δ.

2.2 Significance

Two isoforms of CK1δ, δ1 and δ2, have different kinase activity *in vivo* although they differ by only sixteen residues at the end of a long, intrinsically disordered autoinhibitory tail. Here we show that autoinhibition by the tail requires the extreme C-terminus that is differentially encoded by the isoforms. NMR and HDX-MS studies highlighted isoform-specific differences protein dynamics and kinase activity, identifying several serines on δ1 that are necessary for its more potent autoinhibitory activity *in vitro* and in cells. Beginning to understand mechanisms that regulate CK1δ activity will shed light on its important roles as a key regulator of diverse processes from Wnt signaling and development to the cell cycle and circadian rhythms.

2.3 Introduction

Kinases play a crucial role in biology through their ability to post-translationally modify proteins by phosphorylation, altering the structure and/or activity of their targets. The activity of most kinases is therefore kept under tight regulation, requiring phosphorylation of their activation loops or binding to cofactors or scaffolds to remodel the active site and/or recruit substrates; in

some cases, removal of inhibitory phosphorylation can activate kinases [93, 94]. The kinase domains of Casein Kinase 1δ (CK1δ) and its paralog, CK1ε, are constitutively active, but autophosphorylation of their intrinsically disordered C-terminal extensions, or 'tails', inhibits kinase activity [77, 79]. cK1δ/ε tails are also targeted by a number of other kinases [89, 95] and phosphatases [78, 79, 96, 97] *in vivo,* suggesting a dynamic mode of kinase regulation that is not well understood. Given the involvement of CK1δ/ε in a wide range of pathways from Wnt signaling, cell division, and apoptosis to circadian rhythms [95], understanding how the kinase activity of CK1δ/ε is regulated by phosphorylation of their intrinsically disordered tails could shed light on underappreciated regulatory mechanisms.

In mammalian circadian rhythms, CK1δ/ε regulate the intrinsic timing of the molecular clock through phosphorylation of the Period (PER) proteins [68, 98, 99]. A phosphoswitch involving competing CK1δ/ε-dependent sites on PER2 regulates its half-life [57], wherein phosphorylation of a β-TrCP degron and subsequent protein turnover are counteracted by phosphorylation of several serines located within the Casein Kinase 1 binding domain (CK1BD) that anchors the kinase to PER throughout its daily life cycle [4, 100, 101]. These stabilizing phosphorylation sites occur within the Familial Advanced Sleep Phase (FASP) region, named for a point mutation in human *Per2* (S662G) that eliminates the priming phosphorylation and shortens the clock by ~4 hours, causing an extreme advance in the onset of sleep [16, 17]. We

recently showed that phosphorylation of the PER2 FASP region leads to feedback inhibition of the anchored kinase, utilizing two highly conserved anion binding sites on CK1δ near the active site that bind phosphoserines in the FASP to block access to substrates [102]. This is similar to feedback inhibition of the kinase observed in DNA damage-induced apoptosis [103]. Conservation of these feedback inhibition binding modes establishes the importance of CK1δ/ε anion binding sites for regulation of the kinase by phosphorylated substrates.

Alterations in CK1δ/ε can also shift the balance of the phosphoswitch to have a profound effect on circadian timing in mammals from rodents to humans. These alterations include inherited polymorphisms [14, 15, 55, 73] and alternative splicing of CK1δ [91]. The two CK1δ splice isoforms, δ1 and δ2, are identical throughout the kinase domain and most of the intrinsically disordered tail, differing only in the last 16 amino acids of the C-terminus (Fig. 1A), a region we call here the extreme C-terminus, or XCT. Despite this relatively minor change, the δ1 and δ2 isoforms differentially phosphorylate the PER2 FASP region *in vivo* to control circadian period [91]. Truncating the XCT at residue 400 is sufficient to derepress kinase activity [53], suggesting a crucial role for residues 400-415 in regulation of CK1 activity in cells.

In this study, we examined the biochemical role of the XCT in differential regulation of CK1δ isoform activity. We found that isoform-specific differences in CK1 activity are intrinsic to the kinase *in vitro*; CK1δ1 is autoinhibited to a

greater extent than CK1δ2, and truncation of the isoform-specific extreme C-termini essentially eliminates autoinhibition. NMR assays of the δ1 tail with the kinase domain in *trans* or *cis*, via segmental isotopic labeling, revealed that interaction between the kinase domain and δ1 tail is dependent on phosphorylation and predominantly localized to the δ1-dependent sequence. By contrast, the δ2 tail interacts with the kinase domain even in the absence of phosphorylation, consistent with inhibition data using isolated δ1 or δ2 peptides. Using hydrogen/deuterium exchange mass spectrometry (HDX-MS), we mapped how CK1 isoforms or mutants differentially protect the kinase domain and intrinsically disordered tails from deuterium exchange. Protection of the kinase active site in the presence of an inhibitory phosphoFASP peptide of PER2 [102] mirrors protection by the two CK1δ isoforms, which is largely eliminated by deleting the XCT region with the Δ400 truncation or mutation of the δ1-specific serines to alanines. Our study defines isoform-specific mechanisms of CK1δ regulation and demonstrates a conserved mechanism of kinase inhibition by phosphorylated substrates.

2.4 Results

2.4.1 CK1δ isoforms δ1 and δ2 have different kinase activity *in vitro*

The alternatively spliced CK1δ isoforms, δ1 and δ2, differ only in the last 16 amino acids of the C-terminal tail (Fig. 2.1A). We previously showed that CK1δ1, CK1δ2, and a related isoform, CK1ε, can all catalyze the rate-limiting priming phosphorylation in the PER2 FASP region, with CK1δ2 and CK1ε

demonstrating increased activity compared to CK1δ1 [53]. The kinase domains of CK1δ and CK1ε are highly conserved, and their intrinsically disordered C-terminal tails differ, although the XCT of CK1δ2 and CK1ε are quite similar (Fig. 2.1.1A). This suggested that the differences in activity we observed in cells might be due to their XCT; consistent with this, truncation of the last 16 amino acids of CK1δ1 (Δ400) eliminated its isoform-specific decrease in activity [53].

To determine if isoform-specific differences in activity are intrinsic to the kinase, we established a pipeline to purify dephosphorylated full-length recombinant proteins (Fig. 2.1B). To demonstrate the effect of tail phosphorylation on CK1δ1, we first pre-treated full-length, dephosphorylated CK1δ1 with ATP to induce autophosphorylation and then measured its activity on the PER2 FASP priming site using an ELISA-based kinase assay. As expected, pretreatment of the kinase with ATP before the substrate was added significantly reduced FASP priming phosphorylation compared to untreated CK1δ1 (Fig. 2.1.1B). We then used *in vitro* kinase assays to measure the activity of the two CK1δ isoforms compared to the Δ400 truncation and the fully active, tail-less CK1δΔC kinase domain. Activity was measured on a mutant of the human PER2 FASP region (S665A) that only allows for phosphorylation of the rate-limiting priming serine at S662 (Fig. 2.1C and 2.1.1C-D) [102]. Although differences in activity were modest, the activity of CK1δ1 on the PER2 FASP was significantly lower than CK1δ2 (Fig. 2.1D, E). Truncation of the XCT or the entire tail gave rise to activity that was similar to CK1δ2, recapitulating

differences in the activity we observed in cells [53] and suggesting that CK1δ1 has increased autoinhibition. We also quantified autophosphorylation in these assays. Relative to the full-length proteins, the isolated kinase domain showed minimal autophosphorylation, and the δ1 kinase was significantly less phosphorylated than δ2 or Δ400, despite having more serines and greater inhibition than either (Fig. 2.1.1E). These differences point to an important regulatory role for the CK1δ1 XCT.

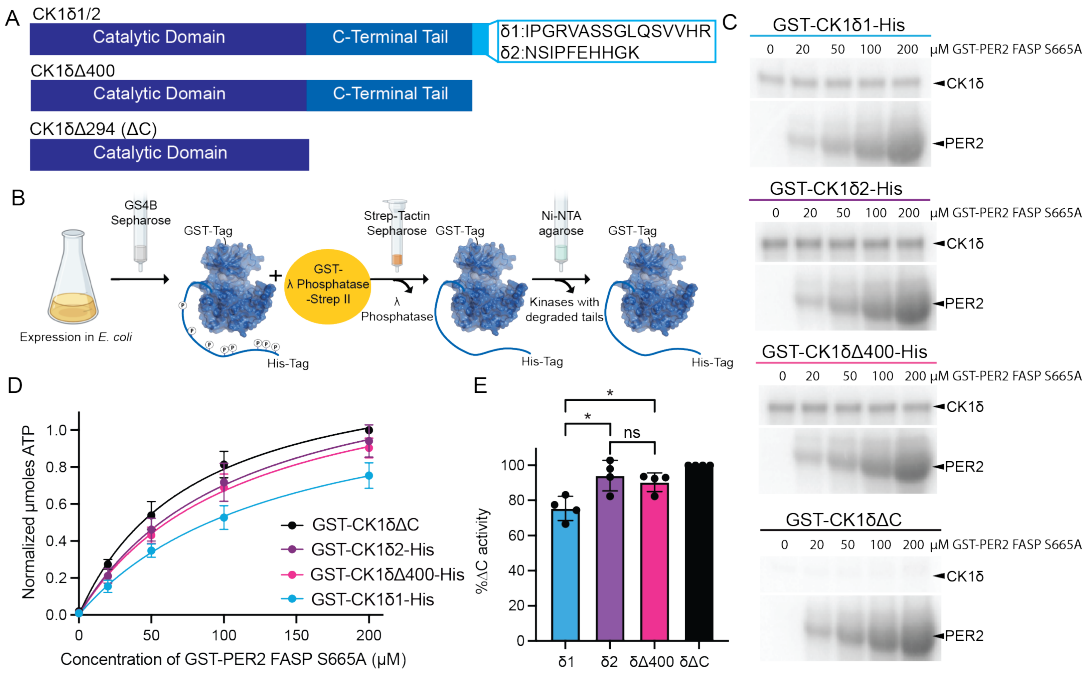


Figure 2.1 CK1δ isoforms alter kinase activity *in vitro*

A, Schematic of CK1δ isoforms and truncations. **B**, Kinase purification scheme with double tag system. **C**, Autoradiograph of ^{32}P kinase assay; top, autophosphorylation of kinase; bottom, priming phosphorylation of the PER2 FASP substrate. Representative autoradiograph from n = 4. **D**, Quantification of priming phosphorylation by CK1δ1 (blue),

CK1δ2 (purple), CK1δΔ400 (pink), or CK1δΔC (black), normalized to CK1δΔC. **E**, Quantification of panel D. Ordinary one-way ANOVA, *, p<0.05 (n=4, mean ± s.d.).

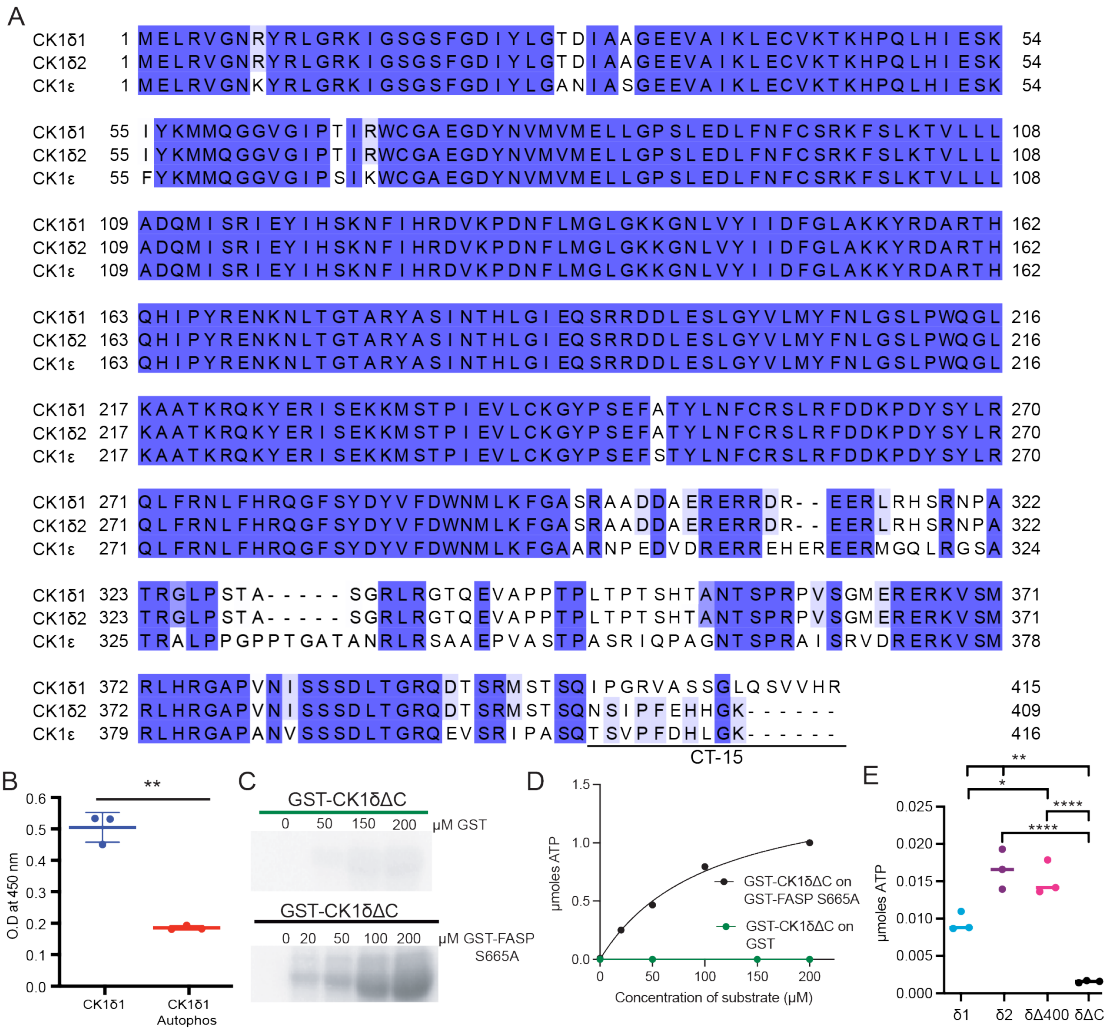


Figure 2.1.1

A, Sequence alignment of human CK1δ isoforms δ1 and δ2 with CK1ε. Kinase domain, residues 1-294. **B**, ELISA-based peptide kinase assay showing phosphorylation of the mouse PER2 FASP priming serine (pS659) with 50 ng CK1δ1 after pretreatment without (blue) or with (red) ATP to allow for kinase autophosphorylation. (n=3 mean ± s.d.). **C**, Representative autoradiograph of ^{32}P kinase assay of CK1δΔC activity on GST-human

PER2 FASP S665A or GST alone (n=1). **D**, Quantification of phosphorylation in panel C. **E**, Quantification of autophosphorylation in Fig 1C. Ordinary one-way ANOVA, * p>0.05; **, p<0.01; ****, p<0.0001 (n=4, mean ± s.d.).

2.4.2 Differences in regulation of the δ1 and δ2 tails by the kinase domain

Possible phosphorylation sites in the tail have been mapped on full-length CK1δ1 *in vitro* [77] and in cells, where a non-phosphorylatable (NP) mutant substituting all 28 serines and threonines in the tail to alanine eliminated its electrophoretic mobility shift and regulation by cellular protein phosphatases [89]. However, these studies did not unambiguously identify phosphorylation sites nor regions required for autoinhibition. The δ1 tail is 125 amino acids long and predicted to be intrinsically disordered, and no part of it was captured in a crystal structure of the full-length kinase [104]. Therefore, we turned to nuclear magnetic resonance (NMR) spectroscopy to monitor backbone chemical shifts on the ^{15}N δ1 tail alone or in the presence of equimolar amounts of the unlabeled kinase domain to better understand potential regulatory mechanisms. The ^{15}N-^{1}H HSQC spectrum of the isolated ^{15}N δ1 tail exhibited limited chemical shift dispersion consistent with an intrinsically disordered protein, and addition of kinase domain in the absence of ATP resulted in very minor chemical shift perturbations (Fig. 2.2A, left), indicating that the tail and kinase domain did not interact in *trans* at these concentrations. However, incubation of the same sample with ATP for 2 hours at 25 ºC resulted in

chemical shift perturbations in the ^{15}N δ1 tail backbone, as well as several new peaks downfield that correspond to phosphorylated residues (Fig. 2.2A, right), demonstrating that the kinase can phosphorylate its tail in *trans.*

Due to issues with peak overlap in the ^{15}N-^{1}H HSQC spectra, we turned to ^{13}C-direct detection experiments, using ^{15}N-^{13}C CON spectra that provide better resolution of the protein backbone in disordered proteins using the amide nitrogen and carboxyl carbon of the peptide bond [105]. We assigned a majority of the peaks in the ^{15}N-^{13}C CON spectrum of the δ1 tail using ^{13}C-direct detection and standard NH-edited methods. Addition of unlabeled kinase to the ^{13}C,^{15}N δ1 tail in the absence of ATP showed minimal chemical shift perturbation in the tail, similar to our ^{15}N-^{1}H HSQC data (Fig. 2.2.1A). After ATP addition, we observed chemical shift perturbations at the same resonances as before (Fig. 2.2B), as well as some peak broadening. Shifts were localized primarily to the XCT of the δ1 tail, and to a lesser extent in a charged region from residues 305-320 that is predicted to have alpha-helical structure (Fig. 2.2C-E). By contrast, ^{15}N-^{1}H HSQC spectra of the isolated δ2 tail showed chemical shift perturbations upon addition of kinase domain alone (Fig. 2.2.1B, left) that were enhanced by the addition of ATP (Fig. 2.2.1B, right), suggesting that the δ2 tail may interact with the kinase domain in *trans* in a manner different from the δ1 tail. Peaks in the CON spectrum of the ^{13}C,^{15}N δ2 tail were significantly broadened compared to the δ1 tail (Fig. 2.2.1C, D), limiting further

NMR analyses of the δ2 tail. These data suggest that the XCT of CK1δ1 is a uniquely favored target for phosphorylation by the kinase, even in *trans*.

To determine how regulation of the δ1 tail is influenced when tethered to the kinase domain, we produced a segmentally isotopically labeled full-length kinase using an NMR invisible, natural abundance kinase domain attached to an ^{15}N-labeled δ1 tail through Sortase A-mediated ligation (Fig. 2.2.1E) [106, 107]. ^{15}N-^{1}H HSQC spectra of the isolated ^{15}N-labeled δ1 tail and the tethered tail appeared to be the same, aside from broadening of a single glycine (G296) near the ligation juncture (Fig. 2.2F), suggesting that the kinase domain and tail do not interact in the unphosphorylated state in *cis* as well. This helps explain why unphosphorylated full length kinase has similar initial activity to the isolated CK1 kinase domain. Addition of ATP to the tethered tail sample resulted in chemical shifts in the ^{15}N-labeled δ1 tethered tail backbone similar to those we observed in *trans* (compare Fig 2.2A and Fig. 2.2.1F), indicating that tethering did not substantially alter kinase domain interaction and phosphorylation of the tail, including activity on the XCT. To confirm this, we generated a minimal δ1 tail construct beginning at residue 376. We obtained chemical shift assignments for the phosphopeaks that correspond to the three phosphosites specific to the δ1 tail (Fig. 2.2G). Phosphorylation of these residues in the full-length CK1δ1 protein was confirmed by LC/MS-MS (Fig. 2.2.1G). Using ^{15}N-edited 1D ^{1}H spectra of the phosphoserine region (Fig. 2.2H), we were able to monitor the kinetics of phosphorylation of the δ1-specific

residue S406. As expected, tethering increased the rate of phosphorylation compared to in *trans* (Fig. 2.2I) [108]. Collectively, our NMR studies revealed differences in how the δ1 and δ2 tails interact with the kinase domain and demonstrated a preference for phosphorylation of the δ1-specific XCT serines by the kinase domain. The preferential phosphorylation of these XCT serines suggests they may play a critical role in the autoinhibition of CK1δ1.

To test if any of the XCT phosphorylation sites are indeed autophosphorylated in cells, we generated an antibody that specifically recognizes a phosphorylated serine in the sequence SVV (pSVV antibody) that corresponds to S411 of CK1δ1 (Fig. 2.2.1H, I). This pSVV antibody detected robust autophosphorylation of purified full-length CK1δ1, which correlated with the band shift of autophosphorylated CK1δ1 (Fig. 2.2J). We then assessed phosphorylation of the XCT region in cells expressing myc-tagged CK1δ1. Global phosphorylation of the CK1δ tail is regulated *in vivo* by an active cycle of autophosphorylation and dephosphorylation [78]. Consistent with this, accumulation of S411 phosphorylation was detected when cells were treated with calyculin A, a broad-spectrum inhibitor of cellular Ser/Thr phosphatases, revealing reversible phosphorylation of CK1δ1 S411 in cells (Fig 2.2K). This is also consistent with reports of CK1δ1 phosphorylation at residues S406, S407, and S411 from large-scale phosphoproteomics screens [109, 110]. Phosphorylation of CK1δ1 S411 in cells was blocked by addition of the CK1δ/ε-specific inhibitor PF 670 (Fig 2.2L), indicating that it is due to

autophosphorylation. These data establish that S411 in the XCT of CK1δ1 is autophosphorylated in cells in a reversible and dynamic manner, consistent with this being a potential regulatory phosphorylation site.

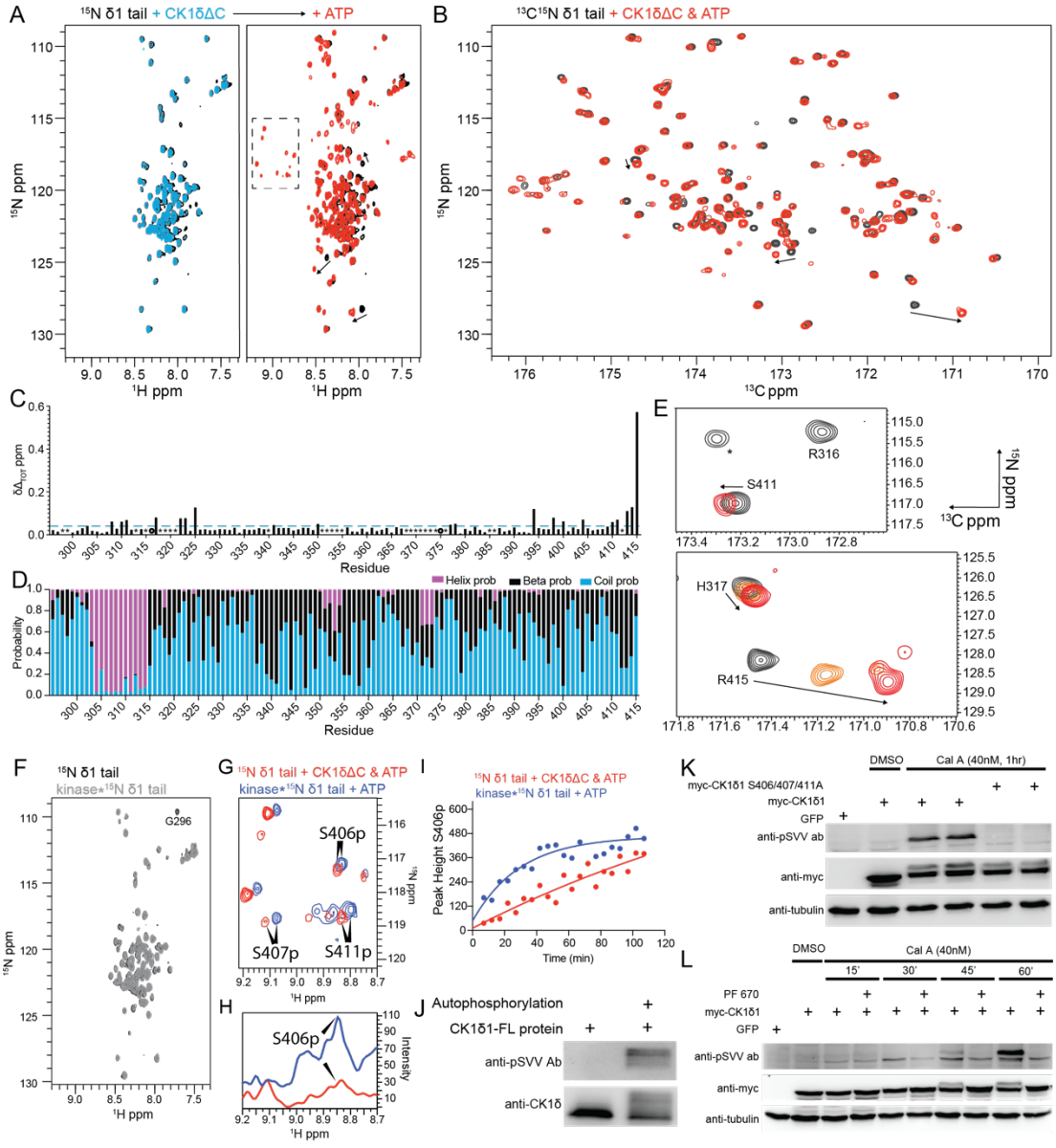
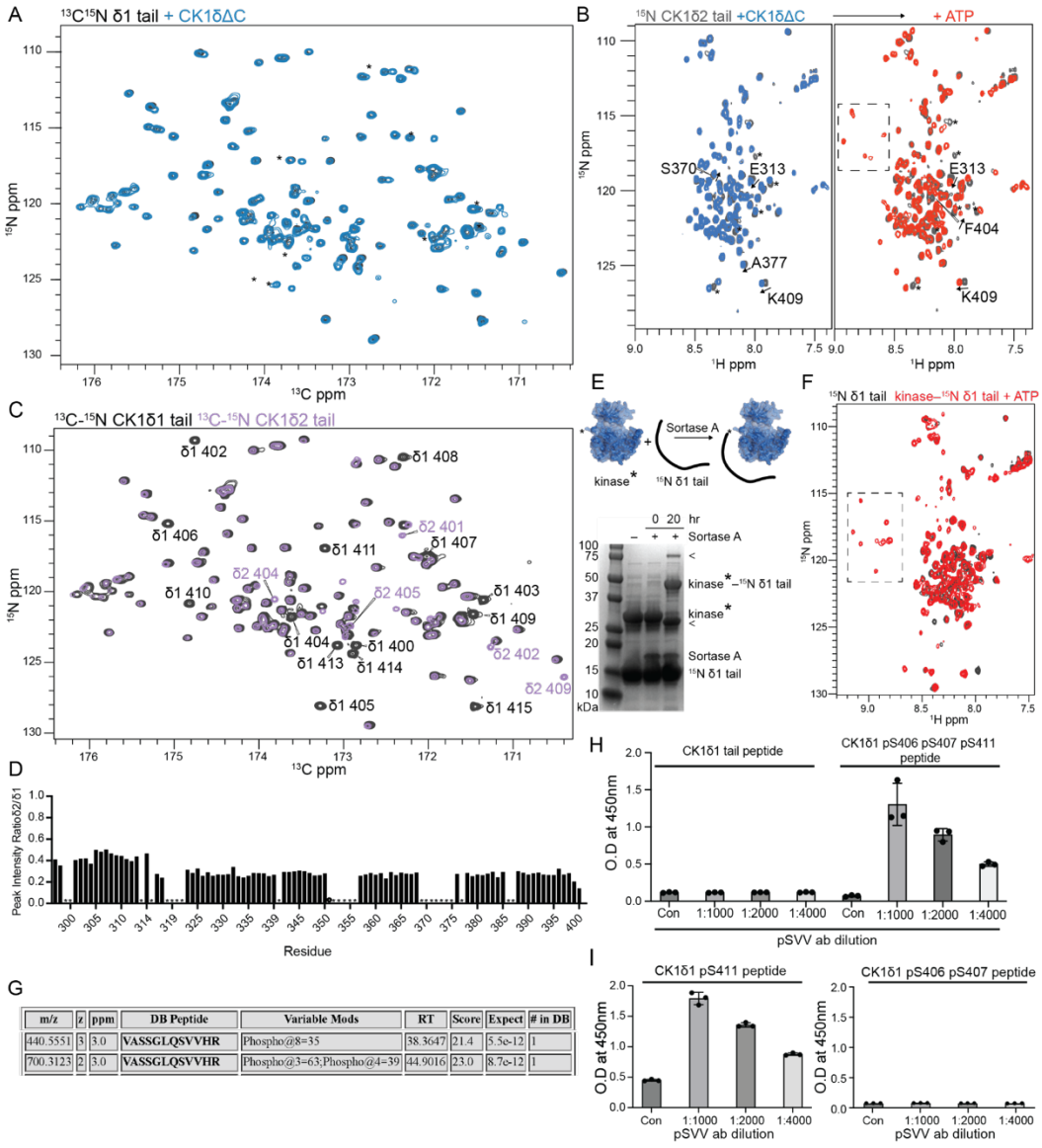


Figure 2.2 Phosphorylation-dependent interaction of the δ1 tail with the kinase domain

A, ^{15}N-^{1}H HSQC spectra of 100 μM ^{15}N δ1 tail alone (black) or in the presence of 100 μM CK1δΔC (blue), or 100 μM CK1δΔC and 2.5 mM ATP for 2 hours at 25 ºC (red); dashed box, peaks corresponding to phosphorylated residues. Arrows, chemical shift perturbation with phosphorylation. **B**, ^{15}N-^{13}C CON spectra of 100 μM ^{13}C,^{15}N δ1 tail alone (black) or in the presence of 100 μM CK1δΔC and 2.5 mM ATP for 2 hours at 25ºC (red). Arrows, chemical shift perturbation of the same peaks from panel B. **C**, Quantification of chemical shift perturbations ($\Delta\delta_{TOT}$, ppm) from the ^{15}N-^{13}C CON spectrum of the δ1 tail alone or after incubation with CK1δΔC and ATP. Dashed line, significance cutoff at 0.05 ppm; open circle, broadened peaks; *, unassigned peaks. **D**, Secondary Structure Propensity (SSP) analysis of the δ1 tail from chemical shift data. Random coil (blue), α-helix (pink), β-strand (black). **E**, View of CON spectra showing phosphorylation-dependent changes in the extreme C-terminus and predicted α-helical region. δ1 tail alone (black), with CK1δΔC and ATP for 2 hours at 25 ºC (red), or an unquenched partial phosphorylation reaction (orange). **F,** ^{15}N-^{1}H HSQC spectra of 34 μM δ1 tail alone (black) or in segmentally labeled kinase (gray). G296, only residue broadened in full-length kinase. **G**, ^{15}N-^{1}H HSQC spectra of ^{15}N δ1 tail with CK1δΔC and ATP in *trans* (red) or in *cis* (blue) with phosphopeak assignments. **H**, ^{15}N-edited 1D ^{1}H spectra of phosphopeaks in panel G. **I**, Quantification of 1D time course measuring phosphorylation of ^{15}N δ1 tail S406 in *trans* (red) or in *cis* (blue) with kinase. **J,** Representative western blot of purified CK1δ1 autophosphorylation detected with pSVV ab (n=3). **K,** Representative western blot of myc-CK1δ1 or myc-CK1δ1 S406/S407/S411A phosphorylation in cells treated with calyculin A (n=3). **L,** Representative western blot of CK1δ1 phosphorylation in cells treated with Cal A and PF 670 (n=3).



m/z	z	ppm	DB Peptide	Variable Mods	RT	Score	Expect	# in DB
440.5551	3	3.0	VASSGLQSVVHR	Phospho@8=35	38.3647	21.4	5.5e-12	1
700.3123	2	3.0	VASSGLQSVVHR	Phospho@3=63;Phospho@4=39	44.9016	23.0	8.7e-12	1

Figure 2.2.1

A, ^{15}N-^{13}C CON spectra of 100 μM ^{13}C,^{15}N δ1 tail alone (black) or in the presence of 100 μM CK1δΔC without ATP (blue). **B**, ^{15}N-^{1}H HSQC spectra of 100 μM ^{15}N δ2 tail alone (black) or in the presence of 100 μM CK1δΔC (blue) and 2.5 mM ATP (red) for 2 hours at 25 ºC; dashed box, peaks corresponding to phosphorylated residues. Arrows, chemical shift perturbation with kinase alone or with phosphorylation. **C**, ^{15}N-^{13}C CON spectra of 400

μM $^{13}C,^{15}N$ δ1 tail alone (black) compared to 400 μM $^{13}C,^{15}N$ δ2 tail alone (purple) with isoform-specific assignments. **D**, Peak intensity of ^{15}N-^{13}C CON δ2 compared to δ1; *, unassigned peaks. **E**, Synthetic scheme for segmental isotopic labeling of the δ1 tail in a full-length kinase. **F**, ^{15}N-^{1}H HSQC spectra of 34 μM ^{15}N δ1 tail alone (black) or the segmentally labeled kinase with 2.5 mM ATP (red) for 2 hours at 25ºC. **G,** Identification of δ1-specific phosphorylation sites from full-length autophosphorylated CK1δ1 after trypsin digest and LC/MS-MS. **H,** ELISA of unphosphorylated CK1δ1 tail peptide or a triply phosphorylated CK1δ1 tail peptide with the pSVV ab (n=3 mean ± s.d.). **I,** ELISA of CK1δ1 pS411 tail peptide or CK1δ1 pS406 pS407 tail peptide with the pSVV ab (n=3 mean ± s.d.).

2.4.3 Structural dynamics of CK1δ by HDX-MS in the presence of nucleotides or inhibitors

Kinase domains interact with a number of ligands, from nucleotides and substrates to products and inhibitors, all of which can exert changes in dynamics or structure across the protein [111]. To better understand autoinhibitory interactions between the kinase domain and tails of different CK1 isoforms, we turned to hydrogen-deuterium exchange mass spectrometry (HDX-MS) to characterize the conformational dynamics of different CK1δ isoforms. HDX-MS has been a powerful tool to provide insights into conformational dynamics in protein kinases [112-114]. We first explored the effects of nucleotide binding on the isolated kinase domain. Deuterium exchange measurements were carried out for CK1δΔC saturated with either ATP or its non-hydrolyzable analogue AMPPNP. Protection against deuterium

incorporation upon binding to either nucleotide was predominantly observed across the N-terminal lobe and active site (Fig. 2.3A, Fig 2.3.1), with lower deuterium exchange in the bound state reflecting decreased solvent accessibility and increased H-bond formation between the nucleotide-interacting residues. At the active site, ATP binding led to stronger protection than AMPPNP binding, highlighting the specificity of their interactions with CK1δΔC (Fig. 2.3B). These effects are similar to those observed for other kinases including PKA, ERK2, IRK, and SRC, as reported by numerous studies [115-118]. In addition to these direct 'orthosteric' effects, the binding of ATP and AMPPNP also elicited distal ('allosteric') conformational changes across the C-terminal lobe. Reduced deuterium exchange was observed across peptides spanning the DFG motif (residues 145-151), the activation loop (151-179), and loop L-EF (208-218), which are all centered around the substrate-binding cleft/groove (Fig 2.3A). No significant protection was detected in other regions of the C-lobe. Comparison of HDX-MS results also revealed the intrinsic dynamics of CK1δΔC, wherein peptides spanning the catalytic cleft and hinge region (residues 56-72) displayed bimodal isotopic distribution of mass spectra (Fig. 2.3C). The two populations indicate the open- and closed-conformations of CK1δΔC and the inherent inter-lobe interactions required for catalytic activity.

To validate HDX-MS as an approach to monitor inhibitory interactions on the kinase domain, we also looked at protection of CK1δΔC from deuterium

exchange in the presence of a quadruple phosphorylated inhibitory peptide from the PER2 FASP region, known as the 4p-FASP. This interaction has been previously characterized by X-ray crystallography [102]. Comparing the HDX behavior between CK1δΔC in the absence and presence of 4p-FASP revealed a reduction in conformational flexibility throughout the kinase domain (Fig. 2.3D), with the largest effects observed at peptides covering the nucleotide-binding site (4-20), hinge region (56-72), activation loop (150-179), and substrate-binding cleft (residues 209-230) (Fig. 2.3E), close to two conserved anion binding sites (located around R178 and K224) that mediate a direct interaction with the 4p-FASP peptide [102]. Comparing the effects of 4p-FASP binding to the HDX profile of ATP-bound CK1δΔC (Fig. 2.3D, E), we found that a larger segment of the activation loop and C-lobe had reduced solvent accessibility with 4p-FASP. These HDX-MS results confirm and extend findings from the co-crystal structure and illustrate how a phosphorylated substrate interacts with the substrate binding domain to substantially impact the conformational flexibility of CK1δ.

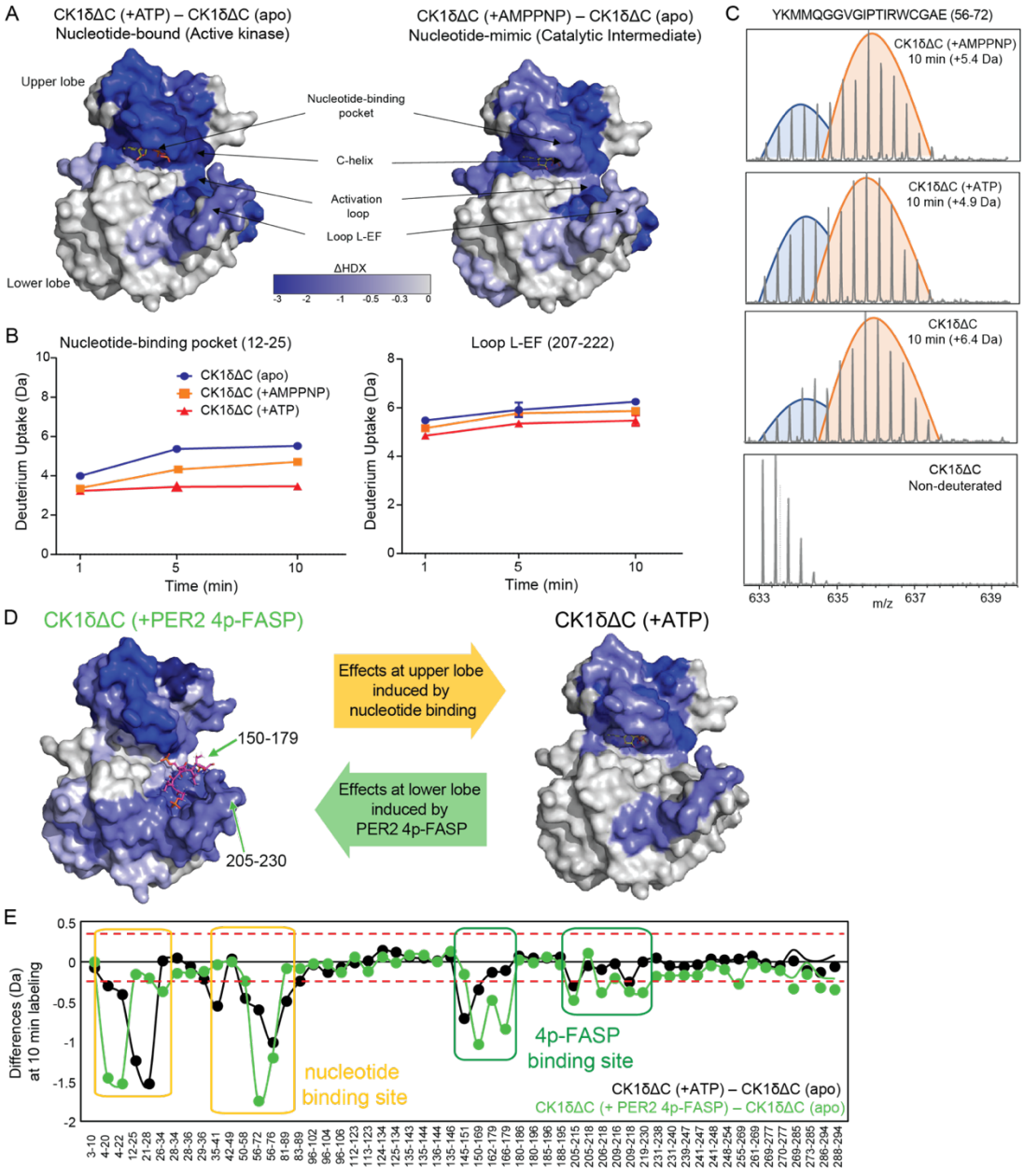


Figure 2.3 HDX-MS reveals dynamics in the CK1δ kinase domain in catalytic intermediate, active, and inhibited states

A, Structural representations of CK1δ kinase domain (PDB: 6PXO, with ATP (yellow) from PDB: 6RU6) indicating the regions with reduced deuterium exchange when saturated with ATP (left) or the ATP-analogue AMPPNP (right). Differences in deuterium exchange at 10

min labeling time are mapped in shades of blue as indicated. **B**, Kinetic plot of relative deuterium uptake for peptides spanning the nucleotide-binding pocket (left) and the catalytic loop (right) for CK1δΔC apo (blue), with ATP (red), or AMPPNP (orange). Average values (n = 6) from technical and biological replicates were used to generate the plot. **C**, Stacked mass spectral plots for peptide containing residues 56-72 with bimodal isotopic distribution at 10 min labeling time with lower (blue) and higher (orange) exchanging populations of CK1δΔC apo, or with ATP or AMPPNP, and a non-deuterated control. **D**, Structural representation of deuterium exchange at 10 min with ATP (right, PDB: 6PXO, with ATP (yellow) from PDB: 6RU6) or 4p-FASP (left, PDB: 8D7O). **E**, Comparison of differences in deuterium exchange of CK1δΔC in the presence and absence of 4p-FASP (green) or ATP (black) after 10 min labeling. Dashed line, significance threshold of ±0.28 Da. The deuterium exchange values are tabulated in Supplementary Table 1.

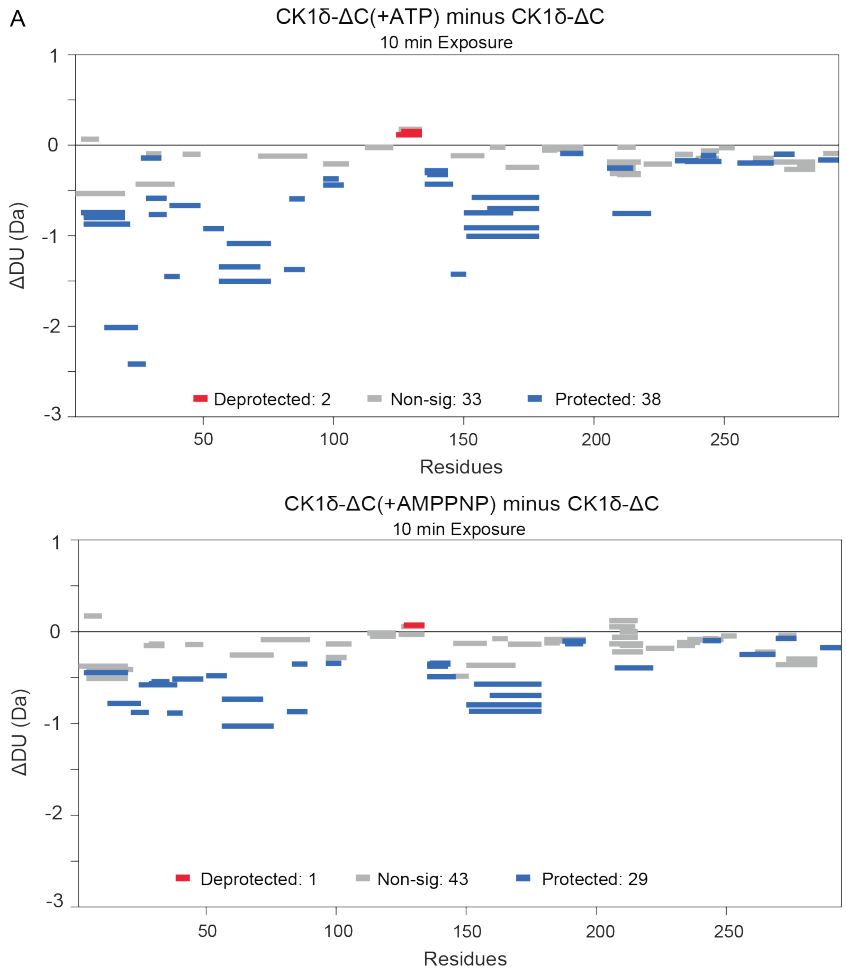


Figure 2.3.1 Conformational changes mediated by nucleotide binding to CK1δ

A, Woods differential plots showing the effect of ATP (top) or AMPPNP (bottom) relative to apo CK1δΔC. Plots generated using Deuteros 2.0. The 99.0% confidence interval was used to highlight peptides with significant deprotection (red), or protection (blue) compared to no change (gray) in deuterium exchange.

2.4.4 HDX-MS reveals the impact of CK1δ isoforms on structural dynamics of the kinase

To explore how differences in isoform phosphorylation affect interactions within CK1δ, we preincubated CK1δΔC, CK1δΔ400, CK1δ1, and CK1δ2 for 15 min at 25 ºC with excess AMPPNP or ATP to block or promote autophosphorylation, respectively, and then compared deuterium exchange profiles by HDX-MS (Fig. 2.4). The HDX-MS results, shown as Woods differential plots, highlight the peptides that undergo significant protection from deuterium exchange in the presence of ATP in blue, while peptides with increased exchange are in red (Fig. 2.4A-D). Similar to the results shown in figure 3, we observed protection in CK1δΔC in peptides covering primarily the N-lobe and substrate-binding groove (Fig. 2.4A). Similar changes in HDX profiles were observed for CK1δΔ400, which lacks the XCT, including across the N-lobe at the hinge region and αC helix, and the activation loop in the C-lobe (Fig. 2.4B). Two peptides at the junction between the kinase domain and the tail (residues 293-312 and 304-320) experienced a small but significant increase in deuterium exchange, indicating increased flexibility of this region upon ATP binding, although no other significant changes were observed along the C-terminal tail of CK1δΔ400 (Fig. 2.4B).

The presence of CK1δ1 and CK1δ2 XCTs led to different degrees of protection comparing autophosphorylation conditions (ATP) versus the non-hydrolyzable analogue AMPPNP (Fig 2.4C, D). In the kinase domain, CK1δ2 differed from CK1δΔ400 by modestly enhancing protection of the nucleotide

binding pocket (Fig 2.4D). However, CK1δ1, with its preferential phosphorylation sites in the XCT, was strikingly different. CK1δ1 autophosphorylation led to a large majority of peptides (71 peptides in CK1δ1 versus only 32 in CK1δ2) showing significant protection against deuterium exchange, spanning the N-lobe and the C-lobe. Peptides spanning the length of the disordered tail on CK1δ1 also showed significant protection, suggesting solvent inaccessibility, possibly due to interdomain interactions. CK1δ2 also had many peptides in the C-terminal tail that showed protection against deuterium exchange, albeit to a lesser extent (Fig. 2.4D, E).

To further understand changes in structural dynamics mediated by phosphorylation, we analyzed residue-specific deuterium exchange by analysis of overlapping peptides, represented as heat maps for each construct (Fig. 2.4E). These provide a comparative overview of time-dependent changes in exchange kinetics across the protein, including the C-terminal tail, and how truncation or splice isoforms influence this. These changes likely reflect both orthosteric effects of nucleotide-binding, as well as allosteric effects induced by the nucleotide and phosphorylation of the C-terminal tail. Lower overall deuterium exchange across CK1δ2 suggests that the δ2 tail has different conformational dynamics and/or interactions with the kinase domain from the δ1 tail, consistent with our NMR data. Furthermore, the effect of different tail lengths on the conformational changes on the kinase domain are clear when monitoring the HDX profiles of CK1δ1 with its other isoforms. With shortening

of tail from CK1δ1 to CK1δΔC, progressive decreases in deuterium exchange were observed across most peptides of the kinase domain (Fig. 2.4.1). The most notable changes were seen for peptides spanning the active site, anion binding sites, and substrate binding cleft. These results highlight the interactions mediated by the tail with the kinase domain to regulate autoinhibition.

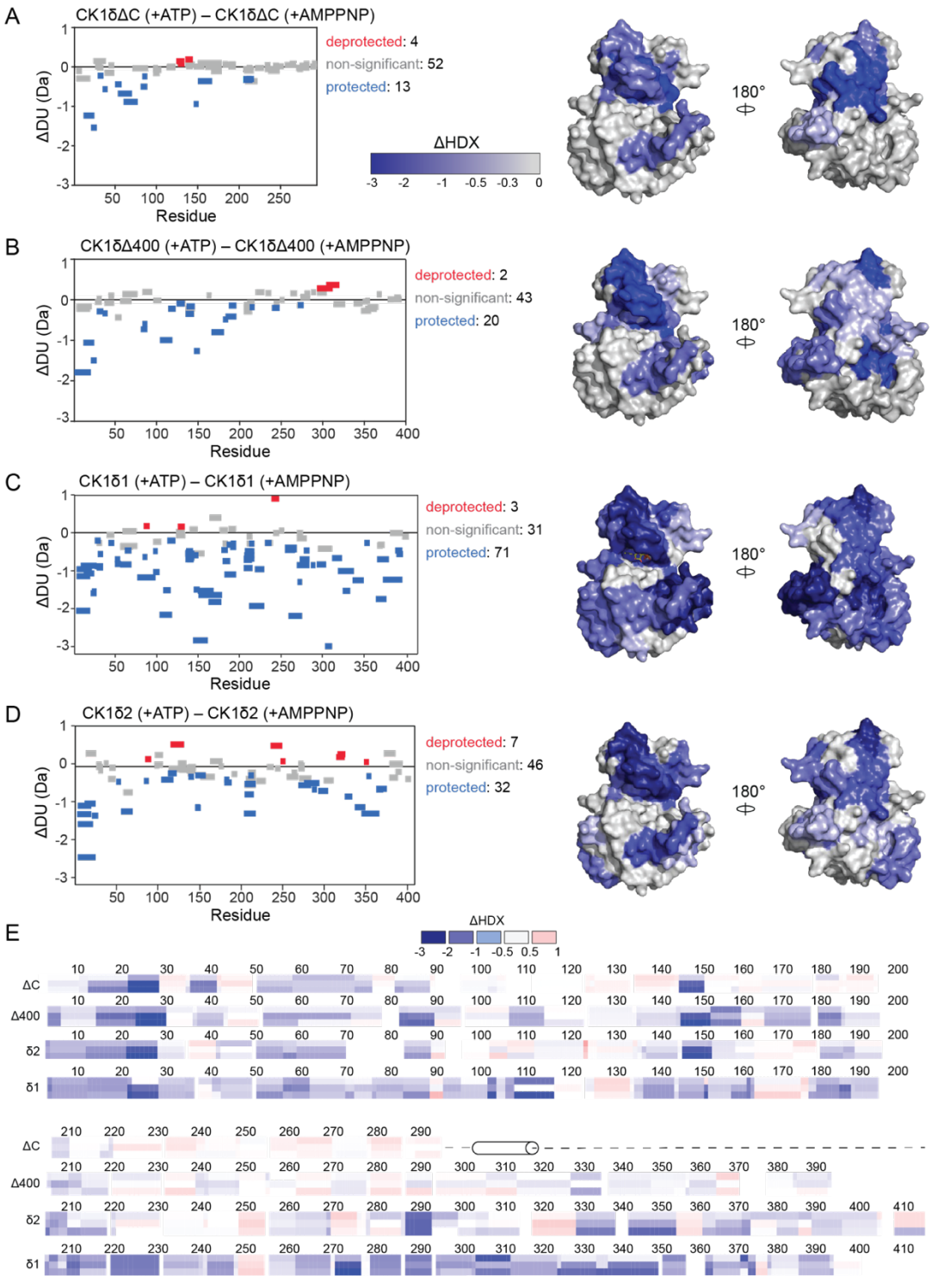


Figure 2.4 Impact of CK1δ tail on structural dynamics of the kinase.

A-D, Left, Woods differential plots showing deuterium exchange between ATP-bound and AMPPNP-bound states of different CK1δ constructs: (**A**) CK1δΔC; (**B**) CK1δΔ400; (**C**) CK1δ1; and (**D**) CK1δ2 at 10 min labeling time. Data available in Supplementary Table 2. Peptides showing significant protection (blue), deprotection (red), or non-significant (gray) deuterium exchange based on ±0.28 Da cutoff (confidence interval 99.0%). Right, corresponding structural representation of deuterium exchange differences at 10 min. **E**, Heat map of changes by residue in relative fractional uptake at 1 (top), 5 (middle), and 10 (bottom)-min labeling times for indicated constructs between ATP-bound and AMPPNP-bound states.

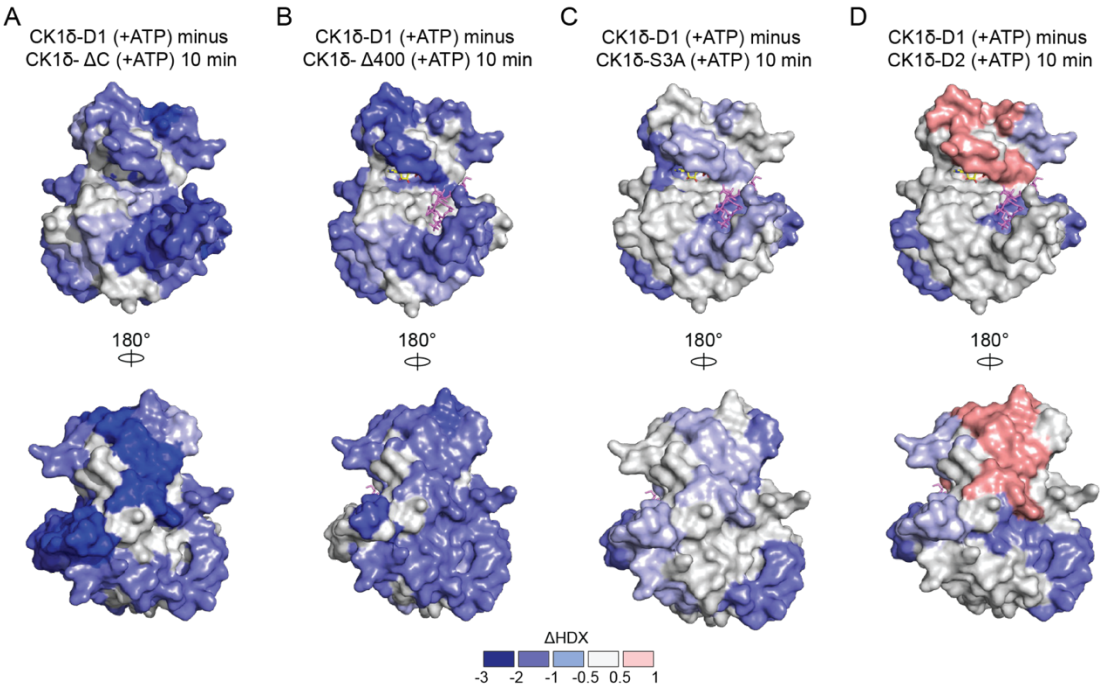


Figure 2.4.1 Comparing the effects of varying tail lengths with full-length CK1δ1

A-D, Structural representations of the differences in relative deuterium uptake values between CK1δ1 with different isoforms **A**, CK1δΔC, **B**, CK1δΔ400, **C**, CK1δ1 S3A, and **D**, CK1δ2 in ATP-bound states. The differences in RFU are mapped onto the kinase domain

(PDB: 6PXO) in front (top) and rear (bottom) views and are calculated as described in methods.

2.4.5 Anion binding sites on the kinase domain play a role in autoinhibition by the δ1 tail

One defining feature of CK1 is two anion binding sites near the active site that participate in substrate positioning and the conformation of the activation loop [55, 104]. The phosphorylated PER2 pFASP peptide docks its phosphate groups into these sites that span the substrate binding cleft of the kinase, thus inhibiting kinase activity (Fig 2.5A, C) [102]. We sought to determine if the phosphorylated XCTs utilize these anion binding sites to inhibit the kinase in a similar manner. First, we tracked changes in deuterium exchange of different kinase constructs by HDX-MS in the presence and absence of ATP, looking specifically at several peptides that are proximal to anion binding site 1 (Fig. 2.5A, B) and site 2 (Fig. 2.5C, D). For anion binding site 1, CK1δ1 showed the greatest protection for all three peptides, with the 4pFASP-bound kinase, CK1δ2, and CK1δΔ400 showing moderate protection compared to CK1δΔC (Fig. 2.5B). For anion binding site 2, CK1δ1 and the 4pFASP-bound kinase showed greater protection than the others (Fig. 2.5D).

To determine if these sites play a role in inhibition by the tails, we looked at the effect of a single, well-characterized point mutation at each anion binding site [55] on the ability of CK1δ1 or CK1δ2 to phosphorylate the PER2 FASP priming serine. Notably, these anion binding site mutations eliminated inhibition

by the phosphorylated PER2 FASP region [102]. The K224D mutation impaired phosphorylation by CK1δ2 and CK1ΔC, which we attribute to impaired substrate positioning based on prior work [55]. Remarkably, the same mutant in CK1δ1 had no decrease in FASP priming (Fig. 2.5E, 2.5.1A-B). Likewise, disruption of site 2 (R127E) had a similar isoform-specific effect on autoinhibition (Fig. 2.5F, 2.5.1C-D), although the effect was more modest than for site 1. We propose that the impaired substrate positioning caused by mutation of the anion binding sites is counter-balanced by relief of autoinhibition in CK1δ1, perhaps due to reduced XCT binding at the mutated anion binding sites.

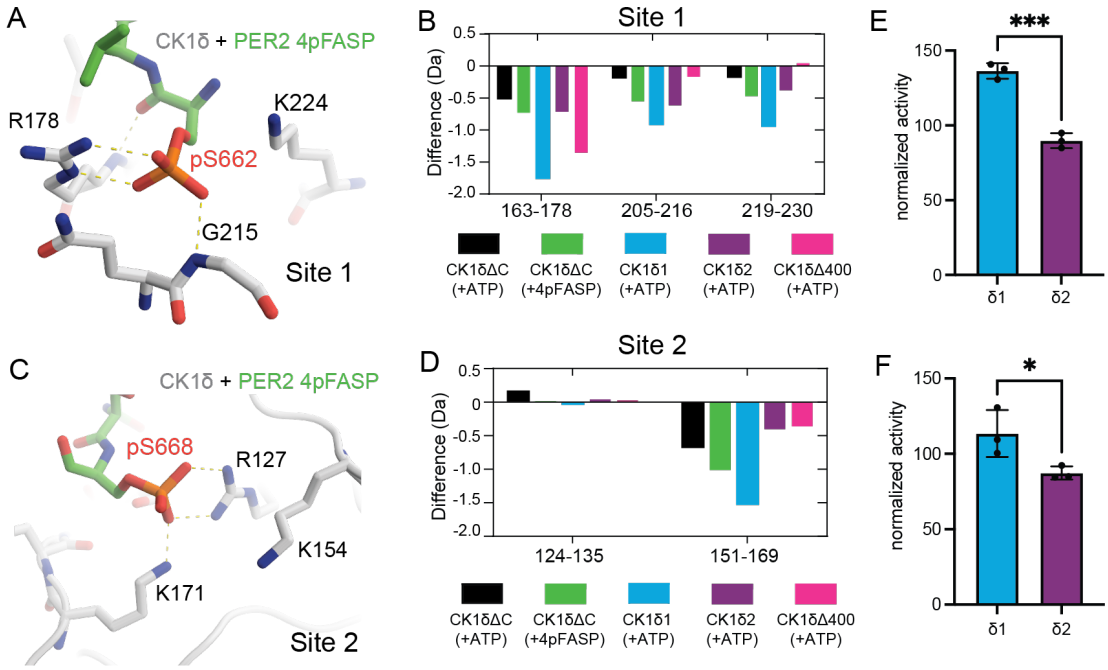


Figure 2.5 Anion binding sites on the kinase are required for autoinhibition by the δ1 tail

A, Structural view of CK1δ bound to the inhibitory PER2 4p-FASP (PDB: 8D70) showing phosphate coordination by anion binding site 1. **B**, Comparison of protection from deuterium exchange of peptides spanning anion binding site 1 with CK1δΔC (black), CK1δΔ400 (pink), CK1δ1 (blue), CK1δ2 (purple), and CK1δ-ΔC:4pFASP (green). **C**, Structural view of CK1δ bound to the inhibitory PER2 4p-FASP (PDB: 8D70) showing phosphate coordination by anion binding site 2. **D**, Comparison of protection from deuterium exchange of peptides spanning anion binding site 2. **E-F**, ^{32}P kinase assays comparing the activity of K224D (**E**) or R127E (**F**) in CK1δ1 (blue) and CK1δ2 (purple) to WT, normalized to the effect of the mutant on the isolated kinase domain (Fig. S5) (n=3, mean ± s.d.). Significance assessed by unpaired two-tailed t-test: *, p<0.05; ***, p<0.001.

