

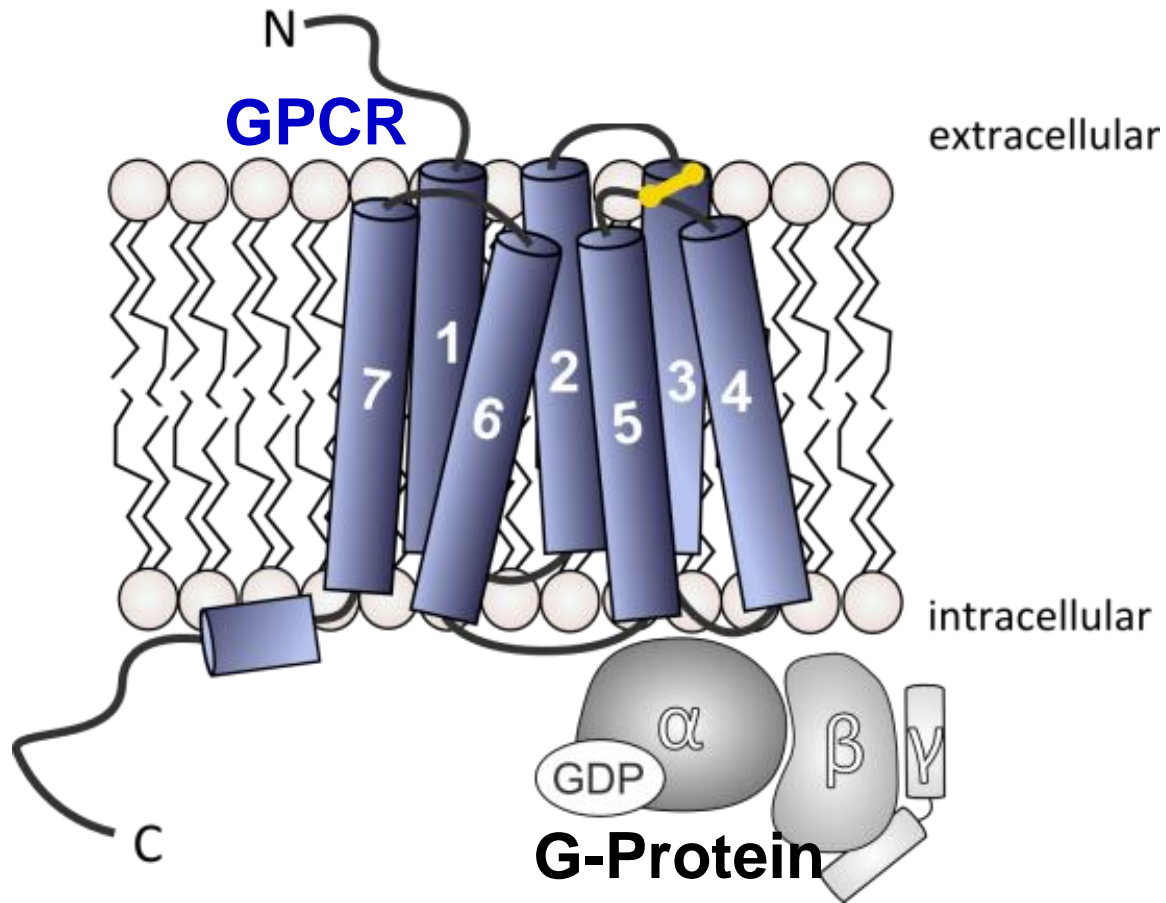
Mapping the binding sites of UDP and prostaglandin E2 glyceryl ester in the nucleotide receptor P2Y6



