Enzymatic Formation of Supramolecular Hydrogels Based on Self-assembly of DNA Derivatives

A Dissertation

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ABSTRACT

Enzymatic Formation of Supramolecular Hydrogels Based on Self-assembly of DNA Derivatives

A thesis presented to Department of Chemistry

Graduate School of Arts and Sciences
Brandeis University
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This thesis describes the design and synthesis of a new hydrogelator Nap-FF-DCp based on the conjugates of oligonucleotide and peptide derivatives. An enzyme is used to control the dephosphorylation of the hydrogelator and to regulate the formation of supramolecular hydrogels. The hydrogelator can also form hydrogels efficiently by adjusting pH = 10. Transmission Electron Microscopy (TEM) images show that compounds 2 and 3 self-assemble into nanofibers. The hydrogelator show the ability to form helical nanofibers within the hydrogels. These results indicate that supramolecular hydrogels, as an expression of the self-assembly of molecules in water, promise a broad range of biomaterials and therapeutics.
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CHAPTER ONE

INTRODUCTION TO ENZYMATIC FORMATION OF
SUPRAMOLECULAR HYDROGELS

1. Supramolecular Hydrogel

The discovery and design of small organic molecules capable of gelling aqueous solvents (hydrogelators) is a rapidly expanding area of research, in particular due to their possible practical applications in tissue engineering\(^1\), vehicles for controlled drug release,\(^2,^3\) and pollutant capture and removal.\(^4\) A gel is a solid, jelly-like material that can have properties ranging from soft and weak to hard and tough. Gels are defined as a substantially dilute cross-linked system, which exhibits no flow when in the steady-state.\(^5\) By weight, gels are mostly liquid, yet they behave like solids due to a three-dimensional cross-linked network within the liquid. It is the cross-links within the fluid that give a gel its structure (hardness) and contribute to stickiness (tack). Flory gave a definition of gel. A gel must possess a continuous structure of some sort, the range of continuity of the structure being of macroscopic dimensions. The continuity of structure must possess a degree of permanency --- at least for a period of time commensurate with the duration of the experiment.\(^6\) Usually, gels are formed by dissolving a small amount (usually 0.1-10 wt %) of gelator in hot solvent (water for hydrogels).\(^7\) Upon cooling below the \(T_{gel}\) (temperature of gelation), the molecules in the solvent will self-assembly to form
immobilized hydrogels (often tested by turning the test tube upside down; if no flow is observed, the solution is said to have gelled).

Figure 1.1. The primary, secondary and tertiary structure of a self-assembled physical gel

The formation of gels is thought to result from the self-assemble of fibers (nano- to micrometer) which become entangled and store solvent via surface tension. To form a gel, the tendency of the molecules to aggregate and to dissolve must be balanced. The key to the design of organic hydrogelators is the control of the balance of hydrophobicity and hydrophilicity of the hydrogelaor. Many studies have shown the formation of gels can be affected by weak interactions such as hydrogen bonding, hydrophobic interactions, salt bridges and transition metal coordination. To understand the mechanism of gel formation, a gel can be broken down into a primary,
secondary and tertiary structure, much like a protein (Figure 1.1). The primary structure (angstrom to nanometer scale) is determined by the molecular level recognition events that promote anisotropic aggregation in one or two dimensions of the gelator molecules.

![Diagram of possible aggregates of amphiphilic molecules](image)

**Figure 1.2.** Schematic illustrations of possible aggregates of amphiphilic molecules in aqueous solution. The arrows represent possible interconversion pathways, dependent on concentration and other solution variables (pH, ionic strength, etc.)

The secondary structure (nano- to micrometer scale) influenced by the molecular structure comes from the morphology of the gels, such as micelles, vesicles, lamellae, and amorphous or crystalline precipitates. (Figure 1.2) To understand the multiple morphologies of the gels, people did lots of studies on the mechanism of aggregation of amphiphilic organic molecules in water. Micelles are fluid species which depends on the structure of the amphiphile. Above this concentration, micelles can convert to ellipsoidal micelles (disks) and then, with further increase in concentration, to cylindrical
micellar fibers (rods). These fibers, however, generally precipitate or display viscoelastic behavior at concentrations above the critical micelle concentration without forming a gel, due to electrostatic repulsion of the charged surfaces.  

