Encapsulation of Nanoparticles with Multifunctional, Cross-Linkable Diblock Copolymers for Biomedicine

C. Schmidtke[*], J. Ostermann[*], H. Tran[*], E. Pöselt[*], H. Kloust[*], J. Niehaus[**], S. Becker[*], A.-M. Kreuziger[*], K. Werner[**], A. Pietsch[*], H. Weller[*,**]

[*] University of Hamburg, Institute of Physical Chemistry
Grindelallee 117, 20146 Hamburg, Germany, schmidtke@chemie.uni-hamburg.de
[**] CAN GmbH, Grindelallee 117, 20146 Hamburg, Germany

ABSTRACT

A phase transfer system for nanoparticles into water using a novel ligand system based on poly(isoprene)-block-poly(ethylene glycol) ligands is described. After the micelle formation surrounds the particle, the poly(isoprene) moiety can be cross-linked. This leads to highly stable water-soluble nanoparticles while retaining physical properties of the particles material. For encapsulated CdSe/CdS/ZnS core/shell/shell quantum dots we obtained fluorescence quantum efficiencies in the 40-55 percent range and extraordinary fluorescence stability in the biological environment. Due to the synthesis of diblock copolymer by living anionic polymerization the chain end can be tailored by different strategies. This allows the control of the end groups, the charge and the bio-conjugation of the nanocontainer.

Keywords: quantum dots, phase transfer, functionalization, diblock copolymer, tumor targeting

1 INTRODUCTION

Fluorescent semiconductor nanoparticles (quantum dots, QDs) are used in biomedicine for imaging, because of their excellent optical properties [1]. In comparison to organic dyes, QDs posses two big advantages due to their high photostability and due to their adjustable emission wavelength by controlling the particle size [2] (see Figure 1). High quality nanoparticles (NPs) with a high crystallinity and monodispersity were produced in organic solvents at high temperatures *via* the bottom-up procedure [3]. CdSe/CdS/ZnS core/shell/shell nanoparticles were synthesized in hexadecylamine [4].

Figure 1: CdSe based QDs under UV irradiation

For biological systems, a phase transfer into aqueous media is essential. Particles tend to aggregate and lose their characteristics without adequate stabilization.
We used a micellular encapsulation method for NPs which is based on a ligand exchange procedure of native ligands with a prepolymer poly(isoprene)-diethylentriamine (PI-N3) and subsequent ligand addition with poly(isoprene)-*b*-poly(ethylene glycol) diblock copolymer (PI-*b*-PEG)) (see Figure 2) [5]. PEG is known for suppressed protein adsorption, lack of toxicity and absence of immunogenicity [6]. As previously reported, ligand systems are usually based on self-organization by hydrophobic effects and [7, 8] / or coordinative bonds. Here, both effects were combined and expanded to include the cross-linkage of the poly(isoprene) moiety of the inner ligand shell. This leads to unique stability in aqueous media with fluorescence quantum efficiencies of up to 55% and ensures rigidity against biodegradation.
PI-*b*-PEG diblock copolymer encapsulated NPs are non-toxic and were used for antibody-mediated imaging of tumors *in vivo* [9].

2 SYNTHESIS AND ENCAPSULATION

CdSe/CdS/ZnS core/shell/shell QDs were synthesized in high boiling organic solvents using trioctyl phosphine (TOP), trioctyl phosphine oxide (TOPO) and hexadecyl amine (HDA) following the well-established protocol of Talapin *et al.* [4]. PI was synthesized by living anionic polymerizationusing *s*-BuLi as an initiator, which leads to the formation of 3,4- and 1,2-isoprene isomer units with terminal –C=C– double bonds mainly. The trans 1,4-isomer was also present, due to the polymerization in tetrahydrofuran (THF) [10]. Subsequently, the PEG block was synthesized *via* anionic ring opening polymerization using PI as a macro-initiator and diphenylmethyl potassium.

