Biophysical studies – key for exploiting the potential of neutrons

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α-synuclein-membrane interactions

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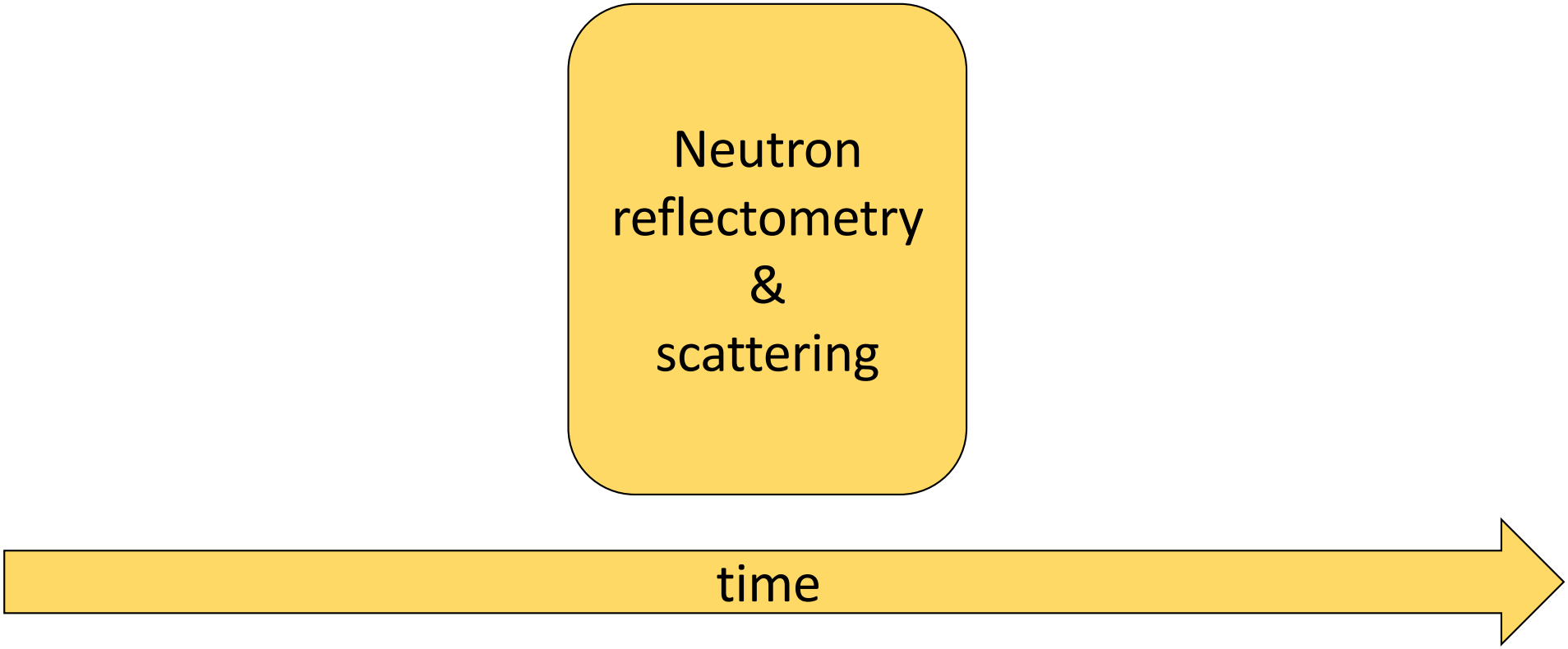
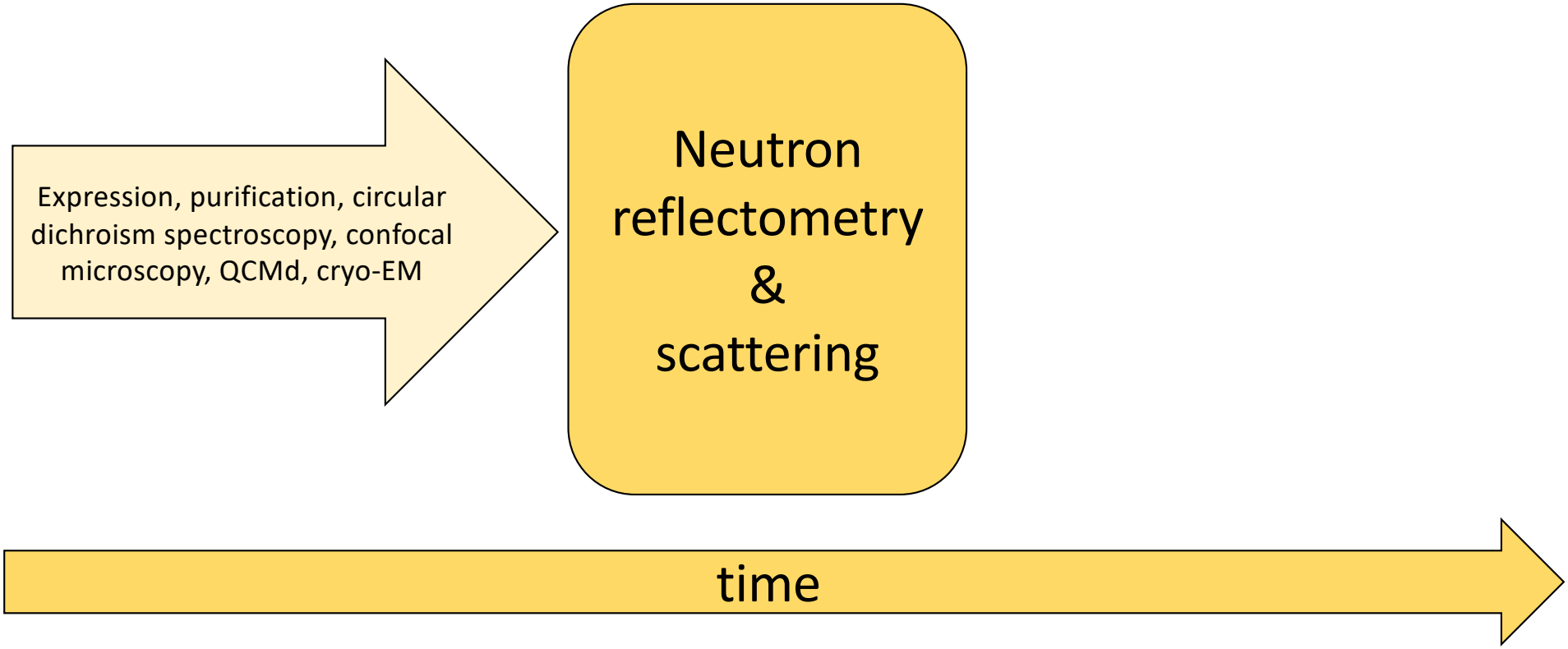
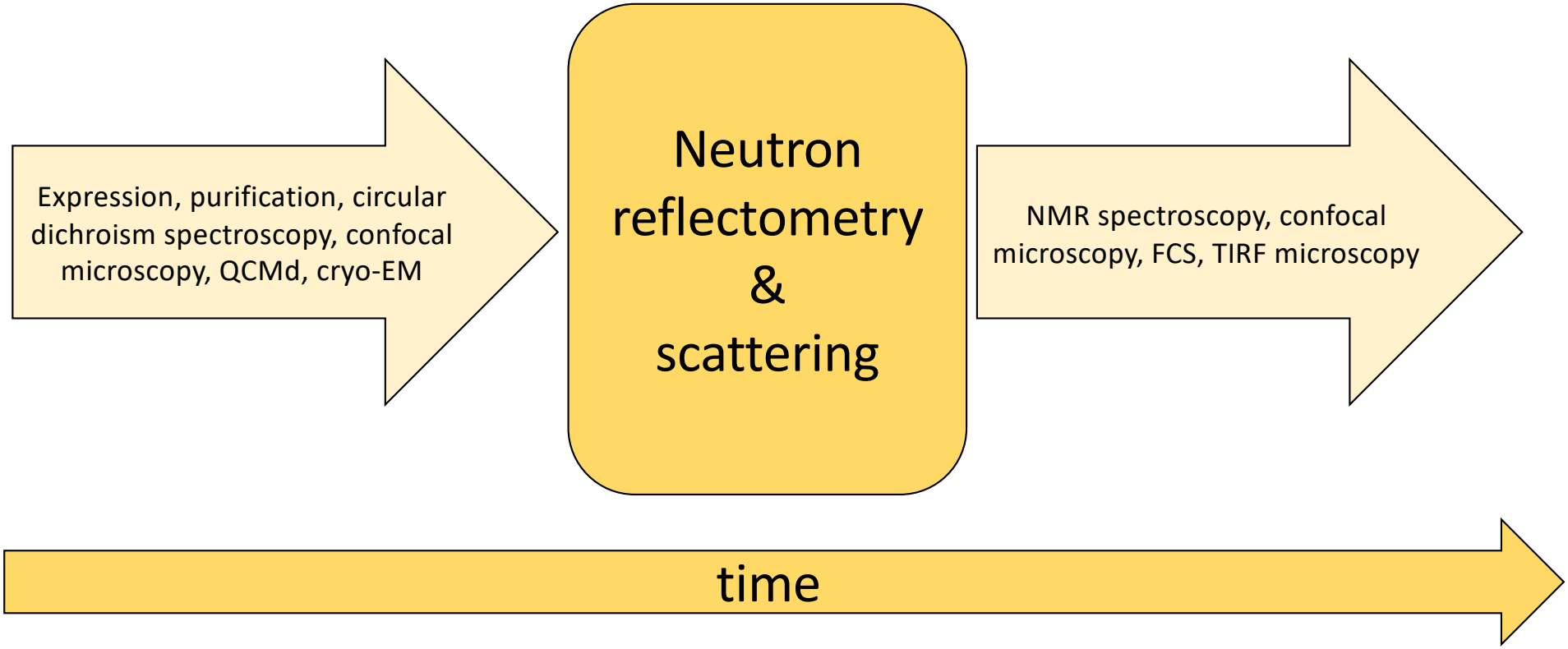
α-synuclein-membrane interactions



α-synuclein-membrane interactions



α-synuclein-membrane interactions



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Trevor Forsyth (ILL)
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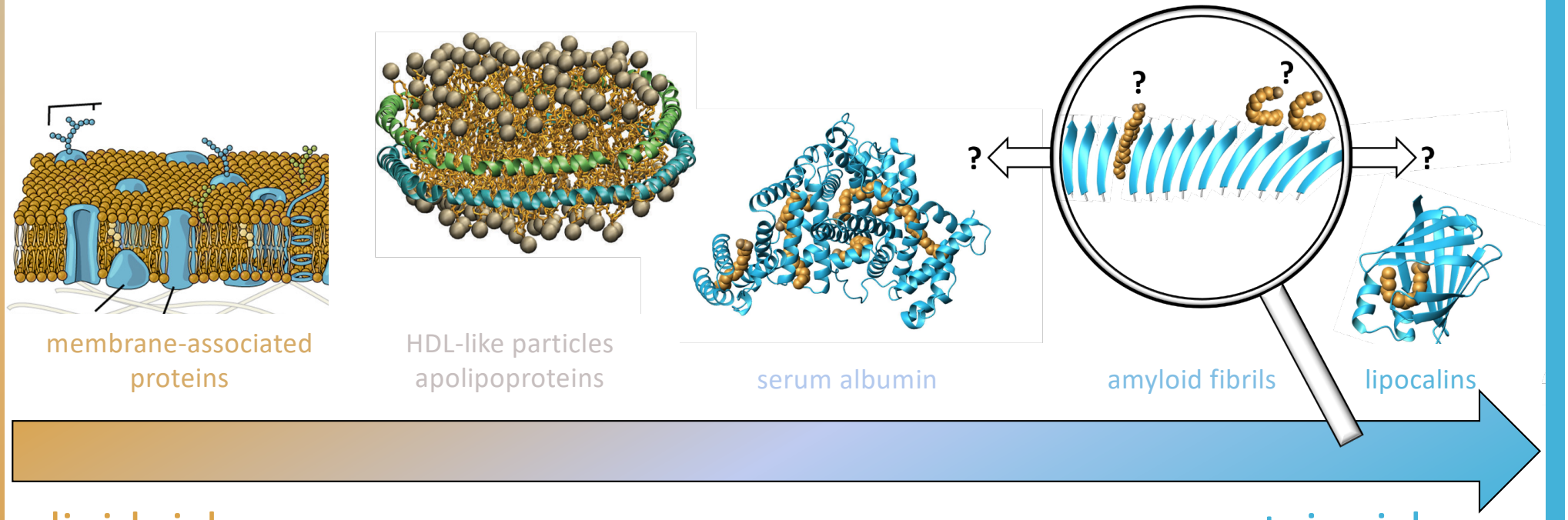
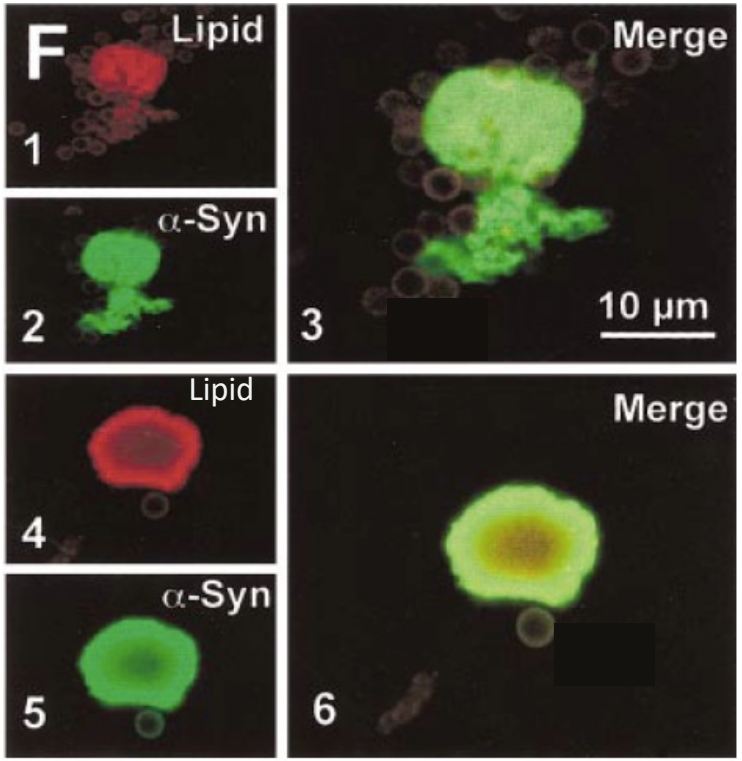
Lipid-protein co-aggregation – from lipid-rich to peptide-rich



Experimental Neurology 166, 324–333 (2000)
doi:10.1006/exnr.2000.7527, available online at http://www.idealibrary.com on IDEAL®

In Situ and *in Vitro* Study of Colocalization and Segregation of α-Synuclein, Ubiquitin, and Lipids in Lewy Bodies

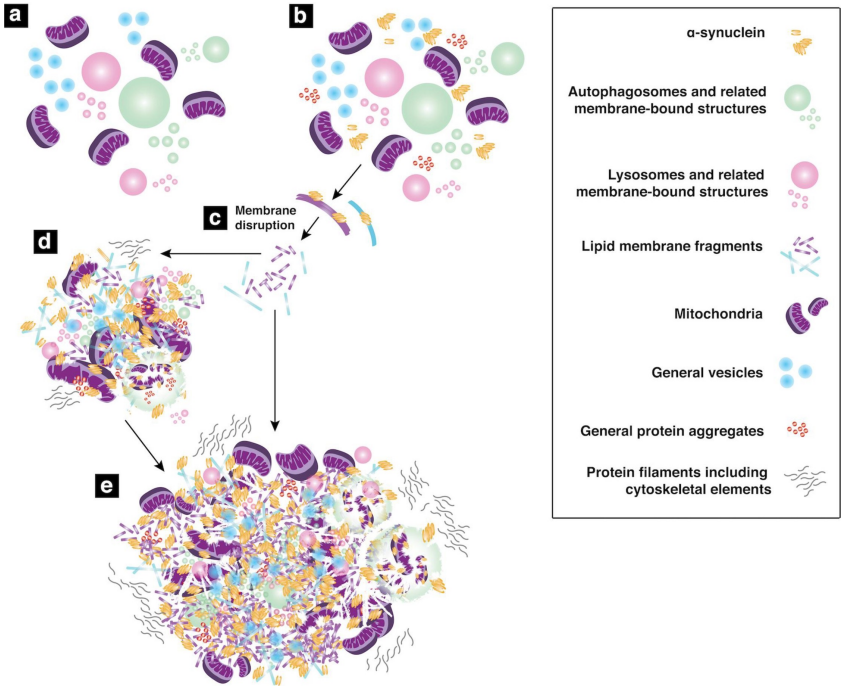
W. P. Gai,* H. X. Yuan,* X. Q. Li,† J. T. H. Power,* P. C. Blumbergs,‡ and P. H. Jensen§



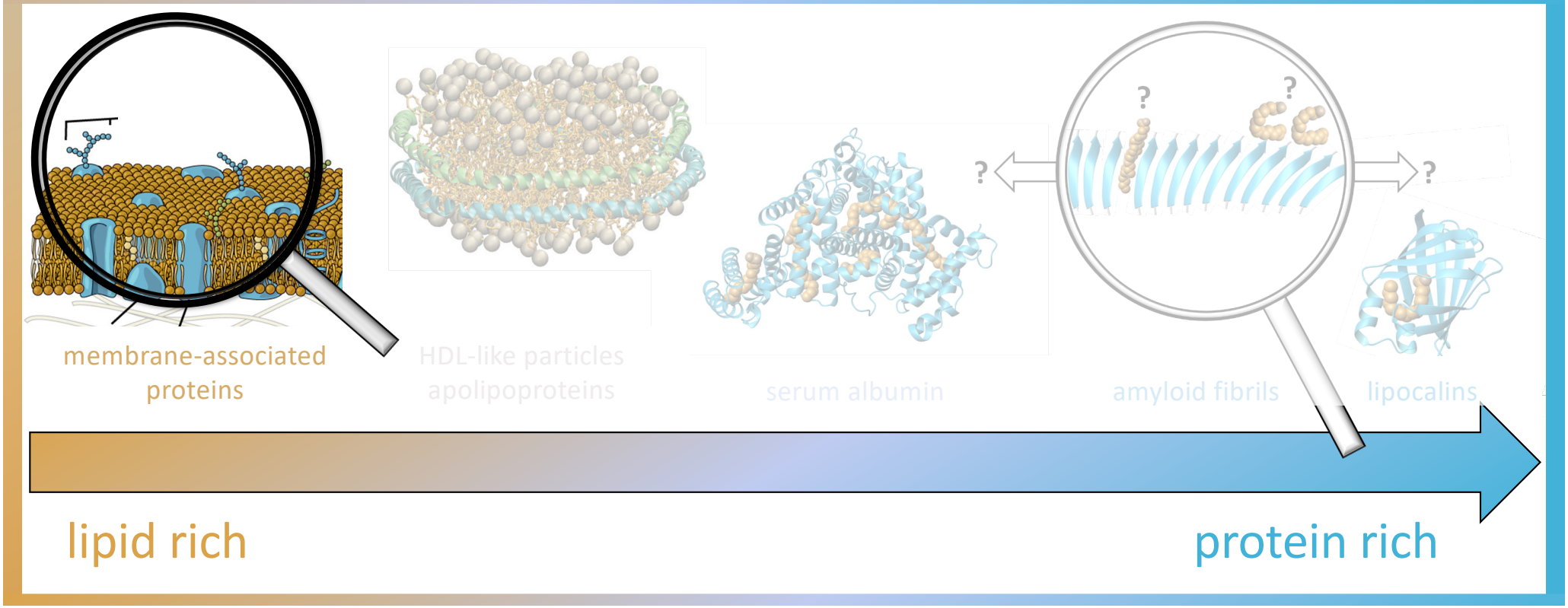
nature neuroscience
ARTICLES
https://doi.org/10.1038/s41593-019-0423-2

Lewy pathology in Parkinson's disease consists of crowded organelles and lipid membranes

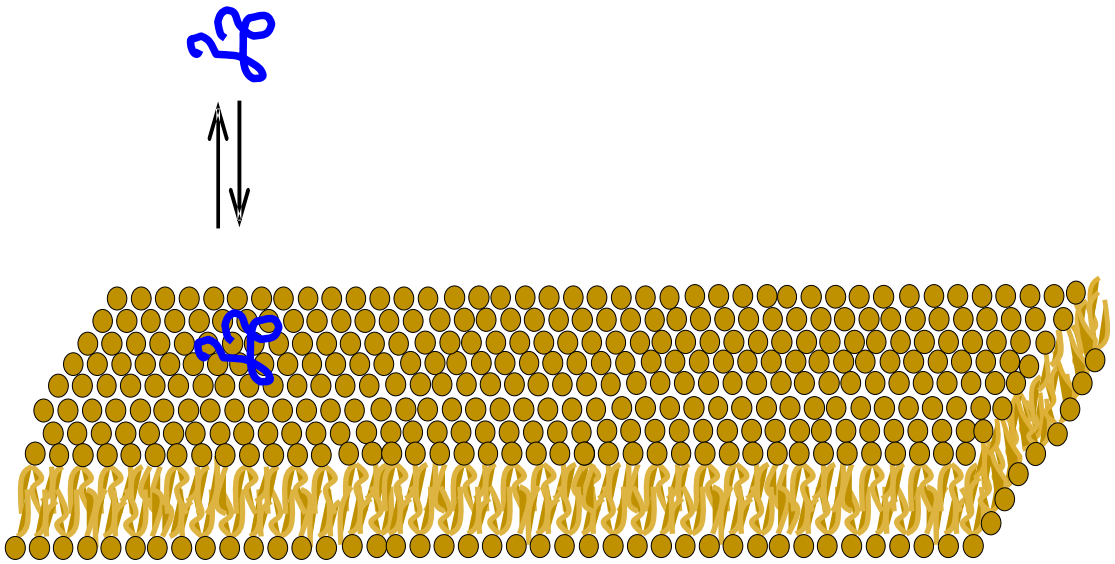
NATURE NEUROSCIENCE | VOL 22 | JULY 2019 | 1099–1109 | www.nature.com/natureneuroscience



Lipid-protein co-aggregation – from lipid-rich to peptide-rich

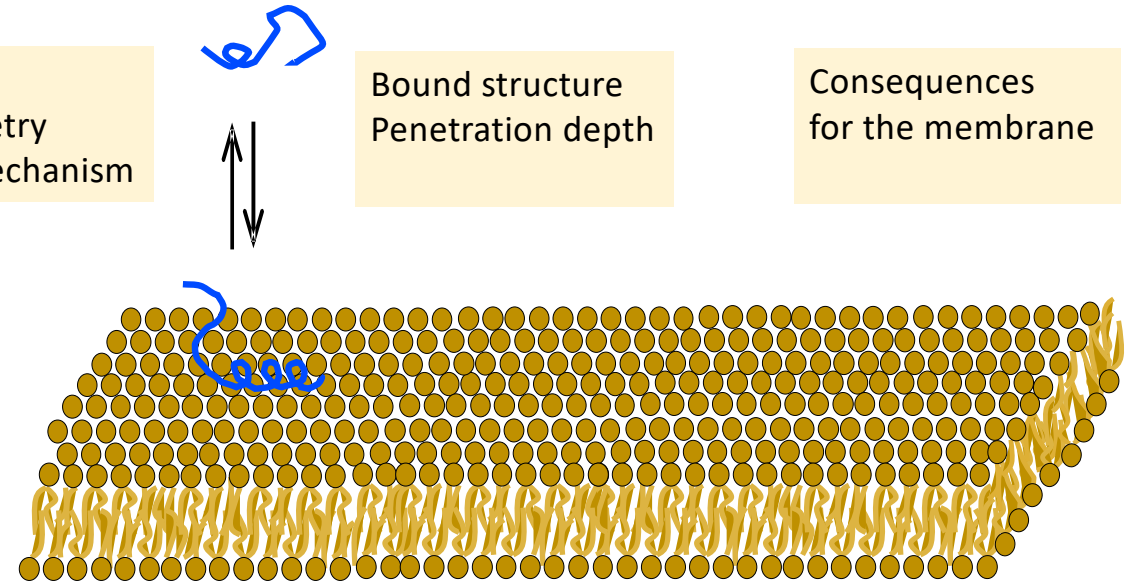


α-synuclein-membrane interactions



α-synuclein-membrane interactions

Affinity
Stoichiometry
Binding mechanism



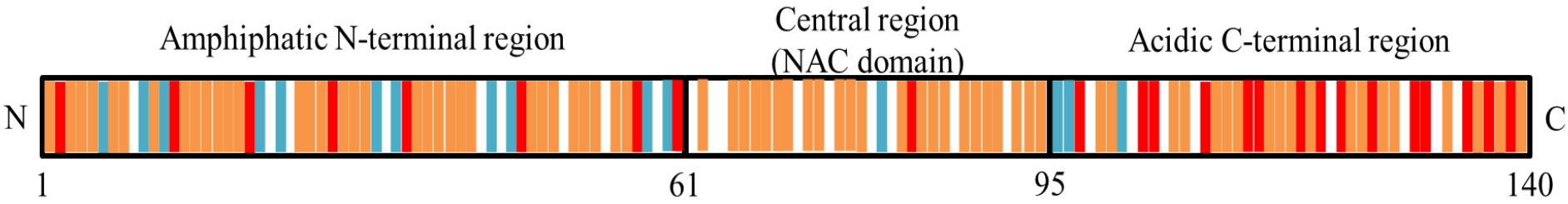
Bound structure
Penetration depth

Consequences
for the membrane

Consequences
for the protein

Biological
Consequences?

