ROUTING AND ACTION

MEMORANDUM

ROUTING

TO:(1) Sciences of Extreme Materials Branch (SEM) (Lambeth, Robert)

Report is available for review

(2) Proposal Files Report No.: Proposal Number: 72960-SM.4

DESCRIPTION OF MATERIAL

CONTRACT OR GRANT NUMBER: W911NF-18-1-0216

INSTITUTION: University of New Hampshire

PRINCIPAL INVESTIGATOR: Erik Berda

TYPE REPORT: Final Report

DATE RECEIVED: 10/1/23 1:10PM

PERIOD COVERED: 7/5/18 12:00AM through 6/30/21 12:00AM

TITLE: Final Report: Synthetic polymers with protein like structure and activity

ACTION TAKEN BY DIVISION

(x) Report has been reviewed for technical sufficiency and IS [x] IS NOT [ ] satisfactory.

( ) Based on my technical review, I have identified no OPSEC or Technology Protection concerns that need to be addressed regarding this report.

(x) Perfomance of the research effort was accomplished in a satisfactory manner and all other technical requirements have been fulfilled.

(x) Based upon my knowledge of the research project, I agree with the patent information disclosed.

Approved by robert.h.lambeth2.civ@mail.mil on 10/4/23 11:40AM

ARO FORM 36-E

REPORT DOCUMENTATION PAGE

Form Approved OMB NO. 0704-0188

1. REPORT DATE (DD-MM-YYYY)	2. REPORT TYPE	3. DATES COVERED (From - To)
01-10-2023	Final Report	5-Jul-2018 - 30-Jun-2021

4. TITLE AND SUBTITLE
Final Report: Synthetic polymers with protein like structure and activity

5a. CONTRACT NUMBER
W911NF-18-1-0216

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER
611102

6. AUTHORS

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAMES AND ADDRESSES
University of New Hampshire
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS (ES)
U.S. Army Research Office
P.O. Box 12211
Research Triangle Park, NC 27709-2211

10. SPONSOR/MONITOR'S ACRONYM(S)
ARO

11. SPONSOR/MONITOR'S REPORT NUMBER(S)
72960-SM.4

12. DISTRIBUTION AVAILIBILITY STATEMENT
Approved for public release; distribution is unlimited.

13. SUPPLEMENTARY NOTES
The views, opinions and/or findings contained in this report are those of the author(s) and should not contrued as an official Department of the Army position, policy or decision, unless so designated by other documentation.

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	15. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Erik Berda
UU	UU	UU	UU		19b. TELEPHONE NUMBER 603-862-1762

RPPR Final Report
as of 04-Oct-2023

Agency Code: 21XD

Proposal Number: 72960SM **Agreement Number: W911NF-18-1-0216**
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DUNS Number: 111089470 EIN: 026000937
Report Date: 30-Sep-2021 Date Received: 01-Oct-2023
Final Report for Period Beginning 05-Jul-2018 and Ending 30-Jun-2021
Title: Synthetic polymers with protein like structure and activity
Begin Performance Period: 05-Jul-2018 **End Performance Period:** 30-Jun-2023
Report Term: 0-Other
Submitted By: Erik Berda Email: erik.berda@unh.edu
Phone: (603) 862-1762
Distribution Statement: 1-Approved for public release; distribution is unlimited.

STEM Degrees: 14 **STEM Participants:** 20

Major Goals: The research goal of this research is to synthesize polymers that mimic the primary, secondary, tertiary, and higher-ordered structure of proteins. In doing so we aim to impart protein-like function, such as controllable assembly/disassembly or enzyme like catalytic activity, into synthetic polymers, thus creating a class of materials with unique properties and unparalleled capabilities. We will accomplish this research goal by examining, in parallel, several design aspects and functions found in protein chemistry as described in the research objectives below:
Research Objective 1 Synthesis of polymers with defined sequences via templated step-growth polymerization: mimicking primary structure
Research Objective 2 Well-defined single-chain nanoparticles (SCNP): mimicking secondary and tertiary structure
Research Objective 3 Controlled, on demand self-assembly/disassembly of polymer nanostructures: mimicking quaternary structure
Research Objective 4 Synthetic enzymes: mimicking protein function

Accomplishments: See upload section

Training Opportunities:
We hosted high school interns, along with a summer undergraduate students.

In addition, we support 3-6 undergrads during each academic year.

These training activities are 2 fold: the young scientist we host benefit from the direct training, but also the graduate students that mentor them gain experience in leadership and mentorship.

In our experience this type of student-coach relationship has been an excellent platform for professional development in out labs.

Results Dissemination: We delivered invited talks (in person and remote), along with publishing 3 papers with 2 more in preparation.

Honors and Awards: One of our Undergraduate students supported by this award, Sawyer Cawthern, won a Goldwater scholarship.

RPPR Final Report
as of 04-Oct-2023

Protocol Activity Status:

Technology Transfer: Nothing to Report

ARTICLES:

Publication Type: Journal Article Peer Reviewed: Y **Publication Status:** 1-Published
Journal: European Polymer Journal
Publication Identifier Type: DOI Publication Identifier: 10.1016/j.eurpolymj.2018.12.046
Volume: 112 Issue: First Page #: 206
Date Submitted: 5/24/19 12:00AM Date Published: 3/1/19 12:00AM
Publication Location:
Article Title: Assessing structure/property relationships and synthetic protocols in the fabrication of poly (oxanorbornene imide) single-chain nanoparticles
Authors: Ruiwen Chen, Sarah J. Benware, Sawyer D. Cawthern, Justin P. Cole, Jacob J. Lessard, Isabelle M. Cr
Keywords: Single-chain nanoparticles, Radical polymerization, Polymer folding, Size exclusion chromatography
Abstract: We present the synthesis of poly(oxanorbornene imide) single-chain nanoparticles (SCNP) by intrachain radical polymerization of pendant methacryloyl units. Well-defined poly(oxanorbornene imide)s were first prepared via ring-opening metathesis polymerization (ROMP) of methacryloyl-containing monomers. Handling and poly- merizing these monomers requires some special care to prevent undesired premature reaction of the metha- cryloyl groups. Addition of AIBN to dilute solution of the ROMP polymers triggers intrachain radical poly- merization of pendant methacryloyl groups, folding the linear polymers into SCNP. Characterization by NMR spectroscopy and SEC confirmed SCNP formation and revealed structure/property relationships related to me- thacryloyl pendent length and percent incorporation.
Distribution Statement: 3-Distribution authorized to U.S. Government Agencies and their contractors
Acknowledged Federal Support: **Y**

Publication Type: Journal Article Peer Reviewed: Y **Publication Status:** 4-Under Review
Journal: Polymer Chemistry
Publication Identifier Type: DOI Publication Identifier: 10.1039/d1py01005k
Volume: Issue: First Page #:
Date Submitted: 8/31/21 12:00AM Date Published:
Publication Location:
Article Title: Probing Secondary Coordination Sphere interactions within Porphyrin-Cored Polymer Nanoparticles
Authors: Brian F. Patenaude, Erik B. Berda, Samuel Pazicni
Keywords: SCNP, synthesis, bio inspired chemistry
Abstract: A suite of zinc porphyrin-cored random coil polymers and polymeric nanoparticles with varying degrees of potential hydrogen bonding character and steric bulk where synthesized and characterized in order to study secondary coordination sphere interactions. The reaction of cyanide with N,N-dimethylformamide in the presence of porphyrin-cored polymeric nanoparticles was monitored via UV-Vis spectroscopy. It is shown that the zinc porphyrin-cored polymers and nanoparticles catalyzed the reaction of cyanide with N,N-dimethylformamide with the highest reactions rates occurring with polymeric nanoparticles with a greater number of potential hydrogen bond donors and greater steric bulk.
Distribution Statement: 2-Distribution Limited to U.S. Government agencies only; report contains proprietary info
Acknowledged Federal Support: **Y**

RPPR Final Report
as of 04-Oct-2023

Publication Type: Journal Article Peer Reviewed: Y **Publication Status:** 1-Published
Journal: ACS Macro Letters
Publication Identifier Type: DOI Publication Identifier: 10.1021/acsmacrolett.0c00774
Volume: 9 Issue: 12 First Page #: 1836
Date Submitted: 8/31/21 12:00AM Date Published: 12/1/20 5:00AM
Publication Location:
Article Title: 100th Anniversary of Macromolecular Science Viewpoint: Re-examining Single-Chain Nanoparticles
Authors: Ruiwen Chen, Erik B. Berda
Keywords: SCNP, synthesis, characterization
Abstract: Single-chain nanoparticles (SCNP) are a class of polymeric nanoparticles obtained from the intramolecular cross-linking of polymers bearing reactive pendant groups. The development of SCNP has drawn tremendous attention since the fabrication of SCNP mimics the self-folding behavior in natural biomacromolecules and is highly desirable for a variety of applications ranging from catalysis, nanomedicine, nanoreactors, and sensors. The versatility of novel chemistries available for SCNP synthesis has greatly expanded over the past decade. Significant progress was also made in the understanding of a structure–property relationship in the single-chain folding process. In this Viewpoint, we discuss the effect of precursor polymer topology on single polymer folding. We summarize the progress in SCNP of complex architectures and highlight unresolved issues in the field, such as scalability and topological purity of SCNP.
Distribution Statement: 1-Approved for public release; distribution is unlimited.
Acknowledged Federal Support: **Y**

Publication Type: Journal Article Peer Reviewed: **Publication Status:** 1-Published
Journal: Polymer Chemistry
Publication Identifier Type: DOI Publication Identifier: 10.1039/d1py01472b
Volume: 13 Issue: 23 First Page #:
Date Submitted: Date Published: 1/1/22 12:00AM
Publication Location:
Article Title: Customizable molecular recognition: advancements in design, synthesis, and application of molecularly imprinted polymers
Authors: Erinn K. Reville, Elizabeth H. Sylvester, Sarah J. Benware, Shreeya S. Negi, Erik B. Berda,
Keywords: Organic Chemistry
Abstract:
Distribution Statement: 1-Approved for public release; distribution is unlimited.
Acknowledged Federal Support:

Partners

,

RPPR Final Report

as of 04-Oct-2023

I certify that the information in the report is complete and accurate:
Signature: Erik B Berda
Signature Date: 10/1/23 1:10PM

Scientific Progress and Accomplishments

Erik Berda, University of New Hampshire, Durham NH 03824

Agreement number **W911NF-18-1-0216**

We made significant progress on the research objectives as outlined in the original proposal and summarized in Fig. 1 below. Despite the setback due to COVID-19, we graduated 4 PhD students, 1 MS student. We published 2 papers and have 2 more in the final stages of preparation. One paper is in revision. Research accomplishments are highlighted below.

