The role of heterogeneous nucleation in the self-assembly of oligothiophenes†

Martin Wolffs,[a] Peter A. Korevaar,[a] Pascal Jonkheijm,[a] Oliver Henze,[b] W. James Feast,[b] Albertus P. H. J. Schenning*[a] and E. W. Meijer*[a]

The cooperative self-assembly of oligothiophenes can be characterized by heterogeneous nucleation caused by trace amounts of impurities leading to a manifold of supramolecular arrangements.

Self-assembly can be used as a bottom-up approach for the development of functional materials.[1] This makes the self-assembly behaviour of molecules a topic of increasing interest.[2] Recently, we have shown that the self-assembly of π-conjugated oligo(p-phenylene vinylene) and trimesic amide derivatives in alkane solvents is governed by an homogeneous nucleation-growth mechanism.[3–5] Here, we report that oligothiophenes also display the self-assembly characteristics of the nucleation-growth mechanism; however, in this case it is extremely difficult, if not impossible, to obtain an homogeneous nucleation. Trace amounts of impurities (less than 0.1 mol%) give rise to a nucleation that is mostly heterogeneous in nature. The molecules under investigation comprise a π-conjugated α,α'-linked sexi (**T6**) or septi (**T7**) thiophene derivative carrying enantiomerically pure chiral oligo(ethyleneoxide) side chains at both the α- and ω-positions (Scheme 1). Previously, we have shown that these molecules can self-assemble in alcoholic solutions into *inter alia*, spherical capsules constructed of a monolayer of the oligothiophenes.[6,7]

We first investigated the effect of impurities on the self-assembly of **T6**. After conventional purification, their sensitivity to air gave rise to impurities at levels less than 2 mol%. These impurities could only be detected by analytical GPC and
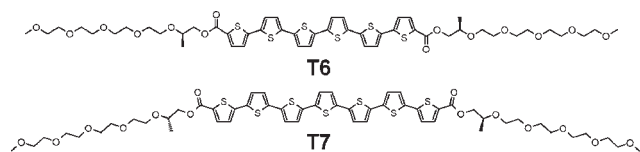
Scheme 1 The molecular structure of **T6** (*R,R* chiral) and **T7** (*S,S* chiral).

[a] *Laboratory of Macromolecular and Organic Chemistry, Eindhoven University of Technology, P.O. Box 513, MB 5600 Eindhoven, The Netherlands. E-mail: a.p.h.j.schenning@tue.nl; e.w.meijer@tue.nl; Fax: +31 40 2451036; Tel: +31 40 2473101*
[b] *The Chemistry Department, University of Durham, South Road, Durham, UK DH1 3LE*

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detailed MALDI-TOF analysis (batch 1, Fig. 1A). Our recent application of recycling gel permeation chromatography to these materials has enabled us to obtain samples of **T6** of an unprecedented purity for π-conjugated oligomers.[5]† Three samples with different purities were used in the work described here: sample A (99.6%), sample B (99.9%) and sample C (99.9 + %), their purities were determined by comparing the peak areas in analytical GPC assuming equal extinction coefficients at λ_{max} = 450 nm,‡ this being the absorption maximum of the sexithiophene core of **T6** in chloroform (Fig. 1A). For all samples MALDI-TOF analysis showed the molecular ion and the sodium adduct of **T6** (Fig. 1B), only in the case of sample A (99.6% pure) could an impurity with a mass corresponding to the loss of one ethylene oxide segment from **T6** be detected.[8]†