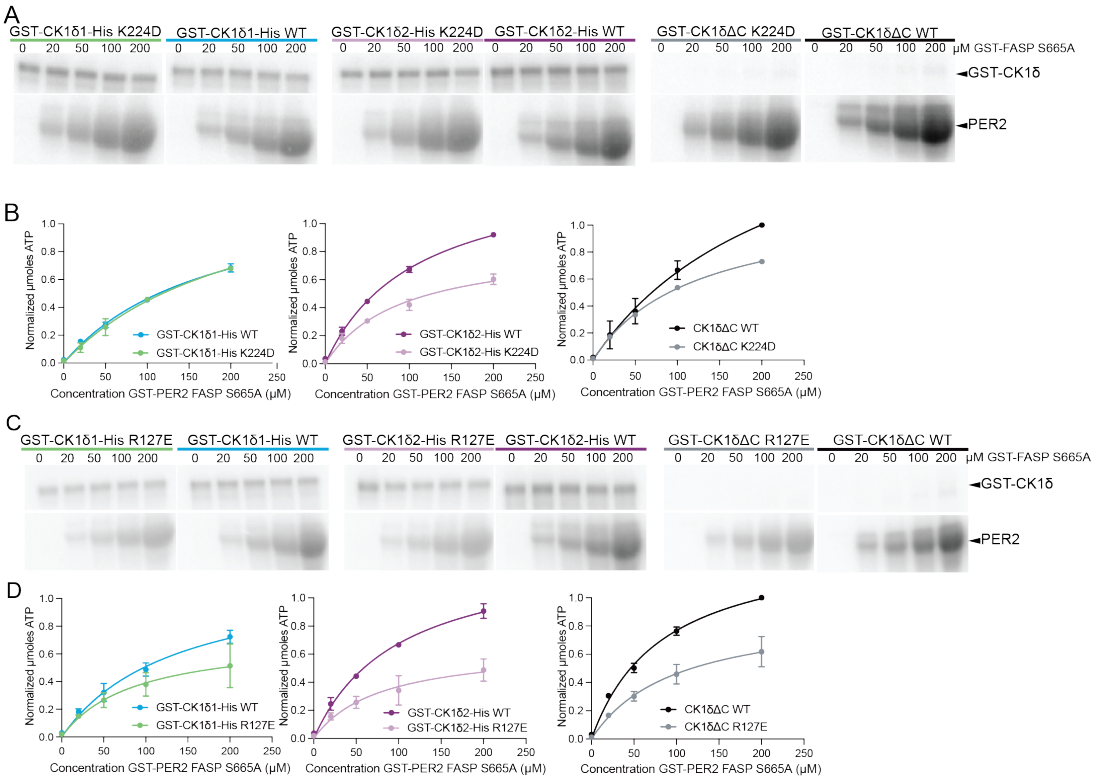


Figure 2.5.1

A, Representative autoradiographs from ^{32}P kinase assays of Site 1 mutant K224D or WT kinase construct as indicated on GST-PER2 FASP S665A (n=3). **B**, ^{32}P kinase assays of anion binding site 1 mutant, K224D, or WT in CK1δΔC (gray/black), CK1δ1 (green/blue), and CK1δ2 (lavender/purple) on PER2 GST-FASP-S665A (n=3, mean ± s.d.). **C**, Representative autoradiographs from ^{32}P kinase assay of Site 2 mutant R127E or WT kinase construct as indicated on GST-PER2 FASP S665A (n=3). **D**, ^{32}P kinase assays comparing activity of anion binding site 2 mutant, R127E, or WT in CK1δ-ΔC (gray/black), CK1δ1 (green/blue), and CK1δ2 (lavender/purple) on PER2 GST-FASP-S665A (n=3, mean ± s.d.)

2.4.6 Isoform-specific sequences of CK1δ differentially inhibit the kinase domain

Prior studies have shown that at least two substrates phosphorylated by CK1, the PER2 FASP region and the phosphorylation activation domain (PAD) of p63, inhibit kinase activity by product inhibition [102, 103]. To test if this model holds for CK1δ tail autoinhibition, where the isoform-specific peptides encoded by the XCT can act as direct inhibitors of the kinase, we added variably phosphorylated δ1 or δ2 peptides to the kinase in *trans* (Fig. 2.6A). To look at their effect on kinase activity, we used ADP-Glo assays with two PER2 substrates, the FASP region and the Degron, measuring activity with excess phosphopeptide compared to a reaction without peptides. For both substrates, inhibition by the δ1 peptides relied more on phosphorylation of the XCT, and the phosphorylated δ1 peptides were generally more potent inhibitors than the

δ2 peptides, although there were some differences in the efficacy for the two substrates (Fig. 2.6B, 2.6.1A).

We extended these findings to full-length CK1δ1 and tested if the δ1-specific phosphosites are necessary for inhibition in the full-length protein *in vitro*. We mutated the three unique phosphosites in δ1 to alanines (S3A: S406A/S407A/S411A) and compared the activity to wild-type CK1δ1 on the PER2 FASP S665A substrate (Fig. 2.6C, 2.6.1B). Consistent with the D1-A peptide data, the CK1δ1 S3A mutant showed a modest but significant increase in activity compared to CK1δ1 (Fig. 2.6C, D), demonstrating that phosphorylation of the extreme C-terminus is important for autoinhibition of CK1δ1.

We next tested if the S3A mutant altered the interaction and dynamics of the tail with the kinase domain as assessed by HDX-MS. Strikingly, we found a large decrease in protected peptides in CK1δ1 S3A relative to the wild-type enzyme ((from 71 to 19) Fig. 2.6E, 2.6.1C), spanning both the kinase domain and the C-terminal tail. Indeed, the protection of the kinase domain by CK1δ1 S3A most closely resembled the HDX-MS behavior for CK1δΔC (Fig 2.4A), suggesting a near-total loss of tail-kinase interaction in the mutant. Hence, phosphorylation of three serines in the CK1δ1 XCT is likely to be a prerequisite for the interaction of the C-terminal tail with the kinase domain.

These findings led us to examine how the CK1δ1 S3A mutation affected PER2 FASP phosphorylation and half-life in cells. FASP phosphorylation

increases the stability of PER2 [57]. Expression of the more active, less autoinhibited forms of CK1δ, CK1δΔC (Fig. 2.1D), significantly increased the stability of PER2-Luc after cycloheximide treatment in HEK293 cells compared to CK1δ1 (Fig. 2.6F). CK1δ1 S3A behaved similarly to CK1δΔC, demonstrating that loss of XCT phosphorylation in δ1 relieved autoinhibition (Fig. 2.6F). As a further test of the effect of the S3A mutation, we measured kinase activity on the FASP priming site in cells. Deletion of the C-terminal tail of CK1δ1 (CK1δΔC) or its S3A mutation markedly increased FASP phosphorylation in full-length PER2 in cells (Fig. 2.6G, H). These results confirm that phosphorylation of the three CK1δ1-specific serines is important for its autoinhibition both *in vitro* and *in vivo*.

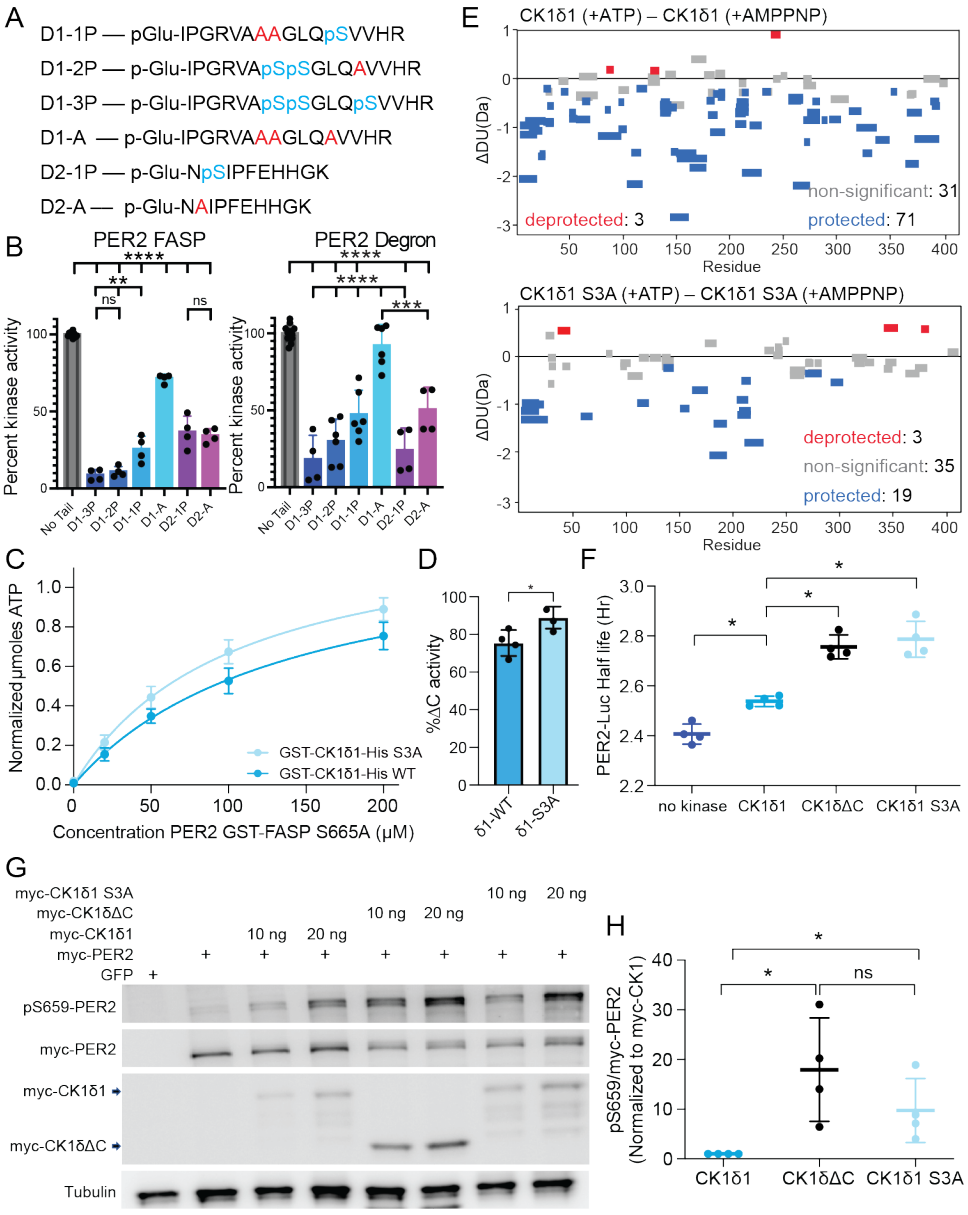


Figure 2.6 Variable C-terminal tail length and mutations impacts kinase activity of CK1-δ on its substrate PER2.

A, Phosphorylated CK1δ C-terminal peptides; blue, phosphorylated serines (pS); red, alanine mutations to block phosphorylation. **B**, ADP-Glo kinase assay with phosphorylated CK1δ C-terminal peptides on PER2 FASP (left) or Degron (right). Significance assessed by ordinary one-way ANOVA **, p<0.01; ****, p<0.0001 (n=2, mean ± s.d.). **C**, ^{32}P kinase

assay of CK1δ1 (blue) or the S3A mutant (light blue) on PER2 GST-FASP-S665A (n=3 mean ± s.d.). **D**, Quantification of panel C (n=3 mean + s.d.) Significance assessed by unpaired two-tailed t-test: *, $p<0.05$. **E**, Woods differential plots showing deuterium exchange between ATP-bound and AMPPNP-bound states of CK1δ1 (top) and CK1δ1 S3A (bottom) at 10 min labeling. Data available in Supplementary Table 2. Peptides showing significant protection (blue), deprotection (red) or non-significant (gray) deuterium exchange based on ±0.28 Da cutoff (confidence interval 99.0%). **F**, Half-life of PER2-Luc after co-transfection with 10 ng of indicated kinase constructs in HEK 293 cells (n=4 with mean ± s.d.). Significance assessed by unpaired two-tailed t-test: *, $p<0.05$. **G**, Representative western blot of mouse PER2 FASP priming phosphorylation (pS659) in HEK 293 lysates expressing indicated proteins (n=4). **H**, Quantification of PER2 FASP priming phosphorylation in panel G (n=4 with mean ± s.d.). Significance assessed by unpaired two-tailed t-test: *, $p<0.05$.

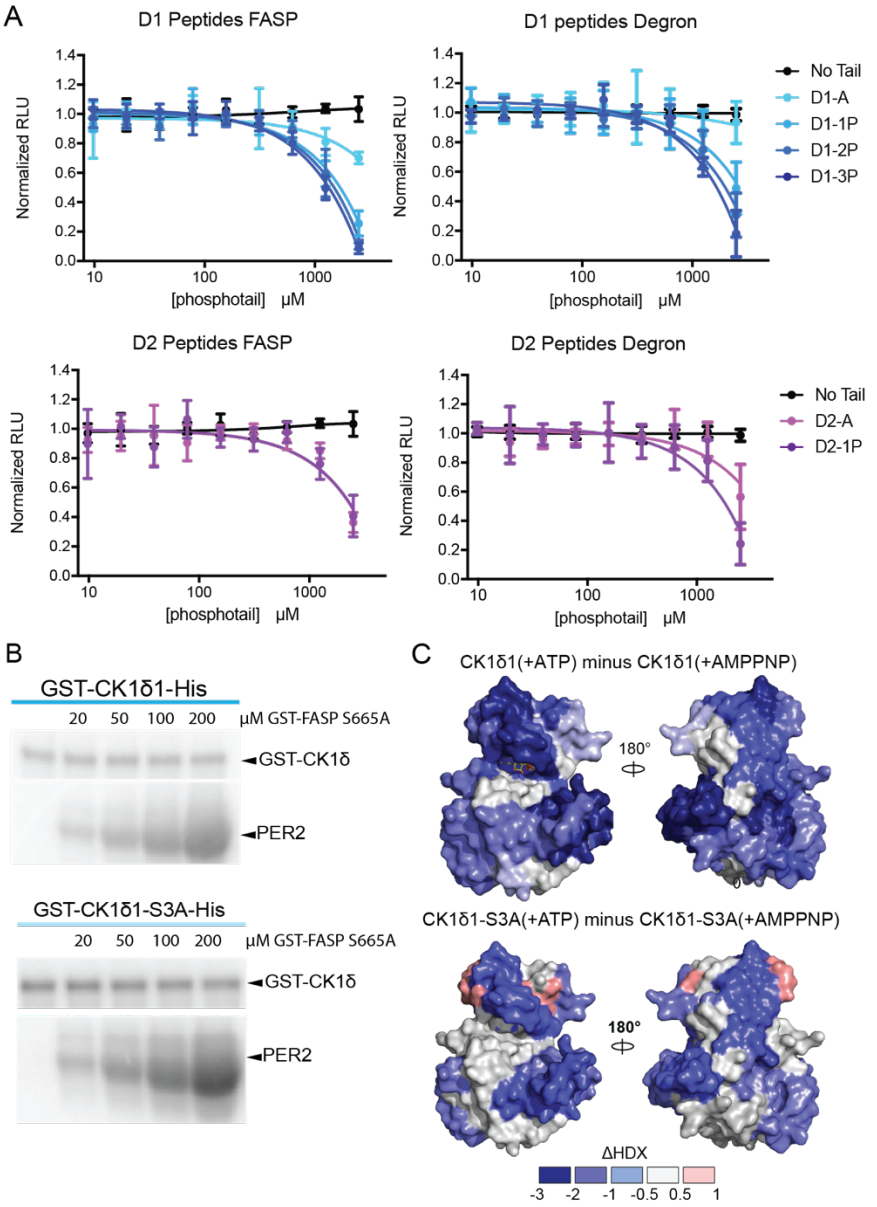


Figure 2.6.1

A, ADP-Glo kinase assay with phosphorylated CK1δ C-terminal peptides, D1 peptides (blue) and D2 peptides (purple), on PER2 FASP (left) or Degron (right). (n=2) **B,** Autoradiograph of ^{32}P kinase assay measuring autophosphorylation of kinase (top) or priming phosphorylation of the PER2 FASP substrate (bottom) for CK1δ1 (blue) and CK1δ1 S3A (light blue). Representative autoradiograph from n = 3. **C,** Structural

representation of the CK1δ kinase domain (PDB: 6PXO, with ATP (yellow) from PDB: 6RU6) indicating the regions with differing deuterium exchange in CK1δ1 (top) and CK1δ1 S3A (bottom) comparing ATP to AMPPNP-bound states (right). Differences in deuterium exchange at 10 min labeling time are mapped in shades of blue as indicated.

2.5 Discussion

CK1δ is considered a constitutively active kinase because it does not require phosphorylation of its activation loop to take on an active conformation. Instead, it relies on autophosphorylation of its C-terminal tail and/or feedback inhibition from phosphorylation of its substrates to regulate kinase activity [77, 79, 102]. While we are beginning to understand mechanisms of feedback inhibition [102, 103], the extent of intrinsic disorder and phosphorylation throughout the tail have impeded mechanistic understanding of autoinhibition. Since the CK1δ tail can be phosphorylated by other kinases to regulate its activity in cells [85-87, 89], the role of autophosphorylation *per se* in the regulation of kinase activity *in vivo* has been unclear. The kinase domain was recently reported to autophosphorylate a conserved threonine near the active site to modestly inhibit its activity and alter substrate selectivity [119]. We observed minimal autophosphorylation of the isolated kinase domain relative to the full-length isoforms, but further studies could look at the integration of regulatory autophosphorylation throughout the kinase. Critically, changes in circadian rhythms with two isoforms of CK1δ that differ only in the tail

demonstrate unambiguously that the tails have an important role *in vivo* [53, 91]. However, it was not clear from these studies if isoform-specific differences in activity occurred due to regulation of the tail by other kinases or if they were intrinsic to CK1δ. Here, we show that CK1δ isoforms have inherent differences in activity arising from interactions of the kinase domain and its disordered tail that are specified by isoform-specific differences in autophosphorylation of the XCT.

NMR spectroscopy allowed us to track site-specific autophosphorylation of the CK1δ disordered tails for the first time. We found that the 3 serines of the δ1-specific XCT are preferentially phosphorylated over other sites in the tail, whether the tail is phosphorylated by the kinase in *trans* or covalently linked to the kinase domain in *cis*. Chemical shift perturbations provided strong evidence for phosphorylation-independent effects of the kinase domain on the δ2 tail, although further analysis of the δ2 tail by NMR was limited due to extensive peak broadening in the absence of the kinase domain. This behavior is consistent with potential “fuzzy” intramolecular interactions within the δ2 tail, where the tail exhibits multiple transient conformations, as has been shown with other intrinsically disordered proteins [120, 121]. Isoform-specific differences in the dynamics of the disordered tails could contribute to decreased inhibition by the CK1δ2 isoform. Due to the sequence similarity of the XCT between CK1δ2 and CK1ε, the differences in tail dynamics we observed here could extend to CK1ε. The presence of disordered C-terminal

tails in orthologs of CK1δ in other species also suggest that this could be a conserved mechanism of autoinhibition.

We recently showed that the phosphorylated PER2 FASP substrate inhibits CK1δ through interaction with its conserved anion binding sites flanking the substrate binding cleft to regulate circadian rhythms [102]. HDX-MS analysis of the kinase domain bound to the inhibitory 4p-FASP phosphopeptide showed increased protection from deuterium exchange near these sites, confirming that HDX-MS could map inhibitory interactions on the kinase domain. Of the two CK1δ isoforms, CK1δ1 showed the greatest deuterium protection across the kinase domain, similar to protection seen with the 4p-FASP peptide. Protection in the kinase domain was moderately reduced in CK1δ2 and essentially eliminated by truncation of the XCT or the S3A mutant that eliminated phosphosites unique to δ1, indicating that phosphorylation of the XCT plays a crucial role in autoinhibition by CK1δ1. We also observed significant protection of the intrinsically disordered tail upon autophosphorylation of both CK1δ1 and CK1δ2 that was eliminated with truncation of the isoform-specific sequences. This suggests that both tails make extensive contact with the kinase domain, although we don't yet understand the specific details of these interactions. A previous study using crosslinking mass spectrometry proposed a possible contact site for part of the CK1ε tail on the kinase domain, although there were few crosslinks [84], suggesting that further optimization is needed. Our HDX-MS data support a model where the

tails make extensive contacts with the kinase domain, but neither the binding sites nor the orientation of the tail is clear yet.

Based on these results, we propose that autophosphorylation of the CK1δ tail dynamically changes its interaction with the kinase domain to regulate kinase activity. Here, we examined the effect of CK1δ autophosphorylation on the PER2 phosphoswitch, which controls PER2 stability and the period of circadian rhythms in mammals, including humans [57, 68]. Recent reports identified a role for CRY-PER-CK1δ complexes in phosphorylating the CLOCK subunit of the core circadian transcription factor CLOCK:BMAL1 to suppress its transcriptional activation of target genes by displacing the complex from DNA [4, 19]. Therefore, regulation of CK1δ activity by its isoform-specific tails could have several roles in regulating circadian rhythmicity, not only through modulating the stability of PER2 but also by instigating transcriptional repression through promoting the release of CLOCK:BMAL1 from DNA. CK1 isoforms also have major roles in pathways beyond circadian rhythms, such as apoptotic signaling [122, 123], Wnt signaling [124-126], and it is overexpressed in several cancers [127, 128]. Therefore, understanding its regulatory mechanisms goes beyond building a mechanistic understanding of circadian rhythms and could help lead to the development of targeted inhibitors that regulate autoinhibition.

2.6 MATERIALS AND METHODS

Protein constructs, expression, and purification

All constructs were expressed from TEV-cleavable vectors generated in-house based off the Parallel vector series [129], and have an N-terminal vector artifact (GAMDPEF) remaining after TEV cleavage. Mutations were introduced using the site-directed mutagenesis protocol in [130] and validated by sequencing.

Full-length human CK1δ isoforms (CK1δ1, residues 1-415; CK1δ2, residues 1-409) and CK1δ Δ400 (residues 1-400) were expressed with an N-terminal TEV-cleavable GST tag and a C-terminal His tag. Different constructs of the CK1δ catalytic domain, CK1δ ΔC (residues 1-294 or 1-317) were expressed with an N-terminal TEV-cleavable His-GST tag. A construct of the kinase domain for Sortase A-mediated ligations, CK1δ ΔC*, containing residues 1-294 with a C-terminal Sortase A recognition site (LPETGG) was expressed with an N-terminal TEV-cleavable His-MBP tag. Constructs of isolated CK1 tails (CK1δ1 tail, residues 295-415; CK1δ2 tail, residues 295-409; CK1δ1 short tail, residues 376-415) were expressed with an N-terminal TEV-cleavable His-NusA tag (HNXL) with an N-terminal tryptophan added for UV detection during purification.

Recombinantly expressed human PER2 peptides (FASP peptide, residues 645–687; Degron peptide, residues 475–505) were expressed with an N-terminal TEV-cleavable His-NusA tag when used for the ADP-Glo peptide

inhibition assays or with an N-terminal TEV-cleavable His-GST tag for the radioactive kinase assay. All recombinant PER2 peptides contain an N-terminal tryptophan for UV detection during purification and a polybasic motif (WRKKK) following the vector artifact, as in [102].

GST-lambda (λ) phosphatase-Strep-tag II was expressed with a TEV-cleavable N-terminal His-GST tag and a C-terminal Strep-tag II. His-Sortase A 7M (Addgene # 51141) and GST-λ phosphatase were expressed in BL21 (DE3) *Escherichia coli*, while all CK1 and PER constructs were expressed in Rosetta2 (DE3) *E. coli*. In general, cells were grown in LB media (for natural abundance growths) or M9 minimal media with the appropriate stable isotopes (i.e., $^{15}N/^{13}C$) for NMR as done before [53] at 37°C with shaking until the OD_{600} reached ~0.6-0.8. Expression was induced with 0.5 M IPTG, and cells were grown for an additional 16-20 hours at 18°C.

For purification of full-length CK1δ, cells were lysed in 50 mM Tris pH 7.5, 500 mM NaCl, 5% glycerol, 1 mM EDTA, 1 mM TCEP, 0.05% Tween using a high-pressure homogenizer. GST-tagged full-length proteins were purified from soluble lysate using Glutathione Sepharose resin (Cytiva) and eluted with buffer containing 50 mM Tris pH 7.5, 500 mM NaCl, 5% glycerol, 1 mM EDTA, 1 mM TCEP, 0.05% Tween, and 25 mM reduced glutathione. To remove phosphorylation from the kinase that may have occurred during expression or

purification, GST-tagged purified proteins were treated with 200 nM GST-λ phosphatase-Strep tag II supplemented with 10 μM $MnCl_2$ in λ phosphatase buffer (50 mM HEPES pH 7.5, 100 mM NaCl, 0.1 mM EDTA, 1 mM TCEP) overnight at 4°C. Strep-Tactin Sepharose resin (Cytiva) was used to remove GST-λ phosphatase-Strep tag II, and dephosphorylated CK1δ was collected with several washes of 50 mM Tris pH 7.5, 500 mM NaCl, 5% glycerol, 1 mM EDTA, 1 mM TCEP, and 0.05% Tween. Dephosphorylated proteins were then further purified by the C-terminal His-tag using Ni-NTA resin (Qiagen). Full-length dephosphorylated proteins were subsequently purified by size-exclusion chromatography on a HiLoad 16/600 Superdex 200 prep grade column (Cytiva) in 50 mM Tris pH 7.5, 200 mM NaCl, 1 mM EDTA, 5 mM β-mercaptoethanol (BME), 5% glycerol, and 0.05% Tween, and then frozen in aliquots for storage at -70°C.

For CK1δ catalytic domain preps, cells were lysed in 50 mM Tris pH 7.5, 500 mM NaCl, 5% glycerol, 1 mM EDTA, 1 mM TCEP, 0.05% Tween using a high-pressure homogenizer. His-GST-tagged proteins were purified from soluble lysate using Glutathione Sepharose resin (Cytiva) and eluted with 50 mM Tris pH 7.5, 500 mM NaCl, 5% glycerol, 1 mM EDTA, 1 mM TCEP, 0.05% Tween containing 25 mM reduced glutathione. To cleave the His-GST tag, His-TEV protease was incubated with the protein at 4°C overnight. Cleaved CK1δ ΔC was purified away from His-GST and His-TEV using Ni-NTA resin (Qiagen) and

size-exclusion chromatography on a HiLoad 16/600 Superdex 75 prep grade column (Cytiva) in 25 mM MOPS pH 7.0, 1 mM EDTA, 100 mM NaCl, 5 mM BME, 11 mM $MgCl_2$, and 0.05% Tween**,** and then frozen in aliquots for storage at -70°C.

For CK1δ ΔC* preps, cells were lysed in 50 mM Tris pH 7.5, 500 mM NaCl, 2 mM TCEP, 5% glycerol and 25 mM imidazole using a high-pressure extruder homogenizer. His-MBP- CK1δ ΔC* was purified using Ni-NTA resin (Qiagen) using standard approaches and eluted from the resin using 50 mM Tris pH 7.5, 500 mM NaCl, 2 mM TCEP, 5% glycerol and 250 mM imidazole. His-TEV protease was added to cleave the His-MBP tag from the CK1δ ΔC* at 4°C overnight. The cleavage reaction was subsequently concentrated and diluted to low imidazole concentration (< 25 mM), and then the kinase was purified away from His-MBP and His-TEV using Ni-NTA resin with 50 mM Tris pH 7.5, 500 mM NaCl, 2 mM TCEP, 5% glycerol and 25 mM imidazole. Protein was collected from the flow-through, then further purified using ion exchange chromatography with a HiTrap SP XL column (Cytiva) preceding size-exclusion chromatography on a HiLoad 16/600 Superdex 75 prep grade column (Cytiva) in 25 mM MOPS pH 7.0, 1 mM EDTA, 100 mM NaCl, 5 mM BME, 11mM $MgCl_2$, and 0.05% Tween**,** and then frozen in aliquots for storage at -70°C.

For CK1δ1 tail and CK1δ2 tail preps, cells were lysed in 50 mM Tris pH 7.5, 500 mM NaCl, 2 mM TCEP, 5% glycerol and 25 mM imidazole using a high-pressure extruder homogenizer. His-NusA-tail fusion proteins were purified using Ni-NTA resin (Qiagen) using standard approaches and His-TEV protease was added to cleave the His-NusA tag from the tail on column at 4°C overnight. Tails were purified away from the resin containing His-NusA and His-TEV with 50 mM Tris pH 7.5, 500 mM NaCl, 2 mM TCEP, 5% glycerol and 25 mM imidazole. Tails were further purified by size exclusion chromatography on a HiLoad 16/600 Superdex 75 prep grade column (Cytiva) using NMR buffer (25 mM MOPS pH 7.0, 1 mM EDTA, 100 mM NaCl, 5 mM BME, 11 mM $MgCl_2$, and 0.05% Tween) and frozen in aliquots for storage at -70°C.

For PER2 peptide preps, cells were lysed in 50 mM Tris pH 7.5, 500 mM NaCl, 2 mM TCEP, 5% glycerol and 25 mM imidazole using a high-pressure extruder homogenizer. His-NusA-FASP or His-NusA-Degron fusion proteins were purified using Ni-NTA resin (Qiagen) using standard approaches and His-TEV protease was added to cleave the His-NusA tag from the PER2 peptides on column at 4°C overnight. Peptides were purified away from the resin containing His-NusA and His-TEV with 50 mM Tris pH 7.5, 500 mM NaCl, 2 mM TCEP, 5% glycerol and 25 mM imidazole. Peptides were purified by size exclusion chromatography on a HiLoad 16/600 Superdex 75 prep grade column (Cytiva) using 1X kinase buffer (25 mM Tris pH 7.5, 100 mM NaCl, 10 mM $MgCl_2$, and

2 mM TCEP). For His-GST-tagged PER2 peptides used in the radioactive kinase assays, cells were lysed in 50 mM Tris pH 7.5, 500 mM NaCl, 5% glycerol, 1 mM EDTA, 1 mM TCEP, 0.05% Tween using a high-pressure homogenizer. His-GST-tagged proteins were purified from soluble lysate using Glutathione Sepharose resin (Cytiva) and eluted with 50 mM Tris pH 7.5, 500 mM NaCl, 5% glycerol, 1 mM EDTA, 1 mM TCEP, 0.05% Tween containing 25 mM reduced glutathione. The peptides were further purified using size-exclusion chromatography on a HiLoad 16/600 Superdex 75 prep grade column (Cytiva) in 25 mM Tris pH 7.5, 100 mM NaCl, 10 mM $MgCl_2$, and 2 mM TCEP, and then frozen in aliquots for storage at -70°C.

For His-Sortase A 7M, cells were lysed using a high-pressure extruder homogenizer followed by brief sonication at 4°C. After clarifying lysate on a centrifuge at 4°C at 140,500 × g for 1 hour, protein was captured using Ni-NTA affinity chromatography (Qiagen). The His-tag was cleaved using His-TEV protease on Ni-NTA resin at 4°C overnight. Cleaved protein was then collected from the flow-through and further purified using size exclusion chromatography on a HiLoad 16/600 Superdex 75 prep grade column (Cytiva) in 50 mM Tris, pH 7.5, 150 mM NaCl, and 10% glycerol, and then frozen in aliquots for storage at -70°C.

For GST-λ phosphatase-Strep tag II, cells were lysed in 50 mM Tris pH 7.5, 500 mM NaCl, 5% glycerol, 1 mM EDTA, 1 mM TCEP, 0.05% Tween using a high-pressure homogenizer. Proteins were purified from soluble lysate using Glutathione Sepharose resin (Cytiva) and eluted with a solution containing 50 mM Tris pH 7.5, 500 mM NaCl, 5% glycerol, 1 mM EDTA, 1 mM TCEP, 0.05% Tween, and 25 mM reduced glutathione. λ-phosphatase was further purified using size-exclusion chromatography on a HiLoad 16/600 Superdex 75 prep grade column (Cytiva) in 50 mM HEPES pH 7.5, 100 mM NaCl, 0.1 mM EDTA, and 1 mM TCEP, and then frozen in aliquots for storage at -70°C.

Solid-phase synthesis of human PER2 and CK1δ peptides

The following peptides were synthesized via solid-phase methods and purified to >90% purity by Biopeptide, Co.: human PER2 4pFASP, GKAEpSVApSLTpSQCpSYA (where pS represents a phosphoserine); human CK1δ D1-1P, pGlu-IPGRVAAAGLQpSVVHR; human CK1δ D1-2P, pGlu-IPGRVApSpSGLQAVVHR; human CK1δ D1-3P, pGlu-IPGRVApSpSGLQpSVVHR; human CK1δ D1-A, pGlu-IPGRVAAAGLQAVVHR; human CK1δ D2-1P, pGlu-NpSIPFEHHGK; human CK1δ D2-A, pGlu-NAIPFEHHGK. Following peptides used for ELISA assay were synthesized by Genscript with ≥95% purity, human CK1-δ1 tail peptide: IPGRVASSGLQSVVHR, human CK1-δ1-3P-tail: IPGRVApSpSGLQpSVVHR.

Segmental labeling with Sortase A

To generate the segmentally isotopically labeled kinase, we utilized Sortase A-mediated ligation reactions [107] between a natural abundance kinase domain (CK1δ ΔC*, harboring the Sortase A recognition motif, LPETGG) and a uniformly ^{15}N-labeled CK1δ1 tail with a free N-terminal glycine left after TEV cleavage. The reaction was incubated and dialyzed overnight at 4°C in (50 mM Tris pH 7.5, 200 mM NaCl, 1 mM EDTA, 5 mM BME, and 0.05% Tween). The His-Sortase A 7M enzyme was purified away from the reaction using Ni-NTA resin (Qiagen) with 50 mM Tris pH 7.5, 500 mM NaCl, 2 mM TCEP, 5% glycerol and 25 mM imidazole, while the ligated CK1δ ΔC*-^{15}N CK1δ1 tail product from the flow-through was further purified by size-exclusion chromatography on a Superdex 75 Increase 10/300 GL analytical grade column (Cytiva) in NMR buffer (25 mM MOPS pH 7.0, 1 mM EDTA, 100 mM NaCl, 5 mM BME, 11 mM $MgCl_2$, and 0.05% Tween) and frozen in aliquots for storage at -70°C.

***In vitro* kinase activity assays**

<u>γ^{32}P-ATP kinase assay</u>

Radioactive kinase assays were performed using human PER2 His-GST-FASP S665A or GST alone as the substrate. Reaction mixtures were prepared in reaction buffer (25 mM Tris pH 7.5, 200 mM NaCl, 10 mM $MgCl_2$, and 1 mM DTT) with 1 μM enzyme and 0-200 μM dilutions of the substrate as indicated at 25°C. Reactions were initiated with the addition of 2 mM ATP containing 2

μCi of ɣ-32p ATP (Perkin Elmer). All reactions were quenched with 2X SDS buffer after an incubation time of 1 hour. SDS-PAGE was performed on samples for each reaction, and the gels were dried at 80°C for 2 hours before being transferred to a storage phosphor screen (Amersham Biosciences) overnight. Images were collected with Typhoon Trio (Amersham Biosciences) and data were analyzed by densitometry using Image J (NIH), Excel (Microsoft), and Prism (GraphPad).

ELISA-based kinase assay

2 μg/mL of FASP-WT peptide was diluted in carbonate buffer, pH 9.5 and coated onto a 96 well plate (100 μL/well). The next day, 50 ng of CK1δ1 full-length (FL) (Signalchem) was incubated in kinase buffer (25 mM Tris pH 7.5, 5 mM beta glycerol phosphate, 2 mM DTT and 0.1 mM sodium orthovanadate) with or without 10 mM magnesium chloride and 200 μM ATP to allow for autophosphorylation for 30 min at 25°C in a reaction mixture of 20 μL. ELISA plate wells were washed thrice with wash buffer (PBS with 0.05% Tween 20, PBS-T) and once with kinase buffer. Reactions containing CK1δ1-FL purified protein with or without autophosphorylation was added to each well and the plate was incubated at 30°C for 1 hour. Next, the reaction mixture was discarded, and wells were washed with wash buffer thrice and incubated with blocking buffer (PBS-T with 5% BSA) for 1 hour at room temperature. Subsequently, wells were incubated with the polyclonal pS659 Ab, an anti-

rabbit antibody conjugated to Biotin, and Streptavidin-HRP for 1 hour each at room temperature with washing as above after each incubation. For signal detection, TMB reagent (1-Step Ultra TMB-ELISA, Thermo Scientific) was added, incubated at room temperature for color development and quenched by addition of STOP solution (Thermo Scientific). The plate was read at 450 nm using an xMark Spectrophotometer plate reader (BioRad). Each sample was analyzed in triplicate.

Autophosphorylation by ELISA and western blotting

For ELISA, 1 µg/ml of indicated tail peptide was diluted in carbonate buffer, pH 9.5 and coated onto a 96 well ELISA plate (100 µl/well) and incubated overnight at 4. The next day, wells were washed with wash buffer (PBS with 0.05% Tween, PBS-T), blocked with blocking buffer for 1 hr (PBS-T with 5% BSA) and incubated with the pSVV antibody for 1 hr at room temperature (RT). Next, wells were incubated with anti-rabbit antibody conjugated to Biotin and further incubated with Streptavidin-HRP for detection. For signal detection, TMB (1-Step Ultra TMB-ELISA, Thermo Scientific) was added, incubated for color development and stopped by addition of STOP solution (Thermo Scientific), which was read at 450 nm using an xMark Spectrophotometer plate reader (BioRad). Each sample was analyzed in triplicate.

For western blot, 75 ng of purified CK1δ1 full-length (FL) protein (Signalchem) was incubated in kinase buffer (25 mM Tris pH 7.5, 5 mM beta glycerol phosphate, 2 mM DTT and 0.1 mM sodium orthovanadate) without or with 10 mM magnesium chloride and 200 μM ATP (for autophosphorylation) for 30 min at 25°C. Then, Lammelli Buffer was added to samples and they were processed for SDS-PAGE and western blot. Either purified protein samples or whole cell extracts of transfected HEK293 cells lysed with cell lysis buffer (50 mM Tris-HCl pH 8.0, 150 mM NaCl, 1% Nonidet P-40, and 0.5% deoxycholic acid containing Complete protease inhibitors (Roche) and PhosStop phosphatase inhibitors (Roche)) were analyzed by denaturing SDS-PAGE, which was transferred to a PVDF membrane (Immobilon, Millipore). The blot was blocked and then probed using indicated primary antibodies and appropriate secondary antibodies conjugated with HRP as described. Signal was detected with ECL reagents from Thermo Fisher Scientific. Densitometric analysis of bands was performed using ImageJ (National Institutes of Health).

ADP-Glo peptide inhibition assay

Kinase reactions were performed on the recombinant human PER2 FASP or Degron using the ADP-Glo kinase assay kit (Promega) according to manufacturer's instructions. Synthetic peptide inhibitors were solubilized in water at a concentration of 10 mM. All kinase reactions were performed in 30 μL reactions in duplicate (n = 3 independent assays) with 100 μM ATP, 0.2 μM

CK1δ ΔC kinase, and 50 μM FASP or 100 μM Degron substrates were incubated in 1X kinase buffer (25 mM Tris pH 7.5, 100 mM NaCl, 2 mM TCEP, 10 mM $MgCl_2$) at room temperature for 1 hour for FASP or 3 hours for Degron in the presence of increasing amounts of synthetic δ1 or δ2 peptides as indicated. 5 μL aliquots were quenched with ADP-Glo reagent after incubation, and luminescence measurements were taken at room temperature with a SYNERGY2 microplate reader (BioTek) in black opaque 384-well microplates. Data analysis was performed using Prism (GraphPad).

PER2::LUC stability assays

Mouse PER2::LUC expression plasmids (10 ng) were transiently transfected alone or with myc-CK1δ1, myc-CK1δΔC, or myc-CK1δ1-S3A (10 ng) in 35 mm dishes of HEK293 cells in phenol red-free DMEM in the presence of 100 mM D-luciferin (#122799, PerkinElmer), 10 mM HEPES and 1.2 g/L sodium bicarbonate. Dishes were sealed with 40 mm cover glasses and vacuum grease and incubated in the LumiCycle (Actimetrics). The next day, 40 μg/mL cycloheximide (Sigma-Aldrich) was added per 35 mm dish. Luminescence data were used to calculate PER2::LUC half-life in Prism (GraphPad) using one-phase decay algorithm as described previously [57]. Briefly, half-lives were calculated using the one-phase decay algorithm in Prism (GraphPad) using the raw luciferase activity, beginning from the point of cycloheximide addition to the plateau at minimum luciferase activity (n = 4).

NMR spectroscopy

NMR spectra were collected on a Varian INOVA 600 MHz, or a Bruker 800 MHz spectrometer equipped with a ^{1}H, ^{13}C, ^{15}N triple resonance z-axis pulsed-field-gradient cryoprobe. Spectra were processed using NMRPipe [131] and analyzed using CCPNmr Analysis [132]. Spectra were collected in NMR buffer (25 mM MOPS pH 7.0, 1 mM EDTA, 100 mM NaCl, 5 mM BME, 11 mM $MgCl_2$, and 0.05% Tween) with 10% D_2O at 25°C. The backbone assignment of the δ1 and δ2 tails was accomplished using standard NH-edited triple-resonance experiments [HN(CA)CO, HNCO, HNCACB, and CBCA(CO)NH] and four-dimensional carbon direct-detection methods such as (HACA)N(CA)CON and (HACA)N(CA)NCO. Phosphopeak assignments of the CK1δ1 short tail were accomplished using standard NH-edited triple-resonance experiments [HN(CA)CO, HNCO, and HNCACB].

Chemical shift perturbation (Δδ) or the change in ^{13}C-^{15}N CON peak position was calculated using the following equation:

$$\Delta\delta = \sqrt{(N\Delta_{ppm}\alpha)^2 + (CO\Delta_{ppm})^2}$$

where Δ_{ppm} is the change in chemical shift and a value of α = 0.3 was used to normalize between ^{15}N and ^{13}C chemical shift ranges. NMR kinase reactions were performed at 25°C with 100 μM ^{13}C,^{15}N CK1δ1 tail or CK1δ2 tail, 2.5 mM ATP and 100 μM CK1δ ΔC. Samples were incubated for 2 hours and quenched

with 20 mM EDTA, then ^{1}H-^{15}N HSQC spectra (total data acquisition = 20 min) and ^{13}C-^{15}N CON spectra (total acquisition time= 9 hr 32 min) were collected. The incubation time to reach saturation of the kinase reaction with CK1δ1 short tail was determined by collecting a series of ^{1}H-^{15}N HSQC spectra (53 20-min spectra for a total acquisition time = 1,060 min) of 100 μM ^{13}C,^{15}N CK1δ1 short tail, 2.5 mM ATP and 100 μM CK1δ ΔC at 25°C. For phosphopeak assignments, the CK1δ1 short tail kinase reaction was then performed with 100 μM ^{13}C,^{15}N CK1δ1 short tail, 2.5 mM ATP and 100 μM CK1δ ΔC incubated overnight at 25°C and quenched with 20 mM EDTA, then ^{1}H-^{15}N HSQC spectra (total data acquisition = 20 min), ^{13}C-^{15}N CON spectra (total acquisition time= 9 hr 32 min), and triple-resonance experiments were collected.

Segmentally labeled NMR kinase reactions were performed at 25°C with 34 μM CK1δ ΔC*-^{15}N CK1δ1 and 2.5 mM ATP. Samples were prepared as described above, and then ^{1}H-^{15}N HSQC spectra (total data acquisition= 2 hours) were collected. For the phosphorylation kinetic assays, reactions were performed at 25°C with 34 μM CK1δ ΔC*-^{15}N CK1δ1 tail, or 34 μM CK1δ ΔC* and 34 μM ^{15}N CK1δ1 tail in *trans*, and then ^{15}N-edited 1D ^{1}H spectra were collected (224 data points at 6 min per spectrum for a total data acquisition= 1,344 min) immediately after the addition of 2.5 mM ATP. Spectra were processed using MestreNova (MestreLab), and data analysis was performed using Excel (Microsoft) and Prism (GraphPad).

Hydrogen-deuterium exchange mass spectrometry

Sample preparation

To determine the effects of the nucleotide binding and monitor catalytic activity, hydrogen-deuterium exchange mass spectrometry (HDX-MS) was performed for different CK1δ constructs in the presence and absence of ATP or AMPPNP. Different CK1δ constructs were incubated with either ATP (~800 μM) or AMPPNP (~1.2 mM), or neither (apo), and 2 mM $MgCl_2$ for 15 min at 25 °C before each hydrogen-deuterium exchange reaction. For HDX of CK1δ ΔC bound to the PER2 4p-FASP peptide, an excess of the peptide substrate (2 mM final concentration) was incubated with CK1δ ΔC in the presence of ADP and $MgCl_2$. Hydrogen-deuterium labeling was initiated by diluting 60-65 pmol of CK1δ saturated with ATP or AMPPNP as indicated above in a reaction buffer prepared in deuterium oxide (D_2O, final concentration of 90%). Pulse-labeling reactions were carried out for 1, 5, and 10-minute timepoints, after which the exchange was minimized by lowering the pH to 2.5 and temperature to 0°C by adding pre-chilled quench solution (0.5 M Guanidinium hydrochloride, 0.1 M TCEP). Non-deuterated controls were carried out by diluting nucleotide-free CK1δ proteins in an aqueous reaction buffer instead of a deuterated buffer.

Mass spectrometry data acquisition

For nanoLC-MS/MS analyses, each quenched sample was subjected to in-line proteolysis using the Enzymate™ immobilized pepsin cartridge (Waters, USA). The digestion was left to proceed for 3 minutes at 12°C, and the resulting

peptide mixtures were analyzed with nanoACQUITY M-class UPLC (Waters, UK) equipped with a trapping column (VanGuard C18, 5 µm particle size) and an analytical column (BEH C18, 75 µm × 100 mm, 1.7 µm particle size) maintained at 3°C to minimize back-exchange [133]. The aqueous solvent was 0.1% formic acid in LC-MS grade water, and the organic solvent was 0.1% formic acid in HPLC-grade acetonitrile. A 10-minute gradient was used to resolve and elute the peptides by reverse-phase chromatography. The peptides were then identified by Synapt G2-Si high-resolution mass spectrometer (Waters, UK). Briefly, the mass spectrometer was operated in positive polarity, data-independent (DIA) fragmentation and ion-mobility ($HDMS^E$) mode separation. Mass spectrometer parameters were as described previously [134]. Glu-fibrinopeptide reference (m/z = 785.8426) was continuously sprayed every 30 seconds during ESI-MS experiments using the lockspray device to ensure mass accuracy.

HDX data analysis

Peak lists for identifying and annotating the peptides from non-deuterated controls were generated by Protein Lynx Global Server v3.0 (Waters, USA), using human CK1δ (Uniprot: P48730) construct-specific protein database and searched against the parameters described previously [134]. Peptide mass measurements for individual peptides were assigned using DynamX v3.0 software (Waters, USA). Peptides with a minimum intensity of 2000, maximum length of 25 amino acids, and mass error within ± 10 ppm were considered for

analysis. Deuterium exchange measurements were determined for all labeling timepoints across different protein states and were manually verified, but the data were not corrected for back-exchange. Only peptides with high signal-to-noise ratio and non-overlapping spectra were considered for final analysis. All measurements were done in biological duplicates and technical triplicates, with statistical analysis done using Deuteros 2.0 [135] and tabulated as Supplementary Tables 1-3.

HDX data representation

Relative fractional deuterium uptake (RFU) is the ratio of the number of deuterons exchanged to the maximum number of available amides per peptide. Differences in deuterium exchange (ΔDU) were calculated as differences in centroid values of a deuterated peptide in protein condition with its corresponding deuterated peptide in a different protein condition. The pepsin digestion maps showing the sequence coverage of various CK1δ constructs and their corresponding deuterium uptake values are provided in Supplementary Tables 1 and 2. HDX profiles between different nucleotide bound CK1δ constructs indicated in Supplementary Figure S4 were compared by subtracting the RFU values for common peptides, as per the following scheme:

$$\Delta DU = RFU_{CK1\delta1(+ATP)} - RFU_{x(+ATP)}$$

where x = CK1δ2, CK1δ1 S3A, CK1δ Δ400, or CK1δ ΔC

Quantification and statistical analysis

All statistical analyses were done using Prism (GraphPad). P-values were calculated using Students t-test, one-way ANOVA with Tukey's multiple comparisons test, or extra sum of squares F-test as indicated in different figures. In all figures, * indicates $p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$, ns, not significant.

Data availability

NMR chemical shift assignments for the human CK1 δ1 and δ2 tails have been deposited at BMRB and will be publicly available upon publication.

Chapter 3

Future Directions

3.1 Anion binding sites on the catalytic domain contribute to isoform-specific autoinhibition

3.1.1 Summary

In this section, I expound upon work on anion binding site mutants that were previously characterized in the context of tailless CK1δ [55]. Here, I use the mutants in the full length CK1δ isoforms in order to probe their role in the tail's autoinhibition. Chapter 2 highlights the role of anion site 1 and 2 in CK1δ1-specific autoinhibition. This section shows a unique role for anion site 3, wherein mutation of this site increases the level of autoinhibition, again specifically for CK1δ1. In order to directly see how the phosphorylated tail is interacting with these sites, we crystallized a phosphorylated δ1 extreme C-terminus tethered to the kinase domain and found density for a phosphoserine in anion site 1. Previous work showed that other inhibitory substrates also bind phosphorylated residues into the anion sites, pointing to a conserved mechanism of CK1 substrate inhibition [102, 103].

3.1.2 Mutation of anion site 3 increases autoinhibition specifically for CK1δ1

Personal correspondence of unpublished data from collaborators showed that anion site 3, and its mutation H165D, plays a role in CK1α activity in the fungus *Neurospora crassa*. This led us to investigate the corresponding

mutation in the human kinase, H162D, and what role it may play in the activity of CK1δ in mammals. I incorporated the mutation into three versions of CK1δ: a tailless CK1δΔC, CK1δ1, and CK1δ2. I then compared the activity of the mutant to the corresponding WT on phosphorylation of either hPER2 FASP S665A (Fig 3.1A, B) or hPER2 Degron (Fig 3.1C, D). H162D in CK1δΔC and CK1δ2 showed very little change in activity compared to WT for either of the substrates, whereas CK1δ1 H162D showed greatly reduced activity, specifically for hPER2 FASP S665A (Fig 3.1 E). HDX-MS analysis of the peptides spanning site 3 showed that CK1δ1, as well as a mutant of its three unique phosphorylation sites discovered in Chapter 2, CK1δ1 S3A, have increased occupancy upon autophosphorylation compared to CK1δ2, CK1δΔ400, and CK1δΔC (Fig 3.1 F). This shows that CK1δ1 has a unique interaction with anion site 3 that does not depend on the phosphorylation of its extreme C-terminus and indicates that anion site 3 may play a role in sequestering the autoinhibitory tail in a substrate-dependent manner. The question remains of what part of the CK1δ1 tail is interacting with this anion site. Since the S3A mutation still shows occupancy at anion site 3, it is not the phosphorylated sites of the δ1 extreme C-terminus that bind there. Also, the differences in how the anion site mutant affects CK1δ1 activity on FASP priming versus degron also brings into question how this interaction may be changing with different substrates.

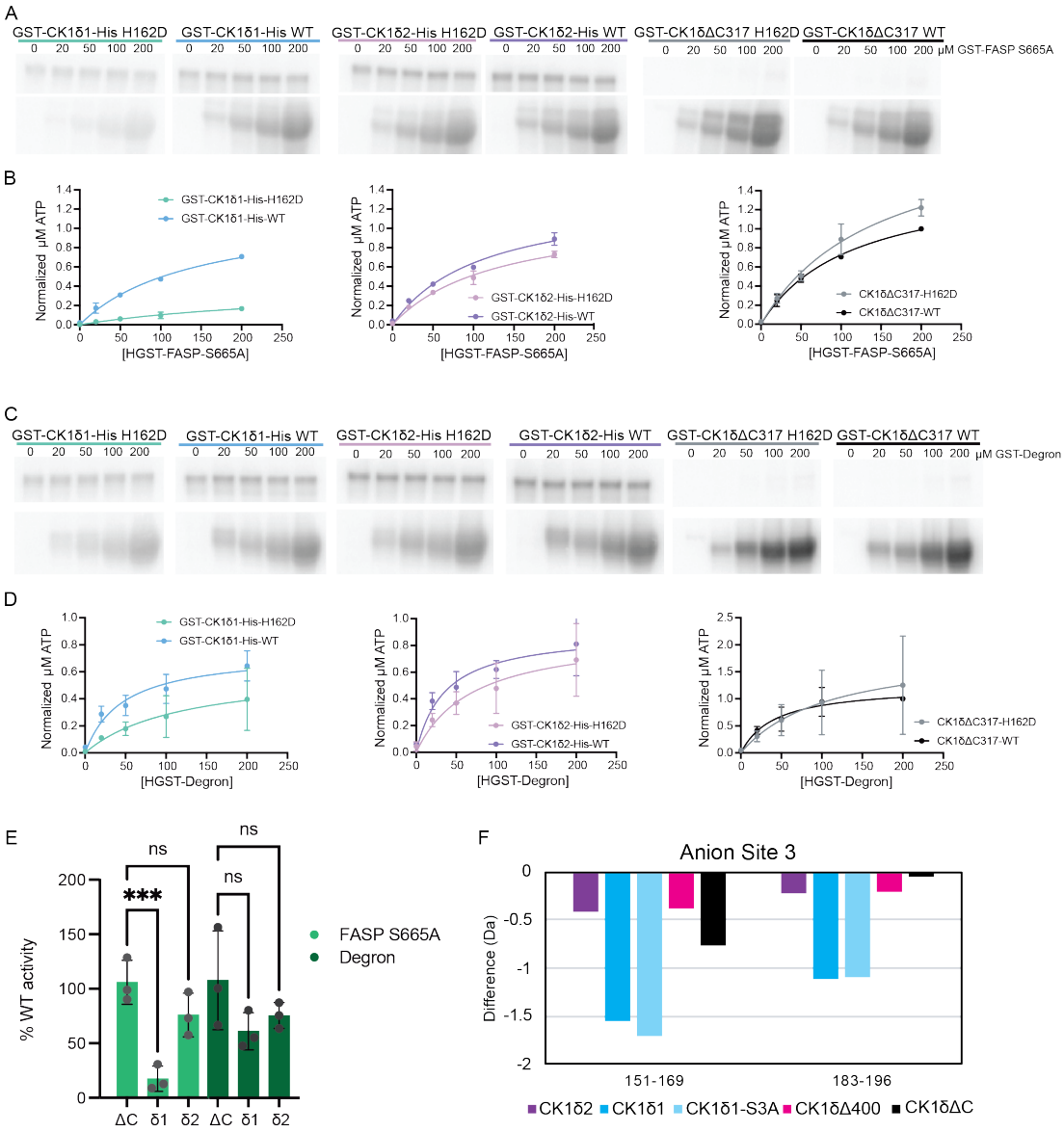


Figure 3.1 Anion Site 3 influences CK1δ1 autoinhibition in a substrate-dependent manner

A, Representative autoradiographs from ^{32}P kinase assays of Site 3 mutant H162D or WT kinase construct as indicated on GST-PER2 FASP S665A (n=3). **B**, ^{32}P kinase assays of anion binding site 3 mutant, H162D, or WT in CK1δΔC (gray/black), CK1δ1 (green/blue), and CK1δ2 (lavender/purple) on PER2 GST-FASP-S665A (n=3, mean ± s.d.). **C**,

Representative autoradiographs from ^{32}P kinase assay of Site 3 mutant H162D or WT kinase construct as indicated on GST-PER2 Degron (n=3). **D**, ^{32}P kinase assays comparing activity of anion binding site 3 mutant, H162D, or WT in CK1δ-ΔC (gray/black), CK1δ1 (green/blue), and CK1δ2 (lavender/purple) on GST-PER2 Degron (n=3, mean ± s.d). **E**, Quantification of the activity of anion site 3 mutant, H162D, compared to the corresponding WT activity on GST-PER2 FASP S665A substrate (light green) or GST-PER2 Degron substrate (dark green) Significance assessed by ordinary two-way ANOVA ***, $p<0.0001$ (n=3, mean ± s.d.). **F,** Comparison of protection from deuterium exchange of peptides spanning anion binding site 3 with CK1δΔC (black), CK1δΔ400 (pink), CK1δ1 (blue), CK1δ2 (purple), and CK1δ1 S3A (light blue).

3.1.3 Crystallization of phosphorylated δ1 tail

Anion binding site mutants utilized in Chapter 2 Fig 2.5 strongly suggested that CK1δ1-specific inhibition relies on these sites. Previous work has shown that inhibitory phosphorylated peptides from substrates can be crystallized with the kinase domain [102, 103]. This led us to try soaking CK1δΔC anion free crystals with δ1 tail peptide phosphorylated at all three sites identified in Chapter 2, D1-3P. Anion free conditions were used so that the phosphorylated residues do not have to compete with sulfate to bind the anion sites, as the binding affinity of these substrates is very low. We also attempted soaking δ2 tail peptides with and without phosphorylation and in anion containing conditions. Unfortunately, density for the δ2 tail peptides was not present for any of our crystals. The δ1 tail peptide, D1-3P, showed some density for a phosphate in anion site 1, but it didn't extend into any backbone density for the peptide (Fig

3.2 D). Since the tail is natively attached to the kinase, thus increasing its local concentration, a construct with the phosphorylated tail still attached to the kinase would be more biologically relevant and potentially allow for crystallization of the phosphorylated tail. Previous studies have tried to crystalize full length CK1δ and saw no density for the tail [104], so a minimal construct of the unique δ1 region of interest with a short native linker to minimize the disorder could aid in capturing the tail in the crystal structure. I then designed a CK1δ-D1 tether construct that contains the catalytic kinase domain with a 10 amino acid linker of the native tail sequence upstream of the extreme C-terminus that comprises the unique δ1 tail (Fig 3.2 A). In ^{32}P kinase assays on PER2 priming phosphorylation, CK1δ-D1 tether shows autophosphorylation and decreased activity on PER2 compared to tailless kinase, CK1δ-ΔC; however, the inhibition is not as extensive as the full length CK1δ1 (Fig 3.2 B, C). This shows that the extreme C-terminus is a major component, but not the sole contributor to autoinhibition and that more needs to be done to explore the role of the upstream tail [77-79]. The crystal structure of CK1δ-D1 tether clearly shows density for a phospho-serine being coordinated in anion site 1, with several of the same contacts exhibited by phosphorylated FASP illustrated in Chapter 2 Fig 2.5 A (Fig 3.2 E). The exact identity of this serine could not be determined, as there is only backbone density around the site and the density does not extend to the C-term of the kinase domain. This likely suggests that several different phospho-serines

(there are 6 serines in the tethered tail) of the tail bind into anion site 1. Anion site 2 did not have enough density to model in the phosphotail. This data agrees with the conclusions of Chapter 2 of the importance on anion site 1 for CK1δ1-specific autoinhibition.

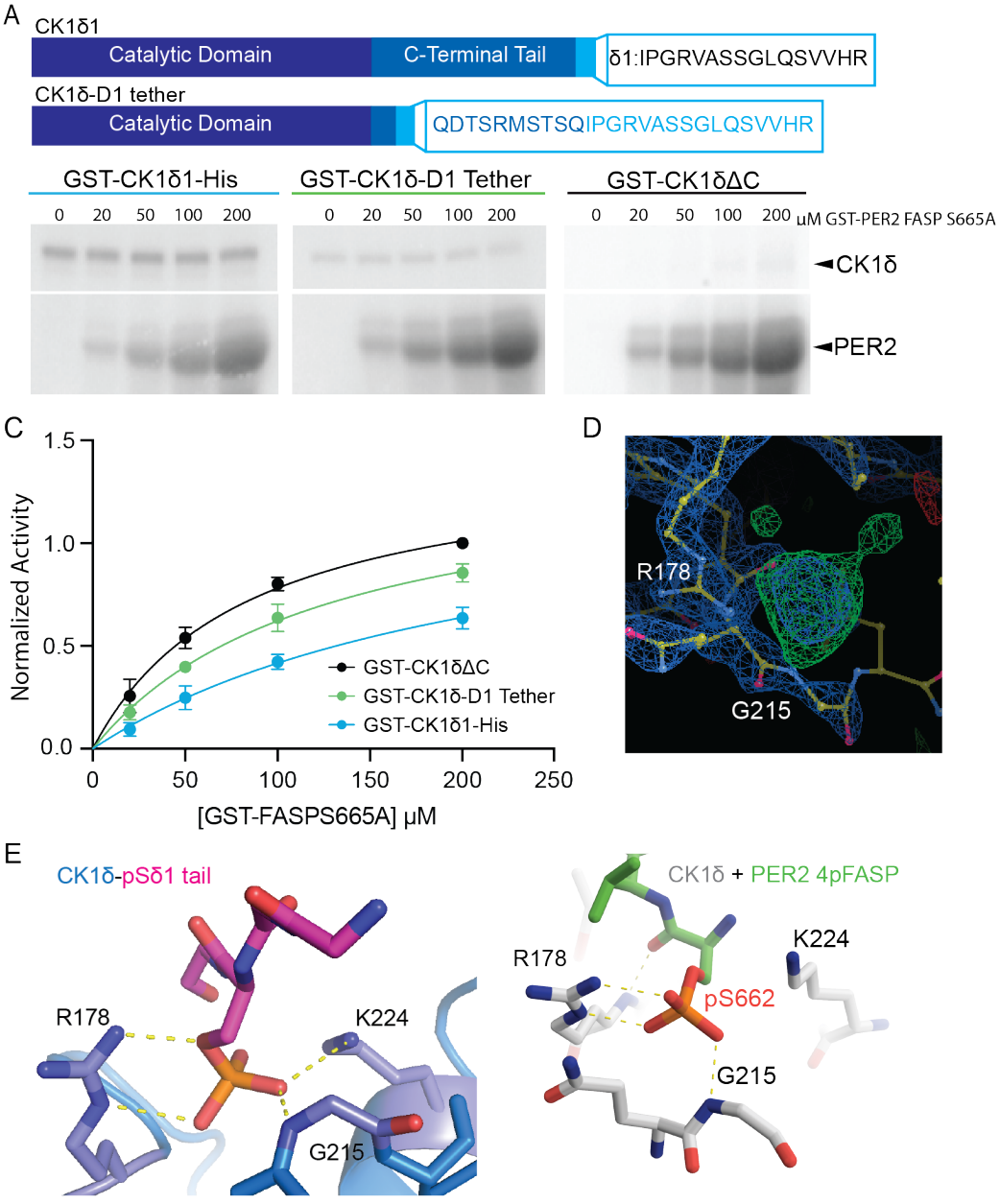


Figure 3.2 CK1δ-D1 tether autoinhibits through coordination of phosphoserine in Anion binding site 1

A, Domain schematic of full length CK1δ1 highlighting the unique extreme C-terminus (top) and the tethered tail construct (bottom) used for crystallography studies highlighting the extreme C-terminus (light blue) and the native tail linker (dark blue). **B,** Representative autoradiographs from ^{32}P kinase assays of full length CK1δ1, CK1δ-D1 tether, and CK1δΔC as indicated on GST-PER2 FASP S665A (n=3). **C**, ^{32}P kinase assays of full length CK1δ1 (blue), CK1δ-D1 tether (green), and CK1δΔC (black) on PER2 GST-FASP-S665A (n=3, mean ± s.d.). **D,** Crystal structure density map of CK1δΔC soaked with D1-3P peptide showing density for a potential phosphoserine in anion site 1 (green) **E,** Structural view of CK1δ-D1 tether showing phosphate coordination by anion binding site 1 (left) and structural view of CK1δ bound to the inhibitory PER2 4p-FASP (PDB: 8D70) showing phosphate coordination by anion binding site 1 (right).

