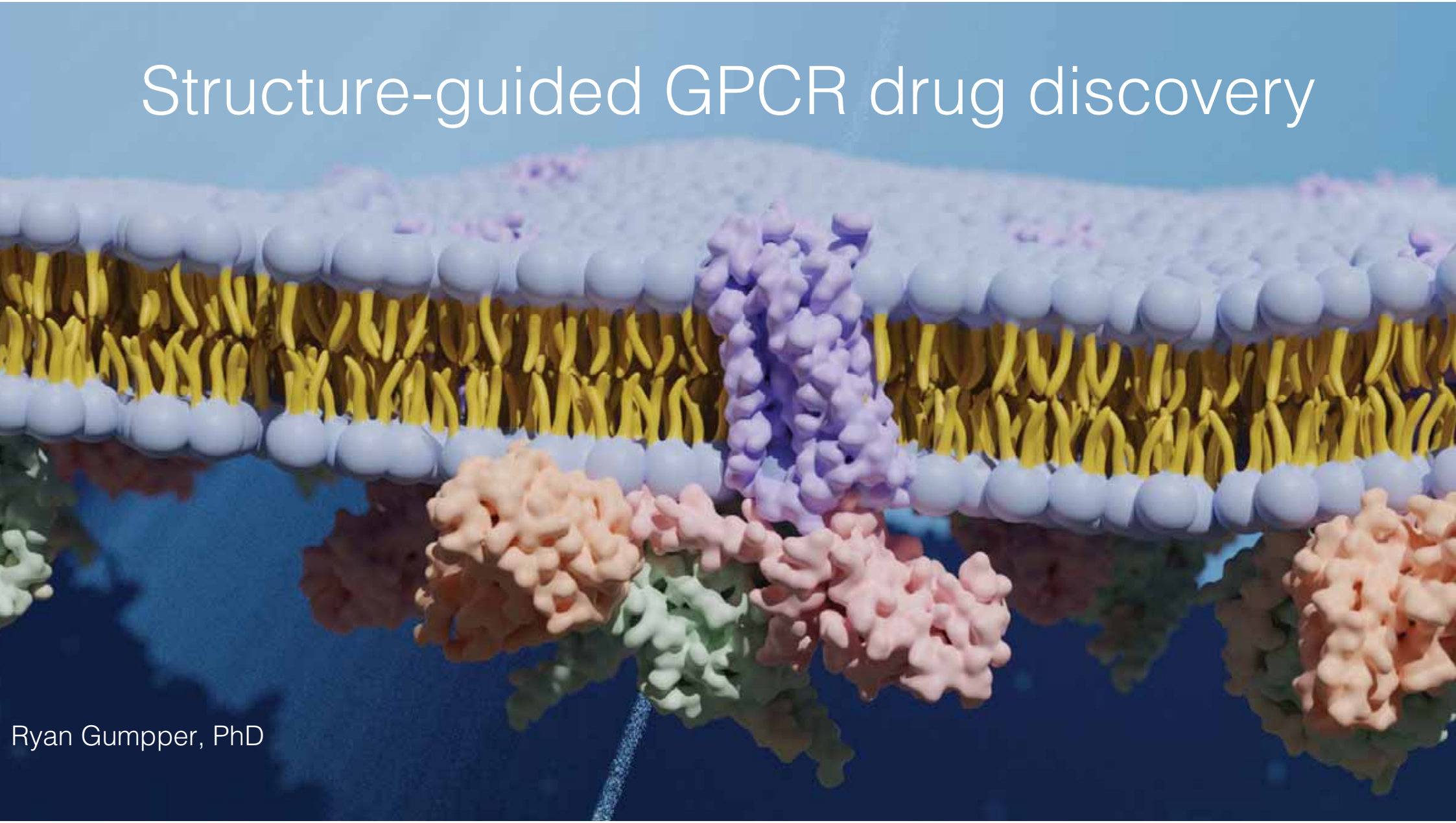
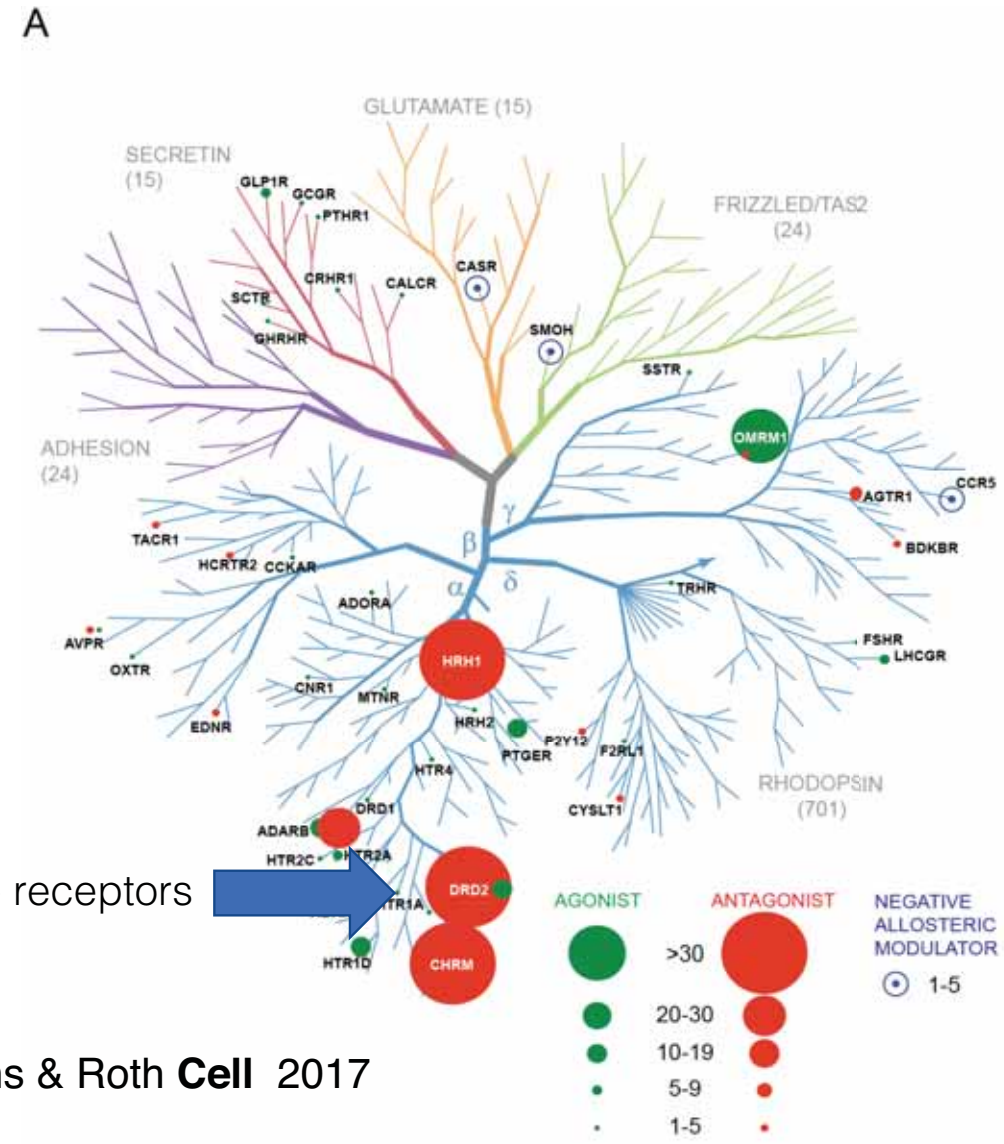
Structure-guided GPCR drug discovery



Ryan Gumpper, PhD

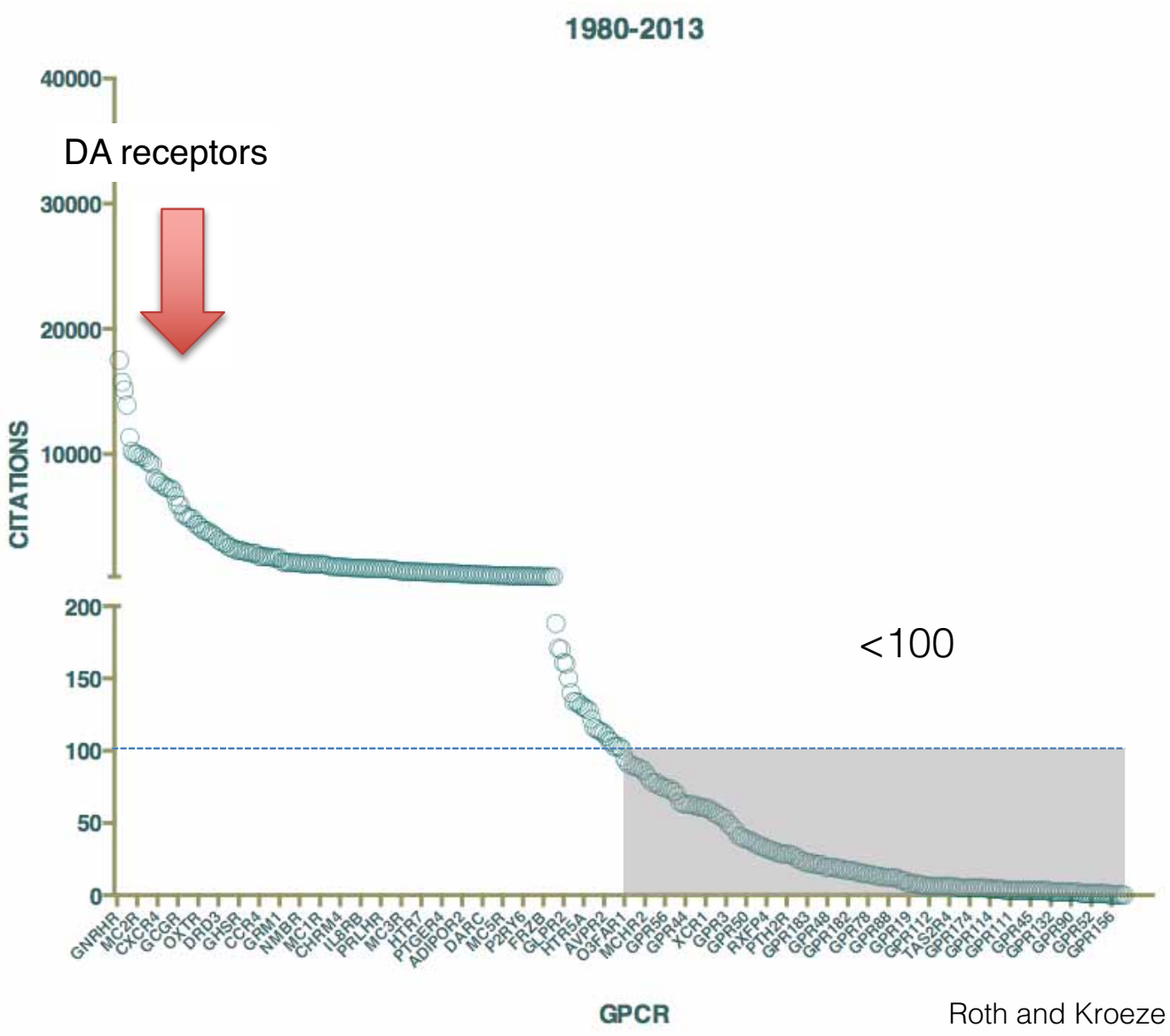
FDA-approved drugs utilize <10% of the druggable GPCR-ome as canonical target



Dopamine receptors

Wacker, Stevens & Roth **Cell** 2017

>50% OF GPCRS ARE UNDER-STUDIED

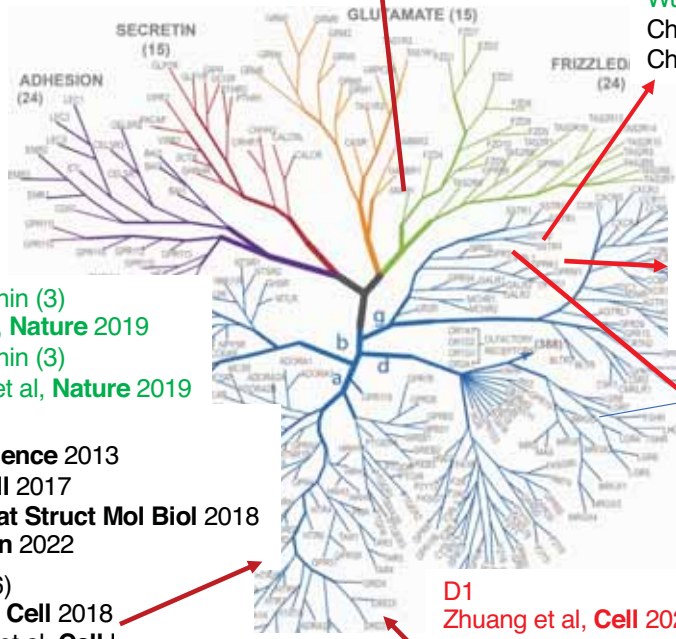


Roth and Kroeze JBC 2015

Smoothened (5)
Wang et al, **Nature** 2013
Wang et al, **Nature Comm 2014**

Rothlab
Stevens and Cherezov
Eric Xu Lab (US and China)

κ-opioid (4)
Wu et al, **Nature** 2012
Che et al, **Cell** 2018
Che et al, **Nature Comm** 2020



δ-opioid—1.8 Å (2)
Fenalti et al, **Nature** 2014
Fenalti et al, **Nat Struc Mol Biol** 2015

MT1 melatonin (3)
Stauch et al, **Nature** 2019
MT2 melatonin (3)
Johanssen et al, **Nature** 2019

MRGPRX1
MRGPRX2
MRGPRX4
Cao, Kang et al, ***Nature*** 2021
Liu, Cao et al, **Nature Chem Bio** 2022

Nociceptin
Thompson et al,

5-HT2B (9)
Wacker et al, **Science** 2013
Wacker et al, **Cell** 2017
McCorvy et al, **Nat Struct Mol Biol** 2018
Cao et al, **Neuron** 2022

