AFM Study of Mixed Carbohydrate/OEG Self-Assembled Monolayers

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Statement of Purpose: Molecular self-assembly of thiols onto gold is a powerful method for surface modification in biomaterials research. For instance, sugar-thiols can be used to form glycan-functionalized surfaces for carbohydrate microarray and biosensor fabrication. In this study, we use a model system consisting of mixed sugar-thiol and oligo(ethylene glycol) thiol. This mixed self-assembled monolayer (SAM) has been used extensively for surface plasmon resonance imaging analysis of carbohydrate bioavailability.[1] Here, we show that atomic force microscopy (AFM) analysis of these mixed SAMs reveals two distinct domains that appear to coalesce and phase separate over time. While the equilibrium has not been identified, our results suggest that clustering of sugar is a thermodynamically favorable process occurring within certain mixed sugar/oligo(ethylene glycol) SAMs.

Methods: *SAM Growth*: Oligo(ethylene glycol) thiol, OEG_2-SH (OEG), and a synthetic tetrasaccharide (a linear polymer of 4 mannose residues) bearing a terminal OEG_2-SH were used for self-assembly.[1] Pieces of gold/mica (prepared via thermal evaporation–BOC Edwards AUTO306 Vacuum Coater) were incubated in an aqueous solution of 100% glycan, 100% OEG, 50% glycan (mixed with OEG by molar ratio), or 33% glycan for 23-25 hours at room temperature in the dark. Prior to AFM imaging, samples were washed with pure ethanol and dried under nitrogen. All samples were stored under a nitrogen atmosphere. *Atomic Force Microscopy (AFM)*: An SPM Dimension 3100 (Veeco) was operated in air-tapping mode using Nanoscope v613r1 software. The images were acquired using an FESP silicon tip (Veeco). The instrument noise floor was 60-75 pm. *Image Processing and Analysis*: The height images were flattened with the 1st order flattening tool in Nanoscope (Veeco). An image threshold tool (ImageJ, NIH) was applied to create a binary (black/white) image and calculate the percent coverage ("analyze particles" function).

Results: The images of 50% and 33% glycan, obtained immediately following self-assembly (day 1), show non-uniform surface features; both samples contain two distinguishable domains. The features coalesce in the 50% sample and form a pattern reminiscent of leopard skin. These domains coarsen over a 13-day time study, but the pattern remains apparent (Figure 1). In the 33% sample, the features appear island-like and larger in diameter, while remaining isolated through the course of study (day 28) (data not shown). The average height difference between the two distinct domains was found to increase from 0.8 nm to 1.5 nm in both 50% and 33% cases. The controls of 100% OEG and 100% glycan were relatively uniform and did not change over time. The percent coverage of elevated domains, as determined from images taken at different locations on the same sample, was higher in 50% than in 33%.

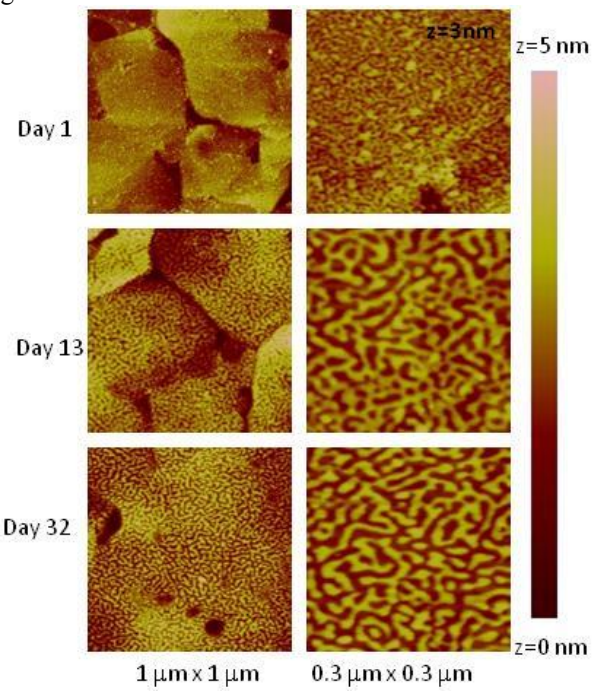

Figure 1. AFM height (z) images after 1st order flattening showing clustering of 50% glycan over time.

Conclusions: The observed clustering behavior of mixed carbohydrate/OEG SAMs appears to be a phase separation phenomena. We theorize that this is driven by self-association of sugar within the mixed SAM through intermolecular interaction. The percent coverage of elevated domains, which are believed to be glycan, shows some proportionality with the ratio of sugar/OEG in the growth solutions. This is supported by our previous study reporting the proportionality between components in mixed sugar/OEG SAMs and the initial solutions.[2] A height difference of 1.5 nm between the sugar and OEG domains also corresponds to the estimated 1.6 nm height of the sugar used in this study.

Mixed self-assembly is an invaluable tool to the biomaterials community, enabling discrete surface modification and the addition of molecular and biological functionality at the materials interface. Given the variety of applications in which SAMs are utilized, including carbohydrate-modified biosensors, the impact of microscopic behavior of the surface must continue to be considered and further studied.

References:

[1]Ratner D.M. et al. *Eur. J. Org. Chem.* **2002**;*5*:826-833.
[2]Dhayal & Ratner *Langmuir* **2009**;*25*(4):2181-2187.