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Folding Processes

Single-Chain Polymeric Nanoparticles by Stepwise Folding**

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In an effort to mimic the folding of natural polymers,[1–3] foldamers, which are oligomers that adopt well-defined secondary structures, have been studied in great detail.[4,5] Dendrimers have also been developed that adopt folded conformations in solution.[6–9] In all of these systems, the conformational synchronization that occurs over extended distances amplifies small energy differences, leading to highly stable, folded macromolecules.[10] The benefits of introducing synchronized conformational motions into macromolecules have been clearly demonstrated by Parquette and co-workers. They showed high enantioselectivity in a catalytic hydrogenation reaction, which resulted from chiral information at the periphery of dendrimers relayed to an achiral catalyst complex in the core.[11]

We are intrigued by the many possibilities of well-defined nanosized objects formed by the folding of a single-chain polymer. These polymers are functionalized in the side chains with recognition units and can, after folding into a well-defined object, express specific functions, such as sensing or catalysis. In that sense, they partially mimic the properties of biomacromolecules, but can be made using the immense number of monomers and polymerization techniques available. Initially, these single-chain polymeric nanoparticles have been obtained by covalent intramolecular cross-linking.[12–20] Using this approach, a variety of nanosized macromolecules in the range of 5–20 nm became available, which showed great potential in drug-delivery systems,[21] (multistep) catalytic conversions,[22–25] and nanotechnology.[14–19] Noncovalent interactions, such as hydrogen bonds, π–π interactions, and hydrophobic interactions, also generate these particles, but now with the ability to respond to external stimuli. This approach has been successfully introduced by our[26,27] and other[28,29] groups using hydrogen-bonding motifs, with the ureidopyrimidinone group[30–33] being a well-known example. The characterization of the nanoparticles has been restricted to size-exclusion chromatography (SEC) and atomic force microscopy (AFM). Both techniques showed a significant collapse of the polymer chains, but no evidence for true folding has been observed to date.

Herein we show that single-chain polymeric nanoparticles (SCPNs) comprising an internal helical architecture can be obtained by noncovalent interactions and that the folding process can be followed by circular dichroism (CD) spectroscopy. Our recognition unit of choice is the chiral benzene-1,3,5-tricarboxamide (BTA) moiety, which we have studied in great detail using a combination of ultraviolet (UV) and CD spectroscopy and which self-assembles into helical stacks stabilized by threefold intermolecular hydrogen bonding.[34–38] The BTAs self-assemble in a cooperative fashion and follow a nucleation–elongation growth mechanism. The transition from the molecularly dissolved state to the aggregated state, indicated by the temperature of elongation T_e, is abrupt and highly concentration-dependent.[36] We anticipated that the cooperative self-assembly of dangling BTAs as side chains would supply us with detailed information on the folding behavior and that the inner structure of the polymeric nanoparticles would be revealed by CD spectroscopy. To follow the folding process, we introduced a photochemical switch to turn on the secondary interactions. The photolabile *o*-nitrobenzyl protecting group is used to avoid solubility issues and allows controlled BTA self-assembly.[39,40] The design of the system and the polymers we studied in this investigation are given in Scheme 1.

For the synthesis of **1**, we used atom-transfer radical polymerization (ATRP) with activators regenerated by electron transfer (ARGET) as the polymerization technique,[41] followed by post-modification using alkyne–azide coupling. Silyl-protected propargyl methacrylate and isobornyl methacrylate were polymerized in a ratio of 80:20, employing benzyl bromoisobutyrate as the initiator and CuBr/TPMA/$Sn(EH)_2$ as the catalyst (see the Supporting Information for details).[41,42] Isobornyl methacrylate was selected to enhance the solubility of the final polymer in apolar solvents.[43] Silyl protection was necessary to prevent interaction of the alkyne function with copper.[44] After precipitation, the number-average molecular weight (M_n) of the polymer was 32 kg mol^{-1}, as determined by ^{1}H NMR spectroscopy, corresponding to an average degree of polymerization of 180. SEC analysis in THF showed a M_n of 22 kg mol^{-1} and a polydispersity index (PDI) of 1.63 (polystyrene standards). The incorporation of propargyl groups was 25%, which was close to the feed ratio of 20%. The deprotection of the silyl group was achieved quantitatively. The enantiomerically pure, azide-functionalized "caged" BTA unit was synthesized in five steps and was fully characterized (see the Supporting Information). The protected BTA azide was coupled to the alkyne-functionalized polymer using Cu^I-catalyzed cycloaddition to afford polymer **1** (Scheme 1, see the Supporting Information for details).[45,46] We functionalized half of the

