Comparison of Three Aminosilanes for Immobilization of Molecular Rotors for Fluid Viscosity Measurements

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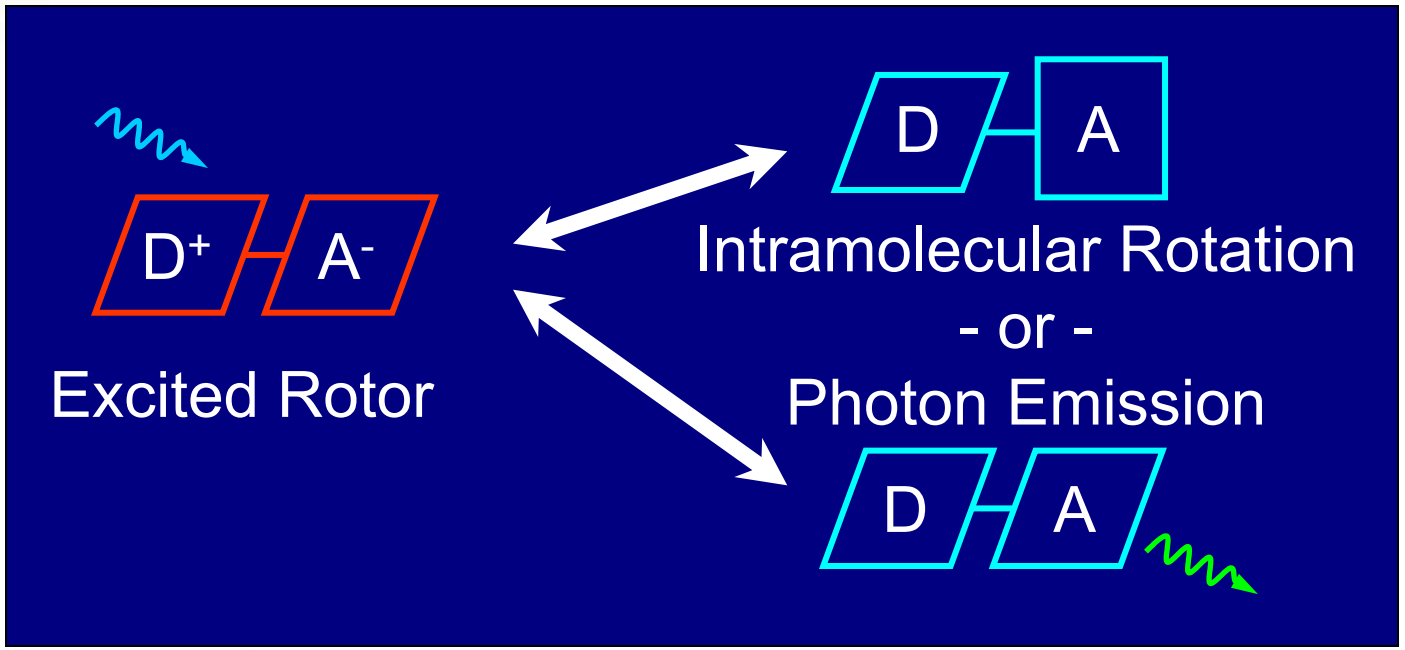
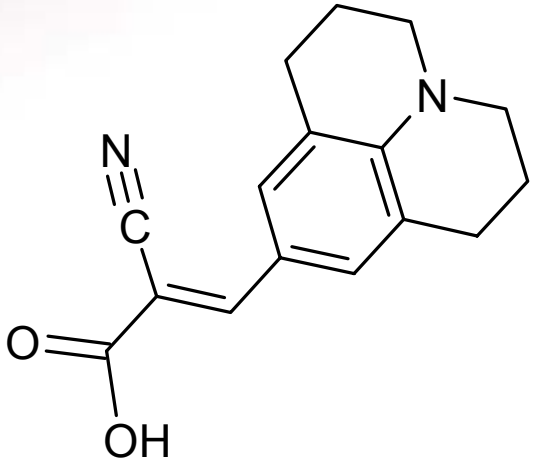
Introduction: Molecular Rotors

