# Single molecule studies of membrane proteins on glass substrates using atomic force microscopy

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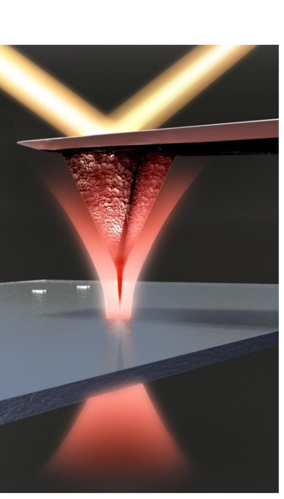
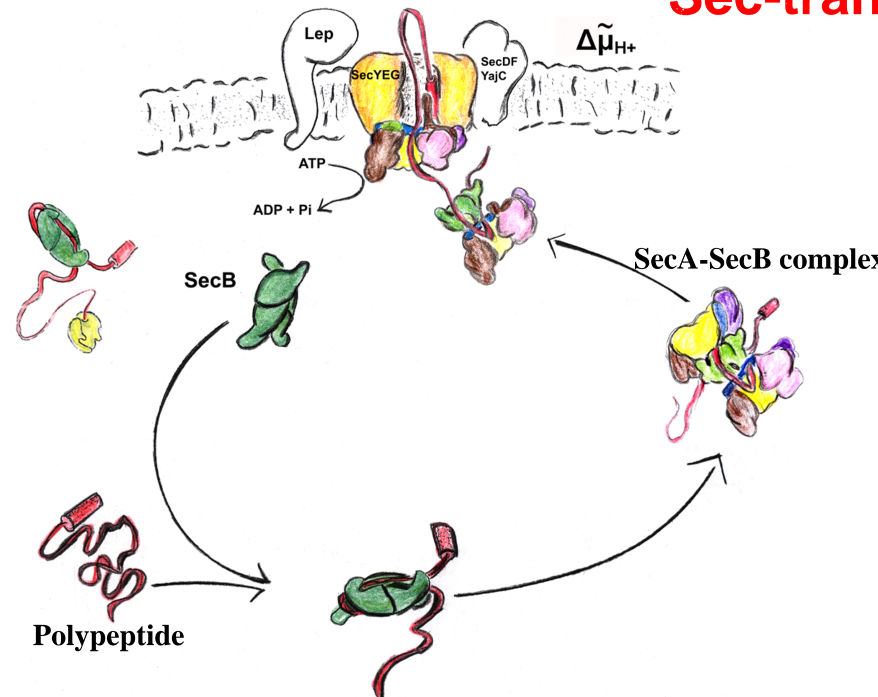



## Abstract

Since its invention in the mid-1980s, the atomic force microscope[1] (AFM) has become an invaluable complementary tool for studying membrane proteins in near-native environments. Historically, mica is the most common substrate utilized for biological AFM. Glass being amorphous, transparent, and optically homogeneous has its own set of advantages over mica and has the potential to broaden the use the AFM into fields that require high quality non-birefringent optical access. The use of silanized glass as AFM substrates has been reported as a means to fine tune surface chemistry. However, such coatings usually require hours of additional preparation time and can lead to increased surface roughness. In this work[2], we present a simple technique for preparing borosilicate glass as a substrate for two membrane systems: non-crystalline translocons (SecYEG) of the general secretary system from *E. coli*, and bacteriorhodopsin (BR) from *H. salinarum*. For both these membrane proteins, quantitative comparisons of the measured protein structures on glass versus mica substrates show agreement. An additional advantage of glass is that lipid coverage is rapid (< 10 minutes) and complete (occupying the entire surface). A goal is to study the bacterial export system using recently developed precision measurement techniques such as ultra-stable AFM.


