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Self-assembling morphologies obtained from helical polycarbodiimide copolymers and their triazole derivatives

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Abstract:	A facile method for the preparation of polycarbodiimide-based secondary structures, e.g., nano-rings, "craters", fibers, looped fibers, fibrous networks, ribbons, worm-like aggregates, toroidal structures, and spherical particles, is described. These aggregates are morphologically influenced by extensive hydrophobic side chain/side chain interactions of the singular polycarbodiimide strands as inferred by atomic force microscopy (AFM) and scanning electron microscopy (SEM) techniques. Polycarbodiimide-g-polystyrene copolymers (PS-PCDs) were prepared by combination of synthetic methods including coordination-insertion polymerization, CuAAC (copper(I)-catalyzed azide alkyne cycloaddition) "click" chemistry, and atom transfer radical polymerization (ATRP). PS-PCDs are found to form specific toroidal architectures at low concentrations in CHCl3. To determine the influence of more polar solvent medium (i.e., THF and THF/EtOH) on polymer aggregation behaviour, a number of representative PS-PCD composites have been tested to show discrete concentration-dependent spherical particles. These fundamental studies are of practical interest to develop experimental procedures for desirable architectures by directed self-assembly in thin film. These architectures may be exploited as drug carriers, whereas other morphological findings represent certain interest in the area of novel functional materials.
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TITLE:
Self-assembling morphologies obtained from helical polycarbodiimide copolymers and their triazole derivatives

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SHORT ABSTRACT:
Here, we present a protocol to prepare and visualize secondary structures (*e.g.*, fibers, toroidal architectures, and nano-spheres) derived from helical polycarbodiimides. The morphology characterized by both atomic force microscopy (AFM) and scanning electron microscopy (SEM) was shown to depend on molecular structure, concentration, and the solvent of choice.

LONG ABSTRACT:
A facile method for the preparation of polycarbodiimide-based secondary structures (*e.g.*, nano-rings, “craters,” fibers, looped fibers, fibrous networks, ribbons, worm-like aggregates, toroidal structures, and spherical particles) is described. These aggregates are morphologically influenced by extensive hydrophobic side chain-side chain interactions of the singular polycarbodiimide strands, as inferred by atomic force microscopy (AFM) and scanning electron microscopy (SEM) techniques. Polycarbodiimide-*g*-polystyrene copolymers (PS-PCDs) were prepared by a combination of synthetic methods, including coordination-insertion

polymerization, copper(I)-catalyzed azide alkyne cycloaddition (CuAAC) "click" chemistry, and atom transfer radical polymerization (ATRP). PS-PCDs were found to form specific toroidal architectures at low concentrations in $CHCl_3$. To determine the influence of a more polar solvent medium (*i.e.,* THF and THF/EtOH) on polymer aggregation behavior, a number of representative PS-PCD composites have been tested to show discrete concentration-dependent spherical particles. These fundamental studies are of practical interest to the development of experimental procedures for desirable architectures by directed self-assembly in thin film. These architectures may be exploited as drug carriers, whereas other morphological findings represent certain interest in the area of novel functional materials.

INTRODUCTION:

The helix is a ubiquitous chiral motif observed in nature. Complex biological systems and their components, such as proteins, polypeptides, and DNA, all utilize the helical structure as a means of performing complex tasks for applications like information storage, tissue molecular transportation support, and localized chemical transformations.

Helical polymeric macromolecules[1] have been a target for the design of functional materials and composites possessing interesting properties, which enabled their practical use in many areas[2-5]. So far, numerous helical scaffolds[6-9], as well as their secondary structure motifs, have been successfully exploited to achieve promising results, both in the field of physical engineering[10-12] and in biological applications[13,14]. Current studies represent a logical extension of our earlier efforts to synthesize optically-active alkyne polycarbodiimides bearing one or two modifiable alkyne moieties per repeat unit[15-17].

Recently, we reported[22] the homo- and co-polymerization of carbodiimide monomers leading to chiral helical macromolecules—a family of (*R*)- and (*S*)-polycarbodiimides with modifiable pendant groups that offer further functionalization through a CuAAC "click" protocol. Br-terminated polycarbodiimides obtained from their respective ethynyl precursors were shown to act as ATRP macroinitiators in *graft*-polymerization with styrene[23].

The specific aim of this manuscript is to provide a practical guide for morphological characterizations (AFM measurements and SEM inspection) of the secondary structures formed from PS-PCDs synthesized from their corresponding ethynyl precursors by using a well-known click protocol[21]. In particular, experimental details, such as the solvent of choice, the temperature, the deposition method, the substrate chosen for deposition, and the polymer structure, were shown to be highly important to obtain specific morphologies (*e.g.*, fibers, including right- and left-handed helical senses; nano-spheres; and nano-rings). They may also be of use for the development of materials with tunable properties based on polycarbodiimides with precisely-controlled chiral architecture.

PROTOCOL:

Note: All reactions were performed in a glove box (or fume hood, when noted) using standard scintillation vials.

1. **Synthesis of the (*R*)- and (*S*)-series of ethynylpolycarbodiimides**

1.1. Place 1.0 g (0.00442 mol) of *N*-(3-ethynylphenyl)-*N*′-hexylcarbodiimide monomer (**ET**) and 0.894 g (0.00442 mol) of *N*-phenyl-*N*′-hexylcarbodiimide monomer (**Ph**) as transparent, viscous liquids in a clean scintillation vial (20 mL) with a magnetic stirring bar (glove box) to obtain a representative ***R*-50-ET-50-Ph** composition.

Note: Use only one monomer to generate the respective homopolymers. Mixing two monomer precursors at different ratios afforded a library of random copolymers[22].

1.2. Weigh out 0.018 g (0.00004 mol) of (*R*)- BINOL Ti(IV) diisopropoxide catalyst as a red (sometimes orange), fine powder material in a glove box (monomer-to-catalyst molar ratio is 250:1) and add it to the scintillation vial.

1.3. Add ~3-5 mL of anhydrous $CHCl_3$ to dissolve both the monomer and the catalyst. Gentle stirring may be required at this step to dissolve the catalyst, which may otherwise form chunks of material. Perform all manipulations with reagents under an inert atmosphere (glove box) at 25 °C.

1.4. Cap the scintillation vial containing all the reagents and allow the reaction mixture to stir overnight at 25 °C in a glove box.

1.5. Remove the magnetic bar and add ~5 mL of additional $CHCl_3$ to re-dissolve the dark red, viscous material (outside the glove box).

1.6. Inject the solution obtained in the previous step into cold MeOH (250 mL) containing 0.5 mL of 1,8-diazabicyclo[2.5.0]undec-7-ene (DBU) to precipitate the polymer material as yellowish fibers.

1.7. Collect the polymer formed by filtration (fritted funnel, 15 mL, 4-8 µm) and wash it with MeOH (~10 mL, 3x).

1.8. Re-dissolve the material obtained from the previous step in $CHCl_3$ and re-precipitate it in MeOH to remove the residual Ti(IV)-BINOL catalyst. Dry the precipitate under high vacuum (200 mTorr) for 24 h to remove the MeOH. Repeat this procedure once to ensure the purity of the resulting polymer.

