Supporting information

Probing Protein–Protein Interactions with Label-Free Mass Spectrometry Quantification in Combination with Affinity Purification by Spin-Tip Affinity Column

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Supplementary methods:

Expression and Purification of Recombinant His_6-tagged proteins

The DNA fragments of NDP52 (10-126), NAP1 (33-75), FYCO1 (882-1231) and FYCO1 (1325-1478) were amplified by PCR from the full-length human cDNA, respectively. All these fragments were cloned into the pET-32M vector (a modified version of pET32a vector containing a His_6-tag) for recombinant protein expressions. Recombinant proteins were expressed in BL21 (DE3) E. coli cells induced by 100 μM IPTG at 16 °C. The bacterial cell pellets were re-suspended in the binding buffer (50 mM Tris, 500 mM NaCl, 5 mM imidazole at pH 7.9), and then lysed by the FB-110XNANO homogenizer machine. Then the lysis was centrifuged at 35000 g for 30 min to remove the debris. His_6-tagged proteins were purified by Ni^{2+}-NTA agarose (GE Healthcare) affinity column. Each recombinant protein was further purified by size-exclusion chromatography or mono-Q ion-exchange chromatography. The N-terminal tag of recombinant protein was cleaved by 3C protease and further removed by size-exclusion chromatography. And all the recombinant proteins were stored at -80 °C before use.

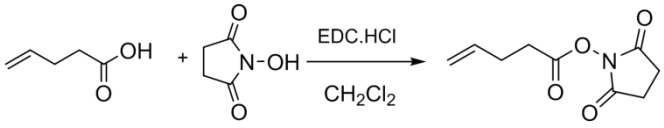
HK293T cell culture and lysis

HEK293T cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Invitrogen) supplemented with 10% fetal bovine serum (FBS, Invitrogen) and incubated at 37 °C in 5% CO_2. HEK293T cells were harvested, washed with PBS buffer and lysed in lysis buffer containing 50 mM Tris (pH 7.4), 150 mM NaCl, 5% (v/v) glycerol, 1% (v/v) NP-40, 1.5 mM $MgCl_2$, 5 mM NaF, 2 mM Na_3VO_4 and protease

inhibitors (EDTA-free, Roche) at 4°C for 1 hour. Cell Lysates were centrifuged at 12000 g for 10 min at 4 °C, and the supernatants were collected for experiments. The total protein concentration of the lysates was determined by BCA protein assay kit. Lysates were immediately used.

Preparation of the functional monomer 4-Pentenoic acid succinimidyl ester (PAS)

The function monomer 4-pentenoic acid succinimidyl ester (PAS) was synthesized by the following method. N-hydroxysuccinimide (3.1498 g, 27.4 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (4.83 g, 25.2 mmol) were added to a solution of 4-pentenoic acid (2.18 mL, 22.2 mmol) in dry dichloromethane (120 mL). The reaction solution was concentrated to dryness under vacuum evaporation after stirring 10 hours at room temperature and then purified by column chromatography (4:1 petroleum ether-ethyl acetate) to give 3.8 g PAS (Yield: 87%). 1H NMR (500MHz, $CDCl_3$) δ 5.90-5.82 (ddt, 1H), 5.14 (dd, 1H), 5.09 (dd, 1H), 2.84 (m, 4H), 2.72 (t, 2H), 2.50 (dt, 2H); MS: calc. M^+=197, observed. $(M+H)^+$=198.



4-PA NHS PAS

Table S1. Optimization of the composition of the polymerization solution for the preparation of m-IMAC columns.

The proportion of PAS	PAS	HEMA	PETA	DMSO	DMF	1-Dodecanol
0.4%	0.1	11.4	14	28	5	41.5
2%	0.5	11	14	28	5	41.5
4%	1	10.5	14	28	5	41.5
12%	3	8.5	14	28	5	41.5

