

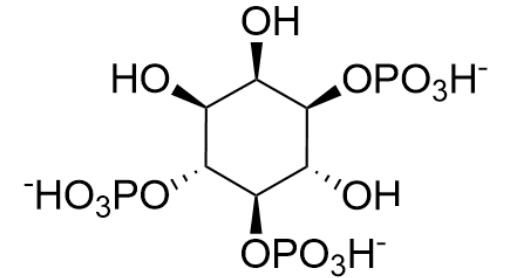
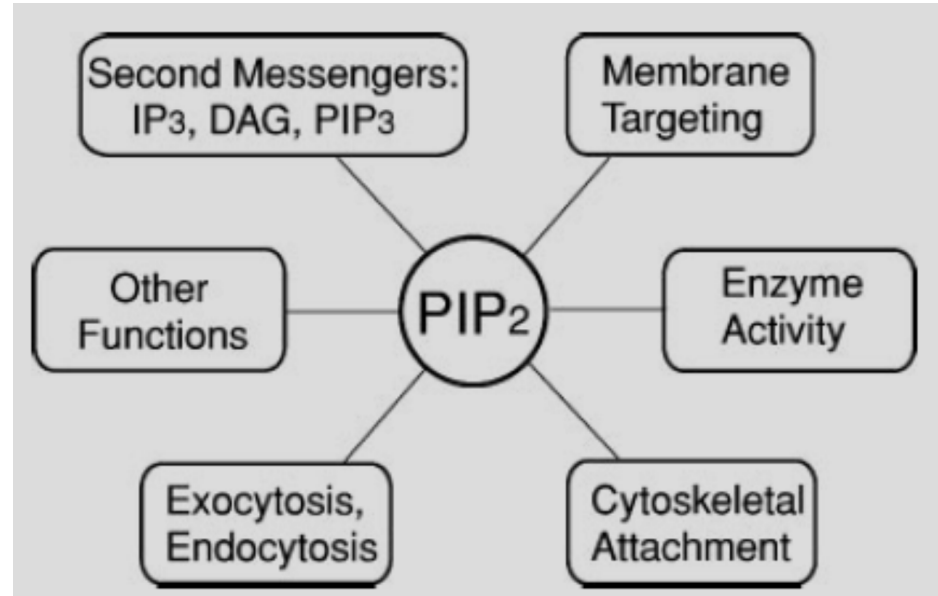
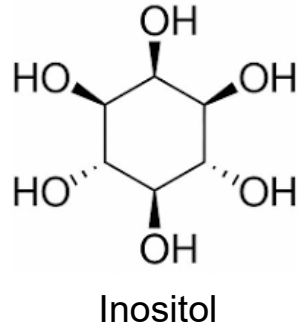
# Ion-Dependent Clustering of PIP<sub>2</sub>

Richard W. Pastor

National Institutes of Health

1. Intro to PIP<sub>2</sub> / Simulation force field
2. 100% PIP<sub>2</sub> monolayers
3. Bilayers with PIP<sub>2</sub> (but still no proteins)
4. Proteins: PIP<sub>2</sub> binding of phospholipase D2 (PLD2)

# 1a. Intro to PIP<sub>2</sub>



Inositol (1,4,5)P<sub>3</sub>  
(IP<sub>3</sub>) second messenger

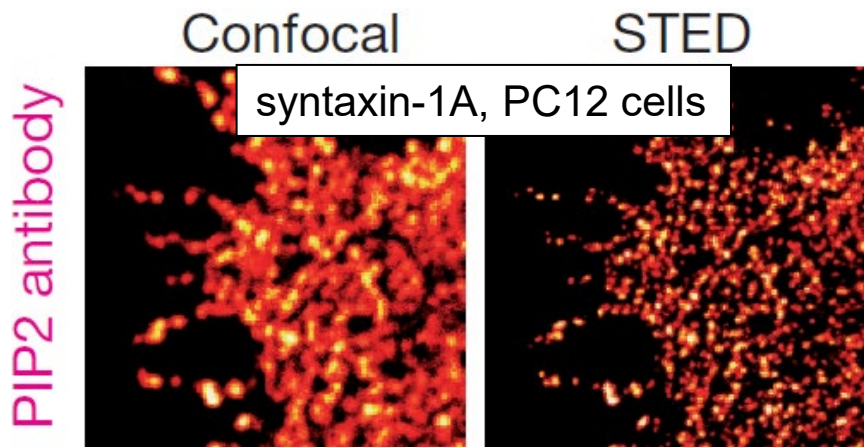
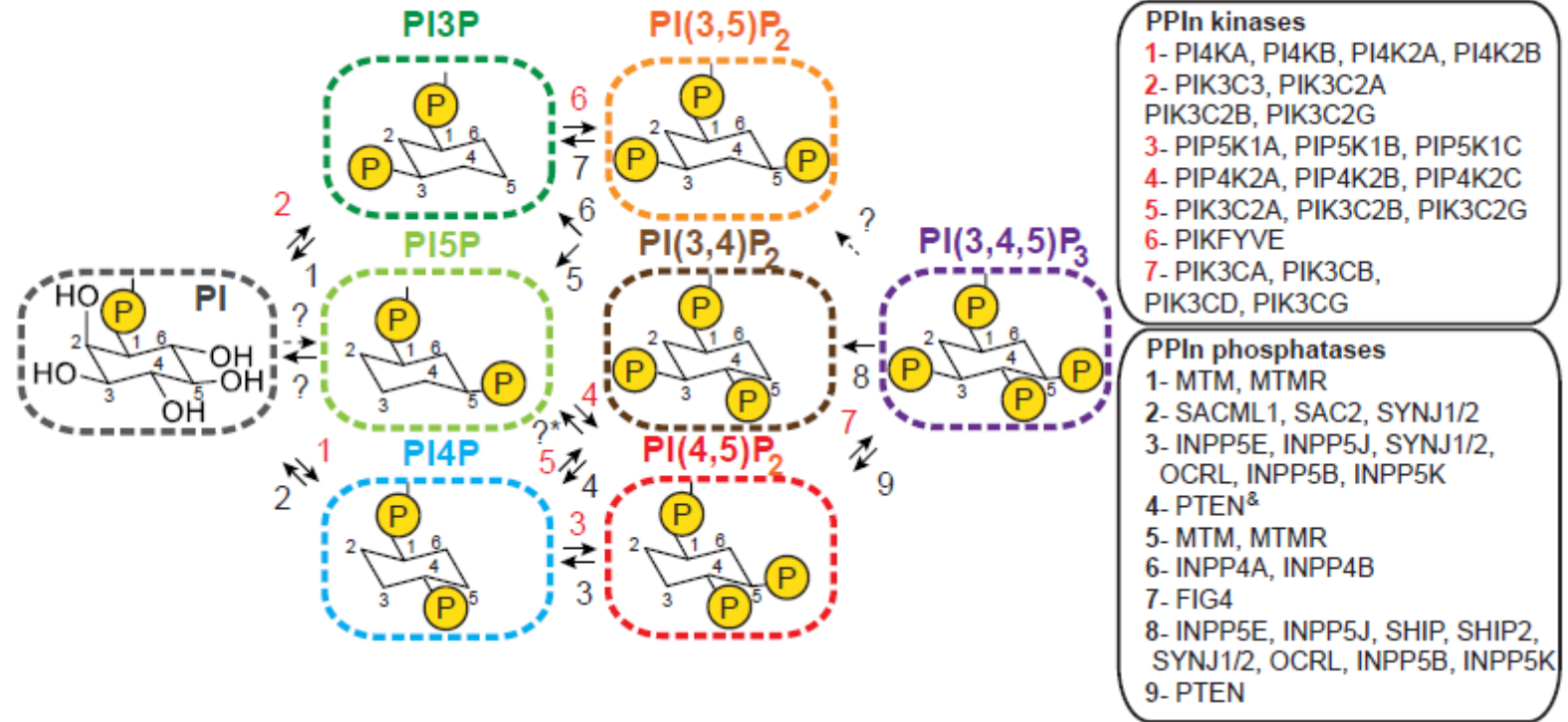
From Mclaughlin and Murray, Nature 2005:

For mammals, life (t=0) begins with PIP<sub>2</sub> hydrolysis:

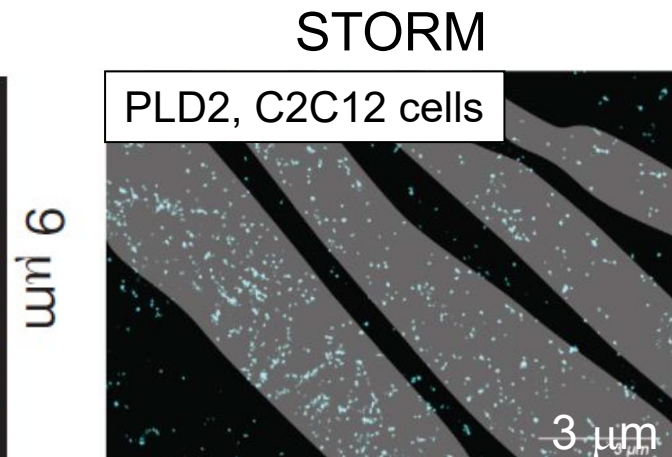
1. fertilizing spermatozoan injects into egg genetic material *and* phospholipase C- $\zeta$
2. Cleavage of PIP(4,5)P<sub>2</sub>  $\rightarrow$  Inositol (1,4,5)P<sub>3</sub>  $\rightarrow$  Ca<sup>2+</sup> oscillations
3.  $\rightarrow$  seals egg membrane to additional sperm, initiate egg activation  $\rightarrow$  you!

