Research Talk

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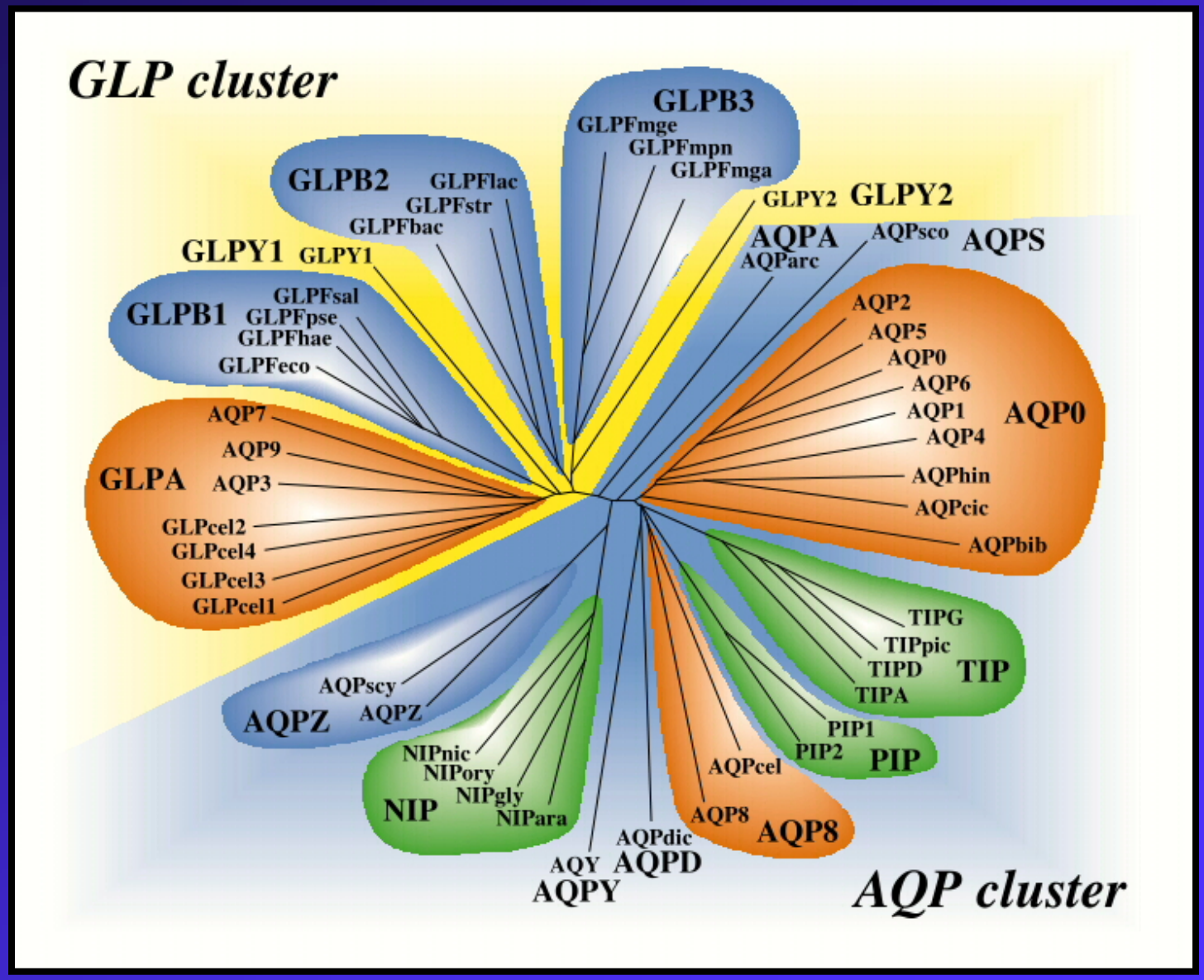
Harvard Medical School

Workshop on Advanced Topics in EM Structure Determination

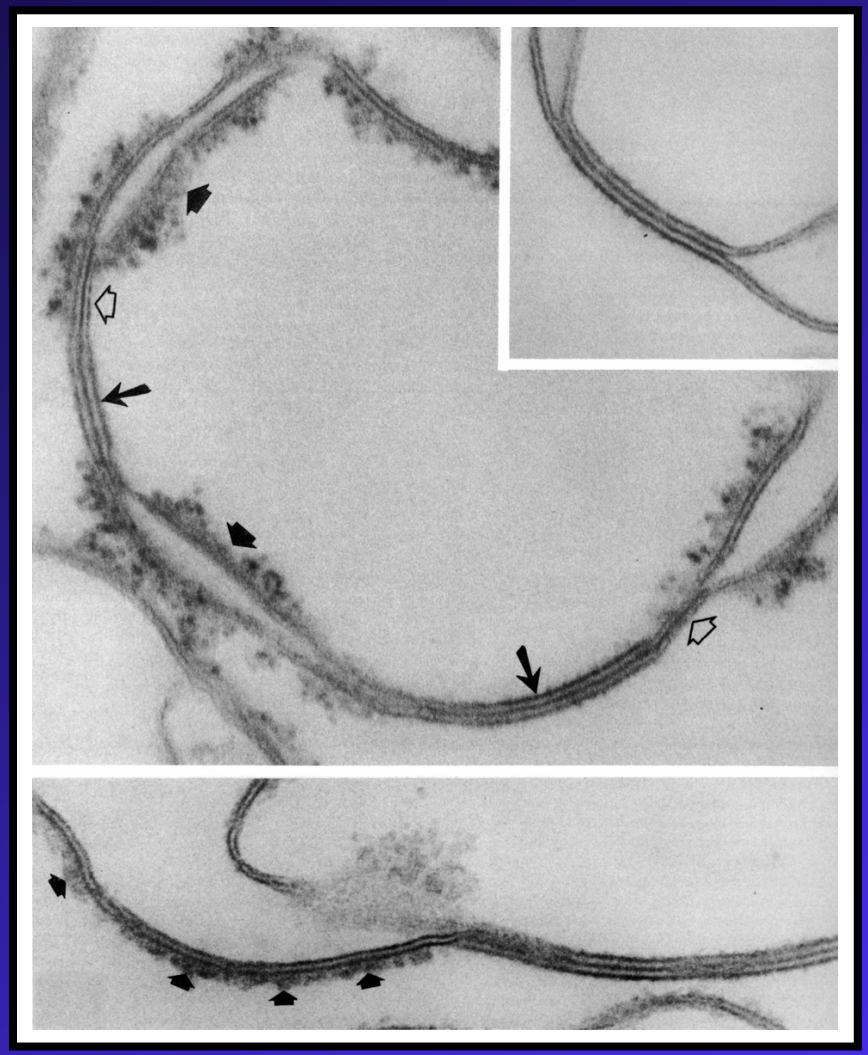
The Scripps Research Institute
La Jolla, November 2007

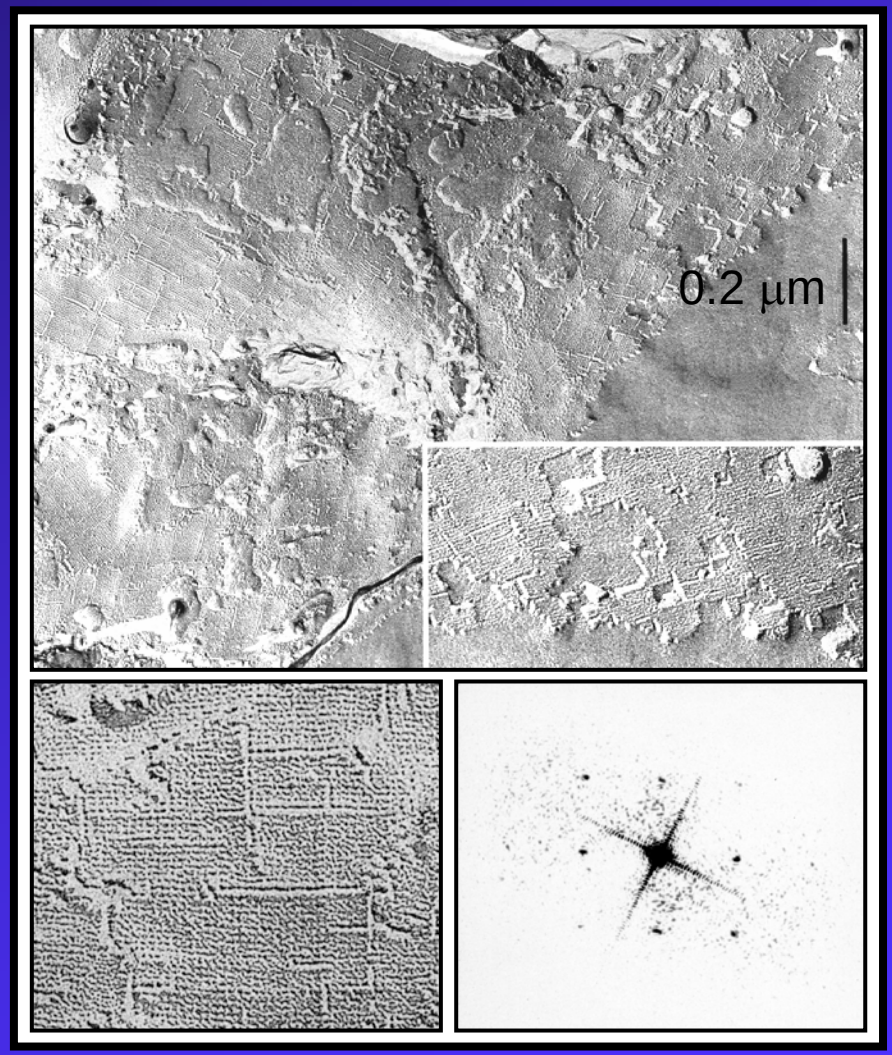
Structure of the Aquaporin-0 Membrane Junction

The aquaporin family of water pores



AQP0 forms thin junctions *in vivo*

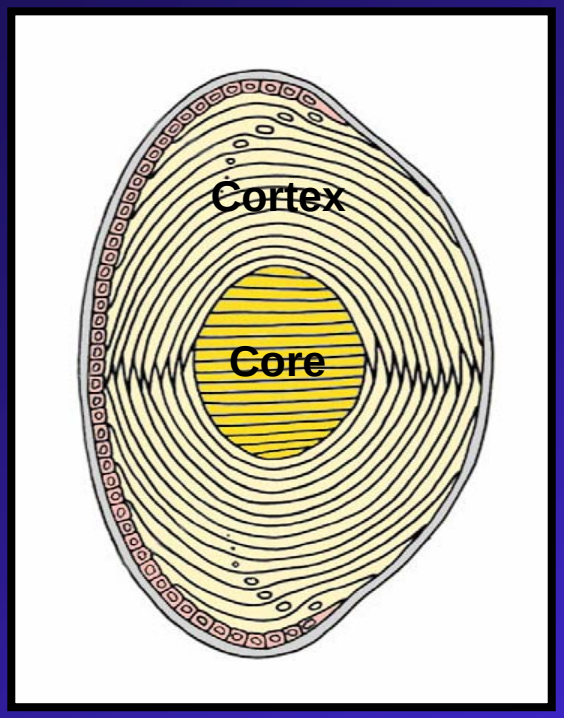




Adapted from:
Paul & Goodenough (1983)
***J. Cell Biol.* 96: 625-632**

Adapted from:
Zampighi et al. (1982)
***J. Cell Biol.* 93: 175-189**

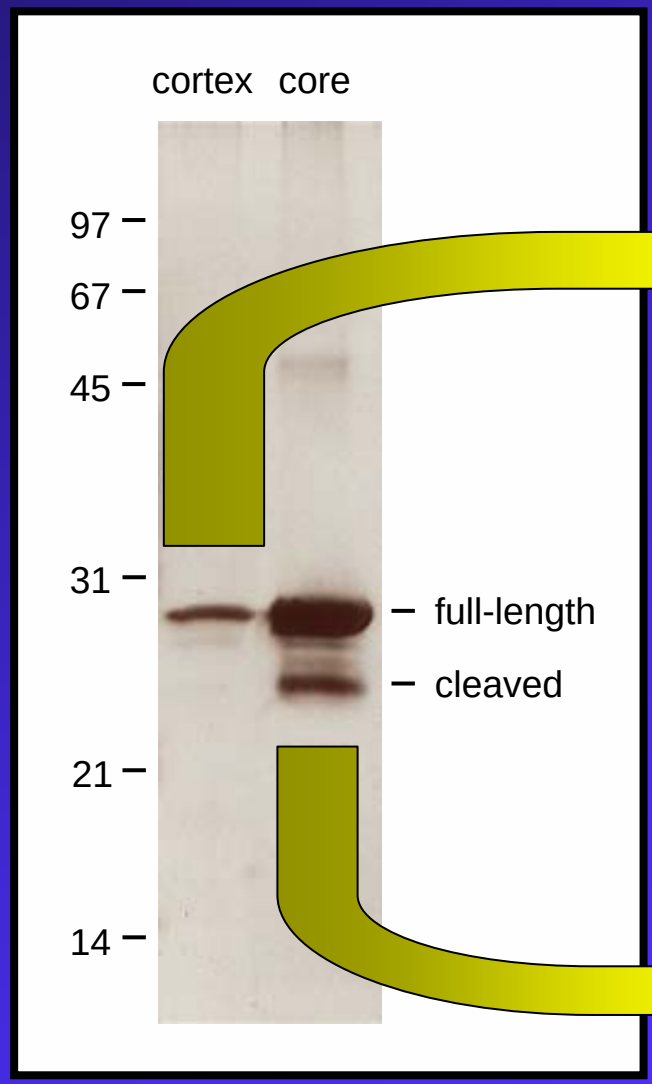
Purification of AQP0 from the lens



Solubilization in 1% DM

Anion exchange (MonoQ)

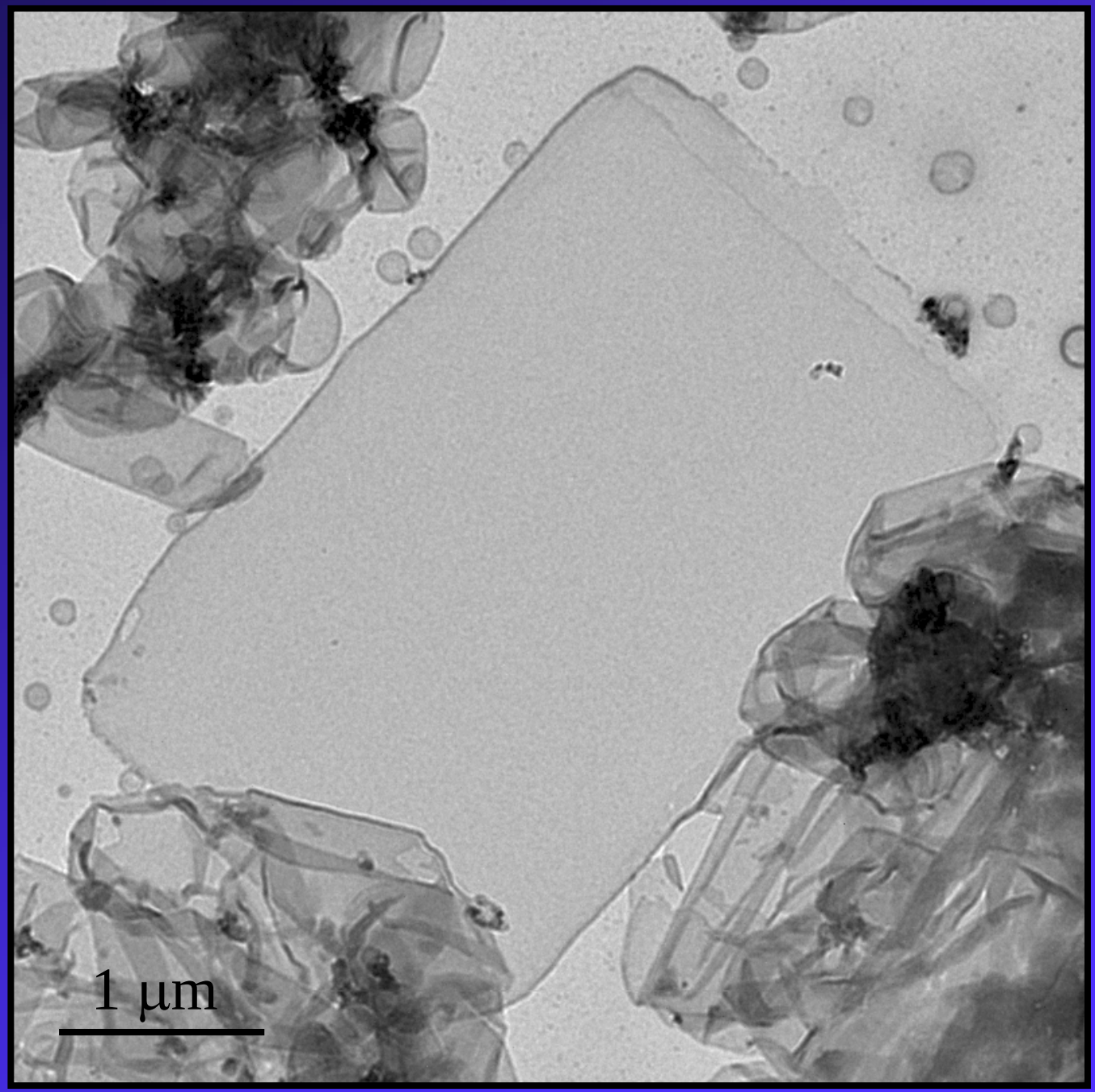
Gel filtration (S12)