Table 3.1 X-ray crystallography data collection and refinement statistics

	CK1δ -D1 tether
Data collection	
Space Group	C 1 2 1
Resolution	45.8-2.5 (2.60-2.50)
a,b,c	55.23, 134.96, 90.67
α, β, γ	90.00 94.30 90.00
R_{merge}	0.208 (0.838)
R_{pim}	0.087 (0.347)
Total Reflections	147510 (16902)
Unique Reflections	22682 (2534)
I/σ	7.3 (2.3)
CC1/2	0.985 (0.691)
Completeness	99.2 (98.8)
Redundancy	6.5 (6.7)
Wilson B-factor	26.74
Refinement	
Resolution	45.796-2.5
R_{work}/R_{free}	21.26/26.56
No. of Atoms	
Protein	
Water	
Ligands	
RMS Deviation	
Bond Lengths	0.009
Bond Angles	1.16
Ramachandran Favored/Outliers	95.5/0.69
Average B-factor	

3.2 CK1δ2 tail has unique intramolecular dynamics

3.2.1 Summary

In this section, I expand upon work done with the CK1δ2 tail shown in Chapter 2 Fig 2.2.1. NMR spectra of the CK1δ2 tail alone showed that it may be exhibiting “fuzzy” intra-molecular interactions because of the signal broadening compared to the CK1δ1 tail alone despite not showing any other signs of aggregation [120, 121, 136]. This signal broadening is indicative of binding or conformational fluctuations that may be happening within the tail. This low signal created challenges in analyses of the data and further experiments are necessary to confirm if there is a “fuzzy” intra-molecular interaction or if the δ2 tail is just unstable in these conditions. Here I will show some of the analyses that we were able to perform with the δ2 tail in the presence of kinase domain alone and kinase with ATP, as well as further dynamics experiments that were attempted to try and track the “fuzzy” interactions of the tail within itself.

3.2.2 CK1δ2 exhibits potential intramolecular “fuzzy” interactions

The CON spectra of CK1δ2 tail alone had drastically reduced signal than CK1δ1 tail alone (Fig 3.3 A, B). Size exclusion chromatography traces show that both CK1δ1 and CK1δ2 tails elute at similar volumes in peak 2 as indicated as they are similar molecular weights: 14kDa and 13.6kDa, respectively (Fig 3.3 C, D). This indicates that CK1δ2 tail is not forming a soluble aggregate that could give rise to the signal broadening seen in the

NMR spectra. In order to see if this broadening is affected by the kinase domain, I then incubated CK1δ2 tail with kinase domain alone or kinase domain and ATP for 2 hours at room temperature. Upon addition of kinase domain, the signal decreased even further causing several peaks to disappear, and very little chemical shift perturbations were detected, potentially indicative of intermediate exchange of the tail with the kinase domain (Fig 3.3 E, F). Even more drastic peak disappearance and signal broadening occurred with kinase domain and ATP (Fig 3.3 E, G). This drastic reduction in signal created challenges in analyzing the interaction of the CK1δ2 tail with the kinase domain. Since the signal of the tail alone was so low, there is a possibility of intramolecular "fuzzy" interactions [120, 121, 136] or that the CK1δ2 tail is forming soluble aggregate as the experiment runs. In order to explore which potential explanation, I created a CK1δ2 tail with a C-terminal cysteine that could then be labeled with a nitroxide spin label, MTSL (S-(1-oxyl-2,2,5,5-tetramethyl-2,5-dihydro-1H-pyrrol-3-yl) methyl methanesulfonothioate). Any residue that comes into proximity to this spin label (<12Å) will have its signal broadened in the HSQC; this effect is called paramagnetic relaxation enhancement (PRE) [137-139]. The HSQC of CK1δ2 tail alone with the nitroxide spin label (Fig 3.3 H, blue) shows broadened peaks compared to the diamagnetic ascorbic acid-treated sample (Fig 3.3 H, pink). Due to low signal in the 3D data and many peaks that should be present based on sequence homology with the CK1δ1 tail were completely

broadened out, assignments of peaks for the CK1δ2 tail are lacking. This lack of extensive assignments lead to rather ambiguous analysis of the PRE (Fig 3.3 E) and further work needs to be done to improve the signal and complete the assignments. Recapitulating this process with CK1δ1 tail or CK1δΔ400 tail would also be necessary to establish this behavior specifically to the region of the tail unique to CK1δ2.

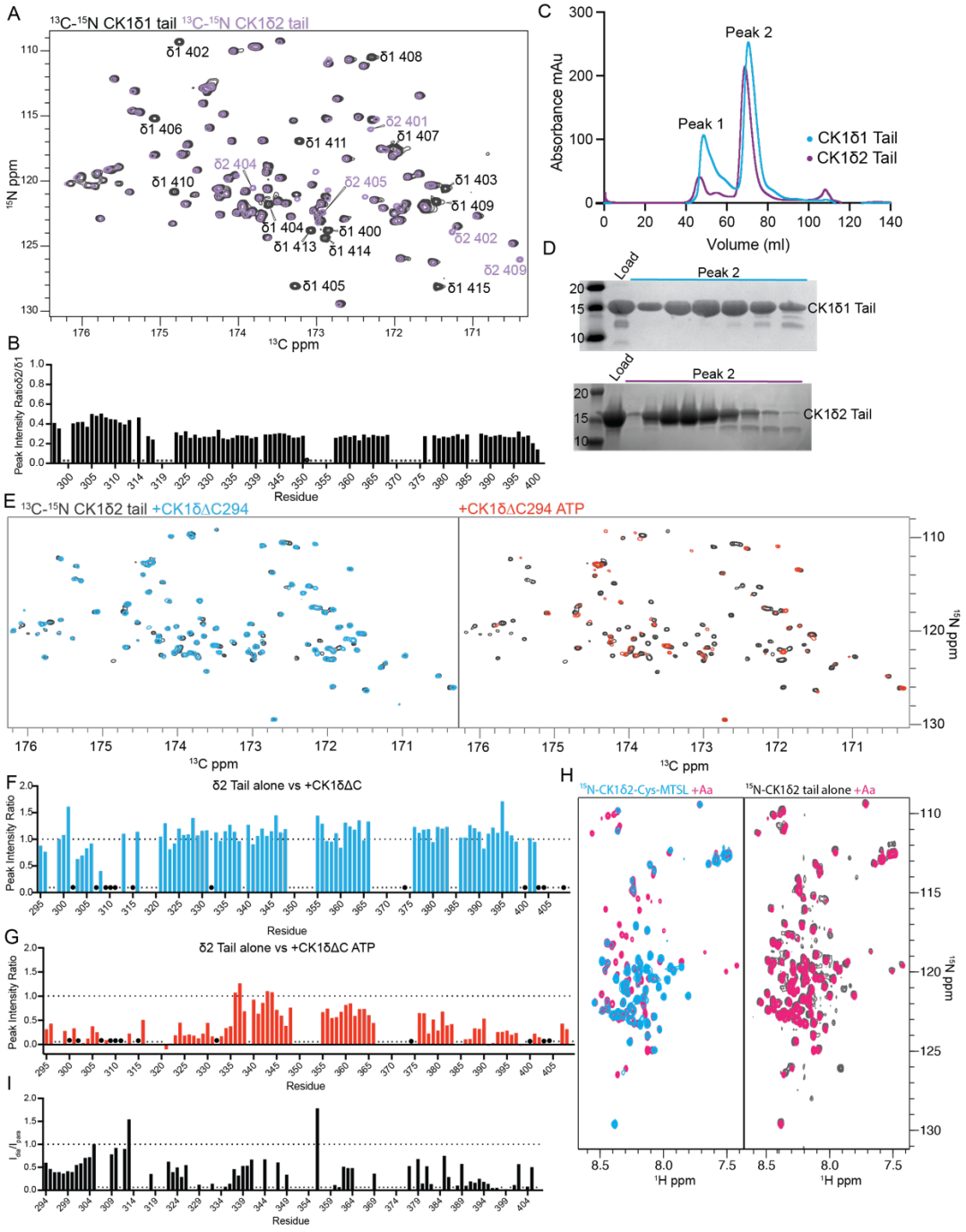


Figure 3.3 Reduced Signal of CK1δ2 tail insinuates potential intramolecular "fuzzy" interactions

A, ^{15}N-^{13}C CON spectra of 400 μM ^{13}C,^{15}N δ1 tail alone (black) compared to 400 μM ^{13}C,^{15}N δ2 tail alone (purple) with isoform-specific assignments. **B,** Peak intensity of ^{15}N-^{13}C CON δ2 compared to δ1; *, unassigned peaks. **C,** Size Exclusion Chromatography trace of CK1δ1 tail (blue) and CK1δ2 tail (purple). **D,** SDS-PAGE gels of Peak 2 for CK1δ1 tail (blue, top) and CK1δ2 tail (purple, bottom). **E,** ^{13}C-^{15}N CON spectra of CK1δ2 tail alone (black), in the presence of kinase domain (left, blue), or kinase domain and ATP (right, red). **F-G,** peak intensity ratio of CK1δ2 tail alone vs with kinase domain (B, blue) or kinase domain and ATP (C, red). *Indicates unassigned peaks and black circles indicate peaks that disappeared. **H,** ^{1}H-^{15}N HSQC spectra of MTSL labeled CK1δ2 tail (black) and ascorbic acid treated CK1δ2 tail (pink). **I,** peak intensity ratio of the diamagnetic ascorbic acid treated CK1δ2 tail vs the paramagnetic MTSL-CK1δ2 tail. *indicates unassigned peaks.

3.3 Initial rates of autophosphorylation are important for isoform-specific autoinhibition

3.3.1 Summary

In this section, I expand upon work done in Chapter 2 on the role of autophosphorylation on isoform-specific autoinhibition. Within the cell, CK1δ is constantly being phosphorylated and de-phosphorylated [78]. As shown in Chapter 2 Fig 2.2, autophosphorylation does not accumulate in cells and phosphatase inhibitors are required to let autophosphorylation accumulate for detection. This begs to question if fully autophosphorylated CK1δ1 and δ2 retain their difference in activity on PER2 substrates. Chapter 2 Fig 2.1 also indicated that CK1δ1 had lower total autophosphorylation than the other

kinase isoforms, although analysis of the autophosphorylation was limited to a single time point. Here, I show the steps that were taken to begin addressing these questions further with gel shift autophosphorylation timecourse assays and ^{32}P ATP assays with pre-autophosphorylated CK1δ isoforms.

3.3.2 Investigating autophosphorylation rates of CK1δ isoforms

In order to expand on the autophosphorylation data shown in Chapter 2 Fig 2.1, I used SDS-PAGE gel shift assays to assess autophosphorylation of the different CK1δ isoforms over time (Fig 3.4). The Coomassie-stained gels show an evident hypermobility shift over time as the kinase becomes autophosphorylated for CK1δ1 WT, CK1δ1 S3A (Fig 3.4 A), CK1δ2, and CK1δΔ400 (Fig 3.4 C); whereas tailless CK1δΔC remains unchanged (Fig 3.4 E) despite having one known autophosphorylation site at T220 [119]. Tracking the band intensity as it shifts up the gel through densitometry, there are distinctly different patterns with CK1δ1 WT forming the weakest hyperphosphorylated band while CK1δ1 S3A and CK1δ2 form the most intense hyperphosphorylated band (Fig 3.4 B, D). Quantification of the disappearance of the unphosphorylated species fails to capture the differences in autophosphorylation rate that appears to be evident in the Coomassie gels (Fig 3.4 G). Quantification of the distance of the hyper-shift also shows that each kinase with a tail shifts to the same extent (Fig 3.4 H). This illustrates the limitations of quantifying from gel hyper-shift data in that the exact amount of autophosphorylation cannot be directly detected. A more

quantifiable approach, such as ^{32}P ATP kinase assay, would yield a more accurate account of the total level of autophosphorylation of each kinase isoform. By directly measuring incorporation of ^{32}P rather than a more ambiguous gel hyper-shift, this could hopefully more accurately illustrate the different extent and rates of CK1δ isoform autophosphorylation.

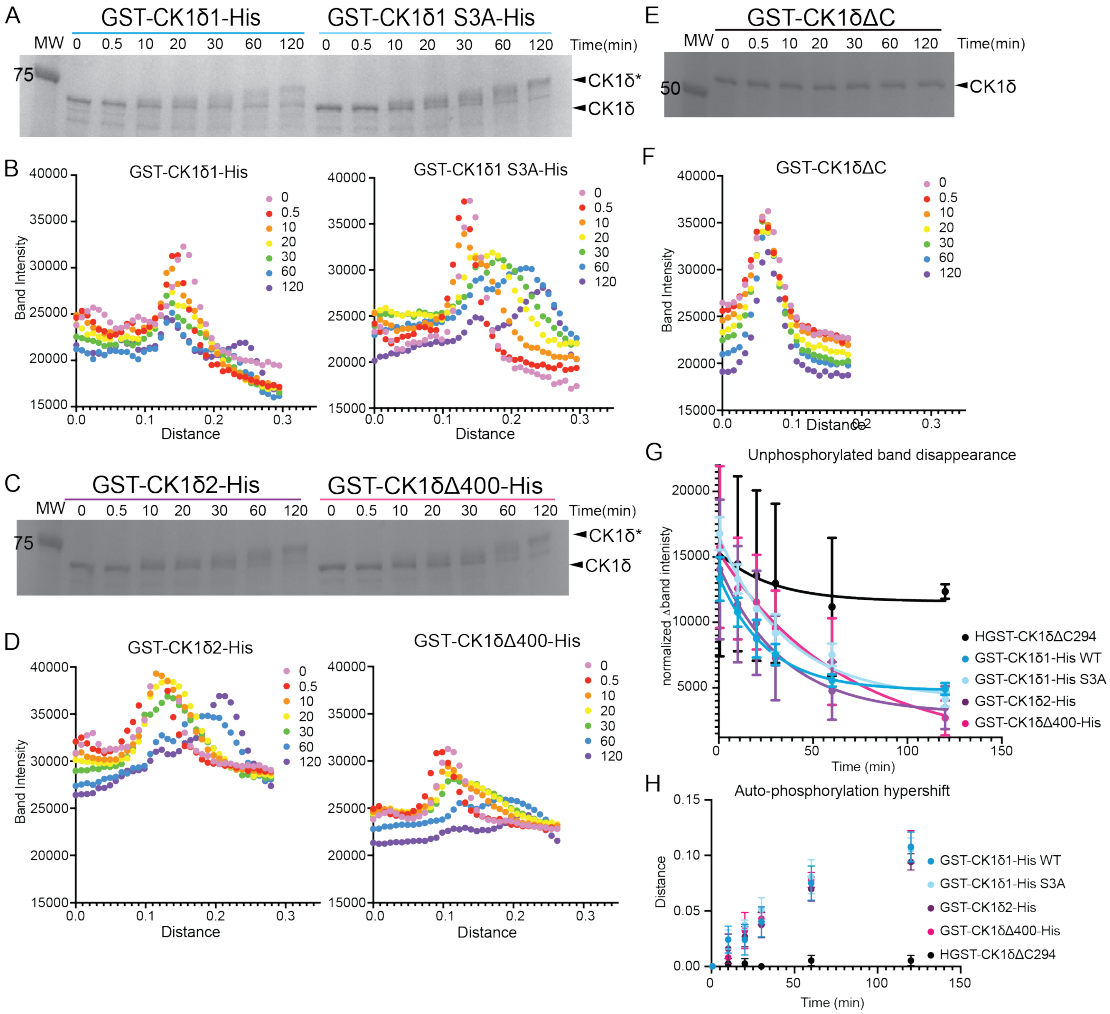


Figure 3.4 Gel Shift assays are insufficient for determining differences in autophosphorylation rates of CK1δ tail variations

A, C, E, Representative SDS-PAGE gels of autophosphorylation timecourse for CK1δ1 WT (A, blue), CK1δ1 S3A (A, light blue), CK1δ2 (C, purple), CK1δΔ400 (C, pink), and CK1δΔC (E, black). CK1δ indicates the unphosphorylated band and CK1δ* the hyperphosphorylated band. **B, D, F,** Quantification of the band intensity and gel shift as kinase becomes autophosphorylated over time for CK1δ1 WT (B, left), CK1δ1 S3A (B, right), CK1δ2 (D, left), CK1δΔ400 (D, right), and CK1δΔC (F). **G,** Quantification of the change in band intensity for the unphosphorylated species over time normalized to the background intensity for CK1δ1 WT (blue), CK1δ1 S3A (light blue), CK1δ2 (purple), CK1δΔ400 (pink), and CK1δΔC (black). **H,** Quantification of the hyper-phosphorylated species gel shift distance over time for CK1δ1 WT (blue), CK1δ1 S3A (light blue), CK1δ2 (purple), CK1δΔ400 (pink), and CK1δΔC (black).

3.3.3 Fully autophosphorylated CK1δ equalizes activity by different isoforms

To explore the differences that may occur with fully autophosphorylated CK1δ isoforms prior to introducing PER2 substrate, I incubated the kinases with ATP and $MgCl_2$ overnight at 4°C rather than dephosphorylating the kinases during protein purification. Full-length CK1δ isoforms come out of *E.coli* already partially phosphorylated and based off of the autophosphorylation timecourse done at room temperature for two hours, an overnight incubation with ATP should have been sufficient to fully autophosphorylate each isoform. I then utilized these pre-autophosphorylated kinases in ^{32}P ATP kinase assays on HGST-PER2 FASP S665A. Some further autophosphorylation

occurred over the course of this assay (Fig 3.5), indicating that the temperature that I incubated the kinases in overnight to fully autophosphorylate them was too low and impeded the kinases activity. Though this time and temperature is sufficient to fully dephosphorylate the kinase with lamda phosphatase, CK1δ is a much less efficient enzyme and this should have been taken into consideration. Both a timecourse with constant concentration of substrate (Fig 3.5 A, B) and a substrate titration as performed in Chapter 2 (Fig 3.5 C, D) was used. The CK1δ constructs containing tails; CK1δ1, CK1δ2, CK1δ1 S3A, and CK1δΔ400 all exhibited very similar activity, unlike what was seen in Chapter 2. In both types of assay, CK1δΔC retained its heightened activity (Fig 3.5), indicating that the tail is responsible for this erasure of differences in activity of the isoforms. Due to the lack of autophosphorylation accumulation in cells and that differences in activity of the isoforms have been detected in cells [53, 78, 79, 91], this suggests that looking at enzyme activity during the initiation of autophosphorylation may be more relevant *in vivo.* This work specifically focuses on the priming phosphorylation of the PER2 FASP substrate (e.g., S662), so more could be done with more canonical primed substrates or on other target substrates to fully explore the contributions of the tail to possible substrate selectivity and modes of autoinhibition.

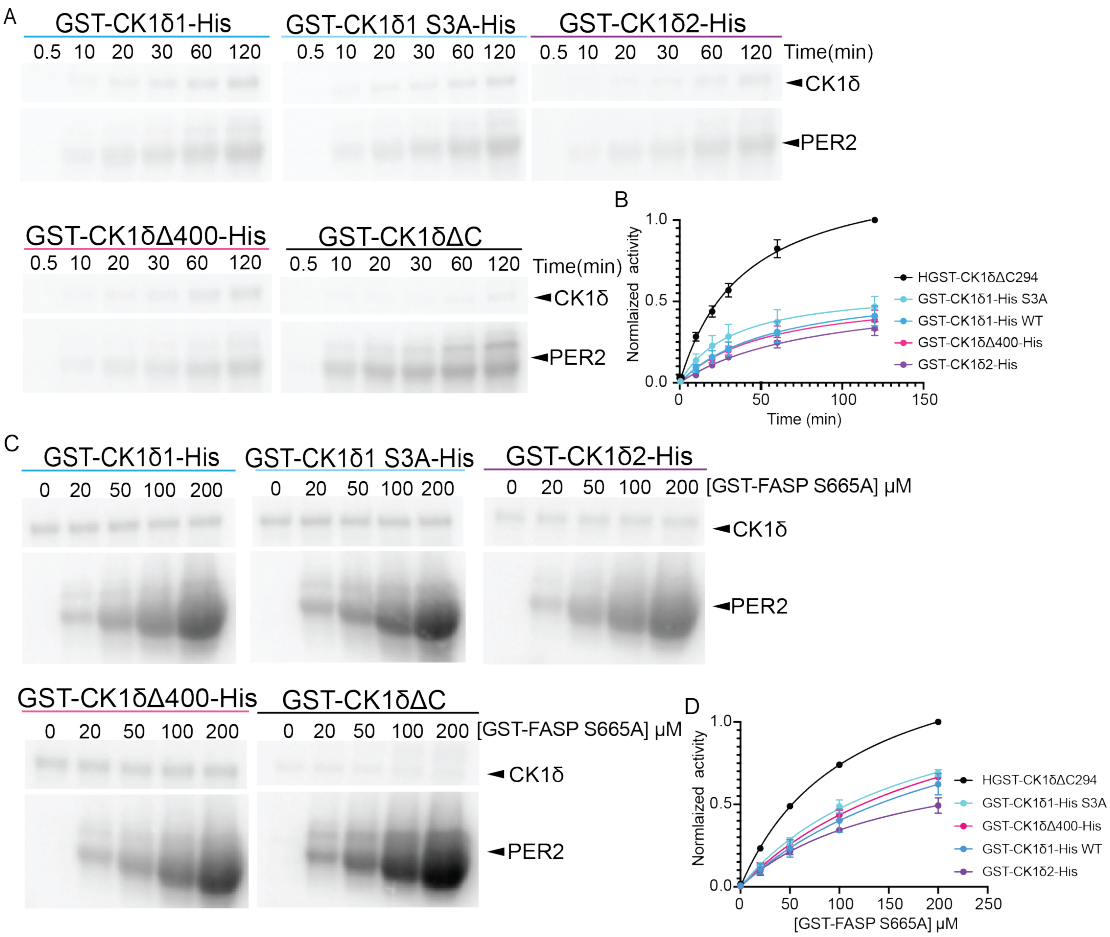


Figure 3.5 Pre-autophosphorylated CK1δ isoforms diminishes differences in activity of kinases with tails

A, Representative autoradiographs from ^{32}P kinase assays of pre-autophosphorylated CK1δ1 WT (blue), CK1δ1 S3A (light blue), CK1δ2 (purple), CK1δΔ400 (pink) and CK1δΔC (black) on HGST-hPER2-FASP S665A over time (n=3). **B,** ^{32}P kinase assays of CK1δ1 WT (blue), CK1δ1 S3A (light blue), CK1δ2 (purple), CK1δΔ400 (pink) and CK1δΔC (black) on PER2 GST-FASP-S665A (n=3, mean ± s.d.) normalized to activity of CK1δΔC. **C,** Representative autoradiographs from ^{32}P kinase assays of pre-autophosphorylated CK1δ1 WT (blue), CK1δ1 S3A (light blue), CK1δ2 (purple), CK1δΔ400 (pink) and CK1δΔC (black) on increasing concentrations of HGST-hPER2-

FASP S665A (n=3). **B,** ^{32}P kinase assays of CK1δ1 WT (blue), CK1δ1 S3A (light blue), CK1δ2 (purple), CK1δΔ400 (pink) and CK1δΔC (black) on increasing concentrations of HGST-hPER2-FASP S665A (n=3, mean ± s.d.) normalized to activity of CK1δΔC.

3.4 Materials and Methods

Protein constructs, expression, and purification

All constructs were expressed from TEV-cleavable vectors generated in-house based off the Parallel vector series [129], and have an N-terminal vector artifact (GAMDPEF) remaining after TEV cleavage. Mutations were introduced using the site-directed mutagenesis protocol in [130] and validated by sequencing.

Full-length human CK1δ isoforms (CK1δ1, residues 1-415; CK1δ2, residues 1-409) and CK1δ Δ400 (residues 1-400) were expressed with an N-terminal TEV-cleavable GST tag and a C-terminal His tag. CK1δ ΔC (residues 1-294) and CK1δ-D1 tether were expressed with an N-terminal TEV-cleavable His-GST tag. Isolated CK1δ2 tail (residues 295-409) and the CK1δ2 tail C-term Cys were expressed with an N-terminal TEV-cleavable His-NusA tag (HNXL) with an N-terminal tryptophan added for UV detection during purification. Recombinantly expressed human PER2 peptides (FASP peptide, residues 645–687; Degron peptide, residues 475–505) were expressed with an N-terminal TEV-cleavable His-GST tag. All recombinant PER2 peptides contain an N-terminal tryptophan for UV detection during purification and a polybasic motif (WRKKK) following the vector artifact, as in [140].

GST-lambda (λ) phosphatase-Strep-tag II was expressed with a TEV-cleavable N-terminal His-GST tag and a C-terminal Strep-tag II. GST-λ phosphatase were expressed in BL21 (DE3) *Escherichia coli*, while all CK1 and PER constructs were expressed in Rosetta2 (DE3) *E. coli*. In general, cells were grown in LB media (for natural abundance growths) or M9 minimal media with the appropriate stable isotopes (i.e., $^{15}N/^{13}C$) for NMR as done before [53] at 37°C with shaking until the OD_{600} reached ~0.6-0.8. Expression was induced with 0.5 M IPTG, and cells were grown for an additional 16-20 hours at 18°C.

For purification of full-length CK1δ, cells were lysed in 50 mM Tris pH 7.5, 500 mM NaCl, 5% glycerol, 1 mM EDTA, 1 mM TCEP, 0.05% Tween using a high-pressure homogenizer. GST-tagged full-length proteins were purified from soluble lysate using Glutathione Sepharose resin (Cytiva) and eluted with buffer containing 50 mM Tris pH 7.5, 500 mM NaCl, 5% glycerol, 1 mM EDTA, 1 mM TCEP, 0.05% Tween, and 25 mM reduced glutathione. To remove phosphorylation from the kinase that may have occurred during expression or purification, GST-tagged purified proteins were treated with 200 nM GST-λ phosphatase-Strep tag II supplemented with 10 mM $MnCl_2$ in λ phosphatase buffer (50 mM HEPES pH 7.5, 100 mM NaCl, 0.1 mM EDTA, 1 mM TCEP) overnight at 4°C. For the pre-autophosphorylated kinases, the proteins were instead treated with 2mM ATP and 10mM $MgCl_2$ overnight at 4°C, skipped to the Ni-NTA resin and were further purified the same as de-

phosphorylated kinase. Strep-Tactin Sepharose resin (Cytiva) was used to remove GST-λ phosphatase-Strep tag II, and dephosphorylated CK1δ was collected with several washes of 50 mM Tris pH 7.5, 500 mM NaCl, 5% glycerol, 1 mM EDTA, 1 mM TCEP, and 0.05% Tween. Dephosphorylated proteins were then further purified by the C-terminal His-tag using Ni-NTA resin (Qiagen). Full-length dephosphorylated proteins were subsequently purified by size-exclusion chromatography on a HiLoad 16/600 Superdex 200 prep grade column (Cytiva) in 50 mM Tris pH 7.5, 200 mM NaCl, 1 mM EDTA, 5 mM β-mercaptoethanol (BME), 5% glycerol, and 0.05% Tween, and then frozen in aliquots for storage at -70°C.

For CK1δ catalytic domain and CK1δ-D1 tether preps, cells were lysed in 50 mM Tris pH 7.5, 500 mM NaCl, 5% glycerol, 1 mM EDTA, 1 mM TCEP, 0.05% Tween using a high-pressure homogenizer. His-GST-tagged proteins were purified from soluble lysate using Glutathione Sepharose resin (Cytiva) and eluted with 50 mM Tris pH 7.5, 500 mM NaCl, 5% glycerol, 1 mM EDTA, 1 mM TCEP, 0.05% Tween containing 25 mM reduced glutathione. To cleave the His-GST tag, His-TEV protease was incubated with the protein at 4°C overnight. Cleaved CK1δ ΔC and CK1δ-D1 tether were purified away from His-GST and His-TEV using Ni-NTA resin (Qiagen). CK1δ-D1 tether was then further purified using ion exchange chromatography with a HiTrap SP XL column (Cytiva) and then phosphorylated overnight at 4°C preceding size-exclusion chromatography on a HiLoad 16/600 Superdex 75 prep grade

column (Cytiva). CK1δ ΔC went straight to size-exclusion chromatography on a HiLoad 16/600 Superdex 75 prep grade column (Cytiva) in 25 mM MOPS pH 7.0, 1 mM EDTA, 100 mM NaCl, 5 mM BME, 11 mM $MgCl_2$, and 0.05% Tween, and then frozen in aliquots for storage at -70°C.

For CK1δ2 tail preps, cells were lysed in 50 mM Tris pH 7.5, 500 mM NaCl, 2 mM TCEP, 5% glycerol and 25 mM imidazole using a high-pressure extruder homogenizer. His-NusA-tail fusion proteins were purified using Ni-NTA resin (Qiagen) using standard approaches and His-TEV protease was added to cleave the His-NusA tag from the tail on column at 4°C overnight. Tails were purified away from the resin containing His-NusA and His-TEV with 50 mM Tris pH 7.5, 500 mM NaCl, 2 mM TCEP, 5% glycerol and 25 mM imidazole. Tails were further purified by size exclusion chromatography on a HiLoad 16/600 Superdex 75 prep grade column (Cytiva) using NMR buffer (25 mM MOPS pH 7.0, 1 mM EDTA, 100 mM NaCl, 5 mM BME, 11 mM $MgCl_2$, and 0.05% Tween) and frozen in aliquots for storage at -70°C for WT δ2 tail and NMR Buffer no reducing agent (25 mM MOPS pH 7.0, 1 mM EDTA, 100 mM NaCl, 11 mM $MgCl_2$, and 0.05% Tween) for δ2 C-term Cys tail. δ2 C-term Cys tail was then labeled with 250 μM MTSL incubated overnight at 4°C. The labeling reaction was then dialyzed (3kDa cutoff) for 4 hours in NMR Buffer no reducing agent (25 mM MOPS pH 7.0, 1 mM EDTA, 100 mM NaCl, 11 mM $MgCl_2$, and 0.05% Tween), and then frozen in aliquots for storage at -70°C. Labeling efficiency was checked by EPR.

For PER2 peptide preps, cells were lysed in 50 mM Tris pH 7.5, 500 mM NaCl, 5% glycerol, 1 mM EDTA, 1 mM TCEP, 0.05% Tween using a high-pressure homogenizer. His-GST-tagged proteins were purified from soluble lysate using Glutathione Sepharose resin (Cytiva) and eluted with 50 mM Tris pH 7.5, 500 mM NaCl, 5% glycerol, 1 mM EDTA, 1 mM TCEP, 0.05% Tween containing 25 mM reduced glutathione. The peptides were further purified using size-exclusion chromatography on a HiLoad 16/600 Superdex 75 prep grade column (Cytiva) in 25 mM Tris pH 7.5, 100 mM NaCl, 10 mM $MgCl_2$, and 2 mM TCEP, and then frozen in aliquots for storage at -70°C.

ɣ^{32}P-ATP kinase assay

Radioactive kinase assays were performed using human PER2 His-GST-FASP S665A or human PER2 His-GST-Degron as the substrate. Reaction mixtures were prepared in reaction buffer (25 mM Tris pH 7.5, 200 mM NaCl, 10 mM $MgCl_2$, and 1 mM DTT) with 1 µM enzyme and 0-200 µM dilutions of the substrate or 20 µM substrate for the time course as indicated at 25°C. Reactions were initiated with the addition of 2 mM ATP containing 2 µCi of ɣ-32p ATP (Perkin Elmer). All reactions were quenched with 2X SDS buffer after an incubation time of 1 hour for the titration or at the indicated time points for the time course. SDS-PAGE was performed on samples for each reaction, and the gels were dried at 80°C for 2 hours before being transferred to a storage phosphor screen (Amersham Biosciences) overnight.

Images were collected with Typhoon Trio (Amersham Biosciences) and data were analyzed by densitometry using Image J (NIH), Excel (Microsoft), and Prism (GraphPad).

Gel Shift Autophosphorylation Assay

Gel shift kinase assays were performed using 1 μM of indicated CK1 isoform in 1X kinase buffer (25 mM Tris pH 7.5, 100 mM NaCl, 2 mM TCEP, 10 mM $MgCl_2$). Reactions were initiated with addition of 2mM ATP and quenched at indicated time points with 2X SDS. SDS-PAGE was performed on samples for each reaction, and the gels were stained with SimplyBlue™ SafeStain (Invitrogen), destained overnight, and then imaged with ChemiDoc Imaging System (Bio-Rad). Analysis was performed with ImageJ.

NMR assays

NMR spectra were collected on a Varian INOVA 600 MHz, or a Bruker 800 MHz spectrometer equipped with a ^{1}H, ^{13}C, ^{15}N triple resonance z-axis pulsed-field-gradient cryoprobe. Spectra were processed using NMRPipe [131] and analyzed using CCPNmr Analysis [132]. Spectra were collected in NMR buffer (25 mM MOPS pH 7.0, 1 mM EDTA, 100 mM NaCl, 5 mM BME, 11 mM $MgCl_2$, and 0.05% Tween) with 10% D_2O at 25°C. The backbone assignment of the δ2 tail was accomplished using standard NH-edited triple-resonance experiments [HN(CA)CO, HNCO, HNCACB, and CBCA(CO)NH]

and four-dimensional carbon direct-detection methods such as (HACA)N(CA)CON and (HACA)N(CA)NCO.

NMR kinase reactions were performed at 25°C with 100 μM ^{13}C,^{15}N CK1δ2 tail, 2.5 mM ATP and 100 μM CK1δ ΔC. Samples were incubated for 2 hours and quenched with 20 mM EDTA, then ^{1}H-^{15}N HSQC spectra (total data acquisition = 20 min) and ^{13}C-^{15}N CON spectra (total acquisition time= 9 hr 32 min) were collected.

PRE NMR experiments were performed with 100 μM ^{15}N CK1δ2 C-term Cys-MTSL tail. ^{1}H-^{15}N HSQC spectra (total data acquisition = 20 min) were collected for untreated sample and sample treated with 10 mM Ascorbic Acid.

Crystallization and structure determination

Crystallization was performed by hanging-drop vapor-diffusion method at 22 °C by mixing an equal volume of protein with reservoir solution. The reservoir solution for CK1δ-D1 tether (5.5 mg/mL) was 0.21 mM succinic acid pH 5.5 and 17% (vol/vol) PEG 3350. The crystals were looped and briefly soaked in a drop of cryopreservant reservoir solution with 20% (vol/vol) glycerol and then flash-frozen in liquid nitrogen for X-ray diffraction data collection. Data sets were collected at the 23-ID-D beamline at the Advanced Photon Source (APS) at the Argonne National Laboratory. Data were indexed, integrated, and merged using CCP4 software suite [141]. Structures were determined by molecular replacement with Phaser MR [142] using anion

free CK1δΔC (PDB: 6PXO). Model building was performed with Coot [143] and structure refinement was performed with PHENIX [144]. All structural models were generated using PyMOL Molecular Graphics System 2.5.4 (Schrödinger).

Hydrogen-deuterium exchange mass spectrometry

Sample preparation

Hydrogen-deuterium exchange mass spectrometry (HDX-MS) was performed for different CK1δ constructs in the presence and absence of ATP or AMPPNP. Different CK1δ constructs were incubated with either ATP (~800 μM) or AMPPNP (~1.2 mM), or neither (apo), and 2 mM MgCl2 for 15 min at 25 °C before each hydrogen-deuterium exchange reaction. Hydrogen-deuterium labeling was initiated by diluting 60-65 pmol of CK1δ saturated with ATP or AMPPNP as indicated above in a reaction buffer prepared in deuterium oxide (D2O, final concentration of 90%). Pulse-labeling reactions were carried out for 1, 5, and 10-minute timepoints, after which the exchange was minimized by lowering the pH to 2.5 and temperature to 0°C by adding pre-chilled quench solution (0.5 M Guanidinium hydrochloride, 0.1 M TCEP). Non-deuterated controls were carried out by diluting nucleotide-free CK1δ proteins in an aqueous reaction buffer instead of a deuterated buffer.

Mass spectrometry data acquisition

For nanoLC-MS/MS analyses, each quenched sample was subjected to in-line proteolysis using the Enzymate^{TM} immobilized pepsin cartridge

(Waters, USA). The digestion was left to proceed for 3 minutes at 12°C, and the resulting peptide mixtures were analyzed with nanoACQUITY M-class UPLC (Waters, UK) equipped with a trapping column (VanGuard C18, 5 µm particle size) and an analytical column (BEH C18, 75 µm × 100 mm, 1.7 µm particle size) maintained at 3°C to minimize back-exchange [133]. The aqueous solvent was 0.1% formic acid in LC-MS grade water, and the organic solvent was 0.1% formic acid in HPLC-grade acetonitrile. A 10-minute gradient was used to resolve and elute the peptides by reverse-phase chromatography. The peptides were then identified by Synapt G2-Si high-resolution mass spectrometer (Waters, UK). Briefly, the mass spectrometer was operated in positive polarity, data-independent (DIA) fragmentation and ion-mobility ($HDMS^E$) mode separation. Mass spectrometer parameters were as described previously [134]. Glu-fibrinopeptide reference (m/z = 785.8426) was continuously sprayed every 30 seconds during ESI-MS experiments using the lockspray device to ensure mass accuracy.

HDX data analysis

Peak lists for identifying and annotating the peptides from non-deuterated controls were generated by Protein Lynx Global Server v3.0 (Waters, USA), using human CK1δ (Uniprot: P48730) construct-specific protein database and searched against the parameters described previously [134]. Peptide mass measurements for individual peptides were assigned using DynamX v3.0 software (Waters, USA). Peptides with a minimum

intensity of 2000, maximum length of 25 amino acids, and mass error within ± 10 ppm were considered for analysis. Deuterium exchange measurements were determined for all labeling timepoints across different protein states and were manually verified, but the data were not corrected for back-exchange. Only peptides with high signal-to-noise ratio and non-overlapping spectra were considered for final analysis. All measurements were done in biological duplicates and technical triplicates, with statistical analysis done using Deuteros 2.0 [135] and tabulated as Supplementary Tables 1-3.

QUANTIFICATION AND STATISTICAL ANALYSIS

All statistical analyses were done using Prism (GraphPad). P-values were calculated using two-way ANOVA with Tukey's multiple comparisons test as indicated. In all figures, * indicates $p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$, ns, not significant.

Chapter 4

Conclusions

4.1 Discussion

In Chapter 1, we highlighted the integral role of PER and CK1δ/ε in circadian timing mechanisms. Specifically, how post-translational modifications, such as phosphorylation by CK1δ/ε, as well as acetylation and O-GlcNAcylation regulate PER stability, and that PER abundance is a critical factor in circadian timing. CK1δ/ε is a constitutively active kinase that does not require activation, so it instead utilizes autoinhibitory phosphorylation of its disordered C-terminal tail to regulate its activity. The work explored here strives to better understand the biochemical details of how that autoinhibition occurs and differs between tail variants.

In Chapter 2, we explored how the extreme c-terminus of the CK1δ splice variants influenced its activity of PER priming phosphorylation. CK1δ1 was determined to have a phospho-dependent inhibition, while CK1δ2 does not appear to be phospho-dependent. However, much more work needs to be done to fully explore how CK1δ2 inhibits kinase activity. Key sites in the extreme c-terminus unique to CK1δ1 were determined to be preferentially autophosphorylated and mutating them to non-phosphorylatable alanine was sufficient to decrease CK1δ1 autoinhibition. HDX-MS showed that the CK1δ1 tail makes more extensive contacts with the kinase domain upon autophosphorylation than CK1δ2 or CK1δΔ400 and the mutant of the

phosphosites essentially abolishes those contacts. Anion site mutants further showed that CK1δ1 utilizes the anion binding sites spanning the substrate binding cleft of the kinase domain to confer its inhibition. Binding to these anion sites has been shown with other phosphorylated inhibitory peptides [102, 103], indicating that this could be a conserved mechanism of inhibition for CK1δ that could be exploited for inhibitor design. The importance of autophosphorylation of the CK1δ1 tail, and specifically the extreme c-terminus, has also recently been shown in other systems [145, 146], showing that this mode of inhibition of CK1δ1 is not specifically to PER substrate and can be applied to the many other systems that CK1δ participates.

In Chapter 3, I expanded on the work done in Chapter 2. Chapter 2 focused on the anion binding sites flanking the substrate binding cleft, but there is a third anion site located further down the C-lobe of the kinase. I showed that CK1δ1 also has a unique interaction with this anion site 3 that counteracts autoinhibition in a phospho-independent manner. Anion site 3 could potentially be sequestering the auto-inhibitory extreme c-terminus of CK1δ1 away from the active site, but the exact mechanism is unclear and needs to be explored further. I also expanded on anion site 1 and showed with a crystal structure that CK1δ1 phosphorylated extreme c-terminus binds a phosphoserine into anion site 1 that is coordinated in a similar manner to the FASP inhibitory peptide. Density was lacking for the surrounding sidechains around the phosphoserine, indicating that multiple different phosphoserines

could be binding into the anion site. This could possibly explain why in Chapter 2 the triply phosphorylated δ1 tail peptide was the most inhibitory compared to the single phosphorylated δ1 tail peptides as there are more possible phosphoserines to bind into the anion site with low affinity, potentially increasing the avidity. Kinase assays with the construct used for crystallization showed that it did autoinhibit, but not to the same extent as full length CK1δ1. This implicates that the extreme c-terminus plays a role in autoinhibition, but it is not completely sufficient to confer full autoinhibition. More needs to be done to fully explore the upstream phosphorylation sites. Differences in total autophosphorylation of the full-length kinases begs to question how the phosphorylation of upstream sites may be changing depending on the identity of extreme c-terminus.

Attempts to further explore the interaction of the CK1δ2 tail with the kinase domain through NMR studies did not yield as conclusive of information as the δ1 tail. I explored whether the CK1δ2 tail could be exhibiting intramolecular “fuzzy” interactions, but the data was inconclusive and seemingly contradictory. The general across the board broadening of the NMR signal indicated that the tail may be forming soluble aggregates, but SEC shows that it behaves similarly to the CK1δ1 tail which gives strong NMR signal. A possible explanation could be that the protein is aggregating over the course of the NMR spectra collection. Checking the SEC trace of the tail after running the experiment or leaving it out at the temperature and for the amount

of time the spectra is collected could help determine if this is the case. Further NMR dynamics studies could also be done to better determine if a “fuzzy” intramolecular interaction is occurring, but more extensive assignments would be needed. Unfortunately, there is not much that can be said about how the CK1δ2 tail inhibition works other than it appears to function differently than the CK1δ1 tail. CK1δ2 mode of inhibition thus remains an open question that could be explored further.

I also looked further into how the rate of autophosphorylation differed in the full length CK1δ isoforms and tail mutants. Unfortunately, the gel hyper-shift data did not give quantitative insight into the differences in autophosphorylation rates despite CK1δ1 visually looking different than the other kinases with a weaker autophosphorylated hyper-shift band. Further work could be done with ^{32}P ATP, ADP-Glo kinase assays, or trypsin digest mass spectrometry to fully characterize what is being autophosphorylated in the different tails and how quickly. Conducting these studies could potentially give insight into the interplay between the unique extreme c-terminus and the phosphorylation of upstream sites in the region of the tail shared by the two splice isoforms. This also led to questioning if the differences in activity of CK1δ isoforms would be exacerbated by allowing the kinase to fully auto-phosphorylate the tail prior to introduction of PER2 substrate. Interestingly, the pre-autophosphorylated CK1δ1, CK1δ2, and the tail mutants CK1δ1 S3A and CK1δΔ400 all exhibited the same level of PER2 priming phosphorylation.

Autophosphorylation does not accumulate in cells and is in a constant cycle with phosphatases and CK1δ1 and CK1δ2 have been shown in cells to shift the circadian period in opposite directions [91]. Our previous work in chapter 2 on fully dephosphorylated CK1δ isoforms showed that CK1δ2 and CK1δΔ400 have greater activity on priming phosphorylation than CK1δ1, a trend that has also been seen in cells [53]. This indicates that in vivo, the initial rates of autophosphorylation are the driving force for the differences in CK1δ1 and CK1δ2 rather than their fully autophosphorylated states. This could be further explored by probing with different substrates, such as more canonical primed substrate, to see if the trends in activity persist or are substrate dependent. Other work has indicated that the tail may play a role in substrate selectivity [146]; however, how that occurs is unclear. Expansion to other substrates that CK1δ acts on in the many other biological systems that is plays a part in could help decipher this mystery. A full understanding of the intricacies that lead to autoinhibition could then lead the way to developing selective inhibitors that could potentially be selective to not only the kinase, but the substrates that it acts upon.

Appendices

Table 2.1 Deuterium exchange values for various pepsin-digested peptides of CK1δΔC in the presence of ATP, AMPPNP, and 4p-FASP at 1, 5, and 10-minute labeling times are tabulated.

Table 2.1

				CK1δ1-ΔC Relative Deuterium Uptake (Da)					
				1 min		5 min		10 min	
S.No.	Peptide	Sequence	Num Exchangers	Uptake	± SD	Uptake	± SD	Uptake	± SD
1	1-20	MELRVGNRYRLGRKIGSGSF	19	6.04	0.54	6.55	0.57	6.82	0.35
2	3-10	LRVGNRYR	7	3.28	0.08	3.51	0.09	3.51	0.05
3	3-20	LRVGNRYRLGRKIGSGSF	17	7.10	0.09	7.59	0.27	7.80	0.08
4	4-20	RVGNRYRLGRKIGSGSF	16	5.85	0.09	6.30	0.31	6.60	0.09
5	4-22	RVGNRYRLGRKIGSGSFGD	18	6.56	0.11	7.02	0.29	7.29	0.13
6	12-25	GRKIGSGSFGDIYL	13	3.99	0.16	5.36	0.05	5.52	0.17
7	21-28	GDIYLGTD	7	1.64	0.02	3.29	0.09	3.77	0.04
8	24-39	YLGTDIAAGEEVAIKL	15	3.08	0.15	3.08	0.40	3.35	0.18
9	26-34	GTDIAAGEE	8	3.60	0.04	3.68	0.02	3.74	0.04
10	28-34	DIAAGEE	6	2.64	0.05	2.78	0.08	2.71	0.01
11	28-36	DIAAGEEVA	8	2.80	0.02	3.03	0.15	3.52	0.16
12	29-36	IAAGEEVA	7	2.30	0.04	2.55	0.10	2.99	0.03
13	35-41	VAIKLEC	6	1.27	0.01	1.90	0.06	2.36	0.01
14	37-49	IKLECVKTKHPQL	11	2.60	0.08	2.73	0.10	2.77	0.04
15	42-49	VKTKHPQL	6	1.68	0.05	1.70	0.08	1.72	0.06
16	50-58	HIESKIYKM	8	1.62	0.08	2.61	0.05	3.03	0.13
17	56-72	YKMMQGGVGIPTIRWCG	15	5.49	0.14	5.57	0.29	6.16	0.15
18	56-76	YKMMQGGVGIPTIRWCGAEGD	19	6.58	0.43	6.25	0.49	7.36	0.05
19	59-76	MQGGVGIPTIRWCGAEGD	16	6.66	0.26	6.77	0.04	7.36	0.18
20	71-90	CGAEGDYNVMVMELLGPSLE	18	0.93	0.07	0.92	0.08	0.95	0.05
21	81-89	VMELLGPSL	7	0.70	0.06	1.12	0.08	1.61	0.11
22	83-89	ELLGPSL	5	0.50	0.07	0.67	0.04	0.82	0.05

23	96-102	CSRKFSL	6	0.85	0.02	0.94	0.09	1.40	0.08
24	96-104	CSRKFSLKT	8	0.95	0.13	1.37	0.09	1.89	0.12
25	96-106	CSRKFSLKTVL	10	0.81	0.03	1.33	0.02	1.57	0.11
26	112-123	MISRIEYIHSKN	11	0.13	0.06	0.28	0.05	0.43	0.05
27	113-123	ISRIEYIHSKN	10	0.05	0.04	0.11	0.05	0.23	0.05
28	124-134	FIHRDVKPDNF	9	0.35	0.02	0.36	0.06	0.54	0.02
29	125-134	IHRDVKPDNF	8	0.24	0.03	0.24	0.10	0.48	0.04
30	126-134	HRDVKPDNF	7	0.18	0.03	0.24	0.03	0.37	0.03
31	135-143	LMGLGKKGN	8	1.95	0.03	2.34	0.09	2.69	0.08
32	135-144	LMGLGKKGNL	9	2.03	0.19	2.54	0.17	2.84	0.15
33	135-146	LMGLGKKGNLVY	11	2.11	0.11	2.55	0.14	2.93	0.10
34	136-144	MGLGKKGNL	8	2.02	0.03	2.36	0.09	2.54	0.04
35	145-151	VYIIDFG	6	0.72	0.10	1.55	0.21	1.75	0.26
36	145-158	VYIIDFGLAKKYRD	13	0.99	0.49	1.42	0.29	1.72	0.33
37	150-169	FGLAKKYRDARTHQHIPYRE	18	4.03	0.24	4.08	0.22	5.19	0.27
38	150-179	FGLAKKYRDARTHQHIPYRENKNLTGTARY	28	7.59	0.11	8.47	0.35	8.75	0.23
39	151-179	GLAKKYRDARTHQHIPYRENKNLTGTARY	27	7.85	0.32	7.81	0.66	8.84	0.35
40	153-179	AKKYRDARTHQHIPYRENKNLTGTARY	25	7.34	0.15	7.52	0.16	7.79	0.06
41	159-179	ARTHQHIPYRENKNLTGTARY	19	6.02	0.14	6.17	0.15	6.64	0.09
42	160-166	RTHQHIP	5	0.86	0.03	1.11	0.05	1.26	0.02
43	166-179	PYRENKNLTGTARY	12	4.99	0.13	5.14	0.30	5.12	0.14
44	180-186	ASINTHL	6	1.47	0.02	2.17	0.09	2.27	0.03
45	180-196	ASINTHLGIEQSRRDDL	16	2.37	0.08	4.02	0.02	4.26	0.06
46	185-196	HLGIEQSRRDDL	11	1.27	0.08	1.83	0.08	2.14	0.05
47	187-196	GIEQSRRDDL	9	1.13	0.02	1.56	0.04	1.71	0.05
48	188-195	IEQSRRDD	7	1.20	0.03	1.26	0.06	1.47	0.03
49	205-215	YFNLGSLPWQG	9	2.62	0.06	3.40	0.12	3.85	0.09
50	205-216	YFNLGSLPWQGL	10	3.10	0.08	3.84	0.17	4.14	0.11
51	205-218	YFNLGSLPWQGLKA	12	4.65	0.03	5.59	0.12	5.83	0.06
52	206-216	FNLGSLPWQGL	9	3.26	0.07	4.06	0.17	4.34	0.02

53	206-218	FNLGSLPWQGLKA	11	4.90	0.17	5.98	0.18	6.34	0.07
54	207-222	NLGSLPWQGLKAATKR	14	5.48	0.06	5.91	0.30	6.25	0.10
55	209-216	GSLPWQGL	6	2.91	0.07	3.36	0.06	3.44	0.05
56	209-218	GSLPWQGLKA	8	4.04	0.05	4.31	0.16	4.68	0.04
57	219-230	ATKRQKYERISE	11	4.46	0.06	4.60	0.05	4.56	0.03
58	231-238	KKMSTPIE	6	2.51	0.09	2.63	0.13	2.62	0.10
59	231-240	KKMSTPIEVL	8	2.61	0.04	2.97	0.07	3.22	0.05
60	235-249	TPIEVLCKGYPSEFA	12	1.73	0.02	2.62	0.03	2.89	0.03
61	239-248	VLCKGYPSEF	8	1.36	0.09	1.77	0.01	2.16	0.06
62	241-247	CKGYPSE	5	1.15	0.02	1.44	0.03	1.74	0.04
63	241-248	CKGYPSEF	6	1.05	0.02	1.35	0.03	1.55	0.02
64	248-254	FATYLNF	6	0.06	0.02	0.12	0.02	0.26	0.02
65	255-269	CRSLRFDDKPDYSYL	13	3.61	0.08	3.90	0.13	4.12	0.09
66	261-269	DDKPDYSYL	7	2.24	0.07	2.22	0.14	2.40	0.14
67	269-277	LRQLFRNLF	8	0.10	0.09	0.45	0.09	0.73	0.08
68	269-285	LRQLFRNLFHRQGFSYD	16	2.55	0.08	2.83	0.25	3.24	0.09
69	270-277	RQLFRNLF	7	0.06	0.07	0.35	0.08	0.66	0.07
70	273-285	FRNLFHRQGFSYD	12	2.36	0.08	2.46	0.19	2.80	0.09
71	278-285	HRQGFSYD	7	2.09	0.11	2.02	0.16	2.26	0.15
72	286-294	YVFDWNMLK	8	3.95	0.07	4.31	0.10	4.44	0.07
73	288-294	FDWNMLK	6	3.02	0.04	3.37	0.09	3.61	0.05

				CK1δ1-ΔC + ATP					
				Relative Deuterium Uptake (Da)					
				1 min		**5 min**		**10 min**	
S.No.	**Peptide**	**Sequence**	**Num Exchangers**	**Uptake**	**± SD**	**Uptake**	**± SD**	**Uptake**	**± SD**
1	1-20	MELRVGNRYRLGRKIGSGSF	19	5.56	0.39	6.00	0.52	6.30	0.35
2	3-10	LRVGNRYR	7	2.97	0.25	3.34	0.10	3.56	0.19
3	3-20	LRVGNRYRLGRKIGSGSF	17	6.20	0.09	6.81	0.17	7.02	0.19
4	4-20	RVGNRYRLGRKIGSGSF	16	5.06	0.07	5.60	0.07	5.78	0.17
5	4-22	RVGNRYRLGRKIGSGSFGD	18	5.77	0.10	6.26	0.09	6.45	0.21
6	12-25	GRKIGSGSFGDIYL	13	3.23	0.07	3.43	0.04	3.46	0.05
7	21-28	GDIYLGTD	7	0.41	0.01	0.94	0.04	1.36	0.07
8	24-39	YLGTDIAAGEEVAIKL	15	2.88	0.06	2.76	0.06	2.92	0.10
9	26-34	GTDIAAGEE	8	3.61	0.06	3.64	0.02	3.60	0.04

10	28-34	DIAAGEE	6	2.69	0.01	2.64	0.10	2.62	0.07
11	28-36	DIAAGEEVA	8	2.75	0.02	2.72	0.07	2.86	0.16
12	29-36	IAAGEEVA	7	2.26	0.03	2.20	0.03	2.22	0.03
13	35-41	VAIKLEC	6	0.84	0.05	0.83	0.11	0.89	0.05
14	37-49	IKLECVKTKH PQL	11	2.40	0.03	2.31	0.05	2.09	0.08
15	42-49	VKTKHPQL	6	1.67	0.05	1.67	0.03	1.62	0.05
16	50-58	HIESKIYKM	8	1.33	0.06	1.98	0.06	2.12	0.05
17	56-72	YKMMQGGV GIPTIRWCG	15	4.46	0.27	4.96	0.34	4.81	0.08
18	56-76	YKMMQGGV GIPTIRWCGA EGD	19	6.39	0.56	6.51	0.73	5.92	0.13
19	59-76	MQGGVGIPTI RWCGAEGD	16	5.92	0.49	5.85	0.38	6.24	0.42
20	71-90	CGAEGDYNV MVMELLGPS LE	18	0.95	0.02	0.92	0.02	0.83	0.10
21	81-89	VMELLGPSL	7	0.19	0.07	0.23	0.05	0.29	0.04
22	83-89	ELLGPSL	5	0.14	0.06	0.16	0.04	0.22	0.04
23	96-102	CSRKFSL	6	0.83	0.01	0.95	0.02	1.04	0.05
24	96-104	CSRKFSLKT	8	1.06	0.11	1.34	0.12	1.48	0.13
25	96-106	CSRKFSLKT VL	10	0.91	0.11	1.24	0.02	1.38	0.03
26	112-123	MISRIEYIHSK N	11	0.16	0.07	0.27	0.06	0.42	0.06
27	113-123	ISRIEYIHSKN	10	0.09	0.06	0.12	0.06	0.19	0.06
28	124-134	FIHRDVKPDN F	9	0.41	0.02	0.48	0.01	0.65	0.02
29	125-134	IHRDVKPDNF	8	0.32	0.10	0.39	0.08	0.66	0.09
30	126-134	HRDVKPDNF	7	0.29	0.03	0.42	0.04	0.52	0.03
31	135-143	LMGLGKKGN	8	2.00	0.03	2.25	0.04	2.39	0.07
32	135-144	LMGLGKKGN L	9	2.16	0.11	2.35	0.13	2.55	0.14
33	135-146	LMGLGKKGN LVY	11	2.15	0.11	2.37	0.11	2.50	0.11
34	136-144	MGLGKKGNL	8	1.97	0.04	2.09	0.03	2.21	0.07
35	145-151	VYIIDFG	6	0.20	0.11	0.34	0.12	0.41	0.11
36	145-158	VYIIDFGLAK KYRD	13	1.32	0.32	1.52	0.07	1.71	0.05
37	150-169	FGLAKKYRD ARTHQHIPY RE	18	4.04	0.27	4.20	0.28	4.43	0.21
38	150-179	FGLAKKYRD ARTHQHIPY RENKNLTGT ARY	28	7.56	0.12	7.66	0.11	7.81	0.26
39	151-179	GLAKKYRDA RTHQHIPYR ENKNLTGTA RY	27	7.82	0.45	7.99	0.26	7.63	0.21
40	153-179	AKKYRDART HQHIPYREN KNLTGTARY	25	7.45	0.13	7.19	0.07	7.20	0.05

41	159-179	ARTHQHIPY RENKNLTGT ARY	19	6.11	0.08	6.00	0.22	5.95	0.11
42	160-166	RTHQHIP	5	0.87	0.03	1.11	0.02	1.24	0.03
43	166-179	PYRENKNLT GTARY	12	5.11	0.21	4.92	0.17	4.89	0.19
44	180-186	ASINTHL	6	1.51	0.03	2.16	0.02	2.22	0.05
45	180-196	ASINTHLGIE QSRRDDL	16	2.87	0.06	3.96	0.04	4.22	0.13
46	185-196	HLGIEQSRR DDL	11	1.40	0.04	1.86	0.07	2.09	0.05
47	187-196	GIEQSRRDD L	9	1.15	0.02	1.51	0.03	1.62	0.03
48	188-195	IEQSRRDD	7	1.13	0.03	1.30	0.03	1.39	0.06
49	205-215	YFNLGSLPW QG	9	2.58	0.06	3.45	0.07	3.60	0.08
50	205-216	YFNLGSLPW QGL	10	3.11	0.09	3.85	0.07	3.94	0.13
51	205-218	YFNLGSLPW QGLKA	12	4.71	0.03	5.45	0.03	5.65	0.10
52	206-216	FNLGSLPWQ GL	9	3.35	0.15	4.10	0.15	4.09	0.13
53	206-218	FNLGSLPWQ GLKA	11	5.01	0.06	5.85	0.07	6.02	0.19
54	207-222	NLGSLPWQG LKAATKR	14	4.85	0.03	5.35	0.04	5.47	0.21
55	209-216	GSLPWQGL	6	3.00	0.03	3.38	0.01	3.42	0.04
56	209-218	GSLPWQGLK A	8	4.05	0.07	4.32	0.05	4.36	0.05
57	219-230	ATKRQKYERI SE	11	4.45	0.13	4.48	0.11	4.37	0.11
58	231-238	KKMSTPIE	6	2.54	0.10	2.56	0.10	2.51	0.11
59	231-240	KKMSTPIEVL	8	2.62	0.04	2.94	0.05	3.04	0.02
60	235-249	TPIEVLCKGY PSEFA	12	2.00	0.03	2.53	0.05	2.71	0.03
61	239-248	VLCKGYPSE F	8	1.34	0.01	1.71	0.04	2.01	0.04
62	241-247	CKGYPSE	5	1.15	0.01	1.40	0.02	1.63	0.05
63	241-248	CKGYPSEF	6	1.07	0.02	1.29	0.02	1.49	0.02
64	248-254	FATYLNF	6	0.08	0.03	0.18	0.04	0.23	0.03
65	255-269	CRSLRFDDK PDYSYL	13	3.65	0.06	3.91	0.06	3.91	0.09
66	261-269	DDKPDYSYL	7	2.30	0.09	2.24	0.08	2.24	0.06
67	269-277	LRQLFRNLF	8	0.12	0.09	0.44	0.09	0.62	0.09
68	269-285	LRQLFRNLF HRQGFSYD	16	2.59	0.09	2.93	0.07	3.04	0.11
69	270-277	RQLFRNLF	7	0.11	0.07	0.39	0.08	0.56	0.09
70	273-285	FRNLFHRQG FSYD	12	2.39	0.06	2.46	0.08	2.55	0.08
71	278-285	HRQGFSYD	7	2.13	0.11	2.04	0.13	2.02	0.12
72	286-294	YVFDWNMLK	8	3.88	0.06	4.23	0.07	4.29	0.10
73	288-294	FDWNMLK	6	3.11	0.04	3.34	0.04	3.52	0.04
				CK1δ1-ΔC + AMPPNP					

