Application of Rosetta to GPCR Comparative Models

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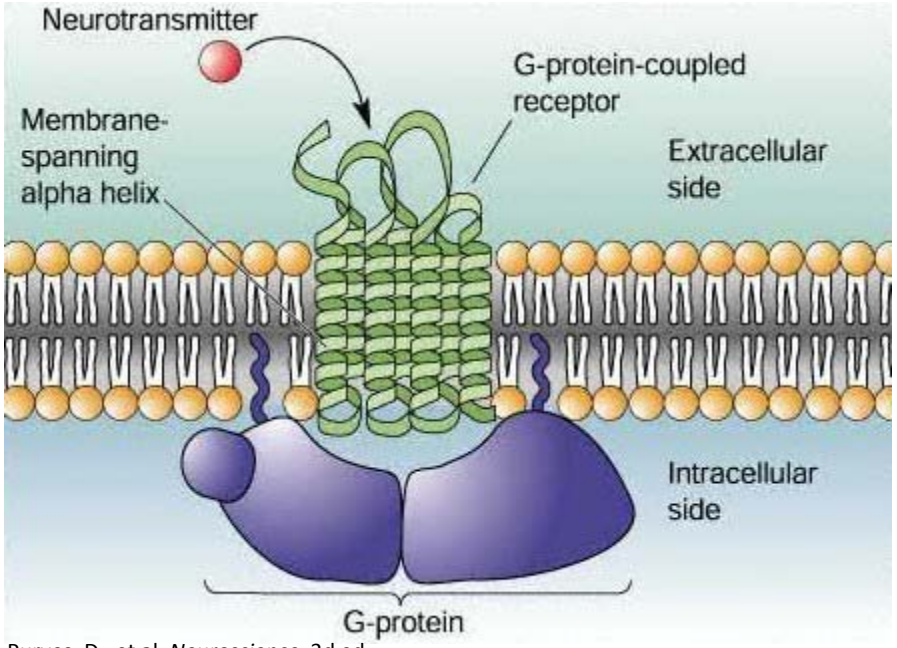
RosettaCon
08.05.10

GPCRs: Ideal therapeutic targets with little structural information



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

NPY receptors:
-breast cancer and obesity therapy

mGlu receptors:
-allosteric modulators target schizophrenia & Fragile X syndrome

Challenges of GPCR Modeling:

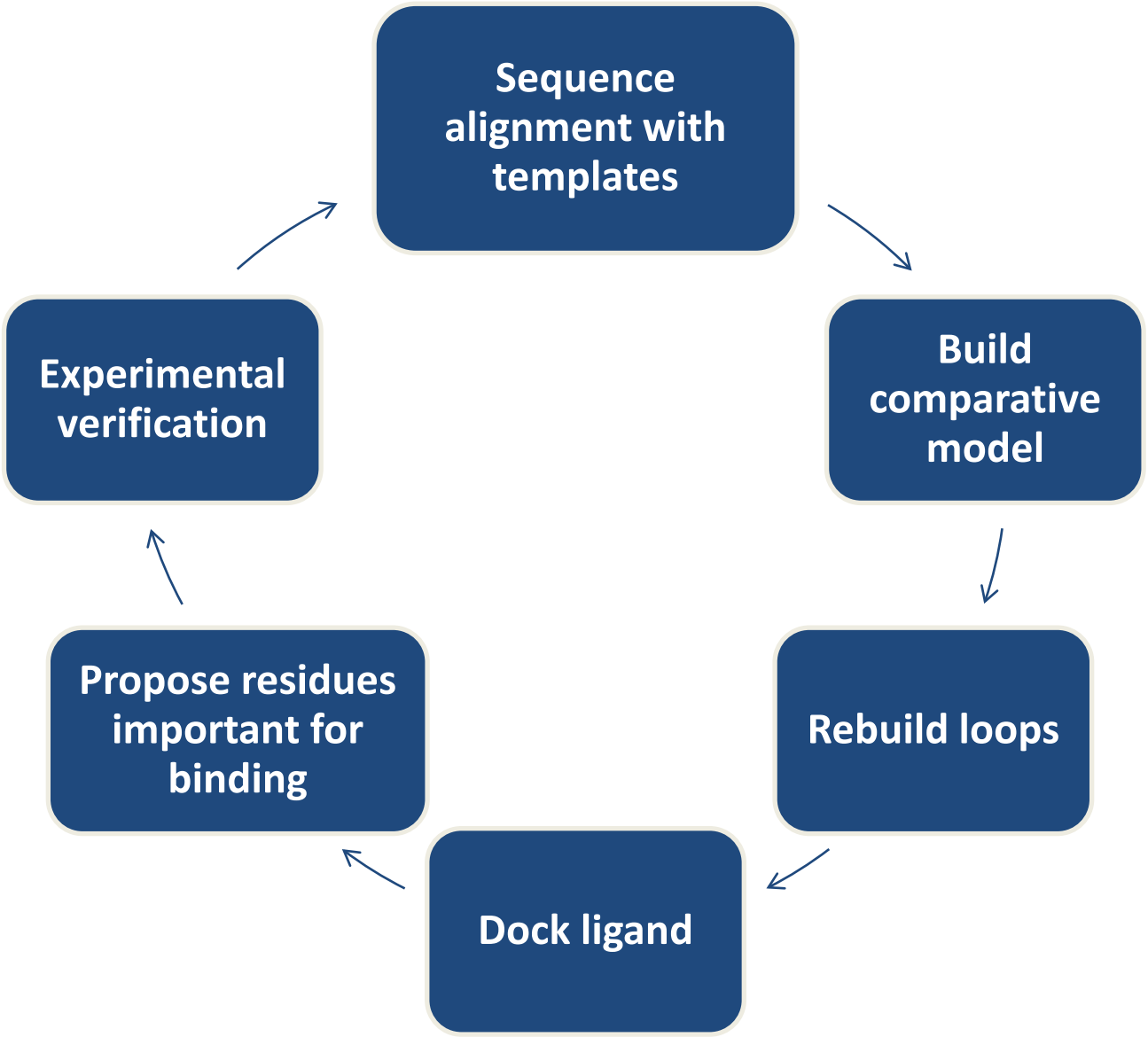
1. membrane protein

-> use the Rosetta membrane potential

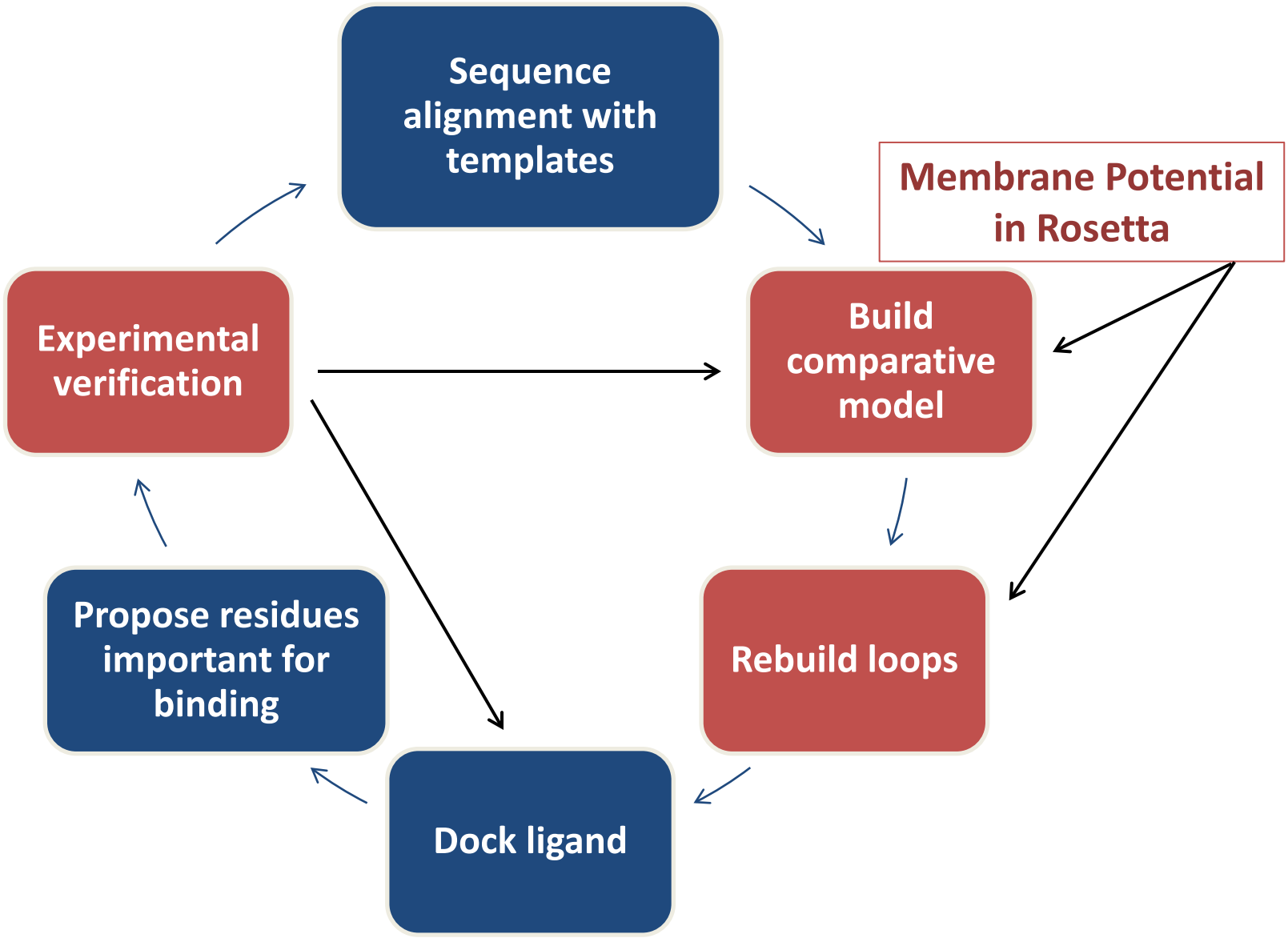
2. low sequence identity between GPCR classes (<20%)

