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Enzymatic Hydrogelation of Small Molecules

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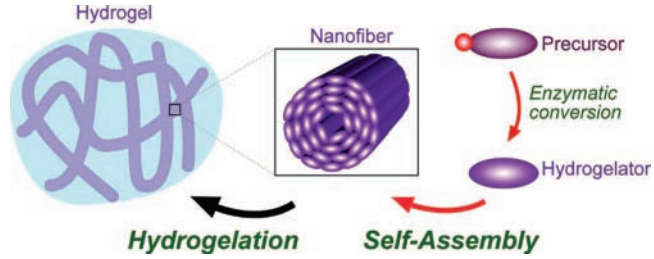

Enzymes, a class of highly efficient and specific catalysts in Nature, dictate a myriad of reactions that constitute various cascades in biological systems. Self-assembly, a process prevalent in Nature, also plays important roles in biology, from maintaining the integrity of cells to performing cellular functions and inducing abnormalities that cause disease. To explore enzyme-regulated molecular self-assembly in an aqueous medium will help to understand and control those important biological processes. On the other hand, certain small organic molecules self-assemble in water to form molecular nanofibers and result in a hydrogel, which is referred to as a "supramolecular hydrogel" (and the small molecules are referred to as "supramolecular hydrogelators"). Supramolecular hydrogelators share common features, such as amphiphilicity and supramolecular interactions ($\pi-\pi$ interactions, hydrogen bonding, and charge interactions among the molecules, among others) that result in nanostructures and form the three-dimensional networks as the matrices of hydrogels.

In this Account, we discuss the use of enzymes to trigger and control the self-assembly of small molecules for hydrogelation, which takes place *in vitro* or *in vivo*, extra- or intracellularly. Using phosphatase, thermolysin, β-lactamase, and phosphatase/kinase as examples, we illustrate the design and application of enzyme-catalyzed or -regulated formation of supramolecular hydrogels that offer a new strategy for detecting the activity of enzymes, screening for enzyme inhibitors, typing bacteria, drug delivery systems, and controlling the fate of cells. Since the expression and distribution of enzymes differ by the types and states of cells, tissues, and organs, using an enzymatic reaction to convert precursors into hydrogelators that self-assemble into nanofibers as the matrices of the hydrogel, one can control the delivery, function, and response of a hydrogel according to a specific biological condition or environment, thus providing an accessible route to create sophisticated materials for biomedicine. Particularly, intracellular enzymatic hydrogelation of small molecules offers a unique means for scientists to integrate molecular self-assembly with inherent enzymatic reactions inside cells for developing new biomaterials and therapeutics at the supramolecular level and improving the basic understanding of dynamic molecular self-assembly in water.

A. Self-Assembly, Supramolecular Hydrogels, and the Biological Inspirations for Enzymatic Hydrogelation

This paper discusses enzymatic hydrogelation of small molecules, which offers a unique and versatile platform for developing new biomaterials. Self-assembly,[1] a process prevalent in Nature, plays important roles in biology, from maintaining the integrity of cells[2,3] to performing cellular functions[4] and inducing abnormalities that cause disease.[5] Cellular nanostructures such as actin filaments, microtubules, vesicles, and micelles are the microscopic presentations of molecular self-assembly in biological systems.[6] Biomacromolecules can form a variety of nanostructures, and likewise certain small organic molecules are capable of self-assembling in a solvent, resulting in a gel.[7–9] If the self-assembly occurs in an aqueous medium, the resulting gel is referred to as a "supramolecular hydrogel",[8] and the small mole-

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cules are referred to as "supramolecular hydrogelators" or "molecular hydrogelators".

Supramolecular hydrogelators share common features with certain self-assembled structures in biology, such as amphiphilicity and supramolecular interactions ($\pi-\pi$ interactions, hydrogen bonding, and charge interactions among the molecules, among others). These noncovalent interactions in particular confer nanostructures and form the three-dimensional networks as the matrices of hydrogels.[8]

Their self-assembly and noncovalent interactions enable supramolecular hydrogels to show rapid responses to a variety of external stimuli, including pH,[10] temperature,[11] ionic strength,[12] and ligand–receptor interactions.[13,14] Most of the small molecule hydrogelators explored so far have been organic or bioactive molecules, and some supramolecular hydrogels also show a good tolerance to the addition of bioactive components into their matrices, thus making them attractive candidates for biomedical applications such as three-dimensional cell cultures,[15,16] screening biomolecules,[17] wound healing,[18] and drug delivery.[19–22] They also exhibit excellent biodegradability because they are composed of water and small molecules. One of the most interesting applications for such self-assemblied materials reported so far is that nanofibers (of the self-assembly of bioactive molecules) selectively promote differentiating the progenitor neuron cells into neurites rather than dendrites.[23] In addition, recent work has shown the control of the fates of cells through intracellular supramolecular hydrogelation.[24,25]

Normally, physical or chemical perturbations initiate the gelation that yields supramolecular hydrogels. For example, if a hydrogelator dissolves in an aqueous solution at a certain pH or temperature, changing pH or temperature alters the solubility of the hydrogelator to form a supersaturated solution and triggers the formation of networks of nanofibers, resulting in a supramolecular hydrogel. Such gelation has been summarized in several excellent and authoritative reviews.[7,8,26,27] This Account focuses instead on enzymatic supramolecular hydrogelation, in which an enzyme catalytically converts a precursor to a hydrogelator and triggers molecular self-assembly in water.[24,25,28–35]

In Nature, enzyme-regulated molecular self-assembly plays a critical role in many cell processes. The formation of microtubules (Figure 1), which governs mitosis, is one example. The polymerization of actins, which governs the focal adhesion of cells, is essentially a self-assembly process of seemingly miraculous sophistication that is regulated by enzymes.[6] These natural self-assemblies inspire the development of enzymatic hydrogelation of small molecules.[35] Compared with physical or conventional chemical perturbations, enzymatic regulation promises a unique opportunity to integrate molecular self-assembly in water with natural biological processes. Moreover, as a new method to make biomaterials, the enzyme-catalyzed formation of hydrogels of small molecules has already shown promise in biomedical applications as diverse as screening enzyme inhibitors,[30] assisting biomineralization,[36] typing bacteria,[33] developing smart drug delivery systems,[31] and controlling the fate of cells.[24,25]

