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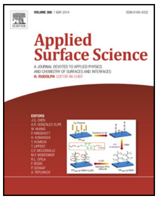

Controlled short-linkage assembly of functional nano-objects☆

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ABSTRACT

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.

1. Introduction

With the increased use of nanomaterials for various kinds of applications, e.g. in biomedical devices [1,2], 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 alkenyl 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, photoconjugation based on perfluorophenyl azide (PFPA) has attracted great interest because of its high reaction rate [19–21]. The use of PFPA-mediated photoconjugation 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 ($\gg$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.

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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 PFPA-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 × 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 × 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% H_2O_2: concentrate H_2SO_4 = 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 N_2. 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 PFPA-NHS in CH_2Cl_2 (2 mg/mL) at room temperature for 12 h [29]. After this process the glass slides were rinsed with CH_2Cl_2 to remove any physisorbed PFPA-NHS and dried with N_2 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 PFPA-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 physisorbed 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 Pilkington 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-^{3}H]-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 perfluorophenylazide (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