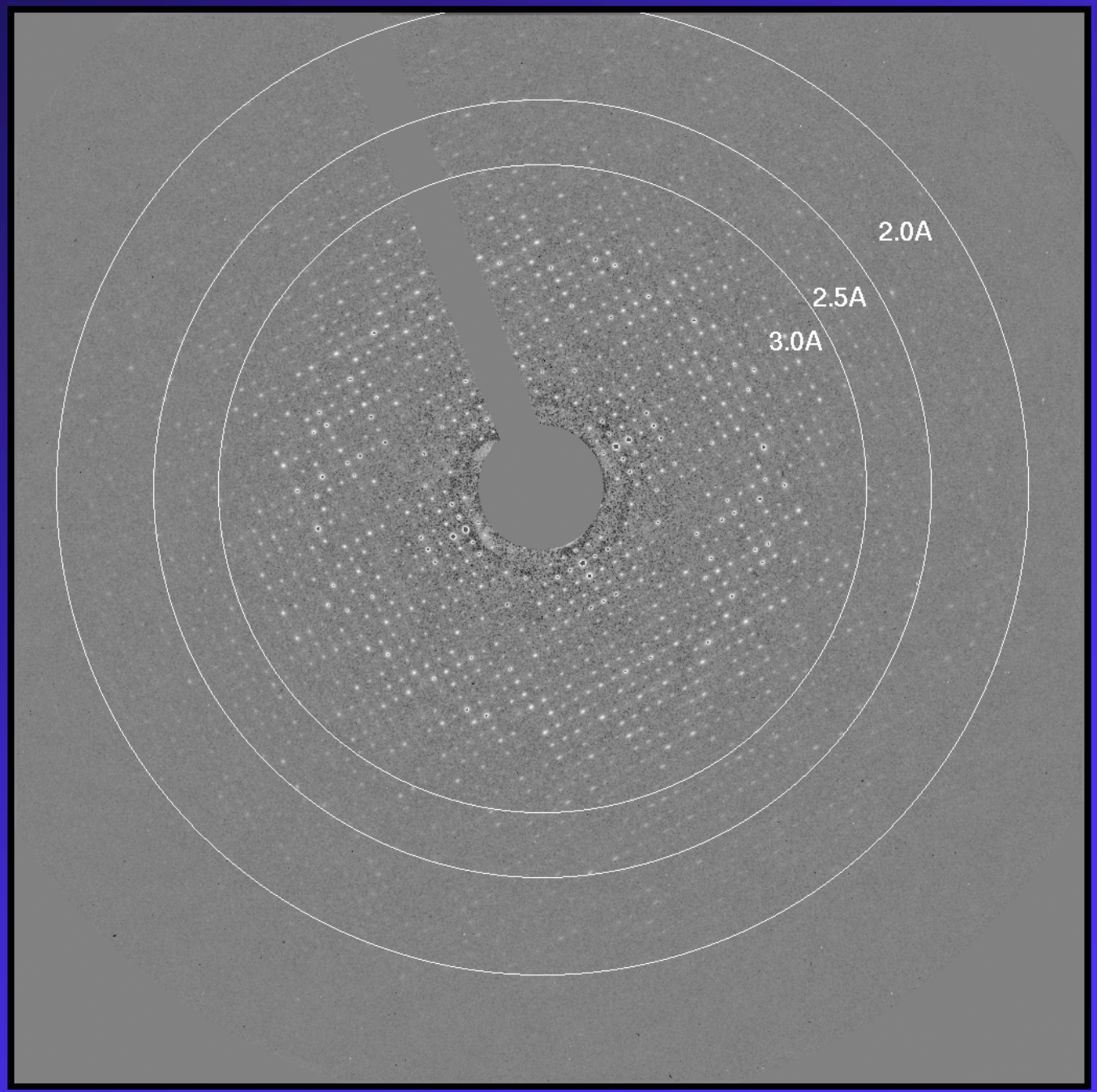
Single-layered 2D crystals

Double-layered 2D crystals

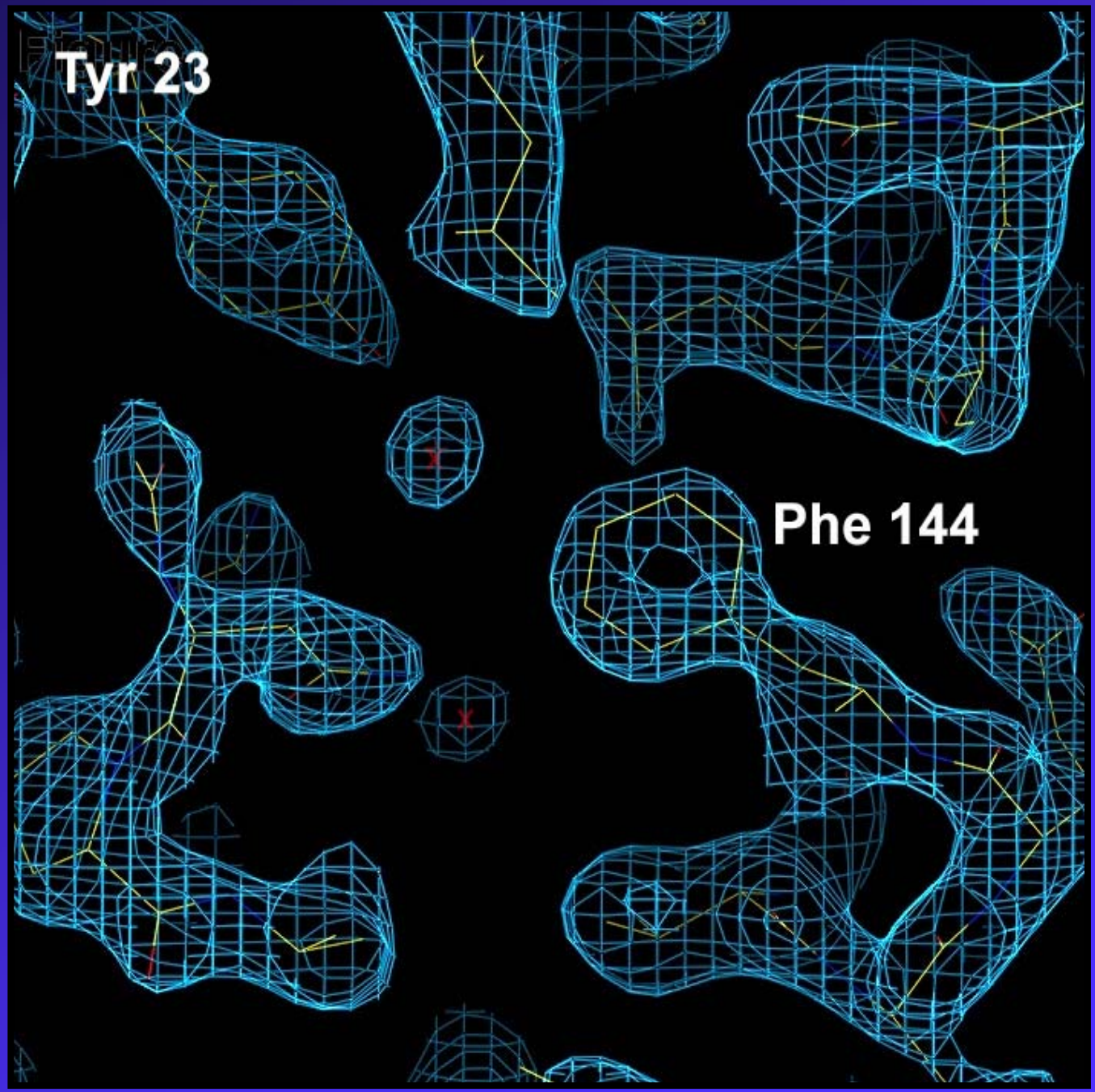
Double-layered 2D crystals of AQP0



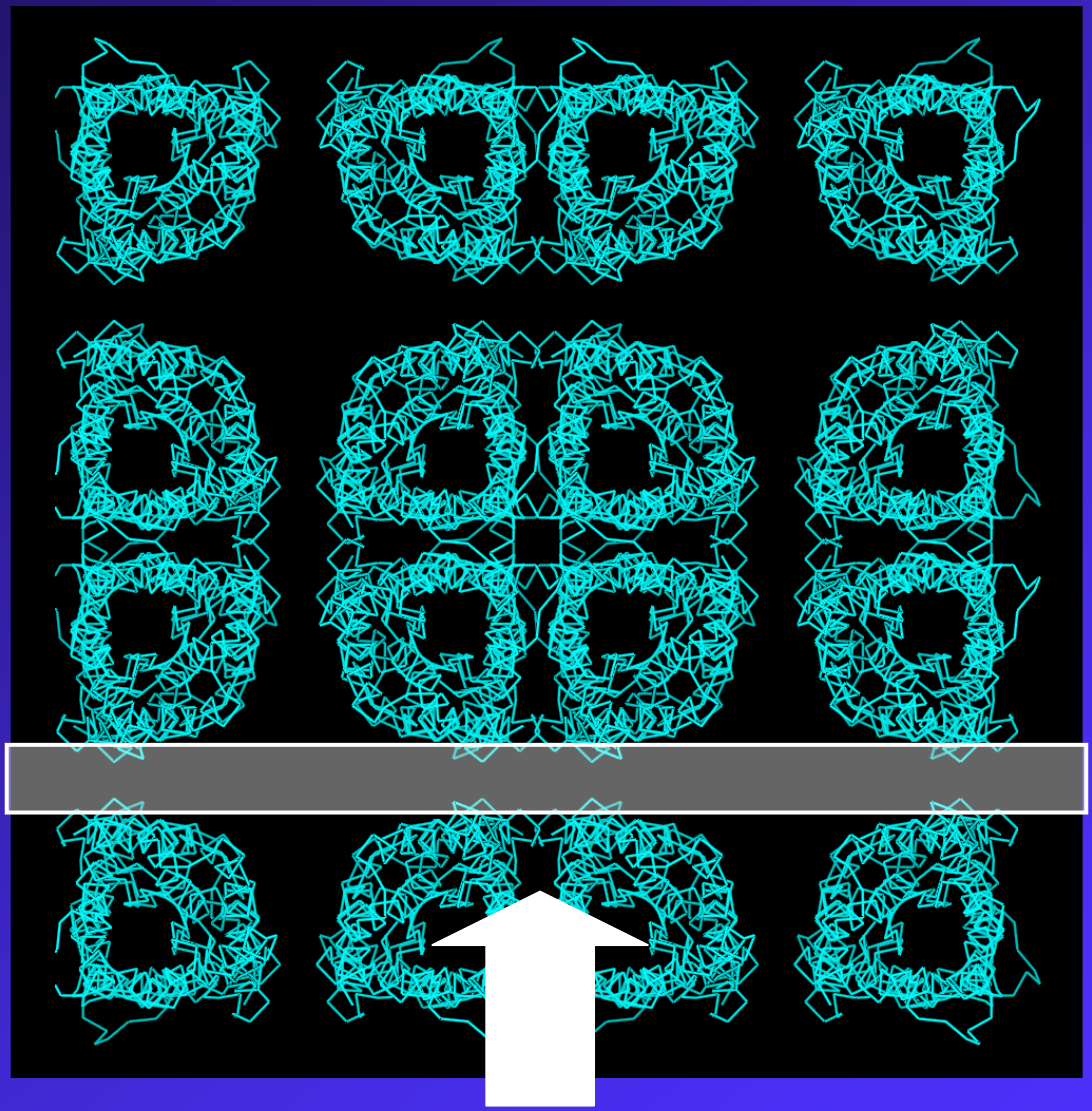
Electron diffraction at liquid He temperature



The 1.9 Å density map



The packing of AQP0 in the 2D crystals



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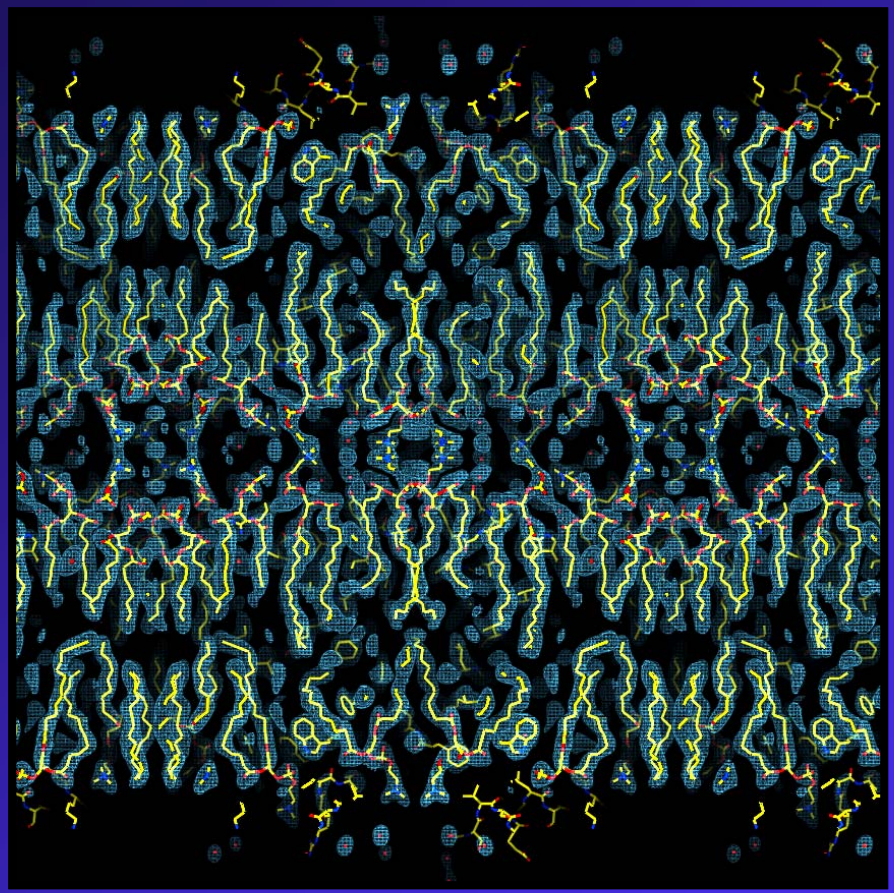

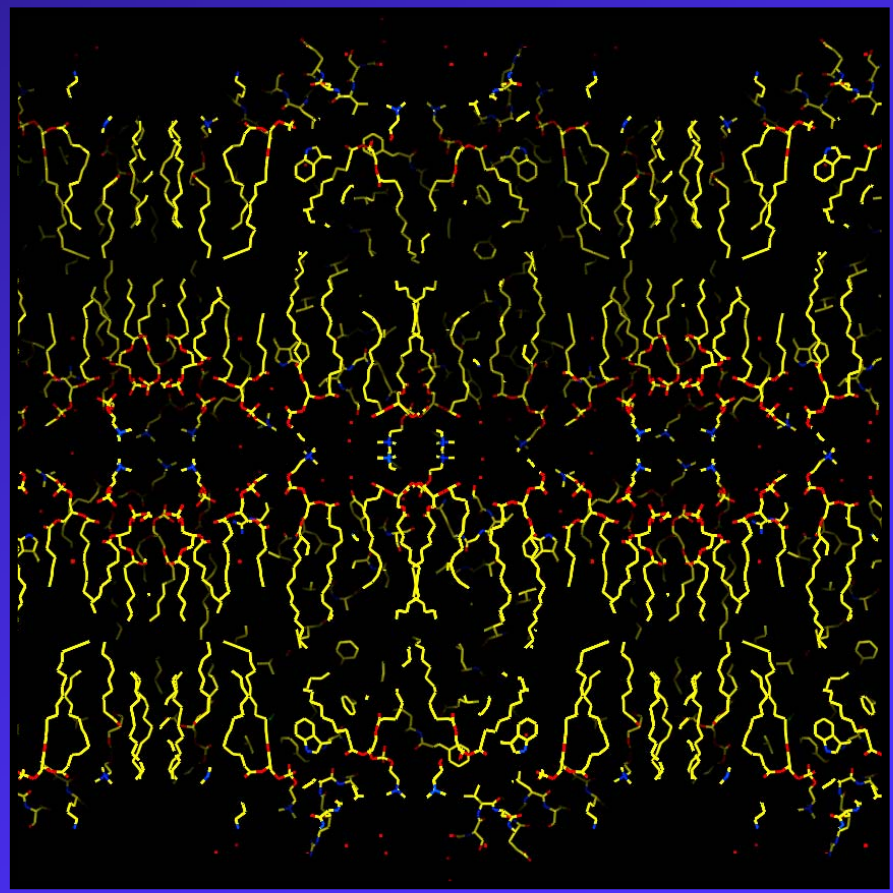

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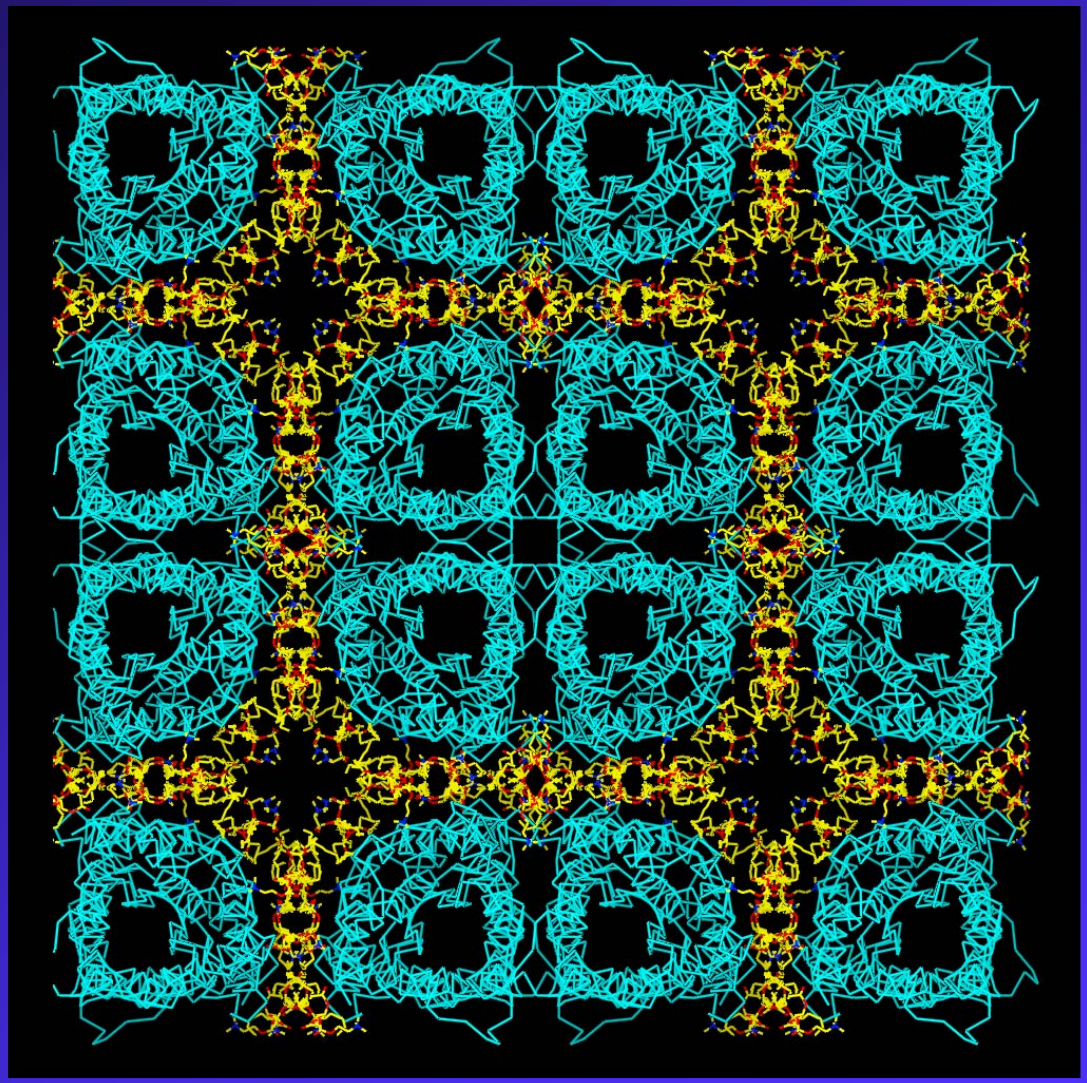
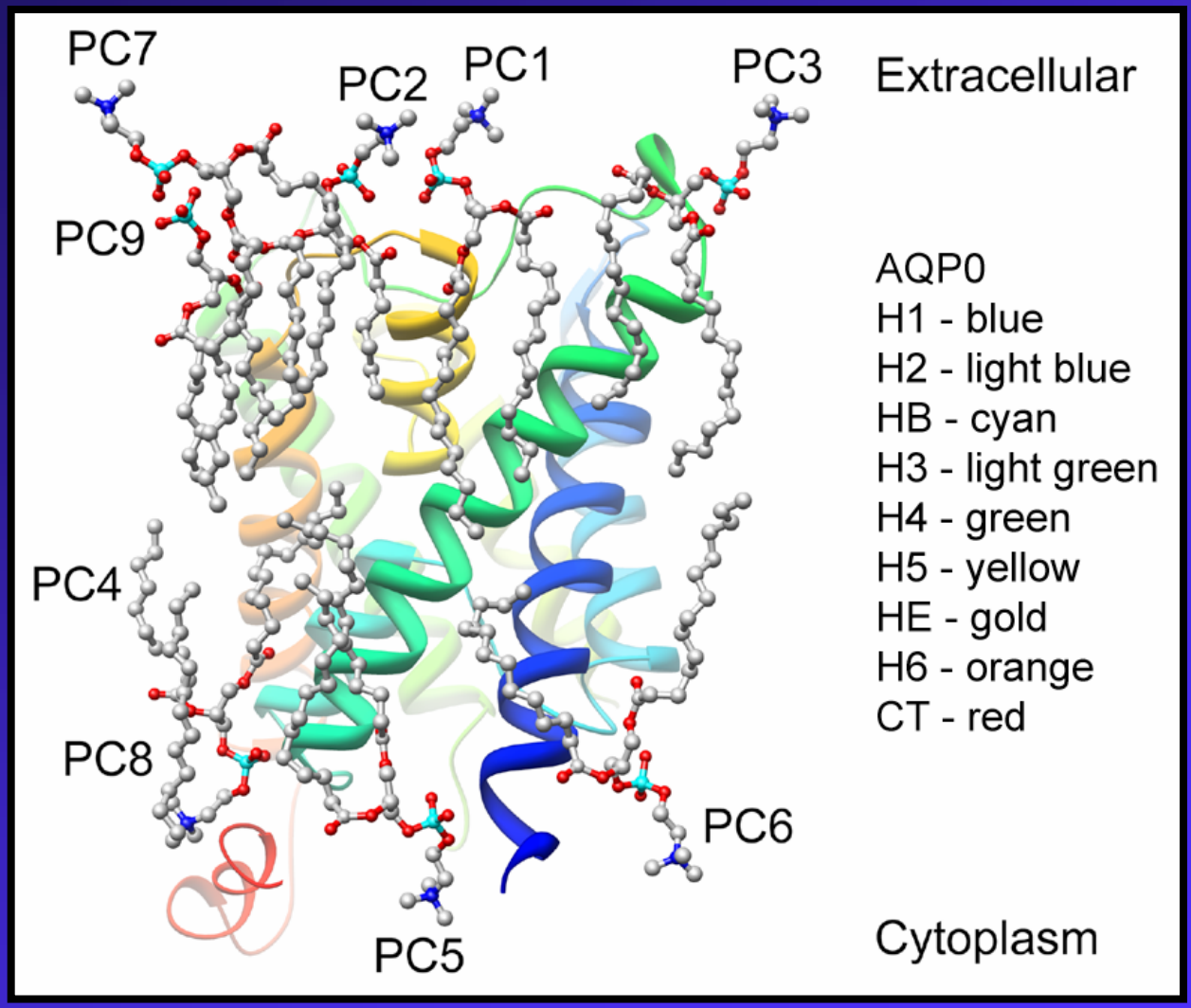
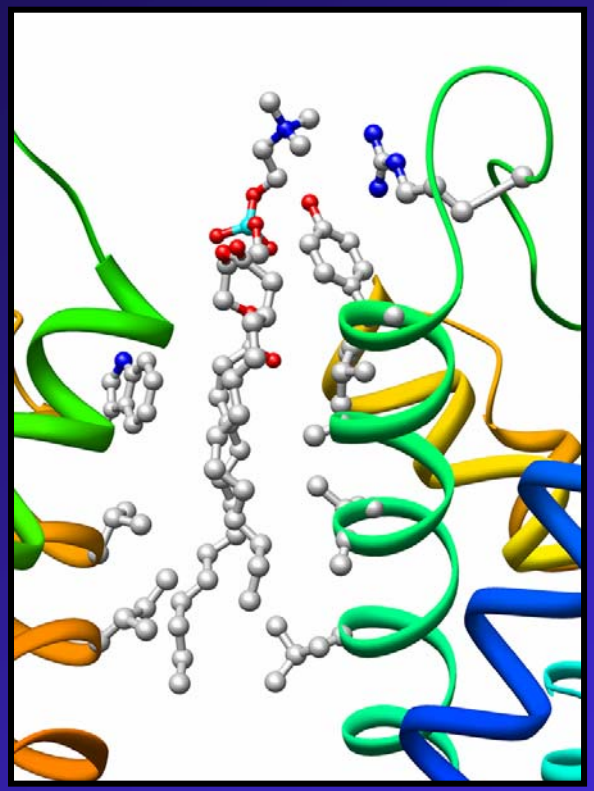


