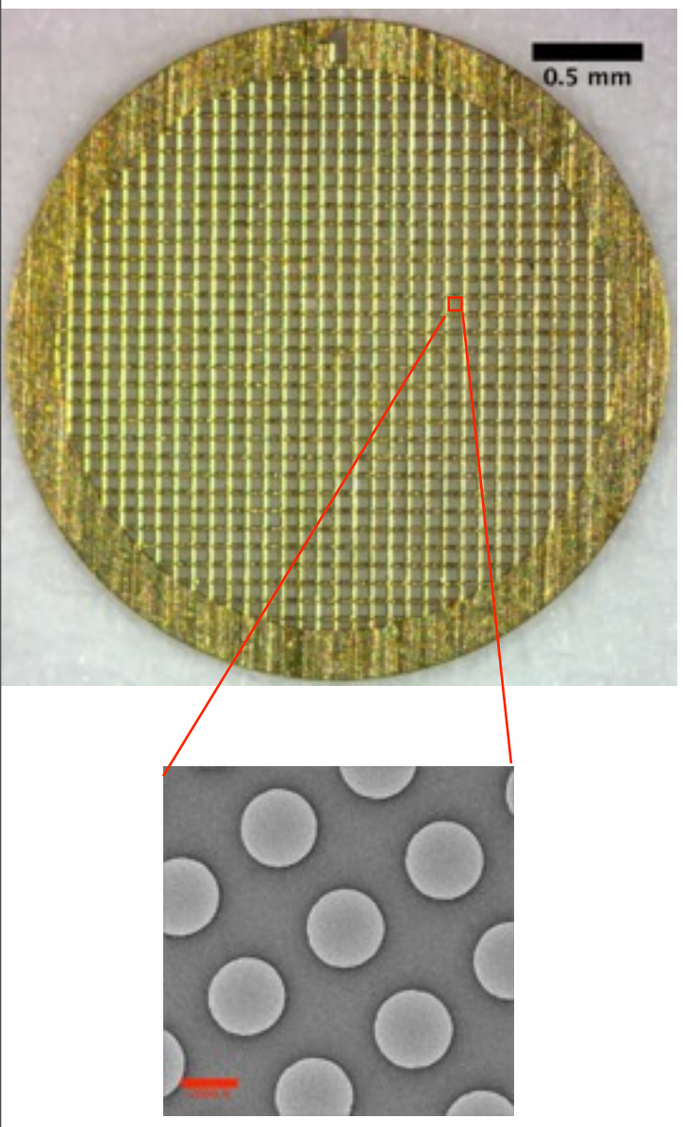
New substrates for electron cryo-microscopy

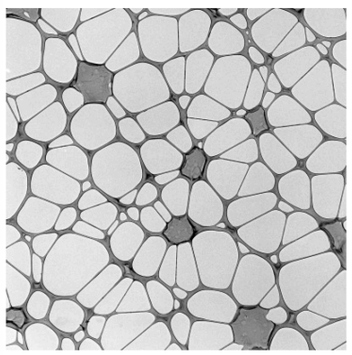
Lori Passmore

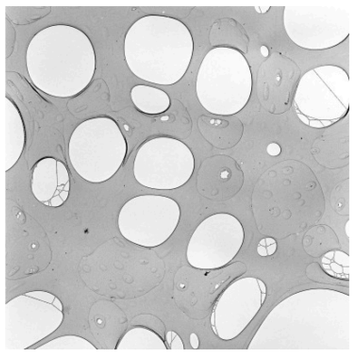
2014 NRAMM Workshop on Advanced Topics in EM Structure Determination