Finally, the tertiary structure of a gel (micro- to millimeter scale) is related with the interaction of individual aggregates which determines the formation of a gel or precipitation of the nanofibers from the solution. The formation of gels results from the self-assemble of nanofibers. The type of cross-linking of fibers included physically branched fibers (interconnected networks) and entangled fibers. Although both types of interactions can form a gel, physically, long, thin, flexible fibers are easier to store solvent and to afford a gel than shorter fibers. For example, Liu, Sawant, and co-workers have examined how the presence of an additive can increase the formation of long, branched fibers and lead to the formation of a gel. The properties of gels can also be affected by other physical conditions, such as the gelation temperature.

Supramolecular hydrogel contains two interpenetrated phases—the solid phase and the liquid phase—that are three dimensional and continuous. The liquid phase of hydrogel is water and the solid phase a network of nanofibers formed by the self-assembly of small molecules (i.e., supramolecular hydrogelators or molecular gelators). Though more than 97% of the hydrogel is water, it behaves as a solid and will not flow without a shear force. Normally, supramolecular hydrogels undergo a phase transition upon the application of an external stimulus because they are formed by the self-assembly of small molecules (normally with molecular weights less than 2,000 g/mol) via weak interactions (e.g., hydrogen bonding and hydrophobic interactions). The unique property of supramolecular hydrogels allows them easily to be biocompatible and
biodegradable, and their resemblance of extracellular matrices have stimulated efforts to
design and synthesize novel supramolecular hydrogelators as materials for biomedical
applications.27-30

Obviously, supramolecular hydrogels formed by bioactive molecules attract
more attentions than the others without biological functions because they can potentially
serve as useful materials for tissue engineering,31,32 biosensing,33,34 and drug delivery.35,36
For examples, hydrogels formed by nanofibers of self-assembled oligopeptides have been
used as scaffold to support the cell survival, assist the cell division, or even promote the
cell differentiation.37 Several excellent reviews22,23,38,39 have provided authoritative and
comprehensive information by summarizing the successful examples of supramolecular
hydrogels from the aspects of synthesis, structure, and characterization. Several research
groups have already developed supramolecular hydrogels based on the small bioactive
molecules and explored the relevant applications. For example, using supramolecular
hydrogels based on the nanofibers of glycosylated amino acetate, Hamachi and
coworkers have developed an elegant approach for the construction of protein arrays.34
While the aqueous cavities in the hydrogel matrix act as an excellent reaction medium for
enzymes, the amphiphilic the nanofibers of the matrix serve as a unique substrate to
enhance the fluorescence of the fluorophore reporters. This novel supramolecular
hydrogel system avoids denature of the proteins, a major drawback of other conventional
protein chips, and it promises useful applications in medical diagnosis and biological
researches.29,33 Hamilton and coworkers have designed and synthesized a series of small
molecular hydrogelators and studied the principle of molecular arrangements in the gel
state.23,40 Shinkai et al. have reported many supramolecular gelators and developed an
elegant approach to synthesize silica nanofibers using the supramolecular gels as templates.\textsuperscript{30,41} Kim et al. have developed the conjugates of amino acid and bioactive molecules to form supramolecular hydrogels.\textsuperscript{35,42} Suzuki et al. have made a series of amino acid derivatives to gel aqueous solution or organic solutions.\textsuperscript{25,28} Smith et al. have developed dendrimers as the supramolecular hydrogelators.\textsuperscript{43} Ulijn et al. have used enzyme to catalyze the formation of oligopeptides as the supramolecular hydrogelators\textsuperscript{44} and used the hydrogels for cell culture.\textsuperscript{32}

2. Enzyme-trigged formation of supramolecular hydrogels based on small peptide derivative

Enzymes, as a class of highly efficient and specific biological catalysts, are needed in almost all process in a biological cell. Since enzymes are selective for their substrates and speed up only a few reactions from among many possibilities, the set of enzymes made in a cell determines which metabolic pathways occur in that cell. So the expression and distribution of enzymes would lead to diverse extracellular and intracellular environments. Because nearly all known enzymes are proteins, it is possible to use enzyme to convert proper peptide derivatives into desired hydrogelators that self-assemble into nanofibers in water and form hydrogels. This enzyme-trigged process can selectively control the formation of supramolecular hydrogel in a cell according to a specific biological condition or environment, thus providing an accessible route to create effective and smart biomaterials.

