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Single particle EM of membrane protein complexes

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TORONTO

Overview of lecture

- Introduction to detergents
- Alternatives to using detergents
    - Amphipols
    - RSC single particle EM
    - Single particles on membranes
- Preparing specimens
    - Negative stain EM of proteins in detergents
    - Cryo-EM of proteins in detergents
- Interpreting images of proteins-detergent complexes
    - Theory
    - Practice
    - Some nice examples
- What does challenging mean for image alignment
- Monitoring alignment accuracy
- Choosing electron dose to get the images you need

Introduction to detergents

Detergents for solubilizing membrane protein complexes

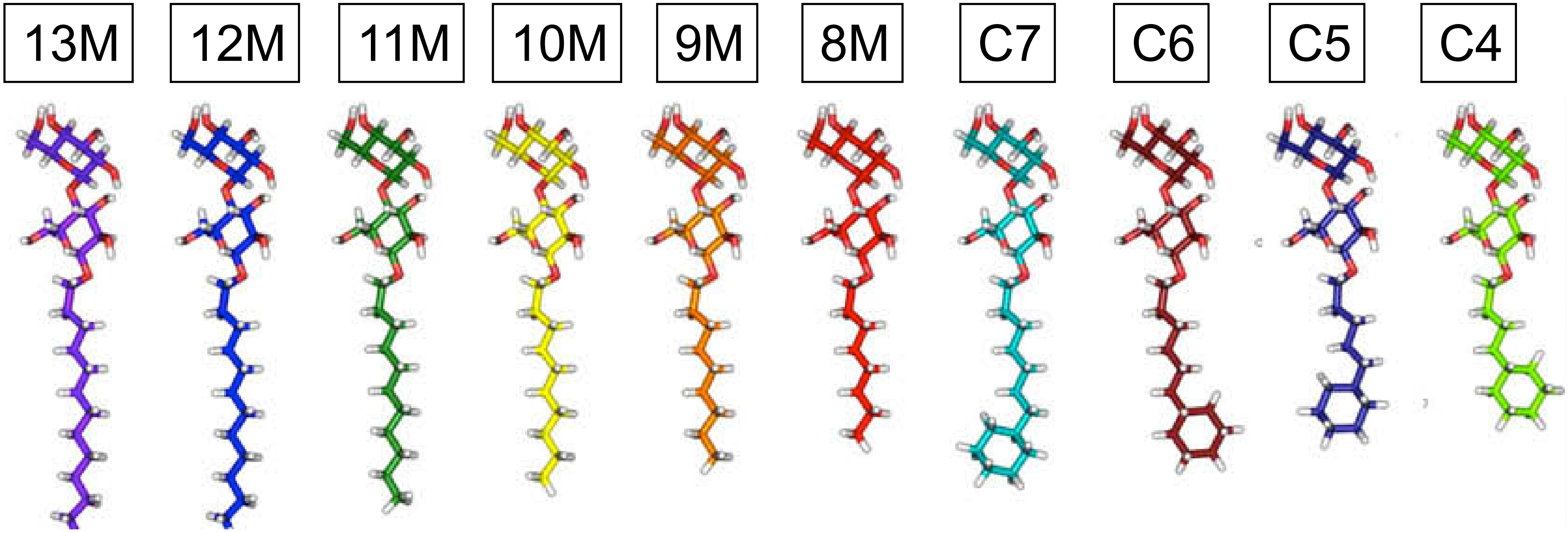
Proteins in lipid bilayer

Detergent solubilized
protein mixture

Purified detergent solubilized
protein particles, suitable for
single particle EM

Figure used with permission from Edmund Kunji (MRC MBU, Cambridge , U.K.)

Detergents (some examples)

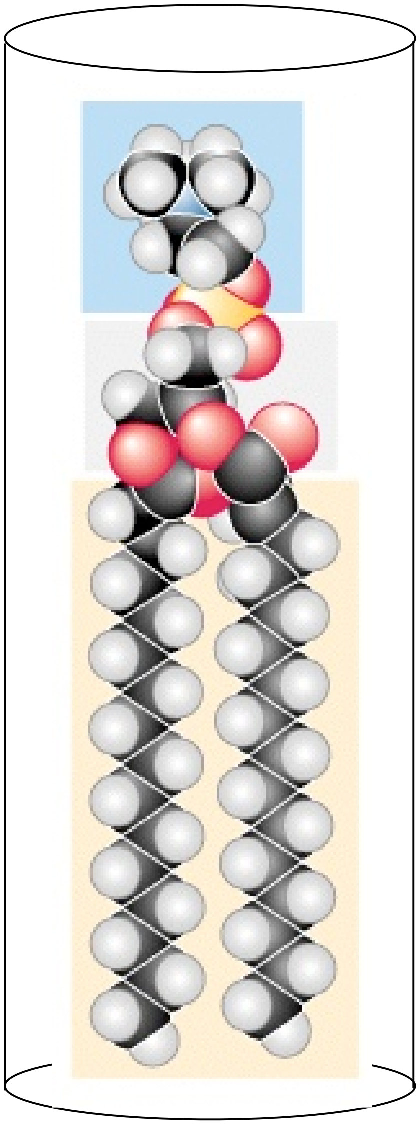


M ≡ Alkyl-maltoside
C ≡ CYMAL (cyclohexyl-containing maltosides)

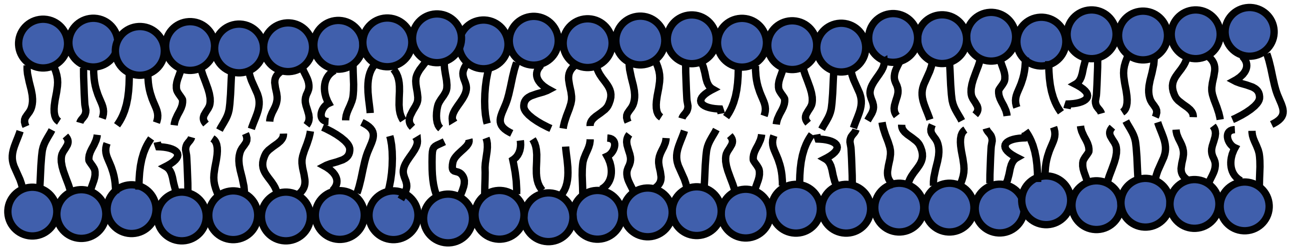
- May have different effects on proteins
- Choice of detergents usually dictated by the protein being studied

Kunji, E.R.S. *et al.* (2008). *Methods* **46**, 62-72.

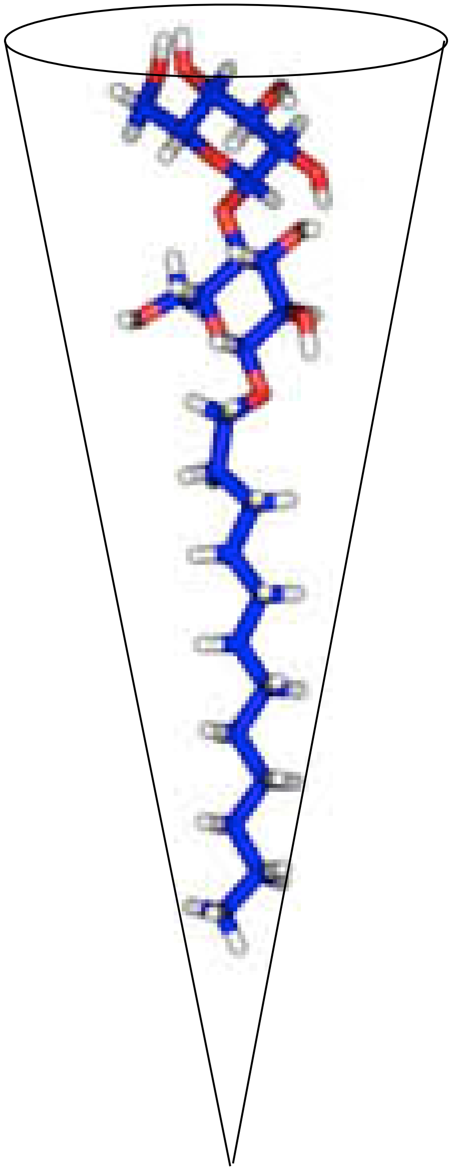
Lipids - structure favors bilayers over micelles



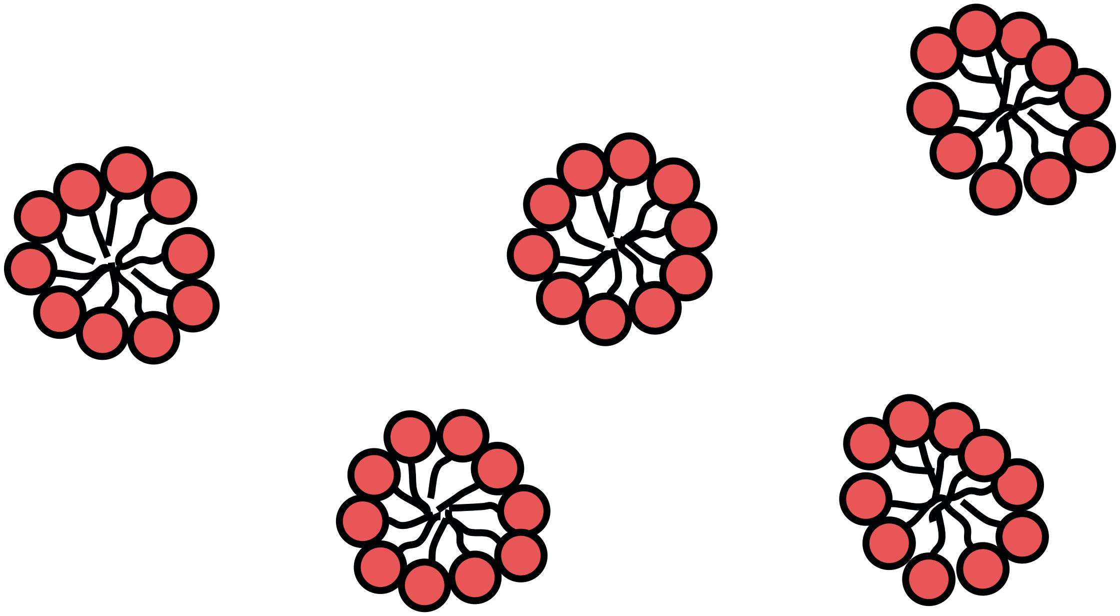
Lipid bilayer:



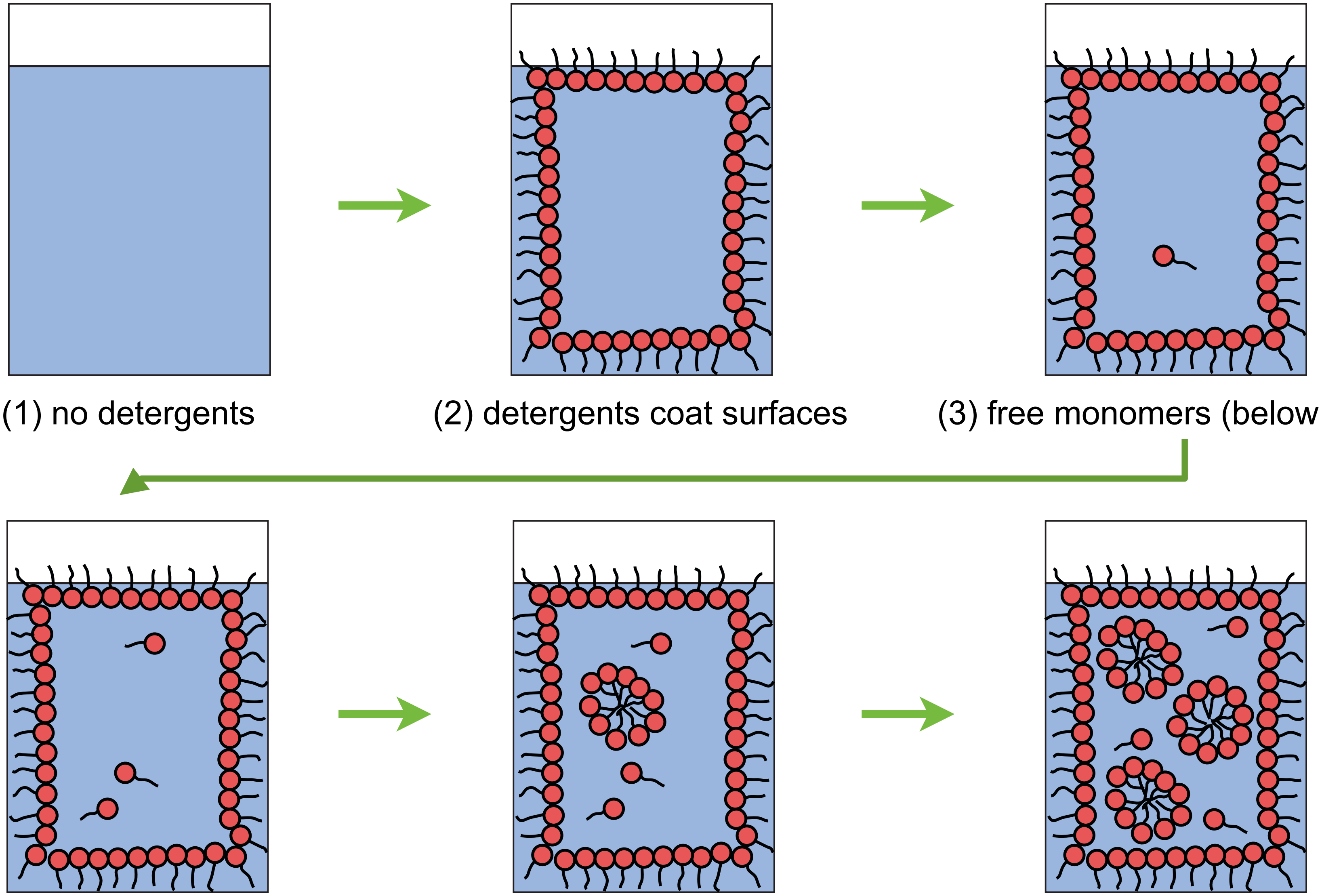
Detergents - structure favors micelles over bilayers



Detergent micelle:



What happens when you add detergents to a solution?



(1) no detergents

(2) detergents coat surfaces

(3) free monomers (below CMC)

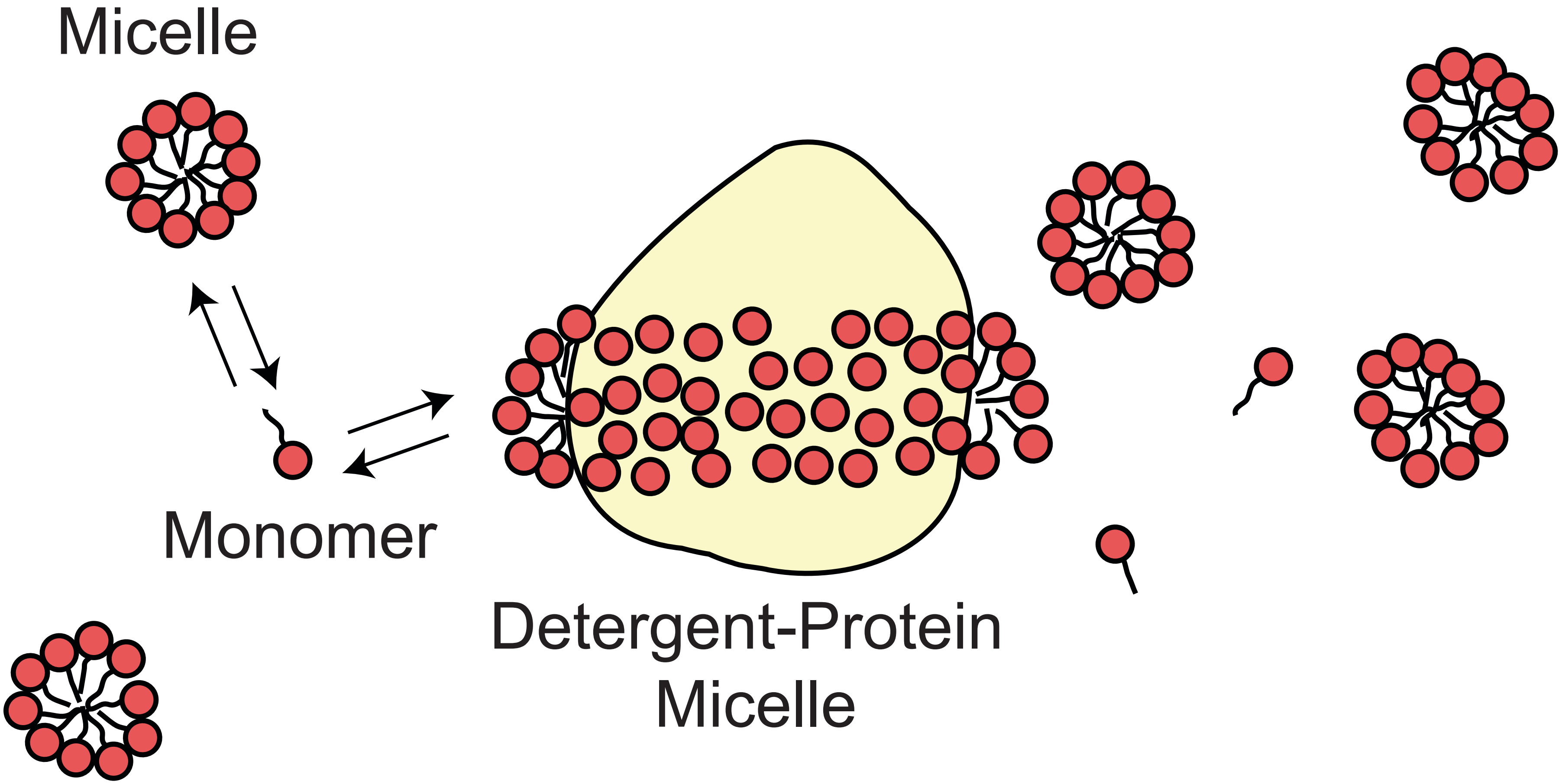
(4) free monomers (at CMC)

(5) micelles form (above CMC)

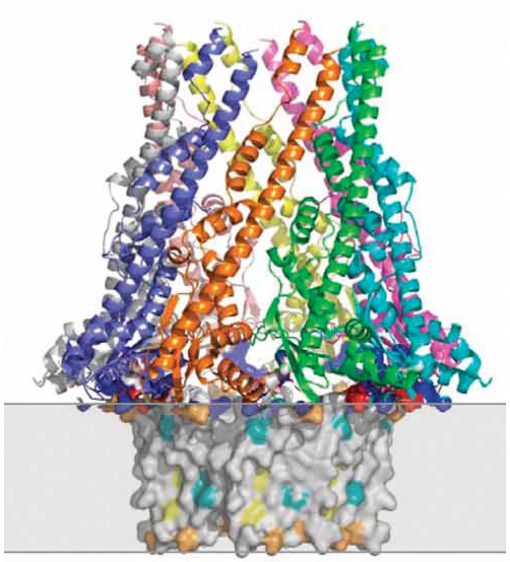
(6) More micelles (far above CMC)

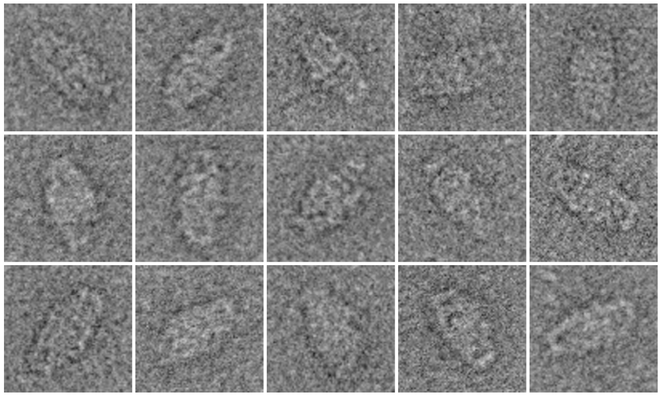
Protein-detergent micelles

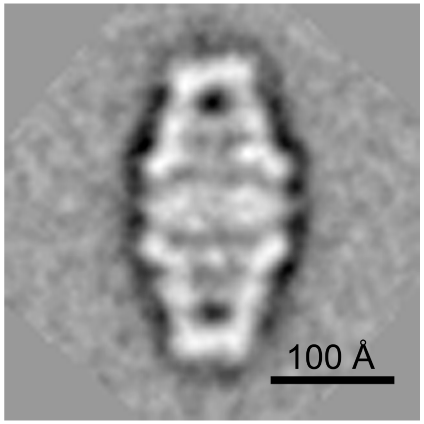
Detergent Equilibrium

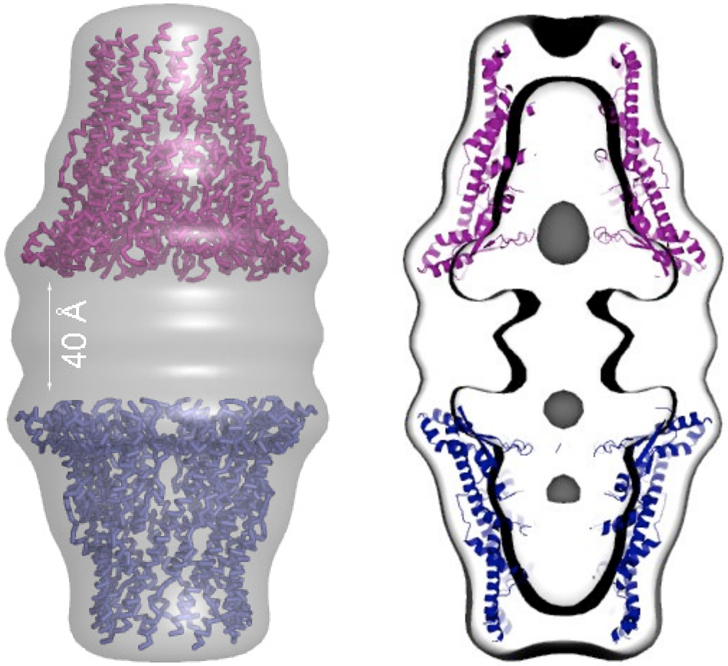


Detergents can do wacky things: e.g. WzzE



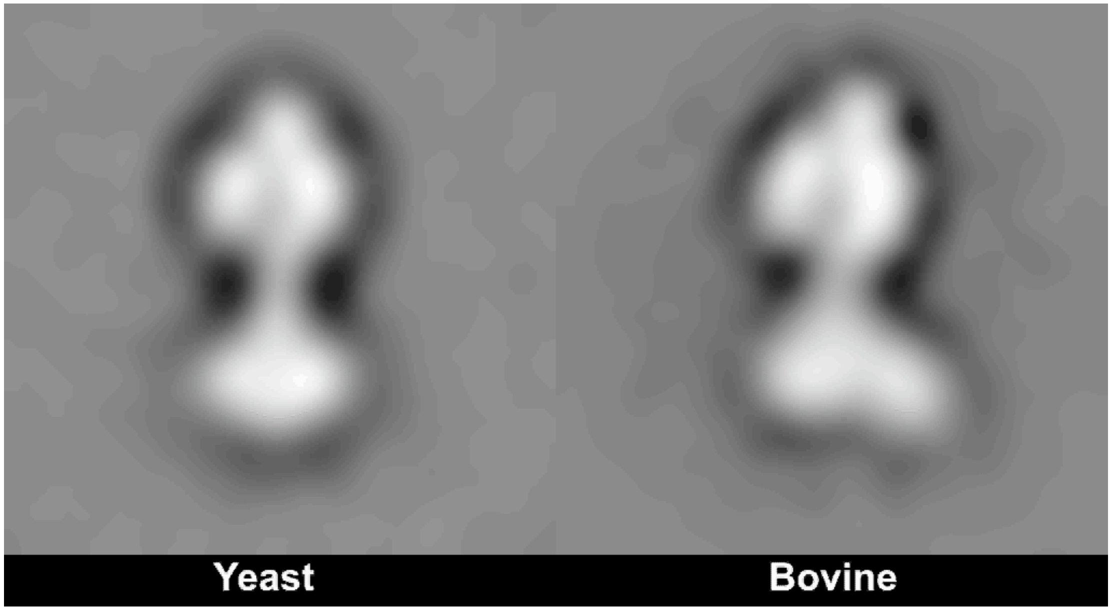


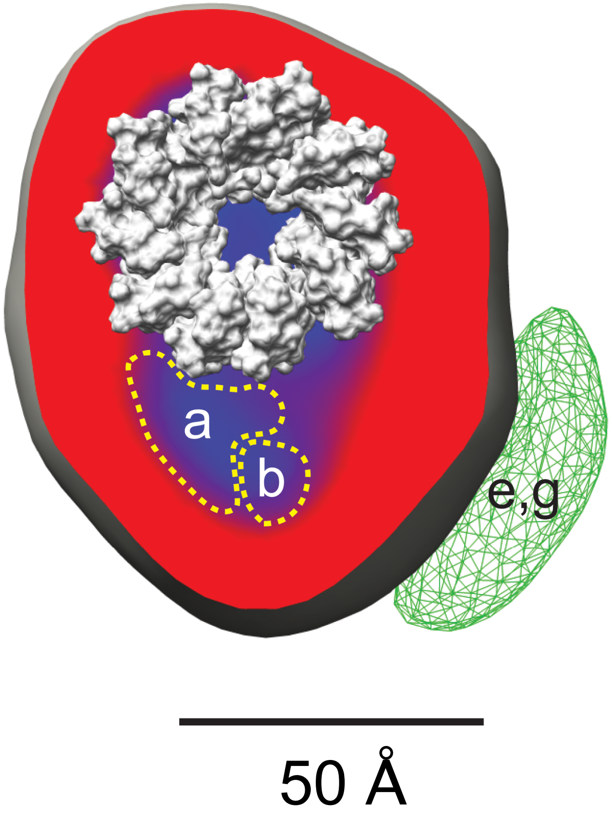




Tocilj, A. *et al.* (2008). *Nature Struct and Mol Biol* **15**, 130-138.