A two-step strategy for th phase transfer of the nanoparticles was used. In the first step, ligand exchange with PI-N3 in chloroform was performed (I in Figure 2). PI-N3 was synthesized by CDI coupling PI to diethylenetriamine (N3). The amines of PI-N3 act as anchor groups and as an organic passivator for the trap states in quantum dots, resulting in higher quantum yields, while the

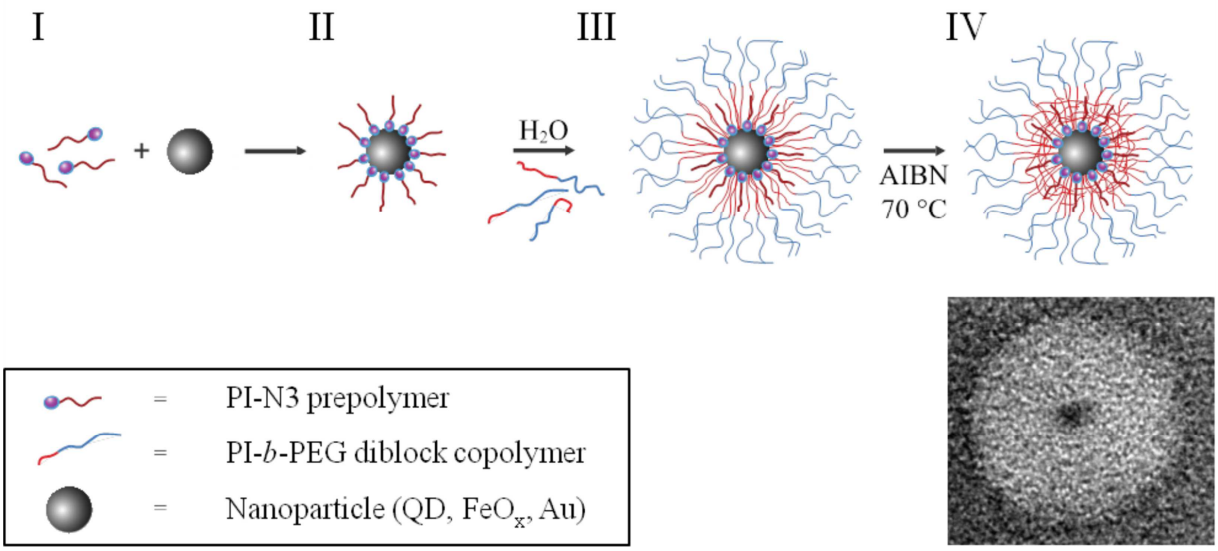

Figure 2: Schematic representation of nanoparticle encapsulation with PI-*b*-PEG diblock copolymer and a phosphotungstic acid-stained TEM image of a QD-micelle with a diameter of 29 nm.

PI part acts as seed for the following micelle formation. After evaporation of chloroform the PI-N3 stabilized nanoparticles (II) were dissolved in THF. In the second step, the amphiphilic PI-*b*-PEG blockcopolymer and a radical initiator azobisisobutyronitrile (AIBN) in THF were added. The THF solution was then injected into water, where spontaneous self-assembly of the PI-*b*-PEG around the hydrophobic PI-N3 coated nanoparticles occurred under micelle formation (III). The hydrophobic radical initiator was co-encapsulated during this process. In the final step, the –C=C– double bonds of the PI blocks were cross-linked *via* thermal radical polymerization (IV). The final size of the micelles could be adjusted by the particle to PI-*b*-PEG ratio during transfer to water.

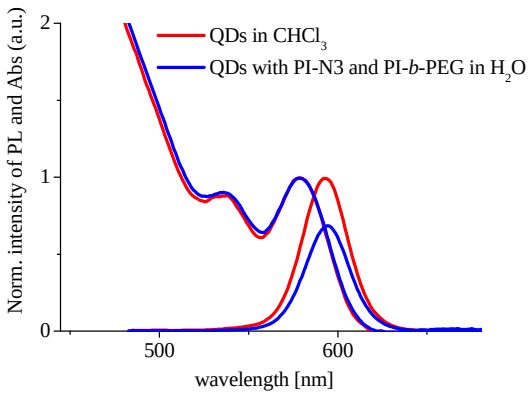

Figure 3: Absorption and emission spectra of the QDs in $CHCl_3$ and after transfer into water.

