Glass: a multi-platform specimen supporting substrate for precision single molecule studies of membrane proteins

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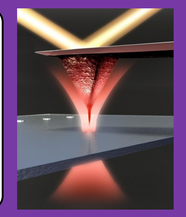
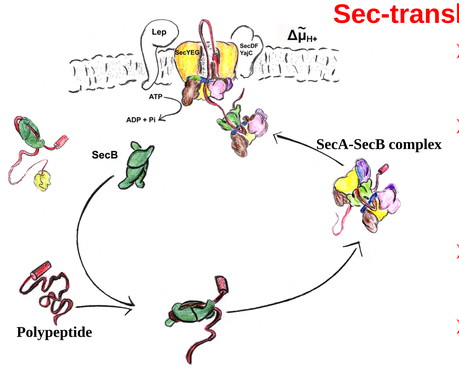


Abstract

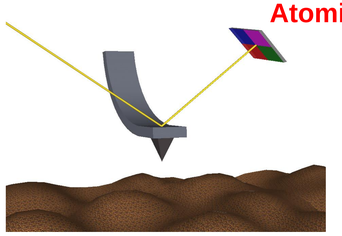
High resolution (∼1 nm lateral resolution) biological AFM imaging has been carried out almost exclusively using freshly cleaved mica as a specimen supporting surface, but mica suffers from a fundamental limitation that has hindered AFM's broader integration with many modern optical methods. Mica exhibits biaxial birefringence; indeed, this naturally occurring material is used commercially for constructing optical wave plates. In general, propagation through birefringent material alters the polarization state and bifurcates the propagation direction of light in a manner which varies with thickness. This makes it challenging to incorporate freshly cleaved mica substrates with modern optical methods, many of which employ highly focused and polarized laser beams passing through the specimen plane. Using bacteriorhodopsin from *Halobacterium salinarum* and the translocon SecYEG from *Escherichia coli,* we demonstrate that faithful images of 2D crystalline and non-crystalline membrane proteins in lipid bilayers can be obtained on common microscope cover glass following a straight-forward cleaning procedure. Direct comparison between data obtained on glass and on mica show no significant differences in AFM image fidelity. This work opens the door for combining high resolution biological AFM with powerful optical methods that require optically isotropic substrates such as ultra-stable[1] and direct 3D AFM[2]. In turn, this capability should enable long timescale conformational dynamics measurements of membrane proteins in near-native conditions.

Sec-translocase



- >30% of proteins are transported from the site of synthesis into or through a membrane[3,4]
- In *E. coli*, the Sec system orchestrates the translocation of polypeptides across membrane before they acquire stable tertiary structure and SecYEG provides the path way[3,4]
- SecA and SecB act as chaperones to maintain newly synthesized polypeptides in a state compatible with transport[6]
- Numerous questions remain regarding the mechanistic details of translocation[3,4,5]

Atomic Force Microscopy (AFM)



- Molecular-scale resolution[3]
- Imaging in near native lipid environment[3]
- Minimal concentration requirements[3]
- Single molecule dynamics[3]
- Drift (>1nm/min on each axis) is a common problem with commercial instruments in biological settings[1]

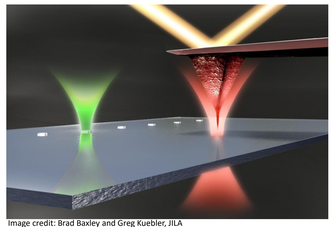
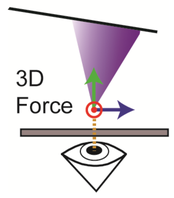


Image credit: Brad Baxley and Greg Kuebler, JILA

Ultra Stable 3D AFM



- Recent techniques[1,2] employ two additional lasers, one focused on fiducial mark and other focused on AFM-tip for tip-sample registration and tip trajectory
- Drift can be reduced to <5pm/min at 25° C
- Direct access to ***F***

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Acknowledgements

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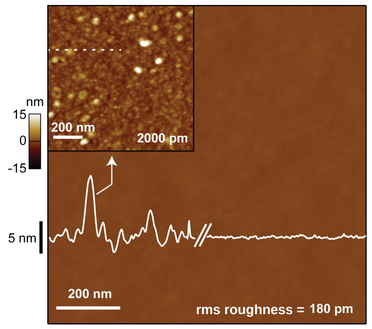
Why use glass as a substrate?

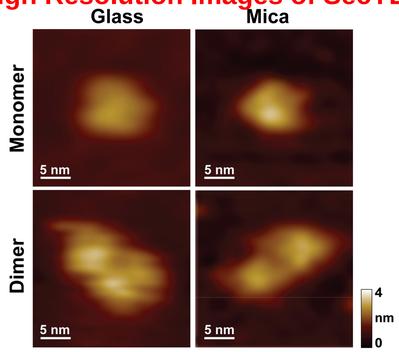
- Typical AFM substrates mica and HOPG exhibit birefringence, complicating optical paths[3]
- Many well established biological and biochemical assays like FRAP, FRET, Fluorescence Microscopy, FIONA and TIRF require transparent and optically homogeneous substrates[3]
- Recently developed techniques like US-AFM[1], 3D AFM[2] and polarization anisotropy methods require transparent substrates and non-birefringence due to polarization based detection
- Amorphous and optically homogeneous substrates like glass enable coupling AFM with advanced imaging techniques[3]

Raw glass vs. KOH treated glass



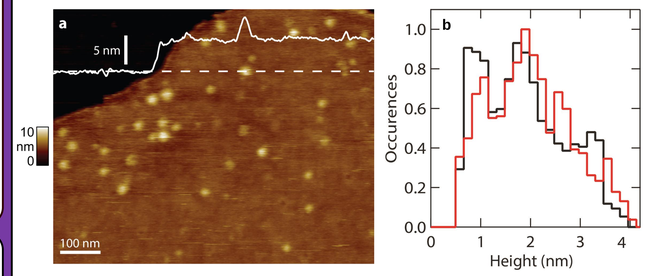
KOH cleaned glass vs. raw glass[3] (inset) shows >10 fold reduction in rms roughness (bottom right corners).