## Sec-translocase




- >30% of proteins are transported from the site of synthesis into or through a membrane[5]
- 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[5,6]
- 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

## Atomic Force Microscopy (AFM)

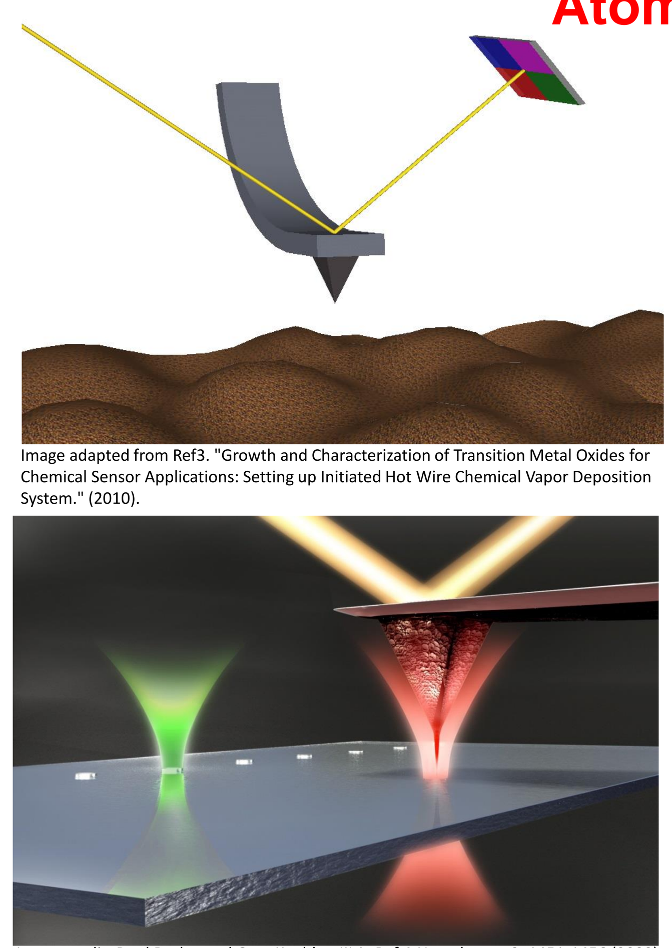
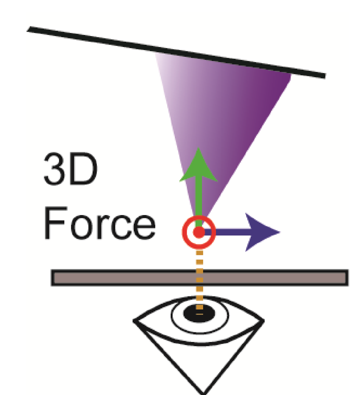


Image adapted from Ref3. "Growth and Characterization of Transition Metal Oxides for Chemical Sensor Applications: Setting up Initiated Hot Wire Chemical Vapor Deposition System." (2010).

Image credit: Brad Baxley and Greg Kuebler, JILA, Ref 4:Nano letters 9, 1451-1456 (2009)

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

## Ultra Stable 3D AFM




- Recent techniques[4] 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***


## References

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## Acknowledgements

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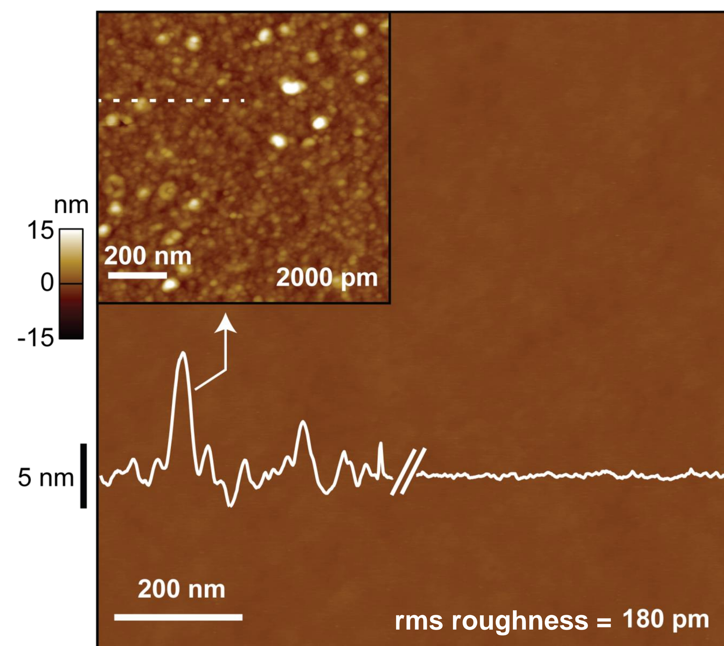
## Why use glass as a substrate?

