Evaluation of thiol acetates (CH_3OSR) and disulfides (RSSR) as lipidic anchors in tethered bilayer membranes (tBLMs)

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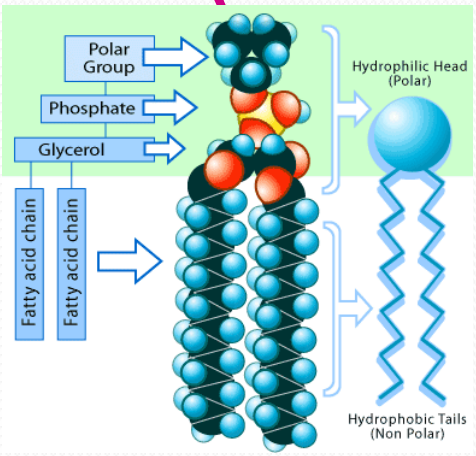
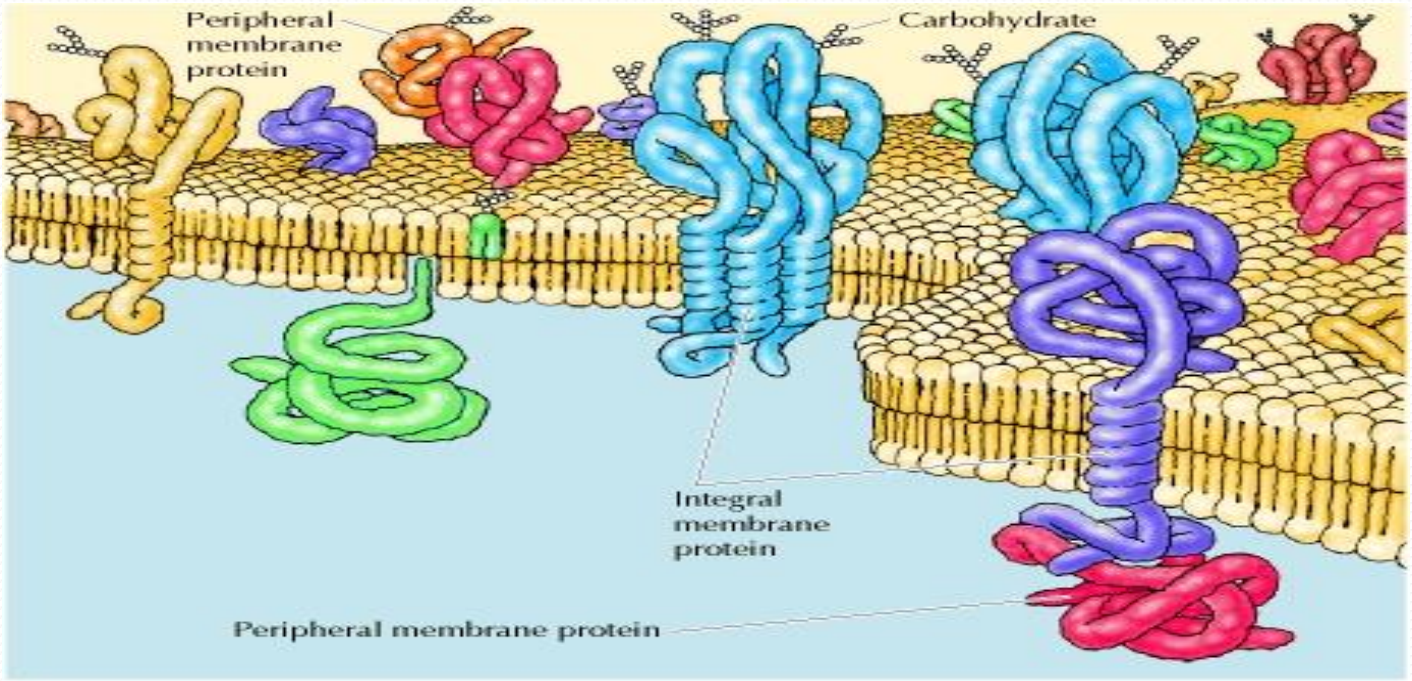
Integral Membrane Proteins (IMPs)

- Cell membranes composed of phospholipid bilayer
  - Hydrophilic and hydrophobic