5-HT5A (4)
Zhang et al, **Nat Struc Bio** 2022

5-HT2C (6)
Peng et al **Cell** 2018
Gumpper et al, **Cell** I

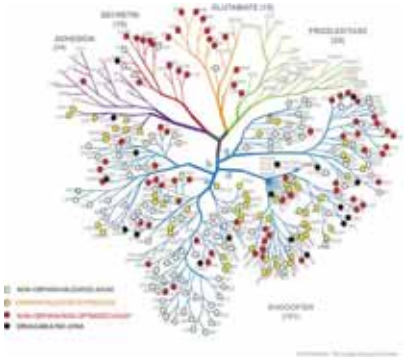
5-HT2A (4)
Kim et al, **Cell** 2020
Levit et al, **Nature** 2022

D4 (2) 1.9Å
Wang et al, **Science** 2017

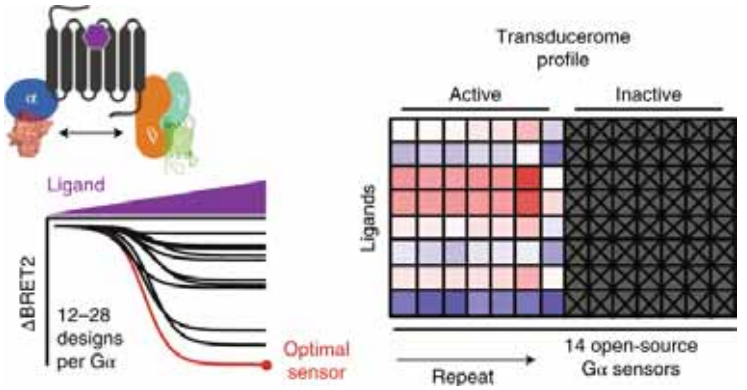
D1
Zhuang et al, **Cell** 2021
D2
Wang et al, **Nature** 2018
Zhuang et al, **Cell** 2021

Barriers to structure-guided GPCR drug discovery for oGPRCs

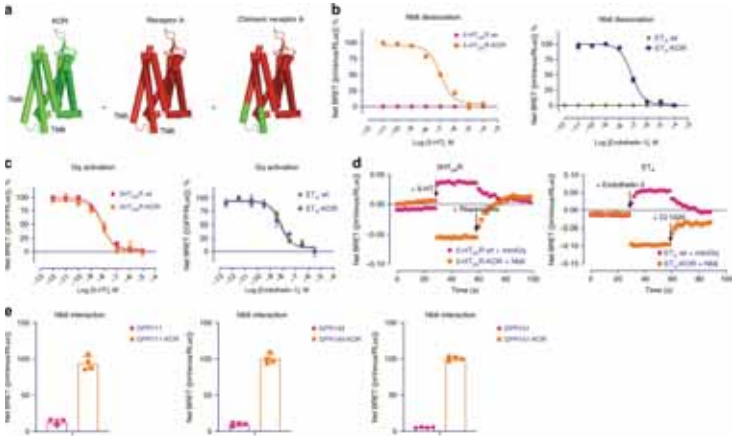
- pM→low nM essential for x-ray structures
- Difficulty with ligand-free structures
- Little sequence identity
- AlphaFold predicted structures not useful



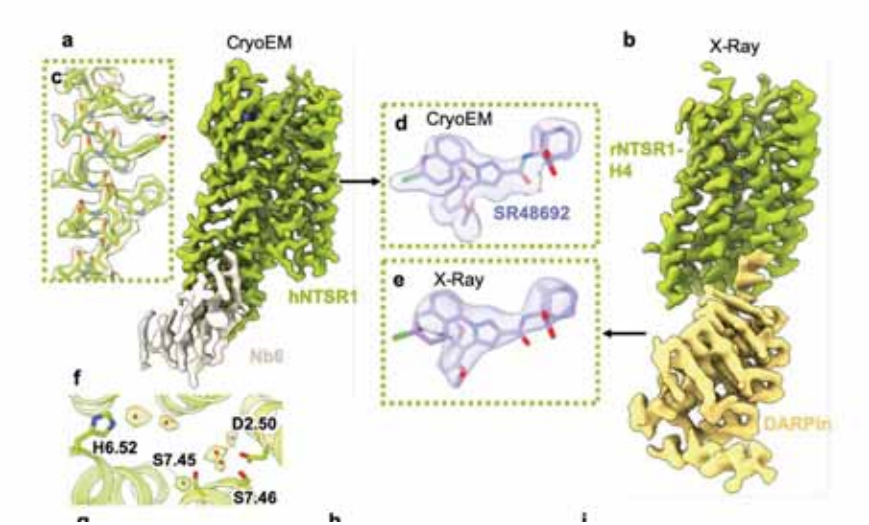
PRESTO-TANGO RESOURCE
320 GPCRS
(Kroeze et al, Nat Struct Mol Bio 2015)



TRUPATH RESOURCE
BRET-based Transducerome Screening
(Olsen, DiBerto et al, Nat Chem Bio 2020)



Nanobody-enabled screening oGPCRs
(Che et al Nature Comm 2020)



GPCR STRUCTURE-GUIDED SMALL MOLECULE TOOL DISCOVERY

Wang et al *Nature* 2013

Article

Docking a bespoke ultra-large tetrahydropyridine library identifies 5-HT2A receptor agonists conferring new biology

https://doi.org/10.1038/s41586-019-0000-0

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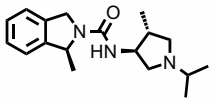
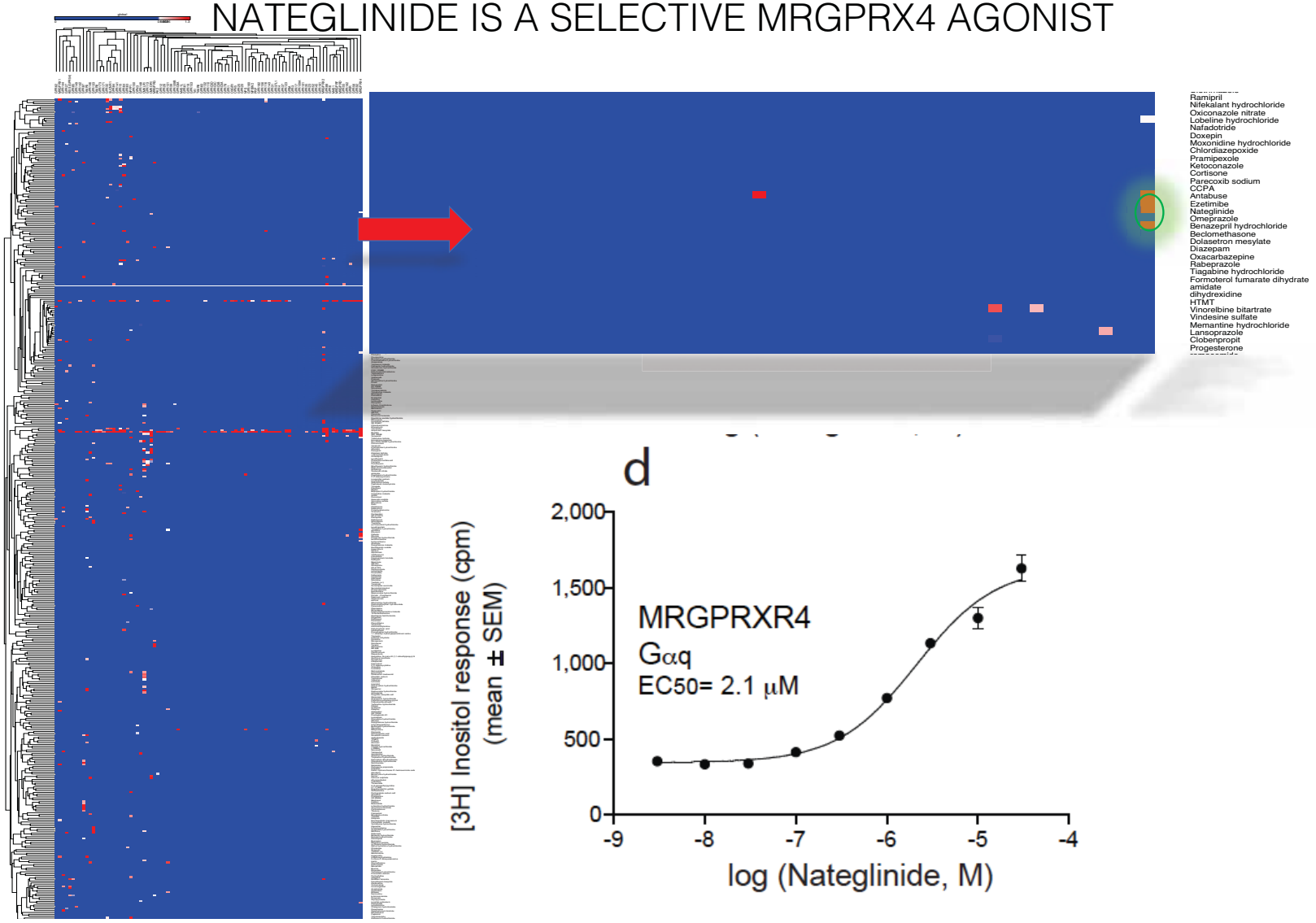
Anat Levit Kaplan†[1], Danielle N. Confair†[2], Kuglae Kim†[3,13], Ximena Barros-Álvarez†[4], Ramona M. Rodriguiz[5,6], Ying Yang[1], Oh Sang Kweon[2], Tao Che[7], John McCorvy[8], David N. Kamber[2], James P. Phelan[2], Luan Carvalho Martins[1,9], Vladimir M. Pogorelov[5], Jeffrey F. DiBerto[3], Samuel T. Slocum[3], Xi-Ping Huang[10], Jain Manish Kumar[3], Michael J. Robertson[4], Ouliana Panova[4], Alpay B. Seven[4], Autumn Q. Wetsel[5], William C. Wetsel[*5,6,11], John J. Irwin[*1], Georgios Skiniotis[*4], Brian K. Shoichet[*1], Bryan L. Roth[*3,12], Jonathan A. Ellman[*2].

D4
Wang et al, *Science* 2017
Lyu, Wang et al, *Nature* 2019



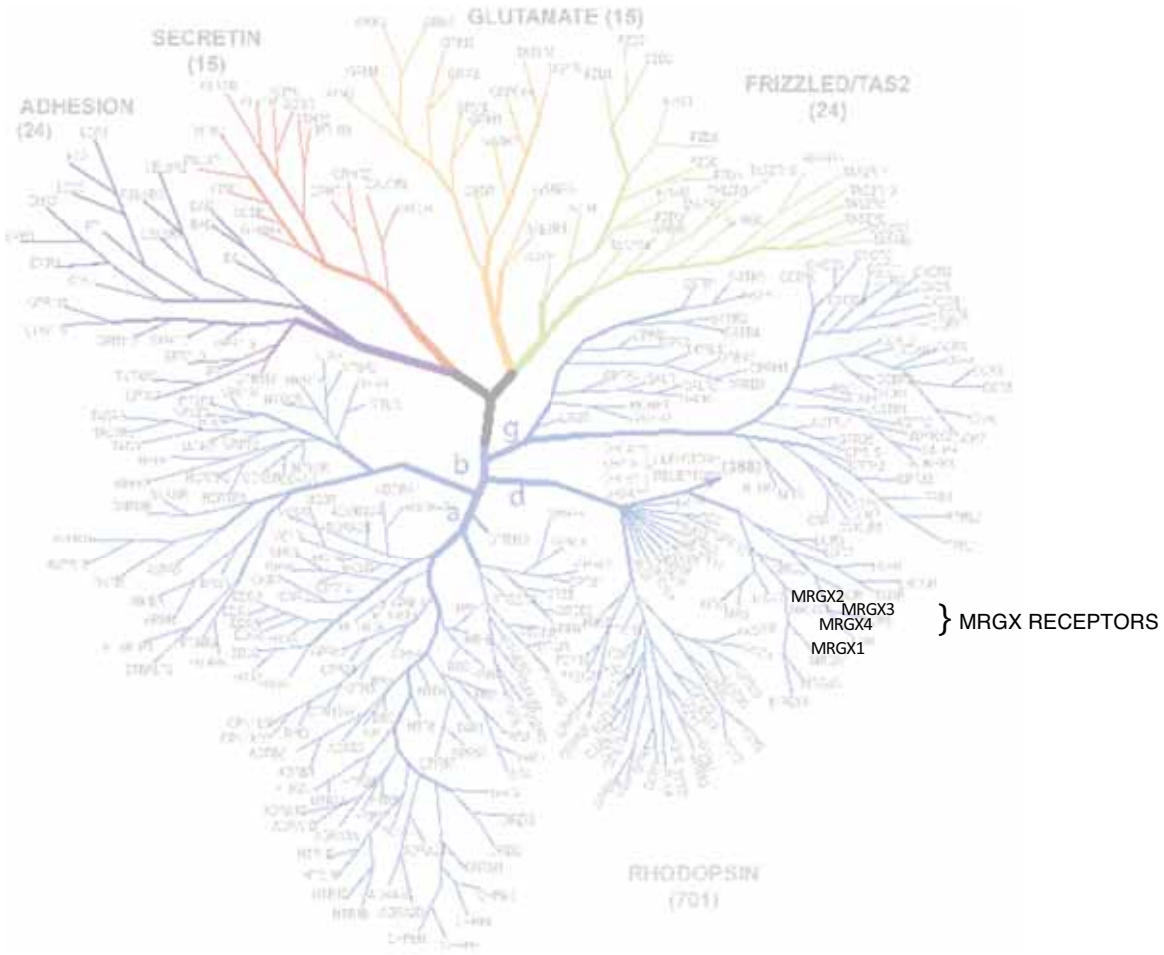
GPR65 and GPR68
Huang et al, *Nature* 2015

