VIP Molecular Recognition

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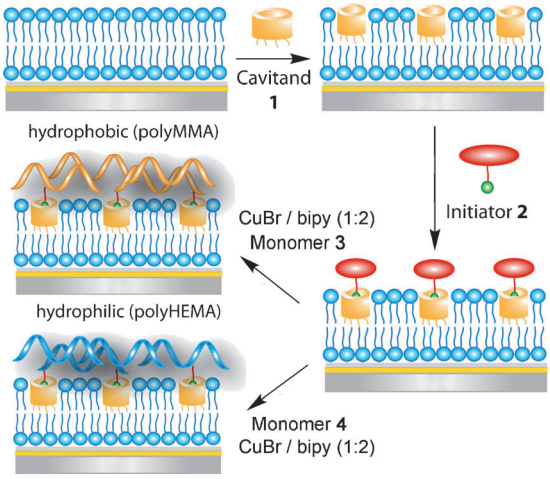
A Membrane-Bound Synthetic Receptor that Promotes Growth of a Polymeric Coating at the Bilayer–Water Interface**

Ying Liu, Michael C. Young, Orly Moshe, Quan Cheng, and Richard J. Hooley**

The central feature of bacterial cell structure is the presence of an outer polysaccharide cell wall surrounding a fluid membrane.[1] This polymeric cell wall provides protection to the sensitive membrane structure, as well as incorporating molecular recognition motifs for cell signaling, transport, and adhesion processes.[2] Synthetic equivalents could be created with preformed polymers that can be covalently attached to vesicles, bilayers, or cells,[3,4] but these methods are limited in scope by the solubility of the polymer. An alternative strategy would be to grow a polymeric coating atop a biomimetic supported lipid bilayer, but this approach has been limited by the poor tolerance of the lipid bilayer to polymer growth conditions. Recent advances, such as atom-transfer radical polymerization (ATRP), are tolerant to aqueous media and can be performed under very mild conditions.[5] Indeed, polymer coatings grown by ATRP (and surface-initiated ATRP) are used as cushions to allow assembly of supported lipid bilayers on surfaces.[6,7] ATRP is also useful for grafting polymers on surfaces and materials.[8] What is unprecedented, however, is to grow a tailored polymeric structure, from individual monomers, on the exterior of an established lipid bilayer. The obvious challenge in this approach is controlling the attachment of the outer polymer coating to the fluid membrane, as opposed to merely growing a polymer in and around the bilayer,[9] or polymerizing the vesicle itself.[10] To grow a polymer coating at the water–bilayer interface, the polymerization initiator must be incorporated in the bilayer itself. Most studies of artificial membrane constructs employ the covalent attachment of the desired motif to a lipid or steroid derivative.[11] Creation of synthetic species that are capable of both selective membrane incorporation and reaction promotion or catalysis is challenging. To prevent the destruction of the lipid bilayer, the reaction conditions must be mild, and the reactive species must be tolerant to the buffered aqueous conditions necessary for bilayer formation. Most examples of reactions at membrane surfaces consist of bio-orthogonal processes,[12] where the targets are covalently linked to steroid or lipid anchors and the reactive units are insensitive to the external media.

Recently, we introduced a different method of displaying functionality at a membrane bilayer surface; the incorporation of a water-soluble deep cavitand that can recognize trimethylammonium-tagged substrates and display the substrate to the exterior milieu, allowing immobilization of a number of species (including proteins) at the membrane surface.[13] Herein we use this system to display a reactive initiator for ATRP[5] for the synthesis and installation of a functionalized polymeric surface at a supported lipid bilayer (SLB) interface through selective molecular recognition (Scheme 1).



Scheme 1. Representation of polymer growth at a cavitand–impregnated supported lipid bilayer.

Tetracarboxylate cavitand **1** (Figure 1) is a water-soluble synthetic receptor that recognizes suitably sized hydrophobic species as well as substituted trimethylammonium salts.[14] The cavitand can be incorporated in either lipid-based micelles[15] or supported lipid bilayers[13] while retaining its recognition properties for NMe_3^+-tagged targets by exploiting cation–π interactions between the faces of aromatic cavitand walls and the charged guest. A significant advantage of cavitands as hosts is their open-ended character: long guests can extend out of the cavity, presenting large functional groups into the bulk solvent. The incorporation of cavitand and bound guests in the system is extremely mild; injected substrates are exposed to water for only 5–10 minutes. With this in mind, we explored the incorporation of a reactive initiator species **2** that is capable of initiating ATRP of methacrylate monomers under mild conditions in aqueous