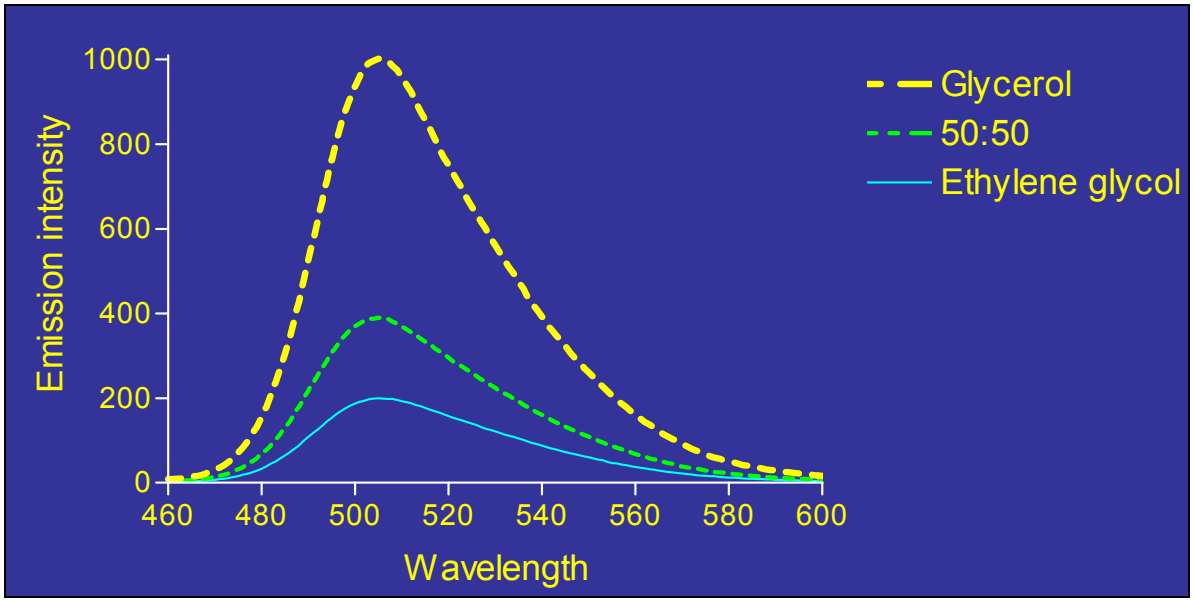
- Special class of molecules which form twisted intramolecular charge-transfer complexes (TICT)
- Dual competing processes of de-excitation – intramolecular rotation or fluorescence emission
- With increasing solution viscosity (or decreasing free volume), probability of fluorescence emission increases



Introduction: Molecular Rotors

- Relationship determined from Förster-Hoffman Equation:
  $\log(\Phi) = C + x \log(\eta)$
- Φ is the quantum yield
- C is a temperature dependent constant
- X is dependent on the structure of the molecular rotor
- η is the viscosity of the solution





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Objectives

1. Is CCVJ still viscosity sensitive after immobilization to glass substrates?

2. Is there a difference in emission signal between silane coatings?

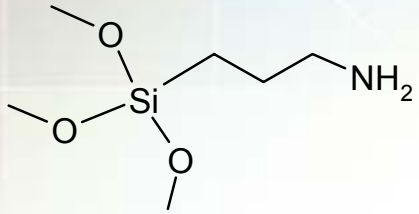
3. If so, one has the highest emission signal resulting in the greatest viscosity sensitivity with CCVJ?