# How does PIP<sub>2</sub> do so many different things?

1. Large number of phosphatidylinositols that react with proteins
2. Forms clusters with proteins ("PIP<sub>2</sub> rafts")



van den Bogaart, ..., Jain, *Nature* **479**, 553 (2011)



Peterson, ..., Hansen, *Nat Commun* **7**, 13873 (2016)

Average diameters 50-90 nm  
 ~5% PIP<sub>2</sub> in clusters (~1% unclustered)  
 → **PIP<sub>2</sub> raft not tightly packed**  
 (very different from Lo phase)  
 Ca<sup>2+</sup> induces clusters (monolayers)  
**Organization of clusters? Ions?**

# 1b. Simulation force field

CHARMM36 *additive all-atom* lipid potential energy function (FF); **no polarizability**

$$V(\hat{R}) = \sum_{\text{bonds}} K_b (b - b_0)^2 + \sum_{\text{angles}} K_\theta (\theta - \theta_0)^2 + \sum_{\text{dihedrals}} \left[ \sum_j K_{\varphi,j} (1 + \cos(n_j \varphi - \delta_j)) \right] + \sum_{\text{nonbond pairs}} \varepsilon_{ij} \left[ \left( \frac{R_{\text{min},ij}}{r_{ij}} \right)^{12} - \left( \frac{R_{\text{min},ij}}{r_{ij}} \right)^6 \right] + \sum_{\text{nonbond pairs}} \frac{q_i q_j}{\varepsilon_D r_{ij}}$$

Klauda, Venable, Freites, O'Connor, Tobias, Mondragon-Ramirez, Vorobyov, MacKerell, Pastor, *J. Phys. Chem. B.*, **114**, 7830 (2010)

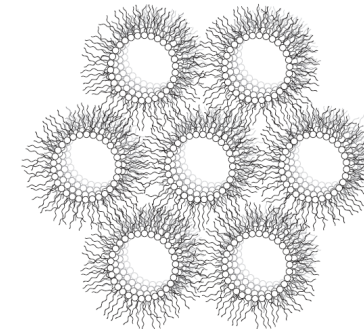
Lipid	Chains	Sim		Expt	
		Kc (k <sub>B</sub> T)	s.e. Kc	X-ray	Flicker
DPPC	16:0,16:0	28.2	0.9	29.8	33.0
DMPC	14:0,14:0	22.6	1.2	25.1	31.2
DOPC	18:1,18:1	21.2	1.0	19.4	26.4
POPC	16:0,18:1	24.7	1.0	24.6	

Lipid	K <sub>θ</sub> (mN/m)	
	MD	X-ray
DPPC	46	44
DMPC	32	43
DOPC	49	89
POPC	44	69

Venable, Brown, Pastor, *Chem. Phys. Lipids*, 192, 60 (2015)

Nagle, *Chemistry and Physics of Lipids*, 205, 18-24 (2017)

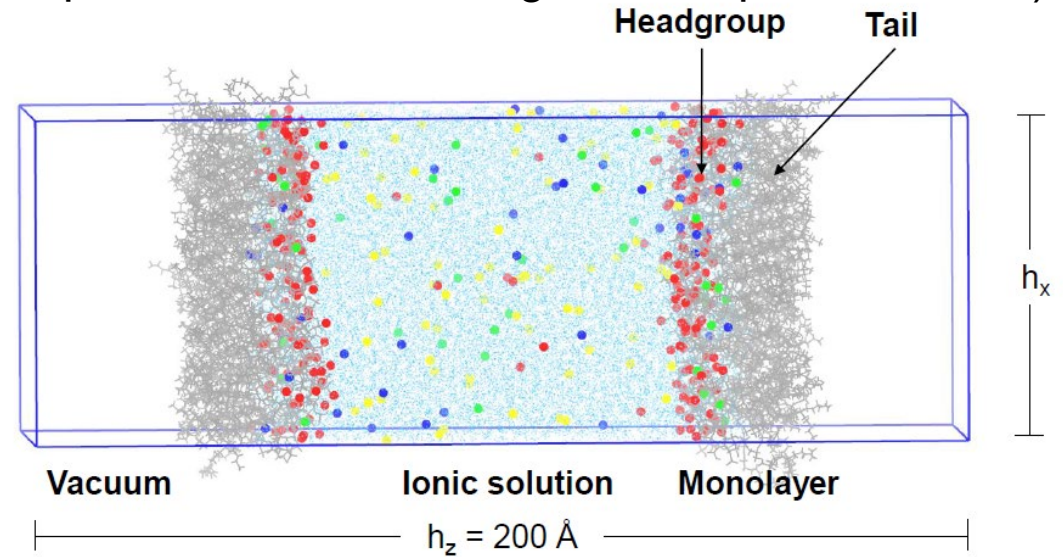
Lipid	Radius of Curvature (Å)			bilayer vs H <sub>II</sub> (exp) %diff in c <sub>0</sub> =1/R <sub>0</sub>
	MD (H <sub>II</sub> )	MD (bilayer)	Expt (H <sub>II</sub> )	
DOPC	-	-108 (5%)	-87.3	19%
SDPE	-	-34 (2%)	-27.5	19%
DOPE	-27 (7%)	-30 (3%)	-28.5	6%
O-lyso-PC	+41 (7%)	-	+38	



## 2. 100% PIP<sub>2</sub> monolayers

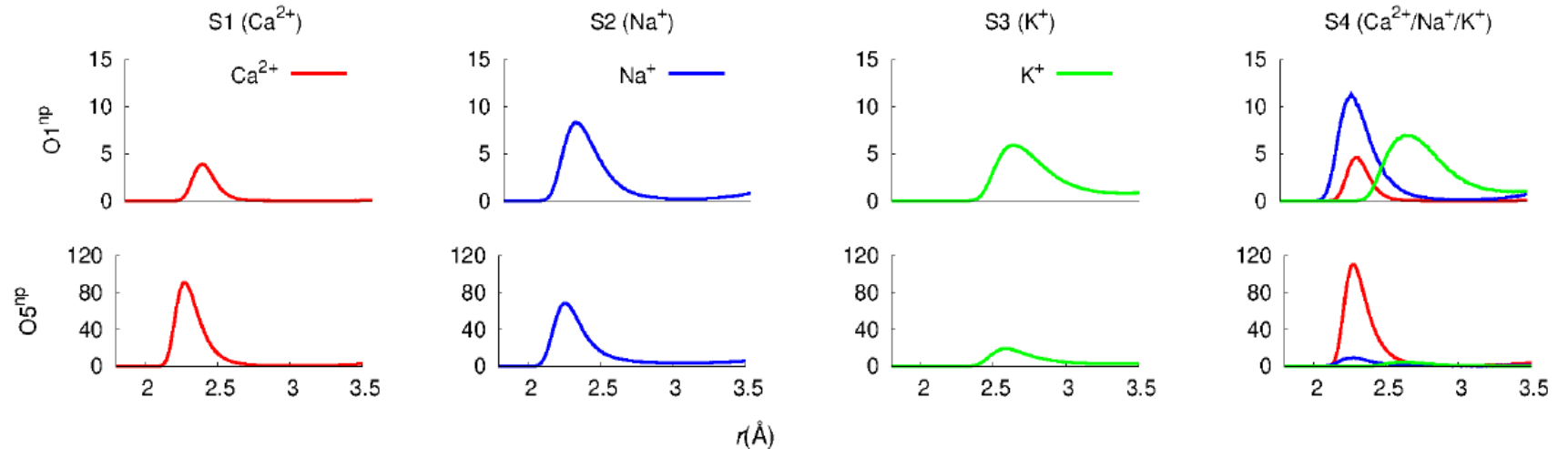
**First:** monomethyl phosphate solutions (adjusted Ca<sup>2+</sup>/phosphate interaction using osmotic pressure data)

system	lipid <sup>a</sup>		ion <sup>b</sup>				bulk concentration
	PIP <sub>2</sub> (64/monolayer)	Ca <sup>2+</sup>	Na <sup>+</sup>	K <sup>+</sup>	Cl <sup>-</sup>	(mM)	
S1	128	298			84	125 (Ca <sup>2+</sup> )	
S2	128		535		23	125 (Na <sup>+</sup> )	
S3	128			535	23	125 (K <sup>+</sup> )	
S4	128	249	45	45	76	41.67 (Ca <sup>2+</sup> ) 41.67 (Na <sup>+</sup> ) 41.67 (K <sup>+</sup> )	

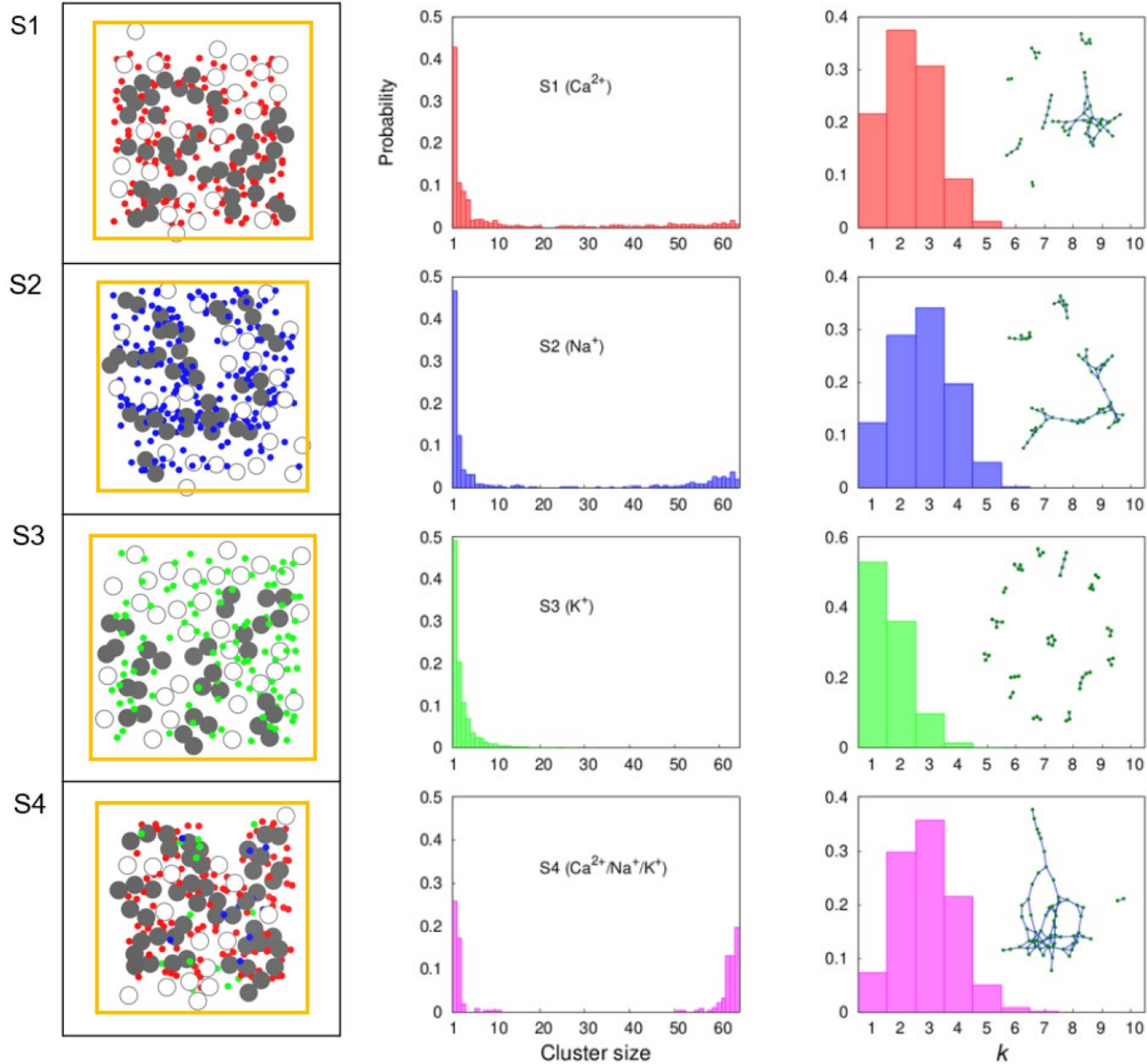


Criteria for cluster based on radial distribution functions of ions and PIP<sub>2</sub> oxygen

