UCSF

UC San Francisco Electronic Theses and Dissertations

Title

Traversing Chemical Space for Opioid Function

Permalink

https://escholarship.org/uc/item/13z491cg

Author

Webb, Chase Marques

Publication Date

2023

Peer reviewed|Thesis/dissertation

eScholarship.org Powered by the California Digital Library
University of California

Traversing chemical space for opioid function

by
Chase Marques Webb

DISSERTATION
Submitted in partial satisfaction of the requirements for degree of
DOCTOR OF PHILOSOPHY

in

Pharmaceutical Sciences and Pharmacogenomics

in the

GRADUATE DIVISION
of the
UNIVERSITY OF CALIFORNIA, SAN FRANCISCO

Approved:

David Julius

Chair

brian shoichet

Aashish Manglik

Mark von Zastrow

Committee Members

Copyright 2023
By

Chase Webb

Dedication

To my loving family: Barbara, Carlos, Chad and Charles.

Acknowledgements

Thanks to the people who supported me and the people who challenged me. Thanks to the people who mentored me and the people I had the pleasure to mentor. Thanks to my parents and family for having me and teaching me how to live. Thanks to Aashish for taking me in and growing with me and teaching me with compassion how to be a scientist. Thanks to Brian for always being positive and enthusiastic and relatable. Thanks to you both for trusting me and facilitating my development. Thanks to my committee member Mark von Zastrow who was always available with a good attitude and some interesting conversation about opioid receptors. Thanks to my committee chair, David Julius who has been on 'the team' since my qualification exam and before 'the prize'. It's been a pleasure to learn and grow from our interactions. Thanks to my team of mentors including Trent Balius, Matt Jacobson, Michael Grabe, Charly Craik, Joe DeRisi, Elyssa Margolis, Howard Fields, Dorit Ron, Jason Sello, Michael Penn, Carol Gross, Kathy Giacomini, Naledi Saul, Dan Lowenstein, and Dr. Metz, who helped me grow and develop. Thanks to Deanna Kroetz for mentoring and guiding me at the start of my journey. Thanks to D'anne Duncan for teaching me strategy, leadership, compassion, self-care, and intentionality. Thanks to my peers, friends, and colleagues for all their help in my development!

Contributions

Chapter 2 of this thesis is a variation of a manuscript submitted for publication:

Webb, C.M., Braz, J; Basbaum, A; Manglik A; Shoichet, B.K; Sampling vast chemical space with large scale molecular docking reveals non-opium derived MOR agonists.

Traversing Chemical Space for Opioid Function

Chase Webb

Abstract

Rational drug design is a multimodal optimization problem where most of the control nodes are at least partially obscured. Fundamentally, rational drug design is predicated on a deep understanding of biological molecular recognition. Arguably, biological molecular recognition is an amalgamation of two principles. Chiefly, biological molecular recognition relies on a so-called metabolic code of logic. This code defines a logic for all the chemical moieties that biological systems use to achieve molecular recognition. At some level, biological interactions are iterations and combinations of only a small set of chemical interactions between a very exclusive set of chemical functional groups. Harnessing this underlying logic to rationally design drugs is the primary investigation of this thesis. This inquiry is supported by another old and well-developed theory of biological interactions, that of preorganization. Preorganization supposes that biological systems that have evolved specific molecular recognition have done so by paying an energetic cost during assembly to pre-organize an energetically unfavorable binding site that relaxes dramatically upon the desired complexation event. One promising approach to tackling this immense problem of drug design by leveraging the metabolic code and preorganization is by employing computational models of ligand-protein interactions using coarse physical models of the complexation event.

The focus of this dissertation is to understand molecular recognition for a clinically relevant and widely generalizable model system, the membrane embedded mu

opioid receptor, a 7-pass transmembrane protein receptor that plays a fundamental role in pain perception and analgesia. In this thesis, I employ molecular docking, a computational approach that assesses physical complementarity between a ligand and a protein target to screen humongous chemical space. I then experimentally characterize several new to the planet opioid small molecules, and even solve a structure of the most potent compound in complex with the human opiate receptor. In so doing, I not only uncover new to the planet opioid therapeutics, but also learn some fundamental new qualities regarding molecular recognition at the human mu opioid receptor.

Also included in this dissertation is a commentary on the state of education in the biomedical enterprise. This PhD experience was unique because it happened amidst a racialized global pandemic and a period of extended social uncertainty and unrest. During this time, student leaders capitalized on the heightened societal focus on social justice. In the final chapter of this dissertation, I share my experiences in this domain and offer some of my personal insights for addressing historical injustices in the biomedical enterprise and making science more representative of our population and more efficacious in solving difficult problems in the domain of human health and wellness.

Table of Contents

Chapter 1: Introduction to opioid pharmacology 1

References 6

Chapter 2: Structure based discovery of novel MOR agonists 9

References 31

Chapter 3: Intentional inclusion in the biomedical enterprise 37

References 43

List of Figures

Figure 2.1 Large Scale Docking 282 million compounds to the μOR yields novel opioid scaffolds. 12

Figure 2.2 Structure elucidation of Z0647. 14

Figure 2.3 Structure based design of Z0647 congeners with varying molecular efficacies. 18

Figure 2.4 In-vivo activity of Z0647. 20

Chapter 1: Introduction to Opioid Pharmacology

Opium has been used by human beings for over six millennia and the crucial clinical utility of opium cannot be overstated[1]. Pain is an essential element of human experience and impacts everyone at some stage of their life. While many classes of drugs exist to relieve pain, none are as effective or widely used as opium and opium derived analgesics, known as opioids[2,3]. The systemic effects of opioids are quite intriguing as they modulate the subjective, integrated sensory experience of pain without truncating other sensory perception such as, touch, temperature, position sense, or discrimination of sharp or dull.

The first documented medical use of opioids is attributed to North African physicians who proliferated its use through trade to the Arabian Peninsula, India and China where they were chiefly employed to ameliorate dysentery.[4] The medical use of opium in China was first recorded in the 1700s, first by the Portuguese and later by the British. Ultimately, this became a political issue and precipitated the Opium Wars[5].

Early opium preparations were oral tinctures or macerations and were primarily used to treat diarrhea associated with dysentery. One such concoction – laudanum, an ethanolic tincture of opium, is still employed today for this end. As the use of opium and opium containing preparations (known as opioids) proliferated, the euphoric and addictive properties became more apparent, as well as their strong potential for misuse and abuse[6]. This was laid bare during the American Civil War, where morphine, a purified opium preparation, was used parenterally for the first time, engendered by the

recent development of the hypodermic syringe. Parenteral administration of morphine proved to enhance all its effects, but none more potently than its euphoric activity[7]. Indeed, morphine abuse became so prolific during the 19th century that numerous international treaties were ratified to limit its trafficking.

Still, the importance of opioids in treating pain has never been questioned[8,9]. Accordingly, a desire to disassociate the analgesia and abuse potential of opioids has generated a monumental effort over the years that has uncovered tens of thousands of opioid analogs as well as several compounds used clinically[10–14]. Although this dissociation has not been achieved by any of these chemical agents, clinical use of some of these drugs has elucidated many characteristics of opioid action.

Whereas opioids have been synthesized and chemically modified for hundreds of years, a modern pharmacologic understanding of opiate action began in 1805 when morphine, the chief bioactive alkaloid of the opium poppy was isolated by the German chemist Serturner[15]. Isolation of morphine demonstrated for the first time that a purified component of a plant derived medicine could faithfully recapitulate the complex pharmacology of the intact plant material. While morphine successfully reduced pain, it retained the undesirable side effects of opium including lethal respiratory depression, and the capacity to induce tolerance physical dependance that contributes to addiction. Over the next two centuries, a concerted effort focused on identification of an 'ideal' opioid where analgesia was separated from these dose-limiting side effects. As synthetic chemistry advanced, thousands or semi- or fully synthetic analogs of morphine

were developed by systematically disconnecting, substituting, or modifying its alkaloid scaffold[4,16]. Notably, this medicinal chemistry campaign emerged from knowledge of morphine and was essentially a ligand-based approach to designing the ideal opioid. Over a century and a half later, the dawn of molecular biology and the realization of the so-called 'opioid receptor' resulted in a paradigm shift for opioid drug development.

In the early 1970s, the molecular target of morphine was identified using a clever combination of radioligand binding studies and subsequently by cloning the genes for the opioid receptors. These studies revealed not one, but 4 endogenous opioid receptors, Mu, Delta, Kappa, Nociceptin, as well as numerous endogenous opioids that exhibit exceptionally complex pharmacology[2]. Activation of these membrane spanning, hepta-helical receptors instigate numerous intracellular signaling cascades. Most proximally to the membrane, opioid receptor activation engenders nucleotide exchange in heterotrimeric inhibitory G proteins, rendering them active. Activated opioid receptors are rapidly desensitized through phosphorylation by G protein Receptor Kinases (GRKs) and internalization by Beta-Arrestins, which act on phosphorylated opioid receptors. The ligand-induced interaction dynamics between opioid receptors and these intracellular transducers gives rise to varied signaling outcomes that can have widely varying organismal phenotypes[17].

Now, in the 21st century, new biophysical methods have enabled a molecular analysis of opioid receptors and their specific interactions with ligands. Importantly, these biophysical methods extend and corroborate years of careful dissection of the

morphine scaffold that have already resulted in robust 'rules' for ligand engagement with the opioid receptors. Specifically, advances in x-ray crystallography, cryo-electron microscopy, and GPCR biochemistry facilitated structure elucidation of all 4 opioid receptors in complex with small molecules that verify the nature of three ligand features: a basic amine to engage with an aspartate in the opioid receptor binding site, and planar aromatic interface, and a substituent extending off the basic amine. Structures further delineate the basis of ligand selectivity amongst the 4 opioid receptor subtypes and some essential differences between agonists, which activate opioid receptors and antagonists which render them inactive[1,18–20]. These monumental observations have primed opioid discovery for a newer modern structure rather than ligand guided approach.

In this thesis, I explore an approach to novel opioid development that relies on chemical complementarity to a physical model of the opioid receptor as the only discriminatory criteria. In so doing, I design a small library of opioid ligands both new to the planet and with emerging pharmacology. Additionally, I describe the chemical space from which these new modulators hail with a hope towards more efficient generation of new opioid chemotypes and better pharmacological control of this signaling system. Finally, I reflect on the composition of the scientific enterprise as well as my own experiences doing science in a pandemic amidst significant social unrest, and outline systems and intentional interventions to address some of the exclusion and systematic bias that permeates both the field of opioid biology and the biomedical enterprise as a whole. In so doing, I establish a blueprint for both a new approach to

opioid drug development as well as a novel way to engage in research that uses equity, justice, inclusion, and diversity as a cornerstone to more efficacious and expedient development of novel therapeutics and scientific knowledge.

