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Intracellular self-assembly of supramolecular gelators to selectively kill cells of interest

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Abstract

The significant progress of supramolecular chemistry since the end of last century includes the development of supramolecular gels. In particular, spatiotemporal self-assembly of synthetic small gelator molecules have attracted increased attention owing to their ability to realize functional properties at a designated space and designated time. Peptides conjugated with hydrophobic moieties are typical examples of a supramolecular gelator (low-molecular-weight gelator, LMWG), which can be designed or programmed to self-assemble to form nanofibers/nanosheets in response to a broad range of stimuli or to microenvironments. In the last decade, several groups reported that the self-assembly of small gelator molecules was achieved inside living cells or on the surfaces of living cells and induced the selective cell death, which would lead to a novel therapeutic approach or a novel cell-selection tool. This focus review outlines the self-assembly of the small gelator molecules inside or around living cells, which controls the cell fates.

1. Introduction

Gels have been widely used in our daily life and in industry. A gel usually consists of a liquid immobilized in a three-dimensional (3D) network. Conventionally, the gel networks are prepared by covalent and noncovalent cross-linking of polymers and inorganic frameworks. Since the end of the last century, the use of molecular self-assemblies formed by noncovalent interactions for the preparation of nanostructured gel networks has been an emerging trend. Gels based on noncovalent interactions (e.g., hydrogen bonding, π - π interactions, van der Waals force and electrostatic interactions) are called supramolecular gels. The gelation based on the noncovalent interactions occurs in organic solvents [1-4], water [5-10] and ionic liquids [11-15]. The main characteristics of a supramolecular gel are its thermoreversibility and rapid response to external stimuli.

Supramolecular gels are divided into two categories: Gels made from a high-molecular-weight polymer or a low-molecular-weight gelator (or low-molecular-mass gelator). A low-molecular-weight gelator (LMWG) can be easily tuned at the molecular level (e.g. molecular structure, estimated interaction with other molecules etc) because of the simple and small molecular structure of the gelator. LMWG molecules self-assemble to form one-dimensional nanofibers and the three-dimensional entanglement (or branching) of the nanofibers induces the gelation of the solvents (Fig. 1). In the last two decades, the synthesis of functional hydrogels using elaborately designed LMWGs has been reported, which indicates their potential use in cell scaffold [16-23], drug carriers [24-28], antimicrobial materials [29-32], catalysts [33, 34], media for organic/inorganic reactions [14, 35-38], biosensors [39-44], emulsifiers [38, 45] and absorbents for pollutant removal from waste water [46,

47]. The large number of the studies on LMWGs give clues for the rational design of a LMWG. There is also a progressive attempt to screen for the aqueous self-assemblies in 8,000 possible tripeptides using a computational tool [48].

Despite a number of reports on LMWGs for hydrogel, there are only a few examples that LMWGs for hydrogel are commercialized (for example, PuraMatrix, 3-D Matrix, Ltd., Tokyo and NANOFIBERGEL®, Nissan Chemical Co., Tokyo). While hydrogels consisted of polymers and inorganic frameworks are inexpensive and mechanically tough, hydrogels of LMWGs are expensive and fragile. These mean that the applications of LMWG hydrogels should be apart from those of polymers and inorganic frameworks. One possible and unique application of LMWGs is the physiological activity induced by their self-assembly. Our group and other groups reported the selective cell death of cancer cells using LMWGs. This focus review summarizes the recent progress on the physiological activity (selective cell death) created by the self-assembly of LMWGs and discuss the future potential.

2. Peptide-based LMWG hydrogel as a novel class of biomaterial

The pioneering works of peptide-based LMWGs were carried out by Zhang's group [16, 49] and Stupp's group [19, 50]. Zhang *et al.* designed various peptides to form β -strand or β -sheet structures and succeeded in the preparation of peptide nanofibers and nanotubes. Their peptides contained neither long alkyl chains nor polyaromatic moieties. Among their studies, they found that *N*-acetylated octapeptides and hexadecapeptides with alternating ionic hydrophilic and hydrophobic

amino acids produced hydrogels appropriate for 2D/3D cultures of nerve cells, endothelial cells and chondrocytes [5].

Stupp *et al.* synthesized the peptide amphiphile (PA), which was composed of a palmitoyl group (C16) and 11 amino acids (**PA1**, Fig. 2). The long alkyl chain was designed to contribute to hydrophobic interaction among the PA molecules in aqueous solution and the peptide segment was to produce hydrogen bonding and also hydrophobic interaction among the molecules. The PA formed a hydrogel through nanofibrous self-assembly upon pH change. The entanglement and branching of the nanofibers induced the hydrogelation of an aqueous solution under physiological conditions. They demonstrated that the hydrogel was utilized as a scaffold for hydroxyapatite mineralization [50]. They also succeeded in culturing neural progenitor cells in the hydrogel of a peptide amphiphile containing a neurite-sprouting epitope (**PA2**, Fig. 2), which allowed rapid differentiation of the cells into neurons [19].

Xu and co-workers reported vancomycin-conjugated pyrene as an antibiotic gelator (**PA3**, Fig. 2) [51]. Vancomycin is a glycopeptide antibiotic having a large and complex structure. They also found novel peptide-based LMWGs having much simpler molecular structures (**PA4**, Fig. 2). They synthesized dipeptides (D-Ala-D-Ala) linked to a 9-fluorenylmethoxycarbonyl (Fmoc) group that formed nanofibrous self-assembly to give a hydrogel [52]. This supramolecular hydrogel exhibited gel-sol transition responsive to an antibiotic (vancomycin) since D-Ala-D-Ala binds to vancomycin. In 2005, Ulijn *et al.* reported the three-dimensional cell culture of bovine chondrocytes in the hydrogel consisted of Fmoc dipeptide [20]. Since fluorene (a part of a Fmoc group) is suspected to be

carcinogenic and Fmoc-protecting group is easily hydrolyzed under alkaline conditions, Xu *et al.* developed dipeptides conjugated with a naphthyl group as another class of LMWG (**PA5**, Fig. 2), which was more biocompatible than Fmoc dipeptide [53].

