



Peptide discs as precursors of supported lipid bilayers with oriented membrane proteins

Alessandra Luchini

Paul Scherrer Institut
Switzerland

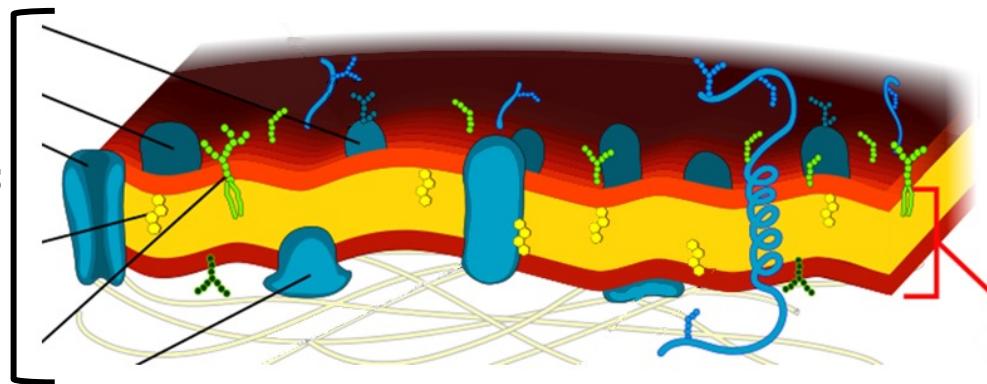
Swedish Neutron Week 2021

Small Sample Polarized Reflectometer



Cell membranes

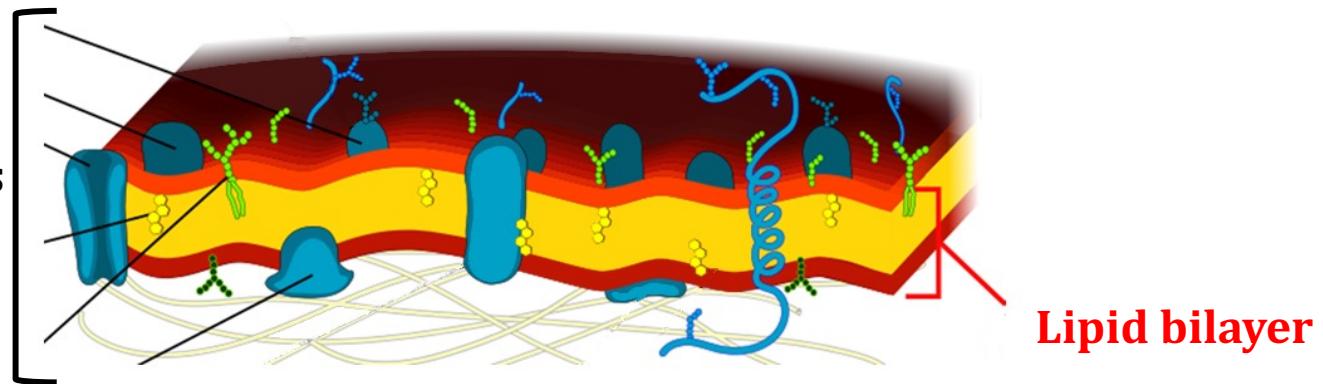
Membrane proteins



Lipid bilayer

Cell membranes

Membrane proteins

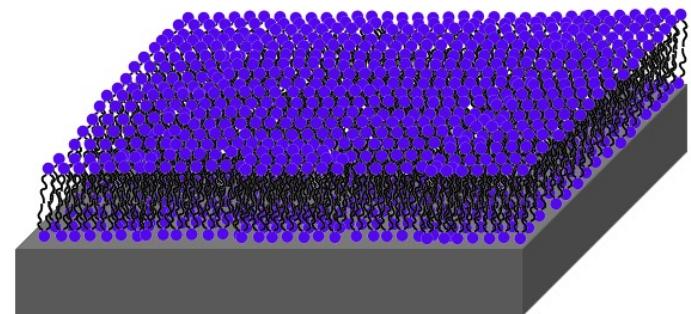


Lipid bilayer

Supported lipid bilayers

- structural scaffold of cell membranes
- tuneable lipid composition
- characterization by means surface-sensitive techniques, e.g. neutron reflectometry

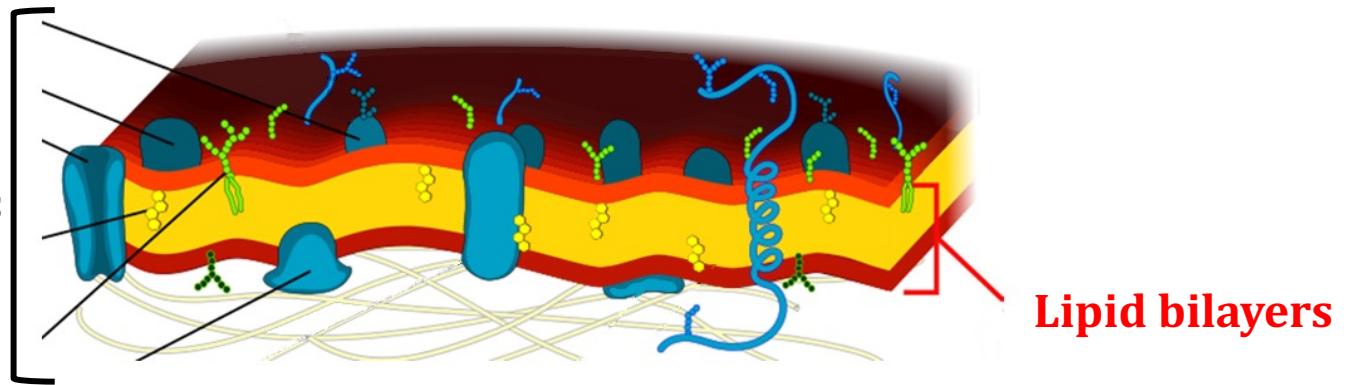
Bulk solvent



Substrate

Cell membranes

Membrane proteins



Lipid bilayers

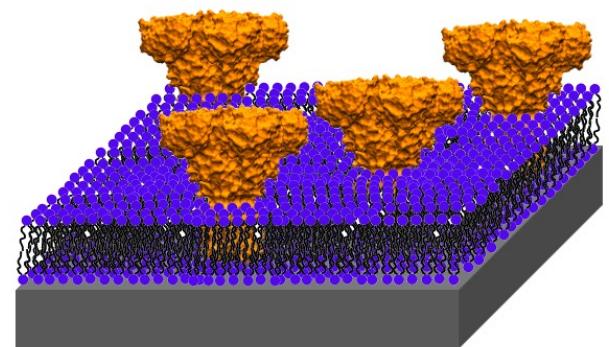
Supported lipid bilayers with membrane proteins

Development of better mimics of cell membranes



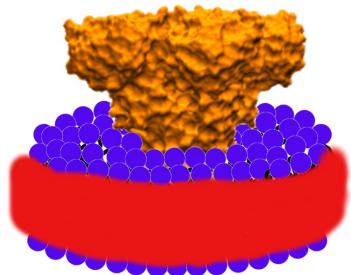
Need for sample preparation protocol for reconstituting membrane proteins in supported lipid bilayers with **controlled orientation**