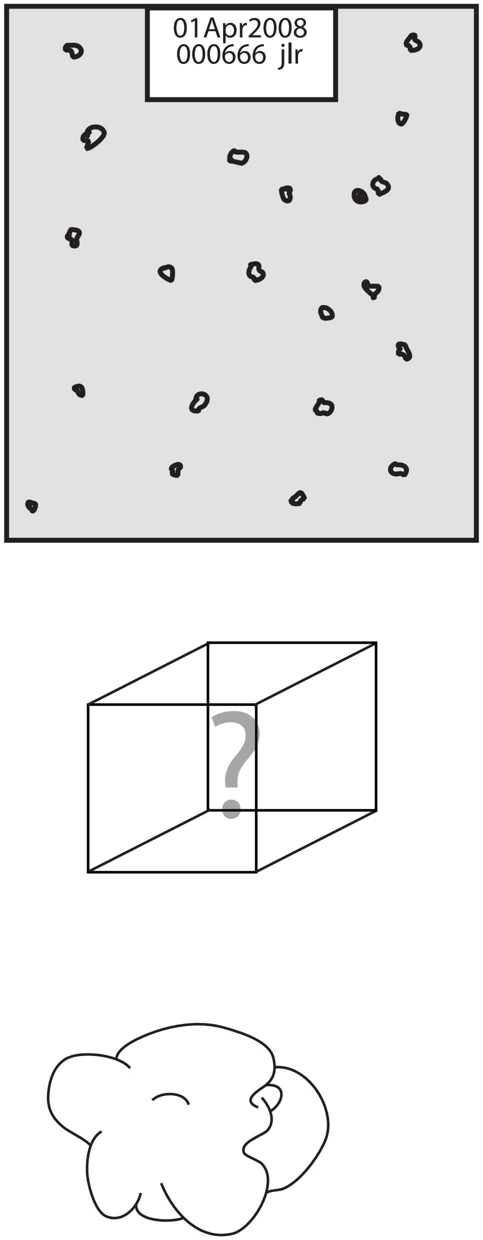
Detergent induced subunit loss in ATP synthase





Lau, Baker and Rubinstein (2008) *J Mol Biol* **382**, 1256-64.

The tragedy of single particle EM



Images of protein
(not necessarily images of your protein, images of folded protein, images of active protein, images of homogeneous protein)

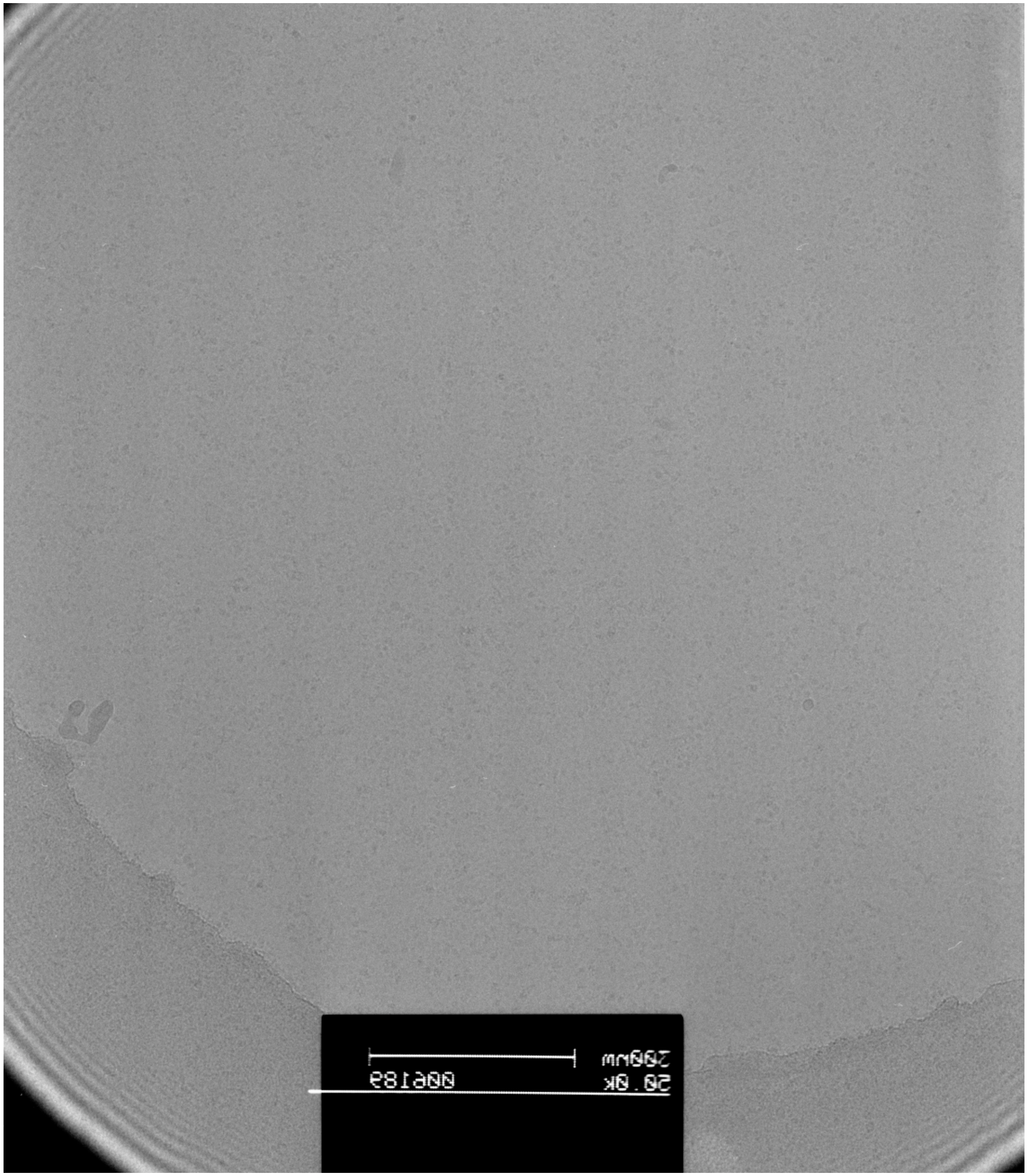
↓

Image analysis software

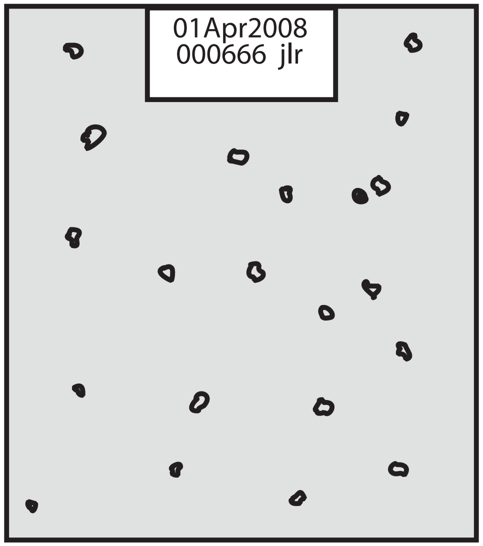
↓

3-D model of structure

This is not protein



The tragedy of single particle EM for membrane proteins



Images of ~~protein~~ something
(not necessarily images of protein, images of your protein, images of folded protein, images of active protein, images of homogeneous protein)

↓

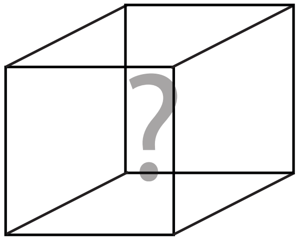
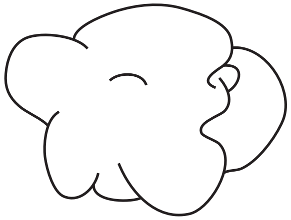


Image analysis software

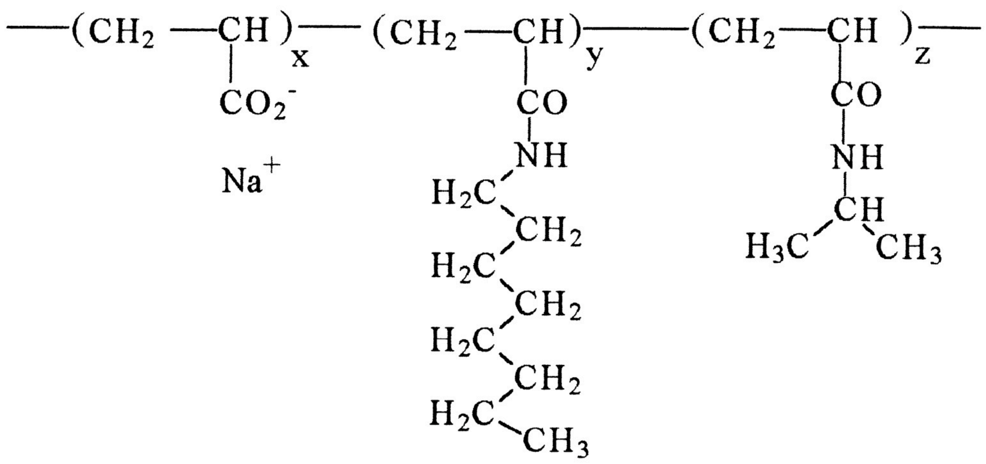
↓

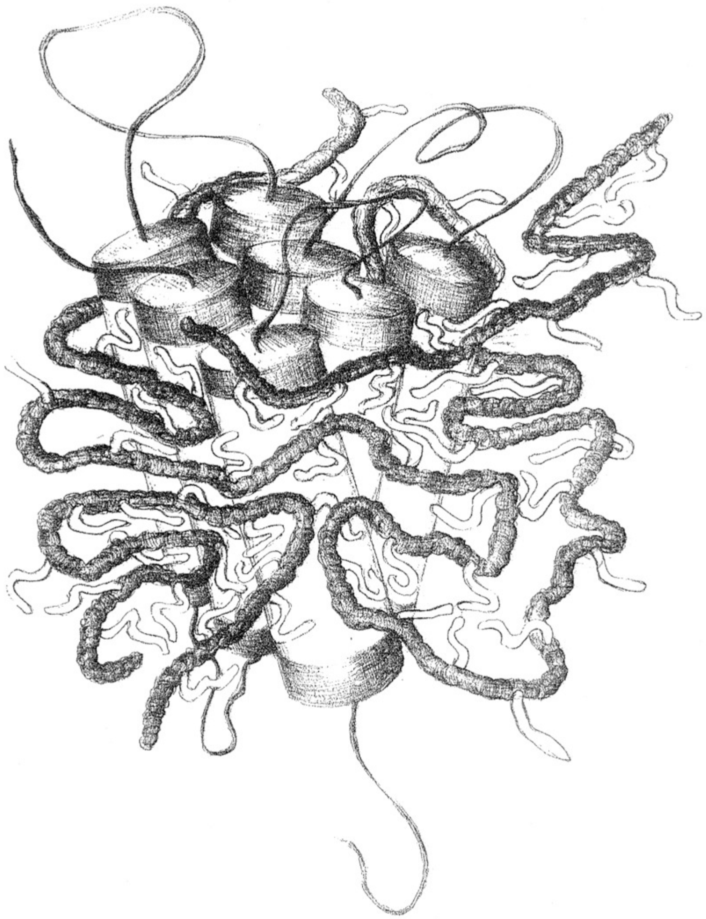


3-D model of structure

Alternatives to detergents in single particle EM

Amphipol interaction with protein

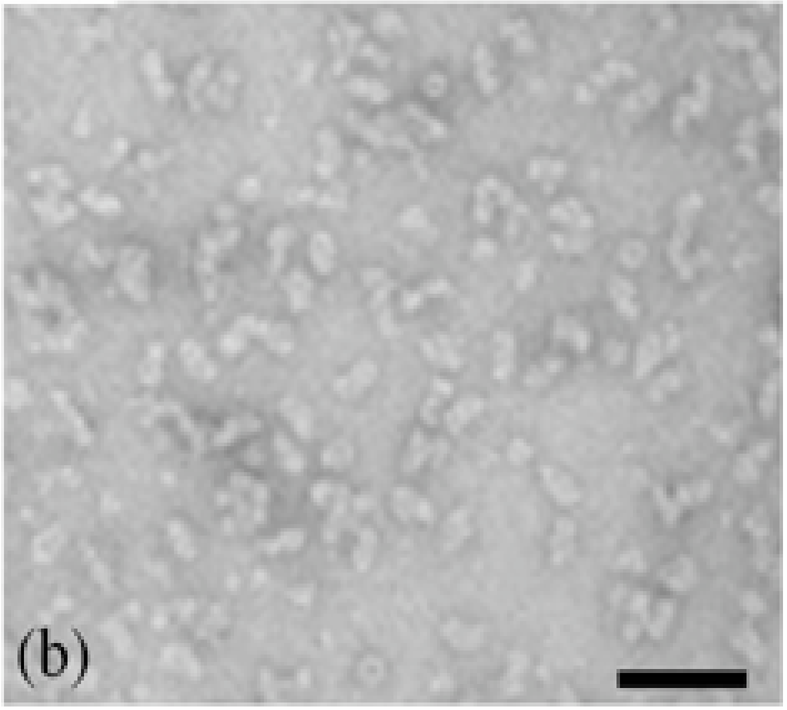




Tribet C *et al.* (1996). *PNAS* **93**,15047-15050

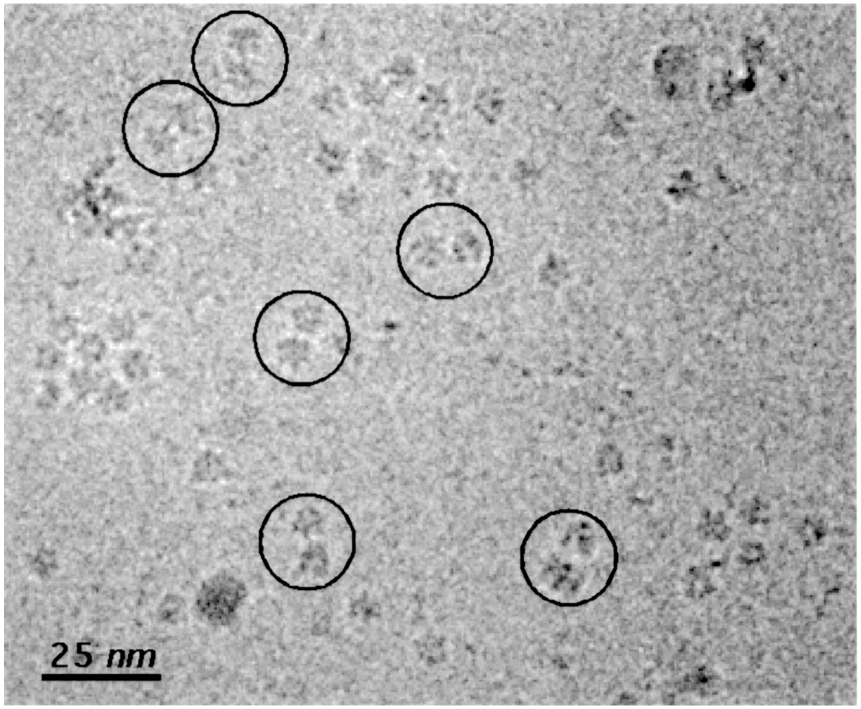
Amphipols for single particle EM

Mitochondrial Complex I
in negative stain



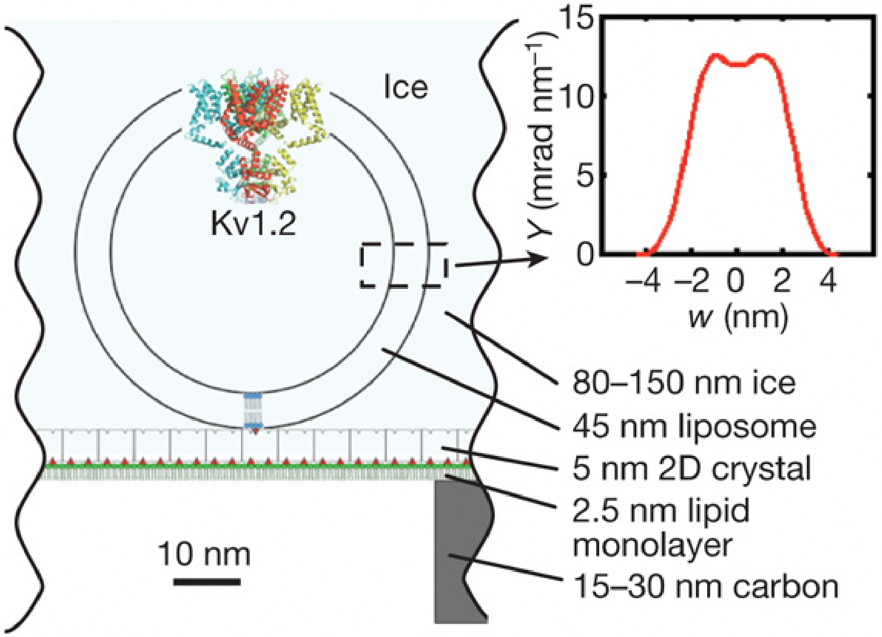
Tribet C. et al. (1996). *PNAS* **93**,15047-15050.

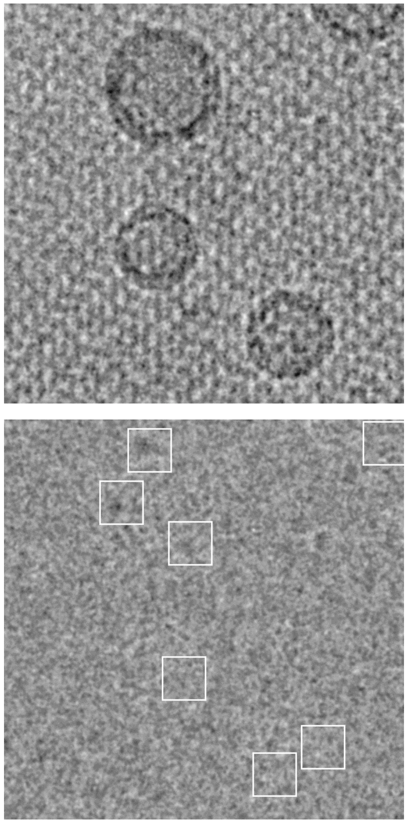
E. coli ATP synthase
in vitreous ice



Wilkens, S. (2000). *J Bioenerg Biomemb* **32,** 333-339.

Random Spherically Constrained (RSC) single particle EM





<u>Advantages:</u>

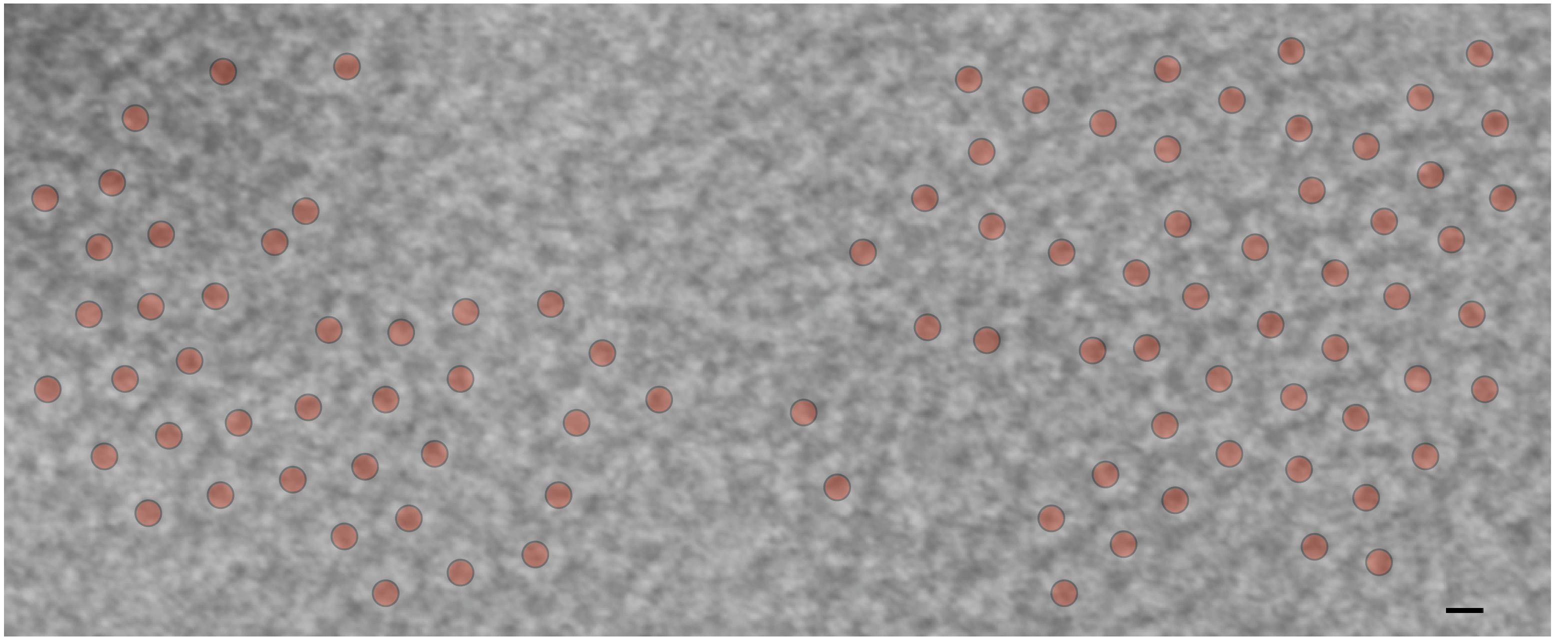
- in lipid environment
- improved determination of Euler angles for small particles

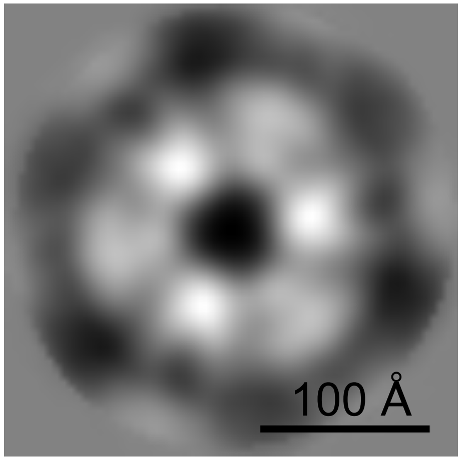
<u>Disadvantages</u>

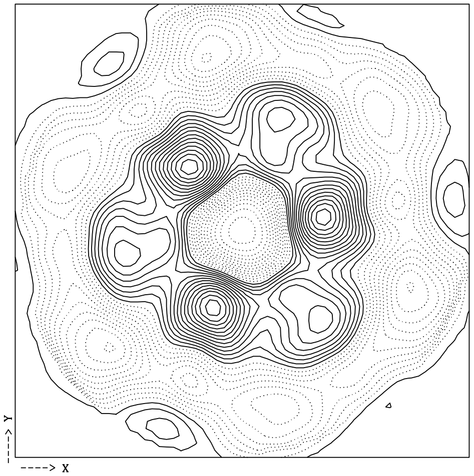
- low density of particles
- thick specimen

Wang and Sigworth (2009). *Nature* **461**, 292-5.

Single particles from a 2-D array





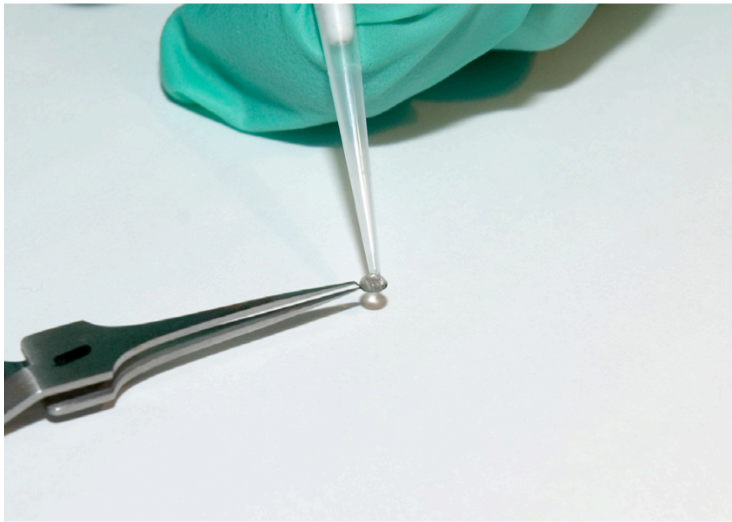


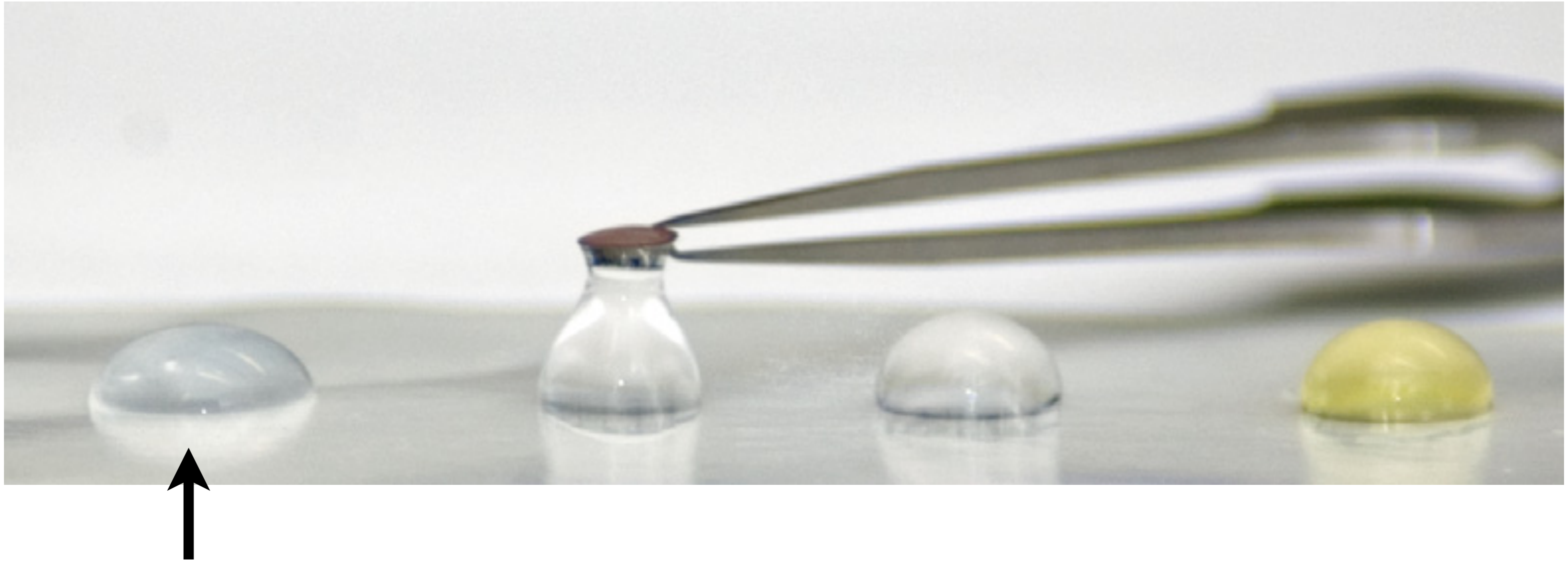
Rujiviphat, J. *et al.* (2009). *J Biol Chem* **In Press.**

Negative stain single particle EM with detergents

Preparing negative stain EM grids with detergents

- Glow discharge in air ~ 15 sec
- Allow protein to adsorb ~ 2 min
- Wash, blot, repeat (3 x total)
- Stain, blot, dry.

