Supporting Information

Synthesis of a high affinity complementary peptide-polymer nanoparticle (NP) pair using phage display

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Table S1. The composition of NPs

Entry	NIPAm	Bis	AAc	TBAm	PAA
1[a]	38 mol%	2 mol%	20 mol%	40 mol%	
2[a]	58 mol%	2 mol%		40 mol%	
3[a]	53 mol%	2 mol%	5 mol%		40 mol%

[a] 693 □M SDS was used during synthesis procedure.

Table S2 Isoelectric points of various proteins

Protein	Isoelectric point (P.I.)
Avidin	10.5
BSA	4.7
IgG	6.4-10
TNF□	6.4
Hemoglobin	6.9

Table S3. Summary of selection conditions

Selections	NPs conc. (μg/mL)	Block protein 0.2%	Incubation Time	Number of Washes
Round 1	100	BSA	1h	5
Round 2	100	Non-Fat Milk	50 min	8
Round 3	80	BSA	40 min	10
Round 4	80	ovalbumin	30 min	10

The nonspecific interaction between NPs and empty phage was evaluated before the selection. Figure S1 shows the interaction between NPs and empty phage. NPs at the concentrations of 80 and 160 μg/mL were chosen as conditions to immobilize NPs on polystyrene microtiter plate wells (Nunc, Maxisorp) by physical adsorption. Six different concentrations of empty phage from 0.049 to 12 nM were used to evaluate the interaction between empty phages and NPs. The results show that the non-specific interaction is low even at 160 μg/mL of NPs and 12 nM empty phage. Thus, NP (100 μg/mL) and phages (12 nM) were used for the first round of selection. Table S3 shows the conditions of each round of selection, which increased in stringency to select for higher affinity phage. The phage titers were calculated from the number of colonies after dilution (Figure S2). In phage display, selection of a high affinity peptide can increase phage titers in the next round of selection. Therefore, the round of selection with markedly higher titers was chosen for the spot assay. Spot assays thus examined individual selectants from the fourth rounds of selection for **NP1**(40TBAm-20AAc) and the third round for **NP3**(40PAA-5AAc).