Bulk solvent

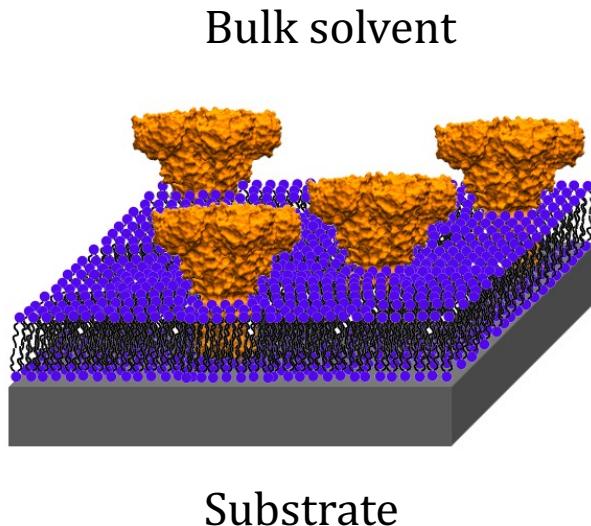


Substrate

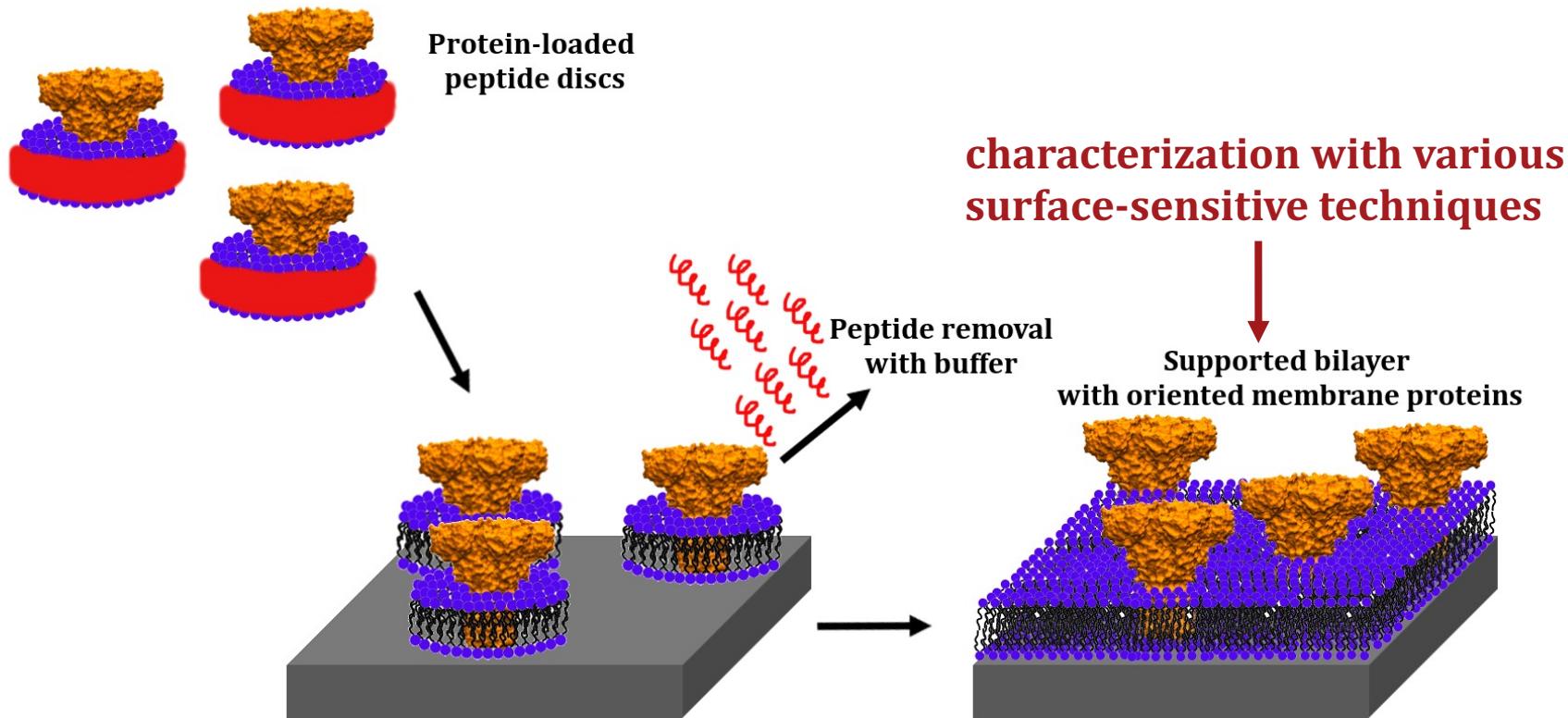
Peptide discs as precursors of supported lipid bilayer with oriented membrane proteins



Peptide discs: discoidal **lipid** aggregates surrounded by self-assembled peptide molecules (**18A**), where **membrane protein** can be reconstituted



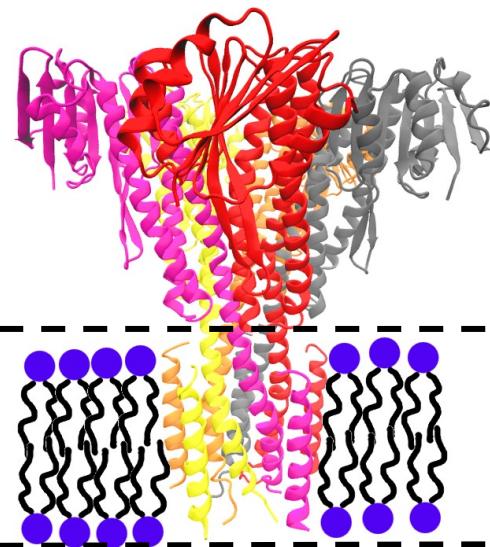
Peptide discs as precursors of supported lipid bilayer with oriented membrane proteins



- **Neutron Reflectometry(NR)**
- Quartz Crystal Microbalance with Dissipation monitoring (QCM-D)
- Surface Plasmon Resonance (SPR)
- Atomic Force Microscopy (AFM)
- Attenuated total reflectance – Fourier transform IR spectroscopy (ATR-FTIR)

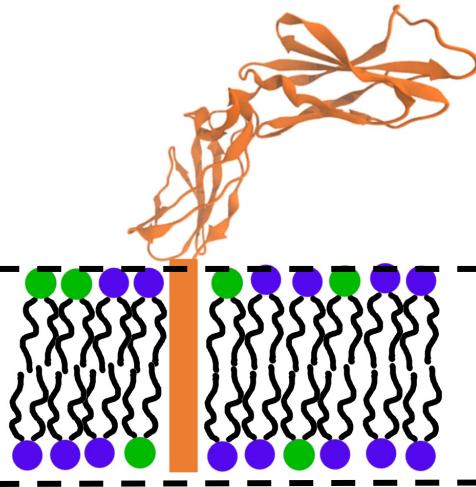
Membrane protein of interest

CorA



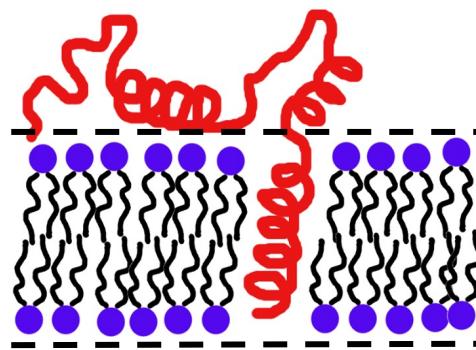
Bacterial magnesium transporter
(homopentamer)
~ 43 kDa (monomer)

Tissue factor (TF)



TF initiates of the blood extrinsic coagulation pathway.
~ 50 kDa

Synaptobrevin (VAMP2)