2. **Synthesis of the (*R*)- and (*S*)-series of triazole polycarbodiimides under a "click" protocol**

2.1. Add 5 mL of anhydrous THF (glove box) and a magnetic stirring bar to the scintillation vial (20 mL) containing 0.25 g (0.00117 mol) of ***R*-50-ET-50Ph** to synthesize a representative ***R*-50-TRZ-50-Ph** composition.

2.2. Weigh out 0.146 g (0.00059 mol) of *N*-(3-azidopropyl)-2-bromo-2-methylpropane

amide[22] in the glove box and add it to the scintillation vial.

2.3. Weigh out 0.022 g (0.00012 mol) of Cu(I) iodide catalyst in the glove box and load it into the scintillation vial. Let the solution stir for 2 min to form a homogeneous suspension.

2.4. Charge the same vial with 0.713 g (0.00468 mol) of DBU, cap the vial, and allow it to stir for 2 h in the glove box at 25 °C (avoid a longer reaction time to prevent hard gel formation).

2.5. Remove the magnetic bar and inject the reaction mixture (greenish gel-like solution) obtained in step 2.4 into cold MeOH (250 mL) containing 0.5 mL of DBU (outside the glove box).

2.6. Collect the formed triazole polymer by filtration (fritted funnel, 15 mL, 10 µm) and wash it with MeOH.

2.7. Repeat purification step 2.6 (*i.e.*, dissolution in THF and precipitation from MeOH) one more time to remove the residual catalyst.

2.8. Dry the product of the "click" reaction under a high vacuum (200 mTorr) for 24 h to remove traces of MeOH.

3. **Synthesis of the (*R*)- and (*S*)-series of polycarbodiimide-*g*-polystyrene copolymers**

3.1. Mix 0.029 g (0.00029 mol) of Cu(I) chloride catalyst with 0.1 g (0.00029 mol) of ***R*-50-TRZ-50Ph** macroinitiator in the scintillation vial (20 mL) containing 0.101 g (0.00058 mol) of *N*,*N*,*N*',*N*',*N*''-pentamethylenediethylenetriamine (PMDETA). Place a magnetic stirring bar into the vial (glove box) to obtain a representative ***R*-50-TRZ-50Ph-*graft*-polystyrene** copolymer.

3.2. Charge a vial from step 3.1 with 1.510 g (0.0145 mol) of freshly-distilled styrene.

3.3. Add ~12 mL of anhydrous toluene (or DMF)[23] into the vial from step 3.2 to dissolve the reagents; seal the vial tightly before taking it out of the glove box.

3.4. Within a fume hood, immerse the sealed vial in an oil bath and increase the temperature. Once the temperature reaches the desired value (temperature may vary from 57 to 100 °C, depending on the particular copolymer)[23], maintain it for 12 h (actual reaction time may range from 6 h to 4 days, depending on the experiment).

3.5. Remove the vial from the hot plate and cool the white, viscous material down to 25 °C.

3.6. Take the reaction vessel with resulting solid out of the glove box.

3.7. Unscrew the vial, remove the stirring bar, and pour the reaction mixture into 250 mL of cold MeOH containing 0.5 mL of DBU.

3.8. Collect the formed flakes of PS-PCDs by filtration (fritted funnel, 15 mL, 4-8 µm) and wash the material with cold MeOH (discard the supernatant left after filtration).

3.9. Repeat purification step 3.8 (*i.e.*, dissolution in DMF and precipitation from MeOH) one more time to remove the residual catalyst.

3.10. Dry the material (white powder) under a high vacuum (200 mTorr) for 24 h to remove the MeOH.

4. **Thin-film preparation for tapping mode atomic force microscopy (TMAFM) measurements**

4.1. Weigh 10 mg of polymeric material and place it in a 5-mL vial.

4.2. Add 1 mL of the solvent of choice (*e.g.*, $CHCl_3$ or THF) into the vial and vortex the polymer suspension to dissolve the material.

Note: Some polymer compositions require an extended period of standing time (~6 h) to completely dissolve the polymer.

4.3. Perform a successive dilution (*i.e.*, using more dilute solutions at each step as the "stock") to prepare a series of stocks of 5.0, 2.5, 1.25, 0.625, 0.313, and 0.156 mg/mL concentrations.

4.4. Filter the stock solution through a 0.45-µm PTFE syringe filter prior to deposition on the silicon wafer (200 µL) with the following specifications (diam.: 25.4 ± 0.5 mm; orientation: 100 ± 0.5°; thickness: 250-300 µm; surface: single-side polished; type: N/Phos).

Note: The deposited solution must cover the entire area of a silicon wafer.

4.5. Use a spin-coating machine immediately after depositing the sample (1 min, 1,000 rpm) to cover the entire wafer surface with a uniform polymeric film).

4.6. Acquire AFM images at 25 °C by using silicon cantilevers with nominal spring constants of 42 N/m, nominal resonance frequencies of 320 kHz, and standard silicon OTESPA or OTESPA-R3 tips (*e.g.*, OTESPA-R3 material: 0.01-0.02 ohm-cm silicon, cantilever: T: 3.7 µm, f_o: 300 kHz, L: 160 µm, k: 26 N/m, W: 40 µm). Vary the amplitude set-point values from 425 to 273 mV, with scan rates of 0.99 and 1.99 Hz, respectively[22,23].

Note: The experimental details for SEM specimen preparation and image acquisition were discussed earlier[23].

REPRESENTATIVE RESULTS:

Figure 1 (upper panel) illustrates BINOL (*R*)- or (*S*)-titanium (IV) catalyst-mediated coordination-insertion polymerization leading to the (*R*)- and (*S*)-series of ethynylpolycarbodiimides with an altering ratio of the repeat units (*i.e.*, aryl- and alkyne aryl). Monomers and catalysts were obtained as described elsewhere[18]. Both (*R*)- and (*S*)-family alkyne random copolymers were selected for "click"-coupling with *N*-(3-azidopropyl)-2-bromo-2-methylpropane amide[22]. The lower panel shows the synthesis of the triazole polycarbodiimides used as macroinitiators in the ATRP reaction to produce polycarbodiimide-*g*-polystyrenes (PS-PCDs)[22,23].

Figure 2 illustrates a model of the polycarbodiimide backbone, depicted as a pink spiral spinning around the yellow axis. Brown and green substituents form a "secondary" helical motif with respect to the pink amidine main chain. Macromolecules can self-assemble in a thin film to form a great variety of complex supramolecular architectures, such as fibers, looped fibers, superhelices, fibrous networks, ribbons, worm-like aggregates, toroidal structures, and craters. A molecular model of the triazole macroinitiator is given in Figure 3 (35-mer segment of a polymer chain with terminal Br atoms represented in red).

Figures 4 and 5 show the representative AFM images of alkyne PCDs, confirming the formation of fiber-like morphologies and their respective diameter sizes (*e.g.,* Figure 5: ~76 nm (panel b), 38-60 nm (panel c), 30-40 nm (panel e), and ~12-20 nm (panel f)). In general, diluting stock solutions resulted in diminishing the size of the aggregated morphologies formed (*e.g.*, "thick," fiber-like networks at relatively high concentrations tend to transform into thin, separated fibers upon dilution).