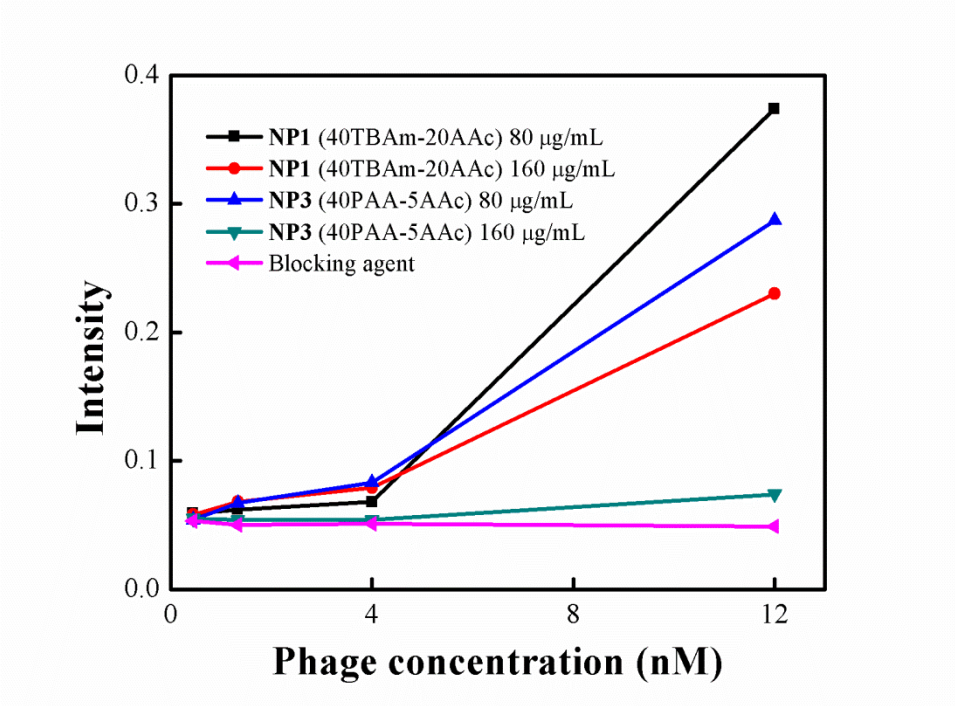

Figure S1. Investigating non-specific interaction between NPs and empty phage

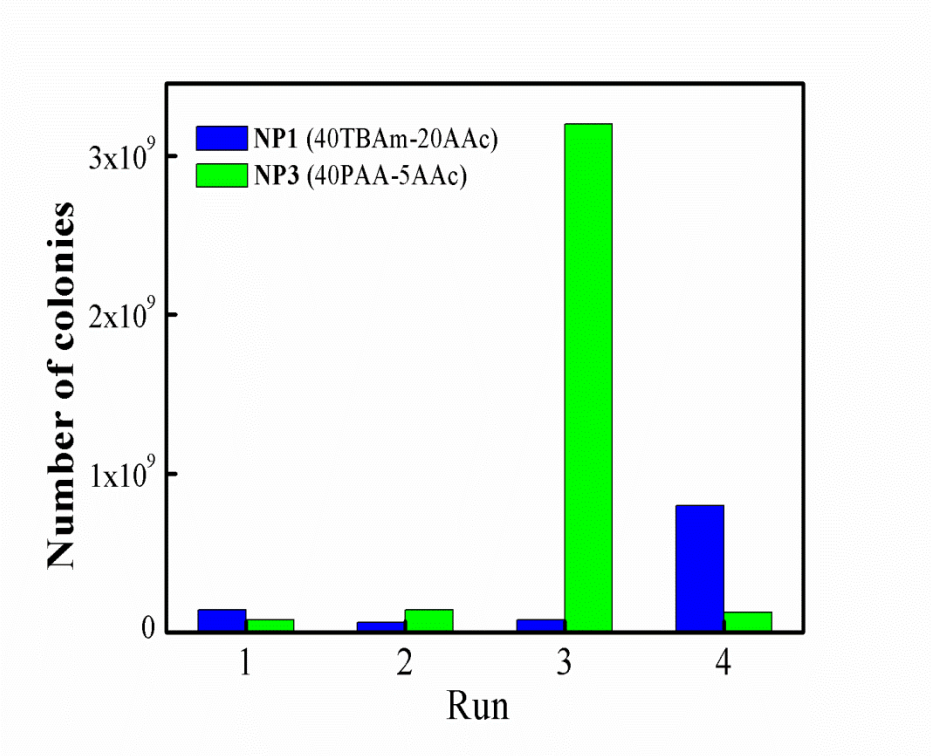

Figure S2. Phage titers after each round of selection.

Table S4. The peptide sequence of phages which have high affinity for **NP3**(20PAA-5AAc).

Phages	**Peptide Sequence**
45[a]	F R L S L C W V A W W P L C G G L L G
47[a]	L P L G L C G W V L V S P L C W I T W L
52[a]	L A V W A S A G W V L V S P L C W I T W L

[a] Cyclic form

Ninety single colonies were selected and evaluated for affinity and specificity to **NP1**(40TBAm-20AAc). Figure S3 represents the affinity between phages and **NP1** (40TBAm-20AAc). Five spots with the highest intensity (spot 14, 20, 22, 26 and 80) were selected and submitted to Sanger DNA sequencing. Table 1 summarizes the peptide sequences of phage with high affinity for **NP1**(40TBAm-20AAc).

For **NP3**(40PAA-5AAc), 58 single colonies were selected, and assessed for their affinity and specificity. Figure S4 depicts relative binding to this NP by individual selectants from the phage-displayed library. Six colonies with the highest relative signals (spots 7, 12, 42, 45, 47 and 52) were selected and submitted to Sanger DNA sequencing. However, phages 7, 12 and 42 did not result in a sequence. Table S4 shows the peptide sequence of the phages which have high affinity with **NP3**(40PAA-5AAc). Positively or negatively charged, hydrophobic and hydrophilic residues are printed in blue, purple, red and green, respectively. Phages 45, 47 and 52 are very hydrophobic, reflecting selection to bind to 40% *N*-phenylacrylamide NPs. Due to potential solubility issues, these sequences were eliminated from further consideration. Therefore, **NP1**(TBAMm20AAc) were chosen for further investigations.