(1) Modes of formation of supramolecular hydrogels.
In general, there are three modes of formation of supramolecular hydrogels—making bonds mode, breaking bonds mode and enzyme-switch mode. In all three modes, a biological process is catalyzed by enzyme and a precursor is converted to a hydrogelator, which self-assembles in an aqueous phase to form nanofibers and results in hydrogelation (Figure 1.3).

**Figure 1.3.** Illustration of the design for enzyme-instructed formation of molecular nanofibers via bond formation or bond cleavage and the macroscopic outcome (i.e., hydrogelation).

The obvious advantage is that since the expression of enzymes usually is specific, the formation of supramolecular hydrogel would exhibit selective response to the biological environments. The attachment of a hydrophilic segment to a hydrogelator by the enzyme-catalyzed bond formation would generate a precursor that is soluble in the aqueous phase. The removal of the hydrophilic segment by the enzyme-catalyzed bond cleavage would convert the precursor back to the hydrogelator, which self-assembles into nanofibers and affords the hydrogel. Such a relatively simple design permits enzyme-triggered formation of supramolecular hydrogel to be applicable on (almost) any gelators.\textsuperscript{45-52} In the following sections, enzyme-instructed formation of supramolecular hydrogel using several enzymes as the examples is summarized.
Scheme 1.1. Molecular structures of the precursor (1), its corresponding hydrogelator (2), and the enzyme-instructed transformation.

Figure 1.4. (A) Transmission electron microscope (TEM) image of the nanofibers of 2 formed by the self-assembly of 2. (B) Scanning electron microscope (SEM) image of bundles of the nanofibers of 2.

(2) Bond-breaking mode.

A phosphatase is an enzyme that removes a phosphate group from its substrate by hydrolyzing phosphoric acid monoesters into a phosphate ion and a molecule with a free
hydroxyl group. Our group used an alkaline phosphatase as the enzyme to remove the hydrophilic phosphate group from the commercially available Fmoc-tyrosine phosphate (1) to control the balance between hydrophilicity and hydrophobicity. As shown in Scheme 1.1, the addition of alkaline phosphatase to the solution converts 1 to a more hydrophobic compound, 2, which creates a small molecular hydrogelator and affords the formation of supramolecular hydrogel.53 (Figure 1.4)

(3) Bond-forming mode.

Thermolysin is the most stable member of a family of metalloproteinases produced by various Bacillus species. These enzymes are widely used for peptide bond formation through the reverse reaction of hydrolysis. Ulijn et al.54 reported the use of thermolysin to link two peptide derivatives 3 and 4 to make a more hydrophobic one 5, which creates a small molecular hydrogelator and affords the formation of supramolecular hydrogel (Scheme 1.2). This strategy could be useful in tissue engineering55 and thermodynamic control of the formation of the nanofibers.56

Scheme 1.2. Structures of 3, 4, and 5 and the enzyme-catalyzed transformation

(4) Enzyme-switch mode.
Since more than one enzyme exists in the biological system, it is quite common for pairs of enzymes to work counteractively to regulate biological functioning in nature. Therefore, it might be possible to use a pair of enzymes to control the self-assembly of small molecules and the formation of supramolecular hydrogels. Phosphatase and kinase are one pair of important enzymes involved in regulating signal transduction in cells. Phosphatase is an enzyme that removes a phosphate group from its substrate (dephosphorylation); kinase is an enzyme that transfers phosphate groups from high-energy donor molecules, such as ATP, to specific substrates (phosphorylation).

Thus, our group designed a compound that be catalyzed by phosphatase and kinase, respectively. As shown in Scheme 1.3, catalyzed by phosphatase, compound 7 can undergo dephosphorylation to give 6 which could self-assembly to form the network of nanofibers and afford the hydrogel. Treating the hydrogel with a tyrosine kinase in the presence of adenosine triphosphates (ATP), the supramolecular hydrogel of 6 will undergo phosphorylation to give the corresponding phosphate (7) to induce a gel-sol phase transition and yield a solution. (Figure 1.5)

Scheme 1.3. (A) Structures of the precursor (6) and the hydrogelator (7) and the corresponding transformations catalyzed by phosphatase and kinase.
3. Extracellular enzymatic formation of supramolecular hydrogels.

