

Covalent assembly of functional inorganic nanoparticles by “click” chemistry in water†

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Huisgen 1,3-dipolar cycloaddition “click” reaction with functional quantum dots and magnetic nanoparticles in water is presented. The introduction of “click” enabling groups on the surface of the nanoparticles is combined into one step with a transfer into water process. Reaction between acetylene and azide groups is used for the fabrication of covalent spherical assemblies in solution.

Aqueous dispersions of functional nanomaterials such as fluorescent nanocrystals (Quantum Dots, QD) or magnetic nanoparticles (MNPs) are of great recent interest due to their applications as biomarkers in medical diagnostics, as signal transducers in sensing, *etc.*¹ Assemblies of these functional systems in water are often required *e.g.* for response signal enhancement. This necessitates the design of coupling strategies in an aqueous environment. Copper(i) catalyzed Huisgen azide/alkyne 1,3-dipolar cycloaddition has made in recent years a spectacular re-entry into the chemical sciences.² This reaction, usually referred to as “click”, is popular whenever a simple, fast and irreversible reaction is necessary for the attachment of structural units.^{3,4} “Click” chemistry in principle lends itself to covalent bonding of functional nanocrystals. However, introducing “click” functional groups to the surface of nanoparticles (NPs) is not a trivial issue. The harsh conditions usually required during NP preparation do not allow for the presence of azide or acetylene functional groups in stabilizing ligands. Despite its known limitations⁵ exchange of the NP capping ligands or chemical ligand modification has been used for azide and acetylene functionalization of QDs,⁶ MNPs,⁷ TiO₂ nanoparticles,⁸ Ag nanoparticles⁹ and Au nanoparticles.^{10,11} Only gold nanoparticles stabilized with PEG derivatized ligands have been used for water-based “click” chemistry.

In this contribution the introduction of “click” functional groups (azide and acetylene) onto water suspendable QDs and MNPs is addressed. The “click” enabling functional groups

are introduced into the NP surface by wrapping the NP with designer amphiphilic polymers *via* supramolecular hydrophobic/hydrophobic interactions. Ligand exchange or ligand chemical modification steps are therefore avoided. Moreover, we present a method for the formation of covalent NP assemblies with sizes ranging from 10 to 200 nm comprising densely packed, though not aggregated, NPs. These “clicked” assemblies display long lasting ability to stay suspended in water and preserve luminescent properties in the case when QDs are used as building blocks.

Core-shell CdSe/ZnS quantum dots¹² and Fe₃O₄ magnetic nanoparticles¹³ were synthesized following the reported protocols. As a result of the synthetic procedures, the NPs were capped with hydrophobic ligands. Suspension of NPs using amphiphilic molecules was reported as a good method to avoid the ligand exchange process.¹⁴ In order to transfer the nanoparticles into water, two new amphiphilic polymers were synthesized by grafting side functional units into a poly-(isobutylene-*alt*-maleic anhydride) backbone. Both polymers carried hydrophobic *n*-octylamine chains for interaction with NPs ligands and hydrophilic carboxylic groups for interaction with water. Additionally, polymer **Pol-1** had a covalently attached amino PEG chain terminated with an azide group, while **Pol-2** carried propargyl amide side groups. The polymers were characterized by ¹H NMR spectroscopy. On average **Pol-1** had 16% of azide groups and **Pol-2** had 22% of acetylene groups attached to the polymer backbone.

Using the two polymers, **Pol-1** and **Pol-2**, hydrophobic CdSe/ZnS QDs and Fe₃O₄ MNPs were suspended in water by mixing the NP solutions in THF with an aqueous solution of a polymer, and subsequent evaporation of THF. Four solutions of suspended NPs were prepared including QD bearing azide groups **QD-Pol-1**, QD bearing acetylene groups **QD-Pol-2**, MNP bearing azide groups **MNP-Pol-1** and MNP bearing acetylene groups **MNP-Pol-2**. “Click” reaction was performed by mixing the NPs solutions in typical catalytic conditions in the presence of Cu(i) catalyst and sodium ascorbate. CdSe/ZnS QDs covalent assemblies were obtained using **QD-Pol-1** and **QD-Pol-2** as substrates; Fe₃O₄ MNP covalent assemblies were obtained using **MNP-Pol-1** and **MNP-Pol-2** (Fig. 1 and 2). Upon “clicking”, the reaction between azides and acetylenes results in a stable 1,2,3-triazole ring.‡