[*] Y. Liu, M. C. Young, O. Moshe, Prof. Q. Cheng, Prof. R. J. Hooley
University of California Riverside, Department of Chemistry
Riverside, CA 92521 (USA)
E-mail: quan.cheng@ucr.edu
richard.hooley@ucr.edu

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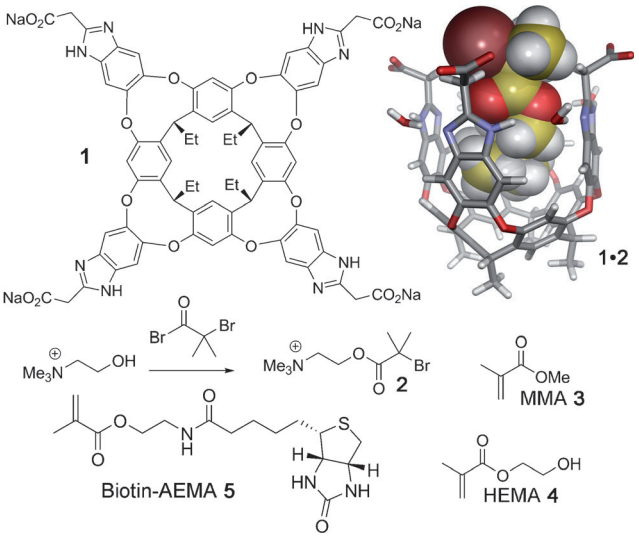

Figure 1. Top: Tetracarboxylate cavitand **1** and the minimized conformation of **1** (SPARTAN) with one bound initiator molecule (**2**) in the cavity. Bottom: Guests and monomers used in this study.

solution.[5,16] By displaying the initiator at the membrane surface, it is possible to create polymers that are noncovalently attached to the top of the bilayer to form a flexible coating. The experiment is illustrated in Scheme 1. The membrane was deposited on a nanoglassified gold chip that was placed in a flow cell (see the Supporting Information).[17] The bilayer was fabricated by the injection of preformed L-α-phosphatidylcholine (PC) vesicles that fuse readily on this chip. Sequential addition of an aqueous solution of cavitand **1** (0.8 mg mL^{-1}, 10% DMSO/water solution) followed by radical initiator **2** (10 mg mL^{-1} aqueous solution) by flowcell injection allows incorporation of the cavitand in the supported lipid bilayer and subsequent display of the initiator at the surface. These events were monitored in real time using surface plasmon resonance (SPR) spectroscopy.

The initial test monomer was methylmethacrylate **3** (MMA). After the injection of a 0.3 M aqueous solution of **3** in the presence of catalytic mixture of CuBr, 2,2′-bipyridyl (bipy), and ascorbic acid (in a 1:2:1.5 ratio), a slow increase in SPR signal was observed (Figure 2), corresponding to the

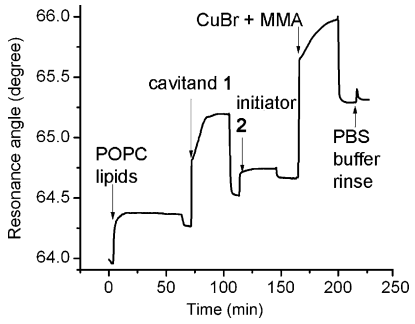

Figure 2. Surface plasmon resonance (SPR) sensorgram for cavitand-mediated synthesis of poly(MMA) atop the supported lipid bilayer (SLB).

formation of poly(MMA) at the membrane surface at room temperature. After 20 min, the excess monomer was rinsed from the flowcell, and reaction (and concomitant SPR signal increase) ceased. The attached polymer was resistant to washings: the system was rinsed with both water and PBS solution, neither of which caused removal of the polymer from the system. Addition of choline chloride as a competitive guest for the initiator anchors did not remove the hydrophobic polymer from the surface, which is most likely due to inaccessibility of the cavitands to the exterior medium after polymerization has occurred. Control experiments were performed in the absence of the cavitand (see the Supporting Information). No incorporation of initiator **2** into the membrane was observed, and no growth of polymer occurred upon addition of MMA and CuBr catalyst, although MMA monomer **3** caused a slight increase in SPR signal owing to weak nonspecific interaction with the SLB. Anchoring of the initiator **2** in the membrane is essential for polymer growth: without the cavitand present, free initiator is washed from the flowcell. The living nature of the polymerization can be tested by repeating polymer growth after excess monomer is removed by washing. Polymer growth can be restarted after removal of monomer by simple reinjection of monomer and catalyst mixture (see the Supporting Information for sensorgrams).