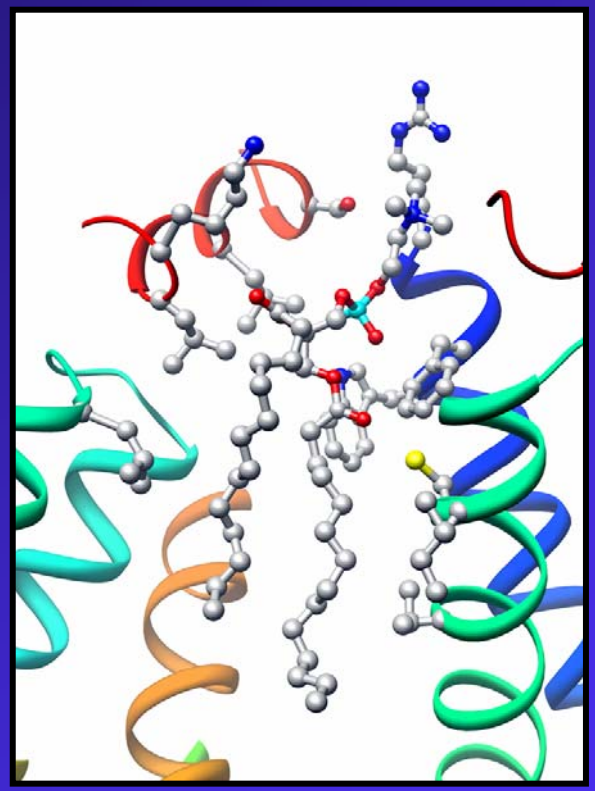
The lipids surrounding an AQP0 monomer



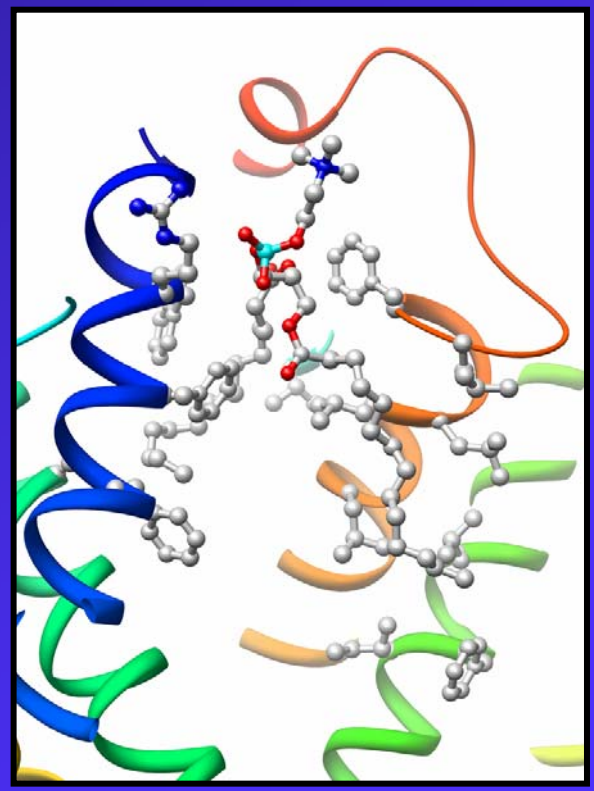
Protein-lipid interactions



PC 1

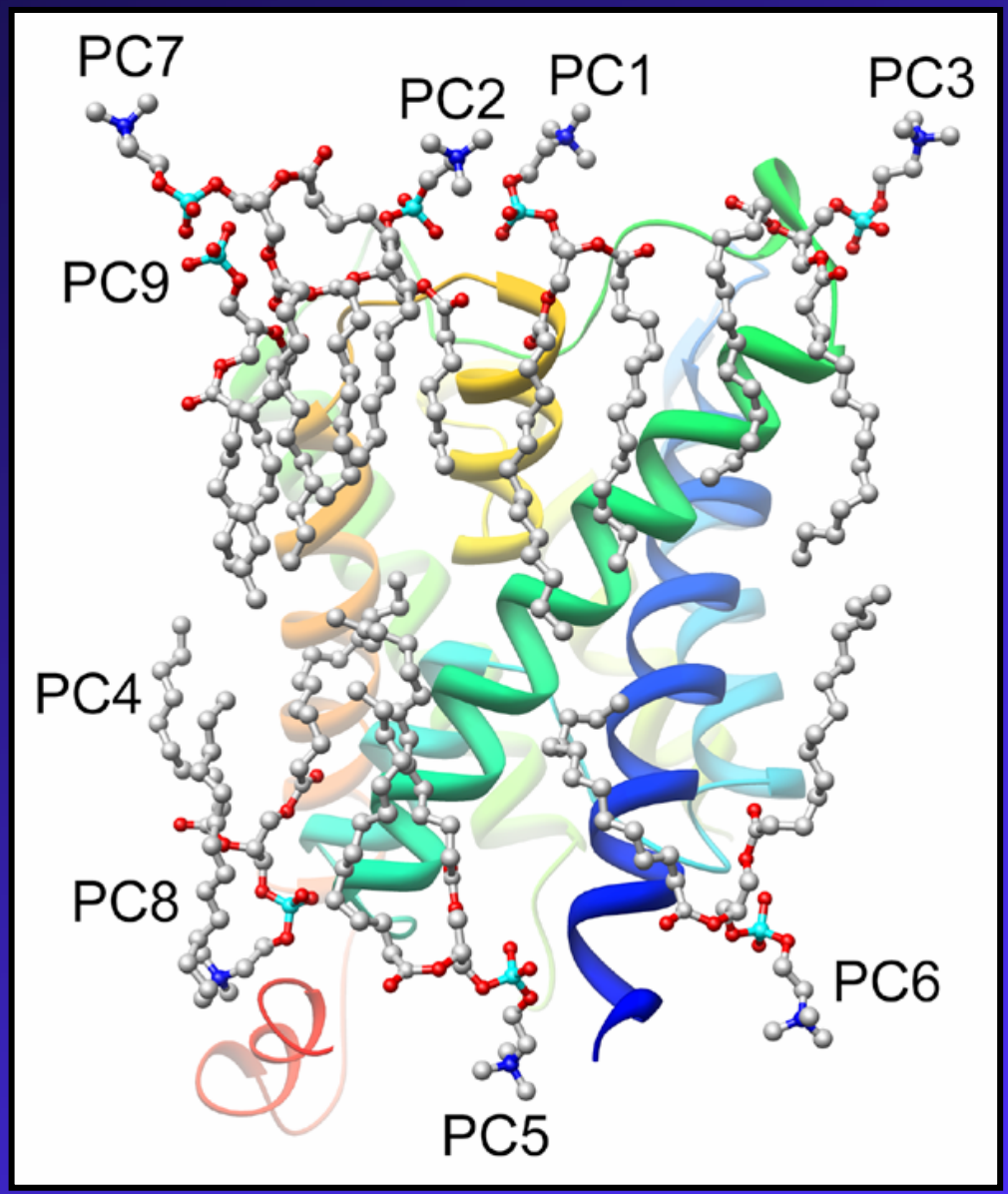


PC 5



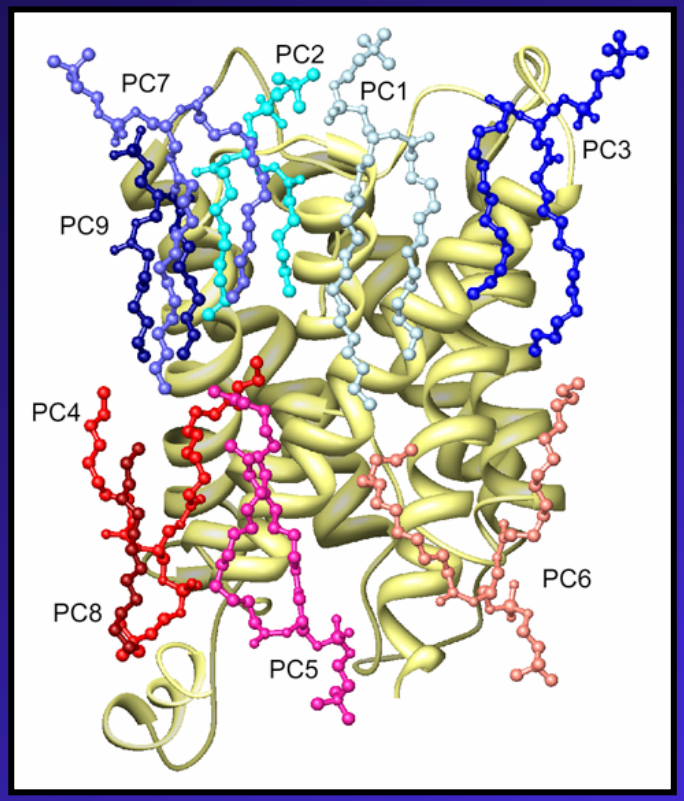
PC 6

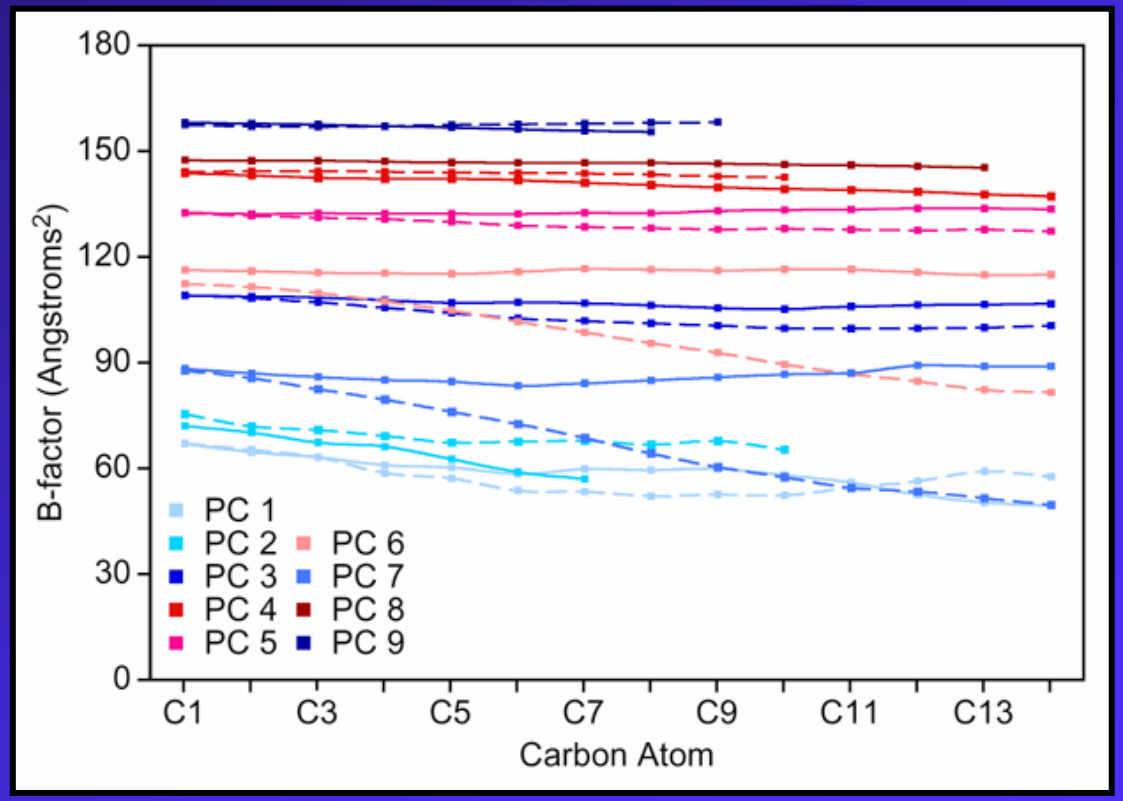
Protein-lipid interactions



Lipid dynamics

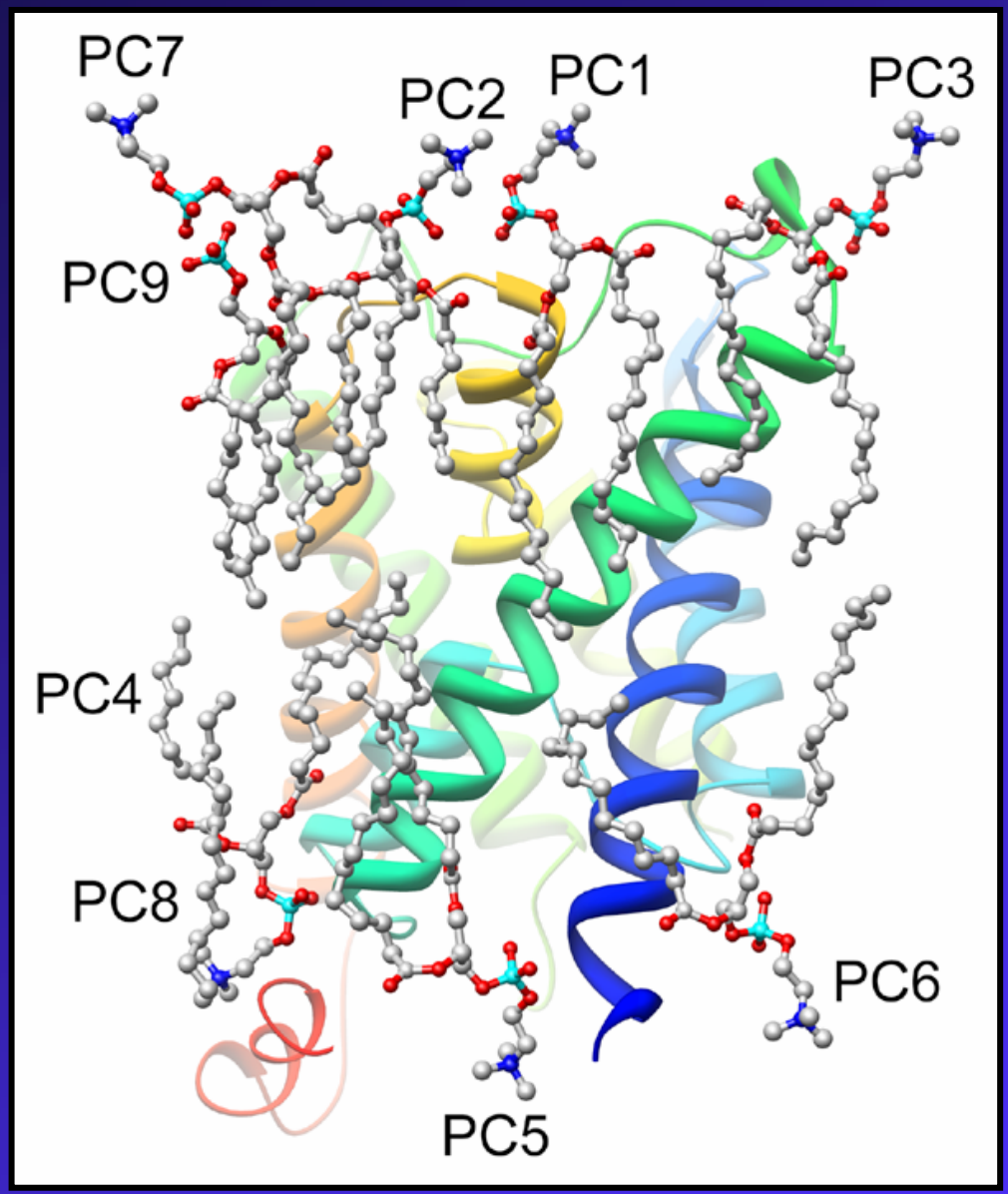
Protein-lipid interactions





Acyl chains appear more constrained in membrane center

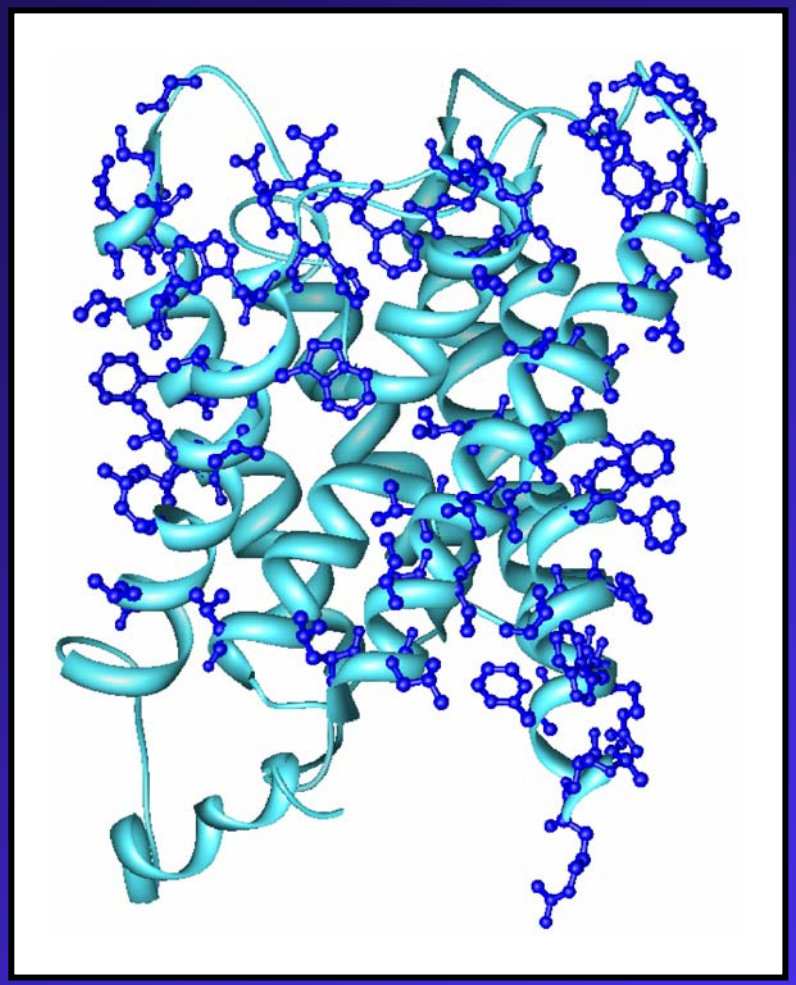
Protein-lipid interactions



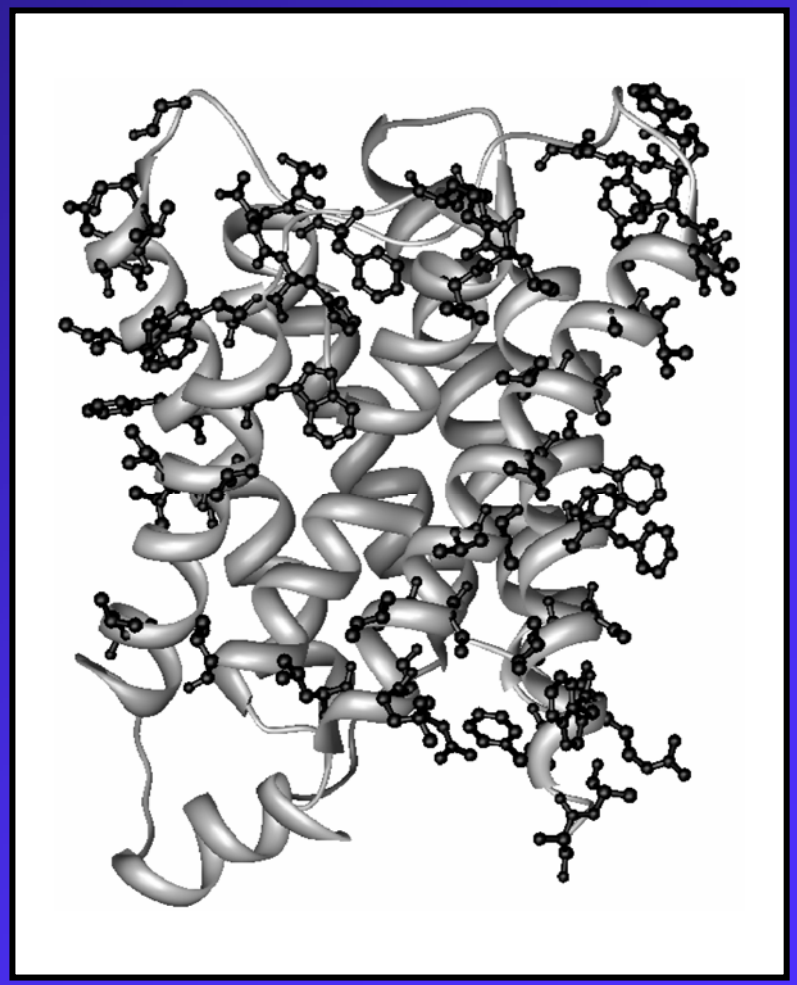
Lipid dynamics

Effects of lipids on protein structure

Protein-lipid interactions



1.9 Å EM structure

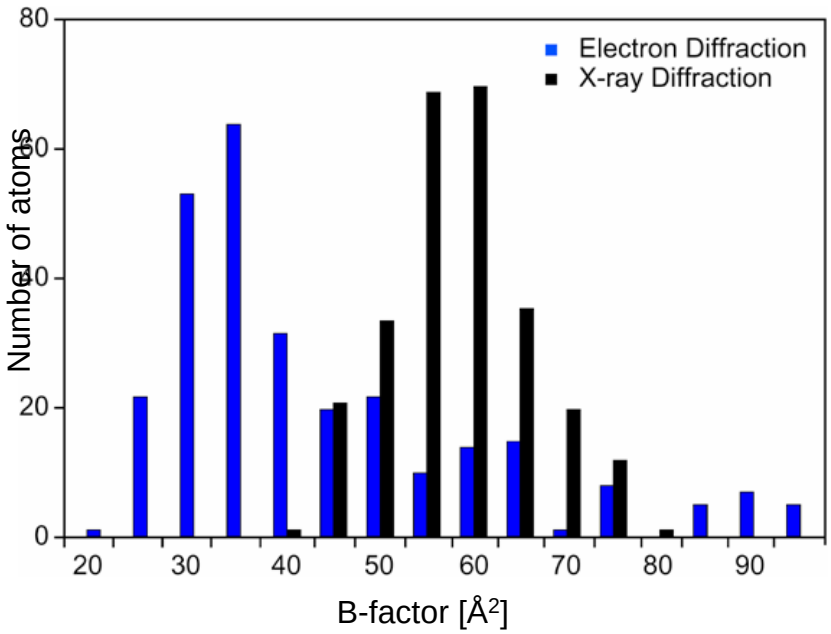


2.2 Å X-ray structure

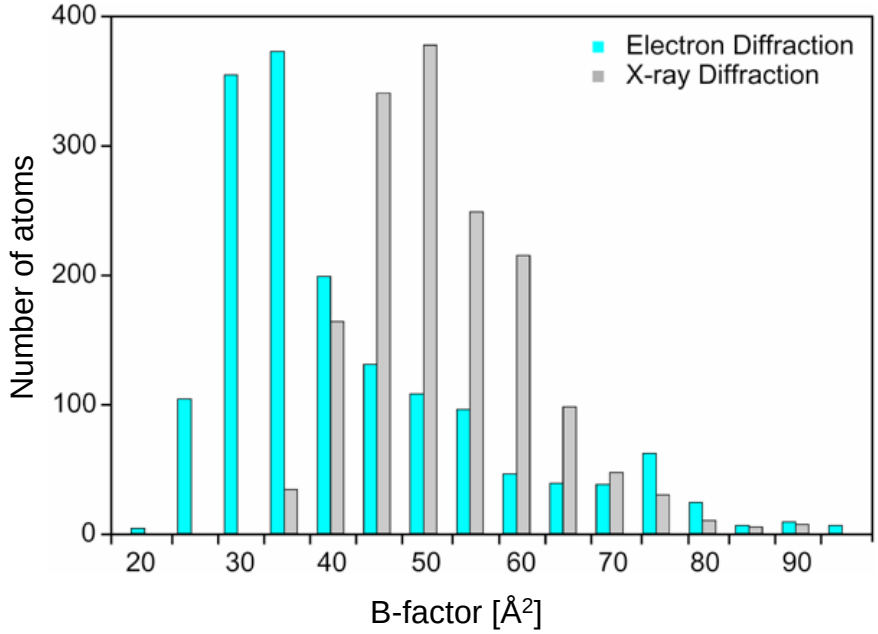
Protein-lipid interactions

B-factors – Electron diffraction *versus* X-ray diffraction

