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Divergent Supramolecular Gelation of Backbone Modified Short Hybrid δ-Peptides

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ABSTRACT

The ordered supramolecular assemblies of short peptides have been recently gaining momentum due to their wide spread applications in biology and materials sciences. In contrast to the α-peptides, limited success has been achieved from the backbone modified peptides. The proteolytic stability and conformational flexibility of the backbone modified peptides composed of β-,γ- and δ-amino acids can be explored to design ordered supramolecular gels and self-assembled materials. In this article, we are reporting the divergent supramolecular gels from a new class of short hybrid dipeptides composed of conformationally flexible new β(O)-δ^5-amino acids. The hybrid dipeptide composed of β^3- and β(O)-δ^5-Phe showed the formation of transparent gels from the aromatic solvents, while the dipeptide composed of β(O)-δ^5-Phe showed the thixotropic gel in phosphate

buffered saline (PBS). In contrast, no organic or hydrogels were observed from the dipeptides composed of alternating α- and β(O)-δ^5-Phe as well as γ^4 and β(O)-δ^5-Phe. The organogelation property displayed by the β^3,β(O)-δ^5-Phe dipeptide was further explored to recover the oil spills from the oil-water mixture. The thixotropic hydrogels displayed by the β(O)-δ^5,β(O)-δ^5-Phe dipeptide was further utilized as matrix along with cell culture medium to grow the cells in 2D-cell culture. Replacing the backbone -CH_2- with "O" in the δ-Phe leads to the drastic change in the supramolecular behavior of δ-peptides. Overall, the short dipeptides from different backbone modified amino acids showed the divergent gelation properties and these properties can be further explored to design new functional biomaterials.

KEYWORDS: δ-Peptides, organo and hydrogels, stimuli responsive, thixotropic, oil-spill recovery, 2D-cell culture, proteolytic stability.

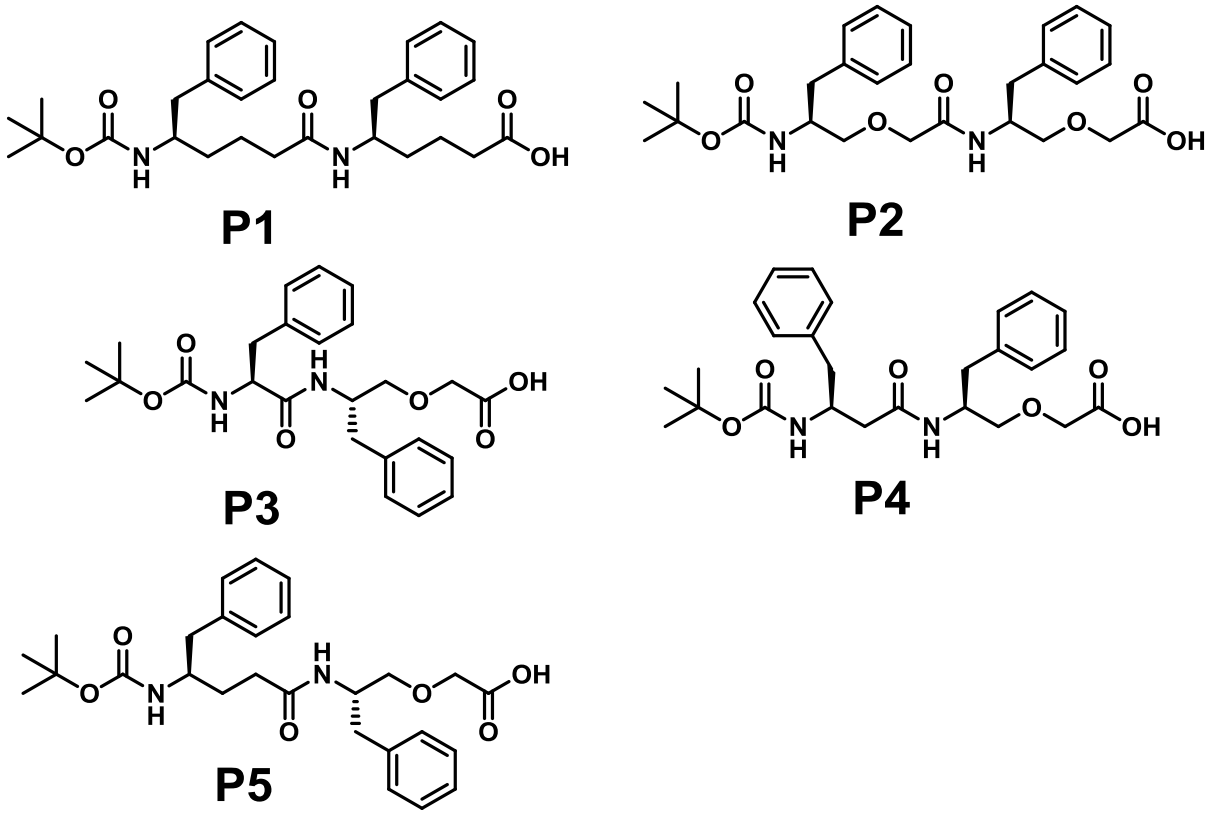
INTRODUCTION

The central theme in the fields of nanoscience and nanotechnology is the formation of highly ordered self-assembled materials in nanoscale.[1] The fascinating properties displayed by the nanostructures have attracted considerable attention in recent years due to their widespread applications in various fields including energytechnologies, catalysis, biomedicaland chemical biology.[2-5] In addition to the numerous types of nano-assemblies derived from the small organic molecules, the biomacromolecules such as lipids, proteins, carbohydrates and nucleic acids, often self-assembled into highly ordered supramolecular architectures.[6-9] The hierarchical supramolecular structures of biomacromolecules are stabilized by various types of non-covalent interactions such as H-bonding, salt bridge interactions, van der Waals interactions and aromatic

pi-stacking.[10] Along with the biomacromolecules, shortpeptides have displayed a rich supramolecular diversity and have been serving as excellent tools to design various types of self-assembled biomaterials.[11-13] In fact, the finding of self-assembled peptide nanotube from a Phe-Phe dipeptide by the Gazit and colleagues has opened new avenues in the generation of soft materials from the short peptides.[14] The dipeptide nanotubes have been explored as templates to cast silver nanowires, as drug delivery agents, light harvesting systems, energy storage materials and antimicrobial candidates etc.[15-18] In addition, the self-assembling properties of peptides have been finding applications in the fields of tissue engineering as well as nanoelectronics.[19, 20]Along with the α-peptides, efforts have also been made in the literature to understand the molecular self-assemblies of β- and γ-peptides.[21,22] A variety of supramolecular architectures such as tapes, fibers, ribbons, polyhedrons, nanotubes and vesicles have also been derived from the supramolecular assemblies of β- and γ-peptides.[23-25]Recently, Malhotra *et al*. demonstrated the antimicrobial properties of self-assembled α,γ-hybrid peptides.[26] The advantage of β- and γ–peptides over the α-peptides is that they are proteolytically stable and required short sequences to attain folded structures.[27]

