

Supporting Information for:

**Rapidly *In Situ* Forming Hydrophobically-Modified Chitosan Hydrogels via
pH-Responsive Nanostructure Transformation**

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CS (viscosity ~ 36 mPa·s, 0.5% in 0.5% acetic acid at 20°C, MW ~ 500 kDa) with a degree of deacetylation of approximately 85% was used in the study. NPCS polymers with different degrees of substitution were prepared via manipulating the ratios of reactants used. The degrees of substitution on NPCS were determined by the ninhydrin assay¹ and the potassium polyvinylsulfate (PVSK) titration method² (see Table S1). A mixture of CS (1 g) and aqueous acetic acid (50 ml, 1% w/v) was stirred for 24 h to ensure total solubility. The pH was adjusted to 6.0 by slow addition of 1N NaOH and the final volume of CS solution was 100 ml. A solution of palmitic acid *N*-hydroxysuccinimide ester (0.1, 0.2, 0.3 or 0.4 g) in absolute ethanol was added drop-wise to the CS solution at 98°C and reacted for 36 h. Subsequently, the prepared solution was cooled at room temperature, added acetone and precipitated by adjusting its pH value to 9.0. The precipitate (NPCS) was then filtered, washed with an excess of acetone and air-dried. The synthesized NPCS was analyzed by ¹H-NMR (Varian Unity Inova 500, USA) and FT-IR (Perkin Elmer, USA) (see Fig. S2 and Fig. S3).

Figure S1

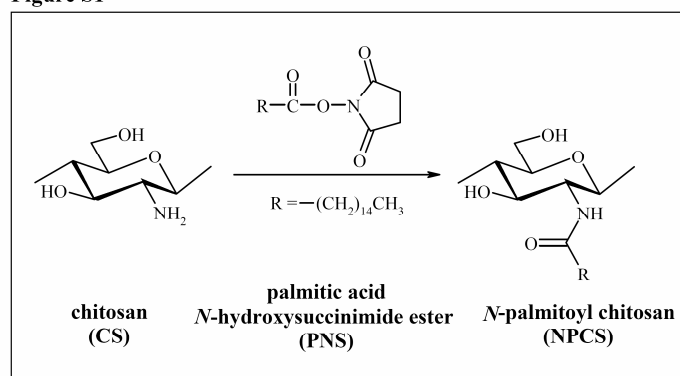


Figure S1. Schematic illustration of the synthesis of *N*-palmitoyl chitosan (NPCS).

Table S1. Degrees of substitution of *N*-palmitoyl chitosan (NPCS) determined by the ninhydrin assay and the potassium polyvinylsulfate (PVSK) titration method ($n = 5$). CS: chitosan; PNS: palmitic acid *N*-hydroxysuccinimide ester.

Samples	Feed Ratio		Degree of Substitution (%)	
	CS (g)	PNS (g)	Ninhydrin Assay	PVSK Titration
NPCS-5%	1.0	0.1	2.1 ± 0.2	5.3 ± 0.1
NPCS-10%	1.0	0.2	10.2 ± 0.1	12.7 ± 0.1
NPCS-15%	1.0	0.3	15.1 ± 0.2	17.0 ± 0.0
NPCS-20%	1.0	0.4	20.5 ± 0.2	20.7 ± 0.0

Figure S2

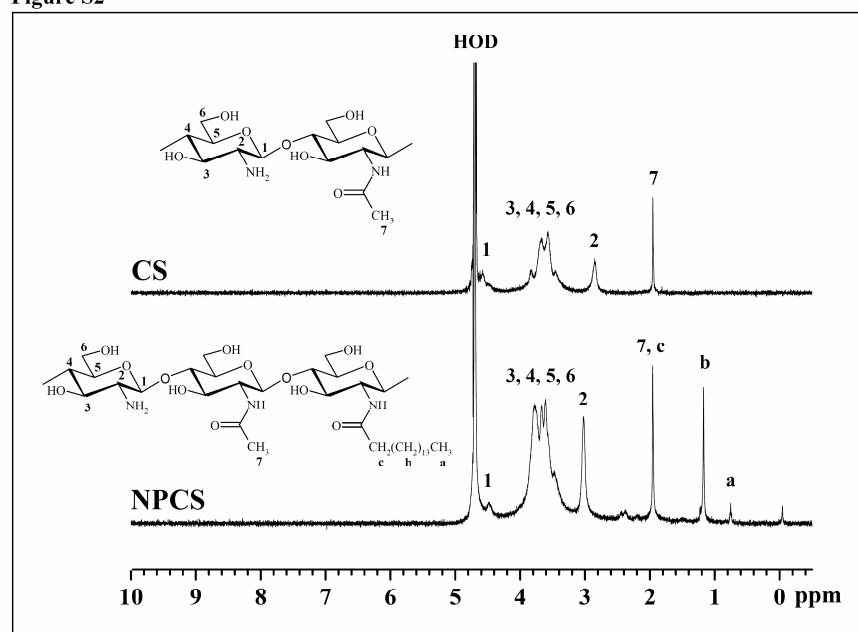


Figure S2. ^1H -NMR of *N*-palmitoyl chitosan (NPCS) and chitosan (CS).