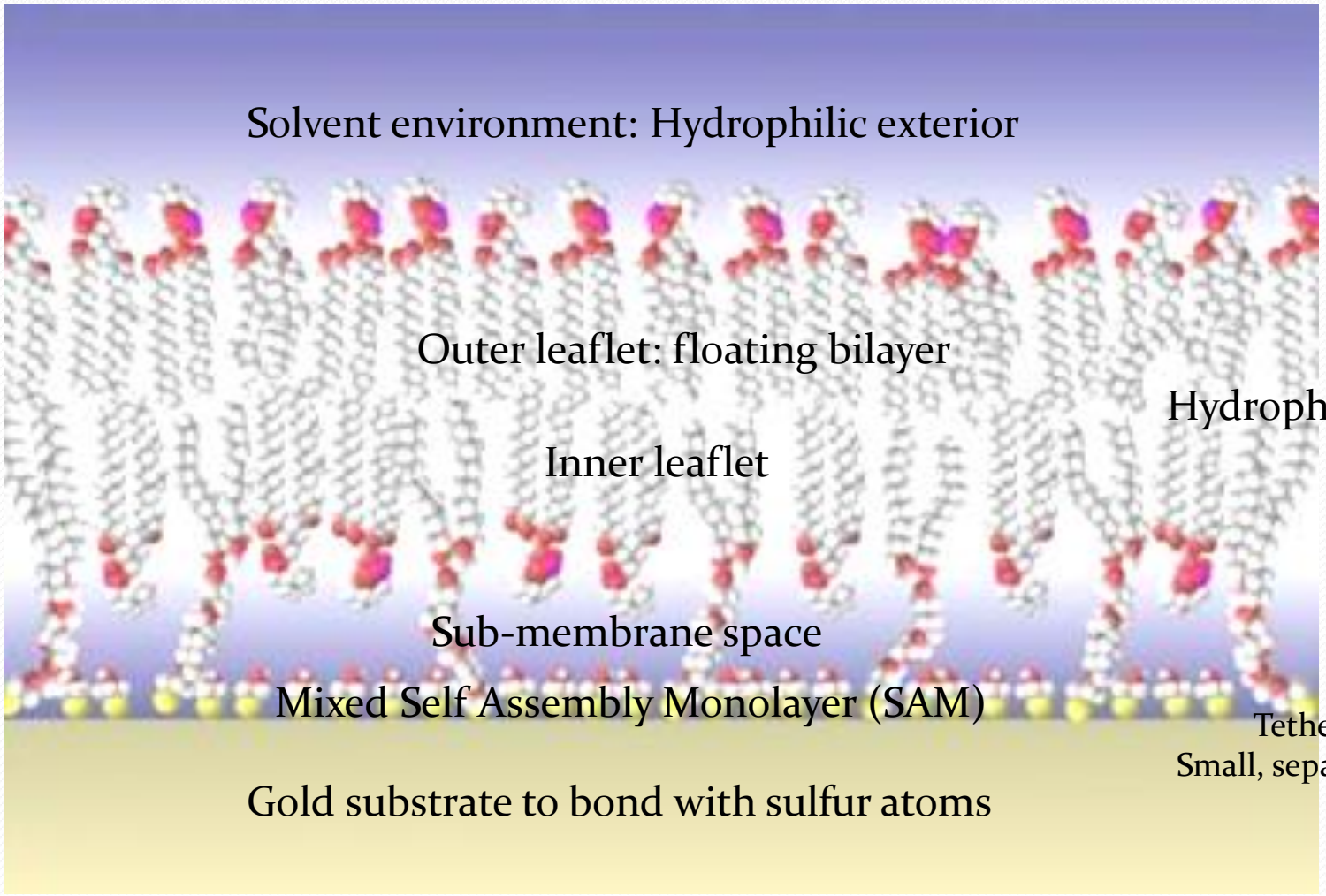
- Proteins carry out a variety of functions
  - Channels
  - Receptors
  - Enzymes
- Structure and function studies



Why we are doing this work

- Native bilayers are complex
  - Many different types of IMPs and lipids
- Structure and function studies are difficult with IMPs
- Tethered Bilayer Lipid Membranes (tBLMs) create a model for studying IMPs
  - Environment stable for days
  - Surface measurements

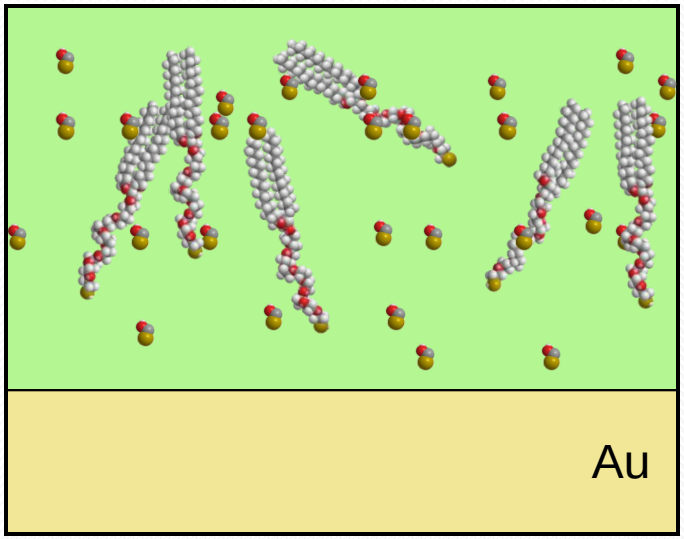
tBLMs


Solvent environment: Hydrophilic exterior
Outer leaflet: floating bilayer
Hydrophobic interior
Inner leaflet
Sub-membrane space
Mixed Self Assembly Monolayer (SAM)
Tether molecule
Small, separation molecule
Gold substrate to bond with sulfur atoms