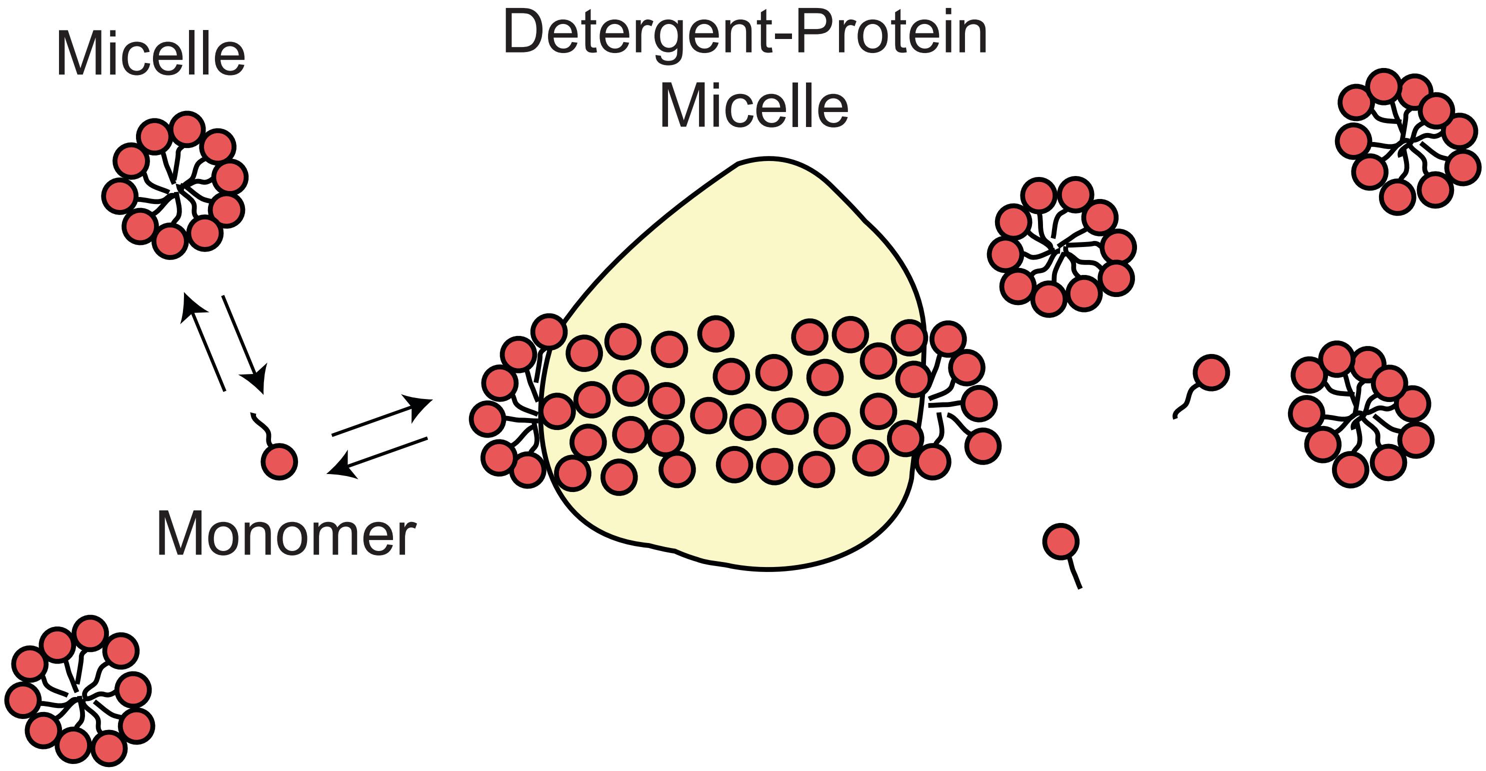




Note the loss of surface tension

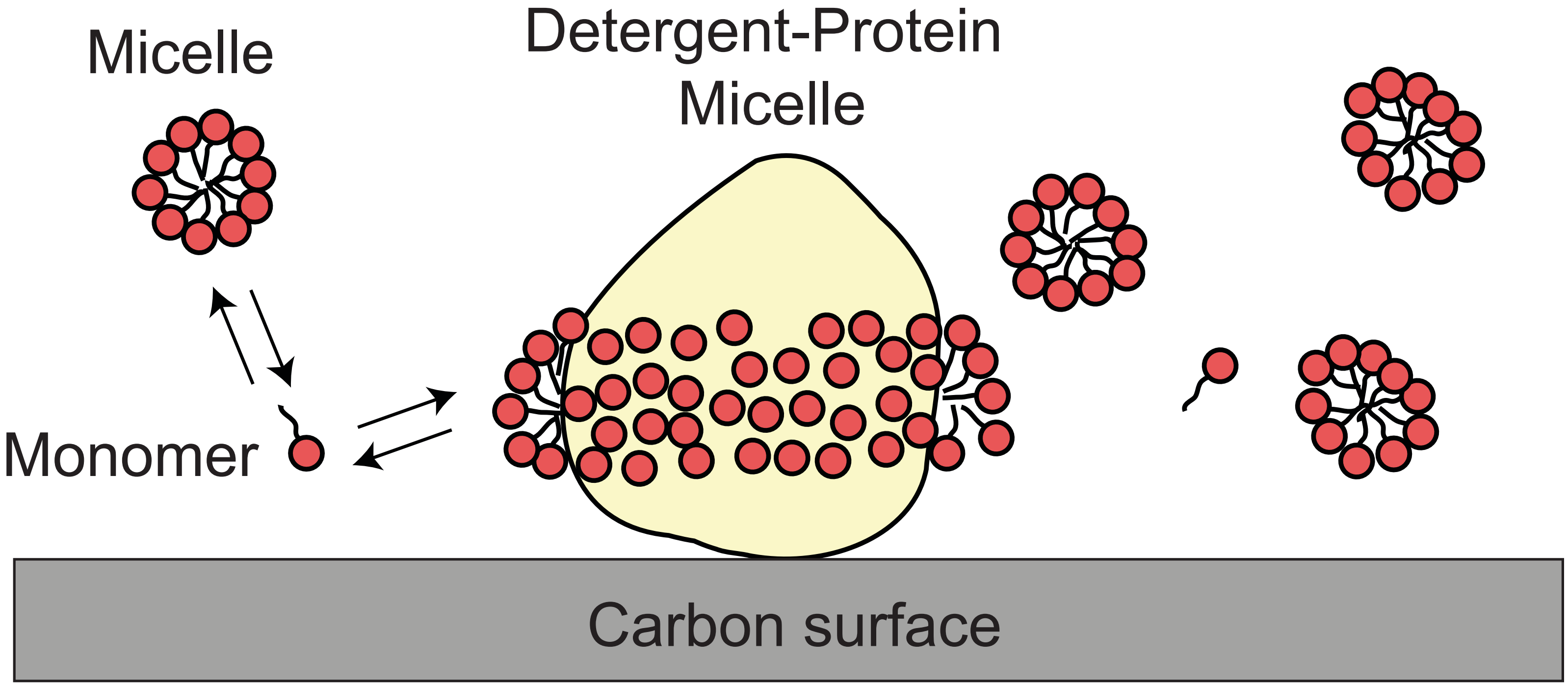
Rubinstein (2007). *Methods* **41**, 409-416.

Staining a membrane protein in detergent



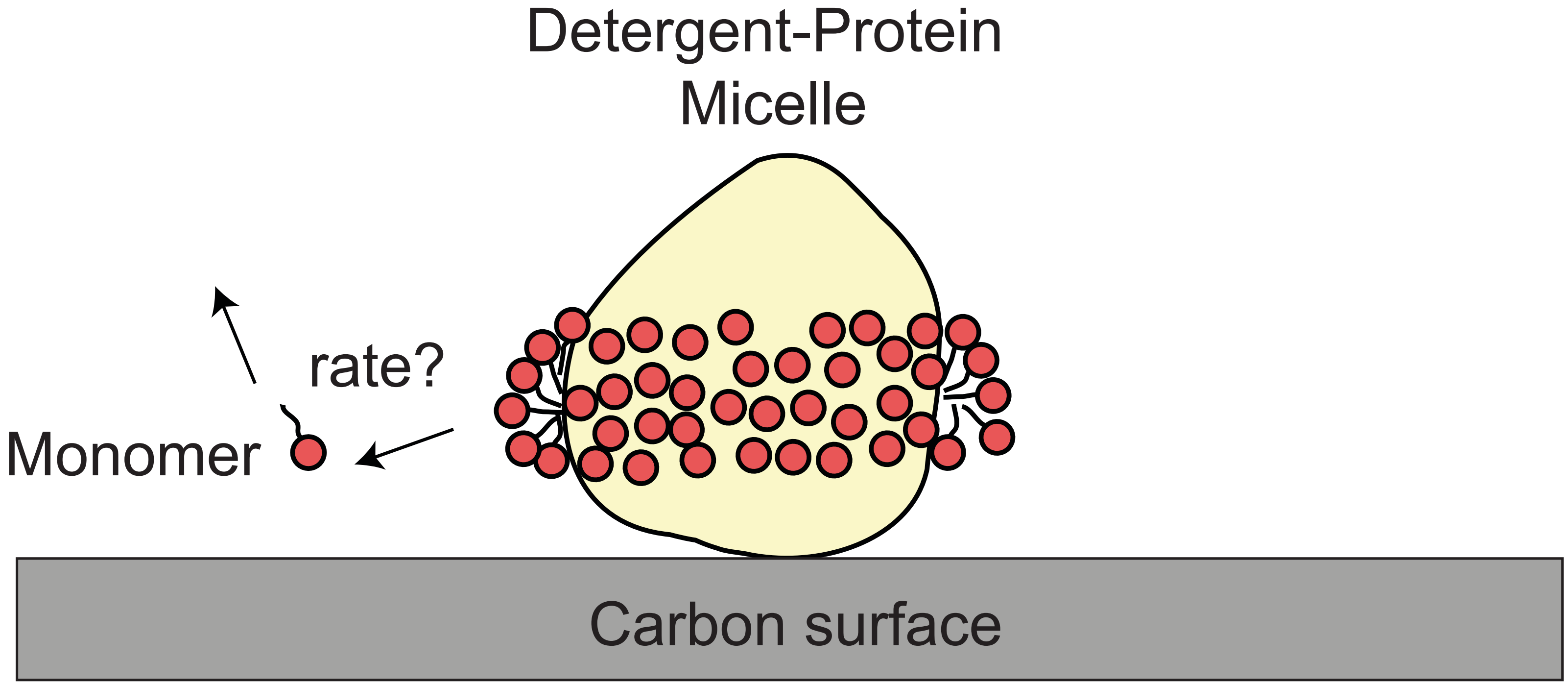
(1) Protein in detergent solution

Staining a membrane protein in detergent



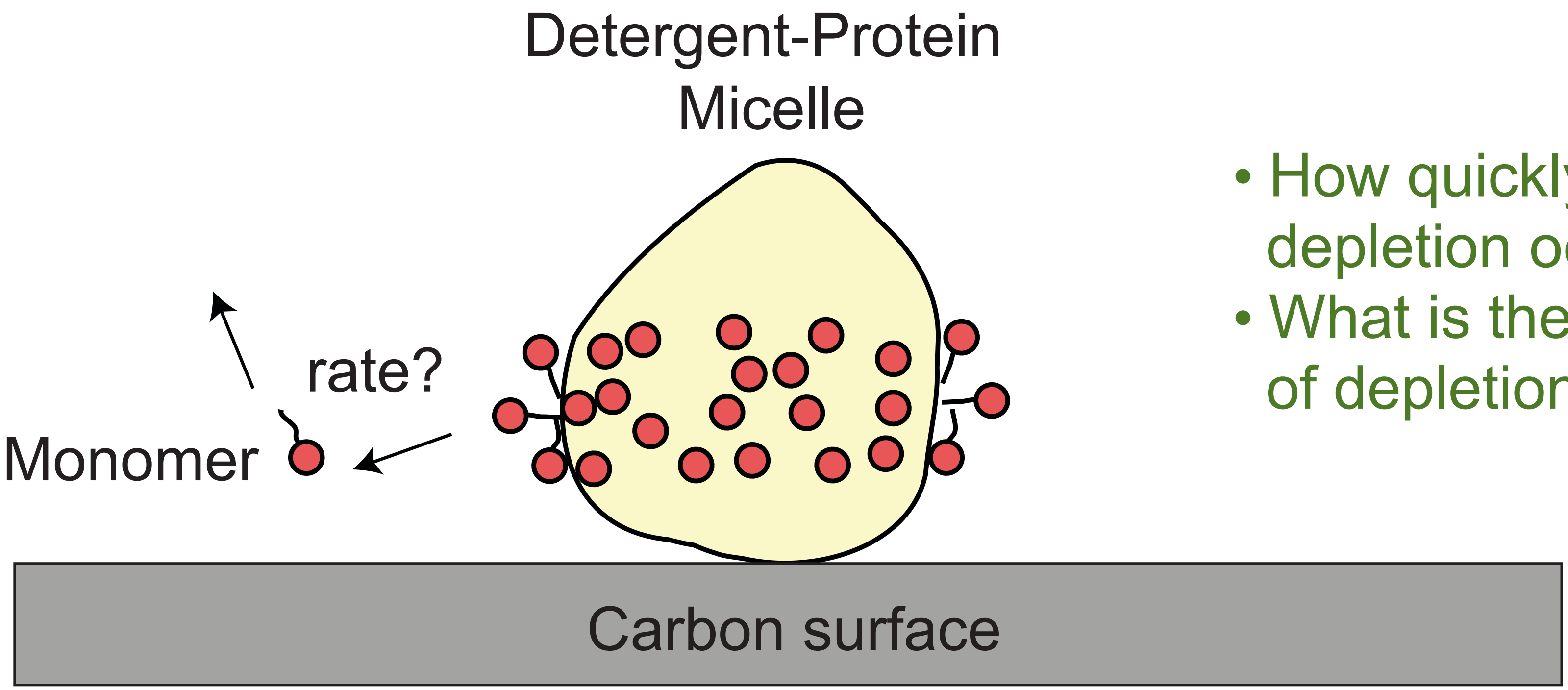
(2) Protein adsorbed to carbon support

Staining a membrane protein in detergent



(3) Grid washed with detergent-free buffer

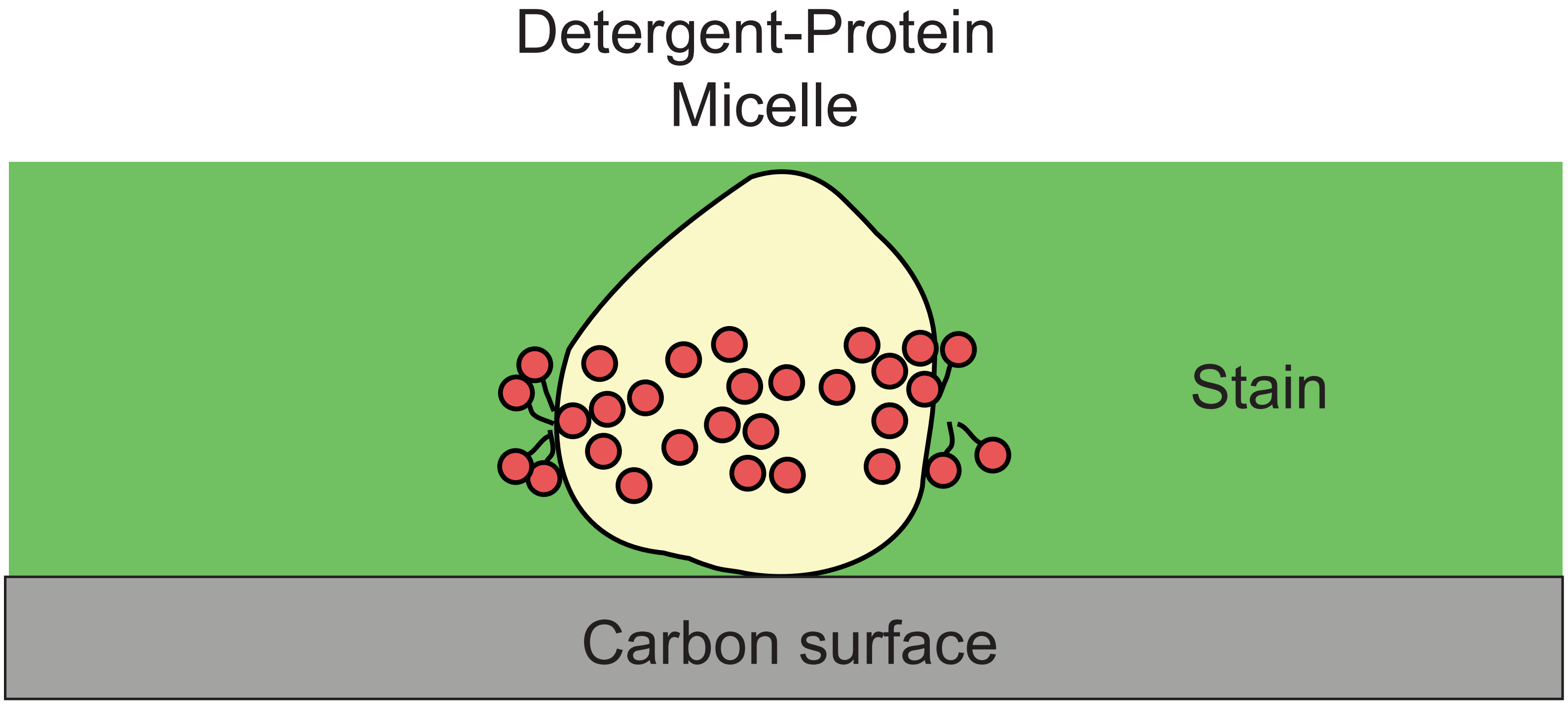
Staining a membrane protein in detergent



- How quickly does depletion occur?
- What is the extent of depletion?

(4) Detergent partially depleted

Staining a membrane protein in detergent



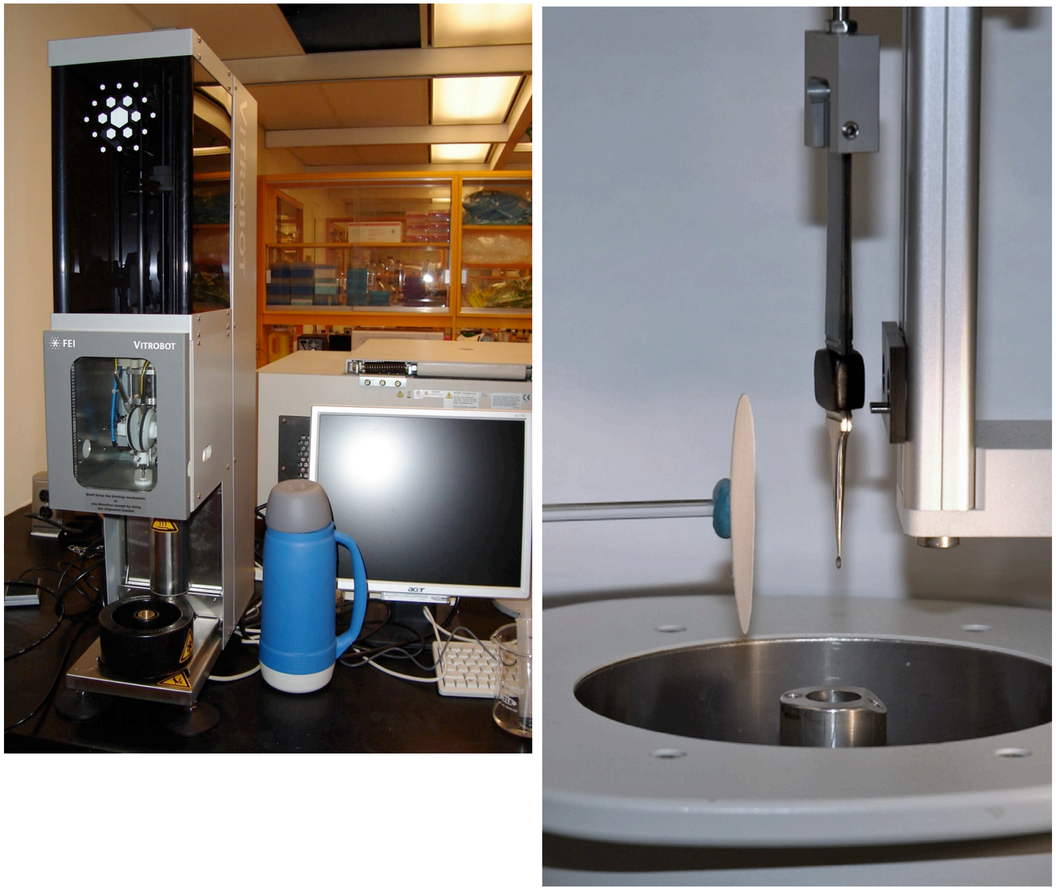
(5) Grid stained

Single particle cryo-EM with detergents

Preparing cryo-EM grids of membrane proteins

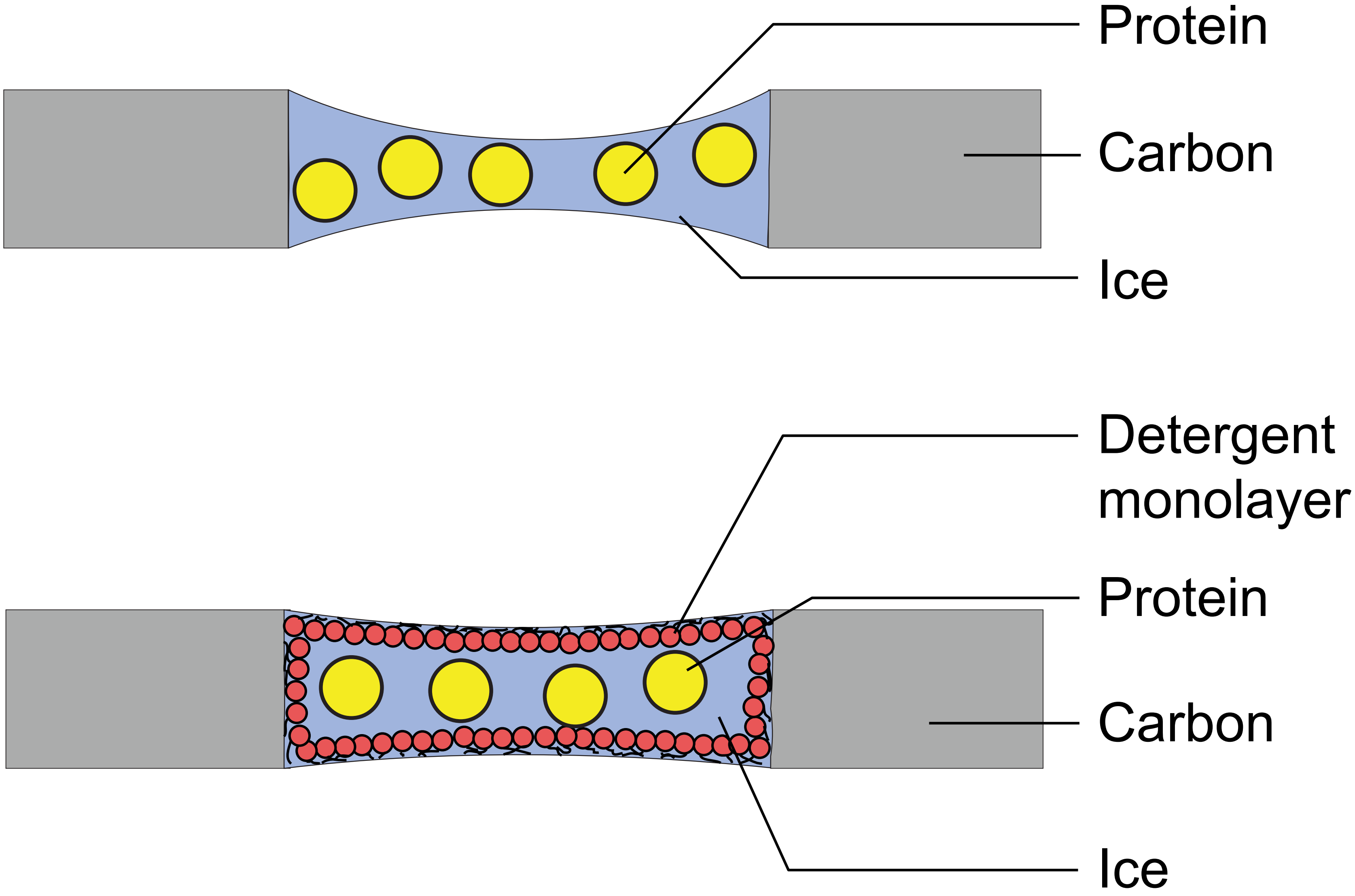
- Glow discharge in air ~ 2 min
- Blot ~ 8 sec
- Plunge into liquid ethane