The self-assembly of these molecules was studied using temperature-dependent UV/Vis and CD spectroscopy. Solutions of samples of A, B, and C in *n*-butanol (2.6 × 10⁻⁵ M) were cooled from 333 to 273 K at a rate of 60 K h⁻¹ (Fig. 2A) and both UV/Vis and CD intensities were monitored simultaneously at λ = 415 nm, which is close to the maxima in UV/Vis and CD associated with H-type self-assembly of **T6** in butanol.[6]† The cooling curves obtained from these techniques are not sigmoidal, but show a sudden change in intensity at a specific temperature known as the temperature of elongation (T_e). This behaviour is indicative of a cooperative self-assembly process according to the nucleation–elongation mechanism.[3] Since the T_e for both UV/Vis and CD techniques is the same, the self-assembly proceeds immediately from monomers *via* helical nuclei to helical assemblies. These cooling curves could not be fitted with the one-dimensional nucleation-growth model used previously,[3] which is probably a
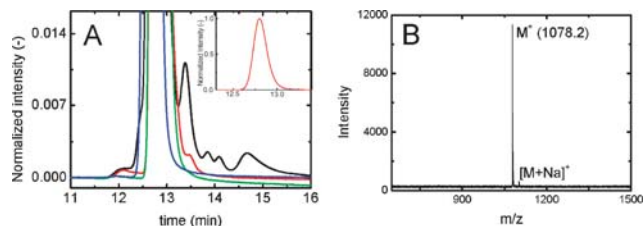
Fig. 1 A. 100× zoom-in of the GPC traces of different **T6** samples: batch 1 (black, 98%), sample A (red, 99.6%), sample B (green, 99.9%) and sample C (blue, 99.9 + %) (the inset shows the original GPC-trace of sample A) at λ_{max} = 450 nm. B. MALDI-TOF spectrum of **T6** (sample C) after purification using recycling GPC.
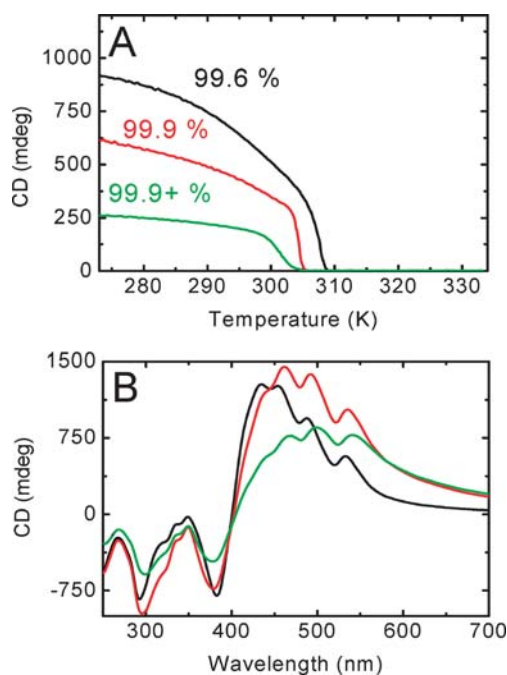
Fig. 2 **T6** in *n*-butanol ($c = 2.6 \times 10^{-5}$ M), sample A (black), B (red) and C (green) A. Temperature-dependent CD spectroscopy ($\lambda = 415$ nm, $\Delta T = -60$ K h^{-1}) and B. CD spectra after cooling at 60 K h^{-1} ($T = 273$ K).

consequence of the formation of large capsules and cylinders (*vide infra*) resulting in linear dichroism (LD).[9]† The shape of the cooling curves is different for all three samples when using the same self-assembly protocol, showing that a tiny amount of impurity has a decisive influence on the process. The most pure sample, C, shows the lowest T_e whilst the least pure sample, A, shows the highest T_e (T_e (C) = 305 K < T_e (B) = 306 K < T_e (A) = 309 K, Fig. 2A). Although the sign of the CD effect appears positive at long wavelengths and negative at short wavelengths, the envelopes and intensities of the CD-spectra of samples A–C at 273 K (Fig. 2B) differ quite dramatically. Heating the first formed solution of the self-assembled **T6** structures to just above the T_e and then slowly cooling the solution for the second time results in a less intense CD-effect in the case of the two least pure samples A and B§†, while for the most pure sample, C, the CD-effect fully recovers. However, when in a third heating-cooling cycle the solution of sample B was heated to a higher temperature (353 K) well above T_e, the system becomes fully reversible.† This effect can be attributed to "seeds" that are only fully disassembled above 333 K and shows that the reversibility of the system is highly dependent on the applied superheating, an effect well established in crystallization studies.[10] The **T6** molecules did not show any degradation in solution during the measurements, however, standing for some time easily lowers the purity of B to that of A.†

When subjecting a solution of the most pure sample, C, to different self-assembly (cooling) protocols; namely, quenching in an ice bath, 60, 4 and 1 K h^{-1} cooling and annealing the solution during 60 K h^{-1} cooling, the CD spectra recorded after assembly are all different.† After correcting for the absorption intensities, similar g_{abs} values are obtained for

the 4 and 1 K h^{-1} cooled solutions, possibly indicating that the thermodynamically most favoured state is reached under these conditions for this sample.†¶ Tapping mode atomic force microscopy (TM–AFM) of drop-cast assemblies of **T6** obtained by cooling solutions of sample B‖ yields capsules in the middle of the evaporated sample while at the edges large cylinders are visible using optical microscopy.† Cooling at 60 K h^{-1} gave much smaller assemblies (~ 5 µm) than cooling at 6 K h^{-1} (~ 100 µm). These results show the importance of purity and cooling protocol on the outcome of the self-assembly process and the nature of the nucleation.