Also shown are the morphologies formed from polycarbodiimide-*g*-polystyrenes spin-coated from $CHCl_3$ stock (Figure 6). Unlike alkyne polycarbodiimide aggregation behaviors in $CHCl_3$, examining PS-PCDs revealed both crater-like assemblies and nano-size toroidal architectures as predominant motifs. The reproducible formation of those morphologies is thought to be driven by concentration changes.

Figures 7 and 8 both illustrate representative AFM images of polycarbodiimide-*g*-polystyrenes indicative of the formation of discrete nano-spheres when applying a THF or binary THF/EtOH (25%, v) solvent system for sample deposition with concentration-dependent particle sizes. Figure 8 shows the assembly of the individual macromolecules into spherical nano-particles of ~84 nm in size matching up closely SEM-measured morphologies (~100 nm, panel e). Remarkably, the greater micron-size aggregates shown in panels a-d may be comprised of individual nano-particles agglomerated together.

Figure 1. Synthesis of alkyne polycarbodiimides and their "grafting from" transformation to PS-PCDs.
(*R*)- and (*S*)-series of ethynylpolycarbodiimides were generated by altering the ratio of carbodiimide precursors (*i.e.*, *N*-phenyl-*N*'-hexylcarbodiimide monomer (**Ph**) and *N*-(3-ethynylphenyl)-*N*'-hexylcarbodiimide (**ET**)), with repeat unit compositions varying in the range: 0:100 (**100-Ph**), 15:85 (**15-ET-85-Ph**), 30:70 (**30-ET-70-Ph**), 50:50 (**50-ET-50-Ph**), 70:30 (**70-ET-30-Ph**), 85:15 (**85-ET-15-Ph**), and 100:0 (**100-ET**). The "click" protocol was used to synthesize their

triazole derivatives, which were further employed as Br-terminated macroinitiators for the ATRP reaction with styrene (either 50 or 100 eq.) to form polycarbodiimide-*g*-polystyrenes. Reprinted with permission[22].

Figure 2. Schematic representation of polycarbodiimides self-assembling into fibrillar motifs.
The panels show the representative helical macromolecule and self-assembly of individual macromolecules to form bundled structures identifiable by AFM analysis. Reprinted with permission[22].

Figure 3. Molecular model of triazole polycarbodiimide.
The triazole polycarbodiimide structure displays the helical motif introduced by specific orientations of Br-terminated side chains. Atom color codes: carbon (green), nitrogen (blue), oxygen (red), and bromine (red).

Figure 4. Evidence for alkyne polycarbodiimide fiber formation by AFM.
Fibrous aggregates appeared to be a common trend for all alkyne composites. Representative AFM micrographs are taken for (*S*)-85-ET-15-Ph-PCD (panels a-c) and (*S*)-100-ET-PCD (panels d–f) polymers deposited from $CHCl_3$. Reprinted with permission[22].

Figure 5. Controlling the size/thickness of fibers through concentration change.
Fibrous aggregates are generated from (*S*)-100-ET-PCD at different concentrations:
5.0 mg/mL (panel a); 2.5 mg/mL (panel b); 1.25 mg/mL (panel c); 0.625 mg/mL (panel d); 0.313 mg/mL (panel e); and 0.156 mg/mL (panel f), deposited from $CHCl_3$. Scan size = 5.0 x 5.0 μm.

Figure 6. Developing PS-PCD nano-ring patterns in the thin film.
Representative concentration series for (*R*)-30-TRZ-70-Ph-STYR(1:100)-PCD polymer: 10.0 mg/mL (panel a); 5.0 mg/mL (panel b); 2.5 mg/mL (panel c); 1.25 mg/mL (panel d); 0.625 mg/mL (panel e); 0.313 mg/mL (panel f); and 0.156 mg/mL (panels g and h). Scan size = 5.0 x 5.0 μm. Reprinted with permission[23].

Figure 7. Polycarbodiimide-*g*-polystyrene nano-particles assembled in the thin film.
Representative height and phase diagrams for (*S*)-50-TRZ-50-Ph-STYR(1:50)-PCD polymer in different solvents: THF (panels a, b) and THF/EtOH binary system (panels c, d). Importantly, appending PS-segments to the polycarbodiimide backbone has a noticeable effect on the self-assembling properties; thus, macromolecules tend to aggregate into “craters” and nano-rings rather than fibers, a predominant motif found for all alkyne PCDs. Reprinted with permission[23].

Figure 8. Schematic representation of polycarbodiimide-*g*-polystyrene nano-spheres as evidenced by SEM-analysis.
Individual nano-particles (panel e) and large aggregates (panels a-d) assembled from (*S*)-70-TRZ-30-Ph-STYR(1:100)-PCD and (*R*)-50-TRZ-50-Ph-STYR(1:100)-PCD polymers, respectively. Proposed self-assembly model for the individual polycarbodiimide-*g*-polystyrene macromolecules with rigid polycarbodiimide backbones (green-yellow) dispersed in the environment and formed by polystyrene lateral chains (light blue). Reprinted with permission[23].

DISCUSSION:

In summary, the spin-coating deposition method represents a convenient way to reproducibly generate multiple-type morphologies, including fiber-like aggregates, ribbons, worm-like structures, fibrillar networks, looped fibers, toroids, and superhelices, from either alkyne polycarbodiimides or from their respective PS-derivatives (*i.e.*, polycarbodiimide-*g*-polystyrenes). Thus, coordination-insertion polymerization, along with further functionalization using a "click" reaction followed by ATRP, provides a unique opportunity to rapidly obtain a series of statistical polycarbodiimides with the *R*- and *S*-configuration of backbones in a nearly quantitative yield.

Alkyne polycarbodiimide formation (Figure 1) appears to be a critical step in polymer synthesis, since those macromolecules are important as substrates for the production of fiber-like aggregates; they also serve as precursors in the synthesis of polycarbodiimide-*g*-polystyrenes. Notably, alkyne compositions with a high n:m ratio tend to produce completely insoluble (or having very limited solubility in organic solvents) material during the course of Cu(I)-catalyzed "click" synthesis, possibly as a result of the cross-linking side reaction. Unlike the most common C_6-residues, longer dodecyl alkyl chains (*i.e.*, C_{12}-) having increased conformational flexibility should be avoided from a practical point of view, since they often lead to the formation of complex dewetting patterns and "craters" rather than distinct individual fibers[22].

Another important practical finding for both alkyne PCD and PS-PCD series is their ability to form secondary structures that are strongly influenced by solvent and concentration (*i.e.*, control over the type and dimensions (or size) of aggregated morphologies), thus opening up possibilities for the structural design and preparation of novel functional materials based on rigid-rod polycarbodiimides. Specifically, toroidal aggregates can be successfully obtained from polycarbodiimide-*g*-polystyrenes when spin-coated from 0.156 mg/mL $CHCl_3$ stock (Figure 6), whereas applying the more polar THF or THF/EtOH (25% by volume) solvent system to the grafted macromolecules induces the formation of spherical aggregates, as shown by the combination of AFM and SEM techniques (Figures 7 and 8). Despite the fact that there is no linear relationship between the concentration and diameter of the fibers/ribbons formed when spin-coated from $CHCl_3$ on an Si wafer, it seemed that decreasing the concentration of stock solution allowed for control of the thickness of the fibrous morphologies for alkyne PCDs (Figure 5).