In addition to the biomaterials, the low molecular weight gelators (LMWG) have also attracted considerable attention due to their applications in oil spill recovery,[28,29] toxicity remediation devices,[30,31] electro-optical displays,[32] drug delivery systems,[33,34] tissue engineering,[35] wound healing[36] etc. The groups of Gazit,[19] Uljin,[38] Xu,[39]Adams[40] and others[41,42] have examined the potential of aromatic-aromatic interactions in the design of different organo and hydrogels from the short peptide sequences. Further, Banerjee and colleagues showed the influence of fatty acids on the supramolecular hydrogelation of Phe-Phe dipeptides.[28,33] We have been interested in understanding the conformational and self-assembling properties of peptides composed of various

types of non-natural β- and γ-amino acids.[43,44] In comparison to β- and γ-amino acids, structural and self-assembling properties of peptides composed of higher homologous amino acids such as δ-amino acids are scarcely examined in the literature. Nevertheless, Hoffman and colleagues investigated the possible conformational space available to the peptides composed of δ-amino acids using theoretical calculations.[45] Apart from the theoretical calculations, the groups of Huc,[46] Sharma[47] and Chakraborty[48] have examined the structural properties of peptides and foldamers



Scheme1: Sequences of the dipeptides under investigations.

containing various types of δ-amino acids. Recently, we have shown the superior and spontaneous supramolecular gelation properties from γ^4Phe-γ^4Phe dipeptide over the β^3Phe-β^3Phe and Phe-Phe dipeptides.[49] As γ^4Phe-γ^4Phe showed excellent gelation over the β- and α-dipeptide counterparts, we have hypothesized that δ^5Phe-δ^5Phe dipeptide motifs can be served as better hydrogelators than

the γ-dipeptide counterpart. More importantly, as the length of the δ^5-amino acids is equal to the length of an α-dipeptide, they can be used as surrogates of α-dipeptides to overcome the proteolytic cleavage. In this context, we have synthesized peptide **P1** and examined its hydrogelation properties in phosphate saline buffer. In sharp contrast to γ^4Phe-γ^4Phe, the new peptide δ^5Phe-δ^5Phe failed to give hydrogels in PBS and aqueous buffers. In order to increase the solubility and conformational flexibility of the peptides composed of δ^5-Phe we considered to introduce heteroatom oxygen (O) into the backbone of δ^5-Phe. We sought to systematically investigate the gelation properties of the hybrid peptides composed of Xxx-β(O)-δ^5-Phe, where Xxx is α, β^3, γ^4 or β(O)-δ^5-Phe (Scheme **1**). Here, we report the formation of the stimuli responsive, injectable, biocompatible hydrogels from the Boc-β(O)-δ^5-Phe-β(O)-δ^5-Phe dipeptide, its utility in 2D-cell culture and organogels from Boc-β^3Phe-β(O)-δ^5-Phe dipeptide and its potential application in oil spill recovery. Under identical conditions, both Boc-αPhe-β(O)-δ^5-Phe and Boc-γ^4Phe-β(O)-δ^5-Phe did not give either organic or hydrogels.

EXPERIMENTAL SECTION

Materials

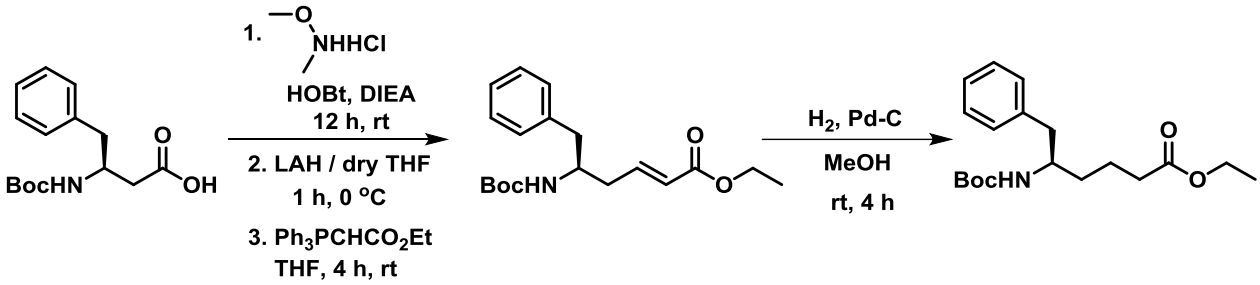
All chemical and reagents including amino acids were purchased from commercial sources. Solvents ethyl acetate and petroleum ether (60−80 °C) were distilled before to use. Solvent tetrahydrofuran (THF) was dried by reflexing on sodium metal wire and distilled before to use. Silica gel 120−200 mesh was used to purify the protected amino acids and peptides in column chromatography. The ^{1}H NMR spectra of protected amino acids and peptides were recorded on 400 MHz (or ^{13}C on 100 MHz) in the solvent deuterated chloroform ($CDCl_3$). The acid labile Boc

and alkyl esters (ethyl or methyl esters) were used for the *N*- and C-terminus protections, respectively. The Boc-(*S*)-β^3-Phe was synthesized starting from Boc-Phe by the reported protocol.[50] The Boc-(*R*)-γ^4-Phe was synthesized starting from Boc-(*S*)-Phe aldehyde through Witting reaction followed by the reduction of double bonds using H_2, Pd/C (10%). Finally, ethyl ester of Boc-γ^4-Phe was subjected to saponification as reported earlier.[43] The β(O)-δ^5-Phe was synthesized starting from Boc-protected amino alcohol using reported protocol.[51] The carbon analogous of β(O)-δ^5-Phe was synthesized from Boc-(*S*)-β^3-Phe. All peptide couplings reactions were achieved using the coupling reagent *N*-ethyl-*N*′-(3-(dimethylaminopropyl)carbodiimide hydrochloride (EDC.HCl) and 1-hydroxybenzotriazole (HOBt) and *N*,*N*-diisopropylethylamine (DIEA). Finally, the pure peptides were characterized by ^{1}H and ^{13}C NMR spectroscopy and authenticated by high resolution mass spectroscopy (HRMS).