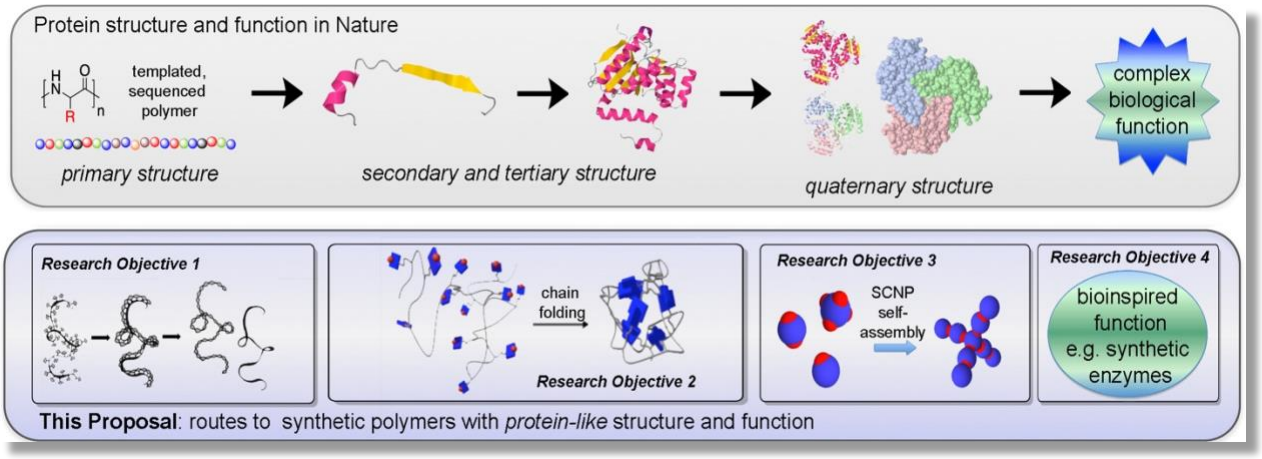
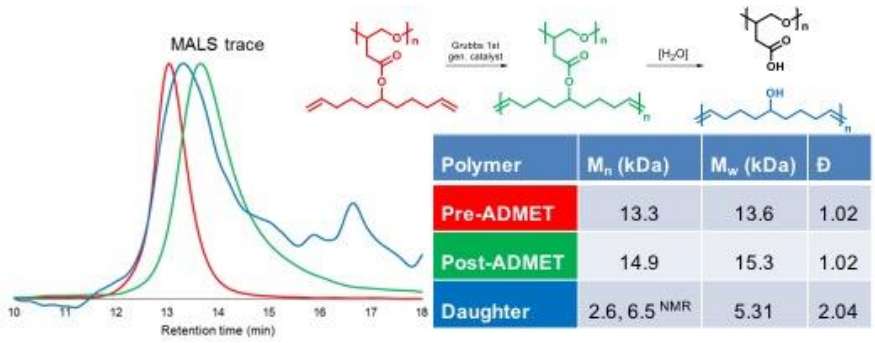


Figure 1: Graphical summary of the 4 research objectives of this effort.

Research Accomplishments

Research Objective One: Mimicking Protein Primary structure via Templated step-growth chemistry.

Although proving to be an exceptionally challenging project, we are continuing to unravel the intricacies associated with intra-chain ADMET polymerization. While the data highlighted in figure 2 does show some ability to translate chain length from parent polymer to daughter polymer, the results aren't ideal. We suspect it is related to a pendant group size mismatch and current efforts are underway to correct and investigate this. One side discover here, relating to overall single-chain nanoparticle research, is that our data confirms nearest neighbor cross-linking is much more likely than global cross-linking (otherwise the translation of chain length to daughter polymer wouldn't work). This has implications in the arena of globular SCNP synthesis and we will look into this as a spin-off of objective one.



Polymer	M_n (kDa)	M_w (kDa)	Đ
Pre-ADMET	13.3	13.6	1.02
Post-ADMET	14.9	15.3	1.02
Daughter	2.6, 6.5 ^{NMR}	5.31	2.04

Figure 2: Overview of recent data on research objective 1. Manuscript currently in preparation.

Research Objective Two: Mimicking Protein Secondary and Tertiary Structure via Well-defined Single-chain Nanoparticles.

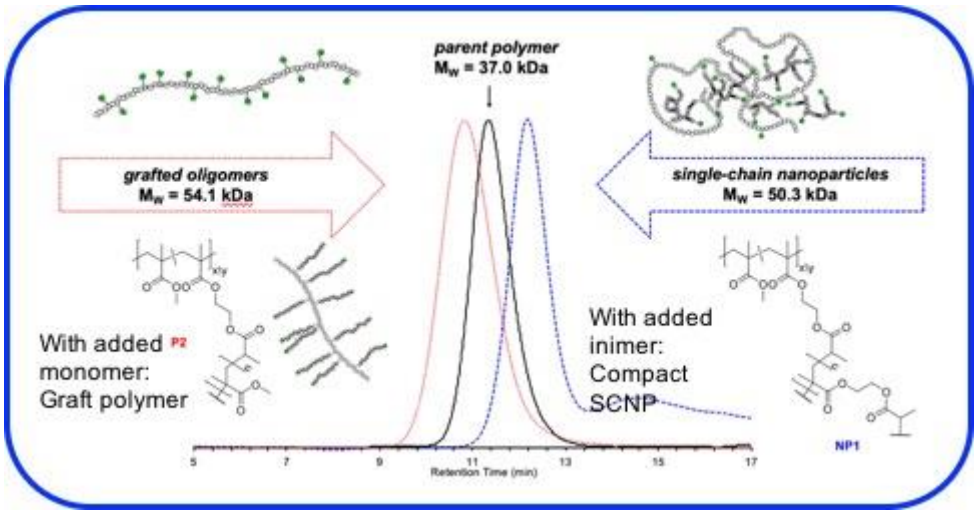


Figure 3: Progress on well-defined globular SCNP using intachain ATRC chemistry

We've made exciting progress on the way toward well-defined, highly compact and globular SCNP. In previous efforts across the SCNP community, typical reports at best show a marginal degree of compaction and densities not appreciably different that the original polymer coil itself. Our current efforts based on our 2017 paper on intrachain ATRC chemistry (*Macromolecules* **2017**, 2996-3003). Figure 3 shows that using our ATRC strategy, while adding a bifunctional small molecule inimer, results in an intrachain, hyper branching polymerization that is templated by the original linear chain. We are expanding on these results and working on a manuscript currently.

We had success with two series of polymers containing either pendant norbornene groups for intrachain ROMP (Figure 4). ROMP was chosen as it is highly functional group tolerant and proceeds rapidly, leading to fast access of nanoparticles. Intramolecular ROMP of a polymer bearing furan-protected maleimide moieties. Herein we provide a more detailed study on chain folding by ROMP. Our results showed that the efficiency of chain folding was related to norbornene content on the polymer precursor, species and feed ratio of Grubbs catalysts, as well as doping effects of fluorinated aromatic comonomers.

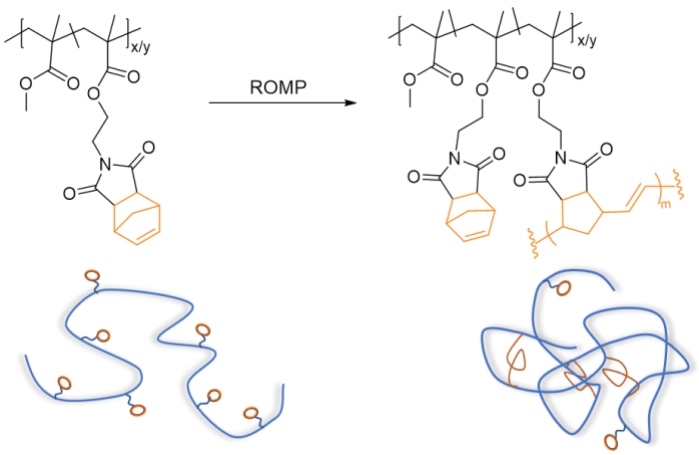
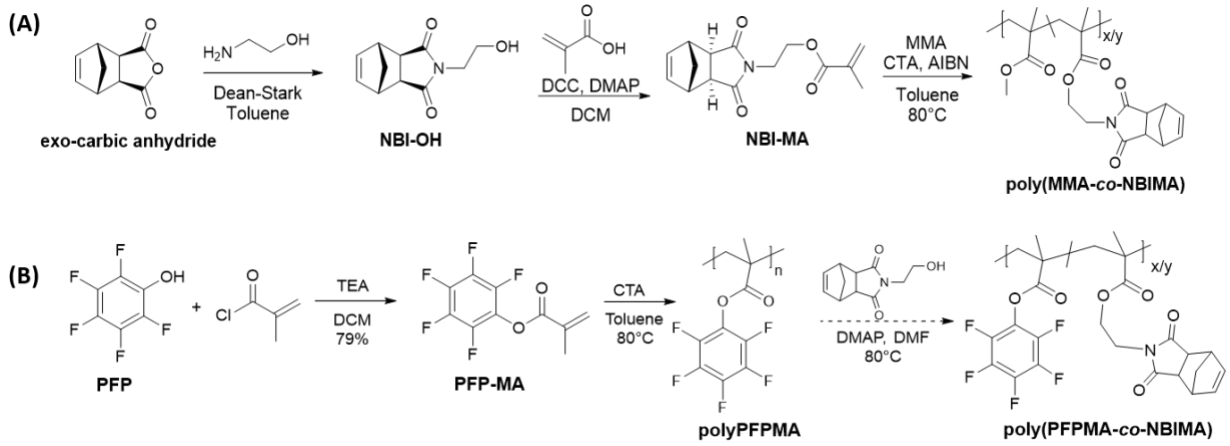


Figure 4. Schematic illustration of intramolecular ROMP of polymers bearing norbornene pendant groups.