-> couple modeling with experimental studies

Iterative comparative modeling with experimental validation



Iterative comparative modeling with experimental validation



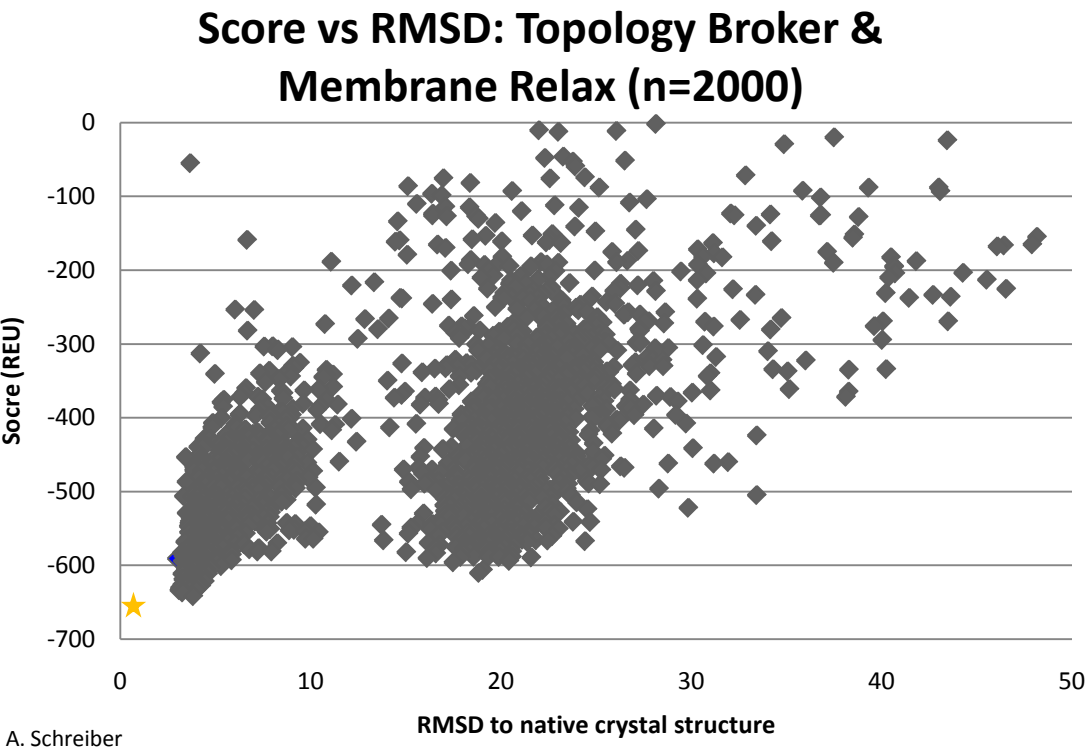
Topology Broker can be used to rebuild flexible regions

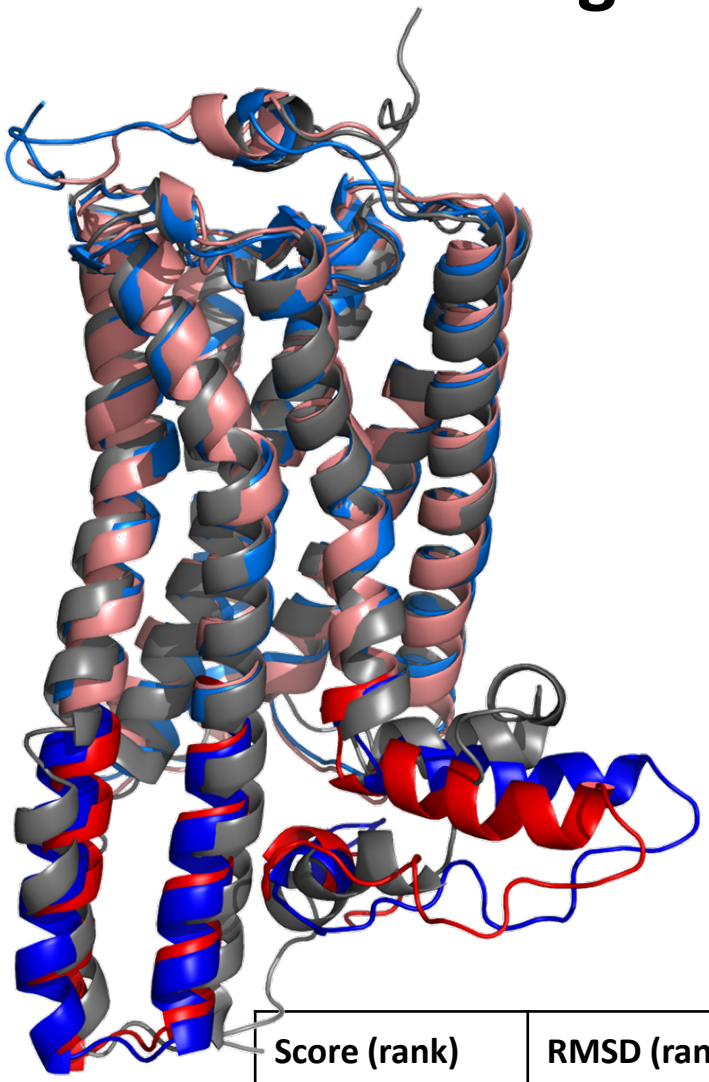
Benchmarking Topology Broker with Squid Rhodopsin:

-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

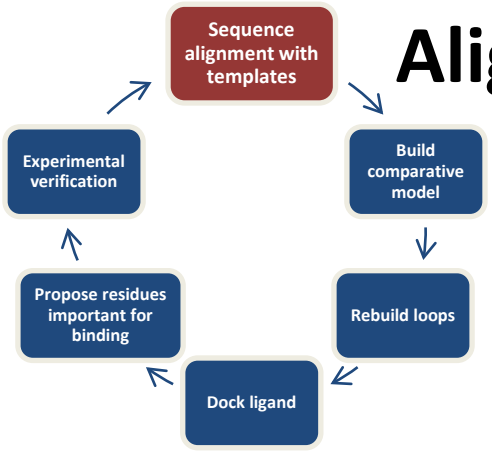




	Score (rank)	RMSD (rank)
relax	-641.7 (1)	3.843Å (95)
rebuild + relax		
relax	-590.76 (146)	2.981Å (1)
rebuild + relax		

A. Schreiber

Align mGlu5 sequence with GPCR templates



Class A GPCRs:

B2adrengeric
Rhodopsin
A2a adenosine

Class C GPCRs:

mGluR1-8

Mustang (structure to structure)

ClustalW (sequence to sequence)

Transmembrane Span 7

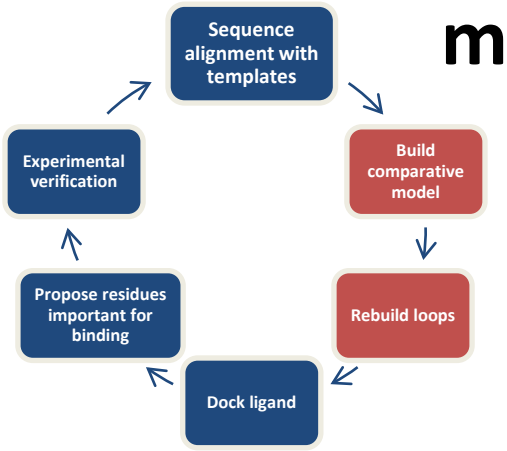
original alignment

```
mGluR5      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
mGluR1      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

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 S P . D F   300
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 M N K Q F   313
```

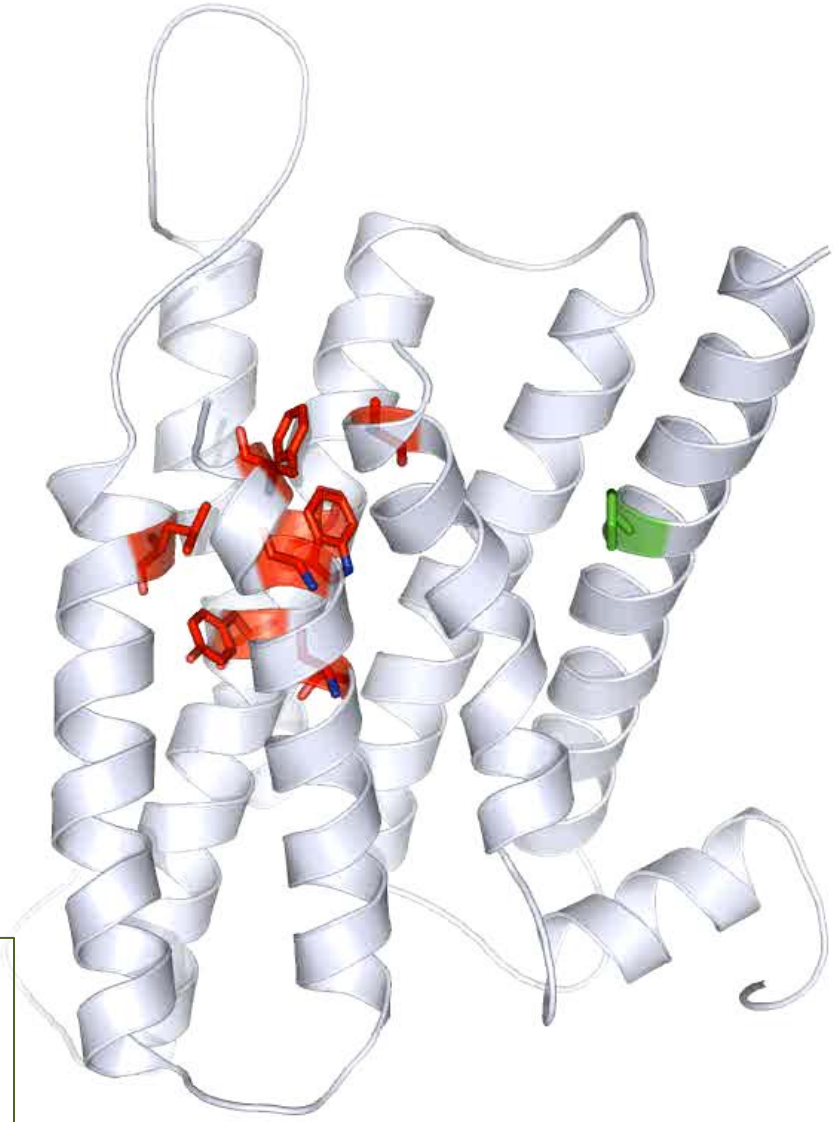
new alignment

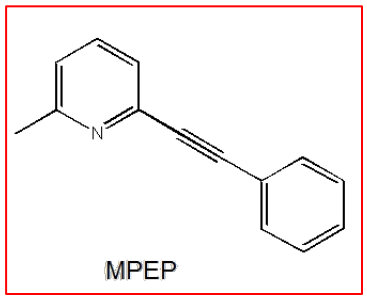
```
mGluR5                  M C F S V S L S A T V A L G C M F V P K V Y I I   825
mGluR1                  T C F A V S L S V T V A L G C M F T P K M Y I I   838

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
```