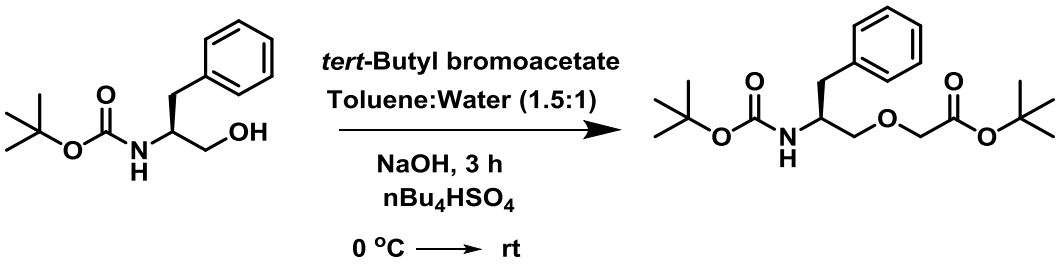
General Procedure for the Solution Phase Peptides (P1-P5) Synthesis:

All peptide (**P1**-**P5**) were synthesized through solution phase peptide chemistry as reported earlier.[49] In brief, 1 mmol of *N*-Boc-protected-amino acid was dissolved in 5 mL of DMF and the solution was to cooled to 0 °C. Subsequently, 1mmol of 1EDC·HCl and 1 mmol of HOBt and 2 mmol of DIEA were added reaction at 0 °C. Immediately, 1.1 mmol of methyl ester of amino acid to the reaction mixture and reaction was allowed to come to the room temperature. The reaction mixture was stirred for about 12 h. The reaction progress was monitored using TLC. After completion, 50 mL of ethyl acetate was added to the reaction mixture followed by 50 mL of saturated sodium chloride solution. The ethyl acetate layer was separated and the aqueous layer was extracted again with ethyl acetate (50 mL × 3). The combined ethyl acetate layer was washed

with 10% Na_2CO_3 (20 mL × 3), 5% HCl (20 mL × 3), water (20 mL × 3), finally with saturated sodium chloride solution (30 mL × 2). The solution was dried over anhydrous sodium sulfate and evaporated under *vacuum* to obtain crude peptides. These crude peptides were purified using column chromatography with EtOAc/hexane solvent system. Finally, peptides were subjected to saponification in methanol solution using 1*N* NaOH. The peptide acid obtained after saponification were directly used gelation studies.



Scheme 2: Synthesis of *N*-Boc-δ^5-Phe-OEt form *N*-Boc-β^3Phe-OH



Scheme3: Synthesis of *N*-Boc-β(O)-δ^5-Phe-O^{t}Bu form *N*-Boc amino alcohol.

Synthesis of *N*-Boc-δ^5-Phe-OH

N-Boc-δ^5-Phe-OH was synthesized starting from *N*-Boc-(*S*)-β^3-Phe-OH. Briefly, *N*-Boc-(*S*)-β^3-Phe-Weinreb amide (1.6 g, 5 mmol) was converted to aldehyde through the reduction of Weinreb amide using LAH (228 mg, 6 mmol) and subjected Wittig reaction to get *N*-Boc-α,β-unsaturated-δ^5-Phe-OEt. The Wittig product was purified using column chromatography by EtOAc/hexane

solvent system. After that, the *N*-Boc-α,β-unsaturated-δ^5-Phe-OEt was reduced using H_2/Pd-C to get *N*-Boc-δ^5-Phe-OEt. Further, the *N*-Boc-δ^5-Phe-OEt was subjected saponification using 1*N* NaOH in EtOH to obtain *N*-Boc-δ^5Phe-OH and directly used for the peptides synthesis without purification.

Synthesis of *N*-Boc-β(O)-δ^5-Phe-O^{t}Bu:

The *N*-Boc-β(O)-δ-Phe was synthesized as reported earlier.[51] Briefly, *tetra*- butylammonium hydrogensulfate (340 mg, 1 mmol) was dissolved in solution of sodium hydroxide (10 g, 250 mmol in 10 mL of water and 15 mL of toluene). To this biphasic reaction mixture, *tert*-butyl bromoacetate (2.2 mL, 15 mmol) was added slowly at room temperature. The reaction mixture was cooled to 0 °C, followed by slow addition of *N*-Boc-phenyl alanine alcohol (2.5 g, 10 mmol) in toluene (15 mL). The reaction was allowed to come to room temperature and the stirring was continued for about 3 h. The organic phase was separated from the aqueous phase. The aqueous solution was again extracted with diethyl ether (30 mL ×2). The combined organic layer was washed with saturated sodium chloride solution (30 mL × 2), dried over anhydrous sodium sulfate and evaporated under reduced pressure. The crude product of *N*-Boc-β(O)-δ^5-Phe-O^{t}Bu was purified by column chromatography using EtOAc/hexane solvent system.

Gelation Study of Peptides P1, P2 and P4

Peptides **P1** and **P4** (10 mg each) were placed in the separate sample vials. To each vial of peptides, 1 mL of toluene was added. The peptide solution in toluene was heated on a hot air gun to dissolve the peptides. The clear solution of peptides in toluene was cooled to room temperature and sonicated for about 10 min. The organo gelation property of peptide **P1** and **P4** was confirmed by

the inverted sample vial experiment. For the hydrogelation, 10 mg of peptide **P2** was dissolved in 1 mL of 1× PBS buffer at pH 7.4 and gently heated on a hot air gun to make it a clear solution and cooled to room temperature. Upon standing for about 12 h, the solution slowly transforms to gel state and the gelation property was further confirmed by the inverted sample vial experiment.

Morphology Study of Peptide P1, P2 and P4

FE-SEM experiment was carried out by drop casting 5μL of gel on SiO_2/Si substrate. The samples were dried at room temperature under *vacuum* and the samples on SiO_2/Si substrate were again coated with gold. The images were taken using scanning electron microscope using tungsten filament as electron source operated at 10 kV.

Rheology Experiment

The rheology experiments were carried out on preformed gel samples of organogel (in toluene) and hydrogel (in 1× PBS) at a concentration of 10 mg/mL using rheometer. All experiments were carried out single time.

Oil Spill Recovery Experiment

Peptide **P4** shows phase selective gelation in hydrocarbon solvent. In a typical experiment the solution of peptide **P4** (30 mg in 3 mL) in toluene was added to the oil-water mixture composed of 10 mL of oil and 30 mL of saturated NaCl solution. To mimic the sea water saturated NaCl solution was used. Instructively, upon treatment of the peptide solution the oil transformed into gels in less than 5 min. The peptide-oil gel was scooped out from the oil/water mixture and subjected to *vacuum* distillation and the oil was recovered from the oil water mixture.