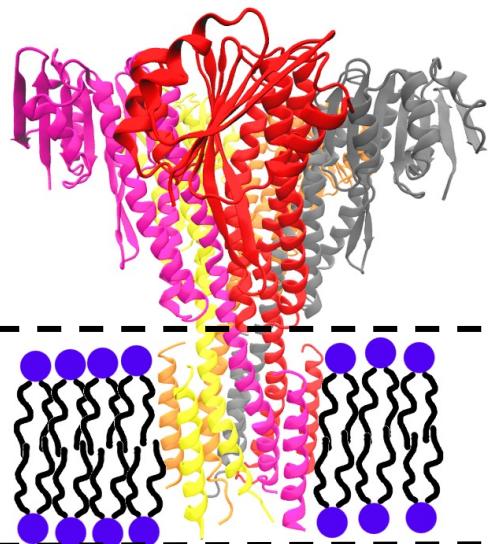


VAMP2 participate to the formation of the SNARE complex, fundamental for neuronal communication
~ 13 kDa

All proteins have asymmetric structure, but different molecular weight

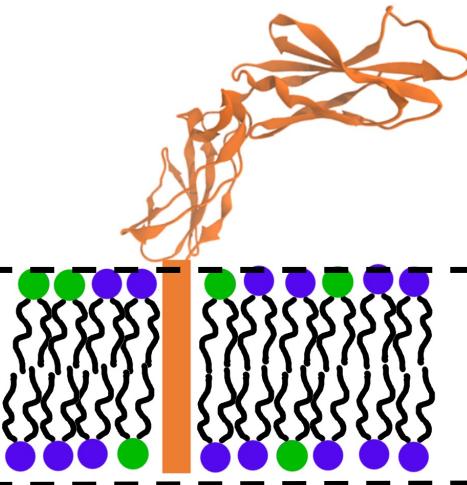
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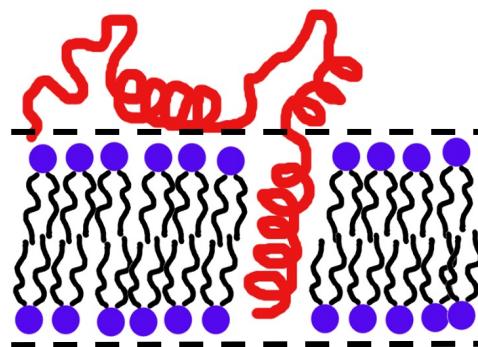
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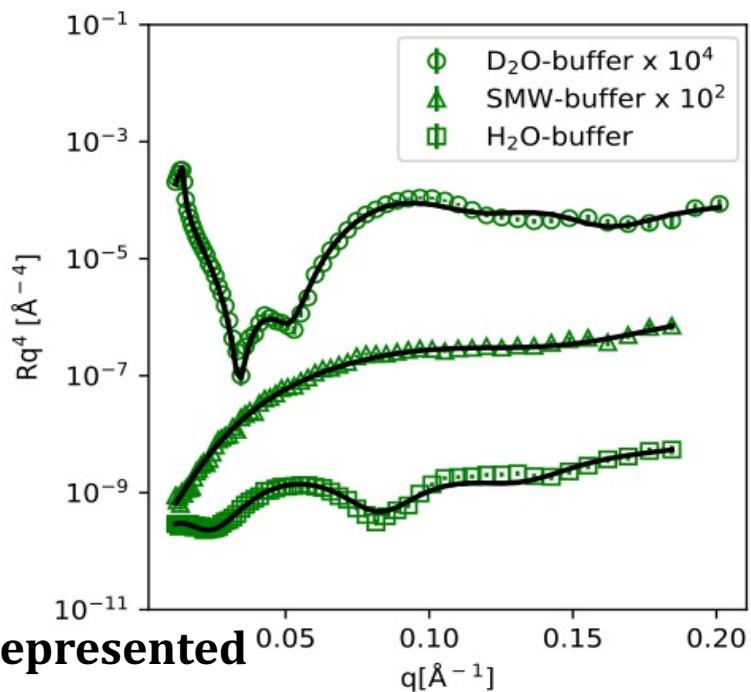


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Peptide discs as precursors of supported lipid bilayer with oriented CorA

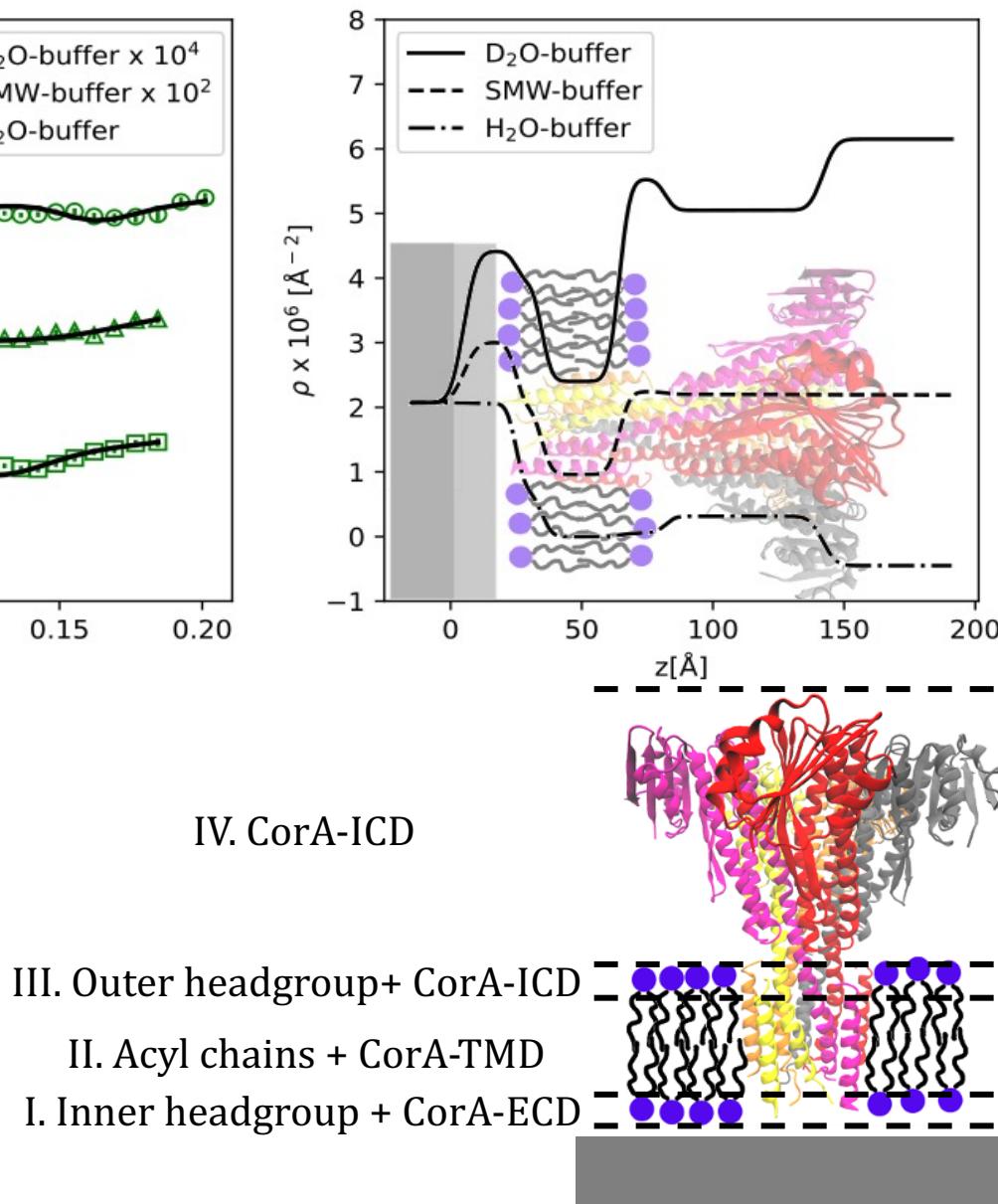
Simultaneous fit of data in different contrasts



