

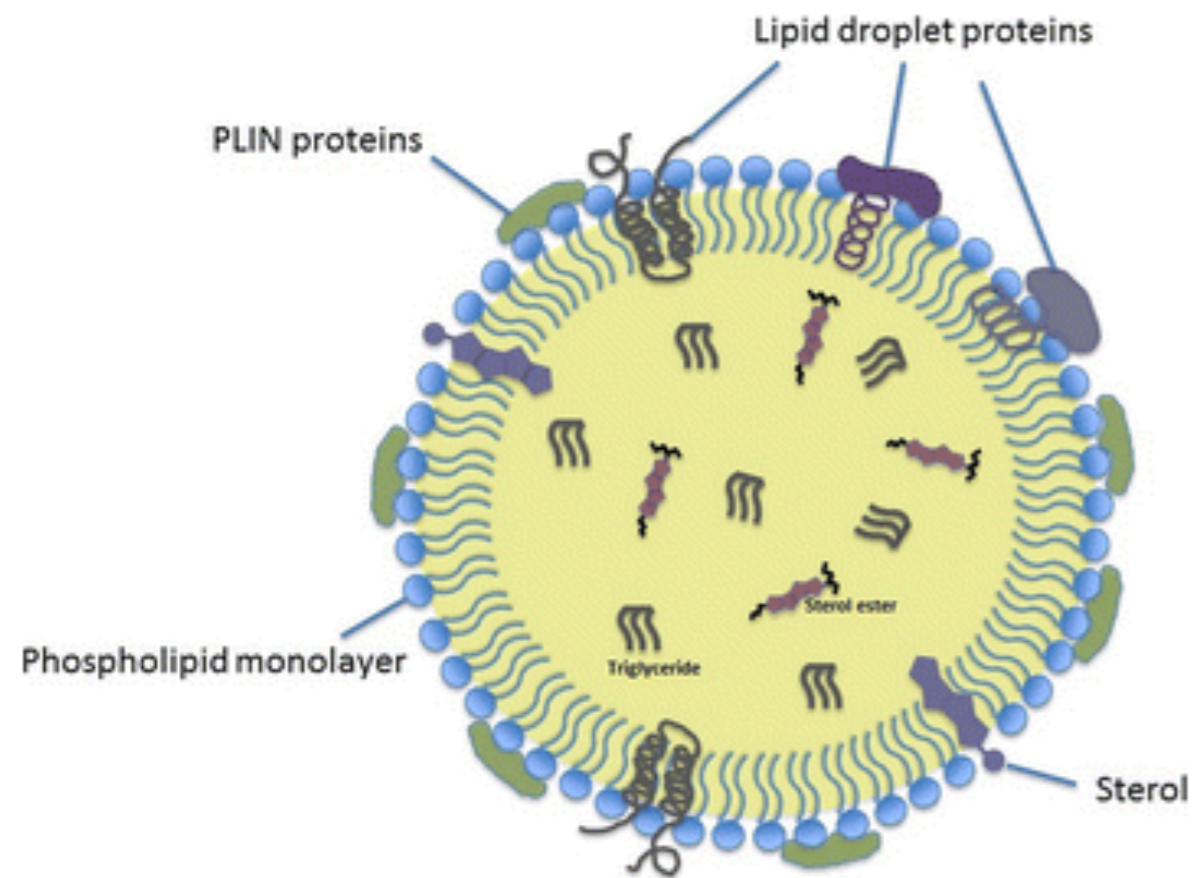
# Molecular simulations of lipid droplets

Luca Monticelli

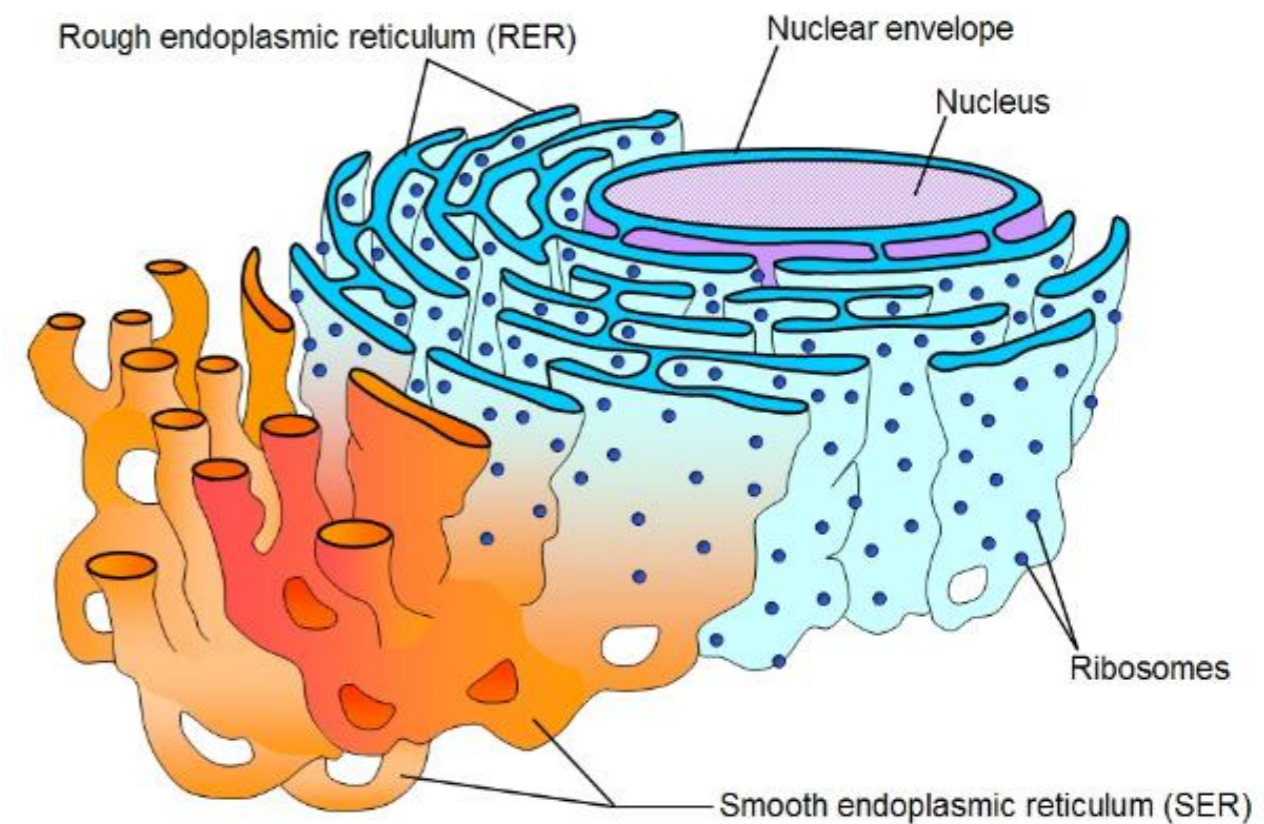
CNRS / U Lyon (France)

# Lipid droplets are emulsions

## Biology: organelles regulating energy metabolism



LD structure



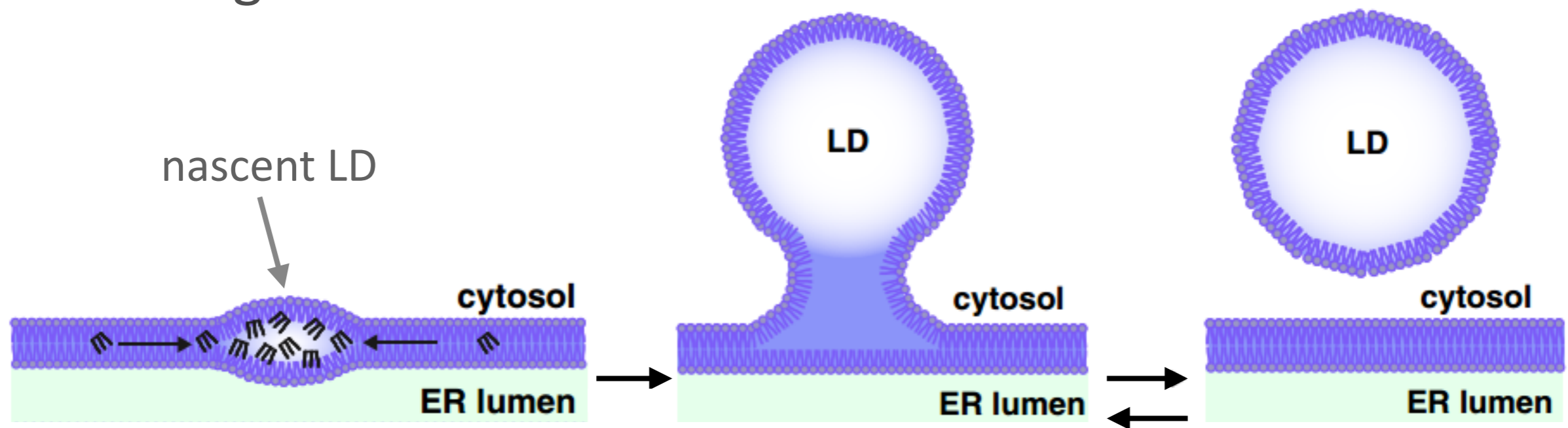
LD formation: in the ER

# Lipid droplet formation: open questions

Nucleation  
and growth

Budding

Fission



Walther & Farese et al., COCB 2014

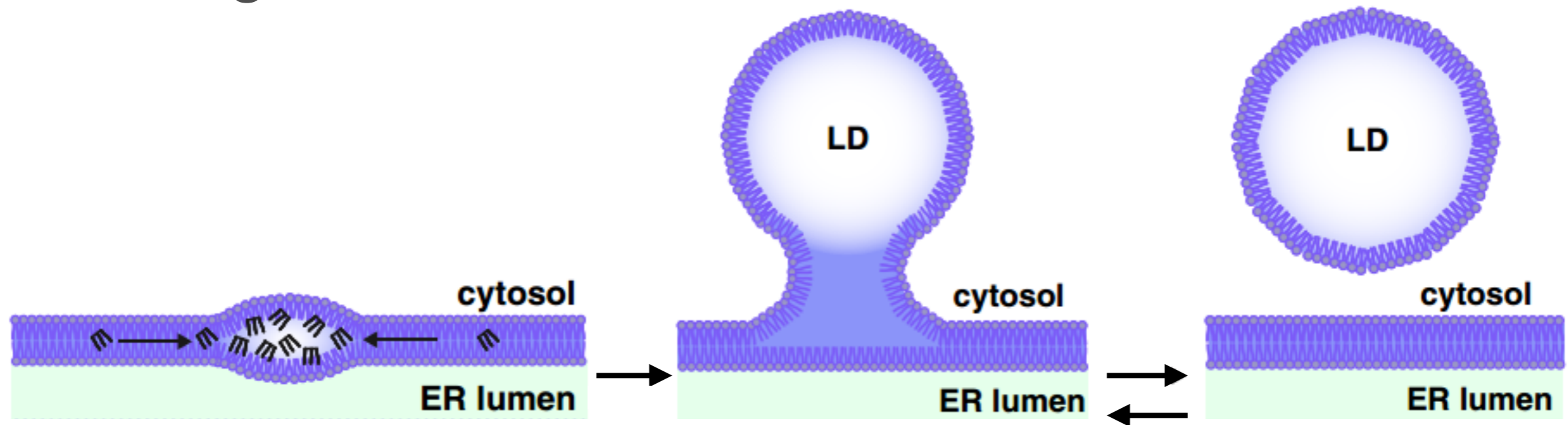
**What is the role of membrane properties?  
Role of lipid composition? Role of proteins?**

# Lipid droplet formation: open questions

Nucleation  
and growth

Budding

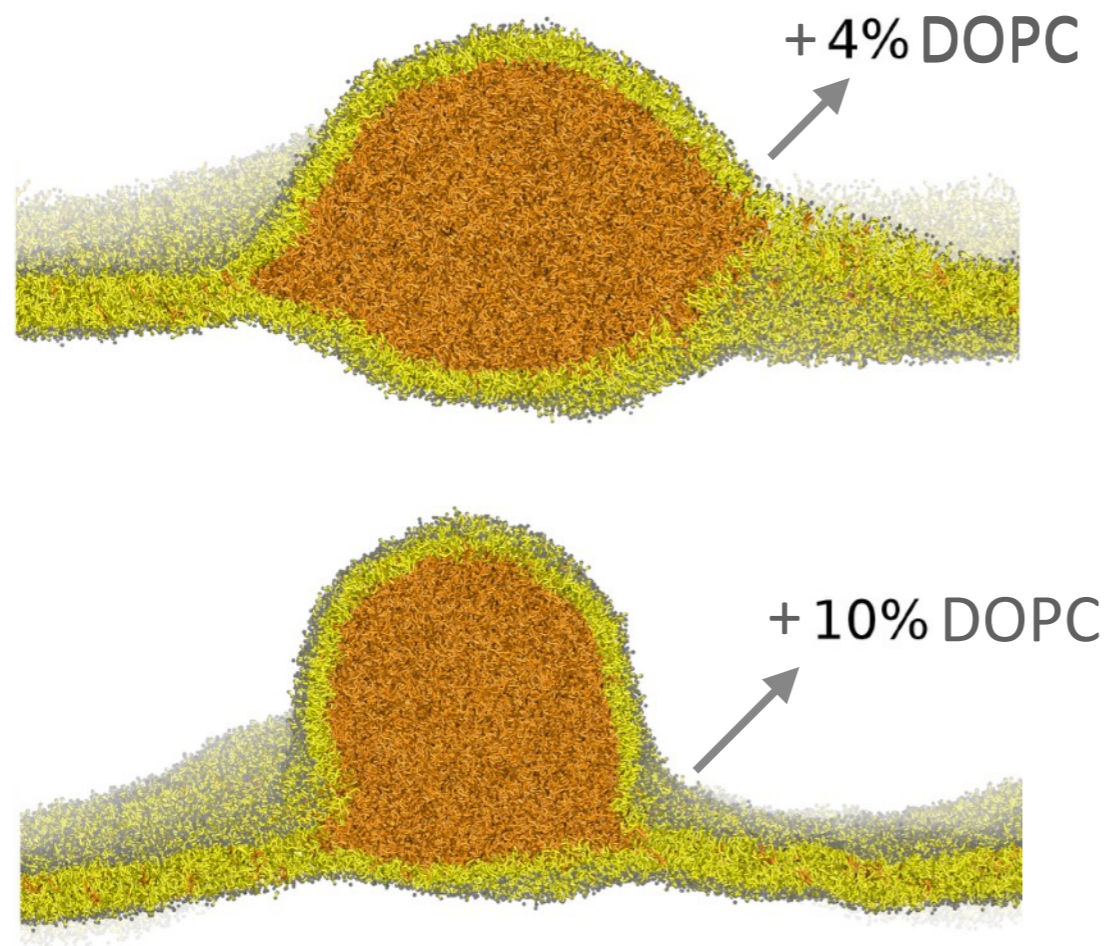
Fission



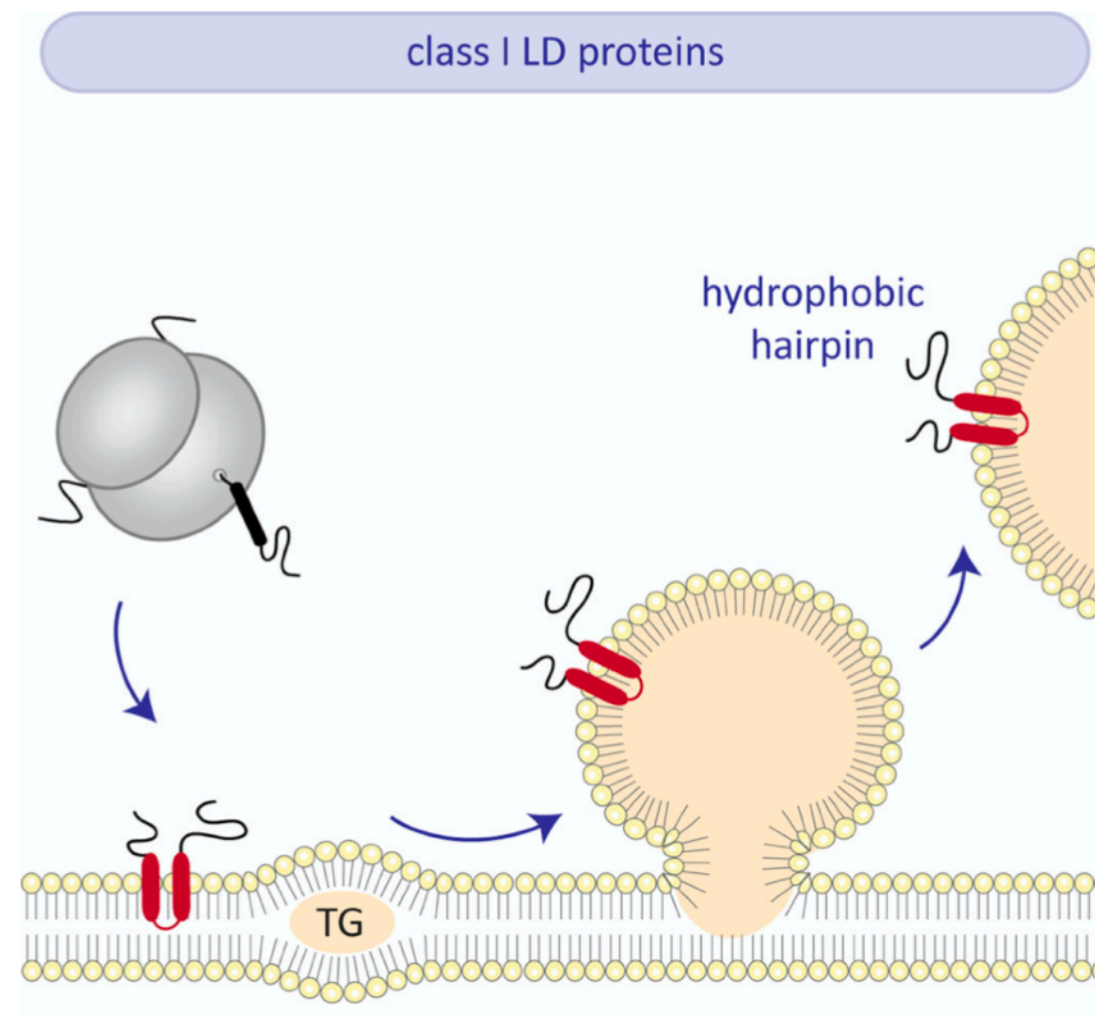
Walther & Farese et al., COCB 2014

**Our goal: to understand the mechanism of LD formation**  
**Impact: biology, metabolic diseases, viral infection, biotech**

# Asymmetric budding (1) and LD proteome (2)

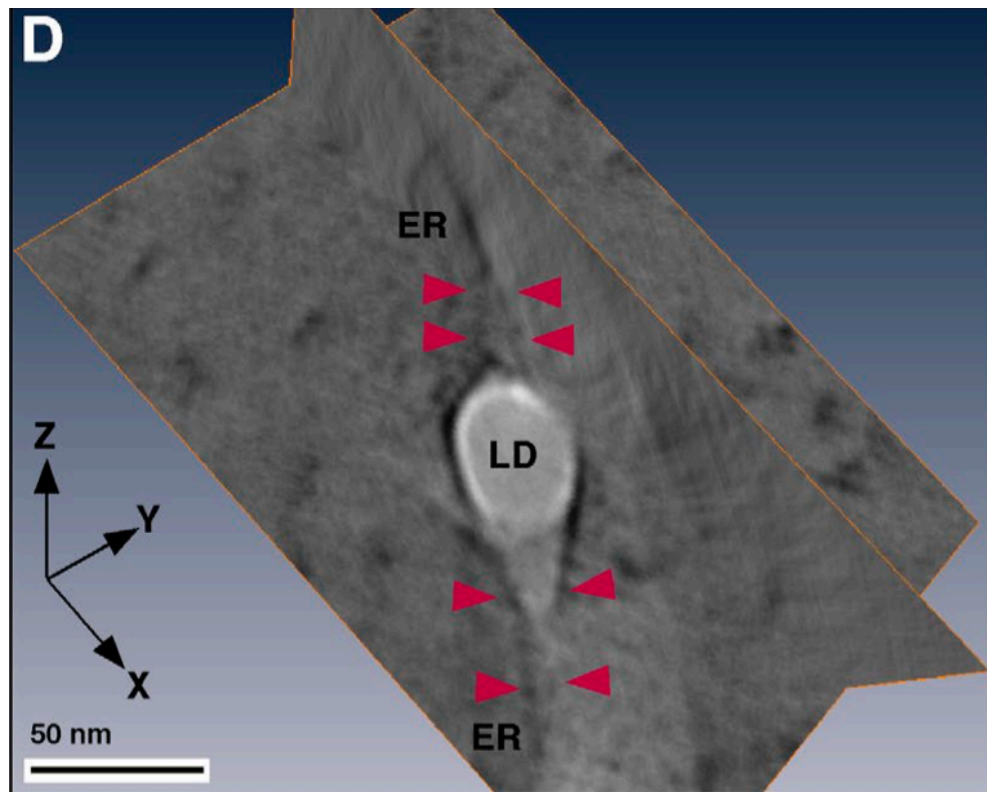


How to study LD biogenesis  
using MD simulations



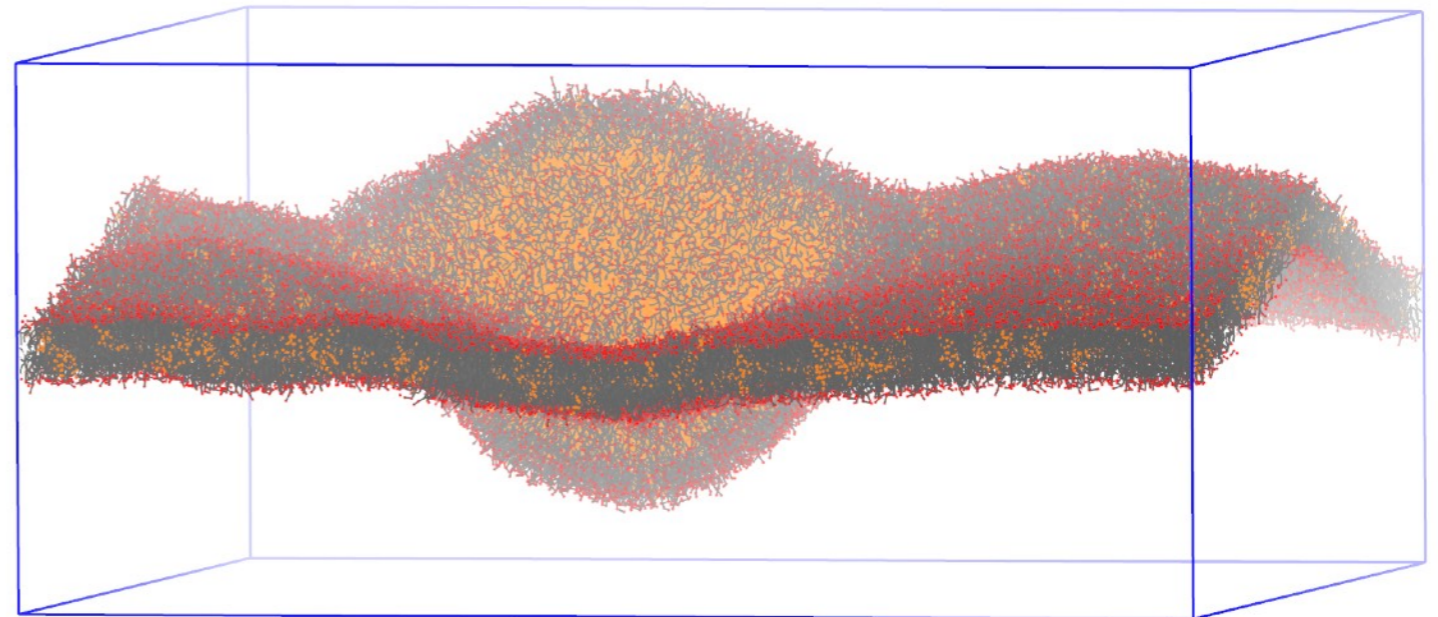
How do LDs get their  
proteins?