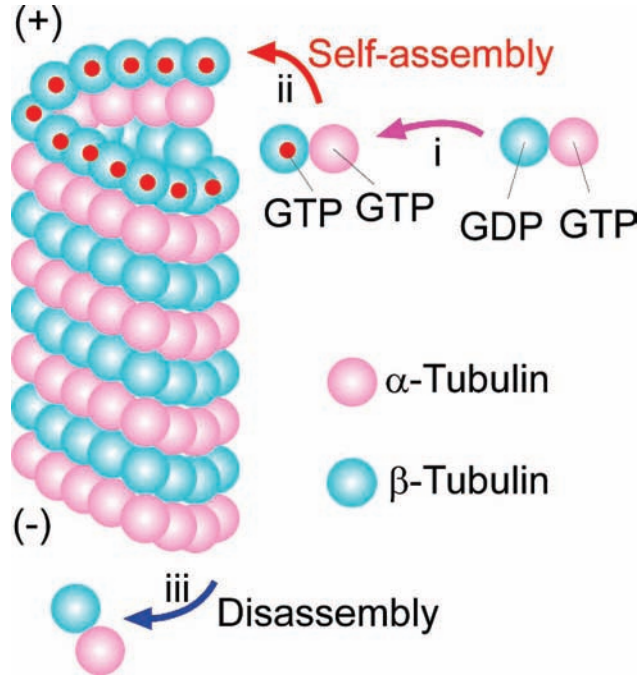

FIGURE 1. An illustration of the process of microtubule assembly: (i) enzyme-catalyzed replacement of the guanosine diphosphate (GDP) on β-tubulin by guanosine triphosphate (GTP); (ii) the GTP bound dimer of α-tubulin and β-tubulin assemble onto the (+) end of a microtubule; (iii) disassembly of the microtubule after enzymatic hydrolysis of GTP to GDP on β-tubulin at the (−) end of the microtubule.

Enzymatic reactions have already been used to generate various biomaterials, including hydrogels. For example, enzymes can catalyze the cross-linking of the polymer chains to form a continuous, three-dimensional matrix for hydrogels, and these polymeric hydrogels have found applications in tissue engineering, wound healing, and drug delivery.[37] In addition, enzyme-responsive surfaces have been created that can direct the attachment of cells, and enzyme-responsive polymeric hydrogel beads have potential as a matrix for drug delivery.[38,39]

Figure 2 shows that the enzymatic hydrogelation of small molecules involves three essential steps. The enzyme first converts a precursor into a hydrogelator (normally via bond

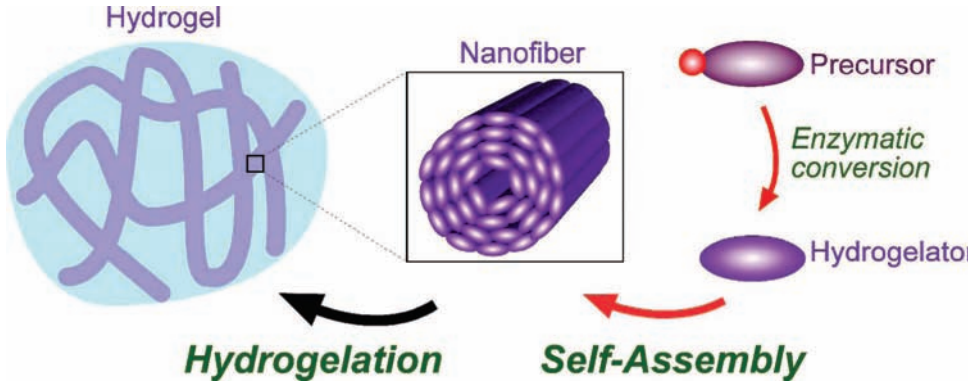

FIGURE 2. The essential steps in the enzymatic hydrogelation of small molecules.

TABLE 1. Examples of Enzymes, Corresponding Substrates, Minimal Gelation Concentration (mgc), and Potential Applications of Enzymatic Gelation of Small Molecules

enzyme(s)	substrate	mgc (wt %)	applications
alkaline phosphatase	Fmoc-Y-P(O)$(OH)_2$ (**1**)	2.0	biomineralization[36]
acid phosphatase	Fmoc-Y-P(O)$(OH)_2$ (**1**)	0.5	inhibitor screening[30]
phosphatase	Nap-βPhg- βPhg-Y-P(O)$(OH)_2$ (**3**)	0.4	drug delivery[34]
tyrosine kinase/phosphatase switch	Nap-FFGEY-P(O)$(OH)_2$ (**7**)	0.15	drug delivery[31]
β-lactamase	Nap-FF-NH$(CH_2)_2$S-ACLH (**9**)	0.1	assay bacteria[33]
thermolysin	Fmoc-F(**12**) and -FF (**13**)	0.22	tissue engineering[28]
esterase	Nap-FF-NH$(CH_2)_2$OCO$(CH_2)_2$COOH (**15**)	0.08	control the fate of cells[24]
human tyrosine phosphatase	Nap-FFY-P(O)$(OH)_2$ (**17**)	0.05	bacterial inhibition[25]

cleavage). The hydrogelator then self-assembles, usually forming nanofibers. The nanofibers then entangle to serve as a matrix for the hydrogel.[29] Besides bond cleavage, there are other ways to generate the hydrogelator using an enzyme. Enzyme-catalyzed bond formation has been reported by Ulijn et al.[28] Given the amphiphilic nature of the hydrogelator, however, the process depicted in Figure 2 should be more versatile in most situations. Table 1 summarizes the enzymes, corresponding substrates, and potential applications.

Unlike polymeric hydrogels formed by the cross-linking of random polymer chains, the self-assembly of small molecules to form a supramolecular hydrogel has three subtle but important features in addition to the characteristics discussed above. (i) Although the nanofibers randomly entangle, the molecular arrangement within the nanofibers (referred to as the secondary structure[8]) displays significant order. (ii) The relatively easy structural modification of the small molecules allows tailoring of the molecular order within the nanofibers. And more importantly, (iii) small molecules are more accessible to enzymes and more easily converted into hydrogelators either extracellularly or intracellularly. This third feature clearly confers a unique merit for exploration and application of enzymatic supramolecular hydrogelation.

B. *In Vitro* or Extracellular Enzymatic Hydrogelation of Small Molecules

B.1. Phosphatase. Phosphatases have a broad spectrum of substrate selectivity and high activity and are easily available. In Nature, they catalyze dephosphorylation in various biological environments. In this section, we introduce recent examples of using phosphatase to form supramolecular hydrogels and their applications.

B.1.1. **Dephosphorylation for Supramolecular Hydrogelation.** The dephosphorylation reaction catalyzed by a phosphatase led to the first example of enzymatic supramolecular hydrogelation.[29] Their easy accessibility and high activity render phosphatases an obvious choice of enzyme to control the self-assembly and hydrogelation of small molecules. As shown in Figure 3, alkali phosphatase turns a readily available precursor **1** (Figure 3A) to a more hydrophobic compound **2** after removing the hydrophilic phosphate group, and **2** forms a supramolecular hydrogel (Figure 3C) whose matrices are made of the self-assembled nanofibers of **2** (Figure 3D,E). This simple enzymatic conversion decreases the solubility of the molecules in solution and triggers the self-assembly of the hydrogelators. Enzymatic hydrogelation normally takes place at physiological conditions, which should help expand the range of its applications in biology and biomedicine.