The sample is represented by a stack of layers

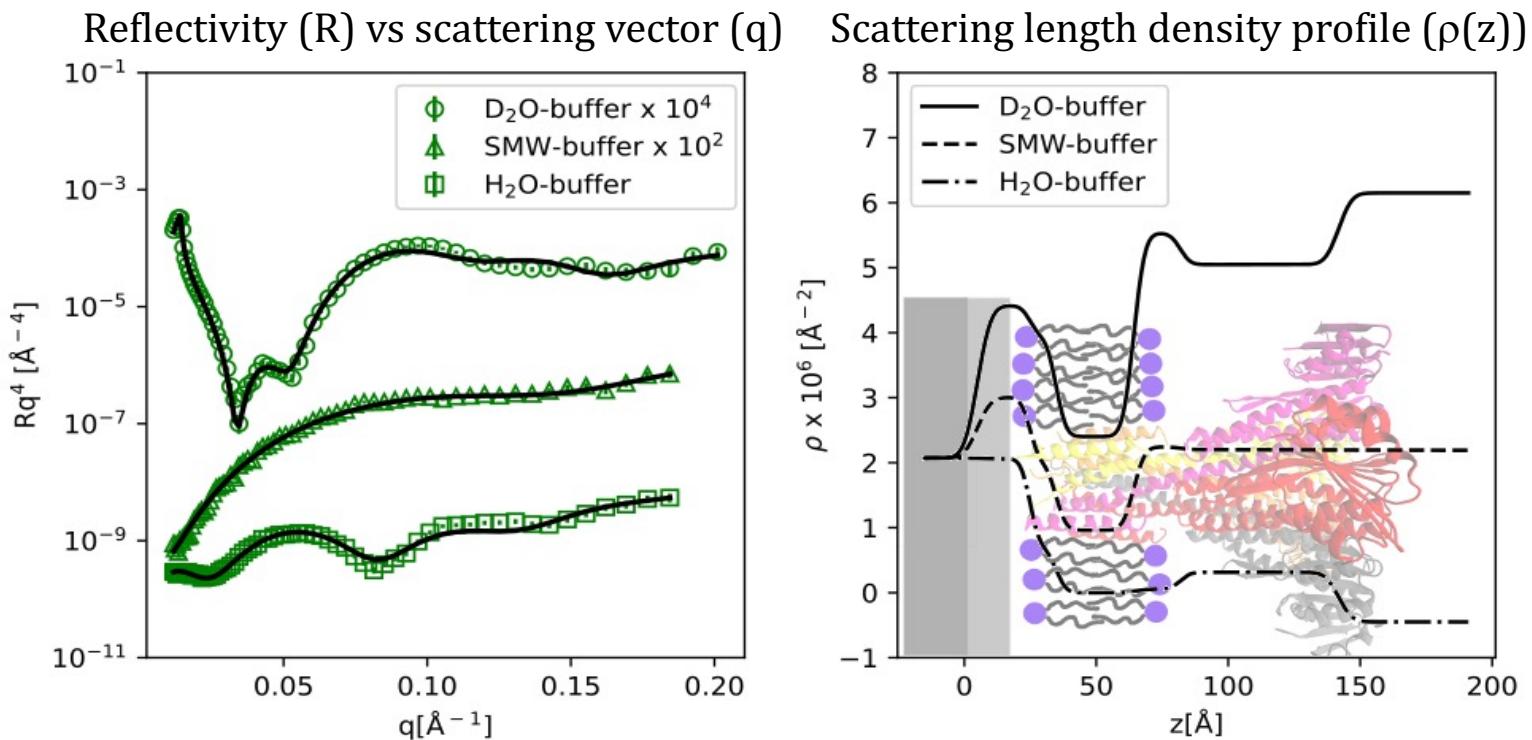
Layer parameters:

- Thickness
- Scattering length density
- Solvent volume fraction
- roughness

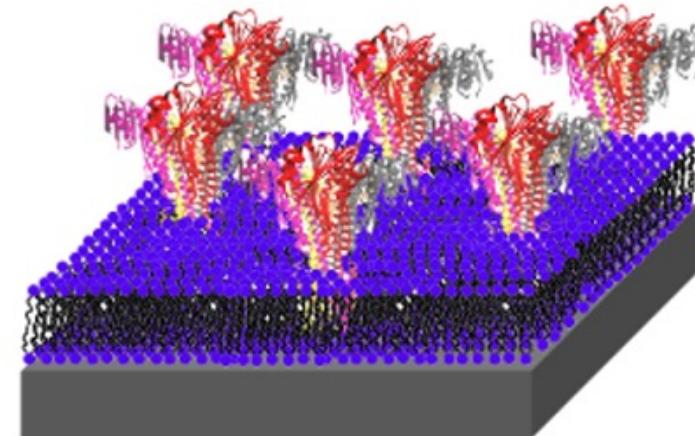


Peptide discs as precursors of supported lipid bilayer with oriented CorA

Simultaneous fit of data in different contrasts

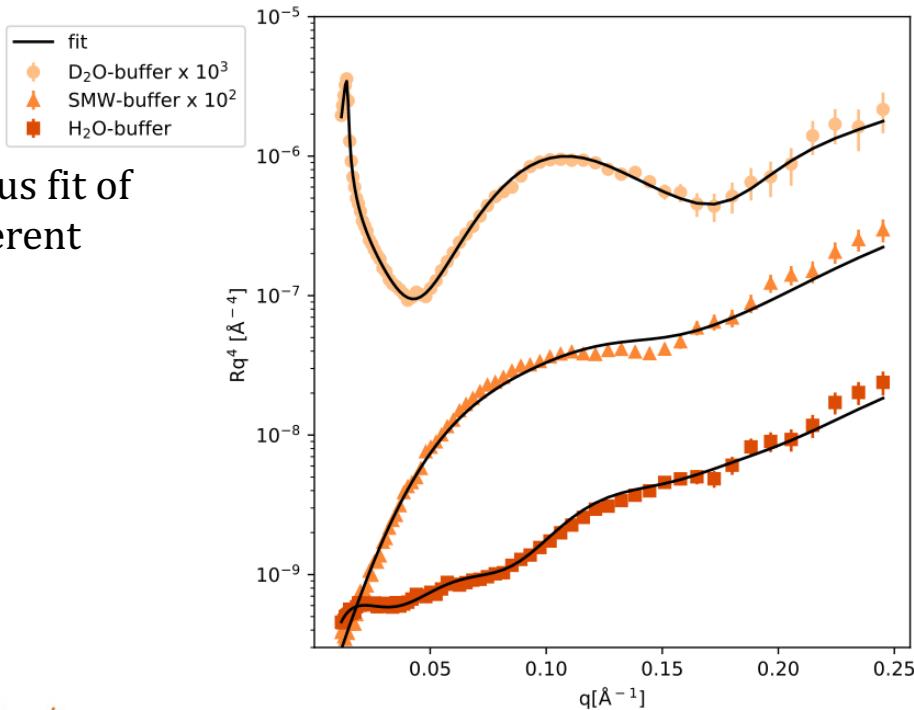


Method validation: all CorA molecules have the same orientation

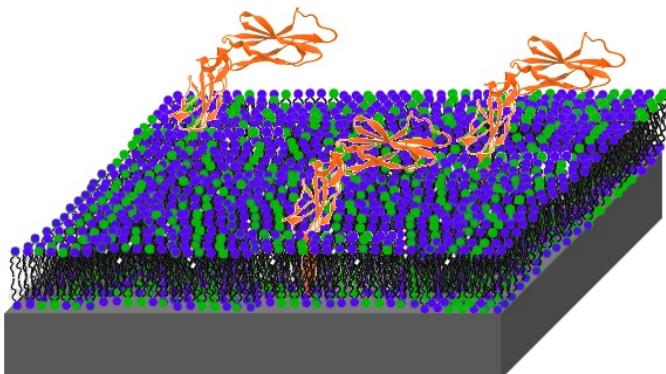
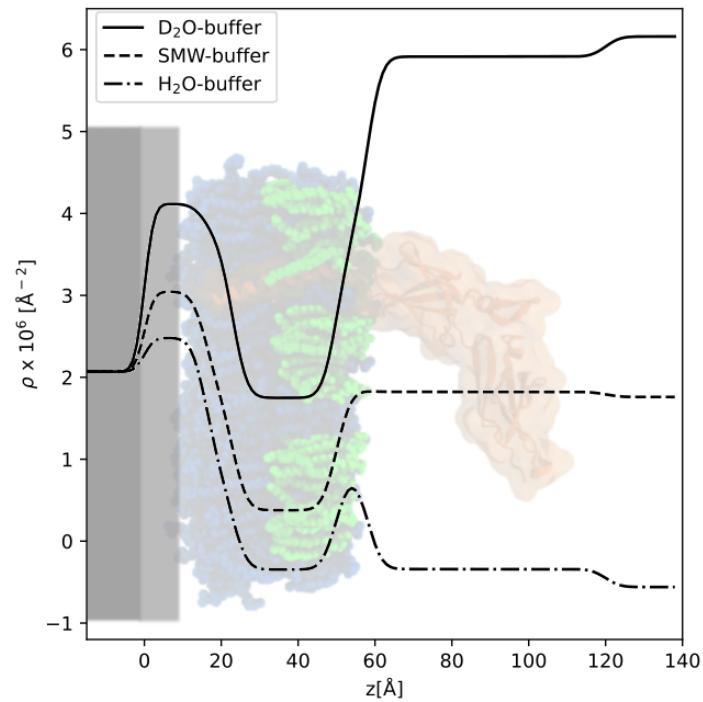


Supported lipid bilayer with oriented TF molecules

Reflectivity (R) vs scattering vector (q)



Scattering length density profile ($\rho(z)$)



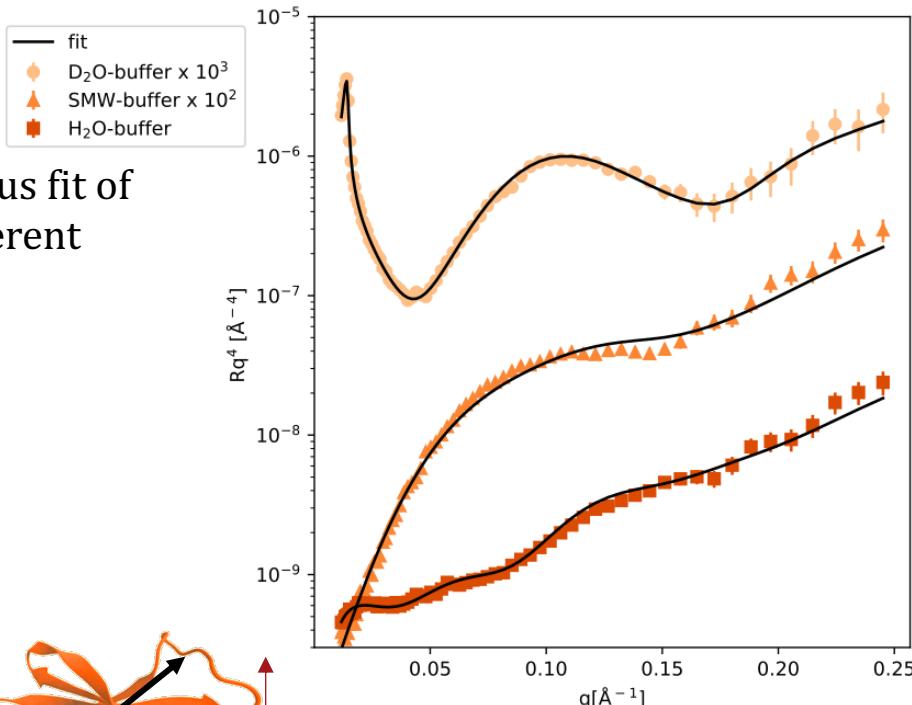
Overall membrane surface coverage $\sim 80\%$

TF-ECD surface coverage $\sim 10\%$

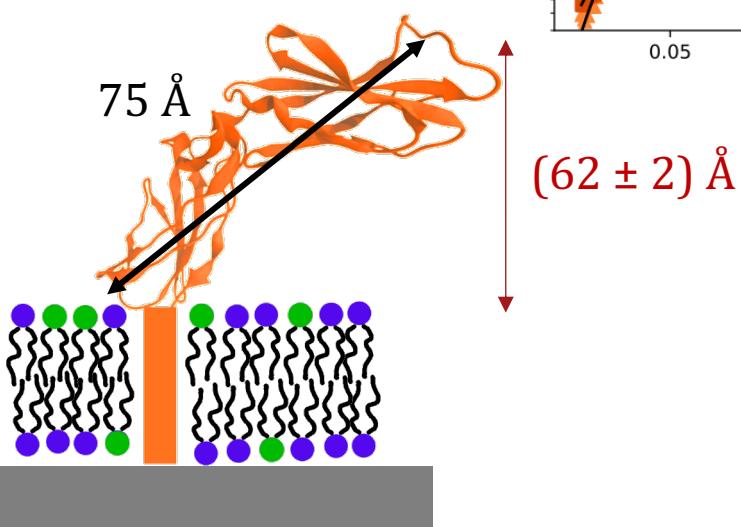
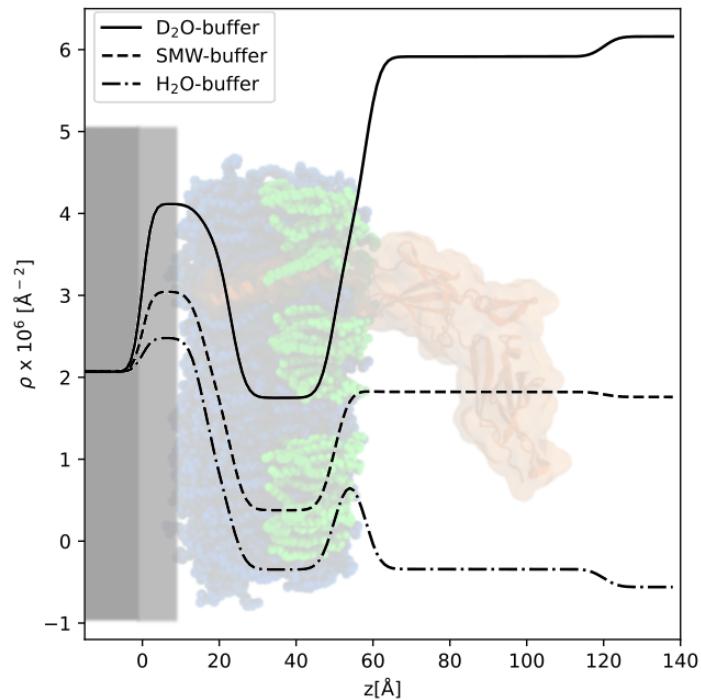
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Supported lipid bilayer with oriented TF molecules