Materials and Methods

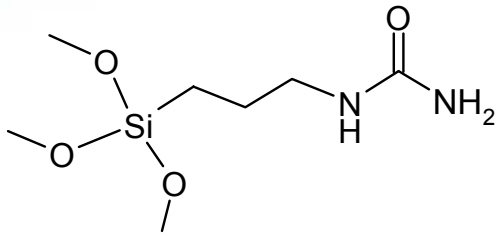
- **Cleaning glass slides**
  - 1:1 HCl and Methanol for 15 minutes
  - 100% Sulfuric acid for 15 minutes
  - Boil for 15 minutes in d.d. H_2O
  - Treat with Corona pen for 1 to 2 minutes before silane incubation

- **Acetic acid/Ethanol Silanization method**
  - 76% Ethanol, 20% Acetic acid, 4 % water
  - Mix silane into the solvents, allow a few minutes to hydrolyze and then add the slides for 5 to 10 minutes
  - Rinse in ethanol, air dry

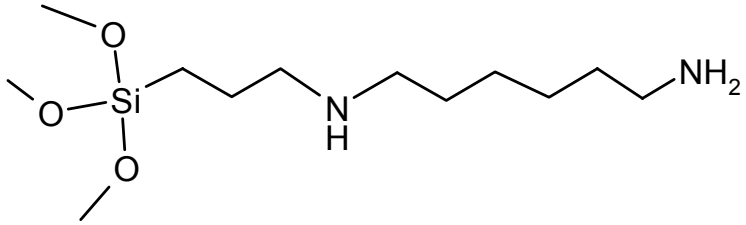
Silane Structures



3-AMINOPROPYLTRIMETHOXYSILANE
SIA0611.0 13822-56-5 $C_6H_{17}NO_3Si$



UREIDOPROPYLTRIMETHOXYSILANE
SIU9058.0 23843-64-3 $C_7H_{18}N_2O_4Si$



N-(6-AMINOHEXYL)AMINOPROPYLTRIMETHOXYSILANE
SIA0594.0 51895-58-0 $C_{12}H_{30}N_2O_3Si$

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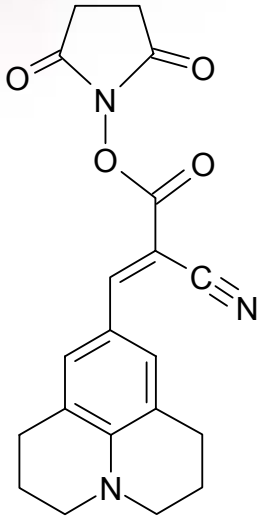
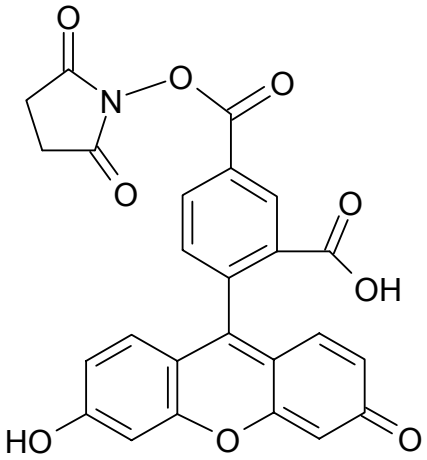
Materials and Methods

- **Covalent attachment of fluorescent dyes to silane surface**
  - 9-(2-carboxy-2-cyanovinyl)julolidine, N-hydroxysuccinimidyl ester (CCVJ)
  - 5-carboxyfluorescein, succinimidyl ester (5-FAM)





–1. Make 25mM stock solutions in DMSO

–2. Prepare 0.1 M sodium bicarbonate solution, pH 8.5

–3. Dilute stock dye into the bicarbonate solution to 0.1mM

–4. Applied immediately to the silanized surface

–5. Incubate for 1 hour at room temperature

–6. Rinse with distilled H_2O followed by ethanol and air dried

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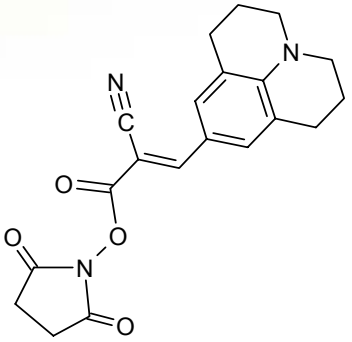
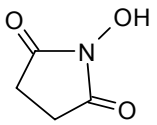
Bonding