Copolymer synthesis



Scheme 1. Synthesis of norbornene-containing copolymers by (A) direct RAFT copolymerization and (B) post-polymerization transesterification.

We sought to synthesize a series of poly(MMA-co-NBIMA) polymers with various amount of norbornene incorporations. (Scheme 1A) Direct copolymerization of MMA and NBIMA by RAFT polymerization led to well-defined polymers only when low NBIMA incorporations were targeted. At higher NBIMA incorporation (≥40%) the polymers started to show higher molecular weight and broader distribution because of interchain cross-linking. The cross-linking reaction was attributed to radical addition on the alkene functionality of norbornene during RAFT polymerization, or it may be caused by the retro Diels-Alder reaction of norbornene imide followed by radical polymerization of the maleimide group. In order to synthesize copolymers with higher NBIMA incorporation we adopted a post-polymerization functionalization strategy which was to attach NBIOH to the polymer after RAFT polymerization of PFPMA *via* a transesterification reaction. (Scheme 1B)

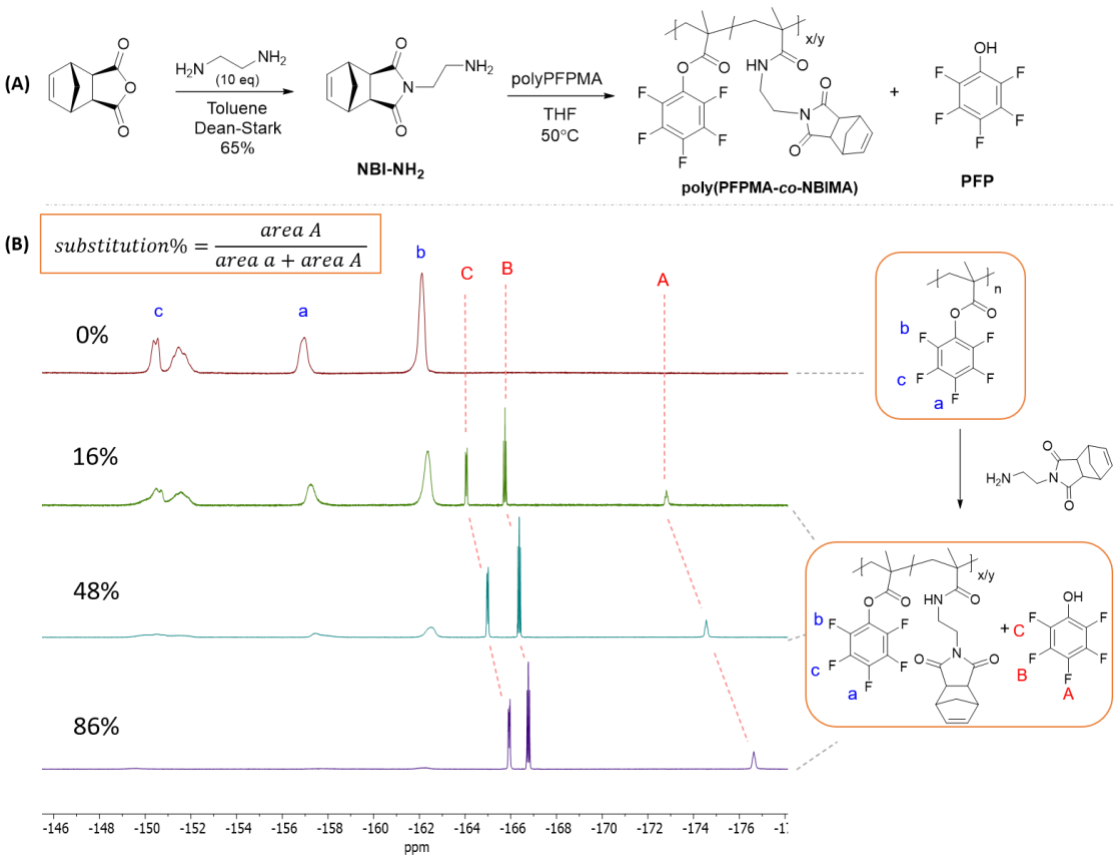


Figure 5. (A) Synthesis of poly(PFPMA-co-NBIMA) by amine-pentafluoroester substitution; (B) Stack of ^{19}F NMR spectrum showing various degree of substitution.

An amine-functionalized norbornene imide NBI-NH_2 was then synthesized and subjected to nucleophilic substitution of polyPFPMA. (Figure 5A) Due to the superior nucleophilicity of primary amine, the reaction proceeded at mild conditions and the degree of substitution could be monitored by ^{19}F NMR. (Figure 5B) The polyPFPMA homopolymer contained three broad peaks at -151, -157 and 162 ppm corresponding to the meta, para and ortho fluorine. The substitution produced pentafluorophenol which showed three new peaks in ^{19}F NMR and the degree of substitution could be calculated based on the ratio of peak integration. As shown in Figure 2B, the polymer peaks became smaller with higher degree of substitution while the pentafluorophenol peaks became larger. A homopolymer of polyNBIMA could be synthesized based on this method when the degree of substitution reached 100%. As seen from Figure 6, the broad fluorine peaks on polyPFPMA completely disappeared and all transformed to pentafluorophenol. After workup to remove pentafluorophenol, no fluorine peak was present indicating all pentafluoroesters substituted with NBI-NH_2.

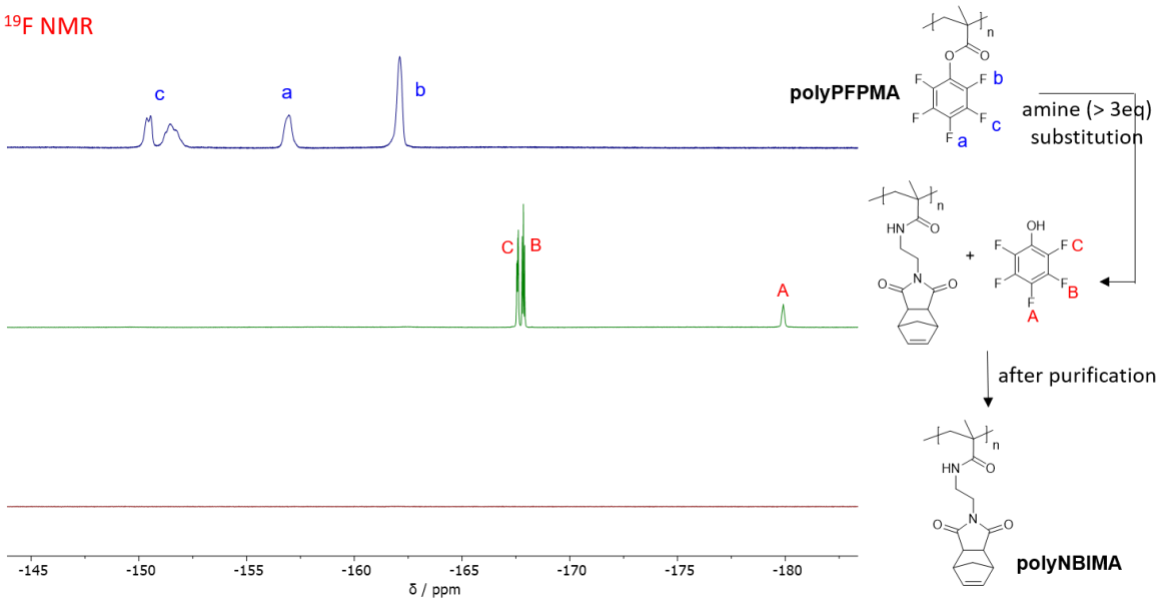


Figure 6. Synthesis of polyNBIMA from polyPFPMA and ^{19}F NMR spectrum of the polymers before and after complete substitution with NBI-NH_2.

Intra- versus intermolecular crosslinking

Polymers P1-P4 were treated with Grubbs 3rd generation catalyst to initiate intra-chain ROMP of pendant norbornene groups. (Figure 7A) Two series of reactions were attempted for each polymer, targeting intra-chain degree of polymerization of 5 and 30. The corresponding nanoparticles were denoted NPx-DP5 and NPx-DP30. In order to promote intra-chain polymerization over inter-chain crosslinking, the reactions were run at a low concentration of 0.75 mg/mL before being quenched with vinyl ether. The nanoparticles obtained were analyzed by GPC and the results are summarized in Figure 7.

