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A pH-responsive Soluble Polymer-based Homogeneous System for Fast and Highly Efficient N-glycoprotein/glycopeptide Enrichment and Identification by Mass Spectrometry

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The molecular weight and pH response of poly-(AA-co-MA), the standard curve of BCA titration of poly-(AA-co-hydrazide) copolymer, UV adsorption analysis of the pH-dependent transparency changes of poly-(AA-co-hydrazide)-asialofetuin conjugates, SDS-PAGE characterization of glycoprotein recovery of poly-(AA-co-hydrazide) based enrichment, the number of glycopeptides and non- glycopeptides identified using different methods were included in the supporting information.

Polymerization time	1h	2h	4h	10h	20h
Mn (g/mol)	15440	36330	63430	144900	214100
Mw/Mn	1.205	1.702	1.183	1.492	1.662

Table S1. Molecular weight of poly-(AA-co-MA) obtained using different polymerization time.

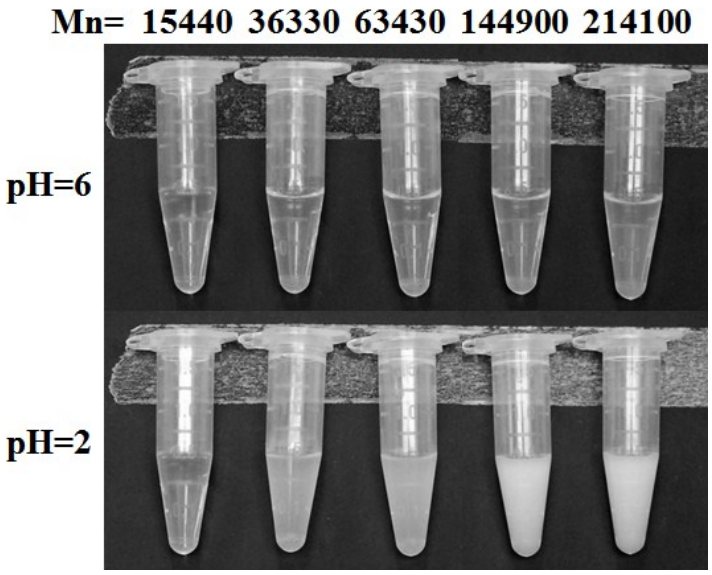

Figure S1. pH response of poly-(AA-co-MA) with molecular weights ranging from 15440 to 214100.

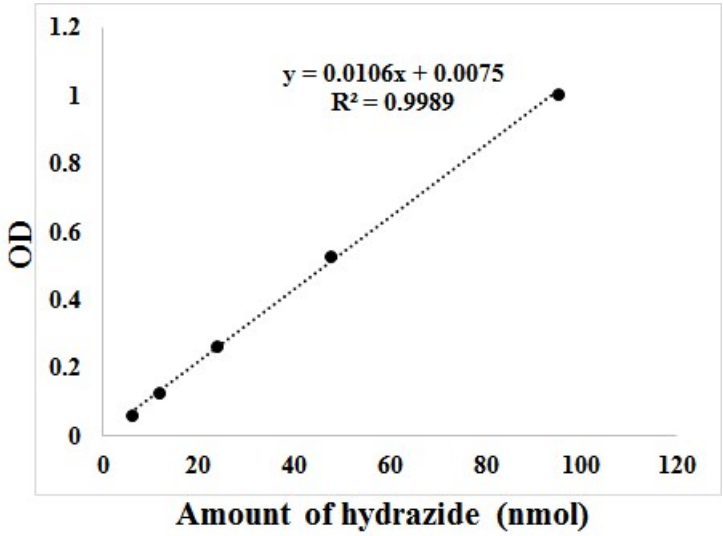

Figure S2. Standard curve of BCA titration of poly-(AA-co-hydrazide) copolymer.

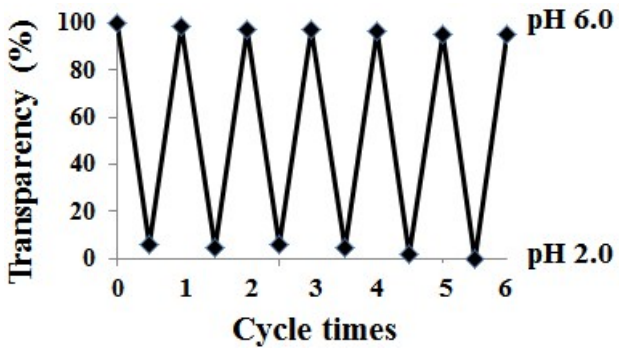

Figure S3. UV absorption analysis of the pH-dependent transparency changes of the poly-(AA-co-hydrazide)-asialofetuin conjugates. The UV absorption of the poly-(AA-co-hydrazide)-asialofetuin conjugates at pH 6.0 before the first cycle was set as 100% transparency and the UV adsorption of the conjugates at pH 2.0 in the sixth cycle was set as 0% transparency.

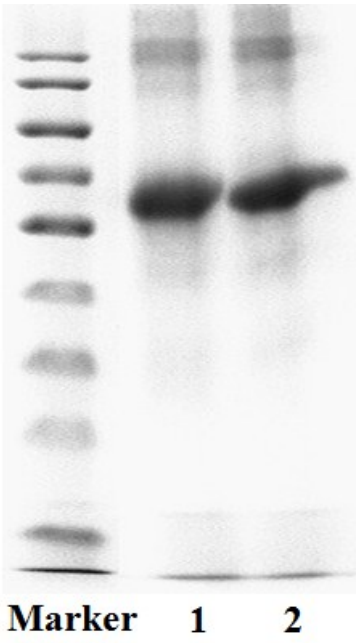

Figure S4. SDS-PAGE characterization of poly-(AA-co-hydrazide)-asialofetuin conjugates (lane 1) and free asialofetuin (lane 2) after PNGase F treatment.

Poly-(AA-co-hydrazide)				
	Test 1	Test 2	Test 3	Total non-redundant
N-glycopeptides	843	748	965	1317
Non-glycopeptides	429	372	472	741
Commercial cross-linked agarose-hydrazide beads				
	Test 1	Test 2	Test 3	Total non-redundant
N-glycopeptides	300	321	286	533
Non-glycopeptides	1128	1136	1359	2135
Non-enriched				
	Test 1	**Test 2**	**Test 3**	Total non-redundant
N-glycopeptides	29	23	26	56

Table S3. The number of identified N-glycopeptides and non-glycopeptides obtained by different methods.