

Application of Rosetta to GPCR Comparative Models

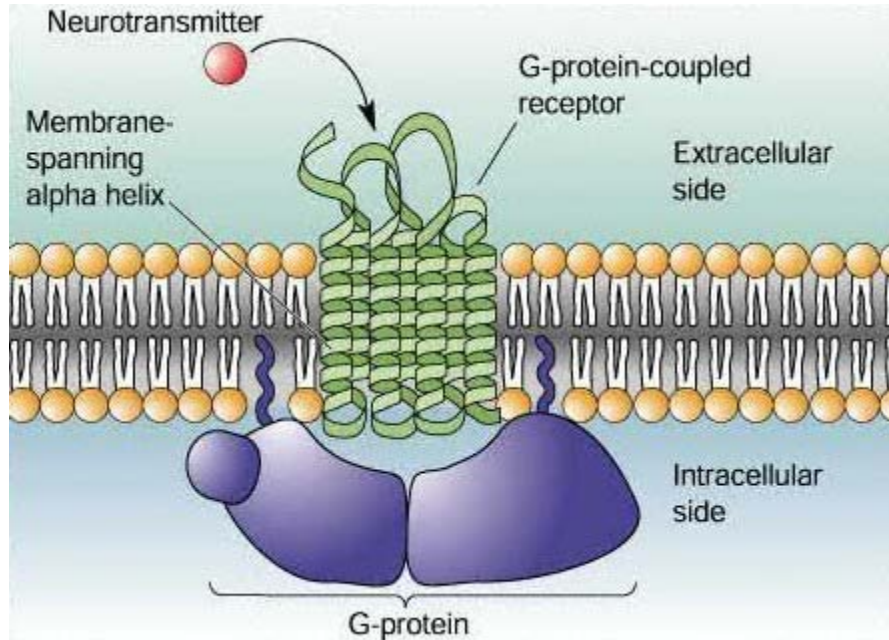
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Meiler Lab, Vanderbilt University

RosettaCon

08.05.10

GPCRs: Ideal therapeutic targets with little structural information



Purves, D., et al. *Neuroscience*, 3rd ed.

NPY receptors:

-breast cancer and obesity therapy

mGlu receptors:

-allosteric modulators target schizophrenia & Fragile X syndrome

Challenges of GPCR Modeling:

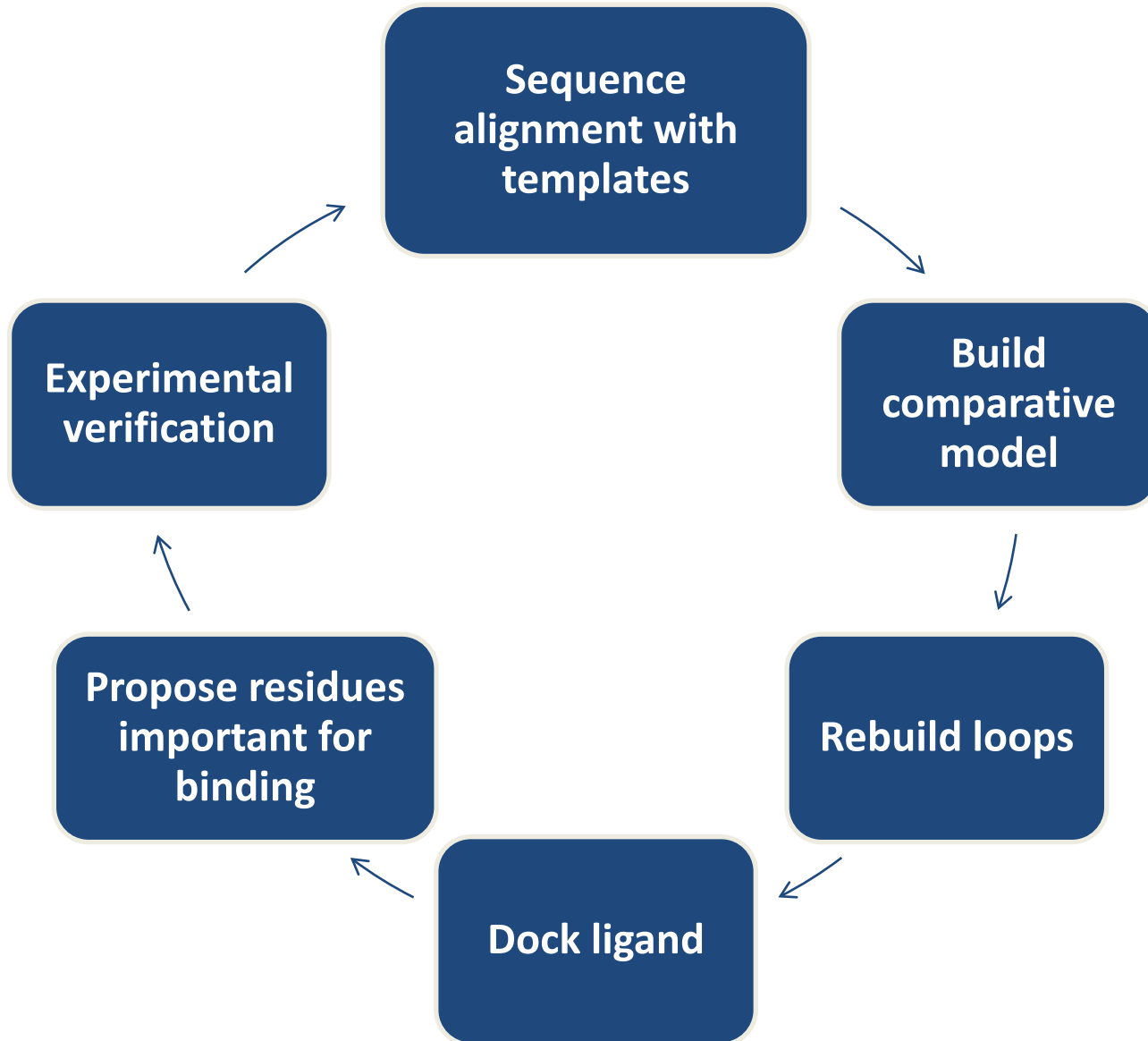
1. membrane protein

-> use the Rosetta membrane potential

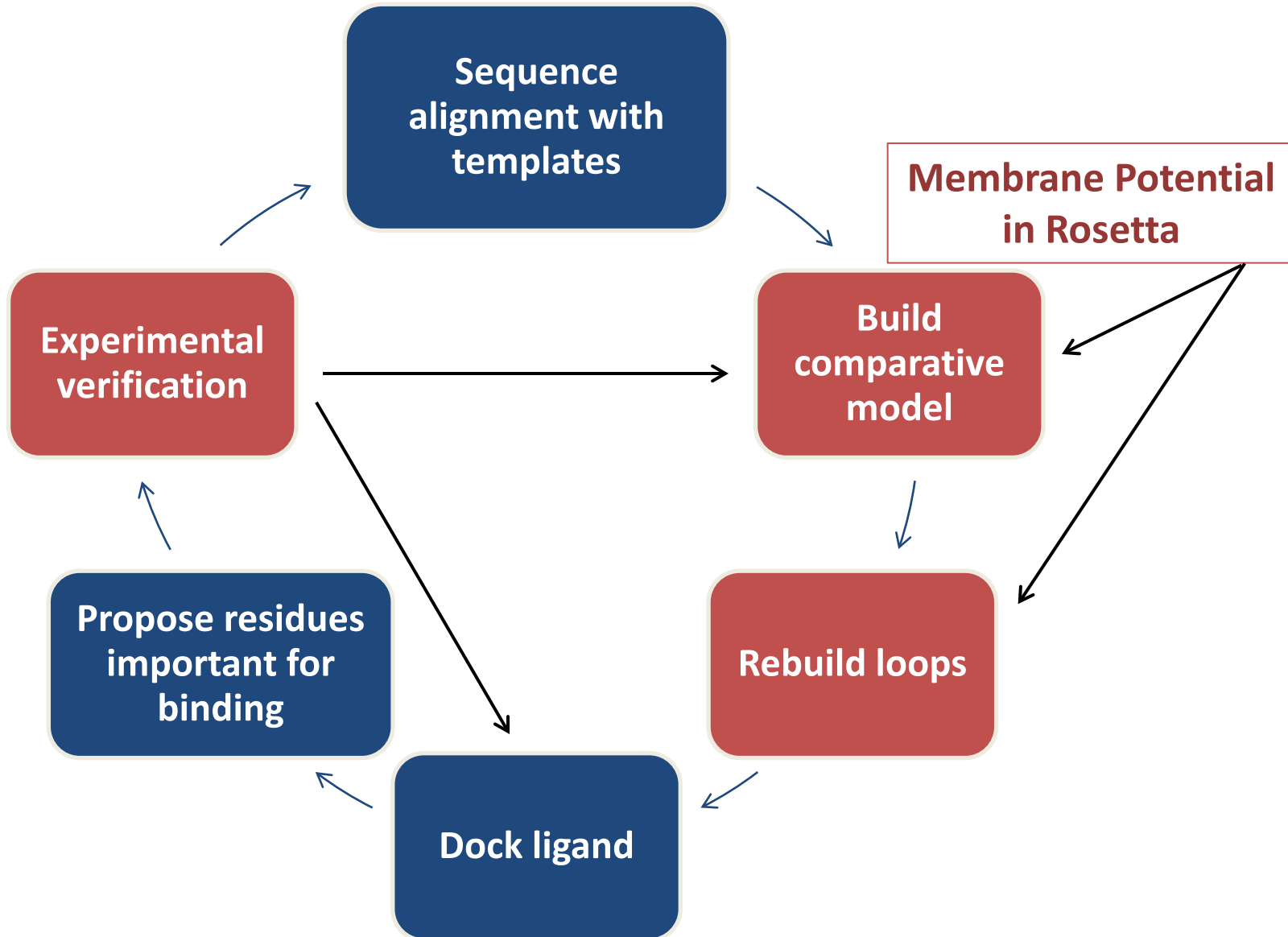
2. low sequence identity between GPCR classes (<20%)