α-synuclein



Repeat 1 Repeat 2 Repeat 3 Repeat 4

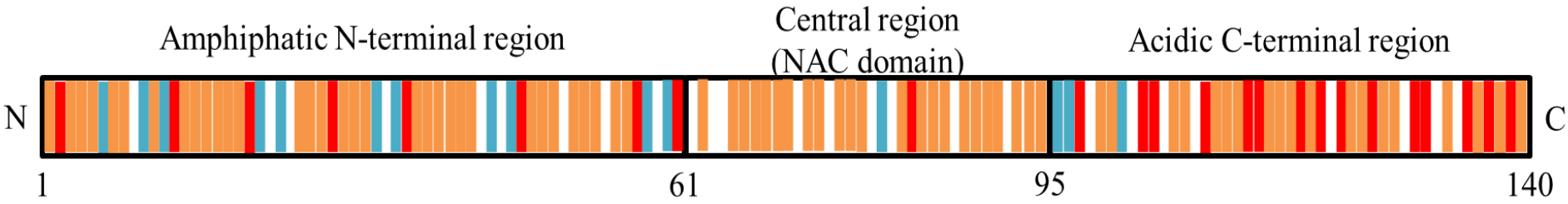
MDVFMKGLSK AKEGVVAAAE KTKQGVAEAA GKTKEGVLYV GSKTKEGVVH (50)

Repeat 5 Repeat 6 Repeat 7

GVATVAEKTK EQVTNVGGAV VTGVTAVAQK TVEGAGSIAA ATGFVKKDQL (100)

GKNEEGAPQE GILEDMPVDP DNEAYEMPSE EGYQDYEPEA (140)

α-synuclein



Repeat 1 Repeat 2 Repeat 3 Repeat 4

MDVFMKGLSK AKEGVVAAAE KTKQGVAEAA GKTKEGVLYV GSKTKEGVVH (50)

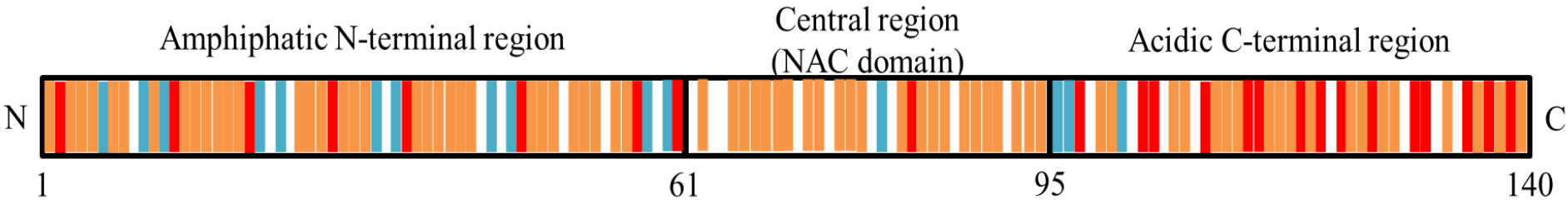
Repeat 5 Repeat 6 Repeat 7

GVATVAEKTK EQVTNVGGAV VTGVTAVAQK TVEGAGSIAA ATGFVKKDQL (100)

GKNEEGAPQE GILEDMPVDP DNEAYEMPSE EGYQDYEPEA (140)

Express in *E. coli* as is with no tags. Purify using boiling, anion exchange and size exclusion chromatography.

α-synuclein

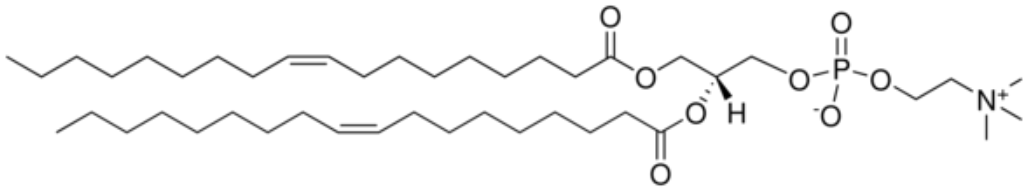


Purify until
No other protein bands seen on silver-stained SDS PAGE
No small molecule contaminants seen by NMR spectrosocpy

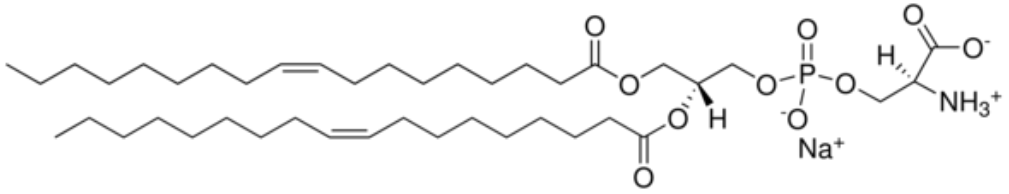
Validate
Correct sequence by mass spectrometry
No modifications by mass spectrometry

Express in *E. coli* as is with no tags. Purify using boiling, anion exchange and size exclusion chromatography.

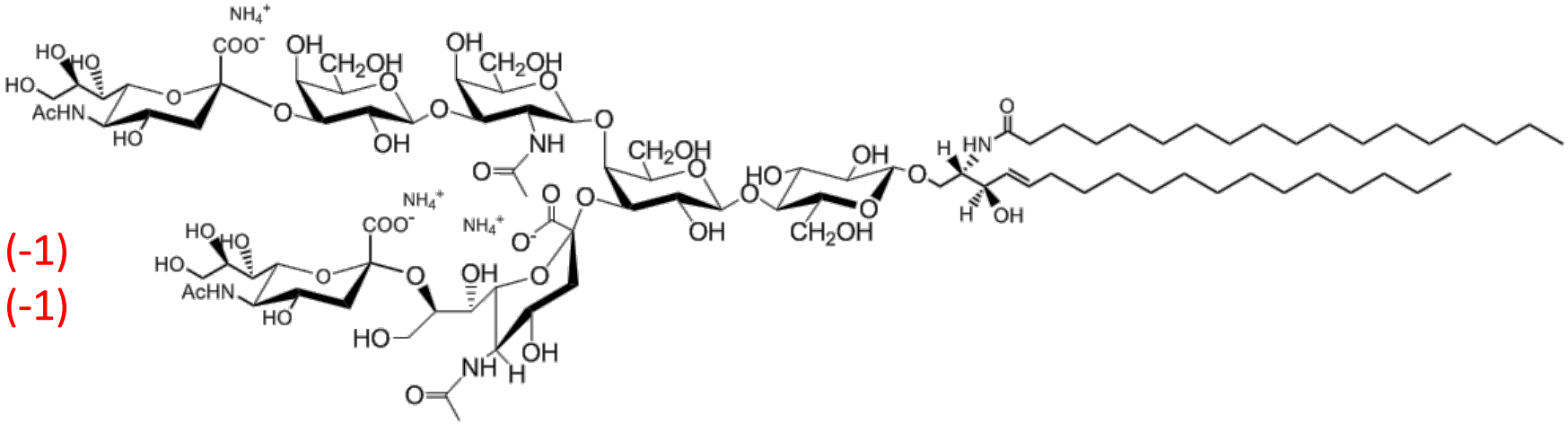
Lipid model systems



DOPC (0)



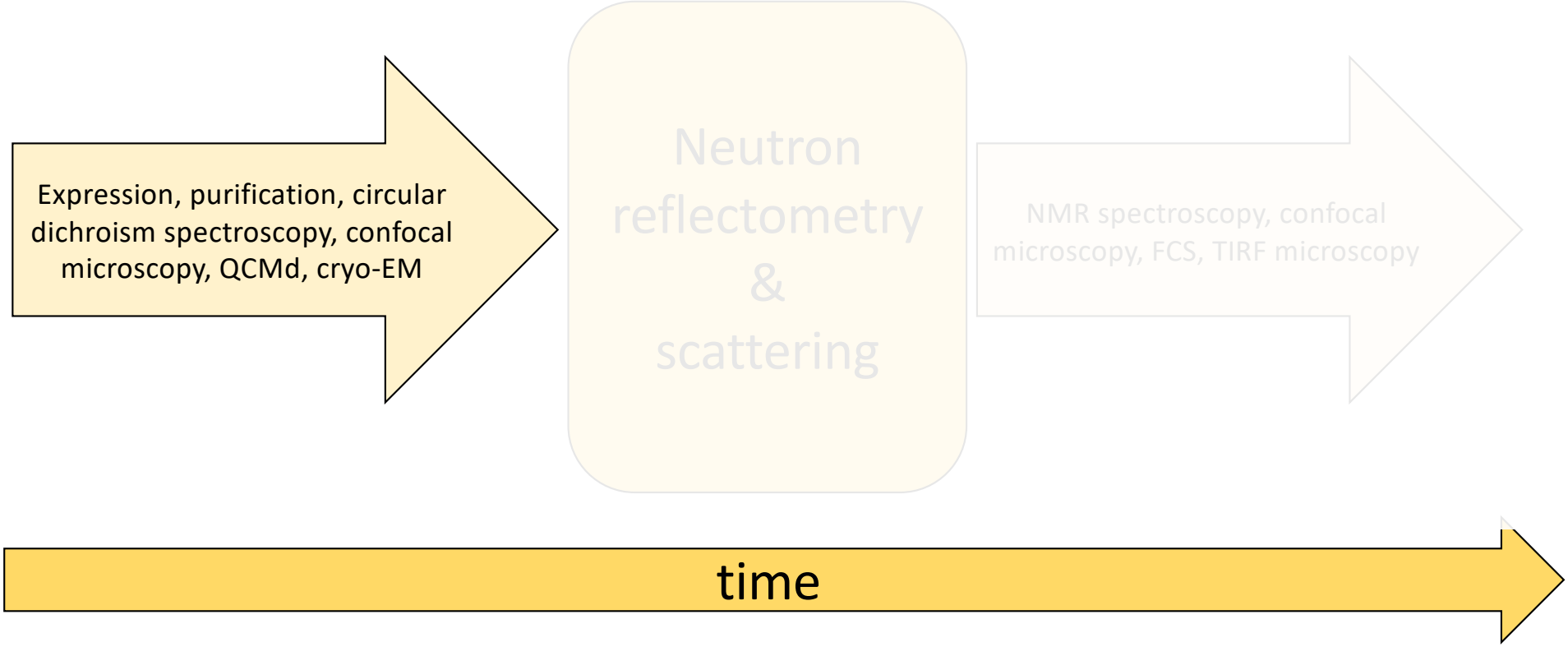
DOPS (-1)



GM1 (-1)

GM3 (-1)

α-synuclein-membrane interactions



Protein adsorption to membranes – before neutrons

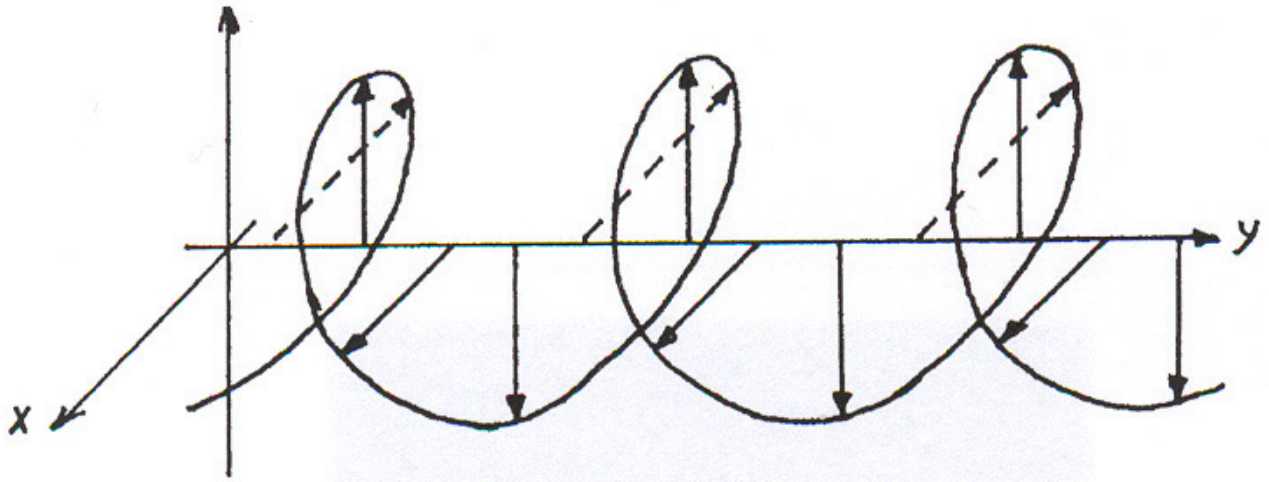
Circular dichroism (CD) spectroscopy

Quartz crystal microbalance

Confocal fluorescence microscopy

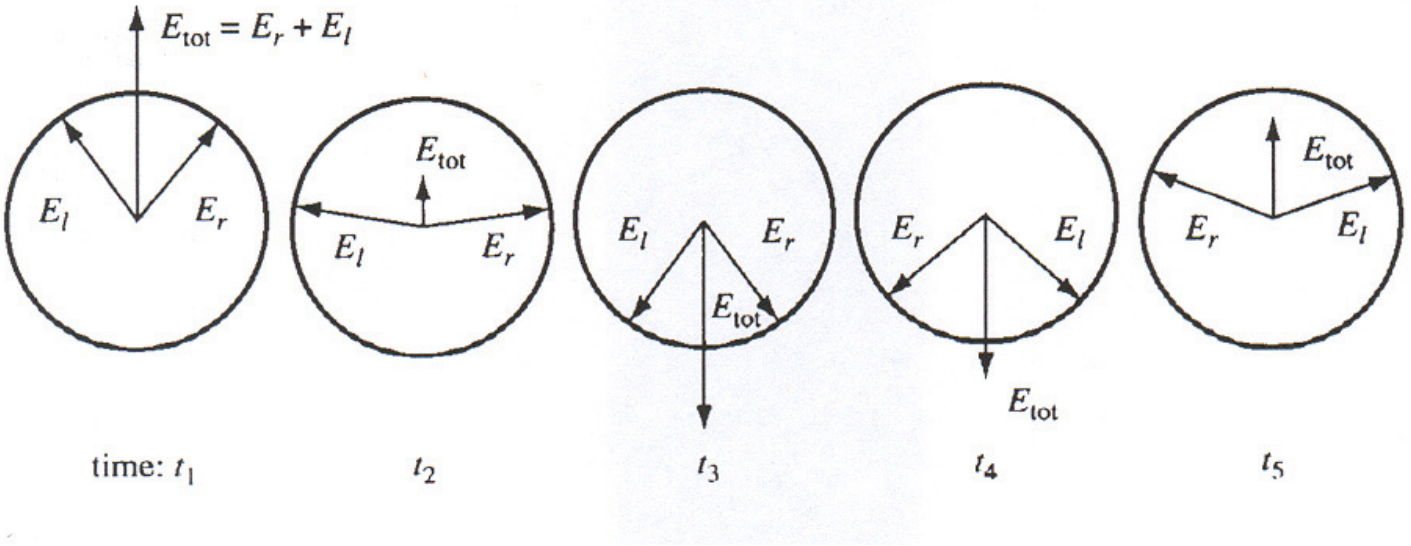
CD

Circularly polarized light

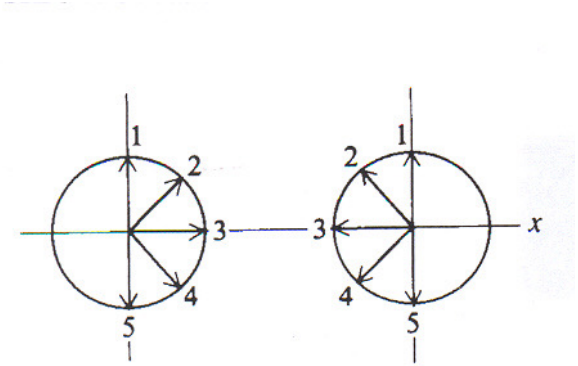


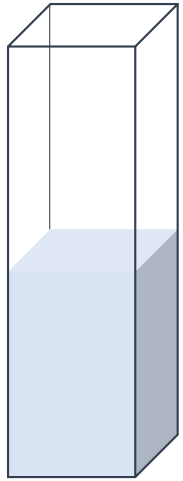
CD

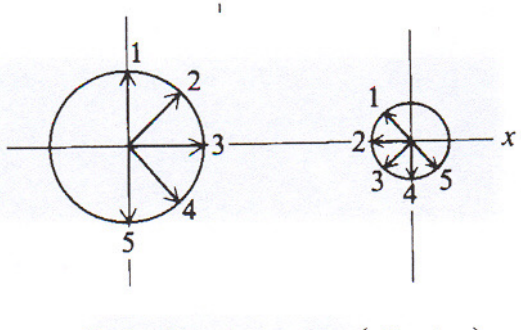
Circularly polarized light

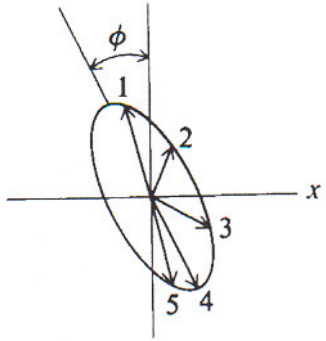


CD









(right) (left) (sum)

