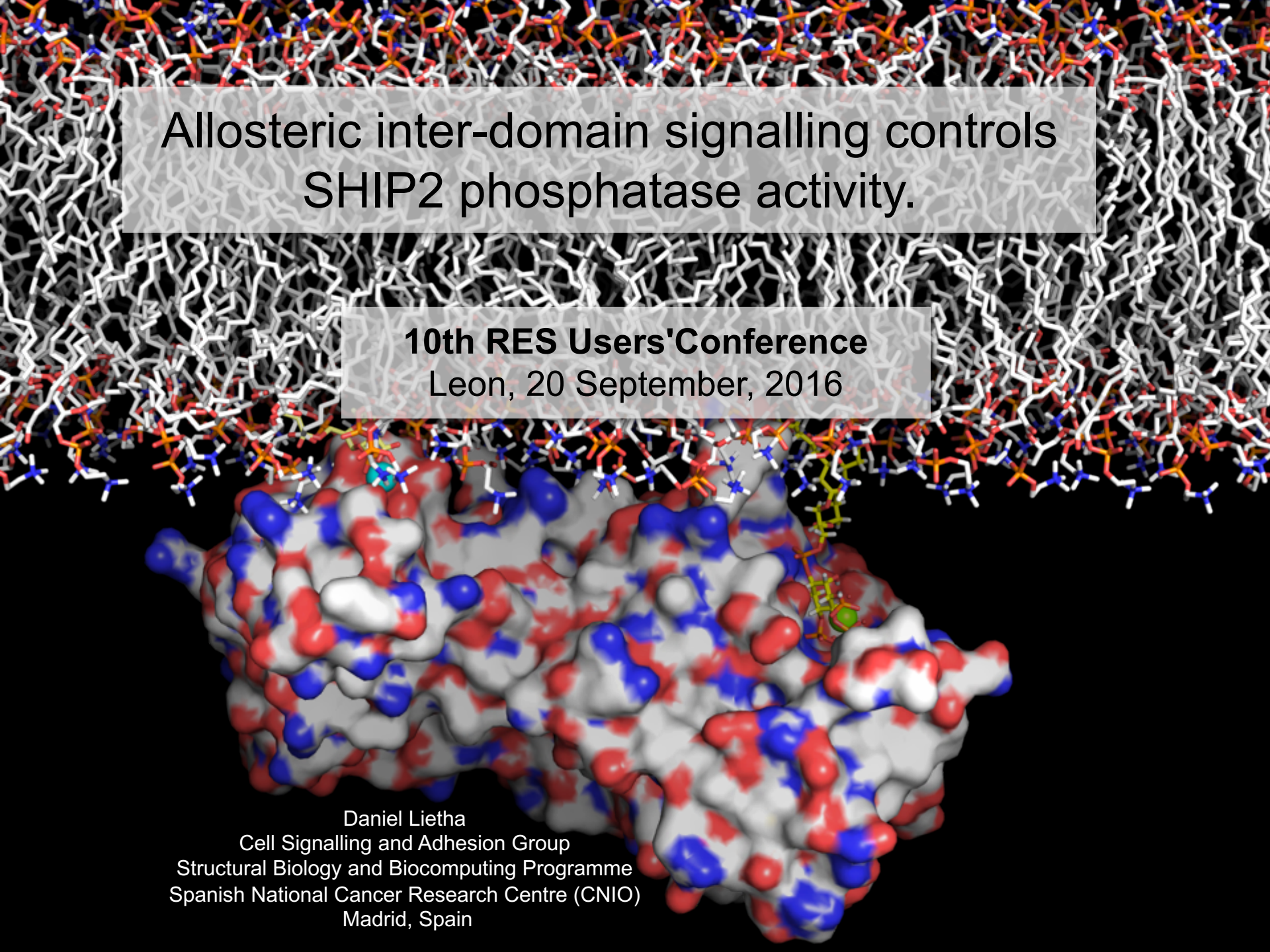
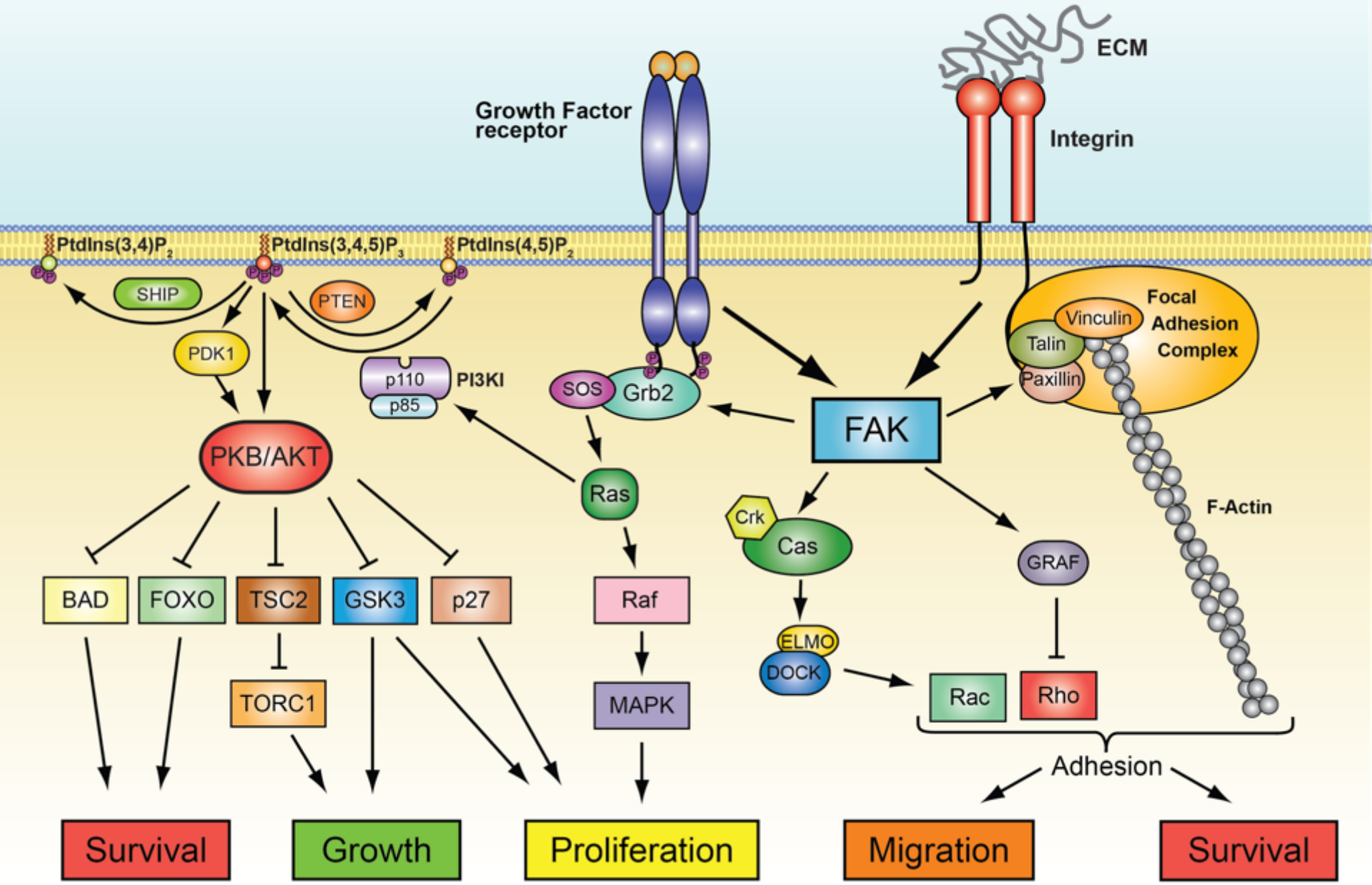
Allosteric inter-domain signalling controls SHIP2 phosphatase activity.

10th RES Users'Conference
Leon, 20 September, 2016

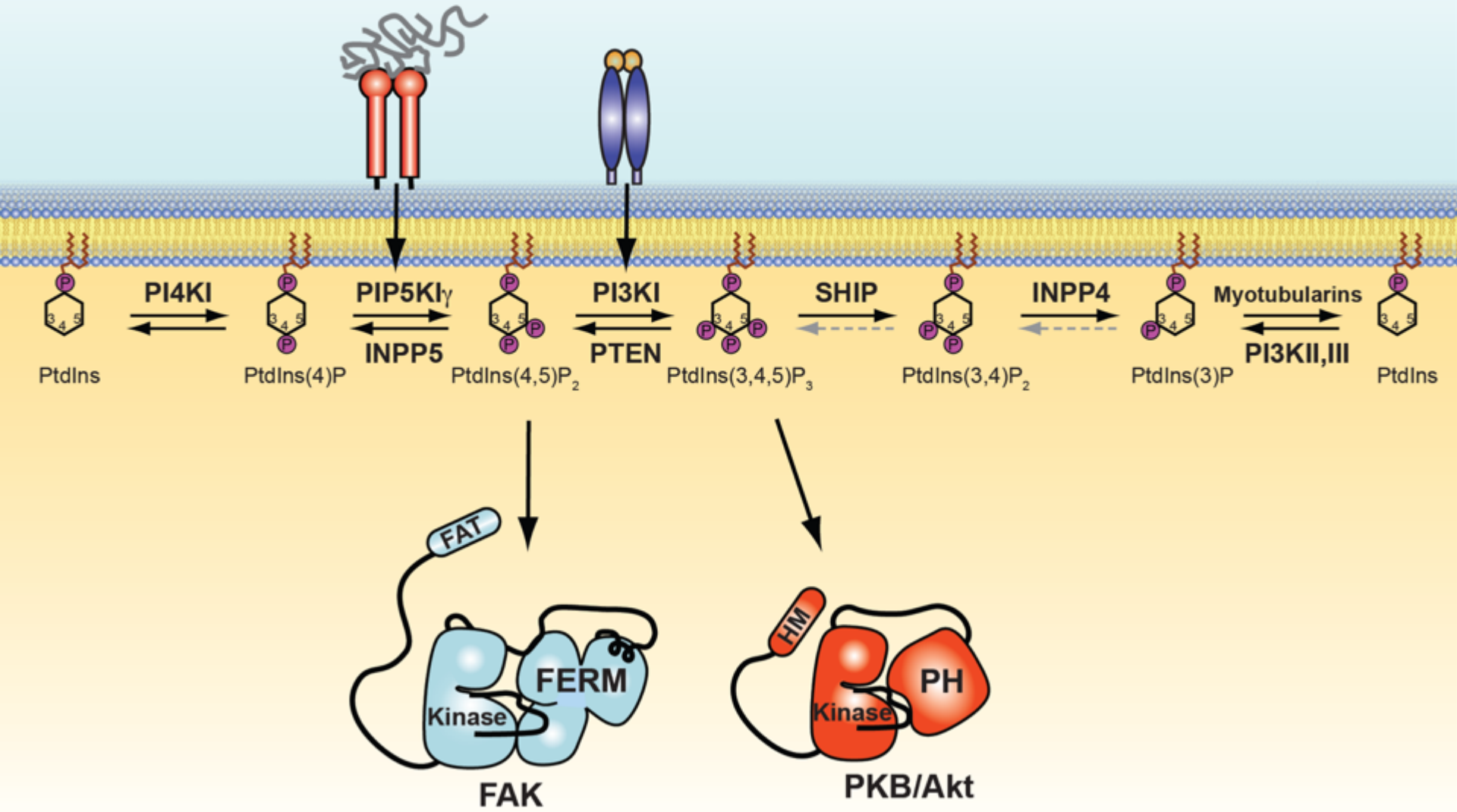


Daniel Lietha
Cell Signalling and Adhesion Group
Structural Biology and Biocomputing Programme
Spanish National Cancer Research Centre (CNIO)
Madrid, Spain

Growth and Adhesion signalling



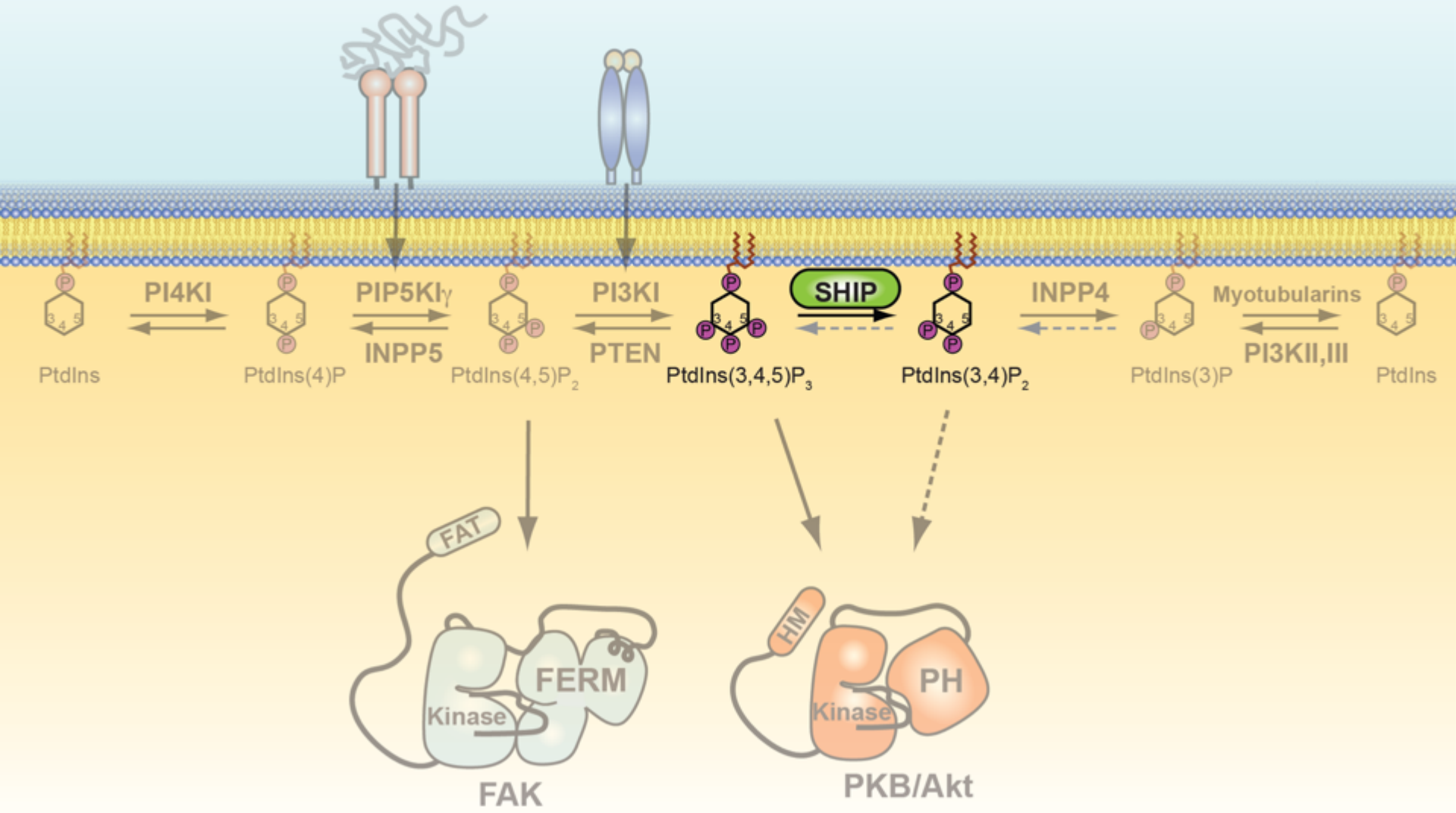
Growth and Adhesion signalling



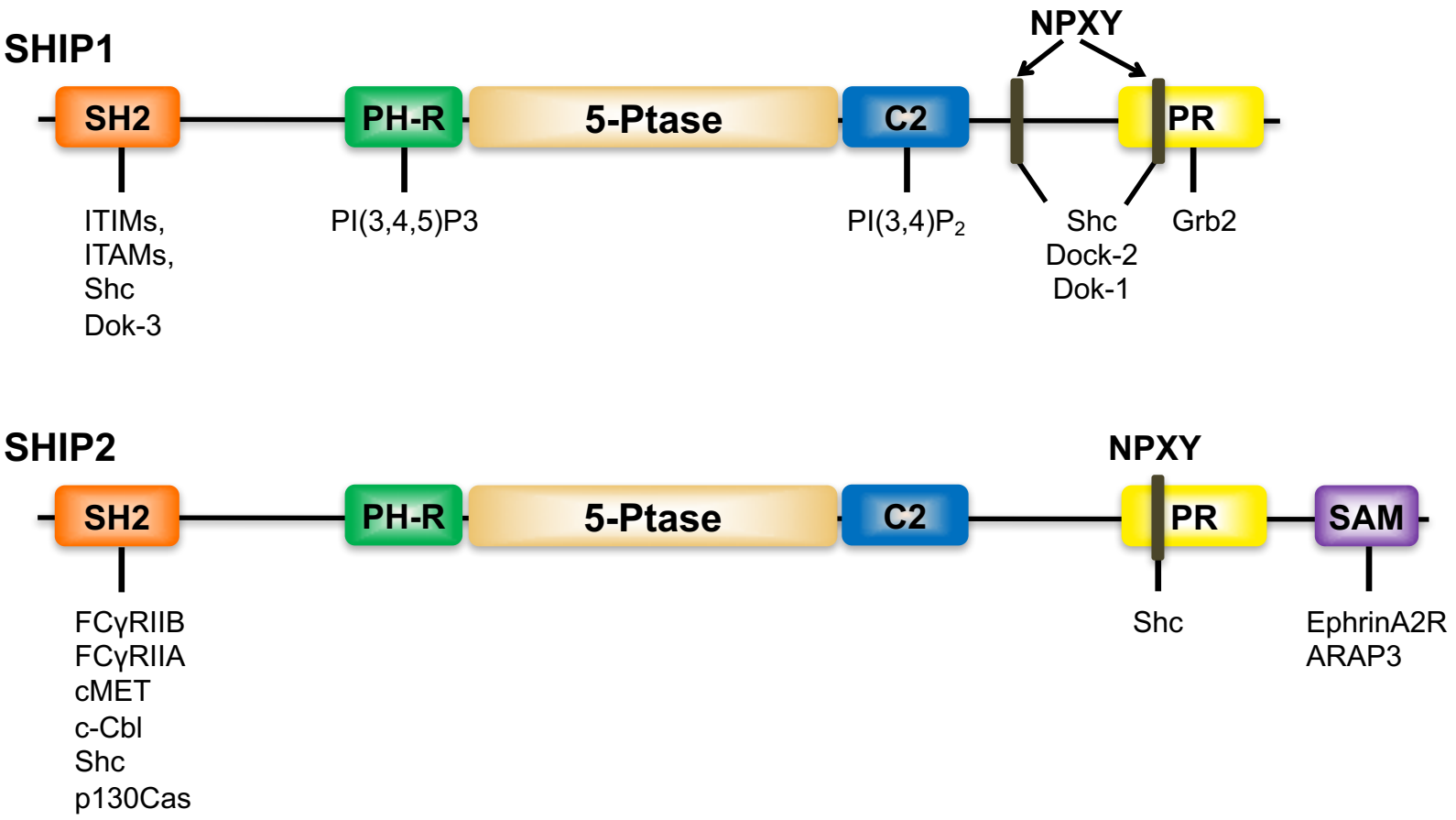
Goñi et al. (2014) *PNAS 111*, 3177

Balzano et al. (2015) *JBC 290,* 24975

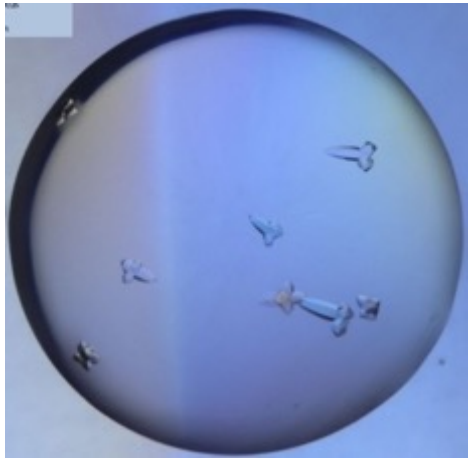
Growth and Adhesion signalling

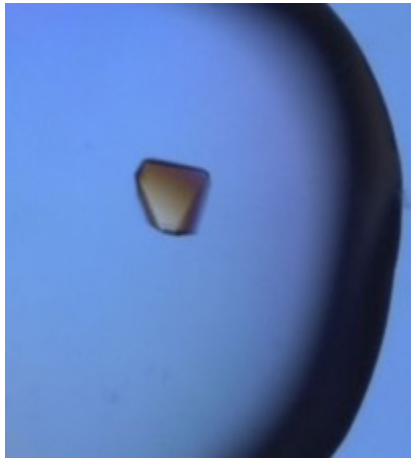


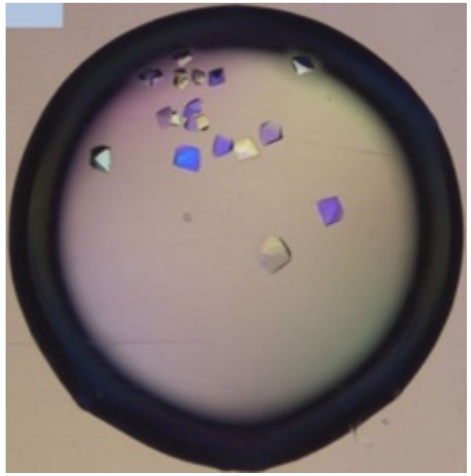
SH2-containing inositol phosphatases (SHIPs)