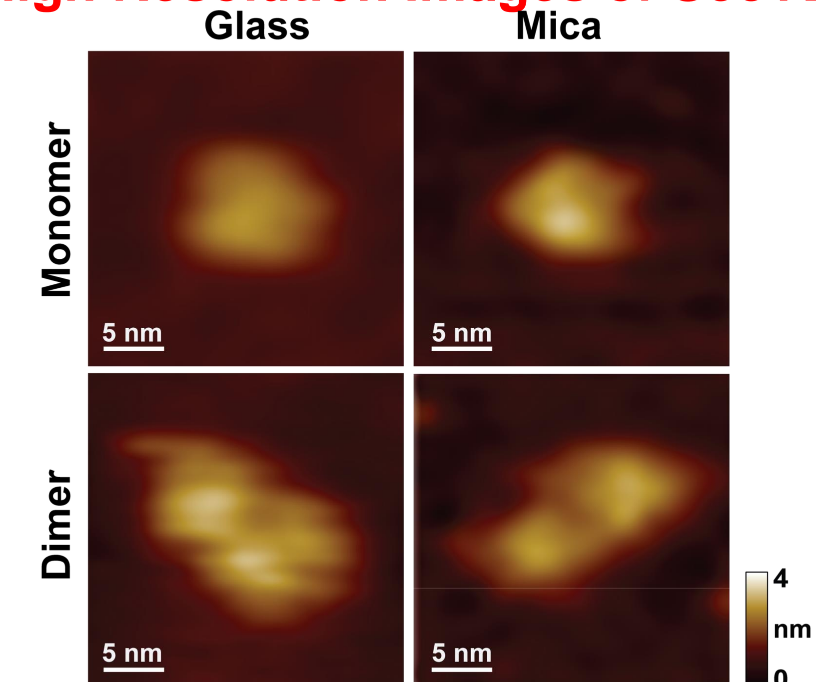
- Typical AFM substrates mica and HOPG exhibit birefringence, complicating optical paths
- Many well established biological and biochemical assays like FRAP, FRET, Fluorescence Microscopy, FIONA and TIRF require transparent and optically homogeneous substrates
- Recently developed techniques like US-AFM[4], 3D AFM 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[2]

## Raw glass vs. KOH treated glass



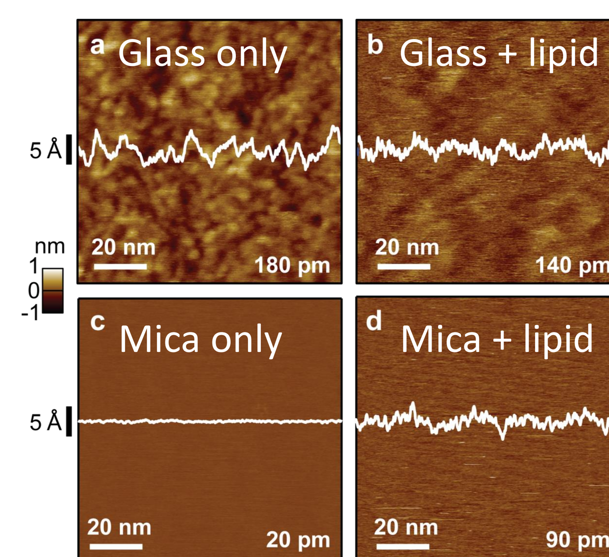

KOH cleaned glass vs. raw glass (inset) shows >10 fold reduction in rms roughness (bottom right corners)[2].

