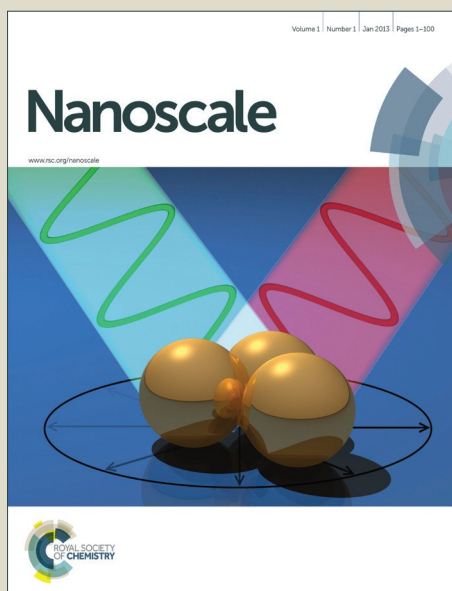


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COMMUNICATION

pH-Responsive reversible self-assembly of gold nanoparticles into nanovesicles†

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A novel system was reported to realize the reversible self-assembly and disassembly of Au nanovesicles (NVs) driven by pH stimuli with commercially available organic molecules, 4-mercaptopbenzoic acid (4-MBA) and oleylamine (OL). Through adjusting deprotonation and protonation of 4-MBA, Au NVs demonstrated a good reversible self-assembly behavior. As a proof-of-concept, Rhodamine was loaded into the vesicles to demonstrate the reversible pH-responsive controlled release.

Among numerous nanoarchitectures, nanovesicles (NVs) have attracted tremendous attention due to their unique hollow structure, showing a promising future in many fields. Among which, inorganic NVs, with components of inorganic nanoparticles (NPs) such as Au NPs,^{1,2} CuS NPs,³ CdSe quantum dots,⁴ would provide unique collective effects and therefore have more possibilities for catalysis,⁴ drug delivery⁵ and bioimaging.⁶ In recent years, externally stimuli-responsive inorganic vesicles are constantly emerging, providing a novel platform for controlled drug delivery. Especially, a number of studies have been reported on the morphological decomposition of inorganic NVs and therefore the release of encapsulated molecules under external stimulus such as pH,^{5,7} heat⁸ and light.^{9,10}

However, there is still room for improvement on such controlled release due to the following two aspects. On one hand, the self-assembly and disassembly of inorganic NVs are sensitive to the organic responsive ligands modified on their surface, such as acid, polyethylene glycol,^{11,12} and azobenzene,¹³ which unfortunately always need complicated organic synthesis. On the other hand, the self-assembly process of inorganic NPs to NVs are usually irreversible. Currently the reversible self-assembly of inorganic NPs could only be triggered between monodisperse NPs and irregular aggregates.^{14,15} As far as we know, almost no reports have demonstrated that disassembled NPs would be able to reassemble

into vesicular structures again, which may require much stable surface ligand. Therefore, it is of great challenge to develop a new system to achieve the reversible self-assembly and disassembly between inorganic NPs and NVs. Responsive release can be more easily achieved if this process can be stimulated by simple and commercially available surface ligands.

Herein, for the first time, reversibly responsive NVs were obtained by self-assembly of Au NPs with commercial organic molecules, 4-mercaptopbenzoic acid (4-MBA) and oleylamine (OL), as ligands on their surface. The self-assembled NVs dissociated into individual NPs in alkaline condition while the dissociated NPs reassembled into NVs again when the solution was brought back to be acidic through adjusting deprotonation and protonation of 4-MBA. The existing studies have demonstrated that pH-responsive inorganic NVs may show potential in controlled release as drug carriers. As a proof-of-concept, Rhodamine (RhB) was loaded in the water-soluble Au NVs which response to alkali and acid rapidly to demonstrate the pH-responsive release.

Monodisperse Au NPs with a size of 3.4 ± 0.3 nm (Fig. S1) capped with OL on the surface were successfully prepared by an established method. In order to fabricate vesicular structures dispersed in water, commercially available 4-MBA was tethered onto the surface of Au NPs using a ligand-exchange method. As shown in Fig. 1, Fourier transform infrared spectroscopy (FTIR) spectra confirmed the co-existence of 4-MBA and OL on the surface of Au NVs. The broad absorption bands from 3000 to 2400 cm^{-1} in the spectrum of 4-MBA originating from intermolecular hydrogen bonds vanished when 4-MBA was modified to Au atoms via coordination.¹⁶ The absorption peaks at 2851 and 2923 cm^{-1} attributed to C-H stretching in the aliphatic chain indicate the existence of OL, while the peak at ca. 1680 cm^{-1} corresponded to typical stretching vibration of C=O confirmed the efficient exchange for 4-MBA on the surface of Au NPs. The co-existence of hydrophobic and hydrophilic ligands on Au NPs, analogous to block copolymers on the whole, provides a possibility towards amphiphilicity-driven self-assembly of inorganic NPs into NVs in water.