# Simulating lipid droplets



W.A. Prinz et al., *J Cell Biol*, 2015

Droplet size: ~38 nm

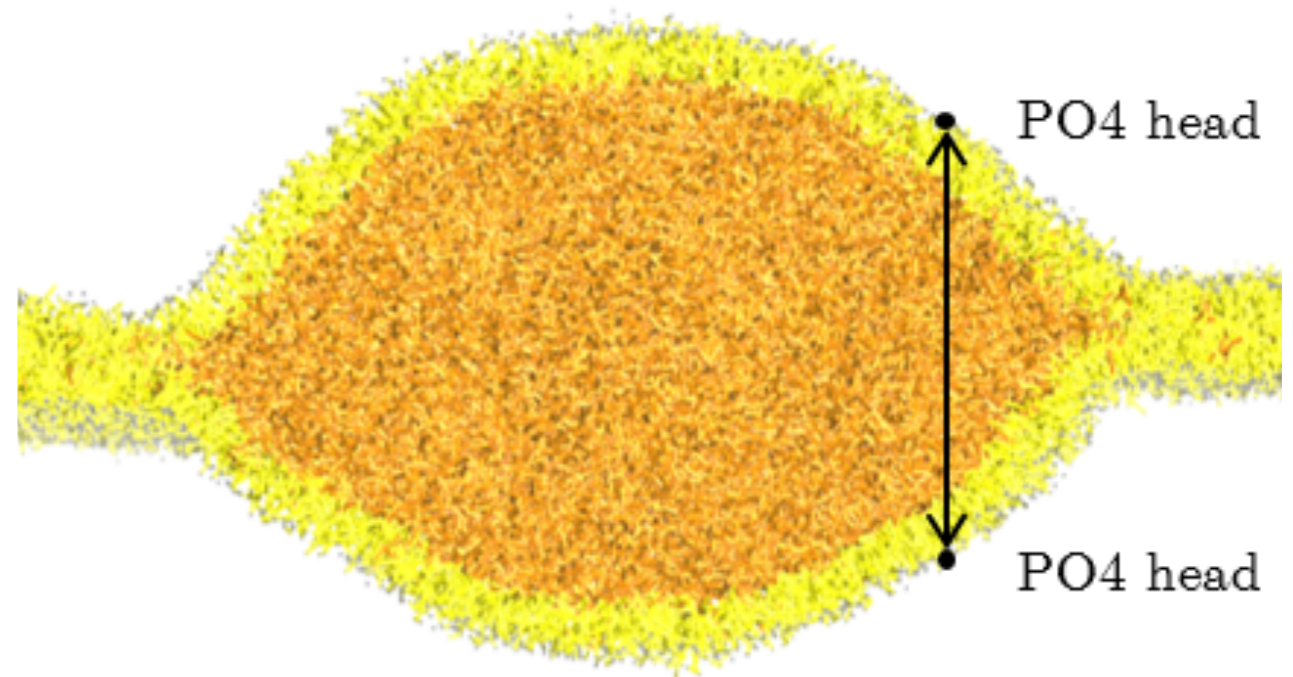
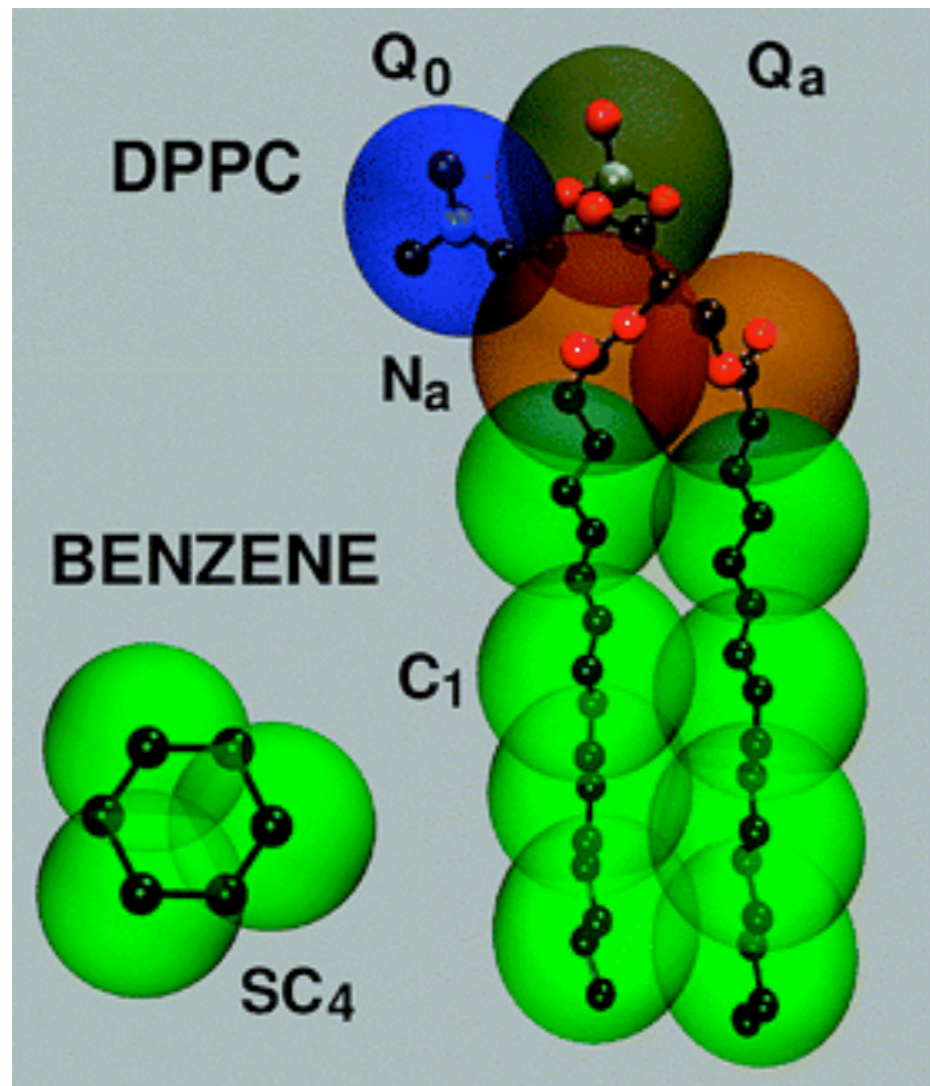


Droplet diameter: ~35 nm

Bilayer size: 78x78 nm

Estimated number of atoms: 20M

# The MARTINI CG model



LD size: 36 nm

Bilayer lateral size: 78 x 78 nm

~2M CG particles (20M atoms)

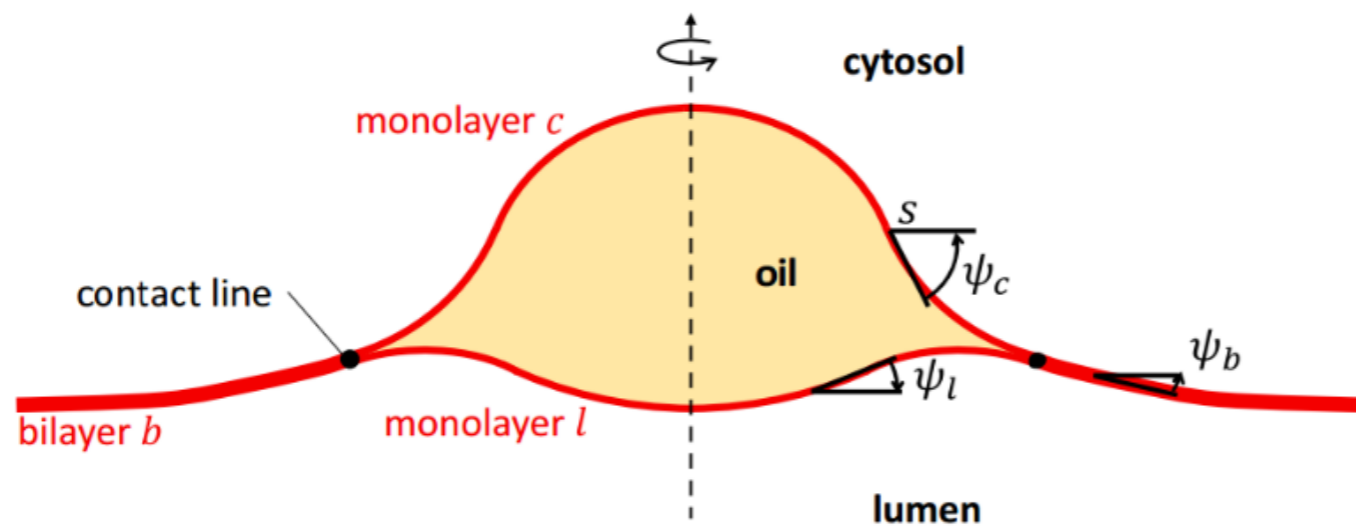
MD simulations: up to 50  $\mu$ s

Marrink *et al.*, *J Phys Chem B* (2007), 111, 7812

Monticelli *et al.*, *JCTC* (2008), 4, 819

Souza *et al.*, *Nature Methods* (2021), 18, 382

# Lipid droplet budding: a theoretical approach



$$F = \sum_{i=\{c,l,b\}} \int_{A_i} \left[ \frac{\kappa}{2} (2H - C_0^i)^2 + \kappa_G^i K \right] dA + \sum_{i=\{c,l,b\}} \gamma_i A_i + 2\pi R\tau - P_l V_l - P_c V_c$$

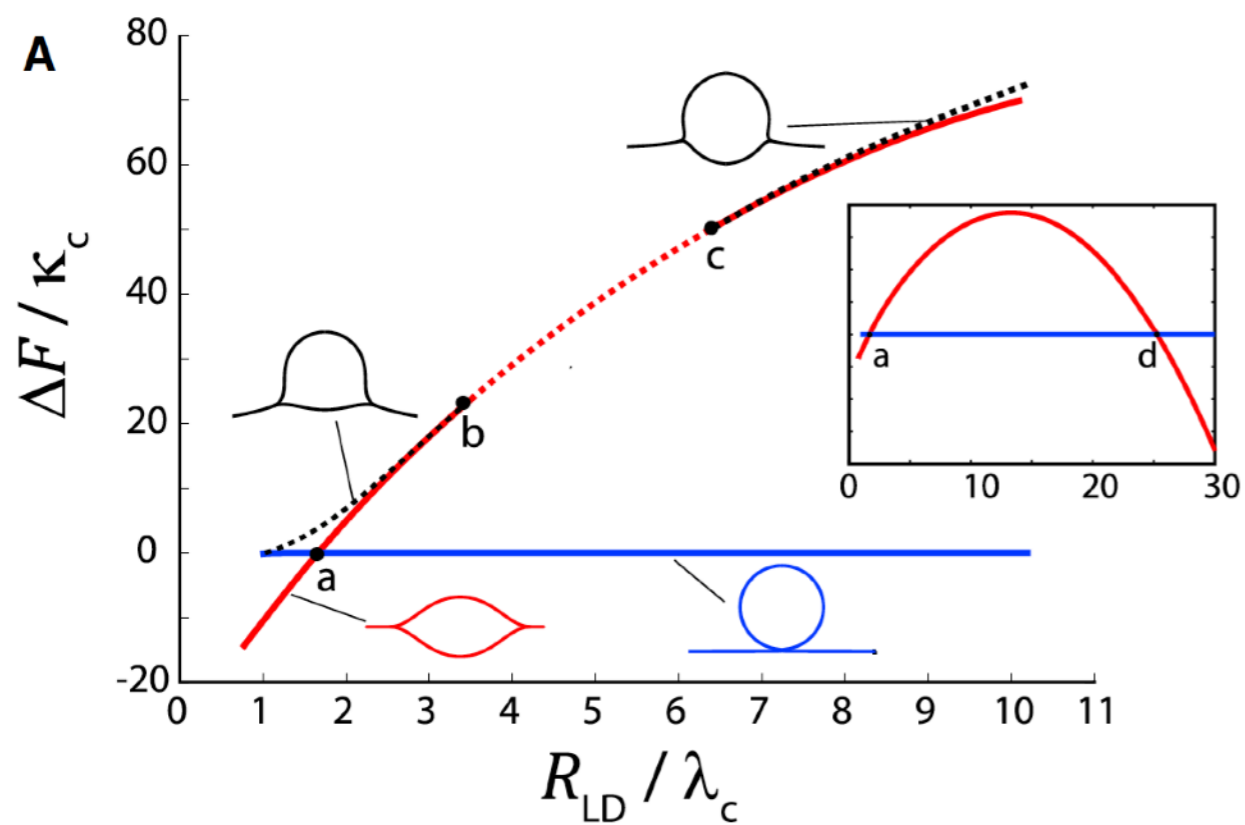
elastic energy
surface energy
line energy

- For a given LD volume  $V$ , the LD *shape* is obtained by minimizing the free energy
- Theory contains approximations and unknowns

Foret *et al*, *Biophys J* 2017

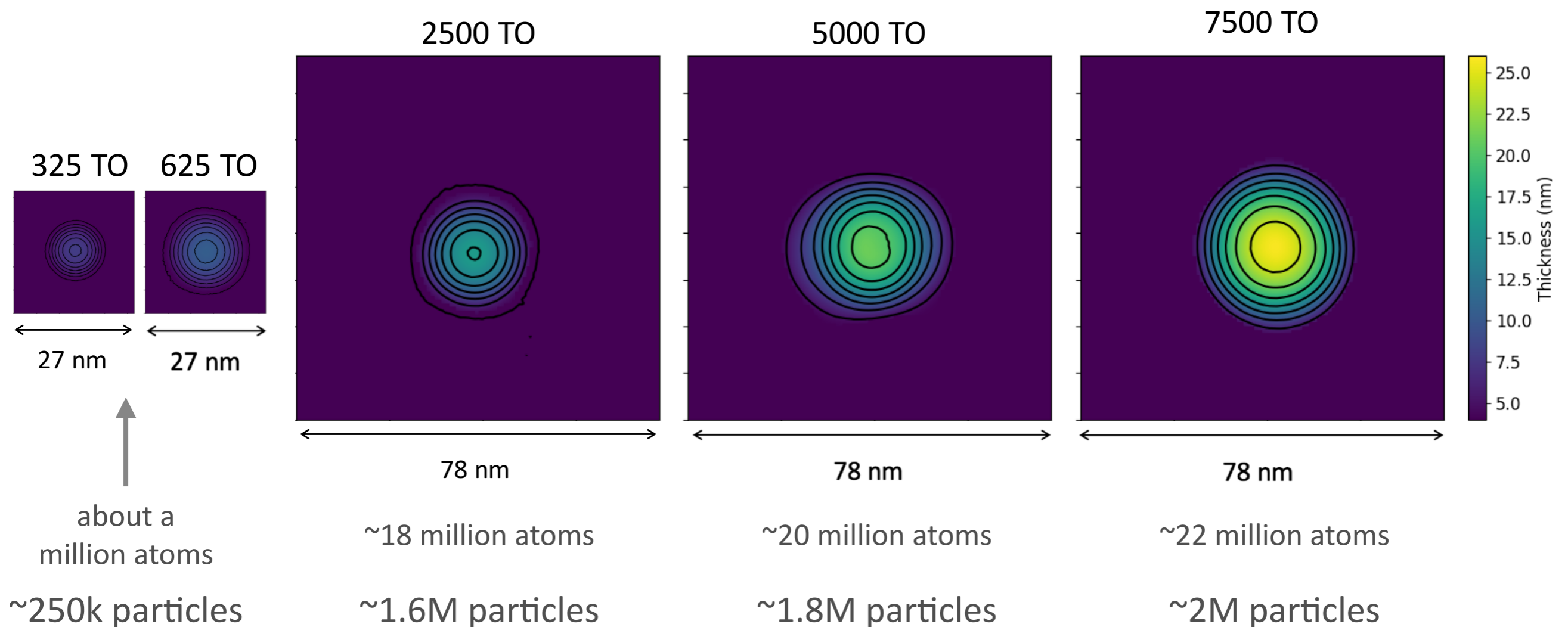


# The theory predicts spontaneous budding, but...

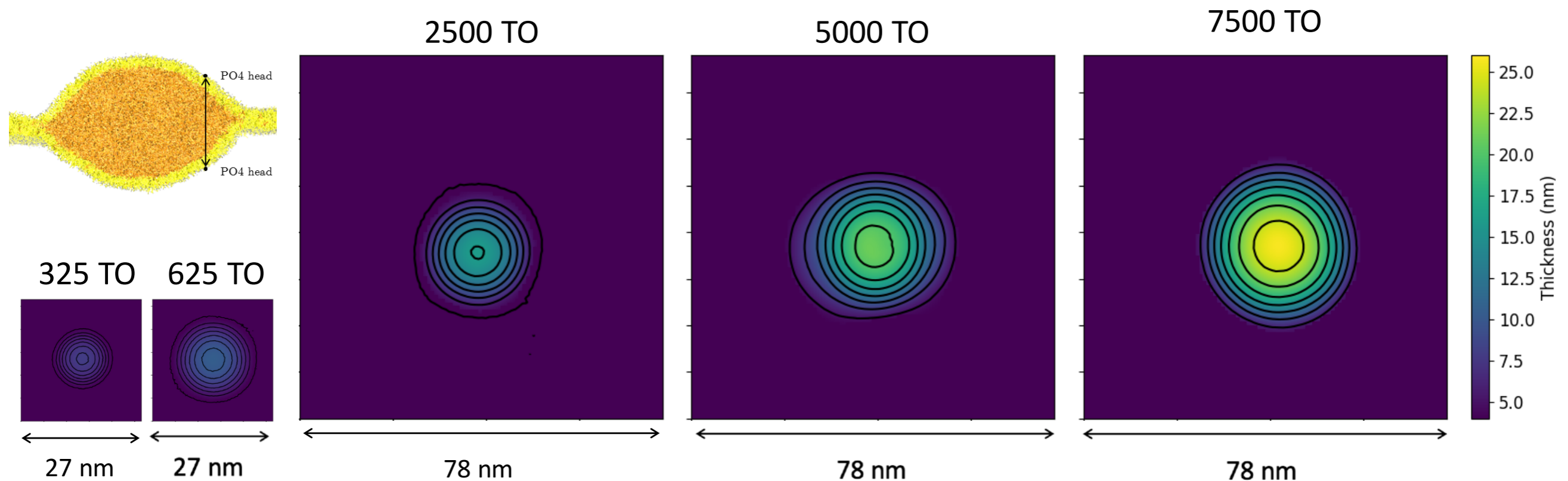


- Small size: symmetric nascent LD (embedded)
- Large size: spherical LDs, should bud out spontaneously; at which size? ( $\lambda_c \sim 10\text{nm}$ ?)
- LD shape can be predicted for any size

