Duplex strand formation using alternating copolymers†

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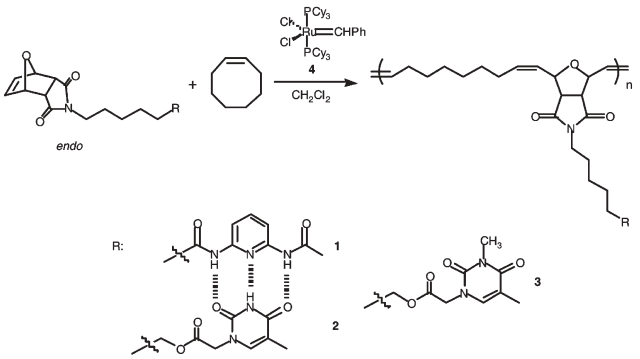
The regular arrangement of complementary diaminopyridine-thymine (DAP-THY) on alternating copolymers permits cooperative binding events and the effective formation of well-controlled micrometre-scale aggregates.

The ability to organize synthetic macromolecules *via* non-covalent interactions provides fundamental materials for encapsulation and delivery media,[1] micro-reactors[2] and biosensors.[3] These materials utilize weak non-covalent interactions similar to those found in biological macromolecules such as proteins, DNA, and RNA, to direct and modulate their three-dimensional topology.[4] Biomacromolecules feature the integration of non-covalent interactions and systematic organization, producing specificity with a cooperative interface and dictating their structure and function. For instance, carbohydrate-binding protein lectins typically show low affinities for simple mono and oligosaccharides whereas lectins cooperate with multiple binding epitopes comprising glycolipids and glycoproteins on the cell surface with high affinity.[5] Polymeric nucleic acids, DNA and RNA, are alternating copolymers of phosphate and nucleoside, producing information storage and replication capability endowed by the nature of the duplex.[6] Thus, the combination of non-covalent interactions and their orderly organization into molecular duplexes is a key to appending further function in the design aspect of bio-inspired macromolecules.[4c,7]

Recently, we have demonstrated the formation of giant vesicular aggregates (recognition induced polymersomes, RIPs), by utilizing the combination of complementary diaminopyridine (DAP) and thymine (THY) functionalized polymers.[8] In these studies, recognition groups are located randomly on the polymer chain. To investigate the effect of registration on self-assembly, alternating copolymers were designed: these copolymers possess specific recognition units in each monomeric unit. To create these copolymers, the DAP and THY functionalized monomers (**1–3**) were synthesized and employed for ring opening metathesis copolymerization (ROMP) (Scheme 1). Recent reports have used ring opening metathesis polymerization (ROMP) to synthesize polymers with recognition units.[9] The solubility of the resultant polymers, however, has precluded control of polymerization and quantitative characterization of the multivalent binding capabilities such as stoichiometry and binding affinity.[10] Herein, we report the design and synthesis of alternating copolymers to show: (a) the formation of duplex supramolecular–copolymer complex relative to stoichiometry, (b) binding affinity between complementary functionalized alternating copolymers (Fig. 1).

Alternating copolymerizations of **1–3** with cyclooctene were carried out in degassed dichloromethane at room temperature using ROMP catalyst **4** (Scheme 1). Molecular weights (M_n) of DAP and THY copolymers were 7400 and 7300 respectively. Alternating unit fractions of the resultant copolymers were estimated by ^{1}H NMR spectroscopy. As reported previously, a combination of catalyst **4** and *endo*-oxanorbornenes along with cyclooctene provided copolymers with high alternating unit percentages (92–98%).[11] The regularly placed nonpolar cyclooctene spacers between polar functionalized oxanorbornene derivatives provide improved solubility to the resulting copolymer in organic solvents. As a control for the recognition dependence of



Scheme 1 Synthesis of alternating copolymers **1–3** with cyclooctene.

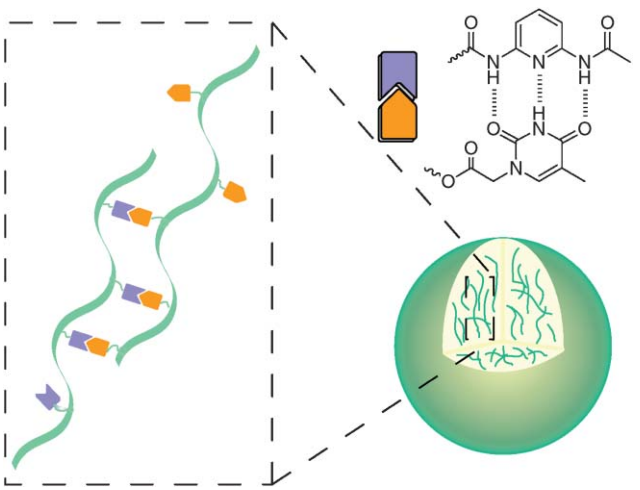


Fig. 1 Illustrative scheme of spherical aggregates using non-covalent specific interaction.

