Programmed Self-Assembly of a Biosensor to Probe Cell Adhesion Interactions

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Abstract

Rarely in Nature are single molecule interactions used for cell adhesion. Rather, several different types of molecules or structures interact in concert to build effective adhesion. We are developing a biosensor that can probe the range of interactions from the single molecule level to the ensemble average of multiple interactions. To achieve this goal we have harnessed specificity to effect programmed self-assembly of magnetic particles. Using polymer linkers, we construct magnetic particle self-assemblies between a ligand and a membrane bound receptor. Through application of an external magnetic field, a force is exerted on the magnetic particles of the assembly. Particles are selectively removed via the external magnetic field based upon their binding force with the functionalized surface of model cell membranes. Using this technique, we report binding forces measured in the piconewton range. We show preliminary data indicating the efficiency of such a biosensor construction and propose its use for both the measurement of ligand-receptor binding strength and its potential application for the elucidation of cell adhesion mechanisms and mechanics.

Introduction

A number of avenues may be utilized to self-assemble particle aggregates both in solution and on surfaces. Many different groups have used Langmuir-Blodgett approaches,[1-3] controlled solvent evaporation,[4,5] biological or polymeric templating,[6-10] and polymer and surfactant-assisted aggregation to prepare self-assembled aggregates.[11,12] However, a limitation of using symmetric components is that the final material will also be symmetric.

In this work we report on methods to specifically surface pattern and self-assemble nanospheres into hierarchical structures with a defined orientation. These methods result in the breaking of symmetry and thus make it possible to achieve highly anisotropic, bottoms-up assembled materials constructs. In our work, nanosphere assembly is achieved by selective polymer cross-bridge formation. To achieve this, difunctional polymer chains were synthesized, where one end of the chain selectively binds to a surface patterned region of the nanosphere while the free end bears complementary groups to form cross-bridges to a second type of nanosphere or patterned surface. We have shown that these methods are essentially universal and may be utilized with a wide variety of particle types and surface chemistries and forces, including those of long range forces such as

magnetic particles. We have constructed tailored aggregates of magnetic particles in the pursuit of developing a biosensor for the detection of ligand-receptor binding events.

We have begun polymer dynamics investigations using the Surface Forces Apparatus (SFA) to explore the interactions between complementary covalent-type bonds such as carboxylic acid-amine, gold-thiol, gold-silver polymer tethered interactions. We are also investigating the interactions between polymer-tethered ligand-receptor systems, such as streptavidin-biotin, to understand the dynamics of reversible bonds using the SFA. In addition to probing these non-covalent bonds via the SFA, we show preliminary data indicating the efficiency of using magnetic particles and external magnetic fields as a method for high precision measurement of piconewton range forces.

Experimental

Preparation and characterization of bi-functional polymer linkers.

All reagents were ACS grade and purchased from Sigma-Aldrich. The general set of reactions for synthesizing functional polymer cross-linking chains is depicted in Figure 1. First, 18-crown-6 (0.95 g, 3.6 mmol), dry THF (30 ml), and acetonitrile (125 µl, 2.40 mmol) were mixed with a solution of potassium naphthalide (2.4 ml, 1 M in THF) to generate an initiator. Next, ethylene oxide (15 ml, 301 mmol) was introduced into the reaction flask via a cooled syringe, and the reaction was stirred at room temperature for 48 h. The growing alkoxide chain end was then terminated by addition of methanesulfonyl chloride (0.37 ml, 4.8 mmol). At this stage, the reaction could be treated with either thioacetic acid (0.52 ml, 10.0 mmol) or NaN_3 (470 mg, 7.2 mmol) to install either mercapto or amino functionality at the ω-chain end. The synthesis of the polyethylene glycol (PEG) linker containing a siloxyl ether linkage will be reported elsewhere.[16] The polymer linkers used in this study had a M_n of about 2,800 g/mol with a polydispersity of 1.13 as determined by gel permeation chromatography and MALDI. IR, MALDI, and ninhydrin tests were used to verify the products of the reaction and creation of bi-functional PEG chains. Further details on the polymer synthesis are described by Barber and coworkers.[19] Amino terminated PEG-thiol (NH2-PEG-SH) chains and carboxy terminated PEG thiol (COOH-PEG-SH) chains were mainly utilized in this work.

