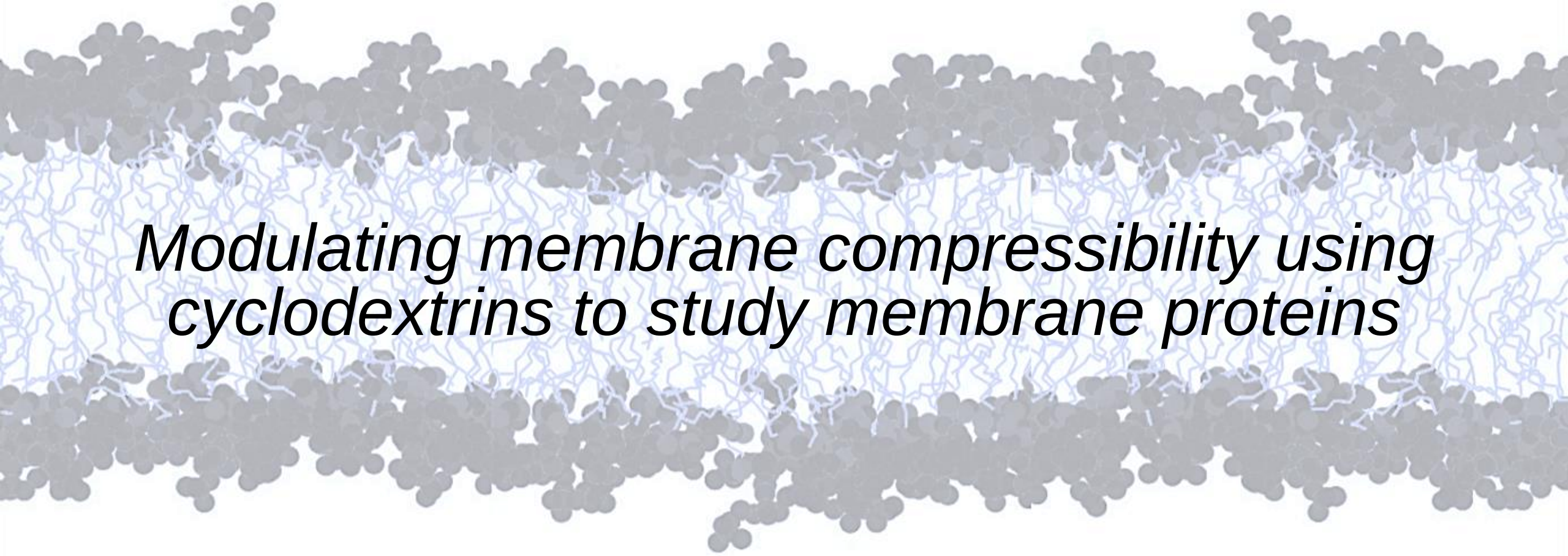
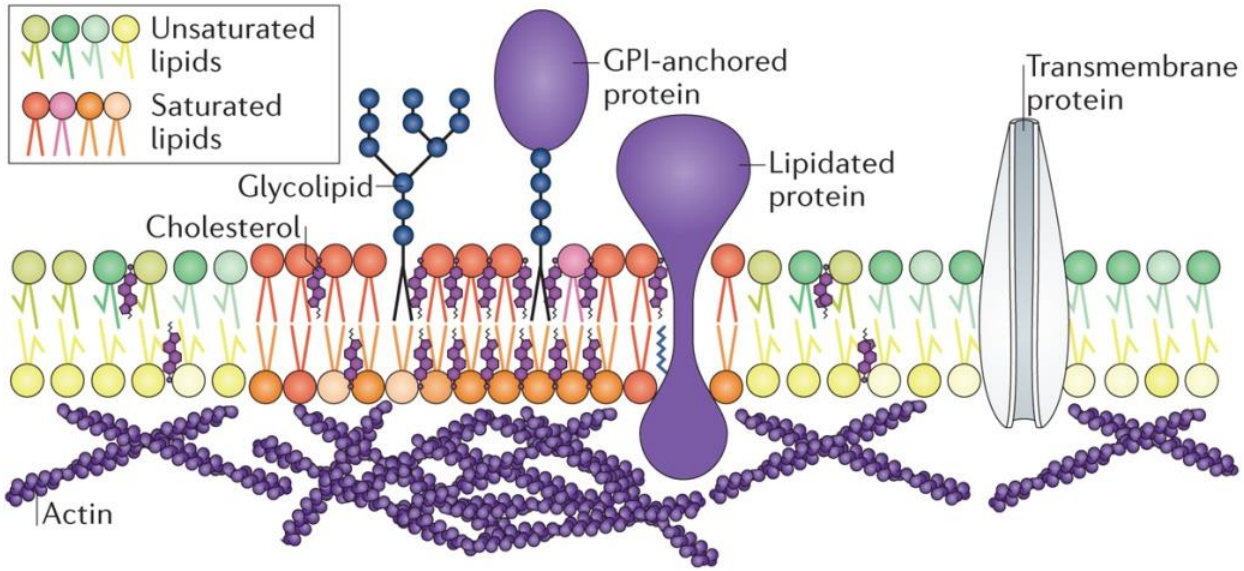


Modulating membrane compressibility using cyclodextrins to study membrane proteins

Dr. Cynthia Alsayyah –Ernst Lab

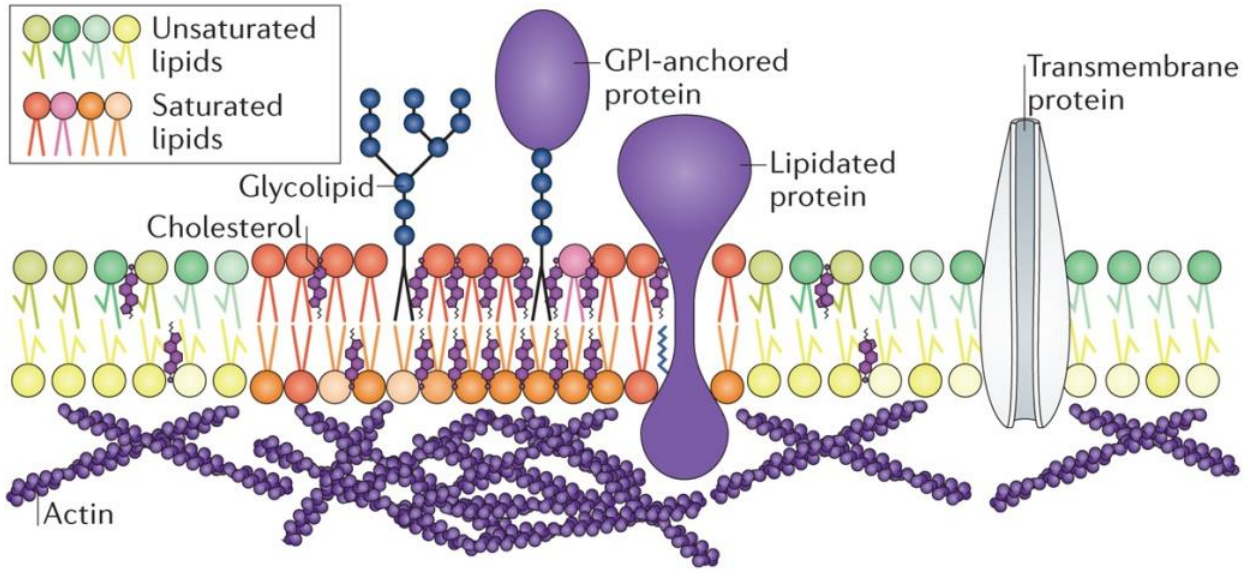
10.9.2024

Biological versus model membranes at a glance

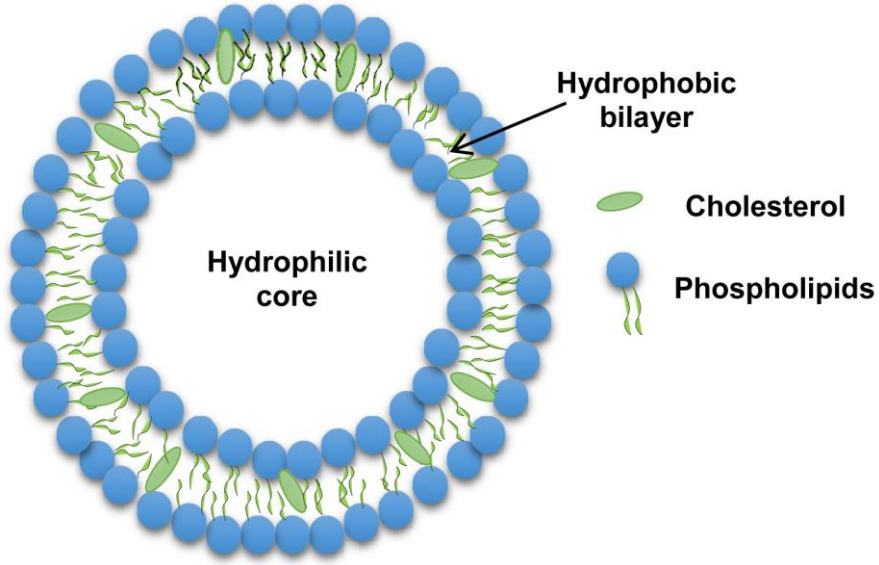


Sezgin E. et al, *Nat Rev Mol Cell Bio* (2017).

