Thiolated PAMAM dendrimer-coated CdSe/ZnSe nanoparticles as protein transfection agents†

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Functionalisation of PAMAM dendrimers with a small number of thiol groups makes them good ligands for CdSe/ZnSe nanoparticles; the particles coated with thiolated dendrimers have good cell permeability and are potent transfection agents.

Semiconductor (*e.g.*, CdSe, CdS, CdTe *etc.*) nanoparticles have recently attracted much attention due to their applications as fluorescent biological probes.[1] The main features of these materials are narrow symmetrical fluorescence spectra, the possibility to fine-tune the colour of fluorescence by modifying particle size, very broad excitation spectra enabling simultaneous multicolour detection, high stability towards photobleaching, relatively simple bioconjugation protocols. For biological applications, nanoparticles must be water-soluble. The most successful nanoparticle synthesis, however, leads to nanoparticles coated with hydrophobic molecules (*e.g.*, trioctylphosphine (TOPO) or dodecylamine).[2] These particles are only soluble in organic solvents. Solubility of nanoparticles in water is usually achieved by coating them with a suitable organic ligand terminated with a hydrophilic functional group. Such ligands must adhere strongly to the nanoparticle surface; thiols, phosphines and amines all have affinity to cadmium halcogenides.

Thiols are by far the strongest ligands for CdSe nanoparticles; unfortunately adsorption of thiols on the nanoparticle surface usually drastically reduces the quantum yield of fluorescence. Many strategies have been used to overcome this problem, including overcoating the nanoparticle with a thin layer of another semiconductor with a wider bandgap,[3] optimisation of the synthesis conditions, design of multipendant phosphine-based ligands for nanoparticles,[4] use of polymers as ligands,[5] *etc*. Some significant achivements have been made and a number of researchers reported preparation of water-soluble, highly fluorescent nanoparticles.[6] Water-soluble nanoparicles for biological applications are also available commercially.[7] Nevertheless, many reported procedures are long and expensive, or difficult to reproduce.

In our search for a reactive, water-soluble and readily available ligand for CdSe nanoparticles, we explored the possibility of using PAMAM dendrimers to protect the nanoparticle surface. PAMAM dendrimers possess a large number of primary and tertiary amine groups at the surface and in the interior of the molecule.[8] Hence, these materials possess strong affinity for transition metal ions.[9] Indeed, preparation of PAMAM dendrimer–CdSe nanocomposites using a solvolytic route has been reported in the literature.[10]

Unfortunately, we were unable to develop a reproducible procedure for replacement of ligands in as-synthesised TOPO-protected CdSe nanoparticles with PAMAM dendrimers. This is probably due to the relatively low affinity of the amine groups for the CdSe particles. In order to improve the binding ability of the PAMAM ligands, we have introduced a small number of thiol groups in the dendrimer structure. Thiol-functionalised PAMAM dendrimers (Fig. 1) were prepared by reacting commercially available PAMAM dendrimers with N-hydroxysuccinimide ester of 3-mercaptopropionic acid.[11]

PAMAM generation 4 dendrimers containing 64 amino groups were thiolated to obtain *ca.* 2 thiol group per dendrimer molecule. The dendrimers thus prepared can readily replace TOPO ligands from the surface of CdSe/ZnSe nanoparticles prepared by a published procedure.[12] Ligand exchange was carried out by addition of dendrimer to TOPO-coated nanoparticles in mixture of methanol with chloroform. The excess dendrimer was removed using extraction.[13] The purity of nanoparticles was confirmed by gel permeation chromatography (GPC).[14] Fig. 2 shows a typical GPC trace. The identification of peaks as nanoparticles (retention time 4.2 min) and excess of free dendrimer (retention time 11.9 min) was achieved by UV-Vis spectroscopy (the corresponding spectra are shown on the insets in Fig. 2).

Dendrimer-protected nanoparticles showed high stability and good fluorescence properties. Partial thiolation is the key to these properties. The multiple terminal amino groups of the dendrimers can stabilise CdSe/ZnSe nanoparticles without adversely affecting their fluorescent properties; on the contrary, amino ligands are known to improve fluorescent properties of semiconductor nanoparticles.[15] However, the poor affinity of amines for CdSe surface does not provide sufficient stabilisation against aggregation. Addition of only a small number of thiol groups significantly improves the affinity of the ligand for nanoparticles and does not significantly affect fluorescent properties. The UV-Vis and fluorescence spectra of dendrimer-coated nanoparticles are shown in Fig. 3.