Limitations in the provided method arise from the technique itself used to generate nano-structures (*i.e.*, motifs easily identifiable by AFM in thin film by tapping mode may or may not retain their structure in solution or in bulk; however, in certain cases, it is possible to corroborate AFM findings with SEM measurements). Another disadvantage of using the AFM technique is the low uniformity of thin-film patterns. Therefore, the end result is often predetermined by a delicate balance of the polymer structure, solvent of choice, deposition method, and substrate. The latter requires thorough and careful screening of multiple specimens in order to define the optimal conditions for each specific morphological motif.

A key advantage of the aforementioned AFM technique is that it offers a reliable and inexpensive way to produce and visualize specific morphologies (*i.e.*, fibers, nano-rings, and spheres) assembled from statistical polycarbodiimide polymers with rigid backbones that are otherwise obtainable by more sophisticated methods, including the blending of chemically-distinct polymers[24], applying surfactants, or using relatively complex machinery like microcapillary devices[25]. Future applications of this method might include developing the electronic nose[26], constructing spherical aggregates as carriers to deliver drug molecules[27], and designing novel liquid crystalline materials[28].

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DISCLOSURES:
The authors have nothing to disclose.

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Figure

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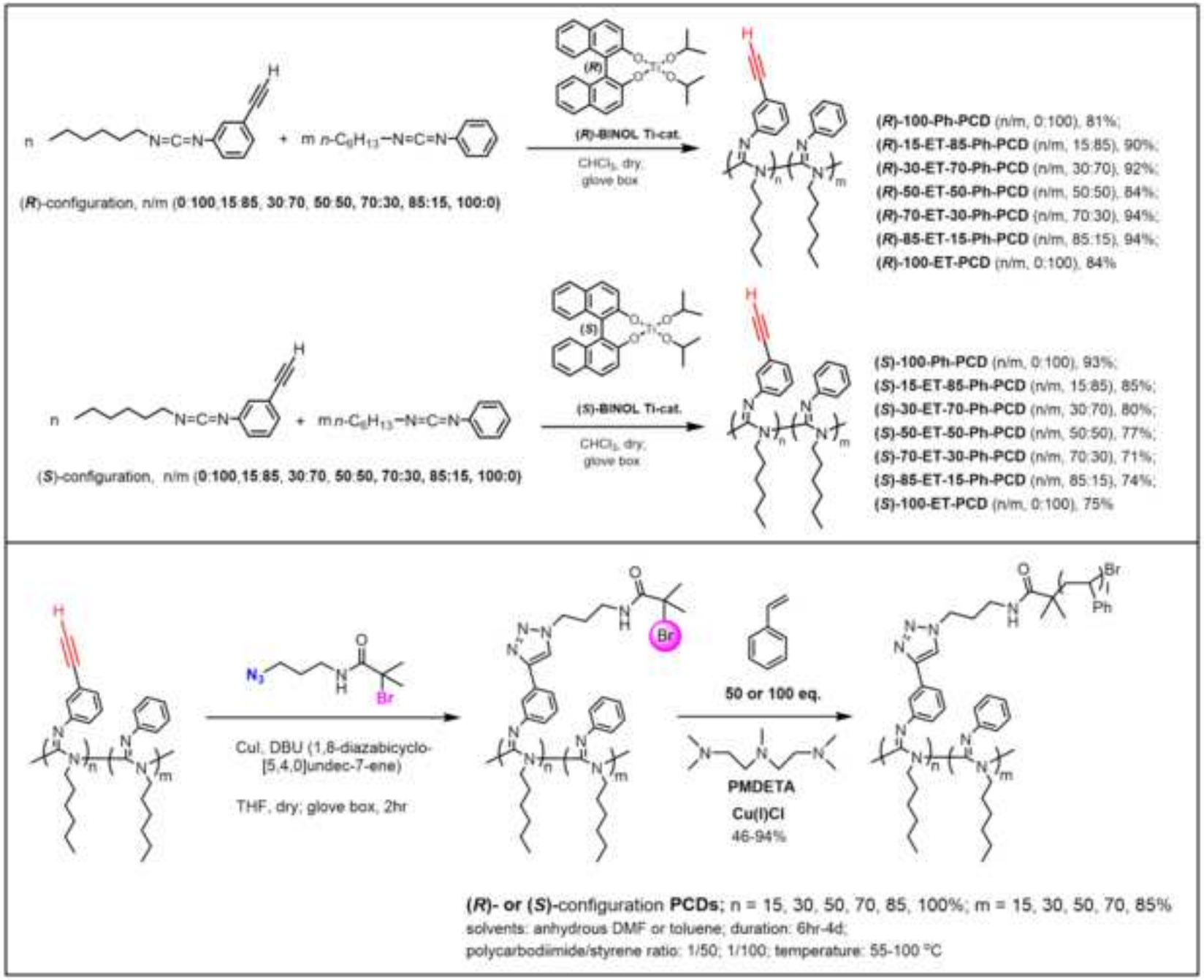

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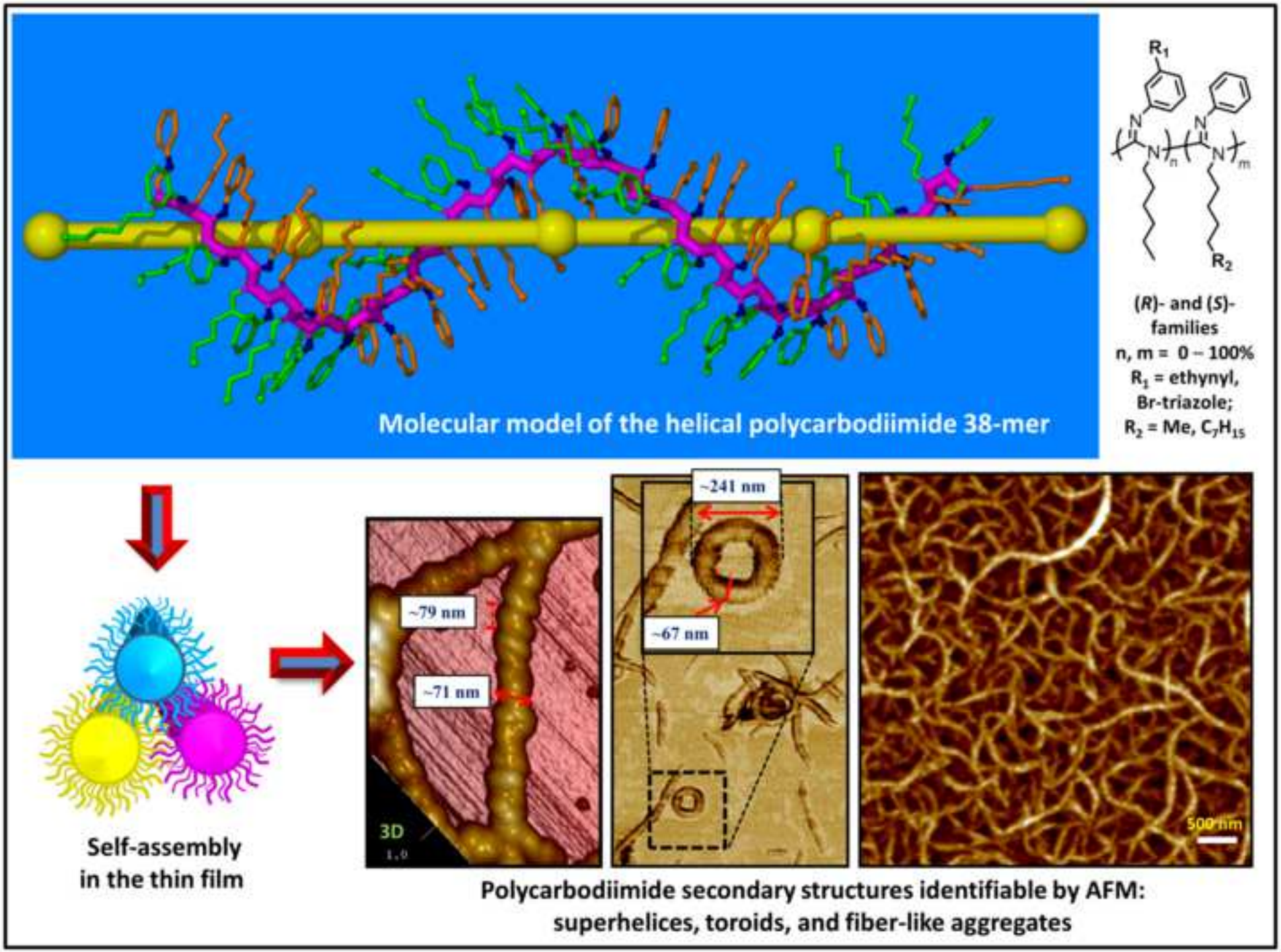