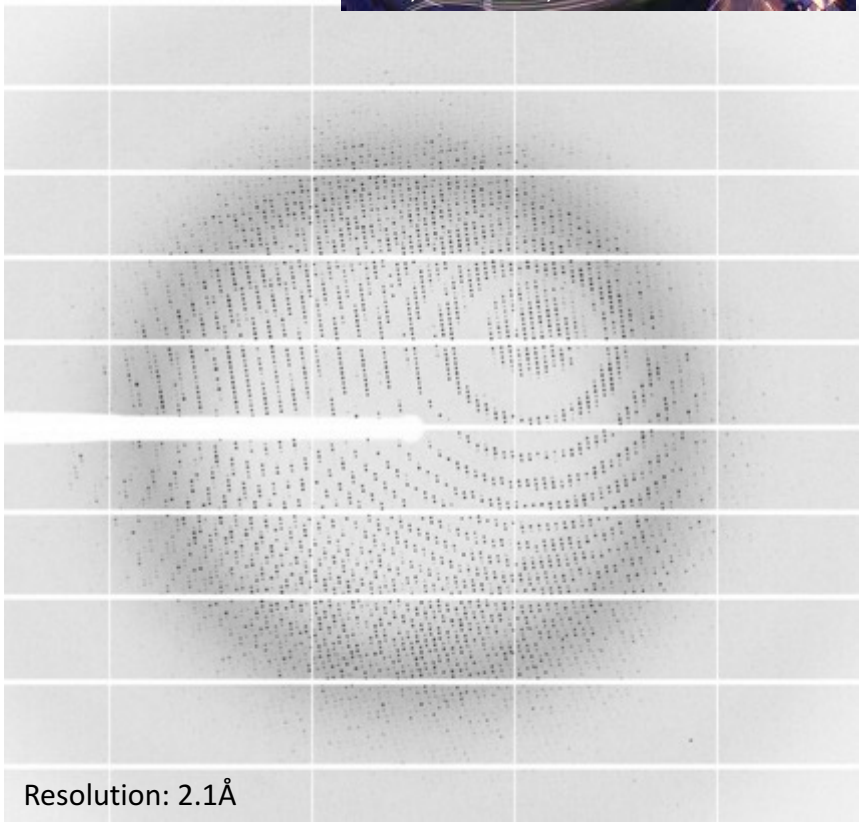


Crystallization of SHIP2 Ptase-C2

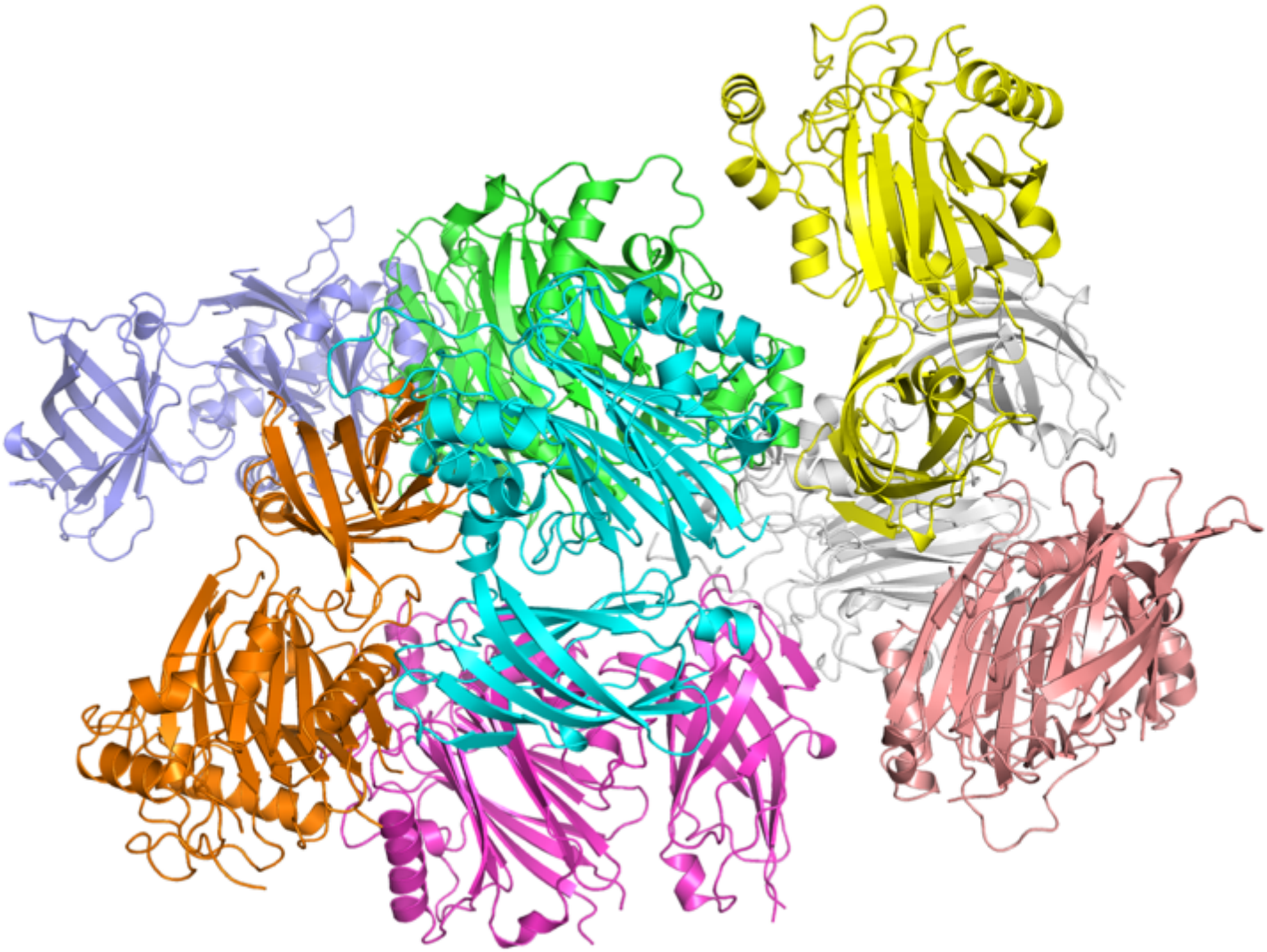




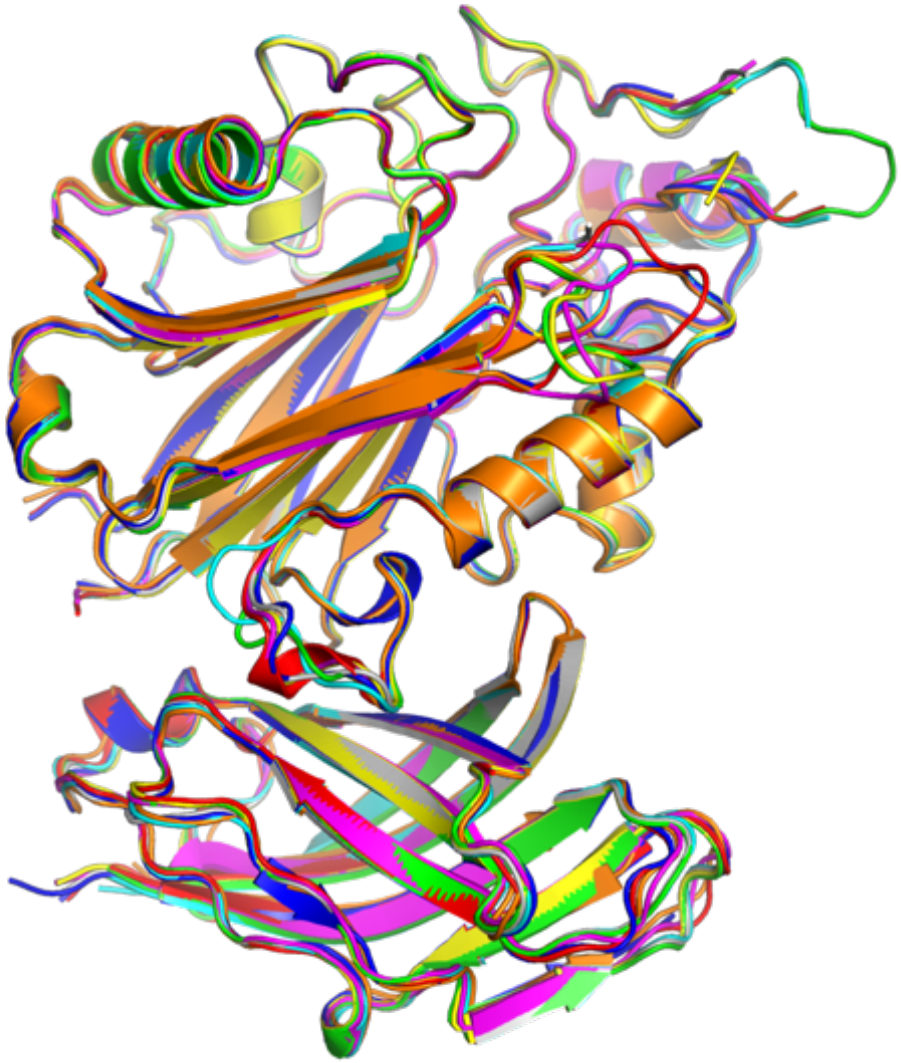




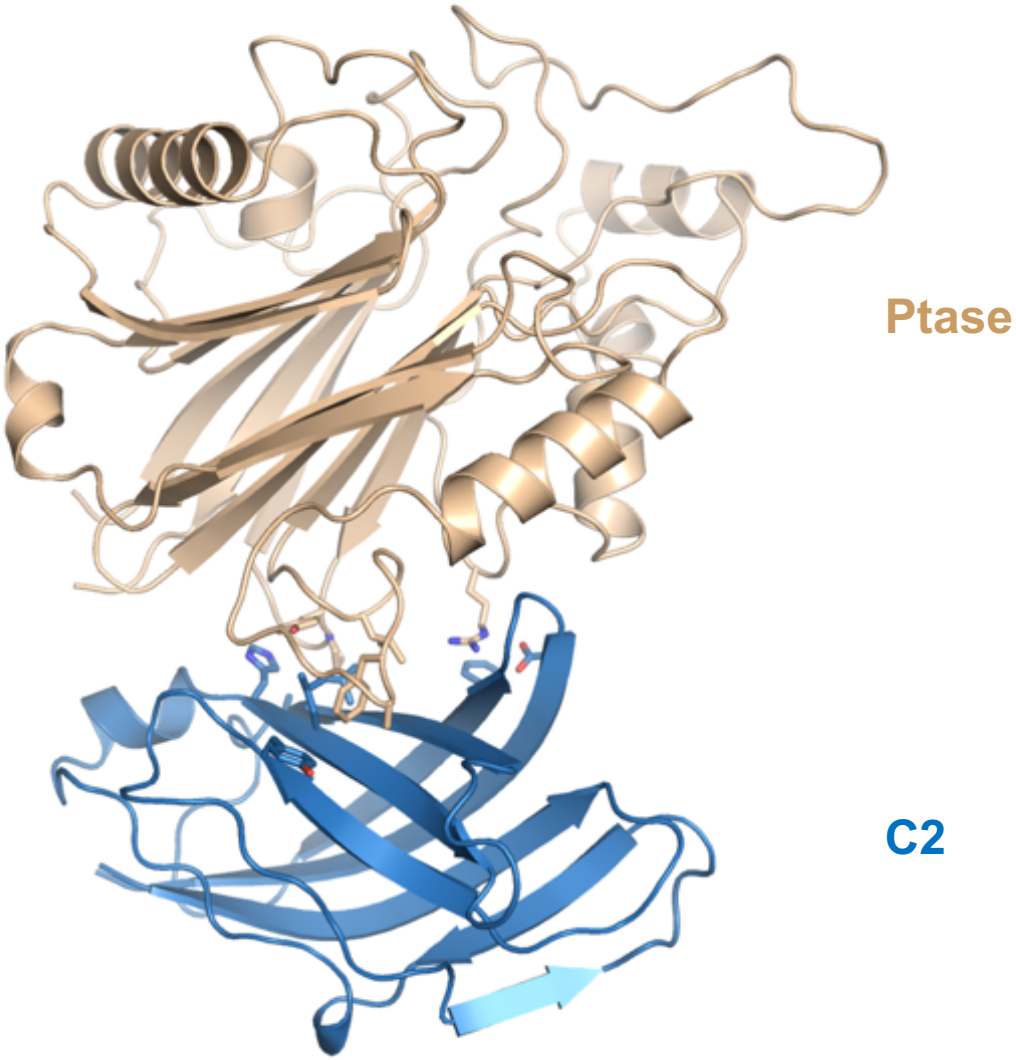
Asymmetric unit cell



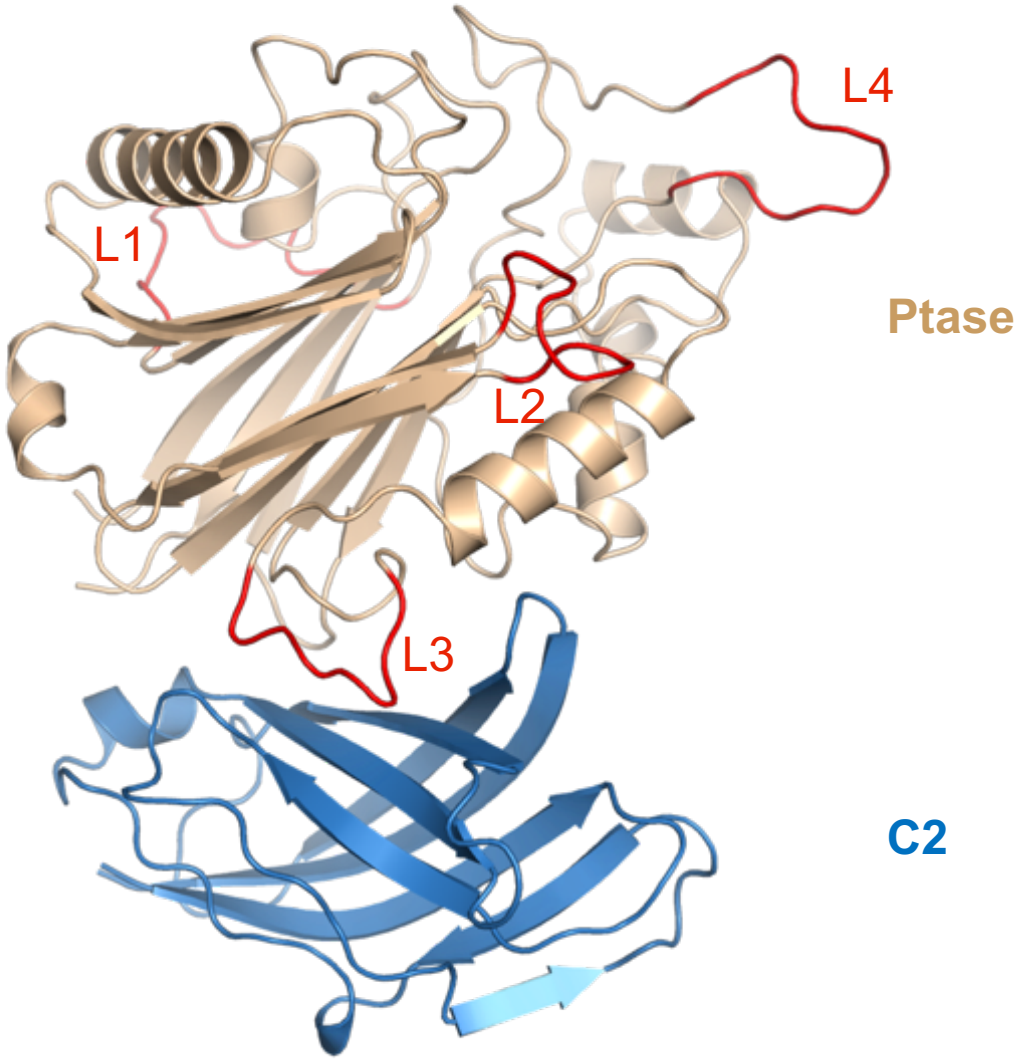
SHIP2 Ptase-C2 superposition



SHIP2 Ptase-C2 structure



SHIP2 Ptase-C2 structure



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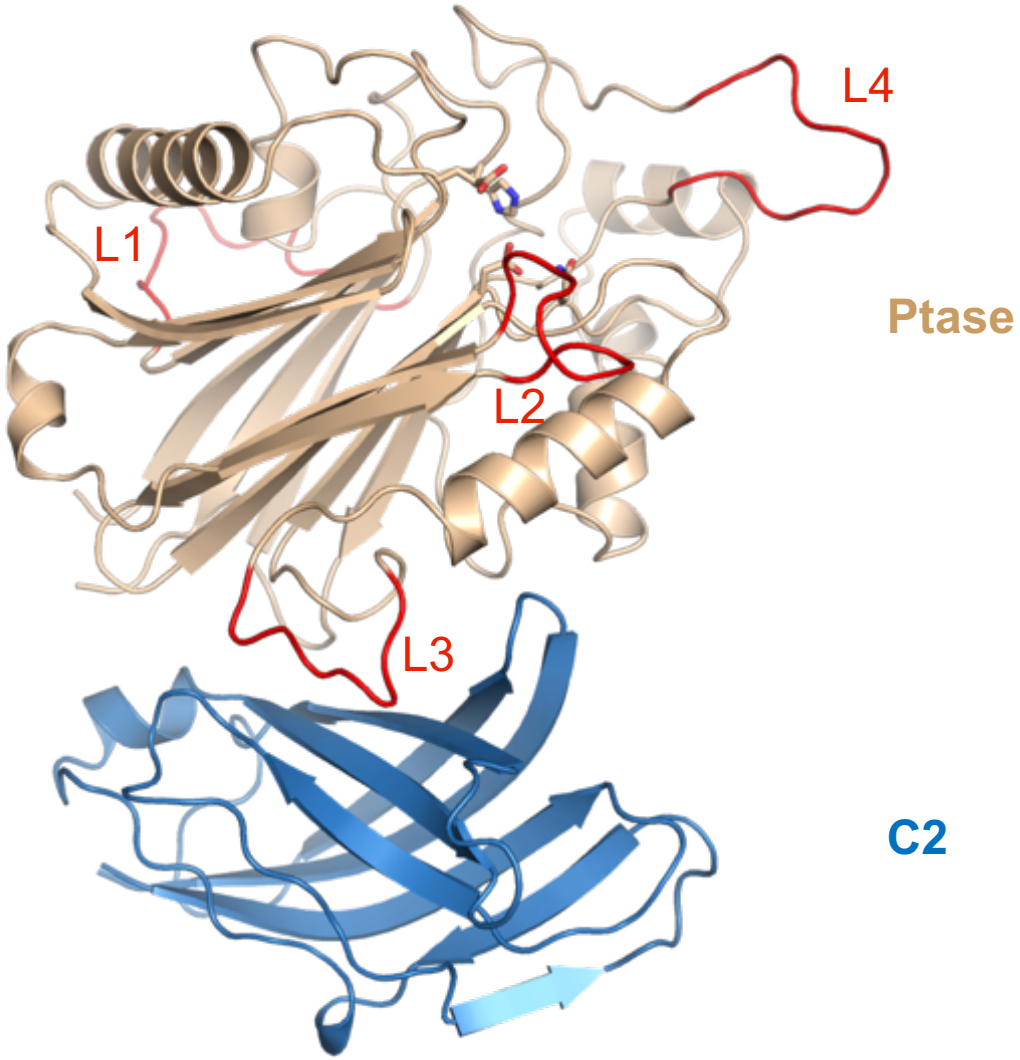
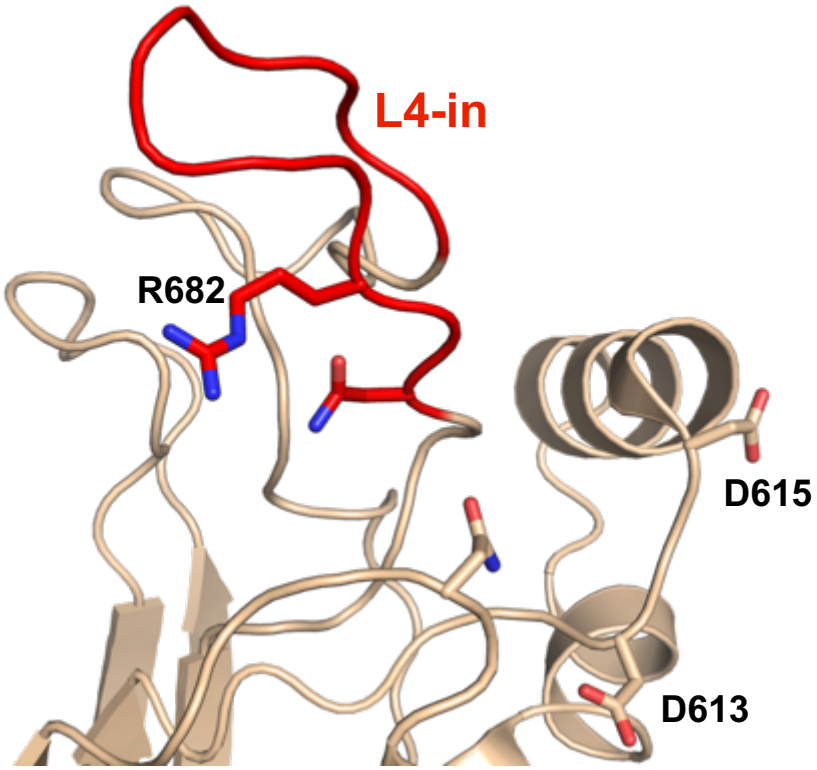
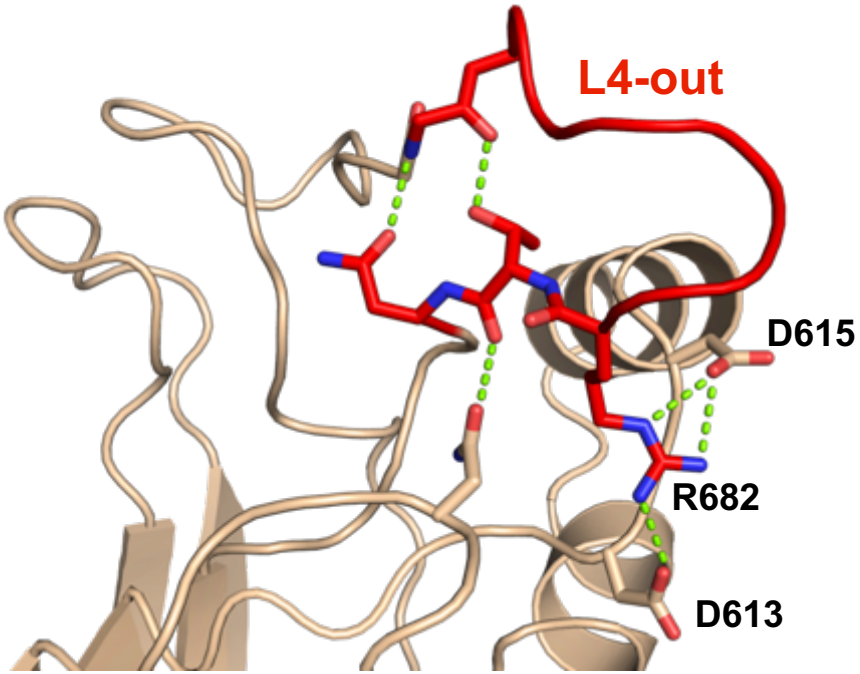


