Study Meeting 5:
The grid & samples in ice

Zuben P. Brown & Prikshat Dadhwal

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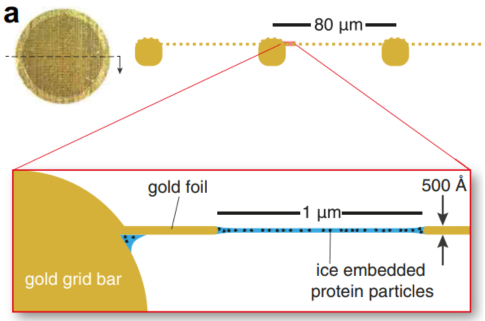
IN THE CITY OF NEW YORK

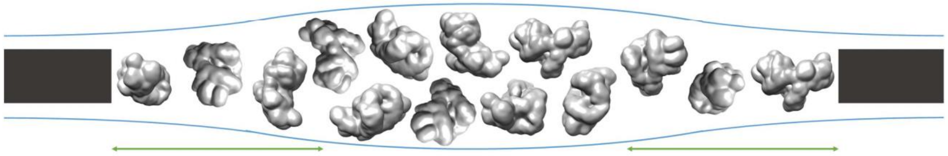
Three interrelated topics

- The specimen support
  - Gold grids
  - Nanowire grids



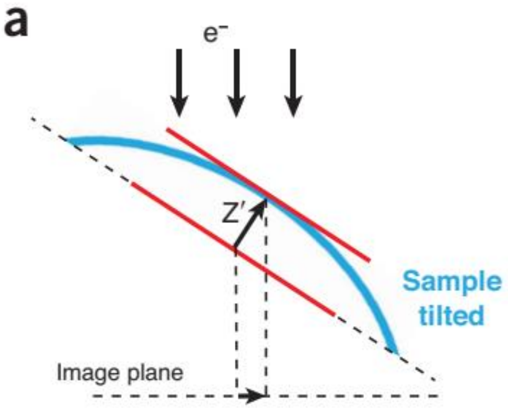
Russo & Passmore (2016)
J. Struc. Bio. 193:33-44

- The sample in ice
  - Description
  - Air-water interface



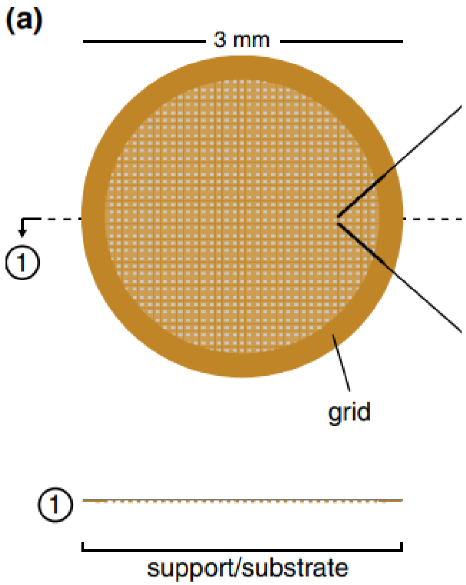
Noble *et al.* (2018)
elife, 7:e34257

- Electron-specimen interactions (next time)



Zheng *et al.* (2017)
Nat. Meth. 14(4):331

Specimen support



Grid materials

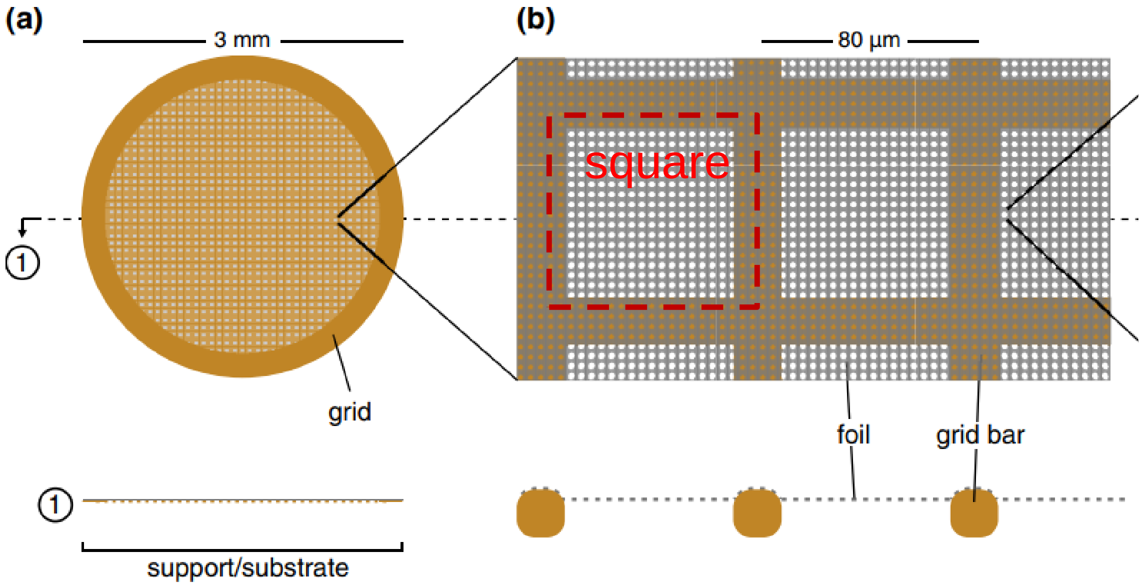
Copper
Nickel
Titanium
Silicon

Gold
CuRh
Molybdenum
Aluminum
Tungsten

- “products are fully specified by 4 parameters”
- Hole diameter, pitch of the foil & material type & mesh type

Russo & Passmore, (2016)
Curr. Opin. Struc. Bio. 37:81-89

Specimen support



Grid materials

Copper
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Foil materials

Amorphous carbon C-flat
Gold
TiSi
SiO_2
SiN
SiC

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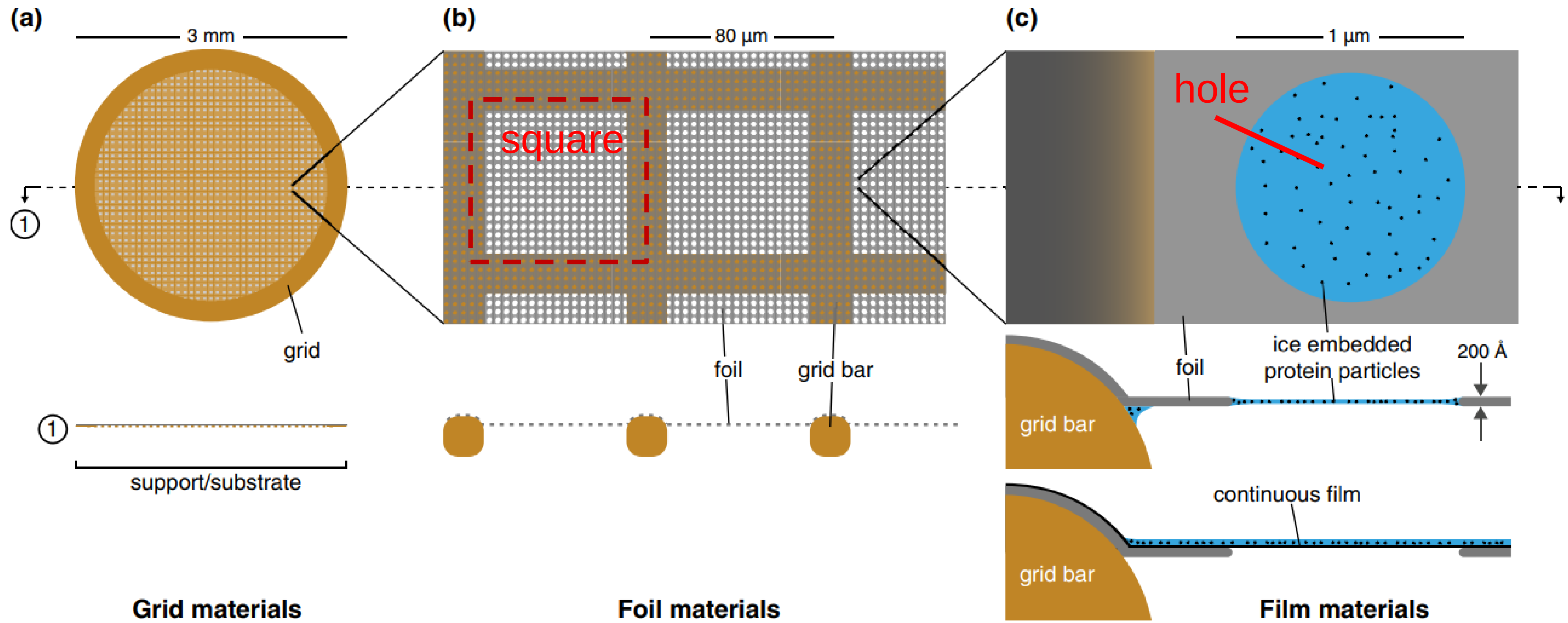

Grid materials

- **Copper**
- Nickel
- Titanium
- Silicon
- **Gold**
- CuRh
- Molybdenum
- Aluminum
- Tungsten

Foil materials

- **Amorphous carbon** C-flat
- Gold
- TiSi
- SiN
- SiO_2
- SiC

Film materials

- **Amorphous carbon**
- Graphene
- Graphene oxide
- TiSi

- “products are fully specified by 4 parameters”
- Hole diameter, pitch of the foil & material type & mesh type

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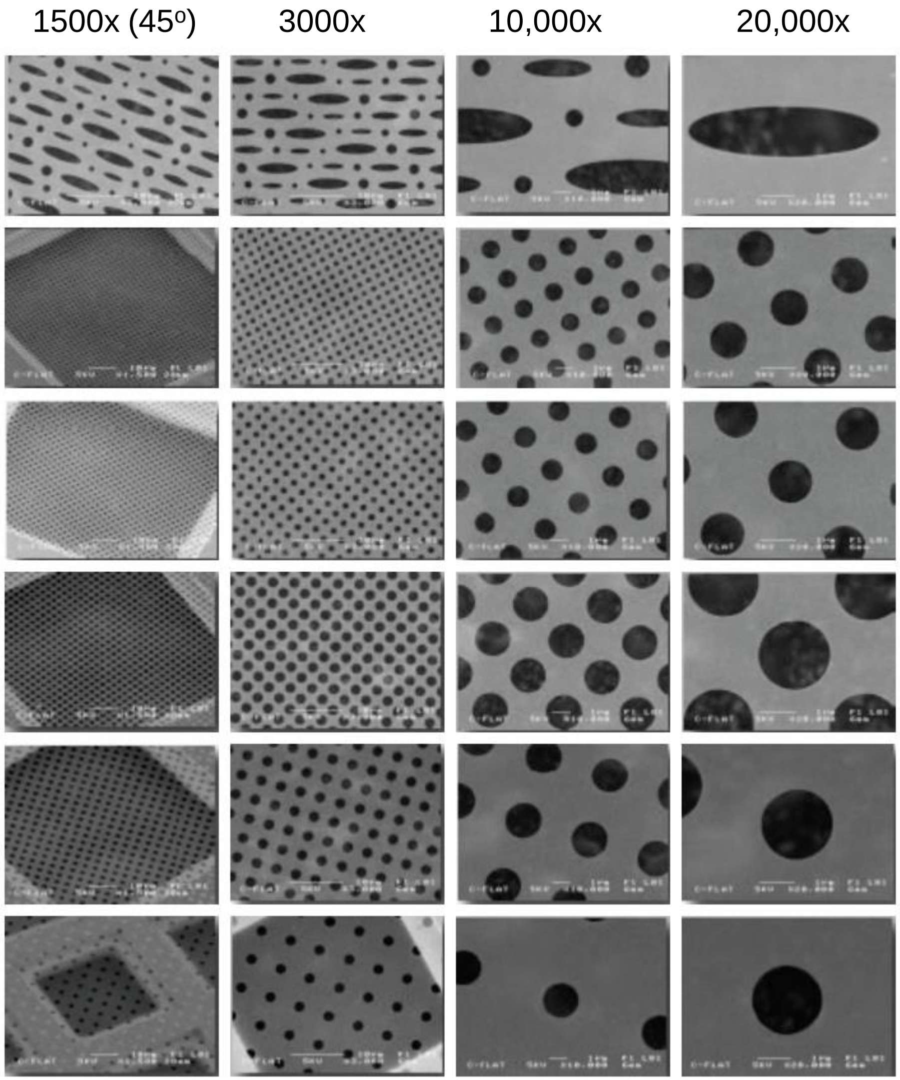
C-flat



CF-MH-2C
CF-MH-4C multi hole and space

CF-1/1-2C
CF-1/1-4C 1.0µm hole, 1.0 µm space

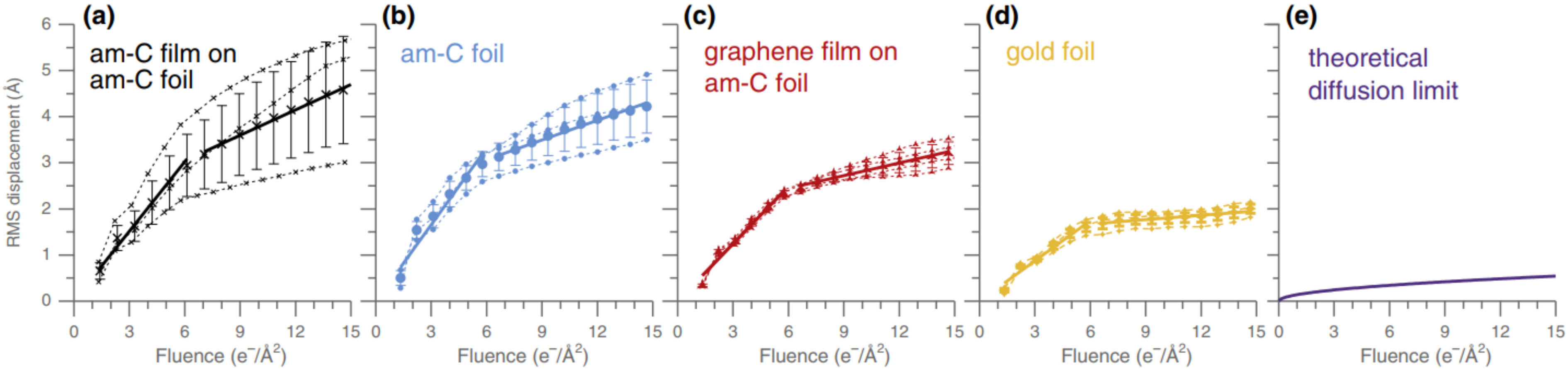
CF-1.2/1.3-2C
CF-1.2/1.3-4C 1.2µm hole, 1.3 µm space

CF-2/1-2C
CF-2/1-4C 2.0µm hole, 1.0µm space

CF-2/2-2C
CF-2/2-4C 2.0µm hole, 2.0µm space

CF-2/4-2C
CF-2/4-4C 2.0µm hole, 4.0µm space

Movement

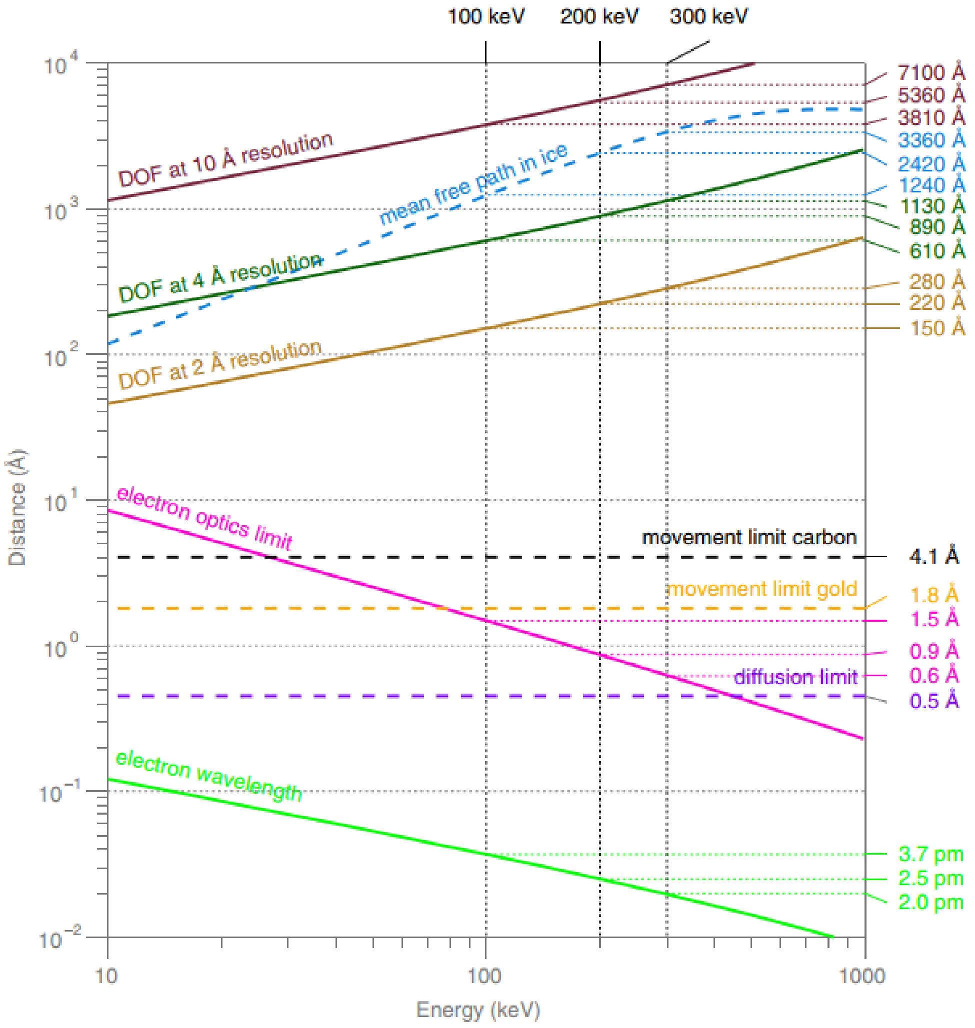


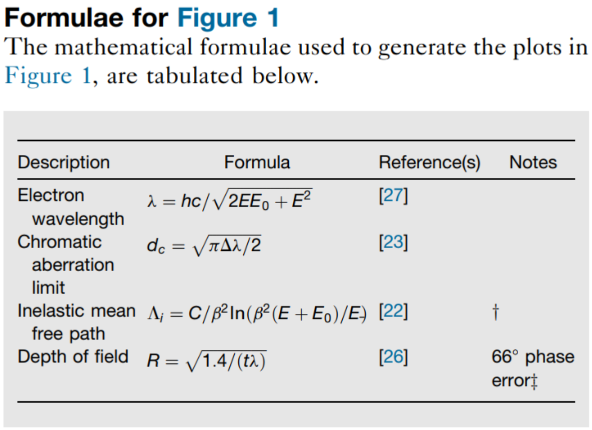
Diffusion limit: "Using the measured variation in the power spectra amplitude with number of electrons per image we deduce that water molecules are randomly displaced by a mean squared distance of ~1.1 Å^2 for every incident 300 keV e/Å^2. … The beam-induced movement of the water molecules generates pseudo-Brownian motion of embedded macromolecules"

[McMullan, Vinothkumar, Henderson (2015) Ultramic. 158:26-32]

Russo & Passmore, (2016)
Curr. Opin. Struc. Bio. 37:81-89

Different hole spacing





Formulae for Figure 1

The mathematical formulae used to generate the plots in Figure 1, are tabulated below.