# Simulating nascent LDs of different sizes, calculating shapes

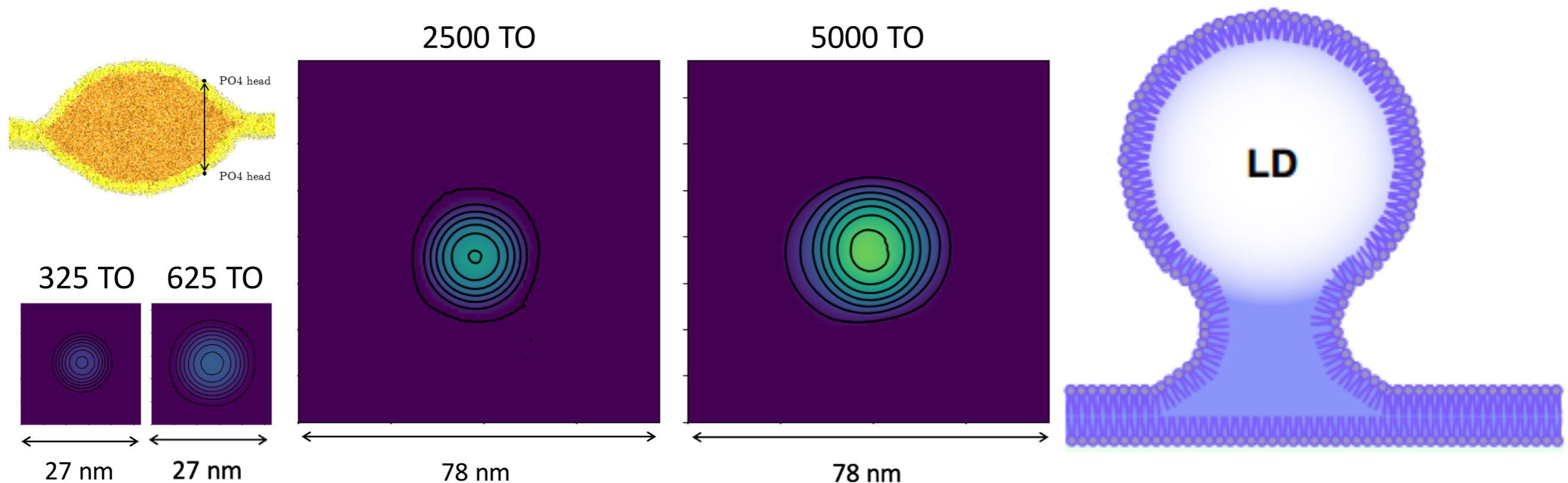


# Nascent LDs up to 35 nm in diameter do not bud out



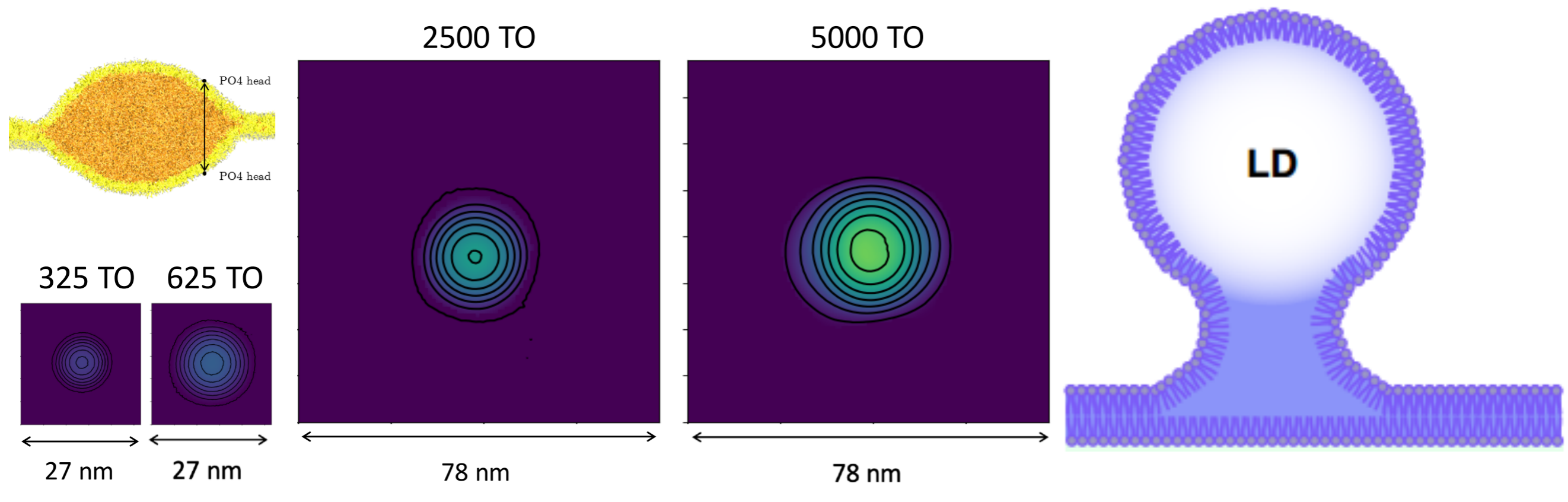
Size of simulated LDs is smaller than budding threshold?  
Problems with the theory? Artifacts in the simulations?

# Nascent LDs up to 35 nm in diameter do not bud out



Size of simulated LDs is smaller than budding threshold?  
Problems with the theory? Artifacts in the simulations?

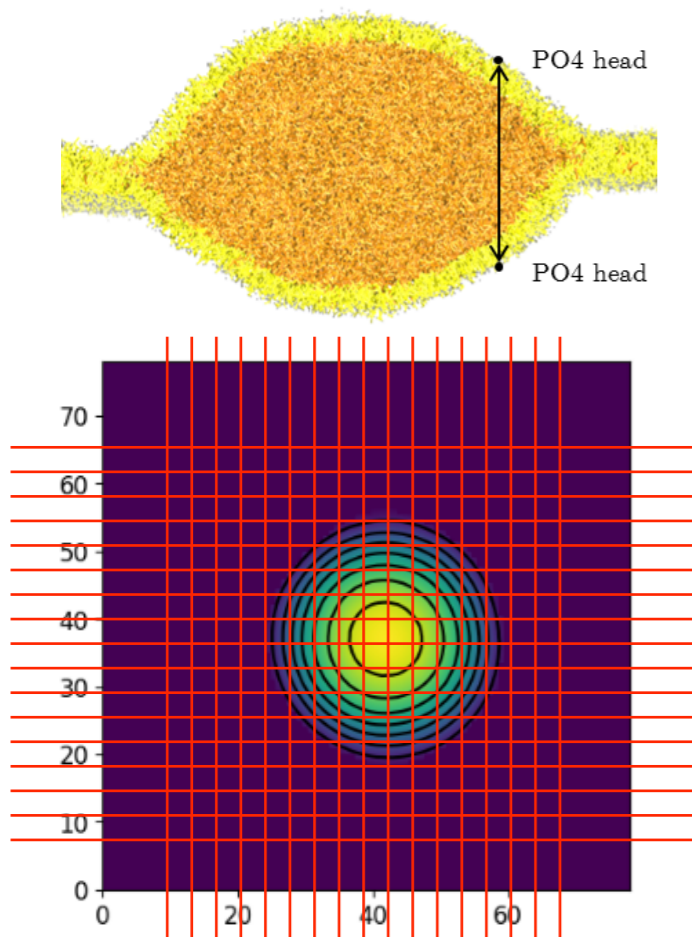
# Nascent LDs up to 35 nm in diameter do not bud out



*Budding requires more PL in one leaflet, that is impossible due to periodic boundary conditions and slow flip-flop*

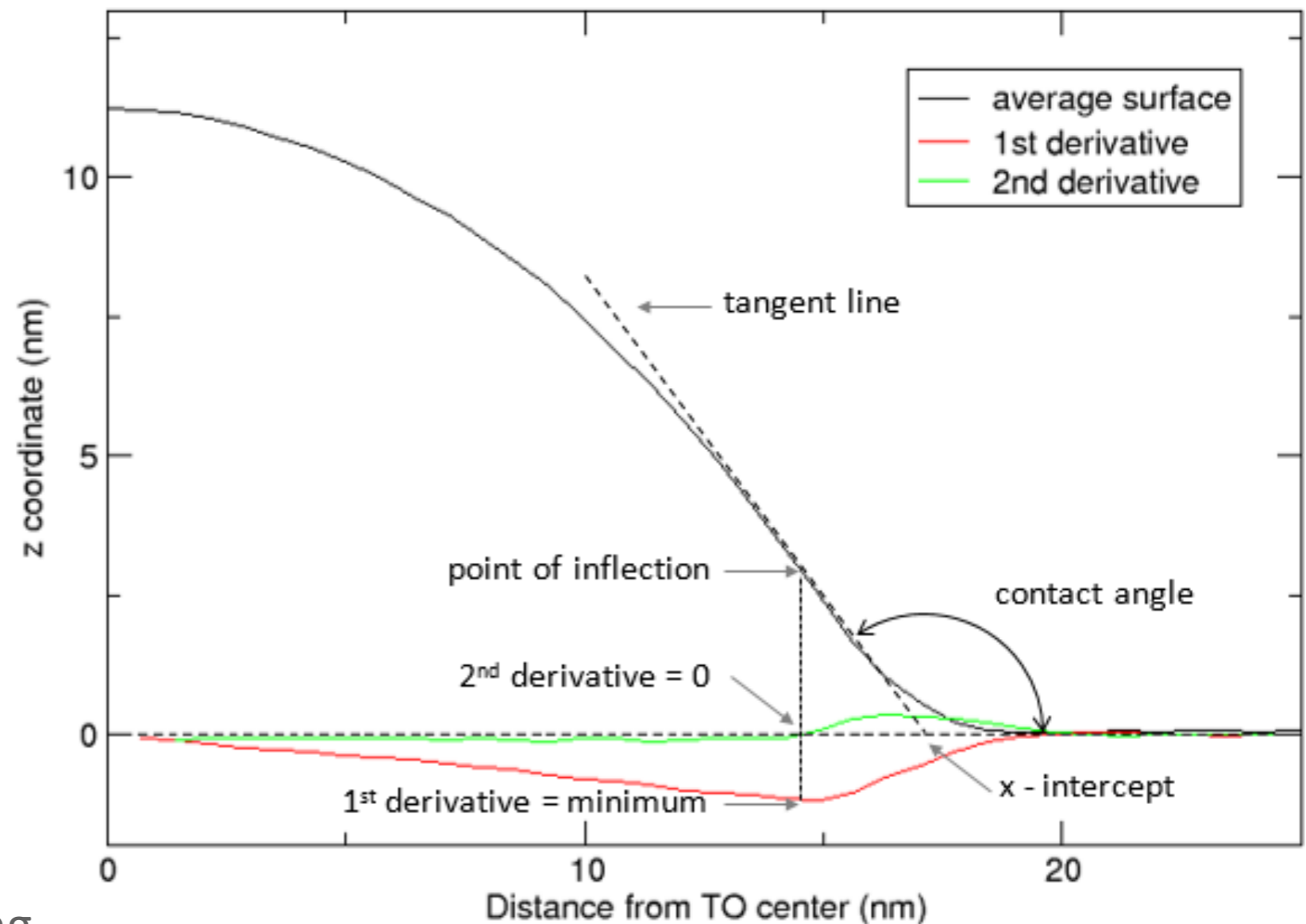
# Analyzing LD shape

1. Trajectory recentering
2. Distance between head groups, on a grid



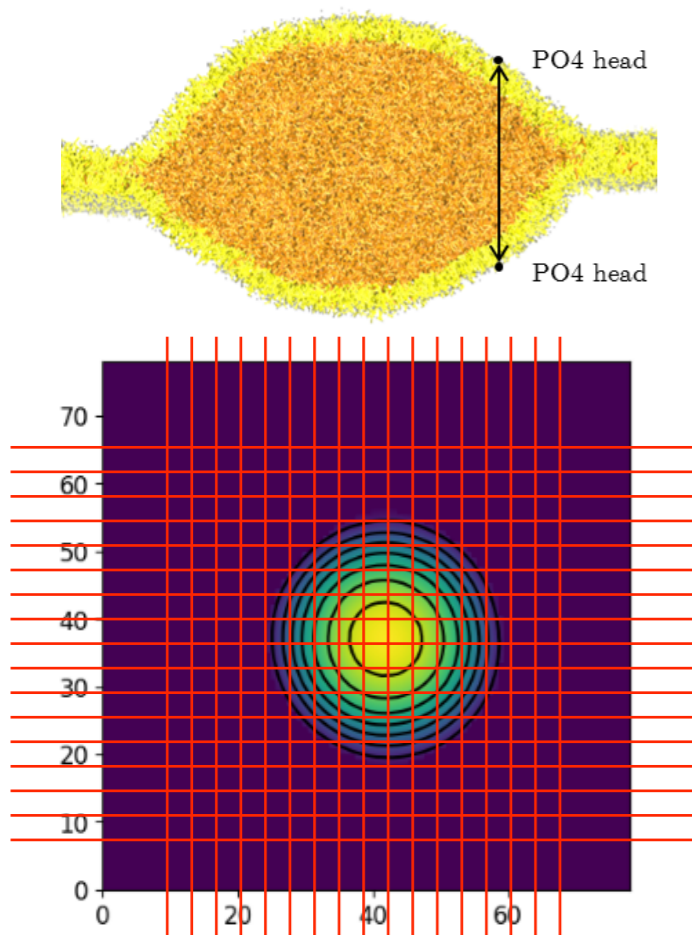
3. time averaging, radial averaging

Definition of contact angle  
by average surface of nascent droplet



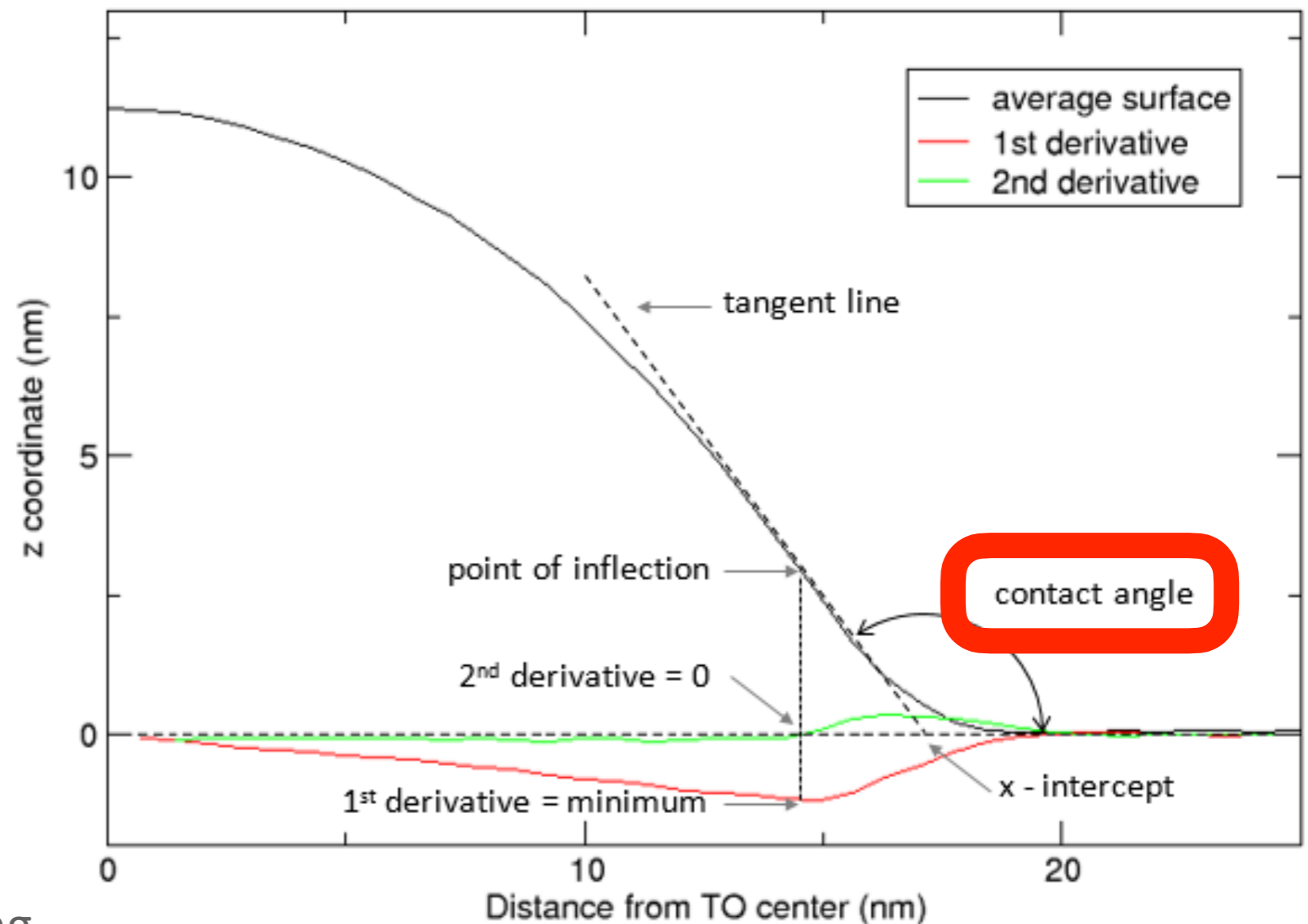
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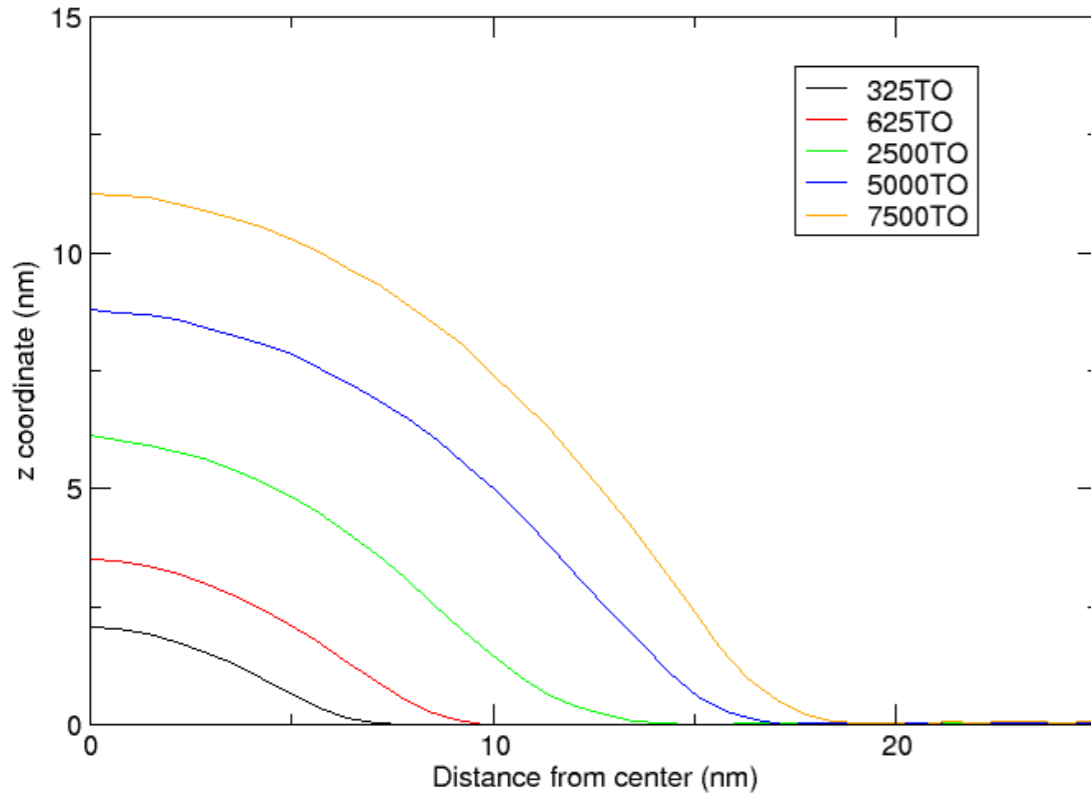
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Definition of contact angle  
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# LD shape vs size