Table S5. Library of Peptides on Phages.

Library Design	Number of Amino Acids	Theoretical Peptide Diversity	Actual Peptide Diversity
CX_5C	7	3.2×10^6	3.2×10^6
CX_5CX	8	6.4×10^7	6.4×10^7
$X_2CX_2CX_2$	8	6.4×10^7	6.4×10^7
X_8	8	2.6×10^{10}	9.4×10^8
XCX_5C	8	6.4×10^7	6.4×10^7
CX_5CX_2	9	1.3×10^9	4.0×10^8
$X_2CX_2CX_3$	9	1.3×10^9	5.2×10^8
$X_2CX_3CX_2$	9	1.3×10^9	8.0×10^8
X_2CX_5C	9	1.3×10^9	9.6×10^8
$X_2CX_4CX_2$	10	2.6×10^{10}	5.3×10^8
$X_2CX_5CX_2$	11	5.1×10^{11}	4.9×10^9
$X_2CX_6CX_2$	12	1.0×10^{13}	7.3×10^8
$X_2CX_7CX_2$	13	2.0×10^{14}	8.0×10^8
$X_2CX_8CX_2$	14	4.1×10^{15}	8.8×10^8
$X_2CX_9CX_2$	15	8.2×10^{16}	7.7×10^8
$X_2CX_{10}CX_2$	16	1.6×10^{18}	3.2×10^8
$X_4CX_2GPX_4CX_4$	18	1.6×10^{18}	9.1×10^8
$X_4CX_{10}CX_4$	20	2.6×10^{23}	6.0×10^8
$X_5CX_8CX_5$	20	2.6×10^{23}	8.8×10^8
$X_5CX_9CX_4$	20	2.6×10^{23}	5.3×10^8
$X_6CX_6CX_6$	20	2.6×10^{23}	1.1×10^9
$X_6CX_7CX_5$	20	2.6×10^{23}	5.0×10^9
$X_7CX_4CX_7$	20	2.6×10^{23}	9.4×10^8
$X_7CX_5CX_6$	20	2.6×10^{23}	2.0×10^9
Total Diversity		1.8×10^{24}	2.5×10^{10}

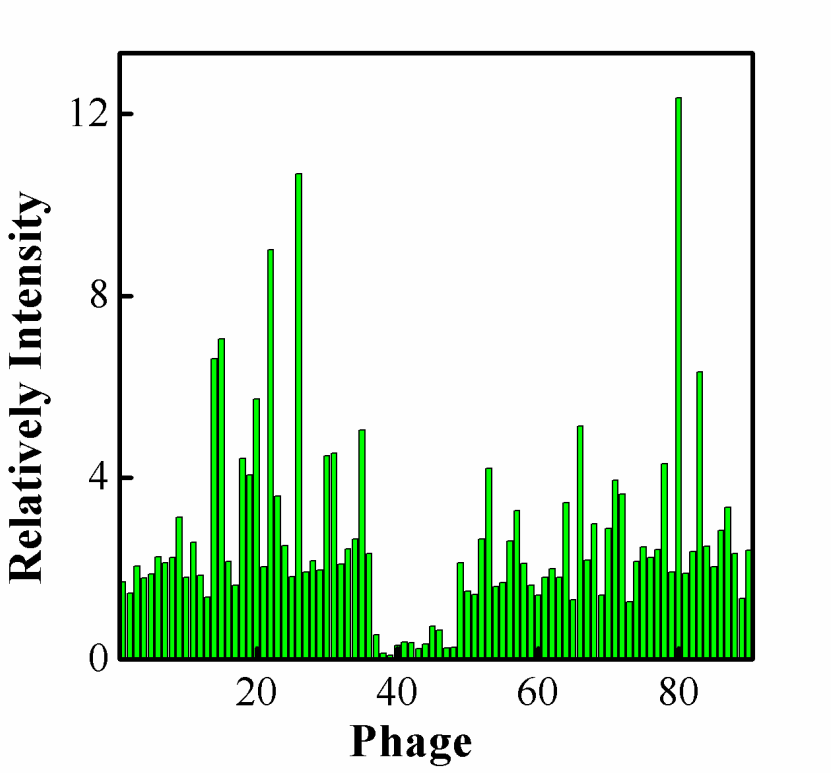

Figure S3. Spot assay for the interaction between **NP1**(40TBAM-20AAc) and phage selectants (colony numbers are indicated on the X-axis).