(need to be careful not to make criteria too broad, or everything in monolayer ends in clustered)



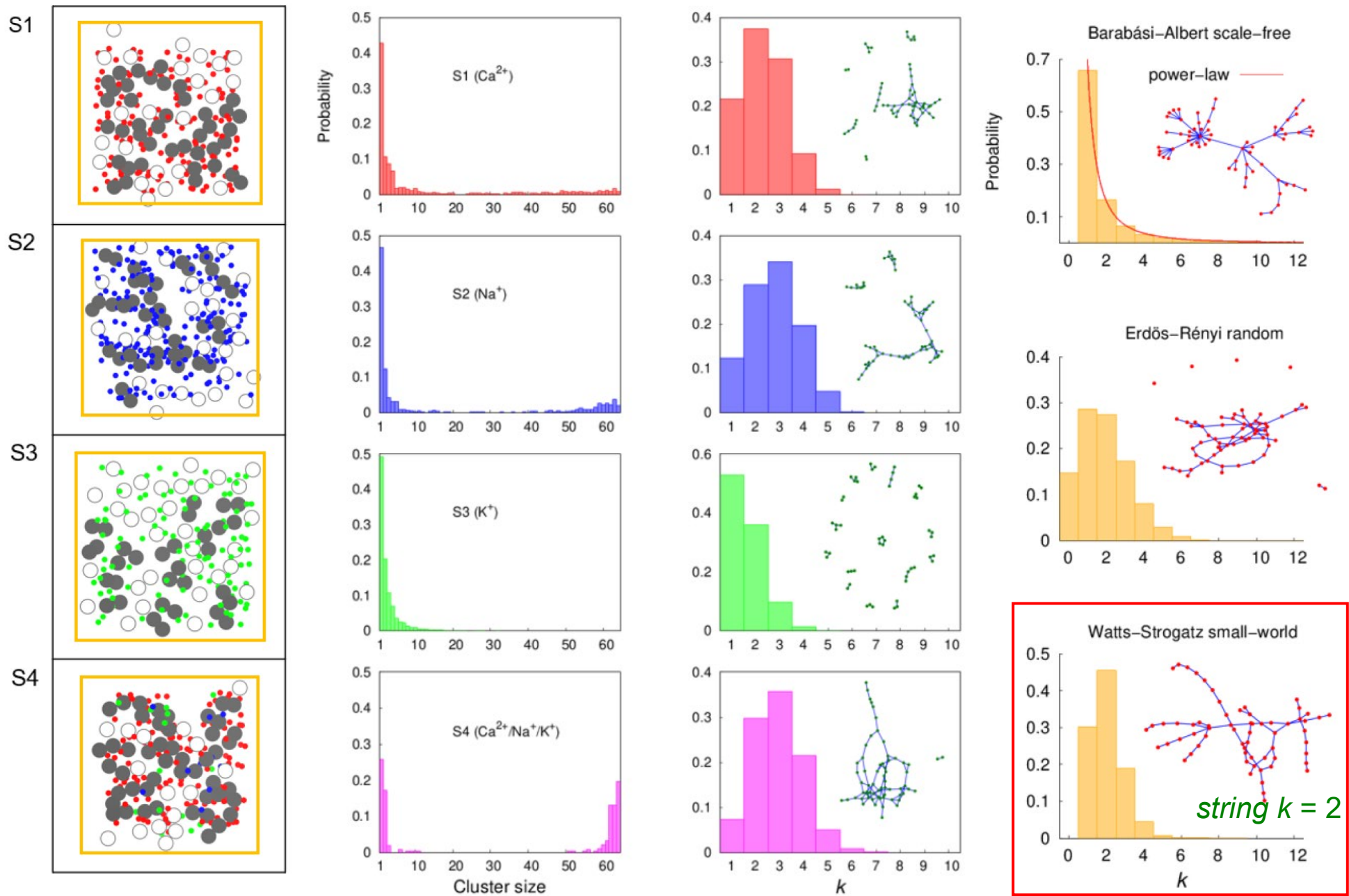
**MMP, Ca<sup>2+</sup>:** Han, ... Pastor, *J. Phys. Chem. B*, **122**, 1494 (2018)  
**Monolayers:** Han, Gericke, Pastor *J. Phys. Chem. B*, **124**, 1183 (2020)



$k = \text{link number/node}$ ; monomer  $k = 0$ , dimer  $= 1$ , long string  $(1+2+2\dots+1)/N \rightarrow 2$ ; clump  $\rightarrow k > 2$

Small values of  $k$  observed (even for large clusters) consistent with strings

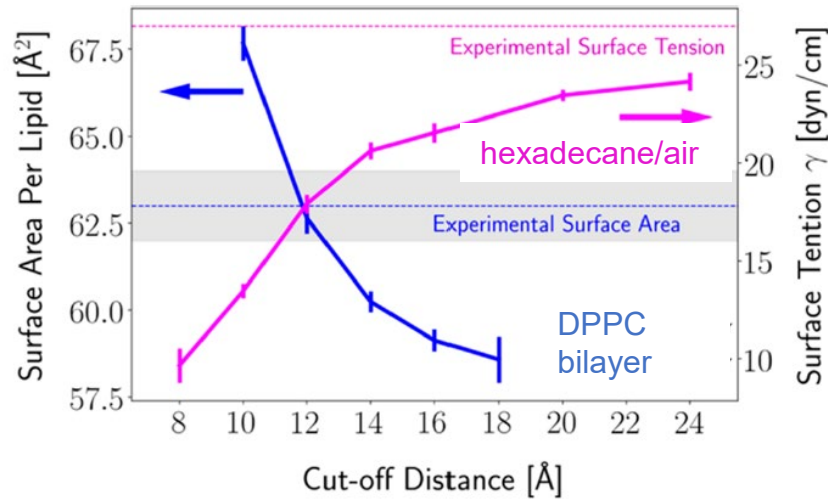
Synergy of K and Ca



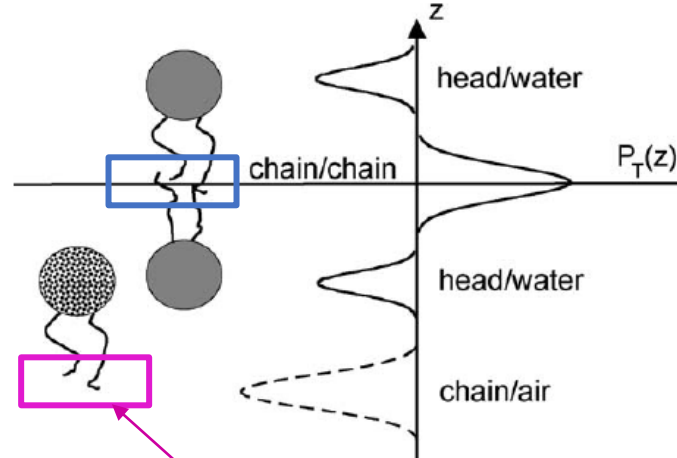
Jensen-Shannon distance with simulation lowest for small-world network

# Simulation issue: Lennard-Jones interactions switched to 0 between 8-12 Å to reduce computational cost

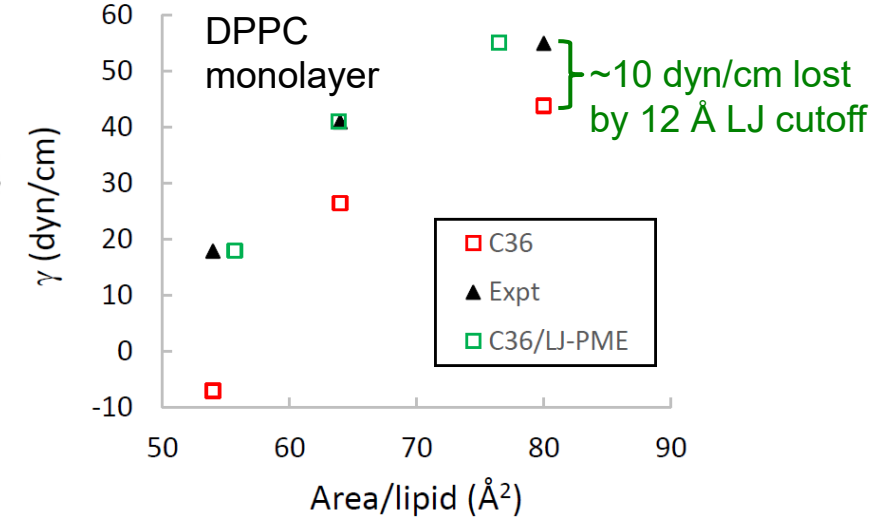
- works for bilayer (optimize FF to expt bilayer surface area)
- bad for hexadecane/air (need much longer cutoff)



→ inconsistency with monolayers



→ incorrect  $\gamma/A$  isotherms



→ automated reoptimization for bilayer *and* monolayer with explicit long-range LJ (LJ-PME)  
 → agreement of expt and simulated monolayer  $\gamma/A$  isotherms (useful for later)

**LJ-PME to CHARMM:** Leonard, Simmonett, Pickard, Huang, Venable, Klauda, Brooks, Pastor, *J. Chem. Theory and Computation*, **14**, 948 (2018)