tBLM Preparation – Rapid Solvent Exchange Method

Step 1: Mixed SAM

Incubation with tether & small molecule

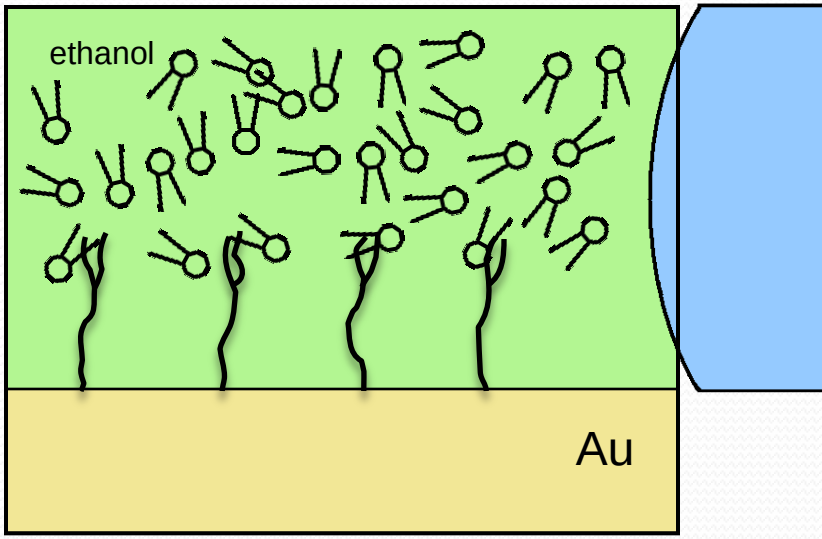


Ng 2008 SURF talk

- Gold substrates sit in solution for 1-5 days

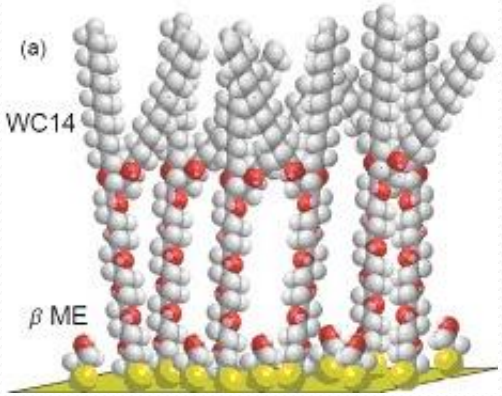
Step 2: Create tBLM

Rapid Solvent Exchange



- Floating bilayer to replicate cell membrane fluidity

The Key Players



- **WC 14**
  - **HS**$(CH_2CH_2O)_6CH_2CH(OR)CH_2(OR)$
    - where R=$C_{14}H_{29}$
- [β-mercaptoethanol (**βME**)]
  - **HS**CH_2CH_2OH

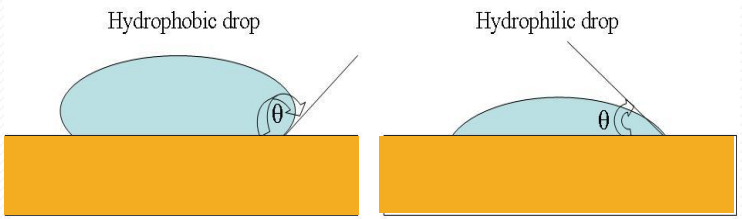
- Thiols lack long term stability!!!!!! Looking for alternatives

- **Thiol acetates**
  - **H_3COS**$(CH_2CH_2O)_6CH_2CH(OR)CH_2(OR)$
  - **H_3COS**$CH_2CH_2OCH_3$ (βME methyl ether = βMEOMe)

- **Disulfides**
  - R'**SS**R', where R' = $(CH_2CH_2O)_6CH_2CH(OR)CH_2(OR)$
  - $HOCH_2CH_2$**SS**CH_2CH_2OH