-> couple modeling with experimental studies

Iterative comparative modeling with experimental validation



Iterative comparative modeling with experimental validation



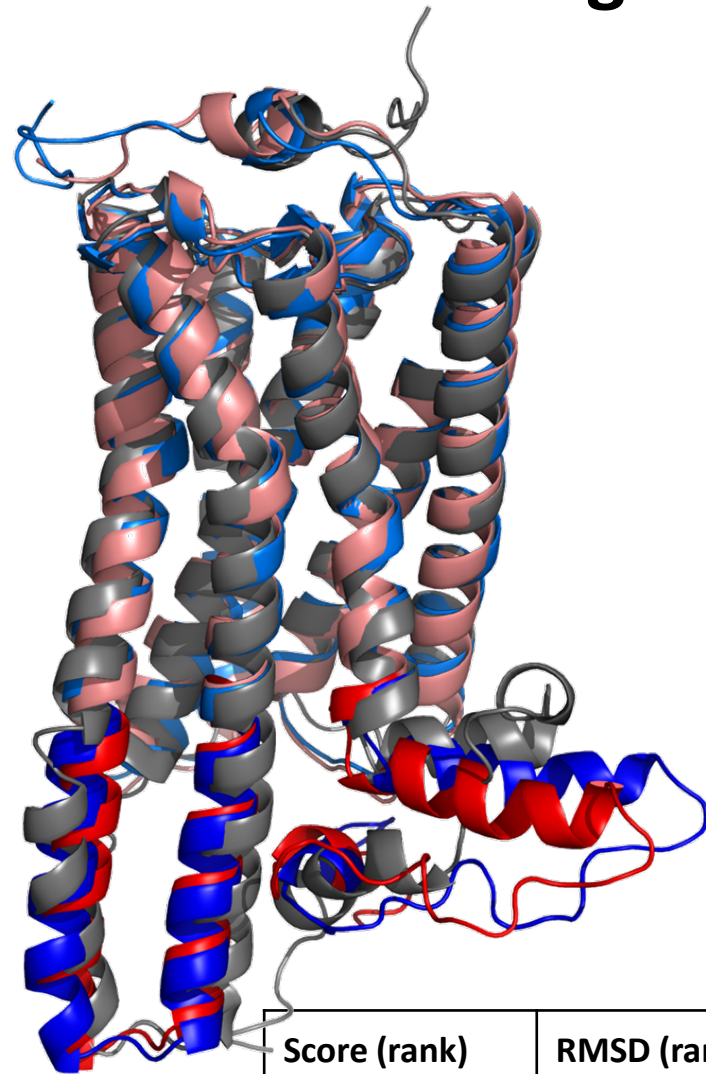
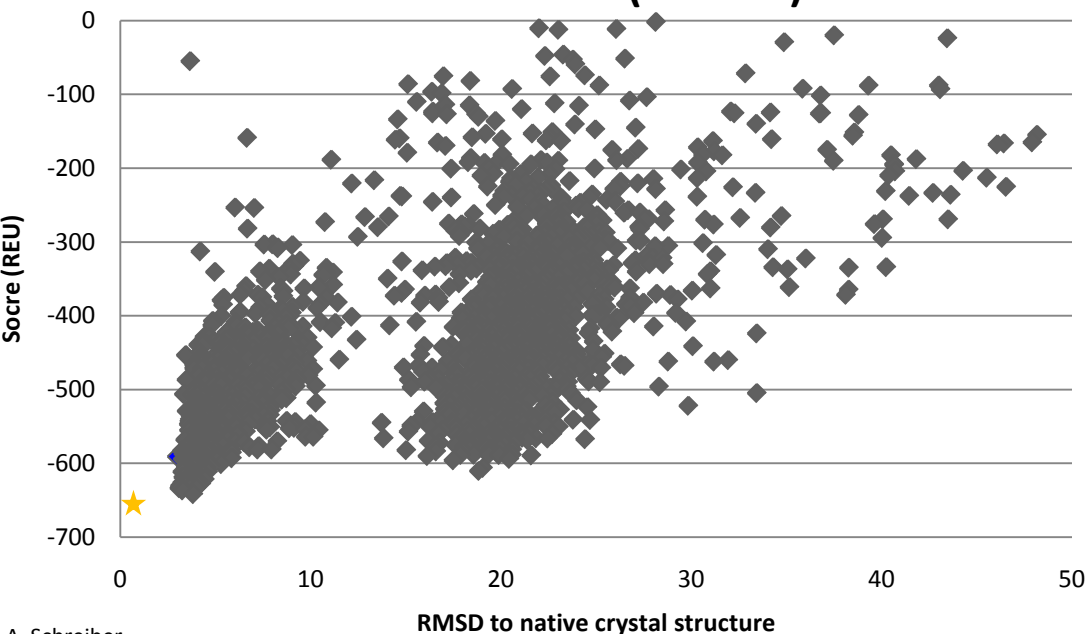
Topology Broker can be used to rebuild flexible regions

Benchmarking Topology Broker with Squid

Rhodopsin:

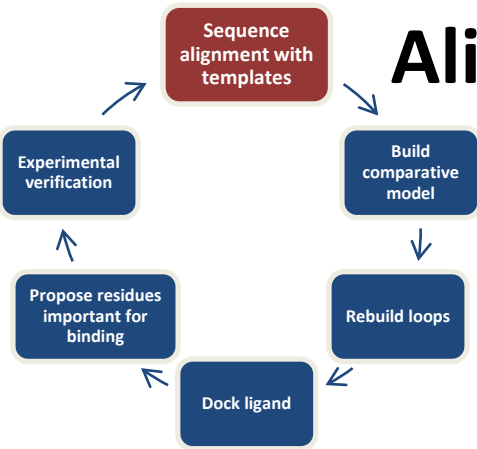
- Constrain *ab initio* folding protocol with claimers to define membrane topology & adjust weights accordingly
- Rebuild intracellular helical region between TM 5 & 6 and C-terminus
- Perform membrane relax on the center of the 2 largest clusters

Score vs RMSD: Topology Broker & Membrane Relax (n=2000)



	Score (rank)	RMSD (rank)
relax		
rebuild + relax	-641.7 (1)	3.843Å (95)
relax		
rebuild + relax	-590.76 (146)	2.981Å (1)

Align mGlu5 sequence with GPCR templates



Class A GPCRs:

B2adrenergic
Rhodopsin
A2a adenosine

Class C GPCRs:

mGluR1-8

Mustang
(structure to structure)

ClustalW
(sequence to sequence)

Transmembrane Span 7

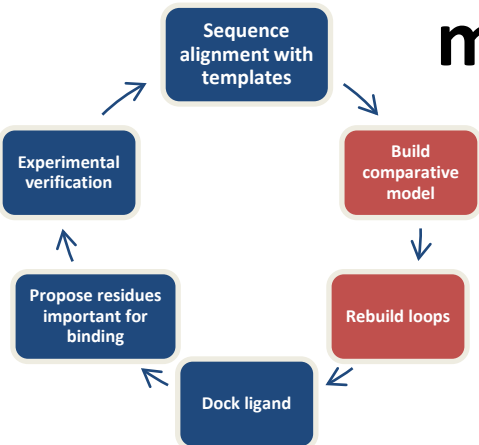
**original
alignment**

<i>mGluR5</i>	Y K I I T M C F S V S L S A T V A L G C M F V P K V Y I I	825
<i>mGluR1</i>	Y K I I T T C F A V S L S V T V A L G C M F T P K M Y I I	838
<i>b2adrenergic (2RH1)</i>	K E V Y I L L N W I G Y V N S G F N P L I Y C R S P . D F	300
<i>rhodopsin (1U19)</i>	P I F M T I P A F F A K T S A V Y N P V I Y I M M N K Q F	313

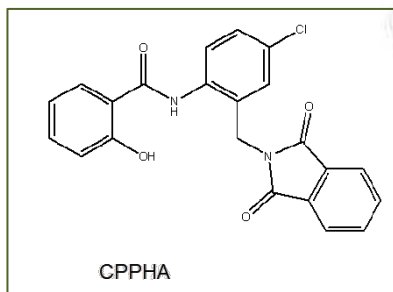
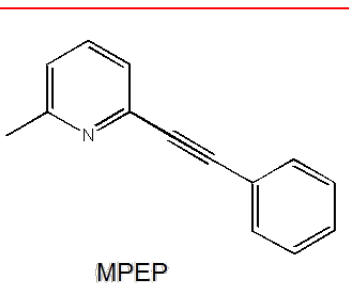
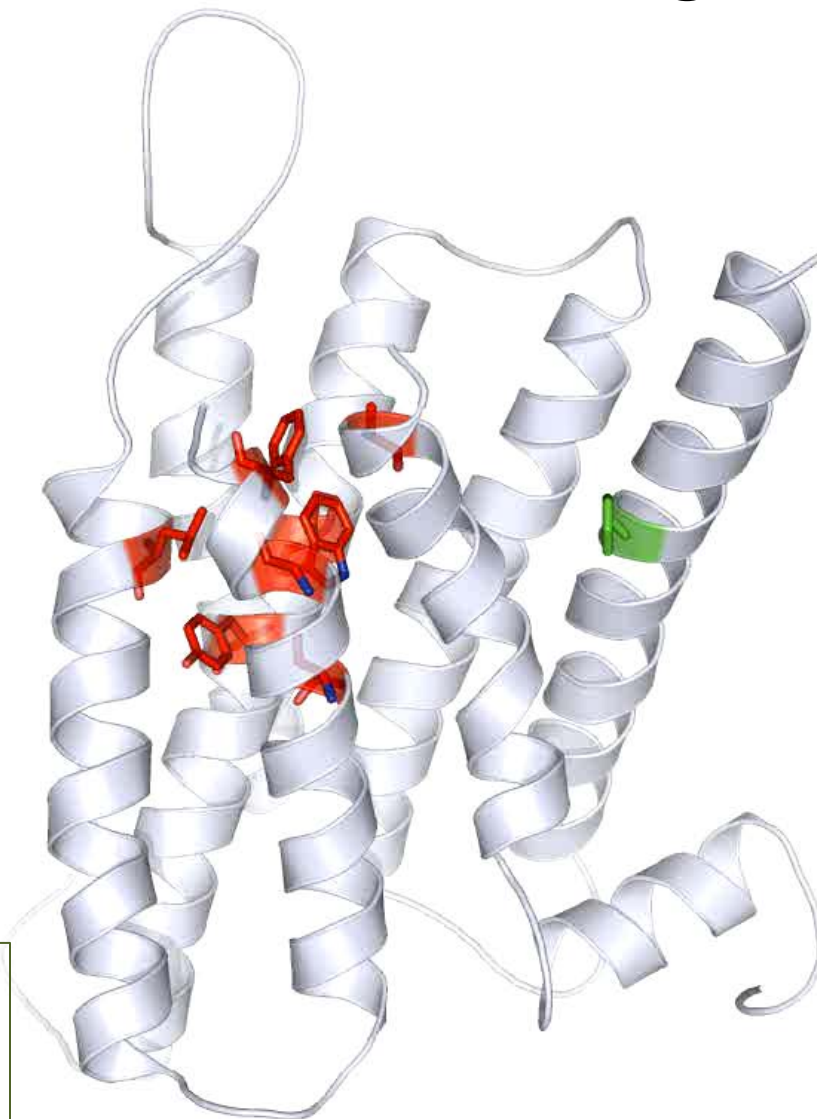
**new
alignment**