Biological versus model membranes at a glance

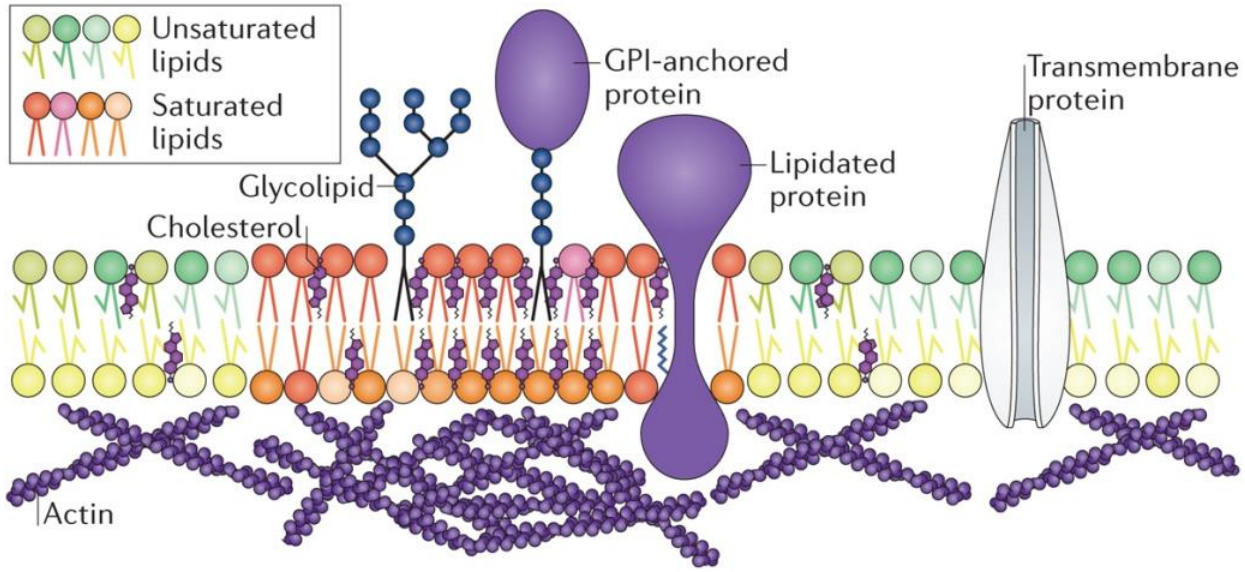


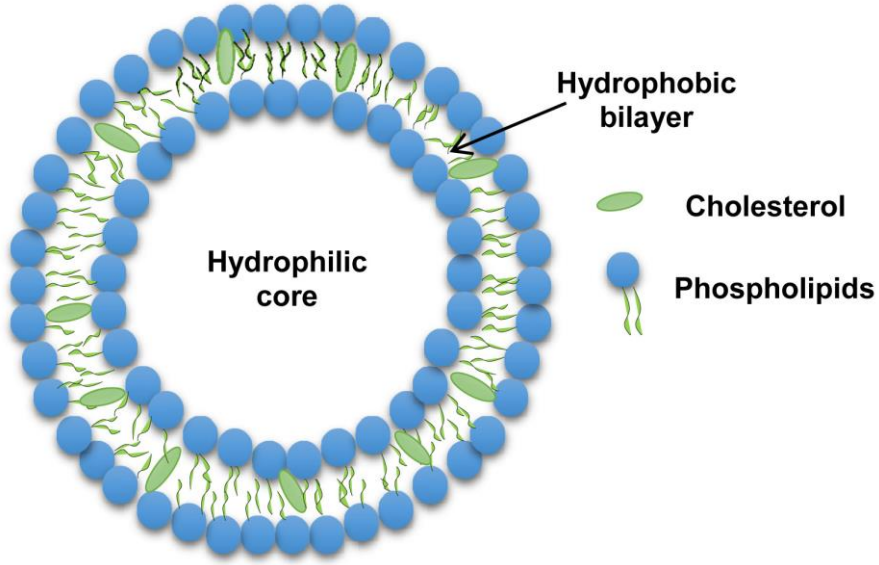
Sezgin E. et al, *Nat Rev Mol Cell Bio* (2017).



Nsairat H. et al, Heliyon (2022).

Biological versus model membranes at a glance





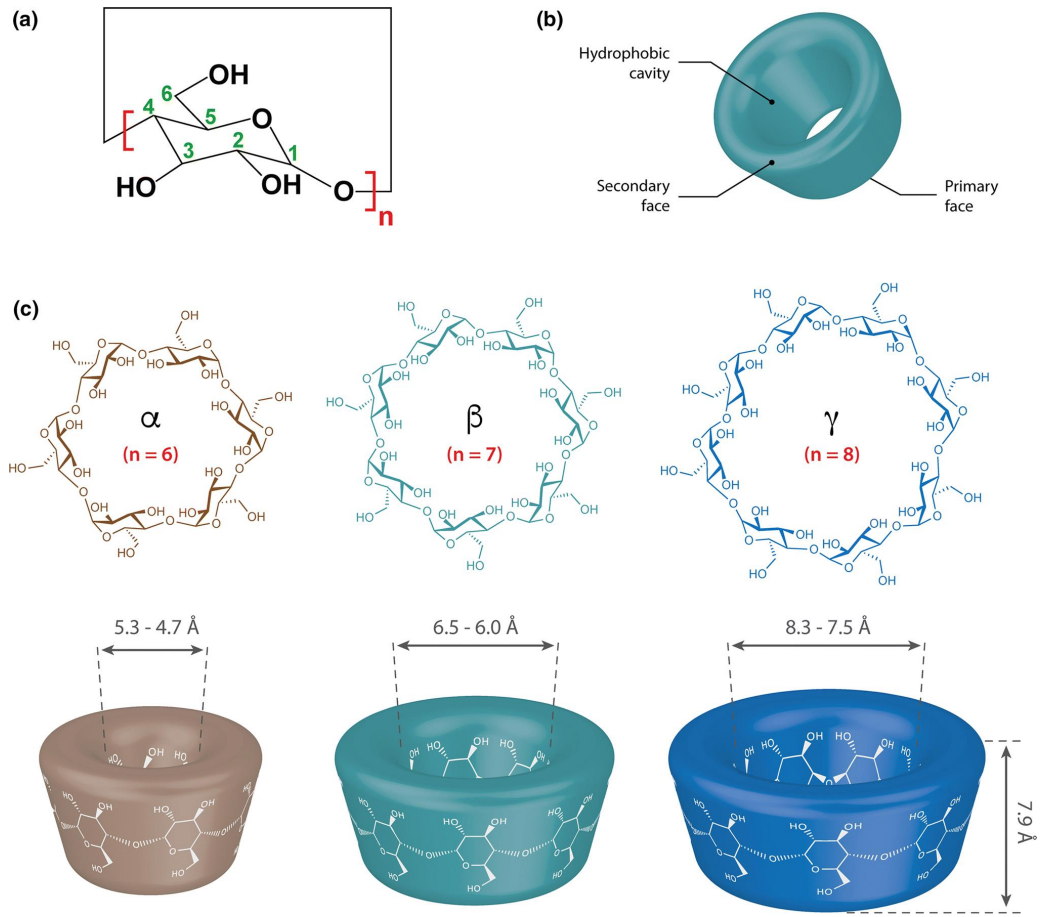
Complex and protein rich

Asymmetry

Natural phase behavior

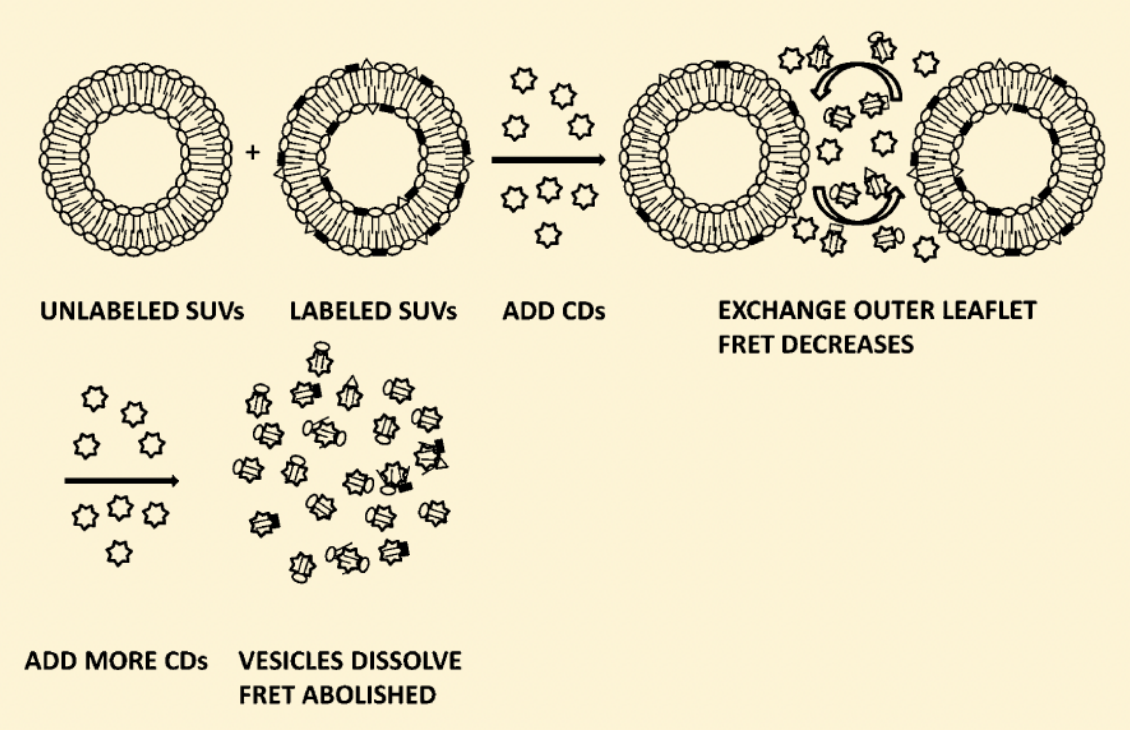
Sezgin E. et al, *Nat Rev Mol Cell Bio* (2017).

Cyclodextrins as tools to transfer lipids

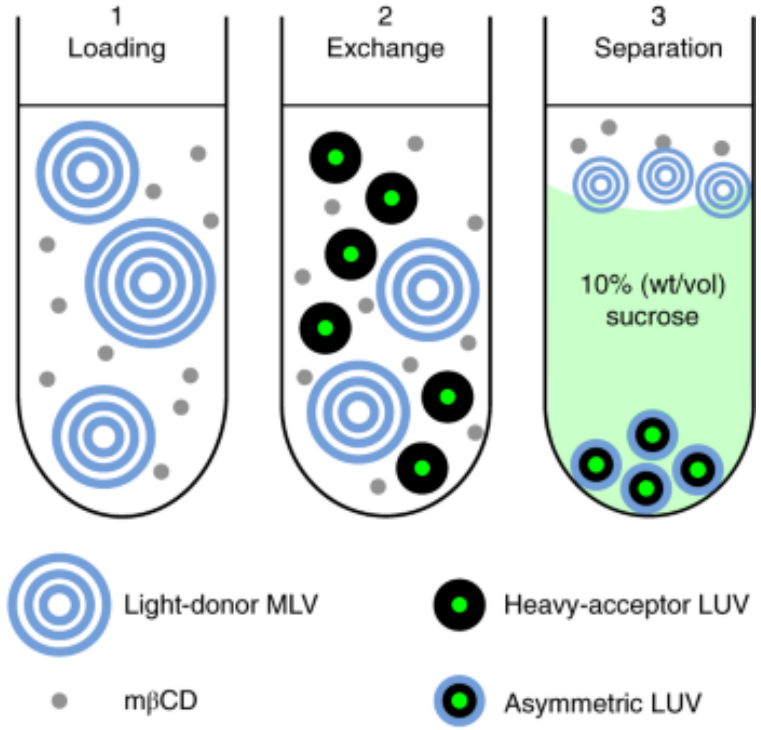


Crini G. et al. *Environ Chem Lett* (2018)

Cyclodextrins as tools to transfer lipids



Zhen Huang and Erwin London - *American Chemical Society* (2013)



Doktorova M. *et al. Nat Protocols* (2018)