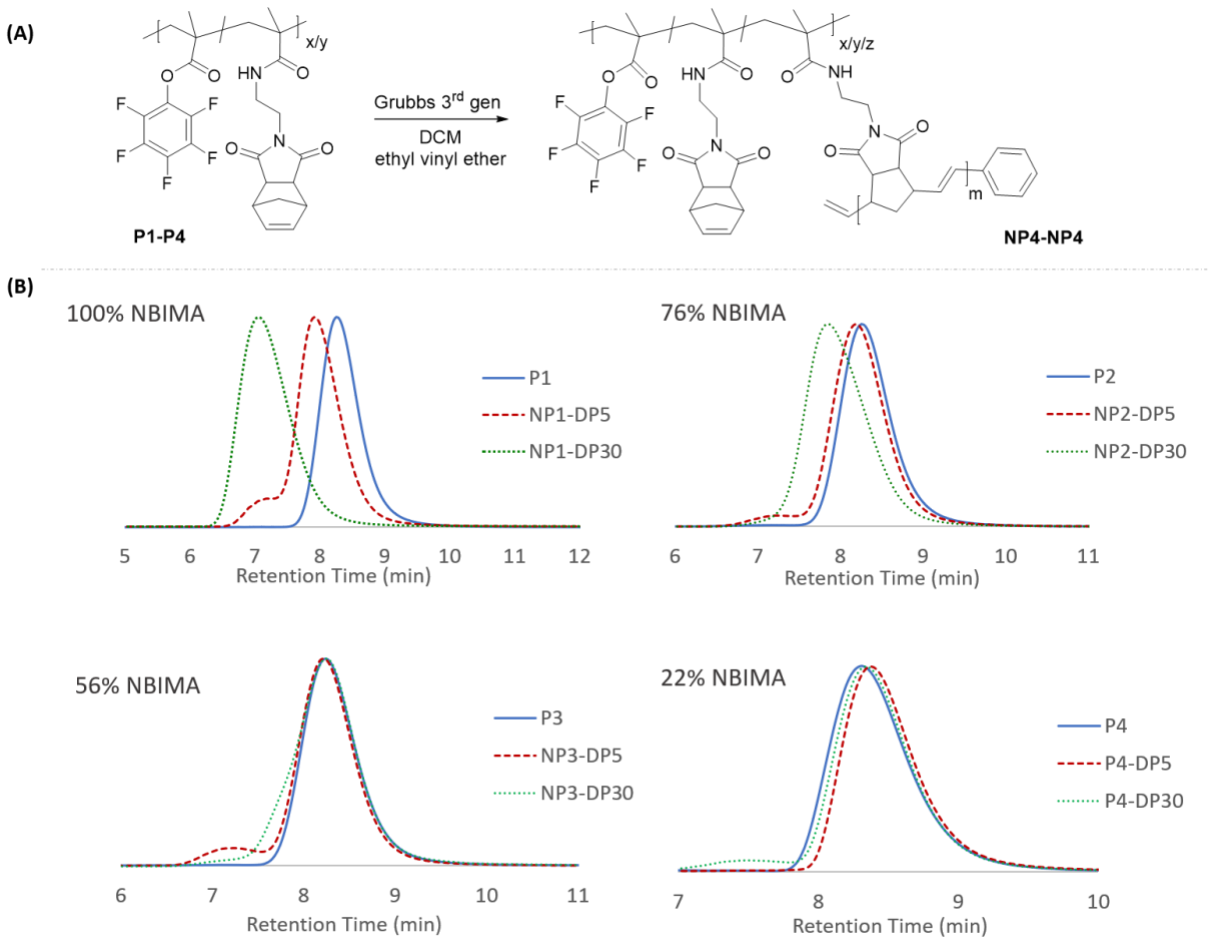


Figure 7. (A) Intra-chain ROMP of P4.1-P4.4; (B) GPC-MALS traces of P4.1-P4.4 and corresponding NP4.1-NP4.4.

The synthesis of SCNP by intra-chain cross-linking reactions is always in competition with inter-chain reactions which lead to a high molecular weight, cross-linked network. The two reactions are chemically equivalent, and the ratio of intra- versus inter- depends solely on the probability of pendant norbornene groups finding each other. When the polymers are dissolved in an ultra-dilute solution, each polymer coil is proposed to be independent. Reactive pendant groups have higher probability of finding a reacting partner along the same chain, so intra-chain reactions within the solvated coil is enhanced. The conformation of a linear polymer precursor becomes denser as a result of intra-chain cross-linking reaction, leading to a nanoparticle with smaller hydrodynamic volume. Hence an SCNP shows longer retention time than its linear precursor when eluted in GPC. When inter-chain cross-linking reactions occur, the molecular weight of a polymer will grow by orders of magnitute. Compared with its linear polymer precursor, inter-chain cross-linked product often exhibits shorter retention times or a shoulder peak on the high molecular weight end. As shown in Figure 7B, when polymers P1 through P4 were subjected to intra-chain ROMP targeting 5 norbornene units per Grubbs catalyst, only P4 gave an SCNP NP4-DP5. The nanoparticle shows longer retention time than its polymer precursor, indicating smaller hydrodynamic volume resulting from intra-chain ROMP. Polymers with high incorporations of NBIMA including P1 and P2 encountered significant inter-chain cross-linking.

The retention times of the resulting nanoparticles NP1-DP5 and NP2-DP5 were shorter than their linear precursors, indicating larger hydrodynamic volume. The GPC traces of P3 and NP3-DP5 were largely overlapped, except that the nanoparticle trace has a small shoulder peak with high molecular weight. This represents a small amount of inter-chain crosslinking during intra-chain ROMP. When the target degree of intra-chain polymerization is 30, similar results were found – only P4 gave single-chain nanoparticles while P1-P3 showed signs of high molecular weight product from inter-chain cross-linking. The degree of inter-chain cross-linking is more significant when the target intra-chain DP is 30, evidenced by the larger shift to short retention time in NP1-DP30 and NP2-DP30. This is attributed to the small feed amount of Grubbs catalyst and fewer propagating chains in the process of polymer folding. The relationship between reactive group incorporation and single chain folding of linear polymer precursors is consistent with our previous findings: high incorporation of reactive pendant groups leads to inter-chain crosslinking and lowers the efficiency of SCNP synthesis. This is because polymer coils in dilute solutions are free to move, and collisions between polymer chains are unavoidable. When collisions occur between two polymer coils, those bearing fewer reactive groups have a smaller chance of having reactive groups in the correct orientation to for crosslinks. A polymer containing more reactive groups is more likely to react with a coupling partner on another polymer chain during collision and form a dimer. This phenomenon is especially prominent for fast reactions such as radical coupling and ROMP.

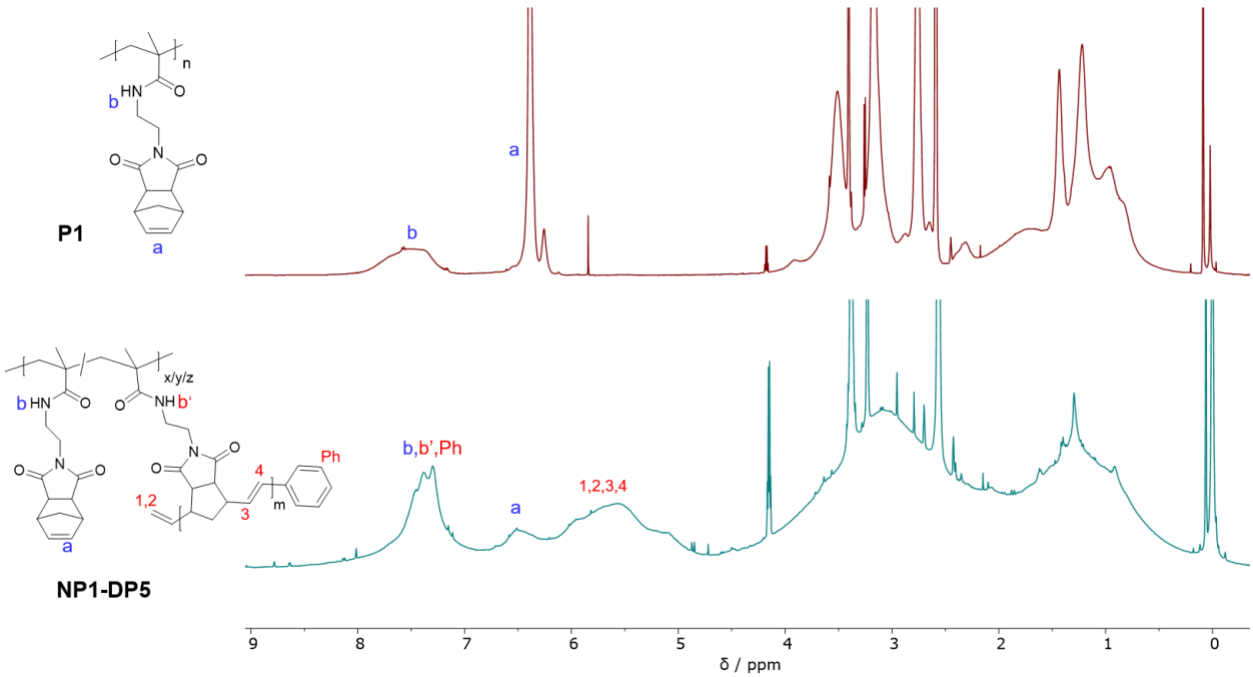
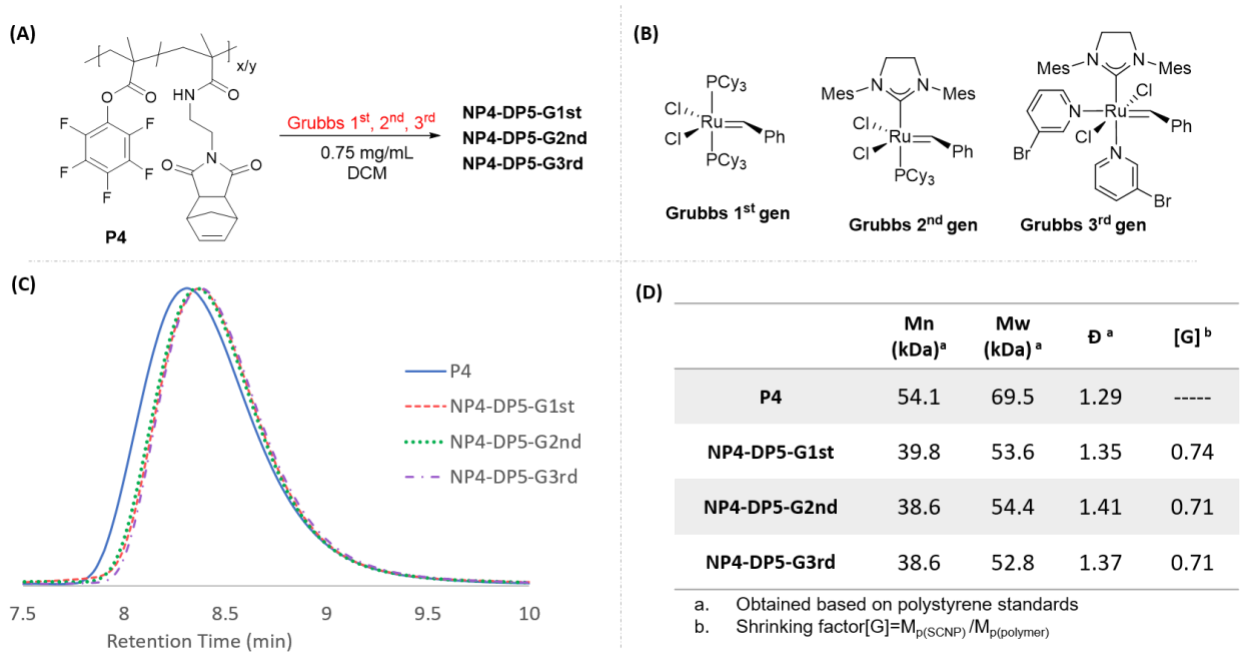


