Adaptive insertion of the hydrophobic anchor into poly(ethylene glycol) host for programmable surface functionalization

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Abstract

Covalent and non-covalent molecular binding represent two strategies to tailor surface properties and functions. However, the lack of responsiveness or the requirement of specific binding groups challenged the spatial-temporal control of surface functionalization. Here, we report the adaptive insertion of the hydrophobic anchor into poly(ethylene glycol) (PEG) host as a new non-covalent binding strategy for surface functionalization. By using polycyclic aromatic hydrocarbons as the hydrophobic anchor, hydrophilic charged and non-charged functional modules were spontaneously loaded onto the polymeric membrane in 2 minutes without the assistance of any catalysts and binding groups. The thermodynamically favorable insertion of hydrophobic anchors can be reversed by pulling the functional modules, enabling the programmable surface functionalization. We anticipate that the adaptive molecular recognition between hydrophobic anchor and PEG will challenge the hydrophilic understanding of PEG and enhance the progress in nanomedicine, advanced materials, and nanotechnology.

Introduction

Spatial-temporal selective surface functionalization represents the key step towards next-generation functional systems. Living organisms have already achieved signal transfer, cargo transport, and photosynthetic energy conversion, *etc.* by controlling the distribution and orientation of functional proteins on cell membrane¹. However, it is still a grand challenge for synthetic materials to realize such precise control due to the lack of adaptive binding chemistry²⁻⁴. Click chemistry provides an efficient binding strategy while sharing the non-responsive nature of covalent binding strategy⁵. Thanks to the development of molecular recognition, biomolecular recognition and host-guest interaction have been used to load therapeutic proteins on polymeric particles, isolate plasma membrane proteins, and load molecular catalysts on the electrode, *etc.*⁶⁻¹⁰ Although proved effective, the loaded functional modules can only be released by adding competitive host/guest molecules or through the photoisomerization of the guest molecule, which challenged the re-loading of functional modules, the recycling of host molecules-modified materials, and the synthesis of guest molecules^{11,12}. Moreover, the host molecules, such as cyclodextrins, and cucurbiturils, have to be installed on the materials' surface in advance¹³. Engineering materials' surface as the host may enable a simple binding strategy.

Polymer and small molecule ligands are widely used to manipulate the surface chemistry of synthetic materials. For instance, PEG, a hydrophilic and non-toxic polymer, has been engineered into stealth corona on nanocarriers, antifouling coating on implanted materials, polymer electrolytes on porous materials, and capping ligand on nanoparticles, *etc.*¹⁴⁻¹⁹ Inspired by "induced fit model" for enzyme-substrate recognition, we believe that flexible polymer chains, similar to the polypeptide chains of enzymes, should be able to fit into the size and shape of guest molecules by changing their conformation and act as the host molecules^{20,21}. However, unlike the existence of a hydrophobic cavity in cyclodextrins and cucurbiturils for binding, PEG chains are well hydrated in an aqueous medium by forming hydrogen bonds with water molecules²²⁻²⁴. The association of a guest molecule and PEG should require the

breakage of these hydrogen bonds and the removal of water molecules from the binding site, just like the protein-ligand binding²⁵⁻²⁷. The dehydration of PEG was thought very difficult, due to its high aqueous solubility²⁸. Thus, PEG, although widely existed on materials' surfaces, has never been explored as the host. Recent simulation and experiment results suggested that the hydration layer of PEG is metastable, which can be removed by changing PEG chain conformation from coil to *helix* or planer-zigzag by stretching the polymer chain (Fig. 1a)²⁹⁻³¹. Hydrophobic graphene composed of sp²-hybridized carbon atoms is noticed to induce the planer-zigzag conformation of PEG by forming multiple van der Waals interactions, including long pair- π and CH- π interaction^{32,33}. We hypothesized that polycyclic aromatic hydrocarbon, bearing a similar chemical structure with graphene, might be able to break the hydration layer and act as the guest molecule (anchor) of PEG. The reversible hydration/dehydration of PEG should allow the adaptive insertion of the anchor.