Overcoming the limitations of model membranes and available protocols

Simplicity and Time

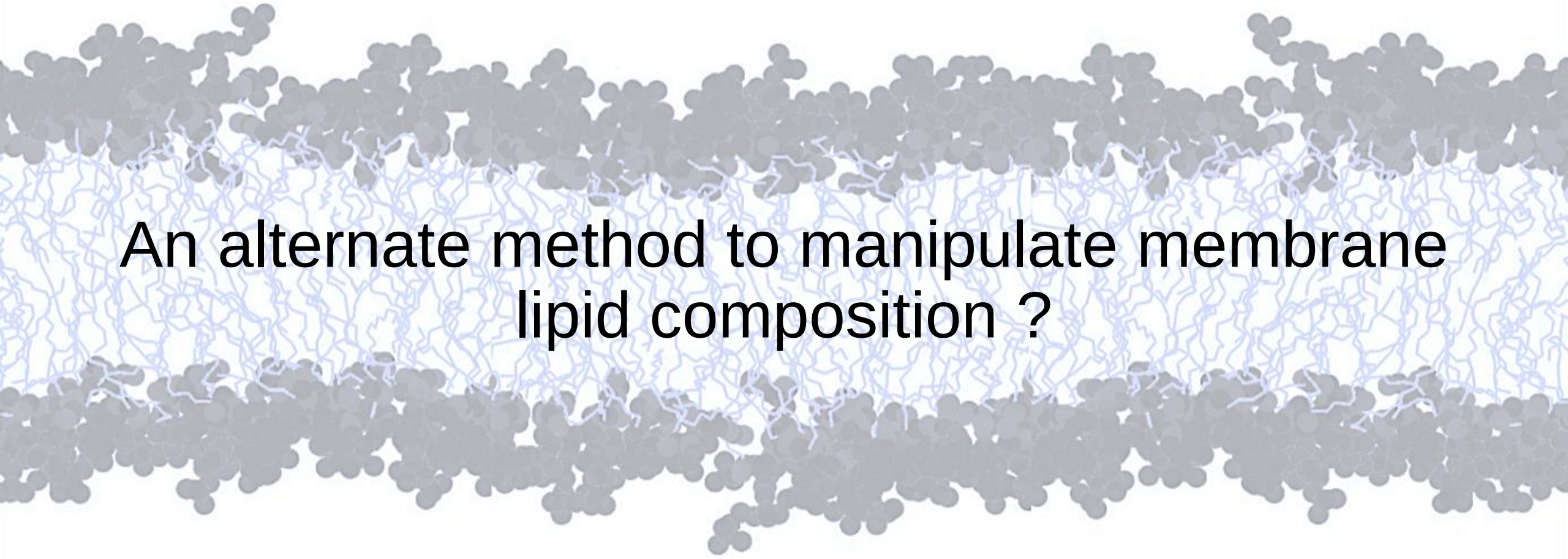
- Limited choice of lipids
- Fixed state studies

Overcoming the limitations of model membranes and available protocols

Simplicity and Time	Hydrophobic mismatch
• Limited choice of lipids • Fixed state studies	• Reconstitution can be difficult in some membrane compositions (example: low compressibility)

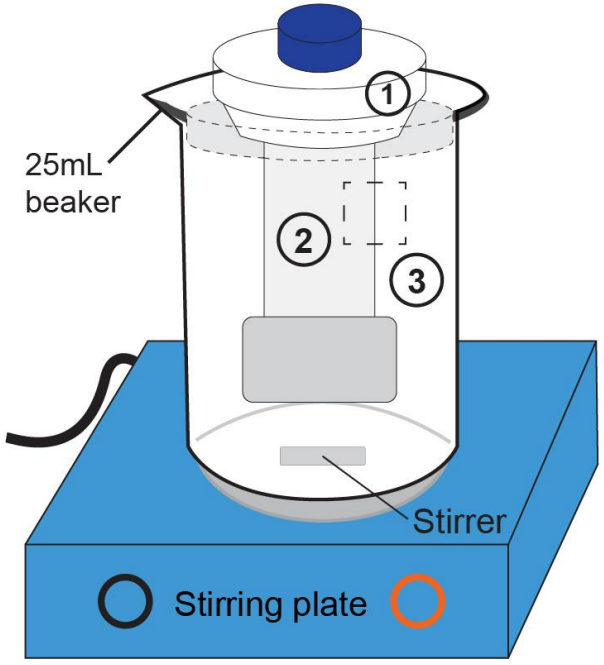
Overcoming the limitations of model membranes and available protocols

Simplicity and Time	Hydrophobic mismatch	Vesicle Mixing
• Limited choice of lipids • Fixed state studies	• Reconstitution can be difficult in some membrane compositions (example: low compressibility)	• Mixing of Acceptor and Donor vesicles requires subsequent separation



An alternate method to manipulate membrane lipid composition ?

An alternate method to manipulate membrane lipid composition ?



1 100kDa dialysis cassette

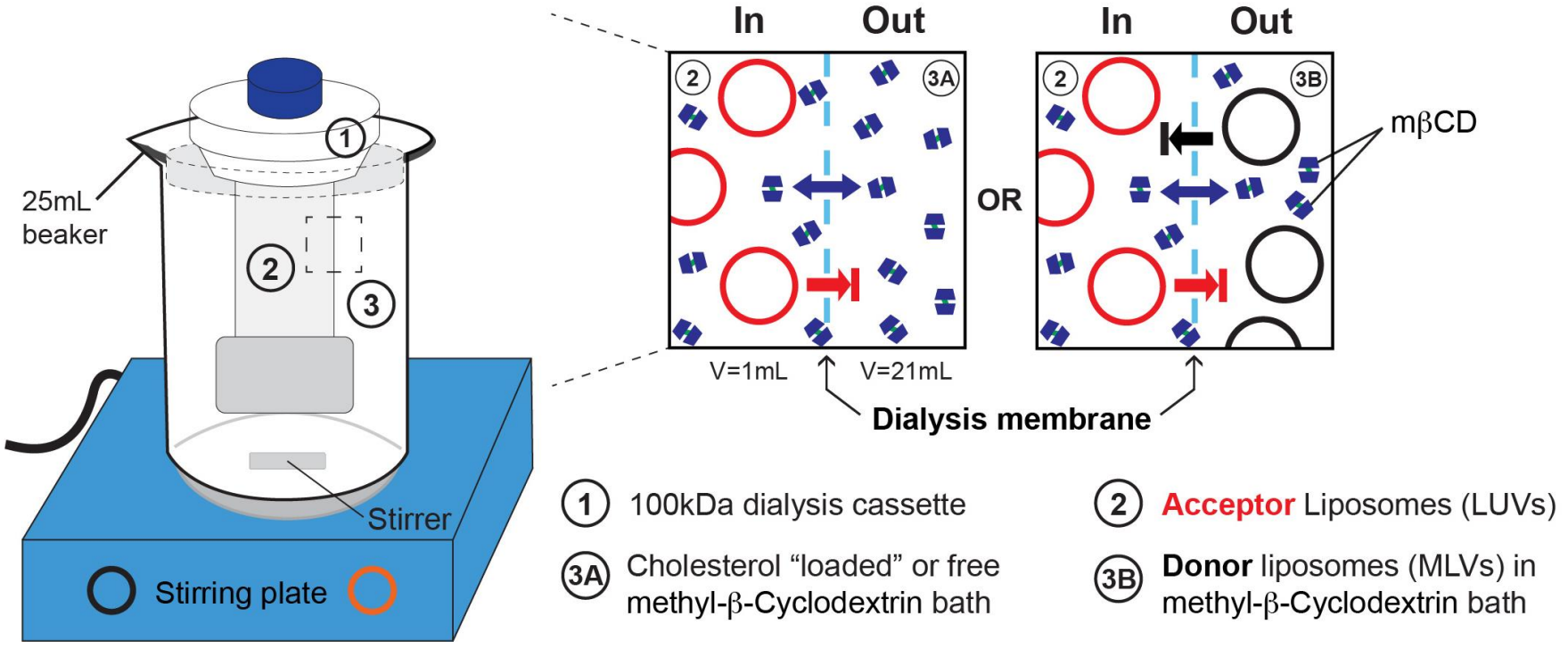
2 **Acceptor** Liposomes (LUVs)

3A Cholesterol “loaded” or free methyl-β-Cyclodextrin bath

3B **Donor** liposomes (MLVs) in methyl-β-Cyclodextrin bath

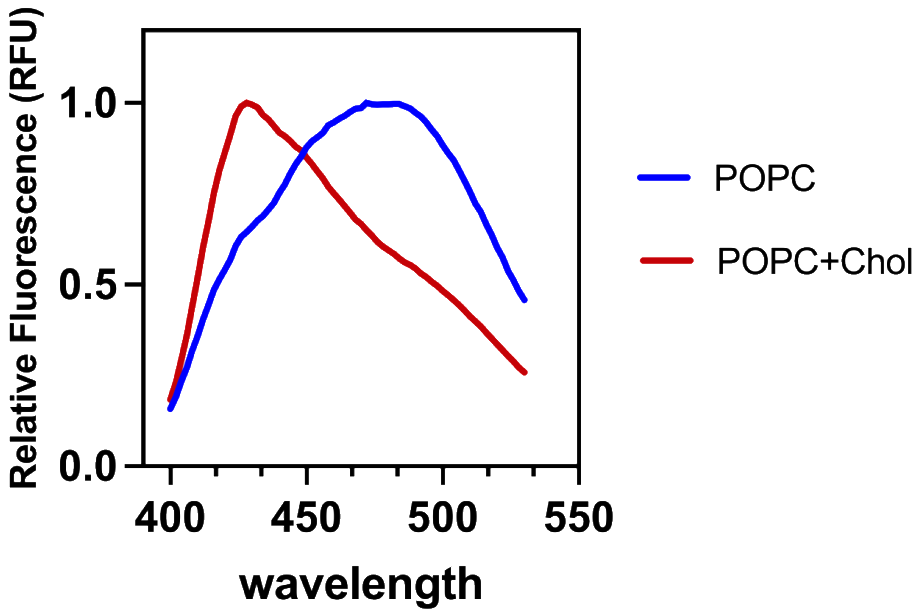
Graphical scheme of the exchange experimental setup

An alternate method to manipulate membrane lipid composition ?



Graphical scheme of the exchange experimental setup

C-Laurdan: a sensitive dye used to report on membrane packing properties

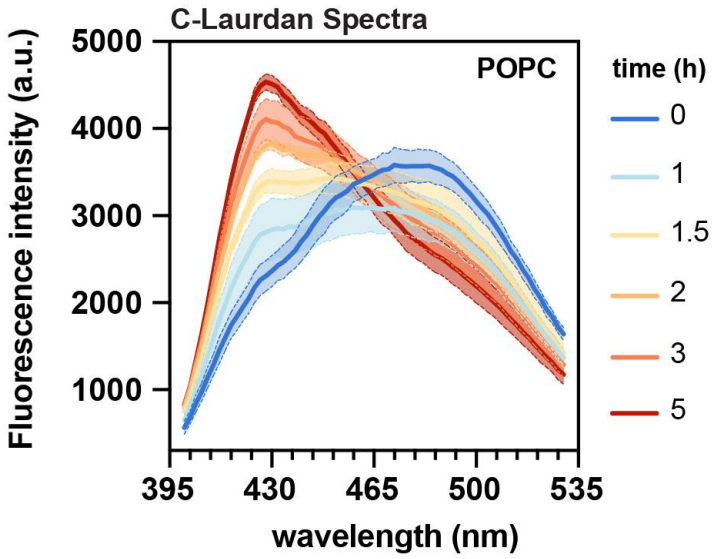


Low GP = loose packing

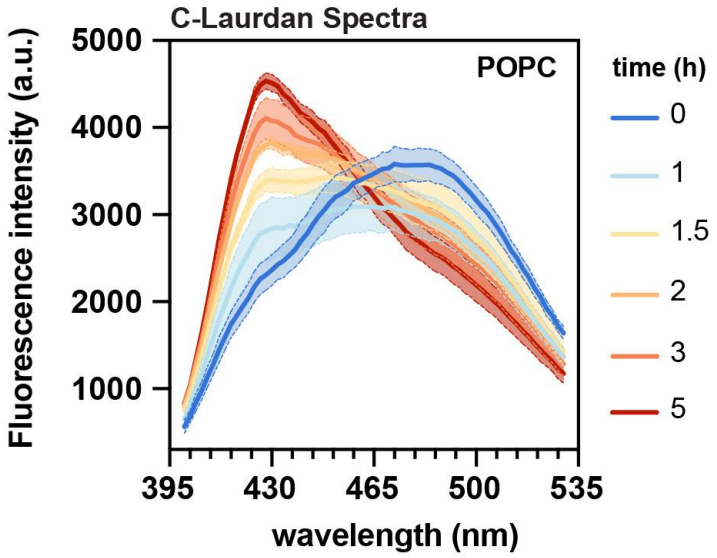
High GP = tight packing

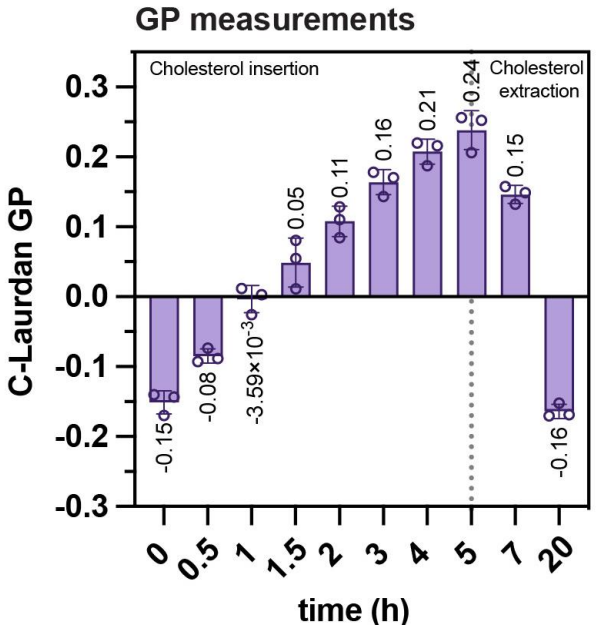
Y. Ko S. et al. *ChemBioChem* (2007)