The morphology of the as-obtained NVs was characterized by

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‡ These authors made equal contributions to this study.

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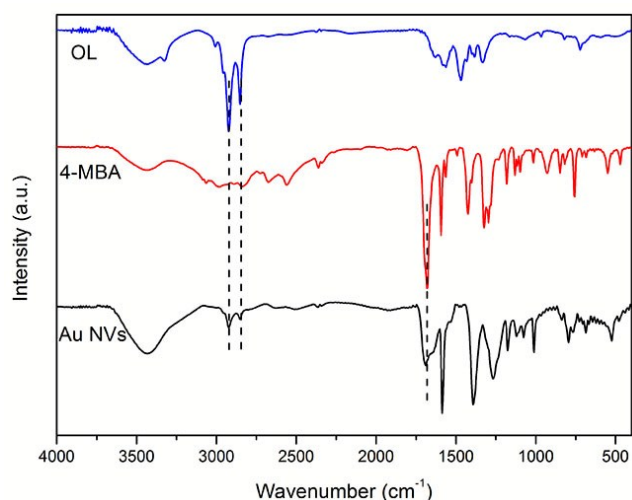


Fig. 1 FTIR spectra of OL, 4-MBA and Au NVs.

transmission electron microscope (TEM). As shown in Fig. 2A, Au NPs capped with both OL and 4-MBA can be well assembled into vesicular nanostructures under ultrasonication. The NVs are composed of multiple layers of Au NPs tightly packed in the shells with a diameter of 89.2 ± 16.8 nm and the voids have an average size of 23.4 ± 6.5 nm (Fig. S2). Under the disturbance of ultrasonication, surface ligands would rearrange to minimize the contact between water and hydrophobic ligands (OL) and to maximize the hydrophilic ligands (4-MBA) density at the NPs/water interface, for reducing the surface energy of the whole system.¹⁷

Noticing the pH-responsibility of 4-MBA, we further studied the structural transformation of Au NVs under the stimuli of pH. Herein, NaOH aqueous solution was employed to tune the pH of the system from 4.8 to 11.7. Once NaOH solution was added into the vesicle solution, the solution colour turned from purple to wine-red immediately. As shown in Fig. 2B, NVs disassembled into monodisperse NPs after addition of NaOH. To well study the effect of pH stimuli, UV-Vis absorption spectroscopy was employed to monitor the structure transformation of the self-assemblies. As shown in Fig. 2D, Au NVs gave a typical surface plasmon resonance (SPR) band centred at 545 nm, which had a red shift compared to that of individual Au NPs due to increased local dielectric permittivity in the vesicular nanostructures. When the pH of the solution increased to 11.7 by addition of NaOH, the SPR band blue shifted back to 513 nm, which was very similar to that of original individual Au NPs, indicating the disassembly of Au NVs to individual Au NPs, agreeing well with the TEM observation (Fig. 2B).

At pH = 11.7, 4-MBA on the surface of Au NPs deprotonated, leading to the formation of negative carboxylate ions (Fig. 3). As a result, hydrogen bonds between neutral carboxylic acids were broken and an electrostatic repulsion between anionic carboxylate groups significantly increased the hydrophilicity of Au NPs and therefore a good dispersion in water. Thus, Au NVs disassembled into monodisperse individual NPs (Fig. 2B). In comparison, when the pH of solution turned back to 4.8, the carboxylate ions received protons, leading to the decrease of hydrophilicity and then the recovery of NVs. The reversible changes were well illustrated by

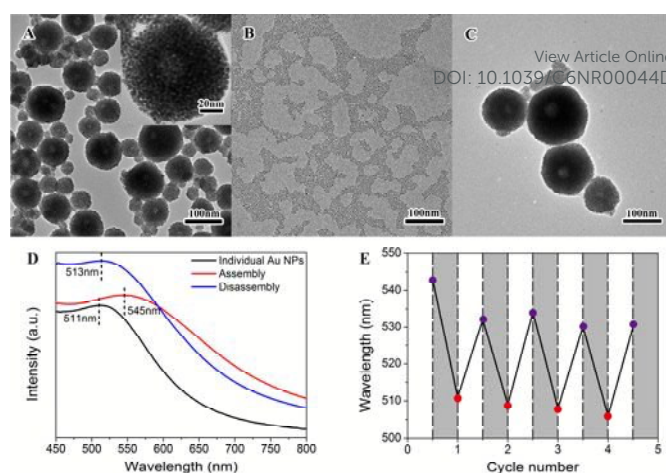


Fig. 2 TEM images of (A) self-assembled Au NVs, (B) disassembled individual Au NPs and (C) reassembled Au NVs. The inset in A is the TEM image of an individual Au NV. (D) UV-Vis absorption spectra of assembled Au NVs, disassembled individual Au NPs and original individual Au NPs. (E) Reversible changes on SPR absorption peak of Au NVs and individual Au NPs in aqueous solution induced by addition of alkali and acid, respectively.