References

(1) Manglik, A. Molecular Basis of Opioid Action: From Structures to New Leads. *Biol. Psychiatry* **2020**, *87* (1), 6–14. https://doi.org/10.1016/j.biopsych.2019.08.028.

(2) Pasternak, G. W.; Pan, Y.-X. Mu Opioids and Their Receptors: Evolution of a Concept. *Pharmacol. Rev.* **2013**, *65* (4), 1257–1317. https://doi.org/10.1124/pr.112.007138.

(3) Snyder, S. H.; Pasternak, G. W. Historical Review: Opioid Receptors. *Trends Pharmacol. Sci.* **2003**, *24* (4), 198–205. https://doi.org/10.1016/S0165-6147(03)00066-X.

(4) Brownstein, M. J. A Brief History of Opiates, Opioid Peptides, and Opioid Receptors. *Proc. Natl. Acad. Sci. U. S. A.* **1993**, *90* (12), 5391–5393. https://doi.org/10.1073/pnas.90.12.5391.

(5) Yu, M.; Davies, B. J. Opium Wars to the Opioid Epidemic: The Same Narcotics Cause Addiction and Kill. *Eur. Urol.* **2020**, *77* (1), 76–77. https://doi.org/10.1016/j.eururo.2019.10.006.

(6) Macht, D. I. THE HISTORY OF OPIUM AND SOME OF ITS PREPARATIONS AND ALKALOIDS. *JAMA* **1915**, *LXIV* (6), 477–481. https://doi.org/10.1001/jama.1915.02570320001001.

(7) Beecher, H. K. Pain in Men Wounded in Battle. *Ann. Surg.* **1946**, *123* (1), 96–105.

(8) Ehrlich, A. T.; Kieffer, B. L.; Darcq, E. Current Strategies toward Safer Mu Opioid Receptor Drugs for Pain Management. *Expert Opin. Ther. Targets* **2019**, *23* (4), 315–326. https://doi.org/10.1080/14728222.2019.1586882.

(9) Chan, H. C. S.; McCarthy, D.; Li, J.; Palczewski, K.; Yuan, S. Designing Safer Analgesics via μ-Opioid Receptor Pathways. *Trends Pharmacol. Sci.* **2017**, *38* (11), 1016–1037. https://doi.org/10.1016/j.tips.2017.08.004.

(10) Faouzi, A.; Wang, H.; Zaidi, S. A.; DiBerto, J. F.; Che, T.; Qu, Q.; Robertson, M. J.; Madasu, M. K.; El Daibani, A.; Varga, B. R.; Zhang, T.; Ruiz, C.; Liu, S.; Xu, J.; Appourchaux, K.; Slocum, S. T.; Eans, S. O.; Cameron, M. D.; Al-Hasani, R.; Pan, Y. X.; Roth, B. L.; McLaughlin, J. P.; Skiniotis, G.; Katritch, V.; Kobilka, B. K.; Majumdar, S. Structure-Based Design of Bitopic Ligands for the μ-Opioid Receptor. *Nature* **2023**, *613* (7945), 767–774. https://doi.org/10.1038/s41586-022-05588-y.

(11) De Neve, J.; Barlow, T. M. A.; Tourwé, D.; Bihel, F.; Simonin, F.; Ballet, S. Comprehensive Overview of Biased Pharmacology at the Opioid Receptors: Biased Ligands and Bias Factors. *RSC Med Chem* **2021**, *12* (6), 828–870. https://doi.org/10.1039/d1md00041a.

(12) Varga, B. R.; Streicher, J. M.; Majumdar, S. Strategies towards Safer Opioid Analgesics-A Review of Old and Upcoming Targets. *Br. J. Pharmacol.* **2021**. https://doi.org/10.1111/bph.15760.

(13) Azzam, A. A. H.; McDonald, J.; Lambert, D. G. Hot Topics in Opioid Pharmacology: Mixed and Biased Opioids. *Br. J. Anaesth.* **2019**, *122* (6), e136–e145. https://doi.org/10.1016/j.bja.2019.03.006.

(14) Valentino, R. J.; Volkow, N. D. Untangling the Complexity of Opioid Receptor Function. *Neuropsychopharmacology* **2018**, *43* (13), 2514–2520. https://doi.org/10.1038/s41386-018-0225-3.

(15) Sertùrner, F. W. Saure Im Opium. *Trommsdorffs Journal der Pharmazie*.

(16) Volkow, N. D.; Blanco, C. The Changing Opioid Crisis: Development, Challenges and Opportunities. *Mol. Psychiatry* **2021**, *26* (1), 218–233. https://doi.org/10.1038/s41380-020-0661-4.

(17) Weis, W. I.; Kobilka, B. K. The Molecular Basis of G Protein-Coupled Receptor Activation. *Annu. Rev. Biochem.* **2018**, *87*, 897–919. https://doi.org/10.1146/annurev-biochem-060614-033910.

(18) Zhuang, Y.; Wang, Y.; He, B.; He, X.; Zhou, X. E.; Guo, S.; Rao, Q.; Yang, J.; Liu, J.; Zhou, Q.; Wang, X.; Liu, M.; Liu, W.; Jiang, X.; Yang, D.; Jiang, H.; Shen, J.; Melcher, K.; Chen, H.; Jiang, Y.; Cheng, X.; Wang, M.-W.; Xie, X.; Xu, H. E. Molecular Recognition of Morphine and Fentanyl by the Human μ-Opioid Receptor. *Cell* **2022**, *185* (23), 4361–4375.e19. https://doi.org/10.1016/j.cell.2022.09.041.

(19) Shim, J.; Coop, A.; MacKerell, A. D. Consensus 3D Model of μ-Opioid Receptor Ligand Efficacy Based on a Quantitative Conformationally Sampled Pharmacophore. *J. Phys. Chem. B* **2011**, *115* (22), 7487–7496. https://doi.org/10.1021/jp202542g.

(20) Huang, W.; Manglik, A.; Venkatakrishnan, A. J.; Laeremans, T.; Feinberg, E. N.; Sanborn, A. L.; Kato, H. E.; Livingston, K. E.; Thorsen, T. S.; Kling, R. C.; Granier, S.; Gmeiner, P.; Husbands, S. M.; Traynor, J. R.; Weis, W. I.; Steyaert, J.; Dror, R. O.; Kobilka, B. K. Structural Insights into \mu-Opioid Receptor Activation. *Nature* **2015**, *524* (7565), 315–321. https://doi.org/10.1038/nature14886.

Chapter 2: Structure based discovery of novel MOR agonists

Abstract

The μ-Opioid receptor (μOR) is the target for clinically used opioid analgesics. Although quite effective for pain management, μOR agonists also induce on-target toxicity that limits clinical utility. Importantly, due to a paucity of diverse chemical matter, it has thus far remained unclear if low intrinsic efficacy, or indeed pathway bias toward G protein versus Beta-Arrestin recruitment is responsible for the higher safety profile of so-called biased agonists. Z0647, discovered by computational docking the largest virtual library ever attempted at the μOR, is a G protein biased agonist with low intrinsic efficacy compared to DAMGO, a full agonist of the μOR. Here we report the cryoEM structure of Z0647 bound μOR in complex with Go protein. The structure reveals a novel pi-pi interaction that could contribute to the previously unrealized pharmacological profile of Z0647. Indeed, Z0647 shows reduced constipation and less aversive withdrawal compared to morphine. Additionally, unlike any opioid known to date, Z0647 fails to manifest cellular tolerance to analgesia. Meanwhile, structure-based evolution led to Z0647 analogs with more pronounced Gi bias and increased efficacy. These vary smoothy in intrinsic efficacy from almost none to very close to DAMGO and could help delineate the contributions of a very weak partial agonist from a highly efficacious G protein biased μOR ligand. Taken together, this study reveals a potential pathway to rationally design next generation opioid therapeutics that are safer and more efficacious.

Introduction

Opiates have been used by humans for over 6000 years. Even before structural elucidation, chemists and pharmacologists attempted to optimize the analgesic effects of opiates, while mitigating the addictive properties and the lethal side effect of respiratory depression. Centuries of systematic dissection and modification of the complex primary opiate alkaloids like thebaine, codeine and morphine have yielded thousands of small molecule opioids[1,2]. Most of these are more efficacious than raw opium, but all still retain dose limiting, on-target toxicities like lethal respiratory depression, tolerance, addiction, and constipation. Interestingly, although there are over thirteen thousand annotated opiates in ChEMBL, these molecules are not extremely diverse, likely due to their shared starting point. (Figure 1A) Not surprisingly, most of these opiates exhibit exceedingly similar molecular pharmacology[3].

With the recent exponential growth in both the size and diversity of readily purchasable virtual chemical libraries, and the determination of crystal and cryo-EM structures of the μOR in both active and inactive states and in the presence of numerous ligands, it has become feasible to explore new non-opium derived chemical scaffolds with the sole criteria being complementarity to the receptor binding site[4–12].

Already, chemically distinct opioid small molecules like PZM21, SR-1701, oliceridine, and mitragynine pseudoindoxyl have demonstrated that chemical diversity can lead to divergent pharmacology at the μOR[4,13–16]. Indeed, these new scaffolds appear to selectively induce G-protein signaling while sparing beta-arrestin recruitment to the activated receptor. Clearly the novel protein-ligand interactions engendered by

these new chemotypes can be leveraged for new cellular effects and indeed are mooted to lead to analgesia with diminished on-target toxicity. Still, it is unclear if this so-called G protein bias is mediated by sub-maximal molecular efficacy at the opiate receptor or a true bias in ligand-induced transducer engagement[17].

Our task was thus twofold. Primarily, we set out to inundate the opiate receptor with novel chemotypes, surmising that novel chemical topologies would facilitate new protein-ligand interactions and thus emerging pharmacology. Secondly, we set out to uncover novel opioid chemotypes with both G protein bias and also a wide range of molecular efficacy to dissect the interplay between the phenomenon of bias versus the reality of partial agonism.

Amid the opioid epidemic, this approach holds promise as a method to design next generation pain killers that could be more efficacious and safer.

Results

Encouraged by the results of large-scale docking campaigns at the Melatonin MT1 receptor, Dopamine D4 receptor, Sigma receptors, Alpha 2A adrenergic receptor, and AmpC beta-lactamase[5,18,19], we turned to prospective prediction of new opioid compounds. Against the active state crystal structure of μOR[7], we docked 282 million molecules from the ZINC15 and ZINC20 virtual ligand databases[20,21] (figure 1B).