Oanh Vu
Meiler lab

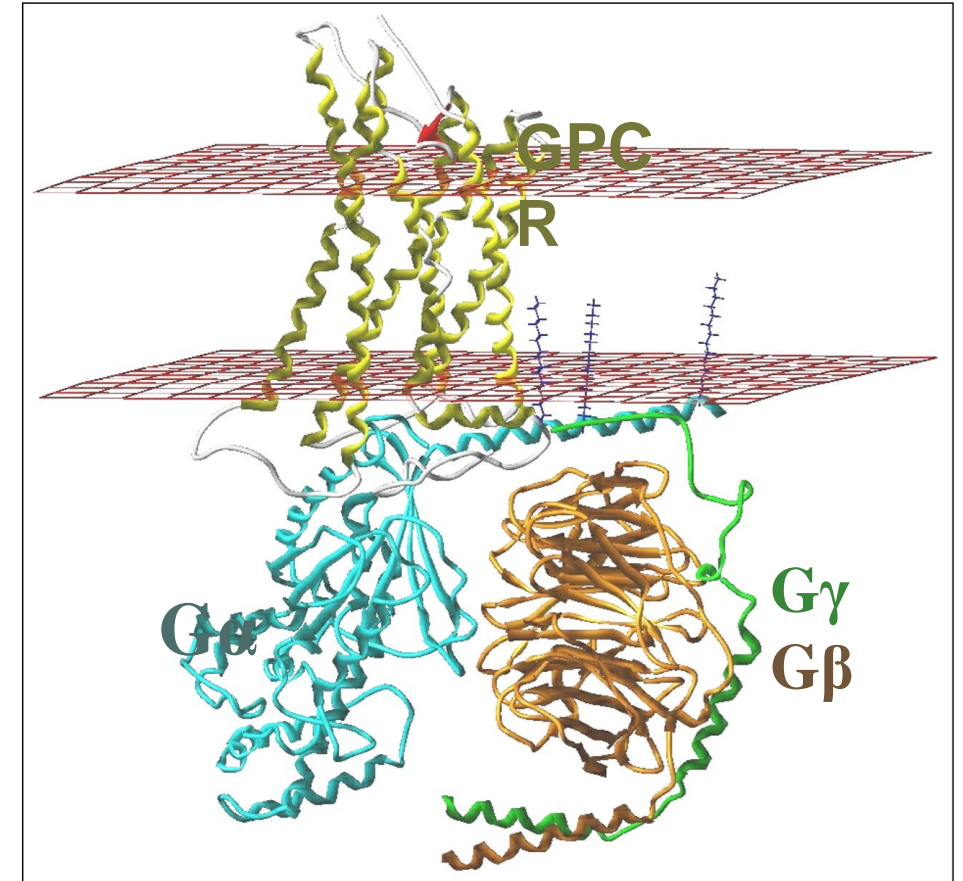
G protein coupled receptors

Schematic structure of a GPCR



<https://biophysik.medizin.uni-leipzig.de/>

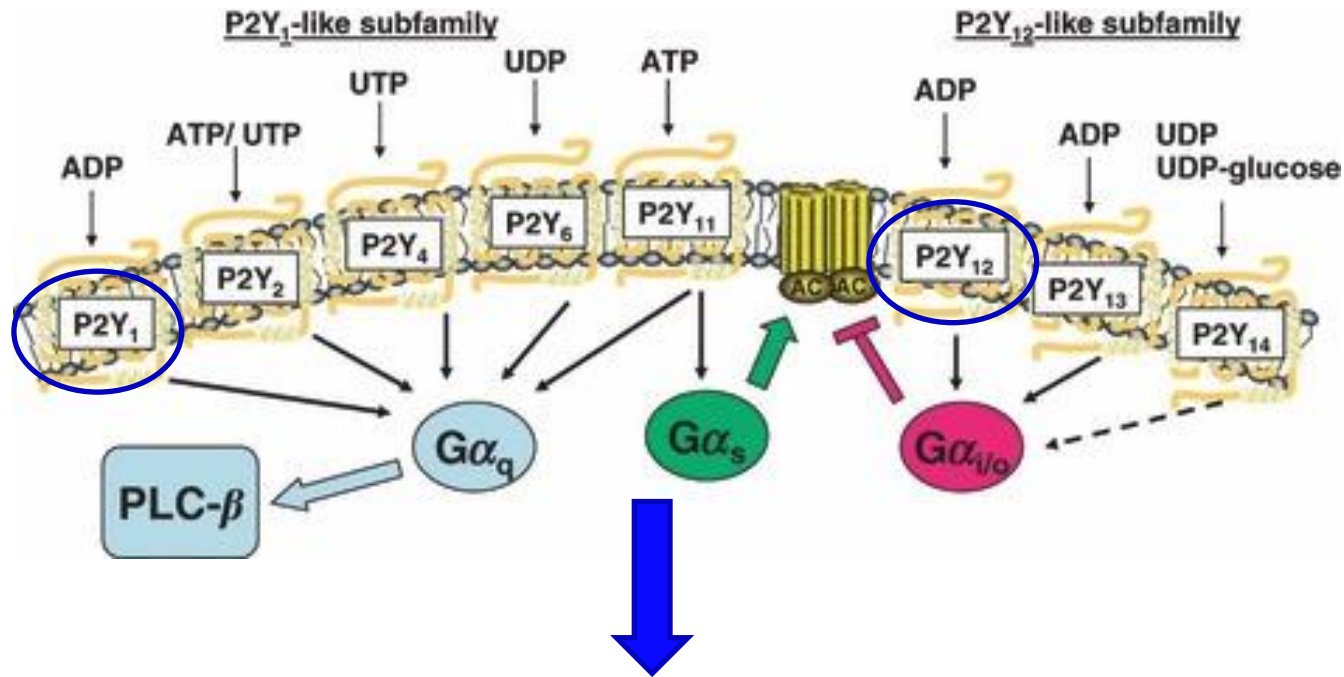
Atomic structure of a GPCR



Heng et al. (2013). *Biotechnology advances*, 31 8, 1676-94.



Metabotropic pyrimidine and purine nucleotide receptors (P2Y receptors)

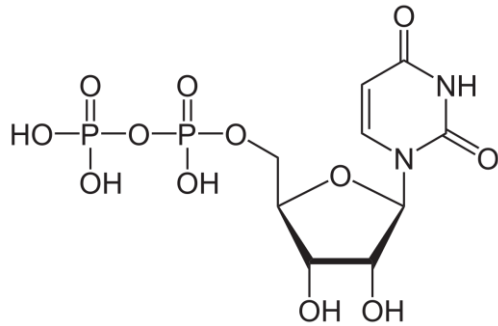


Platelet aggregation,
immune regulation and
inflammation.

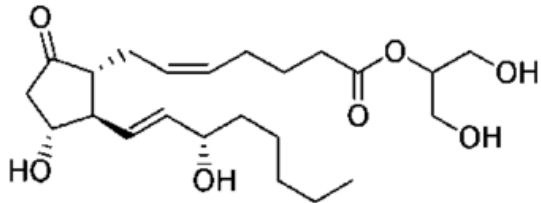
Huang, Z., Xie, N., Illes, P. *et al. Sig Transduct Target Ther* **6**, 162 (2021).



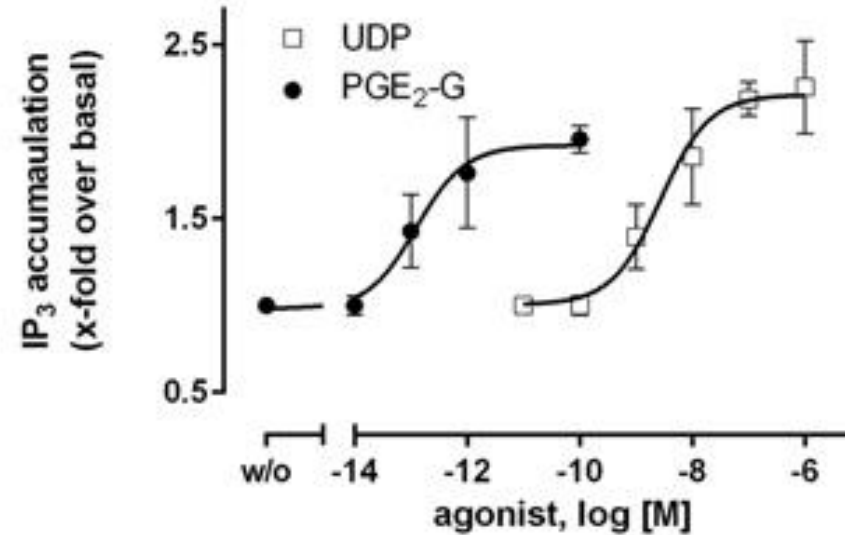
UDP and PGE-2G are native agonists for P2Y6



Uridine diphosphate



Prostaglandin E₂ glyceryl ester

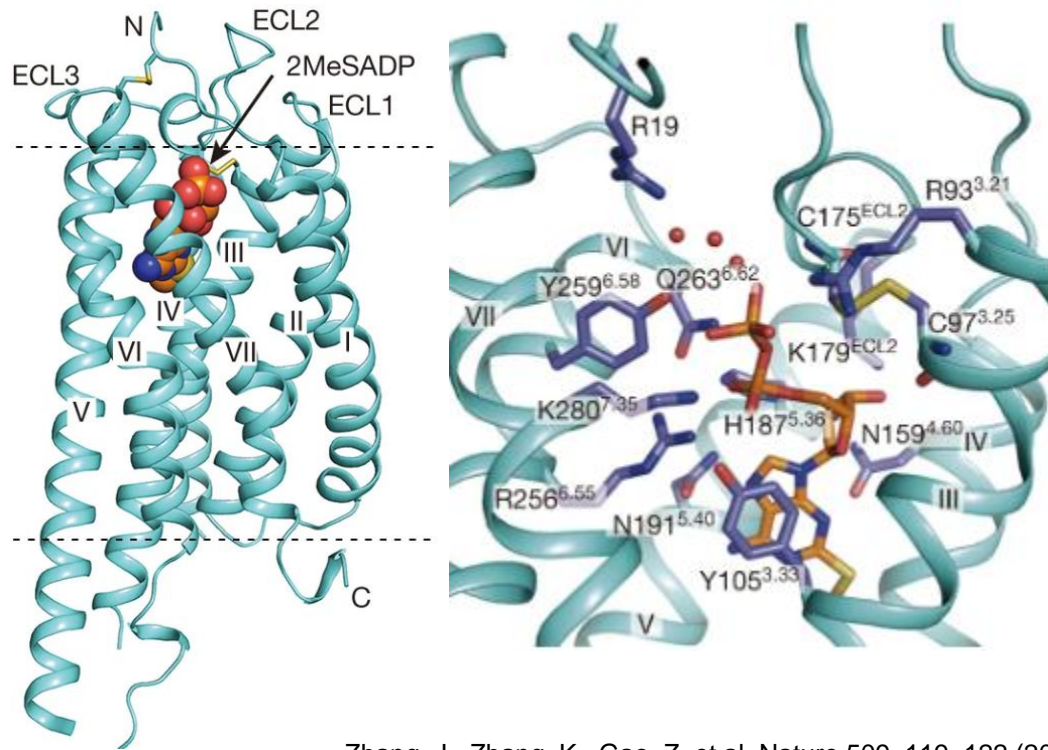


Brüser et al. Sci Rep. 2017; 7: 2380.



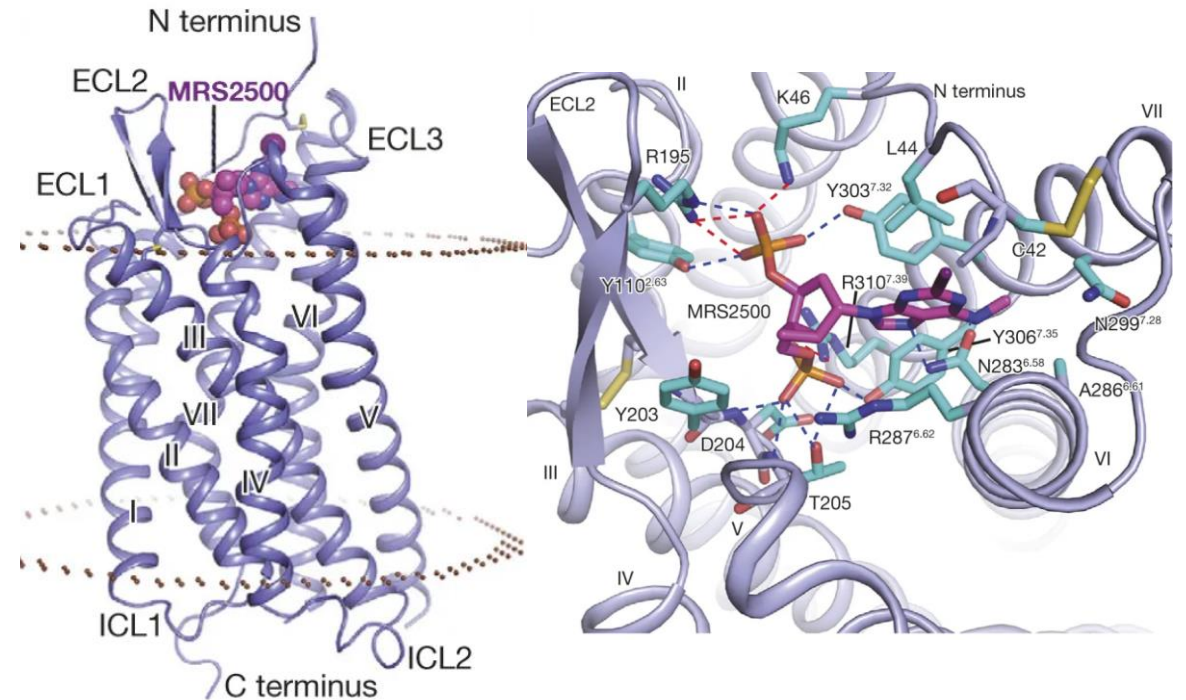
Interaction of P2Y receptors to UDP analogs