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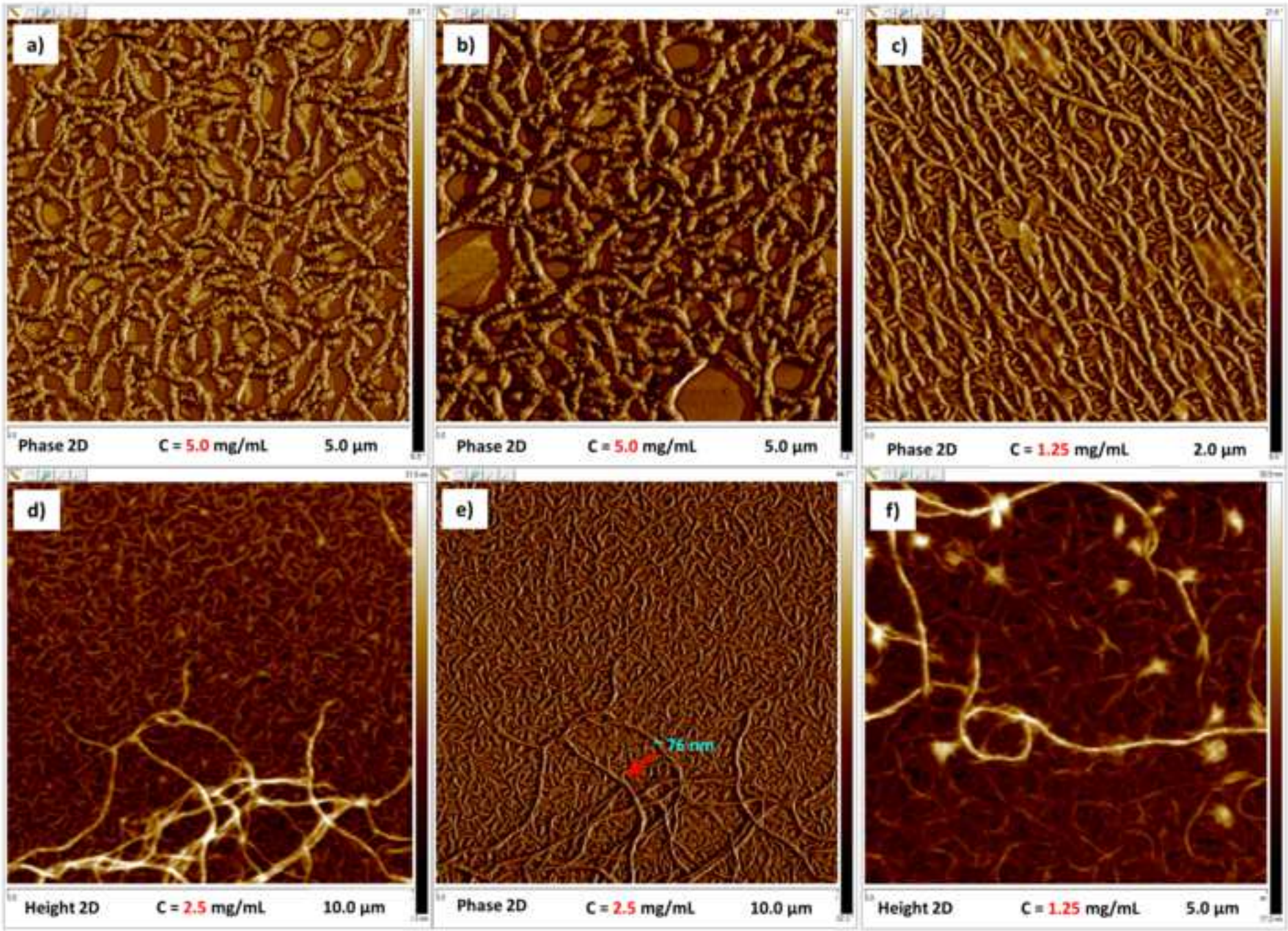