SCREENING 91 oGPCRS AGAINST 446 APPROVED MEDICATIONS REVEALS NATEGLINIDE IS A SELECTIVE MRGPRX4 AGONIST



Kroeze, Sassano, Huang et al, **Nature Structure Mol Biol** 2015

MRGPRX-FAMILY OF GPCRS ARE PRIMATE-SPECIFIC ORPHAN GPCRS



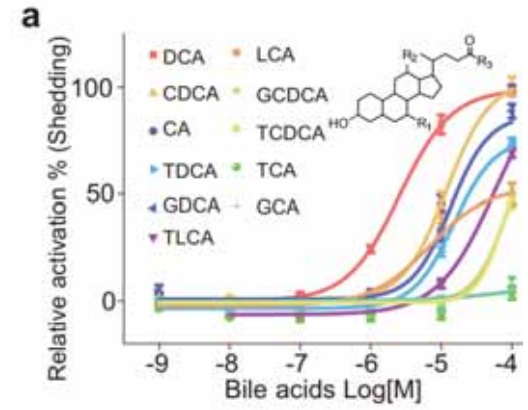
MRGPRX4 is **primate-specific** bile acid receptor for histamine-independent itch in human

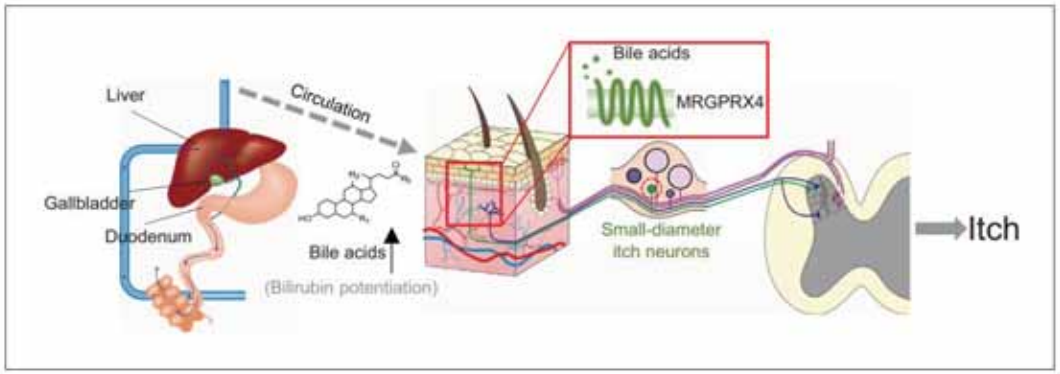
MRGPRX4 is a G protein-coupled receptor activated by bile acids that may contribute to cholestatic pruritus

James Meixiong[a], Chirag Vasavda[a], Solomon H. Snyder[a,b,c,1], and Xinzhong Dong[a,d,e,f,1]

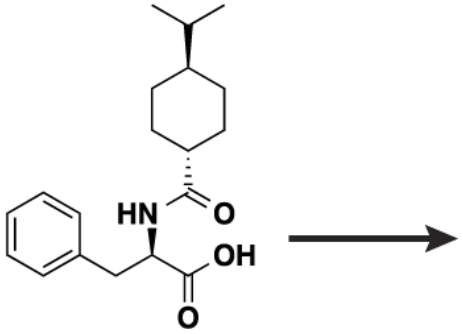
MRGPRX4 is a bile acid receptor for human cholestatic itch

Huasheng Yu[1,2,3], Tianjun Zhao[1,2,3], Simin Liu[1], Qinxue Wu[4], Omar Johnson[4], Zhaofa Wu[1,2], Zihao Zhuang[1], Yaocheng Shi[5], Luxin Peng[5], Renxi He[1,2], Yong Yang[6], Jianjun Sun[7], Xiaoqun Wang[8], Haifeng Xu[9], Zheng Zeng[10], Peng Zou[5], Xiaoguang Lei[2,5], Wenqin Luo[4]*, Yulong Li[1,2,3,11]*





Creation of a more selective MRGPRX4 ligand



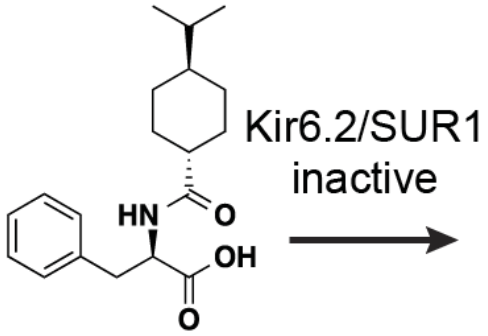
Nateglinide

EC_{50} = 4.7 μM

Lansu and Karpiak et al.
Shinkai et al. 1989

Cao, Kang et al, Nature 2021

Creation of a more selective MRGPRX4 ligand



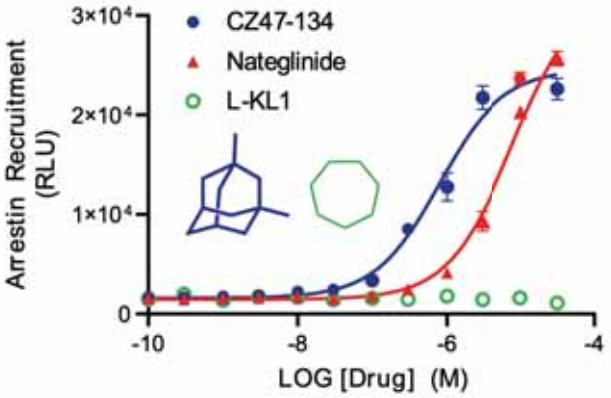
Nateglinide

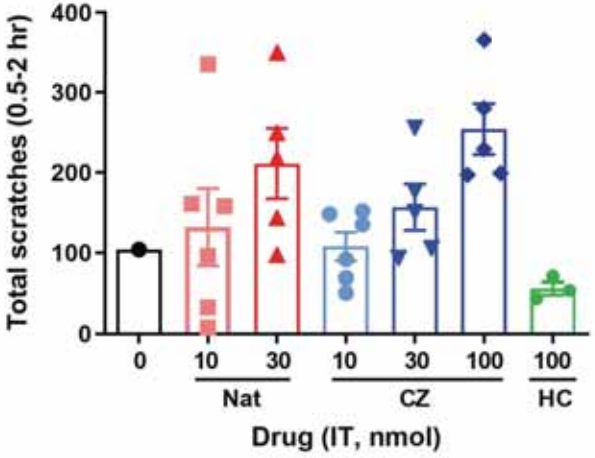
EC_{50} = 4.7 µM

Lansu and Karpiak et al.
Shinkai et al. 1989

Cao, Kang et al, Nature 2021

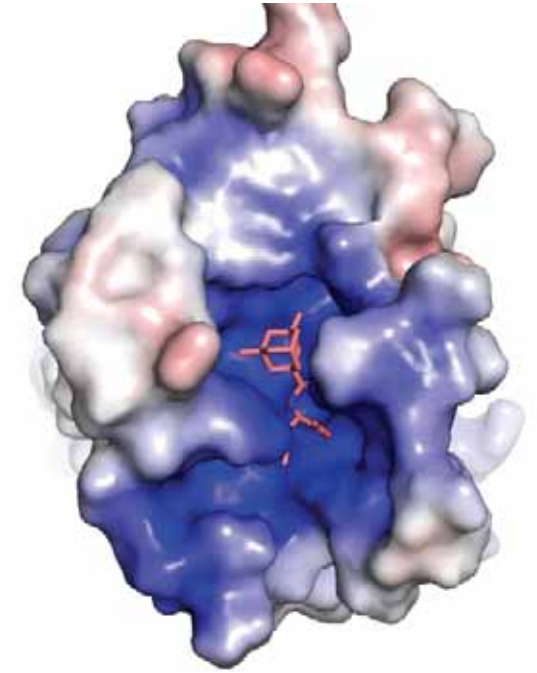
MRGPRX4 agonists induce itch in non-human primate model (Mei-Chuan Ko lab Wake Forest Pharmacology)



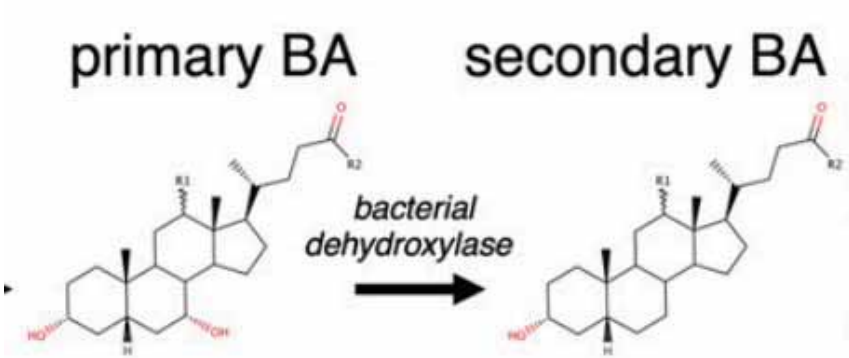


Kate Lansu, Joel Karpiak, Wes Kroeze, Hye Jin Kang,
Reid Olsen, John McCorvy, Justin English, HuiPing Ding, Mei-Chuan Ko

MRGPRX4 has a positively charged extracellular surface



MRGPRX4



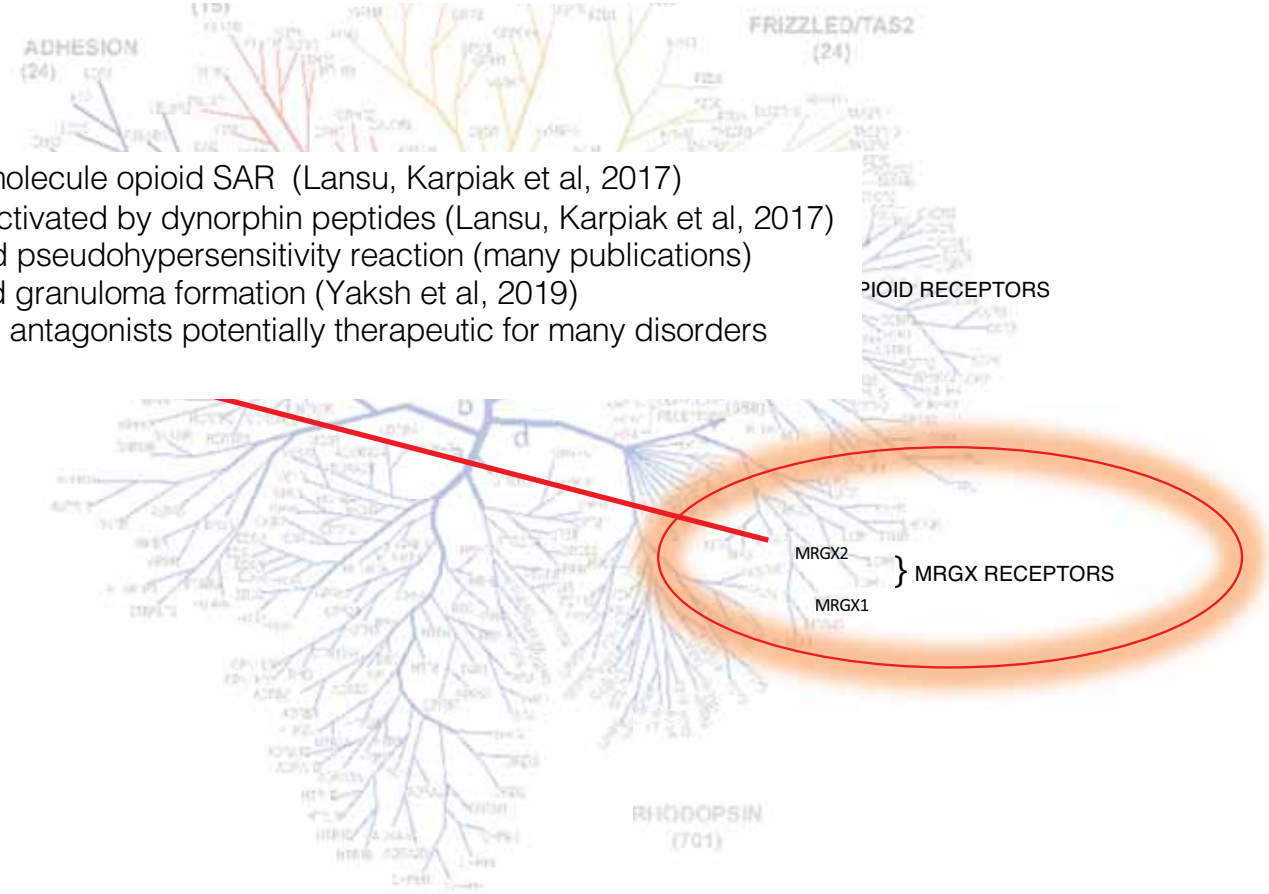
Negatively charged bile acids.

Cao, Kang et al, Nature 2021

What about other MRGPRX-family receptors?

