Study Meeting 5: The grid & samples in ice

Zuben P. Brown & Prikshat Dadhwal
Three interrelated topics

• The specimen support
  – Gold grids
  – Nanowire grids

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

• Electron-specimen interactions
  (next time)
Specimen support

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

Grid materials

<table>
<thead>
<tr>
<th>Copper</th>
<th>Gold</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nickel</td>
<td>CuRh</td>
</tr>
<tr>
<td>Titanium</td>
<td>Molybdenum</td>
</tr>
<tr>
<td>Silicon</td>
<td>Aluminum</td>
</tr>
<tr>
<td></td>
<td>Tungsten</td>
</tr>
</tbody>
</table>

Russo & Passmore, (2016)
Specimen support

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

Russo & Passmore, (2016)
Specimen support

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

Russo & Passmore, (2016)
C-flat

1500x (45°)  3000x  10,000x  20,000x

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 $\sim 1.1 \text{ Å}^2$ for every incident 300 keV $\text{e/Å}^2$. … The beam-induced movement of the water molecules generates pseudo-Brownian motion of embedded macromolecules”


Russo & Passmore, (2016)
Different hole spacing

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

<table>
<thead>
<tr>
<th>Description</th>
<th>Formula</th>
<th>Reference(s)</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electron wavelength</td>
<td>$\lambda = \frac{hc}{\sqrt{2E_0} + E^2}$</td>
<td>[27]</td>
<td></td>
</tr>
<tr>
<td>Chromatic aberration limit</td>
<td>$d_c = \frac{\sqrt{2}\lambda}{2}$</td>
<td>[23]</td>
<td></td>
</tr>
<tr>
<td>Inelastic mean free path limit</td>
<td>$\Lambda_i = C\beta^2\ln(\beta^2E + E_0)/E$</td>
<td>[22]†</td>
<td></td>
</tr>
<tr>
<td>Depth of field</td>
<td>$R = \frac{1.4}{(\xi \lambda)}$</td>
<td>[26]</td>
<td>66° phase error</td>
</tr>
</tbody>
</table>
Different hole spacing

Russo & Passmore, (2016)
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

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

• Electron-specimen interactions (next time)
Three general parameters:

1. Air-water interface
2. Bulk particle behaviour
3. Ice thickness
The specimen-support

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

Noble et al. (2018)
elife, 7:e34257
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
The specimen-support

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

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

Noble et al. (2018)
elife, 7:e34257
Ice thickness & protein spatial arrangement

Thinner ice in the centre, thicker at the edges
Single layer of protein in thinner regions

Noble et al. (2018)
elife, 7:e34257
Best supports for thin ice...

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

Razinkov et al. (2016)
Spatial arrangement is variable

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

Noble et al. (2018)
elife, 7:e34257
CTF estimation & particle position

Defocus variation

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

Table 3
Resolution Limit imposed by inaccuracy of defocus determination.

<table>
<thead>
<tr>
<th>Res. (Å)</th>
<th>100 kV</th>
<th>200 kV</th>
<th>300 kV</th>
<th>400 kV</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.0</td>
<td>54 Å</td>
<td>80 Å</td>
<td>102 Å</td>
<td>122 Å</td>
</tr>
<tr>
<td>3.0</td>
<td>122 Å</td>
<td>179 Å</td>
<td>228 Å</td>
<td>274 Å</td>
</tr>
<tr>
<td>4.0</td>
<td>216 Å</td>
<td>319 Å</td>
<td>406 Å</td>
<td>488 Å</td>
</tr>
<tr>
<td>7.0</td>
<td>662 Å</td>
<td>976 Å</td>
<td>1244 Å</td>
<td>1494 Å</td>
</tr>
</tbody>
</table>

Zhang & Zhou (2011)
Summary

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

- Tomography would give us an absolute range for particle position
- 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

• The specimen support
  – Gold grids
  – Nanowire grids

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

• Electron-specimen interactions (next time)

Russo & Passmore (2016)

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

Zheng et al. (2017)
Nat. Meth. 14(4):331
Exposure to air-water interface (AWI)

- Arbitrarily large water droplet
- Grid bar

Preferred orientation

Blotting

Denaturation

Dissociation

~500 Å
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
Most particles are at the AWI (as Noble et al. showed)

AWI also associated with damage of FAS

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

The AWI causes denaturation

Exposed to the AWI

A: Standard negative staining procedure of FAS sample
B: Sample after gliding over a glass rod
C: Sample collected at the surface of the droplet (upper tweezer)
D: Specimen in C (lower tweezer) after blotting the excess of liquid
Solution

- Graphene
  - Electron conducting
  - Stable
  - Hydrophobic

[Chemical structure of 1-Pyrenecarboxylic acid]
Sub-tomogram averaging (+/-) graphene

- Addition of graphene reduces denaturation

D’Imprima et al. (2018)
BioRxiv, doi.org/10.1101/400432
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 -
Increased noise, but better res.