Description	Formula	Reference(s)	Notes
Electron wavelength	$\lambda = hc/\sqrt{2EE_0 + E^2}$	[27]	
Chromatic aberration limit	$d_c = \sqrt{\pi\Delta\lambda/2}$	[23]	
Inelastic mean free path	$\Lambda_i = C/\beta^2 \ln(\beta^2(E + E_0)/E)$	[22]	†
Depth of field	$R = \sqrt{1.4/(t\lambda)}$	[26]	66° phase error‡

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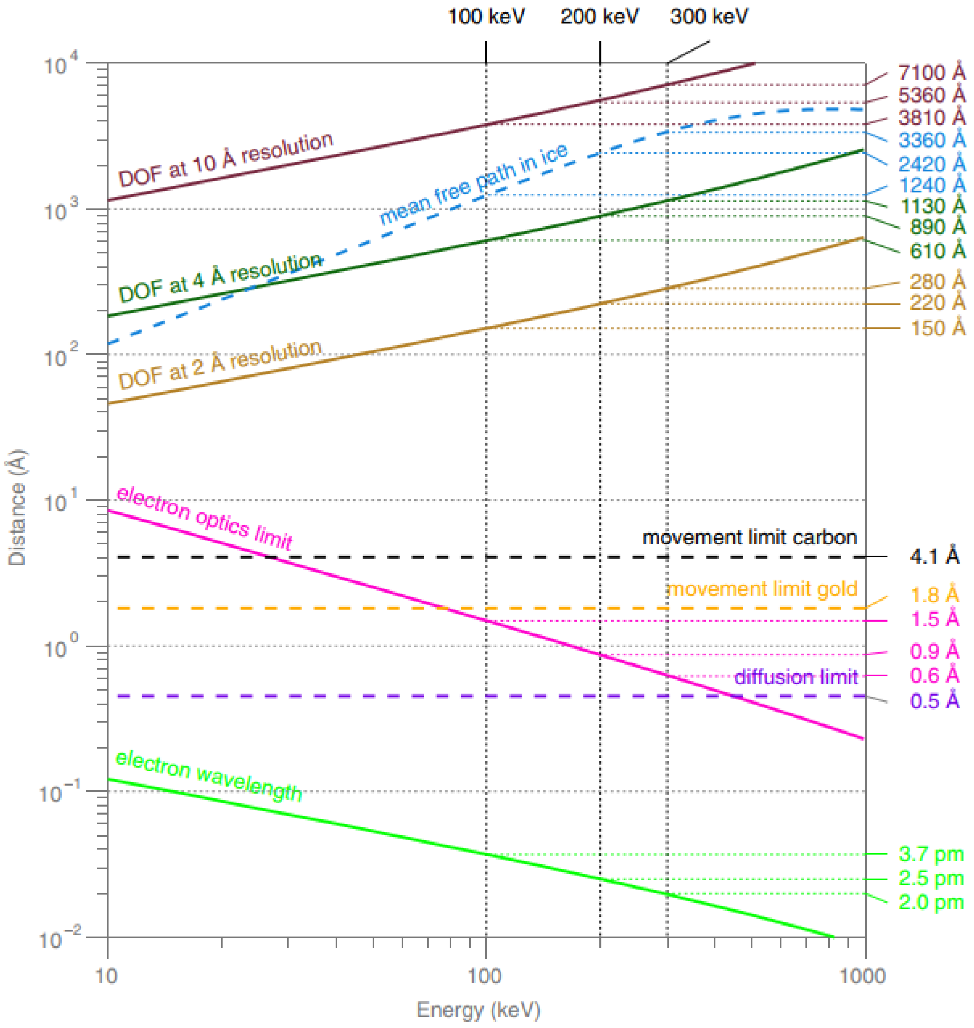

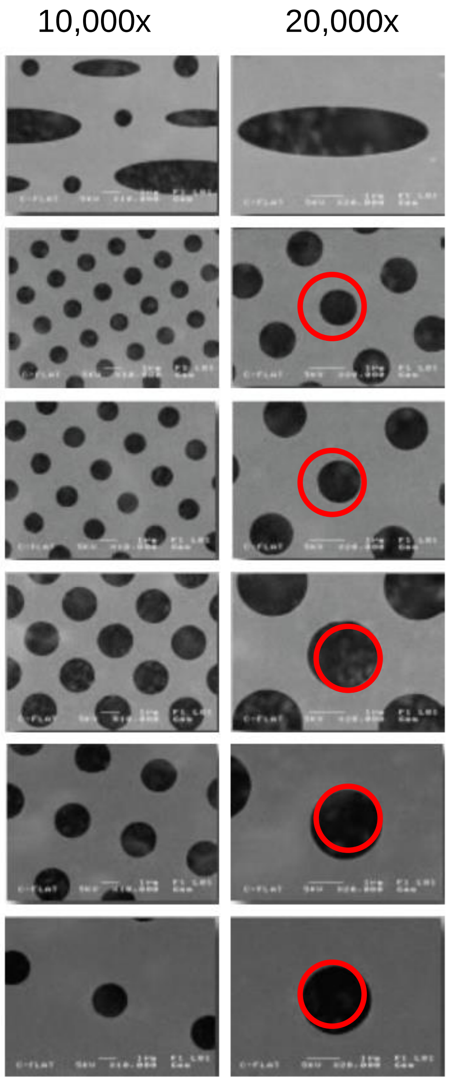

Different ratio of ice:foil

Russo & Passmore, (2016)
Curr. Opin. Struc. Bio. 37:81-89

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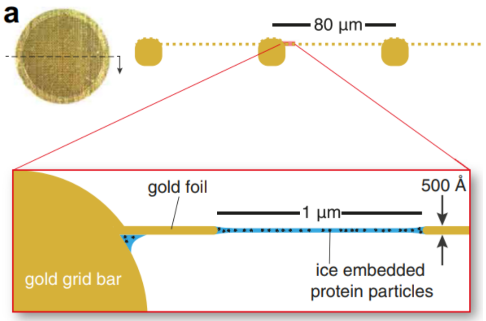
Question

- Does the grid influence movement?
  - Need to balance with protein concentration & behaviour etc
- Could we test this by looking at the MotionCor outputs and seeing movement for different grids?
  - Does anyone have any of these files they would be willing to share?

Three interrelated topics

- The specimen support
  - Gold grids
  - Nanowire grids



Russo & Passmore (2016)
J. Struc. Bio. 193:33-44

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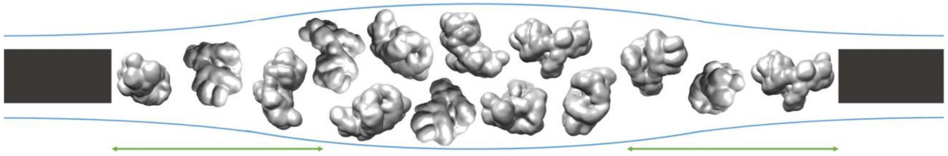
  - Description
  - Air-water interface



Noble *et al.* (2018)
elife, 7:e34257

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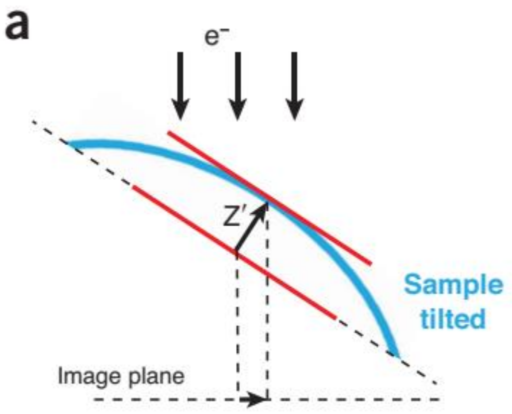

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The specimen-support

- Three general parameters:
  1. Air-water interface
  2. Bulk particle behaviour
  3. Ice thickness

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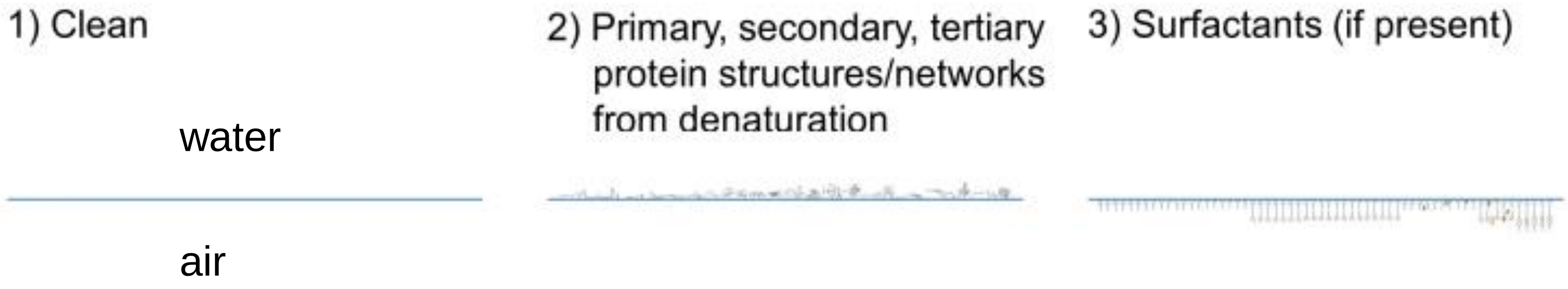
  1. **Air-water interface**
  2. Bulk particle behaviour
  3. Ice thickness



Noble *et al.* (2018)
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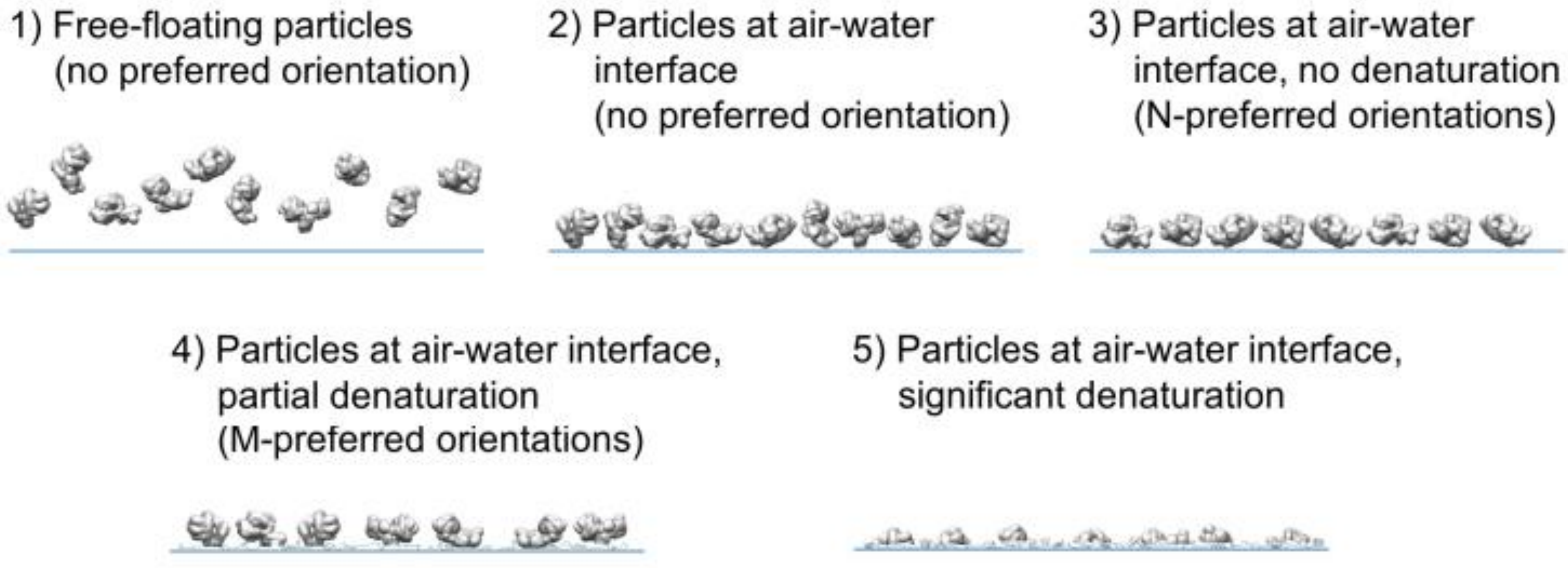
The specimen-support

- Three general parameters:
    1. Air-water interface
    2. **Bulk particle behaviour**
    3. Ice thickness



1) Free-floating particles (no preferred orientation)

2) Particles at air-water interface (no preferred orientation)

3) Particles at air-water interface, no denaturation (N-preferred orientations)

4) Particles at air-water interface, partial denaturation (M-preferred orientations)

5) Particles at air-water interface, significant denaturation

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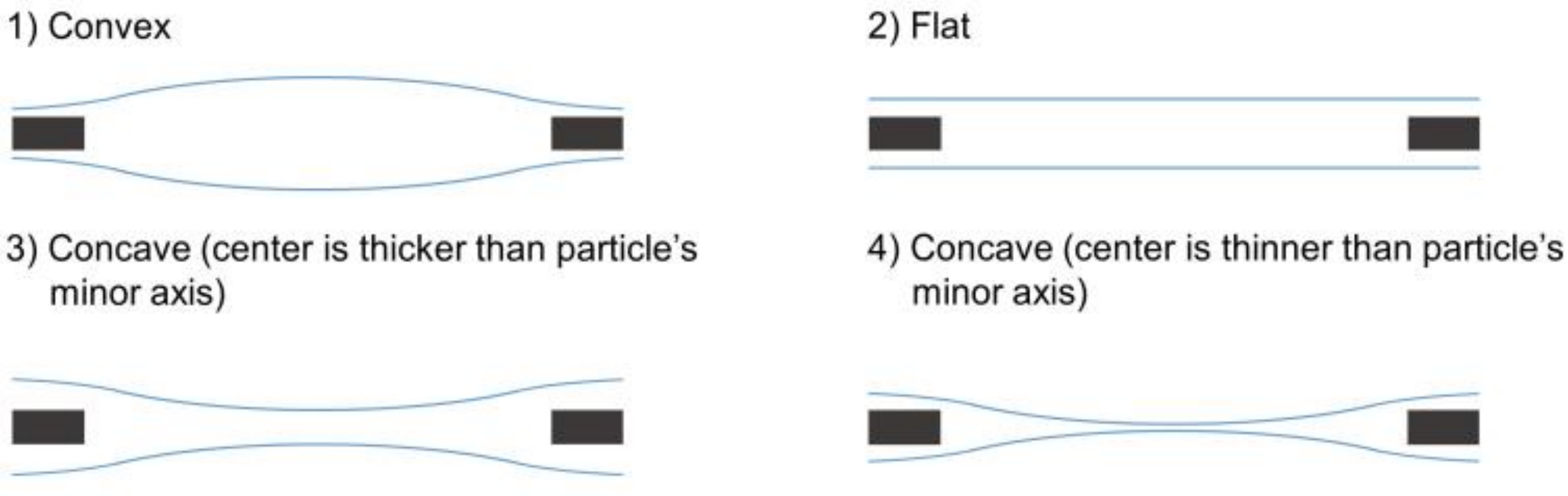
The specimen-support

- Three general parameters:
  1. Air-water interface
  2. Bulk particle behaviour
  3. **Ice thickness**



1) Convex

2) Flat

3) Concave (center is thicker than particle's minor axis)

4) Concave (center is thinner than particle's minor axis)

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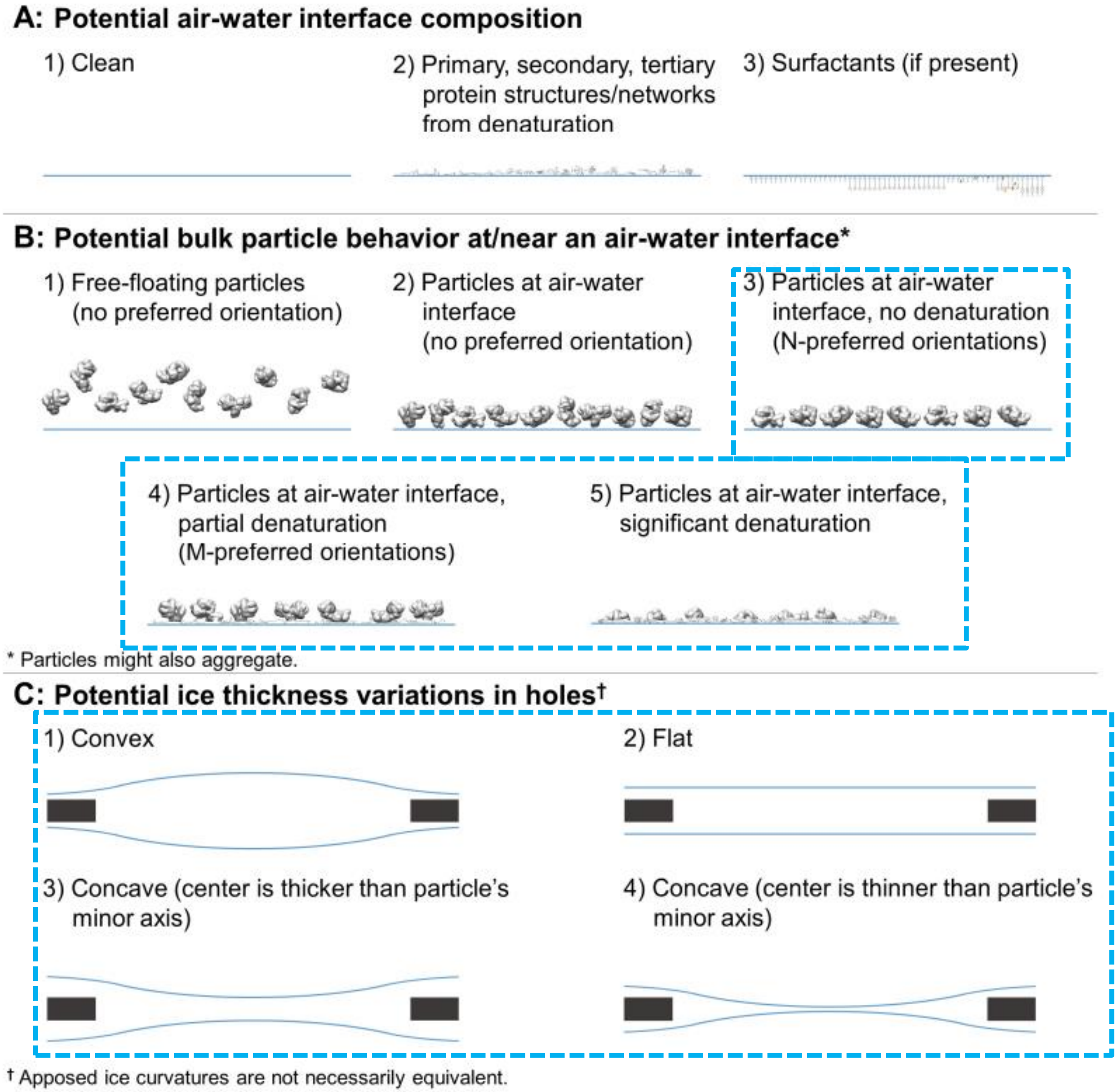
90% of proteins near AWI