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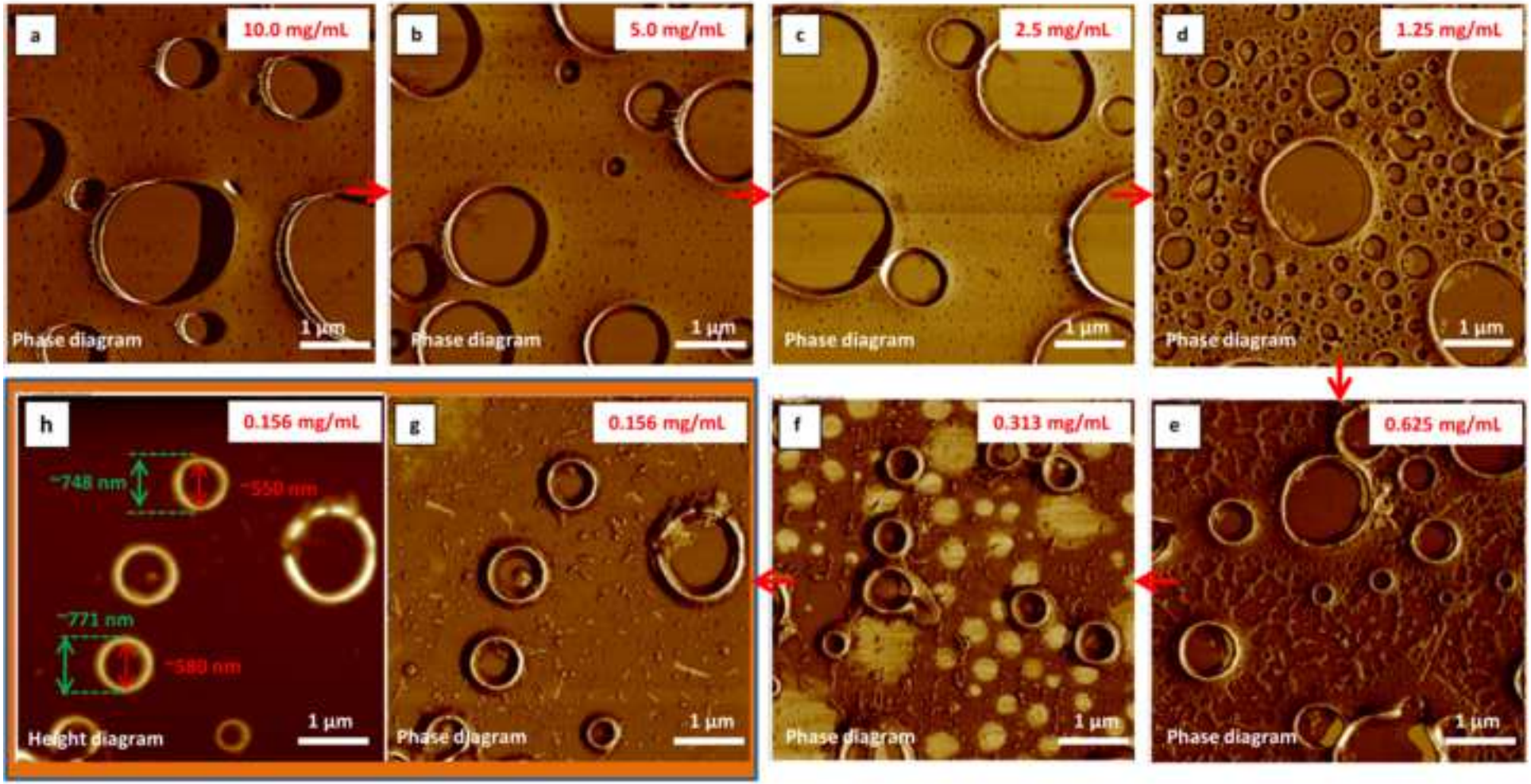

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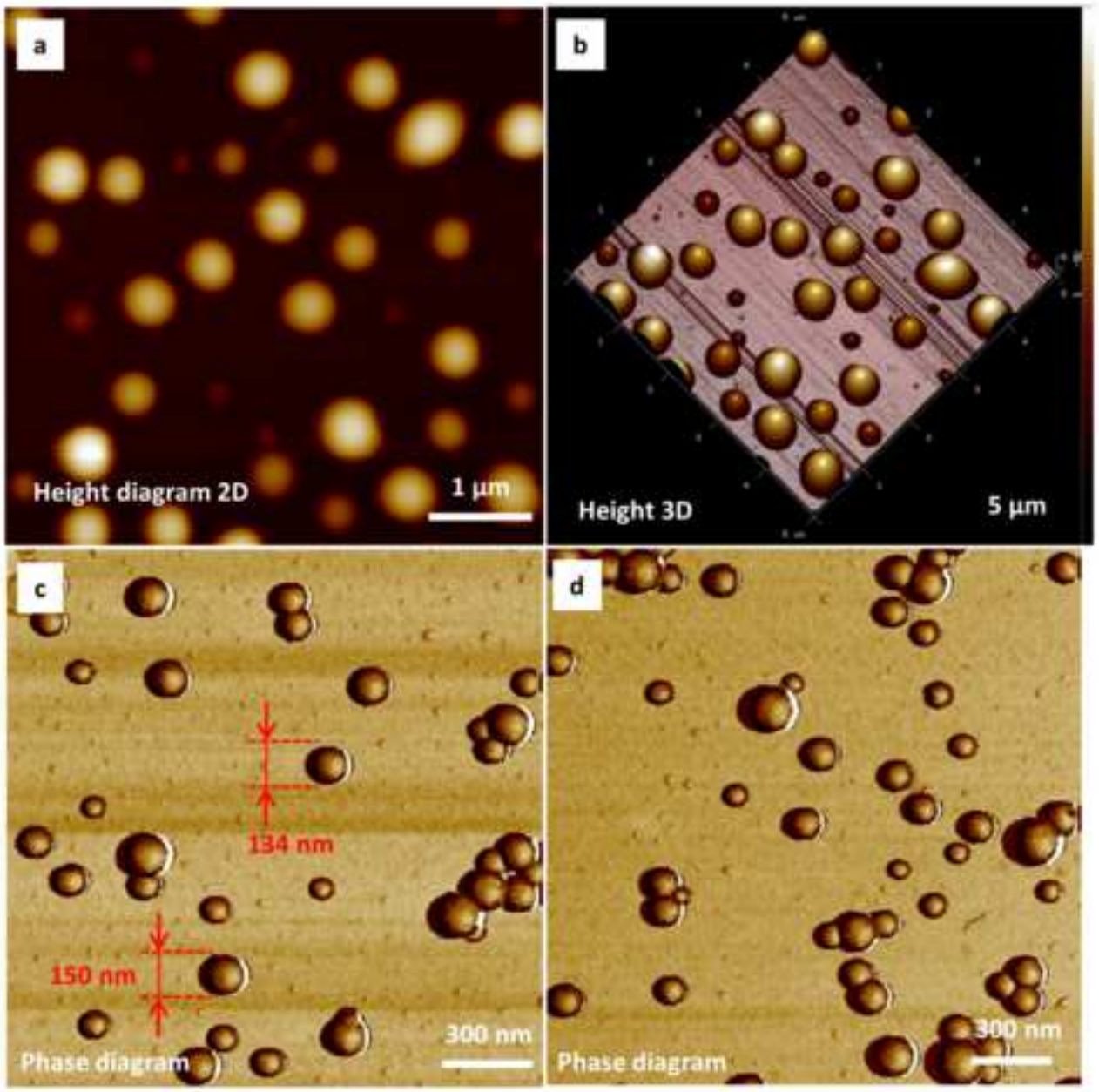