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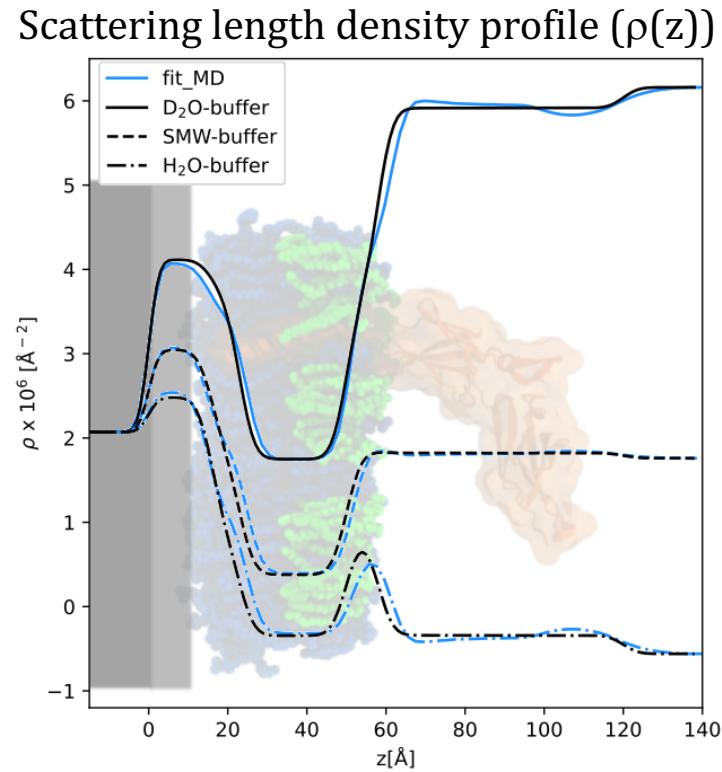
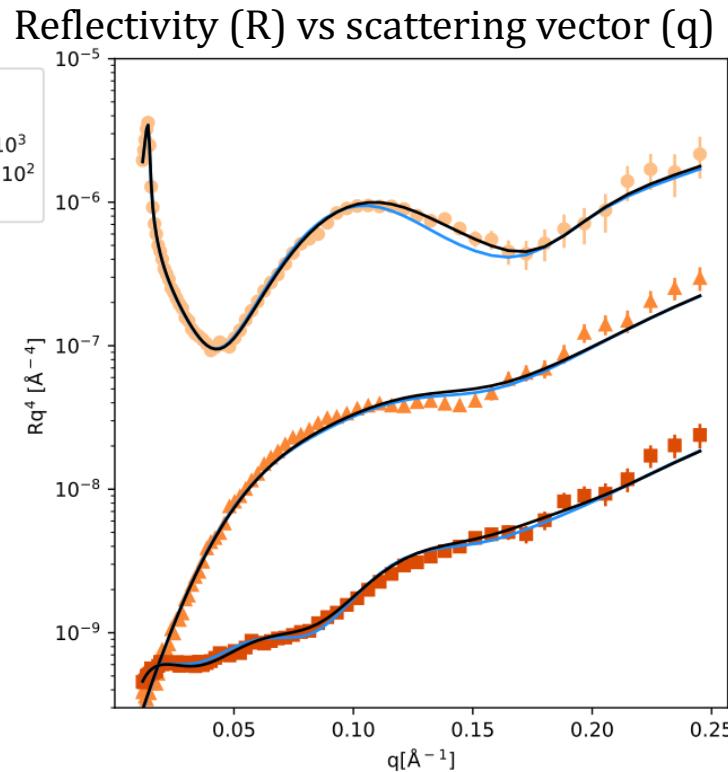
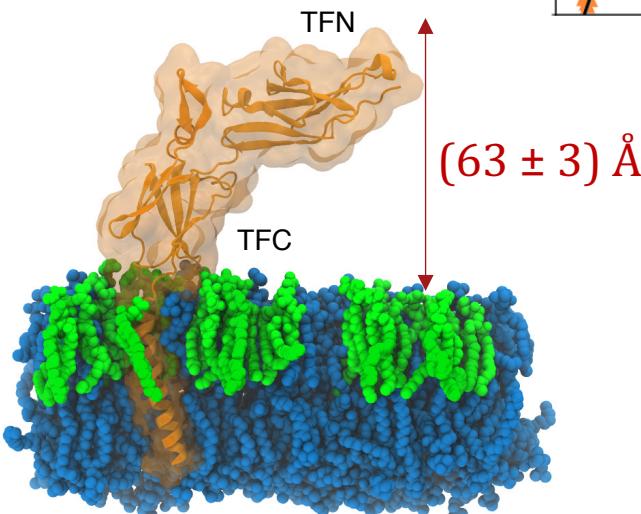
Scattering length density profile ($\rho(z)$)



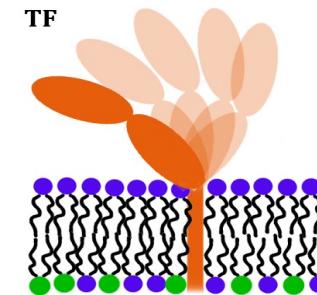
The TF extracellular domain is not perpendicular to the membrane surface

Supported lipid bilayer with oriented TF molecules

Simultaneous fit of data in different contrasts



Insight from Molecular Dynamics simulations:
The ECD experiences different orientations with respect to the membrane plane with an averaged distance consistent with the NR data.



