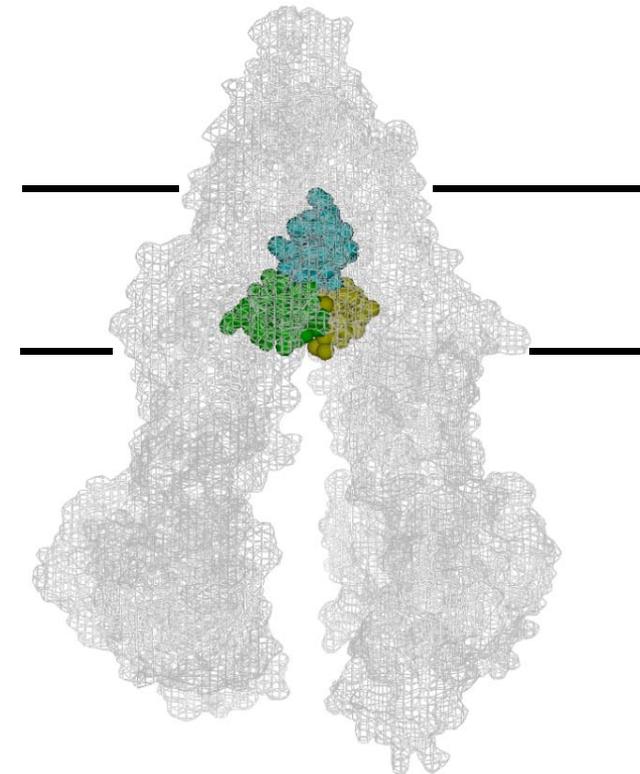
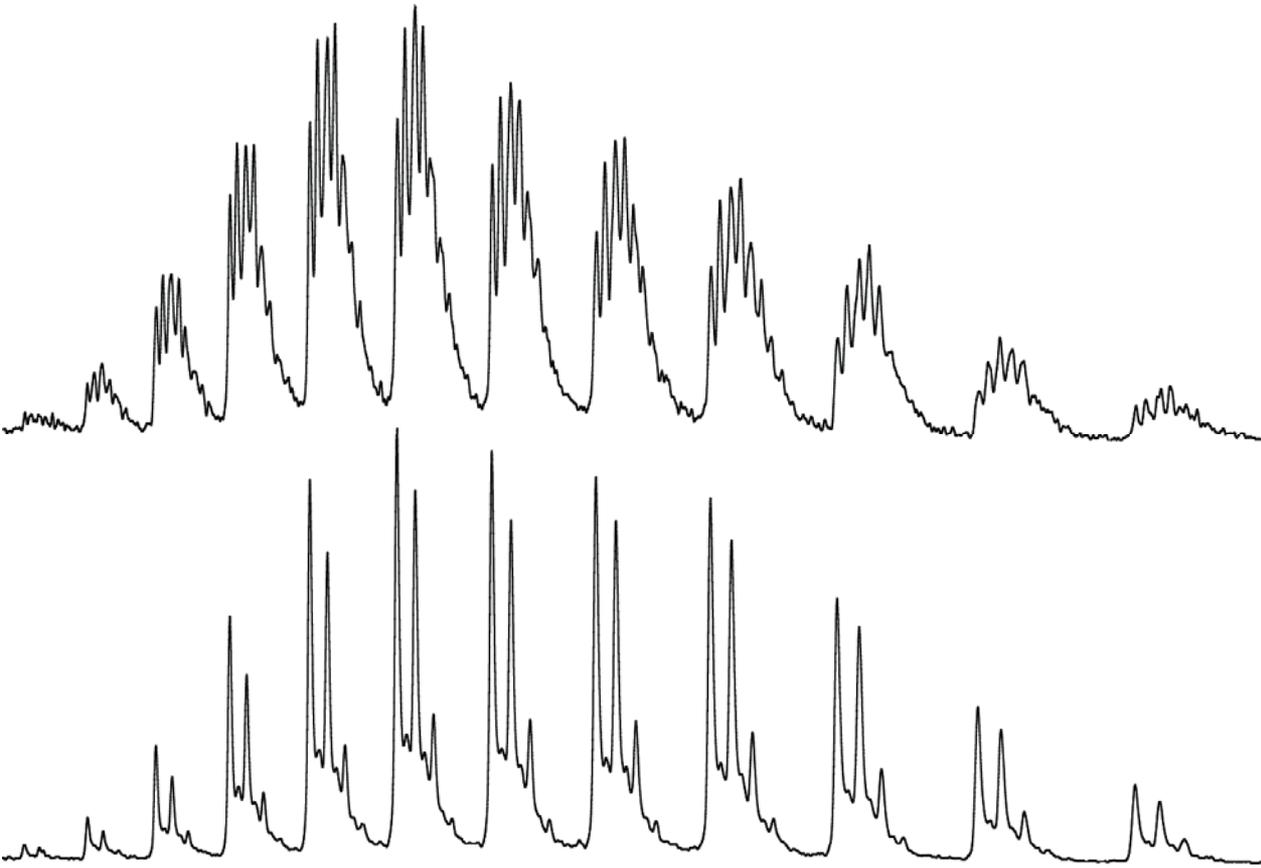


Mass spectrometry reveals drug and lipid induced opening and closing of an efflux pump

Julien Marcoux

Carol Robinson group, University of Oxford



Intro – Detergent – Lipids – Cyclosporin A – Mechanism - Conclusion

Instruments:

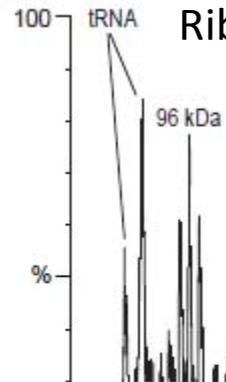
- modified Q-TOF2 (Micromass / Waters)
- modified Synapt 2 and synapt G2

High mass quadrupole (up to m/z 35 000)