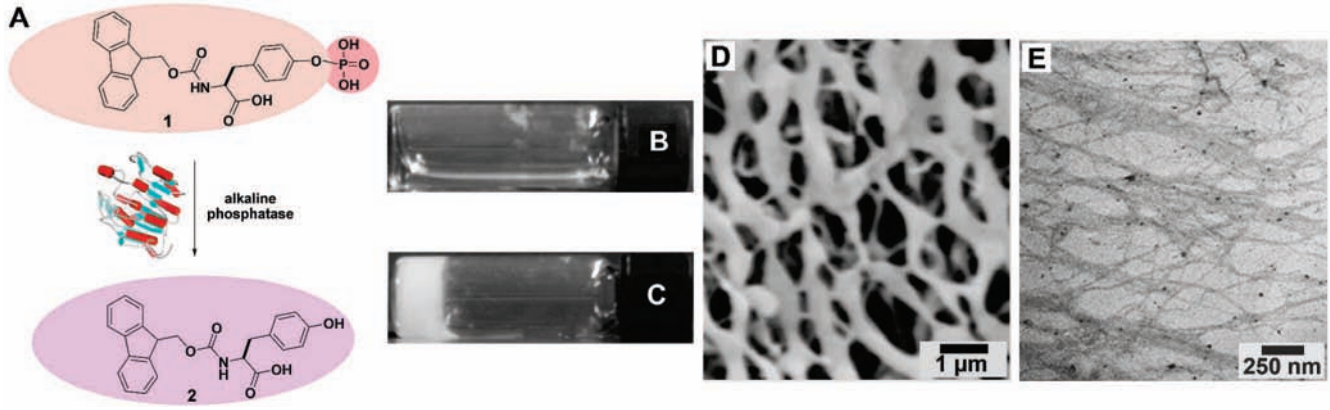

FIGURE 3. (A) Chemical structure of the precursor (**1**) and its corresponding hydrogelator (**2**) and the schematic gelation process, (B) optical images of a solution of **1** in alkali phosphate buffer (pH = 9.8), (C) the hydrogel of **2** formed by adding alkaline phosphatase to a solution of **1**, (D) SEM image of the hydrogel of **2**, and (E) TEM image of the hydrogel of **2**. Adapted with permission from ref 29. Copyright 2004 Wiley-VCH.

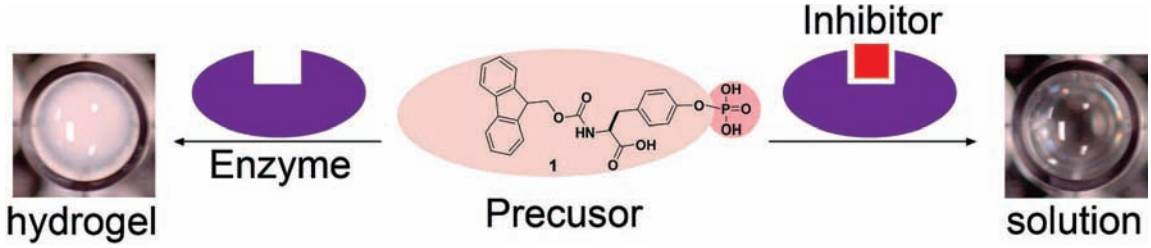

FIGURE 4. An illustration of using enzymatic supramolecular hydrogelation to screen inhibitors of phosphatase. Adapted with permission from ref 30. Copyright 2004 Royal Society of Chemistry.

B.1.2. **Enzymatic Hydrogelation for Screening Inhibitors of an Enzyme.** Many diseases are associated with abnormal activity or overexpression of certain enzymes, so it is important to be able to screen enzyme inhibitors. Since it is easy to distinguish a solution and a hydrogel without the aid of any instrument, one application of enzymatic hydrogelation is as a visual assay to screen for the inhibitor of an enzyme.[30] In the example shown in Figure 4, when the concentration of the inhibitor is lower than the inhibition threshold, acid phosphatase can convert the precursor to a supramolecular hydrogelator, which results in the formation of supramolecular hydrogel. When the concentration of the inhibitor is adequate, the activity of the acid phosphatase will be inhibited and the solution remains fluid. Despite its limited accuracy, this type of assay offers an inexpensive and rapid protocol for screening inhibitors.

B.1.3. **Biomineralization Assisted by Enzymatic Supramolecular Hydrogelation.** In enzymatic hydrogelation catalyzed by phosphatases, the phosphate ions cleaved from the precursor remain in the gel matrix after the formation of the supramolecular hydrogels. Mann and his co-workers have applied enzymatic hydrogelation catalyzed by phosphatase to achieve biomineralization.[36] They showed that adding a solution containing calcium ions above the hydrogel allows the calcium ions to diffuse into the matrices of the gel and form calcium phosphate. The stiffness, critical strain, thermal stability, dynamic storage, and loss moduli of the hydrogel can thus be increased significantly. The resulting hybrid composites (Figure 5) can be fabricated as hydrogels, thin films, or macroporous monoliths. This demonstration indicates that enzymatic hydrogelation can form promising platforms to assist biomineralization.

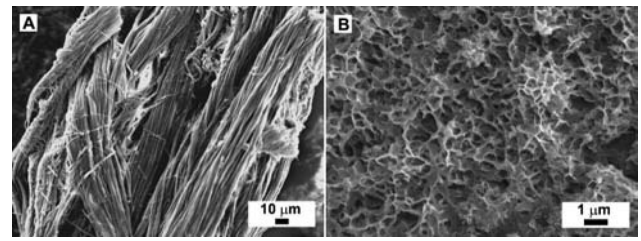

FIGURE 5. SEM images of (A) lightly mineralized and (B) extensively mineralized supramolecular hydrogels formed using the process depicted in Figure 3. Adapted with permission from ref 36. Copyright 2006 Wiley-VCH.

B.1.4. **Enzymatic Formation of Supramolecular Hydrogels with Long-Term Biostability.** Controlled *in vivo* enzymatic hydrogelation promises to allow tailoring the delivery, functions, and responses of a hydrogel according to specific biological conditions. These useful and promising applications, sometimes, demand a hydrogelator with suffi-

