

## Electronic Supplementary Information for Hybrid Gels via Bulk Interfacial Complexation of Supramolecular Polymers and Polyelectrolytes

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### Materials

*Peptide amphiphile synthesis:* The peptide amphiphiles (PAs) C16-VVVAEEEEE-COOH (E3OH) and C16-VVVAEEEEE-K(TAMRA)-CONH<sub>2</sub> were synthesized via standard 9-fluorenyl methoxycarbonyl (Fmoc) solid-phase peptide chemistry on pre-loaded glutamic acid Fmoc-Glu-Wang resin and Rink amide MBHA resin respectively using a CEM Liberty Blue automated microwave peptide synthesizer. Automated coupling reactions were performed using 4 equiv. Fmoc-protected amino acid, 4 equiv. *N,N'*-diisopropylcarbodiimide (DIC), and 8 equiv. ethyl(hydroxyimino)cyanoacetate (Oxyma pure). Removal of the Fmoc groups was achieved with 20% 4-methylpiperidine in DMF. Peptides were cleaved from the resin using standard solutions

of 95% TFA, 2.5% water, 2.5% triisopropylsilane (TIS) for 3 h, precipitated with cold ether, and then purified by reverse-phase HPLC on a Waters Prep150 or Shimadzu Prominence HPLC using a water/acetonitrile (each containing 0.1%  $\text{NH}_4\text{OH}$  v/v) gradient. Eluting fractions containing the desired peptide were confirmed by mass spectrometry using an Agilent 6520 QTOF LCMS. Confirmed fractions were pooled and the acetonitrile was removed by rotary evaporation before freezing and lyophilization. Purity of lyophilized products was tested by LCMS.

For 5-carboxytetramethylrhodamine (TAMRA) labeled PA, the methyltrityl (Mtt) protecting group was removed from the lysine after automated synthesis while still on resin using 3% TFA in DCM with 5% TIS. After washing with DCM and DMF, TAMRA was then coupled to the now free epsilon amine of lysine using 1.2 equiv. carboxy-TAMRA, 1.2 equiv. PyBOP (benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate), and 8 equiv. *N,N*-diisopropylethylamine (DIEA) for approximately 18 h.

*E3OH solutions:* E3OH solutions were prepared by adding deionized water to the PA and adjusting pH using 1 M NaOH. Unless otherwise specified, PA solutions were adjusted to pH 7 and sonicated until all PA was dissolved. Solutions were placed in a water bath at 80 °C for 30 minutes, and then slowly cooled to room temperature.

*TAMRA-E3/E3OH co-assemblies:* TAMRA-E3 co-assembled with E3OH were prepared by mixing a solution of TAMRA-E3 at 0.1 wt% in deionized water with a solution of E3OH such that the final concentration of the TAMRA-E3 relative to E3OH was either 0.1 mol% or 1 mol% depending on how much dye was desired for imaging. The TAMRA-E3 co E3OH mixtures were then sonicated to ensure dissolution and mixing. Solutions were placed in a water bath at 80 °C for 30 minutes and then slowly cooled to room temperature.

*Chitosan:* Medium molecular weight chitosan (190,000-310,000 Da) was purchased from Sigma-Aldrich (#448877). The chitosan was dissolved in 2 vol% acetic acid, the remaining solid was filtered off, and the solution dialyzed in 12-14 kDa molecular weight cutoff Spectra/Por tubing against deionized water and then freeze dried.

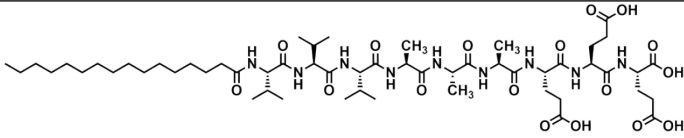
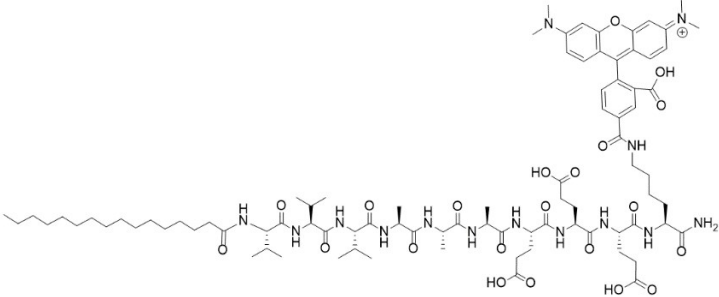
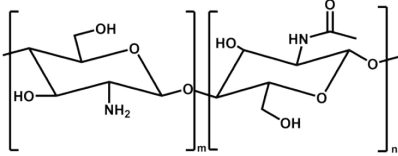
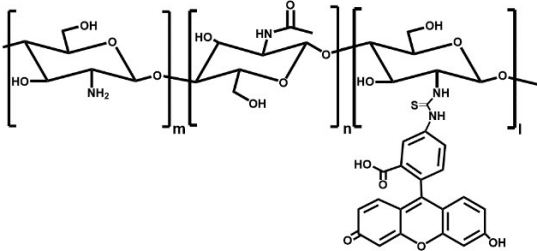
*Chitosan solutions:* Chitosan solutions were prepared by dissolving filtered and lyophilized chitosan at 2 wt% in 2 vol% acetic acid. After the chitosan was dissolved, it was diluted to 1 wt% chitosan with a 500 mM sodium acetate solution.

*FITC-labeled Chitosan:* Filtered and lyophilized chitosan was dissolved in 2 vol% acetic acid, and the solution diluted to 1 wt% using methanol. While the chitosan solution was stirred, a 2 mg/mL fluorescein isothiocyanate (FITC) methanol solution was added to a final volume ratio of chitosan to FITC solution of 2:1. After stirring for 4 hours the chitosan was precipitated by adding 1 M NaOH solution, and the precipitate was washed with aqueous 30% methanol solution. The precipitate was collected using vacuum filtration and redissolved in 1 vol% acetic acid. This solution was dialyzed in Spectra/Por 12-14 kDa molecular weight cutoff tubing against deionized water and then freeze dried.

*FITC-Chitosan solutions:* 2 wt% aqueous FITC-chitosan solutions (filtered and lyophilized) were prepared in 2 vol% acetic acid. Solutions were diluted to 1 wt% chitosan with a 500 mM sodium acetate solution, and they could be mixed with a 1 wt% chitosan solution in order to vary the amount of FITC.