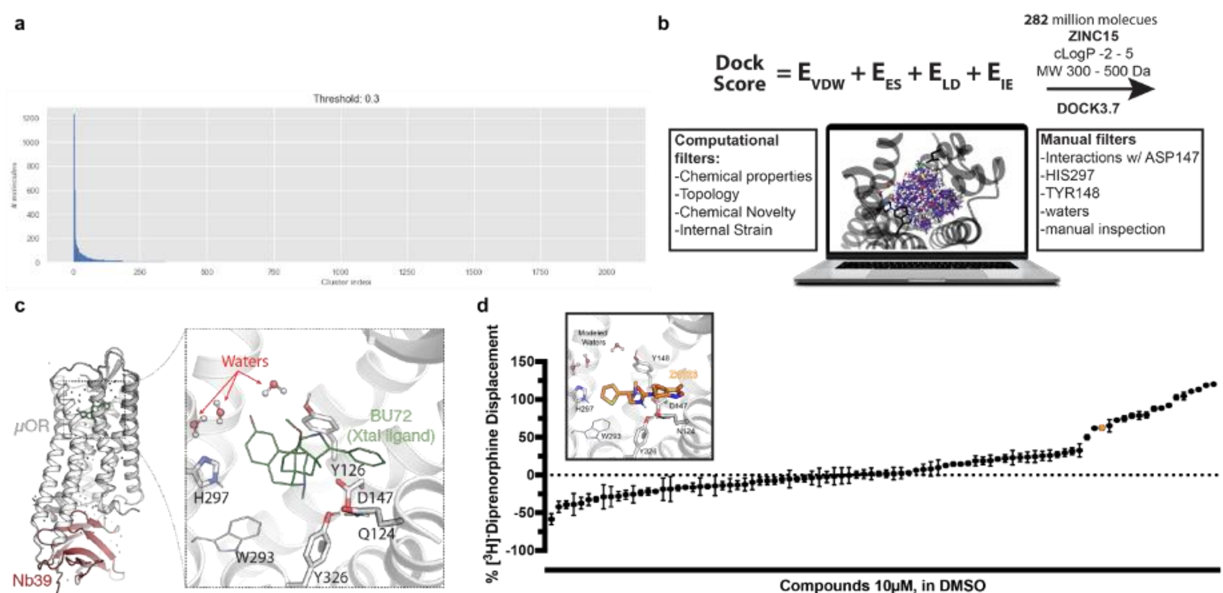

Figure 1. Large Scale Docking 282 million compounds to the μOR yields novel opioid scaffolds. A.) Tanimoto coefficient butina clustering of 1024-bit ECFP4 fingerprints of 13,772 annotated opioids in ChEMBL reveals ~300 clusters with most molecules occupying the same cluster. B.) 282 million small molecules were docked into the activated orthosteric site of the μOR (PDB: 5C1M). After computational filtering, ligands were selected based on molecular diversity and their ability to make interactions with modeled waters, Q124, Y148, and ASP147. d.) Single-point competitive radioligand binding showed 19 ligands that displaced 50% or more tritiated diprenorphine. Inset – the novel opioid scaffold Z4123 was selected for further optimization.

Overall, more than 210 trillion configurations, an average of 500,000 for each molecule were sampled and scored for complementarity using the physics based DOCK3.7[22–25] scoring function. The most highly ranking molecules were filtered for

torsional strain, then the top one million unstrained molecules were clustered by 2-dimensional topological similarity (ECFP4 Tanimoto Coefficient (Tc) > 0.36) to reduce redundancy[26,27]. Molecules were excluded that were too similar to ~13K annotated Mu opioid ligands on chEMBL. 20 thousand top-scoring molecules were manually examined for key polar and hydrophobic contacts with the orthosteric site of the μOR using UCSF Chimera[28–30] including: D147, Y148, Y326, H297, LYS233 and a few structural waters from the active state that are mooted to be involved in receptor activation (Figure 1C)[31–33]. Ultimately, 90 high scoring molecules out of 282 million were subjected to experimental testing for binding to the μOR. Of the 90 tested, 19 displaced tritiated diprenorphine at more than 50% and had MOR binding affinities ranging from 5 μM to 180 nM. This 21% hit rate is amongst the highest ever reported for a screen at the activated μOR[34] (Figure 1D).

The new chemicals are predicted to interact with the MOR in several new ways, and with novel functional groups. All docked ligands recapitulated the canonical interaction with ASP147[8]. Some ligands displayed a rarely seen additional hydrogen bonding interaction near this residue, likely direct, or mediated by a stable water molecule[35]. While the ligands initially uncovered in this screen were quite varied, they possessed modest potencies. The most potent, Z4123 had a Ki of 180 nM and was subjected to a virtual medicinal chemistry optimization campaign.

Due to the structural novelty of Z4123, we sought to increase its molecular efficacy at the MOR and explore chemical structure activity relationships of this scaffold.

Ultimately, 18 thousand analogs of Z4123 were virtually screened and 2 were purchased. The analogs were chosen to explore small chemical modifications of the parent molecule including modification of the disubstituted oxazole moiety present in the hydrophobic vestibule of the binding pocket. From this in-silico design campaign, Z0647 emerged as a potent, single digit nanomolar congener of Z4123 (Figure 2A).

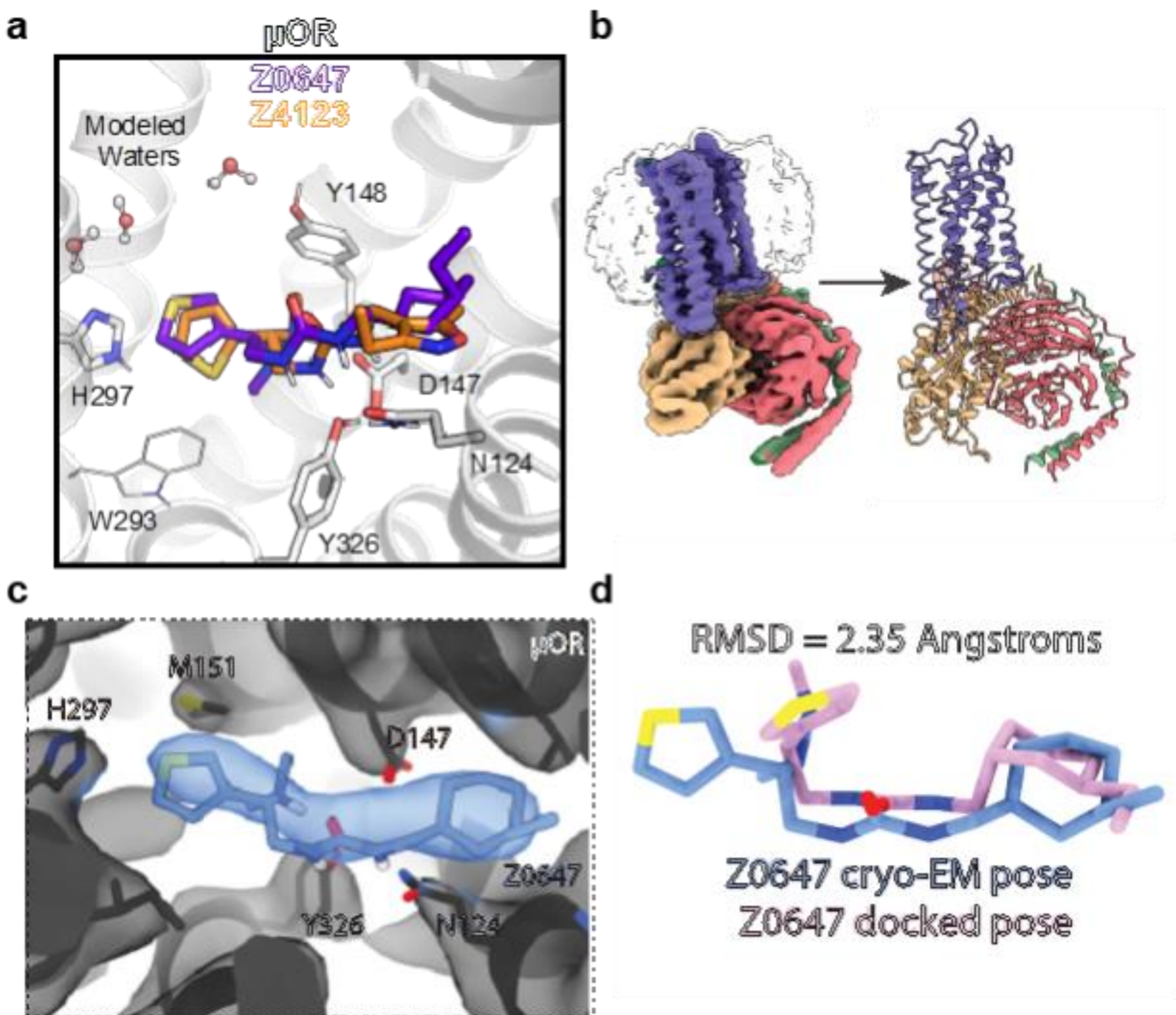

Figure 2. Structure elucidation of Z0647. A.) Overlay of modeled poses of Z4123 and Z0647 in the orthosteric site of the μOR. (Figure caption continued on next page).

(Figure caption continued from previous page). B.) Overall map of Z0647-hMOR-Gbg-ScFv16 complex. C.) Close up of Z0647 coloumbic potential. D.) Comparison of docked pose and empirical pose for Z0647 shows minor deviations.

Because Z0647 was designed from Z4123, which was modeled into the Nb39 bound MOR complexed with the morphinan ligand BU72, structural studies of Z0647 in complex with MOR as well as G protein were undertaken, hoping to understand its novel SAR, perhaps by unveiling novel water positions or protein-ligand interactions in the orthosteric site of the μOR. To this end, a biochemical preparation of full-length human MOR in complex with a mini-G alpha o as well as G beta gamma was purified to homogeneity and imaged cryogenically via electron microscopy. This yielded a high-resolution map of the complex with an overall resolution of 3.18 ang and importantly, 2.23 angstroms in the transmembrane helical domain which includes the orthosteric ligand binding site (figure 2B).

The empirical structure of Z0647 in complex with the Go activated opiate receptor superposes well with the modeled pose directly from docking, deviating by only 2.35 angstroms, mostly caused by the angular displacement of the thiophene moiety which was purposefully modeled to engage with a conserved water network bridging HIS297 and TYR148 (figure 2C). Notably, while Z0647 exists as a diastereomeric mixture, the (S,S,S)- enantiomer fits best within the coulombic potential of the cryo-EM map (figure 2C). While chiral resolution was attempted and failed, this apparent stereospecificity could underlie a reduced observed potency of Z0647, as likely, only a

fraction of the preparation is active. Additionally, the structure reveals that major interactions canonical for opiate receptor activation are recapitulated[33], albeit by new functional groups. Indeed, Z0647 makes a hydrogen bond and an ionic interaction with ASP147 as well as picking up an extra hydrogen bond with the amide portion of the central urea functionality. Z0647 also makes a hydrogen bond with ASN124, common with new-generation MOR chemotypes and hypothesized to underlie G protein activation with attenuated Arrestin translocation. As previously mentioned, Z0647 forgoes the typical phenol mediated engagement with the conserved water network of the opiate receptor and instead makes a novel T-stacking pi-orbital interaction with HIS297 (figure 2C). Moreover, the structure revealed tolerance for aliphatic groups in the traditional 'pendant aromatic sub-pocket' of the opiate receptor orthosteric site where pendent phenyl groups on molecules such as BU72 occupy as well asthe cyclohexyl substituent of Z0647. Such bulky three-dimensional functionalities likely contribute to more favorable hydrophobic packing and displacement of unfavorable trapped water molecules thereby reducing entropic penalties to productive complex formation and simultaneously decreasing the cost of desolvation of the ligand and protein surface to promote appreciable molecular interaction.