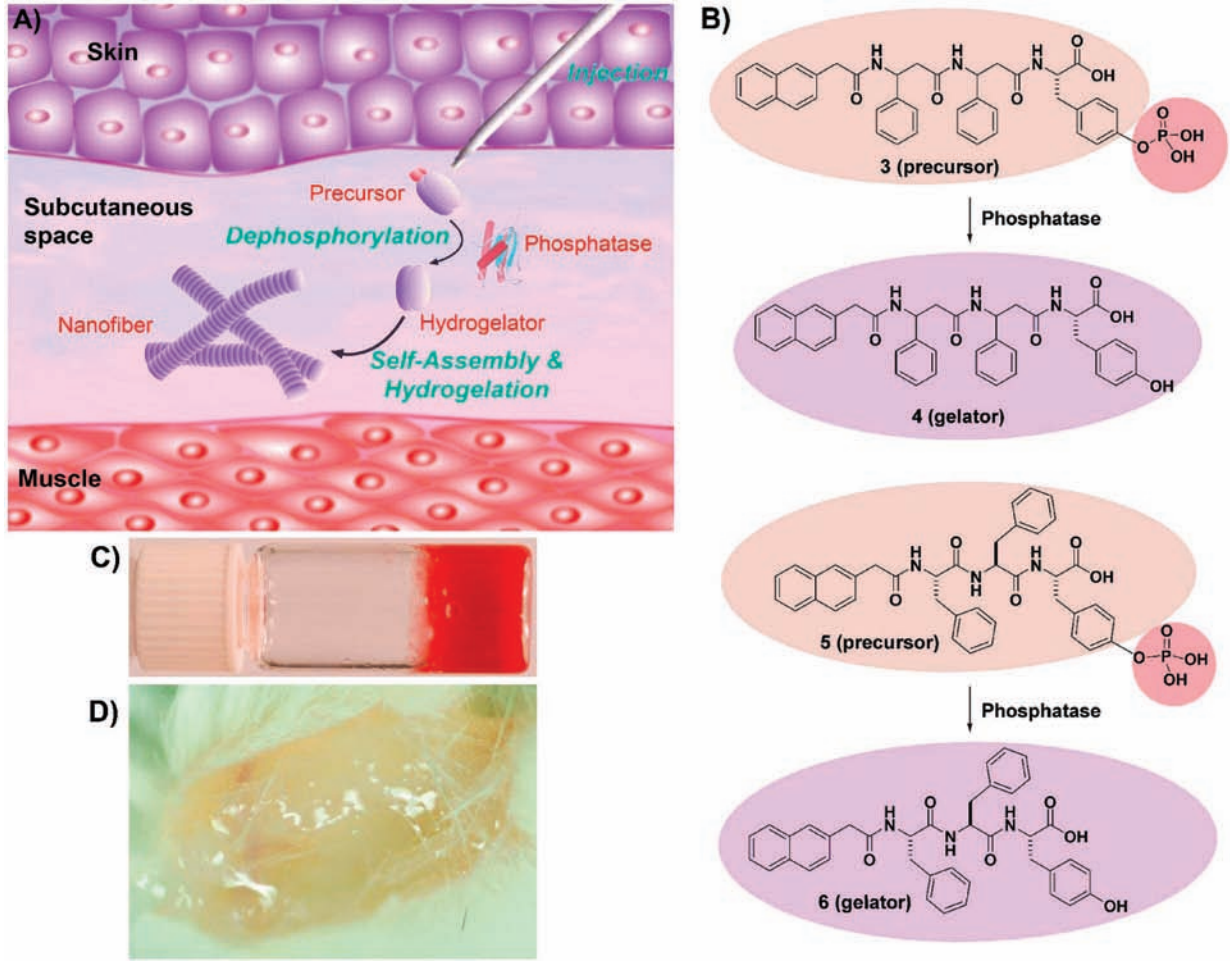

FIGURE 6. (A) An illustration of the process for using phosphatase to control the balance between hydrophilic and hydrophobic interactions that leads to the formation of a hydrogel *in vivo*. (B) The chemical structures of the molecules involved in the hydrogelation and the schematic gelation process for α- or β-peptide derivatives. (C) Optical images of the hydrogels formed by adding alkaline phosphatase to the mixture of blood and phosphate-buffered saline (PBS) containing **3**. (D) Optical images of the hydrogels formed subcutaneously 1 h after injection of a solution containing **3** and alkaline phosphatase under the skin of a mouse. Adapted with permission from ref 34. Copyright 2007 Wiley-VCH.

cient biostability, for example, to resist hydrolytic enzymes. A precursor based on β-amino acid derivatives has been designed with this in mind.[40] As shown in Figure 6, attaching tyrosine phosphate to the C-terminal end of the β-amino acid derivative afforded the precursor (**3**), which served as a substrate for the phosphatases in the hydrogelation. After being treated with a tyrosine phosphatase, **3** hydrolyzed into a hydrogelator. Interestingly, the morphologies of the resulting hydrogels were observed to depend on the rate of hydrogelation: the diameters of the nanofibers were more uniform when less enzyme was used and less uniform when more enzyme was used.[34] Moreover, this enzymatic hydrogelation was able to proceed in complex fluids (e.g., blood, Figure 6C) that contain a variety of proteases and *in vivo* (e.g., the subcutaneous cavity of a mouse, Figure 6D). The β-peptide-based hydrogel exhibited longer half-life than the α-peptide hydrogel (formed by **6**) in the subcutaneous environment.[34] Such excellent biostability *in vivo* renders β-peptide-based hydrogels a potential candidate for long-term biomedical applications. The principle demonstrated in the case of β-peptide (**4**) also implies that other unnatural amino acids can act as substrates for enzymatic hydrogelation, perhaps allowing even longer-term biostability.

B.2. An Enzyme Switch to Regulate Hydrogelation. When enzymatic hydrogelation takes place *in vivo*, more than one enzyme exists in the biological environment. Therefore, it would be helpful to understand the biological regulation involved in using a pair of enzymes to control the self-assembly of small molecules, since it is quite common for pairs of enzymes to work counteractively to regulate biological functioning in Nature.[6] Phosphatase and kinase are one pair of important enzymes involved in regulating signal transduction