<i>mGluR5</i>	M C F S V S L S A T V A L G C M F V P K V Y I I	825
<i>mGluR1</i>	T C F A V S L S V T V A L G C M F T P K M Y I I	838
<i>b2adrenergic (2RH1)</i>	K E V Y I L L N W I G Y V N S G F N P L I Y C R	296
<i>rhodopsin (1U19)</i>	P I F M T I P A F F A K T S A V Y N P V I Y I M	308

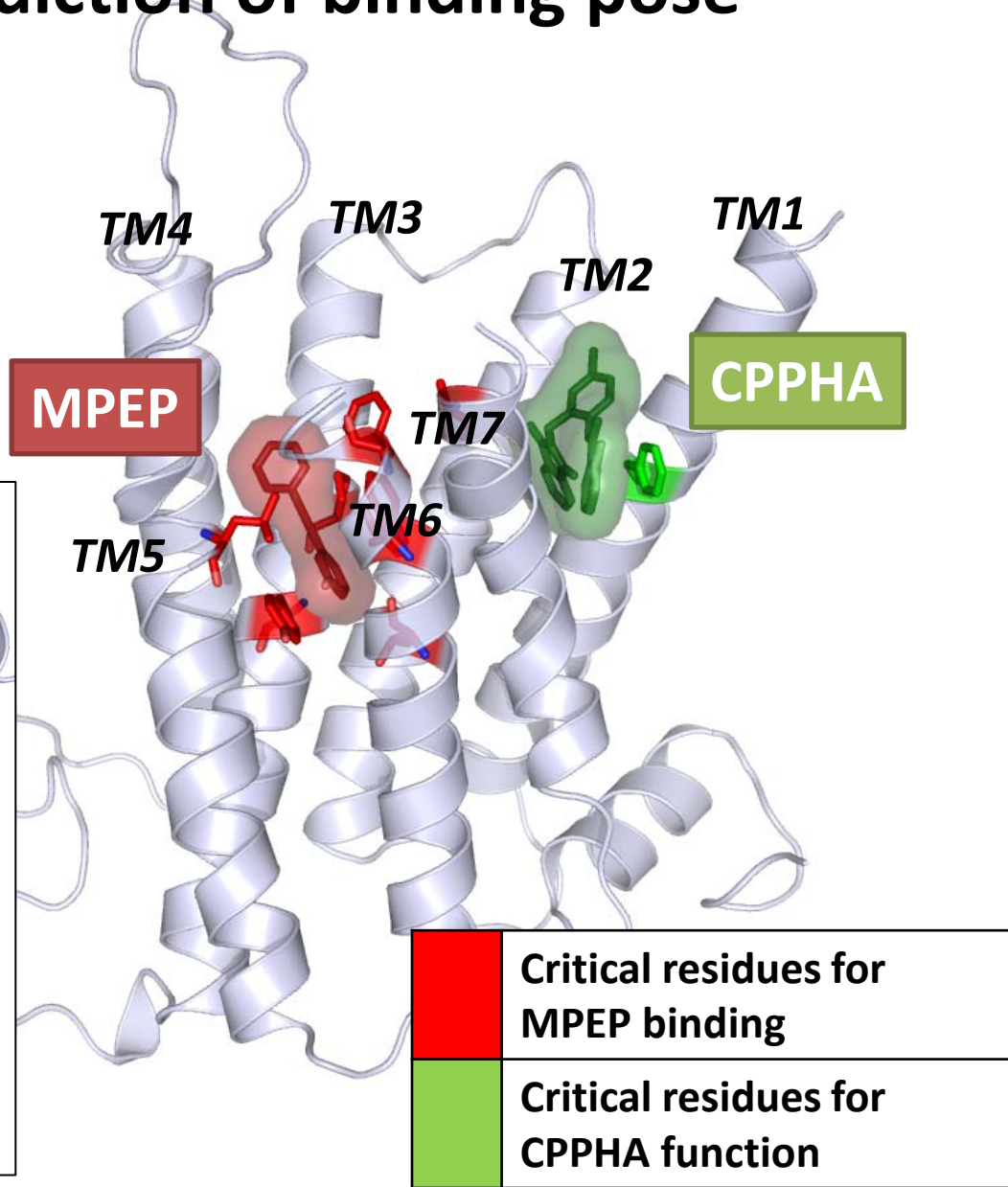
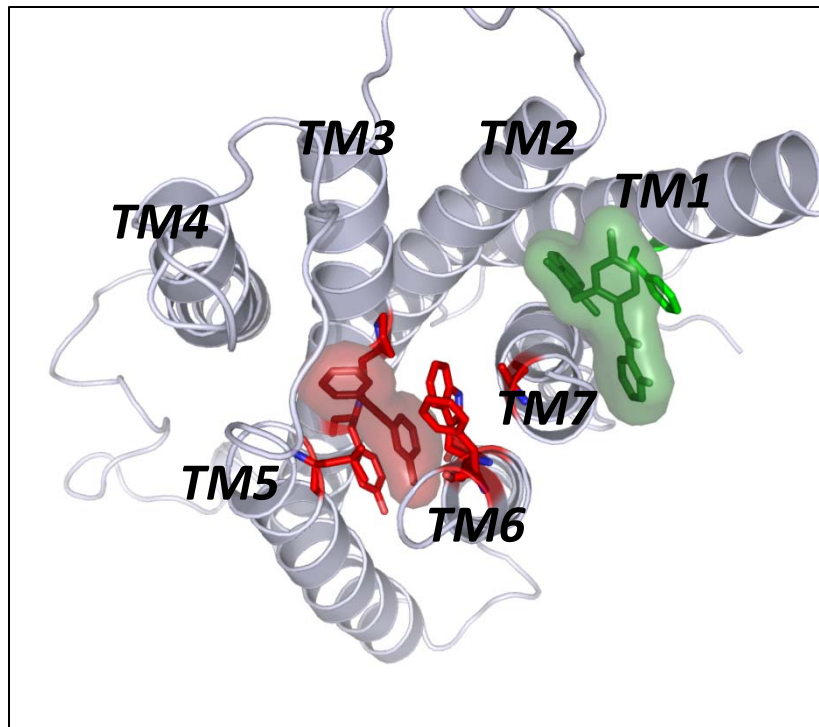
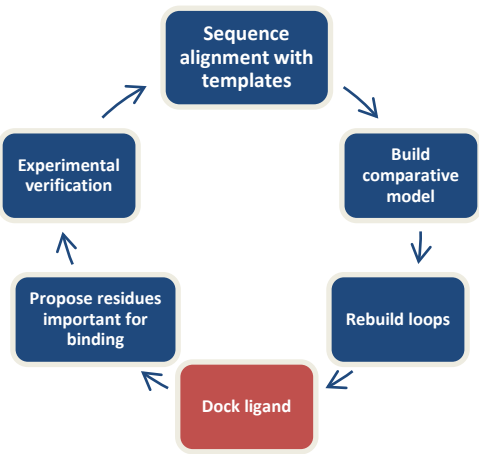
mGlu5 model clusters important residues into a common binding site



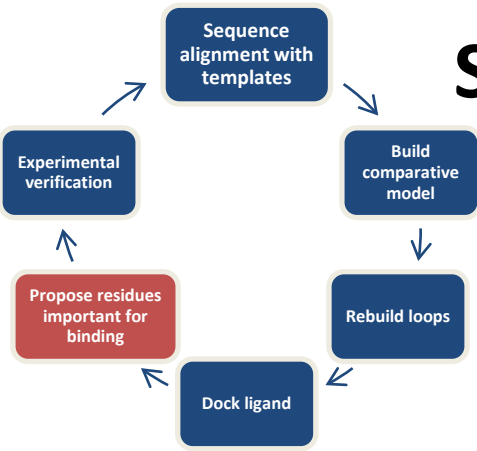
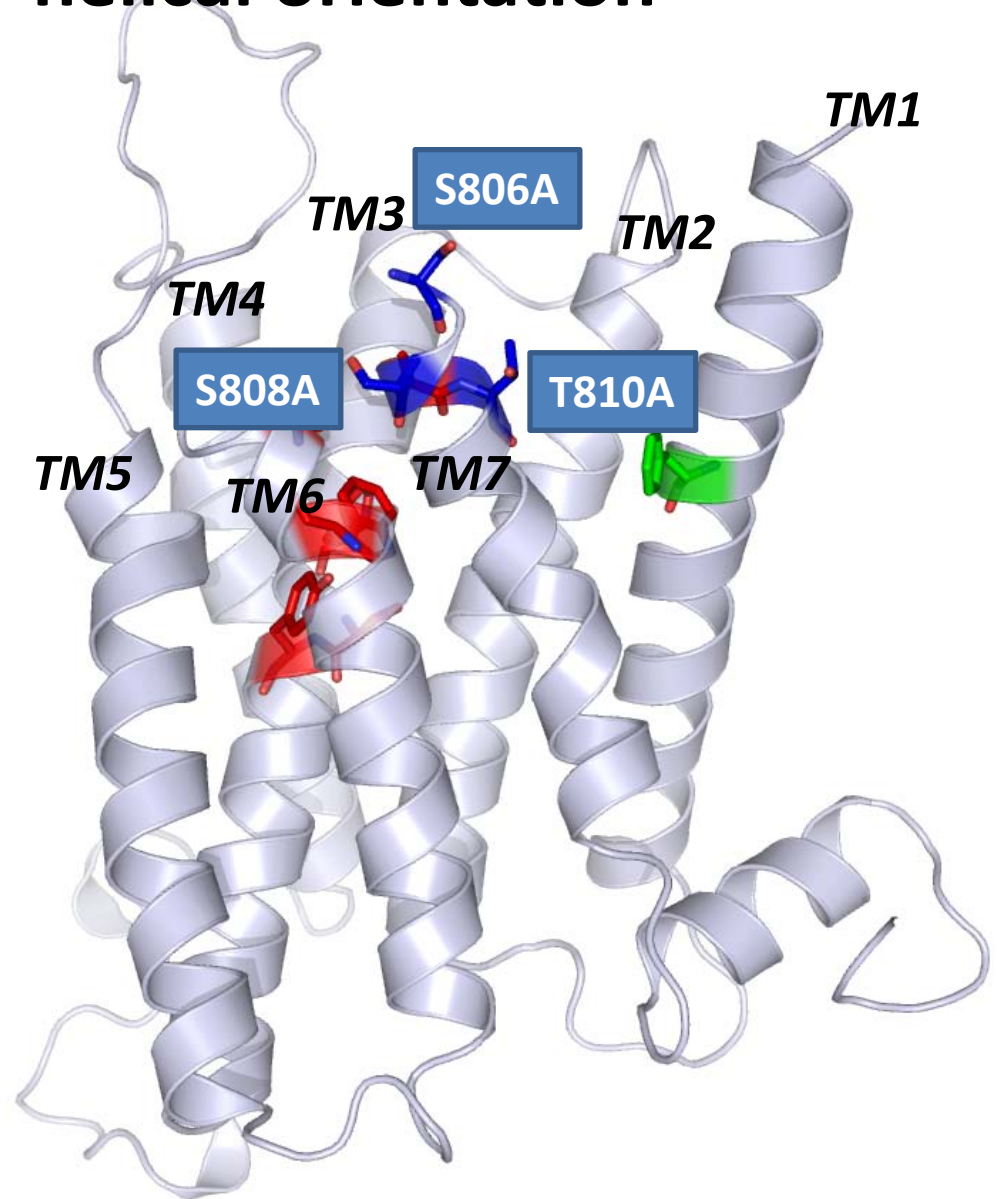
	Critical residues for MPEP binding: <i>TM3: P654, S657, Y658</i> <i>TM6: W784, F787</i> <i>TM7: A809</i>
	Critical residues for CPPHA function: <i>TM1: F585</i>



