Light-controlled self-assembly of non-photoresponsive nanoparticles

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The ability to guide the assembly of nanosized objects reversibly with external stimuli, in particular light, is of fundamental importance, and it contributes to the development of applications as diverse as nanofabrication and controlled drug delivery. However, all the systems described to date are based on nanoparticles (NPs) that are inherently photoresponsive, which makes their preparation cumbersome and can markedly hamper their performance. Here we describe a conceptually new methodology to assemble NPs reversibly using light that does not require the particles to be functionalized with light-responsive ligands. Our strategy is based on the use of a photoswitchable medium that responds to light in such a way that it modulates the interparticle interactions. NP assembly proceeds quantitatively and without apparent fatigue, both in solution and in gels. Exposing the gels to light in a spatially controlled manner allowed us to draw images that spontaneously disappeared after a specific period of time.

Precise manipulation of nanoscale objects with external stimuli is of prime importance for applications that range from nanofabrication1–3 to controlled drug delivery4,5. Of particular interest is the ability to control the behaviour of nanoparticles (NPs) by the use of remote signals that can be delivered to closed systems. In this respect, light and magnetic fields have attracted the most attention. An additional advantage of using these two types of external stimuli is that they can be applied and removed instantaneously, and thus pave the way towards fabrication materials whose structures and properties can be modulated ‘on demand’. For example, magnetic fields have been used to control the movement and assembly of magnetite6–8, cobalt9,10 and other11 superparamagnetic NPs, which gives rise to diverse functions and applications, including magnetically switchable catalysis12,13, water purification14 and the remote control of intracellular signalling15,16. Despite these advances, this strategy requires magnetically responsive components. To overcome this limitation, several groups demonstrated recently an attractive and versatile alternative method based on the use of strongly magnetic environments (for example, ferrofluids or solutions of paramagnetic salts with high magnetic susceptibility), in which ‘non-magnetic’ (that is, diamagnetic) particles self-assemble by opposing the magnetic dipole moments of the surrounding medium. This novel methodology has led to the formulation of concepts such as ‘imaginary magnetic tweezers’17 or ‘virtual magnetic molds’18, and has paved the way towards preparing complex ‘colloidal molecules’19 and magnetically responsive photonic crystals20,21, as well as the precise positioning of diamagnetic particles (including protein molecules) on planar surfaces18.

Compared with a magnetic field, light has several additional advantages as an external stimulus—for example, it can be delivered to a precise location and in the form of different wavelengths. Consequently, the light-directed self-assembly of NPs has been investigated extensively during the past decade, and this has resulted in, for example, photocatalysed22 and high-resolution patterning of surfaces with colloids23. However, these systems are all based on NPs functionalized chemically with monolayers of light-responsive molecular switches (typically azobenzenes24–26 and spiropyran (SPs)27–30), which not only makes their preparation cumbersome, but can also significantly and adversely affect the performance of the switches because of electronic interactions with the metallic substrates31,32. Inspired by recent reports on the magnetic assembly of non-magnetic particles, we envisaged a new methodology to control the assembly of non-photoresponsive NPs with light. We hypothesized that photoresponsive media comprising solutions of light-switchable molecules capable of releasing and capturing H+ could reversibly affect the solution stability of NPs functionalized with pH-sensitive ligands, and thereby induce the assembly of NPs that, on their own, do not respond to light.

An ideal candidate to realize this goal is 1,3,3′-trimethylspiro[chromene-2,2′-indoline], commonly referred to as spiropyran (Fig. 1, and also see Supplementary Fig. 1). Several recent studies demonstrated the ability to modulate the solution pH with light by means of reversible isomerization of different SP derivatives33–35. For example, Shi et al. showed that a water-soluble SP derivative could be used to photoswitch reversibly the solution’s acidity by as much as 2.2 pH units38. This unique property of SP originates from its unusual combination of photochromic and acidochromic properties39. Accordingly, we hypothesized that light-induced proton capture and release could be coupled (Fig. 1) to deprotonation and protonation of ligands on the surfaces of NPs, and thereby reversibly affect the interparticle interactions.