Looked at over 1000 holes with tomography

90% of all particles are within 5-10nm of the air-water interface

(we'll come back to this)

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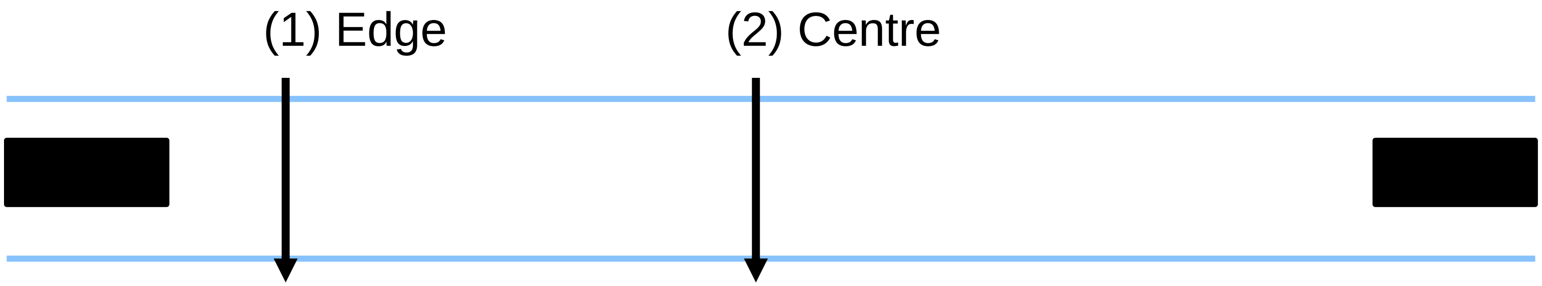
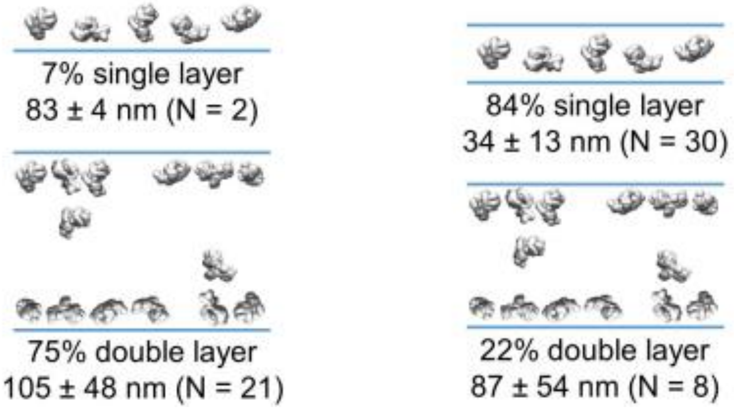
Ice thickness & protein spatial arrangement



(1) Edge

(2) Centre

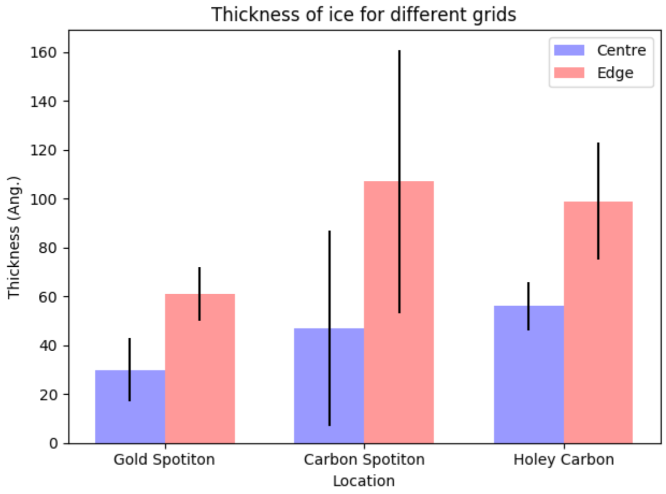


7% single layer
83 ± 4 nm (N = 2)

75% double layer
105 ± 48 nm (N = 21)

84% single layer
34 ± 13 nm (N = 30)

22% double layer
87 ± 54 nm (N = 8)



Thinner ice in the centre, thicker at the edges

Single layer of protein in thinner regions

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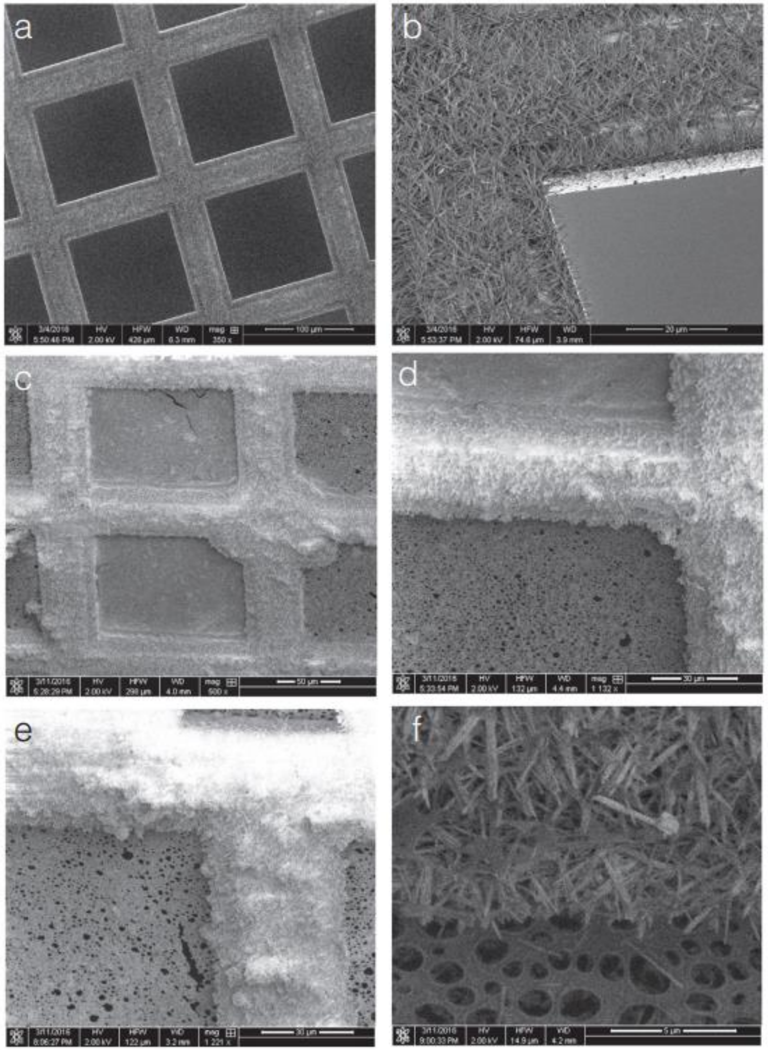
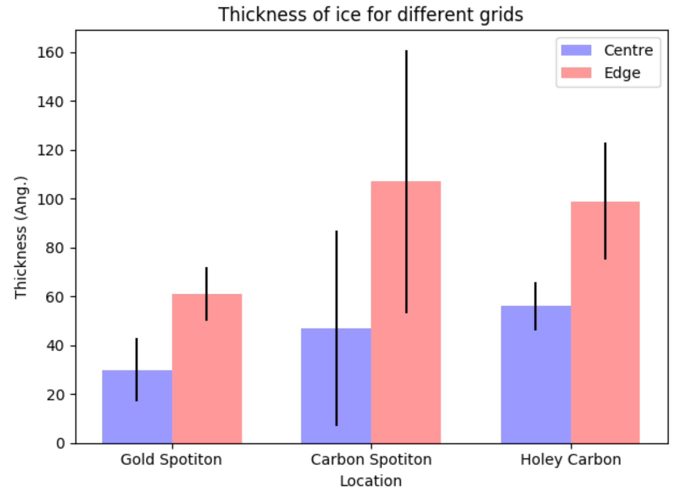
Best supports for thin ice…





Noble *et al.* (2018)
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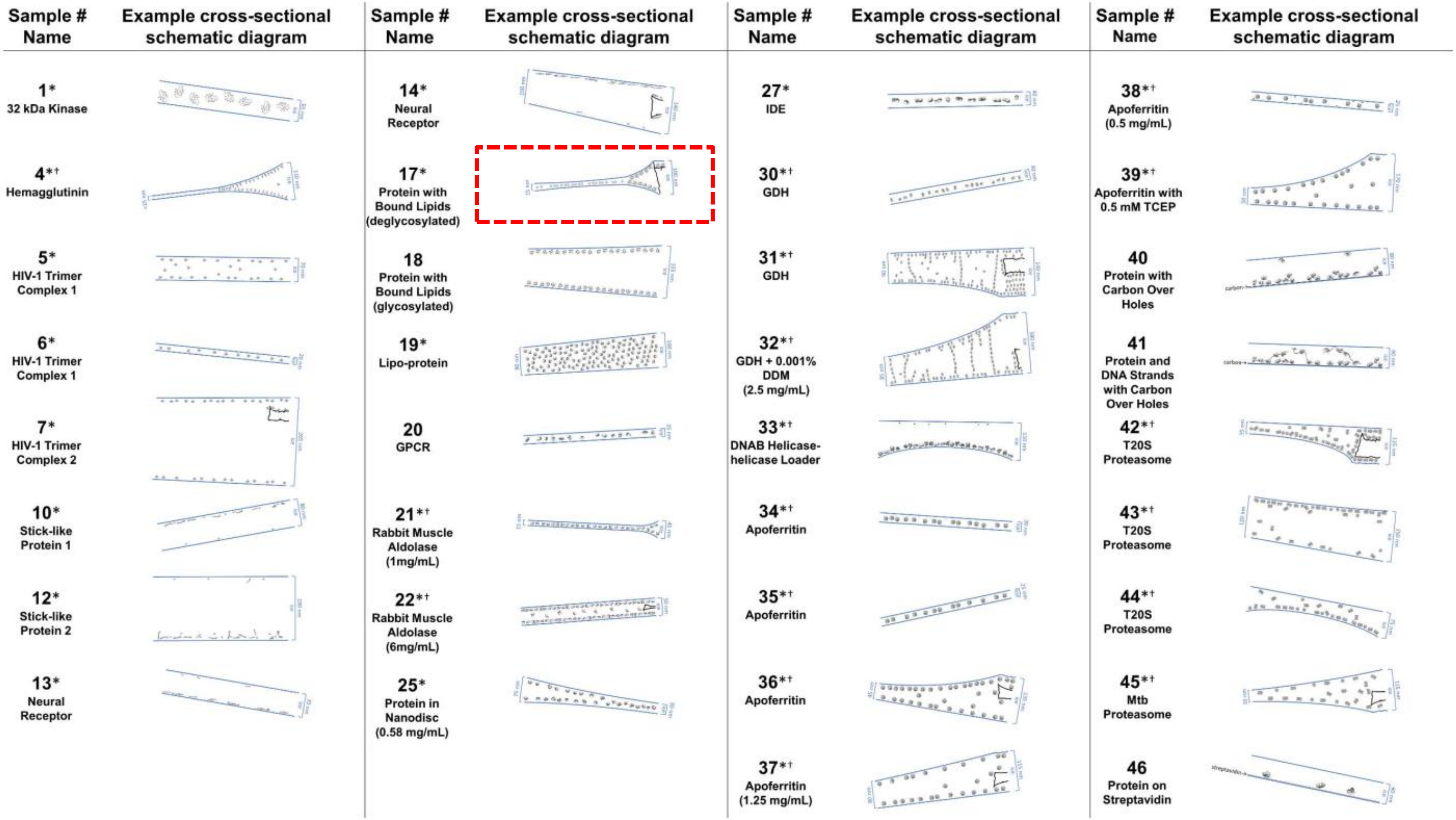
Razinkov *et al.*(2016)
J. Struc. Bio. 195:190-198

Spatial arrangement is variable



Sample # Name	Example cross-sectional schematic diagram	Sample # Name	Example cross-sectional schematic diagram	Sample # Name	Example cross-sectional schematic diagram	Sample # Name	Example cross-sectional schematic diagram
1* 32 kDa Kinase		**14*** Neural Receptor		**27*** IDE		**38*†** Apoferritin (0.5 mg/mL)	
4*† Hemagglutinin		**17*** Protein with Bound Lipids (deglycosylated)		**30*†** GDH		**39*†** Apoferritin with 0.5 mM TCEP	
5* HIV-1 Trimer Complex 1		**18** Protein with Bound Lipids (glycosylated)		**31*†** GDH		**40** Protein with Carbon Over Holes	
6* HIV-1 Trimer Complex 1		**19*** Lipo-protein		**32*†** GDH + 0.001% DDM (2.5 mg/mL)		**41** Protein and DNA Strands with Carbon Over Holes	
7* HIV-1 Trimer Complex 2		**20** GPCR		**33*†** DNAB Helicase-helicase Loader		**42*†** T20S Proteasome	
10* Stick-like Protein 1		**21*†** Rabbit Muscle Aldolase (1mg/mL)		**34*†** Apoferritin		**43*†** T20S Proteasome	
12* Stick-like Protein 2		**22*†** Rabbit Muscle Aldolase (6mg/mL)		**35*†** Apoferritin		**44*†** T20S Proteasome	
13* Neural Receptor		**25*** Protein in Nanodisc (0.58 mg/mL)		**36*†** Apoferritin		**45*†** Mtb Proteasome	
				37*† Apoferritin (1.25 mg/mL)		**46** Protein on Streptavidin	

Noble *et al.* (2018)
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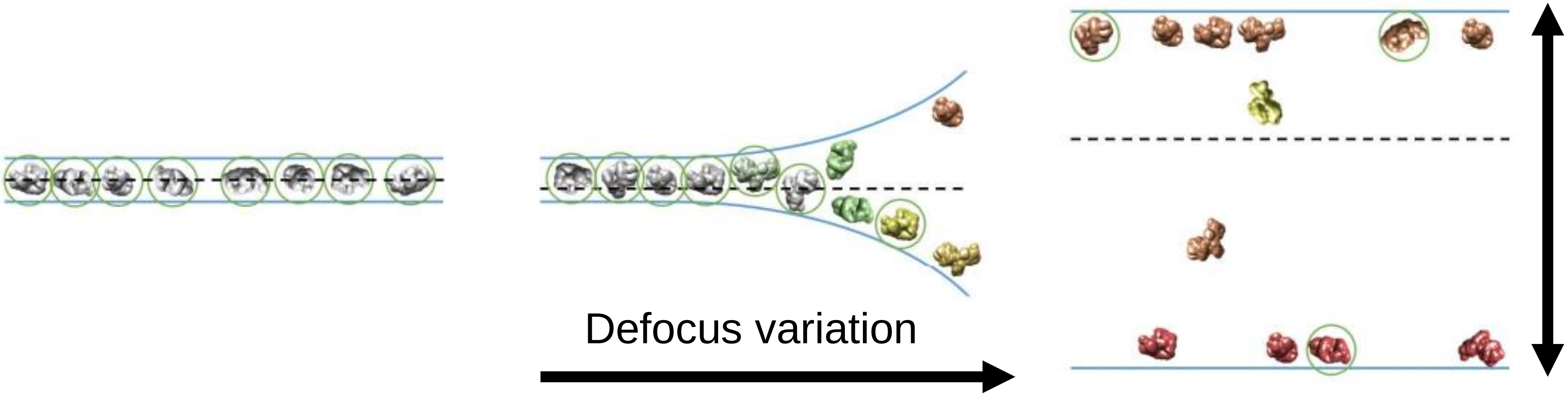
CTF estimation & particle position



Defocus variation

Noble *et al.* (2018)
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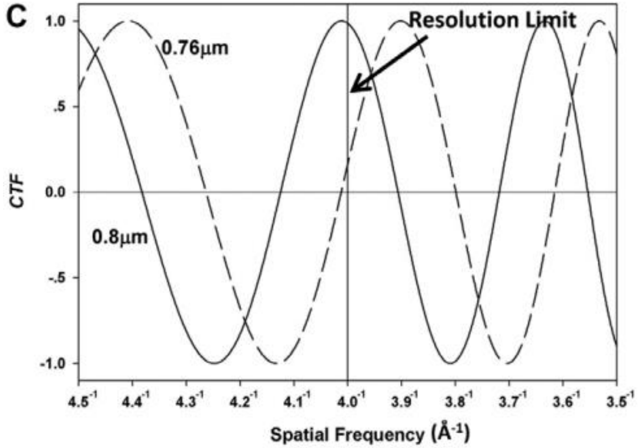

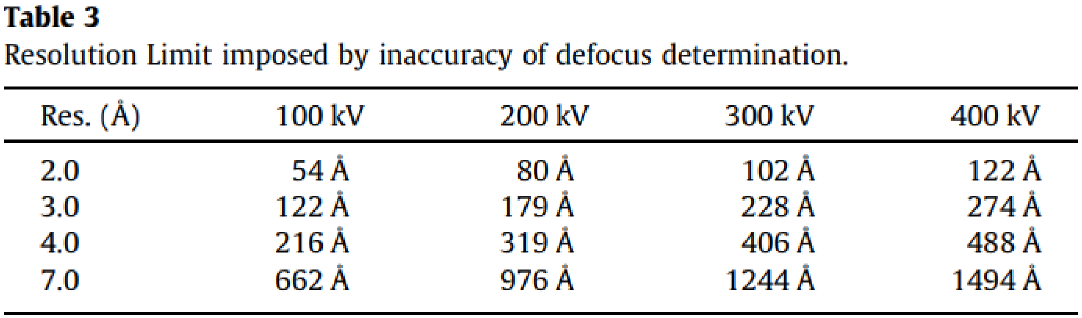

Table 3
Resolution Limit imposed by inaccuracy of defocus determination.

Res. (Å)	100 kV	200 kV	300 kV	400 kV
2.0	54 Å	80 Å	102 Å	122 Å
3.0	122 Å	179 Å	228 Å	274 Å
4.0	216 Å	319 Å	406 Å	488 Å
7.0	662 Å	976 Å	1244 Å	1494 Å

Zhang & Zhou (2011)
J. Struc. Bio. 175:253-263

Summary

- Ice thickness changes with edge/centre
- Proteins double layer
- 90% at AWI

- Tomography would give us an absolute range for particle positon
- Could we generalize it and use it to provide positional information?
- (if we had limitless scope time) could we collect single particle & tomography?