Since there are many enzymes in biological system and the enzymatic process is quite complicated and might be affected by different biological condition, it will be a challenge to use enzyme to trigger supramolecular hydrogels and related changes to control the behavior of cells. To demonstrate enzymatic formation of the supramolecular hydrogels are able to proceed in a biological environment, our group did the following studies in cell lysates or blood, where many enzymes exist and catalyze hydrolysis or other processes to affect the formation of supramolecular hydrogels.

(1) **Supramolecular hydrogels in lysates of bacteria.**

β-lactamases are enzymes produced by some bacteria and are responsible for their resistance to β-antibiotics and have caused widely spread antimicrobial drug resistance.\(^{59,60}\) The β-lactam antibiotics have a common element in their molecular structure: a four-atom ring known as a β-lactam. The lactamase enzyme breaks that ring open, deactivating the molecule's antibacterial properties. Their importance has led us to explore enzymatic molecular self-assembly catalyzed by β-lactamase.\(^{61}\)
Scheme 1.4. Structures of the precursor (8) and the hydrogelator (9) and the β-lactamase-catalyzed transformation.

As shown in Scheme 1.4, when β-lactamase exists, the β-lactam ring of the precursor 8 opens to release the hydrogelator 9, which self-assembles into nanofibers and afford a supramolecular hydrogel. Without β-lactamase, compound 8 cannot be converted to compound 9 and no hydrogel is observed.

Figure 1.6. The formation of nanofibers of 9 in the lysates of *E. Coli* that express different β-lactamases (A: CTX-M13; B: CTX-M14; C: SHV-1; D: TEM-1).
Our group also examined this facile process in the lysates of bacteria and proved that the supramolecular hydrogel of 9 in cell lysates could stay stable. (Figure 1.6) This result provides a possible method to study extracellular self-assembly by exploiting the well-established protocol of controlled-expression of β-lactamases inside cells or in subcellular organelles.62,63

(2) Supramolecular hydrogels in blood and cytoplasma.

To further evaluate the biostability of supramolecular hydrogel in biological environment, our group investigated the enzymatic hydrogelation of 11 in more challenging conditions----in blood and cytoplasm. As shown in Scheme 1.5, after studying the enzymatic hydrogelation of 11 in the buffer solution, our group treated 11 with a tyrosine phosphatase to afford the hydrogelator 12 which self-assembles to nanofibers and forms supramolecular hydrogel.64 Using similar approach, it is also feasible to use the D-amino acids to form peptide nanofibers and hydrogels to achieve long-term biostability.65

![Scheme 1.5. Structures of the precursor (11) and the amphiphile (12) and the enzyme-catalyzed transformation.](image)

4. Intracellular enzymatic formation of supramolecular hydrogels
Enzymatic formation of supramolecular hydrogels provide a unique opportunity to explore the intracellular molecular self-assembly of small molecules, because it can integrate molecular self-assembly with a wide range of biological processes involving enzymes. The use of enzyme to regulate intracellular self-assembly for the formation of supramolecular hydrogels can control the fate of cells or cellular functions. Since small molecules enter cell more easily than polymers, enzymatic supramolecular hydrogelation should be suitable for probing the intracellular processes and developing novel therapeutics.

(1) Formation of supramolecular hydrogels inside HeLa cells.