Before sample

After sample

CD

Spectral ranges for CD

Near-UV CD: 250-320 nm

Aromatic sidechains and disulfide bonds

1 cm cuvette, 1-3 mg/mL

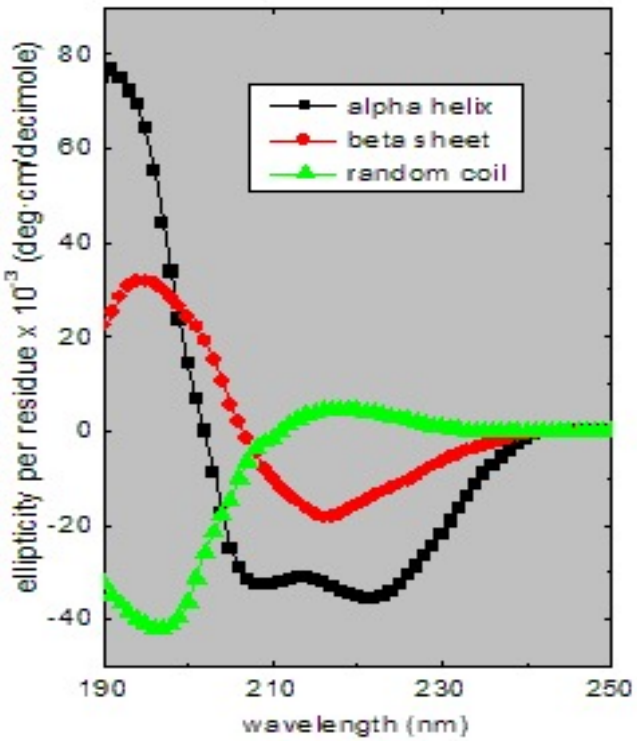
Far-UV CD: 185-250

Peptide backbone

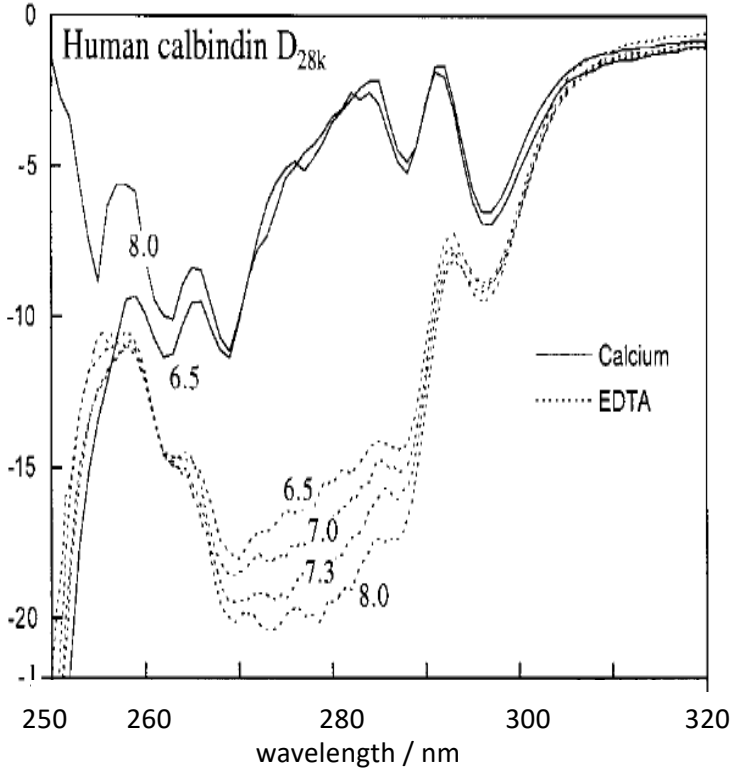
1 mm cuvette, 0.1-0.3 mg/mL

CD

Far-UV CD

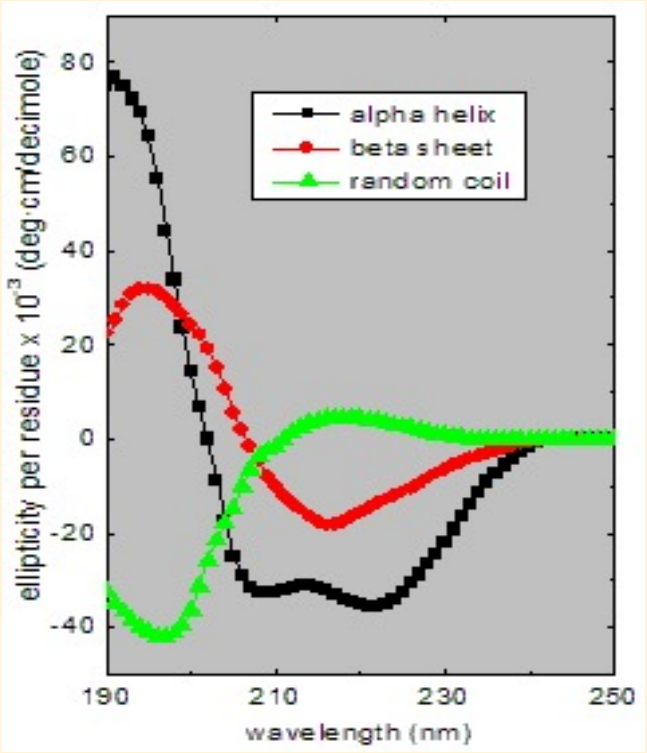


Near-UV CD

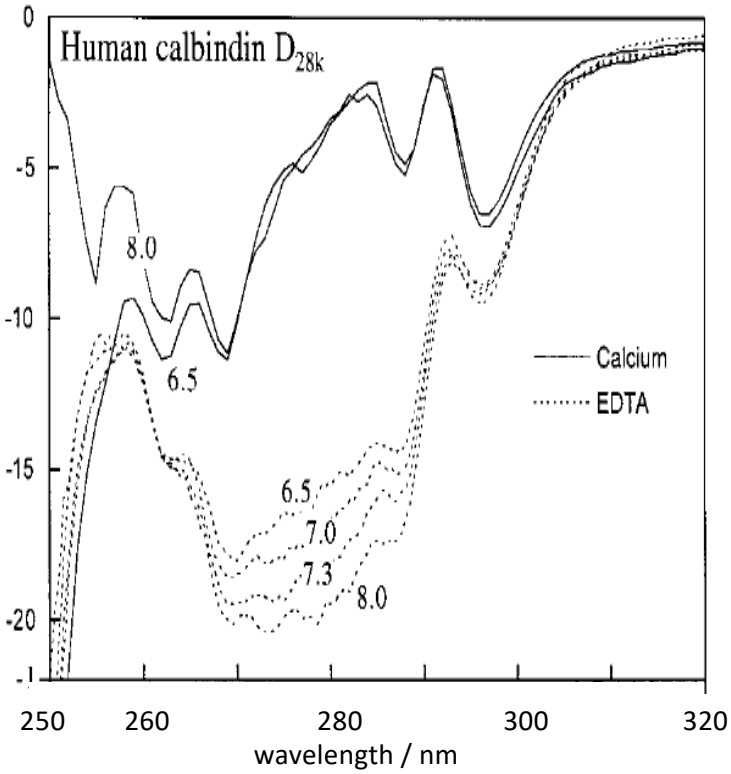


CD

Far-UV CD



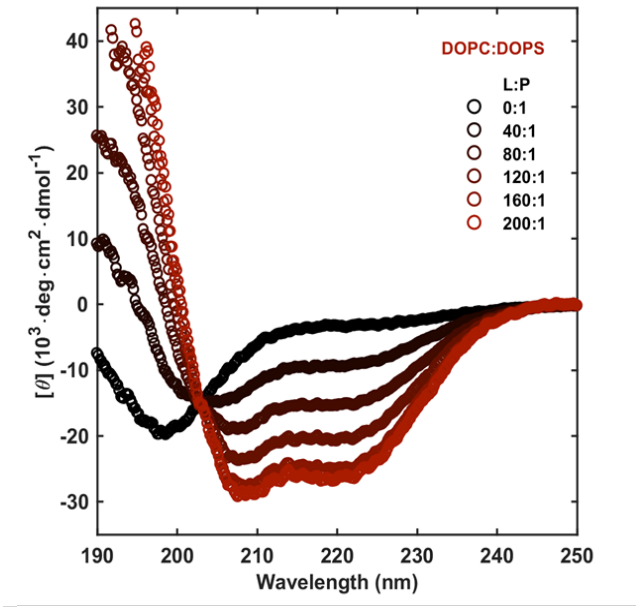
Near-UV CD

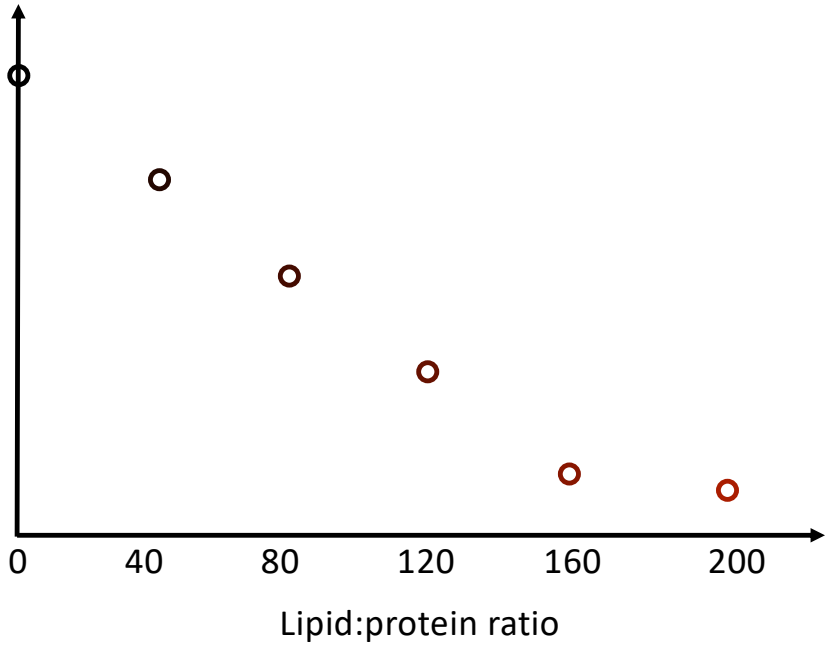


CD

Protein adsorption to membranes

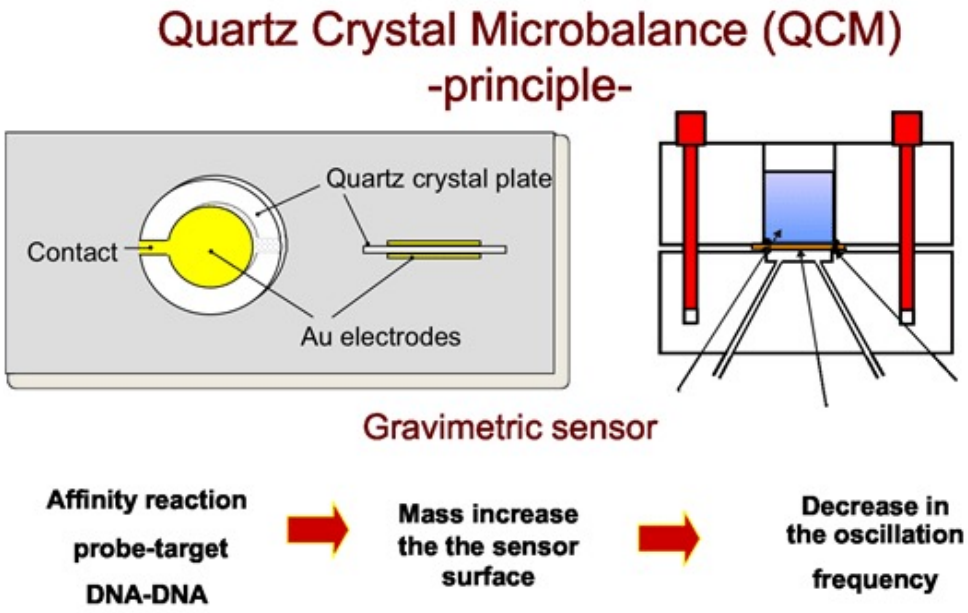
CD spectra of α-syn with gradually increasing amount of membranes

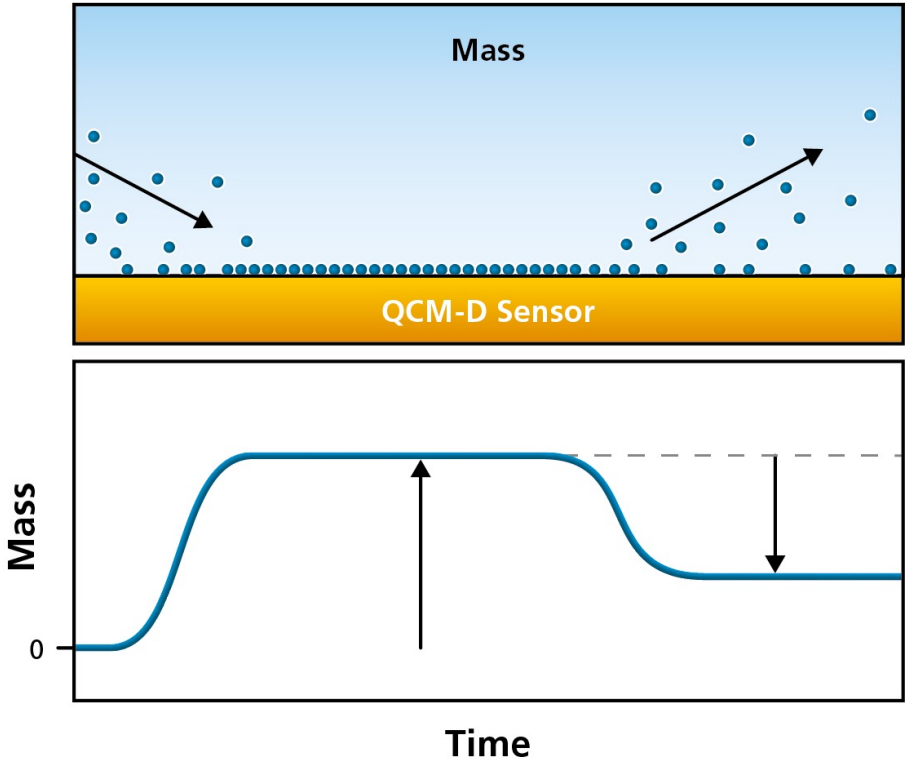




QCMd

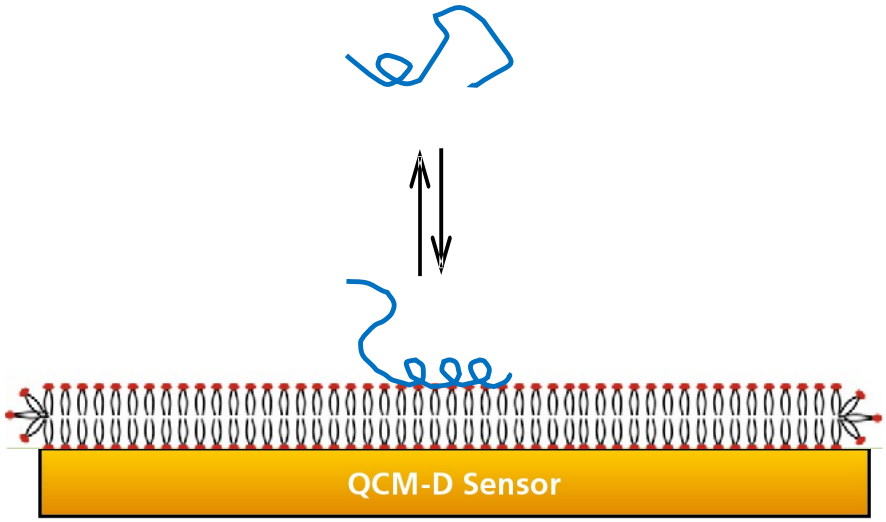
Protein adsorption to membranes

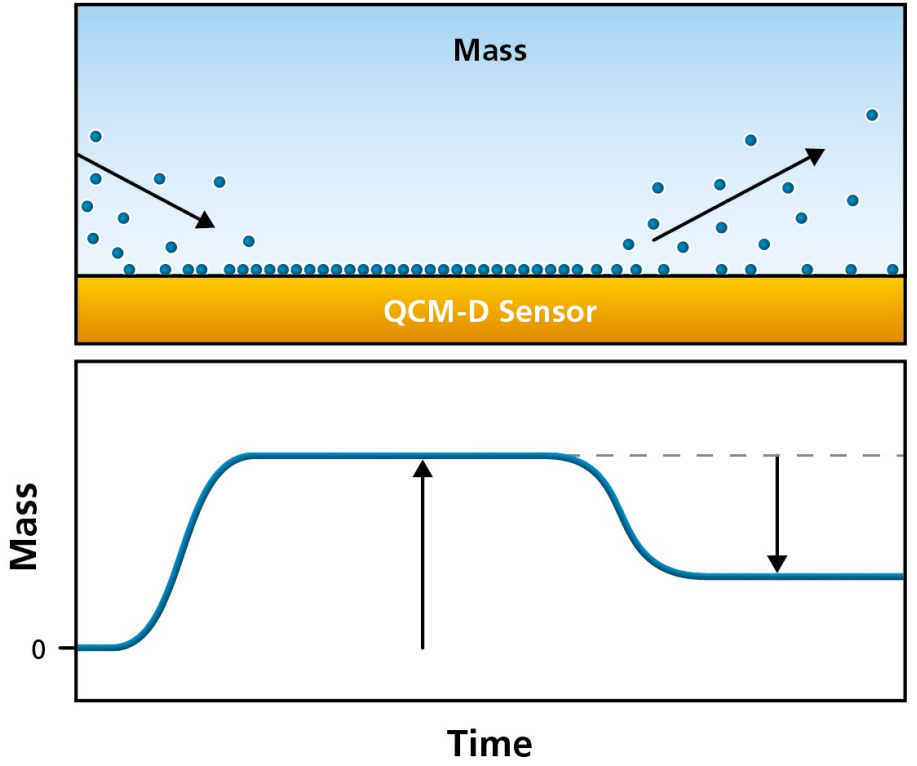




QCMd

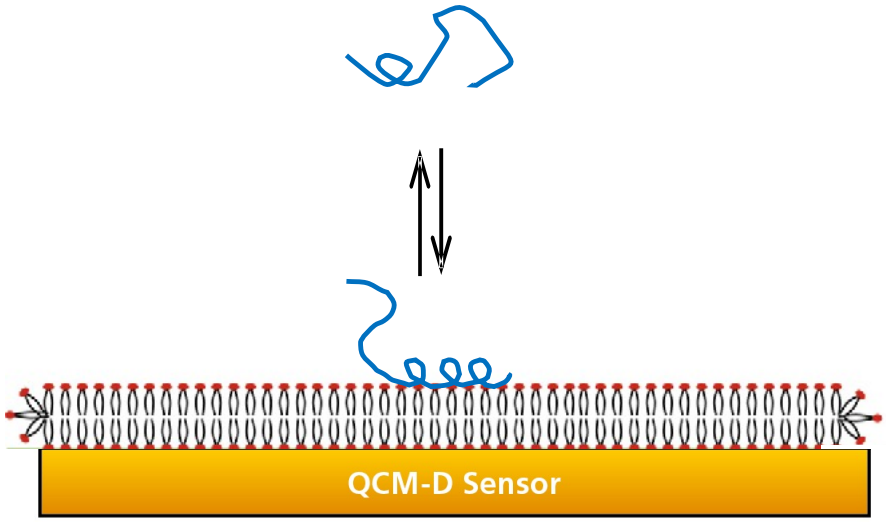
Protein adsorption to membranes



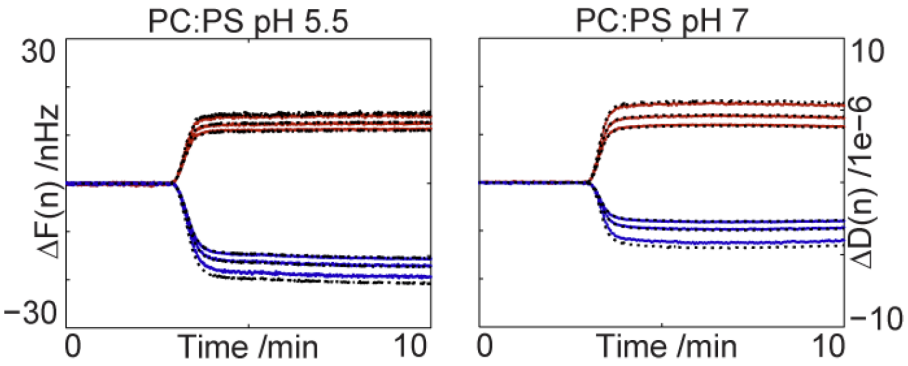


QCMd

Protein adsorption to membranes

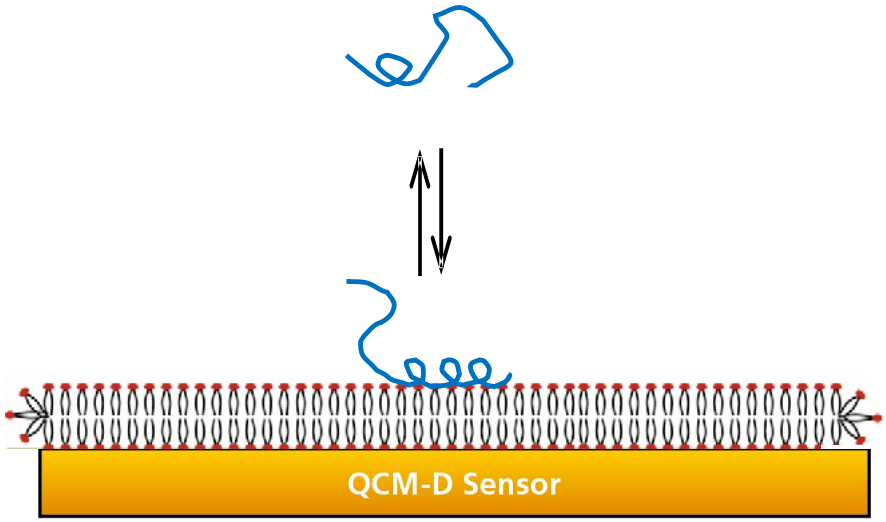


Adsorption to negatively charged membranes

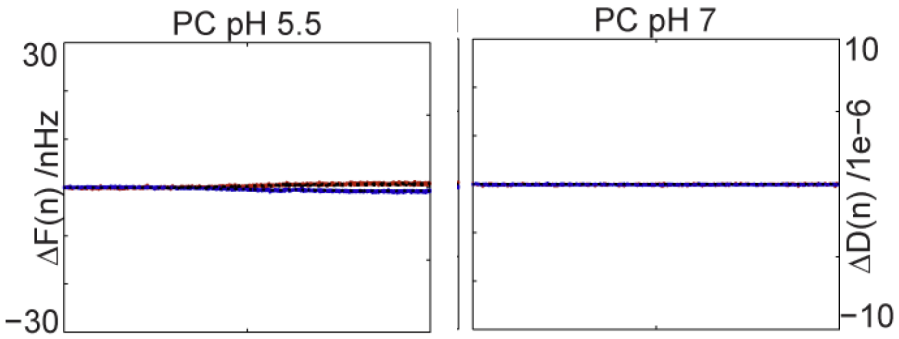


QCMd

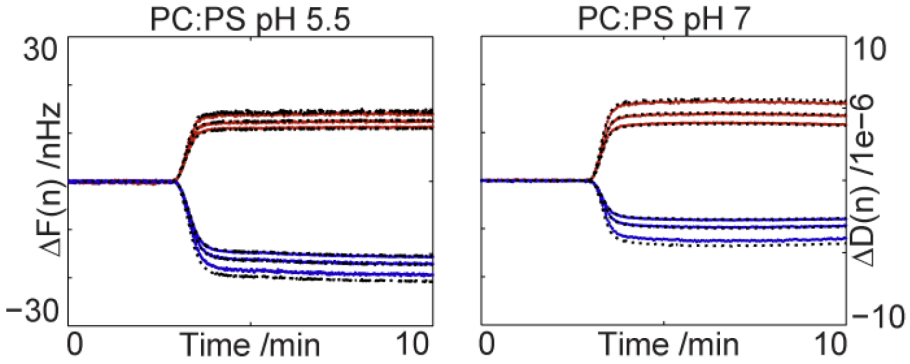
Protein adsorption to membranes



No or little adsorption to neutral membranes

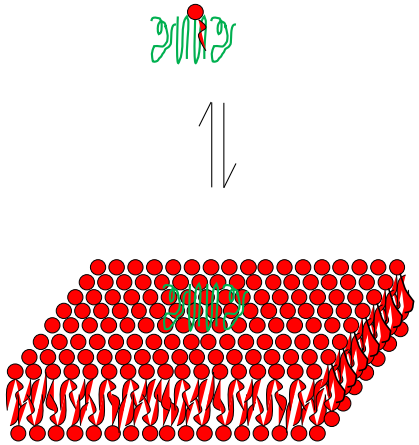


Adsorption to negatively charged membranes

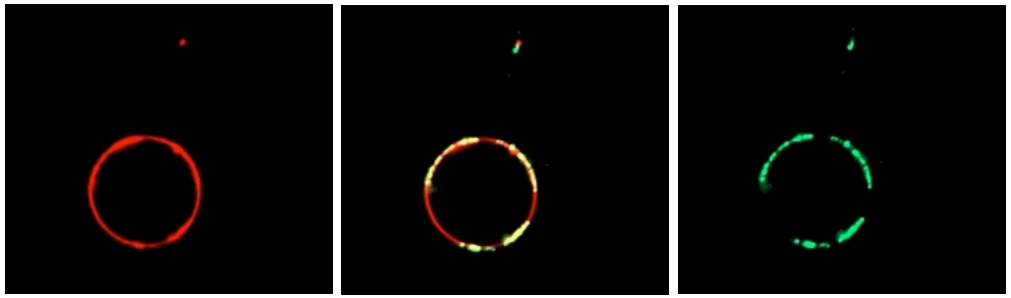


Interactions with model membranes
Confocal microscopy

α-syn on pathway



DOPC + 10% cardiolipin



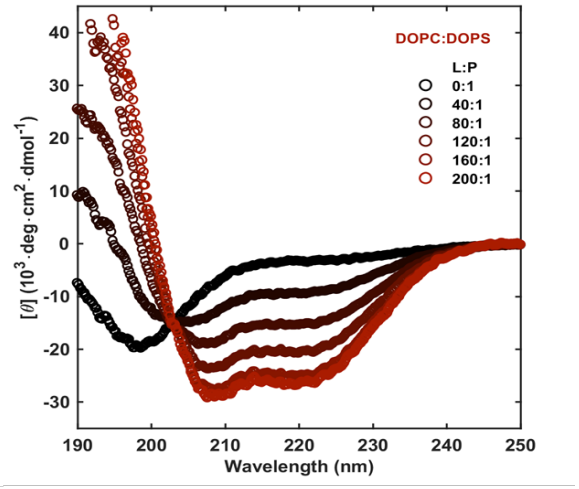
Lipid protein colocalization!