Critical in our hands
but others use less

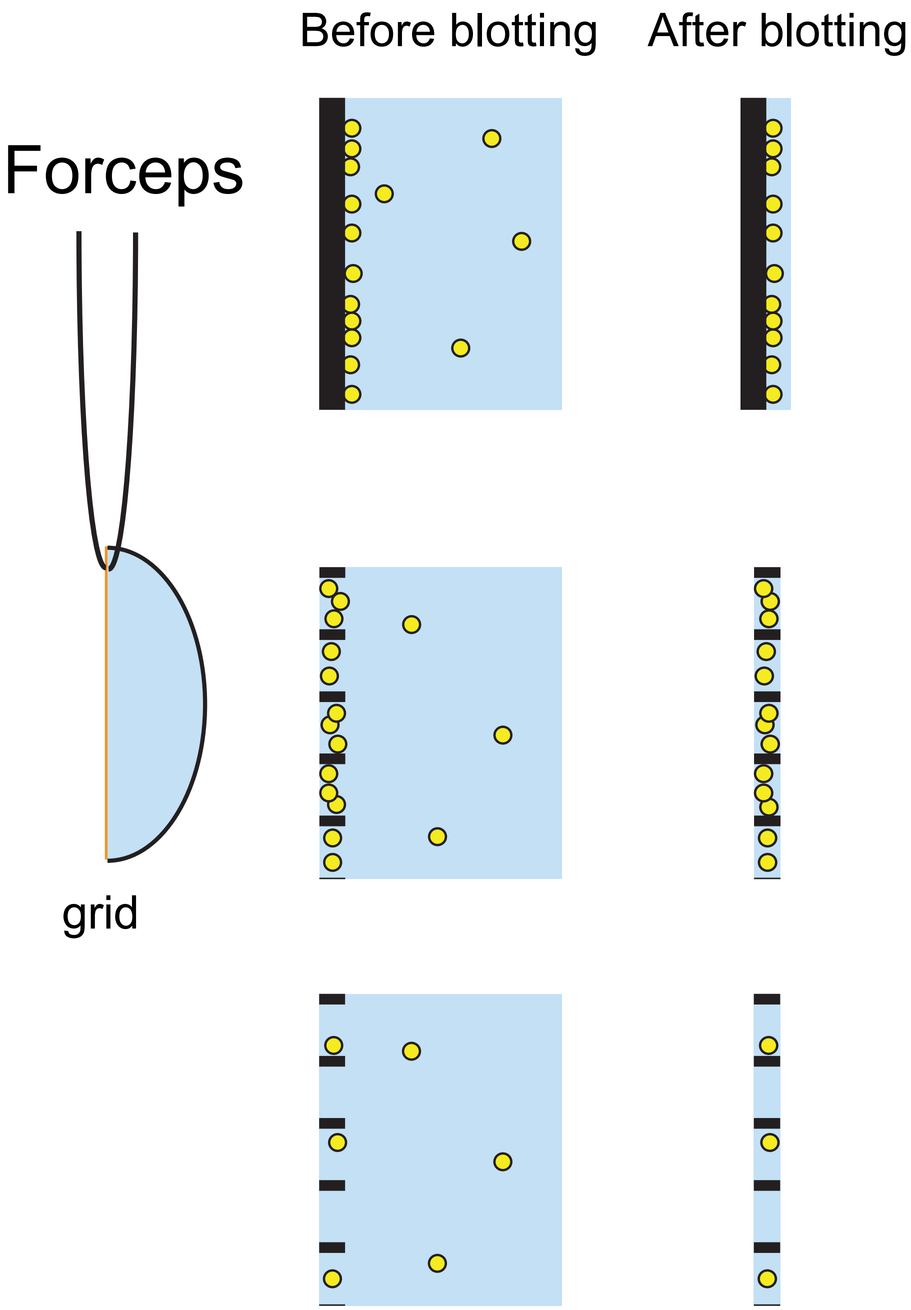


Limit evaporation:
  - Control humidity
  - Reduce temperature

Detergents solutions may tend to make thicker ice



Protein quantity

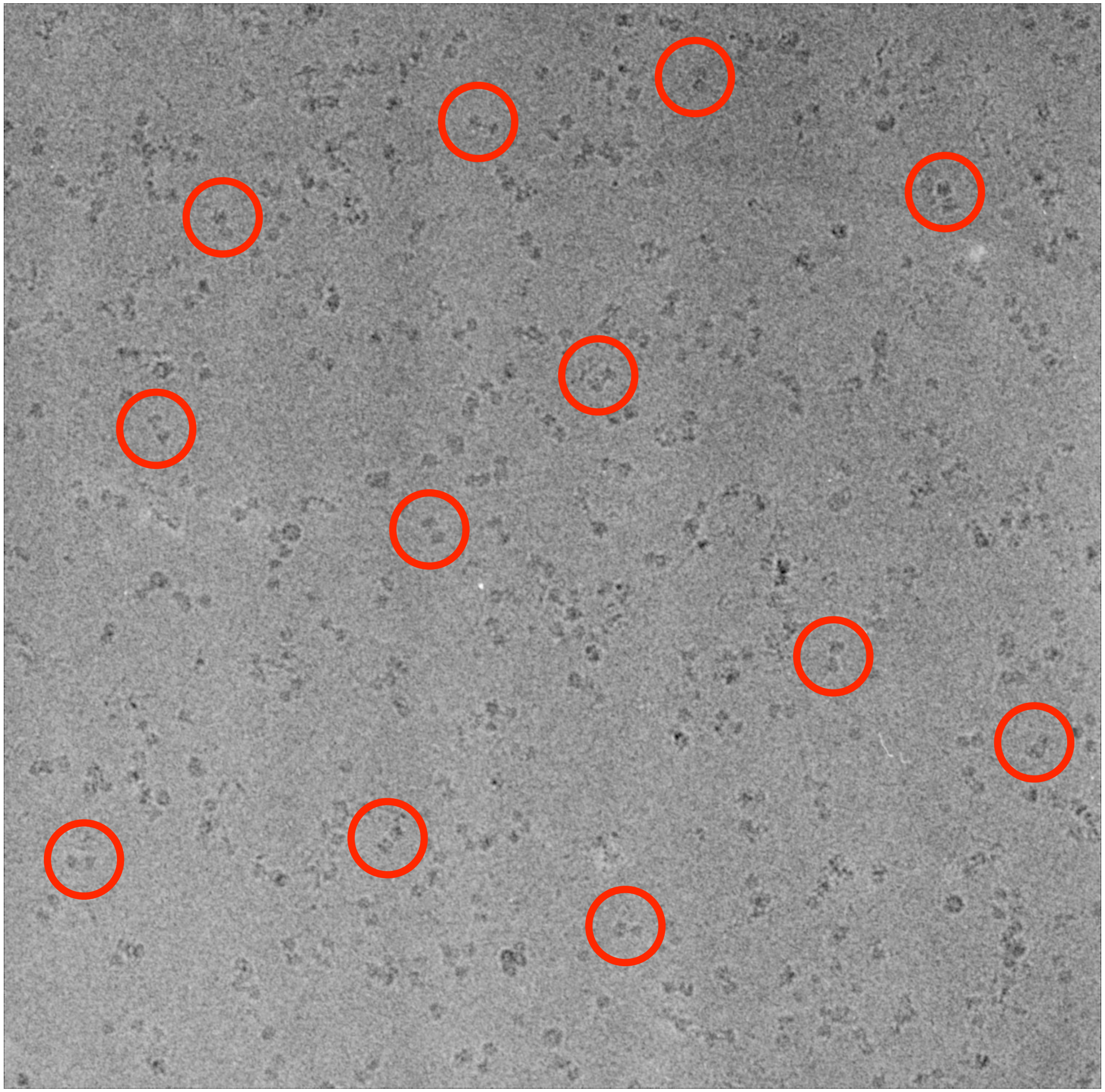


Continuous carbon film
(membrane or soluble protein)

Perforated carbon film
(soluble protein)

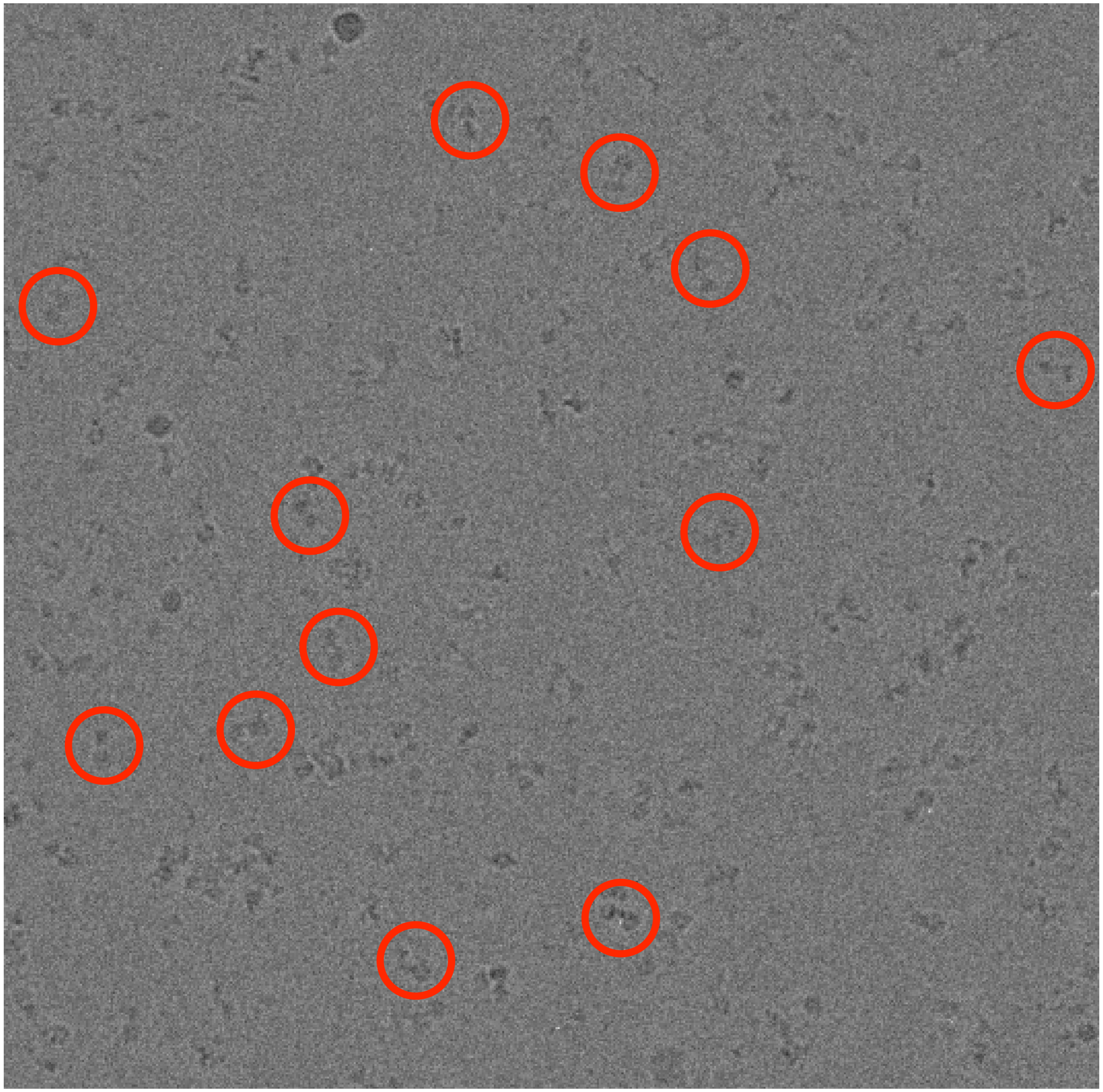
Perforated carbon film
(membrane protein)

Cryo-EM of *T. thermophilus* V-ATPase (W. Lau)



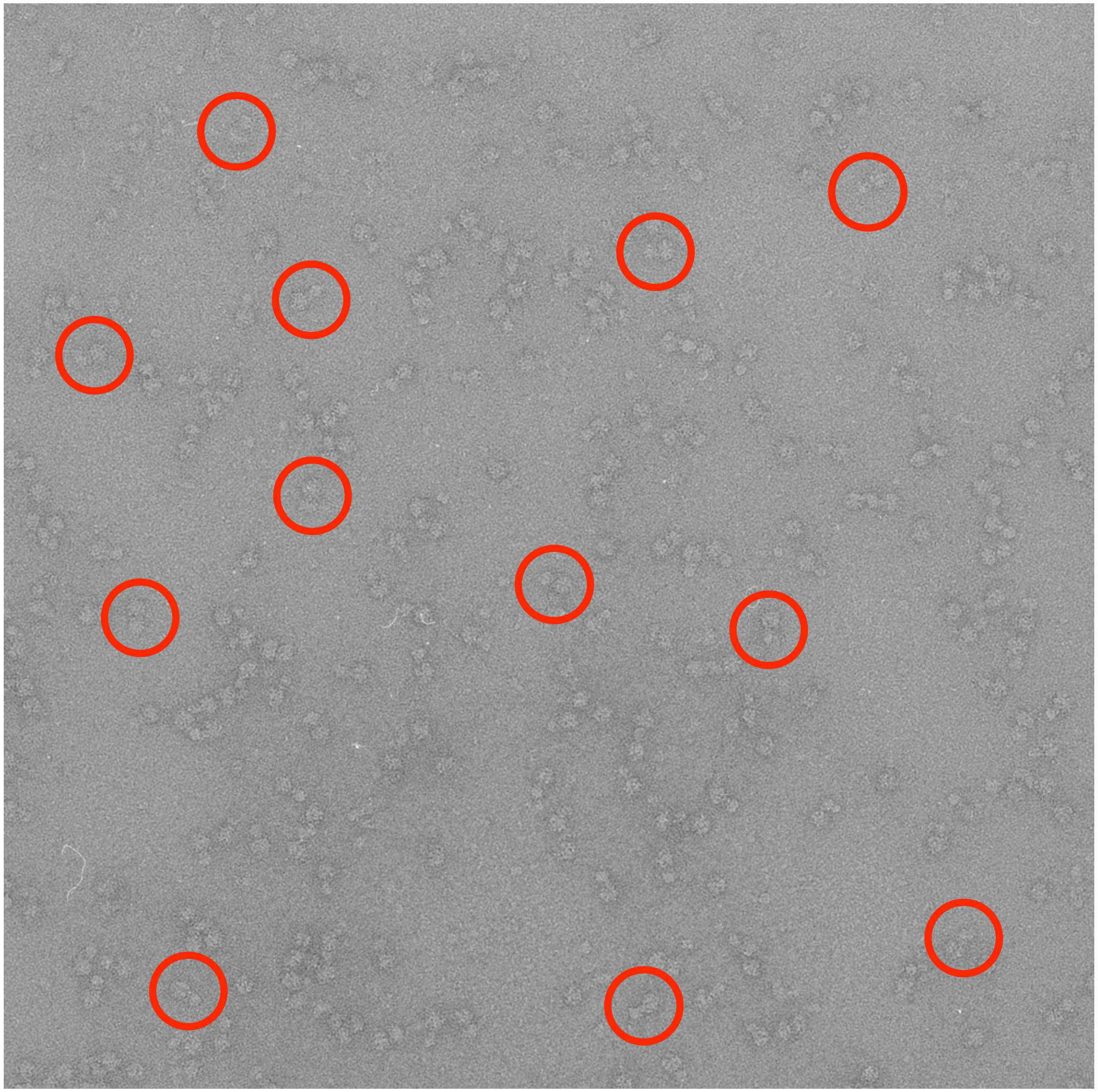
~ 3 mg/ml (5 µM)

Cryo-EM of *B. taurus* ATP synthase (L. Baker)



~ 3 mg/ml (5 µM)

Negative stain of *S. cerevisae* ATP synthase (S. Bueler)



~ 0.01 mg/ml (17 nM)

Support Grid Choices for cryo-EM

Perforated Carbon.

Advantages:
(1) low background
(2) random orientations

Disadvantages:
(1) need more protein
(2) charging?

Continuous Carbon.
(thin supported film)

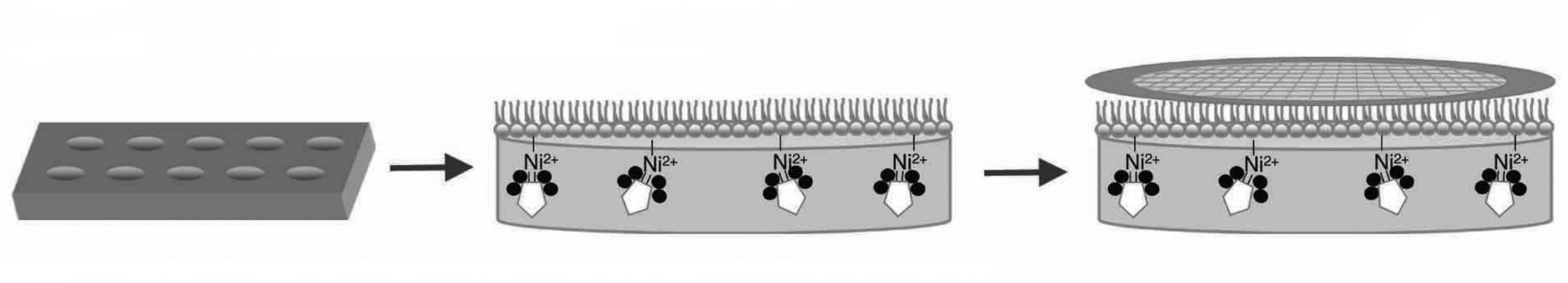
Advantages:
(1) need less protein
(2) less charging?
(3) buffer may be exchanged on the grid after protein bound

Disadvantages:
(1) preferred orientations?
(2) background noise

Are there other options?

Increasing protein quantity for cryo-EM

NiNTA lipid monolayer to concentrate protein (we haven't actually tried this for membrane proteins yet)



Kelly, D. F.,*et al*. *PNAS* **105**, 4703– 4708.

Interpreting images of protein-detergent complexes

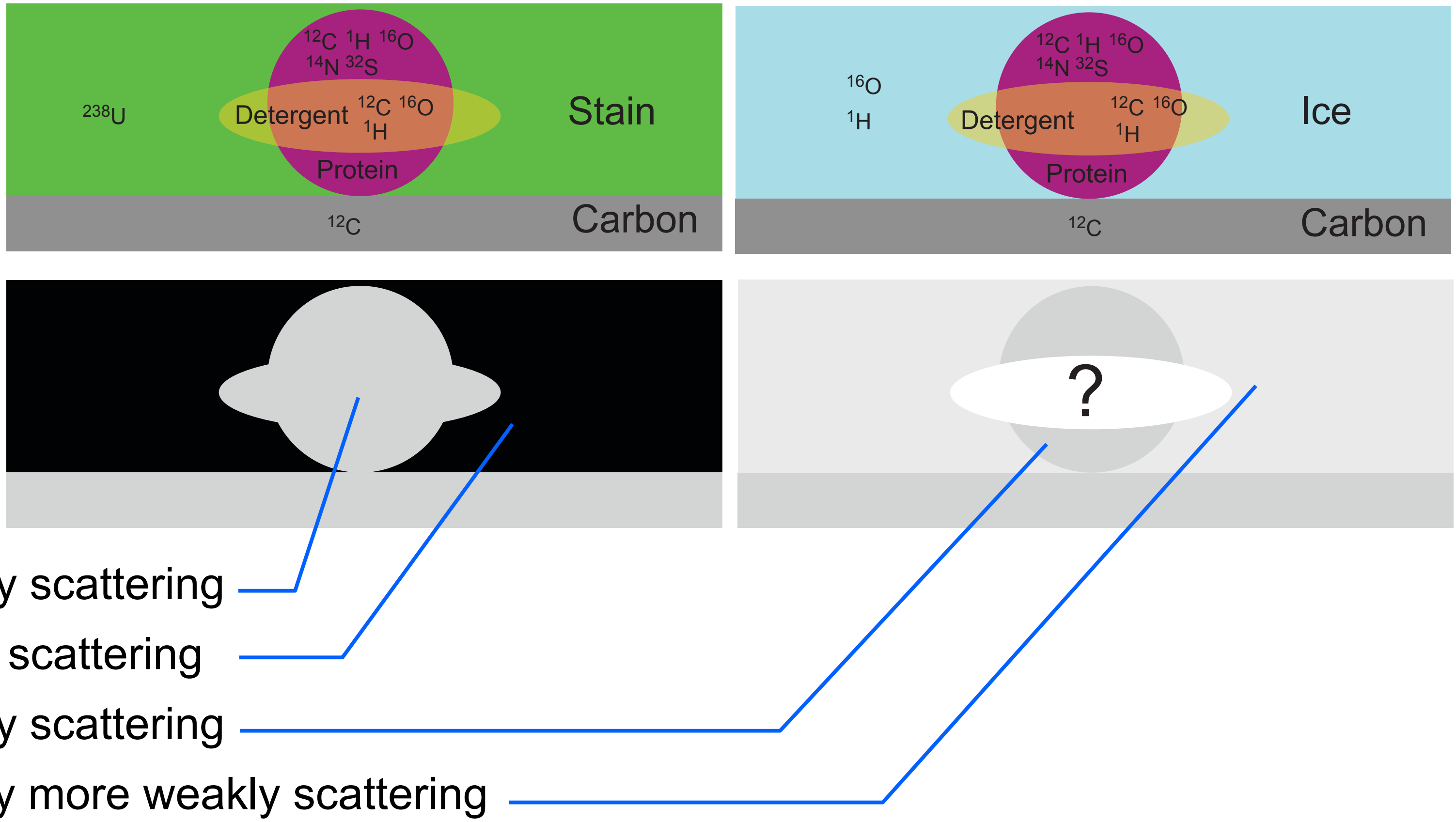
Appearance of detergents in EM

Negative Stain

Frozen, hydrated

Specimen

Image



Weakly scattering

Highly scattering

Weakly scattering

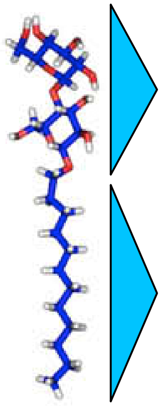
Slightly more weakly scattering

Complication:
detergent may be washed away during staining

Complication:
1) detergent may be washed away if sample is on a cont. grid and grid is washed
2) Detergent may be more or less dense than ice

Densities for different substances

Substance	Density (g/ml)	Reference
Amorphous Ice	0.94	Mishima, O. *et al.* (1985). *Nature* **314**, 76-78.
Protein	~1.36 *	* For why this isn't right, see: H. Fischer, I. *et al.* (2004), Protein Sci **13** 2825–2828.
DDM	1.19	Timmins, P. A, *et al.* (1988). *FEBS Lett* **2**, 361- 368
LDAO	0.882	Timmins, P. A, *et al.* (1988). *FEBS Lett* **2**, 361- 368
Triton X-100	1.10	Ganong, B. R. , *et al.* (1989). *Anal Biochem* **179**, 66–71.
OG	1.16	le Maire, M. *et al.* (2000). *BBA* **1508**, 86-111.
CHAPS	1.23	le Maire, M. *et al.* (2000). *BBA* **1508**, 86-111.
Deoxycholic acid	1.29	le Maire, M. *et al.* (2000). *BBA* **1508**, 86-111.
Cholic acid	1.30	le Maire, M. *et al.* (2000). *BBA* **1508**, 86-111.
SDS	1.16	le Maire, M. *et al.* (2000). *BBA* **1508**, 86-111.