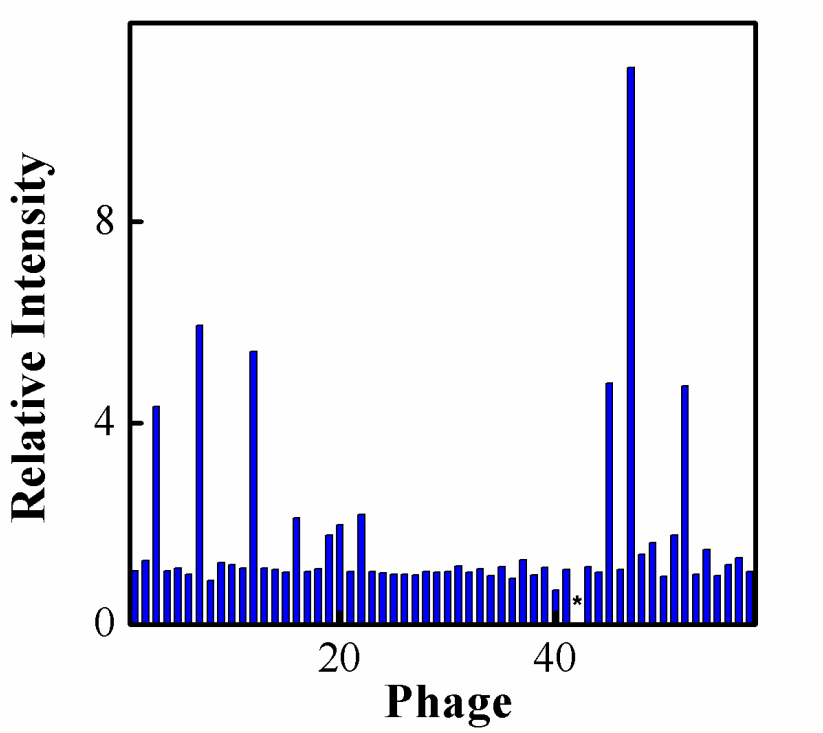

Figure S4. The interaction between **NP3**(20PAA-5AAc) and the phages. *The signal is too high over the instrument detection limit.