Alternative loop 4 conformations

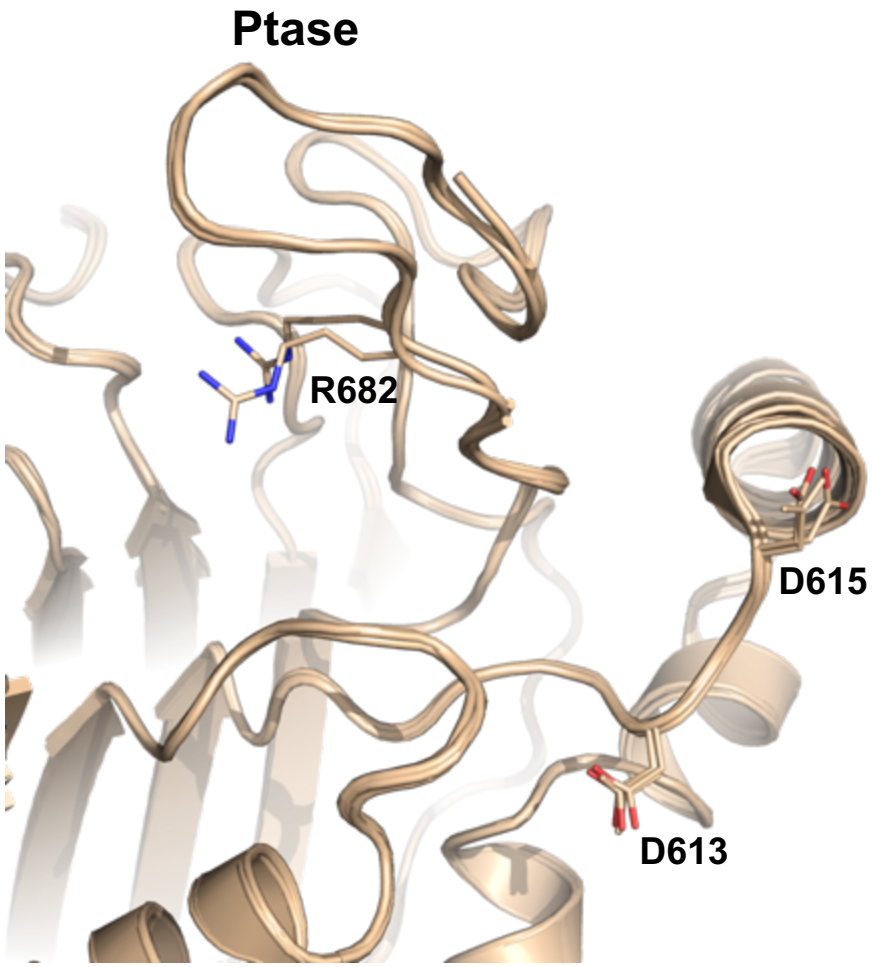


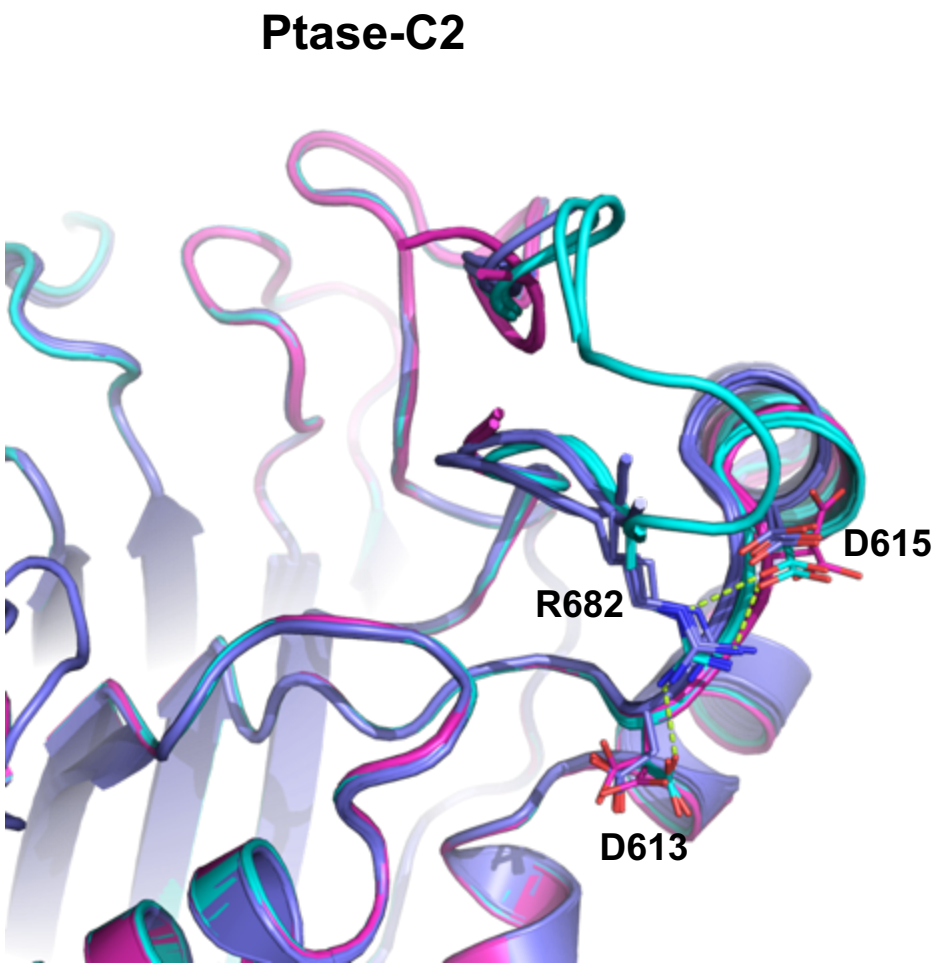
Ptase



Ptase-C2

SHIP2 Ptase-C2 structure

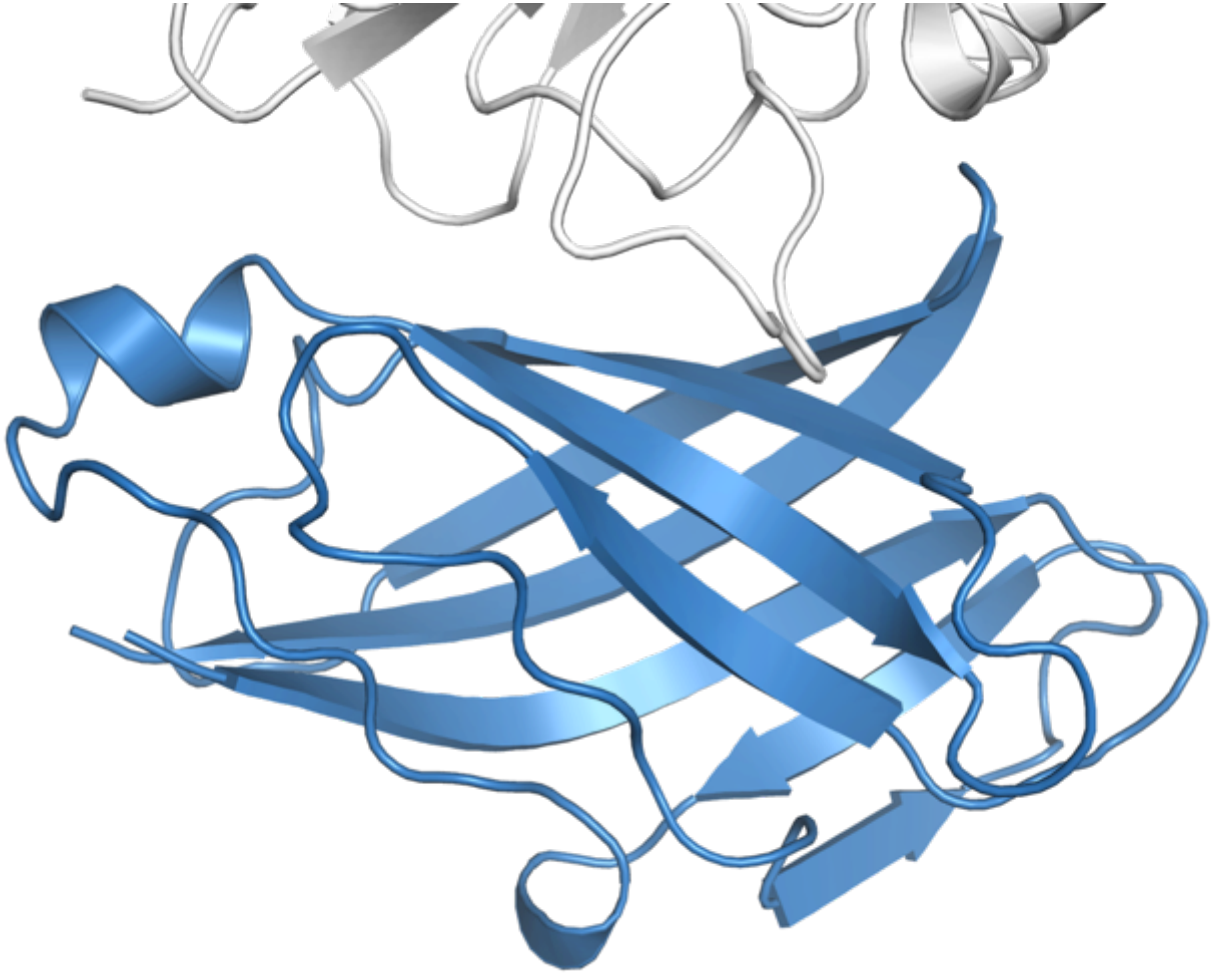




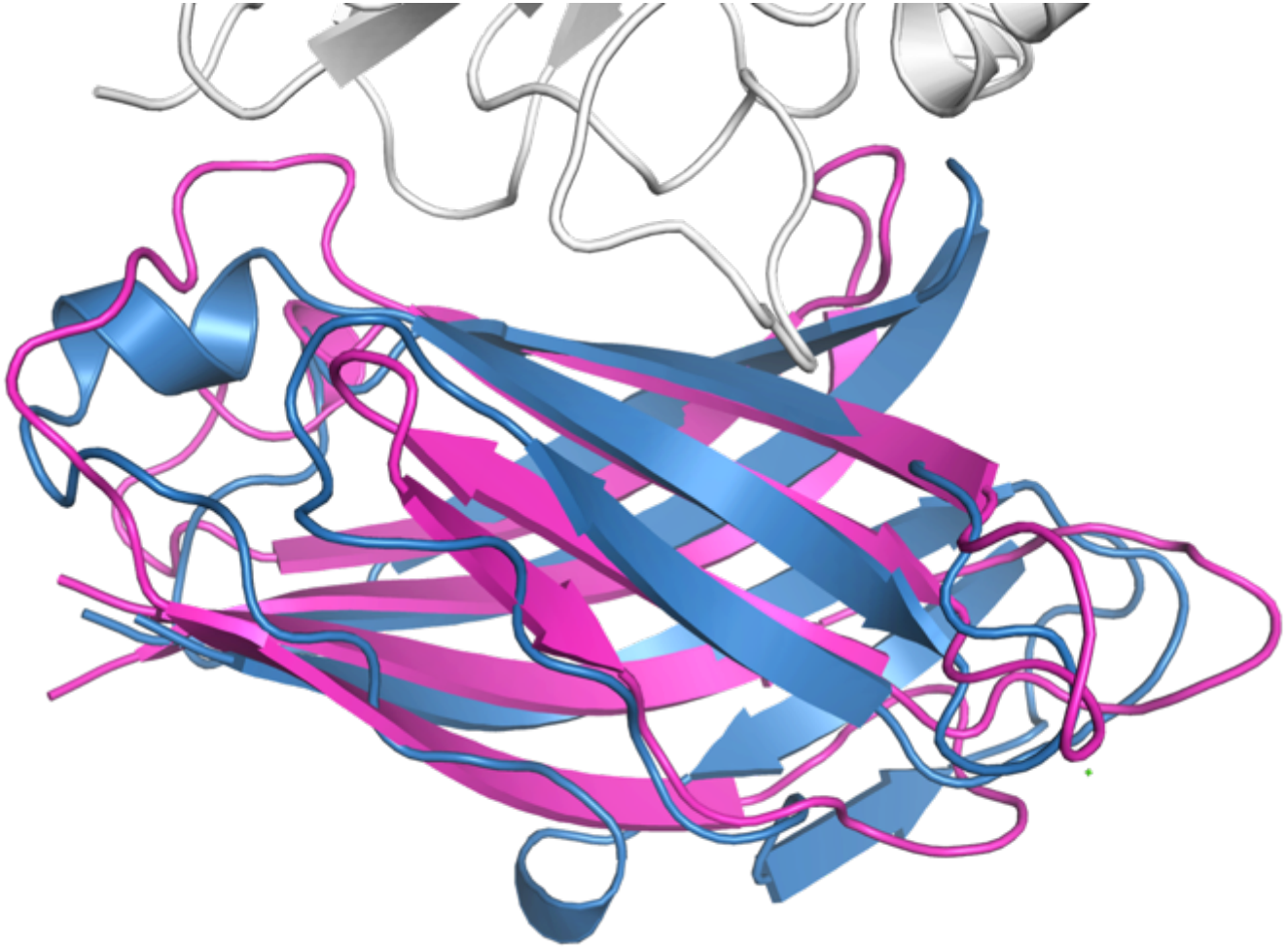
4x R682 unbound

2x R682 unbound
4x R682 singly bound to D615
2x R682 doubly bound to D613 and D615

SHIP2 C2 domain

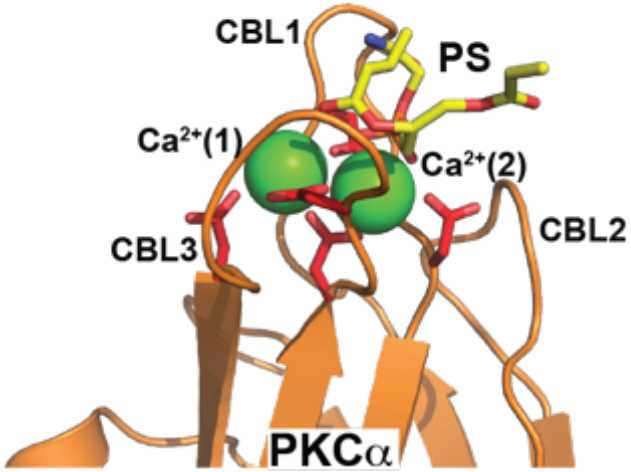


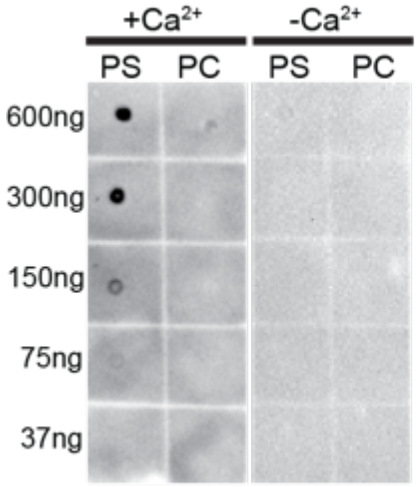
SHIP2 C2 domain is closest to PKC-type

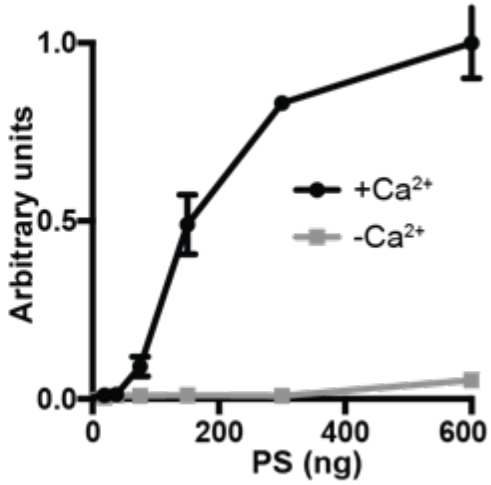


Dysferlin (PDB 4IHB, rmsd=2.5, %ID =15)

SHIP2 C2 domain binds PS lipid and Ca^{2+}

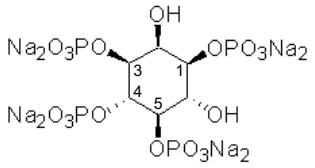




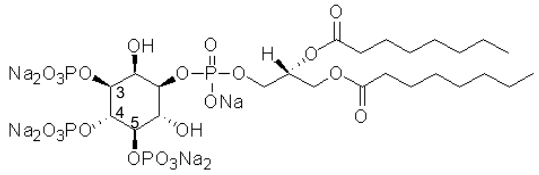


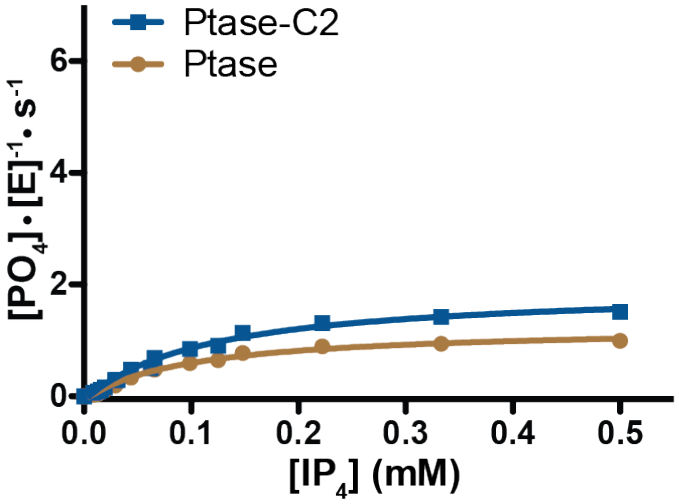
SHIP2 Enzyme activity

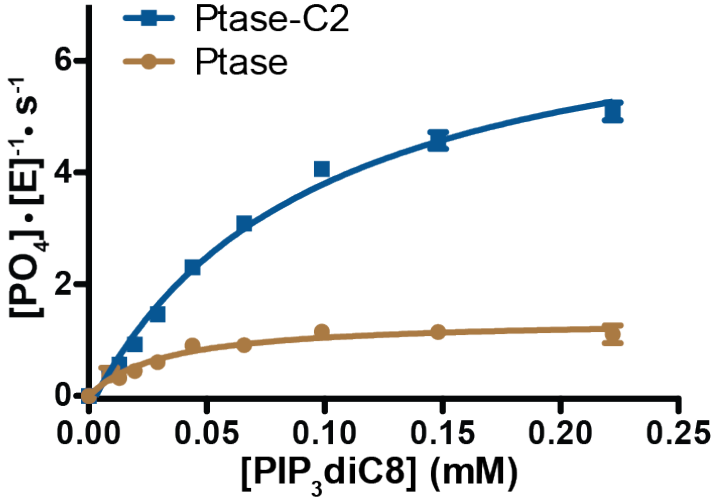
IP_4 (head group)



PIP_3diC8

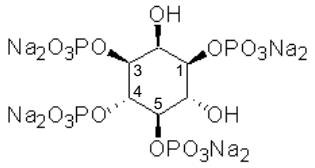


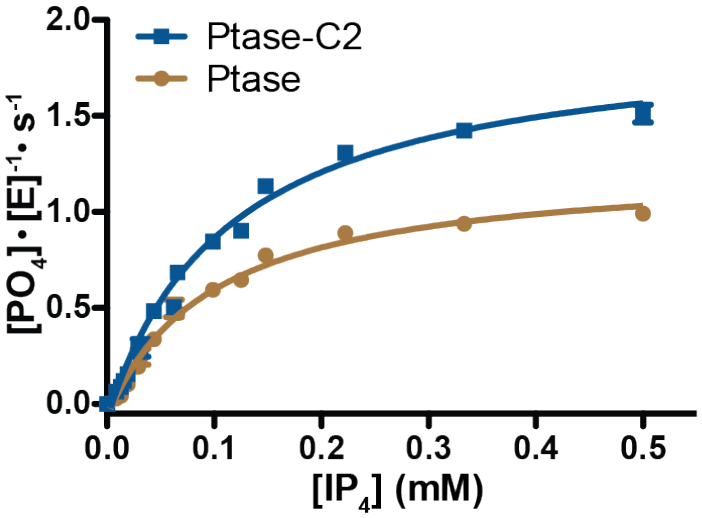




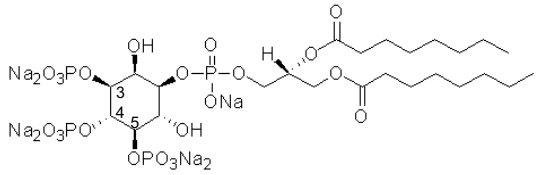
SHIP2 Enzyme activity

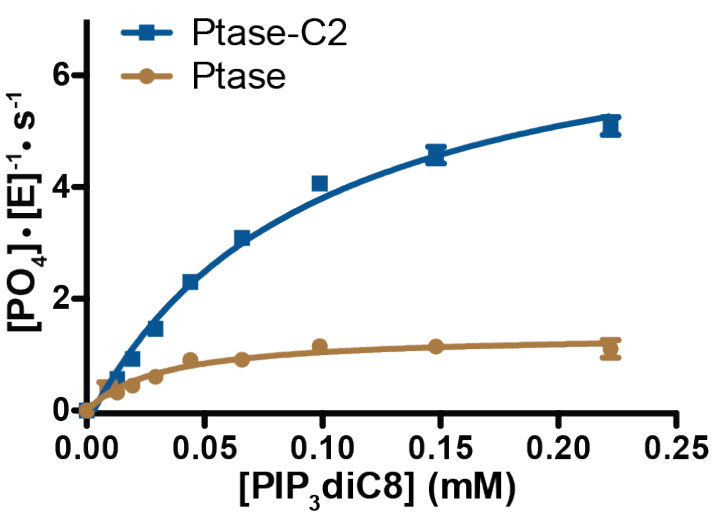
IP4 (head group)