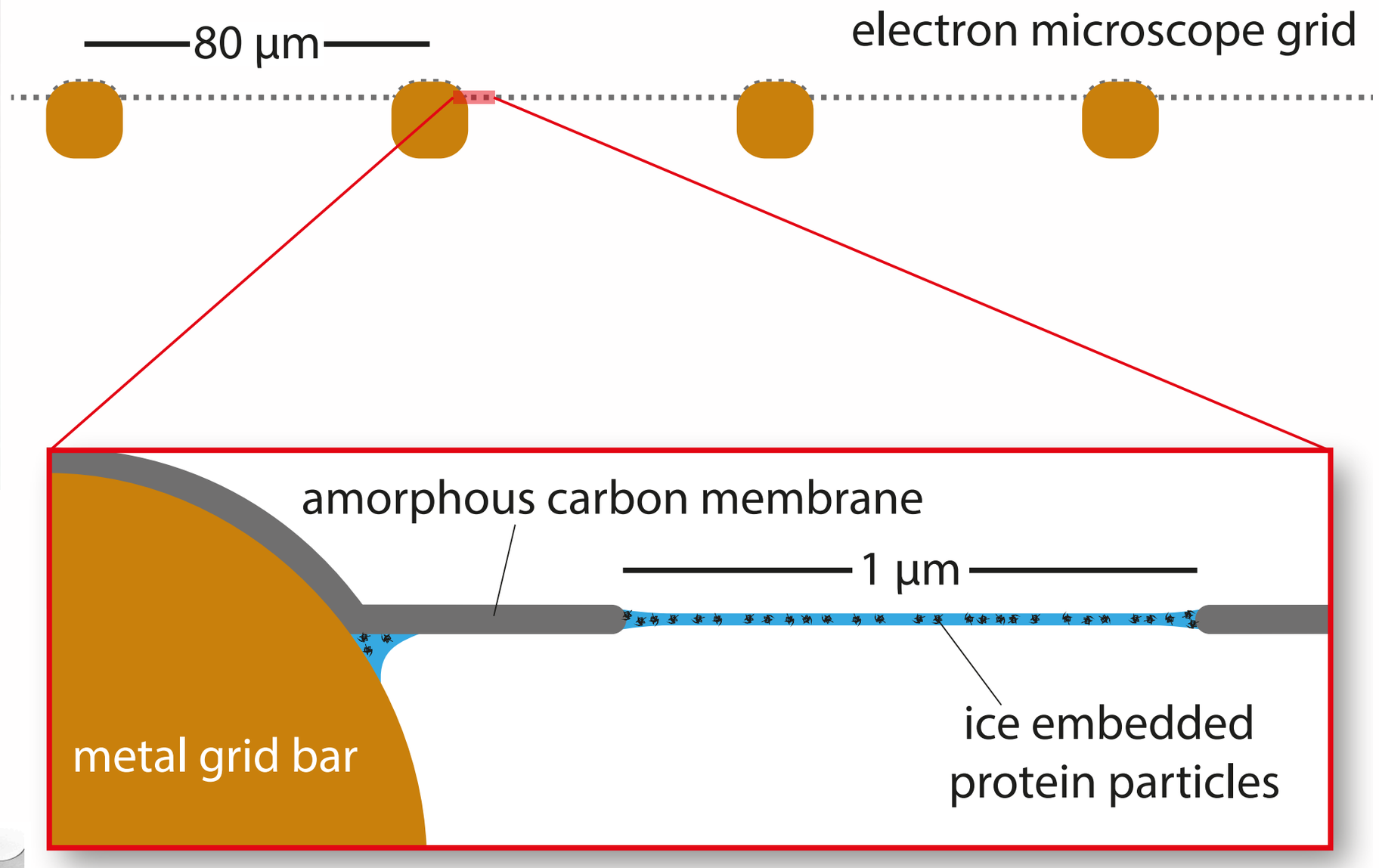
MRC Laboratory of Molecular Biology

Traditional substrates for cryo-EM



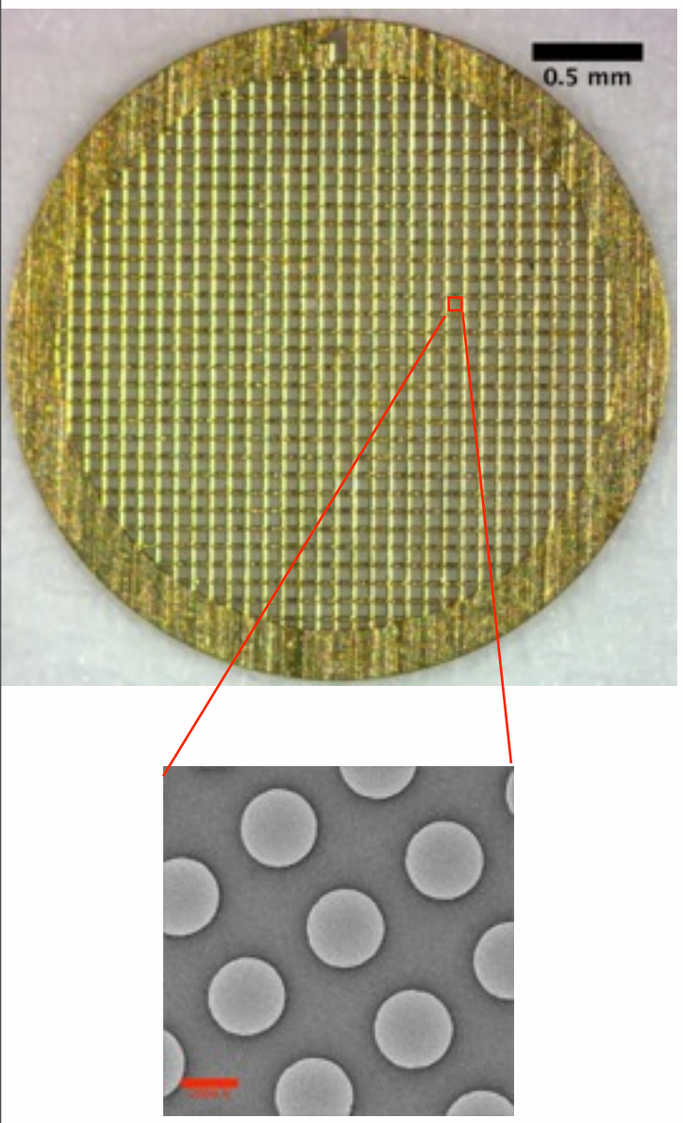


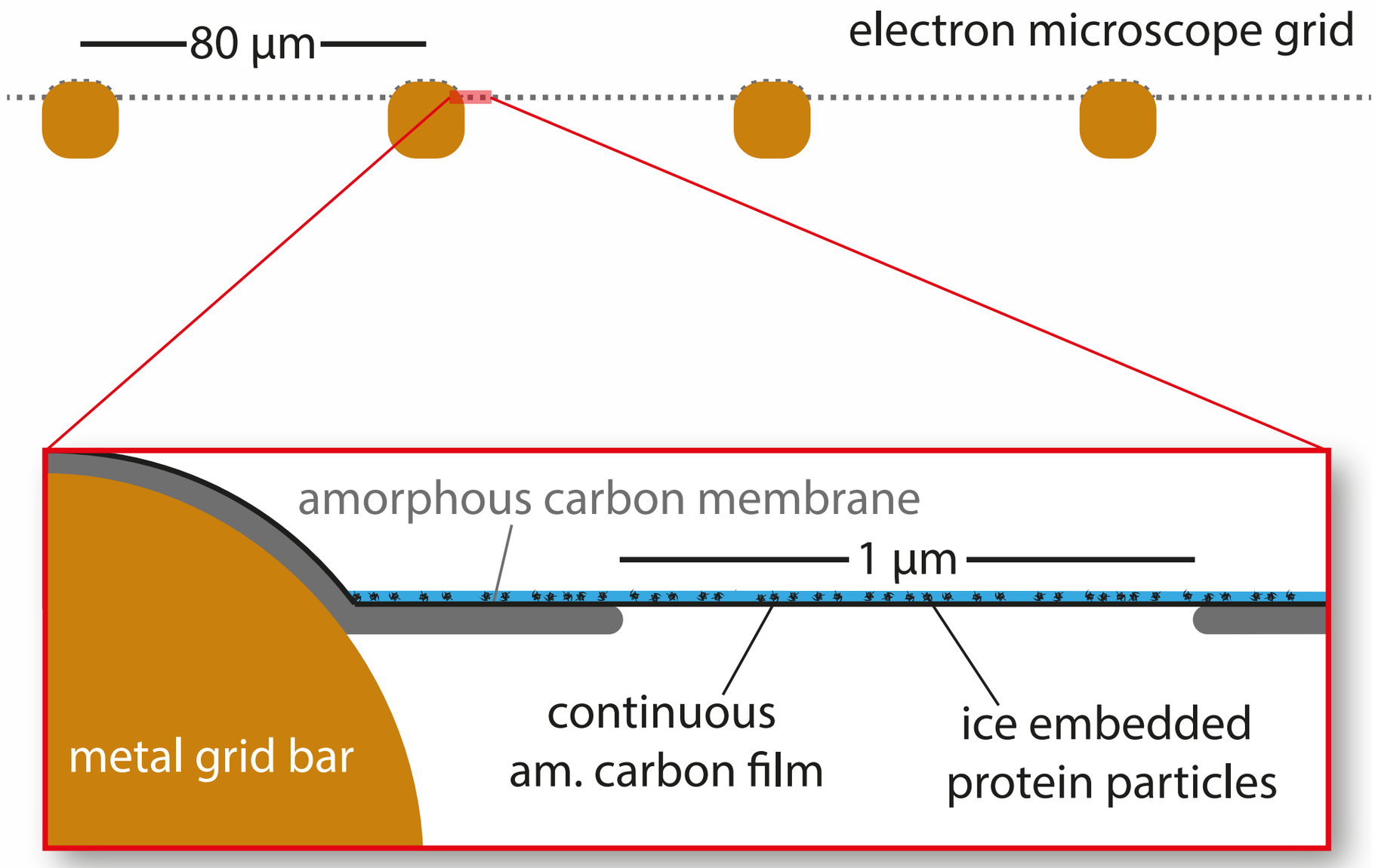


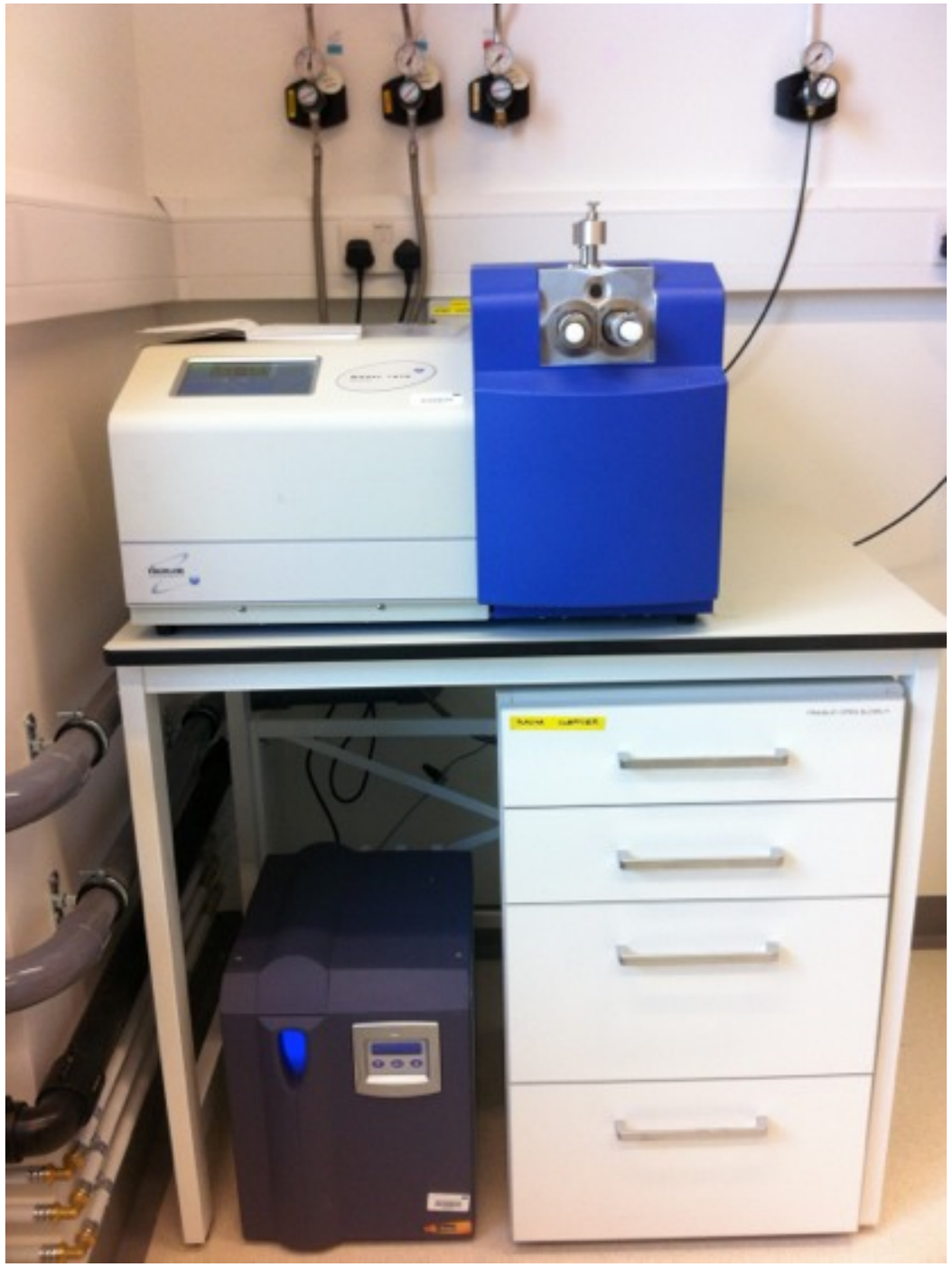


Quantifoil, C-flat
Cryomesh

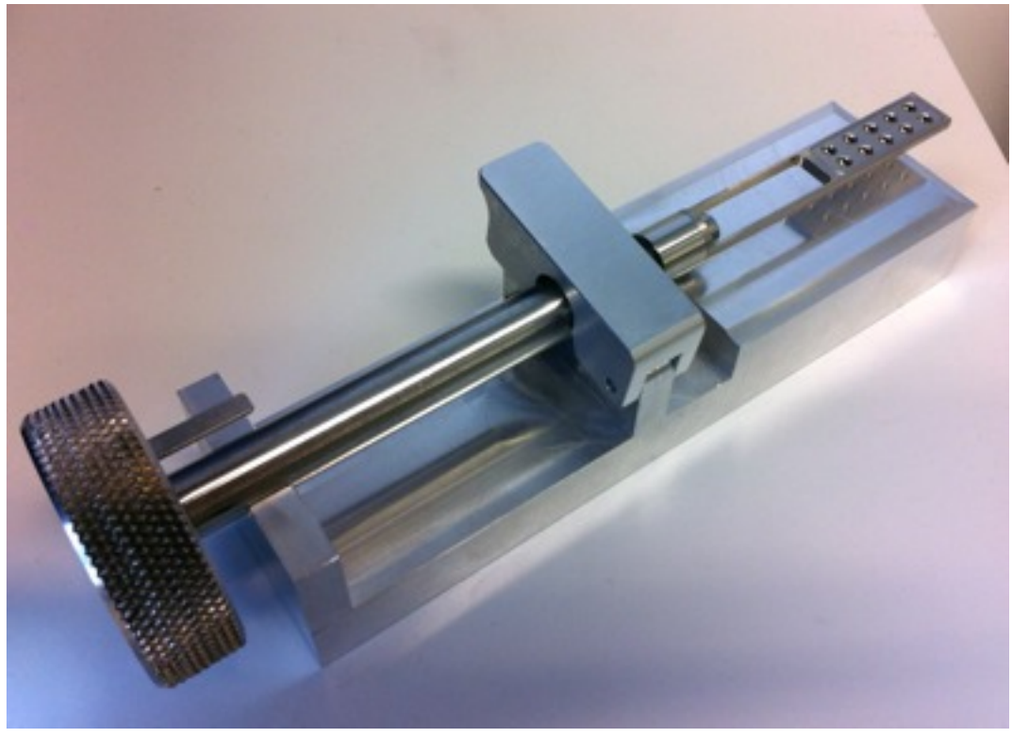
Traditional substrates for cryo-EM







Plasma chamber



Plasma created by ionisation of a gas under low vacuum
E.g. in air (glow discharge), oxygen, argon, hydrogen