3. Self-assembly of peptide amphiphile that kills cells

The peptide sequence in the peptide amphiphile played an important role in the self-assembly and supramolecular hydrogelation. The discovery of the peptide sequence is a key step for the study of supramolecular hydrogel prepared from peptide amphiphiles. We fortuitously found a simple peptide amphiphile, *N*-palmitoylated tetrapeptide (C16-Gly-Gly-Gly-His), as a good hydrogelator (Fig. 3a) [54], when we were studying the recycling of precious metal ions using peptides and proteins [55, 56]. In addition to its simple molecular structure, C16-Gly-Gly-Gly-His gelled physiological media (around pH 7.5) at a remarkably low concentration (0.03 wt%). The hydrogel prepared with 0.1 wt% C16-Gly-Gly-Gly-His had nanofibers with a diameter of 20 nm (Fig. 3b), which were formed by the self-assembly of C16-Gly-Gly-Gly-His. Based on our findings, the more simplified peptide amphiphile was commercialized as a supramolecular hydrogelator by Nissan Chemical Industries, Ltd [57].

Although the works described in the previous section indicated the high potential of hydrogels consisted of peptide-based LMWGs, C16-Gly-Gly-Gly-His exhibited the intense cytotoxicity to animal cells (e.g. human cancer cells). Xu *et al.* also reported the remarkable cytotoxicity of naphthyl tripeptide (and dipeptide), containing Tyr and repeated Phe residues, to bacteria and animal cells, in

which the self-assembly of the naphthyl peptide inside living cells gave critical damage to the cells [58, 59]. These imply that the peptide sequence and length (also the length of an acyl group) in PA plays a critical role in the biocompatibility.

Xu *et al.* further demonstrated that the PA self-assembled to form nanofibers inside living cells, which was thought to account for the cytotoxicity of the PA [58, 59]. They synthesized the PA precursors that were transformed by intracellular enzymes (esterase and phosphatase) to hydrogelators (Fig. 4). One of the precursors was naphthyl tripeptide containing phosphorylated Tyr (**PA6** precursor, Fig. 4a). The other was naphthyl dipeptide containing an ester bond (**PA7** precursor, Fig. 4b). These precursors were soluble in aqueous solution and did not form nanofibrous self-assembly in the absence of the enzymes. In ref 44, they genetically transformed *E. coli* to overexpress phosphatase upon addition of isopropyl- β -d-thiogalactopyranoside (IPTG). They incubated *E. coli* with **PA6** precursor and observed the inhibition of the *E. coli* growth only when IPTG was added to the culture medium, indicating the self-assembly of the PA inside living cells and also the regulation of the cell death. In ref 45, they used HeLa cells and mouse fibroblast cells to explore the intracellular self-assembly of the PA. **PA7** precursor exhibited remarkable cytotoxicity to HeLa cells but to fibroblast cells. The nanofibrous self-assembly of **PA7** was found inside the dead HeLa cells. They attributed the difference in cytotoxicity to the expression level of esterase because HeLa cells produced esterase more than fibroblast cells.

A peptide sequence can be readily designed as required. Peptides are substrates of many enzymes. Inspired by the excellent works of Xu *et al.*, we used matrix metalloproteinase-7 (MMP-7) to trigger

supramolecular hydrogelation of C16-Gly-Gly-Gly-His. MMP-7 is excessively produced and secreted by various cancer cells, which is considered to play an important role in the tumor invasion [60-62]. MMP-7 recognizes -Pro-Leu-Gly-Leu- as a substrate sequence [63]. We then designed and synthesized a hydrogelator precursor (**ER-C16**), in which the Pro residue was expected to produce a curved structure to prevent the formation of nanofibers and -Arg-Lys-NH₂ was expected to provide electrostatic repulsion between the precursor molecules owing to its cationic charges (Fig. 5a). The precursor (**ER-C16**) was soluble in an aqueous solution and formed a micelle-like self-assembly. When MMP-7 was added to the precursor solution, the solution was transformed to a translucent hydrogel within 2 h (Fig. 5b and c). A drastic morphological change at the nanoscale was observed in the precursor solution after the addition of MMP-7 (Fig. 5d and e).

The cytotoxicity tests on human cancer cells and normal cells revealed that both **ER-C16** and **G-C16** induced the death of cancer cells (Fig. 6a and c), whereas normal cells remained alive in the presence of **ER-C16** but were killed by **G-C16** (Fig. 6b and d) [64]. The quantitative assay of the cell viability revealed that the precursor (**ER-C16**) was cytotoxic to all the tested types of cancer cells but only to two kinds of normal cells (Fig. 6e). All five different kinds of the cancer cells tested secreted more MMP-7 compared with that of normal cells (Fig. 6f). This study revealed that the selective death of cancer cells was correlated to the production of a LMWG (**G-C16**) by MMP-7. **G-C16** was taken by the cancer cells and that **G-C16** self-assembled to form nanofibers inside the cells, leading to a high viscosity of the cytoplasm in the cancer cells. Kuimova *et al.* studied the remarkable relationship between cell death and high intracellular viscosity [65]. There is also a report describing that amyloid-

like protein aggregation inside a living cell interfered with the intracellular transport of protein and RNA, resulting in the critical damage on the cellular functions [66]. These studies suggested that intracellular self-assembly or hydrogelation by the LMWG gave a serious damage to cells, leading to the cell death.

Xu *et al.* also reported the selective killing of cancer cells using enzyme-triggered intracellular self-assembly of naphthyl peptides (termed enzyme-instructed self-assembly) [67-69]. They used alkaline phosphatase and carboxylesterase, which were overexpressed in some of cancer cell lines, to trigger the intracellular self-assembly. They further carried out *in vivo* experiments using mice and succeeded in the inhibition of tumor growth, in which they observed tumor apoptosis without harming normal tissues [70].