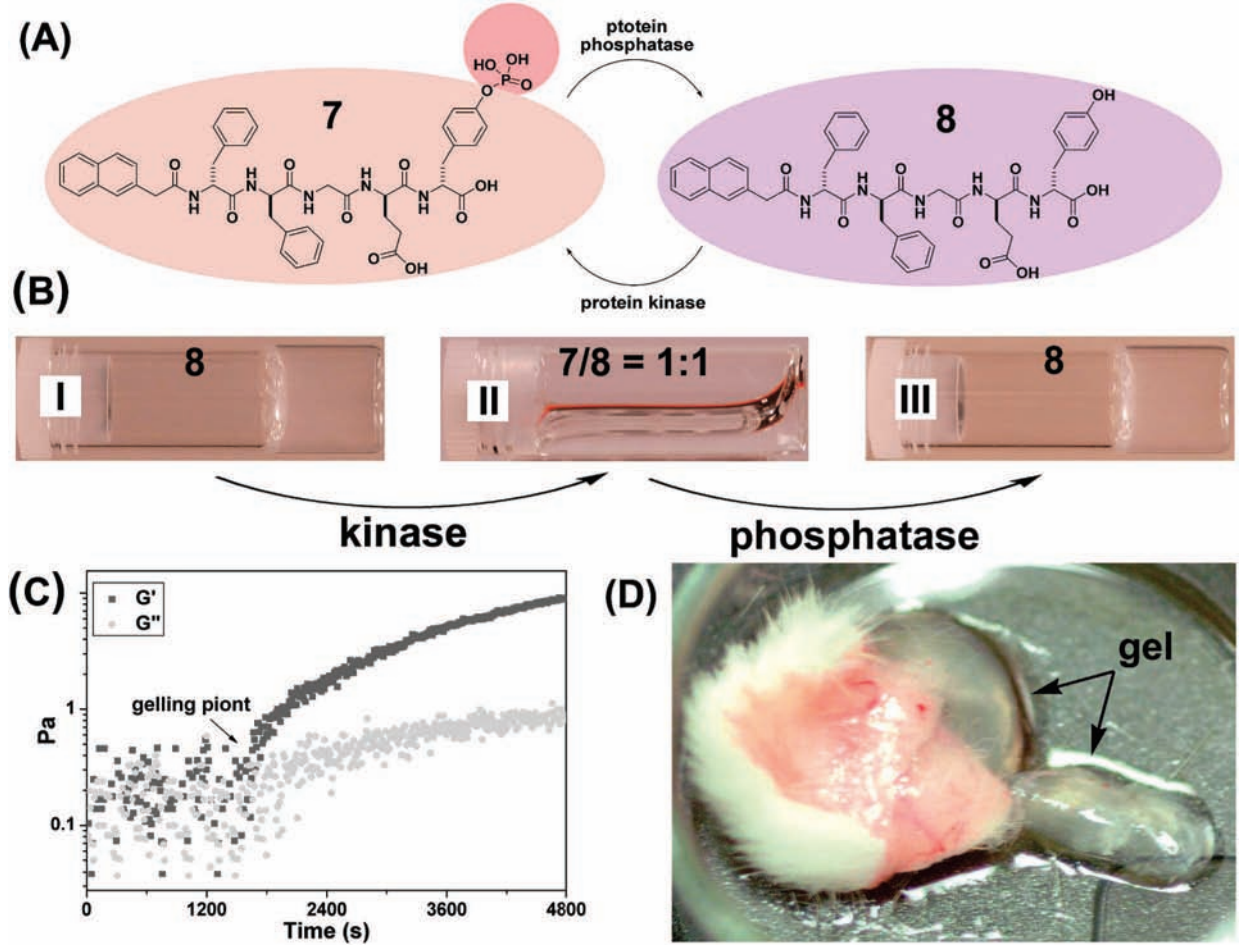

FIGURE 7. (A) Chemical structures of the precursor (**7**) and its corresponding hydrogelator (**8**) and the diagram of a phosphatase/kinase enzyme switch. (B) Optical images of the hydrogel (panel I) formed by **8** in 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer by adjusting the pH, the solution (panel II) obtained by treating the hydrogel with a kinase (at 50% conversion), and the hydrogel (panel III) restored by adding phosphatase into the solution. (C) Dynamic time sweep of a solution containing **7** and **8** after the addition of the phosphatase. The arrow indicates the apparent gelation point. (D) An optical image of a hydrogel formed subcutaneously one hour after injection of **8** into mice. Adapted with permission from ref 31. Copyright 2006 American Chemical Society.

in cells. Kinases catalyze the attachment of phosphate groups to a protein (phosphorylation), and phosphatases remove them (dephosphorylation).[6] Thus, we designed a compound that can undergo phosphorylation and dephosphorylation catalyzed by phosphatase and kinase.[31] As shown in Figure 7, the hydrogelator (**8**) self-assembles into nanofibers and forms a supramolecular hydrogel in an aqueous solution. As a substrate of tyrosine kinase, **8** undergoes phosphorylation catalyzed by tyrosine kinase in the presence of ATP to yield a more hydrophilic compound (**7**), which leads to the gel−sol transition. Upon the addition of alkaline phosphatase, the dephosphorylation of **7** restores the hydrogel (indicated by the rheological data shown in Figure 7C). This was the first example of using a pair of enzymes to regulate a supramolecular hydrogel. Subcutaneous injection of **7** in mice led to the formation of a supramolecular hydrogel *in vivo* (Figure 7D). The combination of the enzymatic switch with small molecular hydrogelation certainly deserves further exploration. It could lead to a new way to make and apply biomaterials for therapeutic interventions because many diseases (e.g., cancer, diabetes, Alzheimer's disease, and multiple sclerosis) are associated with the abnormal activity of kinases or phosphatases or other types of enzymatic switches.

B.3. β-Lactamases Catalyzing Formation of Supramolecular Hydrogels. The β-lactamases are an important family of enzymes that catalyze the hydrolysis of β-lactam antibiotics and have caused wide-spread antimicrobial drug resistance.[41,42] Their importance has led us to explore enzymatic hydrogelation catalyzed by β-lactamase.[33] A typical scheme is shown in Figure 8. The precursor (**9**), consisting of a cephem nucleus as the linker connecting a hydrophilic group and a hydrogelator, is too soluble to form a hydrogel. Upon the action of a β-lactamase, the β-lactam ring opens to release the hydrogelator, which self-assembles into nanofi-