To test this hypothesis, we designed a molecular probe comprising of hydrophobic pyrenyl (Py) group as the anchor, osmium complex (Os) as the model functional module³⁴, and PEG as the spacer (Py-PEG₄-Os, Fig. 1b). Such design can help us elucidate the association of Py and PEG: 1) the polarity-sensitive fluorescence of Py can reflect the position of the anchor in PEG host³⁵, 2) the visible-light absorbance of Os can demonstrate the loading process of molecular probe onto PEG host (Supplementary Fig. 1), 3) photoinduced electron transfer from Os to Py can quench the fluorescence of Py and validate the conformation of loaded molecular probe (Supplementary Fig. 2). As for the PEG host, we are interested in the PEG corona of polymersomes with glassy polystyrene (PS) core due to their well-defined structure and high stability (Fig. 1c)³⁶⁻³⁹. We envisioned that the molecular probe can be loaded onto polymersomes by inserting its hydrophobic anchor into PEG corona by forming van der Waals interaction. The non-covalent interaction and reversible hydration/dehydration of PEG should allow the adaptive insertion of the anchor for programmable surface functionalization (Fig. 1d).

Results And Discussion

Insertion of the hydrophobic anchor into PEG corona. We first tested the loading of molecular probes onto the PEG corona of polymersomes. Here, functional groups with increased hydrophobicity in the order of hydroxyl (Hy) isopropyl (Ip) phenyl (Ph) naphthyl (Na) Py were used as the anchor (Fig. 2a and Supplementary Fig. 3-7). Once linked to -PEG₄-Os with ether group, these anchors can be easily dissolved in water, even for the most hydrophobic Py (Fig. 2b and Supplementary Fig. 8). This should arise from the 1) intramolecular van der Waals interaction between PEG spacer and Py, which reduced the unfavorable interaction between Py and water, and 2) intermolecular electrostatic repulsion between positive charged Os, which inhibited the aggregation of Py in water (Supplementary Fig. 8 and Section 1). The solubilized Py is partially covered by the PEG spacer, resulting in the folded conformation of Py-PEG₄-Os in water (Fig. 2b, Supplementary Fig. 9 and Section 2). Once added into the aqueous dispersion of polymersomes, Ip-/Ph-/Na-/Py-PEG₄-Os were loaded onto polymersomes in 2 minutes, while no loading was detected for Hy-PEG₄-Os (Fig. 2c). This suggested that the hydrophobic anchor, instead of the

hydrophilic -PEG₄-Os, was interacting with PEG corona. The successful loading was proved by the color change of precipitated polymersomes, cryogenic electron microscopy (cryo-EM), zeta potential, and elemental mapping characterizations (Fig. 2d, Fig. 2e and Supplementary Fig. 10).

To unravel the loading mechanism, the interaction between the hydrophobic anchor and polymersomes was characterized by isothermal titration calorimetry (ITC). When the aqueous solution of Ip-/Ph-/Na-/Py-PEG₄-Os was injected into the aqueous dispersion of polymersomes, positive entropy change ($\Delta S > 0$) and negative enthalpy change ($\Delta H < 0$) were detected, which should be caused by the release of water molecules bound to PEG corona and the formation of van der Waals interaction between the hydrophobic anchor and PEG corona, respectively (Fig. 2f). This contributed to the thermodynamically favorable loading of Ip-/Ph-/Na-/Py-PEG₄-Os onto polymersomes ($\Delta G < 0$). For Hy-PEG₄-Os, no interaction was detected ($\Delta H=0$, $\Delta S=0$), again validating that only hydrophobic anchors can break the hydration layer and interact with PEG corona. Moreover, with the increase of anchor-hydrophobicity, the saturated loading number and binding stoichiometry of molecular probes per polymersome became significantly increased (Fig. 2c and Supplementary Table 1). This demonstrated that the more hydrophobic anchor would have a higher binding affinity to the PEG corona. The position of the anchor on polymersomes was investigated by monitoring the fluorescence of Py. Here, we used Py-PEG₄-imidazole (Py-PEG₄-Im), the precursor of Py-PEG₄-Os, as the molecular probe to exclude the quenching of Py by Os (Supplementary Fig. 11). During the loading process, the fluorescence intensity of Py decreased by ~80%, and the ratio of first and third fluorescence peaks (I_1/I_3) decreased from 1.73 to 1.43 (Supplementary Fig. 12), which indicated a dramatic decrease of polarity around Py⁴⁰. After removing the residual Py-PEG₄-Im in solution, I_1/I_3 was determined to be 1.32, consistent with the literature value of Py in the PEG phase ($I_1/I_3 = 1.35$) and much larger than Py in PS ($I_1/I_3 = 0.95$)^{41,42}. This confirmed that Py was inserted in PEG corona while not PS core. To further validate that hydrophobic anchor was associating with PEG, porous PEG hydrogel microparticle was explored as the host. After the addition of Py-PEG₄-Im, PEG hydrogel microparticles start to emit fluorescence in 2 minutes, suggesting the rapid loading of Py-PEG₄-Im (Supplementary Movie 1 and Supplementary Fig. 13). Due to the porous structure of PEG hydrogel microparticles, Py-PEG₄-Im was homogeneously distributed over the whole microparticles⁴³. These results demonstrated that PEG was acting as the host of the hydrophobic anchor.

