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Invited feature article

Significantly improved stability of silver nanodots via nanoparticles encapsulation

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ABSTRACT

Luminescent silver nanodots are bright, silver cluster-based emitters with tunable emission windows from the blue to near-IR. Their stability and photophysical properties depend highly on the protection group that forms coordinate bonds with the cluster core. The coordinate nature of such protections suggests that silver nanodots are vulnerable to any materials that competitively bind to the silver-cluster core, resulting in deterioration of nanodots. Given the excellent photophysical properties of silver nanodots, it is necessary to investigate methods to stabilize silver nanodots. While nanoparticles offer diverse platforms to protect silver nanodots and adequate room to build smart, robust, and multi-functional silver nanodot-nanoparticle hybrids, we examined the construction of nanoparticle-encapsulated silver nanodots in reverse micelles, liposomes, and silica nanoparticles. Charges of surfactants in organic nanoparticles strongly influence the stability of silver nanodots. Both reverse micelles and liposomes built of charged surfactants destabilize silver nanodots, but silver nanodots are stable in non-ionic reverse micelles. However, it is difficult to encapsulate a silica layer on top of silver nanodots due to electrostatic repulsions between the DNA molecules and hydrolyzed tetraethyl orthosilicate. Such repulsions are overcome by introducing an amino silane to cross-link silver species and orthosilicate and to initiate the growth of silica surrounding silver nanodots. This optimized protocol can be applied to any silver nanodot, yielding multi-color, chemically and photophysically stable silica nanoparticle-encapsulated silver nanodots in PBS.

1. Introduction

Silver nanodots (AgNDs) are defined as few-atom clusters of reduced silver atoms [1]. Such clusters are vulnerable, thus, rare gas matrices at cryogenic temperatures were initially utilized to prevent their oxidation and further agglomeration in the early stage of silver cluster investigation [2–6]. Luminescent silver nanodots became applicable until the synthesis of stable, water-soluble nanodots with a wide range of protection groups, such as dendrimers, microgels, peptides, mercapto small molecules, and single-stranded DNA (ssDNA) molecules [7–11]. Optimizations done by adjusting DNA sequences and preparation conditions led to improved emitter purity, ranging from the blue to the near-IR, with luminescence quantum yields up to 60% [12–14]. Moreover, the two-photon absorption capability of silver nanodots is about 100-fold better than that of typical organic dyes [13,15]. Exhibiting large molar extinction coefficients, outstanding luminescence quantum yield, and excellent photostability, silver nanodots produce high emission brightness, illustrating great potential as biological imaging agents [12,16,17]. However, the coordinate bonding nature between silver nanodots and their protection groups suggests that they might be vulnerable to chemical quenching in aqueous solutions due to destabilization of nanodots by competition coordination. Furthermore, the low K_{sp} of silver halide and the high affinity of silver ions for some biological molecules induce low chemical stability of silver nanodots in physiological conditions [18]. Even though some silver nanodots, such as peptide-protected red emitters and the 20mer polycytosine-protected green emitter, are stable in chloride-rich solutions,

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the majority of silver nanodots deteriorate instantly even in phosphate-buffered saline (PBS).

However, nanoparticles offer diverse platforms to protect silver nanodots as well as adequate room to build smart, robust, and multi-functional silver nanodot-nanoparticle hybrids. The introduction of nanoparticles enables not only assembling multiple silver nanodots into a single particle to greatly increase their brightness but also conjugating targeting groups to the nanoparticles to effectively enhance specific cargo delivery [19]. Moreover, a combination of therapeutic and diagnostic capabilities in one dose, termed theranostics, has especially attracted attention in the last decade [20]. Liposomes, polymer gels, and silica nanoparticles are potentially useful for the fabrication of the above hybrids [21,22]. Silica nanoparticles acting as either a carrier or a protection layer are widely used for the construction of functional nanoparticles [23–25]. Organic dyes, gold clusters, and silver nanoparticles have been encapsulated in silica nanoparticles [26–28]. However, silver nanodot-silica nanoparticle hybrids have rarely been reported. It is hard to directly encapsulate large organic molecules into silica nanoparticles by hydrolysis of tetraethyl orthosilicate [24]. Given that most protection groups of silver nanodots, such as ssDNA molecules and poly(acrylic acid) sodium salt, are negatively charged at pH above 7, it is difficult to build a silica layer on the top of silver nanodots due to the electrostatic repulsion between silver nanodots and hydrolysis derivatives of orthosilicate. We have successfully initialized a silica coating to the negatively-charged silver nanodots and the resulting silica nanoparticles show excellent stability in PBS. In this report, we investigated the factors that influence the stability of silver nanodots and examined several nanoparticle platforms in order to construct chemically stable silver nanodots. We further significantly improved the chemical stability of silver nanodots via anchor-induced encapsulation in silica nanoparticles.