Thickness  
DOPC+TO 300K

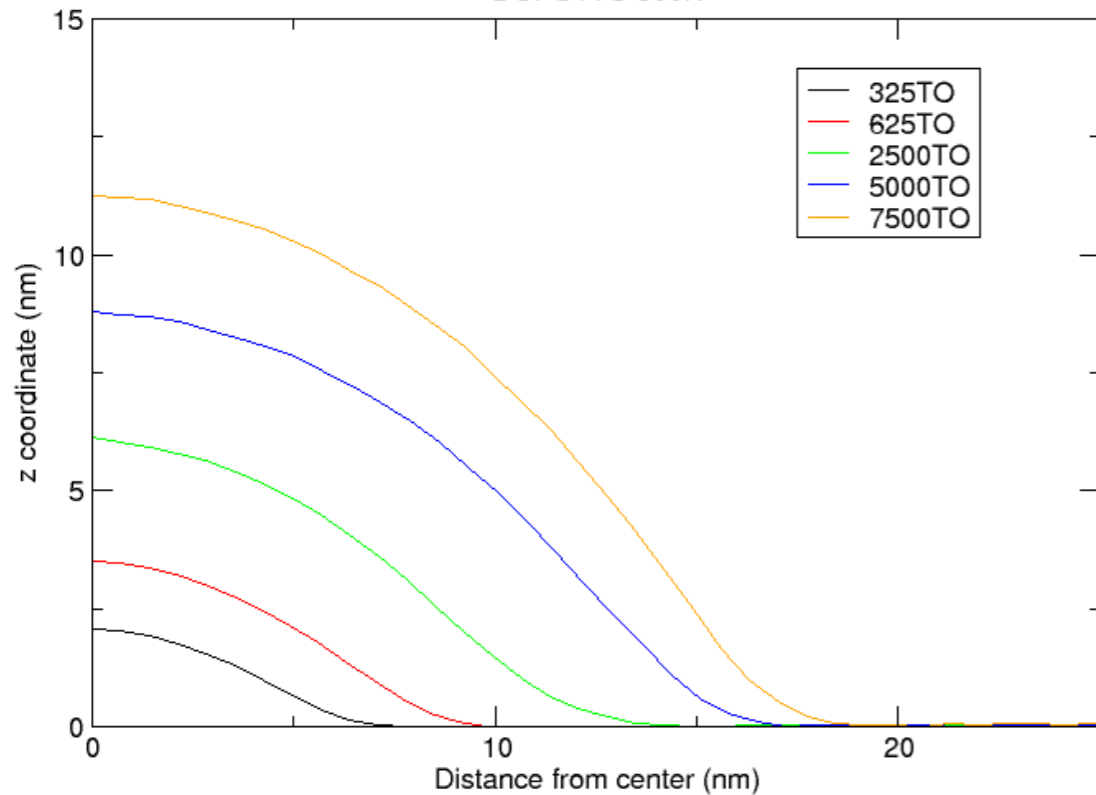


# DOPC	# TO	contact angle	x-intercept (nm)
2016	325	$154.4 \pm 0.1$	$6.3 \pm 0.1$
2016	625	$148.8 \pm 0.2$	$8.5 \pm 0.1$
18144	2500	$140.3 \pm 0.3$	$11.8 \pm 0.1$
18144	5000	$131.2 \pm 0.8$	$14.6 \pm 0.2$
18144	7500	$130.2 \pm 0.9$	$17.1 \pm 0.3$



# LD shape vs size

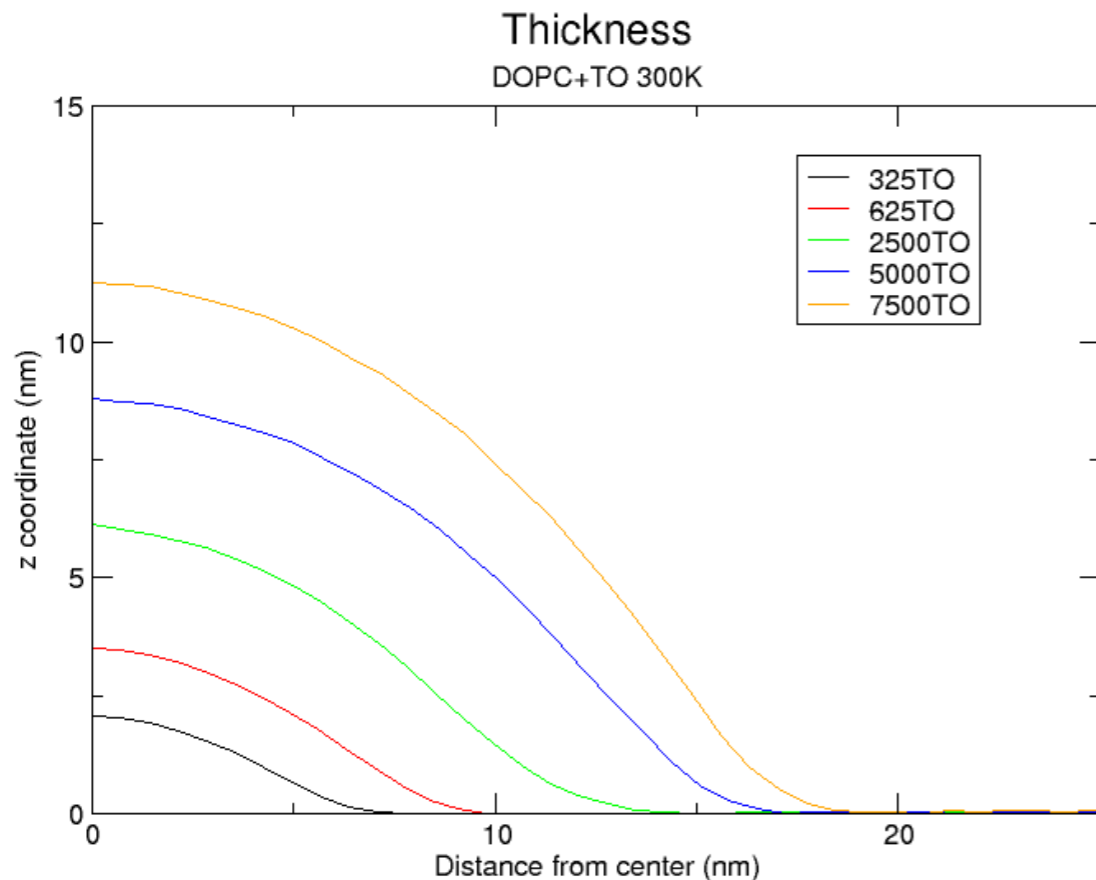
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DOPC+TO 300K



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- All simulated LDs are *far from spherical shape (budding)*

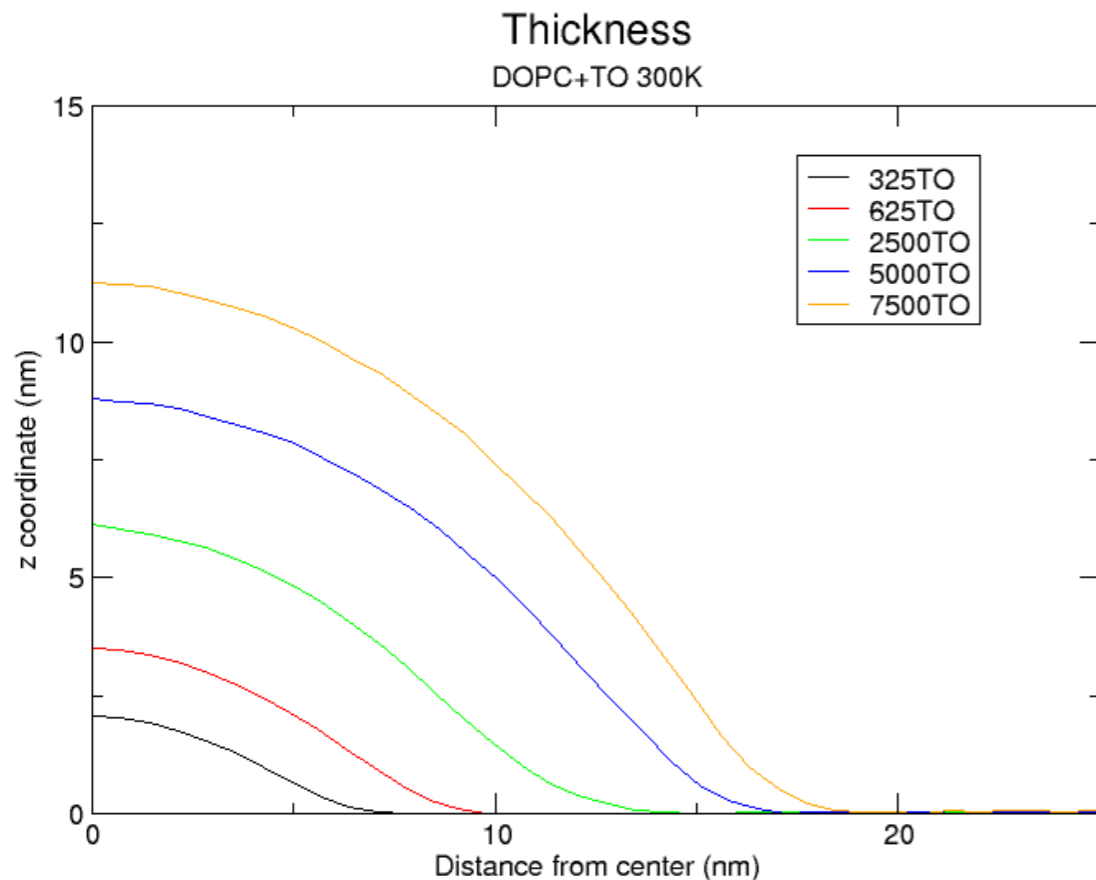
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- All simulated LDs are *far from spherical shape (budding)*
- Larger LDs become spherical to minimize interfacial energy  
→ *surface tension determines LD shape*

# LD shape vs size

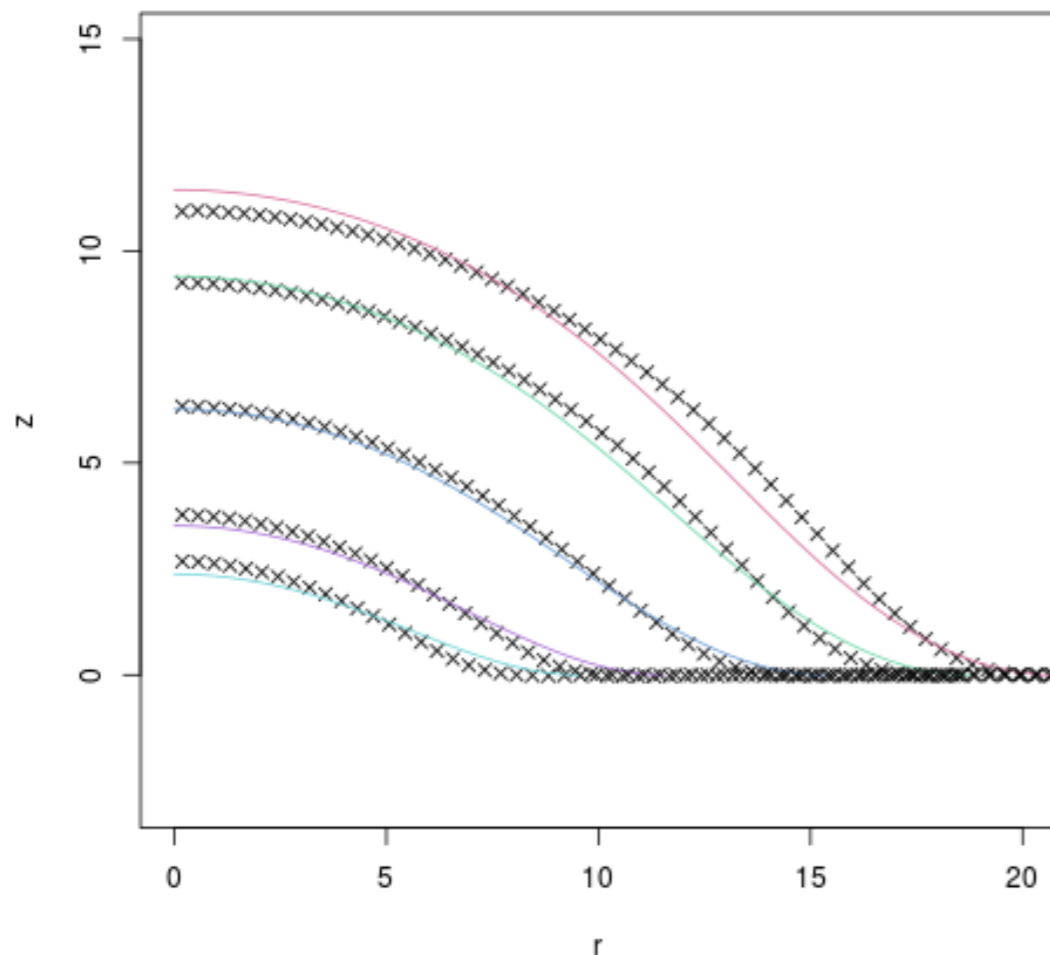


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- All simulated LDs are *far from spherical shape (budding)*
- Larger LDs become spherical to minimize interfacial energy  
→ *surface tension determines LD shape*
- Budding can be controlled by *tuning the surface tension*

# Fitting simulated shapes with the theoretical shape

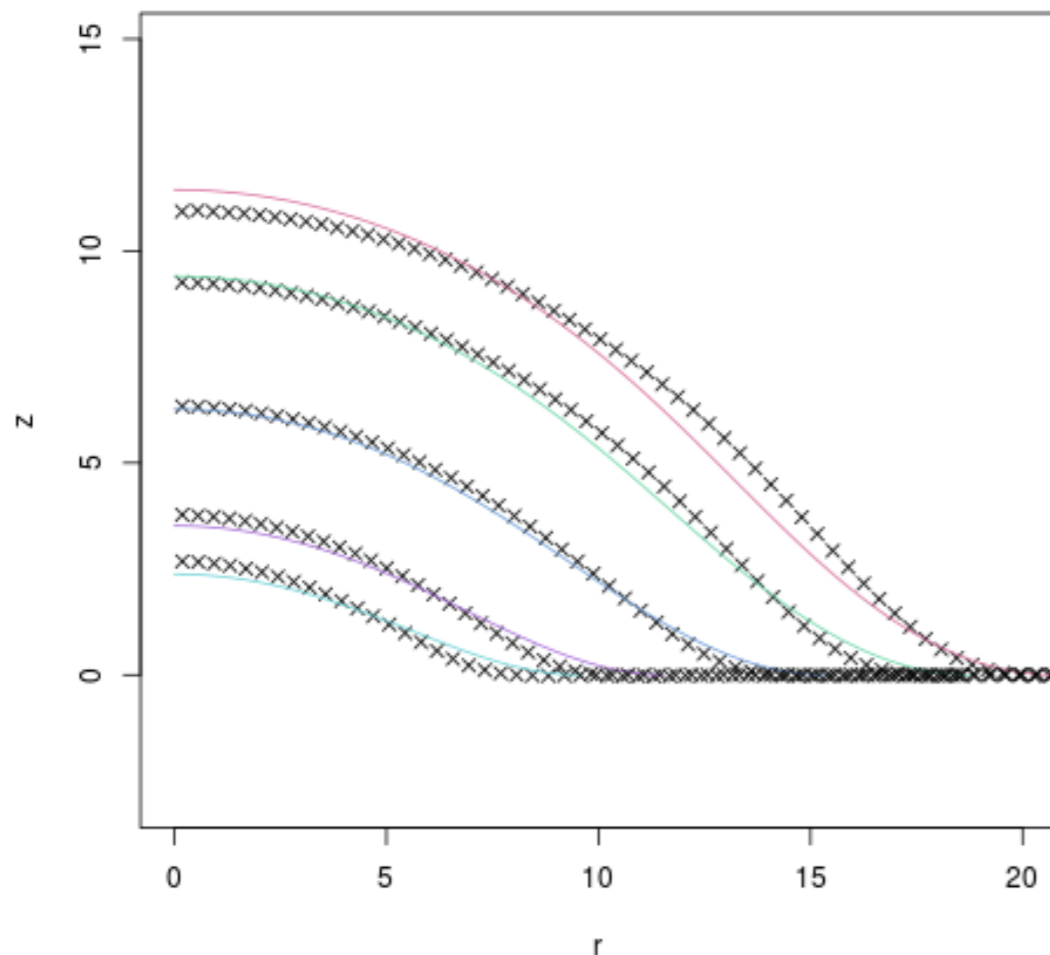
Imposing zero tension in bilayer



**Bad fit**, does not improve by  
changing elastic parameters

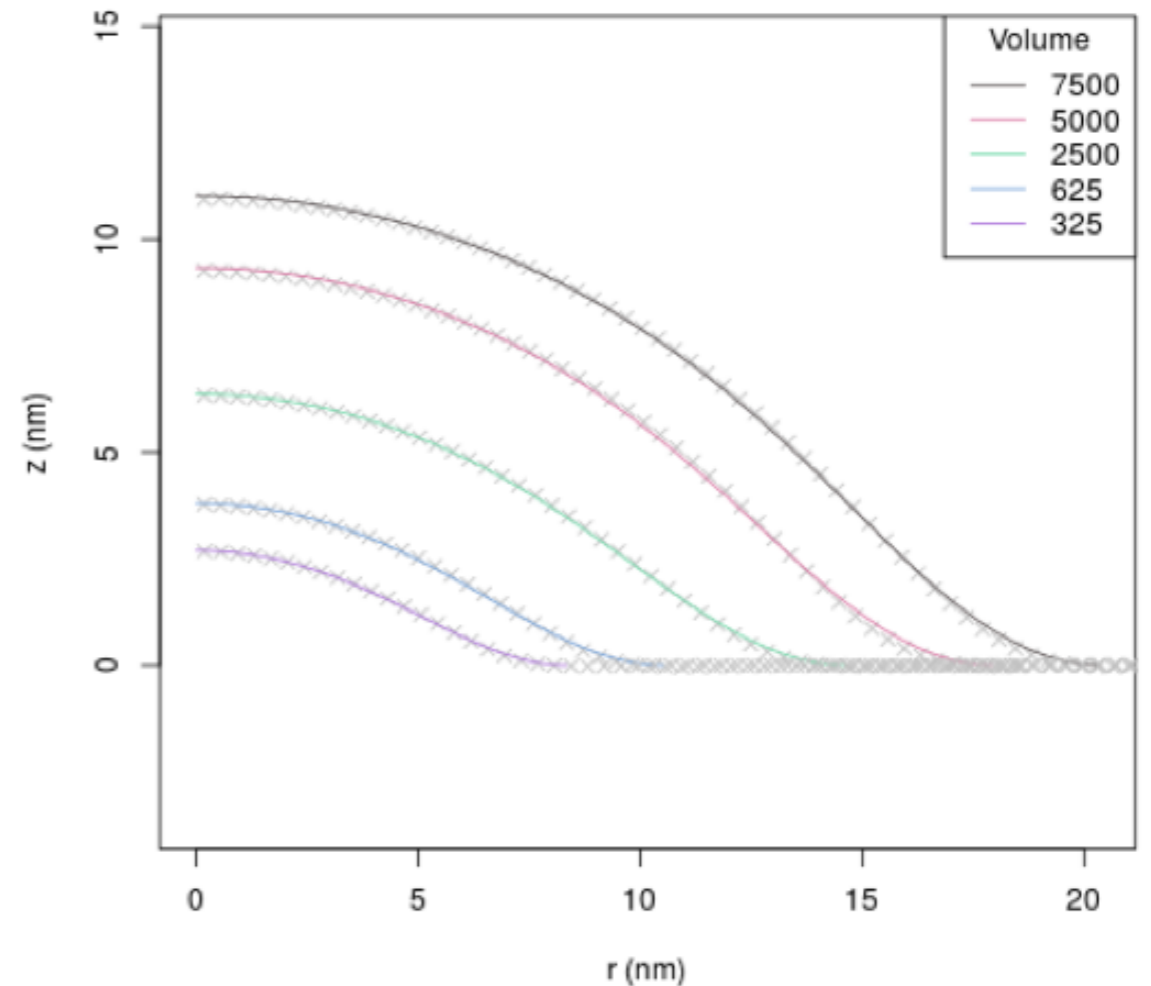
# Fitting simulated shapes with the theoretical shape