Results and discussion

To test this hypothesis, we worked with 5.5 nm Au NPs (5.5 nm indicates the diameter of the metallic core) functionalized with monolayers of 11-mercaptoundecanoic acid (MUA). Interestingly, we found that these NPs were insoluble in pure methanol (or any other common solvent), presumably because of strong interparticle interactions that involve multiple40 hydrogen bonds between the terminal COOH groups. However, the addition of a small excess of a strong acid (for example, 2 equiv. methanolic HCl with respect to the number of MUA groups) breaks the hydrogen-bonded bridges...
and stabilizes individual particles, which results in NP suspensions that are stable for more than six months (for more dilute NP solutions, a larger excess of HCl, typically ~7 equiv., is necessary). We titrated this acid-stabilized solution with SP, which acts as a base and competes with the surface-bound COOH groups for protons. Once a critical number of the extra protons had been removed from the NPs (the addition of >1.0 equiv. SP with respect to HCl is necessary), a rapid assembly of NPs commenced and eventually led to the quantitative removal of the NPs from the solution (no stirring or shaking was applied). The resulting mixture was stable in the dark and under ambient lighting conditions (fluorescent laboratory light), and represents a unique example of a system that features the reversible control of the assembly of non-photoresponsive NPs using light, as described below.

The yellow colour of the supernatant can be attributed to protonated merocyanine (MCH+ in Fig. 1), whose presence was confirmed by the characteristic optical response of the solution (a pronounced band at ~420 nm (see the yellow curve in Fig. 2a)). When the sample was placed near a visible light source, we observed a rapid (within ten seconds) redissolution of the precipitate (gentle shaking was applied), which gave rise to a red solution, indicative of a stable dispersion of NPs (see the red curve in Fig. 2a; the red solution and the ~520 nm band result from the localized surface plasmon resonance (SPR) of Au NPs). To achieve this, typically we used a common 50 W fluorescent bulb (with a light intensity ~1 mW cm−2), although less-intense light sources, such as those generated by flashlights of mobile phones, could also be used to redisperse the NPs quickly and quantitatively. We tested a variety of non-photoresponsive NPs using light, as described below.

Reversibility and scope of the method. Once the first cycle has completed, the disassembly–assembly sequence can be repeated. Figure 2c shows five cycles, whereby the wavelength of the maximum absorption of light by NPs can be toggled reversibly between ~523 nm and ~538 nm (because of the free and aggregated Au NPs, respectively) simply by turning the visible light on and off (see also Supplementary Fig. 3). We also used dynamic light scattering (DLS) and transmission electron microscopy (TEM) to confirm the reversible nature of the process (for example, see Fig. 2d,e). At first, this excellent reversibility may appear surprising given the pronounced fatigue effects typically associated with the SP switches39. However, the gradual photodegradation of many systems based on SPs is usually associated with the use of ultraviolet light (λ ≈ 365 nm) to induce the isomerization reaction. In our case, switching is achieved exclusively with blue light, a much less destructive stimulus. Consequently, we observed no appreciable changes in the samples even after performing 100 disassembly–assembly cycles. Another striking feature is the quantitative yield of both the assembly and disassembly steps—careful inspection of the aggregated samples by TEM revealed the absence of any free NPs. This result is in sharp contrast to previously reported systems based on NPs functionalized with switchable molecules, in which residual non-assembled NPs are typically observed25,27,41.

To verify the scope of our methodology, we synthesized 5.5 nm Au NPs functionalized with a shorter COOH-terminated thiol, namely 6-mercaptohexanoic acid (MHA). As shown in
Supplementary Figs 4 and 5, these particles behaved in nearly the same way as the original MUA-coated NPs. We also prepared MUA-decorated Au NPs having a markedly larger diameter, namely 11 nm (Supplementary Fig. 6). Similar to their smaller counterparts, these NPs were stable in methanol in the presence of a small excess of HCl, and they quantitatively precipitated on the addition of SP. Once precipitated, the particles could be redispersed readily when exposed to visible light and they reassembled in the absence of an intense light source over many cycles without appreciable fatigue. The only major difference between the 11 nm and the 5.5 nm NPs was that the 11 nm NPs could be removed from the solution much faster (within several minutes via sedimentation) because of their larger masses. We also synthesized MUA-capped Au NPs with diameters of 2.5 nm and found that they were stable in methanol even without the addition of extra HCl, presumably because the high curvature of the small particles prevents the formation of a sufficient number of hydrogen-bonded crosslinks between the terminal COOH groups.

Curvature-dependent properties of NPs. Despite the analogous properties of the 5.5 nm and 11 nm MUA-functionalized NPs, we speculated that subtle differences in their behaviour might arise from the different curvatures of their surfaces. Recently, it was shown that the chemical, electrochemical and photophysical properties of molecules attached to surfaces depend on the curvature of the underlying surface. Of particular relevance to our study is the demonstration of the curvature-dependent acidity of carboxylic acids (deposited as o-functionalized alkanethiols on gold).