				Relative Deuterium Uptake (Da)					
				1 min		5 min		10 min	
S.No.	Peptide	Sequence	Num Exchangers	Uptake	± SD	Uptake	± SD	Uptake	± SD
1	1-20	MELRVGNRYRLGRKIGSGSF	19	5.52	0.59	6.01	0.53	6.33	0.30
2	3-10	LRVGNRYR	7	2.99	0.09	3.46	0.18	3.64	0.24
3	3-20	LRVGNRYRLGRKIGSGSF	17	6.56	0.14	7.21	0.08	7.37	0.09
4	4-20	RVGNRYRLGRKIGSGSF	16	5.27	0.20	5.83	0.05	6.09	0.05
5	4-22	RVGNRYRLGRKIGSGSFGD	18	6.02	0.21	6.73	0.33	6.87	0.34
6	12-25	GRKIGSGSFGDIYL	13	3.37	0.21	4.32	0.05	4.71	0.03
7	21-28	GDIYLGTD	7	0.88	0.01	2.26	0.05	2.89	0.02
8	24-39	YLGTDIAAGEEVAIKL	15	2.60	0.19	2.65	0.09	2.77	0.08
9	26-34	GTDIAAGEE	8	3.54	0.06	3.58	0.01	3.59	0.01
10	28-34	DIAAGEE	6	2.59	0.07	2.57	0.01	2.58	0.03
11	28-36	DIAAGEEVA	8	2.70	0.04	2.78	0.03	2.92	0.10
12	29-36	IAAGEEVA	7	2.21	0.05	2.29	0.03	2.44	0.03
13	35-41	VAIKLEC	6	1.03	0.04	1.42	0.11	1.45	0.06
14	37-49	IKLECVKTKHPQL	11	2.17	0.06	2.13	0.02	2.26	0.04
15	42-49	VKTKHPQL	6	1.61	0.10	1.54	0.05	1.58	0.02
16	50-58	HIESKIYKM	8	1.50	0.06	2.32	0.07	2.58	0.07
17	56-72	YKMMQGGVGIPTIRWCG	15	5.23	0.07	5.53	0.02	5.42	0.16
18	56-76	YKMMQGGVGIPTIRWCGAEGD	19	6.08	0.60	6.56	0.15	6.35	0.18
19	59-76	MQGGVGIPTIRWCGAEGD	16	6.28	0.42	6.48	0.10	7.25	0.47
20	71-90	CGAEGDYNVMVMELLGPSLE	18	0.67	0.02	0.81	0.05	0.86	0.03
21	81-89	VMELLGPSL	7	0.21	0.04	0.50	0.05	0.79	0.04
22	83-89	ELLGPSL	5	0.17	0.04	0.36	0.05	0.47	0.05
23	96-102	CSRKFSL	6	0.81	0.05	0.98	0.01	1.07	0.01
24	96-104	CSRKFSLKT	8	1.11	0.12	1.40	0.12	1.62	0.19
25	96-106	CSRKFSLKTVL	10	0.83	0.02	1.27	0.02	1.45	0.05
26	112-123	MISRIEYIHSKN	11	0.17	0.06	0.26	0.06	0.43	0.06
27	113-123	ISRIEYIHSKN	10	0.08	0.06	0.14	0.07	0.17	0.07
28	124-134	FIHRDVKPDNF	9	0.30	0.05	0.40	0.02	0.51	0.01
29	125-134	IHRDVKPDNF	8	0.22	0.05	0.44	0.14	0.55	0.06
30	126-134	HRDVKPDNF	7	0.26	0.03	0.34	0.03	0.44	0.03

31	135-143	LMGLGKKGN	8	1.82	0.08	2.10	0.01	2.33	0.04
32	135-144	LMGLGKKGNL	9	1.99	0.13	2.24	0.11	2.51	0.14
33	135-146	LMGLGKKGNLVY	11	1.96	0.14	2.26	0.11	2.44	0.11
34	136-144	MGLGKKGNL	8	1.79	0.05	1.97	0.04	2.20	0.03
35	145-151	VYIIDFG	6	0.41	0.10	1.08	0.15	1.32	0.13
36	145-158	VYIIDFGLAKKYRD	13	1.20	0.35	1.55	0.05	1.70	0.05
37	150-169	FGLAKKYRDARTHQHIPYRE	18	3.83	0.41	4.17	0.37	4.77	0.48
38	150-179	FGLAKKYRDARTHQHIPYRENKNLTGTARY	28	7.10	0.35	7.59	0.17	7.95	0.17
39	151-179	GLAKKYRDARTHQHIPYRENKNLTGTARY	27	7.11	0.30	7.59	0.30	7.81	0.36
40	153-179	AKKYRDARTHQHIPYRENKNLTGTARY	25	6.80	0.46	6.97	0.13	7.19	0.08
41	159-179	ARTHQHIPYRENKNLTGTARY	19	5.60	0.04	5.81	0.07	5.96	0.08
42	160-166	RTHQHIP	5	0.86	0.04	1.09	0.05	1.19	0.02
43	166-179	PYRENKNLTGTARY	12	5.19	0.07	4.80	0.13	5.00	0.07
44	180-186	ASINTHL	6	1.69	0.06	2.12	0.03	2.15	0.02
45	180-196	ASINTHLGIEQSRRDDL	16	2.72	0.09	3.87	0.06	4.17	0.06
46	185-196	HLGIEQSRRDDL	11	1.32	0.07	1.75	0.06	2.04	0.08
47	187-196	GIEQSRRDDL	9	1.18	0.04	1.43	0.03	1.61	0.02
48	188-195	IEQSRRDD	7	1.00	0.02	1.25	0.03	1.34	0.03
49	205-215	YFNLGSLPWQG	9	2.85	0.15	3.68	0.07	3.90	0.06
50	205-216	YFNLGSLPWQGL	10	3.42	0.06	4.24	0.06	4.26	0.06
51	205-218	YFNLGSLPWQGLKA	12	4.75	0.03	5.48	0.04	5.70	0.03
52	206-216	FNLGSLPWQGL	9	3.42	0.18	4.29	0.05	4.31	0.15
53	206-218	FNLGSLPWQGLKA	11	5.04	0.11	5.91	0.07	6.12	0.07
54	207-222	NLGSLPWQGLKAATKR	14	5.16	0.11	5.77	0.10	5.87	0.04
55	209-216	GSLPWQGL	6	3.00	0.08	3.37	0.03	3.44	0.03
56	209-218	GSLPWQGLKA	8	4.04	0.12	4.39	0.09	4.61	0.04
57	219-230	ATKRQKYERISE	11	4.41	0.18	4.25	0.07	4.37	0.13
58	231-238	KKMSTPIE	6	2.43	0.10	2.43	0.10	2.47	0.09
59	231-240	KKMSTPIEVL	8	2.52	0.08	2.89	0.03	3.10	0.02
60	235-249	TPIEVLCKGYPSEFA	12	1.86	0.04	2.49	0.04	2.80	0.04

61	239-248	VLCKGYPSEF	8	1.36	0.06	1.74	0.06	2.07	0.05
62	241-247	CKGYPSE	5	1.13	0.03	1.38	0.03	1.67	0.02
63	241-248	CKGYPSEF	6	1.00	0.02	1.26	0.02	1.45	0.02
64	248-254	FATYLNF	6	0.11	0.03	0.18	0.03	0.21	0.03
65	255-269	CRSLRFDDKPDYSYL	13	3.49	0.12	3.74	0.09	3.87	0.07
66	261-269	DDKPDYSYL	7	2.14	0.15	2.17	0.02	2.17	0.09
67	269-277	LRQLFRNLF	8	0.14	0.09	0.47	0.08	0.65	0.08
68	269-285	LRQLFRNLFHRQGFSYD	16	2.37	0.12	2.76	0.07	2.89	0.07
69	270-277	RQLFRNLF	7	0.12	0.08	0.41	0.07	0.61	0.07
70	273-285	FRNLFHRQGFSYD	12	2.22	0.17	2.42	0.09	2.52	0.07
71	278-285	HRQGFSYD	7	1.95	0.15	1.92	0.12	1.96	0.12
72	286-294	YVFDWNMLK	8	3.82	0.07	4.23	0.06	4.27	0.06
73	288-294	FDWNMLK	6	3.04	0.06	3.35	0.04	3.44	0.04
				CK1δ1-ΔC					
				Relative Fractional Deuterium Uptake (%)					
				1 min		**5 min**		**10 min**	
S.No.	**Peptide**	**Sequence**	**Num Exchangers**	**Uptake**	**± SD**	**Uptake**	**± SD**	**Uptake**	**± SD**
1	1-20	MELRVGNRYRLGRKIGSGSF	19	31.78	2.82	34.46	2.98	35.91	1.86
2	3-10	LRVGNRYR	7	46.83	1.09	50.11	1.29	50.12	0.75
3	3-20	LRVGNRYRLGRKIGSGSF	17	41.76	0.51	44.62	1.56	45.85	0.48
4	4-20	RVGNRYRLGRKIGSGSF	16	36.59	0.58	39.35	1.92	41.23	0.59
5	4-22	RVGNRYRLGRKIGSGSFGD	18	36.43	0.63	39.01	1.59	40.49	0.75
6	12-25	GRKIGSGSFGDIYL	13	30.72	1.22	41.26	0.42	42.43	1.27
7	21-28	GDIYLGTD	7	23.46	0.35	46.97	1.26	53.90	0.54
8	24-39	YLGTDIAAGEEVAIKL	15	20.53	1.00	20.57	2.68	22.32	1.17
9	26-34	GTDIAAGEE	8	45.02	0.54	45.96	0.19	46.72	0.47
10	28-34	DIAAGEE	6	44.05	0.79	46.37	1.25	45.17	0.22
11	28-36	DIAAGEEVA	8	35.02	0.28	37.92	1.86	44.05	2.00
12	29-36	IAAGEEVA	7	32.82	0.60	36.49	1.36	42.68	0.40
13	35-41	VAIKLEC	6	21.10	0.25	31.73	1.03	39.32	0.12
14	37-49	IKLECVKTKHPQL	11	23.68	0.69	24.84	0.94	25.23	0.41
15	42-49	VKTKHPQL	6	28.08	0.80	28.38	1.37	28.72	0.98
16	50-58	HIESKIYKM	8	20.21	1.03	32.67	0.58	37.90	1.57
17	56-72	YKMMQGGVGIPTIRWCG	15	36.60	0.91	37.14	1.95	41.05	1.01

18	56-76	YKMMQGGVGIPTIRWCGAEGD	19	34.63	2.27	32.89	2.59	38.76	0.27
19	59-76	MQGGVGIPTIRWCGAEGD	16	41.62	1.63	42.32	0.24	46.02	1.15
20	71-90	CGAEGDYNVMVMELLGPSLE	18	5.17	0.37	5.12	0.47	5.27	0.28
21	81-89	VMELLGPSL	7	9.95	0.81	16.02	1.14	23.02	1.54
22	83-89	ELLGPSL	5	10.01	1.34	13.42	0.86	16.35	0.93
23	96-102	CSRKFSL	6	14.13	0.29	15.69	1.55	23.32	1.26
24	96-104	CSRKFSLKT	8	11.83	1.60	17.16	1.17	23.59	1.55
25	96-106	CSRKFSLKTVL	10	8.08	0.31	13.27	0.15	15.70	1.13
26	112-123	MISRIEYIHSKN	11	1.14	0.52	2.57	0.41	3.91	0.49
27	113-123	ISRIEYIHSKN	10	0.50	0.43	1.07	0.47	2.27	0.47
28	124-134	FIHRDVKPDNF	9	3.86	0.22	4.00	0.63	5.95	0.19
29	125-134	IHRDVKPDNF	8	3.03	0.40	3.06	1.24	6.05	0.55
30	126-134	HRDVKPDNF	7	2.58	0.44	3.42	0.36	5.30	0.36
31	135-143	LMGLGKKGN	8	24.38	0.33	29.23	1.10	33.67	1.02
32	135-144	LMGLGKKGNL	9	22.57	2.11	28.22	1.86	31.61	1.69
33	135-146	LMGLGKKGNLVY	11	19.17	1.03	23.16	1.27	26.66	0.94
34	136-144	MGLGKKGNL	8	25.22	0.32	29.56	1.12	31.76	0.53
35	145-151	VYIIDFG	6	12.03	1.68	25.77	3.44	29.12	4.28
36	145-158	VYIIDFGLAKKYRD	13	7.59	3.75	10.91	2.19	13.25	2.52
37	150-169	FGLAKKYRDARTHQHIPYRE	18	22.38	1.34	22.68	1.20	28.84	1.49
38	150-179	FGLAKKYRDARTHQHIPYRENKNLTGTARY	28	27.11	0.40	30.23	1.25	31.27	0.81
39	151-179	GLAKKYRDARTHQHIPYRENKNLTGTARY	27	29.08	1.18	28.94	2.46	32.73	1.30
40	153-179	AKKYRDARTHQHIPYRENKNLTGTARY	25	29.36	0.62	30.07	0.63	31.15	0.24
41	159-179	ARTHQHIPYRENKNLTGTARY	19	31.69	0.75	32.47	0.78	34.96	0.48
42	160-166	RTHQHIP	5	17.13	0.60	22.14	0.95	25.28	0.44
43	166-179	PYRENKNLTGTARY	12	41.62	1.12	42.84	2.48	42.70	1.14
44	180-186	ASINTHL	6	24.46	0.26	36.09	1.56	37.83	0.57
45	180-196	ASINTHLGIEQSRRDDL	16	14.82	0.52	25.12	0.10	26.62	0.38
46	185-196	HLGIEQSRRDDL	11	11.56	0.76	16.62	0.75	19.43	0.42
47	187-196	GIEQSRRDDL	9	12.52	0.25	17.38	0.45	18.95	0.55

48	188-195	IEQSRRDD	7	17.13	0.42	17.94	0.88	20.97	0.40
49	205-215	YFNLGSLPWQG	9	29.08	0.69	37.77	1.30	42.74	1.01
50	205-216	YFNLGSLPWQGL	10	30.98	0.82	38.42	1.72	41.42	1.12
51	205-218	YFNLGSLPWQGLKA	12	38.75	0.26	46.58	1.04	48.62	0.46
52	206-216	FNLGSLPWQGL	9	36.23	0.81	45.10	1.89	48.20	0.17
53	206-218	FNLGSLPWQGLKA	11	44.50	1.50	54.39	1.60	57.67	0.60
54	207-222	NLGSLPWQGLKAATKR	14	39.14	0.41	42.22	2.17	44.64	0.69
55	209-216	GSLPWQGL	6	48.58	1.21	56.01	1.04	57.40	0.91
56	209-218	GSLPWQGLKA	8	50.51	0.57	53.91	1.97	58.49	0.52
57	219-230	ATKRQKYERISE	11	40.57	0.52	41.83	0.47	41.41	0.24
58	231-238	KKMSTPIE	6	41.89	1.54	43.77	2.13	43.64	1.74
59	231-240	KKMSTPIEVL	8	32.60	0.46	37.16	0.87	40.21	0.59
60	235-249	TPIEVLCKGYPSEFA	12	14.45	0.21	21.84	0.27	24.08	0.26
61	239-248	VLCKGYPSEF	8	17.01	1.12	22.07	0.11	27.02	0.72
62	241-247	CKGYPSE	5	23.10	0.39	28.70	0.65	34.82	0.87
63	241-248	CKGYPSEF	6	17.49	0.35	22.45	0.50	25.82	0.35
64	248-254	FATYLNF	6	1.06	0.33	1.95	0.32	4.34	0.31
65	255-269	CRSLRFDDKPDYSYL	13	27.75	0.60	29.99	0.99	31.69	0.72
66	261-269	DDKPDYSYL	7	32.04	1.05	31.67	1.99	34.26	2.04
67	269-277	LRQLFRNLF	8	1.29	1.08	5.64	1.10	9.06	1.03
68	269-285	LRQLFRNLFHRQGFSYD	16	15.92	0.51	17.70	1.55	20.26	0.55
69	270-277	RQLFRNLF	7	0.87	0.98	4.95	1.16	9.36	1.04
70	273-285	FRNLFHRQGFSYD	12	19.66	0.66	20.46	1.56	23.34	0.71
71	278-285	HRQGFSYD	7	29.91	1.56	28.90	2.31	32.29	2.19
72	286-294	YVFDWNMLK	8	49.39	0.84	53.91	1.24	55.56	0.86
73	288-294	FDWNMLK	6	50.35	0.59	56.19	1.56	60.21	0.83

				CK1δ1-ΔC + ATP					
				Relative Fractional Deuterium Uptake (%)					
				1 min		**5 min**		**10 min**	
S.No.	**Peptide**	**Sequence**	**Num Exchangers**	**Uptake**	**± SD**	**Uptake**	**± SD**	**Uptake**	**± SD**
1	1-20	MELRVGNRYRLGRKIGSGSF	19	29.25	2.07	31.56	2.74	33.17	1.83
2	3-10	LRVGNRYR	7	42.38	3.54	47.65	1.49	50.91	2.68
3	3-20	LRVGNRYRLGRKIGSGSF	17	36.47	0.55	40.07	0.99	41.32	1.10
4	4-20	RVGNRYRLGRKIGSGSF	16	31.62	0.42	35.01	0.41	36.15	1.05

5	4-22	RVGNRYRLGRKIGSGSFGD	18	32.06	0.56	34.76	0.53	35.86	1.17
6	12-25	GRKIGSGSFGDIYL	13	24.88	0.54	26.40	0.27	26.63	0.41
7	21-28	GDIYLGTD	7	5.83	0.13	13.45	0.52	19.39	0.96
8	24-39	YLGTDIAAGEEVAIKL	15	19.20	0.41	18.37	0.39	19.49	0.66
9	26-34	GTDIAAGEE	8	45.17	0.73	45.52	0.19	44.95	0.48
10	28-34	DIAAGEE	6	44.86	0.21	44.05	1.71	43.63	1.12
11	28-36	DIAAGEEVA	8	34.37	0.29	34.05	0.89	35.76	1.98
12	29-36	IAAGEEVA	7	32.35	0.40	31.40	0.41	31.73	0.39
13	35-41	VAIKLEC	6	13.96	0.75	13.84	1.83	14.88	0.77
14	37-49	IKLECVKTKHPQL	11	21.82	0.31	21.00	0.42	19.02	0.69
15	42-49	VKTKHPQL	6	27.79	0.90	27.89	0.55	26.96	0.90
16	50-58	HIESKIYKM	8	16.63	0.76	24.80	0.70	26.51	0.58
17	56-72	YKMMQGGVGIPTIRWCG	15	29.76	1.82	33.03	2.28	32.09	0.56
18	56-76	YKMMQGGVGIPTIRWCGAEGD	19	33.61	2.93	34.26	3.86	31.14	0.66
19	59-76	MQGGVGIPTIRWCGAEGD	16	37.03	3.04	36.55	2.37	38.98	2.66
20	71-90	CGAEGDYNVMVMELLGPSLE	18	5.28	0.09	5.12	0.09	4.63	0.54
21	81-89	VMELLGPSL	7	2.75	1.01	3.22	0.69	4.18	0.63
22	83-89	ELLGPSL	5	2.82	1.17	3.26	0.86	4.47	0.83
23	96-102	CSRKFSL	6	13.91	0.17	15.77	0.39	17.33	0.84
24	96-104	CSRKFSLKT	8	13.21	1.41	16.80	1.44	18.53	1.59
25	96-106	CSRKFSLKTVL	10	9.12	1.10	12.36	0.25	13.77	0.28
26	112-123	MISRIEYIHSKN	11	1.50	0.60	2.49	0.57	3.81	0.51
27	113-123	ISRIEYIHSKN	10	0.90	0.58	1.23	0.60	1.87	0.59
28	124-134	FIHRDVKPDNF	9	4.52	0.22	5.38	0.08	7.25	0.17
29	125-134	IHRDVKPDNF	8	4.02	1.24	4.93	0.97	8.29	1.15
30	126-134	HRDVKPDNF	7	4.10	0.37	6.00	0.63	7.43	0.43
31	135-143	LMGLGKKGN	8	25.05	0.35	28.09	0.54	29.85	0.89
32	135-144	LMGLGKKGNL	9	24.00	1.20	26.15	1.47	28.34	1.58
33	135-146	LMGLGKKGNLVY	11	19.58	0.99	21.54	1.02	22.74	1.04
34	136-144	MGLGKKGNL	8	24.63	0.53	26.13	0.41	27.64	0.83
35	145-151	VYIIDFG	6	3.39	1.87	5.65	2.01	6.90	1.84
36	145-158	VYIIDFGLAKKYRD	13	10.13	2.44	11.67	0.52	13.15	0.41
37	150-169	FGLAKKYRDARTHQHIPYRE	18	22.47	1.52	23.34	1.55	24.58	1.14

38	150-179	FGLAKKYRDARTHQHIPYRENKNLTGTARY	28	27.00	0.42	27.37	0.39	27.91	0.93
39	151-179	GLAKKYRDARTHQHIPYRENKNLTGTARY	27	28.97	1.66	29.60	0.95	28.27	0.79
40	153-179	AKKYRDARTHQHIPYRENKNLTGTARY	25	29.81	0.53	28.74	0.29	28.78	0.21
41	159-179	ARTHQHIPYRENKNLTGTARY	19	32.17	0.41	31.60	1.18	31.31	0.60
42	160-166	RTHQHIP	5	17.44	0.60	22.22	0.48	24.86	0.63
43	166-179	PYRENKNLTGTARY	12	42.58	1.74	41.03	1.38	40.72	1.56
44	180-186	ASINTHL	6	25.18	0.47	36.08	0.41	36.93	0.78
45	180-196	ASINTHLGIEQSRRDDL	16	17.96	0.39	24.73	0.22	26.35	0.84
46	185-196	HLGIEQSRRDDL	11	12.68	0.40	16.87	0.68	19.03	0.46
47	187-196	GIEQSRRDDL	9	12.74	0.27	16.75	0.38	17.97	0.34
48	188-195	IEQSRRDD	7	16.09	0.42	18.50	0.42	19.92	0.87
49	205-215	YFNLGSLPWQG	9	28.62	0.67	38.38	0.74	39.97	0.91
50	205-216	YFNLGSLPWQGL	10	31.07	0.86	38.54	0.73	39.41	1.26
51	205-218	YFNLGSLPWQGLKA	12	39.24	0.26	45.44	0.26	47.08	0.86
52	206-216	FNLGSLPWQGL	9	37.22	1.65	45.56	1.71	45.48	1.41
53	206-218	FNLGSLPWQGLKA	11	45.52	0.59	53.15	0.61	54.77	1.70
54	207-222	NLGSLPWQGLKAATKR	14	34.65	0.24	38.20	0.27	39.06	1.53
55	209-216	GSLPWQGL	6	49.98	0.51	56.33	0.17	56.96	0.72
56	209-218	GSLPWQGLKA	8	50.61	0.86	53.97	0.66	54.49	0.65
57	219-230	ATKRQKYERISE	11	40.47	1.17	40.69	0.99	39.68	1.01
58	231-238	KKMSTPIE	6	42.27	1.61	42.62	1.64	41.85	1.84
59	231-240	KKMSTPIEVL	8	32.74	0.52	36.78	0.60	38.03	0.30
60	235-249	TPIEVLCKGYPSEFA	12	16.68	0.25	21.05	0.39	22.57	0.23
61	239-248	VLCKGYPSEF	8	16.75	0.08	21.39	0.48	25.15	0.48
62	241-247	CKGYPSE	5	22.92	0.20	28.00	0.43	32.55	1.01
63	241-248	CKGYPSEF	6	17.83	0.41	21.53	0.35	24.77	0.32
64	248-254	FATYLNF	6	1.38	0.48	2.95	0.62	3.88	0.50
65	255-269	CRSLRFDDKPDYSYL	13	28.09	0.44	30.09	0.49	30.10	0.66
66	261-269	DDKPDYSYL	7	32.88	1.26	31.94	1.15	32.02	0.81
67	269-277	LRQLFRNLF	8	1.45	1.19	5.47	1.15	7.80	1.14
68	269-285	LRQLFRNLFHRQGFSYD	16	16.17	0.57	18.31	0.44	19.00	0.70

69	270-277	RQLFRNLF	7	1.53	1.01	5.52	1.13	8.03	1.27
70	273-285	FRNLFHRQGFSYD	12	19.90	0.48	20.47	0.63	21.24	0.69
71	278-285	HRQGFSYD	7	30.38	1.61	29.19	1.87	28.89	1.71
72	286-294	YVFDWNMLK	8	48.52	0.81	52.82	0.88	53.63	1.23
73	288-294	FDWNMLK	6	51.92	0.64	55.63	0.70	58.74	0.58
				CK1δ1-ΔC + AMPPNP					
				Relative Fractional Deuterium Uptake (%)					
				1 min		**5 min**		**10 min**	
S.No.	**Peptide**	**Sequence**	**Num Exchangers**	**Uptake**	**± SD**	**Uptake**	**± SD**	**Uptake**	**± SD**
1	1-20	MELRVGNRYRLGRKIGSGSF	19	29.08	3.08	31.61	2.81	33.33	1.59
2	3-10	LRVGNRYR	7	42.64	1.23	49.44	2.63	52.00	3.36
3	3-20	LRVGNRYRLGRKIGSGSF	17	38.58	0.84	42.42	0.47	43.34	0.53
4	4-20	RVGNRYRLGRKIGSGSF	16	32.93	1.26	36.46	0.29	38.07	0.33
5	4-22	RVGNRYRLGRKIGSGSFGD	18	33.42	1.18	37.37	1.83	38.16	1.87
6	12-25	GRKIGSGSFGDIYL	13	25.95	1.60	33.21	0.39	36.20	0.23
7	21-28	GDIYLGTD	7	12.57	0.13	32.30	0.70	41.29	0.32
8	24-39	YLGTDIAAGEEVAIKL	15	17.35	1.26	17.68	0.57	18.48	0.53
9	26-34	GTDIAAGEE	8	44.19	0.73	44.78	0.17	44.85	0.17
10	28-34	DIAAGEE	6	43.11	1.25	42.75	0.25	42.93	0.45
11	28-36	DIAAGEEVA	8	33.74	0.56	34.76	0.34	36.47	1.30
12	29-36	IAAGEEVA	7	31.53	0.77	32.68	0.41	34.90	0.40
13	35-41	VAIKLEC	6	17.20	0.69	23.72	1.82	24.17	1.06
14	37-49	IKLECVKTKHPQL	11	19.69	0.59	19.32	0.16	20.53	0.36
15	42-49	VKTKHPQL	6	26.85	1.58	25.72	0.78	26.39	0.36
16	50-58	HIESKIYKM	8	18.79	0.78	29.06	0.89	32.26	0.84
17	56-72	YKMMQGGVGIPTIRWCG	15	34.85	0.48	36.87	0.15	36.12	1.09
18	56-76	YKMMQGGVGIPTIRWCGAEGD	19	32.02	3.17	34.55	0.79	33.44	0.93
19	59-76	MQGGVGIPTIRWCGAEGD	16	39.23	2.60	40.53	0.61	45.29	2.93
20	71-90	CGAEGDYNVMVMELLGPSLE	18	3.74	0.12	4.51	0.29	4.78	0.14
21	81-89	VMELLGPSL	7	3.04	0.62	7.11	0.77	11.26	0.50
22	83-89	ELLGPSL	5	3.38	0.77	7.13	1.08	9.40	0.97
23	96-102	CSRKFSL	6	13.57	0.79	16.42	0.17	17.86	0.18
24	96-104	CSRKFSLKT	8	13.91	1.50	17.51	1.55	20.21	2.37

25	96-106	CSRKFSLKTVL	10	8.33	0.15	12.67	0.17	14.52	0.47
26	112-123	MISRIEYIHSKN	11	1.54	0.54	2.41	0.50	3.91	0.54
27	113-123	ISRIEYIHSKN	10	0.76	0.56	1.39	0.69	1.71	0.68
28	124-134	FIHRDVKPDNF	9	3.34	0.53	4.44	0.27	5.63	0.08
29	125-134	IHRDVKPDNF	8	2.75	0.59	5.50	1.74	6.83	0.72
30	126-134	HRDVKPDNF	7	3.70	0.48	4.84	0.39	6.32	0.36
31	135-143	LMGLGKKGN	8	22.70	1.02	26.26	0.18	29.06	0.47
32	135-144	LMGLGKKGNL	9	22.07	1.40	24.91	1.21	27.85	1.51
33	135-146	LMGLGKKGNLVY	11	17.79	1.25	20.52	1.02	22.23	1.03
34	136-144	MGLGKKGNL	8	22.37	0.64	24.62	0.55	27.49	0.42
35	145-151	VYIIDFG	6	6.86	1.65	17.93	2.51	21.98	2.22
36	145-158	VYIIDFGLAKKYRD	13	9.23	2.67	11.92	0.41	13.08	0.41
37	150-169	FGLAKKYRDARTHQHIPYRE	18	21.26	2.25	23.15	2.04	26.51	2.67
38	150-179	FGLAKKYRDARTHQHIPYRENKNLTGTARY	28	25.37	1.23	27.11	0.61	28.40	0.59
39	151-179	GLAKKYRDARTHQHIPYRENKNLTGTARY	27	26.33	1.13	28.11	1.10	28.94	1.33
40	153-179	AKKYRDARTHQHIPYRENKNLTGTARY	25	27.22	1.85	27.88	0.53	28.75	0.33
41	159-179	ARTHQHIPYRENKNLTGTARY	19	29.48	0.19	30.56	0.37	31.39	0.44
42	160-166	RTHQHIP	5	17.11	0.87	21.71	1.09	23.70	0.47
43	166-179	PYRENKNLTGTARY	12	43.22	0.56	39.97	1.09	41.66	0.56
44	180-186	ASINTHL	6	28.19	0.92	35.39	0.57	35.80	0.34
45	180-196	ASINTHLGIEQSRRDDL	16	17.01	0.56	24.20	0.37	26.09	0.37
46	185-196	HLGIEQSRRDDL	11	12.02	0.63	15.93	0.59	18.57	0.76
47	187-196	GIEQSRRDDL	9	13.09	0.42	15.93	0.33	17.83	0.26
48	188-195	IEQSRRDD	7	14.23	0.27	17.81	0.40	19.10	0.37
49	205-215	YFNLGSLPWQG	9	31.68	1.70	40.89	0.74	43.31	0.66
50	205-216	YFNLGSLPWQGL	10	34.20	0.58	42.41	0.61	42.62	0.58
51	205-218	YFNLGSLPWQGLKA	12	39.61	0.26	45.64	0.33	47.54	0.26
52	206-216	FNLGSLPWQGL	9	38.05	2.03	47.66	0.53	47.86	1.67
53	206-218	FNLGSLPWQGLKA	11	45.78	1.00	53.74	0.62	55.66	0.62
54	207-222	NLGSLPWQGLKAATKR	14	36.88	0.80	41.22	0.69	41.90	0.29

55	209-216	GSLPWQGL	6	50.02	1.34	56.09	0.56	57.34	0.53
56	209-218	GSLPWQGLKA	8	50.46	1.52	54.91	1.13	57.68	0.55
57	219-230	ATKRQKYERISE	11	40.06	1.64	38.64	0.64	39.73	1.16
58	231-238	KKMSTPIE	6	40.46	1.74	40.58	1.60	41.14	1.57
59	231-240	KKMSTPIEVL	8	31.45	1.04	36.07	0.37	38.71	0.27
60	235-249	TPIEVLCKGYPSEFA	12	15.54	0.34	20.73	0.32	23.37	0.37
61	239-248	VLCKGYPSEF	8	17.03	0.77	21.75	0.75	25.88	0.60
62	241-247	CKGYPSE	5	22.54	0.56	27.57	0.56	33.32	0.38
63	241-248	CKGYPSEF	6	16.60	0.32	21.02	0.28	24.21	0.34
64	248-254	FATYLNF	6	1.91	0.47	3.00	0.55	3.57	0.57
65	255-269	CRSLRFDDKPDYSYL	13	26.85	0.95	28.79	0.68	29.79	0.54
66	261-269	DDKPDYSYL	7	30.55	2.10	30.94	0.27	30.94	1.27
67	269-277	LRQLFRNLF	8	1.72	1.18	5.89	1.03	8.17	1.02
68	269-285	LRQLFRNLFHRQGFSYD	16	14.84	0.72	17.28	0.42	18.05	0.42
69	270-277	RQLFRNLF	7	1.78	1.10	5.82	1.03	8.75	1.00
70	273-285	FRNLFHRQGFSYD	12	18.47	1.43	20.14	0.74	20.97	0.56
71	278-285	HRQGFSYD	7	27.88	2.13	27.44	1.70	28.04	1.69
72	286-294	YVFDWNMLK	8	47.71	0.85	52.93	0.78	53.44	0.78
73	288-294	FDWNMLK	6	50.63	0.93	55.80	0.71	57.35	0.60

				CK1δ1-ΔC					
				Relative Deuterium Uptake (Da)					
				1 min		**5 min**		**10 min**	
S.No.	**Peptide**	**Sequence**	**Num Exchangers**	**Uptake**	**± SD**	**Uptake**	**± SD**	**Uptake**	**± SD**
1	4-11	RVGNRYRL	7	2.38	0.10	2.36	0.03	2.48	0.04
2	4-20	RVGNRYRLGRKIGSGSF	16	5.51	0.10	6.15	0.05	6.19	0.17
3	4-22	RVGNRYRLGRKIGSGSFGD	18	5.82	0.04	6.43	0.02	6.59	0.11
4	4-25	RVGNRYRLGRKIGSGSFGDIYL	21	5.83	0.19	6.92	0.11	7.45	0.02
5	8-24	RYRLGRKIGSGSFGDIY	16	1.86	0.16	2.49	0.05	2.34	0.11
6	12-25	GRKIGSGSFGDIYL	13	4.23	0.09	4.96	0.07	5.34	0.01
7	21-27	GDIYLGT	6	1.95	0.19	2.83	0.04	3.12	0.08
8	21-28	GDIYLGTD	7	1.97	0.19	3.09	0.07	3.65	0.01
9	25-34	LGTDIAAGEE	9	3.85	0.11	4.16	0.08	4.04	0.03
10	26-33	GTDIAAGE	7	3.10	0.05	3.13	0.04	3.24	0.05
11	26-34	GTDIAAGEE	8	3.75	0.07	3.77	0.03	3.90	0.03
12	26-36	GTDIAAGEEVA	10	4.29	0.14	4.46	0.05	4.83	0.03

13	28-34	DIAAGEE	6	2.62	0.04	2.63	0.02	2.65	0.06
14	28-36	DIAAGEEVA	8	2.85	0.06	3.14	0.05	3.53	0.07
15	29-36	IAAGEEVA	7	2.23	0.06	2.53	0.05	2.82	0.06
16	35-41	VAIKLEC	6	1.86	0.04	2.11	0.08	3.03	0.10
17	40-49	ECVKTKHPQL	8	2.00	0.14	2.12	0.06	2.00	0.10
18	42-49	VKTKHPQL	6	1.68	0.04	1.73	0.07	1.76	0.08
19	50-56	HIESKIY	6	1.52	0.07	1.73	0.04	1.89	0.04
20	50-58	HIESKIYKM	8	1.89	0.08	2.66	0.04	2.85	0.09
21	56-76	YKMMQGGVGIPTIRWCGAEGD	19	5.25	0.28	6.41	0.39	7.05	0.08
22	56-77	YKMMQGGVGIPTIRWCGAEGDY	20	4.96	0.42	4.28	0.09	4.85	0.34
23	59-72	MQGGVGIPTIRWCG	12	3.55	0.15	3.92	0.08	4.13	0.00
24	81-89	VMELLGPSL	7	0.78	0.16	0.95	0.06	1.23	0.06
25	82-89	MELLGPSL	6	0.41	0.15	0.66	0.03	0.92	0.03
26	83-89	ELLGPSL	5	0.41	0.13	0.50	0.02	0.62	0.02
27	96-102	CSRKFSL	6	1.14	0.12	1.05	0.10	1.03	0.11
28	96-104	CSRKFSLKT	8	1.47	0.15	1.37	0.02	1.44	0.12
29	96-106	CSRKFSLKTVL	10	1.05	0.21	1.08	0.09	1.20	0.09
30	96-107	CSRKFSLKTVLL	11	1.11	0.18	1.13	0.06	1.24	0.07
31	108-114	LADQMIS	6	2.14	0.07	2.29	0.09	2.54	0.11
32	113-123	ISRIEYIHSKN	10	0.56	0.12	0.16	0.03	0.12	0.02
33	114-123	SRIEYIHSKN	9	0.97	0.04	0.72	0.12	0.76	0.04
34	119-134	IHSKNFIHRDVKPDNF	14	2.27	0.05	1.82	0.12	2.02	0.04
35	124-134	FIHRDVKPDNF	9	0.36	0.14	0.34	0.06	0.35	0.02
36	124-135	FIHRDVKPDNFL	10	0.42	0.28	0.25	0.05	0.27	0.04
37	135-143	LMGLGKKGN	8	1.68	0.10	2.00	0.07	2.29	0.10
38	135-144	LMGLGKKGNL	9	2.01	0.15	2.12	0.09	2.44	0.09
39	135-145	LMGLGKKGNLV	10	2.04	0.16	2.21	0.14	2.52	0.14
40	135-146	LMGLGKKGNLVY	11	1.71	0.14	1.97	0.04	2.18	0.09
41	136-144	MGLGKKGNL	8	1.96	0.10	2.14	0.06	2.36	0.10
42	136-146	MGLGKKGNLVY	10	1.95	0.10	2.16	0.14	2.37	0.10
43	145-151	VYIIDFG	6	1.12	0.03	1.58	0.02	1.67	0.02
44	150-169	FGLAKKYRDARTHQHIPYRE	18	3.54	0.09	4.34	0.07	4.65	0.25
45	162-179	HQHIPYRENKNLTGTARY	16	6.63	0.14	6.49	0.25	6.50	0.28
46	166-179	PYRENKNLTGTARY	12	5.06	0.13	5.46	0.17	5.62	0.08

47	170-179	NKNLTGTARY	9	4.07	0.10	4.17	0.04	4.31	0.13
48	180-186	ASINTHL	6	1.41	0.08	2.03	0.04	2.13	0.06
49	180-196	ASINTHLGIEQSRRDDL	16	2.49	0.25	3.33	0.04	3.75	0.11
50	183-196	NTHLGIEQSRRDDL	13	2.10	0.05	2.75	0.05	3.00	0.05
51	185-196	HLGIEQSRRDDL	11	1.21	0.18	1.44	0.03	1.62	0.05
52	189-196	EQSRRDDL	7	0.94	0.13	0.99	0.02	1.09	0.03
53	203-217	LMYFNLGSLPWQGLK	13	2.64	0.14	2.94	0.02	3.34	0.03
54	205-218	YFNLGSLPWQGLKA	12	4.70	0.21	5.18	0.16	5.34	0.14
55	207-218	NLGSLPWQGLKA	10	4.76	0.10	5.55	0.07	5.92	0.09
56	209-216	GSLPWQGL	6	2.71	0.03	3.23	0.07	3.36	0.09
57	209-218	GSLPWQGLKA	8	3.91	0.05	4.52	0.05	4.71	0.05
58	209-230	GSLPWQGLKAATKRQKYERISE	20	4.72	0.06	5.46	0.09	5.74	0.02
59	212-230	PWQGLKAATKRQKYERISE	17	7.22	0.07	7.60	0.06	8.00	0.11
60	219-230	ATKRQKYERISE	11	3.67	0.01	4.09	0.09	4.21	0.09
61	231-238	KKMSTPIE	6	2.26	0.01	2.51	0.08	2.59	0.04
62	231-240	KKMSTPIEVL	8	2.41	0.05	2.66	0.06	3.01	0.14
63	239-247	VLCKGYPSE	7	1.57	0.13	1.93	0.11	2.41	0.10
64	241-247	CKGYPSE	5	1.15	0.07	1.49	0.03	1.79	0.03
65	241-248	CKGYPSEF	6	0.87	0.10	1.09	0.04	1.31	0.04
66	248-254	FATYLNF	6	0.28	0.10	0.13	0.02	0.16	0.01
67	255-267	CRSLRFDDKPDYS	11	3.02	0.08	3.29	0.07	3.52	0.04
68	255-268	CRSLRFDDKPDYSY	12	3.59	0.03	4.02	0.08	4.23	0.06
69	255-269	CRSLRFDDKPDYSYL	13	3.31	0.05	3.54	0.01	3.76	0.09
70	261-268	DDKPDYSY	6	2.68	0.06	2.77	0.05	2.85	0.06
71	261-269	DDKPDYSYL	7	2.35	0.05	2.43	0.02	2.46	0.06
72	268-277	YLRQLFRNLF	9	0.07	0.02	0.48	0.03	0.75	0.03
73	268-285	YLRQLFRNLFHRQGFSYD	17	2.23	0.07	2.66	0.06	2.93	0.06
74	269-277	LRQLFRNLF	8	0.15	0.06	0.42	0.05	0.63	0.03
75	269-285	LRQLFRNLFHRQGFSYD	16	2.14	0.01	2.47	0.06	2.65	0.07
76	270-277	RQLFRNLF	7	0.17	0.08	0.41	0.04	0.59	0.04
77	272-285	LFRNLFHRQGFSYD	13	2.33	0.02	2.56	0.03	2.66	0.03
78	273-285	FRNLFHRQGFSYD	12	2.15	0.07	2.37	0.07	2.49	0.06
79	286-294	YVFDWNMLK	8	4.18	0.13	4.50	0.03	4.63	0.04
80	288-294	FDWNMLK	6	3.32	0.05	3.58	0.05	3.75	0.07

				CK1δ1-ΔC + 4p-FASP Relative Deuterium Uptake (Da)					
				1 min		5 min		10 min	
S.No.	Peptide	Sequence	Num Exchangers	Uptake	± SD	Uptake	± SD	Uptake	± SD
1	4-11	RVGNRYRL	7	2.23	0.07	2.48	0.03	2.48	0.04
2	4-20	RVGNRYRLG RKIGSGSF	16	4.44	0.04	4.88	0.06	4.74	0.06
3	4-22	RVGNRYRLG RKIGSGSFG D	18	4.78	0.14	5.34	0.06	5.05	0.06
4	4-25	RVGNRYRLG RKIGSGSFG DIYL	21	5.47	0.05	6.84	0.07	6.90	0.12
5	8-24	RYRLGRKIG SGSFGDIY	16	1.77	0.10	2.08	0.10	2.18	0.10
6	12-25	GRKIGSGSF GDIYL	13	3.90	0.06	4.65	0.06	5.18	0.02
7	21-27	GDIYLGT	6	1.21	0.19	2.67	0.07	2.94	0.14
8	21-28	GDIYLGTD	7	1.46	0.02	2.86	0.13	3.44	0.01
9	25-34	LGTDIAAGEE	9	3.87	0.04	3.72	0.03	3.79	0.04
10	26-33	GTDIAAGE	7	2.79	0.02	2.81	0.04	2.72	0.05
11	26-34	GTDIAAGEE	8	3.41	0.02	3.40	0.03	3.52	0.03
12	26-36	GTDIAAGEEV A	10	3.62	0.03	4.05	0.02	4.32	0.02
13	28-34	DIAAGEE	6	2.53	0.02	2.51	0.01	2.50	0.03
14	28-36	DIAAGEEVA	8	2.61	0.04	3.06	0.05	3.39	0.01
15	29-36	IAAGEEVA	7	2.15	0.03	2.45	0.03	2.70	0.03
16	35-41	VAIKLEC	6	1.41	0.02	1.76	0.10	2.99	0.07
17	40-49	ECVKTKHPQ L	8	1.84	0.09	1.65	0.15	1.67	0.26
18	42-49	VKTKHPQL	6	1.80	0.01	1.73	0.04	1.76	0.08
19	50-56	HIESKIY	6	1.24	0.02	1.54	0.05	1.77	0.03
20	50-58	HIESKIYKM	8	1.70	0.04	2.45	0.02	2.61	0.05
21	56-76	YKMMQGGV GIPTIRWCGA EGD	19	3.82	0.15	4.44	0.13	5.83	0.33
22	56-77	YKMMQGGV GIPTIRWCGA EGDY	20	3.37	0.04	2.88	0.23	3.33	0.25
23	59-72	MQGGVGIPTI RWCG	12	2.62	0.12	2.47	0.25	2.38	0.09
24	81-89	VMELLGPSL	7	0.48	0.08	0.91	0.09	1.14	0.06
25	82-89	MELLGPSL	6	0.20	0.03	0.62	0.06	0.83	0.05
26	83-89	ELLGPSL	5	0.20	0.02	0.46	0.02	0.54	0.02
27	96-102	CSRKFSL	6	0.67	0.07	0.83	0.10	1.01	0.10
28	96-104	CSRKFSLKT	8	0.92	0.10	1.33	0.02	1.42	0.05
29	96-106	CSRKFSLKT VL	10	0.55	0.05	0.87	0.05	1.07	0.06
30	96-107	CSRKFSLKT VLL	11	0.73	0.08	1.11	0.11	1.27	0.04

31	108-114	LADQMIS	6	2.06	0.09	2.27	0.08	2.44	0.02
32	113-123	ISRIEYIHSKN	10	0.07	0.02	0.15	0.03	0.18	0.03
33	114-123	SRIEYIHSKN	9	0.30	0.09	0.40	0.03	0.64	0.11
34	119-134	IHSKNFIHRDVKPDNF	14	1.68	0.24	1.93	0.04	2.16	0.13
35	124-134	FIHRDVKPDNF	9	0.31	0.01	0.36	0.02	0.41	0.03
36	124-135	FIHRDVKPDNFL	10	0.08	0.03	0.13	0.03	0.25	0.06
37	135-143	LMGLGKKGN	8	1.68	0.06	2.04	0.08	2.38	0.04
38	135-144	LMGLGKKGNL	9	1.91	0.04	2.24	0.08	2.53	0.05
39	135-145	LMGLGKKGNLV	10	1.88	0.13	2.29	0.14	2.62	0.16
40	135-146	LMGLGKKGNLVY	11	1.72	0.04	2.08	0.07	2.32	0.04
41	136-144	MGLGKKGNL	8	1.83	0.03	2.10	0.05	2.35	0.04
42	136-146	MGLGKKGNLVY	10	1.84	0.03	2.18	0.08	2.46	0.07
43	145-151	VYIIDFG	6	0.66	0.03	1.35	0.01	1.51	0.02
44	150-169	FGLAKKYRDARTHQHIPYRE	18	3.12	0.11	3.59	0.02	3.62	0.13
45	162-179	HQHIPYRENKNLTGTARY	16	6.42	0.13	6.37	0.10	6.02	0.11
46	166-179	PYRENKNLTGTARY	12	4.96	0.16	5.06	0.05	4.78	0.14
47	170-179	NKNLTGTARY	9	4.13	0.05	4.10	0.04	4.16	0.09
48	180-186	ASINTHL	6	1.39	0.04	2.06	0.04	2.15	0.04
49	180-196	ASINTHLGIEQSRRDDL	16	2.39	0.05	3.41	0.07	3.74	0.06
50	183-196	NTHLGIEQSRRDDL	13	2.01	0.02	2.68	0.07	3.06	0.02
51	185-196	HLGIEQSRRDDL	11	0.95	0.02	1.38	0.06	1.57	0.02
52	189-196	EQSRRDDL	7	0.76	0.01	0.97	0.06	1.05	0.03
53	203-217	LMYFNLGSLPWQGLK	13	1.83	0.21	2.76	0.06	2.85	0.14
54	205-218	YFNLGSLPWQGLKA	12	4.26	0.10	5.20	0.08	5.45	0.05
55	207-218	NLGSLPWQGLKA	10	4.57	0.11	5.33	0.12	5.54	0.07
56	209-216	GSLPWQGL	6	2.76	0.04	3.04	0.05	3.15	0.04
57	209-218	GSLPWQGLKA	8	3.88	0.05	4.18	0.04	4.33	0.07
58	209-230	GSLPWQGLKAATKRQKYERISE	20	4.75	0.01	5.49	0.05	5.57	0.10
59	212-230	PWQGLKAATKRQKYERISE	17	7.07	0.04	7.13	0.14	7.30	0.05
60	219-230	ATKRQKYERISE	11	3.67	0.06	3.93	0.06	3.82	0.09
61	231-238	KKMSTPIE	6	2.26	0.01	2.33	0.02	2.40	0.07
62	231-240	KKMSTPIEVL	8	2.24	0.04	2.54	0.04	2.83	0.03
63	239-247	VLCKGYPSE	7	1.31	0.10	1.82	0.02	2.24	0.14

64	241-247	CKGYPSE	5	1.11	0.02	1.39	0.02	1.62	0.02
65	241-248	CKGYPSEF	6	0.82	0.04	1.06	0.04	1.27	0.04
66	248-254	FATYLNF	6	0.03	0.01	0.07	0.01	0.07	0.02
67	255-267	CRSLRFDDK PDYS	11	2.85	0.06	3.14	0.05	3.25	0.07
68	255-268	CRSLRFDDK PDYSY	12	3.51	0.07	3.76	0.07	3.85	0.06
69	255-269	CRSLRFDDK PDYSYL	13	3.20	0.01	3.41	0.04	3.49	0.04
70	261-268	DDKPDYSY	6	2.69	0.02	2.75	0.02	2.98	0.03
71	261-269	DDKPDYSYL	7	2.30	0.02	2.44	0.02	2.44	0.02
72	268-277	YLRQLFRNLF	9	0.11	0.03	0.46	0.03	0.71	0.02
73	268-285	YLRQLFRNLF HRQGFSYD	17	2.14	0.07	2.59	0.04	2.73	0.07
74	269-277	LRQLFRNLF	8	0.10	0.04	0.39	0.02	0.56	0.03
75	269-285	LRQLFRNLF HRQGFSYD	16	2.15	0.02	2.49	0.02	2.66	0.02
76	270-277	RQLFRNLF	7	0.05	0.07	0.32	0.06	0.49	0.06
77	272-285	LFRNLFHRQ GFSYD	13	2.33	0.04	2.77	0.07	2.83	0.03
78	273-285	FRNLFHRQG FSYD	12	2.02	0.12	2.26	0.06	2.48	0.12
79	286-294	YVFDWNMLK	8	3.97	0.05	4.35	0.02	4.44	0.06
80	288-294	FDWNMLK	6	3.17	0.04	3.46	0.04	3.53	0.04

				CK1δ1-ΔC					
				Relative Fractional Deuterium Uptake (%)					
				1 min		**5 min**		**10 min**	
S.No.	**Peptide**	**Sequence**	**Num Exchangers**	**Uptake**	**± SD**	**Uptake**	**± SD**	**Uptake**	**± SD**
1	4-11	RVGNRYRL	7	33.94	1.48	33.66	0.39	35.40	0.53
2	4-20	RVGNRYRLG RKIGSGSF	16	34.46	0.63	38.43	0.30	38.72	1.04
3	4-22	RVGNRYRLG RKIGSGSFG D	18	32.31	0.25	35.73	0.09	36.61	0.59
4	4-25	RVGNRYRLG RKIGSGSFG DIYL	21	27.77	0.91	32.96	0.50	35.46	0.10
5	8-24	RYRLGRKIG SGSFGDIY	16	11.60	1.01	15.57	0.32	14.59	0.70
6	12-25	GRKIGSGSF GDIYL	13	32.56	0.66	38.13	0.52	41.07	0.10
7	21-27	GDIYLGT	6	32.56	3.24	47.10	0.69	51.95	1.34
8	21-28	GDIYLGTD	7	28.21	2.67	44.13	1.04	52.08	0.16
9	25-34	LGTDIAAGEE	9	42.83	1.19	46.28	0.93	44.91	0.37
10	26-33	GTDIAAGE	7	44.31	0.68	44.74	0.61	46.31	0.67
11	26-34	GTDIAAGEE	8	46.85	0.93	47.10	0.31	48.70	0.42
12	26-36	GTDIAAGEEV A	10	42.90	1.37	44.56	0.48	48.26	0.33
13	28-34	DIAAGEE	6	43.59	0.75	43.88	0.32	44.16	1.05
14	28-36	DIAAGEEVA	8	35.66	0.74	39.26	0.66	44.15	0.85

15	29-36	IAAGEEVA	7	31.92	0.90	36.13	0.67	40.35	0.83
16	35-41	VAIKLEC	6	30.94	0.73	35.22	1.31	50.57	1.63
17	40-49	ECVKTKHPQL	8	25.01	1.77	26.46	0.75	24.95	1.25
18	42-49	VKTKHPQL	6	28.02	0.66	28.88	1.09	29.40	1.32
19	50-56	HIESKIY	6	25.26	1.09	28.84	0.72	31.48	0.68
20	50-58	HIESKIYKM	8	23.62	1.06	33.24	0.48	35.65	1.08
21	56-76	YKMMQGGVGIPTIRWCGAEGD	19	27.62	1.45	33.75	2.05	37.08	0.43
22	56-77	YKMMQGGVGIPTIRWCGAEGDY	20	24.79	2.12	21.38	0.46	24.23	1.72
23	59-72	MQGGVGIPTIRWCG	12	29.55	1.23	32.69	0.68	34.40	0.04
24	81-89	VMELLGPSL	7	11.12	2.23	13.57	0.84	17.61	0.85
25	82-89	MELLGPSL	6	6.87	2.50	10.95	0.52	15.38	0.49
26	83-89	ELLGPSL	5	8.30	2.67	9.93	0.33	12.40	0.38
27	96-102	CSRKFSL	6	19.05	2.07	17.56	1.75	17.24	1.81
28	96-104	CSRKFSLKT	8	18.39	1.93	17.13	0.29	17.98	1.47
29	96-106	CSRKFSLKTVL	10	10.46	2.08	10.77	0.91	11.99	0.89
30	96-107	CSRKFSLKTVLL	11	10.06	1.63	10.28	0.58	11.31	0.68
31	108-114	LADQMIS	6	35.62	1.21	38.17	1.44	42.31	1.91
32	113-123	ISRIEYIHSKN	10	5.64	1.20	1.59	0.26	1.17	0.19
33	114-123	SRIEYIHSKN	9	10.83	0.39	8.01	1.32	8.42	0.46
34	119-134	IHSKNFIHRDVKPDNF	14	16.24	0.34	13.00	0.88	14.43	0.28
35	124-134	FIHRDVKPDNF	9	3.96	1.60	3.79	0.72	3.86	0.18
36	124-135	FIHRDVKPDNFL	10	4.17	2.79	2.48	0.53	2.67	0.41
37	135-143	LMGLGKKGN	8	21.01	1.25	25.05	0.86	28.62	1.28
38	135-144	LMGLGKKGNL	9	22.36	1.71	23.61	1.04	27.16	1.01
39	135-145	LMGLGKKGNLV	10	20.36	1.55	22.06	1.43	25.17	1.42
40	135-146	LMGLGKKGNLVY	11	15.53	1.31	17.91	0.35	19.82	0.80
41	136-144	MGLGKKGNL	8	24.48	1.31	26.72	0.80	29.47	1.23
42	136-146	MGLGKKGNLVY	10	19.46	1.03	21.60	1.37	23.67	0.96
43	145-151	VYIIDFG	6	18.64	0.50	26.26	0.29	27.84	0.33
44	150-169	FGLAKKYRDARTHQHIPYRE	18	19.69	0.48	24.11	0.37	25.85	1.40
45	162-179	HQHIPYRENKNLTGTARY	16	41.45	0.86	40.58	1.54	40.63	1.72
46	166-179	PYRENKNLTGTARY	12	42.14	1.12	45.52	1.39	46.87	0.70
47	170-179	NKNLTGTARY	9	45.26	1.06	46.33	0.40	47.89	1.49

48	180-186	ASINTHL	6	23.51	1.37	33.81	0.69	35.53	1.01
49	180-196	ASINTHLGIEQSRRDDL	16	15.57	1.57	20.84	0.25	23.46	0.70
50	183-196	NTHLGIEQSRRDDL	13	16.14	0.39	21.12	0.41	23.07	0.39
51	185-196	HLGIEQSRRDDL	11	11.01	1.63	13.09	0.30	14.70	0.42
52	189-196	EQSRRDDL	7	13.48	1.92	14.10	0.23	15.54	0.36
53	203-217	LMYFNLGSLPWQGLK	13	20.29	1.09	22.65	0.16	25.70	0.21
54	205-218	YFNLGSLPWQGLKA	12	39.14	1.74	43.20	1.33	44.53	1.13
55	207-218	NLGSLPWQGLKA	10	47.58	0.99	55.48	0.71	59.17	0.89
56	209-216	GSLPWQGL	6	45.24	0.49	53.76	1.19	56.03	1.43
57	209-218	GSLPWQGLKA	8	48.84	0.58	56.45	0.56	58.82	0.64
58	209-230	GSLPWQGLKAATKRQKYERISE	20	23.58	0.29	27.28	0.43	28.72	0.09
59	212-230	PWQGLKAATKRQKYERISE	17	42.48	0.42	44.73	0.35	47.07	0.65
60	219-230	ATKRQKYERISE	11	33.36	0.14	37.21	0.80	38.27	0.82
61	231-238	KKMSTPIE	6	37.65	0.10	41.85	1.38	43.15	0.62
62	231-240	KKMSTPIEVL	8	30.15	0.63	33.21	0.75	37.66	1.80
63	239-247	VLCKGYPSE	7	22.44	1.86	27.52	1.63	34.37	1.42
64	241-247	CKGYPSE	5	22.92	1.30	29.76	0.68	35.81	0.62
65	241-248	CKGYPSEF	6	14.49	1.68	18.15	0.59	21.88	0.72
66	248-254	FATYLNF	6	4.60	1.73	2.14	0.34	2.65	0.21
67	255-267	CRSLRFDDKPDYS	11	27.43	0.71	29.88	0.68	32.00	0.32
68	255-268	CRSLRFDDKPDYSY	12	29.93	0.27	33.46	0.68	35.24	0.53
69	255-269	CRSLRFDDKPDYSYL	13	25.45	0.37	27.22	0.10	28.92	0.67
70	261-268	DDKPDYSY	6	44.60	1.07	46.25	0.80	47.43	0.96
71	261-269	DDKPDYSYL	7	33.56	0.69	34.74	0.25	35.18	0.89
72	268-277	YLRQLFRNLF	9	0.79	0.24	5.36	0.36	8.31	0.32
73	268-285	YLRQLFRNLFHRQGFSYD	17	13.12	0.39	15.66	0.33	17.23	0.33
74	269-277	LRQLFRNLF	8	1.91	0.76	5.21	0.60	7.82	0.42
75	269-285	LRQLFRNLFHRQGFSYD	16	13.40	0.07	15.41	0.40	16.57	0.42
76	270-277	RQLFRNLF	7	2.49	1.21	5.93	0.52	8.49	0.58
77	272-285	LFRNLFHRQGFSYD	13	17.92	0.18	19.69	0.23	20.47	0.26
78	273-285	FRNLFHRQGFSYD	12	17.92	0.57	19.78	0.60	20.76	0.54
79	286-294	YVFDWNMLK	8	52.27	1.61	56.26	0.39	57.83	0.50
80	288-294	FDWNMLK	6	55.40	0.89	59.68	0.78	62.42	1.14
				CK1δ1-ΔC + 4p-FASP **Relative Fractional Deuterium Uptake (%)**					

				1 min		5 min		10 min	
S.No.	Peptide	Sequence	Num Exchangers	Uptake	± SD	Uptake	± SD	Uptake	± SD
1	4-11	RVGNRYRL	7	31.83	0.93	35.38	0.46	35.44	0.61
2	4-20	RVGNRYRLG RKIGSGSF	16	27.72	0.25	30.49	0.39	29.61	0.40
3	4-22	RVGNRYRLG RKIGSGSFG D	18	26.57	0.77	29.67	0.32	28.08	0.33
4	4-25	RVGNRYRLG RKIGSGSFG DIYL	21	26.04	0.23	32.56	0.35	32.86	0.58
5	8-24	RYRLGRKIG SGSFGDIY	16	11.08	0.65	13.01	0.60	13.65	0.61
6	12-25	GRKIGSGSF GDIYL	13	30.03	0.48	35.78	0.50	39.84	0.13
7	21-27	GDIYLGT	6	20.17	3.22	44.46	1.22	49.07	2.25
8	21-28	GDIYLGTD	7	20.81	0.31	40.88	1.79	49.16	0.14
9	25-34	LGTDIAAGEE	9	42.98	0.46	41.35	0.34	42.14	0.47
10	26-33	GTDIAAGE	7	39.86	0.24	40.21	0.58	38.87	0.73
11	26-34	GTDIAAGEE	8	42.58	0.26	42.49	0.31	43.97	0.36
12	26-36	GTDIAAGEEV A	10	36.19	0.26	40.47	0.16	43.19	0.20
13	28-34	DIAAGEE	6	42.23	0.28	41.78	0.14	41.66	0.45
14	28-36	DIAAGEEVA	8	32.63	0.49	38.22	0.60	42.35	0.18
15	29-36	IAAGEEVA	7	30.70	0.40	35.02	0.37	38.52	0.44
16	35-41	VAIKLEC	6	23.42	0.31	29.33	1.69	49.86	1.15
17	40-49	ECVKTKHPQ L	8	22.96	1.09	20.57	1.86	20.90	3.22
18	42-49	VKTKHPQL	6	30.05	0.25	28.91	0.60	29.36	1.29
19	50-56	HIESKIY	6	20.59	0.33	25.61	0.91	29.46	0.57
20	50-58	HIESKIYKM	8	21.31	0.53	30.56	0.20	32.64	0.62
21	56-76	YKMMQGGV GIPTIRWCGA EGD	19	20.11	0.77	23.35	0.66	30.70	1.76
22	56-77	YKMMQGGV GIPTIRWCGA EGDY	20	16.87	0.19	14.41	1.15	16.66	1.26
23	59-72	MQGGVGIPTI RWCG	12	21.83	0.98	20.60	2.09	19.83	0.77
24	81-89	VMELLGPSL	7	6.80	1.08	13.02	1.21	16.34	0.87
25	82-89	MELLGPSL	6	3.31	0.53	10.27	1.05	13.87	0.90
26	83-89	ELLGPSL	5	4.09	0.37	9.18	0.34	10.73	0.47
27	96-102	CSRKFSL	6	11.11	1.25	13.79	1.69	16.76	1.74
28	96-104	CSRKFSLKT	8	11.46	1.30	16.69	0.28	17.69	0.67
29	96-106	CSRKFSLKT VL	10	5.49	0.47	8.67	0.47	10.66	0.57
30	96-107	CSRKFSLKT VLL	11	6.61	0.71	10.10	1.01	11.53	0.35
31	108-114	LADQMIS	6	34.34	1.54	37.84	1.31	40.60	0.31
32	113-123	ISRIEYIHSKN	10	0.75	0.22	1.47	0.33	1.80	0.33

33	114-123	SRIEYIHSKN	9	3.29	0.99	4.39	0.39	7.12	1.21
34	119-134	IHSKNFIHRDVKPDNF	14	12.03	1.70	13.81	0.29	15.41	0.95
35	124-134	FIHRDVKPDNF	9	3.47	0.12	4.03	0.21	4.52	0.34
36	124-135	FIHRDVKPDNFL	10	0.82	0.31	1.32	0.31	2.52	0.57
37	135-143	LMGLGKKGN	8	20.99	0.72	25.56	1.05	29.71	0.49
38	135-144	LMGLGKKGNL	9	21.18	0.45	24.94	0.90	28.13	0.59
39	135-145	LMGLGKKGNLV	10	18.76	1.31	22.94	1.40	26.25	1.64
40	135-146	LMGLGKKGNLVY	11	15.65	0.34	18.90	0.67	21.06	0.35
41	136-144	MGLGKKGNL	8	22.83	0.37	26.28	0.57	29.38	0.46
42	136-146	MGLGKKGNLVY	10	18.39	0.34	21.79	0.80	24.56	0.71
43	145-151	VYIIDFG	6	10.97	0.48	22.46	0.23	25.21	0.32
44	150-169	FGLAKKYRDARTHQHIPYRE	18	17.34	0.62	19.94	0.09	20.12	0.72
45	162-179	HQHIPYRENKNLTGTARY	16	40.11	0.81	39.83	0.64	37.61	0.71
46	166-179	PYRENKNLTGTARY	12	41.36	1.29	42.19	0.41	39.82	1.13
47	170-179	NKNLTGTARY	9	45.89	0.59	45.52	0.44	46.24	0.97
48	180-186	ASINTHL	6	23.19	0.69	34.37	0.69	35.91	0.68
49	180-196	ASINTHLGIEQSRRDDL	16	14.91	0.28	21.33	0.44	23.37	0.40
50	183-196	NTHLGIEQSRRDDL	13	15.49	0.18	20.59	0.51	23.55	0.18
51	185-196	HLGIEQSRRDDL	11	8.65	0.17	12.59	0.55	14.30	0.21
52	189-196	EQSRRDDL	7	10.80	0.17	13.88	0.88	14.96	0.42
53	203-217	LMYFNLGSLPWQGLK	13	14.11	1.58	21.25	0.47	21.96	1.08
54	205-218	YFNLGSLPWQGLKA	12	35.47	0.86	43.33	0.67	45.44	0.41
55	207-218	NLGSLPWQGLKA	10	45.72	1.12	53.28	1.24	55.35	0.68
56	209-216	GSLPWQGL	6	46.06	0.62	50.61	0.85	52.51	0.69
57	209-218	GSLPWQGLKA	8	48.55	0.57	52.28	0.49	54.17	0.92
58	209-230	GSLPWQGLKAATKRQKYERISE	20	23.73	0.07	27.47	0.23	27.87	0.48
59	212-230	PWQGLKAATKRQKYERISE	17	41.58	0.22	41.96	0.80	42.96	0.27
60	219-230	ATKRQKYERISE	11	33.33	0.54	35.77	0.54	34.73	0.79
61	231-238	KKMSTPIE	6	37.70	0.22	38.80	0.36	40.07	1.22
62	231-240	KKMSTPIEVL	8	27.96	0.55	31.76	0.52	35.40	0.43
63	239-247	VLCKGYPSE	7	18.69	1.38	26.04	0.31	32.02	1.95
64	241-247	CKGYPSE	5	22.20	0.36	27.75	0.34	32.49	0.41
65	241-248	CKGYPSEF	6	13.63	0.64	17.72	0.74	21.14	0.62

66	248-254	FATYLNF	6	0.54	0.14	1.23	0.23	1.10	0.41
67	255-267	CRSLRFDDK PDYS	11	25.89	0.55	28.51	0.45	29.52	0.64
68	255-268	CRSLRFDDK PDYSY	12	29.27	0.60	31.29	0.56	32.05	0.48
69	255-269	CRSLRFDDK PDYSYL	13	24.60	0.07	26.26	0.30	26.81	0.34
70	261-268	DDKPDYSY	6	44.86	0.29	45.85	0.29	49.61	0.56
71	261-269	DDKPDYSYL	7	32.81	0.29	34.84	0.28	34.83	0.30
72	268-277	YLRQLFRNLF	9	1.24	0.28	5.10	0.31	7.90	0.27
73	268-285	YLRQLFRNLF HRQGFSYD	17	12.57	0.42	15.25	0.25	16.06	0.44
74	269-277	LRQLFRNLF	8	1.23	0.47	4.86	0.30	6.94	0.43
75	269-285	LRQLFRNLF HRQGFSYD	16	13.44	0.09	15.58	0.15	16.62	0.09
76	270-277	RQLFRNLF	7	0.71	0.98	4.53	0.81	6.96	0.89
77	272-285	LFRNLFHRQ GFSYD	13	17.93	0.30	21.29	0.51	21.78	0.23
78	273-285	FRNLFHRQG FSYD	12	16.82	1.00	18.84	0.52	20.66	1.03
79	286-294	YVFDWNMLK	8	49.60	0.66	54.33	0.31	55.50	0.78
80	288-294	FDWNMLK	6	52.79	0.73	57.61	0.71	58.89	0.61

Table 2.2 Deuterium exchange values for various pepsin-digested peptides of CK1δ1, CK1δ2, CK1δ1 S3A, and CK1δ1ΔC in the presence of ATP, AMPPNP, and 4p-FASP at 1, 5, and 10-minute labeling times are tabulated.

