Ion-Dependent Clustering of PIP$_2$

Richard W. Pastor
National Institutes of Health

1. Intro to PIP$_2$ / Simulation force field
2. 100% PIP$_2$ monolayers
3. Bilayers with PIP$_2$ (but still no proteins)
4. Proteins: PIP$_2$ binding of phospholipase D2 (PLD2)
1a. Intro to PIP$_2$

From Mclaughlin and Murray, Nature 2005:

For mammals, life (t=0) begins with PIP$_2$ hydrolysis:

1. fertilizing spermatozoan injects into egg genetic material and phospholipase C-ζ
2. Cleavage of PIP(4,5)P$_2$ → Inositol (1,4,5)P$_3$ → Ca$^{2+}$ oscillations
3. → seals egg membrane to additional sperm, initiate egg activation → you!

Charge = -4 at pH 7

Second Messengers:
- IP$_3$, DAG, PIP$_3$
- Membrane Targeting
- Other Functions
- Enzyme Activity
- Exocytosis, Endocytosis
- Cytoskeletal Attachment

Inositol (1,4,5)P$_3$ (IP$_3$) second messenger

Inositol
How does PIP$_2$ do so many different things?

1. Large number of phosphatidyl inositols that react with proteins

2. Forms clusters with proteins ("PIP$_2$ rafts")

Average diameters 50-90 nm

~5% PIP$_2$ in clusters (~1% unclustered)

→ PIP$_2$ raft not tightly packed

(very different from Lo phase)

Ca$^{2+}$ induces clusters (monolayers)

Organization of clusters? Ions?


1b. Simulation force field

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

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


<table>
<thead>
<tr>
<th>Lipid</th>
<th>Chains</th>
<th>Sim Kc (k_BT)</th>
<th>s.e. Kc</th>
<th>Expt X-ray</th>
<th>Expt Flicker</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPPC</td>
<td>16:0,16:0</td>
<td>28.2</td>
<td>0.9</td>
<td>29.8</td>
<td>33.0</td>
</tr>
<tr>
<td>DMPC</td>
<td>14:0,14:0</td>
<td>22.6</td>
<td>1.2</td>
<td>25.1</td>
<td>31.2</td>
</tr>
<tr>
<td>DOPC</td>
<td>18:1,18:1</td>
<td>21.2</td>
<td>1.0</td>
<td>19.4</td>
<td>26.4</td>
</tr>
<tr>
<td>POPC</td>
<td>16:0,18:1</td>
<td>24.7</td>
<td>1.0</td>
<td>24.6</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Lipid</th>
<th>K_θ (mN/m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPPC</td>
<td>46</td>
</tr>
<tr>
<td>DMPC</td>
<td>32</td>
</tr>
<tr>
<td>DOPC</td>
<td>49</td>
</tr>
<tr>
<td>POPC</td>
<td>44</td>
</tr>
</tbody>
</table>

2. 100% PIP$_2$ monolayers

First: monomethyl phosphate solutions (adjusted Ca$^{2+}$/phosphate interaction using osmotic pressure data)

Criteria for cluster based on radial distribution functions of ions and PIP$_2$ oxygen

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

Synergy of K and Ca

where \( k \) = link number/node; monomer \( k = 0 \), dimer = 1, long string \((1+2+2\ldots+1)/N \rightarrow 2\); clump \( \rightarrow k > 2\)

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

Synergy of K and Ca

*JPCB, 124, 1183 (2020)*
Jensen-Shannon distance with simulation lowest for small-world network.

string $k = 2$

*JPCB, 124, 1183 (2020)*
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 γ/A isotherms

~10 dyn/cm lost by 12 Å LJ cutoff

DPPC monolayer

DPPC bilayer

like hexadecane/air

→ automated reoptimization for bilayer and monolayer with explicit long-range LJ (LJ-PME)

→ agreement of expt and simulated monolayer g/A isotherms (useful for later)


C36/LJ-PME for lipid bilayers II (application): Yu, Krämer, Venable, Brooks, Klauda, and Pastor, *ibid.*, 1581
3. Bilayers with PIP$_2$ (but still no proteins)

<table>
<thead>
<tr>
<th>Leaflet</th>
<th>PIP$_2$</th>
<th>chol</th>
<th>POPC</th>
<th>POPE</th>
<th>POPA</th>
<th>PSM</th>
<th>total</th>
</tr>
</thead>
<tbody>
<tr>
<td>upper</td>
<td>0</td>
<td>210</td>
<td>240</td>
<td>30</td>
<td>0</td>
<td>120</td>
<td>600</td>
</tr>
<tr>
<td>lower</td>
<td>60 (10%)</td>
<td>180</td>
<td>90</td>
<td>150</td>
<td>60</td>
<td>60</td>
<td>600</td>
</tr>
</tbody>
</table>

60 PIP$_2$ in one leaflet, 150 mM cation

150 mM K$^+$

150 mM Ca$^{2+}$
• Inside cell: K⁺ concentration high/Ca²⁺ low → low aggregation
• Need clusters? Pump in Ca²⁺ (at 3.5 µs)

50 mM Ca²⁺ added to bulk water region; K⁺ (bulk) = 150 mM → 25 mM Ca²⁺ bulk; rest bound to PIP₂; K⁺ (bulk) = 250 mM