Proteolytic Stability of Peptide P2

Proteolytic stability of peptide **P2** was carried out using reported protocol.[37] The peptide **P2** (1 mM) was dissolved in 1 mL of HEPES buffer (pH 7.4) containing 5% DMSO. Then, 10μL of 5μM chymotrypsin enzyme was added to the peptide solution and incubated at 37 ˚C for about 24 h. After that, 50 μL from the above mentioned stock solution was injected in RP-HPLC. The HPLC was carried out using ACN/H_2O at flow rate of 1 mL/min. Same procedure was followed to understand the proteolytic stability of peptide Boc-Phe-Phe-OH.

Effect of Hydrogel from Peptide P2 on Cell Viability

Cells were cultured in complete growth media (DMEM/F12) with 10% (v/v) (fetal bovine serum) FBS and 100 U/mL penicillin in humidified 5% (v/v) CO_2 at 37 °C. Gel was prepared by diluting peptide**P2** dissolved in PBS to 10-fold using DMEM cell culture medium. This was coated on the 96-well cell culture plate and incubated further for about 3 h. Cells suspension in complete growth medium was pipetted into each well (pre-coated with gel) at a count of 10,000 cells/well. The plate was further incubated for 18 h at 37 °C under 5% of CO_2. After 18 h of incubation complete growth media was removed and cells were washed with PBS. Cell viability assay was carried out after 18 h using calcein staining. Calcein AM stain at 2 μM final concentration was added to each cells and incubated for 45 min and then images were taken at 10× with fluorescence microscope. Experiments were performed as four independent replicates. The data represented in bar graph are further analyzed. Significance values indicated by * for $p < 0.05$, ns ~ non-significant for $p > 0.05$; in comparison to the respective control cell samples.

RESULT AND DISCUSSION

Gelation Study

The sequences of δ- and hybrid δ-peptides are given in the Scheme **1**. The β^3- and γ^4-Phe were synthesized as reported earlier.[50,43] The δ^5-Phe was synthesized starting from the Boc-β^3-Phe as shown in the Scheme **2**. The new β(O)-δ^5-Phe was synthesized starting from β-amino alcohol as shown in the Scheme **3**. To understand their ordered self-aggregation properties, the purified peptide **P1** was subjected to the gelation studies in 1× phosphate buffer saline (1×PBS) at pH=7.4 as well as in aromatic organic solvents. Peptide **P1** failed to give hydrogels in aqueous buffer however it gelates various aromatic organic solvents. The organogels of **P1** in various aromatic solvents are shown in the Figure **1**. In contrast, peptide **P2** gave stable gels in 1× PBS at pH 7.4 at the concentration of 10 mg/mL dissolved upon gently heating and standing for about 12 h. Inspired by the spontaneous hydrogelation of **P2**, we have systematically probed the gelation properties of hybrid dipeptides consisting Xxx-β(O)-δ^5-Phe sequences (**P3-P5**, Scheme **1**). Among the all hybrid peptides, **P4** has shown excellent gelation property in a variety of aromatic solvents such as toluene, benzene, xylene and mesitylene at the concentration of 10 mg/mL. Other peptides (**P3** and **P5**) were found to be either insoluble or not inducing the gelation in aromatic organic solvents. All hybrid peptides (**P3, P4** and **P5)** were found to be insoluble in 1× PBS and failed to give hydrogels. The gels derived from the peptides **P1**, **P2** and **P4** were found to be external stimuli responsive. The transition of gel to sol can be achieved by either mechanical forces or by increasing the temperature. In contrast to **P3** and **P5**, the hybrid peptide **P4** consisting of β- and β(O)-δ^5-Phe may be reached the threshold of hydrophobicity and hydrophilicity which probably responsible for the gelation of aromatic organic solvents.[28] Being side-chains were constant among all the peptides, the backbone conformational flexibility is probably responsible for the gelation properties of the peptides.[28] Further, FT-IR spectroscopy was used to study the conformation of peptide **P1**, **P2** and **P4** in the xerogel state. The C=O stretching (amide I) and NH bending (amide

II) frequencies around ~1650 cm^{-1} and ~1700 cm^{-1} respectively suggest the β-sheet character of peptides in the gel state (See ESI).[33]

Morphology Study

To understand supramolecular assembly formed by the peptides **P1**, **P2** and **P4**, the FE-SEM was carried out. As both **P3** and **P5** did not show any gelation properties, we have not subjected them for the morphology studies. Though the peptides **P1, P2** and **P4** displayed the fibrillar morphology however they are different from one another. Peptide **P1** and **P4** displayed densely packed fibrillar network compared to peptide **P2**.