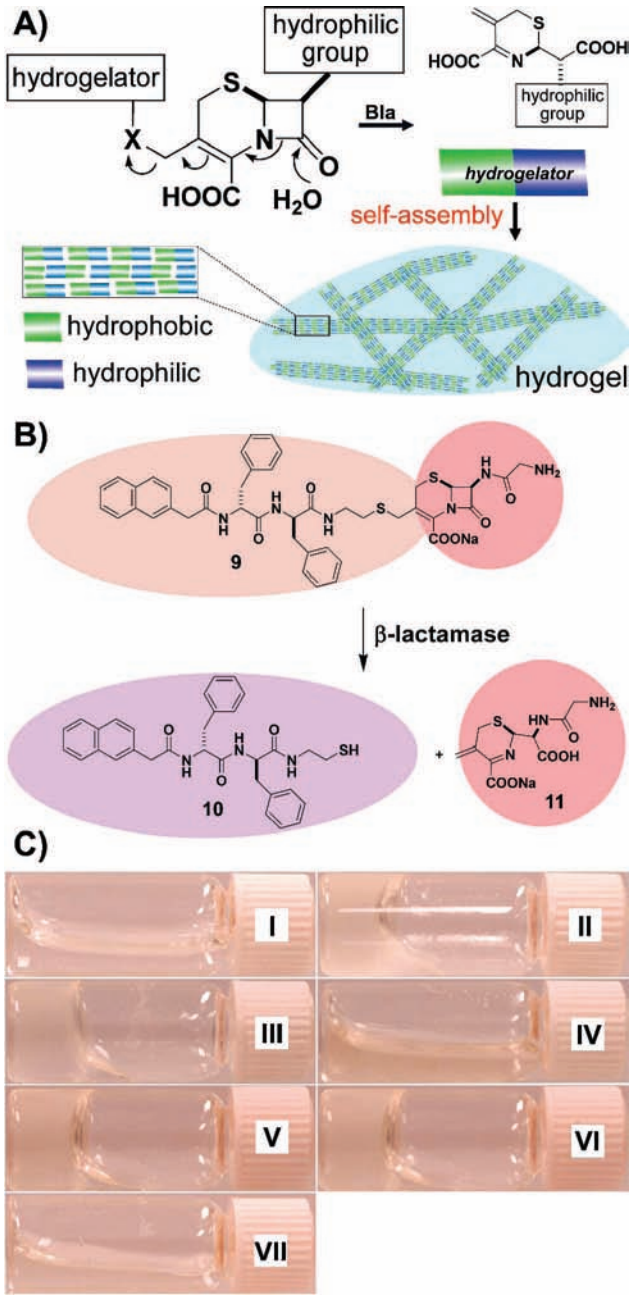

FIGURE 8. (A) An illustration of the design of using β-lactamase to form supramolecular hydrogels. (B) The chemical structures of the compounds involved in the β-lactamase-catalyzed hydrogelation. (C) The results of adding different types of cell lysates into the solution of **9** (the final concentration of **9** is 0.35 wt %, pH = 8.0): (I) *E. coli* C600 (β-lactamase negative), (II) *E. coli* with CTX-M13 (extended-spectrum β-lactamase), (III) *E. coli* with CTX-M14 (extended-spectrum β-lactamase), (IV) *E. coli* JP995 (β-lactamase negative), (V) *E. coli* with SHV-1 (broad-spectrum β-lactamase), (VI) *E. coli* with TEM-1 (broad-spectrum β-lactamase), and (VII) water. Adapted with permission from ref 33. Copyright 2007 American Chemical Society.

bers to afford a hydrogel. This facile process could detect β-lactamase in the lysates of bacteria. β-lactamase in a bacterial lysate could convert the precursor (**9**) to its corresponding hydrogelator (**10**), resulting in the formation of a supramolecular hydrogel (II, III, V, and VI in Figure 8C). Without β-lactamase (I, IV, and VII in Figure 8C), no gel would form because of the lack of **10** to support hydrogelation.

As in screening inhibitors of acid phosphatase by enzymatic hydrogelation, this process can screen for inhibitors of β-lactamase: a gel does not form when the β-lactamase is inhibited by other molecules. We envision that β-lactamase might be used to control the self-assembly of small molecules and that it might offer a general platform for the study of intracellular hydrogelation (*vide infra*) by exploiting the well-established vectors for controlled-expression of β-lactamases inside cells or in subcellular organelles.[43–45]

B.4. Thermolysin Catalyzing Formation of Supramolecular Hydrogels. Unlike phosphatase, which breaks a covalent bond to trigger hydrogelation, thermolysin catalyzes the formation of a covalent bond between two compounds to form a supramolecular hydrogelator (**12** and **13** leading to **14** in Figure 9A), which self-assembles into a three-dimensional fibril network to support the hydrogel (Figure 9B).[28] Since thermolysin catalyzes the reverse hydrolysis of many substrates, especially hydrophobic amino acids and peptides, it should expand the scope of enzymatic hydrogelation. It has already been developed into a useful tool for forming hydrogels as scaffolds in tissue engineering.[15]

C. Intracellular Enzymatic Hydrogelation of Small Molecules

Enzymatic hydrogelation can integrate molecular self-assembly with a wide range of biological processes involving enzymes, and this approach provides a unique opportunity to explore intracellular hydrogelation. The ability to form self-assembled intracellular artificial structures should offer a convenient means of examining the dynamics of the molecular superstructures inside cells and help elucidate the functions of small molecules at a new level of complexity, supramolecular and intracellular.

C.1. Supramolecular Hydrogelation inside Mammalian Cells. To form a hydrogel within a cell, an endogenous enzyme should convert a soluble precursor, which does not necessarily self-assemble and gel extracellularly, into a hydrogelator that self-assembles into nanofibers or some other ordered nanostructures. To meet this requirement, we designed and synthesized **15** as an esterase substrate (Figure 10).[24] Mammalian cells take in **15** by diffusion, their endogenous esterases convert **15** to **16**, and the molecules of **16** self-assemble to form nanofibers, resulting in a supramolecular hydrogel inside the cells. The gelation changes the vis-

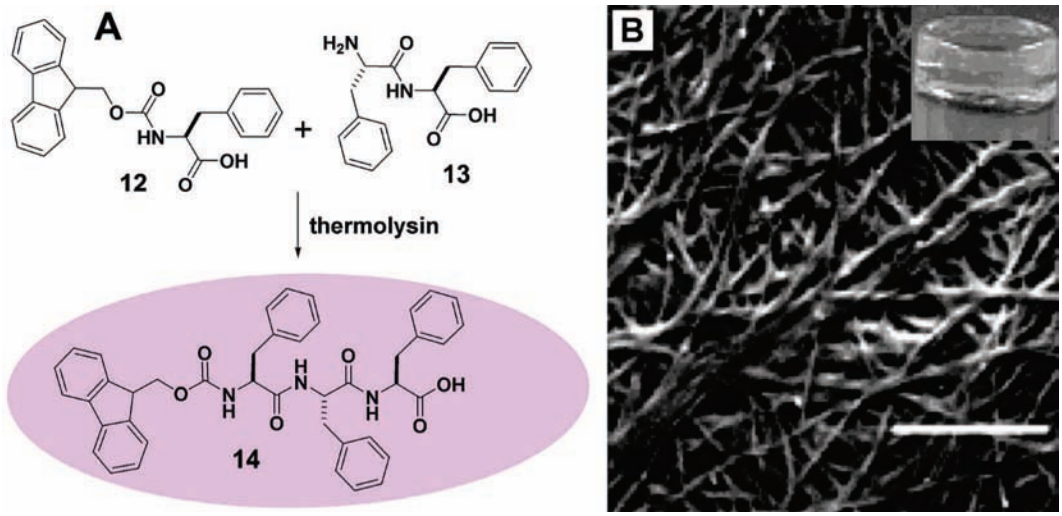

FIGURE 9. (A) Chemical structures of **12**, **13**, and **14** and a schematic diagram of hydrogelator formation and (B) SEM and optical (inset) images of the corresponding gel (scale bar is 0.5 μm). Adapted with permission from ref 28. Copyright 2006 American Chemical Society.

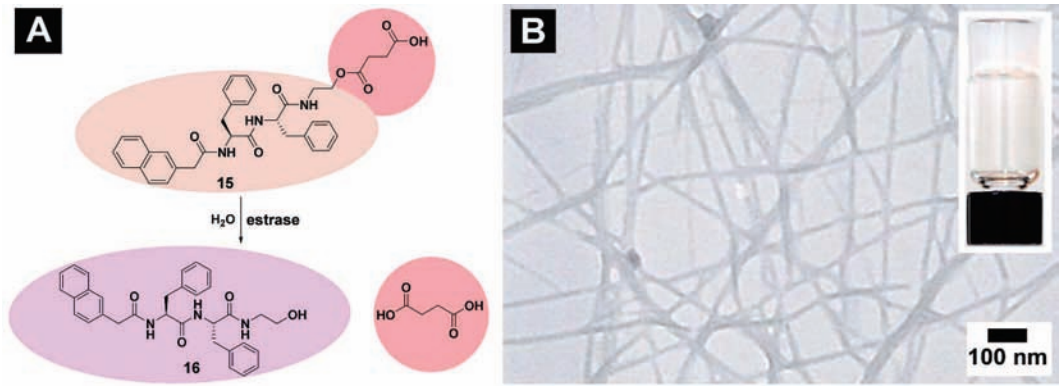

FIGURE 10. (A) An illustration of using an esterase to convert precursor molecule (**15**) to the hydrogelator (**16**), thus resulting in supramolecular hydrogelation and (B) TEM of the hydrogel (inset, optical image).