Docking allosteric modulators allows prediction of binding pose



Site-directed mutagenesis studies validate helical orientation

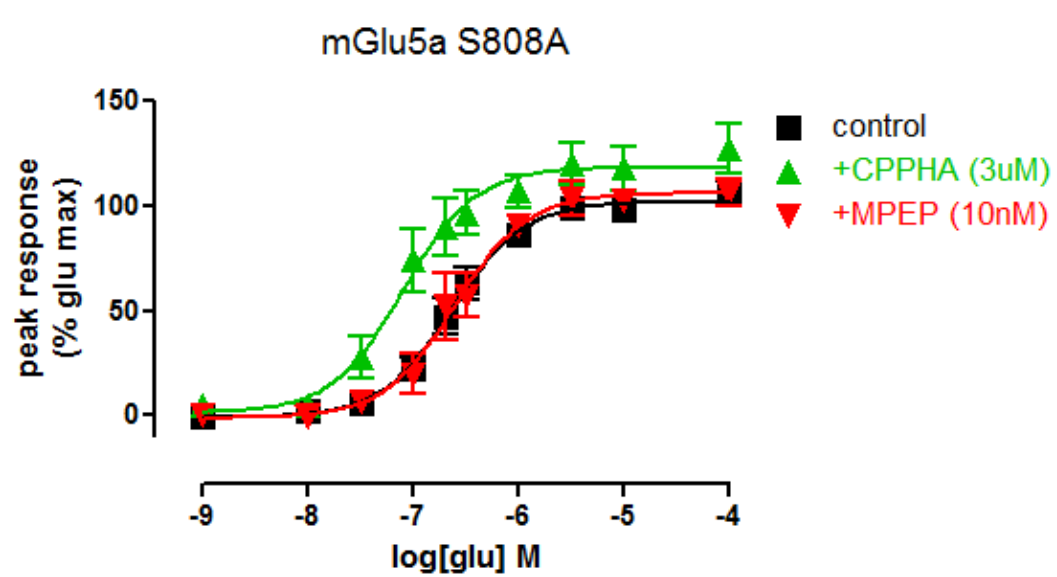
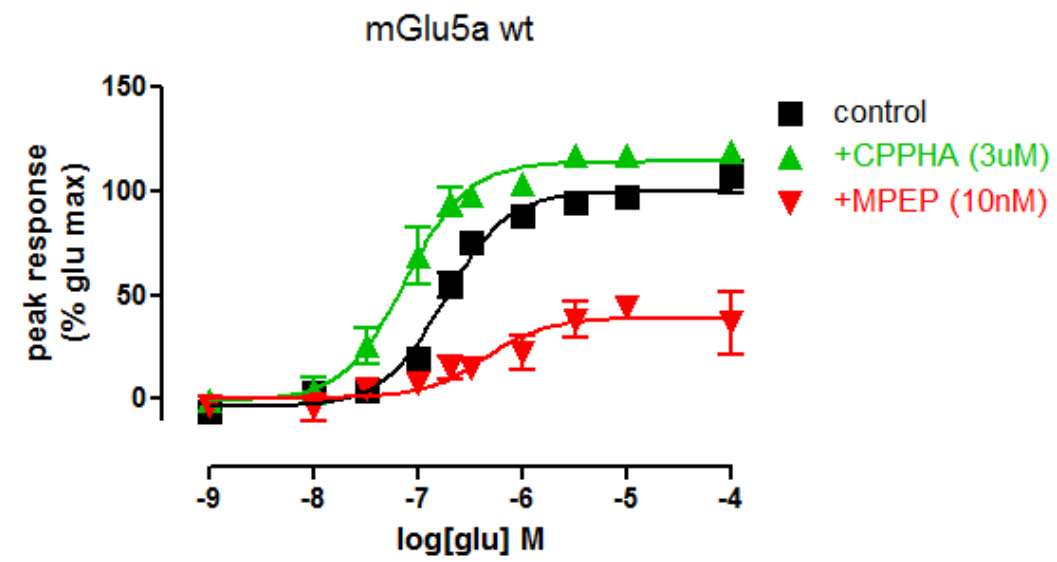
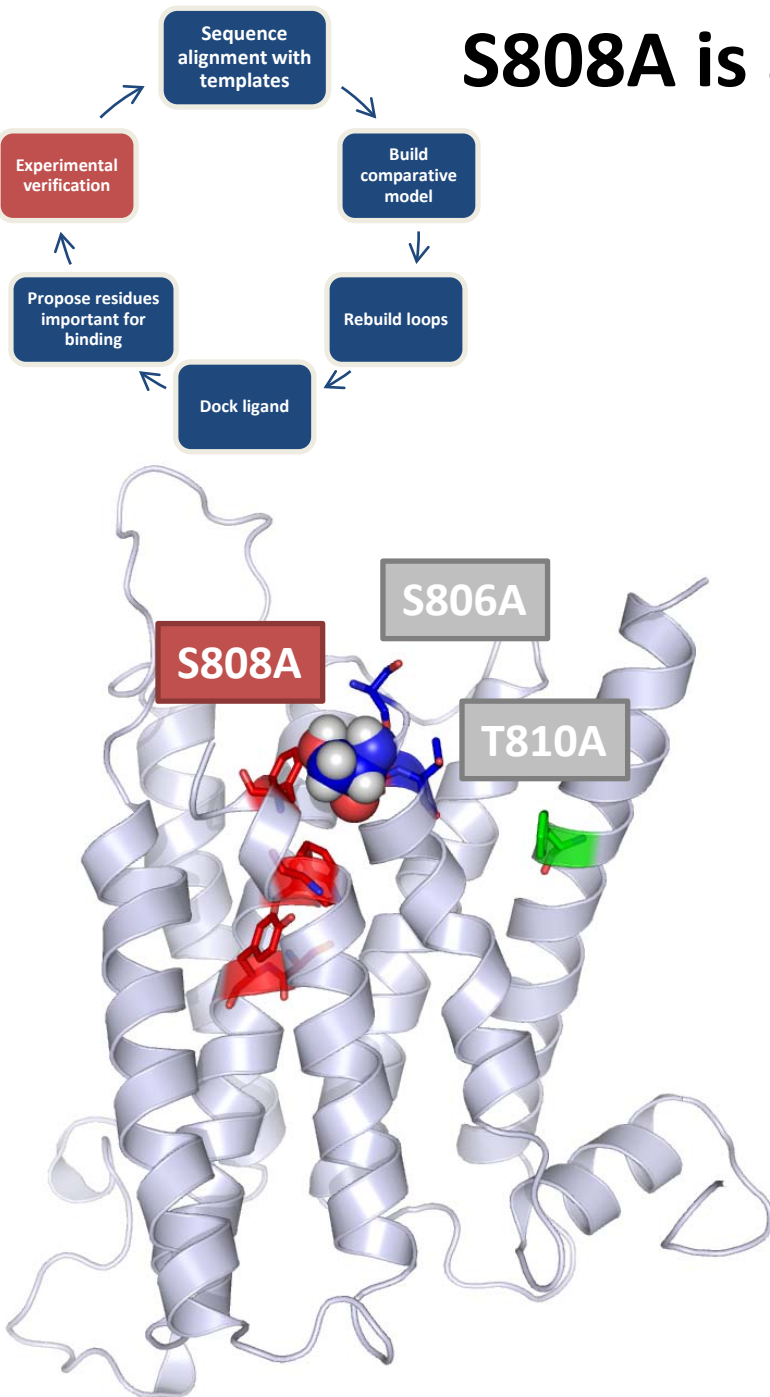


Goal of experimental validation:

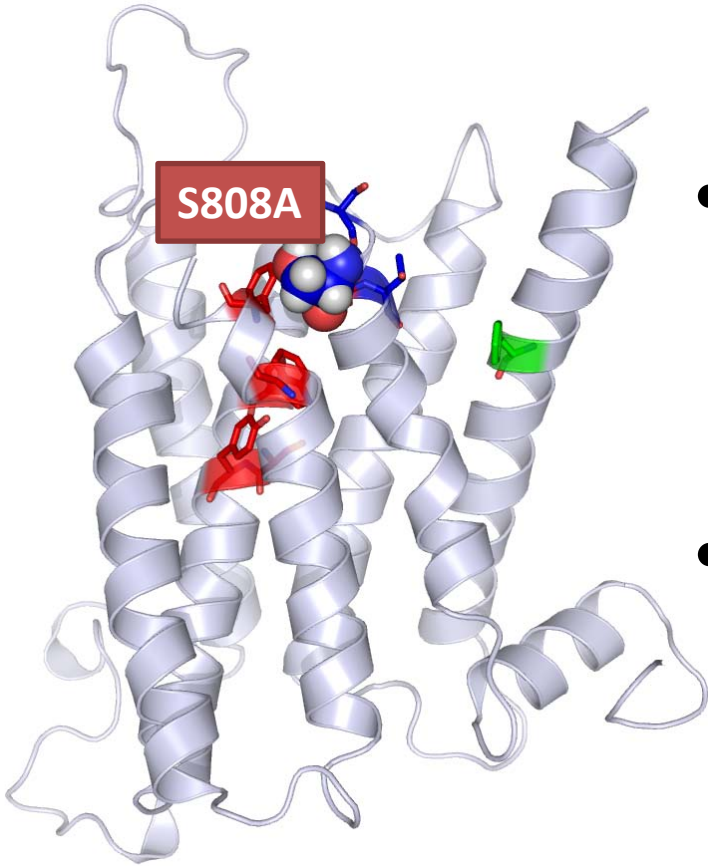
-verify orientation of TM7 resulting from PxxY alignment

	Critical residues for MPEP binding
	Critical residues for CPPHA function
	Residues proposed for site-directed mutagenesis

S808A is a functionally important residue for MPEP



Conclusions



- Topology Broker in Rosetta can be used to rebuild flexible regions of a membrane protein
- Experimental verification is valuable and necessary for building comparative models of GPCRs

Acknowledgements

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Meiler Lab

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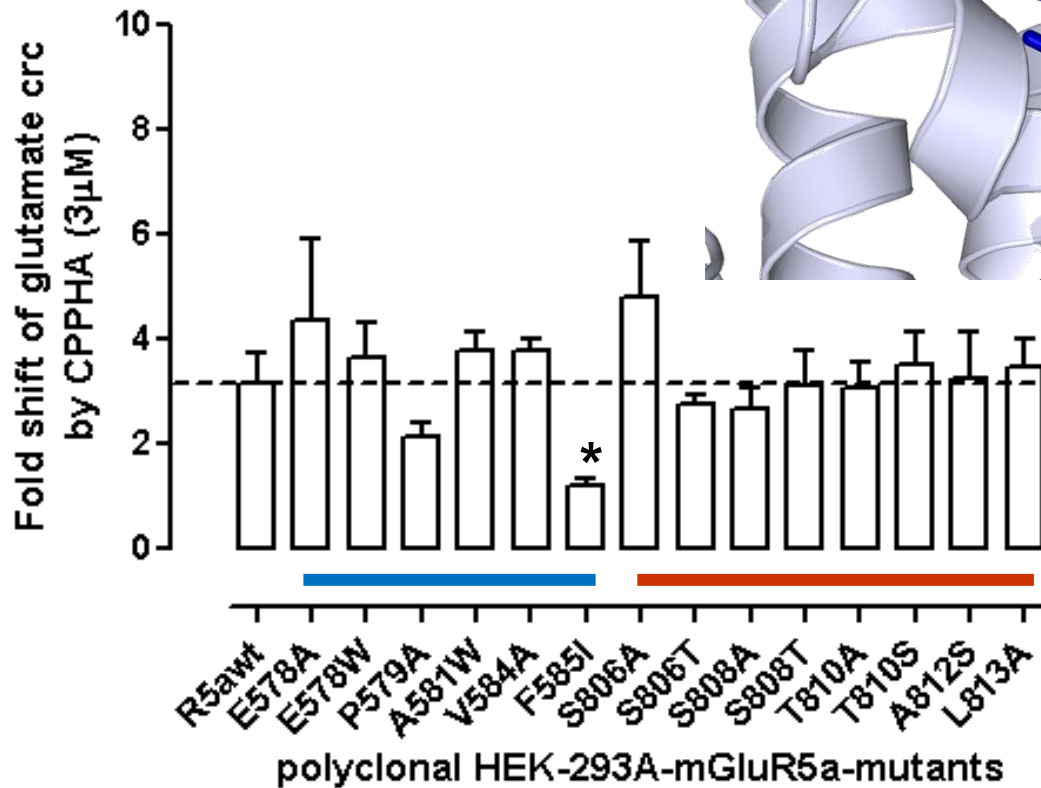
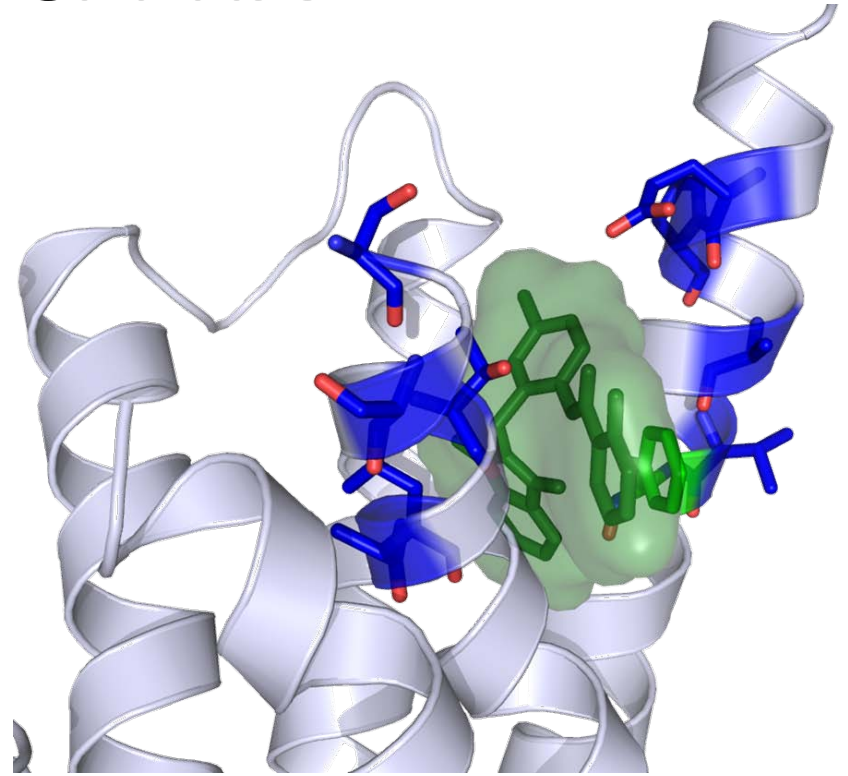
Vladimir Yarov-Yarovoy



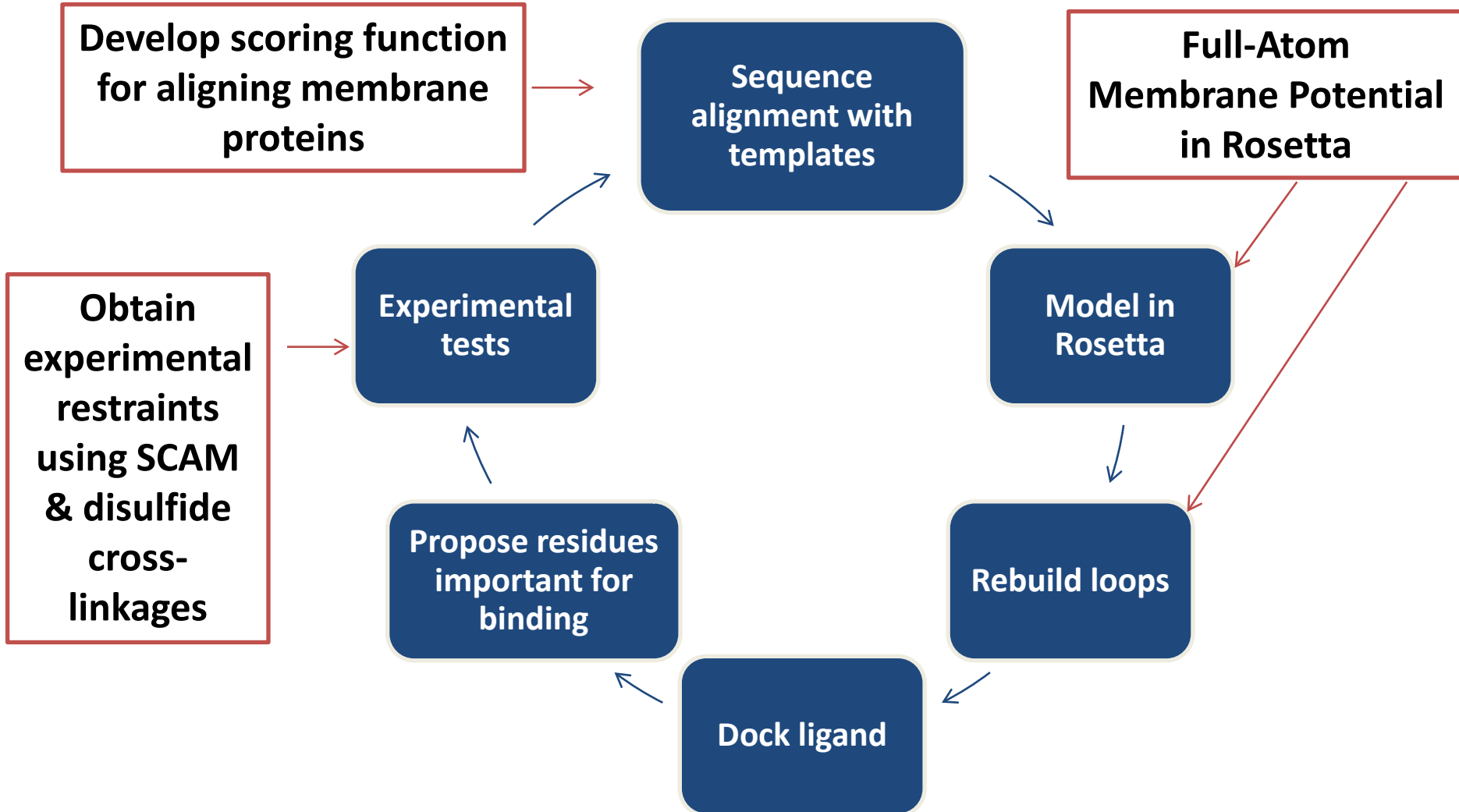
Funding

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CPPHA



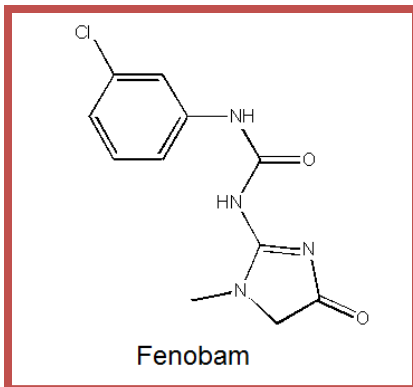
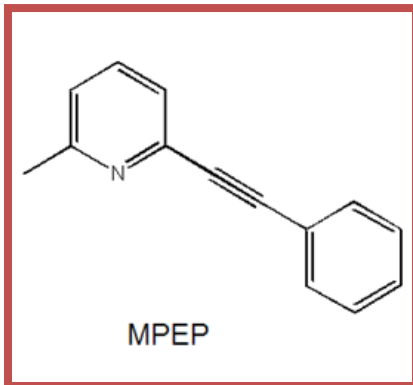
Future Directions



