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Supramolecular copolymerization as a strategy to control the stability of self-assembled nanofibers

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ABSTRACT: One of the major challenges to be addressed in supramolecular polymerizations is the ability to control the stability of the polymers formed, i.e. to control the rate of monomer exchange in the equilibrium between monomer and polymer. We here show that the exchange dynamics of supramolecular polymers based on the benzene-1,3,5-tricarboxamide (BTA) motif can be regulated by copolymerizing molecules with dendronized (dBTA) and linear (nBTA) ethylene glycol-based water-soluble side chains. Whereas nBTAs form long nanofibers in water, dBTAs do not polymerize and form small spherical aggregates only. In contrast, the copolymerization of the two BTAs results in long nanofibers up to an equimolar ratio of both components. Intriguingly, the exchange dynamics of both the BTA monomers in the copolymer are significantly slowed down in the mixed systems, leading to a more stable copolymer, while the morphology and spectroscopic signature of the copolymers are identical to that of the homopolymer of nBTA. In many ways, this copolymerization represents the supramolecular counterpart of the copolymerization of styrene and maleic anhydride.

Slow exchange
Fast exchange
+ Tunable dynamic behaviour
Multicomponent self-assembly is a unique strategy in the development of functional supramolecular systems, as it provides ways to tune the physical behavior of these systems.\textsuperscript{[1–6]} Introducing such a concept in the area of supramolecular polymers will expand their potential in applications and demonstrate the advantage of a modular, non-covalent synthetic approach.\textsuperscript{[7–10]} In the last two decades, these supramolecular polymers have been extensively studied to understand the mechanisms behind their polymerization process,\textsuperscript{[11]} with the aim to develop new motifs that could result in polymers with superior properties. Also, significant progress has been made in the area of supramolecular copolymerizations to control the microstructure of the copolymers formed.\textsuperscript{[12–15]} A major challenge to be addressed in these supramolecular copolymers is the ability to control their stability and/or dynamic behavior in the equilibrium exchange dynamics between monomers and polymers.\textsuperscript{[16–20]} In addition, it will unveil the similarities and differences between supramolecular polymers and their covalent macromolecular counterparts.

In classical covalent copolymer formation, in which the copolymerization of different monomers results in random, block, gradient or precision copolymers, the copolymer properties can be accurately tuned by tuning the reactivity ratios of the monomers in combination with advanced polymerization methods.\textsuperscript{[21–23]} An intriguing example is the copolymerization of styrene with maleic anhydride. The latter does not polymerize, but when copolymerized with styrene, a very stable – high $T_g$ – copolymer is obtained. We envisage that the supramolecular counterpart, i.e. copolymerizing supramolecular monomers with distinct differences in their dynamic behavior and steric demands could provide a handle to control the overall exchange dynamics of the supramolecular copolymer as well, and hereby their stability and applicability.

Herein, we report the supramolecular copolymerization of two benzene-1,3,5-tricarboxamide-based molecules (\textbf{nBTA} and \textbf{dBTA}, Scheme 1), of which \textbf{dBTA} is unable to polymerize on its own. We apply hydrogen-deuterium exchange mass spectrometry (HDX-MS) to monitor the exchange dynamics of supramolecular polymers and copolymers, as it has recently been proved to be a powerful label-free method to elucidate dynamic processes in supramolecular polymers.\textsuperscript{[24]} Remarkably, the exchange dynamics of both monomers are slowed down upon copolymerization, a results that is seconded by molecular dynamics simulations.
All BTAs were synthesized according to standard procedures.\textsuperscript{[25–27]} The synthetic details and molecular characterization of newly synthesized dBTA, d1BTA and d2BTA are given in the Supporting Information (Figures S1-9). The supramolecular polymerization of nBTA in water has been well documented and is typically studied by spectroscopic (UV-vis and FT-IR), microscopic (cryogenic transmission electron microscopy, cryoTEM) and scattering techniques (dynamic light scattering, DLS, and small angle X-ray scattering, SAXS) in combination with molecular dynamics simulation studies and HDX-MS exchange dynamics.\textsuperscript{[24,28–31]} To ensure that the supramolecular polymers formed by BTAs and their mixtures were in a thermodynamically stable state, all aqueous solutions were prepared by adding the appropriate amount of milliQ water to the desired BTA or mixtures of BTAs weighted into a sample vial. This mixture was heated at 75°C for 1 h and vortexed for after 30 min. The solutions were then cooled to room temperature.

The formation of supramolecular polymers stabilized by intermolecular hydrogen bonding of nBTA in water is characterized by two absorption maxima at 211 nm and 228 nm in the UV spectrum.\textsuperscript{[30]} In contrast, when molecularly dissolved, nBTA shows one maximum at 204 nm.\textsuperscript{[27]} The presence of intermolecular hydrogen bonds between the amides at the BTA core in water is evidenced by a shift in the amide I band from 1648 cm\textsuperscript{−1} in MeOH-d4 to 1635 cm\textsuperscript{−1} in MeOH-d4 to 1635 cm\textsuperscript{−1} in
The dendritic dBTA displays a different absorption spectrum with a maximum at 197 nm (Figure 1A). At higher concentrations, the FT-IR spectrum of dBTA does resemble that of nBTA in water (Figure 1B). CryoTEM analysis of nBTA shows the formation of one-dimensional nanofibers (Figure S10). CryoTEM images of dBTA suggest the formation of small spherical aggregates (Figure S11), which was corroborated by DLS measurements showing particle sizes of around 7 nm (Figure S12). Thus, whereas nBTA homopolymerizes in water into long cylindrical aggregates, dBTA is unable to do so.