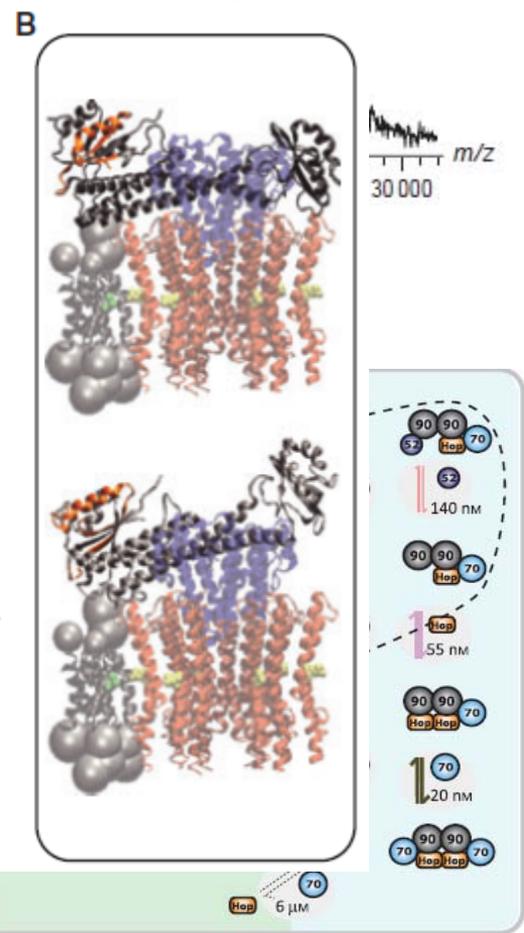
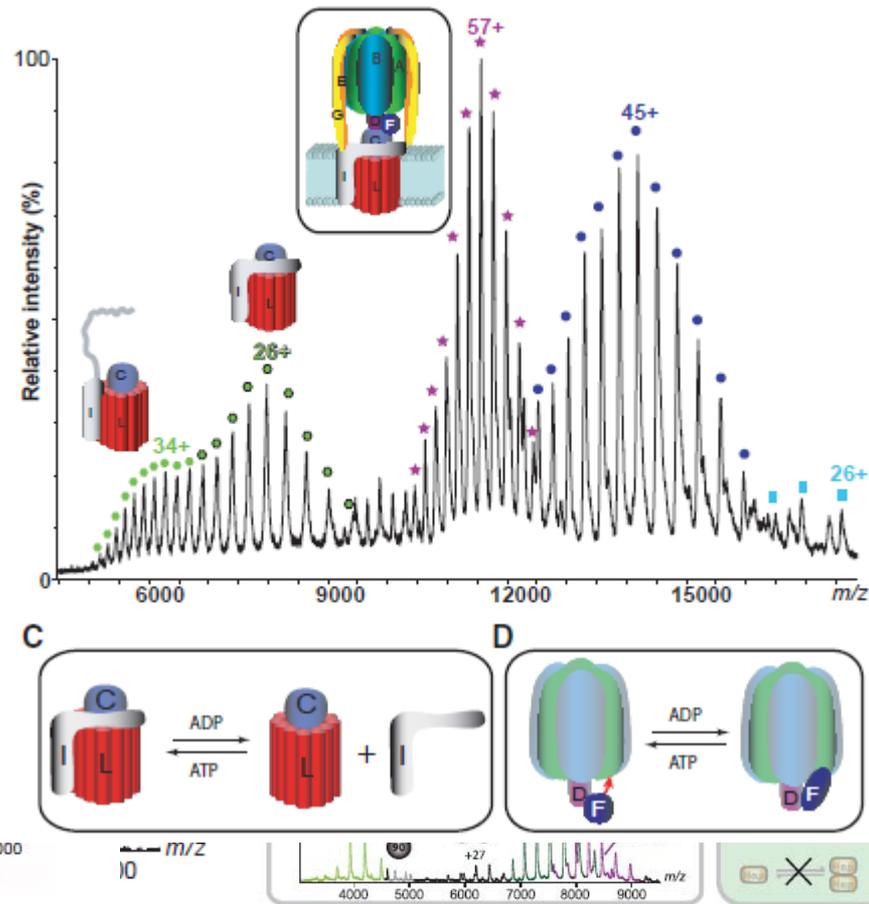
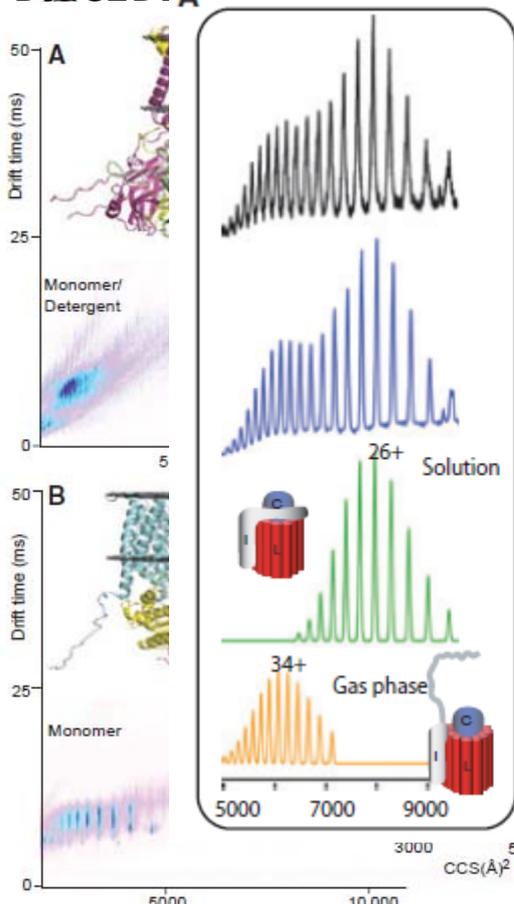
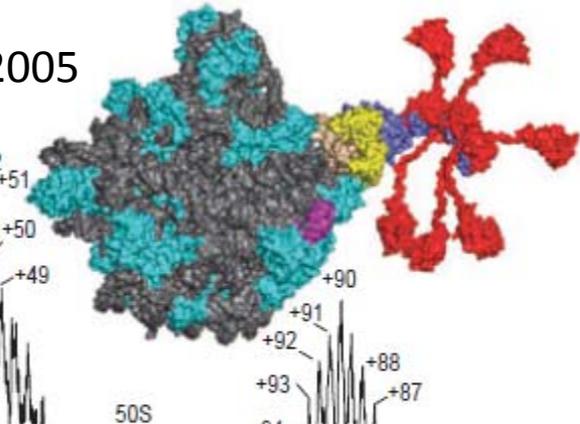
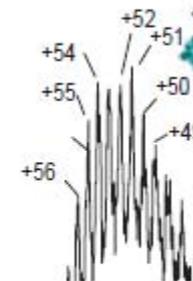
Increased backpressure

Nanospray

BtuC2D2 vATPase (2011)



Rib. 2.3 mDa / 2005



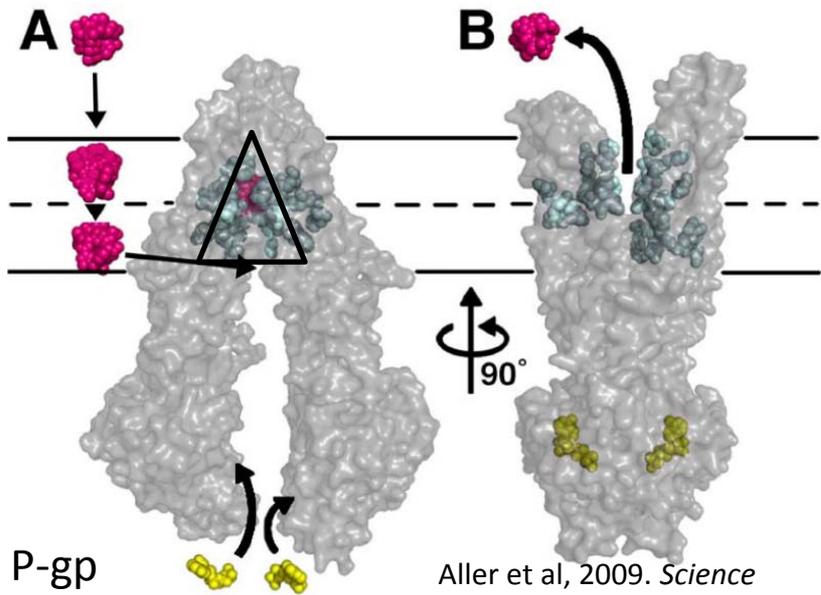
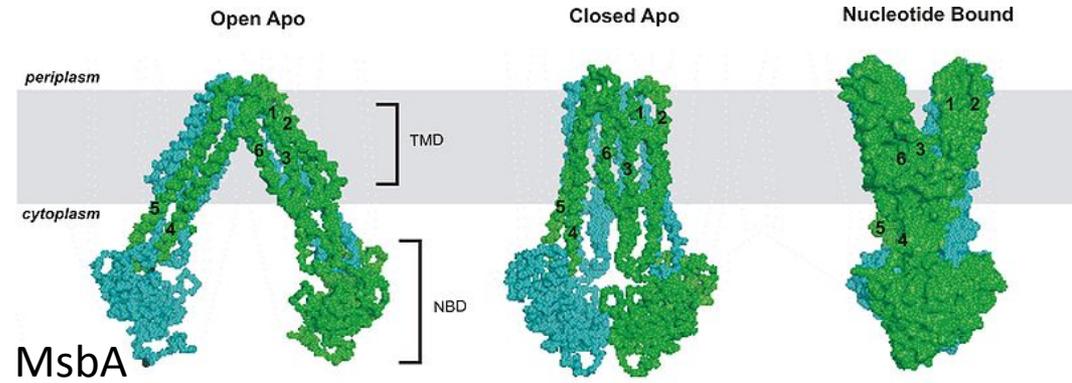
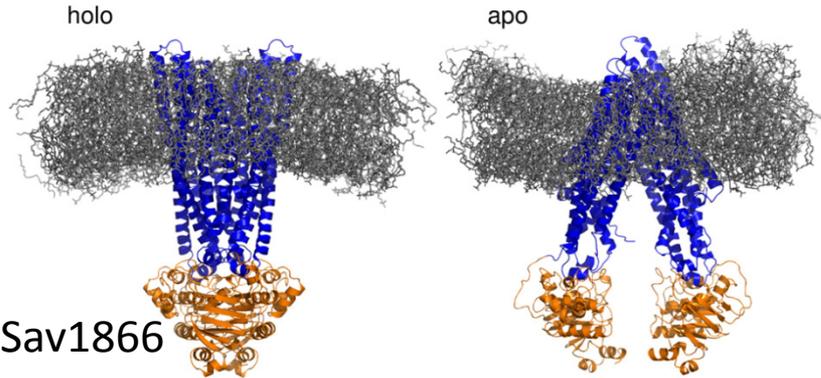
ABC transporters

48 genes in human genome / Mainly importers or exporters

Topology: transmembrane domain & 2 nucleotide binding sites

Tetramer (BtuC₂D₂), dimer (BmrA, MsbA, Sav1866) or monomer (**P-glycoprotein**)