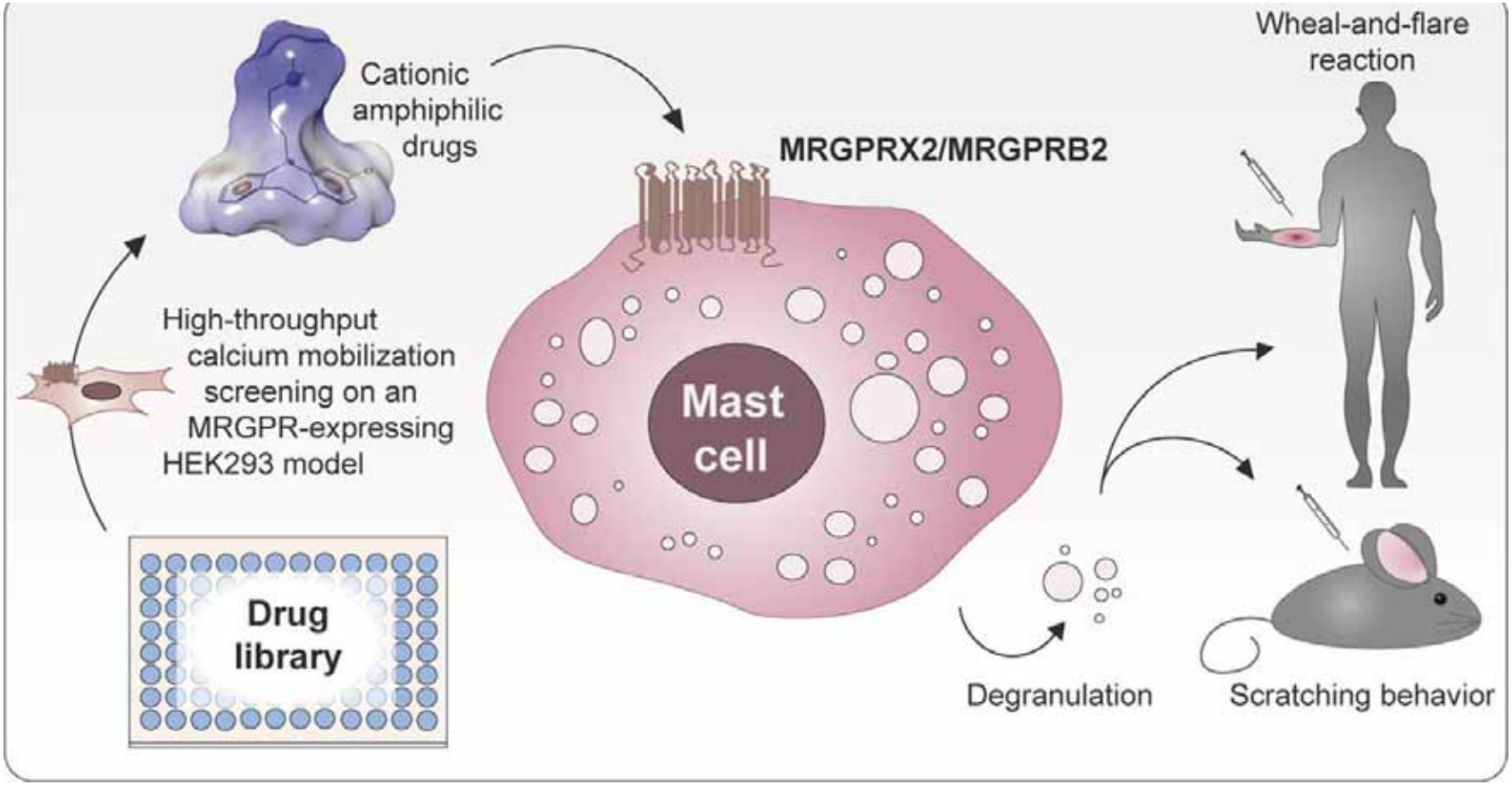
Two GPCR Families Mediate Actions of Opioid Small Molecules and Peptides

- Unique small molecule opioid SAR (Lansu, Karpiak et al, 2017)
- Preferentially activated by dynorphin peptides (Lansu, Karpiak et al, 2017)
- Opioid-induced pseudohypersensitivity reaction (many publications)
- Opioid induced granuloma formation (Yaksh et al, 2019)
- Small molecule antagonists potentially therapeutic for many disorders

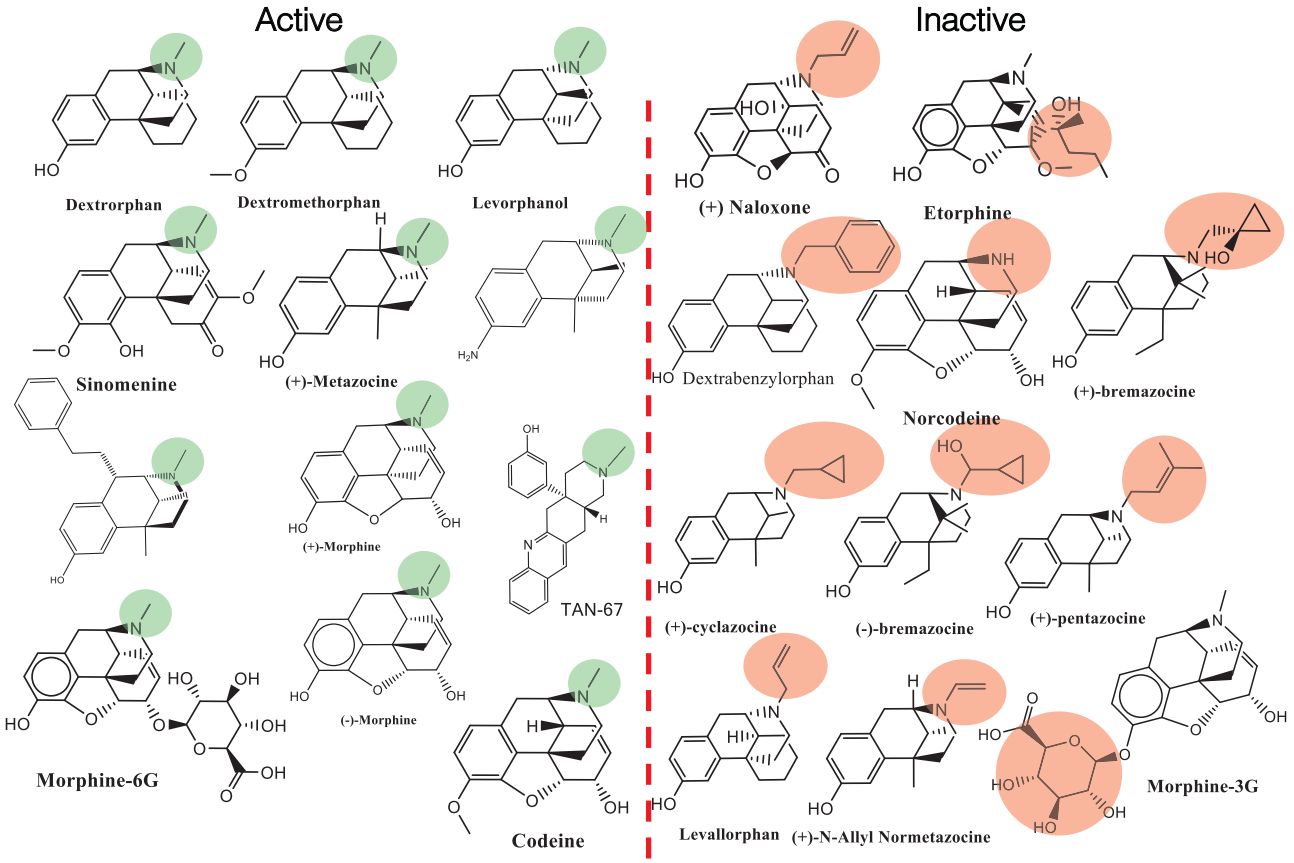


Considerable controversy exists regarding G protein pathway downstream of MRGPRX2

- Gq vs Gi?
- Monomers, Dimers, Hetereo-oligomers?
- How do positively charged peptides and drugs interact?
- Can we create antagonists for MRGPR-family receptors as potential therapeutics?



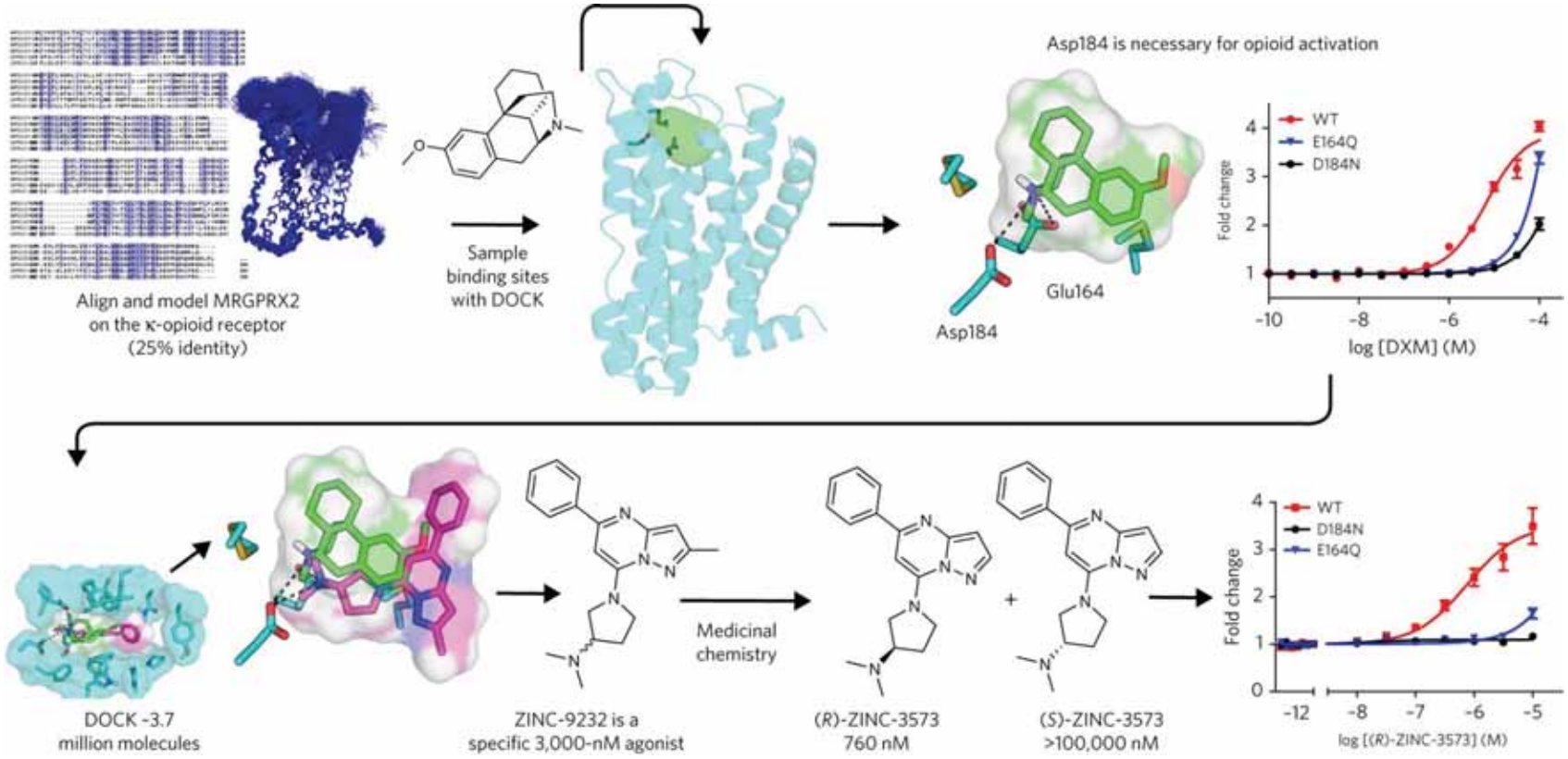
Opioids have unique structure activity relationships at MRRGX2



Lansu, Karpiak et al, Nature Chem Bio 2017

7:09:01 AM

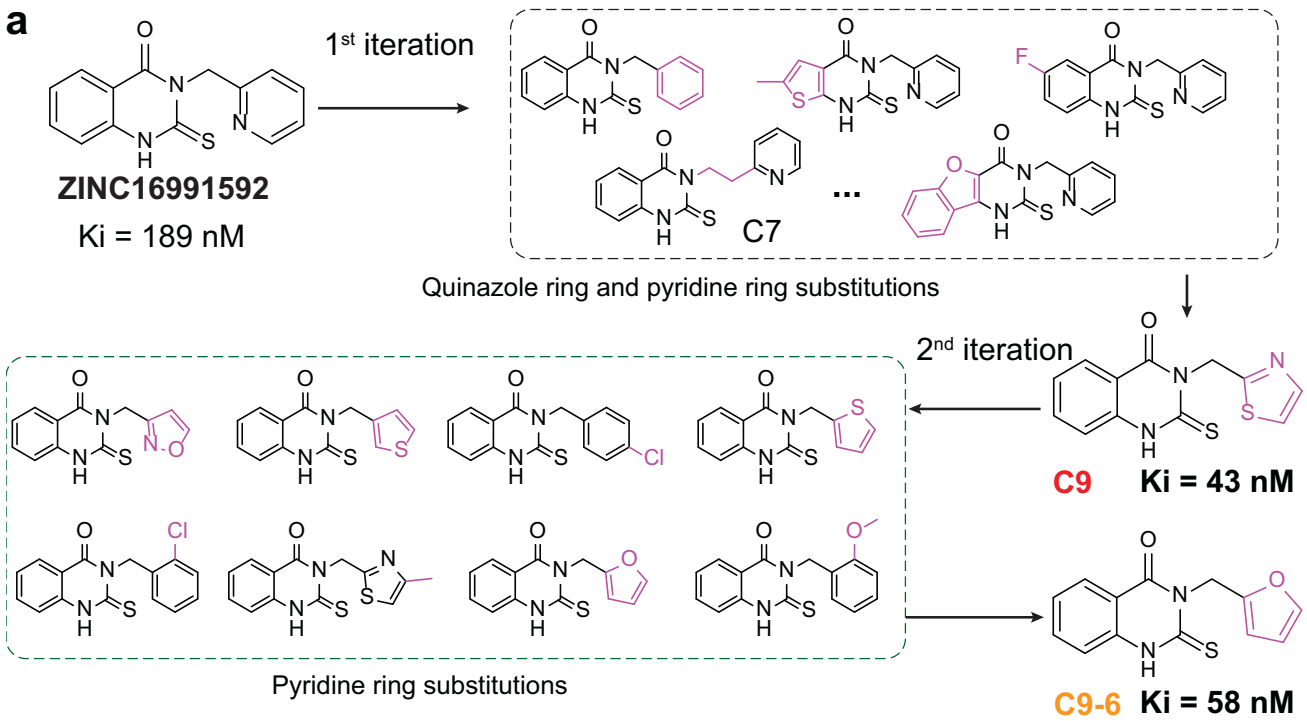
Docking for a specific tool → matched stereoisomeric pair, active & inactive, specific for MRGPRX2, no off-targets