P2Y12 and agonists : 2MeSADP and 2MeSATP



Zhang, J., Zhang, K., Gao, Z. et al. *Nature* 509, 119–122 (2014)

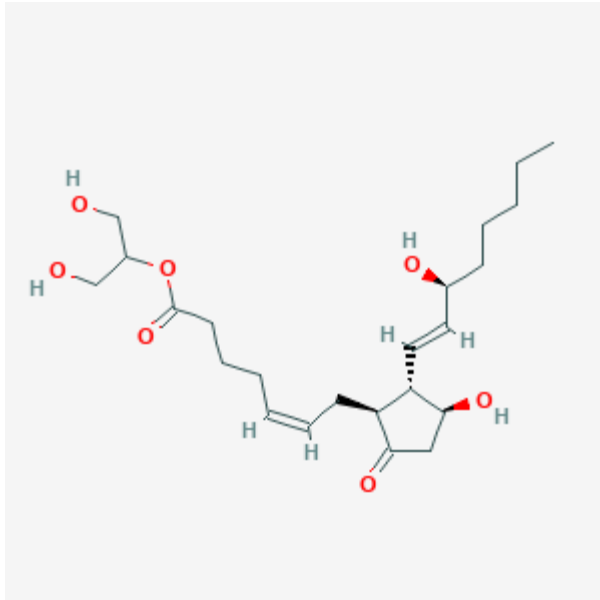
P2Y1 and antagonist: MRS2500



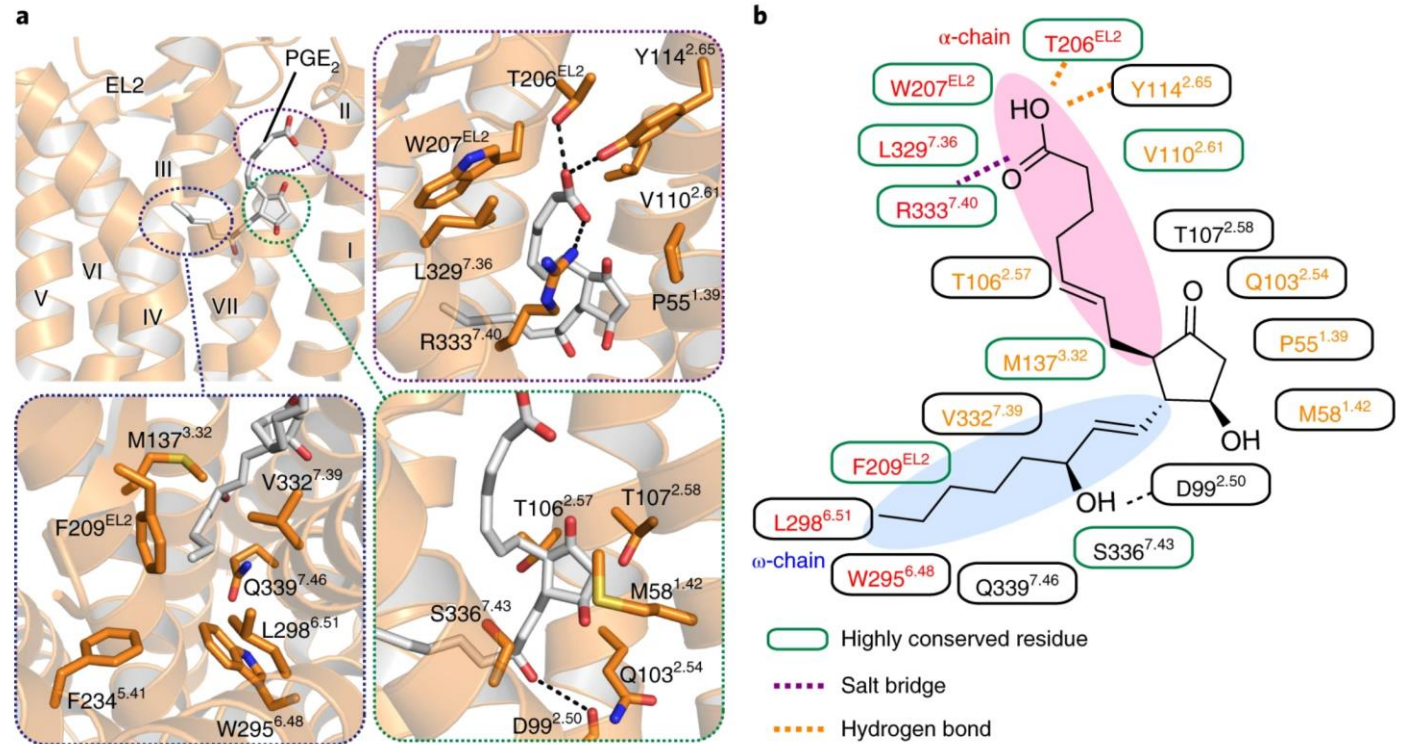
Zhang, D., Gao, Z., Zhang, K. et al. *Nature* 520, 317–321 (2015).