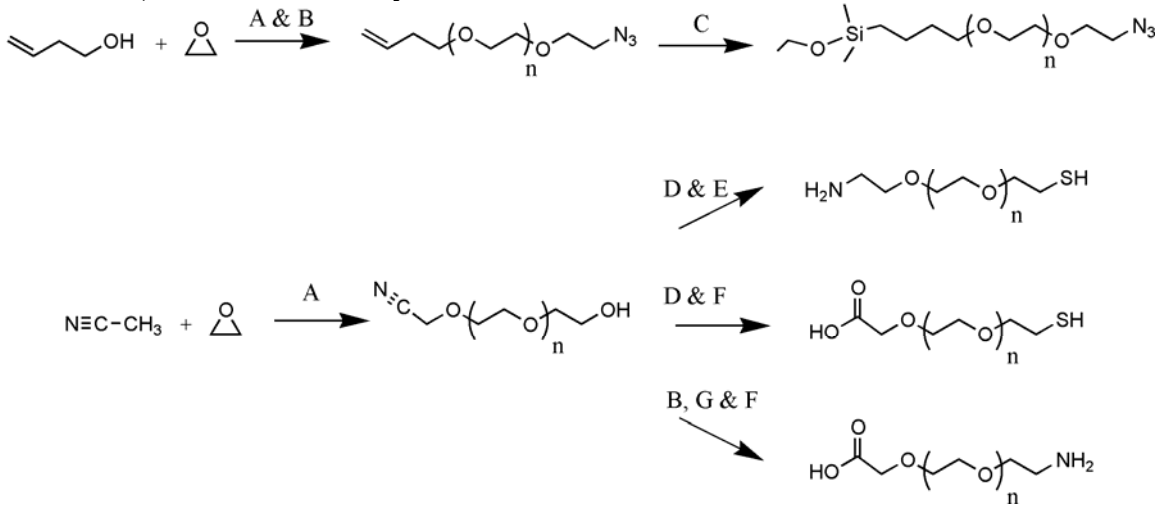

Figure 1. Synthetic route for the preparation of PEG linkers. Conditions: A) K / Naphthalenide; B) MsCl, Et_3N then NaN_3; C) $HSi(CH_3)_2OEt$, [Pt]; D) MsCl, Et_3N then CH_3COSH; E) $LiAlH_4$, THF; F) HCl, H_2O, Δ; G) PPh_3, H_3PO_4.[19]

Preparation and characterization of bilateral nanospheres and aggregates.

SiO_2, carboxylate-functionalized polystyrene and non-functionalized polystyrene particles were purchased from Polysciences, Inc. and Bangs Laboratories, Inc. Beads were cleaned prior to use following manufacturer's guidelines. Typically, a monolayer of particles was prepared on a substrate (glass, mica, or silicon) by either slow evaporation of solvent or by using Langmuir-Blodgett techniques. Next, the spheres were made anisotropic by coating with Au or Ag layers. The metal layers were evaporated onto the substrate-supported particles, either by thermal evaporation or sputter coating [13-15]. The metal-coated particles were released from the substrate by either sonication or by physically scraping the particles from the surface. The morphology of the metal surface coating (e.g. Au or Ag) was varied by the use or absence of adhesion layers (e.g., chromium), and manipulating the deposition rate, the substrate temperature, and the operating pressure in the deposition chamber. Nanospheres down to 40nm diameters have been patterned in this manner.[19] The bilateral nanospheres were then selectively functionalized with the desired PEG by incubating in ethanol for a minimum of 8 hours. After rinsing to remove non-reacted material, complementary nanospheres dispersed in ethanol were added to construct nanospheres aggregates. The mixture was incubated for another 8 hours and subsequently rinsed. For SEM imaging, dilute droplets of the ethanol solution containing PEG cross-linked aggregates were placed on copper grids. Digital SEM images were obtained using an FEI XL-30 SFEG operated at 5kV.