Surface Measurements

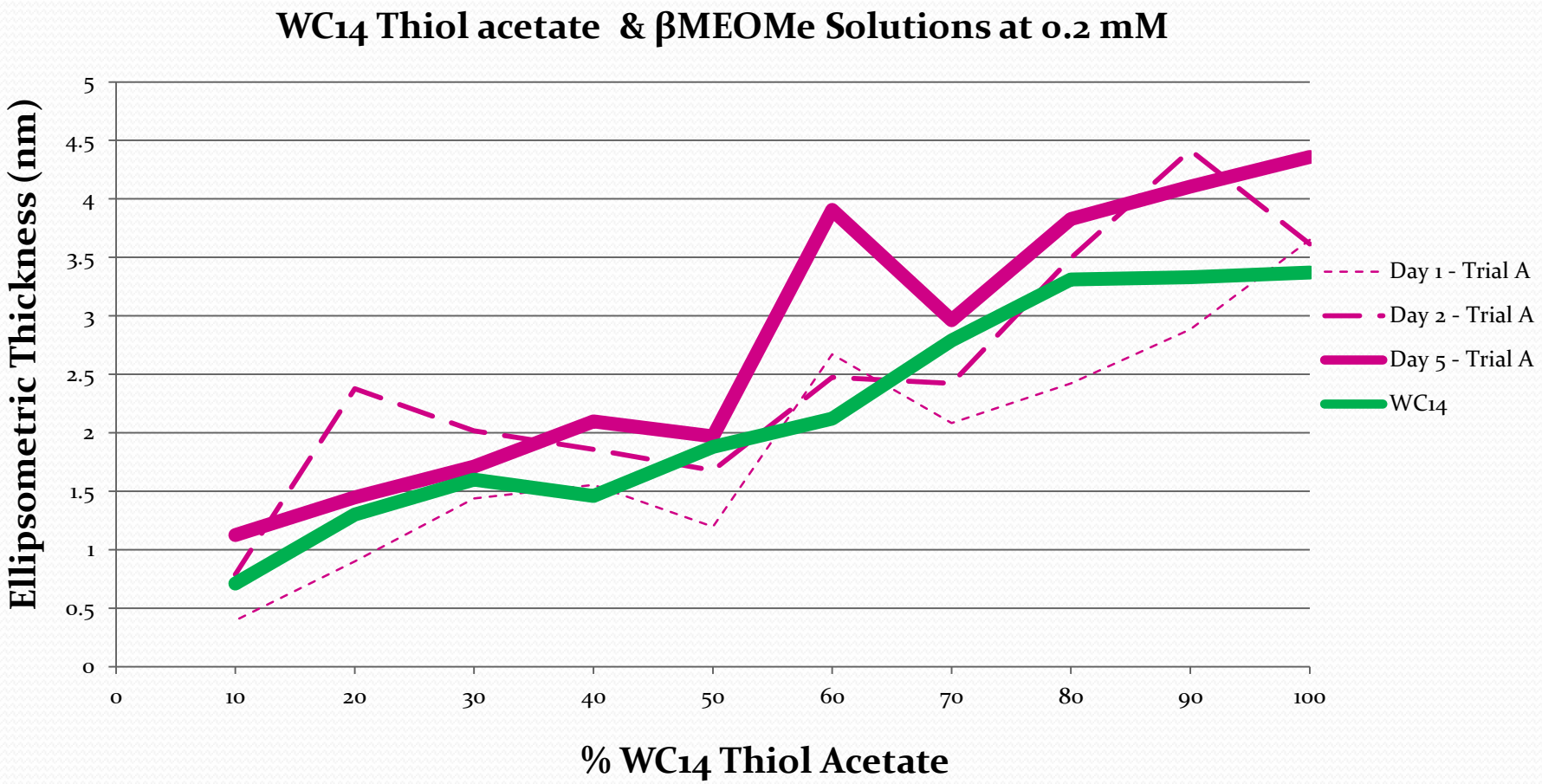
- Ellipsometry
  - An optical metrology that allows the determination of thin film thicknesses
- Contact Angle
  - A technique that allows the determination of the surface energy (hydrophobicity/hydrophilicity)



- Neutron Reflectivity
  - A scattering technique that allows the determination of the structure of films
- Electrochemical Impedance Spectroscopy
  - An electrochemical technique that measures the resistance and capacitance of films as a function of frequency to describe the bilayer and its imperfections

