Polymer-Functionalised Surfaces for Microarray Applications

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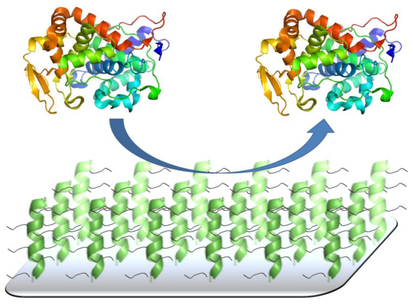
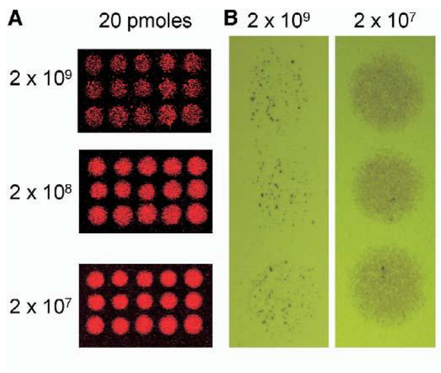
1. The Need to Improve Technologies for Polymer Surface Modification

Conventional Microarrays

- Ligands immobilised onto glass slides
- Fluorescent-labelled proteins or whole uni-cellular organisms added to assess binding, see right
- Whole bacteria and isolated proteins exhibit non-specific binding to the surface, see below
- This reduces resolution and gives rise to false positives



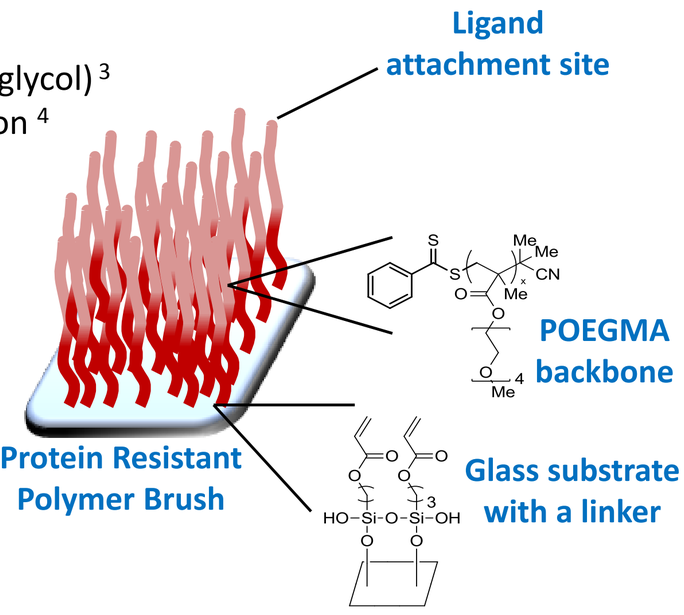
Protein resistant surfaces are used to reduce non-specific binding [2]



Carbohydrate microarray platforms can be used for bacterial detection, giving the binding 'fingerprint' for a specific species [1]

Polymer Coated Arrays

- Polymers of oligo(ethyleneglycol) [3]
- Inhibit non-specific adhesion [4]
- Traditional 'grafting to' strategies require a gold surface but microarrays require glass
- Therefore, we want to develop scalable, efficient methods to immobilise polymers onto glass microarrays

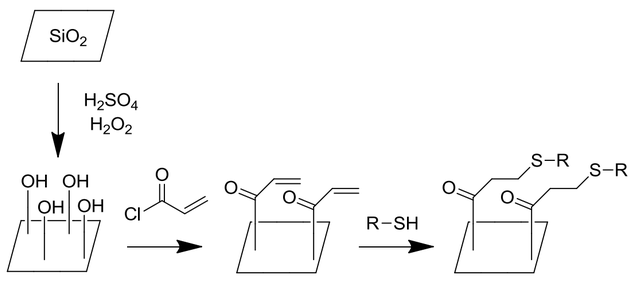


[1] Disney, MD. et al., Chemistry and Biology, **2004**, 11, 1701-1707; [2] Wang, J., Gibson, MI. et al., Macro. Rap. Commun., **2009**, 30, 845-850; [3] Jones, MW., Gibson, MI., et al., Polym Chem., **2011**, 2, 572-574; [4] Whitesides, GM., et al., Langmuir, **2001**, 17, 5605-5620.

