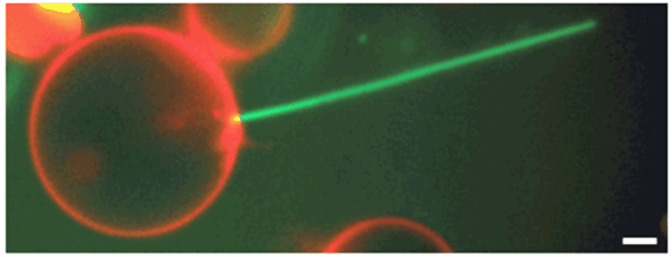
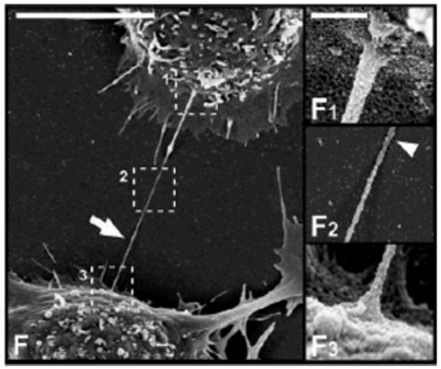
Designing Lipids to Target Specific Membrane Phases and Enable Actuation of Membrane Curvature

SAND2014-18198PE





Darryl Y. Sasaki
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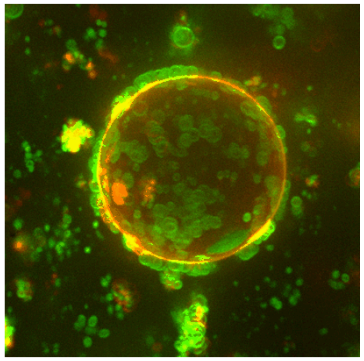
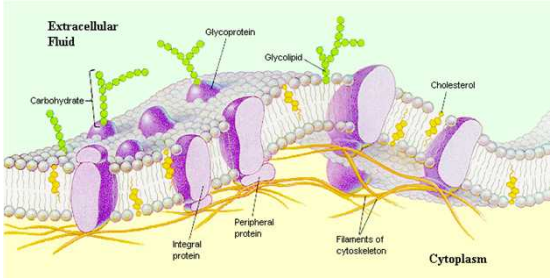
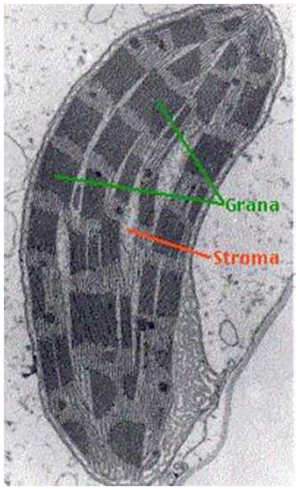


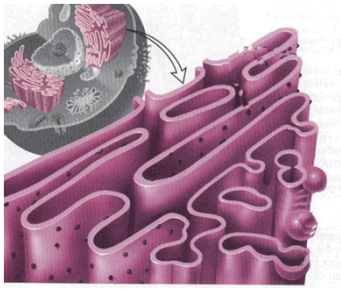
Plasma membrane -
Cell communication, encapsulation

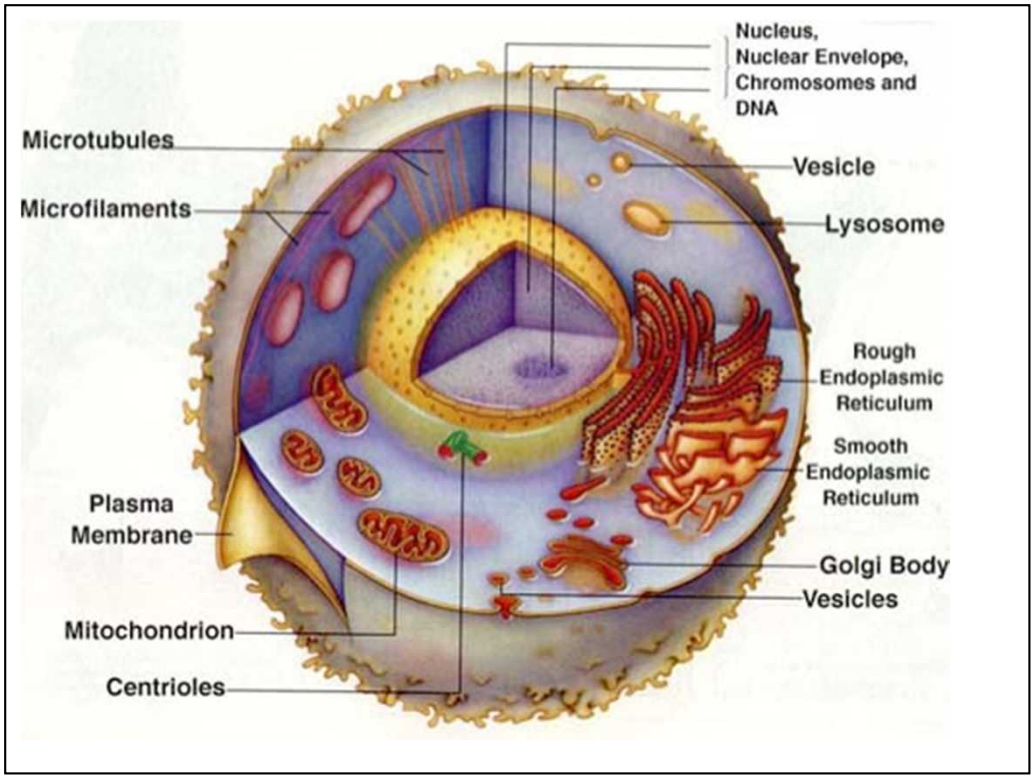


Chloroplasts -
photosynthesis

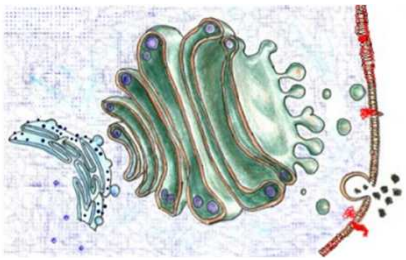


Endoplasmic reticulum -
Synthesis of proteins, lipids, carbohydrates

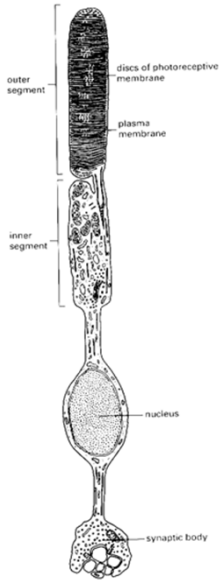




Golgi appartus -
Protein transport and distribution

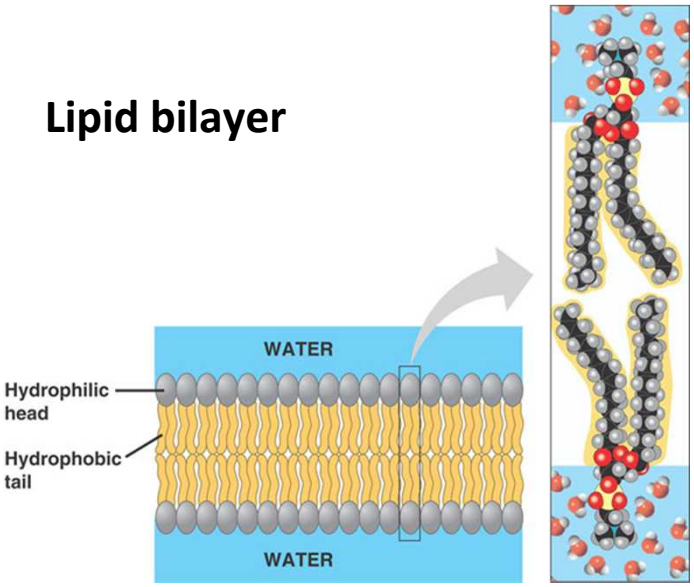


Photoreceptor cells -
light capture for vision

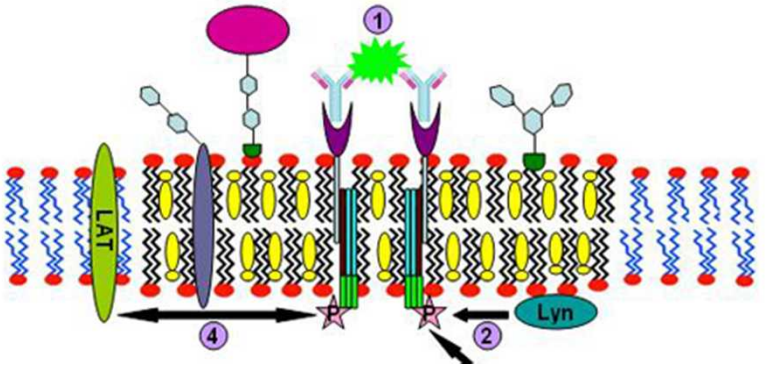


Biology utilizes the fluidity and defined architecture of the lipid membrane to build complex functional systems

Lipid bilayer

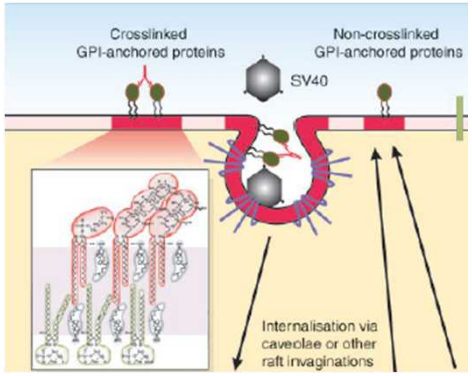


Phase separation

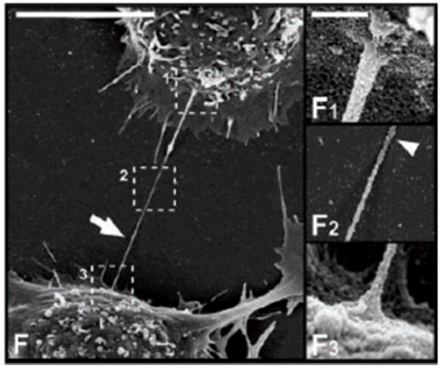


Molecular concentration, membrane mechanics

Dimensional transformation

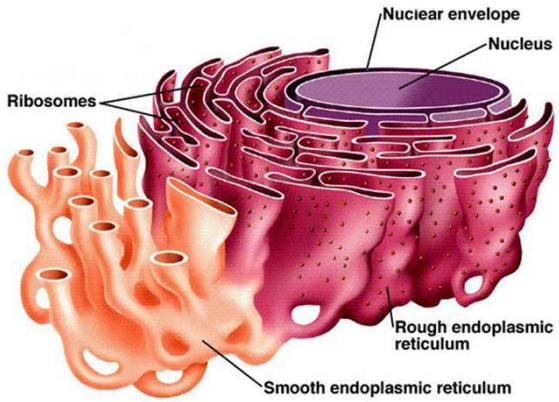


Budding, endocytosis



Cellular nanotubes

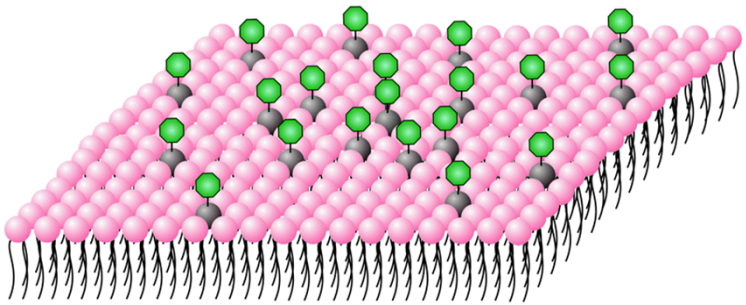
Hierarchical assemblies



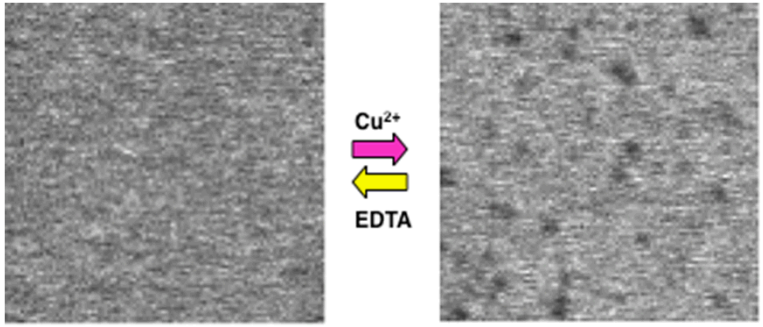
Functional composite materials

Synthetic lipid bilayers provides a molecularly dynamic matrix to study cell membrane-mimetic processes