Table 2.2

				CK1δ1 Relative Deuterium Uptake (Da)					
				1 min		5 min		10 min	
S.No.	Peptide	Sequence	Num Exchangers	Uptake	± SD	Uptake	± SD	Uptake	± SD
1	3-20	LRVGNRYRL GRKIGSGSF	17	6.38	0.24	7.27	0.13	7.68	0.12
2	4-20	RVGNRYRLG RKIGSGSF	16	5.24	0.15	5.88	0.02	6.19	0.10
3	4-22	RVGNRYRLG RKIGSGSFG D	18	5.41	0.29	6.36	0.06	6.74	0.16
4	9-28	YRLGRKIGS GSFGDIYLGT D	19	7.02	0.05	7.28	0.08	7.40	0.09
5	12-25	GRKIGSGSF GDIYL	13	3.77	0.23	4.73	0.25	4.75	0.22
6	21-28	GDIYLGTD	7	1.42	0.06	2.55	0.02	3.03	0.04

7	22-28	DIYLGTD	6	1.08	0.09	2.12	0.09	2.48	0.12
8	26-33	GTDIAAGE	7	2.88	0.07	2.88	0.01	2.99	0.06
9	26-36	GTDIAAGEEV A	10	3.79	0.05	4.00	0.05	4.32	0.07
10	28-34	DIAAGEE	6	2.48	0.06	2.47	0.04	2.45	0.09
11	28-36	DIAAGEEVA	8	2.79	0.01	3.07	0.04	3.34	0.06
12	29-36	IAAGEEVA	7	2.09	0.04	2.32	0.04	2.55	0.04
13	37-49	IKLECVKTKH PQL	11	1.97	0.17	2.61	0.09	2.92	0.07
14	40-49	ECVKTKHPQ L	8	1.88	0.17	1.75	0.20	1.74	0.11
15	42-49	VKTKHPQL	6	1.34	0.10	1.36	0.05	1.41	0.05
16	50-56	HIESKIY	6	1.10	0.06	1.53	0.02	1.67	0.02
17	50-58	HIESKIYKM	8	1.47	0.16	2.57	0.14	2.95	0.09
18	56-70	YKMMQGGV GIPTIRW	13	3.89	0.15	4.61	0.15	4.77	0.05
19	56-72	YKMMQGGV GIPTIRWCG	15	3.68	0.22	3.87	0.15	3.73	0.20
20	56-77	YKMMQGGV GIPTIRWCGA EGDY	20	4.54	0.23	4.72	0.10	4.64	0.05
21	59-76	MQGGVGIPTI RWCGAEGD	16	4.69	0.40	4.31	0.37	4.65	0.05
22	62-70	GVGIPTIRW	7	1.67	0.08	2.04	0.04	2.17	0.06
23	77-99	YNVMVMELL GPSLEDLFN FCSRK	21	9.16	0.05	9.67	0.03	9.89	0.05
24	83-89	ELLGPSL	5	0.50	0.02	0.67	0.02	0.80	0.02
25	85-92	LGPSLEDL	6	0.43	0.04	0.50	0.07	0.49	0.05
26	96-102	CSRKFSL	6	0.87	0.10	0.86	0.06	1.15	0.08
27	96-104	CSRKFSLKT	8	1.61	0.21	1.56	0.03	2.24	0.04
28	96-106	CSRKFSLKT VL	10	0.81	0.04	1.01	0.04	1.16	0.04
29	104-119	TVLLLADQMI SRIEYI	15	3.67	0.02	4.85	0.04	5.54	0.05
30	108-117	LADQMISRIE	9	3.09	0.30	3.38	0.15	3.61	0.01
31	113-123	ISRIEYIHSKN	10	0.37	0.04	0.45	0.02	0.59	0.02
32	124-134	FIHRDVKPDN F	9	0.29	0.04	0.34	0.04	0.42	0.05
33	124-135	FIHRDVKPDN FL	10	0.22	0.03	0.28	0.01	0.40	0.03
34	126-134	HRDVKPDNF	7	0.14	0.03	0.19	0.02	0.27	0.04
35	135-143	LMGLGKKGN	8	1.66	0.11	2.12	0.10	2.34	0.13
36	135-144	LMGLGKKGN L	9	1.81	0.07	2.25	0.01	2.48	0.08
37	135-145	LMGLGKKGN LV	10	1.88	0.07	2.41	0.05	2.61	0.04
38	135-146	LMGLGKKGN LVY	11	1.63	0.07	2.10	0.02	2.36	0.09
39	136-144	MGLGKKGNL	8	1.88	0.05	2.25	0.02	2.51	0.02
40	137-144	GLGKKGNL	7	1.49	0.04	1.91	0.01	2.09	0.04

41	137-146	GLGKKGNLVY	9	1.69	0.06	2.09	0.02	2.46	0.09
42	140-150	KKGNLVYIIDF	10	0.36	0.07	0.15	0.00	0.12	0.01
43	144-162	LVYIIDFGLAKKYRDARTH	18	2.04	0.03	4.86	0.10	6.48	0.07
44	145-151	VYIIDFG	6	0.66	0.08	1.53	0.12	1.50	0.05
45	145-152	VYIIDFGL	7	0.84	0.02	1.71	0.09	2.07	0.06
46	145-158	VYIIDFGLAKKYRD	13	1.21	0.05	1.45	0.01	1.69	0.01
47	150-169	FGLAKKYRDARTHQHIPYRE	18	3.12	0.08	3.66	0.03	4.06	0.05
48	151-169	GLAKKYRDARTHQHIPYRE	17	3.65	0.03	3.95	0.21	4.45	0.03
49	153-179	AKKYRDARTHQHIPYRENKNLTGTARY	25	6.74	0.18	6.62	0.18	7.07	0.24
50	163-178	QHIPYRENKNLTGTAR	14	3.06	0.06	4.22	0.05	5.10	0.08
51	164-178	HIPYRENKNLTGTAR	13	1.43	0.01	1.74	0.25	1.87	0.01
52	176-186	TARYASINTHL	10	0.21	0.07	0.27	0.06	0.32	0.05
53	178-186	RYASINTHL	8	0.89	0.02	1.59	0.22	2.06	0.01
54	180-186	ASINTHL	6	0.71	0.07	1.33	0.07	1.71	0.07
55	180-189	ASINTHLGIE	9	1.22	0.05	2.53	0.01	3.18	0.05
56	180-196	ASINTHLGIEQSRRDDL	16	1.41	0.07	2.61	0.07	3.19	0.11
57	182-196	INTHLGIEQSRRDDL	14	1.26	0.05	2.18	0.04	2.75	0.11
58	185-196	HLGIEQSRRDDL	11	0.81	0.06	1.26	0.06	1.47	0.09
59	187-196	GIEQSRRDDL	9	0.81	0.11	1.12	0.13	1.29	0.13
60	189-196	EQSRRDDL	7	0.78	0.04	1.01	0.09	1.19	0.08
61	190-196	QSRRDDL	6	0.63	0.05	0.98	0.05	1.07	0.04
62	204-217	MYFNLGSLPWQGLK	12	1.28	0.04	2.22	0.09	2.85	0.11
63	205-215	YFNLGSLPWQG	9	2.34	0.05	2.85	0.04	3.05	0.09
64	205-216	YFNLGSLPWQGL	10	2.70	0.13	3.39	0.10	3.58	0.17
65	205-218	YFNLGSLPWQGLKA	12	4.08	0.11	4.63	0.02	4.98	0.08
66	206-218	FNLGSLPWQGLKA	11	4.37	0.14	5.07	0.14	5.31	0.16
67	207-216	NLGSLPWQGL	8	2.69	0.08	3.25	0.08	3.51	0.10
68	207-218	NLGSLPWQGLKA	10	4.20	0.12	4.81	0.02	5.09	0.07
69	209-216	GSLPWQGL	6	2.49	0.10	2.75	0.11	2.94	0.11
70	209-218	GSLPWQGLKA	8	3.35	0.13	3.51	0.07	3.66	0.13
71	212-218	PWQGLKA	5	2.39	0.05	2.50	0.04	2.74	0.02
72	219-230	ATKRQKYERISE	11	4.31	0.14	4.47	0.03	4.60	0.12

73	231-239	KKMSTPIEV	7	2.44	0.07	2.83	0.08	3.03	0.03
74	231-240	KKMSTPIEVL	8	2.15	0.07	2.56	0.04	2.79	0.04
75	239-248	VLCKGYPSEF	8	1.06	0.08	1.43	0.06	1.80	0.11
76	241-247	CKGYPSE	5	1.14	0.05	1.45	0.02	1.74	0.05
77	241-248	CKGYPSEF	6	0.83	0.05	1.05	0.02	1.34	0.04
78	248-254	FATYLNF	6	0.16	0.05	0.14	0.02	0.19	0.02
79	255-267	CRSLRFDDKPDYS	11	2.77	0.04	3.04	0.05	3.28	0.04
80	255-268	CRSLRFDDKPDYSY	12	3.40	0.09	3.85	0.11	4.28	0.22
81	255-269	CRSLRFDDKPDYSYL	13	3.18	0.12	3.45	0.08	3.66	0.10
82	259-276	RFDDKPDYSYLRQLFRNL	16	3.55	0.03	5.58	0.23	6.86	0.07
83	261-269	DDKPDYSYL	7	2.21	0.10	2.20	0.04	2.24	0.10
84	269-277	LRQLFRNLF	8	0.21	0.04	0.50	0.05	0.81	0.03
85	269-281	LRQLFRNLFHRQG	12	2.12	0.09	2.27	0.07	2.55	0.07
86	269-285	LRQLFRNLFHRQGFSYD	16	2.33	0.13	2.70	0.09	3.00	0.12
87	270-276	RQLFRNL	6	1.09	0.09	1.82	0.19	2.17	0.11
88	270-277	RQLFRNLF	7	0.27	0.06	0.50	0.07	0.75	0.02
89	270-285	RQLFRNLFHRQGFSYD	15	2.05	0.04	NaN	NaN	2.81	0.06
90	273-285	FRNLFHRQGFSYD	12	2.11	0.13	2.36	0.06	2.57	0.11
91	278-285	HRQGFSYD	7	1.90	0.13	1.88	0.03	2.01	0.12
92	286-292	YVFDWNM	6	2.33	0.06	2.57	0.05	2.68	0.07
93	293-302	LKFGASRAAD	9	3.32	0.15	3.42	0.10	3.68	0.10
94	293-305	LKFGASRAADDAE	12	4.26	0.18	4.36	0.12	4.61	0.15
95	294-302	KFGASRAAD	8	2.98	0.10	3.00	0.12	3.02	0.04
96	303-312	DAERERRDRE	9	2.74	0.22	3.14	0.25	3.27	0.28
97	312-324	EERLRHSRNPATR	11	4.00	0.17	4.04	0.06	4.11	0.09
98	319-338	RNPATRGLPSTASGRLRGTQ	17	3.88	0.28	4.73	0.45	6.20	0.43
99	325-334	GLPSTASGRL	8	0.68	0.12	1.28	0.09	1.73	0.08
100	337-350	TQEVAPPTPLTPTS	9	0.81	0.00	1.88	0.00	2.66	0.00
101	351-357	HTANTSP	5	0.97	0.03	1.03	0.04	1.10	0.03
102	362-375	GMERERKVSMRLHR	13	2.17	0.18	3.13	0.16	3.94	0.04
103	364-371	ERERKVSM	7	3.01	0.09	3.26	0.22	3.38	0.21
104	364-378	ERERKVSMRLHRGAP	13	6.07	0.04	7.01	0.06	7.69	0.03
105	365-373	RERKVSMRL	8	2.87	0.11	2.71	0.14	2.93	0.10
106	371-394	MRLHRGAPVNISSSDLTGRQDTSR	22	7.52	0.29	7.63	0.23	8.06	0.07

107	379-395	VNISSSDLTG RQDTSRM	16	5.15	0.12	5.31	0.09	5.49	0.01
108	386-394	LTGRQDTSR	8	4.66	0.10	4.96	0.01	5.03	0.09
109	387-395	TGRQDTSRM	8	2.32	0.03	2.76	0.07	2.71	0.05
110	394-402	RMSTSQIPG	7	0.77	0.02	0.80	0.02	0.82	0.04
				CK1δ1 + ATP					
				Relative Deuterium Uptake (Da)					
				1 min		**5 min**		**10 min**	
S.No.	**Peptide**	**Sequence**	**Num Exchangers**	**Uptake**	**± SD**	**Uptake**	**± SD**	**Uptake**	**± SD**
1	3-20	LRVGNRYRL GRKIGSGSF	17	5.31	0.20	5.93	0.00	6.17	0.00
2	4-20	RVGNRYRLG RKIGSGSF	16	4.45	0.02	5.09	0.05	5.15	0.09
3	4-22	RVGNRYRLG RKIGSGSFG D	18	4.84	0.06	5.57	0.08	5.72	0.03
4	9-28	YRLGRKIGS GSFGDIYLGT D	19	6.25	0.16	6.60	0.11	6.61	0.14
5	12-25	GRKIGSGSF GDIYL	13	2.60	0.01	3.50	0.01	3.65	0.01
6	21-28	GDIYLGTD	7	0.52	0.06	1.02	0.10	1.27	0.04
7	22-28	DIYLGTD	6	0.68	0.01	0.85	0.01	0.93	0.01
8	26-33	GTDIAAGE	7	2.72	0.03	2.51	0.00	2.77	0.00
9	26-36	GTDIAAGEEV A	10	3.36	0.05	3.64	0.02	3.43	0.02
10	28-34	DIAAGEE	6	2.24	0.06	2.27	0.06	2.21	0.05
11	28-36	DIAAGEEVA	8	2.48	0.09	2.56	0.05	2.53	0.06
12	29-36	IAAGEEVA	7	1.80	0.07	1.92	0.08	1.85	0.06
13	37-49	IKLECVKTKH PQL	11	1.67	0.15	1.91	0.04	2.91	0.04
14	40-49	ECVKTKHPQ L	8	1.59	0.05	1.61	0.13	1.61	0.13
15	42-49	VKTKHPQL	6	1.11	0.03	1.15	0.02	1.14	0.02
16	50-56	HIESKIY	6	0.73	0.03	0.97	0.07	0.92	0.06
17	50-58	HIESKIYKM	8	1.14	0.04	1.76	0.03	2.12	0.03
18	56-70	YKMMQGGV GIPTIRW	13	2.94	0.43	3.98	0.09	3.92	0.03
19	56-72	YKMMQGGV GIPTIRWCG	15	4.08	0.37	4.30	0.07	3.39	0.02
20	56-77	YKMMQGGV GIPTIRWCGA EGDY	20	4.47	0.32	4.54	0.05	3.88	0.29
21	59-76	MQGGVGIPTI RWCGAEGD	16	5.08	0.32	4.52	0.05	4.64	0.05
22	62-70	GVGIPTIRW	7	1.47	0.03	1.59	0.03	1.71	0.05
23	77-99	YNVMVMELL GPSLEDLFN FCSRK	21	8.72	0.08	8.77	0.47	8.80	0.31
24	83-89	ELLGPSL	5	0.15	0.05	0.24	0.06	0.17	0.03
25	85-92	LGPSLEDL	6	0.48	0.02	0.37	0.04	0.65	0.02

26	96-102	CSRKFSL	6	0.57	0.17	0.61	0.06	0.73	0.22
27	96-104	CSRKFSLKT	8	1.03	0.11	1.20	0.05	1.20	0.03
28	96-106	CSRKFSLKT VL	10	0.66	0.06	0.74	0.28	0.92	0.02
29	104-119	TVLLLADQMI SRIEYI	15	2.21	0.02	3.20	0.02	3.37	0.01
30	108-117	LADQMISRIE	9	1.96	0.05	2.06	0.01	2.09	0.01
31	113-123	ISRIEYIHSKN	10	0.30	0.06	0.42	0.01	0.38	0.01
32	124-134	FIHRDVKPDN F	9	0.24	0.04	0.27	0.03	0.47	0.04
33	124-135	FIHRDVKPDN FL	10	0.27	0.04	0.38	0.06	0.35	0.03
34	126-134	HRDVKPDNF	7	0.43	0.02	0.43	0.02	0.43	0.02
35	135-143	LMGLGKKGN	8	1.49	0.08	1.68	0.06	1.82	0.06
36	135-144	LMGLGKKGN L	9	1.59	0.02	1.83	0.01	1.99	0.04
37	135-145	LMGLGKKGN LV	10	1.68	0.04	1.95	0.04	2.09	0.08
38	135-146	LMGLGKKGN LVY	11	1.36	0.02	1.67	0.01	1.83	0.02
39	136-144	MGLGKKGNL	8	1.60	0.02	1.83	0.02	2.01	0.02
40	137-144	GLGKKGNL	7	1.15	0.02	1.29	0.01	1.39	0.01
41	137-146	GLGKKGNLV Y	9	1.43	0.01	1.76	0.01	1.88	0.06
42	140-150	KKGNLVYIID F	10	0.22	0.03	0.18	0.04	0.20	0.00
43	144-162	LVYIIDFGLAK KYRDARTH	18	1.20	0.03	2.92	0.14	3.65	0.19
44	145-151	VYIIDFG	6	0.13	0.09	0.67	0.01	0.91	0.01
45	145-152	VYIIDFGL	7	0.57	0.02	0.57	0.02	0.57	0.02
46	145-158	VYIIDFGLAK KYRD	13	0.91	0.13	0.96	0.36	1.08	0.17
47	150-169	FGLAKKYRD ARTHQHIPY RE	18	1.81	0.02	2.27	0.02	2.52	0.02
48	151-169	GLAKKYRDA RTHQHIPYR E	17	2.50	0.04	2.93	0.02	2.80	0.02
49	153-179	AKKYRDART HQHIPYREN KNLTGTARY	25	5.33	0.15	5.11	0.15	5.43	0.15
50	163-178	QHIPYRENK NLTGTAR	14	2.39	0.10	3.10	0.02	3.33	0.02
51	164-178	HIPYRENKNL TGTAR	13	1.39	0.02	1.99	0.16	2.25	0.01
52	176-186	TARYASINTH L	10	0.58	0.21	0.46	0.07	0.38	0.05
53	178-186	RYASINTHL	8	0.88	0.03	1.38	0.01	1.60	0.01
54	180-186	ASINTHL	6	0.54	0.04	1.06	0.07	1.30	0.11
55	180-189	ASINTHLGIE	9	1.17	0.10	1.81	0.00	2.46	0.00
56	180-196	ASINTHLGIE QSRRDDL	16	1.32	0.06	2.07	0.28	2.15	0.48
57	182-196	INTHLGIEQS RRDDL	14	0.98	0.04	1.53	0.22	1.64	0.04
58	185-196	HLGIEQSRR DDL	11	0.69	0.03	1.01	0.11	1.21	0.16

59	187-196	GIEQSRRDDL	9	0.86	0.04	1.15	0.07	1.21	0.09
60	189-196	EQSRRDDL	7	0.56	0.06	0.82	0.04	0.84	0.07
61	190-196	QSRRDDL	6	0.50	0.04	0.74	0.03	0.80	0.04
62	204-217	MYFNLGSLPWQGLK	12	1.01	0.10	1.50	0.02	1.90	0.02
63	205-215	YFNLGSLPWQG	9	1.30	0.02	2.38	0.02	2.33	0.02
64	205-216	YFNLGSLPWQGL	10	2.22	0.06	2.73	0.05	2.65	0.01
65	205-218	YFNLGSLPWQGLKA	12	3.45	0.15	4.02	0.18	4.19	0.04
66	206-218	FNLGSLPWQGLKA	11	3.97	0.07	4.55	0.09	4.75	0.04
67	207-216	NLGSLPWQGL	8	2.40	0.18	2.99	0.03	3.02	0.03
68	207-218	NLGSLPWQGLKA	10	3.80	0.03	4.26	0.02	4.61	0.02
69	209-216	GSLPWQGL	6	1.99	0.06	2.17	0.13	2.35	0.19
70	209-218	GSLPWQGLKA	8	2.97	0.11	3.23	0.06	3.35	0.03
71	212-218	PWQGLKA	5	2.11	0.05	2.19	0.02	2.45	0.02
72	219-230	ATKRQKYERISE	11	3.57	0.07	3.72	0.02	3.65	0.02
73	231-239	KKMSTPIEV	7	2.17	0.06	2.48	0.04	2.76	0.04
74	231-240	KKMSTPIEVL	8	1.92	0.03	2.32	0.09	2.51	0.03
75	239-248	VLCKGYPSEF	8	0.96	0.06	1.35	0.07	1.59	0.07
76	241-247	CKGYPSE	5	0.93	0.04	1.08	0.02	1.34	0.02
77	241-248	CKGYPSEF	6	0.79	0.05	0.97	0.08	1.22	0.03
78	248-254	FATYLNF	6	0.20	0.01	0.20	0.02	0.26	0.09
79	255-267	CRSLRFDDKPDYS	11	2.33	0.07	2.73	0.04	2.82	0.04
80	255-268	CRSLRFDDKPDYSY	12	2.97	0.13	3.32	0.03	3.43	0.03
81	255-269	CRSLRFDDKPDYSYL	13	2.75	0.09	3.08	0.07	3.09	0.09
82	259-276	RFDDKPDYSYLRQLFRNL	16	3.42	0.02	4.56	0.08	4.64	0.01
83	261-269	DDKPDYSYL	7	1.67	0.11	1.76	0.08	1.78	0.03
84	269-277	LRQLFRNLF	8	0.15	0.08	0.57	0.10	0.66	0.17
85	269-281	LRQLFRNLFHRQG	12	2.16	0.21	2.48	0.15	2.45	0.10
86	269-285	LRQLFRNLFHRQGFSYD	16	1.82	0.32	1.96	0.24	2.09	0.21
87	270-276	RQLFRNL	6	0.58	0.11	0.84	0.13	0.86	0.04
88	270-277	RQLFRNLF	7	0.24	0.10	0.50	0.10	0.58	0.16
89	270-285	RQLFRNLFHRQGFSYD	15	1.69	0.11	2.11	0.04	1.93	0.04
90	273-285	FRNLFHRQGFSYD	12	1.69	0.09	1.89	0.01	1.94	0.03
91	278-285	HRQGFSYD	7	1.30	0.06	1.41	0.02	1.49	0.03
92	286-292	YVFDWNM	6	1.61	0.08	2.05	0.10	1.93	0.08
93	293-302	LKFGASRAAD	9	2.87	0.11	2.91	0.10	2.96	0.10

94	293-305	LKFGASRAADDAE	12	3.86	0.08	3.74	0.05	3.82	0.10
95	294-302	KFGASRAAD	8	2.65	0.05	2.68	0.02	2.74	0.06
96	303-312	DAERERRDRE	9	1.97	0.03	2.88	0.03	2.51	0.03
97	312-324	EERLRHSRNPATR	11	3.62	0.04	3.42	0.03	3.43	0.03
98	319-338	RNPATRGLPSTASGRLRGTQ	17	3.36	0.32	3.95	0.25	4.00	0.28
99	325-334	GLPSTASGRL	8	0.33	0.13	0.19	0.05	0.20	0.07
100	337-350	TQEVAPPTPLTPTS	9	0.97	0.06	1.19	0.00	1.40	0.00
101	351-357	HTANTSP	5	0.64	0.14	0.84	0.17	0.79	0.07
102	362-375	GMERERKVSMRLHR	13	1.48	0.10	2.02	0.27	2.35	0.32
103	364-371	ERERKVSM	7	2.90	0.04	2.67	0.04	3.01	0.04
104	364-378	ERERKVSMRLHRGAP	13	6.01	0.06	6.92	0.30	7.21	0.43
105	365-373	RERKVSMRL	8	1.74	0.09	2.19	0.04	2.66	0.15
106	371-394	MRLHRGAPVNISSSDLTGRQDTSR	22	6.54	0.02	6.90	0.02	6.81	0.02
107	379-395	VNISSSDLTGRQDTSRM	16	5.29	0.06	5.26	0.06	5.48	0.06
108	386-394	LTGRQDTSR	8	4.18	0.13	4.67	0.03	4.56	0.05
109	387-395	TGRQDTSRM	8	2.09	0.02	2.49	0.02	2.15	0.02
110	394-402	RMSTSQIPG	7	0.76	0.10	0.85	0.07	0.73	0.03
				CK1δ1 + AMPPNP					
				Relative Deuterium Uptake (Da)					
				1 min		**5 min**		**10 min**	
S.No.	**Peptide**	**Sequence**	**Num Exchangers**	**Uptake**	**± SD**	**Uptake**	**± SD**	**Uptake**	**± SD**
1	3-20	LRVGNRYRLGRKIGSGSF	17	6.72	0.06	7.62	0.04	7.50	0.12
2	4-20	RVGNRYRLGRKIGSGSF	16	5.42	0.14	6.22	0.06	6.25	0.13
3	4-22	RVGNRYRLGRKIGSGSFGD	18	5.87	0.06	6.71	0.06	6.84	0.15
4	9-28	YRLGRKIGSGSFGDIYLGTD	19	7.38	0.01	7.71	0.04	7.49	0.08
5	12-25	GRKIGSGSFGDIYL	13	3.56	0.16	4.47	0.04	4.46	0.13
6	21-28	GDIYLGTD	7	0.98	0.03	2.01	0.04	2.50	0.04
7	22-28	DIYLGTD	6	0.66	0.16	1.49	0.13	1.81	0.04
8	26-33	GTDIAAGE	7	2.86	0.04	2.97	0.10	2.94	0.09
9	26-36	GTDIAAGEEVA	10	3.77	0.05	3.92	0.09	3.97	0.02
10	28-34	DIAAGEE	6	2.47	0.04	2.56	0.05	2.52	0.05
11	28-36	DIAAGEEVA	8	2.79	0.05	3.02	0.02	2.96	0.04
12	29-36	IAAGEEVA	7	2.11	0.04	2.23	0.03	2.25	0.03

13	37-49	IKLECVKTKHPQL	11	2.01	0.18	1.86	0.29	2.82	0.08
14	40-49	ECVKTKHPQL	8	2.02	0.15	1.96	0.12	1.69	0.10
15	42-49	VKTKHPQL	6	1.37	0.04	1.35	0.04	1.36	0.05
16	50-56	HIESKIY	6	0.91	0.05	1.29	0.05	1.42	0.04
17	50-58	HIESKIYKM	8	1.67	0.13	2.54	0.06	2.58	0.13
18	56-70	YKMMQGGVGIPTIRW	13	3.88	0.03	5.16	0.03	4.78	0.03
19	56-72	YKMMQGGVGIPTIRWCG	15	4.26	0.33	4.80	0.32	4.49	0.09
20	56-77	YKMMQGGVGIPTIRWCGAEGDY	20	5.18	0.23	5.54	0.36	5.19	0.09
21	59-76	MQGGVGIPTIRWCGAEGD	16	5.23	0.30	5.25	0.25	5.08	0.19
22	62-70	GVGIPTIRW	7	1.59	0.03	1.93	0.10	2.01	0.03
23	77-99	YNVMVMELLGPSLEDLFNFCSRK	21	9.46	0.07	9.99	0.05	9.75	0.03
24	83-89	ELLGPSL	5	0.31	0.03	0.48	0.03	0.60	0.03
25	85-92	LGPSLEDL	6	0.41	0.15	0.44	0.15	0.33	0.05
26	96-102	CSRKFSL	6	0.59	0.01	0.85	0.01	0.99	0.04
27	96-104	CSRKFSLKT	8	1.82	0.07	1.83	0.20	2.50	0.03
28	96-106	CSRKFSLKTVL	10	0.90	0.09	1.04	0.02	1.15	0.02
29	104-119	TVLLLADQMISRIEYI	15	3.19	0.12	4.32	0.04	4.43	0.05
30	108-117	LADQMISRIE	9	2.92	0.10	NaN	NaN	3.64	0.21
31	113-123	ISRIEYIHSKN	10	0.28	0.02	0.32	0.02	0.37	0.02
32	124-134	FIHRDVKPDNF	9	0.37	0.03	0.42	0.03	0.47	0.02
33	124-135	FIHRDVKPDNFL	10	0.36	0.01	0.33	0.03	0.45	0.00
34	126-134	HRDVKPDNF	7	0.23	0.03	0.24	0.02	0.32	0.02
35	135-143	LMGLGKKGN	8	1.68	0.13	2.03	0.07	2.09	0.11
36	135-144	LMGLGKKGNL	9	1.81	0.04	2.11	0.01	2.30	0.03
37	135-145	LMGLGKKGNLV	10	1.87	0.12	2.24	0.06	2.47	0.05
38	135-146	LMGLGKKGNLVY	11	1.66	0.03	1.96	0.01	2.17	0.02
39	136-144	MGLGKKGNL	8	1.85	0.08	2.20	0.02	2.22	0.05
40	137-144	GLGKKGNL	7	1.52	0.04	1.83	0.02	1.93	0.01
41	137-146	GLGKKGNLVY	9	1.60	0.06	1.87	0.01	2.13	0.01
42	140-150	KKGNLVYIIDF	10	0.72	0.00	0.15	0.01	0.11	0.01
43	144-162	LVYIIDFGLAKKYRDARTH	18	1.67	0.03	4.01	0.03	5.29	0.10
44	145-151	VYIIDFG	6	0.37	0.04	1.00	0.04	1.31	0.09
45	145-152	VYIIDFGL	7	0.41	0.04	1.29	0.03	1.58	0.02
46	145-158	VYIIDFGLAKKYRD	13	1.26	0.01	1.47	0.01	1.50	0.01

47	150-169	FGLAKKYRDARTHQHIPYRE	18	3.60	0.10	4.03	0.05	3.91	0.09
48	151-169	GLAKKYRDARTHQHIPYRE	17	4.12	0.09	4.54	0.14	4.52	0.22
49	153-179	AKKYRDARTHQHIPYRENKNLTGTARY	25	7.14	0.34	7.41	0.28	7.56	0.19
50	163-178	QHIPYRENKNLTGTAR	14	3.12	0.05	4.09	0.04	4.25	0.03
51	164-178	HIPYRENKNLTGTAR	13	1.53	0.01	1.81	0.20	1.81	0.04
52	176-186	TARYASINTHL	10	0.27	0.06	0.26	0.05	0.30	0.06
53	178-186	RYASINTHL	8	1.15	0.02	1.58	0.21	2.17	0.04
54	180-186	ASINTHL	6	0.87	0.07	1.53	0.04	1.75	0.04
55	180-189	ASINTHLGIE	9	1.49	0.00	2.85	0.04	3.29	0.02
56	180-196	ASINTHLGIEQSRRDDL	16	1.59	0.16	2.85	0.08	3.20	0.09
57	182-196	INTHLGIEQSRRDDL	14	1.47	0.11	2.57	0.05	2.84	0.10
58	185-196	HLGIEQSRRDDL	11	0.80	0.05	1.37	0.08	1.48	0.07
59	187-196	GIEQSRRDDL	9	0.93	0.15	1.30	0.12	1.26	0.12
60	189-196	EQSRRDDL	7	0.84	0.09	1.08	0.07	1.08	0.07
61	190-196	QSRRDDL	6	0.75	0.06	1.01	0.07	1.02	0.04
62	204-217	MYFNLGSLPWQGLK	12	1.46	0.12	2.46	0.11	2.84	0.13
63	205-215	YFNLGSLPWQG	9	2.15	0.05	2.89	0.02	2.97	0.03
64	205-216	YFNLGSLPWQGL	10	2.73	0.08	3.47	0.04	3.61	0.04
65	205-218	YFNLGSLPWQGLKA	12	4.03	0.03	4.80	0.02	4.88	0.03
66	206-218	FNLGSLPWQGLKA	11	4.45	0.18	5.22	0.22	5.29	0.18
67	207-216	NLGSLPWQGL	8	2.75	0.08	3.13	0.16	3.55	0.14
68	207-218	NLGSLPWQGLKA	10	4.29	0.03	4.94	0.08	5.06	0.04
69	209-216	GSLPWQGL	6	2.55	0.10	2.85	0.11	2.92	0.15
70	209-218	GSLPWQGLKA	8	3.31	0.04	3.58	0.07	3.63	0.03
71	212-218	PWQGLKA	5	2.46	0.09	2.60	0.04	2.64	0.02
72	219-230	ATKRQKYERISE	11	4.68	0.19	4.83	0.17	4.71	0.09
73	231-239	KKMSTPIEV	7	2.49	0.07	2.89	0.05	3.00	0.05
74	231-240	KKMSTPIEVL	8	2.20	0.07	2.64	0.04	2.75	0.04
75	239-248	VLCKGYPSEF	8	1.13	0.08	1.51	0.06	1.76	0.06
76	241-247	CKGYPSE	5	1.19	0.04	1.47	0.04	1.71	0.03
77	241-248	CKGYPSEF	6	0.88	0.04	1.11	0.03	1.30	0.03
78	248-254	FATYLNF	6	0.08	0.03	0.04	0.03	0.06	0.02
79	255-267	CRSLRFDDKPDYS	11	2.72	0.09	3.02	0.04	3.09	0.04

80	255-268	CRSLRFDDK PDYSY	12	3.54	0.03	4.04	0.04	3.97	0.17
81	255-269	CRSLRFDDK PDYSYL	13	3.22	0.11	3.53	0.09	3.54	0.09
82	259-276	RFDDKPDYS YLRQLFRNL	16	3.44	0.01	5.39	0.01	5.79	0.10
83	261-269	DDKPDYSYL	7	2.15	0.11	2.22	0.03	2.16	0.04
84	269-277	LRQLFRNLF	8	0.24	0.06	0.47	0.04	0.73	0.05
85	269-281	LRQLFRNLF HRQG	12	2.26	0.08	2.46	0.06	2.49	0.08
86	269-285	LRQLFRNLF HRQGFSYD	16	2.39	0.13	2.82	0.09	2.89	0.11
87	270-276	RQLFRNL	6	0.93	0.06	1.72	0.14	2.05	0.08
88	270-277	RQLFRNLF	7	0.27	0.02	0.47	0.03	0.61	0.02
89	270-285	RQLFRNLFH RQGFSYD	15	2.19	0.05	2.58	0.07	2.59	0.07
90	273-285	FRNLFHRQG FSYD	12	2.23	0.12	2.39	0.02	2.49	0.04
91	278-285	HRQGFSYD	7	1.90	0.14	2.00	0.04	2.03	0.09
92	286-292	YVFDWNM	6	2.19	0.06	2.53	0.04	2.52	0.03
93	293-302	LKFGASRAA D	9	3.43	0.13	3.65	0.11	3.54	0.25
94	293-305	LKFGASRAA DDAE	12	4.48	0.10	4.60	0.09	4.52	0.10
95	294-302	KFGASRAAD	8	3.16	0.10	3.30	0.11	3.07	0.03
96	303-312	DAERERRDR E	9	3.36	0.06	3.40	0.20	3.42	0.08
97	312-324	EERLRHSRN PATR	11	4.04	0.10	4.17	0.05	4.18	0.07
98	319-338	RNPATRGLP STASGRLRG TQ	17	3.77	0.15	4.35	0.26	6.19	0.49
99	325-334	GLPSTASGR L	8	0.44	0.06	0.86	0.06	1.09	0.02
100	337-350	TQEVAPPTP LTPTS	9	1.08	0.00	2.06	0.00	2.50	0.12
101	351-357	HTANTSP	5	1.08	0.03	1.14	0.02	1.13	0.04
102	362-375	GMERERKVS MRLHR	13	2.13	0.17	3.26	0.08	3.79	0.23
103	364-371	ERERKVSM	7	3.03	0.05	3.11	0.08	3.33	0.35
104	364-378	ERERKVSMR LHRGAP	13	6.10	0.07	6.66	0.11	6.84	0.04
105	365-373	RERKVSMRL	8	2.93	0.08	2.76	0.29	2.71	0.18
106	371-394	MRLHRGAPV NISSSDLTGR QDTSR	22	7.47	0.02	8.16	0.02	7.18	0.02
107	379-395	VNISSSDLTG RQDTSRM	16	5.62	0.16	5.52	0.02	5.61	0.01
108	386-394	LTGRQDTSR	8	4.73	0.03	5.03	0.08	4.97	0.05
109	387-395	TGRQDTSRM	8	2.38	0.06	2.64	0.09	2.61	0.13
110	394-402	RMSTSQIPG	7	0.76	0.04	0.79	0.02	0.76	0.03
				CK1δ1					
				Relative Fractional Deuterium Uptake (%)					
				1 min		**5 min**		**10 min**	

S.No.	Peptide	Sequence	Num Exchangers	Uptake	± SD	Uptake	± SD	Uptake	± SD
1	3-20	LRVGNRYRL GRKIGSGSF	17	37.51	1.41	42.74	0.79	45.16	0.70
2	4-20	RVGNRYRLG RKIGSGSF	16	32.76	0.95	36.75	0.10	38.68	0.65
3	4-22	RVGNRYRLG RKIGSGSFG D	18	30.05	1.60	35.34	0.31	37.45	0.87
4	9-28	YRLGRKIGS GSFGDIYLGT D	19	36.97	0.27	38.31	0.41	38.96	0.49
5	12-25	GRKIGSGSF GDIYL	13	29.00	1.75	36.38	1.94	36.51	1.71
6	21-28	GDIYLGTD	7	20.35	0.90	36.49	0.33	43.25	0.53
7	22-28	DIYLGTD	6	17.94	1.54	35.27	1.43	41.25	1.93
8	26-33	GTDIAAGE	7	41.17	1.01	41.21	0.17	42.66	0.83
9	26-36	GTDIAAGEEV A	10	37.86	0.55	39.99	0.49	43.24	0.66
10	28-34	DIAAGEE	6	41.26	1.00	41.17	0.68	40.87	1.49
11	28-36	DIAAGEEVA	8	34.90	0.10	38.38	0.49	41.70	0.69
12	29-36	IAAGEEVA	7	29.88	0.59	33.10	0.56	36.39	0.50
13	37-49	IKLECVKTKH PQL	11	17.91	1.51	23.69	0.81	26.51	0.67
14	40-49	ECVKTKHPQ L	8	23.55	2.14	21.83	2.51	21.75	1.36
15	42-49	VKTKHPQL	6	22.33	1.60	22.59	0.80	23.55	0.77
16	50-56	HIESKIY	6	18.32	0.93	25.45	0.38	27.82	0.31
17	50-58	HIESKIYKM	8	18.34	1.95	32.16	1.79	36.87	1.13
18	56-70	YKMMQGGV GIPTIRW	13	29.91	1.15	35.46	1.19	36.70	0.35
19	56-72	YKMMQGGV GIPTIRWCG	15	24.51	1.47	25.77	1.02	24.89	1.36
20	56-77	YKMMQGGV GIPTIRWCGA EGDY	20	22.71	1.17	23.58	0.52	23.18	0.26
21	59-76	MQGGVGIPTI RWCGAEGD	16	29.32	2.49	26.94	2.30	29.07	0.31
22	62-70	GVGIPTIRW	7	23.80	1.21	29.19	0.51	31.02	0.79
23	77-99	YNVMVMELL GPSLEDLFN FCSRK	21	43.61	0.25	46.06	0.13	47.11	0.24
24	83-89	ELLGPSL	5	10.00	0.37	13.45	0.46	15.92	0.40
25	85-92	LGPSLEDL	6	7.17	0.73	8.26	1.09	8.13	0.79
26	96-102	CSRKFSL	6	14.53	1.61	14.33	1.04	19.20	1.41
27	96-104	CSRKFSLKT	8	20.16	2.65	19.54	0.41	28.06	0.55
28	96-106	CSRKFSLKT VL	10	8.08	0.43	10.12	0.41	11.61	0.44
29	104-119	TVLLLADQMI SRIEYI	15	24.47	0.12	32.36	0.28	36.94	0.35
30	108-117	LADQMISRIE	9	34.31	3.34	37.58	1.64	40.13	0.12
31	113-123	ISRIEYIHSKN	10	3.75	0.36	4.52	0.25	5.86	0.25
32	124-134	FIHRDVKPDN F	9	3.22	0.39	3.74	0.41	4.66	0.50

33	124-135	FIHRDVKPDNFL	10	2.24	0.29	2.83	0.14	4.01	0.33
34	126-134	HRDVKPDNF	7	2.07	0.38	2.76	0.32	3.85	0.55
35	135-143	LMGLGKKGN	8	20.77	1.39	26.50	1.25	29.22	1.67
36	135-144	LMGLGKKGNL	9	20.15	0.79	24.96	0.11	27.60	0.88
37	135-145	LMGLGKKGNLV	10	18.76	0.66	24.10	0.54	26.14	0.36
38	135-146	LMGLGKKGNLVY	11	14.79	0.67	19.10	0.19	21.48	0.80
39	136-144	MGLGKKGNL	8	23.47	0.57	28.12	0.26	31.32	0.28
40	137-144	GLGKKGNL	7	21.33	0.64	27.29	0.19	29.84	0.51
41	137-146	GLGKKGNLVY	9	18.79	0.66	23.21	0.25	27.32	1.04
42	140-150	KKGNLVYIIDF	10	3.63	0.74	1.55	0.03	1.16	0.14
43	144-162	LVYIIDFGLAKKYRDARTH	18	11.33	0.15	27.00	0.54	35.99	0.37
44	145-151	VYIIDFG	6	10.92	1.32	25.45	2.03	25.03	0.88
45	145-152	VYIIDFGL	7	11.95	0.30	24.48	1.36	29.56	0.86
46	145-158	VYIIDFGLAKKYRD	13	9.31	0.37	11.17	0.07	12.97	0.08
47	150-169	FGLAKKYRDARTHQHIPYRE	18	17.33	0.46	20.31	0.17	22.55	0.27
48	151-169	GLAKKYRDARTHQHIPYRE	17	21.46	0.16	23.26	1.22	26.21	0.19
49	153-179	AKKYRDARTHQHIPYRENKNLTGTARY	25	26.94	0.70	26.49	0.72	28.27	0.95
50	163-178	QHIPYRENKNLTGTAR	14	21.84	0.46	30.17	0.38	36.45	0.56
51	164-178	HIPYRENKNLTGTAR	13	10.97	0.05	13.40	1.95	14.35	0.05
52	176-186	TARYASINTHL	10	2.13	0.66	2.71	0.57	3.16	0.53
53	178-186	RYASINTHL	8	11.10	0.22	19.86	2.74	25.80	0.18
54	180-186	ASINTHL	6	11.86	1.10	22.10	1.11	28.58	1.13
55	180-189	ASINTHLGIE	9	13.58	0.55	28.08	0.14	35.29	0.53
56	180-196	ASINTHLGIEQSRRDDL	16	8.82	0.42	16.30	0.46	19.92	0.69
57	182-196	INTHLGIEQSRRDDL	14	9.02	0.38	15.55	0.29	19.61	0.79
58	185-196	HLGIEQSRRDDL	11	7.33	0.54	11.43	0.52	13.39	0.82
59	187-196	GIEQSRRDDL	9	8.97	1.26	12.46	1.49	14.37	1.39
60	189-196	EQSRRDDL	7	11.17	0.61	14.45	1.32	16.94	1.14
61	190-196	QSRRDDL	6	10.46	0.78	16.29	0.76	17.91	0.65
62	204-217	MYFNLGSLPWQGLK	12	10.69	0.34	18.47	0.75	23.71	0.92
63	205-215	YFNLGSLPWQG	9	26.01	0.55	31.64	0.49	33.90	0.96
64	205-216	YFNLGSLPWQGL	10	26.98	1.28	33.88	0.98	35.81	1.68

65	205-218	YFNLGSLPWQGLKA	12	33.97	0.88	38.61	0.14	41.49	0.69
66	206-218	FNLGSLPWQGLKA	11	39.77	1.24	46.08	1.29	48.25	1.45
67	207-216	NLGSLPWQGL	8	33.61	0.98	40.65	1.05	43.83	1.29
68	207-218	NLGSLPWQGLKA	10	42.01	1.15	48.10	0.22	50.94	0.71
69	209-216	GSLPWQGL	6	41.50	1.65	45.82	1.76	48.93	1.86
70	209-218	GSLPWQGLKA	8	41.85	1.66	43.83	0.91	45.80	1.59
71	212-218	PWQGLKA	5	47.82	1.09	50.07	0.73	54.76	0.31
72	219-230	ATKRQKYERISE	11	39.16	1.29	40.66	0.23	41.85	1.05
73	231-239	KKMSTPIEV	7	34.92	1.03	40.46	1.15	43.27	0.49
74	231-240	KKMSTPIEVL	8	26.88	0.89	31.99	0.52	34.81	0.54
75	239-248	VLCKGYPSEF	8	13.20	0.98	17.82	0.76	22.48	1.33
76	241-247	CKGYPSE	5	22.76	1.07	29.05	0.48	34.85	1.00
77	241-248	CKGYPSEF	6	13.84	0.90	17.58	0.41	22.31	0.70
78	248-254	FATYLNF	6	2.60	0.80	2.27	0.29	3.20	0.26
79	255-267	CRSLRFDDKPDYS	11	25.16	0.33	27.67	0.46	29.79	0.33
80	255-268	CRSLRFDDKPDYSY	12	28.35	0.78	32.06	0.88	35.68	1.81
81	255-269	CRSLRFDDKPDYSYL	13	24.47	0.92	26.53	0.62	28.12	0.74
82	259-276	RFDDKPDYSYLRQLFRNL	16	22.20	0.19	34.87	1.46	42.90	0.46
83	261-269	DDKPDYSYL	7	31.57	1.45	31.42	0.53	31.96	1.46
84	269-277	LRQLFRNLF	8	2.67	0.53	6.25	0.65	10.11	0.34
85	269-281	LRQLFRNLFHRQG	12	17.71	0.73	18.90	0.62	21.23	0.56
86	269-285	LRQLFRNLFHRQGFSYD	16	14.55	0.81	16.86	0.57	18.74	0.76
87	270-276	RQLFRNL	6	18.09	1.48	30.37	3.19	36.23	1.90
88	270-277	RQLFRNLF	7	3.80	0.82	7.17	1.02	10.71	0.34
89	270-285	RQLFRNLFHRQGFSYD	15	13.66	0.26	NaN	NaN	18.74	0.39
90	273-285	FRNLFHRQGFSYD	12	17.55	1.05	19.64	0.53	21.45	0.95
91	278-285	HRQGFSYD	7	27.15	1.89	26.89	0.37	28.70	1.67
92	286-292	YVFDWNM	6	38.75	1.01	42.90	0.79	44.59	1.23
93	293-302	LKFGASRAAD	9	36.88	1.62	38.05	1.12	40.84	1.09
94	293-305	LKFGASRAADDAE	12	35.50	1.54	36.37	0.96	38.41	1.29
95	294-302	KFGASRAAD	8	37.19	1.25	37.52	1.49	37.73	0.55
96	303-312	DAERERRDRE	9	30.39	2.46	34.85	2.81	36.34	3.10
97	312-324	EERLRHSRNPATR	11	36.36	1.50	36.70	0.57	37.32	0.86
98	319-338	RNPATRGLPSTASGRLRGTQ	17	22.83	1.62	27.84	2.63	36.45	2.51

99	325-334	GLPSTASGRL	8	8.44	1.44	15.99	1.15	21.62	1.05
100	337-350	TQEVAPPTPLTPTS	9	9.00	0.04	20.92	0.04	29.57	0.04
101	351-357	HTANTSP	5	19.37	0.63	20.65	0.73	22.01	0.67
102	362-375	GMERERKVSMRLHR	13	16.73	1.41	24.10	1.22	30.31	0.32
103	364-371	ERERKVSM	7	43.03	1.27	46.60	3.10	48.32	2.97
104	364-378	ERERKVSMRLHRGAP	13	46.73	0.29	53.96	0.46	59.16	0.24
105	365-373	RERKVSMRL	8	35.88	1.42	33.81	1.74	36.60	1.20
106	371-394	MRLHRGAPVNISSSDLTGRQDTSR	22	34.18	1.30	34.70	1.03	36.63	0.31
107	379-395	VNISSSDLTGRQDTSRM	16	32.17	0.76	33.18	0.58	34.28	0.05
108	386-394	LTGRQDTSR	8	58.23	1.21	62.05	0.19	62.83	1.09
109	387-395	TGRQDTSRM	8	29.06	0.44	34.44	0.88	33.91	0.66
110	394-402	RMSTSQIPG	7	11.04	0.32	11.37	0.27	11.75	0.56

				CK1δ1 + ATP					
				Relative Fractional Deuterium Uptake (%)					
				1 min		**5 min**		**10 min**	
S.No.	**Peptide**	**Sequence**	**Num Exchangers**	**Uptake**	**± SD**	**Uptake**	**± SD**	**Uptake**	**± SD**
1	3-20	LRVGNRYRLGRKIGSGSF	17	31.22	1.17	34.85	0.02	36.32	0.02
2	4-20	RVGNRYRLGRKIGSGSF	16	27.79	0.11	31.84	0.34	32.21	0.54
3	4-22	RVGNRYRLGRKIGSGSFGD	18	26.90	0.33	30.94	0.45	31.75	0.18
4	9-28	YRLGRKIGSGSFGDIYLGTD	19	32.91	0.83	34.73	0.55	34.80	0.74
5	12-25	GRKIGSGSFGDIYL	13	20.04	0.09	26.90	0.09	28.05	0.09
6	21-28	GDIYLGTD	7	7.50	0.92	14.64	1.38	18.21	0.53
7	22-28	DIYLGTD	6	11.26	0.17	14.09	0.17	15.42	0.20
8	26-33	GTDIAAGE	7	38.87	0.48	35.81	0.03	39.51	0.03
9	26-36	GTDIAAGEEVA	10	33.60	0.47	36.44	0.16	34.29	0.16
10	28-34	DIAAGEE	6	37.36	0.98	37.85	0.92	36.78	0.83
11	28-36	DIAAGEEVA	8	31.02	1.13	32.01	0.57	31.63	0.71
12	29-36	IAAGEEVA	7	25.74	1.00	27.38	1.19	26.45	0.81
13	37-49	IKLECVKTKHPQL	11	15.16	1.36	17.38	0.32	26.46	0.32
14	40-49	ECVKTKHPQL	8	19.85	0.60	20.10	1.60	20.11	1.65
15	42-49	VKTKHPQL	6	18.54	0.45	19.11	0.41	18.94	0.41
16	50-56	HIESKIY	6	12.22	0.53	16.21	1.25	15.33	1.00
17	50-58	HIESKIYKM	8	14.19	0.45	21.97	0.43	26.54	0.43
18	56-70	YKMMQGGVGIPTIRW	13	22.58	3.34	30.59	0.67	30.18	0.25

19	56-72	YKMMQGGV GIPTIRWCG	15	27.21	2.50	28.70	0.46	22.59	0.10
20	56-77	YKMMQGGV GIPTIRWCGA EGDY	20	22.35	1.61	22.71	0.26	19.40	1.43
21	59-76	MQGGVGIPTI RWCGAEGD	16	31.78	1.97	28.28	0.31	28.98	0.31
22	62-70	GVGIPTIRW	7	21.07	0.48	22.70	0.46	24.45	0.65
23	77-99	YNVMVMELL GPSLEDLFN FCSRK	21	41.54	0.38	41.74	2.24	41.91	1.48
24	83-89	ELLGPSL	5	2.92	0.97	4.87	1.17	3.49	0.63
25	85-92	LGPSLEDL	6	8.01	0.37	6.16	0.70	10.86	0.27
26	96-102	CSRKFSL	6	9.42	2.77	10.13	0.99	12.18	3.68
27	96-104	CSRKFSLKT	8	12.93	1.32	15.05	0.67	14.94	0.41
28	96-106	CSRKFSLKT VL	10	6.59	0.57	7.45	2.82	9.23	0.24
29	104-119	TVLLLADQMI SRIEYI	15	14.76	0.15	21.34	0.10	22.44	0.09
30	108-117	LADQMISRIE	9	21.78	0.54	22.87	0.12	23.27	0.12
31	113-123	ISRIEYIHSKN	10	3.03	0.62	4.15	0.07	3.77	0.10
32	124-134	FIHRDVKPDN F	9	2.69	0.41	2.98	0.36	5.22	0.48
33	124-135	FIHRDVKPDN FL	10	2.70	0.42	3.85	0.64	3.51	0.30
34	126-134	HRDVKPDNF	7	6.13	0.32	6.09	0.32	6.10	0.32
35	135-143	LMGLGKKGN	8	18.66	0.98	21.03	0.75	22.73	0.74
36	135-144	LMGLGKKGN L	9	17.61	0.25	20.31	0.11	22.08	0.50
37	135-145	LMGLGKKGN LV	10	16.81	0.36	19.45	0.39	20.87	0.77
38	135-146	LMGLGKKGN LVY	11	12.34	0.19	15.18	0.13	16.61	0.21
39	136-144	MGLGKKGNL	8	20.00	0.30	22.90	0.25	25.13	0.25
40	137-144	GLGKKGNL	7	16.40	0.26	18.49	0.08	19.87	0.08
41	137-146	GLGKKGNLV Y	9	15.92	0.06	19.54	0.09	20.85	0.62
42	140-150	KKGNLVYIID F	10	2.24	0.34	1.78	0.44	2.01	0.00
43	144-162	LVYIIDFGLAK KYRDARTH	18	6.66	0.15	16.22	0.77	20.27	1.04
44	145-151	VYIIDFG	6	2.18	1.42	11.16	0.20	15.12	0.20
45	145-152	VYIIDFGL	7	8.15	0.25	8.11	0.25	8.12	0.25
46	145-158	VYIIDFGLAK KYRD	13	7.04	0.98	7.37	2.78	8.28	1.34
47	150-169	FGLAKKYRD ARTHQHIPY RE	18	10.07	0.10	12.62	0.10	13.99	0.10
48	151-169	GLAKKYRDA RTHQHIPYR E	17	14.72	0.21	17.21	0.12	16.49	0.12
49	153-179	AKKYRDART HQHIPYREN KNLTGTARY	25	21.32	0.61	20.45	0.61	21.71	0.61
50	163-178	QHIPYRENK NLTGTAR	14	17.10	0.69	22.13	0.16	23.79	0.16

51	164-178	HIPYRENKNL TGTAR	13	10.71	0.12	15.30	1.24	17.30	0.05
52	176-186	TARYASINTH L	10	5.80	2.09	4.59	0.73	3.76	0.54
53	178-186	RYASINTHL	8	10.96	0.43	17.30	0.11	19.99	0.11
54	180-186	ASINTHL	6	9.01	0.66	17.62	1.11	21.61	1.89
55	180-189	ASINTHLGIE	9	13.02	1.12	20.09	0.03	27.31	0.03
56	180-196	ASINTHLGIE QSRRDDL	16	8.26	0.38	12.96	1.77	13.41	3.02
57	182-196	INTHLGIEQS RRDDL	14	7.02	0.30	10.95	1.55	11.73	0.29
58	185-196	HLGIEQSRR DDL	11	6.25	0.30	9.21	1.00	11.01	1.45
59	187-196	GIEQSRRDD L	9	9.53	0.45	12.81	0.73	13.49	1.00
60	189-196	EQSRRDDL	7	8.05	0.91	11.75	0.61	12.04	1.07
61	190-196	QSRRDDL	6	8.38	0.66	12.29	0.56	13.38	0.59
62	204-217	MYFNLGSLP WQGLK	12	8.40	0.83	12.53	0.20	15.83	0.20
63	205-215	YFNLGSLPW QG	9	14.45	0.22	26.48	0.22	25.92	0.22
64	205-216	YFNLGSLPW QGL	10	22.18	0.59	27.30	0.50	26.51	0.08
65	205-218	YFNLGSLPW QGLKA	12	28.71	1.22	33.50	1.52	34.91	0.30
66	206-218	FNLGSLPWQ GLKA	11	36.08	0.62	41.36	0.83	43.14	0.34
67	207-216	NLGSLPWQG L	8	30.04	2.30	37.38	0.35	37.77	0.35
68	207-218	NLGSLPWQG LKA	10	37.96	0.29	42.59	0.21	46.12	0.21
69	209-216	GSLPWQGL	6	33.20	1.02	36.19	2.19	39.15	3.24
70	209-218	GSLPWQGLK A	8	37.18	1.36	40.38	0.76	41.93	0.37
71	212-218	PWQGLKA	5	42.18	1.08	43.74	0.31	48.98	0.31
72	219-230	ATKRQKYERI SE	11	32.46	0.65	33.85	0.22	33.15	0.22
73	231-239	KKMSTPIEV	7	31.06	0.79	35.42	0.56	39.41	0.56
74	231-240	KKMSTPIEVL	8	23.97	0.42	29.01	1.16	31.42	0.41
75	239-248	VLCKGYPSE F	8	11.96	0.73	16.88	0.93	19.88	0.89
76	241-247	CKGYPSE	5	18.64	0.77	21.53	0.36	26.71	0.36
77	241-248	CKGYPSEF	6	13.25	0.78	16.16	1.37	20.38	0.48
78	248-254	FATYLNF	6	3.31	0.24	3.32	0.36	4.28	1.58
79	255-267	CRSLRFDDK PDYS	11	21.14	0.62	24.86	0.33	25.66	0.33
80	255-268	CRSLRFDDK PDYSY	12	24.74	1.05	27.70	0.22	28.58	0.22
81	255-269	CRSLRFDDK PDYSYL	13	21.15	0.67	23.69	0.54	23.79	0.67
82	259-276	RFDDKPDYS YLRQLFRNL	16	21.37	0.11	28.52	0.47	29.00	0.03
83	261-269	DDKPDYSYL	7	23.86	1.53	25.07	1.14	25.37	0.50
84	269-277	LRQLFRNLF	8	1.91	1.05	7.09	1.30	8.23	2.13