2. Material and methods

2.1. Chemicals

Silver nitrate (99.9999%), phosphate buffer saline (PBS), sodium borohydride, Triton X-100, poly(acrylic acid), phosphatidylcholine from soybean, sodium bis(2-ethylhexyl) sulfosuccinate (AOT), cyclohexane, chloroform, (3-mercaptopropyl)trimethoxysilane, tetraethyl orthosilicate (TEOS), N-[3-(Trimethoxysilyl)propyl]ethylenediamine (NED), (3-Glycidyloxypropyl)trimethoxysilane (Epoxyl silane), cholesterol, egg lecithin (egg PC, ≥30%), n-hexanol, cyclohexane, and phosphatidylglycerol (PG) were purchased from Sigma-Aldrich and used as received. ssDNAs were synthesized in Integrated DNA Technologies.

2.2. Instruments

HRTEM images were obtained on a JEM 3010 high resolution transmission electron microscope. The size distribution of nanoparticles was obtained by counting the size of nanoparticles in multiple TEM images. UV–vis absorption spectra and emission spectra were obtained on an S-4100 (SCINCO) and a QM-40 (Photon Technology International, Inc.), respectively. Microscopic emission images were obtained on an Olympus XI-81 microscope with a ×60 objective (NA 1.35) and an Andor Luca^{EM} S 658M camera. Eppendorf cooling centrifuge 5415 R was used to collect silica nanoparticles.

2.3. Liposome preparation

Liposomes, AOT-based reverse micelles, and Triton X-100-based reverse micelles were prepared following literature procedures [29–31]. Chloroform (0.37 mL) solutions of egg PC (30 mg), PG (1 mg), and cholesterol (5 mg) were placed into vials to form phospholipid films by removing chloroform under a nitrogen stream followed by evaporation under vacuum for 12 h. Dry films were hydrated by adding concentrated solutions of silver nanodots to keep a lipid concentration of 20 mM. Dispersions were homogenized with vortex mixing, and emission spectra were examined with a fluorometer.

2.4. Reverse micelle preparation

AOT reverse micelles were prepared by dissolving AOT in isooctane followed by the addition of concentrated aqueous silver nanodots to obtain the desired value of w_o. The concentration of AOT in isooctane was kept at 0.1 M throughout the experiments. With respect to Triton-based reverse micelles, Triton X-100 and hexanol were mixed at a ratio of 4:1 v/v, followed by the addition of cyclohexane to yield reverse micelles with a final Triton X-100 concentration of 0.126 M. Subsequently, concentrated aqueous silver nanodots were added to obtain the desired value of w_o.

2.5. Silver nanodots preparation

Different silver nanodot emitters were prepared according to published data [13,32,33]. ssDNA (50 μM) and silver ions were mixed at a DNA base/Ag^+ ratio of 2:1, followed by the reduction with aqueous sodium borohydride (1 mg/mL, 50 μL). Silver nanodots were used as probes a day after chemical reduction of the mixture. ssDNA for the 560-emitter, the 615-emitter, the 670-emitter and the 700-emitter were $ATATC_{12}ATAT$, $CGCGC_{12}CGCG$, $GGGGC_8CCCC$, and CCCTAACTCCCC, respectively [12,34].

2.6. Poly(acrylic acid) protected silver nanodots

$AgNO_3$ (29 μmol) was mixed with NED (58 μmol) in methanol (2 mL), stirred at room temperature in dark for 2 h. 10 μL of this solution was added into an aqueous solution of poly(acrylic acid) (MW 2100, 81 μM) and reduced with sodium borohydride (0.02 mg). This solution was stirred in the dark overnight, yielding a pink solution with red emission [35].

2.7. Silver nanodot-encapsulated silica nanoparticle preparation

In the early stage of AgND@SiO_2 preparation, aqueous silver nanodots (125 μM based on ssDNA), the required NED, and the required TEOS were mixed in an aqueous solution and left at room temperature overnight. In the optimized protocol, aqueous silver nanodots (125 μM based on ssDNA) and the required NED were mixed in an aqueous solution and left at room temperature for two hours, followed by the addition of TEOS (16.7 mM). 24 h later, the solution was centrifuged at 16,000 rpm to collect silica nanoparticles. The 700-emitter@SiO_2 was prepared after one more TEOS coating (6.3 mM) for 12 h.

2.8. Surface modification of AgND@SiO2

Aqueous AgND@SiO_2 solution was diluted with ethanol (water/ethanol 1:1). AgND@SiO_2 was then collected by centrifugation and then redispersed in ethanol. Extra silane such as NED (70 μM) or epoxy silane (70 μM, 350 μM and 700 μM, respectively) was added into the new solution and the mixture was left at room temperature overnight.

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