Ulijn *et al.* also demonstrated the death of cancer cells using the self-assembly of LMWG molecules (Fmoc glucosamine-6-phosphate) on the surfaces of target cancer cells. Alkaline phosphatase, which was bound to a cell membrane of specific cancer cells, converted a simple carbohydrate phosphate derivative to the self-assembling hydrogelator [71]. Yang *et al.* employed the combination of intracellular enzyme (alkaline phosphatase) and intracellular glutathione to induce the intracellular self-assembly of LMWG molecules, resulting in the death of liver cancer cells. These studies provide a proof-of-concept that spatiotemporal molecular self-assembly can be programmed in the molecular structure of a LMWG [38], which sets a LMWG hydrogel apart from a polymer gel.

Based on the above studies, Saito *et al.* proposed the practical application of selective cell-death induced by the self-assembly [72]. Although induced pluripotent stem cells (iPSCs) are the powerful tool in the regenerative medicine, there is a risk of residual undifferentiated iPSCs in the use of iPSCs in clinical applications because of their potential tumorigenicity. Since ecto-alkaline phosphatase is excessively expressed on the cell surfaces of undifferentiated iPSCs, Saito *et al.* used the phosphorylated PA (phosphorylated naphthyl-D-peptides) for the self-assembly on the cell surfaces. They succeeded in the selective removal of undifferentiated iPSCs from iPSC-derived differentiated cells.

4. Conclusions and outlook

In this focus review, we have summarized the recent progress in the use of LMWGs for the selective cell death. The distinct feature of the cell death induced by the LMWGs is that the self-assembly of LMWG molecules exhibit remarkable cytotoxicity, whereas a single molecule of a LMWG does not have cytotoxicity. To this end, there were several requirements: to deliver a substantial amount of LMWG molecules from the outside to the inside of a living cell, to allow their self-assembly under molecular crowding conditions and to inhibit their self-assembly outside a cell. Fortunately, LMWG molecules have small molecular weights that allow the rapid cell uptake of the LMWG molecules. It is easy to design the molecular structures of LMWGs to impart desired functional properties. The rapid response of their self-assembly to external stimuli amplifies a

nanoscale molecular event of a chemical or biochemical reaction to induce a macroscopic event [44, 52, 73-75]. These characteristics realized the spatiotemporal control of the self-assembly of LMWG molecules, leading to the selective cell death. The physiological activity induced by their self-assembly would provide a novel approach in drug discovery and therapeutics, and also in a novel tool for cell selection in tissue engineering and regenerative medicine [72].

Conflict of interest

There are no conflicts to declare.

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Tatsuo Maruyama received his PhD at the Department of Chemistry & Biotechnology of The University of Tokyo in 2002 under the supervision of Prof. Minoru Seki. He started his academic carrier as an assistant professor in Prof. Masahiro Goto's group at Kyushu University in 2002 and moved to Kobe University in 2007 as an associate professor. He was promoted to full professor in 2019. He received the SCEJ Award for Outstanding Young Researcher from The Society of Chemical Engineering Japan in 2006 and the Kao Scientists' Prize from The Kao Foundation for Arts and Sciences in 2018. His research interests lie in the areas of polymer-surface engineering and supramolecular gelator.



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References

1. Terech P, Weiss RG. Low molecular mass gelators of organic liquids and the properties of their gels. *Chem Rev.* 1997;97:3133-59.
2. Dastidar P. Supramolecular gelling agents: Can they be designed? *Chem Soc Rev.* 2008;37:2699-715.
3. Hanabusa K, Suzuki M. Development of low-molecular-weight gelators and polymer-based gelators. *Polymer J.* 2014;46:776-82.
4. Hanabusa K, Suzuki M. Physical gelation by low-molecular-weight compounds and development of gelators. *Bull Chem Soc Jpn.* 2016;89:174-82.
5. Zhang SG. Fabrication of novel biomaterials through molecular self-assembly. *Nat Biotechnol.* 2003;21:1171-8.
6. Das D, Dasgupta A, Roy S, Mitra RN, Debnath S, Das PK. Water gelation of an amino acid-based amphiphile. *Chem Eur J.* 2006;12:5068-74.
7. Dasgupta A, Mondal JH, Das D. Peptide hydrogels. *RSC Adv.* 2013;3:9117-49.
8. Fleming S, Ulijn RV. Design of nanostructures based on aromatic peptide amphiphiles. *Chem Soc Rev.* 2014;43:8150-77.
9. Du X, Zhou J, Shi J, Xu B. Supramolecular hydrogelators and hydrogels: From soft matter to molecular biomaterials. *Chem Rev.* 2015;115:13165-307.
10. Krieg E, Bastings MMC, Besenius P, Rybtchinski B. Supramolecular polymers in aqueous media. *Chem Rev.* 2016;116:2414-77.
11. Ikeda A, Sonoda K, Ayabe M, Tamaru S, Nakashima T, Kimizuka N, Shinkai S. Gelation of ionic liquids with a low molecular-weight gelator showing t-gel above 100 degrees c. *Chem Lett.* 2001;1154-5.
12. Kimizuka N, Nakashima T. Spontaneous self-assembly of glycolipid bilayer membranes in sugar-philic ionic liquids and formation of ionogels. *Langmuir.* 2001;17:6759-61.
13. Hanabusa K, Fukui H, Suzuki M, Shirai H. Specialist gelator for ionic liquids. *Langmuir.* 2005;21:10383-90.