## Imposing zero tension in bilayer



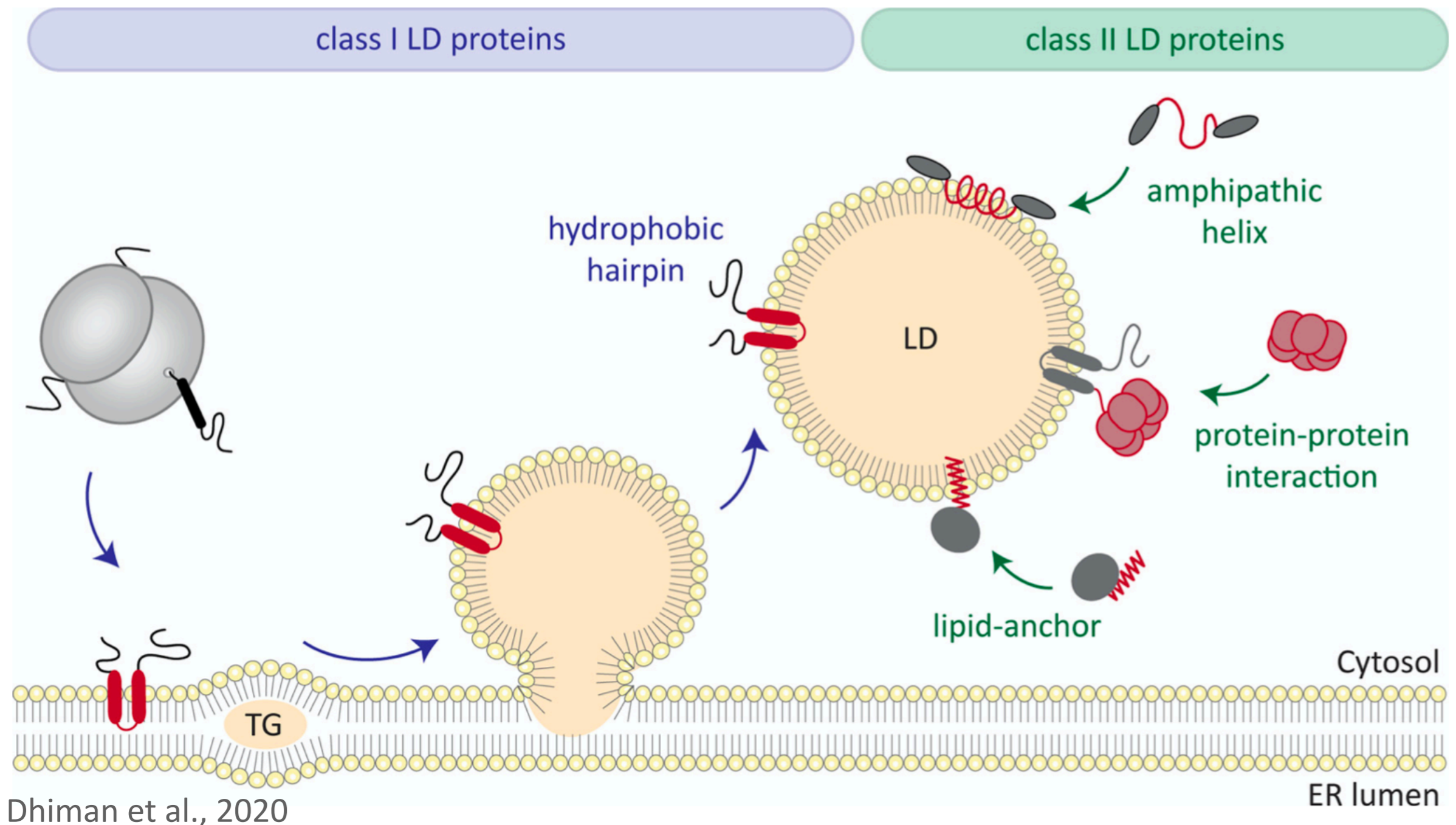
**Bad fit**, does not improve by changing elastic parameters

## Allowing tension in the bilayer



Best fit for  $\gamma_m=3.8$  mN/m,  $\gamma_b=3$  mN/m  
**Unrealistically high  $\gamma_b$  -> artifact?**

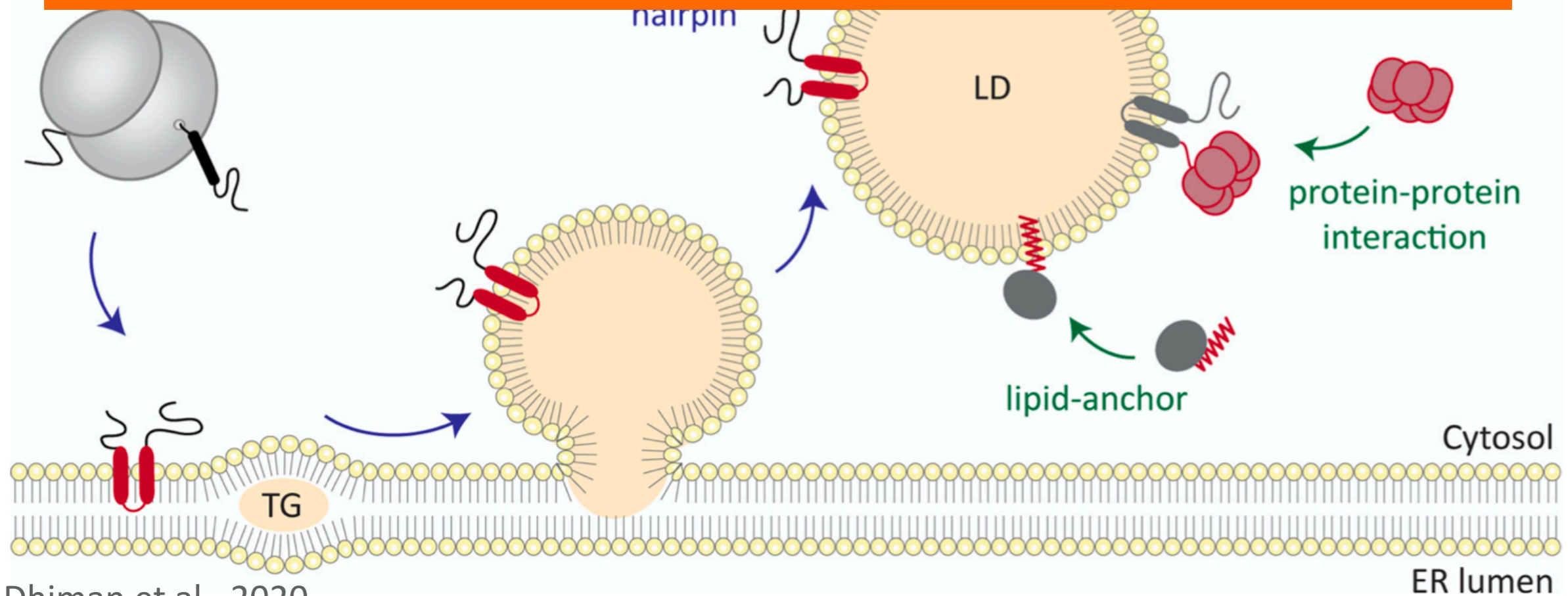
# Proteins are essential components of LDs



Dhiman et al., 2020

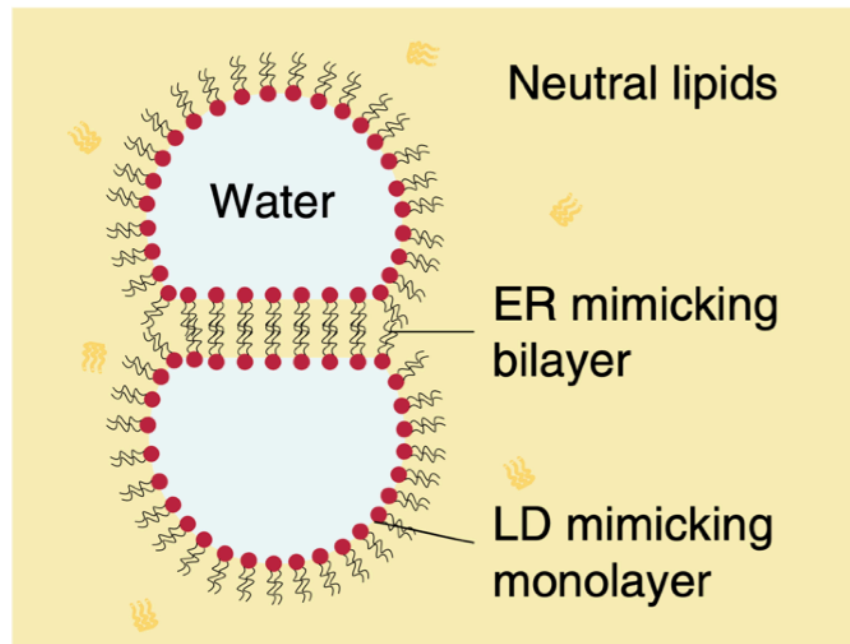
# Proteins are essential components of LDs

...but what determines which proteins bind to LDs?



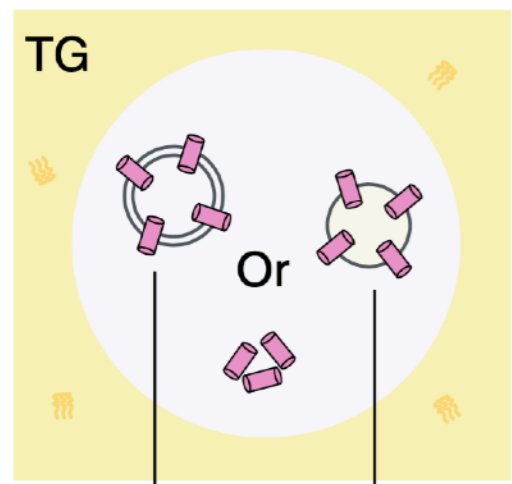
Dhiman et al., 2020

# Measuring protein partitioning: experimental setup

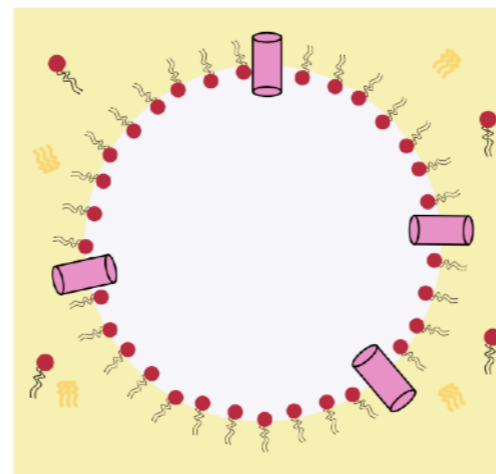


## Droplet interface bilayer (DIB)

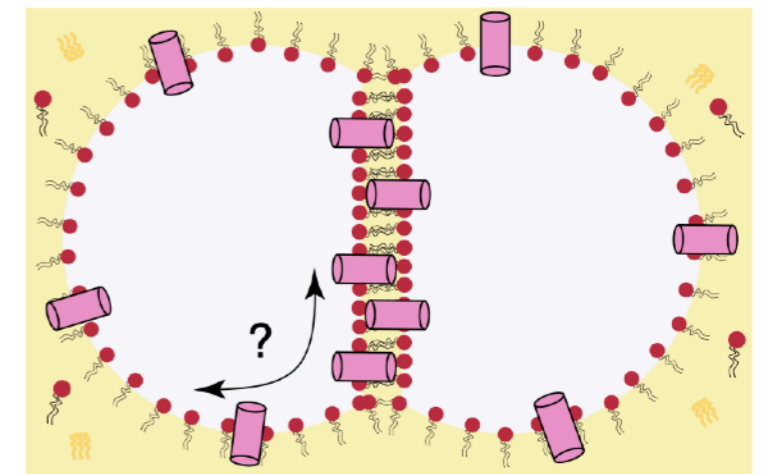
- mimic the ER-LD contact
- can incorporate proteins (but not all proteins)



Mixing  
+  
Phospholipids

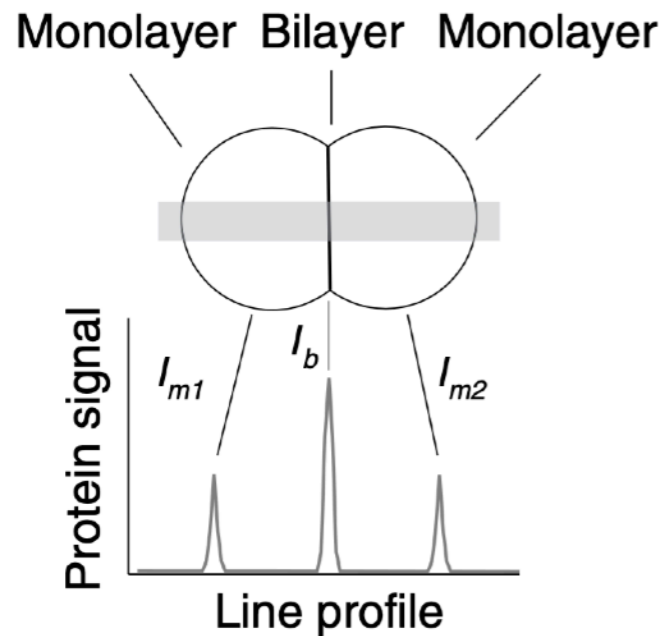


Pairing





# Measuring protein partitioning: experimental setup



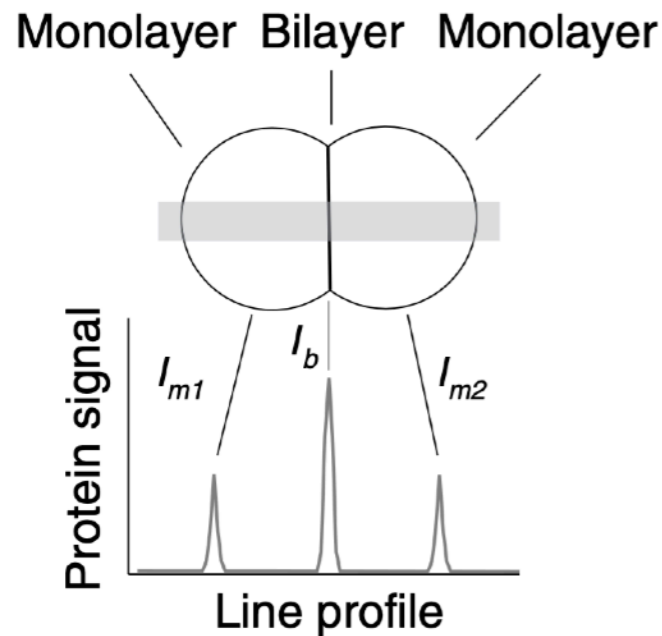
Partition coefficient

$$P = \frac{I_b}{I_{m1} + I_{m2}}$$

>1, bilayer preference

<1, monolayer preference

# Measuring protein partitioning: experimental setup



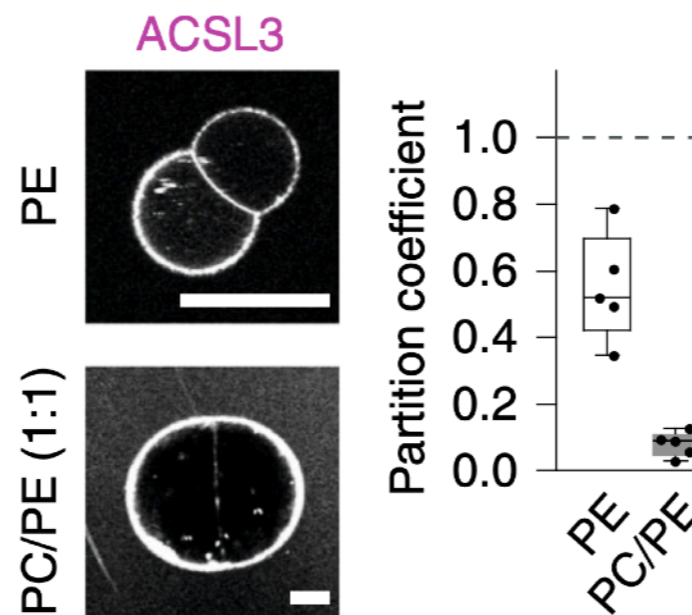
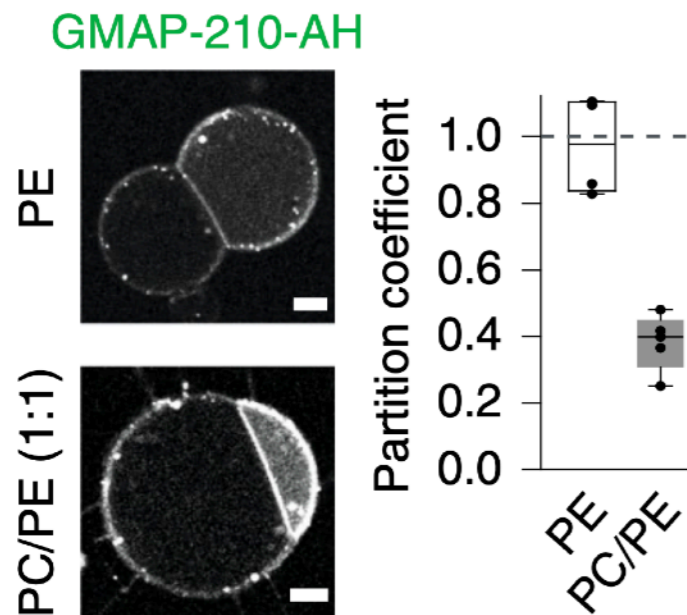
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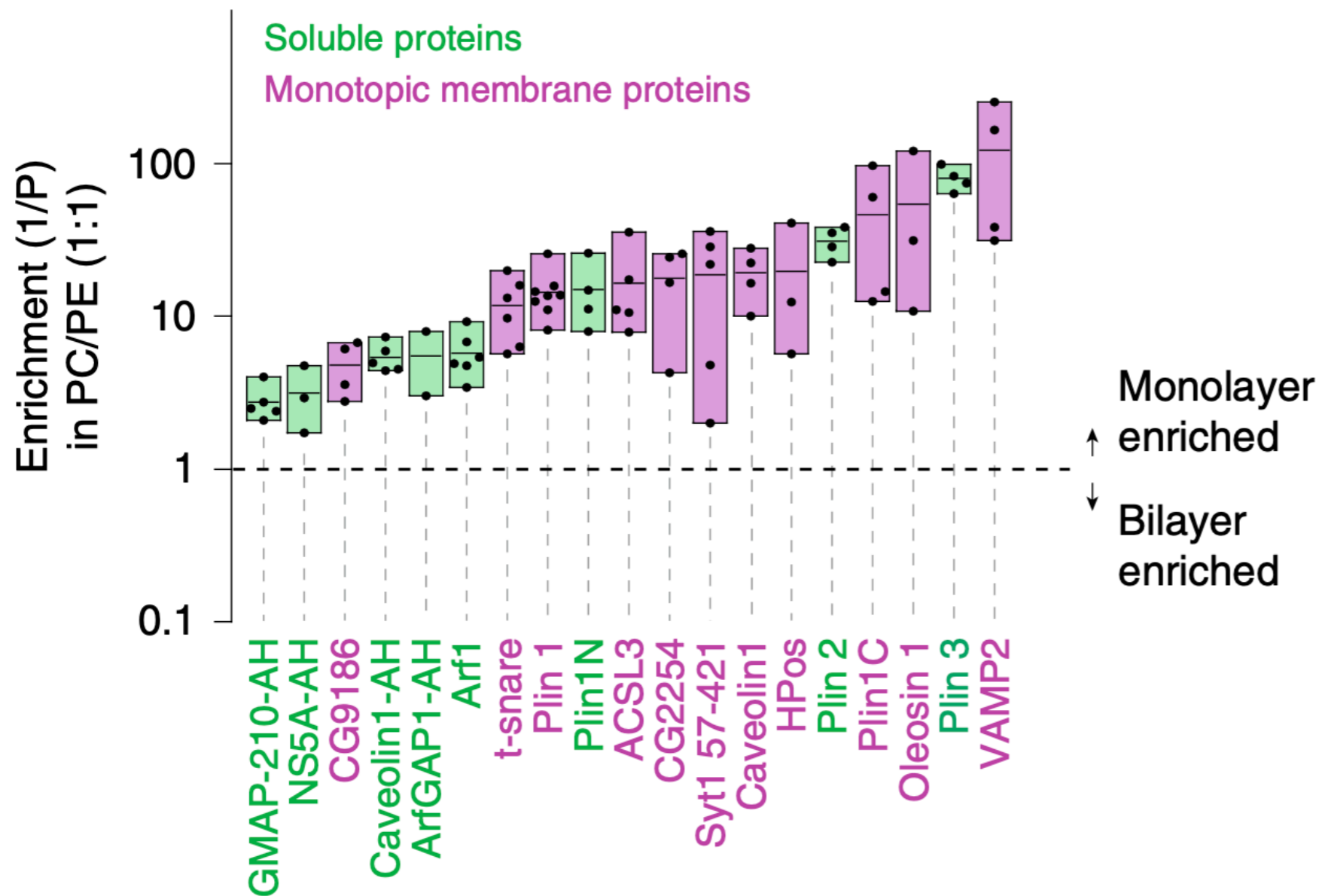
>1, bilayer preference

<1, monolayer preference

We tested 2 types of proteins:  
**soluble with amphipathic helices (AH)** and **monotopic with hydrophobic domains (HD)**