Figure 8. ^{1}H NMR spectrum of P1 and NP1-DP5 in d6-DMSO.

The polymers and nanoparticles were characterized by ^{1}H NMR. (Figure 8) Amide protons on the polymers appear at 7.1-7.9 ppm. Olefin groups on pendant norbornene show up at 6.1-6.6 ppm. After ROMP of norbornene groups, some unreacted norbornene peaks remained. New peaks showed up at a broad range of 4.9-6.2 ppm corresponding to the polymerized norbornene units, as well as vinyl end groups from quenching agent ethyl vinyl ether. Phenyl end groups from Grubbs initiator are overlapped within amide proton peaks. Although the degree of ROMP cannot be quantified from integration due to the broadness and overlap of peaks after crosslinking, the emergence of polymerized norbornene peaks confirms successful polymerization of pendant norbornene groups on linear polymer precursors.

Grubbs catalysts

We compared the efficiency of 1st, 2nd and 3rd generation Grubbs catalyst in the self-folding of polymer P4 by intra-chain ROMP. (Figure 9) Grubbs 3rd gen catalyst is the most used for ROMP of cyclic olefins. The dissociation of electron-deficient bromopyridine ligands is extremely rapid and rebinding is slow, leading to fast initiation of polymerization and narrow distribution of the resulting polymers. The N-heterocyclic carbene ligand on the catalyst improves the complex's selectivity for binding olefinic substrates in the presence of free phosphine ligands, hence increases the turnover of the intermediate. To investigate whether Grubbs 3rd gen catalyst exhibit superior behavior for intra-chain ROMP of norbornene pendants on linear polymer precursors, we subjected polymer P4 to 1st, 2nd and 3rd gen Grubbs catalysts under the same conditions. A shrinking factor defined as $[G] = M_{p(SCNP)} / M_{p(polymer)}$ where M_p represents the peak molecular weight of the sample was used to evaluate the level of compaction.[35] It was found that all three catalysts successfully initiated intra-chain ROMP and folded the linear polymers to smaller single-chain nanoparticles. The GPC peak retention and peak shape of the nanoparticles were about the same. The shrinking factors of the three SCNP were 0.74, 0.71 and 0.71, respectively. The nanoparticle synthesized from 1st gen Grubbs catalyst is slightly less compacted than the other two, but overall, the level of compaction from the linear polymer precursor were similar for all 3 catalysts.



	Mn (kDa)[a]	Mw (kDa)[a]	Đ [a]	[G] [b]
P4	54.1	69.5	1.29	-----
NP4-DP5-G1st	39.8	53.6	1.35	0.74
NP4-DP5-G2nd	38.6	54.4	1.41	0.71
NP4-DP5-G3rd	38.6	52.8	1.37	0.71

a. Obtained based on polystyrene standards
b. Shrinking factor[G]=$M_{p(SCNP)}$ /$M_{p(polymer)}$

Figure 9. Intra-chain ROMP of P4 by Grubbs 1st, 2nd and 3rd gen catalyst (A, B); GPC-MALS traces (C) and molecular weight data (D) of P4 and corresponding nanoparticles.

Doping effect of fluorinated aromatic comonomers

Researchers have found that fluorinated aromatic hydrocarbons (FAH) exhibit doping effects on second and third generation Grubbs' catalyst.[36-38] When a pentafluoroester moiety was attached to a sufficiently long spacer unit on a ROMP active monomer, Grubbs 2nd and 3rd gen catalysts demonstrated extremely fast and uncontrolled rates of propagation while Grubbs 1st gen catalyst gave well-controlled polymers. The actual mechanism of this phenomenon is under debate, but it is widely accepted that the doping effects of FAH on ruthenium centers is caused by strong, stabilizing π-π interactions. Since the norbornene-bearing copolymers we used contain pentafluoroesters, intra-chain ROMP might be affected by doping effects as well. The comparison between 3 generations of Grubbs catalysts on single chain folding of P4 revealed that all 3 catalysts led to SCNP with shrinking factors of around 0.7. Specific doping effects of FAH on 2nd and 3rd gen Grubbs catalysts were not obvious when performing ROMP intramolecularly. We further substituted the residual pentafluoesters on polymer P4 with n-hexylamine to afford polymer P5. (Figure 10A) P5 was folded with 3rd gen Grubbs catalyst and the results were compared with P4. As shown in Figure 10B, from P5 to NP5 there was a very small shift to longer retention time indicating successful intramolecular folding of the linear polymer precursor by ROMP. Although P4 and P5 contain the same molar incorporation of the ROMP-active norbornene pendants, and the two polymers were subjected to the exact same procedures synthesizing SCNP, the resulting nanoparticles NP4 and NP5 showed different levels of compaction. The shrinking factor of NP5 was 0.94, larger than NP4 which was 0.71, indicating NP5 less compacted than NP4. This could be caused by the doping effects of pentafluoroesters on P4 to the 3rd gen Grubbs catalyst, causing a faster intra-chain propagation which led to a more compacted single-chain nanoparticle.

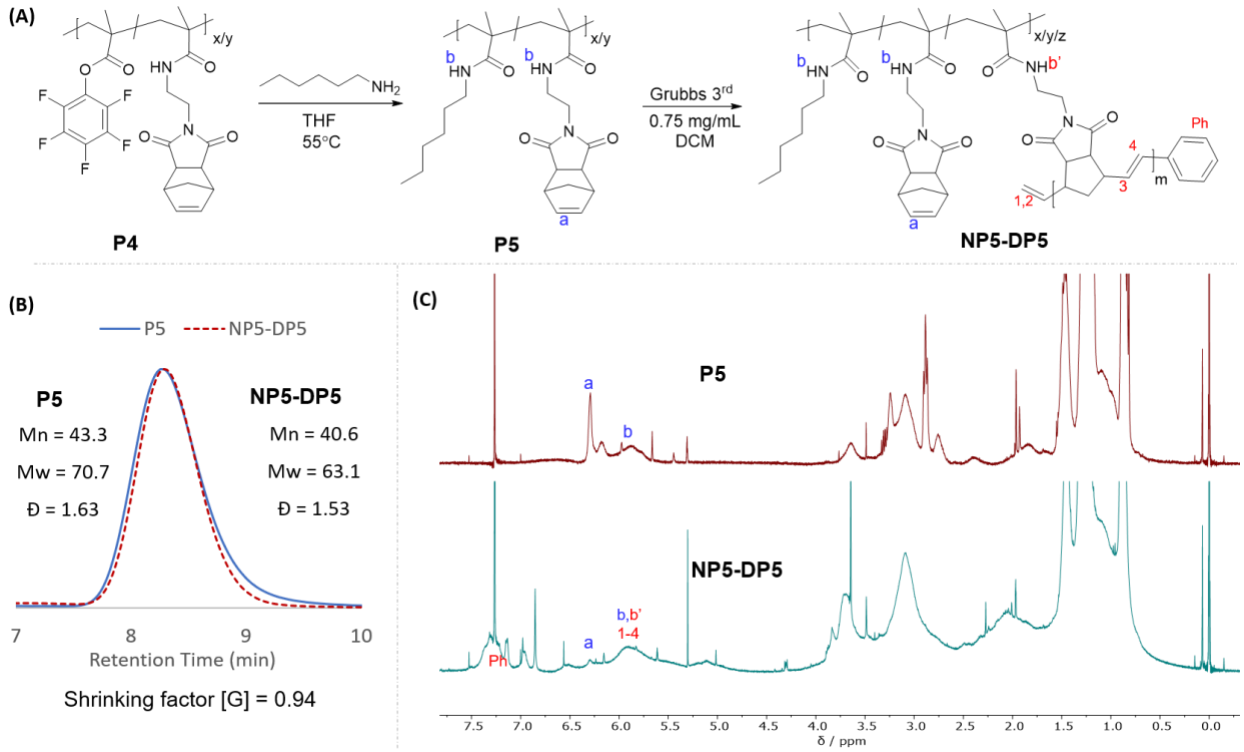


Figure 10. (A) Synthesis of P5 and NP5-DP5; (B) GPC-MALS traces of P5 and NP5-DP5; (C) ^{1}H NMR spectrum of .5 and NP5-DP5.

