Supporting Information

Peptide-crosslinked molecularly imprinted polymers for efficient separation of immunoglobulin G from human serum

Qiujing Dong[a], *Mengmeng Yang*[a], *Yafei Wang*,[a] *Ying Guan* [a,*], *Wangqing Zhang*[a,*], *Yongjun Zhang*[b,*]

[a] Key Laboratory of Functional Polymer Materials and State Key Laboratory of Medicinal Chemical Biology, Institute of Polymer Chemistry, College of Chemistry, Nankai University, Tianjin 300071, China. [b] School of Chemistry, Tiangong University, Tianjin 300387, China

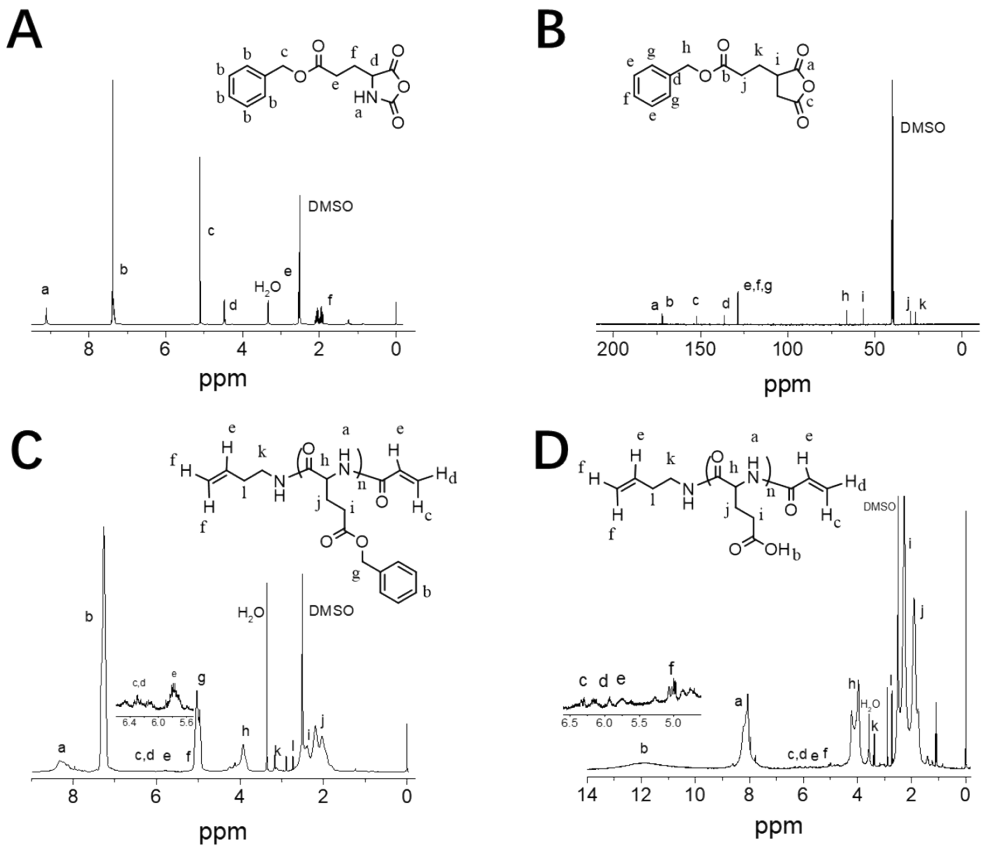

Fig. S1. (A, B) ^{1}H (A) and ^{13}C NMR spectra (B) of BLG-NCA monomer. (C, D) ^{1}H NMR spectra of PBLG intermediate (C) and PLGA crosslinker (D). Solvent: DMSO-d_6.