An important property of PAMAM dendrimers is their ability to permeate cell walls. Due to their cell permeability properties, PAMAM dendrimers are commercially used as transfection agents (SuperFect, available from Qiagen). Cell permeability is highest for high generation dendrimers, and can be further improved by partial thermal degradation of these materials.[16] We reasoned that dendrimer-coated nanoparticles could mimic higher generation

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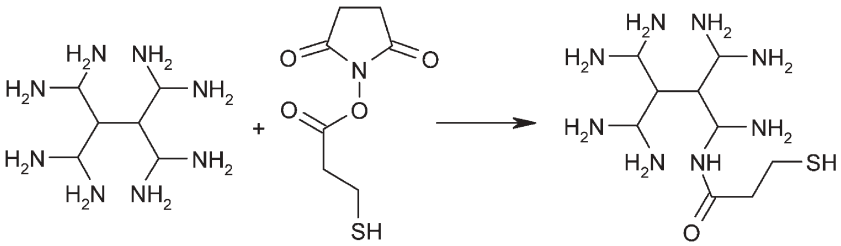

Fig. 1 Preparation of thiolated PAMAM dendrimers. The structure of the dendrimers is shown schematically for brevity.

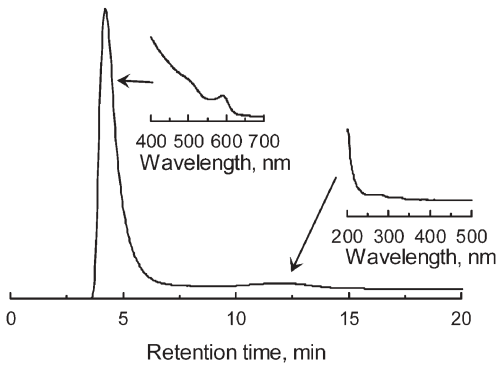

Fig. 2 GPC trace of CdSe/ZnSe nanoparticles coated with partially thiolated PAMAM dendrimers. The insets show UV-Vis spectra of the two main peaks.

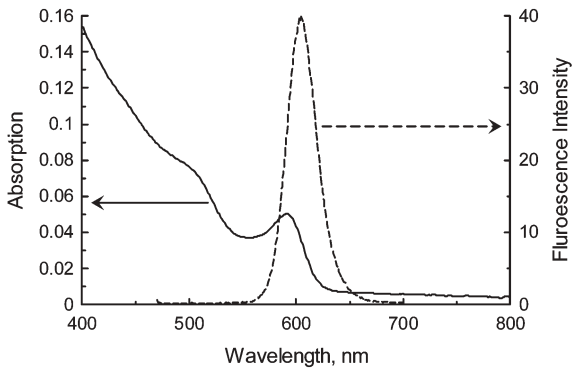

Fig. 3 UV-Vis (left) and fluorescence (right) spectra of thiolated PAMAM dendrimer-protected CdSe/ZnSe nanoparticles.

dendrimers. Indeed, orange-fluorescing CdSe/ZnSe nanoparticles are *ca*. 4 nm in diameter.[17] Dendrimer coating adds *ca.* 3–4 nm organic shell. The size of the whole assembly is hence *ca.* 11 nm. This can be compared with the diameter of generation 9 PAMAM dendrimer (11 nm).[18] Like dendrimers, the coated nanoparticles have spherical shape with the surface dominated by the amino groups.

In order to check if the dendrimer-stabilised nanoparticles can indeed permeate the cell wall, we incubated live non-differentiated teratocarcinoma NT2 cells[19] with the buffered solutions of nanoparticles.[20] After washing with the buffer, the live cells were analysed using confocal microscopy.[21] The image in Fig. 4a is the fluorescence micrograph, whereas the image in Fig. 4b is the visible micrograph. The nanoparticles were mostly localised in the cytoplasm, as evidenced by the analysis of z-stacked confocal images.

