Supplementary Information for

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Well-defined nanostructured surface-imprinted polymers for highly selective enrichment of low-abundance protein in mammalian cell extract

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1. Synthetic route of ARPCs

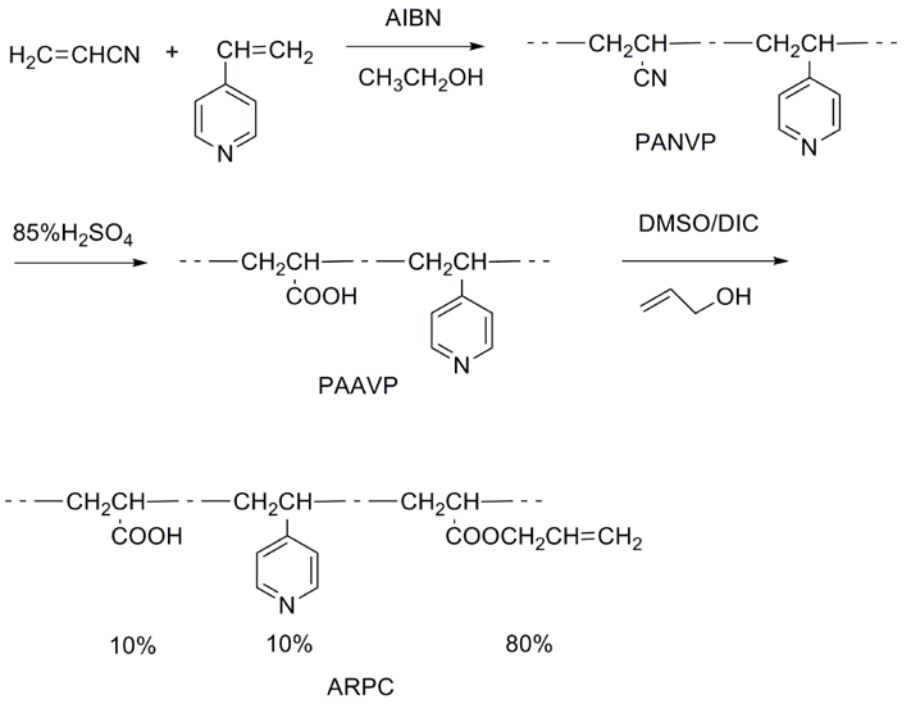

Fig. S1. Synthetic route of ARPCs

2. ^{1}H NMR spectra of ARPCs

The polymer products PANVP, PAAVP, and the ARPCs were analyzed in DMSO, using ^{1}H NMR with a UNITY-plus-400 NMR instrument (Fig. S2). The spectrum for PANVP showed peaks at 2.04 and 3.14 ppm, which were attributed to the hydrogen in methylene, and methane-adjoining acrylonitrile. Additional peaks at 7.28 and 8.60 ppm were attributed to the hydrogen in the pyridine ring. In the spectrum for PAAVP, the peak at 10.26 ppm was attributed to the hydrogen in carboxyl groups. Compared the two spectra, the carboxyl group can form intra-molecular and intermolecular hydrogen bonds. The broadening of the peaks in the range from 2.13 ppm to 1.14 ppm was therefore attributed to hydrogen in the methylene groups in the backbone of the polymer. The spectrum for the ARPCs showed a peak at 3.36 ppm, which was attributed to hydrogen in the methylene-adjoining ester group, and peaks at 5.09, 5.22, and 5.89 ppm, which were attributed to hydrogen in the alkenes.