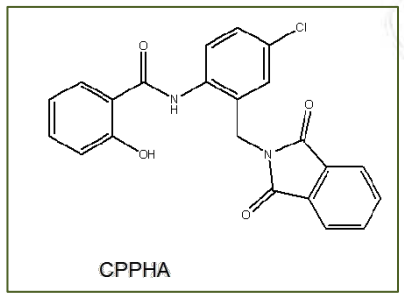
mGlu5 model clusters important residues into a common binding site



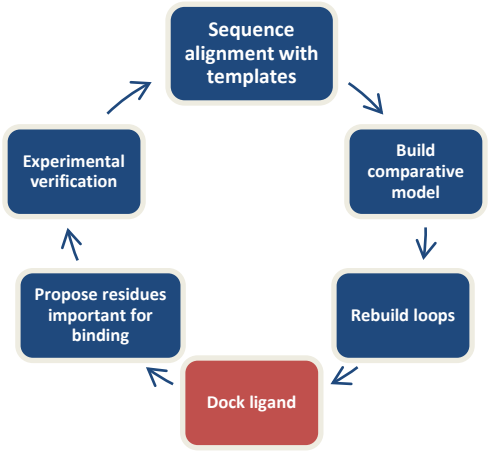
	Critical residues for MPEP binding: *TM3: P654, S657, Y658* *TM6: W784, F787* *TM7: A809*
	Critical residues for CPPHA function: *TM1: F585*