*Alexa Fluor 647-Labeled Lysozyme:* 20 mg of lysozyme from chicken egg white was obtained from Sigma Aldrich and dissolved in 0.1 M sodium bicarbonate to achieve a lysozyme concentration of 10 mg/mL. Alexa Fluor 647 carboxylic acid succinimidyl ester (1 mg) was

dissolved in 100  $\mu$ L of dimethylformamide and added to the stirring lysozyme solution at room temperature. After reacting for 1 hour while stirring, the reaction was quenched by adding 200  $\mu$ L of 1 M hydroxylamine in water (pH 8) and stirred for 1 additional hour. The Alexa Fluor 647-labeled lysozyme was purified by dialyzing for 48 hours in deionized water using Spectra/Por 3.5 kDa molecular weight cutoff tubing and then freeze dried and stored at -20  $^{\circ}$ C.

E3OH	
TAMRA-E3	
Chitosan	
FITC-Chitosan	

ESI Table 1: Chemical structures of E3OH, TAMRA-E3, chitosan, and FITC-chitosan

## Methods

*Negative stain TEM:* E3OH samples were prepared at 2 wt% with one of the samples having 1.1x molar equivalents of NaOH to E3OH added to achieve pH 7, and the other sample having 3.3x molar equivalents of NaOH to E3OH added to achieve pH 10. Samples were diluted to ~1 mM concentration with water immediately prior to sample preparation. Carbon coated TEM grids (CF300-Cu-UL, Electron Microscopy Sciences) were glow discharged using a PELCO Easi-Glow instrument with 15 mA current for 30 s. 5  $\mu$ L of diluted sample was applied on a grid and the excess solution wicked with a filter paper after 30s. 5  $\mu$ L of 1 wt% uranyl acetate solution, filtered with a 200 nm PTFE syringe filter before use, was applied on the grid. After 10 s, the excess staining solution was wicked with a filter paper, and the sample was allowed to dry. TEM images were obtained using a JEOL ARM300F microscope operated at 300 kV acceleration voltage. The images were recorded with a Gatan OneView IS detector in imaging mode, with real-time drift correction.

*Formation of Gels Containing Protein for Confocal Microscopy:* Gels were prepared by dissolving either Alexa Fluor 647-labeled BSA from Invitrogen or Alexa Fluor 647-labeled lysozyme in either the PA solution or the chitosan solution. For gels with protein in the PA solution, the protein was dissolved at 1 mg/mL in a pre-annealed TAMRA-E3 co-assembled with E3OH (1.5 wt% solution). The TAMRA-E3 was 0.1 mol% relative to the total amount of PA in solution. The protein loaded PA solution was injected at the same time as a 10:1 chitosan to FITC chitosan solution by weight in opposite sides of the vial, and solutions were immediately vortex mixed for 10 seconds. For gels with protein in the chitosan solution, the protein was dissolved at 1 mg/mL in a 10:1 chitosan to FITC chitosan solution. The protein loaded chitosan solution was injected at the same time as a pre-annealed TAMRA-E3 co-assembled with E3OH

(1.5 wt% solution). The TAMRA-E3 was 0.1 mol% relative to the total amount of PA in solution. The gels were placed in Electron Microscopy Sciences microporous specimen capsules and solvent exchanged with deionized water.

*Quantification of chitosan in excluded fluid:* A 10:1 chitosan to FITC-chitosan solution by weight was prepared by mixing 1 wt% chitosan solution with 1 wt% FITC-chitosan solution. A calibration curve was obtained by diluting the 10:1 chitosan to FITC-chitosan solution with deionized water. The absorbance of three 100  $\mu$ L solutions at each concentration were measured at 490 nm using a Cytation 3 plate reader, and the measured values were then averaged for each concentration. Gels were prepared by vortex mixing the 1 wt% 10:1 chitosan to FITC-chitosan solution with equal volumes of 1, 1.5, and 2 wt% E3OH solutions for 10 s. The excluded fluid was collected, and the volumes were recorded. The solutions were then centrifuged, and the absorbance of 100  $\mu$ L of the supernatant was measured at 490 nm using the Cytation 3 plate reader, and the calibration curve was used to calculate the concentration of the chitosan in solution.

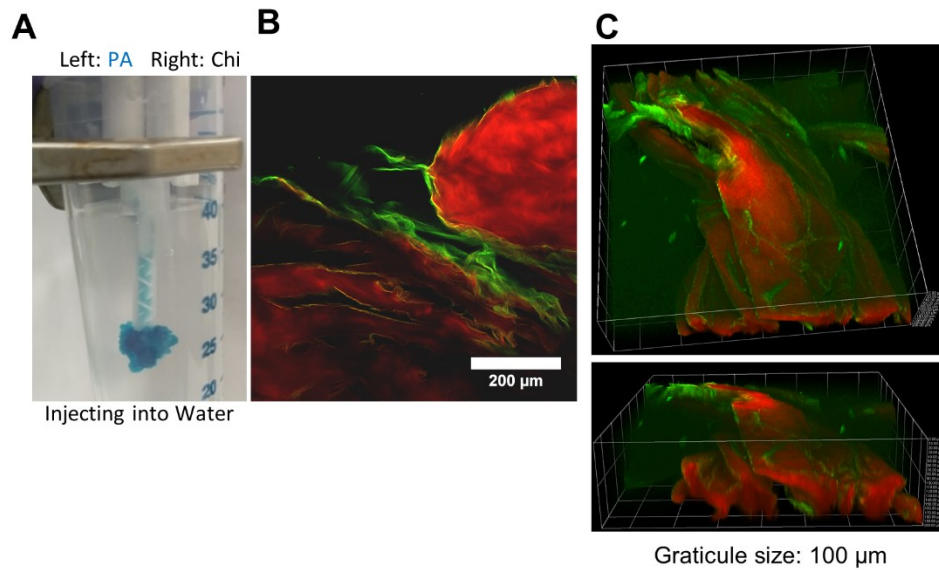
*Quantification of protein encapsulation:* A stock solution of 0.45 wt% chitosan solution was prepared by diluting 1 wt% chitosan with deionized water, and FITC labeled BSA was dissolved in the 0.45 wt% solution at 1 mg/mL. A calibration curve was obtained by diluting the 1 mg/mL FITC-BSA solution with a 0.45 wt% chitosan solution. The fluorescence intensity of three 100  $\mu$ L solutions at each concentration was measured with excitation at 490 nm and emission at 520 nm using a Cytation 3 plate reader. The absorbance of 100  $\mu$ L solutions at 1, 0.75, 0.5, 0.25, 0.1, 0.075, and 0.05 mg/mL was measured at 490 nm using a Cytation 3 plate reader, and the measured values were then averaged over three samples for each concentration. Protein loaded gels were prepared by dissolving FITC-BSA at 1 mg/mL in either a pre-annealed 1.5 wt% E3OH solution or a 1 wt% chitosan solution. Gels with FITC-BSA in the PA solution were then prepared by vortex mixing a