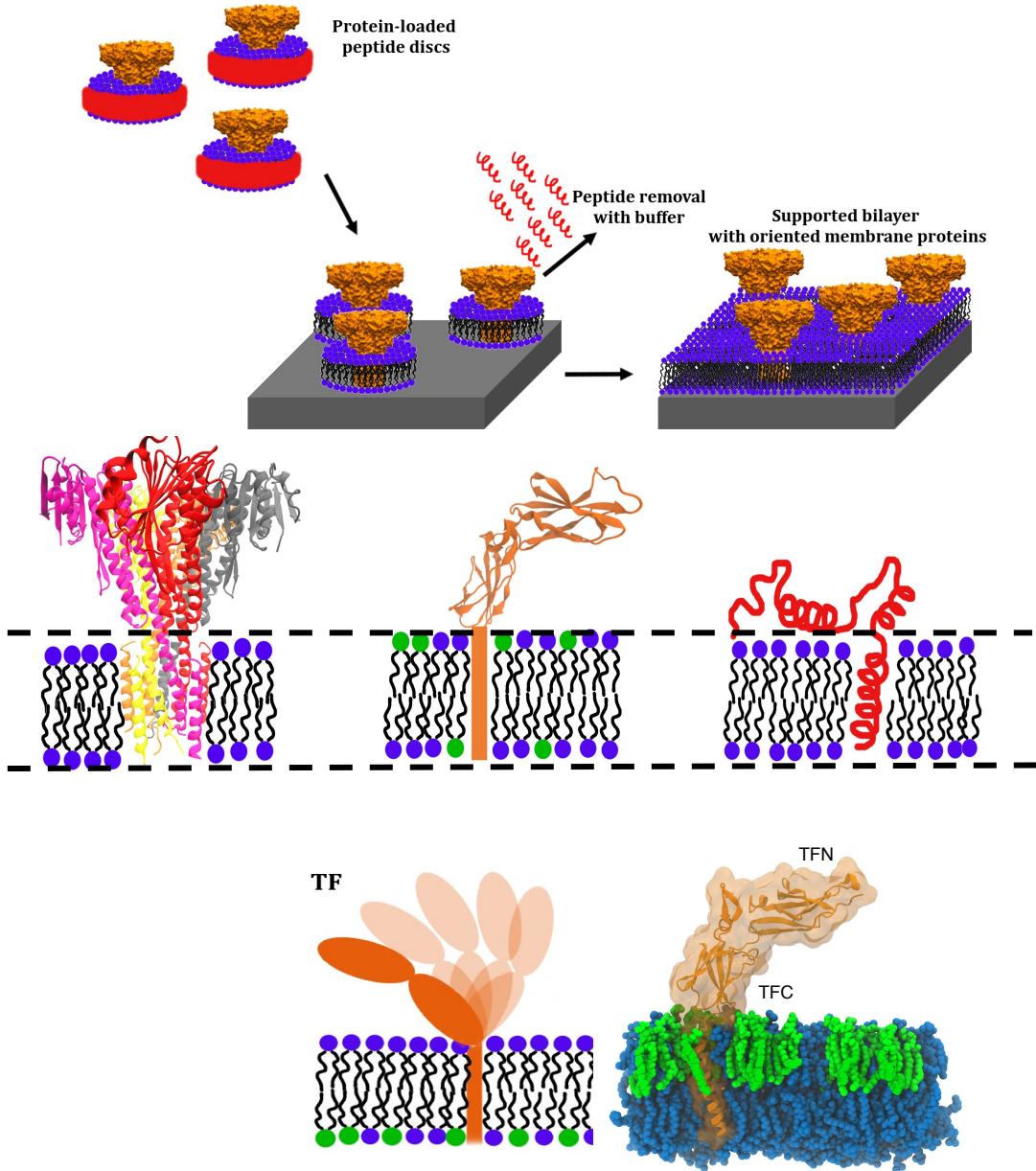
Conclusions

Peptide discs can be used as precursors of supported lipid bilayers and supported lipid bilayers incorporating membrane proteins of different sizes

The method is compatible with different surface sensitive techniques, e.g. QCMD, ATR-FTIR, SPR, AFM, NR

NR provided the location of the protein molecules with respect of the bilayer surface

MD simulation can support NR data analysis and provide complementary information



Acknowledgments



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Raul Araya Secchi



Mario Campana

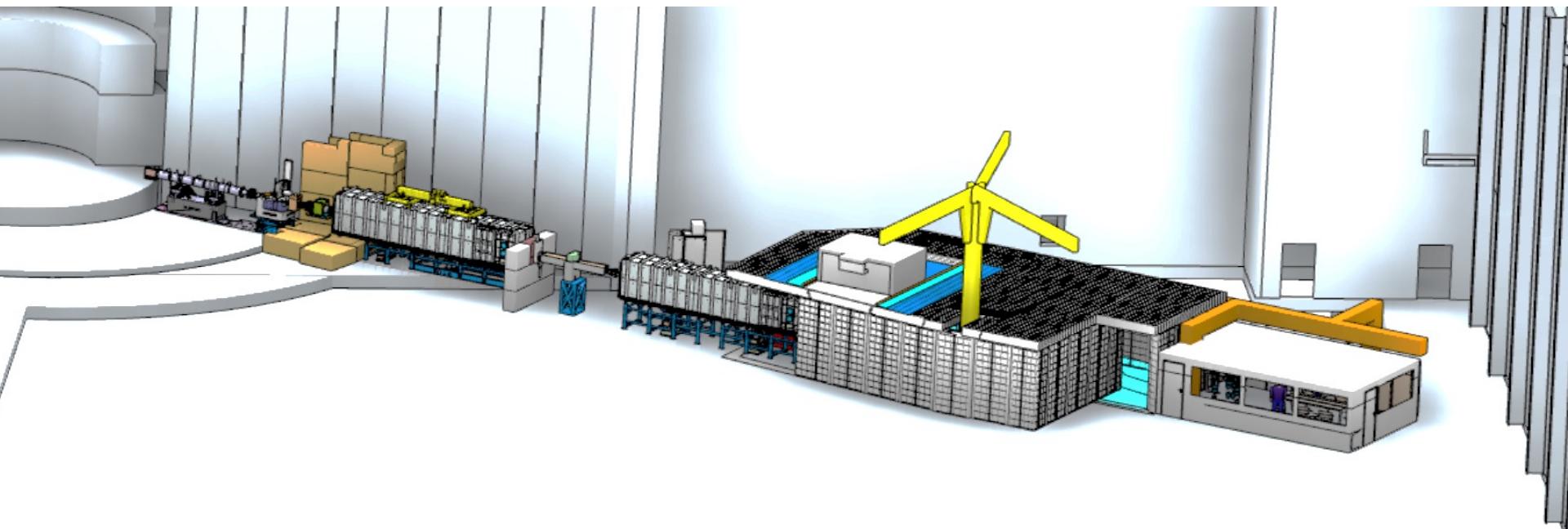


Better lives through
new knowledge

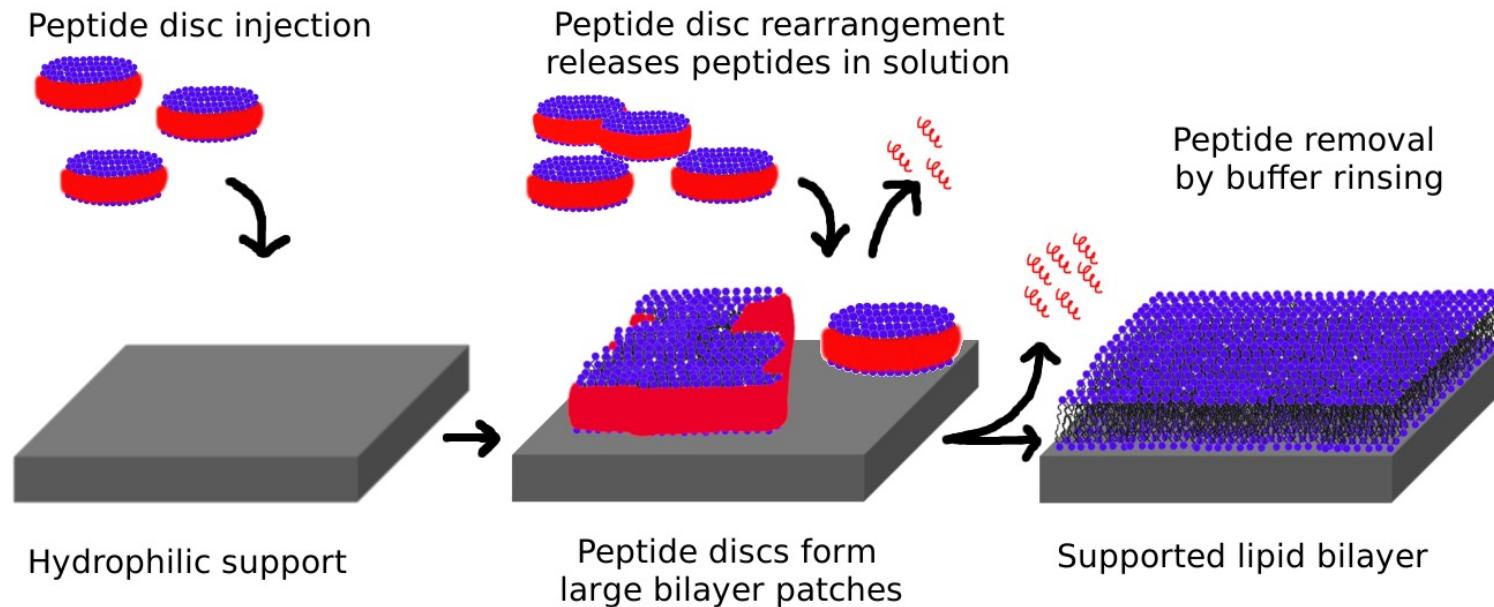


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Small Sample Polarized Reflectometer