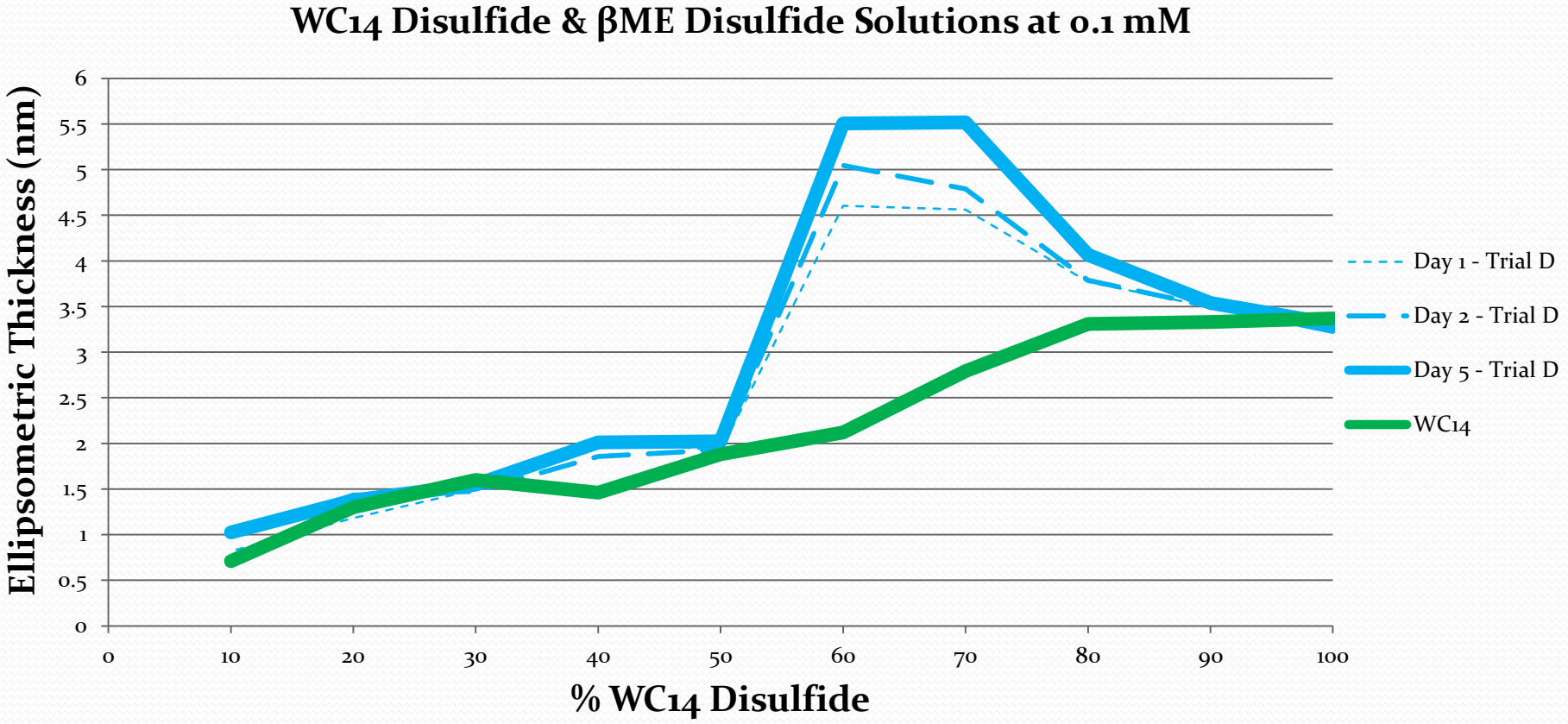
Ellipsometry Data: thiol acetates

- Refresher: determination of thin film thicknesses
- Graph of 1 of 3 data sets
- SAM growth as a function of increasing tether concentration and time