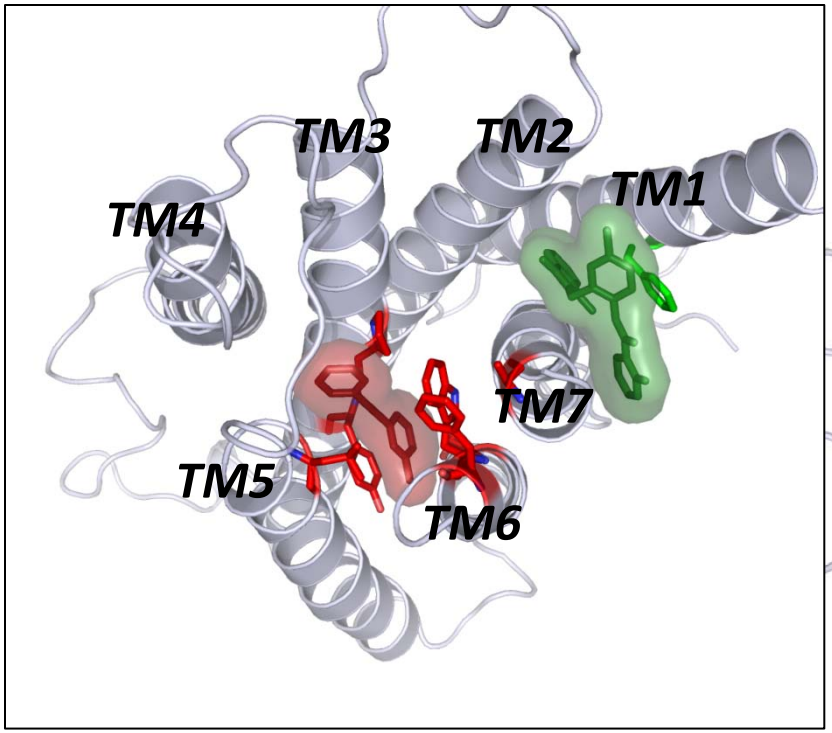


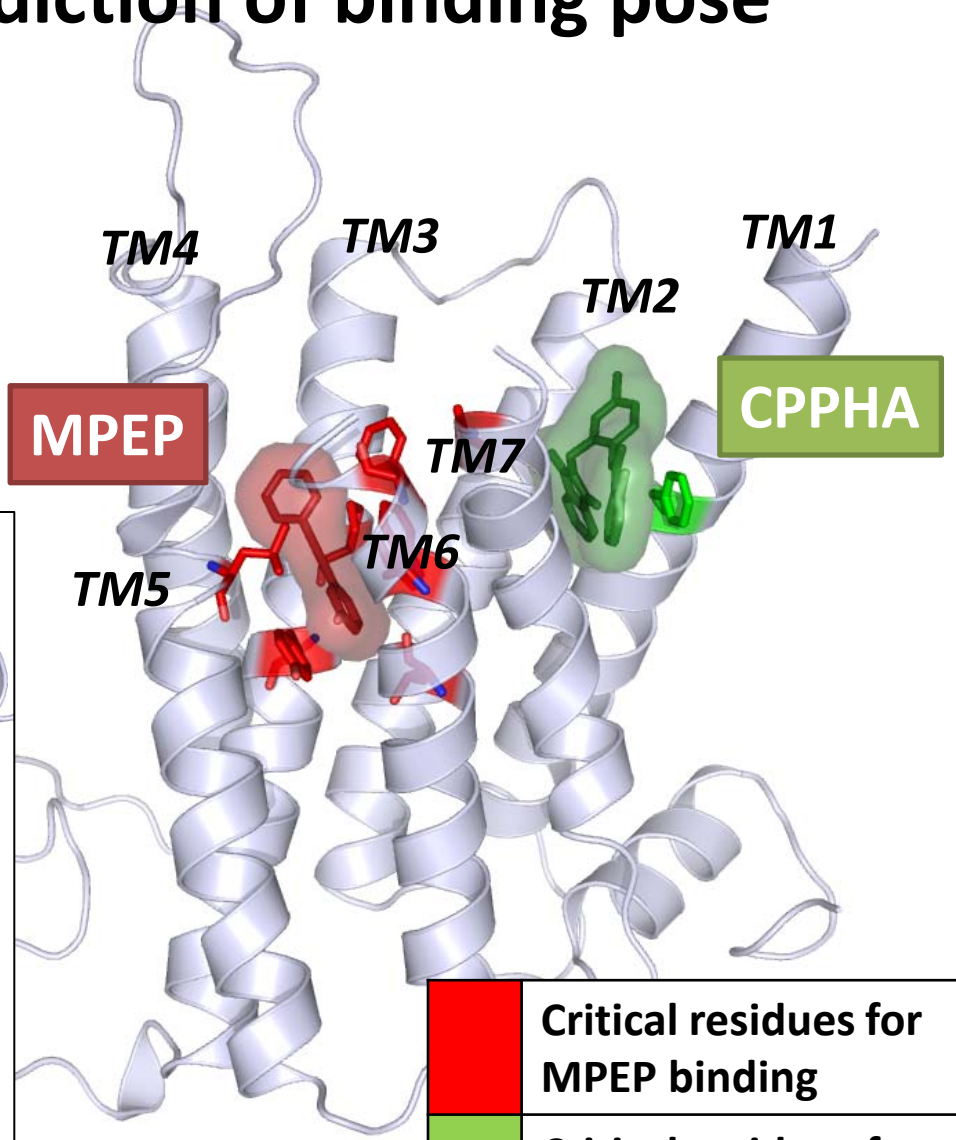




Docking allosteric modulators allows prediction of binding pose

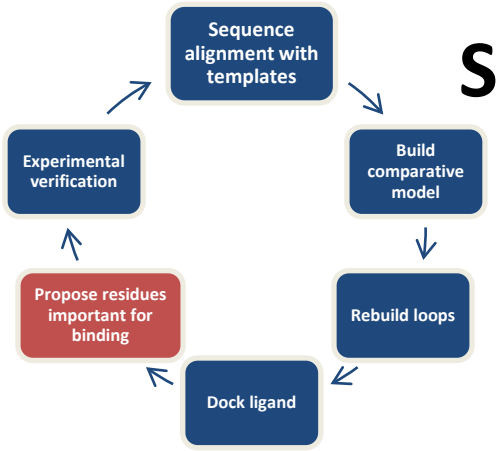






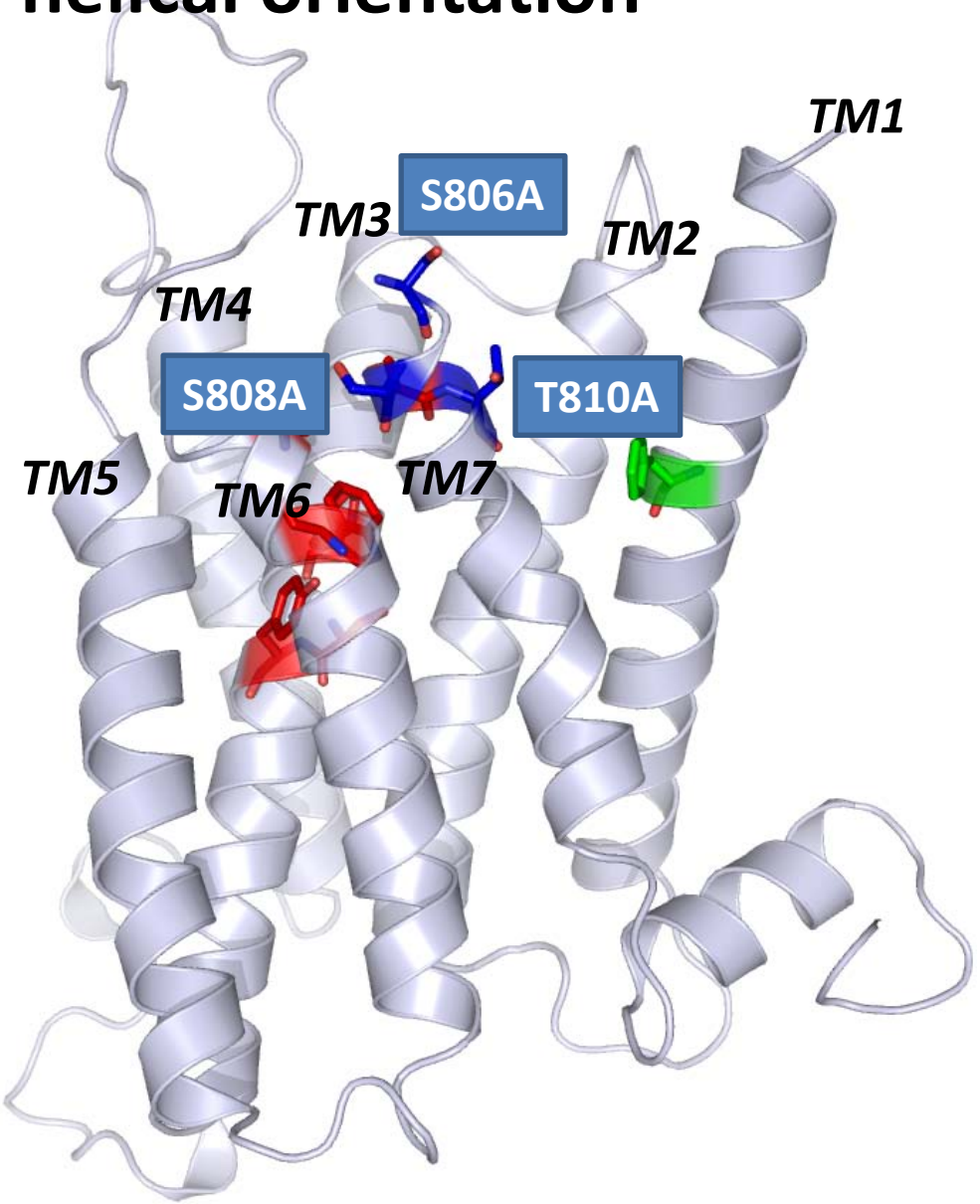
	Critical residues for MPEP binding
	Critical residues for CPPHA function

Site-directed mutagenesis studies validate helical orientation

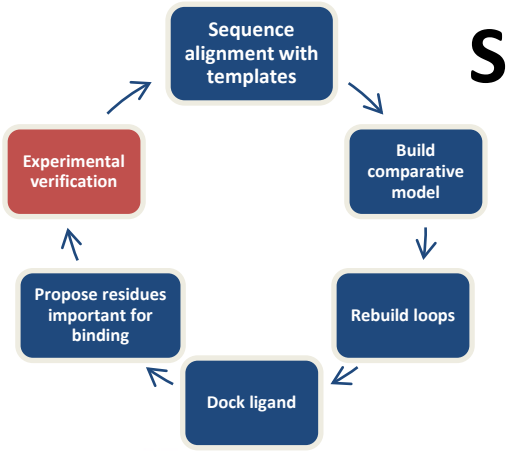


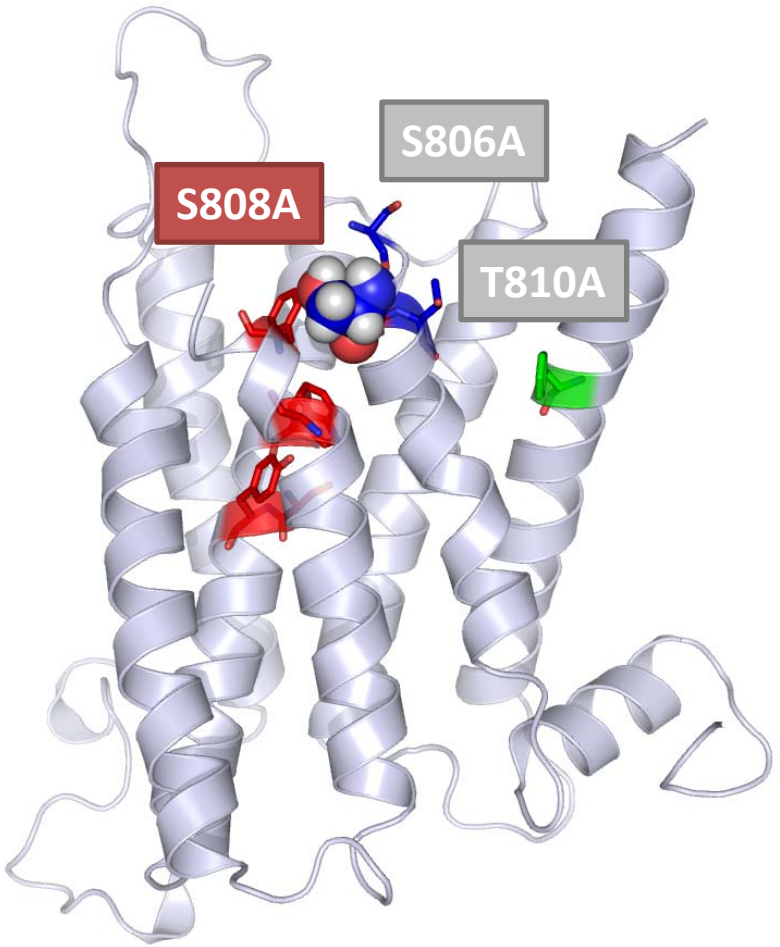
Goal of experimental validation:
-verify orientation of TM7 resulting from PxxY alignment

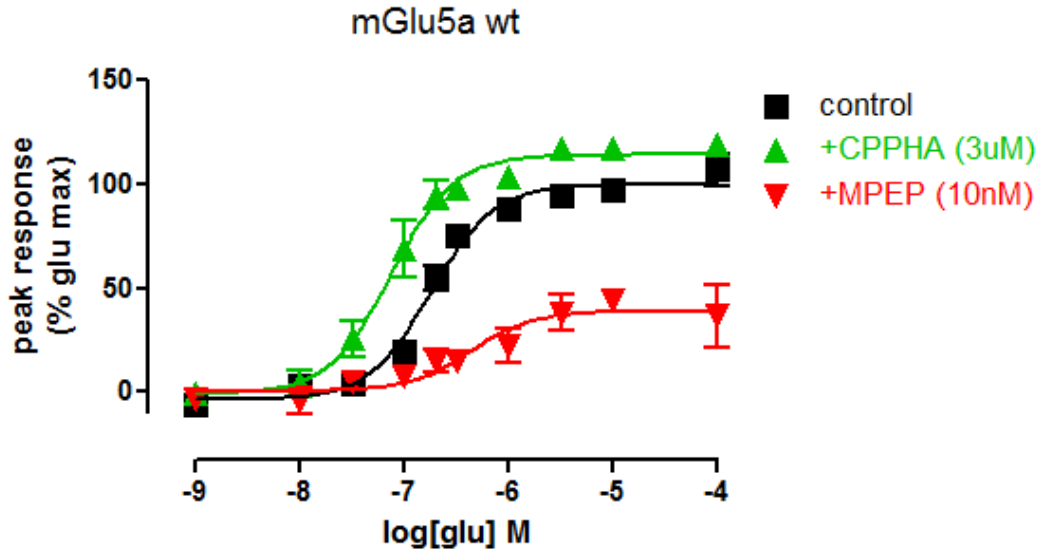
	Critical residues for MPEP binding
	Critical residues for CPPHA function
	Residues proposed for site-directed mutagenesis