1 wt% chitosan solution with equal volumes of the FITC-BSA E3OH solutions for ten seconds. Gels with FITC-BSA in the chitosan solution were prepared by vortex mixing the FITC-BSA chitosan solution with equal volumes of the 1.5wt% E3OH solutions for ten seconds. The excluded fluid was collected, and the volumes were recorded. The solutions were then centrifuged, and the fluorescence intensity of 100  $\mu$ L of the supernatant was measured with an excitation at 490 nm and an emission at 520 nm using a Cytation 3 plate reader. The calibration curve was used to calculate the concentration of the FITC-BSA in solution. For gels prepared with the FITC-BSA in the chitosan solution, the fluorescence intensity of the excluded fluid was too high to reliably quantify the concentration using fluorescence, so absorbance was used instead. The concentration of FITC-BSA in solution was used to calculate the remaining amount of FITC-BSA in the gel.

*Quantification of protein release:* FITC-labeled BSA was dissolved at 1 mg/mL in a phosphate buffered saline solution. A calibration curve was obtained by diluting the 1 mg/mL FITC-BSA solution with PBS. The absorbance of three 100  $\mu$ L solutions at each concentration was measured at 490 nm using a Cytation 3 plate reader. Protein loaded gels were prepared by dissolving FITC-BSA at 1 mg/mL in either a pre-annealed 1.5 wt% E3OH solution or a 1 wt% chitosan solution. Gels with FITC-BSA in the PA solution were prepared by vortex mixing a 1 wt% chitosan solution with equal volumes of the FITC-BSA E3OH solutions for ten seconds. Gels with FITC-BSA in the chitosan solution were prepared by vortex mixing the FITC-BSA chitosan solution with equal volumes of the 1.5 wt% E3OH solutions for ten seconds. Gels were collected and washed for 24 hours in a PBS solution to remove excess chitosan and salt. Gels were placed in PBS and a portion of the supernatant was removed, centrifuged, and the absorbance spectra of 100  $\mu$ L of the centrifuged supernatant was measured using a Cytation 3 plate reader (Rayleigh background scattering was subtracted so that remaining gel fragments did not affect release analysis). The

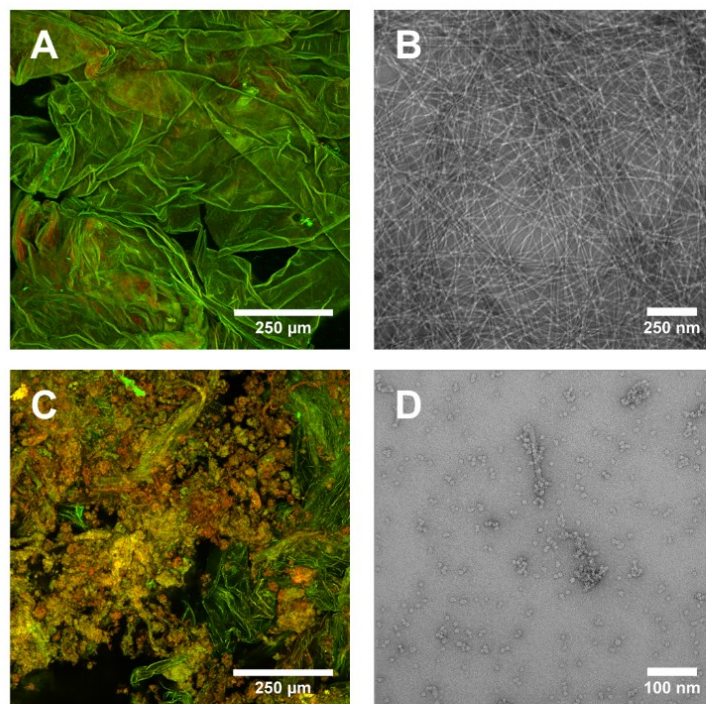
calibration curve was used to calculate the concentration of the FITC-BSA in solution by comparing sample values at their absorbance values at 490 nm. After measuring absorbance, the supernatant that was removed was put back into the respective samples, and this measurement protocol was used at each time point (N=4 samples were used for each sample condition, samples were held at 37 °C).

### Supplementary Figures:

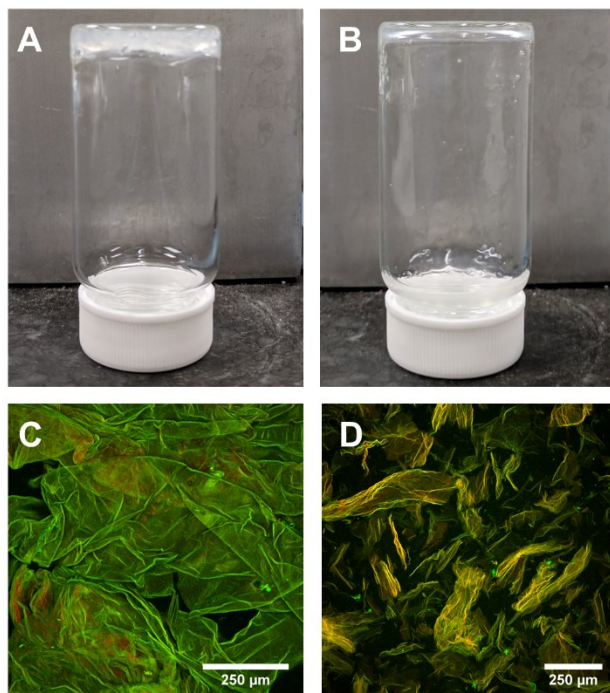


**Fig. S1:** (A) 1.5% PA solution with blue food dye for contrast in left barrel of dual barrel syringe, 1 wt% chitosan solution in right barrel were injected through a mixing head attachment into water to form a gel. Water bath does not take part in gelation and was used for demonstration purposes. (B) confocal micrographs of gel prepared with dual-barrel syringe mixer (FITC chitosan in green, TAMRA-E3 in red) confocal micrograph shows similar interfacial gel structure as seen in vortex mixing. (C) 3D reconstruction of confocal microscopy z-stack of gel prepared with dual barrel syringe (FITC chitosan in green, TAMRA-E3 in red)