Addressable component
- Chemical interaction
- Temperature
- Electric field

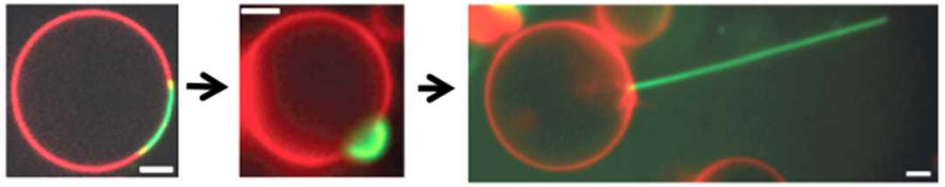


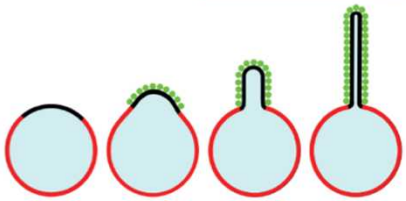
Phase separation



Molecular concentration, membrane mechanics

Dimensional transformation

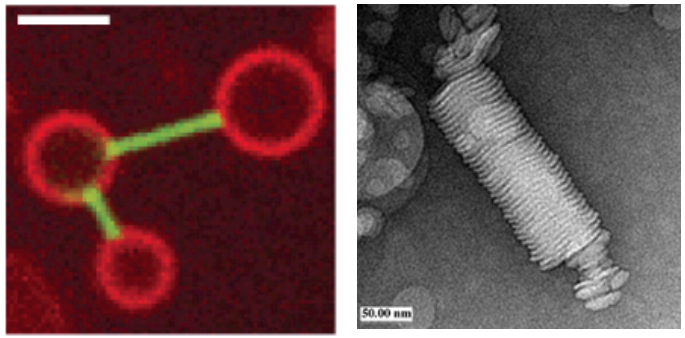




Budding, endocytosis

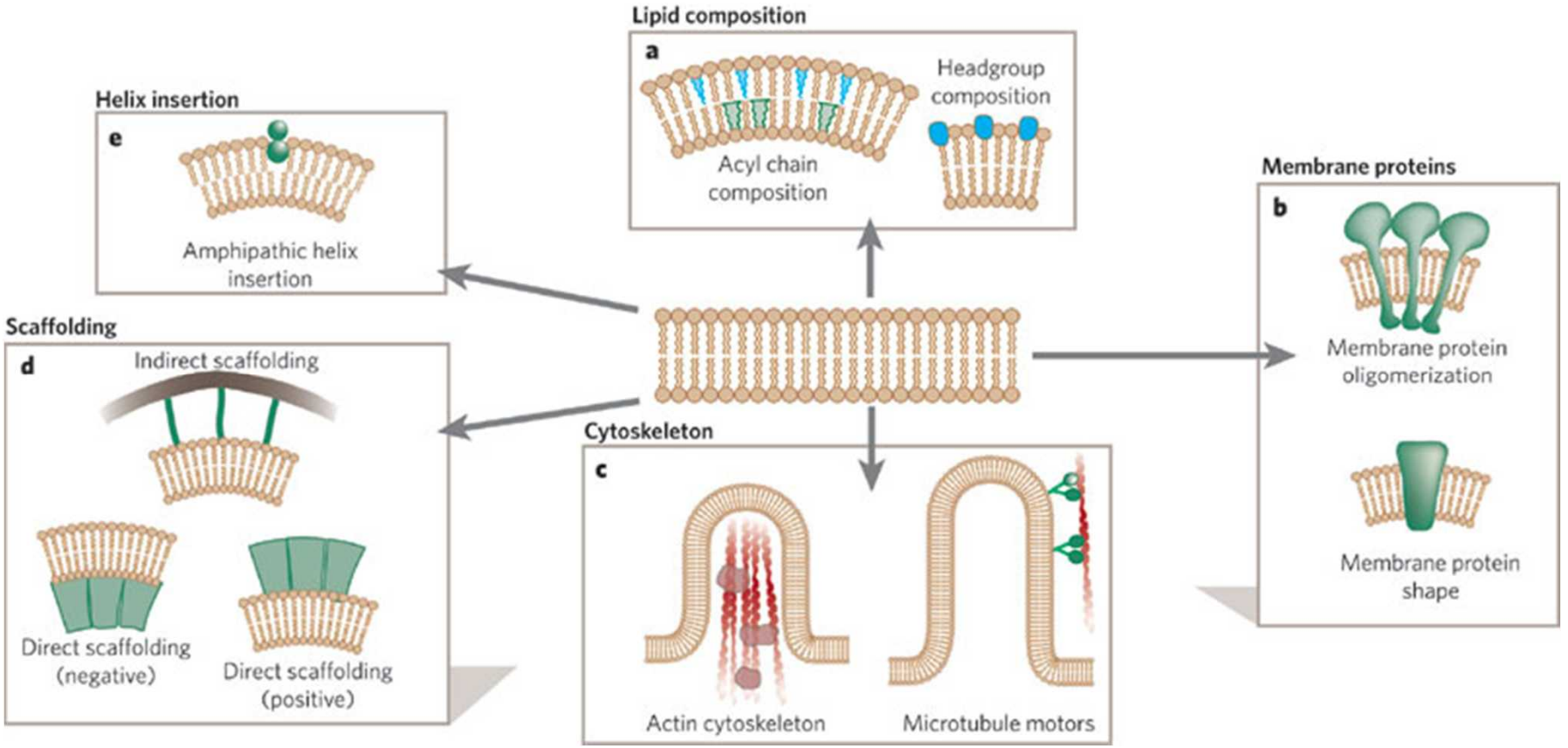
Cellular nanotubes

Hierarchical assemblies



Building complex composites

Curvature inducing pathways in cellular membranes



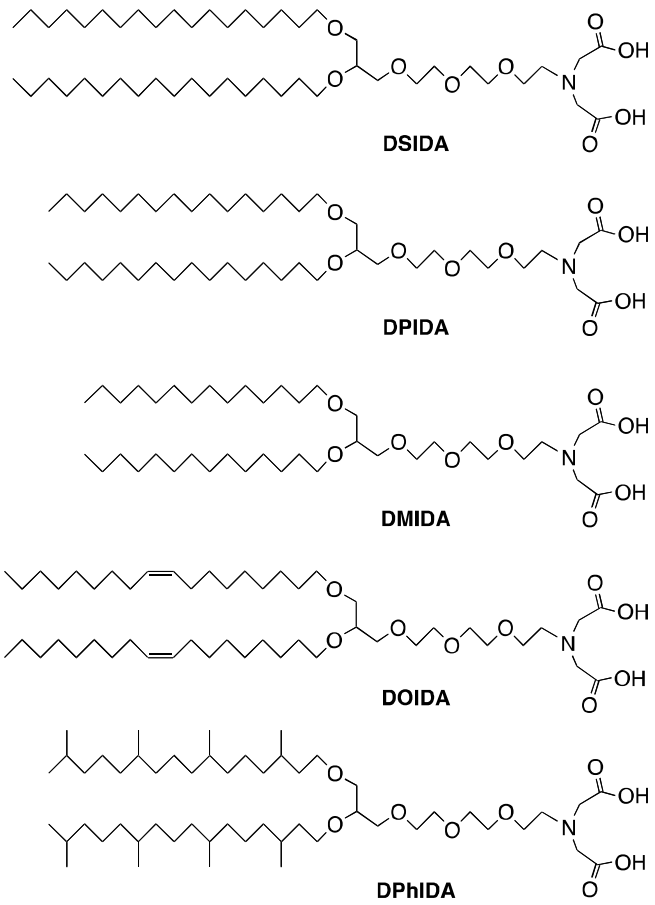
From McMahon, Gallop *Nature* **2005**, *438*, 590.

- Chemical recognition enables site specificity of bending events
- Curvature is facilitated through *pulling forces*, *steric interactions*, and *templates*

Tail structure

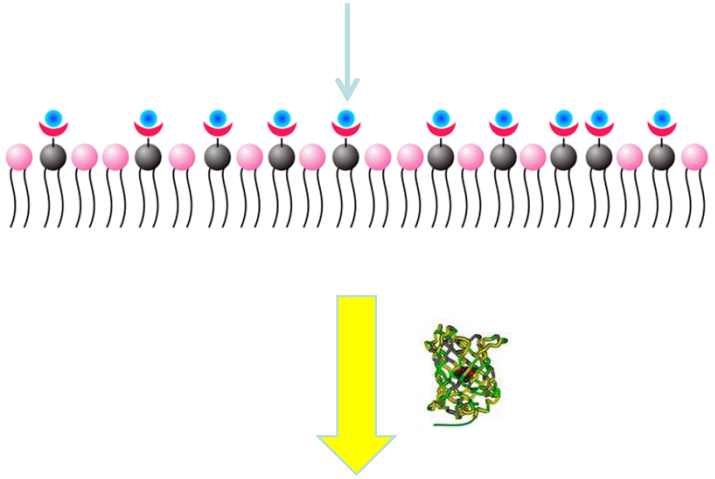
Chemical recognition on lipid membranes induces structural and functional transformation

Iminodiacetic acid lipids

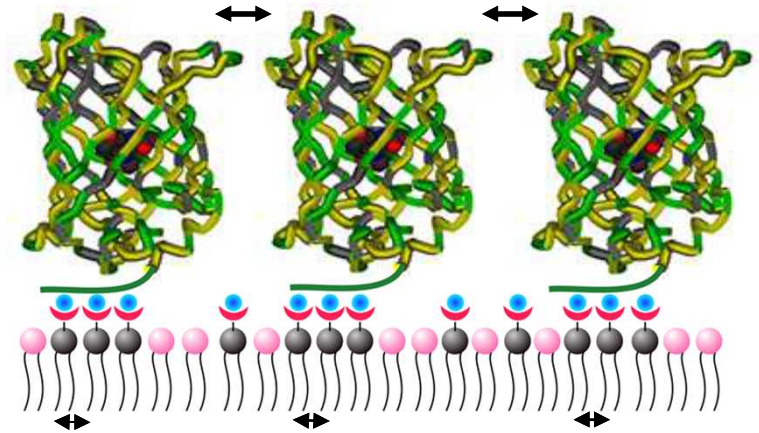


Control over membrane domain formation, bending rigidity, ligand affinity, spontaneous curvature

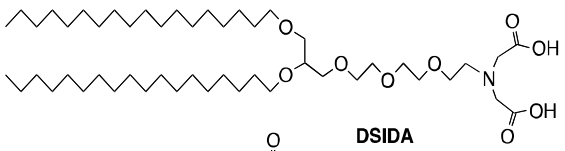
His-tag affinity sites

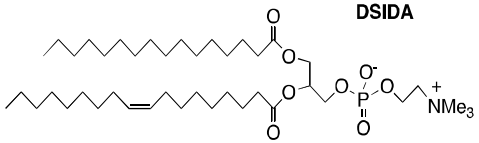


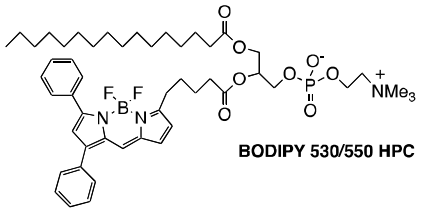
Steric interactions



Microdomain formation driven by metal ion recognition



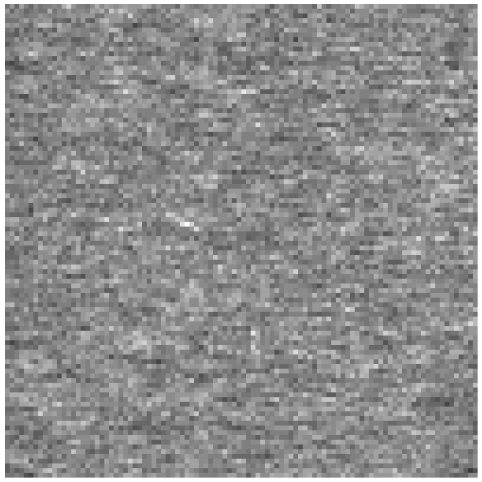




T_g

65 ° C

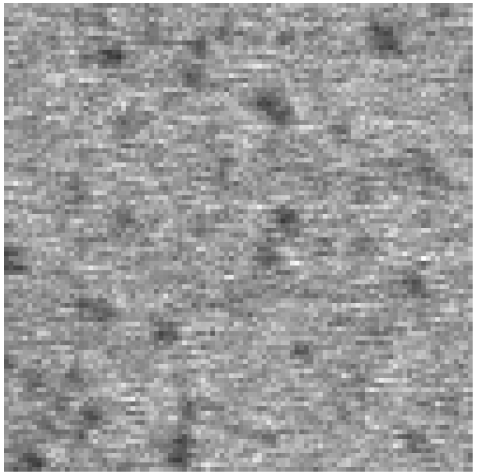
-2 ° C



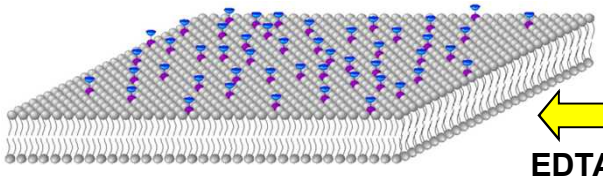
Supported lipid bilayer on glass

Cu^{2+}

EDTA

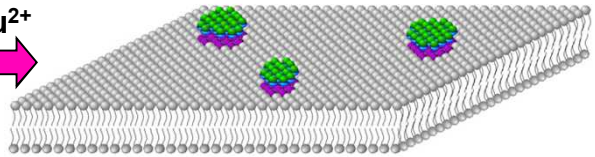


Membrane ~30 min after addition of ~15 uM $CuCl_2$



Cu^{2+}

EDTA

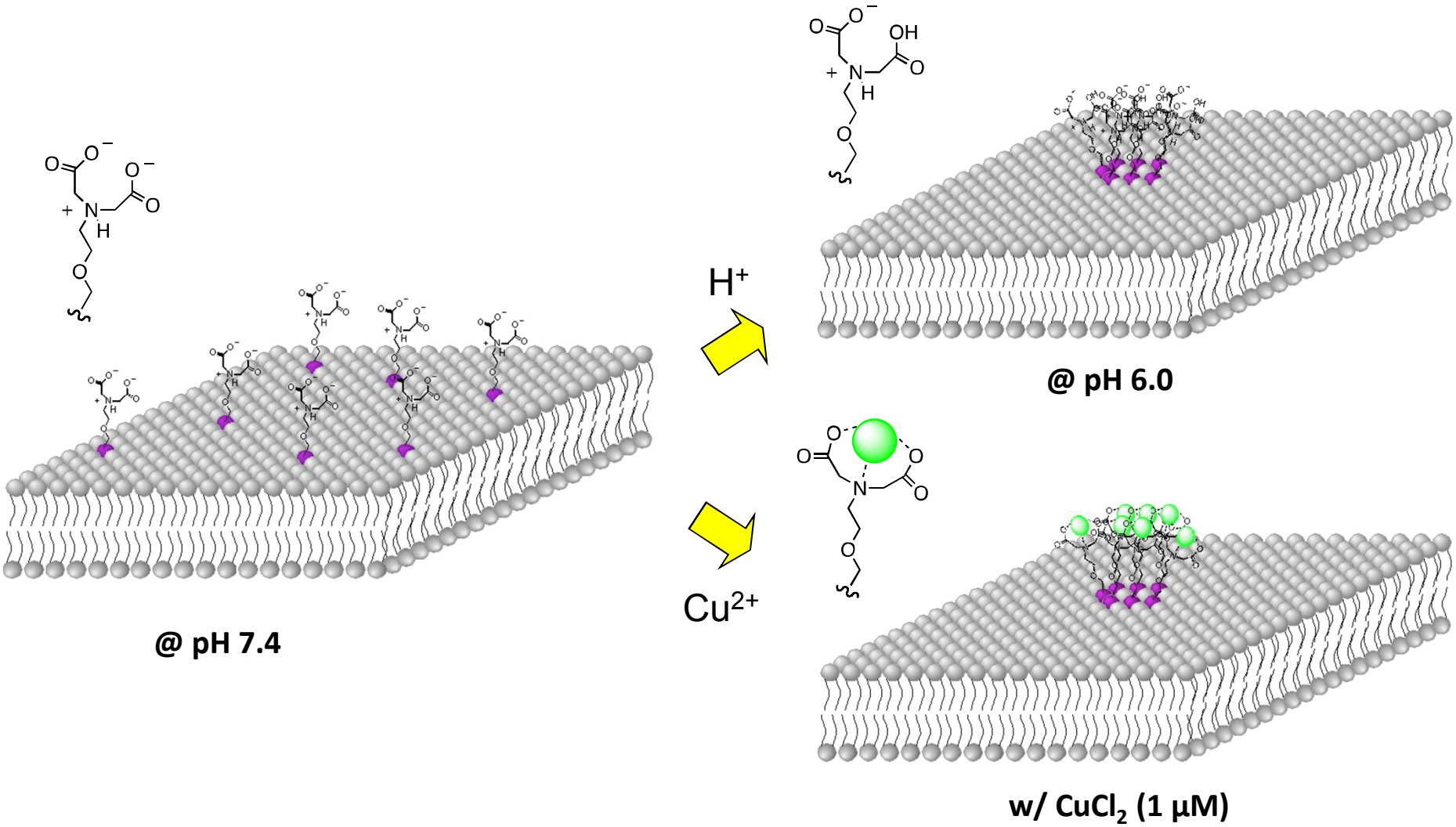


Miscible lipid system

Metal ion-induced phase separation

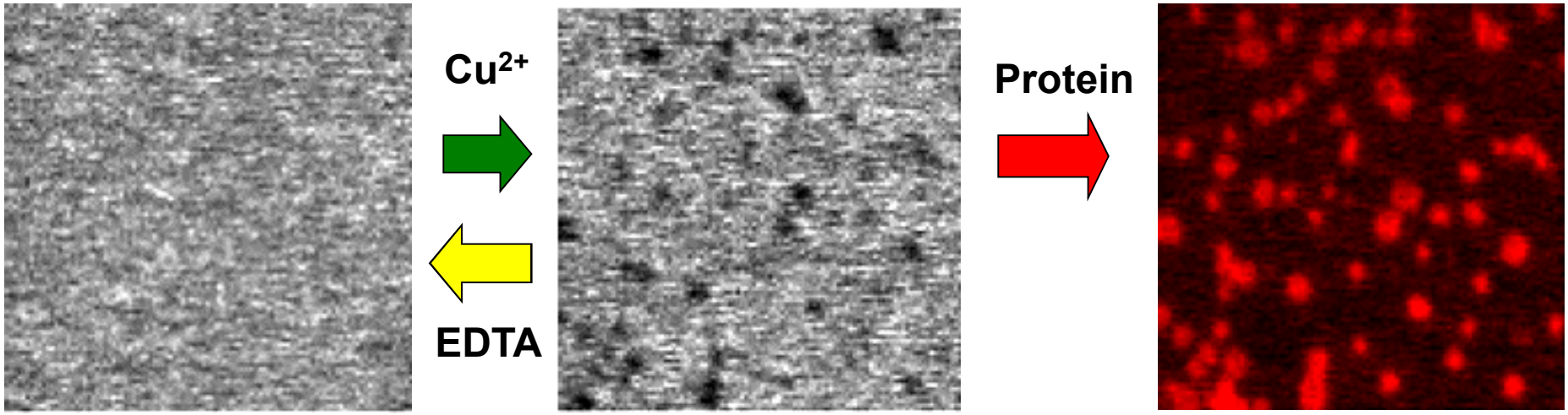
- Microdomains (1 - 2 μM dia.) exhibit mobility rates consistent with gel phase structure
- Formation rate of domains diffusion limited, while domain architecture stable with time
- System is completely reversible with addn. of EDTA (~ 10 μM)

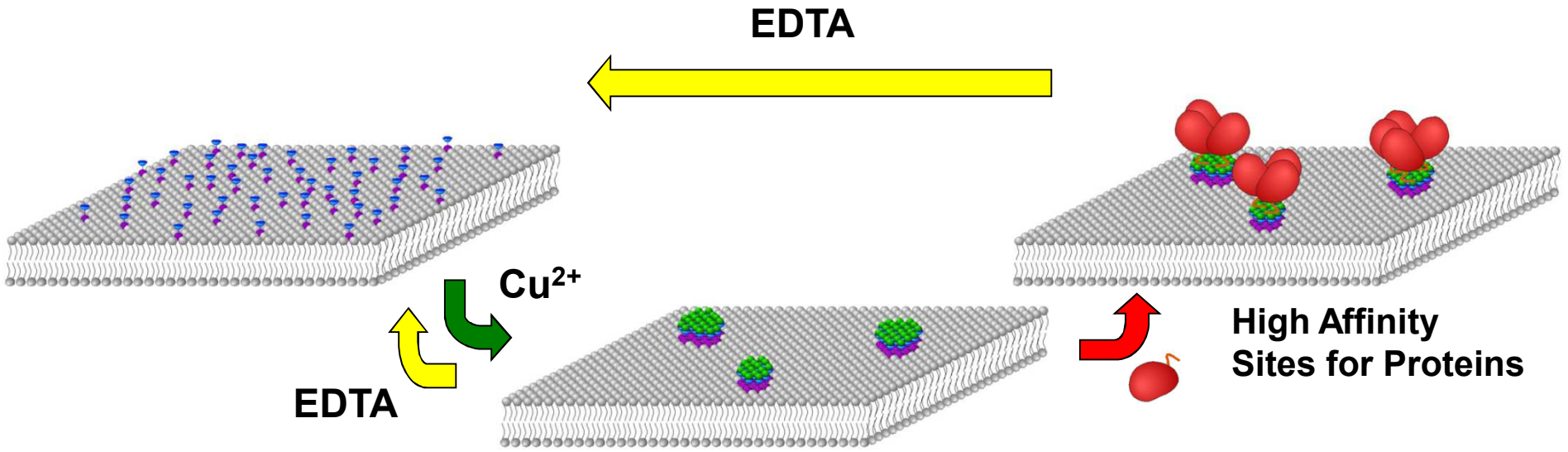
Mechanism of domain formation - neutralize charged headgroup via ion binding



- Electrostatics and packing interactions can be used to direct the aggregation/dispersion of membrane components

Domains bind his-tagged proteins *reversibly*

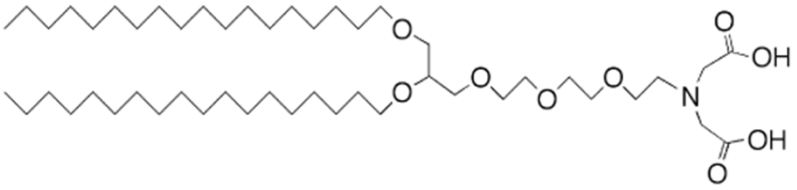




Hayden, C. C.; Sasaki, D. Y.; et al *J. Am. Chem. Soc.* **2009**, *131*, 8728.

