A Novel Self-Assembling Nucleobase Scaffold Coating with Nano-Scale Control

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Statement of Purpose: Synergistic peptide sequences in a coating promote cellular spreading[1] especially when the peptide spacing mimics *in vivo* distances. To generate a new scaffold material that can surface assemble on a hydrophobic surface and allow nano-scale positioning of biological residues, we focused on a supramolecular system composed of small molecular weight monomers. By tuning the distances within the assembly, the scaffold could be used to recreate epitope structures and/or construct arrays of biological residues on a surface.

Methods: Surface self-assembly experiments of bolaform monomers with a hydrocarbon core and end capped guanines connected by a short PNA[2] segment (Figure 1a) were carried out on highly oriented pyrolytic graphite, HOPG, (Grade ZYB) and imaged under fluid tapping mode AFM (Multimode, Veeco). Cantilever tips (Si_3N_4) with an average spring constant of 0.58 N/m were used. A water (Millipore) droplet was placed on the HOPG surface and monomer dissolved in DMSO (nmol/mL concentrations) was introduced into the water droplet. For two monomer experiments, each monomer solution was pre-mixed before introducing it into the water droplet to reduce any surface concentration gradients (i.e., **G-a$_{12}$-G** assembling in one area and **G-a$_{18}$-G** assembling in another). The surface was imaged with optimal gain and setpoint values. Representative images were captured from different areas of the surface and averaged to obtain characteristic lengths and surface concentration values. Small scan sizes were necessary to differentiate between the bands formed by each monomer to calculate surface mole fraction. In single monomer experiments, power spectrum density analysis was performed using the function in Nanoscope v6.11 software. The bands were measured in both the single and the mixing monomer experiments by cross-sectional analysis and other lateral measurement tools with the software. The areas were summed and a percentage mole fraction was calculated. Analysis images had > 95% coverage of monomer bands.

Molecular modelling (Insight II, Accelrys) of 9 individual monomer molecules on a simulated graphene sheet was carried out using the BUILDER and DISCOVER modules and computed on an SGI machine. Parameters were optimized to simulate an aqueous environment by setting a high dielectric constant. Steepest Descent and Conjugate methods were used to minimize the energy (Class I forcefield; CVFF).

Results/Discussion:

Single monomer characterizations of **G-a$_n$-G** (with hydrocarbon length **n=12, 18**) were assembled on HOPG under water. **G-a$_{12}$-G** and **G-a$_{18}$-G** formed bands with a characteristic spacings of 3.8 and 4.8 nm respectively. Modeling studies suggest that there is intermolecular alignment of the hydrocarbons, allowing the guanines to form a unique surface hydrogen bond network. We believe the various steric constraints within this system directs the surface assembly to produce the unique guanine network that is shown in our molecular models (Figure 1b). In a two monomer system (**G-a$_{12}$-G** and **G-a$_{18}$-G)**, the assembly phase separates into two separate bands (Figure 1c); the difference in monomer length would not favour a single band that contained both monomers on account of the disruption of inter-chain surface hydrogen bonding that would result from mismatched monomer lengths. To ensure both of the chain-end guanines are involved in the hydrogen bond network, the different monomers (**G-a$_{12}$-G** and **G-a$_{18}$-G**) need to phase-separate into different bands, resulting in a two component surface assembly with phase separation at the nano-scale. Since both monomers have the same guanine binding motif, there is no binding specificity; the order of the different sized bands is random. Interestingly, we have found that surface concentration of the two monomers is almost identical to the solution concentration in a 1:1 ratio, even though one monomer has six additional methylene groups; we believe this occurs as a result of a combination of the strong hydrogen bonding tape motif and the hydrophobic effect.

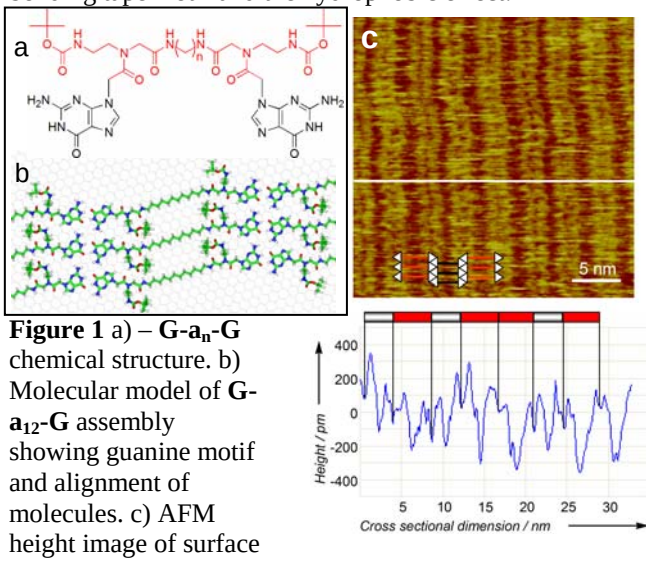

Figure 1 a) – **G-a$_n$-G** chemical structure. b) Molecular model of **G-a$_{12}$-G** assembly showing guanine motif and alignment of molecules. c) AFM height image of surface phase separation of two monomers (**G-a$_{12}$-G** and **G-a$_{18}$-G** – grey and red, respectively) on HOPG with cross section identifying each monomer band.

Conclusions: The surface assembly patterns of small molecular weight novel nucleobase materials can be tuned at the nano-scale by molecular design. By using the **G-a$_{12}$-G** and **G-a$_{18}$-G** two monomer system, we can phase separate at the nano-scale and, interestingly, the surface concentrations of the two monomers are identical to their solution concentrations even though they have a different number of methylene groups.

References:

1 Ochsenhirt SE et al. *Biomat.* **2006**;27:3863-3874.
2 Nielson PE. *Acc. Chem. Res.* **1999;**32:624-630.