Insertion and extraction of cholesterol to/from membranes using methyl-β-cyclodextrin

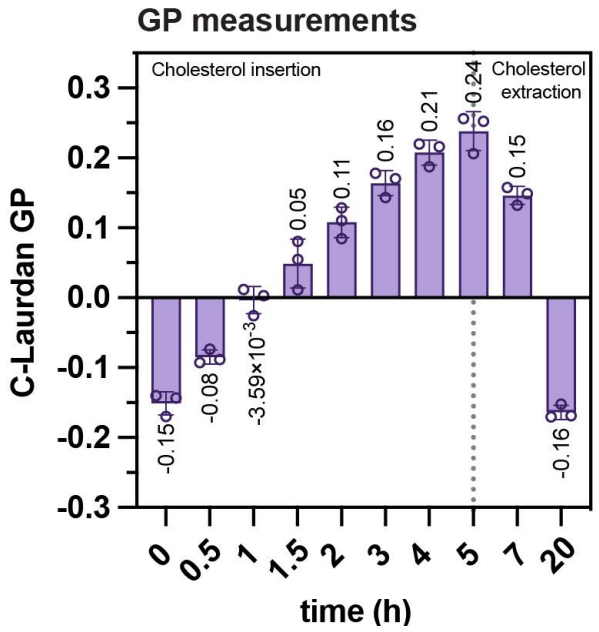


Insertion and extraction of cholesterol to/from membranes using methyl-β-cyclodextrin

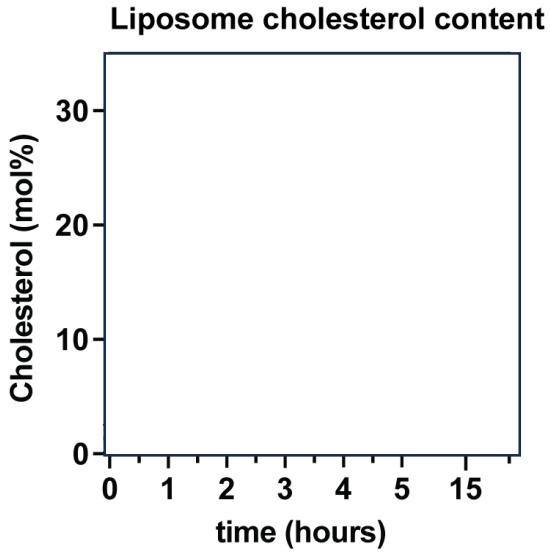




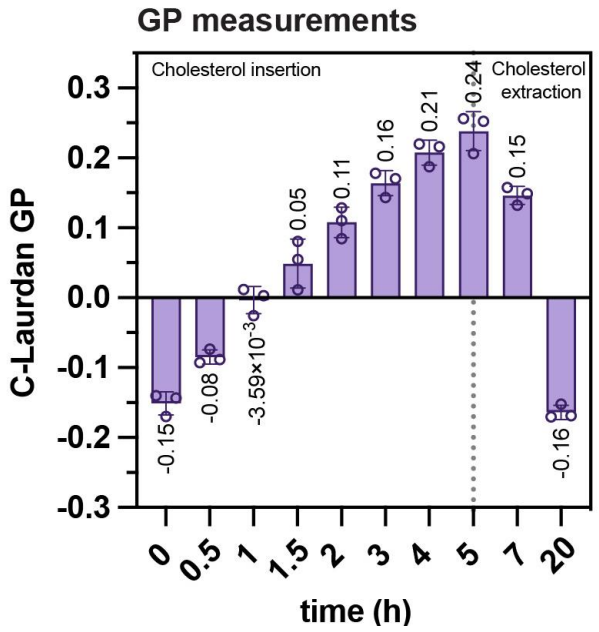
Insertion and extraction of cholesterol to/from membranes using methyl-β-cyclodextrin



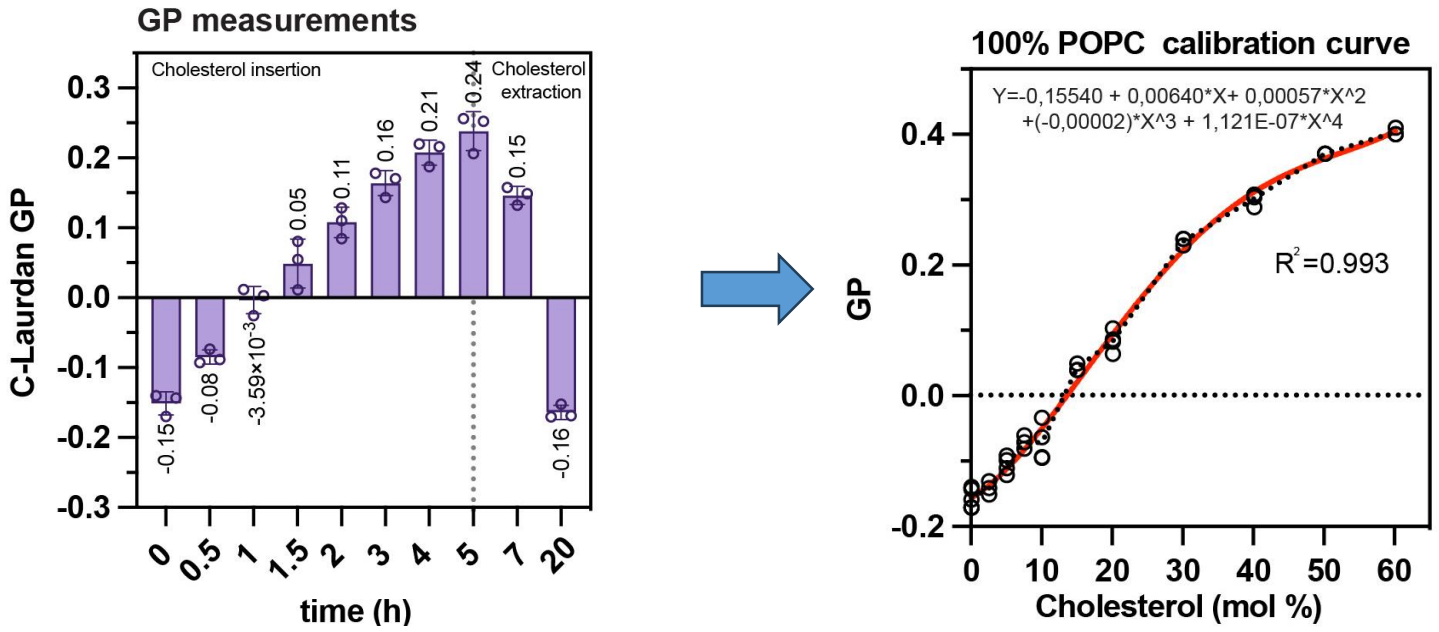
?



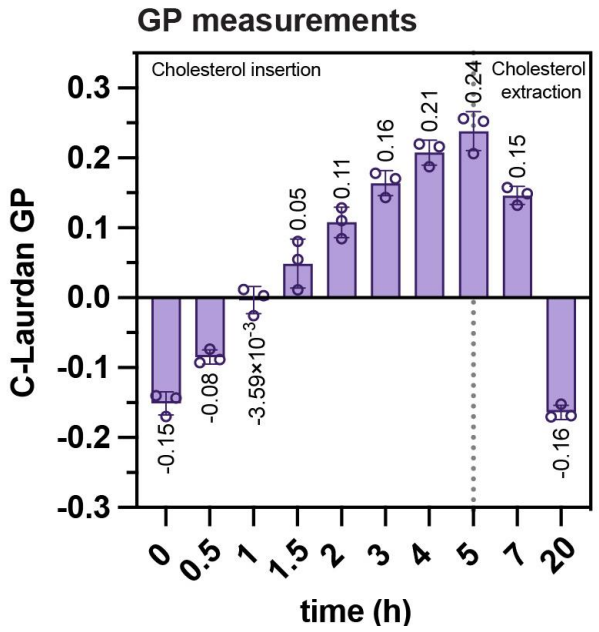
Insertion and extraction of cholesterol to/from membranes using methyl-β-cyclodextrin

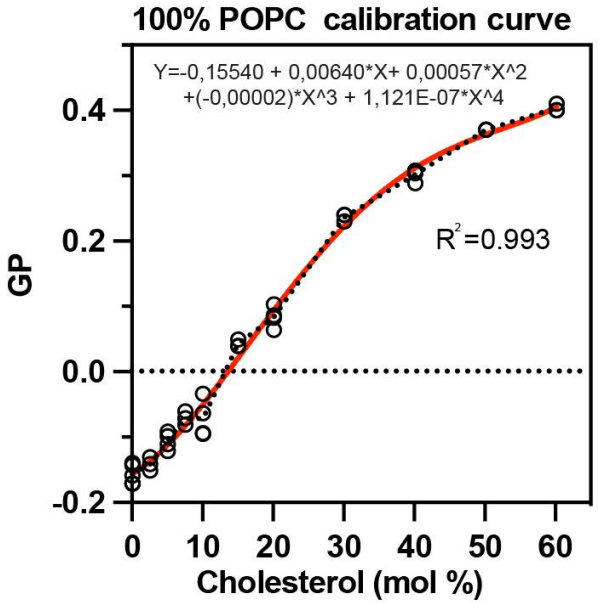


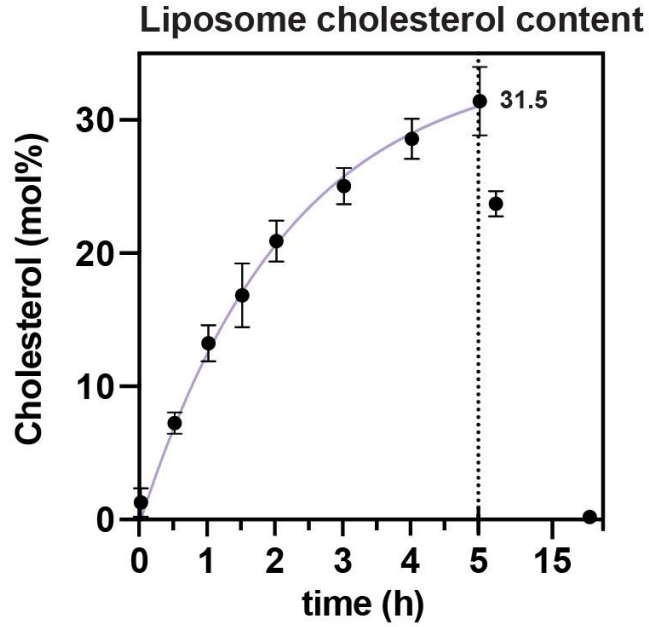
Insertion and extraction of cholesterol to/from membranes using methyl-β-cyclodextrin