Domains can localize *lipid-protein binding*

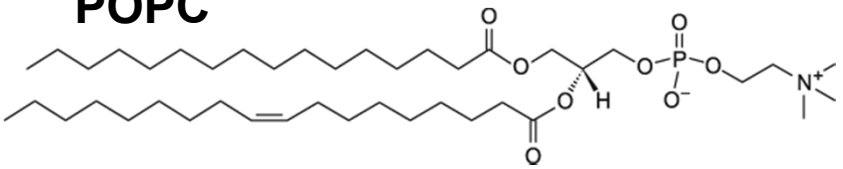
DSIDA T_m ~65 ° C



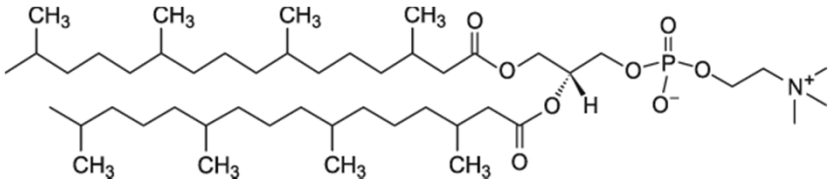
+

Matrix lipids T_m < Room Temp

POPC



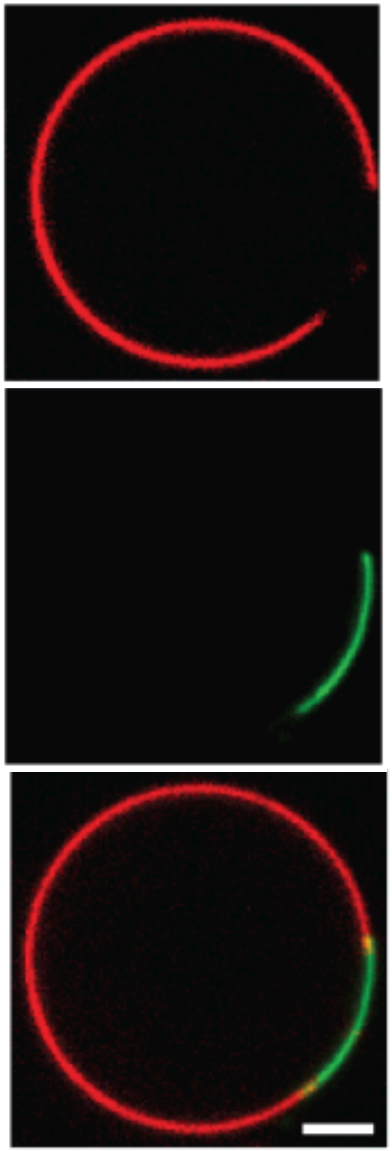
DPhPC



+

Histidine-tagged proteins

Giant unilamellar vesicle



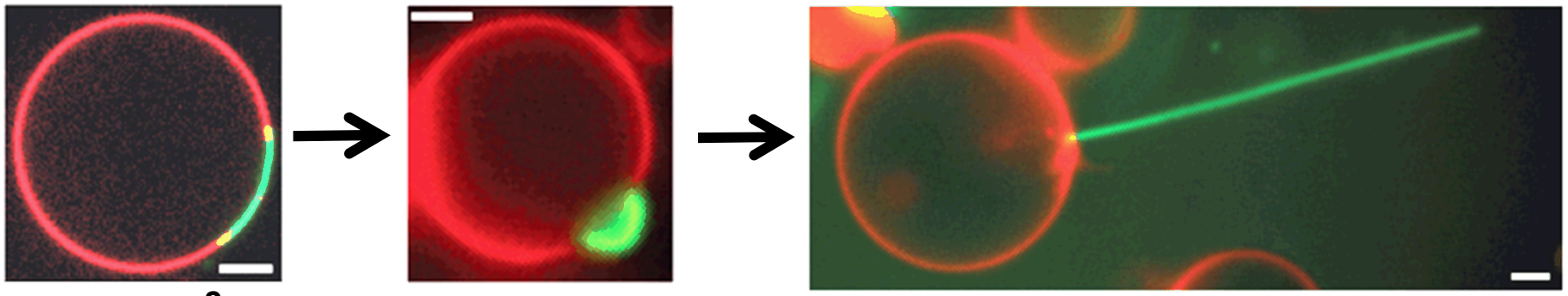
Membrane

Protein

Merge

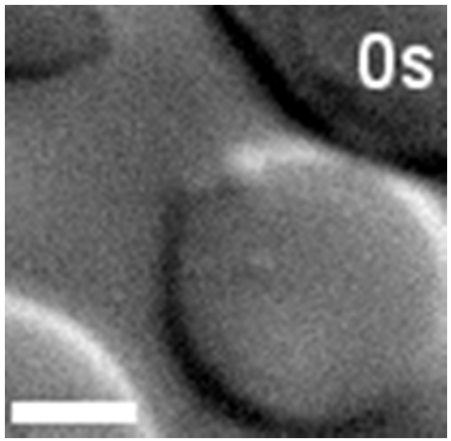
2 µm

Localized protein binding *bends lipid membranes*



2 µm

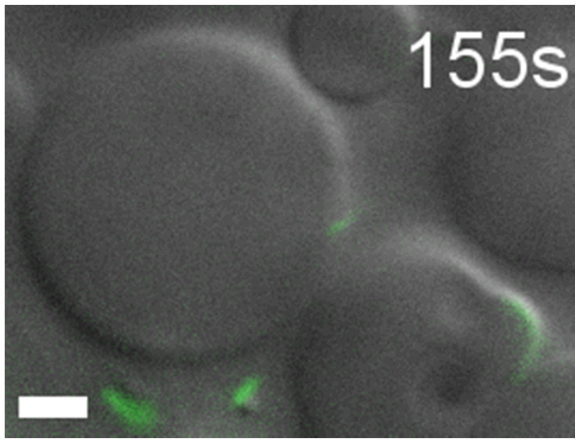
Domain deformation



5 µm

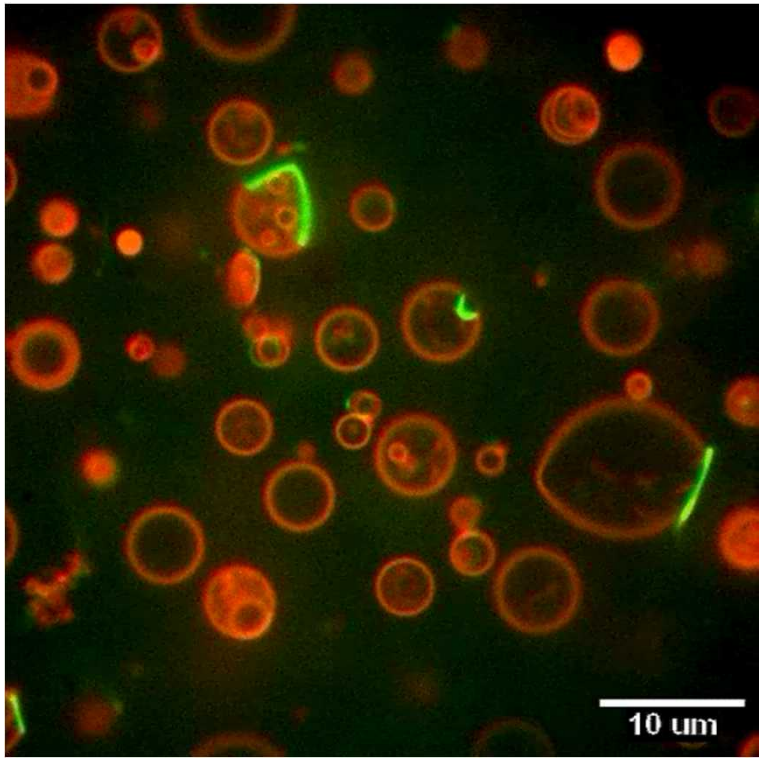
time-lapse

Tubule extension



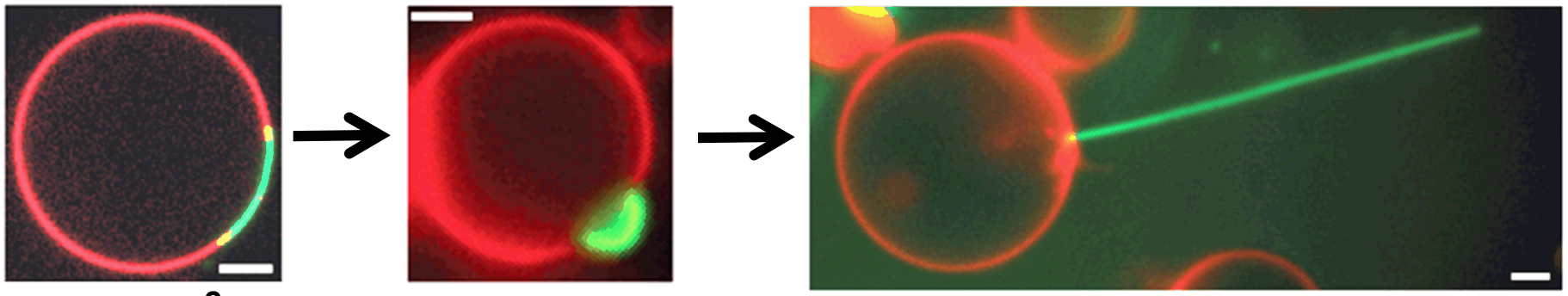
5 µm

time-lapse

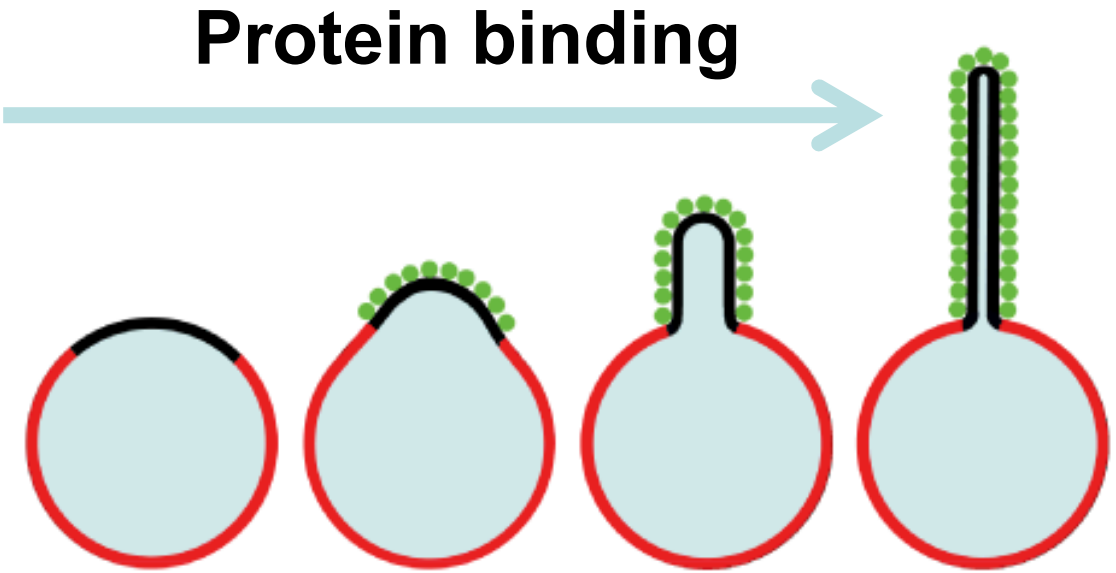


z-stack

Localized protein binding *bends lipid membranes*

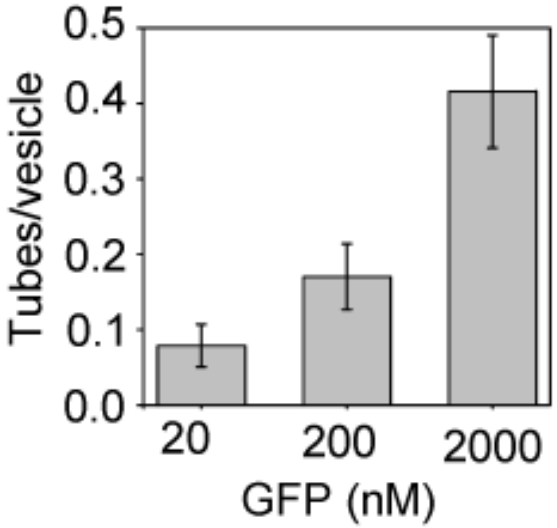


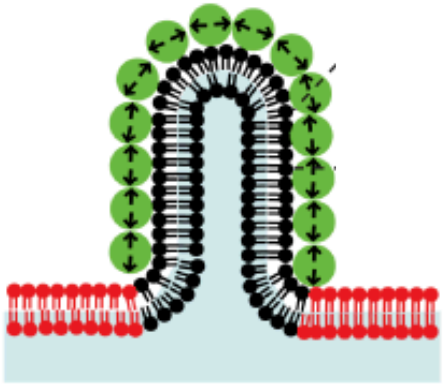
As proteins bind, domain curvature increases, eventually forming a lipid tubule.



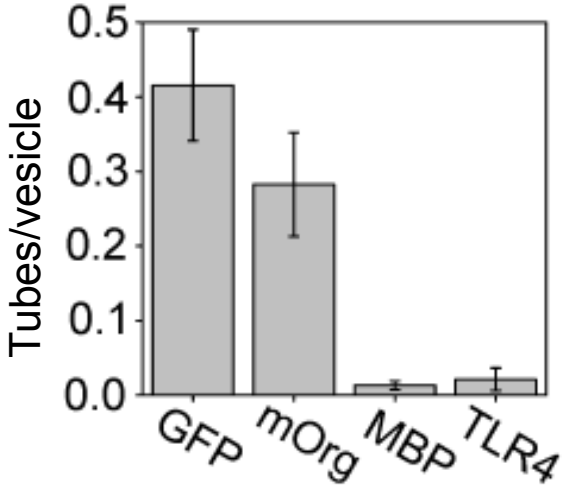
Protein crowding bends membranes

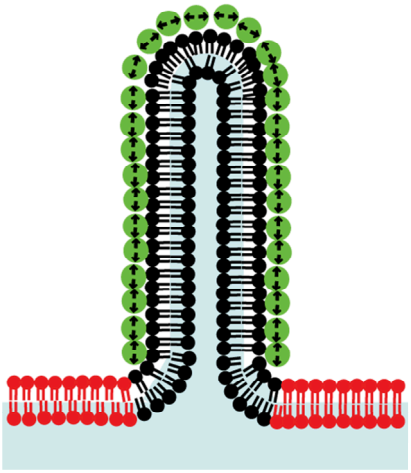
Tubule formation increases with protein concentration

