Mixed Self-Assembled Monolayers on Bi-Functional Magnetic Microcarriers

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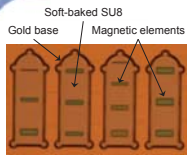
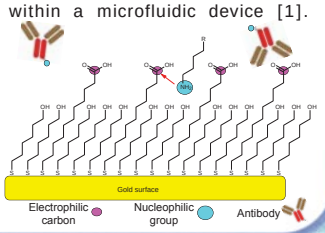
Introduction

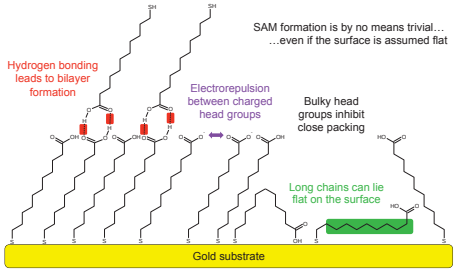


A new generation of magnetic microcarrier is being developed, enabling the functionalisation of two separate surfaces (gold and SU8 polymer). Magnetic elements of varying coercivity provide the unique advantage of being individually rewriteable within a microfluidic device [1]. Microcarriers can be mass produced and prepared with probe molecules [2] for a particular bioassay (e.g. point-of-care diagnostics), and screened in-flow within a lab-on-a-chip platform [3]. By using different fluorescent labels on each surface the microcarriers can provide a positive control in binding assays.

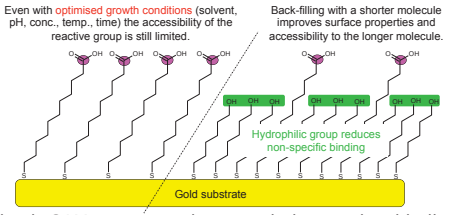


Here, we focus on optimising the chemical functionalisation of the gold side of such microcarriers. Two Quartz Crystal Microbalance (QCM) setups (in-beaker and in-flow) were used to study the effects of solvent, pH, conc. and temp. on the formation kinetics of Self-Assembled Monolayers (SAMs) of 11-mercaptoundecanoic acid (MUA) and 6-mercapto-1-hexanol (6-MCH) and their mixtures in real-time. Mixed SAMs have shown greater binding efficiency due to increased accessibility to functional groups [4]. The shorter hydroxyl (–OH) terminated 6-MCH are intended to act as spacer molecules to the longer carboxylic (–COOH) terminated MUA.

Mixed Self-Assembled Monolayers



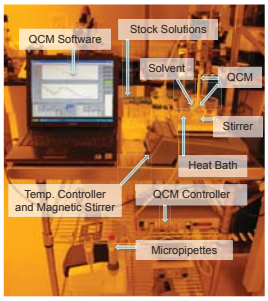
Monolayer quality is dependent on a variety of factors, ranging from surface defects to solvent properties. A SAM consisting of one type is often restricted in binding efficiency, especially for large molecules, where a fractional surface coverage can actually provide more probe binding than a full monolayer [4].



Mixed SAMs are used to optimise probe binding efficiency, where spacer molecules get adsorbed between bulky head-groups to increase their accessibility from packed ($\sqrt{3}\times\sqrt{3}$)R30° formations.

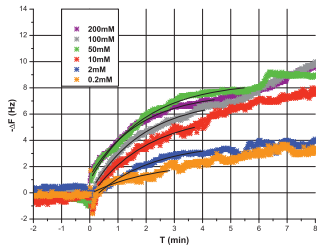
In-Beaker QCM Measurements

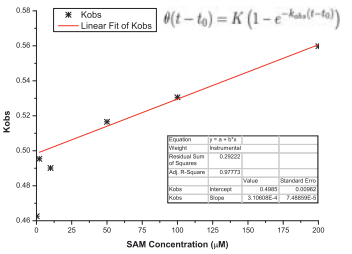
The in-beaker QCM setup recorded Langmuir type adsorption of alkanethiolate (MUA) concs. varying from 0.2μM to 200μM in high grade ethanol at room temp. (RT). Langmuir behaviour holds for the first 2-6mins, after which the monolayer undergoes more complex and lengthy reordering phases. The solvent reaches a critical density at around 50μM, with a ratio of available molecules to possible binding sites of approximately 3500:1, concs. below which will require longer formation times.



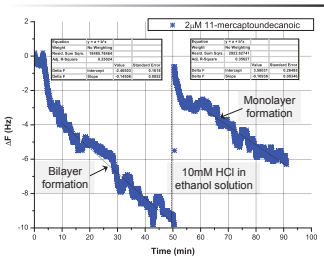
For stable QCM measurements the setup needs to guarantee:

- Temp. stability (ΔT<0.1°C)
- No mechanical vibrations
- No EM-noise pickup
- No solvent contaminants (e.g. Cu, PDMS, etc.)
- Agitation of reaction beaker
- Clean gold substrate (e.g. use fresh piranha acid)
- Diluted stock solutions to minimize mixing in beaker





In fitting each trace to the Langmuir time course of monolayer formation [5], one can plot the observed exponential growth constant k_{obs} vs. solvent conc. Considering $k_{obs}=k_aC+k_d$ and the obtained trend line, we find the free energy of monolayer formation to be ΔG_{ads}=-4.3kcal/mol.