85	269-281	LRQLFRNLF HRQG	12	18.01	1.75	20.64	1.29	20.45	0.84
86	269-285	LRQLFRNLF HRQGFSYD	16	11.38	2.02	12.25	1.50	13.04	1.32
87	270-276	RQLFRNL	6	9.64	1.88	13.98	2.15	14.41	0.59
88	270-277	RQLFRNLF	7	3.41	1.43	7.10	1.39	8.35	2.22
89	270-285	RQLFRNLFH RQGFSYD	15	11.26	0.76	14.10	0.26	12.84	0.26
90	273-285	FRNLFHRQG FSYD	12	14.11	0.79	15.76	0.12	16.14	0.23
91	278-285	HRQGFSYD	7	18.53	0.87	20.12	0.22	21.24	0.49
92	286-292	YVFDWNM	6	26.86	1.41	34.16	1.65	32.15	1.31
93	293-302	LKFGASRAA D	9	31.92	1.19	32.32	1.13	32.94	1.07
94	293-305	LKFGASRAA DDAE	12	32.19	0.70	31.15	0.44	31.83	0.81
95	294-302	KFGASRAAD	8	33.12	0.62	33.54	0.26	34.22	0.81
96	303-312	DAERERRDR E	9	21.89	0.33	32.01	0.33	27.90	0.33
97	312-324	EERLRHSRN PATR	11	32.90	0.39	31.09	0.27	31.14	0.27
98	319-338	RNPATRGLP STASGRLRG TQ	17	19.75	1.91	23.21	1.48	23.51	1.62
99	325-334	GLPSTASGR L	8	4.18	1.67	2.39	0.68	2.46	0.92
100	337-350	TQEVAPPTP LTPTS	9	10.83	0.70	13.18	0.04	15.60	0.04
101	351-357	HTANTSP	5	12.77	2.70	16.86	3.35	15.76	1.47
102	362-375	GMERERKVS MRLHR	13	11.39	0.79	15.54	2.09	18.06	2.50
103	364-371	ERERKVSM	7	41.49	0.52	38.16	0.52	42.98	0.52
104	364-378	ERERKVSMR LHRGAP	13	46.25	0.44	53.26	2.30	55.45	3.34
105	365-373	RERKVSMRL	8	21.76	1.07	27.34	0.54	33.21	1.92
106	371-394	MRLHRGAPV NISSSDLTGR QDTSR	22	29.71	0.07	31.35	0.07	30.96	0.07
107	379-395	VNISSSDLTG RQDTSRM	16	33.09	0.40	32.85	0.39	34.24	0.39
108	386-394	LTGRQDTSR	8	52.25	1.57	58.39	0.37	56.97	0.58
109	387-395	TGRQDTSRM	8	26.16	0.26	31.14	0.26	26.89	0.26
110	394-402	RMSTSQIPG	7	10.82	1.42	12.16	1.05	10.50	0.48

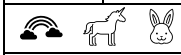
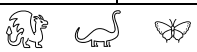
				CK1δ1 + AMPPNP					
				Relative Fractional Deuterium Uptake (%)					
				1 min		**5 min**		**10 min**	
S.No.	**Peptide**	**Sequence**	**Num Exchangers**	**Uptake**	**± SD**	**Uptake**	**± SD**	**Uptake**	**± SD**
1	3-20	LRVGNRYRL GRKIGSGSF	17	39.54	0.38	44.84	0.21	44.14	0.69
2	4-20	RVGNRYRLG RKIGSGSF	16	33.89	0.88	38.86	0.37	39.08	0.81
3	4-22	RVGNRYRLG RKIGSGSFG D	18	32.64	0.34	37.30	0.35	38.02	0.82

4	9-28	YRLGRKIGSGSFGDIYLGTD	19	38.83	0.05	40.56	0.20	39.43	0.40
5	12-25	GRKIGSGSFGDIYL	13	27.40	1.22	34.38	0.33	34.28	1.00
6	21-28	GDIYLGTD	7	13.99	0.42	28.78	0.52	35.65	0.59
7	22-28	DIYLGTD	6	11.00	2.67	24.75	2.16	30.11	0.59
8	26-33	GTDIAAGE	7	40.82	0.51	42.45	1.40	42.02	1.23
9	26-36	GTDIAAGEEVA	10	37.65	0.52	39.21	0.91	39.66	0.16
10	28-34	DIAAGEE	6	41.17	0.62	42.74	0.79	41.95	0.87
11	28-36	DIAAGEEVA	8	34.84	0.59	37.76	0.30	37.05	0.54
12	29-36	IAAGEEVA	7	30.13	0.63	31.89	0.37	32.13	0.44
13	37-49	IKLECVKTKHPQL	11	18.26	1.67	16.94	2.65	25.65	0.74
14	40-49	ECVKTKHPQL	8	25.31	1.86	24.50	1.54	21.10	1.26
15	42-49	VKTKHPQL	6	22.78	0.64	22.44	0.72	22.70	0.80
16	50-56	HIESKIY	6	15.21	0.82	21.44	0.75	23.69	0.59
17	50-58	HIESKIYKM	8	20.91	1.61	31.72	0.77	32.26	1.64
18	56-70	YKMMQGGVGIPTIRW	13	29.88	0.25	39.68	0.25	36.81	0.25
19	56-72	YKMMQGGVGIPTIRWCG	15	28.37	2.19	32.03	2.16	29.90	0.62
20	56-77	YKMMQGGVGIPTIRWCGAEGDY	20	25.90	1.15	27.70	1.79	25.93	0.43
21	59-76	MQGGVGIPTIRWCGAEGD	16	32.71	1.85	32.82	1.54	31.73	1.20
22	62-70	GVGIPTIRW	7	22.77	0.46	27.52	1.42	28.70	0.46
23	77-99	YNVMVMELLGPSLEDLFNFCSRK	21	45.06	0.33	47.56	0.24	46.44	0.13
24	83-89	ELLGPSL	5	6.25	0.67	9.55	0.60	12.09	0.52
25	85-92	LGPSLEDL	6	6.76	2.46	7.29	2.43	5.51	0.89
26	96-102	CSRKFSL	6	9.84	0.10	14.20	0.10	16.42	0.63
27	96-104	CSRKFSLKT	8	22.74	0.83	22.87	2.52	31.30	0.41
28	96-106	CSRKFSLKTVL	10	8.98	0.86	10.42	0.21	11.47	0.22
29	104-119	TVLLLADQMISRIEYI	15	21.24	0.78	28.81	0.27	29.51	0.32
30	108-117	LADQMISRIE	9	32.43	1.14	NaN	NaN	40.47	2.37
31	113-123	ISRIEYIHSKN	10	2.78	0.16	3.17	0.23	3.65	0.25
32	124-134	FIHRDVKPDNF	9	4.09	0.32	4.65	0.37	5.20	0.26
33	124-135	FIHRDVKPDNFL	10	3.63	0.09	3.32	0.29	4.51	0.01
34	126-134	HRDVKPDNF	7	3.22	0.50	3.45	0.32	4.60	0.32
35	135-143	LMGLGKKGN	8	20.94	1.61	25.36	0.90	26.11	1.37
36	135-144	LMGLGKKGNL	9	20.06	0.43	23.43	0.11	25.52	0.38
37	135-145	LMGLGKKGNLV	10	18.74	1.23	22.43	0.56	24.72	0.45

38	135-146	LMGLGKKGNLVY	11	15.13	0.25	17.83	0.07	19.69	0.16
39	136-144	MGLGKKGNL	8	23.13	1.00	27.55	0.25	27.79	0.62
40	137-144	GLGKKGNL	7	21.75	0.54	26.10	0.22	27.52	0.18
41	137-146	GLGKKGNLVY	9	17.73	0.70	20.77	0.06	23.71	0.08
42	140-150	KKGNLVYIIDF	10	7.24	0.00	1.52	0.14	1.08	0.10
43	144-162	LVYIIDFGLAKKYRDARTH	18	9.27	0.19	22.30	0.19	29.40	0.58
44	145-151	VYIIDFG	6	6.24	0.66	16.58	0.69	21.92	1.48
45	145-152	VYIIDFGL	7	5.79	0.51	18.38	0.37	22.58	0.36
46	145-158	VYIIDFGLAKKYRD	13	9.66	0.07	11.28	0.07	11.54	0.11
47	150-169	FGLAKKYRDARTHQHIPYRE	18	19.99	0.58	22.38	0.28	21.74	0.52
48	151-169	GLAKKYRDARTHQHIPYRE	17	24.21	0.51	26.72	0.84	26.61	1.30
49	153-179	AKKYRDARTHQHIPYRENKNLTGTARY	25	28.55	1.34	29.66	1.12	30.23	0.77
50	163-178	QHIPYRENKNLTGTAR	14	22.32	0.36	29.23	0.31	30.39	0.25
51	164-178	HIPYRENKNLTGTAR	13	11.76	0.05	13.95	1.55	13.92	0.28
52	176-186	TARYASINTHL	10	2.69	0.56	2.60	0.52	2.98	0.63
53	178-186	RYASINTHL	8	14.39	0.28	19.78	2.60	27.17	0.45
54	180-186	ASINTHL	6	14.49	1.11	25.56	0.61	29.16	0.61
55	180-189	ASINTHLGIE	9	16.59	0.05	31.62	0.46	36.59	0.20
56	180-196	ASINTHLGIEQSRRDDL	16	9.94	0.97	17.84	0.51	19.97	0.57
57	182-196	INTHLGIEQSRRDDL	14	10.48	0.78	18.34	0.34	20.31	0.68
58	185-196	HLGIEQSRRDDL	11	7.25	0.44	12.47	0.72	13.45	0.67
59	187-196	GIEQSRRDDL	9	10.29	1.67	14.46	1.32	13.96	1.30
60	189-196	EQSRRDDL	7	12.04	1.34	15.46	1.03	15.47	1.07
61	190-196	QSRRDDL	6	12.53	1.03	16.89	1.15	16.94	0.67
62	204-217	MYFNLGSLPWQGLK	12	12.16	1.00	20.53	0.90	23.64	1.12
63	205-215	YFNLGSLPWQG	9	23.86	0.55	32.12	0.28	33.00	0.32
64	205-216	YFNLGSLPWQGL	10	27.33	0.75	34.70	0.42	36.08	0.42
65	205-218	YFNLGSLPWQGLKA	12	33.59	0.23	40.01	0.14	40.66	0.29
66	206-218	FNLGSLPWQGLKA	11	40.49	1.60	47.43	1.95	48.11	1.65
67	207-216	NLGSLPWQGL	8	34.35	1.02	39.13	2.05	44.43	1.73
68	207-218	NLGSLPWQGLKA	10	42.88	0.32	49.41	0.82	50.58	0.45
69	209-216	GSLPWQGL	6	42.53	1.59	47.43	1.84	48.59	2.43

70	209-218	GSLPWQGLKA	8	41.35	0.45	44.69	0.92	45.41	0.38
71	212-218	PWQGLKA	5	49.17	1.76	52.07	0.81	52.71	0.49
72	219-230	ATKRQKYERISE	11	42.50	1.75	43.93	1.54	42.78	0.85
73	231-239	KKMSTPIEV	7	35.62	0.93	41.30	0.73	42.87	0.78
74	231-240	KKMSTPIEVL	8	27.46	0.91	32.97	0.55	34.36	0.48
75	239-248	VLCKGYPSEF	8	14.07	0.99	18.86	0.77	22.06	0.70
76	241-247	CKGYPSE	5	23.82	0.74	29.42	0.71	34.15	0.67
77	241-248	CKGYPSEF	6	14.63	0.68	18.50	0.43	21.69	0.52
78	248-254	FATYLNF	6	1.31	0.50	0.71	0.58	1.02	0.42
79	255-267	CRSLRFDDKPDYS	11	24.69	0.81	27.44	0.41	28.08	0.37
80	255-268	CRSLRFDDKPDYSY	12	29.53	0.25	33.69	0.37	33.09	1.38
81	255-269	CRSLRFDDKPDYSYL	13	24.74	0.85	27.17	0.69	27.22	0.68
82	259-276	RFDDKPDYSYLRQLFRNL	16	21.48	0.04	33.67	0.04	36.20	0.62
83	261-269	DDKPDYSYL	7	30.77	1.54	31.70	0.45	30.85	0.52
84	269-277	LRQLFRNLF	8	3.03	0.71	5.93	0.55	9.07	0.59
85	269-281	LRQLFRNLFHRQG	12	18.87	0.64	20.52	0.54	20.74	0.63
86	269-285	LRQLFRNLFHRQGFSYD	16	14.96	0.81	17.63	0.59	18.05	0.67
87	270-276	RQLFRNL	6	15.53	1.07	28.61	2.29	34.20	1.39
88	270-277	RQLFRNLF	7	3.89	0.35	6.76	0.42	8.73	0.22
89	270-285	RQLFRNLFHRQGFSYD	15	14.63	0.33	17.23	0.47	17.24	0.50
90	273-285	FRNLFHRQGFSYD	12	18.57	1.03	19.90	0.13	20.72	0.34
91	278-285	HRQGFSYD	7	27.18	2.01	28.58	0.54	29.03	1.29
92	286-292	YVFDWNM	6	36.56	1.01	42.15	0.62	42.00	0.56
93	293-302	LKFGASRAAD	9	38.06	1.41	40.53	1.21	39.37	2.78
94	293-305	LKFGASRAADDAE	12	37.30	0.87	38.31	0.74	37.67	0.87
95	294-302	KFGASRAAD	8	39.45	1.22	41.25	1.43	38.37	0.37
96	303-312	DAERERRDRE	9	37.29	0.64	37.83	2.19	37.97	0.90
97	312-324	EERLRHSRNPATR	11	36.70	0.89	37.93	0.46	38.00	0.60
98	319-338	RNPATRGLPSTASGRLRGTQ	17	22.19	0.90	25.61	1.56	36.39	2.88
99	325-334	GLPSTASGRL	8	5.55	0.81	10.79	0.80	13.60	0.31
100	337-350	TQEVAPPTPLTPTS	9	12.00	0.04	22.87	0.04	27.81	1.29
101	351-357	HTANTSP	5	21.57	0.67	22.81	0.49	22.58	0.76
102	362-375	GMERERKVSMRLHR	13	16.38	1.32	25.04	0.59	29.12	1.79
103	364-371	ERERKVSM	7	43.22	0.76	44.42	1.09	47.63	4.98

104	364-378	ERERKVSMRLHRGAP	13	46.96	0.51	51.23	0.86	52.62	0.32
105	365-373	RERKVSMRL	8	36.68	1.04	34.52	3.63	33.88	2.25
106	371-394	MRLHRGAPVNISSSDLTGRQDTSR	22	33.96	0.07	37.10	0.07	32.62	0.07
107	379-395	VNISSSDLTGRQDTSRM	16	35.12	0.99	34.53	0.10	35.04	0.05
108	386-394	LTGRQDTSR	8	59.13	0.41	62.93	0.94	62.18	0.59
109	387-395	TGRQDTSRM	8	29.72	0.78	33.00	1.15	32.61	1.62
110	394-402	RMSTSQIPG	7	10.88	0.50	11.28	0.29	10.85	0.45

				CK1δ2 Relative Deuterium Uptake (Da)					
				1 min		5 min		10 min	
S.No.	Peptide	Sequence	Num Exchangers	Uptake	± SD	Uptake	± SD	Uptake	± SD
1	3-20	LRVGNRYRLGRKIGSGSF	17	6.61	0.07	6.70	0.06	6.81	0.04
2	4-20	RVGNRYRLGRKIGSGSF	16	5.33	0.06	5.77	0.16	5.48	0.09
3	4-22	RVGNRYRLGRKIGSGSFGD	18	5.83	0.05	6.31	0.13	5.94	0.09
4	4-25	RVGNRYRLGRKIGSGSFGDIYL	21	5.74	0.06	6.73	0.18	6.90	0.11
5	12-25	GRKIGSGSFGDIYL	13	4.05	0.03	4.20	0.05	4.14	0.06
6	13-26	RKIGSGSFGDIYLG	13	1.24	0.10	1.72	0.05	1.70	0.06
7	21-28	GDIYLGTD	7	1.43	0.02	2.43	0.20	2.73	0.03
8	26-33	GTDIAAGE	7	2.87	0.04	2.93	0.07	2.74	0.03
9	26-34	GTDIAAGEE	8	3.51	0.03	3.50	0.08	3.26	0.02
10	28-34	DIAAGEE	6	2.55	0.03	2.49	0.06	2.36	0.04
11	35-41	VAIKLEC	6	0.83	0.02	1.09	0.09	1.63	0.01
12	40-49	ECVKTKHPQL	8	1.99	0.06	1.88	0.11	1.68	0.10
13	42-49	VKTKHPQL	6	1.80	0.06	1.76	0.09	1.62	0.07
14	50-56	HIESKIY	6	1.07	0.04	1.40	0.08	1.55	0.06
15	56-70	YKMMQGGVGIPTIRW	13	3.76	0.17	4.81	0.41	4.88	0.10
16	56-72	YKMMQGGVGIPTIRWCG	15	3.98	0.17	4.13	0.35	4.35	0.03
17	59-70	MQGGVGIPTIRW	10	3.61	0.17	4.11	0.27	4.21	0.19
18	83-89	ELLGPSL	5	0.48	0.03	0.63	0.02	0.70	0.03
19	85-92	LGPSLEDL	6	0.13	0.05	0.23	0.06	0.27	0.04
20	96-102	CSRKFSL	6	0.77	0.03	0.89	0.04	1.15	0.06
21	96-106	CSRKFSLKTVL	10	0.76	0.05	1.07	0.05	1.25	0.10
22	97-106	SRKFSLKTVL	9	0.72	0.13	1.05	0.07	1.14	0.07

23	104-119	TVLLLADQMISRIEYI	15	3.81	0.06	4.54	0.07	5.23	0.06
24	112-123	MISRIEYIHSKN	11	0.23	0.03	0.31	0.06	0.49	0.05
25	113-123	ISRIEYIHSKN	10	0.25	0.03	0.30	0.04	0.32	0.04
26	113-134	ISRIEYIHSKNFIHRDVKPDNF	20	0.53	0.05	0.56	0.07	0.63	0.06
27	115-132	RIEYIHSKNFIHRDVKPD	16	3.20	0.01	3.53	0.07	3.82	0.01
28	124-134	FIHRDVKPDNF	9	0.31	0.07	0.35	0.07	0.38	0.07
29	124-135	FIHRDVKPDNFL	10	0.27	0.05	0.33	0.05	0.39	0.05
30	126-134	HRDVKPDNF	7	0.24	0.03	0.28	0.04	0.31	0.02
31	126-135	HRDVKPDNFL	8	0.48	0.05	0.51	0.04	0.50	0.01
32	135-144	LMGLGKKGNL	9	1.87	0.03	2.17	0.07	2.25	0.13
33	135-145	LMGLGKKGNLV	10	1.87	0.01	2.16	0.09	2.12	0.02
34	135-146	LMGLGKKGNLVY	11	1.73	0.03	2.06	0.05	1.99	0.02
35	136-144	MGLGKKGNL	8	1.71	0.03	1.99	0.04	1.98	0.03
36	137-144	GLGKKGNL	7	1.59	0.02	1.86	0.07	1.78	0.04
37	137-146	GLGKKGNLVY	9	1.78	0.02	2.05	0.06	2.02	0.01
38	145-151	VYIIDFG	6	0.70	0.04	1.30	0.04	1.38	0.06
39	145-152	VYIIDFGL	7	0.95	0.06	1.63	0.07	1.79	0.04
40	145-158	VYIIDFGLAKKYRD	13	1.18	0.06	1.32	0.11	1.49	0.08
41	151-169	GLAKKYRDARTHQHIPYRE	17	4.16	0.09	4.01	0.18	3.60	0.03
42	163-178	QHIPYRENKNLTGTAR	14	3.16	0.12	4.17	0.08	5.17	0.06
43	173-179	LTGTARY	6	0.76	0.01	1.11	0.13	1.22	0.04
44	180-186	ASINTHL	6	0.68	0.06	1.32	0.06	1.52	0.04
45	180-189	ASINTHLGIE	9	1.16	0.03	2.22	0.30	2.89	0.02
46	180-196	ASINTHLGIEQSRRDDL	16	1.37	0.08	2.32	0.25	2.86	0.10
47	183-196	NTHLGIEQSRRDDL	13	1.05	0.03	1.66	0.12	1.82	0.05
48	185-196	HLGIEQSRRDDL	11	0.86	0.01	1.30	0.09	1.35	0.04
49	189-196	EQSRRDDL	7	0.86	0.06	1.13	0.07	1.13	0.06
50	204-218	MYFNLGSLPWQGLKA	13	4.64	0.04	5.22	0.12	5.17	0.04
51	205-215	YFNLGSLPWQG	9	2.15	0.06	2.61	0.17	2.82	0.04
52	205-216	YFNLGSLPWQGL	10	2.62	0.07	3.10	0.21	2.93	0.07
53	205-218	YFNLGSLPWQGLKA	12	4.07	0.05	4.49	0.20	4.44	0.11
54	206-218	FNLGSLPWQGLKA	11	4.53	0.02	5.02	0.16	4.96	0.07
55	207-216	NLGSLPWQGL	8	2.55	0.07	3.07	0.15	2.89	0.14

56	207-218	NLGSLPWQGLKA	10	4.34	0.04	4.73	0.21	4.73	0.24
57	209-216	GSLPWQGL	6	2.47	0.07	2.59	0.11	2.55	0.12
58	209-218	GSLPWQGLKA	8	3.36	0.08	3.45	0.13	3.23	0.06
59	212-218	PWQGLKA	5	2.45	0.04	2.58	0.09	2.43	0.01
60	219-230	ATKRQKYERISE	11	4.55	0.09	4.49	0.20	4.23	0.05
61	220-230	TKRQKYERISE	10	4.07	0.17	4.62	0.03	4.22	0.03
62	231-238	KKMSTPIE	6	2.38	0.03	2.44	0.09	2.34	0.08
63	231-240	KKMSTPIEVL	8	2.13	0.02	2.47	0.08	2.51	0.05
64	236-250	PIEVLCKGYPSEFAT	12	1.20	0.05	2.04	0.20	2.55	0.16
65	239-248	VLCKGYPSEF	8	1.01	0.05	1.39	0.05	1.60	0.08
66	241-248	CKGYPSEF	6	0.84	0.02	1.05	0.03	1.15	0.02
67	248-254	FATYLNF	6	0.20	0.03	0.23	0.03	0.18	0.02
68	255-269	CRSLRFDDKPDYSYL	13	3.27	0.03	3.42	0.09	3.39	0.05
69	261-268	DDKPDYSY	6	2.26	0.04	2.26	0.09	2.15	0.06
70	261-269	DDKPDYSYL	7	2.15	0.07	2.11	0.10	1.92	0.07
71	268-277	YLRQLFRNLF	9	0.30	0.16	0.55	0.15	0.79	0.13
72	269-275	LRQLFRN	6	1.19	0.08	1.94	0.03	2.40	0.09
73	269-277	LRQLFRNLF	8	0.24	0.03	0.50	0.02	0.75	0.04
74	269-285	LRQLFRNLFHRQGFSYD	16	2.45	0.04	2.74	0.09	2.64	0.04
75	270-276	RQLFRNL	6	0.12	0.03	0.40	0.02	0.57	0.03
76	270-277	RQLFRNLF	7	0.25	0.06	0.48	0.07	0.62	0.06
77	270-285	RQLFRNLFHRQGFSYD	15	2.20	0.05	2.52	0.02	2.37	0.03
78	276-295	LFHRQGFSYDYVFDWNMLKF	19	8.96	0.04	9.15	0.09	9.03	0.01
79	278-285	HRQGFSYD	7	1.96	0.09	1.92	0.13	1.72	0.10
80	286-292	YVFDWNM	6	2.61	0.01	2.74	0.07	2.40	0.02
81	290-298	WNMLKFGAS	8	3.79	0.40	4.06	0.24	4.16	0.17
82	293-302	LKFGASRAAD	9	3.36	0.12	3.26	0.14	3.05	0.12
83	293-305	LKFGASRAADDAE	12	4.46	0.09	4.40	0.14	3.95	0.08
84	300-312	AADDAERERRDRE	12	3.05	0.07	3.34	0.06	3.20	0.06
85	315-325	LRHSRNPATRG	9	0.47	0.01	0.50	0.02	0.44	0.01
86	316-326	RHSRNPATRGL	9	0.48	0.04	0.57	0.04	0.62	0.03
87	325-334	GLPSTASGRL	8	0.73	0.05	1.26	0.05	1.60	0.06
88	337-350	TQEVAPPTPLTPTS	9	1.05	0.04	1.54	0.03	2.11	0.13
89	345-367	PLTPTSHTANTSPRPVSGMERER	18	6.92	0.04	6.75	0.17	6.48	0.19

90	348-354	PTSHTAN	5	0.77	0.02	0.78	0.03	0.69	0.02
91	351-357	HTANTSP	5	1.11	0.02	1.10	0.03	0.94	0.01
92	363-371	MERERKVSM	8	3.74	0.14	3.93	0.17	3.85	0.13
93	363-376	MERERKVSM RLHRG	13	2.15	0.01	3.33	0.03	4.24	0.01
94	365-371	RERKVSM	6	2.31	0.12	2.35	0.10	2.20	0.04
95	372-386	RLHRGAPVNI SSSDL	13	4.20	0.08	4.20	0.06	3.67	0.08
96	379-389	VNISSSDLTG R	10	1.37	0.05	2.25	0.03	2.57	0.02
97	379-395	VNISSSDLTG RQDTSRM	16	5.34	0.05	5.15	0.04	4.87	0.03
98	386-394	LTGRQDTSR	8	4.62	0.06	4.89	0.05	4.89	0.05
99	398-405	SQNSIPFE	6	1.48	0.11	1.49	0.15	1.31	0.11
				CK1δ2 + ATP					
				Relative Deuterium Uptake (Da)					
				1 min		**5 min**		**10 min**	
S.No.	**Peptide**	**Sequence**	**Num Exchangers**	**Uptake**	**± SD**	**Uptake**	**± SD**	**Uptake**	**± SD**
1	3-20	LRVGNRYRL GRKIGSGSF	17	5.39	0.04	6.08	0.18	6.11	0.04
2	4-20	RVGNRYRLG RKIGSGSF	16	4.38	0.04	5.00	0.21	5.00	0.04
3	4-22	RVGNRYRLG RKIGSGSFG D	18	5.05	0.01	5.26	0.28	5.00	0.01
4	4-25	RVGNRYRLG RKIGSGSFG DIYL	21	4.68	0.03	5.45	0.22	5.48	0.03
5	12-25	GRKIGSGSF GDIYL	13	2.77	0.03	3.11	0.03	3.23	0.03
6	13-26	RKIGSGSFG DIYLG	13	1.29	0.04	1.64	0.09	2.21	0.04
7	21-28	GDIYLGTD	7	0.44	0.02	0.89	0.02	1.03	0.02
8	26-33	GTDIAAGE	7	2.77	0.03	2.86	0.03	2.81	0.03
9	26-34	GTDIAAGEE	8	3.31	0.02	3.42	0.11	3.35	0.02
10	28-34	DIAAGEE	6	2.41	0.03	2.39	0.06	2.26	0.03
11	35-41	VAIKLEC	6	0.87	0.01	1.04	0.11	1.64	0.01
12	40-49	ECVKTKHPQ L	8	1.30	0.03	1.57	0.20	1.28	0.03
13	42-49	VKTKHPQL	6	1.38	0.01	1.71	0.07	1.55	0.01
14	50-56	HIESKIY	6	0.70	0.04	0.87	0.07	0.94	0.04
15	56-70	YKMMQGGV GIPTIRW	13	3.01	0.05	3.67	0.14	3.39	0.05
16	56-72	YKMMQGGV GIPTIRWCG	15	NaN	NaN	NaN	NaN	NaN	NaN
17	59-70	MQGGVGIPTI RW	10	3.17	0.04	3.08	0.04	3.35	0.04
18	83-89	ELLGPSL	5	0.17	0.01	0.20	0.03	0.10	0.01
19	85-92	LGPSLEDL	6	0.13	0.04	0.15	0.05	0.37	0.04
20	96-102	CSRKFSL	6	0.78	0.01	0.83	0.01	0.81	0.01

21	96-106	CSRKFSLKTVL	10	0.78	0.04	0.98	0.05	1.05	0.04
22	97-106	SRKFSLKTVL	9	0.81	0.06	0.79	0.06	1.23	0.06
23	104-119	TVLLLADQMISRIEYI	15	3.42	0.01	4.31	0.08	4.03	0.01
24	112-123	MISRIEYIHSKN	11	0.09	0.03	0.24	0.05	0.34	0.02
25	113-123	ISRIEYIHSKN	10	NaN	NaN	NaN	NaN	NaN	NaN
26	113-134	ISRIEYIHSKNFIHRDVKPDNF	20	0.40	0.05	0.52	0.08	0.54	0.05
27	115-132	RIEYIHSKNFIHRDVKPD	16	4.11	0.01	4.60	0.03	5.03	0.01
28	124-134	FIHRDVKPDNF	9	0.33	0.09	0.40	0.09	0.34	0.09
29	124-135	FIHRDVKPDNFL	10	0.33	0.04	0.40	0.05	0.45	0.04
30	126-134	HRDVKPDNF	7	0.16	0.01	0.33	0.02	0.20	0.01
31	126-135	HRDVKPDNFL	8	0.64	0.01	0.42	0.01	0.41	0.01
32	135-144	LMGLGKKGNL	9	1.62	0.01	1.86	0.07	2.09	0.01
33	135-145	LMGLGKKGNLV	10	1.59	0.00	1.83	0.04	2.07	0.00
34	135-146	LMGLGKKGNLVY	11	1.40	0.04	1.74	0.09	1.91	0.04
35	136-144	MGLGKKGNL	8	1.59	0.02	1.48	0.05	1.81	0.02
36	137-144	GLGKKGNL	7	1.35	0.02	1.50	0.03	1.51	0.02
37	137-146	GLGKKGNLVY	9	1.63	0.01	1.80	0.12	1.98	0.01
38	145-151	VYIIDFG	6	0.14	0.03	0.18	0.06	0.07	0.03
39	145-152	VYIIDFGL	7	0.11	0.04	0.21	0.04	0.58	0.04
40	145-158	VYIIDFGLAKKYRD	13	0.91	0.06	1.24	0.06	1.28	0.06
41	151-169	GLAKKYRDARTHQHIPYRE	17	2.85	0.03	3.39	0.12	3.18	0.03
42	163-178	QHIPYRENKNLTGTAR	14	3.14	0.04	3.96	0.04	4.44	0.04
43	173-179	LTGTARY	6	1.01	0.01	1.22	0.02	1.22	0.01
44	180-186	ASINTHL	6	0.69	0.03	1.12	0.03	1.25	0.03
45	180-189	ASINTHLGIE	9	1.18	0.02	2.00	0.11	2.57	0.02
46	180-196	ASINTHLGIEQSRRDDL	16	1.48	0.07	2.21	0.07	2.35	0.07
47	183-196	NTHLGIEQSRRDDL	13	0.97	0.02	1.58	0.06	1.60	0.02
48	185-196	HLGIEQSRRDDL	11	1.27	0.01	1.24	0.01	NaN	NaN
49	189-196	EQSRRDDL	7	0.80	0.06	1.01	0.06	0.88	0.06
50	204-218	MYFNLGSLPWQGLKA	13	3.80	0.01	4.22	0.01	4.00	0.01
51	205-215	YFNLGSLPWQG	9	1.42	0.04	1.89	0.04	2.28	0.04
52	205-216	YFNLGSLPWQGL	10	1.80	0.05	2.58	0.18	2.30	0.05
53	205-218	YFNLGSLPWQGLKA	12	3.40	0.05	4.00	0.15	4.03	0.05

54	206-218	FNLGSLPWQGLKA	11	4.01	0.01	4.61	0.13	4.61	0.01
55	207-216	NLGSLPWQGL	8	1.90	0.05	2.56	0.08	2.31	0.05
56	207-218	NLGSLPWQGLKA	10	3.96	0.03	4.65	0.03	4.42	0.03
57	209-216	GSLPWQGL	6	2.22	0.04	2.50	0.08	2.21	0.07
58	209-218	GSLPWQGLKA	8	3.00	0.05	3.20	0.10	3.13	0.05
59	212-218	PWQGLKA	5	2.25	0.01	2.35	0.01	2.24	0.01
60	219-230	ATKRQKYERISE	11	3.74	0.05	4.04	0.25	3.84	0.05
61	220-230	TKRQKYERISE	10	3.87	0.02	4.38	0.11	NaN	NaN
62	231-238	KKMSTPIE	6	NaN	NaN	NaN	NaN	NaN	NaN
63	231-240	KKMSTPIEVL	8	2.13	0.02	2.45	0.06	2.58	0.02
64	236-250	PIEVLCKGYPSEFAT	12	1.39	0.01	2.31	0.01	3.18	0.01
65	239-248	VLCKGYPSEF	8	0.87	0.04	1.45	0.08	1.63	0.06
66	241-248	CKGYPSEF	6	0.80	0.03	1.02	0.03	1.21	0.02
67	248-254	FATYLNF	6	0.16	0.02	0.23	0.04	0.19	0.02
68	255-269	CRSLRFDDKPDYSYL	13	2.99	0.01	3.27	0.10	3.17	0.01
69	261-268	DDKPDYSY	6	2.04	0.03	2.09	0.12	2.03	0.03
70	261-269	DDKPDYSYL	7	1.69	0.06	1.85	0.18	1.53	0.06
71	268-277	YLRQLFRNLF	9	0.16	0.09	0.34	0.16	0.32	0.09
72	269-275	LRQLFRN	6	1.46	0.01	2.06	0.03	2.27	0.01
73	269-277	LRQLFRNLF	8	0.23	0.03	0.51	0.04	0.58	0.03
74	269-285	LRQLFRNLFHRQGFSYD	16	1.98	0.04	2.35	0.11	2.30	0.04
75	270-276	RQLFRNL	6	0.08	0.02	0.42	0.03	0.57	0.02
76	270-277	RQLFRNLF	7	0.20	0.07	0.50	0.07	0.54	0.07
77	270-285	RQLFRNLFHRQGFSYD	15	1.90	0.02	2.13	0.06	2.18	0.02
78	276-295	LFHRQGFSYDYVFDWNMLKF	19	8.52	0.01	8.92	0.11	8.93	0.01
79	278-285	HRQGFSYD	7	1.60	0.07	1.89	0.07	1.91	0.07
80	286-292	YVFDWNM	6	1.96	0.01	2.15	0.15	2.10	0.01
81	290-298	WNMLKFGAS	8	3.52	0.17	3.79	0.17	NaN	NaN
82	293-302	LKFGASRAAD	9	3.01	0.11	3.17	0.19	2.84	0.12
83	293-305	LKFGASRAADDAE	12	3.94	0.04	4.08	0.23	3.93	0.04
84	300-312	AADDAERERRDRE	12	2.86	0.02	3.24	0.10	2.94	0.02
85	315-325	LRHSRNPATRG	9	0.50	0.01	0.75	0.06	0.67	0.01
86	316-326	RHSRNPATRGL	9	0.87	0.02	0.95	0.03	0.82	0.02
87	325-334	GLPSTASGRL	8	0.18	0.02	0.28	0.11	0.11	0.02

88	337-350	TQEVAPPTP LTPTS	9	0.90	0.03	1.33	0.03	1.44	0.03
89	345-367	PLTPTSHTAN TSPRPVSGM ERER	18	6.13	0.04	5.95	0.13	5.58	0.04
90	348-354	PTSHTAN	5	0.85	0.01	0.87	0.01	0.84	0.01
91	351-357	HTANTSP	5	0.92	0.01	0.92	0.06	0.74	0.01
92	363-371	MERERKVSM	8	3.32	0.06	3.28	0.09	3.24	0.06
93	363-376	MERERKVSM RLHRG	13	1.80	0.01	2.91	0.01	2.93	0.01
94	365-371	RERKVSM	6	1.98	0.02	2.23	0.08	2.14	0.02
95	372-386	RLHRGAPVNI SSSDL	13	3.66	0.06	3.99	0.25	4.33	0.06
96	379-389	VNISSSDLTG R	10	1.08	0.07	1.67	0.10	1.93	0.07
97	379-395	VNISSSDLTG RQDTSRM	16	5.49	0.02	5.13	0.08	5.14	0.02
98	386-394	LTGRQDTSR	8	4.40	0.04	4.67	0.05	4.73	0.04
99	398-405	SQNSIPFE	6	1.34	0.00	1.39	0.02	1.06	0.00

				CK1δ2 + AMPPNP					
				Relative Deuterium Uptake (Da)					
				1 min		**5 min**		**10 min**	
S.No.	**Peptide**	**Sequence**	**Num Exchangers**	**Uptake**	**± SD**	**Uptake**	**± SD**	**Uptake**	**± SD**
1	3-20	LRVGNRYRL GRKIGSGSF	17	6.66	0.14	7.19	0.08	7.36	0.05
2	4-20	RVGNRYRLG RKIGSGSF	16	5.23	0.05	5.83	0.08	6.06	0.12
3	4-22	RVGNRYRLG RKIGSGSFG D	18	5.62	0.10	6.39	0.03	6.56	0.15
4	4-25	RVGNRYRLG RKIGSGSFG DIYL	21	5.36	0.21	6.74	0.08	7.49	0.17
5	12-25	GRKIGSGSF GDIYL	13	3.44	0.04	4.12	0.07	4.36	0.19
6	13-26	RKIGSGSFG DIYLG	13	1.31	0.06	1.66	0.06	1.87	0.16
7	21-28	GDIYLGTD	7	0.88	0.05	1.92	0.05	2.37	0.11
8	26-33	GTDIAAGE	7	2.83	0.03	2.89	0.05	2.82	0.09
9	26-34	GTDIAAGEE	8	3.40	0.10	3.42	0.08	3.41	0.12
10	28-34	DIAAGEE	6	2.45	0.03	2.48	0.04	2.40	0.09
11	35-41	VAIKLEC	6	0.73	0.06	1.01	0.12	1.56	0.01
12	40-49	ECVKTKHPQ L	8	1.63	0.03	1.75	0.06	1.64	0.08
13	42-49	VKTKHPQL	6	1.66	0.05	1.70	0.07	1.65	0.12
14	50-56	HIESKIY	6	0.94	0.10	1.24	0.07	1.36	0.09
15	56-70	YKMMQGGV GIPTIRW	13	3.45	0.31	4.19	0.21	4.57	0.21
16	56-72	YKMMQGGV GIPTIRWCG	15	3.20	0.18	3.50	0.08	3.07	0.25
17	59-70	MQGGVGIPTI RW	10	3.39	0.27	3.67	0.22	4.04	0.18

18	83-89	ELLGPSL	5	0.32	0.02	0.46	0.03	0.49	0.03
19	85-92	LGPSLEDL	6	0.19	0.06	0.19	0.04	0.18	0.07
20	96-102	CSRKFSL	6	0.78	0.09	0.82	0.01	0.78	0.09
21	96-106	CSRKFSLKTVL	10	0.83	0.13	1.05	0.08	1.02	0.12
22	97-106	SRKFSLKTVL	9	0.72	0.12	0.98	0.09	0.94	0.12
23	104-119	TVLLLADQMISRIEYI	15	3.15	0.03	4.10	0.05	4.37	0.05
24	112-123	MISRIEYIHSKN	11	0.24	0.04	0.34	0.08	0.44	0.06
25	113-123	ISRIEYIHSKN	10	0.19	0.03	0.23	0.04	0.23	0.03
26	113-134	ISRIEYIHSKNFIHRDVKPDNF	20	0.44	0.06	0.51	0.04	0.56	0.05
27	115-132	RIEYIHSKNFIHRDVKPD	16	2.99	0.07	3.84	0.05	4.43	0.07
28	124-134	FIHRDVKPDNF	9	0.24	0.10	0.35	0.08	0.37	0.08
29	124-135	FIHRDVKPDNFL	10	0.26	0.05	0.33	0.05	0.37	0.07
30	126-134	HRDVKPDNF	7	0.23	0.04	0.25	0.06	0.25	0.02
31	126-135	HRDVKPDNFL	8	0.49	0.07	0.52	0.02	0.52	0.04
32	135-144	LMGLGKKGNL	9	1.72	0.04	2.01	0.03	2.13	0.10
33	135-145	LMGLGKKGNLV	10	1.68	0.06	2.01	0.05	2.16	0.09
34	135-146	LMGLGKKGNLVY	11	1.61	0.04	1.87	0.04	2.04	0.13
35	136-144	MGLGKKGNL	8	1.61	0.05	1.87	0.03	2.01	0.12
36	137-144	GLGKKGNL	7	1.45	0.04	1.69	0.05	1.82	0.07
37	137-146	GLGKKGNLVY	9	1.58	0.01	1.90	0.04	2.02	0.13
38	145-151	VYIIDFG	6	0.43	0.05	1.06	0.09	1.14	0.06
39	145-152	VYIIDFGL	7	0.49	0.05	1.16	0.04	1.71	0.08
40	145-158	VYIIDFGLAKKYRD	13	1.20	0.08	1.52	0.06	1.55	0.09
41	151-169	GLAKKYRDARTHQHIPYRE	17	3.07	0.17	3.36	0.08	3.46	0.37
42	163-178	QHIPYRENKNLTGTAR	14	3.09	0.09	3.91	0.05	4.29	0.16
43	173-179	LTGTARY	6	0.84	0.12	1.16	0.04	1.23	0.12
44	180-186	ASINTHL	6	0.77	0.06	1.38	0.05	1.62	0.10
45	180-189	ASINTHLGIE	9	1.35	0.03	2.53	0.04	3.00	0.05
46	180-196	ASINTHLGIEQSRRDDL	16	1.48	0.10	2.59	0.07	2.90	0.16
47	183-196	NTHLGIEQSRRDDL	13	1.03	0.05	1.74	0.03	1.92	0.05
48	185-196	HLGIEQSRRDDL	11	0.89	0.02	1.28	0.02	1.41	0.07
49	189-196	EQSRRDDL	7	0.74	0.11	1.10	0.08	1.09	0.09
50	204-218	MYFNLGSLPWQGLKA	13	4.44	0.01	5.06	0.09	5.34	0.23

51	205-215	YFNLGSLPWQG	9	2.02	0.10	2.59	0.12	2.81	0.13
52	205-216	YFNLGSLPWQGL	10	2.39	0.16	2.99	0.10	3.12	0.21
53	205-218	YFNLGSLPWQGLKA	12	3.85	0.10	4.41	0.07	4.56	0.23
54	206-218	FNLGSLPWQGLKA	11	4.32	0.07	4.92	0.05	5.06	0.32
55	207-216	NLGSLPWQGL	8	2.42	0.16	3.06	0.10	2.70	0.07
56	207-218	NLGSLPWQGLKA	10	4.13	0.05	4.62	0.06	4.92	0.14
57	209-216	GSLPWQGL	6	2.31	0.11	2.52	0.09	2.68	0.11
58	209-218	GSLPWQGLKA	8	2.96	0.05	3.27	0.14	3.38	0.20
59	212-218	PWQGLKA	5	2.31	0.13	2.46	0.08	2.59	0.12
60	219-230	ATKRQKYERISE	11	4.10	0.19	4.23	0.13	4.23	0.23
61	220-230	TKRQKYERISE	10	4.07	0.04	4.22	0.05	3.91	0.06
62	231-238	KKMSTPIE	6	2.30	0.06	2.39	0.03	2.41	0.11
63	231-240	KKMSTPIEVL	8	2.11	0.06	2.48	0.03	2.61	0.15
64	236-250	PIEVLCKGYPSEFAT	12	1.24	0.01	2.23	0.01	2.71	0.12
65	239-248	VLCKGYPSEF	8	0.93	0.05	1.32	0.10	1.65	0.09
66	241-248	CKGYPSEF	6	0.79	0.03	1.02	0.03	1.23	0.05
67	248-254	FATYLNF	6	0.03	0.03	0.04	0.03	0.07	0.03
68	255-269	CRSLRFDDKPDYSYL	13	3.23	0.07	3.45	0.02	3.49	0.16
69	261-268	DDKPDYSY	6	2.23	0.08	2.22	0.03	2.19	0.13
70	261-269	DDKPDYSYL	7	2.09	0.12	2.05	0.07	1.99	0.12
71	268-277	YLRQLFRNLF	9	0.20	0.13	0.51	0.13	0.70	0.13
72	269-275	LRQLFRN	6	1.25	0.05	1.94	0.11	2.20	0.08
73	269-277	LRQLFRNLF	8	0.20	0.04	0.52	0.03	0.63	0.04
74	269-285	LRQLFRNLFHRQGFSYD	16	2.40	0.09	2.75	0.06	2.81	0.04
75	270-276	RQLFRNL	6	0.14	0.04	0.38	0.02	0.55	0.05
76	270-277	RQLFRNLF	7	0.17	0.07	0.46	0.07	0.52	0.07
77	270-285	RQLFRNLFHRQGFSYD	15	2.19	0.05	2.51	0.02	2.54	0.16
78	276-295	LFHRQGFSYDYVFDWNMLKF	19	9.00	0.13	9.33	0.01	9.35	0.08
79	278-285	HRQGFSYD	7	1.89	0.12	1.89	0.10	1.79	0.16
80	286-292	YVFDWNM	6	2.65	0.07	2.90	0.02	2.84	0.21
81	290-298	WNMLKFGAS	8	3.99	0.38	3.96	0.24	4.36	0.29
82	293-302	LKFGASRAAD	9	3.16	0.14	3.18	0.13	3.05	0.30
83	293-305	LKFGASRAADDAE	12	4.21	0.20	4.20	0.13	4.42	0.06
84	300-312	AADDAERERRDRE	12	2.89	0.09	3.19	0.05	3.38	0.14

85	315-325	LRHSRNPATRG	9	0.41	0.03	0.43	0.02	0.42	0.01
86	316-326	RHSRNPATRGL	9	0.47	0.03	0.54	0.06	0.52	0.09
87	325-334	GLPSTASGRL	8	0.46	0.04	0.78	0.10	0.91	0.05
88	337-350	TQEVAPPTPLTPTS	9	1.11	0.10	1.94	0.03	2.51	0.03
89	345-367	PLTPTSHTANTSPRPVSGMERER	18	6.45	0.11	6.88	0.04	6.82	0.04
90	348-354	PTSHTAN	5	0.78	0.03	0.78	0.03	0.73	0.02
91	351-357	HTANTSP	5	1.04	0.05	1.04	0.02	1.03	0.08
92	363-371	MERERKVSM	8	3.59	0.07	3.87	0.11	3.88	0.14
93	363-376	MERERKVSMRLHRG	13	2.31	0.03	3.17	0.01	3.45	0.14
94	365-371	RERKVSM	6	2.23	0.23	2.17	0.04	2.16	0.11
95	372-386	RLHRGAPVNISSSDL	13	4.29	0.06	4.02	0.12	4.00	0.07
96	379-389	VNISSSDLTGR	10	1.37	0.10	2.06	0.10	2.26	0.17
97	379-395	VNISSSDLTGRQDTSRM	16	5.16	0.05	5.29	0.02	5.19	0.11
98	386-394	LTGRQDTSR	8	4.63	0.05	4.84	0.04	4.87	0.05
99	398-405	SQNSIPFE	6	1.18	0.13	1.23	0.05	1.40	0.04

				CK1δ2					
				Relative Fractional Deuterium Uptake (%)					
				1 min		**5 min**		**10 min**	
S.No.	**Peptide**	**Sequence**	**Num Exchangers**	**Uptake**	**± SD**	**Uptake**	**± SD**	**Uptake**	**± SD**
1	3-20	LRVGNRYRLGRKIGSGSF	17	38.85	0.40	39.40	0.34	40.03	0.21
2	4-20	RVGNRYRLGRKIGSGSF	16	33.32	0.38	36.04	0.97	34.25	0.54
3	4-22	RVGNRYRLGRKIGSGSFGD	18	32.40	0.29	35.05	0.71	33.00	0.53
4	4-25	RVGNRYRLGRKIGSGSFGDIYL	21	27.33	0.29	32.03	0.86	32.84	0.51
5	12-25	GRKIGSGSFGDIYL	13	31.14	0.24	32.32	0.40	31.85	0.44
6	13-26	RKIGSGSFGDIYLG	13	9.55	0.75	13.23	0.39	13.08	0.47
7	21-28	GDIYLGTD	7	20.45	0.36	34.77	2.88	38.94	0.45
8	26-33	GTDIAAGE	7	41.01	0.60	41.88	1.05	39.15	0.43
9	26-34	GTDIAAGEE	8	43.89	0.35	43.72	0.96	40.75	0.24
10	28-34	DIAAGEE	6	42.45	0.50	41.58	0.97	39.36	0.65
11	35-41	VAIKLEC	6	13.77	0.27	18.19	1.54	27.11	0.13
12	40-49	ECVKTKHPQL	8	24.86	0.77	23.50	1.33	20.98	1.25
13	42-49	VKTKHPQL	6	29.97	1.02	29.40	1.48	26.93	1.16
14	50-56	HIESKIY	6	17.88	0.70	23.27	1.35	25.89	0.98

15	56-70	YKMMQGGV GIPTIRW	13	28.91	1.32	36.99	3.12	37.50	0.80
16	56-72	YKMMQGGV GIPTIRWCG	15	26.56	1.12	27.50	2.32	28.99	0.20
17	59-70	MQGGVGIPTI RW	10	36.13	1.72	41.14	2.73	42.06	1.94
18	83-89	ELLGPSL	5	9.51	0.60	12.60	0.48	14.06	0.54
19	85-92	LGPSLEDL	6	2.16	0.82	3.90	1.03	4.48	0.67
20	96-102	CSRKFSL	6	12.76	0.56	14.88	0.64	19.20	0.95
21	96-106	CSRKFSLKT VL	10	7.56	0.46	10.68	0.52	12.49	1.03
22	97-106	SRKFSLKTVL	9	8.04	1.40	11.72	0.78	12.70	0.76
23	104-119	TVLLLADQMI SRIEYI	15	25.40	0.40	30.29	0.45	34.87	0.43
24	112-123	MISRIEYIHSK N	11	2.10	0.28	2.83	0.55	4.42	0.45
25	113-123	ISRIEYIHSKN	10	2.53	0.33	2.97	0.42	3.20	0.41
26	113-134	ISRIEYIHSKN FIHRDVKPDN F	20	2.64	0.24	2.81	0.34	3.15	0.30
27	115-132	RIEYIHSKNFI HRDVKPD	16	19.99	0.07	22.06	0.47	23.89	0.08
28	124-134	FIHRDVKPDN F	9	3.48	0.76	3.84	0.79	4.24	0.75
29	124-135	FIHRDVKPDN FL	10	2.66	0.54	3.30	0.54	3.90	0.46
30	126-134	HRDVKPDNF	7	3.43	0.37	4.05	0.56	4.38	0.29
31	126-135	HRDVKPDNF L	8	6.01	0.57	6.37	0.45	6.19	0.12
32	135-144	LMGLGKKGN L	9	20.76	0.39	24.06	0.83	25.02	1.46
33	135-145	LMGLGKKGN LV	10	18.70	0.14	21.64	0.85	21.16	0.15
34	135-146	LMGLGKKGN LVY	11	15.68	0.29	18.68	0.46	18.10	0.15
35	136-144	MGLGKKGNL	8	21.33	0.37	24.87	0.49	24.74	0.44
36	137-144	GLGKKGNL	7	22.72	0.22	26.51	0.98	25.48	0.52
37	137-146	GLGKKGNLV Y	9	19.74	0.20	22.77	0.63	22.45	0.10
38	145-151	VYIIDFG	6	11.64	0.61	21.63	0.66	22.95	1.08
39	145-152	VYIIDFGL	7	13.61	0.92	23.25	0.95	25.57	0.60
40	145-158	VYIIDFGLAK KYRD	13	9.08	0.47	10.15	0.84	11.47	0.65
41	151-169	GLAKKYRDA RTHQHIPYR E	17	24.47	0.53	23.60	1.07	21.20	0.19
42	163-178	QHIPYRENK NLTGTAR	14	22.58	0.84	29.77	0.59	36.91	0.43
43	173-179	LTGTARY	6	12.66	0.23	18.57	2.17	20.33	0.59
44	180-186	ASINTHL	6	11.41	1.07	21.92	1.03	25.32	0.61
45	180-189	ASINTHLGIE	9	12.86	0.30	24.70	3.34	32.11	0.19
46	180-196	ASINTHLGIE QSRRDDL	16	8.54	0.52	14.49	1.54	17.87	0.60
47	183-196	NTHLGIEQSR RDDL	13	8.10	0.25	12.75	0.92	14.02	0.38

48	185-196	HLGIEQSRRDDL	11	7.78	0.12	11.85	0.81	12.31	0.32
49	189-196	EQSRRDDL	7	12.30	0.92	16.09	1.02	16.17	0.93
50	204-218	MYFNLGSLPWQGLKA	13	35.67	0.29	40.13	0.89	39.74	0.33
51	205-215	YFNLGSLPWQG	9	23.93	0.64	29.01	1.84	31.33	0.49
52	205-216	YFNLGSLPWQGL	10	26.20	0.75	31.05	2.05	29.30	0.74
53	205-218	YFNLGSLPWQGLKA	12	33.90	0.40	37.44	1.63	36.97	0.89
54	206-218	FNLGSLPWQGLKA	11	41.20	0.15	45.60	1.49	45.13	0.68
55	207-216	NLGSLPWQGL	8	31.90	0.88	38.33	1.83	36.18	1.79
56	207-218	NLGSLPWQGLKA	10	43.41	0.36	47.25	2.11	47.26	2.39
57	209-216	GSLPWQGL	6	41.12	1.12	43.13	1.90	42.55	1.96
58	209-218	GSLPWQGLKA	8	41.96	0.99	43.13	1.68	40.37	0.70
59	212-218	PWQGLKA	5	48.98	0.90	51.61	1.78	48.54	0.24
60	219-230	ATKRQKYERISE	11	41.32	0.83	40.85	1.78	38.49	0.45
61	220-230	TKRQKYERISE	10	40.74	1.70	46.19	0.27	42.25	0.26
62	231-238	KKMSTPIE	6	39.75	0.51	40.60	1.47	39.00	1.25
63	231-240	KKMSTPIEVL	8	26.69	0.26	30.87	0.95	31.34	0.61
64	236-250	PIEVLCKGYPSEFAT	12	10.01	0.43	16.98	1.64	21.22	1.33
65	239-248	VLCKGYPSEF	8	12.67	0.59	17.32	0.63	19.94	1.06
66	241-248	CKGYPSEF	6	14.05	0.36	17.47	0.49	19.14	0.40
67	248-254	FATYLNF	6	3.26	0.43	3.82	0.52	3.01	0.40
68	255-269	CRSLRFDDKPDYSYL	13	25.14	0.22	26.30	0.66	26.09	0.41
69	261-268	DDKPDYSY	6	37.68	0.73	37.74	1.55	35.87	0.94
70	261-269	DDKPDYSYL	7	30.67	1.05	30.19	1.37	27.43	1.04
71	268-277	YLRQLFRNLF	9	3.38	1.73	6.14	1.66	8.72	1.49
72	269-275	LRQLFRN	6	19.83	1.33	32.36	0.46	39.95	1.48
73	269-277	LRQLFRNLF	8	2.97	0.42	6.23	0.31	9.34	0.53
74	269-285	LRQLFRNLFHRQGFSYD	16	15.34	0.25	17.10	0.59	16.48	0.22
75	270-276	RQLFRNL	6	1.99	0.56	6.64	0.40	9.45	0.44
76	270-277	RQLFRNLF	7	3.56	0.90	6.79	0.96	8.85	0.86
77	270-285	RQLFRNLFHRQGFSYD	15	14.64	0.36	16.77	0.13	15.80	0.17
78	276-295	LFHRQGFSYDYVFDWNMLKF	19	47.13	0.20	48.15	0.46	47.55	0.05
79	278-285	HRQGFSYD	7	28.00	1.22	27.36	1.89	24.63	1.48
80	286-292	YVFDWNM	6	43.57	0.16	45.67	1.22	40.08	0.29
81	290-298	WNMLKFGAS	8	47.32	5.02	50.71	3.01	52.03	2.17

82	293-302	LKFGASRAAD	9	37.36	1.36	36.20	1.57	33.84	1.38
83	293-305	LKFGASRAADDAE	12	37.13	0.77	36.67	1.15	32.94	0.68
84	300-312	AADDAERERRDRE	12	25.40	0.56	27.83	0.51	26.66	0.54
85	315-325	LRHSRNPATRG	9	5.23	0.10	5.59	0.24	4.90	0.12
86	316-326	RHSRNPATRGL	9	5.28	0.41	6.38	0.50	6.90	0.34
87	325-334	GLPSTASGRL	8	9.17	0.68	15.70	0.62	19.95	0.74
88	337-350	TQEVAPPTPLTPTS	9	11.66	0.45	17.14	0.28	23.40	1.43
89	345-367	PLTPTSHTANTSPRPVSGMERER	18	38.43	0.25	37.52	0.97	35.99	1.07
90	348-354	PTSHTAN	5	15.37	0.38	15.62	0.67	13.84	0.36
91	351-357	HTANTSP	5	22.30	0.34	21.90	0.52	18.89	0.19
92	363-371	MERERKVSM	8	46.71	1.76	49.18	2.17	48.16	1.68
93	363-376	MERERKVSMRLHRG	13	16.54	0.08	25.64	0.23	32.65	0.08
94	365-371	RERKVSM	6	38.53	2.06	39.11	1.72	36.59	0.60
95	372-386	RLHRGAPVNISSSDL	13	32.27	0.59	32.33	0.47	28.23	0.60
96	379-389	VNISSSDLTGR	10	13.74	0.55	22.50	0.27	25.69	0.18
97	379-395	VNISSSDLTGRQDTSRM	16	33.39	0.32	32.18	0.28	30.45	0.16
98	386-394	LTGRQDTSR	8	57.75	0.80	61.14	0.59	61.11	0.61
99	398-405	SQNSIPFE	6	24.63	1.75	24.81	2.49	21.90	1.76

				CK1δ2 + ATP					
				Relative Fractional Deuterium Uptake (%)					
				1 min		**5 min**		**10 min**	
S.No.	**Peptide**	**Sequence**	**Num Exchangers**	**Uptake**	**± SD**	**Uptake**	**± SD**	**Uptake**	**± SD**
1	3-20	LRVGNRYRLGRKIGSGSF	17	31.73	0.21	35.78	1.06	35.95	0.21
2	4-20	RVGNRYRLGRKIGSGSF	16	27.38	0.23	31.23	1.32	31.23	0.23
3	4-22	RVGNRYRLGRKIGSGSFGD	18	28.05	0.05	29.21	1.58	27.76	0.05
4	4-25	RVGNRYRLGRKIGSGSFGDIYL	21	22.30	0.16	25.95	1.05	26.11	0.16
5	12-25	GRKIGSGSFGDIYL	13	21.30	0.24	23.89	0.24	24.85	0.24
6	13-26	RKIGSGSFGDIYLG	13	9.91	0.34	12.61	0.73	17.00	0.34
7	21-28	GDIYLGTD	7	6.29	0.24	12.66	0.30	14.74	0.24
8	26-33	GTDIAAGE	7	39.62	0.36	40.92	0.36	40.17	0.36
9	26-34	GTDIAAGEE	8	41.41	0.24	42.77	1.36	41.89	0.24
10	28-34	DIAAGEE	6	40.16	0.48	39.78	1.07	37.63	0.48
11	35-41	VAIKLEC	6	14.51	0.13	17.40	1.78	27.37	0.13