14. Dutta S, Das D, Dasgupta A, Das PK. Amino acid based low-molecular-weight ionogels as efficient dye-adsorbing agents and templates for the synthesis of tio(2) nanoparticles. *Chem Eur J*. 2010;16:1493-505.
15. Minakuchi N, Hoe K, Yamaki D, Ten-No S, Nakashima K, Goto M, Mizuhata M, Maruyama T. Versatile supramolecular gelators that can harden water, organic solvents and ionic liquids. *Langmuir*. 2012;28:9259-66.
16. Holmes TC, de Lacalle S, Su X, Liu G, Rich A, Zhang S. Extensive neurite outgrowth and active synapse formation on self-assembling peptide scaffolds. *Proc Natl Acad Sci USA*. 2000;97:6728-33.
17. Hartgerink JD, Beniash E, Stupp SI. Peptide-amphiphile nanofibers: A versatile scaffold for the preparation of self-assembling materials. *Proc Natl Acad Sci USA*. 2002;99:5133-8.
18. Holmes TC. Novel peptide-based biomaterial scaffolds for tissue engineering. *Trends Biotechnol*. 2002;20:16-21.
19. Silva GA, Czeisler C, Niece KL, Beniash E, Harrington DA, Kessler JA, Stupp SI. Selective differentiation of neural progenitor cells by high-epitope density nanofibers. *Science*. 2004;303:1352-5.
20. Jayawarna V, Ali M, Jowitt TA, Miller AE, Saiani A, Gough JE, Ulijn RV. Nanostructured hydrogels for three-dimensional cell culture through self-assembly of fluorenylmethoxycarbonyl-dipeptides. *Advanced Materials*. 2006;18:611-+.
21. Schneider A, Garlick JA, Egles C. Self-assembling peptide nanofiber scaffolds accelerate wound healing. *PloS one*. 2008;3.
22. Lin BF, Megley KA, Viswanathan N, Krogstad DV, Drews LB, Kade MJ, Qian YC, Tirrell MV. Ph-responsive branched peptide amphiphile hydrogel designed for applications in regenerative medicine with potential as injectable tissue scaffolds. *J Mater Chem*. 2012;22:19447-54.
23. Mujeeb A, Miller AF, Saiani A, Gough JE. Self-assembled octapeptide scaffolds for in vitro chondrocyte culture. *Acta Biomater*. 2013;9:4609-17.
24. Mahler A, Reches M, Rechter M, Cohen S, Gazit E. Rigid, self-assembled hydrogel composed of a modified aromatic dipeptide. *Advanced Materials*. 2006;18:1365-+.
25. Gao Y, Kuang Y, Guo ZF, Guo Z, Krauss IJ, Xu B. Enzyme-instructed molecular self-assembly confers nanofibers and a supramolecular hydrogel of taxol derivative. *J Am Chem Soc*. 2009;131:13576-7.
26. Liang GL, Yang ZM, Zhang RJ, Li LH, Fan YJ, Kuang Y, Gao Y, Wang T, Lu WW, Xu B. Supramolecular hydrogel of a d-amino acid dipeptide for controlled drug release in vivo. *Langmuir*. 2009;25:8419-22.
27. Nanda J, Banerjee A. Beta-amino acid containing proteolitically stable dipeptide based hydrogels: Encapsulation and sustained release of some important biomolecules at physiological ph and temperature. *Soft Matter*. 2012;8:3380-6.
28. Restu WK, Nishida Y, Yamamoto S, Ishii J, Maruyama T. Short oligopeptides for biocompatible and biodegradable supramolecular hydrogels. *Langmuir*. 2018;in press.

29. Hughes M, Debnath S, Knapp CW, Ulijn RV. Antimicrobial properties of enzymatically triggered self-assembling aromatic peptide amphiphiles. *Biomaterials Science*. 2013;1:1138-42.
30. Debnath S, Shome A, Das D, Das PK. Hydrogelation through self-assembly of fmoc-peptide functionalized cationic amphiphiles: Potent antibacterial agent. *J Phys Chem B*. 2010;114:4407-15.
31. Fukushima K, Liu S, Wu H, Engler AC, Coady DJ, Maune H, Pitera J, Nelson A, Wiradharma N, Venkataraman S, Huang Y, Fan W, Ying JY, Yang YY, Hedrick JL. Supramolecular high-aspect ratio assemblies with strong antifungal activity. *Nat Commun*. 2013;4:2861.
32. Bai JK, Gong ZY, Wang JX, Wang CD. Enzymatic hydrogelation of self-assembling peptide i4k2 and its antibacterial and drug sustained-release activities. *RSC Adv*. 2017;7:48631-8.
33. Guler MO, Stupp SI. A self-assembled nanofiber catalyst for ester hydrolysis. *J Am Chem Soc*. 2007;129:12082-3.
34. Fang WW, Zhang Y, Wu JJ, Liu C, Zhu HB, Tu T. Recent advances in supramolecular gels and catalysis. *Chem Asian J*. 2018;13:712-29.
35. Love CS, Chechik V, Smith DK, Wilson K, Ashworth I, Brennan C. Synthesis of gold nanoparticles within a supramolecular gel-phase network. *Chem Commun*. 2005:1971-3.
36. Adhikari B, Biswas A, Banerjee A. Graphene oxide-based supramolecular hydrogels for making nanohybrid systems with au nanoparticles. *Langmuir*. 2012;28:1460-9.
37. Wu ZX, Wang J, Han LL, Lin RQ, Liu HF, Xin HLL, Wang DL. Supramolecular gel-assisted synthesis of double shelled co@coo@n-c/c nanoparticles with synergistic electrocatalytic activity for the oxygen reduction reaction. *Nanoscale*. 2016;8:4681-7.
38. Nishida Y, Tanaka A, Yamamoto S, Tominaga Y, Kunikata N, Mizuhata M, Maruyama T. In situ synthesis of a supramolecular hydrogelator at an oil/water interface for stabilization and stimuli-induced fusion of microdroplets. *Angew Chem Int Ed*. 2017;56:9410-4.
39. Yoshimura I, Miyahara Y, Kasagi N, Yamane H, Ojida A, Hamachi I. Molecular recognition in a supramolecular hydrogel to afford a semi-wet sensor chip. *Journal of the American Chemical Society*. 2004;126:12204-5.
40. Yamaguchi S, Yoshimura L, Kohira T, Tamaru S, Hamachi I. Cooperation between artificial receptors and supramolecular hydrogels for sensing and discriminating phosphate derivatives. *Journal of the American Chemical Society*. 2005;127:11835-41.
41. Koshi Y, Nakata E, Yamane H, Hamachi I. A fluorescent lectin array using supramolecular hydrogel for simple detection and pattern profiling for various glycoconjugates. *Journal of the American Chemical Society*. 2006;128:10413-22.
42. Wada A, Tamaru S, Ikeda M, Hamachi I. Mcm-enzyme-supramolecular hydrogel hybrid as a fluorescence sensing material for polyanions of biological significance. *Journal of the American Chemical Society*. 2009;131:5321-30.
43. Ikeda M, Ochi R, Wada A, Hamachi I. Supramolecular hydrogel capsule showing prostate specific antigen-responsive function for sensing and targeting prostate cancer cells. *Chem Sci*. 2010;1:491-8.