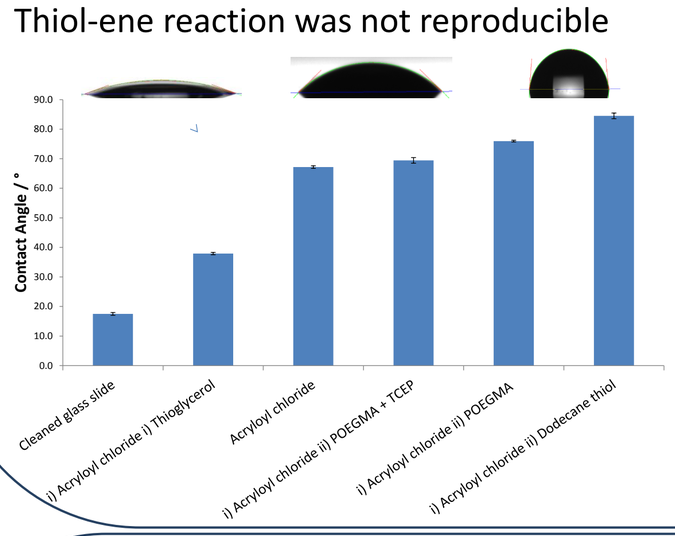
2. 'Grafting to' Functionalisation of Non-Gold Substrates

A. Functionalisation with Acyl Chloride

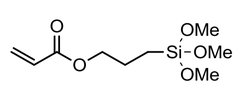
- Direct coupling of acryloyl chloride with silanol groups
- Working on both glass and silicon



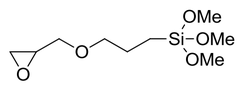
- Contact angle indicated functionalisation
- Thiol-ene reaction was not reproducible



B. Functionalisation with Silanes

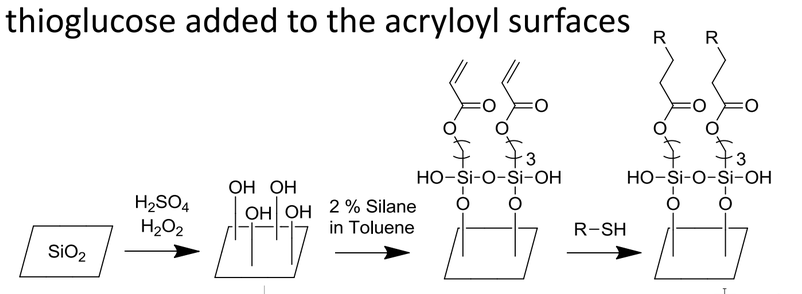


3-(Trimethyoxysilyl) propyl acrylate

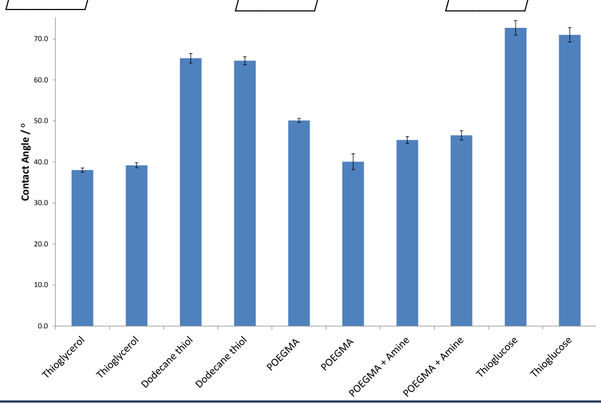


(3-Glycidyloxypropyl) trimethoxysilane

- Optimal functionalisation time of 30 minutes found
- Glycidyl silane was found to be less reactive towards thiols than acryloyl silane
- POEGMA, dodecane thiol, thioglycerol and thioglucose added to the acryloyl surfaces