Traditional substrates for cryo-EM

- Proteins interact with surfaces present during the blotting process

  ➡ Denaturation of proteins, preferential orientations

- Electron radiation induces motion of the particles and substrates

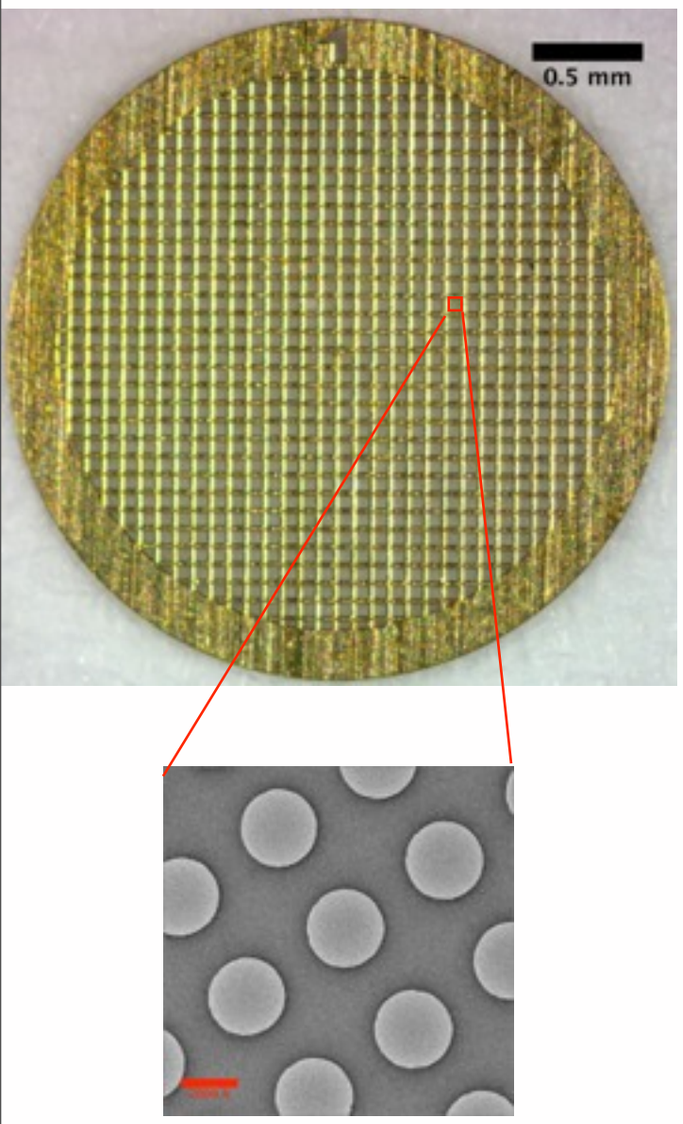
  ➡ Image blurring

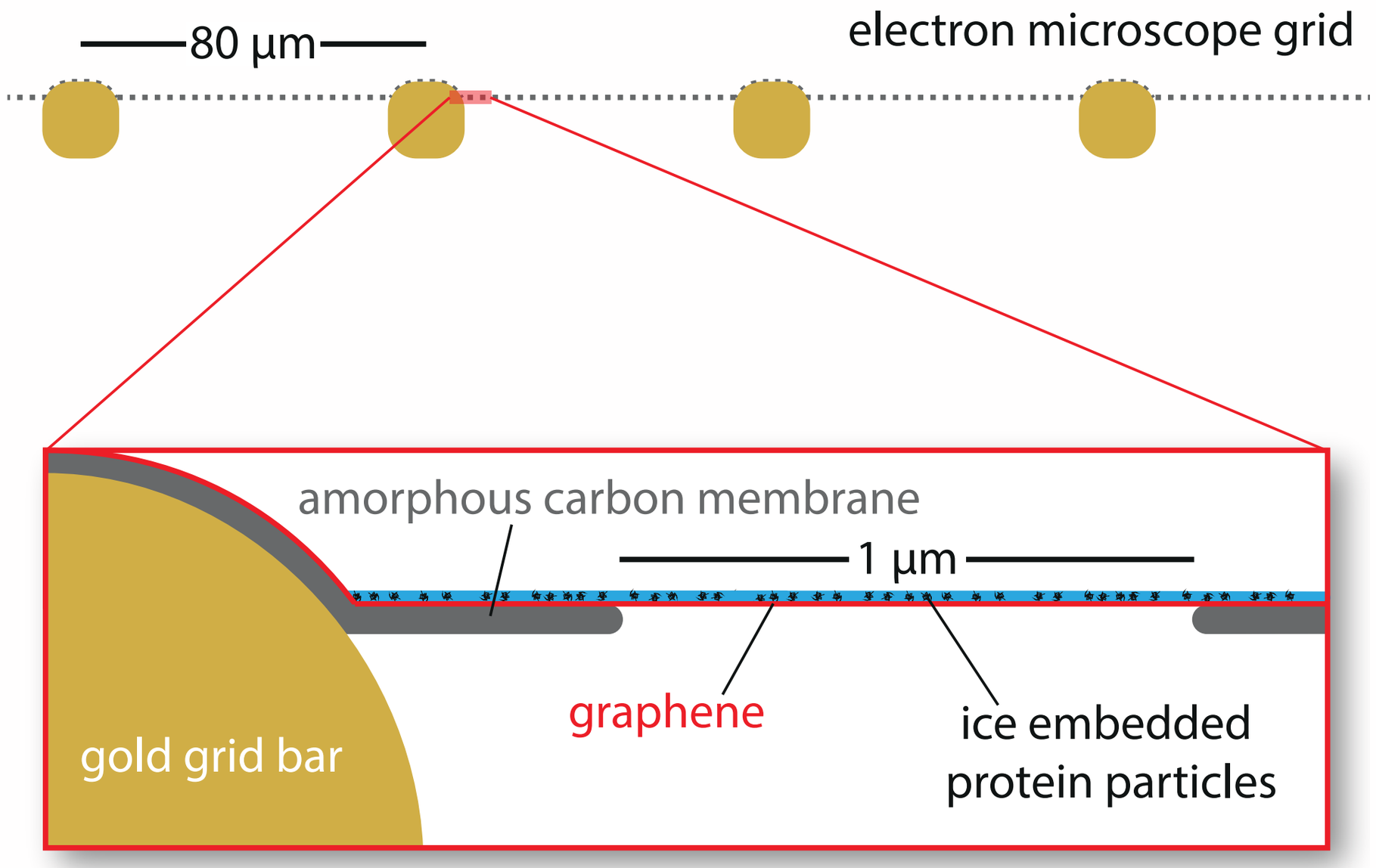
- Additional layer of carbon reduces signal to noise per particle