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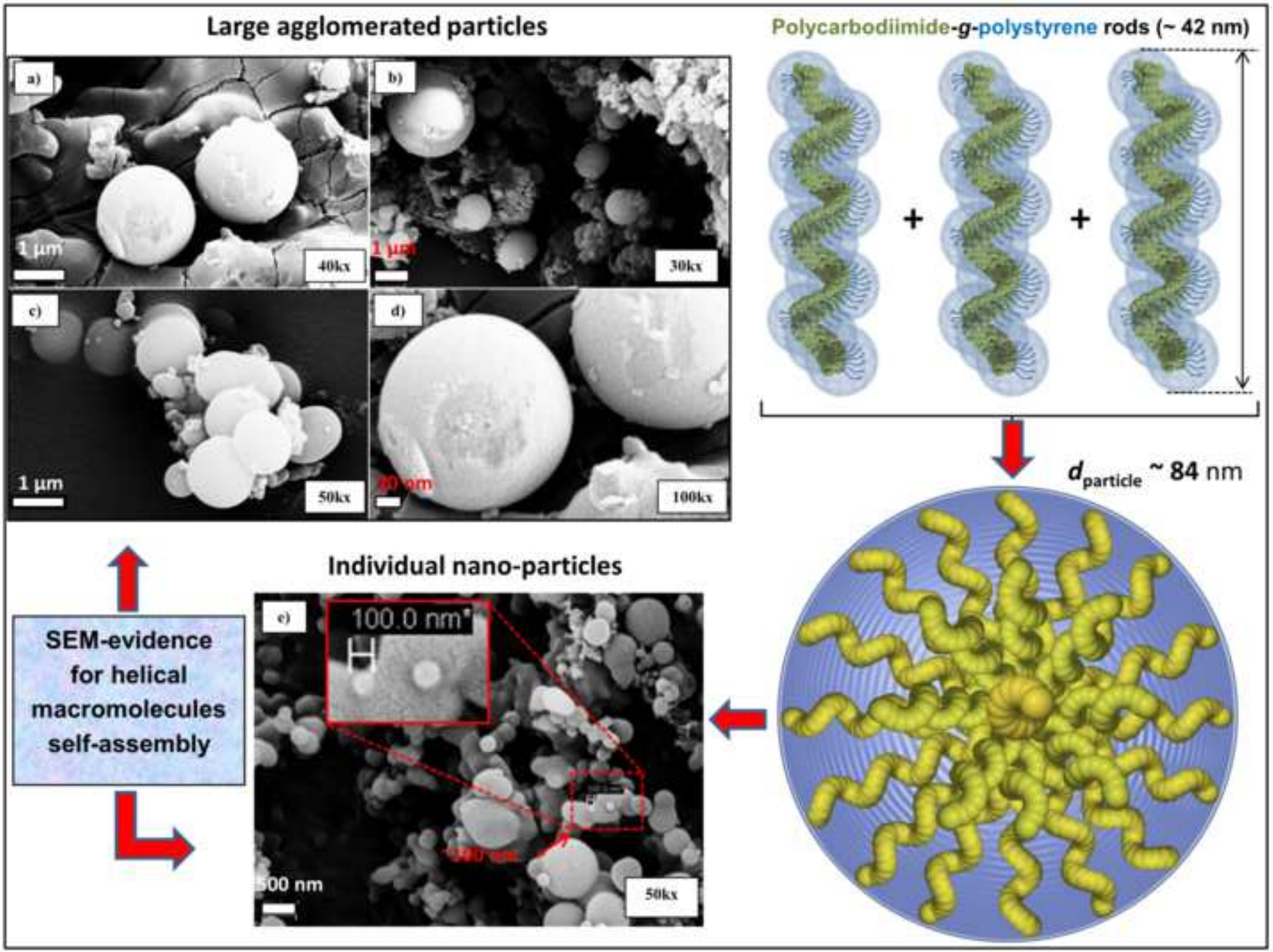

Figure-3

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Excel Spreadsheet- Table of Materials/Equipment

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JoVE_Materials-Oleg.xls

Name of Material/ Equipment	Company	Catalog Number	Comments/Description
styrene	Sigma-Aldrich	S4972-1L	reagent
N,N,N′,N′′,N′′-Pentamethyldiethylenetriamine (PMDETA)	Sigma-Aldrich	369497-250ML	reagent
Copper(I) iodide	Sigma-Aldrich	215554-5G	reagent
Copper(I) chloride	Alfa-Aesar	14644, 5G	reagent
1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU)	Sigma-Aldrich	139009-100G	reagent
N,N-dimethylformamide (DMF)	Sigma-Aldrich	227056-100mL	solvent
Tetrahydrofuran (THF)	Acros-Organics	B0320346	solvent
Chloroform	Sigma-Aldrich	372978-100mL	solvent
Methanol	Fisher-Chemical	A411-20	solvent
20 mL glass scintillation vials	Cole-Palmer	UX-08918-03	glassware
1-Dram vials (15 x 45 mm)	Kimble-Chase	KIM-60965D-1	glassware
13 mm syringe filter with 0.45µm PTFE membrane	VWR International	28145-493	membrane filter
Silicon wafer disks (25.4± .5 mm)	Wafer World, Inc	S076453	AFM substrate
Corning Stirrer/Hot Plate	Hot Plate	PC-420	heating device

single stage Unilab mBraun glove box	Unilab	12-109	glove box
Nanoscope IV-Multimode Veeco AFM-machine	Veeco	3100 Dimension V Atomic Probe Microscope	AFM-instrument

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Title of Article: Self-assembling morphologies obtained from the helical polycarbodiimide copolymers and their triazole derivatives

Author(s): Kulikov, Oleg V.*; Siriwardane, Dumindika A.;McCandless, Gregory T.; Mahmood, Samsuddin F.; Novak, Bruce M.*

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September 4, 2016

JoVE Chemistry
Editorial Board

Dear Editor,

Following your email of 2nd August 2016 and the reviewers` report enclosed therein, please find attached a revision of manuscript 55124_R1_070516 in accordance with the suggested changes.

1. Formatting:
-Please format the font size correctly. For some paragraphs, the font is scaled up by 108% (compare the introduction, paragraphs 1, 2, & 4 to paragraph 3).

We are thankful for this suggestion. As specified by Standard Manuscript Template, we used font 12 pt Calibri throughout the entire document. Currently, font size is scaled by 100%.

-2.7, 3.9 – Please indicate the steps that are to be repeated for the purification by step number.
-Once abbreviations have been defined, do not use the entire name of the chemical at subsequent occurrences (see DBU).

Purifications steps that need to be repeated are now indicated (i.e. for step 2.7, line 145: "Repeat purification step 2.6", for step 3.9, line 177: "Repeat purification step 3.8"). Corrected - full chemical name for DBU is used only once in the main text (line 115, page 3).

-References – Please include DOI where available.

Appropriate DOI numbers are included for all the references in the list.

2. Please copyedit the manuscript for numerous grammatical errors, some of which are indicated below. Such editing is required prior to acceptance and should be performed by a native English speaker to eliminate awkward phrasing and correct article usage (a, an, the).

-Title – Please delete "the".
-Long Abstract – Please break up the first sentence for clarity.

As suggested by editor, main text was checked for errors and appropriate corrections have been made in the title, long abstract, and throughout the text.