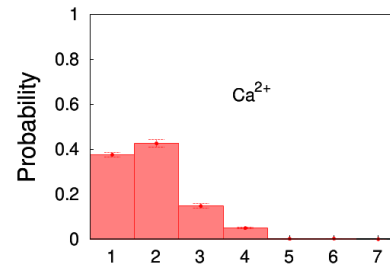
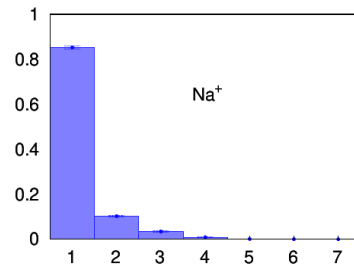
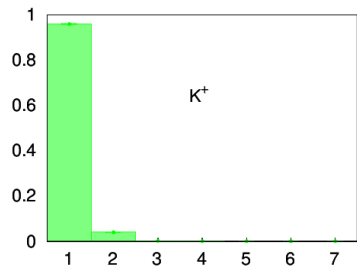
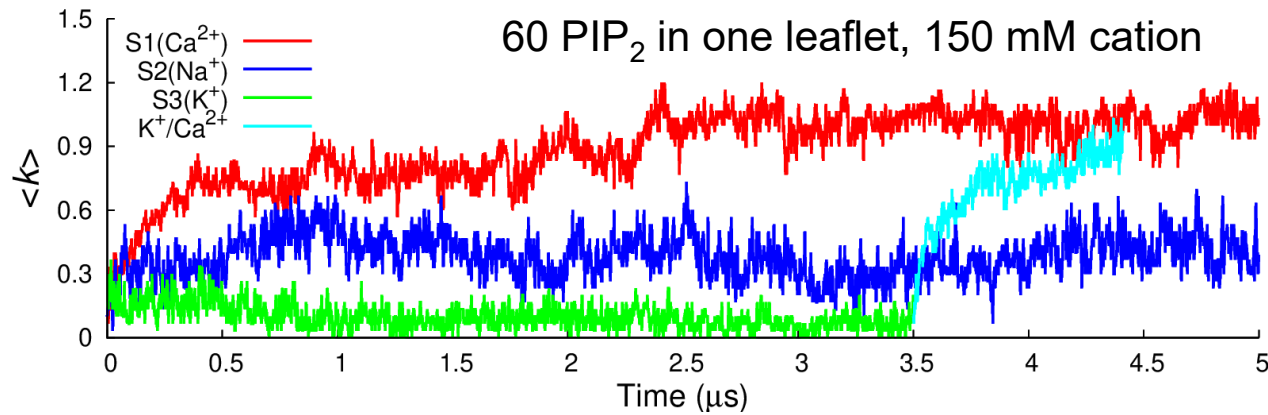
**C36/LJ-PME for lipid bilayers I (theory/techniques):** Yu, Krämer, Venable, Simmonett, MacKerell, Klauda, Pastor, Brooks, *J. Chem. Theory and Computation*, **17**, 1562 (2021)

**C36/LJ-PME for lipid bilayers II (application):** Yu, Krämer, Venable, Brooks, Klauda, and Pastor, *ibid.*, 1581

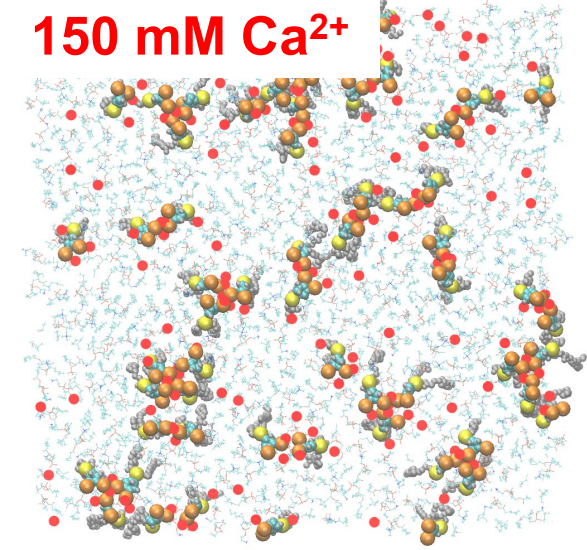
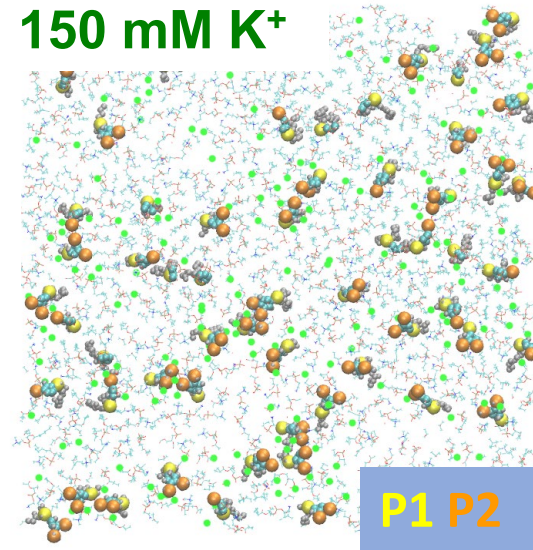


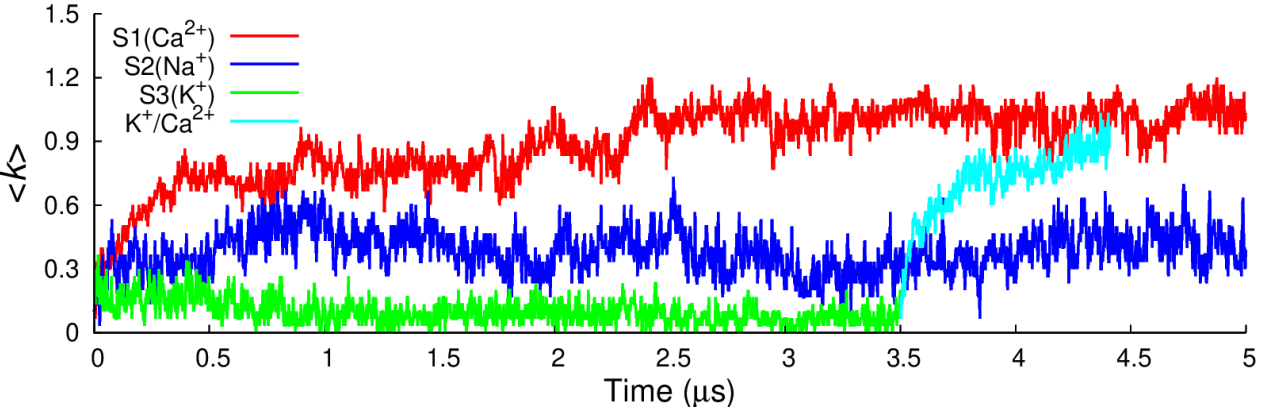
### 3. Bilayers with PIP<sub>2</sub> (but still no proteins)

Leaflet	PIP <sub>2</sub>	chol	POPC	POPE	POPA	PSM	total
upper	0	210	240	30	0	120	600
lower	<b>60 (10%)</b>	180	90	150	60	60	600



Cluster size



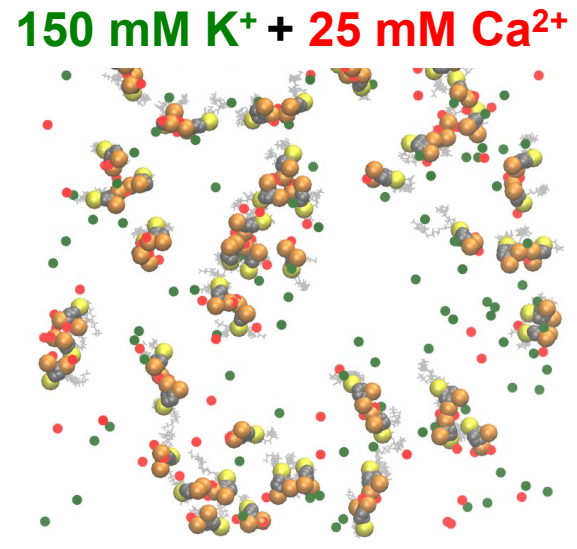
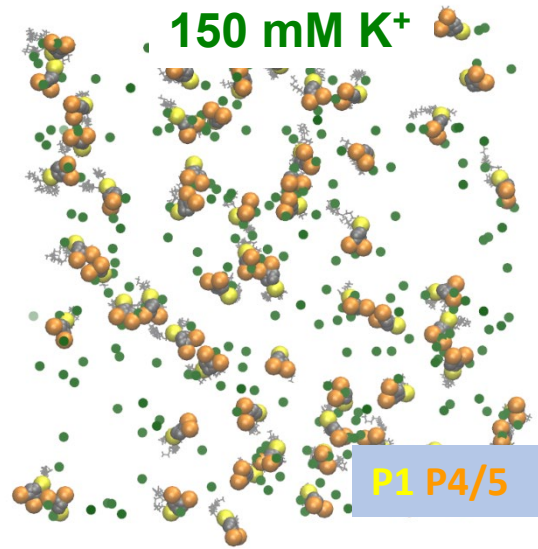
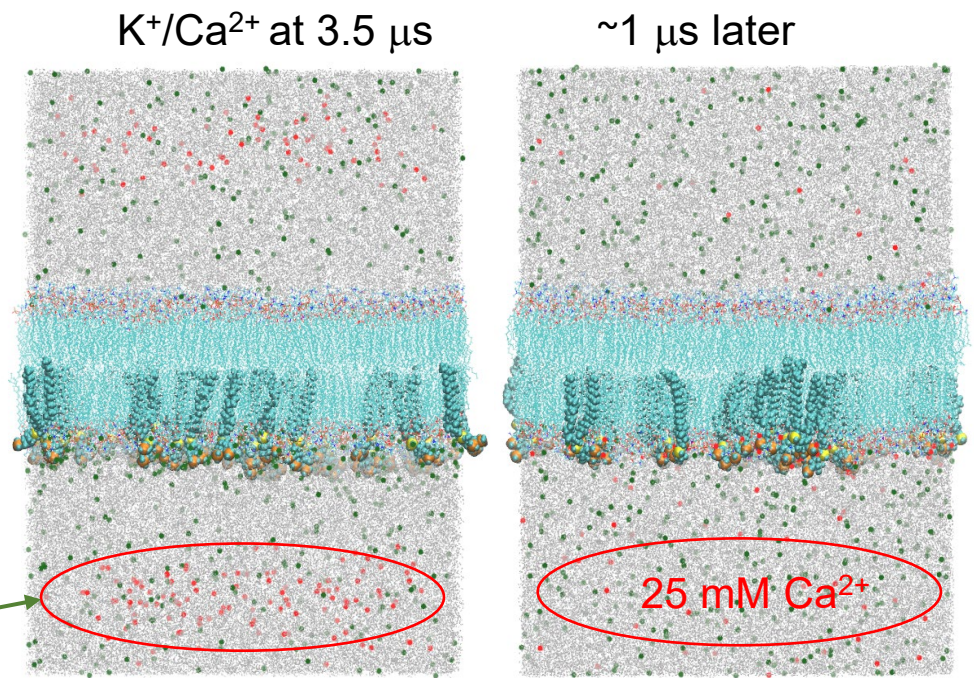


