Electronic Supplementary Material (ESI) for Soft Matter.

Rational Design of a Supramolecular Hydrogel with Customizable pH-Responsiveness on Basis of the pH-induced Ionization/Protonation Transition of BSA

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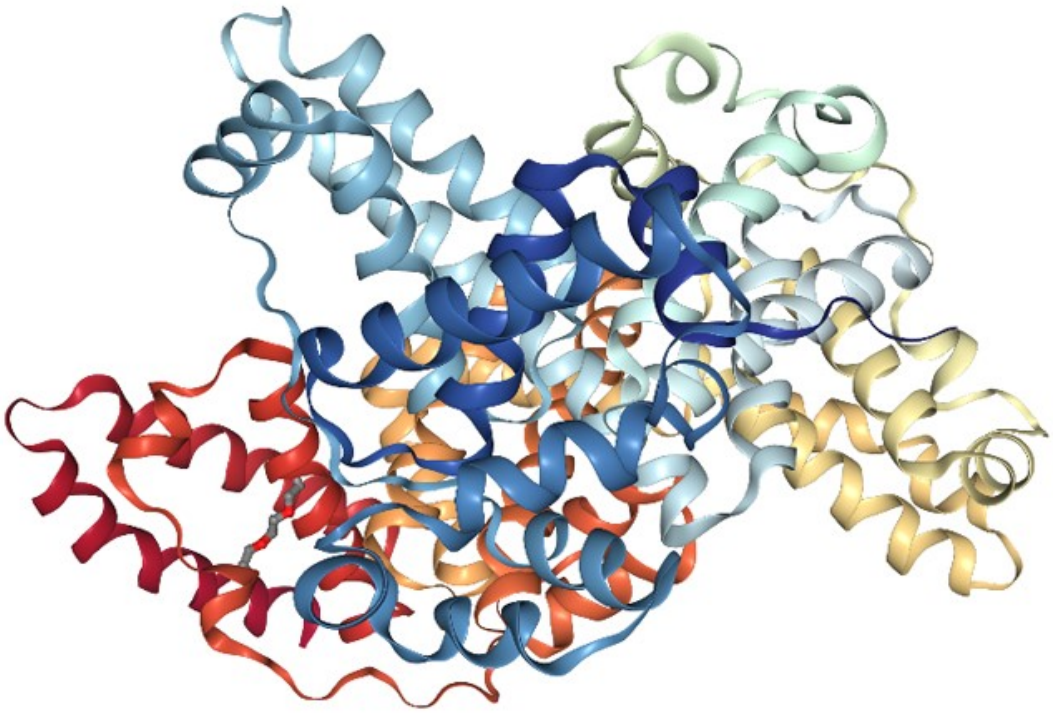

1 DTHKSEIAHR 11 FKDLGEEHFK 21 GLVLIAFSQY 31 LQQCPFDEHV
41 KLVNELTEFA 51 KTCVADESHA 61 GCEKSLHTLF 71 GDELCKVASL
81 RETYGDMADC 91 CEKQEPERNE 101 CFLSHKDDSP 111 DLPKLKPDPN
121 TLCDEFKADE 131 KKFWGKYLYE 141 IARRHPYFYA 151 PELLYYANKY
161 NGVFQECCQA 171 EDKGACLLPK 181 IETMREKVLT 191 SSARQRLRCA
201 SIQKFGERAL 211 KAWSVARLSQ 221 KFPKAEFVEV 231 TKLVTDLTKV
241 HKECCHGDLL 251 ECADDRADLA 261 KYICDNQDTI 271 SSKLKECCDK
281 PLLEKSHCIA 291 EVEKDAIPEN 301 LPPLTADFAE 311 DKDVCKNYQE
321 AKDAFLGSFL 331 YEYSRRHPEY 341 AVSVLLRLAK 351 EYEATLEECC
361 AKDDPHACYS 371 TVFDKLKHLV 381 DEPQNLIKQN 391 CDQFEKLGEY
401 GFQNALIVRY 411 TRKVPQVSTP 421 TLVEVSRSLG 431 KVGTRCCTKP
441 ESERMPCTED 451 YLSLILNRLC 461 VLHEKTPVSE 471 KVTKCCTESL
481 VNRRPCFSAL 491 TPDETYVPKA 501 FDEKLFTFHA 511 DICTLPDTEK
521 QIKKQTALVE 531 LLKHKPKATE 541 EQLKTVMENF 551 VAFVDKCCAA
561 DDKEACFAVE 571 GPKLVVSTQT 581 ALA