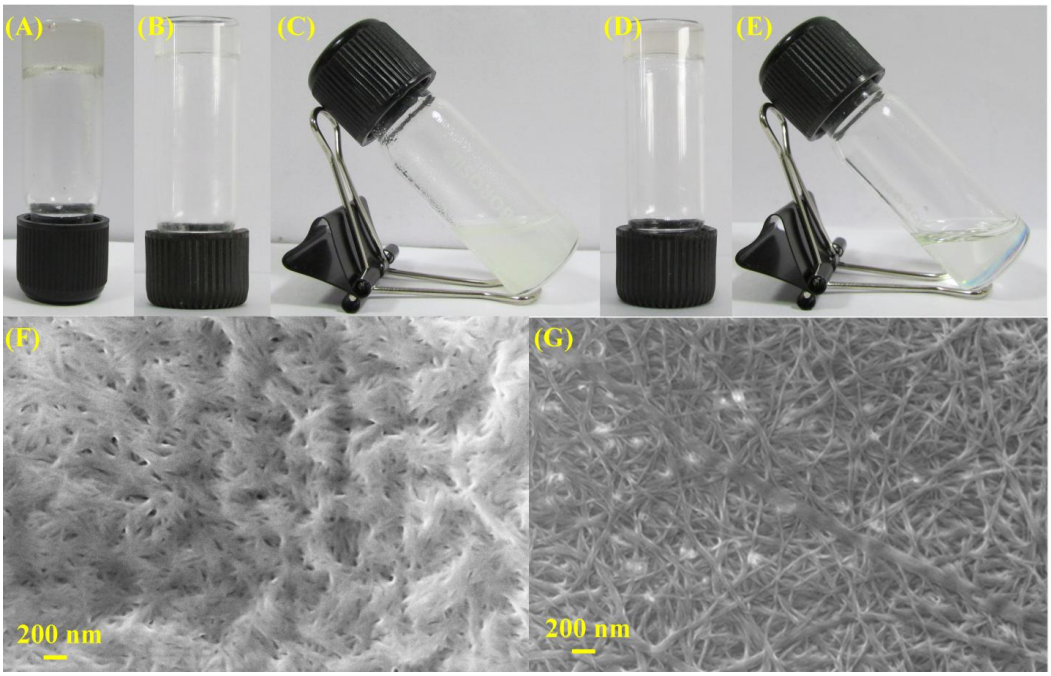


Figure 1: Examination for the formation of gel in (A) Peptide **P1** (conc. 10 mg/mL) in toluene (B) Peptide **P2** (conc. 10 mg/mL) in 1× PBS at pH 7.4. (C) Peptide **P3** (conc. 10 mg/mL) in toluene. (D) Peptide **P4** (conc. 10 mg/mL) in toluene. (E) Peptide **P5** (conc. 10 mg/mL) in toluene. (F) FE-SEM images of the organogel of peptide **P4** in toluene. (G) FE-SEM images of the hydrogel of peptide **P2** in 1× PBS.

Viscoelastic Property

To gain insight into the viscoelasticity and self-healing properties of organogels of **P1** and **P4** and hydrogels of **P2**, we carried out the rheological measurements. These results are shown in Figure **2**. The larger value of the storage modulus (G') compared to the loss modulus (G") in the strain sweep experiment at constant angular frequency, signifying the elastic nature of both organogels (**P1** and **P4**) and hydrogels (**P2**). The constant values of G' and G" in a frequency sweep experiments further support the stable organic and hydrogels from these peptides.

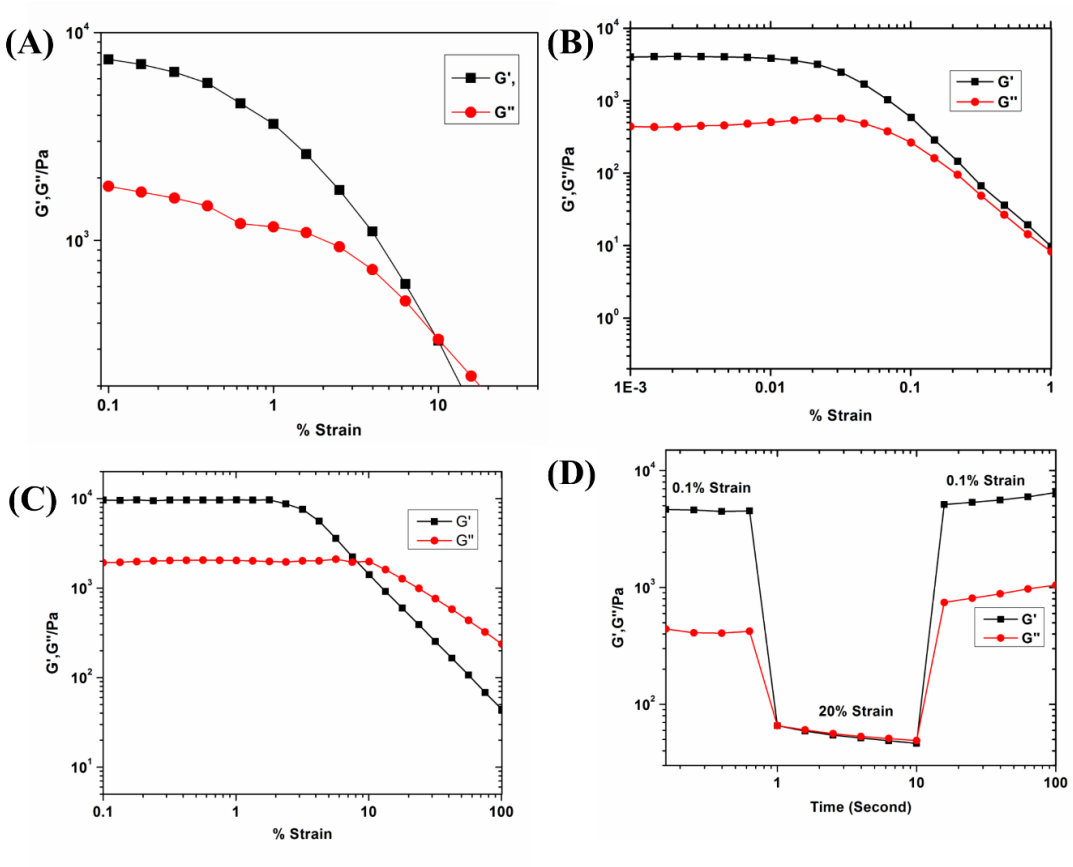


Figure 2: (A) Strain sweep rheological analysis of **P1** organogel in toluene (10 mg/mL) at constant angular frequency (1 rad/sec). (B) Strain sweep rheological analysis of the **P2** hydrogel in 1 × PBS (10 mg/mL) at constant angular frequency (1 rad/Sec). (C) Strain sweep rheological

analysis of **P4** organogel in toluene (10 mg/mL) at constant angular frequency (1 rad/Sec). (D) Step-strain rheology experiment of peptide **P2** hydrogel (10 mg/mL) at constant frequency (1 rad/Sec).

Stimuli Responsive Properties of Organo and Hydrogels

We further noticed that the organogels of the peptides **P1** and **P4** and hydrogel of peptide **P2** were found to be external stimuli responsive. The transition from gel to sol can be achieved using either