- *Inside cell:* K<sup>+</sup> concentration high/Ca<sup>2+</sup> low → low aggregation
- *Need clusters?* Pump in Ca<sup>2+</sup> (at 3.5 μs)

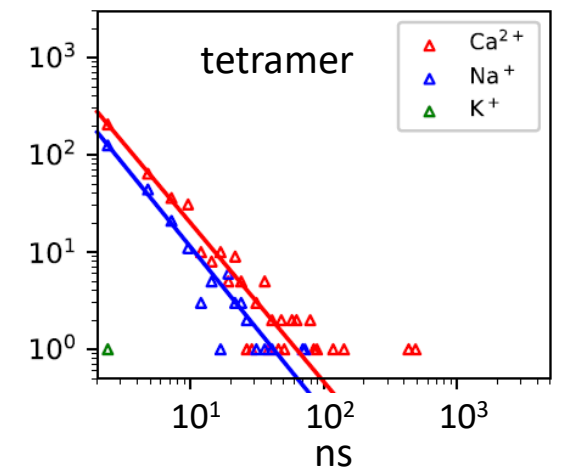
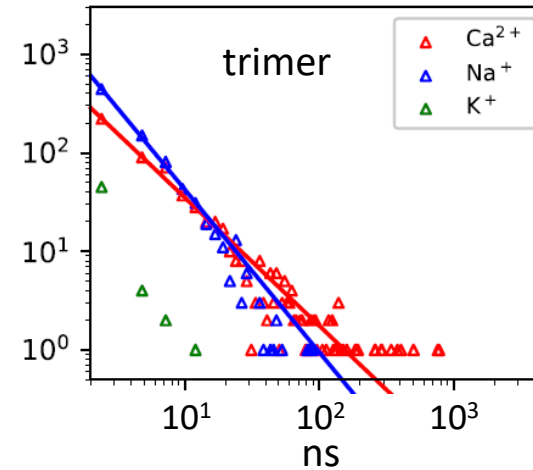
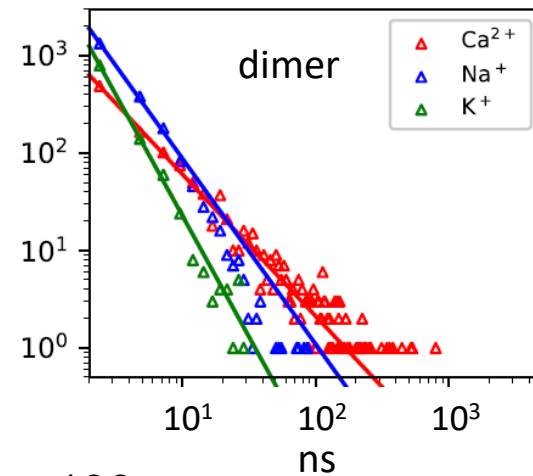
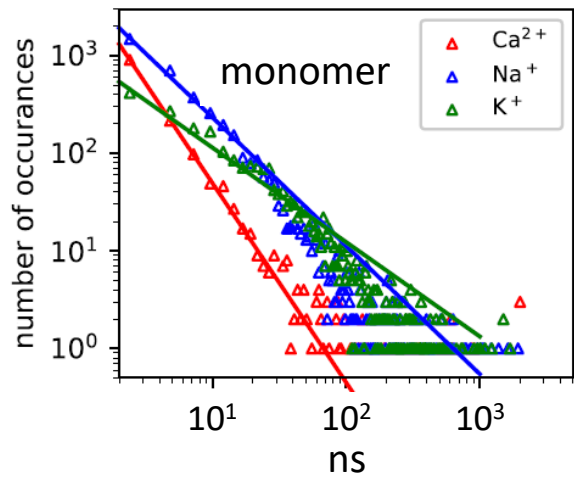
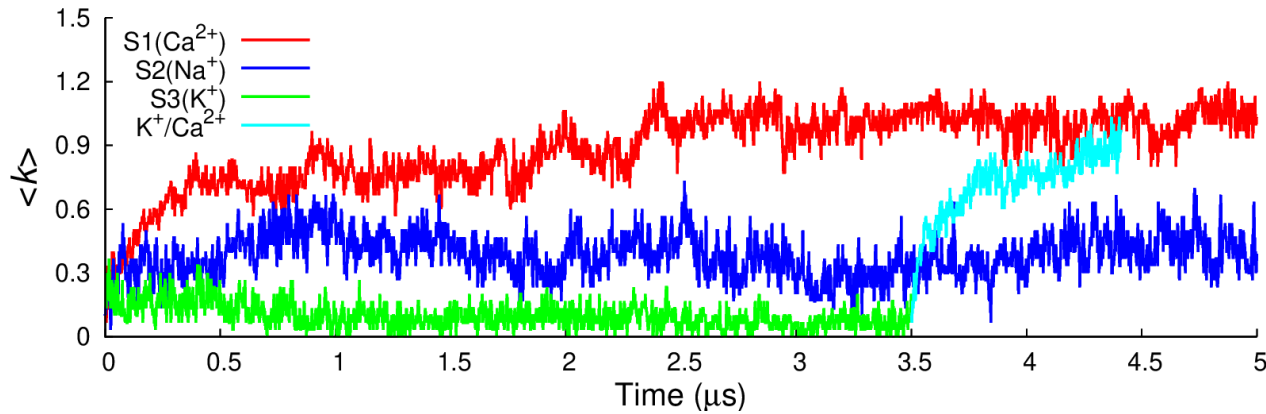
50 mM Ca<sup>2+</sup> added to bulk water region; K<sup>+</sup> (bulk) = 150 mM → 25 mM Ca<sup>2+</sup> bulk; rest bound to PIP<sub>2</sub>; K<sup>+</sup> (bulk) = 250 mM

- K + 25 mM Ca ≈ 150 mM Ca; synergy (as for monolayers)
- *small string-like* clusters with Ca<sup>2+</sup> → many “hot ends”

PIP<sub>2</sub> bilayers: Han, Kim, Venable, Pastor, in prep.



# Lifetimes of clusters follow expected trend



PIP<sub>2</sub> in K<sup>+</sup> remain monomers for 100s ns

Clusters in Ca<sup>2+</sup> stable for up to 1  $\mu\text{s}$ , but most are around 100 ns

Simulations on simpler symmetric bilayers:

- POPC
- 0.15 PIP<sub>2</sub>/0.85 POPC
- 0.15 PIP<sub>2</sub>/0.375 POPE/0.475 POPC

320 lipids, 310 K; 150 mM Ca<sup>2+</sup> or 300 mM K<sup>+</sup>

Lipids	Cation	Area (Å <sup>2</sup> /lipid)	K <sub>A</sub> (dyn/cm)	D (10 <sup>-8</sup> cm <sup>2</sup> /s)		
				POPC	PIP <sub>2</sub>	POPE
POPC	none	66.5	249	14.5		
POPC	K <sup>+</sup>	65.9	223	16.9		
POPC/PIP <sub>2</sub>	K <sup>+</sup>	66.2	268	11.9	10.2	
POPC/PIP <sub>2</sub> /POPE	K <sup>+</sup>	62.7	251	11.7	10.4	10.6
POPC	Ca <sup>2+</sup>	66.0	247	12.0		
POPC/PIP <sub>2</sub>	Ca <sup>2+</sup>	65.4	275	7.9	5.1	
POPC/PIP <sub>2</sub> /POPE	Ca <sup>2+</sup>	61.9	246	7.1	3.5	6.5

Diffusion constant not very sensitive to ion and lipid composition

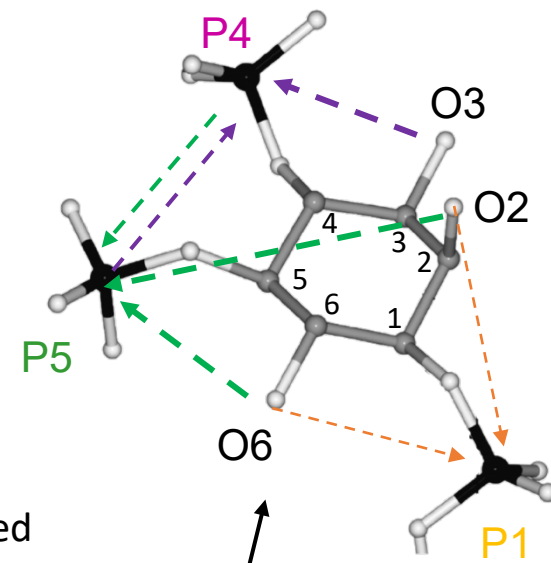
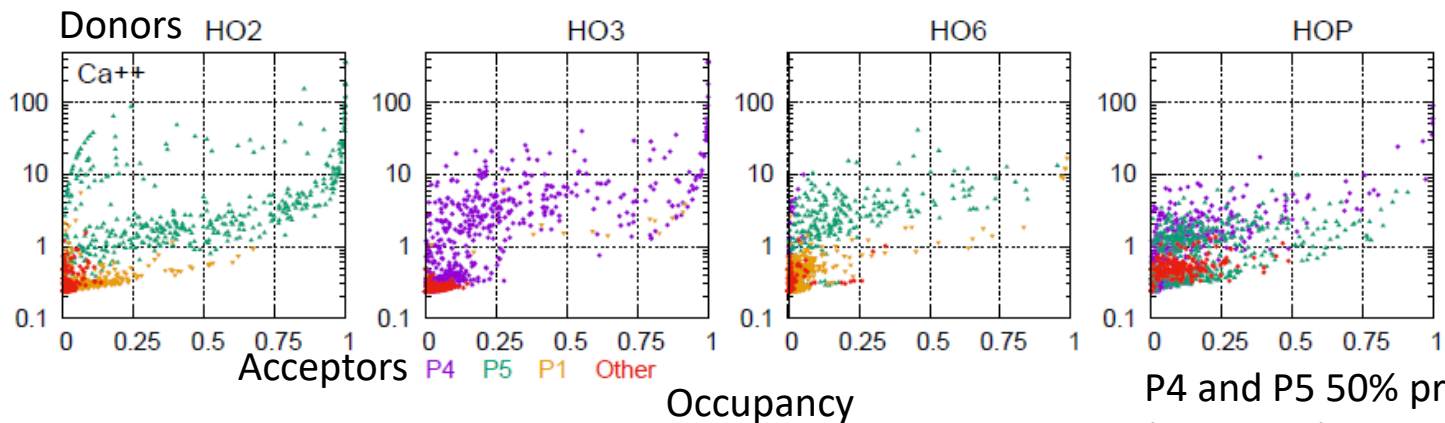
Big changes with PIP<sub>2</sub> and Ca<sup>2+</sup>

POPE lowers D of PIP<sub>2</sub>

Nothing too interesting

Back to the complex asymmetric bilayer (10% PIP<sub>2</sub>, 600 lipids/leaflet)

## Hydrogen bonds in Ca<sup>2+</sup> solutions (other ions similar):



P4 and P5 50% protonated (-1 charge) so total charge of each PIP<sub>2</sub> is -4.