Fig.S1 Three-dimensional crystal structure and sequence of BSA

Table. S1 Amino acid composition of BSA

Amino acid	R group	Number
Ala	$-CH_3$	48
Cys	$-CH_2-SH$	35
Asp	$-CH_2-COOH$	41
Glu	$-(CH_2)_2-COOH$	58
Phe	$-CH_2-C_6H_5$	30
Gly	-H	17
His	$-CH_2-C=CH-NH-CH=N$	16
Ile	$-CH(CH_3)-CH_2CH_3$	16
Lys	$-(CH_2)_4-NH_2$	60
Leu	$-CH_2-CH(CH_3)_2$	65
Met	$-(CH_2)-S-CH_3$	5
Asn	$-CH_2-CONH_2$	14
Pro	$-C_3H_6$	28
Gln	$-(CH_2)_2-CONH_2$	21
Arg	$-(CH_2)_3-NH-C(NH_2)=NH$	26
Ser	$-CH_2-OH$	32
Thr	$-CH(CH_3)-OH$	34
Val	$-CH-(CH_3)_2$	38
Trp	$-C_8NH_6$	3
Tyr	$-CH_2-C_6H_4-OH$	21

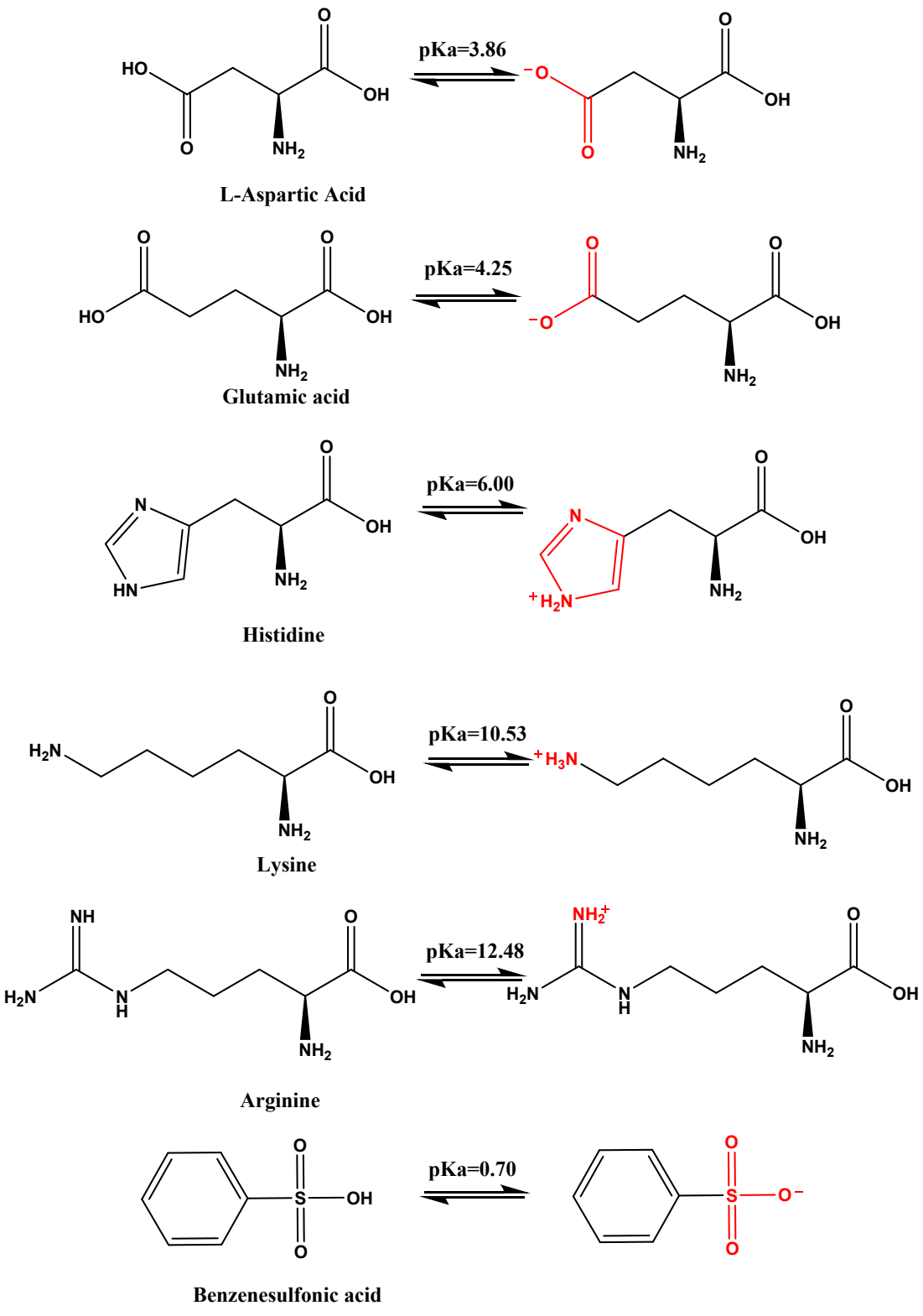

Fig. S2 Schematic diagrams of ionization of carboxyl groups (Asp and Glu) and protonation of X groups (His, Lys, and Arg) and benzenesulfonic acid.