Guided by the empirical structure of μOR in complex with Z0647, a series of congeners were designed to explore structure activity relationships within the orthosteric site. This analysis yielded three notable observations (figure 3A). Primarily, we observed that modifications to the thiophene of Z0647 were tolerated even though they were modeled to abrogate interactions of the ligand with either HIS297, or a conserved

water network bridging H297 and Y128. Secondly, although Z0647 was modeled to engage in a bidentate hydrogen bond to D147, methylation of the urea group was tolerated, suggesting that this additional bidentate interaction is beneficial, but not necessary to promote ligand engagement to the MOR. Finally, modulation of the substituted cyclohexyl- portion of the molecule tolerated both aromatic and aliphatic moieties, suggesting lots of flexibility for hydrophobic engagement in the pendant phenyl sub-pocket of the MOR orthosteric site (figure 3B). These congeners showed smooth variation in molecular efficacy for G-protein activation while displaying a range of efficacy in Arrestin translocation to the receptor (figure 3C). Interestingly, none of these congeners appreciably induce internalization of the µOR. These compounds, some of which are nearly as efficacious as DAMGO in inducing G protein activation, will likely be useful chemical probe molecules for dissecting the relative contribution of molecular efficacy to overall signaling properties of a given small molecule opioid.

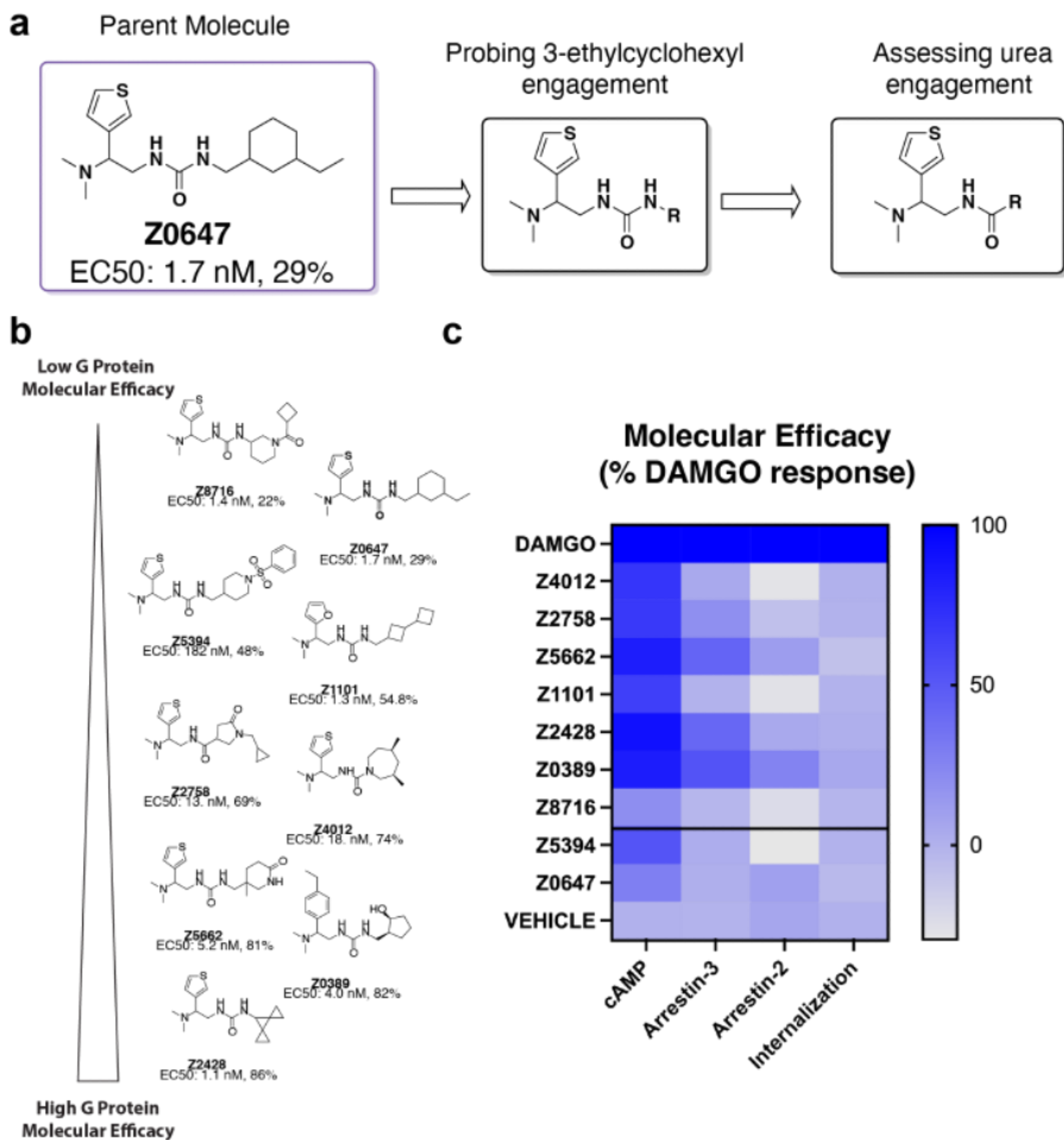

Figure 3. Structure based design of Z0647 congeners with varying molecular efficacies. A.) Z0647 was modified by changing both the cyclohexyl- and urea moieties. (Figure caption continued on next page).

(Figure caption continued from previous page). B.) Analogs of Z0647 have a range of molecular efficacy for activating G proteins and explore three-dimensional functional groups in place of the cyclohexyl substituent in the parent molecule. C.) Additionally, Z0647 analogs have varying molecular efficacies in arrestin2/3 recruitment.

Additionally, consistent with its in-vitro pharmacological characterization as an MOR partial agonist, Z0647 induces dose dependent analgesia in both a tail flick and hot plate analgesia assay (figure 4), suggesting that it effectively quells pain both spinally and supraspinally.

Additionally, Z0647 induces less constipation than morphine at equianalgesic doses (figure 4), an effect that could be related to its extremely low potency and efficacy Arrestin recruitment and low efficacy partial agonism of Gi/o/z activation in-vitro. Perhaps most interestingly, Z0647 induces no cellular tolerance to analgesia when administered repeatedly, even though it exhibits cross tolerance to morphine, a completely unprecedented molecular phenotype. In addition, in line with the absence of cellular tolerance to analgesia, Z0647 also induces drastically reduced withdrawal symptoms, such as prototypical jumping behaviors.

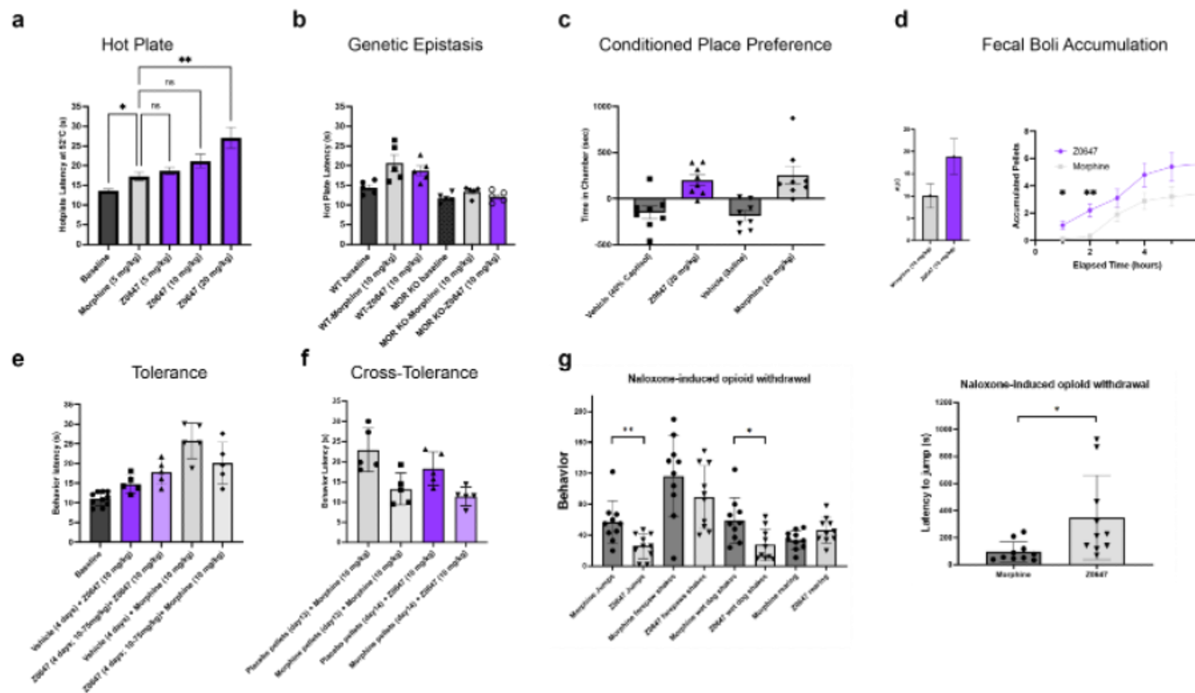

Figure 4. In-vivo activity of Z0647. A.) Hot plate analgesia assay. B.) Genetic epistasis in MOR-KO mice. C.) Conditioned place preference assay without scented chambers. D.) Fecal boli accumulation. E.) Tolerance induced by dose escalation over 4 days from 10-75 mpk. F.) Cross tolerance to morphine after administration to morphine tablets. G.) Naloxone precipitated withdrawal and aversive behaviors.

as well as other well documented aversive behaviors to withdrawal. This is exceptionally interesting given that Z0647 does appear to be reinforcing in a conditioned place preference paradigm. Finally, in a MOR-KO mouse, Z0647 analgesia is completely abrogated, suggesting that Z0647 analgesia is mediated by MOR activation alone.