12	40-49	ECVKTKHPQL	8	16.23	0.33	19.61	2.51	15.98	0.33
13	42-49	VKTKHPQL	6	22.94	0.14	28.44	1.16	25.80	0.14
14	50-56	HIESKIY	6	11.73	0.73	14.48	1.13	15.64	0.73
15	56-70	YKMMQGGVGIPTIRW	13	23.15	0.39	28.25	1.09	26.04	0.39
16	56-72	YKMMQGGVGIPTIRWCG	15	NaN	NaN	NaN	NaN	NaN	NaN
17	59-70	MQGGVGIPTIRW	10	31.66	0.36	30.79	0.36	33.48	0.36
18	83-89	ELLGPSL	5	3.33	0.27	3.94	0.63	2.01	0.27
19	85-92	LGPSLEDL	6	2.15	0.65	2.48	0.78	6.22	0.65
20	96-102	CSRKFSL	6	13.05	0.12	13.79	0.16	13.42	0.12
21	96-106	CSRKFSLKTVL	10	7.83	0.41	9.75	0.47	10.49	0.41
22	97-106	SRKFSLKTVL	9	9.03	0.69	8.80	0.69	13.66	0.69
23	104-119	TVLLLADQMISRIEYI	15	22.81	0.09	28.76	0.51	26.85	0.09
24	112-123	MISRIEYIHSKN	11	0.81	0.24	2.20	0.46	3.07	0.22
25	113-123	ISRIEYIHSKN	10	NaN	NaN	NaN	NaN	NaN	NaN
26	113-134	ISRIEYIHSKNFIHRDVKPDNF	20	1.99	0.25	2.58	0.38	2.72	0.27
27	115-132	RIEYIHSKNFIHRDVKPD	16	25.66	0.07	28.78	0.21	31.41	0.07
28	124-134	FIHRDVKPDNF	9	3.71	1.02	4.40	1.02	3.76	1.02
29	124-135	FIHRDVKPDNFL	10	3.26	0.45	3.95	0.47	4.49	0.45
30	126-134	HRDVKPDNF	7	2.32	0.12	4.72	0.27	2.87	0.12
31	126-135	HRDVKPDNFL	8	7.97	0.12	5.19	0.12	5.18	0.12
32	135-144	LMGLGKKGNL	9	17.95	0.15	20.72	0.75	23.21	0.15
33	135-145	LMGLGKKGNLV	10	15.94	0.03	18.32	0.43	20.75	0.03
34	135-146	LMGLGKKGNLVY	11	12.74	0.33	15.84	0.79	17.37	0.33
35	136-144	MGLGKKGNL	8	19.82	0.30	18.44	0.63	22.57	0.30
36	137-144	GLGKKGNL	7	19.24	0.22	21.45	0.36	21.57	0.22
37	137-146	GLGKKGNLVY	9	18.14	0.10	19.97	1.35	22.05	0.10
38	145-151	VYIIDFG	6	2.35	0.54	2.93	1.05	1.11	0.54
39	145-152	VYIIDFGL	7	1.64	0.59	3.01	0.62	8.27	0.59
40	145-158	VYIIDFGLAKKYRD	13	7.00	0.46	9.55	0.47	9.88	0.46
41	151-169	GLAKKYRDARTHQHIPYRE	17	16.79	0.18	19.95	0.71	18.71	0.18
42	163-178	QHIPYRENKNLTGTAR	14	22.45	0.27	28.26	0.27	31.68	0.27
43	173-179	LTGTARY	6	16.76	0.23	20.41	0.27	20.34	0.23
44	180-186	ASINTHL	6	11.42	0.48	18.60	0.53	20.83	0.48
45	180-189	ASINTHLGIE	9	13.09	0.19	22.25	1.24	28.58	0.19

46	180-196	ASINTHLGIEQSRRDDL	16	9.27	0.41	13.79	0.41	14.69	0.41
47	183-196	NTHLGIEQSRRDDL	13	7.48	0.17	12.17	0.44	12.32	0.17
48	185-196	HLGIEQSRRDDL	11	11.52	0.08	11.30	0.08	NaN	NaN
49	189-196	EQSRRDDL	7	11.39	0.91	14.46	0.91	12.60	0.91
50	204-218	MYFNLGSLPWQGLKA	13	29.20	0.04	32.49	0.04	30.74	0.04
51	205-215	YFNLGSLPWQG	9	15.76	0.48	21.05	0.48	25.33	0.48
52	205-216	YFNLGSLPWQGL	10	18.00	0.54	25.82	1.79	22.96	0.54
53	205-218	YFNLGSLPWQGLKA	12	28.37	0.38	33.31	1.26	33.57	0.38
54	206-218	FNLGSLPWQGLKA	11	36.49	0.09	41.90	1.18	41.94	0.09
55	207-216	NLGSLPWQGL	8	23.70	0.60	32.04	1.05	28.90	0.60
56	207-218	NLGSLPWQGLKA	10	39.58	0.34	46.54	0.34	44.18	0.34
57	209-216	GSLPWQGL	6	37.07	0.62	41.64	1.41	36.91	1.19
58	209-218	GSLPWQGLKA	8	37.47	0.64	39.99	1.29	39.10	0.64
59	212-218	PWQGLKA	5	44.99	0.21	47.04	0.21	44.82	0.21
60	219-230	ATKRQKYERISE	11	33.99	0.45	36.70	2.26	34.88	0.45
61	220-230	TKRQKYERISE	10	38.71	0.17	43.82	1.09	NaN	NaN
62	231-238	KKMSTPIE	6	NaN	NaN	NaN	NaN	NaN	NaN
63	231-240	KKMSTPIEVL	8	26.58	0.25	30.59	0.79	32.22	0.25
64	236-250	PIEVLCKGYPSEFAT	12	11.56	0.05	19.23	0.05	26.49	0.05
65	239-248	VLCKGYPSEF	8	10.83	0.55	18.10	0.96	20.34	0.78
66	241-248	CKGYPSEF	6	13.34	0.49	17.07	0.58	20.23	0.40
67	248-254	FATYLNF	6	2.64	0.32	3.79	0.63	3.19	0.32
68	255-269	CRSLRFDDKPDYSYL	13	22.96	0.11	25.14	0.77	24.39	0.11
69	261-268	DDKPDYSY	6	33.98	0.49	34.83	1.99	33.82	0.49
70	261-269	DDKPDYSYL	7	24.20	0.89	26.40	2.56	21.86	0.89
71	268-277	YLRQLFRNLF	9	1.79	1.03	3.83	1.80	3.61	1.03
72	269-275	LRQLFRN	6	24.33	0.23	34.29	0.53	37.83	0.23
73	269-277	LRQLFRNLF	8	2.92	0.34	6.35	0.54	7.29	0.34
74	269-285	LRQLFRNLFHRQGFSYD	16	12.37	0.22	14.70	0.69	14.38	0.22
75	270-276	RQLFRNL	6	1.40	0.39	6.95	0.56	9.48	0.39
76	270-277	RQLFRNLF	7	2.86	0.94	7.11	0.98	7.66	0.94
77	270-285	RQLFRNLFHRQGFSYD	15	12.64	0.11	14.19	0.41	14.50	0.11
78	276-295	LFHRQGFSYDYVFDWNMLKF	19	44.87	0.05	46.95	0.60	47.00	0.05
79	278-285	HRQGFSYD	7	22.90	1.07	27.07	1.07	27.26	1.07

80	286-292	YVFDWNM	6	32.74	0.12	35.88	2.51	35.02	0.12
81	290-298	WNMLKFGAS	8	43.99	2.11	47.35	2.11	NaN	NaN
82	293-302	LKFGASRAA D	9	33.42	1.27	35.27	2.09	31.51	1.30
83	293-305	LKFGASRAA DDAE	12	32.86	0.36	34.03	1.94	32.72	0.36
84	300-312	AADDAERER RDRE	12	23.81	0.13	27.03	0.85	24.53	0.13
85	315-325	LRHSRNPAT RG	9	5.61	0.09	8.37	0.66	7.43	0.09
86	316-326	RHSRNPATR GL	9	9.68	0.27	10.54	0.31	9.16	0.27
87	325-334	GLPSTASGR L	8	2.19	0.24	3.48	1.35	1.43	0.24
88	337-350	TQEVAPPTP LTPTS	9	10.05	0.28	14.75	0.28	15.99	0.28
89	345-367	PLTPTSHTAN TSPRPVSGM ERER	18	34.08	0.25	33.05	0.72	31.02	0.25
90	348-354	PTSHTAN	5	16.96	0.18	17.35	0.24	16.87	0.18
91	351-357	HTANTSP	5	18.37	0.13	18.34	1.28	14.76	0.13
92	363-371	MERERKVSM	8	41.53	0.71	41.00	1.13	40.48	0.71
93	363-376	MERERKVSM RLHRG	13	13.87	0.08	22.35	0.08	22.52	0.08
94	365-371	RERKVSM	6	33.02	0.36	37.14	1.26	35.62	0.36
95	372-386	RLHRGAPVNI SSSDL	13	28.18	0.47	30.73	1.92	33.32	0.47
96	379-389	VNISSSDLTG R	10	10.82	0.66	16.73	1.01	19.34	0.66
97	379-395	VNISSSDLTG RQDTSRM	16	34.30	0.10	32.06	0.49	32.15	0.10
98	386-394	LTGRQDTSR	8	55.02	0.49	58.34	0.65	59.09	0.49
99	398-405	SQNSIPFE	6	22.31	0.00	23.14	0.28	17.63	0.02

				CK1δ2 + AMPPNP					
				Relative Fractional Deuterium Uptake (%)					
				1 min		**5 min**		**10 min**	
S.No.	**Peptide**	**Sequence**	**Num Exchangers**	**Uptake**	**± SD**	**Uptake**	**± SD**	**Uptake**	**± SD**
1	3-20	LRVGNRYRL GRKIGSGSF	17	39.20	0.81	42.32	0.45	43.31	0.27
2	4-20	RVGNRYRLG RKIGSGSF	16	32.68	0.30	36.45	0.47	37.88	0.75
3	4-22	RVGNRYRLG RKIGSGSFG D	18	31.23	0.57	35.53	0.15	36.45	0.82
4	4-25	RVGNRYRLG RKIGSGSFG DIYL	21	25.54	1.01	32.11	0.39	35.66	0.83
5	12-25	GRKIGSGSF GDIYL	13	26.45	0.28	31.69	0.55	33.54	1.49
6	13-26	RKIGSGSFG DIYLG	13	10.07	0.43	12.81	0.45	14.41	1.24
7	21-28	GDIYLGTD	7	12.60	0.69	27.40	0.67	33.84	1.62
8	26-33	GTDIAAGE	7	40.49	0.48	41.24	0.76	40.22	1.30
9	26-34	GTDIAAGEE	8	42.49	1.23	42.80	0.96	42.65	1.48

10	28-34	DIAAGEE	6	40.90	0.51	41.32	0.65	39.98	1.51
11	35-41	VAIKLEC	6	12.11	0.97	16.88	1.93	25.92	0.13
12	40-49	ECVKTKHPQL	8	20.39	0.38	21.92	0.80	20.48	0.98
13	42-49	VKTKHPQL	6	27.68	0.75	28.31	1.10	27.46	1.94
14	50-56	HIESKIY	6	15.60	1.74	20.61	1.18	22.66	1.45
15	56-70	YKMMQGGVGIPTIRW	13	26.51	2.40	32.22	1.64	35.12	1.64
16	56-72	YKMMQGGVGIPTIRWCG	15	21.31	1.17	23.31	0.55	20.48	1.69
17	59-70	MQGGVGIPTIRW	10	33.90	2.66	36.68	2.23	40.42	1.80
18	83-89	ELLGPSL	5	6.41	0.43	9.17	0.56	9.77	0.60
19	85-92	LGPSLEDL	6	3.12	0.97	3.13	0.65	3.08	1.09
20	96-102	CSRKFSL	6	13.05	1.47	13.62	0.24	12.92	1.54
21	96-106	CSRKFSLKTVL	10	8.27	1.30	10.47	0.77	10.23	1.18
22	97-106	SRKFSLKTVL	9	7.95	1.38	10.93	0.98	10.42	1.31
23	104-119	TVLLLADQMISRIEYI	15	20.98	0.19	27.32	0.35	29.16	0.31
24	112-123	MISRIEYIHSKN	11	2.18	0.37	3.13	0.72	3.99	0.58
25	113-123	ISRIEYIHSKN	10	1.86	0.30	2.32	0.38	2.29	0.26
26	113-134	ISRIEYIHSKNFIHRDVKPDNF	20	2.20	0.31	2.53	0.21	2.82	0.25
27	115-132	RIEYIHSKNFIHRDVKPD	16	18.67	0.42	24.01	0.33	27.71	0.45
28	124-134	FIHRDVKPDNF	9	2.62	1.06	3.89	0.94	4.14	0.87
29	124-135	FIHRDVKPDNFL	10	2.62	0.46	3.34	0.51	3.67	0.65
30	126-134	HRDVKPDNF	7	3.27	0.54	3.57	0.86	3.56	0.35
31	126-135	HRDVKPDNFL	8	6.18	0.92	6.45	0.26	6.47	0.46
32	135-144	LMGLGKKGNL	9	19.07	0.42	22.38	0.32	23.70	1.12
33	135-145	LMGLGKKGNLV	10	16.85	0.63	20.12	0.54	21.59	0.89
34	135-146	LMGLGKKGNLVY	11	14.63	0.38	17.01	0.38	18.55	1.14
35	136-144	MGLGKKGNL	8	20.12	0.61	23.34	0.34	25.15	1.48
36	137-144	GLGKKGNL	7	20.72	0.52	24.13	0.69	25.96	1.07
37	137-146	GLGKKGNLVY	9	17.57	0.10	21.12	0.41	22.49	1.48
38	145-151	VYIIDFG	6	7.12	0.83	17.72	1.51	19.08	0.94
39	145-152	VYIIDFGL	7	6.94	0.72	16.55	0.62	24.43	1.21
40	145-158	VYIIDFGLAKKYRD	13	9.24	0.60	11.66	0.46	11.95	0.72
41	151-169	GLAKKYRDARTHQHIPYRE	17	18.08	1.01	19.75	0.46	20.37	2.20
42	163-178	QHIPYRENKNLTGTAR	14	22.06	0.66	27.93	0.37	30.61	1.13
43	173-179	LTGTARY	6	14.00	2.03	19.40	0.75	20.43	1.96

44	180-186	ASINTHL	6	12.91	1.05	22.99	0.78	27.00	1.65
45	180-189	ASINTHLGIE	9	15.04	0.34	28.16	0.48	33.28	0.51
46	180-196	ASINTHLGIE QSRRDDL	16	9.26	0.61	16.22	0.45	18.11	0.99
47	183-196	NTHLGIEQSR RDDL	13	7.94	0.35	13.39	0.24	14.79	0.41
48	185-196	HLGIEQSRR DDL	11	8.07	0.20	11.67	0.17	12.84	0.67
49	189-196	EQSRRDDL	7	10.63	1.58	15.64	1.09	15.63	1.30
50	204-218	MYFNLGSLP WQGLKA	13	34.14	0.04	38.90	0.72	41.11	1.81
51	205-215	YFNLGSLPW QG	9	22.42	1.11	28.78	1.30	31.22	1.48
52	205-216	YFNLGSLPW QGL	10	23.95	1.58	29.86	0.98	31.19	2.15
53	205-218	YFNLGSLPW QGLKA	12	32.08	0.87	36.75	0.59	38.01	1.91
54	206-218	FNLGSLPWQ GLKA	11	39.27	0.63	44.68	0.46	45.98	2.90
55	207-216	NLGSLPWQG L	8	30.23	2.02	38.25	1.29	33.79	0.86
56	207-218	NLGSLPWQG LKA	10	41.32	0.49	46.22	0.63	49.19	1.38
57	209-216	GSLPWQGL	6	38.55	1.81	41.97	1.43	44.60	1.86
58	209-218	GSLPWQGLK A	8	37.03	0.67	40.82	1.79	42.29	2.51
59	212-218	PWQGLKA	5	46.12	2.51	49.20	1.53	51.81	2.48
60	219-230	ATKRQKYERI SE	11	37.27	1.71	38.42	1.19	38.45	2.06
61	220-230	TKRQKYERIS E	10	40.69	0.42	42.20	0.46	39.08	0.61
62	231-238	KKMSTPIE	6	38.35	1.00	39.80	0.54	40.24	1.76
63	231-240	KKMSTPIEVL	8	26.31	0.70	31.01	0.40	32.68	1.83
64	236-250	PIEVLCKGYP SEFAT	12	10.37	0.11	18.54	0.08	22.55	0.96
65	239-248	VLCKGYPSE F	8	11.66	0.68	16.47	1.27	20.62	1.13
66	241-248	CKGYPSEF	6	13.22	0.44	17.08	0.53	20.58	0.81
67	248-254	FATYLNF	6	0.52	0.47	0.67	0.53	1.11	0.45
68	255-269	CRSLRFDDK PDYSYL	13	24.82	0.53	26.54	0.19	26.85	1.21
69	261-268	DDKPDYSY	6	37.22	1.30	37.04	0.56	36.50	2.13
70	261-269	DDKPDYSYL	7	29.85	1.65	29.33	0.98	28.42	1.71
71	268-277	YLRQLFRNLF	9	2.26	1.42	5.66	1.43	7.74	1.45
72	269-275	LRQLFRN	6	20.86	0.88	32.27	1.84	36.59	1.38
73	269-277	LRQLFRNLF	8	2.49	0.51	6.44	0.36	7.92	0.54
74	269-285	LRQLFRNLF HRQGFSYD	16	15.03	0.54	17.17	0.39	17.56	0.24
75	270-276	RQLFRNL	6	2.33	0.58	6.33	0.40	9.14	0.89
76	270-277	RQLFRNLF	7	2.37	0.99	6.54	0.94	7.40	1.00
77	270-285	RQLFRNLFH RQGFSYD	15	14.58	0.35	16.77	0.11	16.91	1.07

78	276-295	LFHRQGFSYDYVFDWNMLKF	19	47.37	0.66	49.12	0.06	49.23	0.40
79	278-285	HRQGFSYD	7	27.07	1.78	27.03	1.49	25.51	2.30
80	286-292	YVFDWNM	6	44.10	1.10	48.29	0.40	47.30	3.57
81	290-298	WNMLKFGAS	8	49.86	4.74	49.52	2.95	54.50	3.66
82	293-302	LKFGASRAAD	9	35.10	1.56	35.30	1.46	33.89	3.35
83	293-305	LKFGASRAADDAE	12	35.08	1.67	35.01	1.11	36.83	0.52
84	300-312	AADDAERERRDRE	12	24.05	0.73	26.59	0.40	28.16	1.19
85	315-325	LRHSRNPATRG	9	4.53	0.30	4.74	0.24	4.63	0.15
86	316-326	RHSRNPATRGL	9	5.22	0.38	6.01	0.64	5.81	0.96
87	325-334	GLPSTASGRL	8	5.75	0.46	9.75	1.21	11.40	0.67
88	337-350	TQEVAPPTPLTPTS	9	12.35	1.13	21.61	0.28	27.93	0.28
89	345-367	PLTPTSHTANTSPRPVSGMERER	18	35.83	0.61	38.20	0.25	37.91	0.25
90	348-354	PTSHTAN	5	15.57	0.55	15.51	0.61	14.55	0.33
91	351-357	HTANTSP	5	20.72	0.92	20.73	0.38	20.65	1.62
92	363-371	MERERKVSM	8	44.89	0.89	48.35	1.37	48.50	1.77
93	363-376	MERERKVSMRLHRG	13	17.74	0.21	24.36	0.08	26.56	1.06
94	365-371	RERKVSM	6	37.15	3.86	36.10	0.64	36.03	1.79
95	372-386	RLHRGAPVNISSSDL	13	32.96	0.47	30.95	0.94	30.74	0.54
96	379-389	VNISSSDLTGR	10	13.71	1.03	20.58	0.95	22.56	1.72
97	379-395	VNISSSDLTGRQDTSRM	16	32.22	0.34	33.05	0.10	32.47	0.68
98	386-394	LTGRQDTSR	8	57.90	0.64	60.47	0.49	60.88	0.67
99	398-405	SQNSIPFE	6	19.62	2.21	20.57	0.84	23.40	0.69

				CK1δ1-Δ400					
				Relative Deuterium Uptake (Da)					
				1 min		**5 min**		**10 min**	
S.No.	**Peptide**	**Sequence**	**Num Exchangers**	**Uptake**	**± SD**	**Uptake**	**± SD**	**Uptake**	**± SD**
1	1-21	MELRVGNRYRLGRKIGSGSFG	20	5.22	0.03	7.00	0.03	7.32	0.03
2	4-20	RVGNRYRLGRKIGSGSF	16	5.71	0.14	6.18	0.12	6.46	0.04
3	4-22	RVGNRYRLGRKIGSGSFGD	18	6.14	0.22	6.84	0.11	7.10	0.03
4	12-25	GRKIGSGSFGDIYL	13	4.64	0.11	5.14	0.21	5.36	0.25
5	21-28	GDIYLGTD	7	1.53	0.02	2.74	0.04	2.80	0.14
6	26-34	GTDIAAGEE	8	3.53	0.07	3.50	0.09	3.38	0.06
7	28-34	DIAAGEE	6	2.66	0.02	2.61	0.03	2.54	0.02

8	28-36	DIAAGEEVA	8	2.99	0.02	3.22	0.02	3.20	0.08
9	35-41	VAIKLEC	6	1.13	0.03	1.65	0.08	1.78	0.01
10	37-49	IKLECVKTKH PQL	11	2.43	0.07	2.58	0.11	2.46	0.02
11	42-49	VKTKHPQL	6	1.74	0.09	1.80	0.08	1.77	0.11
12	50-58	HIESKIYKM	8	1.58	0.11	2.45	0.05	2.37	0.00
13	56-70	YKMMQGGV GIPTIRW	13	4.01	0.31	5.08	0.35	5.51	0.03
14	59-77	MQGGVGIPTI RWCGAEGD Y	17	5.82	0.05	5.43	0.19	5.35	0.23
15	81-89	VMELLGPSL	7	0.96	0.07	1.45	0.11	1.81	0.08
16	83-89	ELLGPSL	5	0.62	0.03	0.81	0.03	0.82	0.02
17	83-95	ELLGPSLEDL FNF	11	4.31	0.12	4.58	0.04	4.69	0.07
18	96-106	CSRKFSLKT VL	10	1.02	0.09	1.47	0.04	1.51	0.02
19	104-119	TVLLLADQMI SRIEYI	15	3.93	0.05	5.16	0.08	5.79	0.09
20	114-123	SRIEYIHSKN	9	0.31	0.02	0.38	0.03	0.42	0.02
21	124-134	FIHRDVKPDN F	9	0.38	0.10	0.29	0.09	0.30	0.11
22	124-135	FIHRDVKPDN FL	10	0.43	0.04	0.52	0.04	0.46	0.09
23	135-144	LMGLGKKGN L	9	2.03	0.13	2.45	0.09	2.32	0.24
24	135-145	LMGLGKKGN LV	10	2.27	0.10	2.71	0.09	2.66	0.10
25	135-146	LMGLGKKGN LVY	11	1.89	0.12	2.32	0.06	2.16	0.25
26	136-144	MGLGKKGNL	8	2.05	0.02	2.15	0.06	2.02	0.16
27	136-146	MGLGKKGNL VY	10	2.06	0.11	2.51	0.15	2.35	0.28
28	145-152	VYIIDFGL	7	0.94	0.12	1.92	0.08	2.04	0.06
29	151-160	GLAKKYRDA R	9	4.39	0.09	4.79	0.09	4.62	0.09
30	160-166	RTHQHIP	5	0.88	0.03	1.27	0.07	1.58	0.02
31	163-178	QHIPYRENK NLTGTAR	14	3.64	0.03	4.33	0.03	4.95	0.12
32	180-186	ASINTHL	6	0.81	0.04	1.45	0.04	1.54	0.16
33	183-196	NTHLGIEQSR RDDL	13	1.36	0.06	2.14	0.02	2.40	0.09
34	187-196	GIEQSRRDD L	9	1.02	0.05	1.46	0.03	1.59	0.02
35	204-218	MYFNLGSLP WQGLKA	13	5.22	0.01	5.58	0.01	5.28	0.01
36	205-215	YFNLGSLPW QG	9	2.56	0.04	3.29	0.13	3.45	0.10
37	205-216	YFNLGSLPW QGL	10	2.95	0.14	3.68	0.08	3.79	0.15
38	205-218	YFNLGSLPW QGLKA	12	4.39	0.12	5.12	0.21	5.04	0.42
39	206-216	FNLGSLPWQ GL	9	2.98	0.10	3.83	0.08	3.92	0.09
40	206-218	FNLGSLPWQ GLKA	11	4.83	0.14	5.44	0.15	5.64	0.28
41	209-216	GSLPWQGL	6	2.71	0.08	2.96	0.07	3.19	0.10

42	209-218	GSLPWQGLKA	8	3.80	0.13	3.91	0.15	4.00	0.11
43	212-218	PWQGLKA	5	2.76	0.04	2.80	0.04	2.86	0.05
44	219-230	ATKRQKYERISE	11	4.57	0.11	4.56	0.06	4.50	0.01
45	231-238	KKMSTPIE	6	2.44	0.07	2.51	0.07	2.48	0.10
46	231-240	KKMSTPIEVL	8	2.36	0.13	2.77	0.13	2.61	0.12
47	239-248	VLCKGYPSEF	8	1.06	0.04	1.53	0.03	1.55	0.24
48	241-247	CKGYPSE	5	1.11	0.02	1.37	0.06	1.60	0.08
49	241-248	CKGYPSEF	6	0.99	0.07	1.25	0.04	1.36	0.19
50	242-259	KGYPSEFATYLNFCRSLR	16	5.12	0.07	5.31	0.05	5.23	0.17
51	248-254	FATYLNF	6	0.15	0.03	0.17	0.04	0.18	0.04
52	255-269	CRSLRFDDKPDYSYL	13	3.38	0.06	3.70	0.12	3.54	0.22
53	261-269	DDKPDYSYL	7	2.33	0.11	2.32	0.10	2.16	0.13
54	269-277	LRQLFRNLF	8	0.31	0.13	0.63	0.12	0.67	0.14
55	270-277	RQLFRNLF	7	0.32	0.03	0.63	0.04	0.61	0.12
56	273-285	FRNLFHRQGFSYD	12	2.34	0.18	2.58	0.12	2.82	0.23
57	278-285	HRQGFSYD	7	2.18	0.08	2.21	0.06	2.02	0.12
58	286-292	YVFDWNM	6	2.27	0.14	2.52	0.03	2.64	0.07
59	293-305	LKFGASRAADDAE	12	4.79	0.18	4.84	0.08	4.89	0.02
60	293-312	LKFGASRAADDAERERRDRE	19	7.71	0.08	7.73	0.04	7.66	0.12
61	304-320	AERERRDREERLRHSRN	16	5.71	0.07	5.64	0.28	5.56	0.03
62	313-327	ERLRHSRNPATRGLP	12	1.98	0.06	2.06	0.02	2.06	0.02
63	316-335	RHSRNPATRGLPSTASGRLR	17	5.06	0.10	6.22	0.09	6.25	0.30
64	337-348	TQEVAPPTPLTP	7	1.78	0.07	1.81	0.07	1.92	0.09
65	346-358	LTPTSHTANTSPR	10	3.74	0.15	3.51	0.05	3.71	0.00
66	348-354	PTSHTAN	5	0.56	0.03	0.57	0.03	0.52	0.06
67	351-357	HTANTSP	5	1.18	0.03	1.16	0.04	1.18	0.07
68	351-365	HTANTSPRPVSGMER	12	4.76	0.12	4.67	0.11	4.43	0.04
69	354-367	NTSPRPVSGMERER	11	4.86	0.16	5.45	0.07	5.59	0.15
70	365-371	RERKVSM	6	2.47	0.09	2.56	0.06	2.54	0.09
71	378-385	PVNISSSD	6	0.75	0.04	0.77	0.02	0.70	0.05
72	379-395	VNISSSDLTGRQDTSRM	16	5.84	0.15	5.74	0.03	5.55	0.18
73	386-394	LTGRQDTSR	8	4.35	0.11	4.80	0.12	4.90	0.02
				CK1δ1-Δ400 + ATP **Relative Deuterium Uptake (Da)**					

				1 min		5 min		10 min	
S.No.	Peptide	Sequence	Num Exchangers	Uptake	± SD	Uptake	± SD	Uptake	± SD
1	1-21	MELRVGNRYRLGRKIGSGSFG	20	5.03	0.00	5.52	0.24	5.62	0.00
2	4-20	RVGNRYRLGRKIGSGSF	16	5.34	0.04	5.89	0.06	6.16	0.26
3	4-22	RVGNRYRLGRKIGSGSFGD	18	6.04	0.20	6.44	0.10	6.90	0.05
4	12-25	GRKIGSGSFGDIYL	13	4.18	0.09	3.88	0.08	4.01	0.06
5	21-28	GDIYLGTD	7	0.65	0.03	0.89	0.03	1.27	0.10
6	26-34	GTDIAAGEE	8	3.36	0.08	3.63	0.05	3.61	0.04
7	28-34	DIAAGEE	6	2.54	0.02	2.58	0.01	2.61	0.05
8	28-36	DIAAGEEVA	8	2.88	0.03	2.83	0.05	2.79	0.03
9	35-41	VAIKLEC	6	0.84	0.06	0.87	0.03	0.91	0.03
10	37-49	IKLECVKTKHPQL	11	2.23	0.02	2.41	0.04	2.42	0.07
11	42-49	VKTKHPQL	6	1.71	0.07	1.75	0.06	1.83	0.08
12	50-58	HIESKIYKM	8	1.26	0.10	1.79	0.11	2.01	0.14
13	56-70	YKMMQGGVGIPTIRW	13	4.04	0.16	3.84	0.01	4.58	0.30
14	59-77	MQGGVGIPTIRWCGAEGDY	17	4.88	0.19	4.87	0.17	5.49	0.04
15	81-89	VMELLGPSL	7	0.36	0.08	0.28	0.04	0.41	0.05
16	83-89	ELLGPSL	5	0.30	0.02	0.22	0.02	0.23	0.03
17	83-95	ELLGPSLEDLFNF	11	4.06	0.09	4.33	0.16	4.52	0.04
18	96-106	CSRKFSLKTVL	10	0.97	0.04	1.19	0.02	1.37	0.02
19	104-119	TVLLLADQMISRIEYI	15	2.81	0.12	3.63	0.10	4.05	0.06
20	114-123	SRIEYIHSKN	9	0.32	0.01	0.32	0.01	0.23	0.02
21	124-134	FIHRDVKPDNF	9	0.21	0.11	0.30	0.11	0.32	0.09
22	124-135	FIHRDVKPDNFL	10	0.43	0.04	0.46	0.06	0.50	0.04
23	135-144	LMGLGKKGNL	9	2.05	0.13	2.31	0.09	2.38	0.07
24	135-145	LMGLGKKGNLV	10	2.23	0.13	2.48	0.14	2.69	0.10
25	135-146	LMGLGKKGNLVY	11	1.94	0.10	2.27	0.07	2.48	0.05
26	136-144	MGLGKKGNL	8	1.88	0.06	2.10	0.07	2.22	0.08
27	136-146	MGLGKKGNLVY	10	2.13	0.15	2.34	0.12	2.60	0.12
28	145-152	VYIIDFGL	7	0.29	0.16	0.32	0.14	0.35	0.14
29	151-160	GLAKKYRDAR	9	3.94	0.09	4.56	0.10	4.24	0.09
30	160-166	RTHQHIP	5	0.99	0.09	1.28	0.02	1.51	0.01
31	163-178	QHIPYRENKNLTGTAR	14	2.72	0.11	3.24	0.18	3.37	0.32

32	180-186	ASINTHL	6	0.75	0.04	1.21	0.03	1.50	0.05
33	183-196	NTHLGIEQSRRDDL	13	1.38	0.08	1.88	0.03	2.20	0.09
34	187-196	GIEQSRRDDL	9	1.03	0.05	1.28	0.02	1.46	0.04
35	204-218	MYFNLGSLPWQGLKA	13	4.99	0.05	5.29	0.01	NaN	NaN
36	205-215	YFNLGSLPWQG	9	2.50	0.06	2.92	0.15	3.30	0.21
37	205-216	YFNLGSLPWQGL	10	2.85	0.09	3.55	0.01	3.60	0.17
38	205-218	YFNLGSLPWQGLKA	12	4.43	0.20	4.80	0.19	5.18	0.17
39	206-216	FNLGSLPWQGL	9	3.01	0.09	3.48	0.13	3.86	0.18
40	206-218	FNLGSLPWQGLKA	11	4.76	0.06	5.24	0.08	5.60	0.04
41	209-216	GSLPWQGL	6	2.68	0.09	2.83	0.04	3.01	0.04
42	209-218	GSLPWQGLKA	8	3.62	0.09	3.83	0.13	4.07	0.10
43	212-218	PWQGLKA	5	2.66	0.06	2.71	0.02	2.74	0.05
44	219-230	ATKRQKYERISE	11	4.19	0.09	4.44	0.13	4.57	0.12
45	231-238	KKMSTPIE	6	2.44	0.02	2.47	0.07	2.52	0.07
46	231-240	KKMSTPIEVL	8	2.41	0.13	2.75	0.12	2.98	0.13
47	239-248	VLCKGYPSEF	8	1.18	0.06	1.52	0.03	1.83	0.11
48	241-247	CKGYPSE	5	1.14	0.01	1.31	0.03	1.57	0.06
49	241-248	CKGYPSEF	6	1.04	0.04	1.22	0.05	1.44	0.07
50	242-259	KGYPSEFATYLNFCRSLR	16	4.96	0.33	5.30	0.06	5.47	0.27
51	248-254	FATYLNF	6	0.06	0.03	0.10	0.04	0.14	0.05
52	255-269	CRSLRFDDKPDYSYL	13	NaN	NaN	3.75	0.07	3.93	0.08
53	261-269	DDKPDYSYL	7	2.25	0.12	2.17	0.12	2.24	0.08
54	269-277	LRQLFRNLF	8	0.27	0.12	0.49	0.11	0.68	0.09
55	270-277	RQLFRNLF	7	0.23	0.03	0.39	0.03	0.57	0.04
56	273-285	FRNLFHRQGFSYD	12	2.28	0.15	2.49	0.14	2.63	0.14
57	278-285	HRQGFSYD	7	2.13	0.09	2.19	0.12	2.17	0.09
58	286-292	YVFDWNM	6	2.21	0.14	2.41	0.12	2.66	0.11
59	293-305	LKFGASRAADDAE	12	5.10	0.10	5.11	0.12	5.15	0.17
60	293-312	LKFGASRAADDAERERRDRE	19	8.25	0.06	8.17	0.04	8.23	0.07
61	304-320	AERERRDREERLRHSRN	16	5.54	0.04	5.68	0.04	6.11	0.05
62	313-327	ERLRHSRNPATRGLP	12	1.78	0.03	2.10	0.03	2.27	0.02
63	316-335	RHSRNPATRGLPSTASGRLR	17	4.73	0.12	5.31	0.09	5.63	0.18
64	337-348	TQEVAPPTPLTP	7	1.72	0.13	1.76	0.10	1.74	0.04

65	346-358	LTPTSHTANTSPR	10	3.64	0.00	3.69	0.00	3.69	0.00
66	348-354	PTSHTAN	5	0.53	0.03	0.51	0.03	0.55	0.05
67	351-357	HTANTSP	5	1.13	0.02	1.20	0.03	1.17	0.03
68	351-365	HTANTSPRPVSGMER	12	4.98	0.08	5.08	0.01	4.94	0.05
69	354-367	NTSPRPVSGMERER	11	4.82	0.10	5.30	0.07	5.65	0.10
70	365-371	RERKVSM	6	2.56	0.09	2.44	0.04	2.54	0.05
71	378-385	PVNISSSD	6	0.75	0.02	0.79	0.03	0.81	0.03
72	379-395	VNISSSDLTGRQDTSRM	16	5.86	0.06	6.15	0.10	6.27	0.05
73	386-394	LTGRQDTSR	8	4.29	0.12	4.67	0.17	4.92	0.08
				CK1δ1-Δ400 + AMPPNP					
				Relative Deuterium Uptake (Da)					
				1 min		**5 min**		**10 min**	
S.No.	**Peptide**	**Sequence**	**Num Exchangers**	**Uptake**	**± SD**	**Uptake**	**± SD**	**Uptake**	**± SD**
1	1-21	MELRVGNRYRLGRKIGSGSFG	20	5.95	0.10	7.33	0.06	7.39	0.16
2	4-20	RVGNRYRLGRKIGSGSF	16	5.72	0.07	6.33	0.09	6.38	0.11
3	4-22	RVGNRYRLGRKIGSGSFGD	18	6.38	0.07	7.17	0.14	7.07	0.07
4	12-25	GRKIGSGSFGDIYL	13	4.95	0.03	5.31	0.12	5.05	0.03
5	21-28	GDIYLGTD	7	1.13	0.07	2.40	0.05	2.78	0.11
6	26-34	GTDIAAGEE	8	3.69	0.03	3.74	0.04	3.68	0.06
7	28-34	DIAAGEE	6	2.62	0.04	2.58	0.05	2.58	0.06
8	28-36	DIAAGEEVA	8	2.94	0.03	3.05	0.03	3.08	0.06
9	35-41	VAIKLEC	6	0.96	0.03	1.14	0.08	1.24	0.09
10	37-49	IKLECVKTKHPQL	11	2.42	0.07	2.55	0.07	2.51	0.08
11	42-49	VKTKHPQL	6	1.77	0.06	1.74	0.05	1.72	0.01
12	50-58	HIESKIYKM	8	1.38	0.26	2.37	0.11	2.45	0.05
13	56-70	YKMMQGGVGIPTIRW	13	4.24	0.17	4.77	0.01	4.71	0.01
14	59-77	MQGGVGIPTIRWCGAEGDY	17	5.18	0.10	5.34	0.04	5.35	0.04
15	81-89	VMELLGPSL	7	0.58	0.12	1.00	0.09	1.20	0.16
16	83-89	ELLGPSL	5	0.42	0.05	0.70	0.03	0.83	0.04
17	83-95	ELLGPSLEDLFNF	11	4.35	0.06	4.54	0.04	4.33	0.05
18	96-106	CSRKFSLKTVL	10	0.87	0.04	1.34	0.02	1.39	0.06
19	104-119	TVLLLADQMISRIEYI	15	3.46	0.08	4.64	0.07	4.98	0.08
20	114-123	SRIEYIHSKN	9	0.29	0.04	0.33	0.01	0.42	0.06
21	124-134	FIHRDVKPDNF	9	0.32	0.09	0.39	0.09	0.41	0.09

22	124-135	FIHRDVKPDNFL	10	0.40	0.04	0.56	0.06	0.59	0.05
23	135-144	LMGLGKKGNL	9	2.18	0.08	2.56	0.12	2.70	0.10
24	135-145	LMGLGKKGNLV	10	2.52	0.09	2.86	0.18	3.03	0.09
25	135-146	LMGLGKKGNLVY	11	2.17	0.04	2.56	0.08	2.70	0.06
26	136-144	MGLGKKGNL	8	1.98	0.06	2.32	0.08	2.42	0.04
27	136-146	MGLGKKGNLVY	10	2.21	0.12	2.55	0.13	2.71	0.13
28	145-152	VYIIDFGL	7	0.45	0.08	1.28	0.17	1.70	0.07
29	151-160	GLAKKYRDAR	9	4.82	0.09	5.08	0.09	5.12	0.09
30	160-166	RTHQHIP	5	1.16	0.01	1.32	0.09	1.41	0.06
31	163-178	QHIPYRENKNLTGTAR	14	3.32	0.03	3.99	0.11	4.27	0.03
32	180-186	ASINTHL	6	1.02	0.04	1.78	0.06	1.97	0.03
33	183-196	NTHLGIEQSRRDDL	13	1.55	0.02	2.45	0.08	2.60	0.03
34	187-196	GIEQSRRDDL	9	1.05	0.03	1.49	0.04	1.60	0.03
35	204-218	MYFNLGSLPWQGLKA	13	5.21	0.01	5.77	0.03	5.95	0.02
36	205-215	YFNLGSLPWQG	9	2.63	0.02	3.51	0.01	3.58	0.09
37	205-216	YFNLGSLPWQGL	10	3.10	0.04	3.85	0.15	3.74	0.10
38	205-218	YFNLGSLPWQGLKA	12	4.51	0.09	5.36	0.13	5.37	0.10
39	206-216	FNLGSLPWQGL	9	3.31	0.13	3.97	0.09	3.96	0.09
40	206-218	FNLGSLPWQGLKA	11	4.87	0.04	5.58	0.12	5.69	0.07
41	209-216	GSLPWQGL	6	2.89	0.11	3.13	0.07	3.11	0.04
42	209-218	GSLPWQGLKA	8	3.85	0.12	4.11	0.14	4.10	0.10
43	212-218	PWQGLKA	5	2.72	0.04	2.78	0.03	2.92	0.02
44	219-230	ATKRQKYERISE	11	4.40	0.30	4.48	0.20	4.40	0.06
45	231-238	KKMSTPIE	6	2.37	0.04	2.47	0.08	2.35	0.06
46	231-240	KKMSTPIEVL	8	2.60	0.12	3.00	0.12	3.12	0.12
47	239-248	VLCKGYPSEF	8	1.31	0.04	1.77	0.06	2.05	0.05
48	241-247	CKGYPSE	5	1.16	0.04	1.44	0.03	1.65	0.05
49	241-248	CKGYPSEF	6	1.09	0.05	1.39	0.07	1.55	0.05
50	242-259	KGYPSEFATYLNFCRSLR	16	5.39	0.22	5.39	0.31	5.32	0.06
51	248-254	FATYLNF	6	0.03	0.03	0.10	0.03	0.14	0.03
52	255-269	CRSLRFDDKPDYSYL	13	3.64	0.05	3.90	0.09	3.93	0.08
53	261-269	DDKPDYSYL	7	2.18	0.10	2.19	0.16	2.08	0.09
54	269-277	LRQLFRNLF	8	0.30	0.12	0.62	0.12	0.81	0.12
55	270-277	RQLFRNLF	7	0.29	0.04	0.52	0.04	0.70	0.05

56	273-285	FRNLFHRQGFSYD	12	2.35	0.17	2.50	0.22	2.55	0.14
57	278-285	HRQGFSYD	7	2.03	0.14	2.13	0.18	2.00	0.13
58	286-292	YVFDWNM	6	2.25	0.17	2.57	0.10	2.50	0.08
59	293-305	LKFGASRAADDAE	12	5.06	0.02	5.30	0.02	4.99	0.19
60	293-312	LKFGASRAADDAERERRDRE	19	7.83	0.12	7.92	0.06	7.94	0.05
61	304-320	AERERRDREERLRHSRN	16	5.87	0.04	5.82	0.06	5.74	0.04
62	313-327	ERLRHSRNPATRGLP	12	1.79	0.02	2.36	0.04	2.38	0.02
63	316-335	RHSRNPATRGLPSTASGRLR	17	5.44	0.19	NaN	NaN	6.80	0.21
64	337-348	TQEVAPPTPLTP	7	1.81	0.07	1.88	0.09	1.83	0.09
65	346-358	LTPTSHTANTSPR	10	3.71	0.03	3.70	0.00	3.96	0.00
66	348-354	PTSHTAN	5	0.58	0.03	0.55	0.04	0.59	0.04
67	351-357	HTANTSP	5	1.19	0.01	1.25	0.02	1.18	0.03
68	351-365	HTANTSPRPVSGMER	12	5.18	0.07	5.17	0.09	5.16	0.01
69	354-367	NTSPRPVSGMERER	11	4.93	0.08	5.60	0.08	5.83	0.10
70	365-371	RERKVSM	6	2.37	0.06	2.46	0.05	2.41	0.04
71	378-385	PVNISSSD	6	0.78	0.02	0.81	0.01	0.81	0.02
72	379-395	VNISSSDLTGRQDTSRM	16	6.28	0.04	6.45	0.14	6.26	0.04
73	386-394	LTGRQDTSR	8	4.42	0.09	4.85	0.03	4.83	0.19

				CK1δ1-Δ400					
				Relative Fractional Deuterium Uptake (%)					
				1 min		**5 min**		**10 min**	
S.No.	**Peptide**	**Sequence**	**Num Exchangers**	**Uptake**	**± SD**	**Uptake**	**± SD**	**Uptake**	**± SD**
1	1-21	MELRVGNRYRLGRKIGSGSFG	20	26.10	0.15	34.98	0.15	36.62	0.15
2	4-20	RVGNRYRLGRKIGSGSF	16	35.66	0.88	38.62	0.74	40.37	0.25
3	4-22	RVGNRYRLGRKIGSGSFGD	18	34.09	1.21	37.99	0.60	39.46	0.15
4	12-25	GRKIGSGSFGDIYL	13	35.67	0.83	39.54	1.61	41.20	1.94
5	21-28	GDIYLGTD	7	21.89	0.23	39.20	0.59	40.03	1.93
6	26-34	GTDIAAGEE	8	44.12	0.92	43.72	1.17	42.29	0.75
7	28-34	DIAAGEE	6	44.41	0.35	43.51	0.51	42.28	0.30
8	28-36	DIAAGEEVA	8	37.41	0.24	40.22	0.23	40.02	0.98
9	35-41	VAIKLEC	6	18.91	0.52	27.50	1.32	29.66	0.15
10	37-49	IKLECVKTKHPQL	11	22.07	0.68	23.48	0.97	22.36	0.18
11	42-49	VKTKHPQL	6	28.99	1.53	29.98	1.37	29.46	1.78

12	50-58	HIESKIYKM	8	19.69	1.35	30.61	0.66	29.57	0.00
13	56-70	YKMMQGGV GIPTIRW	13	30.82	2.38	39.07	2.71	42.36	0.24
14	59-77	MQGGVGIPTI RWCGAEGD Y	17	34.25	0.27	31.94	1.11	31.46	1.35
15	81-89	VMELLGPSL	7	13.75	0.93	20.72	1.52	25.82	1.15
16	83-89	ELLGPSL	5	12.43	0.69	16.30	0.58	16.42	0.49
17	83-95	ELLGPSLEDL FNF	11	39.16	1.06	41.67	0.37	42.64	0.63
18	96-106	CSRKFSLKT VL	10	10.23	0.86	14.67	0.40	15.11	0.24
19	104-119	TVLLLADQMI SRIEYI	15	26.19	0.36	34.40	0.54	38.63	0.60
20	114-123	SRIEYIHSKN	9	3.42	0.17	4.25	0.36	4.70	0.19
21	124-134	FIHRDVKPDN F	9	4.27	1.11	3.26	1.03	3.31	1.23
22	124-135	FIHRDVKPDN FL	10	4.25	0.38	5.16	0.44	4.56	0.85
23	135-144	LMGLGKKGN L	9	22.52	1.48	27.17	0.95	25.78	2.63
24	135-145	LMGLGKKGN LV	10	22.65	1.04	27.14	0.93	26.63	0.97
25	135-146	LMGLGKKGN LVY	11	17.18	1.06	21.06	0.50	19.64	2.23
26	136-144	MGLGKKGNL	8	25.57	0.22	26.88	0.74	25.27	2.00
27	136-146	MGLGKKGNL VY	10	20.56	1.13	25.10	1.49	23.48	2.84
28	145-152	VYIIDFGL	7	13.47	1.70	27.37	1.13	29.08	0.90
29	151-160	GLAKKYRDA R	9	48.79	1.00	53.25	1.00	51.34	1.00
30	160-166	RTHQHIP	5	17.67	0.51	25.35	1.35	31.69	0.38
31	163-178	QHIPYRENK NLTGTAR	14	26.00	0.20	30.92	0.20	35.37	0.86
32	180-186	ASINTHL	6	13.51	0.61	24.11	0.74	25.61	2.59
33	183-196	NTHLGIEQSR RDDL	13	10.50	0.46	16.48	0.18	18.49	0.71
34	187-196	GIEQSRRDD L	9	11.32	0.52	16.25	0.31	17.62	0.27
35	204-218	MYFNLGSLP WQGLKA	13	40.16	0.11	42.90	0.11	40.65	0.11
36	205-215	YFNLGSLPW QG	9	28.42	0.50	36.54	1.44	38.34	1.09
37	205-216	YFNLGSLPW QGL	10	29.46	1.39	36.79	0.84	37.87	1.50
38	205-218	YFNLGSLPW QGLKA	12	36.58	0.97	42.67	1.72	42.04	3.53
39	206-216	FNLGSLPWQ GL	9	33.07	1.14	42.59	0.90	43.60	0.97
40	206-218	FNLGSLPWQ GLKA	11	43.88	1.25	49.49	1.35	51.23	2.52
41	209-216	GSLPWQGL	6	45.23	1.29	49.28	1.21	53.10	1.72
42	209-218	GSLPWQGLK A	8	47.50	1.62	48.88	1.86	50.02	1.32
43	212-218	PWQGLKA	5	55.12	0.82	55.98	0.71	57.12	0.94
44	219-230	ATKRQKYERI SE	11	41.55	1.04	41.43	0.51	40.95	0.12

45	231-238	KKMSTPIE	6	40.66	1.09	41.80	1.22	41.34	1.70
46	231-240	KKMSTPIEVL	8	29.52	1.64	34.63	1.62	32.68	1.56
47	239-248	VLCKGYPSE F	8	13.20	0.50	19.14	0.35	19.42	2.96
48	241-247	CKGYPSE	5	22.27	0.44	27.46	1.24	31.92	1.61
49	241-248	CKGYPSEF	6	16.57	1.09	20.77	0.71	22.70	3.17
50	242-259	KGYPSEFAT YLNFCRSLR	16	32.03	0.43	33.22	0.31	32.70	1.05
51	248-254	FATYLNF	6	2.54	0.53	2.77	0.73	3.00	0.64
52	255-269	CRSLRFDDK PDYSYL	13	26.00	0.45	28.44	0.95	27.21	1.70
53	261-269	DDKPDYSYL	7	33.28	1.57	33.18	1.49	30.93	1.84
54	269-277	LRQLFRNLF	8	3.89	1.63	7.88	1.45	8.40	1.78
55	270-277	RQLFRNLF	7	4.50	0.38	8.94	0.63	8.72	1.70
56	273-285	FRNLFHRQG FSYD	12	19.52	1.52	21.49	1.03	23.49	1.91
57	278-285	HRQGFSYD	7	31.12	1.15	31.62	0.83	28.91	1.73
58	286-292	YVFDWNM	6	37.77	2.41	41.97	0.45	44.08	1.13
59	293-305	LKFGASRAA DDAE	12	39.89	1.48	40.37	0.67	40.75	0.14
60	293-312	LKFGASRAA DDAERERRD RE	19	40.60	0.42	40.70	0.23	40.33	0.64
61	304-320	AERERRDRE ERLRHSRN	16	35.69	0.42	35.26	1.72	34.72	0.22
62	313-327	ERLRHSRNP ATRGLP	12	16.50	0.54	17.17	0.18	17.18	0.18
63	316-335	RHSRNPATR GLPSTASGR LR	17	29.75	0.57	36.60	0.55	36.77	1.78
64	337-348	TQEVAPPTP LTP	7	25.37	0.94	25.92	0.97	27.38	1.22
65	346-358	LTPTSHTANT SPR	10	37.42	1.53	35.13	0.53	37.15	0.00
66	348-354	PTSHTAN	5	11.27	0.60	11.44	0.57	10.32	1.17
67	351-357	HTANTSP	5	23.61	0.52	23.26	0.78	23.69	1.43
68	351-365	HTANTSPRP VSGMER	12	39.70	1.03	38.94	0.95	36.95	0.32
69	354-367	NTSPRPVSG MERER	11	44.22	1.49	49.56	0.68	50.83	1.34
70	365-371	RERKVSM	6	41.12	1.53	42.59	0.99	42.32	1.43
71	378-385	PVNISSSD	6	12.54	0.66	12.78	0.28	11.66	0.84
72	379-395	VNISSSDLTG RQDTSRM	16	36.50	0.92	35.90	0.18	34.69	1.14
73	386-394	LTGRQDTSR	8	54.42	1.36	60.01	1.49	61.26	0.21
				CK1δ1-Δ400 + ATP					
				Relative Fractional Deuterium Uptake (%)					
				1 min		**5 min**		**10 min**	
S.No.	**Peptide**	**Sequence**	**Num Exchangers**	**Uptake**	**± SD**	**Uptake**	**± SD**	**Uptake**	**± SD**
1	1-21	MELRVGNRY RLGRKIGSG SFG	20	25.14	0.01	27.61	1.20	28.08	0.01

2	4-20	RVGNRYRLG RKIGSGSF	16	33.40	0.25	36.80	0.37	38.49	1.62
3	4-22	RVGNRYRLG RKIGSGSFG D	18	33.53	1.13	35.80	0.56	38.34	0.27
4	12-25	GRKIGSGSF GDIYL	13	32.19	0.69	29.85	0.62	30.81	0.49
5	21-28	GDIYLGTD	7	9.22	0.47	12.65	0.46	18.11	1.46
6	26-34	GTDIAAGEE	8	42.05	0.96	45.41	0.64	45.16	0.47
7	28-34	DIAAGEE	6	42.33	0.26	42.93	0.23	43.45	0.78
8	28-36	DIAAGEEVA	8	36.04	0.40	35.43	0.67	34.92	0.40
9	35-41	VAIKLEC	6	13.95	1.06	14.56	0.44	15.23	0.52
10	37-49	IKLECVKTKH PQL	11	20.27	0.17	21.91	0.36	22.00	0.62
11	42-49	VKTKHPQL	6	28.49	1.13	29.10	1.06	30.52	1.29
12	50-58	HIESKIYKM	8	15.78	1.22	22.38	1.37	25.12	1.73
13	56-70	YKMMQGGV GIPTIRW	13	31.11	1.20	29.57	0.10	35.24	2.30
14	59-77	MQGGVGIPTI RWCGAEGD Y	17	28.71	1.14	28.66	0.99	32.27	0.25
15	81-89	VMELLGPSL	7	5.14	1.19	4.07	0.58	5.91	0.65
16	83-89	ELLGPSL	5	5.94	0.43	4.46	0.46	4.63	0.58
17	83-95	ELLGPSLEDL FNF	11	36.88	0.84	39.37	1.50	41.12	0.37
18	96-106	CSRKFSLKT VL	10	9.71	0.39	11.87	0.25	13.65	0.21
19	104-119	TVLLLADQMI SRIEYI	15	18.74	0.78	24.20	0.66	27.03	0.38
20	114-123	SRIEYIHSKN	9	3.55	0.12	3.58	0.12	2.61	0.28
21	124-134	FIHRDVKPDN F	9	2.31	1.22	3.35	1.19	3.59	0.96
22	124-135	FIHRDVKPDN FL	10	4.33	0.43	4.56	0.60	5.01	0.44
23	135-144	LMGLGKKGN L	9	22.72	1.47	25.65	1.04	26.40	0.83
24	135-145	LMGLGKKGN LV	10	22.30	1.35	24.77	1.40	26.93	1.05
25	135-146	LMGLGKKGN LVY	11	17.64	0.93	20.61	0.65	22.57	0.43
26	136-144	MGLGKKGNL	8	23.49	0.75	26.21	0.91	27.78	0.99
27	136-146	MGLGKKGNL VY	10	21.32	1.45	23.42	1.22	26.00	1.24
28	145-152	VYIIDFGL	7	4.13	2.32	4.50	1.98	5.05	1.93
29	151-160	GLAKKYRDA R	9	43.80	1.00	50.72	1.06	47.10	1.01
30	160-166	RTHQHIP	5	19.83	1.74	25.52	0.47	30.10	0.25
31	163-178	QHIPYRENK NLTGTAR	14	19.45	0.78	23.13	1.26	24.06	2.28
32	180-186	ASINTHL	6	12.42	0.67	20.12	0.43	24.95	0.81
33	183-196	NTHLGIEQSR RDDL	13	10.63	0.63	14.44	0.26	16.95	0.69
34	187-196	GIEQSRRDD L	9	11.47	0.53	14.18	0.28	16.17	0.40

35	204-218	MYFNLGSLPWQGLKA	13	38.41	0.35	40.70	0.11	NaN	NaN
36	205-215	YFNLGSLPWQG	9	27.78	0.65	32.40	1.63	36.64	2.29
37	205-216	YFNLGSLPWQGL	10	28.52	0.93	35.49	0.11	36.02	1.70
38	205-218	YFNLGSLPWQGLKA	12	36.89	1.63	40.02	1.60	43.13	1.43
39	206-216	FNLGSLPWQGL	9	33.45	1.02	38.65	1.43	42.94	2.02
40	206-218	FNLGSLPWQGLKA	11	43.31	0.59	47.67	0.75	50.90	0.41
41	209-216	GSLPWQGL	6	44.72	1.55	47.19	0.59	50.19	0.60
42	209-218	GSLPWQGLKA	8	45.24	1.15	47.83	1.65	50.87	1.24
43	212-218	PWQGLKA	5	53.11	1.12	54.24	0.43	54.74	0.91
44	219-230	ATKRQKYERISE	11	38.05	0.78	40.38	1.15	41.52	1.08
45	231-238	KKMSTPIE	6	40.62	0.26	41.18	1.16	42.03	1.15
46	231-240	KKMSTPIEVL	8	30.17	1.58	34.37	1.55	37.23	1.67
47	239-248	VLCKGYPSEF	8	14.74	0.77	19.02	0.36	22.87	1.33
48	241-247	CKGYPSE	5	22.71	0.17	26.22	0.58	31.44	1.16
49	241-248	CKGYPSEF	6	17.28	0.68	20.37	0.85	24.05	1.18
50	242-259	KGYPSEFATYLNFCRSLR	16	31.02	2.06	33.15	0.35	34.19	1.66
51	248-254	FATYLNF	6	1.06	0.58	1.73	0.64	2.32	0.78
52	255-269	CRSLRFDDKPDYSYL	13	NaN	NaN	28.82	0.52	30.24	0.62
53	261-269	DDKPDYSYL	7	32.09	1.72	31.02	1.74	31.98	1.15
54	269-277	LRQLFRNLF	8	3.40	1.55	6.17	1.37	8.53	1.17
55	270-277	RQLFRNLF	7	3.26	0.39	5.55	0.46	8.08	0.56
56	273-285	FRNLFHRQGFSYD	12	19.03	1.28	20.76	1.14	21.90	1.19
57	278-285	HRQGFSYD	7	30.40	1.35	31.31	1.70	31.01	1.28
58	286-292	YVFDWNM	6	36.75	2.29	40.13	1.95	44.34	1.79
59	293-305	LKFGASRAADDAE	12	42.54	0.86	42.60	1.03	42.89	1.42
60	293-312	LKFGASRAADDAERERRDRE	19	43.41	0.30	43.01	0.24	43.33	0.35
61	304-320	AERERRDREERLRHSRN	16	34.63	0.23	35.53	0.23	38.19	0.32
62	313-327	ERLRHSRNPATRGLP	12	14.80	0.21	17.51	0.28	18.93	0.18
63	316-335	RHSRNPATRGLPSTASGRLR	17	27.84	0.72	31.22	0.50	33.14	1.04
64	337-348	TQEVAPPTPLTP	7	24.59	1.88	25.07	1.40	24.83	0.51
65	346-358	LTPTSHTANTSPR	10	36.43	0.00	36.91	0.00	36.95	0.00
66	348-354	PTSHTAN	5	10.50	0.60	10.27	0.61	10.91	0.98
67	351-357	HTANTSP	5	22.55	0.37	24.05	0.64	23.40	0.60

68	351-365	HTANTSPRP VSGMER	12	41.48	0.68	42.30	0.11	41.20	0.44
69	354-367	NTSPRPVSG MERER	11	43.85	0.89	48.16	0.68	51.36	0.87
70	365-371	RERKVSM	6	42.67	1.45	40.65	0.60	42.38	0.85
71	378-385	PVNISSSD	6	12.46	0.33	13.15	0.52	13.58	0.57
72	379-395	VNISSSDLTG RQDTSRM	16	36.61	0.40	38.46	0.65	39.16	0.33
73	386-394	LTGRQDTSR	8	53.62	1.45	58.36	2.10	61.48	0.97
				CK1δ1-Δ400 + AMPPNP					
				Relative Fractional Deuterium Uptake (%)					
				1 min		**5 min**		**10 min**	
S.No.	**Peptide**	**Sequence**	**Num Exchangers**	**Uptake**	**± SD**	**Uptake**	**± SD**	**Uptake**	**± SD**
1	1-21	MELRVGNRY RLGRKIGSG SFG	20	29.73	0.52	36.64	0.29	36.97	0.78
2	4-20	RVGNRYRLG RKIGSGSF	16	35.78	0.44	39.58	0.56	39.86	0.71
3	4-22	RVGNRYRLG RKIGSGSFG D	18	35.46	0.38	39.83	0.80	39.26	0.38
4	12-25	GRKIGSGSF GDIYL	13	38.05	0.23	40.85	0.94	38.88	0.23
5	21-28	GDIYLGTD	7	16.08	0.95	34.36	0.68	39.65	1.56
6	26-34	GTDIAAGEE	8	46.18	0.34	46.76	0.46	45.94	0.79
7	28-34	DIAAGEE	6	43.71	0.73	42.93	0.88	43.00	0.96
8	28-36	DIAAGEEVA	8	36.71	0.38	38.07	0.35	38.49	0.76
9	35-41	VAIKLEC	6	15.97	0.49	19.01	1.34	20.59	1.52
10	37-49	IKLECVKTKH PQL	11	21.97	0.67	23.18	0.63	22.78	0.68
11	42-49	VKTKHPQL	6	29.44	0.96	28.98	0.83	28.63	0.20
12	50-58	HIESKIYKM	8	17.26	3.23	29.58	1.37	30.58	0.62
13	56-70	YKMMQGGV GIPTIRW	13	32.61	1.31	36.71	0.10	36.24	0.10
14	59-77	MQGGVGIPTI RWCGAEGD Y	17	30.49	0.61	31.43	0.25	31.46	0.25
15	81-89	VMELLGPSL	7	8.29	1.65	14.34	1.24	17.12	2.33
16	83-89	ELLGPSL	5	8.42	1.07	13.96	0.58	16.66	0.86
17	83-95	ELLGPSLEDL FNF	11	39.54	0.52	41.27	0.37	39.39	0.44
18	96-106	CSRKFSLKT VL	10	8.67	0.36	13.37	0.21	13.89	0.59
19	104-119	TVLLLADQMI SRIEYI	15	23.10	0.54	30.93	0.49	33.20	0.51
20	114-123	SRIEYIHSKN	9	3.23	0.42	3.63	0.12	4.70	0.67
21	124-134	FIHRDVKPDN F	9	3.57	0.96	4.36	0.97	4.56	0.97
22	124-135	FIHRDVKPDN FL	10	4.03	0.42	5.62	0.62	5.87	0.46
23	135-144	LMGLGKKGN L	9	24.22	0.84	28.44	1.33	29.98	1.13