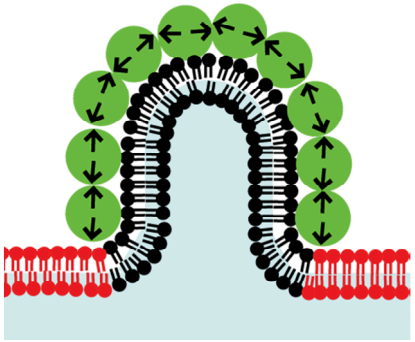




Proteins of smaller molecular weight form more tubules

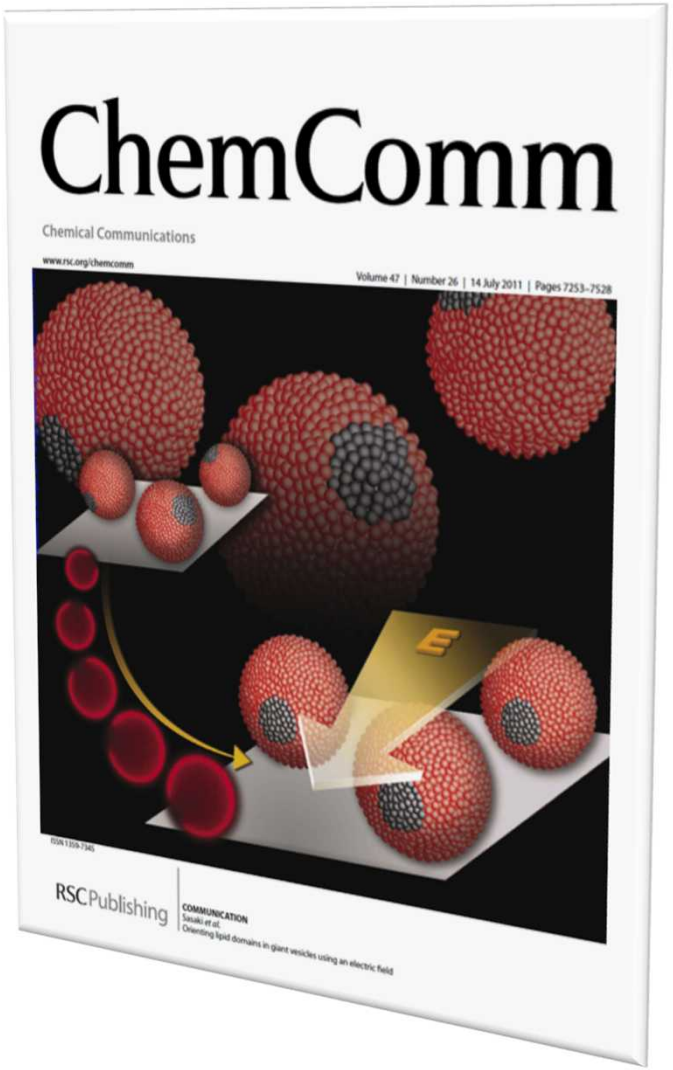


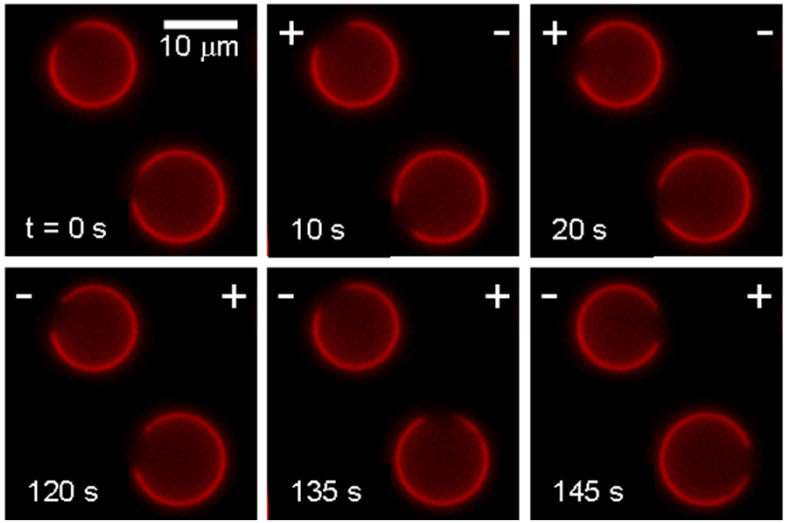


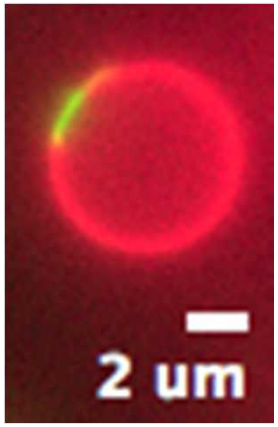


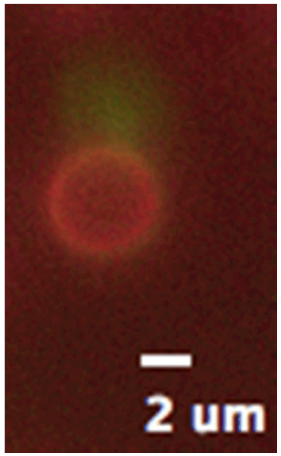
Stachowiak, J. C.; Hayden, C. C.; Sasaki, D. Y. *Proc. Nat. Acad. Sci.* **2010**, *107*, 7781.

Electric Field Orientation of Lipid Domains





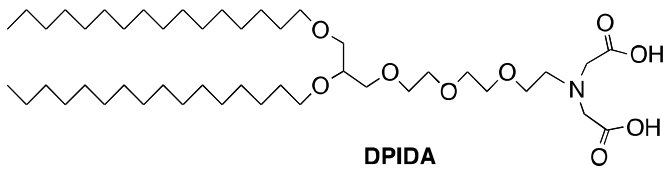




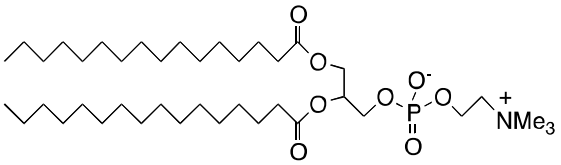
Domain orientation directs nanotube orientation

Zendejas, F. J.; Meagher, R. J.; Stachowiak, J. C.; Hayden, C. C.; Sasaki, D. Y. *ChemComm* **2011**, *47(26)*, 7320 – 7322.

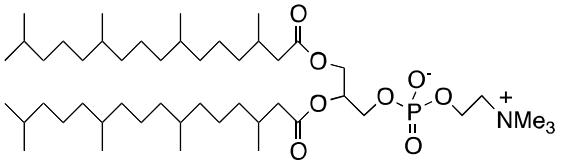
Liquid ordered and liquid disordered domains can be prepared with Cu-IDA lipids



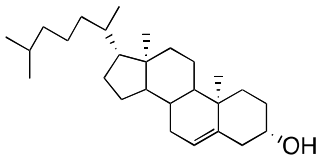
DPIDA



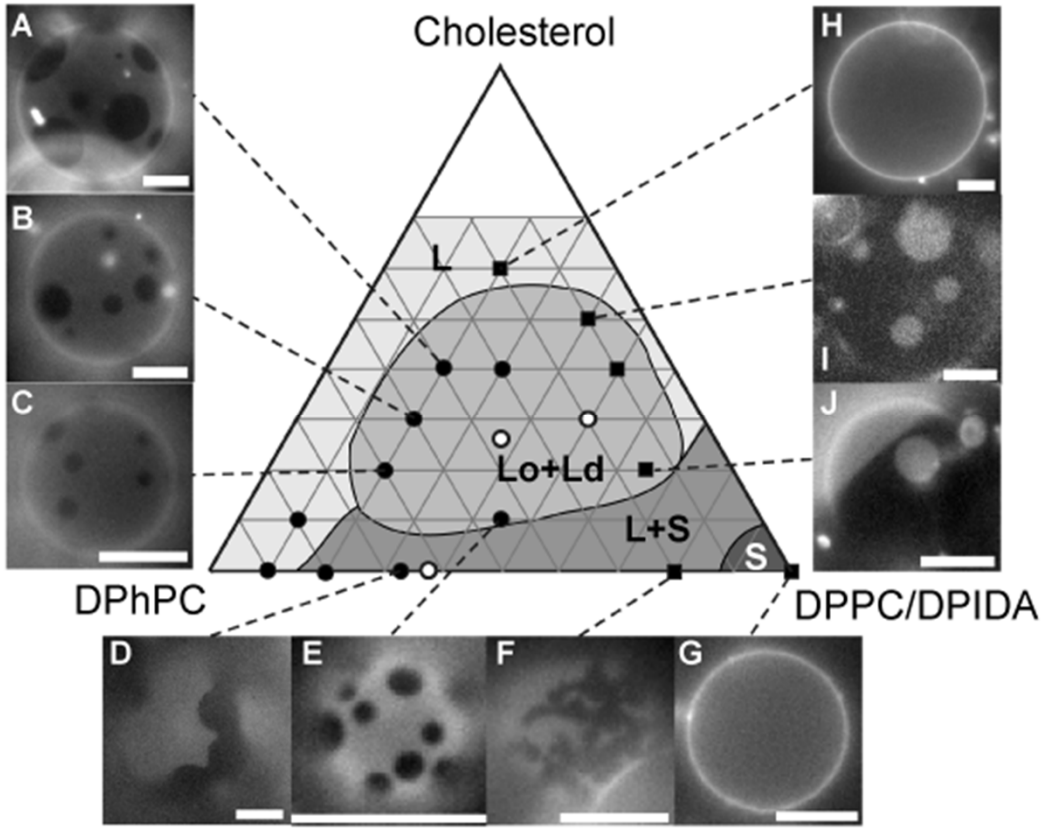
DPPC



DPhPC

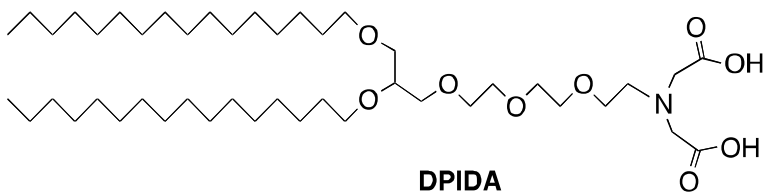


Cholesterol

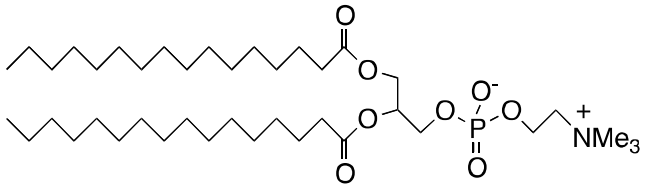


Stachowiak, J. C.; Hayden, C. C.; Sanchez, M. A. A.; Wang, J.; Bunker, B. C.; Voigt, J. A.; Sasaki, D. Y. *Langmuir* **2011**, *27*, 1457.

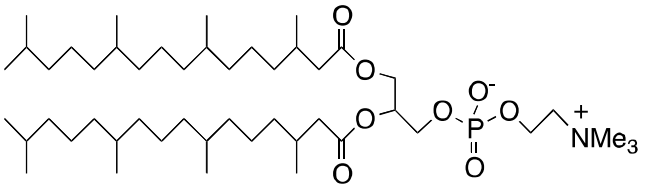
Domain phase and size tailored by membrane composition and processing condition



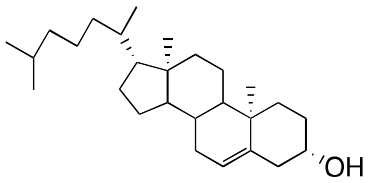
DPIDA



DPPC

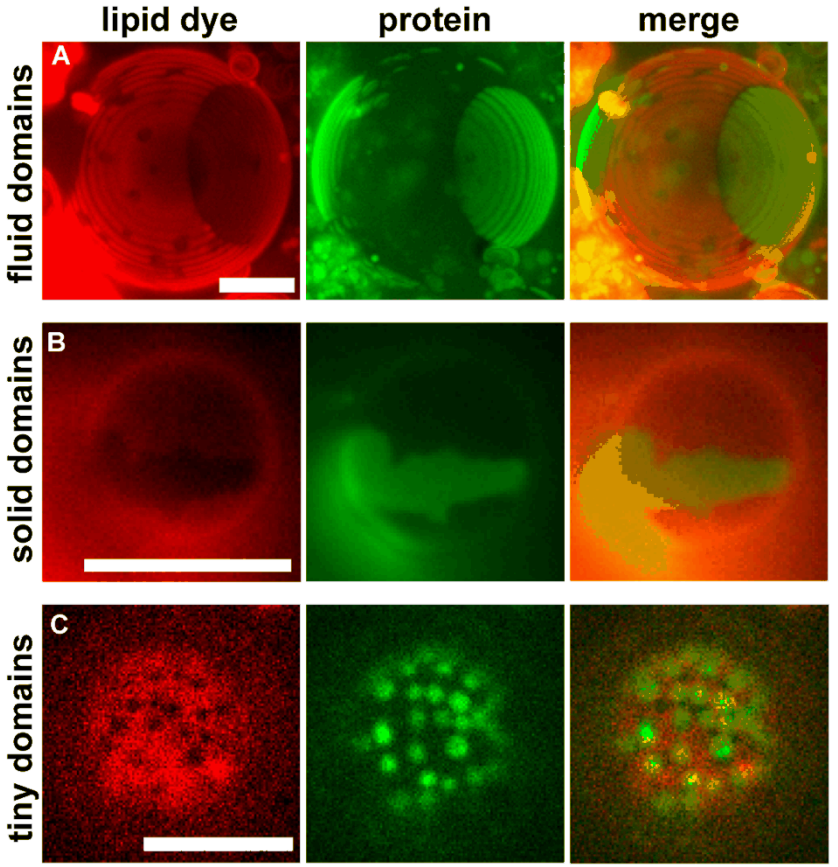


DPhPC



Cholesterol

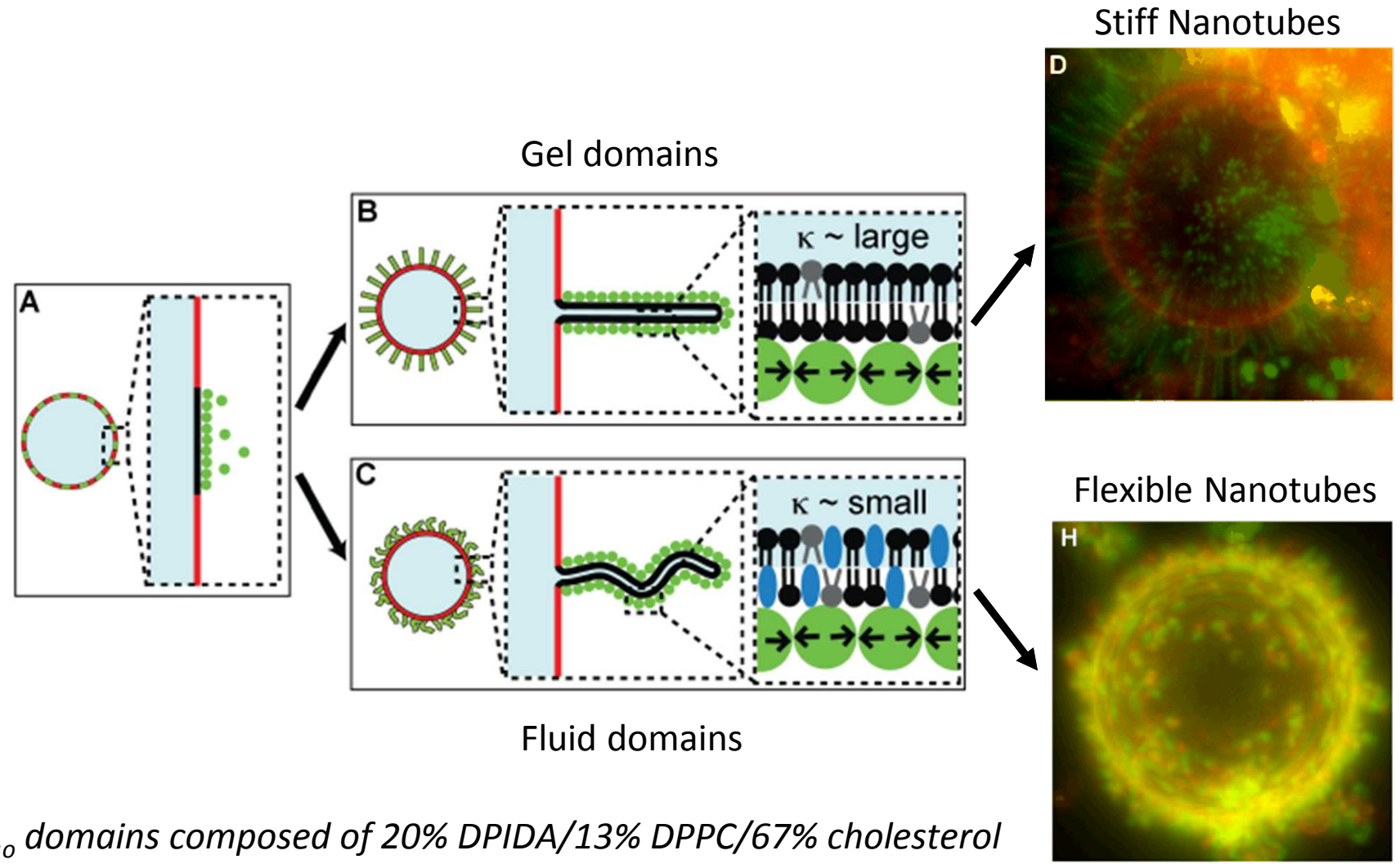
Protein selective domains in liquid ordered and gel phases



Scale bars – 10 μm

Stachowiak, J. C.; Hayden, C. C.; Sanchez, M. A. A.; Wang, J.; Bunker, B. C.; Voigt, J. A.; Sasaki, D. Y. *Langmuir* **2011**, *27*, 1457.

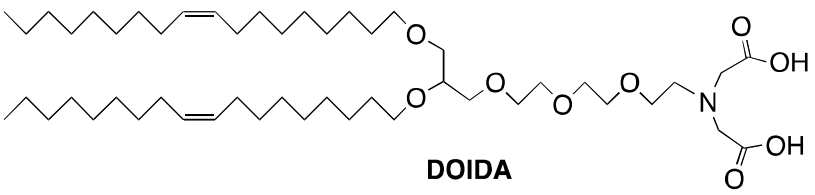
Nanotube structure controlled by the bending energy and size of the host domain



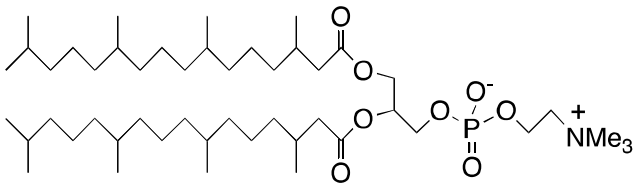
L_o domains composed of 20% DPIDA/13% DPPC/67% cholesterol

Stachowiak, J. C.; Hayden, C. C.; Sanchez, M. A. A.; Wang, J.; Bunker, B. C.; Voigt, J. A.; Sasaki, D. Y. *Langmuir* **2011**, *27*, 1457.