Three interrelated topics

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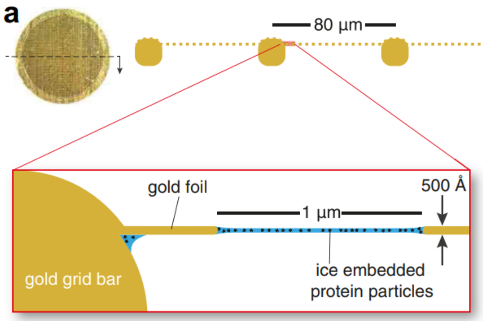
  - Nanowire grids



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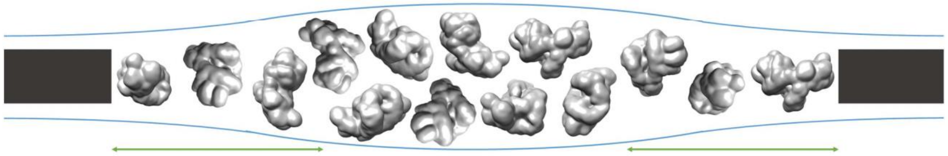

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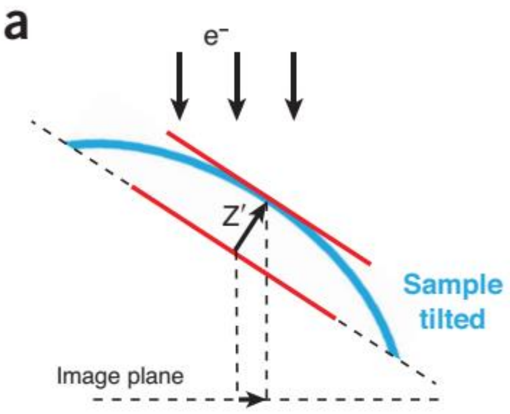

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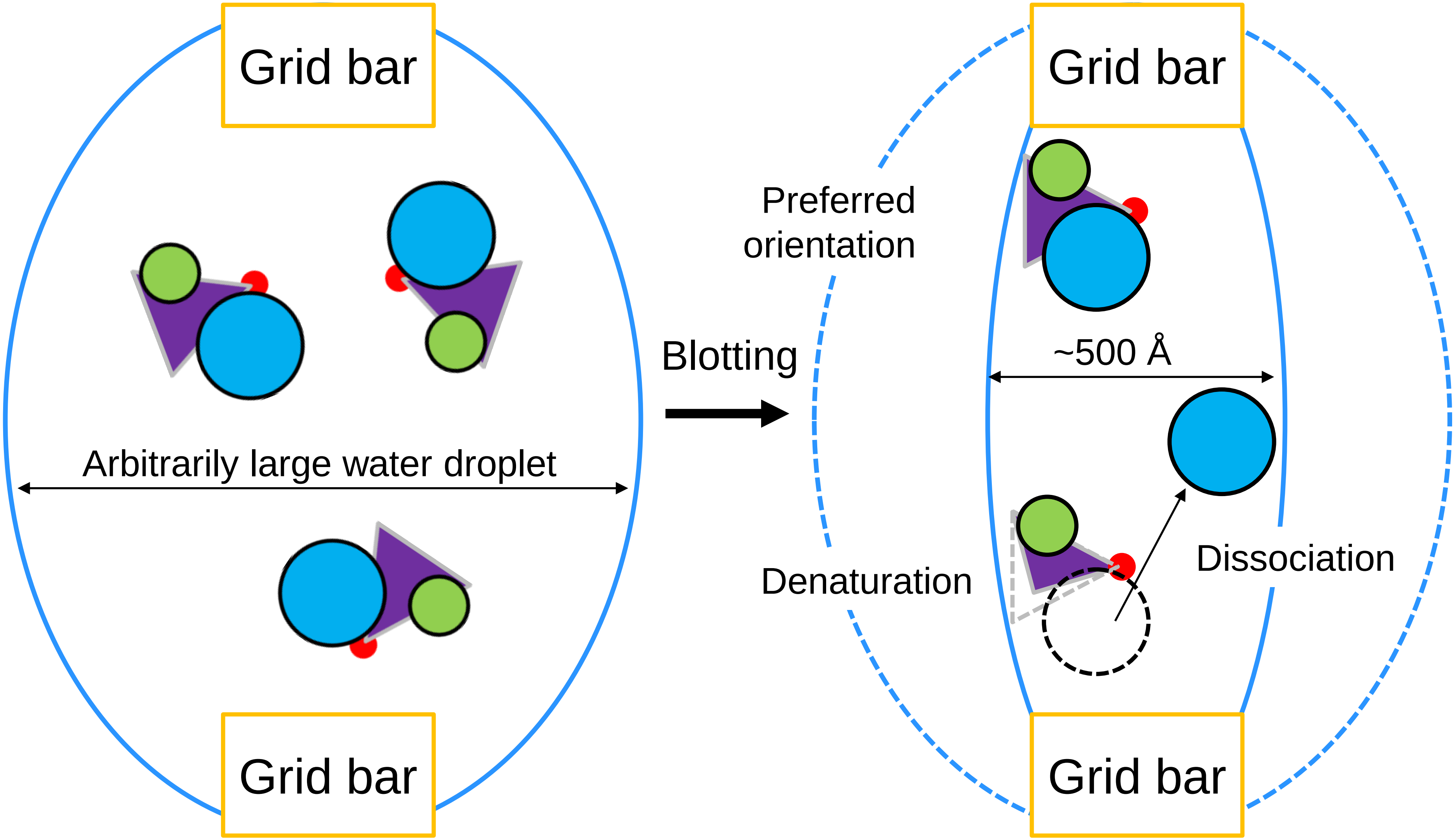
Exposure to air-water interface (AWI)



Grid bar

Arbitrarily large water droplet

Grid bar

Blotting

Preferred orientation

Grid bar

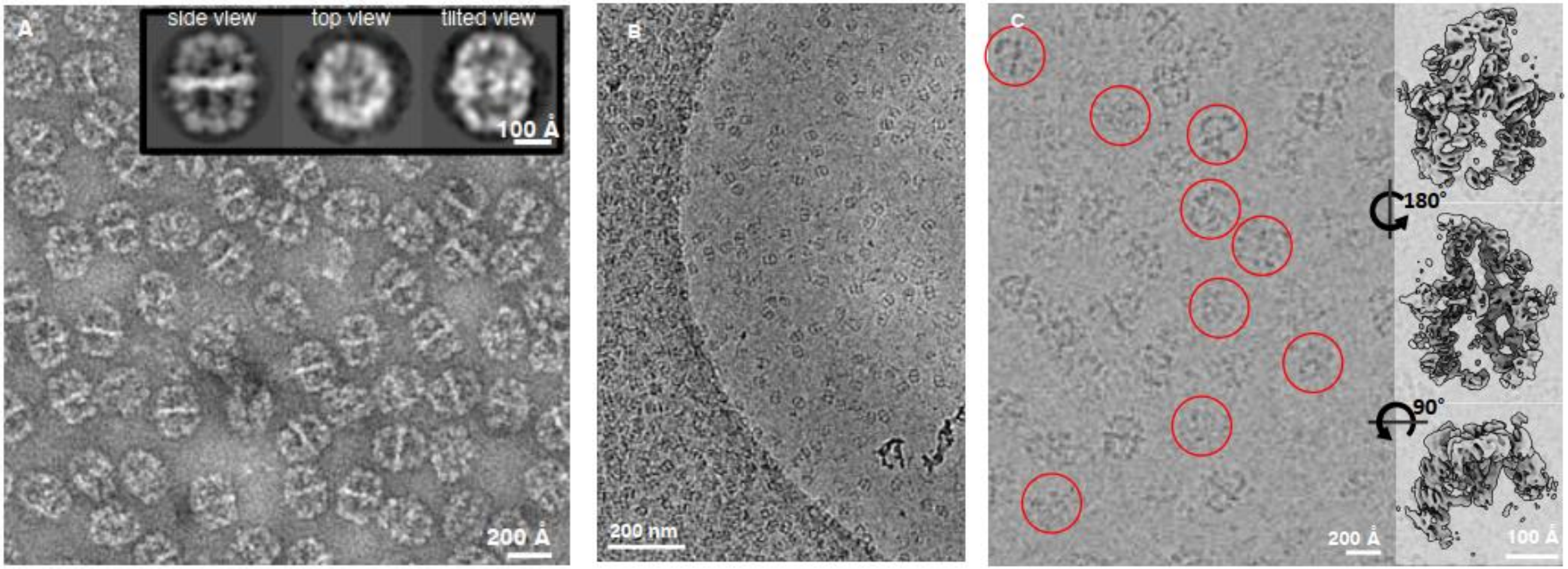
~500 Å

Denaturation

Dissociation

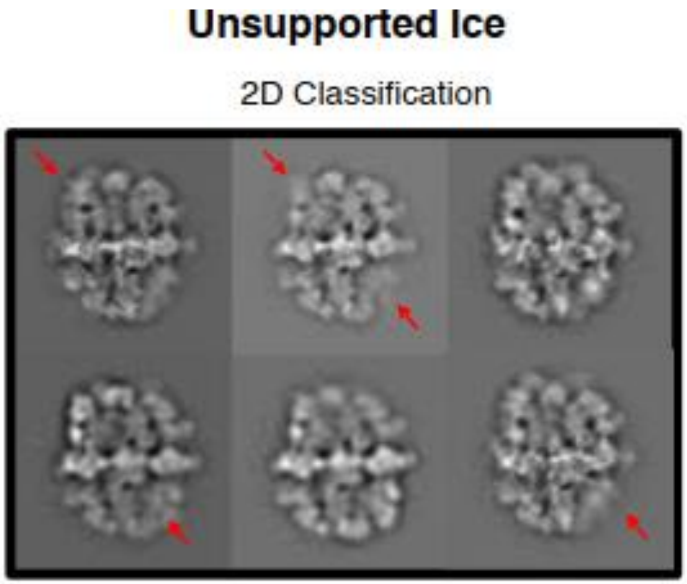
Grid bar

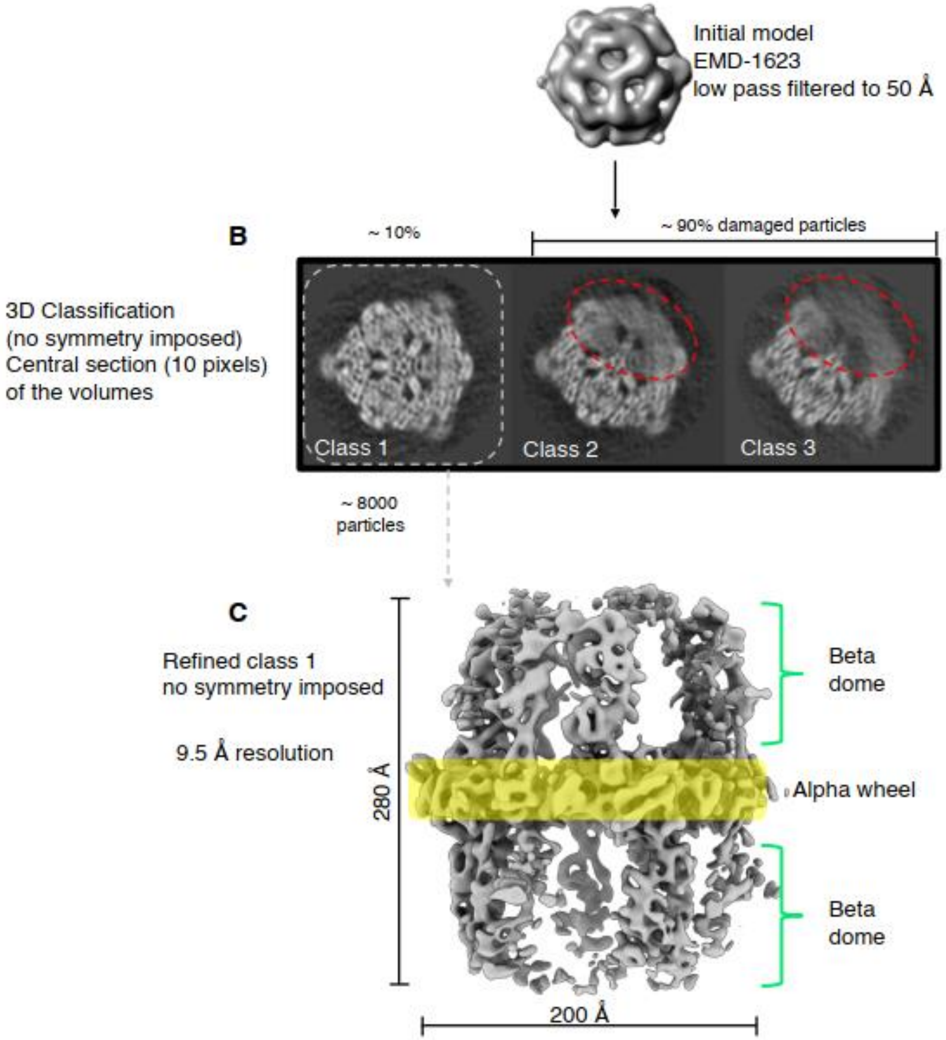
Negative stain vs. cryoEM



D’Imprima *et al*. (2018)
BioRxiv, doi.org/10.1101/400432

Majority of FAS are damaged

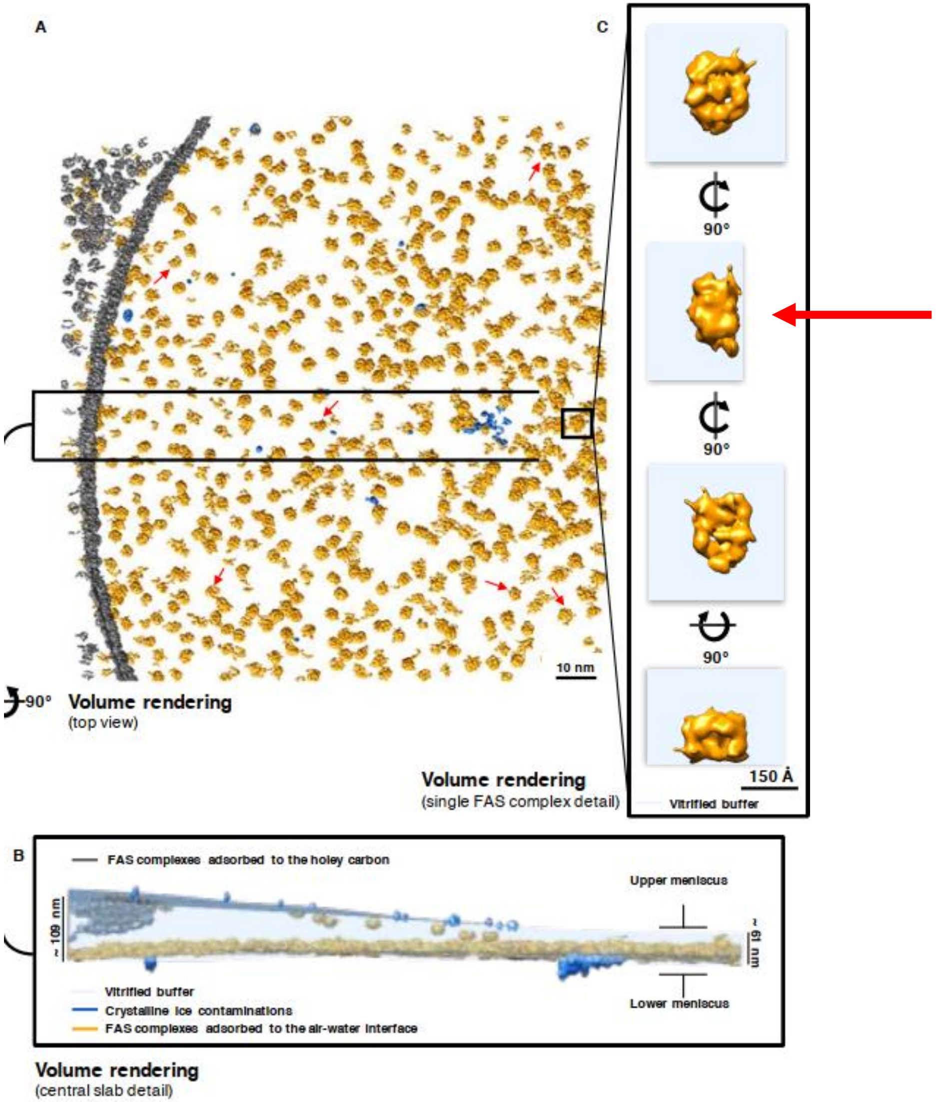




- 90% of particles damaged… where have we heard that before?

D’Imprima *et al.* (2018)
BioRxiv, doi.org/10.1101/400432

AWI & damaged particles



- Most particles are at the AWI (as Noble *et al.* showed)
- AWI also associated with damage of FAS

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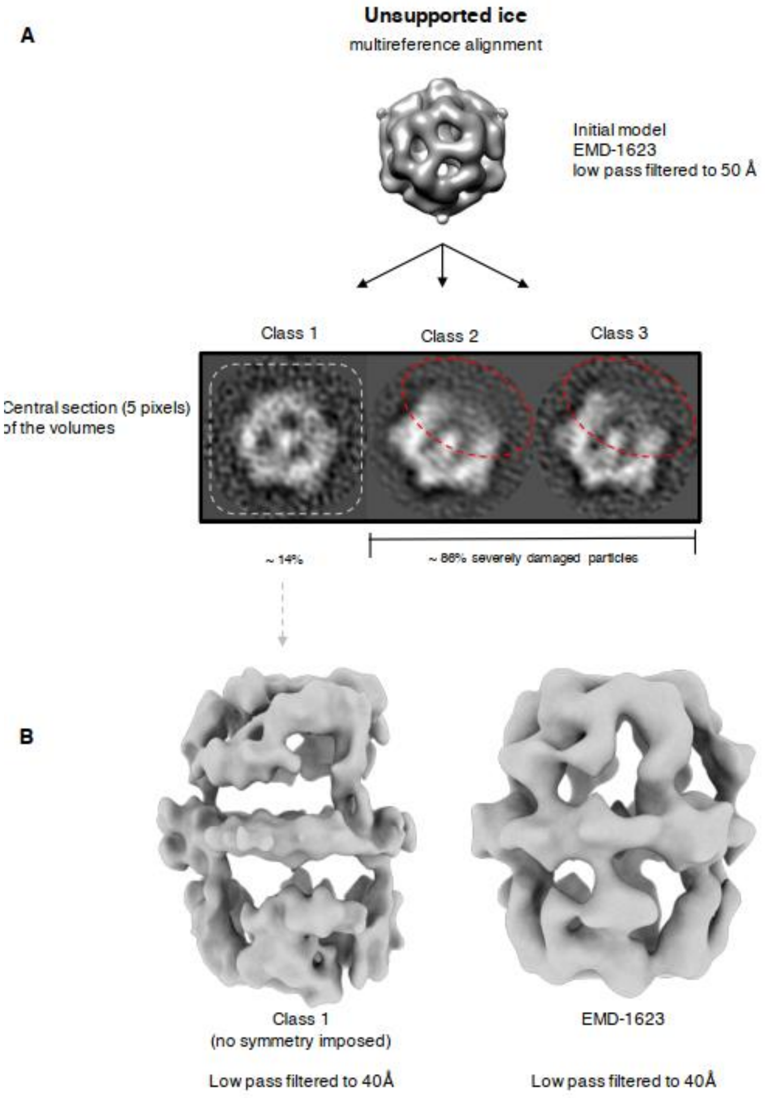
D'Imprima *et al.* (2018)
BioRxiv, doi.org/10.1101/400432

Reconstruction of damaged particles

- Reconstruction shows particle damage associated with AWI
- Is the AWI the cause?
    - Next slide