Based on the above results, we proposed the schematic loading mechanism of Py-PEG₄-Os onto PEG corona of polymersome (Fig. 2g). In water, Py-PEG₄-Os formed a folded conformation due to the van der Waals interaction between Py and PEG spacer to reduce the unfavorable interaction between Py and water. The multiple PEG chains of PEG corona can interact more strongly with Py than a single PEG spacer, which induced the insertion of Py into PEG corona and the coordinative unfolding of Py-PEG₄-Os. The insertion of Py would induce the dehydration of PEG, which may change PEG chain conformation from coil to planer-zigzag at the binding site.^{32,33} When Py was completely inserted into PEG corona, the unfolded hydrophilic -PEG₄-Os was left on the corona surface. The electrostatic repulsion between Os restricted the packing density of Py-PEG₄-Os on PEG corona, resulting

in an average separation distance of 6.4 ± 0.3 nm (Supplementary Fig. 14 and Fig. 15). The multiple van der Waals interaction between Py and PEG corona can stabilize the loaded Py-PEG₄-Os in both water and salt solution.

PEG_n-Os (ester) was the same for different PEG spacers (n=4, 11, 21). **b**, Schematic fluorescence quenching of inserted Py by photoinduced electron transfer from Os to Py. **c**, The influence of packing density of molecular probes on the surface accessibility of Os. Salt-induced extension of PEG spacer by screening electrostatic repulsion between Os. **d**, Fluorescence change of Py-PEG₄-Os or Py-PEG_n-Os (ester) loaded polymersomes by adding NaCl. **e**, Scheme of electrode prepared by drop-casting the mixture of Os-polymersomes and ITO nanoparticles onto FTO coated glass. **f,g**, Cyclic voltammograms (**f**) and peak current (**g**) of Os-polymersomes/ITO modified electrode. Error bars in panels **d** and **g** represent the standard deviation obtained from two independent measurements.

Conformation of loaded molecular probes on PEG corona. The conformation of loaded molecular probes, especially the surface accessibility of functional modules, would influence their functions.^{44,45} Here, to validate the conformation, we designed another molecular probe by linking Py and -PEG_n-Os with the ester group (Supplementary Fig. 9 and 16). As-synthesized Py-PEG_n-Os (ester) had more available PEG spacers (n=4, 11, 22) and exhibited a higher packing density than Py-PEG₄-Os on PEG corona (Supplementary Fig. 17). This allowed us to investigate the conformation by manipulating the length of the PEG spacer and evaluate the influence of packing density on the surface accessibility of Os. As shown in Figure 3a, the fluorescence intensity of loaded Py-PEG_n-Os became visibly increased with the length of the PEG spacer. Since Os can quench the fluorescence of Py by photoinduced electron transfer, the increased fluorescence should be caused by the enlarged distance between Os and Py by the longer PEG spacer (Fig. 3b). Such distance-sensitive fluorescence of inserted Py permitted us to investigate the conformation of loaded molecular probes by the fluorescent spectrometer. Since electrostatic repulsion between Os has been validated to restrict the packing density of Py-PEG₄-Os on PEG corona, the higher packing density of Py-PEG₄-Os (ester) suggested the increased binding affinity of Py by ester linkage (Supplementary Section 3). This might induce the bending of the PEG spacer to keep the distance between Os, which would decrease the surface accessibility of Os (Fig. 3c). Here, we used NaCl to screen the electrostatic repulsion and manipulate the surface accessibility of Os. For loaded Py-PEG_n-Os (ester), the fluorescence intensity got increased after adding NaCl. This corresponded to the increased distance between Py and Os, which should be caused by the extension of the bent PEG spacer (Fig. 3d). For Py-PEG₄-Os with a lower packing density than Py-PEG_n-Os (ester), the fluorescence intensity kept almost the same, suggesting that all of the PEG spacers are extended. Salt-induced extension of the PEG spacer allowed us to control the surface accessibility of Os under different packing densities (Fig. 3c). To further confirm the surface accessibility, Py-PEG_n-Os (ester) loaded polymersomes (Os-polymersomes) and ITO nanoparticles were mixed and drop-casted on fluorine-doped