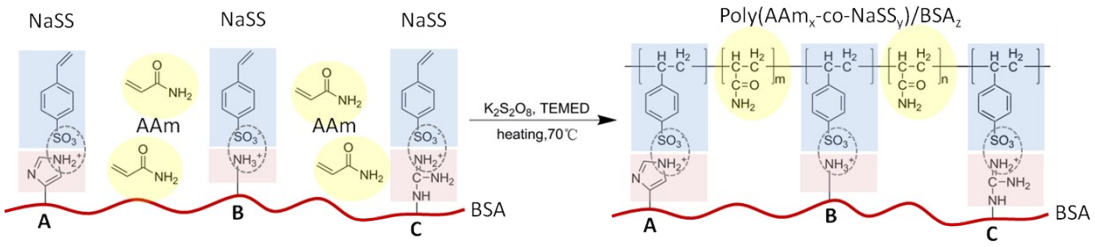

Fig.S3 Thermal-induced free radical co-polymerization between AAm and NaSS (m, $n \geq 0$)

Herein, thermal-induced free radical polymerization was utilized to synthesize the poly(AAm_x-co-$NaSS_y$)/BSA_z, so it is a random copolymer instead of an alternative copolymer. After mixing NaSS, BSA, and AAm, NaSS units are firstly be absorbed and gathered around the three kinds of protonated side groups (**Fig. S2**) of BSA, while AAm units are homogeneously dispersed in the prepared solution. The function of AAm is to connect discrete NaSS units through free radical copolymerization. Therefore, after adding the initiator ($K_2S_2O_8$) and the accelerator (TEMED), the solution is heated at 70°C to initiate free radical polymerization. The "A", "B", "C" in Fig.S3 represent the protonated side groups of the three amino acids (His, Lys, Arg) in BSA, which can form electrostatic attraction with the NaSS monomers. In poly(AAm_x-co-$NaSS_y$)/BSA_z, the "m" and "n" represent the molar amount of AAm monomer required to connect two adjacent NaSS monomers, which also represents the myriad possibilities of random free radical polymerization.

Table. S2 Feeding composition of a series of poly(AAm_x-co-$NaSS_y$)/BSA_z supramolecular hydrogels

Sample	[BSA] (mg/mL)	Mole Ratio (AAm/NaSS)	[Monomer][b] (mmol/mL)	NaSS[c] (g)	AAm (g)
poly($AAm_{2.515}$-co-$NaSS_{0.042}$)/BSA_{50}	50	60:1	2.557	0.0432	0.8937
poly($AAm_{2.934}$-co-$NaSS_{0.042}$)/BSA_{50}	50	70:1	2.976	0.0432	1.0427
poly($AAm_{3.353}$-co-$NaSS_{0.042}$)/BSA_{50}	50	80:1	3.395	0.0432	1.1916
poly($AAm_{3.772}$-co-$NaSS_{0.042}$)/BSA_{50}	50	90:1	3.814	0.0432	1.3406
poly($AAm_{4.191}$-co-$NaSS_{0.042}$)/BSA_{50}	50	100:1	4.233	0.0432	1.4895
poly($AAm_{3.387}$-co-$NaSS_{0.008}$)/BSA_{10}	10	404:1	3.395	0.0086	1.2036
poly($AAm_{3.370}$-co-$NaSS_{0.025}$)/BSA_{30}	30	134:1	3.395	0.0259	1.1976
poly($AAm_{3.336}$-co-$NaSS_{0.059}$)/BSA_{70}	70	57:1	3.395	0.0605	1.1857
poly($AAm_{3.320}$-co-$NaSS_{0.075}$)/BSA_{90}	90	44:1	3.395	0.0778	1.1797

[a]"x", "y" represent the molar concentration of AAm and NaSS, mmol/mL; z represents the mass concentration of BSA, mg/mL

[b][Monomer] represents the total molar concentration of NaSS monomer and AAm monomer

[c]the feeding amount of NaSS is on basis of the concentration of BSA. In order to quantify the feeding amount of different ingredients, an ideal model is formulated: at pH of 4.0, the ionization of Glu unit (21) was completely restricted and the Asp (24) unit was completely ionized while His (16), Lys (60), and Arg (26) units were completely protonated. To keep the proton-electron charge balance of the whole hydrogel system, the molar number of NaSS is designed equal to 57× of that of BSA.