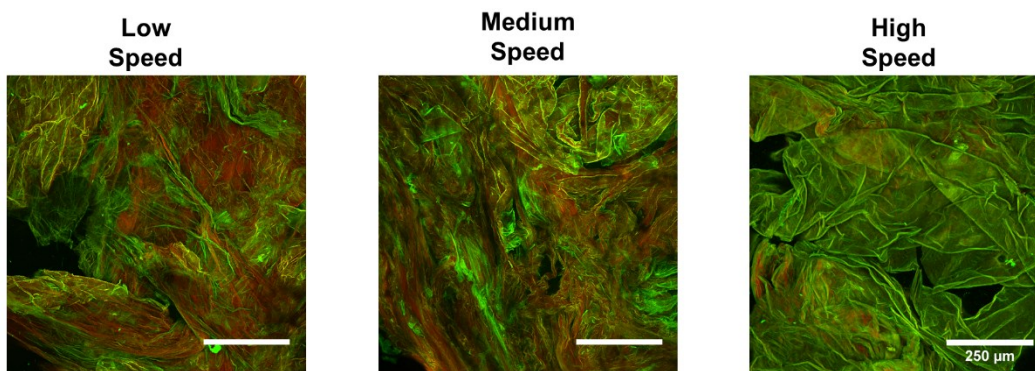




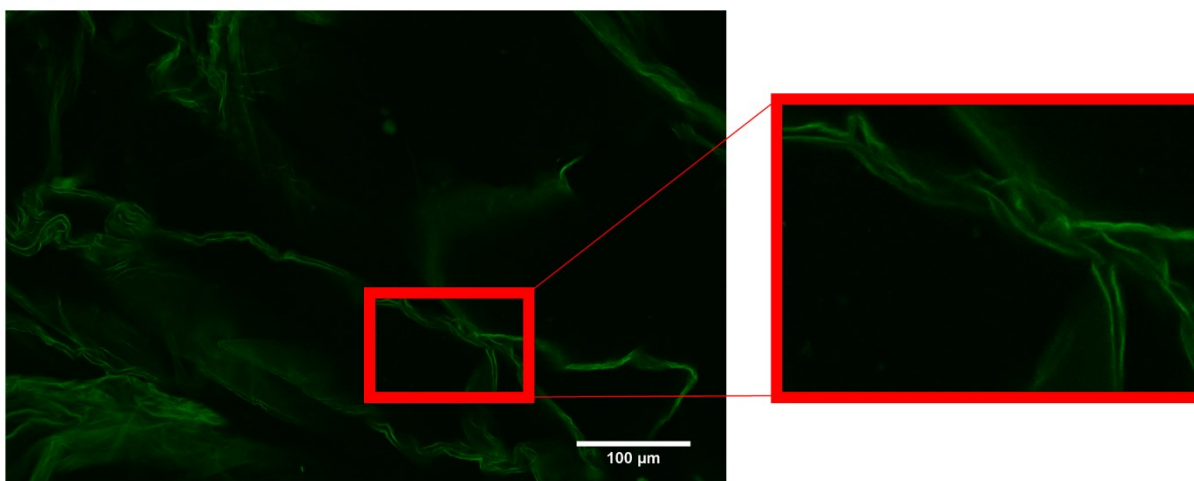
**Fig. S2:** (A) Composite channel maximum intensity projection of confocal z-stack of PA-chitosan gel with the FITC-chitosan in green and the TAMRA-PA in red. Gel was prepared by mixing a 1 wt% solution of 10:1 chitosan/FITC-chitosan with an equal volume of a 2 wt% E3OH solution (pH 7) containing 0.1 mol% TAMRA-E3. (B) TEM image of PA nanofibers from an E3OH solution of pH 7. (C) Composite channel maximum intensity projection of confocal z-stack of PA-chitosan precipitates with the FITC-chitosan in green and the TAMRA-PA in red. Precipitates were created by mixing a 1 wt% solution of 10:1 chitosan/FITC-chitosan with an equal volume of a 2 wt% E3OH solution (pH 10) containing 0.1 mol% TAMRA-E3. (D) TEM image of PA micelles and nanofibers from an E3OH solution of pH 10.



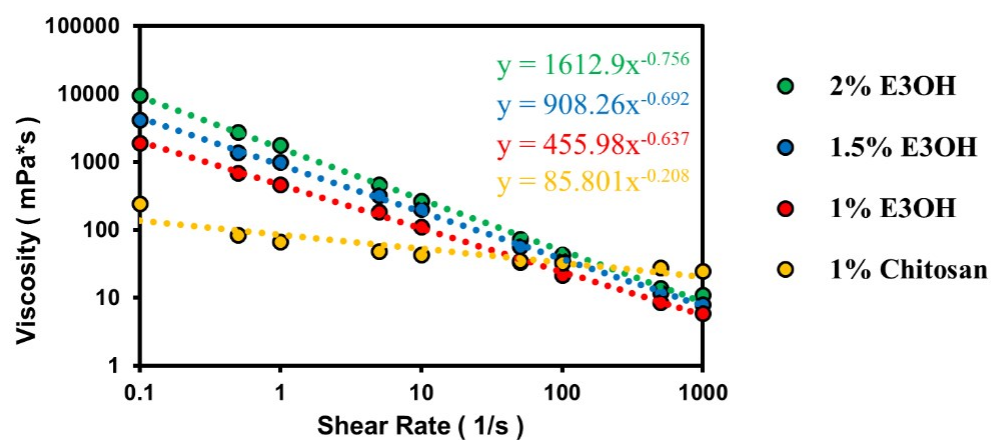
**Fig. S3:** (A) image of a gel undergoing a tilt test, gel was prepared by mixing a 1 wt% solution of chitosan with an equal volume of a 2 wt% E3OH solution (pH 7). (B) image of a sample undergoing a tilt test with the samples contents, gel particles, sliding down the vial, the gel particles were prepared by mixing a 1 wt% solution of chitosan with an equal volume of a 0.5 wt% E3OH solution (pH 7). (C) Composite channel maximum intensity projection of confocal z-stack of PA-chitosan gel with the FITC-chitosan in green and the TAMRA-PA in red. Gel was prepared by mixing a 1 wt% solution of 10:1 chitosan/FITC-chitosan with an equal volume of a 2 wt% E3OH solution (pH 7) containing 0.1 mol% TAMRA-E3. (D) Composite channel maximum intensity projection of confocal z-stack of PA-Chitosan gel particles with the FITC-Chitosan in green and the TAMRA-PA in red. Particles were created by mixing a 1 wt% solution of 10:1 Chitosan/FITC-Chitosan with an equal volume of a 0.5 wt% E3OH solution (pH 7) containing 0.1 mol% TAMRA-E3.