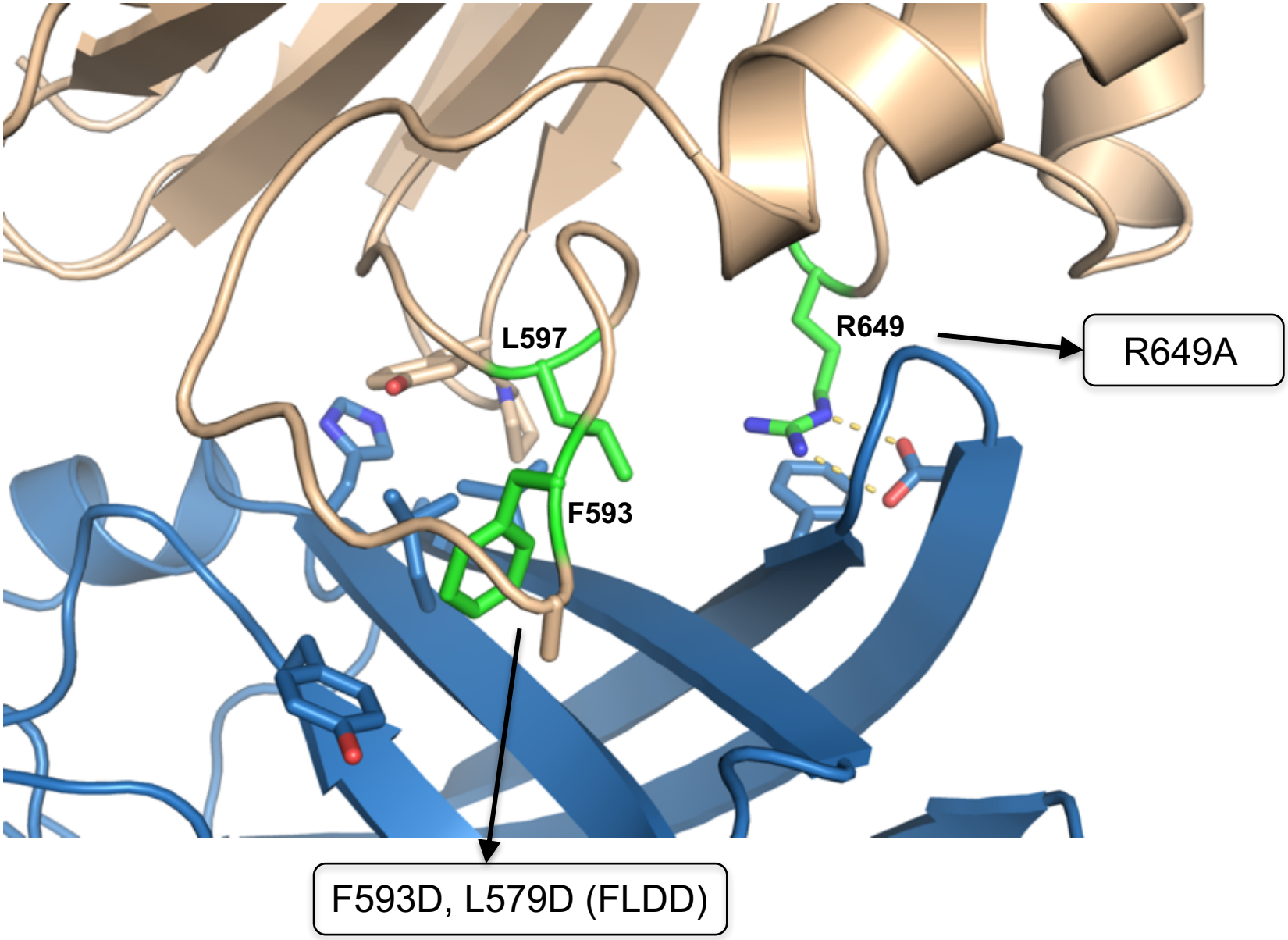


PIP_3diC8

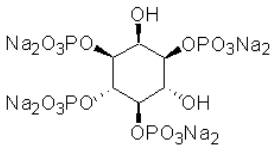


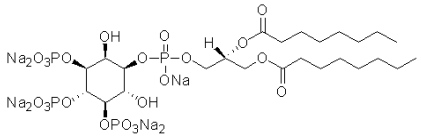


Interface mutations



SHIP2 Enzyme activity





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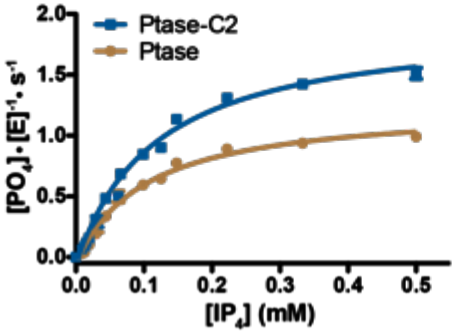

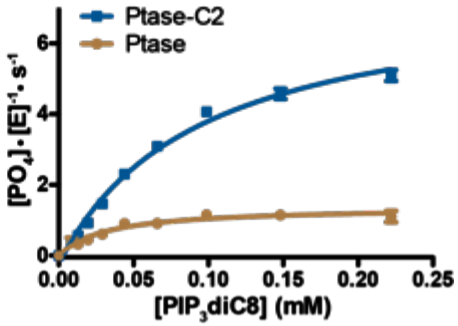

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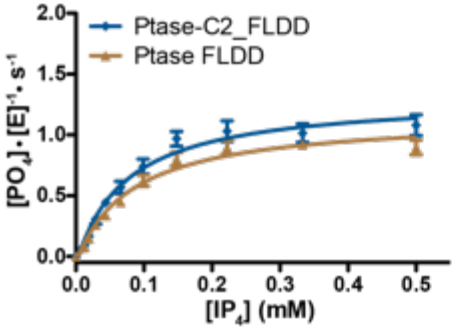

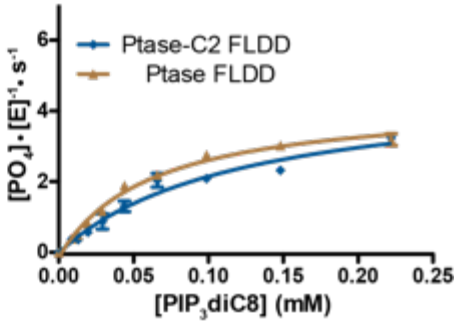

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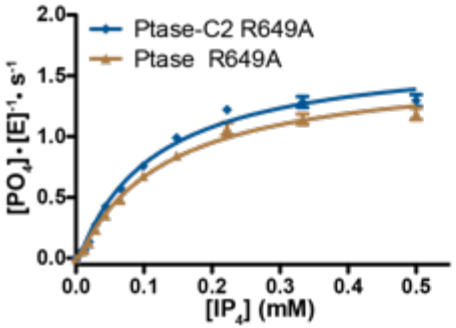
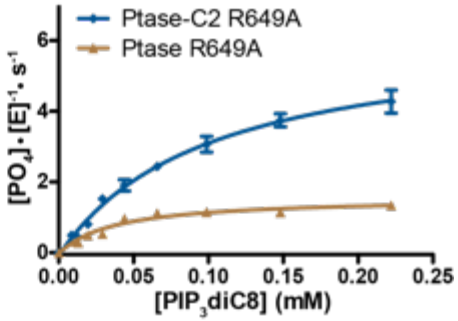
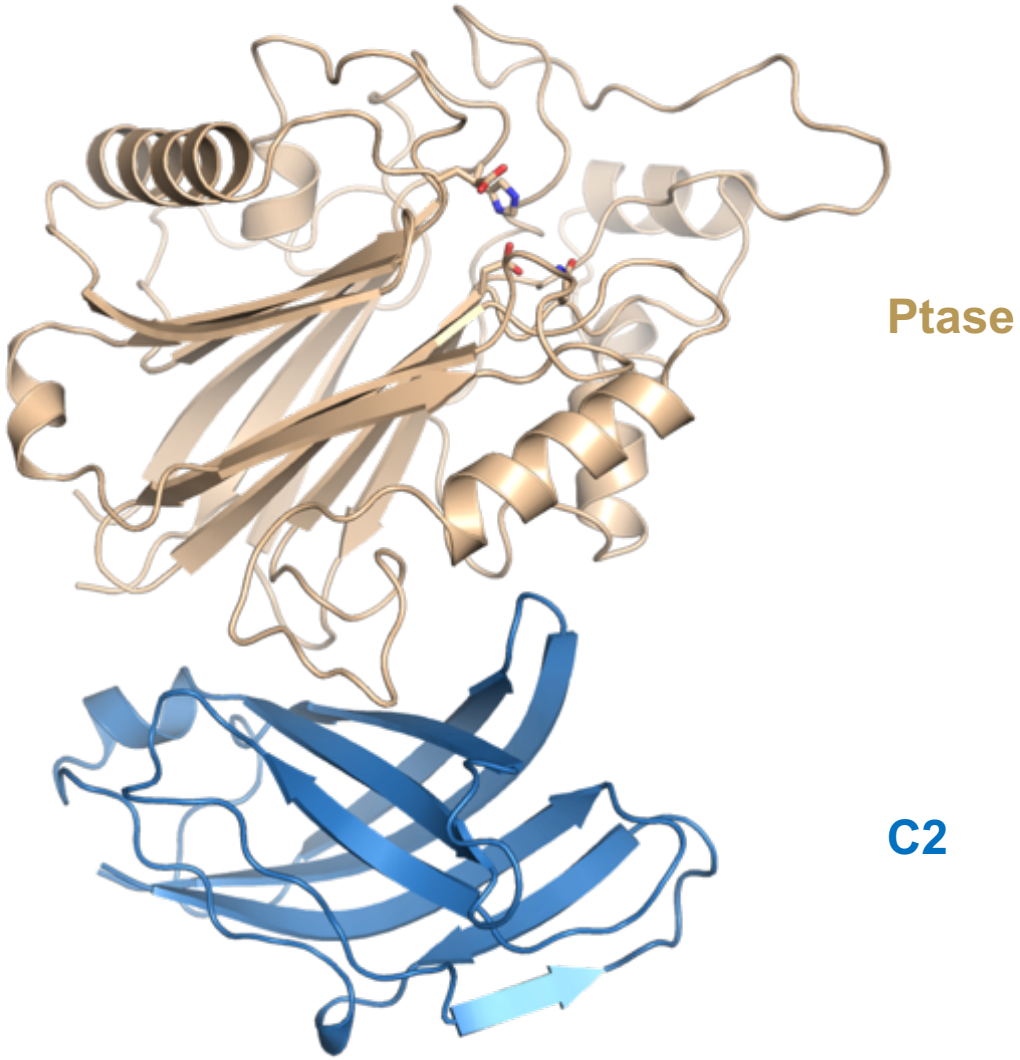




How does C2 domain communicate to active site?

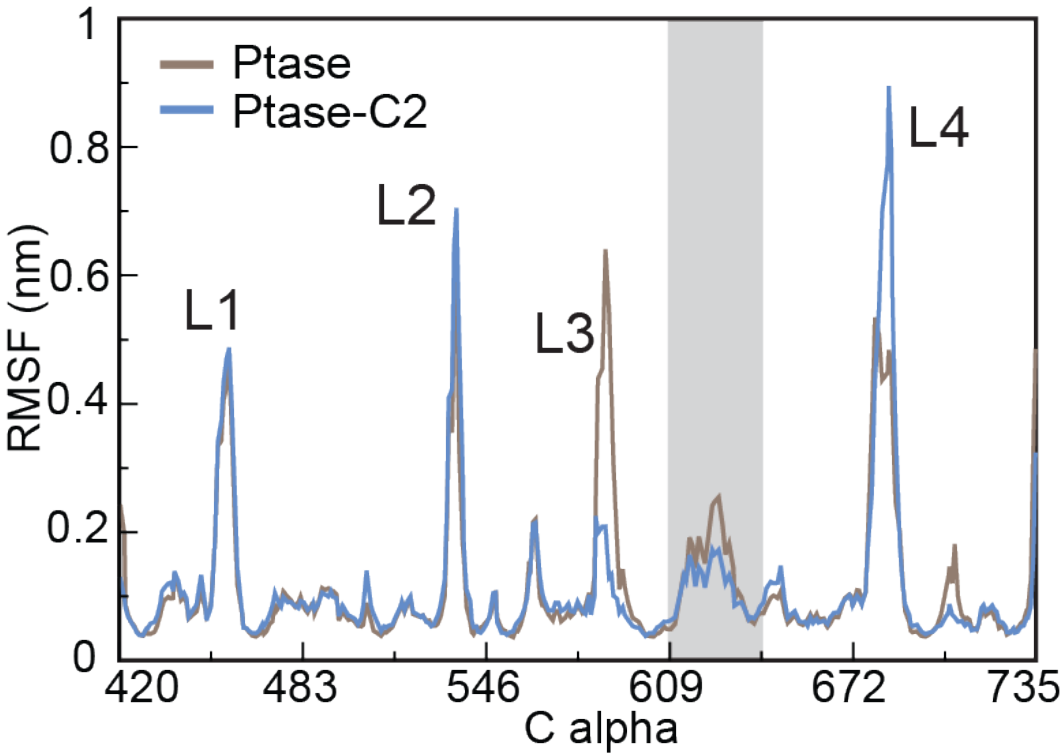


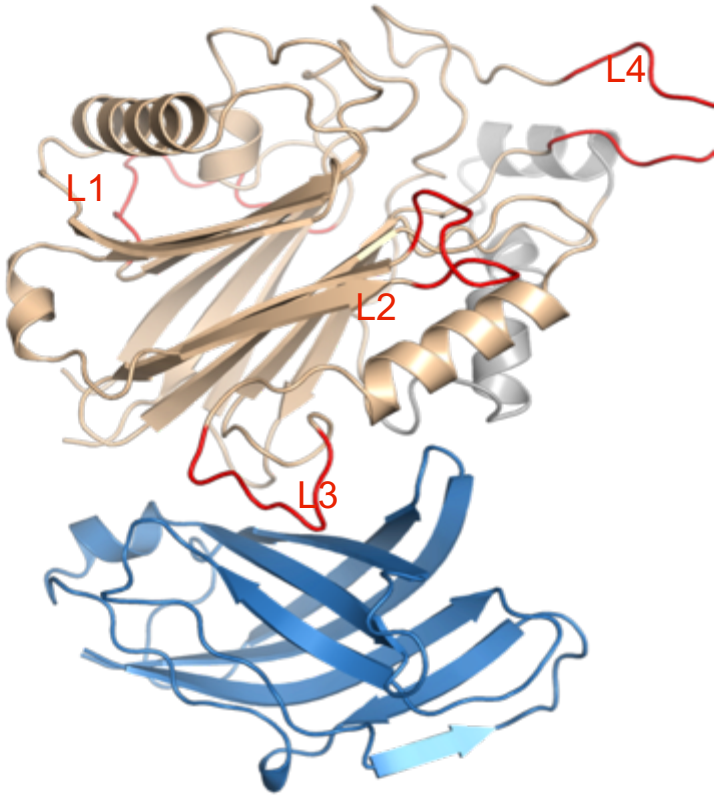
Molecular dynamics simulations

Marta Camacho
PhD student
Cell Signalling and Adhesion Group,
CNIO, Spain

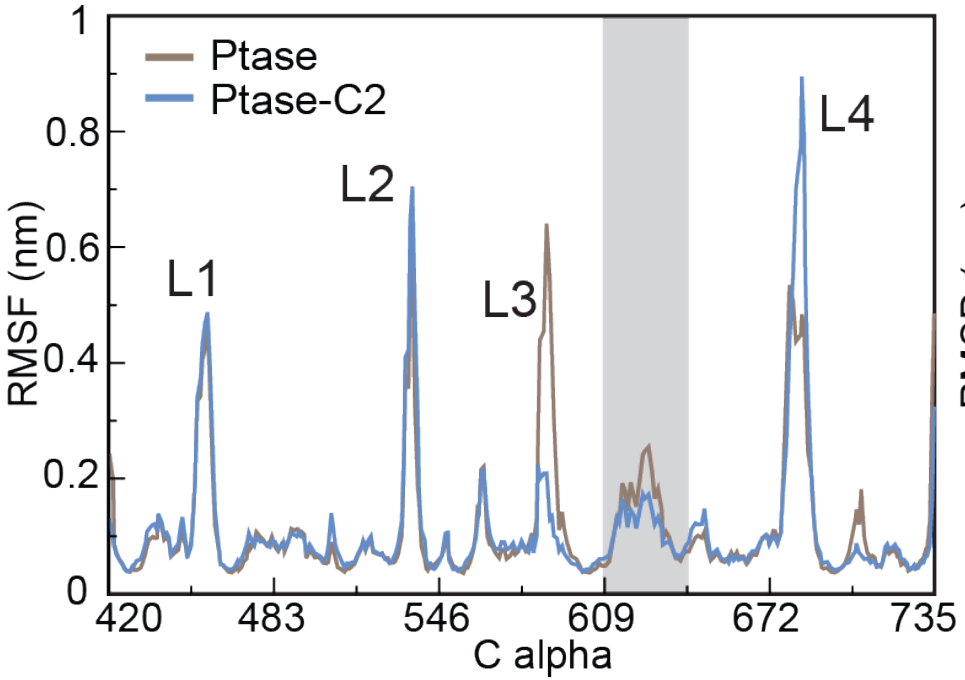
Nicole Dolker
Senior Scientist with Alfonso Valencia
Structural Computational Biology Group
CNIO, Spain