24	135-145	LMGLGKKGNLV	10	25.18	0.86	28.55	1.76	30.27	0.94
25	135-146	LMGLGKKGNLVY	11	19.71	0.40	23.27	0.73	24.53	0.51
26	136-144	MGLGKKGNL	8	24.80	0.69	29.05	0.95	30.25	0.44
27	136-146	MGLGKKGNLVY	10	22.06	1.16	25.48	1.28	27.15	1.26
28	145-152	VYIIDFGL	7	6.46	1.14	18.26	2.45	24.35	1.02
29	151-160	GLAKKYRDAR	9	53.52	1.03	56.39	1.00	56.87	1.00
30	160-166	RTHQHIP	5	23.15	0.15	26.33	1.87	28.11	1.25
31	163-178	QHIPYRENKNLTGTAR	14	23.73	0.20	28.52	0.77	30.49	0.20
32	180-186	ASINTHL	6	17.00	0.59	29.62	0.98	32.85	0.55
33	183-196	NTHLGIEQSRRDDL	13	11.92	0.17	18.82	0.59	20.02	0.24
34	187-196	GIEQSRRDDL	9	11.65	0.31	16.60	0.49	17.76	0.33
35	204-218	MYFNLGSLPWQGLKA	13	40.04	0.11	44.36	0.20	45.73	0.14
36	205-215	YFNLGSLPWQG	9	29.18	0.19	38.98	0.16	39.73	1.01
37	205-216	YFNLGSLPWQGL	10	30.97	0.42	38.55	1.55	37.42	0.96
38	205-218	YFNLGSLPWQGLKA	12	37.60	0.74	44.67	1.06	44.75	0.84
39	206-216	FNLGSLPWQGL	9	36.77	1.45	44.13	0.99	44.05	1.03
40	206-218	FNLGSLPWQGLKA	11	44.24	0.36	50.72	1.06	51.75	0.65
41	209-216	GSLPWQGL	6	48.13	1.84	52.14	1.13	51.80	0.70
42	209-218	GSLPWQGLKA	8	48.07	1.51	51.42	1.79	51.20	1.25
43	212-218	PWQGLKA	5	54.49	0.73	55.69	0.54	58.31	0.40
44	219-230	ATKRQKYERISE	11	39.96	2.73	40.76	1.77	40.00	0.54
45	231-238	KKMSTPIE	6	39.57	0.59	41.09	1.35	39.22	0.93
46	231-240	KKMSTPIEVL	8	32.50	1.49	37.49	1.46	38.98	1.52
47	239-248	VLCKGYPSEF	8	16.35	0.44	22.18	0.69	25.64	0.62
48	241-247	CKGYPSE	5	23.16	0.73	28.82	0.55	32.94	1.00
49	241-248	CKGYPSEF	6	18.18	0.86	23.24	1.17	25.78	0.77
50	242-259	KGYPSEFATYLNFCRSLR	16	33.66	1.35	33.68	1.96	33.23	0.35
51	248-254	FATYLNF	6	0.45	0.47	1.70	0.58	2.34	0.47
52	255-269	CRSLRFDDKPDYSYL	13	27.96	0.35	29.98	0.71	30.20	0.60
53	261-269	DDKPDYSYL	7	31.11	1.38	31.26	2.25	29.69	1.33
54	269-277	LRQLFRNLF	8	3.75	1.46	7.74	1.47	10.18	1.50
55	270-277	RQLFRNLF	7	4.09	0.53	7.42	0.56	10.01	0.72
56	273-285	FRNLFHRQGFSYD	12	19.58	1.40	20.85	1.83	21.25	1.14
57	278-285	HRQGFSYD	7	29.03	2.03	30.46	2.50	28.52	1.81
58	286-292	YVFDWNM	6	37.56	2.79	42.83	1.66	41.73	1.31

59	293-305	LKFGASRAADDAE	12	42.14	0.14	44.17	0.14	41.56	1.58
60	293-312	LKFGASRAADDAERERRDRE	19	41.20	0.65	41.70	0.34	41.79	0.26
61	304-320	AERERRDREERLRHSRN	16	36.66	0.28	36.39	0.40	35.89	0.25
62	313-327	ERLRHSRNPATRGLP	12	14.95	0.18	19.70	0.29	19.82	0.18
63	316-335	RHSRNPATRGLPSTASGRLR	17	32.00	1.10	NaN	NaN	39.99	1.25
64	337-348	TQEVAPPTPLTP	7	25.91	1.07	26.88	1.27	26.10	1.30
65	346-358	LTPTSHTANTSPR	10	37.07	0.32	37.02	0.00	39.63	0.00
66	348-354	PTSHTAN	5	11.59	0.67	11.01	0.80	11.76	0.75
67	351-357	HTANTSP	5	23.83	0.21	24.94	0.49	23.66	0.51
68	351-365	HTANTSPRPVSGMER	12	43.13	0.60	43.08	0.79	42.99	0.09
69	354-367	NTSPRPVSGMERER	11	44.86	0.76	50.95	0.70	53.04	0.94
70	365-371	RERKVSM	6	39.46	0.94	41.07	0.85	40.08	0.63
71	378-385	PVNISSSD	6	13.02	0.27	13.53	0.24	13.44	0.35
72	379-395	VNISSSDLTGRQDTSRM	16	39.25	0.22	40.31	0.88	39.10	0.22
73	386-394	LTGRQDTSR	8	55.22	1.12	60.63	0.40	60.41	2.33

				CK1δ1-S3A					
				Relative Deuterium Uptake (Da)					
				1 min		**5 min**		**10 min**	
S.No.	**Peptide**	**Sequence**	**Num Exchangers**	**Uptake**	**± SD**	**Uptake**	**± SD**	**Uptake**	**± SD**
1	1-20	MELRVGNRYRLGRKIGSGSF	19	6.15	0.27	6.76	0.23	6.74	0.23
2	1-21	MELRVGNRYRLGRKIGSGSFG	20	5.66	0.34	7.29	0.29	7.29	0.22
3	3-20	LRVGNRYRLGRKIGSGSF	17	5.79	0.09	6.40	0.02	6.03	0.54
4	3-22	LRVGNRYRLGRKIGSGSFGD	19	6.09	0.05	6.68	0.19	6.80	0.21
5	3-24	LRVGNRYRLGRKIGSGSFGDIY	21	5.85	0.48	7.24	0.08	7.55	0.15
6	4-20	RVGNRYRLGRKIGSGSF	16	5.16	0.05	5.67	0.14	5.83	0.14
7	4-22	RVGNRYRLGRKIGSGSFGD	18	5.77	0.21	6.24	0.22	6.25	0.23
8	12-25	GRKIGSGSFGDIYL	13	4.22	0.15	4.55	0.11	5.50	0.29
9	21-28	GDIYLGTD	7	1.47	0.12	2.01	0.09	2.85	0.28
10	26-34	GTDIAAGEE	8	3.29	0.03	3.36	0.03	3.07	0.25
11	28-34	DIAAGEE	6	2.32	0.07	2.39	0.07	2.19	0.06
12	28-36	DIAAGEEVA	8	2.65	0.06	2.90	0.04	3.15	0.06

13	29-36	IAAGEEVA	7	1.99	0.03	2.27	0.03	2.34	0.03
14	37-49	IKLECVKTKHPQL	11	2.84	0.08	2.73	0.37	3.05	0.34
15	42-49	VKTKHPQL	6	1.25	0.06	1.27	0.07	1.21	0.10
16	56-72	YKMMQGGVGIPTIRWCG	15	4.40	0.43	4.07	0.46	3.66	0.04
17	59-70	MQGGVGIPTIRW	10	4.32	0.20	4.33	0.43	4.34	0.44
18	83-89	ELLGPSL	5	0.56	0.02	0.75	0.02	0.84	0.04
19	96-102	CSRKFSL	6	0.87	0.02	1.03	0.10	1.27	0.02
20	96-106	CSRKFSLKTVL	10	0.95	0.05	1.22	0.10	1.40	0.11
21	103-117	KTVLLLADQMISRIE	14	4.34	0.02	4.97	0.02	5.59	0.11
22	104-119	TVLLLADQMISRIEYI	15	3.55	0.23	4.52	0.10	5.24	0.05
23	112-123	MISRIEYIHSKN	11	0.26	0.05	0.41	0.10	1.17	0.03
24	113-123	ISRIEYIHSKN	10	0.13	0.08	0.22	0.09	0.28	0.06
25	113-134	ISRIEYIHSKNFIHRDVKPDNF	20	0.56	0.07	0.77	0.11	1.01	0.07
26	124-134	FIHRDVKPDNF	9	0.26	0.01	0.30	0.02	0.36	0.01
27	124-135	FIHRDVKPDNFL	10	0.26	0.02	0.29	0.02	0.41	0.03
28	135-143	LMGLGKKGN	8	1.54	0.12	1.88	0.10	2.07	0.14
29	135-144	LMGLGKKGNL	9	1.69	0.14	1.95	0.14	2.18	0.16
30	135-146	LMGLGKKGNLVY	11	1.64	0.11	1.97	0.10	2.26	0.11
31	136-146	MGLGKKGNLVY	10	1.73	0.11	2.12	0.09	2.25	0.13
32	137-144	GLGKKGNL	7	1.48	0.08	1.84	0.17	1.97	0.20
33	152-161	LAKKYRDART	9	1.25	0.01	1.49	0.17	1.89	0.02
34	163-178	QHIPYRENKNLTGTAR	14	2.99	0.08	3.39	0.03	4.55	0.12
35	175-186	GTARYASINTHL	11	1.69	0.30	1.50	0.26	1.81	0.29
36	180-186	ASINTHL	6	0.74	0.07	1.35	0.06	1.53	0.06
37	180-196	ASINTHLGIEQSRRDDL	16	1.54	0.03	2.58	0.02	3.07	0.06
38	183-196	NTHLGIEQSRRDDL	13	1.34	0.04	2.40	0.08	2.70	0.10
39	187-196	GIEQSRRDDL	9	0.73	0.05	1.15	0.07	1.23	0.14
40	192-211	RRDDLESLGYVLMYFNLGSL	19	2.61	0.11	2.95	0.10	3.22	0.23
41	205-216	YFNLGSLPWQGL	10	3.18	0.08	3.76	0.13	4.03	0.15
42	205-218	YFNLGSLPWQGLKA	12	4.37	0.10	4.93	0.12	5.10	0.17
43	206-218	FNLGSLPWQGLKA	11	4.61	0.05	5.16	0.06	5.23	0.09
44	207-218	NLGSLPWQGLKA	10	4.22	0.09	4.98	0.13	5.10	0.16

45	209-218	GSLPWQGLKA	8	3.61	0.05	3.76	0.11	3.92	0.13
46	212-230	PWQGLKAATKRQKYERISE	17	5.84	0.14	7.00	0.19	7.10	0.09
47	231-240	KKMSTPIEVL	8	2.22	0.09	2.60	0.10	2.77	0.12
48	239-248	VLCKGYPSEF	8	1.00	0.04	1.28	0.05	1.71	0.03
49	241-247	CKGYPSE	5	0.97	0.02	1.19	0.04	1.44	0.06
50	241-248	CKGYPSEF	6	0.83	0.02	1.18	0.05	1.25	0.04
51	242-259	KGYPSEFATYLNFCRSLR	16	4.52	0.28	4.66	0.11	4.49	0.24
52	248-254	FATYLNF	6	0.07	0.01	0.15	0.02	0.20	0.02
53	255-268	CRSLRFDDKPDYSY	12	3.53	0.21	3.60	0.18	4.19	0.16
54	255-269	CRSLRFDDKPDYSYL	13	3.28	0.16	3.42	0.08	3.45	0.05
55	261-269	DDKPDYSYL	7	2.26	0.09	2.24	0.11	2.20	0.14
56	268-277	YLRQLFRNLF	9	0.18	0.06	0.58	0.07	0.86	0.06
57	269-277	LRQLFRNLF	8	0.23	0.02	0.49	0.02	0.74	0.02
58	269-281	LRQLFRNLFHRQG	12	2.09	0.07	2.07	0.04	2.40	0.16
59	269-285	LRQLFRNLFHRQGFSYD	16	2.28	0.10	2.64	0.10	2.84	0.14
60	270-276	RQLFRNL	6	1.59	0.02	2.31	0.09	2.50	0.07
61	272-285	LFRNLFHRQGFSYD	13	2.34	0.01	2.47	0.07	2.62	0.13
62	273-285	FRNLFHRQGFSYD	12	2.31	0.11	2.37	0.12	2.45	0.16
63	278-285	HRQGFSYD	7	1.88	0.11	1.79	0.11	1.79	0.16
64	286-292	YVFDWNM	6	2.66	0.14	2.91	0.16	2.86	0.18
65	293-305	LKFGASRAADDAE	12	4.12	0.11	4.00	0.13	4.15	0.13
66	304-320	AERERRDREERLRHSRN	16	4.62	0.27	4.58	0.15	4.69	0.17
67	312-324	EERLRHSRNPATR	11	3.76	0.14	3.49	0.28	3.52	0.11
68	313-327	ERLRHSRNPATRGLP	12	1.47	0.09	1.69	0.10	1.89	0.07
69	315-325	LRHSRNPATRG	9	0.44	0.05	0.42	0.14	0.61	0.04
70	322-336	ATRGLPSTASGRLRG	13	1.81	0.24	1.89	0.18	2.14	0.29
71	325-334	GLPSTASGRL	8	0.76	0.08	0.98	0.22	1.50	0.12
72	338-349	QEVAPPTPLTPT	7	1.74	0.16	1.64	0.10	1.60	0.07
73	343-351	PTPLTPTSH	5	1.20	0.14	1.64	0.04	1.94	0.19
74	343-356	PTPLTPTSHTANTS	10	0.34	0.01	0.34	0.07	0.35	0.03
75	344-355	TPLTPTSHTANT	9	1.85	0.08	1.88	0.07	1.76	0.06
76	349-367	TSHTANTSPRPVSGMERER	16	4.28	0.11	4.10	0.23	3.99	0.18
77	363-376	MERERKVSMRLHRG	13	2.39	0.07	3.32	0.12	3.71	0.08

78	372-384	RLHRGAPVNISSS	11	2.45	0.09	2.72	0.10	2.69	0.03
79	378-385	PVNISSSD	6	1.01	0.05	0.91	0.06	1.07	0.05
80	385-405	DLTGRQDTSRMSTSQIPGRVA	19	2.39	0.07	2.74	0.13	2.91	0.20
81	402-413	GRVAAAGLQAVV	11	1.73	0.29	2.02	0.22	2.04	0.03
				CK1δ1-S3A + ATP					
				Relative Deuterium Uptake (Da)					
				1 min		**5 min**		**10 min**	
S.No.	**Peptide**	**Sequence**	**Num Exchangers**	**Uptake**	**± SD**	**Uptake**	**± SD**	**Uptake**	**± SD**
1	1-20	MELRVGNRYRLGRKIGSGSF	19	NaN	NaN	NaN	NaN	NaN	NaN
2	1-21	MELRVGNRYRLGRKIGSGSFG	20	4.09	0.56	5.31	0.08	5.90	0.44
3	3-20	LRVGNRYRLGRKIGSGSF	17	5.50	0.21	5.98	0.19	6.10	0.29
4	3-22	LRVGNRYRLGRKIGSGSFGD	19	4.23	0.02	5.20	0.35	5.94	0.02
5	3-24	LRVGNRYRLGRKIGSGSFGDIY	21	5.08	0.33	5.28	0.15	5.82	0.07
6	4-20	RVGNRYRLGRKIGSGSF	16	4.68	0.19	5.05	0.43	5.20	0.31
7	4-22	RVGNRYRLGRKIGSGSFGD	18	5.05	0.20	5.26	0.12	5.97	0.15
8	12-25	GRKIGSGSFGDIYL	13	3.89	0.08	3.59	0.15	3.98	0.39
9	21-28	GDIYLGTD	7	0.41	0.12	0.87	0.11	1.24	0.11
10	26-34	GTDIAAGEE	8	3.27	0.02	3.18	0.02	3.40	0.02
11	28-34	DIAAGEE	6	1.76	0.14	1.82	0.15	1.66	0.05
12	28-36	DIAAGEEVA	8	2.40	0.16	2.72	0.20	3.02	0.21
13	29-36	IAAGEEVA	7	1.78	0.12	1.74	0.10	2.01	0.14
14	37-49	IKLECVKTKHPQL	11	2.86	0.16	3.21	0.19	3.18	0.06
15	42-49	VKTKHPQL	6	0.83	0.18	0.95	0.02	0.97	0.11
16	56-72	YKMMQGGVGIPTIRWCG	15	3.94	0.04	3.64	0.04	3.75	0.04
17	59-70	MQGGVGIPTIRW	10	3.13	0.26	2.86	0.13	2.81	0.06
18	83-89	ELLGPSL	5	0.21	0.10	0.41	0.18	0.71	0.07
19	96-102	CSRKFSL	6	0.56	0.09	0.75	0.12	0.96	0.24
20	96-106	CSRKFSLKTVL	10	0.78	0.18	1.07	0.05	1.12	0.13
21	103-117	KTVLLLADQMISRIE	14	3.74	0.00	4.00	0.00	4.31	0.00
22	104-119	TVLLLADQMISRIEYI	15	2.19	0.13	3.06	0.04	3.57	0.11
23	112-123	MISRIEYIHSKN	11	0.60	0.33	0.75	0.29	0.86	0.24

24	113-123	ISRIEYIHSKN	10	0.36	0.25	0.40	0.02	0.23	0.06
25	113-134	ISRIEYIHSKN FIHRDVKPDN F	20	0.20	0.05	0.55	0.03	0.66	0.06
26	124-134	FIHRDVKPDN F	9	0.26	0.04	0.32	0.05	0.40	0.11
27	124-135	FIHRDVKPDN FL	10	0.47	0.08	0.50	0.09	0.60	0.06
28	135-143	LMGLGKKGN	8	1.57	0.11	1.66	0.08	1.97	0.14
29	135-144	LMGLGKKGN L	9	1.62	0.14	1.81	0.11	2.09	0.17
30	135-146	LMGLGKKGN LVY	11	1.65	0.13	1.77	0.09	2.04	0.13
31	136-146	MGLGKKGNL VY	10	1.73	0.15	1.83	0.11	2.03	0.12
32	137-144	GLGKKGNL	7	1.42	0.16	1.52	0.13	1.65	0.05
33	152-161	LAKKYRDAR T	9	0.23	0.05	0.14	0.03	0.19	0.05
34	163-178	QHIPYRENK NLTGTAR	14	2.54	0.05	3.01	0.06	3.49	0.04
35	175-186	GTARYASINT HL	11	2.17	0.22	2.01	0.02	2.04	0.02
36	180-186	ASINTHL	6	0.68	0.02	1.09	0.05	1.33	0.08
37	180-196	ASINTHLGIE QSRRDDL	16	1.70	0.08	1.33	0.02	1.53	0.34
38	183-196	NTHLGIEQSR RDDL	13	0.93	0.03	1.32	0.26	1.61	0.03
39	187-196	GIEQSRRDD L	9	0.90	0.17	1.16	0.07	1.23	0.11
40	192-211	RRDDLESLG YVLMYFNLG SL	19	2.68	0.16	2.90	0.10	3.11	0.13
41	205-216	YFNLGSLPW QGL	10	1.93	0.24	2.27	0.61	3.05	0.01
42	205-218	YFNLGSLPW QGLKA	12	3.21	0.19	3.62	0.35	3.53	0.07
43	206-218	FNLGSLPWQ GLKA	11	3.68	0.00	4.21	0.00	4.18	0.01
44	207-218	NLGSLPWQG LKA	10	3.75	0.11	4.35	0.04	4.20	0.21
45	209-218	GSLPWQGLK A	8	3.38	0.13	3.39	0.10	3.56	0.17
46	212-230	PWQGLKAAT KRQKYERISE	17	5.98	0.15	6.15	0.10	6.23	0.07
47	231-240	KKMSTPIEVL	8	2.22	0.14	2.50	0.12	2.75	0.12
48	239-248	VLCKGYPSE F	8	1.05	0.09	1.36	0.06	1.66	0.05
49	241-247	CKGYPSE	5	0.85	0.11	1.22	0.09	1.60	0.05
50	241-248	CKGYPSEF	6	1.08	0.05	1.26	0.01	1.48	0.05
51	242-259	KGYPSEFAT YLNFCRSLR	16	4.28	0.15	3.96	0.06	4.24	0.16
52	248-254	FATYLNF	6	0.33	0.16	0.23	0.03	0.54	0.01
53	255-268	CRSLRFDDK PDYSY	12	3.37	0.16	3.49	0.12	3.62	0.35
54	255-269	CRSLRFDDK PDYSYL	13	3.15	0.12	3.16	0.04	3.39	0.09
55	261-269	DDKPDYSYL	7	2.00	0.18	1.75	0.10	1.81	0.20
56	268-277	YLRQLFRNLF	9	NaN	NaN	NaN	NaN	NaN	NaN

57	269-277	LRQLFRNLF	8	0.20	0.03	0.73	0.07	0.63	0.03
58	269-281	LRQLFRNLF HRQG	12	2.06	0.20	2.15	0.20	2.46	0.08
59	269-285	LRQLFRNLF HRQGFSYD	16	2.08	0.12	2.09	0.08	2.41	0.12
60	270-276	RQLFRNL	6	1.34	0.07	2.06	0.04	2.33	0.05
61	272-285	LFRNLFHRQ GFSYD	13	2.21	0.08	2.24	0.01	2.46	0.01
62	273-285	FRNLFHRQG FSYD	12	1.97	0.18	1.86	0.08	2.12	0.17
63	278-285	HRQGFSYD	7	1.75	0.11	1.51	0.18	1.59	0.24
64	286-292	YVFDWNM	6	NaN	NaN	NaN	NaN	NaN	NaN
65	293-305	LKFGASRAA DDAE	12	3.99	0.18	3.71	0.15	3.80	0.23
66	304-320	AERERRDRE ERLRHSRN	16	4.52	0.10	4.18	0.11	4.43	0.20
67	312-324	EERLRHSRN PATR	11	3.73	0.29	3.46	0.28	3.31	0.06
68	313-327	ERLRHSRNP ATRGLP	12	1.23	0.11	1.61	0.12	1.85	0.11
69	315-325	LRHSRNPAT RG	9	0.30	0.08	0.37	0.03	0.43	0.06
70	322-336	ATRGLPSTA SGRLRG	13	1.53	0.04	1.61	0.33	2.03	0.04
71	325-334	GLPSTASGR L	8	0.59	0.03	0.58	0.03	0.59	0.03
72	338-349	QEVAPPTPL TPT	7	1.25	0.02	1.04	0.02	1.39	0.02
73	343-351	PTPLTPTSH	5	1.13	0.08	1.09	0.04	1.29	0.08
74	343-356	PTPLTPTSHT ANTS	10	1.16	0.10	1.04	0.19	1.01	0.07
75	344-355	TPLTPTSHTA NT	9	1.63	0.11	2.05	0.09	1.79	0.01
76	349-367	TSHTANTSP RPVSGMERE R	16	3.63	0.12	3.51	0.12	3.56	0.18
77	363-376	MERERKVSM RLHRG	13	2.00	0.08	2.90	0.09	3.03	0.15
78	372-384	RLHRGAPVNI SSS	11	2.17	0.03	2.38	0.13	2.61	0.13
79	378-385	PVNISSSD	6	1.18	0.06	1.15	0.05	1.23	0.05
80	385-405	DLTGRQDTS RMSTSQIPG RVA	19	2.50	0.04	2.99	0.07	3.21	0.15
81	402-413	GRVAAAGLQ AVV	11	1.69	0.03	1.81	0.03	2.15	0.08

				CK1δ1-S3A + AMPPNP					
				Relative Deuterium Uptake (Da)					
				1 min		**5 min**		**10 min**	
S.No.	**Peptide**	**Sequence**	**Num Exchangers**	**Uptake**	**± SD**	**Uptake**	**± SD**	**Uptake**	**± SD**
1	1-20	MELRVGNRY RLGRKIGSG SF	19	6.31	0.23	7.00	0.19	7.08	0.20
2	1-21	MELRVGNRY RLGRKIGSG SFG	20	5.93	0.26	6.43	0.27	7.28	0.09

3	3-20	LRVGNRYRL GRKIGSGSF	17	6.17	0.08	6.54	0.07	6.93	0.31
4	3-22	LRVGNRYRL GRKIGSGSF GD	19	6.40	0.49	6.74	0.35	7.24	0.32
5	3-24	LRVGNRYRL GRKIGSGSF GDIY	21	5.81	0.08	6.53	0.53	7.21	0.07
6	4-20	RVGNRYRLG RKIGSGSF	16	5.29	0.05	5.86	0.15	6.22	0.07
7	4-22	RVGNRYRLG RKIGSGSFG D	18	5.94	0.26	6.37	0.18	6.76	0.27
8	12-25	GRKIGSGSF GDIYL	13	4.69	0.08	4.42	0.28	5.08	0.36
9	21-28	GDIYLGTD	7	1.05	0.02	1.83	0.05	2.58	0.03
10	26-34	GTDIAAGEE	8	2.69	0.23	3.23	0.22	2.97	0.21
11	28-34	DIAAGEE	6	2.37	0.22	2.21	0.06	2.25	0.09
12	28-36	DIAAGEEVA	8	2.77	0.02	2.24	0.14	3.04	0.11
13	29-36	IAAGEEVA	7	2.01	0.02	1.94	0.01	2.13	0.04
14	37-49	IKLECVKTKH PQL	11	2.49	0.49	3.00	0.03	2.49	0.21
15	42-49	VKTKHPQL	6	1.33	0.11	1.24	0.07	1.25	0.12
16	56-72	YKMMQGGV GIPTIRWCG	15	4.94	0.47	5.08	0.16	4.26	0.31
17	59-70	MQGGVGIPTI RW	10	3.27	0.48	4.57	0.22	4.02	0.38
18	83-89	ELLGPSL	5	0.35	0.02	0.53	0.03	0.64	0.01
19	96-102	CSRKFSL	6	0.76	0.04	0.93	0.11	0.94	0.03
20	96-106	CSRKFSLKT VL	10	0.83	0.05	1.19	0.10	1.25	0.04
21	103-117	KTVLLLADQ MISRIE	14	3.80	0.10	4.33	0.01	4.72	0.02
22	104-119	TVLLLADQMI SRIEYI	15	2.92	0.05	3.87	0.16	4.23	0.26
23	112-123	MISRIEYIHSK N	11	0.77	0.03	1.05	0.29	1.82	0.03
24	113-123	ISRIEYIHSKN	10	0.21	0.09	0.15	0.09	0.21	0.09
25	113-134	ISRIEYIHSKN FIHRDVKPDN F	20	0.43	0.13	0.58	0.12	0.87	0.06
26	124-134	FIHRDVKPDN F	9	0.25	0.01	0.21	0.04	0.32	0.05
27	124-135	FIHRDVKPDN FL	10	0.38	0.06	0.34	0.04	0.49	0.05
28	135-143	LMGLGKKGN	8	1.58	0.12	1.77	0.09	2.04	0.13
29	135-144	LMGLGKKGN L	9	1.73	0.13	1.94	0.13	2.22	0.13
30	135-146	LMGLGKKGN LVY	11	1.72	0.10	1.97	0.11	2.20	0.10
31	136-146	MGLGKKGNL VY	10	1.78	0.14	1.93	0.30	2.28	0.21
32	137-144	GLGKKGNL	7	1.58	0.09	1.76	0.16	2.03	0.11
33	152-161	LAKKYRDAR T	9	0.95	0.03	1.39	0.03	1.33	0.34
34	163-178	QHIPYRENK NLTGTAR	14	2.94	0.07	3.49	0.03	4.20	0.03

35	175-186	GTARYASINTHL	11	2.39	0.02	1.64	0.28	1.77	0.02
36	180-186	ASINTHL	6	0.79	0.02	1.38	0.06	1.70	0.03
37	180-196	ASINTHLGIEQSRRDDL	16	1.53	0.15	2.11	0.02	3.50	0.02
38	183-196	NTHLGIEQSRRDDL	13	1.49	0.15	2.27	0.03	2.99	0.11
39	187-196	GIEQSRRDDL	9	0.66	0.06	1.00	0.08	1.17	0.08
40	192-211	RRDDLESLGYVLMYFNLGSL	19	2.32	0.25	2.90	0.13	3.12	0.08
41	205-216	YFNLGSLPWQGL	10	3.09	0.19	3.60	0.05	3.97	0.10
42	205-218	YFNLGSLPWQGLKA	12	4.32	0.10	4.87	0.13	5.23	0.10
43	206-218	FNLGSLPWQGLKA	11	4.18	0.07	5.06	0.16	5.43	0.08
44	207-218	NLGSLPWQGLKA	10	4.23	0.22	4.86	0.13	5.18	0.09
45	209-218	GSLPWQGLKA	8	3.54	0.13	3.71	0.10	3.96	0.05
46	212-230	PWQGLKAATKRQKYERISE	17	6.75	0.15	7.78	0.19	8.01	0.02
47	231-240	KKMSTPIEVL	8	2.27	0.09	2.57	0.09	2.87	0.09
48	239-248	VLCKGYPSEF	8	1.07	0.04	1.32	0.07	1.68	0.08
49	241-247	CKGYPSE	5	0.95	0.07	1.19	0.03	1.43	0.02
50	241-248	CKGYPSEF	6	1.00	0.02	1.17	0.07	1.37	0.02
51	242-259	KGYPSEFATYLNFCRSLR	16	4.02	0.36	4.41	0.21	4.44	0.44
52	248-254	FATYLNF	6	0.09	0.06	0.16	0.03	0.24	0.02
53	255-268	CRSLRFDDKPDYSY	12	3.42	0.12	3.69	0.10	3.92	0.19
54	255-269	CRSLRFDDKPDYSYL	13	3.22	0.24	3.44	0.10	3.66	0.09
55	261-269	DDKPDYSYL	7	2.08	0.12	2.07	0.12	2.14	0.08
56	268-277	YLRQLFRNLF	9	0.15	0.12	0.49	0.12	0.91	0.06
57	269-277	LRQLFRNLF	8	0.21	0.05	0.52	0.04	0.79	0.05
58	269-281	LRQLFRNLFHRQG	12	2.15	0.15	2.16	0.14	2.12	0.04
59	269-285	LRQLFRNLFHRQGFSYD	16	2.27	0.07	2.56	0.07	2.92	0.08
60	270-276	RQLFRNL	6	1.47	0.07	2.14	0.08	2.47	0.09
61	272-285	LFRNLFHRQGFSYD	13	2.30	0.12	2.62	0.13	2.81	0.02
62	273-285	FRNLFHRQGFSYD	12	2.05	0.15	2.28	0.08	2.44	0.09
63	278-285	HRQGFSYD	7	1.65	0.20	1.75	0.14	1.73	0.12
64	286-292	YVFDWNM	6	2.33	0.22	2.83	0.23	2.83	0.18
65	293-305	LKFGASRAADDAE	12	4.14	0.16	3.84	0.21	4.36	0.11
66	304-320	AERERRDREERLRHSRN	16	4.55	0.22	4.76	0.18	5.16	0.06
67	312-324	EERLRHSRNPATR	11	3.69	0.29	3.90	0.37	3.63	0.06

68	313-327	ERLRHSRNPATRGLP	12	1.36	0.03	1.51	0.01	1.79	0.03
69	315-325	LRHSRNPATRG	9	0.29	0.06	0.50	0.06	0.60	0.06
70	322-336	ATRGLPSTASGRLRG	13	1.81	0.21	1.96	0.10	2.19	0.17
71	325-334	GLPSTASGRL	8	0.37	0.08	0.65	0.10	0.80	0.13
72	338-349	QEVAPPTPLTPT	7	1.51	0.07	1.49	0.05	1.79	0.15
73	343-351	PTPLTPTSH	5	1.11	0.05	1.16	0.12	1.65	0.07
74	343-356	PTPLTPTSHTANTS	10	0.46	0.01	0.39	0.02	0.36	0.02
75	344-355	TPLTPTSHTANT	9	1.54	0.11	1.94	0.08	1.92	0.04
76	349-367	TSHTANTSPRPVSGMERER	16	4.02	0.19	3.84	0.14	3.98	0.14
77	363-376	MERERKVSMRLHRG	13	2.20	0.07	2.83	0.21	3.43	0.09
78	372-384	RLHRGAPVNISSS	11	2.56	0.01	2.70	0.05	2.89	0.05
79	378-385	PVNISSSD	6	0.65	0.04	0.61	0.05	0.67	0.04
80	385-405	DLTGRQDTSRMSTSQIPGRVA	19	2.65	0.13	2.95	0.19	3.37	0.01
81	402-413	GRVAAAGLQAVV	11	1.61	0.09	1.82	0.08	2.03	0.06

				CK1δ1-S3A					
				Relative Fractional Deuterium Uptake (%)					
				1 min		**5 min**		**10 min**	
S.No.	**Peptide**	**Sequence**	**Num Exchangers**	**Uptake**	**± SD**	**Uptake**	**± SD**	**Uptake**	**± SD**
1	1-20	MELRVGNRYRLGRKIGSGSF	19	6.15	0.27	6.76	0.23	6.74	0.23
2	1-21	MELRVGNRYRLGRKIGSGSFG	20	5.66	0.34	7.29	0.29	7.29	0.22
3	3-20	LRVGNRYRLGRKIGSGSF	17	5.79	0.09	6.40	0.02	6.03	0.54
4	3-22	LRVGNRYRLGRKIGSGSFGD	19	6.09	0.05	6.68	0.19	6.80	0.21
5	3-24	LRVGNRYRLGRKIGSGSFGDIY	21	5.85	0.48	7.24	0.08	7.55	0.15
6	4-20	RVGNRYRLGRKIGSGSF	16	5.16	0.05	5.67	0.14	5.83	0.14
7	4-22	RVGNRYRLGRKIGSGSFGD	18	5.77	0.21	6.24	0.22	6.25	0.23
8	12-25	GRKIGSGSFGDIYL	13	4.22	0.15	4.55	0.11	5.50	0.29
9	21-28	GDIYLGTD	7	1.47	0.12	2.01	0.09	2.85	0.28
10	26-34	GTDIAAGEE	8	3.29	0.03	3.36	0.03	3.07	0.25
11	28-34	DIAAGEE	6	2.32	0.07	2.39	0.07	2.19	0.06
12	28-36	DIAAGEEVA	8	2.65	0.06	2.90	0.04	3.15	0.06

13	29-36	IAAGEEVA	7	1.99	0.03	2.27	0.03	2.34	0.03
14	37-49	IKLECVKTKHPQL	11	2.84	0.08	2.73	0.37	3.05	0.34
15	42-49	VKTKHPQL	6	1.25	0.06	1.27	0.07	1.21	0.10
16	56-72	YKMMQGGVGIPTIRWCG	15	4.40	0.43	4.07	0.46	3.66	0.04
17	59-70	MQGGVGIPTIRW	10	4.32	0.20	4.33	0.43	4.34	0.44
18	83-89	ELLGPSL	5	0.56	0.02	0.75	0.02	0.84	0.04
19	96-102	CSRKFSL	6	0.87	0.02	1.03	0.10	1.27	0.02
20	96-106	CSRKFSLKTVL	10	0.95	0.05	1.22	0.10	1.40	0.11
21	103-117	KTVLLLADQMISRIE	14	4.34	0.02	4.97	0.02	5.59	0.11
22	104-119	TVLLLADQMISRIEYI	15	3.55	0.23	4.52	0.10	5.24	0.05
23	112-123	MISRIEYIHSKN	11	0.26	0.05	0.41	0.10	1.17	0.03
24	113-123	ISRIEYIHSKN	10	0.13	0.08	0.22	0.09	0.28	0.06
25	113-134	ISRIEYIHSKNFIHRDVKPDNF	20	0.56	0.07	0.77	0.11	1.01	0.07
26	124-134	FIHRDVKPDNF	9	0.26	0.01	0.30	0.02	0.36	0.01
27	124-135	FIHRDVKPDNFL	10	0.26	0.02	0.29	0.02	0.41	0.03
28	135-143	LMGLGKKGN	8	1.54	0.12	1.88	0.10	2.07	0.14
29	135-144	LMGLGKKGNL	9	1.69	0.14	1.95	0.14	2.18	0.16
30	135-146	LMGLGKKGNLVY	11	1.64	0.11	1.97	0.10	2.26	0.11
31	136-146	MGLGKKGNLVY	10	1.73	0.11	2.12	0.09	2.25	0.13
32	137-144	GLGKKGNL	7	1.48	0.08	1.84	0.17	1.97	0.20
33	152-161	LAKKYRDART	9	1.25	0.01	1.49	0.17	1.89	0.02
34	163-178	QHIPYRENKNLTGTAR	14	2.99	0.08	3.39	0.03	4.55	0.12
35	175-186	GTARYASINTHL	11	1.69	0.30	1.50	0.26	1.81	0.29
36	180-186	ASINTHL	6	0.74	0.07	1.35	0.06	1.53	0.06
37	180-196	ASINTHLGIEQSRRDDL	16	1.54	0.03	2.58	0.02	3.07	0.06
38	183-196	NTHLGIEQSRRDDL	13	1.34	0.04	2.40	0.08	2.70	0.10
39	187-196	GIEQSRRDDL	9	0.73	0.05	1.15	0.07	1.23	0.14
40	192-211	RRDDLESLGYVLMYFNLGSL	19	2.61	0.11	2.95	0.10	3.22	0.23
41	205-216	YFNLGSLPWQGL	10	3.18	0.08	3.76	0.13	4.03	0.15
42	205-218	YFNLGSLPWQGLKA	12	4.37	0.10	4.93	0.12	5.10	0.17
43	206-218	FNLGSLPWQGLKA	11	4.61	0.05	5.16	0.06	5.23	0.09
44	207-218	NLGSLPWQGLKA	10	4.22	0.09	4.98	0.13	5.10	0.16

45	209-218	GSLPWQGLKA	8	3.61	0.05	3.76	0.11	3.92	0.13
46	212-230	PWQGLKAATKRQKYERISE	17	5.84	0.14	7.00	0.19	7.10	0.09
47	231-240	KKMSTPIEVL	8	2.22	0.09	2.60	0.10	2.77	0.12
48	239-248	VLCKGYPSEF	8	1.00	0.04	1.28	0.05	1.71	0.03
49	241-247	CKGYPSE	5	0.97	0.02	1.19	0.04	1.44	0.06
50	241-248	CKGYPSEF	6	0.83	0.02	1.18	0.05	1.25	0.04
51	242-259	KGYPSEFATYLNFCRSLR	16	4.52	0.28	4.66	0.11	4.49	0.24
52	248-254	FATYLNF	6	0.07	0.01	0.15	0.02	0.20	0.02
53	255-268	CRSLRFDDKPDYSY	12	3.53	0.21	3.60	0.18	4.19	0.16
54	255-269	CRSLRFDDKPDYSYL	13	3.28	0.16	3.42	0.08	3.45	0.05
55	261-269	DDKPDYSYL	7	2.26	0.09	2.24	0.11	2.20	0.14
56	268-277	YLRQLFRNLF	9	0.18	0.06	0.58	0.07	0.86	0.06
57	269-277	LRQLFRNLF	8	0.23	0.02	0.49	0.02	0.74	0.02
58	269-281	LRQLFRNLFHRQG	12	2.09	0.07	2.07	0.04	2.40	0.16
59	269-285	LRQLFRNLFHRQGFSYD	16	2.28	0.10	2.64	0.10	2.84	0.14
60	270-276	RQLFRNL	6	1.59	0.02	2.31	0.09	2.50	0.07
61	272-285	LFRNLFHRQGFSYD	13	2.34	0.01	2.47	0.07	2.62	0.13
62	273-285	FRNLFHRQGFSYD	12	2.31	0.11	2.37	0.12	2.45	0.16
63	278-285	HRQGFSYD	7	1.88	0.11	1.79	0.11	1.79	0.16
64	286-292	YVFDWNM	6	2.66	0.14	2.91	0.16	2.86	0.18
65	293-305	LKFGASRAADDAE	12	4.12	0.11	4.00	0.13	4.15	0.13
66	304-320	AERERRDREERLRHSRN	16	4.62	0.27	4.58	0.15	4.69	0.17
67	312-324	EERLRHSRNPATR	11	3.76	0.14	3.49	0.28	3.52	0.11
68	313-327	ERLRHSRNPATRGLP	12	1.47	0.09	1.69	0.10	1.89	0.07
69	315-325	LRHSRNPATRG	9	0.44	0.05	0.42	0.14	0.61	0.04
70	322-336	ATRGLPSTASGRLRG	13	1.81	0.24	1.89	0.18	2.14	0.29
71	325-334	GLPSTASGRL	8	0.76	0.08	0.98	0.22	1.50	0.12
72	338-349	QEVAPPTPLTPT	7	1.74	0.16	1.64	0.10	1.60	0.07
73	343-351	PTPLTPTSH	5	1.20	0.14	1.64	0.04	1.94	0.19
74	343-356	PTPLTPTSHTANTS	10	0.34	0.01	0.34	0.07	0.35	0.03
75	344-355	TPLTPTSHTANT	9	1.85	0.08	1.88	0.07	1.76	0.06
76	349-367	TSHTANTSPRPVSGMERER	16	4.28	0.11	4.10	0.23	3.99	0.18
77	363-376	MERERKVSMRLHRG	13	2.39	0.07	3.32	0.12	3.71	0.08

78	372-384	RLHRGAPVNISSS	11	2.45	0.09	2.72	0.10	2.69	0.03
79	378-385	PVNISSSD	6	1.01	0.05	0.91	0.06	1.07	0.05
80	385-405	DLTGRQDTSRMSTSQIPGRVA	19	2.39	0.07	2.74	0.13	2.91	0.20
81	402-413	GRVAAAGLQAVV	11	1.73	0.29	2.02	0.22	2.04	0.03
				CK1δ1-S3A + ATP					
				Relative Fractional Deuterium Uptake (%)					
				1 min		**5 min**		**10 min**	
S.No.	**Peptide**	**Sequence**	**Num Exchangers**	**Uptake**	**± SD**	**Uptake**	**± SD**	**Uptake**	**± SD**
1	1-20	MELRVGNRYRLGRKIGSGSF	19	NaN	NaN	NaN	NaN	NaN	NaN
2	1-21	MELRVGNRYRLGRKIGSGSFG	20	20.45	2.78	26.56	0.39	29.49	2.20
3	3-20	LRVGNRYRLGRKIGSGSF	17	32.38	1.22	35.15	1.14	35.90	1.73
4	3-22	LRVGNRYRLGRKIGSGSFGD	19	22.26	0.09	27.35	1.84	31.27	0.09
5	3-24	LRVGNRYRLGRKIGSGSFGDIY	21	24.18	1.57	25.13	0.74	27.71	0.35
6	4-20	RVGNRYRLGRKIGSGSF	16	29.25	1.17	31.59	2.66	32.53	1.96
7	4-22	RVGNRYRLGRKIGSGSFGD	18	28.06	1.12	29.22	0.65	33.17	0.85
8	12-25	GRKIGSGSFGDIYL	13	29.95	0.60	27.62	1.17	30.58	2.99
9	21-28	GDIYLGTD	7	5.84	1.72	12.50	1.64	17.67	1.58
10	26-34	GTDIAAGEE	8	40.82	0.27	39.75	0.27	42.55	0.27
11	28-34	DIAAGEE	6	29.39	2.36	30.25	2.56	27.75	0.89
12	28-36	DIAAGEEVA	8	29.96	2.01	33.96	2.48	37.78	2.57
13	29-36	IAAGEEVA	7	25.39	1.71	24.83	1.43	28.76	2.07
14	37-49	IKLECVKTKHPQL	11	26.03	1.45	29.15	1.77	28.87	0.52
15	42-49	VKTKHPQL	6	13.76	2.98	15.75	0.40	16.13	1.77
16	56-72	YKMMQGGVGIPTIRWCG	15	26.29	0.24	24.26	0.24	24.97	0.24
17	59-70	MQGGVGIPTIRW	10	31.33	2.63	28.55	1.30	28.14	0.65
18	83-89	ELLGPSL	5	4.12	2.06	8.27	3.51	14.12	1.44
19	96-102	CSRKFSL	6	9.39	1.47	12.57	2.06	16.06	3.99
20	96-106	CSRKFSLKTVL	10	7.82	1.84	10.73	0.50	11.18	1.28
21	103-117	KTVLLLADQMISRIE	14	26.70	0.00	28.60	0.00	30.76	0.00
22	104-119	TVLLLADQMISRIEYI	15	14.59	0.86	20.41	0.28	23.82	0.74
23	112-123	MISRIEYIHSKN	11	5.43	2.99	6.79	2.62	7.83	2.15

24	113-123	ISRIEYIHSKN	10	3.59	2.49	4.00	0.15	2.27	0.59
25	113-134	ISRIEYIHSKN FIHRDVKPDN F	20	0.98	0.27	2.75	0.16	3.32	0.31
26	124-134	FIHRDVKPDN F	9	2.86	0.40	3.52	0.53	4.48	1.17
27	124-135	FIHRDVKPDN FL	10	4.73	0.85	5.00	0.94	6.02	0.56
28	135-143	LMGLGKKGN	8	19.68	1.40	20.76	0.94	24.64	1.77
29	135-144	LMGLGKKGN L	9	17.99	1.56	20.06	1.19	23.27	1.87
30	135-146	LMGLGKKGN LVY	11	15.01	1.19	16.10	0.84	18.55	1.14
31	136-146	MGLGKKGNL VY	10	17.31	1.50	18.27	1.12	20.28	1.24
32	137-144	GLGKKGNL	7	20.26	2.30	21.67	1.83	23.62	0.64
33	152-161	LAKKYRDAR T	9	2.56	0.55	1.61	0.36	2.07	0.54
34	163-178	QHIPYRENK NLTGTAR	14	18.16	0.37	21.50	0.43	24.92	0.30
35	175-186	GTARYASINT HL	11	19.77	1.97	18.26	0.18	18.53	0.18
36	180-186	ASINTHL	6	11.30	0.42	18.16	0.84	22.19	1.30
37	180-196	ASINTHLGIE QSRRDDL	16	10.64	0.51	8.33	0.11	9.54	2.10
38	183-196	NTHLGIEQSR RDDL	13	7.18	0.25	10.16	1.97	12.39	0.25
39	187-196	GIEQSRRDD L	9	9.95	1.91	12.92	0.76	13.65	1.22
40	192-211	RRDDLESLG YVLMYFNLG SL	19	14.09	0.84	15.27	0.51	16.35	0.70
41	205-216	YFNLGSLPW QGL	10	19.33	2.44	22.69	6.13	30.49	0.13
42	205-218	YFNLGSLPW QGLKA	12	26.77	1.57	30.18	2.92	29.45	0.60
43	206-218	FNLGSLPWQ GLKA	11	33.43	0.03	38.28	0.03	38.00	0.09
44	207-218	NLGSLPWQG LKA	10	37.48	1.05	43.47	0.41	42.01	2.09
45	209-218	GSLPWQGLK A	8	42.25	1.60	42.38	1.25	44.47	2.17
46	212-230	PWQGLKAAT KRQKYERISE	17	35.17	0.90	36.21	0.61	36.65	0.39
47	231-240	KKMSTPIEVL	8	27.75	1.79	31.23	1.52	34.36	1.46
48	239-248	VLCKGYPSE F	8	13.11	1.18	17.00	0.69	20.72	0.61
49	241-247	CKGYPSE	5	16.94	2.14	24.47	1.76	32.06	1.05
50	241-248	CKGYPSEF	6	18.01	0.86	21.00	0.17	24.74	0.77
51	242-259	KGYPSEFAT YLNFCRSLR	16	26.73	0.94	24.78	0.39	26.52	1.03
52	248-254	FATYLNF	6	5.51	2.74	3.76	0.53	9.05	0.23
53	255-268	CRSLRFDDK PDYSY	12	28.06	1.30	29.10	1.04	30.19	2.89
54	255-269	CRSLRFDDK PDYSYL	13	24.26	0.89	24.34	0.31	26.04	0.69
55	261-269	DDKPDYSYL	7	28.54	2.54	24.98	1.44	25.79	2.80
56	268-277	YLRQLFRNLF	9	NaN	NaN	NaN	NaN	NaN	NaN

57	269-277	LRQLFRNLF	8	2.44	0.41	9.16	0.81	7.92	0.43
58	269-281	LRQLFRNLF HRQG	12	17.20	1.65	17.89	1.65	20.54	0.71
59	269-285	LRQLFRNLF HRQGFSYD	16	12.99	0.78	13.05	0.47	15.07	0.72
60	270-276	RQLFRNL	6	22.33	1.12	34.41	0.73	38.91	0.81
61	272-285	LFRNLFHRQ GFSYD	13	16.97	0.65	17.26	0.04	18.93	0.04
62	273-285	FRNLFHRQG FSYD	12	16.43	1.46	15.51	0.65	17.69	1.45
63	278-285	HRQGFSYD	7	24.97	1.57	21.62	2.57	22.76	3.38
64	286-292	YVFDWNM	6	NaN	NaN	NaN	NaN	NaN	NaN
65	293-305	LKFGASRAA DDAE	12	33.26	1.54	30.92	1.23	31.64	1.89
66	304-320	AERERRDRE ERLRHSRN	16	28.24	0.60	26.15	0.70	27.68	1.25
67	312-324	EERLRHSRN PATR	11	33.89	2.65	31.42	2.53	30.09	0.51
68	313-327	ERLRHSRNP ATRGLP	12	10.21	0.92	13.42	1.01	15.40	0.90
69	315-325	LRHSRNPAT RG	9	3.32	0.93	4.12	0.34	4.74	0.71
70	322-336	ATRGLPSTA SGRLRG	13	11.78	0.31	12.36	2.52	15.63	0.31
71	325-334	GLPSTASGR L	8	7.38	0.39	7.29	0.39	7.36	0.39
72	338-349	QEVAPPTPL TPT	7	17.89	0.36	14.92	0.36	19.80	0.36
73	343-351	PTPLTPTSH	5	22.67	1.51	21.76	0.76	25.85	1.52
74	343-356	PTPLTPTSHT ANTS	10	11.64	0.98	10.38	1.93	10.09	0.73
75	344-355	TPLTPTSHTA NT	9	18.08	1.27	22.83	0.99	19.89	0.15
76	349-367	TSHTANTSP RPVSGMERE R	16	22.71	0.78	21.96	0.73	22.23	1.11
77	363-376	MERERKVSM RLHRG	13	15.37	0.63	22.33	0.70	23.35	1.14
78	372-384	RLHRGAPVNI SSS	11	19.70	0.26	21.65	1.16	23.72	1.20
79	378-385	PVNISSSD	6	19.74	1.02	19.16	0.85	20.48	0.79
80	385-405	DLTGRQDTS RMSTSQIPG RVA	19	13.17	0.21	15.75	0.37	16.89	0.81
81	402-413	GRVAAAGLQ AVV	11	15.33	0.30	16.44	0.30	19.53	0.73
				CK1δ1-S3A + AMPPNP					
				Relative Fractional Deuterium Uptake (%)					
				1 min		**5 min**		**10 min**	
S.No.	**Peptide**	**Sequence**	**Num Exchangers**	**Uptake**	**± SD**	**Uptake**	**± SD**	**Uptake**	**± SD**
1	1-20	MELRVGNRY RLGRKIGSG SF	19	33.20	1.21	36.82	1.00	37.29	1.04
2	1-21	MELRVGNRY RLGRKIGSG SFG	20	29.66	1.31	32.14	1.36	36.40	0.46

3	3-20	LRVGNRYRL GRKIGSGSF	17	36.28	0.48	38.49	0.39	40.78	1.80
4	3-22	LRVGNRYRL GRKIGSGSF GD	19	33.69	2.58	35.46	1.85	38.13	1.69
5	3-24	LRVGNRYRL GRKIGSGSF GDIY	21	27.64	0.40	31.12	2.51	34.35	0.34
6	4-20	RVGNRYRLG RKIGSGSF	16	33.04	0.31	36.62	0.96	38.86	0.46
7	4-22	RVGNRYRLG RKIGSGSFG D	18	33.00	1.46	35.36	1.00	37.58	1.51
8	12-25	GRKIGSGSF GDIYL	13	36.09	0.60	34.02	2.15	39.05	2.76
9	21-28	GDIYLGTD	7	15.04	0.28	26.21	0.75	36.84	0.37
10	26-34	GTDIAAGEE	8	33.60	2.84	40.42	2.73	37.10	2.62
11	28-34	DIAAGEE	6	39.42	3.69	36.78	0.99	37.51	1.56
12	28-36	DIAAGEEVA	8	34.64	0.20	27.97	1.70	37.95	1.36
13	29-36	IAAGEEVA	7	28.71	0.26	27.68	0.19	30.41	0.63
14	37-49	IKLECVKTKH PQL	11	22.64	4.42	27.25	0.26	22.66	1.92
15	42-49	VKTKHPQL	6	22.20	1.79	20.69	1.16	20.84	1.94
16	56-72	YKMMQGGV GIPTIRWCG	15	32.90	3.11	33.85	1.04	28.40	2.08
17	59-70	MQGGVGIPTI RW	10	32.69	4.77	45.68	2.22	40.23	3.75
18	83-89	ELLGPSL	5	7.05	0.37	10.69	0.55	12.86	0.26
19	96-102	CSRKFSL	6	12.69	0.71	15.54	1.78	15.70	0.48
20	96-106	CSRKFSLKT VL	10	8.32	0.53	11.87	0.96	12.47	0.40
21	103-117	KTVLLLADQ MISRIE	14	27.14	0.71	30.91	0.06	33.71	0.16
22	104-119	TVLLLADQMI SRIEYI	15	19.50	0.35	25.82	1.06	28.18	1.76
23	112-123	MISRIEYIHSK N	11	6.96	0.24	9.55	2.67	16.51	0.24
24	113-123	ISRIEYIHSKN	10	2.13	0.89	1.50	0.86	2.13	0.89
25	113-134	ISRIEYIHSKN FIHRDVKPDN F	20	2.16	0.67	2.89	0.61	4.36	0.30
26	124-134	FIHRDVKPDN F	9	2.83	0.16	2.35	0.49	3.50	0.56
27	124-135	FIHRDVKPDN FL	10	3.77	0.61	3.40	0.40	4.91	0.52
28	135-143	LMGLGKKGN	8	19.69	1.47	22.16	1.15	25.44	1.61
29	135-144	LMGLGKKGN L	9	19.26	1.47	21.52	1.42	24.70	1.43
30	135-146	LMGLGKKGN LVY	11	15.63	0.89	17.90	0.96	20.03	0.89
31	136-146	MGLGKKGNL VY	10	17.80	1.37	19.28	2.97	22.75	2.14
32	137-144	GLGKKGNL	7	22.55	1.32	25.18	2.22	28.96	1.55
33	152-161	LAKKYRDAR T	9	10.51	0.29	15.46	0.29	14.78	3.75
34	163-178	QHIPYRENK NLTGTAR	14	21.02	0.49	24.90	0.24	30.01	0.24

35	175-186	GTARYASINTHL	11	21.75	0.18	14.91	2.52	16.09	0.19
36	180-186	ASINTHL	6	13.23	0.41	22.95	0.92	28.41	0.49
37	180-196	ASINTHLGIEQSRRDDL	16	9.55	0.96	13.16	0.11	21.85	0.15
38	183-196	NTHLGIEQSRRDDL	13	11.44	1.17	17.44	0.26	22.97	0.88
39	187-196	GIEQSRRDDL	9	7.38	0.65	11.08	0.88	13.01	0.84
40	192-211	RRDDLESLGYVLMYFNLGSL	19	12.23	1.31	15.27	0.70	16.40	0.44
41	205-216	YFNLGSLPWQGL	10	30.94	1.85	35.98	0.52	39.68	0.96
42	205-218	YFNLGSLPWQGLKA	12	35.99	0.81	40.61	1.05	43.57	0.83
43	206-218	FNLGSLPWQGLKA	11	38.00	0.61	46.02	1.49	49.38	0.72
44	207-218	NLGSLPWQGLKA	10	42.35	2.19	48.60	1.27	51.81	0.94
45	209-218	GSLPWQGLKA	8	44.22	1.59	46.44	1.28	49.54	0.65
46	212-230	PWQGLKAATKRQKYERISE	17	39.72	0.85	45.74	1.11	47.14	0.14
47	231-240	KKMSTPIEVL	8	28.32	1.17	32.11	1.16	35.94	1.11
48	239-248	VLCKGYPSEF	8	13.35	0.47	16.45	0.84	20.99	1.05
49	241-247	CKGYPSE	5	18.93	1.37	23.84	0.63	28.64	0.45
50	241-248	CKGYPSEF	6	16.69	0.26	19.48	1.10	22.77	0.38
51	242-259	KGYPSEFATYLNFCRSLR	16	25.12	2.24	27.57	1.29	27.72	2.76
52	248-254	FATYLNF	6	1.48	1.06	2.75	0.44	3.92	0.39
53	255-268	CRSLRFDDKPDYSY	12	28.53	1.03	30.77	0.85	32.65	1.57
54	255-269	CRSLRFDDKPDYSYL	13	24.75	1.86	26.47	0.80	28.13	0.66
55	261-269	DDKPDYSYL	7	29.67	1.78	29.60	1.65	30.60	1.11
56	268-277	YLRQLFRNLF	9	1.71	1.35	5.43	1.34	10.07	0.66
57	269-277	LRQLFRNLF	8	2.60	0.62	6.50	0.51	9.91	0.62
58	269-281	LRQLFRNLFHRQG	12	17.93	1.27	18.01	1.13	17.65	0.33
59	269-285	LRQLFRNLFHRQGFSYD	16	14.16	0.42	15.98	0.43	18.27	0.48
60	270-276	RQLFRNL	6	24.56	1.24	35.69	1.26	41.16	1.52
61	272-285	LFRNLFHRQGFSYD	13	17.70	0.96	20.14	0.97	21.58	0.13
62	273-285	FRNLFHRQGFSYD	12	17.12	1.27	19.02	0.64	20.36	0.79
63	278-285	HRQGFSYD	7	23.53	2.90	24.97	1.95	24.72	1.73
64	286-292	YVFDWNM	6	38.88	3.74	47.18	3.80	47.14	3.03
65	293-305	LKFGASRAADDAE	12	34.52	1.37	32.00	1.77	36.32	0.89
66	304-320	AERERRDREERLRHSRN	16	28.47	1.37	29.73	1.14	32.24	0.38
67	312-324	EERLRHSRNPATR	11	33.54	2.61	35.49	3.35	32.99	0.51

68	313-327	ERLRHSRNPATRGLP	12	11.37	0.27	12.59	0.11	14.96	0.21
69	315-325	LRHSRNPATRG	9	3.26	0.67	5.60	0.68	6.68	0.68
70	322-336	ATRGLPSTASGRLRG	13	13.89	1.58	15.10	0.77	16.82	1.33
71	325-334	GLPSTASGRL	8	4.67	1.06	8.14	1.25	10.02	1.62
72	338-349	QEVAPPTPLTPT	7	21.53	1.02	21.31	0.77	25.52	2.17
73	343-351	PTPLTPTSH	5	22.29	0.99	23.17	2.39	33.03	1.32
74	343-356	PTPLTPTSHTANTS	10	4.60	0.14	3.93	0.24	3.58	0.19
75	344-355	TPLTPTSHTANT	9	17.10	1.22	21.60	0.92	21.38	0.43
76	349-367	TSHTANTSPRPVSGMERER	16	25.15	1.19	24.03	0.91	24.86	0.89
77	363-376	MERERKVSMRLHRG	13	16.94	0.51	21.77	1.59	26.36	0.69
78	372-384	RLHRGAPVNISSS	11	23.23	0.12	24.57	0.49	26.31	0.44
79	378-385	PVNISSSD	6	10.78	0.65	10.20	0.82	11.19	0.63
80	385-405	DLTGRQDTSRMSTSQIPGRVA	19	13.95	0.70	15.50	1.00	17.73	0.08
81	402-413	GRVAAAGLQAVV	11	14.63	0.81	16.56	0.76	18.50	0.57

Table 2.3 HDX metadata table depicting the experimental conditions for different CK1δ1 isoforms, and the corresponding analysis is represented.

Table 2.3								
State	**CK1δ1**	**CK1δ1 +ATP**	**CK1δ1+ AMPPNP**	**CK1δ-ΔC**	**CK1δ-ΔC+ATP**	**CK1δ-ΔC+AMPPNP**	**CK1δ-ΔC + 4p-FASP**	**CK1δ-Δ400**
HDX Time Course (min)	0, 1, 5, 10	0, 1, 5, 10	0, 1, 5, 10	0, 1, 5, 10	0, 1, 5, 10	0, 1, 5, 10	0, 1, 5, 10	0, 1, 5, 10
Back-exchange	NA	NA	NA	NA	NA	NA	NA	NA
Number of peptides	110	110	110	80	73	73	80	73
Sequence Coverage	93.73%	93.73%	93.73%	93.88%	93.88%	93.88%	93.88%	94.25%
Average peptide length/redundancy	11.94/3.44	11.94/3.44	11.94/3.44	11.45 / 3.32	12.22/3.23	12.22/3.23	11.45 / 3.32	11.41/2.21
Replicates (biological, technical)	2,3	2,3	2,3	2,3	2,3	2,3	2,3	2,3
Repeatability (Average SD)	0.057	0.054	0.05	0.061	0.054	0.056	0.045	0.045
State	**CK1δ-Δ400+ATP**	**CK1δ-Δ400+AMPPNP**	**CK1δ2**	**CK1δ2+ ATP**	**CK1δ2+ AMPPNP**	**CK1δ1-S3A**	**CK1δ1-S3A+ATP**	**CK1δ1-S3A+AMPPNP**
HDX Time Course (min)	0, 1, 5, 10	0, 1, 5, 10	0, 1, 5, 10	0, 1, 5, 10	0, 1, 5, 10	0, 1, 5, 10	0, 1, 5, 10	0, 1, 5, 10
Back-exchange	NA	NA	NA	NA	NA	NA	NA	NA
Number of peptides	73	73	99	96	99	80	77	79
Sequence Coverage	94.25%	94.25%	92.18%	91.69%	92.18%	91.10%	90.84%	88.23%
Average peptide length/ redundancy	11.41/2.21	11.41/2.21	11.38/2.99	11.36/2.91	11.38/2.99	12.59/2.66	12.69/2.59	12.48/2.72
Replicates (biological, technical)	2,3	2,3	2,3	2,3	2,3	2,3	2,3	2,3
Repeatability (Average SD)	0.059	0.049	0.051	0.032	0.06	0.061	0.069	0.062

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