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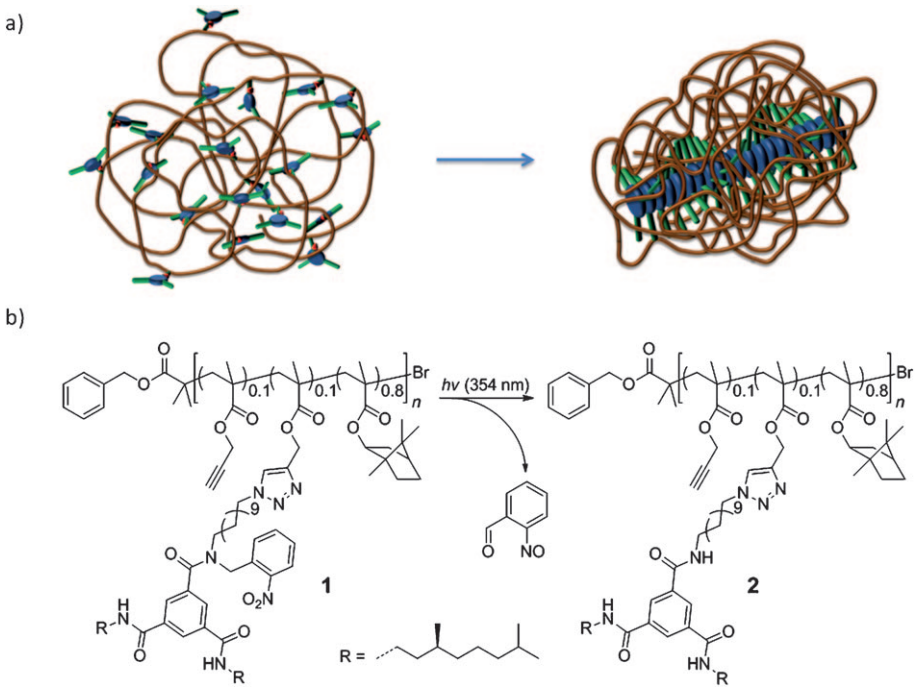


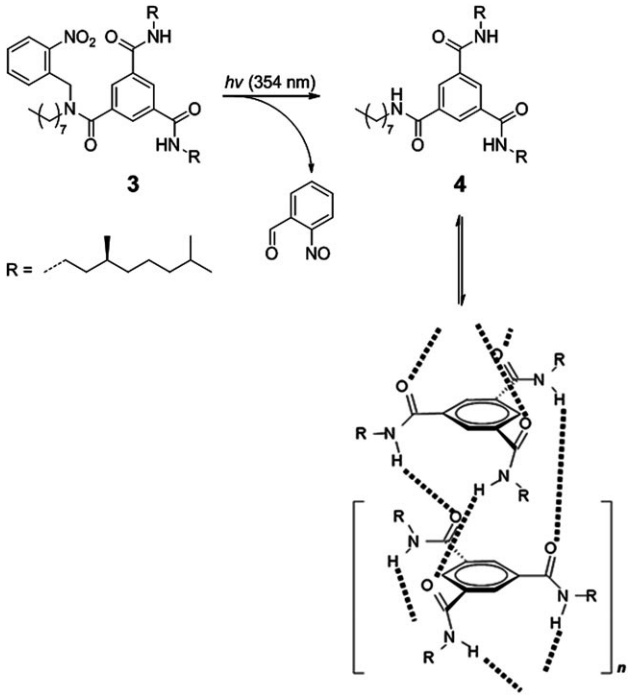
Scheme 1. a) Representation of a random-coil polymer that folds into an ordered chiral single-chain polymeric nanoparticle. b) Chemical structures of the polymers that can be triggered to fold. Upon irradiation of **1**, the *o*-nitrobenzyl group is cleaved to afford **2**, allowing self-assembly under selected conditions.