High Resolution Images of SecYEG



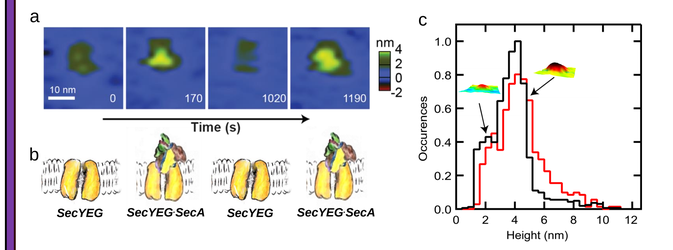
SecYEG heights and volumes on glass agree well with that on mica and X-ray crystallography[3]

Heights of SecYEG



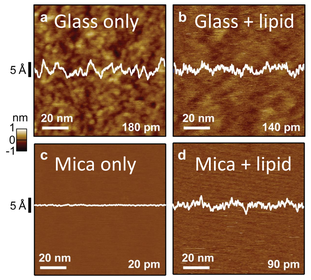
(a) Image of SecYEG in lipid bilayer on glass with a cross section profile (solid white line), and (b) SecYEG heights on glass (*red*) and mica (*black*)[3].

SecYEG-SecA interactions

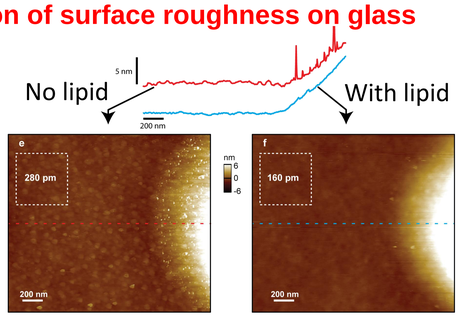


Direct visualization of native protein-protein interactions in lipid[3]. (a) Binding and unbinding of SecA to SecYEG observed in membrane. Cartoon depiction (b) of the events observed in panel (a). Height histogram (c) of SecYEG·SecA complex on glass (*red*) and mica (*black*) show overall agreement. The peak at 4 nm has been attributed to a highly active state of the translocase[3,6].

Direct visualization of reduction of surface roughness on glass

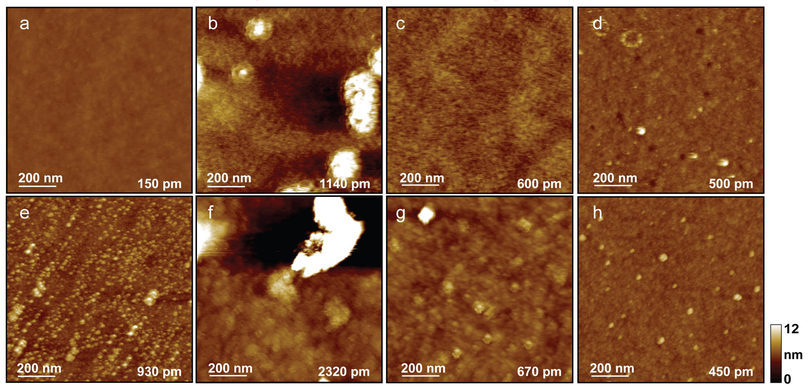


KOH treated glass surfaces become smoother (20% reduction, *N*=340) upon lipid coating. In contrast, mica becomes >4 fold rougher[3].



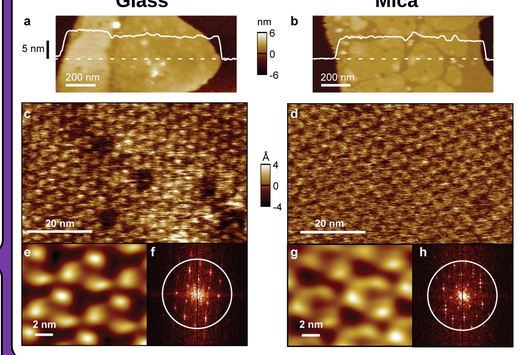
Upon lipid deposition KOH treated glass shows a ~40% reduction in rms roughness. Here the same area is shown before and after lipid coating[3].

Evaluating Different Cleaning Treatments



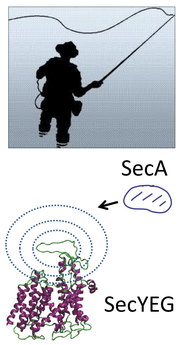
Glass treated with KOH (a), HF (b&c), NH_4F (d&e), KOH followed by HF (f&g) and KOH followed by NH_4F solution(h). rms roughness indicted at bottom right corner of each image[3]

Bacteriorhodopsin on Glass and Mica



BR patch on glass (a), mica (b), BR lattice on glass (c) and mica (d) with insets showing the arrangement of trimers (e&g) and FFT(f&h) with circle of nm^{-1} radius[3].

Potential Applications



- Novel AFM modalities could measure molecular "fly fishing"[5]
- Map trajectories of protein domains in 3D
- Dynamic Loops of SecYEG, linking transmembrane helices 6-7 and 8-9, play a vital role in capturing SecA and in translocation
- Probing translocation of precursor through Sec-translocase