Most PIP<sub>2</sub> H-bonds intramolecular

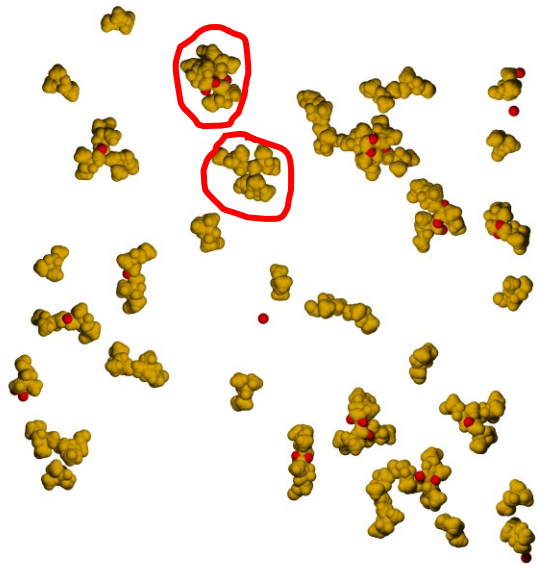
POPE most common acceptor lipid for PIP<sub>2</sub>;  
POPE also most common donor to PIP<sub>2</sub> (not shown)

Second most to water

Acceptor	PIP <sub>2</sub> Donor Atoms			
	OH2	OH3	OH6	PO4H
intra	0.659	0.474	0.432	0.598
PIP <sub>2</sub>	0.009	0.010	0.004	0.013
Chol	0.000	0.002	0.001	0.001
POPA	0.007	0.014	0.041	0.013
POPC	0.007	0.012	0.036	0.020
POPE	0.036	0.034	0.129	0.056
PSM	0.016	0.007	0.019	0.017
Water	0.223	0.424	0.321	0.240

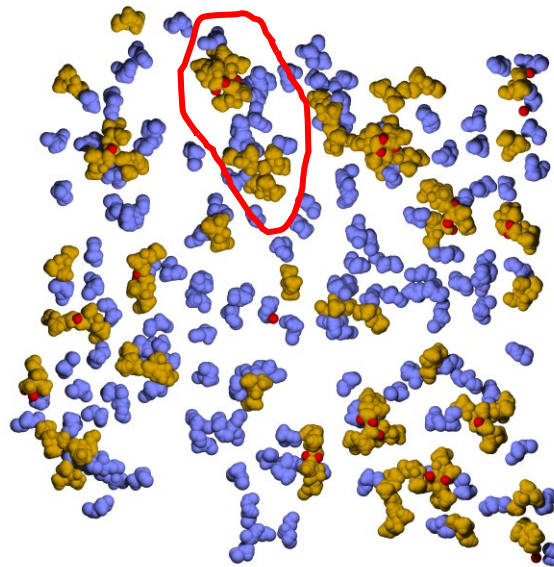
Can POPE link PIP<sub>2</sub> clusters? *Maybe, and with some help from POPA*

PIP<sub>2</sub> (orange)

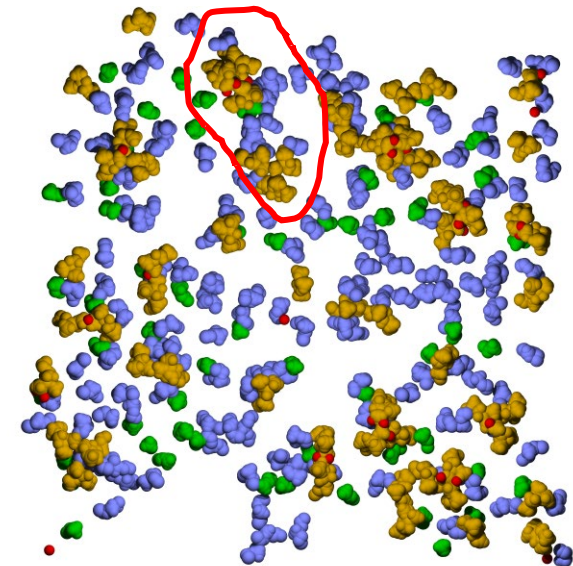


2.8 μs

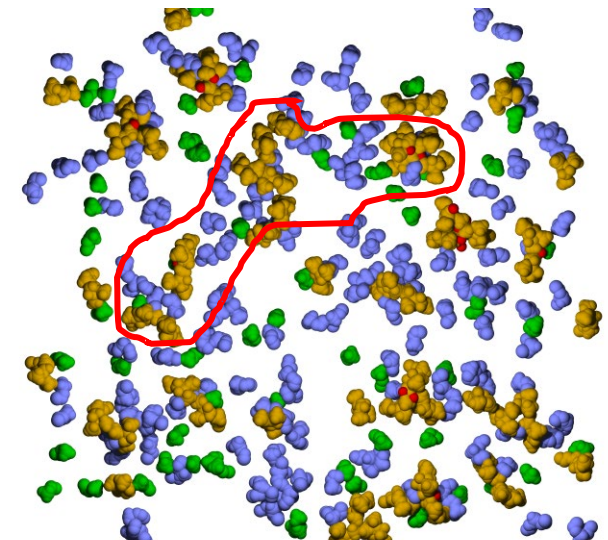
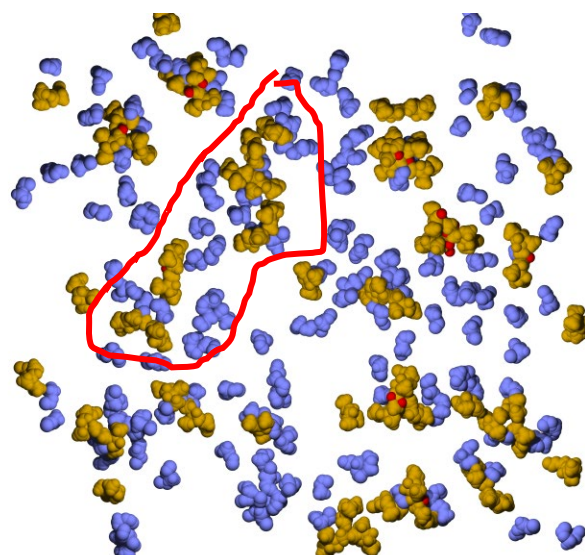
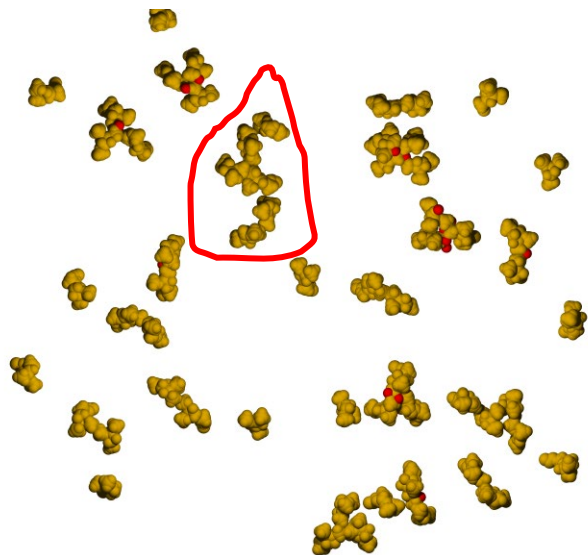
PIP<sub>2</sub> + POPE



PIP<sub>2</sub> + POPE + POPA

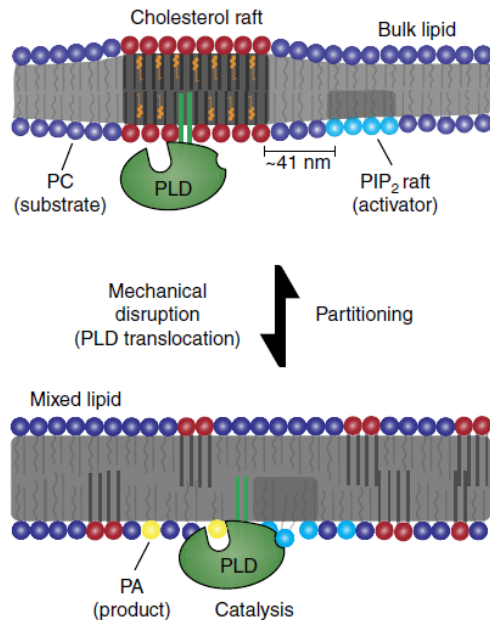
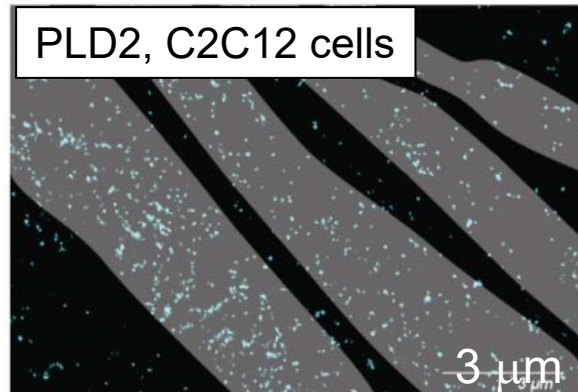


