New substrates for electron cryo-microscopy

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Traditional substrates for cryo-EM

- Quantifoil, C-flat
- Cryomesh

- Electron microscope grid
- Metal grid bar
- Amorphous carbon membrane
- Ice embedded protein particles

Dimensions:
- 80 μm
- 1 μm
Traditional substrates for cryo-EM

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Traditional substrates for cryo-EM.
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

- gold grid bar
- amorphous carbon membrane
- ice embedded protein particles
- electron microscope grid

- graphene
- 80 μm
- 1 μm
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?


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

\[ \text{H}^+ + e^- \rightarrow \text{H}_2^+ \]

Graphene 21 eV bond

no graphene  
graphene + 10 s hydrogen  
graphene + 20 s hydrogen  
graphene + 40 s hydrogen
Apoferitin on graphene vs. no graphene.
20 thousand particles
5.2 Å without motion correction, 5.0 Å with
Ribosome speed plots

- Amorphous carbon on quantifoil
  - RMS displacement (Å)
  - Exposure time (ms)
  - Fluence (e\(^{-}/\text{Å}^2\))
  - 0.18 Å/e\(^{-}/\text{Å}^2\)
  - 0.47 Å/e\(^{-}/\text{Å}^2\)

- Unsupported ice on quantifoil
  - RMS displacement (Å)
  - Exposure time (ms)
  - Fluence (e\(^{-}/\text{Å}^2\))
  - 0.14 Å/e\(^{-}/\text{Å}^2\)
  - 0.50 Å/e\(^{-}/\text{Å}^2\)

- Graphene on quantifoil
  - RMS displacement (Å)
  - Exposure time (ms)
  - Fluence (e\(^{-}/\text{Å}^2\))
  - 0.092 Å/e\(^{-}/\text{Å}^2\)
  - 0.41 Å/e\(^{-}/\text{Å}^2\)
• 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