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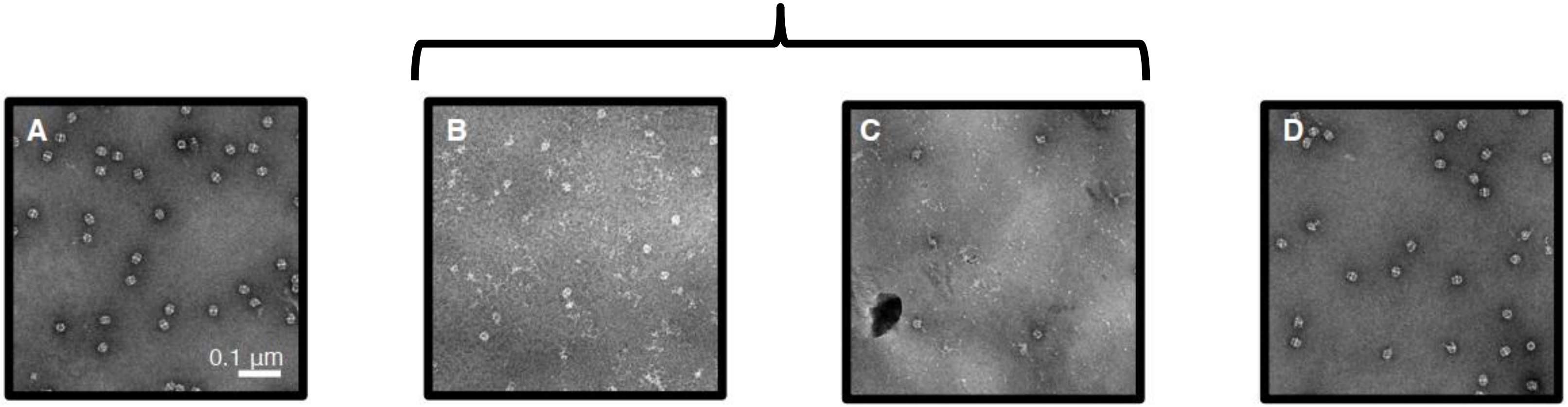
The AWI causes denaturation

Exposed to the AWI

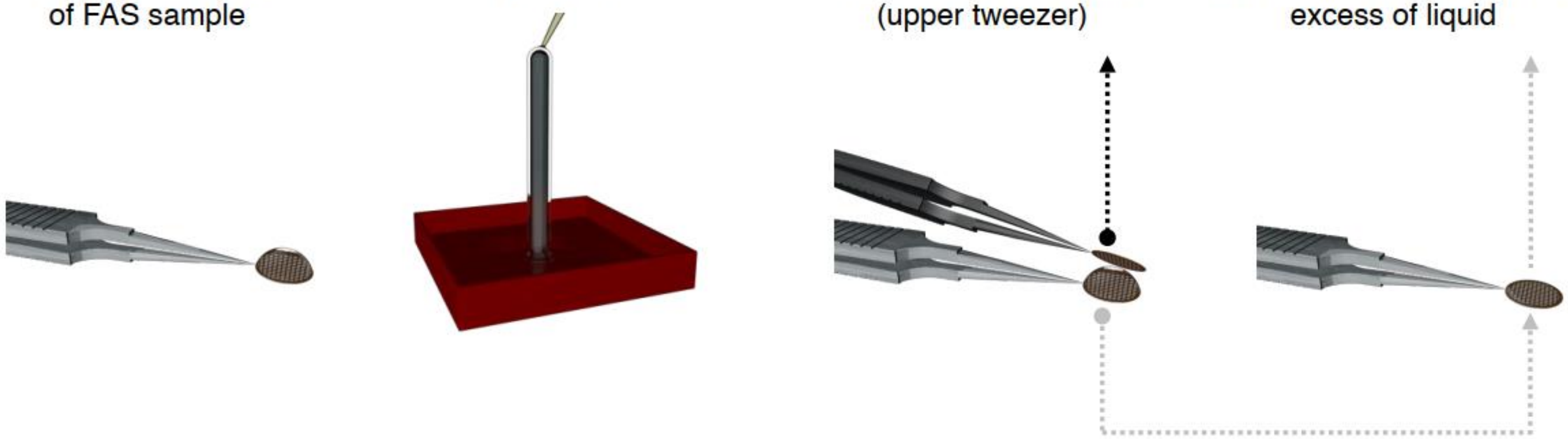


Standard negative staining procedure of FAS sample

Sample after gliding over a glass rod

Sample collected at the surface of the droplet (upper tweezer)

Specimen in C (lower tweezer) after blotting the excess of liquid



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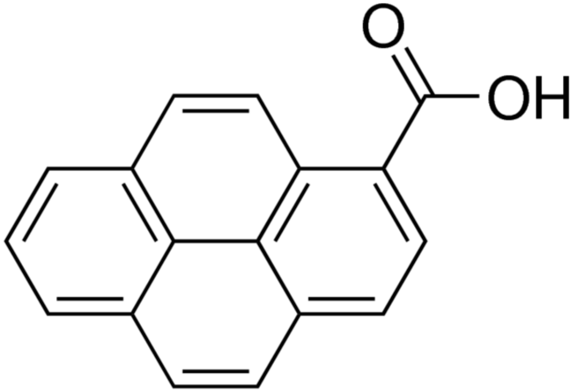
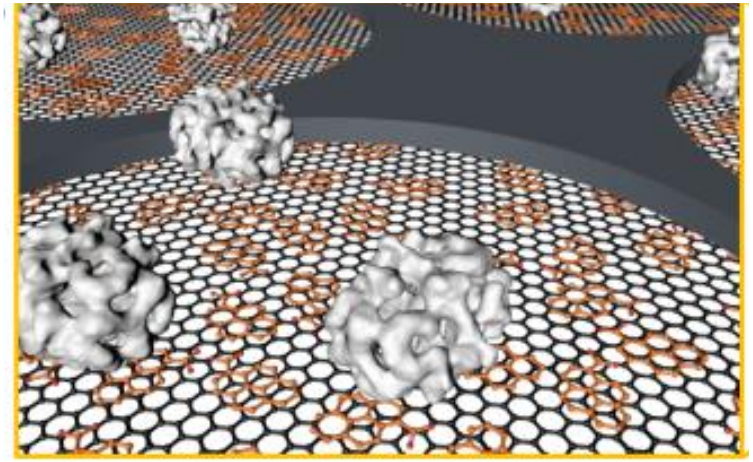
D’Imprima *et al.* (2018)
BioRxiv, doi.org/10.1101/400432

Solution

- Graphene
  - Electron conducting
  - Stable
  - Hydrophobic



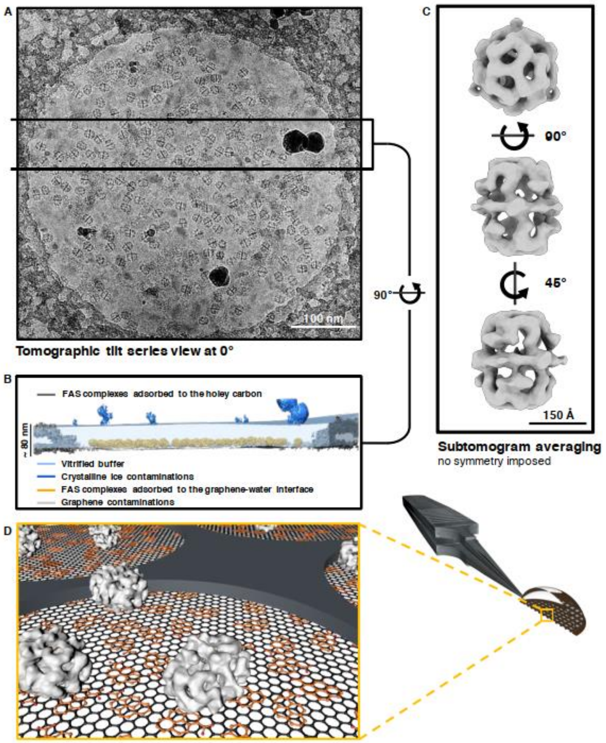
1-Pyrenecarboxylic acid



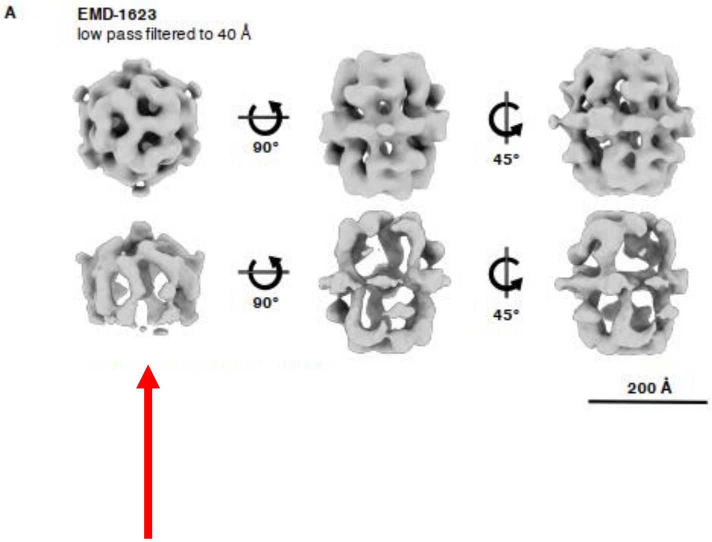
Sub-tomogram averaging (+/-) graphene

- Addition of graphene reduces denaturation

hydrophilized graphene +



hydrophilized graphene -



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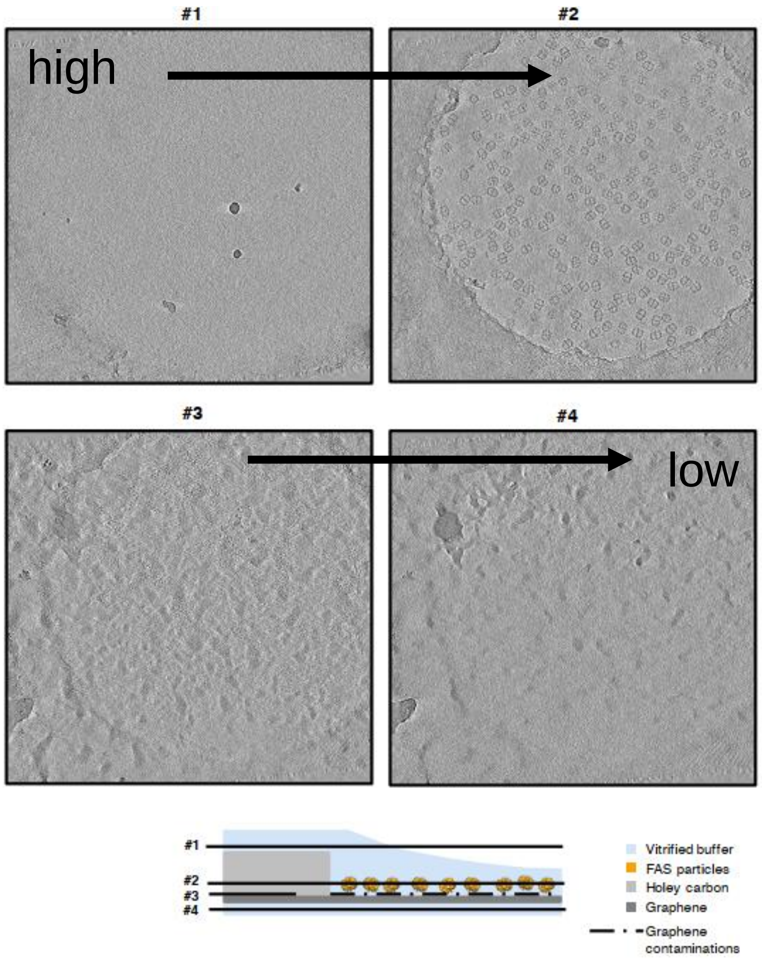
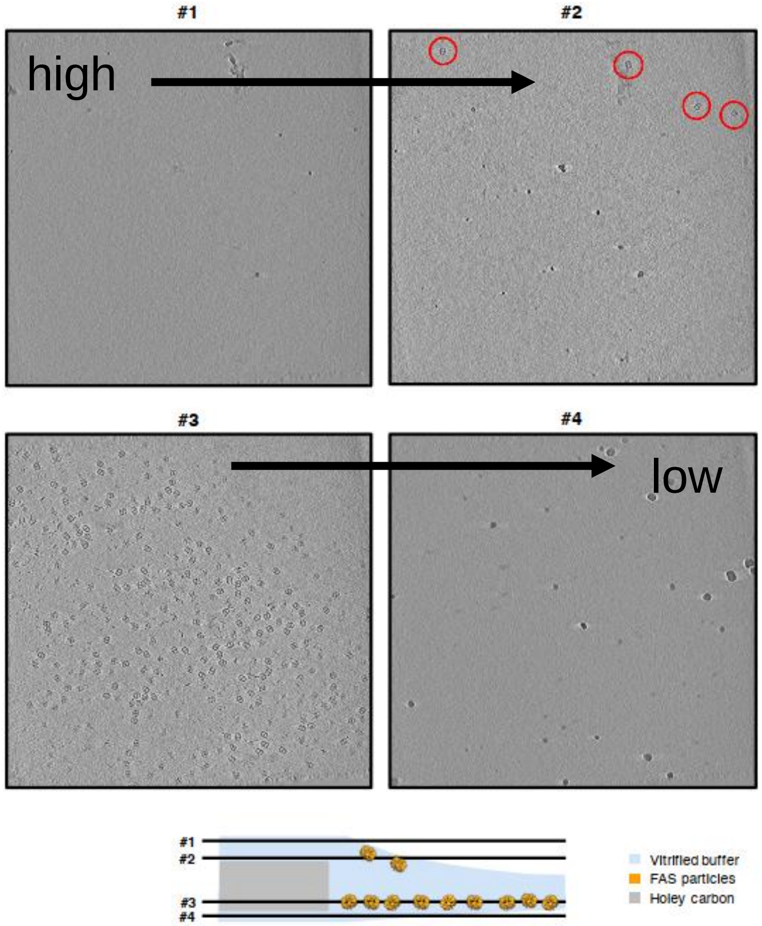
Hydrophilized graphene changes spatial distribution

hydrophilized graphene +



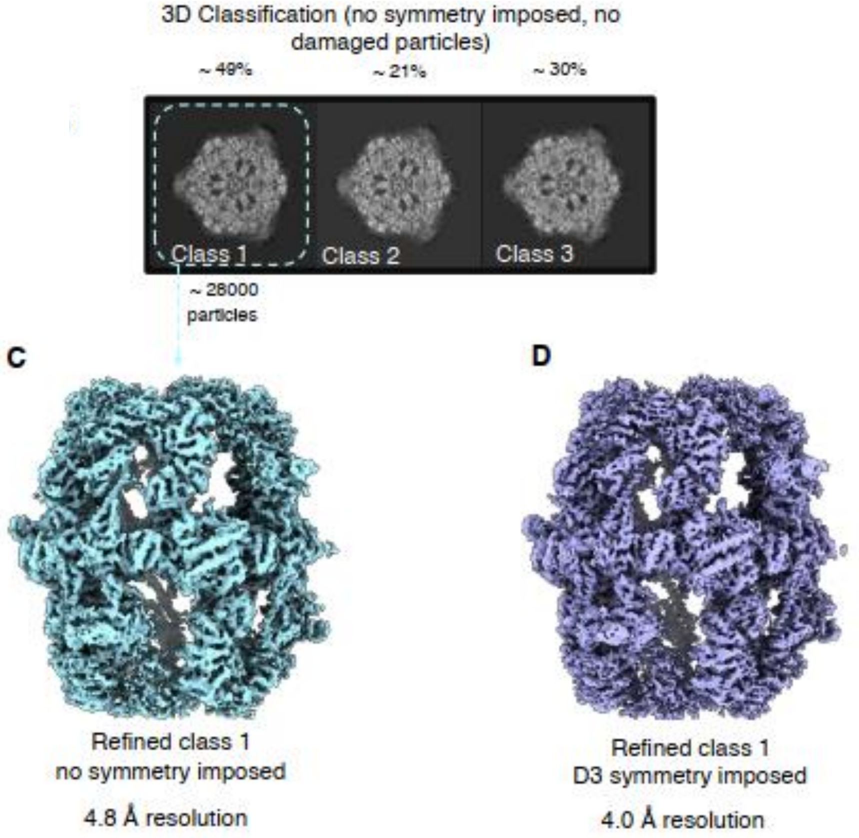
hydrophilized graphene -



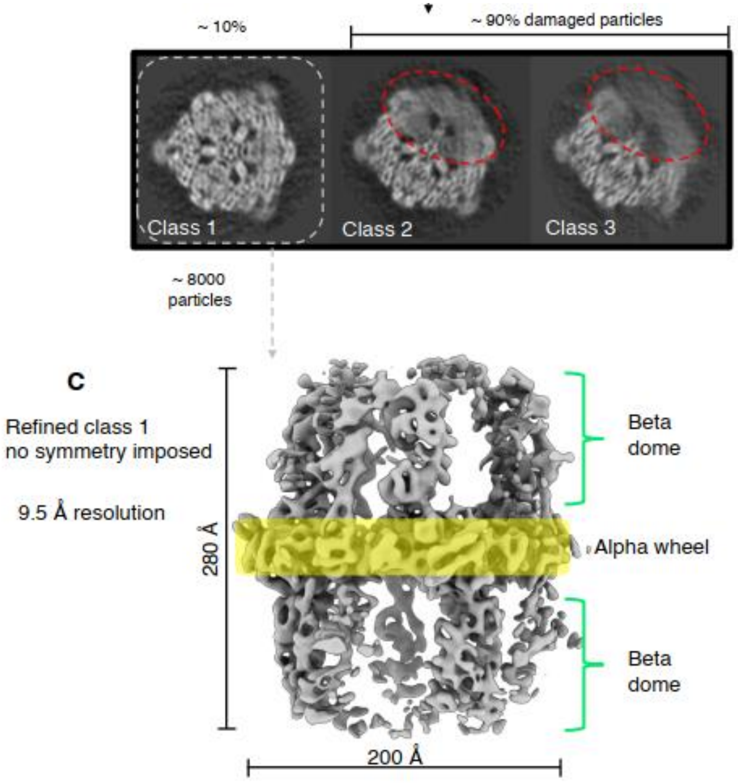
D’Imprima *et al*. (2018)
BioRxiv, doi.org/10.1101/400432

Increased undamaged particles

hydrophilized graphene +



hydrophilized graphene -



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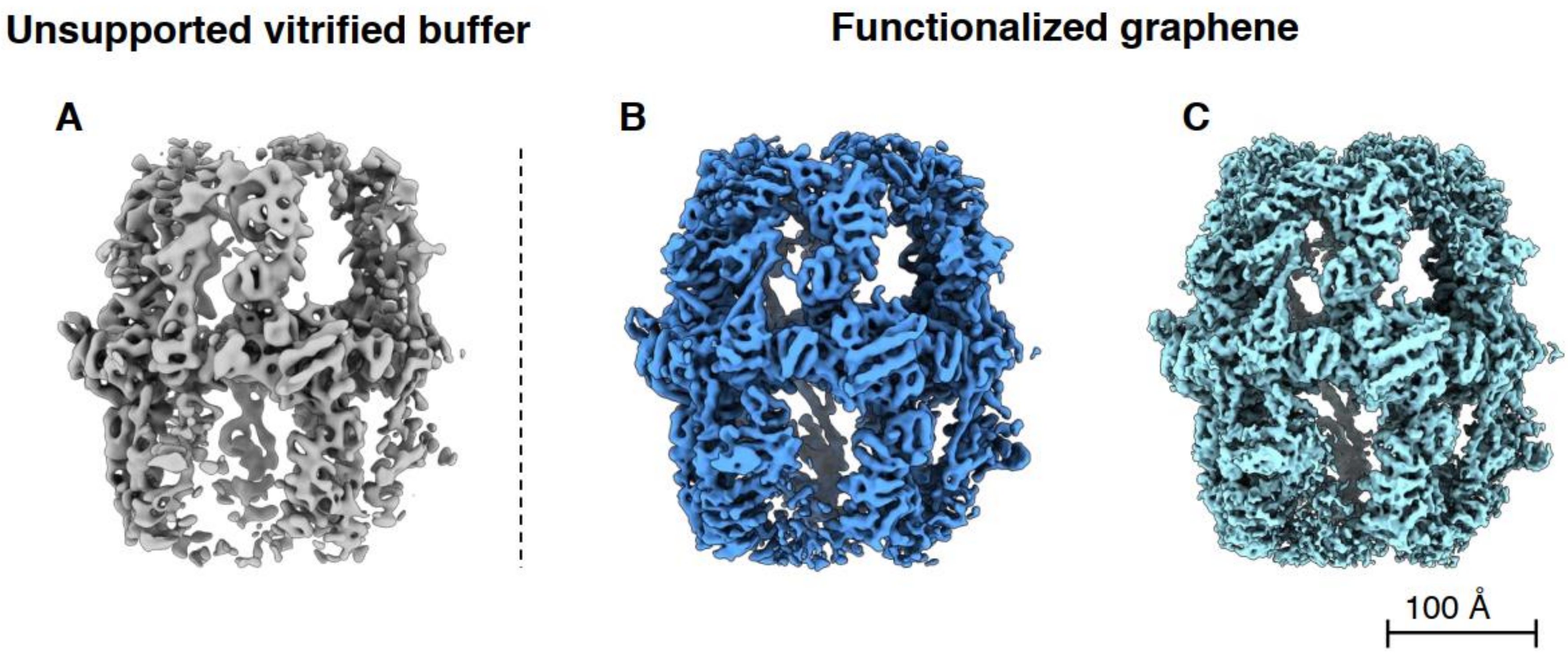
Increased noise, but better res.