Involved in multidrug resistance



Usual drug binding techniques

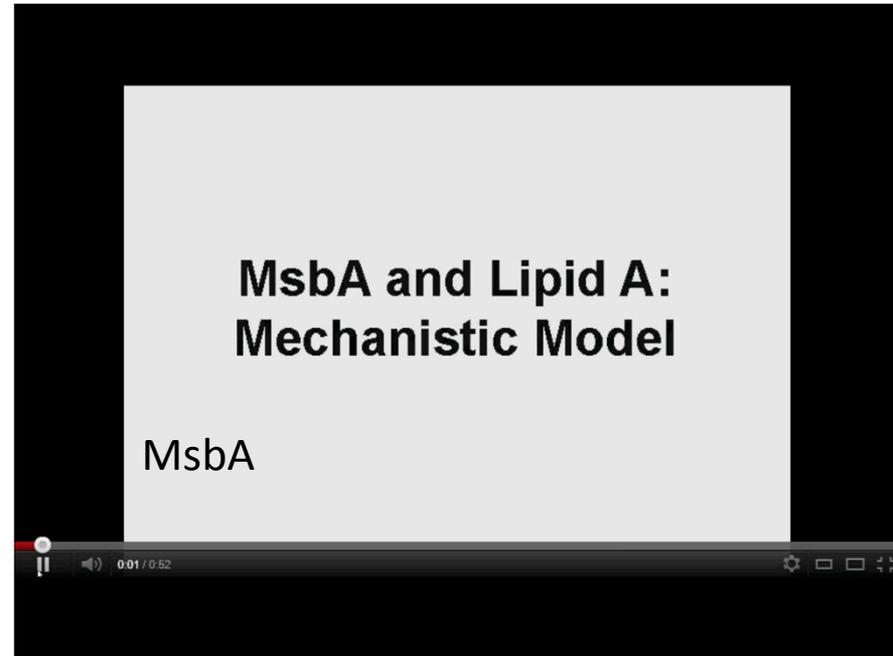
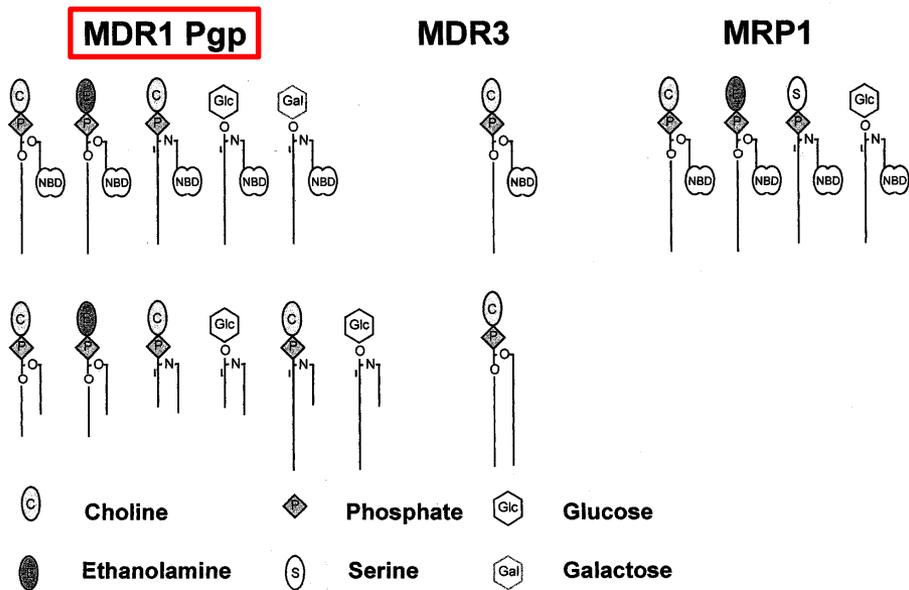
- photoaffinity labelling
- fluorescence quenching

1 to 4 binding sites (channel?)

Kd ranging from ~25 nM to ~250 mM

Some drugs activate, inhibit or both

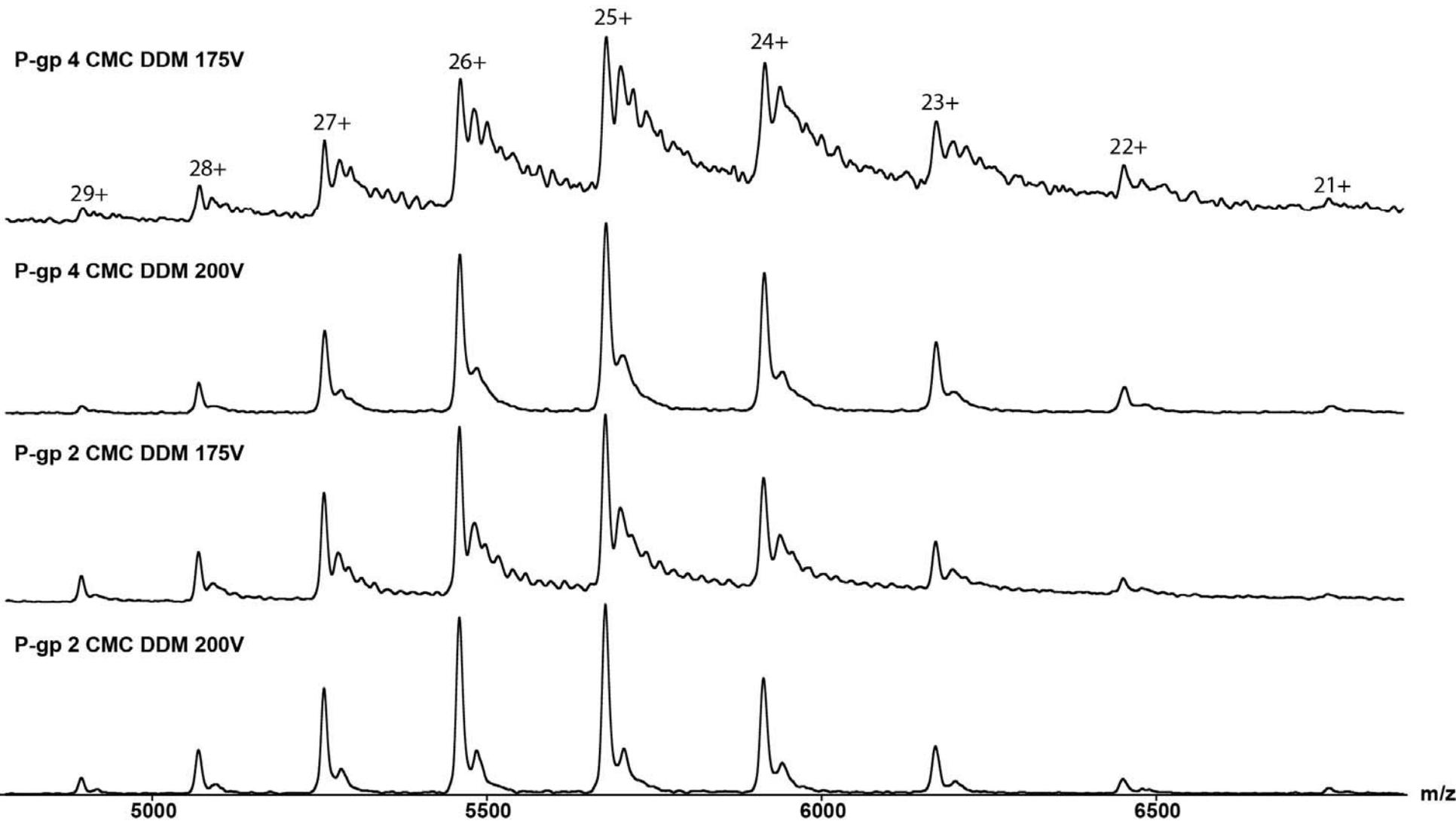
Lipid translocation by multidrug transporters