tin oxide (FTO) coated glass (Fig. 3e and Supplementary Fig. 18). Redox peaks of Os were successfully detected using the salt solution as the electrolyte, suggesting that Os could be reached by ITO nanoparticles (Fig. 3f). Moreover, the peak current was not influenced by the length of the PEG spacer, indicating the excellent surface accessibility of Os (Fig. 3g). Electrostatic repulsion between Os and van der Waals interaction between Py and PEG corona enabled the unique conformation of molecular probes on PEG corona.

Adaptive insertion for programmable surface functionalization. The non-covalent interaction and the reversible hydration/dehydration of PEG should allow the adaptive insertion of hydrophobic anchor for programmable surface functionalization (Fig. 1d). Here, we first investigated the substitution of the loaded molecular probes. Py-PEG₄-Im and Na-PEG₄-Os were chosen for the substitution experiment owing to their complementary absorbance and different binding affinity to PEG corona. When Py-PEG₄-Im was added into the dispersion of polymersomes loaded with Na-PEG₄-Os, the absorbance of Os in the supernatant increased, while the one of Py decreased (Fig. 4a). This validated the successful substitution of the loaded Na-PEG₄-Os by Py-PEG₄-Im. However, no release of the loaded Py-PEG₄-Os was detected by adding Py-PEG₄-Im (Fig. 4b). This demonstrated that the substitution can only occur between molecular probes with different hydrophobic anchors. Py of Py-PEG₄-Im has a higher binding affinity to PEG corona than Na of Na-PEG₄-Os. During substitution, Py-PEG₄-Im should be first loaded onto the blank region between two loaded Na-PEG₄-Os, and then ‘push’ Na-PEG₄-Os into solution by steric repulsion (Fig. 4c).

To enable programmable surface functionalization, we investigated the on/off insertion switch of the hydrophobic anchor by introducing reagents to compete with PEG corona (Fig. 4d to f). Unlike β -cyclodextrin, the addition of free PEG could not detach the loaded Py-PEG₄-Os, which should be caused by the well hydration of PEG chains. After testing different reagents, we found that poly(sodium 4-styrenesulfonate) (PSS) with hydrophobic phenyl group and hydrophilic sulfonate could ‘pull’ nearly all of the loaded Py-PEG₄-Os into solution⁹. This should be attributed to the π - π interaction and electrostatic attraction between PSS and Py-PEG₄-Os (Fig. 4d and Supplementary Fig. 19). By adding salts to screen the electrostatic attraction, the detached Py-PEG₄-Os could be re-inserted into PEG corona (Fig. 4e). Ca²⁺ was found to be more efficient in triggering the re-insertion of Py-PEG₄-Os than Na⁺ due to its stronger interaction with PSS⁴⁶. Above all, PSS could switch off the insertion of Py-PEG₄-Os by wrapping around it via π - π and electrostatic interaction, and CaCl₂ could switch on the insertion of Py-PEG₄-Os by screening electrostatic interaction between Py-PEG₄-Os and PSS (Fig. 4f). By manipulating the interaction between PSS and Py-PEG₄-Os, we enabled the on/off insertion switch of the hydrophobic anchor.

