Electronic Supplementary Information

Covalent assembly of functional inorganic nanoparticles by "click" chemistry in water

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Experimental procedures:

1. Synthesis of CdSe/ZnS quantum dots.[1]

Cadmium oxide (0.026 g, 0.20 mmol) and stearic acid (0.25 g, 0.88 mmol) were dried in vacuum and subsequently heated in N_2 atmosphere (230 °C) until the solution became transparent. The mixture was cooled to room temperature and TOPO (4.0 g, 10 mmol) followed by n-hexadecylamine (2.5 g, 10 mmol) were added. The mixture was warmed to 200°C and a solution of Se in TOP (0.2 mL, 0.2 mmol) was added. To grow the passivating ZnS layer around the CdSe core, the solution of S in TOP (0.2 mL, 0.2 mmol) was added alternately with a solution of Et_2Zn in heptane/TOP (1 mL, 0.2 mmol) in few small portions. After cooling to room temperature QDs were purified by triple centrifugation of the particles in $CHCl_3$ / MeOH.

2. Synthesis of oleic acid-capped Fe_3O_4 magnetic nanoparticles.[2]

Iron (III)-oleate complexes were prepared firstly by reaction of iron chloride ($FeCl_3 \cdot 6H_2O$) (5.4 g, 20 mmol) with sodium oleate (18.25 g, 60 mmol) in a mixture of ethanol, water and hexane at 70°C for 4 h (ethanol / water / hexane = 40 mL / 30 mL / 70 mL). After removal of solvents, the as-obtained iron-oleate complexes were dissolved in 50 g ODE as stock solution for the synthesis of Fe_3O_4 MNPs. A typical synthesis is as follows: 25 g ODE containing 10 mmol iron-oleate complexes was mixed with oleic acid (1.43 g, 5 mmol) followed by addition of extra 25 g ODE at room temperature. The reaction mixture was heated to 320°C within 20 min, and then kept at that temperature for 30 min. At 320°C, the decomposition reaction occurred and the initial transparent solution became turbid and brownish black. The reaction mixture was then cooled to room temperature followed by adding 100 mL of ethanol to precipitate the nanoparticles. MNPs were purified by triple centrifuging of particles in $CHCl_3$ / MeOH.

3. Synthesis of polymer with azide functional group **Pol-1**

To the solution of 2 g poly(isobutylene-*alt*-maleic anhydride) (M_w = 6000 g/mol) in 400 mL of dry THF, *n*-octylamine (0.80 mL, 4.2 mmol) and DIPEA (1.7 mL) were added and the mixture was stirred for 1h in 50°C. Subsequently, the temperature was lowered to room temperature and 1.7 mL of 11-azido-3,6,9-trioxaundecan-1-amine (0.9 mL, 4.8 mmol) was added with stirring continued for the next 15 h. After THF evaporation the material was suspended in water with small excess of NaOH with respect to the carboxylic groups in the polymer backbone. After evaporation of water and DIPEA, the remaining residue was dissolved in water and dialyzed against 0.01M solution of NaOH and pure water for a few days (membrane cut-off 6000D) to obtain polymer in a sodium salt form. Yield: 3.1 g.

NMR: δ_H(400 MHz; D_2O): 7.21 (11 H, br), 3.77 – 3.35 (198 H, m), 3.35 – 1.61 (223 H, m), 1.47 (68 H, br), 1.22 (186 H, br), 1.12 – 0.65 (274 H, m). Composition – percentage of functional groups: carboxylic 64%, *n*-octylamide 20%, azide 16% (l = 16, m = 10, n = 12); To characterize the polymer, NMR spectra were compared with the spectra of poly(isobutylene-*alt*-maleic anhydride) with the anhydride groups opened to carboxylates by treatment with stoichiometric amounts of NaOH. NMR spectrum integration was normalized to the backbone 6000 g/mol (n = 38). The signals from the PEG linker visible in the 3.77 – 3.35 ppm range were used to calculate the number of azide groups, the alkyl signal CH_2 at 1.22 ppm was used to find the number of *n*-octylamide chains. The CH_3 terminal signals at 0.65 - 1.12 ppm served as a backbone reference. The average number molar mass of the polymer, calculated based on the NMR was 12 000 g/mol.