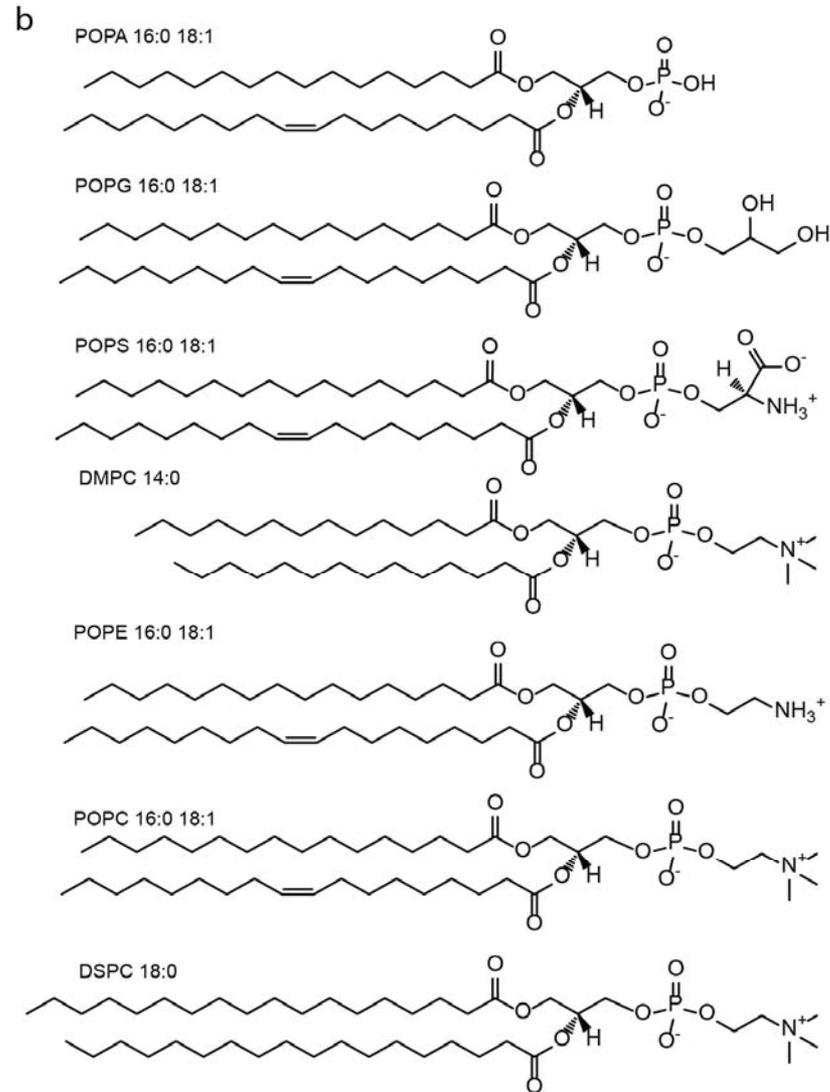
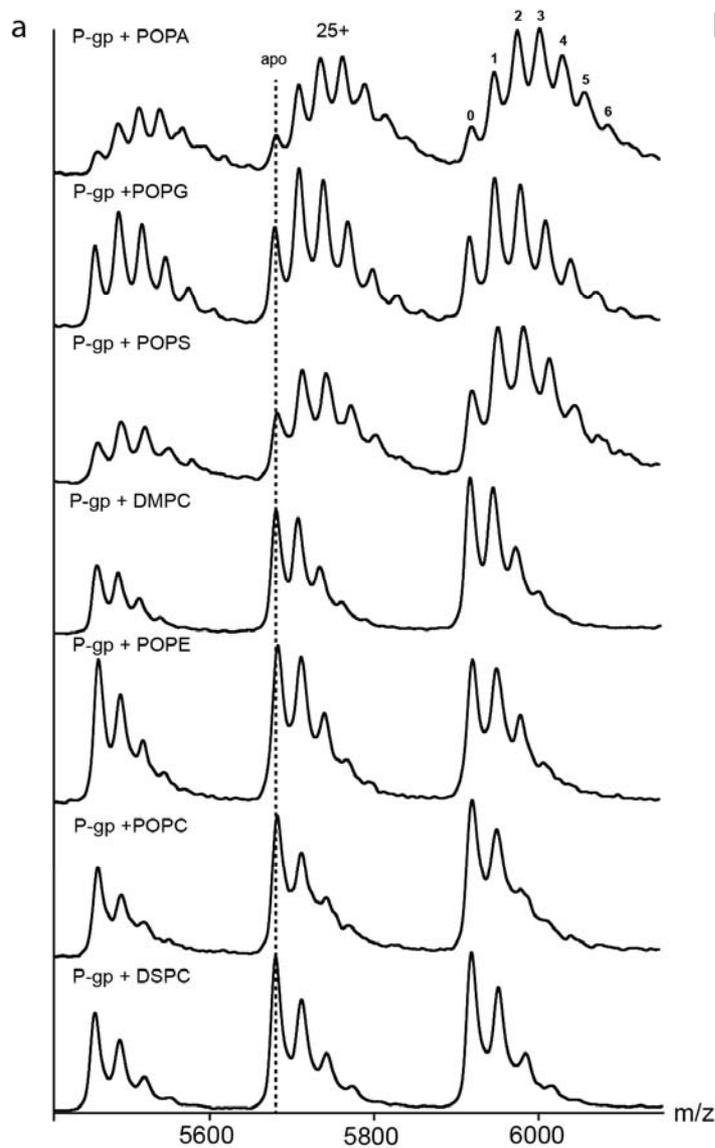
Borst et al, 2000. BBA

- P-gp binds and translocates phospho/sphingo/glycolipids
 - Flippase activity inhibited by P-gp inhibitors
 - Lipid environment modulates drug binding
- drugs and lipids use same path (competitive inhibition)

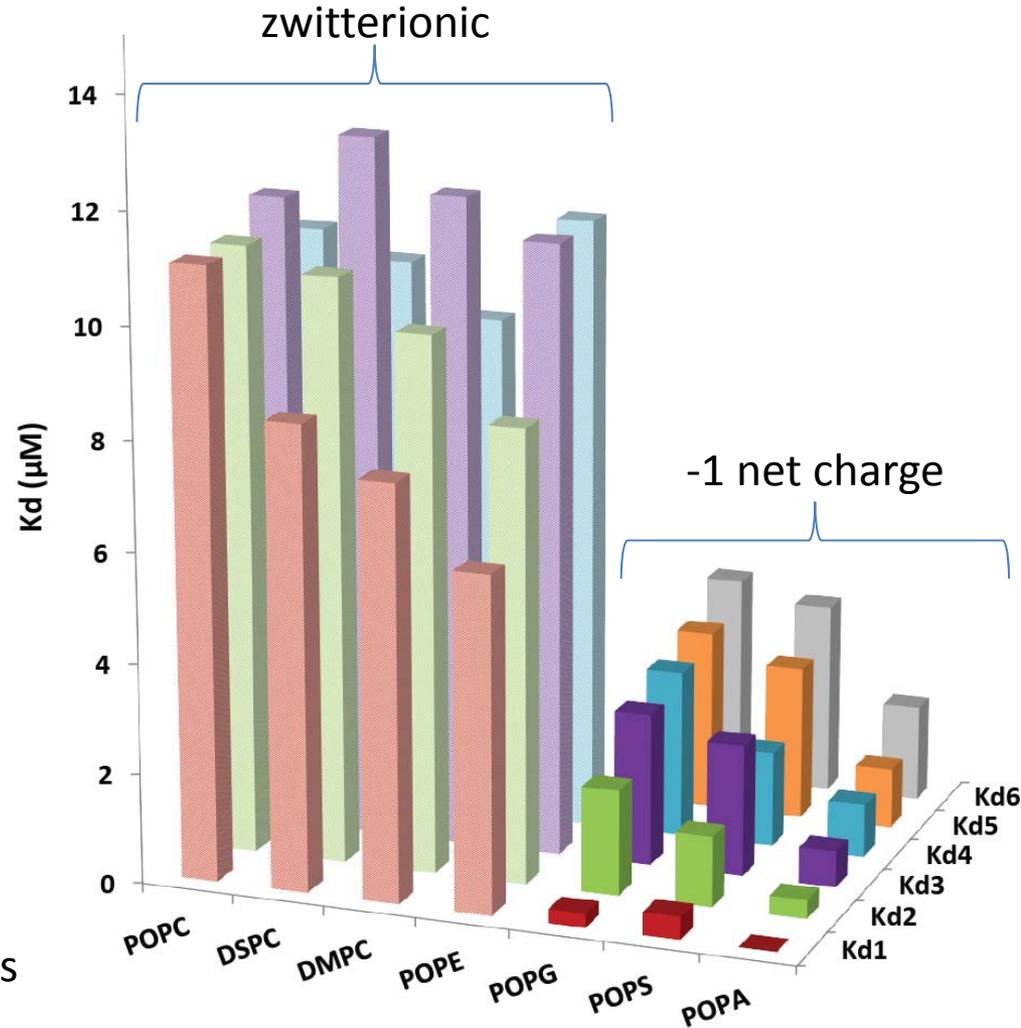
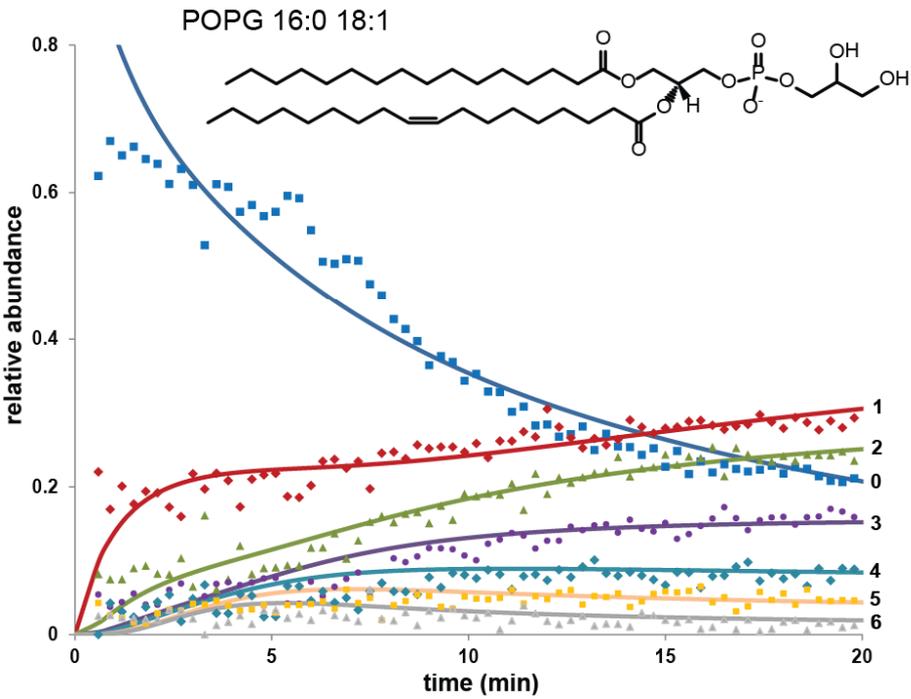
Biochimica et Biophysica Acta 1788 (2009) 2335–2344