Unlike for gold nanoparticles as observed by Jiang *et al.*,¹¹ using a different approach, in our case the NP assemblies did not precipitate upon completion of the reaction. The aqueous suspension remained stable for several months following the

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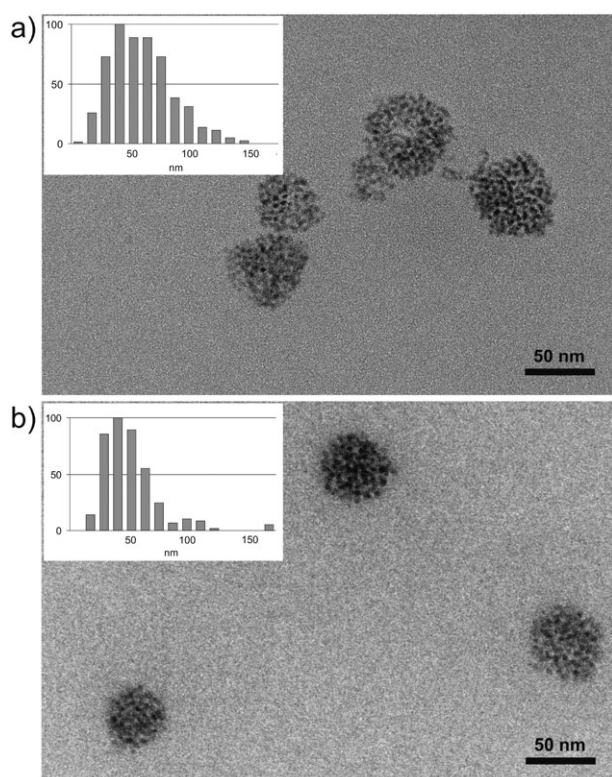


Fig. 1 TEM images of covalent NP assemblies with the normalized size distribution shown in the insets. (a) CdSe/ZnS QD assemblies formed by the reaction between **QD-Pol-1** and **QD-Pol-2**. (b) Fe_3O_4 MNP assemblies formed by the reaction between **MNP-Pol-1** and **MNP-Pol-2**.

experiments. From the clarity of the solutions after the reaction, the NPs were obviously not forming extended network structures, as expected, but some smaller entities. This was confirmed by transmission electron microscopy (TEM) imaging. It is likely that charges accumulated at the surface of the covalent assemblies owing to the presence of carboxylic groups are responsible for the colloidal stability and prevent formation of larger structures, *e.g.* networks. Spherical covalent NP assemblies obtained in both experiments had similar shapes, while a slightly broader size distribution was obtained in the case of the “clicked” QDs. The value of the median of

the QD covalent assemblies’ diameters was 56 nm (inset of Fig. 1a) while for MNPs it was 46 nm (inset of Fig. 1b). The size distribution was relatively broad, which is understandable when considering that the Huisgen reaction is an irreversible process, and in such a case it is hard to build equilibrium structures of monodisperse objects.

For the reactions reported, the presence of the Cu(I) catalyst is critical. Mixing of NPs suspended with both polymers (**QD-Pol-1** and **QD-Pol-2**), however without Cu(I), did not yield covalent assemblies, as evidenced by TEM (see supporting information†). The luminescence of the QDs is very sensitive to the presence of the catalyst. Addition of Cu(I) acetate to the mixture of QDs results in strong and fast luminescence quenching reaching a minimum within a few minutes following the reaction initiation. Quite different behavior was observed when sodium ascorbate was added to the NPs mixture before the addition of the Cu catalyst. The quenching is much slower and the remaining emission is at a higher level (Fig. 3a). We attribute this behavior to the quenching of QDs by Cu(II) ¹⁵ ions present in the catalyst solution and their QD-mediated photoreduction to Cu(I).¹⁶ Importantly, the luminescence can be restored by washing out the Cu ions with NaOH solution and subsequent centrifugation. Thus, the problem of Cu quenched emission is addressed and 70–90% of the initial