release of N-hydroxysuccinimide

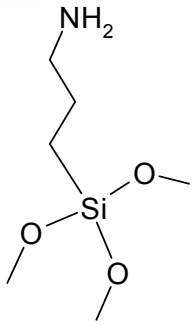
NHS reaction,
amine to amide linkage





Hydrolysis-Condensation

Byproduct Methanol



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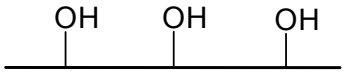
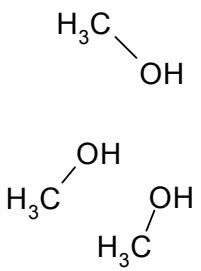
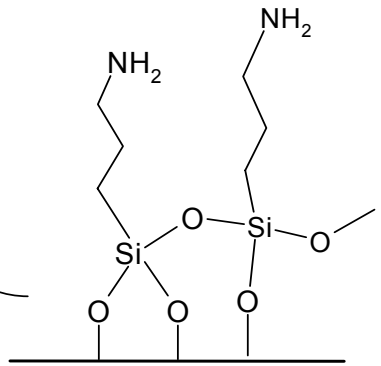
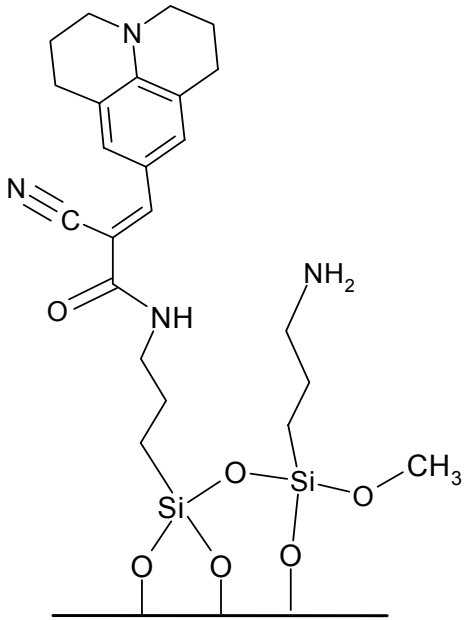
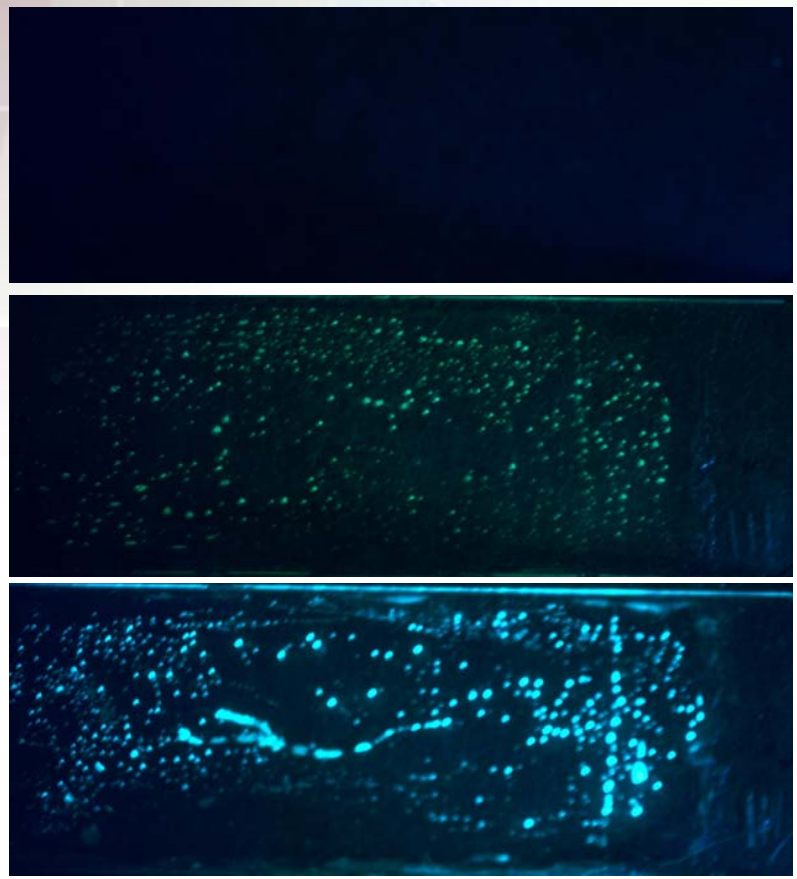








