Supporting Information

Nanoscale clustering of carbohydrate thiols in mixed SAMs on gold

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Figure S1. Characterizing the height histogram data of the 'tall' domain, 'short' domain, and inter-domain transition region. A raw AFM image (**R**, a 4:1 sugar:OEG Day 13 sample) was binarized using the Auto Local Threshold plugin for ImageJ. Bernsen thresholding with a radius of 25 pixels (14.6 nm) was selected to produce **B**. The ImageJ *erode* and *dilate* functions were then applied to expand and contract the high domain regions to produce **D** and **E** (Note: ImageJ dilation adds black pixels to the edges of existing black pixels; erosion does the opposite). The intersection between **D** and the negative of **E** produced **T**, representing the inter-domain transition region. The binary images were then used as masks for the original raw image (given by the operation **R** • mask), and the histograms of the results were plotted. The following operators were used: $\cap = AND$, ! = NOT, $\bullet = Multiply$. The analysis was performed on a 300 nm x 300 nm image. The images shown have been cropped to 150 nm x 150 nm for enhanced detail.

The plots indicate that the height histogram for mixed sugar+OEG glycoSAMs can be described by the sum of three roughly Gaussian curves: two outer peaks that correspond to the 'tall' (red) and 'short' (blue) domains, and a middle peak corresponding to the inter-domain transition regions (green).



Figure S2. (a) Height histogram data from a representative set of 'low' sugar samples (2:3 molar ratio sugar (**3**):OEG (**2**)) from Day 0 through Day 28. The horizontal axis is given as depth, where larger depth values correspond to lower SAM features (NOTE: depth is the native output of the AFM imager in tapping mode and is relative between samples). (b-f) Raw histogram data (blue fill) overlaid with the resulting distribution fits. The 'short' domain and 'tall' domain peaks are shown in green, the inter-domain transition region peak in cyan, and the overall fit in red. Distance from green to green peak maxima is the 'short' domain to 'tall' domain height difference estimate (inter-domain height differential, Δ , illustrated in f).



Figure S3. (a) Height histogram data from a representative set of 'high' sugar samples (4:1 molar ratio sugar(3):OEG (2)) from Day 0 through Day 28. The horizontal axis is given as depth, where larger values correspond to lower features. (b-f) Raw histogram data (blue fill) for the representative samples shown in (a) overlaid with the resulting distribution fits. The 'short' domain and 'tall' domain peaks are shown in green, the inter-domain transition region peak in cyan, and the overall fit in red. Distance from green to green peak maxima is the 'short' domain to 'tall' domain height difference estimate (inter-domain height differential, Δ , illustrated in f).



Figure S4. Five-month time-course of 1:2 ('Low' sugar) and 3:1 ('High' sugar) molar ratio of sugar (3) to OEG (2) mixed SAMs. Clustering behavior is consistent with observations of other molar ratios of sugar to OEG—lower concentration sugar SAMs result in an island-like pattern and higher concentrations result in a more continuous pattern. Images are 300 nm x 300 nm.