In a next step, the supramolecular copolymerization of nBTA and dBTA was investigated at different mixing ratios. UV-vis spectra of these mixtures resulted in absorption spectra similar to nBTA up to a mixing ratio of 1:1 (Figure 1A). Above 50 mol% of dBTA present in the mixtures, a UV spectrum different from the parent molecules was observed. This observation suggests that up to a 1:1 ratio, the organization of hydrogen bonds within the supramolecular copolymers of nBTA and dBTA is similar to that of pure nBTA. The morphology of the supramolecular copolymers measured through electron microscopy
experiments corroborated this; cryoTEM images indicated the formation of nanofibers similar to nBTA polymer for both 2:1 and 1:1 mixtures of nBTA and dBTA (Figure 1C-D). For 1:2 mixtures of nBTA and dBTA, nanofibers as well as small spherical aggregates were observed in the cryoTEM imaging (Figure S11).

Monomers d1BTA, with one dendritic wedge, and d2BTA with two dendritic wedges, are the perfect comparison to the copolymers with 2:1 and 1:2 ratio of nBTAs and dBTA. Whereas d1BTA showed a UV spectrum similar to nBTA, d2BTA displayed a similar UV profile as that of dBTA (Figure S13). The new analogs are not only similar in their spectroscopic signature to nBTA and dBTA, but their morphologies are also similar; d1BTA results in a 1D nanofiber and d2BTA gives smaller aggregates as evidenced by TEM measurements (Figure S14).

Next, we performed HDX-MS experiments to compare the exchange dynamics of both the individual aggregates with those of the copolymers. Hereto, 500 μM solutions of nBTA, dBTA, or mixtures thereof were prepared in water and 100 times diluted in D2O (Figure S15A). Upon dilution, the nature of the aggregates did not change (Figure S16). Figure S15B shows the ESI-MS spectrum of nBTA taken 1 h after dilution in D2O. Two populations in the isotope distributions can be distinguished, one corresponding to three times deuterated species (nBTA3D) and one corresponding to six times deuterated species (nBTA6D). In contrast, dilution of dBTA into D2O shows the presence of only one isotopic distribution that corresponds to a mass increase of 15 units (dBTA15D) immediately after diluting (Figure S15C). The significant differences in morphologies formed by nBTA and dBTA are therefore also reflected in the H/D exchange rates of the amide protons. HDX-MS experiments on a 2:1 mixture of nBTA and dBTA by the same protocol (Figure S15D) after 1 h showed, surprisingly, the presence of the isotopic mass distribution of dBTA12D (Figure S15C), indicative that the amide protons in dBTA are, at least to a certain degree, hydrophobically shielded from the surrounding water in the supramolecular copolymer.

More quantitative data were collected from kinetic measurements of the HDX-MS experiments (Figure 2A and 2B, 1:1 mixture, red data points). Immediately after dilution of the solutions in D2O, the percentages of both nBTA3D and dBTA12D are 100%, since all OH are instantaneously exchange to OD. When in time NH exchanges to ND, the percentage of nBTA3D and dBTA12D decreases. By quantifying the intensities of the dBTA12D and
nBTA3D isotope peaks as a function of time, we observe very fast decreases in the first hour which slow down significantly afterwards. Strikingly, we notice that the mass spectra of nBTA show that nBTA3D species are more abundant in the 1:1 mixture compared to pure nBTA (Figure 2A, black data points). This indicates that fewer amides are exposed to the surrounding water and as a result, the monomers are more stably incorporated into the supramolecular copolymers of the 1:1 mixture.

Figure 2. HDX-MS of nBTA, dBTA and their mixtures followed as a function of time in which the percentage of A) nBTA3D or B) dBTA12D is probed.

HDX-MS experiments were conducted also for higher and lower nBTA:dBTA ratios as a function of time (Figure 2A,B). At 2:1 and 1:1 nBTA:dBTA, the copolymers showed higher percentages of remaining nBTA3D compared to the homopolymer of nBTA, which indicates a higher shielding/ordering of the amides and therefore decrease in dynamics. At 1:2 nBTA:dBTA ratio, however, nBTA displayed a considerably faster decay at the early phase of the measurement, and slowed down dramatically after a few hours after which the percentage of nBTA3D remained almost constant. A similar trend was also observed for the decay of dBTA12D. A more quantitative approach to elucidate the dynamic features of the copolymers was performed by fitting the decay data by a tri-exponential fit (see Supporting Information for details). The fits clearly indicate three distinct decay processes that occur in these systems with different rate constants ($k_{\text{initial}}$, $k_{\text{fast}}$ and $k_{\text{slow}}$, Table S1, Figure S17). The results unambiguously show an increase in the fraction of the slowest exchange process with the dBTA:nBTA ratio up to 1:1, at the expense of the fastest one, indicating an increase in the stability of the copolymer.
nanofibers. Similar trends are also observed for dBTA data (Table S2, Figure S17). All the HDX experiments indicate the formation of an increase in the stability of the supramolecular copolymers compared to the homopolymers.