Conclusion

In summary, we reported the adaptive molecular recognition between the hydrophobic anchor and PEG host for programmable surface functionalization. This non-covalent binding strategy is distinctive in its

high efficiency, short functionalization time, mild conditions, controlled molecular orientation, and capability of dynamic loading. We envision that the new molecular recognition mode will enable the design of next-generation functional systems and enhance the progress in nanomedicine, nanotechnology, and advanced materials.

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Methods

Preparation of polymersomes. 10 mg poly(ethylene glycol)-*b*-polystyrene (PEG₄₄-*b*-PS₁₇₈) was dissolved in 1 mL mixture of THF and 1,4-dioxane (THF: 1,4-dioxane = 4:1 by volume). After stirring for 30 min, 1 mL water was injected at a rate of 1 mL/h. Then, 10 mL of water was added to quench the PS. After centrifugation and water wash (3 times), the polymersomes were dispersed in water. The concentration of polymersomes was measured by NanoSight LM10.

The loading of the molecular probe onto polymersomes. The aqueous solution of molecular probes (Hy-, Ip-, Ph-, Na-, Py-PEG₄-Os) was added into the aqueous dispersion of polymersomes. The concentration of molecular probes was ~21 μ M and the concentration of polymersomes was 3.80×10^{-4} μ M in the final mixture. After shaking for a certain time, 300 μ L of the mixture was collected and centrifuged to remove the polymersomes. The supernatant was diluted 2 times with water and measured with the UV-vis spectrometer. The collected polymersomes were washed with water for 3 times and used for other characterizations.

ITC measurements. An aqueous solution of the molecular probe was injected into the aqueous dispersion of polymersomes for ITC measurements. The concentration of polymersomes was 2.05×10^{-3} μ M. The concentration of molecular probe was 0.49 mM for Hy-PEG₄-Os, 0.35 mM for Ip-PEG₄-Os, 0.35 mM for Ph-PEG₄-Os, 0.70 mM for Na-PEG₄-Os, and 1.34 mM for Py-PEG₄-Os. 2 μ L aqueous solution of molecular probes was injected into the dispersion of polymersomes in 10 s for 20 times. The injection interval was 200 s. The stirring speed was 750 rpm. Reference power was 10 μ cal s⁻¹.

Fluorescence measurements. The binding process between Py-PEG₄-Im and polymersomes was monitored by JASCO FP-8300ST Spectrofluorometer. 5 μ L aqueous dispersion of polymersomes

(5.56×10^{-3} μM) or water was added into Py-PEG₄-Im (4.5 μM , 1.2 mL) for 20 times. The excitation wavelength was 342 nm. Excitation and emission bandwidth was 2.5 nm.

Electrochemical characterization. Cyclic voltammograms were performed with a three-electrode system using Ag/AgCl (saturated KCl) as the reference electrode and platinum foil (1.0 cm $\times$ 1.0 cm $\times$ 0.1 cm) as the counter electrode. The working electrodes were prepared by drop-casting the mixture of Py-PEG₄-Os or Py-PEG_n-Os (ester) loaded polymersomes (from 0.1 mg PEG₄₄-*b*-PS₁₇₈) and ITO nanoparticles (0.4 mg) onto FTO coated glass with Parafilm as spacers for a constant surface area of 1.0 $\times$ 1.0 cm². The electrolyte solution was composed of 40 mM MES and 50 mM KCl (pH=6.5). Without ITO nanoparticles, no obvious redox peaks of Os could be detected.

Substitution. Polymersomes (from 4 mg PEG₄₄-*b*-PS₁₇₈) were modified with an excess amount of Na-PEG₄-Os. After being washed with water for 3 times, the polymersomes were dispersed in 0.8 mL water. 0.4 mL of Py-PEG₄-Im solution ($\sim$ 145 μM) or water was added. After shaking for 10 min, polymersomes were removed by centrifugation, and the supernatant was analyzed with a UV-vis spectrometer to determine the amount of the released Na-PEG₄-Os.