Interactions of EP3 GPCR receptor to PGE-2G analog



PGE-2G



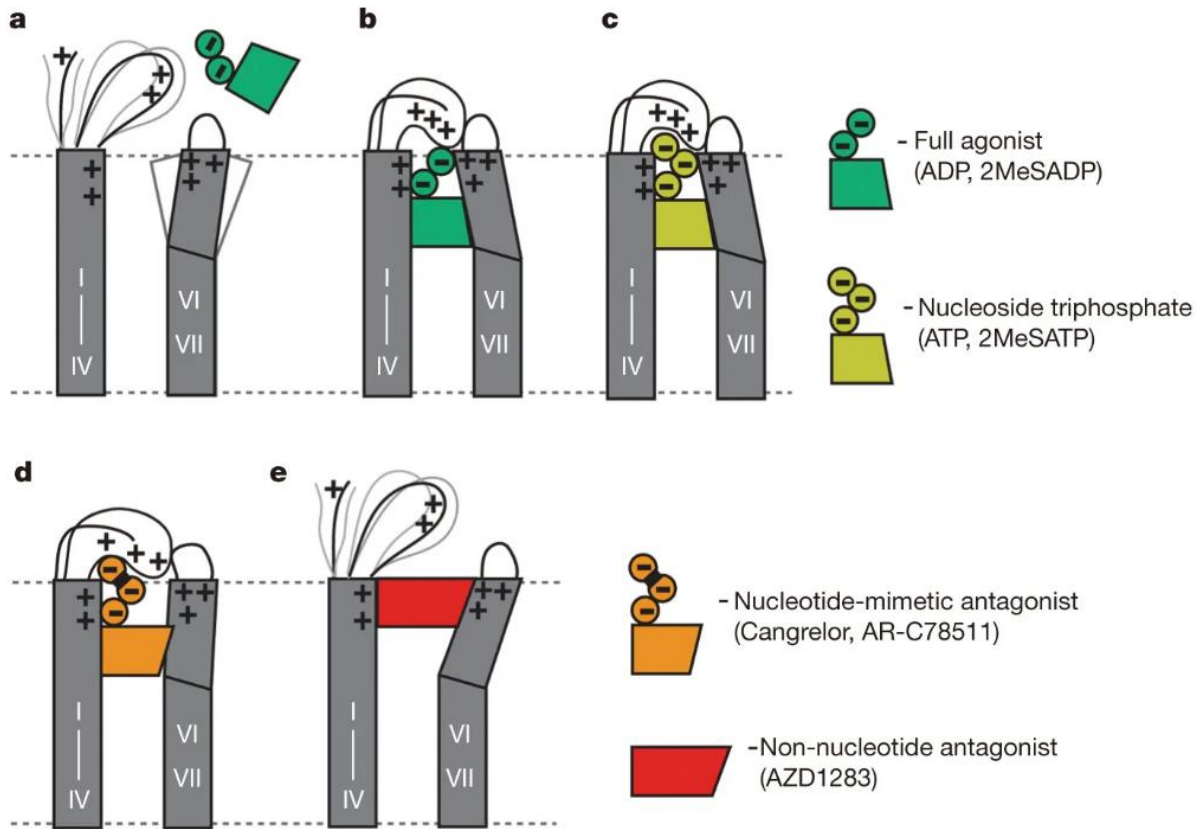
PGE₂ - EP3 co-crystal structure

Morimoto et al. (2019) Nat. Chem. Biol. 15: 8-10



Hypothesis of P2Y receptor activation

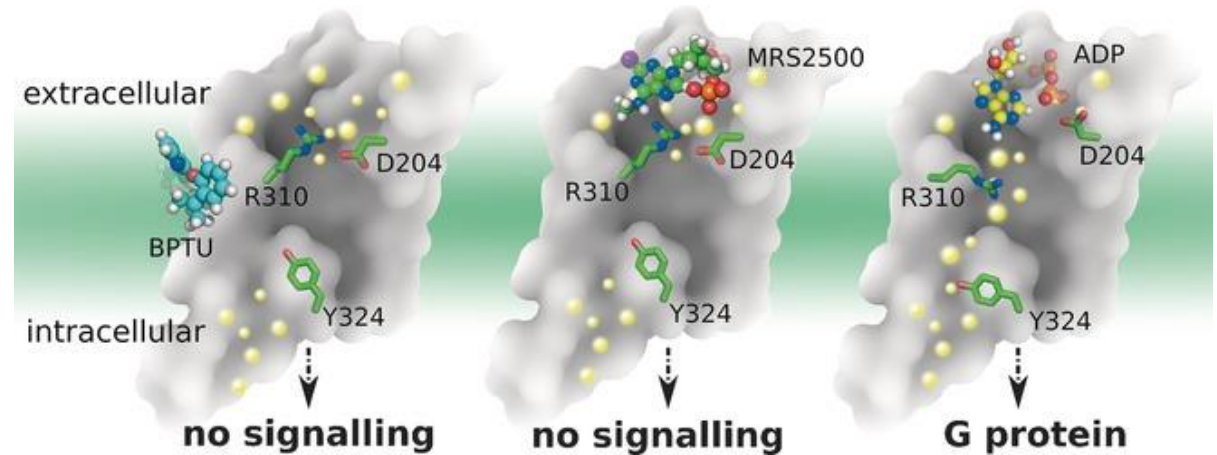
Activation mechanism of P2Y₁₂



Zhang, J., Zhang, K., Gao, Z. et al. Nature 509, 119–122 (2014)

- Conserved across three P2Y receptors:
The binding pockets have an upper half of cluster of basic side chains and lower half of hydrophobic sidechains

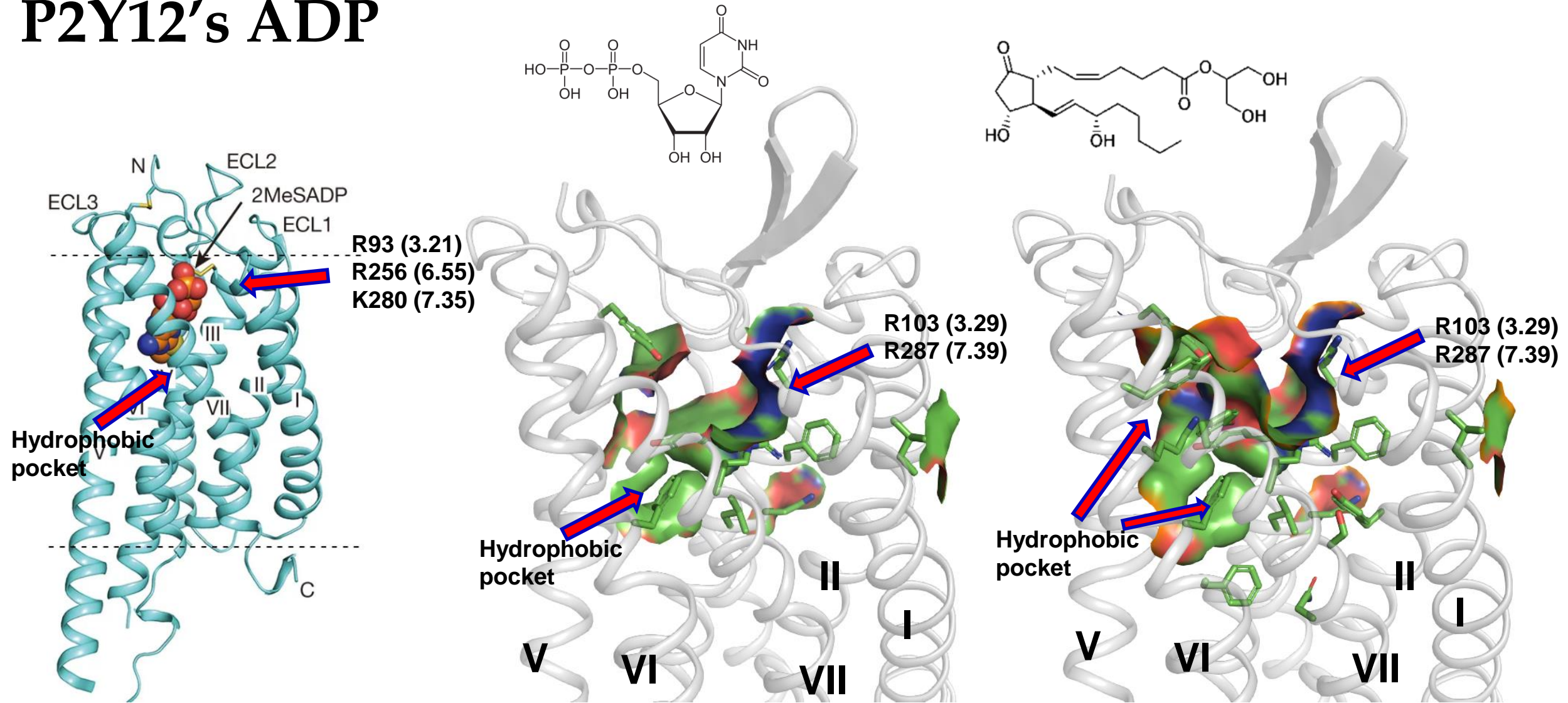
P2Y₁: disruption of D45.52 & R7.39 salt bridge