Grey et al, JPD 2011

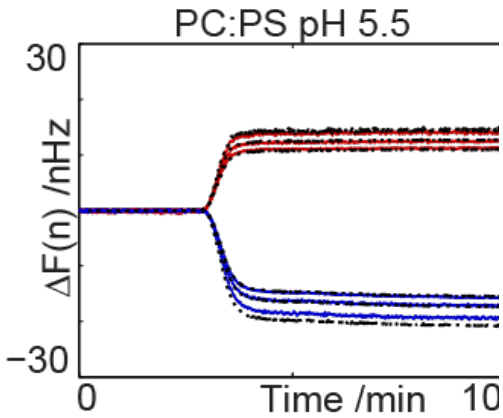
Protein adsorption

- Adsorption to anionic membranes. No adsorption detected for zwitterionic bilayers
- Protein forms an α-helix at the membrane
- Only N-terminal and central part of the protein in contact with the membrane (NMR)
- C-terminal part of the protein disordered and more extended into solution

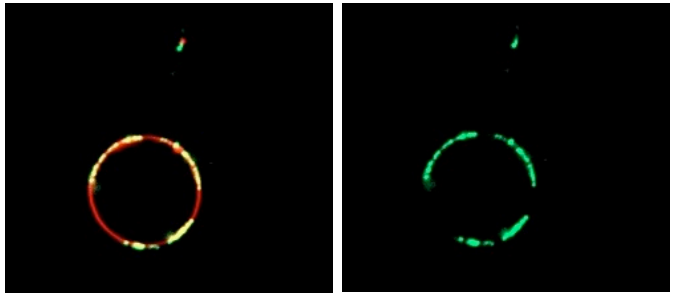
CD spectra



Quartz crystal microbalance

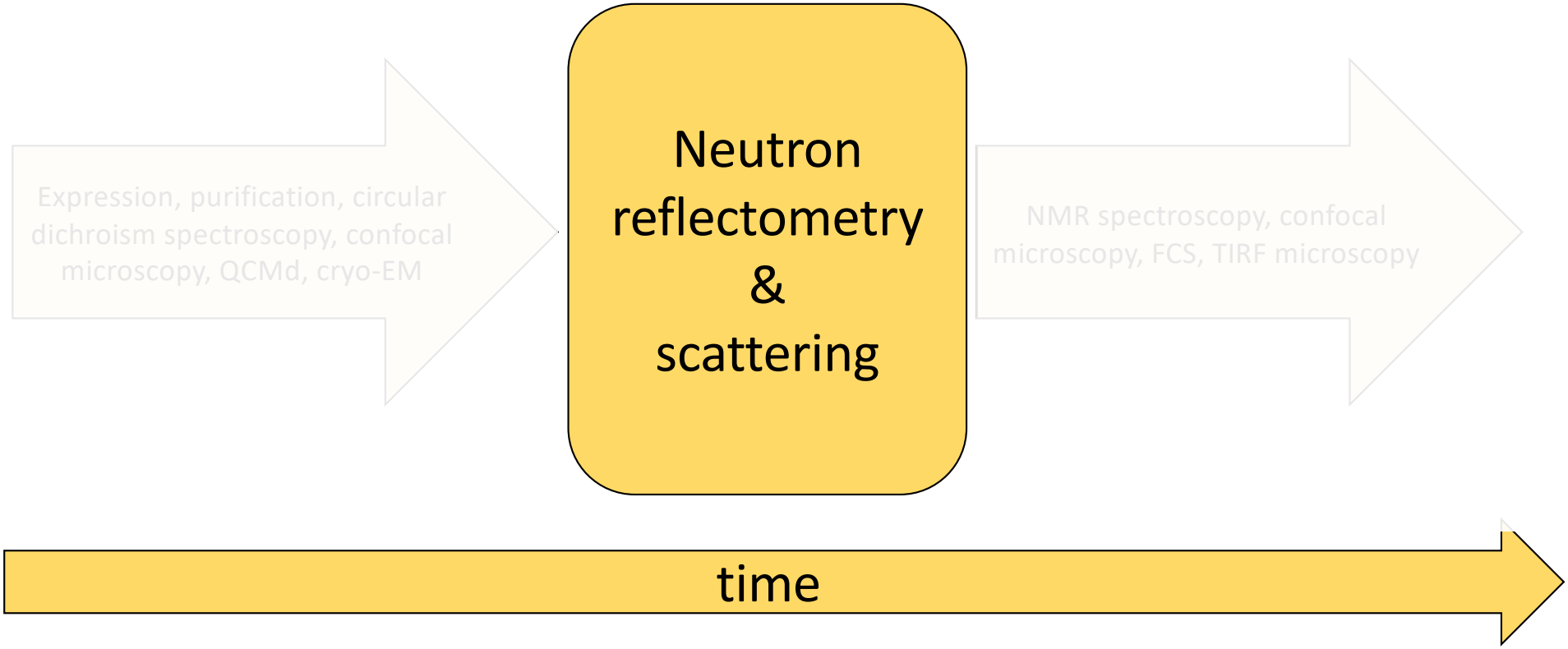


Confocal fluorescence microscopy



Hellstrand et al. ACS Neuroscience (2013); Gaspar et al, BBA (2018), Gaspar et al. (Manuscript)

α-synuclein-membrane interactions

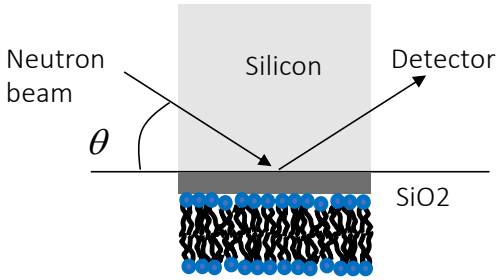


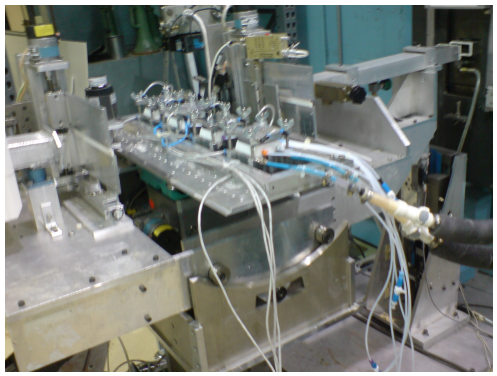
Neutron scattering and neutron reflectometry studies of protein-lipid co-assembly

- Structure including penetration depth?
- How to address with neutrons?
- How to address with other methods?
- Consequences?

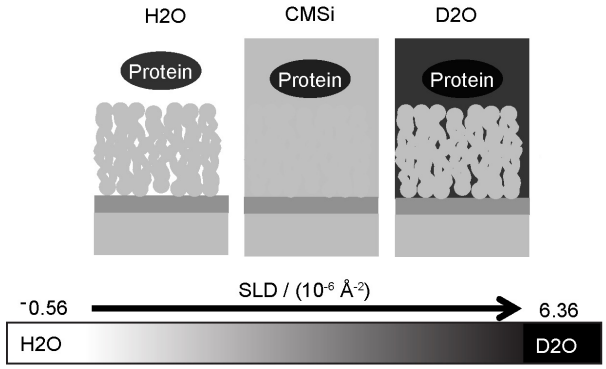
Penetration depth and membrane perturbation?
(ISIS, ILL & SNS)

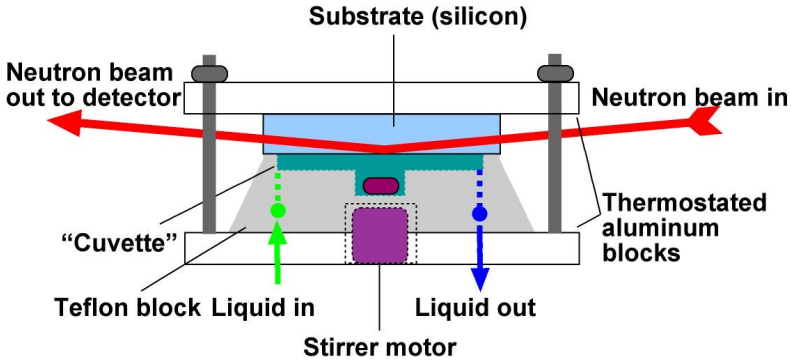
Neutron reflectometry on supported lipid bilayer





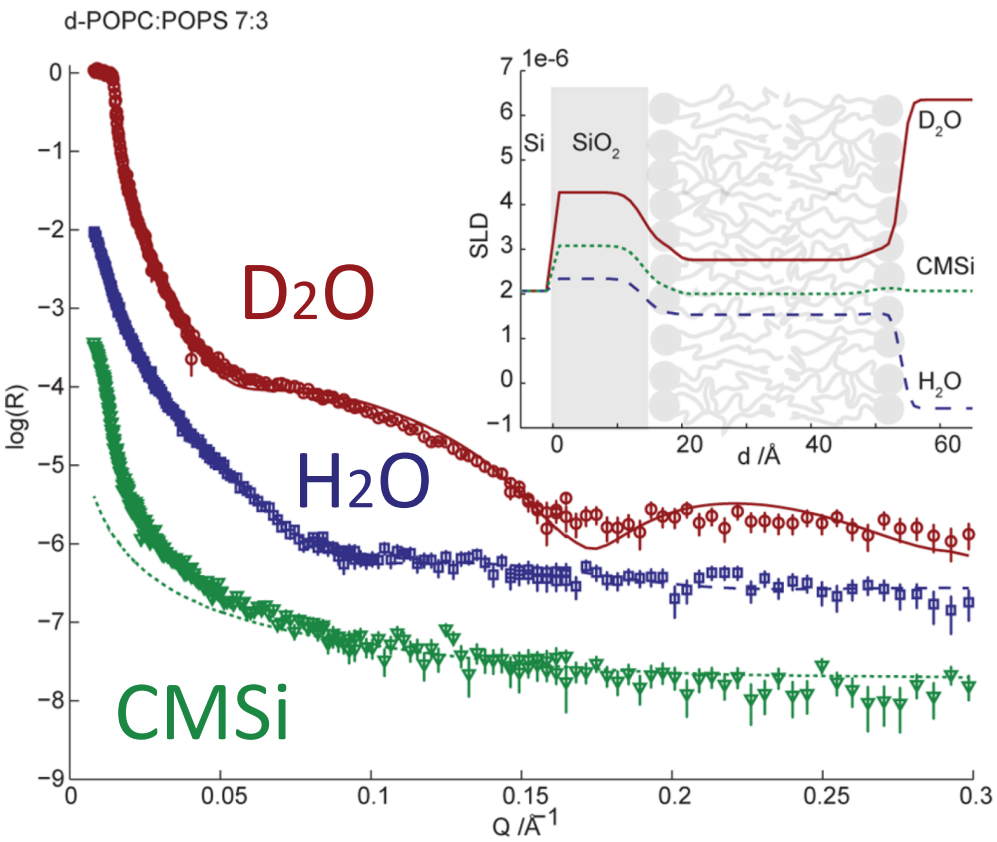
Contrast matching

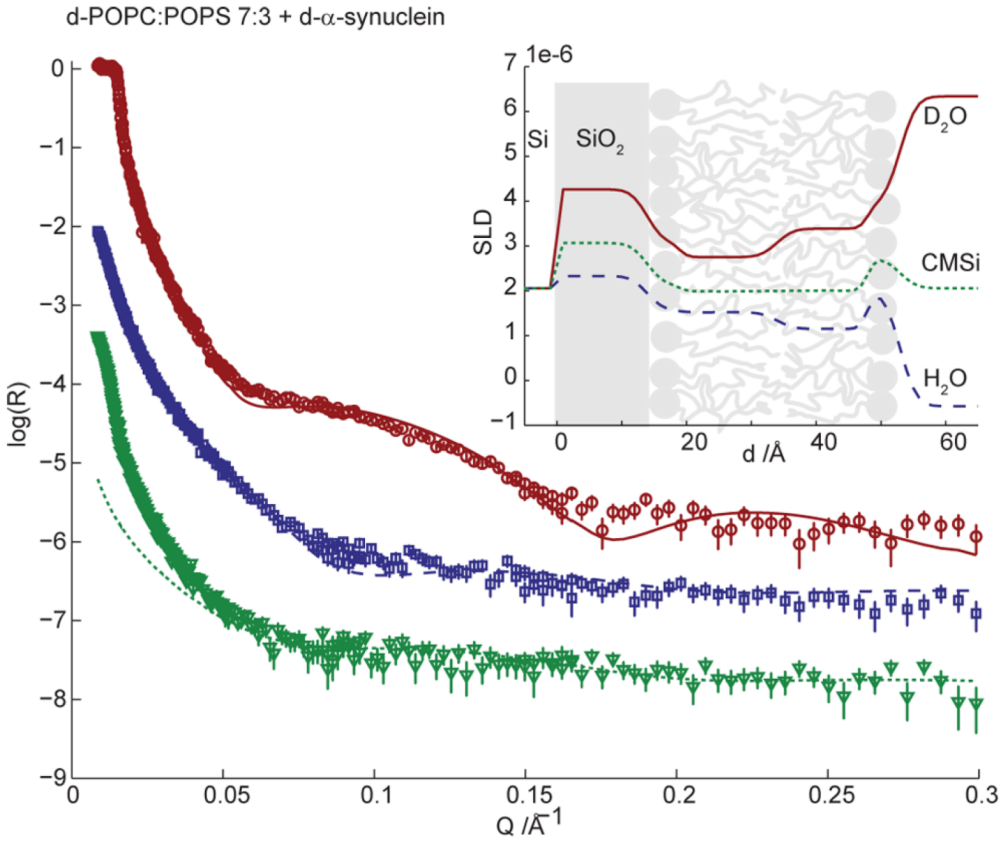




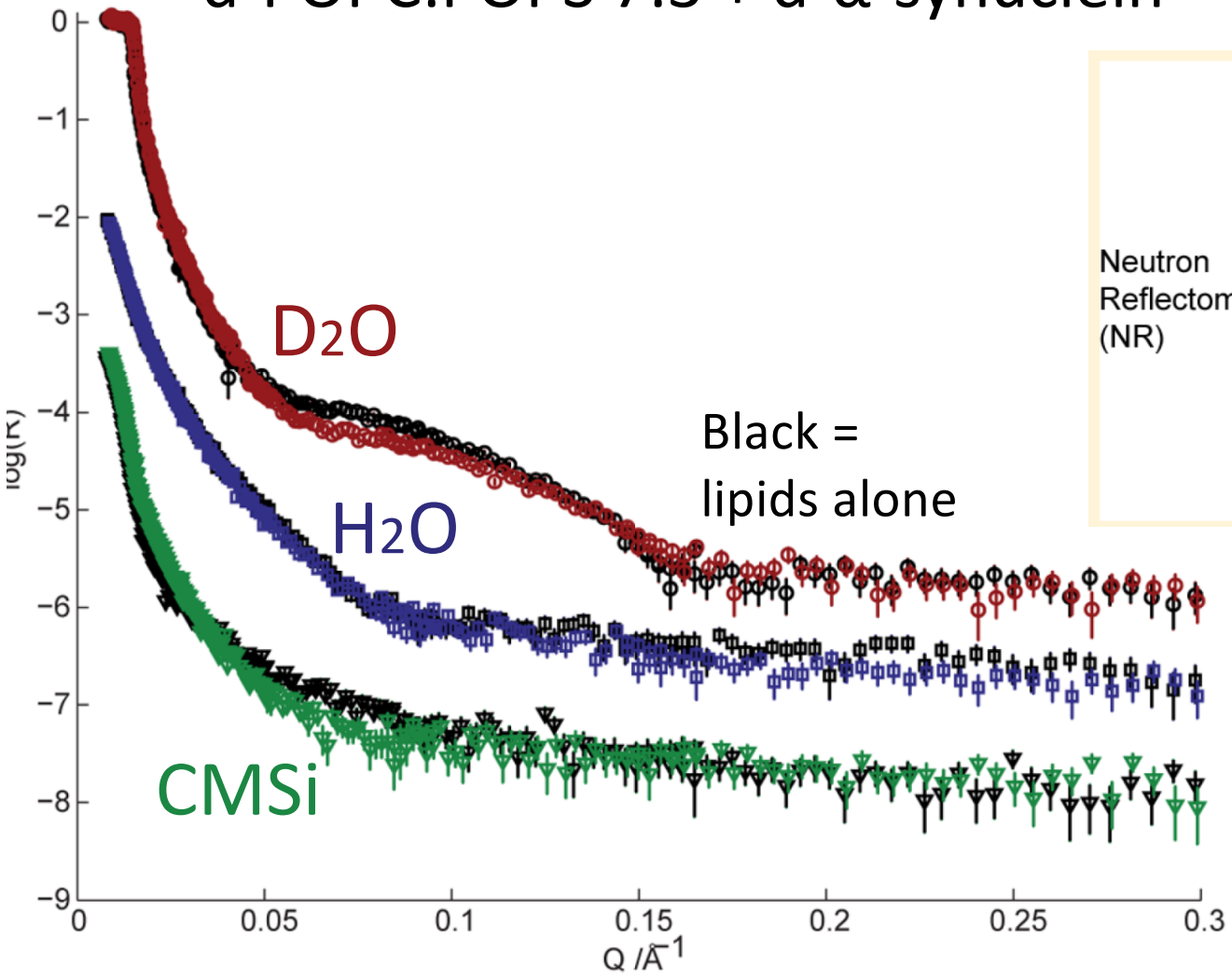
membrane alone

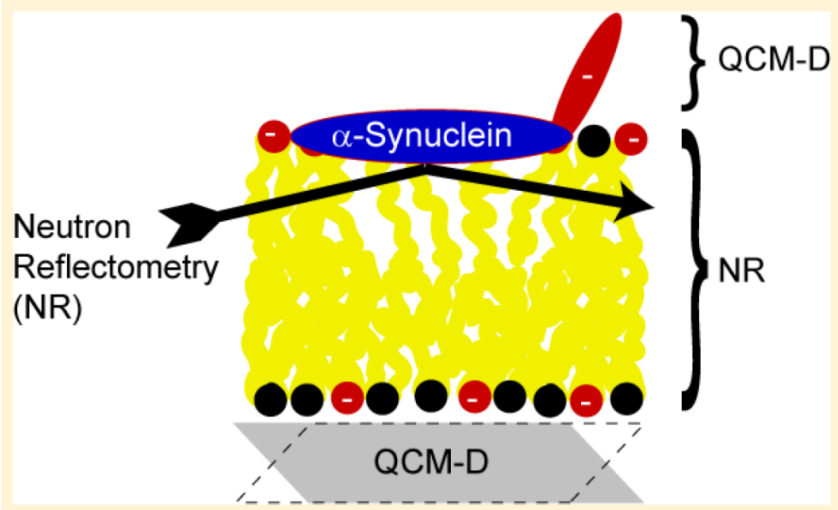
membrane + protein





d-POPC:POPS 7:3 + d-α-synuclein

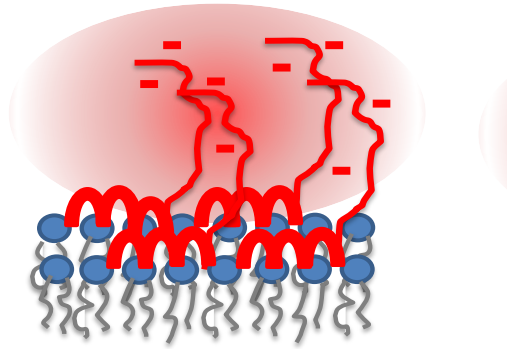




Hellstrand et al
ACS Chem Neurosci 2013

Protein adsorption

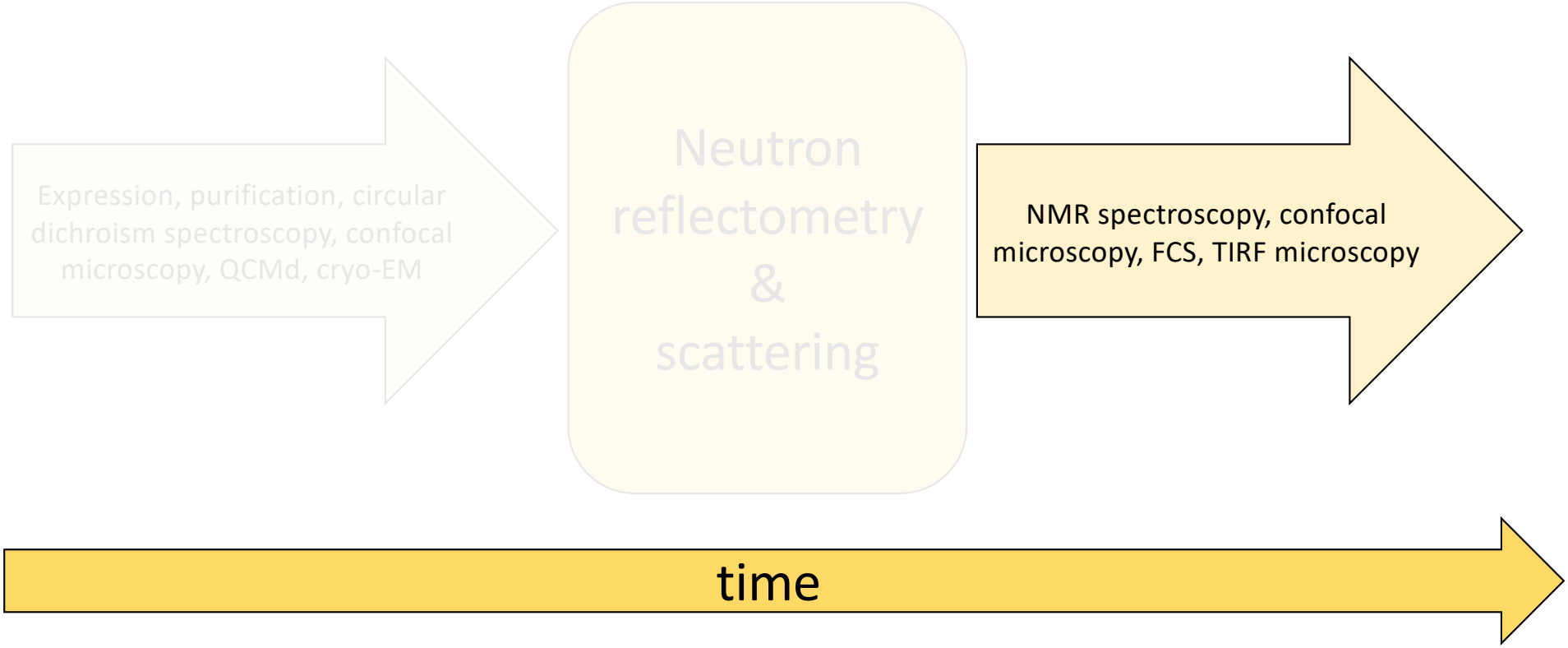
- Adsorption to anionic membranes. No adsorption detected for neutral zwitterionic bilayers
- Protein forms an α-helix at the membrane
- Only N-terminal and central part of the protein in contact with the membrane
- C-terminal part of the protein disordered and extended in the solution
- Protein located in the headgroup and upper acyl layer, not penetrating deeper