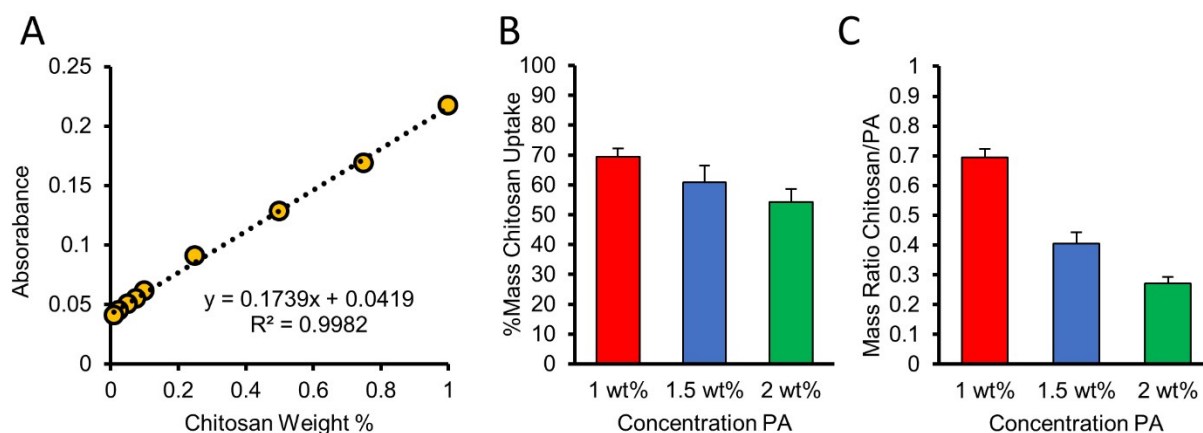
**Fig. S4:** Composite channel maximum intensity projection of confocal z-stack of PA-Chitosan gel with the FITC-chitosan in green and the TAMRA-PA in red. Gel was prepared at low, medium, and high mixing speeds with a 1 wt% solution of 10:1 Chitosan/FITC-Chitosan and an equal volume of a 2 wt% E3OH solution (pH 7) containing 0.1 mol% TAMRA-E3.



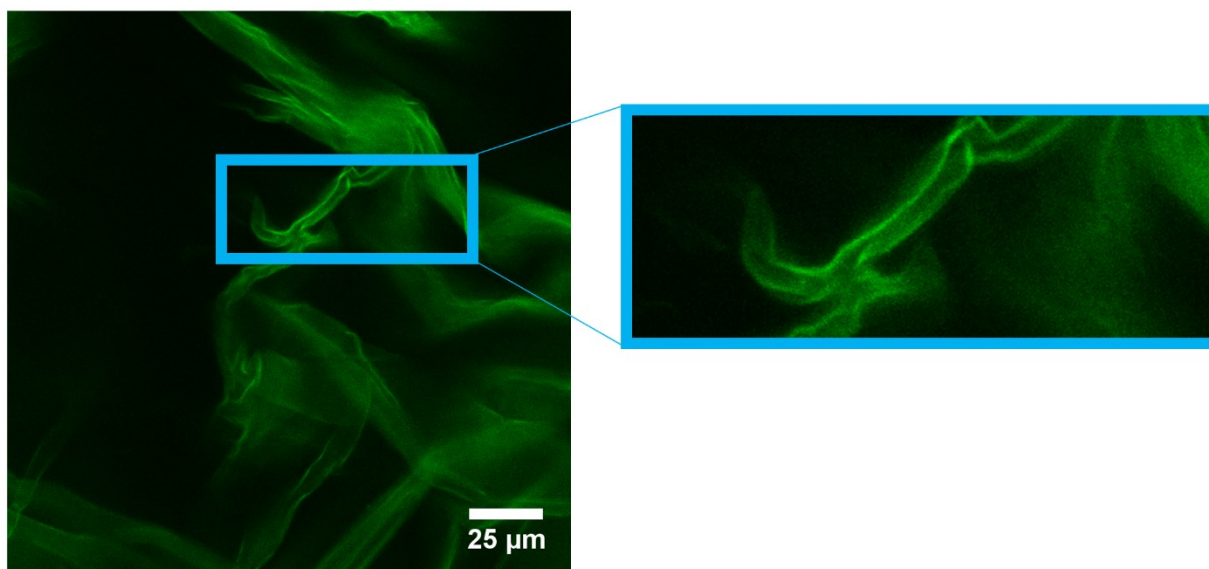
**Fig. S5:** Confocal image of gel produced by mixing 1 wt% E3OH solution with 1 wt% FITC-chitosan solution. The layered structure is clearly observed showing that it is not an artifact of dye interactions in samples containing both dye-labeled PAs and chitosan.



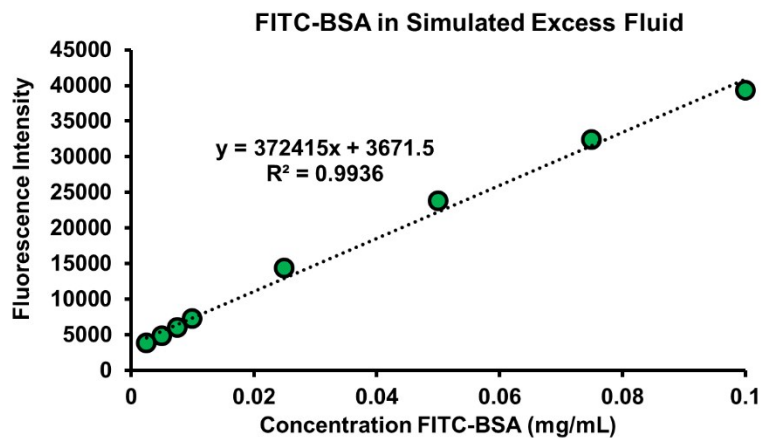
**Fig. S6:** Flow curve of PA solutions at varied concentrations with power law fits and a chitosan solution at 1 wt% with a power law fit.