44. Ikeda M, Tanida T, Yoshii T, Kurotani K, Onogi S, Urayama K, Hamachi I. Installing logic-gate responses to a variety of biological substances in supramolecular hydrogel-enzyme hybrids. *Nat Chem*. 2014;6:511-8.
45. Scott GG, McKnight PJ, Tuttle T, Ulijn RV. Tripeptide emulsifiers. *Adv Mater*. 2016;28:1381-13816.
46. Ray S, Das AK, Banerjee A. Ph-responsive, bolaamphiphile-based smart metallo-hydrogels as potential dye-adsorbing agents, water purifier, and vitamin b-12 carrier. *Chem Mater*. 2007;19:1633-9.
47. Adhikari B, Palui G, Banerjee A. Self-assembling tripeptide based hydrogels and their use in removal of dyes from waste-water. *Soft Matter*. 2009;5:3452-60.
48. Frederix P, Scott GG, Abul-Haija YM, Kalafatovic D, Pappas CG, Javid N, Hunt NT, Ulijn RV, Tuttle T. Exploring the sequence space for (tri-) peptide self-assembly to design and discover. *Nat Chem*. 2015;7:30-7.
49. Zhang S, Holmes TC, DiPersio CM, Hynes RO, Su X, Rich A. Self-complementary oligopeptide matrices support mammalian cell attachment. *Biomaterials*. 1995;16:1385-93.
50. Hartgerink JD, Beniash E, Stupp SI. Self-assembly and mineralization of peptide-amphiphile nanofibers. *Science*. 2001;294:1684-8.
51. Xing BG, Yu CW, Chow KH, Ho PL, Fu DG, Xu B. Hydrophobic interaction and hydrogen bonding cooperatively confer a vancomycin hydrogel: A potential candidate for biomaterials. *Journal of the American Chemical Society*. 2002;124:14846-7.
52. Zhang Y, Gu HW, Yang ZM, Xu B. Supramolecular hydrogels respond to ligand-receptor interaction. *J Am Chem Soc*. 2003;125:13680-1.
53. Yang ZM, Liang GL, Ma ML, Gao Y, Xu B. Conjugates of naphthalene and dipeptides produce molecular hydrogelators with high efficiency of hydrogelation and superhelical nanofibers. *J Mater Chem*. 2007;17:850-4.
54. Koda D, Maruyama T, Minakuchi N, Nakashima K, Goto M. Proteinase-mediated drastic morphological change of peptide-amphiphile to induce supramolecular hydrogelation. *Chem Commun*. 2010;46:979-81.
55. Maruyama T, Matsushita H, Shimada Y, Kamata I, Hanaki M, Sonokawa S, Kamiya N, Goto M. Proteins and protein-rich biomass as environmentally friendly adsorbents selective for precious metal ions. *Environ Sci Technol*. 2007;41:1359-64.
56. Maruyama T, Terashima Y, Takeda S, Okazaki F, Goto M. Selective adsorption and recovery of precious metal ions using protein-rich biomass as efficient adsorbents. *Process Biochem*. 2014;49:850-7.
57. WO/2010/013555.
58. Yang Z, Liang G, Guo Z, Guo Z, Xu B. Intracellular hydrogelation of small molecules inhibits bacterial growth. *Angew Chem Int Ed*. 2007;46:8216-9.
59. Yang ZM, Xu KM, Guo ZF, Guo ZH, Xu B. Intracellular enzymatic formation of nanofibers results in hydrogelation and regulated cell death. *Adv Mater*. 2007;19:3152-6.