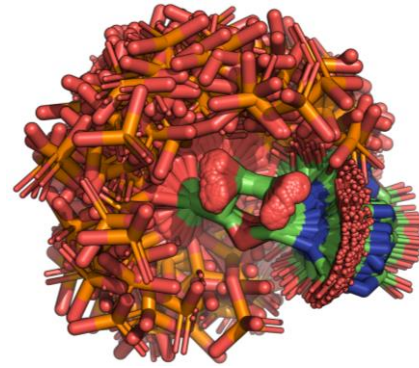
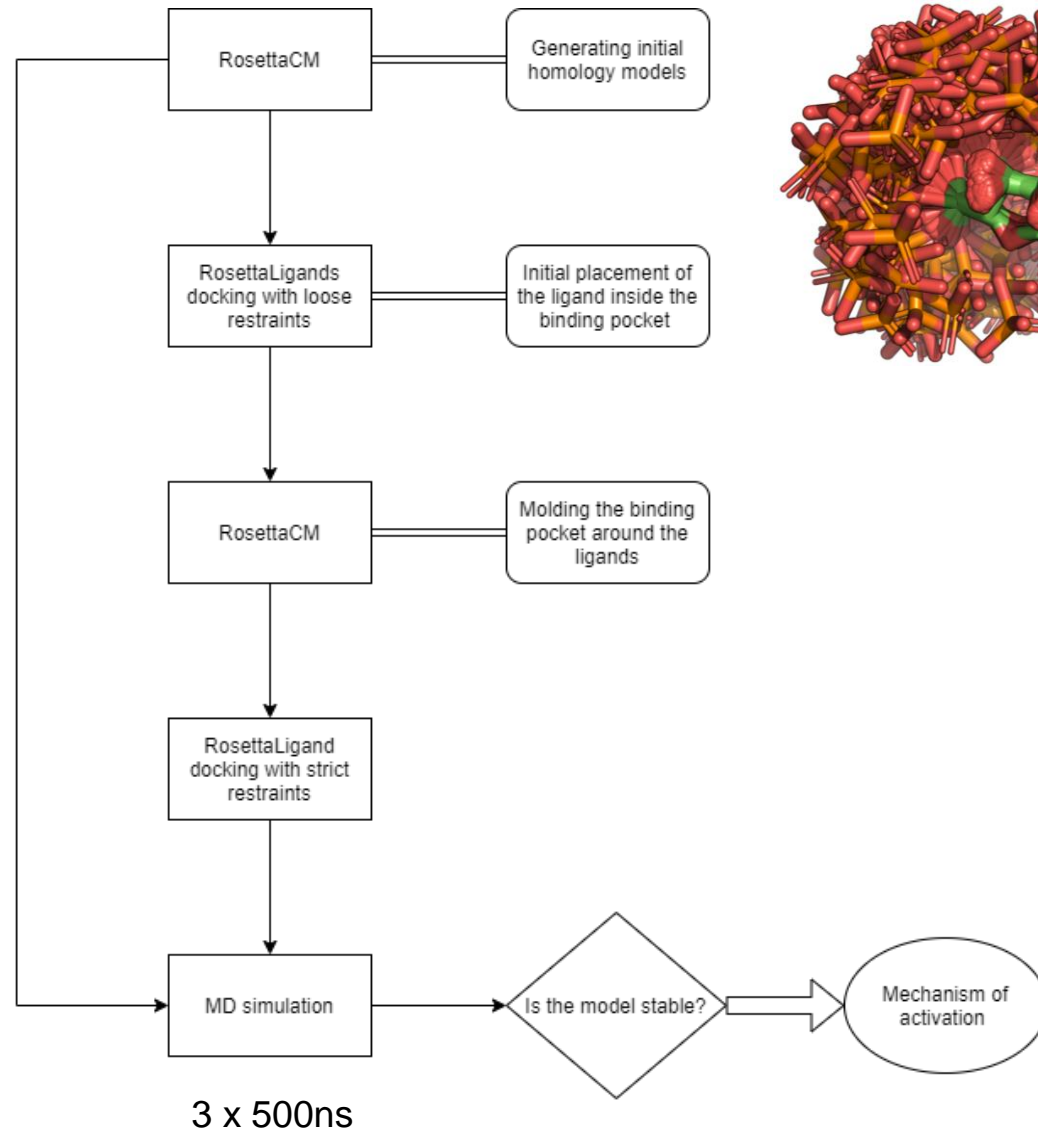
Yuan et al. (2016). Angewandte Chemie International Edition in English. 55.



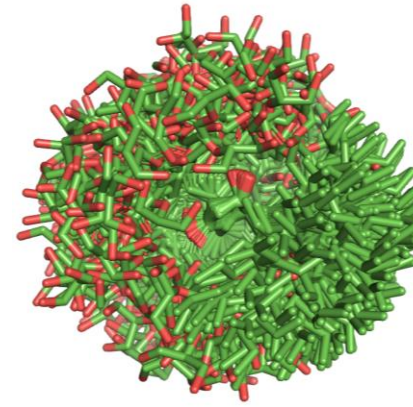
Mutagenesis data suggest similar binding pockets to P2Y12's ADP



Protocol



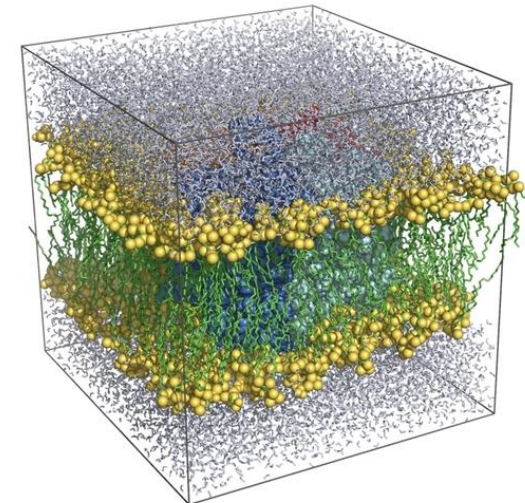
Rotamer libraries



+

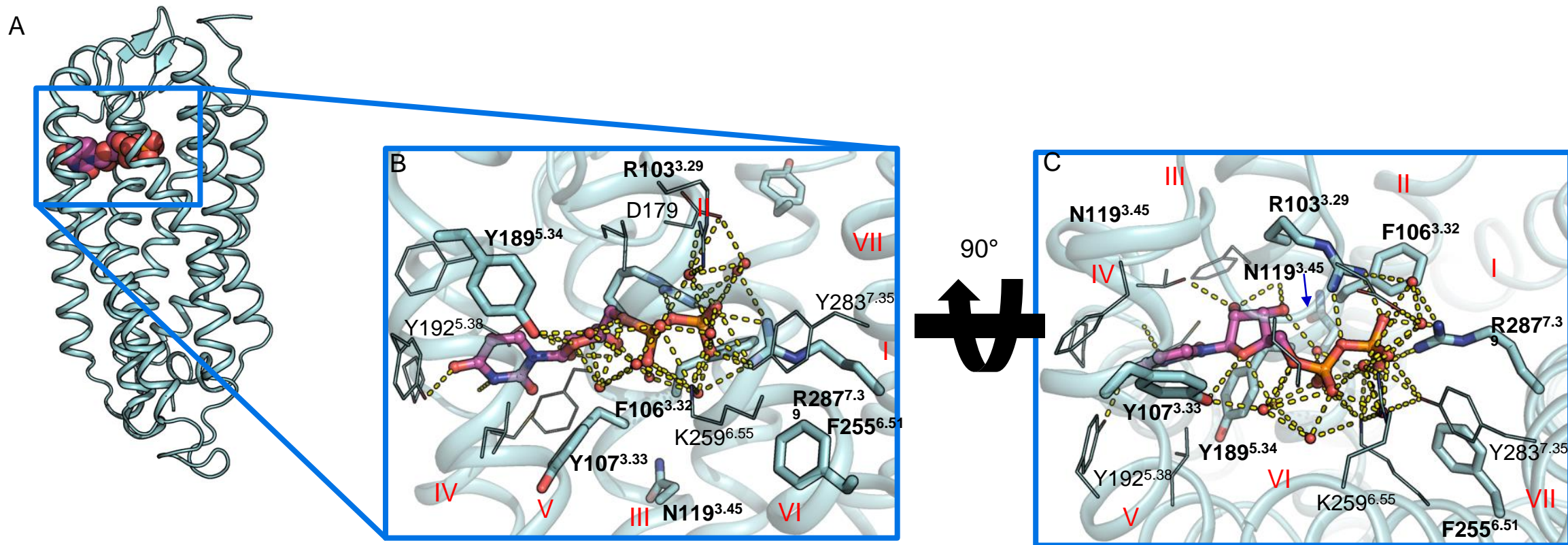


Receptor ensemble



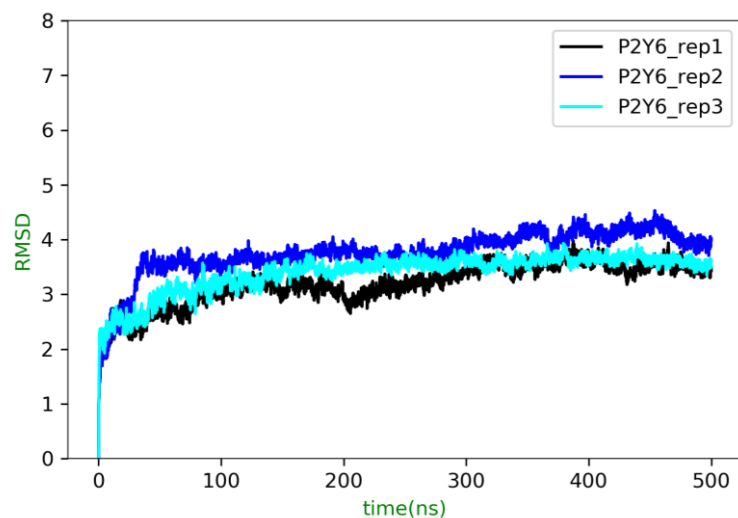
P2Y6-UDP MD-refined docked model

UDP was docked to P2Y6 homology models, then the selected docked model was further refined with total of 1.5 μ s of molecular dynamics.