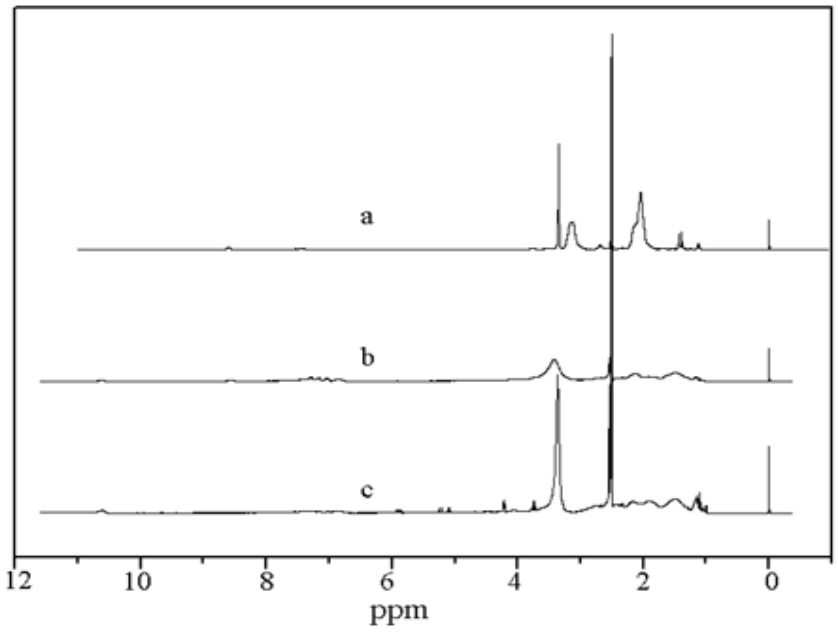

Fig. S2. ^{1}H NMR spectra of PANVP (a), PAAVP (b) and ARPCs (c).

3. FT-IR spectra of ARPCs

FT-IR spectra were recorded for PANVP, PAAVP, and the ARPCs with a Bio-Rad FTS 6000 spectrometer, using KBr pellets (Fig. S3). The spectrum of PANVP showed a narrow band at 2242.76 cm^{-1}, which was attributed to the stretching vibrations of CN. In the spectrum for PAAVP, the wide band at about 3181.7 cm^{-1} was attributed to the stretching vibrations of O–H. This showed that the nitrile groups completely transformed to carboxyl groups. In the spectrum for the ARPCs, the wide band at about 3194.6 cm^{-1} corresponded to the stretching vibrations of O–H, because some carboxyl groups still existed, and the band at 3089.96 cm^{-1} (attributed to the stretching vibrations of C–H) showed that carboxyl groups were partly substituted by ester groups.

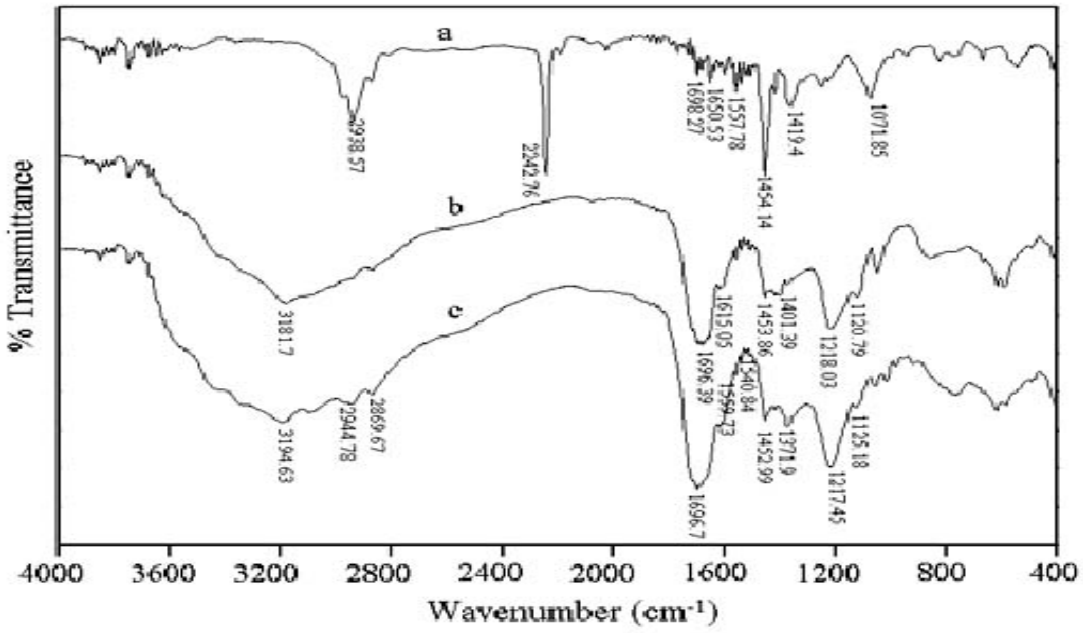

Fig. S3. FT-IR spectra of PANVP (a), PAAVP (b) and ARPCs (c).

4. GPC analysis of the synthesized PANVP

The backbone of the assistant recognition chains was analyzed using a Waters 410 GPC system; as determined from Fig. S4, Mn ¼ = 1999, and Mw/Mn¼ = 1.43. These parameters met the required specifications for the ARPCs.