Magnetic particle aggregates were formed from 7 μm and 1 μm magnetic particles from Spherotech, Inc. The 7 μm particles were functionalized with streptavidin and the 1 μm particles were functionalized with Goat anti-Rabbit IgG. Bilateral nanospheres were formed utilizing the same methods described above. In the case of the magnetic aggregates, a 3,400 M_w SH-PEG-SH polymer was purchased from Nektar. The polymer was diluted to a concentration of 0.1 mg/ml in ethanol and filtered with a 0.1 μm Anotop filter prior to addition to the magnetic particles. The two types of magnetic particles and polymer solution were incubated in a vial for a few hours and then several droplets of the particle-polymer solution were placed on Cu TEM grids for SEM analysis.

Binding force measurement with magnetic particles.

Binding force measurements were carried out utilizing the 7 μm streptavidin-functionalized magnetic particles. Glass microscope slides were coated with OTS to render them hydrophobic. Subsequently a monolayer consisting of 95 mol % DSPE/2 mol % DSPE-PEG_{2000}-Biotin/3 mol % DSPE-PEG_{5000} lipids was deposited onto the slide using a Langmuir-Blodgett trough to create a model membrane surface.[20] Once deposited, the slide was maintained in phosphate buffer in a petri dish. For binding assays, 600 μL of the magnetic particle suspension at 0.1% w/v, were added to the dish and allowed to incubate a maximum of 8 hours at 25°C.

The force required to remove the streptavidin-functionalized particles from the model membrane surface was applied by varying the perpendicular distance of a permanent ring magnet from the surface of the microscope slide. The height of the permanent magnet was altered to tune the external magnetic field and thus the force applied to the magnetic particles. Figure 2 illustrates the distance dependence of the external field of the permanent ring magnet used. The data was measured using a LDJ Model 101B Gaussmeter with model HR-701 Hall probe. Based upon the distance of the ring magnet from the substrate, the calibration curve was used to determine the force applied to the particles. Images as a function of applied force were taken using a Nikon stereoscope with a CoolSNAP Pro_{cf} monochrome digital camera and Simple PCI version 5.1 image capture software. The number of beads bound and subsequently dislodged as a function of applied force were counted and used to estimate the ligand-receptor bond strength.

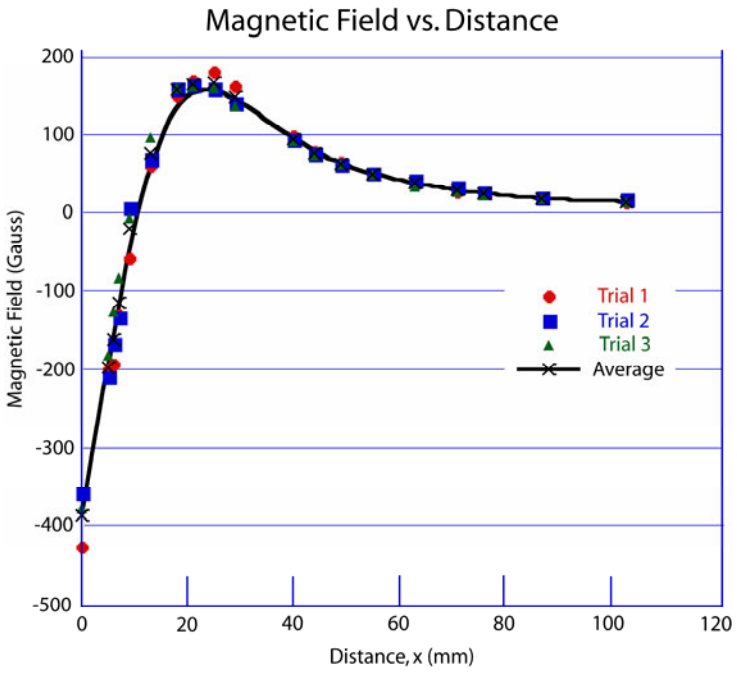

Figure 2. Magnetic field of the permanent ring magnet as a function of distance, x (mm), away from the surface.

Results and Discussion

Controlled Aggregate Assembly.

Figure 3(A) illustrates a cross-linking reaction between 400 nm SiO_2 particles and 5 μm polystyrene particles. The polystyrene particles were hemispherically coated with Ag and functionalized with HS-PEG-NH_2. The SiO_2 particles were hemispherically coated with Au and functionalized with HS-PEG-COOH. In this case, the cross-bridge formation occurred between the ends of two different PEG chains to form the aggregate structure.