Phospholipids

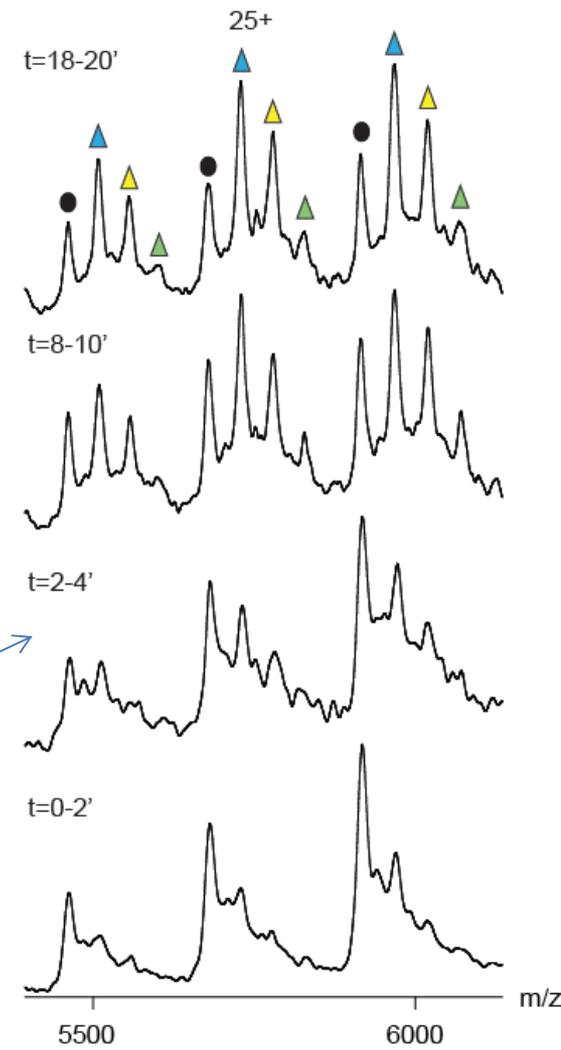
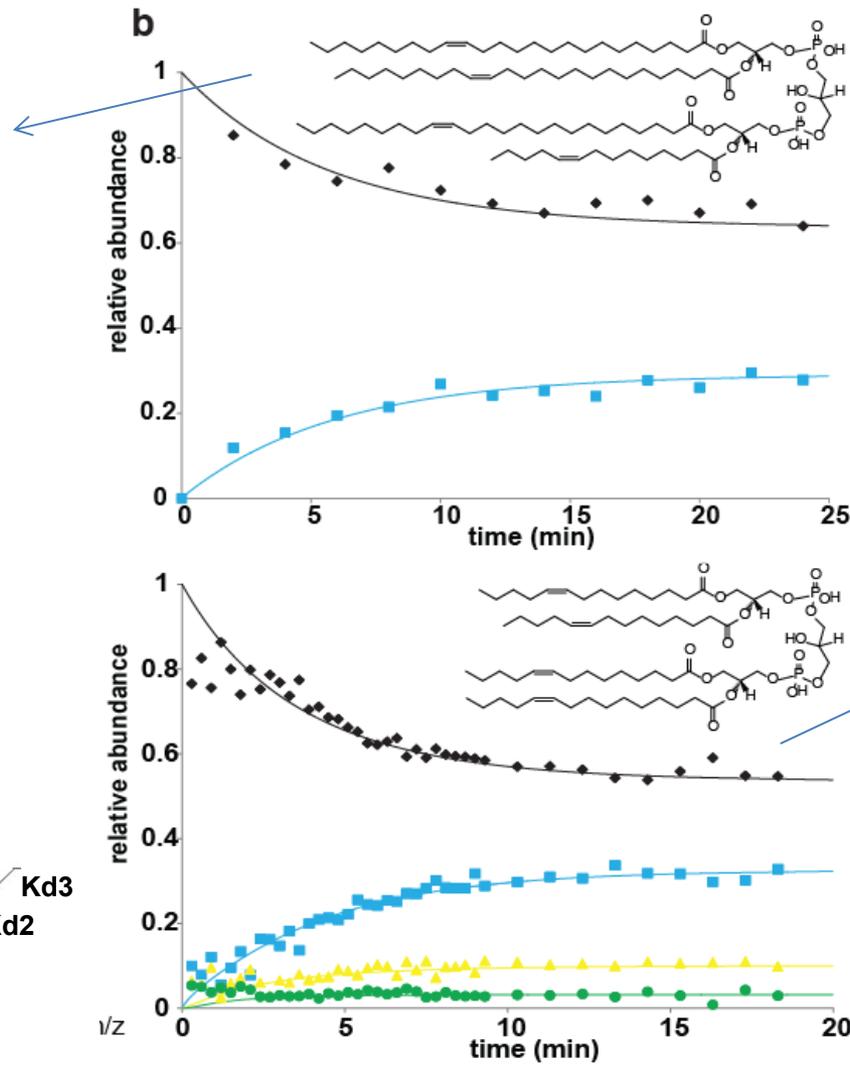
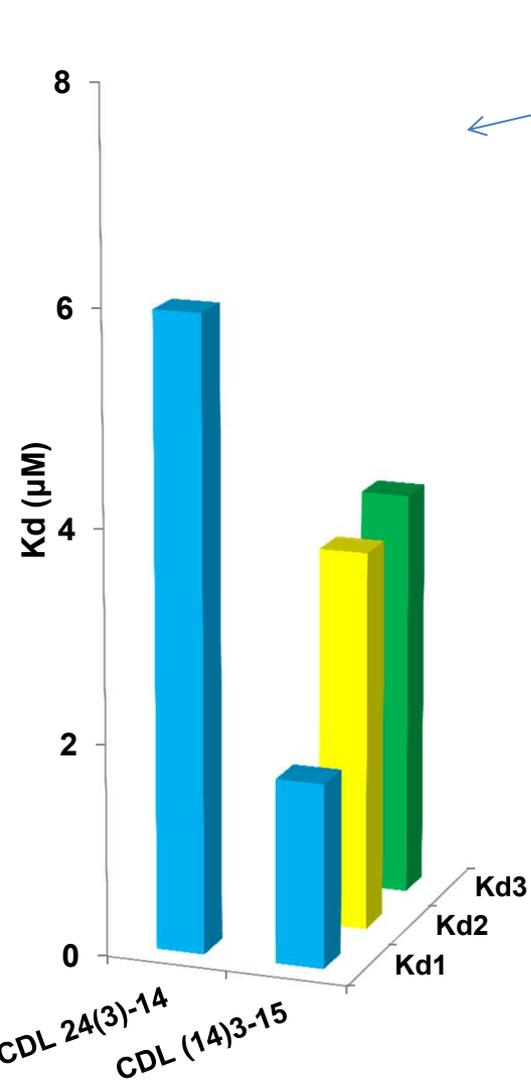