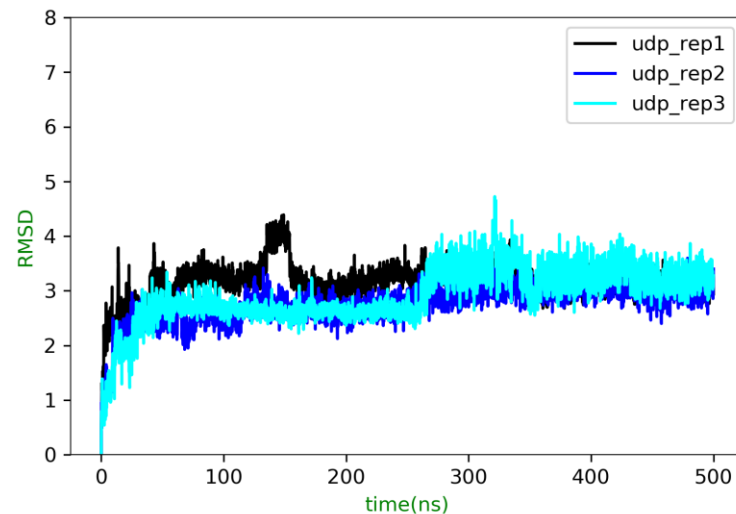


RMSD to the starting docked model and RMSF

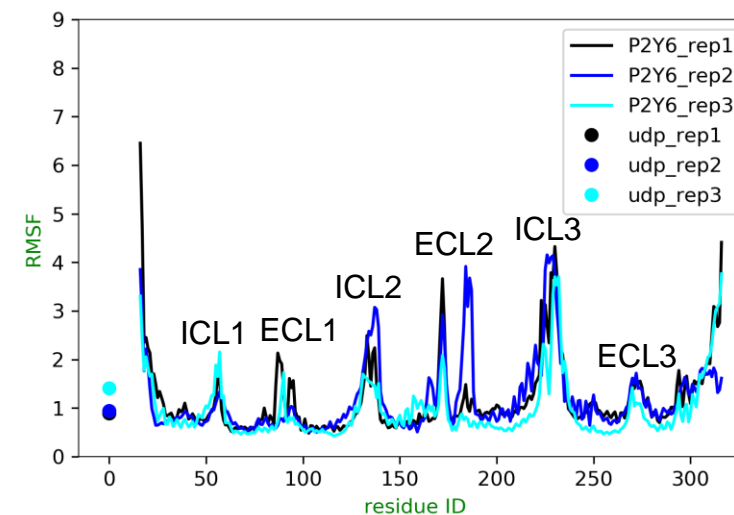
P2Y6 RMSD



UDP RMSD

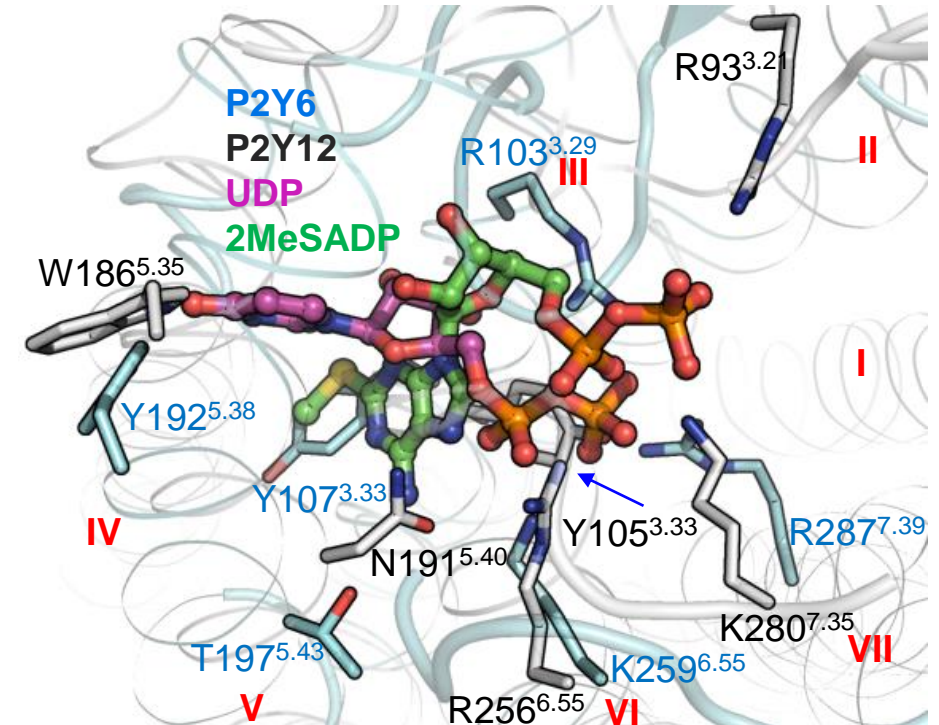
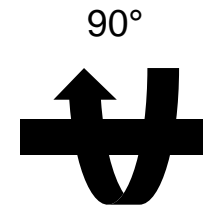
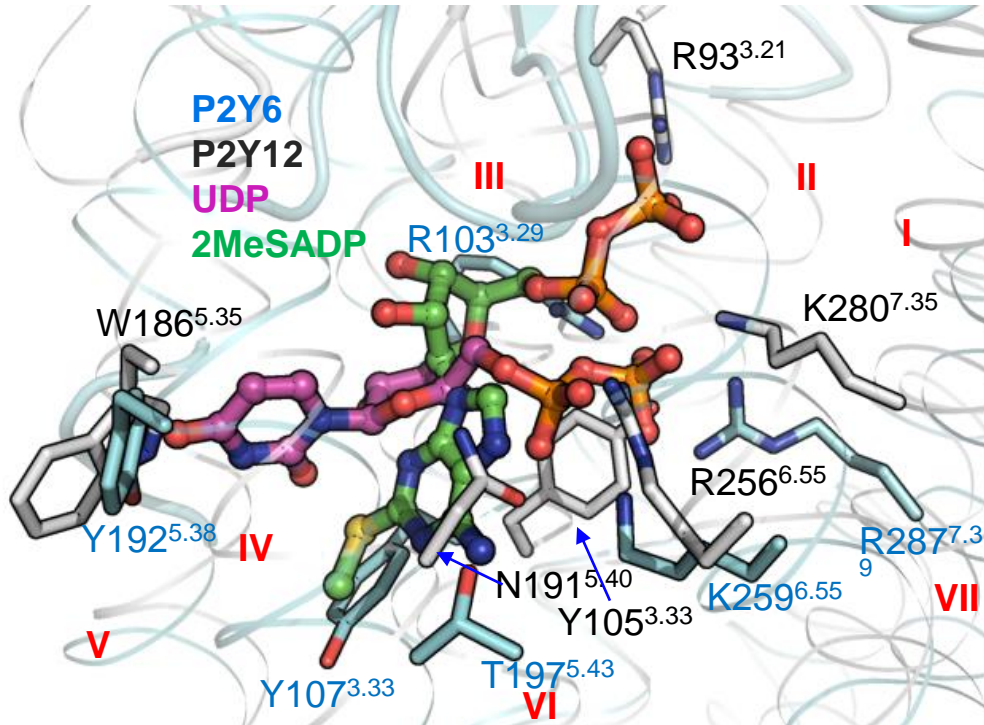