Anionic Lipid Membrane

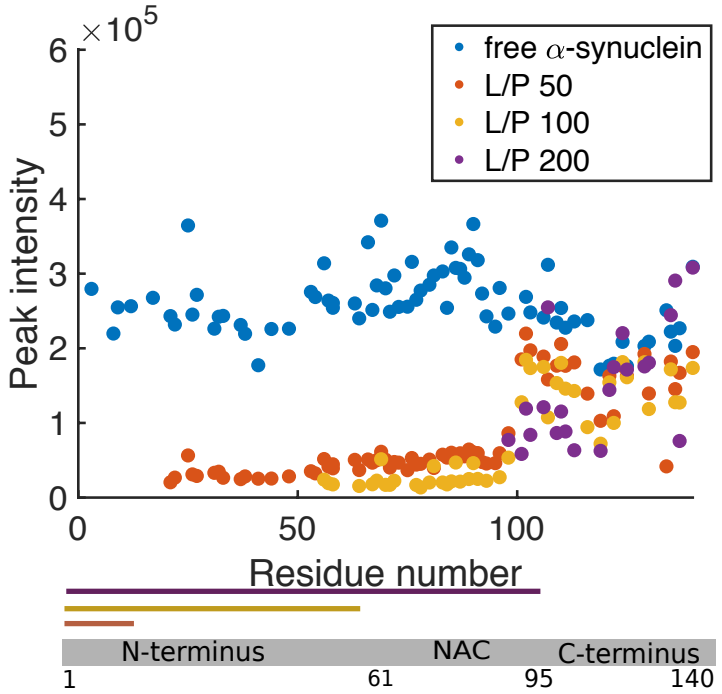
Hellstrand et al. ACS Neuroscience (2013); Gaspar et al, BBA (2018), Gaspar et al. (Manuscript)

α-synuclein-membrane interactions



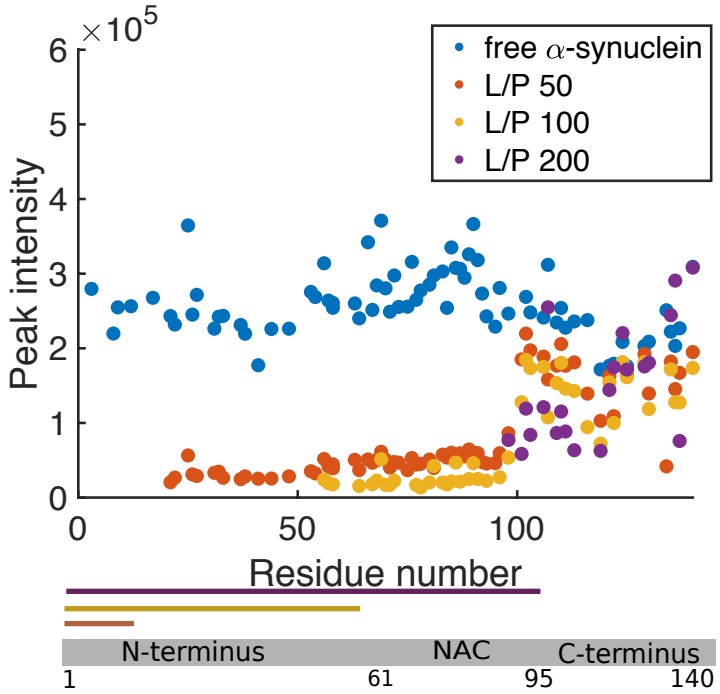
Protein adsorption strongly depend on the proportions between lipids and proteins

NMR sepctroscopy

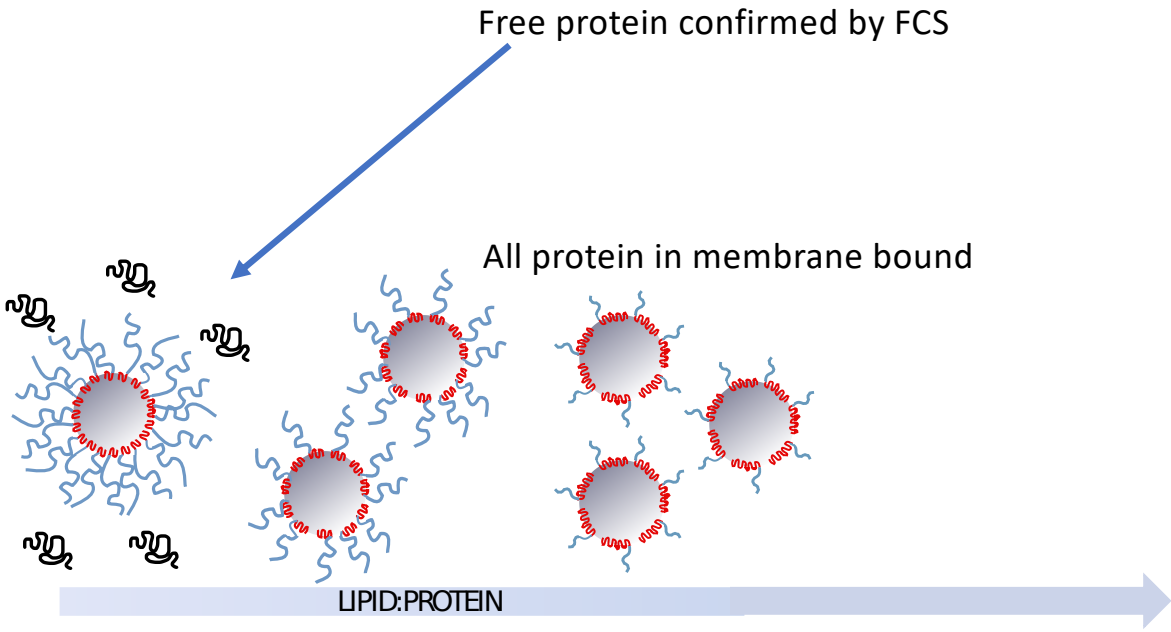


Makasewicz et al (manuscript)

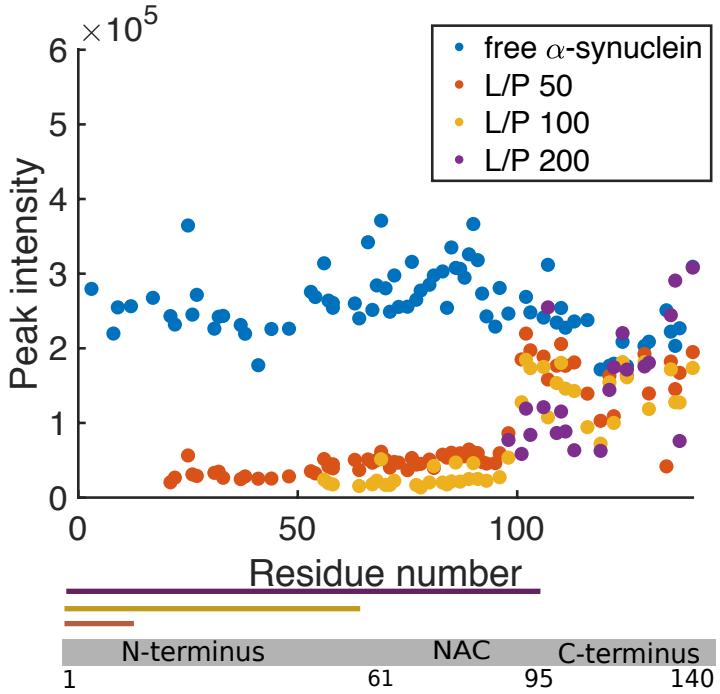
Protein adsorption strongly depend on the proportions between lipids and proteins

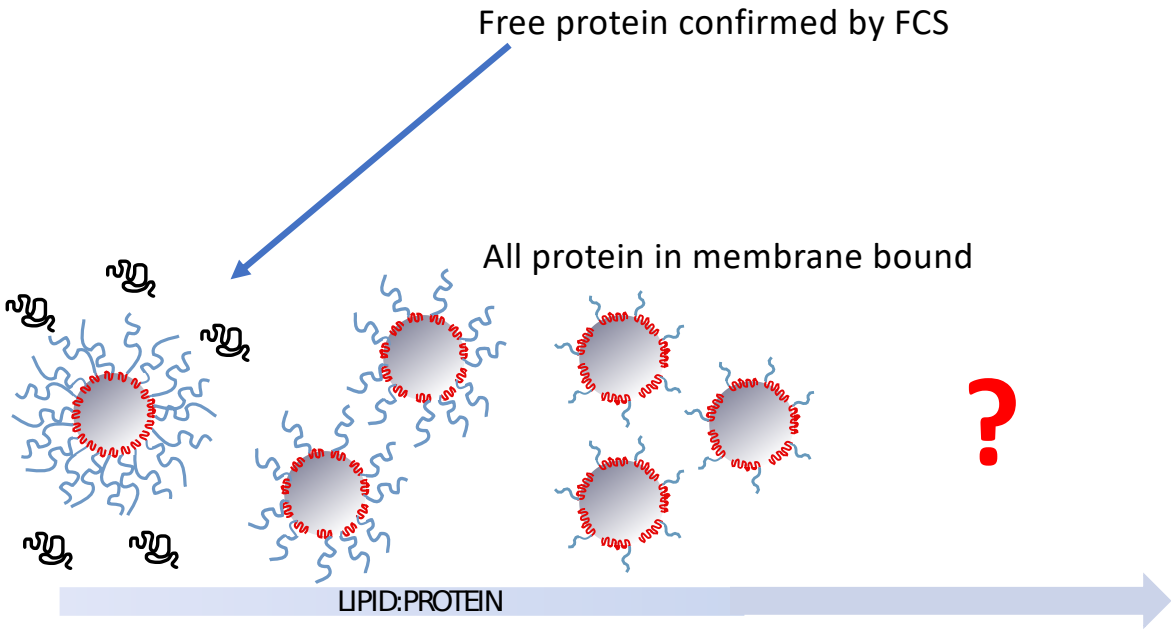


Makasewicz et al (manuscript)



Protein adsorption strongly depend on the proportions between lipids and proteins

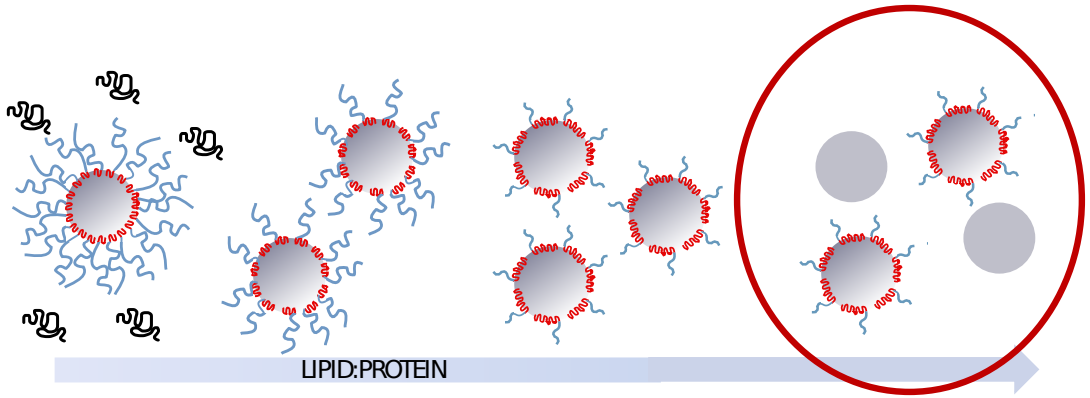




Makasewicz et al (manuscript)

Cooperative binding

conditions with excess membrane compared to protein



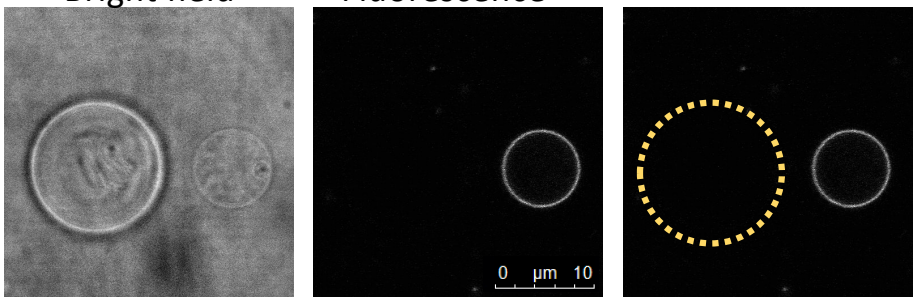
Makasewicz et al (under revision)

Cooperative binding of α-synuclein to membranes

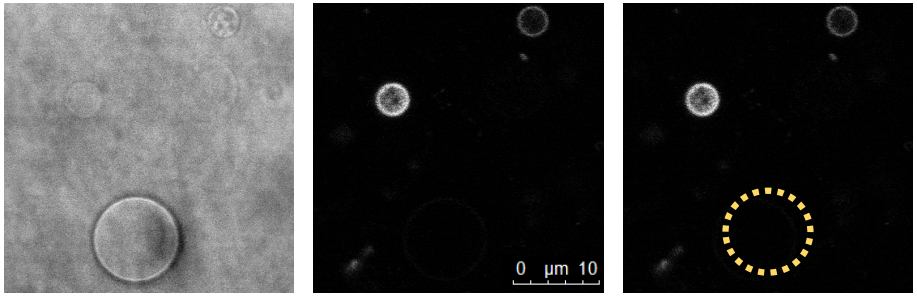
Confocal microscopy

Bright field Fluorescence

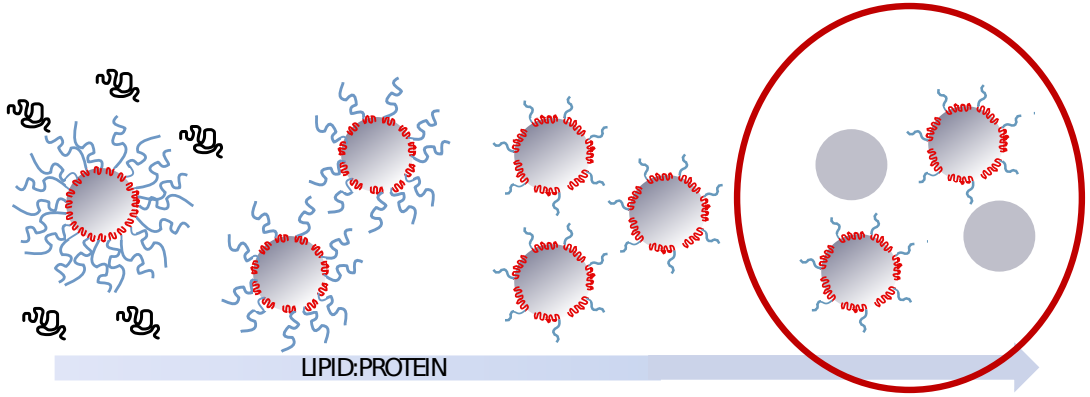
A



B



excess membrane compared to protein



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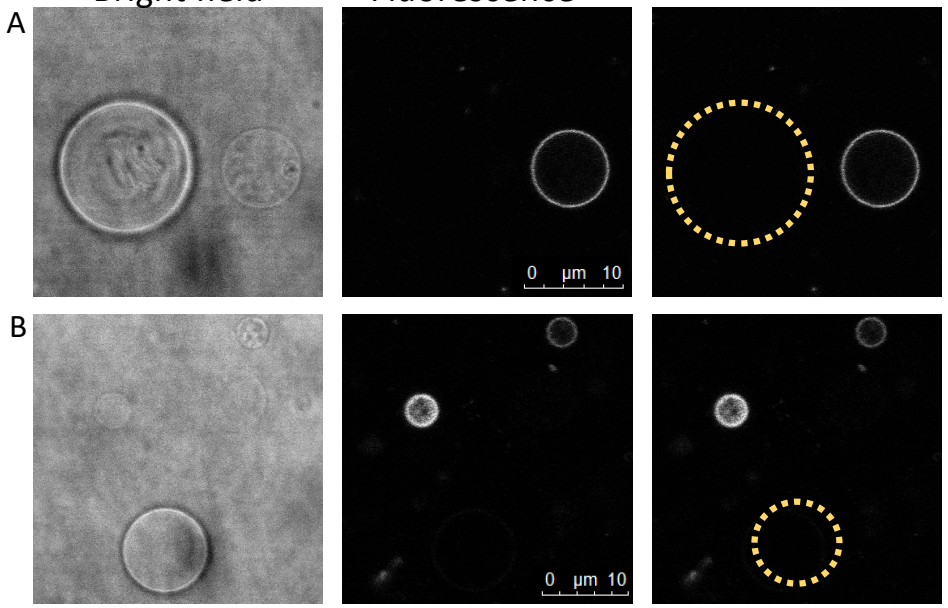
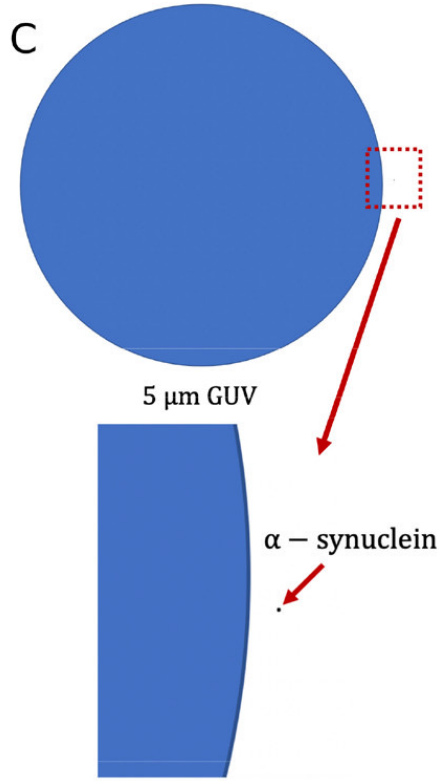


excess membrane compared to protein



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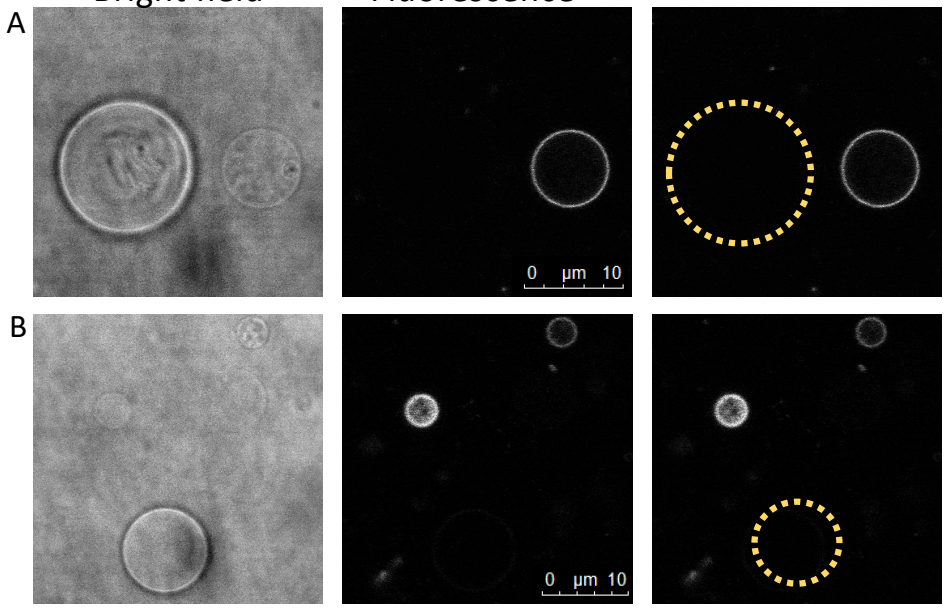
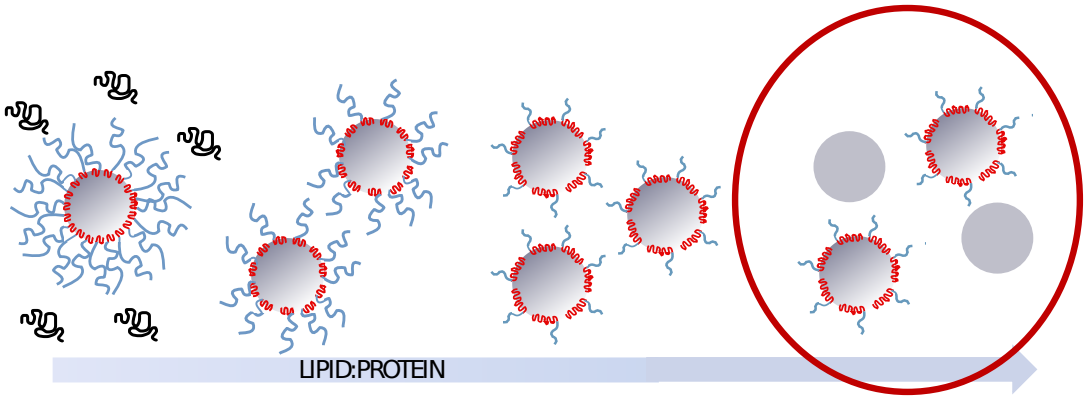


excess membrane compared to protein

Some vesicle bare, some vesicles filled.
Confirmed by FCS.