60. Yamashita K, Azumano I, Mai M, Okada Y. Expression and tissue localization of matrix metalloproteinase 7 (matrilysin) in human gastric carcinomas. Implications for vessel invasion and metastasis. *Int J Cancer*. 1998;79:187-94.
61. Egeblad M, Werb Z. New functions for the matrix metalloproteinases in cancer progression. *Nat Rev Cancer*. 2002;2:161-74.
62. Nabeshima K, Inoue T, Shimao Y, Sameshima T. Matrix metalloproteinases in tumor invasion: Role for cell migration. *Pathol Int*. 2002;52:255-64.
63. Welch AR, Holman CM, Browner MF, Gehring MR, Kan CC, Van Wart HE. Purification of human matrilysin produced in escherichia coli and characterization using a new optimized fluorogenic peptide substrate. *Arch Biochem Biophys*. 1995;324:59-64.
64. Tanaka A, Fukuoka Y, Morimoto Y, Honjo T, Koda D, Goto M, Maruyama T. Cancer cell death induced by the intracellular self-assembly of an enzyme-responsive supramolecular gelator. *J Am Chem Soc*. 2015;137:770-5.
65. Kuimova MK, Botchway SW, Parker AW, Balaz M, Collins HA, Anderson HL, Suhling K, Ogilby PR. Imaging intracellular viscosity of a single cell during photoinduced cell death. *Nat Chem*. 2009;1:69-73.
66. Woerner AC, Frottin F, Hornburg D, Feng LR, Meissner F, Patra M, Tatzelt J, Mann M, Winklhofer KF, Hartl FU, Hipp MS. Cytoplasmic protein aggregates interfere with nucleocytoplasmic transport of protein and rna. *Science*. 2016;351:173-6.
67. Zhou J, Du XW, Yamagata N, Xu B. Enzyme-instructed self-assembly of small d-peptides as a multiple-step process for selectively killing cancer cells. *J Am Chem Soc*. 2016;138:3813-23.
68. Wang HM, Feng ZQQ, Wu DD, Fritzsche KJ, Rigney M, Zhou J, Jiang YJ, Schmidt-Rohr K, Xu B. Enzyme-regulated supramolecular assemblies of cholesterol conjugates against drug-resistant ovarian cancer cells. *J Am Chem Soc*. 2016;138:10758-61.
69. Feng ZQQ, Wang HM, Chen XY, Xu B. Self-assembling ability determines the activity of enzyme-instructed self-assembly for inhibiting cancer cells. *J Am Chem Soc*. 2017;139:15377-84.
70. Feng Z, Han X, Wang H, Tang T, Xu B. Enzyme-instructed peptide assemblies selectively inhibit bone tumors. *Chem*. 2019;5:2442-9.
71. Pires RA, Abul-Haija YM, Costa DS, Novoa-Carballal R, Reis RL, Ulijn RV, Pashkuleva I. Controlling cancer cell fate using localized biocatalytic self-assembly of an aromatic carbohydrate amphiphile. *J Am Chem Soc*. 2015;137:576-9.
72. Kuang Y, Miki K, Parr CJC, Hayashi K, Takei I, Li J, Iwasaki M, Nakagawa M, Yoshida Y, Saito H. Efficient, selective removal of human pluripotent stem cells via ecto-alkaline phosphatase-mediated aggregation of synthetic peptides. *Cell Chem Biol*. 2017;24:685-94 e4.
73. Yang ZM, Gu HW, Fu DG, Gao P, Lam JK, Xu B. Enzymatic formation of supramolecular hydrogels. *Adv Mater*. 2004;16:1440-4.
74. Ikeda M, Tanida T, Yoshii T, Hamachi I. Rational molecular design of stimulus-responsive supramolecular hydrogels based on dipeptides. *Adv Mater*. 2011;23:2819-22.
75. Ikeda M. Stimuli-responsive supramolecular systems guided by chemical reactions. *Polymer J*.

2019;51:371-80.

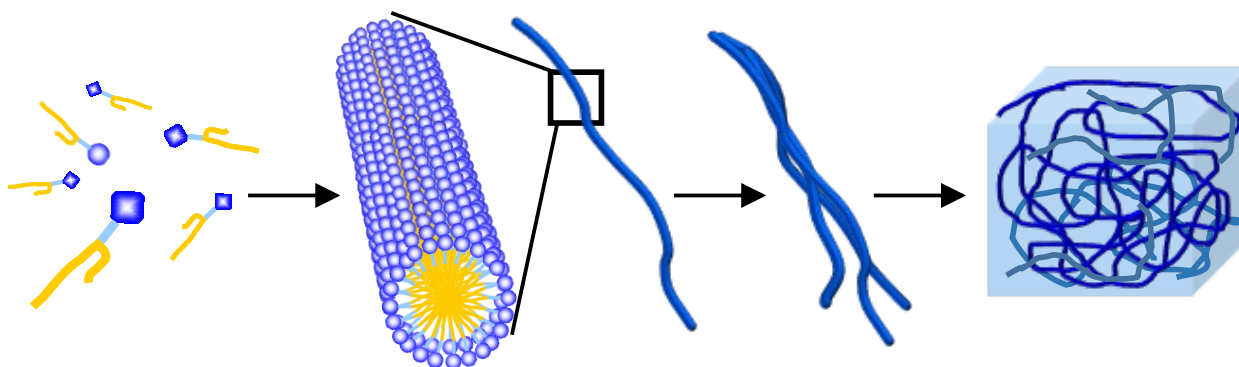


Fig. 1 Schematic illustration of fibrous self-assembly of LMWG molecules to form a hydrogel.

PA1: C16-Cys-Cys-Cys-Cys-Gly-Gly-Gly-Ser-Arg-Gly-Asp

PA2: C16-Ala-Ala-Ala-Ala-Gly-Gly-Gly-Glu-Ile-Lys-Val-Ala-Val

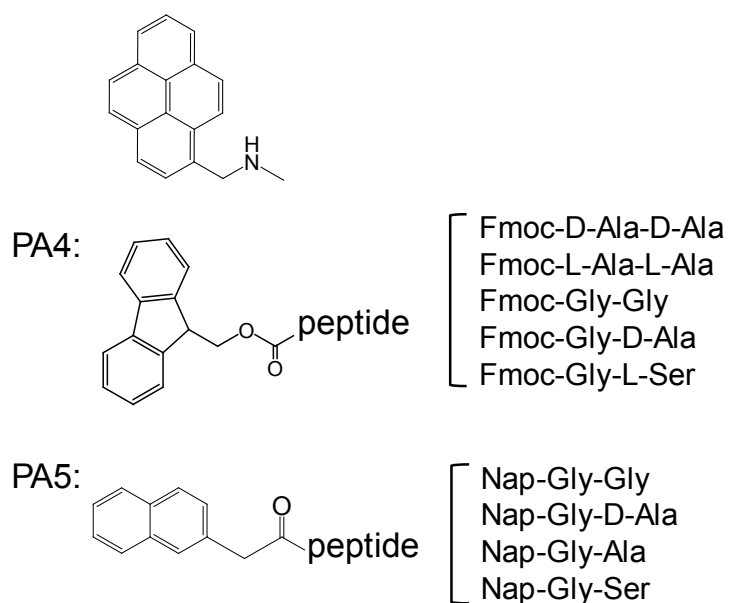
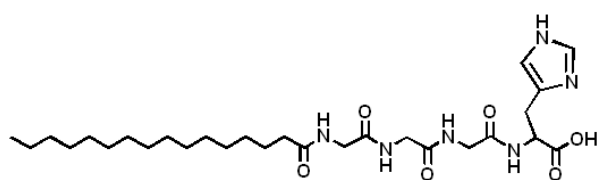


Fig. 2 Chemical structures of representative peptide-based LMWGs.