-1.1 – "having magnetic stirring bar in it" – awkward phrasing

-1.1 – *now reads as "with magnetic stirring bar" (line 93, page 3).*

-1.2, 2.2, 2.3 – "using analytical balances" *is removed for clarity.*

-2.6 – "10 μ" – *corrected to* "10 μm" *(line 142, page 4).*

-3.5 – "to a room temperature" – *corrected to* "25 ^{o}C" *(line 167, page 4).*
-3.4 – "reached" – *corrected to "reaches".*
-3.5 – "bar got stuck in it" – *removed.*
-Line 255 – "Animated model of triazole macroinitiator" – *changed to "molecular model" (line 225, page 6).*
-Line 316 – "what posed significant experimental challenge for purification and further use" – *removed for clarity.*
-Line 340 – "the outcome result" – *replaced with "end result" (line 339, page 8).*
-Line 352 – ", etc.)" – lists should not be ended this way. – *removed for clarity.*

3. Additional detail is required:
-1.8 – What vacuum pressure is used? – *missing information is added (i.e. 200 mTorr, line 121, page 3).*
-2.3 – How long is the solution stirred? – *2 min. (line 134, page 4).*
-2.4 – Is this a separate vial? – *indicated in text that this is the same vial (line 136, page 4).*
-2.5 – Which reaction mixture? From step 2.3 or 2.4? – *indicated in text that reaction mixture was taken from the step 2.4 (line 140, page 4).*
-3.2 – Is the styrene added to the mixture from step 3.1 or is this a separate vial as indicated? – *from step 3.1 (line 157, page 4).*
-3.3 – Which vials is this added to? – *from step 3.2 (line 159, page 4).*
-4.4 – What volume of solution is added to the wafer? – *200 μL (line 195, page 5).*
-Please include a citation for how to perform AFM as insufficient detail is provided to replicate the experiment. – *citations are already included (i.e. references 22, 23).*
-Please add a step for SEM, which is discussed in the abstracts and introduction, but not included in the protocol. A citation can be included in lieu of detail, and this step does not need to be highlighted for filming. – *experimental details for SEM acquisition are given in the ref. 23. Please see lines 207, 208 – "Note: experimental details for SEM specimens preparation and images acquisition are discussed earlier.[23]"*

4. Discussion: Please include independent citations when discussing significance of the technique.
– *As requested by Editor, independent citations have been included in discussion section (please see lines 347-351, pages 8, 9).*

Reviewers' comments:
Reviewer #1:
1) Some description should be improved or modified.
a) "Polycarbodiimide-g-polystyrene copolymers to introduce polystyrene moieties" in Abstract: Polycarbodiimide-g-polystyrene can not be further modified with polystyrene moieties.
As requested by Reviewer 1, this sentence in Long Abstract was rephrased. Now reads as "Polycarbodiimide-g-polystyrene copolymers (PS-PCDs) were prepared by combination of synthetic methods including coordination-insertion polymerization, CuAAC "click" chemistry, and atom transfer radical polymerization (ATRP)."Lines 43-46, pages 1, 2.

b) "So far, a large variety of demonstrating unique properties helical scaffolds6-9 and based on them multiple-type supramolecular architectures" in Line 64 and 65 (page 2).

Changed to "So far, numerous helical scaffolds,[6-9] as well as their secondary structure motifs, have been successfully exploited to achieve promising results both in the field of physical engineering[10-12] and in biological applications[13,14]", lines 63-65, page 2.

c) "a structure of polymer" in Line 82 (page 2) – "polymer structure".
Corrected to "polymer structure" (lines 79, 80, page 2).

d) "in the different ratio" in Line 99 (page 3) → "at the different ratio".
Changed to "at the different ratio" (line 97, page 3).

e) "Cu(I)I catalyst" in Line 135 (page 4).
Corrected to "Cu(I) iodine catalyst" (line 133, page 4).

f) The description of synthesis protocol is too detailed.

Respectfully disagree with Reviewer`s opinion. Authors must provide as much as possible detailed explanation about their experiment to ensure its reproducibility. Moreover, in previous version we were asked by Editor to add even more details (e.g. "Please add more details to your protocol steps. Please ensure you answer the "how" question, i.e., how is the step performed?" – quoted from Editorial comments for manuscript JoVE55124, Jun16, 2016).

g) "a catalyst" in Line 215 (page 5): in fact two catalysts with R and S configuration were used.

We are thankful for this comment. Indeed, we have used two different forms Ti(IV)-BINOL catalysts (i.e. having R- and S-configuration). Corrected to "catalysts"(line 214, page 5).

h) "stock solutions dilution" in Line 230 and 231 (page 6) → "diluting stock solutions".
Corrected to "diluting stock solutions"(lines 230-231, page 6).

i) As for "spin-coating deposition method combined with AFM-visualization represents a 303 convenient way to reproducibly generate multiple-type morphologies": AFM-visualization can observe but not generate morphologies.

"AFM-visualization" was removed to avoid ambiguity. Now reads as "In summary, spin-coating deposition method represents a convenient way to reproducibly generate multiple-type morphologies including fiber-like aggregates, ribbons, worm-like structures, fibrillar networks, looped fibers, toroids, and superhelices from either alkyne polycarbodiimides or from their respective PS-derivatives (i.e., polycarbodiimide-g-polystyrenes)", lines 302-306, pages 7-8.

2) "Figure 3" in Line 225 (page 6): In JoVE55124-R1 manuscript, Figure 3 was not offered.
In current version of manuscript, Figure 3 is to display animated cartoon of polycarbodiimide scaffold. We apologize that some cartoons/schemes from the original manuscript have been combined and/or modified.

3) As for Figure 4 and 5, which solvent was used to obtain different morphologies.
In both cases solvent was chloroform. Now it is indicated in figures' legends (lines 273 and 278, page 7).

4) "their respective sizes" in Line 229 (page 6): specify the size to diameter or length.

These sizes (Figure 5) reflect diameter, but not the length of fibrous aggregated morphologies (line 230, page 6).

Reviewer #2:

1). The authors do not discuss characterization of the polymers through traditional polymer characterization techniques, such as NMR, GPC, etc. It would be useful if the authors described the results of these characterization as a method to verify the synthesis procedure worked.

*ESI sections of both references 22 (Macromolecules, **48**, 4088-4103, 2015) and 23 (Polymer, **92**, 94-101 , 2016) contain very detailed polymers characterization including ^{1}H NMR and GPC data. To avoid duplication (plagiarism), in current submission this information was not presented (also, there are already eight figures to illustrate the manuscript main text).*

2. Details of the AFM measurement are not provided. AFM can be sensitive to various experimental parameters, and this informaiton should be provided to make it easy for others to reproduce the study. In the materials list, the authors could also provide the types of tips they use in their analysis.

Details of AFM acquisition are given in the main text (please see lines 203-208, page 5).

3. The authors should describe the advantages of AFM over other techniques (such as electron microscopy) for studying these polymer assemblies.

*Appropriate discussion of the advantages of AFM-technique is given in the main text (please see lines 344-348, page 8) and SEM analysis was only used to corroborate AFM results (i.e. fibers and spheres formation). In general, electron microscopy techniques (TEM, SEM) require different specimen preparation conditions that can alter the results, so their direct comparison with AFM-findings may be misleading. However, TEM was successfully used to provide evidence for the fibrous morphologies (please see reference 22, Macromolecules, **48**, 4088-4103, 2015).*

Once again we thank Reviewers for a critical reading and for their valuable suggestions. We believe that corrections made in manuscript would be helpful for reader`s analysis and understanding. We hope that revised manuscript would be suitable for the publication in JoVE Chemistry.

If there is any other information required, please let me know.

Sincerely,

Bruce M. Novak
Dean, Natural Sciences & Mathematics