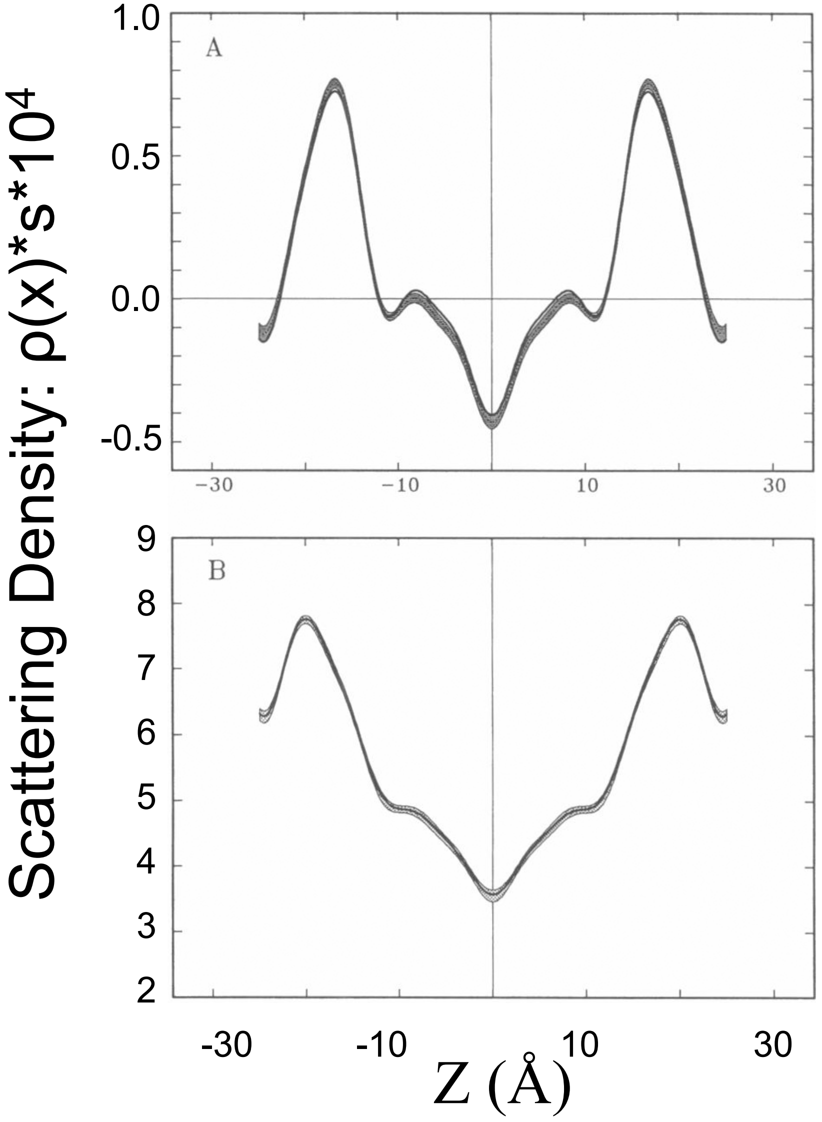


High density

Low density

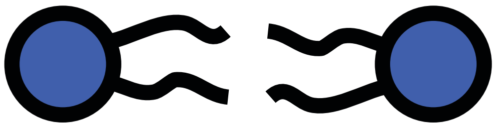
See le Maire, M. *et al.* (2000). *BBA* **1508**, 86-111. for many others

The density of lipids



Neutron density profile
(DOPC bilayer)

X-ray density profile
(DOPC bilayer)

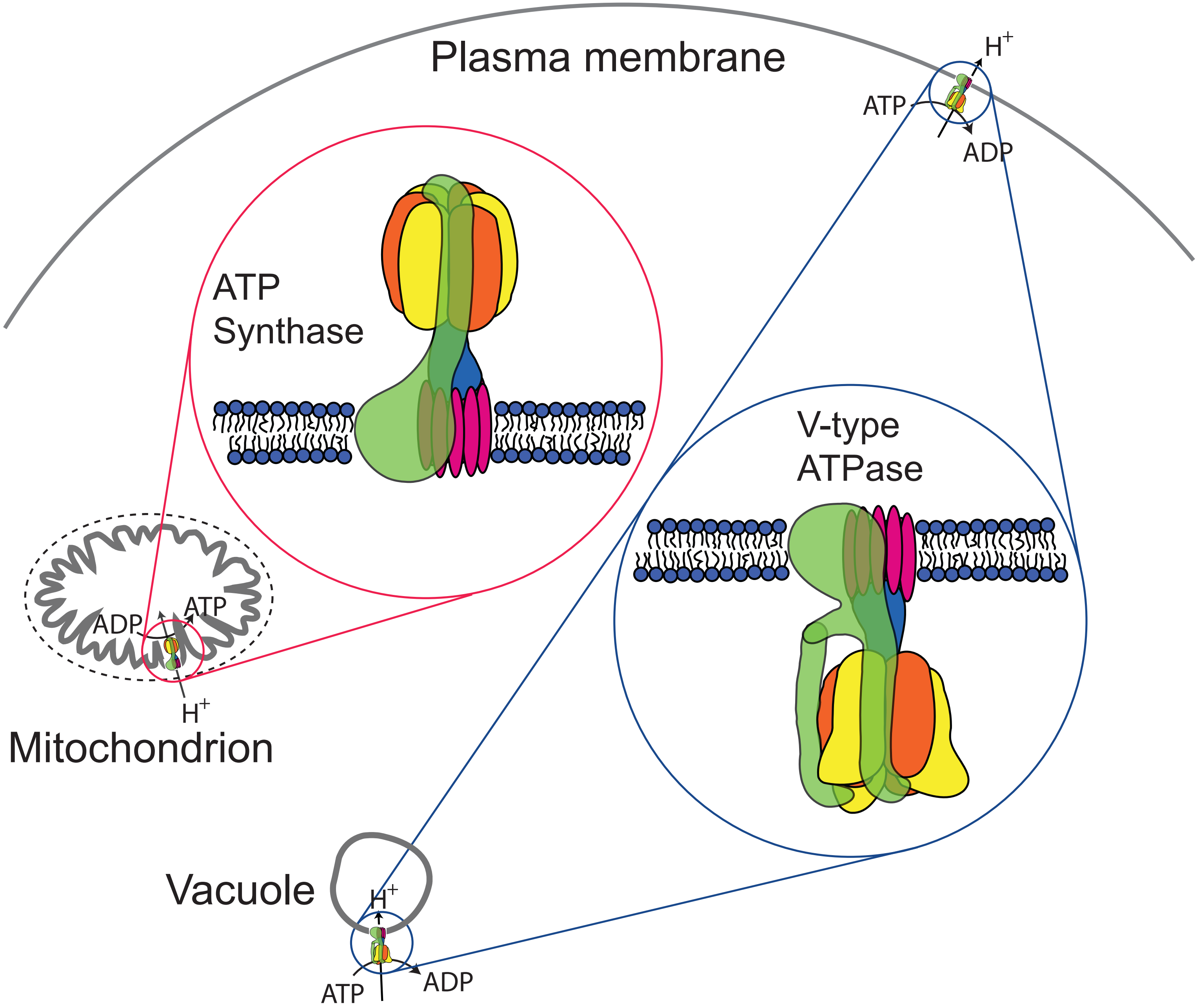


Membrane structure

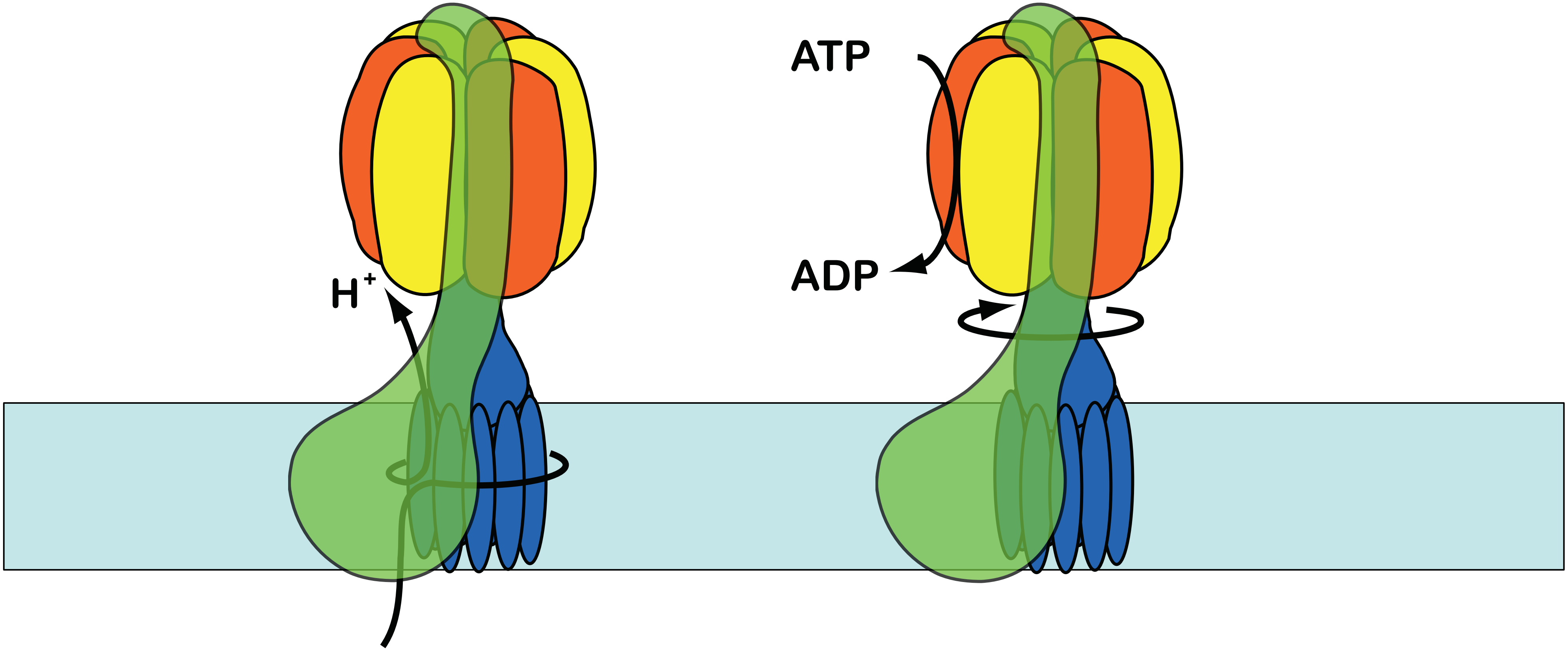
Wiener and White (1992). *Biophysical J.* **61**, 434-447.