Accordingly, we prepared and studied the behaviour of solutions of MUA-functionalized gold NPs of diameter 5.5 nm and 11 nm,
Each solution containing the same concentrations of HCl, SP and surface-bound COOH groups. Specifically, we exposed both samples to visible light to obtain solutions of free NPs, and followed the kinetics of assembly and simultaneously monitored the extent of the SP + H⁺ → MCH⁺ reaction. In the graphs in Fig. 3, the blue curves correspond to the concentration of the MCH⁺ species and the red curves represent NP absorbance (A) at the wavelength of maximum A (A_{NP@λmax}), where the abrupt increase (for example, at t ≈ 2 min in Fig. 3a) in A denotes the onset of NP assembly. We found that the assembly of 5.5 nm NPs commenced when the concentration of MCH⁺ reached ~85% of its maximum value (Fig. 3a). However, as much as ~95% MCH⁺ must be generated to induce the assembly of 11 nm NPs (Fig. 3b).

This seemingly counterintuitive result, whereby (under otherwise identical conditions) the small NPs assemble first, can be explained by considering the different propensities of MUA on differently sized NPs to interact with one another and bind the extra protons (that originate from HCl):

1. The relatively high curvature of the 5.5 nm NP positions neighbouring COOH groups at large distances, which favours the formation of interparticle (versus intraparticle) hydrogen-bonded links between the COOH moieties. Hence, after gradually deprotonating separate batches of large and small NPs, the small NPs begin to assemble first (that is, after losing a smaller number of the extra protons).

2. However, the large diameter of the 11 nm NPs forces the terminal COOH groups to be near each other, which results in relatively strong interactions with the extra H⁺; this explains the slower increase in the concentration of MCH⁺ (see Fig. 3b, blue curve).

To gain further insight into the assembly process, we studied mixtures of differently sized NPs. To this end, we prepared solutions that contained 5.5 nm and 11 nm NPs (both with the same concentrations of surface-bound MUA) and found that the onset of assembly was between those expected from free 5.5 nm and 11 nm NPs (specifically, the assembly was induced when [MCH⁺] reached ~90% of its maximum value (Fig. 3c)). This result suggests that there is a rapid exchange of protons between the small and the large NPs; consequently, TEM images of the samples collected at the onset of assembly revealed that NPs of both sizes were present in the aggregates. Of interest is the linear nature of the NP assemblies formed at this early stage of the assembly (Supplementary Fig. 7). Such linear structures have been observed previously for interacting particles functionalized with charged ligands. This suggests that at the onset of self-assembly, the NPs still bear a partial positive charge.

**Reversible trapping and release.** Given that the self-assembly (and ultimately precipitation) in our system is driven by the formation of multiple hydrogen-bonded bridges between the NPs, we speculated that as they assemble the particles might be able to interact strongly with selected molecules, and thus effectively remove them from solution. To verify this hypothesis, we decided to work with a nitrrobenezoxadiazole (NBD)-based dye (Fig. 4a and also see Supplementary Fig. 8), which, in addition to incorporating several atoms than can act as potential hydrogen bond donors and acceptors, has a high molar extinction coefficient (ε ≈ 21,000 M⁻¹ cm⁻¹ at λ_{max} = 470 nm), and hence can be quantified spectrophotometrically. In these experiments, we first exposed our photoswitchable medium to visible light to redisolve the NPs, and then added a specific amount of NBD, where θ indicates the number ratio of NBD molecules to 5.5 nm NPs. We found that the presence of the dye did not affect the aggregation behaviour of the NPs, and that the aggregating NPs displayed a strong affinity for NBD. Specifically, the trapping efficiency, defined as the percentage of NBD removed from solution with the NPs, was 33.7 ± 2.4% for θ = 400, 52.1 ± 6.7% for θ = 200 and practically quantitative (98.6 ± 1.3%) for θ = 100 (Fig. 4b). In other words, each particle could, on average, trap as many as 100 dye molecules, a reasonable result taking into account that a single NP of this size is functionalized with ~400 MUA ligands. Importantly, trapping was reversible: subsequent exposure to visible light caused the NPs to redisolve and the trapped molecules to be released into the solution; as shown in Fig. 4c, the process could be repeated.