Also, the supramolecular polymers of the d1BTA and d2BTA-based aggregates were analyzed (Figure S18). Polymers of d1BTA show a similar decay curve to that obtained for the nBTA polymers. In contrast, H-D exchange for all OH and NH protons occurred instantaneously in case of d2BTA, which is identical to the results obtained for dBTA. Although the molecular composition of d1BTA is comparable to the composition of copolymers of nBTA and dBTA with 2:1 and 1:2 ratio, respectively, their dynamic behavior is not. The most pronounced difference is present between d2BTA – showing instantaneous H/D exchange – and the 2:1 dBTA/nBTA mixture in which complete exchange takes days. An interesting next question will address the possibility to capture any structural difference between an nBTA:dBTA copolymer and a nBTA polymer capable to explain such a dynamic difference at a molecular level.

To help answer this, we performed all-atom molecular dynamics (MD) simulations on a copolymer composed of a mixture of nBTA:dBTA in a 2:1 ratio (Figure 3A). For details of the method, the reader is referred to the Supporting Information. Our earlier simulation studies indicated the fibers are not perfect and extended in water but undergo folding due to hydrophobic effects to shield the interaction between the hydrophobic parts of the structure with water. Such folding produces a level of disorder \[^{17,29}\] and the radial distribution function (g(r)) between the monomer cores as a function of the inter-core distances provides an indication of the level of stacking order in the polymers.\[^{17,29,31,32}\] Higher g(c) peaks (relative probability for finding cores at stacking distance c = 3.4 Å from the neighbors) indicate higher persistence/regularity of the stacking. The g(r) of the cores was extracted from the equilibrated phase MD trajectory of the 2:1 nBTA:dBTA copolymer (last 100 ns of MD simulation) and compared to that obtained for nBTA (Figure 3B).\[^{29,31}\] A higher persistence/order (~15%) of the core-core stacking in the equilibrated copolymer compared to nBTA homopolymer is observed. Moreover, we calculated the g(r) between the nBTA cores and water (probability of finding water molecules as a function of the distance between the nBTA cores) from the MD simulations (Figure 3C). This data show that the level of hydration of the cores is reduced in the copolymer compared to the nBTA homopolymer (less contacts between cores and water molecules). In particular, the g(r) curves of Figure 3C show that the penetration of water molecules in the interior of the copolymer (g(r) at
distance <1 nm from the monomer cores) is decreased by ~20% compared to the nBTA fiber. This lower solvation fits well with the slower dynamics seen in the copolymers."

**Figure 3**: All-atom molecular dynamics (MD) simulations. (A) Initial configuration for the 2:1 nBTA:dBTA copolymer, which was then relaxed and equilibrated in water. (B) Core-core radial distribution functions ($g(r)$) for the nBTA homopolymer (black) and the 2:1 nBTA:dBTA copolymer (green). (C) Core-water radial distribution functions ($g(r)$) in the nBTA homopolymer (black) and 2:1 nBTA:dBTA copolymer (green). (D) Solvent accessible surface area (SASA) for the nBTA monomers in the nBTA homopolymer (black) and 2:1 nBTA:dBTA copolymer (green). The average for nBTA monomers in the assemblies is identified in red.

Next, we analyzed the solvent-accessible surface area (SASA) of the BTAs within the fibers. As seen in Figure 3D, the SASA data for the nBTA monomers in the copolymer (green) are uniformly distributed around the average (red line) different from those in the homopolymer (black), where at least one large defected domain is evidenced in our fiber model (i.e., in a fiber section of 48 monomers). This suggests that the incorporation of dBTA monomers, with their larger surface, into the copolymer improves the regularity in the monomer-monomer packing and reduces the probability of creating defects (exchange hot spots) along the fiber, which fits well with the reduced nBTA exchange dynamics seen experimentally in these copolymers.

In conclusion, the morphologies and dynamic behavior of supramolecular homo- and copolymers based on nBTA and dBTA were investigated. The supramolecular copolymerization
of nBTA and dBTA up to a 1:1 ratio afforded nanofibers with the same spectroscopic signature and morphology as that of nBTA, but significantly reduced exchange behavior of the monomers in the copolymer as compared to the individual aggregates. Molecular dynamics simulations performed on a 2:1 nBTA:dBTA copolymer revealed that the degree of order between monomers in the supramolecular polymer was increased when dBTA monomers were added to a nBTA homopolymer. This increase in order was corroborated by a decreased tendency to form discontinuity points/defects that may work as “exchange hot spots”, which likely decreases the exchange dynamics of nanofibers. As this study shows a behavior similar to what is reported on the covalent styrene - maleic anhydride co-polymerization, it illustrates remarkable and unforeseen similarities between macromolecules and supramolecular polymers.

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