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Effect of thermosensitive poly(N-isopropylacrylamide) on blood coagulation

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1 Zeta potential and size of PNIPAM aggregates in water

The zeta potential and size of PNIPAM aggregates in water were measured with a zeta potential analyzer (Zetasizer Nano ZS, Malvern Instruments Ltd., UK). The data were listed in Table S1.

Table S1 Zeta potential and size of PNIPAM aggregates in water (n=3).

Temperature	Zeta potential (mV)	Size (nm)
25°C	-0.4203	39.17
32°C	-0.1048	191.23
37°C	-0.6953	657.1

2 Hemolysis of PNIPAM

2.1 Method

RBC suspension (50 μL, 16% v/v in PBS) was incubated with PBS or PNIPAM solutions (1 mL) at room temperature or 37°C, respectively. After incubation for predetermined time, the RBC suspension was centrifuged at 1000×g for 5 min. The resulting supernatants were collected, 200 μL of which were measured at 540 nm with a 96-well plate reader (Multiskan MK3, Thermo Scientific) to detect hemoglubin release from

the RBCs. Complete (100%) hemolysis was prepared by adding the RBC suspension (50 μL, 16% v/v in PBS) to 1 mL of pure water. The hemolysis rate of PNIPAM-treated RBCs was calculated by comparing their A540nm values to that of the 100% hemolysis sample.

2.2 Result

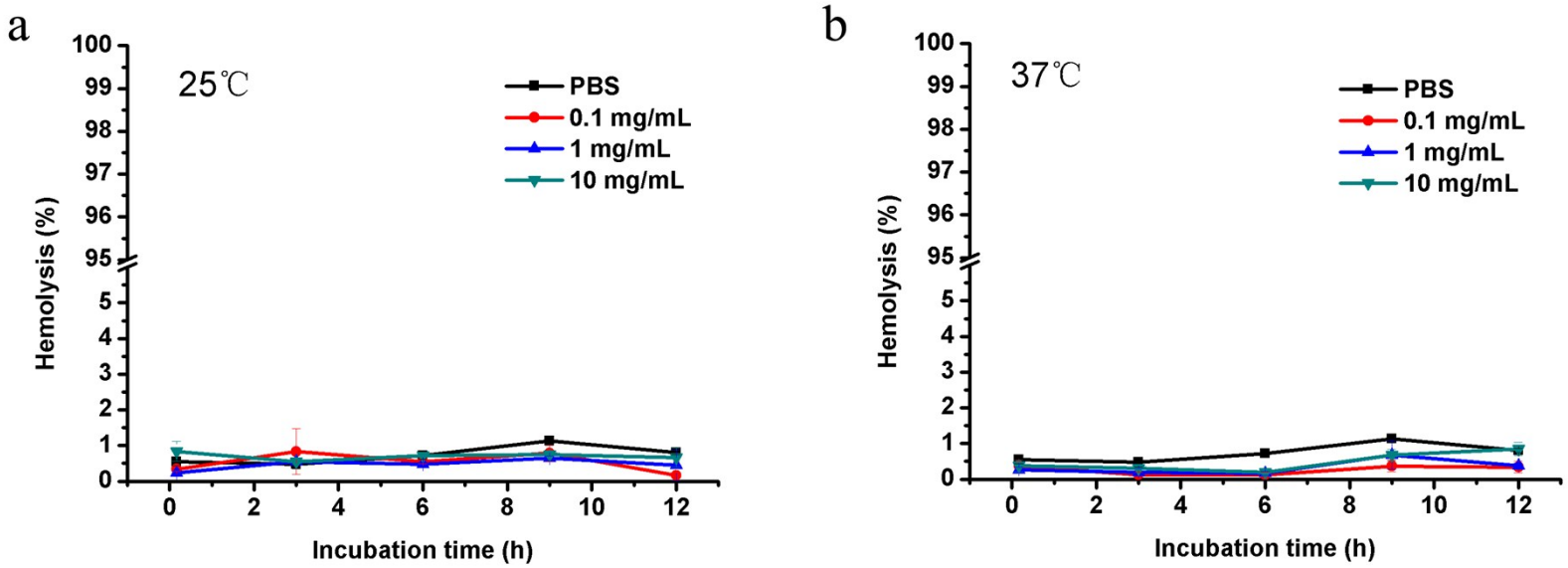

Figure 1S Hemolysis of PNIPAM at 25 or 37°C (n=5).