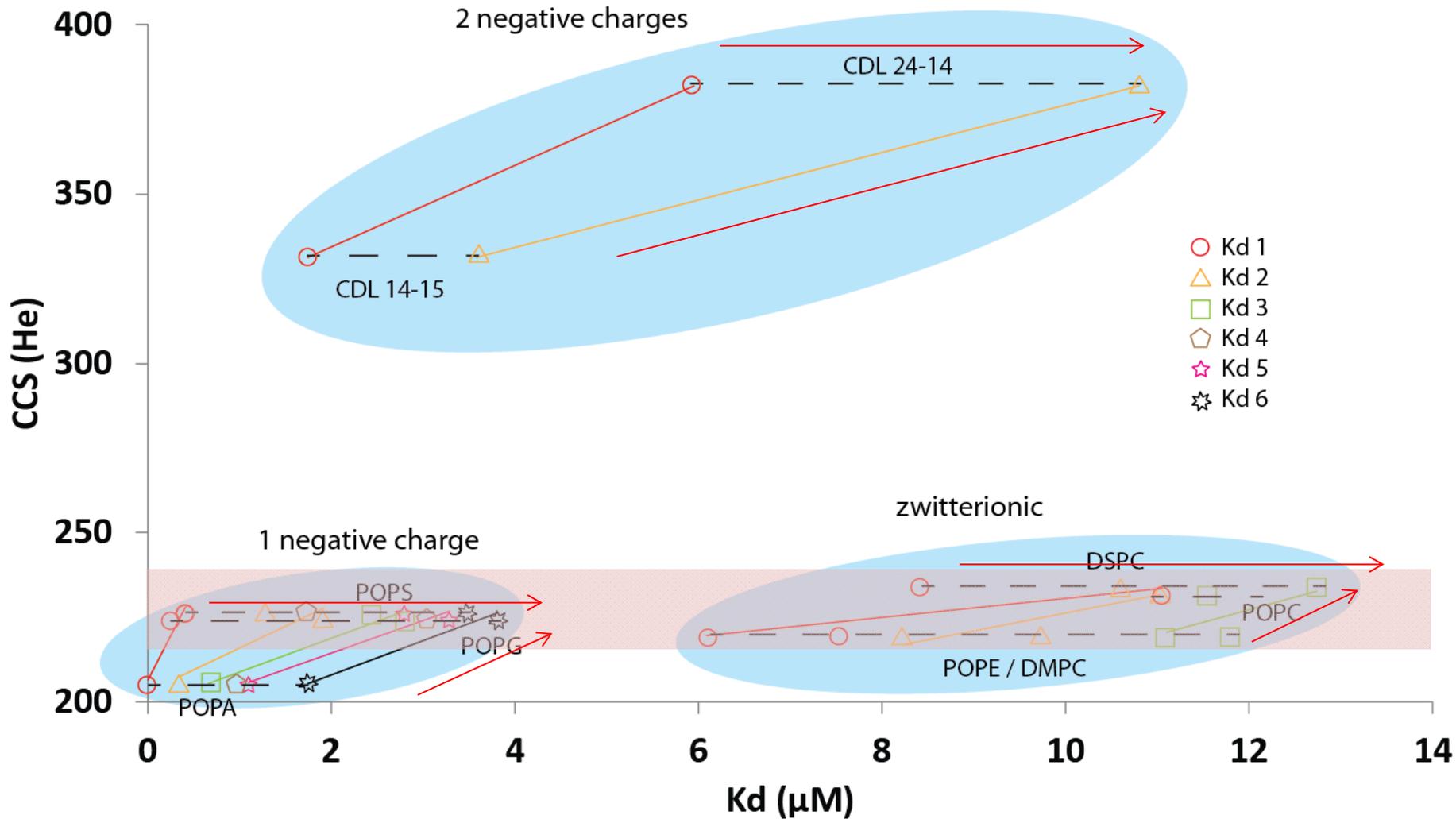
Phospholipids



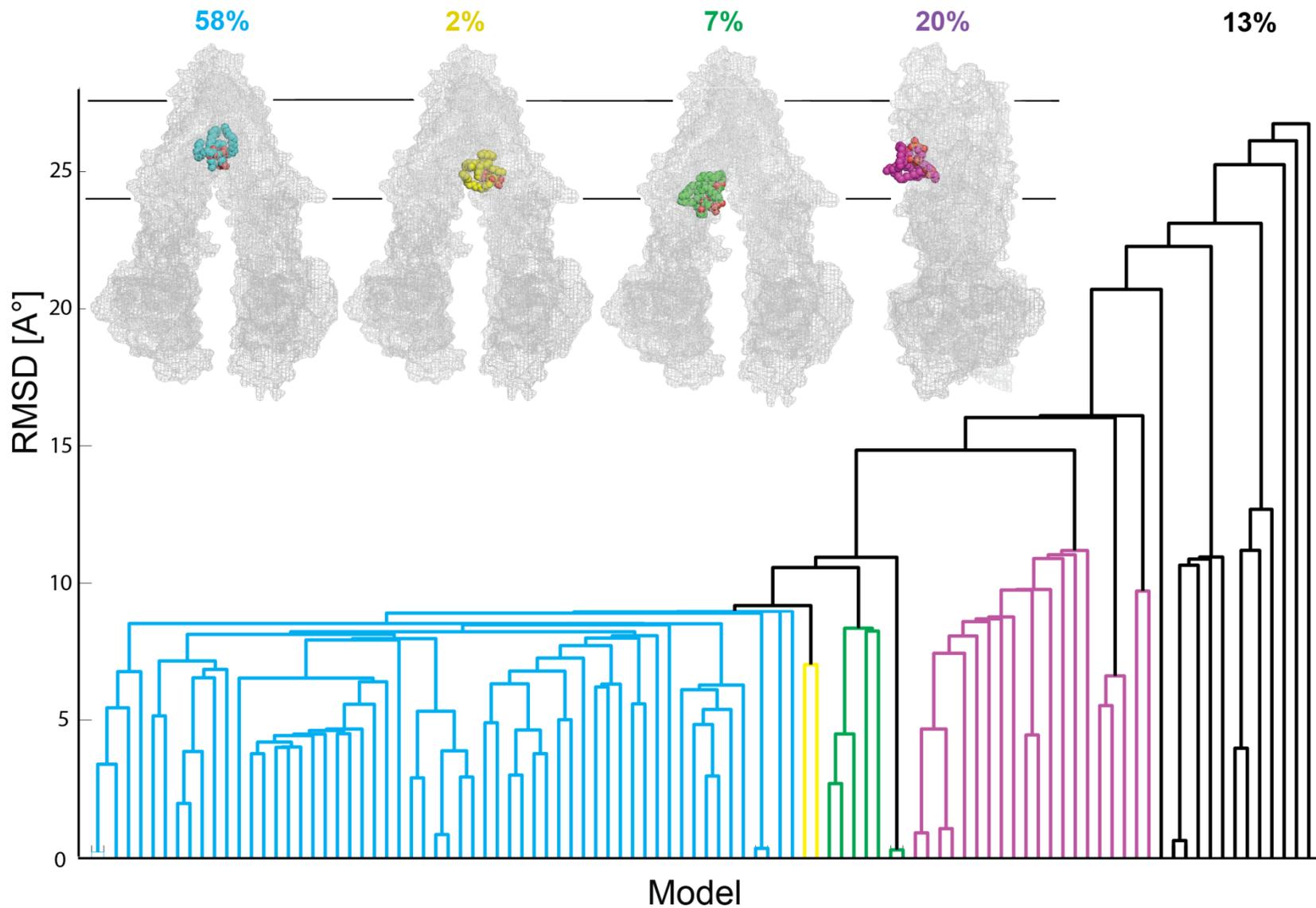
Clear preference for negatively charged lipids