**Creating self-erasing images.** Finally, having established that the assembly/disassembly sequence leads to a pronounced and reversible colour change (from yellow to red (see Fig. 2a, inset)), we hypothesized that by performing the reaction in thin films of polymer gels by irradiating them locally (for example, through a mask), we might be able to create patterns that would disappear
spontaneously when the light source was removed. To test this hypothesis, we first identified poly(ethylene glycol) (PEG) as an ideal material to form the gel: our crosslinked PEG gels were robust (Fig. 5b and also see Supplementary Fig. 9), they had a high affinity for methanol and, quite strikingly, could be prepared in the presence of all the components, including MUA-functionalized Au NPs, SP and HCl, in a free-radical polymerization process that did not appreciably affect any of them, in other words, no post-polymerization soaking with NPs was necessary (see Methods for details). Under ambient conditions, the gels are yellow, which indicates the presence of MCH\(^+\) (when aggregated and precipitated, the NPs, which are used in small quantities, do not contribute to the colour of the gel). To demonstrate the proof-of-concept, we irradiated a \(6\,\text{cm} \times 4\,\text{cm} \times 1\,\text{mm}\) piece of gel with blue light through a mask that featured the Cheshire Cat of Alice’s Adventures in Wonderland by Lewis Carroll (Fig. 5a). The colour in the exposed regions turned red, which indicated NP dissolution accompanied by the fading of MCH\(^+\) (generating the colourless SP). Similar to their solution behaviour, the free NPs were ‘metastable’ and assembled spontaneously. Consequently, the image vanished within three minutes, and the original gel was regenerated in which a new image could be created. Figure 5c shows a series of flags created in the same piece of gel—again, no deterioration in the performance of the system could be observed even after about 100 write–erase cycles, which suggests potential applications in reversible, time-sensitive information storage.

**Conclusions**

We have developed a general method that allows the light-induced, reversible assembly of NPs that, by themselves, are not photosensitive. Our method is based on the use of a photoswitchable medium, whose acidity can be modulated reversibly with visible light and so affects NP interaction potentials to the extent that they are either quantitatively assembled or fully dispersed. The use of a non-invasive stimulus in the form of low-intensity blue light renders our photoswitchable medium virtually fatigue-free. We envision that our strategy can be extended readily to NPs of other compositions, sizes and shapes. Furthermore, particles with terminal functionalities other than carboxylic acids (for example, sulfonic acids, phosphonic acids and phenols) are expected to behave analogously, but the use of more/less basic photosensitive media might be necessary. Nevertheless, the pK\(_a\) values of SPs can be modulated readily by decorating them with different electron-withdrawing and -donating substituents\(^{39}\)—for example, placing the NO\(_2\) group at the para position with respect to the phenolic oxygen would significantly decrease the basicity of the photosensitive medium. Moreover, it should be easy to adapt this strategy to work in water; in fact, several watersoluble SP derivatives have been reported recently\(^{36,38}\). Here, however, we expect the opposite behaviour: given that MUA-functionalized NPs are insoluble in water but can readily be solubilized on deprotonation of the terminal COOH groups, we presume that under ambient conditions NPs will be highly soluble, but their assembly will be triggered by blue light. Access to such ‘inverse’ systems would further expand the versatility of our methodology.

The ability to deliver/remove NPs rapidly and quantitatively to and from solutions could have many interesting consequences. For example, different inorganic NPs are active catalysts for various reactions\(^{32}\); we assume that the catalytic activity can be modulated, depending on whether the particles are dispersed or strongly interact with one another. Next, the surface chemistries of our self-assembling NPs can be tailored (via the formation of bi- or multicomponent self-assembled monolayers) so that they display affinity towards specific small molecules present in solution, to allow the reversible capture and release of these molecules, which potentially is of major importance for water purification\(^{34}\).
Importantly, these and other proof-of-concept experiments could be carried out in microfluidic set-ups, in which reversible assembly/disassembly could be triggered at desired locations by exposing these regions to blue light. On a more fundamental level, it seems particularly interesting to consider the light-induced assembly of various protein molecules whose solubility depends on the pH of the surrounding medium in a similar way to that of MUA-coated NPs. Finally, we want to investigate the co-assembly of different types of NPs, in which context it would be interesting to study the behaviour of mixtures of proteins and inorganic NPs.