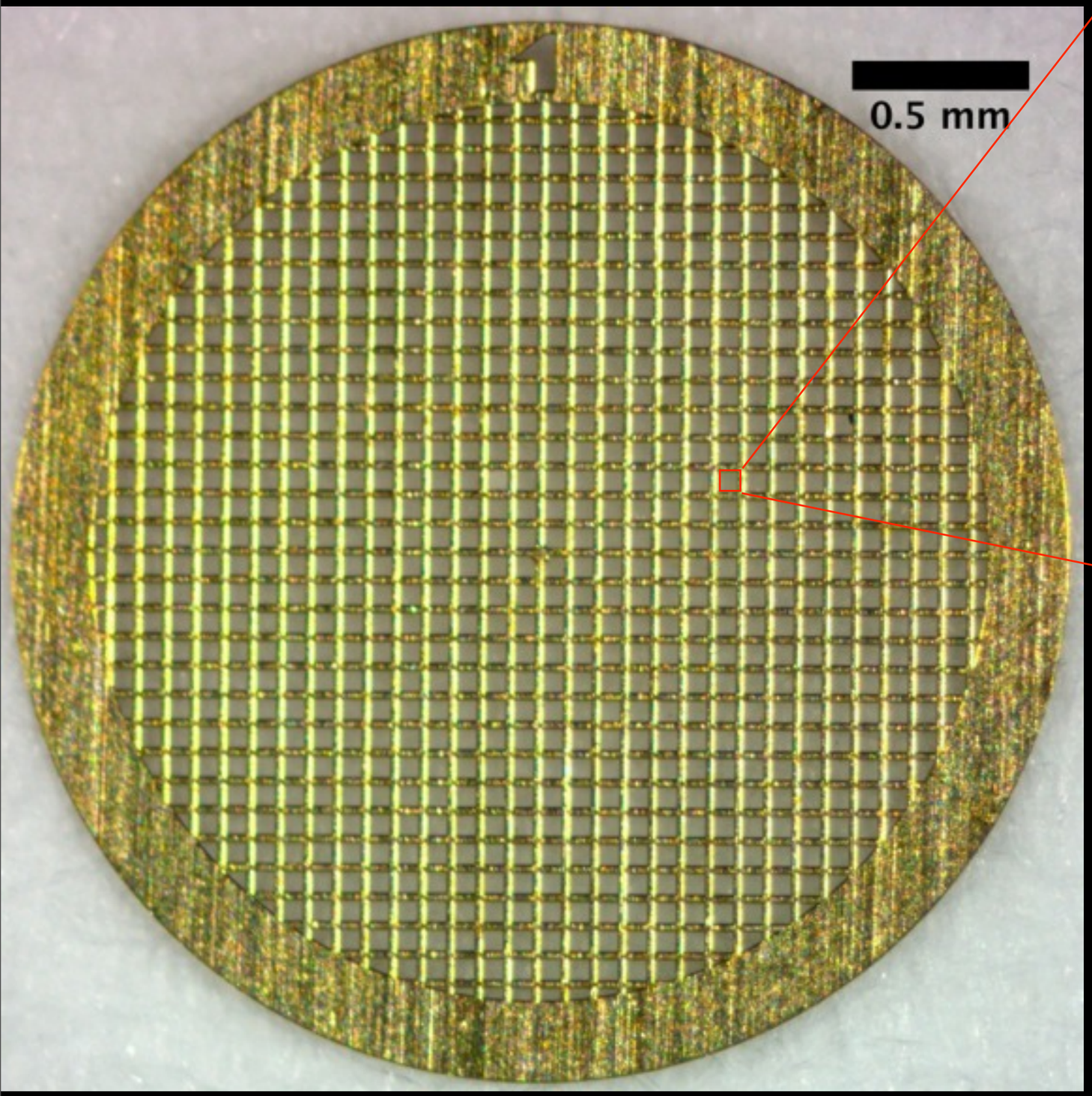
  ➡ alignment more difficult

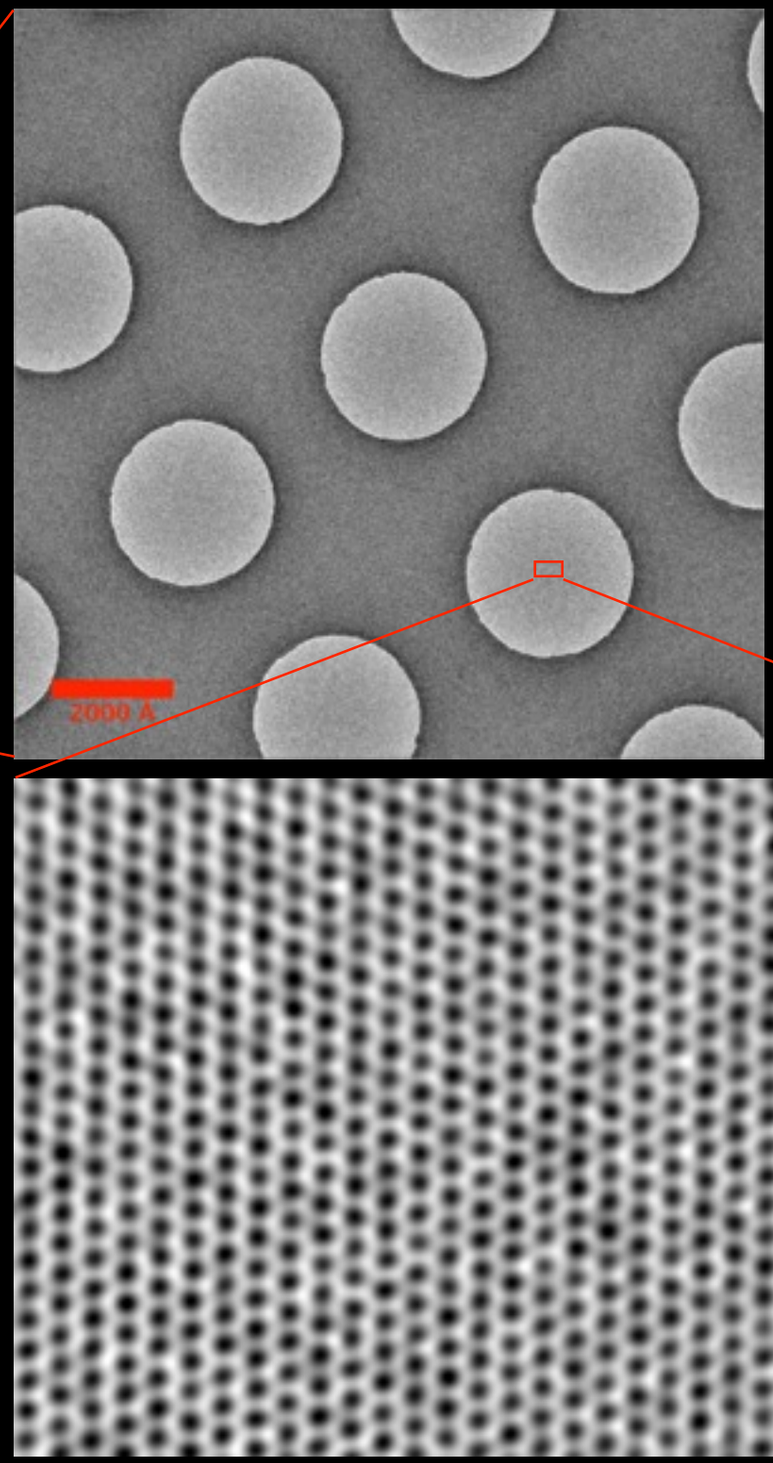
- Overall lack of reproducibility from grid to grid

Graphene substrates for cryo-EM

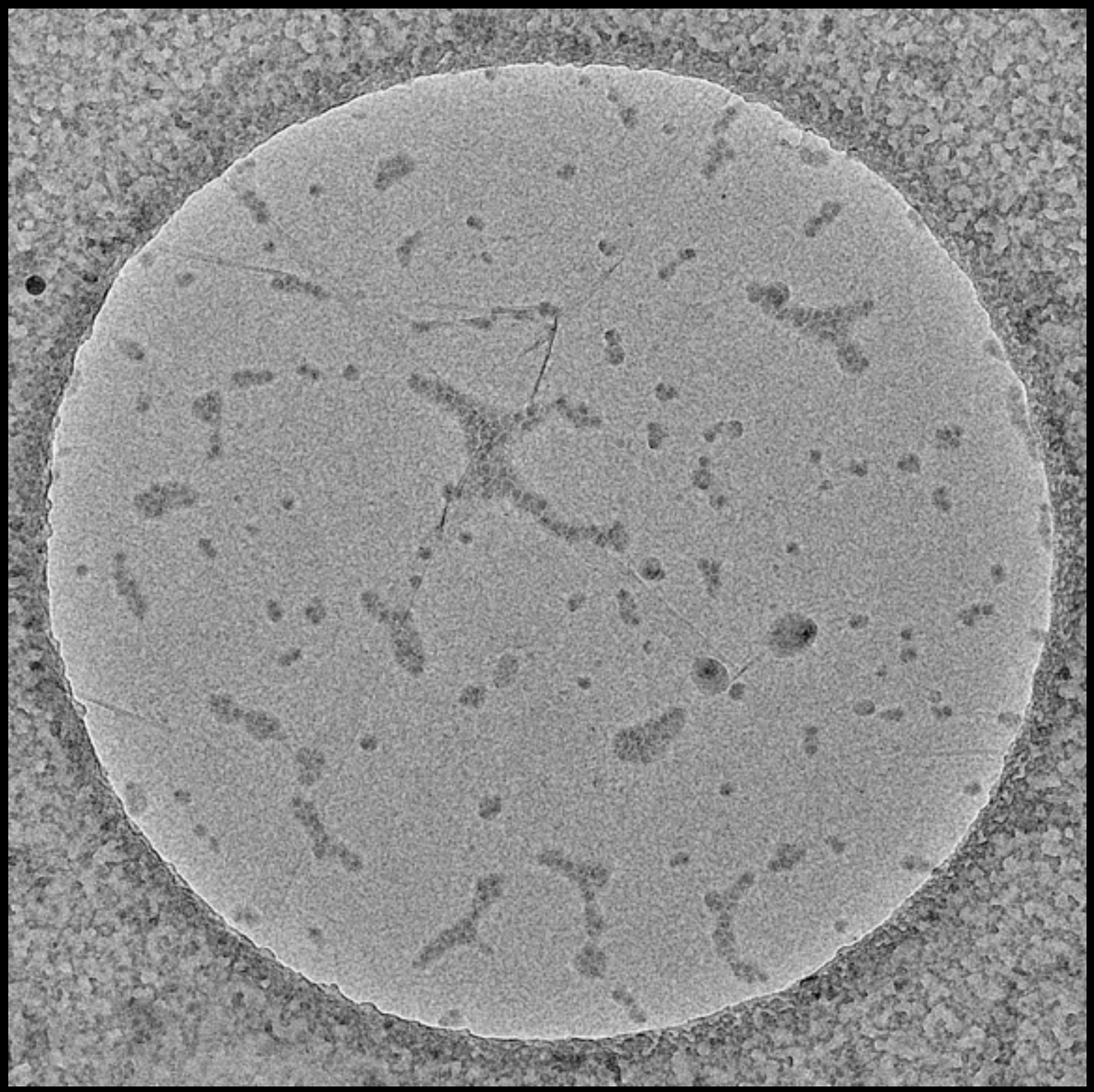





0.5 mm


2000 Å

10 Å



70S Ribosomes on graphene as synthesised

1.2 μm hole

So how do we make graphene more hydrophilic so we can use it for cryoEM?

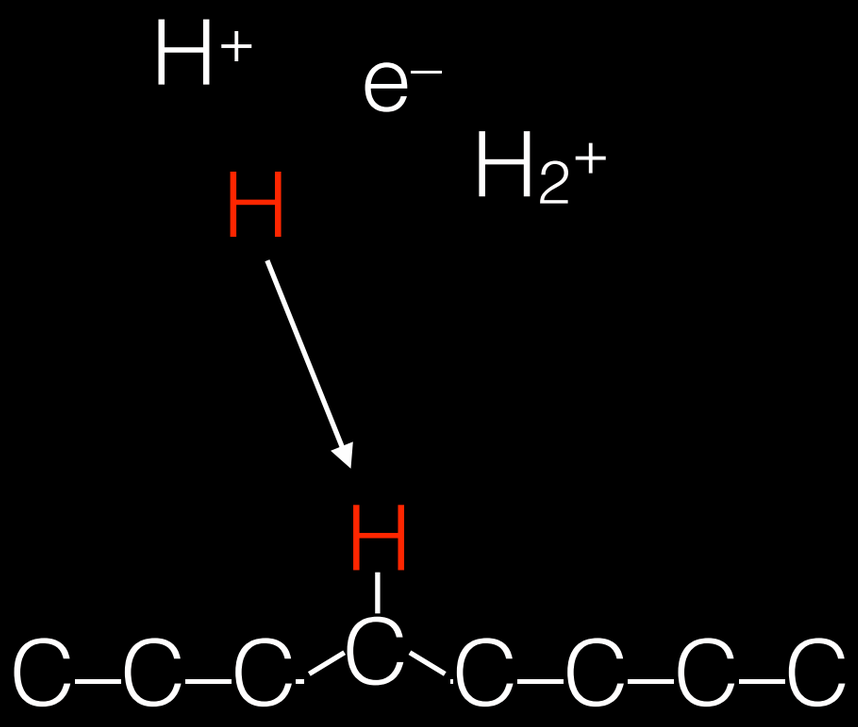
Partial hydrogenation: Russo and Passmore (2014) Nature Methods

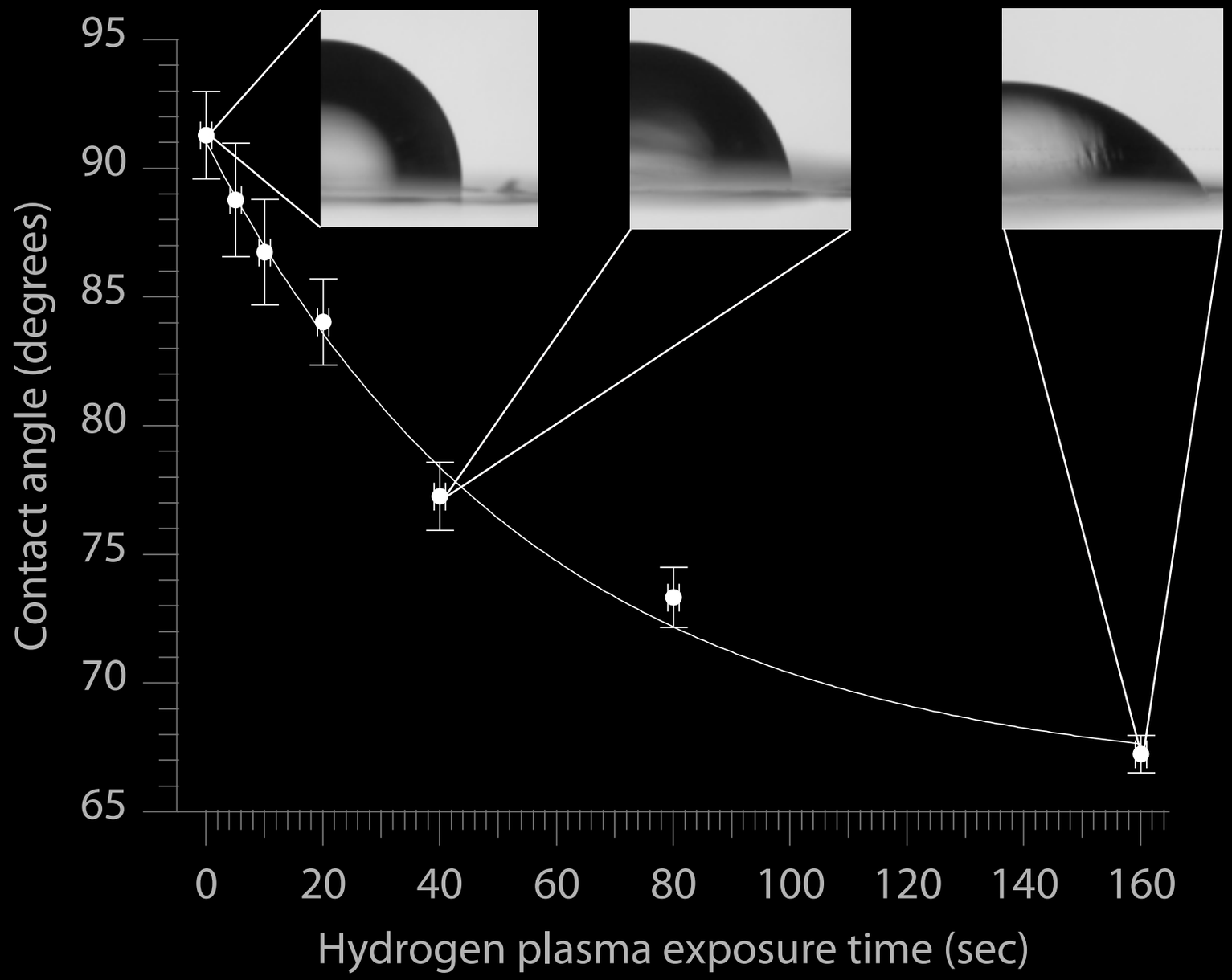
Graphene oxide: Pantelic, Stahlberg et al (2010) JSB, (2011) JSB, (2011) Nano Lett