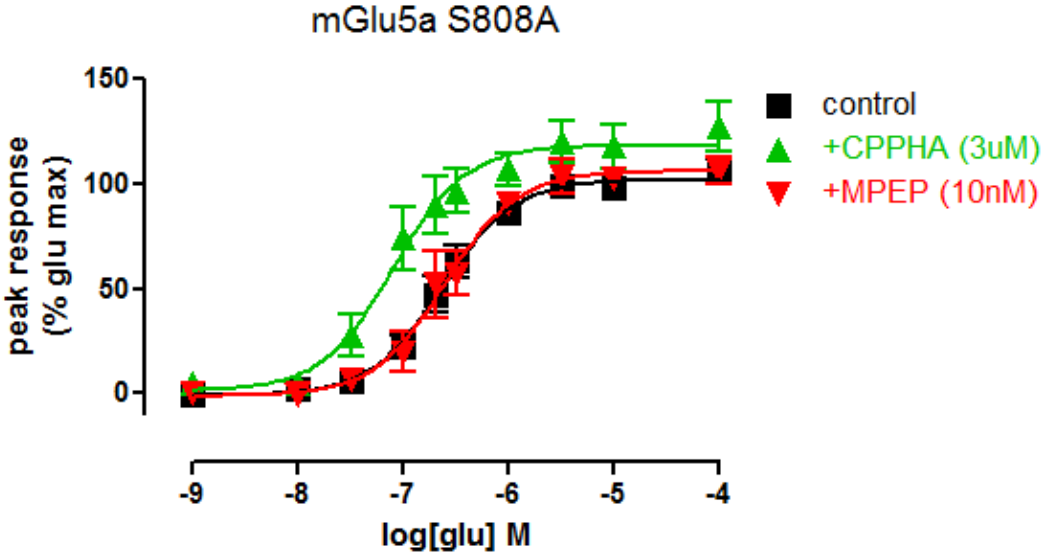


S808A is a functionally important residue for MPEP

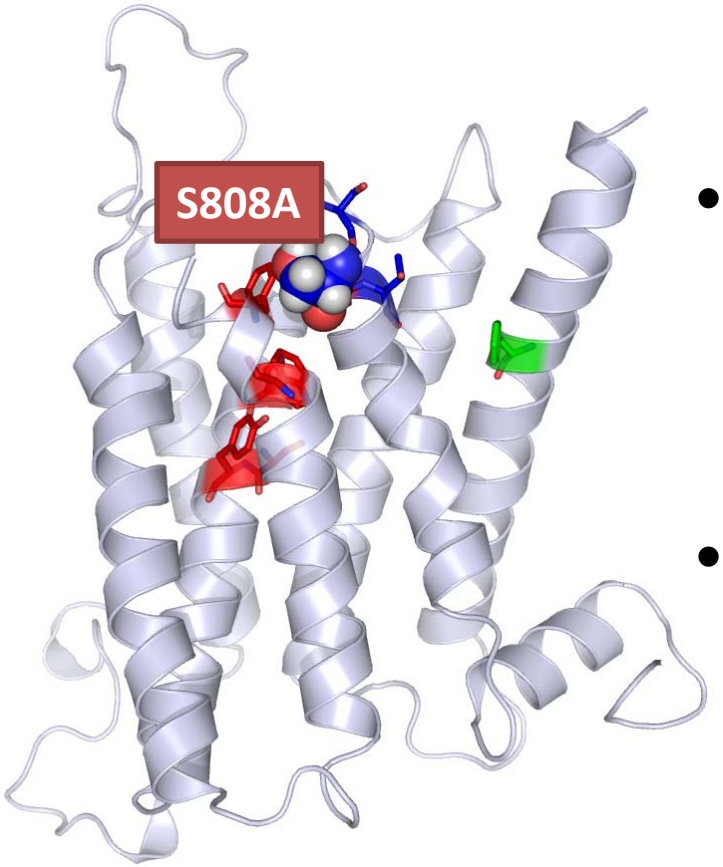








Conclusions



- 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

Jens Meiler
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Anette Schreiber
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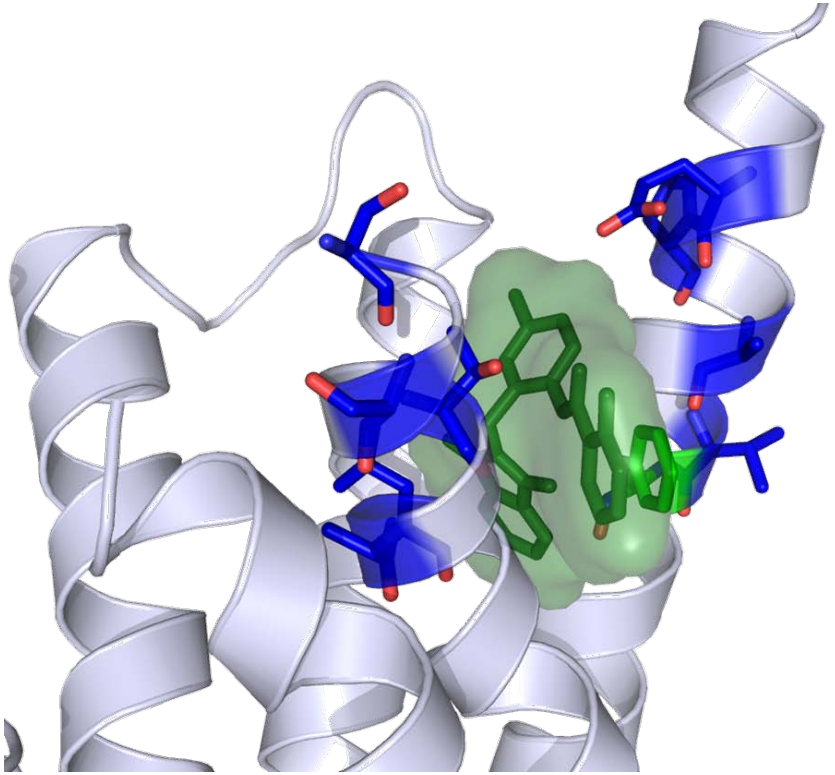
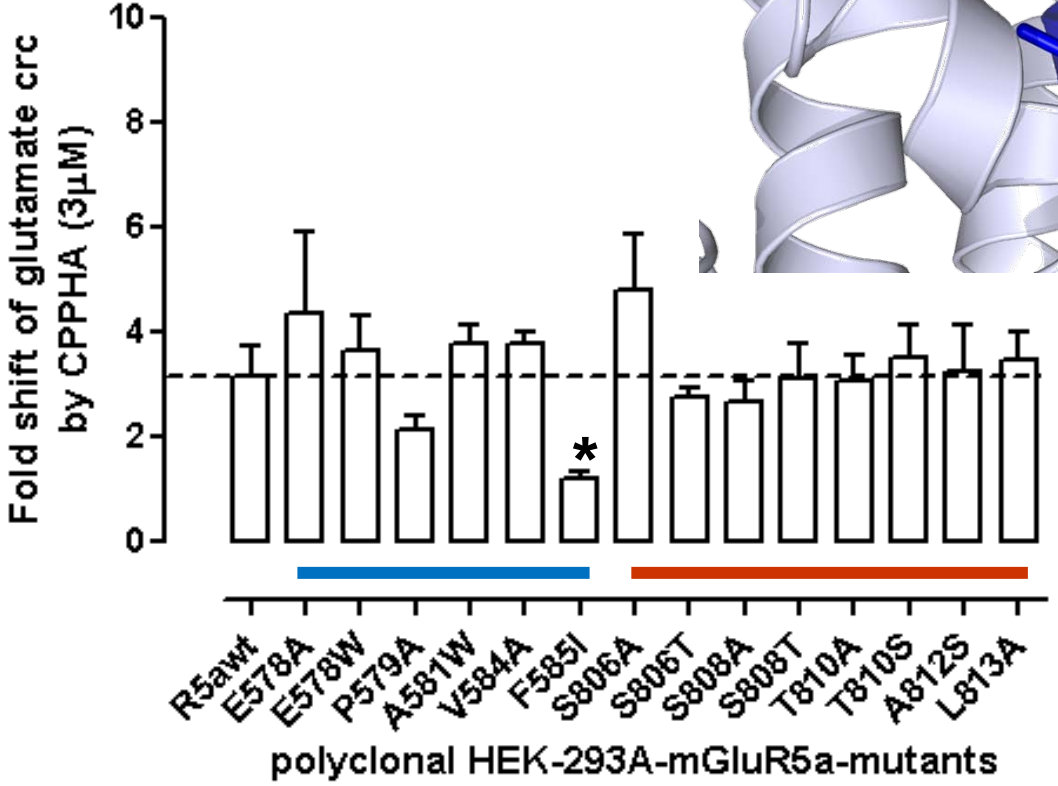
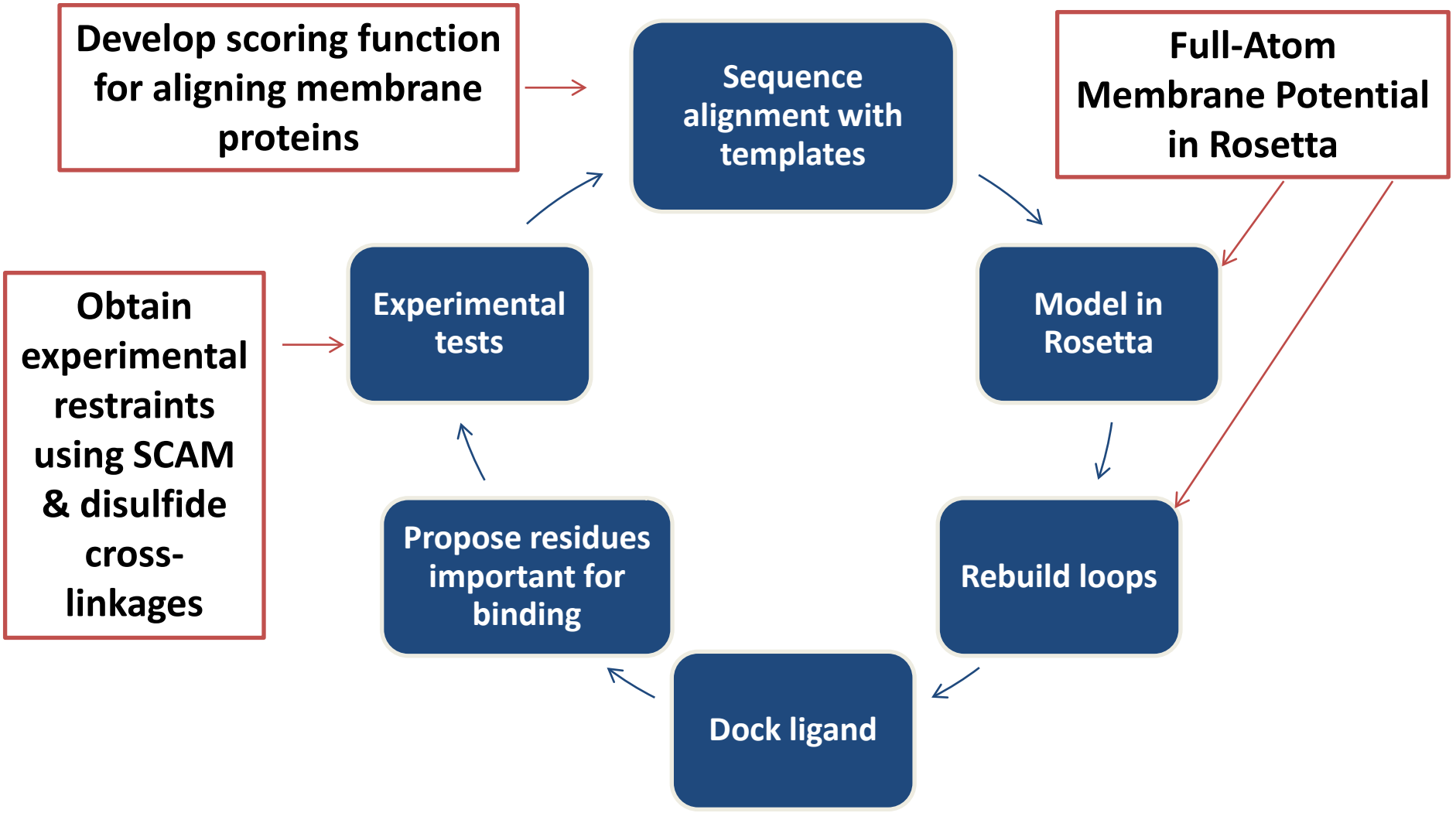
CPPHA