5.0 μs

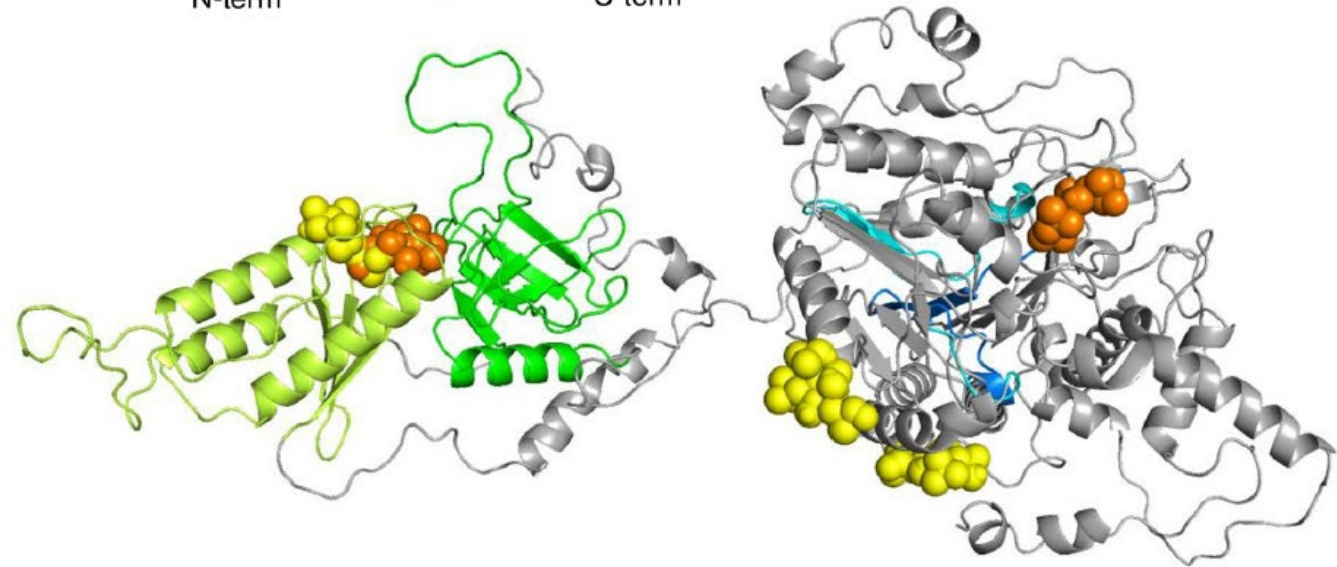
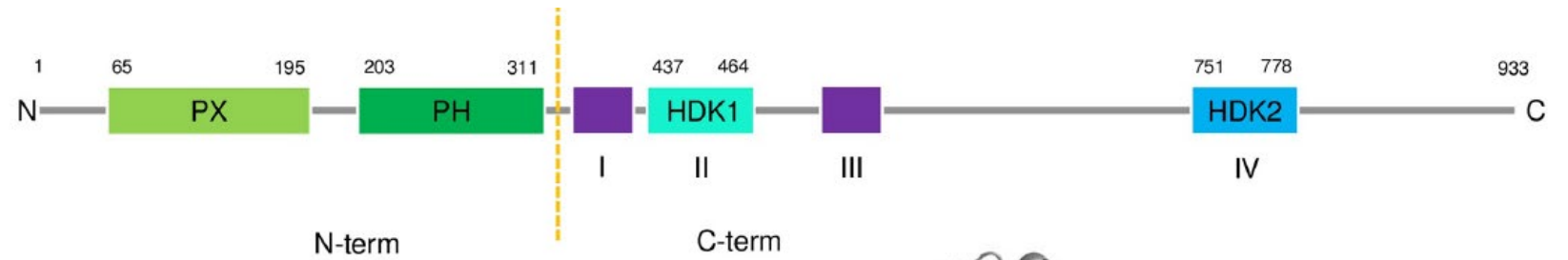


## 4. Proteins: PIP<sub>2</sub> binding of phospholipase D2 (PLD2)

STORM

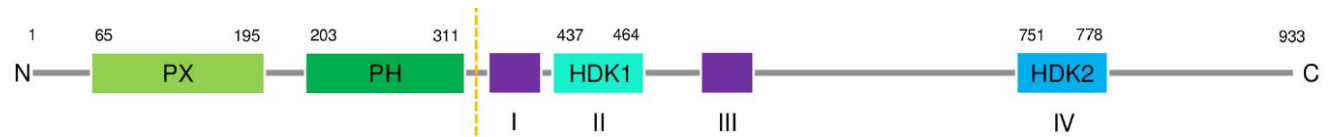
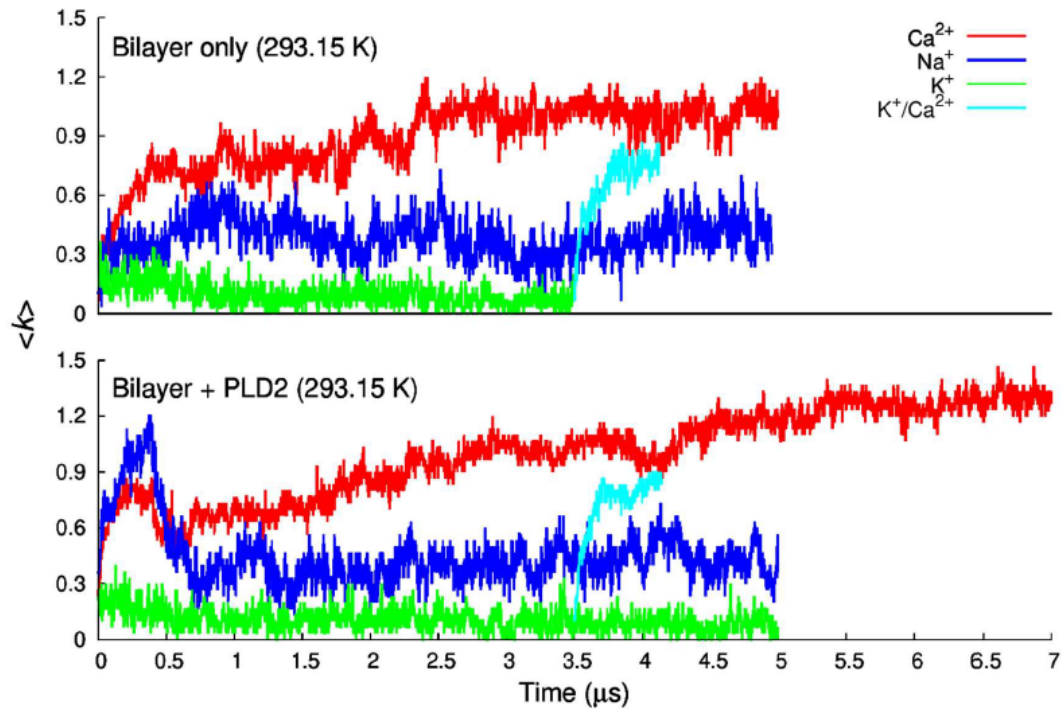


Domain structure of human PLD2

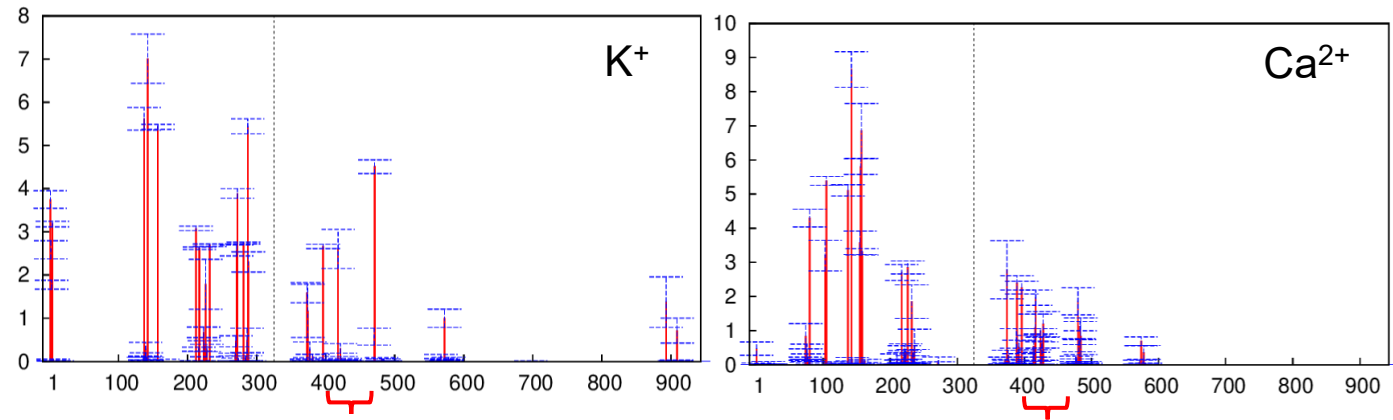


Model-built structure of human PLD2

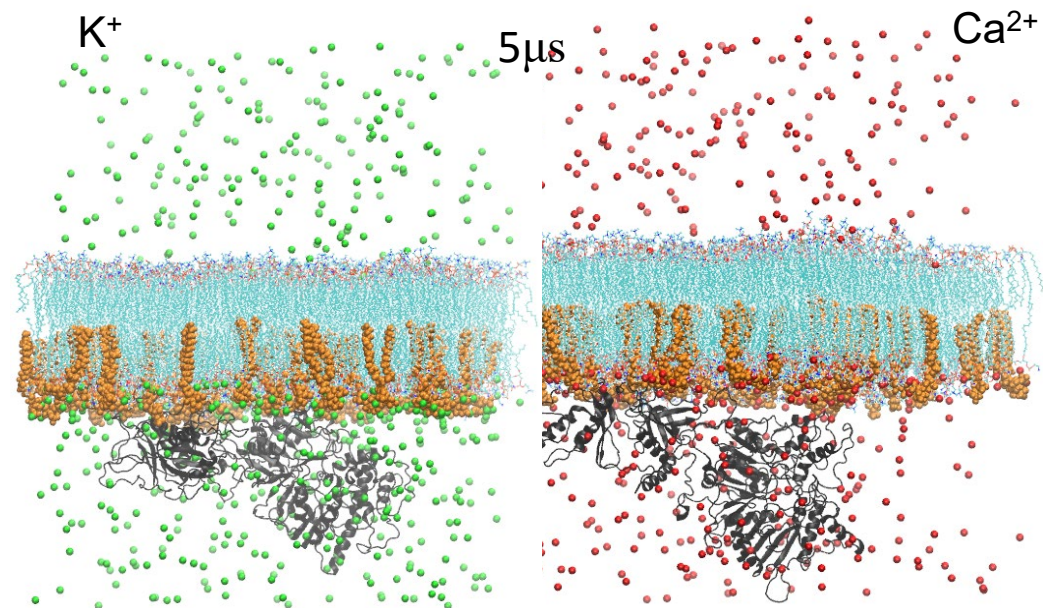
Han, Pastor, Fenollar-Ferrer, *PLoS One*, **15**, e0236201 (2020)