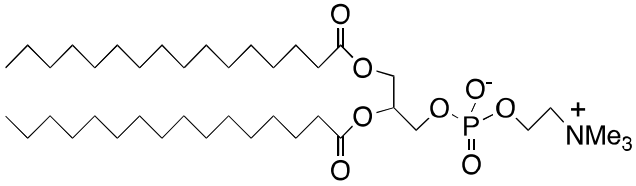
Liquid disordered domains with affinity for his-tagged proteins prepared with DOIDA



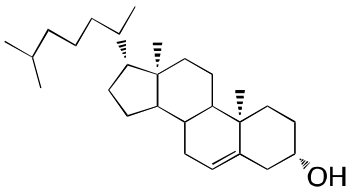
DOIDA



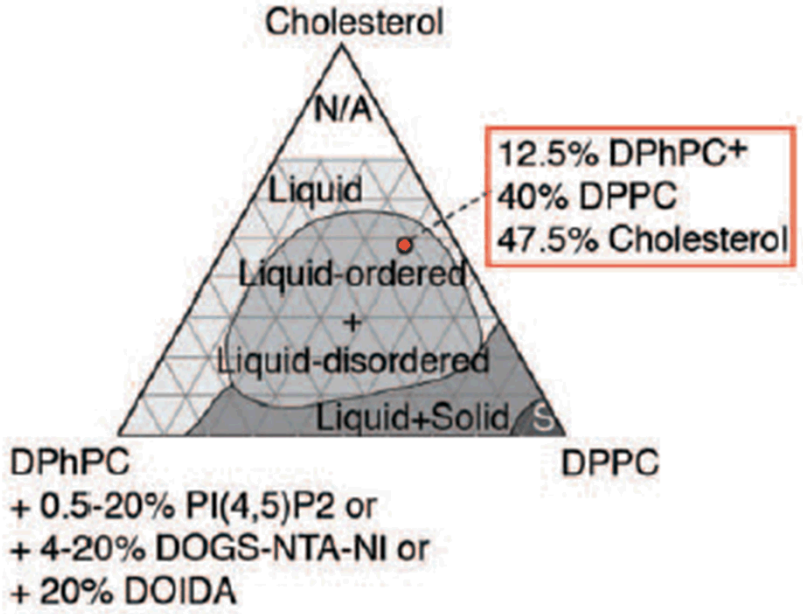
DPhPC

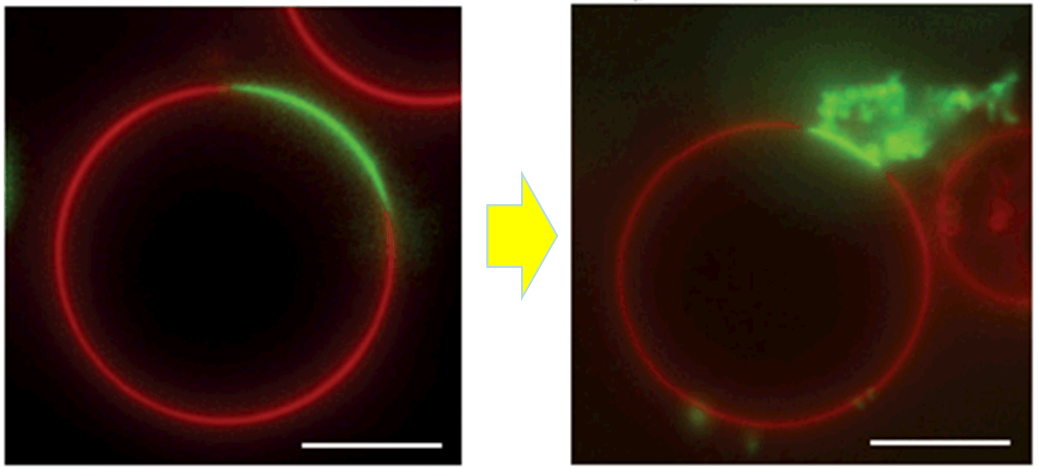


DPPC



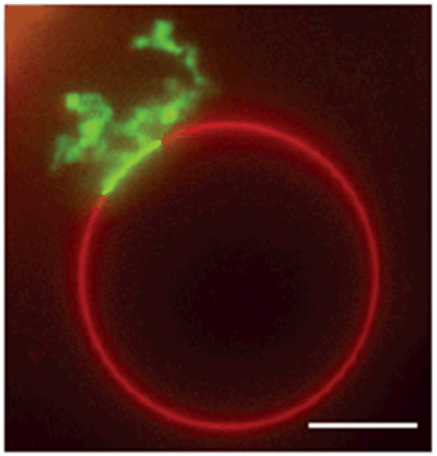
Cholesterol

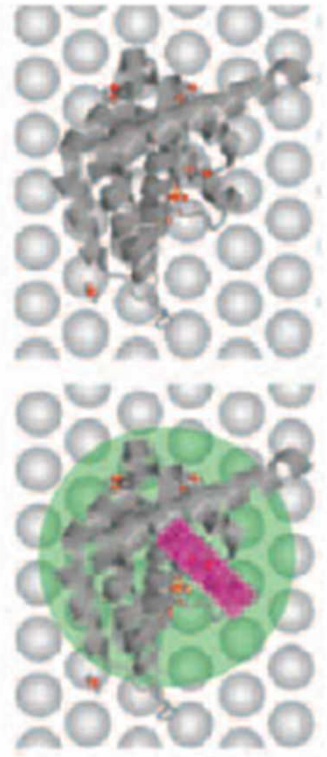


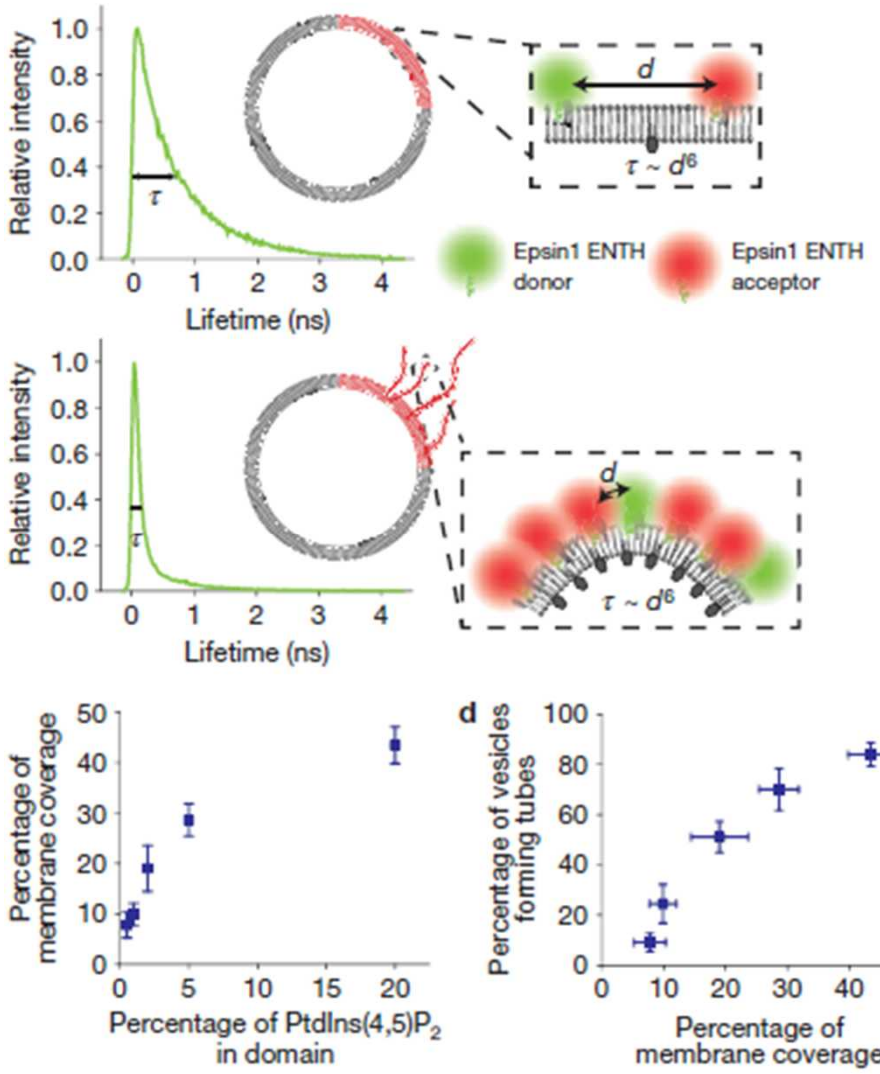


Stachowiak, J. C.; Schmid, E. M.; Ryan, C. J.; Ann, H. S.; Sasaki, D. Y.; Sherman, M. B.; Geissler, P. L.; Fletcher, D. A.; Hayden, C. C. *Nature Cell*

FRET measurements determine protein coverage on domain to understand dependency on tubulation

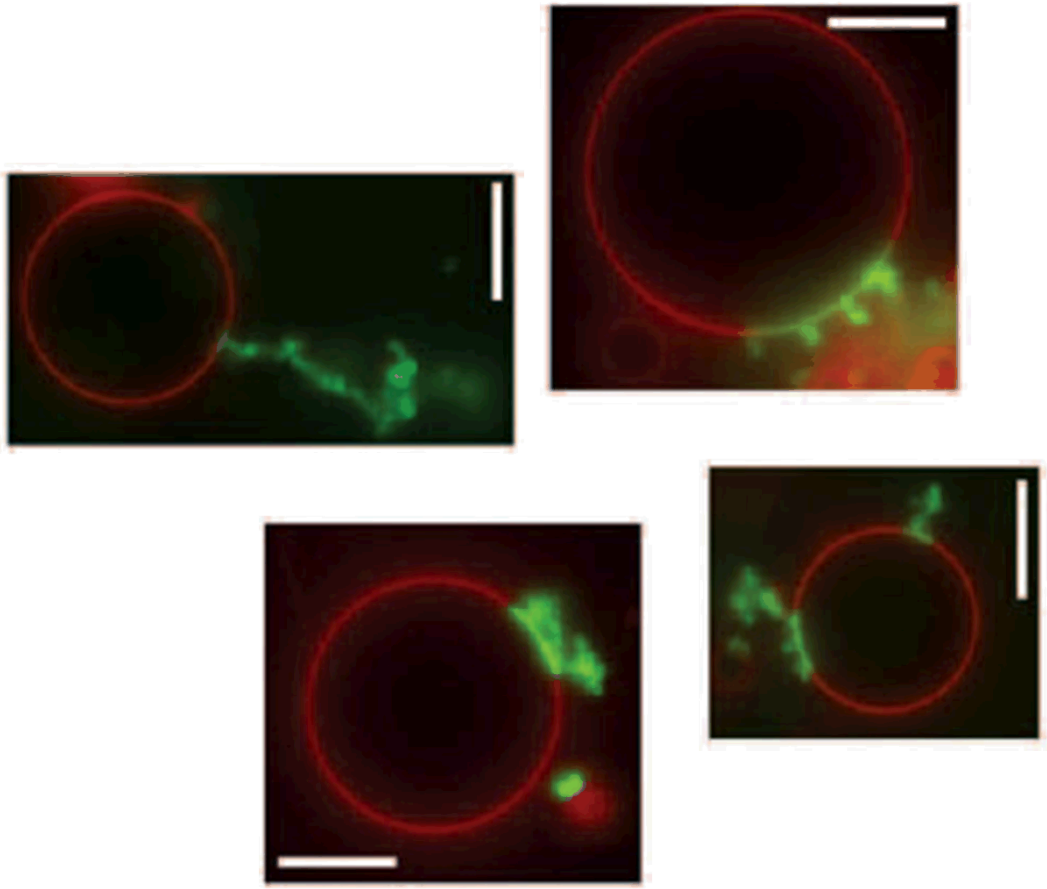


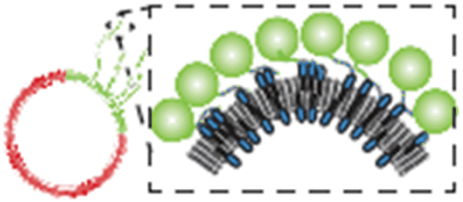


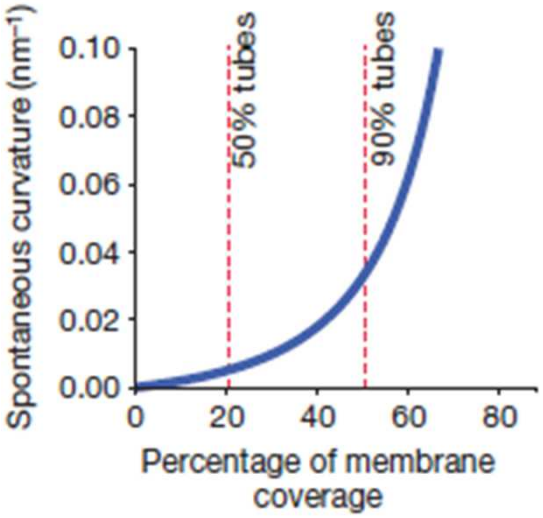


Stachowiak, J. C.; Schmid, E. M.; et al. *Nature Cell Biology* **2012**,

Low membrane coverage of proteins to membrane tubulation suggests interfacial crowding



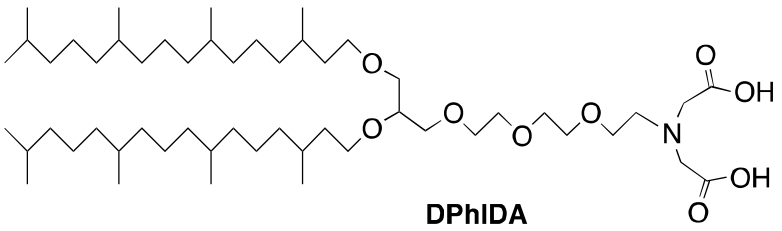




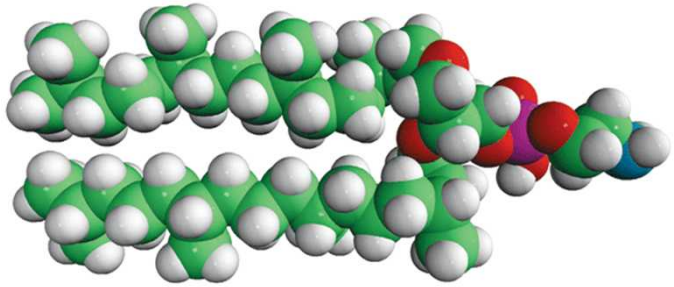
Low bending rigidity of L_d domain allows multiple tubules to form

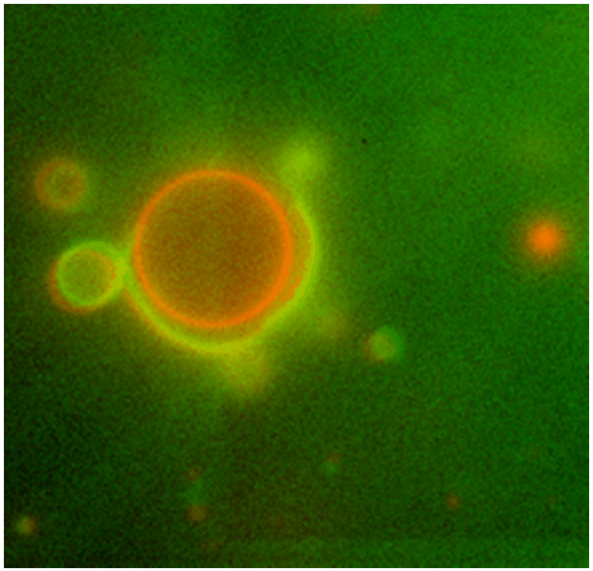
Stachowiak, J. C.; Schmid, E. M.; et al. *Nature Cell Biology* **2012**,

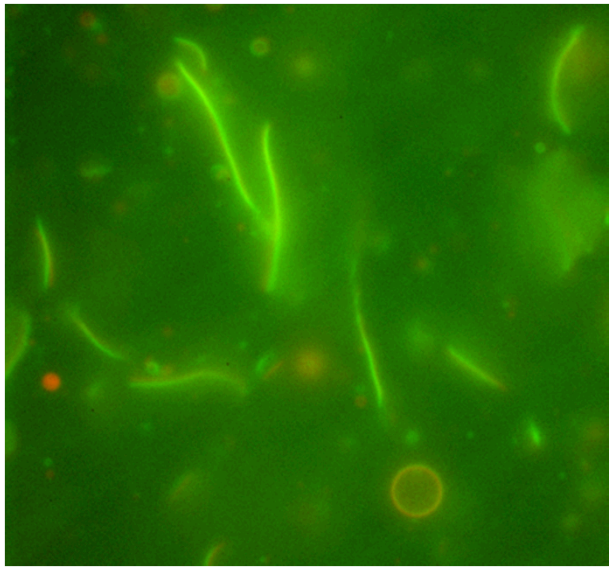
Conical shape of diphytanyl lipid with IDA headgroup to impart negative curvature