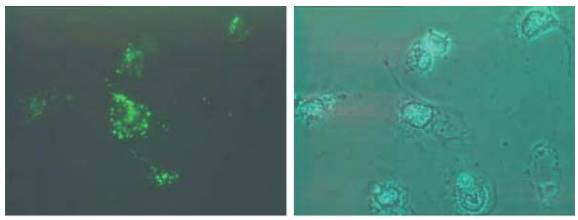

Fig. 4 Confocal fluorescence (*a*) and visible (*b*) images of live NT2 cells incubated with dendrimer-coated CdSe/ZnSe nanoparticles.

In order to probe whether the dendrimer-coated nanoparticles can help transfect other molecules across the cell membrane, we studied transport of an EF-hand calcium-binding protein S100A4 in NT2 cells. S100A4 protein possesses important features such as conformational changes in the presence of calcium and the ability to disassemble myosin filaments and possibly other protein oligomers.[22] This protein is thought to be a perfect tool to influence cellular proliferation, differentiation and apoptosis. The calcium dependent binding to its targets and also the ability to form heterotetramers with other S100 proteins[23] might help to use this protein as a potential drug delivery vector.

To help independently visualise the protein and the nanoparticles, the protein was labelled using an Alexa Fluor 488 protein labelling kit (Molecular Probes). The labelled protein was then incubated with the cells in the presence of dendrimer-coated nanoparticles.[24] The cells were fixed and treated with DAPI stain in order to visualise the nuclei. The fluorescence confocal images are shown in Fig. 5. The blue channel corresponds to the DAPI stain, the green channel is the Alexa Fluor dye, and the red channel is CdSe/ZnSe nanoparticles.

It is clearly seen that the nanoparticles successfully transported the S100A4 protein into the cell. Most protein localised in the cytoplasm, with very little penetration into the nucleus. Control experiments showed that the level of protein transfection without nanoparticles is significantly lower (see the electronic supplementary information†). Transfection by using particles as a delivery vesicles is the most efficient way to transfer proteins into the cell that has a long lasting effect and may also help to avoid interaction and activation of extracellular receptors when protein interacts directly with the cellular surface.

It is interesting to note the co-localisation of the protein and the nanoparticles in the cytoplasm. We believe that this is due to the strong binding of protein to the polycationic polyamine surface of the dendrimer-coated nanoparticles.

In conclusion, we have shown that partial thiolation of PAMAM dendrimers makes them good ligands for CdSe/ZnSe

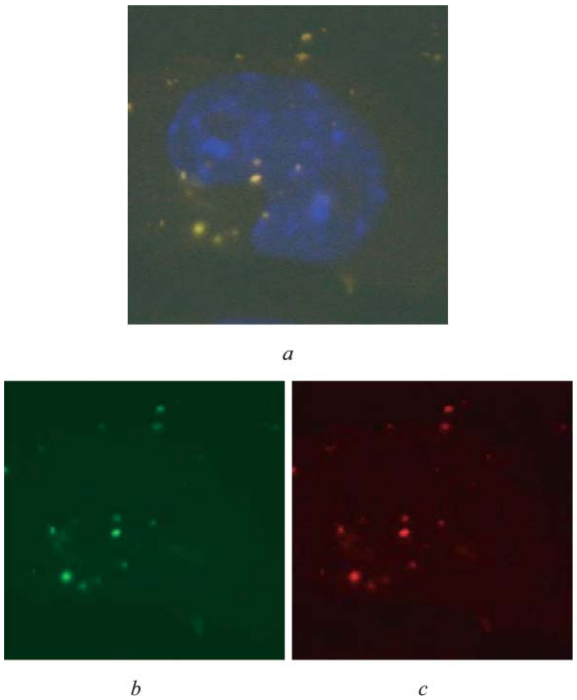

Fig. 5 Confocal images of NT2 cells incubated with Alexa Fluor-labelled S100A4 protein and CdSe/ZnSe nanoparticles. The blue channel in combined image (*a*) is DAPI stain. Images (*b*) and (*c*) show Alexa Fluor dye (green channel) and CdSe/ZnSe nanoparticles (red channel), respectively.

nanoparticles. The dendrimer-protected nanoparticles are soluble in water and have good stability. The dendrimer-coated nanoparticles can transport other molecules across the cell wall and hence are promising fluorescent transfecting agents.

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