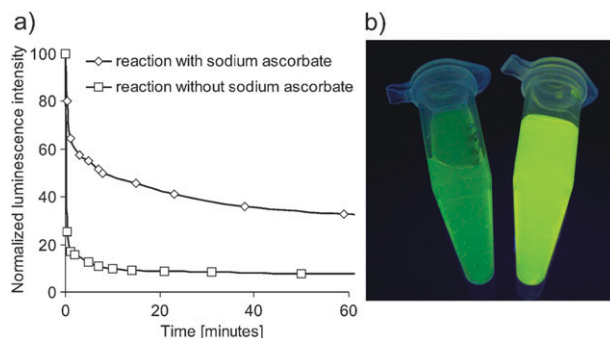


Fig. 3 (a) Normalized luminescence intensity of QDs as a function of the reaction time. The luminescence decreases faster when no sodium ascorbate is added to the reaction. (b) Vials with suspensions of covalent QD assemblies before (left) and after (right) washing off the catalyst; 70–90% of original luminescence is recovered.

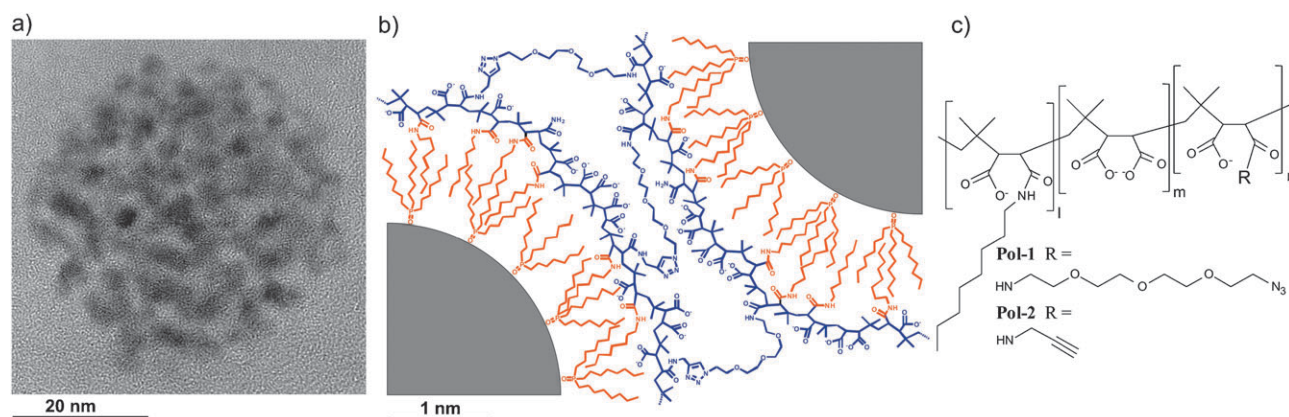


Fig. 2 (a) TEM image of a covalently “clicked” assembly of CdSe/ZnS QDs. (b) Scheme of two NPs “clicked” together *via* the amphiphilic polymer bearing azide and acetylene functionalities. (c) Polymers used for introduction of “click” functional groups at the NP surface.

luminescence from the QDs substrate is recovered in the final assemblies (Fig. 3b).

In conclusion, for the first time, the “click” chemistry carried out with QDs and MNPs in water is presented. This approach leads to the formation of spherical, covalent NPs assemblies with diameter values in the range of 20–200 nm. The QD assemblies can be obtained without a loss of the luminescent properties. The presented method may also be applied to many other hydrophobically functionalized nanoparticles and nanomaterials.

Notes and references

‡ Besides TEM and fluorescence data the reaction progress was followed by ^1H NMR. This procedure was, however, applied only when empty micelles were used in a model reaction of **Pol-1** with **Pol-2** (see ESI†).

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