Figure 3(B) illustrates the formation of the "raspberry" type morphology utilizing 14 nm gold nanoparticles and 500 nm polystyrene particles. The gold nanoparticles were treated with an HS-PEG-NH_2 and the polystyrene nanoparticles contained carboxylic acid moieties already built into the surface. Here the PEG-NH_2 needed to only interact with a carboxylic acid surface site to create a cross-link.

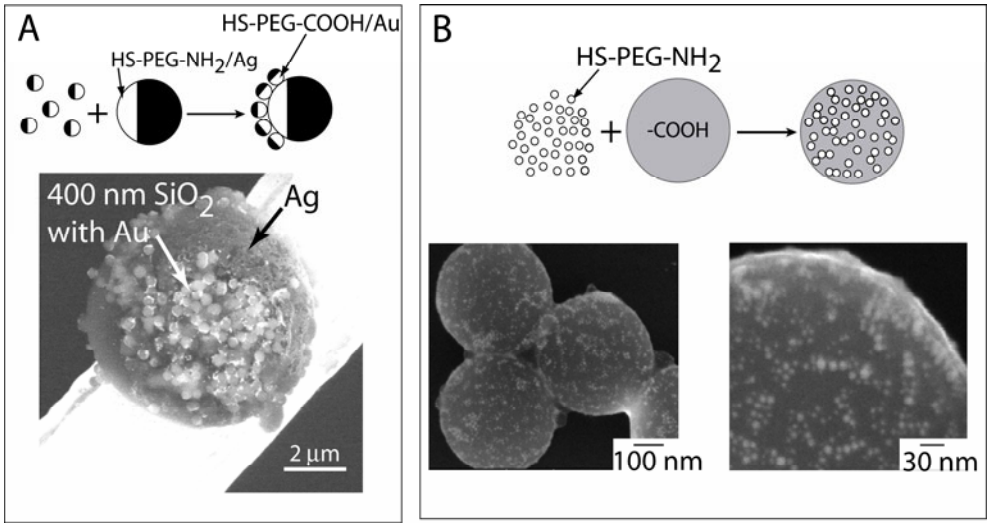

Figure 3. (A) SEM image of "half-raspberries" assembly of two complimentary-functionalized SiO_2 particles. (B) SEM image of "whole-raspberries" assembly of complimentary-functionalized Au nanoparticles and polystyrene particles.[19]

In addition to self-assemblies of SiO_2 and polymer particles, we have demonstrated the ability to specifically orient magnetic particles in dumbbell configurations. Figure 4 illustrates an example of such an arrangement. The larger particle is a 7-µm streptavidin-coated magnetic particle with a hemispherical coating of Ag. The smaller particle is a 1-µm hemispherically gold-coated paramagnetic particle. A 3,400 M_w PEG chain with dithiol functionality (HS-PEG-HS) links the particles together in a highly specific orientation such that the Ag and Au coated portions of the particles are facing each other (See the top of Figure 4).

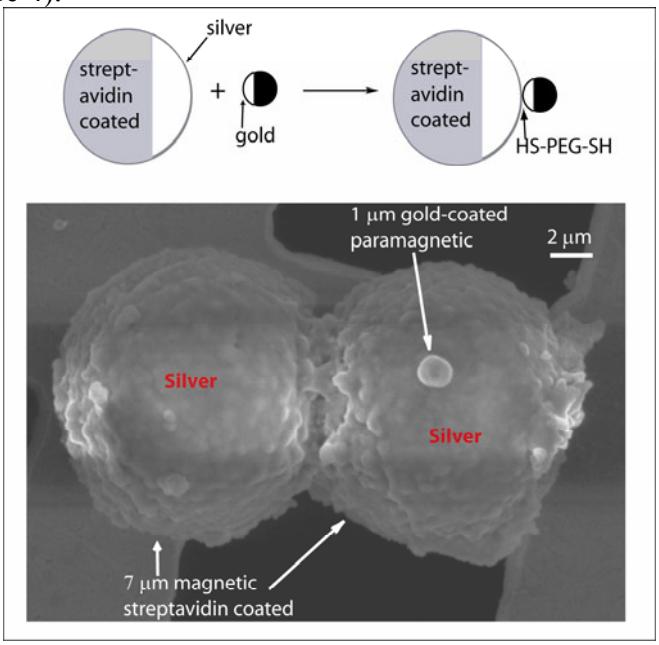

Figure 4. SEM image of (right) a 7-µm and a 1-µm magnetic particle. The orientation of the particles (top) suggests that the smaller particle is specifically bound to the larger particle by polymer cross-bridges.