In this supramolecular hydrogel system, BSA acts as a pH-responsive element, while NaSS and AAm together form the polymer matrix. Then, when exploring the effect of the AAm/NaSS ratio on the supramolecular hydrogel, the BSA concentration was kept constant at 50 mg/ml; on the other hand, when exploring the effect of the BSA concentration, the total molar concentration of the two monomers, was kept constant. It should be noted that as the BSA concentration changes, its molar amount changes, and the corresponding NaSS molar amount also changes. Then, under the condition that the total amount of monomers remains unchanged, the molar amount of AAm also changes, so the actual AAm/NaSS ratio also changes. Here, the two monomers are seen as a whole, rather than simply controlling their proportions.

S1. Mathematical modeling and release kinetics

In order to elucidate the in vitro drug release mechanism from hydrogels, various kinetic models were used to analyze the cumulative release of CAP. The Higuchi model, Korsemeyer-Peppas model and Kopcha model were selected to fit the drug release results for describing the mechanism of CAP release from the hydrogel. The Higuchi model (2) illustrates the drug release from an insoluble matrix is based on Fickian diffusion:

$$Q_t = Q_0 + k_H t^{1/2} \quad (1)$$

where Q_t is the cumulative amount of drug release at time "t", Q_0 is the initial amount of drug, k_H is the Higuchi constant.

The Korsemeyer-Peppas model (3) describes drug release from the polymeric system:

$$Q_t = K_{KP} t^n \quad (2)$$

where K_{KP} is the Korsmeyer-Peppas constant, n is the release exponent which defines the drug release mechanisms. When the Korsmeyer-Peppas model is used to thin films, the release index n ($n = 0.5$ or $n \leq 0.5$) conforms to Fickian diffusion, while the value of n ($0.5 < n < 1$) is related to non-Fickian release, meaning that the drug release follows both erosion and diffusion mechanisms. And $n = 1$ corresponds to the zero order release which defines the drug release is independent of time.

The Kopcha model (4) defines the relative contribution of diffusion and polymer relaxation to drug release:

$$Q_t = At^{1/2} + Bt \quad (3)$$

where A is the diffusion constant and B is the erosional exponent. If the ratio of A/B is high (A is much greater than B), implying the drug release will follow the diffusion mechanism; and if A/B is low (B is much greater than A), meaning that the polymer erosion or relaxation plays a dominant role in drug release.

The model that best fits the release results was chose based on the correlation coefficient (R^2) in models described above. The model that gives the highest R^2 value is considered as the best fit of the drug release data.

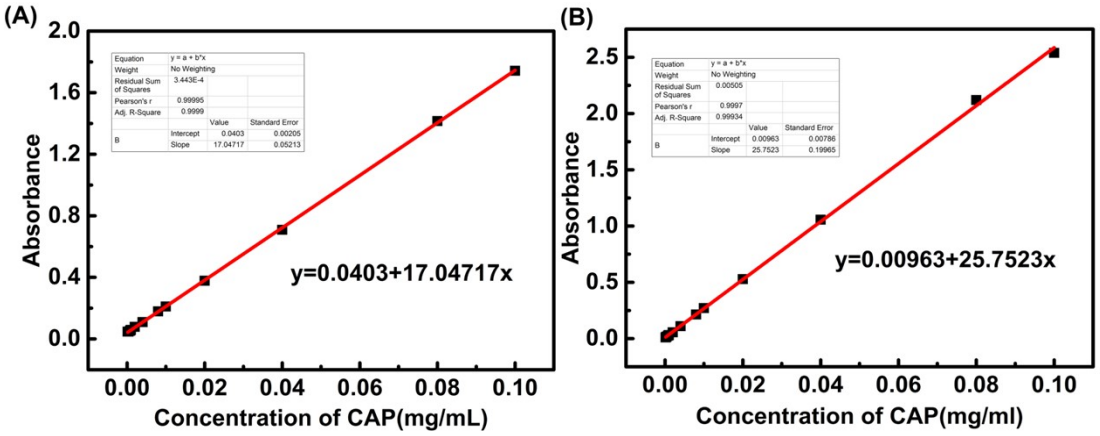

Fig.S4 The standard curve of capecitabine (CAP) in (A) simulated gastric fluid (SGF) and (B) simulated intestinal fluid (SIF)