Lipid-contacting atoms



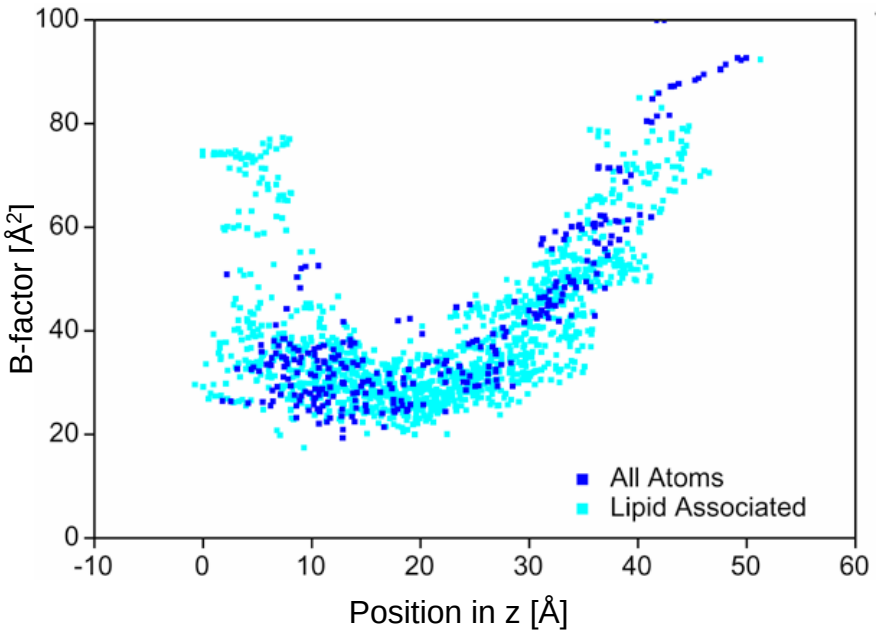
All atoms



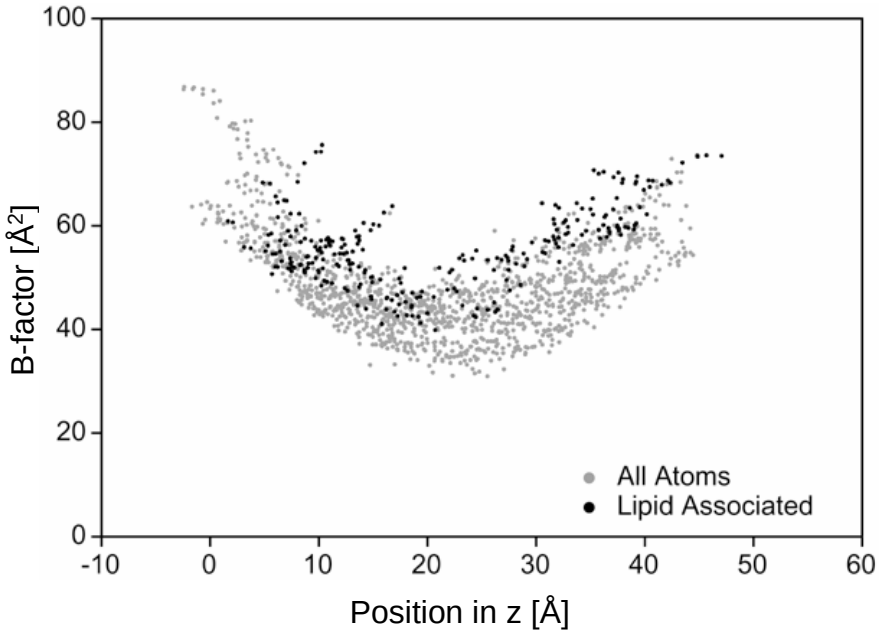
Protein-lipid interactions

B-factors – Electron diffraction *versus* X-ray diffraction

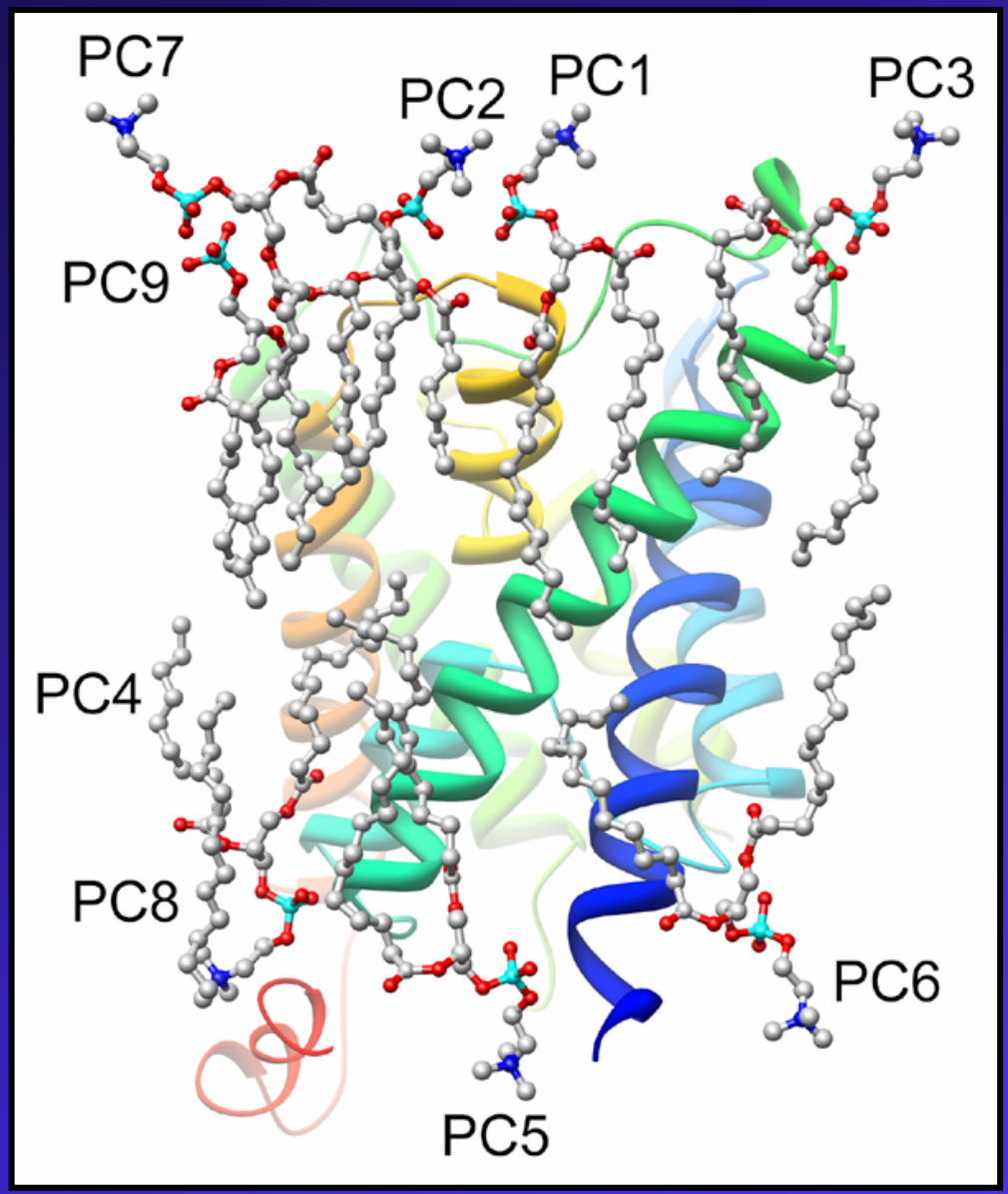
EM structure



X-ray structure



Protein-lipid interactions

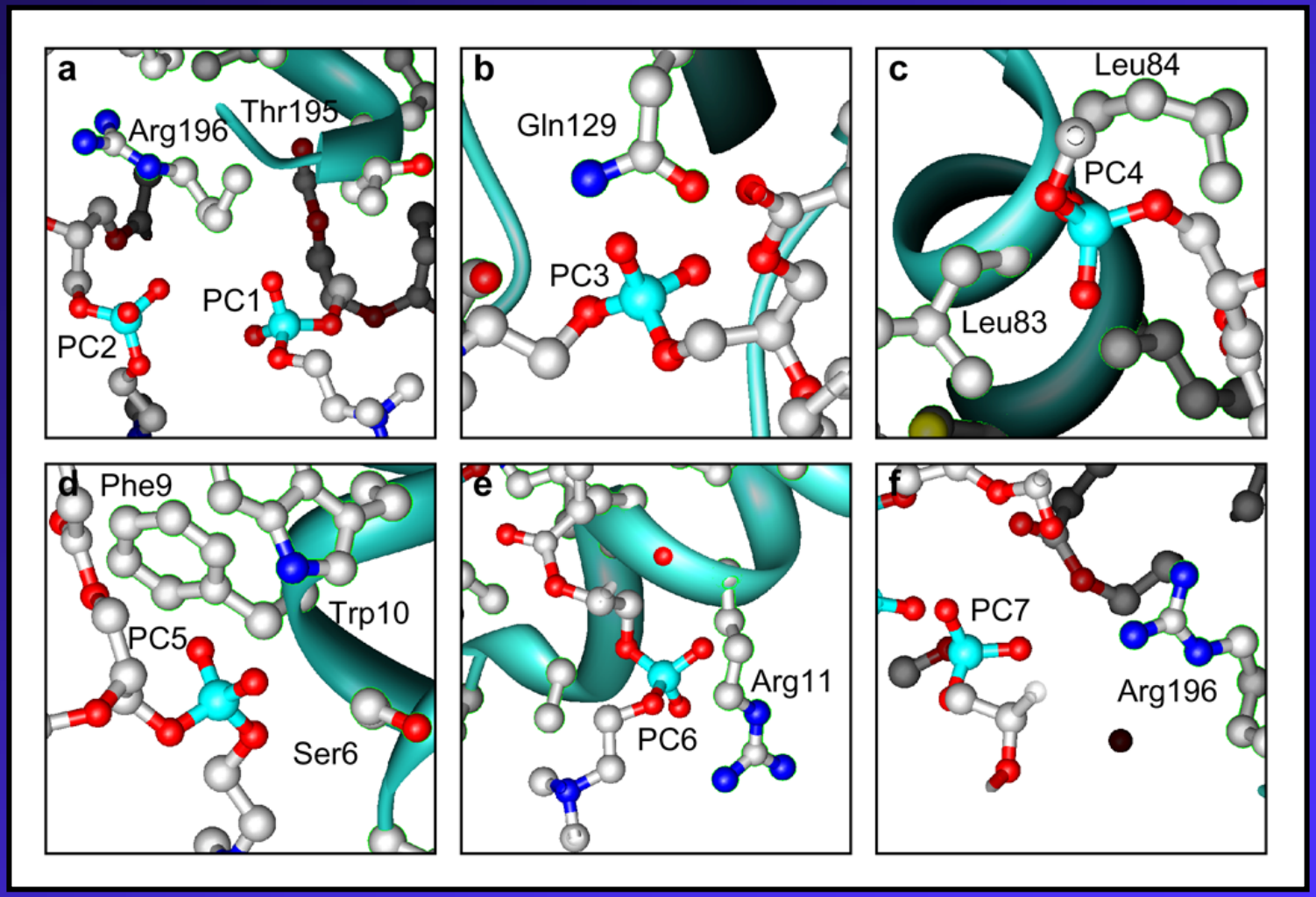


Lipid dynamics

Effects of lipids on protein structure

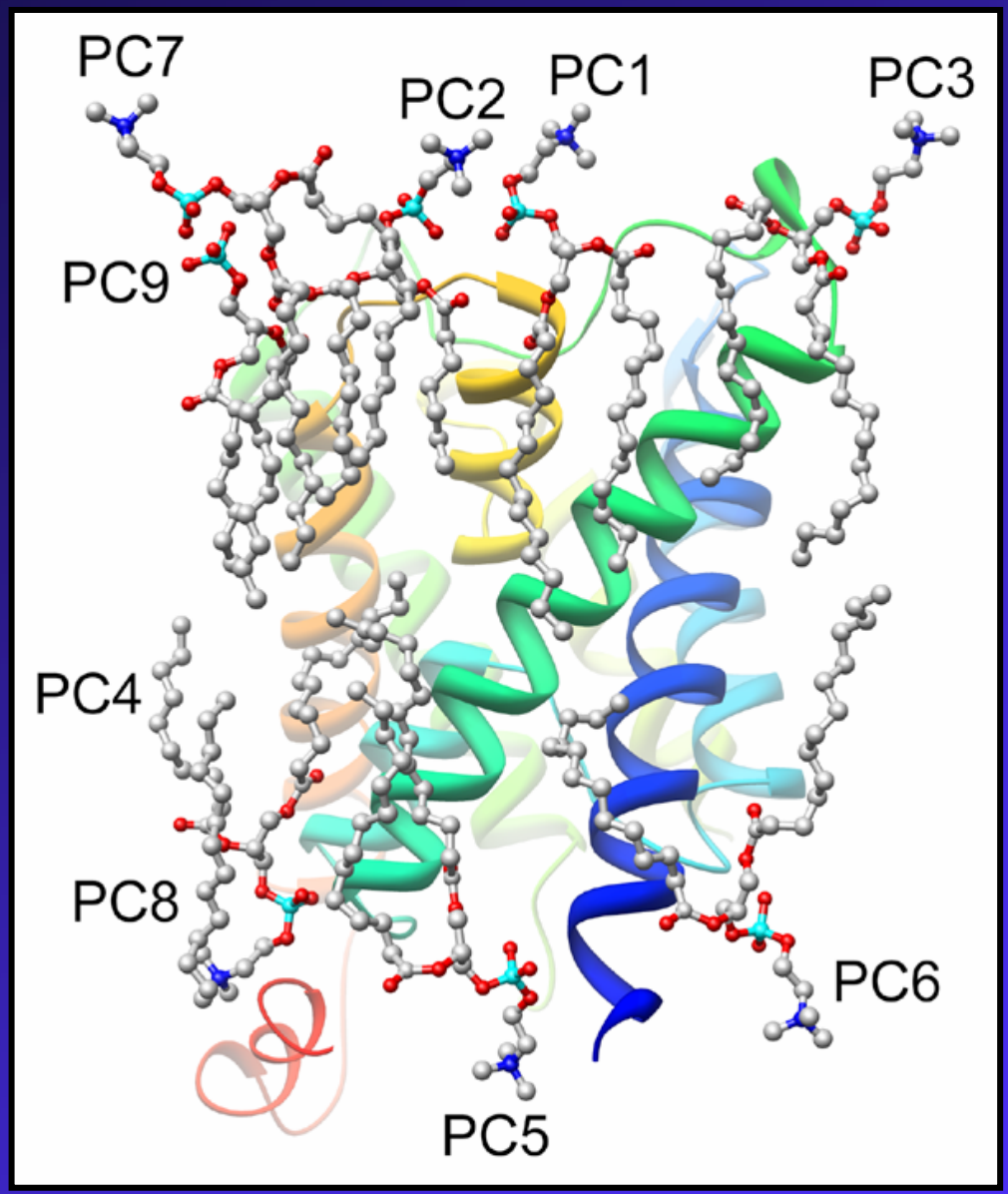
Lipid binding motifs

Protein-lipid interactions



No obvious binding motif for PC phosphodiester group

Protein-lipid interactions



Lipid dynamics

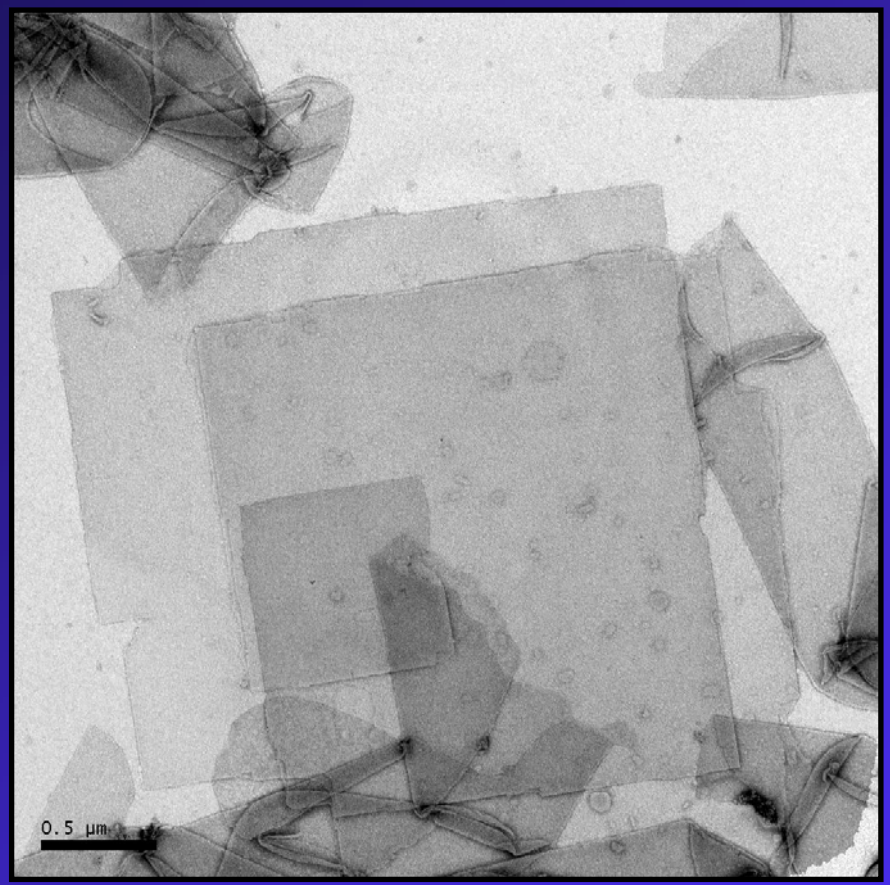
Effects of lipids on protein structure

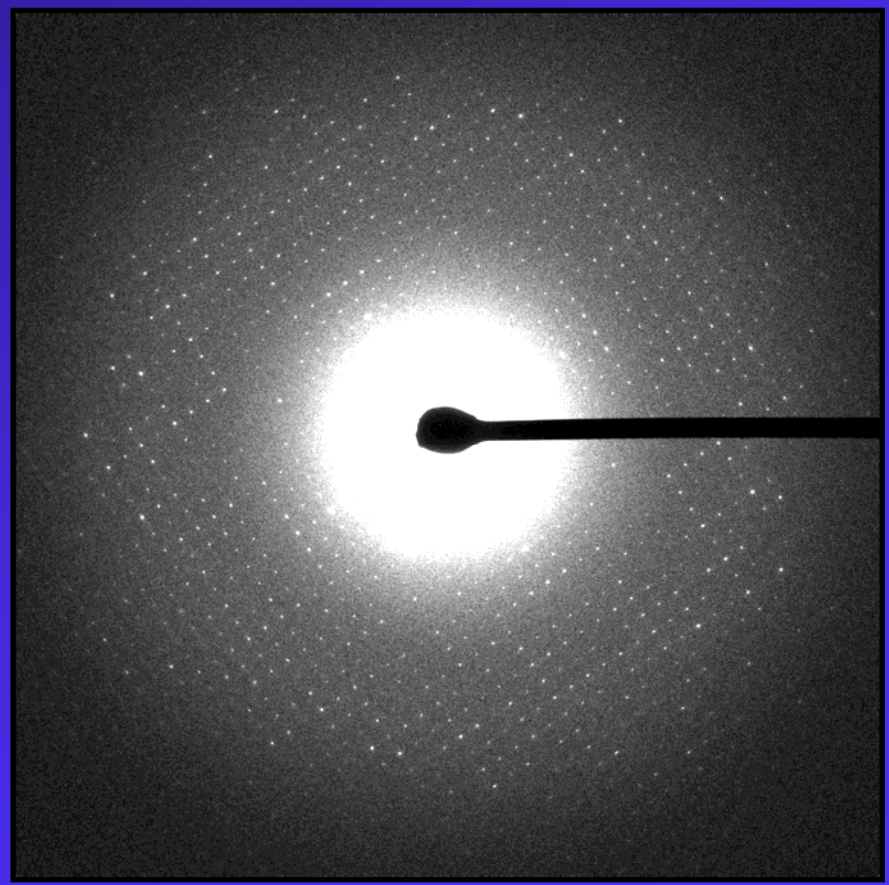
Lipid binding motifs

Different lipids

Protein-lipid interactions

2D crystals in *E. coli* polar lipids (67% PE, 23% PG, 10% cardiolipin)