Measurement of Specific Binding Interactions.

By the application of an external magnetic field, forces in the piconewton range were applied to the 7 μm streptavidin functionalized magnetic particles as they were interacting with a biotin-functionalized membrane. Figure 5 is a schematic drawing illustrating the interaction that the functionalized magnetic particle will have with the self-assembled monolayer on the microscope slide. Figure 6 shows images before and after the application of a 0.35 pN and 4 pN force to two different regions on the model membrane. It is interesting to note that earlier work by Ota and coworkers found similar binding strengths of 3.6 pN to 5.4 pN at a loading rate of 7.7 pN/s with an optical trapping system.[18] These proof of concept measurements demonstrate that a simple two-dimensional biosensor platform can be constructed to measure specific binding of ligand-receptor interactions using functionalized magnetic beads and a low resolution imaging system.

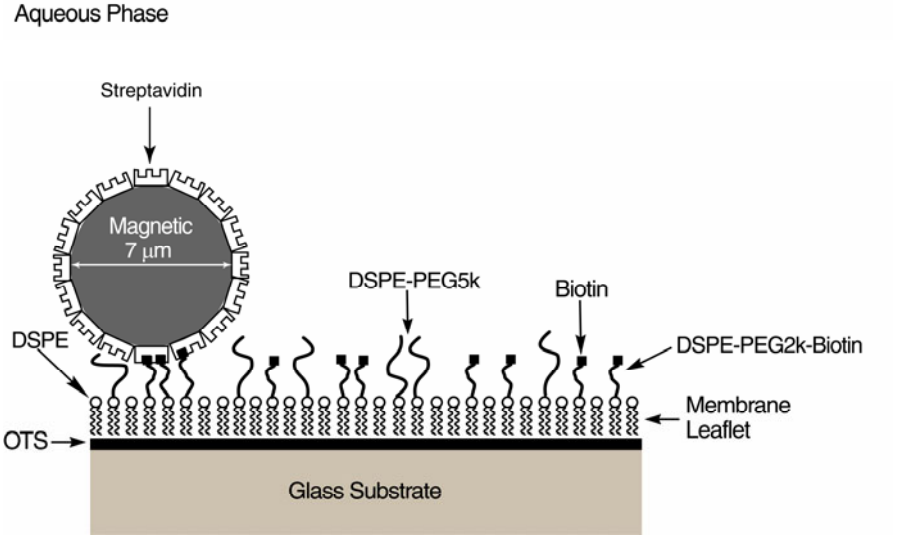

Figure 5. Schematic drawing to illustrate the interaction between the streptavidin-coated magnetic particles and the biotinylated model membrane surfaces in Figure 6.

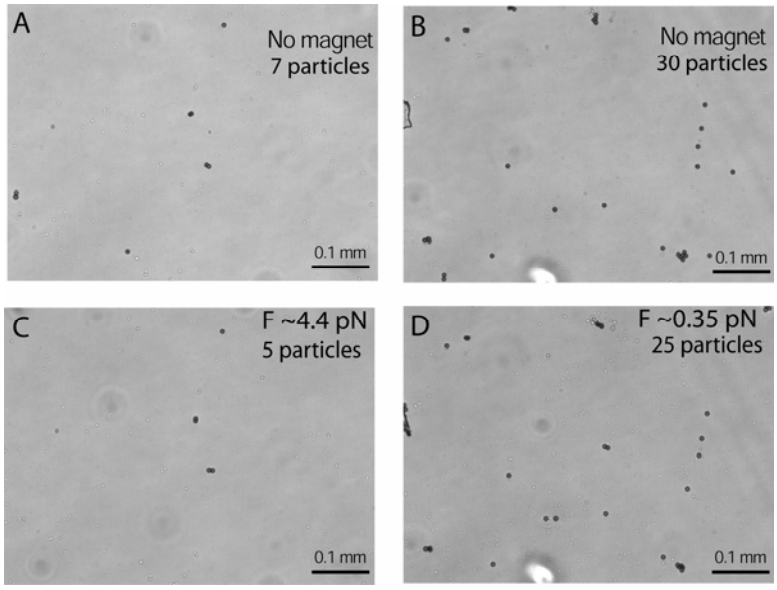

Figure 6. Images of streptavidin-coated magnetic particles interacting with biotinylated membranes on a microscope slide in the absence or presence of an eternal magnetic field. (A) & (B) No magnetic field, (C) 4.4 pN and (D) 0.35 pN.