Lansu, Karpiak et al, Nature Chem Bio 2017

High resolution cryo-EM structures of Gq- and Gi-coupled MRGPRX2 obtained

Cao, Kang et al, Nature 2021



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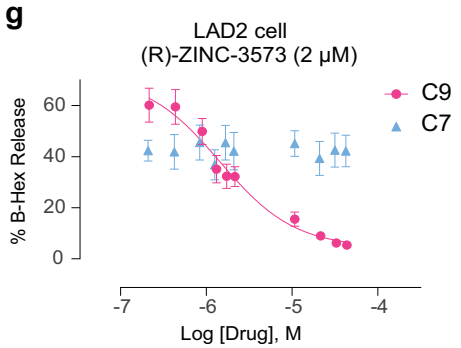
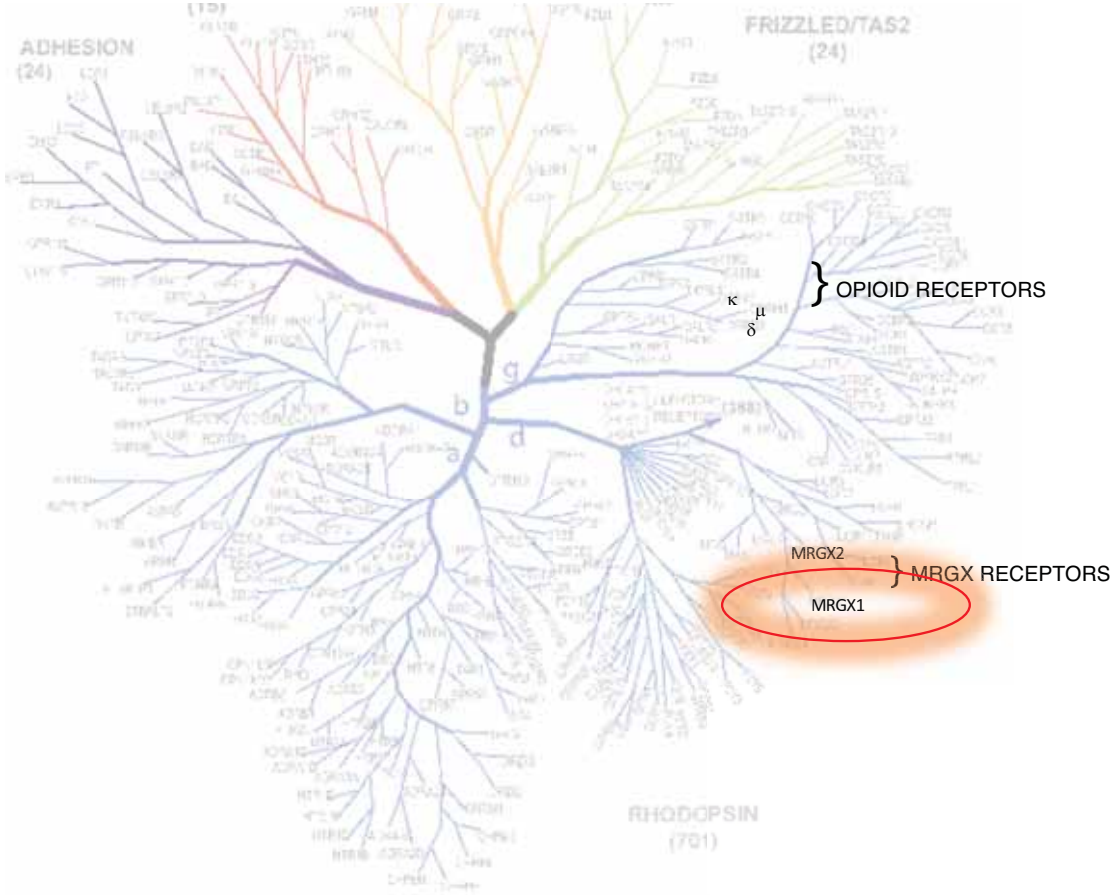


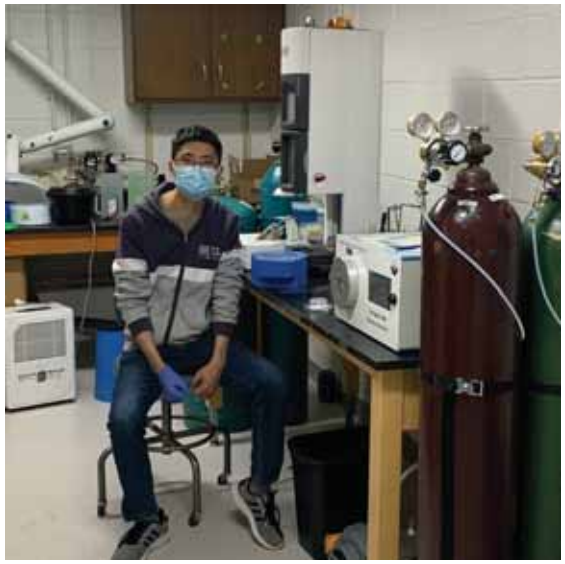
Isha Singh (Shoichet lab)
Can Cao and Hye Jin Kang (Roth lab)
Soman Abraham lab (Duke)

Cao, Kang et al, Nature 2021

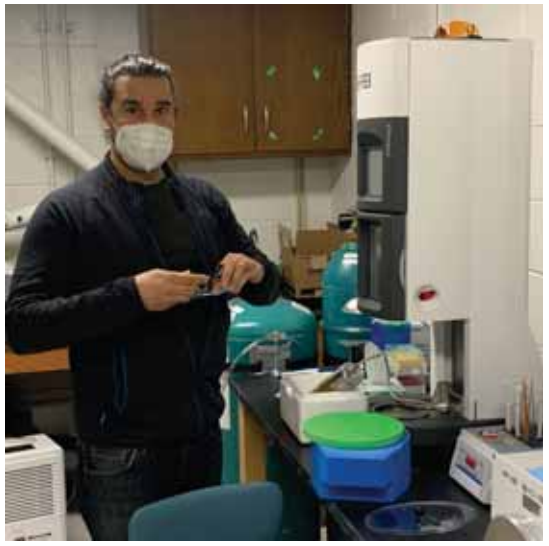
Two GPCR Families Mediate Actions of Opioid Small Molecules and Peptides



Yongfeng Liu, PhD

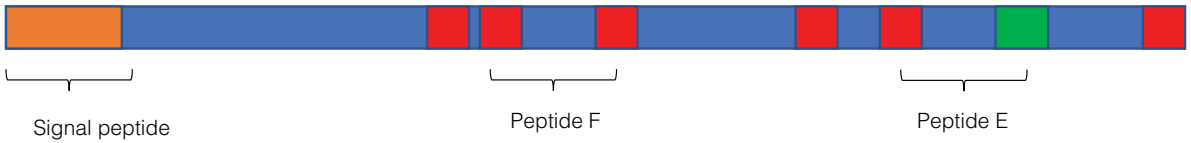


Can Cao, PhD



Jonathan Fay, PhD

Proenkephalin → Met- and Leu-enkephalin



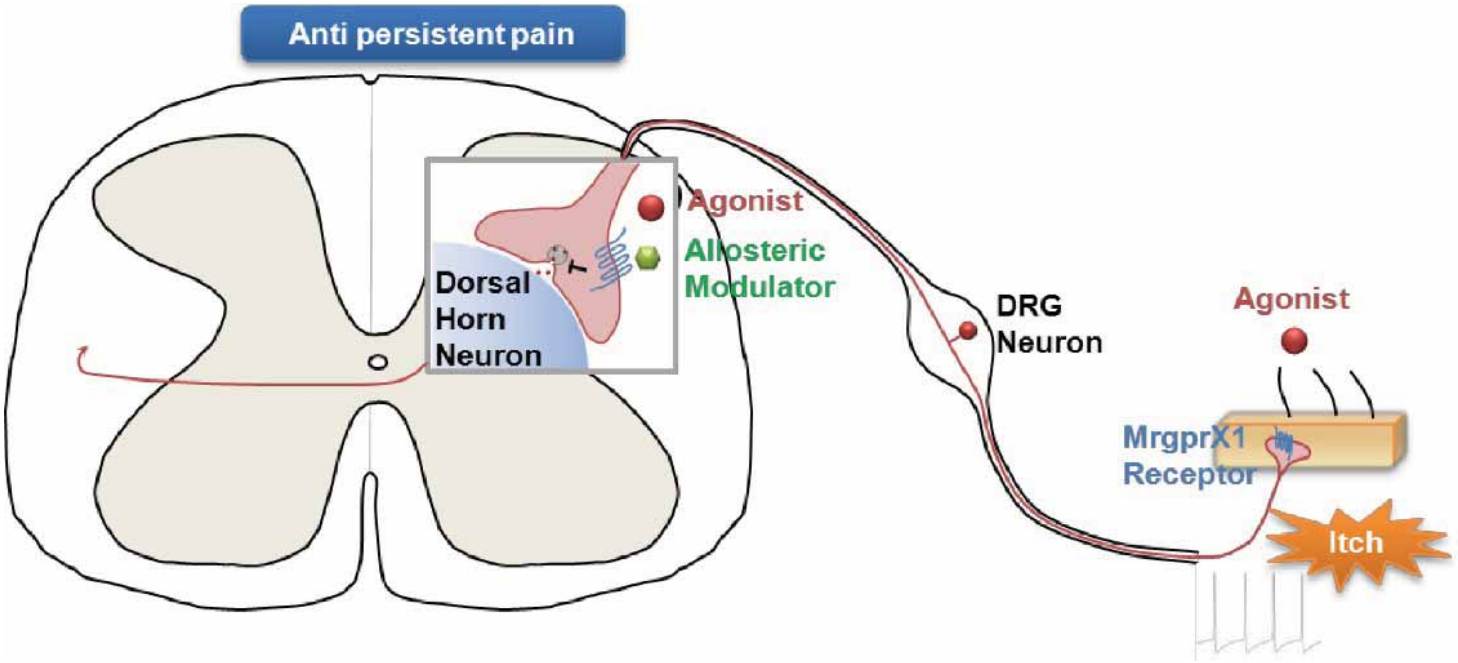
YGGFMRRVGRPEWWMDYQKRYG

BAM1-22

VGRPEWWMDYQKRYG

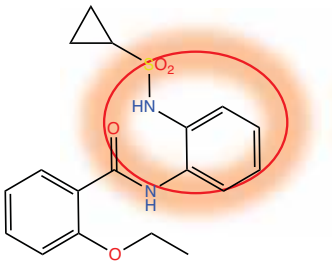
BAM8-22

MRGX1 involved in nociceptive and pruritogenic sensations

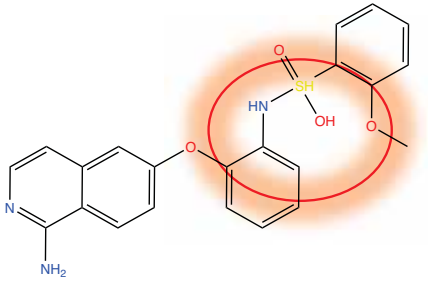


Li et al, 2019

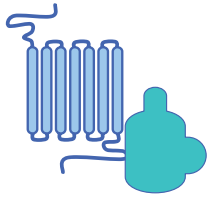
Ambiguous pharmacology and SAR



ML382
(PAM)



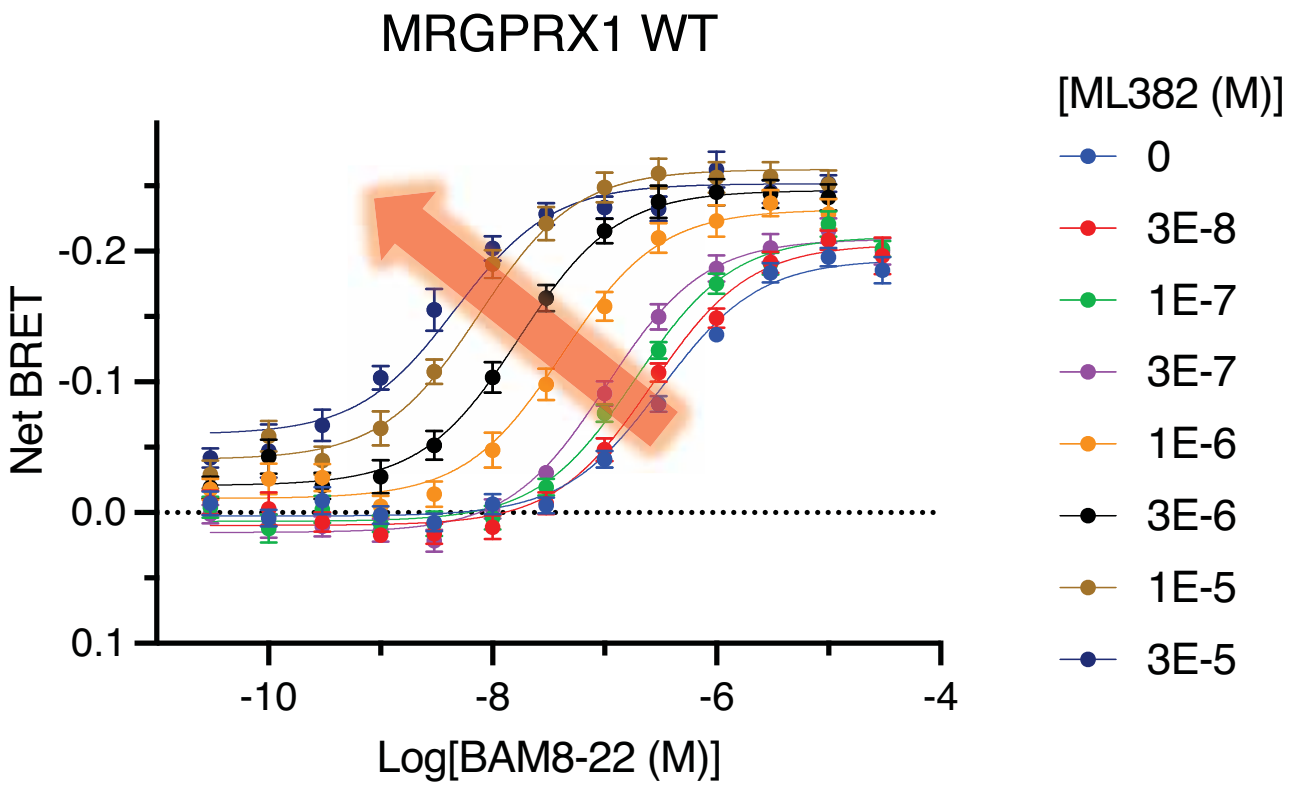
C1
(ORTHOSTERIC AGONIST?)