Figure S3

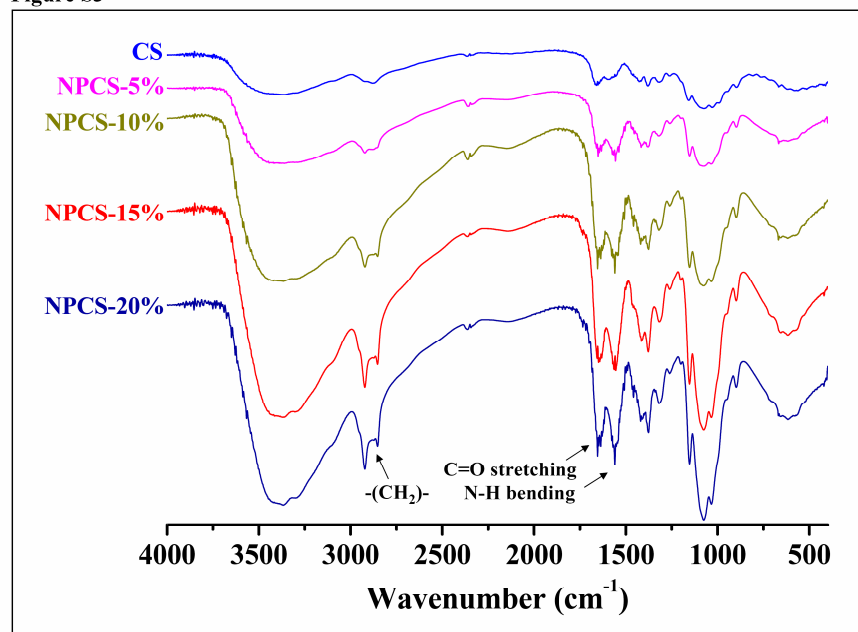


Figure S3. FT-IR of *N*-palmitoyl chitosan (NPCS) and chitosan (CS).

Rheology Study

Rheology experiments were performed at 25°C (Haake RS 600 rheometer, USA), equipped with either a cone-plate (35 mm plate diameter and 2° cone angle) or a parallel plate geometry (20 mm plate diameter). The viscosity reported here was taken from the value in the Newtonian plateau when it existed or at the lowest accessible shear rate. The gel strength at different pH values was monitored via frequency sweep measurements at a fixed strain amplitude of 1% (linear viscoelastic region) to measure the hydrogel elastic (G') and viscous (G'') moduli. Dynamic frequency spectra were obtained in the linear viscoelastic regime of the samples, as determined by dynamic strain sweep experiments. The influence of temperature on G' and G'' was investigated via temperature sweep measurements over a temperature range of 4°C–50°C at a ramp rate of 0.038°C/s and an oscillatory strain amplitude of 1% and a frequency of 0.1 Hz.

Figure S4

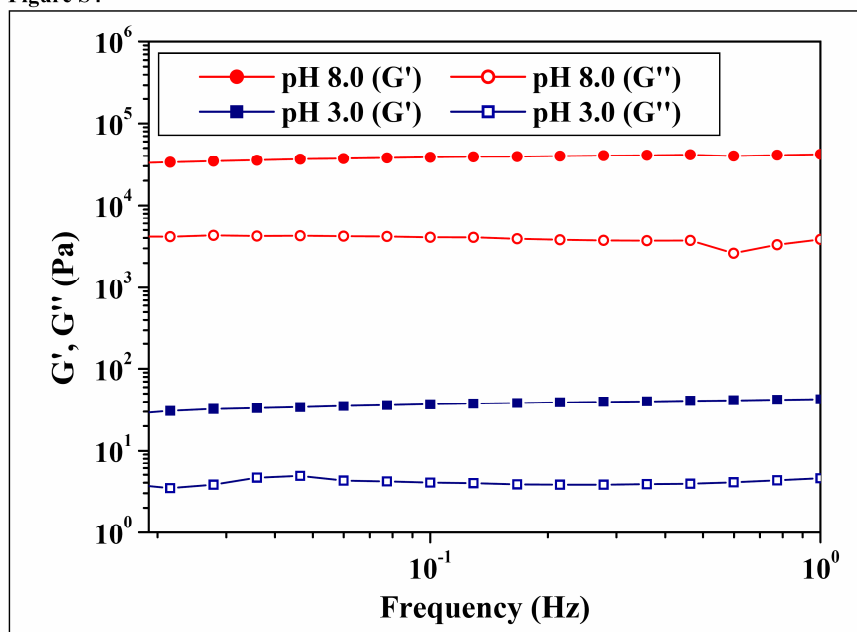


Figure S4. Dynamic frequency sweeps of NPCS-15% as a function of pH (corresponding to Fig. 3a in the main text).