Allosteric modulators of mGlu₅ have therapeutic value

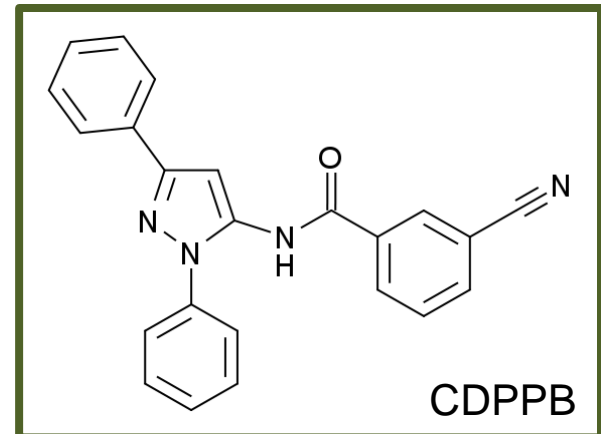
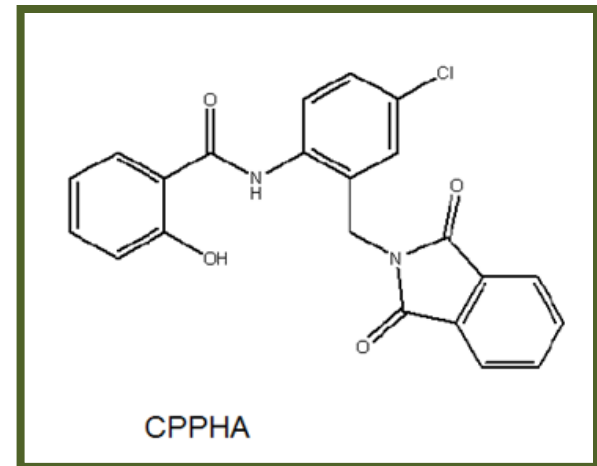
mGluR5 NAMs:

Anxiety, Fragile X Syndrome, chronic pain, depression, migraine, Parkinson's disease levodopa-induced dyskinesia



mGluR5 PAMs:

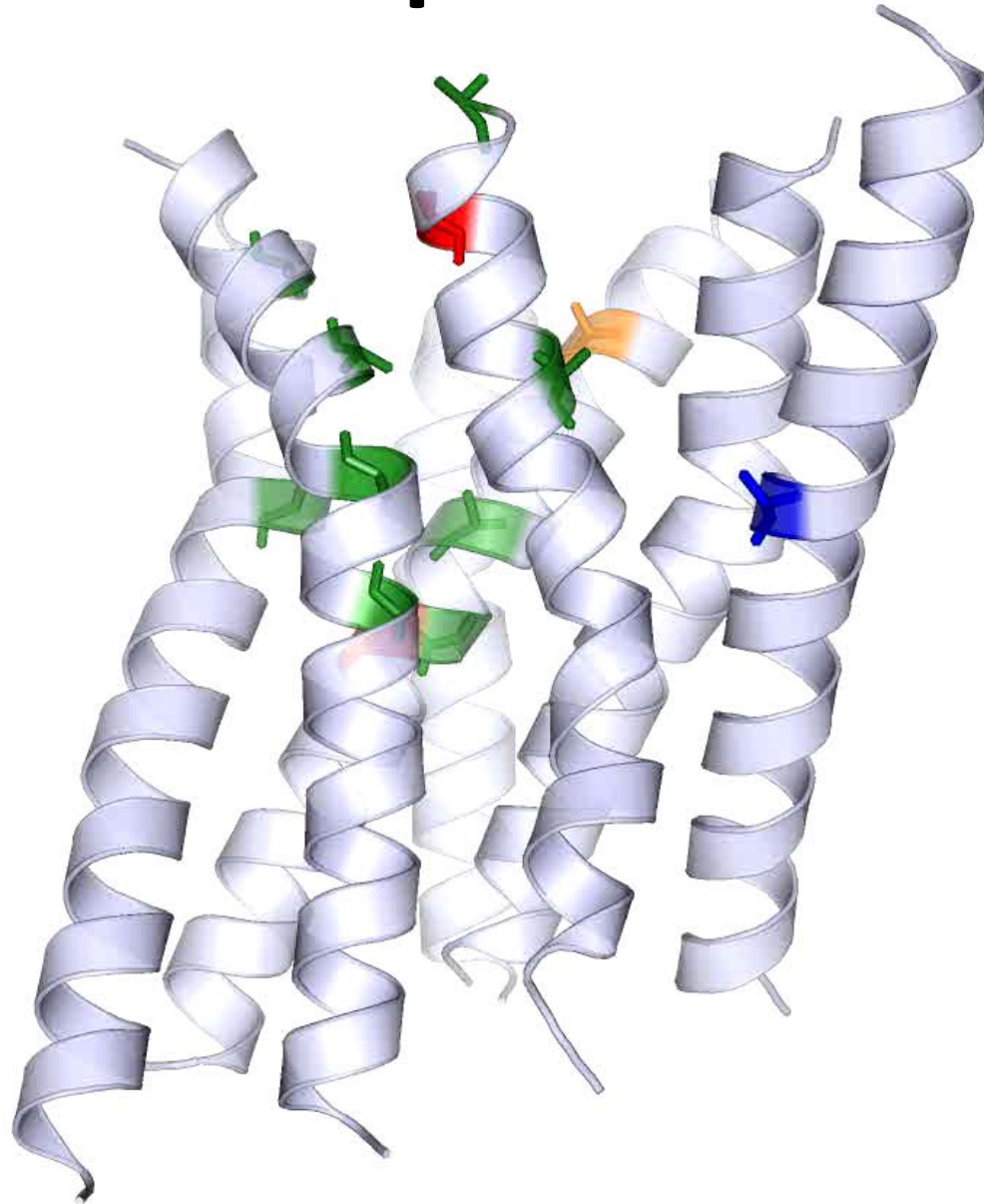
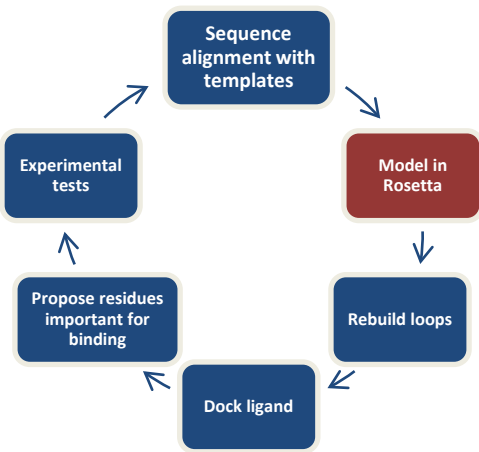
Schizophrenia, cognition disorders



Current treatments for schizophrenia have negative side effects

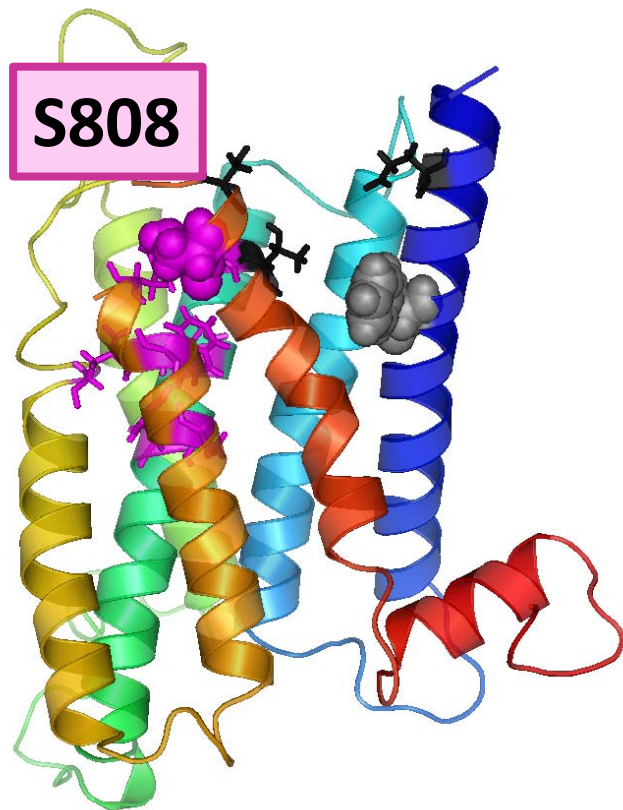
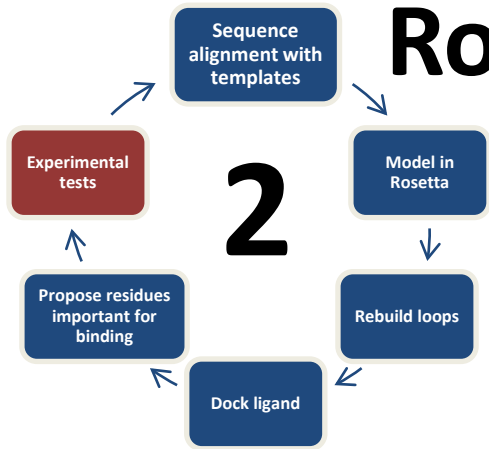
- *Chlorpromazine*: painful twisting and contractions of muscles
- *Haloperidol*: high incidence of permanent tardive dyskinesia, especially in females over 45 years old (repetitive, involuntary, purposeless movements - grimacing, tongue protrusion, lip smacking, puckering and pursing of the lips, and rapid eye blinking)
- *Aripiprazole (Abilify)*: weight gain of 2.2 lbs a year, hyperglycemia could lead to coma/death