3 Adsorption of AA to PNIPAM at 25 or 37ºC

3.1 Method

For convenient isolation of PNIPAM, PNIPAM immobilized magnetic beads were introduced into experiment of AA adsorption. Preparation of beads coated with PNIPAM was same as procedures in plasma protein absorption study. The AA adsorption measurement was performed as followed. Firstly, 200 μL aliquots of magnetic beads suspension(10 mg/mL) were mixed with 500 μL AA aqueous solution and incubated at 37ºC and 25ºC respectively by shaking 2 h. Secondly, Prior to removing the supernatants, magnetic beads were collected and washed with 37ºC and 25ºC PBS twice respectively. Subsequently, adsorbed AA were eluted from beads surface by shaking 1 h with elution buffer (200μL PBS, containing 0.1% Tween-20) at room temperature and the eluted samples were centrifuged after removing the magnetic beads. Finally supernatants were collected and diluted with 10-fold, subjected to UV spectra scanning with a UV-2550 spectrophotometer (Shimadzu Corporation, Japan) from 200 nm to 500 nm.

3.2 Result

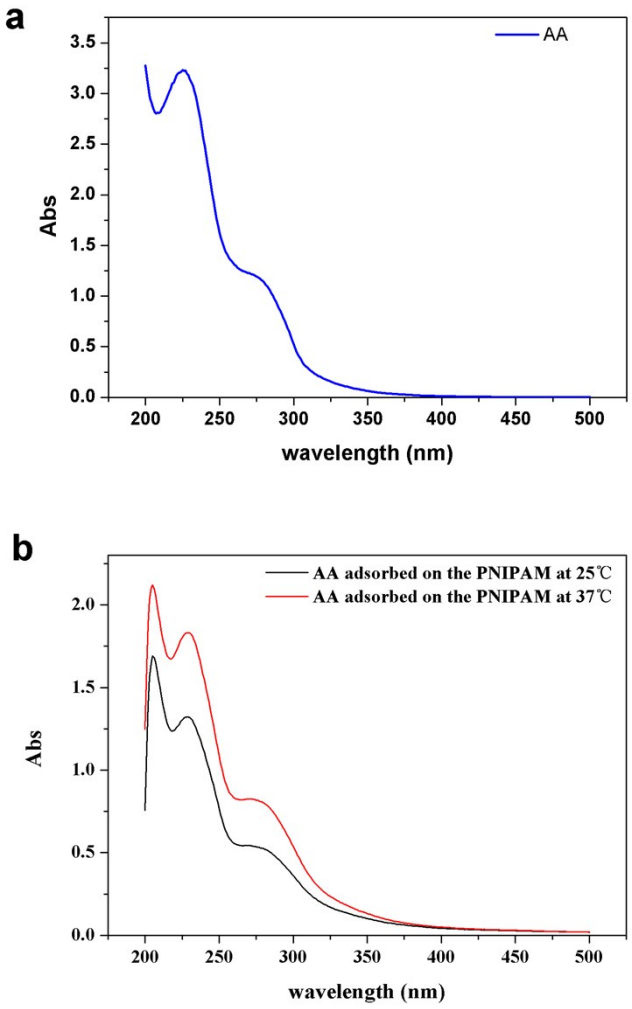
a
AA
Abs
wavelength (nm)
b
AA adsorbed on the PNIPAM at 25℃
AA adsorbed on the PNIPAM at 37℃
Abs
wavelength (nm)

Figure 2S (a) UV spectrum of AA (not adsorbed on PNIPAM); (b) UV absorption spectra of AA adsorbed on the PNIPAM immobilized magnetic beads at 25 or 37ºC.