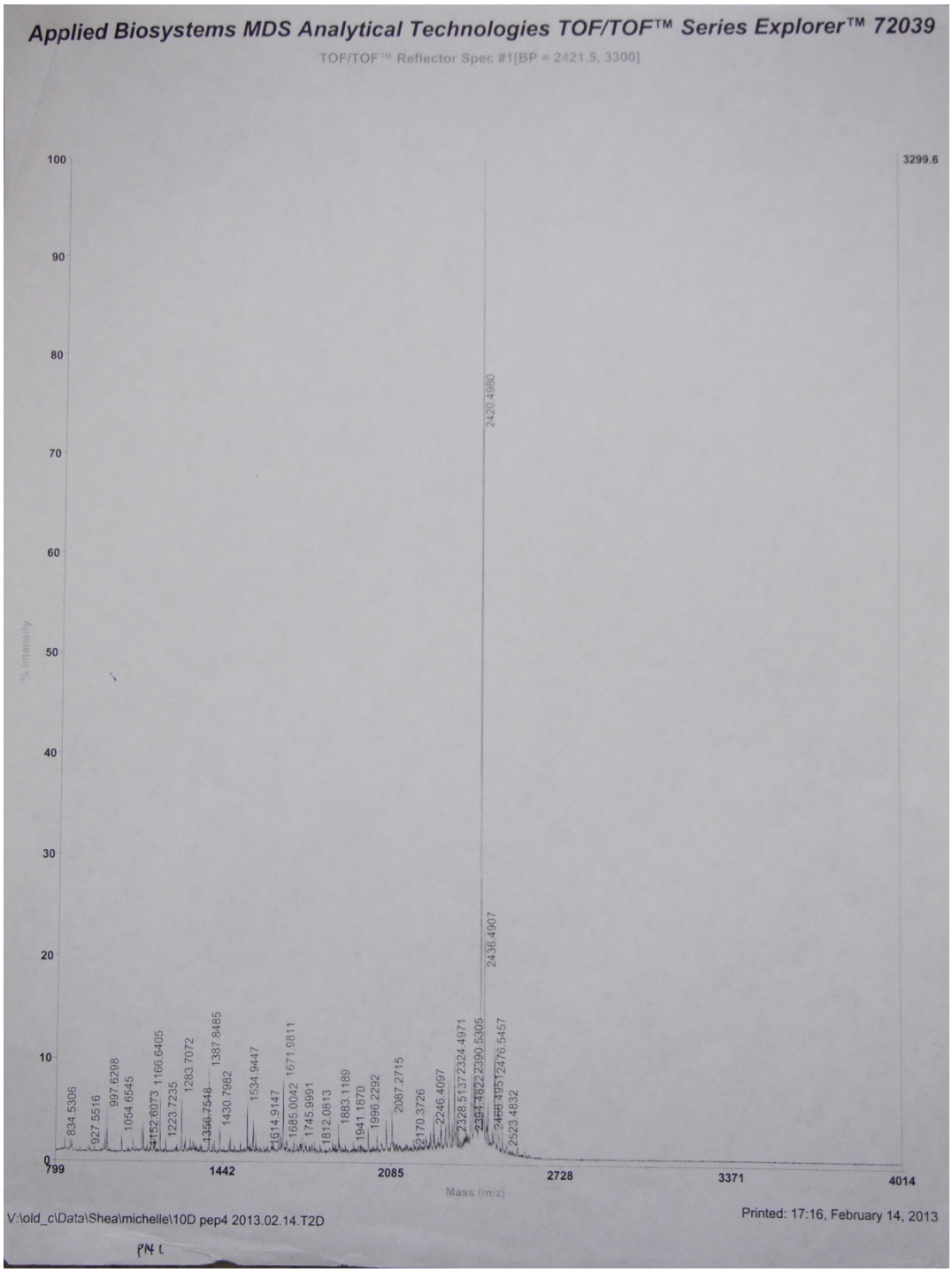

Figure S5. MALDI-TOF-MS spectrum of **P14** dissolved in the solution containing 50% acetonitrile in water