## High Resolution Images of SecYEG

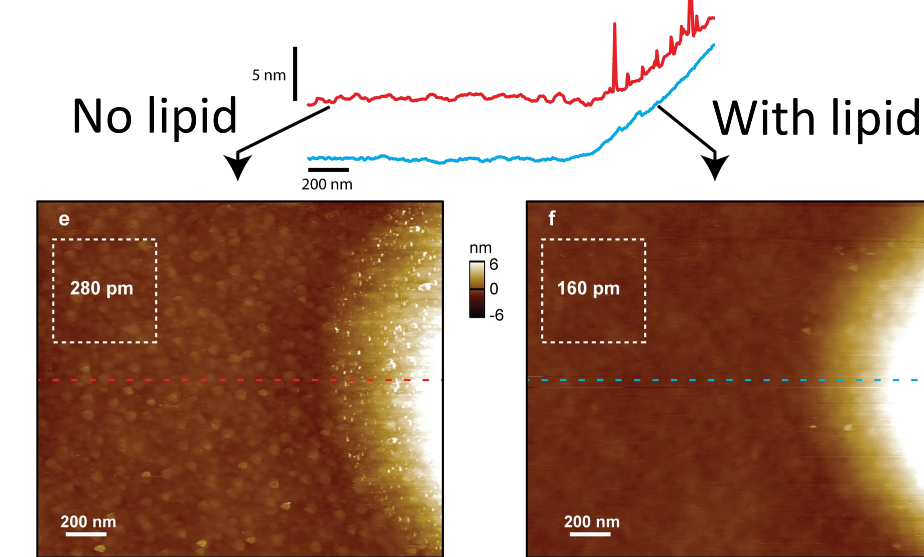



SecYEG heights and volumes on glass agree well with that on mica and X-ray crystallography[2]