4 Proteomics analysis of the adsorbed plasma proteins onto the PNIPAM-coated beads

Table S2: Identification of the plasma proteins adsorbed at different temperatures.

NO.	Protein Types	Accession	Gene name	Proteins on PNIPAM at 37ºC	Proteins on PNIPAM at 25ºC
1	Immune response related proteins	P00751	CFB	Complement factor B	
2		P00738	HP	Haptoglobin	
3		P01024	C3	Complement C3	
4		P01859	IGHG2	Ig gamma-2 chain C region	
5		P01857	IGHG1	Ig gamma-1 chain C region	
6		P01871	IGHM	Ig mu chain C region	Ig mu chain C region
7		P01861	IGHG4	Ig gamma-4 chain C region	
8		P81605	DCD	Dermcidin	Dermcidin
9	Blood coagulation related proteins	P02671	FGA	Fibrinogen alpha chain	Fibrinogen alpha chain
10		P03951	F11	Coagulation factor XI	
11		P05106	ITGB3	Integrin beta-3	
12		P00488	F13A1	Coagulation factor XIII A chain	
13	Transport/Binding proteins	P02768	ALB	Serum albumin	Serum albumin
14		P02647	APOA1	Apolipoprotein A-I	Apolipoprotein A-I
15		P02649	APOE	Apolipoprotein E	Apolipoprotein E

16		P04004	VTN	Vitronectin	Vitronectin
17		P02787	TF	Serotransferrin	
18		P02788	LTF	Lactotransferrin	
19		P14923	JUP		Junction plakoglobin
20		Q08554	DSC1		Desmocollin-1
21		Q02413	DSG1		Desmoglein-1
22		Q5SW24	DACT2		Dapper homolog 2
23		Q8TER0	SNED1		Sushi, nidogen and EGF-like domain-containing protein 1
24		Q9BT23	LIMD2		LIM domain-containing protein 2
25		Q9UK10	ZNF221		Zinc finger protein 225
26		Q9H6R4	NOL6		Nucleolar protein 6
27		Q9UPQ0	LIMCH1		LIM and calponin homology domains-containing protein 1
28	Cytoskeletal proteins	P60709	ACTB	Actin, cytoplasmic 1	
29		P46821	MAP1B	Microtubule-associated protein 1B	
30		P35321	SPRR1A	Cornifin-A	Cornifin-A
31		Q8TD57	DNAH3	Dynein heavy chain 3, axonemal	Dynein heavy chain 3, axonemal
32		Q86YZ3	HRNR	Hornerin	Hornerin
33		Q5T749	KPRP	Keratinocyte proline-rich protein	
34		P22528	SPRR1B		Cornifin-B
35		Q08AD1	CAMSAP2		Calmodulin-regulated spectrin-associated protein 2

36		Q70YC5	ZNF365		Protein ZNF365
37		Q9BYE4	SPRR2G		Small proline-rich protein 2G
38	Other proteins	Q8TCG1	KIAA1524	Protein CIP2A	
39		Q14410	GK2	Glycerol kinase 2	
40		P0C869	PLA2G4B		Cytosolic phospholipase A2 beta
41		Q6UWP8	SBSN		Suprabasin
42		Q6IPM2	IQCE		IQ domain-containing protein E
43		Q9GZM5	YIPF3		Protein YIPF3
44		Q16629	SRSF7	Serine/arginine-rich splicing factor 7	
45		O60882	MMP20	Matrix metalloproteinase-20	

5 Morphology of PNIPAM aggregates above LCST

The morphology of PNIPAM aggregates in water at different temperatures was observed by TEM (FEI Tecnai Osiris, FEI Instruments Ltd., USA).