Conclusions

In summary, we have coupled synthesis and self-assembly to produce, *in situ*, complex higher-order structures of nanoparticle and particle aggregates. We have developed anisotropic nanoparticles and chain end reactive polymers to program the nanoparticle surface for assembly. We have extended this work to investigate selective adhesion mediated by discrete ligand-receptor binding interactions. Preliminary work demonstrates that magnetic bead biosensors can be easily constructed to probe binding strengths in the piconewton range.

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References

1. Burghard, M., Philipp, G., Roth, S., von Klitzing, R., Pugin, R., Schmid, G. *Adv. Mater.* **1998**, *10*, 842.
2. Heath, J. R., Knobler, C. M., Leff, D. V. *J. Phys. Chem. B* **1997**, *101*, 198.
3. Nakahama, K.; Kawaguchi, H.; Fujimoto, K. *Langmuir* **2000**, *16*, 7882-7886.
4. Wang, Z. L. *Adv. Mater.* **1998**, *10*, 13.
5. Micheletto, R.; Fukuda, H.; Ohtsu, M. *Langmuir* **1995**, *11*, 3333-3336.
6. Claridge, S. A., Goh, S. L., Frechet, J. M. J. Williams, S. C., Micheel, C. M., Alivisatos, A. P. *Chem. Mater.* **2005**, *17*, 7, 1628-1635.
7. Boal, A. K., Ilhan, F., DeRouchey, J., Thurn-Albrecht, T. Russell, T. P., Rotello, V. M. *Nature* **2000**, *404*, 746.
8. Wang, S., Yan, J., Chen, L. *Mater. Lett.* **2005**, *59*, 1383.
9. Costanzo, P. J., Patten, T. E., Seery, T. A. P. *Chem. Mater.* **2004**, *16*, 1775.
10. Hiddessen, A. L.; Rodgers, S. D.; Weitz, D. A.; Hammer, D. A. *Langmuir* **2000**, *16*, 9744-9753.
11. Ramos, L.; Lubensky, T. C.; Dan, N.; Nelson, P.; Weitz, D. A. *Science* **1999**, *286*, 2325-2328.
12. Ramos, L.; Weitz, D. A. *Langmuir* **2001**, *17*, 2275-2277.
13. Haynes, C. L.; Van Duyne, R. P. *Journal of Physical Chemistry B* **2001**, *105*, 5599-5611.
14. Haynes, C. L.; McFarland, A. D.; Smith, M. T.; Hulteen, J. C.; Van Duyne, R. P. *Journal of Physical Chemistry B* **2002**, *106*, 1898-1902.

15. Whitesides, G. M.; Boncheva, M. *Proceedings of the National Academy of Sciences of the United States of America* **2002**, *99*, 4769-4774.
16. Costanzo, P. J., Patten, T. E., Seery T. A. P. *Chemistry of Materials* **2004** *16*, 1775-1785.
17. Kuhl, T. L.; Leckband, D. E.; Lasic, D. D.; Israelachvili, J. N. *Biophys. J.* **1994**, *66*, 1479-1488.
18. Ota, T.; Sugiura, T; Kawata, S, *Applied Physics Letters* **2005**, *87*, 043901-043904.
19. Barber, S. M.; Costanzo, P. J.; Moore, N. W.; Patten, T.E.; Lancaster, K.S.; Lebrilla, C.B.; Kuhl, T. L., *Journal of Physical Chemistry A* **2006**, *110*, 4538-4542.
20. Kuhl, T. L.; Majewski, J.; Wong, J. Y.; Steinberg, S.; Leckband, D. E.; Israelachvili, J. N.; Smith, G. S., *Biophysical Journal* **1998**, *75*, 2352-2362.