available alkyne groups with the BTA moiety, yielding a polymer containing around 20 BTA molecules per chain. According to the ^{1}H NMR spectrum, M_n was estimated to be 47 kg mol^{-1}, while SEC analysis in chloroform revealed a M_n of 26 kg mol^{-1} and a PDI of 1.7 (polystyrene standards).

To confirm the feasibility of our approach to "cage" the BTA moieties by one UV labile *o*-nitrobenzyl group, we first investigated the self-assembly of model compounds **3** and **4** (Scheme 2). Irradiation of **3** in [D$_4$]MeOH with UV light (λ = 354 nm, T = 20 °C, c = 5 mg mL^{-1}) in a photoreactor resulted in full conversion of **3** into **4** within two hours of irradiation, as determined by ^{1}H NMR (Supporting Information, Figure S1). Irradiation did not result in degradation of the BTA motif. Both **3** and **4** are molecularly dissolved in [D$_4$]MeOH, preventing the formation of helical supramolecular polymers.[47] Next, we deprotected **3** in methylcyclohexane (MCH), a solvent in which **3** should be molecularly dissolved and **4** self-assembles into supramolecular polymers with preferred *M* helicity.[35,36] Indeed, CD spectroscopy of **3** in MCH ($c = 1.2 \times 10^{-5}$ M) showed no Cotton effect. A Cotton effect arose upon irradiating the solution, which showed saturation after about 60 min of irradiation (Figure 1). The magnitude (−16 mdeg at λ = 223 nm), sign, and shape of the CD spectrum is similar to those found in other chiral BTAs dissolved in MCH at a similar concentration and temperature.[47] This result indicates that *o*-nitrosobenzaldehyde, which is released during the reaction,[39] does not interfere with the self-assembly process and that supramolecular polymers with a preferred helicity are formed upon deprotection.

Next, the feasibility to fully deprotect polymer **1** to polymer **2** (Scheme 1) was evaluated. Irradiation of polymer **1** (c = 3 mg mL^{-1} in [D$_2$]tetrachloroethane) with UV light (λ = 354 nm) resulted in full photolytic cleavage of the *o*-nitrobenzyl group within 20 min, as seen by the disappearance of the benzylic signal in ^{1}H NMR spectrum (Supporting Information, Figure S2).[48] The infrared spectrum of polymer **2** in the solid state after workup (Supporting Information, Figure S3) showed vibrations at positions typical for threefold helical intermolecular hydrogen bonding in BTAs.[49]

In dilute solutions, the nature of the solvent is of high importance to induce self-assembly of the BTA moieties in polymer **2**. While the helical self-assembly of model BTA **4** was demonstrated in MCH, polymer **1** and **2** were found to be insoluble in this solvent. To reconcile the solvent preferences of the polymethacrylate backbone and BTA self-assembly, we decided to apply solvent mixtures, more specifically a mixture of 1,2-dichloroethane (DCE) and MCH for the deprotection



Scheme 2. Design of a "caged" BTA. Upon irradiation of **3**, the *o*-nitrobenzyl group is cleaved to afford **4**, allowing self-assembly under selected conditions.