DPhIDA

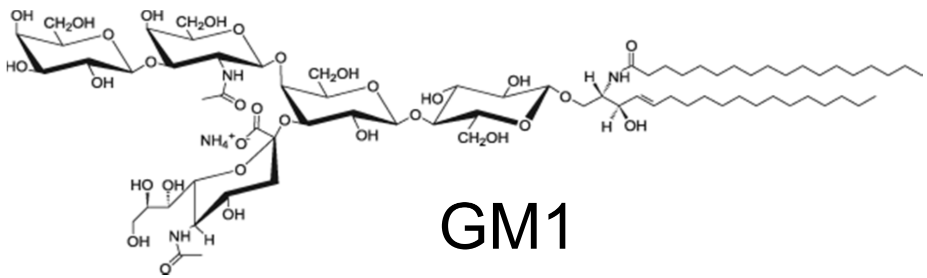




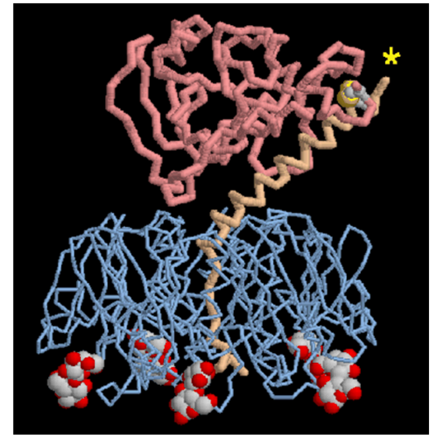


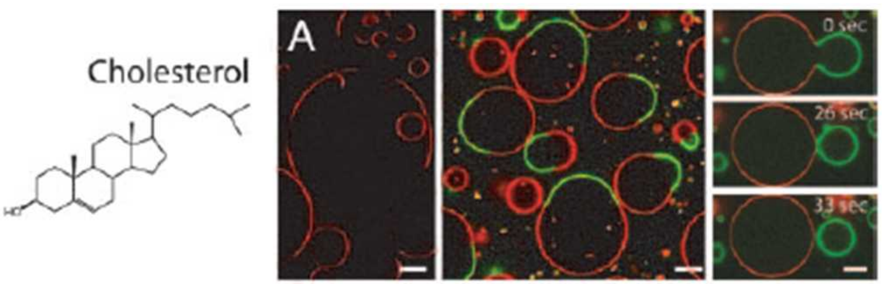
- In the presence of his-GFP, GUVs of 10% DPhIDA/POPC appear structurally frustrated with a series of vesicle opening and closing events
- Resultant stable structures are flat membrane sheets adhered on edge to substrate

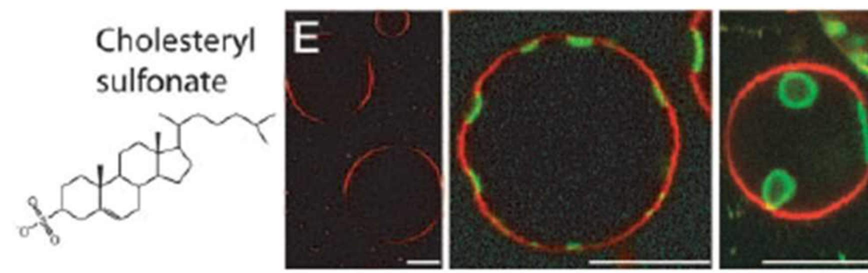
Membrane curvature induced by binding of cholera toxin to GM1 loaded vesicles



GM1







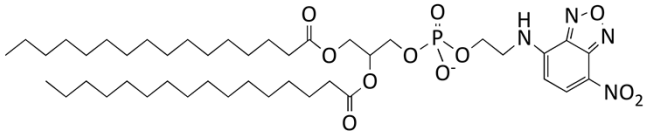
Bacia, K.; Schwille, P.; Kurzchalia, T. *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 3272

- Curvature induced in liquid ordered domains of DOPC/sphingomyelin/cholesterol GUVs upon binding of CTxB dependent
- However, curvature sign was dependent upon sterol structure and chemistry

Headgroup

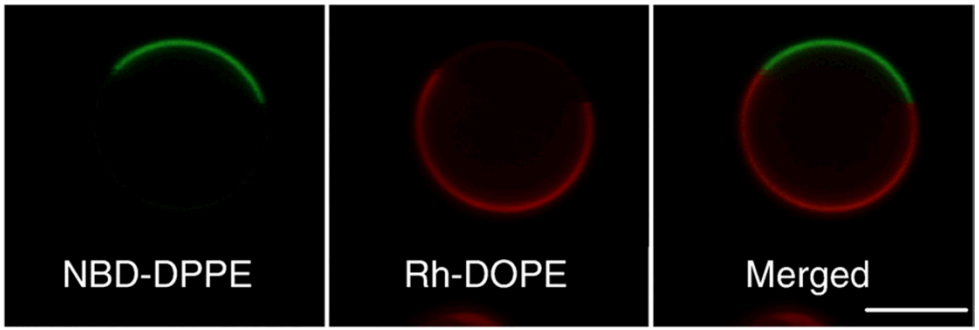
Lipid tail structure is important but headgroup also plays a decisive role in phase partitioning

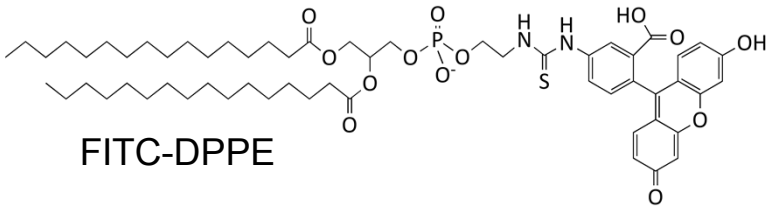
Phase selectivity



NBD-DPPE

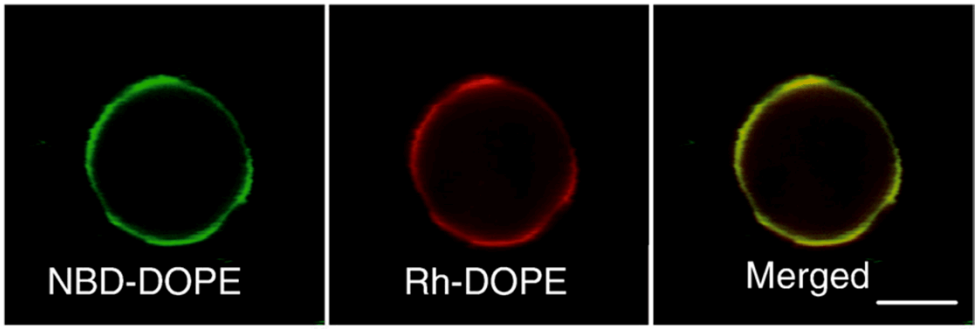
L_o

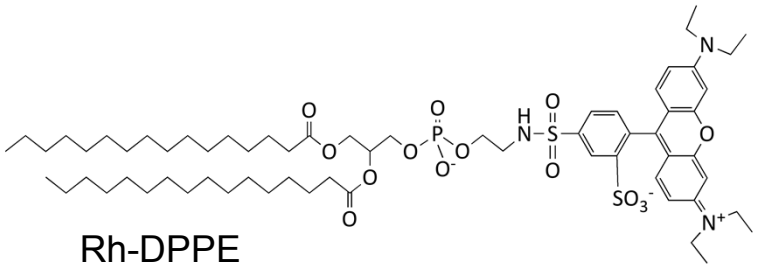




FITC-DPPE

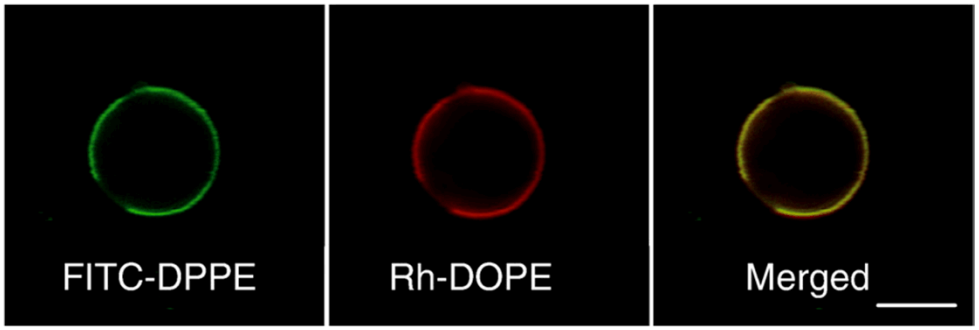
L_d





Rh-DPPE

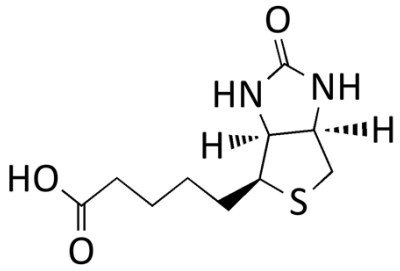
L_d



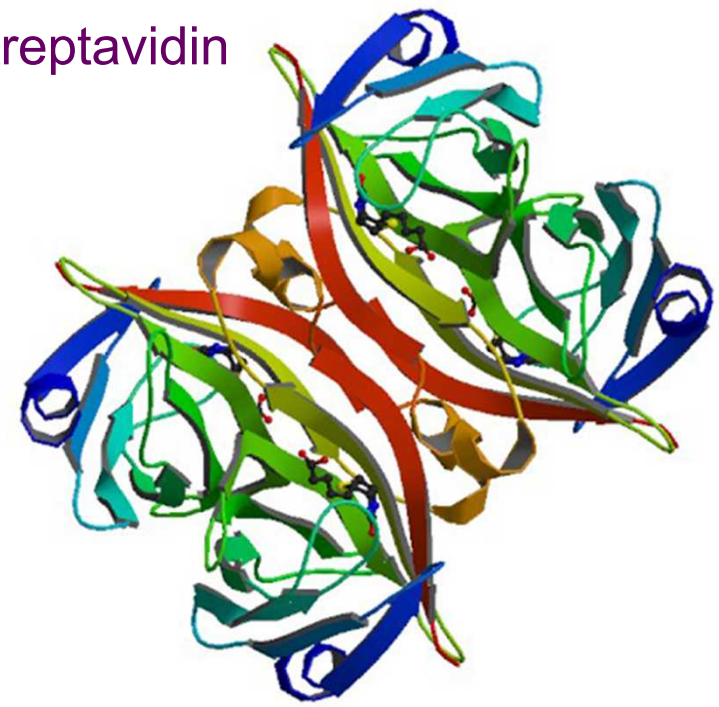
Sengupta, P.; Hammond, A.; Holowka, D.; Baird, B. *Biochim. Biophys. Acta* **2008**, *1778*, 20.

Biotin-streptavidin interaction - labeling and functionalization of membranes

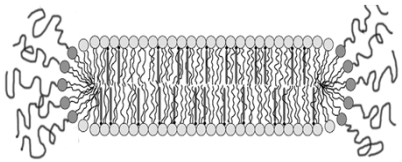
Biotin



Streptavidin

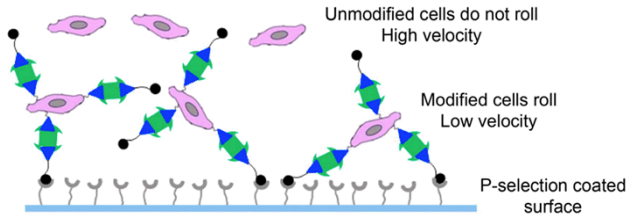


Biosensors



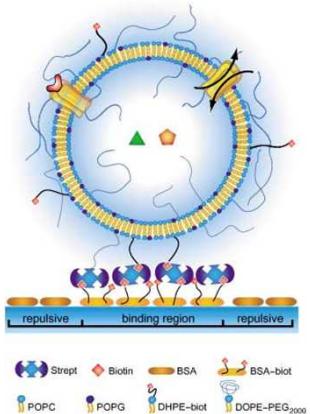
Lundquist, A.; Hansen, S. B.; Nordström, H.; Danielson, U. H.; Edwards, K. *Anal. Biochem.* **2010**, *405*, 153.

Cell surface engineering



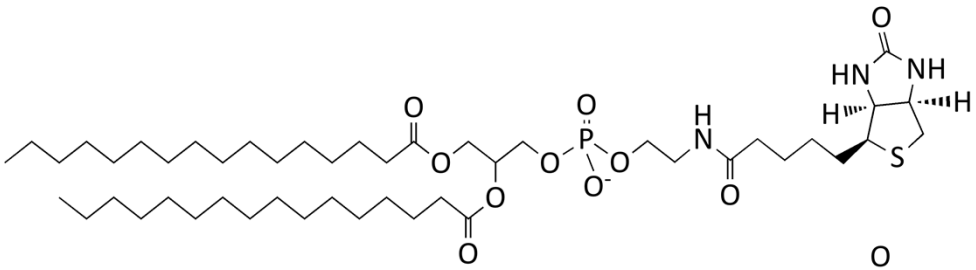
Sarkar, D.; Vemula, P. K.; Zhao, W.; Gupta, A.; Karnik, R.; Karp, J. M. *Biomaterials* **2010**, *31*, 5266

Surface patterning of nanoscale reaction vessels

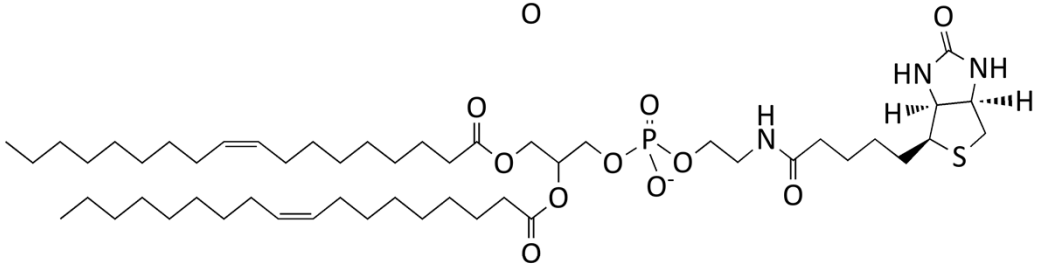


Stamou, D.; Duschl, C.; Delamarche, E.; Vogel, H. *Angew. Chem. Int. Ed.* **2003**, *42*, 5580.

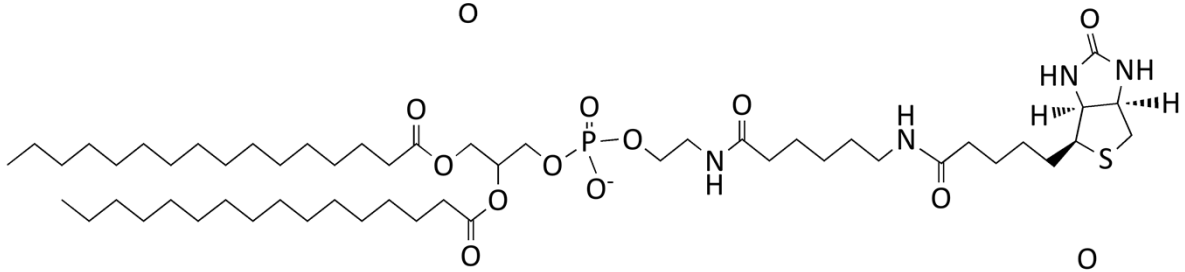
Biotinylated lipids



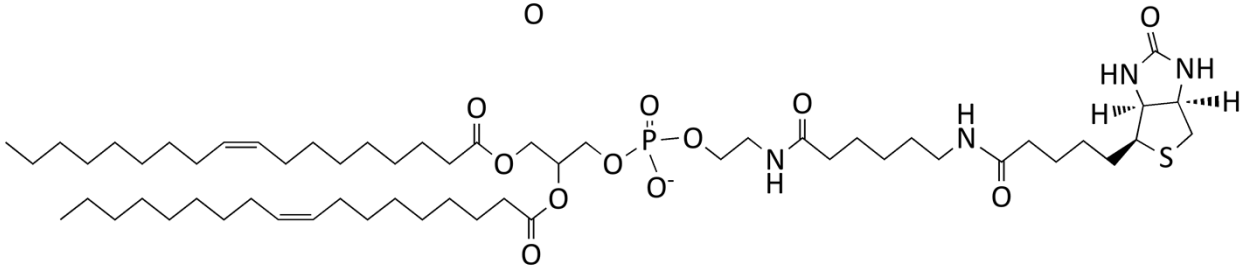
DPPE-biotin



DOPE-biotin



DPPE-cap-biot

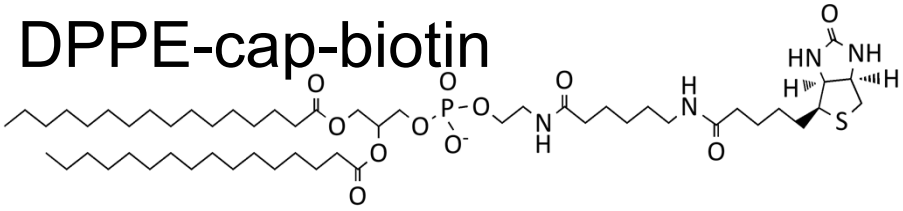


DOPE-cap-bio

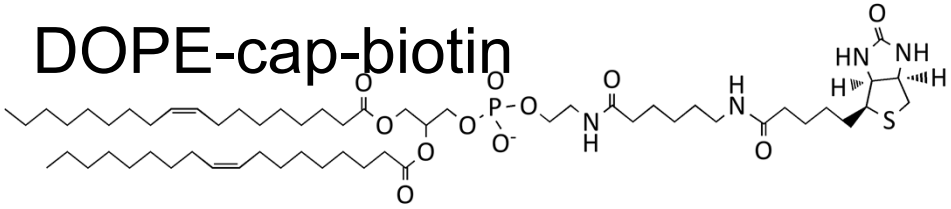
Avanti polar lipids

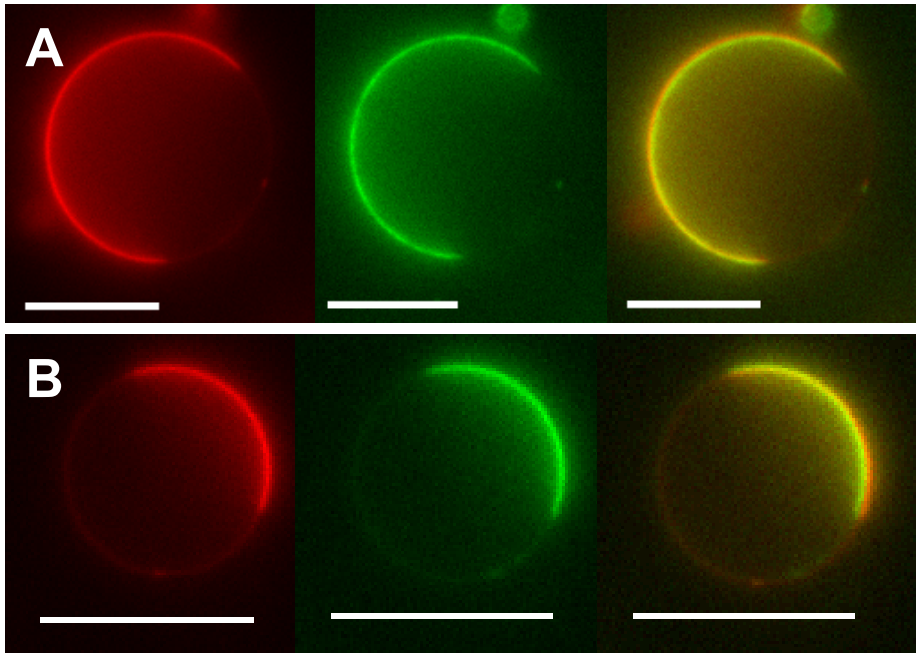
DPPE-cap-biotin partitions selectively to L_d phase in GUV solution phase studies