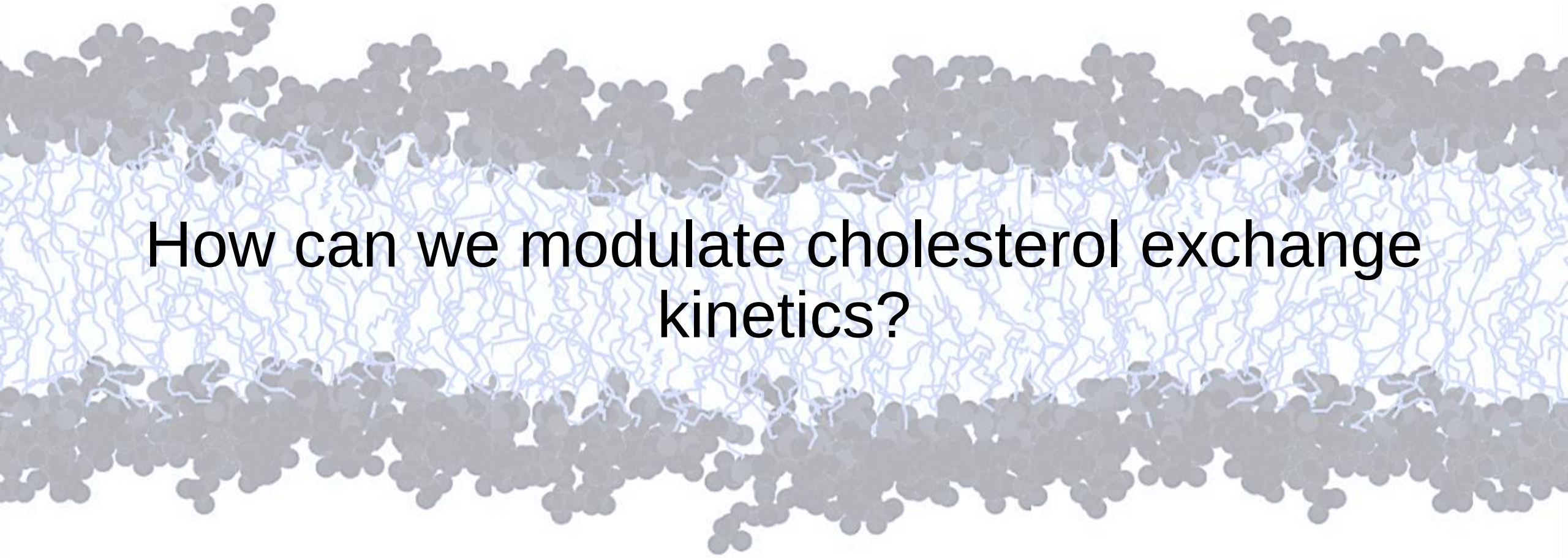
Insertion and extraction of cholesterol to/from membranes using methyl-β-cyclodextrin







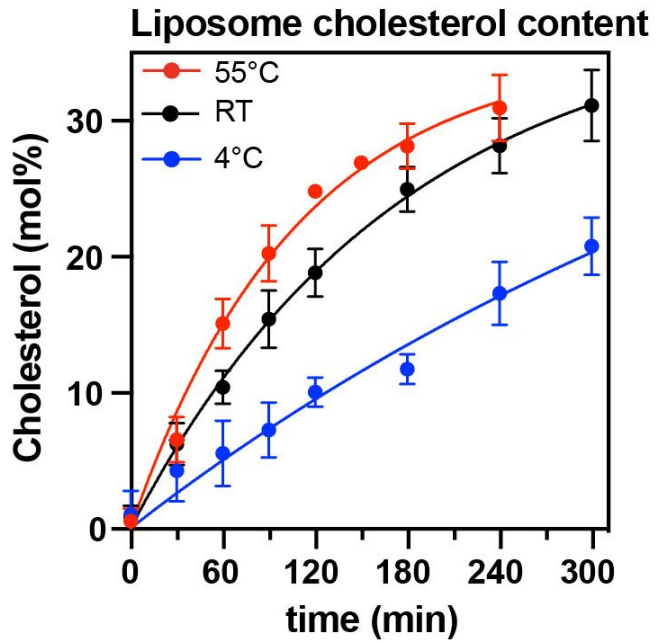
Using calibration curves we can determine the exact amount of cholesterol in our liposomes throughout the exchange.

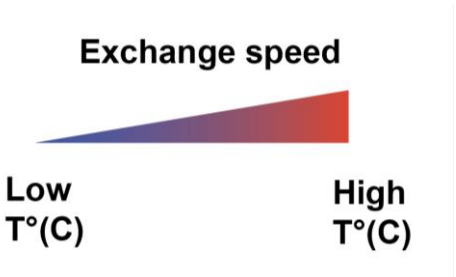


How can we modulate cholesterol exchange kinetics?

Using biophysical parameters to modulate exchange kinetics

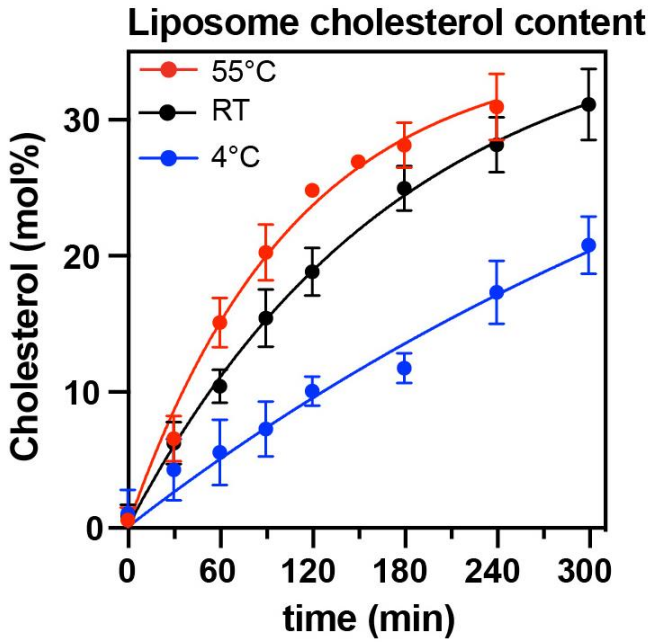
Cholesterol insertion at different temperatures

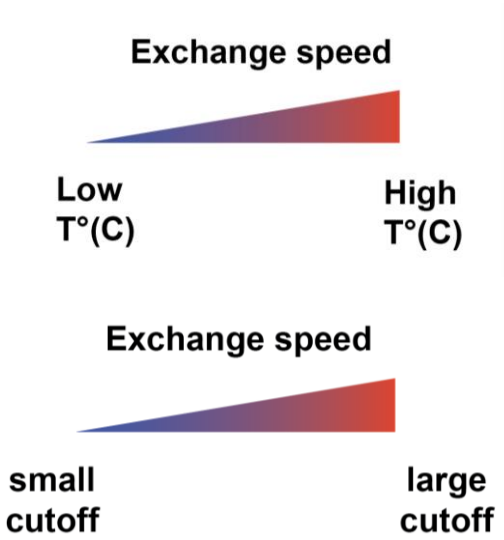




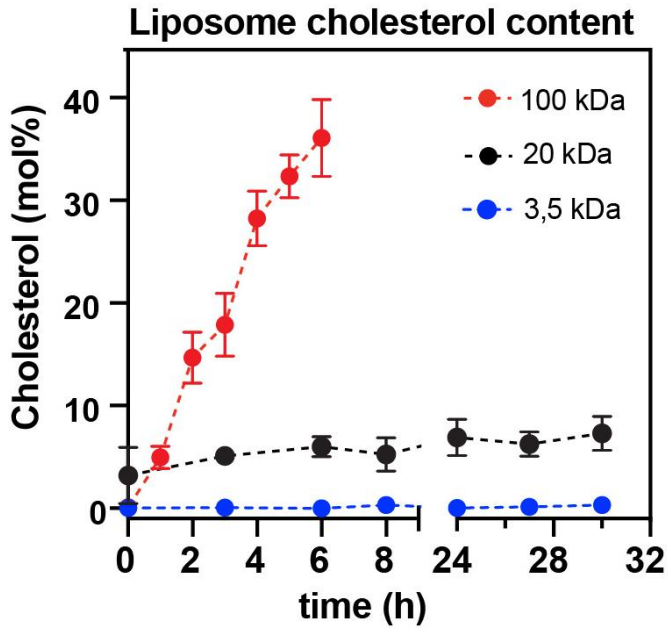
Using biophysical parameters to modulate exchange kinetics

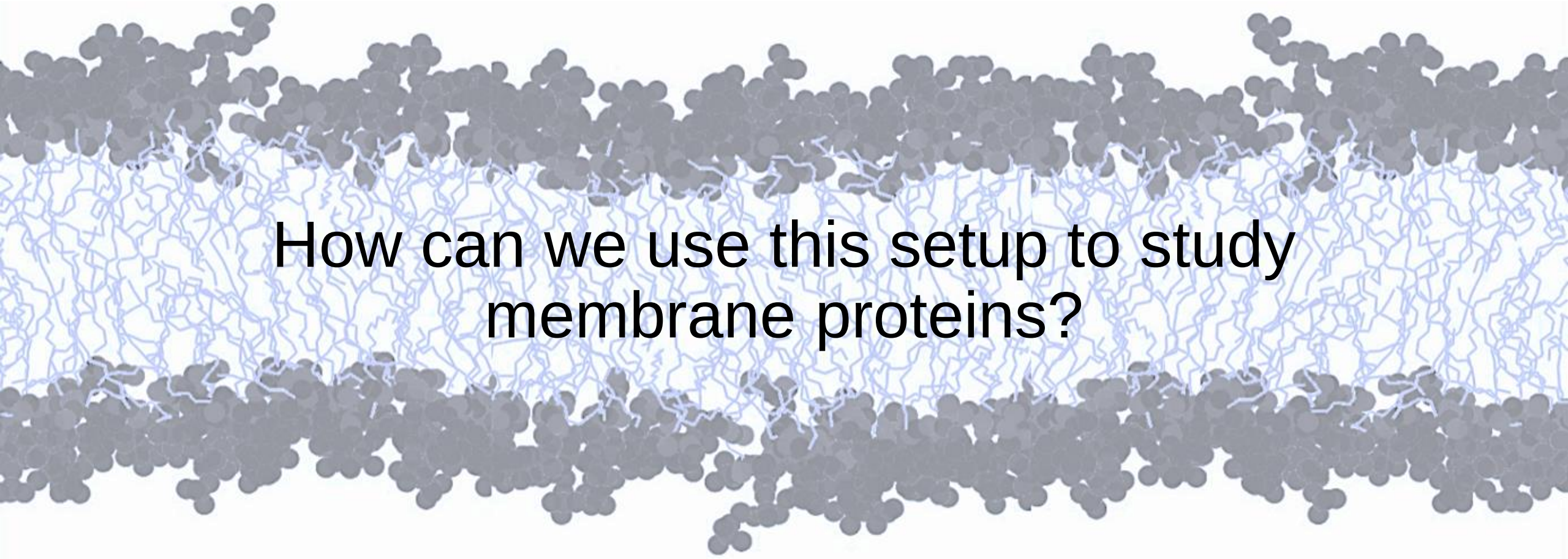
Cholesterol insertion at different temperatures