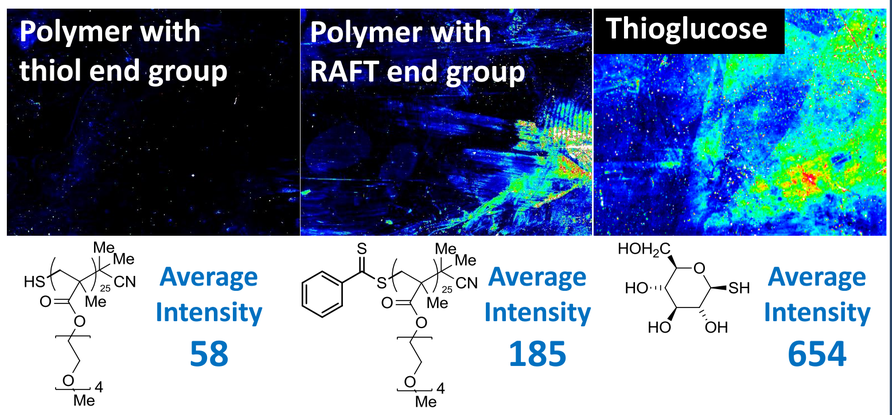


Contact angle data shows reproducible surface modification



C. Protein-Binding Studies

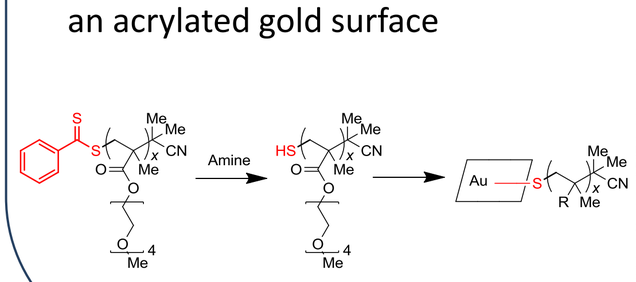
- Fluorescently labelled Con A (glucose binding lectin) was added to the functionalised surfaces
- Binding was visualised using a fluorescence scanner

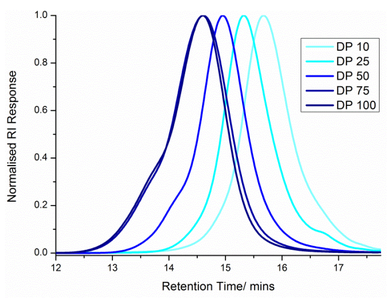


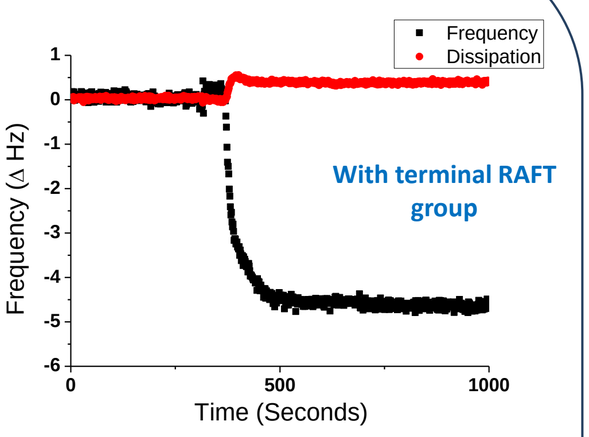
- POEGMA *without* free thiol terminus did not bind to acrylate. Non-specific protein adsorption was observed
- POEGMA *with* free thiol terminus did bind to the slide and no protein binding was observed
- Con A showed significant binding to glucose surface.
- Demonstrates the proof of principle that grafting polymers/bioactives to glass slides is efficient, simple and cheaper than gold

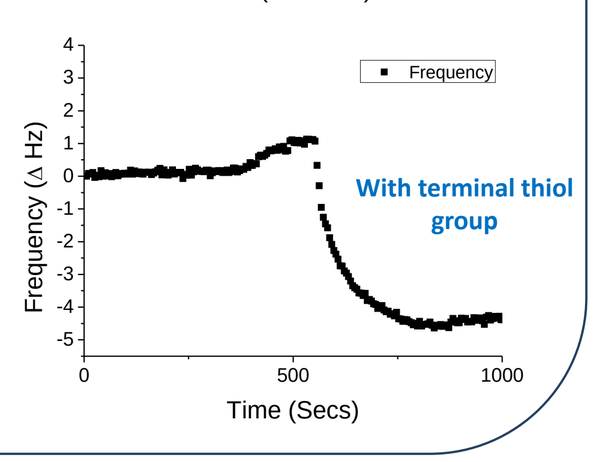
3. Gold Functionalisation

- Grafting to gold is the current standard methodology, but not suitable for microarrays
- However, to compare the efficiency of our new method, control experiments on gold are needed
- Addition of POEGMA to the gold surface was monitored in real time using **Quartz Crystal Microbalance with Dissipation (QCM-D)**
- Actual binding was lower than expected
- Average Δf was slightly larger for the binding of POEGMAs with a thiol terminus
- Experiments are continuing, along with a comparison to an acrylated gold surface









4. Conclusions

- Although initially successful at changing the surface hydrophobicity, the acryloyl chloride functionalisation proved irreproducible and further surface analysis (XPS, IR) was unobtainable
- Silane functionalisation has proved successful and reproducible. The acrylated silane seen to give more even coatings but further surface analysis is in progress
- Protein binding and gold QCM-D studies have shown the advantages of RAFT end group cleavage to increase polymer-surface binding and produce more even coatings
- Further work is in progress to investigate the effect of different lengths of POEGMA. QCM-D is being used to determine surface thickness and the microarray scanner to deduce the optimum length for protein resistance

Acknowledgements

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