VGRPEWWMDYQKRYG

BAM 8-22
(ENDOGENOUS AGONIST?)

ML382 is a potent and efficacious MRGPRX1 PAM



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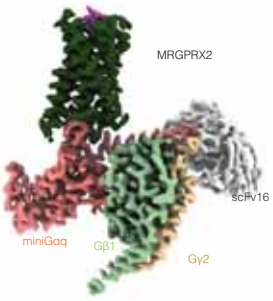
Liu, Cao et al, Nat Chem Bio in press

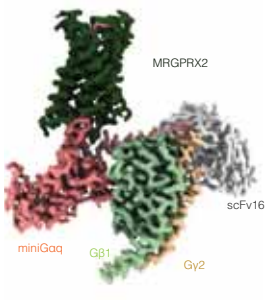
MRGPRX4

MRGPRX4-Gq-MS47134

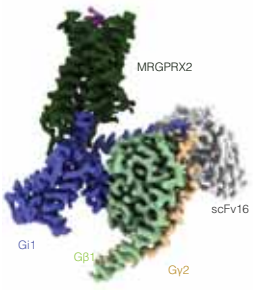
MRGPRX2



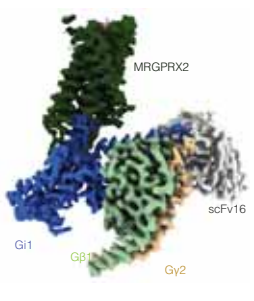
MRGPRX2-Gq-ZIN3573



MRGPRX2-Gq-C14

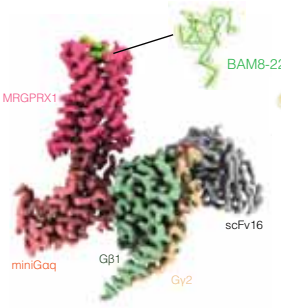


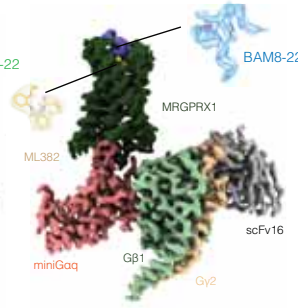
MRGPRX2-Gi-ZIN3573

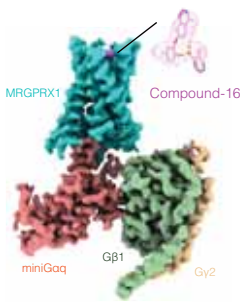


MRGPRX2-Gi-C14

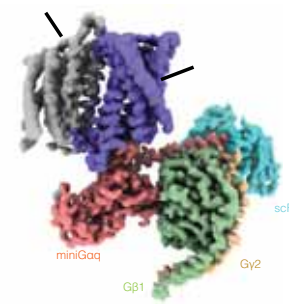
MRGPRX1



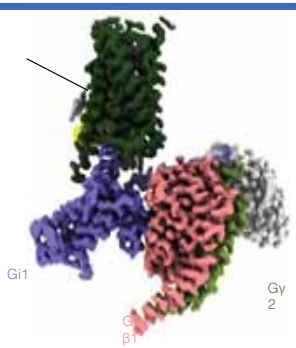




oGPCR #1



oGPCR#2



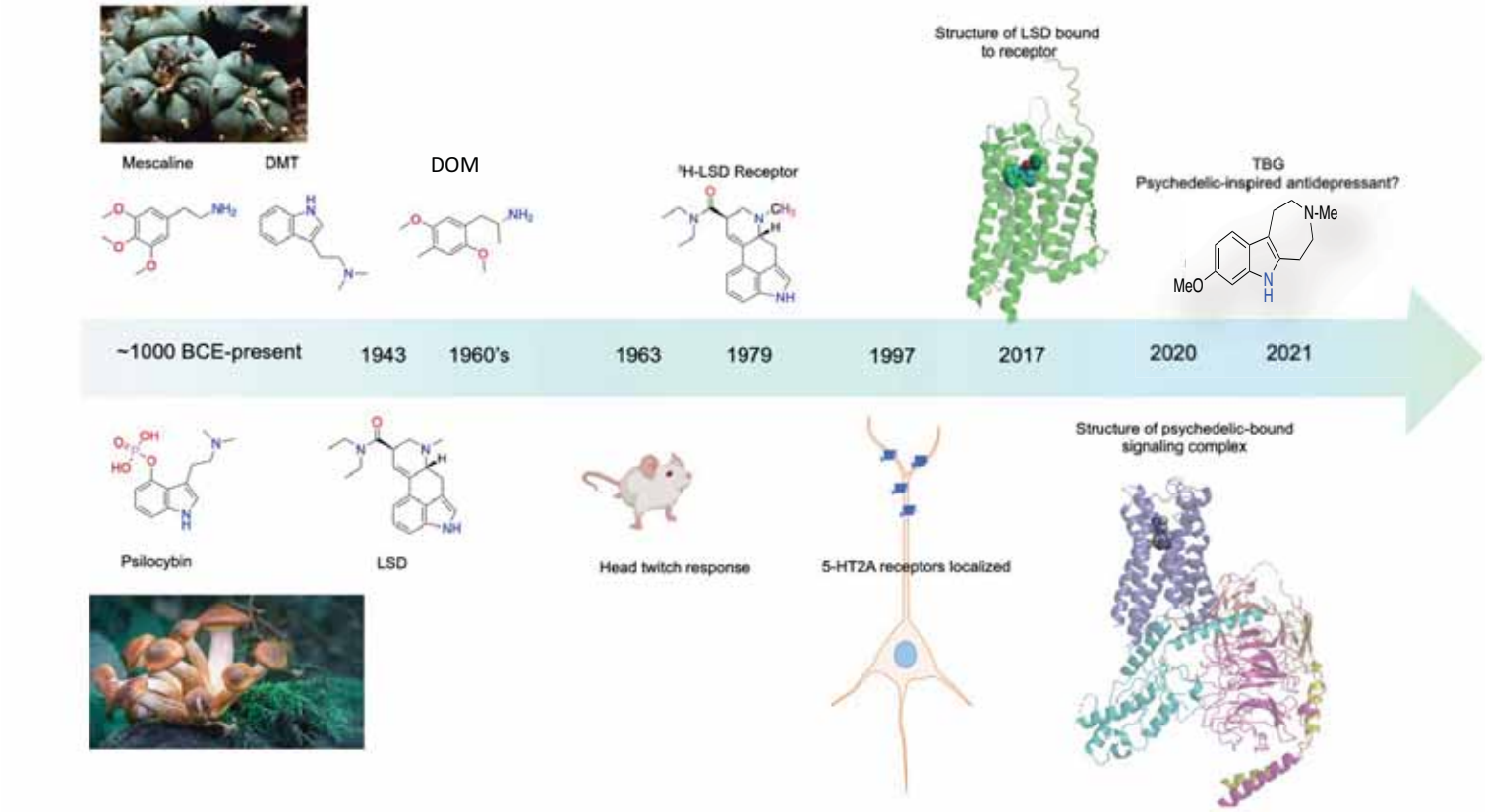
oGPCR#3

oGPCR #3 Apo

Can Cao, PhD
Yongfeng Liu, PhD
Brian Krumm, PhD
Jonathan Fay, PhD

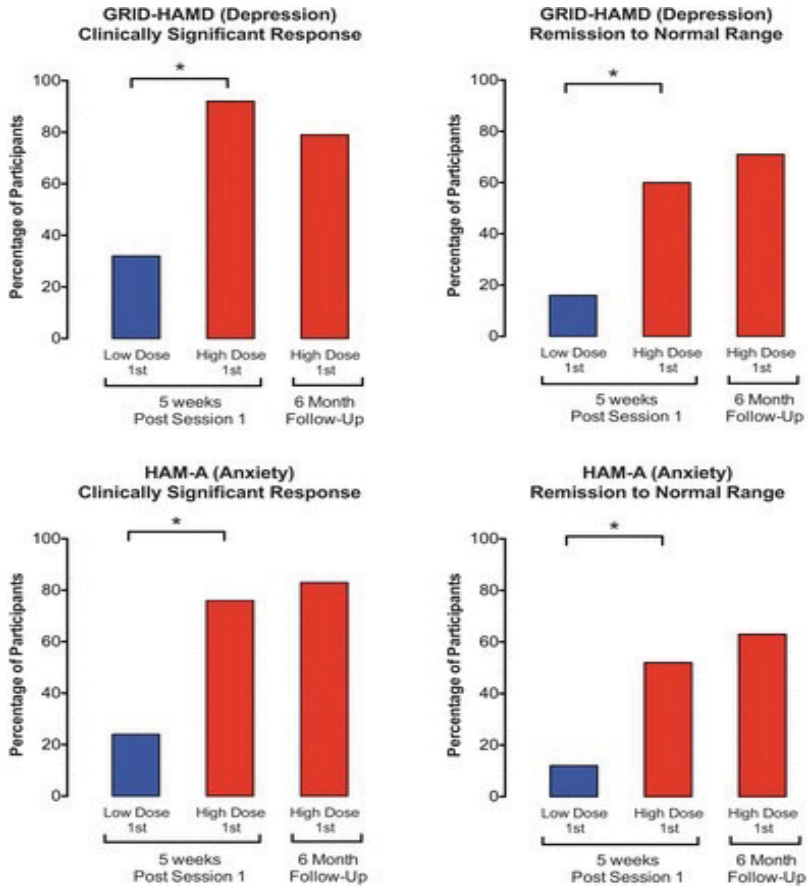
(Cao et al, Nature 2021; Liu et al Nat Chem Bio 2022)