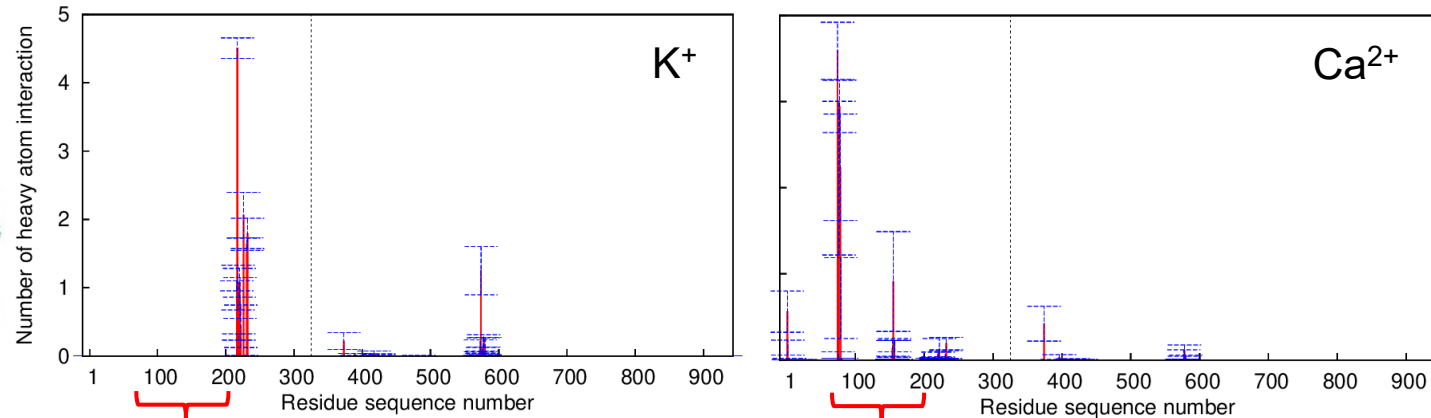
### PIP<sub>2</sub>-PLD2 Interactions



More binding to HKD1 domain (437-464) in Ca<sup>2+</sup>

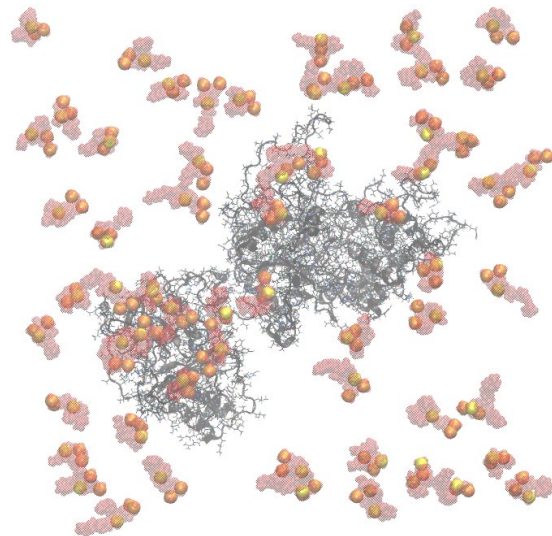
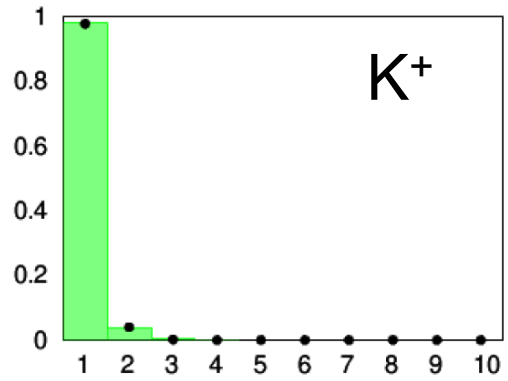


### POPA-PLD2 Interactions

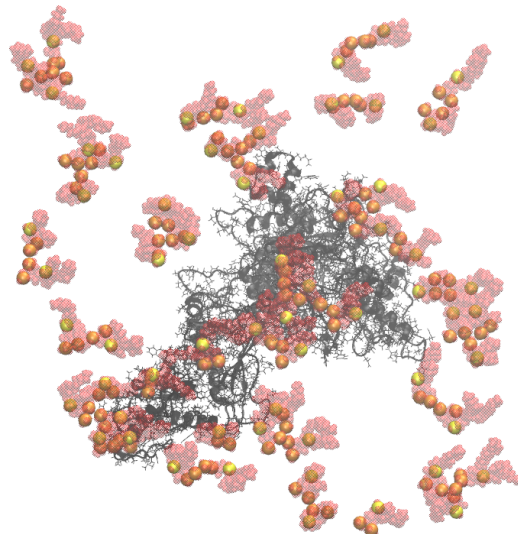
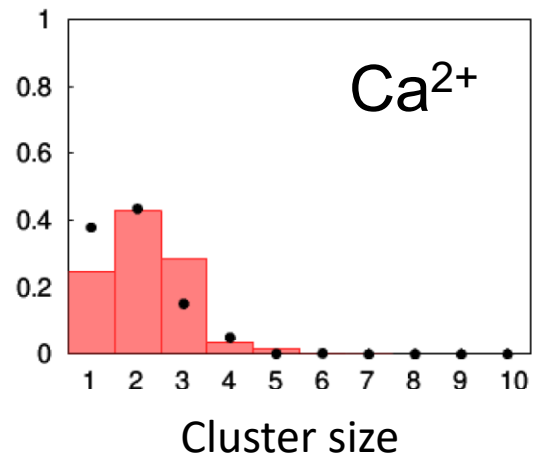
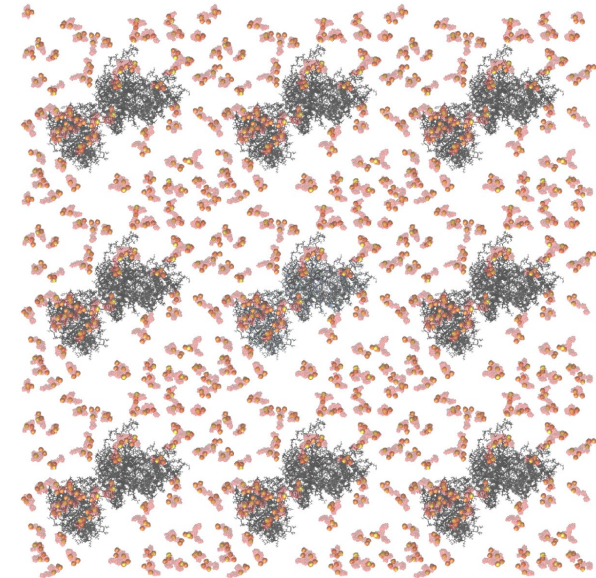


No binding to PX domain (65-195) in K<sup>+</sup>; binding in Ca<sup>2+</sup>

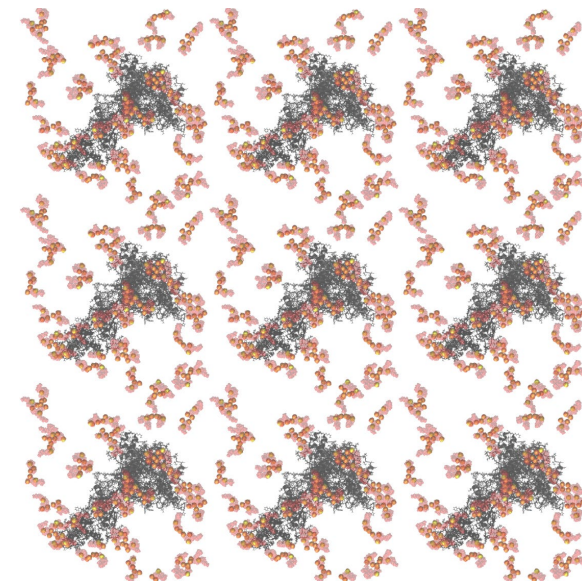
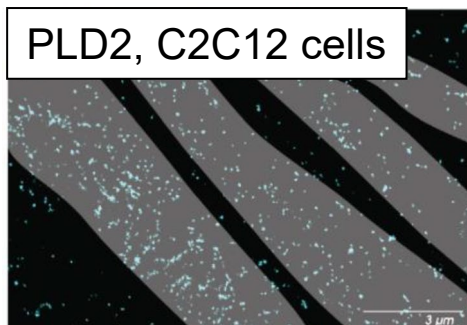




Expand to show  
8 image cells



to check for PIP<sub>2</sub>  
“strings” between  
images (none)

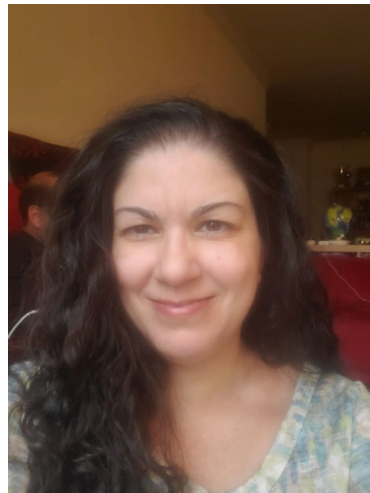


## Conclusions/Questions

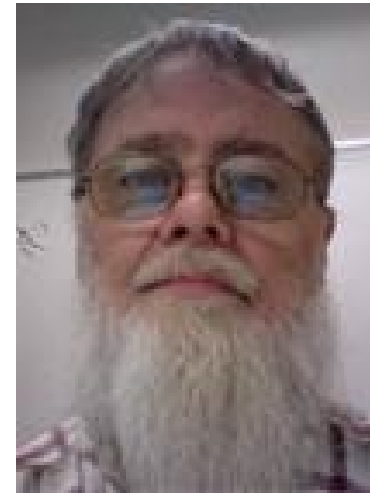
- Clustering highly ion dependent (mechanism of control in cells)
- Small string-like clusters in 10% PIP<sub>2</sub> bilayers; synergy of K<sup>+</sup> and Ca<sup>2+</sup> (also for monolayers)
- Clusters short lived (< μs), consistent with expt diffusion constant data and present sims
- Possible role of POPE and POPA in stabilization of clusters?
- Ca<sup>2+</sup> enhances PLD2 binding. Some stabilization of clusters by PLD2
- Organization a larger length scale?? Will small world graph (found for monolayers) hold?
- Test with polarizable force fields (revision of CHARMM Drude FF in progress)



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