Two example biological systems: the ATP synthase and V-type ATPase

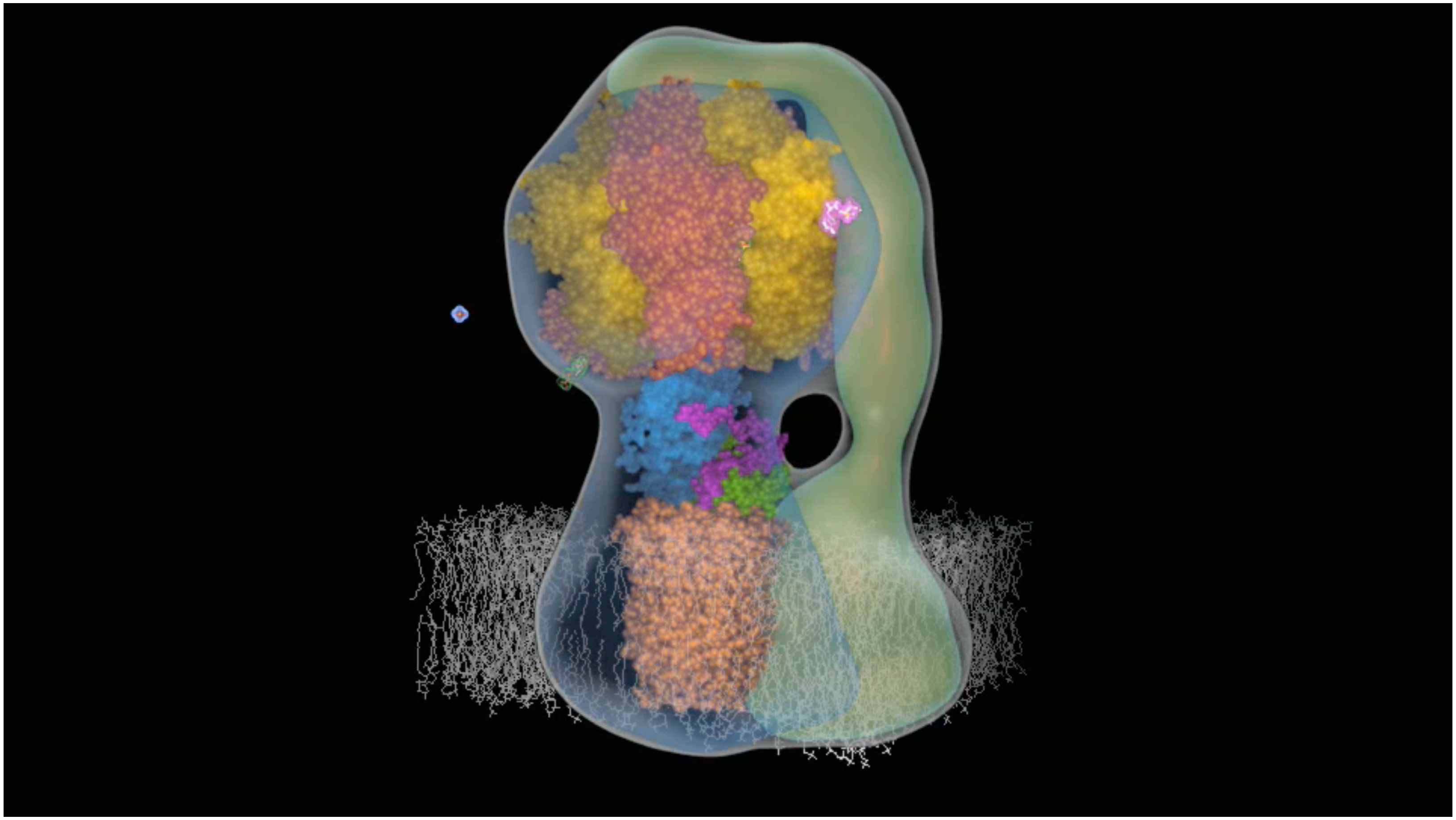
Two example membrane protein complexes



Rotary catalysis: The ATP synthase

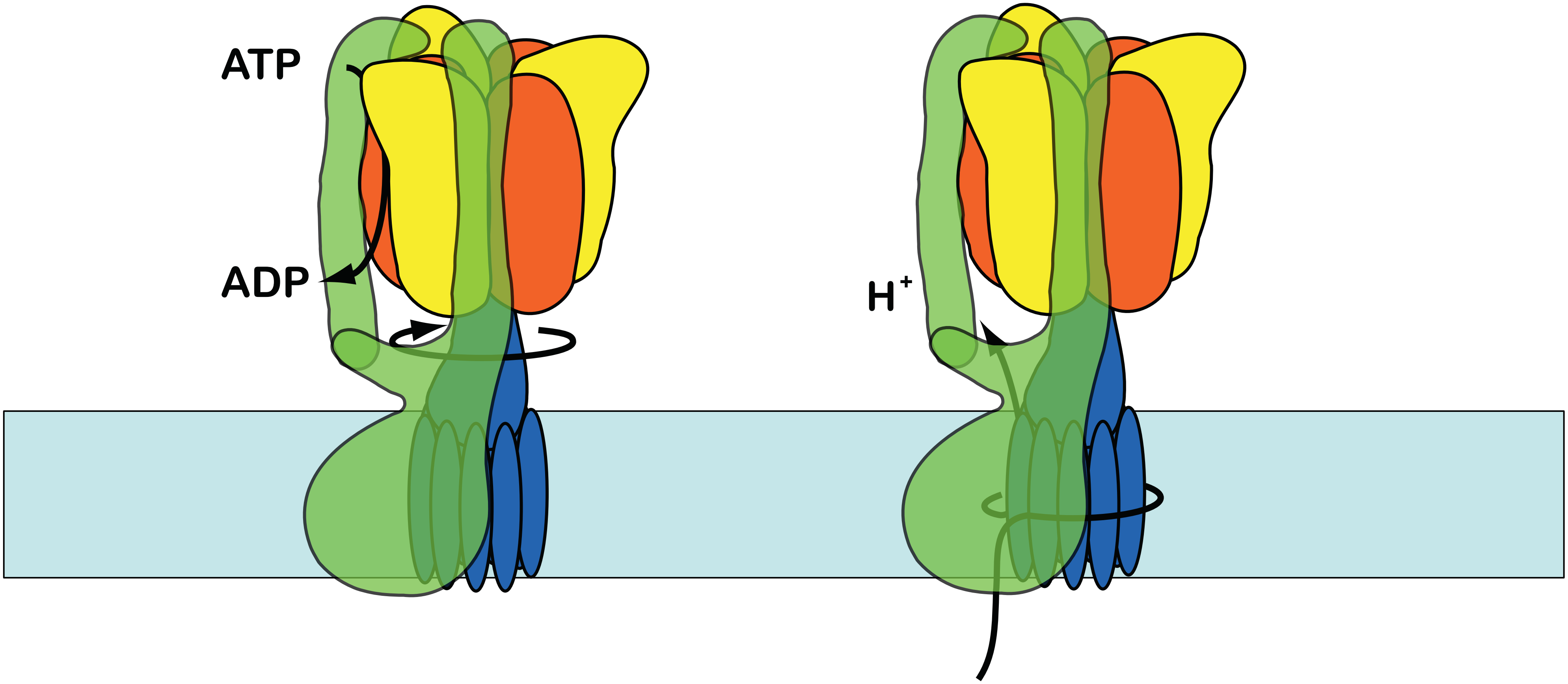


ATP synthase rotary mechanism

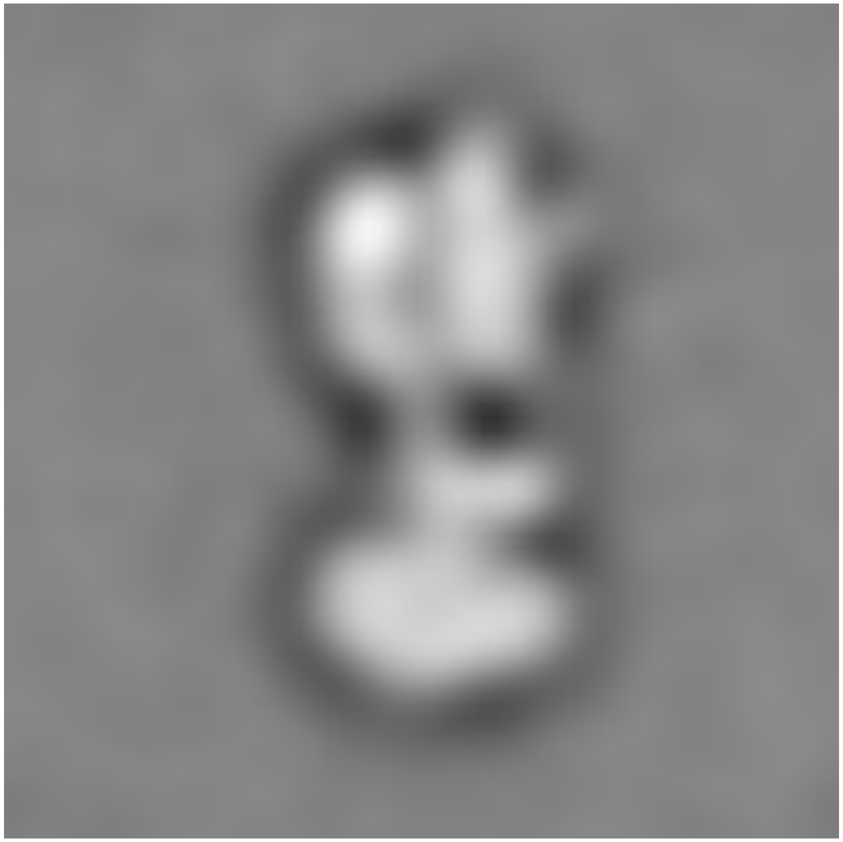


www.mrc-mbu.cam.ac.uk

Rotary catalysis: The V-ATPase

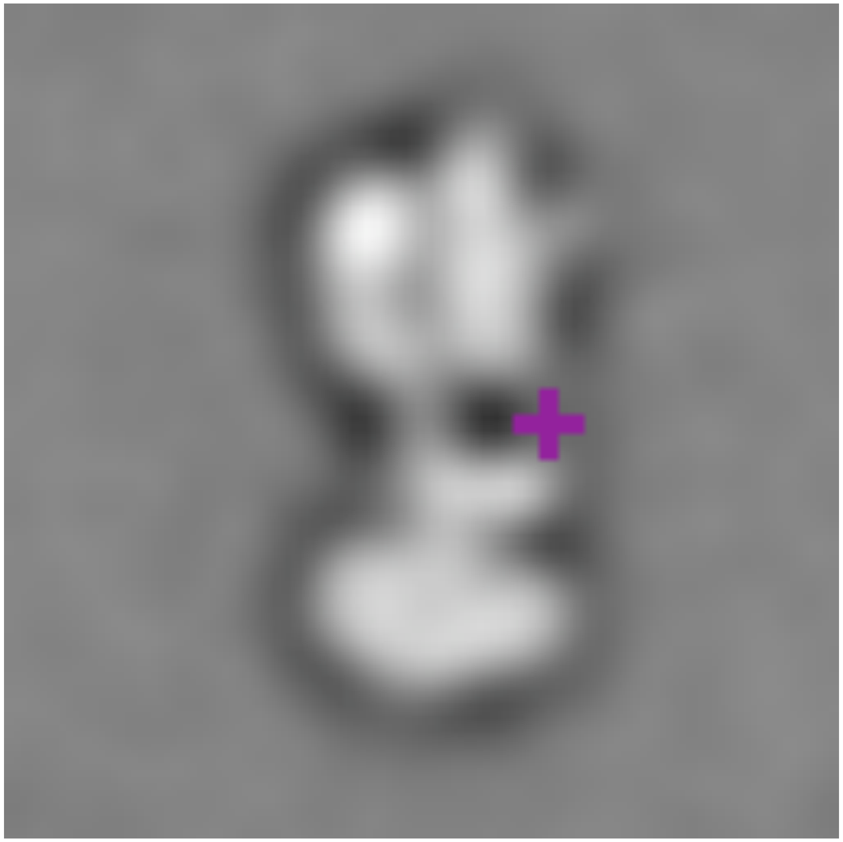


Class average images of V-ATPase



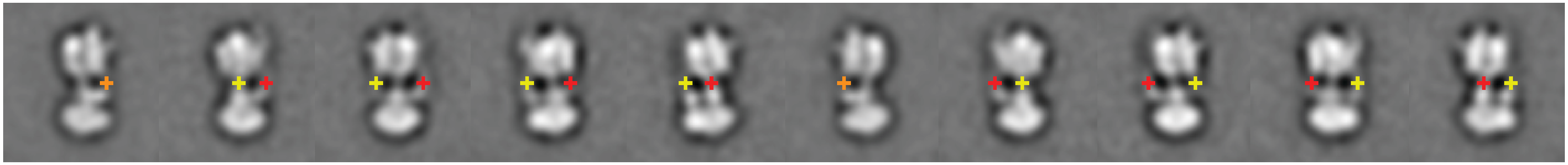
Lau and Rubinstein, Unpublished

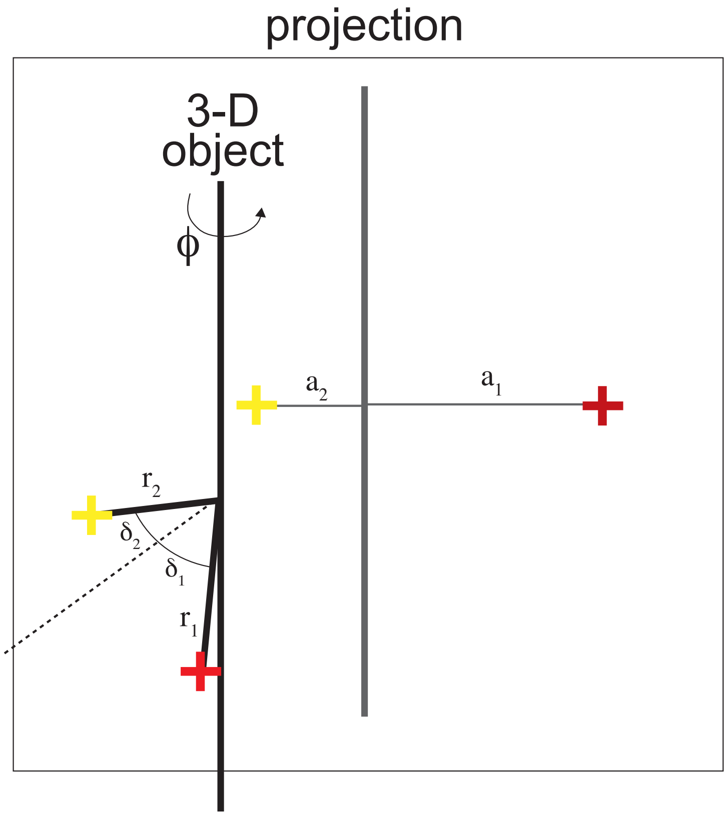
Class average images of V-ATPase

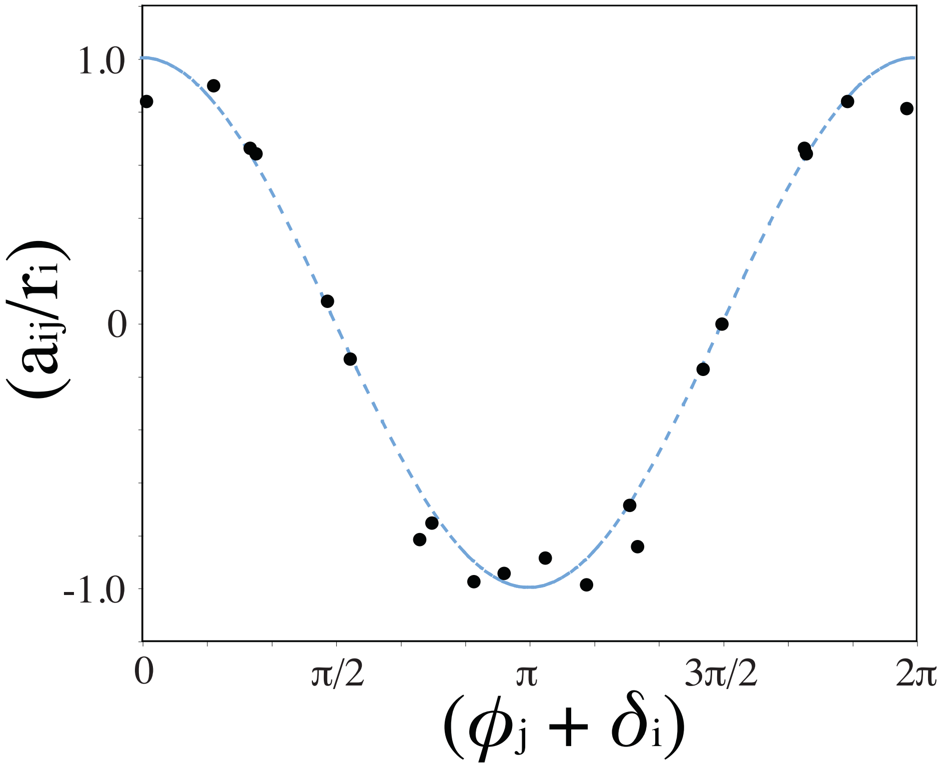


Lau and Rubinstein, Unpublished

Rotational Analysis of V-ATPase



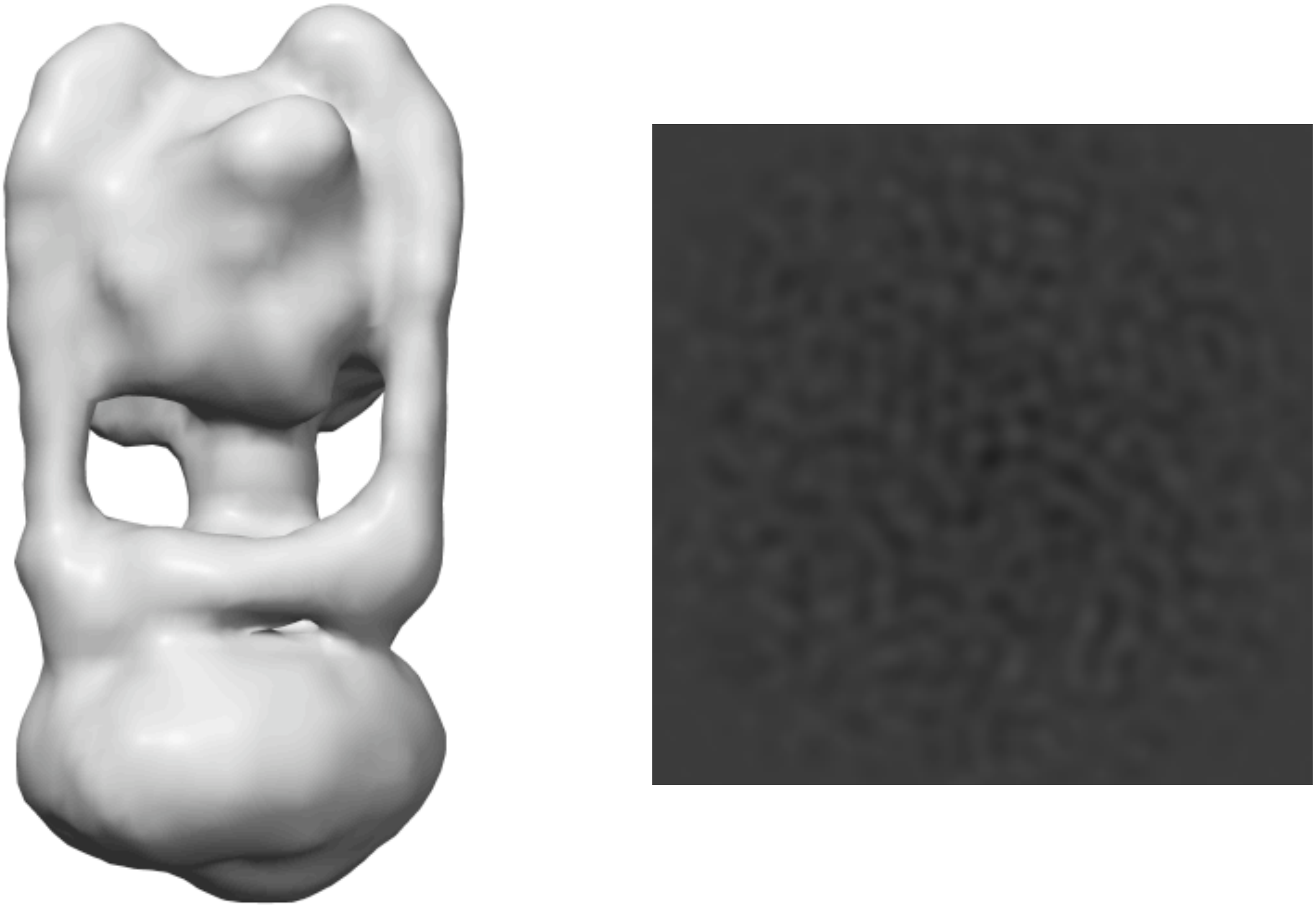




- Conjugate gradients minimization of the error function $f = \sum(r_i\cos(\phi_j+\delta_i) - a_{i,j})^2$ where $a_{i,j}/r_i = \cos(\phi_j+\delta_i)$

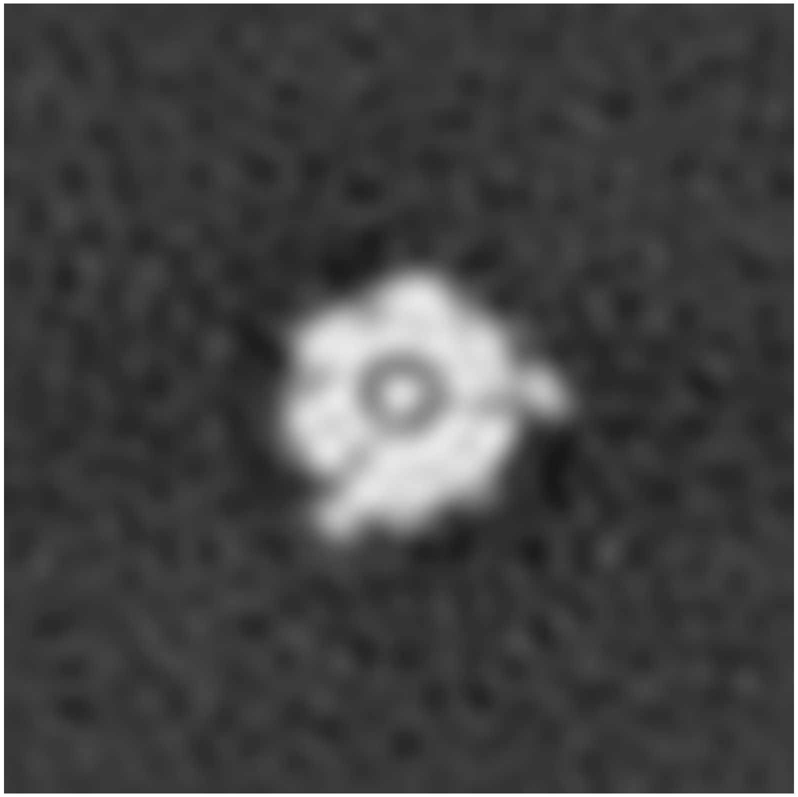
Baker and Rubinstein (2008) *J Struct Biol* **162**, 260-70.