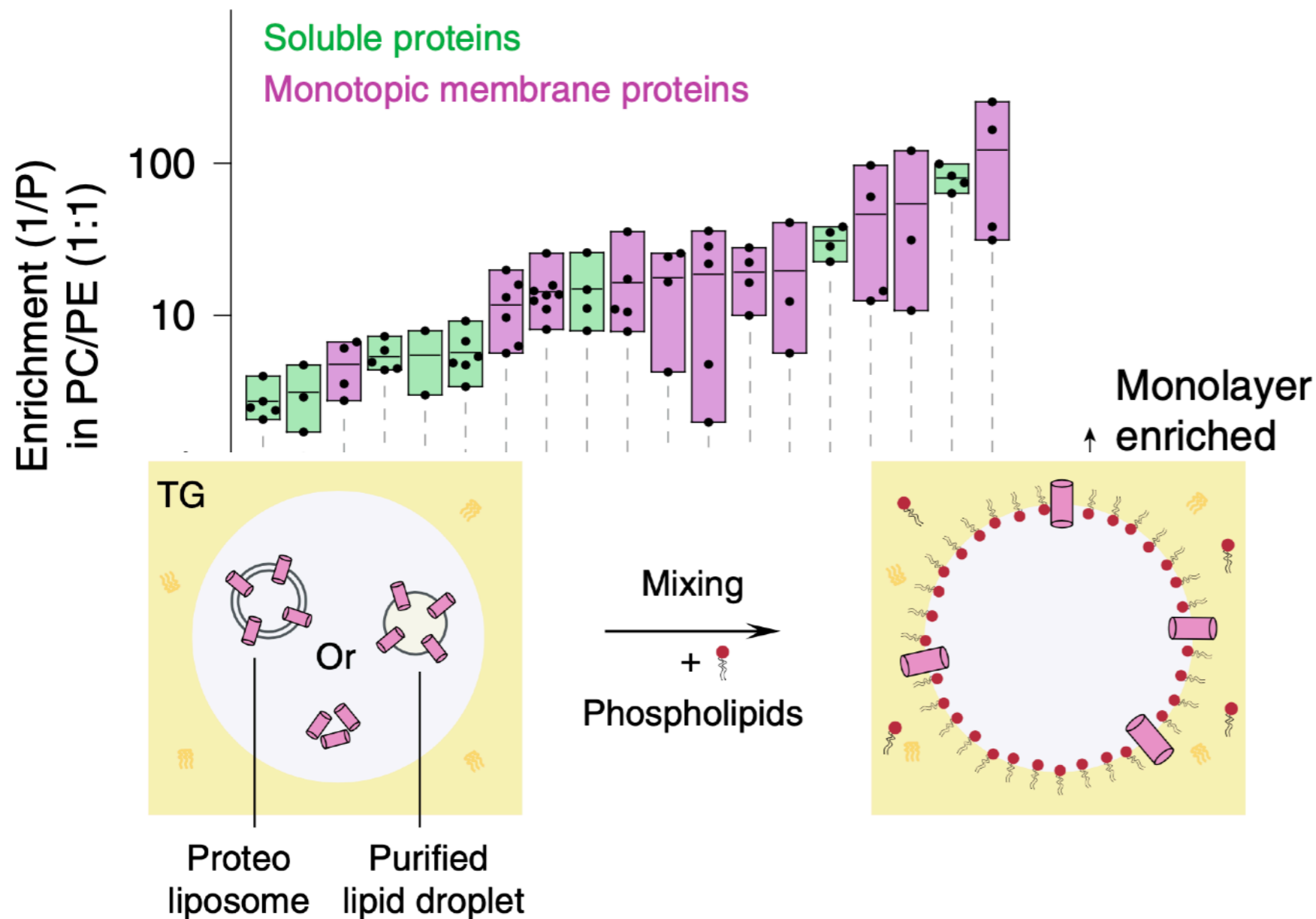


# Soluble proteins with AH and monotonic proteins with HD



All tested proteins partition preferentially to the monolayer

# Soluble proteins with AH and monotonic proteins with HD

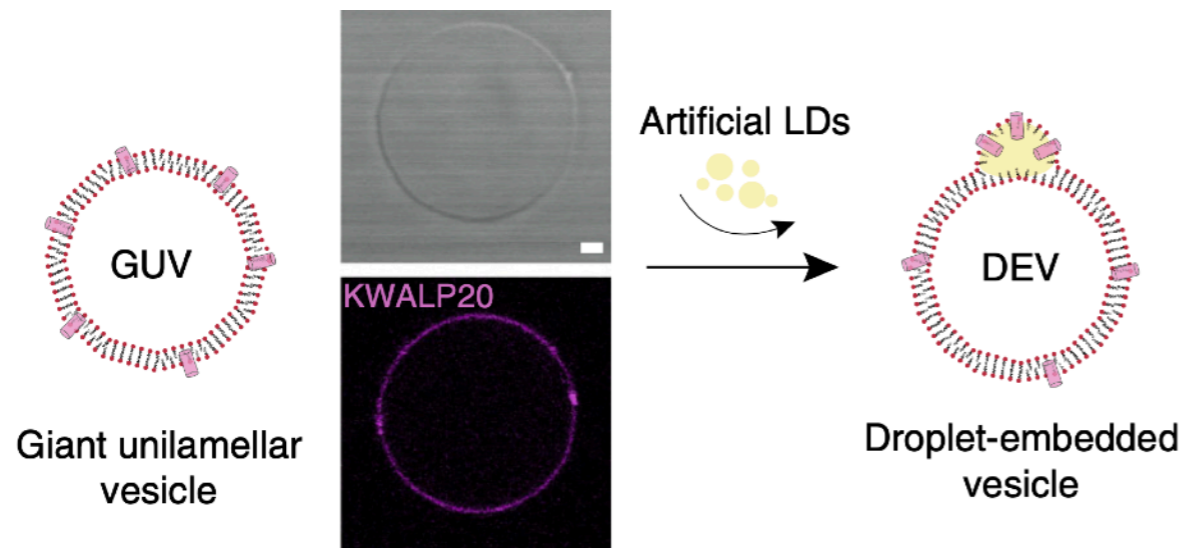
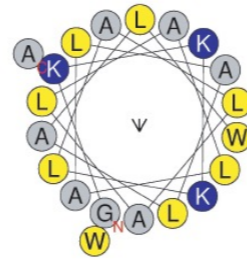


Drawbacks:

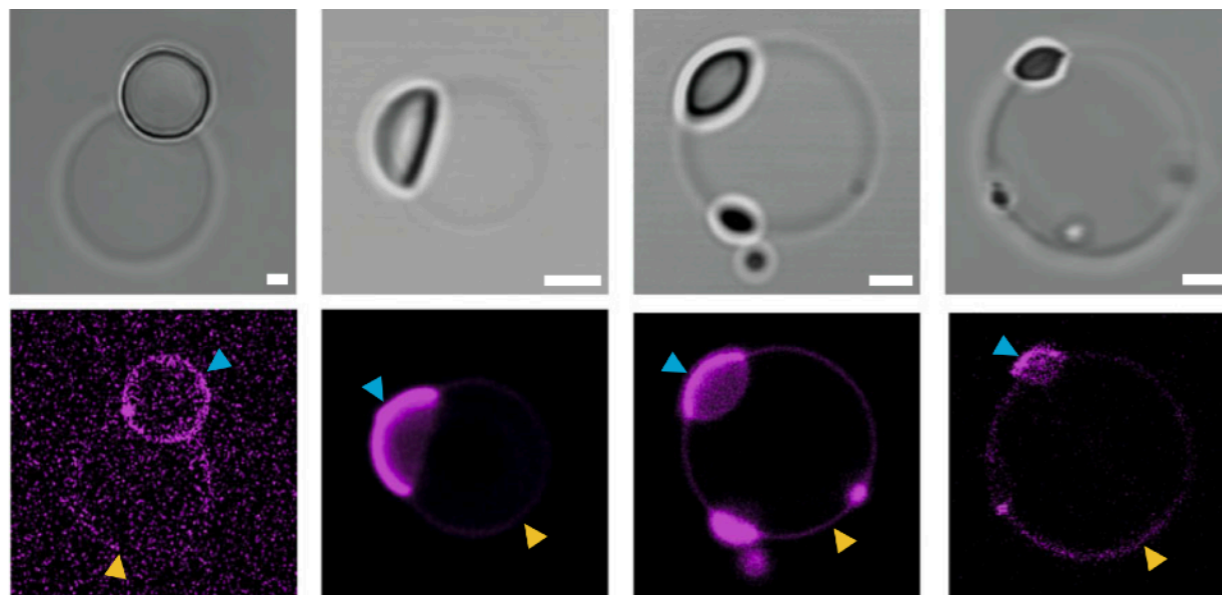
- other proteins may be present
- multiple/mixed AHs and HDs
- why do they go there?

# KWALP partitions to nascent LDs

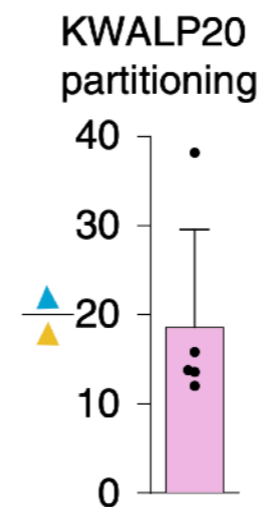
RhB-GKKKLALALALALALALWVA-NH<sub>2</sub>



KWALP20 partitions strongly to the LD surface (both in DIBs and in DEVs)

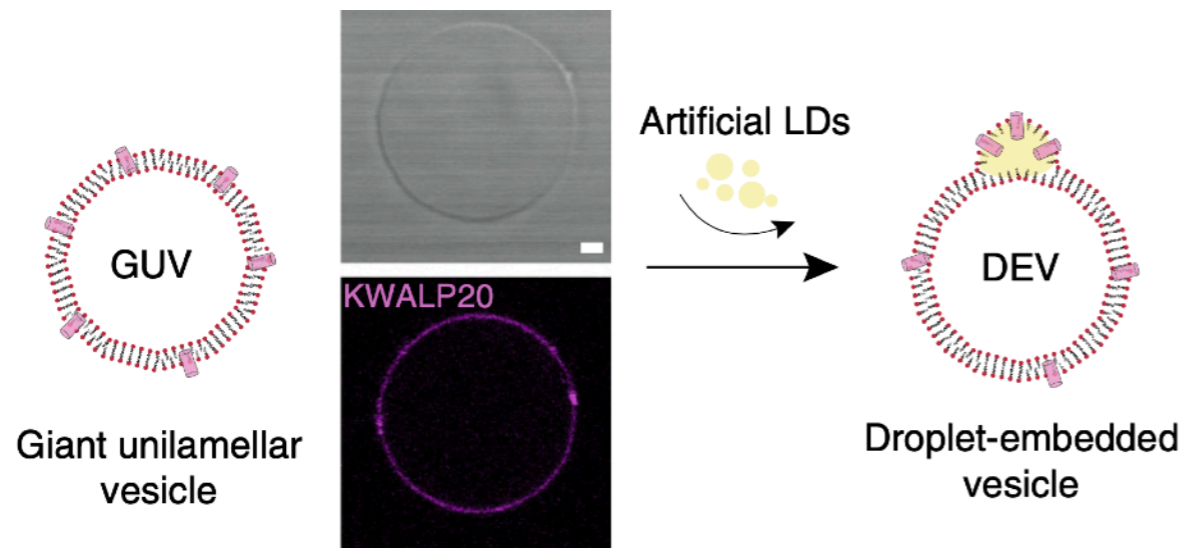
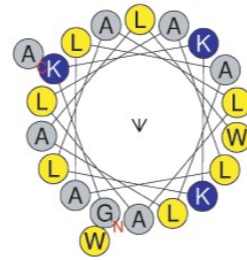


▲ Monolayer      ▲ Bilayer

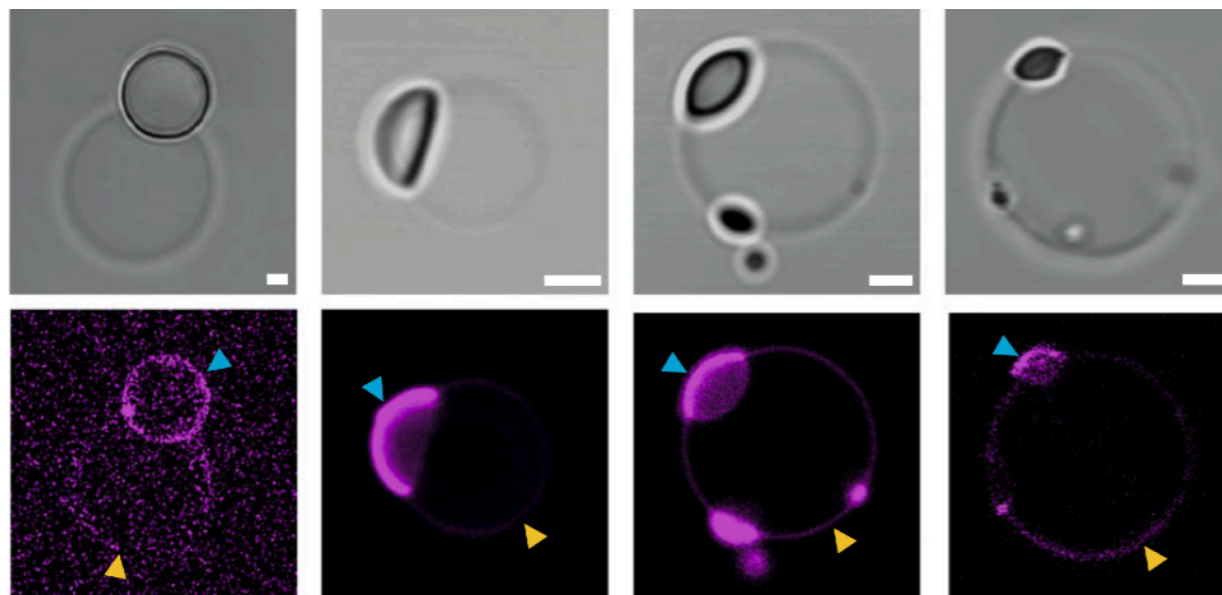


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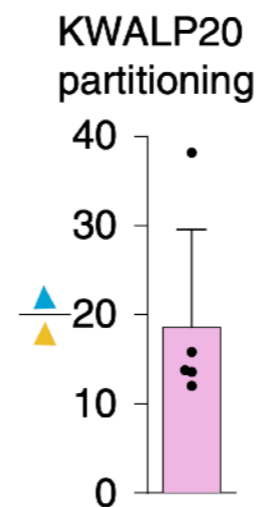
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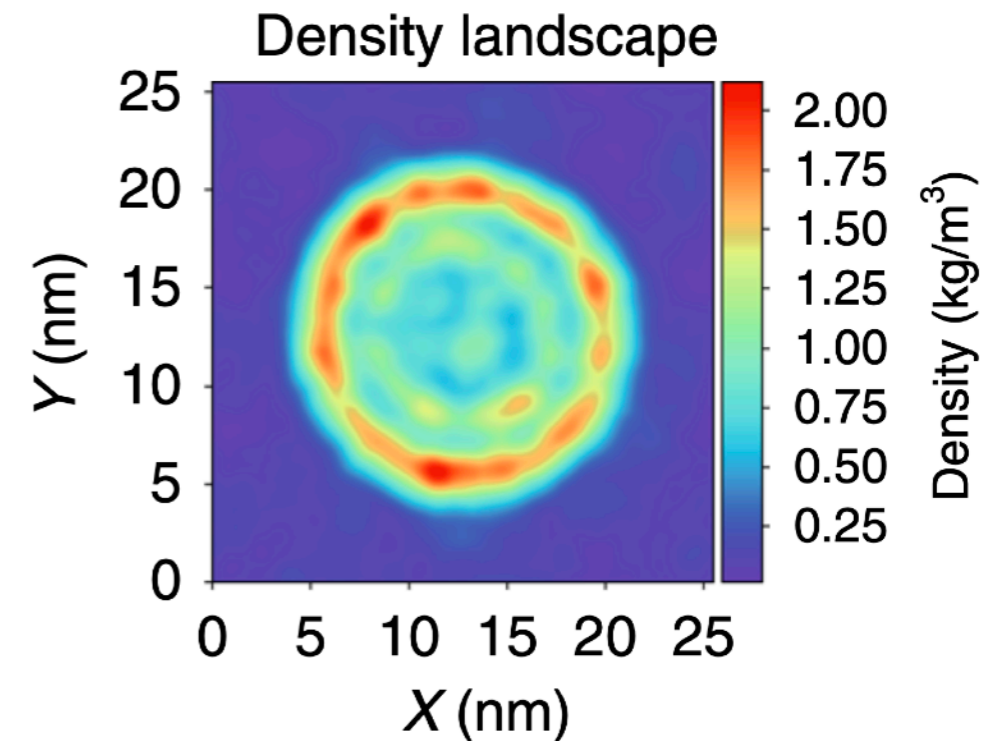
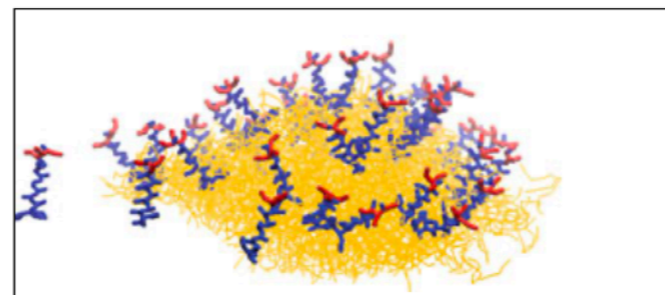
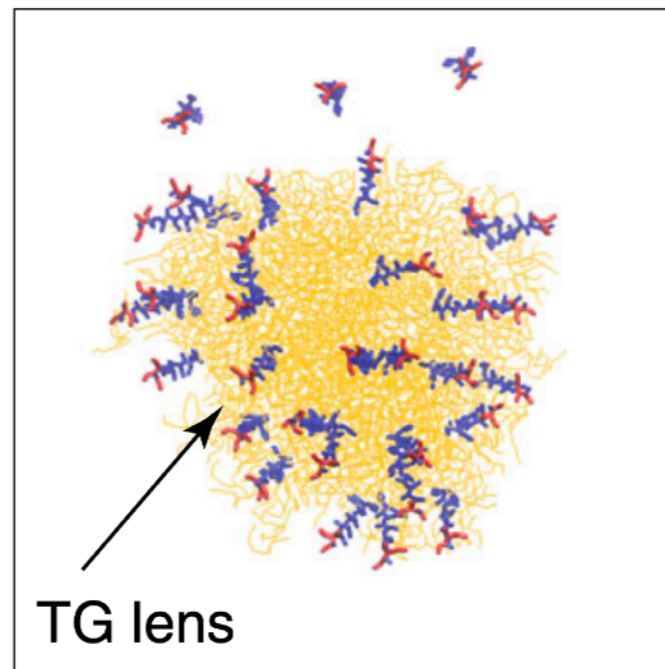
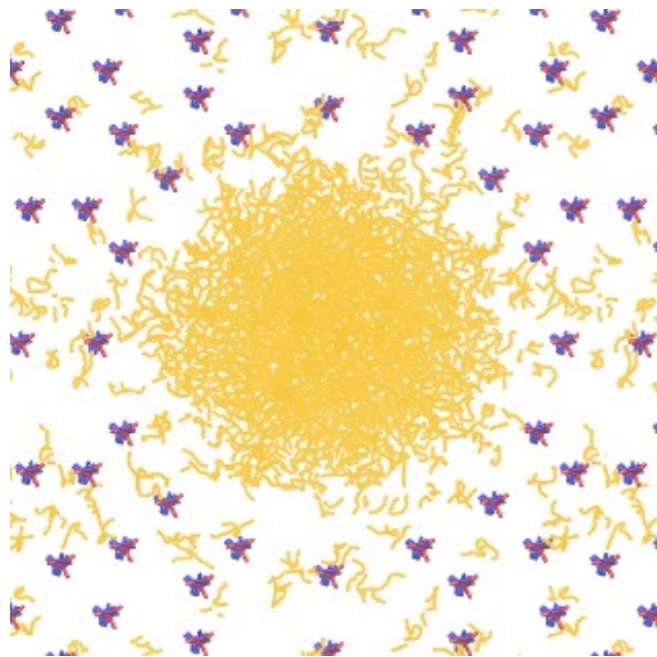


▲ Monolayer    ▲ Bilayer



***Why does KWALP go to the LD surface?***

# Simulating KWALP distribution



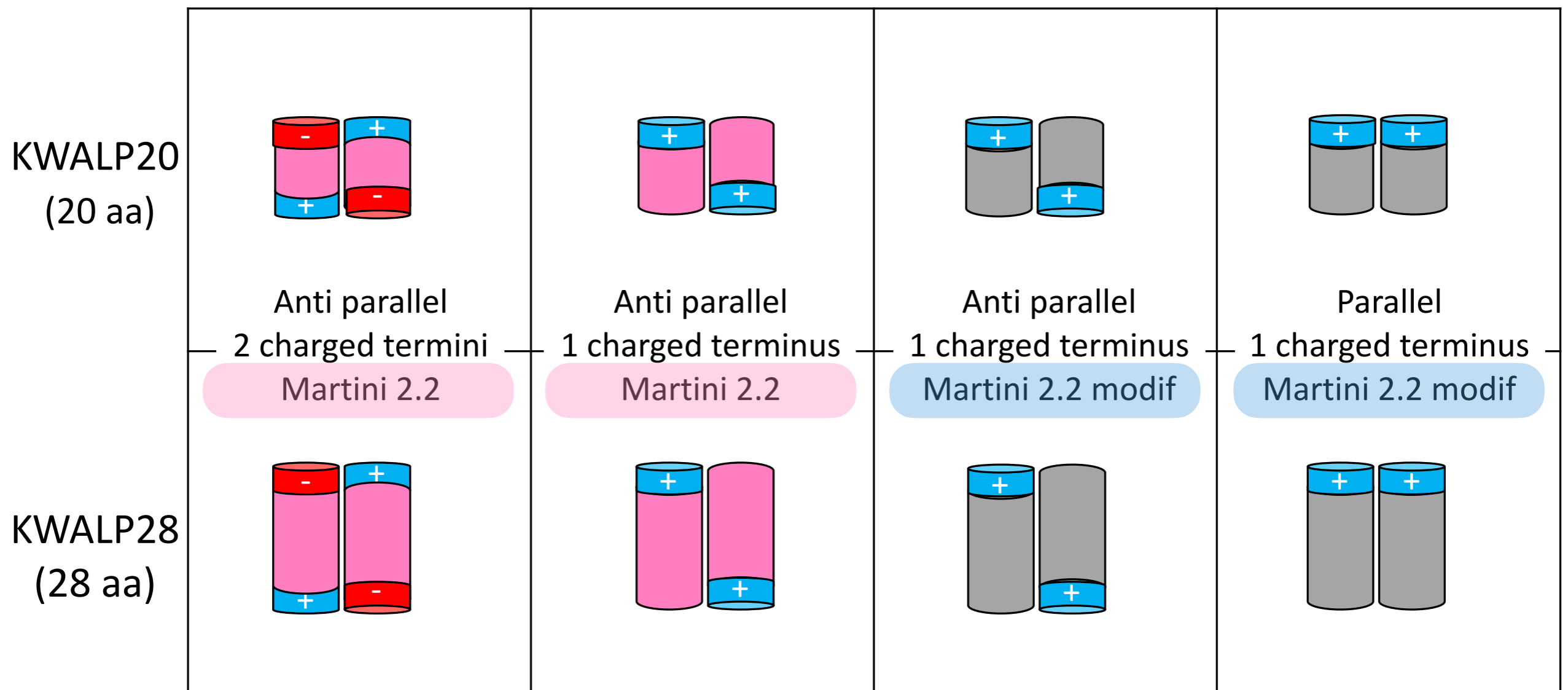
Initial configuration:

- 625 TO molecules in DOPC bilayer (LD radius: ~10 nm)
- box size: 26 nm × 26 nm × 20 nm
- 16 or 32 peptides
- Martini 2.2 (and modifications)
- Unbiased MD, 20-40 μs

Caillon et al., *Nature Comm.* 2020

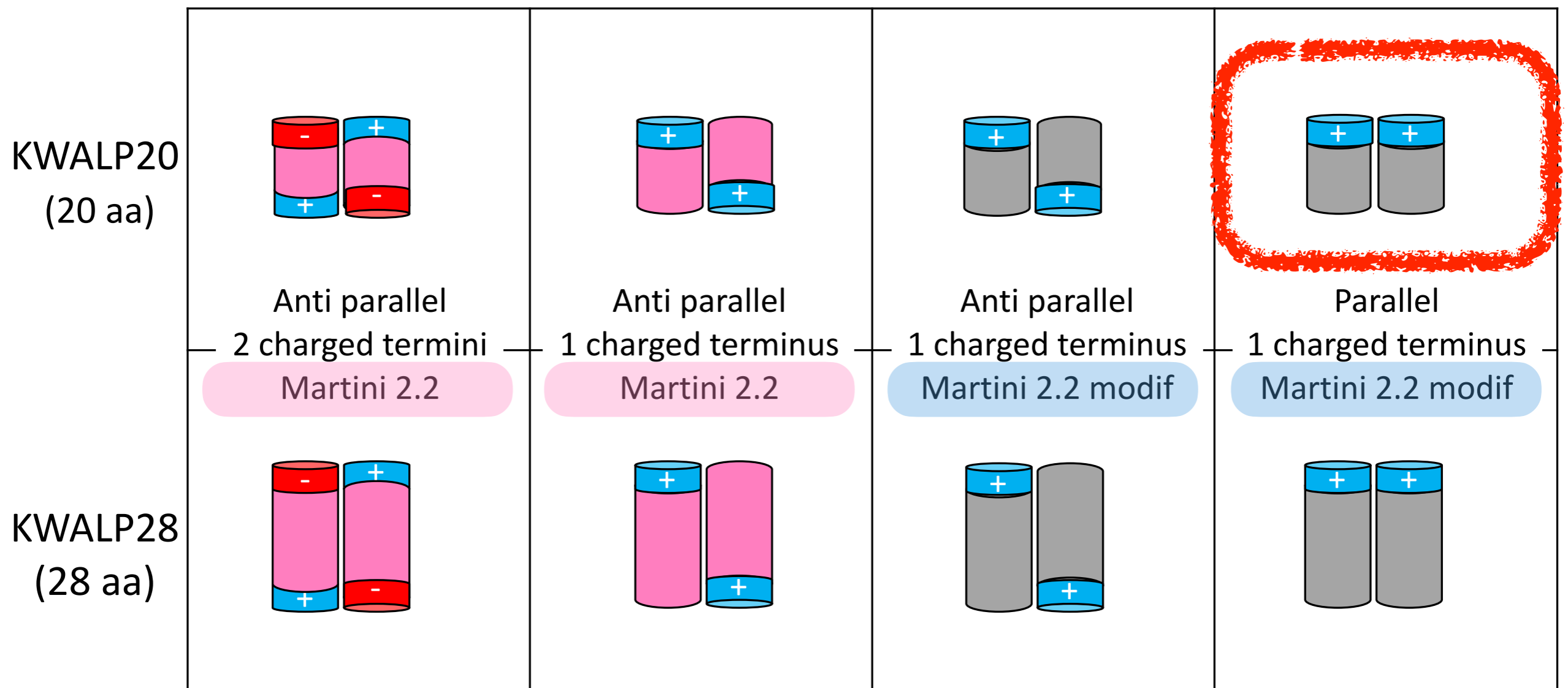
Simulations reproduce experimental results (qualitatively)

# Effect of terminal charges, orientation, length, and protein-protein attraction

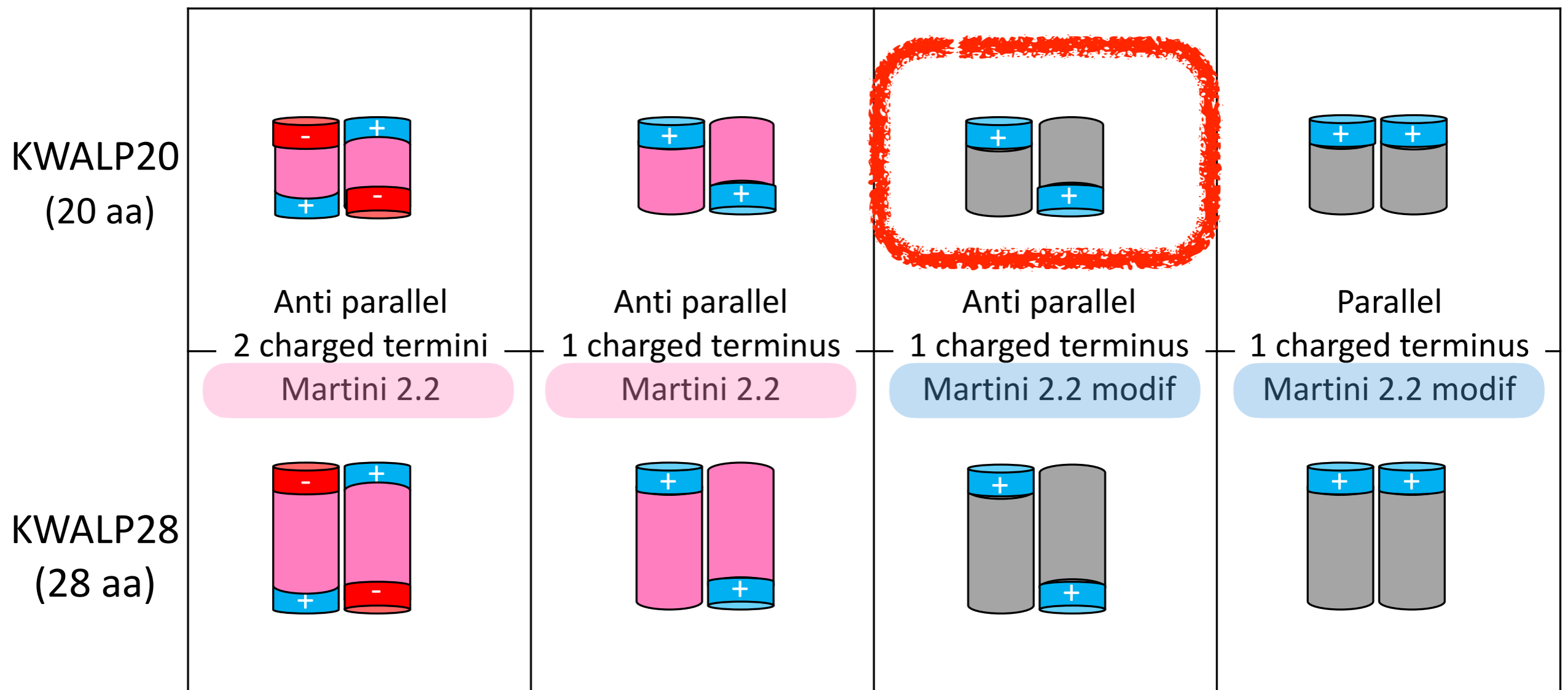




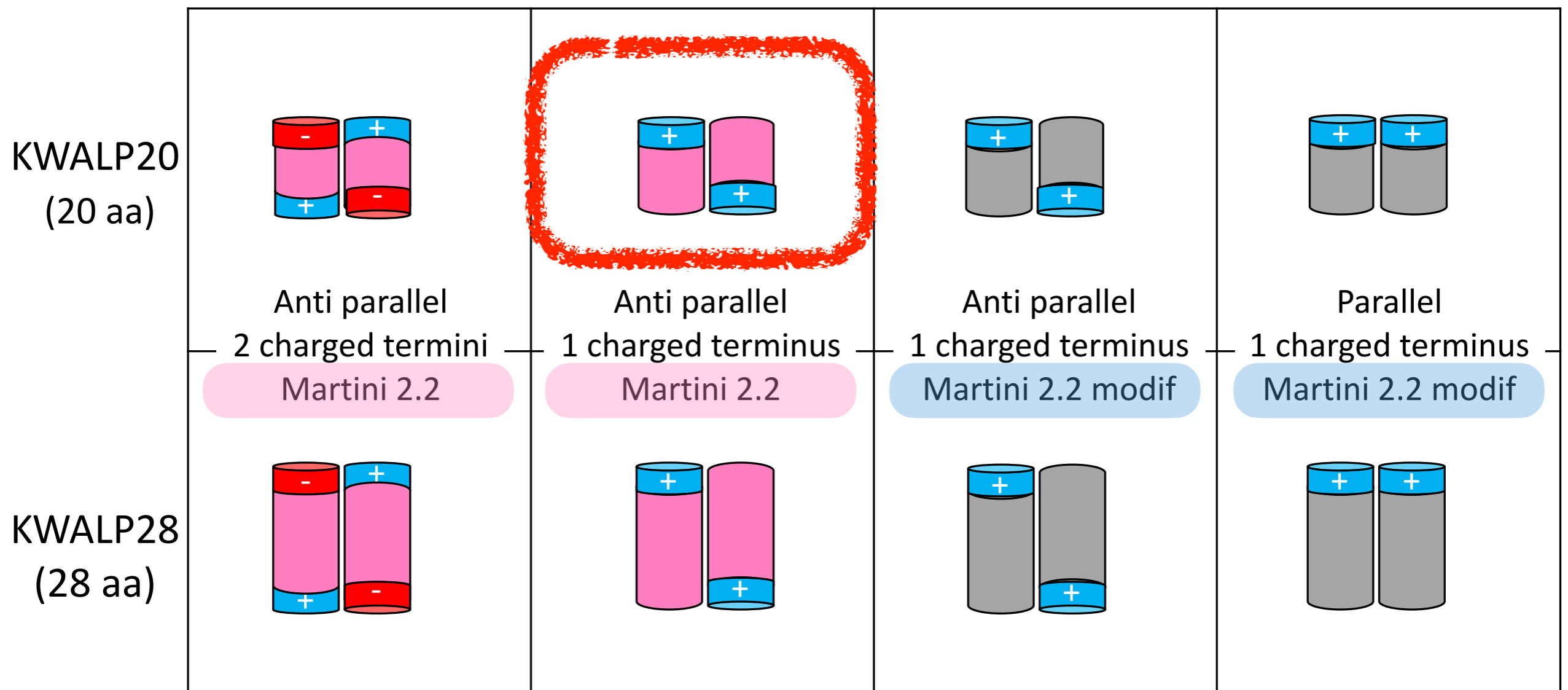
# Effect of terminal charges, orientation, length, and protein-protein attraction



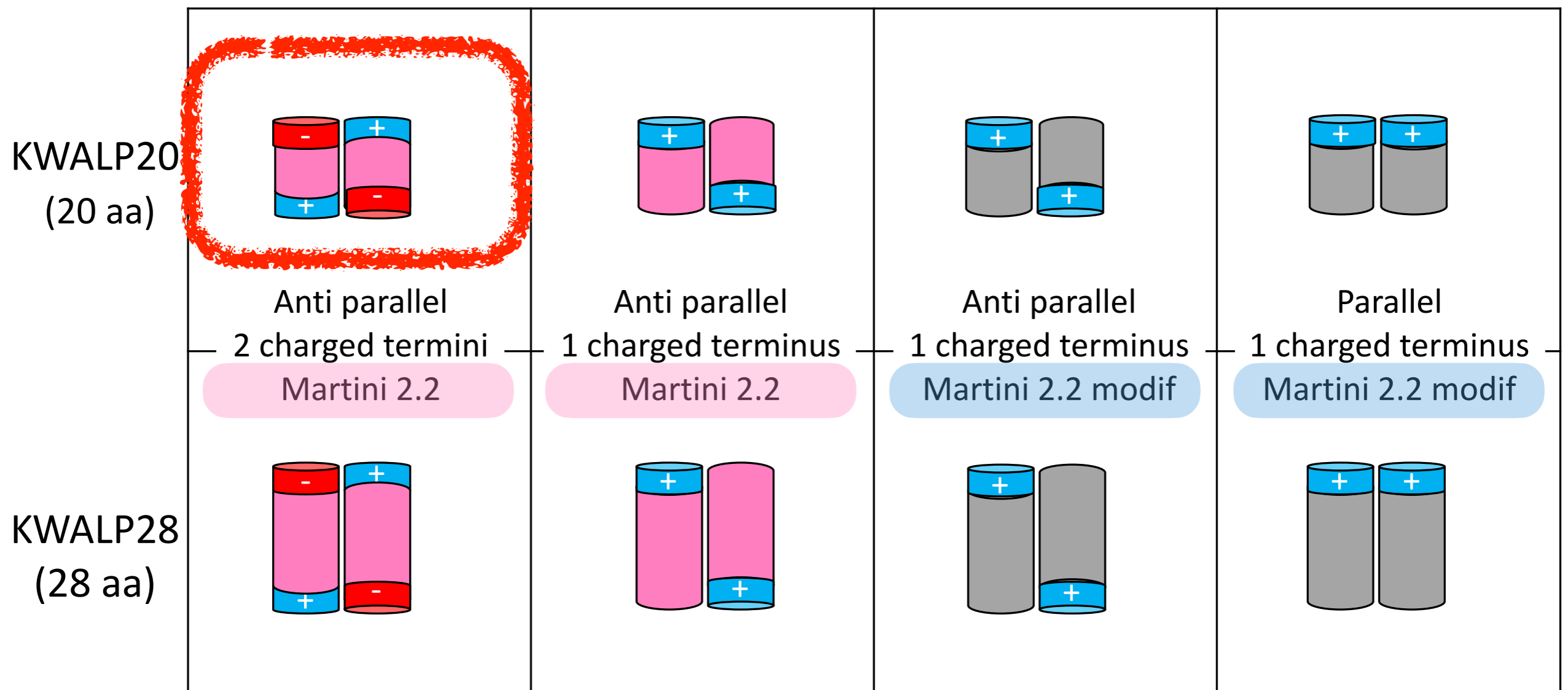
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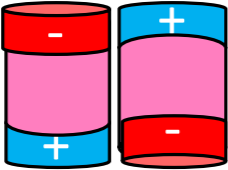
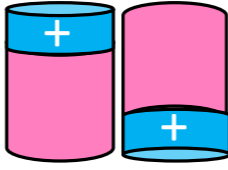
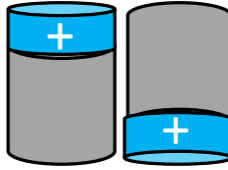
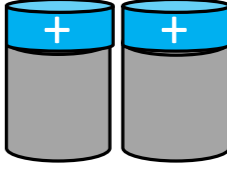
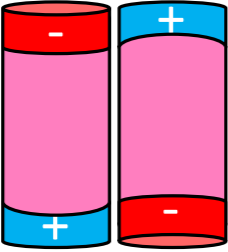
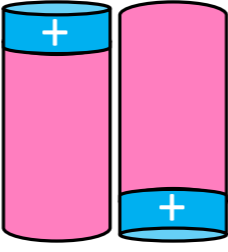
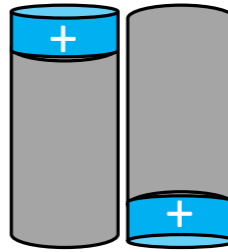
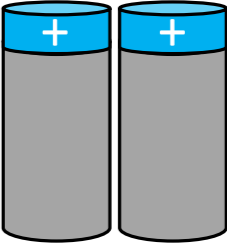
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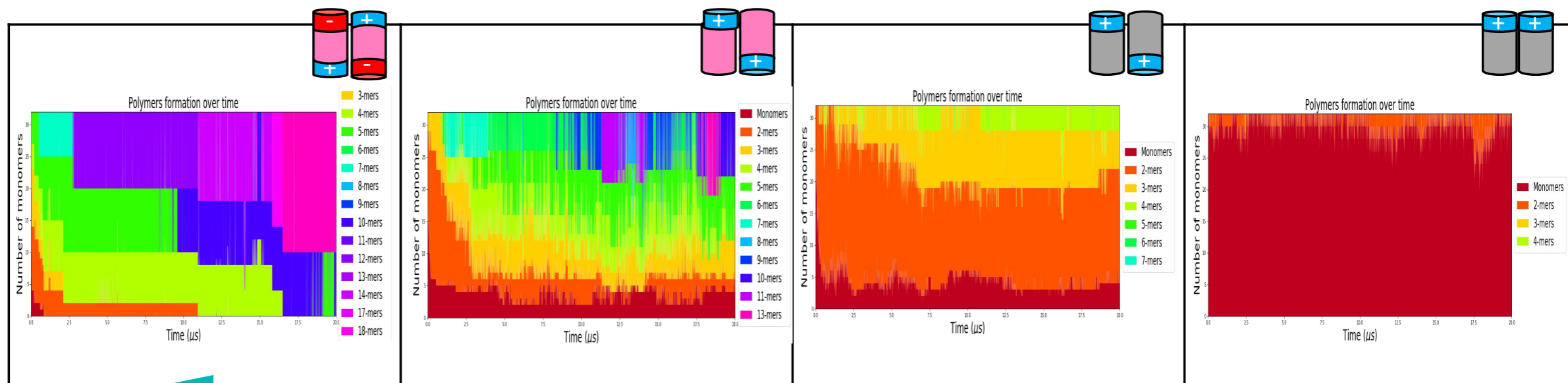


# Effect of terminal charges, orientation, length, and protein-protein attraction

KWALP20 (20 aa)				
	Anti parallel 2 charged termini Martini 2.2	Anti parallel 1 charged terminus Martini 2.2	Anti parallel 1 charged terminus Martini 2.2 modif	Parallel 1 charged terminus Martini 2.2 modif
KWALP28 (28 aa)				