Qualitative examination



Blank slide

5-FAM bound to Ureido silane

CCVJ bound to Ureido silane

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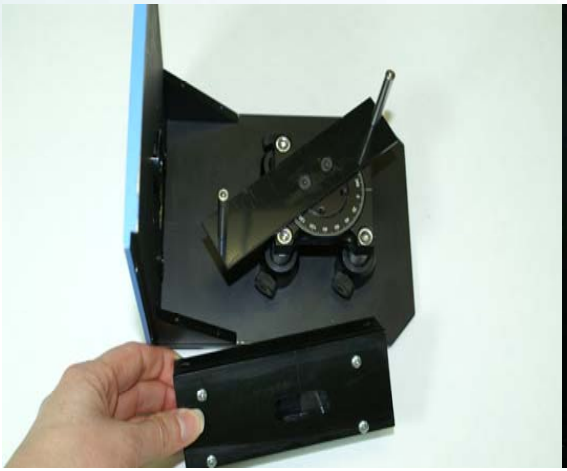
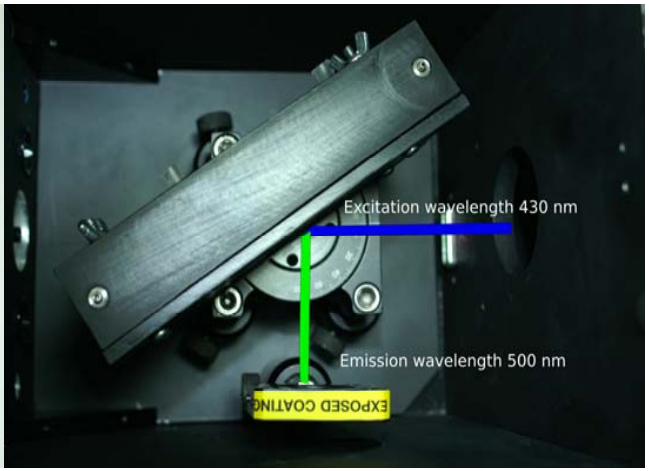
Fluorescent measurements