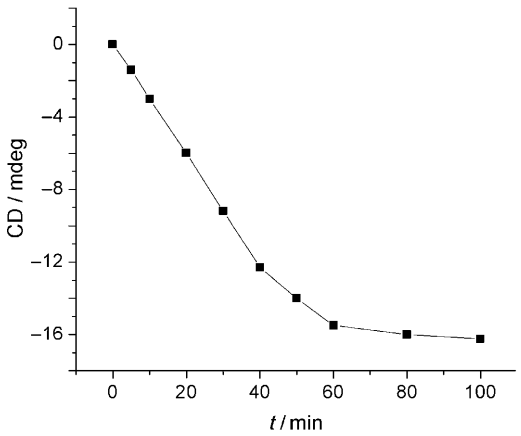


Figure 1. Development of the Cotton effect in the conversion of compound **3** into **4** as measured at 223 nm as a function of irradiation time ($T = 20\,^{\circ}C$, $c = 1.2 \times 10^{-5}$ M in MCH).

reaction of **1**. Importantly, the CD spectra were measured after heating the sample to 80 °C and slow cooling to room temperature. A pronounced negative Cotton effect in deprotected polymer **2** was observed with a maximum at $\lambda = 225$ nm (Supporting Information, Figure S4), typical of helical hydrogen bonded BTA aggregates, when measured in a mixture of DCE/MCH 65:35 vol% (BTA content $c_{BTA} = 7.5 \times 10^{-5}$ mol L^{-1}, $T = 20\,^{\circ}C$).[51] A systematic investigation of the effect of solvent composition on the absolute CD intensity resulted in the data summarized in Figure 2. We used the concentration-independent anisotropy value (g_{value}) of the Cotton effect at $\lambda = 225$ nm. Interestingly, in pure DCE, polymer **2** already showed a negative Cotton effect, which increased upon increasing the MCH concentration. A maximum value of the Cotton effect was found in a 70:30 DCE/MCH mixture. Increasing the amount of MCH results in a decrease of the CD intensity. Therefore, the solvent (combination) has to balance the backbone solubility of both the protected and deprotected polymer and the self-assembly capability of the chiral BTA unit.[49,51]

Having established an optimal solvent mixture, the deprotection reaction of **1** ($c_{BTA} = 7.5 \times 10^{-5}$ mol L^{-1}, DCE/MCH 70:30 vol%) was followed as a function of time using UV spectroscopy (Supporting Information, Figure S5). Already after 10 min of irradiation, the deprotection reaction was complete, as evidenced by saturation of the UV absorption signal. CD analysis of the sample that was deprotected at room temperature for 20 min showed a very small Cotton effect of −2 mdeg at $\lambda = 225$ nm (Figure 3a,

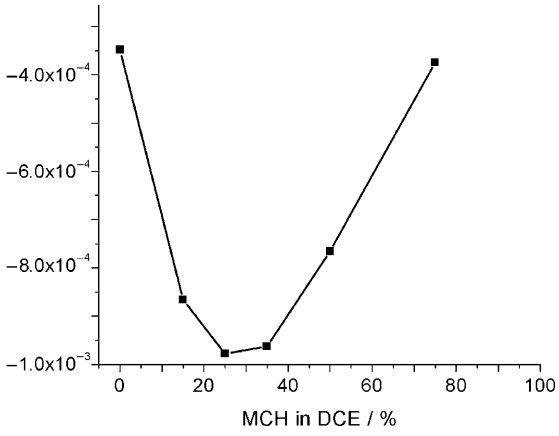


Figure 2. Dependence of the g_{value} of **2** on the solvent composition ($c_{BTA} = 7.5 \times 10^{-5}$ M, $\lambda = 225$ nm, $T = 20\,^{\circ}C$).