Aromatic functionalisation: Pantelic et al (2014) Appl Phys Lett

Amorphous carbon: Sader, Rosenthal et al (2013) JSB

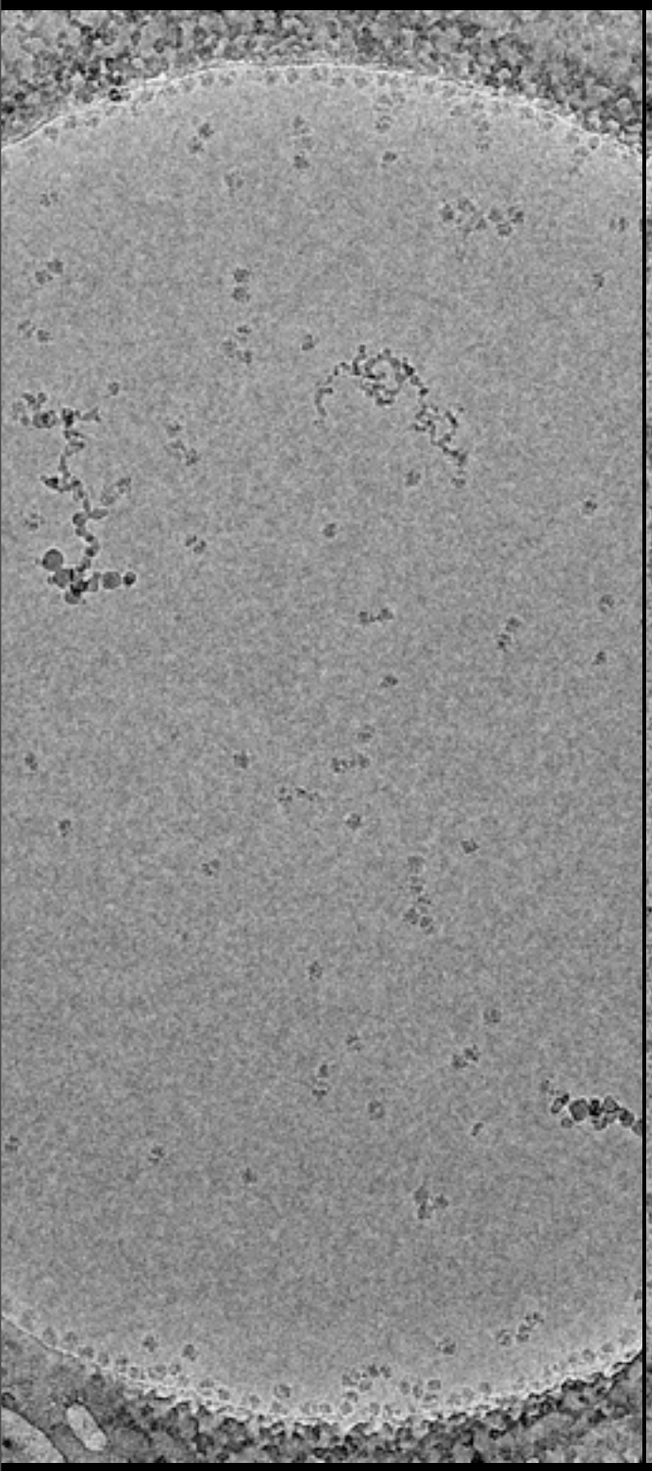
Hydrogen plasma



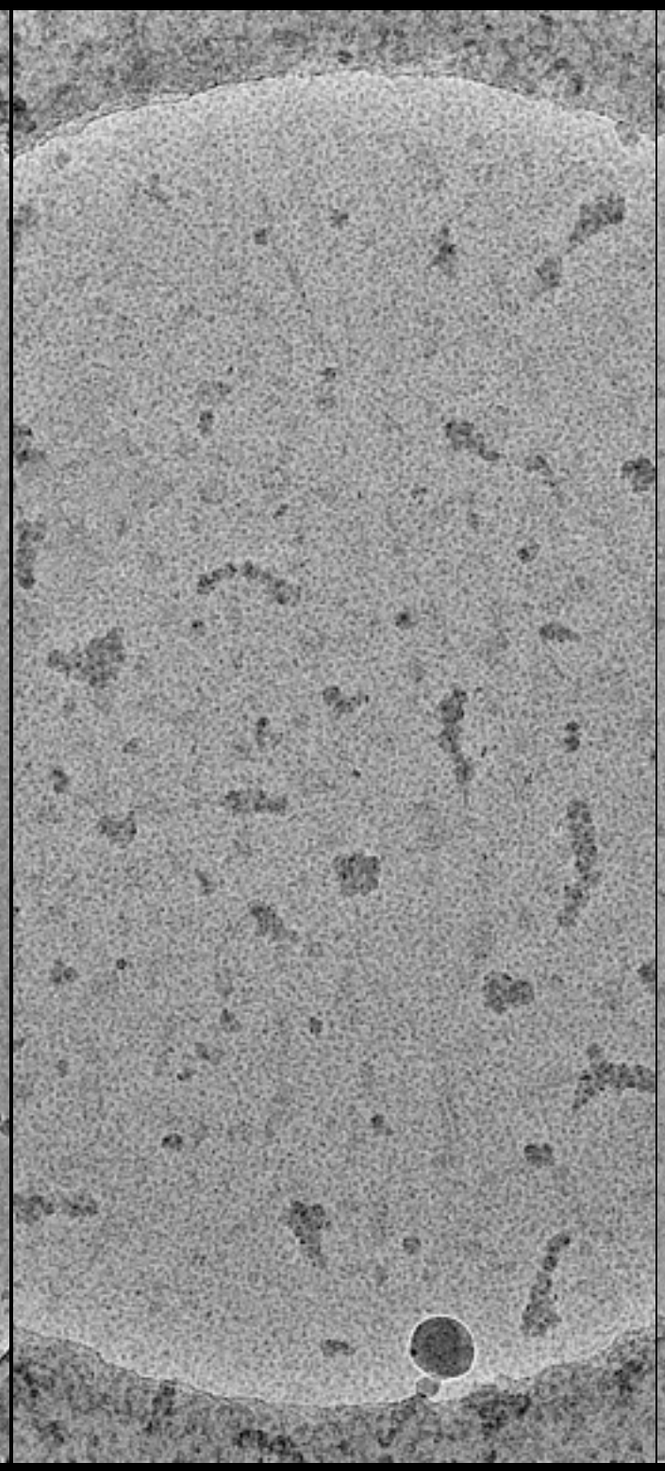


Graphene 21 eV bond

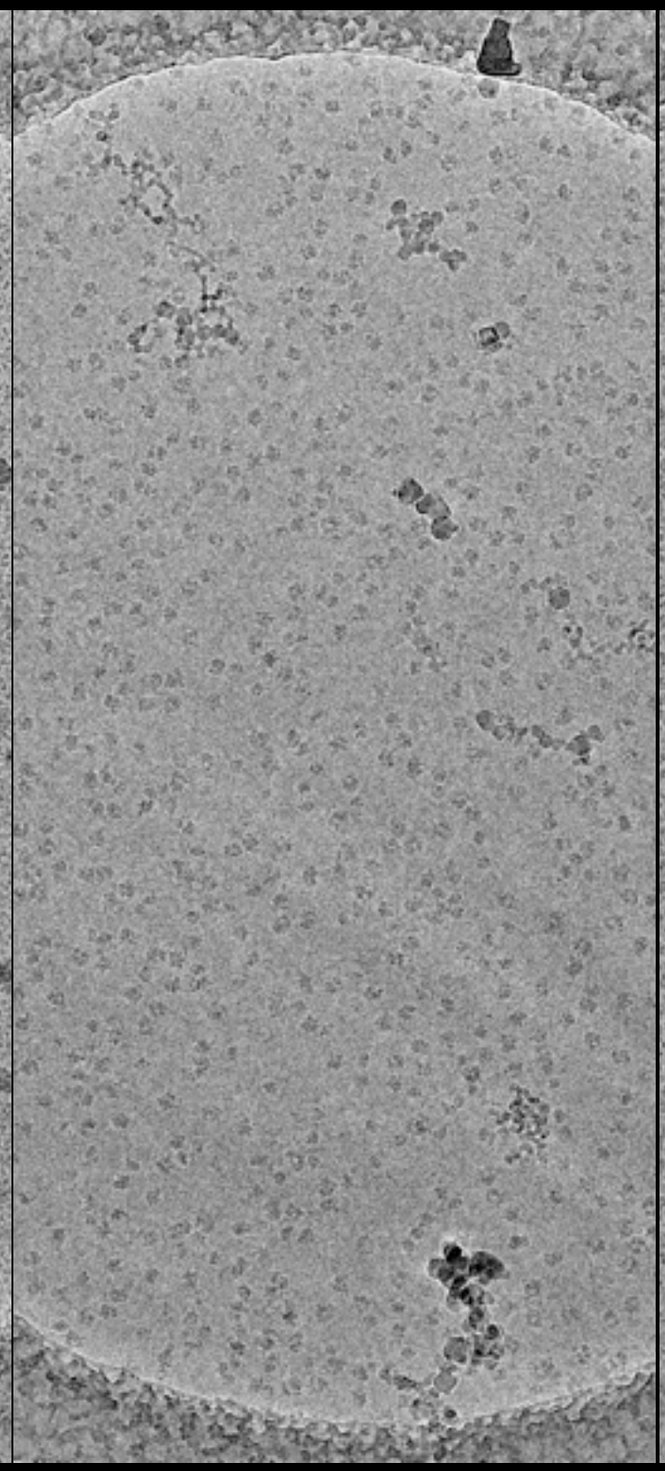
Russo & Passmore (2014) Nature Methods



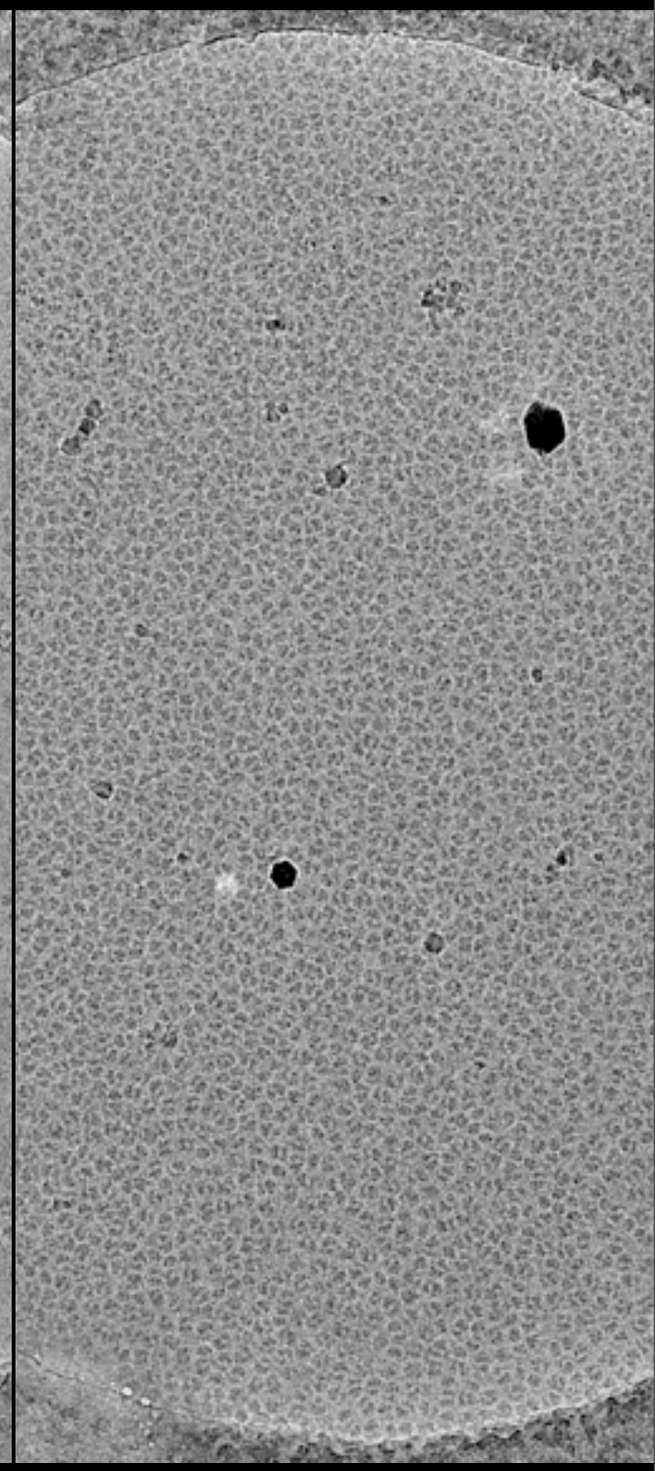