Monolayer Purification

Structure determination by single particle EM

Biochemical purification

↓

Specimen preparation

↓

Low-dose imaging

↓

Image processing

↓

3D reconstruction

} not automated
time-consuming

} more or less automated
time-efficient

Purification of macromolecular complexes

Challenges

– unstable, heterogeneous

– low expression, low yield

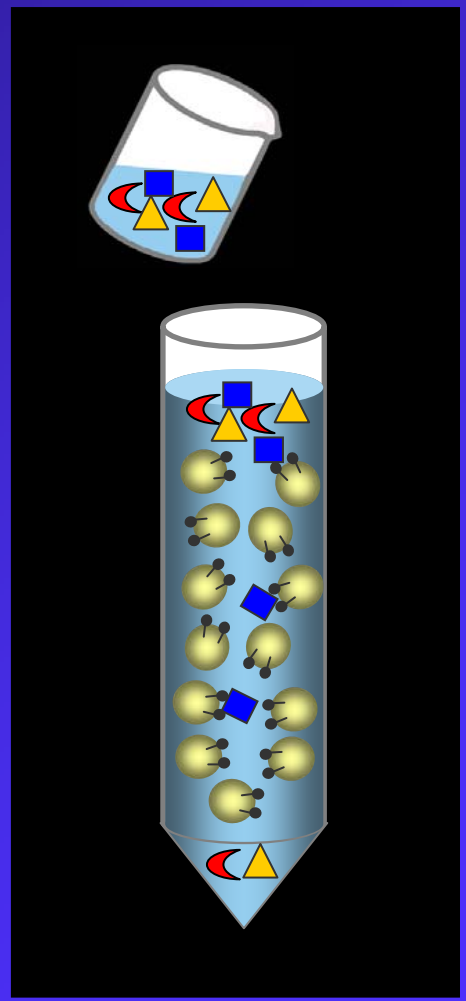
– high purity

Commonly used affinity tags

– His tag

– FLAG tag

– TAP tag



Lichty *et al.*, Protein Expression and Purification (2005)

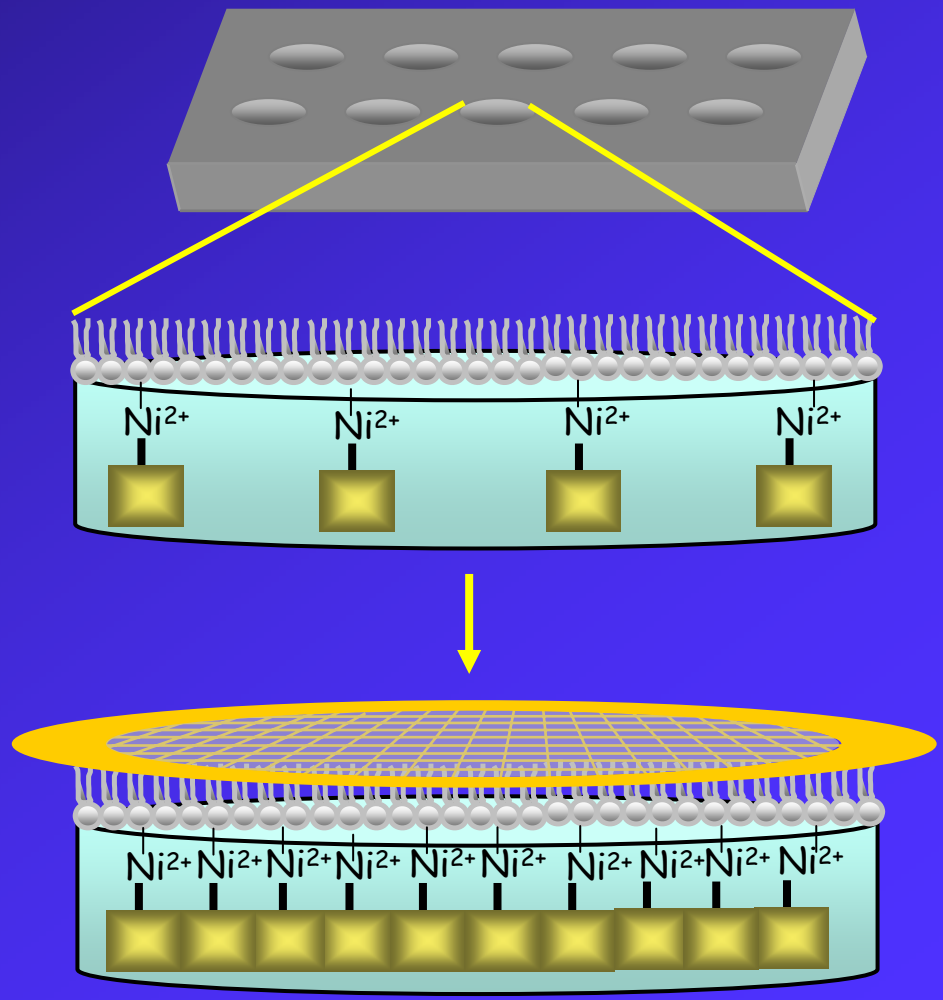
2D crystallization of His-tagged proteins on lipid monolayers

Kubalek *et al.* (1994)

- Ni-NTA lipid
- His-tagged HIV1 RT
- 2D, negative stain

Kelly *et al.* (2006)

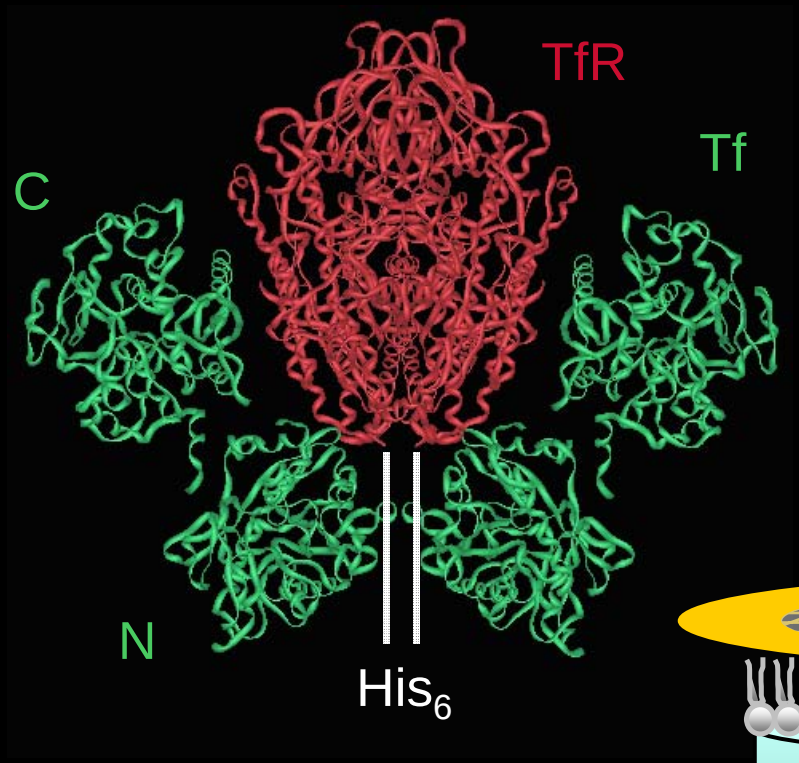
- Ni-NTA lipid
- ß1-integrin:α-actinin vinculin$_{D1}$ complex
- 3D, cryo-EM



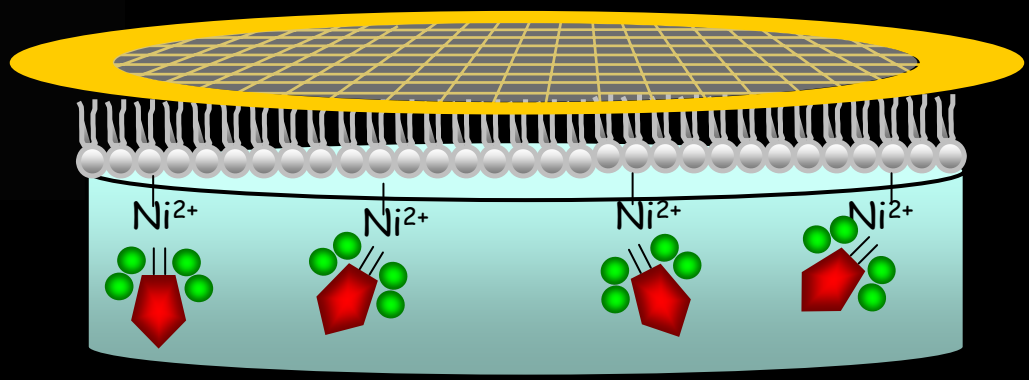
A combinatorial approach for protein purification and sample preparation for single particle EM studies

Establish whether Ni-NTA lipid monolayers can be used as a tool to purify macromolecular complexes

The test system

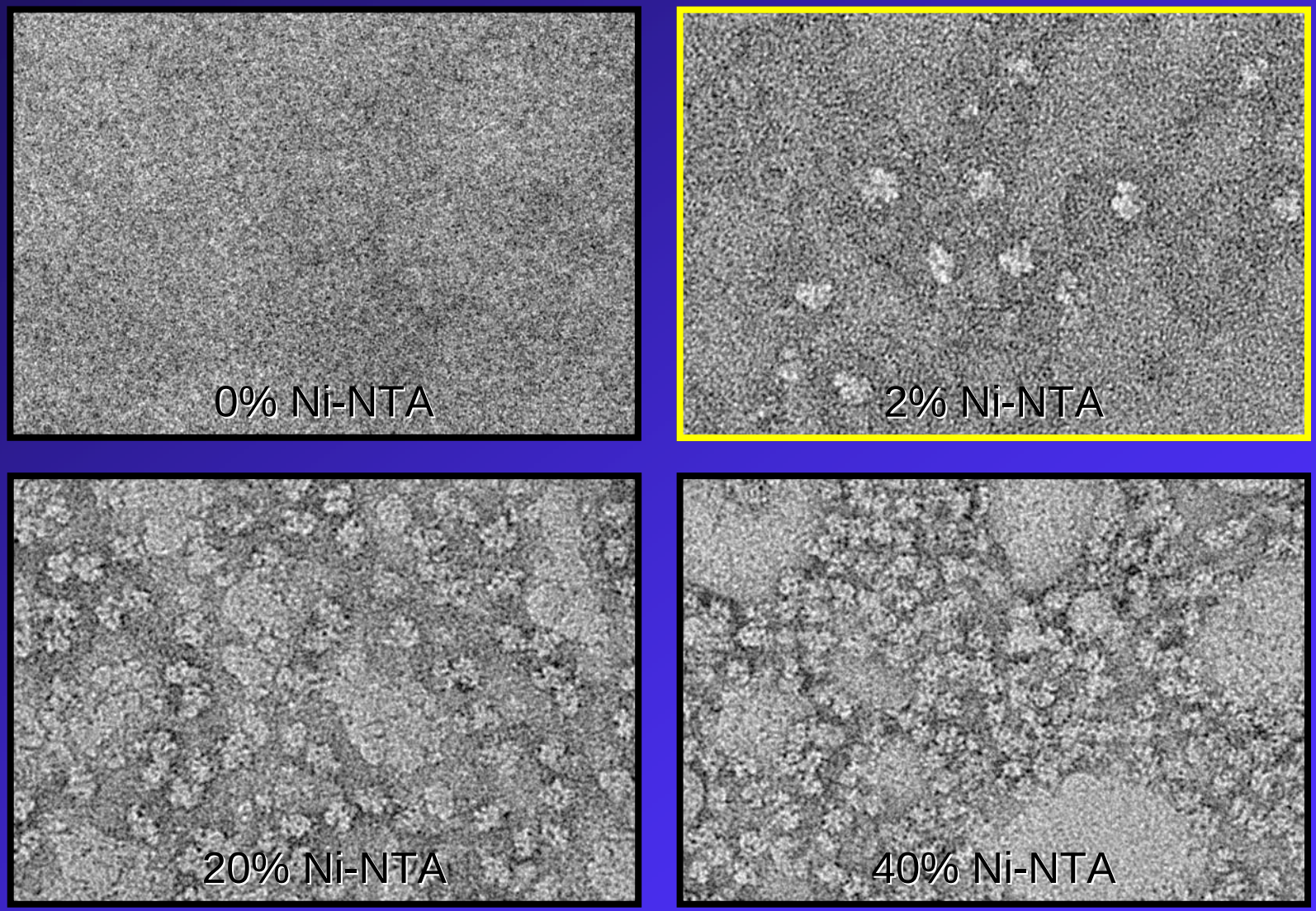


Transferrin – transferrin receptor (Tf-TfR) complex

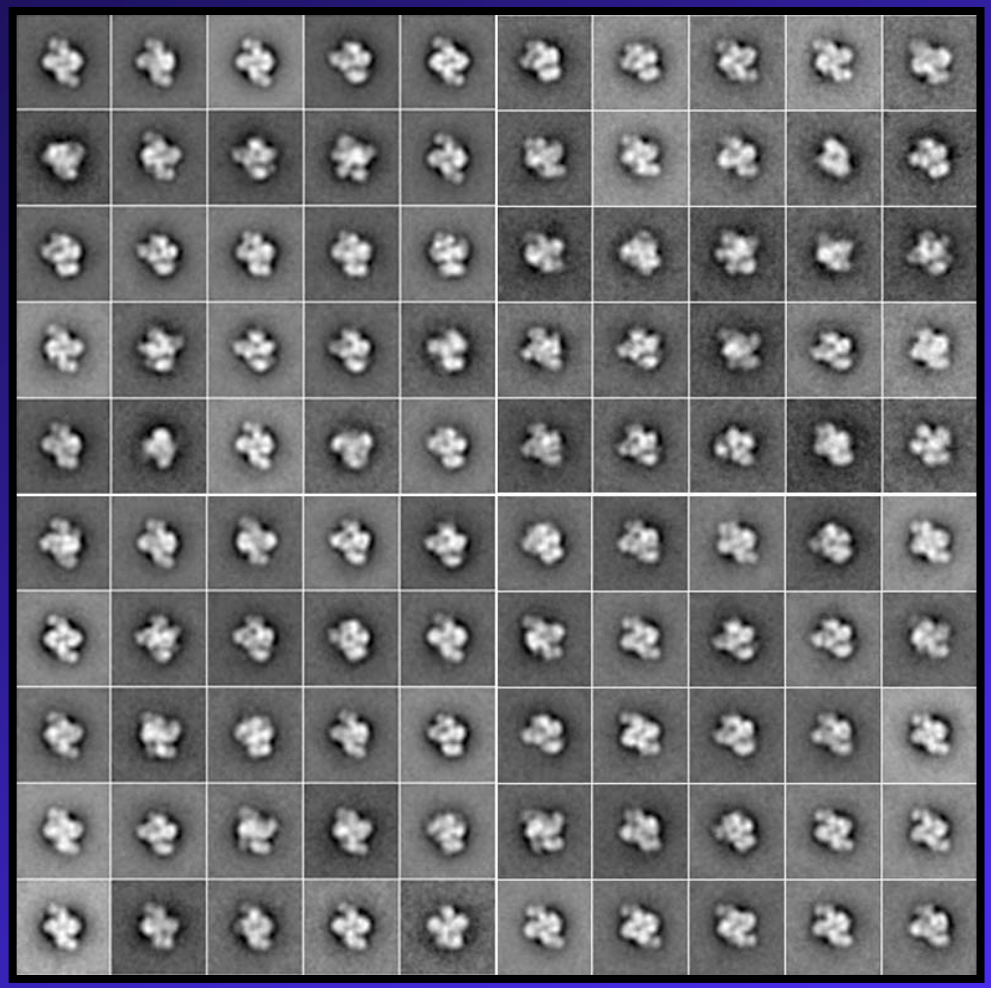


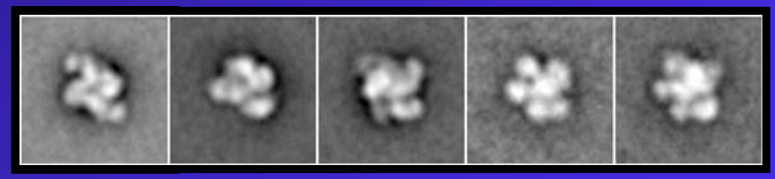
Cheng *et al.* (2004)