Initial Comparative Model

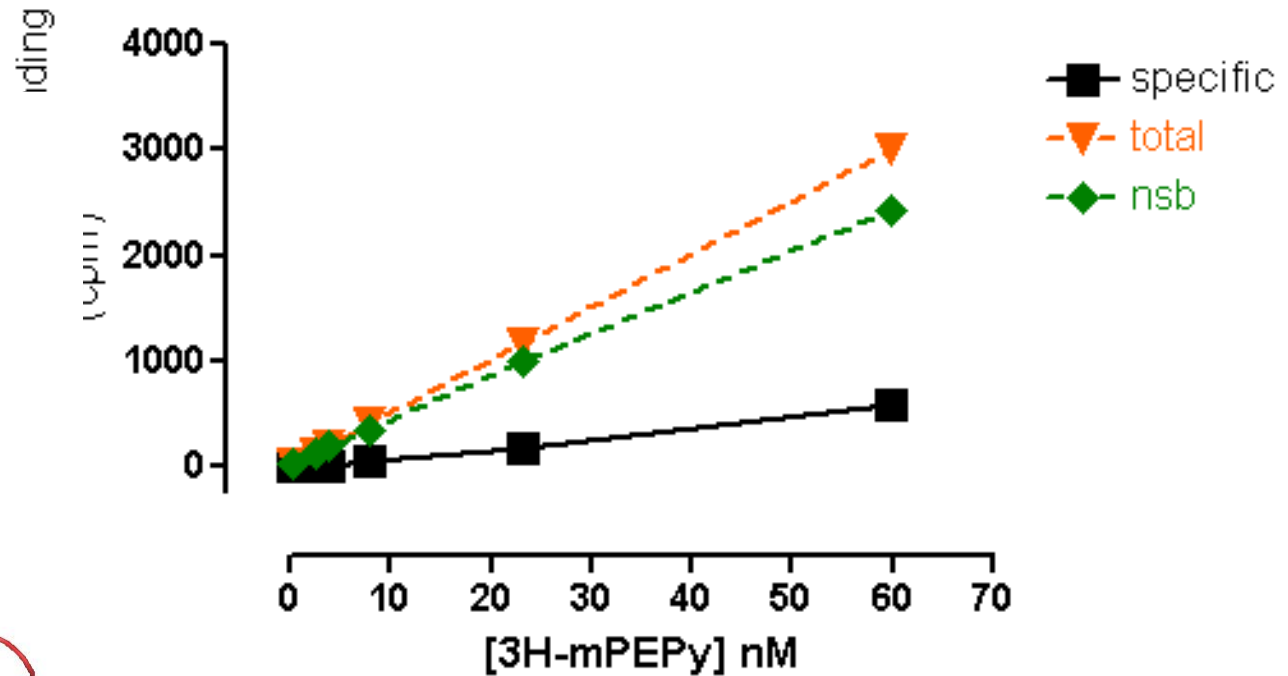


	MPEP Binding
	MPEP function
	CPPHA function

Round 2: Experimental validation

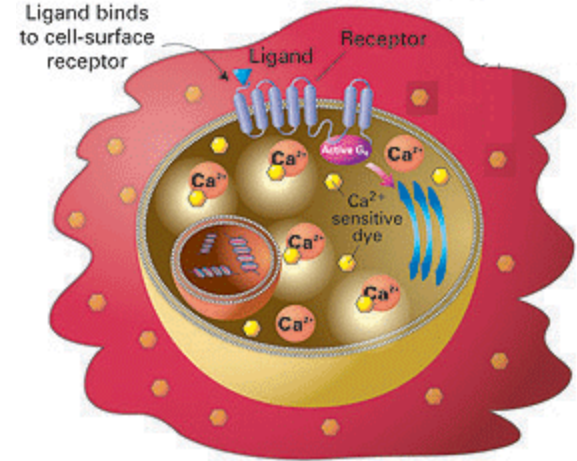


HEK-293A-R5a-S808A, polyclonal, p8, 10/2/10
20 μ g

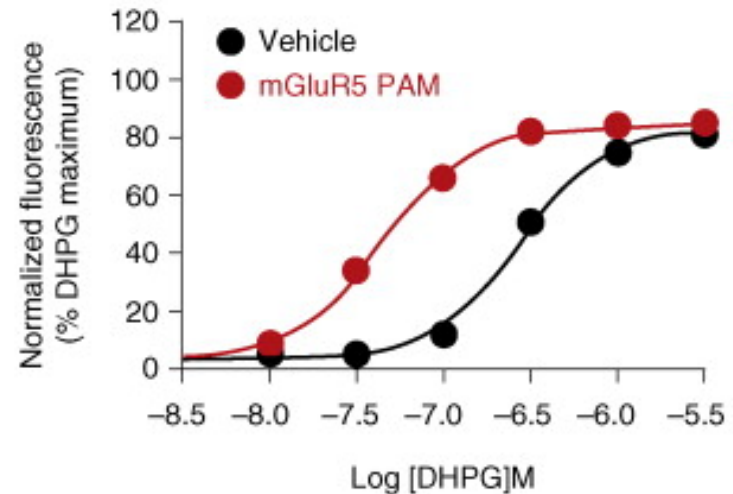
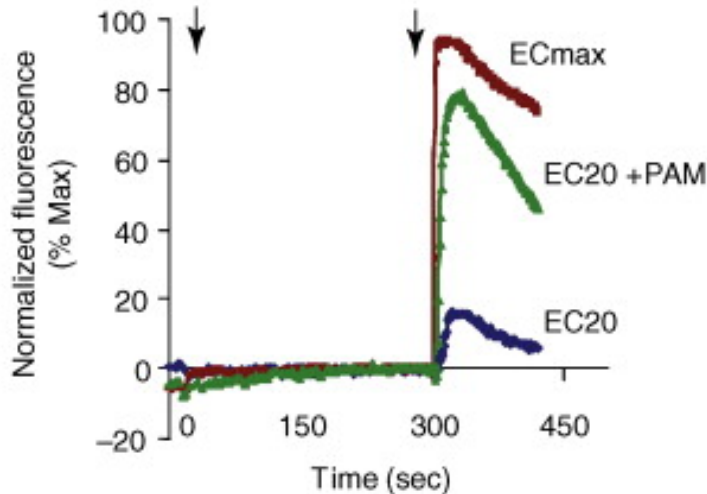


Using calcium mobilization as a functional assay

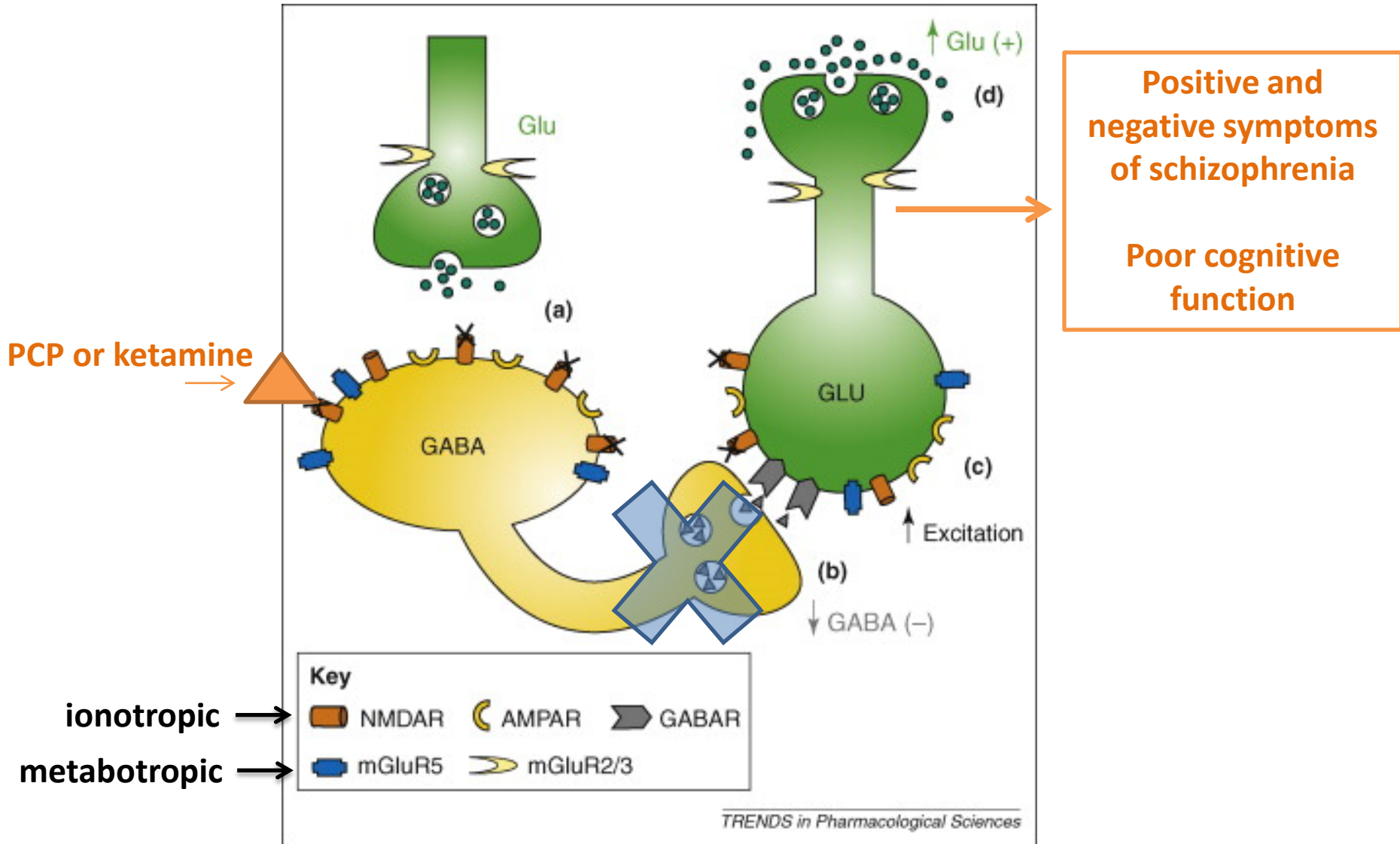
1. Transfect cells with wild-type or mutant mGluR5 complementary DNA
2. Load cells with calcium-sensitive dye
3. Add allosteric modulators
4. Add glutamate
5. Record peak calcium response to glutamate
6. Normalize to maximum response elicited by supra-maximal dose of glutamate (100 μM)