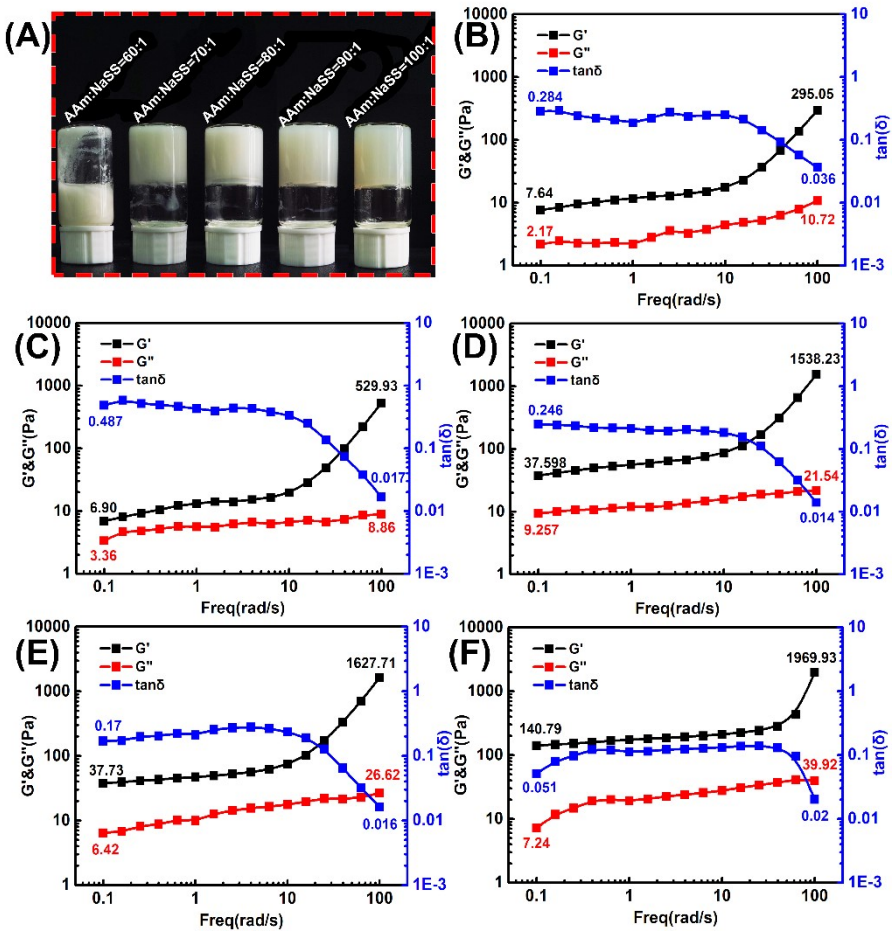

Fig. S5 Digital photographs (A) and frequency-dependent (γ_0 = 0.5%, 25 °C) oscillatory shear rheology of (B) sample poly($AAm_{2.515}$-co-$NaSS_{0.042}$)/BSA_{50}, (C) sample poly($AAm_{2.934}$-co-$NaSS_{0.042}$)/BSA_{50}, (D) sample poly($AAm_{3.353}$-co-$NaSS_{0.042}$)/BSA_{50}, (E) sample poly($AAm_{3.772}$-co-$NaSS_{0.042}$)/BSA_{50}, and (F) sample poly($AAm_{4.191}$-co-$NaSS_{0.042}$)/BSA_{50}. When the BSA concentration is kept at 50 mg/mL, the G′ and G″ values of the poly(AAm_x-co-$NaSS_y$)/BSA_z supramolecular hydrogel are gradually enhanced with the increase of AAm/ NaSS ratio. Compared to the 70:1 and 60:1 group, the 80:1 group demonstrates significantly increased G' value.