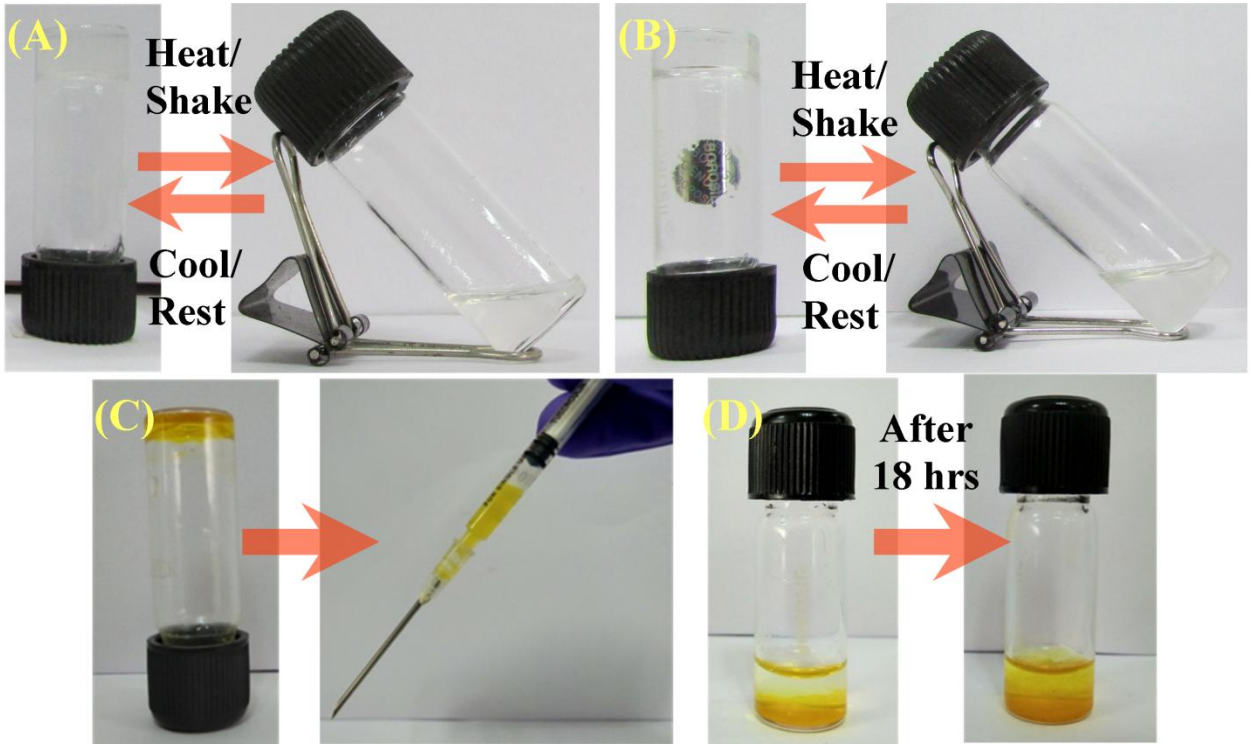


Figure 3: Stimuli responsive nature of the (A) organogel of peptide **P4** and (B) hydrogel of peptide **P2**. (C) Injectable nature of the hydrogel from peptide **P2**. (D) Slow release of the dye from hydrogel matrix to the 1× PBS over about 18 h.

heat or mechanical shaking. Upon cooling and resting, the sol state slowly transformed into the gel state. By applying the mechanical strain, the hydrogel was transformed to sol and after

removing the strain the sol slowly transformed again into the gel state. This thixotropic nature of the gels was further supported by the rheology experiments (Figure **2D**). As these types of injectable hydrogels have been finding applications in the drug delivery, we have examined the potential of **P2** hydrogel as a drug delivery system. We encapsulated 500µL of 1mM proflavine solution in the gel matrix and 500µL of 1× PBS was added on the top of the hydrogel matrix. The slow release of the drug was monitored using UV-Vis spectroscopy. The release kinetics suggested that ~35% of the drug was released from gel to the buffer in 18 h (See ESI).

Oil Spill Recovery by Organogel from Peptide P4

Inspired by the gelation of peptide **P4** in various aromatic solvents, we sought to investigate whether it can be used to selectively gelate the crude oil from the oil-water mixture. The large scale separation of oil from oil-water mixture has been one of the big challenges in worldwide due

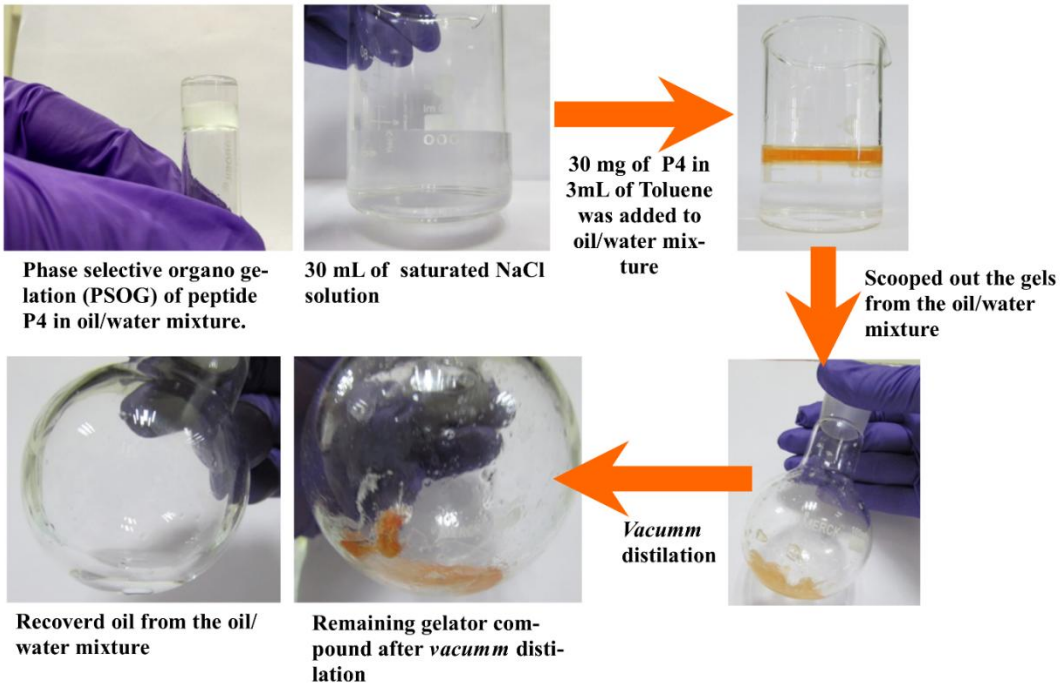


Figure 4: Phase selective gelation in oil/water mixture and oil spill recovery.

to the increasing release of oil contaminated waste water and marine oil spills to the seas which severely damage the marine eco-system.[52-53] There are numerous methods available for the oil-spill recovery, however peptide based amphiphiles attracted considerable attention in recent years in oil spill recovery.[54] Recently Basak *et al*. have demonstrated the oil spill recovery using ethanolic solution of small peptides composed of aromatic amino acids.[28] The schematic process of oil spill recovery by the peptide **P4** is shown in the Figure **4**. To understand the selective gelation of crude oil, the solution of peptide **P4** (30 mg in 3 mL) in toluene was added to the oil-water mixture composed of 10 mL of oil and 30 mL of saturated NaCl solution. Instructively, upon treatment of the peptide solution, the oil transformed into gel in less than 5 min. The peptide-oil gel was scooped out from the oil/water mixture and subjected for *vacuum* distillation. Through this process we have successfully recovered the oil from the oil-salt water mixture. Overall, the self-assembling nature of the dipeptide **P4** was found to be an excellent alternative for recovering the oil in oil spills.