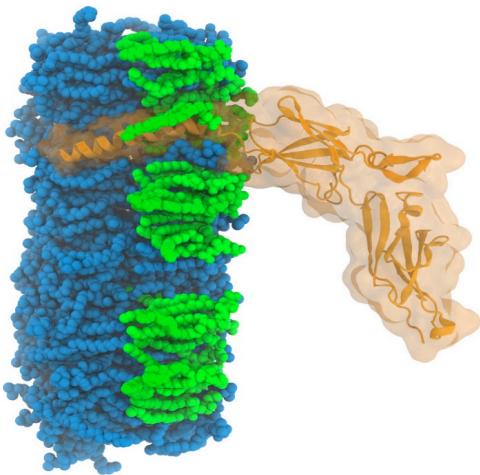
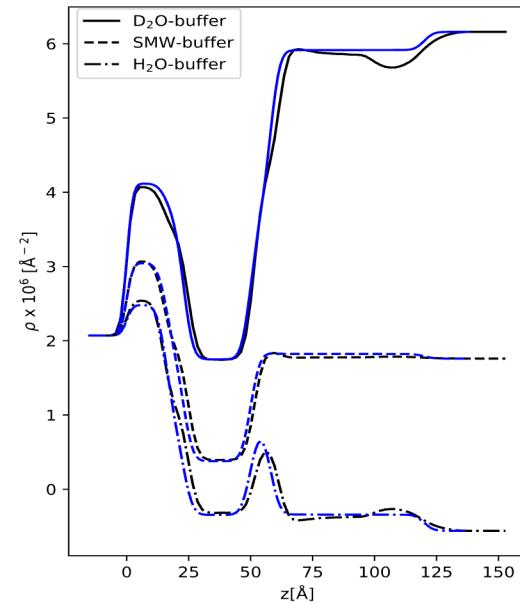
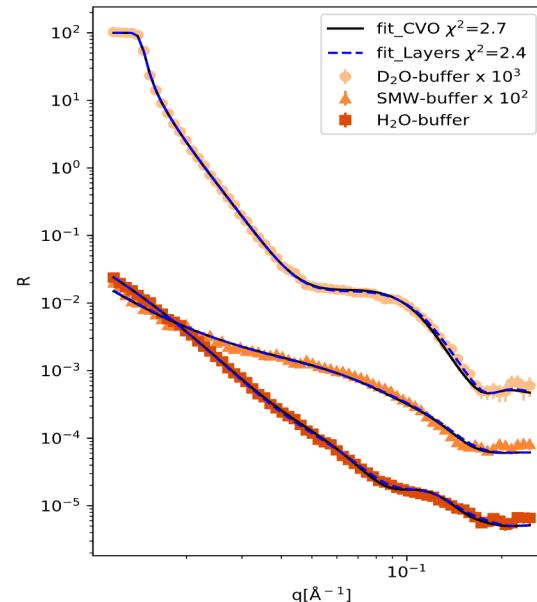
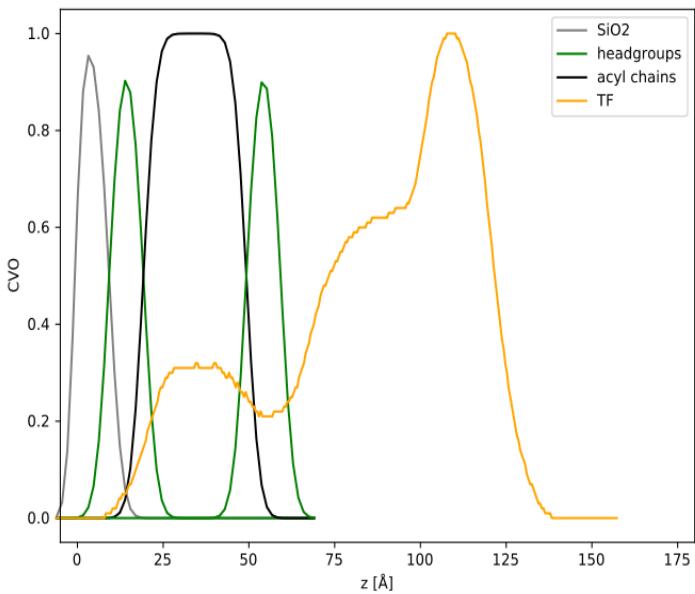
Peptide disc mediated formation of supported lipid bilayer with oriented membrane proteins



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- Surface Plasmon Resonance (SPR)
- Atomic Force Microscopy (AFM)
- **Attenuated total reflectance – Fourier transform IR spectroscopy (ATR-FTIR)**

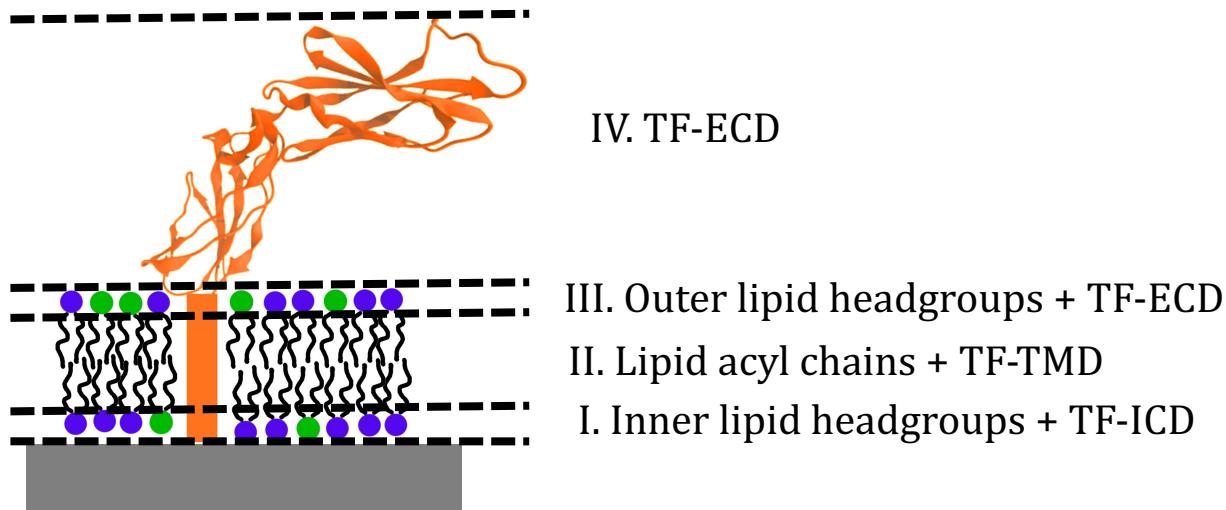
Combining MD and NR

MD simulation can support NR data analysis and provide complementary information



Supported lipid bilayer with oriented TF molecules

Using independent information about sample structure and composition in NR model



Layer parameters:

- Thickness
- **Scattering length density**
- Solvent volume fraction
- roughness

In layer II $\longrightarrow \rho_{II} = \phi_{acylchain}\rho_{acylchain} + \phi_{TF-TMD}\rho_{TF-TMD} + \phi_{solvent}\rho_{solvent}$

In layer IV $\longrightarrow \rho_{IV} = \phi_{TF-ECD}\rho_{TF-ECD} + \phi_{solvent}\rho_{solvent}$

$$\phi_{TF-ECD} = \frac{n_{TF}V_{ECD}}{V_{IV}}$$
$$\phi_{TF-TMD} = \frac{n_{TF}V_{TMD}}{V_{II}} = \frac{V_{TMD}t_{IV}\phi_{TF-ECD}}{V_{ECD}t_{II}}$$

Same number of TF molecules in layer IV and II

Supported lipid bilayer with oriented TF molecules