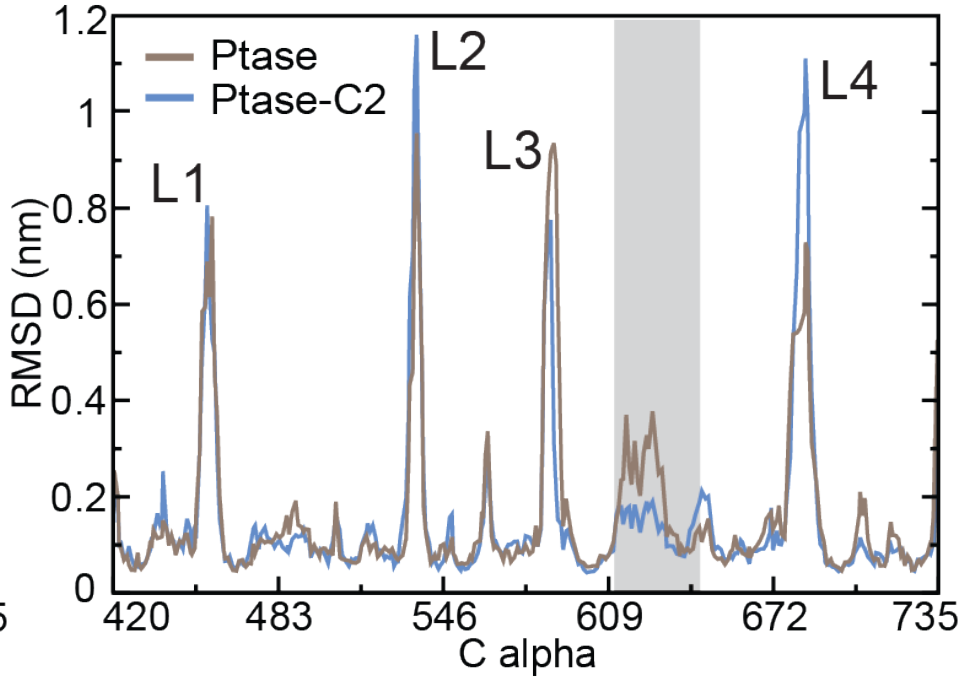
Fluctuations



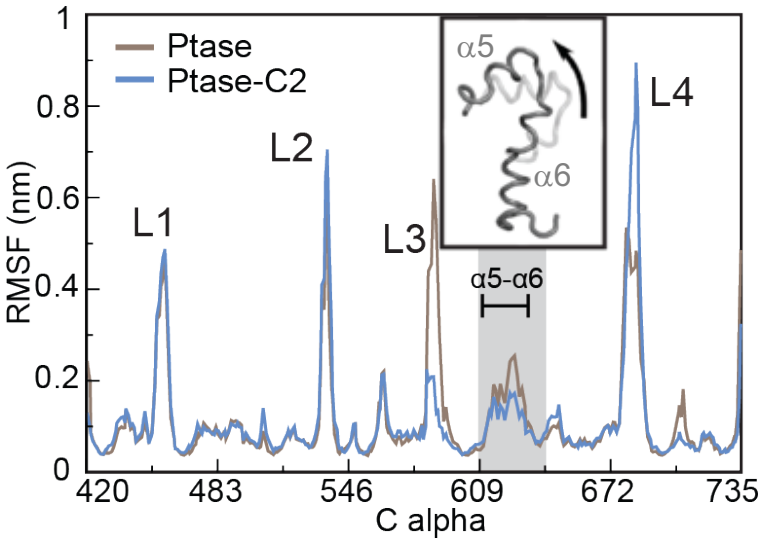


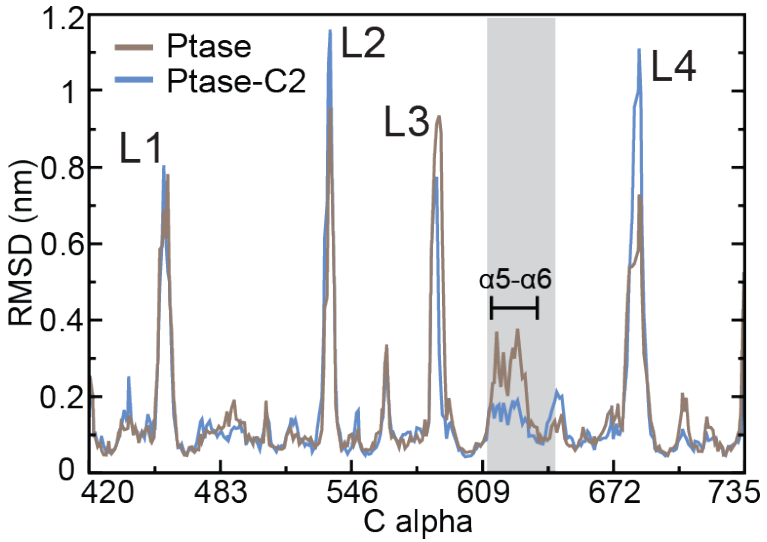
Fluctuations and Deviations

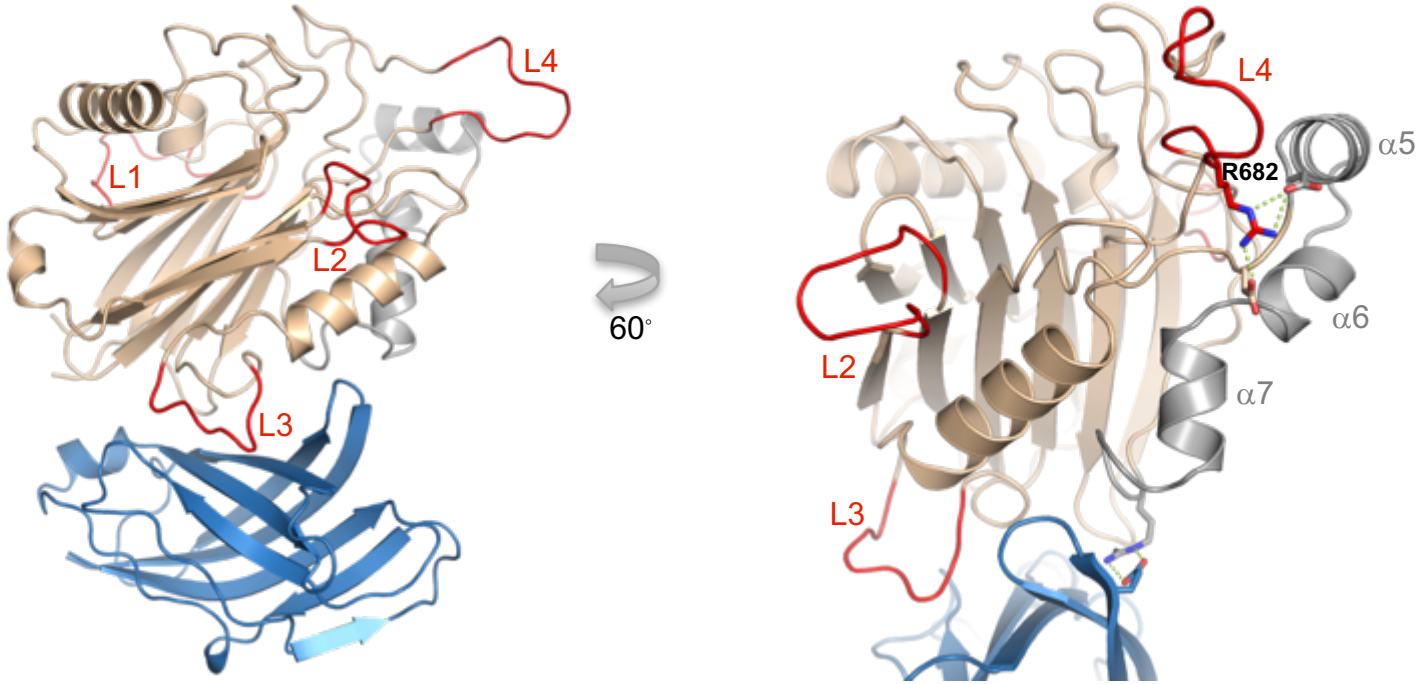




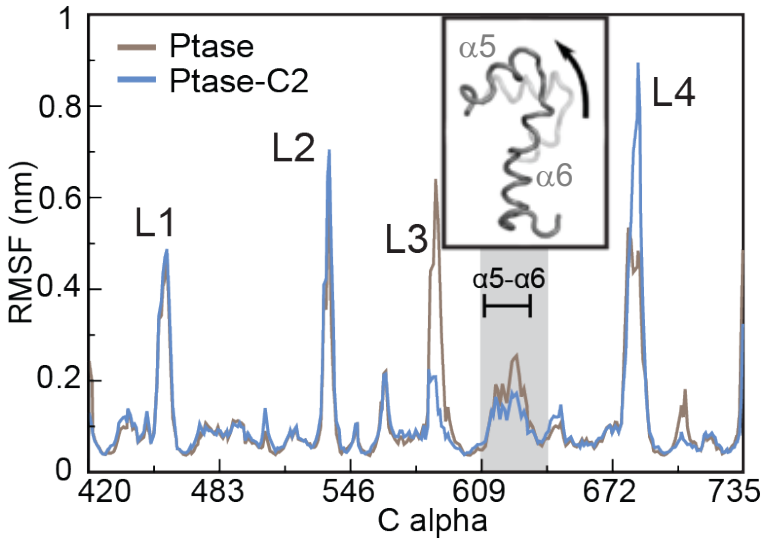
Fluctuations in helices α5 and α6

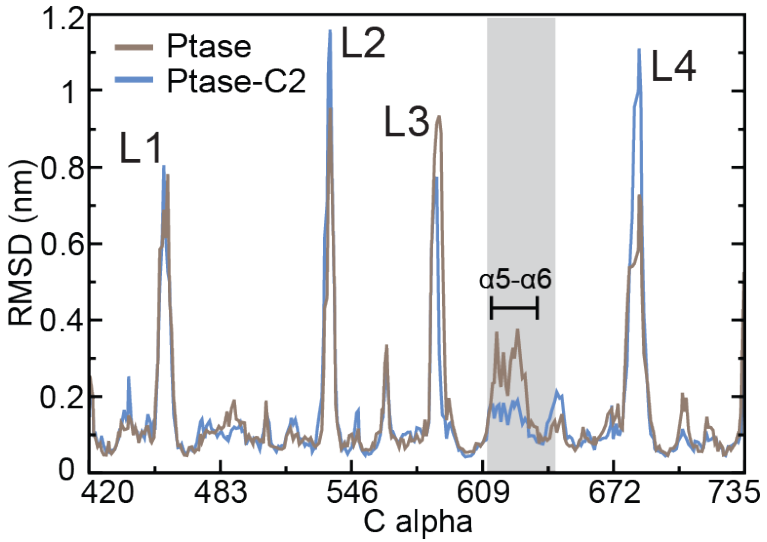


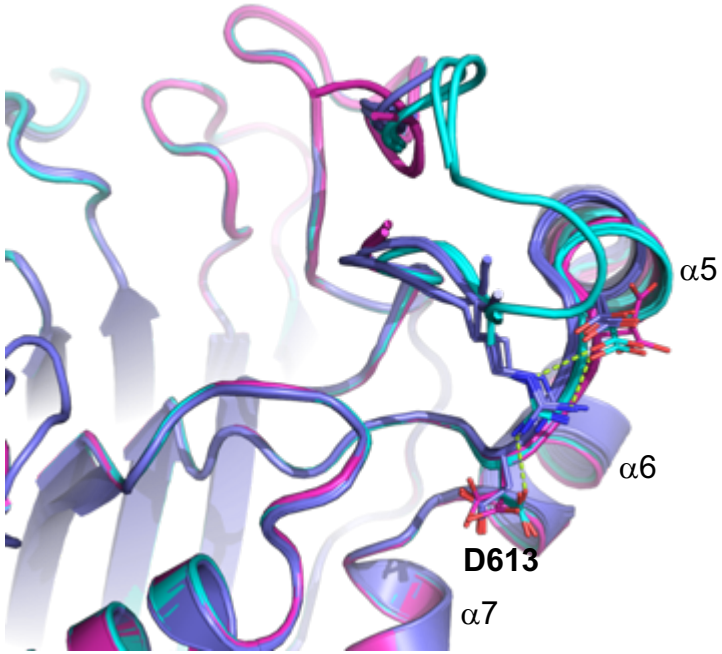


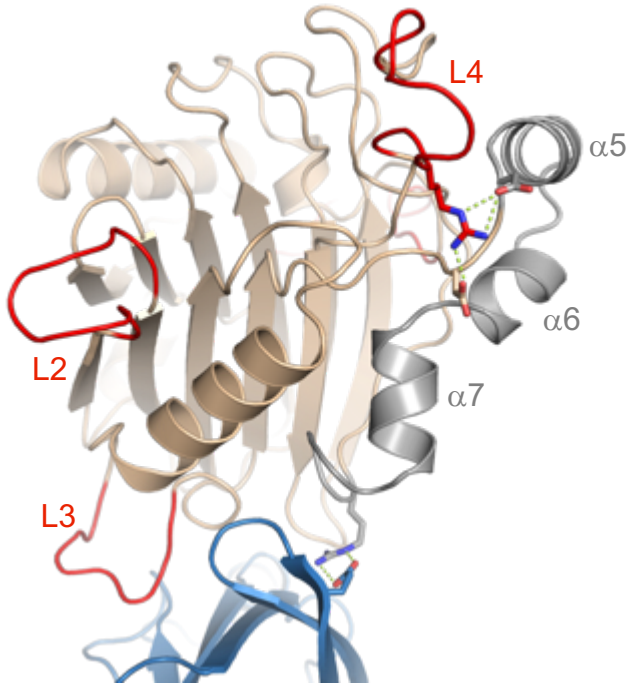


Fluctuations in helices α5 and α6

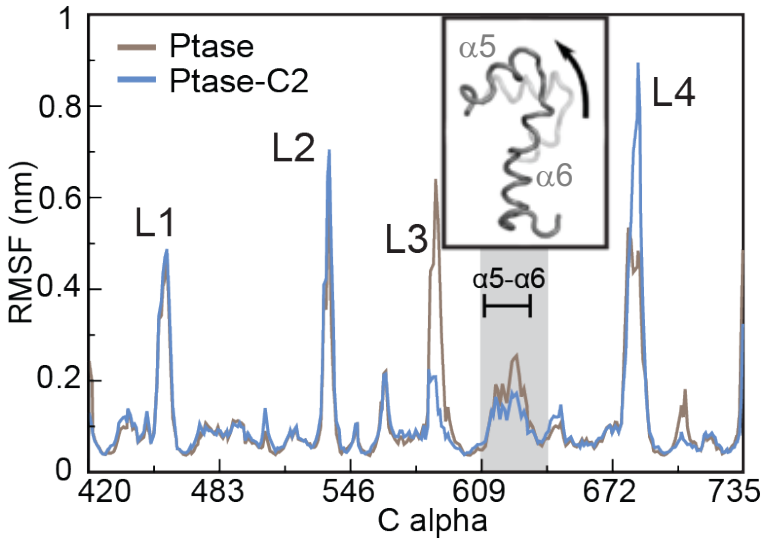


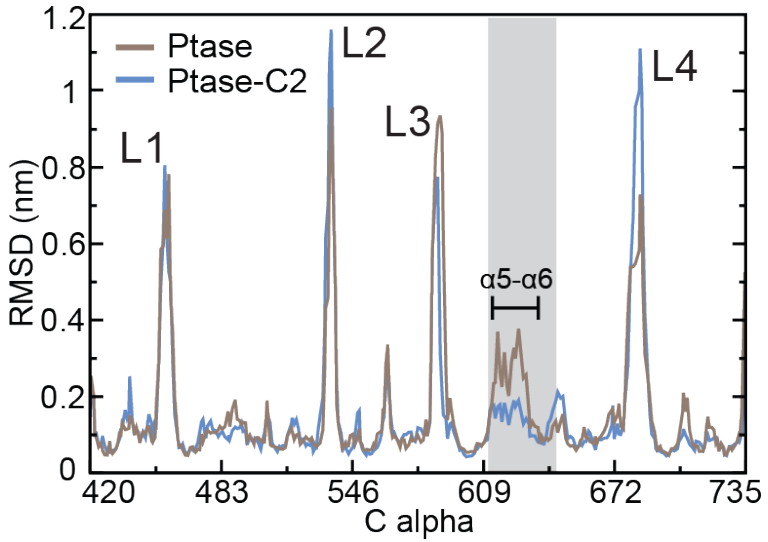


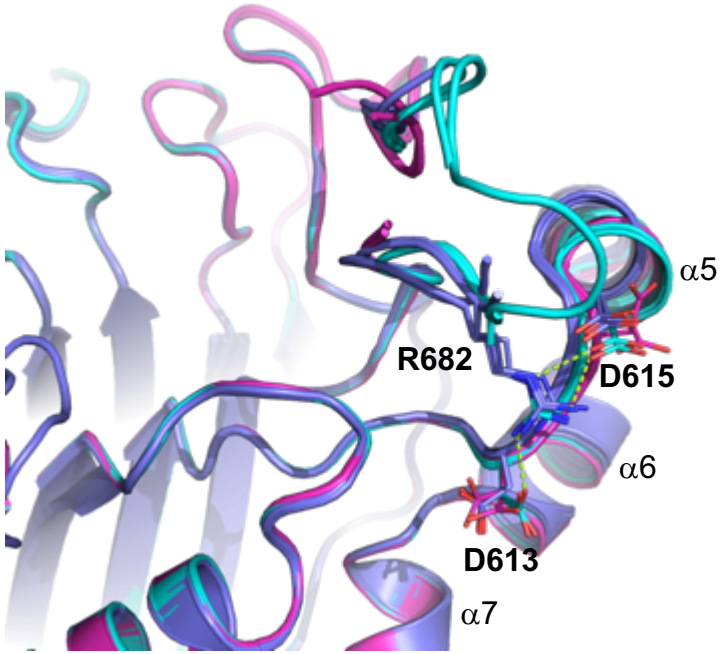




Fluctuations in helices α5 and α6







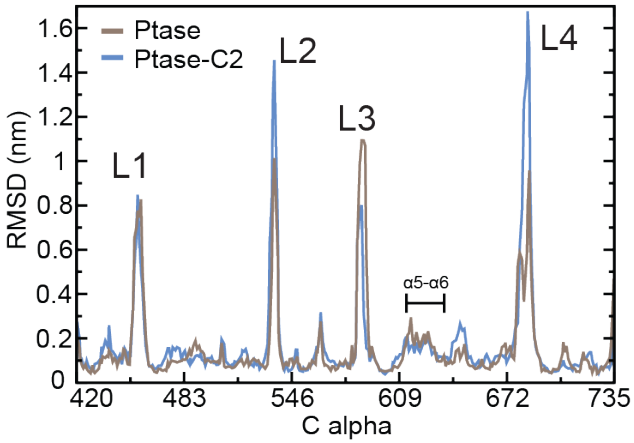
R682 unbound

R682 singly bound to D615

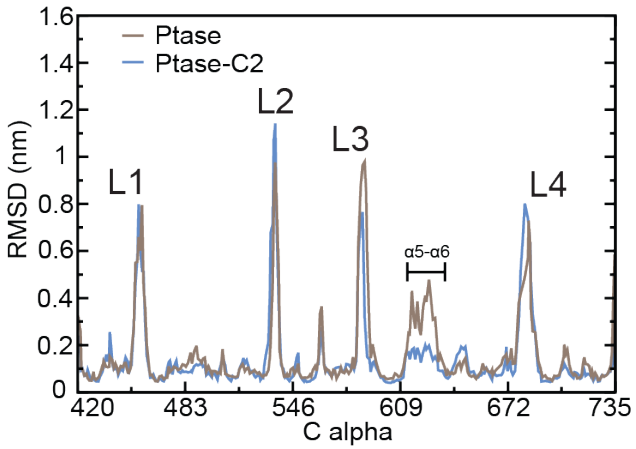
R682 doubly bound to D613 and D615

α5 and α6 fluctuations depend on R682 state

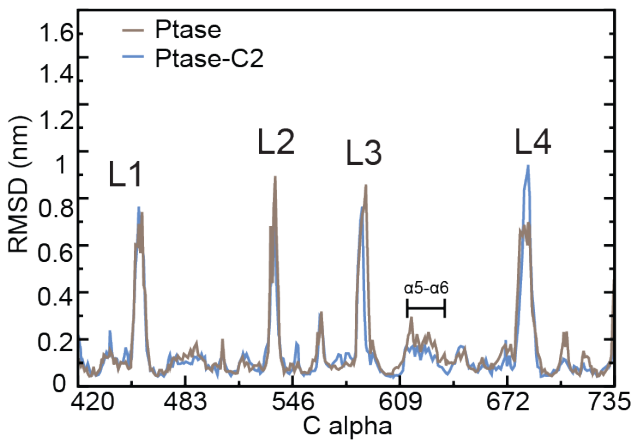
R682 unbound

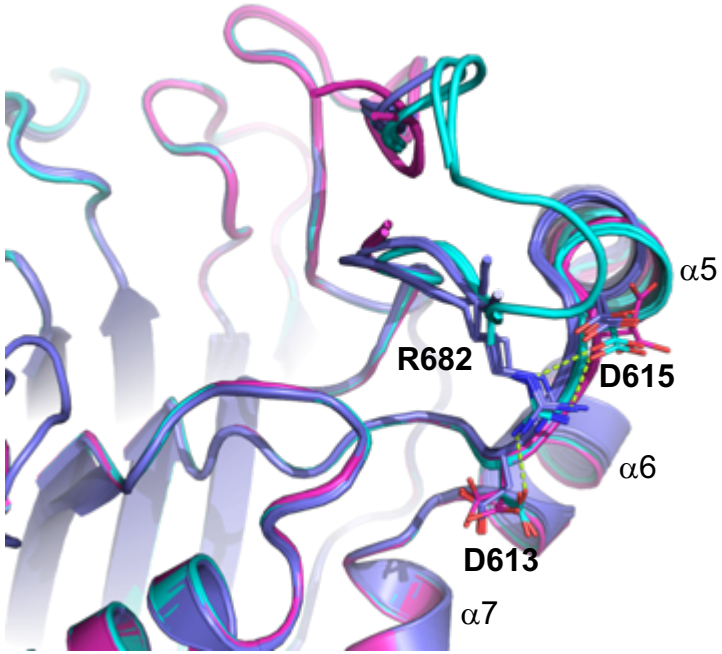


R682 singly bound to D613 or D615



R682 doubly bound to D613 and D615





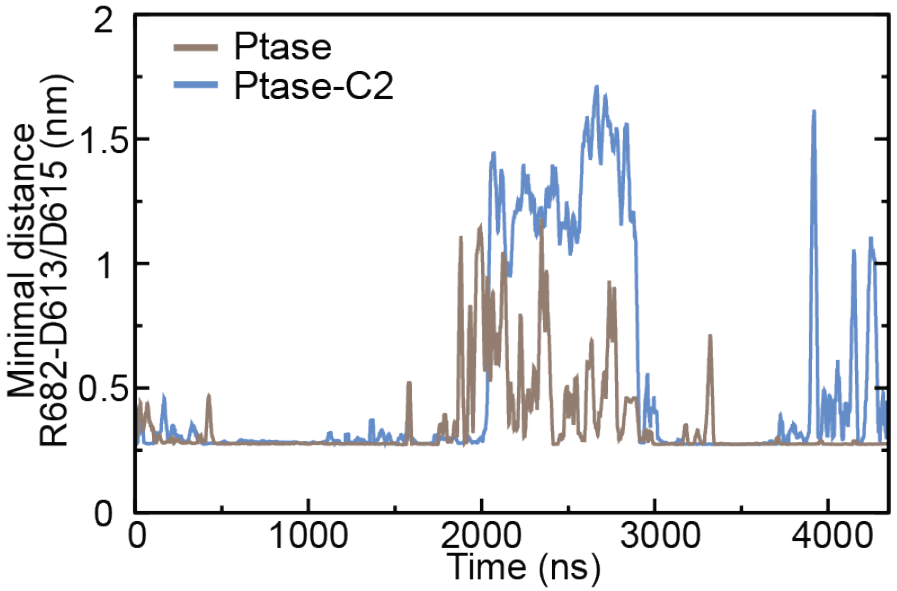
R682 unbound

R682 singly bound to D613 or D615

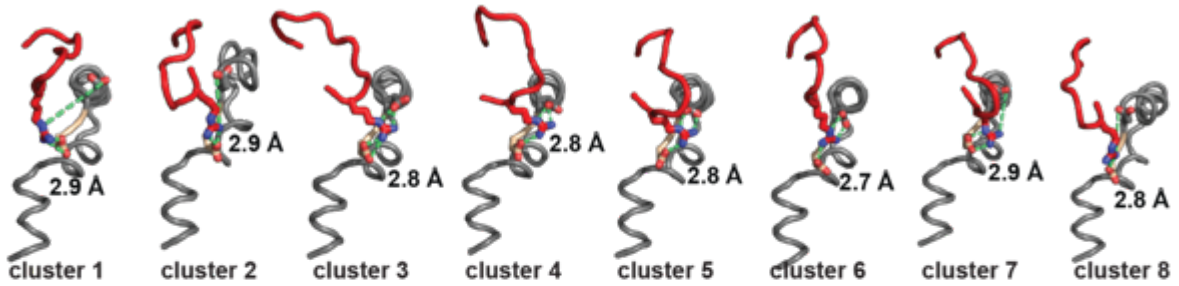
R682 doubly bound to D613 and D615

Hypothesis:
C2 stabilizes L4-out conformation

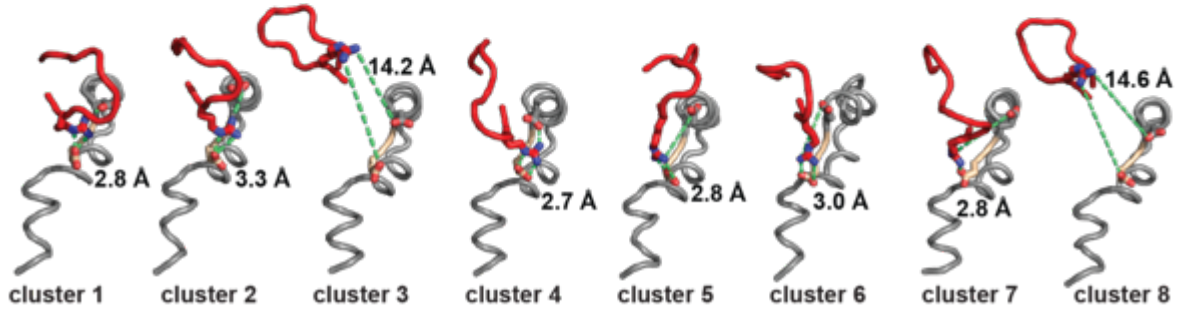
C2 domain helps L4-out to L4-in transition