V-ATPase 3-D model



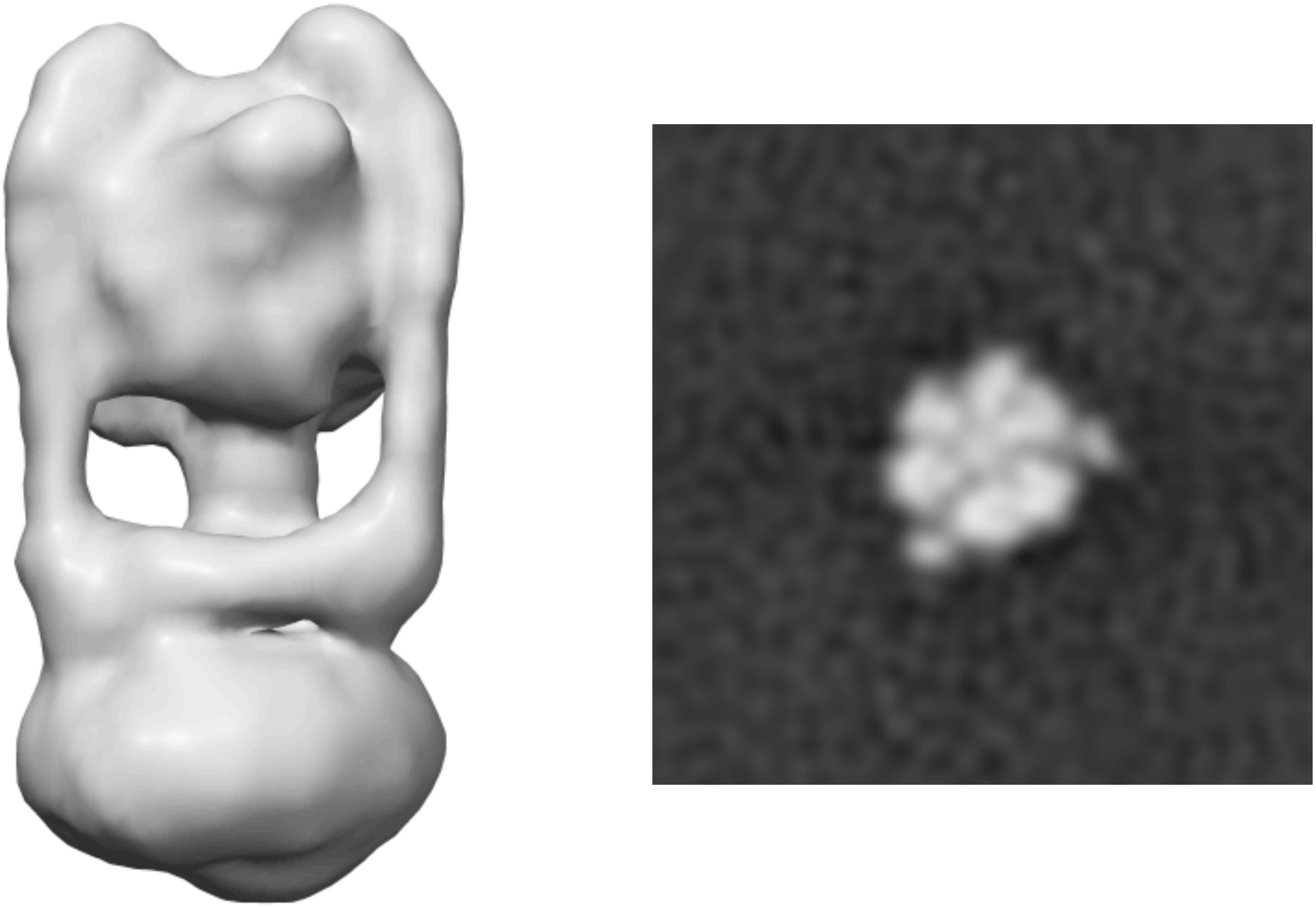
Lau and Rubinstein, Unpublished

V-ATPase 3-D model



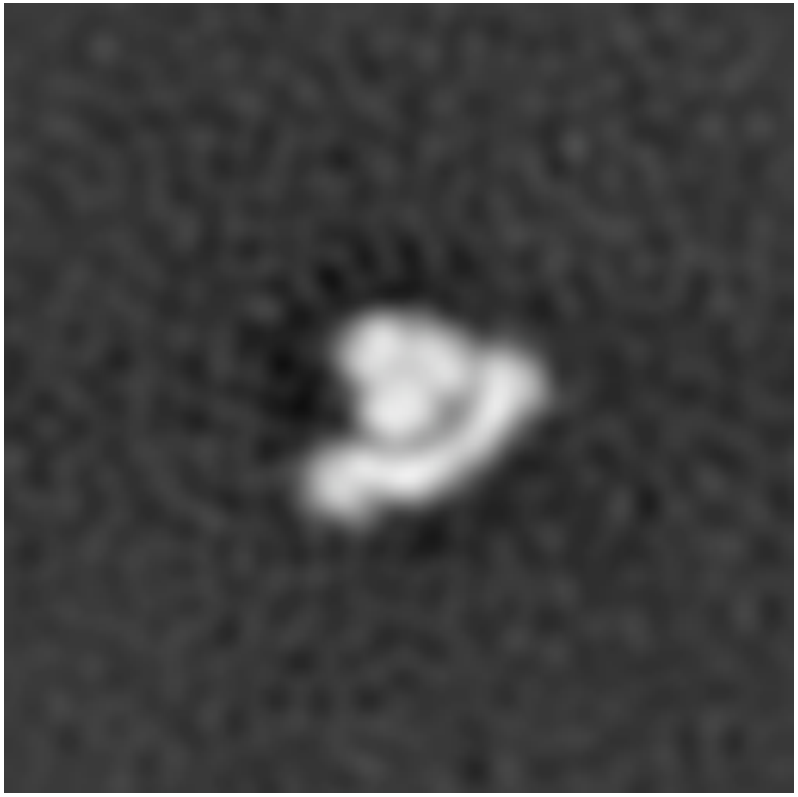
Lau and Rubinstein, Unpublished

V-ATPase 3-D model



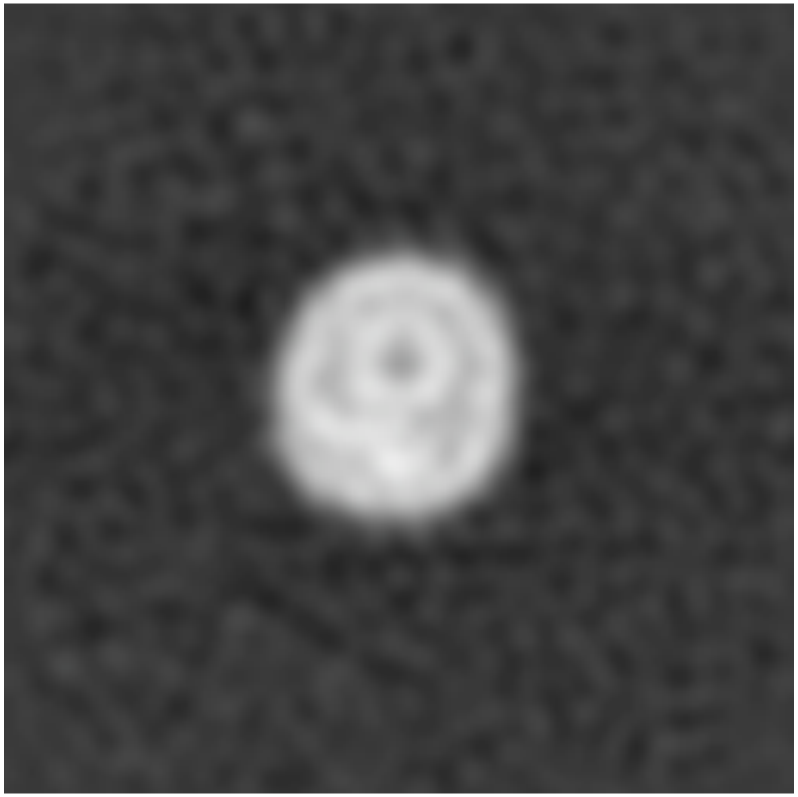
Lau and Rubinstein, Unpublished

V-ATPase 3-D model



Lau and Rubinstein, Unpublished

V-ATPase 3-D model

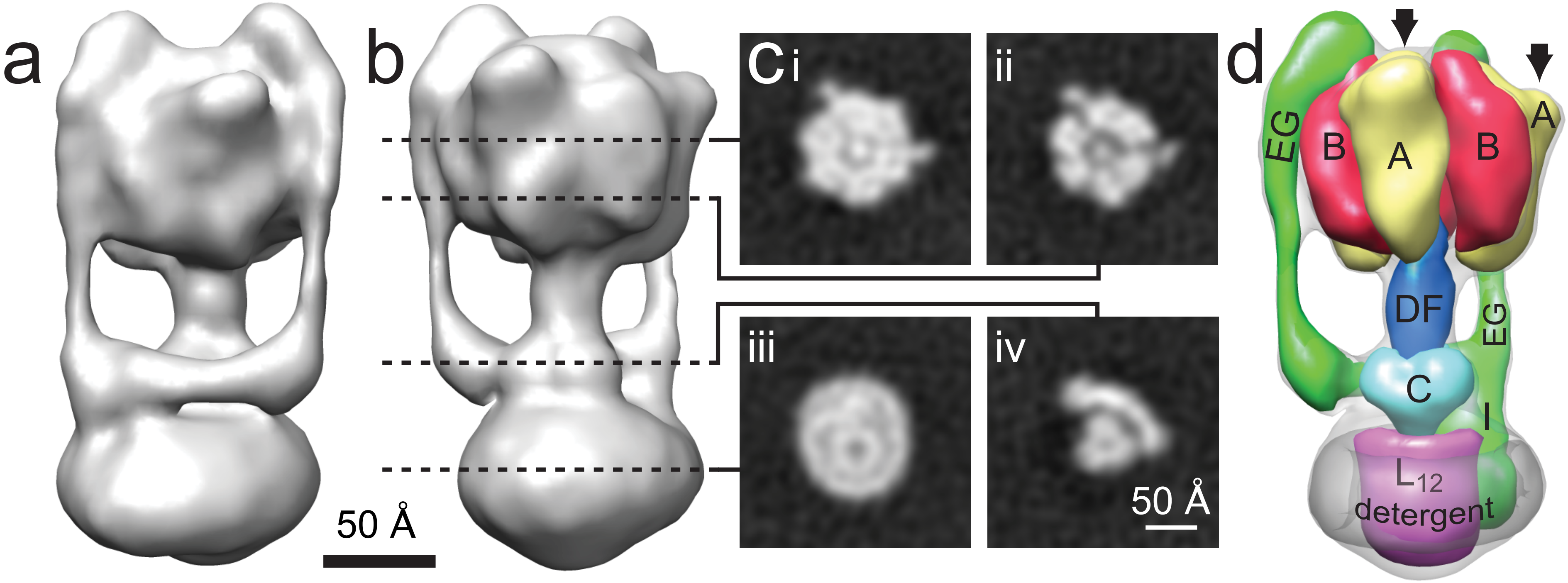


Lau and Rubinstein, Unpublished

V-ATPase 3-D model

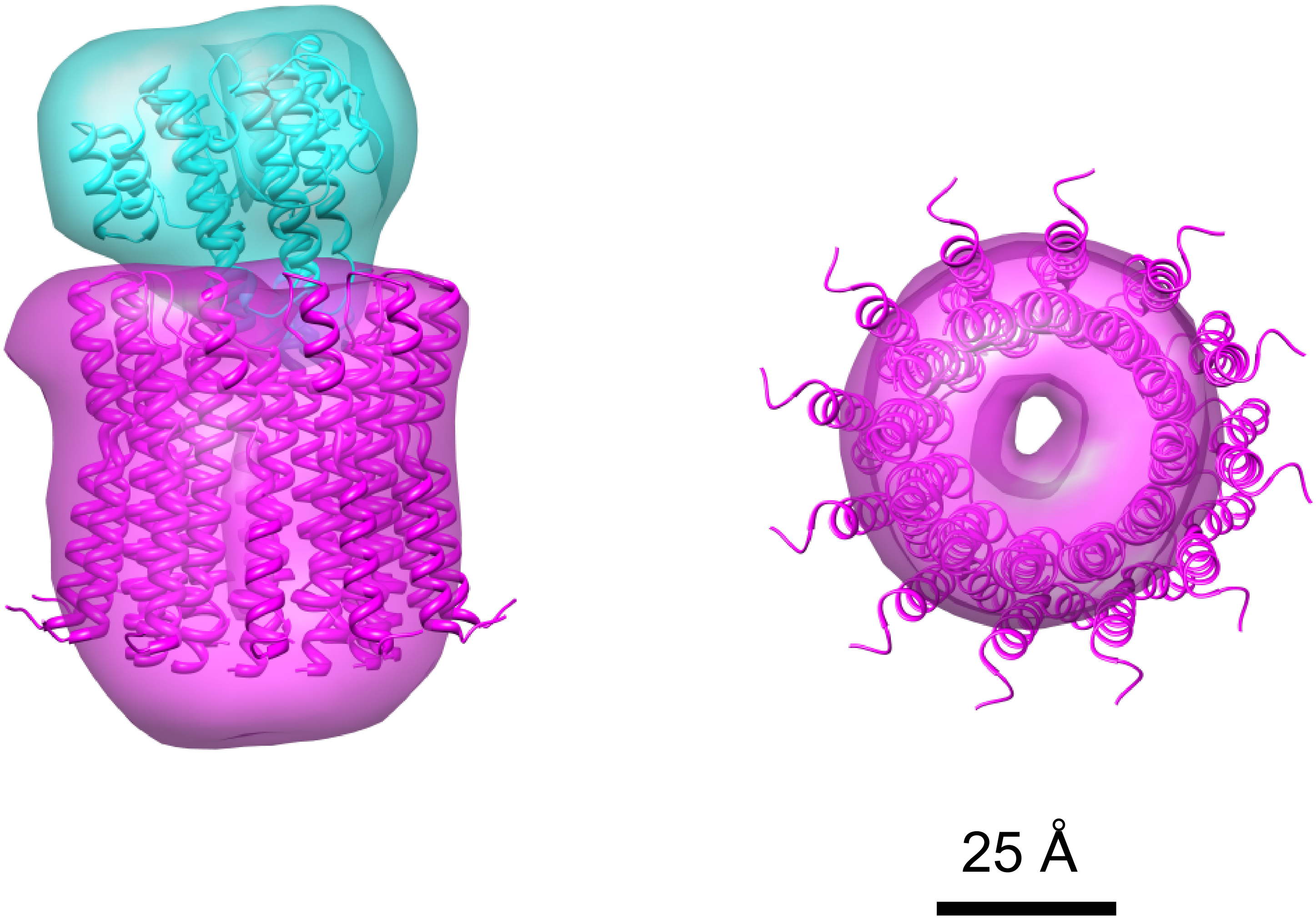
Lau and Rubinstein, Unpublished

V-ATPase 3-D model



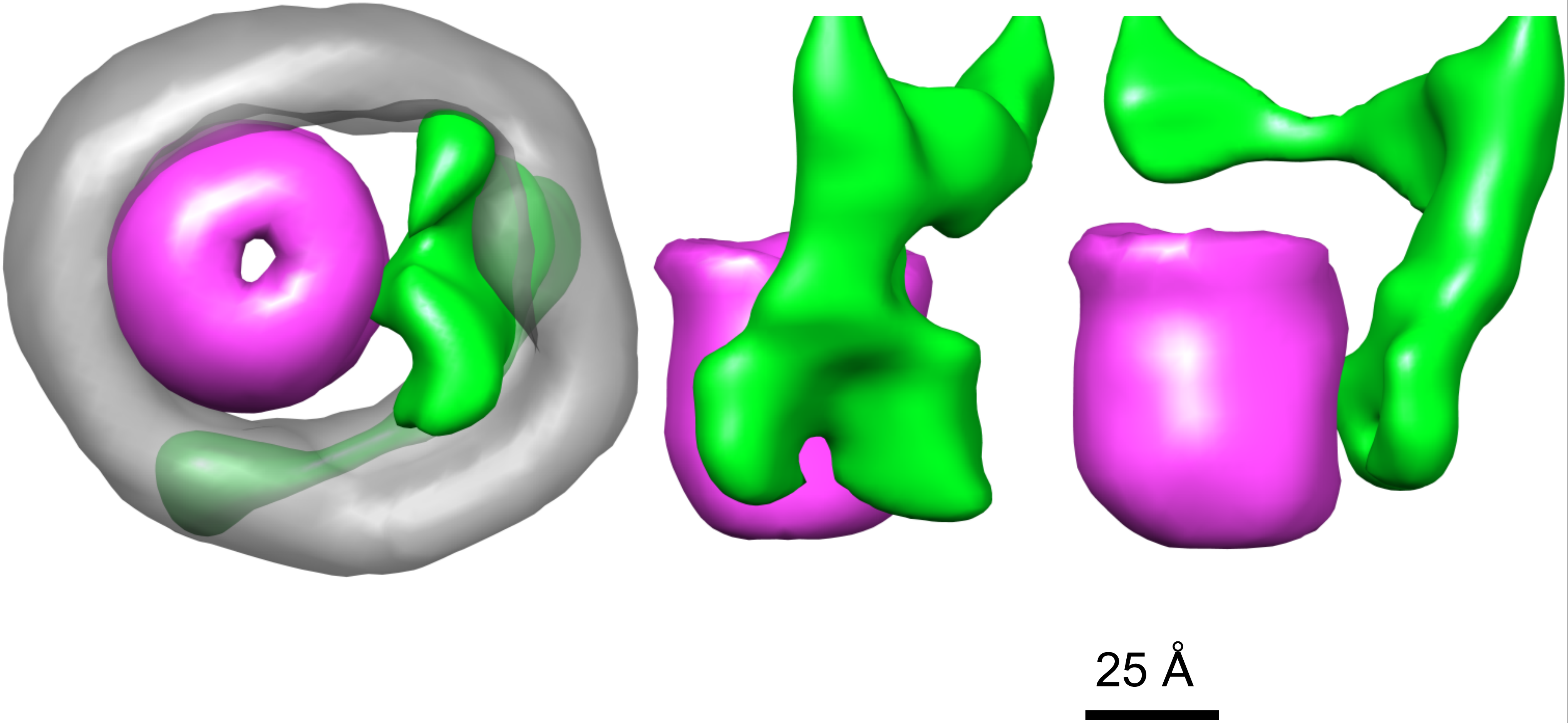
Lau and Rubinstein, Unpublished

The membrane region: known structures



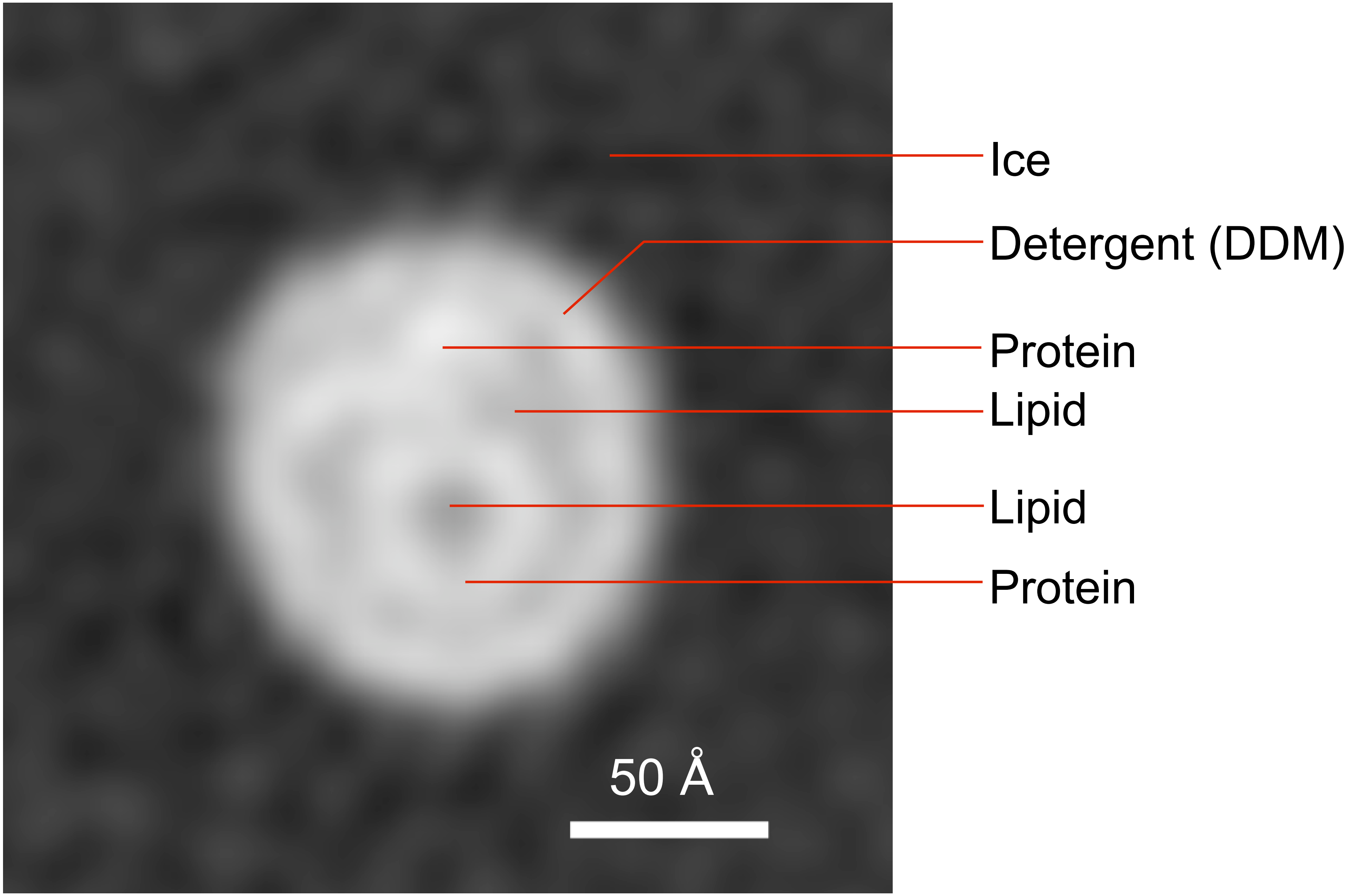
Lau and Rubinstein, Unpublished

The membrane region: unknowns structures

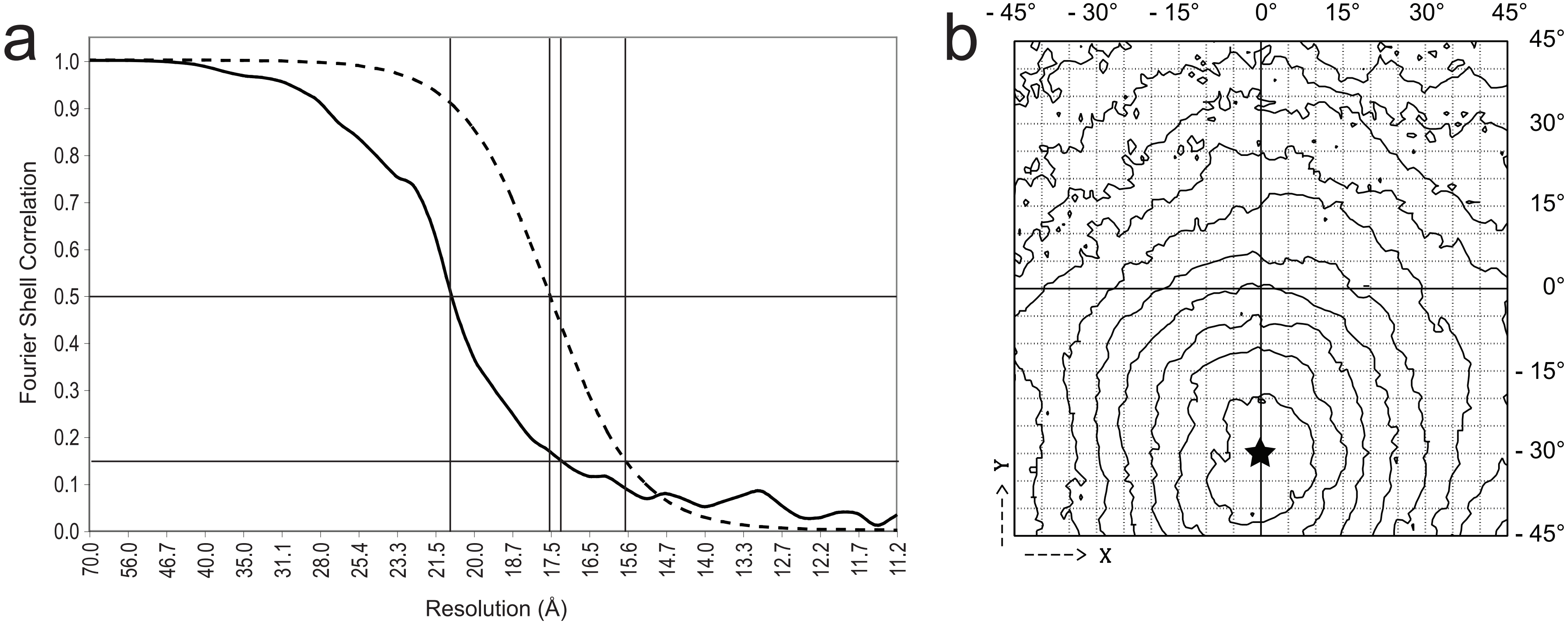


Lau and Rubinstein, Unpublished

A mid-membrane segment of the map

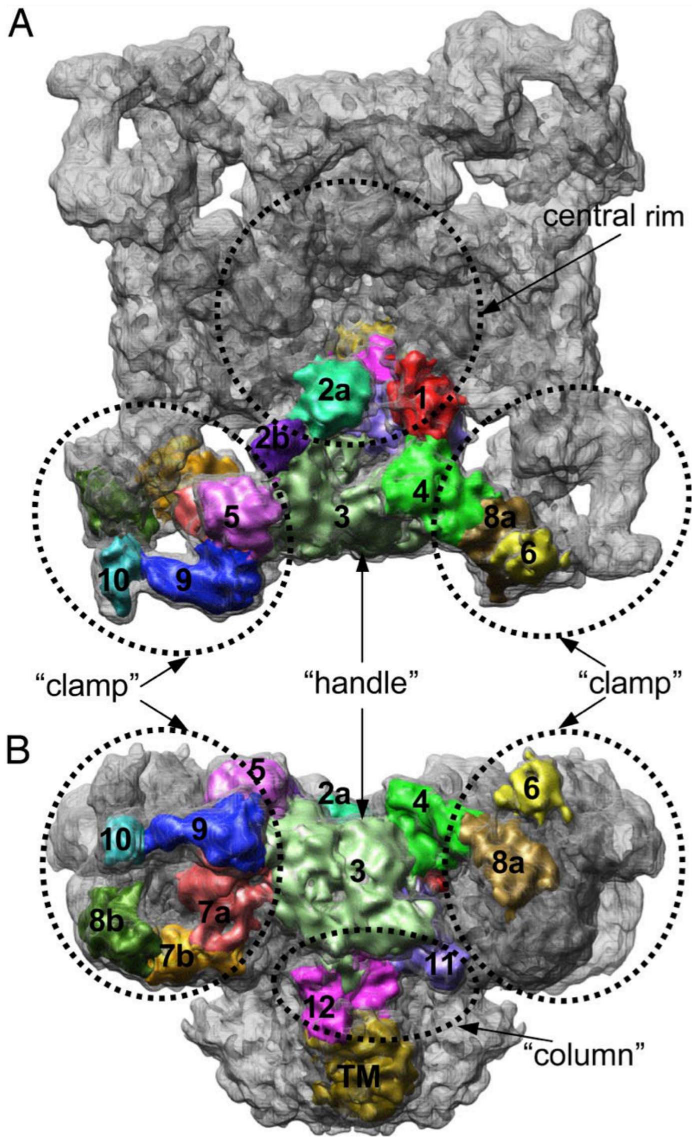


Model Validation



Can we do well with single particle cryo-EM in detergents?

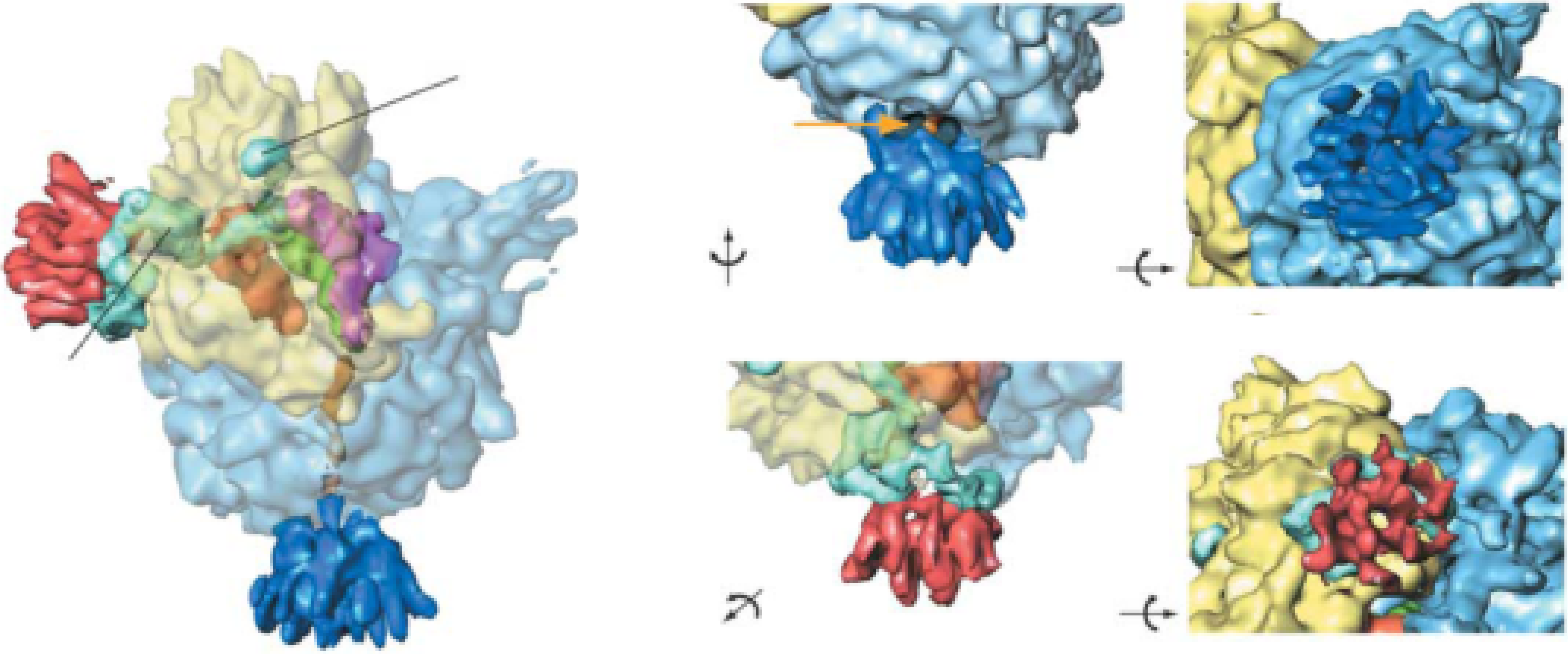
Ryanodine Receptor - Chiu Group



Detergent: CHAPS (?)
Support: Continuous carbon (?)

Serysheva, I. I., *et al.* (2008). *PNAS* **105**, 9610 –9615.

SecYEG in complex with 70S ribosome - Frank Group



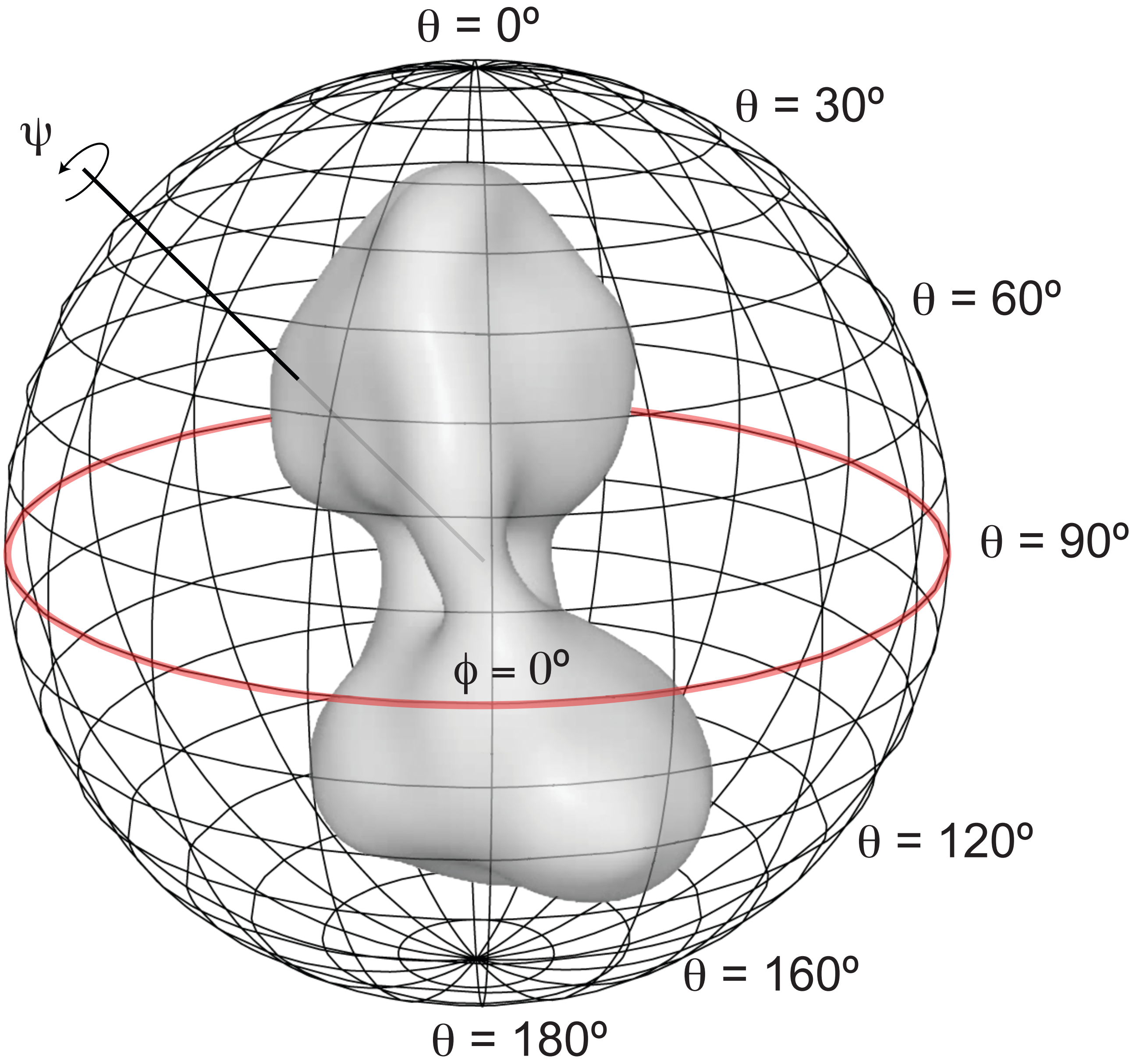
Detergent: CHAPS (?)

Support: Continuous carbon (?)

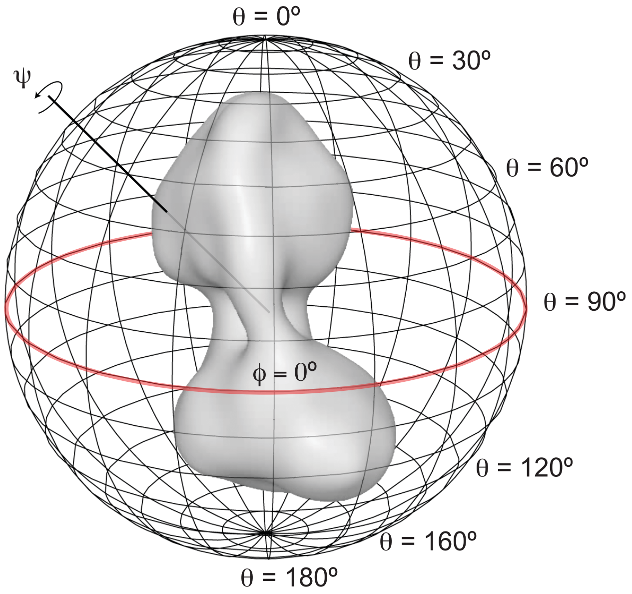
Mitra, K., *et al.* (2005). *Nature* **438**, 318-324.

Dealing with alignment problems to make models better

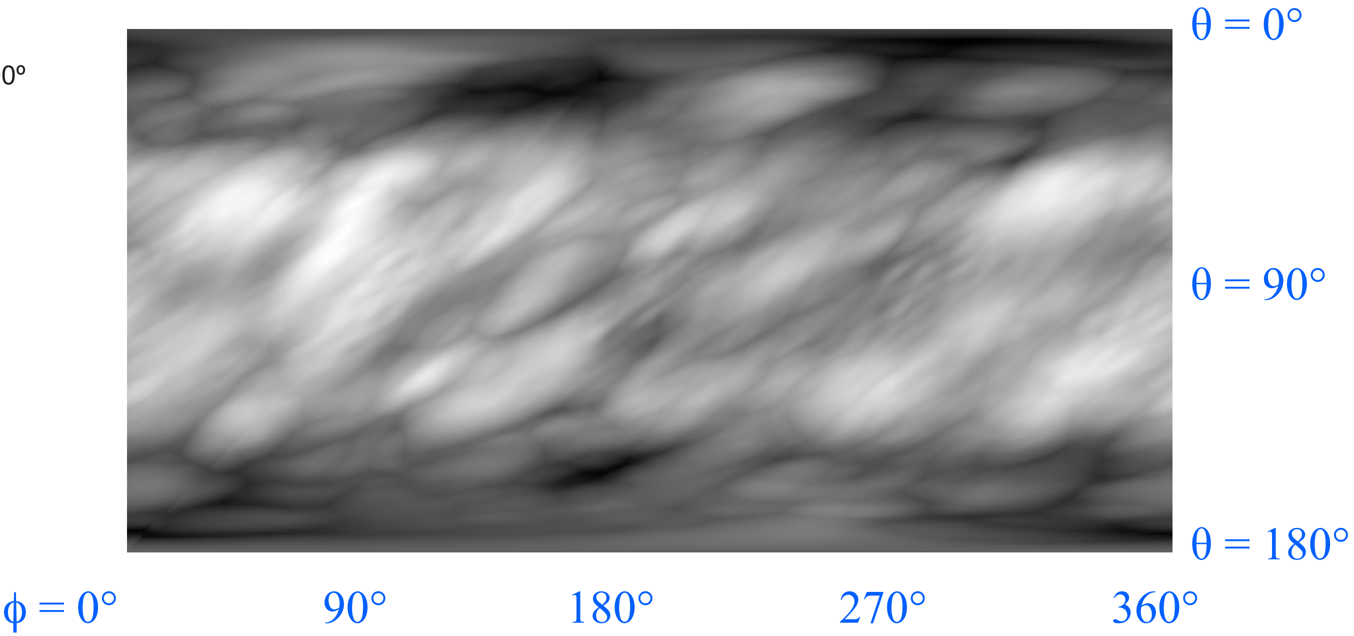
Accuracy of particle alignment



Accuracy of particle alignment



CC(θ,φ)



ψ, Δx, Δy all adjusted to give maximum cross-correlation

Optimizing alignments to improve resolution (L. Baker)

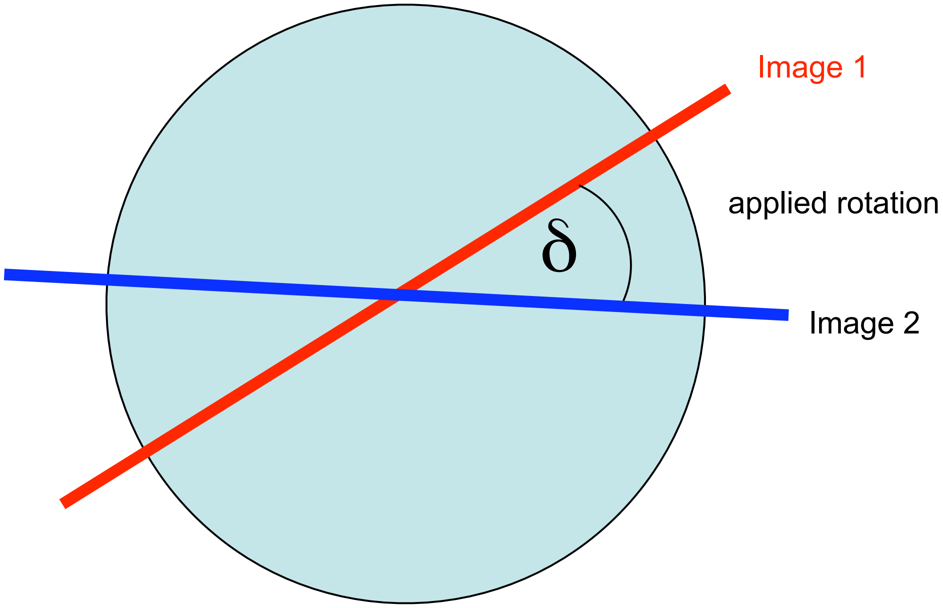


Image 1

$R_{\phi 1} R_{\Theta 1} R_{\psi 1} = R_{TOT1}$

Image 2

$R_{\phi 2} R_{\Theta 2} R_{\psi 2} = R_{TOT2}$

Ideally: $R_{\delta} R_{TOT1} = R_{TOT2}$

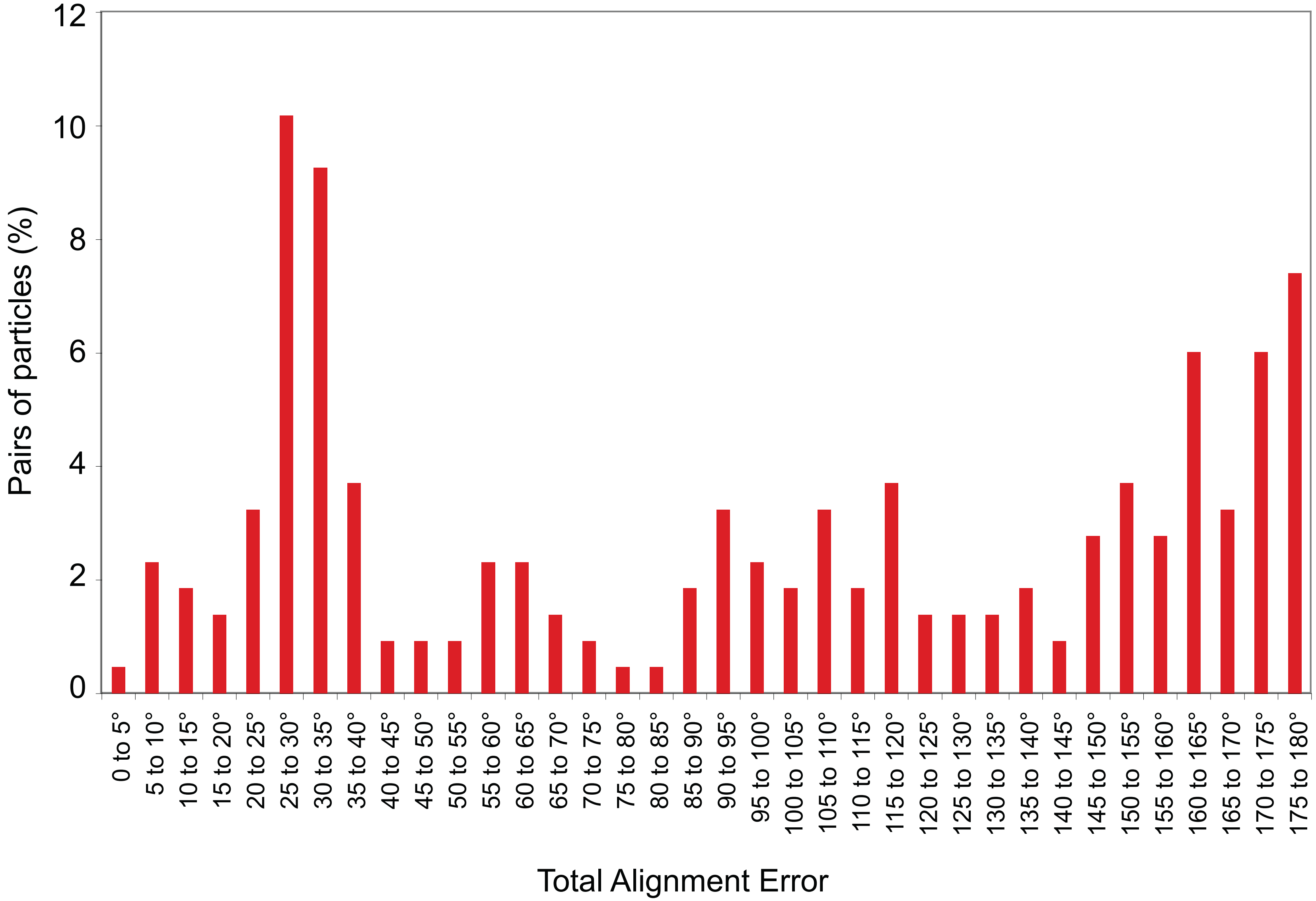
In practice: $R_{error} R_{\delta} R_{TOT1} = R_{TOT2}$