Research Objective Three: Mimicking Protein Quaternary Structure via Controlled Assembly and Disassembly of Polymer Nanostructures.

Research Objective three is perhaps the most technologically complex amoung the work outlined for this effort. As it requires extremely complicated syntheses, most of the work over the previous reporting period centered on this. Figure 11 outlines the basic scope of this research objective.

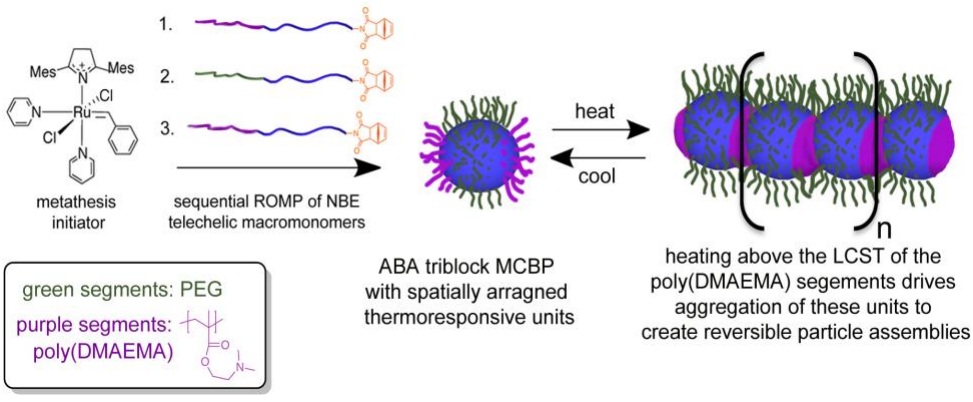


Figure 11: Design principles behind the progress made on Objective 3.

Figure 12 shows some of the progress made towards these ends. We are continuing synthesis as well as focusing on improved NMR characterization using the new 750 MHz instrument now available at UNH.

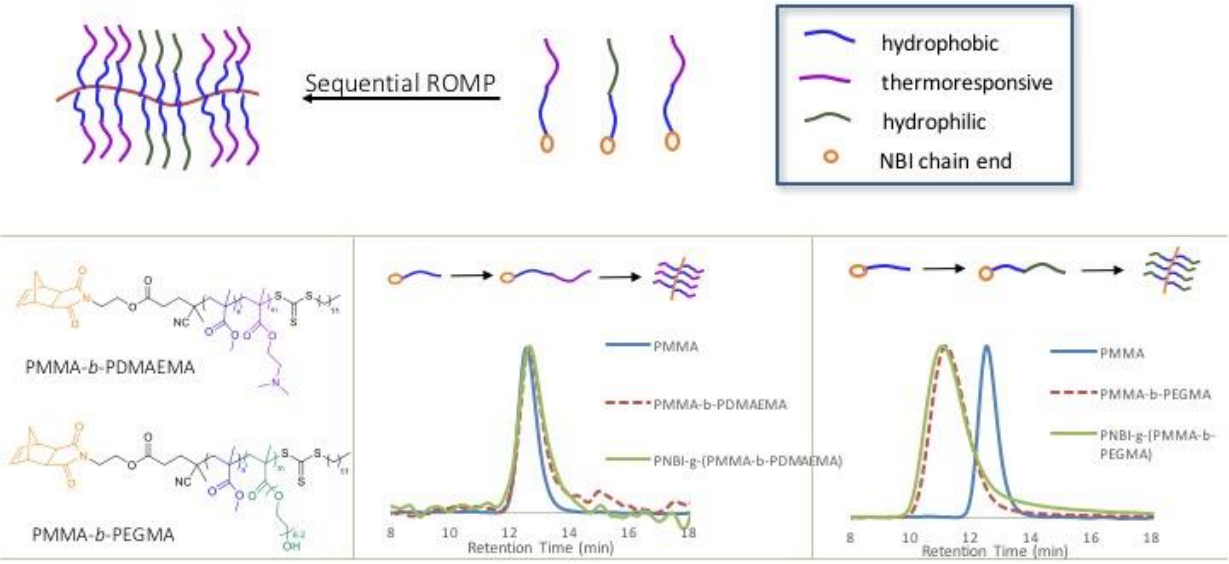


Figure 12: Structures and corresponding GPC data for materials under exploration for this research objective.

Research Objective 4: Mimicking Protein Function via Synthetic Enzymes

Work on this research area primarily focused on exploring design parameters and logistics associated with making rudimentary synthetic enzymes. This resulted in one submitted paper (in revision at RSC Polymer Chemistry) and one in preparation.

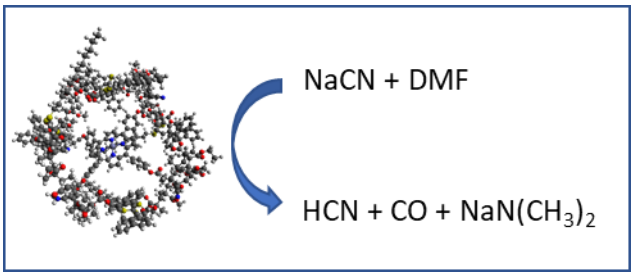
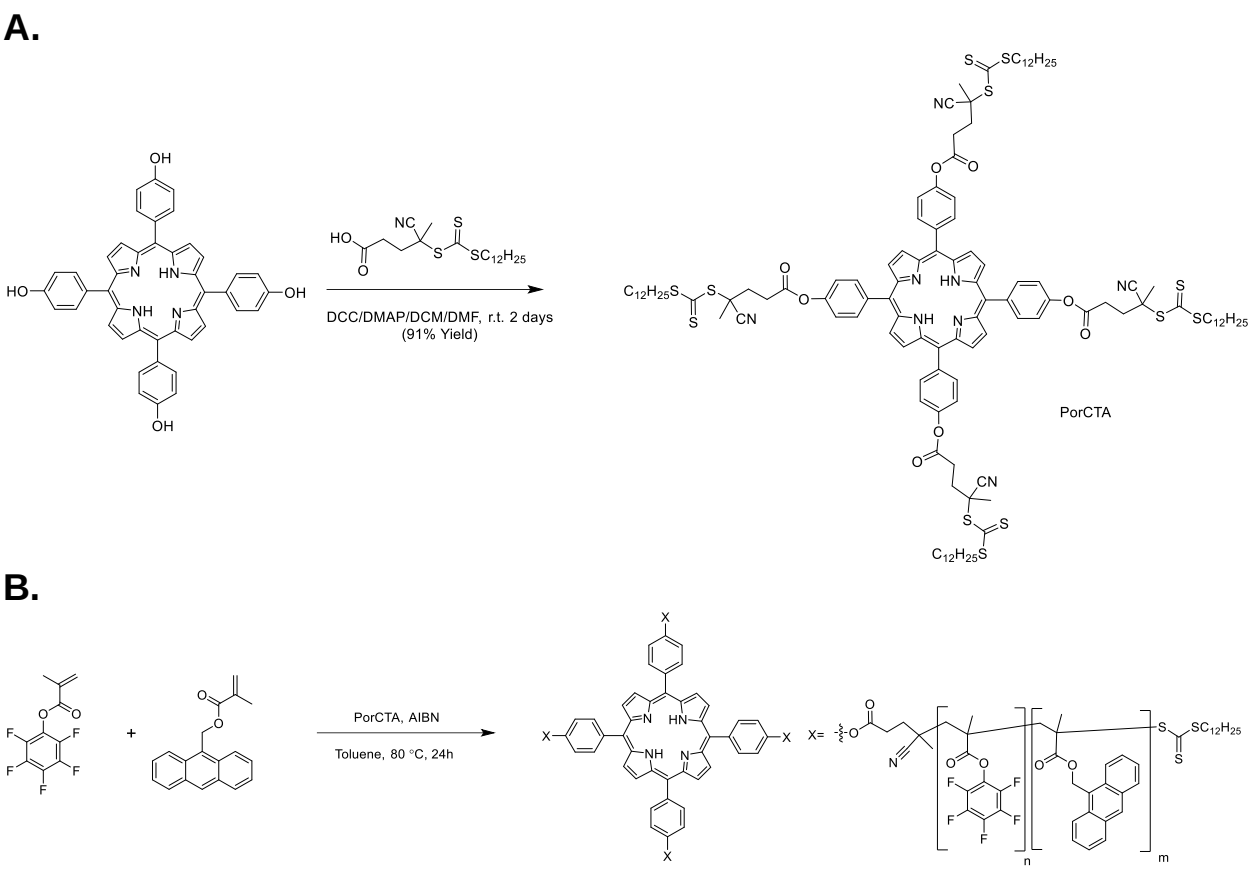


Figure 13: PCPN enzyme models

Figure one shows a schematic of one artificial enzyme system we explored. Here, a suite of zinc porphyrin-cored random coil polymers and porphyrin-cored polymeric nanoparticles (NCPN) with varying degrees of potential hydrogen bonding character and steric bulk where synthesized and characterized in order to study secondary coordination sphere interactions. The reaction of cyanide with N,N-dimethylformamide in the presence of porphyrin-cored polymeric nanoparticles was monitored via UV-Vis spectroscopy. It is shown that the zinc porphyrin-cored polymers and nanoparticles catalyzed the reaction of cyanide with N,N-dimethylformamide with the highest reactions rates occurring with polymeric nanoparticles with a greater number of potential hydrogen bond donors and greater steric bulk.