4. Synthesis of polymer with acetylene functional group **Pol-2**

To the solution of 2 g poly(isobutylene-*alt*-maleic anhydride) (M_w = 6000 g/mol) in 400 mL of dry THF, *n*-octylamine (0.80 mL, 4.8 mmol) and DIPEA (1.7 mL) were added and the mixture was stirred for 1h in 50°C. Subsequently, the temperature was lowered to room temperature and propalgyl amine (0.23 mL, 4.8 mmol) was added with stirring continued for the next 15h. After THF evaporation the material was suspended in water with small excess of NaOH with respect to the carboxylic groups in the polymer backbone. Subsequently, water and DIPEA were evaporated and the remaining residue was dissolved in water and dialyzed against 0.01M NaOH and later pure water for a few days (membrane cut-off 6000D) to obtain polymer in a sodium salt form. Yield: 2.0 g. NMR: δ_H(400 MHz; D_2O): 7.21 (9 H, br), 3.93 – 3.72 (33 H, m), 3.20 – 1.63 (206 H, m), 1.46 (70 H, br), 1.22 (215 H, br), 1.08 – 0.65 (279 H, m). Composition – percentage of functional groups: carboxylic 56%, *n*-octylamide 22%, acetylene 22% (l = 17, m = 5, n = 16); To characterize the polymer, NMR spectra were compared with the spectra of poly(isobutylene-*alt*-maleic anhydride) with the anhydride groups opened to carboxylates by treatment with stoichiometric amounts of NaOH. NMR spectrum integration was normalized to the backbone 6000 g/mol (n = 38). Signals from CH_2 propalgyl linker visible in the 3.93 – 3.72 ppm range were used to calculate the number of acetylene groups, the alkyl signal CH_2 at 1.22 ppm was used to find the number of *n*-octylamide chains. The CH_3 terminal signals at 1.08 – 0.65 ppm served as a backbone reference. The average number molar mass of polymer, calculated based on the NMR was 10 000 g/mol.

5. Transfer of hydrophobic NPs into water using amphiphilic polymers, synthesis of **QD-Pol-1**, **QD-Pol-2**, **MNP-Pol-1** and **MNP-Pol-2**.

10 mg of purified QDs or NPs were suspended in THF (20 mL) and 20 mg of **Pol-1** or **Pol-2** in 10 mL water solution was added. The mixture was concentrated with a rotary evaporator to 5 mL volume by removing THF and water. Water evaporation was performed for a long period of time with a flask not immersed in a water bath, allowing for the evaporating solvents to cool the solution to below 10°C. The water suspension obtained was filtered trough a 0.22 μm MILEX PES membrane filter and the filter was washed with 2 mL of pure water resulting in a clear aqueous solution of the QDs/polymer micellar assemblies. The solution can be concentrated with a rotary evaporator to achieve appropriate volume or concentration. A typical solution used was 5 mL in volume and was carrying 10 mg of NPs and 20 mg of appropriate polymer (NPs concentration 2 mg / mL).

6. Synthesis of covalently assembled NPs structures by “click”.

In a typical experiment 1 mL of water solution containing 2 mg of NPs suspended with 4 mg **Pol-1** (**QD-Pol-1** or **MNP-Pol-1**) was mixed with 1 mL of solution containing 2 mg of NPs suspended with 4 mg **Pol-2** (**QD-Pol-2** or **MNP-Pol-2**). 60 μL of 7mM solution of Na ascorbate was added followed by 20 μL of 2mM solution of Cu(I) acetate. The mixture was left overnight in 5°C and as a result covalent assemblies were formed. In case of QDs to restore the luminescence partly quenched due to the catalyst influence, 50 μL of 1M NaOH was added to the solution and the mixture was left at r.t. for 1 h. Subsequently, high speed centrifuging was carried out (30000 g) for 2h. The supernatant was discarded and the precipitate was resuspended in pure water resulting in a bright, fluorescent and transparent solution. Luminescent properties of QDs before and after "click" are presented at Fig. 2.