## Direct visualization of reduction of surface roughness on glass

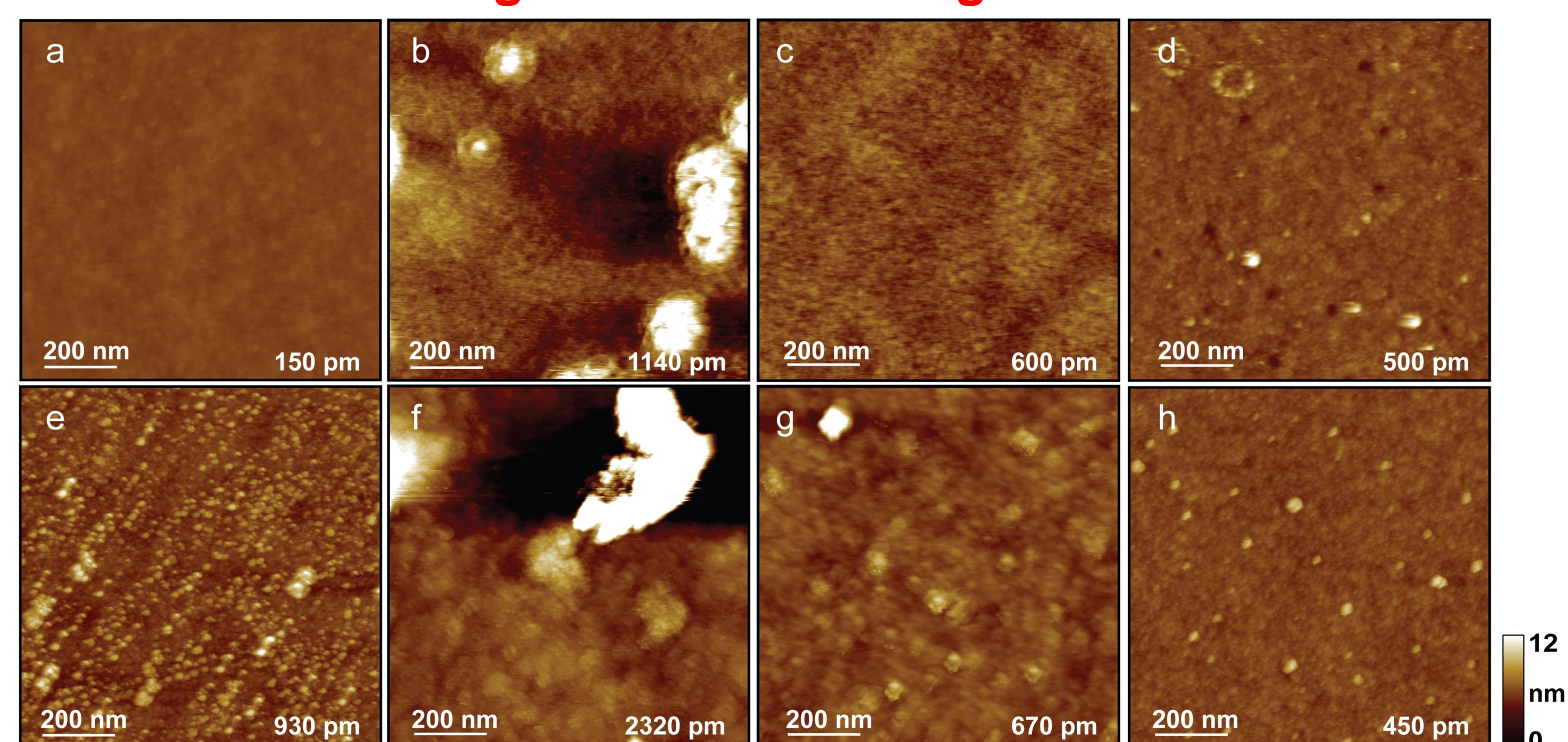



KOH treated glass surfaces become smoother (20% reduction, $N$=340) upon lipid coating[2]. In contrast, mica becomes >4 fold rougher.



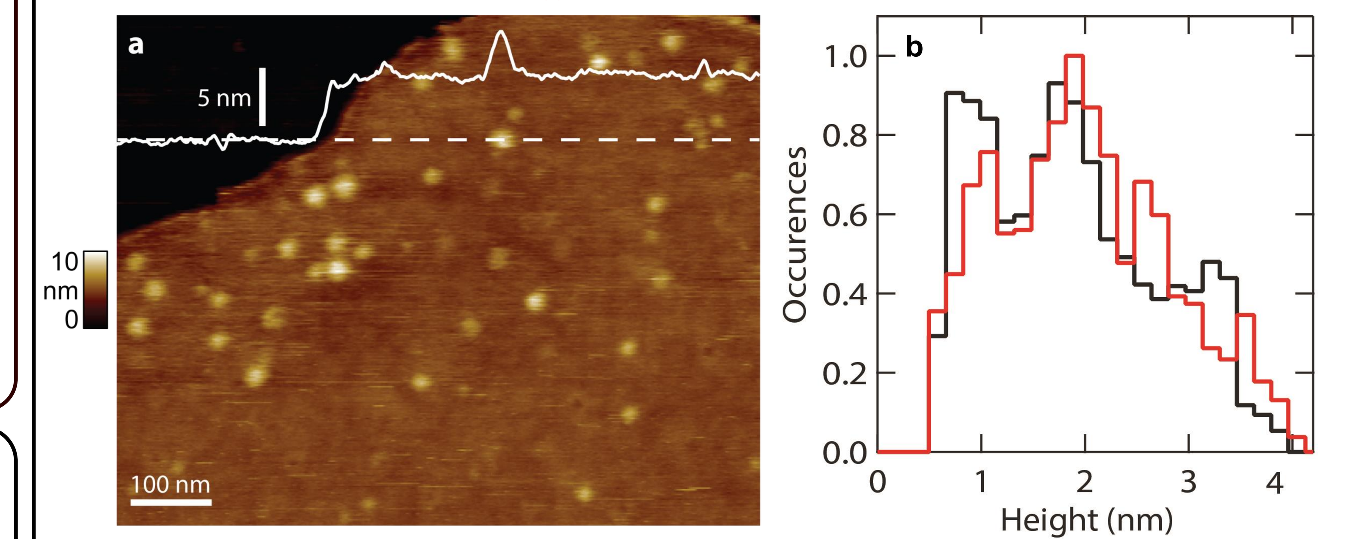

Upon lipid deposition KOH treated glass shows a ~40% reduction in rms roughness[2]. Here the same area is shown before and after lipid coating.