Observed for pure POPS and POPC/POPS

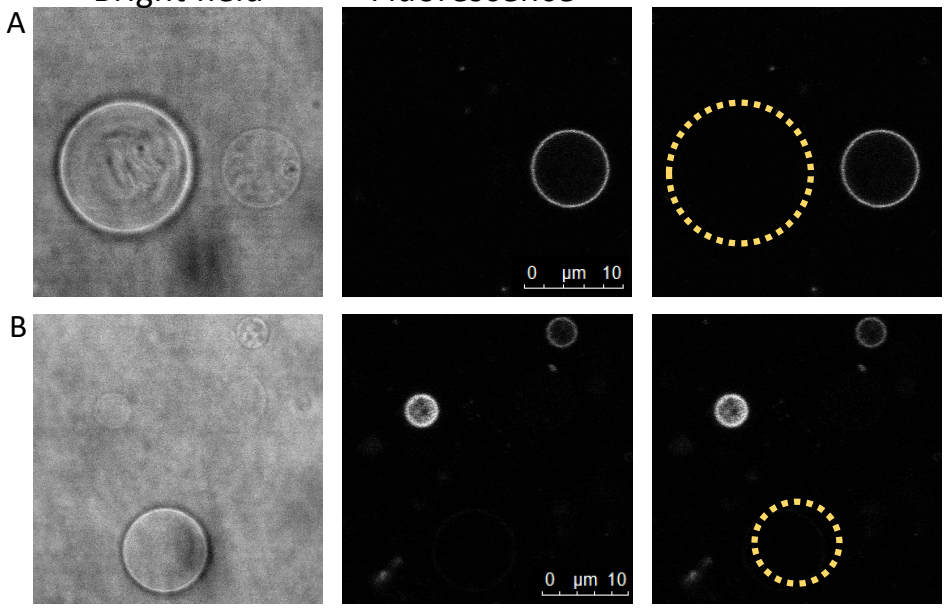
When more protein is added, all vesicles filled



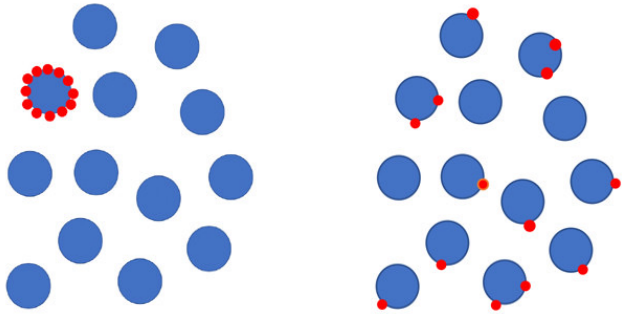
Cooperative binding of α-synuclein to membranes

Confocal microscopy

Bright field Fluorescence



excess membrane compared to protein

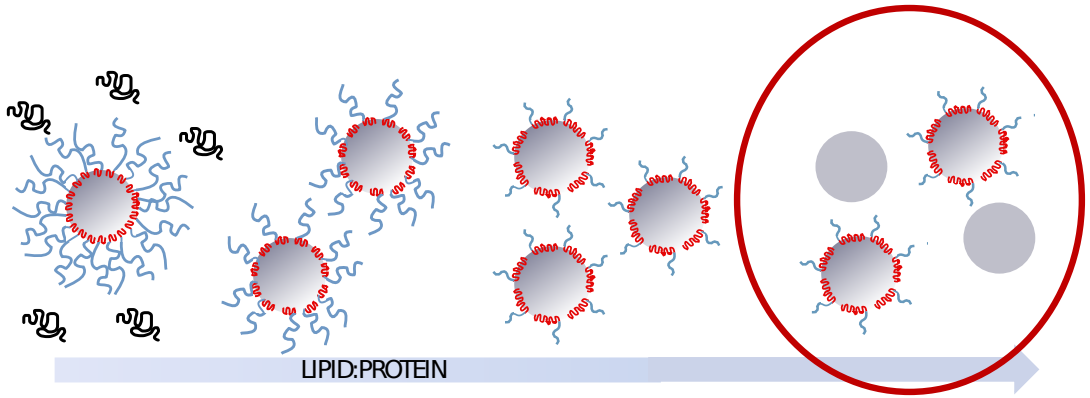


cooperative binding independent binding

Some vesicle bare, some vesicles filled.
Confirmed by FCS.

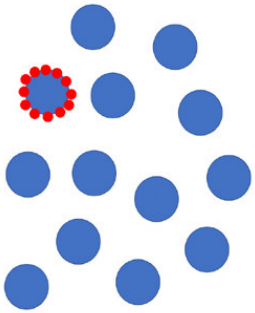
Observed for pure POPS and POPC/POPS

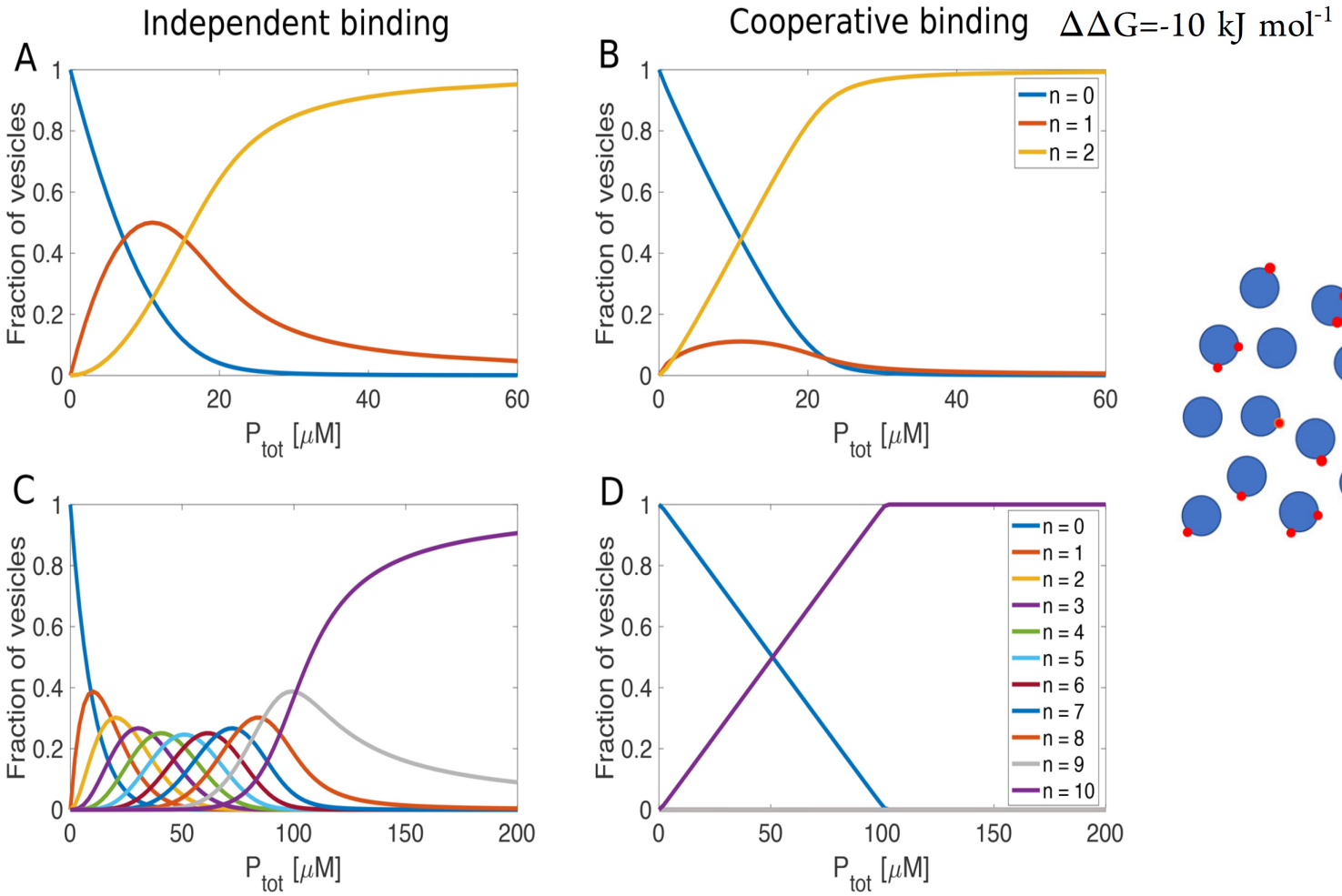
When more protein is added, all vesicles filled

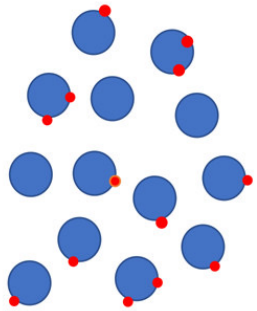


Makasewicz *et al* ACS Chem Neurosci. 2021

Cooperative binding of α-synuclein to membranes

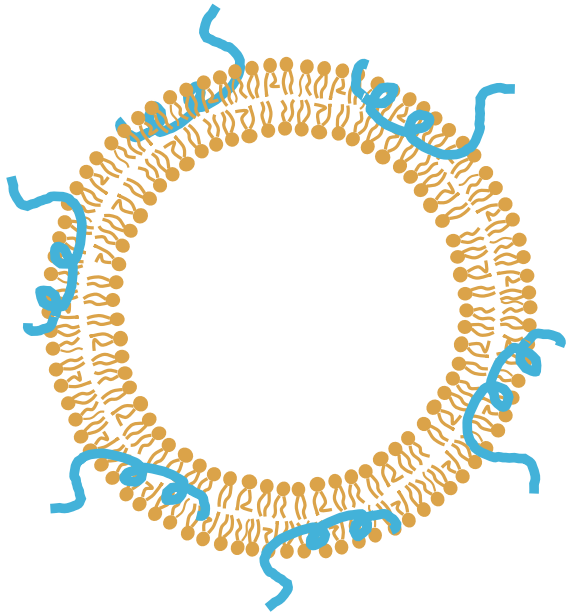






Vesicle shape?

?



?

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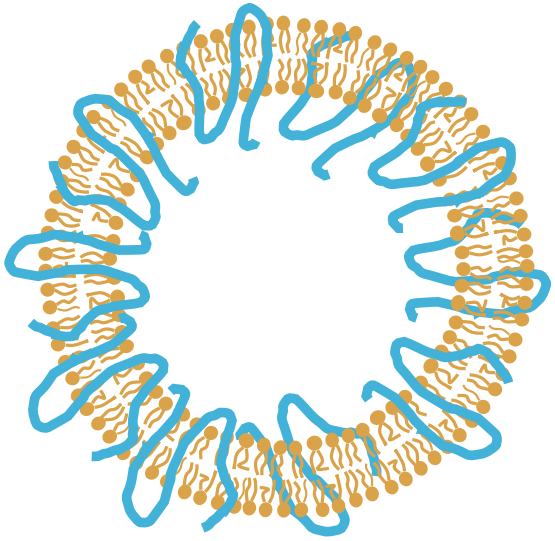

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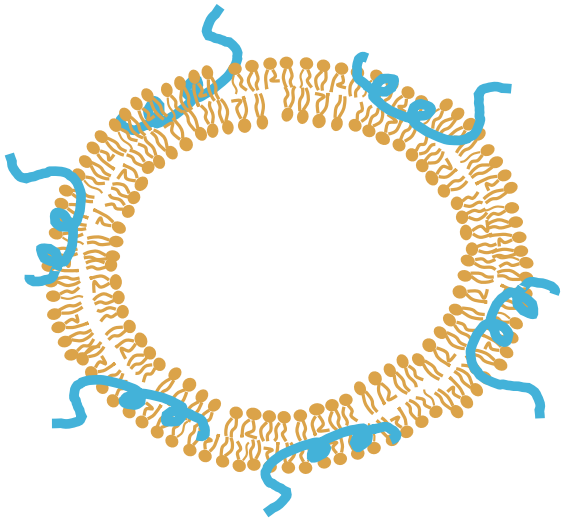

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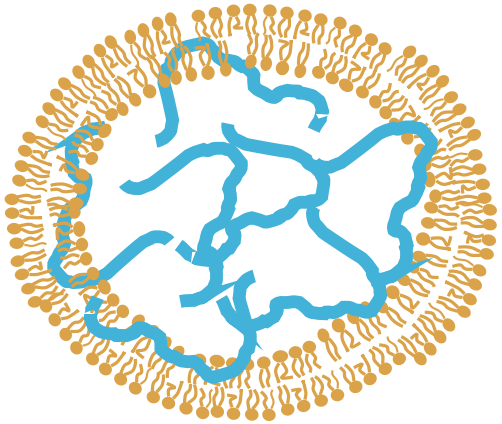

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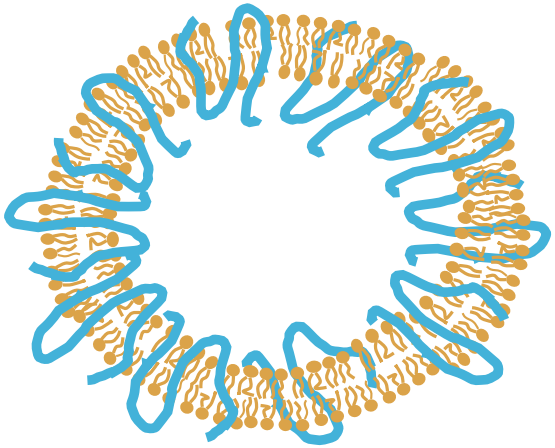
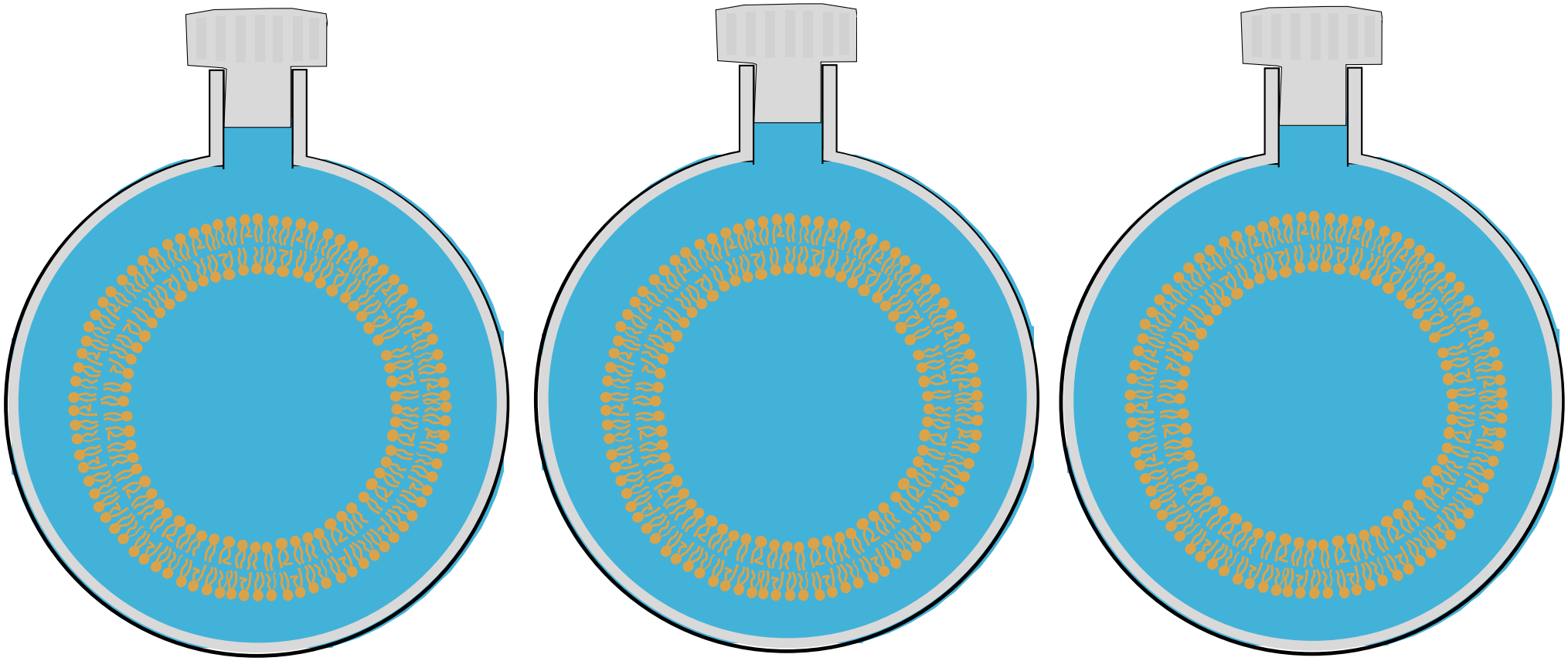
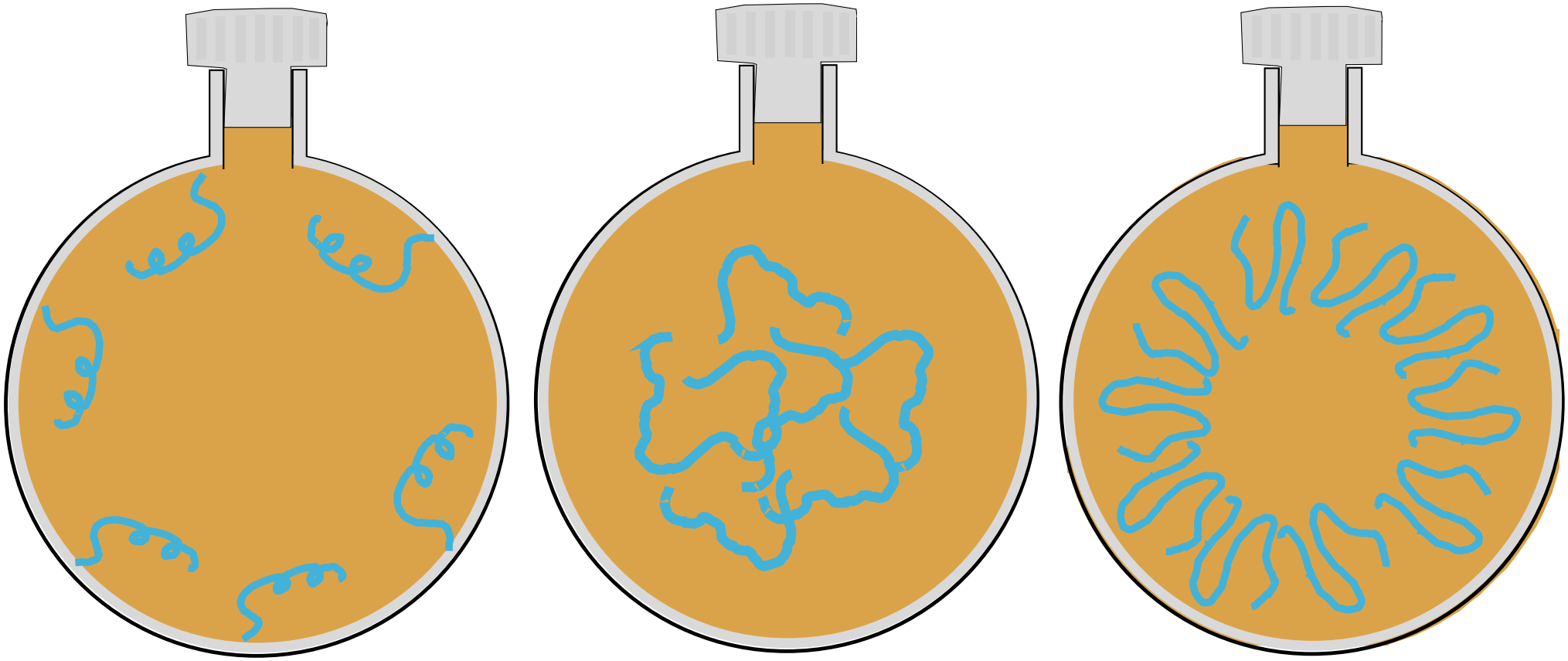
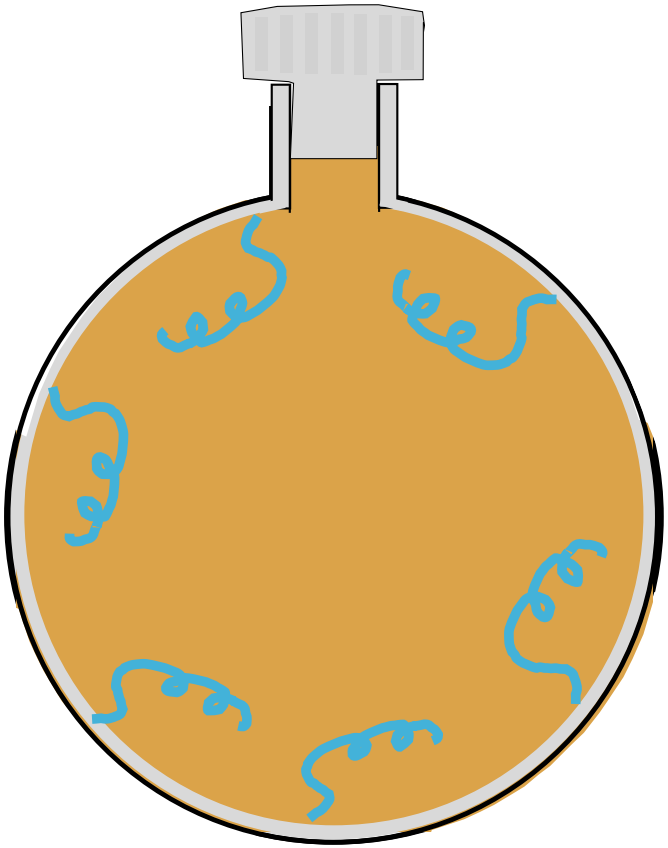


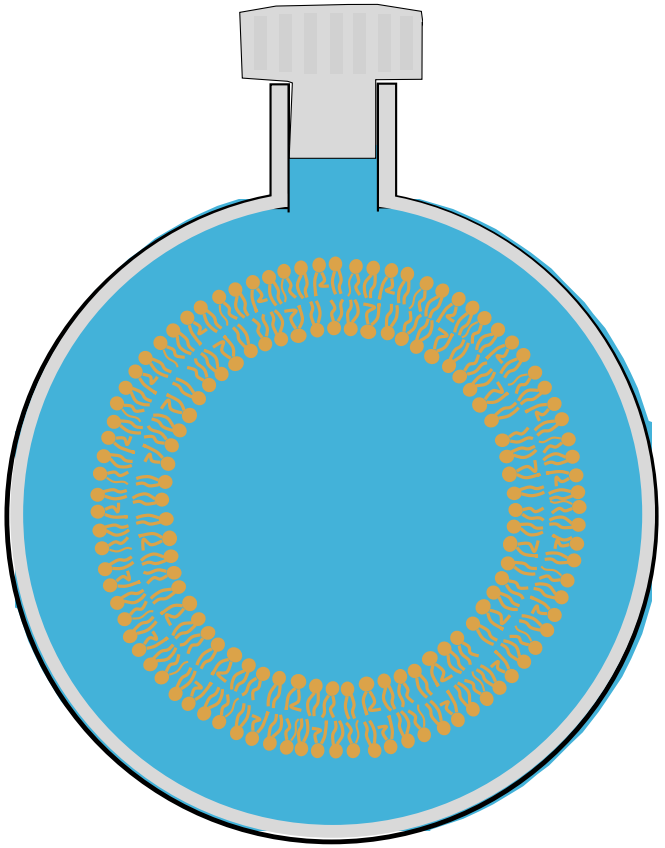
Contrast matching of protein (100% D_2O)



Contrast matching of lipid (12% D_2O)







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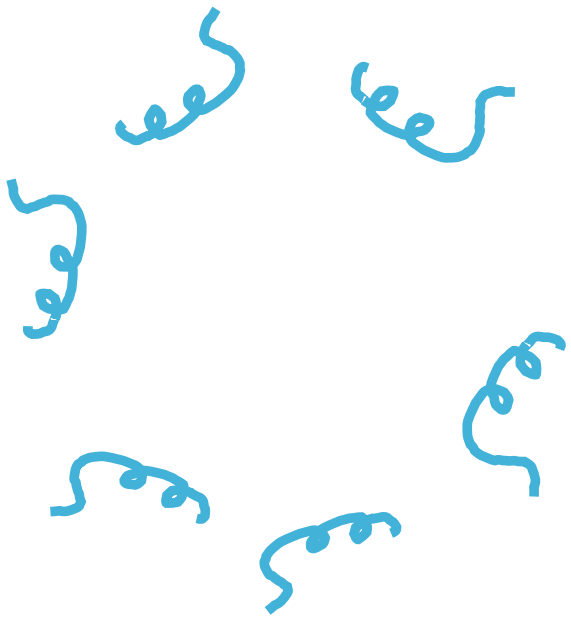