As shown in Figure 1.9, compound 13 is a soluble precursor, which doesn’t self-assemble and form nanofibers extracellularly, but it can be converted to a hydrogelator by an intracellular enzyme and form the molecular nanofibers within a cell. Our group investigated the conversion of 13 to 14 by the endogenous esterases in Mammalian cells. (Scheme 1.6) The results show that the molecules of 14 can self-assemble to form nanofibers, result in hydrogelation inside the cells and cause the cell death. As shown in Figure 1.7, most HeLa cells died at day three after the addition of 13 to the culture medium, while most of NIH3T3 cells remained alive and dividing. Because HeLa cells likely have higher esterase activities or expression levels and are able to convert more 13 to 14 than NIH3T3 cells do, so the more hydrogels form inside the HeLa cells and cause their death. Inside the NIH3T3 cells, the concentration of esterases is too low to convert enough 13 to 14, so no hydrogel form in NIH3T3 cells to cause cell death. The fluorescence assay of esterase in the two cell lines and the Congo red staining of the cells also support the mechanism. Though other factors (e.g., differences
in the uptake of 13 by HeLa and NIH3T3 cells) might also contribute to the apparent low toxicity of 13 to NIH3T3 cells, this result indicates that the kinetics of formation of intercellular nanostructure are specific to different types of cells, which may offer a new approach for control the fate of cells.

**Scheme 1.6.** An esterase to convert precursor (13) to the amphiphile (14).

Figure 1.7. (A) TEM of the nanofiber formed by 14 (inset: optical image the hydrogel). MTT assays of (B) NIH3T3 cells and (C) HeLa cells treated with 13 at concentrations of 0.08 wt%, 0.04 wt%, and 0.02 wt%.

**(2) Formation of supramolecular hydrogels inside BL21 cells.**

Some recent studies To further prove that intracellular enzymatic formation of supramolecular hydrogel can control the fate of cells, our group examined two types of E. coli strains: the wild type BL21 (as the control) and a BL21 strain (BL21(P+)) that overexpress human tyrosine phosphatase (hPTP). Since the only difference between the two strains is the expression of phosphatase, any discrepancy in the uptake of the precursor was minimized. The E. coli cells take in the precursor 16 by diffusion, the
endogenous phosphatase converts the precursor 16 into the hydrogelator 17 which self-assemble into nanofibers and results in the formation of supramolecular hydrogel (Scheme 1.7). The BL21(P+) bacteria stops growing upon the addition of 16 (IC$_{50}$ = 20 μg/mL), but the wild type BL21 bacteria grew normally (IC$_{50}$ > 2000 μg/mL) under the same condition. The formation of nanofibers of 17 inside BL21(P+) cells can be supported by TEM, HPLC analysis and Congo red staining. It is the first time to use intracellular enzymatic formation of supramolecular hydrogel to inhibit bacterial growth. The principle and the strategy demonstrated in this work could lead to a new class of therapeutic agents that take advantage of the kinetics of enzyme catalysis rather than tight ligand-receptor binding.

Scheme 1.7. A schematic representation of intracellular nanofiber formation and the inhibition of bacterial growth.
References and Notes


7. For some hydrogelators, a small amount of polar cosolvent (DMSO, methanol, ethanol, etc.) is required to dissolve the compound before addition of water.


44. S. Toledano, R. J. Williams, V. Jayawarna, and R. V. Ulijn, *Journal of the*
American Chemical Society, 2006, 128, 1070.


CHAPTER TWO

Enzymatic Formation of Supramolecular Hydrogels Based on Self-assembly of Oligonucleotide-peptide Conjugates