DPPE-cap-biotin



DOPE-cap-biotin



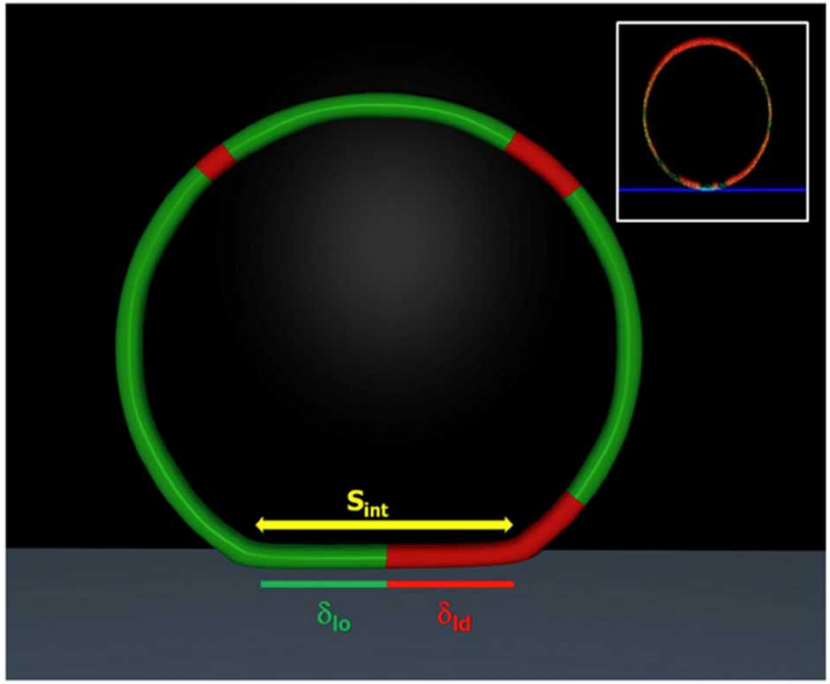


FITC-labeled streptavidin binds to L_d phase of membrane – suggests preferential partitioning of biotin lipid to L_d phase

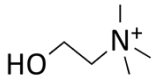
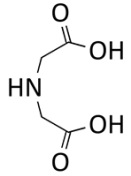
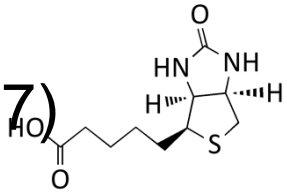
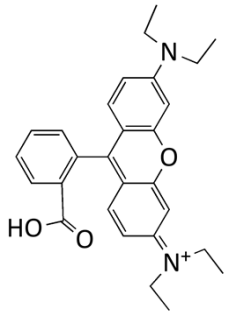
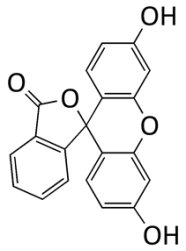
Membrane phase partitioning of biotinylated lipids in GUV-surface binding study (Sarmento, et al.)

	K_p (L_d/L_o)
DOPE-Cap-biotin	7.4
DPPE-Cap-biotin	3.8

- *L_d vs. L_o phase affinity measured on streptavidin-coated surface*
- *Cap-biotin lipids partition strongly to L_d phase regardless of tail structure*



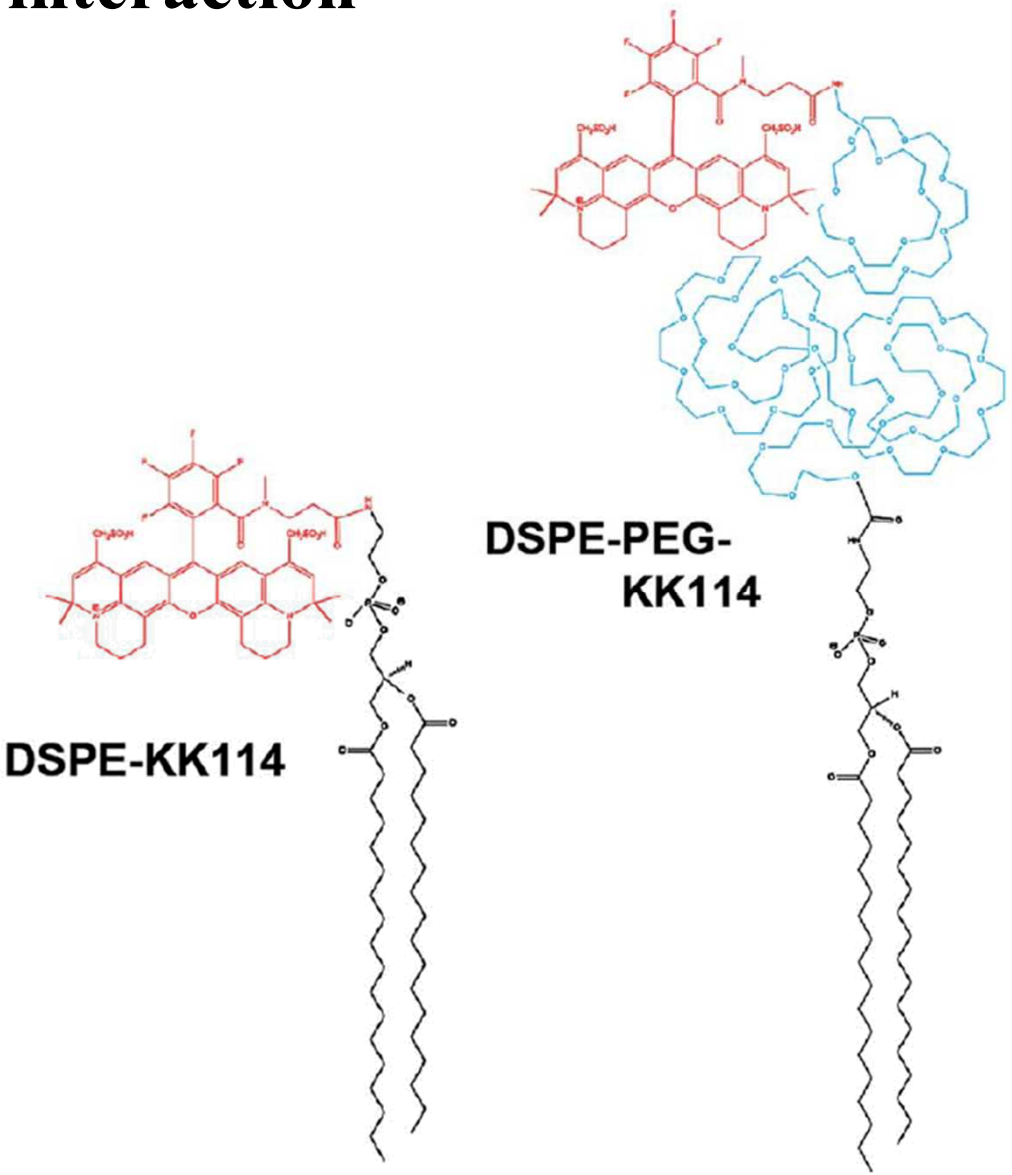
Sarmento, M. J.; Prieto, M.; Fernandes, F. *Biochim. Biophys. Acta* **2012**, *1818*, 2605.

		Log p	Octanol/water
Choline		-5.16	1:6,920,000
Iminodiacetic acid		-3.27	1:54,000
Biotin (vitamin B7)		0.39	2.5:1
Rhodamine		1.95	89:1
Fluorescein		3.35	2200:1

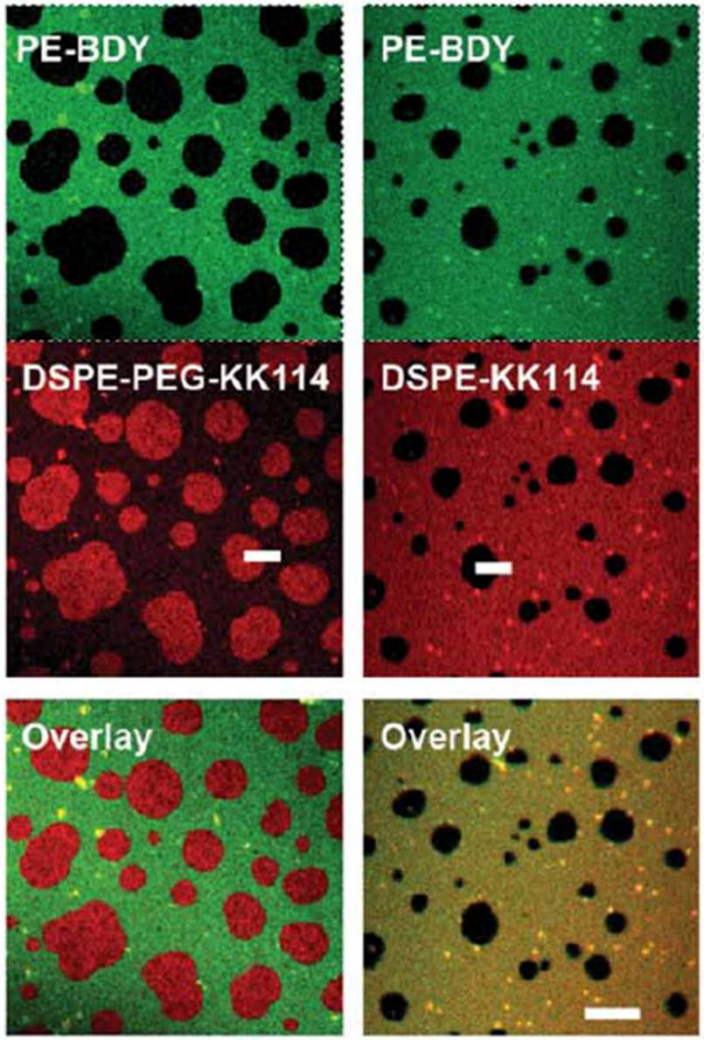
http://www.toxnet.nlm.nih.gov/index.html

Spacer

PEG spacer decouples headgroup-membrane interaction

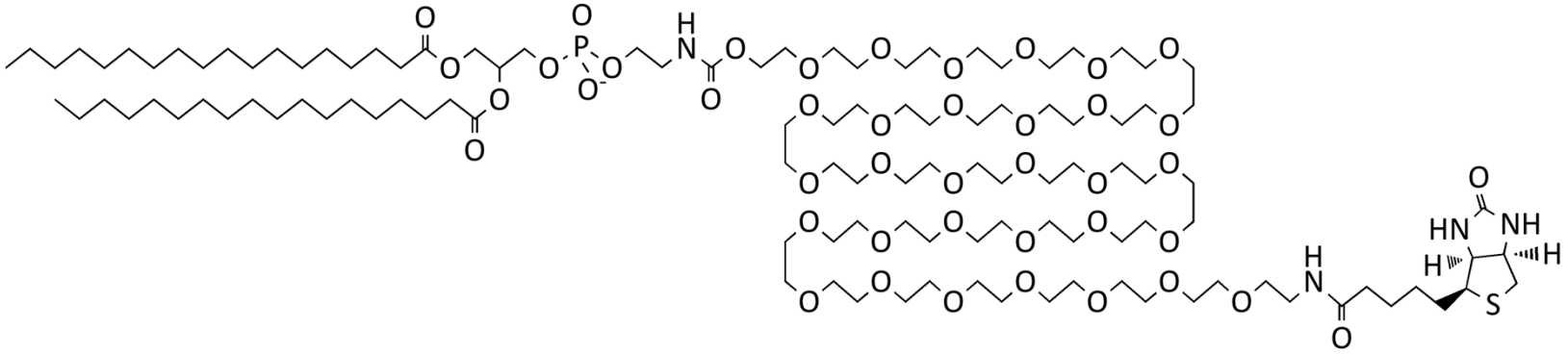


DPPC/cholesterol/DPhP



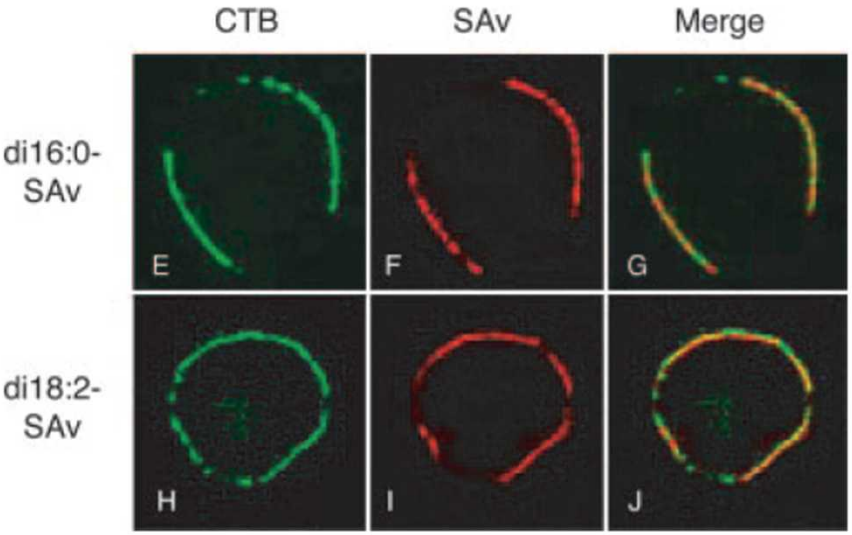
Honigmann, A.; Mueller, V.; Hell, S. W.; Eggeling, C. *Farad. Discuss.* **2012**, *161*, 77.

PEG spacer improves phase selectivity for lipid rafts of biotinylated lipid

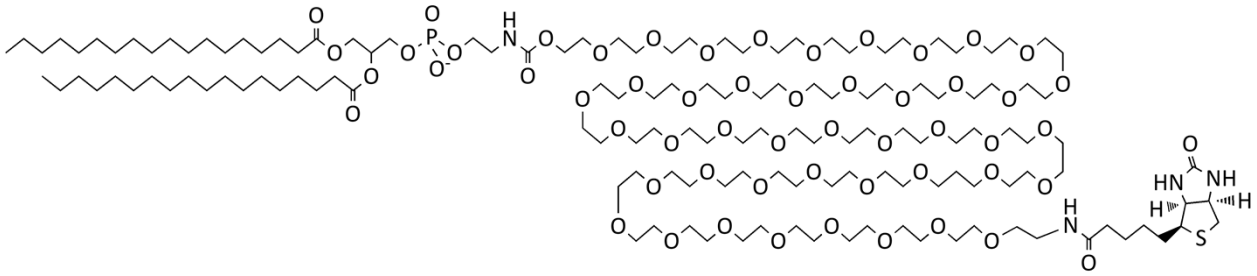


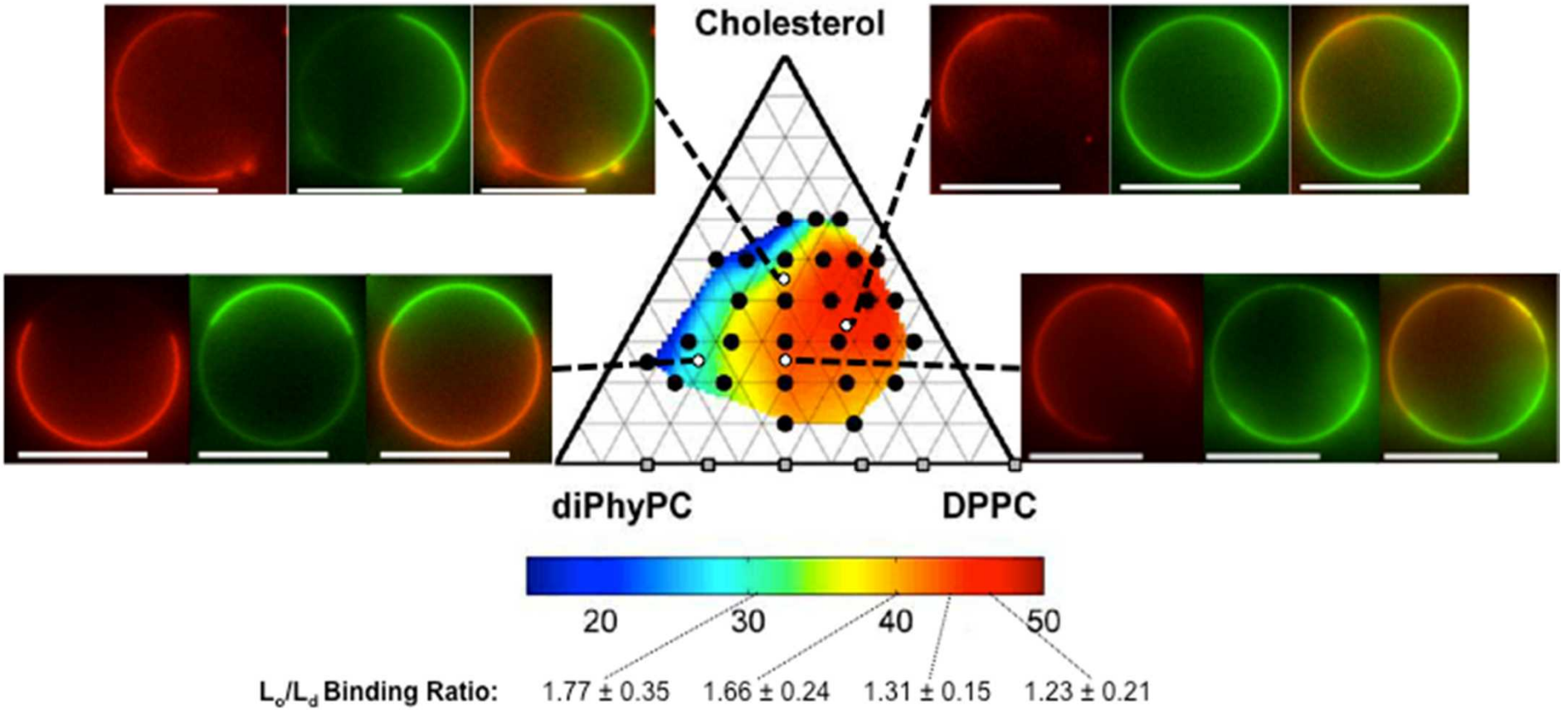
Wang, T.-Y.; Leventis, R.; Silvius, J. R. *J. Biol. Chem.* **2005**, *280*, 22839.