Methods
Synthesis and functionalization of 5.5 nm Au NPs. First, a stock solution of didodecyldimethylammonium bromide (DDAB) was prepared by dissolving DDAB (833 mg, 1.80 mmol) in 18 ml toluene (with sonication). Then 450 mg dodecylamine (DDA) and 50 mg of HAuCl₄·3H₂O were dissolved in 12.5 ml of the stock solution with sonication. Gold(0) was then reduced by the rapid injection of 125 mg tetraethylammonium bromide in 5 ml DDAB stock solution under vigorous stirring. The resulting solution of small NPs (’seeds’) was aged for 24 hours. Growth solution was then prepared by adding to 50 ml toluene the following reagents in the following order: (1) 1.00 g DDAB, (2) 1.85 g DDA, (3) 200 mg HAuCl₄·3H₂O and (4) 7 ml of the aged seed solution. Finally, 131 μl NH₄Cl·H₂O dissolved in 20 ml DDAB stock solution was added dropwise (~1 drop s⁻¹) to the vigorously stirred growth solution. The reaction mixture was slowly stirred overnight, which resulted in a monodisperse batch of 5.5 nm NPs. Next, 3 ml of the 5.5 nm Au NP solution was mixed with the same volume of methanol, and the mixture was left on an orbital shaker (at a slower shaking rate) for about one hour. The resulting black precipitate was collected by decantation and redissolved in 3 ml pure toluene. Solid MUA (4.1 mg) (used in excess; 10 equiv. with respect to the number of binding sites on gold, calculated assuming that a single thiolate ligand on gold occupies an area of 21.4 Å²) was added and the mixture was stirred for several hours (we separately functionalized the NPs with a four- and 25-fold excess of MUA and saw no noticeable difference in the morphology or the behaviour of the resulting NPs). The resulting black precipitate was collected by centrifugation and washed extensively with toluene (12 ml) to remove the excess unbound MUA. Finally, modified Au NPs were redisolved in 3 ml MeOH that contained 3 μl of a 1.25 M methanolic solution of HCl. For the synthesis and functionalization of 2.5 nm and 11 nm Au NPs, see Supplementary page 2.

Trapping experiments. In a typical experiment, a methanolic solution of MUA-functionalized 5.5 nm Au NPs stabilized with a small excess of HCl (2 equiv. with respect to surface-bound MUA) and containing SP (3 equiv. with respect to surface-bound MUA) was exposed to blue light for 90 seconds to solubilize the NPs. A specific amount of NBD was added (100, 200 or 400 molecules per NP) and the mixture was kept in the dark for five minutes, which resulted in the quantitative precipitation of NPs. The UV-vis absorption spectrum of the supernatant was then recorded to estimate the concentration of free NBD in solution. In the studies of reversible trapping and release of NBD, UV-vis spectra were recorded after alternating exposure to blue light for 90 seconds and dark incubation for five minutes.

Preparation of photoresponsive gel. PEG methyl ether methacrylate (M₅₀ = 950 (Aldrich Catalogue No. 447951)) and PEG diacrylate (M₇₀ = 700 (Aldrich Catalogue No. 455008)) were used as received. PEG methyl ether methacrylate (2.55 g, 2.68 mmol) was added to 5 ml of a methanolic solution of MUA-protected Au NPs (d = 5.5 nm, c₅₀ = 7.26 nm, stabilized with 7.5 μl of 1.25 M methanolic solution of HCl) and the mixture was vortexed until it was homogeneous. Crosslinker (PEG diacrylate, 400 μl, 450 mg, 0.643 mmol), SP (16 mg, 0.057 mmol) and photoinitiator (diphenyl (2,4,6-trimethylbenzoyl)phosphine oxide, 6 mg, 0.017 mmol) were then added and the resulting solution was placed between two glass slides separated by 1 mm, and it was exposed to long-wave (λ ≈ 365 nm) ultraviolet radiation (hand-held lamp, intensity ≈ 0.7 mW cm⁻²) for 25 minutes.

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References


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**Author contributions**

R.K. conceived and designed the experiments. P.K.K., D.S., R.L., B.M. and M.B. performed the experiments. H.Z., T.U. and D.M. contributed materials and/or analysis tools. R.K. wrote the manuscript. All the authors discussed the results and commented on the manuscript.

**Additional information**

Supplementary information is available in the online version of the paper. Reprints and permissions information is available online at www.nature.com/reprints. Correspondence and requests for materials should be addressed to R.K.

**Competing financial interests**

The authors declare no competing financial interests.