Controlled short-linkage assembly of functional nano-objects

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A B S T R A C T
In this work, we report a method that allows the deterministic, photo-controlled covalent assembly of nanoparticles directly on surface. As a model system, we study the conjugation of molecularly imprinted polymer (MIP) nanoparticles on a glass surface and confirm that the immobilized nanoparticles maintain their molecular recognition functionality. The glass slide was first modified with perfluorophenylazide and then used to bind MIP nanoparticles under UV irradiation. After each step the surface was analyzed by water contact angle measurement, fluorescence microscopy, scanning electron microscopy, and/or synchrotron-based X-ray photoelectron spectroscopy. The MIP nanoparticles immobilized on the glass surface remained stable and maintained specific binding for the template molecule, propranolol. The method developed in this work allows MIP nanoparticles to be directly coupled to a flat surface, offering a straightforward means to construct robust chemical sensors. Using the reported photo conjugation method, it is possible to generate patterned assembly of nanoparticles using a photomask. Since perfluorophenylazide-based photochemistry works with all kinds of organic material, the method developed in this work is expected to enable immobilization of not only MIPs but also other kinds of organic and inorganic–organic core–shell particles for various applications involving photon or electron transfer.

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1. Introduction

With the increased use of nanomaterials for various kinds of applications, e.g. in biomedical devices [12], nanoelectronics [3], dye sensitized solar cells [4–6], and nano-sensors [7–10], there is an increasing demand for reliable and well-controlled routes to the assembly of nano-objects at predefined locations. Moreover, minimizing the physical distance between the nano-objects and their binding sites is of utmost importance for many of these applications in particular in cases where efficient energy transfer, e.g. in the form of electrons, is pivotal. Immobilization of nanoparticles is an important area of research and development [11,12]. Many methods have been reported and suggested for the conjugation (immobilization) of nanoparticles, e.g. non-covalent binding through electrostatic interaction between amine and carboxyl groups [13], multiple H-bond interactions [14], and more sophisticated covalent binding realized by the nucleophilic reaction between amine and epoxide groups [15], amine-anhydride reaction [16], and the hydrosilylation of alkynyl groups with H-terminated silicon surface [17].

A more interesting strategy is based on photochemical conjugation, which is easy to perform, requires only a short reaction time, and provides the possibility to generate nanoparticle patterns using a suitable photomask [18]. In particular, photocoujugation based on perfluorophenyl azide (PFPA) has attracted great interest because of its high reaction rate [19–21]. The use of PFPA-mediated photocoujugation to immobilize small organic molecules is straightforward and well documented [22]. It has been shown that an intermediate polymer layer can be used to provide a high density of PFPA on a surface, to maximize the contact to nanoparticles and facilitate their fixation [23,24]. However, this approach has the significant drawback that a large gap (≥1 nm) between the nanoparticles and the surface is introduced because of the presence of the polymer layer, which can result in poor energy transfer characteristics [25,26]. An approach that would allow for direct immobilization of organic nanoparticles via PFPA would therefore be highly desirable to minimize these deleterious effects.
Here, we report a simple and generic route for controlled short-linkage assembly — via a single small organic molecule as the bridging unit — of nanoparticles on a surface without the need for an intermediate layer. We demonstrate that the assembly route is non-destructive to the function of the nanoparticles. As a model system, we study the conjugation of molecularly imprinted polymer (MIP) nanoparticles on a glass surface and confirm that the immobilized nanoparticles maintain their molecular recognition functionality. We chose MIP nanoparticles as a model system since they are being increasingly used as enzyme and receptor mimics to develop catalytic systems [27] and chemical sensors. Since PPFA-based photochemistry works with all kinds of organic material, the method developed in this work is expected to enable immobilization of not only MIPs but also other kinds of particles, e.g. organic and inorganic–organic core–shell particles.