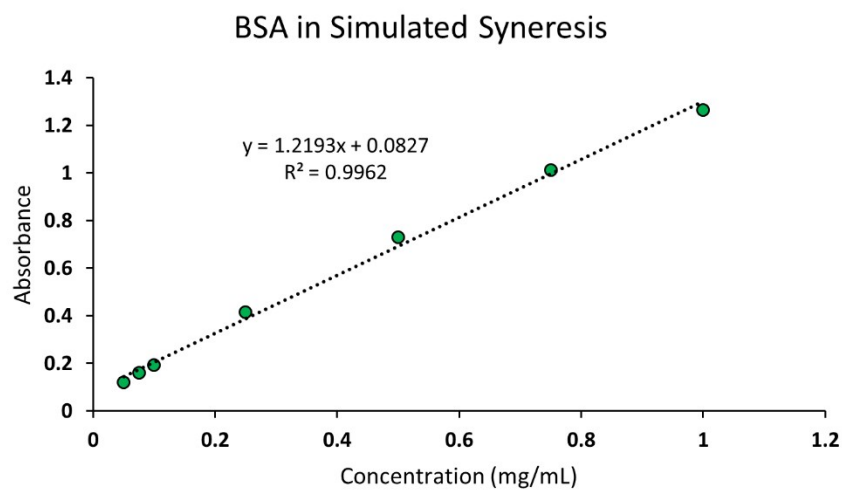
**Fig. S7:** (A) Absorbance calibration curve of 10:1 chitosan/FITC-chitosan. (B) Mass percent of chitosan incorporated into gels during the gelation of PA solutions at various concentrations with a 1 wt% chitosan solution. (C) Mass ratio of chitosan to PA in gels prepared via the gelation of PA solutions at various concentrations with a 1 wt% chitosan solution.



**Fig. S8:** Confocal micrograph of PA-chitosan gel containing FITC-BSA. Gel was prepared by mixing a 1 wt% solution of chitosan with an equal volume of 1.5 wt% E3OH solution loaded with 1 mg/mL FITC-BSA. The layered structure is clearly observed, as is the high concentration in the contact layer where chitosan has a high concentration as well. This shows that findings were not an artifact of dye interactions in samples containing labeled PAs, dyed protein, and dyed chitosan.