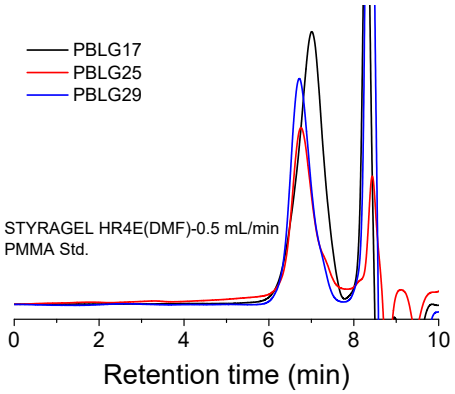

Fig. S2. GPC traces of PBLG intermediates in DMF solution at flow rate of 0.5 mL/min using STYRAGEL HR4E column and PMMA standards.

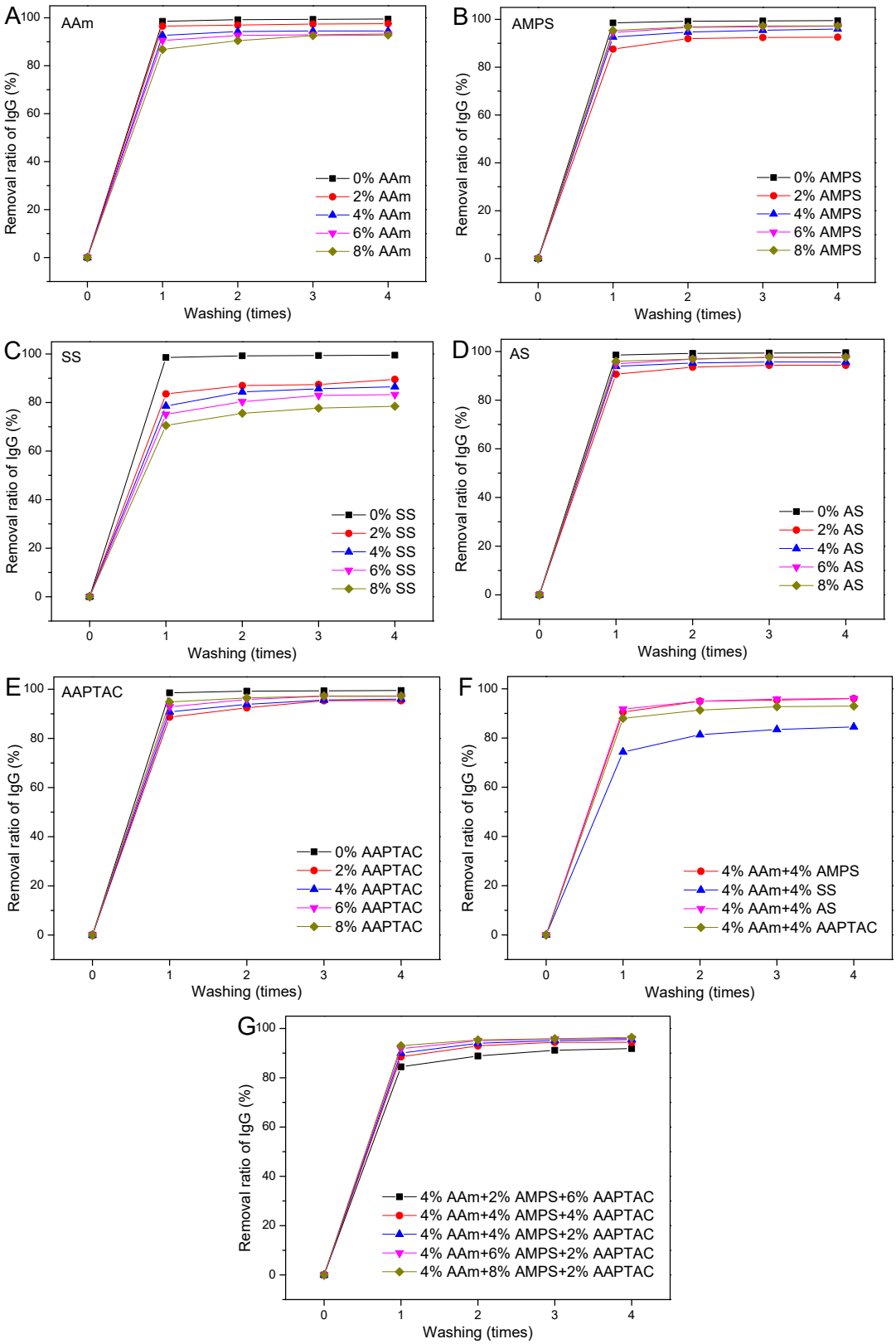

Fig. S3. Elution efficiency of 2% PLGA25-crosslinked MIP in 20 mM pH 7.4 PBS containing 154 mM NaCl at 37 °C. (A) AAm functional monomer content from 0% to 8%. (B) AMPS functional monomer content from 0% to 8%. (C) SS functional monomer content from 0% to 8%. (D) AS functional monomer content from 0% to 8%. (E) AAPTAC functional monomer content from 0% to 8%. (F) Combination of two functional monomers. (G) Combination of three functional monomers. IgG used in the preparation of the MIP was 45 mg.