2. Materials and methods

2.1. Synthesis of molecularly imprinted polymer nanoparticles

Molecularly imprinted polymer (MIP) nanoparticles were synthesized using propranolol as template following a procedure described in our previous publication [28]. Briefly, the template molecule, (R,S)-propranolol (137 mg, 0.53 mmol) was dissolved in 40 mL of acetonitrile in a 150 mm x 25 mm borosilicate glass tube equipped with a screw cap. Methacrylic acid (113 mg, 1.31 mmol), trimethylolpropane trimethacrylate (648 mg, 2.02 mmol) and azobis-isobutyronitrile (28 mg) were then added. The solution was purged with a gentle flow of nitrogen for 5 min and then sealed. Polymerization was carried out by rotating the borosilicate glass tube horizontally in a Stovall HO-10 Hybridization Oven (Greensboro, NC, USA) at a speed of 20 rpm, at 60 °C for 24 h. After polymerization, the polymer particles were collected by centrifugation. The template was removed by washing with methanol containing 10% acetic acid (v/v), until no template could be detected from the washing solvent using UV spectrometric measurement. The polymer particles were finally washed with acetone and dried in a vacuum chamber. For comparison, non-imprinted polymer (NIP) nanoparticles were synthesized under the same condition except that no template was added in the reaction mixture.

2.2. Functionalization of glass slides

Microscope glass slides (10 mm x 10 mm) were cleaned with 2 M NaOH for 1 min and rinsed with distilled water. To ensure their cleanliness, the slides were kept in 0.5 M HCl for 2 h, washed with distilled water, immersed in piranha solution (30% H2O2: concentrate H2SO4 = 3:7, v:v) at 75 °C for 40 min (Caution: Piranha is highly corrosive solution so extreme safety precautions should be taken), then rinsed thoroughly with water and dried with N2. The glass slides were then immersed immediately in a toluene solution of APTES (6%, v/v) at 50 °C for 12 h. The glass slides were rinsed three times with toluene and then three times with ethanol. Finally, the glass slides were kept at 100 °C in an oven for 24 h.

The amine-functionalized glass slides were treated with PPFA-NHS in CH2Cl2 (2 mg/mL) at room temperature for 12 h [29]. After this process the glass slides were rinsed with CH2Cl2 to remove any physiosorbed PPFA-NHS and dried with N2 gas.

2.3. Photoconjugation of nanoparticles

Nitrogen-free polymer nanoparticles were suspended in acetonitrile (8 mg/mL) and sonicated briefly to give a stable colloidal solution. The nanoparticles were deposited on the PPFA-modified glass surface by drop casting 30 µL of the colloidal solution. After the solvent was evaporated, the glass slides were irradiated with a UV lamp (8 W, 366 nm) for 30 min to initiate the photocoupling reaction. The distance between the UV lamp and the sample surface was kept at 8 cm. The UV-treated glass slides were washed three times and sonicated in acetonitrile to remove any physiosorbed nanoparticles. For comparison, a reference slide was prepared in the same way, except that no UV irradiation was used.

2.4. XPS measurements

The XPS measurements were performed at the ambient pressure X-ray photoelectron spectroscopy end station of beamline I511 of the MAX IV Laboratory in Lund, Sweden [30]. The base pressure of the analysis chamber is 10−10 mbar, but increased to 10−9 mbar after introduction of the samples due to degassing. For the XPS measurements Pilkinson TEC fluorine tin oxide coated glass was used. All spectra were calibrated with respect to the Si 2p peak. The XPS results were verified twice for each sample (the difference is summarized in Tables S1 and S2 in the Supporting information).

2.5. Radioligand binding analysis

For the radioligand binding analysis nanoparticle-coated glass slides were incubated in 1 mL of acetonitrile containing 0.5% acetic acid (v/v) and 246 fmol of (S)-[4-3H]-propranolol on a rocking table for 16 h. After incubation the glass slides were removed and transferred into 1 mL of eluting solvent (methanol containing 10% acetic acid) and gently stirred on a rocking table for 16 h. From the liquid phases collected 500 µL was transferred into 10 mL of scintillation cocktail, and the radioactivity of the mixture was measured by liquid scintillation counting using a Perkin Elmer Tri-card 2810TR liquid scintillation analyzer. The amount of the labeled propranolol bound to the surfaces was calculated from the radioactivity remaining in the liquid phase.

3. Results and discussion

The MIP nanoparticles used in this work were synthesized by precipitation polymerization using propranolol as a template [28]. The nanoparticles were immobilized on glass surface as depicted in Scheme 1. The immobilization procedure consisted of three steps: (1) amino group modification of the glass slide surface using (3-aminopropyl)-triethoxysilane (APTES), (2) azide-modification of the surface by reaction with N-hydroxysuccinimide–functionalized perfluorophenylzilane (PFPA-NHS) [29,31] and (3) drop-casting of the MIP nanoparticles onto the azide-modified surface followed by photoactivated conjugation.