C16-Gly-Gly-Gly-His

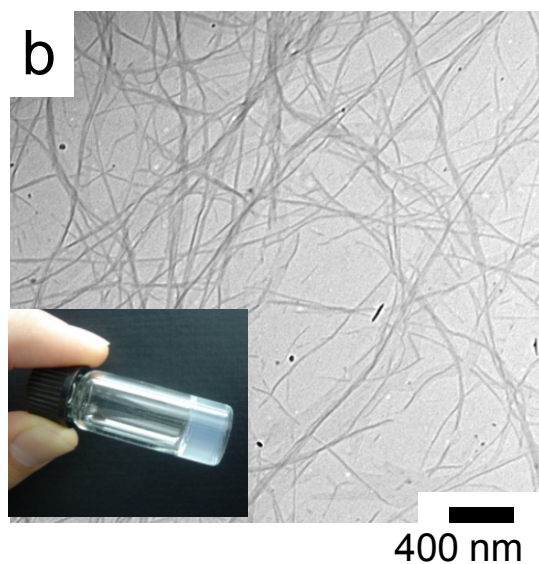
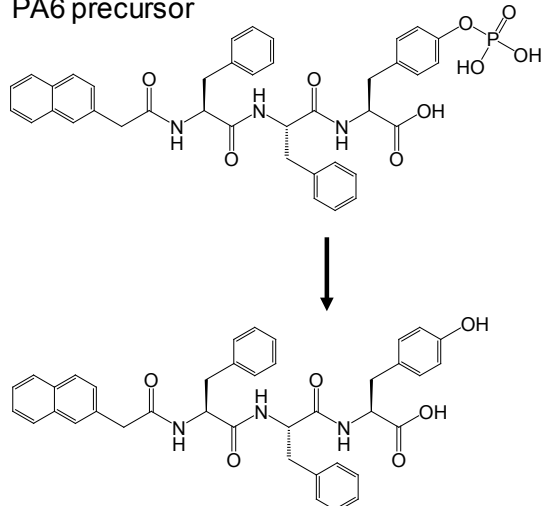


Fig. 3 a) Molecular structure of C16-Gly-Gly-Gly-His. b) TEM image of freeze-dried C16-Gly-Gly-Gly-His hydrogel and a macroscopic image of the hydrogel (0.2 wt%) in pH 7.5 phosphate buffer (inset). Reproduced from ref. 54.

PA6 precursor



b PA7 precursor

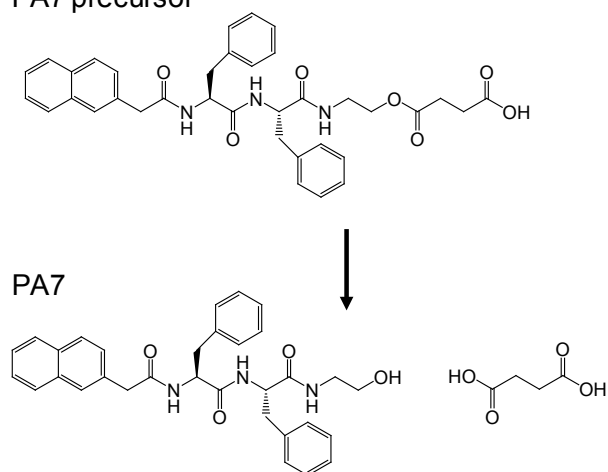


Fig. 4 Enzyme-mediated production of self-assembling PAs from PA precursors [58, 59].