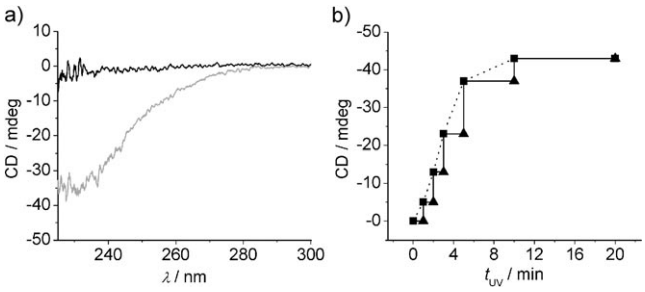


Figure 3. a) Fully deprotected polymer **2** before (black curve) and after heating to 80 °C and cooling to 20 °C (gray curve). $c_{BTA} = 7.5 \times 10^{-5}$ M, DCE/MCH 70:30 vol%, $T = 20\,^{\circ}C$, 20 min UV irradiation. b) Evolution of the Cotton effect of **1** as a function of photoirradiation time t_{UV} with intermittent heating to 80 °C and cooling to 20 °C. Heating and cooling step: vertical lines, deprotection step: horizontal lines; •••••■••••• heated sample, ▲ deprotected sample. $c_{BTA} = 7.5 \times 10^{-5}$ M, DCE/MCH 70:30 vol%, $\lambda = 225$ nm, $T = 20\,^{\circ}C$.

black line). This value is significantly lower than the maximum value of −40 mdeg ($g_{value} = -9.8 \times 10^{-4}$) found for polymer **2** in the same solvent mixture (Figure 2). The latter result was obtained after heating and cooling the sample. Gratifyingly, heating the solution to 80 °C followed by cooling to 20 °C at a rate of 5 K min^{-1} resulted in a pronounced Cotton effect of −37 mdeg at $\lambda = 225$ nm (Figure 3a, gray line). This interesting behavior was evaluated in further detail by performing CD measurements on a solution of **1** ($c_{BTA} = 7.5 \times 10^{-5}$ mol L^{-1}, DCE/MCH 70:30 vol%) after irradiating the solution for short time intervals. CD spectra were measured immediately after UV irradiation and also after heating to 80 °C and cooling to 20 °C (Figure 3b). For each individual deprotection step, it was observed that the Cotton effects before and after irradiation were of similar size. Heating to 80 °C resulted in complete disappearance of the Cotton effect. However, upon cooling, the Cotton effect increased proportionally to the amount of deprotected BTAs.[43] Interestingly, the CD cooling curves of **1** after 2 and 5 min of irradiation corresponding to 60 and 95% of deprotected BTAs, respectively, showed an onset of aggregation that shifted from approximately 55 °C to 75 °C (Figure 4a).

To experimentally test our concept of the single-chain polymer nanoparticles by stepwise folding, it is important to exclude intermolecular self-assembly. Therefore, we compared the self-assembly and the ability to transfer chiral information by the "sergeants and soldiers" principle of BTAs present in polymer **2**, with the "free" C_3-symmetrical BTAs that have been studied before. Where the latter showed a strong concentration dependent temperature of elongation (Supporting Information, Figure S6), polymer **2** showed a

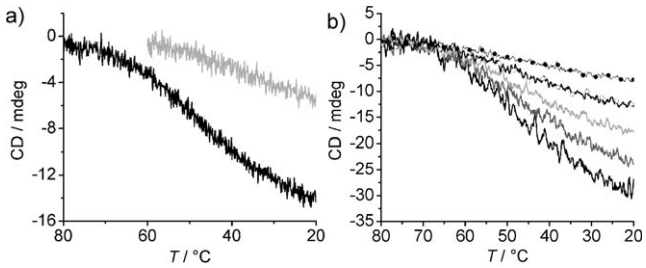


Figure 4. a) CD spectra of **2** versus temperature after 2 (gray curve) and 5 min of irradiation (black curve) $c_{BTA} = 3.25 \times 10^{-5}$ M, DCE/MCH 70:30 vol%, $\lambda = 225$ nm. b) CD spectra of **2** versus temperature at different concentrations. From top to bottom: 1.5, 3.0, 4.5, 6.0, 7.5×10^{-5} M. DCE/MCH 70:30 vol%, $\lambda = 225$ nm.

concentration-independent onset of aggregation (Figure 4b), and the increase of the CD intensity was a more gradual process. Normalizing the curves in Figure 4b for concentration by plotting the g_{value} as a function of temperature (Supporting Information, Figure S7) reveals that all cooling curves can be superimposed. The onset of aggregation was studied by changing the BTA incorporation (Figure 4a and Supporting Information, Figure S8). As the onset strongly depends on the local BTA concentration and not on the overall concentration, the aggregation process is predominantly a single-chain folding process. More evidence that single-chain folding is operative was shown by the absence of chirality transfer from chiral to achiral BTA polymers, indicating the absence of intermolecular self-assembly (Supporting Information, Figure S9).