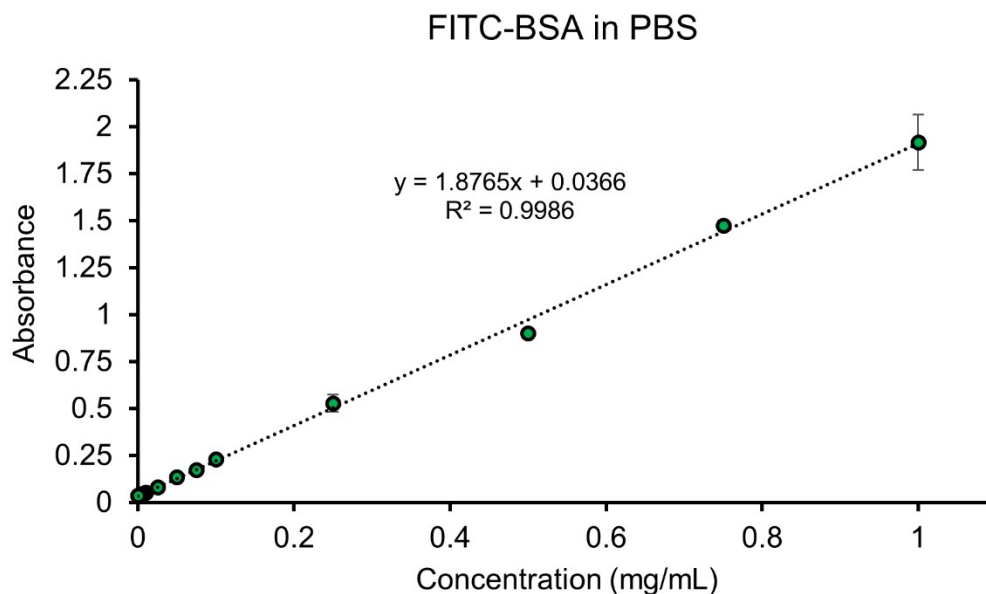


**Fig. S9:** Fluorescence calibration curve of FITC-labeled BSA in a 0.45 wt% chitosan solution.

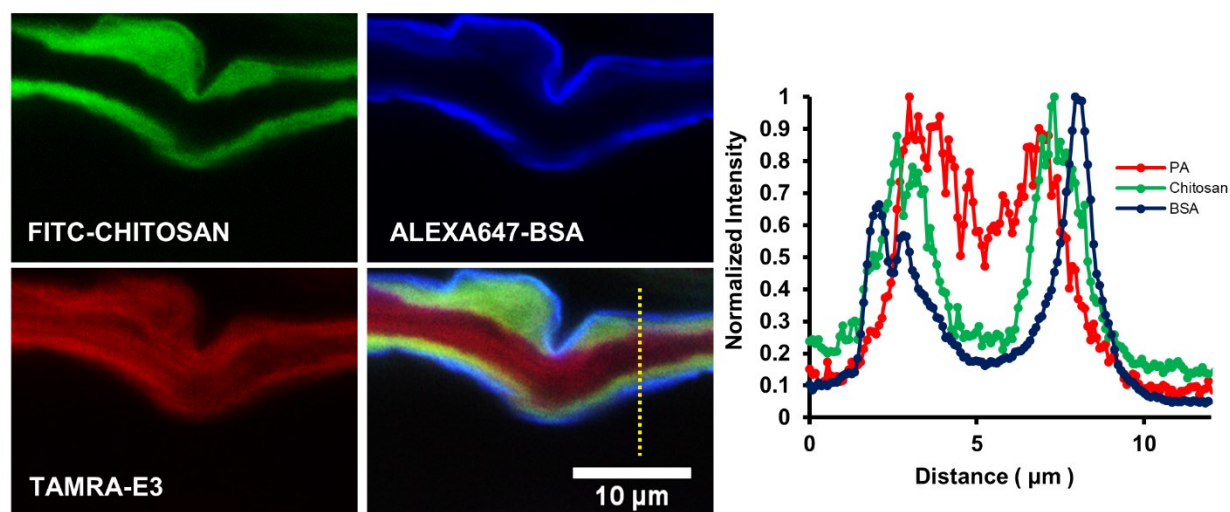


**Fig. S10:** Absorbance calibration curve of FITC-labeled BSA in a 0.45 wt% chitosan solution.

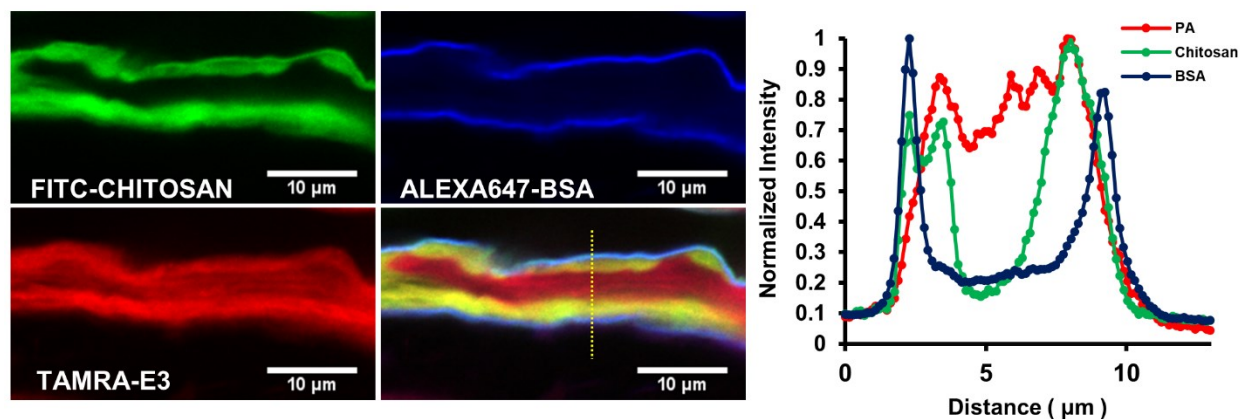




**Fig. S11:** Absorbance calibration curve of FITC-labeled BSA in phosphate buffered saline.

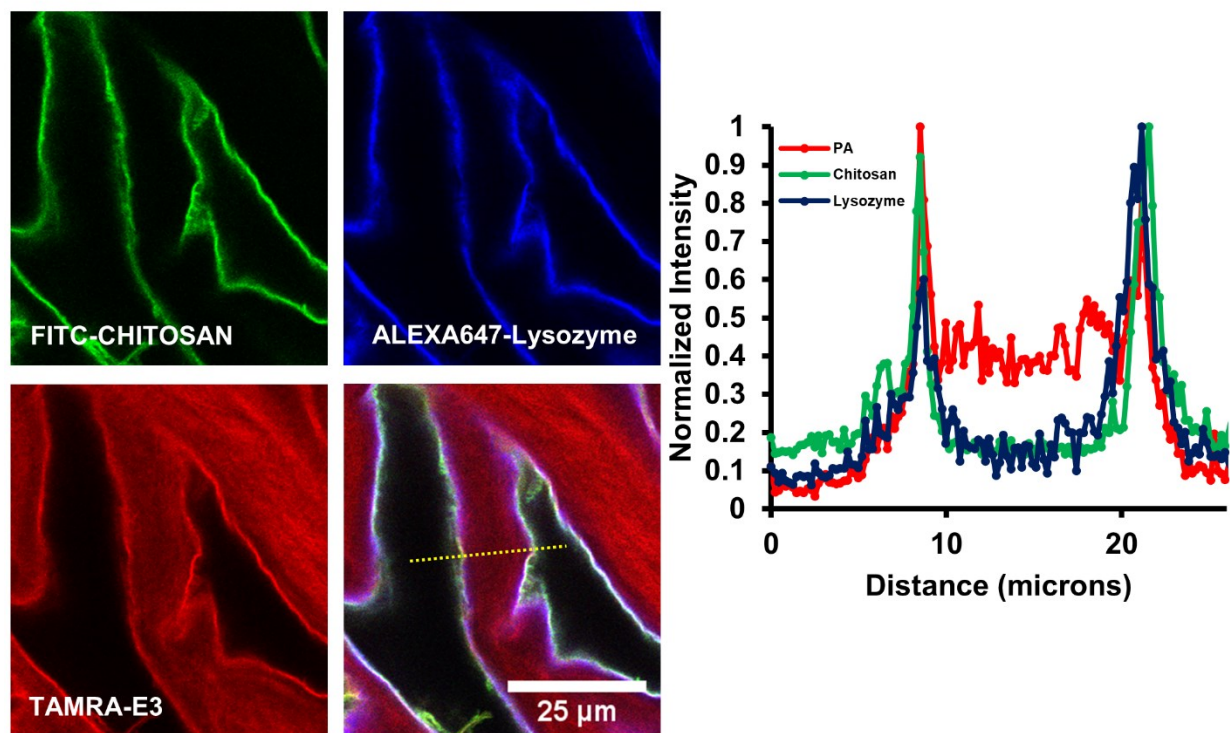


**Fig. S12:** (Left) confocal micrographs of each channel (FITC chitosan in green, TAMRA-E3 in red, and ALEXA-647 BSA in blue) for a PA-chitosan gel containing BSA. The ALEXA-647 BSA was dissolved in the chitosan solution prior to mixing (Right) shows a line cut with normalized intensity values of each channel (FITC chitosan in green, TAMRA-E3 in red, and ALEXA-647 BSA in blue) for the line cut (yellow dashed line) of the composite confocal image to the left.