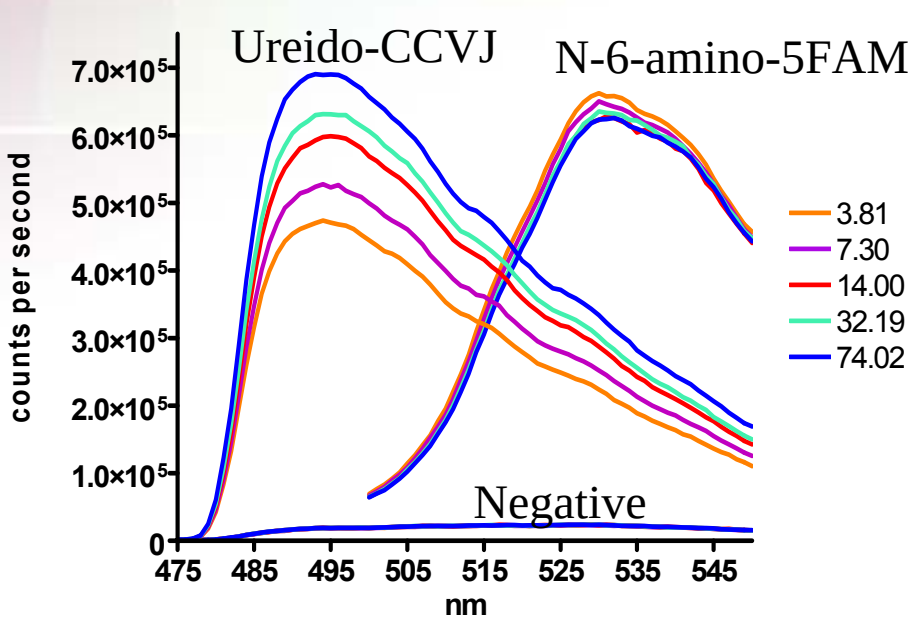


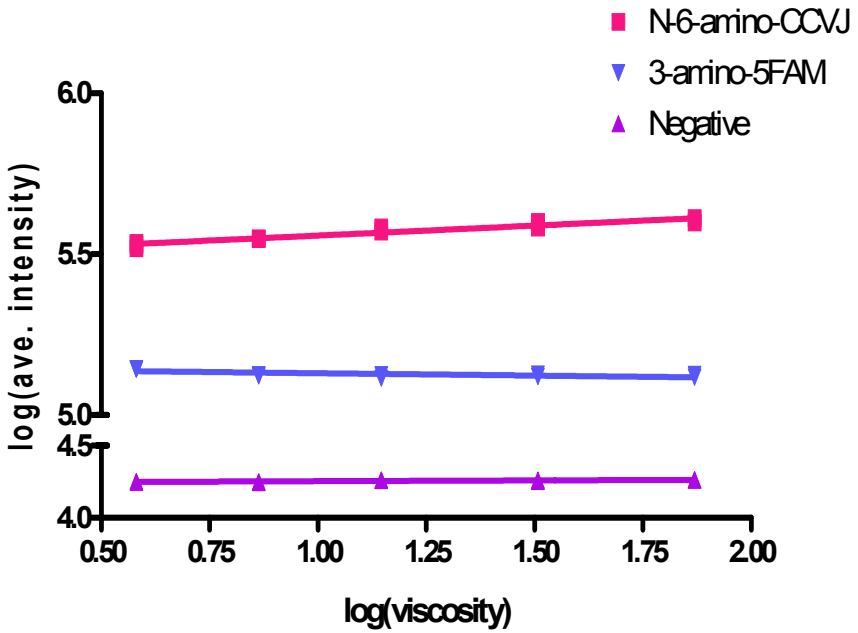
- Slides are inserted into a custom designed stage for the Fluoromax 3 SPEX
- Solvents consisted of glycerol, ethylene glycol and methanol mixtures

Solvent #	% Mixture	Solvent	mPa sec	log(viscosity)
1	40/60	Glycerol/Ethylene Glycol	74.02	1.869
2	20/80	Glycerol/Ethylene Glycol	32.19	1.508
3	100	Ethylene Glycol	14.00	1.146
4	80/20	Ethylene Glycol/Methanol	7.30	0.863
5	60/40	Ethylene Glycol/Methanol	3.81	0.581