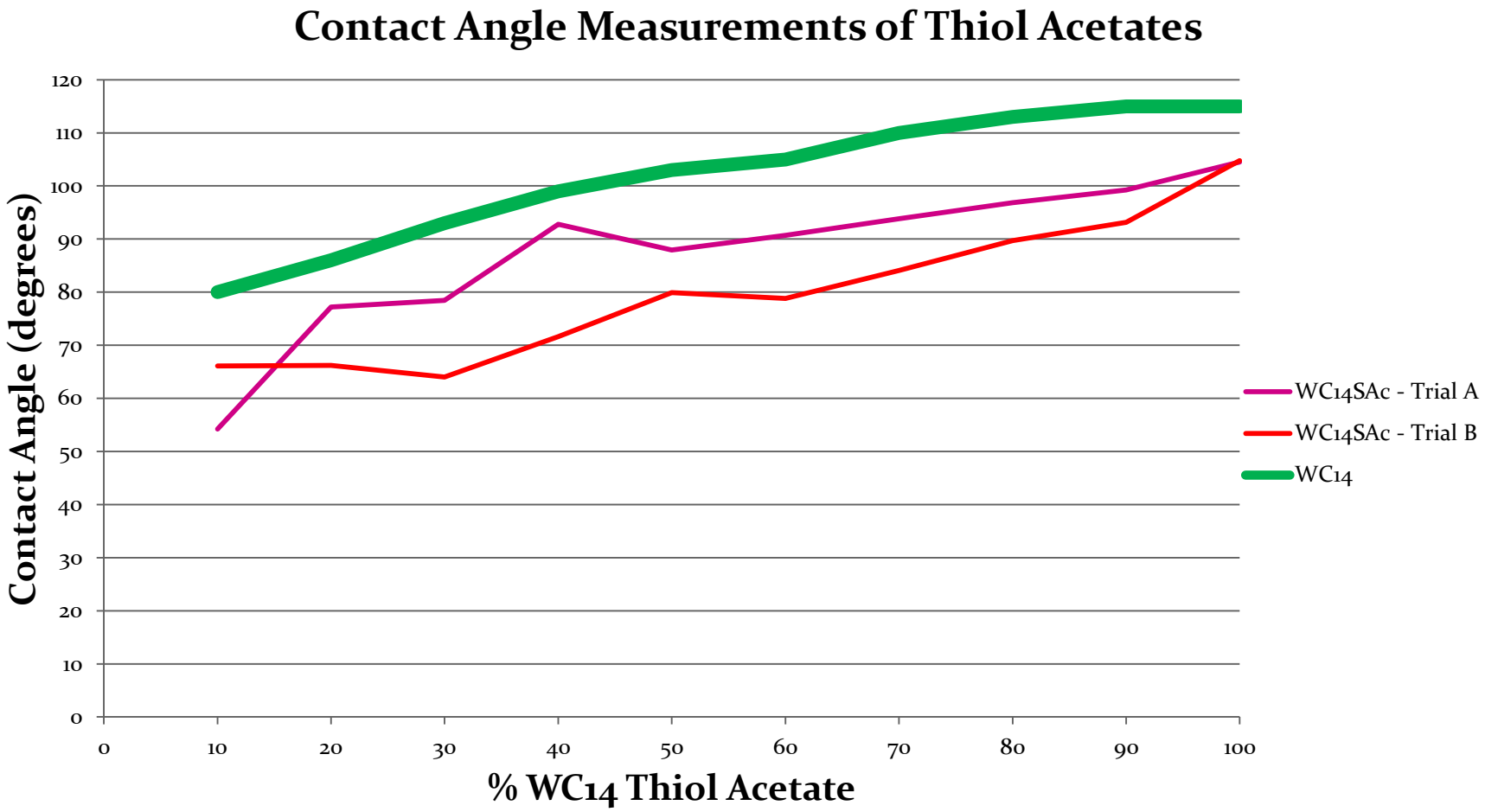
Ellipsometry Data: R'SSR'/[βMES]$_2$

- Graph of 1 of 2 data sets
- SAM growth as a function of increasing tether concentration and time
- Anomalies could be due to large molecules laying down on the surface → "blanket theory"



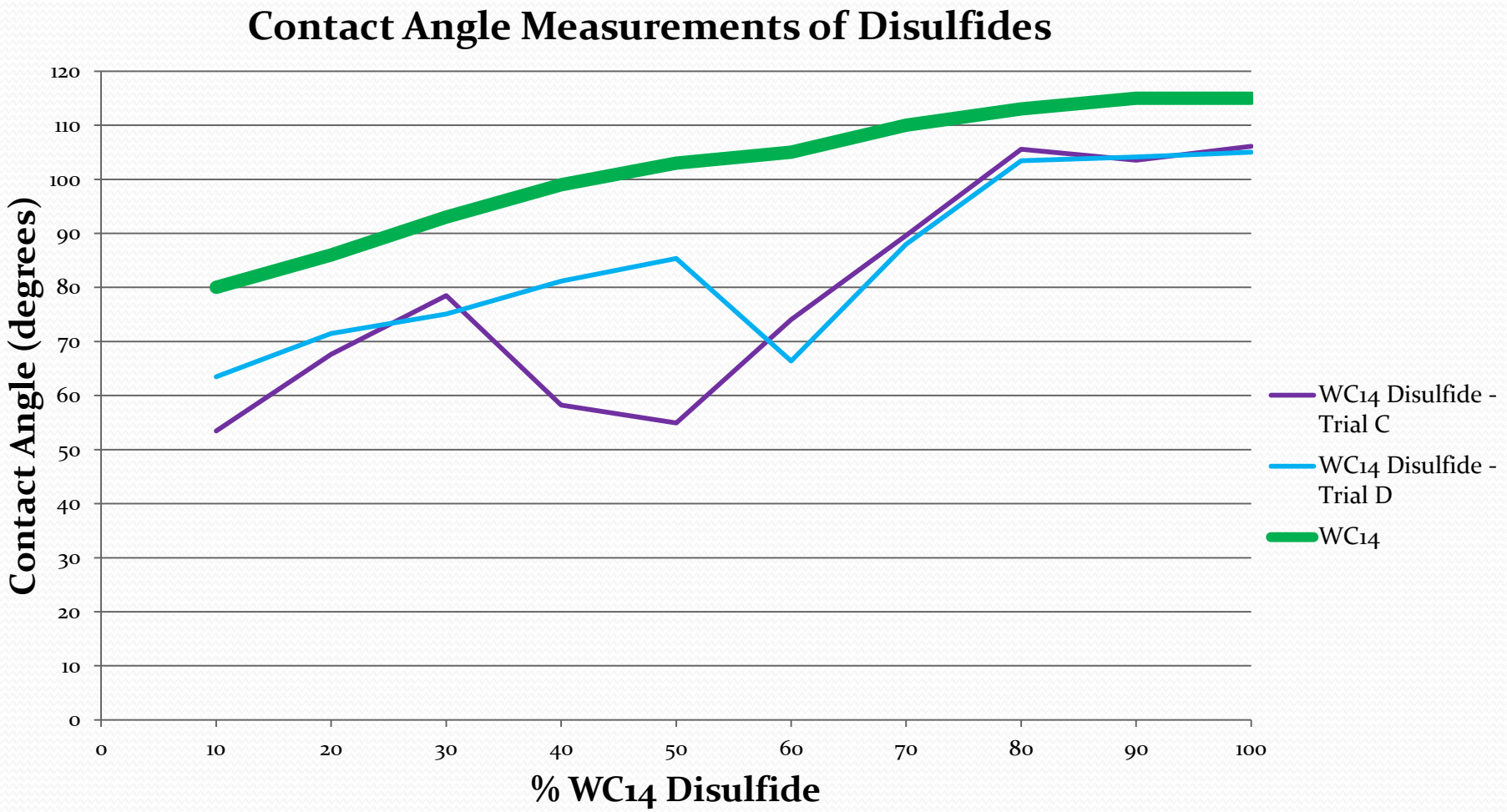
Contact Angle Data: thiol acetates

- Refresher: determination of the hydrophobicity/hydrophilicity of the surface
- Contact Angle less than WC14 thiol→ surface is less ordered