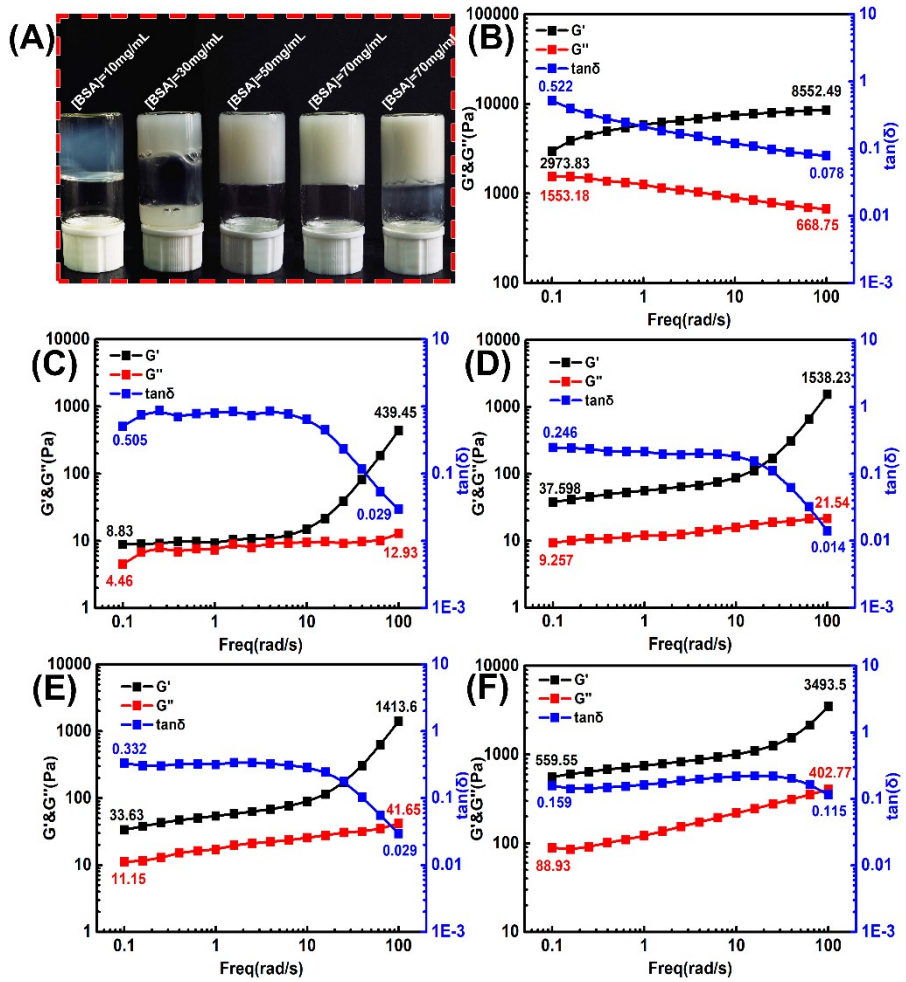

Fig. S6 Digital photographs (A) and frequency-dependent (γ_0 = 0.5%, 25 °C) oscillatory shear rheology of (B) sample poly($AAm_{3.387}$-co-$NaSS_{0.008}$)/BSA_{10}, (C) sample poly($AAm_{3.370}$-co-$NaSS_{0.025}$)/BSA_{30}, (D) sample poly($AAm_{3.353}$-co-$NaSS_{0.042}$)/BSA_{50}, (E) sample poly($AAm_{3.336}$-co-$NaSS_{0.059}$)/BSA_{70}, and (F) sample poly($AAm_{3.320}$-co-$NaSS_{0.075}$)/BSA_{90}. In this scheme, the total molar amount of concentration is kept at 3.395 mmol/mL, which is the same as sample poly($AAm_{3.353}$-co-$NaSS_{0.042}$)/BSA_{50}. When the BSA concentration is kept at 10 mg/mL, sample poly($AAm_{3.387}$-co-$NaSS_{0.008}$)/BSA_{10} seems to be unnormal (much higher than that of other groups), which can be attributed to the explosive polymerization of monomers. Sample poly($AAm_{3.353}$-co-$NaSS_{0.042}$)/BSA_{50}, poly($AAm_{3.336}$-co-$NaSS_{0.059}$)/BSA_{70} and poly($AAm_{3.320}$-co-$NaSS_{0.075}$)/BSA_{90} maintain high structural stability while sample poly($AAm_{3.370}$-co-$NaSS_{0.025}$)/BSA_{30} can't form stable supramolecular structure.

S2. The Mechanism of Explosive Polymerization of Monomers

Free radical polymerization is a kind of chain reaction. It could be separated into four stages: chain initiation, chain propagation, chain termination, and chain transfer. The polymerization rate could be expressed using the following equation:

$$R_p = k_p(\frac{fk_d}{k_t})^{1/2}[I]^{1/2}[M] \quad (4)$$

In this equation, R_p is the polymerization rate, k_p is the polymerization rate constant, f is the initiation efficiency constant of initiator, k_d is initiation rate constant of initiator, k_t is the termination rate constant, *[I]* is the concentration of the initiator, *[M]* is the monomer concentration. In some cases (for instance the concentration of monomer is too high, the reaction solution is too vicious, or the local heat transfer is restricted), the viscosity of the solution will be gradually increased with the progress of the reaction, then the translation motion of free radicals will be restricted and chain termination will be restricted (*i.e.,* k_t will be decreased). As a result, the polymerization rate, namely, R_p will be greatly enhanced, the temperature of the system will greatly increase and gelation phenomenon (also known as self-accelerating phenomenon) correspondingly occurs *(i.e.,* explosive polymerization).