the fluctuation of SPR absorption of Au NPs and the TEM observation. As shown in Fig. 2E, the SPR absorption of Au NVs kept larger than 530 nm, while monodisperse Au NPs exhibited an absorption centred at 510 nm with a small blue-shift tendency over 5 repeated cycles. Direct TEM observation also confirmed the reversible self-assembly and disassembly (Fig. 2C and S3).

The pH-responsive Au NVs with excellent water dispersion and good reversible self-assembly and disassembly behaviour are ideal candidates for controlled drug release. As a proof-of-concept, a fluorescent molecule, Rhodamine B (RhB), was loaded into Au NVs during self-assembly and then released by addition of NaOH. The fluorescence emission of RhB centred at 575 nm was employed to monitor the release of RhB. As shown in Fig. 4A, the fluorescence intensity increased with addition of NaOH. The increment diminished gradually and finally the fluorescence intensity did not

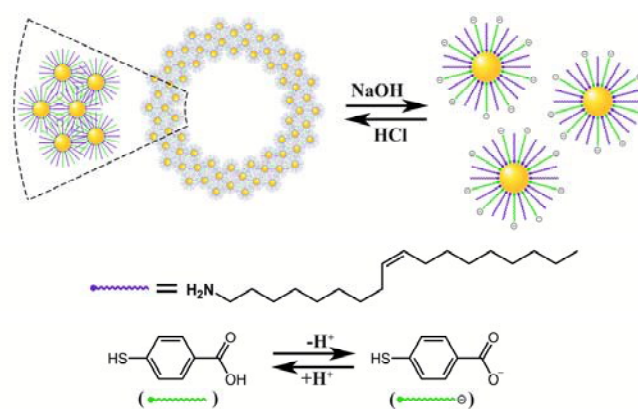


Fig. 3 Schematic illustration of pH-responsive reversible self-assembly of Au NPs.

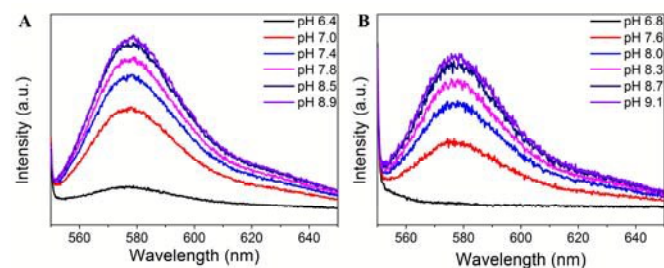


Fig. 4 Fluorescence emission spectra of RhB ($\lambda_{\text{ex}} = 540$ nm) released from (A) self-assembled Au NVs and (B) reassembled Au NVs with the increase of pH by addition of NaOH in batches.

change with the increase of NaOH which signified that RhB molecules were released entirely. Furthermore, in order to study the dynamic property of this release process, the fluorescence intensity was detected every minute at a fixed addition of NaOH. As shown in Fig. S4, once the addition of NaOH, the fluorescence intensity increased immediately and did not change with time. It meant that the release of RhB was rapid and not time-dependent. Based on the above results, it can be speculated that hydroxyl ions rapidly reacted with carboxyl groups, leading to the disassembly of Au NVs and the following release of the imbedded RhB. Due to the reversible response, the disassembled Au NPs were employed to reassemble into NVs again to load RhB. The repeated release of RhB is similar to that before, indicating a good reversible release behaviour of Au NVs (Fig. 4B).

Conclusions

In summary, we have successfully developed a novel system to realize the reversible self-assembly and disassembly of Au NVs in aqueous solution driven by pH stimuli with commercially available organic molecules, 4-MBA and OL. Monodisperse Au NPs was assembled into NVs below pH 4.8, while the reversible dissociation into individual NPs took place at pH higher than 11.7 due to deprotonation of carboxyl groups of 4-MBA. As a proof-of-concept, RhB was loaded into Au NVs which exhibited a good reversible pH-responsive release. The Au NVs with reversible pH-responsive behaviour could act as promising candidates for controlled drug release, biosensor, catalysis, and chemical reactor.

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