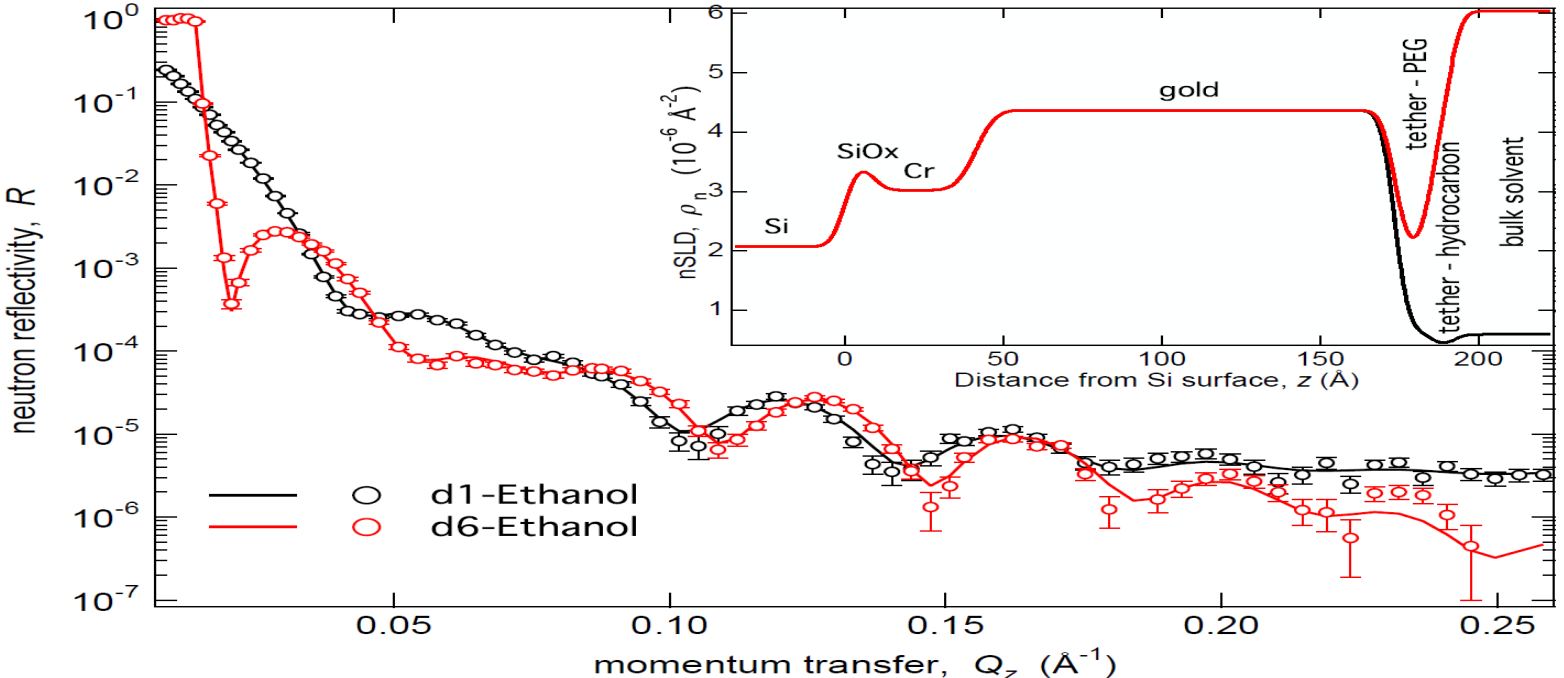
Contact Angle Data: disulfides

- Same results for disulfides as thiol acetates: not as ordered as thiols



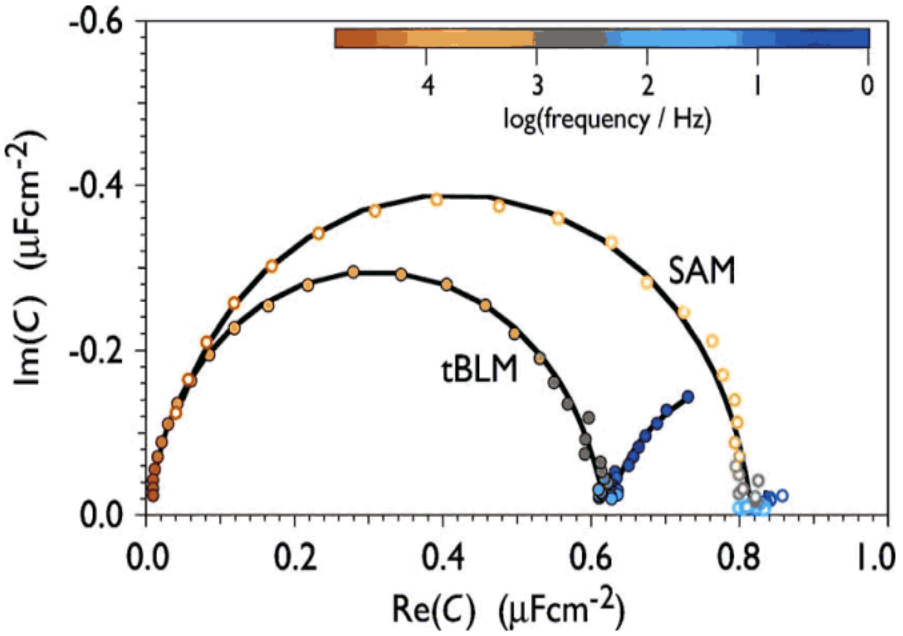
Neutron Reflectometry

- Refresher: determination of structure of films based on scattering of a reflected neutron beam
- Using a known scattering length of each nuclei and a known number of atoms
  - calculate material density (SLD)
- Sample: 70:30 tether composition → 50% less SAM seen than WC14,
  - NOT well ordered
- Only incubated for 24 hours
  - Would have told us about the sub-membrane space if timing was not an issue

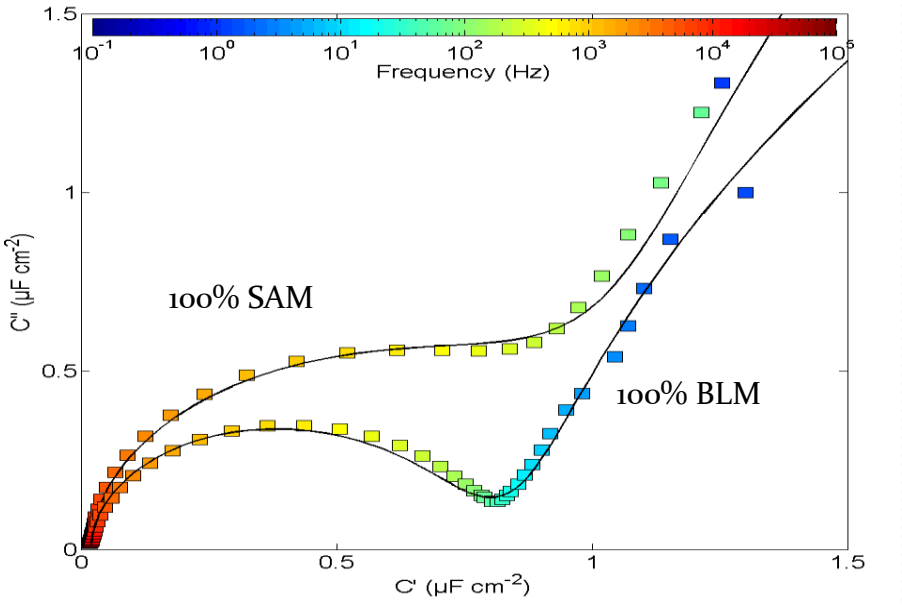


Electrochemical Impedance Spectroscopy (EIS)

WC14 Capacitance



WC14SAc Capacitance (100%)



- Refresher: measures the resistance and capacitance of films as a function of frequency
- Performed on 4 samples of WC14SAc:βMEOME: (A) 20: 80 (B) 30:70 (C) 40:60, (D) 100:0 (3 days)