Introduction

Self-assembly occurs at all scales, and it plays important roles in biology, from maintaining the integrity of cells to performing cellular functions, and to inducing abnormalities that cause disease. Cellular nanostructures such as actin filaments, microtubules, vesicles, and micelles are the microscopic presentations of molecular self-assembly in biological systems. Since the establishment of the concept of supramolecular chemistry and the key role of weak interactions (e.g., Van der Waals, ionic, and hydrogen bonds) in biology and materials sciences, molecular self-assembly has become a powerful strategy to create new materials and develop new technologies. The examples include formation of supramolecular polymers by the self-assembly of molecular components, formation of liquid crystals, self-assembly of colloids, and self-assembled monolayer on metal surfaces. These conceptual and technological advances suggest the possibility to use molecular self-assembly for creating nanofibers. Being held together by non-covalent interactions, supramolecular hydrogels are
inherently biodegradable and are being explored as a new type of biomaterials, especially when the hydrogelators are bioactive molecules (e.g., oligonucleotides and their derivatives), for applications such as tissue engineering by using the hydrogels as the matrices of cell culture. Moreover, the self-assembled three dimensional fibril networks allow the entrapment of not only water molecules but also the other bioactive molecules (drugs, nutrients, and proteins) or therapeutic agents. The supramolecular nature also makes the hydrogels to exhibit rapid response to external stimuli (e.g., pH, ionic strength, or temperature changes) because the self-assembled three dimensional networks are susceptible to physical or chemical perturbations. These two features, therefore, render the hydrogels an excellent carrier for applications such as controlled drug releases. Nucleotides are some of the most complex among the small biological molecules since they contain the greatest variety of hydrogen-bonding functional groups. Therefore, nucleotides can easily catch water molecules and are generally hydrated in the crystalline state; about 45% of their crystal structures are hydrates, presenting a striking contrast to 20% for carbohydrates. Nucleotides can also be very useful as a programmable headgroup when we make use of their multiple complementary hydrogen bonds to obtain nanometer-sized structures through self-assembly. Considering the promising importance of both supramolecular hydrogels and nucleotides as new materials the fusion of these two concepts should afford interesting features applicable to a wide variety of biological and medical uses such as biocompatible materials, gels for electrophoresis and gene engineering.
Results and Discussion

1. Design and Synthesis.

I designed Nap-FF-DCp (1, Scheme 2.1.) as the hydrogelator because (i) FF is prone to self-assembly, (ii) Nap-FF gels water effectively (at 0.8% wt %) (iii) the residue of Deoxycytidine monophosphate can dephosphorylate in the presence of alkaline phosphatase.

![Scheme 2.1. Chemical structure of Nap-FF-DCp](image)

One of the motivations to use naphthalene (Nap) rather than N-(fluorenyl-methoxycarboxylic) (FMOC) is that Nap should be more biocompatible, as evidenced by several clinical drugs consisting of a Nap motif (eg., propranolol, naphazoline, nafronyl). I choose to use ester bond to connect Deoxycytidine monophosphate with Nap-FF because the ester bond can hydrolyze at pH = 10 to afford Nap-FF which could form hydrogel. This would make Nap-FF-DCp a multifunction hydrogelator: (1) Enzymatic: Alkaline Phosphatase is used to control the dephosphorylation of Nap-FF-DCp and to regulate the formation of supramolecular
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hydrogels. (Scheme 2.2.) (2) Physical: Nap-FF-DCp could also afford a hydrogel after adjusting pH = 10. (Scheme 2.3.)

Scheme 2.2. Schematic process of the formation of the hydrogelator

Scheme 2.3. Schematic process of the formation of the hydrogelator

2. Gelation Behavior.

Prevailingy existing in cells, tissues, and organs, phosphatases represent a large family of enzymes that catalyze the removal of phosphate groups from a substrate and play an important role in intra- and intercellular signaling. Because of its ability to catalyze the removal of the hydrophilic phosphate group, alkaline phosphatase became the enzyme of choice to control the balance between hydrophilicity and hydrophobicity, convert a precursor to a hydrogelator, and trigger the formation of hydrogels. Here, I report the use of an enzymatic reaction to convert an oligonucleotide-peptide conjugate
into a neutral group, which creates a small-molecular hydrogelator and leads to the formation of supramolecular hydrogels. This gelation process utilizes an alkaline phosphatase - one of the components of kinase/phosphatase switches that regulate protein activity - to dephosphorylate the PO$_4^{3-}$ of Nap-FF-DCp (Figure 2.1.). Unlike previously reported enzymatic gelation process by our group, this process, which involves bond breaking of oligonucleotide-peptide conjugate rather than bond breaking of peptide, adjusts the balance of the hydrophobicity and hydrophilicity of the precursor to yield a hydrogelator. Since dephosphorylation is a common yet important biological reaction existing in many organisms, its coupling with hydrogelation may lead to a general and useful way to generate and utilize biomaterials based on supramolecular hydrogels. I also found that this Nap-FF-DCp could also afford a hydrogel after adjusting pH = 10. (Figure 2.2.)