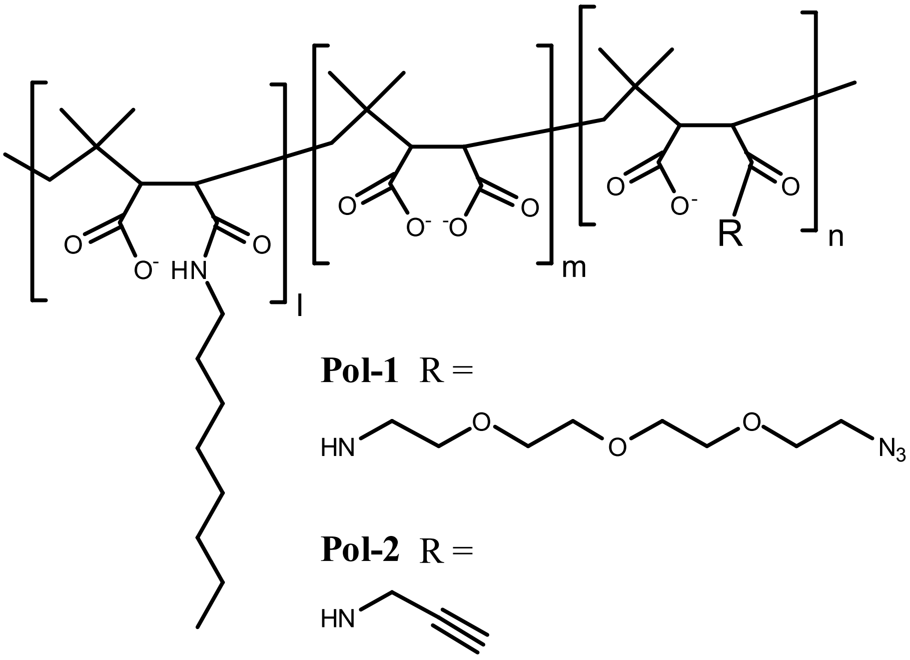

Fig. 1. Chemical structure of polymers **Pol-1** and **Pol-2** used for transferring hydrophobic nanoparticles into water. For "click" reactions, **Pol-1** carries a side PEG chain terminated with azide groups, while **Pol-2** carries propargyl side groups.