Fold shift of glutamate crc by CPPHA (3µM)
10
8
6
4
2
0
*
R5awt
E578A
E578W
P579A
A581W
V584A
F585I
S806A
S806T
S808A
S808T
T810A
T810S
A812S
L813A
polyclonal HEK-293A-mGluR5a-mutants

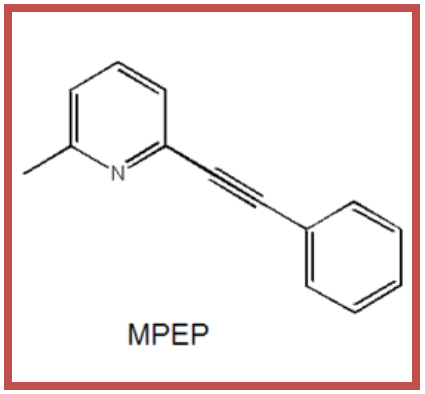
Future Directions

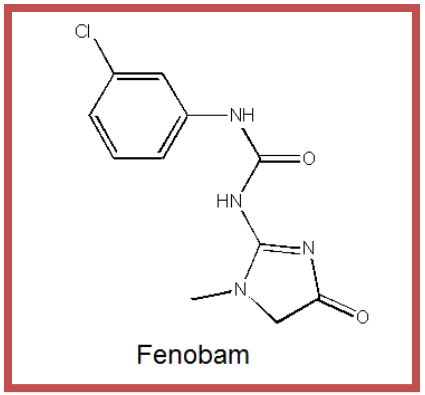


Allosteric modulators of $mGlu_5$ have therapeutic value

mGluR5 NAMs:

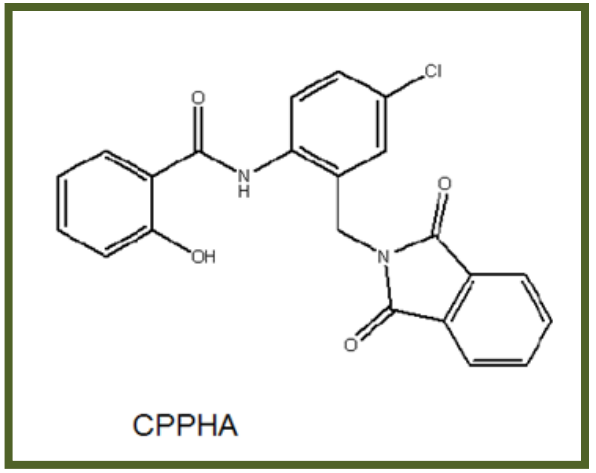
Anxiety, Fragile X Syndrome, chronic pain, depression, migraine, Parkinson's disease levodopa-induced dyskinesia

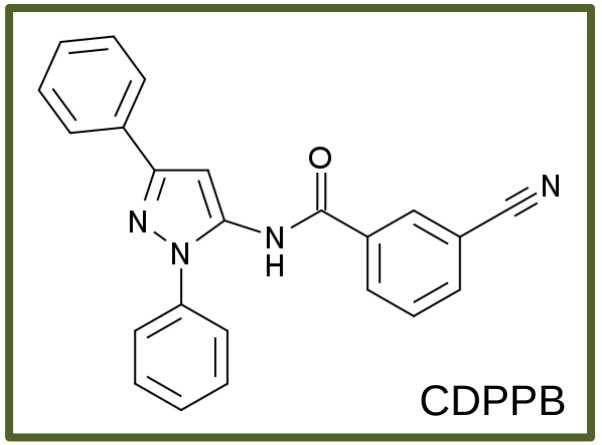




mGluR5 PAMs:

Schizophrenia, cognition disorders

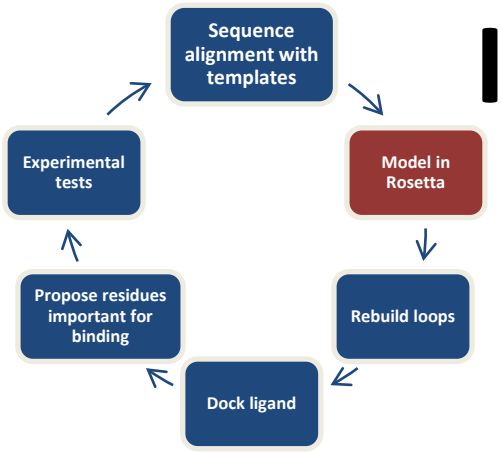




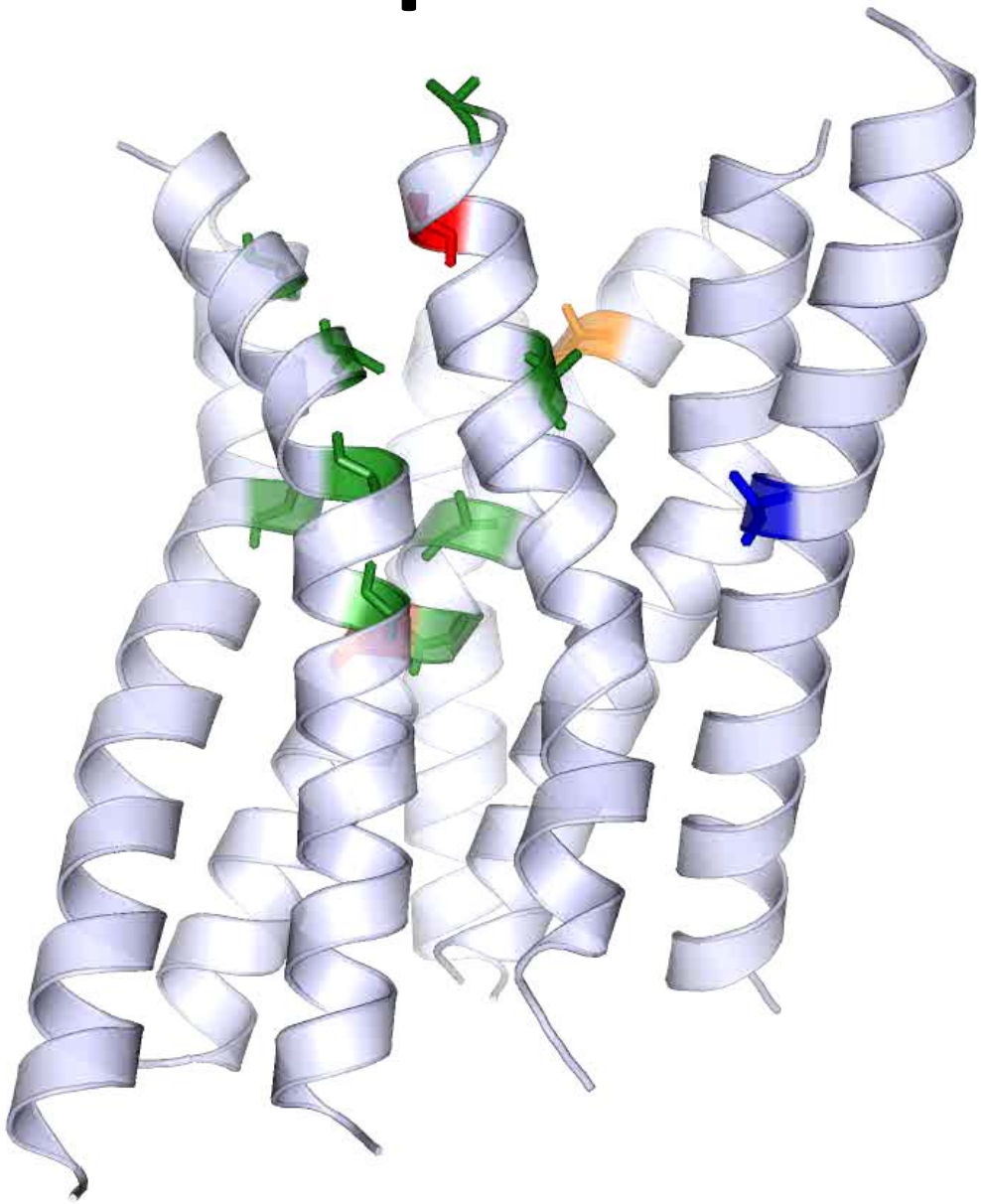
Current treatments for schizophrenia have negative side effects