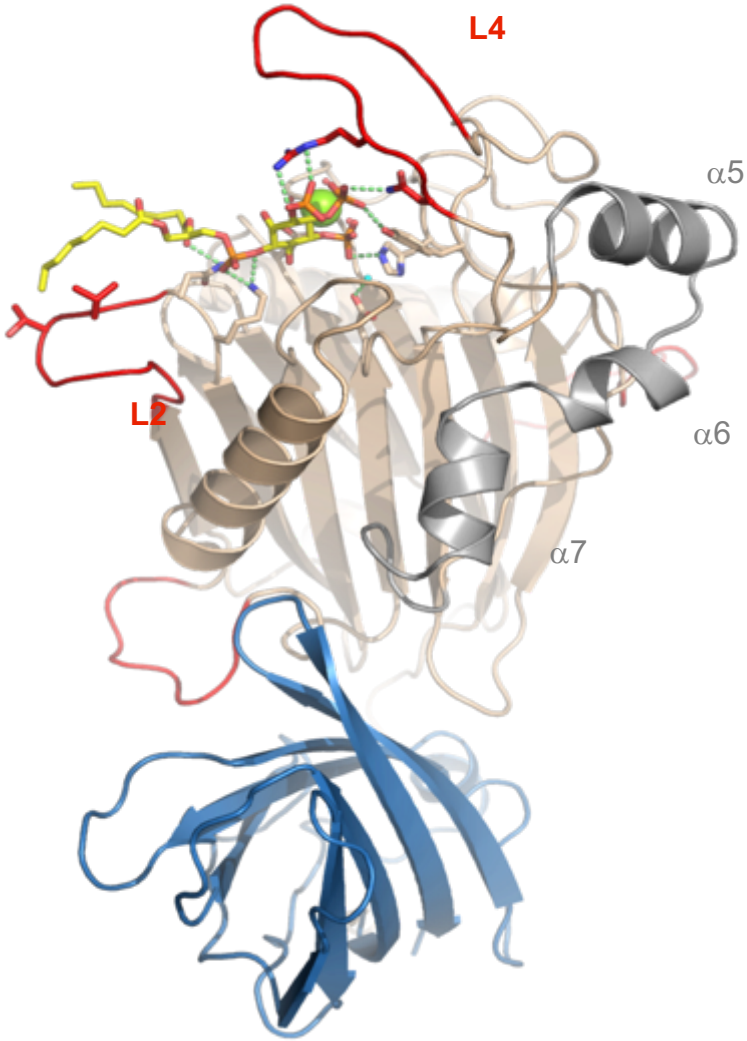
Ptase



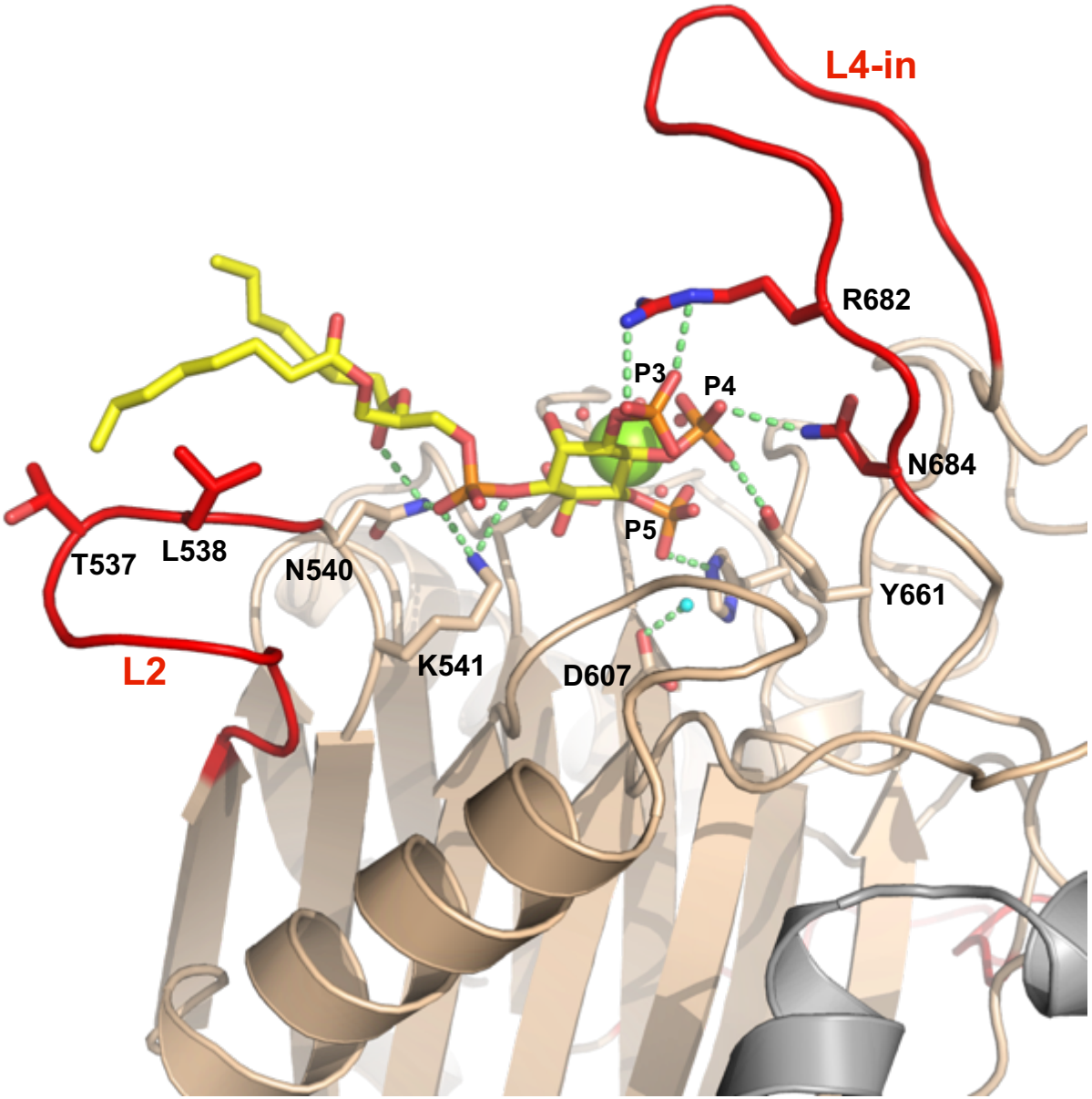
Ptase-C2



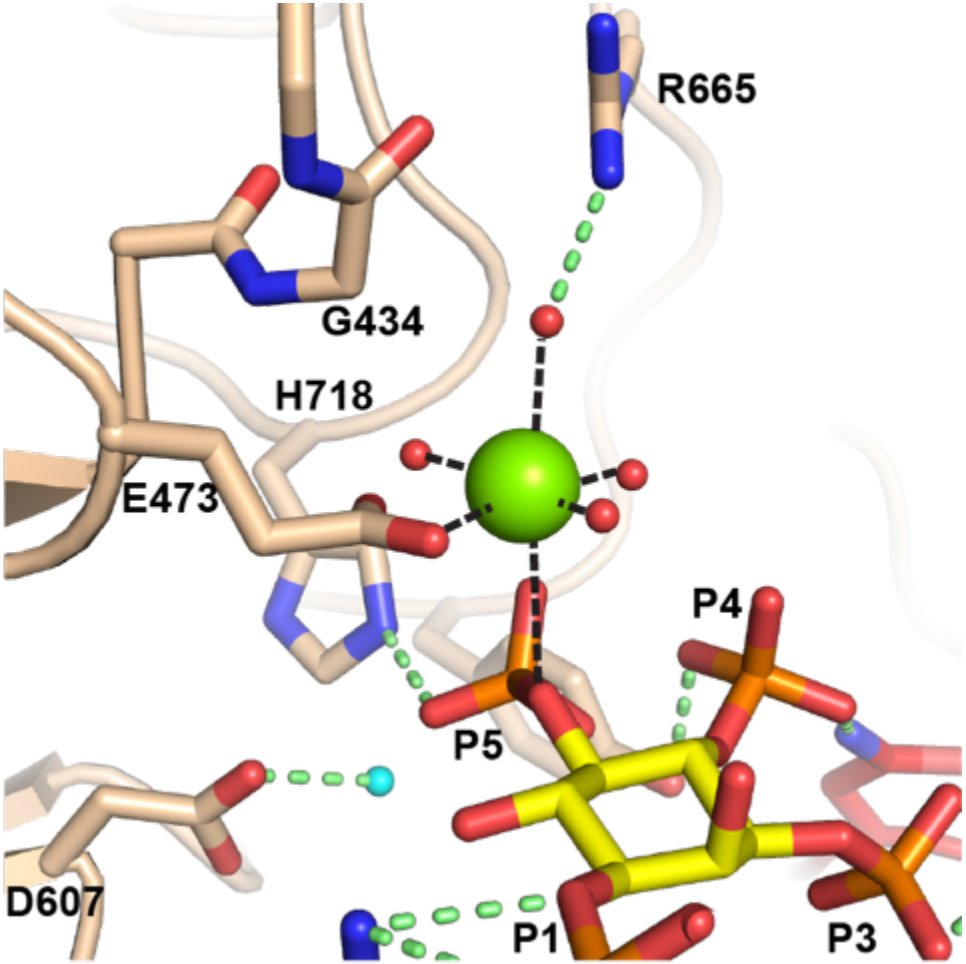
SHIP2 bound to substrate



SHIP2 bound to substrate

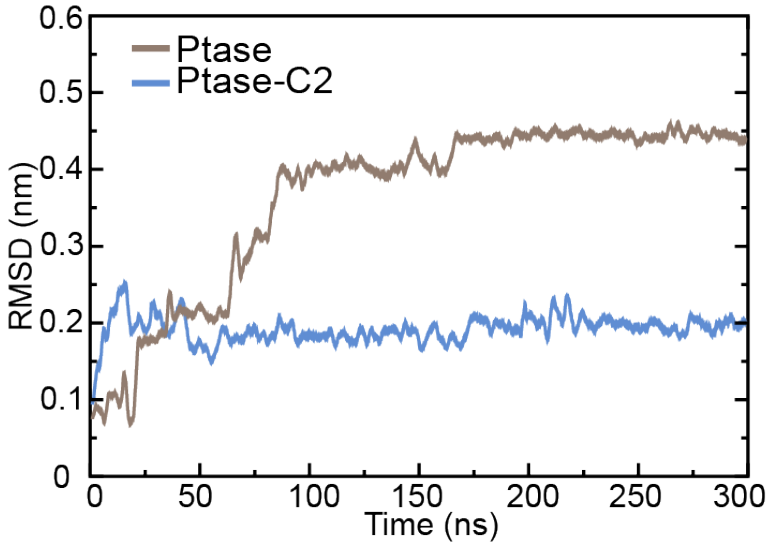


The catalytic center

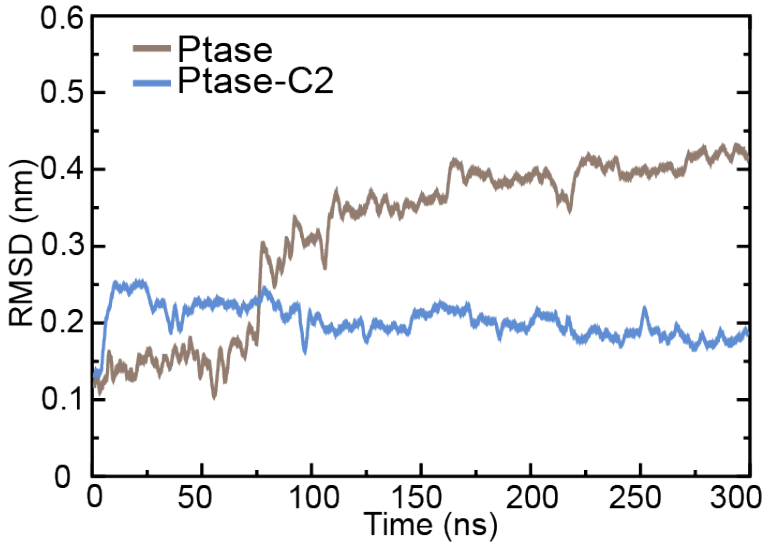


The C2 domain stabilizes substrate

IP_4



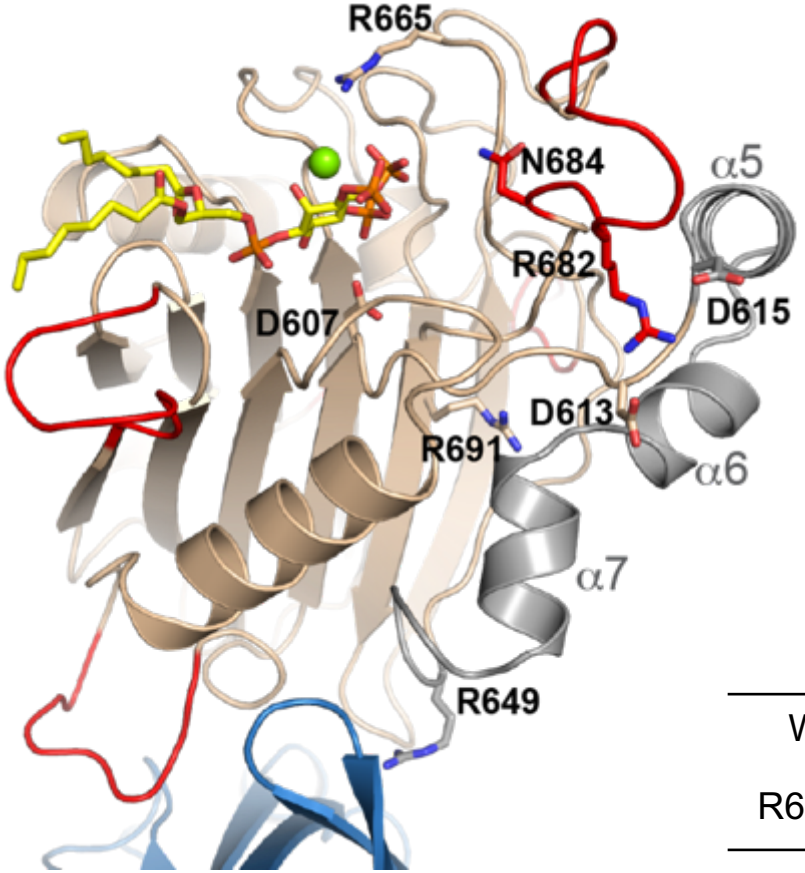
PIP_3

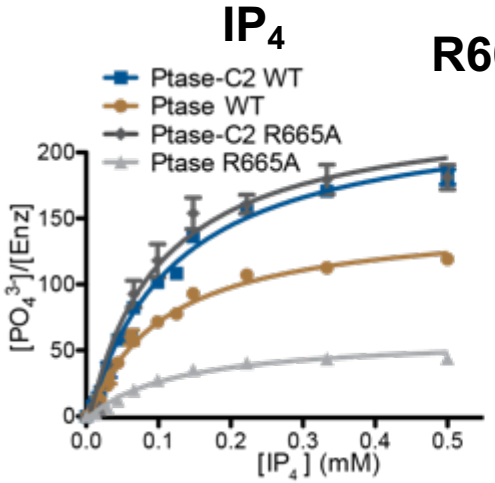


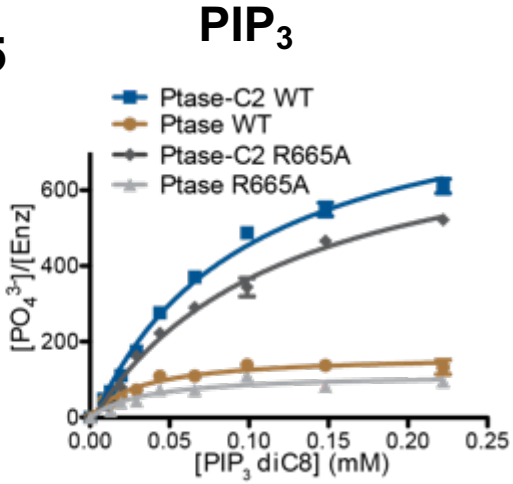
Summary

- C2 domain communicates via hydrophobic interface (F593, L597) to PIP_3
- C2 domain communicates via polar contact (R649) to IP_4
- The “polar path” leads via helices α5-7 to loop 4 and affects its dynamics
- C2 domain has overall stabilizing effect on active site

Active site mutants

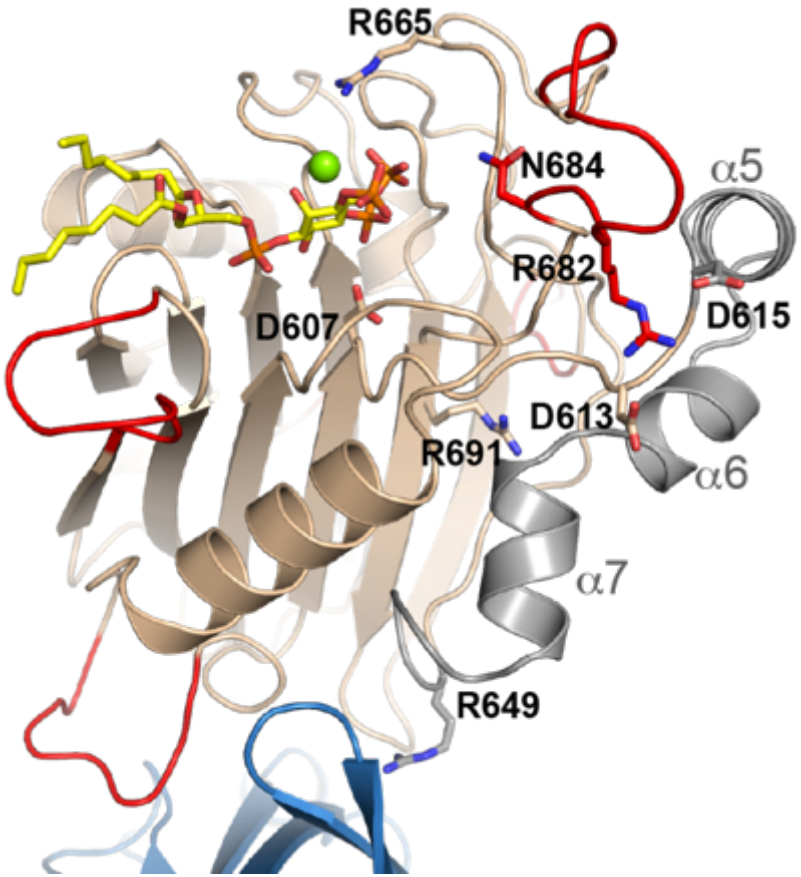


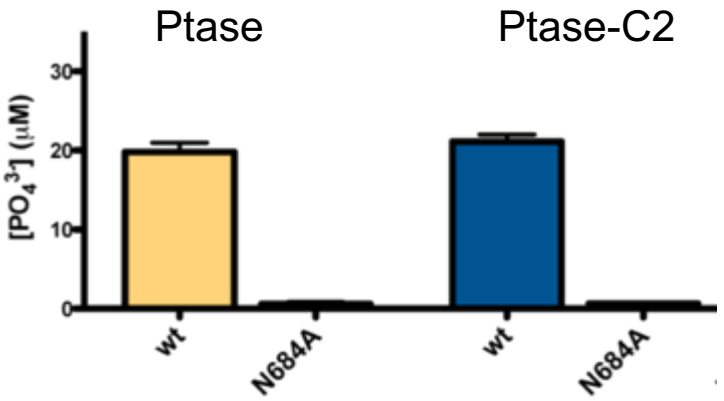




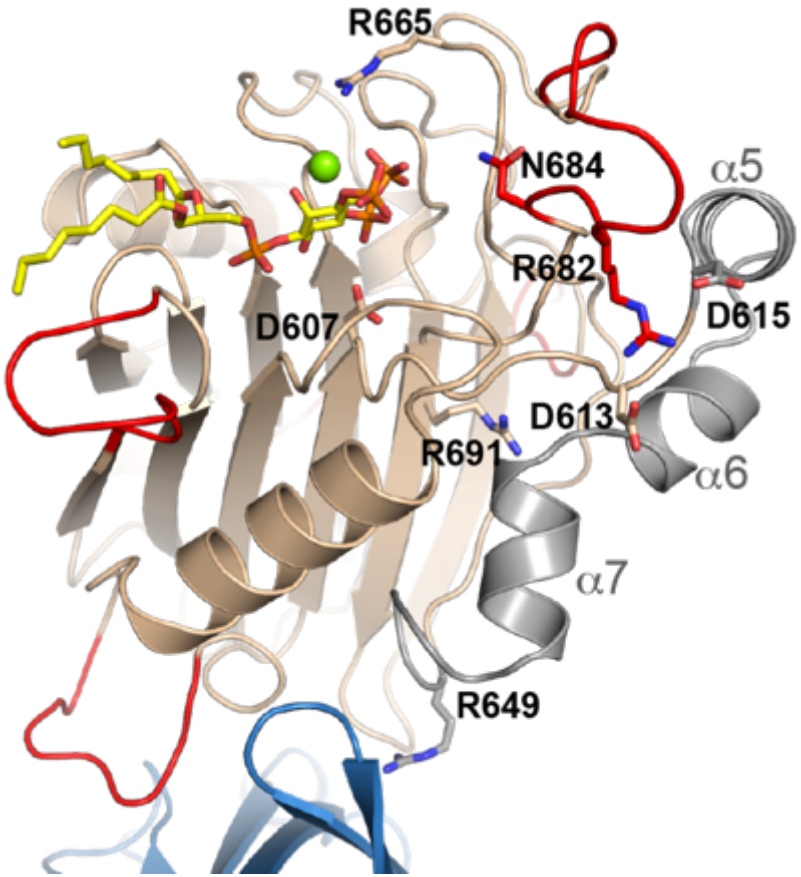
	k_{cat} (IP_4) [s^{-1}]		k_{cat} (PIP_3) [s^{-1}]	
	Ptase	Ptase-C2	Ptase	Ptase-C2
WT	1.31 ± 0.02	2.02 ± 0.04	0.69 ± 0.02	7.83 ± 0.03
R685	0.53 ± 0.03	2.07 ± 0.09	0.23 ± 0.02	7.02 ± 0.37