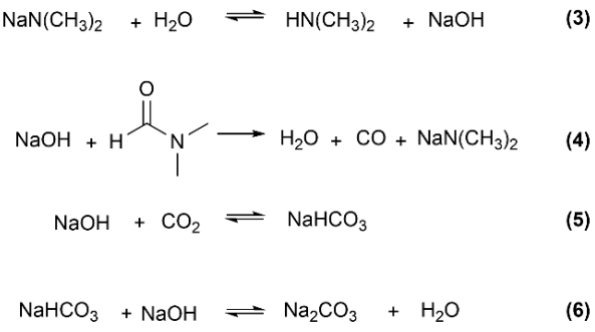


Scheme 2: Synthesis of PCPN precursors

Scheme 2 above describes the general path we took to afford these materials. First, porphyrin is decorated with 4 RAFT chain transfer agents (**A**). Subsequent polymerization from each of these sites using pentafluorophenyl methacrylate (PFMA) as a functional handle, and anthracenyl methacrylate (AMA) as a photo cross-linking agent, results in the precursor star polymer (**B**). Coordination with Zn provides a catalytically active unit (**C**). Subsequent substitution of the PFMA

unit tunes the amount of hydrogen bonding character available while dilution and irradiation result in PCPN formation.

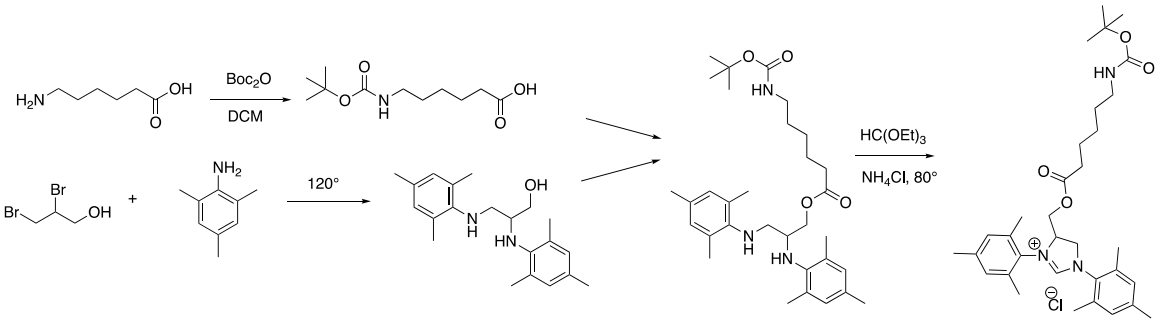
Through a series of UV vis and NMR experiments, we discovered that these PCPN are able to catalyze the series of reactions highlighted in scheme 3.



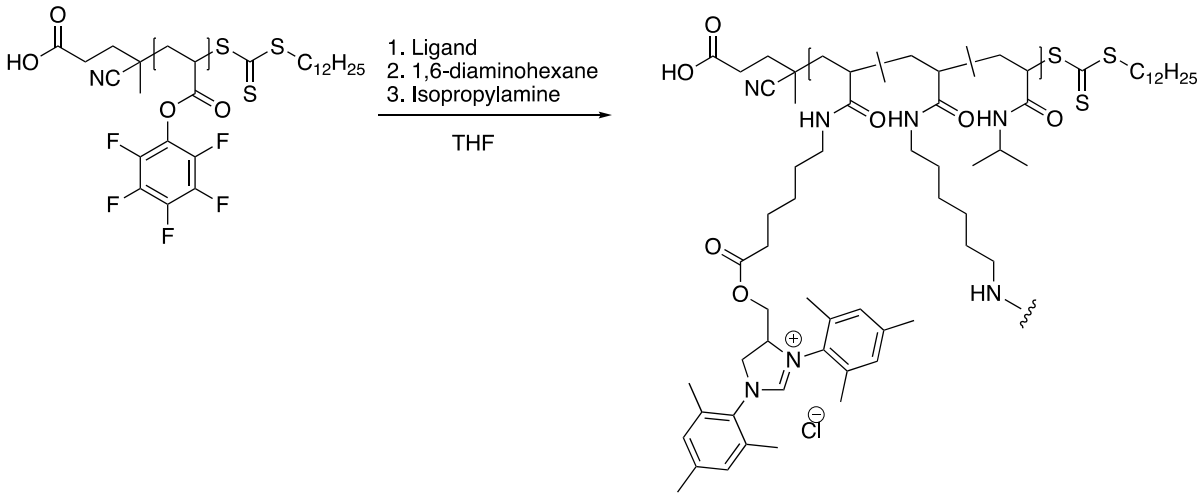
Scheme 3: Reaction of NaCN with DMF catalyzed by PCPN

We also made progress on a Ruthenium functionalized SCNP with the goal of making an "olefin metathase" enzyme mimic that can perform aqueous olefin metathesis. The synthetic scheme below outlines the current progress.

A.

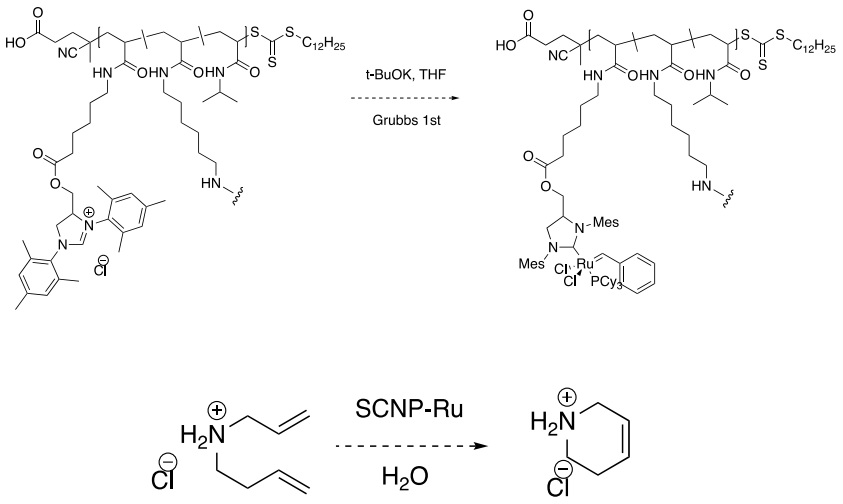


B.



Scheme 4: Synthesis of "metathase" synthetic enzyme. (A) Pendant NHC ligand synthesis. (B) NHC functionalized SCNP synthesis

After optimizing the reaction conditions, we can synthesize these materials in gram quantities. Coordination of ruthenium and subsequent catalysis are ongoing (Scheme 5)



Scheme 5: Ongoing metal coordination and catalysis experiments.

DNA bottle-brush polymers

Background information and hypothesis.

The scope of synthetic methods for preparing DNA bottlebrushes (BBPs) stands to benefit from continued innovation. Current literature examples focus on incorporating poly (ethylene glycol) (PEG) to impart increased control over hierarchal assemblies and stability for gene delivery strategies. However, most reported examples use a carbon-carbon backbone, limiting biomedical efficacy and leveraging synthetically derived DNA. We utilized biologically derived pUC19 plasmid DNA (pDNA) as the backbone. Having pUC19 as the backbone allowed unimolecular, high-molecular-weight BBPs with inherent degradability and tunability over DNA-polymer architecture.

Preliminary Data.

Inspired by the available chemistries for directly alkylating DNA and our group's expertise in chemical biology - we leverage biologically derived pUC19 plasmid DNA (pDNA) - which serves as a backbone for synthetically made poly (ethylene glycol) methyl ether mustargen (mPEG-CEA) to alkylate under biologically relevant conditions. We can generate a variety of linear and macrocyclic bottlebrush polymers by varying the molecular weight of mPEG-CEA and the concentration relative to the nucleobases of pDNA (Figure 1). We employed atomic force microscopy (AFM) and agarose gel electrophoresis to visualize the formed bottlebrushes. This

method is a facile route to achieve PEGylated DNA materials, which can significantly improve DNA's ability as a functional material.

Through direct visualization using AFM, the results of these experiments are summarized in Figure 2. We observe conformational changes in the plasmid isoforms by varying the molecular weight of PEG alkylated to pUC19 (Mw = 2000 g/mol or Mw = 750 g/mol). Figure 2a-b shows linearized pUC19 with PEG attached throughout the backbone of pUC19. In contrast, Figure 2c showcases that when alkylating native pUC19 (containing supercoiled and open circular isoforms), a more open circular structure when compared to non-PEGylated native pUC19 (Figure 3). Opening of the supercoiled DNA is partly due to the instability of the supercoiled isoform. Interestingly, differences when PEGylating with PEG_{750} show a more uniform distribution of PEG throughout the backbone, possibly owing to a higher graft density (Figure 2D). Upon visualizing supercoiled pUC19-PEG750, large aggregations were observed, possibly demonstrating some ability for these PEGylated plasmids to self-assemble in some manner.