On/off insertion switch. Polymersomes (from 2 mg PEG₄₄-*b*-PS₁₇₈) were modified with an excess amount of Py-PEG₄-Os. After being washed with water for 3 times, the polymersomes were dispersed in 0.9 mL water. 0.1 mL of 100 mM PSS, 100 mM PEG, 100 mM PAA, or 100 mM Na₂SO₄ was added into the above dispersion of polymersomes (The concentration of PSS, PEG, and PAA referred to the concentration of monomeric units without special notification). After shaking for 10 min, the polymersomes were collected by centrifugation. The supernatant was analyzed with the UV-vis spectrometer to determine the amount of released Py-PEG₄-Os.

The released Py-PEG₄-Os can be easily re-loaded onto polymersome by adding NaCl or CaCl₂ into the above mixture of polymersomes and PSS. After shaking for 10 min, polymersomes were collected by centrifugation and the supernatant was analyzed with the UV-vis spectrometer to determine the amount of re-loaded Py-PEG₄-Os. By manipulating the concentration of salt, a different molar ratio of cation/sulfonate(PSS) was achieved.

The adsorption of PSS on polymersomes in water or NaCl solution was excluded by incubating PSS and polymersomes. The concentration of PSS in the supernatant after incubation was measured with the UV-vis spectrometer.

Declarations

Data availability

All the data supporting the conclusion are available in the paper and/or Supplementary Information.

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Author contributions

S.Z. and D.A.W. conceived the concept. S.Z. designed the experiments and wrote the first draft of the paper. W.L. and D.W. performed structural characterization of polymersomes. J.L. prepared and characterized the dendrimersome. J.S. performed fluorescence characterization. **S.P.T.** prepared and characterized the PEG hydrogel microparticles. All authors discussed the results and commented on the manuscript.

Competing interests

The authors declare no competing interests.

Figures

Figure 1

Adaptive insertion of the hydrophobic anchor into PEG host for programmable surface functionalization. a, Schematic breakage of the surface hydration layer of PEG corona by changing PEG chain conformation from coil to planer-zigzag. b, Design of molecular probe. c, The loading of the molecular probe onto PEG corona of polymersomes. d, Programmable surface functionalization by adaptive insertion: substitution and on/off insertion switch.

Figure 2

Design and loading of molecular probes onto PEG corona. a, Manipulation of the hydrophobicity of anchor. b, Optical image and conformation of Py-PEG4-Os dissolved in water. c, The loading process of molecular probes onto polymersomes. Error bar represents the standard deviation obtained from two independent measurements. d, Optical images of precipitated polymersomes before and after the modification by Py-PEG4-Os. e, Cryo-EM image of polymersomes loaded with Py-PEG4-Os. f, ITC analysis

of the interaction between molecular probes and polymersomes. g, Schematic loading of Py-PEG4-Os onto PEG corona of polymersomes.

Figure 3

The conformation of loaded molecular probes on PEG corona. a, Fluorescence of Py-PEGn-Os (ester) loaded polymersomes. The loading amount of Py-PEGn-Os (ester) was the same for different PEG spacer lengths (n=4, 11, 21). b, Schematic fluorescence quenching of inserted Py by photoinduced electron

transfer from Os to Py. c, The influence of packing density of molecular probes on the surface accessibility of Os. Salt-induced extension of PEG spacer by screening electrostatic repulsion between Os. d, Fluorescence change of Py-PEG4-Os or Py-PEG_n-Os (ester) loaded polymersomes by adding NaCl. e, Scheme of electrode prepared by drop-casting the mixture of Os-polymersomes and ITO nanoparticles onto FTO coated glass. f,g, Cyclic voltammograms (f) and peak current (g) of Os-polymersomes/ITO modified electrode. Error bars in panels d and g represent the standard deviation obtained from two independent measurements.

Figure 4

Adaptive insertion for programmable surface functionalization. a,b,c, Substitution of loaded molecular probes. a, Substitution of loaded Na-PEG4-Os by Py-PEG4-Im. b, Comparison of substitutional performance of loaded Na-PEG4-Os and loaded Py-PEG4-Os by adding Py-PEG4-Im. d,e,f, On/off insertion switch by alternately adding PSS and CaCl₂. d, De-insertion of loaded Py-PEG4-Os by adding PSS, PEG, poly(acrylic acid sodium salt), or Na₂SO₄. e, Re-insertion of Py-PEG4-Os by adding CaCl₂ or NaCl. Error bars in panels b and e represent the standard deviation obtained from two independent measurements.

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