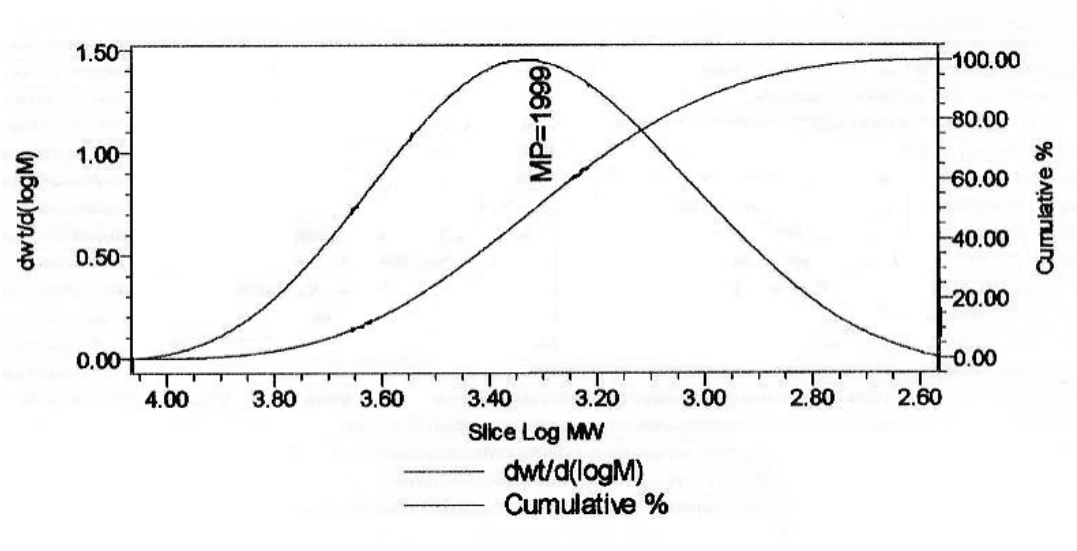

Fig. S4. GPC analysis of synthesized PANVP.

5. Optimization of eluting buffers

In this work, we used the standard solutions of the pure cloned target protein for investigating the eluting buffer. After absorption, the MIP/NIP were washed with different eluting buffers containing100 mM KCl, 150mM KCl, 500mM KCl and 2M KCl for three times, respectively. As shown in Fig. S5, the protein in lane 1-6 was collected with low-concentration-salt buffer (100 mM and 150mM KCl), which were nonspecific absorbed protein, and the specific adsorbed protein in lanes 7-9 was collected with high-concentration-salt buffer (500 mM KCl). When using 2 M KCl buffer to wash the MIP/NIP, there was no protein eluted in the eluting buffer. According to the result, we used the synthesized MIP and NIP to adsorb the protein mixture. Hence, the phosphate buffer solution containing100 mM KCl, 150mM KCl

and 500mM KCl was employed in the following studies.

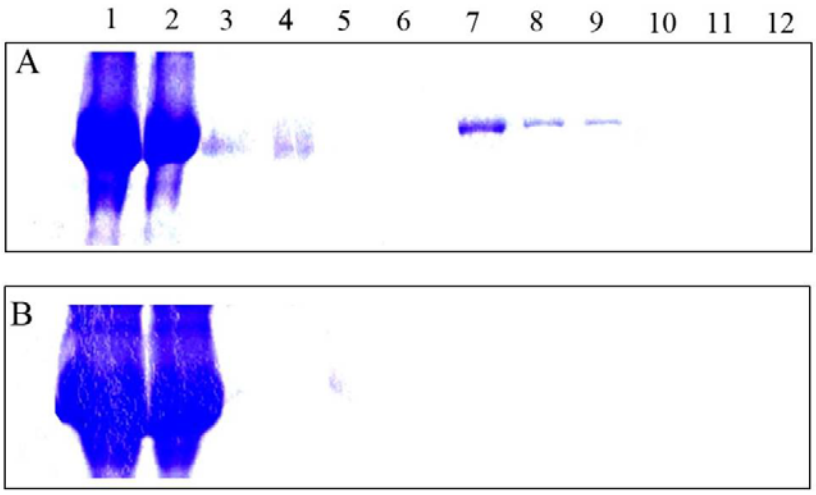

Fig. S5. Gel electrophoretic analysis of elution buffer using MIP (A) and NIP (B). Concentration of KCl in elution buffer: lanes 1-3, 100 mM; lanes 4-6, 150 mM; lanes 7-9, 500 mM; lane 10-12, 2 M.