Cardiolipins

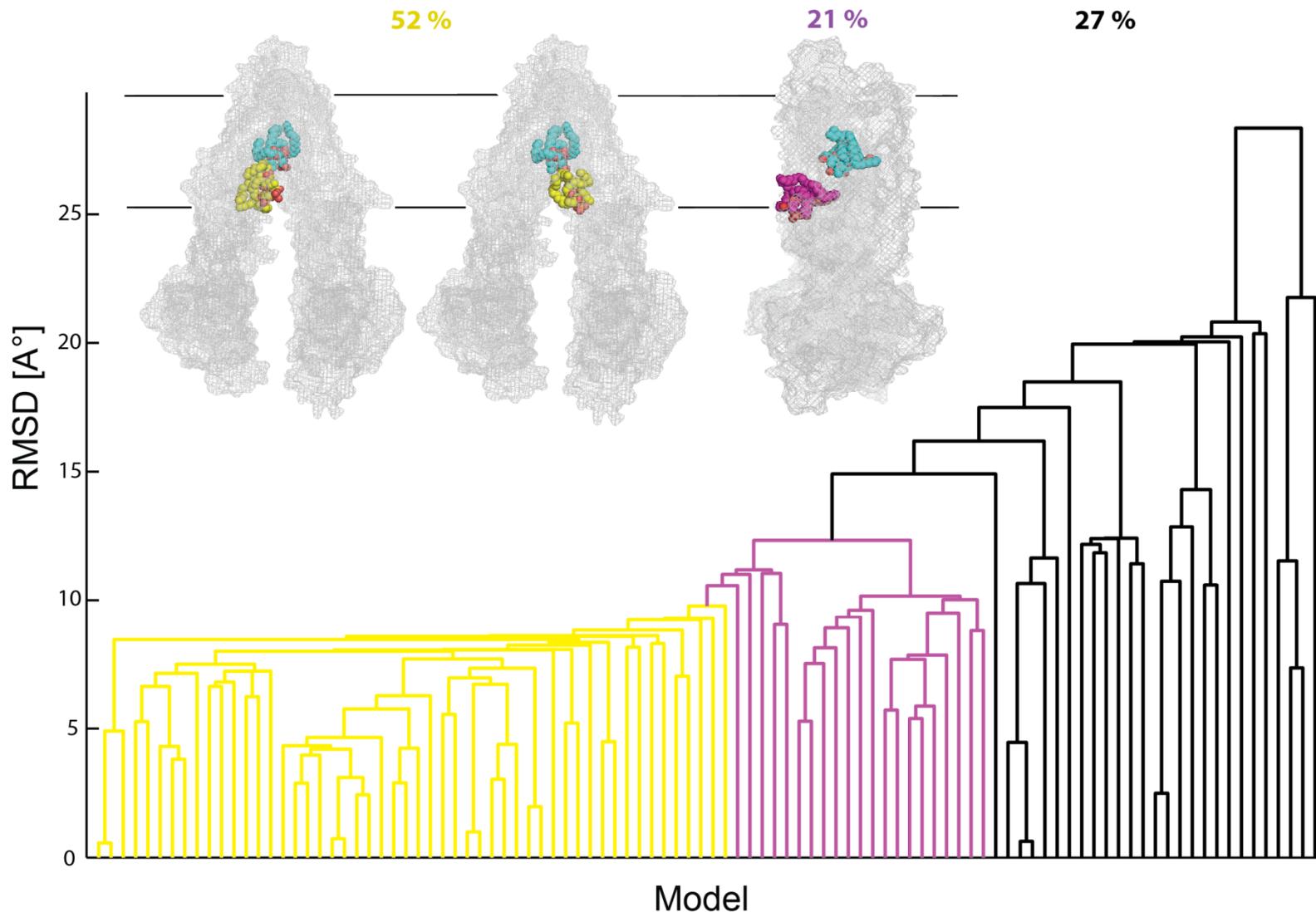




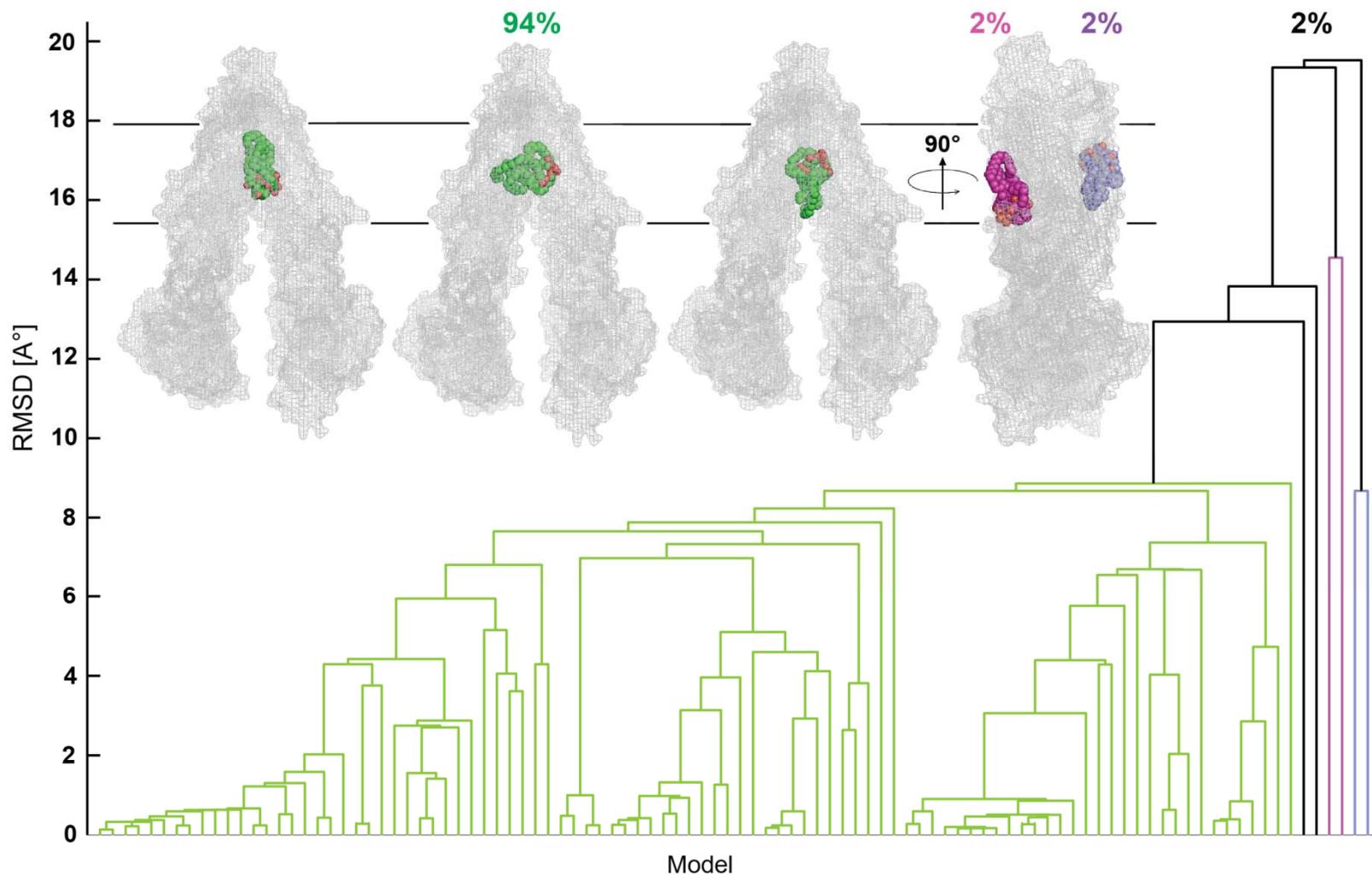
Modelling 1st CDL14-15



Modelling 2nd CDL14-15



Modelling 1st CDL24-14

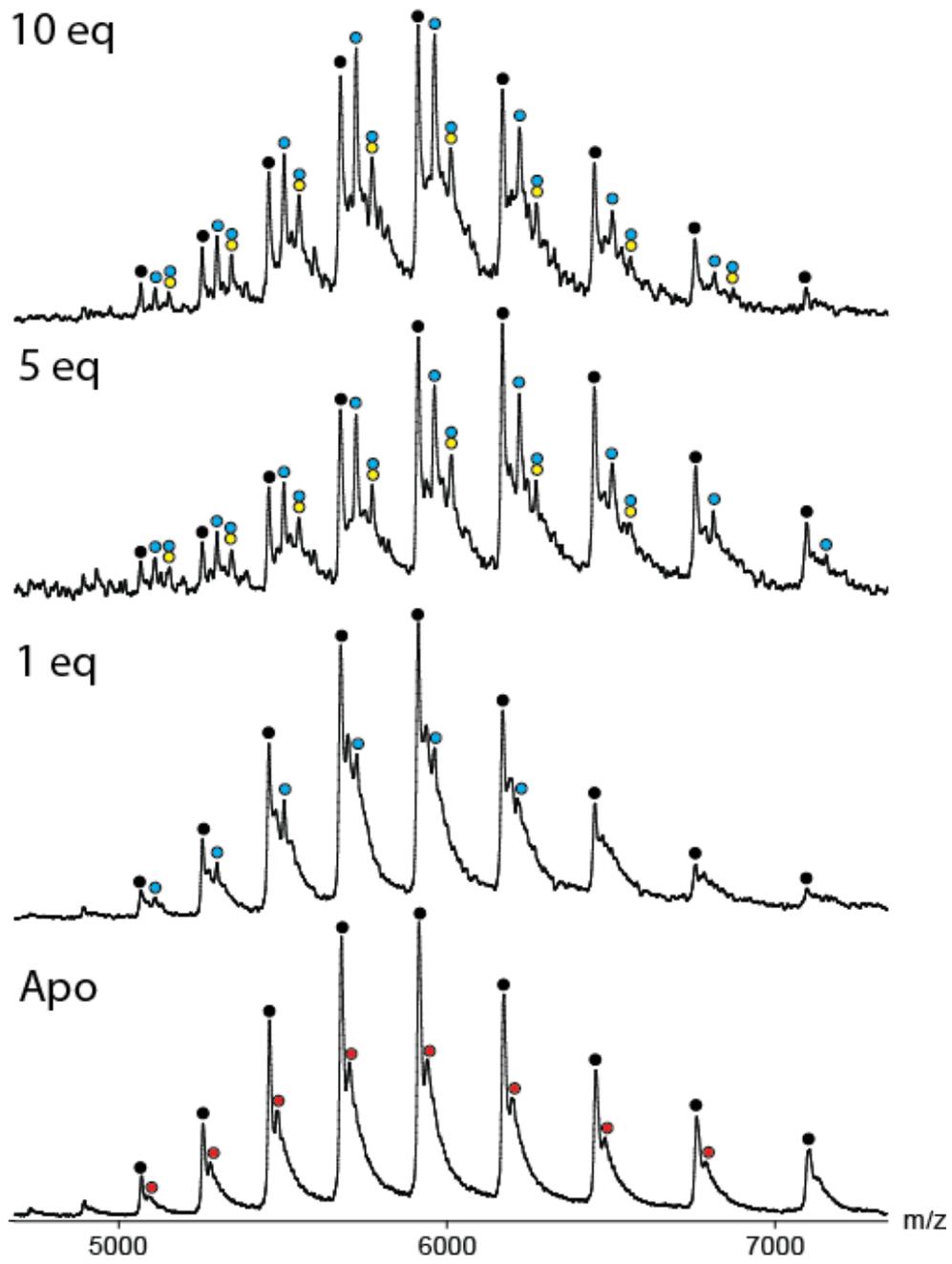
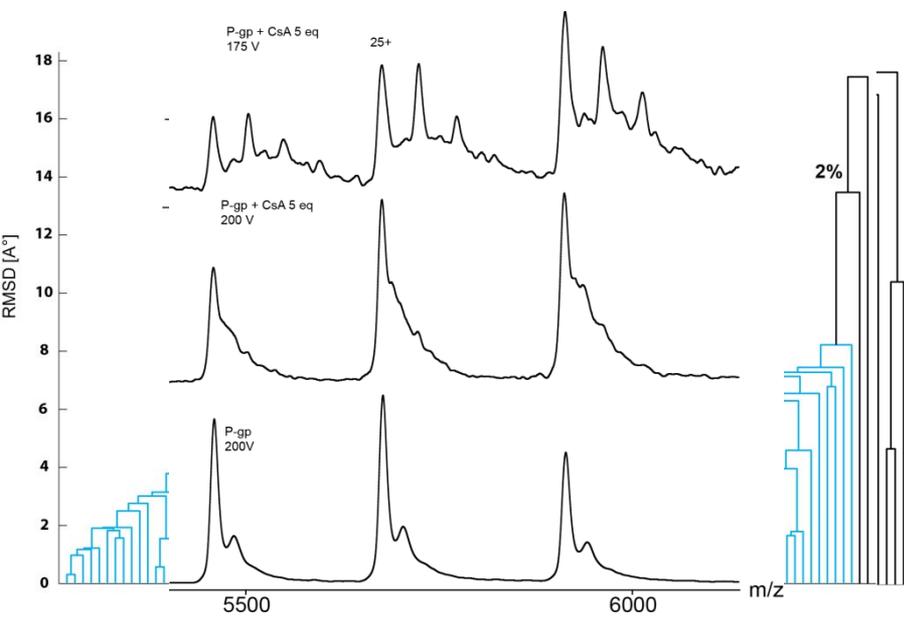
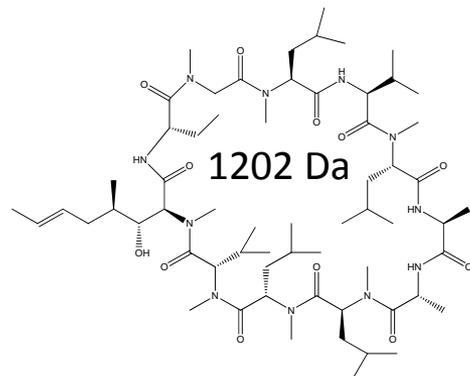