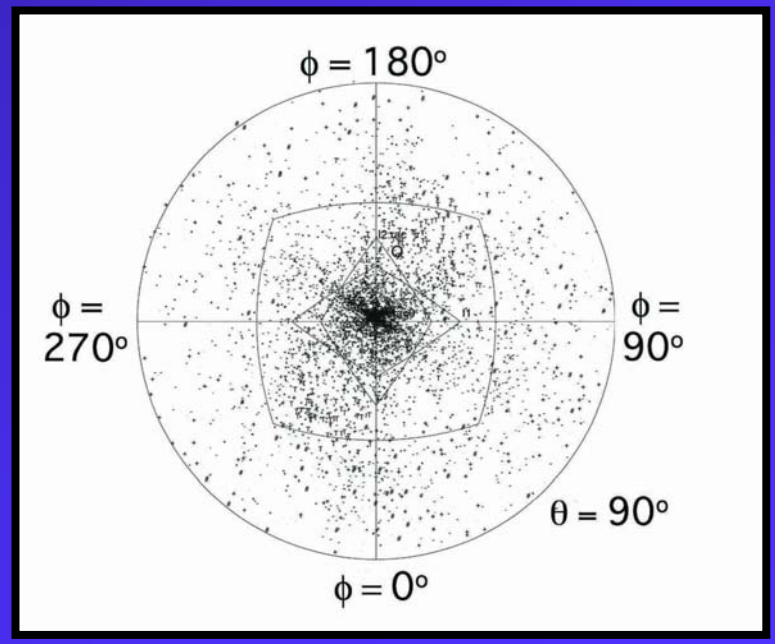
Tf-TfR complex on Ni-NTA monolayer



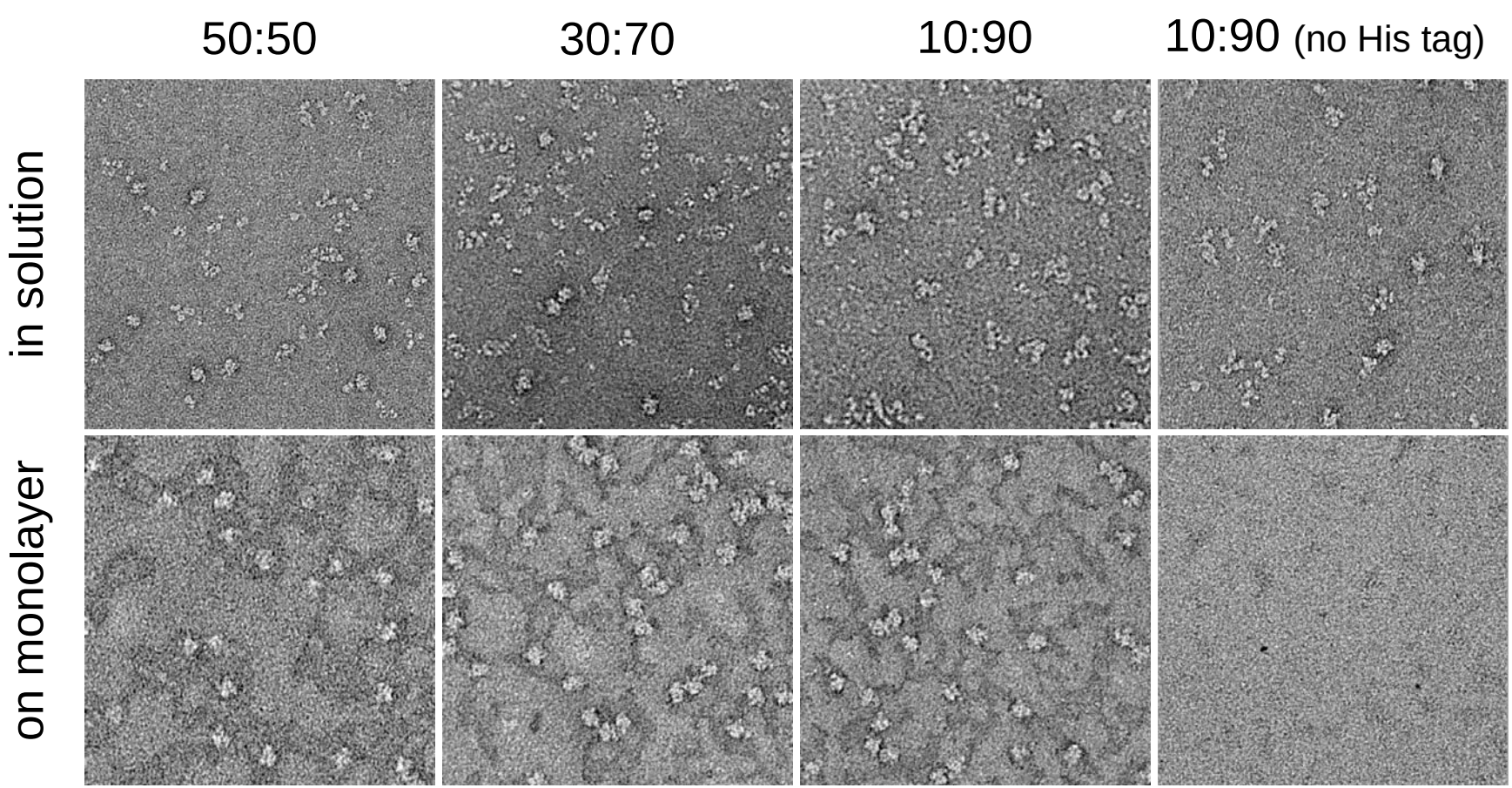
Tf-TfR complex on Ni-NTA monolayer



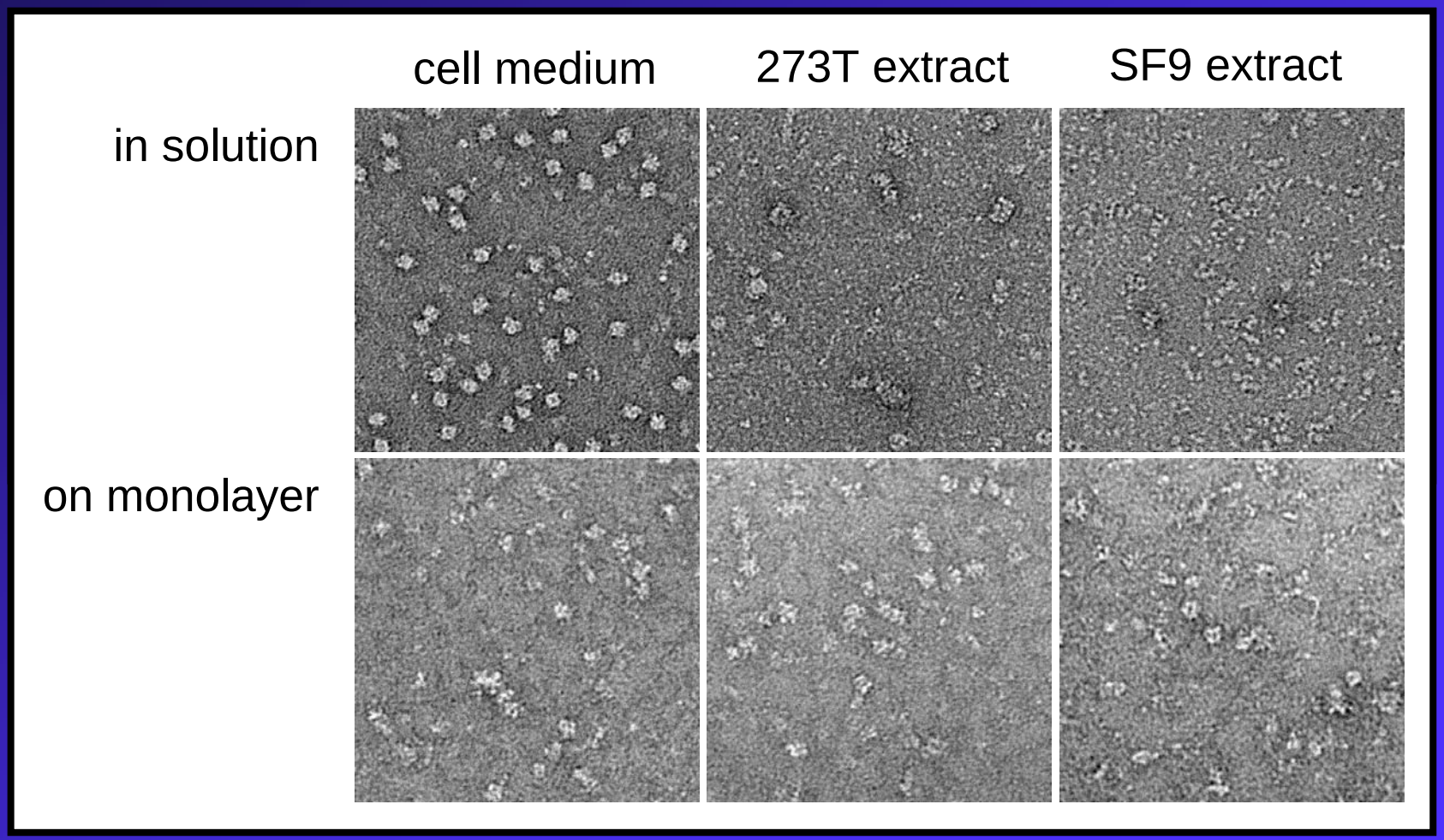




Monolayer purification of the Tf-TfR complex from a defined protein mixture



Monolayer purification of the Tf-TfR complex from cell extracts



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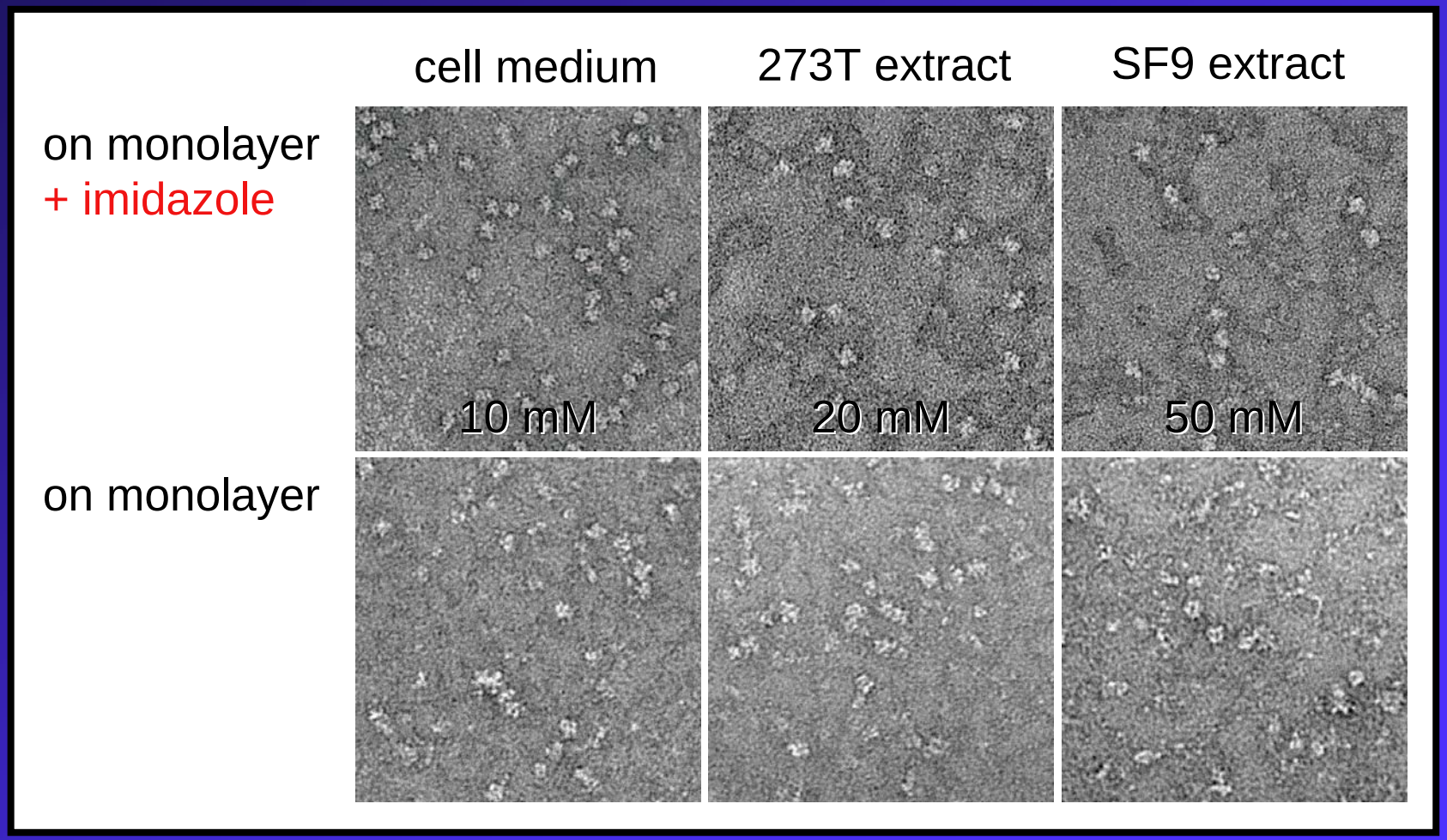
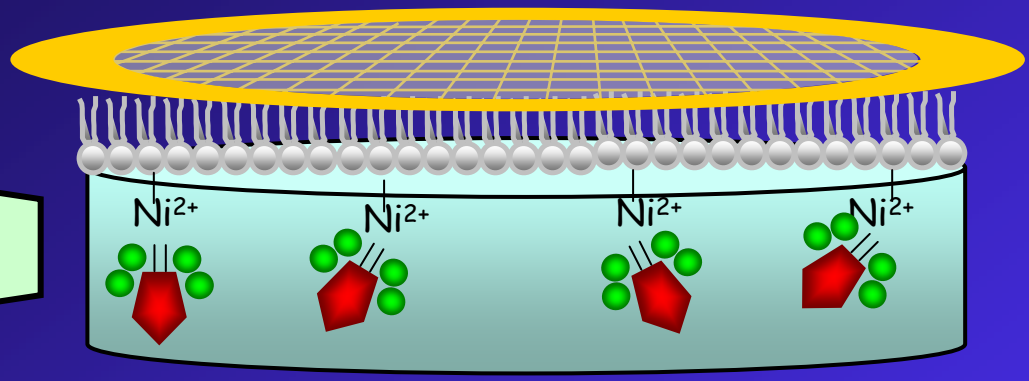
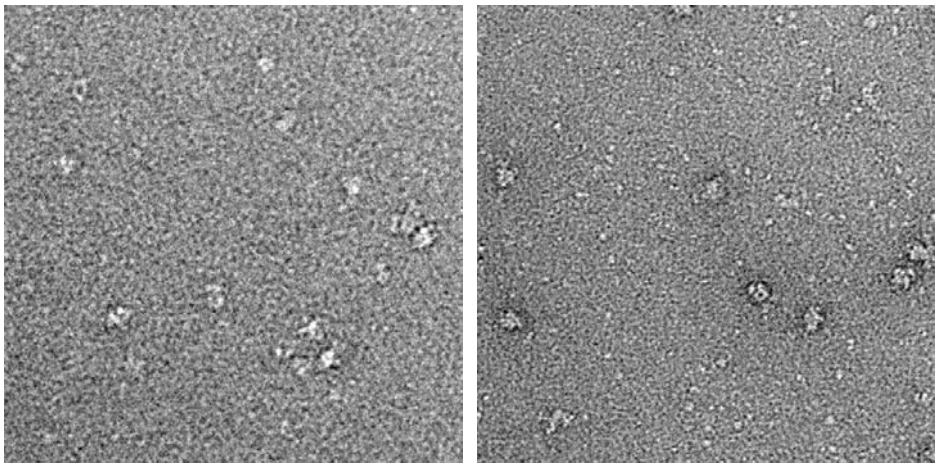


Characterization of purified complexes

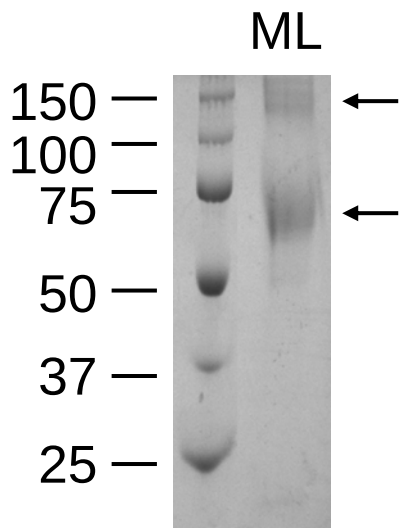


Successively elute 20 ML samples with 300 mM imidazole



ML elution

Ni-NTA column

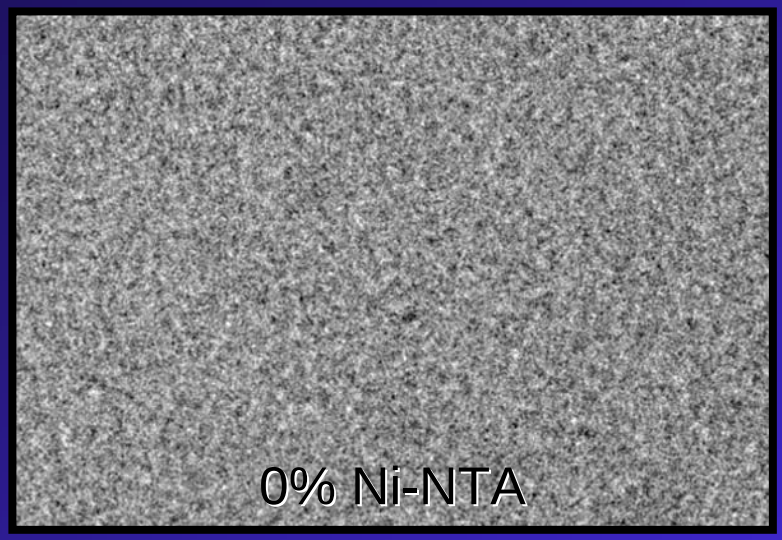


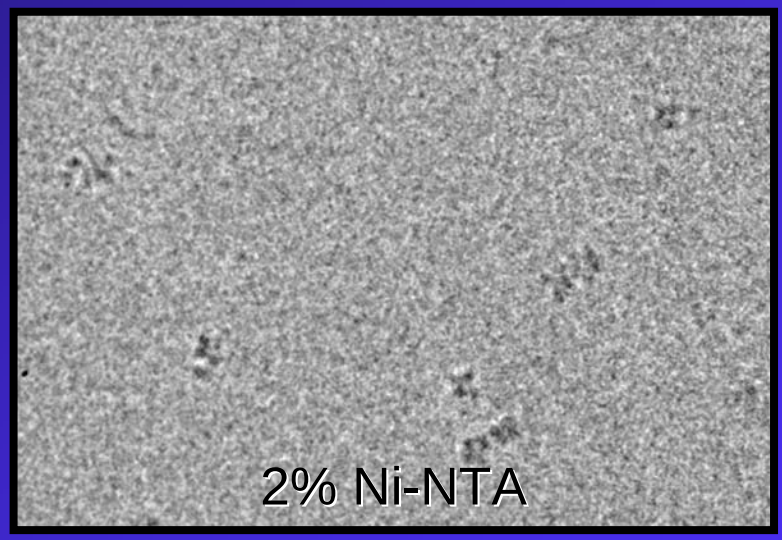
A combinatorial approach for protein purification and sample preparation for single particle EM studies

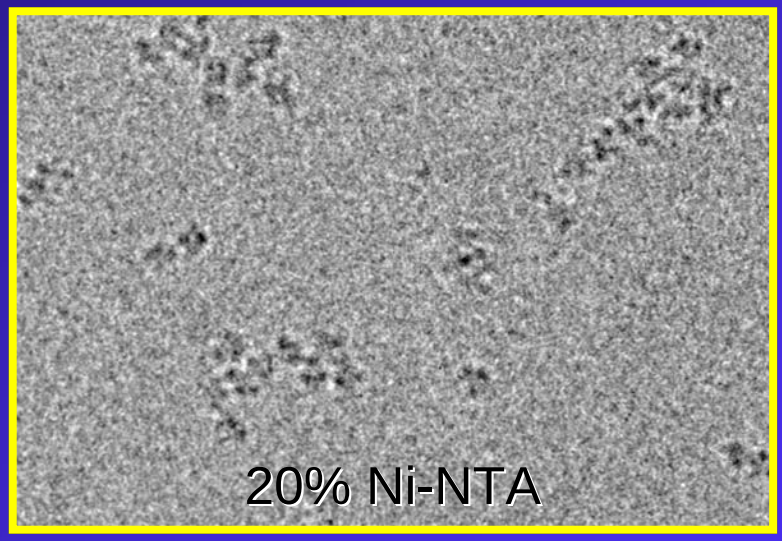
Establish whether Ni-NTA lipid monolayers can be used as a tool to purify macromolecular complexes

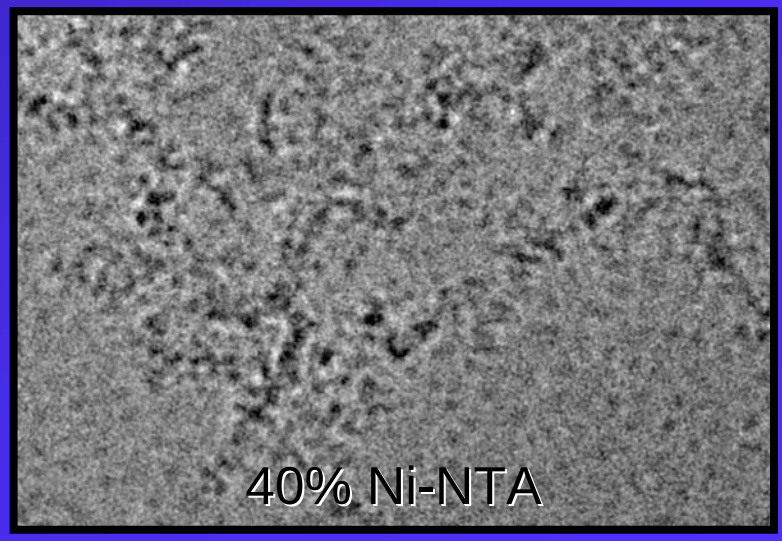
Establish whether lipid monolayer samples can be used for structure determination by single particle EM