Biotinyated DPPE with PEG spacer (MW 1450) found to reside within detergent resistant membranes (DRMs) in mammalian cells



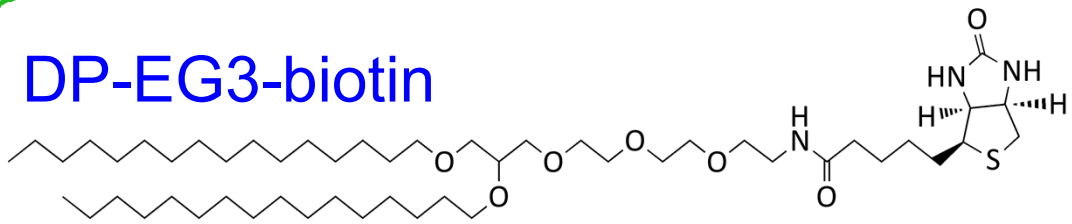
Phase selectivity of DPPE-PEG(2000)-biotin



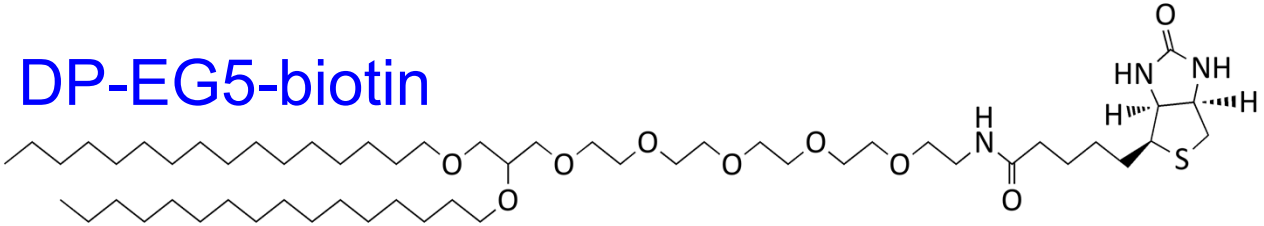


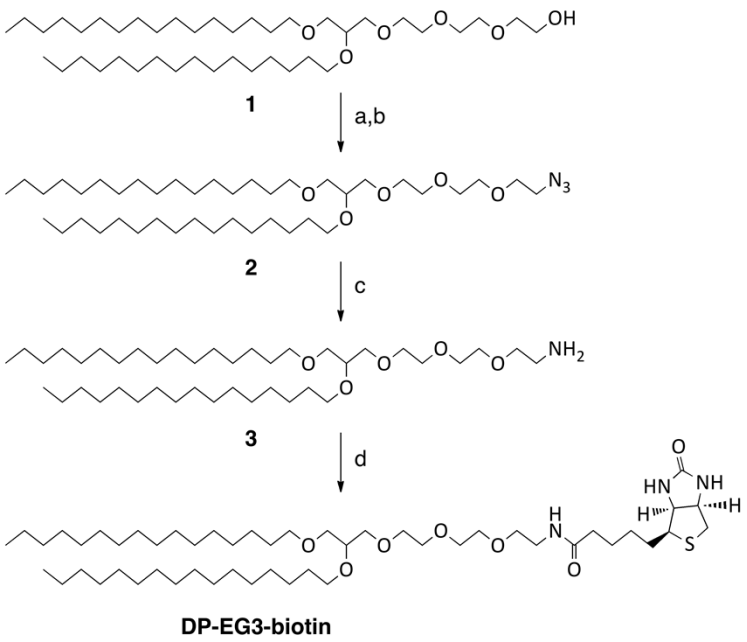
Biotinylated lipids with oligoethylene glycol spacer – spacer length and chemistry

DP-EG3-biotin



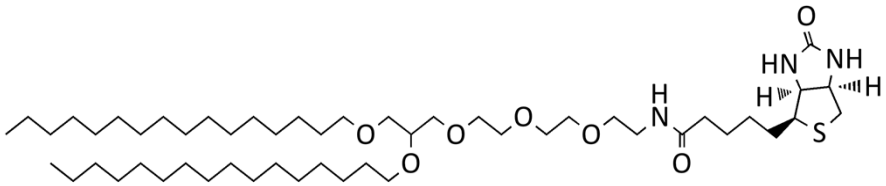
DP-EG5-biotin

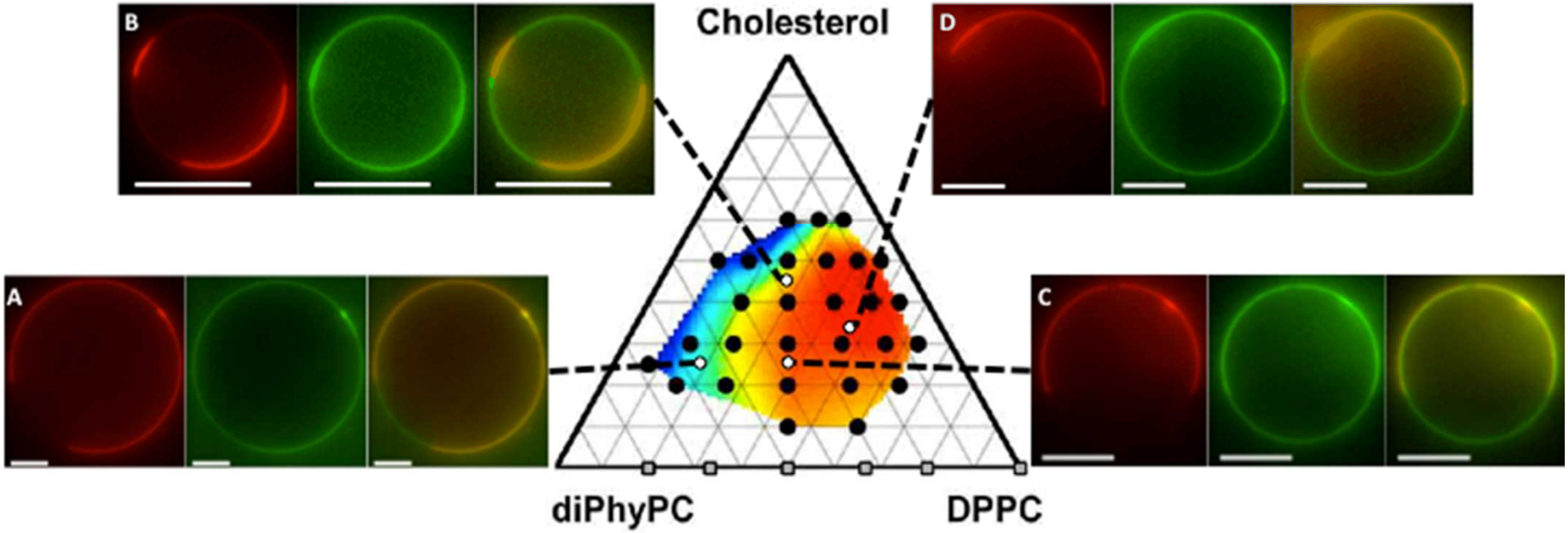




a) MsCl, NEt_3, THF, 0 ° C, 1h
b) NaN_3, DMF, r.t., overnight
c) LAH, THF, reflux, 15 min.
d) Biotin-NHS, DMF/THF, r.t., overnight

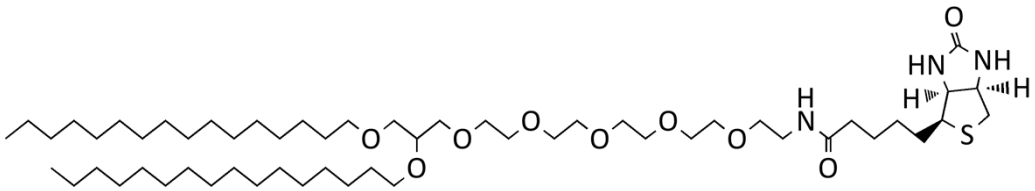
Phase selectivity of DP-EG3-biotin

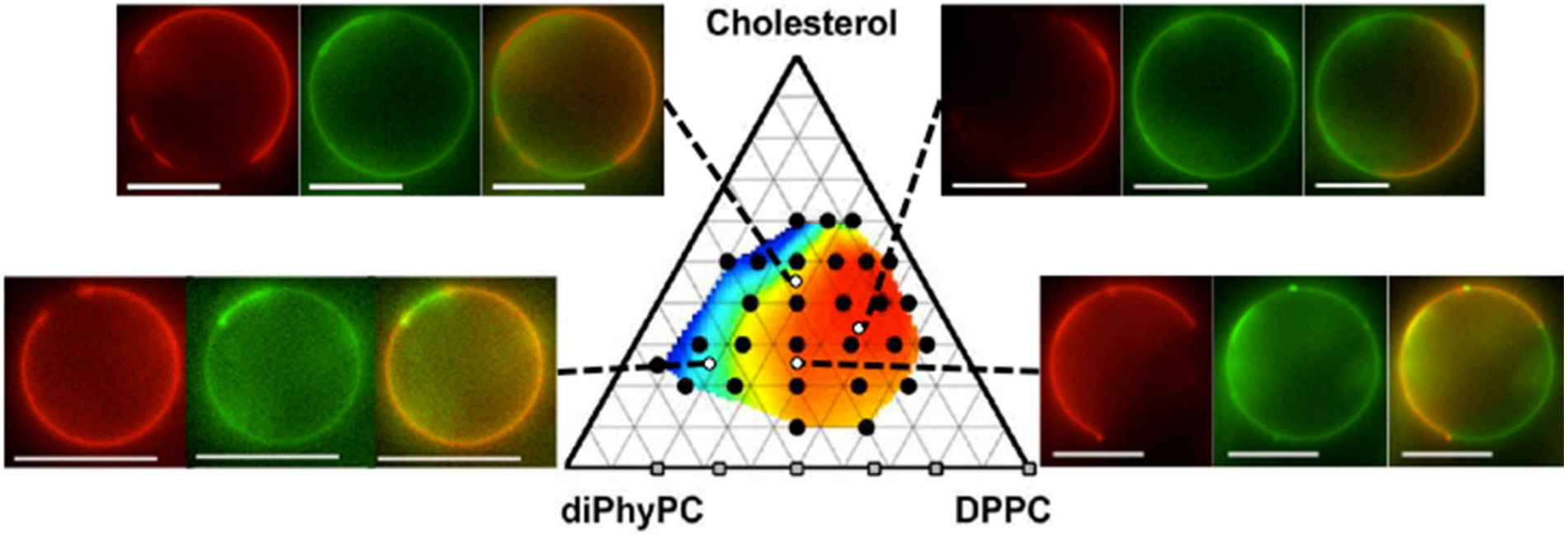




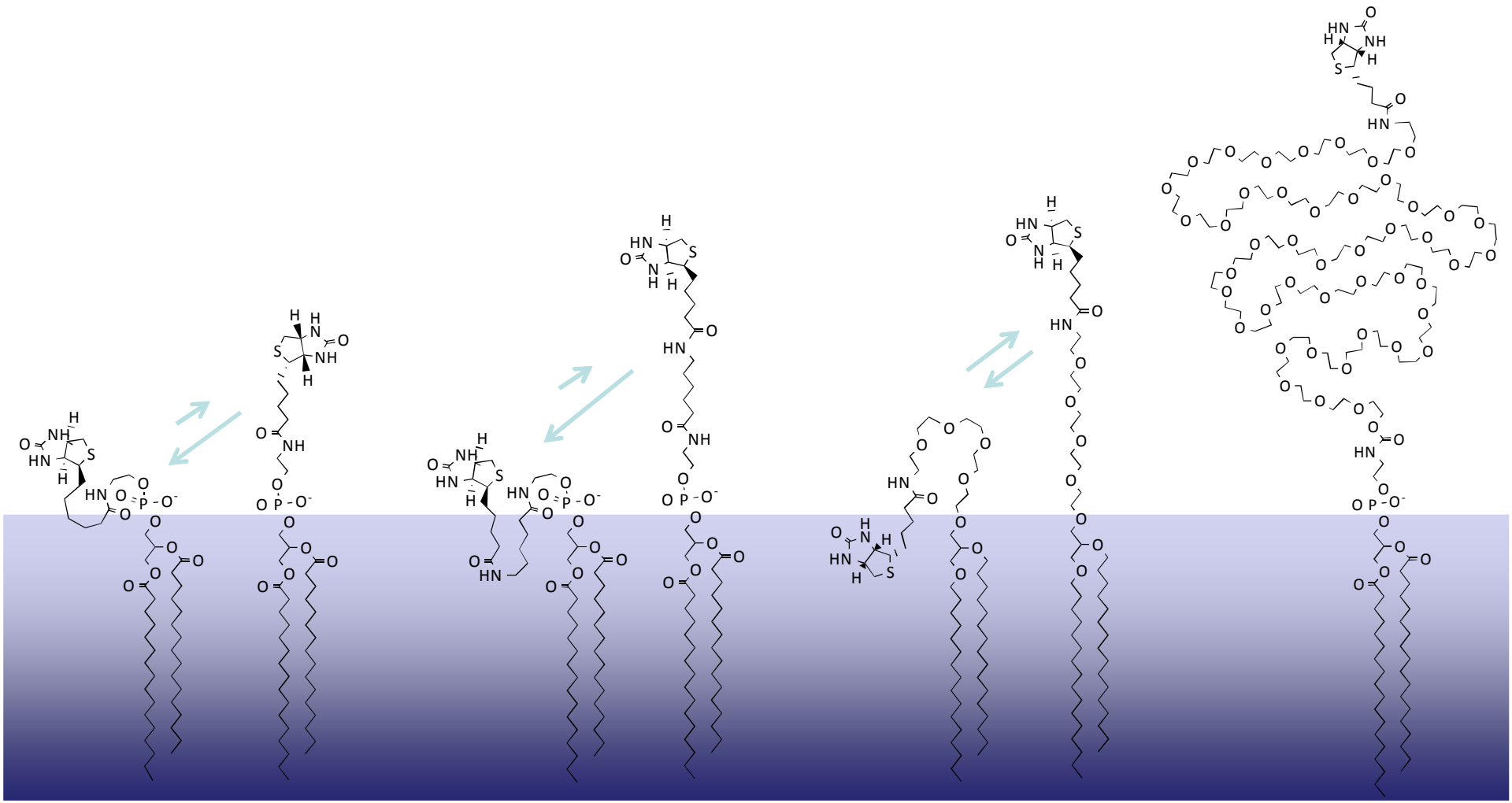
L_o/L_d Binding Ratio: 0.92 ± 0.21 0.97 ± 0.16 1.01 ± 0.12 0.93 ± 0.09

Phase selectivity of DP-EG5-biotin





Headgroup and spacer disrupts lipid packing interactions: biotin and cap spacer membrane insertion



Summary

- Spatial confinement enables control over the structure and mechanics of membrane assemblies
- Tubulation/budding results from steric pressure of bound proteins as well as lipid aggregation with his-tag binding
- Through tailoring of lipid structure we hope to understand role of line tension and how to control membrane asymmetry

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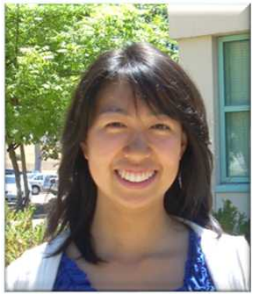


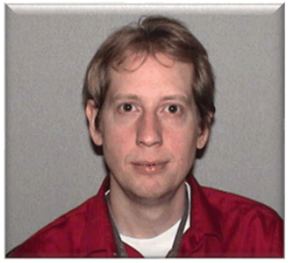

DOE Office of Basic Energy Sciences, Division of Materials Science and Engineering and Division of Chemical Science, Geoscience, and Biosciences

Sandia National Labs - LDRD Program

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