After each step the resulting surface was analyzed by water contact angle measurement, fluorescence microscopy, scanning electron microscopy (SEM), and/or synchrotron-based X-ray photoelectron spectroscopy (XPS).

3.1. Optimization of surface functionalization with APTES

The concentration of APTES during the first step in our immobilization procedure was optimized to achieve a high density of amino groups, which act as binding groups for the PFPA-NHS, on the surface while avoiding aggregation of the silane [32]. Indeed, in agreement with literature [33–38], we found that an increase of the APTES concentration from 2% to 8% resulted in an increased hydrophobicity of the surface, as observed from an increase of the water contact angle from 40° to 80° (Fig. 1). Fluorescence microscopy performed after labeling of the surface with fluorescein isothiocyanate also showed an increase in amino group density (Fig. 2), actually to the degree that aggregation was observed for
treatment with 8% APTES solution. In contrast, for a 6% concentration of APTES we found the fluorescence to be high and relatively evenly distributed. Thus, this concentration was used in all subsequent experiments.

3.2. Surface characterization by XPS

XPS measurements were performed to confirm the success of the single preparation steps. The experiments were carried out using conductive fluorine-doped tin oxide (FTO) rather than standard glass slides to avoid the shift and broadening of the spectral features, which on non-conductive surfaces are induced by the interaction of the X-ray induced surface charge with the outgoing photoelectrons. Analysis of the N 1s and C 1s X-ray photoelectron (XP) spectra for the APTES-modified slides proves the attachment of APTES to the surface (Fig. 3(a) and (d)). In agreement with literature [38–42] two peaks are seen in the N 1s spectra (Fig. 3(a)), one at 400.12 eV binding energy related to free amines and one at 401.35 eV due to protonated amines. In the C 1s spectrum (Fig. 3(d)) we find a peak at 285.30 eV due to saturated C atoms and one at 286.30 eV due to amino-linked C atoms. A major part of the peak arising from saturated C atoms can be assigned to contamination from exposure of the sample to air (XPS is highly sensitive to the minute amounts of contamination.

Fig. 1. Water contact angle on glass surface after treatment with 2% (a), 4% (b), 6% (c) and 8% (d) APTES. The water contact angle was 40°, 55°, 60° and 80° in (a), (b), (c) and (d), respectively.
found on almost any surface due to the adsorption of carbon-containing compounds present in air), but the nitrogen and carbon C–N features clearly indicate the attachment of APTES to the surface [41].

Characterization after PFPA-NHS treatment of the APTES-modified surface in step 2 of the immobilization procedure (Scheme 1) revealed that the surface modification was successful. Step 2 led to an increase in the water contact angle of the surface.
from 60° to 80° (Fig. 1(a)), which, as PFPA is more hydrophobic than APTES, confirms the PFPA termination of the surface. In the corresponding N 1s XP spectrum (Fig. 3(b)), the peaks at highest binding energy at 401.86 and 405.05 eV are related to the two terminal and one middle N atoms of the azide group [43]. The low binding energy peak at 400.36 eV is attributed to a partial amine decomposition product of the azide [42]. In the C 1s spectra (Fig. 3(e)) the features at 285.11, 286.40, and 288.00 eV are attributed to C atoms in C–C, C–N, and C–F and N–C=O bonds, respectively. Carbon atoms in C–F and N–C=O bonds are expected to have similar C 1s binding energies, and both contribute to the highest binding energy peak [43]. The observed features correspond to the expected spectra for PFPA, and thus the spectra corroborate that the surface modification of FTO by APTES and PFPA was successful. Since the same reaction has been used between the surface-bound APTES (confirmed by fluorescence microscopy, Fig. 2) and PFPA for both the glass and FTO slides, the XPS results have bearing also on the treatment of the standard glass slides and suggest that the chemical modification works in the same way for both types of system.