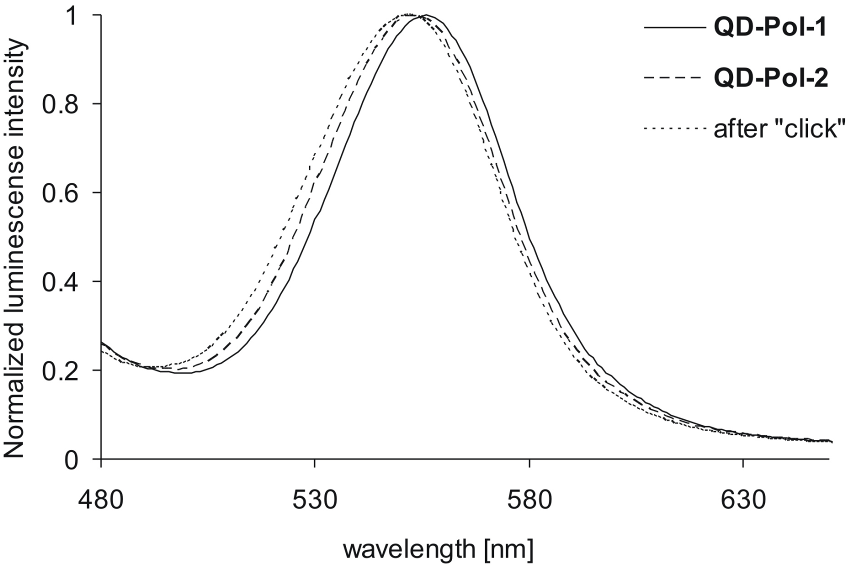

Fig. 2. Emission spectra of aqueous solutions of CdSe/ZnS QDs with azide (QD-Pol-1) and acetylene (QD-Pol-2) functionalized amphiphilic polymeric coatings before the reaction, and after the "click" reaction in water.

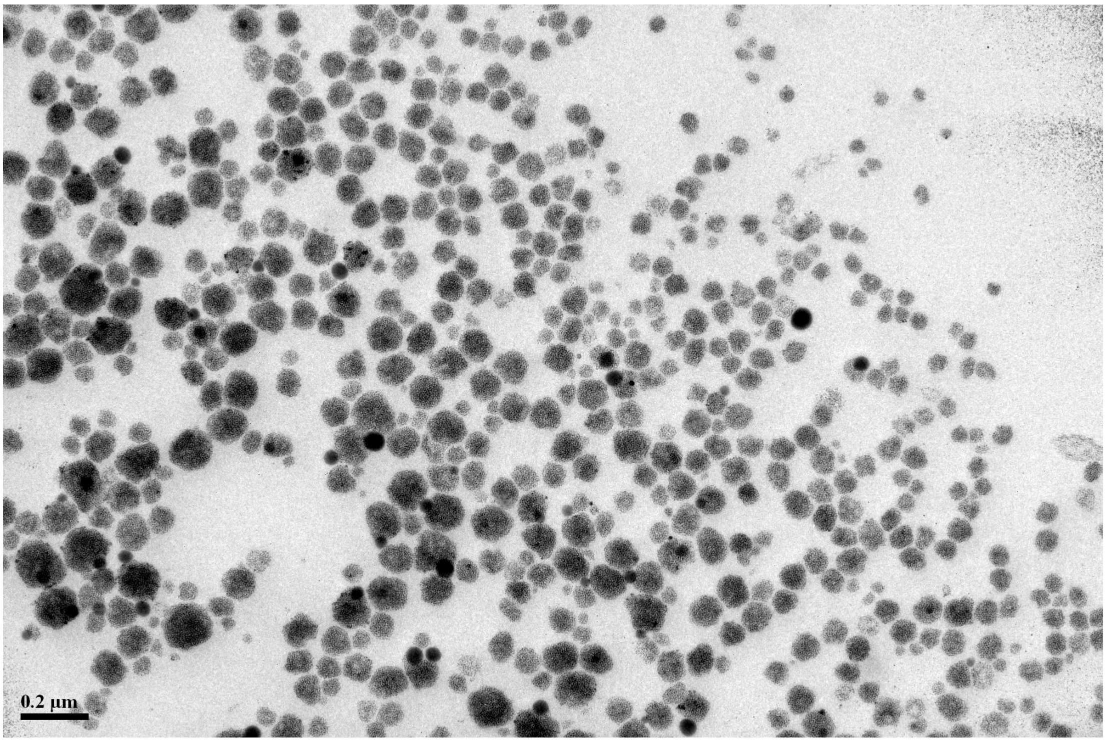

Fig. 3. QDs covalent assemblies prepared by reaction of **QD-Pol-1** with **QD-Pol-2**. Representative image used for the determination of the size distribution.

