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Surface plasmon resonance as a tool for investigation of non-covalent nanoparticle interactions in heterogeneous self-assembly & disassembly systems

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ABSTRACT

Biomolecule-driven assembly of nanoparticles is a powerful and convenient approach for development of advanced nanosensors and theranostic agents with diverse "on-demand" composition and functionality. While a lot of research is being devoted to fabrication of such agents, the development of non-invasive analytical tools to monitor self-assembly/disassembly processes in real-time substantially lags behind. Here, we demonstrate the capabilities of localized surface plasmon resonance (SPR) phenomenon to study non-covalent interactions not just between plasmonic particles, but between gold nanoparticles (AuNP) and non-plasmonic ones. We show its potential to investigate assembly and performance of a novel type of advanced smart materials, namely, biocomputing agents. These agents, self-assembled from nanoparticles via biomolecular interfaces such as proteins, DNA, etc., can analyze presence of biomolecular inputs according to Boolean logic and undergo the input-induced disassembly in order to implement the proper output action. Using UV–Vis spectroscopy to monitor the assembly/disassembly processes of the basic YES-gate structure that consists of a polymer core particle with a multitude of associated gold nanoparticles, we found that the structure transformations are well-characterized by pronounced difference in SPR spectral band position (shifting up to 50 nm). This SPR shift correlates remarkably well with biochemical estimation of the assembly/disassembly extent, and can provide valuable real-time kinetic analysis. We believe that the obtained data can be easily extended to other non-plasmonic nanoparticle systems having similar chemical and colloidal properties. SPR method can become a valuable addition to analytical toolbox for characterization of self-assembled smart nanosystems used in biosensing, imaging, controlled release and other applications.

1. Introduction

Biomedical agents based on self-assembly of nanoparticles via non-covalent biomolecular interfaces promise to become an important milestone in development of personalized nanomedicine due to easily adjustable composition, size and functionality (Couvreur and Vauthier, 2006; Evans et al., 2015). To date, a great variety of nanostructures were constructed via biomolecule-driven self-assembly for such applications as in vitro diagnostics (Hu et al., 2011), multimodal bioimaging (Padmanabhan et al., 2016; Aghayeva et al., 2013), drug delivery (Torchilin, 2014), gene therapy (Hart, 2010), etc. In addition, smart systems with controlled architecture for application in biosensing and controlled drug release responsive to a diversity of biochemical factors such as pH (Lu and Unsworth, 2016), proteolytic enzymes (Von Maltzahn et al., 2007), temperature (Kotsuchibashi et al., 2015), as well as combination of these parameters (Bai et al., 2015; Chen et al., 2012) have been developed in the last decade. Many of these systems are based on specially designed homo- and block-copolymers that are capable of self-assembling into gel scaffolds or micelle-like structures, which change their properties depending on the external perturbation. Recently, we have shown even more versatile agents based on nanoparticle self-assembly via biomolecular interfaces (e.g., proteins, DNA, etc.), which can be programmed to simultaneously analyze the levels of multiple molecular inputs (of virtually any nature) according to the rules of Boolean logic, and implement a variety of output actions based on the biocomputation results (Nikitin et al., 2014).

One of the crucial challenges in fabrication of such systems is a thorough non-invasive control of assembly/disassembly processes in real-time. In this respect, current analytical toolbox for characterization of these nanosystems is quite limited. For example, electron

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microscopy provides precise and reliable information about the structure and morphology of nanosystems, however, it is barely applicable for characterization of colloid systems assembled via relatively weak non-covalent interactions. Indeed, the latter type is often prone to disassembly and/or aggregation because of shear forces arising upon sample preparation (e.g. sample drying), which may compromise the real picture of the initial structure. Another common tool to study nanoobjects, Dynamic Light Scattering (DLS), is essentially free of this problem and is capable of measuring size and size distribution of the particles in nearly real environment (Bhattacharjee, 2016). Unfortunately, the major obstacle for its wide application to study assembly/disassembly of nanostructures is its relatively low resolution, which hampers distinguishing a nanostructure against its constituents. Another technique, the Nanoparticle Tracking Analysis (NTA), powered by dark field optic microscopy manages to substantially increase the resolution but still inherits limitation on the working concentration of the particles in solution as a common problem of optical microscopy methods.

Meanwhile, localized surface plasmon resonance (SPR) is well known to be an extremely useful tool to study colloidal transformations of metallic particles (Li et al., 2015). The spectral shift of SPR band upon gold nanoparticles (AuNP) aggregation/dispersion accompanied by visual color change is the main principle in fabrication of AuNP-based (bio)sensors and colorimetric logic gates (Giljohann et al., 2010; Nie et al., 2014). The non-invasive real-time character of the method is of great demand to study gold nanoparticles transformations, including their aggregation mediated by cross-linking by means of DNA (Elghanian, 1997), proteins (Aslan et al., 2004; Srivastava et al., 2005) and covalent interactions (Westcott et al., 1999); or by disruption of nanoparticle colloidal stability as a result of external factors like change of ionic strength of the solution (Sato et al., 2003).

However, until now SPR-based investigation of non-covalent self-assembly systems was limited to interactions of plasmonic nanoparticles with other plasmonic entities or biomolecules (Ament et al., 2012; Adato and Altug, 2013), while expanding its applicability to study interactions of metallic particles with non-metallic ones seems very promising.