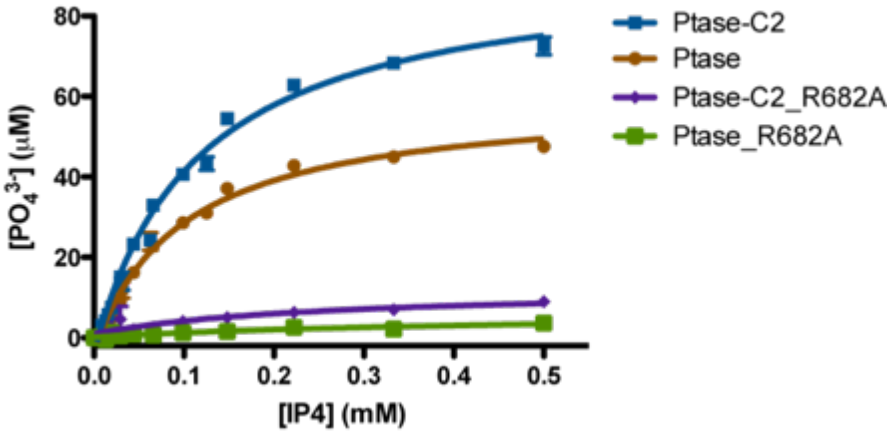
Active site mutants



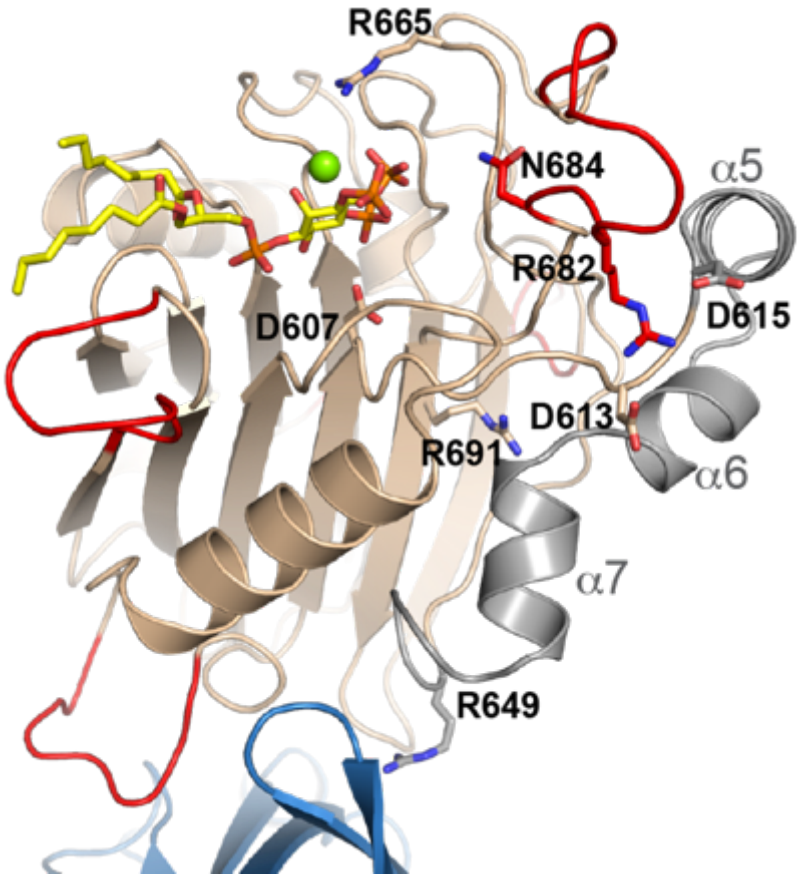


Probing the allosteric path via polar interaction



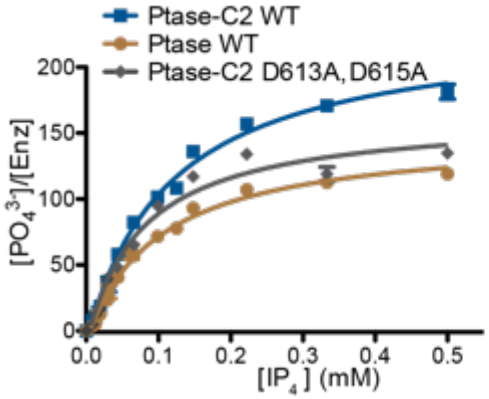


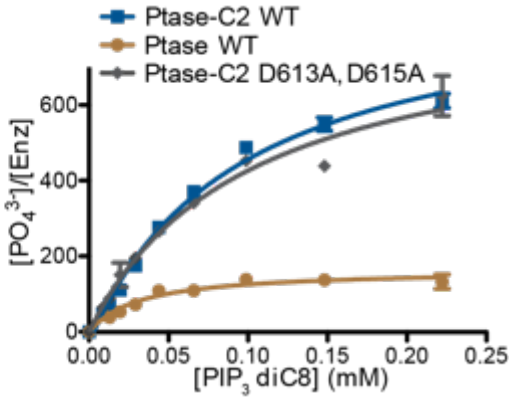
Probing the allosteric path via polar interaction



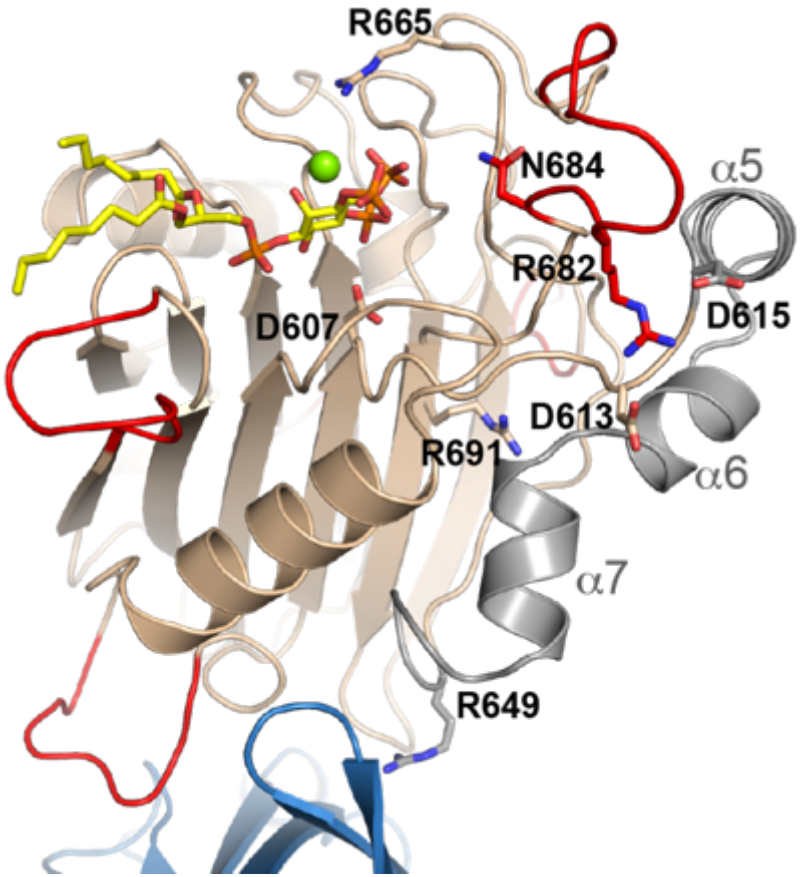
IP_4 **PIP_3**

D613A, D615A





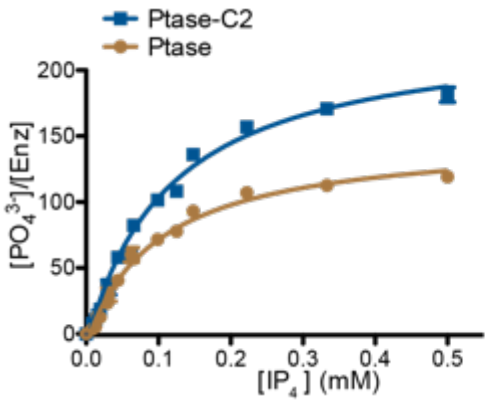
Probing the allosteric path via polar interaction