Psychedelic History

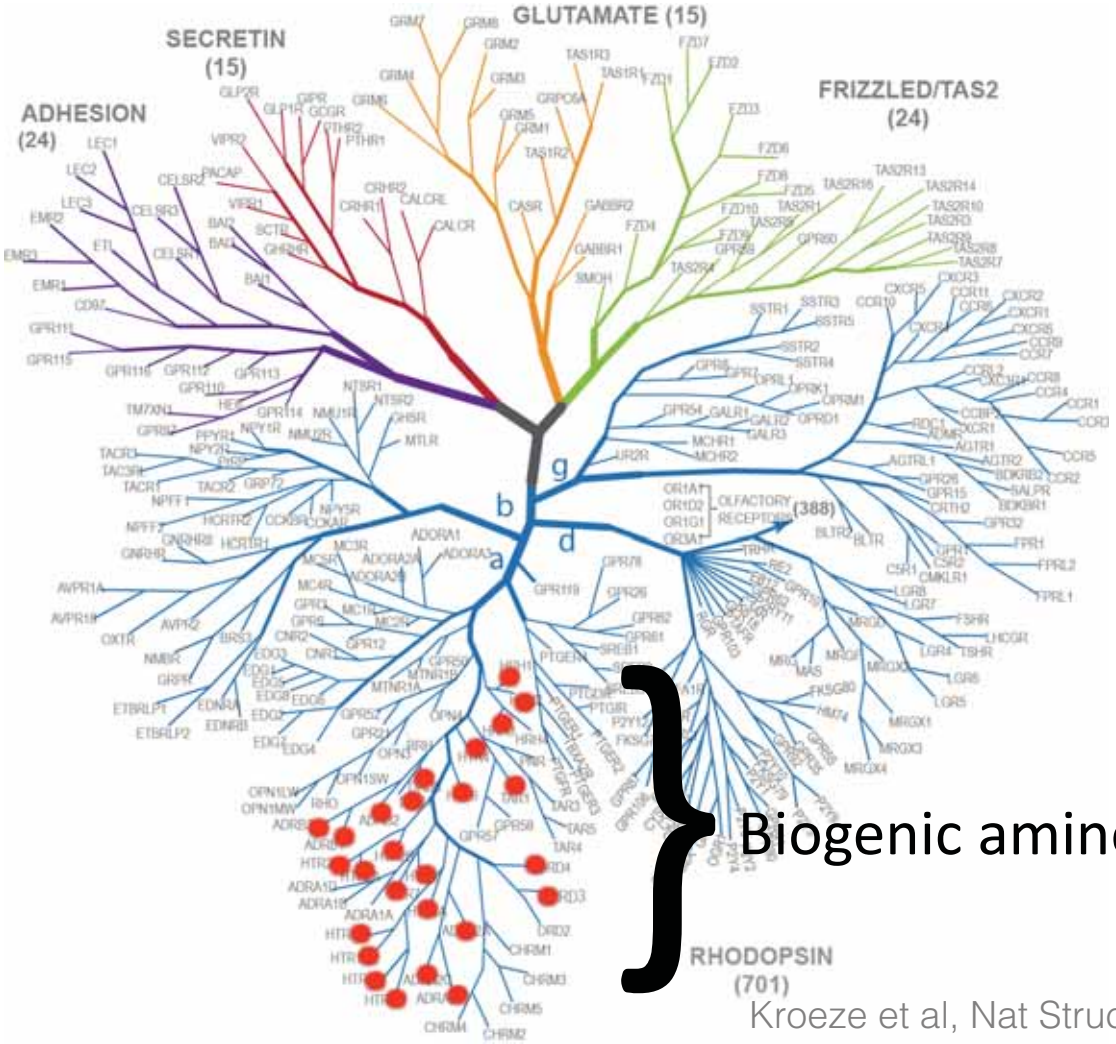


Brady-McClure and Roth *Nature Reviews Drug Discovery, 2022*

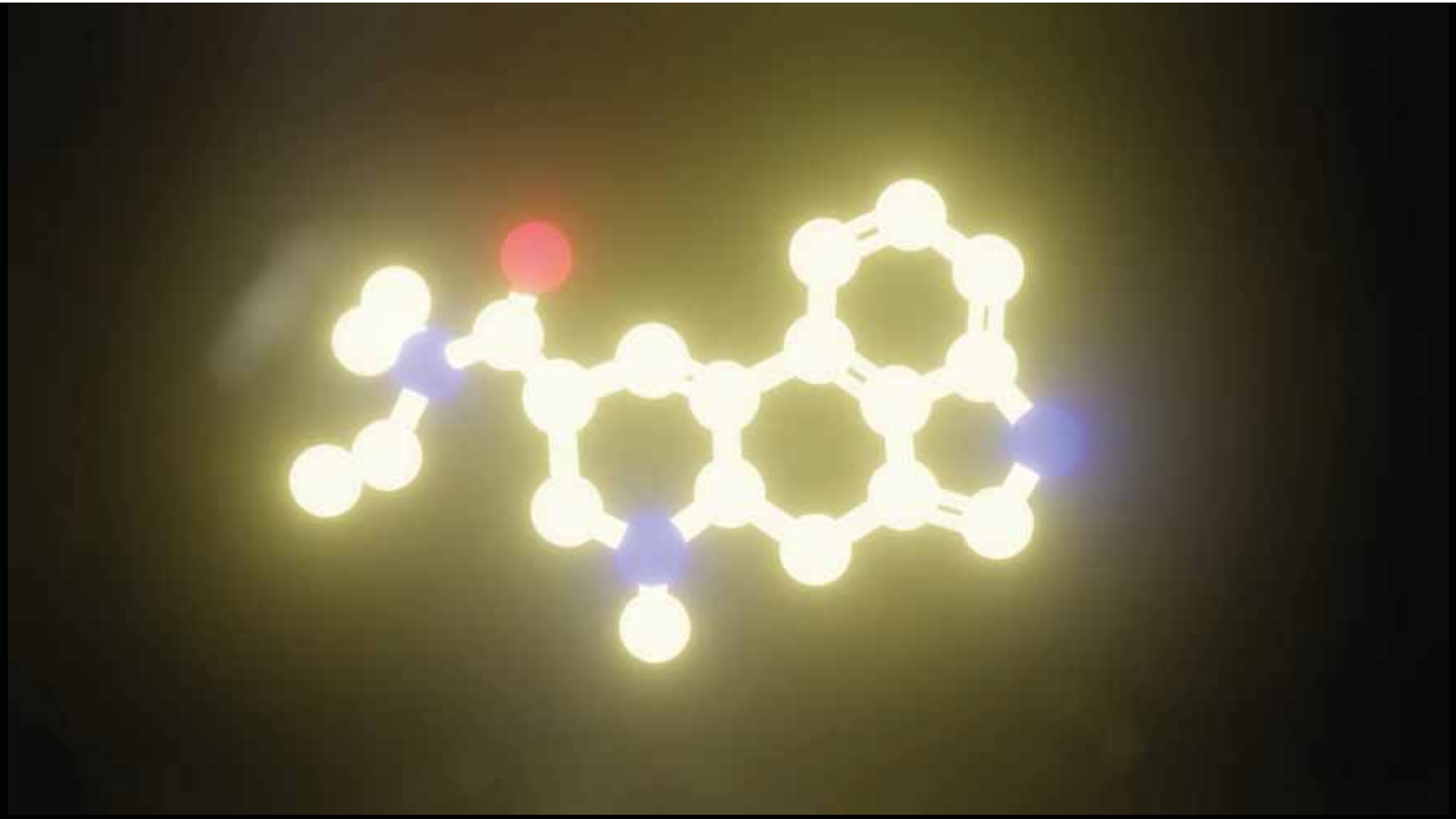
Single dose psilocybin rapidly induces antidepressant and anxiolytic response (Griffiths et al, J Psychopharmacol 2016)



LSD has a complex pharmacology



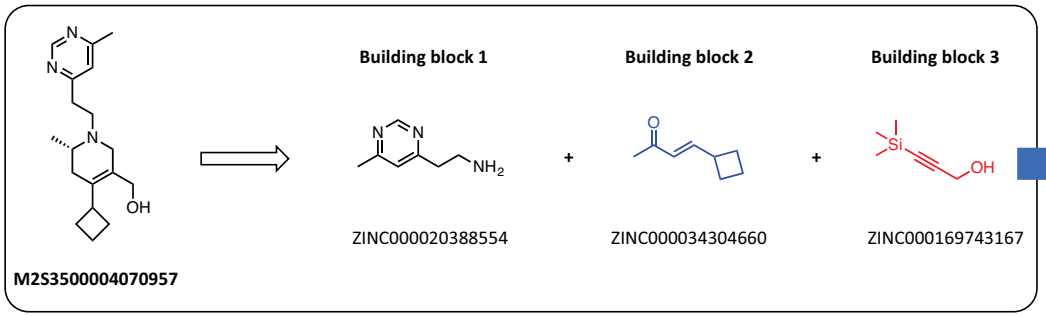
Kroeze et al, Nat Struct Mo Bio 2015



Gabriel Aeschlimann of Ribosome Studio and
Hamilton's Pharmacopoeia

Wacker et al, **Cell** 2017
Kim et al, **Cell** 2020

Design of a large virtual Tetrahydropyridine library



75 million virtual tetrahydropyridines

Ellman lab, Yale | Irwin lab, UCSF

Levit et al, Nature in. press

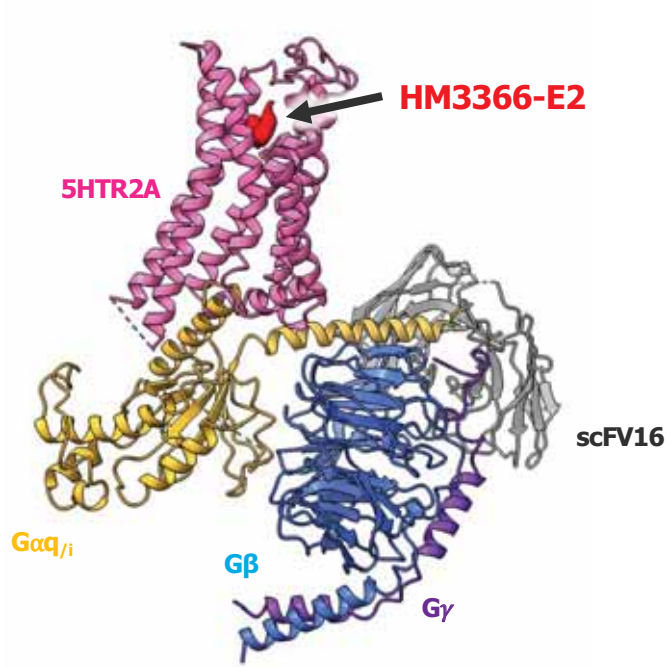
Ultra-large-scale docking of tetrahydropyridine library

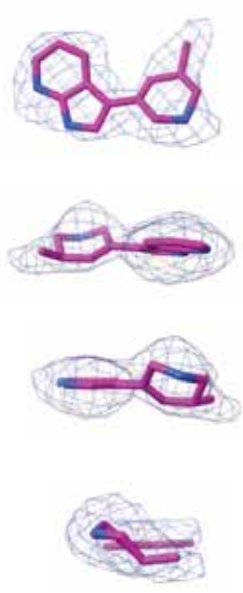
- 75 million compounds
- Iterative cycle of docking and synthetic elaboration and optimization
- ‘3366 revealed as 22 nM potent, selective Gq biased 5-HT2A agonist
- Ultra-LSD previously used in: Wang et al, ***Science*** 2017; Lyu et al, ***Nature*** 2019; Stein et al, ***Nature*** 2020; Sadybekov et al, ***Nature 2022*****, Alon et al, *Nature*** 2021.

Levit et al, Nature in. press

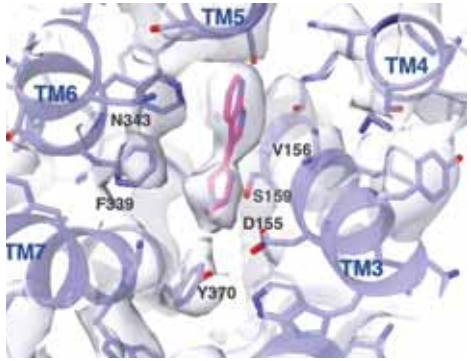
Levit et al,Nature in p ress

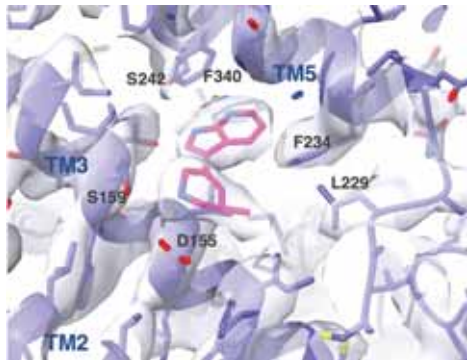
CryoEM structure of 5-HTR$_{2A}$R/Gq with the novel, selective, Gq biased agonist HM3366





3.1 Å resolution



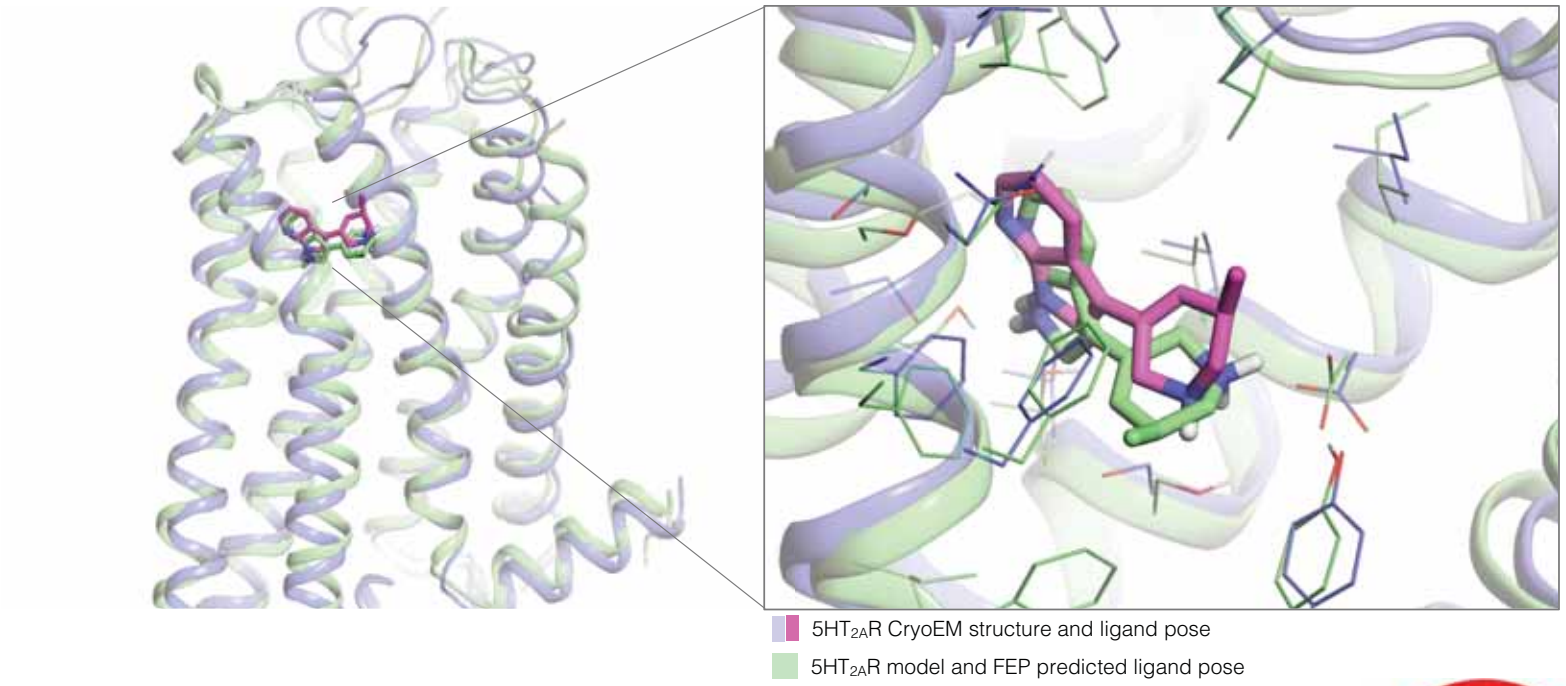


Roth lab, UNC | Skiniotis lab, Stanford

Docking an ultra-large library of THPs for selective 5HT2AR agonists

Levit et al, Nature in. press

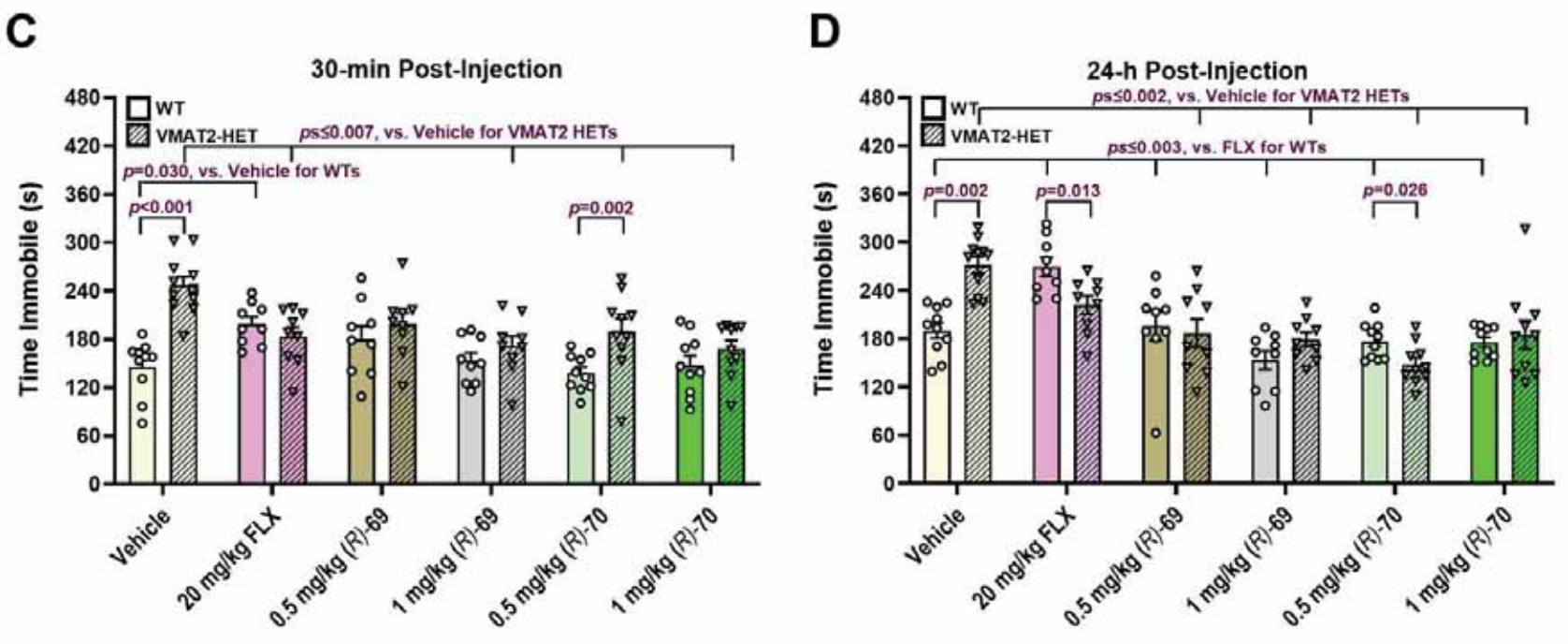
cryo EM structure superposes well with computational prediction