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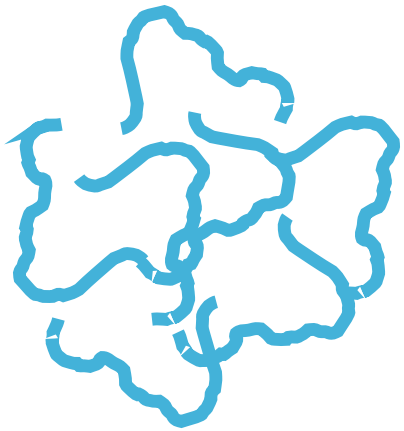

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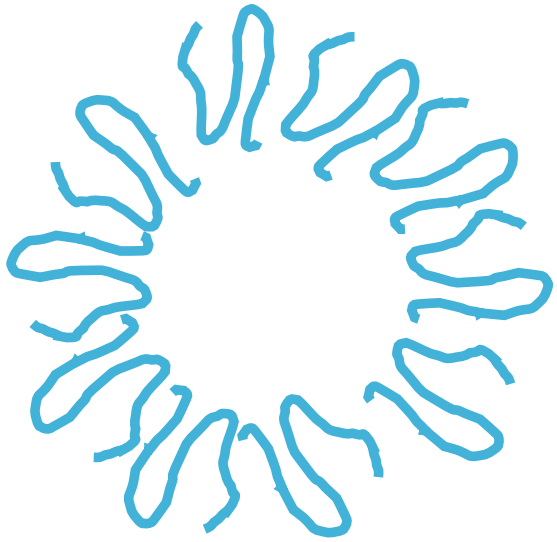

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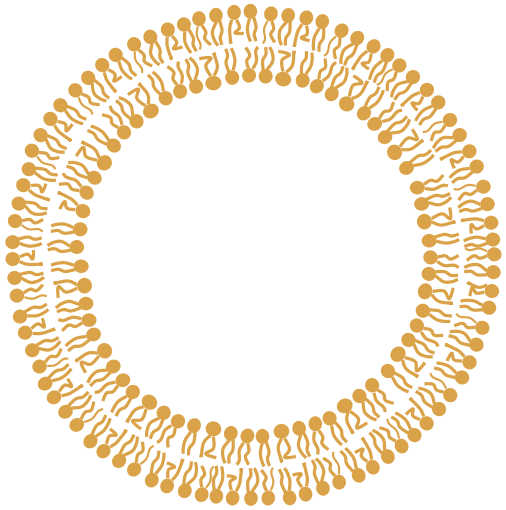

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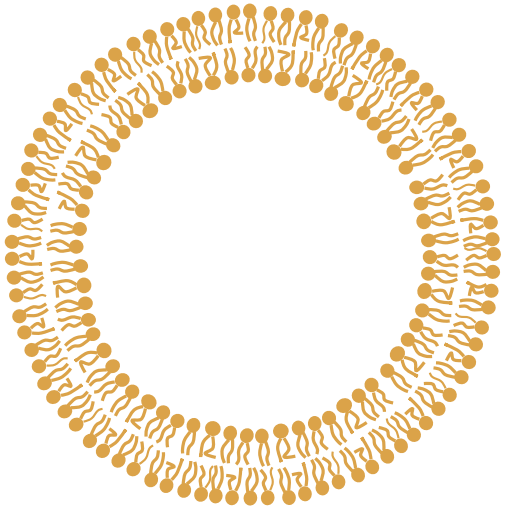

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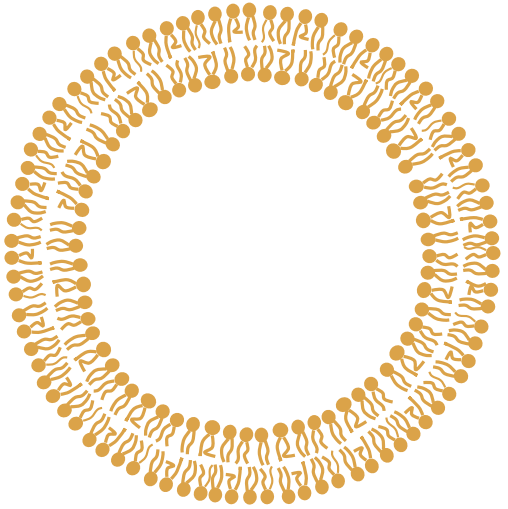
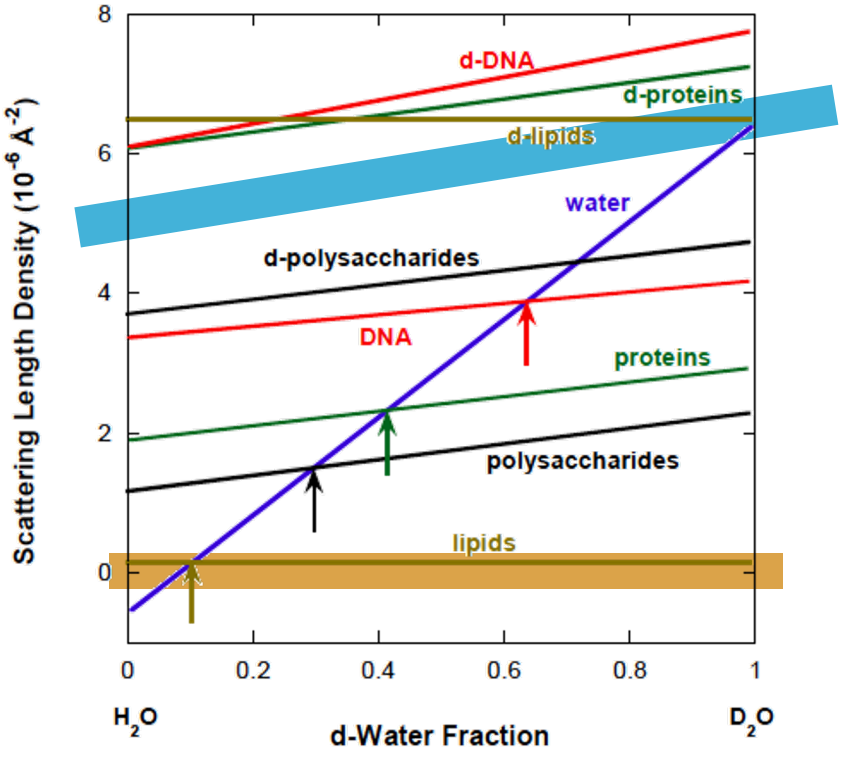


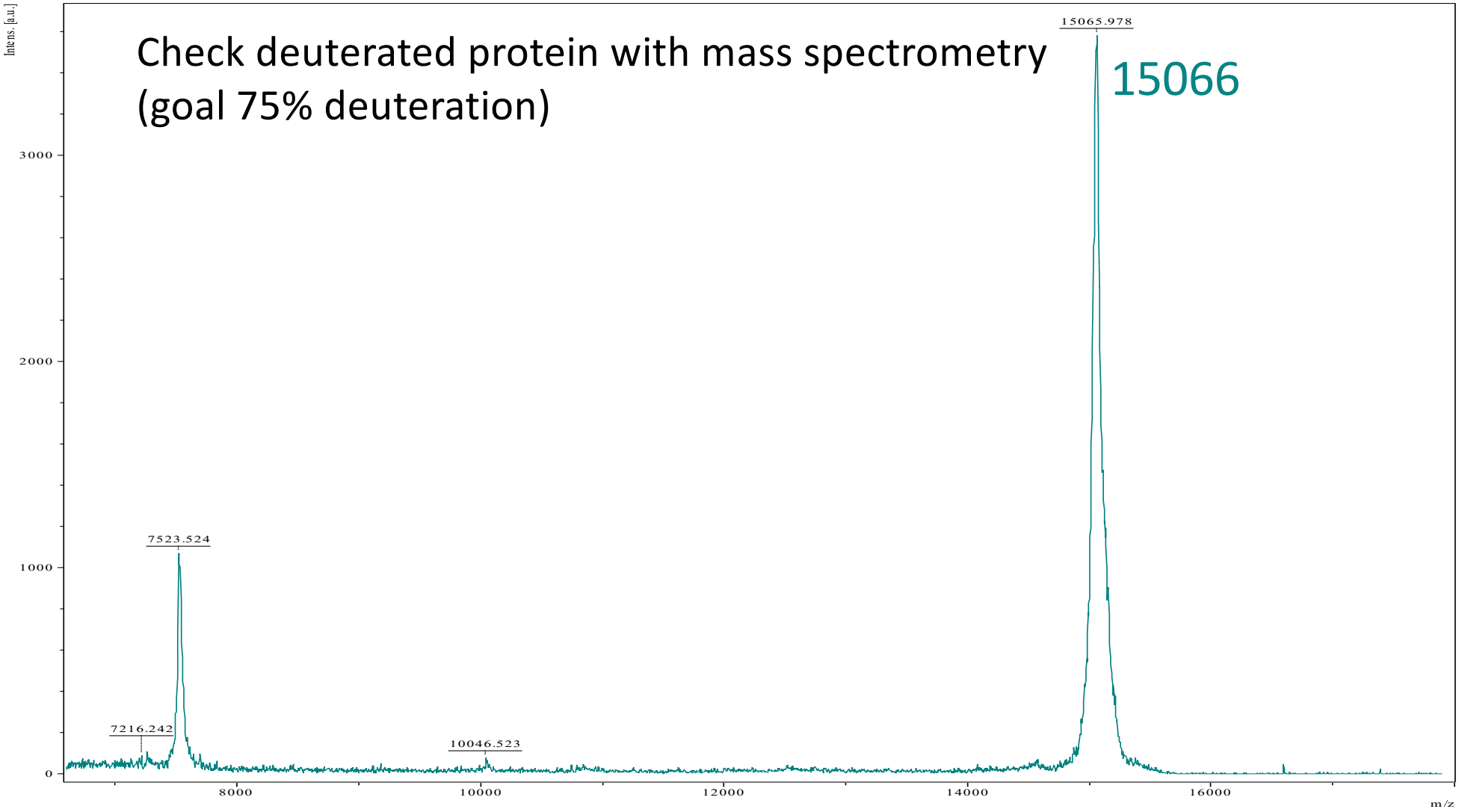
Contrast variation



75% deuterated

Check deuterated protein with mass spectrometry (goal 75% deuteration)

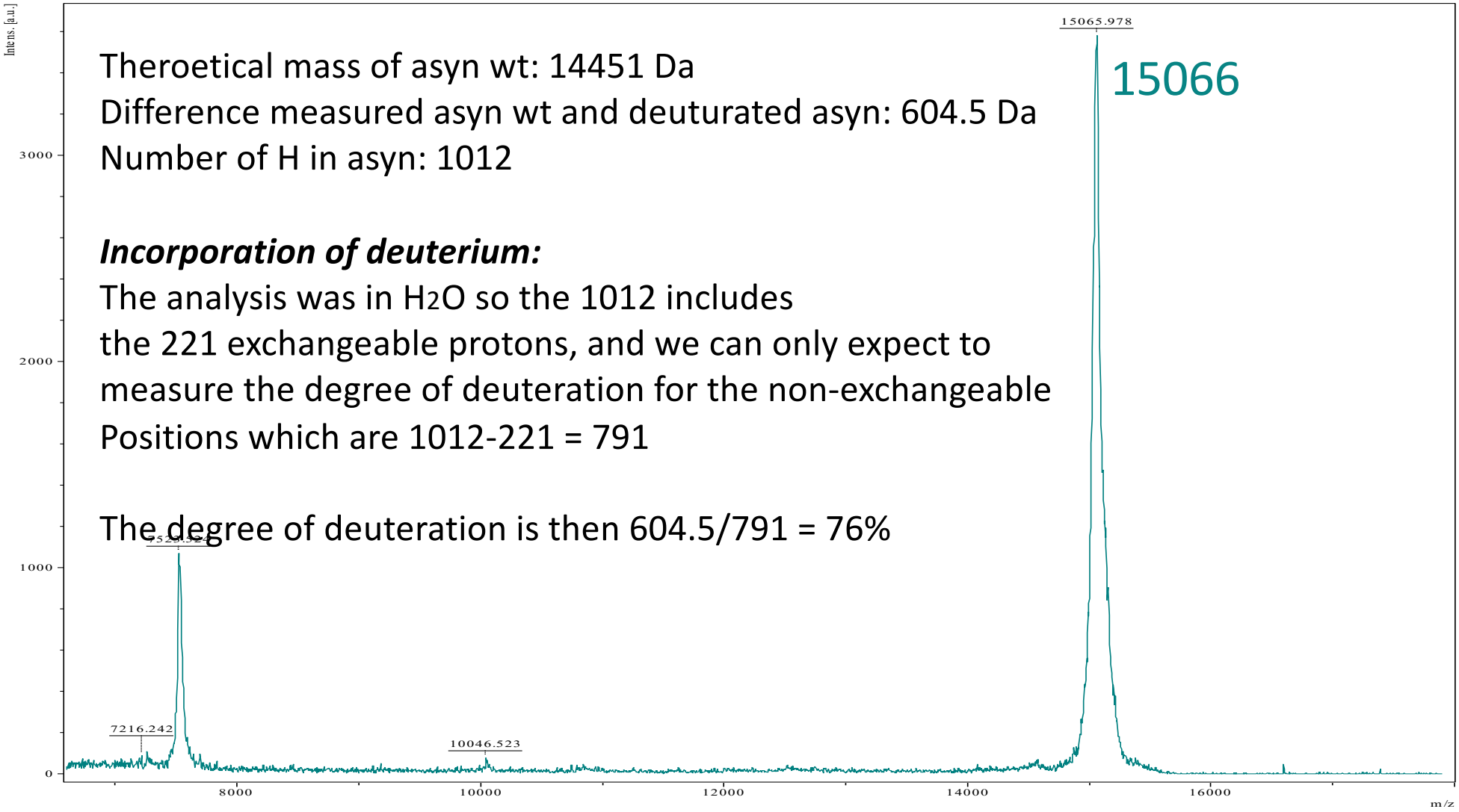
15066



Theroetical mass of asyn wt: 14451 Da
Difference measured asyn wt and deuturated asyn: 604.5 Da
Number of H in asyn: 1012

Incorporation of deuterium:
The analysis was in H_2O so the 1012 includes
the 221 exchangeable protons, and we can only expect to measure the degree of deuteration for the non-exchangeable
Positions which are 1012-221 = 791

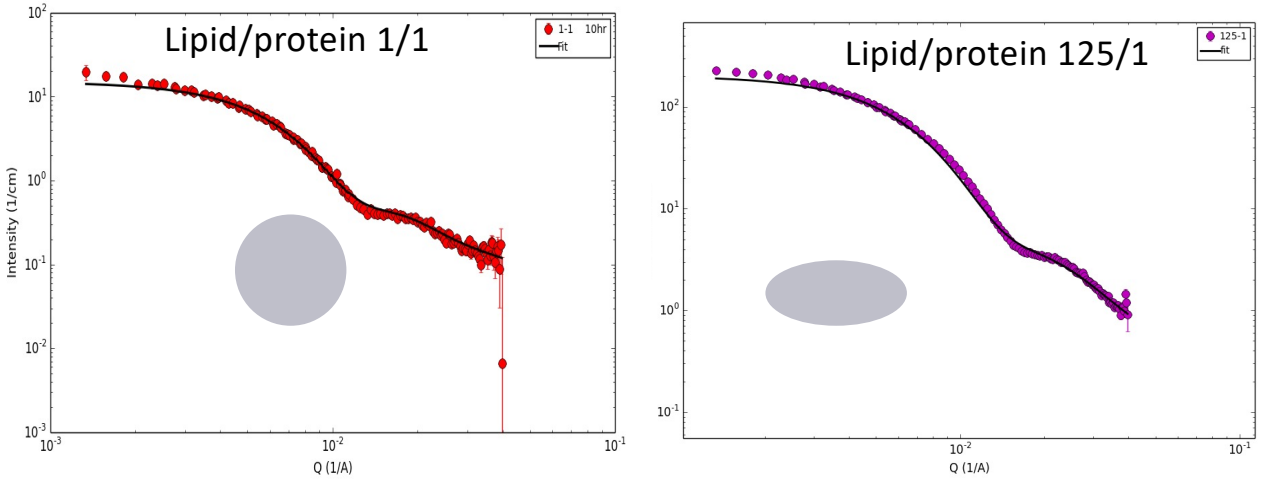
The degree of deuteration is then 604.5/791 = 76%



Vesicle shape

Deformation in conditions with excess membrane compared to protein

SANS (ILL). POPC/POPS vesicles with D-a-syn. Match out the protein



Idini et al (manuscript)

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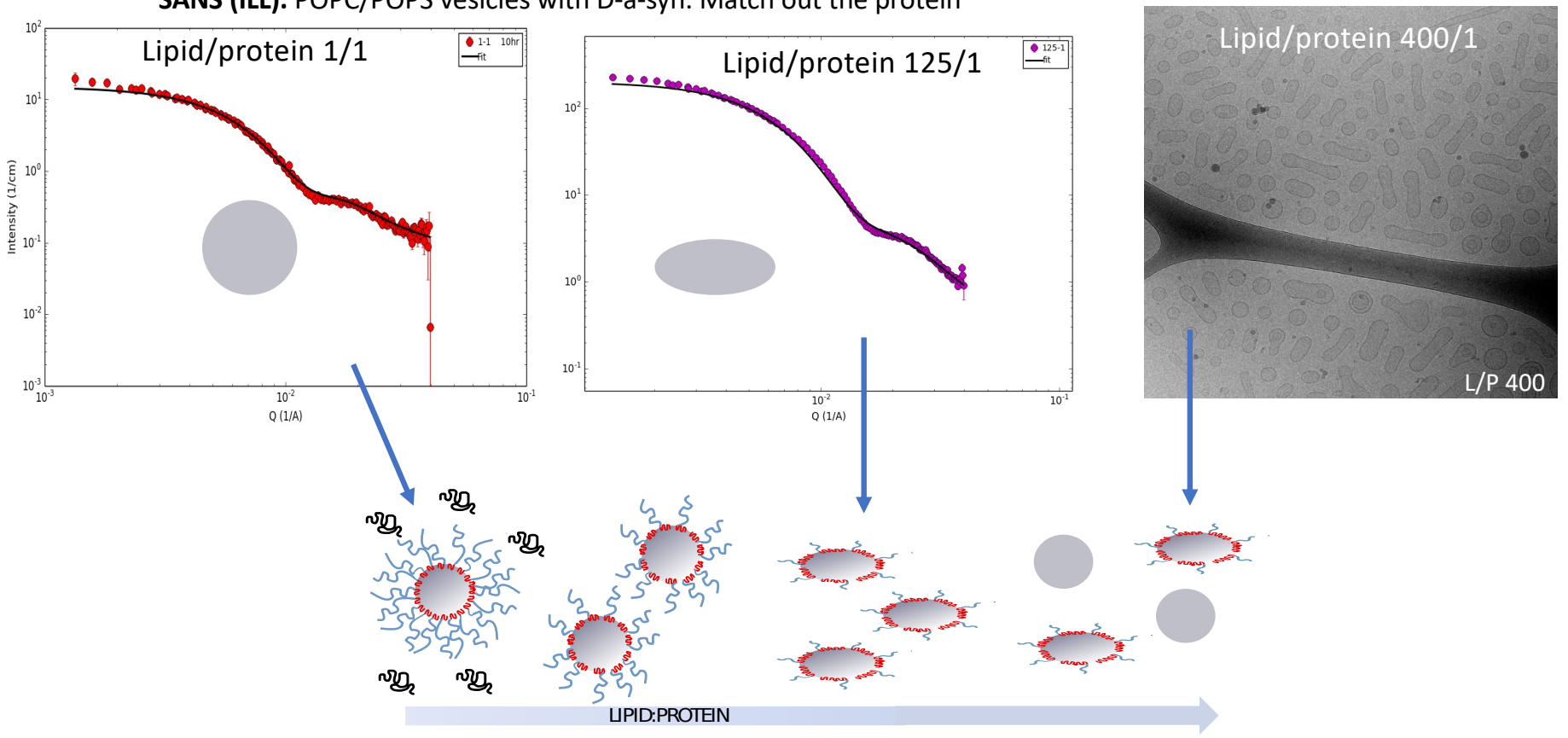
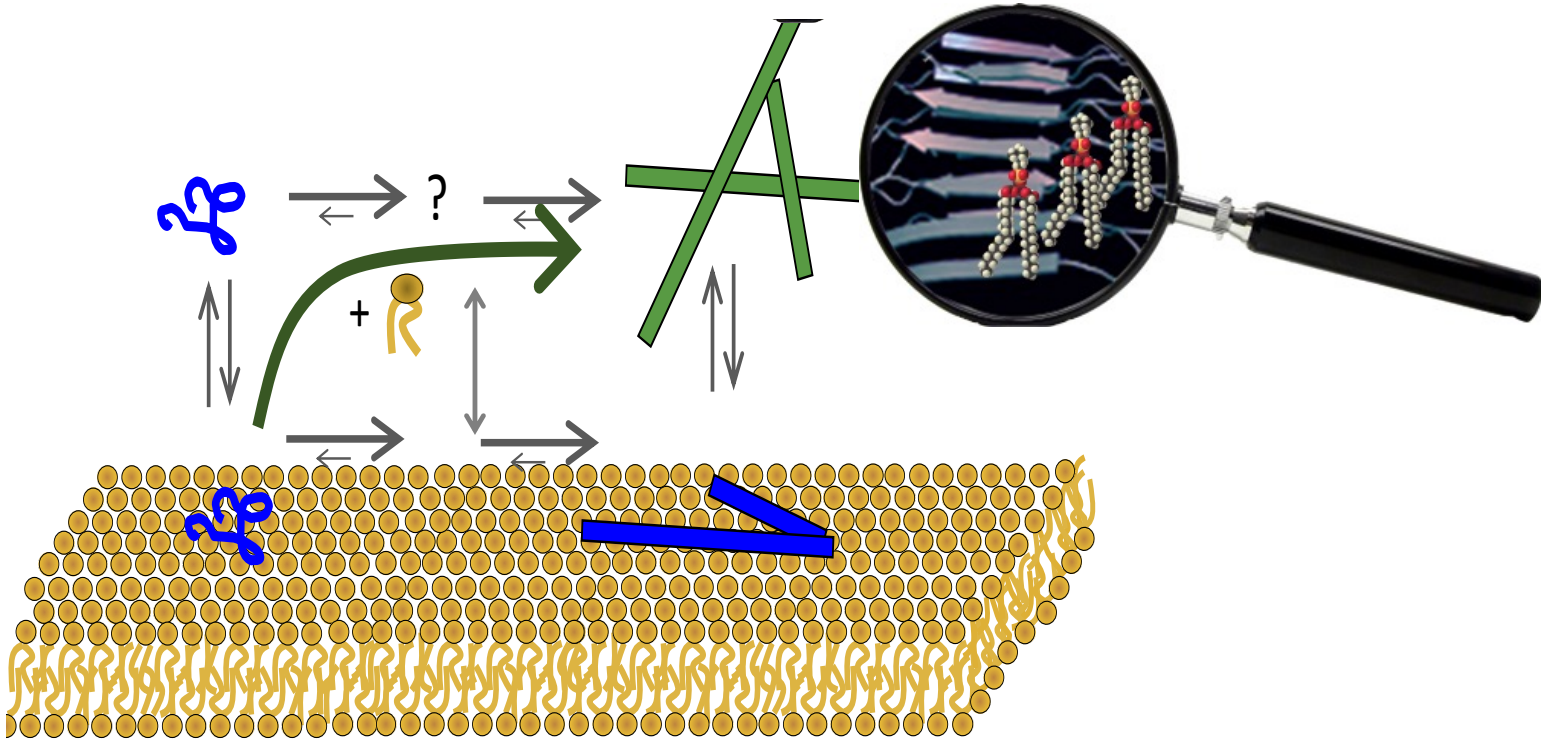


Idini et al (manuscript)

Makasewicz et al (under revision)

Lipid-protein co-aggregation – from lipid-rich to peptide-rich



Neutron scattering and neutron reflectometry studies of protein-lipid co-assembly

Questions re co-aggregates

Composition?