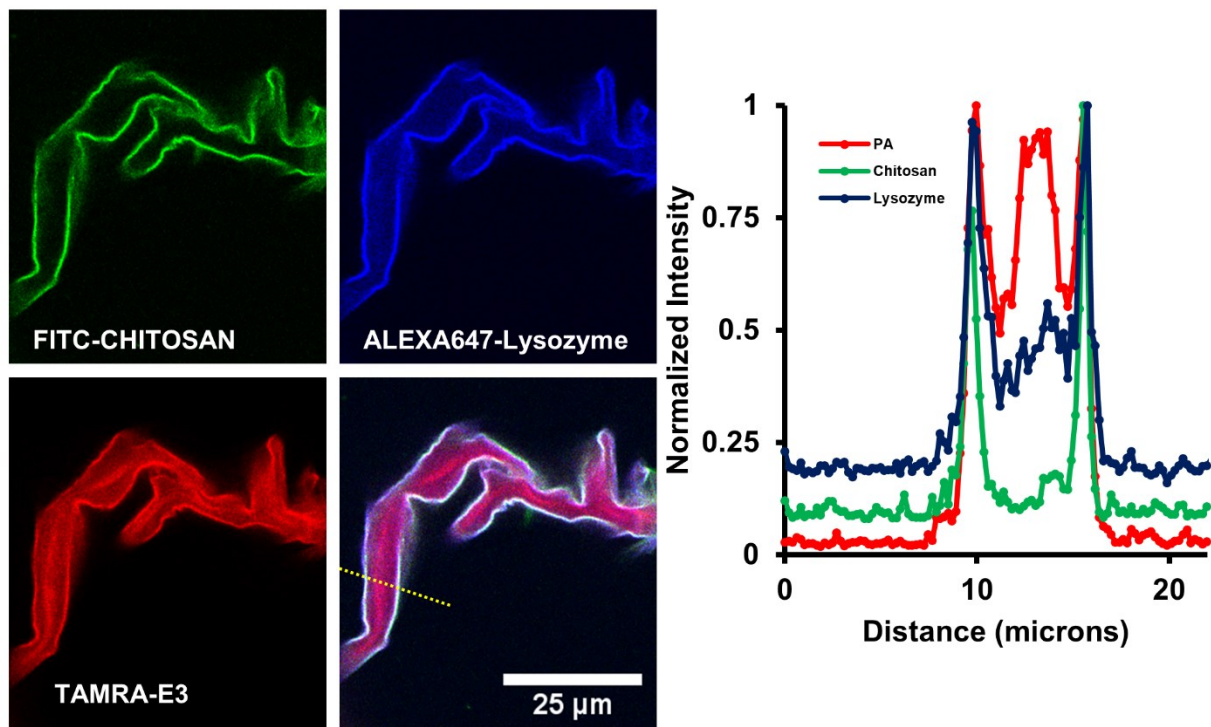


**Fig. S13:** (Left) Confocal micrographs of each channel (FITC chitosan in green, TAMRA-E3 in red, and ALEXA-647 BSA in blue) for a PA-chitosan gel containing BSA. The ALEXA-647 BSA was dissolved in the PA solution prior to mixing. (Right) shows a line cut with normalized intensity values of each channel (FITC chitosan in green, TAMRA-E3 in red, and ALEXA-647 BSA in blue) for the line cut (yellow dashed line) of the composite confocal image to the left.

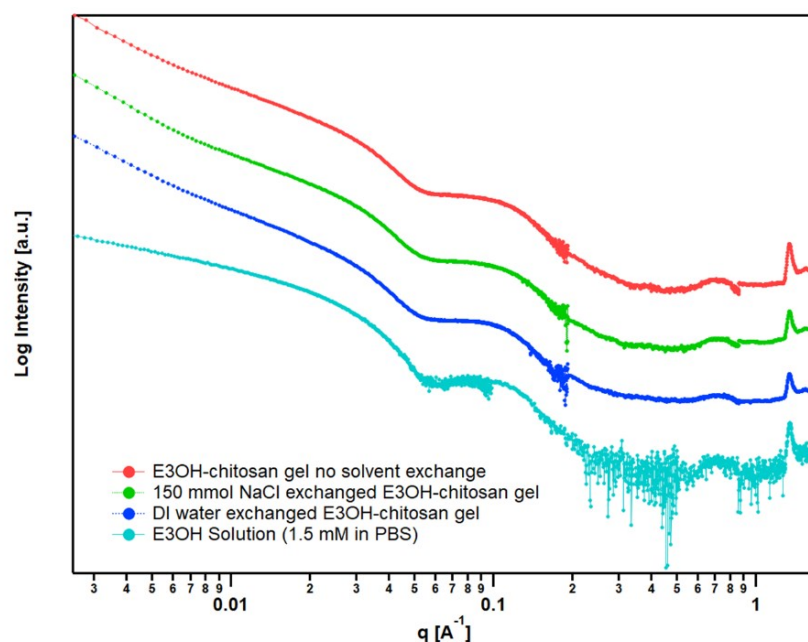




**Fig. S14:** (Left) Confocal micrographs of a PA-chitosan gel containing ALEXA-647-labeled lysozyme (FITC chitosan in green, TAMRA-E3 in red, and lysozyme in blue). The ALEXA-647 lysozyme was dissolved in the chitosan solution prior to mixing (Right) Plot of the normalized intensity values of each channel (FITC chitosan in green, TAMRA-E3 in red, and ALEXA-647 lysozyme in blue) for the line cut (yellow dashed line) of the composite confocal image on the left.



**Fig. S15:** (Left) Confocal micrographs of PA-chitosan gel containing ALEXA-647-labeled lysozyme (FITC chitosan in green, TAMRA-E3 in red, and lysozyme in blue). The ALEXA-647 lysozyme was dissolved in the PA solution prior to mixing. (Right) Plot of the normalized intensity values of each channel (FITC chitosan in green, TAMRA-E3 in red, and ALEXA-647 lysozyme in blue) for the line cut (yellow dashed line) of the composite confocal image on the left.



**Fig. S16:** X-ray scattering patterns of gels with no solvent exchange (red) as well as gels solvent exchanged into a 150 mM NaCl solution (green) and DI water solution (dark blue). Additionally, there is a scattering curve of an E3OH solution (light blue). The dark blue and light blue traces are repeated from Figure 2D. Comparing the scattering curve of the solution to the gels shows that the gels have the same molecular packing ( $\beta$ -sheet) and similar fiber diameter. This shows that complexation and solvent exchange does not disrupt the peptide amphiphile fiber structure.