† Electronic Supplementary Information (ESI) available: Monomers and copolymers synthesis, ^{1}H NMR spectra and GPC profiles. See http://www.rsc.org/suppdata/cc/b5/b502929e/

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the fabrication process, *N*-methyl THY functionalized monomer **3** incapable of forming the specific hydrogen bonding was also copolymerized with cyclooctene.

To examine the formation of the supramolecular–copolymer complex, complementary alternating copolymers (**1** and **2**) were mixed in $CHCl_3$ and THF. Combination of copolymers **1** and **2** in $CHCl_3$ (1 mg ml^{-1}) led to a turbid solution. However, copolymers **1** and **3** incorporating *N*-methylthymine (1 mg ml^{-1}) did not result in turbidity. This apparent distinction demonstrates that the presence of specific three-point hydrogen bonds induces the formation of complex. The effect of recognition unit regularity was clearly exhibited in hydrogen competitive solvents such as THF. Turbidity was significantly observed upon the mixing of **1** and **2** even in THF (1 mg ml^{-1}). To investigate the formation of macroscopic aggregates quantitatively, optical density at 700 nm was used to determine the turbidity of the mixture with varied fractions of THY group (Fig. 2). In $CHCl_3$ and THF, both cases showed increased tendency upon titration, which maximized $\sim$1 equivalent of THY added.

To determine the stoichiometry of the supramolecular–copolymer complex, Job plots were obtained using ^{1}H NMR and optical density (Fig. 3).[12] A symmetric plot with a maximum at molar ratio *ca*. 0.5 was obtained *via* ^{1}H NMR, indicating the duplex formation of supramolecular–polymer complexes relative to the stoichiometry. Further evidence for the macroscopic formation of supramolecular–copolymer complex was obtained from the Job plot in optical density at 700 nm. Likewise the plot also showed a maximum at molar ratio 0.5, but with a dissymmetric profile. This can be explained by relatively strong two-point hydrogen bonding THY–THY triggering the formation of a macroscopic non-duplex complex with an excess of THY copolymer.[13]

Quantitative data for binding affinity of duplex complex was obtained *via* ^{1}H NMR titration. The association constants (K_a) for alternating copolymer and monomer systems were characterized by 400 MHz ^{1}H NMR at 20 °C. Addition of DAP guest (10 mM) to THY host in $CDCl_3$ (1 mM) results in substantial down-field shifts of the THY imide peak (Fig. 4).[14] Association constants were estimated by fitting a curve for the 1 : 1 binding isotherm with the least-squares method. K_as of 24 000 and 820 M^{-1} were obtained for alternating copolymer and monomer systems

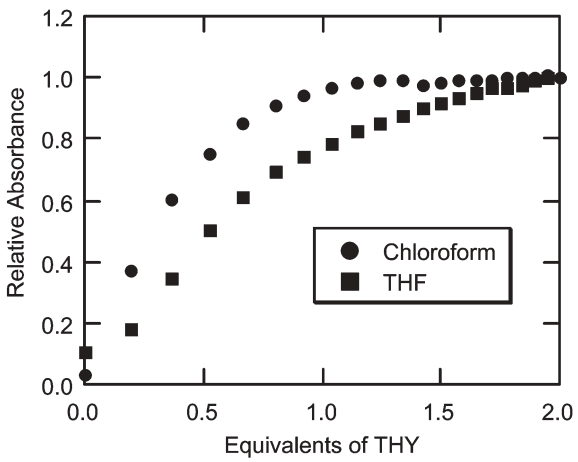

Fig. 2 Formation of supramolecular–polymer complex in $CHCl_3$ (●) and THF (■) as determined by the turbidity measured at 700 nm.

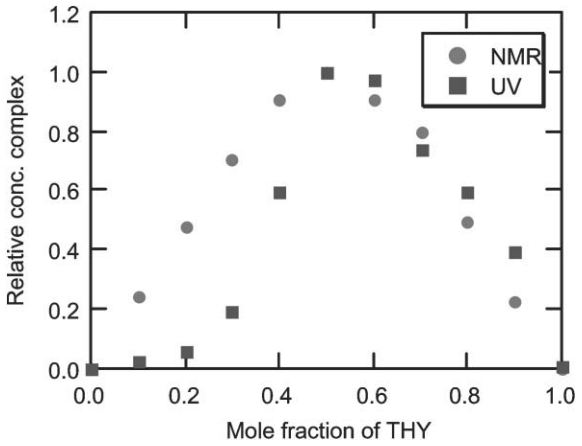

Fig. 3 Job plots obtained in ^{1}H NMR (●) and optical density (■) in $CDCl_3$ (total conc. = 1 mM) and $CHCl_3$ (total conc. = 2 mM) respectively.