Figure S5

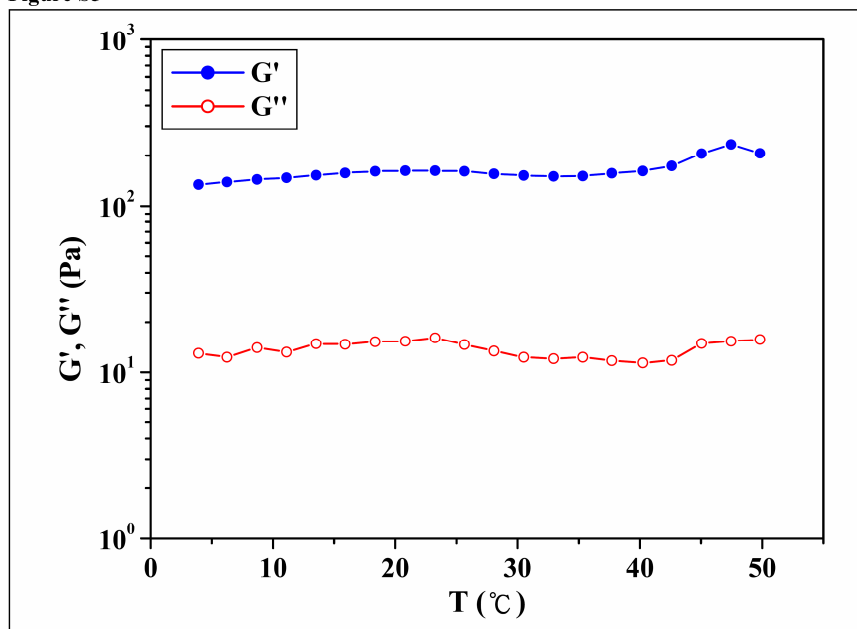


Figure S5. Dynamic temperature sweeps of NPCS-15% (1% w/v, pH 6.5) at an oscillatory strain amplitude of 1% and a frequency of 0.1 Hz.

The SAXS experiments were performed using the BL17B3 beamline at the National Synchrotron Radiation Research Center (NSRRC), Hsinchu, Taiwan. The energy of beam source was 8 keV and its corresponding wavelength was 1.55 Å. The scattering intensity was collected using a two-dimensional MarCCD detector with 512×512 pixel resolution. The sample-to-detector distance was 1734.9 mm. The intensity profile was output as the plot of the scattering intensity (I) vs. the scattering vector, $q = (4\pi/\lambda)\sin(\theta/2)$ (θ = scattering angle). The SAXS profiles were corrected for the absorption, the air scattering, and the background arising from thermal diffused scattering.

Modeling of SAXS Profiles

The SAXS profile of aqueous NPCS-15% at pH 3.0 was fitted quantitatively by the following scattering function for a fractal object composing of cylindrical building blocks.

$$I(q) \sim S(q)P(q)$$

$S(q)$ is the structure factor given by^{3,4}

$$S(q) = 1 + \frac{D \exp[\Gamma(D-1) \sin(D-1) \tan^{-1}(q\xi)]}{(qR_e)^D [1 + (q\xi)^{-2}]^{(D-1)/2}} \quad (S1)$$

where D is the fractal dimension; ξ is the correlation length that can be used as a measure for the size of the fractal network aggregate and R_e is the effective radius of the building block. $P(q)$ is the form factor of randomly oriented cylinders (with the radius and length of R and L , respectively) given by

$$P(q) = V^2 \int_0^{\pi/2} \left(\frac{\sin(\frac{qL}{2} \cos \varphi)}{\frac{qL}{2} \cos \varphi} \right)^2 \left(\frac{2J_1(qR \sin \varphi)}{qR \sin \varphi} \right)^2 \sin \varphi d\varphi \quad (S2)$$

The fitted result is displayed by the solid curve in Fig. 4a in the main text. The numerical values of the parameters obtained from the fitting were $R = 0.48 \pm 0.22$ nm; $L = 2.81 \pm 0.05$

***In Vivo* Hydrogel Formation**

Animal care and use was performed in compliance with the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources, National Research Council, and published by the National Academy Press, revised in 1996 and approved by the institutional review board (IRB, National Tsing Hua University, Hsinchu, Taiwan). Balb/C mice (male, 10–12 weeks old) were used in the study. Mice were anesthetized using pentobarbital prior to experiment. After shaving and disinfection, 500 μ l of the sterilized aqueous NPCS-15% (1% w/v, pH 6.5) was injected subcutaneously. After 30 min, mice were sacrificed and the hydrogel and surrounding tissue were isolated and photographed.

References

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