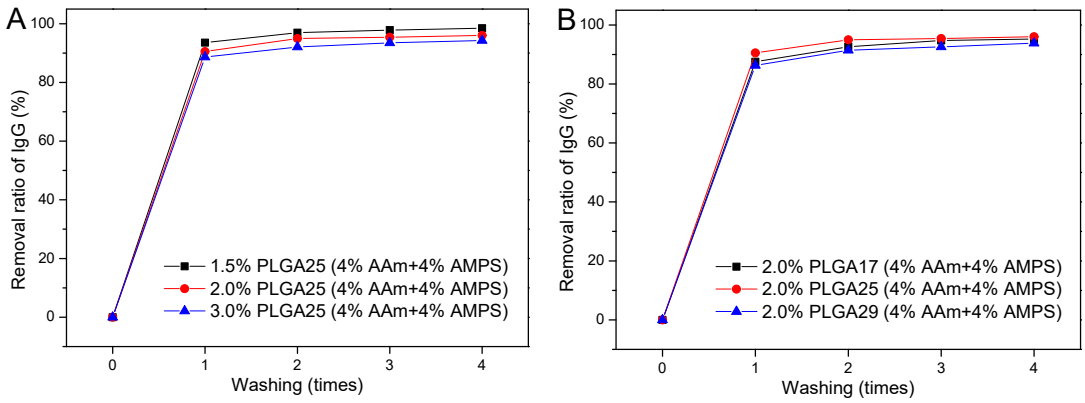

Fig. S4. Elution efficiency of PLGA-crosslinked MIP in 20 mM pH 7.4 PBS containing 154 mM NaCl at 37 °C. (A) Different amount of PLGA25 from 1.5% to 3.0% with 4% AAm and 4% AMPS functional monomers. (B) Different molecular weight of 2% PLGA with 4% AAm and 4% AMPS functional monomers. IgG used in the preparation of the MIP was 45 mg.

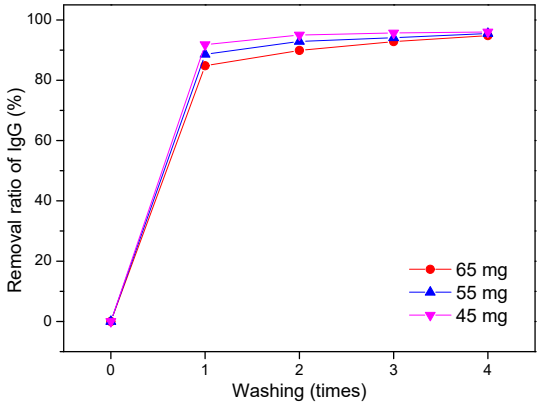

Fig. S5. Elution efficiency of 2% PLGA25-crosslinked MIP in 20 mM pH 7.4 PBS containing 154 mM NaCl at 37 °C. The functional monomers were consisting of 4% AAm, 6% AMPS and 2% AAPTAC. IgG used in the preparation of the MIP was 45 mg, 55 mg and 65 mg.