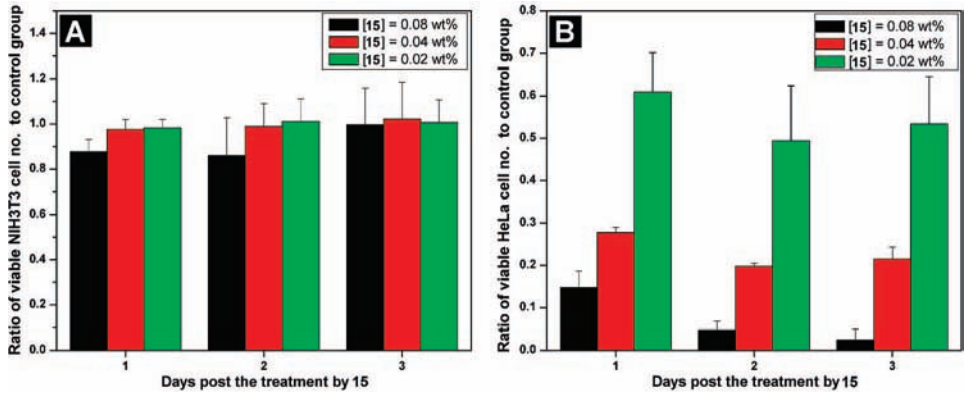

FIGURE 11. MTT assays of (A) NIH-3T3 cells and (B) HeLa cells treated with **15** at concentrations of 0.08, 0.04, and 0.02 wt %.

cosity of the cytoplasm and causes cell death. Interestingly, at a certain concentration of the precursor (e.g., [**15**] = 0.04 wt %), most HeLa cells died at day three after the addition of **15** to the culture medium, while most of NIH-3T3 cells remained alive and dividing at the same concentration of **15** (Figure 11). One possible explanation would be that the levels of expression of esterases (i.e., E1 in Figure 12) between these two cell lines are different. HeLa cells likely have higher esterase expression levels and are able to convert more **15** to the hydrogelator (**16**) than NIH-3T3 cells do, so a gel could form inside the HeLa cells. Inside the NIH-3T3 cells, the concentration of **16** was too low to reach the minimum gelation concentration due to the lower concentration of esterases, so no gel formed. This mechanism was supported by the results of

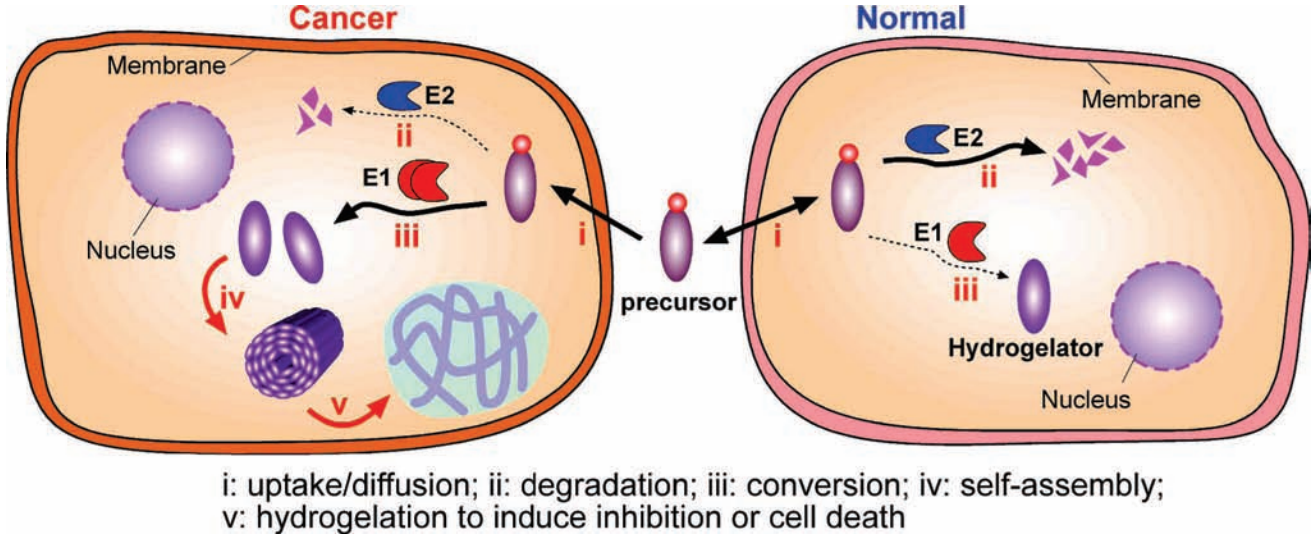

FIGURE 12. A possible mechanism for the formation of intracellular nanofibers leading to hydrogelation and cell death (E1 = phosphatase; E2 = other enzymes): (i) the precursor (**15**) is taken up by the cells; (ii) some **15** molecules degrade, catalyzed by other enzymes; (iii:)some **15** molecules are converted into the hydrogelator, **16**; (iv) **16** self-assembles into nanofibers; (v) the nanofibers form three-dimensional networks and result in a gel, which induces inhibition or cell death.

a fluorescence assay of esterase in the cells.[24] Though other factors (e.g., differences in the uptake of **15** by HeLa and NIH-3T3 cells) might also contribute to the apparent low toxicity of **15** for NIH-3T3 cells, the above result indicates that the formation kinetics of the intracellular nanostructure are specific to different types of cells. Whether intracellular hydrogelation leads to apoptosis or necrosis or both has yet be determined, but this approach clearly offers a new way to control the fate of cells.

C.2. Supramolecular Hydrogelation inside Bacteria. To further demonstrate that hydrogelation inside cells can control their fate, we designed the precursors for enzymatic hydrogelation inside bacteria. Two types of *Escherichia coli* strains were used in the experiments: the wild-type BL21 (as the control) and a BL21 strain bred to overexpress human tyrosine phosphatase (BL21(P+)). Since the only difference between the two strains was their phosphatase expression, any discrepancy in the uptake of the precursor was minimized. After the diffusion of the precursor (**17**) into the *E. coli*, the phosphatase converted **17** into **18**, which resulted in the formation of three-dimensional networks of nanofibers and triggered supramolecular hydrogelation (Figure 13A). The BL21(P+) bacteria were observed to stop growing upon the addition of **17** (IC_{50} = 20 μg/mL), but the wild-type BL21 bacteria grew normally (IC_{50} > 2000 μg/mL).[25] HPLC showed that **18** accumulated significantly in the BL21(P+) (Figure 13B). After the bacteria had been isolated and broken apart by sonication, hydrogelation could be observed only in the sample prepared from the BL21(P+) and treated with **17** (Figure 13C). The formation of nanofibers in the hydrogels was also evident in TEM images (Figure 13D). These results confirm that enzymatic hydrogelation inside the bacteria inhibited their growth. Since small molecules enter cells easily, it should be possible to design a substrate susceptible to multiple enzymes to achieve more sophisticated control. The principle and the strategy demonstrated here could lead to a new class of therapeutic agents that take advantage of the kinetics of enzymatic reactions rather than tight ligand–receptor binding.[42]