Sample poly($AAm_{3.387}$-co-$NaSS_{0.008}$)/BSA_{10} in **Table S2**, is a typical product of explosive polymerization. The comparison in **Fig. S6** is to control the total molar quantity of NaSS and AAm monomers unchanged, and change the BSA concentration. With the change of BSA concentration, its molar amount changed, and the molar amount of NaSS also changed due to the fixed stoichiometric ratio of BSA/NaSS (1/57). Since the total amount of monomer (AAm and NaSS) is fixed, then the molar amount of AAm and the actual AAm/NaSS also changed. When the BSA concentration is 10 mg/ml, the stoichiometric ratio of AAm/NaSS is 404/1 rather than 80/1, then the AAm monomer concentration in the system is relatively too high, which results in the above-mentioned explosive polymerization and great G' values (**Fig. S6B)** resulting from the high concentration of monomer.

S3. The thermal-induced free radical copolymerization yield

From Fig. S5&S6, sample poly($AAm_{3.353}$-co-$NaSS_{0.042}$)/BSA_{50} (BSA/NaSS=1:57;

AAm/NaSS=80:1; BSA=50 mg/ml) was chosen for subsequent characterization. In the drug release experiment, the BSA/NaSS ratio was changed to 1/102, and the sample was named as poly($AAm_{6.000}$-co-$NaSS_{0.075}$)/BSA_{50}. Herein, the thermal-induced free radical copolymerization yields of these two poly(AAm_x-co-$NaSS_y$)/BSA_z hydrogels were measured.

We utilized as alternative method to measure the copolymerization yield of the two samples. Firstly, the dry quality of different reactants can be ascertained (AAm (M_{AAm}), NaSS (M_{NaSS}), and BSA (M_{BSA}); then the poly(AAm_x-co-$NaSS_y$)/BSA_z hydrogel was synthesized at pH 4.0 and dialyzed against dd water (cut-off: 1000 D) for removing unreacted monomers, $K_2S_2O_8$, and TEMED; finally, the dry quality of final hydrogel sample was weighed (Ms). Thus, the copolymerization yield could be roughly calculated using the following equation:

$$yield = \frac{M_s - M_{BSA}}{M_{AAm} + M_{NaSS}} \quad (5)$$

All relevant data are presented in the table below:

Table. S3 Feeding composition and copolymerization yield of the poly($AAm_{3.353}$-co-$NaSS_{0.042}$)/BSA_{50} and poly($AAm_{6.000}$-co-$NaSS_{0.075}$)/BSA_{50}

BSA/NaSS	M_{BSA} (g)	M_{NaSS} (g)	M_{AAm} (g)	Total dry weight M_S(g)	Copolymerization yield (%)
1:57	0.25	0.0432	1.1916	1.11±0.01	69.44±1.21
1:102	0.25	0.0773	1.8659	1.87±0.08	83.51±4.44

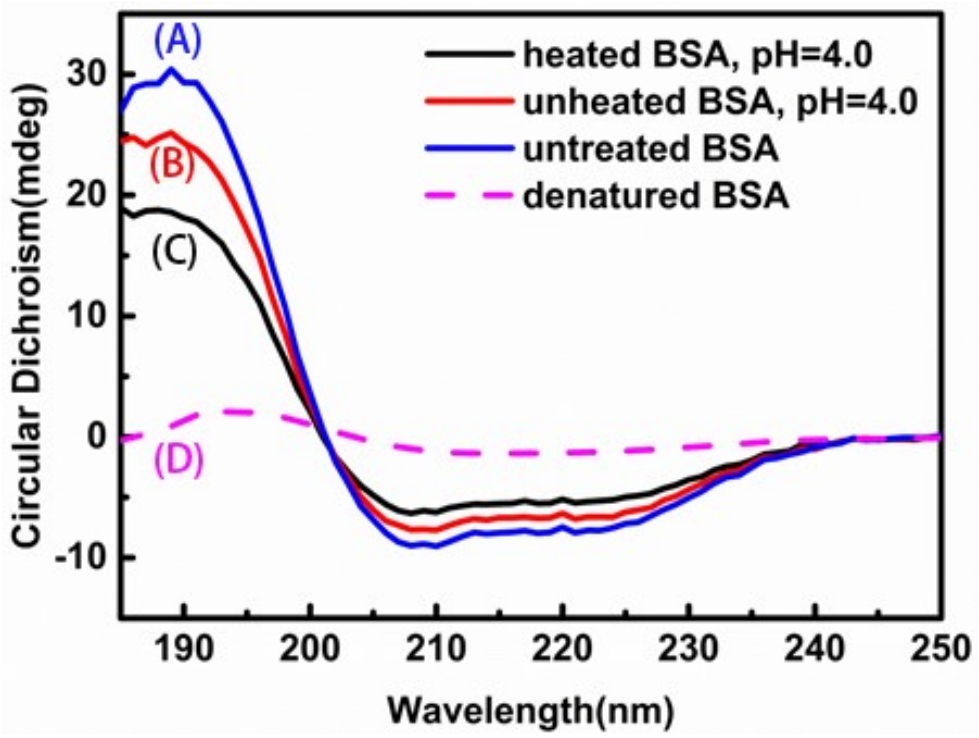

Fig. S7 The circular dichroism of (A) pristine BSA, (B) acid-treated BSA (pH 4.0), and (C) heat-treated BSA (pH 4.0, 70 ℃), and (D) denatured BSA.