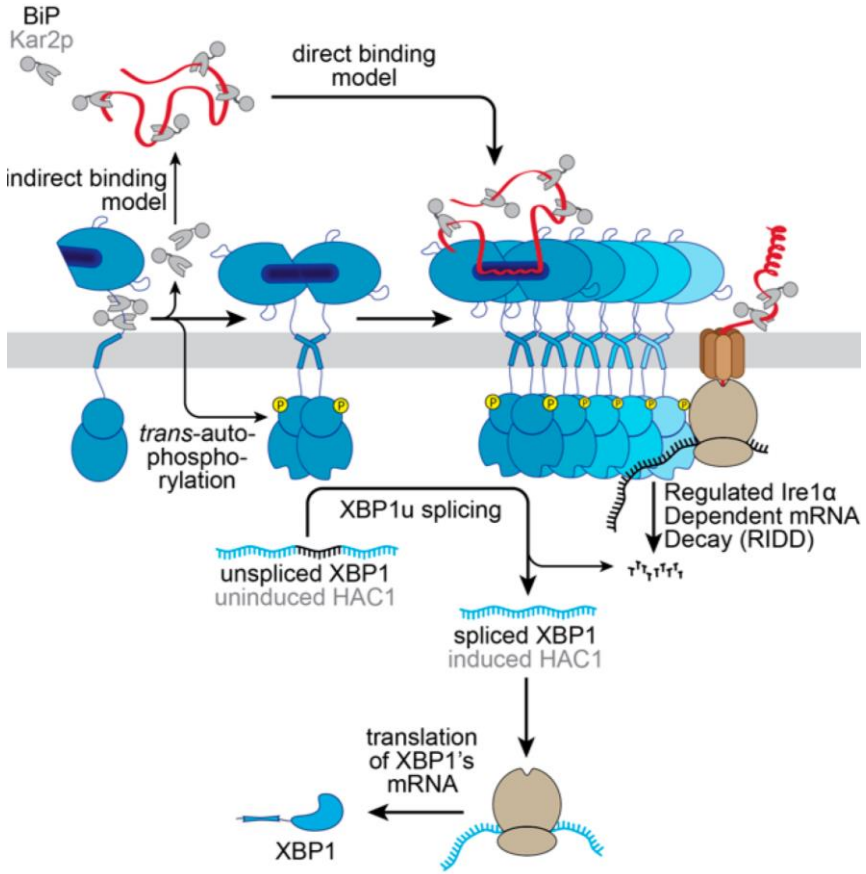
Cholesterol insertion with different membrane cutoffs





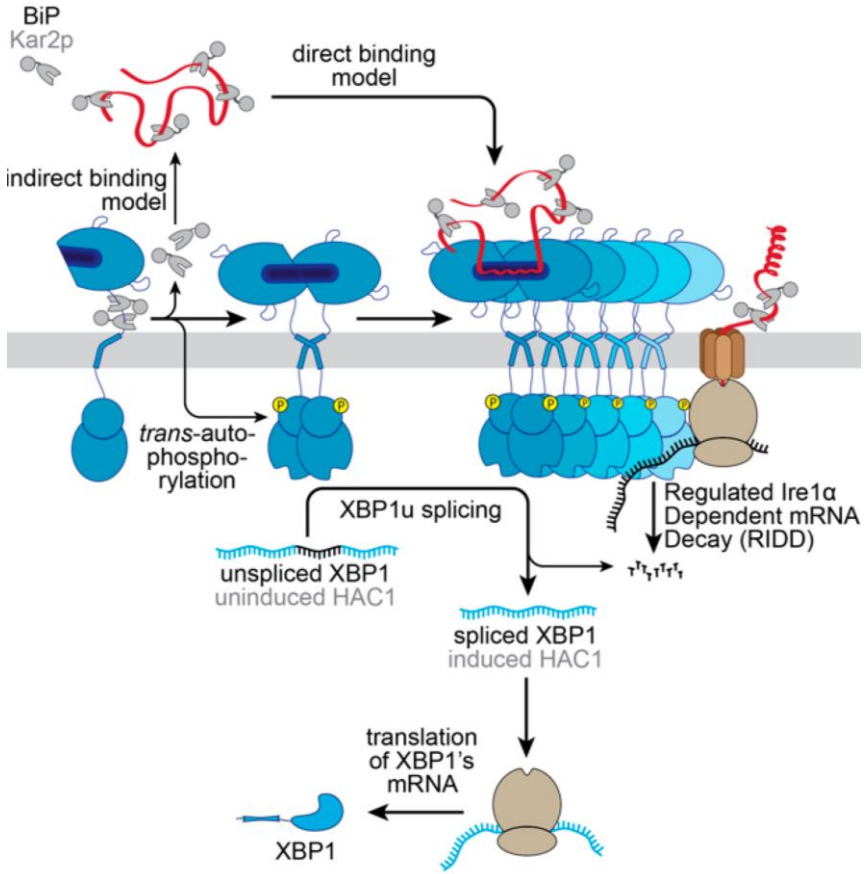
How can we use this setup to study membrane proteins?

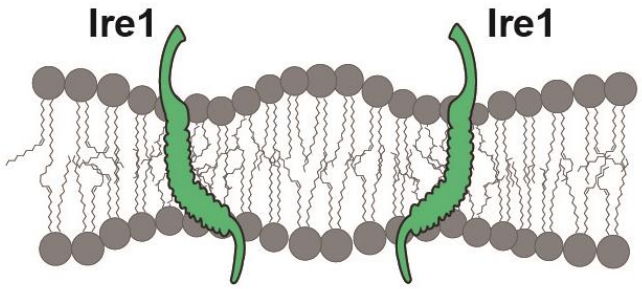
Ire1 is a conserved transducer of ER stress



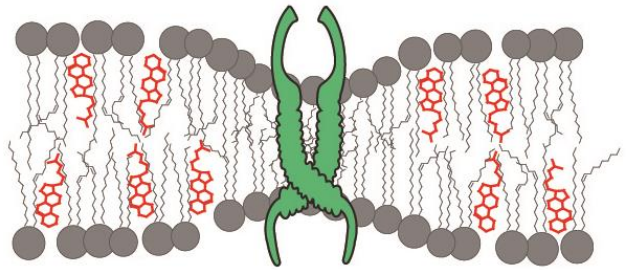
Radanovic et al. *Cells* (2021)

Ire1 is a conserved transducer of ER stress





Unstressed

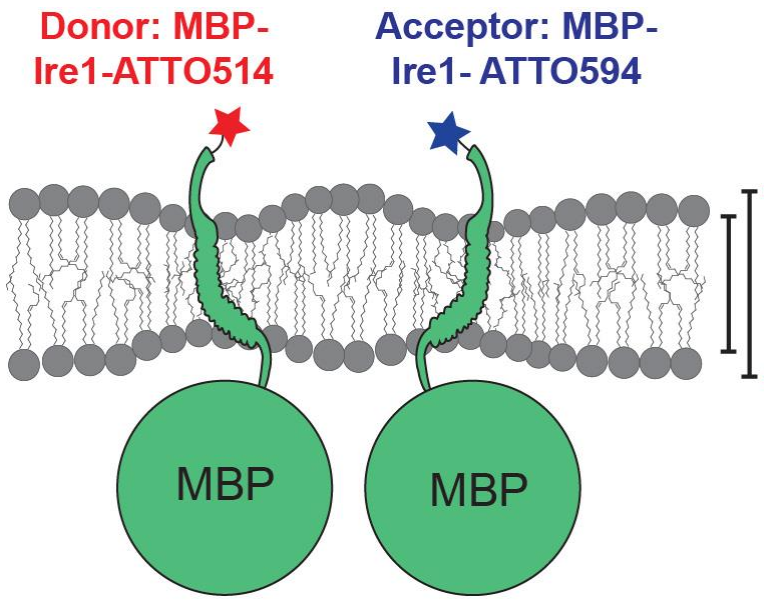


Lipid bilayer stress

Radanovic et al. *Cells* (2021)

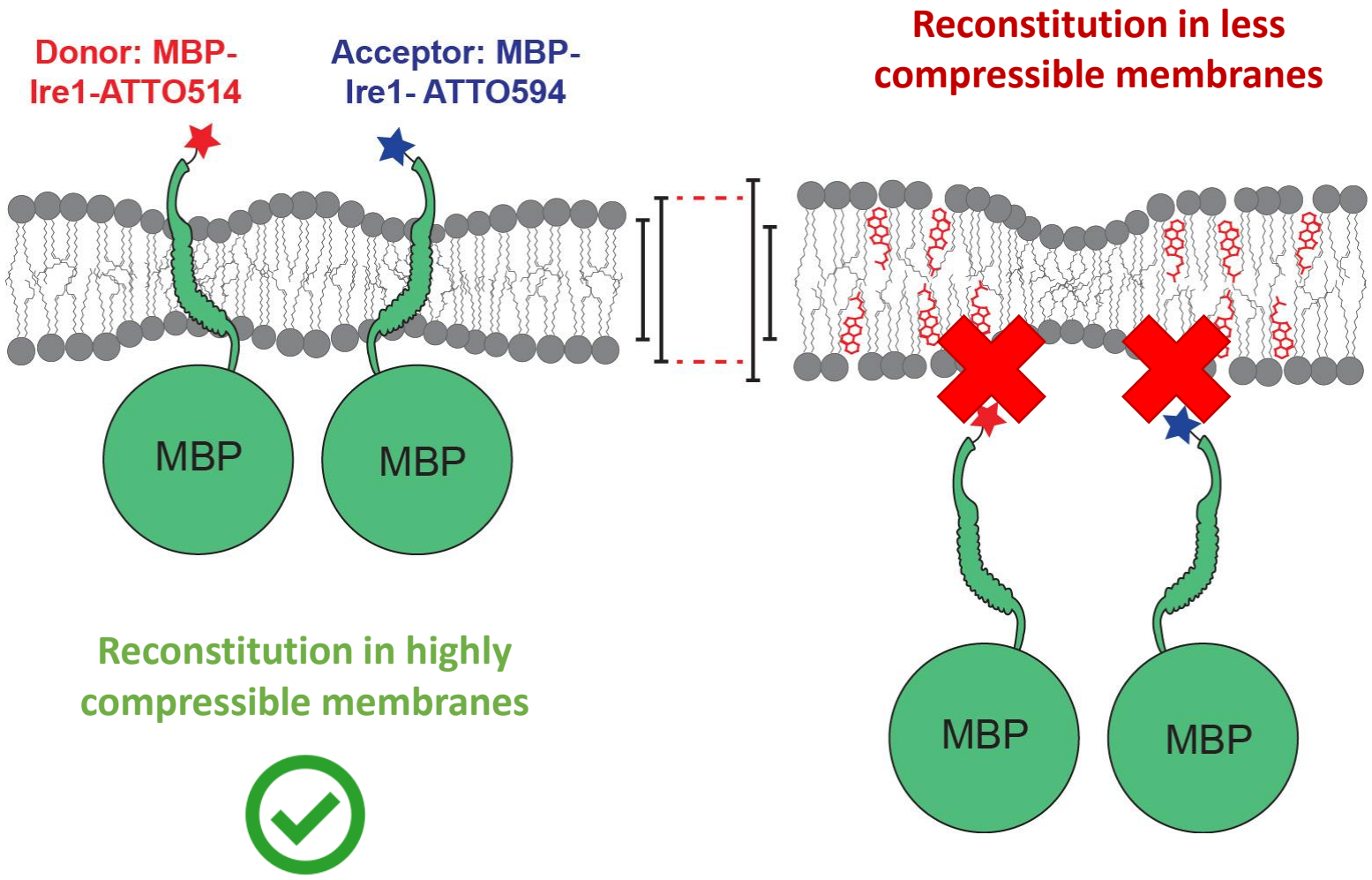
Halbleib K. et al. *Mol Cell* (2017)