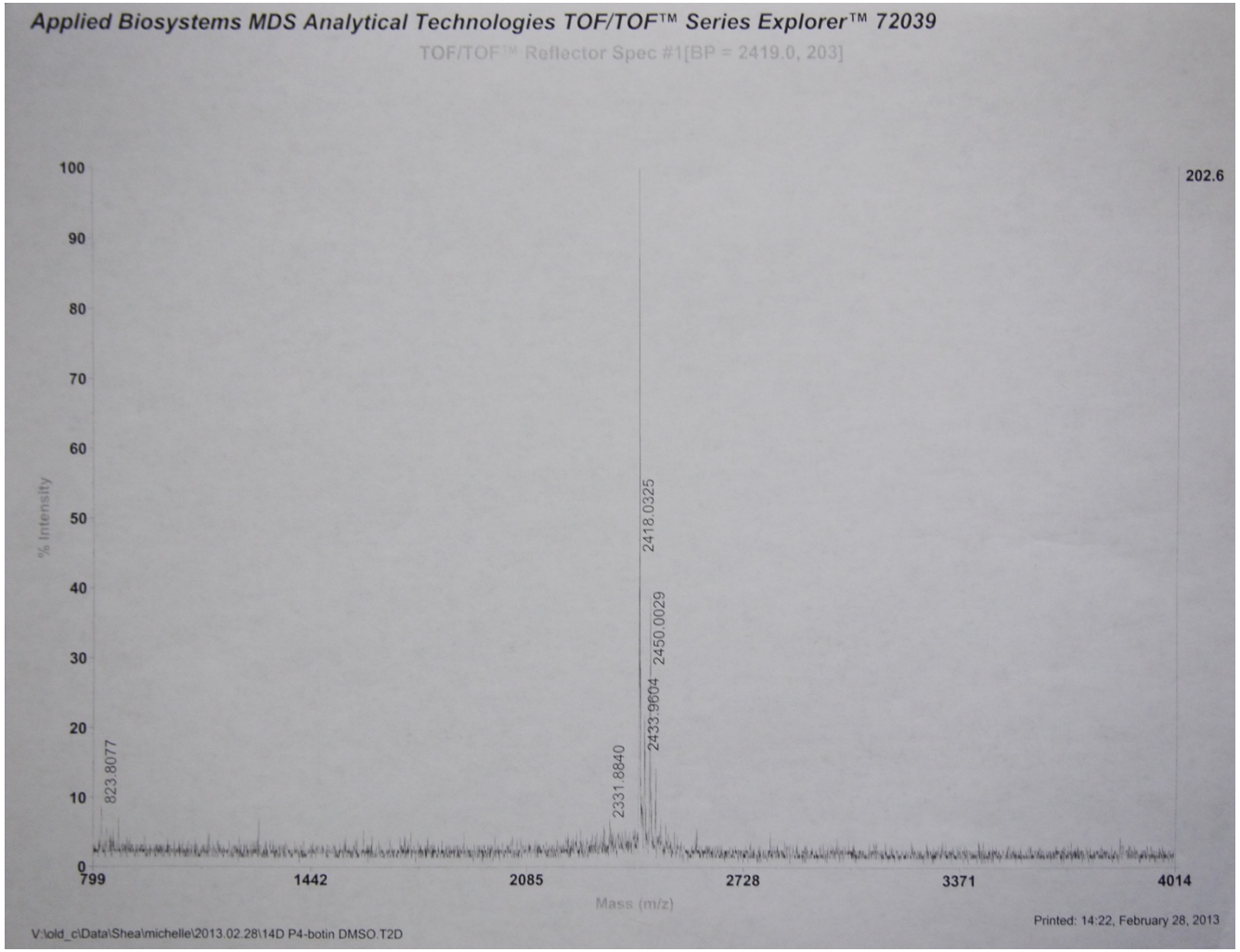

Figure S6. MALDI-TOF-MS spectrum of **P14** dissolved in a solution of 20% DMSO in 10 mM HEPES