Cryo-EM of the Tf-TfR complex from Sf9 cell extract

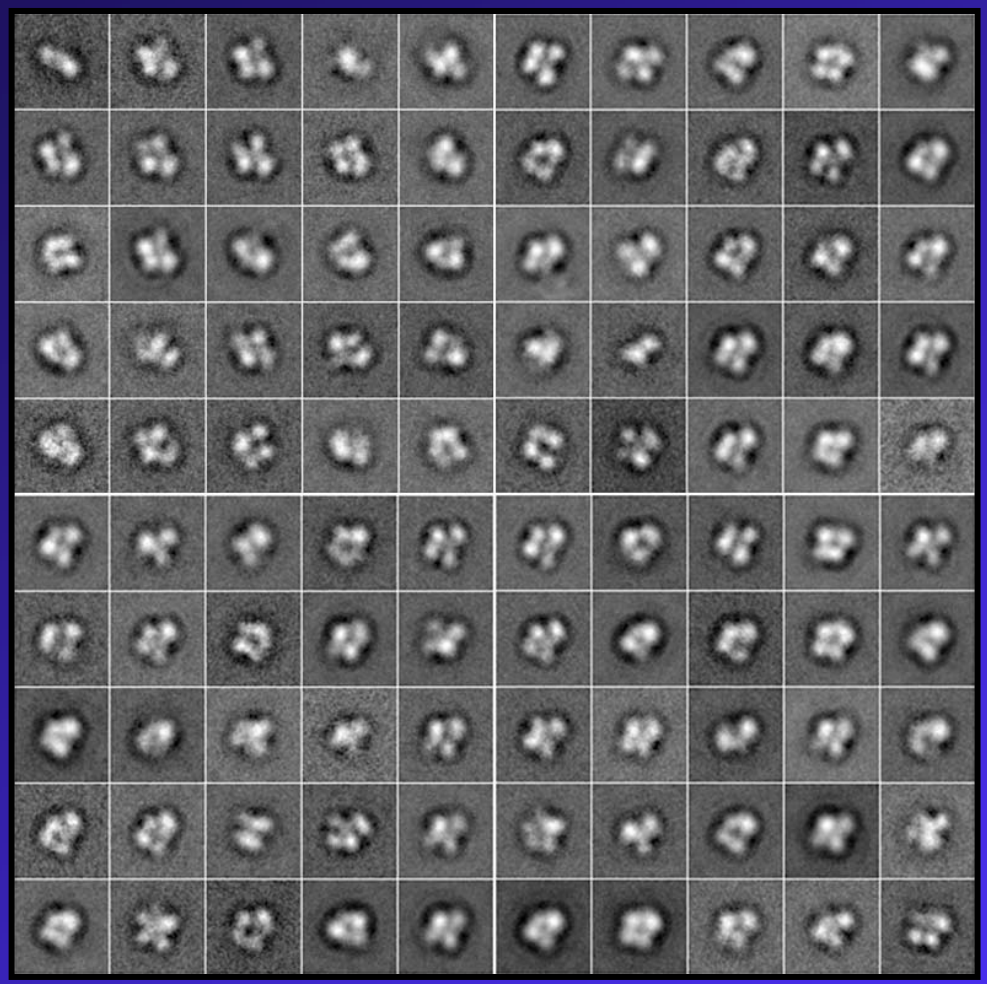


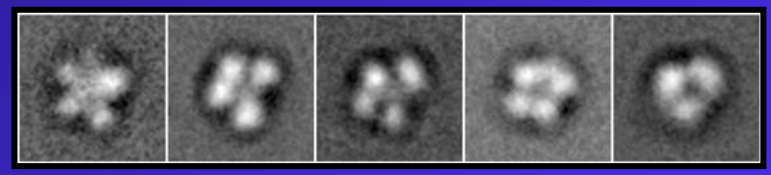


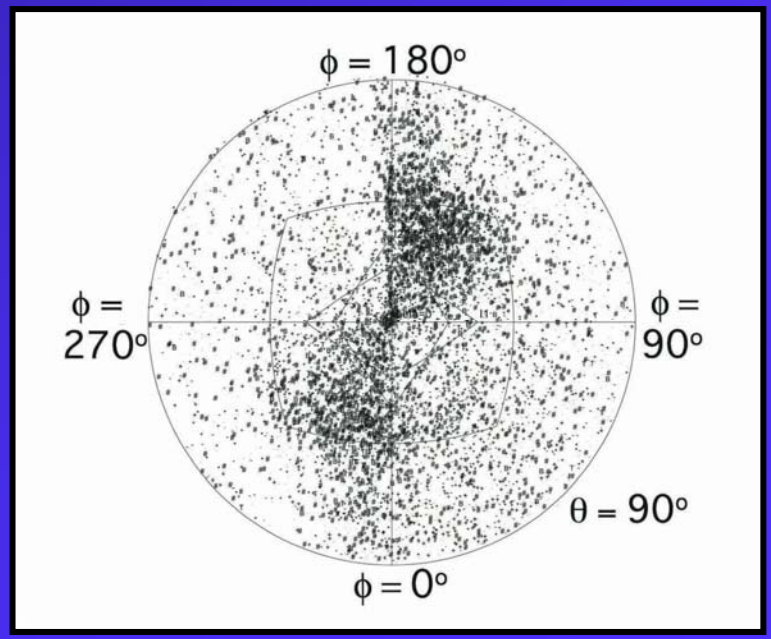




Cryo-EM of the Tf-TfR complex from Sf9 cell extract







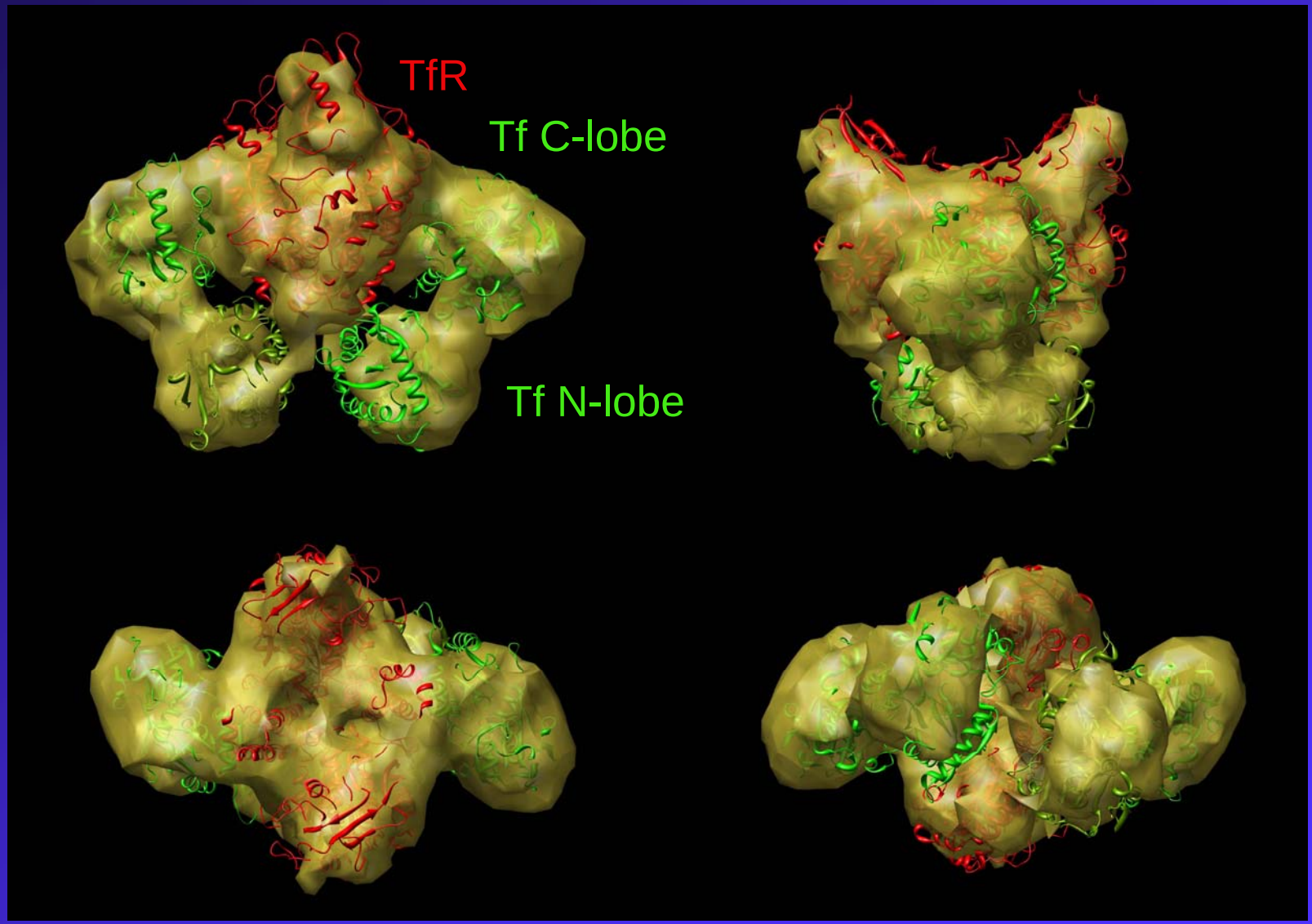
3D reconstruction of the Tf-TfR complex in vitrified ice on lipid monolayer

Initial model from pdb-file
(Cheng *et al.*, 2004)
filtered to 30 Å resolution

↓

FREALIGN (Grigorieff, 2007)
– refine orientation parameters
– correct for CTF
– calculate 3D reconstruction

3D density map of the Tf-TfR complex



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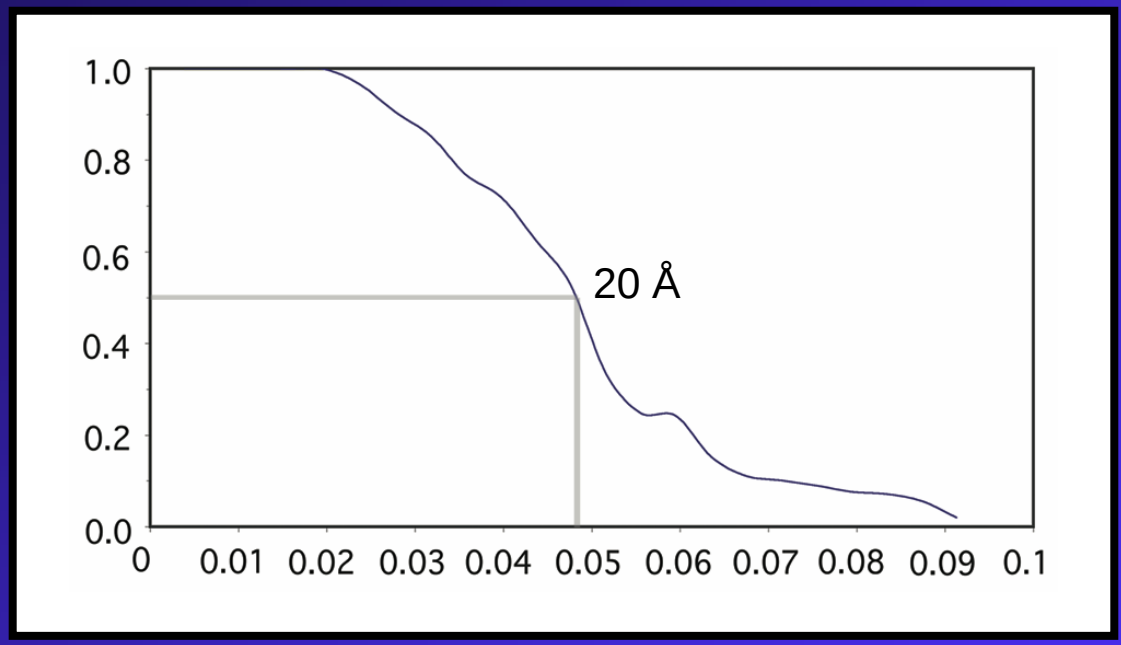
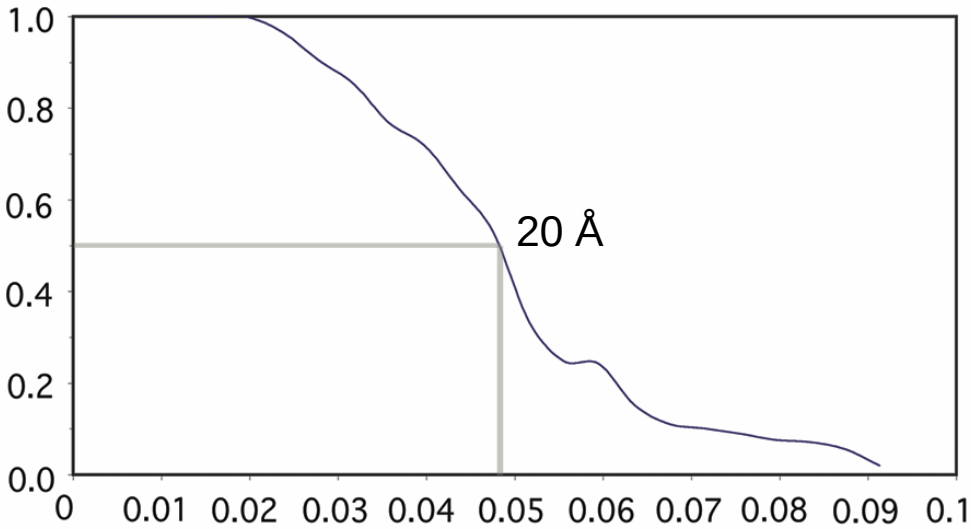
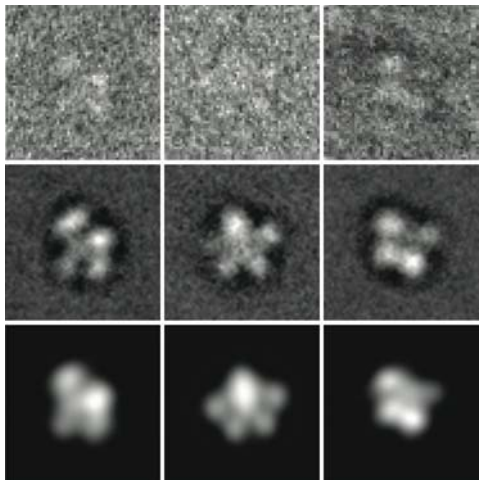


3D density map of the Tf-TfR complex





Raw images

Class averages

Re-projections

A combinatorial approach for protein purification and sample preparation for single particle EM studies

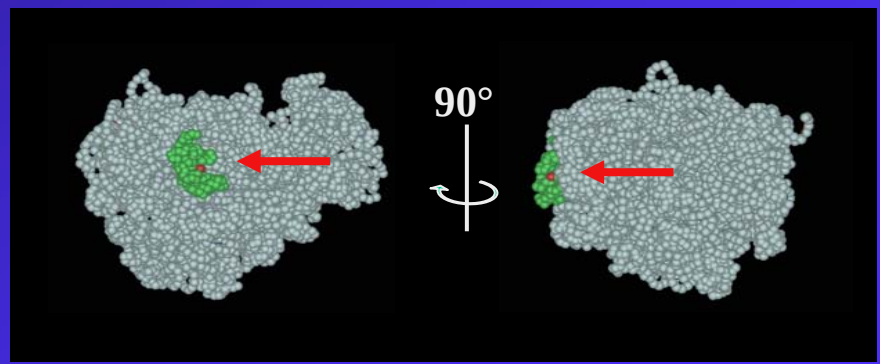
Establish whether Ni-NTA lipid monolayers can be used as a tool to purify macromolecular complexes

Establish whether lipid monolayer samples can be used for structure determination by single particle EM

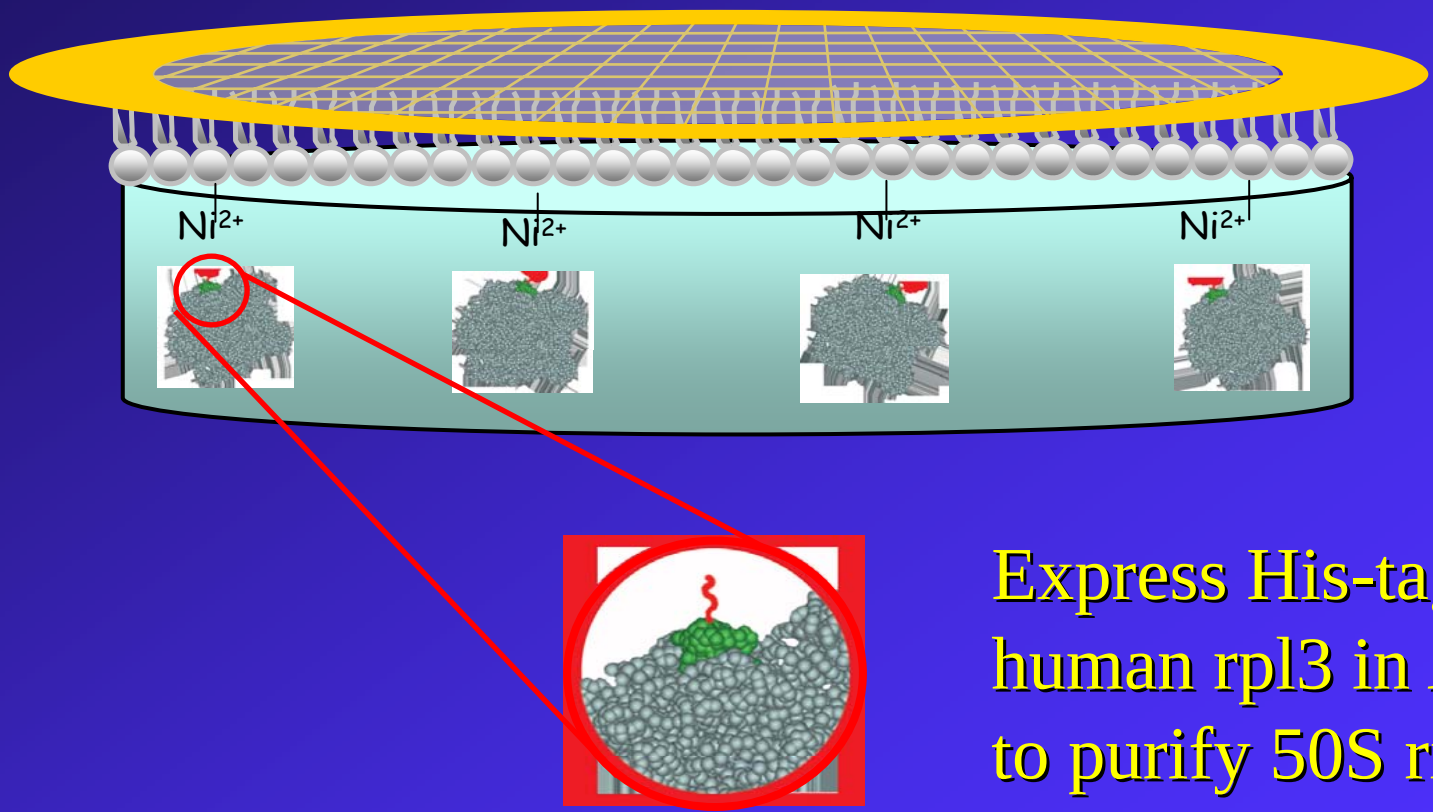
Apply the method to a real system

Monolayer purification of ribosomal complexes from *E. coli* extract

His-tagged hEx1 clone (from RZPD library)
for rpl3 (60S ribosomal protein)
(has 47 additional residues at N-terminus
compared to *E. coli* homolog)



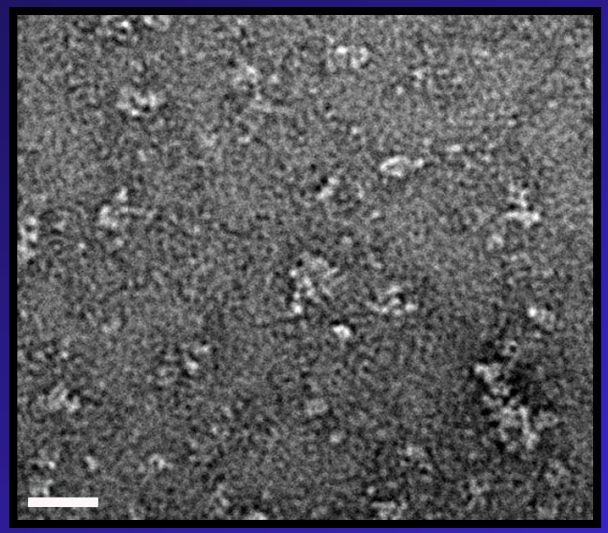
Monolayer purification of ribosomal complexes from *E. coli* extract



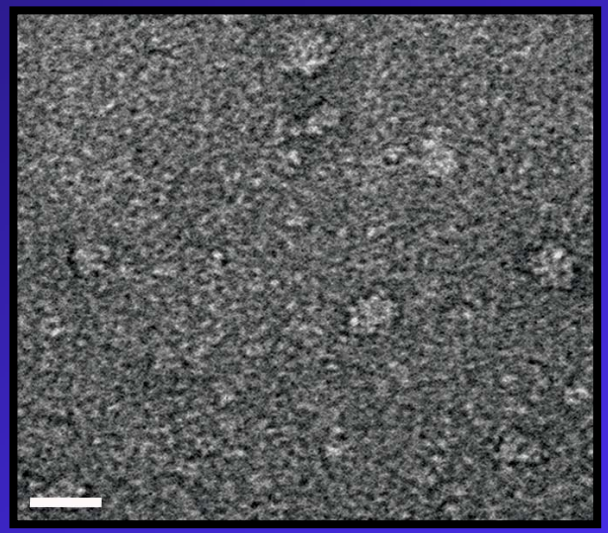
Express His-tagged human rpl3 in *E. coli* to purify 50S ribosome from extract