Biocompatibility Studies of the Peptide P2 Hydrogel:

As many thixotropic peptides based hydrogels have been using as a cell culture matrix, we sought to examine the biocompatibility of the peptide **P2** hydrogel and its utility as matrix in the 2D cell culture. To understand the biocompatibility, peptide gel was mixed with DMEM media at a ratio of 1:10 and the cells were grown over the gel matrix. We have examined biocompatibility of the **P2** hydrogel using both cancer cell line LN229 and normal cell line HEK293T. After 18h, the live cells were stained with calcein AM and imaged using fluorescence microscope. The difference in the growth of the cells over the gel matrix compared to the control experiment without peptide gel are shown in the Figure **5**. These results suggest that hydrogel from δ-peptideis biocompatible and this type of peptides can be used as matrix along with the culture media. As the δ-amino acid can

serve as a dipeptide mimetic, we have examined the proteolytic stability of peptide **P2** along with the control α-dipeptide Boc-Phe-Phe-OH using the protease chymotrypsin. These results are shown in Figure **6**. In contrast to α-dipeptide, the HPLC analysis suggests that peptide **P2** was found to be stable to chymotrypsin.

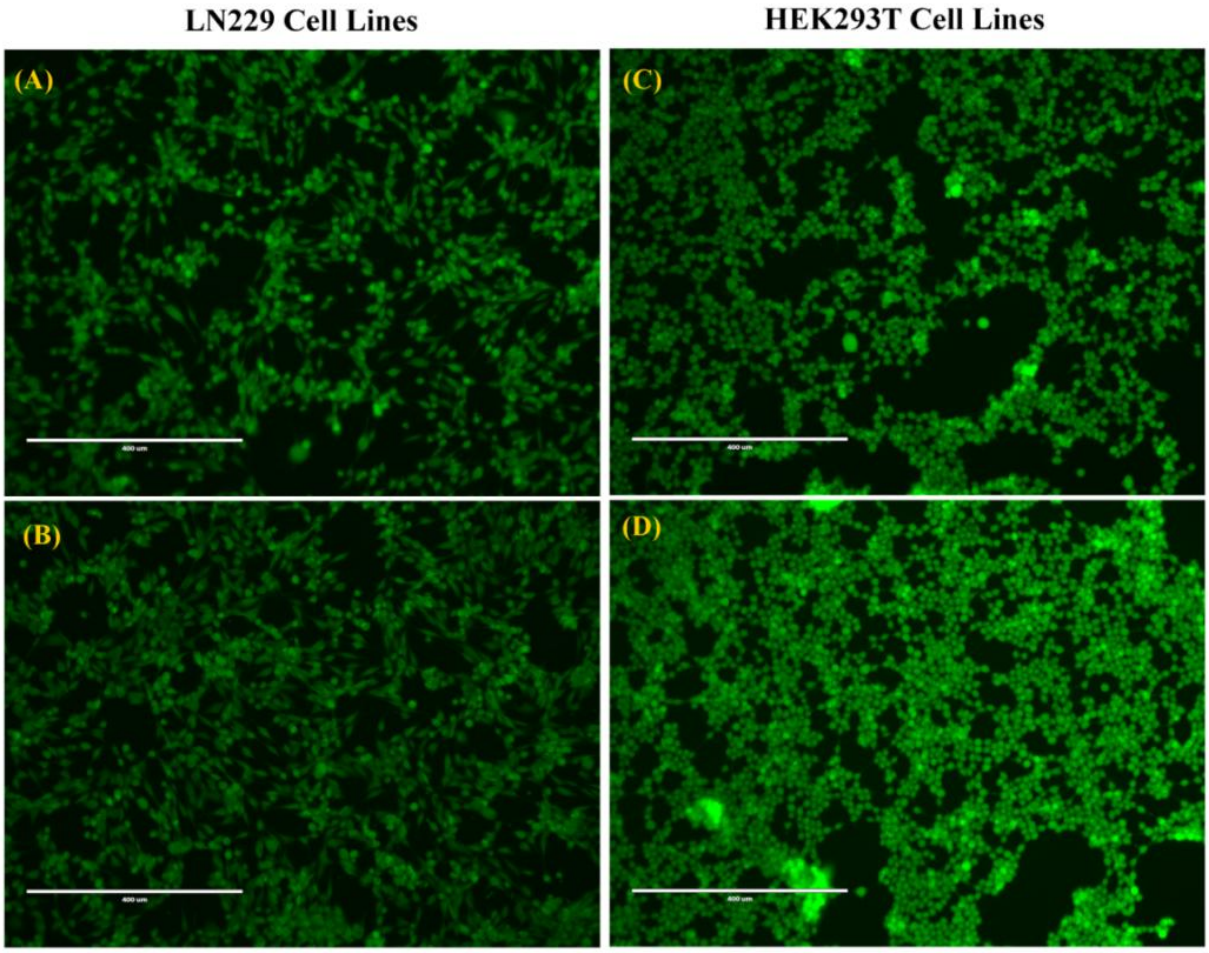


Figure 5: Growth of the cells over gel matrix after 18 h. (A) and (C) are the growth of the cells over gel matrix. (B) and (D) are the control experiment without any hydrogel. The viable cells are stained with calcein AM stain. (Scale bar for all images are 400 μm)