Measurements-Calculations

- Emission Peak is averaged
- N = 3 for each solvent





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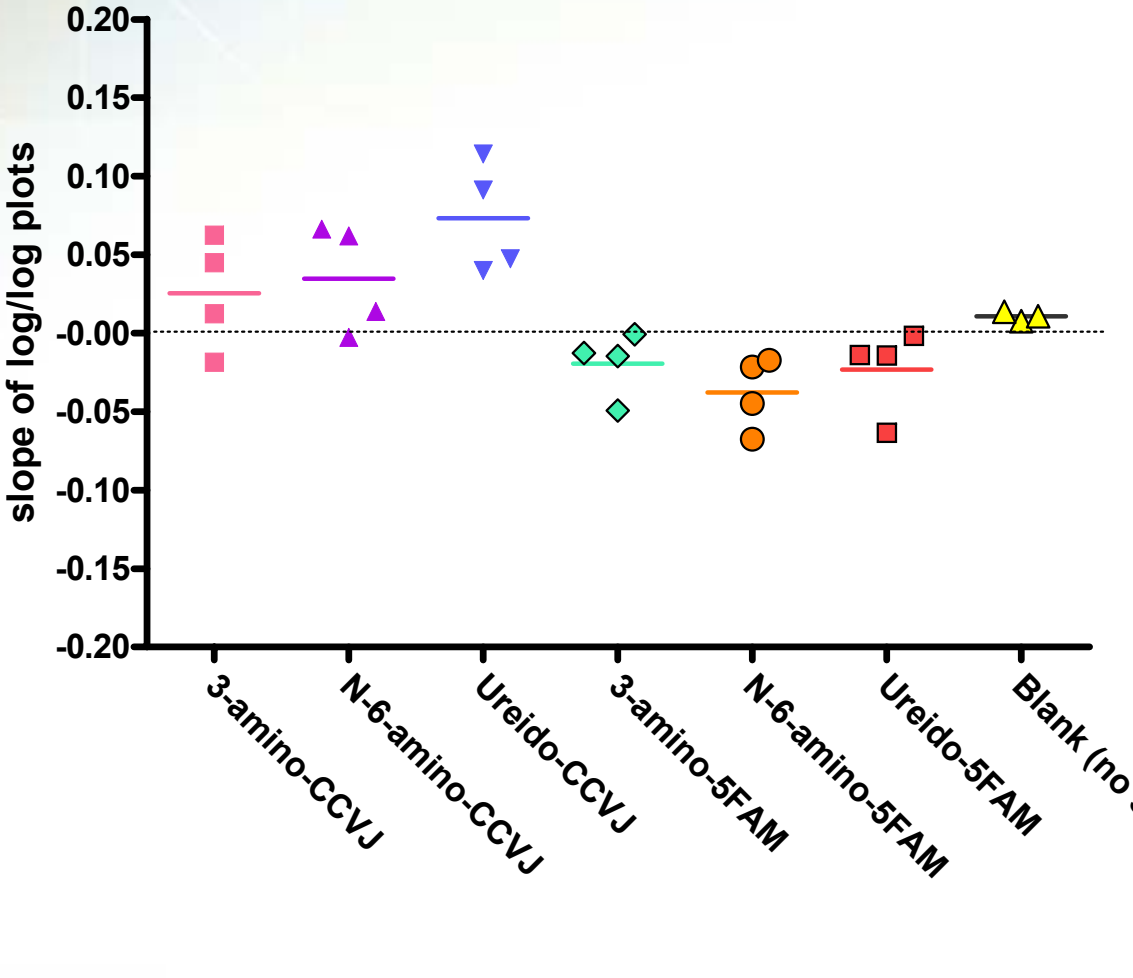
Results



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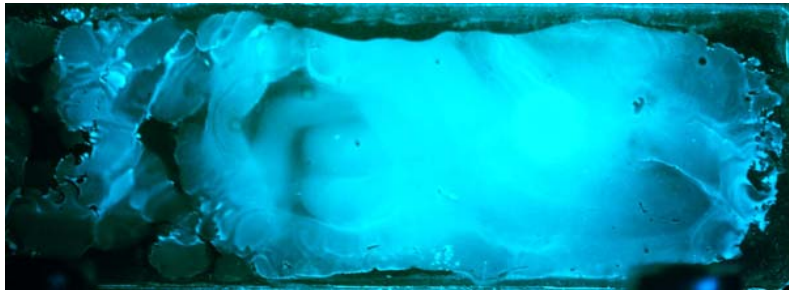
Current work

CCVJ bound to Ureido silane



Toluene solvent,
4 day incubation time
Heated after rinsing

Methanol solvent,
20 minute incubation time
Heated after rinsing

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Conclusions

- CCVJ is viscosity sensitive after immobilization
  - Reduction in sensitivity is observed
- The highest viscosity sensitivity is found with the Ureidopropyltrimethoxysilane
  - Median slope of 0.07
- Optimization of the silane coating results improves immobilization density
  - Sensitivity only marginally improved

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