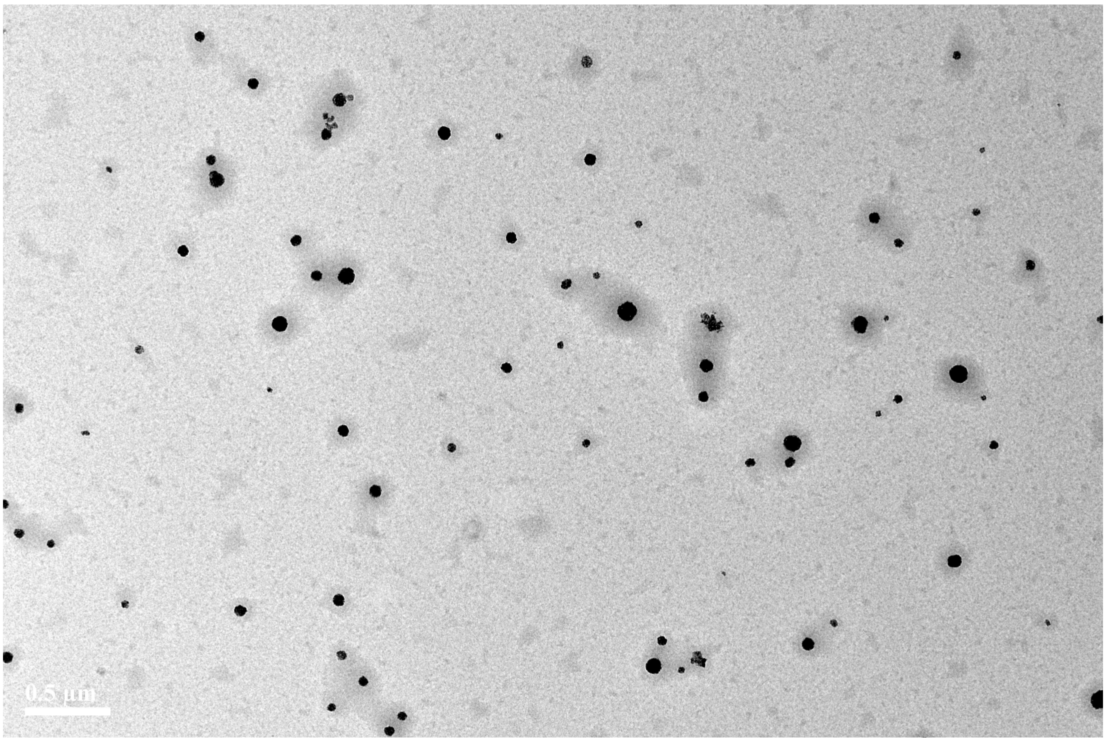

Fig. 4. MNPss covalent assemblies prepared by reaction of **MNP-Pol-1** with **MNP-Pol-2**. Representative image used for the determination of the size distribution.