Some caveats deserve highlight. Although structure-based discovery successfully identified novel scaffolds for the opiate receptor, some of the properties of

Z0647 could be circumstantial. Biased signaling between G proteins and Arrestins is a highly controversial hypothesis and the effects of Z0647 on GRK translocation and receptor phosphorylation cannot be ruled out as causative for its unique in-vivo pharmacology. Furthermore, without knowledge of these specific conformations, we focused on designing novel chemical interactions within the orthosteric site of the activated opiate receptor. Here, a wealth of structure activity data was leveraged to extend our modeling, but this is not an approach that generalizes to less well studied GPCRs. Additionally, the failure to chirally resolve the active (S,S,S)- enantiomer of Z0647 obfuscates the potential divergent pharmacology of enantiomers, which has been observed previously in our inquiries and others. Finally, several aspects of the pharmacology presented herein remain preliminary, including the cellular tolerance and withdrawal phenotypes.

Discussion & Conclusions:

Notwithstanding the aforementioned caveats, this study supports a structure-based approach for opioid drug discovery. Whereas this method cannot yet reliably find leads with precisely tailored pharmacological profiles, it's encouraging that most of the ligands uncovered in this study were agonists, and all of them were topologically distinct from one another and known opiates. Furthermore, targeting subpockets in the receptor can reliably identify novel chemotypes that can make novel interactions, the basis to new pharmacological profiles and biological effects. Here, we optimized an initial docking hit, Z4123, 440-fold to the final lead molecule, Z0647 by empirically evaluating fewer than 30 molecules. Additionally, structural elucidation confirmed the accuracy of

the docking prediction and facilitated rational design of analogs that can be used to dissect the relationship between in-vitro pharmacological profile and organismal in-vivo analgesia phenotypes. Though this compound was inspired by recent successful drug discovery efforts against the opiate receptor, importantly this structure-based approach used only complementarity with the active state opiate receptor as a discriminatory criterion to evaluate new chemical matter. Ultimately, this unbiased search through a vast chemical space yielded a new lead, Z0647, as well as several interesting tool compounds with interesting and divergent biological effects. These compounds could ultimately delineate the interplay between G protein activation, GRK translocation and substrate phosphorylation, beta-arrestin translocation and activation, and receptor internalization. Indeed, Z0647 exhibits significant opiate mediated analgesia while sparing dose-limiting constipation and cellular tolerance which is mooted to play a role in addiction. The interesting pharmacological properties of Z0647 make it and its congeners useful tool compounds to sort out the role of G protein, arrestin, and GRK translocation/activation in µOR signaling. More broadly, the in-vitro and in-vivo results of this large-scale assessment of chemical complementarity to the active state opiate receptor could represent a new approach to developing safer opiate analgesics.

Methods

Chemicals, reagents, and cell lines. Chemicals and reagents in this study were purchased from commercial sources (Sigma, Tocris, Fisher Scientific, Spectrum, and ZINC database suppliers) or synthesized as outlined in supplementary information. HEK293 (ATCC CRL-1573; 60113019; certified mycoplasma free and authentic by

ATCC) and HEK293-T (HEK293T; ATCC CRL-11268; 59587035; certified mycoplasma free and authentic by ATCC) cells were from the ATCC and are well validated for signaling studies. CHO-K1 cells were from Perkin Elmer and are well validated for radioligand binding and signaling studies.

Molecular docking and analogue selection. The active state murine μ-opioid receptor structure (PDB: 5C1M) was used as input for receptor preparation with DOCK Blaster (http://blaster.docking.org). 45 chemical hot-spots were used based on the modeled binding pose of morphinan ligands and PZM21. Waters were modeled based on high occupancy in MD simulations and precedence amongst type A GPCRs. The ligand sampling parameters were set with bin size, bin overlap, and distance tolerances of 0.4 Å, 0.1 Å, and 1.5 Å, respectively, for both the chemical hot-spots and the docked molecules. Ligand poses were scored by summing the receptor-ligand electrostatics and van der Waals interaction energy corrected for ligand desolvation and ligand internal energy. Receptor atom partial charges were used from the united AMBER force field except for one of the modeled water molecules where the dipole moment was increased as previously described (cite). Over 282 million commercially available molecules from the ZINC (http://zinc20.docking.org) lead-like set were docked into the receptor using DOCK3.7 (http://dock.compbio.ucsf.edu). Among the top ranking, 0.5% were inspected and XX were selected for experimental testing in the primary screen. A resource to perform these docking studies is publicly available (tldr.docking.org).

For a secondary screen, the top hit from the primary screen was computationally analogued via the ZINC database using a similarity score of 0.7 (as defined in the ZINC search utility). Additionally, substructure searches were performed using portions of the

parent hit molecule. The searches yielded 18.5 thousand purchasable compounds which were then docked as in the primary screen. Analogues were manually inspected for interactions and further experimental testing.

Radioligand binding studies. For a primary screen of selected molecules, binding to the μOR was assessed by measuring competitive binding against human MOR in membranes prepared from CHO-K1 cells against the radioligand tritiated-diprenorphine (3H-DPN) or tritiated Naltrexone (3H-NTX). Each compound was initially tested at 33 μM and was incubated with 3H-DPN at a concentration equal to the empirical K_D of the radioligand. The reaction contained 5 μg of membranes, and was incubated in a buffer consisting of 50 mM HEPES, 0.1 mM EDTA, 10 mM MgCl2, 0.1% (w/v) BSA, pH with KOH to 7.4 covered and protected from light for 2 hr at 25 °C. To separate free from bound radioligand, reactions were rapidly filtered over Whatman GF/B filters with the aid of a Perkin Elmer Cell Harvester and 3H-DPN or 3H-NTX counts were measured via scintillation using meltilex wax based scintillant and a Perkin Elmer BetaMax scintillation counter. Compounds with more than 50% displacement of 3H-DPN or 3H-NTX were further tested in full dose-response to determine their affinity in CHO-K1 membranes. Subsequently, the analogues were tested in full dose-response for affinity at the μOR.

In vivo behavioral studies. Animal experiments were approved by the UCSF Institutional Animal Care and Use Committee and were conducted in accordance with the NIH Guide for the Care and Use of Laboratory animals (protocol #AN195657). Adult (8-10 weeks old) male C56BL/6 mice (strain # 664) were purchased from the Jackson Laboratory. Mu-opioid knockout mice were kindly provided by Dr Kevin Yackle at UCSF

(Liu et al., 2021). Mice were housed in cages on a standard 12:12 hour light/dark cycle with food and water ad libitum.

For all behavioral tests, the experimenter was always blind to treatment. Behavioral tests were conducted 30 minutes after the compound injection. Briefly, the hindpaw thermal sensitivity was measured by placing the mouse on a 52oC hotplate or, for the tail flick assay, by immersing its tail into a 50oC water bath. For the ambulatory (rotarod) test, mice were first trained on an accelerating rotating rod, 3 times for 5 min, before testing with the compound.

Constipation assay. Mice had access to food and water ad libitum prior to the test. On the test day, mice received an i.p. injection of a solution (100µL) containing saline, 10 mg/kg morphine or 10 mg/kg Z0647 and then individually placed in a clean cage, with no access to food or water. Fecal pellets were collected and counted every hour, up to 6 hours.

Open Field Test. Mice were injected with the compound and 30 minutes later placed in the center of a round open-field (2 feet diameter) and their exploratory behavior (distance traveled) recorded over the next 15 minutes.

Conditioned Place Preference. To determine if Z0647 was inherently rewarding we used the conditioned place paradigm as described previously (Juarez-Salinas et al., 2018). Briefly, mice were habituated to the test apparatus, on 2 consecutive days, and their preference for each chamber recorded for 30 minutes (Pretest). On the 3rd and 4th days, mice were injected with the vehicle control or the compound, placed in a cylinder for 30 minutes and then restricted for 30 minutes in the preferred (vehicle) or non-preferred (compound) chamber, respectively (2 conditioning days). On the 5th day (test

day), mice were allowed to roam freely between the 3 chambers of the apparatus and their preference for each chamber recorded for 30 minutes. To calculate the CPP score, we subtracted the time spent in each chamber of the box on the Pretest day from that of the test day (CPP score = Test - Pretest).

Opioid Withdrawal Symptoms Assay. To investigate the effect of the novel opioid on naloxone-precipitated opioid withdrawal symptoms, we first generated mice tolerant to the opioid compounds, as previously described (Singh et al., 2023). Briefly, mice received 8 escalating doses of morphine or Z0647 (IP) over 4 days (10, 15, 20, 30, 50, 60, 70 and 75 mg/kg; twice daily, i.p.). On the 5th day, mice received a single dose of morphine or Z0647 (20 mg/kg, i.p.), followed 2h later by a single dose of naloxone (10 mg/kg; i.p.) and were immediately video recorded for the next 20 minutes. To document withdrawal, we scored the number of naloxone-precipitated jumps, forepaw shakes, wet dog shakes and rearing over the next 20 minutes, as well as the latency for the first jump.

Pharmacokinetics of Z0647. Studies were performed by Bienta, a subsidiary of Enamine Ltd.

Preparation of Z0647-μOR-miniGo complex. The human WT μOR was cloned into pCDNA3.1_zeo_tetO vector with an N-terminal FLAG tag and a C-terminal fusion to a mini Go protein. This construct was expressed in Expi293 TetR cells using the Expifectamine transfection system (ThermoFisher Scientific). Cells were transfected at a density of 3 x 10^6 cells per mL at 37 °C. Expression was induced 18 hr later with the addition of 1 mg/mL doxycycline and the enhancers supplied in the expifectamine transfection kit. 24 hr post induction, cells were harvested and frozen at -80 °C overnight. Receptor was extracted and purified from frozen cell pellets as previously described for

μOR. Briefly, the receptor was purified with FLAG affinity chromatography and size exclusion chromatography as previously described[35] in the presence of 10 μM Z0647. The monomeric fractions of receptor-miniGo complex were collected and concentrated to 20 mg/mL, freshly purified Z0647-bound μOR-miniGo was used for complex formation with Gβ1γ2.