A: Refined class 1
(8000 particles)
no symmetry imposed

9.5 Å resolution

B: Refined class 1
(8000 particles)
no symmetry imposed

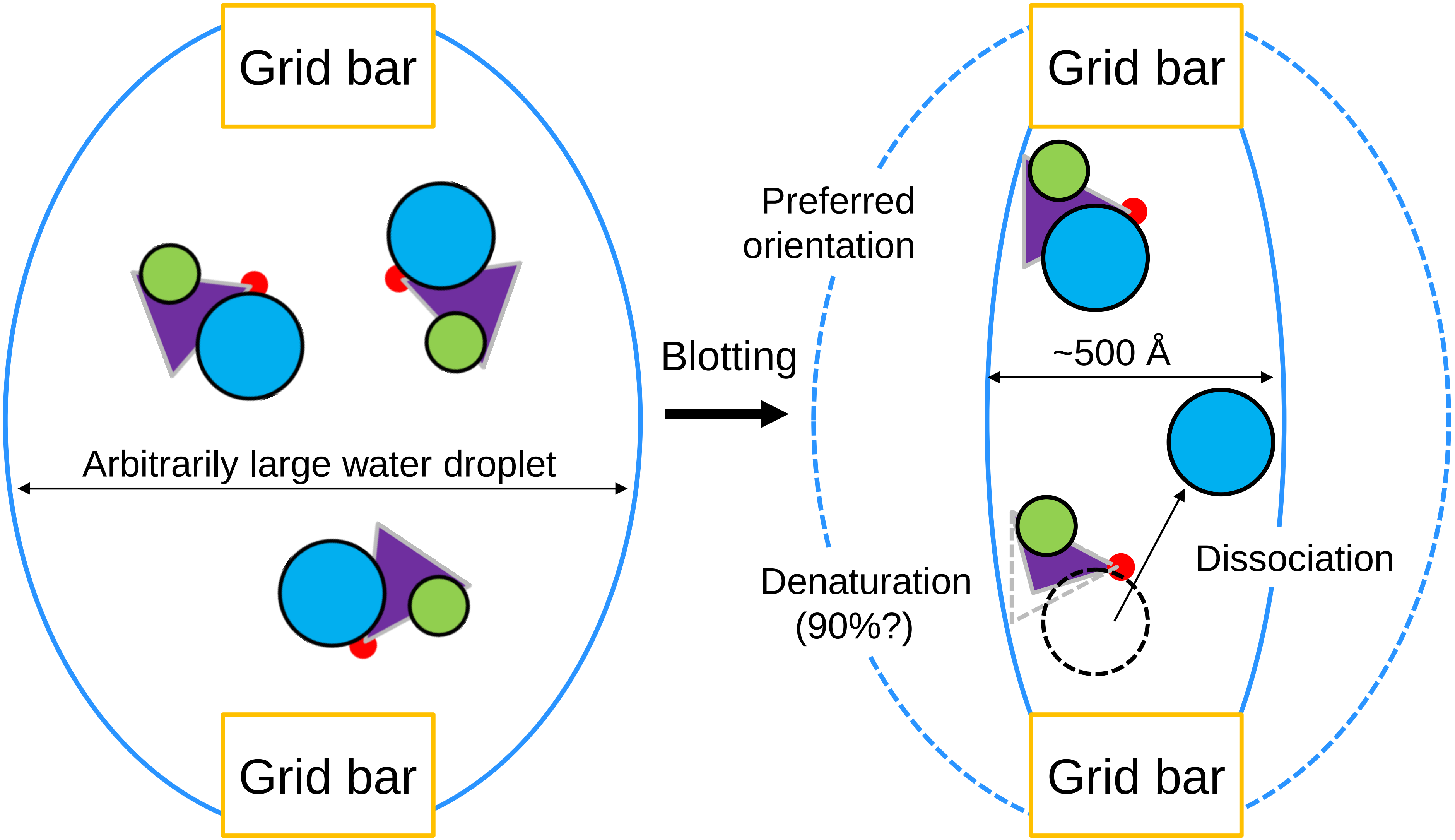
6.4 Å resolution

C: Refined class 1
(28000 particles)
no symmetry imposed

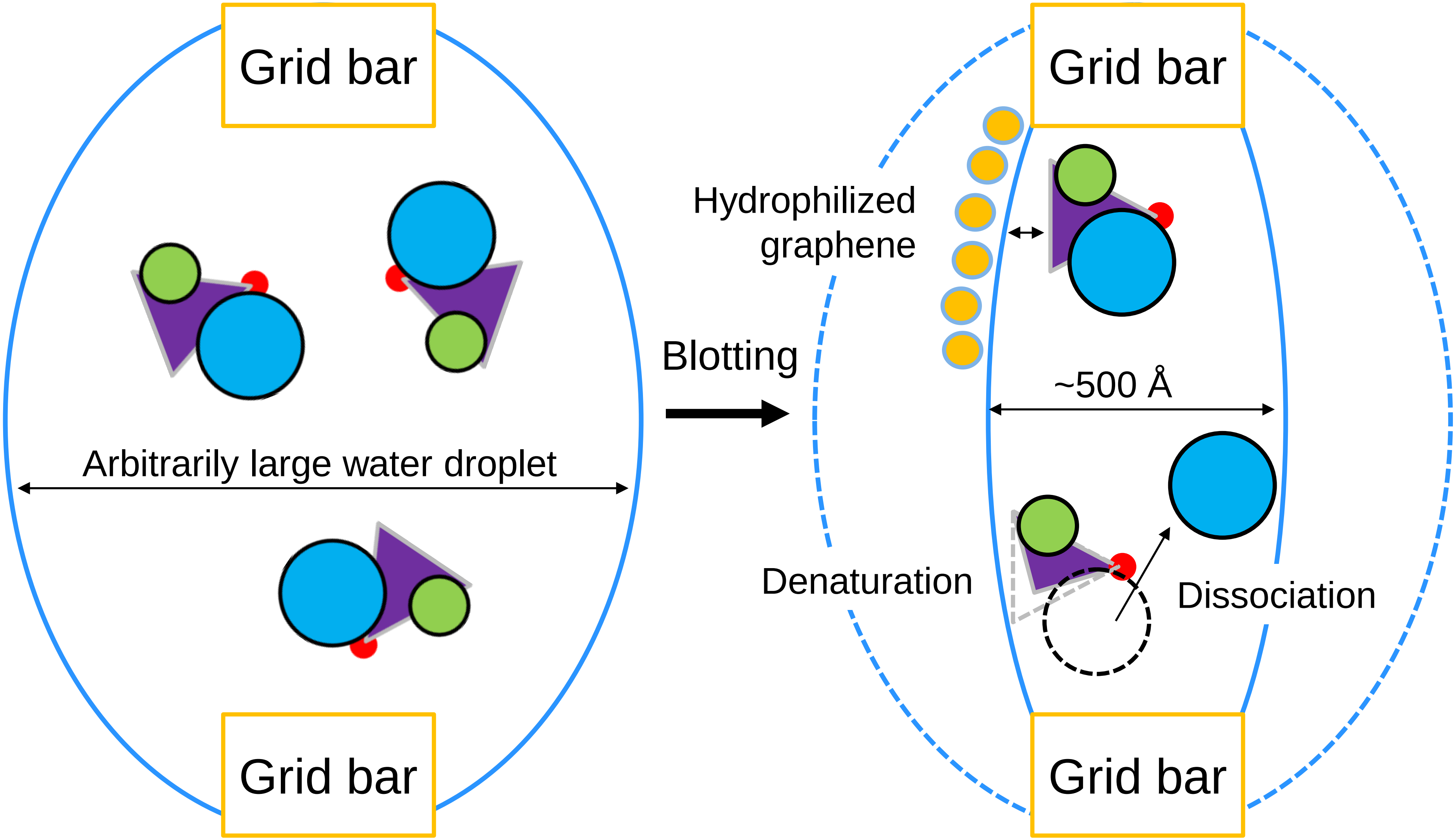
4.8 Å resolution

D’Imprima *et al*. (2018)
BioRxiv, doi.org/10.1101/400432

Exposure to air-water interface (AWI)

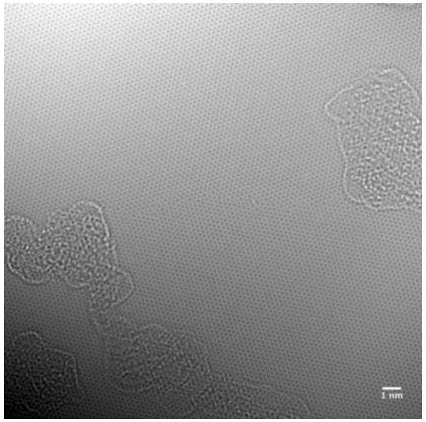


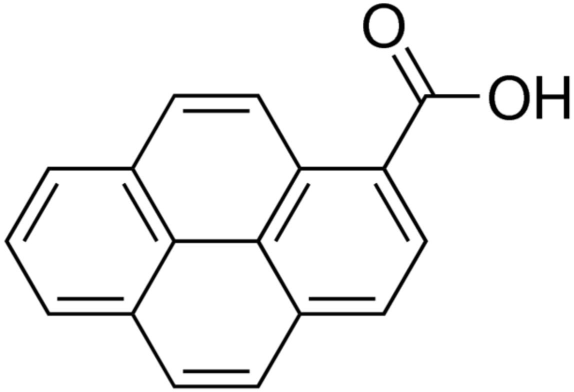
Exposure to air-water interface (AWI)



Graphene & 1-pyrCA

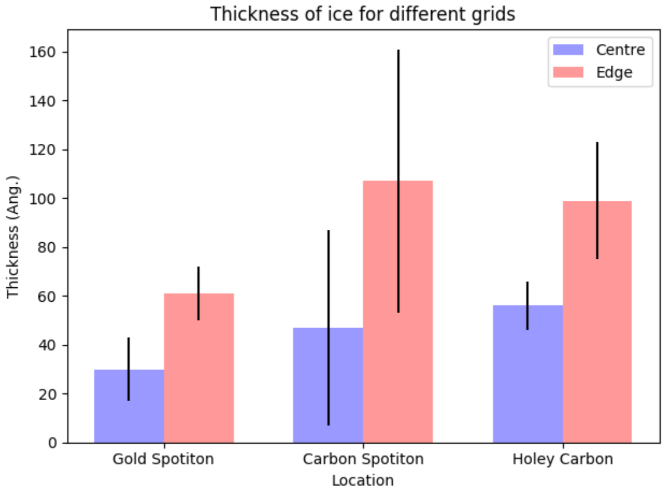
- Graphene (Graphena):
  - $76
  - 10 mm x 10 mm
- 1-Pyrenecarboxylic acid (Sigma)
  - $71.50
  - 1 g
- Can we try this?
  - Oxidises quickly so need to make girds just prior to use
  - Higher noise





Summary

- Au-Au best grid (or nanowire grid)
  - Does hole spacing have any effect?
  - Can anyone share with me some MC2 log files & grid type



- Most proteins (90%) are at the AWI
- Majority of proteins damaged by this?
  - At least FAS is (90%)
- Can we use graphene & 1-pyrCA to improve stability & get some nice structures?

Group meeting

More than 90 days?

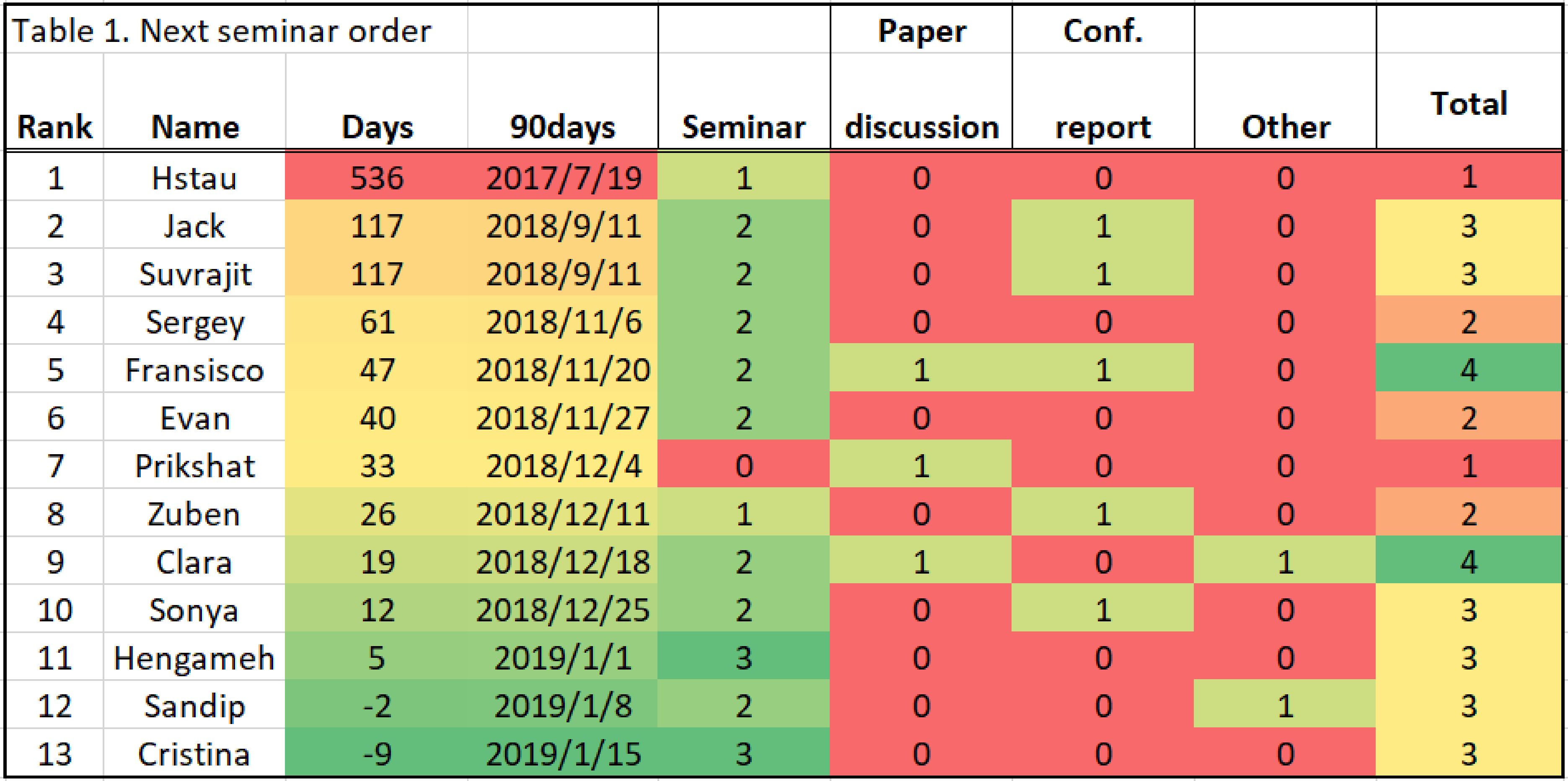
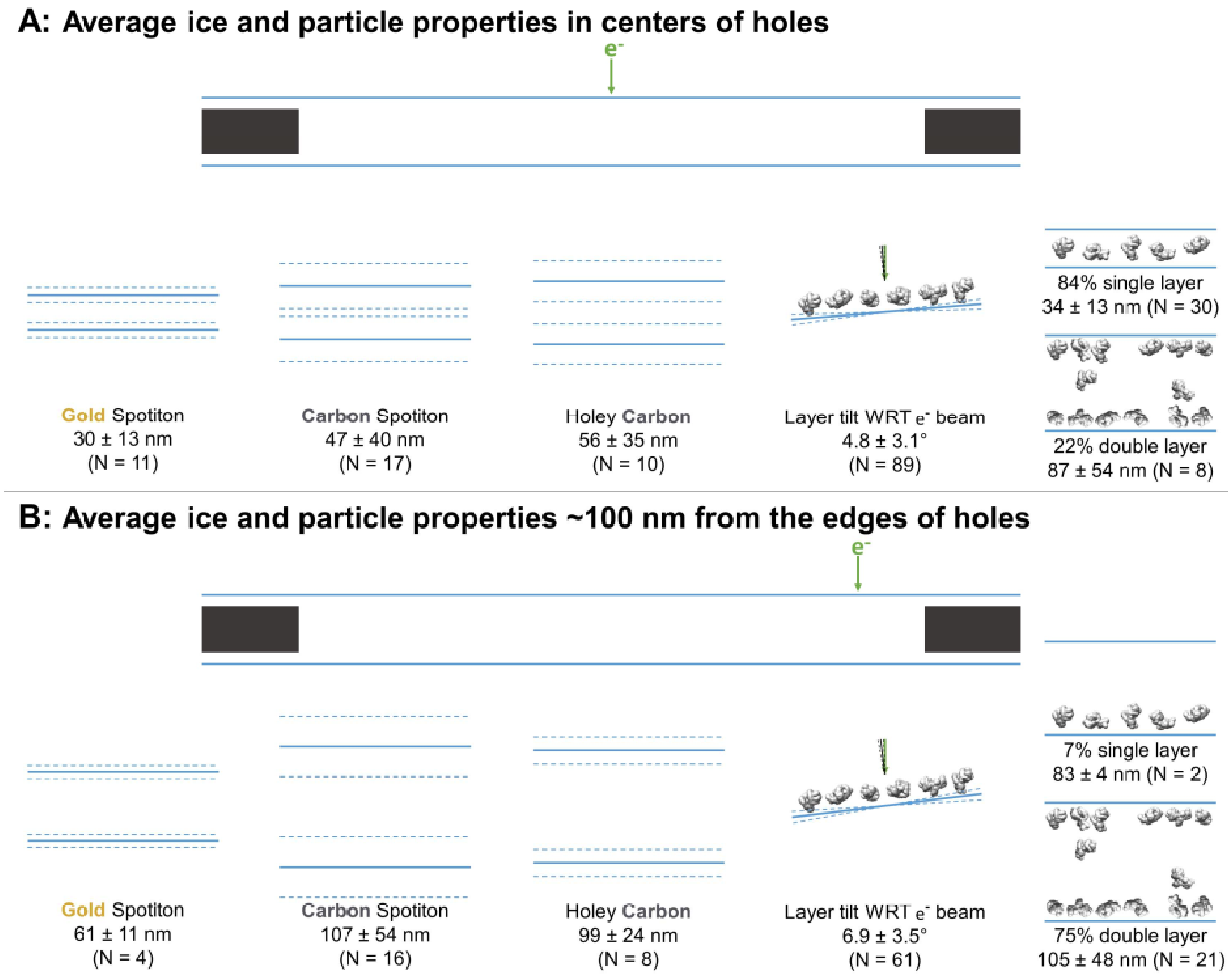