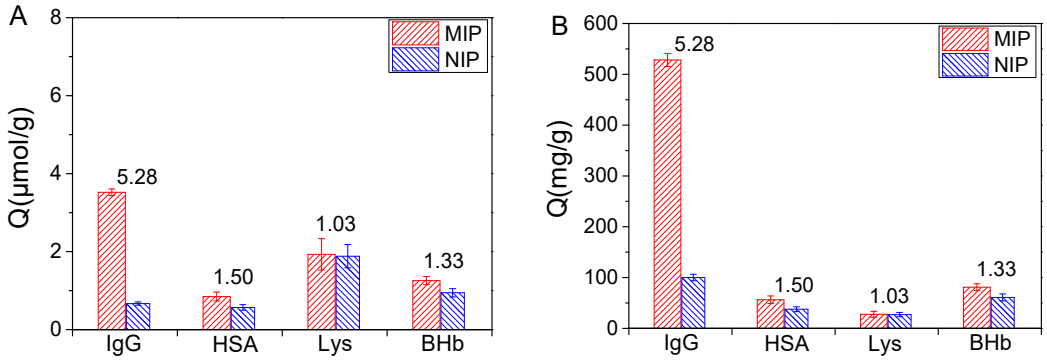

Fig. S6. (A) Adsorption of different proteins onto IgG-PLGA25-MIP1 and IgG-PLGA25-NIP1. $C_{0,\ protein}$ = 3.33 μmol/L. The binding amounts of proteins were expressed in μmol/g (A) and mg/g (B).

Table S1. Synthesis and GPC characterization of PBLG intermediates.

Sample	[M]/[I][a)]	DP [b)]	Mn[Da] [b)]	Mw/Mn [b)]
PBLG17	20/1	17	3874	1.55
PBLG25	27/1	25	5535	1.47
PBLG29	32/1	29	6437	1.39

[a)] Feeding molar ratio of monomer/initiator; [b)] Determined by GPC.

Table S2. Recipes of the IgG MIPs for the study of effect of pH, the length of the peptide crosslinker, and crosslink density.

	Crosslink density (mol%)	IgG (mg)	NIPAM (mg)	AAm (mg)	AMPS (mg)	AAPTAC (μL)
IgG-PLGA25-MIP2	2	45.0	113.0	2.8	8.3	0
IgG-PLGA17-MIP2	2	45.0	113.0	2.8	8.3	0
IgG-PLGA29-MIP2	2	45.0	113.0	2.8	8.3	0
IgG-PLGA25-MIP3	1.5	45.0	113.0	2.8	8.3	0
IgG-PLGA25-MIP4	3	45.0	113.0	2.8	8.3	0

Table S3. PLGA25-crosslinked MIPs using AAm as functional monomer.

	PLGA25 (mol%)	IgG (mg)	NIPAM (mg)	AAm (mg)
0% AAm	2	45.0	113.0	0
2% AAm	2	45.0	113.0	1.4
4% AAm	2	45.0	113.0	2.8
6% AAm	2	45.0	113.0	4.2
8% AAm	2	45.0	113.0	5.6

Table S4. PLGA25-crosslinked MIPs using AMPS as functional monomer.

	PLGA25 (mol%)	IgG (mg)	NIPAM (mg)	AMPS (mg)
0% AMPS	2	45.0	113.0	0
2% AMPS	2	45.0	113.0	4.1
4% AMPS	2	45.0	113.0	8.3
6% AMPS	2	45.0	113.0	12.4
8% AMPS	2	45.0	113.0	16.6

Table S5. PLGA25-crosslinked MIPs using SS as functional monomer.

	PLGA25 (mol%)	IgG (mg)	NIPAM (mg)	SS (mg)
0% SS	2	45.0	113.0	0
2% SS	2	45.0	113.0	4.1
4% SS	2	45.0	113.0	8.2
6% SS	2	45.0	113.0	12.4
8% SS	2	45.0	113.0	16.5

Table S6. PLGA25-crosslinked MIPs using AS as functional monomer.