Structure?

Selectivity?

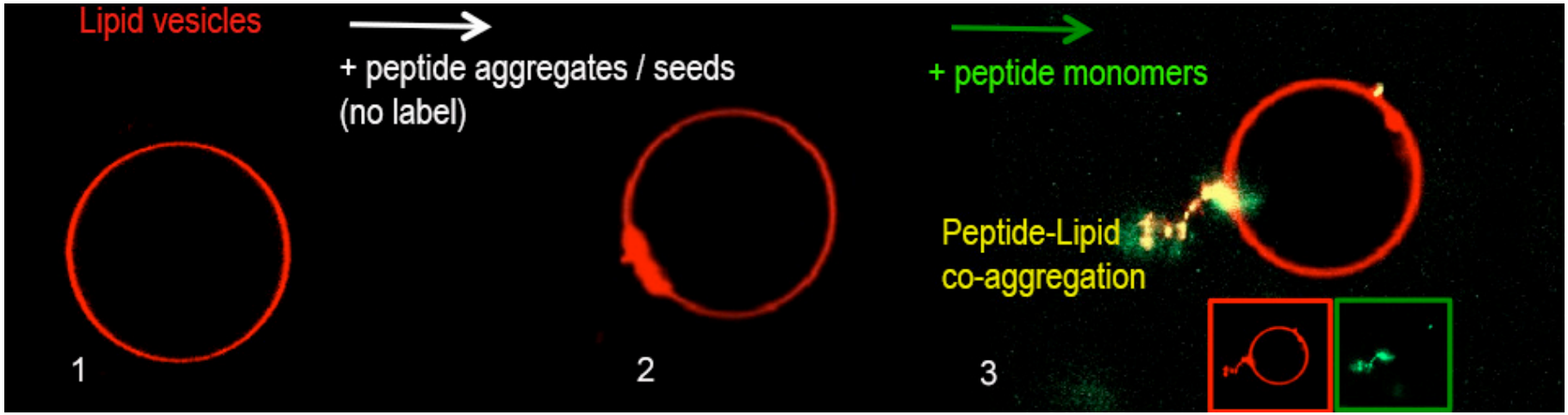
Mechanism?

Consequences?

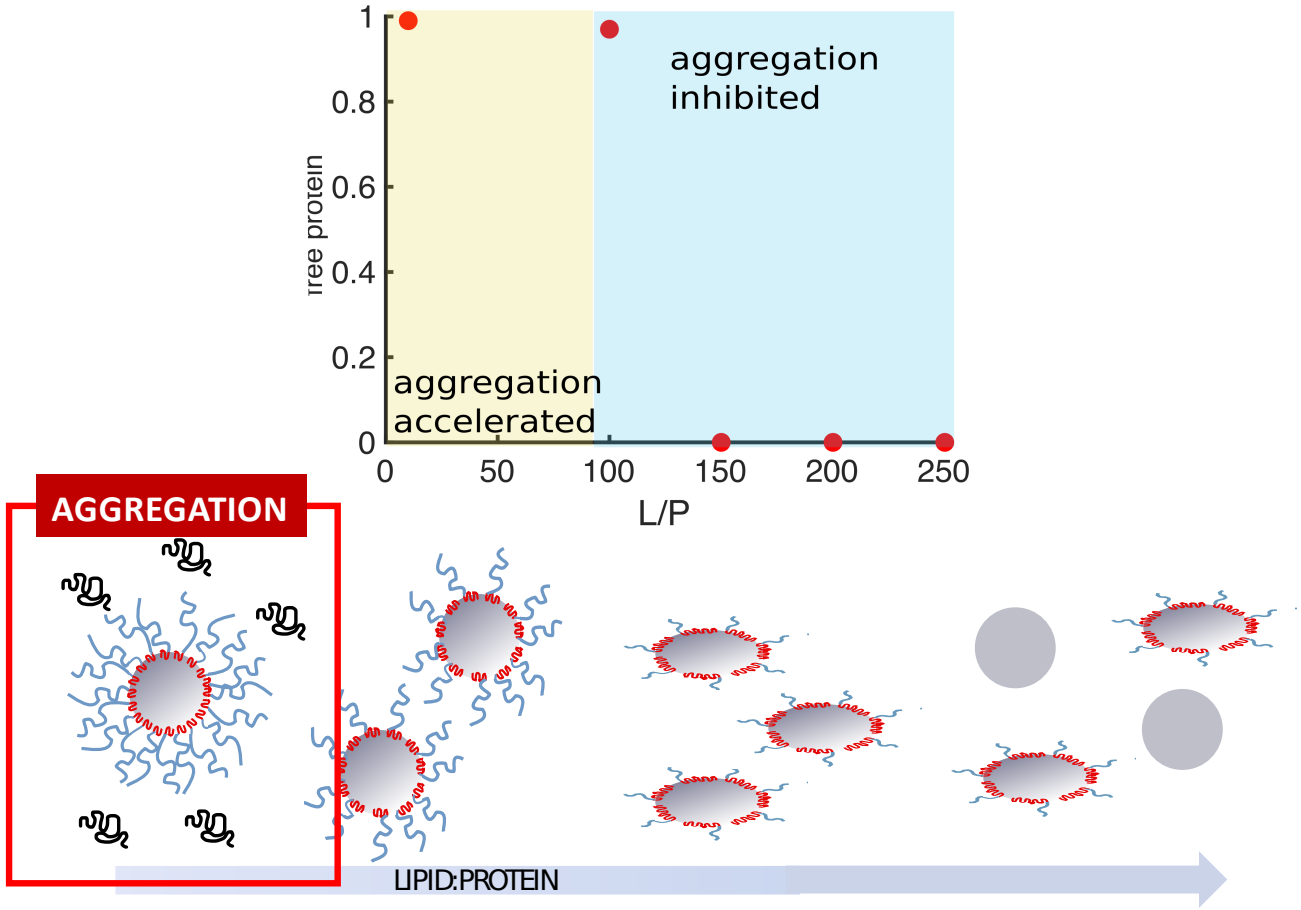
Journal of Parkinson's Disease 1 (2011) 359–371
DOI 10.3233/JPD-2011-11067
IOS Press

Membrane Interaction of α-Synuclein in Different Aggregation States

Marie Grey[a,d,*], Sara Linse[b], Hanna Nilsson[c], Patrik Brundin[d] and Emma Sparr[a]

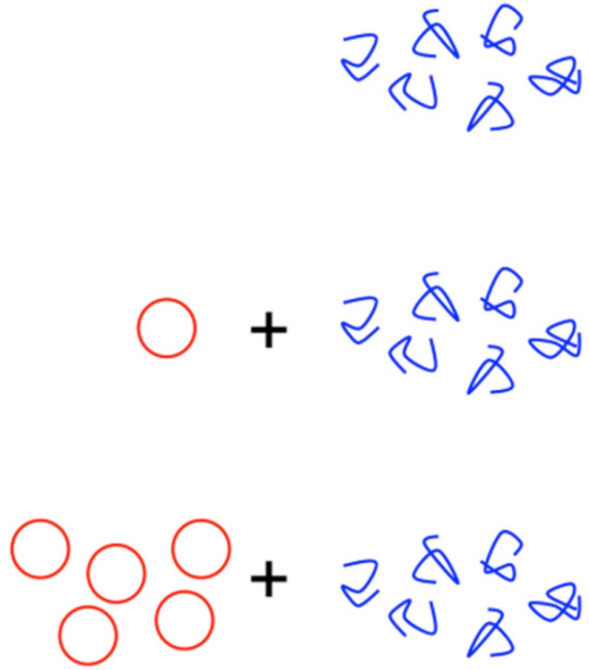


Aggregation only in conditions where vesicles are saturated and there is free protein in the solution



cryo-EM

vesicles α-syn



cryo-EM

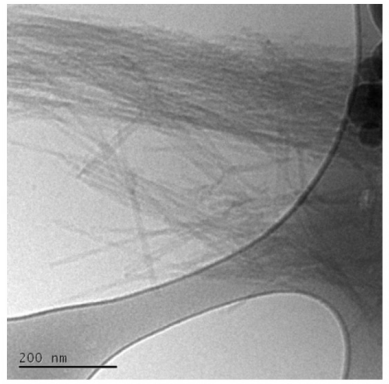
vesicles

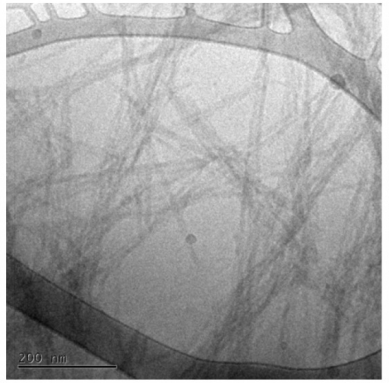
α-syn

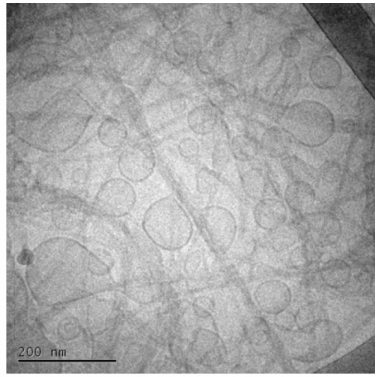
Δt

Δt

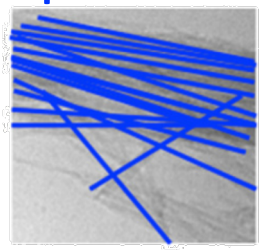
Δt



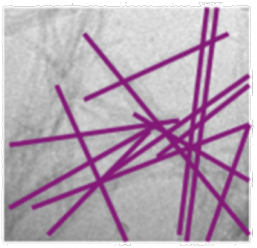




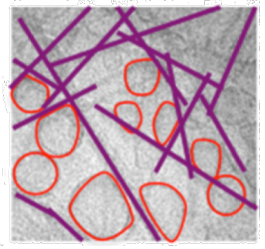
protein

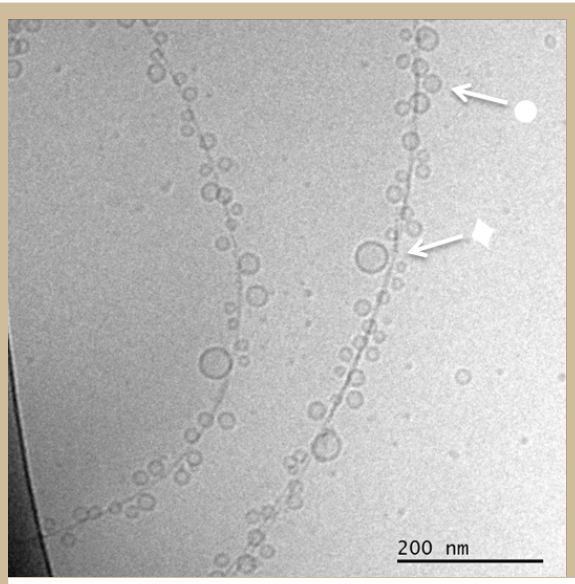


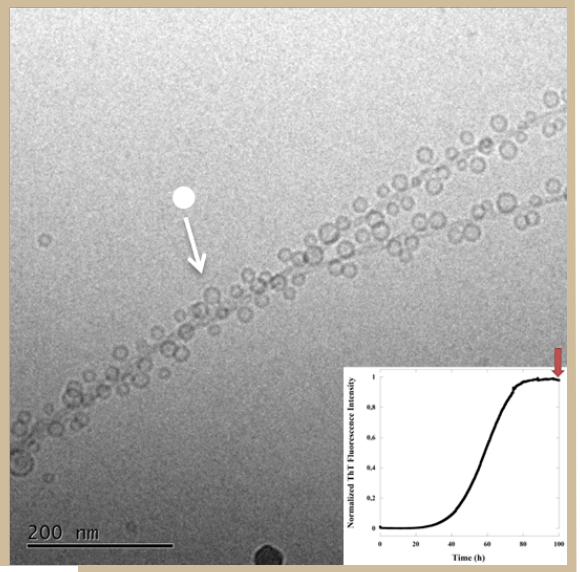
mix

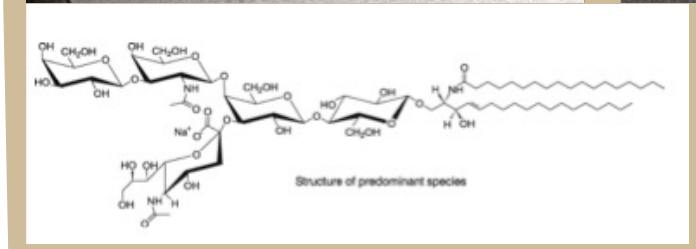


mix + lipid

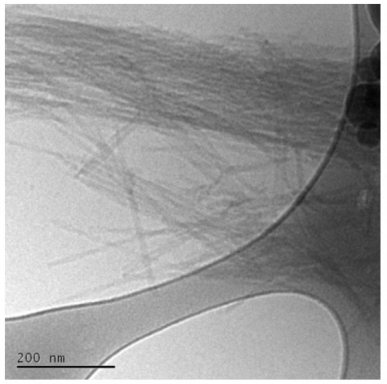




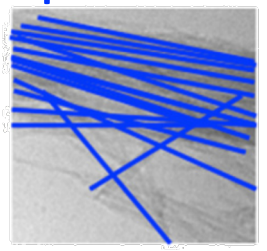


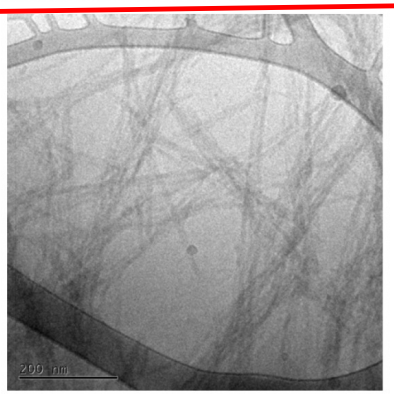


DOPC:ganglioside

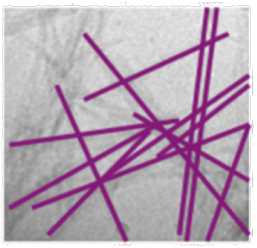


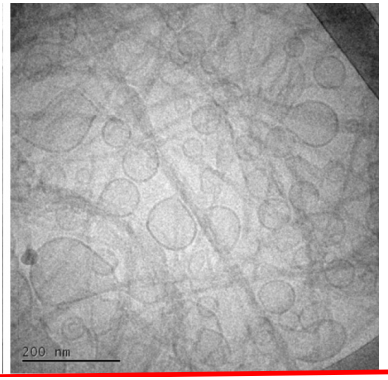
protein



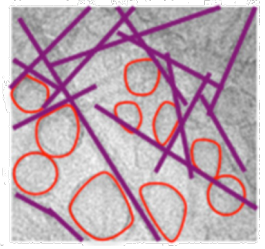


mix

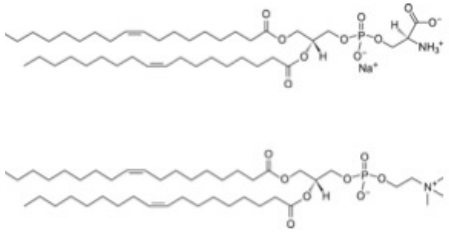




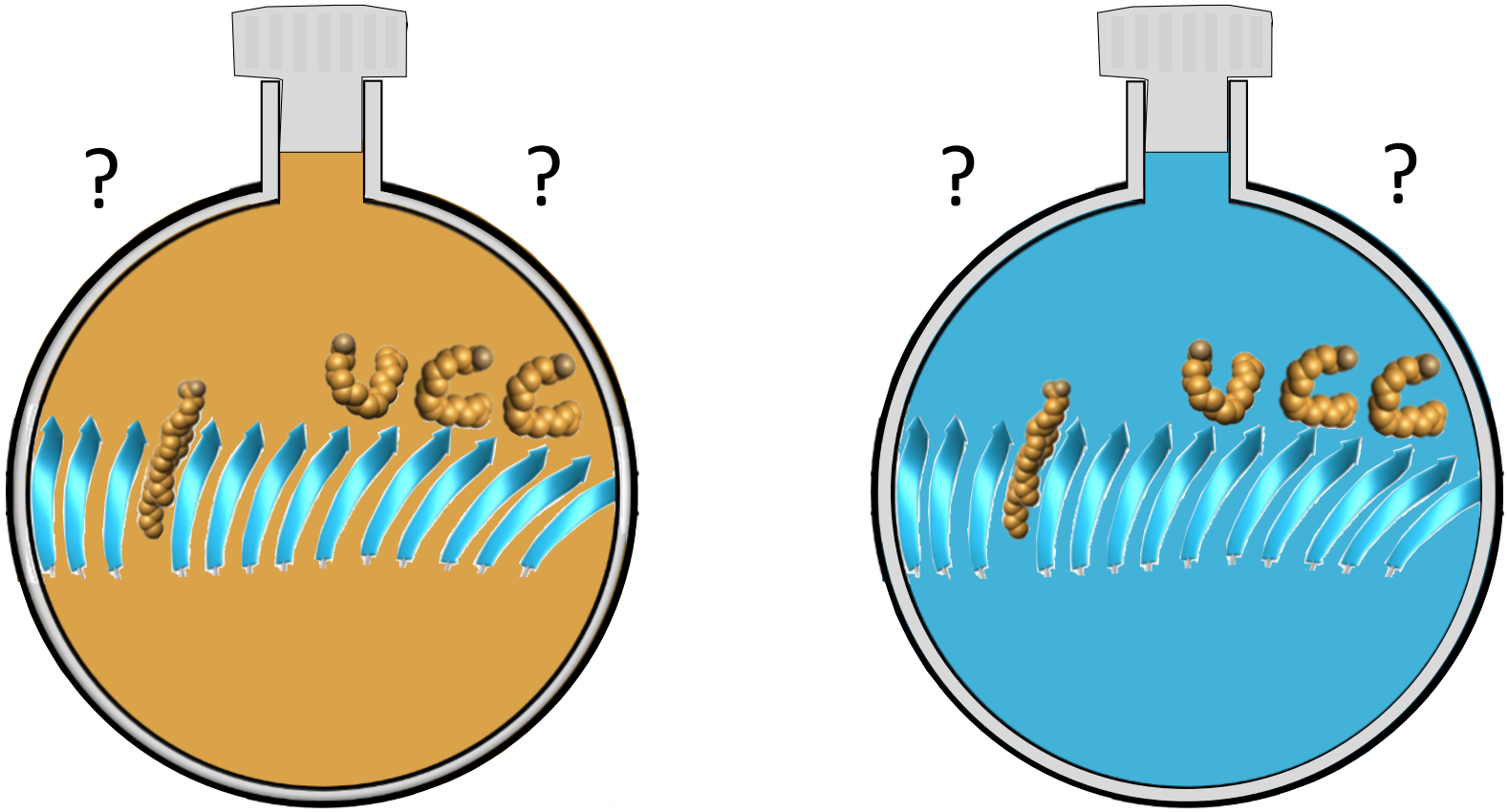
mix + lipid



DOPC:DOPS



Neutron scattering with contrast variation



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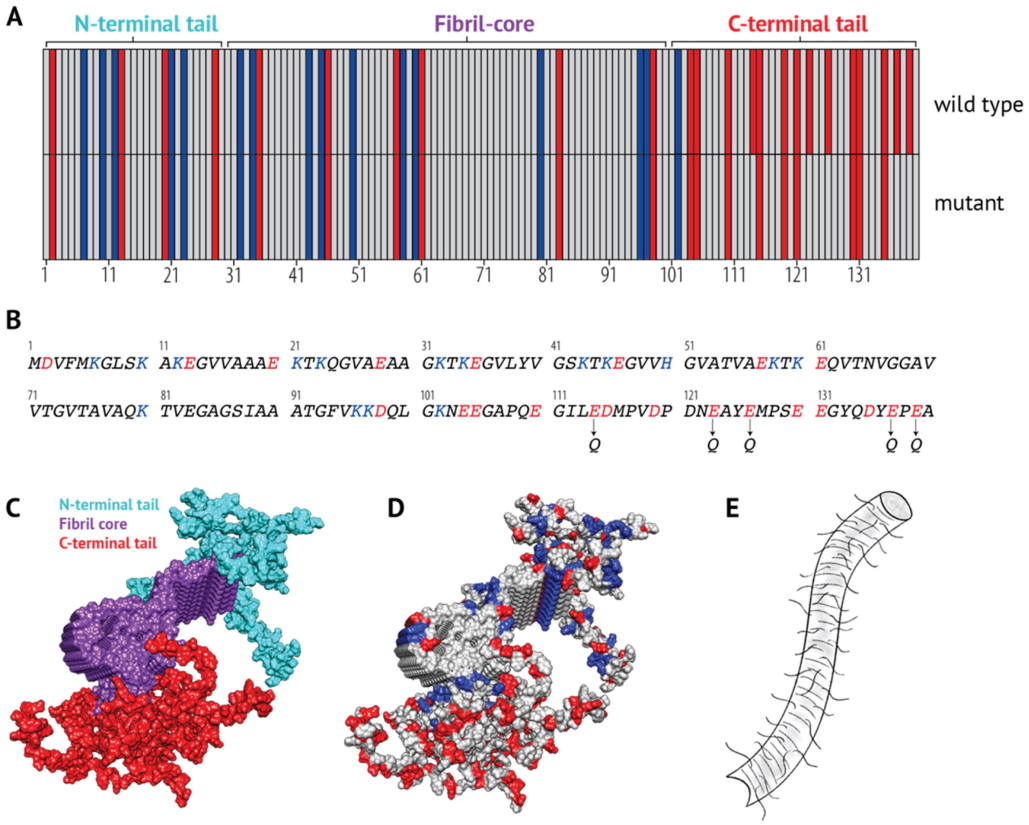

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