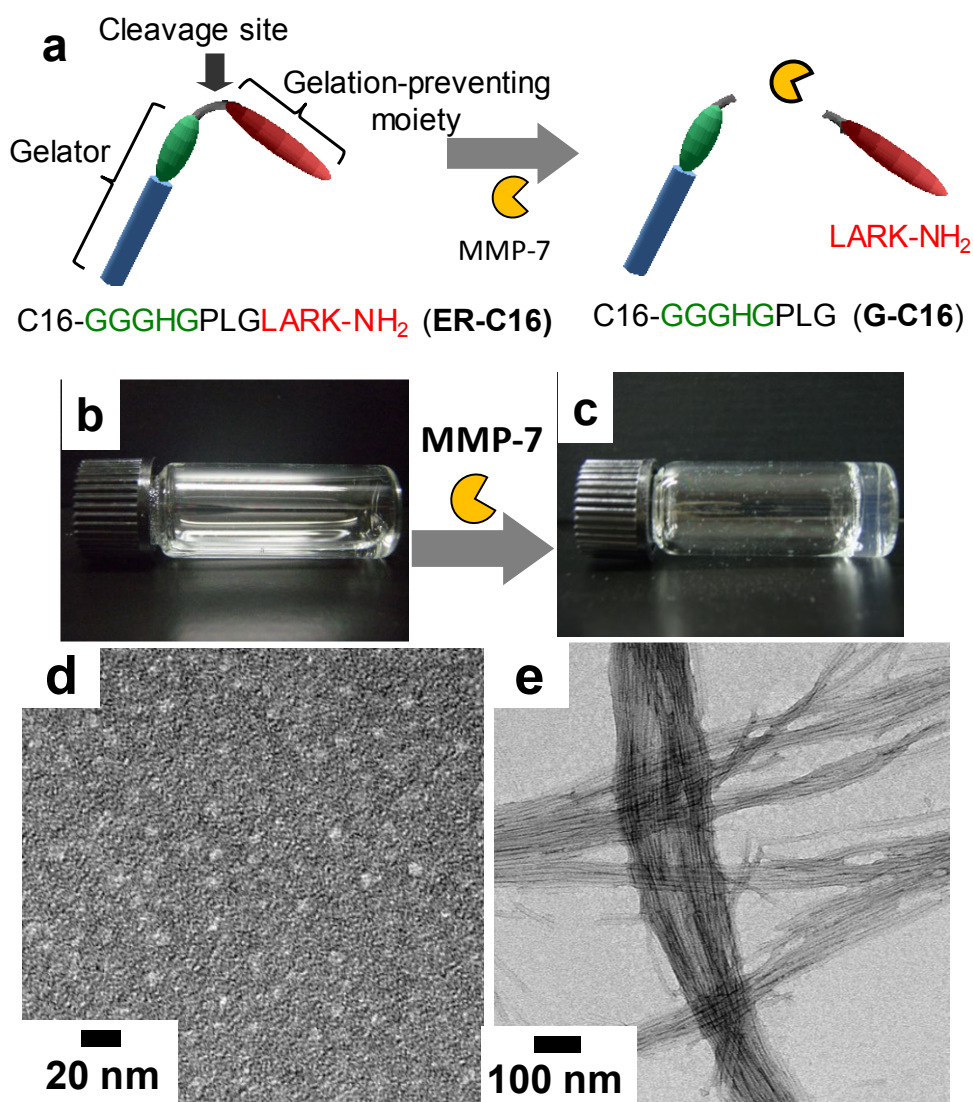


Fig. 5 (a) Schematic illustration of MMP-7-mediated hydrolysis of a gelator precursor (ER-C18). (b, c) Optical images of the 0.2 wt% precursor solution in Tris-HCl buffer (b), the hydrogel obtained after adding MMP-7 (2 µg/mL) to the solution (reaction time of 2 h) (c). (d, e) TEM images of the freeze-dried 0.2 wt% precursor solution (d) and hydrogel (e) obtained after adding 1 µg/mL MMP-7 to the solution [54].

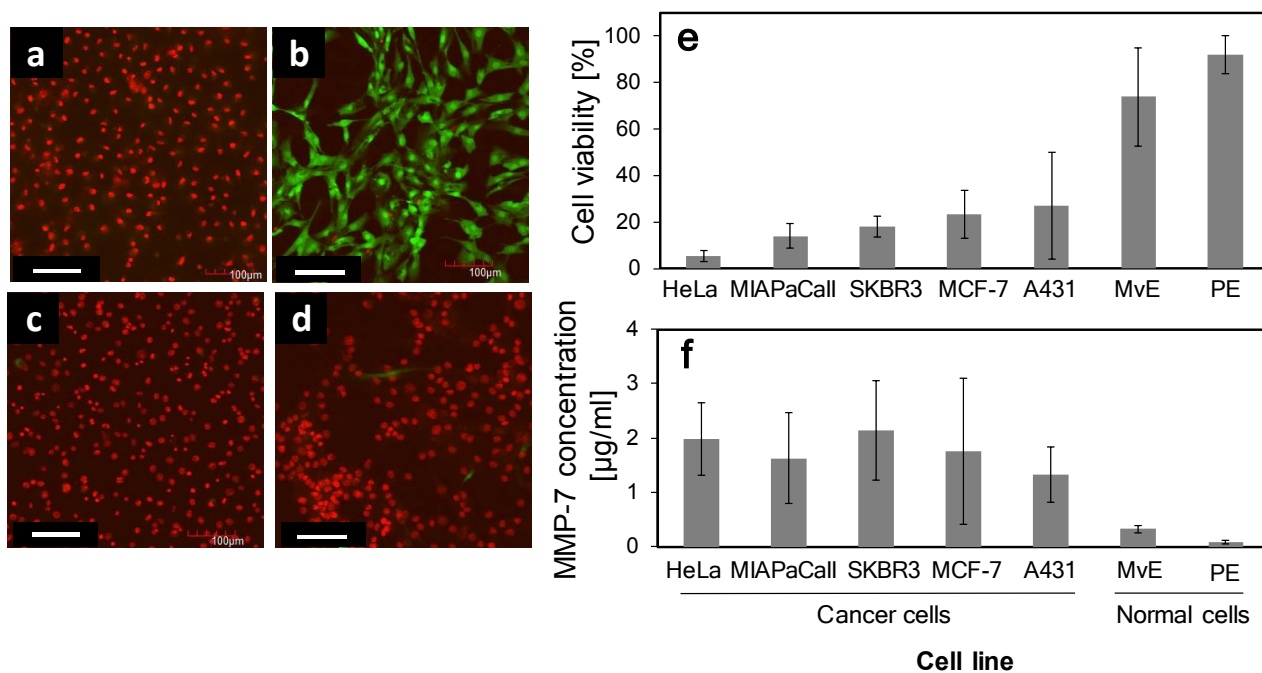


Fig. 6 (a–d) Live/dead assays of HeLa cells (a, c) and MvE cells (b, d) after incubation for 18 h with **ER-C16** and its derivatives. Cells cultured on a microplate were observed using a confocal laser scanning microscope (CLSM). Living cells are dyed green (bright) and dead cells are dyed red (dark). (a, b) The gelator precursor (**ER-C16**, 0.02 wt%) and (c, d) the gelator (**G-C16**, 0.02 wt%). Scale bars represent 100 μm . (e) Viability assays of cancer cells and normal human cells after incubation with **ER-C16** (0.025 wt%). (f) MMP-7 concentration in the culture media after culturing the cells. MvE cells represents normal human dermal microvascular endothelial cells and PE represents primary human pancreatic epithelial cells. Reproduced from ref. 64.