In the final step of immobilization procedure MIP particles were drop-casted onto the azide-modified slides and irradiated with UV light to induce photoconjugation. The treatment led to a decrease of the water contact angle to 60° (Fig. 4(b)). This formation of a more hydrophilic surface can be explained by the partial COOH groups on the nanoparticles. The XP spectra in Fig. 3(c) and (f) confirm the successful deposition of MIP particles; the azide-related peaks in the N 1s spectra have disappeared, and in the C 1s spectra the nitrogen–bonded carbon component has increased significantly in intensity. Additional new features can be attributed to oxygen–bonded carbon atoms (COOH and C=O). In the O 1s spectrum (Fig. 5), the peaks at 532.59, 534.41, and 535.97 eV are attributed to the oxygen atoms in the ester (532.59 eV) and carboxyl groups (534.41 and 535.97 eV) [44] that are abundant in the MIP nanoparticles.

3.3. Stability of the immobilized nanoparticles

To verify the importance of the photoactivated conjugation reaction for the covalent immobilization of the MIP nanoparticles in our procedure, we compared SEM images of a fully treated surface with a control surface, prepared by depositing the MIP nanoparticles onto a plain glass slide followed by UV irradiation (Fig. 6). While only a few particles are observed on the control slide, the fully treated surface shows immobilization of a high density of nanoparticles. Another control experiment showed that, if the surface was fully treated with APTES and PFPA before deposition of MIP particles but the UV irradiation step was omitted, the nanoparticles could easily be removed from the glass surface by brief sonication in acetonitrile.

The SEM characterization also reveals an agglomeration of nanoparticles (Fig. 6). Similar agglomeration has been observed previously for other systems [23,24]. To obtain a monolayer of immobilized nanoparticles, further optimization of the nanoparticle deposition before photoconjugation will be needed.

3.4. Verification of the functionality of the immobilized nanoparticles

To verify that use of our photoconjugation method does not sacrifice function, we immobilized MIP nanoparticles and non-imprinted polymer (NIP) nanoparticles on glass slides using the same photoconjugation protocol and compared the two surfaces’ uptake of tritium-labeled propranolol. In the radioligand binding analysis we found that the NIP conjugated surface did not show any propranolol binding, while the MIP conjugated surface was able to bind 26.6 fmol of labeled propranolol. Under the same condition, we found that 1 mg of the MIP nanoparticles could bind 108 fmol of labeled propranolol (which was 4.3 times higher than that could be achieved with the NIP nanoparticles). Considering that the amount of MIP nanoparticles that can be immobilized on a 1 cm² flat surface is much smaller than 1 mg, the radioligand binding achieved by the MIP conjugated surface is significant. These results show that the MIP nanoparticles conjugated on the surface via the short linkage had negligible non-specific binding, and that they maintained their intrinsic template affinity, which is the most critical

![Fig. 4. Water contact angle measurement on glass surface modified with PFPA (a) and after photoconjugation of MIP nanoparticles (b). The water contact angle was 80° in (a) and 60° in (b).](image01.png)

![Fig. 5. O 1s XP spectrum of FTO treated with APTES followed by attachment of PFPA and immobilization of MIP nanoparticles.](image02.png)
function of imprinted materials [45–48]. As the photoconjugation reaction only involves a small portion of the utmost organic layer, the method described here is general for nanoparticle immobilization and will not affect the functional properties of, e.g., magnetic nanoparticles, quantum dots, and catalytic TiO₂. In addition to the model substrates (glass and FTO) used in this work, the photoconjugation procedure could also be carried out on other surfaces, e.g., Au and Ag, on which the photoactive PFPA could be introduced through a self-assembled monolayer of amine-terminated thiols.

4. Conclusions

To conclude, we have demonstrated and characterized a versatile photoactivated conjugation method to immobilize molecularly imprinted nanoparticles. In addition, the method also opens up avenues for well-controlled patterned directed assembly of complex structures. We have shown that the method is non-destructive and allows the immobilized particles to maintain specific properties, in this case molecular recognition. Since PFPA-based photochemistry works with all kinds of organic material [22], the method developed in this work provides a generic approach to immobilizing all kinds of particles, i.e., not only MIPs but also other kinds of organic and inorganic–organic core–shell particles. Furthermore, it could also be used for assembly of nano-objects on a surface. In comparison to other approaches our method is simple and fast and results in very short linkage between the conjugated objects, which is essential for many kinds of applications where energy transfer is of great importance. Examples of possible applications include sensors, catalysis, and utilization of quantum dots in organic–inorganic hybrid solar cells.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.apsusc.2014.01.174.
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