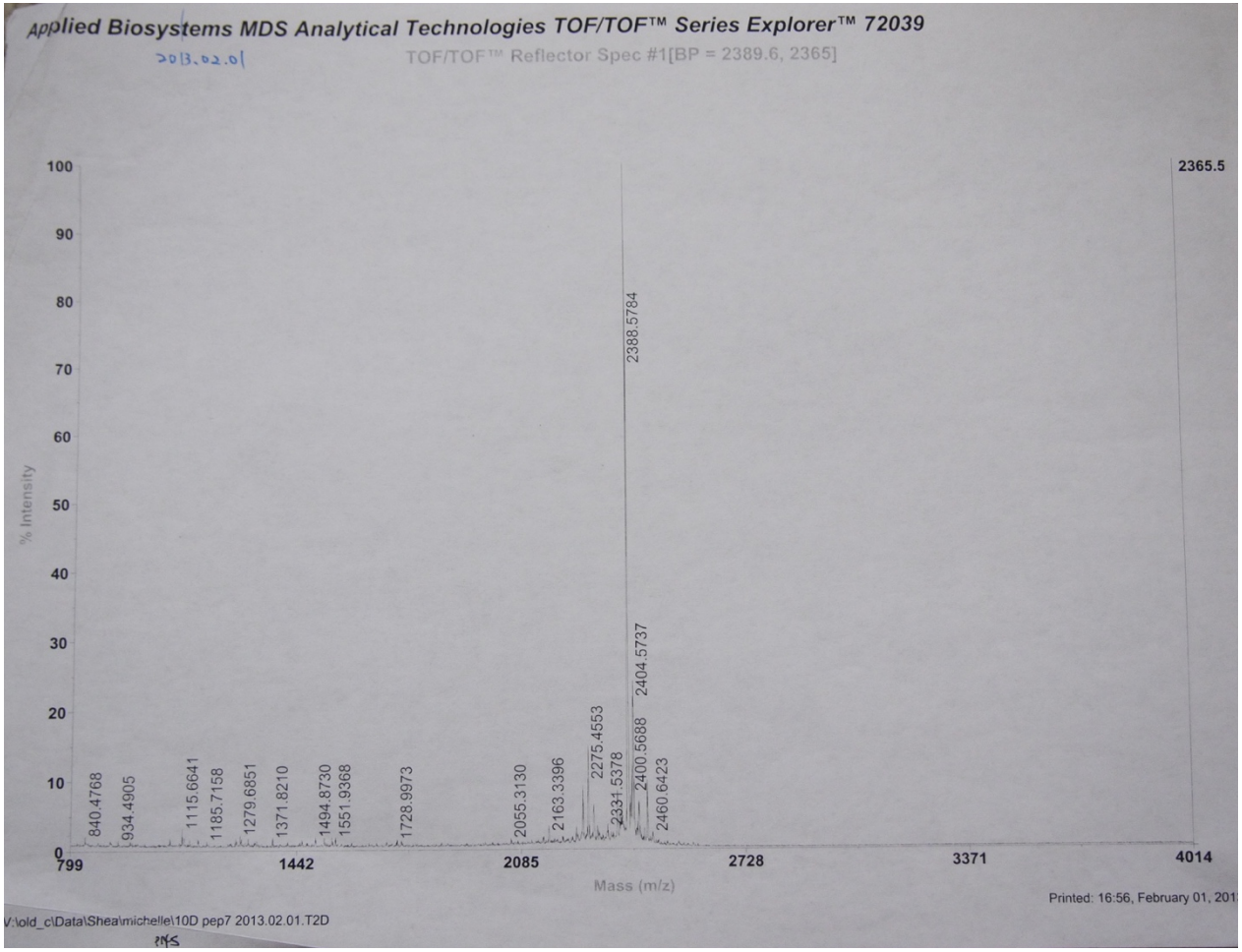

Figure S7 MALDI-TOF-MS spectrum of **P14S** dissolved in the solution containing 50% acetonitrile in water