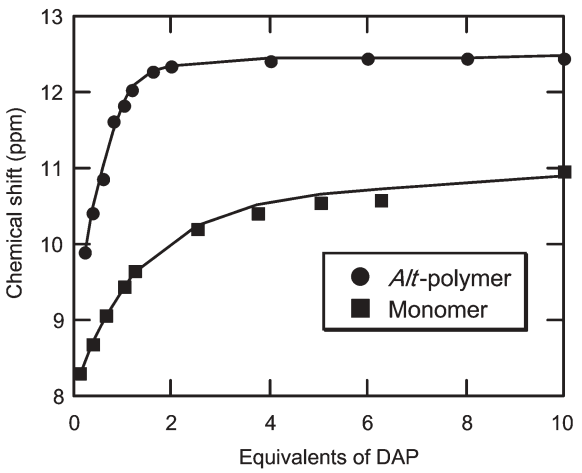

Fig. 4 ^{1}H NMR titration of **2** with **1**: alternating copolymers (●) and monomers (■). The chemical shift of the NH group of THY was monitored.

respectively, providing evidence for cooperative binding events on polymeric scaffolds. Slow exchange was observed during the early stage of the titration for polymeric system, likely indicating significantly tight binding events of the duplex.[15]

Observation of the enhanced and tight binding events for copolymeric scaffolds clearly suggests the importance of systematic positioning of recognition units in the formation of supramolecular–copolymer duplex. As expected in a copolymeric system, once a pair of complementary recognition units forms to acquire the specific hydrogen bonding, 'zipper' like binding events are organized for further interactions inherent to alternating placement of the recognition units. Significant energetic differences were observed for this 'zipper' effect, namely cooperativity: estimated free energy difference between copolymeric and monomer systems ($\Delta\Delta G$) based on ^{1}H NMR titration is 2.0 kcal mol^{-1}.

Macroscopic morphology and structures of the duplexes were established by differential interference contrast (DIC) and laser scanning confocal microscopy (LSCM). All duplexes assemble into spherical structures. An average diameter of freshly prepared

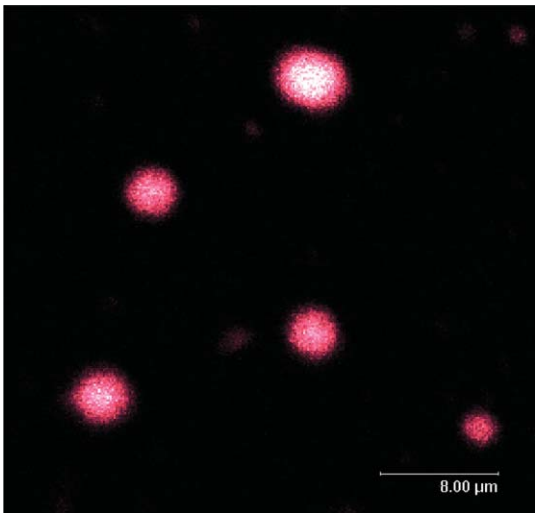

Fig. 5 Representative LCSM image of supramolecular–copolymer complex formed by alternating copolymers **1** and **2** in $CHCl_3$ (1 mg ml^{-1}).

supramolecular copolymer assemblies in $CHCl_3$ (1 mg ml^{-1}) was 3.4 ± 1.1 μm estimated by DIC. To provide direct evidence for the morphology of observed spherical structure, fluorescent tagged copolymers were synthesized using flavin tagged monomer for LSCM.[16] Uniform spherical aggregates were observed by flavin fluorescence light (511 nm). Fluorescence intensities inside these spheres diminished gradually from the center to edge, revealing that the spherical assemblies have filled morphology (Fig. 5). Highly localized fluorescence obtained in the micrographs is indicative of high yield of aggregate formation.

In summary, we have demonstrated the formation of supramolecular–copolymer complex using complementary functionalized alternating copolymers. The 1 : 1 stoichiometry in Job plots reveals the formation of duplex strand. Cooperative effects were observed upon the combination of the complementary functionalized alternating copolymers, resulting in 'zipper' like binding. This quantitative understanding has improved the versatility of recognition unit functionalized copolymers that can contribute to the creation of highly specific and controllable, templates and building blocks in nanoscale material science. The effect of backbone flexibility on the binding affinity subsequent to reducing double bonds to single bonds and solution annealing experiment is currently being investigated and will be reported in due course.

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