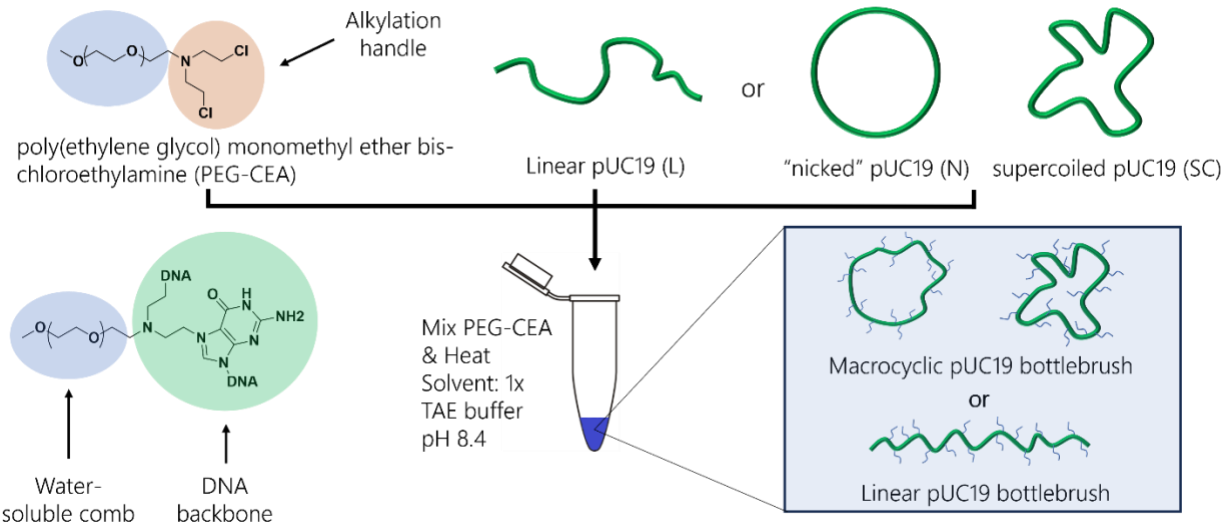


Figure 1. Schematic representation of pUC19 bottlebrush synthesis

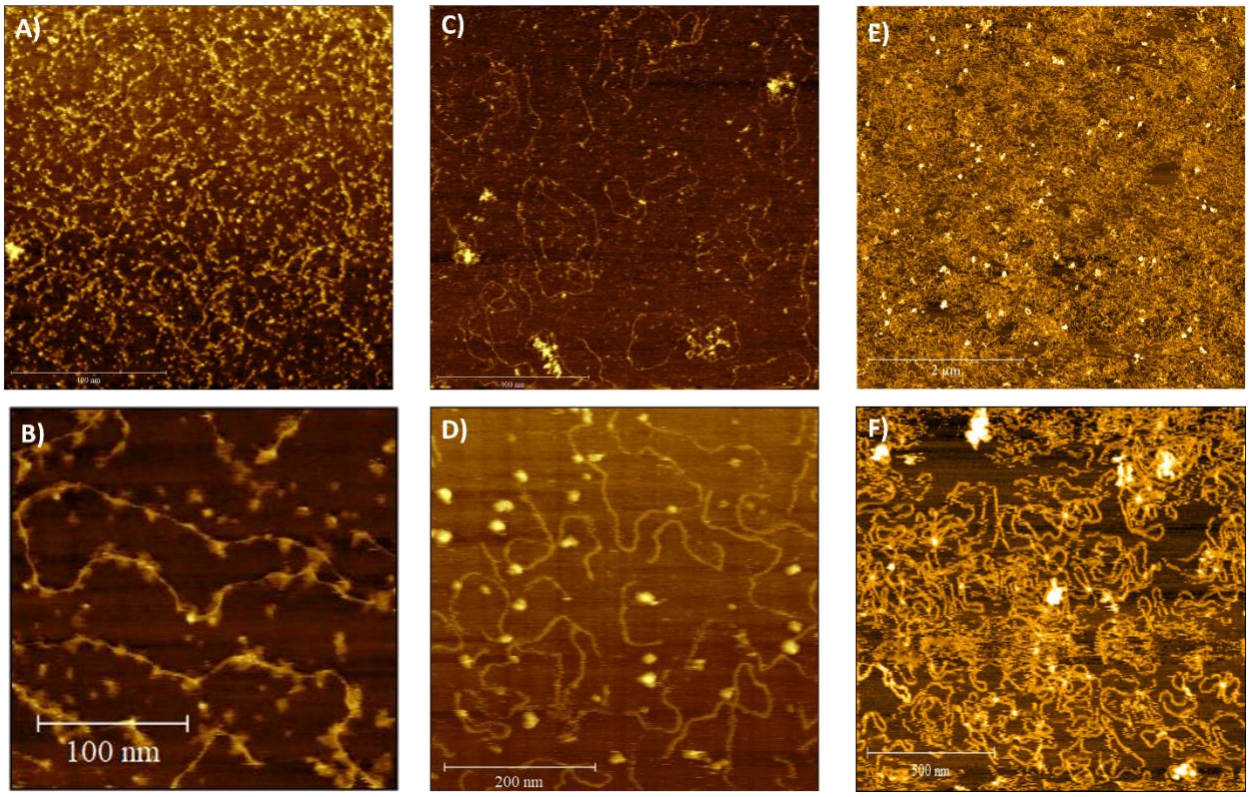


Figure 2. A) 1 µm scan of linear pUC19-PEG_{2000} B) 254 nm scan of linear pUC19-PEG_{2000} C) 5 µm scan of native $pUC19_{2000}$ D) 512 nm scan of linear pUC19-PEG_{750} E) 5 µm scan of native pUC19-PEG750 F) 1.5 µm scan of native pUC19-PEG750

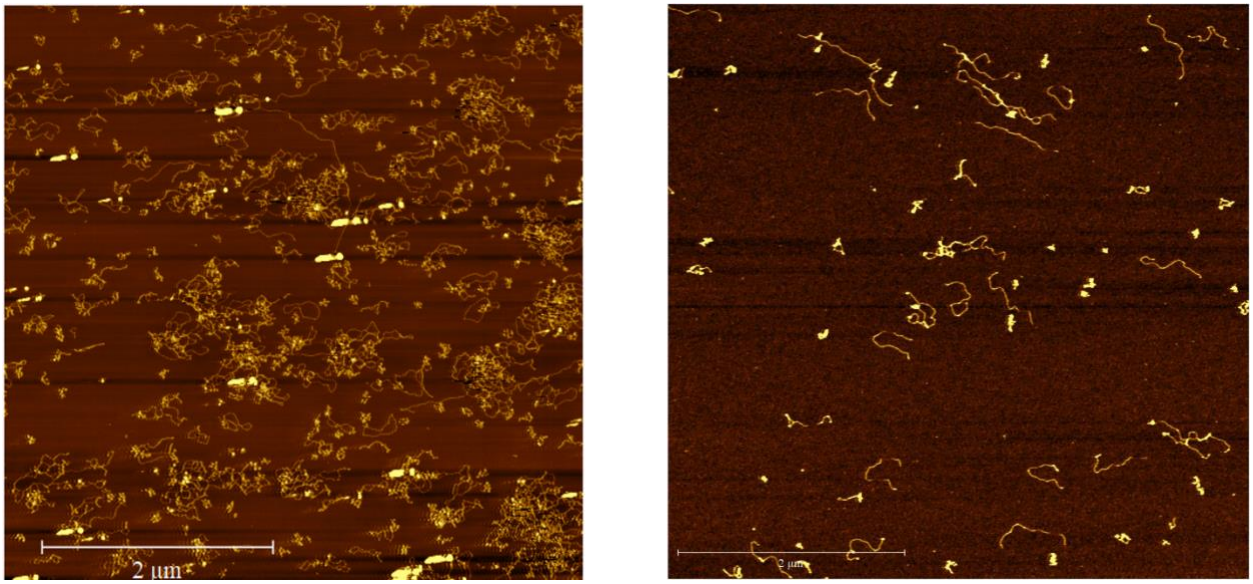


Figure 3. A) 5 µm scan of linear pUC19 B) 5 µm scan of native pUC19

Biologically derived SCNPs.

Inspired by the preliminary results, we are actively plan synthetic routes for generating SCNPs using biologically derived pUC19 plasmids (Figure 4). SCNPs would be a natural extension of the project of a bifunctional PEG-CEA to form intramolecular crosslinks under the same experimental conditions we have optimized for the plasmid bottlebrushes. To our knowledge, there are no reports of using DNA as a polymeric backbone to generate an SCNP. Controlling the number of crosslinks could lead to predictable collapsing structures by tuning PEG-CEA concentration relative to the number of nucleobases present. Accessing collapsed isoforms of plasmid DNA could be vital for pioneering new gene delivery strategies and drug delivery methods.

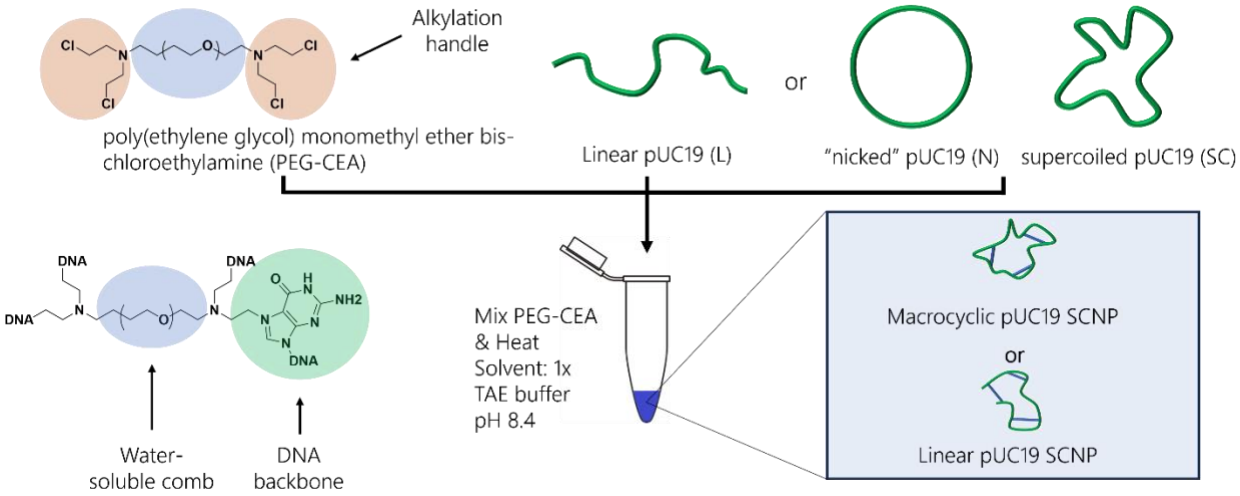


Figure 4. Schematic representation of pUC19 SCNP synthesis