	PLGA25 (mol%)	IgG (mg)	NIPAM (mg)	AS (mg)
0% AS	2	45.0	113.0	0
2% AS	2	45.0	113.0	2.9
4% AS	2	45.0	113.0	5.8
6% AS	2	45.0	113.0	8.7
8% AS	2	45.0	113.0	11.6

Table S7. Preparation formula of PLGA25-crosslinked MIP using AAPTAC as a functional monomer

	PLGA25 (mol%)	IgG (mg)	NIPAM (mg)	AAPTAC (mg)
0% AAPTAC	2	45.0	113.0	0
2% AAPTAC	2	45.0	113.0	2.9
4% AAPTAC	2	45.0	113.0	5.8
6% AAPTAC	2	45.0	113.0	8.7
8% AAPTAC	2	45.0	113.0	11.6

Table S8. PLGA25-crosslinked MIPs with two functional monomers.

	PLGA25 (mol%)	IgG (mg)	NIPAM (mg)	AAm (mg)	AMPS (mg)	SS (mg)	AS (mg)	AAPTAC (μL)
4% AAm+4% AMPS (IgG-PLGA25-MIP2)	2	45.0	113.0	2.8	8.3			
4% AAm+4% SS	2	45.0	113.0	2.8		8.2		
4% AAm+4% AS	2	45.0	113.0	2.8			5.8	
4% AAm+4% AAPTAC	2	45.0	113.0	2.8				10.0

Table S9. PLGA25-crosslinked MIPs with three functional monomers.

	PLGA25 (mol%)	IgG (mg)	NIPAM (mg)	AAm (mg)	AMPS (mg)	SS (mg)	AS (mg)	AAPTAC (μL)
4% AAm+4% AMPS+4% AAPTAC	2	45.0	113.0	2.8	8.3			10.0
4% AAm+4% AMPS+2% AAPTAC	2	45.0	113.0	2.8	8.3			5.0
4% AAm+6% AMPS+2% AAPTAC	2	45.0	113.0	2.8	12.4			5.0
4% AAm+8% AMPS+2% AAPTAC	2	45.0	113.0	2.8	16.6			5.0
4% AAm+2% AMPS+6% AAPTAC	2	45.0	113.0	2.8	4.1			15.0

Table S10. Recipes of the IgG MIPs with different feeding amount of IgG.

	PLGA25 (mol%)	IgG (mg)	NIPAM (mg)	AAm (mg)	AMPS (mg)	AAPTAC (μL)
IgG-PLGA25-MIP1	2	55.0	113.0	2.8	12.4	5.0
IgG-PLGA25-MIP5	2	45.0	113.0	2.8	12.4	5.0
IgG-PLGA25-MIP6	2	65.0	113.0	2.8	12.4	5.0

Table S11. Performance of MIPs using different polymerization times. The formula of IgG-PLGA25-MIP1 was used for the MIPs.

Polymerization time (h)	Elution Efficiency (%)	Q_{MIP} (mg/g)	Q_{NIP} (mg/g)	IF
6	98.9	327.8	90.5	3.62
12	96.2	502.2	98.8	5.08
18	95.8	520.9	99.6	5.23
24	95.5	528.3	100.1	5.28

Q_{MIP} or Q_{NIP}: rebinding capacity of IgG on MIP or NIP. $C_{0,\ IgG}$ = 0.5 mg/mL, 20 mM pH 5.0 phosphate buffer, T = 37°C.

Table S12. Optimization of the separation procedure.

Swelling Equilibrium Time (h)	Rebinding Time (h)	Elution Time (h)	Purity (%)	Separation Yield (%)
24	24	24	91	78
24	18	24	92	77
24	12	24	90	75
24	6	24	84	53
18	24	24	86	72
12	24	24	65	59
24	24	18	89	73
24	24	12	90	62