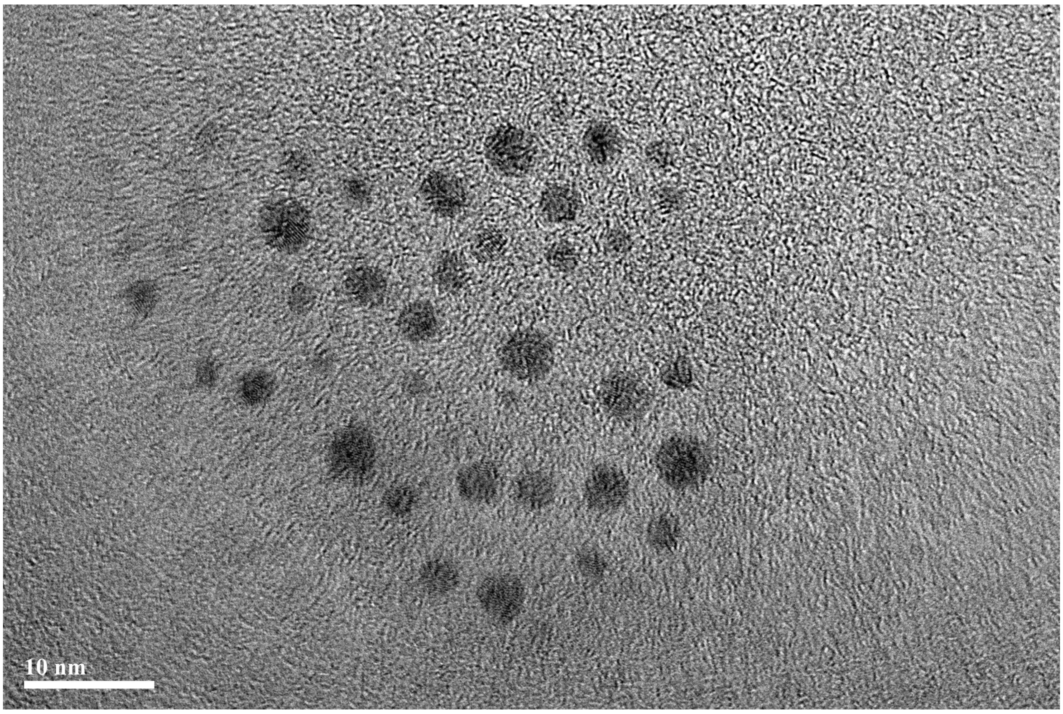

Fig. 5. TEM image of **QD-Pol-1** mixed with **QD-Pol-2** without the Cu (I) catalyst. The distance between the QDs is much larger indicating that no reaction has occurred.

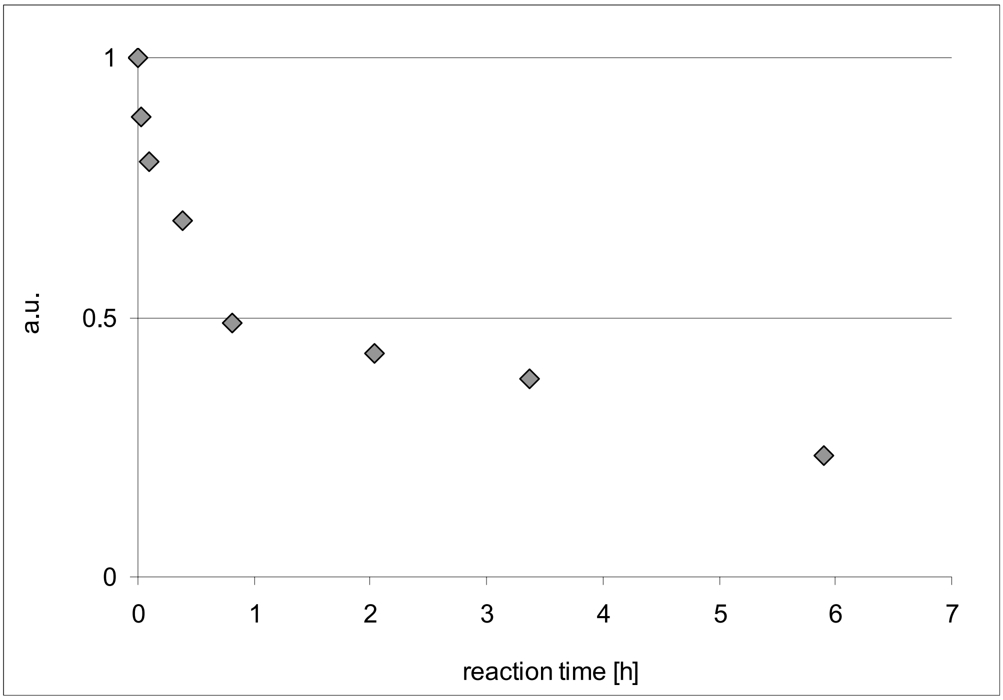

Fig. 6. Decrease of ^{1}H NMR acetylene group signal height visible at 3.97 - 3.88 ppm indicates “click” reaction progress. A model reaction carried between micelles of **Pol-1** and **Pol-2**.