Finally, we investigated the influence of additives on the stability of the folded structures. We prepared a solution of **2** ($c_{BTA} = 8.5 \times 10^{-5}$ mol L^{-1}, DCE/MCH 70:30 vol%) and added various amounts of hexafluoroisopropanol (HFIP) (Figure 5). The Cotton effect gradually disappeared upon the addition of HFIP. After the addition of 1000 equivalents, the Cotton effect was completely absent. In contrast, the addition of 15 equivalents of HFIP is sufficient to fully disrupt aggregation of the "free" C_3-symmetrical BTAs (Supporting Information, Figure S10). The folding of polymer **2** was reversible as the Cotton effect was almost completely recovered after the addition of > 1000 equivalents of quinuclidine (Supporting Information, Figure S11).

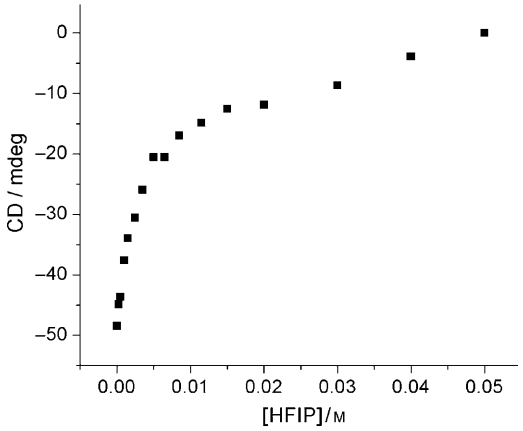


Figure 5. Cotton effect of **4** versus HFIP concentration c_{BTA} 8.5×10^{-5} M, $\lambda = 225$ nm, $T = 20$ °C.

The results presented herein introduce single-chain polymeric nanoparticles by stepwise folding, which is controlled by side-chain aggregation. By careful selection of the solvent (a mixture of DCE and MCH), we are able to balance the solubility of the main chain with the hydrogen bonding required for folding of polymer **2**. Folding is achieved by controlled cooling of the solution of polymer **2** to arrive at the thermodynamically stable state. When polymer **2** is formed from **1** by irradiation at room temperature, the system resides in a kinetically trapped or collapsed state; that is, a unimolecular glass or "molten globule" state. The more thermodynamically favorable folded state is achieved by an additional heating/cooling step. Temperature plays a decisive role: At high temperatures, BTAs are not aggregated, the polymer is unfolded, and at the same time the polymer backbone is flexible. Slowly reducing the temperature induces BTA aggregation because there is sufficient mobility within the polymer to adopt an optimal conformation for self-assembly to occur.

We anticipate that the self-assembly occurs within a single chain, as the onset of self-assembly is determined by the local concentration of dangling BTAs on the chain and is independent of the overall concentration of the polymer. Moreover, unfolding experiments with the hydrogen-bond breaking solvent HFIP shows a significant increase in stability of the BTA-helix in the single-chain polymer nanoparticle compared to the same helix of unbound or "free" BTAs. The observed aggregation behavior in the SCPNs described herein partly mimics the folding of proteins, and has also been described in synthetic systems, such as foldamers and DNA origami structures.[4,52,53] The latter also require small temperature jumps to arrive at full self-assembly.

In conclusion, we have been able to follow and control the folding of polymers into an ordered chiral single-chain polymer nanoparticle. The high stability and chiral conformation of the folded particles make them excellent candidates for compartmentalized catalytic systems.[54] Our current work is focused on the folding kinetics and on the development of water-based systems with catalytically active groups embedded in the hydrophobic compartment.

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