The intriguing subtleties observed in the self-assembly of **T6** prompted an examination of a **T7** derivative. Septithiophene **T7** was purified to 98% purity, after which the self-assembly process in *n*-butanol ($c = 2.6 \times 10^{-5}$ M) was investigated using temperature-dependent CD and UV/Vis spectroscopy. This system also shows all the characteristics of an heterogeneous nucleation–elongation mechanism discussed above for **T6**. Subjecting the solution of **T7** to two cooling rates, quenching and 60 K h^{-1}, gave self-assembled structures having opposite helicity as revealed by CD measurements. Since all the optical data measured for **T7** were free from LD and scattering effects,† the differences can be attributed to a molecular effect. To investigate the possibility of helix reversal (stereomutation), a quenched solution of **T7** was annealed at 320 K.[11] Initially the CD effect decreased for 1 h, after which the stereomutation started and was complete after 4 h (Fig. 3A). However, the CD effect corresponding to the final state of the annealing process does not resemble the CD effect obtained after cooling the solution at 60 K h^{-1}. The observed stereomutation in CD is accompanied by a hypsochromic shift and a decrease in oscillator strength in the UV/Vis spectrum (Fig. 3B); indicating that during the annealing process yet another organization is formed.

Why is there such a dramatic influence of impurities and why are a manifold of supramolecular arrangements found in the self-assembly of both **T6** and **T7**? In the past, manifolds of self-assembled structures have been produced by a variety of preparation methods.[1] With the exception of a few examples,[11,12] it is usually assumed that molecular components self-assemble into the thermodynamically stable aggregate.[13,14] It is only recently that kinetically controlled aggregates have been taken into account.[11,14] However, proteins are well known to fold into different conformational and aggregated local minima, often called "substates".[15,16] The connection of all these local minima creates an energy landscape for the
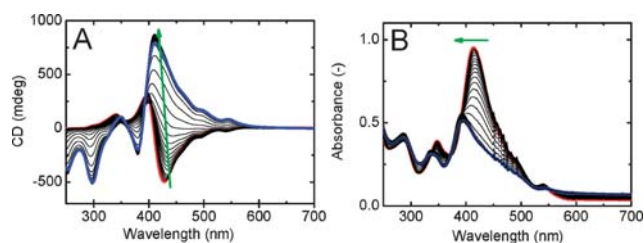

Fig. 3 Time-dependent CD (A) and UV/Vis (B) spectra during annealing where the initial spectrum is indicated by red and the final spectrum by blue. The green arrow indicates the increasing time ($c = 2.6 \times 10^{-5}$ M, $\Delta t = 5$ min, $T = 320$ K).

protein-folding process. In crystal engineering of small molecules and polymers, similar local energy minima are encountered called polymorphs.[17–19] A particular crystal form is not always the most stable one and conversion between the different polymorphs is possible.[17] Temperature, cooling rate and the presence of impurities *inter alia* play a significant role in polymorphism. Impurities, even at the parts per million level can act as seeds for heterogeneous nucleation and can influence the crystallization kinetics and packing of the molecules.[10,20] It is reasonable to expect that analogues of the polymorphism observed in crystallization and energy landscapes in protein folding will be encountered in the self-assembly of π-conjugated oligomers and polymers. The self-assembly of both **T6** and **T7** is highly dependent on the purity of the sample, showing heterogeneous nucleation at even 0.1 mol% concentration of impurity. Although heterogeneous nucleation is well established in the field of crystallization, within self-assembly processes it has not been identified previously.

Only in its most pure form, does evidence emerge that **T6** can self-assemble homogeneously, however, the smallest traces of impurities give rise to the presence of multiple CD signatures for the **T6** and **T7** assemblies. This behaviour shows the existence of a complex energy landscape for the self-assembly of these materials. Previous studies showed that the thiophene segments are most stable in a cofacial parallel orientation with no handedness.[6] However, the steric hindrance of the chiral substituent in the oligo(ethyleneoxide) side chain present in the oligomers investigated in this study hinders the optimum packing.[6] These counteracting forces could ensure that multiple orientations of **T6** and **T7** are possible, resulting in a multiplicity of local minima in the energy landscape *i.e.* multiple self-assembled structures. These counteracting forces also hamper easy homogenous nucleation, but make the compounds very susceptible to heterogeneous nucleation with different structures resulting. Systems that do not contain such counteracting forces, but in which the different forces are complementary, are likely to show a simple energy landscape, with easy to control self-assembly protocols, without a profound influence of impurities. Our oligo(*p*-phenylene vinylene) ureidotriazine and trimesic amide derivatives are examples of that class of materials.[3,4]

In the current work we have demonstrated that trace amounts of impurities have a dramatic effect on molecular packing, self-assembly and the size distribution of the assemblies formed. Besides the influence of impurities, the resulting self-assembled structures are also dependent on the self-assembly protocol adopted. These results are relevant to the controlled construction of self-assembled structures and functional materials in general. Our findings reveal that self-assembly has remarkable similarities to both the crystallization of molecules on the one hand and to the dynamics of protein folding and aggregation on the other hand.

Notes and references

§ A different sample, sample B′, was used, which has a similar purity to sample B.
¶ Remarkably, the cooling curves for the slowest cooling rates are positive at high temperatures and become negative at lower temperatures, however the full spectra does not change sign.
‖ Since the purity of sample C is very difficult to obtain, we performed the TM-AFM measuerment on sample B.

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