Cyclosporin A

Immunosuppressant and antifungal antibiotic

Inhibition of calcein transport: $IC_{50} = 3 \mu M$

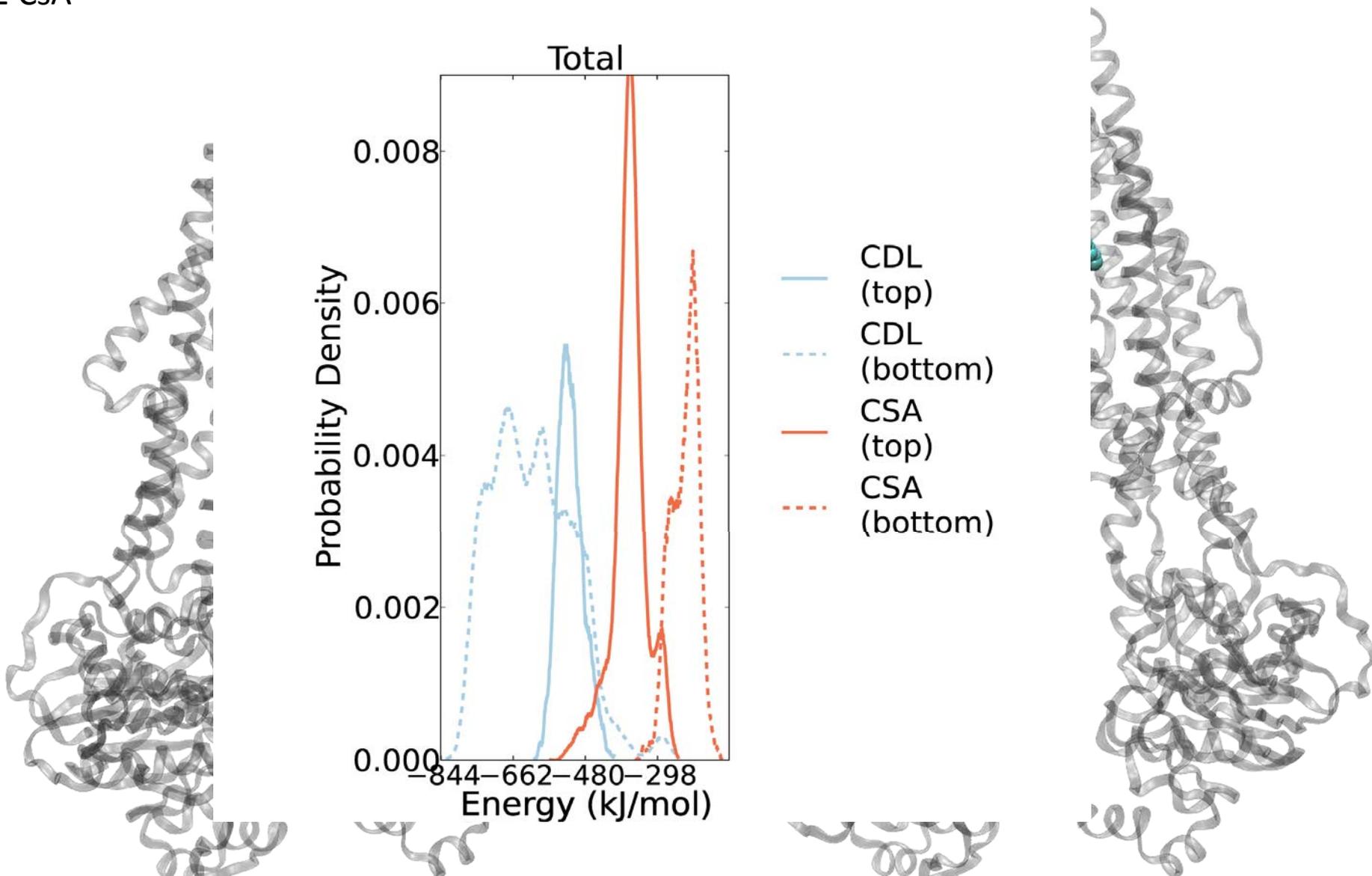
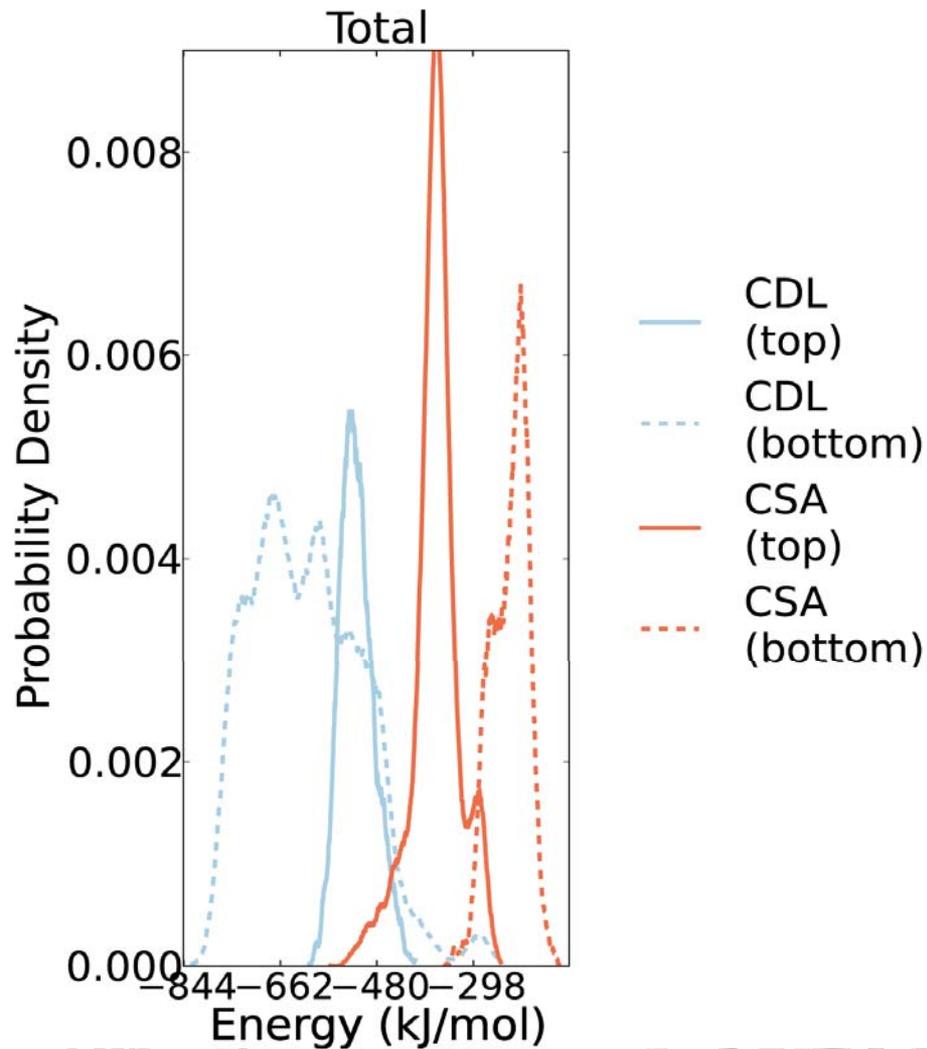
$K_d = 20$ to 200 nM



Molecular dynamics

Interaction energies between Pgp and ligands

2 CsA



Conclusion

- First use of native MS to detect specific interaction between a membrane protein and substrates.
→ confirmation of the binding of 2 nucleotides at the same time
- Binding kinetics provide Kds for phospholipids and CDLs.
→ preference for negatively charged lipids (interaction with positive residues?)
- Modelling of 2 short and 1 long CDL
→ size related difference explained by molecular docking
- Binding and modelling of 2 CsA
→ confirmation of multiple binding for CsA, lower interaction explained by MD.
- IM-MS shows that substrates can generate a smaller outward conformation

More generally:

ability of MS to probe the effects of small molecules on the equilibrium between open and closed states of a membrane pump

Many thanks

University of Oxford

CVR Group:

Sheila Wang

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Houchao Tao

Qinghai Zhang