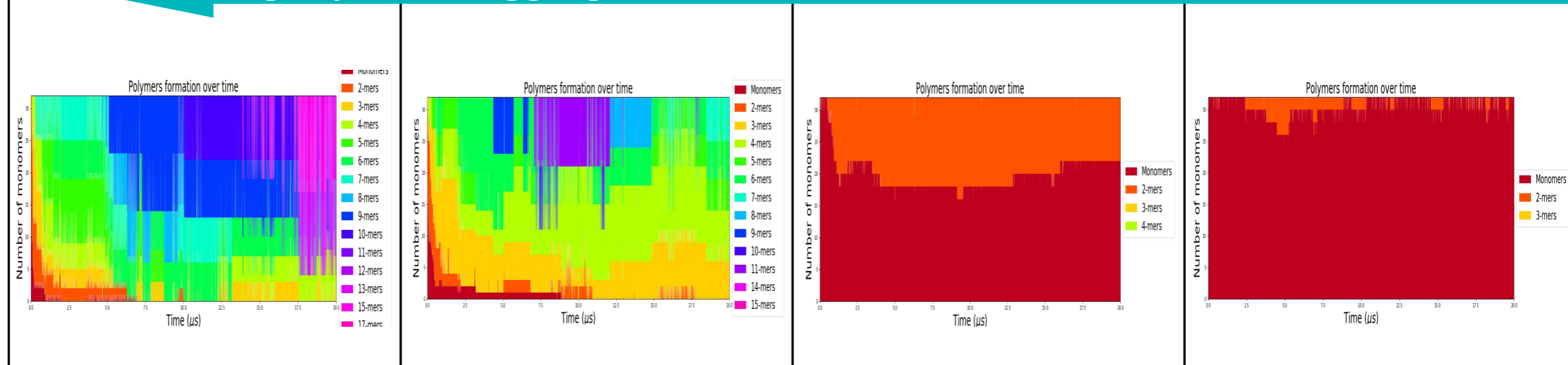
# Tuning oligomerization

KWALP20



Stronger protein aggregation

KWALP28

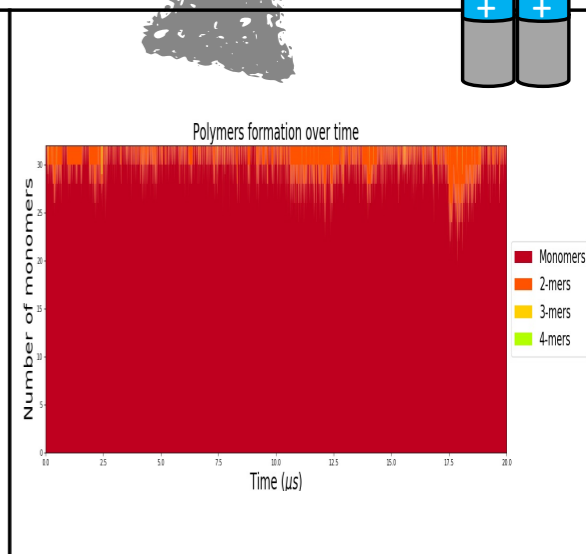
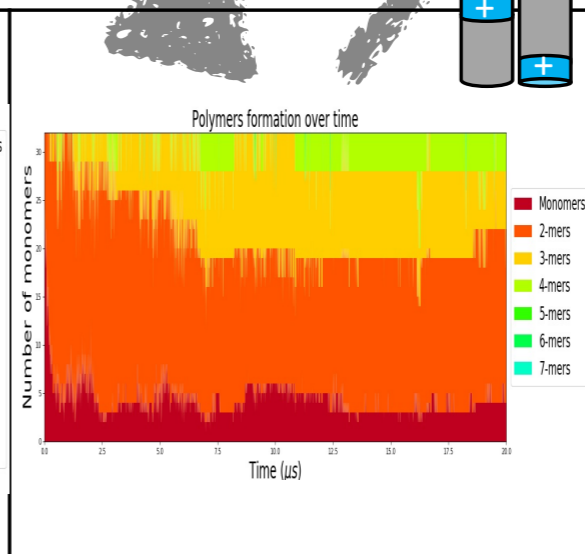
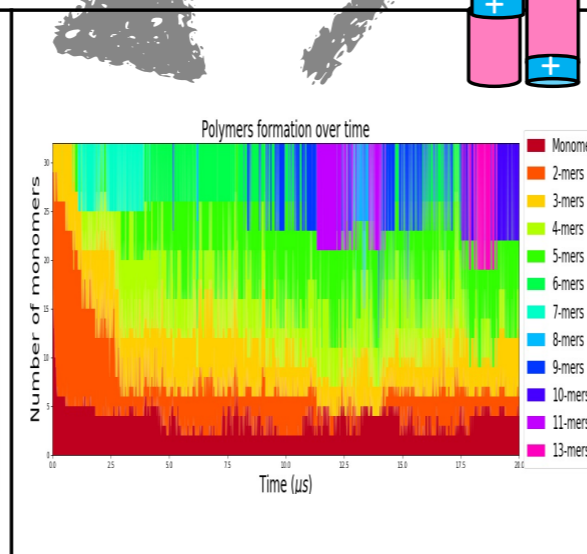
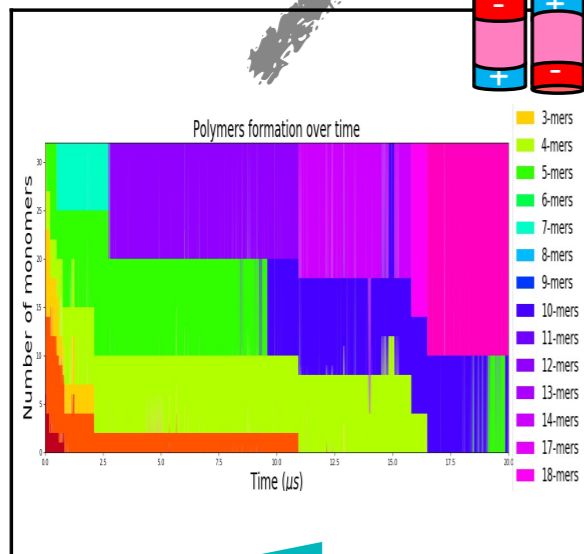
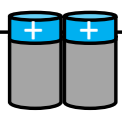
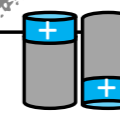
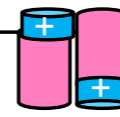
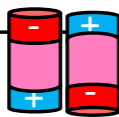


# Tuning oligomerization

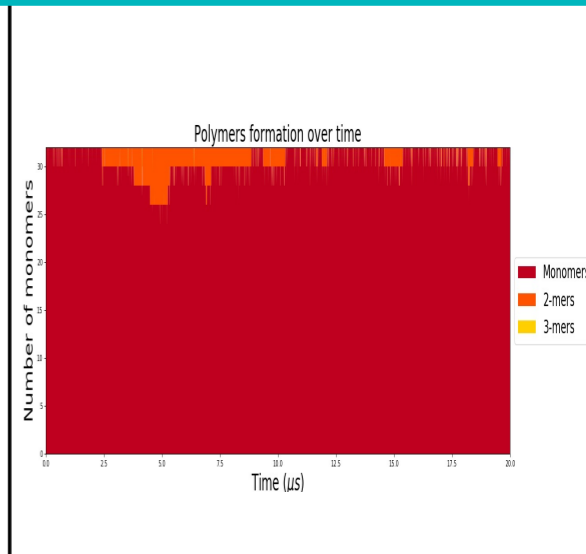
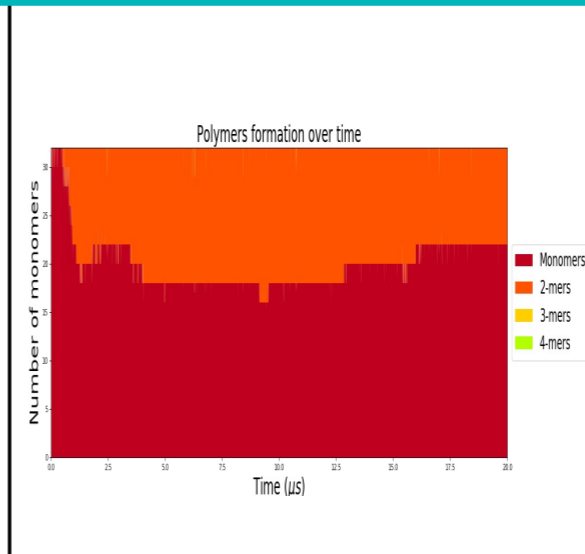
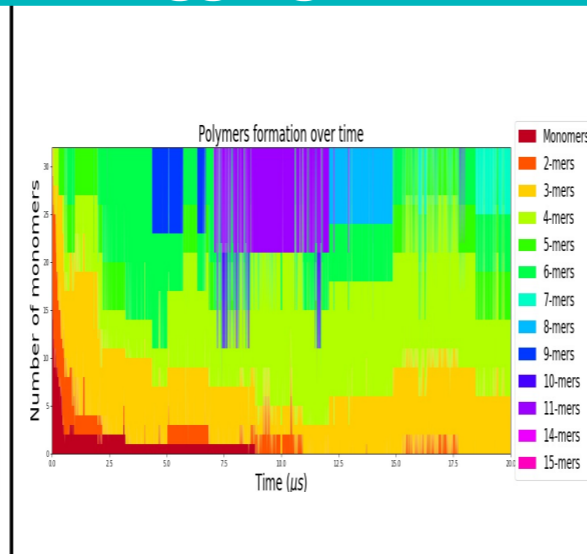
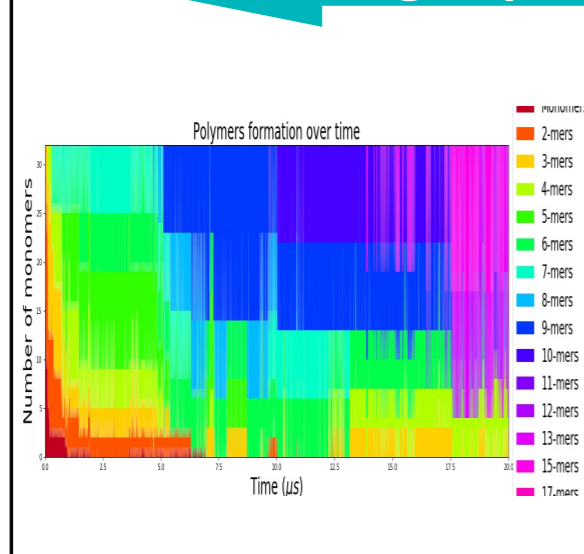
Eliminating the C-terminal charge

Modifying the force field

Parallel orientation



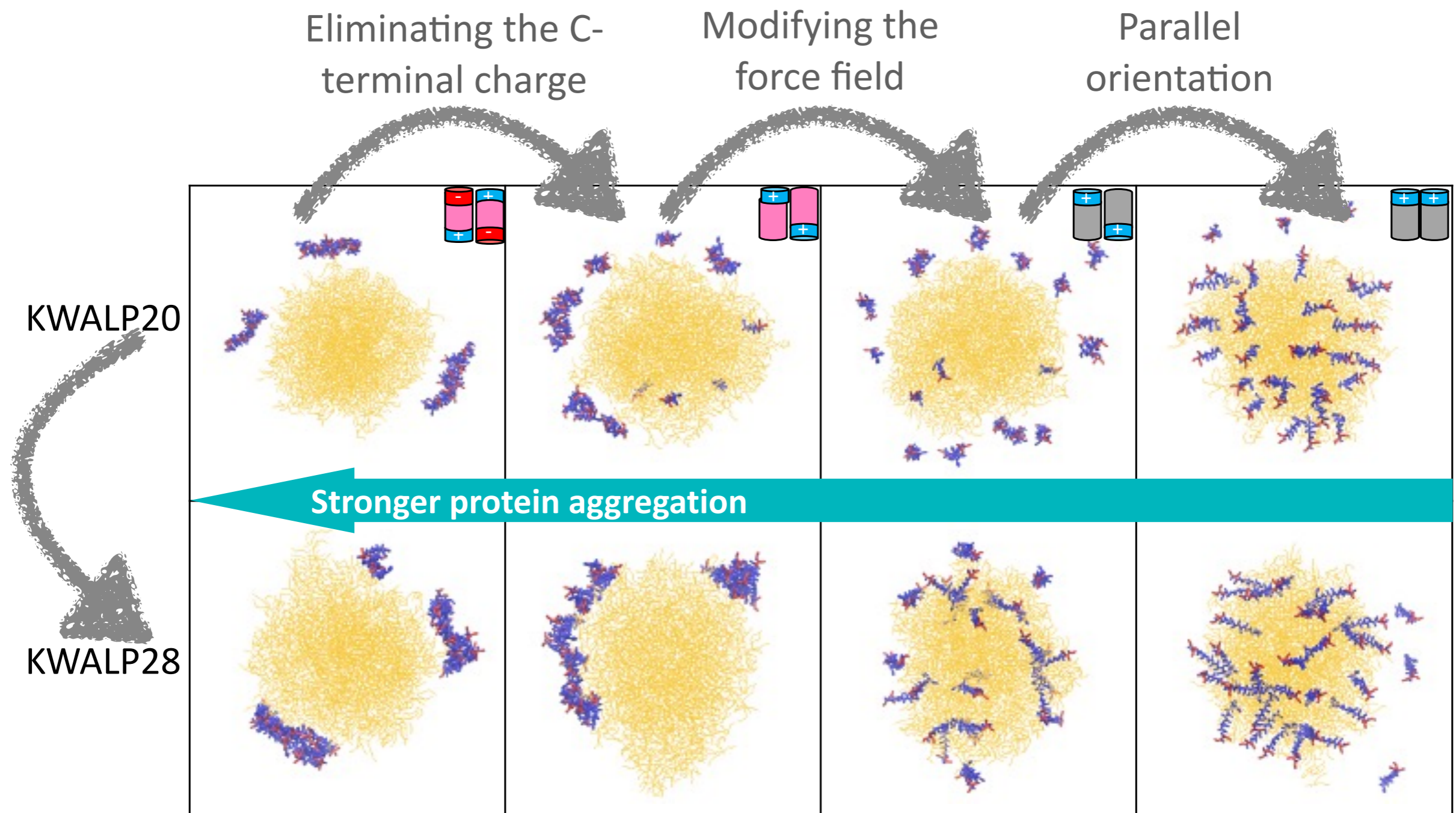
Stronger protein aggregation



KWALP20

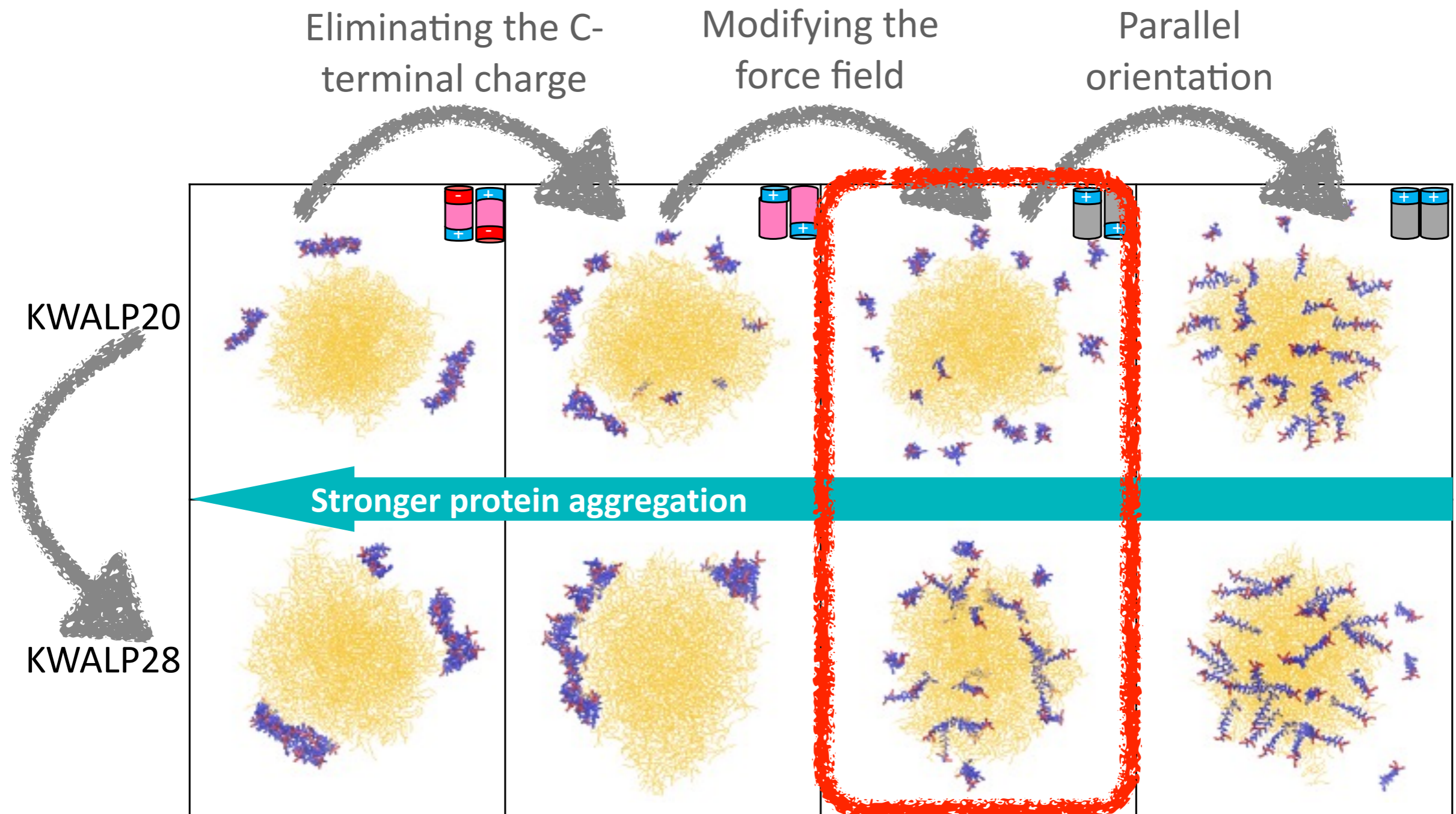
KWALP28

# Charge and oligomerization affect partitioning

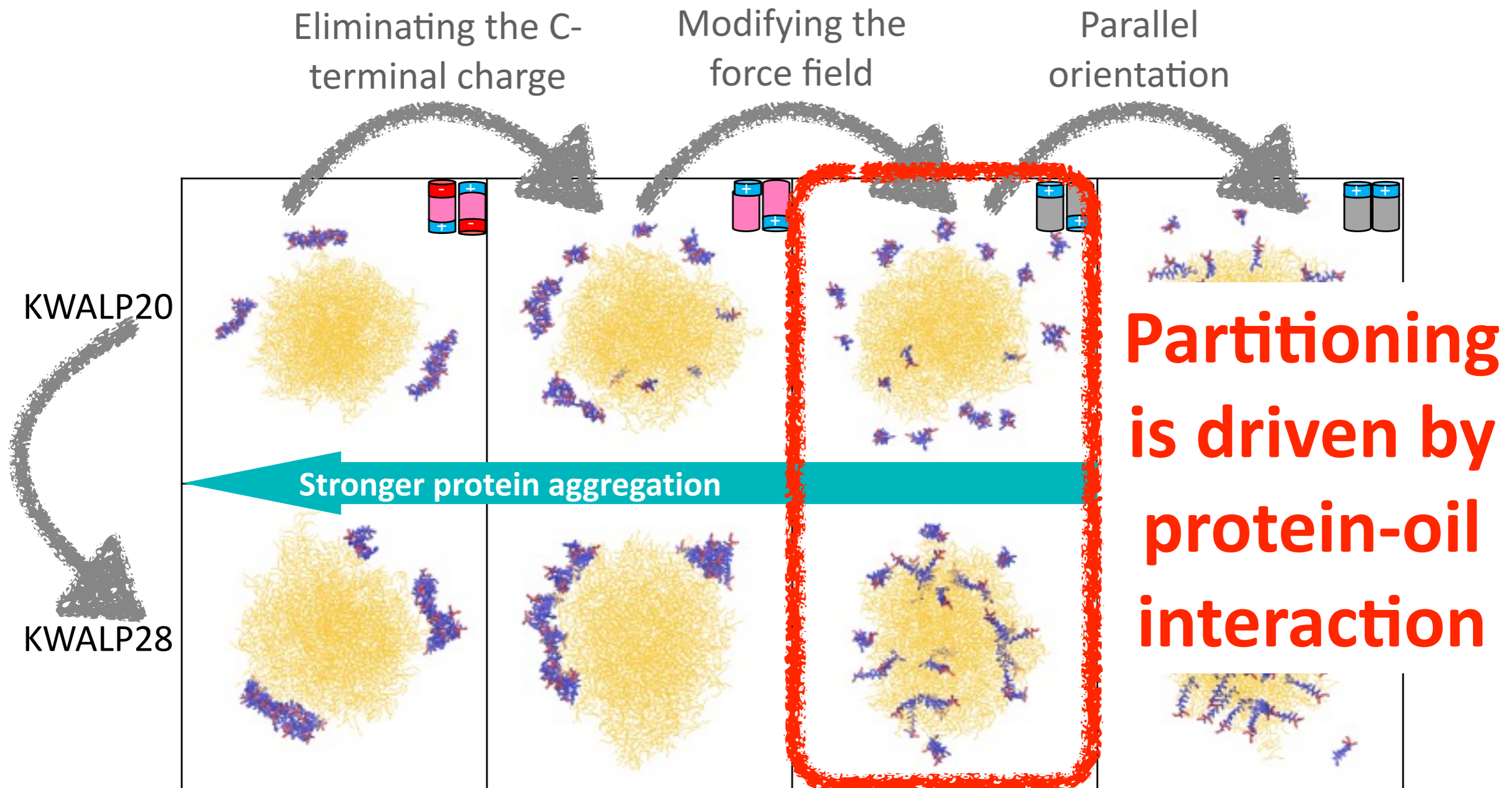




# Charge and oligomerization affect partitioning



# Charge and oligomerization affect partitioning



# Acknowledgements

V Nieto MMSB, CNRS (France)

AR Thiam, L Caillon, L Foret, A  
Chorlay ENS-Paris (France)

## MOBI team



[www.mad.ibcp.fr](http://www.mad.ibcp.fr)

MARTini Database

⚠ This is a beta version of MAD service. If you have any suggestions or problems, please contact us at [mad-support@ibcp.fr](mailto:mad-support@ibcp.fr) or use contact page.

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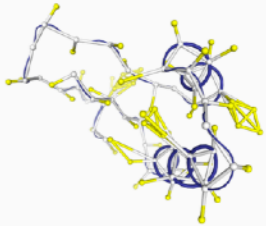
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Force fields

### General information

**1CRN**  
1CRN  
Proteins  
prepared with martinize2  
Version 1.0 created at 2020-11-27 17:17.  
Last modified at 2021-01-28 11:54.



### Details

Created for force field **martini304** (Manually).

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