Table 1. Next seminar order

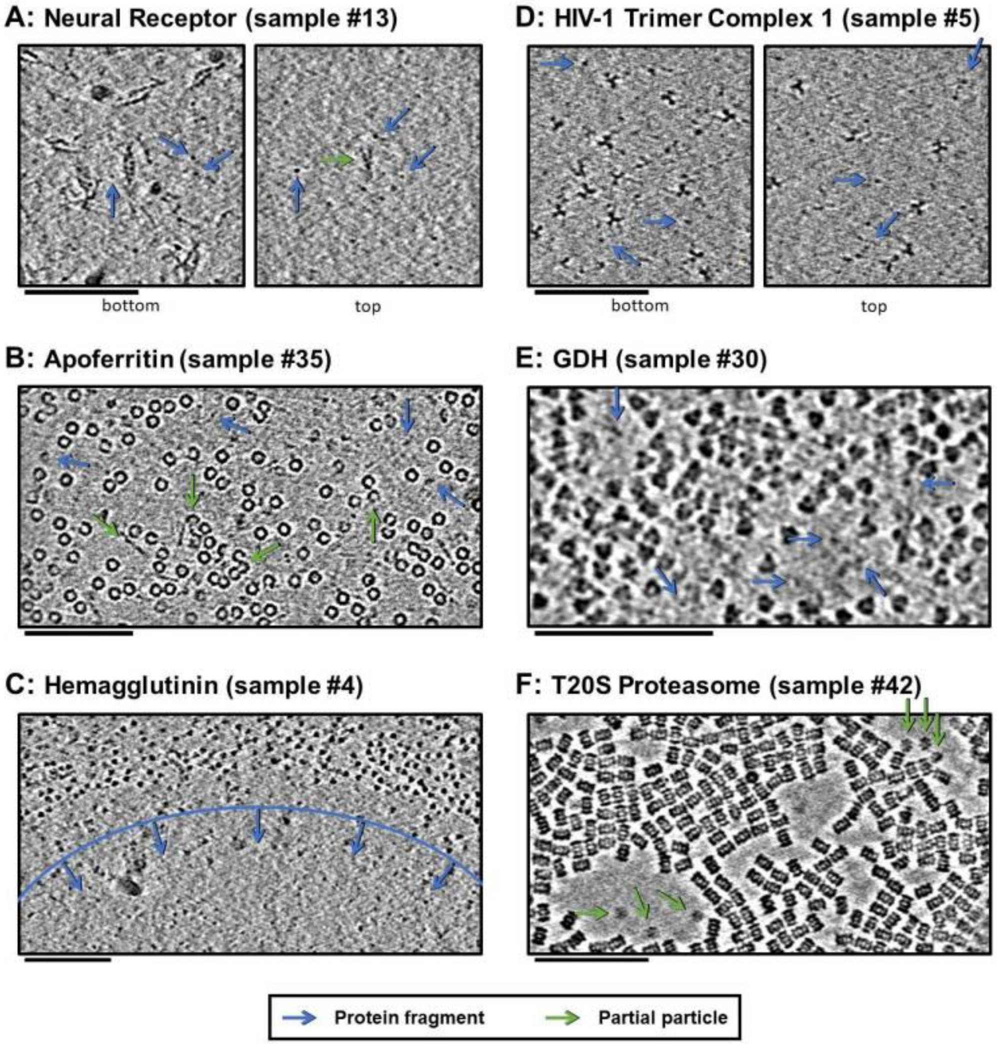
Rank	Name	Days	90days	Seminar	Paper discussion	Conf. report	Other	Total
1	Hstau	536	2017/7/19	1	0	0	0	1
2	Jack	117	2018/9/11	2	0	1	0	3
3	Suvrajit	117	2018/9/11	2	0	1	0	3
4	Sergey	61	2018/11/6	2	0	0	0	2
5	Fransisco	47	2018/11/20	2	1	1	0	4
6	Evan	40	2018/11/27	2	0	0	0	2
7	Prikshat	33	2018/12/4	0	1	0	0	1
8	Zuben	26	2018/12/11	1	0	1	0	2
9	Clara	19	2018/12/18	2	1	0	1	4
10	Sonya	12	2018/12/25	2	0	1	0	3
11	Hengameh	5	2019/1/1	3	0	0	0	3
12	Sandip	-2	2019/1/8	2	0	0	1	3
13	Cristina	-9	2019/1/15	3	0	0	0	3

Noble: Fig. 3



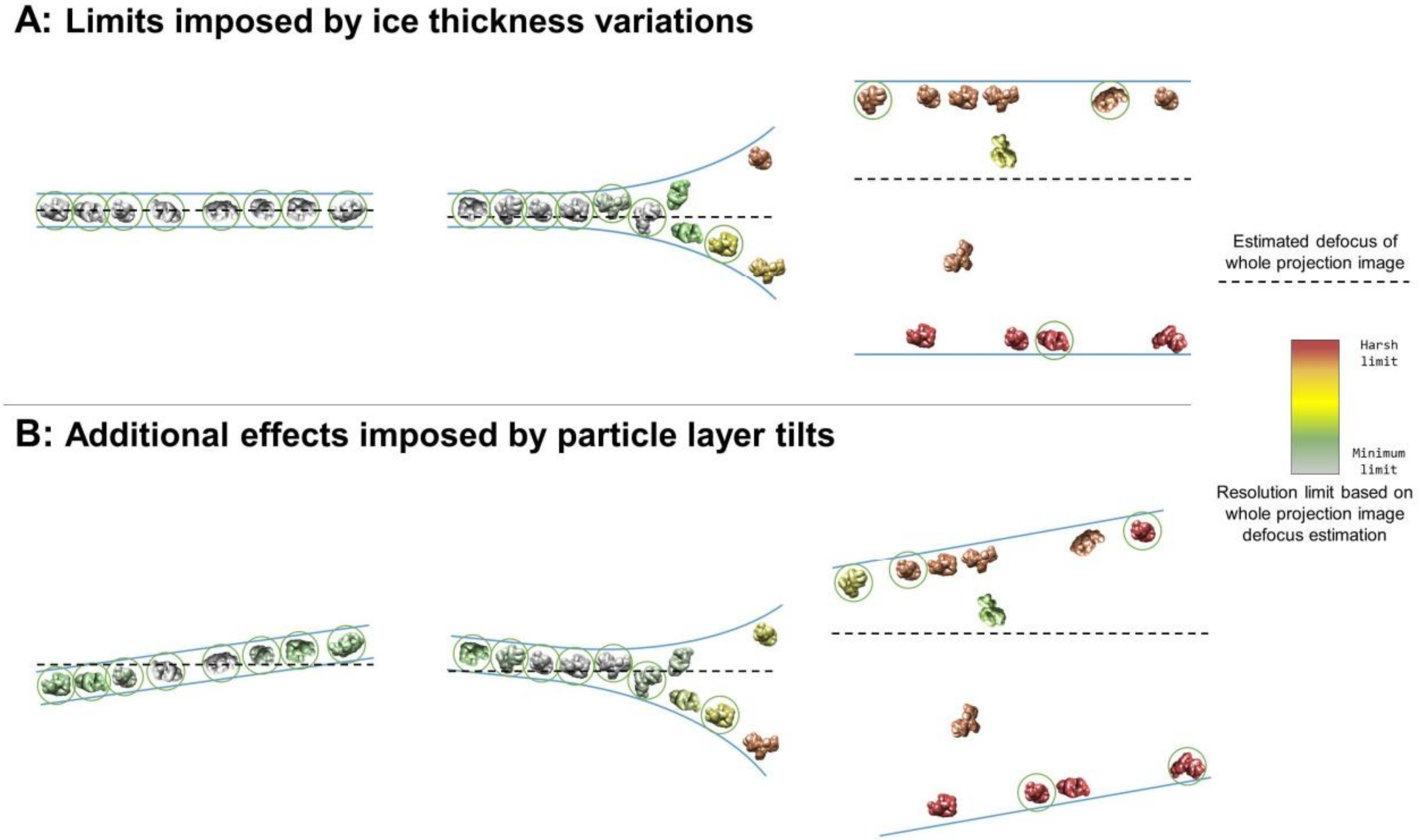
Noble *et al.* (2018)
elife, 7:e34257

Noble: Fig. 5



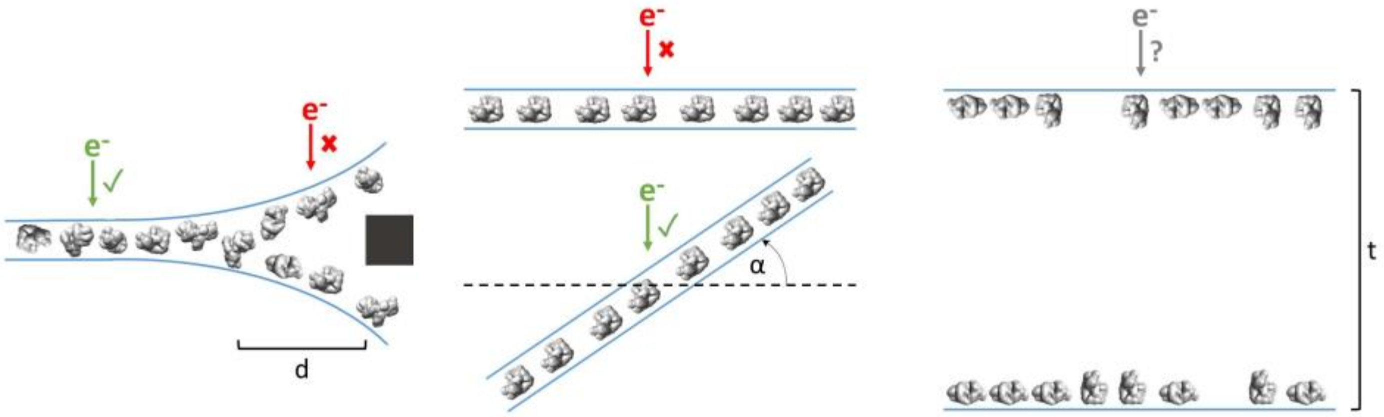
Noble *et al.* (2018)
elife, 7:e34257

Noble: Fig. 6



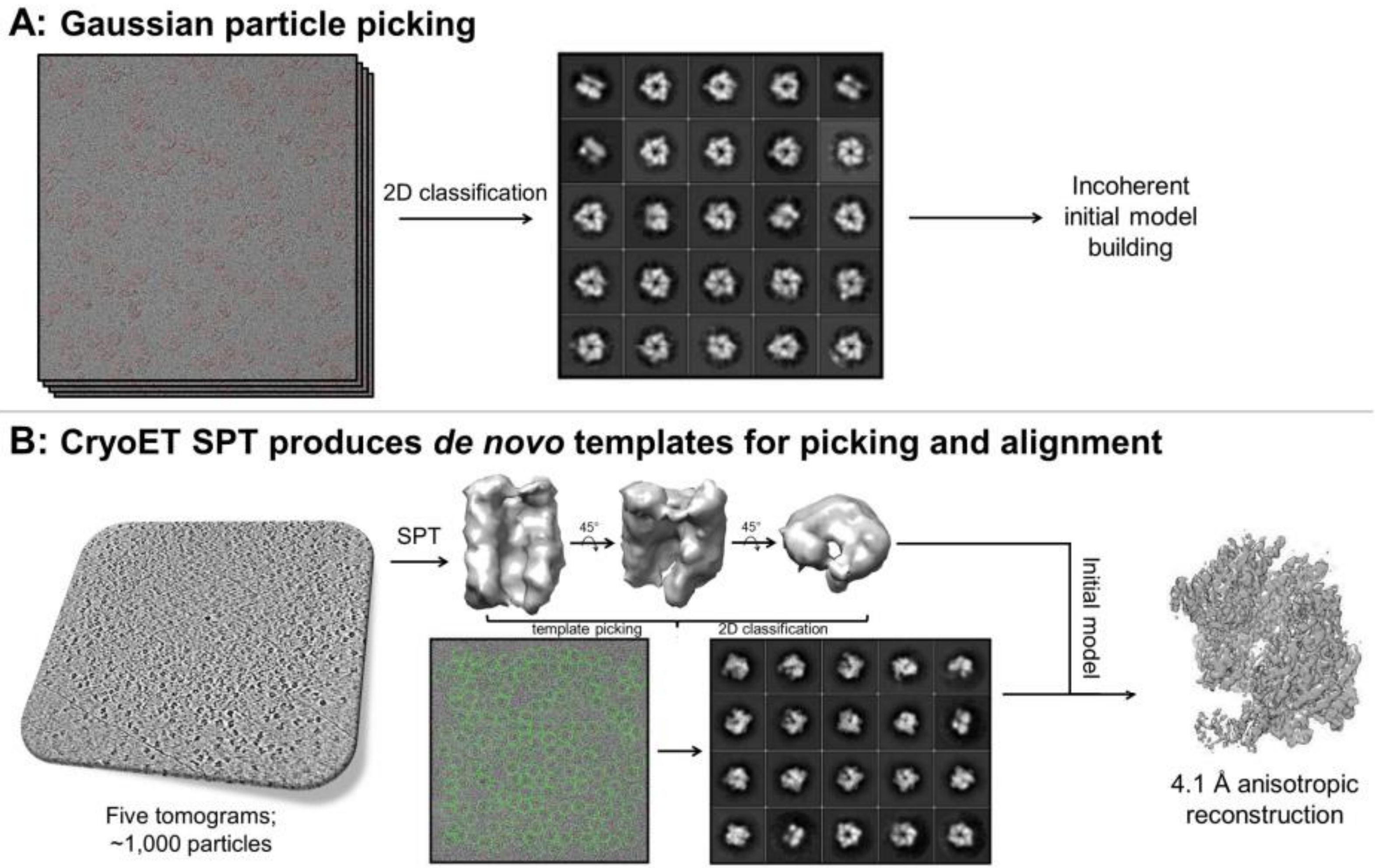
Noble *et al.* (2018)
elife, 7:e34257

Noble: Fig. 7



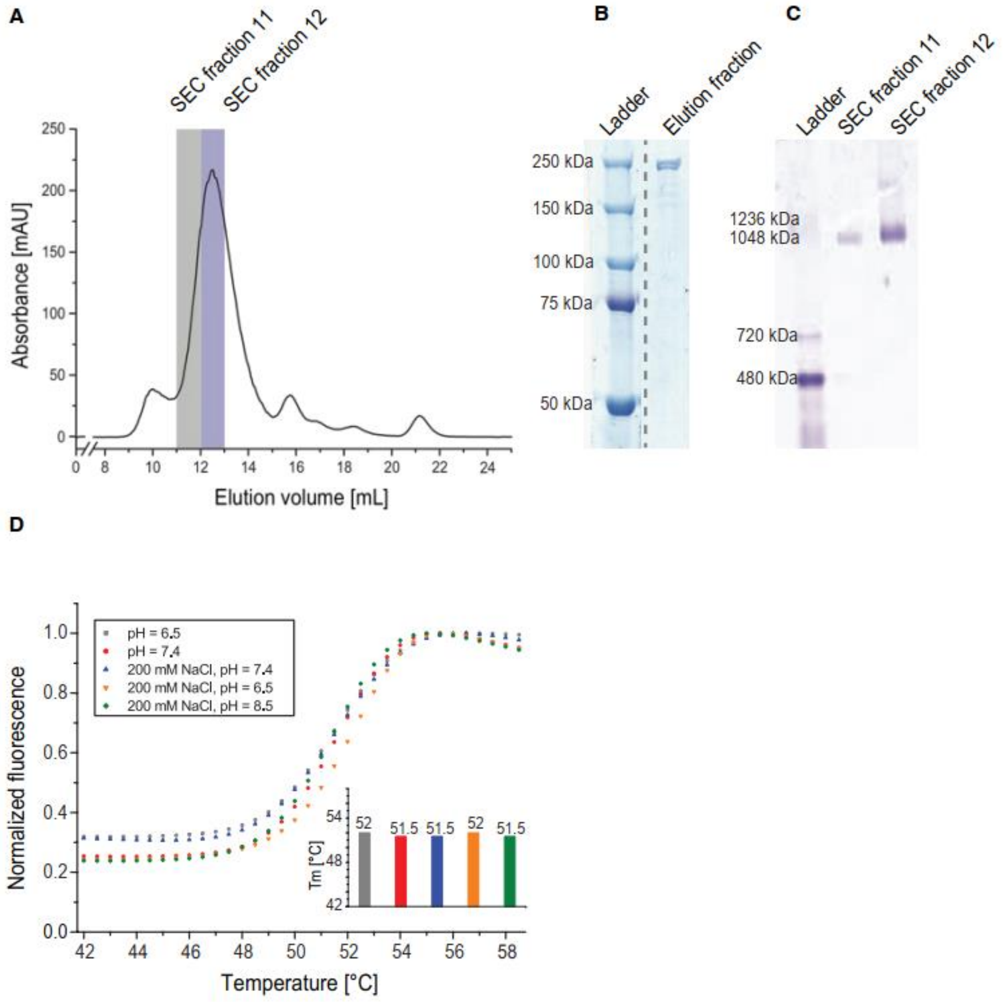
Noble *et al.* (2018)
elife, 7:e34257

Tomography & SPA (fig. 8)