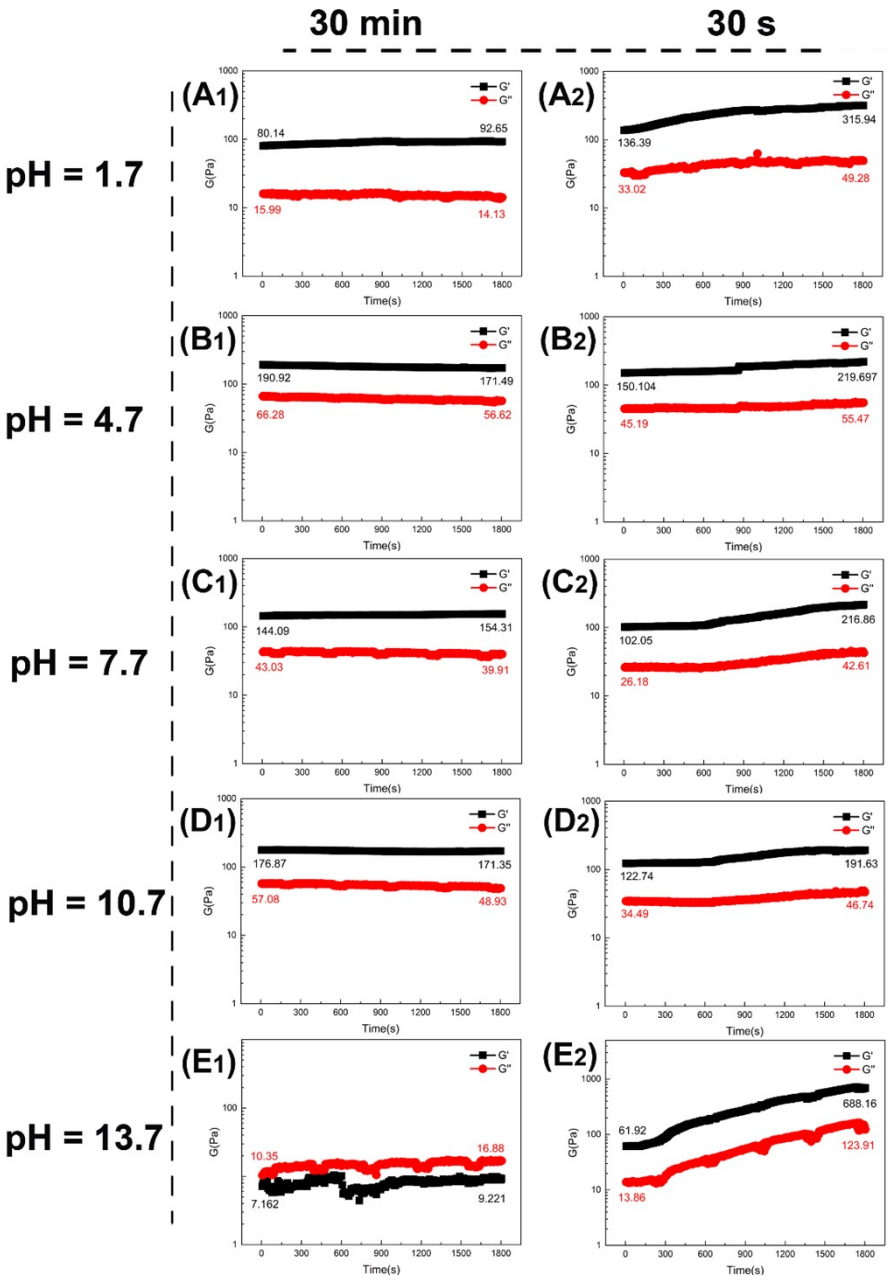

Fig. S8 Time-dependent (6.0 rad/s, 25 °C) oscillatory shear rheology of the poly($AAm_{3.353}$-co-$NaSS_{0.042}$)/BSA_{50} supramolecular hydrogel after being soaked in aqueous solutions with pH values of 1.7, 4.7, 7.7, 10.7, and 13.7 for 30 min (left) and 30 s (right). After being soaked for 30 min, all the samples reach an internal swelling balance and electric charge rearrangement was also finished except the group of pH=13.7 (E_1), which has become a sol and is completely dissolved in the aqueous solution. After being soaked for 30 s, electric charge rearrangement is not finished for every group. Thus, their G′ values are gradually increased with time.