Expression and purification of Gβ1γ2. Human Gβ1γ2 heterodimer was expressed in Trichoplusia ni Hi5 insect cells (unauthenticated and untested for mycoplasma contamination, Expression Systems) using a single baculovirus generated in Spodoptera frugiperda Sf9 insect cells (unauthenticated and untested for mycoplasma contamination; Expression Systems). A bicistronic pVLDual construct contained the Gβ1 subunit with a N-terminal 6× His tag, and an untagged human Gγ2 subunit. For expression, Hi5 insect cells were transduced with baculovirus at a density of ~3.0 × 106 cells per ml, grown with 27 °C shaking at 130 rpm. 48 h post-transduction, cells were collected and washed in a hypotonic buffer containing 20 mM HEPES, pH 8.0, 5 mM β-mercaptoethanol (β-ME), and protease inhibitors (20 μg ml−1 leupeptin, 160 μg ml−1 benzamidine). The membrane fraction was then separated by centrifugation and solubilized with 20 mM HEPES pH 8.0, 100 mM sodium chloride, 1.0% sodium cholate, 0.05% dodecylmaltoside (Anatrace), and 5 mM β-mercaptoethanol (β-ME). Solubilized Gβ1γ2 heterodimer was then incubated with HisPur Ni-NTA resin (Thermo Scientific) in batch. Bound Gβ1γ2 heterodimer was washed extensively and detergent was slowly exchanged to 0.1% (w/v) lauryl maltose neopentyl glycol (L-MNG, Anatrace) and 0.01% CHS before elution with 20 mM HEPES pH 7.5, 100 mM NaCl, 0.1% L-MNG, 0.01% CHS, 270 mM imidazole, 1 mM dithiothreitol (DTT), and protease inhibitors. Eluted Gβ1γ2 heterodimer was pooled and rhinovirus 3C

protease was added to cleave the N-terminal 6× His tag during overnight dialysis in 20 mM HEPES pH 7.5, 100 mM NaCl, 0.02% L-MNG, 0.002% CHS, 1 mM DTT, and 10 mM imidazole. To remove uncleaved Gβ1γ2, dialysed material was incubated with HisPur Ni-NTA resin in batch. The unbound fraction was then incubated for 1 h at 4 °C with lambda phosphatase (New England Biolabs), calf intestinal phosphatase (New England Biolabs), and Antarctic phosphatase (New England Biolabs) for dephosphorylation. Final anion exchange chromatography was performed using a MonoQ 4.6/100 PE (Cytiva) column to purify only geranylgeranylated heterodimer. The resulting protein was pooled and dialysed overnight in 20 mM HEPES pH 7.5, 100 mM NaCl, 0.02% L-MNG, and 100 μM TCEP, and concentrated with a 3 kDa centrifugal concentrator to a final concentration of . Glycerol was added to a final concentration of 20%, and the protein was flash frozen in liquid nitrogen and stored at −80 °C until further use.

Preparation of μOR-Z0647-miniGo-ScFv16- Gβ1γ2 complex. To prepare the Z0647-μOR complex, purified μOR-mini-Go was incubated with a 2-fold molar excess of purified Gβ1γ2, ScFv16, and 10 μM Z0647 overnight at 4 °C. After incubation, complexed material was concentrated with a 100 kDa MWCO Amicon filter and injected into a Superdex200 Increase 10/300 GL gel filtration column equilibrated in 50 mM HEPES, 150 mM NaCl, 0.005% GDN (w/v), 10 μM Z0647, pH 7.5. Monodisperse fractions were concentrated with a 100 kDa MWCO Amicon filter immediately prior to cryo-EM grid preparation.

Cryo-EM vitrification, data collection and processing. The Z0647-bound μOR-Go complex was concentrated to 4 mg/mL and 3 μL was applied to a glow-discharged 300 mesh 1.2/1.3 gold grid covered in a holey gold film (UltrAufoil). After a 30 s hold at 4 °C, excess sample was removed with a blotting time of 5 s and a blotting force of 0 prior to

plunge freezing in liquid ethane using a Vitrobot Mark IV (ThermoFisher). A total of 10,068 super-resolution movies were recorded using a K3 detector on a Titan Krios (ThermoFisher) microscope operated at 300 keV with a BioQuantum post-column energy filter set to a zero-loss energy selection slit width of 10 eV. The 66-frame movies were recorded for 2 s at a nominal magnification of 130,000x (physical pixel size of 0.664 Å per pixel) and a defocus range of −0.8 to − 2.2 μm for a total dose of 77 e− Å−2. Exposure areas were acquired with automated scripts in a 3 × 3 image shift collection strategy using SerialEM 3.8.

Super resolution movies of the Z0647-μOR-Go complex were motion-corrected, binned to physical pixel size, and dose-fractionated on-the-fly during data collection using UCSF MotionCor2. Corrected micrographs were imported to CryoSPARC v3.1 for CTF estimation employing the Patch CTF estimation job type. Templates for particle picking were generated from projections of the same μOR complex reconstructed from a previous 200 keV imaging session. Particle picking templates were low-pass filtered to 20 Å and used to pick 6,706,062 particles. After picking, estimated CTF fit resolution >5 Åand relative ice thickness measurements were used to remove low-quality micrographs before further processing. 5,766,358 curated particles were extracted in a 288-pixel box and fourier cropped to 72 pixels before undergoing a round of 3D classification with alignment utilizing one 20 Å low-pass filtered reconstruction and 3 ‘random’ reconstructions generated from a prematurely truncated ab-initio reconstruction job. 639,485 particles classified into the μOR-Z0647-Go complex class were extracted in a 288-pixel box fourier cropped to 144 pixels for 2 additional rounds of 3D classification with alignment, using the same class distributions as previously described. From these 2 rounds of 3D

classification, 596,197 particles belonging to the μOR-Z0647-Go complex class were extracted into a 288-pixel box and subjected to one round of non-uniform refinement in cryoSPARC using a 7TM domain mask and a further round of alignment-free 3D classification in Relion v3.1.2. Particles in qualitatively good-looking classes were re-imported into cryoSPARC for focused refinements. Particles from the 7TM domain masked 3D classification were subjected to two rounds of focused refinement using the same mask as in RELION, decreasing mask pixel dilation between rounds. For each focused refinement, pose/shift gaussian priors (7° standard deviation of prior (rotation), 4 Å standard deviation of prior (shifts)) were used to limit large deviations from the initially determined poses.

Model Building and Refinement. We modeled the Z0647 bound-μOR-Go complex in a composite cryo-EM map. For μOR we started with murine μOR (PDB:5C1M) for the remainder of the protein, we used the structure of NTSR-miniGo- Gβ1γ2-ScFv16 which had high structural agreement with the construct employed in this study. After truncating unresolved regions, the 7TM domain in this model was fit into the composite cryo-EM map of the Z0647 bound-μOR-Go complex using UCSF Chimera v1.3. This template model was rebuilt in Coot for rigid body fitting of the remainder of the protein. For Z0647, parameters were generated for all possible stereoisomers using Phenix and the (s,s,s,)-configuration isomer was used for modeling due to its optimal fit in the coulombic potential. Each of these components were individually fit into the cryo-EM coulombic potential with ChimeraX 1.2.5. We subsequently iteratively refined the model with manual refinement in Coot and ISOLDE v1.0b3 and real space refinement in Phenix.

References

(1) Snyder, S. H.; Pasternak, G. W. Historical Review: Opioid Receptors. *Trends Pharmacol. Sci.* **2003**, *24* (4), 198–205. https://doi.org/10.1016/S0165-6147(03)00066-X.

(2) Sertùrner, F. W. Saure Im Opium. *Trommsdorffs Journal der Pharmazie*.

(3) Roth, B. L.; Irwin, J. J.; Shoichet, B. K. Discovery of New GPCR Ligands to Illuminate New Biology. *Nat. Chem. Biol.* **2017**, *13* (11), 1143–1151. https://doi.org/10.1038/nchembio.2490.

(4) Manglik, A.; Lin, H.; Aryal, D. K.; McCorvy, J. D.; Dengler, D.; Corder, G.; Levit, A.; Kling, R. C.; Bernat, V.; Hübner, H.; Huang, X.-P.; Sassano, M. F.; Giguère, P. M.; Löber, S.; Da Duan; Scherrer, G.; Kobilka, B. K.; Gmeiner, P.; Roth, B. L.; Shoichet, B. K. Structure-Based Discovery of Opioid Analgesics with Reduced Side Effects. *Nature* **2016**, *537* (7619), 185–190. https://doi.org/10.1038/nature19112.

(5) Lyu, J.; Wang, S.; Balius, T. E.; Singh, I.; Levit, A.; Moroz, Y. S.; O'Meara, M. J.; Che, T.; Algaa, E.; Tolmachova, K.; Tolmachev, A. A.; Shoichet, B. K.; Roth, B. L.; Irwin, J. J. Ultra-Large Library Docking for Discovering New Chemotypes. *Nature* **2019**, *566* (7743), 224–229. https://doi.org/10.1038/s41586-019-0917-9.

(6) Bender, B. J.; Gahbauer, S.; Luttens, A.; Lyu, J.; Webb, C. M.; Stein, R. M.; Fink, E. A.; Balius, T. E.; Carlsson, J.; Irwin, J. J.; Shoichet, B. K. A Practical Guide to Large-Scale Docking. *Nat. Protoc.* **2021**. https://doi.org/10.1038/s41596-021-00597-z.

(7) Huang, W.; Manglik, A.; Venkatakrishnan, A. J.; Laeremans, T.; Feinberg, E. N.; Sanborn, A. L.; Kato, H. E.; Livingston, K. E.; Thorsen, T. S.; Kling, R. C.; Granier, S.; Gmeiner, P.; Husbands, S. M.; Traynor, J. R.; Weis, W. I.; Steyaert, J.; Dror, R. O.;

Kobilka, B. K. Structural Insights into \mu-Opioid Receptor Activation. *Nature* **2015**, *524* (7565), 315–321. https://doi.org/10.1038/nature14886.

(8) Manglik, A. Molecular Basis of Opioid Action: From Structures to New Leads. *Biol. Psychiatry* **2020**, *87* (1), 6–14. https://doi.org/10.1016/j.biopsych.2019.08.028.

(9) Manglik, A.; Kruse, A. C.; Kobilka, T. S.; Thian, F. S.; Mathiesen, J. M.; Sunahara, R. K.; Pardo, L.; Weis, W. I.; Kobilka, B. K.; Granier, S. Crystal Structure of the \mu-Opioid Receptor Bound to a Morphinan Antagonist. *Nature* **2012**, *485* (7398), 321–326. https://doi.org/10.1038/nature10954.

(10) Faouzi, A.; Wang, H.; Zaidi, S. A.; DiBerto, J. F.; Che, T.; Qu, Q.; Robertson, M. J.; Madasu, M. K.; El Daibani, A.; Varga, B. R.; Zhang, T.; Ruiz, C.; Liu, S.; Xu, J.; Appourchaux, K.; Slocum, S. T.; Eans, S. O.; Cameron, M. D.; Al-Hasani, R.; Pan, Y. X.; Roth, B. L.; McLaughlin, J. P.; Skiniotis, G.; Katritch, V.; Kobilka, B. K.; Majumdar, S. Structure-Based Design of Bitopic Ligands for the μ-Opioid Receptor. *Nature* **2023**, *613* (7945), 767–774. https://doi.org/10.1038/s41586-022-05588-y.