RMSF



Compare to 2MeSADP-P2Y12 crystal structure

Agonists' negatively charged diphosphate group forms hydrogen bonds/salt bridges to residues on the extracellular half of both P2Y6 and P2Y12 receptors, stabilizing the proximity between TMs 3-4 and TMs 6-7 (the “closed state”)

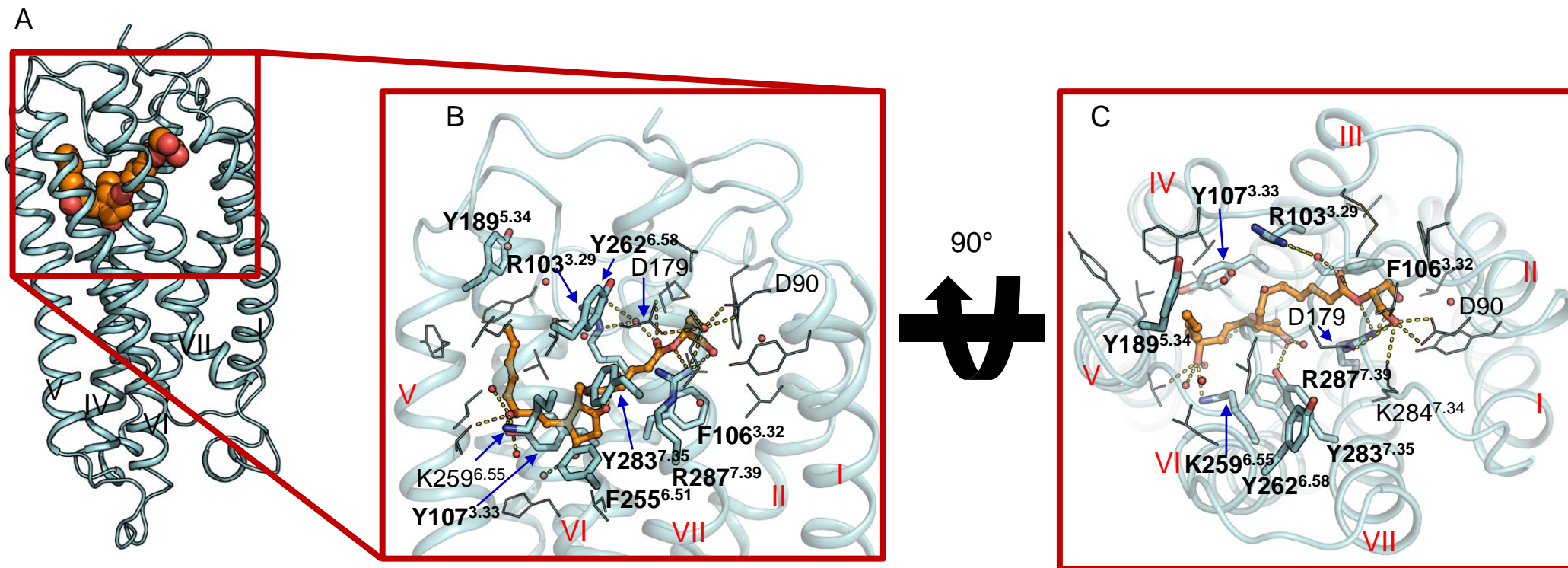


Zhang, J., Zhang, K., Gao, Z. et al. Nature 509, 119–122 (2014)



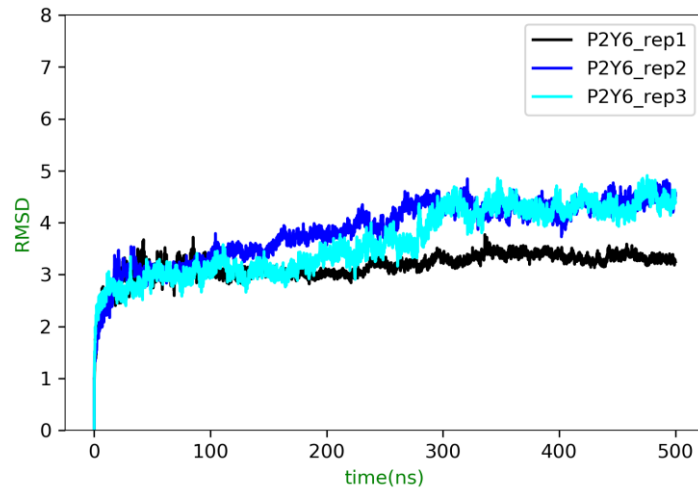
P2Y6-PGE₂-G MD-refined docked model

PGE₂-G was docked to P2Y6 homology models, then the selected docked model was further refined with total of 1.5 μ s of molecular dynamics.