Stringent test: if either particle image misaligns, the pair will have a large R_{error}

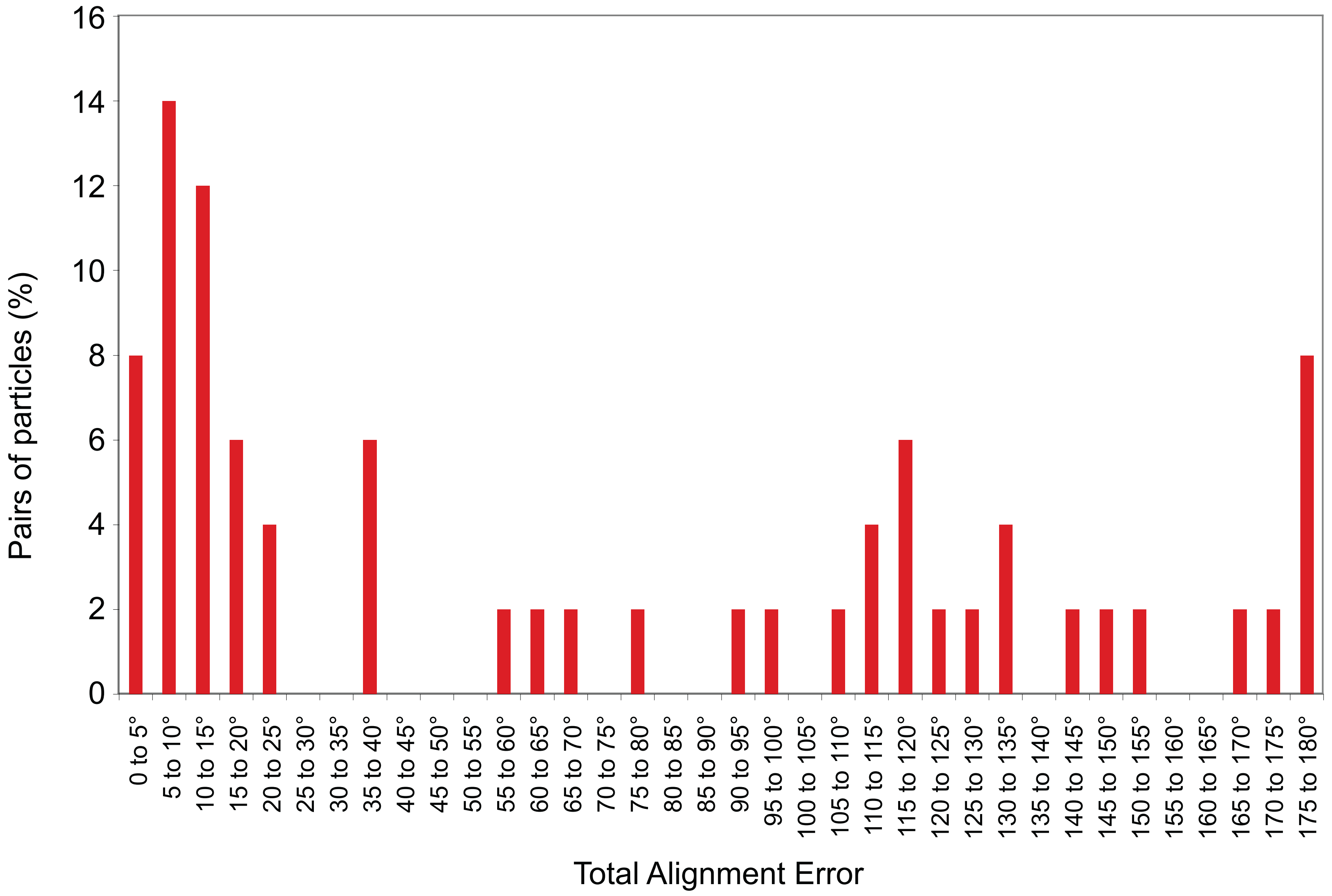
Choose alignment conditions (parameters, merit function, program) to minimize R_{error}

Inspired by: Rosenthal and Henderson (2003). *J Mol Biol* **333**, 721-45.

Alignment errors for bovine ATP synthase



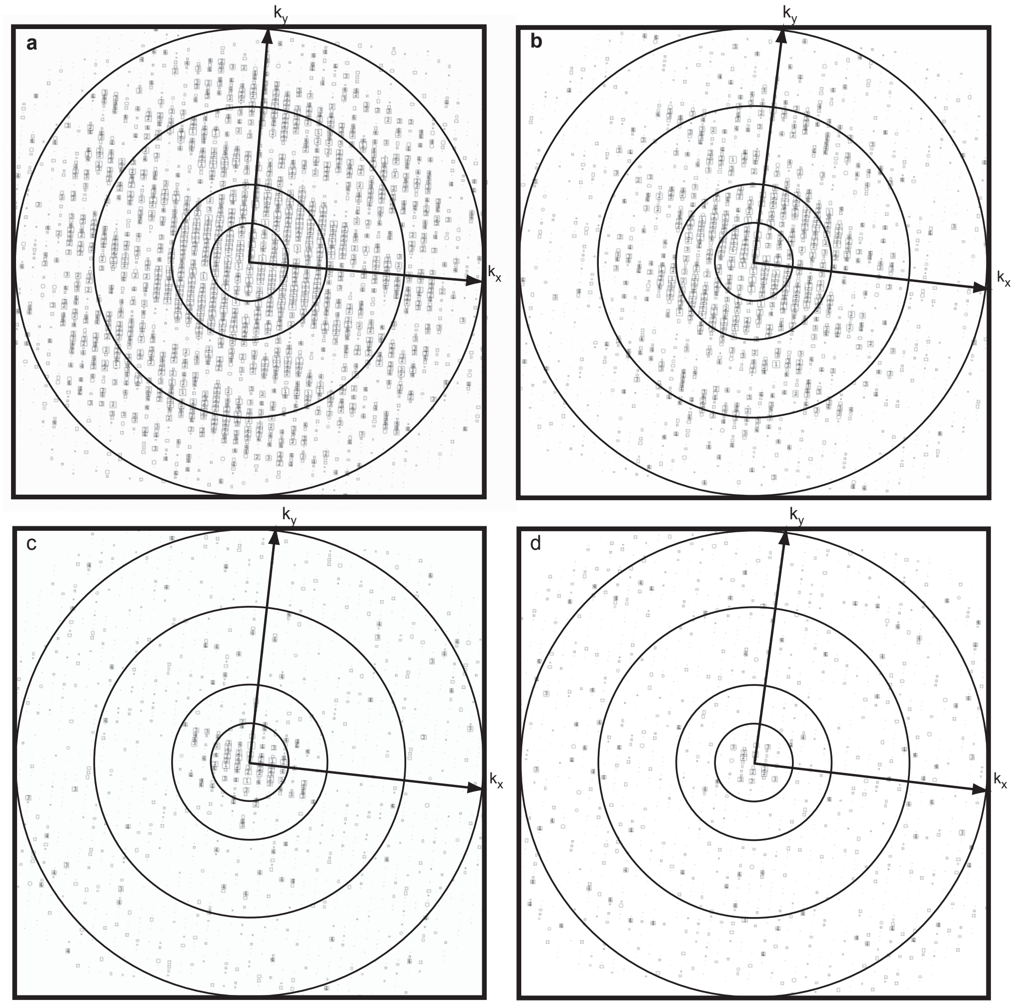
Alignment errors for *T. thermophilus* V-ATPase



Optimizing dose to improve alignment accuracy

Optimizing signal-to-noise ratios in images

0-8 $e^-/Å^2$ 8-16 $e^-/Å^2$



46-54 $e^-/Å^2$ 78-96 $e^-/Å^2$

$$\left|f_{\vec{k}}(N)\right|^2 = \left|f_{\vec{k}}(0)\right|^2 e^{\frac{-N}{N_e(\vec{k})}}$$

- Critical Dose, N_e, is dose at which intensity fades to 1/e times initial value (Unwin and Henderson, 1975)

- Optimal dose, N_{opt}, in <u>imaging</u> is ~2.5 x Critical dose, N_e (Hayward and Glaeser, 1979)

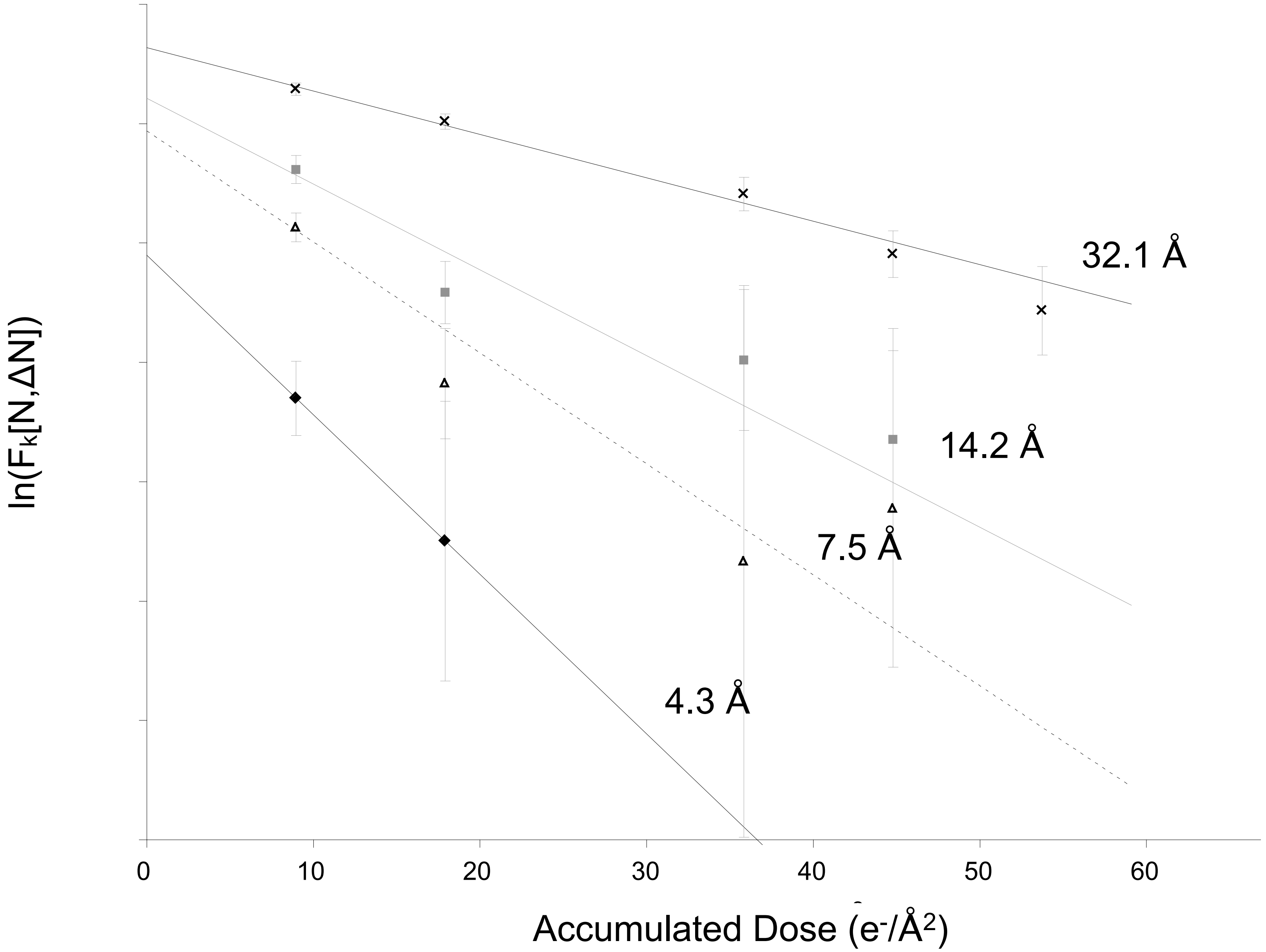
Baker, Smith, Bueler and Rubinstein, Unpublished

At 200 kV and 50,000 x magnification, a dose of ~ 12 $e^-/Å^2$ on the specimen focused to an image captured on SO-163 film will give an OD of ~1.0 (12 min. in D19)

If you use more electrons:
1) Higher signal-to-noise ratios at low spatial frequencies may allow more accurate alignment and improve your model resolution
2) Lower signal-to-noise ratios at high spatial frequencies may limit your model resolution

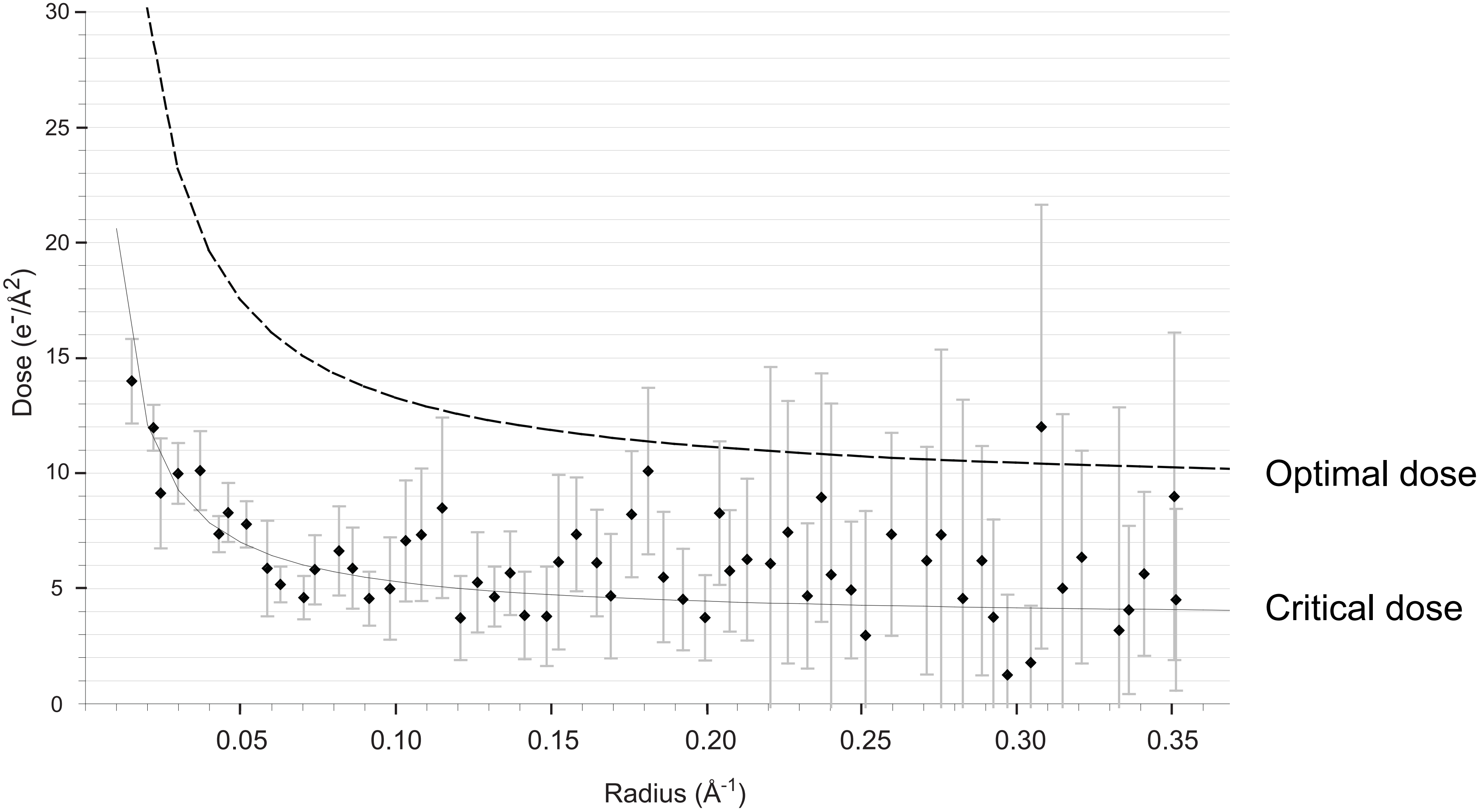
If you use fewer electrons:
1) Better signal-to-noise ratios at high spatial frequencies may improve your model resolution
2) Lower signal-to-noise ratios at low spatial frequencies may limit your alignment accuracy and limit your model resolution

Optimizing signal-to-noise ratios in images



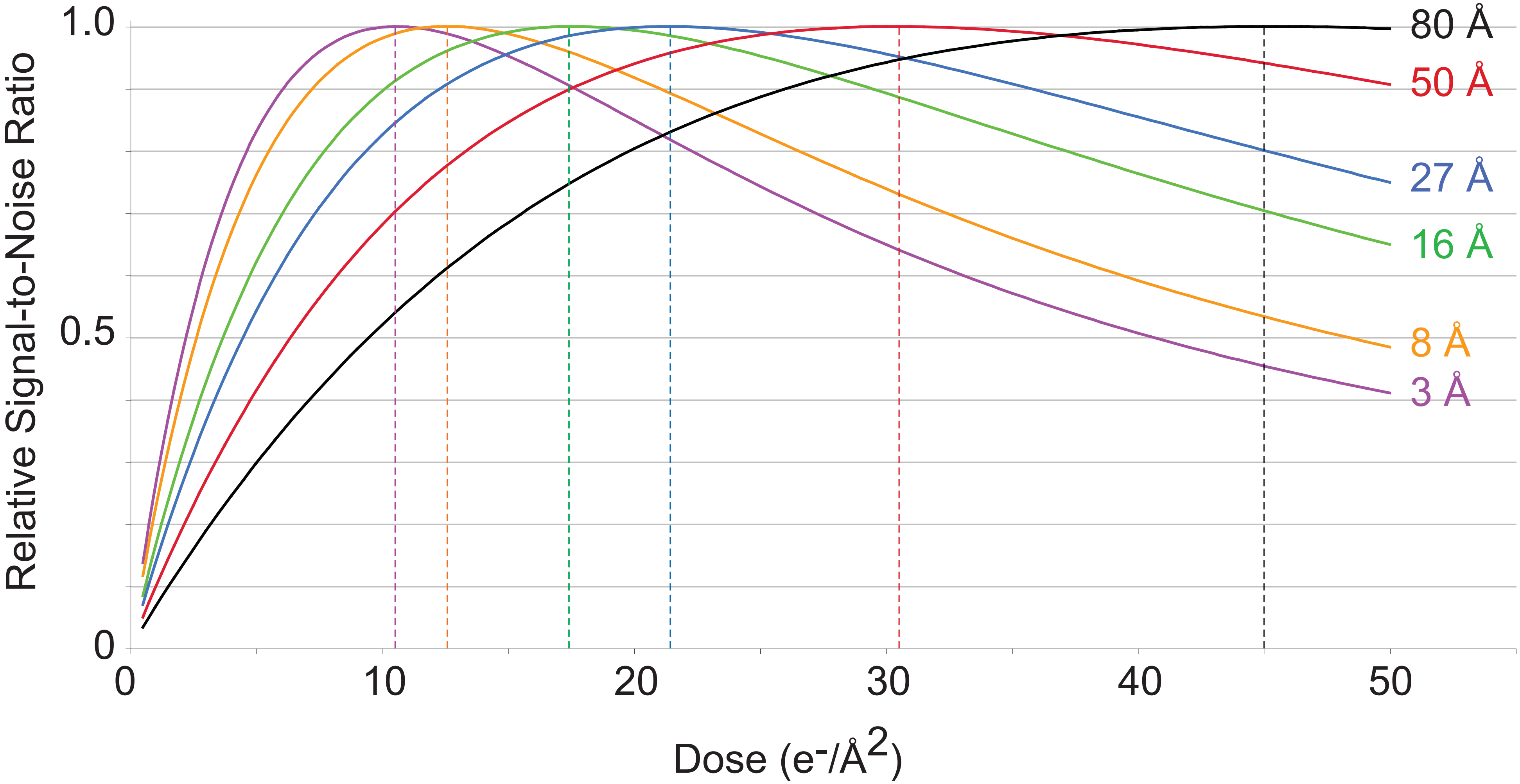
Baker, Smith, Bueler and Rubinstein, Unpublished

Optimizing signal-to-noise ratios in images



Baker, Smith, Bueler and Rubinstein, Unpublished

Optimizing signal-to-noise ratios in images

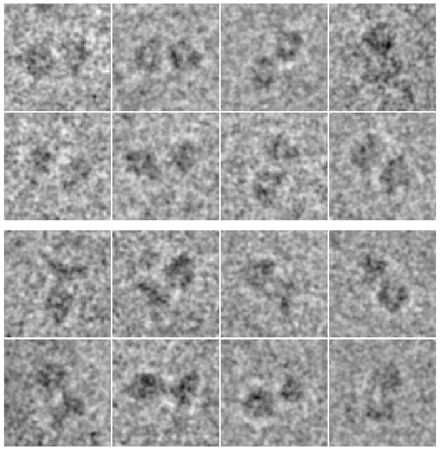


Baker, Smith, Bueler and Rubinstein, Unpublished

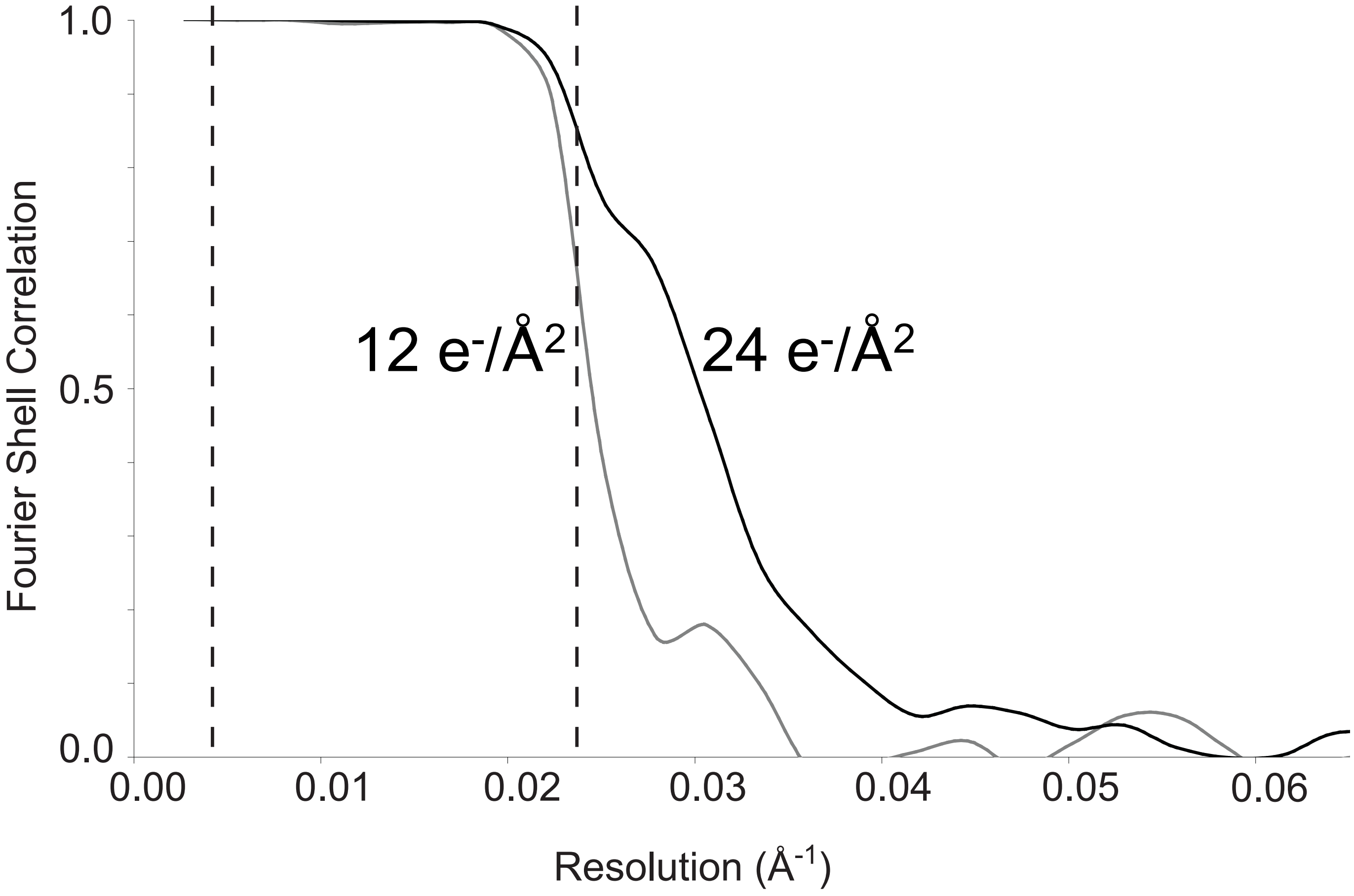
Aligning particles acquired at different doses

- From 5000 particle images:

12 e$^-$/Å^2



24 e$^-$/Å^2



Overview of lecture

- Introduction to detergents
- Alternatives to using detergents
  - Amphipols
  - RSC single particle EM
  - Single particles on membranes
- Preparing specimens
  - Negative stain EM of proteins in detergents
  - Cryo-EM of proteins in detergents
- Interpreting images of proteins-detergent complexes
  - Theory
  - Practice
  - Some nice examples
- What does challenging mean for image alignment
- Monitoring alignment accuracy
- Choosing electron dose to get the images you need

Acknowledgements:

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