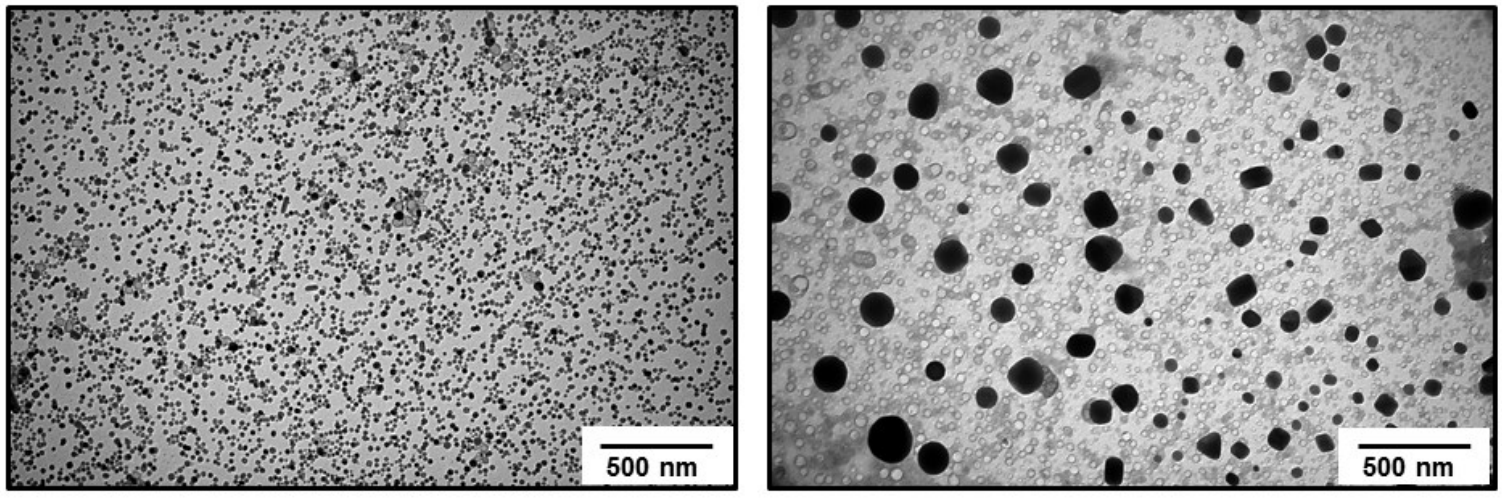

Figure 3S Morphology of PNIPAM aggregates in water.

6 Effect of PNIPAM on secondary structure of fibrinogen detected by circular dichroism

Table S3: Quantification of secondary structure of fibrinogen in the presence of PNIPAM

Samples	***α helix***	***β sheet***	***β turn***	***Random coil***
Fibrinogen	30.40%	20.50%	19.50%	29.60%
Fibrinogen - PNIPAM（0.01 mg/ml）	26.10%	21.60%	18.70%	33.60%
Fibrinogen - PNIPAM（0.05 mg/ml）	25.90%	21.80%	18.80%	33.50%
Fibrinogen - PNIPAM（0.1 mg/ml）	24.70%	22.10%	18.60%	34.60%
Fibrinogen - PNIPAM（0.15 mg/ml）	24.70%	21.40%	17.80%	36.10%
Fibrinogen - PNIPAM（0.2 mg/ml）	24.70%	21.40%	17.80%	36.10%
Fibrinogen - PNIPAM（0.4 mg/ml）	24.00%	21.70%	17.70%	36.60%
Fibrinogen - PNIPAM（0.6 mg/ml）	24.60%	21.40%	17.80%	36.20%
Fibrinogen - PNIPAM（0.8 mg/ml）	23.10%	22.10%	17.80%	37.00%
Fibrinogen - PNIPAM（1 mg/ml）	23.20%	22.20%	17.90%	36.70%
Fibrinogen - PNIPAM（2 mg/ml）	22.10%	23.10%	18.30%	36.50%