Figure 2.1. Optical images of (A) 8 % wt of Nap-FF-DCp solution and (B) gel of 2 formed by adding 1 μL of alkaline phosphatase (50 U ml$^{-1}$) to solution of 1
Figure 2.2. Optical images of (A) 10 % wt of Nap-FF-DCp solution and (B) gel of 3 formed by adding 1M NaOH solution into solution of 1 to adjust pH = 10

3. Circular Dichroism

As shown in Figure 2.3., both gel 2 and gel 3 have almost the same bands between 180 nm and 250 nm.

Figure 2.3. The CD spectra of the hydrogels
The negative 210-nm and the positive 192-nm bands result from the excitation splitting of the lowest peptide $\pi\pi^*$ transition\(^{40-43}\) which indicates the $\alpha$-helix secondary structure. The positive band at about 310 nm comes from the $\pi\pi^*$ transition of the base of Deoxycitidine. $\pi\pi^*$ transition of Naphthyl aromatics leads to the negative band near 287 nm.

4. Microscopic Observation of the Hydrogels.

As shown in Figure 2.4A, Transmission Electron Microscopy (TEM) images of gel 2 exhibit entangled irregular fibers, which provide the matrix for the hydrogel. Gel 3 has a slightly different morphology - the fibers, compared to those in gel 2, are wider (Figure 2.4B).
5. Conclusion and Outlook

In summary, enzymatic supramolecular hydrogelation represents a simple, yet powerful strategy to control the balance of hydrophobicity and hydrophilicity of a molecule for its self-assembly in water. In essence, a hydrophilic and enzyme accessible group attaches to a supramolecular hydrogelator to make a precursor that dissolves in an aqueous environment and the enzyme converts the precursor back to a hydrogelator via enzymatic catalysis, thus resulting in self-assembly and hydrogelation. Though I have reported this successful example, there are still challenges to be met. For example, what are the responses of biological systems upon hydrogelation catalyzed by an enzyme or enzymes, particularly in an intracellular environment? How would a precursor respond to
multiple enzymes? How would one improve the sensitivity and selectivity of enzymatic hydrogelation as a reporting platform for the detection of a specific enzyme and inhibitor-screening of enzymes? It is becoming evident that enzymatic supramolecular hydrogelation may lead to a new paradigm for modeling cellular processes, managing artificial nanostructures in a biological environment, and developing new therapeutic agents for nanomedicines, and it is certainly worthwhile to address the above challenges in the future research of enzymatic hydrogelation.
Experimental Section

1. Materials and General Methods

2-Naphthylacetic Acid, N-Hydroxysuccinimide, N,N’-Dicyclohexylcarbodiimide, L-Phenylalanine were purchased from Alfa Aesar. 2-Deoxycytidine 5-monophosphate was purchased from Sigma Aldrich. Chloroactonitrile was purchased from Acros Organics. All the reagents and solvents were used as received from commercial sources. The structures of final products were confirmed by $^1$H NMR, $^{31}$P NMR and LC-MS. $^1$H NMR, $^{31}$P NMR spectra were recorded at 400 MHz using DMSO-d$_6$ as the solvent; LC-MS were measured on Waters Acquity UPLC-mircromass ZQ Mass Spectrometer; circular dichroism spectra were taken on JASCO J-810 Spectropolarimeter; Transmission electron micrograph (TEM) was done on Morgagni 268; The synthetic route to compounds 1-5 is shown in Scheme 2.4.

Enzyme: Alkaline Phosphatase: 1 U = cleaving 1 µmol of phosphate group from Nap-FF-DCp/minute

Scheme 2.4. (i) N-Hydroxysuccinimide, N,N’-Dicyclohexylcarbodiimide,
L-Phenylalanine; (ii) N-Hydroxysuccinimide, N,N’-Dicyclohexylcarbodiimide, L-Phenylalanine; (iii) Chloroacetonitrile, TEA; (iv) 2-Deoxycytidine 5-phosphate Tetrabutylammonium Salt, DMF.

2. Synthesis of Nap-FF

L-Phenylalanine (1 mmol) reacts with N-Hydroxysuccinimide (NHS) activated ester of 2-Naphthylacetic Acid (1 mmol) to afford compound 2 (Nap-F). And then L-Phenylalanine (1 mmol) reacts with N-Hydroxysuccinimide (NHS) activated ester of compound 2 (1 mmol) to give compound 3 (Nap-FF). 60% yield.