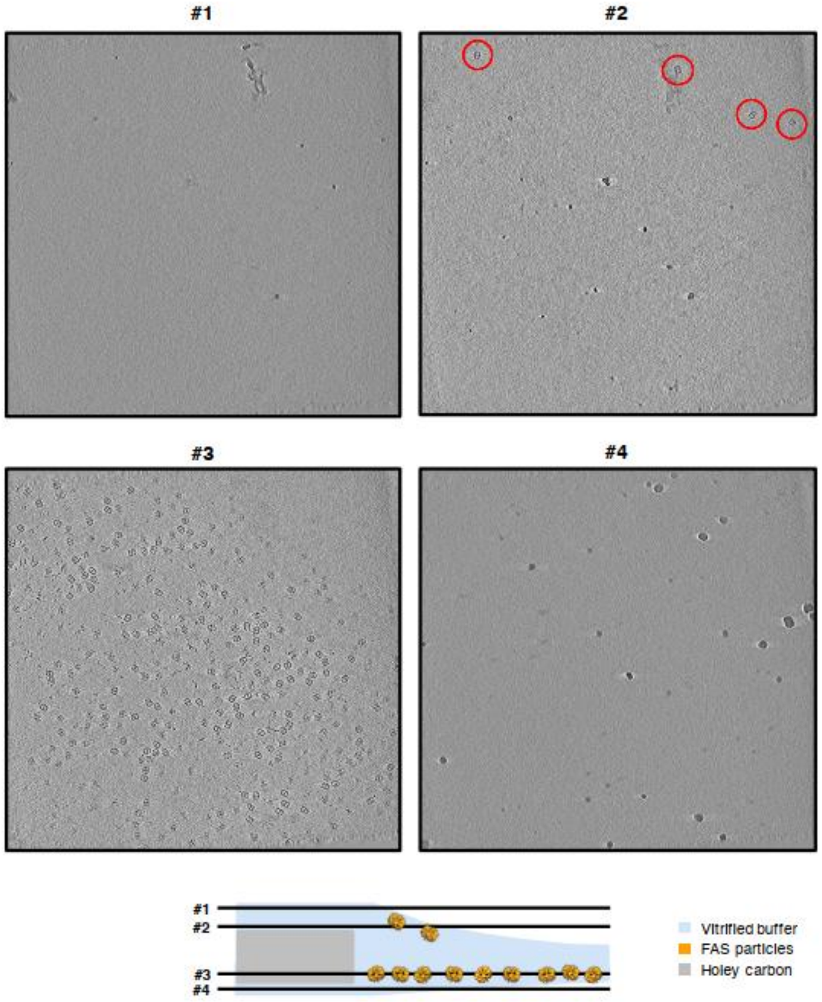
Noble *et al.* (2018)
elife, 7:e34257

FAS production



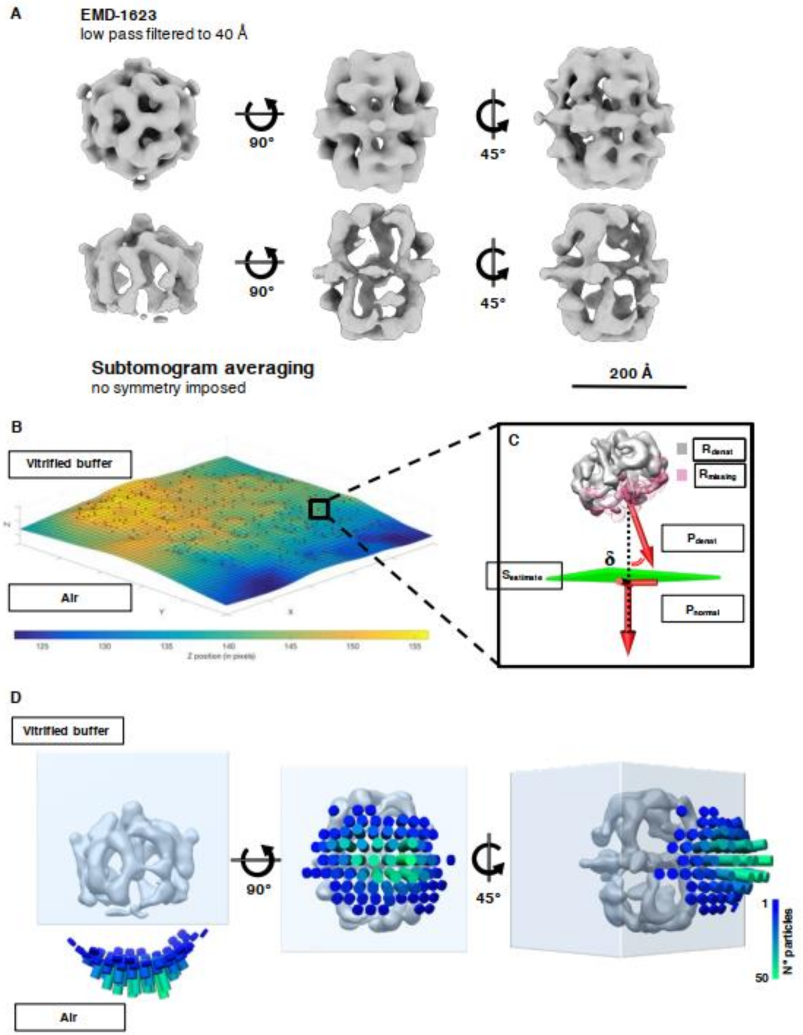
D’Imprima *et al*. (2018)
BioRxiv, doi.org/10.1101/400432

Tomography slices



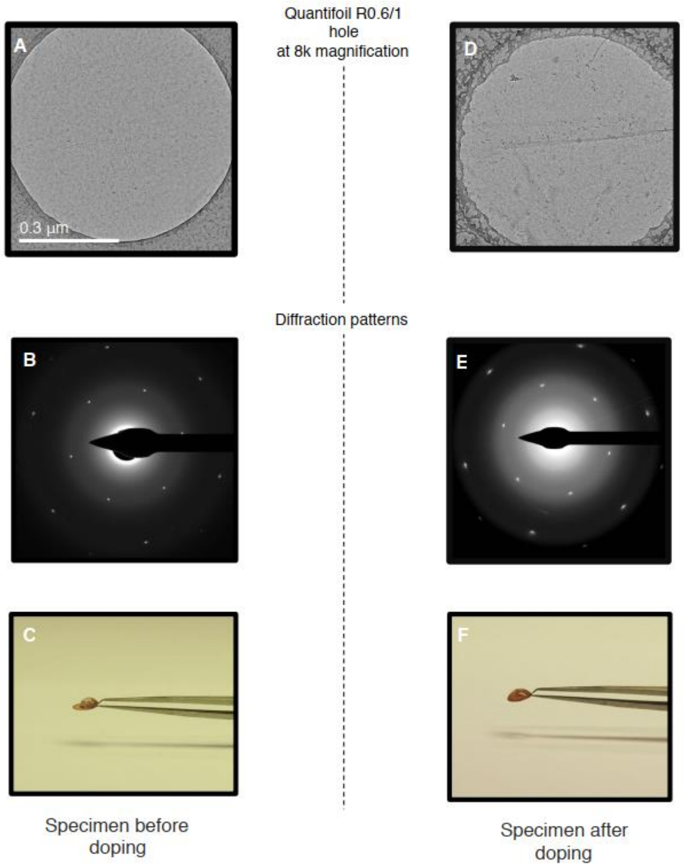
D’Imprima *et al*. (2018)
BioRxiv, doi.org/10.1101/400432

Fig. 3



D’Imprima *et al*. (2018)
BioRxiv, doi.org/10.1101/400432

Doping with graphene: Fig. 5



D'Imprima *et al*. (2018)
BioRxiv, doi.org/10.1101/400432

Fig. 6

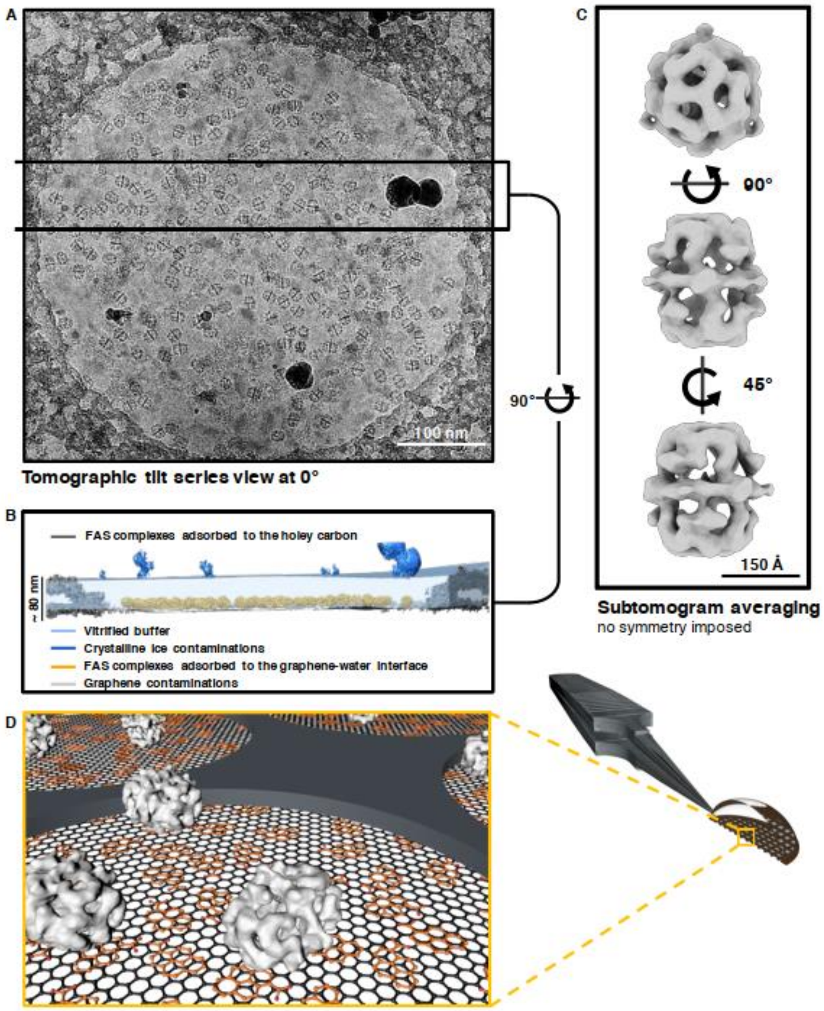


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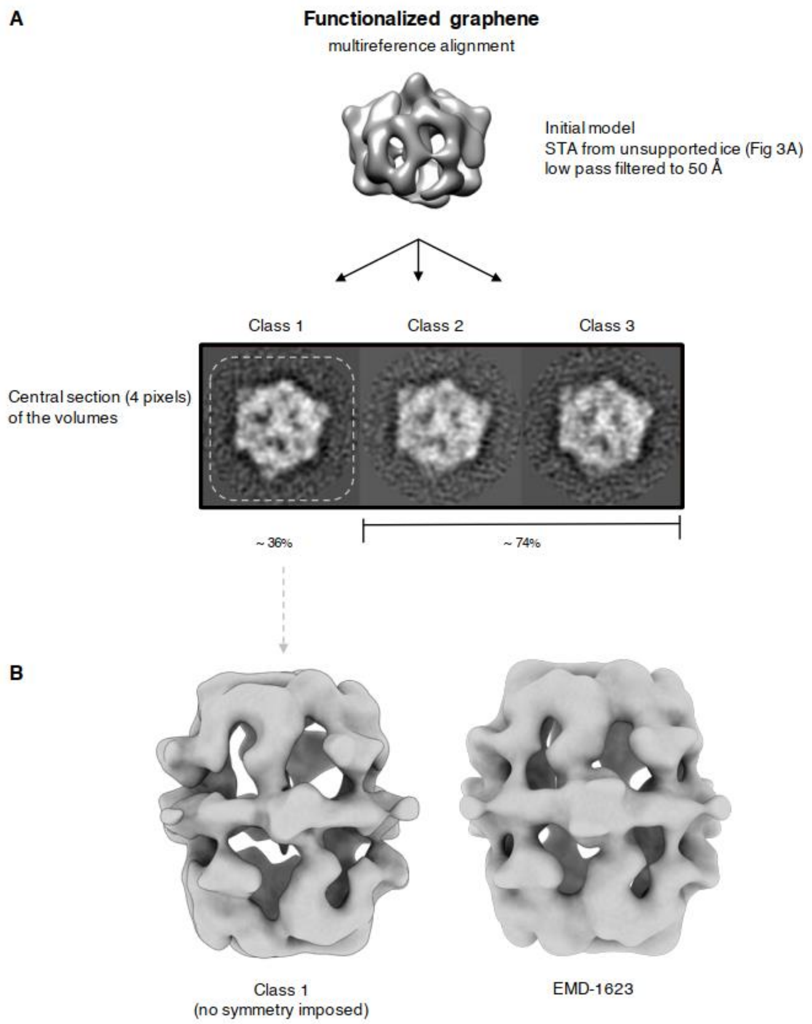
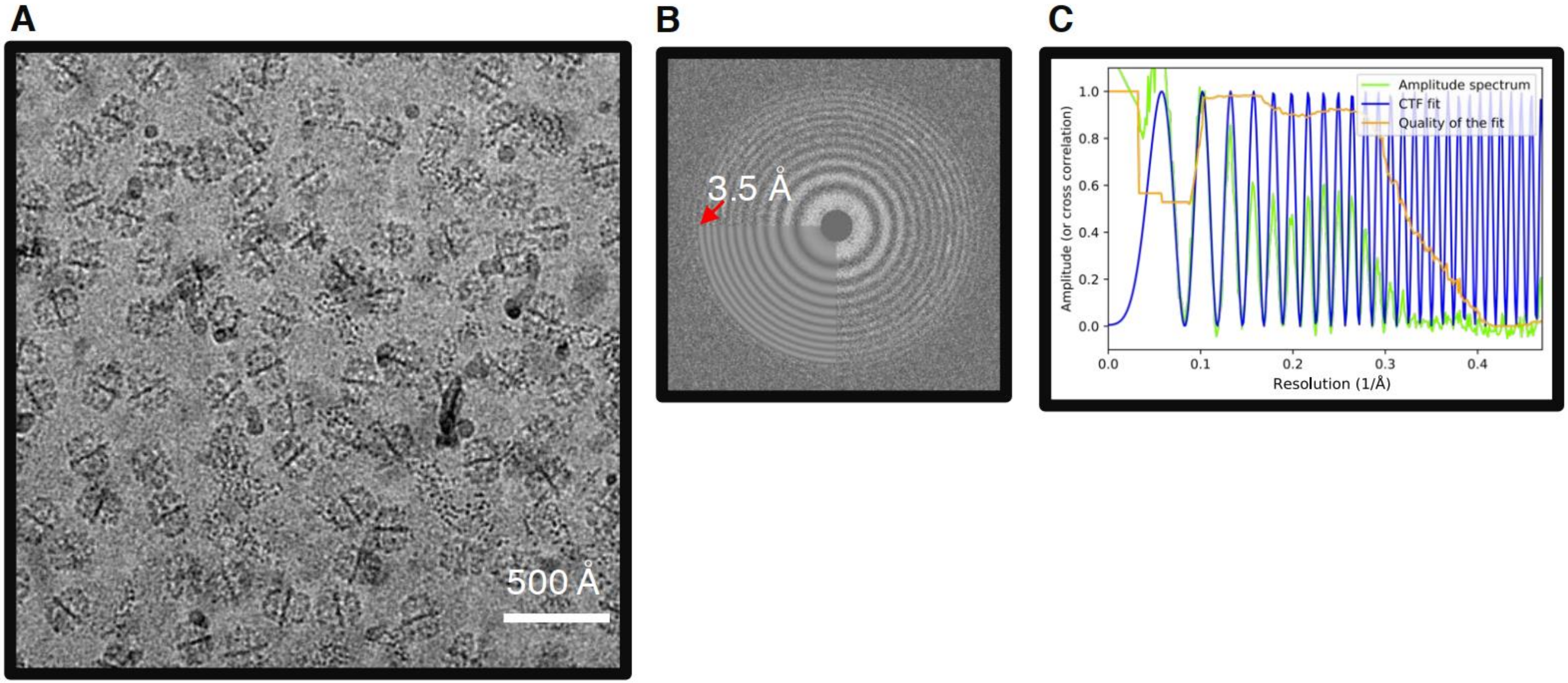
Fig. 6 sup. 2



D’Imprima *et al*. (2018)
BioRxiv, doi.org/10.1101/400432

Fig. 7



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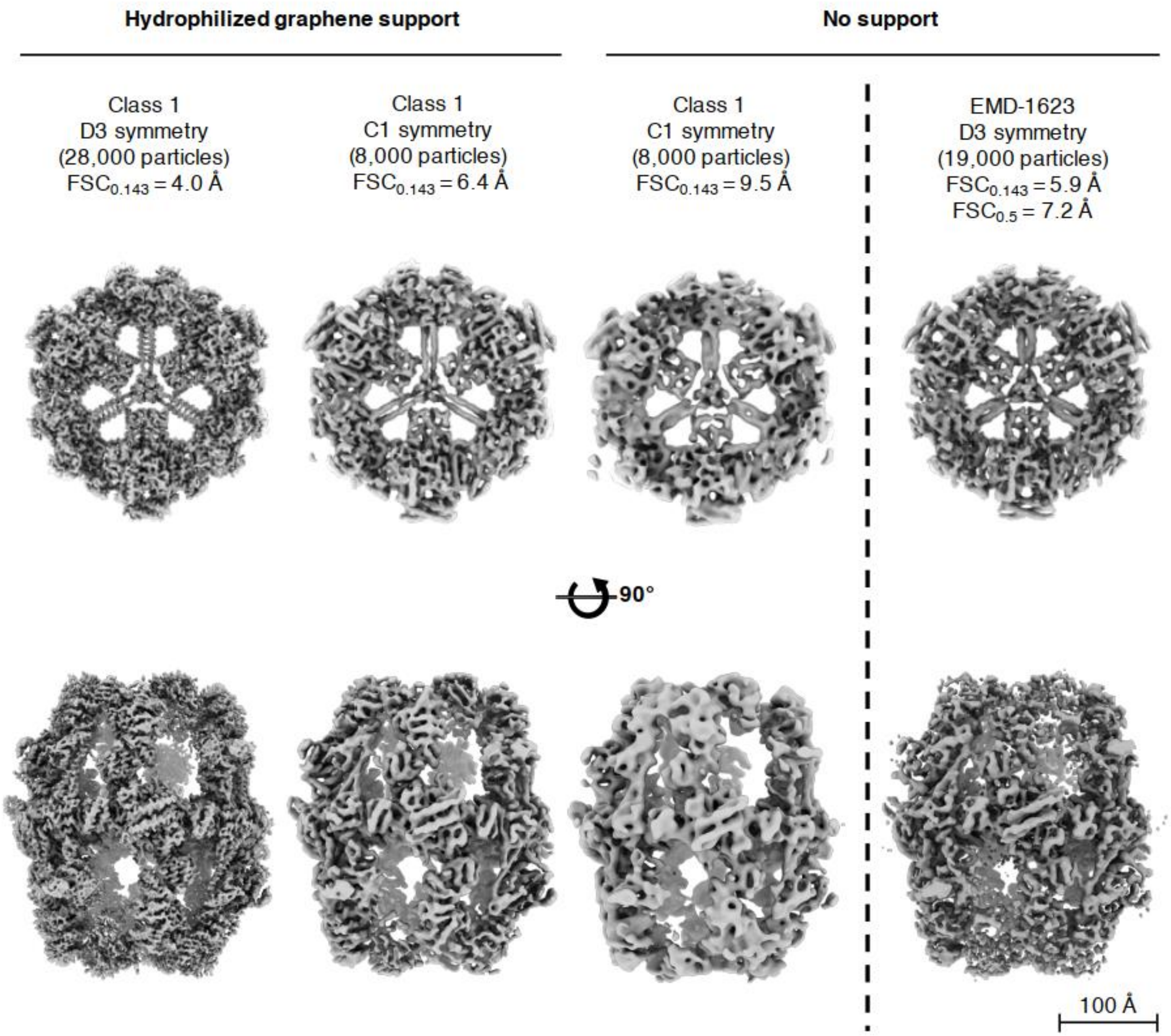
BioRxiv, doi.org/10.1101/400432

Fig. 8 sup. 3



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BioRxiv, doi.org/10.1101/400432