(11) Zhuang, Y.; Wang, Y.; He, B.; He, X.; Zhou, X. E.; Guo, S.; Rao, Q.; Yang, J.; Liu, J.; Zhou, Q.; Wang, X.; Liu, M.; Liu, W.; Jiang, X.; Yang, D.; Jiang, H.; Shen, J.; Melcher, K.; Chen, H.; Jiang, Y.; Cheng, X.; Wang, M.-W.; Xie, X.; Xu, H. E. Molecular Recognition of Morphine and Fentanyl by the Human μ-Opioid Receptor. *Cell* **2022**, *185* (23), 4361–4375.e19. https://doi.org/10.1016/j.cell.2022.09.041.

(12) Wang, Y.; Zhuang, Y.; DiBerto, J. F.; Zhou, X. E.; Schmitz, G. P.; Yuan, Q.; Jain, M. K.; Liu, W.; Melcher, K.; Jiang, Y.; Roth, B. L.; Xu, H. E. Structures of the Entire Human Opioid Receptor Family. *Cell* **2023**, *186* (2), 413–427.e17. https://doi.org/10.1016/j.cell.2022.12.026.

(13) Schmid, C. L.; Kennedy, N. M.; Ross, N. C.; Lovell, K. M.; Yue, Z.; Morgenweck, J.; Cameron, M. D.; Bannister, T. D.; Bohn, L. M. Bias Factor and Therapeutic Window Correlate to Predict Safer Opioid Analgesics. *Cell* **2017**, *171* (5), 1165–1175.e13. https://doi.org/10.1016/j.cell.2017.10.035.

(14) Pedersen, M. F.; Wróbel, T. M.; Märcher-R\orsted, E.; Pedersen, D. S.; M\oller, T. C.; Gabriele, F.; Pedersen, H.; Matosiuk, D.; Foster, S. R.; Bouvier, M.; Bräuner-Osborne, H. Biased Agonism of Clinically Approved μ-Opioid Receptor Agonists and TRV130 Is Not Controlled by Binding and Signaling Kinetics. *Neuropharmacology* **2020**, *166*, 107718. https://doi.org/10.1016/j.neuropharm.2019.107718.

(15) Gillis, A.; Gondin, A. B.; Kliewer, A.; Sanchez, J.; Lim, H. D.; Alamein, C.; Manandhar, P.; Santiago, M.; Fritzwanker, S.; Schmiedel, F.; Katte, T. A.; Reekie, T.; Grimsey, N. L.; Kassiou, M.; Kellam, B.; Krasel, C.; Halls, M. L.; Connor, M.; Lane, J. R.; Schulz, S.; Christie, M. J.; Canals, M. Low Intrinsic Efficacy for G Protein Activation Can Explain the Improved Side Effect Profiles of New Opioid Agonists. *Sci. Signal.* **2020**, *13* (625). https://doi.org/10.1126/scisignal.aaz3140.

(16) Bhowmik, S.; Galeta, J.; Havel, V.; Nelson, M.; Faouzi, A.; Bechand, B.; Ansonoff, M.; Fiala, T.; Hunkele, A.; Kruegel, A. C.; Pintar, J. E.; Majumdar, S.; Javitch, J. A.; Sames, D. Site Selective C-H Functionalization of Mitragyna Alkaloids Reveals a Molecular Switch for Tuning Opioid Receptor Signaling Efficacy. *Nat. Commun.* **2021**, *12* (1), 3858. https://doi.org/10.1038/s41467-021-23736-2.

(17) Williams, J. T.; Ingram, S. L.; Henderson, G.; Chavkin, C.; von Zastrow, M.; Schulz, S.; Koch, T.; Evans, C. J.; Christie, M. J. Regulation of μ-Opioid Receptors:

Desensitization, Phosphorylation, Internalization, and Tolerance. *Pharmacol. Rev.* **2013**, *65* (1), 223–254. https://doi.org/10.1124/pr.112.005942.

(18) Alon, A.; Lyu, J.; Braz, J. M.; Tummino, T. A.; Craik, V.; O'Meara, M. J.; Webb, C. M.; Radchenko, D. S.; Moroz, Y. S.; Huang, X.-P.; Liu, Y.; Roth, B. L.; Irwin, J. J.; Basbaum, A. I.; Shoichet, B. K.; Kruse, A. C. Structures of the σ2 Receptor Enable Docking for Bioactive Ligand Discovery. *Nature* **2021**, *600* (7890), 759–764. https://doi.org/10.1038/s41586-021-04175-x.

(19) Fink, E. A.; Xu, J.; Hübner, H.; Braz, J. M.; Seemann, P.; Avet, C.; Craik, V.; Weikert, D.; Schmidt, M. F.; Webb, C. M.; Tolmachova, N. A.; Moroz, Y. S.; Huang, X.-P.; Kalyanaraman, C.; Gahbauer, S.; Chen, G.; Liu, Z.; Jacobson, M. P.; Irwin, J. J.; Bouvier, M.; Du, Y.; Shoichet, B. K.; Basbaum, A. I.; Gmeiner, P. Structure-Based Discovery of Nonopioid Analgesics Acting through the α_{2A}-Adrenergic Receptor. *Science* **2022**, *377* (6614), eabn7065. https://doi.org/10.1126/science.abn7065.

(20) Irwin, J. J.; Tang, K. G.; Young, J.; Dandarchuluun, C.; Wong, B. R.; Khurelbaatar, M.; Moroz, Y. S.; Mayfield, J.; Sayle, R. A. ZINC20-A Free Ultralarge-Scale Chemical Database for Ligand Discovery. *J. Chem. Inf. Model.* **2020**, *60* (12), 6065–6073. https://doi.org/10.1021/acs.jcim.0c00675.

(21) Sterling, T.; Irwin, J. J. ZINC 15--Ligand Discovery for Everyone. *J. Chem. Inf. Model.* **2015**, *55* (11), 2324–2337. https://doi.org/10.1021/acs.jcim.5b00559.

(22) Meng, E. C.; Shoichet, B. K.; Kuntz, I. D. Automated Docking with Grid-Based Energy Evaluation. *J. Comput. Chem.* **1992**, *13* (4), 505–524. https://doi.org/10.1002/jcc.540130412.

(23) Lorber, D. M.; Shoichet, B. K. Flexible Ligand Docking Using Conformational Ensembles. *Protein Sci.* **1998**, *7* (4), 938–950. https://doi.org/10.1002/pro.5560070411.

(24) Mysinger, M. M.; Shoichet, B. K. Rapid Context-Dependent Ligand Desolvation in Molecular Docking. *J. Chem. Inf. Model.* **2010**, *50* (9), 1561–1573. https://doi.org/10.1021/ci100214a.

(25) Coleman, R. G.; Carchia, M.; Sterling, T.; Irwin, J. J.; Shoichet, B. K. Ligand Pose and Orientational Sampling in Molecular Docking. *PLoS One* **2013**, *8* (10), e75992. https://doi.org/10.1371/journal.pone.0075992.

(26) Rogers, D.; Hahn, M. Extended-Connectivity Fingerprints. *J. Chem. Inf. Model.* **2010**, *50* (5), 742–754. https://doi.org/10.1021/ci100050t.

(27) Maggiora, G.; Vogt, M.; Stumpfe, D.; Bajorath, J. Molecular Similarity in Medicinal Chemistry. *J. Med. Chem.* **2014**, *57* (8), 3186–3204. https://doi.org/10.1021/jm401411z.

(28) Goddard, T. D.; Huang, C. C.; Meng, E. C.; Pettersen, E. F.; Couch, G. S.; Morris, J. H.; Ferrin, T. E. UCSF ChimeraX: Meeting Modern Challenges in Visualization and Analysis. *Protein Sci.* **2018**, *27* (1), 14–25. https://doi.org/10.1002/pro.3235.

(29) Pettersen, E. F.; Goddard, T. D.; Huang, C. C.; Couch, G. S.; Greenblatt, D. M.; Meng, E. C.; Ferrin, T. E. UCSF Chimera\textemdasha Visualization System for Exploratory Research and Analysis. *J. Comput. Chem.* **2004**, *25* (13), 1605–1612. https://doi.org/10.1002/jcc.20084.

(30) Goddard, T. D.; Huang, C. C.; Ferrin, T. E. Visualizing Density Maps with UCSF Chimera. *J. Struct. Biol.* **2007**, *157* (1), 281–287. https://doi.org/10.1016/j.jsb.2006.06.010.

(31) Topiol, S. Current and Future Challenges in GPCR Drug Discovery. *Methods Mol. Biol.* **2018**, *1705*, 1–21. https://doi.org/10.1007/978-1-4939-7465-8_1.

(32) Venkatakrishnan, A. J.; Ma, A. K.; Fonseca, R.; Latorraca, N. R.; Kelly, B.; Betz, R. M.; Asawa, C.; Kobilka, B. K.; Dror, R. O. Diverse GPCRs Exhibit Conserved Water Networks for Stabilization and Activation. *Proc. Natl. Acad. Sci. U. S. A.* **2019**, *116* (8), 3288–3293. https://doi.org/10.1073/pnas.1809251116.

(33) Shim, J.; Coop, A.; MacKerell, A. D. Consensus 3D Model of μ-Opioid Receptor Ligand Efficacy Based on a Quantitative Conformationally Sampled Pharmacophore. *J. Phys. Chem. B* **2011**, *115* (22), 7487–7496. https://doi.org/10.1021/jp202542g.

(34) Kieffer, B. L. Drug Discovery: Designing the Ideal Opioid. *Nature* **2016**, *537* (7619), 170–171. https://doi.org/10.1038/nature19424.

(35) Koehl, A.; Hu, H.; Maeda, S.; Zhang, Y.; Qu, Q.; Paggi, J. M.; Latorraca, N. R.; Hilger, D.; Dawson, R.; Matile, H.; Schertler, G. F. X.; Granier, S.; Weis, W. I.; Dror, R. O.; Manglik, A.; Skiniotis, G.; Kobilka, B. K. Structure of the \mu-Opioid Receptor-Gi Protein Complex. *Nature* **2018**, *558* (7711), 547–552. https://doi.org/10.1038/s41586-018-0219-7.

Chapter 3: Creating community and building representation in the biomedical enterprise

Diversity, equity and inclusion are often charged terms that bring about lengthy institutional statements and incessant virtue signaling[1–3]. Indeed, at UCSF, the racial reckoning precipitated by the state-sanctioned murder of George Floyd prompted some deep soul-searching replete with a frantic initiation on institutional directives, action plans, diversity statements, and town halls. Ultimately, as the US population moved on from the uprisings engendered by the killing of George Floyd, UCSF followed suit[2]. Anti-racism, once a rally point for justice minded members of the academy, diminished in popularity and many of the taskforces and action plans that were hastily assembled crumbled just as hastily[4–6]. Still, student driven initiatives were able to foment lasting structural change at UCSF. Two such changes and how they were accomplished are described below.