Here we demonstrate the use of SPR for real-time non-invasive investigation of non-covalent biointerface-mediated assembly of AuNP with non-plasmonic colloid objects, namely, 230-nm polystyrene-based microspheres. We use the SPR spectrum shift phenomenon for investigation of the extent of the assembly processes (e.g., surface coverage parameters) and kinetics of assembly and controlled disassembly. As the model object for assembly/disassembly investigations, we use the new type of advanced smart materials, namely, a biocomputing structure that implements a Boolean YES logic gate for a specific biomolecular input. This type of nanoparticles self-assemblies has been successfully employed as agents in biosensing and target specific drug delivery applications (Nikitin et al., 2014) and we believe that SPR, as a readily available, non-destructive method, can become an essential tool in characterization of such systems.

2. Materials and methods

2.1. Materials

The following reagents were used: streptavidin (STR), horseradish peroxidase (HRP) (ThermoFisher Scientific, USA); chloramphenicol (CAP), bovine serum albumin (BSA), phosphate-buffered saline (PBS), acetate buffer, 2-(N-morpholino)ethanesulfonic acid (MES) hydrate, N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC), N-hydroxysulfosuccinimide sodium salt (sulfo-NHS), Biotin N-hydroxysuccinimide ester (BIO-NHS), 3,3′,5,5′-Tetramethylbenzidine (TMB) (Sigma-Aldrich, Germany); Mouse anti- fluorescein isothiocyanate FC11 antibody (anti-FITC), Mouse anti-chloramphenicol CAPB10 antibody (anti-CAP) (Russian Research Center for Molecular Diagnostics and Therapy, Russia); Estapor MH1-020/50 230-nm carboxylated polystyrene coated magnetic beads (Merck Millipore, France); chloroauric acid (Dragtsvetmet, Russia). Conjugates of biotin (BIO-BSA) and chloramphenicol (CAP-BSA) with BSA were prepared as previously (Nikitin et al., 2014). All the other auxiliary reagents were of “analytical grade” quality.

2.2. Instruments

Absorption spectra as well as absorbance density measurements were performed using CLARIOstar® multimode microplate reader (BMG Labtech, Germany). SEM (scanning electron microscopy) images were obtained using JEOL JSM-7001F transmission electron microscope (JEOL, USA).

2.3. Nanoparticle synthesis

Ferrihydrite particles (FH) were synthesized according to the previously reported method (Nikitin et al., 2014). AuNP (40 ± 5 nm) were prepared by citrate reduction of $HAuCl_4$ solution according to modified standard protocol (Turkevich et al., 1951).

2.4. Nanoparticle-protein conjugates

2.4.1. Magnetic particles (MP)

The conjugates of magnetic core nanoparticles with CAP-BSA and BIO-BSA (MP-CAP-BSA: BIO-BSA) were obtained as described previously (Orlov et al., 2016). 30 mg EDC and 12 mg sulfo-NHS in 125 μL of MES buffer (50 mM, pH 5.0) were added to 2 mg of Estapor MH1 magnetic nanoparticles and incubated 20 min with sonication. After magnetic separation, the following protein mixtures were added 20 μL of borate buffer (0.1 M, pH 8.6): i) 32 μg CAP-BSA, 8 μg BIO-BSA, no BSA; ii) 10 μg CAP-BSA, 8 μg BIO-BSA, 22 μg BSA; iii) 3.3 μg CAP-BSA, 8 μg BIO-BSA, 28.7 μg BSA; iv) 1 μg CAP-BSA, 8 μg BIO-BSA, 31 μg BSA; v) 0.3 μg CAP-BSA, 8 μg BIO-BSA, 31.7 μg BSA. The total protein concentration in every mixture was 2 g/L. After overnight incubation in a shaker, the MP-CAP-BSA: BIO-BSA conjugates were magnetically washed with 1% BSA in PBS buffer (pH 7.4) thrice.

2.4.2. Ferrihydrite nanoparticles (FH)

The conjugates of ferrihydrite nanoparticles with STR and HRP (FH-STR: HRP) were obtained as follows. 20 mg of EDC and 12 mg of sulfo-NHS in 60 μL of MES buffer (50 mM, pH 5.0) was added to 1 mg of FH and incubated for 20 min with sonication. After separation by centrifugation (15 min at 15,000*g*), 60 μg of STR and 90 μg of HRP in 60 μL of HEPES buffer (10 mM, pH 4.6) was added to the particles and incubated overnight in a shaker. Then the FH-STR: HRP conjugates were washed thrice by centrifugation (15 min at 15,000*g*) with 1% BSA in PBS buffer (pH 7.4).

2.4.3. Gold nanoparticles (AuNP)

The conjugates of gold nanoparticles with anti-CAP antibodies (AuNP-anti-CAP) and the non-specific anti-FITC mouse IgG (AuNP-anti-FITC) were obtained as follows. 48.6 μg of antibodies were added to 1.6 mL of gold nanoparticle suspension (OD=2.0 at 530 nm) with pH=9.0 (adjusted with NaOH), and the resulting mixture was incubated for 2 min. After addition of 0.4 mL of 5% BSA in PBS, the AuNP conjugates were washed four times with 1% BSA in PBS by centrifugation (10 min at 10,000*g*).

2.5. Assembly and performance of biocomputing structures

Biocomputing structures were constructed as follows. 66.0 ng of