The absorption and emission spectra of the quantum dots in $CHCl_3$ and after transfer into water are shown in Figure 3. There is almost no observable spectral shift, which is attributed to the fact that the local environment of the QDs did not change significantly during the phase transfer into water, since they remain in the hydrophobic micelle core. It is seen that the fluorescence intensity was decreased in water. By optimizing the phase transfer to water (rigorous mixing), fluorescence quantum efficiencies in water in the 40-55 percent region were obtained.

3 FUNCTIONALIZATION

Micellular encapsulated nanoparticles for targeted imaging or for targeted drug release must be equipped with affinity molecules / specific functionality. The use of the amphiphilic PI-*b*-PEG diblock copolymer enables the straightforward attachment of a multiplicity of functional groups allowing a linkage of biological recognition molecules. Depending on the stage when the functionalization takes place, we distinguish between: 1.) prior to the ligand addition (pre-assembly) by terminating the polymerization or after the polymerization and 2.) after the ligand addition (post-assembly) (see Figure 4).

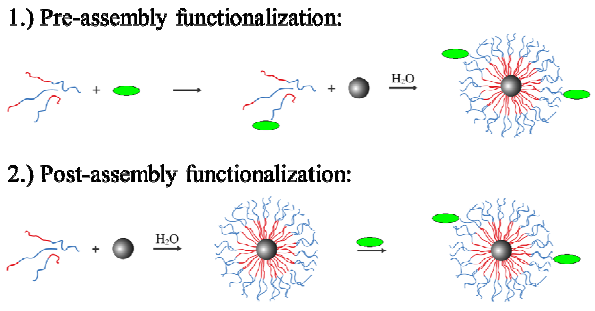

Figure 4: Schematic illustration of pre- and post-assembly functionalization (specific molecule = green object).

3.1 Pre-Assembly Functionalization

Due to the synthesis of PI-*b*-PEG by living anionic polymerization the chain end can be designed by adding various terminating agents. The potassium alkoxide end group is a good nucleophile for ring-opening (e.g. with succinic anhydride) or substitution reactions with halide compounds (e.g. 2-(boc-amino)ethyl bromide, epichlorohydrin, propargyl bromide etc.).

Conventional termination of the living anionic polymerization with acetic acid leads to just one hydroxyl group per polymer. After the polymerization this hydroxyl group can be easily transformed into amine, carboxy, alkyne, aldehyde and biotin end groups using common functionalization strategies for PEG [6, 11]. The groups could be verified by using NMR and GPC. These modifications open up the possibility to couple diverse biomolecules (antibodies, carbohydrates, peptides, DNA) *via* different chemical linkages (CDI, Click chemistry, EDC/NHS, Schiff-base) in aqueous or organic media.

3.1 Post-Assembly Functionalization

Post-assembly functionalization of PI-*b*-PEG encapsulated NPs is useful to couple sensitive affinity molecules like antibodies *via* EDC/NHS, cyanogen bromide, SMCC or biotin/streptavidin.

4 BIO APPLICATIONS

The encapsulation of nanoparticles (QDs and SPIOs) with the modified PI-*b*-PEG-X (X = amine, carboxy, hydroxy, aldehyde) diblock copolymers was successful. The resulting micelles have proven to be non-cytotoxic assessed by water soluble tetrazolium (WST8) and lactate dehydrogenase (LDH) toxicity assays on human alveolar epithelial cell line A549 in the investigated concentration regime of 0.001 to 1 µM. This range represents a cell to NP ratio of 1 to $7.5 \cdot 10^{10}$ up to $7.5 \cdot 10^{13}$.

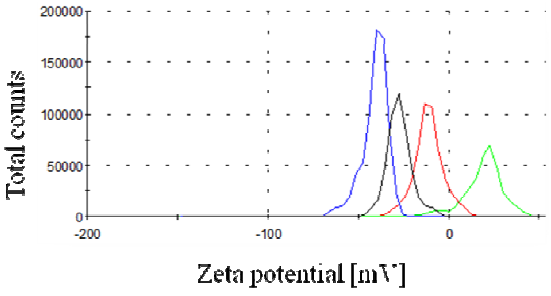

Figure 5: Zeta potential of PI-*b*-PEG-X (X = -COOH (blue); -CHO (black); -OH (red) and -NH_2 (green)) encapsulated QDs in water.