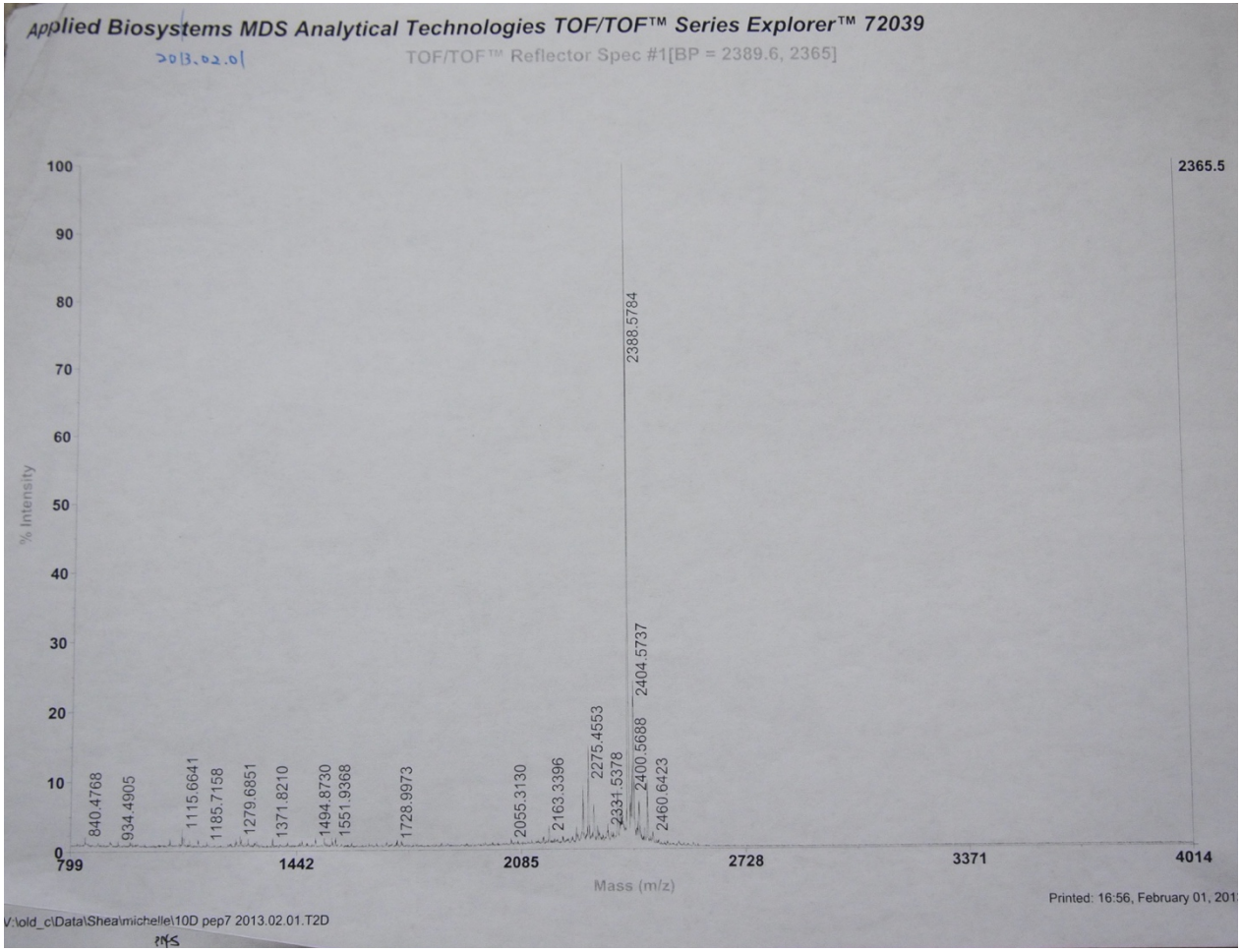

Figure S8. MALDI-TOF-MS spectrum of **P14M** dissolved in a solution of 50% acetonitrile in water

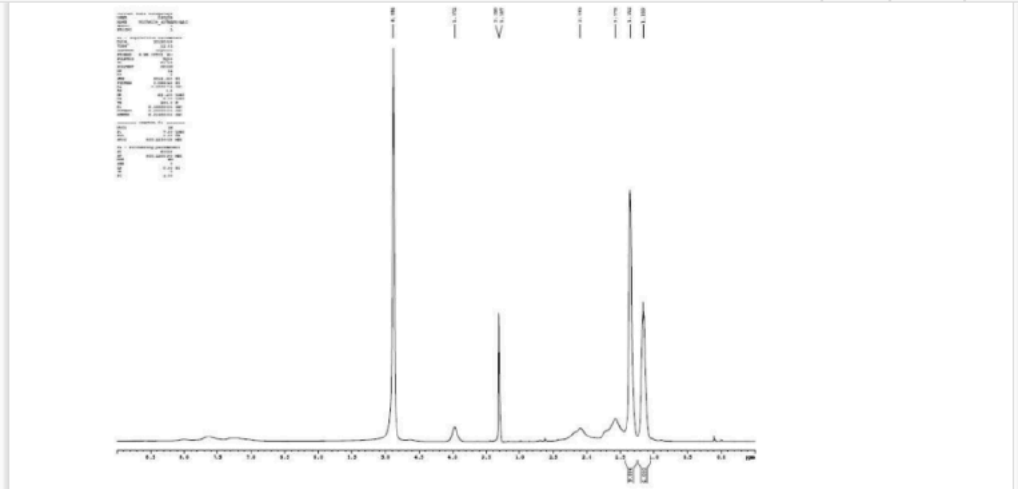

Figure S10. ^{1}H NMR spectra of NP7

Figure S9. ^{1}H NMR of NP1(40TBAm-20AAc).