• K⁺ + 25 mM Ca ≈ 150 mM Ca; synergy (as for monolayers)
• small string-like clusters with Ca²⁺ → many “hot ends”

PIP₂ bilayers: Han, Kim, Venable, Pastor, in prep.
Lifetimes of clusters follow expected trend

Clusters in Ca\textsuperscript{2+} stable for up to 1 µs, but most are around 100 ns

PIP\textsubscript{2} in K\textsuperscript{+} remain monomers for 100s ns
Simulations on simpler symmetric bilayers:

- POPC
- 0.15 PIP$_2$/0.85 POPC
- 0.15 PIP$_2$/0.375 POPE/0.475 POPC

320 lipids, 310 K; 150 mM Ca$^{2+}$ or 300 mM K$^+$

<table>
<thead>
<tr>
<th>Lipids</th>
<th>Cation</th>
<th>Area (Å$^2$/lipid)</th>
<th>$K_A$ (dyn/cm)</th>
<th>D ($10^{-8}$ cm$^2$/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>POPC</td>
<td>none</td>
<td>66.5</td>
<td>249</td>
<td>POPC 14.5</td>
</tr>
<tr>
<td>POPC</td>
<td>K$^+$</td>
<td>65.9</td>
<td>223</td>
<td>POPC 16.9</td>
</tr>
<tr>
<td>POPC/PIP$_2$</td>
<td>K$^+$</td>
<td>66.2</td>
<td>268</td>
<td>POPC/PIP$_2$ 11.9</td>
</tr>
<tr>
<td>POPC/PIP$_2$/POPE</td>
<td>K$^+$</td>
<td>62.7</td>
<td>251</td>
<td>POPC/PIP$_2$/POPE 11.7</td>
</tr>
<tr>
<td>POPC</td>
<td>Ca$^{2+}$</td>
<td>66.0</td>
<td>247</td>
<td>POPC 12.0</td>
</tr>
<tr>
<td>POPC/PIP$_2$</td>
<td>Ca$^{2+}$</td>
<td>65.4</td>
<td>275</td>
<td>POPC/PIP$_2$ 7.9</td>
</tr>
<tr>
<td>POPC/PIP$_2$/POPE</td>
<td>Ca$^{2+}$</td>
<td>61.9</td>
<td>246</td>
<td>POPC/PIP$_2$/POPE 7.1</td>
</tr>
</tbody>
</table>

- Diffusion constant not very sensitive to ion and lipid composition
- Big changes with PIP$_2$ and Ca$^{2+}$
- POPE lowers D of PIP$_2$

Nothing too interesting
Back to the complex asymmetric bilayer (10% PIP₂, 600 lipids/leaflet)

Hydrogen bonds in Ca²⁺ solutions (other ions similar):

Most PIP₂ H-bonds intramolecular

POPE most common acceptor lipid for PIP₂;
POPE also most common donor to PIP₂ (not shown)

Second most to water

<table>
<thead>
<tr>
<th>Acceptor</th>
<th>OH2</th>
<th>OH3</th>
<th>OH6</th>
<th>PO4H</th>
</tr>
</thead>
<tbody>
<tr>
<td>intra</td>
<td>0.659</td>
<td>0.474</td>
<td>0.432</td>
<td>0.598</td>
</tr>
<tr>
<td>PIP₂</td>
<td>0.009</td>
<td>0.010</td>
<td>0.004</td>
<td>0.013</td>
</tr>
<tr>
<td>Chol</td>
<td>0.000</td>
<td>0.002</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>POPA</td>
<td>0.007</td>
<td>0.014</td>
<td>0.041</td>
<td>0.013</td>
</tr>
<tr>
<td>POPC</td>
<td>0.007</td>
<td>0.012</td>
<td>0.036</td>
<td>0.020</td>
</tr>
<tr>
<td>POPE</td>
<td>0.036</td>
<td>0.034</td>
<td>0.129</td>
<td>0.056</td>
</tr>
<tr>
<td>PSM</td>
<td>0.016</td>
<td>0.007</td>
<td>0.019</td>
<td>0.017</td>
</tr>
<tr>
<td>Water</td>
<td>0.223</td>
<td>0.424</td>
<td>0.321</td>
<td>0.240</td>
</tr>
</tbody>
</table>
Can POPE link PIP$_2$ clusters? *Maybe, and with some help from POPA*
4. Proteins: PIP$_2$ binding of phospholipase D2 (PLD2)

**Domain structure of human PLD2**

Model-built structure of human PLD2

Han, Pastor, Fenollar–Ferrer, *PLOS One*, 15, e0236201 (2020)

Ca^{2+} and K^+

**PIP_2-PLD2 Interactions**

- More binding to HKD1 domain (437-464) in Ca^{2+}

**POPA-PLD2 Interactions**

- No binding to PX domain (65-195) in K^+; binding in Ca^{2+}
Conclusions/Questions

• Clustering highly ion dependent (mechanism of control in cells)
• Small string-like clusters in 10% PIP$_2$ bilayers; synergy of K$^+$ and Ca$^{2+}$ (also for monolayers)
• Clusters short lived (< $\mu$s), consistent with expt diffusion constant data and present sims
• Possible role of POPE and POPA in stabilization of clusters?
• Ca$^{2+}$ 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)