On June 9 2020, many watched in sheer terror as Derrick Chauvin pinned an unarmed and visibly distressed George Floyd on the concrete with his knee directly pressing into Floyd's carotid artery. George Floyd gasped and exclaimed that he could not breathe numerous times to no avail. While Chauvin committed this senseless act of state sanctioned brutality, his fellow 'peace officers' restrained concerned bystanders. After nearly 10 minutes, Chauvin successfully squeezed the life out of Floyd and EMTs placed Floyd's lifeless body into an ambulance to be taken to the morgue. Regrettably, this horrific act of violence was captured on video and went mega-viral on social media. When I finally got the opportunity, I watched the video and was deeply emotionally

affected by the callousness of the murder. Many of my peers, Black graduate students at UCSF were also deeply affected, having perceived that they could also be randomly subjected to such a horrific and violent act just because they, like Floyd, were Black Americans. As I tried to make sense of the killing, I suffered immense emotional turmoil. I could not sleep due to night terrors and entered a catatonic state of fear and depression. Law enforcement all over the US became more militant and hostile due to their well-deserved negative perception. I no longer felt safe on the UCSF Mission Bay campus, which is highly overpoliced and where Black skin is actively criminalized.

Soon, I learned I was not alone in these sentiments. Encouraged by Dr. D'anne Duncan, the Dean of Diversity and Learner Success, I began organizing with other Black PhD students to hold space for community building and refuge from the incessant racial insensitivity of a primarily white institution like UCSF. While organizations existed in other schools for Black students to find community, the graduate division at UCSF lacked a central voice for Black learners. As such, I collaborated with other senior graduate students to found and serve as the inaugural co-president of Black Excellence in Science, Technology, Engineering, and Mathematics (BE-STEM). BE-STEM served as a central voice to both build community for Black learners, and also as a nucleation point for Black outreach and activism both inside the institution, and with the San Francisco community at large.

Accordingly, BE-STEM leadership got to work crafting a specific, time-constrained and justice focused list of demands to the administration of UCSF on behalf

of Black graduate students pursing PhDs. Amazingly, at this time, the list of demands has largely been adopted by UCSF administration. This speaks to the efficacy of community organizing and my direct impact as an inaugural co-president. BE-STEM focused on more than just advocacy during its formative year and extended significant time and resources to community building[7]. These efforts consisted of leadership panels, regular community building events graciously sponsored by labs that were friendly to the cause, informal scientific talks to build a scholarly community, and practice qualifying exams to help Black students overcome increasingly unfair stigma and prejudice. Ultimately, BE-STEM helped many Black students cope with unending racial microaggressions[1] at UCSF and allowed these learners to contribute to the scientific mission of UCSF in a more holistic fashion.

In addition to the internal community building efforts, BE-STEM worked tirelessly to recruit and retain Black individuals at all levels of the institution[8]. To this end, BE-STEM members participated on faculty and senior administrator hiring panels. Perhaps most encouragingly, I served on the hiring panel for the Vice Provost of Student Academic Affairs and Dean of the Graduate Division. This hiring panel, which included C-suite university officials, was largely influenced by my contributions and my unending commitment to racial justice and equity. Ultimately, a Black woman was deemed most qualified for the position and Dean Nicquet Blake joined the administration of UCSF in late 2021.

As BE-STEM grew and became more recognized on campus, I focused my presidential duties on training the next generation of student leaders, both at UCSF and in the neglected and historically marginalized majority Black community of Bayview-Hunters Point. To this end, I founded and designed an outreach program called the Bay Area Youth Science Program, or BAYS to welcome historically excluded Black and Indigenous members of our Bay Area community into UCSF. BAYS is a collaboration of internal and external stakeholders that begins with a 12-week comprehensive virtual lecture series at one of the most resource starved schools in the SF Bay Area, KIPP SF College Prep Academy. Kipp is a majority Black/Brown high school that is exceptionally resource starved[9]. Teacher turnover is high, there are not enough textbooks for each pupil, and over 95% of students there are on governmental free and reduced lunch programs. After the lecture series, a number of interested and engaged students are selected for an intensive 8-week laboratory internship at UCSF where these students apply the skills they learned in lecture and experience laboratory research first hand. Over the course of the summer, BAYS interns spend Monday-Thursday conducting independent research projects in UCSF labs under the guidance of graduate student and postdoc mentors. During their time on campus, students also engage in journal clubs, lab meetings, and community building events. On Friday's students participate in a collaborative course taught by BE-STEM graduate students and CSEO personnel that covers research basics, aspects of the hidden curriculum, life skills, financial competency, career path guidance, and college preparation. The internship culminates in a formal presentation of their research project that is open to all of UCSF. Throughout the experience, we create structured time for near-peer mentors with shared identities

to engage in informal mentorship in addition to the student's lab mentors. These mentors are essential to guide the BAYS scholars through unfamiliar circumstances, serve as strong role models with shared identities, and provide firsthand perspectives on navigating the hidden curriculum[10,11]. To truly foment transformative change, we further invest in our BAYS scholars by providing an intensive college application bootcamp. In these sessions, graduates of the BAYS internship gain skills in the college application process. The college preparation period is from September-March following the internship. Interns work virtually on their college applications, scholarships, and financial aid applications for 2-6 hours per week and attend weekly sessions with the BAYS Program cohort & coordinators. In so doing, BAYS works to address the 'leaky' talent pipeline from secondary school to PhD/Professional degree by removing barriers to accessing the academy, promoting community engagement, building learner confidence, and slowly breaking down systems of otherness that have perpetuated the lack of representation of some groups at UCSF[12].

One interesting operational approach that BAYS utilizes is a student-led, administrator supported paradigm. In crafting the BAYS program, I intentionally sought out a career administrator to work with graduate student leaders to lessen the burden of managing a full-blown outreach program in addition to a full course load of demanding graduate studies. This leadership structure has provided an opportunity to bank institutional knowledge and facilitates the input of multiple student leaders who are by definition transient members of the academy.

In sum, cultivating community amongst historically excluded individuals has been an essential and wholly required aspect of my journey through graduate education. While I find it shameful that I, a personal victim of intentional marginalization, must work on behalf of a hostile institution like UCSF to make it safer for people like me, I gladly approach this work as I find it intrinsic to the mission of the academy. Indeed, as an institution of higher education bound by PRIDE values, it is the responsibility of UCSF to address its long and torturous history of intentional marginalization by welcoming historically excluded individuals into its hallowed halls. Clearly, in including those who have been intentionally left out in the past, we can bolster our ability to make cutting edge biomedical discoveries. It was one of my deepest pleasures to be a leader at UCSF in this domain during my graduate education.

References

(1) Valandra; Fields, L.; Sebree, W.; Sober, W. Navigating an Anti-Black Campus Climate: #black@pwi. *Race Ethnicity and Education* **2022**, 1–18. https://doi.org/10.1080/13613324.2022.2088720.

(2) Aouragh, M. “White Privilege” and Shortcuts to Anti-Racism. *Race Cl.* **2019**, *61* (2), 3–26. https://doi.org/10.1177/0306396819874629.

(3) Gosztyla, M. L.; Kwong, L.; Murray, N. A.; Williams, C. E.; Behnke, N.; Curry, P.; Corbett, K. D.; DSouza, K. N.; Gala de Pablo, J.; Gicobi, J.; Javidnia, M.; Lotay, N.; Prescott, S. M.; Quinn, J. P.; Rivera, Z. M. G.; Smith, M. A.; Tang, K. T. Y.; Venkat, A.; Yamoah, M. A. Responses to 10 Common Criticisms of Anti-Racism Action in STEMM. *PLoS Comput. Biol.* **2021**, *17* (7), e1009141. https://doi.org/10.1371/journal.pcbi.1009141.

(4) Joseph, A. J.; Janes, J.; Badwall, H.; Almeida, S. Preserving White Comfort and Safety: The Politics of Race Erasure in Academe. *Social Identities* **2020**, *26* (2), 166–185. https://doi.org/10.1080/13504630.2019.1671184.

(5) Yi, J.; Neville, H. A.; Todd, N. R.; Mekawi, Y. Ignoring Race and Denying Racism: A Meta-Analysis of the Associations between Colorblind Racial Ideology, Anti-Blackness, and Other Variables Antithetical to Racial Justice. *J. Couns. Psychol.* **2022**. https://doi.org/10.1037/cou0000618.

(6) Mehta, D. Lab Heads Should Learn to Talk about Racism. *Nature* **2018**, *559* (7713), 153. https://doi.org/10.1038/d41586-018-05646-4.

(7) Allen-Ramdial, S.-A. A.; Campbell, A. G. Reimagining the Pipeline: Advancing STEM Diversity, Persistence, and Success. *Bioscience* **2014**, *64* (7), 612–618. https://doi.org/10.1093/biosci/biu076.

(8) Gasman, M. *Doing the Right Thing: How Colleges and Universities Can Undo Systemic Racism in Faculty Hiring*; Princeton University Press, 2022. https://doi.org/10.1515/9780691229447.

(9) Shange, S. *Progressive Dystopia: Abolition, Antiblackness, and Schooling in San Francisco*; Duke University Press, 2019.

(10) Lescak, E. A.; O'Neill, K. M.; Collu, G. M.; Das, S. Ten Simple Rules for Providing a Meaningful Research Experience to High School Students. *PLoS Comput. Biol.* **2019**, *15* (4), e1006920. https://doi.org/10.1371/journal.pcbi.1006920.

(11) Arif, S.; Massey, M. D. B.; Klinard, N.; Charbonneau, J.; Jabre, L.; Martins, A. B.; Gaitor, D.; Kirton, R.; Albury, C.; Nanglu, K. Ten Simple Rules for Supporting Historically Underrepresented Students in Science. *PLoS Comput. Biol.* **2021**, *17* (9), e1009313. https://doi.org/10.1371/journal.pcbi.1009313.

(12) Kulis, S.; Chong, Y.; Shaw, H. DISCRIMINATORY ORGANIZATIONAL CONTEXTS AND BLACK SCIENTISTS ON POSTSECONDARY FACULTIES. *Res. High. Educ.* **1999**, *40* (2), 115–148. https://doi.org/10.1023/A:1018778412377.

Publishing Agreement

It is the policy of the University to encourage open access and broad distribution of all theses, dissertations, and manuscripts. The Graduate Division will facilitate the distribution of UCSF theses, dissertations, and manuscripts to the UCSF Library for open access and distribution. UCSF will make such theses, dissertations, and manuscripts accessible to the public and will take reasonable steps to preserve these works in perpetuity.

I hereby grant the non-exclusive, perpetual right to The Regents of the University of California to reproduce, publicly display, distribute, preserve, and publish copies of my thesis, dissertation, or manuscript in any form or media, now existing or later derived, including access online for teaching, research, and public service purposes.

DocuSigned by:
Chase Webb
45D52C1BC79541...

Author Signature

11/27/2023

Date