- *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

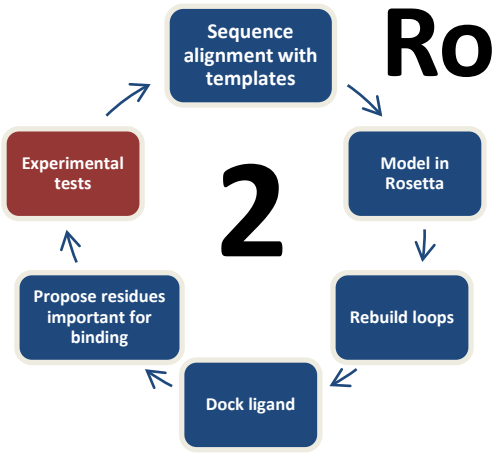
Initial Comparative Model

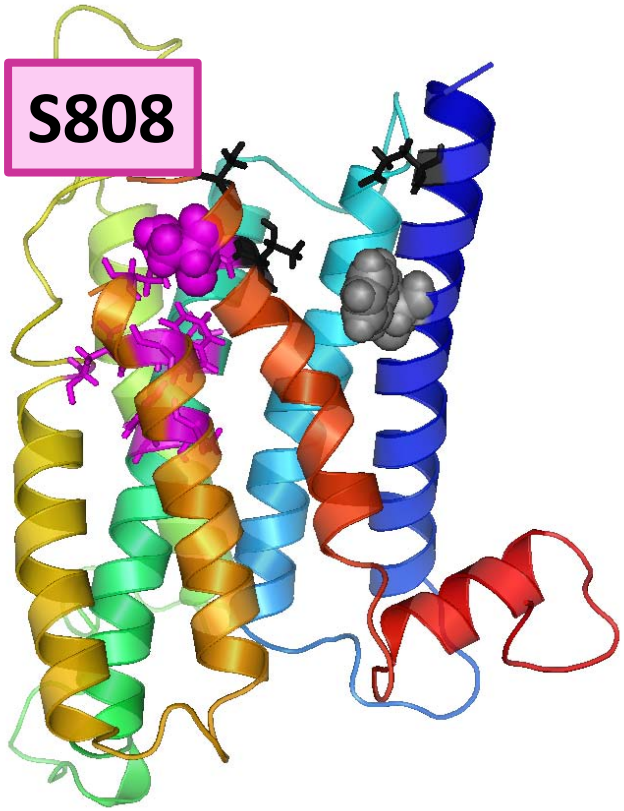


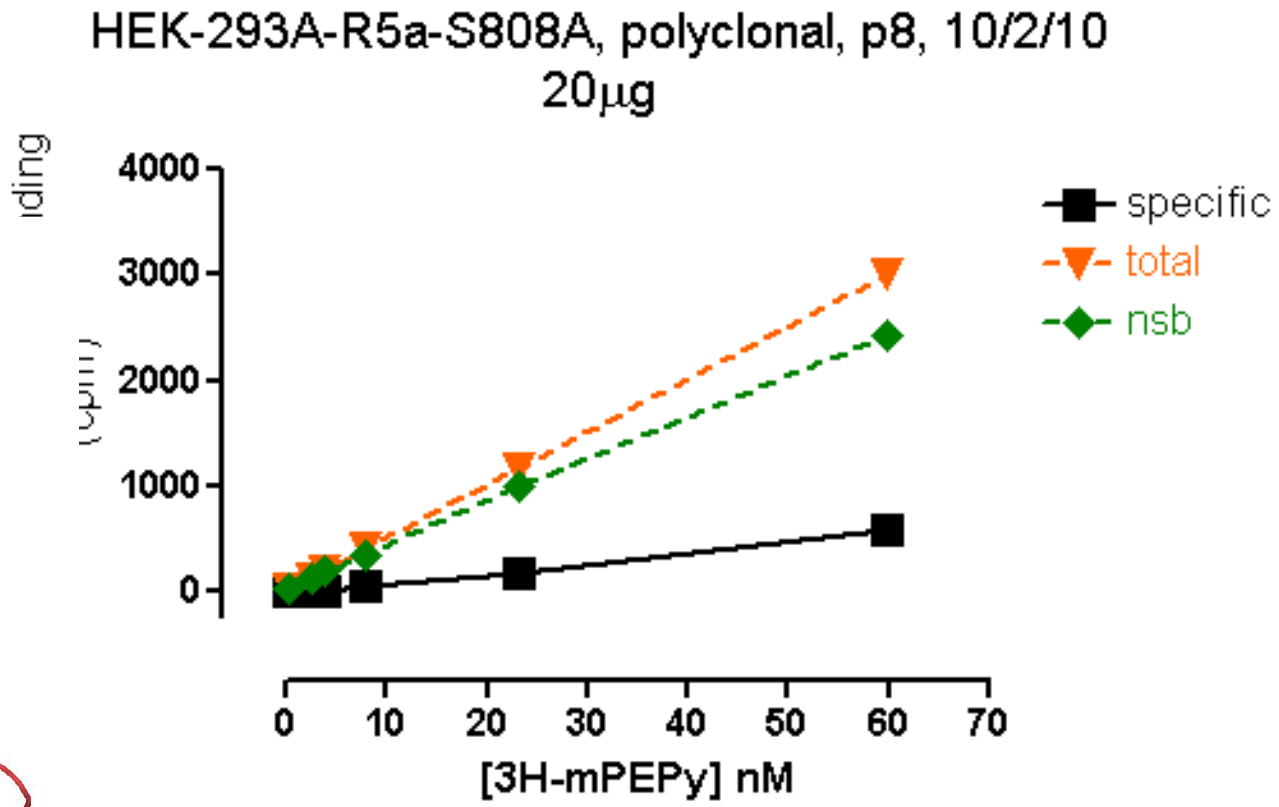
	MPEP Binding
	MPEP function
	CPPHA function



Round 2: Experimental validation

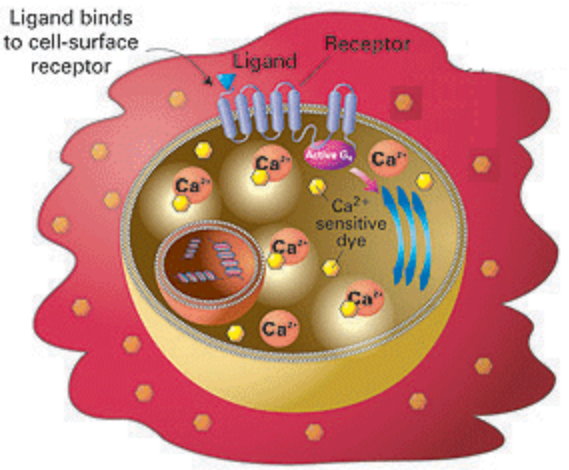




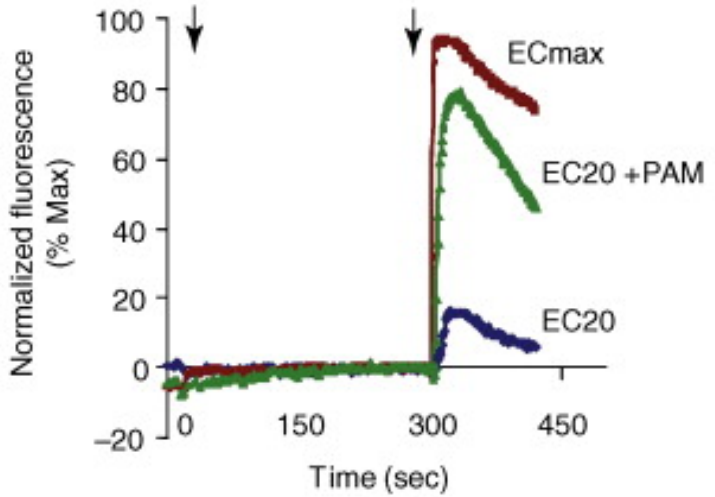


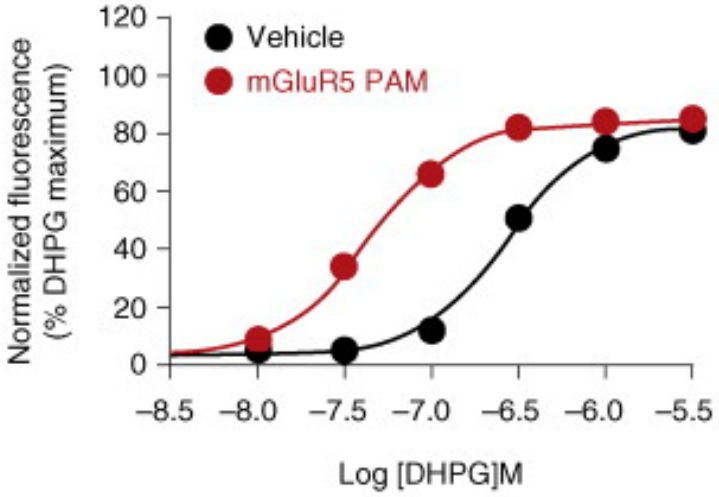
Using calcium mobilization as a functional assay

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 Ca^{2+} can be detected by FLIPR or FlexStation microplate readers using calcium-sensitive dye indicators