An *in vitro* model to study Ire1 oligomerization in response to changes in membrane compressibility

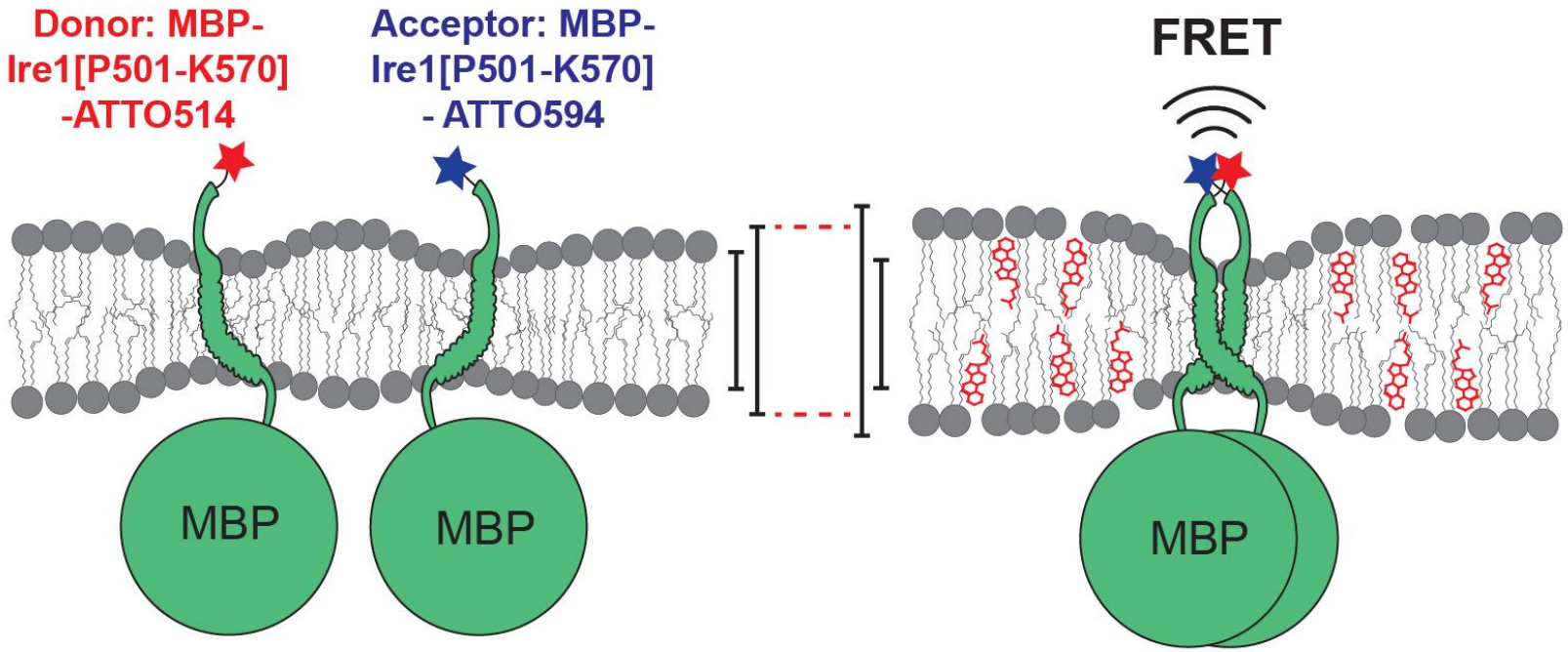


Reconstitution in highly compressible membranes

An *in vitro* model to study Ire1 oligomerization in response to changes in membrane compressibility

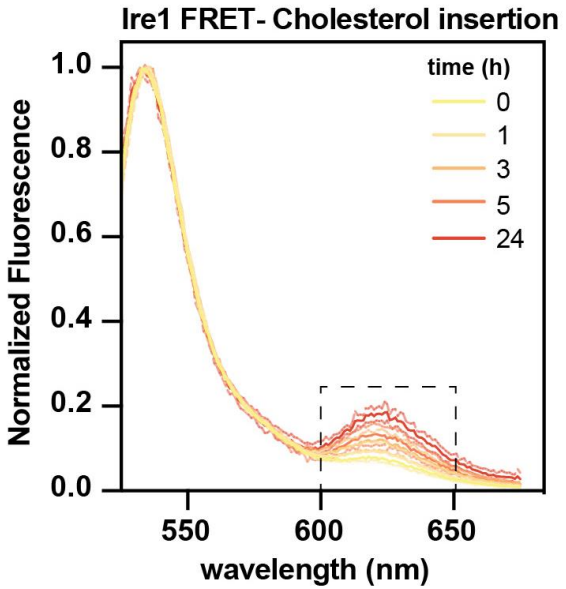


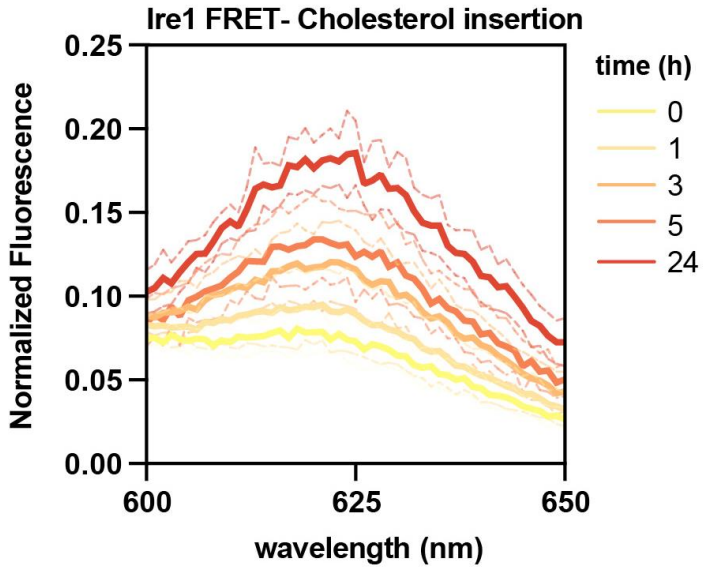
An *in vitro* model to study Ire1 oligomerization in response to changes in membrane compressibility