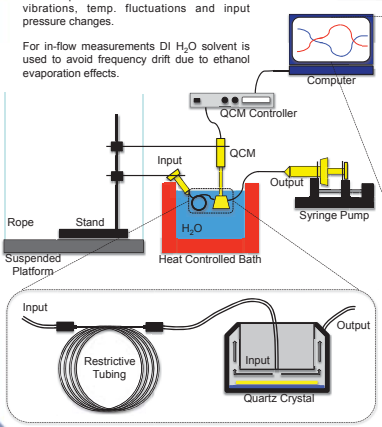


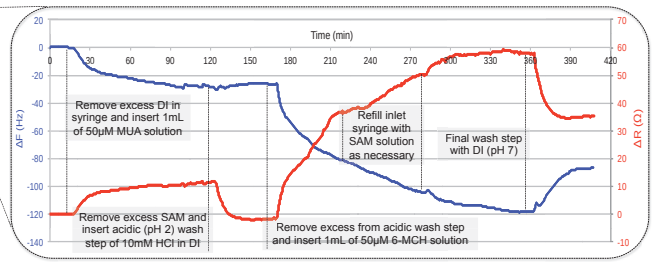
By acidifying the neutral ethanol solvent to pH 2 (HCl: 10mM) the carboxy terminus become protonated, which restricts hydrogen bonding and bilayer formation. The growth rate in the intermediate density phase (after Langmuir adsorption) is less steep at pH 2, suggesting monolayer formation. Slight changes to the reaction solution (e.g. density, temp., etc.) cause the observed QCM frequency offset.

In-Flow QCM Measurements

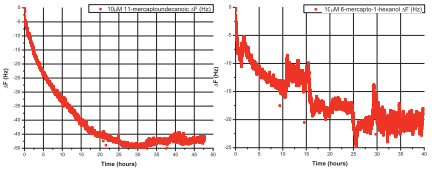
The setup minimises the effects of external vibrations, temp. fluctuations and input pressure changes.

For in-flow measurements DI H_2O solvent is used to avoid frequency drift due to ethanol evaporation effects.





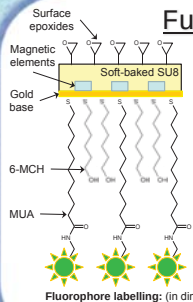
The in-flow QCM setup measures formation kinetics of mixed SAMs in a single run. For faster formation kinetics the 50μM solution (in DI H_2O) is assembled at an elevated and stabilised temp. of 40°C. By comparing the DI H_2O frequency level at the beginning and end of the run, a total drop of 85Hz is observed. This agrees with the overall frequency drop produced in the two separate >30hr measurements (for each alkanethiol) in the in-beaker setup using a 10μM conc.



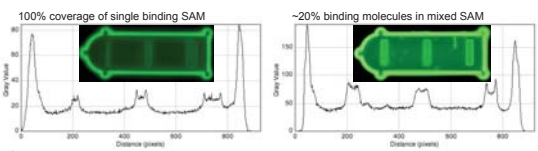
To study the properties of different SAM mixing ratios, full growth characterisations are made. A 10μM MUA solution requires ~30hrs to form a full monolayer (-48Hz) at RT. After which the QCM crystal was placed into an acidic wash bath (pH 2) before being put into a 10μM 6-MCH spacer SAM solution, which again saturates (-20Hz) after ~30hrs at RT.

It is interesting to note, that although the carboxylic terminated SAM saturated at a 48Hz frequency drop, the smaller hydroxyl terminated molecules were still able to produce a further 20Hz drop.

Functionalisation of Microcarriers



By understanding mixed SAMs we can optimise probe binding onto the gold side of the bi-functional magnetic microcarriers. Typically microcarriers functionalised with mixed SAMs show higher fluorescence levels than those using a single SAM.



Fluorophore labelling: (in dimethylformamide)

- Activation of carboxylic groups using n,n'-diisopropylcarbodiimide and Oxyma Pure
- Binding aminomethylfluorescein to SAM on gold substrate

Conclusions

- A new magnetic microcarrier is introduced that enables bi-functionality.
- Probe binding efficiencies are optimised using mixed SAMs. The effects of solvent, pH, conc. and temp. on the monolayer formation kinetics are investigated in real-time using in-flow and in-beaker QCM setups. Guidelines for mixed SAM growth are outlined.
- Mixed SAMs show higher fluorescence levels on functionalised microcarriers.

References & Acknowledgements

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[2] J.J. Palfreyman, C.H.W. Barnes *et al.*, AIP Conf. Proc. **1311** (1) 184 (2010)
[3] K.N. Vyas, C.H.W. Barnes *et al.*, IEEE Trans. Mag. **47** (6) 1571 (2011)
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[5] D.S. Karpovich, G.J. Blanchard, Langmuir **10** (9) 3315 (1994)

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