no graphene



graphene +
10 s hydrogen

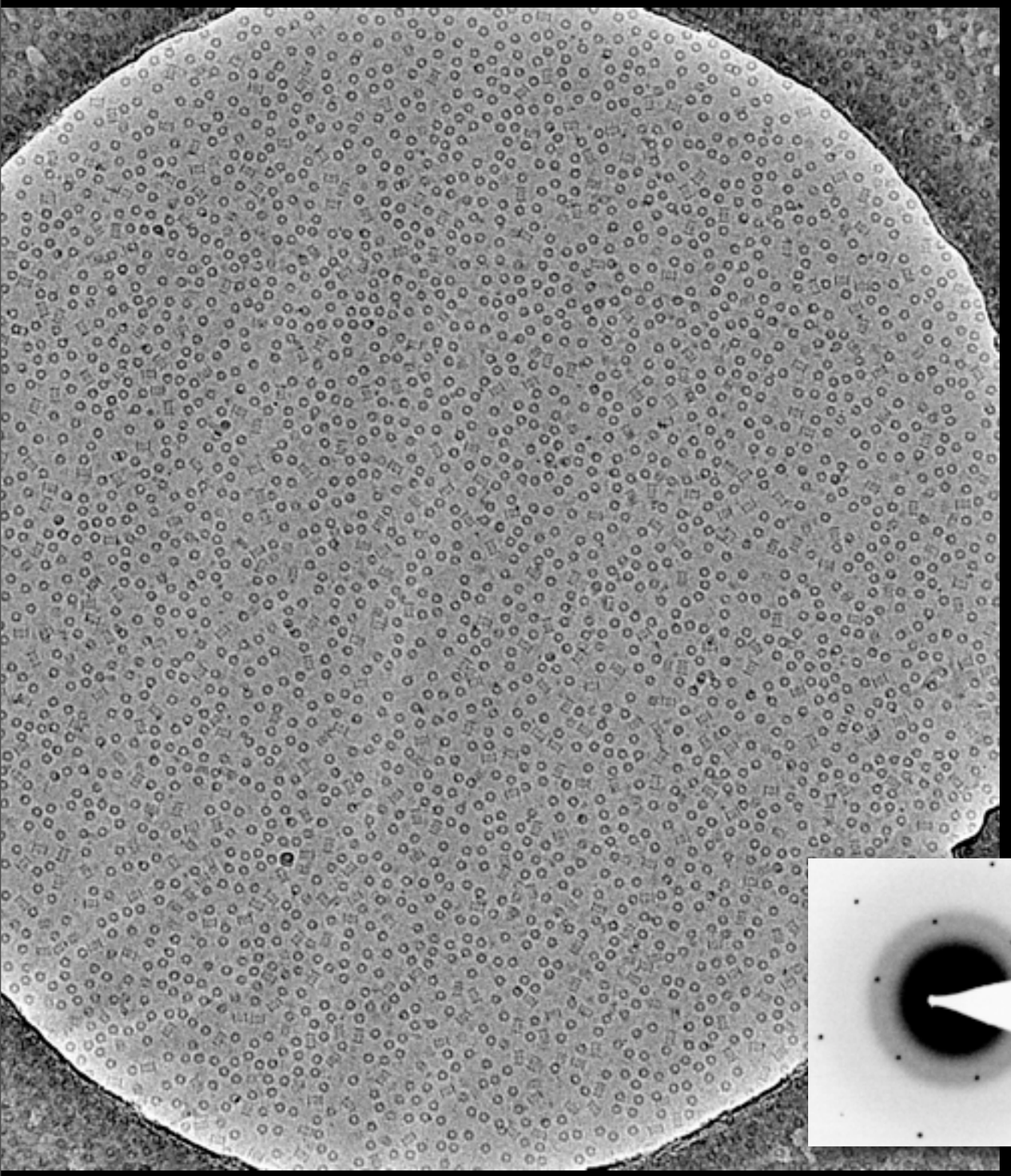


graphene +
20 s hydrogen

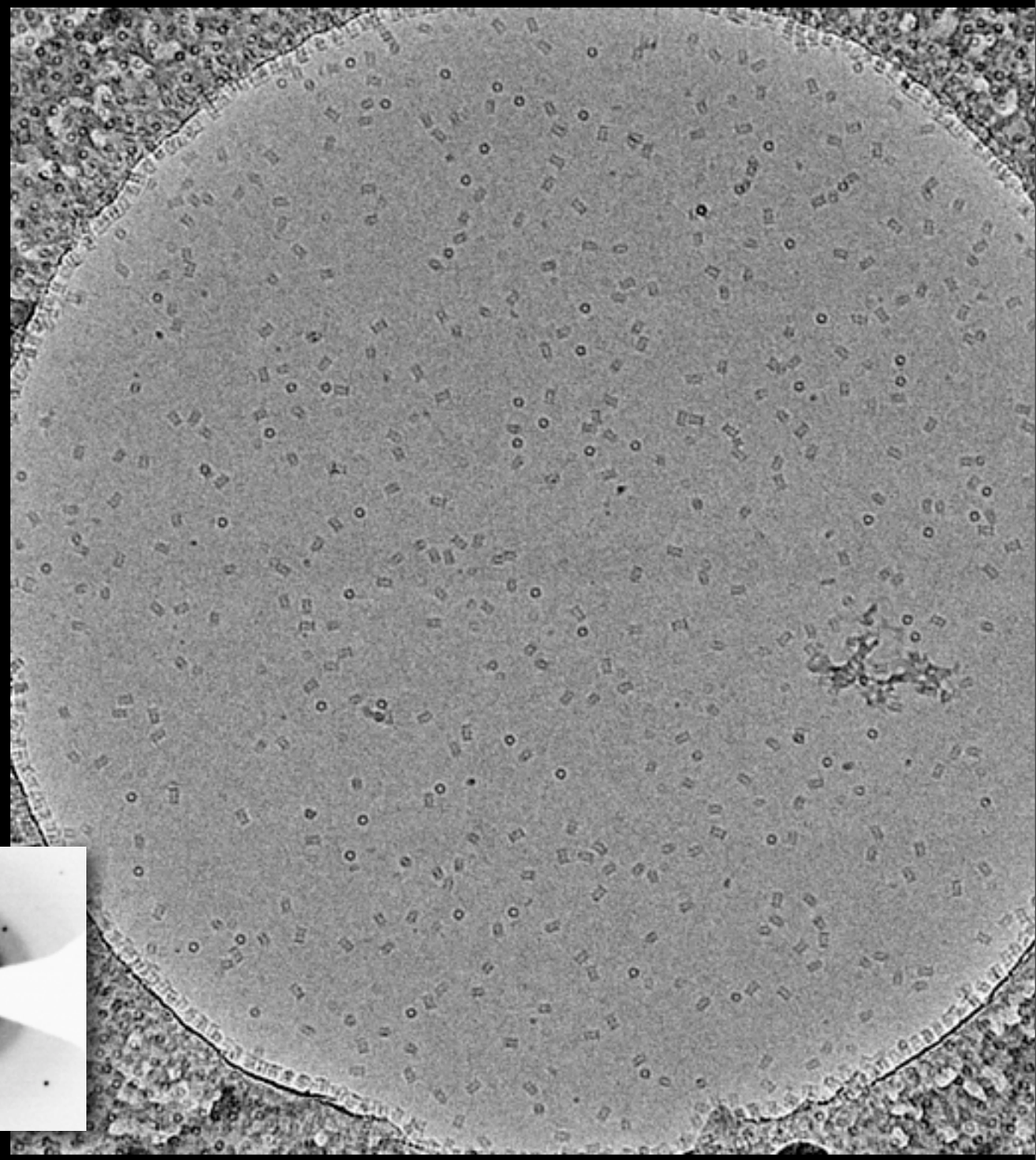


graphene +
40 s hydrogen

Human 20S proteasome

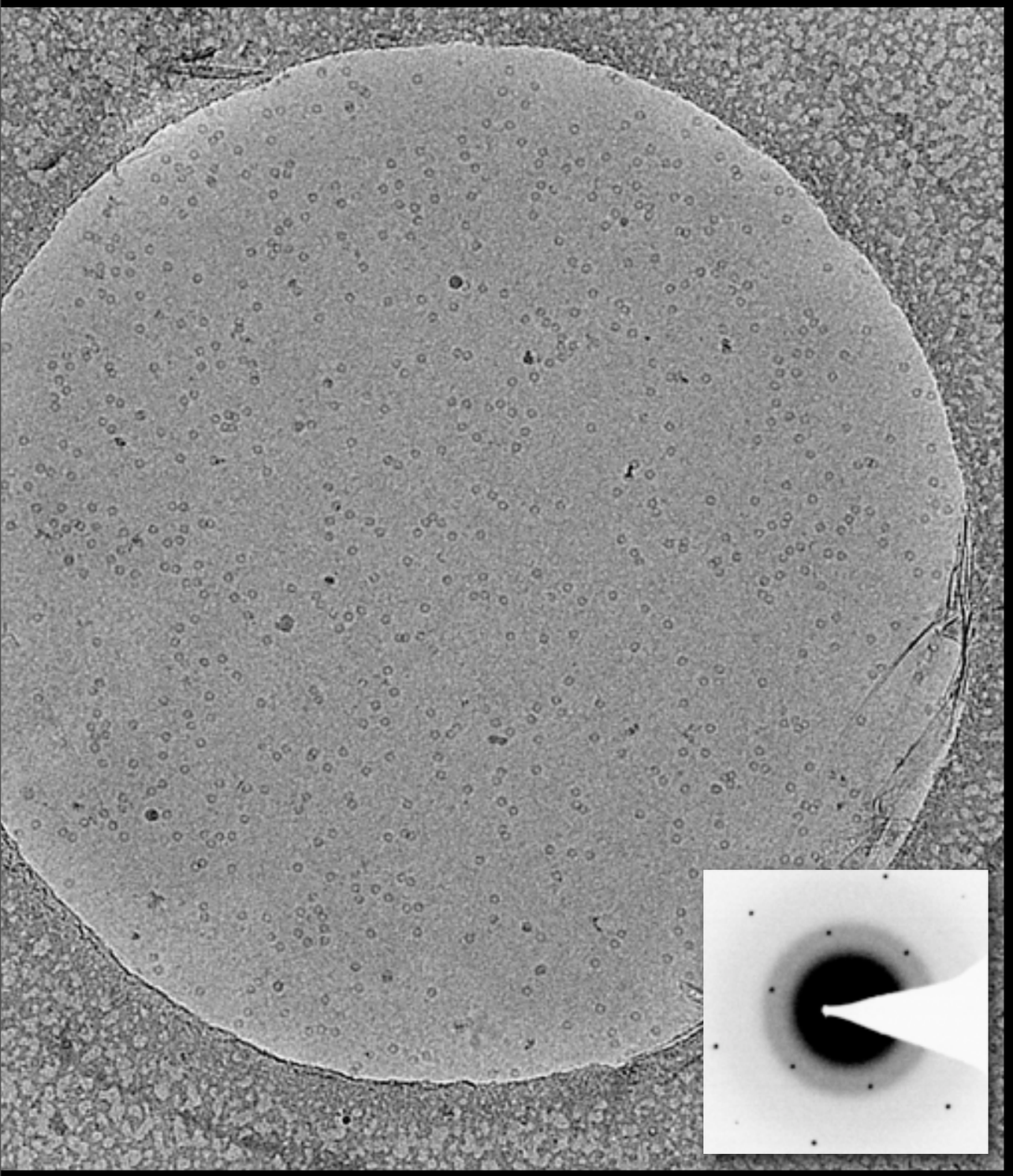


on graphene

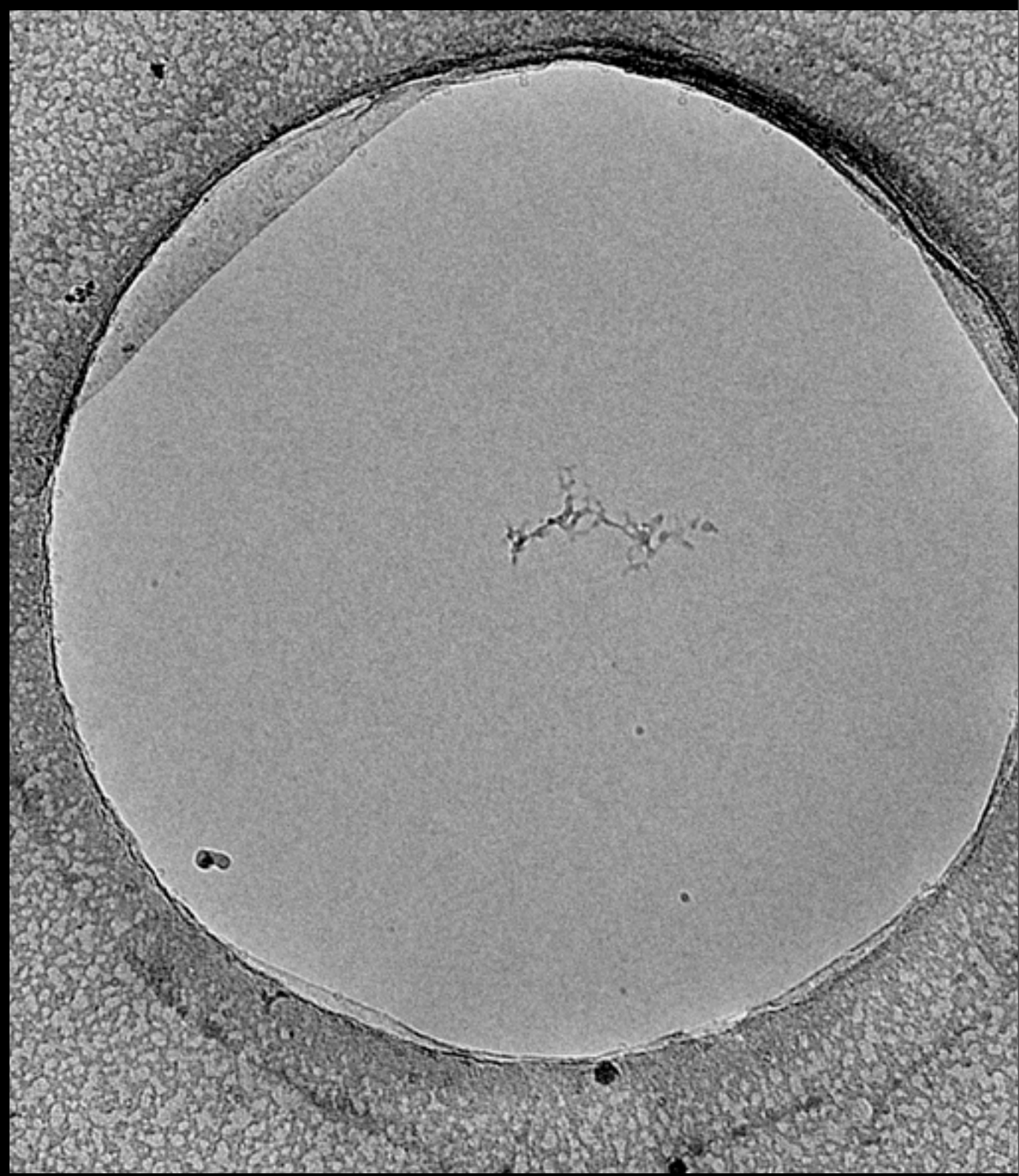


no graphene

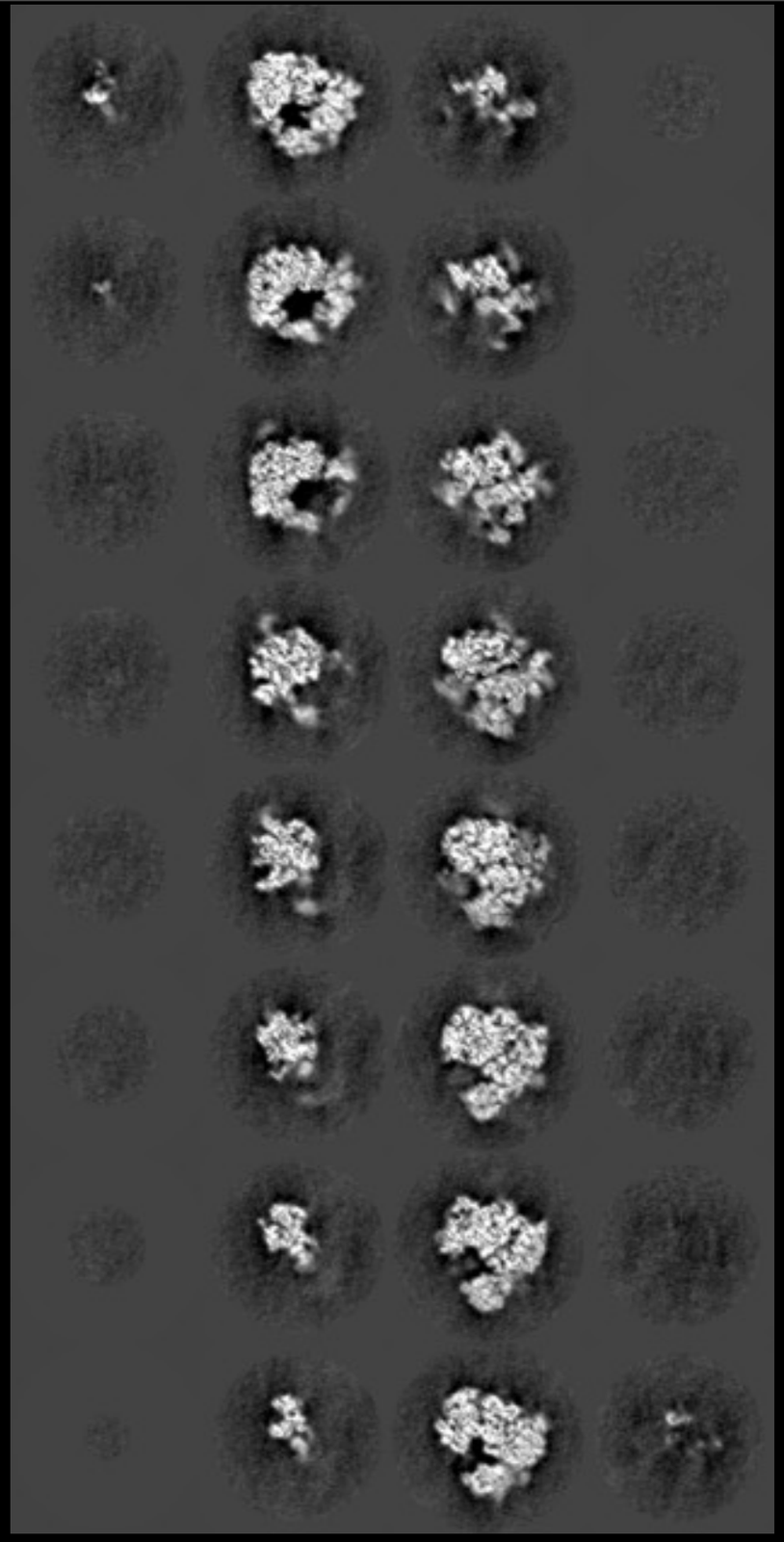
Apoferritin