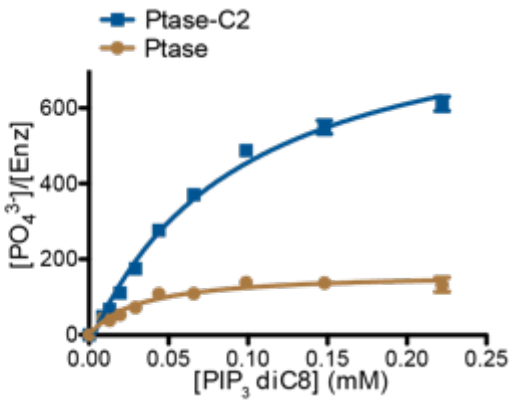


IP_4

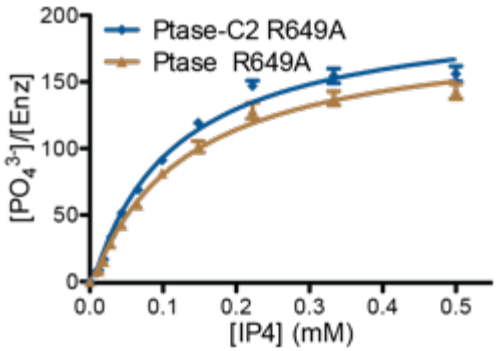
PIP_3

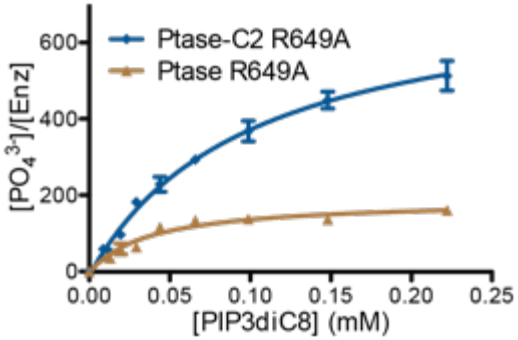
WT



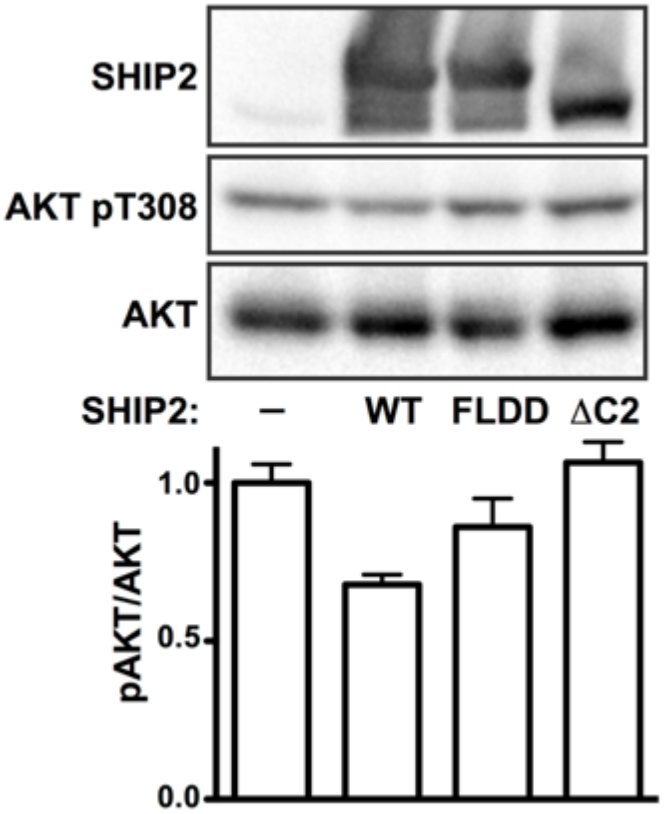


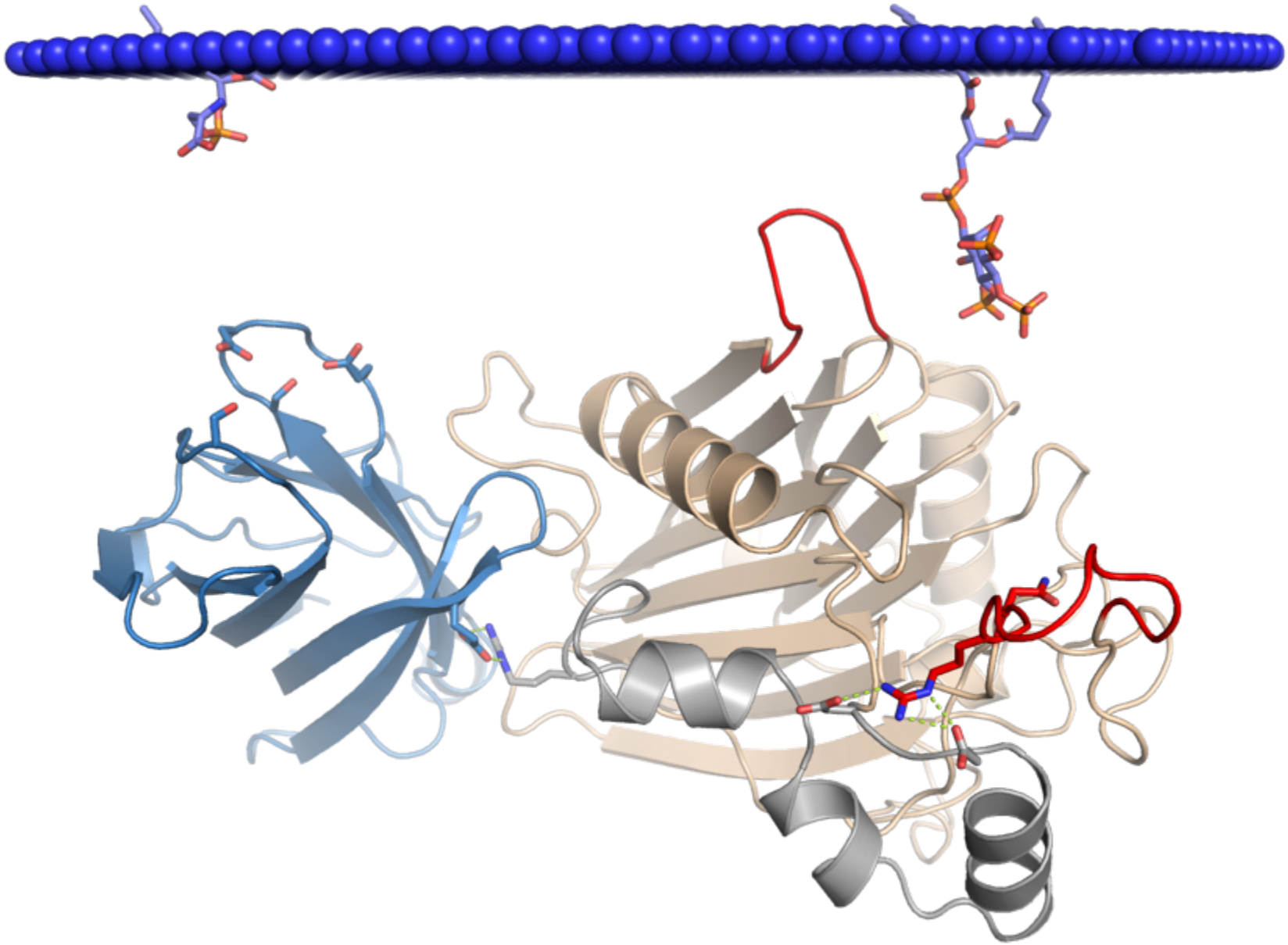
R649A

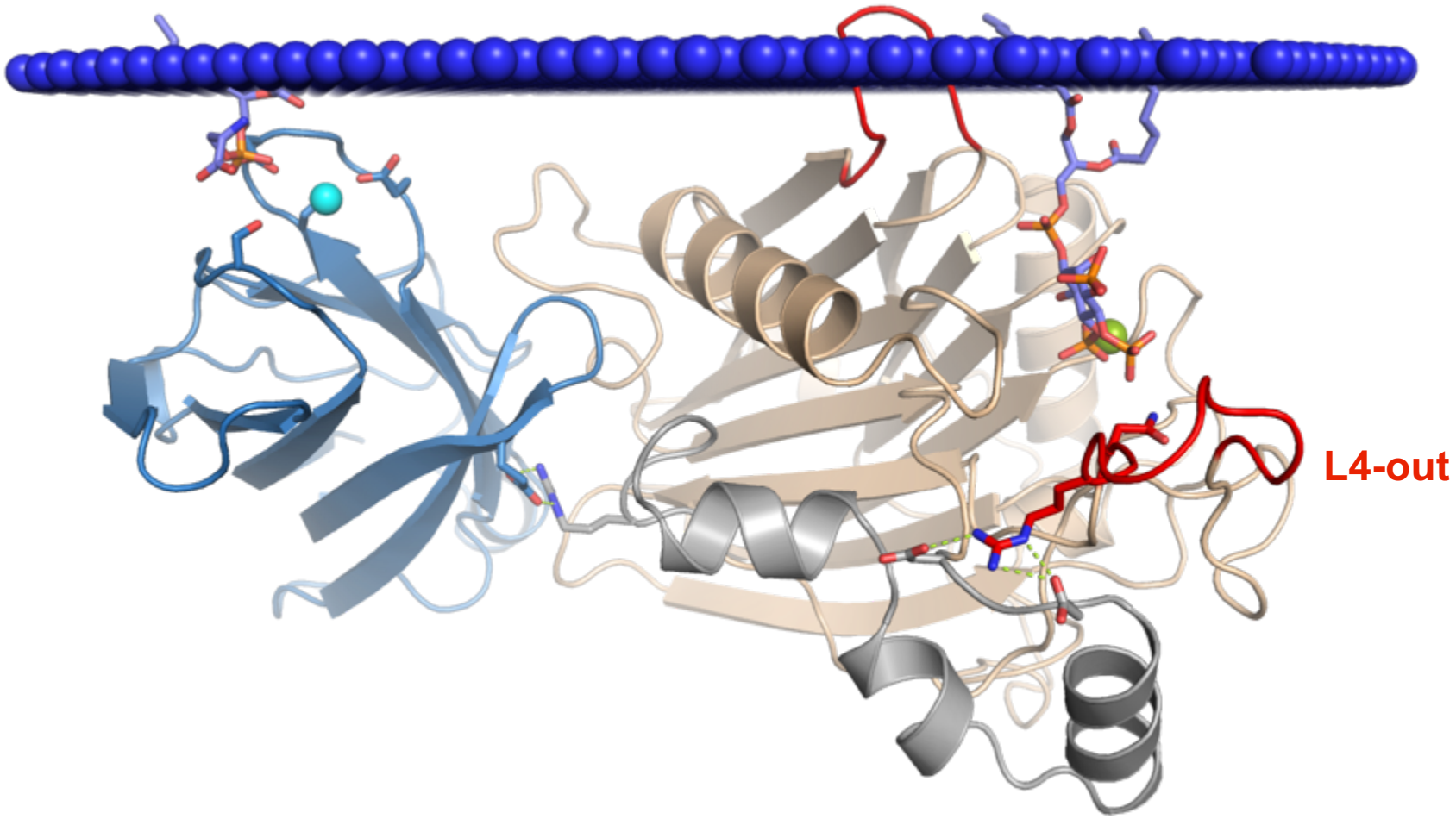


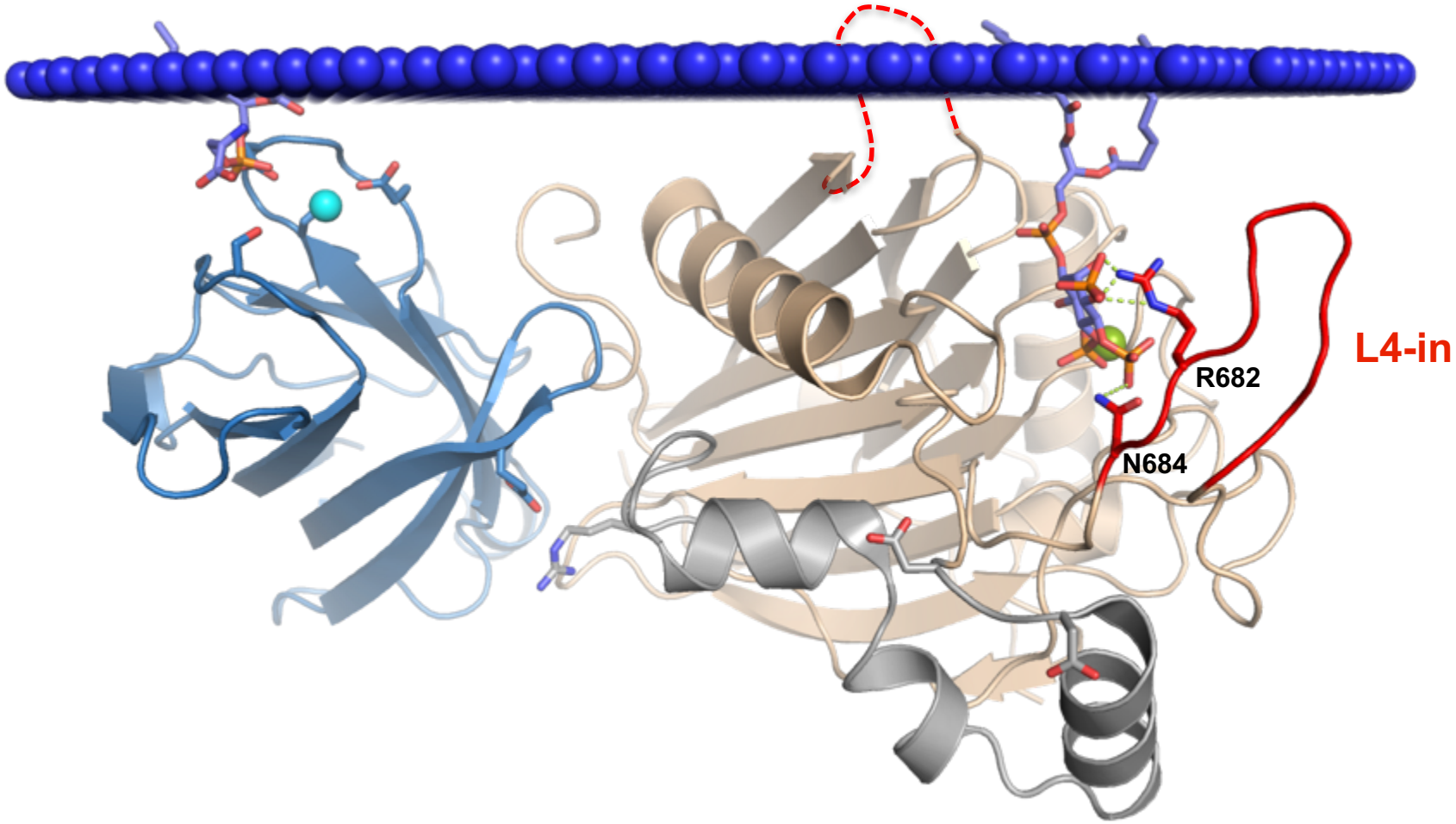


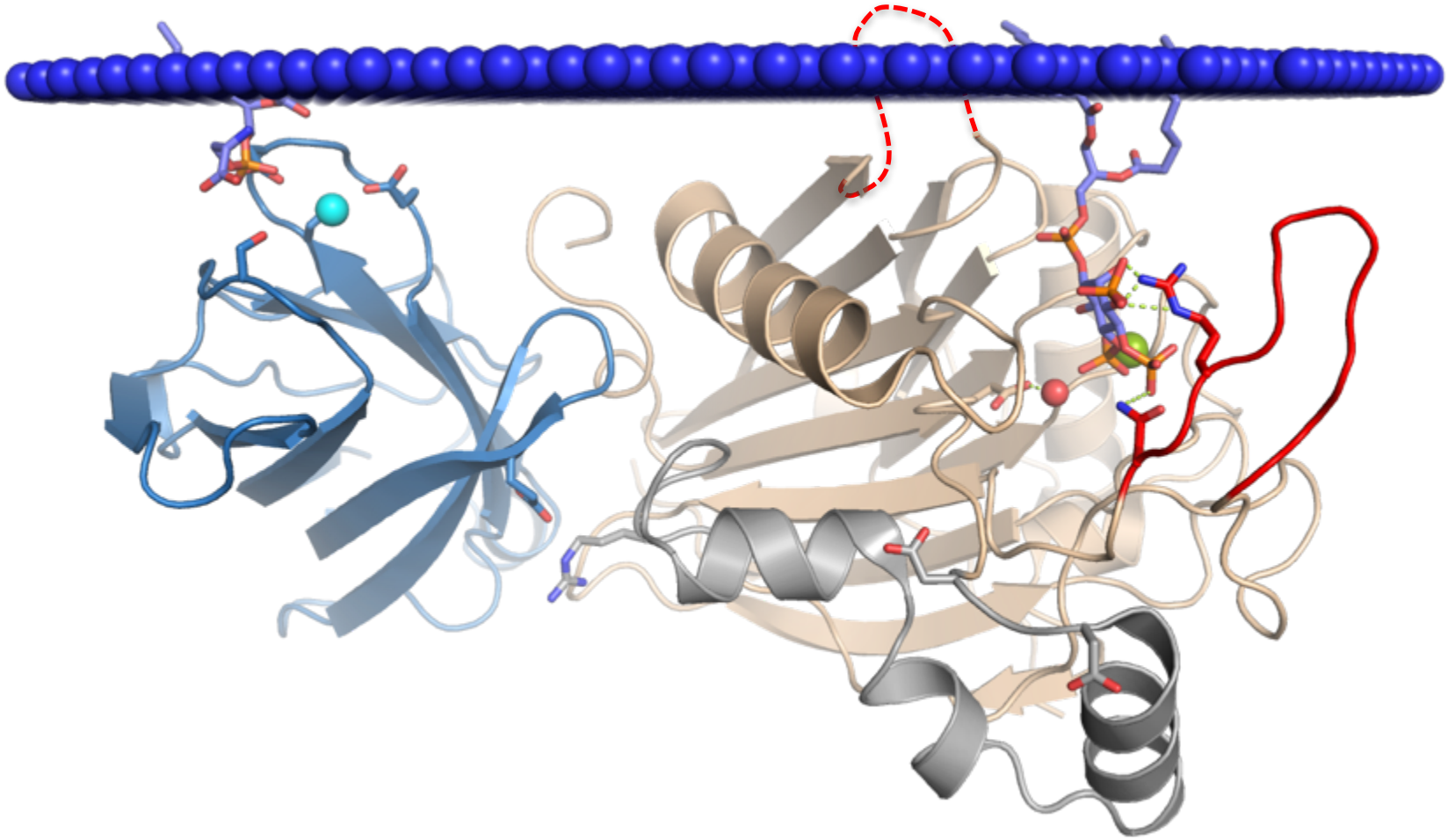
Cellular activity of SHIP2

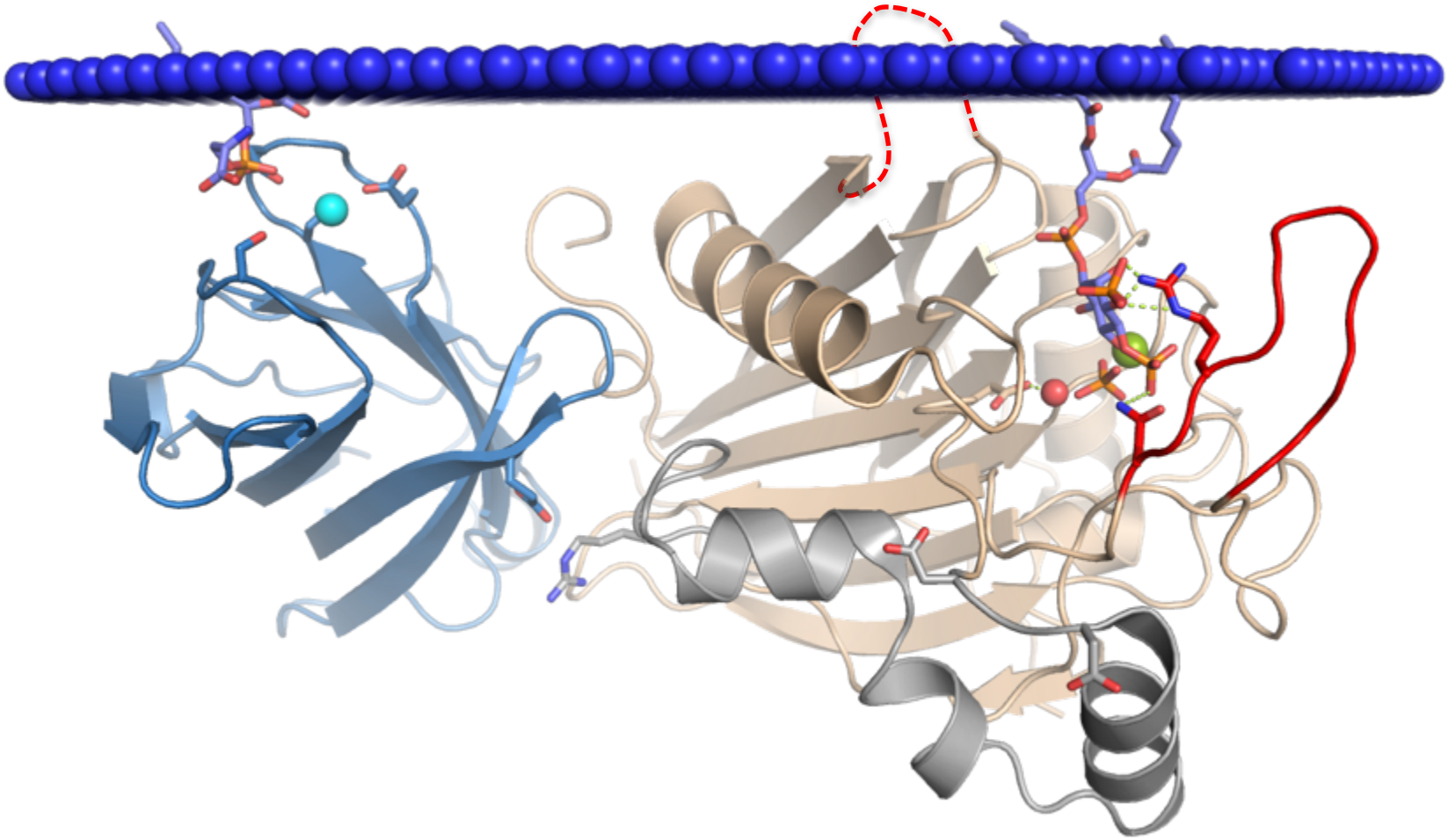


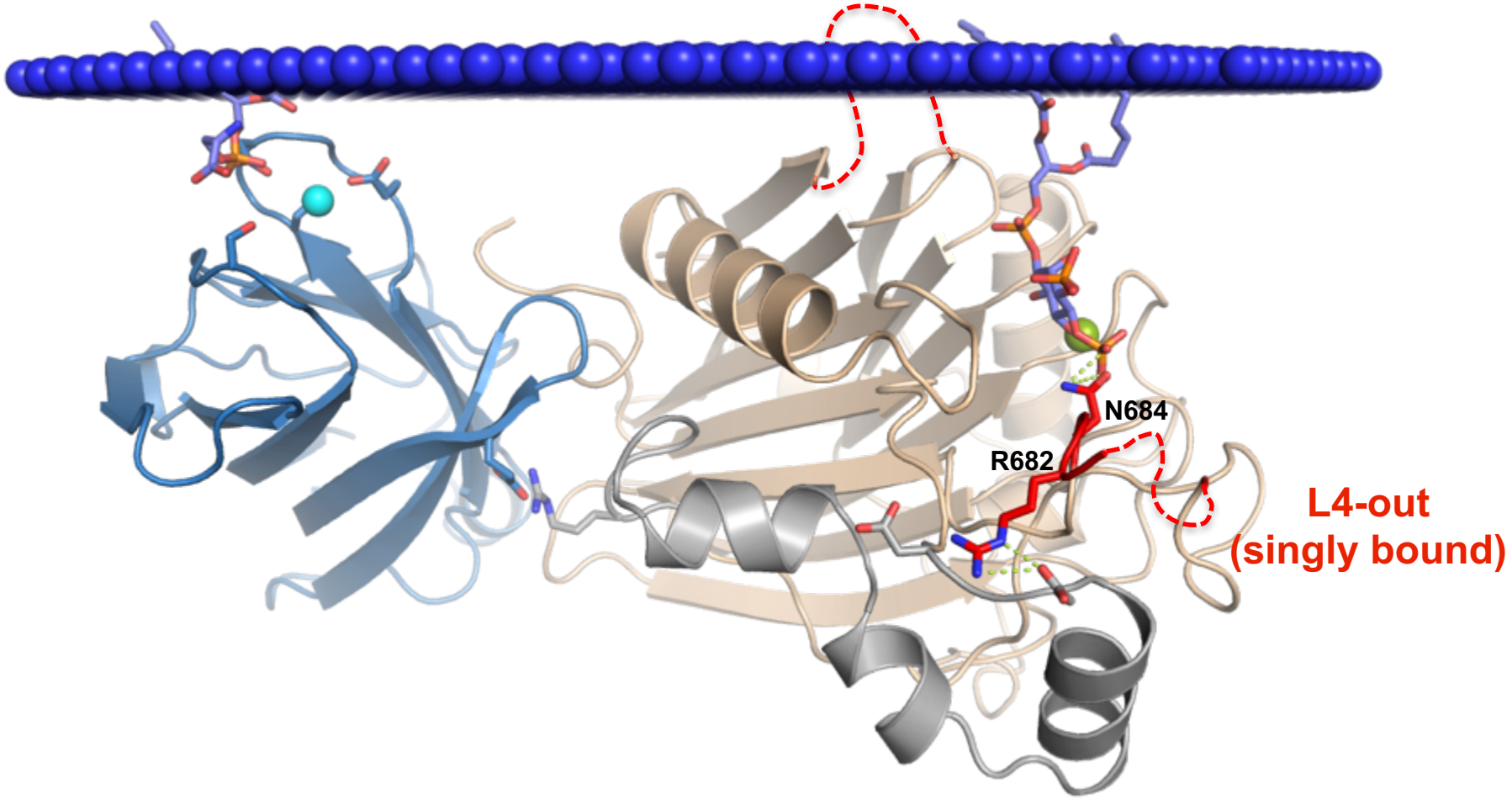


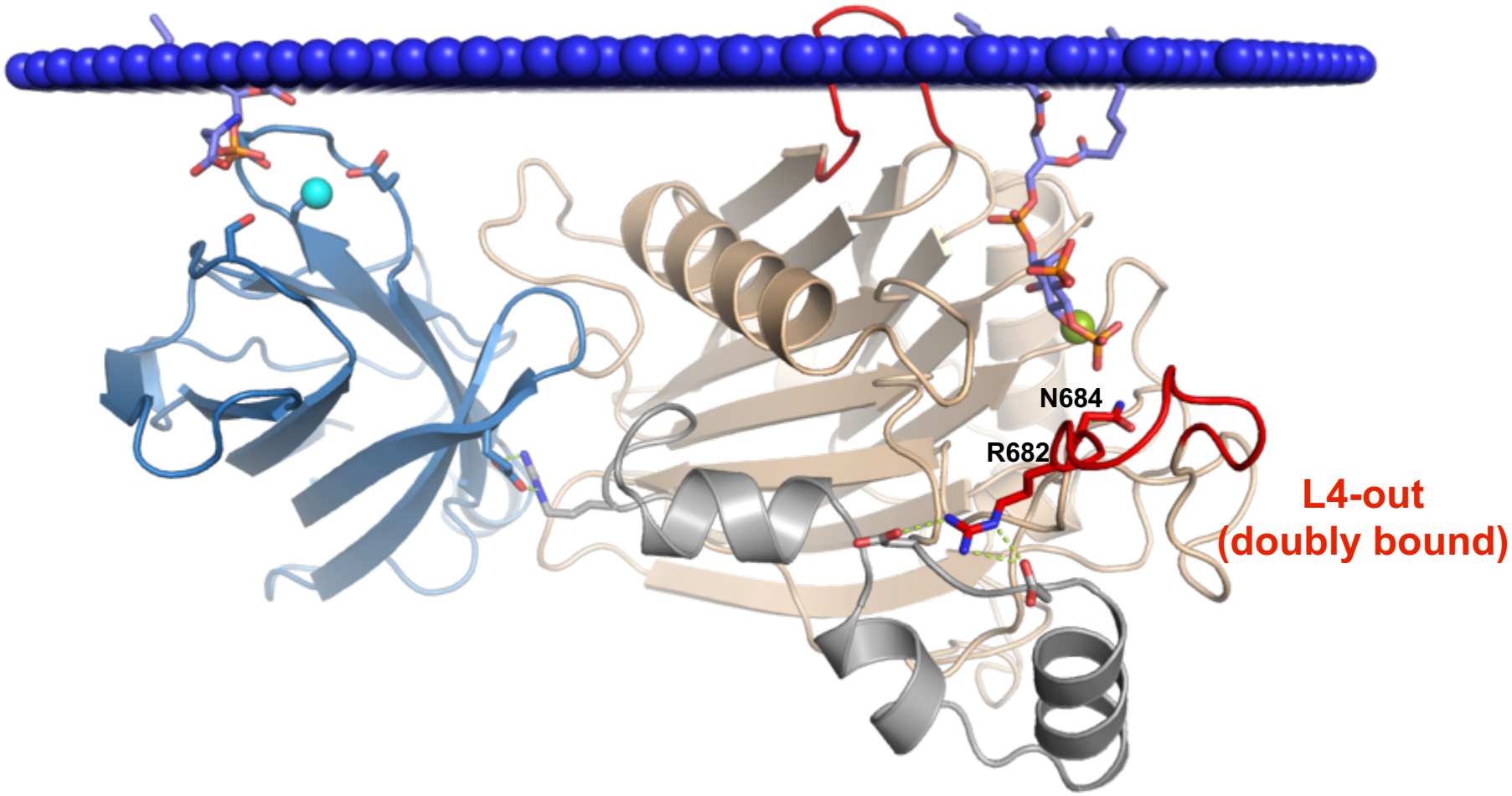

L4-out

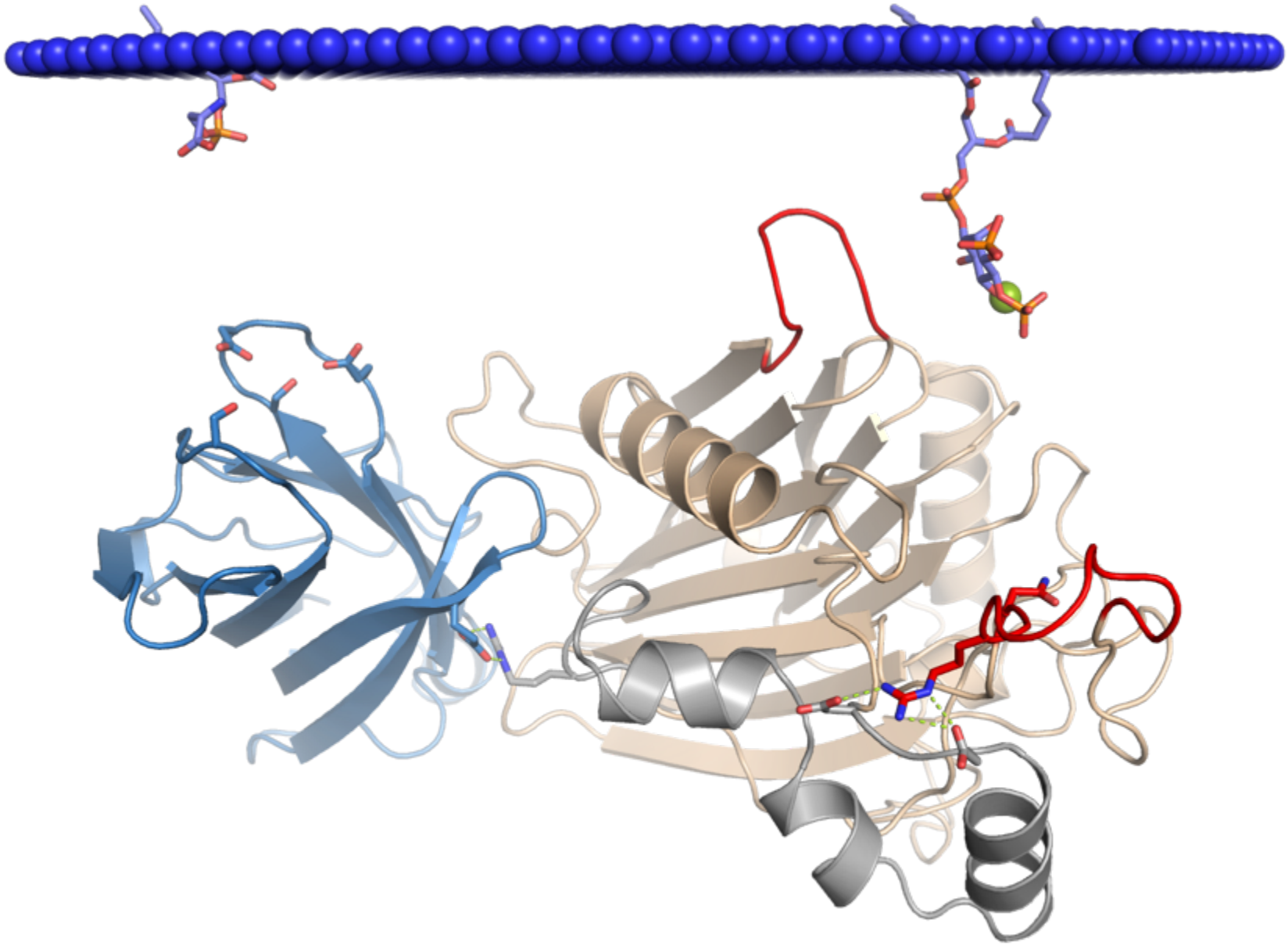

L4-in
R682
N684






N684
R682
L4-out
(singly bound)


N684
R682
L4-out
(doubly bound)



Acknowledgements

Cell Signalling and Adhesion Group

Past members
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Collaborator

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Barcelona Supercomputing Center
Centro Nacional de Supercomputación

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"la Caixa"
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formerly known as AICR