As seen in Figure 5, it is also possible to control the surface-charge of the encapsulated nanoparticle by using modified polymers. The nanocontainer is negatively (carboxy), slightly negatively (aldehyde), neutrally (hydroxy) or positively charged (amine) depending on the polymers terminating group. This is due to the fact that the end groups have different charges in water / in biological environment.

PI-*b*-PEG coated QDs show non-specific cell adhesion on A549 cells at incubation times of 50 minutes after washing. We have also investigated the cellular response on amine, carboxy, hydroxy, aldehyde terminated PI-*b*-PEG particles after 17 hours of incubation time followed by a lavage. After that time an uptake is observable, which is stronger in the case of the carboxy and amine terminated coating. Interestingly, no toxic effects were observable after the same incubation times, indicating that the particle shell still prevents direct contact or dissolution of the quantum dots and the cytosol. We also noticed that the uptake is not equal for all cells. Some show relative strong uptake and others almost none. This suggests that the uptake might occur during cell proliferation (the cell cycle time of A549 cells is 40 hours). Other authors also reported similar findings [12].

In cooperation with University Medical Center Hamburg-Eppendorf antibody-mediated dual imaging (optical and magnetic imaging) of tumours *in vivo* was successfully performed [9].

5 CONCLUSION

We have presented a new strategy for micellular-encapsulating nanoparticles with functionalizable PI-*b*-PEG diblock copolymers. The micelles show a high stability and inertness in the biological environment. It is possible to control the surface properties of the resulting nanocontainers and to use them for biomedical imaging.

6 ACKNOWLEDGEMENTS

We thank K. Peldschus, J. Salamon, C. Maßlo, M. Heine, G. Adam, U. Schumacher and C. Wagener for bio-imaging experiments.

REFERENCES

[1] C. W. Chan Warren, S. Nie, Science, 281, 2016-2018, 1998.
[2] C. Burda, X. Chen, R. Narayanan, M. El-Sayed, Chem. Rev., 105, 1025-1102, 2005.
[3] Zhang, E. Lees, F. Amin, P. Rivera_Gil, F. Yang, P. Mulvaney, W. Parak, small, 7, 3113-3127, 2011.
[4] D. Talapin, I. Mekis, S. Götzinger, A. Kornowski, O. Benson, H. Weller, J. Phys. Chem. B, 108, 18826-18831, 2004.

[5] E. Pöselt, S. Fischer, S. Förster, H. Weller, C. Schmidtke, “A method for the manufacture of a crosslinked micellar nanoparticle complex and products thereof”, PCT Int. Appl., 82, WO 2010100108 (A1), 2010.
[6] M. S. Thompson, T. P. Vadala, M. L. Vadala, Y. Lin, J. S. Riffle, Polymer, 49, 345-373, 2008.
[7] K. Susumu, H. Uyeda, I. Medintz, T. Pons, J. Delehanty, H. Mattoussi, J. Am. Chem. Soc., 129, 13987-13996, 2007.
[8] R. Jin, Angew. Chem., 120, 6852-6855, 2008.
[9] E. Pöselt, C. Schmidtke, S. Fischer, K. Peldschus, J. Salamon, H. Kloust, H. Tran, A. Pietsch, M. Heine, G. Adam, U. Schumacher, C. Wagener, S. Förster, H. Weller, ACS Nano, Articles ASAP, 2012.
[10] M. M. F. AI-Jarrah, R. L. Apikian, E. Ahmed, Polym. Bull., 12, 433-436, 1984.
[11] S. Zalipsky, Bioconjugate Chem., 6, 150–165, 1995.
[12] J. A. Kim, C. Åberg, A. Salvati, K. A. Dawson, Nat. Nanotechnol., 7, 62-68, 2012.