Unsupported vitrified buffer

Refined class 1
(8000 particles)
no symmetry imposed

9.5 Å resolution

Functionalized graphene

Refined class 1
(8000 particles)
no symmetry imposed

6.4 Å resolution

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)

- Arbitrarily large water droplet
- Grid bar
- Preferred orientation
- Blotting
- Denaturation (90%?)
- Dissociation
- Grid bar
- ~500 Å
Exposure to air-water interface (AWI)

Arbitrarily large water droplet

Hydrophilized graphene

Blotting

Denaturation

Dissociation

Grid bar

~500 Å
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?
## Table 1. Next seminar order

<table>
<thead>
<tr>
<th>Rank</th>
<th>Name</th>
<th>Days</th>
<th>90days</th>
<th>Seminar</th>
<th>Paper</th>
<th>Conf.</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Hstau</td>
<td>536</td>
<td>2017/7/19</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>Jack</td>
<td>117</td>
<td>2018/9/11</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>Suvrajit</td>
<td>117</td>
<td>2018/9/11</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>Sergey</td>
<td>61</td>
<td>2018/11/6</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>Fransisco</td>
<td>47</td>
<td>2018/11/20</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>6</td>
<td>Evan</td>
<td>40</td>
<td>2018/11/27</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>7</td>
<td>Prikshat</td>
<td>33</td>
<td>2018/12/4</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>Zuben</td>
<td>26</td>
<td>2018/12/11</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>9</td>
<td>Clara</td>
<td>19</td>
<td>2018/12/18</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>10</td>
<td>Sonya</td>
<td>12</td>
<td>2018/12/25</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>11</td>
<td>Hengameh</td>
<td>5</td>
<td>2019/1/1</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>12</td>
<td>Sandip</td>
<td>-2</td>
<td>2019/1/8</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>13</td>
<td>Cristina</td>
<td>-9</td>
<td>2019/1/15</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
</tbody>
</table>
Noble: Fig. 3

A: Average ice and particle properties in centers of holes

- **Gold Spotiton**: 30 ± 13 nm (N = 11)
- **Carbon Spotiton**: 47 ± 40 nm (N = 17)
- **Holey Carbon**: 56 ± 35 nm (N = 10)

Layer tilt WRT $e^-$ beam: 4.8 ± 3.1° (N = 89)

- 84% single layer: 34 ± 13 nm (N = 30)
- 22% double layer: 87 ± 54 nm (N = 8)

B: Average ice and particle properties ~100 nm from the edges of holes

- **Gold Spotiton**: 61 ± 11 nm (N = 4)
- **Carbon Spotiton**: 107 ± 54 nm (N = 16)
- **Holey Carbon**: 99 ± 24 nm (N = 8)

Layer tilt WRT $e^-$ beam: 6.9 ± 3.5° (N = 61)

- 7% single layer: 83 ± 4 nm (N = 2)
- 75% double layer: 105 ± 48 nm (N = 21)
Noble: Fig. 5

A: Neural Receptor (sample #13)

B: Apoferritin (sample #35)

C: Hemagglutinin (sample #4)

D: HIV-1 Trimer Complex 1 (sample #5)

E: GDH (sample #30)

F: T20S Proteasome (sample #42)

Protein fragment
Partial particle

Noble et al. (2018)
elife, 7:e34257
A: Limits imposed by ice thickness variations

B: Additional effects imposed by particle layer tilts

Noble et al. (2018)
elife, 7:e34257
A: Gaussian particle picking

2D classification

Incoherent initial model building

B: CryoET SPT produces de novo templates for picking and alignment

Five tomograms: ~1,000 particles

SPT

template picking

2D classification

Initial model

4.1 Å anisotropic reconstruction

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

D’Imprima et al. (2018)
BioRxiv, doi.org/10.1101/400432
Fig. 8 sup. 3

Hydrophilized graphene support

<table>
<thead>
<tr>
<th>Class 1</th>
<th>Class 1</th>
<th>No support</th>
</tr>
</thead>
<tbody>
<tr>
<td>D3 symmetry</td>
<td>C1 symmetry</td>
<td>EMD-1623</td>
</tr>
<tr>
<td>(28,000 particles)</td>
<td>(8,000 particles)</td>
<td>D3 symmetry</td>
</tr>
<tr>
<td>FSC (_{0.143} = 4.0 \text{ Å} )</td>
<td>FSC (_{0.143} = 6.4 \text{ Å} )</td>
<td>(19,000 particles)</td>
</tr>
<tr>
<td></td>
<td>FSC (_{0.143} = 9.5 \text{ Å} )</td>
<td>FSC (_{0.143} = 5.9 \text{ Å} )</td>
</tr>
<tr>
<td></td>
<td>FSC (_{0.5} = 7.2 \text{ Å} )</td>
<td></td>
</tr>
</tbody>
</table>

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