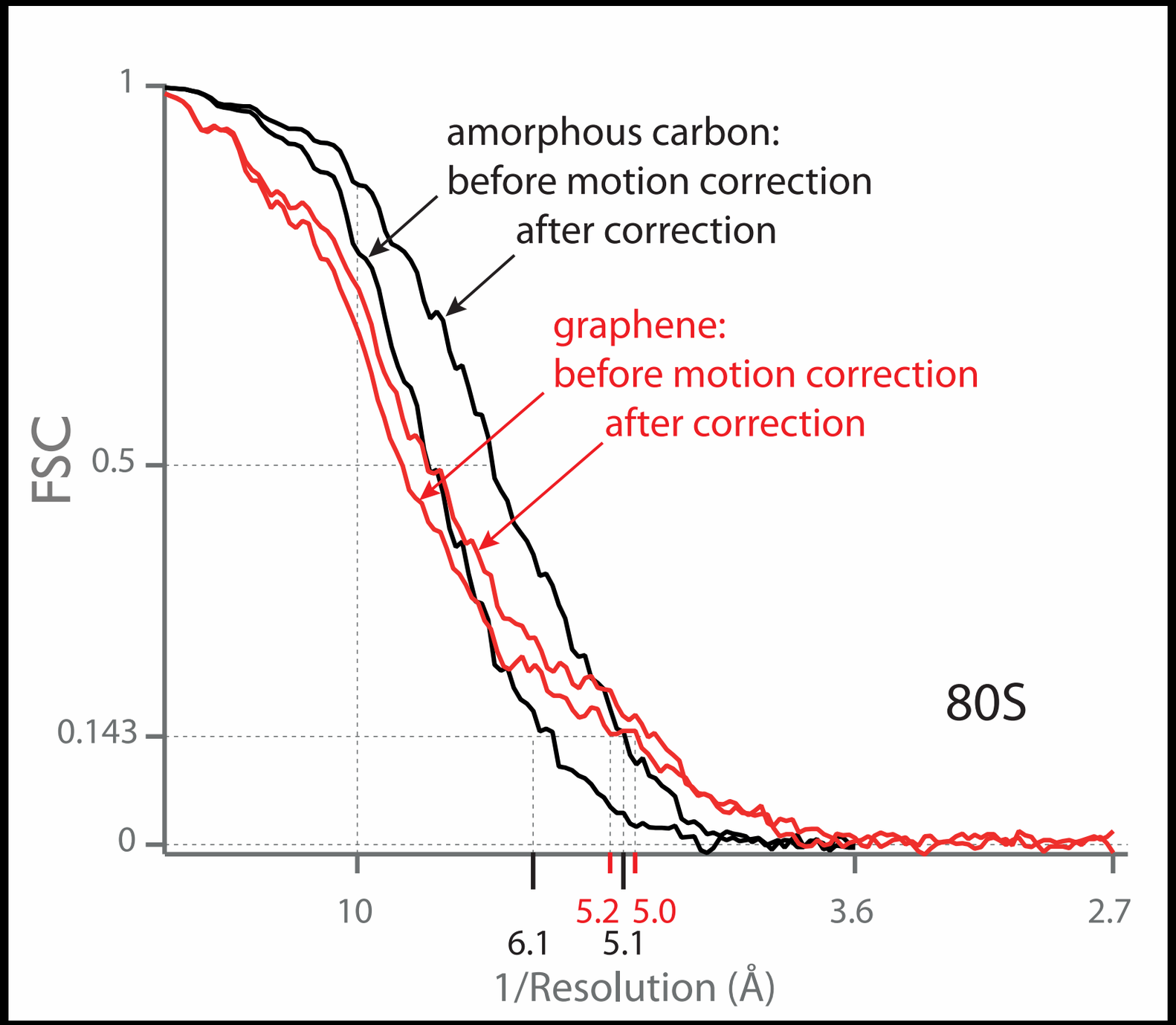


on graphene



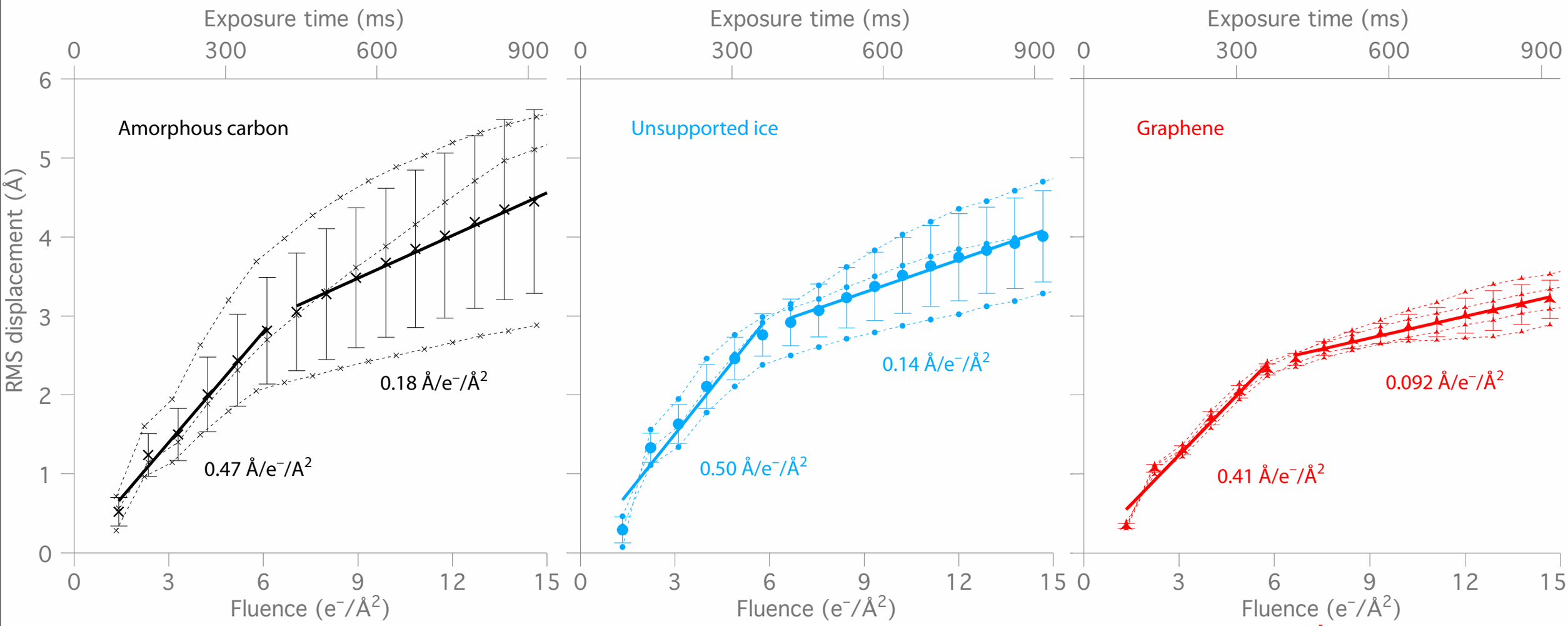
no graphene





20 thousand particles
5.2 Å without motion correction, 5.0 Å with

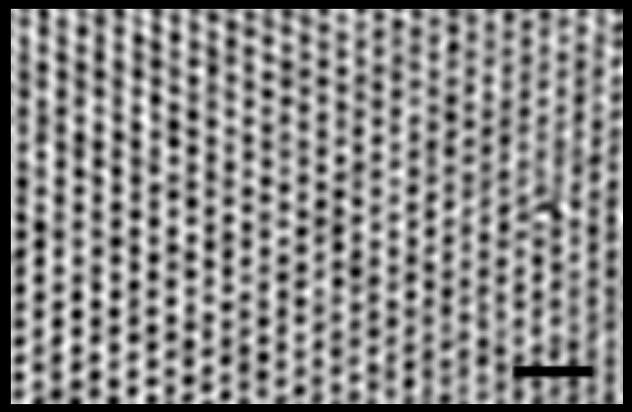
Ribosome speed plots

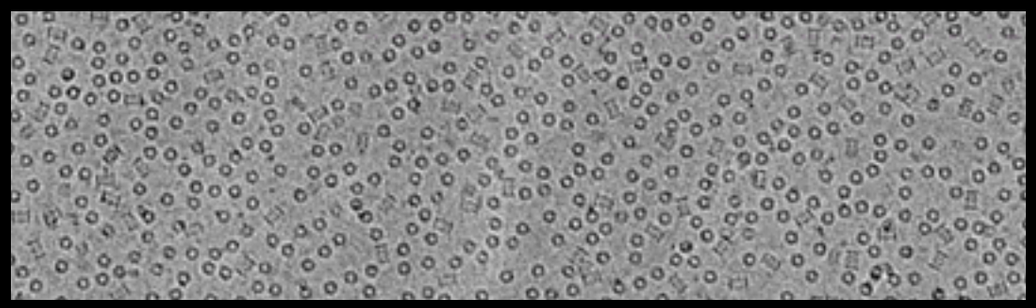


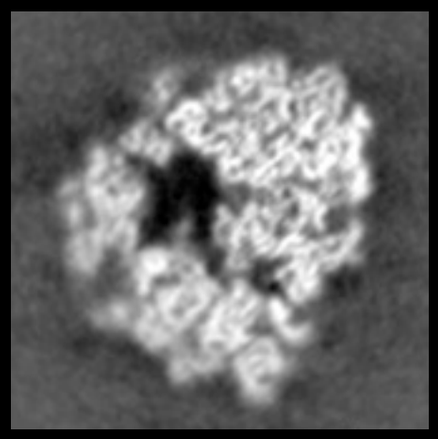
amorphous carbon on quantifoil

quantifoil

graphene on quantifoil







- Graphene is an excellent support material for cryo-EM, particularly as an alternative to thin amorphous carbon

- We can modify and control the surface properties of graphene with low-energy plasmas

- Using graphene instead of amorphous carbon reduces noise and radiation induced motion

Russo & Passmore (2014) Nature Methods