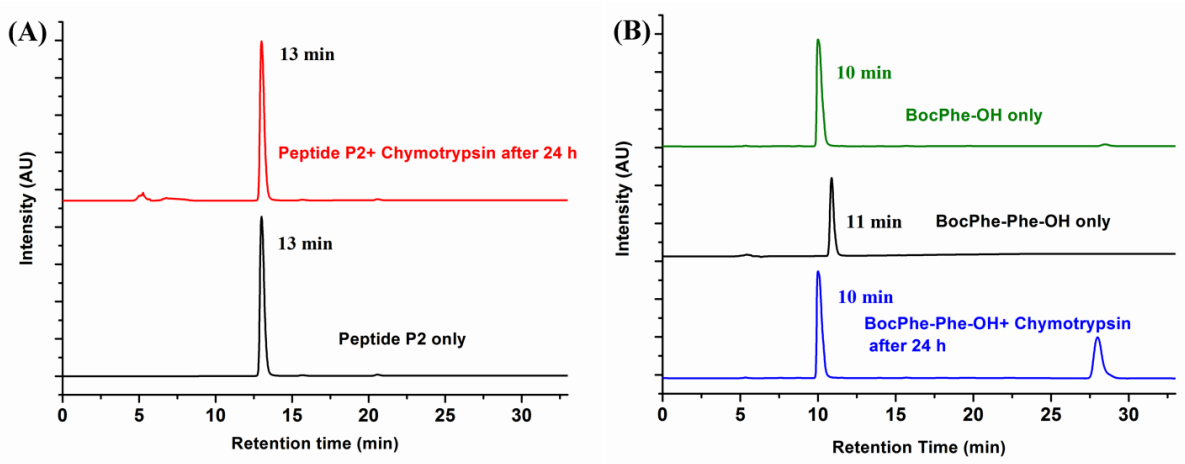


Figure 6: HPLC analysis depicting the stability of peptides (A) **P2** and (B) control peptide Boc-Phe-Phe-OH against the protease chymotrypsin.

CONCLUSION

In conclusion, we have demonstrated the formation of self-assembled divergent gels from a new class of short peptides consisting of δ-Phe. These δ-amino acids may serve as surrogates of α-dipeptide. The dipeptide (**P1**) composed of backbone homologated δ^5-Phe was found to be insoluble in aqueous buffers, however gave stable gels in aromatic organic solvents. In contrast, the dipeptide composed β(O)-δ^5-Phe showed remarkable, transparent, thixotropic hydrogels from the phosphate saline. Replacing backbone $–CH_2-$ with "O" atom showed phenomenal change in their supramolecular gelation properties. The subtle change in the backbone leads to a drastic change in the molecular behavior of these peptides. Among the hybrid dipeptides**P3**, **P4** and **P5**, surprisingly only peptide **P4** (β^3/β(O)-δ^5) gave organogels in aromatic solvents. Further, we have demonstrated the utility of β^3/β(O)-δ^5-hybrid peptide **P4** in the oil spill recovery through a phase

selective gelation process. In addition, we have shown the potential applications of the dipeptide **P2** hydrogel as drug delivery system as well as matrix to grow the cells. Both **P2** and **P4** were found to be sensitive to the external heat as well as mechanical shaking. The biocompatibility, proteolytic stability and thixotropic nature of the new δ-peptide may serve as potential alternatives to the existing peptide gels and it can be used as injectable and drug delivery agents. Overall, the study reported here can open new avenues for the design of biomaterials, injectable gels and drug delivery agents.

ASSOCIATED CONTENT

Supporting Information

Details of the peptides and amino acids characterization, gelation property, ^{1}H, ^{13}C NMR and HRMS spectra are available in the supporting information.

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ABBREVIATIONS

TLC, thin layer chromatography; PBS, phosphate-buffered saline; FE-SEM, field emission scanning electron microscope; NMR, nuclear magnetic resonance.

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Divergent Supramolecular Gelation of Backbone Modified Short Hybrid δ-Peptides

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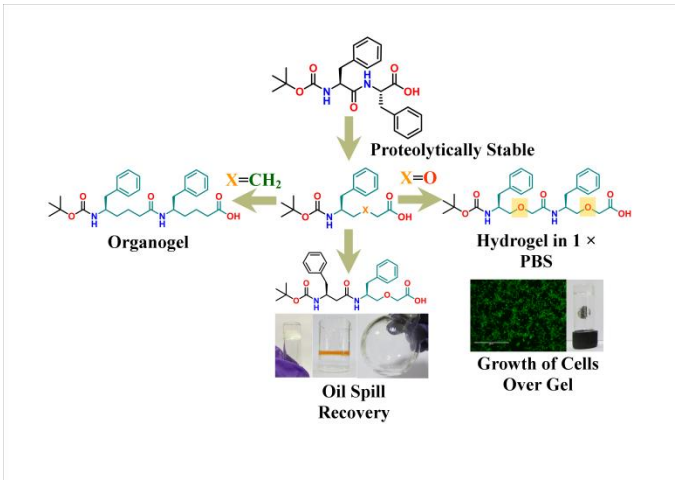

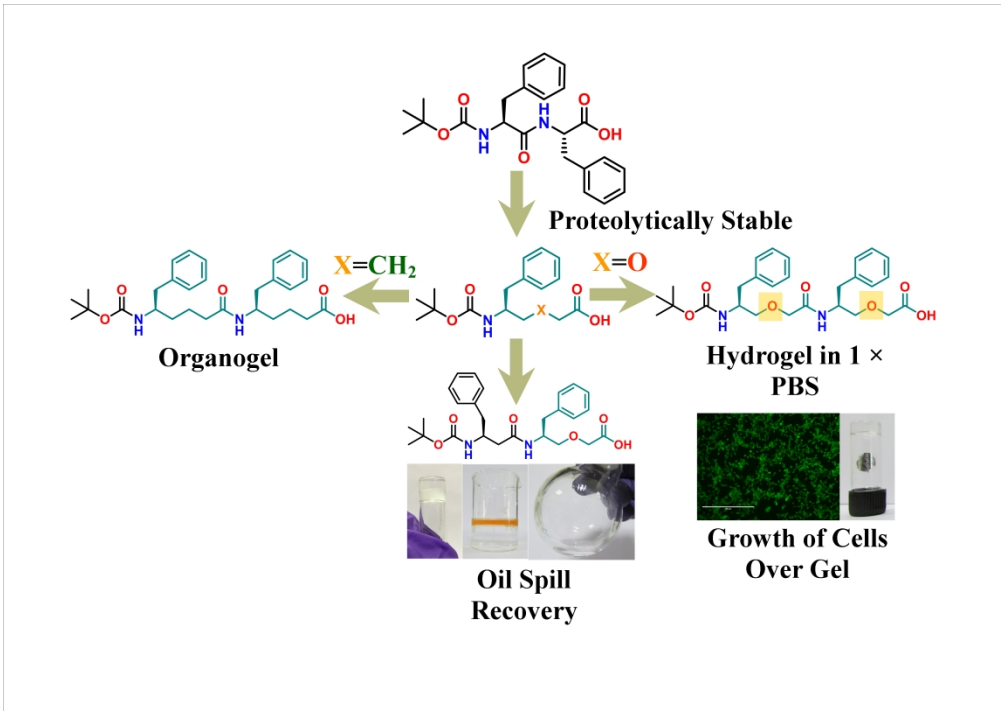

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