Monolayer purification of ribosomal complexes from *E. coli* extract

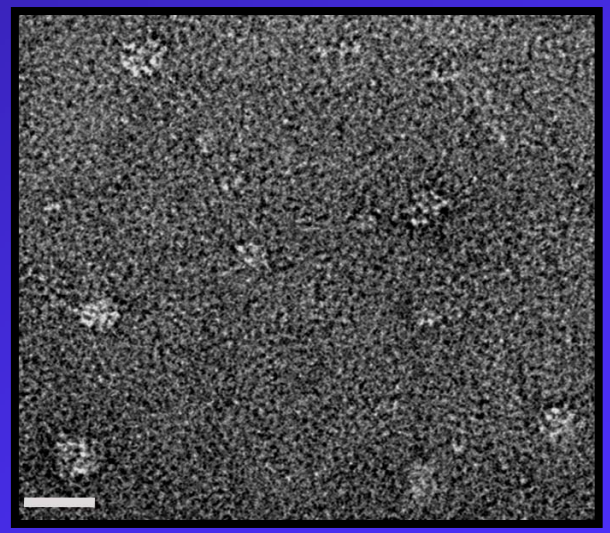
cell extract



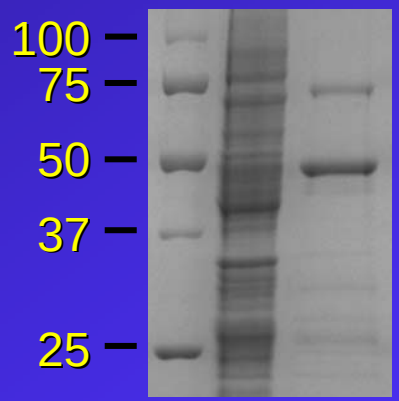
Ni-NTA column



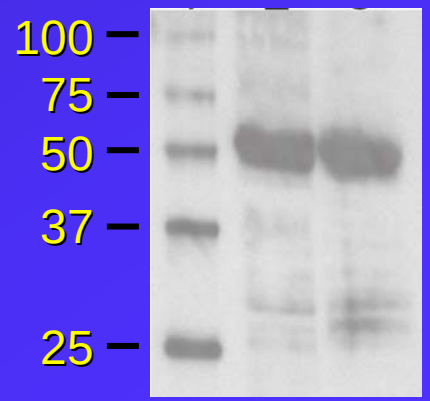
Ni-NTA monolayer



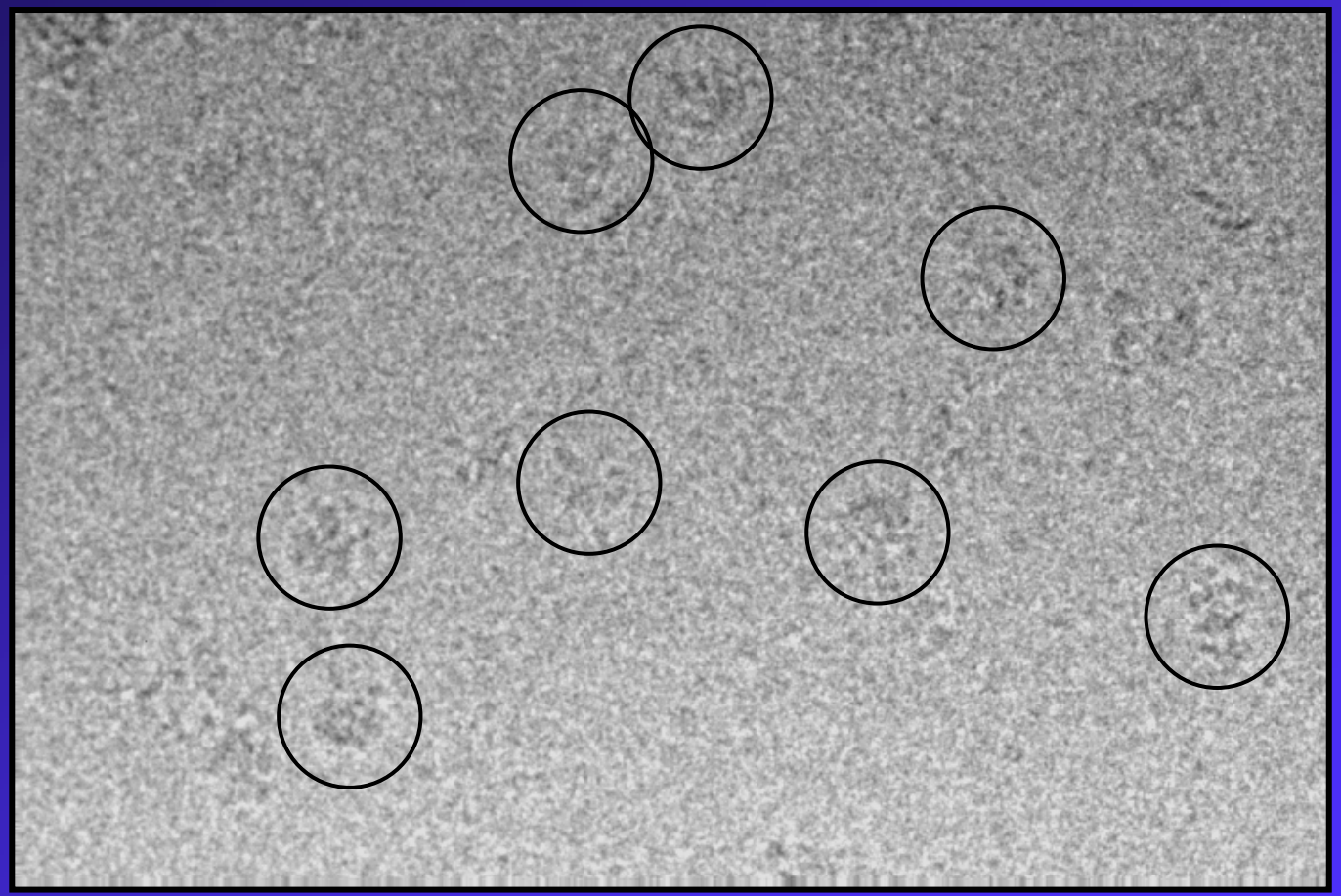
SDS-PAGE gel



Western blot

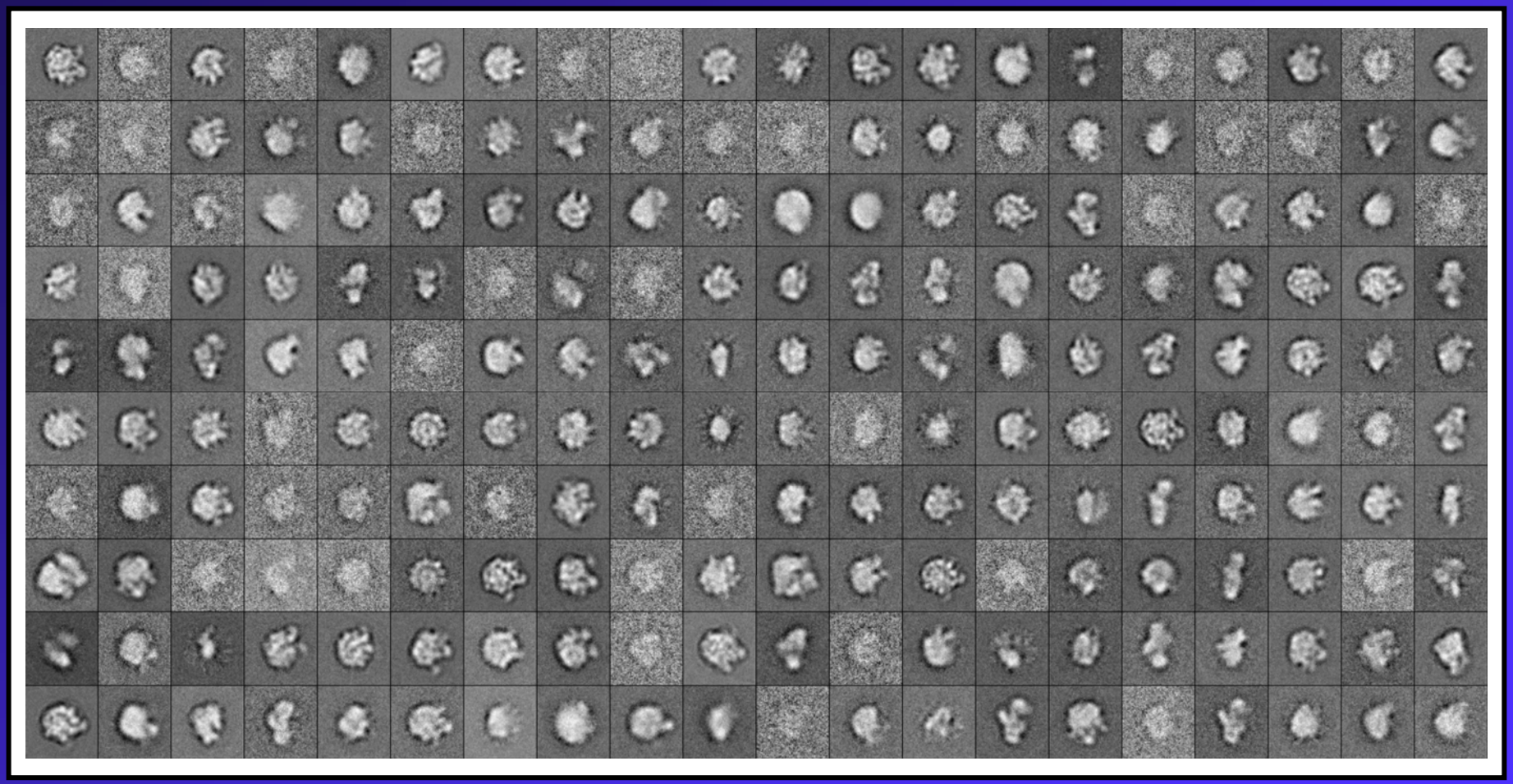


Monolayer purification of ribosomal complexes from *E. coli* extract



Vitrified specimen

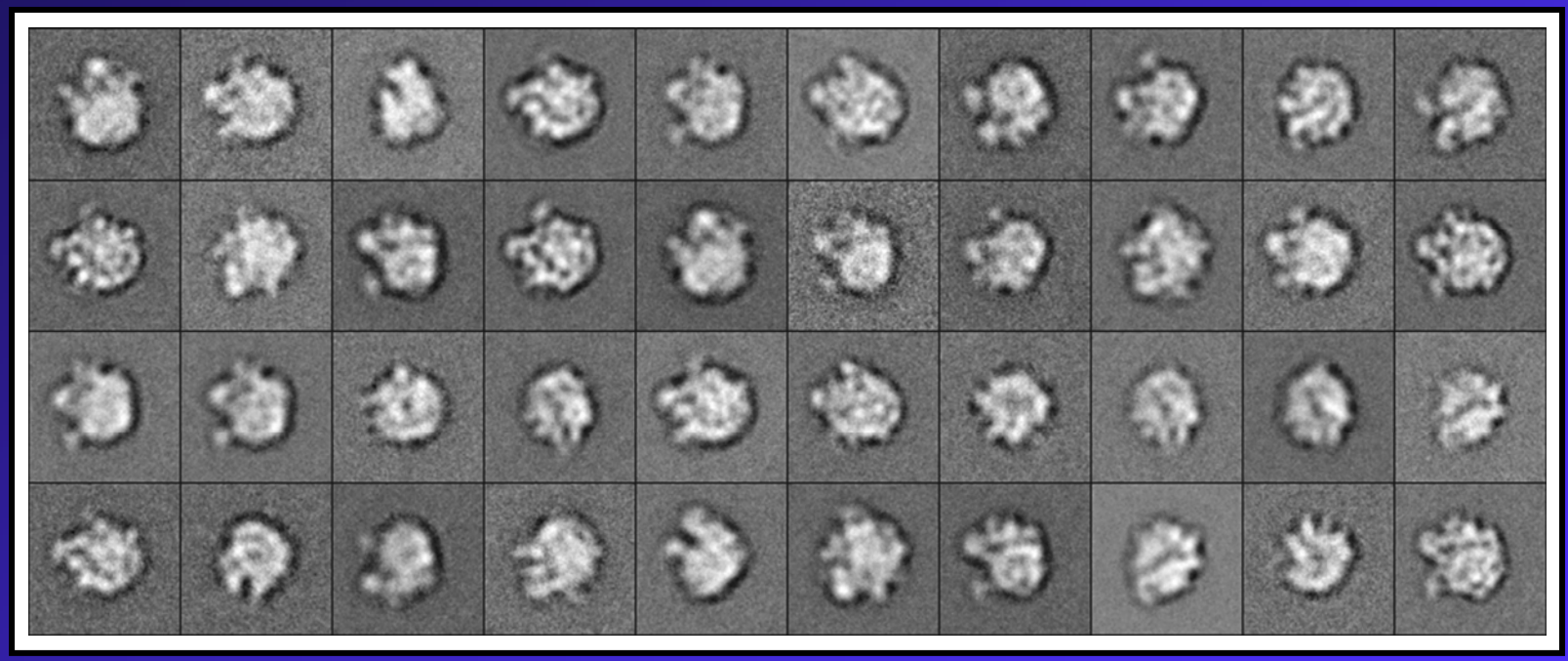
Monolayer purification of ribosomal complexes from *E. coli* extract



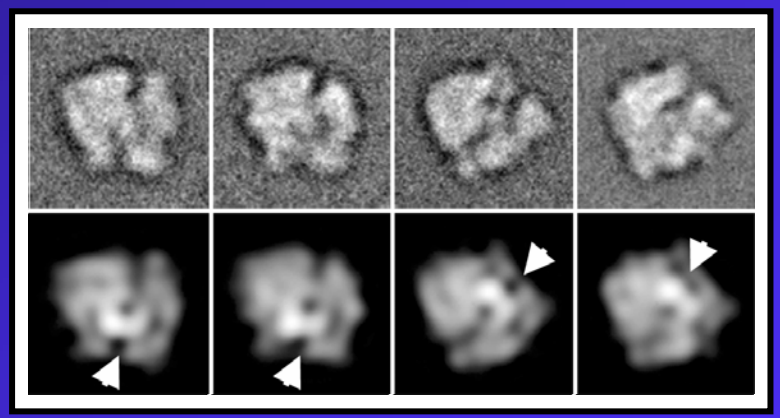
45,444 particles in 200 classes

Monolayer purification of ribosomal complexes from *E. coli* extract

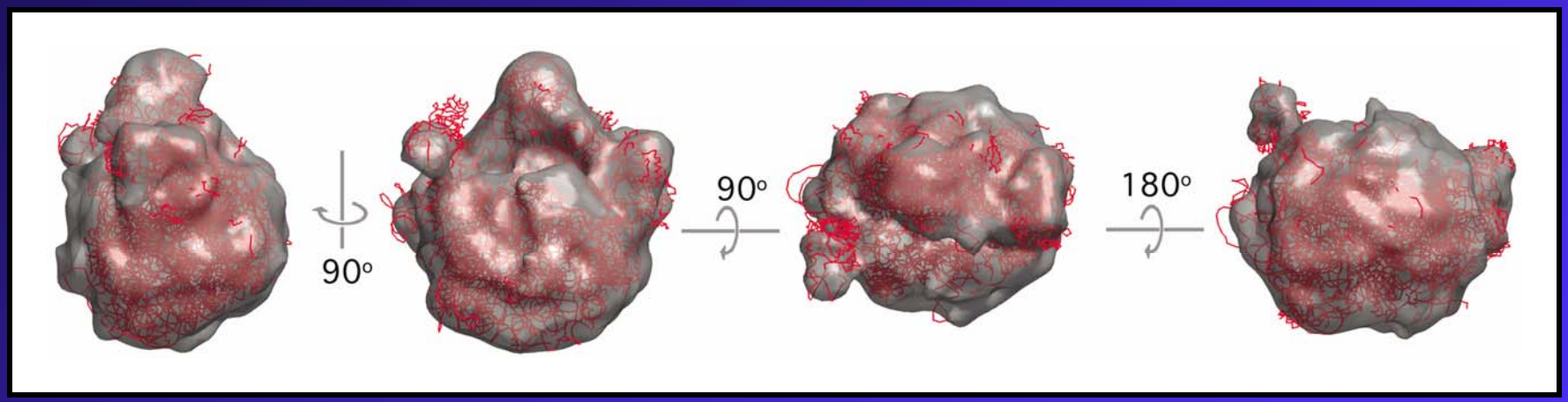
50S



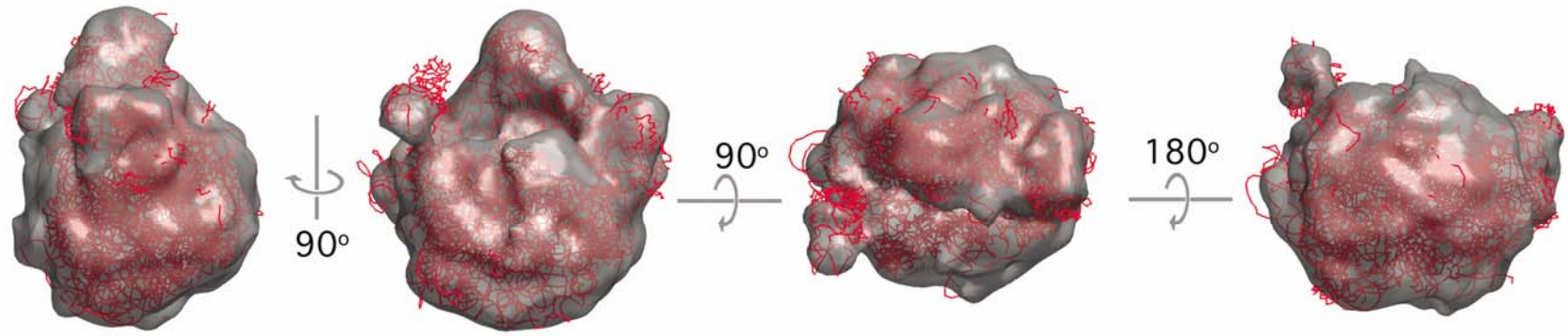
70S



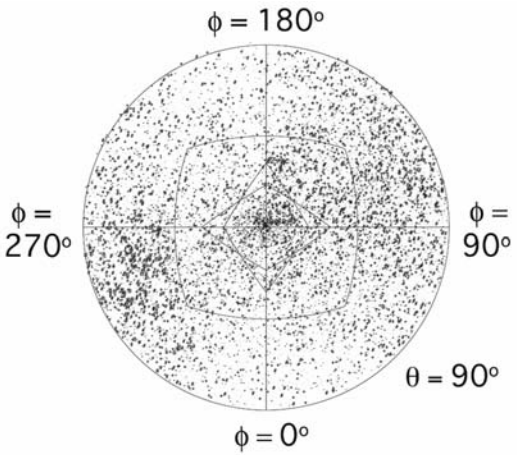
Monolayer purification of ribosomal complexes from *E. coli* extract



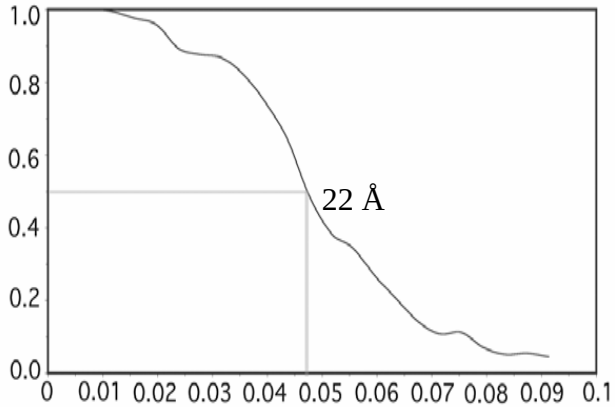
Monolayer purification of ribosomal complexes from *E. coli* extract



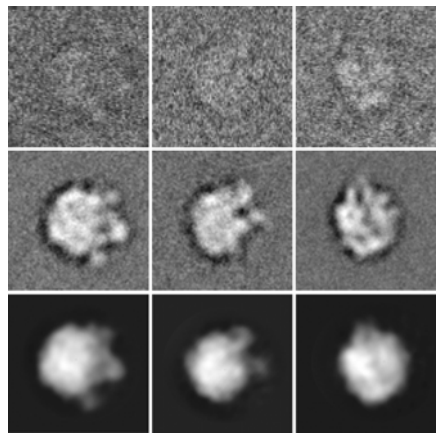
Angular distribution



FSC curve



Comparison with re-projections



Advantages of monolayer purification

- fast
  → ideal for unstable and transient complexes
- one-step procedure that needs little material
  → ideal for low-abundance and low-yield complexes
- easy to vitrify because of lipid monolayer
  easy to adjust particle concentration
- purification of all complexes that contain tagged subunit
  (assembly intermediates, alternative complexes etc.)
- tagging of transient subunits, activators or substrates
  would allow imaging of specific complexes
- combination with libraries of tagged constructs
  → potential for high-throughput studies

Future directions

- use of His-tagged calmodulin
  → suitable for TAP-tagged constructs

- use of His-tagged protein A
  → suitable for any tagged construct in combination with antibodies against the tag

- use of fluorinated lipids
  → suitable for membrane proteins

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