X Alvarez, K Kim, Y Yang; Skiniotis/Roth/Shoichet

Levit et al, Nature in. press

VMAT2-HET Mice Respond to *(R)*-69 and *(R)*-70 in Tail Suspension Assays



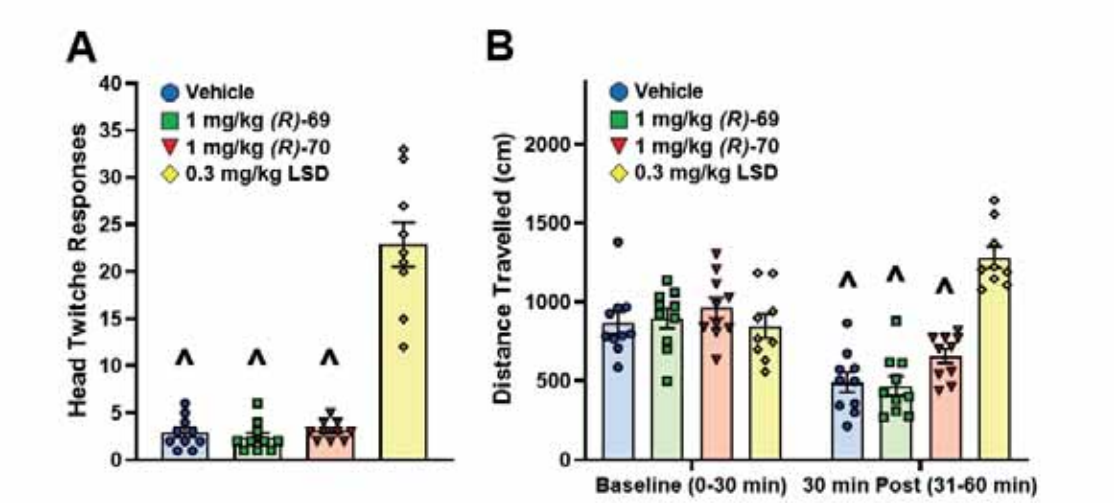
n = 7-9 mice/genotype/treatment
* p<0.05 compared to WT
+ p<0.05 compared to Vehicle within genotype
RMANOVA with Bonferroni corrected *post-hoc*

Levit et al, Nature in. press

Ramona Rodriquez and Bill Wetsel, Duke

(R)-69 and *(R)*-70 Do Not Affect Head Twitch Responses or Locomotion in C57BL/6J Mice

- (A) C57BL/6J mice do not engage in significant head twitch responses (HTRs) with *(R)*-69 or *(R)*-70.
- (B) Locomotion in the open field is unaffected with acute administration of *(R)*-69 or *(R)*-70.
- (C) With 0.3 mg/kg LSD, HTRs and locomotion are markedly increased.

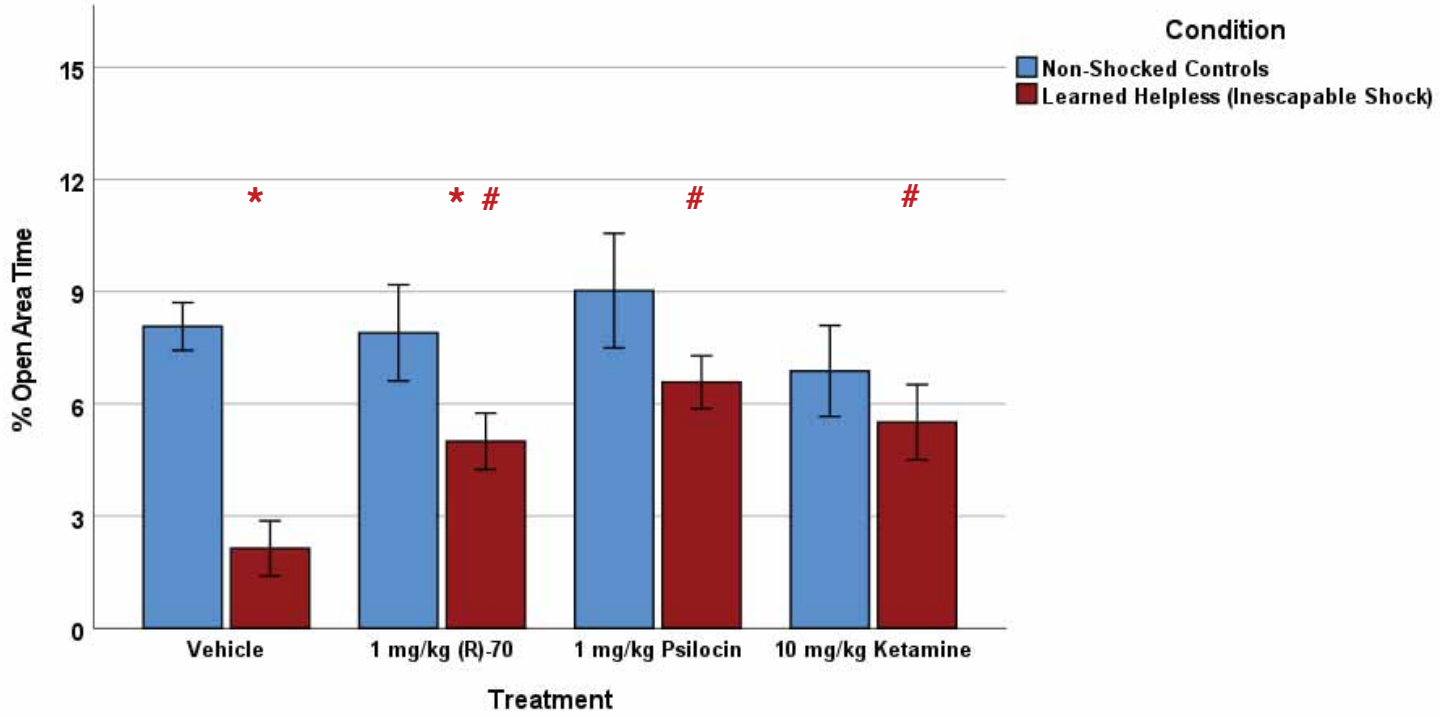


n = 8-10 mice/treatment
^ p<0.05 compared to LSD
ANOVA with Bonferroni corrected *post-hoc*

Levit et al, Nature in. press

; Ramona Rodriquez and Bill Wetsel, Duke

C57BL/6J Mice (Learned Helplessness): Mice Exposed to Chronic Inescapable Foot-Shock Spend Less Time in the Open Areas of an Elevated Zero Maze; a Single Treatment with *R*-(70), Psilocin or Ketamine Increases Open Area Time up to 13 Days After Treatment.



Data analyzed by ANOVA;
*p<0.05, Learned Helpless vs Non-Shock Controls; #p<0.05, vs. vehicle control

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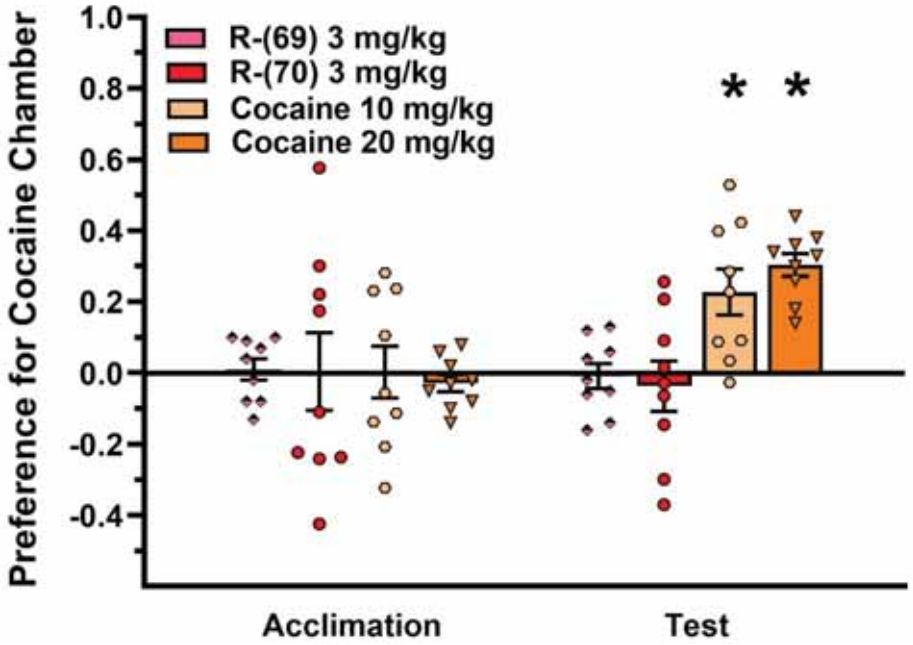
C57BL/6J Mice: No Group Differences Found at Acclimatization; Relative to Cocaine, Neither *R*-(69) Nor *R*-(70) showed Conditioned Place Preference.



Data analyzed by repeated measures ANOVA. *p<0.05, vs. 10 or 20 mg/kg cocaine.

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Take home message

- Many new technologies for GPCR biology: TRUPATH (Olsen, DiBerto et al, *Nature Chem Bio* 2020; Che et al, *Nature Comm* 2020) for interrogating transducers and nanobodies for inactive state structures (Che et al, *Nature Comm* 2020)

- ULTRA-LSD as a platform for discovery of useful and novel chemical matter for molecular targets (Lyu, Wang et al, *Nature* 2019; Stein, Kang et al, *Nature* 2020; Alon et al, *Nature* 2021; Sadybekov et al, *Nature* 2022)

- Structure-guided and structure-informed drug discovery for oGPCRs feasible (Huang et al, Nature 2015; Lansu et al, *Nat Chem Bio* 2017; Cao et al, *Nature* 2021; Zhang et al, *Nature Struct Mol Bio* 2022; Levitt, Ku et al, *Nature* *in press*)

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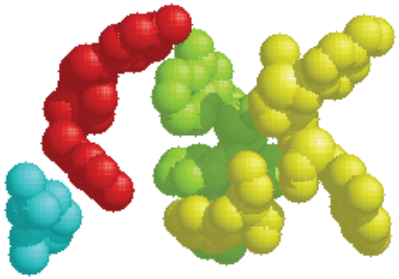
  - John McCorvy, PhD→Medical College Wisconsin
  - Tao Che, PhD→Washington University
  - Alex Tribo→Hopkins PhD Student
  - Kate Lansu→Eli Lilly
  - Reid Olsen→Exscientia
  - XP Huang, PhD
  - Brian Krumm, PhD
  - Estela Lopez
  - Hye Jin Kang, PhD→Sungkyunkwan University
  - Dave Nichols, PhD
  - Justin English, PhD→University of Utah
  - Kuglae Kim, PhD→Yonsei University
  - Kunjie Hua, MS
  - Roger Zou
  - Wes Kroeze, PhD
  - Yi-Ting Chiu, PhD
  - Noah Sciaky, PhD
  - Can Cao, PhD
  - Jeff DeBerto: PhD student
  - Sam Slocum, PhD→Onsero Pharmaceuticals
  - Ryan Strachan, PhD→Septerna Pharmaceuticals
  - Yongfeng Liu, PhD
- Jonathan Fay, PhD (UNC Biochemistry)

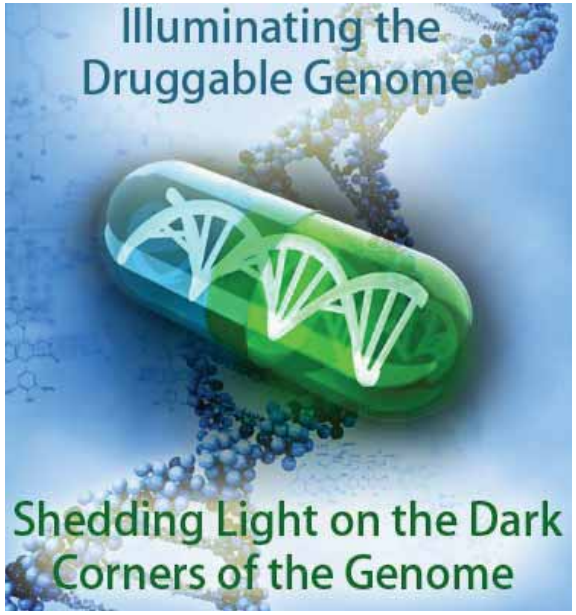
- Brian Shoichet lab (UCSF)
  - Anat Levit, PhD
  - Joel Karpiak, PhD
  - Shuo Gu, PhD
  - Reed Stein
  - John Irwin, PhD

- Mei-Chuan Ko lab (Wake Forest)

- Jian Jin Lab (MSSM)
  - He Chen, PhD
  - Chengwei Zhang, PhD
  - Jing Liu, PhD



NIMH Psychoactive Drug Screening Program



NIDA
NATIONAL INSTITUTE
ON DRUG ABUSE

NIMH
National Institute
of Mental Health