D. Perspectives and Prospects

Benefiting from advances in supramolecular chemistry and from the research of molecular self-assembly,[1,46–48] the fundamental understanding of how to form molecular gels has improved considerably in the past decade.[8,26] The substantial progress in identifying low molecular weight gelators[7–9,26,49–57] has motivated and underpinned the exploration of enzymatic hydrogelation of small molecules. Enzymatic hydrogelation can provide new systems that mimic and elucidate the self-assembly behavior of macromolecules in biological systems. They may thus constitute a new platform from which to control the self-assembly of small molecules for biomedical applications and a new approach to solving complex problems such as cancer and multiple drug resistance.

It is conceivable that a substrate for multiple enzymes might be designed allowing sophisticated control over the formation of supramolecular hydrogels inside cells. In essence, the target of intracellular hydrogelation is the cytosol, whose gelation or

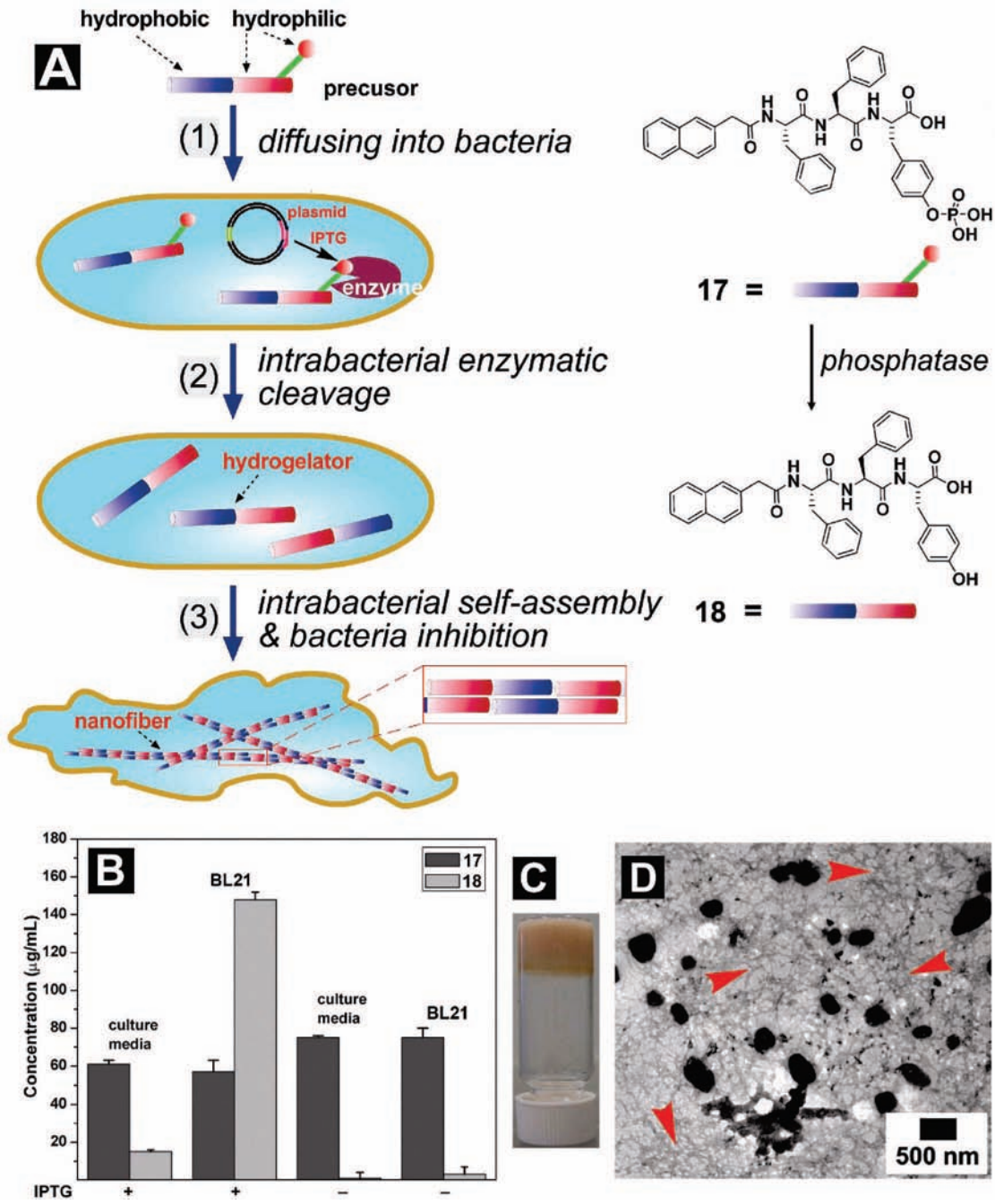

FIGURE 13. (A) A schematic representation of intracellular nanofiber formation leading to hydrogelation and the inhibition of bacterial growth, the chemical structures, and graphic representations of the precursor (**17**) and the corresponding hydrogelator (1**8**), (B) concentrations of **17** and **18** in the culture medium and within the cells (BL21, IPTG+ = overexpression of phosphatases or IPTG− = normal expression of phosphatases), and (C) optical and (D) TEM images of the hydrogel formed inside the bacteria after culturing with **17** for 24 h (arrows indicate the nanofibers formed by **18**). The high electron density areas (dark black areas) likely are intracellular inclusions (polyphosphate bodies and carboxysomes among others[59]). Adapted with permission from ref 25. Copyright 2007 Wiley-VCH.

change of viscosity will promise to allow control of many cellular processes (or pathways) via the kinetics of enzymatic reactions rather than specific and tight ligand–receptor binding.

A remaining challenge is that enzymatic hydrogelation requires rather high concentrations (micromolar to millimolar) of gelators. These might be lowered by choosing hydrogelators of specific functions[22,58] or by exploiting hydrogelation within subcellular organelles.

Enzymatically formed molecular hydrogelators would be a reasonable starting point for investigating the self-assembly of small molecules inside cells, which remains a little-explored area that might hold great promise.

Enzymatic regulation of gelation, as a versatile platform for developing smart biomaterials, offers not only the advantageous features of supramolecular soft matter (e.g., programmability, reversibility, and dynamics) but also a new approach to integrating the processes of biological systems with the formation of hierarchical nanostructures. The collaboration of scientists from chemistry, biology, medicine, and engineering will probably be required to advance in this nascent research direction, but the results could lead to a new paradigm for managing cellular processes, probing cellular functions, and developing new biomaterials and therapies.

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BIOGRAPHICAL INFORMATION

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FOOTNOTES

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