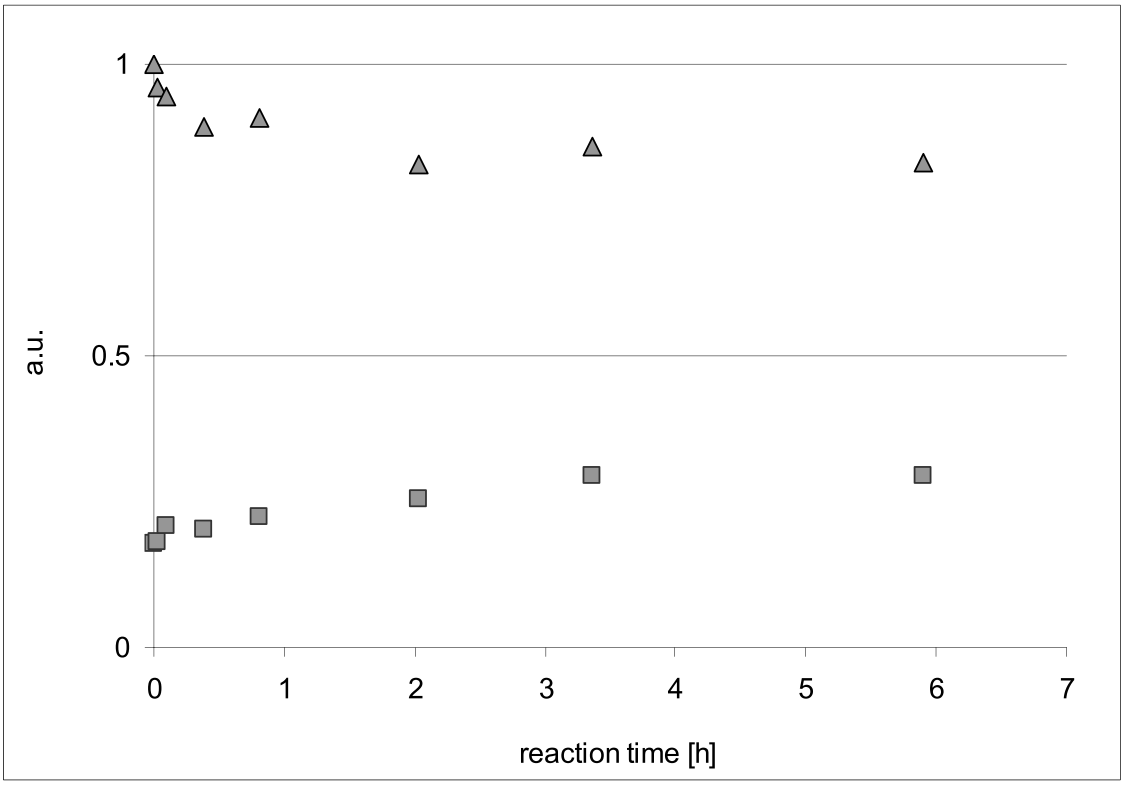

Fig. 7. Decrease in integrated intensity of ^{1}H NMR PEG linker sharp signal visible at 3.75 ppm (triangles) and increase in integrated intensity of broad PEG linker signal visible at 3.71 - 3.57 ppm (squares) indicates changes in a mobility of PEG linker and the progress of the reaction. The model reaction was carried out using micelles of **Pol-1** and **Pol-2**.

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(1) Jańczewski, D.; Tomczak, N.; Khin, Y. W.; Han, M. Y.; Vancso, G. J. *Eur. Polym. J.* **2009**, *45*, 3-9.

(2) (a) Park, J.; An, K. J.; Hwang, Y. S.; Park, J. G.; Noh, H. J.; Kim, J. Y.; Park, J. H.; Hwang, N. M.; Hyeon, T. *Nat. Mater.* **2004**, *3*, 891-895. (b) Jana, N. R.; Chen, Y. F.; Peng, X. G. *Chem. Mater.* **2004**, *16*, 3931-3935.