$^1$H NMR of Nap-FF (400 MHz, DMSO-d$_6$) $\delta$ (ppm):
8.36-8.40 (d, 1H), 8.29-8.34 (d, 1H), 7.82-7.88 (d, 1H), 7.71-7.80 (m, 2H), 7.58 (s, 1H), 7.42-7.51 (m, 2H), 7.10-7.29 (m, 1H), 3.44-3.61 (q, 2H), 2.66-3.11 (m, 4H).

3. Synthesis of Nap-FF Cyanomethyl Ester

Chloroacetonitrile (3 mmol) was added to a mixture of compound 3 (1 mmol) and triethylamine (2 mmol) in dry acetonitrile (20 ml). The reaction mixture was stirred at room temperature for 12 h. After removal of the solvent under reduced pressure, the product was purified by silica gel column chromatography with Hexanes and Ethyl Acetate (1:1) to give compound 4. 40% yield.

$^1$H NMR of Nap-FF Cyanomethyl Ester (400 MHz, DMSO-d$_6$) $\delta$ (ppm):
8.69-8.75 (d, 1H), 8.34-8.41 (d, 1H), 7.82-7.88 (d, 1H), 7.71-7.80 (m, 2H), 7.58 (s, 1H), 7.42-7.51 (m, 2H), 7.10-7.29 (m, 11H), 4.97 (s, 2H), 4.50-4.63 (m, 2H), 3.44-3.61 (q, 2H),
2.66-3.11 (m, 4H).

4. Preparation of 2-Deoxycytidine 5-phosphate Tetrabutylammonium Salt

2-Deoxycytidine 5-monophosphate was converted to tetrabutylammonium form using a Dowex 50 W resin (Tetrabutylammonium form, 3 ml, eluted with pyridine/water = 1/1, 12 ml). The eluted solution was evaporated and dried under vacuum.

$^1$H NMR of 2-Deoxycytidine 5-phosphate Tetrabutylammonium Salt (400 MHz, DMSO-d$_6$) $\delta$ (ppm):
7.73-7.79 (d, 1H), 7.28-7.41 (s, 2H), 6.14-6.22 (t, 1H), 5.72-5.78 (d, 1H), 4.20-4.27 (m, 1H), 3.82-3.91 (m, 3H), 3.11-3.20 (t, 3H), 2.04-2.13 (m, 1H), 1.88-1.99 (m, 1H), 1.49-1.62 (m, 3H), 1.22-1.36 (m, 3H), 0.89-0.97 (t, 4.5H)

$^{31}$P NMR of 2-Deoxycytidine 5-phosphate Tetrabutylammonium Salt (400 MHz, DMSO-d$_6$) $\delta$ (ppm): 0.14

5. Synthesis of Deoxycytidine-phosphate Derivative

Compound 4 (0.5 mmol) was added to a dry DMF solution of 2-Deoxycytidine 5-phosphate Tetrabutylammonium salt (0.1 mmol) in a microtube. The reaction mixture was incubated at room temperature for 12 h. After the reaction, the mixture was diluted with 50 mM ammonium acetate solution (pH 4.5, 1ml), and the product was purified by HPLC. The product was concentrated under reduced pressure and then lyophilized. 35% yield. ESI-MS (negative), m/z calcd for Nap-FF-DCp 769.2 [M-H]-, found 768.4

$^1$H NMR of Deoxycytidine-phosphate Derivative (400 MHz, DMSO-d$_6$) $\delta$ (ppm):
7.81-7.88 (d, 1H), 7.69-7.80 (m, 3H), 7.58 (s, 1H), 7.36-7.51 (m, 4H), 7.00-7.29 (m, 11H),
6.12-6.27 (t, 1H), 5.71-5.77 (d, 1H), 5.17-5.30 (m, 1H), 4.33-4.63 (m, 4H), 3.73-3.86 (m, 1H), 3.44-3.61 (q, 2H), 2.66-3.11 (m, 4H), 2.09-2.20 (m, 1H).

$^{31}$P NMR of Deoxycytidine-phosphate Derivative (400 MHz, DMSO-$d_6$) δ (ppm): 0.14
References and Notes