## Evaluating Different Cleaning Treatments



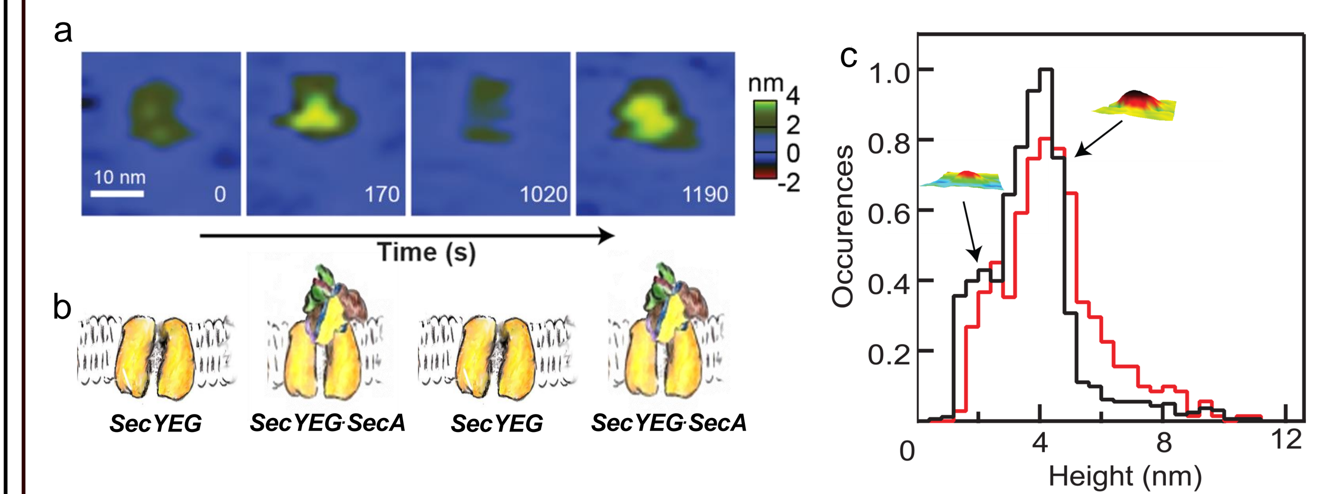

Glass treated with KOH (a), HF (b&c), $NH_4F$ (d&e), KOH followed by HF (f&g) and KOH followed by $NH_4F$ solution(h). rms roughness indicted at bottom right corner of each image[2]