Further evidence for the attachment of the polymer to the bilayer surface is shown by contact angle experiments and SPR analysis (see the Supporting Information). Addition of a water drop to the hydrophilic bilayer causes spreading across the surface with a contact angle of 10.5°. After poly(MMA) formation, a fully formed drop is observed at the surface with a substantially increased contact angle of 53.9°. The SPR sensorgrams allow calculation of surface coverage upon adaptation of Jung's formula.[18] The surface coverage of cavitand **1** (and by extension, initiator **2**) was estimated to be 1.53×10^{-10} mol cm^{-2}. The surface coverage of freshly grown poly(MMA) was determined to be 1.58×10^{-8} mol cm^{-2}, with a calculated thickness of approximately 17 nm (see the Supporting Information).

Characterization of the membrane underneath the polymer was achieved by FRAP (fluorescence recovery after photobleaching) experiments. A POPC membrane doped with 5% fluorescent NBD-PC was formed in a flow cell on a cleaned substrate. Cavitand **1** was introduced and a poly(MMA) film grown atop the membrane. FRAP measurements were taken of both the pristine membrane and the polymer-coated bilayer film. The diffusion coefficient for the pristine lipid membrane was (3.98 ± 0.85) μm^2 s^{-1}, which is consistent with the literature results for similar systems.[19] For the polymer-coated bilayer membrane, however, the fluorescence intensity was considerably lower, and the diffusion coefficient was reduced to (0.09 ± 0.02) μm^2 s^{-1}. This indicates that the presence of the polymeric surface substantially rigidifies the membrane bilayer underneath it, limiting the lateral mobility and diffusion coefficient of the lipids.

The nature of the polymeric coating can be varied. When 2-hydroxyethyl methacrylate (HEMA) **4** was used as monomer, a hydrophilic polymer (poly(HEMA)) was formed at the membrane surface, and the process was again monitored by

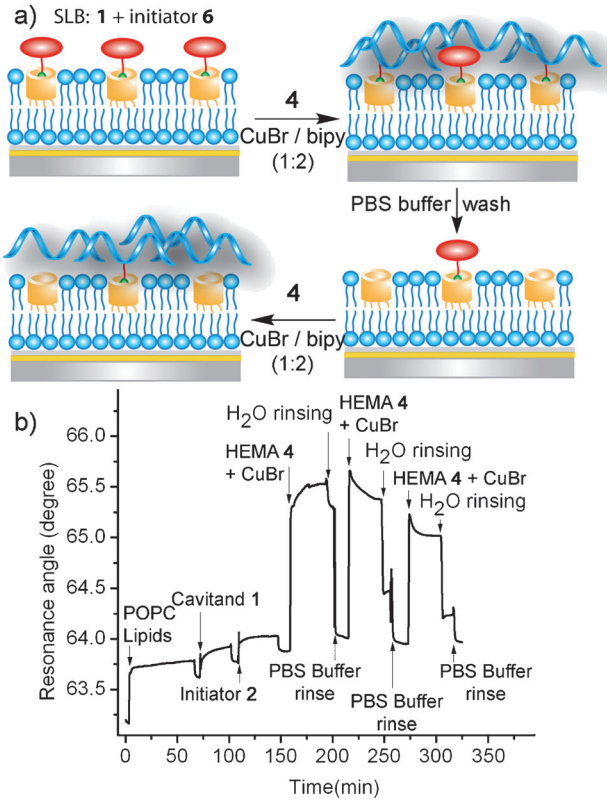

Figure 3. a) Reversible polymer growth; b) SPR sensorgram indicating the repeated removal/construction process with poly(HEMA).