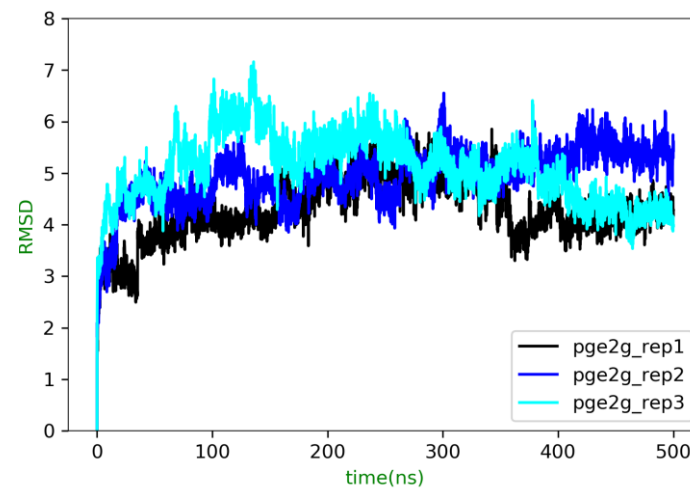


RMSD to the starting docked model and RMSF

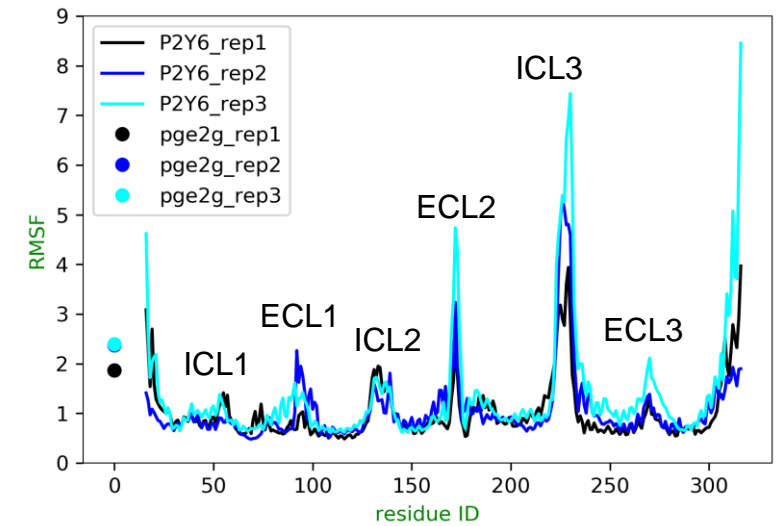
P2Y6 RMSD



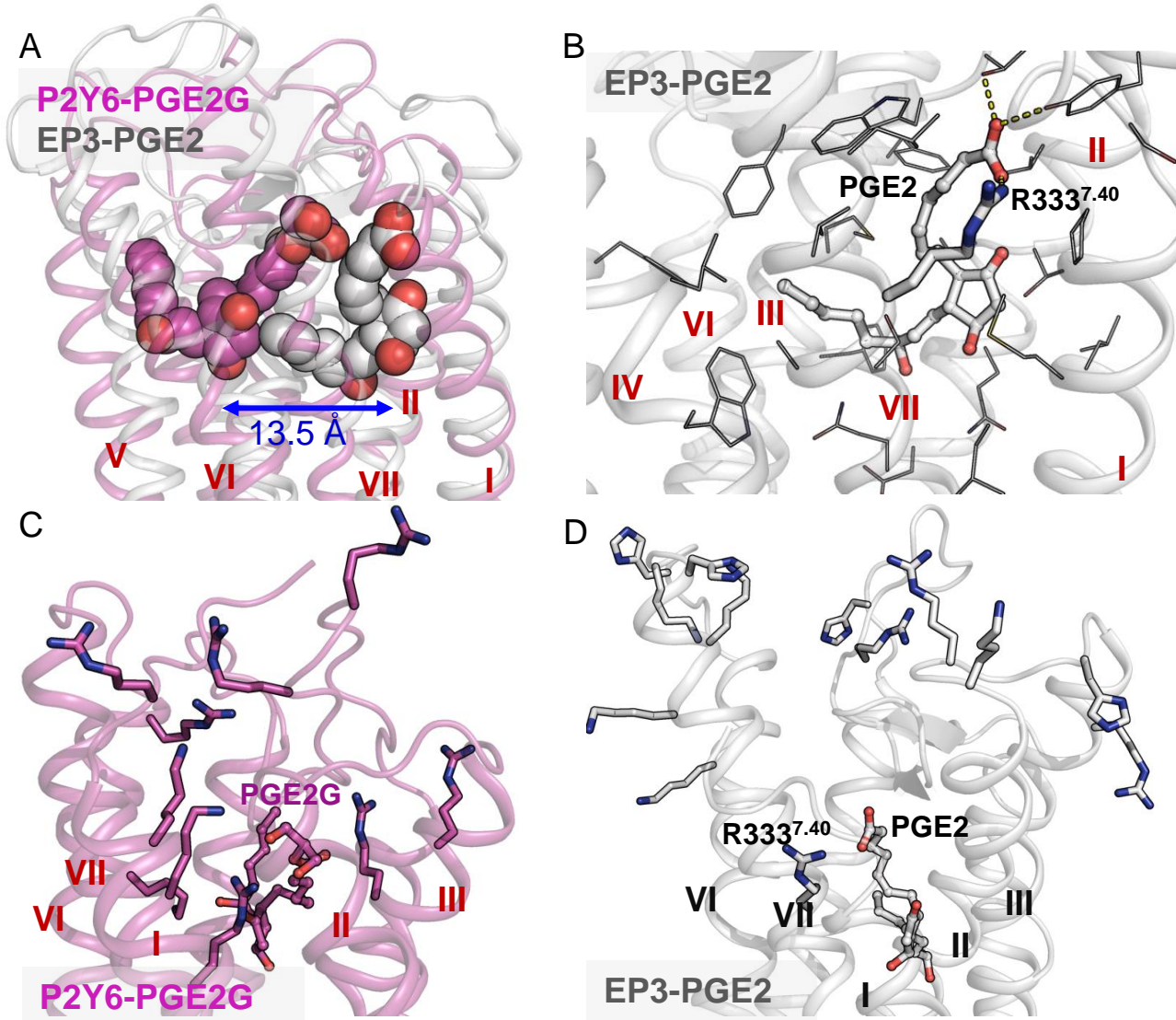
PGE2G RMSD



RMSF



Comparison to the EP3-PGE2 complexes



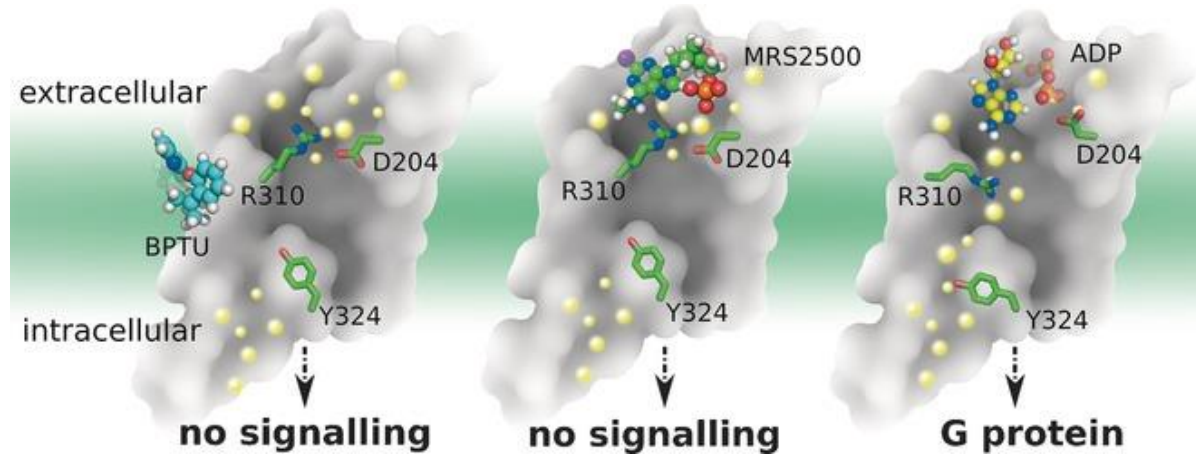
The ring of PGE2-G shifts around 13.5Å toward the TM5 compared to that of PGE2.

The transmembrane region of the P2Y6 has more positively charged sidechains than does EP3, enabling the shift and elongation of the binding pose of PGE2-G.

In contrast, the only positively charged residue in the extracellular half of the transmembrane region of EP3 is R333 on TM7.

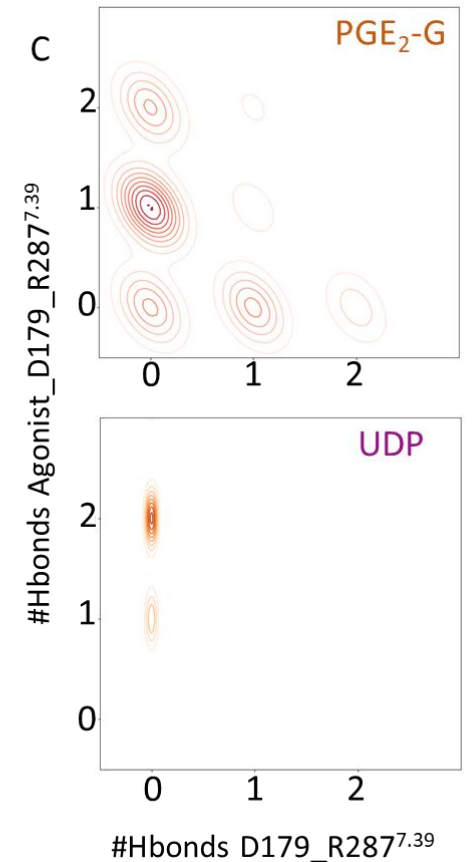
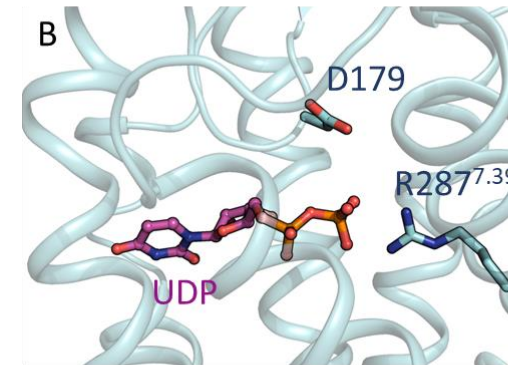
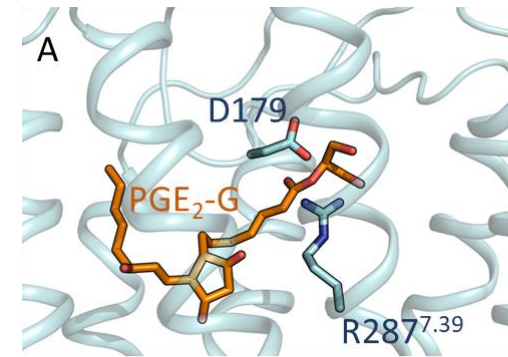


Conservation in agonism and D45.52 & R7.39 saltbridge



Yuan et al. (2016). Angewandte Chemie International Edition in English. 55.

- Previous MD study on P2Y1 suggested that agonist disrupted the ionic lock between D^{45.52} (ECL2) and R^{7.39}
- Throughout MD simulations, UDP completely blocked the saltbridge between D179^{45.52} (ECL2) and R287^{7.39}, while PGE₂-G frequently interfered this ionic interactions.



Acknowledgment

Meiler lab

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- Gregory Sliwoski
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