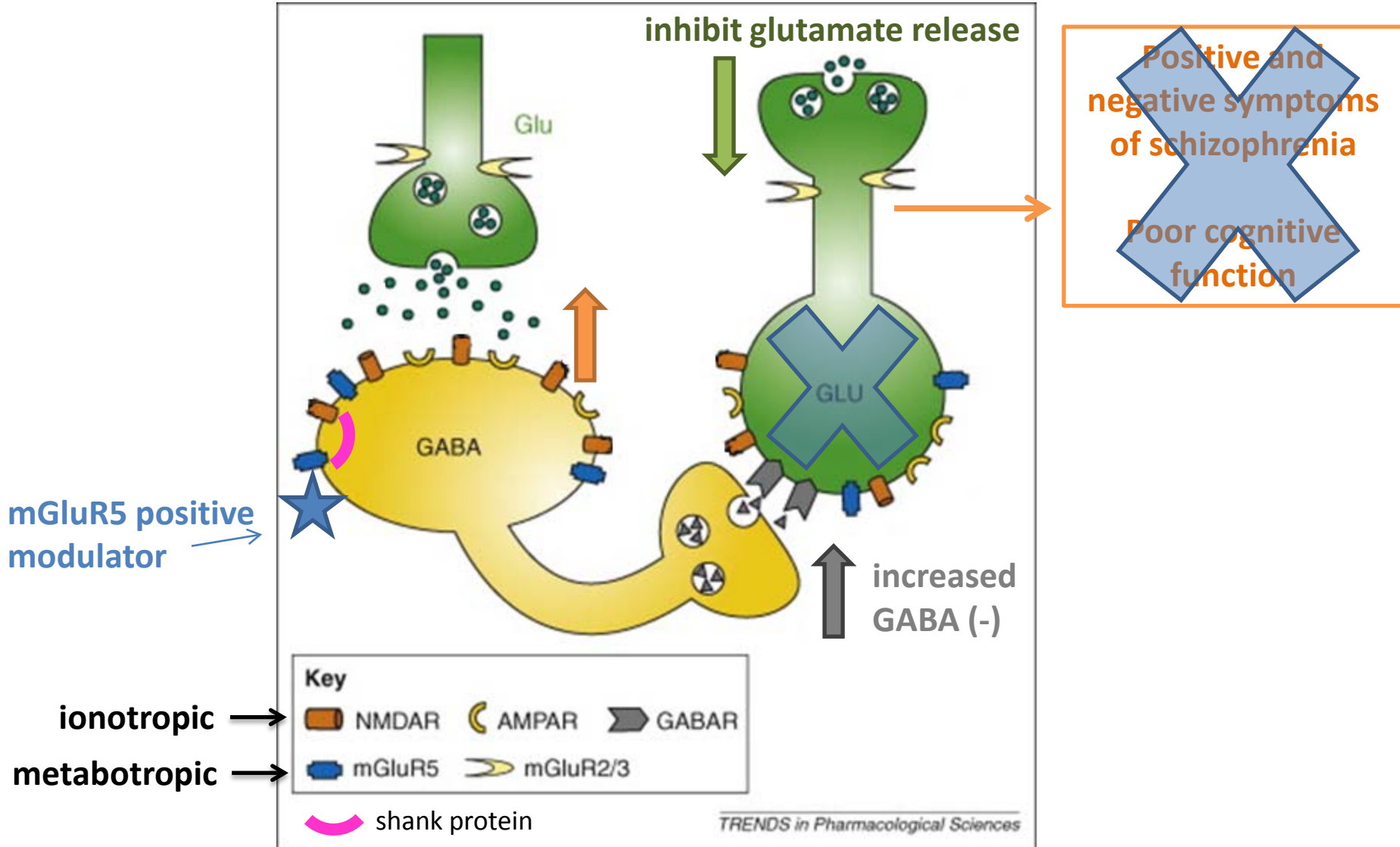
Increase in cytosolic Ca²⁺ can be detected by FLIPR or FlexStation microplate readers using calcium-sensitive dye indicators



NMDA antagonists produce symptoms of schizophrenia



Activating mGluR5 can reduce symptoms of schizophrenia



TM1

mGluR5 Y L R W G D P E P I A A V V F A C L G L L A T L F V T V V F I I
mGluR1 Y L E W S N I E S I I A I A F S C L G I L V T L F V T L I F V L

b2adrenergic (2RH1) D E V W V V G M G I V M S L I V L A I V F G N V L V I T A I A K
rhodopsin (1U19) E P W Q F S M L A A Y M F L L I M L G F P I N F L T L Y V T V Q

TM2

603 S S R E L C Y I I L A G I F L G Y V C P F T L I A K P T . 653
 616 S G R E L C Y I L L G G V F L C Y C M T F I F I A K P S . 628
 S G R E L C Y I L L F G V G L S Y C M T F F F I A K P S . 637
 60 S G R E L C S Y V L L A G I F L C Y A T T F L M I A E P D . 648
 64 S S R E L C Y I I L A G I C L G Y L C T F C L I A K P K . 640
 S G R E L C S Y V L L T G I F L I Y A I T F L M V A E P G . 646
 S G R E L S Y V L L T G I F L C Y I I T F L M I A K P D . 651
 A S G R E L S Y V L L T G I F L C Y S I T F L M I A A P D . 644
 A T N R E L S Y L L L F S L L C C F S S S L F F I G E P Q . 673

V T N Y F I T S L A C A D L V M G L A V V P F G A A H I L M 96
 P L N Y I L L N L A V A D L F M V F G G F T T T L Y T S L H 100

TM3

mGluR1 T T S C Y L Q R L L V G L S S A M C Y S A L V T K T N R I A R I L A 687
mGluR2 T A V C T L R R L G L G T A F S V C Y S A L L T K T N R I A R I F G 662
mGluR3 P V I C A L R R L G L G S S F A I C Y S A L L T K T N C I A R I F D 671
mGluR4 L G T C S L R R I F L G L G M S I S Y A A L L T K T N R I Y R I F E 682
mGluR5 Q I Y C Y L Q R I G I G L S P A M S Y S A L V T K T N R I A R I L A 674
mGluR6 A A V C A A R R L F L G L G T T L S Y S A L L T K T N R I Y R I F E 680
mGluR7 V A V C S F R R V F L G L G M C I S Y A A L L T K T N R I Y R I F E 685
mGluR8 T I I C S F R R V F L G L G M C F S Y A A L L T K T N R I H R I F E 678
CaSR D W T C R L R Q P A F G I S F V L C I S C I L V K T N R V L V F E 707

TM4

I A S I L I S V Q L T L V V T L I I M E P P M P I 733
 I C L A L I L S G Q L L I V V A W L V E A P G T G 706
 I C L G L I L V Q I V M V S V W L I L E A P G T R 715
 I T F S L I S L Q L L G I C V W F V D P S H S V 726
 I A F I L I C I Q L G I V A L F I M E P P D I M 720
 I T F S L T S L Q V V G M I A W L G A R P P H S V 724
 I T S S L I S V Q L L G V F I W F G V D P P N I I 729
 I T F S L I S V Q L L G V F V W F V D P P H I I 722
 L V F L C T F M Q I V I C V I W L Y T A P P S S Y 751

b2adrenergic (2RH1) N F W C E F W T S I D V L C V T A S I E T L C V I A V D R Y F A I T 136
rhodopsin (1U19) P T G C N L E G F F A T L G G E I A L W S L V V L A I E R Y V V V C 140

K N K A R V I I L M V W I V S G L T S F L P I Q M 171
 E N H A I M G V A F T W V M A L A C A A P L V G 174

TM5

mGluR1 G V V A P L G Y N G L L I M S C T Y Y A F K T R N V P . 778
mGluR2 S M L G S L A Y N V L L I A L C T L Y A F K T R K C P . 753
mGluR3 S M L I S L T Y D V I L V I L C T V Y A F K T R K C P . 762
mGluR4 S L I C L L G Y S M L L M V T C T V Y A I K T R G V P . 778
mGluR5 G V V T P L G Y N G L L I L S C T F Y A F K T R N V P . 765
mGluR6 S L I G C L G Y S L L L M V T C T V Y A I K A R G V P . 776
mGluR7 Q I I C S L G Y S I L L M V T C T V Y A I K T R G V P . 781
mGluR8 S L I C S L G Y S I L L M V T C T V Y A I K T R G V P . 774
CaSR A L G F L I G Y T C L L A A I C F F F A F K S R K L P . 798

TM6

A N F N E A K Y I A F T M Y T T C I I W L A F V P I Y F G S N . 809
 E N F N E A K F I G F T M Y T T C I I W L A F L P I F Y V T S S 785
 E N F N E A K F I G F T M Y T T C I I W L A F L P I F Y V T S S 794
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 A N F N E A K Y I A F T M Y T T C I I W L A F V P I Y F G S N . 796
 E T F N E A K P I G F T M Y T T C I I W L A F V P I F F G T A Q 808
 E N F N E A K P I G F T M Y T T C I V W L A F I P I F F G T A Q 813
 E T F N E A K P I G F T M Y T T C I I W L A F I P I F F G T A Q 830
 E N F N E A K F I T F S M L I F F I V W I S F I P A Y A S T Y G 633

b2adrenergic (2RH1) Q A Y A I A S S I V S F Y V P L V I M V F V Y S R V F Q E A K R Q L 230
rhodopsin (1U19) S . F V I Y M F V V H F I I P L I V I F F C Y G Q L V F T V K E A A 234

K E H K A L K T L G I I M G T F T L C W L P F F I V N I V H V I 298
 A E K E V T R M V I I M V I A F L I C W L P Y A G V A F Y I F T 277

TM7

mGluR1 T C F A V S L S V T V A L G C M F T P K M Y I I 838
mGluR2 M C V S V S L S G S V V L G C L F A P K L H I I 817
mGluR3 M C I S V S L S G F V V L G C L F A P K V H I I 826
mGluR4 L T V S V S L S A S V S L G M L Y M P K V Y I I 845
mGluR5 M C F S V S L S A T V A L G C M F V P K V Y I I 825
mGluR6 L T V S L S L S A S V S L G M L Y V P K T Y V I 843
mGluR7 L T I S M N L S A S V A L G M L Y M P K V Y I I 848
mGluR8 L T V S M S L S A S V S L G M L Y M P K V Y I I 841
CaSR E V I A I L A A S F G L L A C I F F N K I Y I I 860

b2adrenergic (2RH1) K E V Y I L L N W I G Y V N S G F N P L I Y C R 296
rhodopsin (1U19) P I F M T I P A F F A K T S A V Y N P V I Y I M 308