SPR (Figure 3). No loss of polymer was observed upon washing with water, but the poly(HEMA) patch was significantly less intractable than the poly(MMA) in salt solution and could be removed from the bilayer interface by a simple wash with PBS buffer (Figure 3). Cavitand **1** is well-known to display lower binding affinities for its targets in high salt conditions,[14b] and it appears that even the multiple anchors from initiation are not sufficient to hold the hydrophilic poly(HEMA) at the bilayer surface under the shear forces applied in the flowcell. The hydrophilic polymer can therefore be selectively removed by this treatment. This experiment allows analysis of the state of the bilayer membrane after polymerization, which is not possible with the immovable poly(MMA). SPR analysis indicates that the supported lipid bilayer remains fully intact throughout the experiment. We therefore carried out additional polymer growth on the same membrane after washing with PBS buffer to remove the membrane-bound polymer. Initiator **2** was injected into the freshly regenerated chip, followed by a HEMA **4**/CuBr solution. Polymer growth was observed again at the bilayer interface, indicating that the cavitands were not removed from the bilayer and remained bound and functional in the membrane (see the Supporting Information). In fact, repeated poly(HEMA) growth did not require addition of further initiator molecules (Figure 3b). When HEMA **4** and CuBr were injected to the system without new addition of initiators, growth of poly(HEMA) could again be observed, albeit to a smaller extent. The degree of polymerization can be tracked and estimated by SPR measurements: after the initial reaction (and aqueous wash), an increase in resonance angle of 1.37° was observed. This increase was lessened to 0.47° for the second growth after poly(HEMA) removal, and to 0.27° after the third experiment. Evidently not all bound initiator molecules are used up in the initial polymerization, and remain intact, bound in the cavitands underneath the growing polymer. Upon washing, the "used" initiators are removed from the cavitand along with the polymer, leaving fresh initiator at the membrane surface, which is suitable for future reaction (Figure 3a). If a new batch of initiators was added to the system after each washing, the polymer growth occurs at its maximal levels (see the Supporting Information). While the initiators are used in the reaction, the cavitands are not and remain in the membrane, ready for refilling with new reactants.

Functional polymers can also be created at the membrane interface. Biotin-tagged AEMA **5** was synthesized by amide coupling of biotin with 2-aminoethylmethacrylate hydrochloride (AEMA). Monomer **5** is only sparingly water-soluble, and was introduced to the bilayer/**1**/**2** complex with catalytic CuBr as before in a 10:1 water/DMSO solution (see the Supporting Information for sensorgrams). Poly(Biotin-AEMA) was resistant to removal from the surface by PBS washing, indicating its relative insolubility in water. The bioadhesive properties of the poly(Biotin-AEMA) surface are characterized by treating the membrane-bound polymer with avidin and monitoring the capture of protein at the polymer surface. SPR analysis carried out using fluorescently-tagged avidin shows that upon injection of 0.25 mg mL^{-1} avidin–fluorescein conjugate, a change in resonance angle of 0.71° was observed (see the Supporting Information). On the plain POPC membrane, there was a slight nonspecific binding of the avidin–fluorescein conjugate, but the observed resonance angle change was minimal. The adhesion process has also been studied by confocal fluorescence microscopy (Figure 4). The microscopic image illustrates the immobilization of the avidin–fluorescein conjugate by the bioadhesive polymer. The white line delineates the edge of the chip, showing no protein adhesion in the absence of the bioadhesive polymer.

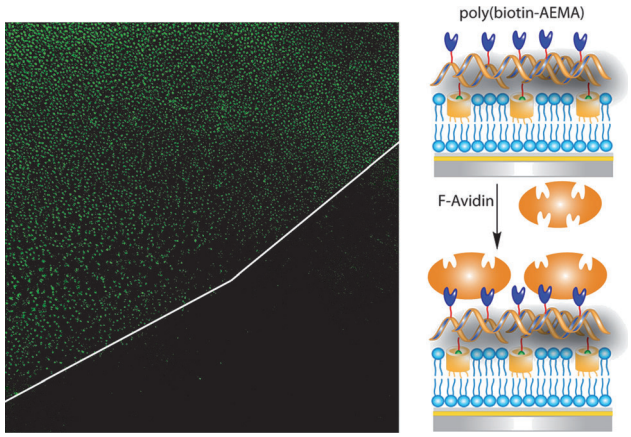

Figure 4. Confocal microscopy image of poly(biotin-AEMA) grown at the bilayer interface, immobilizing fluorescently labeled avidin. The white line indicates the interface between SLB and bare chip surface.

In summary, we have shown that a membrane-bound water-soluble deep cavitand can be used to anchor an ATRP initiator that can initiate polymer growth at the membrane–water interface of a supported lipid bilayer. The initiator molecule cannot be incorporated into the membrane directly, therefore polymer growth occurs only in the presence of cavitand. Both hydrophobic and hydrophilic polymers, as well as bioadhesive polymers, can be synthesized by simple alteration of the monomer. For hydrophilic poly(HEMA), the polymerization reaction can be repeated several times after polymer removal by simple washing. The supported lipid bilayer remains intact and the cavitands remain incorporated inside throughout the reaction, and formation of the polymeric coating considerably reduces the lateral mobility of lipid components in the membrane. The mild experimental conditions and anchored polymer growth provide a crucial breakthrough for installation of functional polymers on a fluid lipid membrane.

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