## Heights of SecYEG



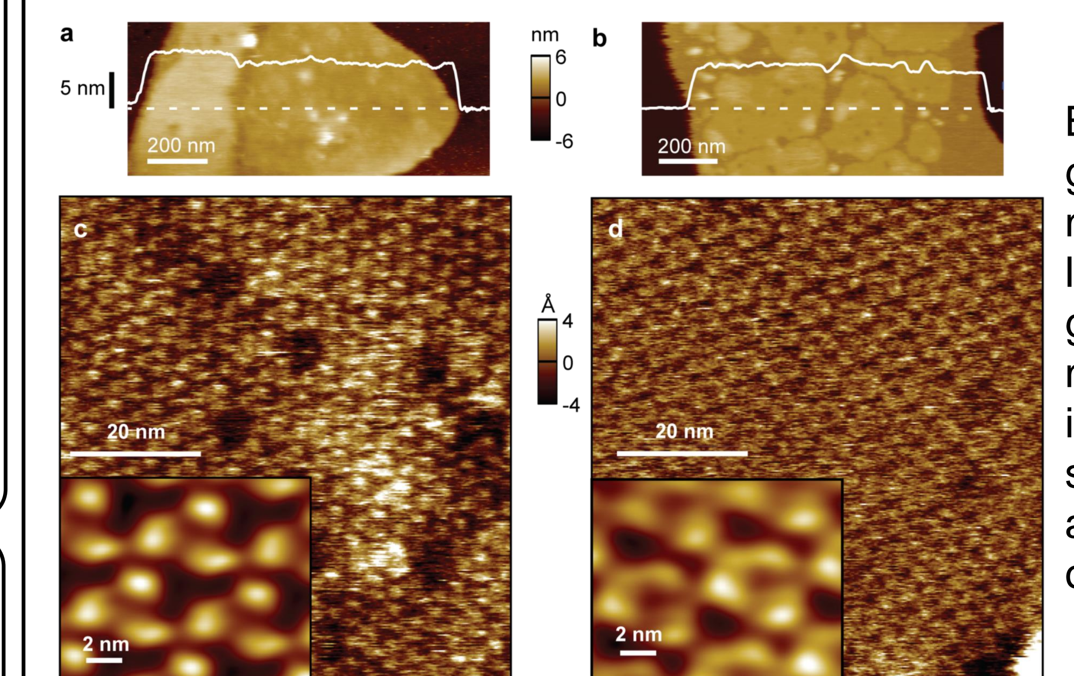

(a) Image of SecYEG in lipid bilayer on glass with a cross section profile[2] (solid white line), and (b) SecYEG heights on glass (*red*) and mica (*black*)[2].

## SecYEG-SecA interactions



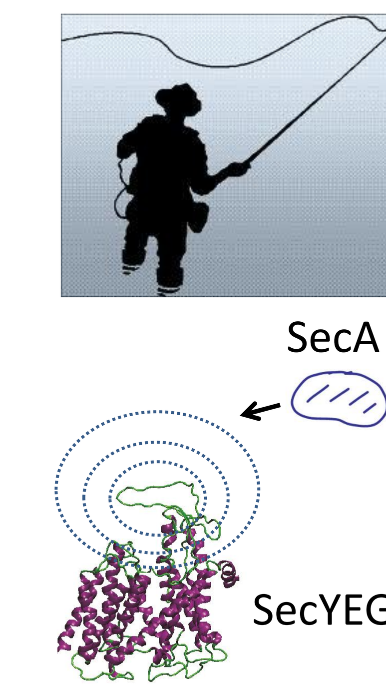

Direct visualization of native protein-protein interactions in lipid[2]. (a) Binding and unbinding of SecA to SecYEG observed in membrane. Cartoon depiction (b) of the events observed in panel (a). Height histogram of SecYEG·SecA complex on glass (*red*) and mica (*black*) show overall agreement. The peak at 4 nm has been attributed to a highly active state of the translocase.

## 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[2]

## Potential Applications




- Novel AFM modalities could measure molecular "fly fishing"[6]
- 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

# 3RD MIDWEST SINGLE MOLECULE WORKSHOP

UNIVERSITY OF ILLINOIS
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**AUGUST 4-5, 2014**

University of Illinois – Physics Department – 320 Loomis Lab, 1110 W. Green Street – Urbana-Champaign, Illinois 61801 – 217-333-3393

## Poster II-9: Real Time Observation of Lipid-Protein Interactions in Crude Cell Lysates and with Single-Molecule Resolution

*Edwin Arauz, **Vasudha Aggarwal**, Ankur Jain, Taekjip Ha, and Jie Chen*
University of Illinois at Urbana-Champaign

Lipid-protein interactions play key roles in signal transduction. Obtaining new mechanistic insights of these interactions is obligatory for a better understanding of biological processes. Here we use a single-molecule pull-down assay (SiMPull) to probe lipid-protein interactions in crude cell lysates. We demonstrate the applicability of this assay by showing specific interaction between several signaling lipids and their lipid-binding partners. We perform intensive single-molecule data analysis to quantitatively describe the assembly lipid-binding proteins on their target lipids. Importantly, this assay is applicable to full-length proteins expressed in crude cell lysates, as show for the protein kinase AKT which binds to PI(3,4,5)3 lipid specifically. This new assay lays the foundation to study the interaction of large macromolecular complexes with lipids second messengers in cell lysates, avoiding the need of harsh and lengthy procedures used during protein purification.

## Poster II-10: Single-molecule Studies of Membrane Proteins on Glass Substrates Using Atomic Force Microscopy

***Nagaraju Chada**, Krishna P. Sigdel, Tina R. Matin, Raghavendar Reddy Sanganna Gari, Chunfeng Mao, Linda L. Randall, and Gavin M. King*
University of Missouri

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