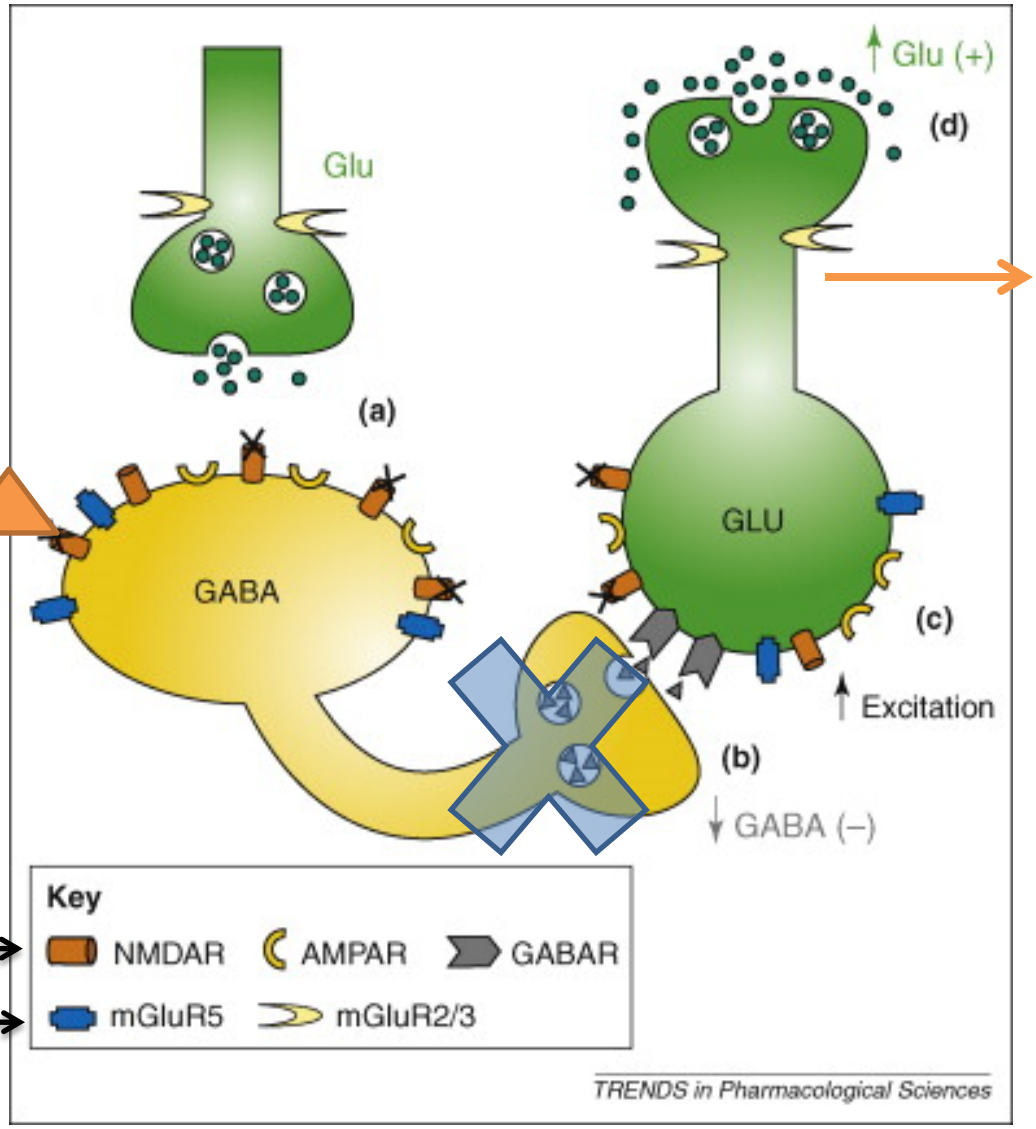




Conn, P. J. et al (2009). *Trends in Pharmacological Sciences*, *30*(1), 25-31.

http://www.moleculardevices.com/pages/reagents/cal4_kit.html

NMDA antagonists produce symptoms of schizophrenia



Positive and negative symptoms of schizophrenia

Poor cognitive function

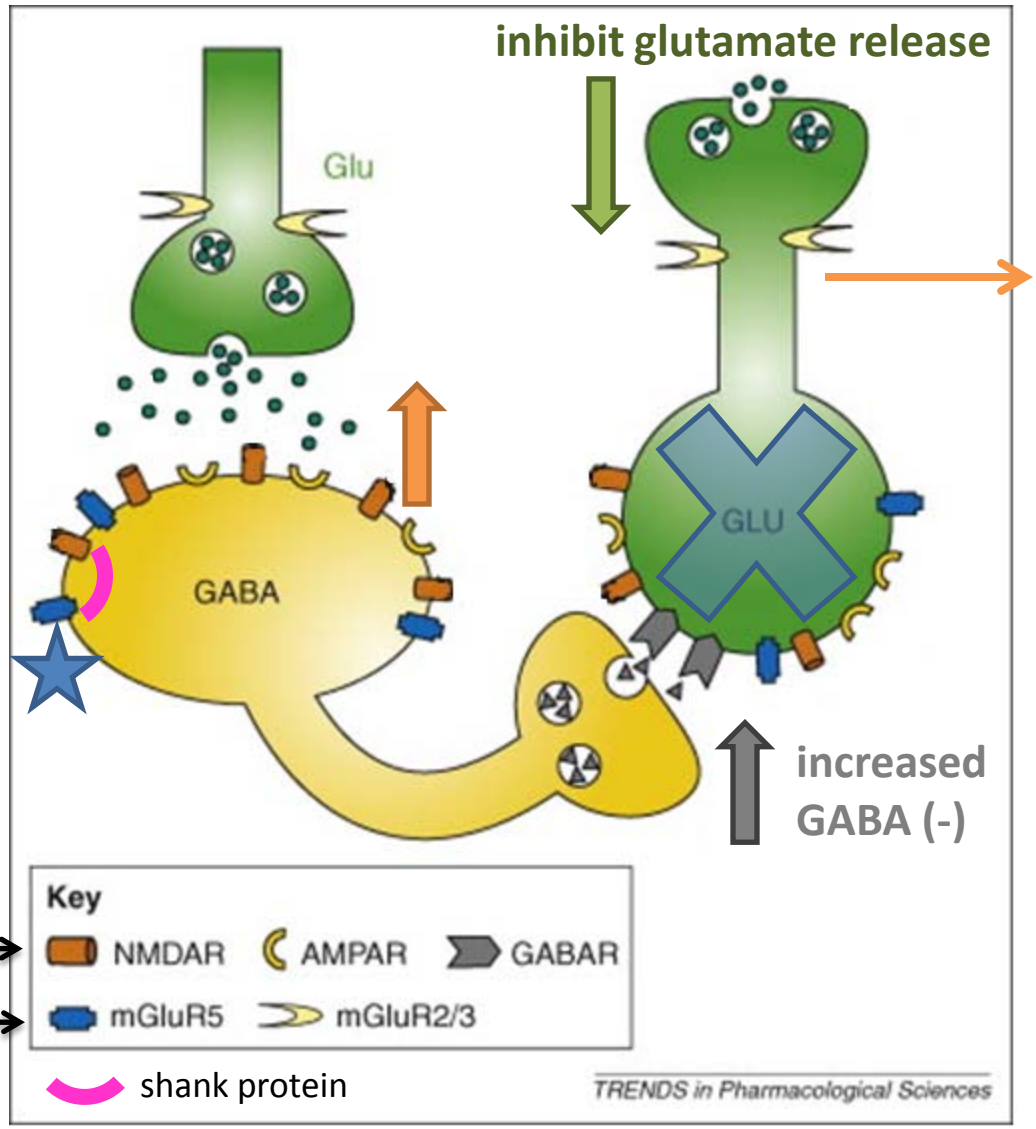
PCP or ketamine

ionotropic

metabotropic

Conn, P. J. et al (2009). *Trends in Pharmacological Sciences*, *30*(1), 25-31.

Activating mGluR5 can reduce symptoms of schizophrenia



Positive and negative symptoms of schizophrenia

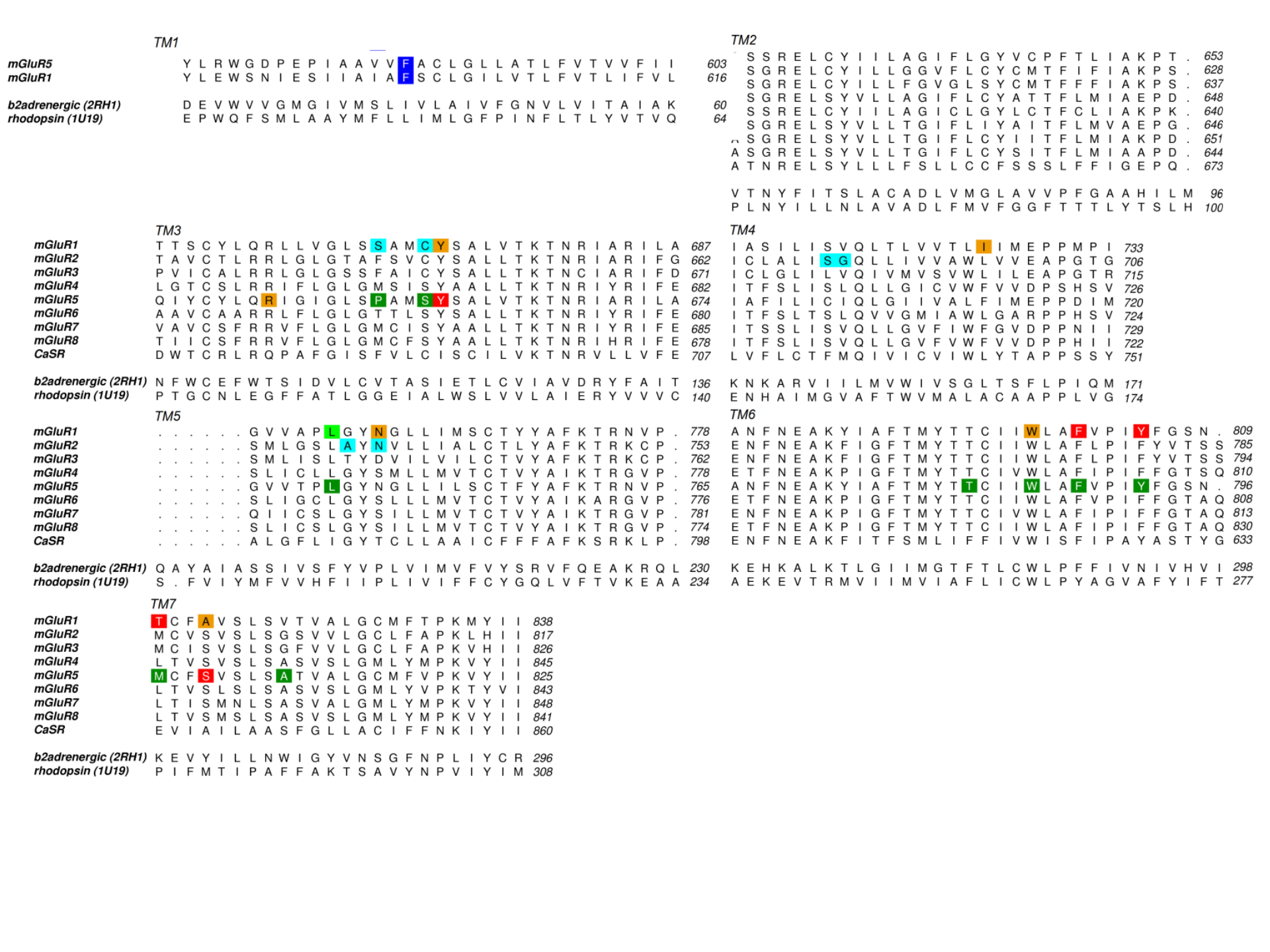
Poor cognitive function

mGluR5 positive modulator

ionotropic

metabotropic

Conn, P. J. et al (2009). *Trends in Pharmacological Sciences*, *30*(1), 25-31.


TM1
TM2
TM3
TM4
TM5
TM6
TM7
mGluR5
mGluR1
mGluR2
mGluR3
mGluR4
mGluR6
mGluR7
mGluR8
CaSR
b2adrenergic (2RH1)
rhodopsin (1U19)