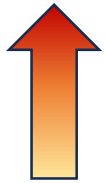


Gradual (reversible) evolution from high to low compressibility

Ire1 oligomerization in response to changes in membrane composition



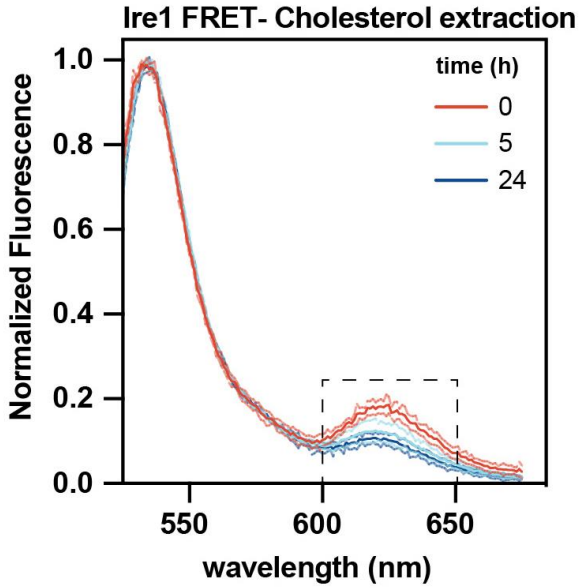


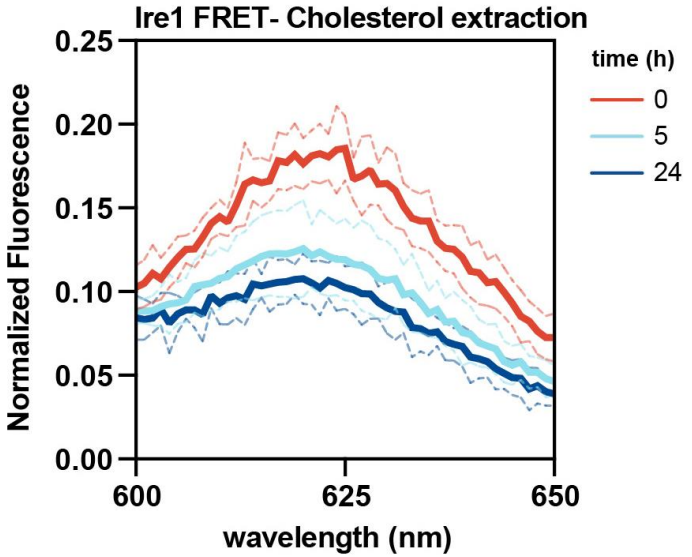


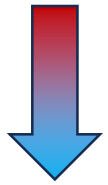
Cholesterol insertion

with Emmanuel Rodrigues

Ire1 oligomerization in response to changes in membrane composition



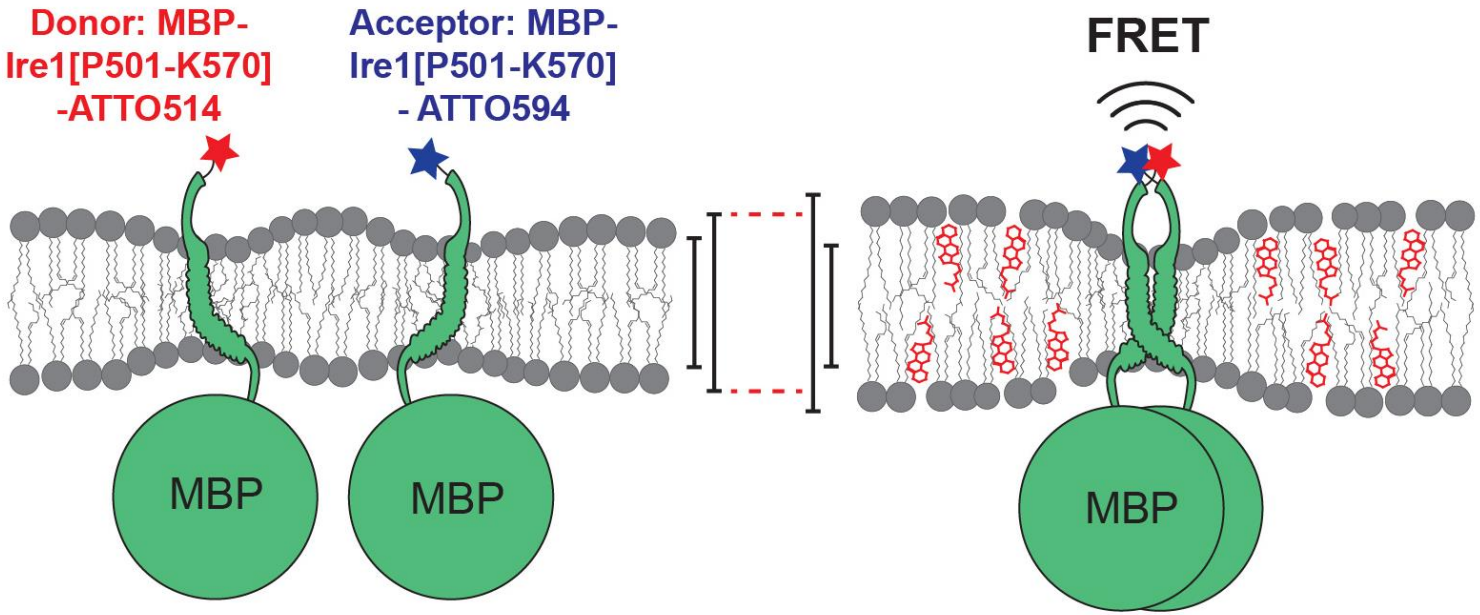


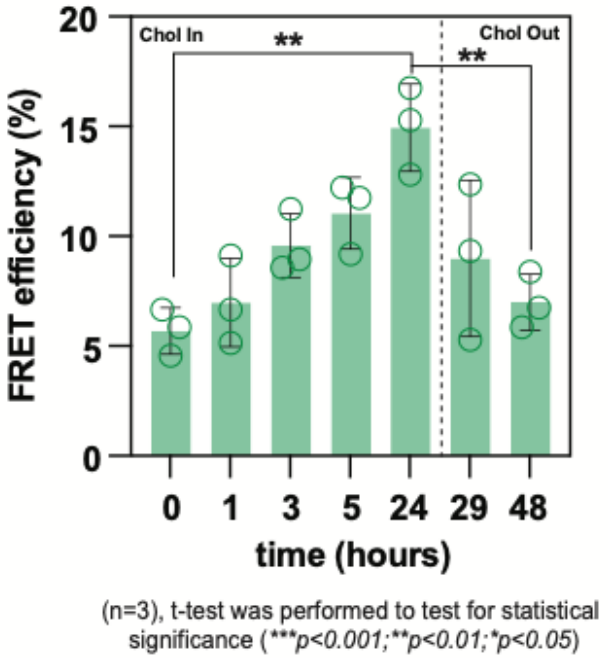


Cholesterol removal

with Emmanuel Rodrigues

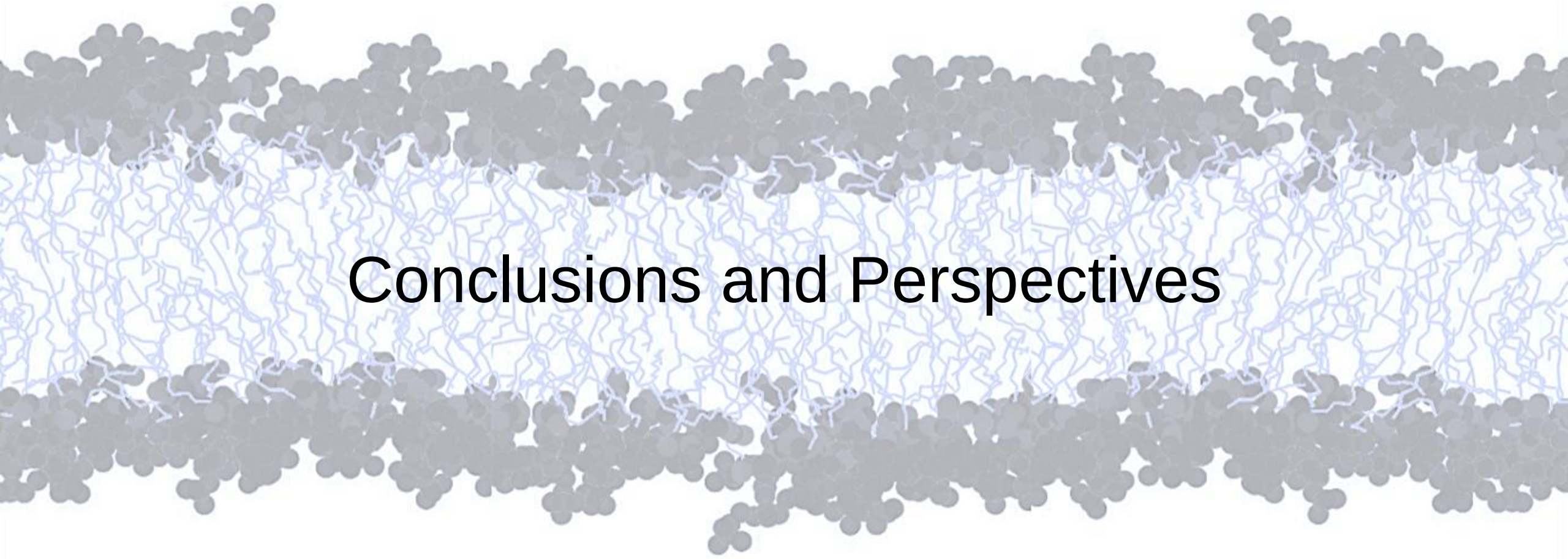
Ire1 oligomerization in response to changes in membrane composition





(n=3), t-test was performed to test for statistical significance (***p<0.001;**p<0.01;*p<0.05)

This technique can be used to study the response of different transmembrane proteins to changes in membrane composition



Conclusions and Perspectives

Conclusions and Perspectives

Simplicity and Time

- Limited choice of lipids
- Fixed state studies

Hydrophobic mismatch

- Reconstitution can be difficult in some membrane compositions

Vesicle Mixing

- Mixing of Donor and Acceptor liposomes requires separation

Conclusions and Perspectives

Simplicity and Time

- Limited choice of lipids
- Fixed state studies

Gradually modify lipid composition.
Fully reversible and allows time resolved measurements.

Hydrophobic mismatch

- Reconstitution can be difficult in some membrane compositions

Vesicle Mixing

- Mixing of Donor and Acceptor liposomes requires separation

Conclusions and Perspectives

Simplicity and Time

- Limited choice of lipids
- Fixed state studies

Gradually modify lipid composition.
Fully reversible and allows time resolved measurements.

Hydrophobic mismatch

- Reconstitution can be difficult in some membrane compositions

Requires a single reconstitution in a favorable lipid composition limiting heterogeneity.

Vesicle Mixing

- Mixing of Donor and Acceptor liposomes requires separation

Conclusions and Perspectives

Simplicity and Time

- Limited choice of lipids
- Fixed state studies

Gradually modify lipid composition.
Fully reversible and allows time resolved measurements.

Hydrophobic mismatch

- Reconstitution can be difficult in some membrane compositions

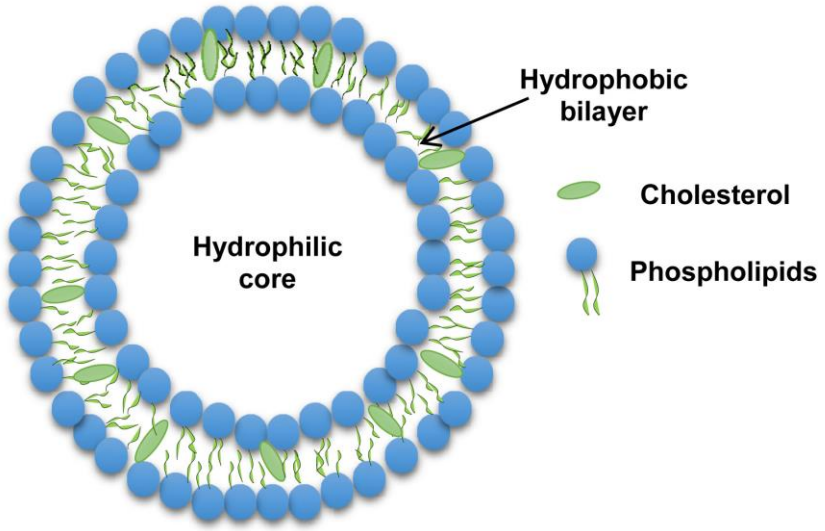
Requires a single reconstitution in a favorable lipid composition limiting heterogeneity.

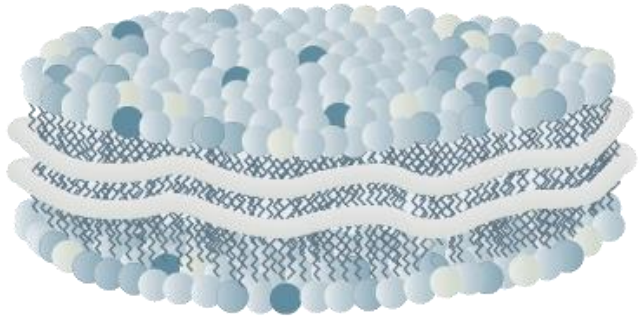
Vesicle Mixing

- Mixing of Donor and Acceptor liposomes requires separation

Acceptors and donors kept in separate compartments throughout the exchange

Conclusions and Perspectives



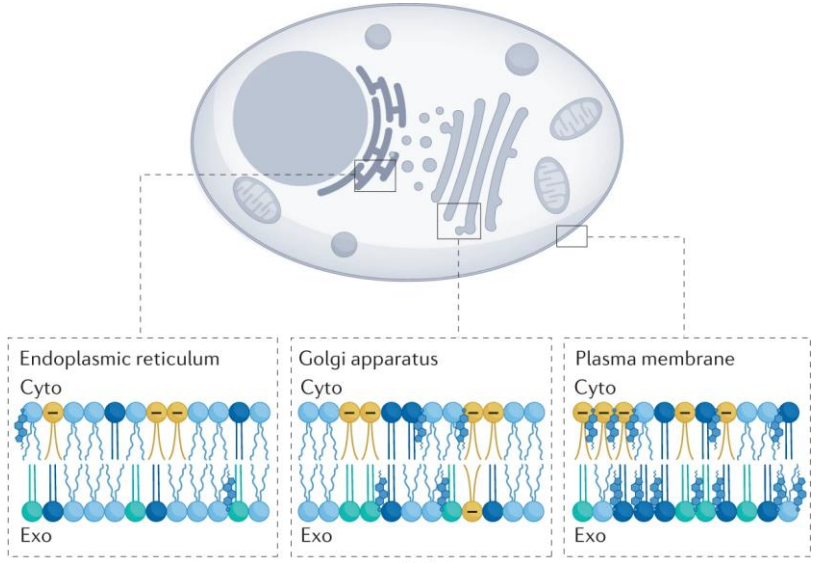


Structural Biology

Complex and protein rich

Asymmetry

Natural phase behavior



Remodeling of biological membranes

Nsairat H. et al, Heliyon (2022).

Levental, I. et al. *Nat Rev Mol Cell Biol* (2023).

European Research Council
Established by the European Commission

UNIVERSITÄT DES SAARLANDES

Ernst Lab

Prof. Dr. Robert Ernst
Dr. Claudia Götz
Dr. Alex von der Malsburg
Dr. Mike Renne
Viola Schuck
Heike Stumpf
Julia Hach
Jona Causemann
Aamna Jain
Konstancja Porzycka
Alexa Cogan
Dr. Cynthia Alsayyah (cynthia.alsayyah@uks.eu)

Past interns: Emmanuel Rodrigues