  - Lower concentrations: bilayers did not form consistently
  - 100:0 SAM is reasonable and indicates good coverage, 100:0 BLM (bottom curve)
  - Reasonable, but higher capacitance than obtain with thiols → leaky (less ordered, more defects)
- Overall, there's variability however the data indicates the formation of SAMs and tBLMs with thiol acetates

Conclusions and Upcoming Research

- Can thiol acetates and/or disulfides replace thiols in tBLMs?
- Maybe…
  - Less ordered
    - Lower contact angles
    - Increased capacitance
- So we are close but no cigar…yet
  - Collect more data on thiol acetates and disulfides
  - Especially with disulfides
    - Thicknesses very comparable to WC14 at high tether concentrations
- However, demonstrated the formation of a tBLM formation with thiol acetates
  - Look at long term stability

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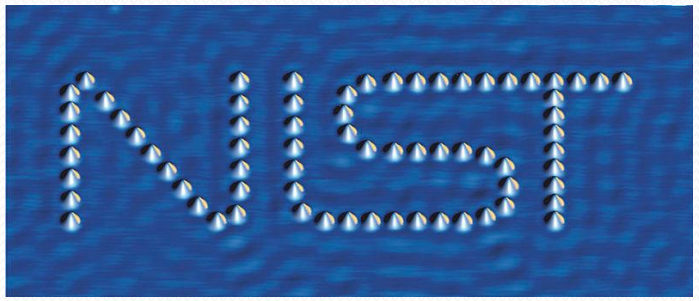

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Questions?