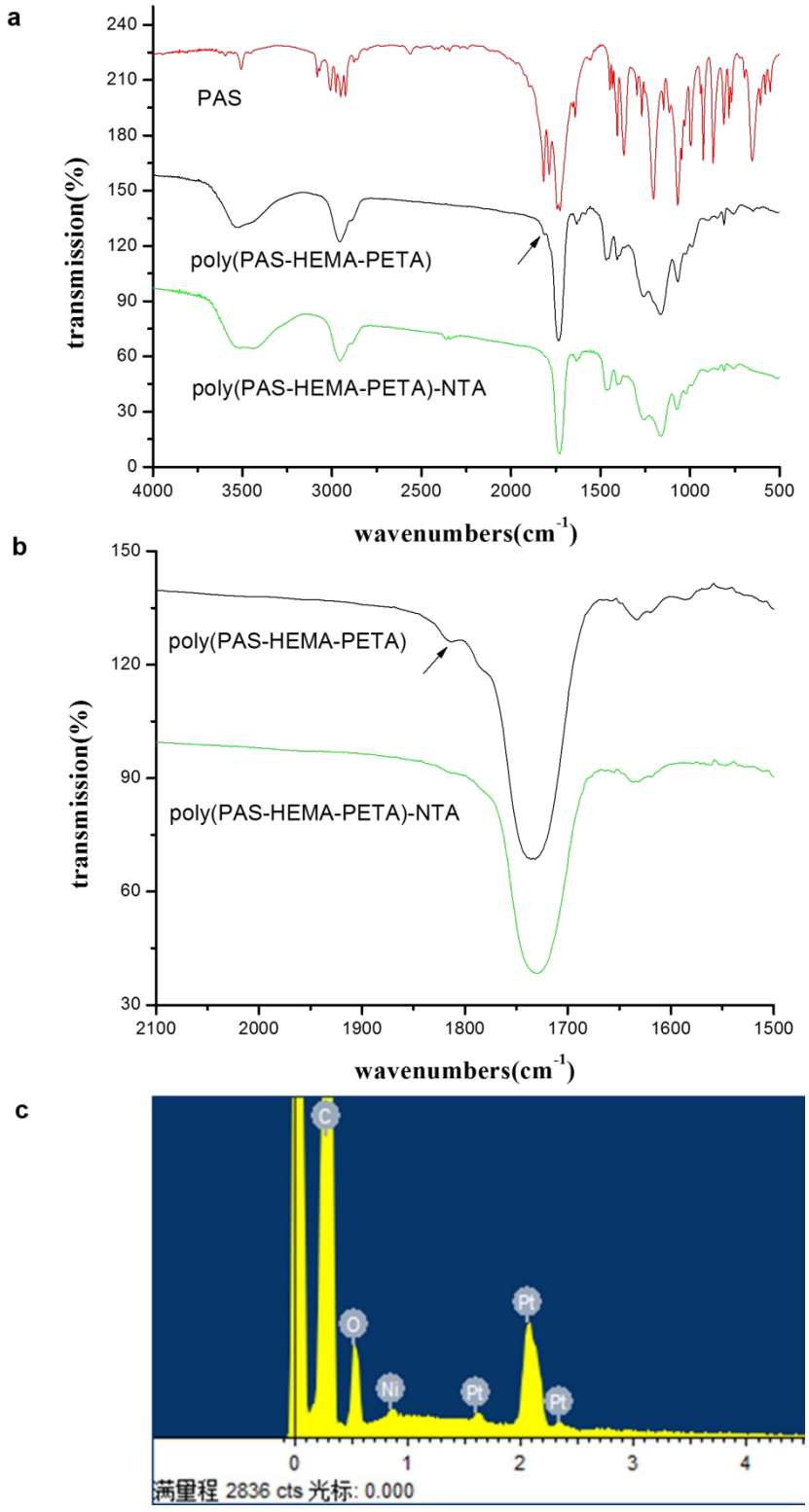


Figure S1. IR spectra demonstrating the functionalization of the m-IMAC Spin-Tip monolithic column (a, b). The EDS spectra of the monolithic column with 4%wt PAS demonstrating the successful loading of the Ni^{2+}(c).

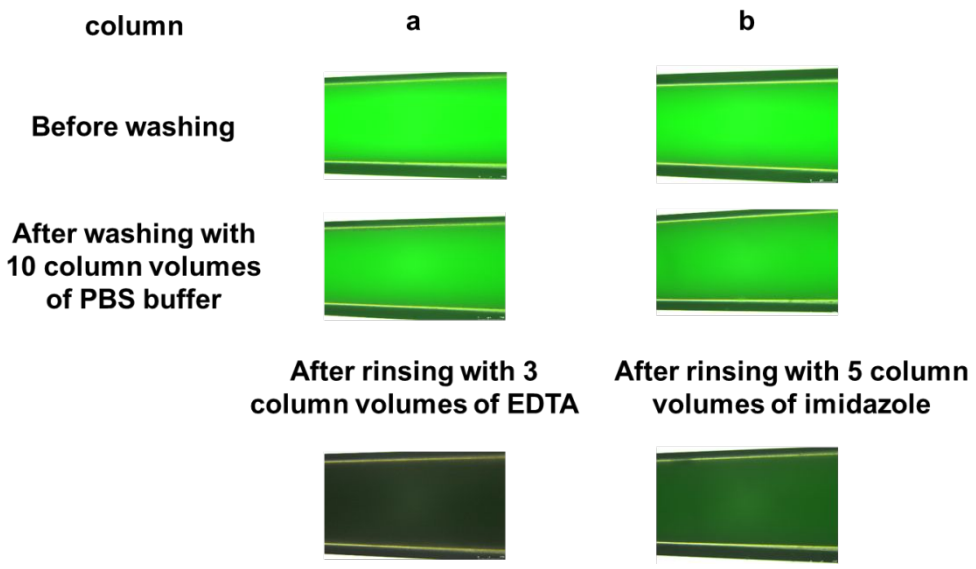


Figure S2. Fluorescence images of the m-IMAC columns demonstrate that the His_6-tagged GFP can be immobilized on the column in the IMAC manner. (a) rinsing the column with 3 column volumes of EDTA (100 mM), (b) rinsing the column with 5 column volumes of imidazole (500 mM).

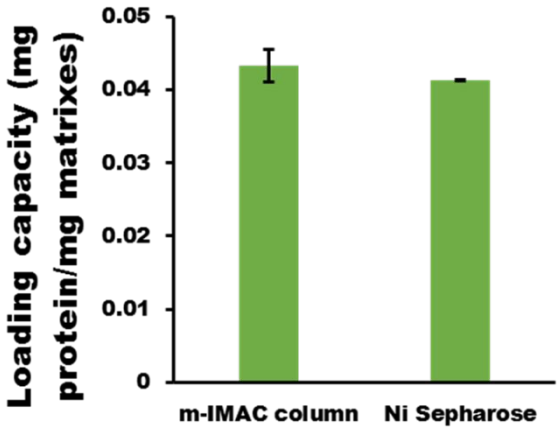


Figure S3. Comparison of the loading capacity (mg His_6-tagged GFP/mg matrix) between our monolithic affinity matrix and the commercially available Ni Sepharose.

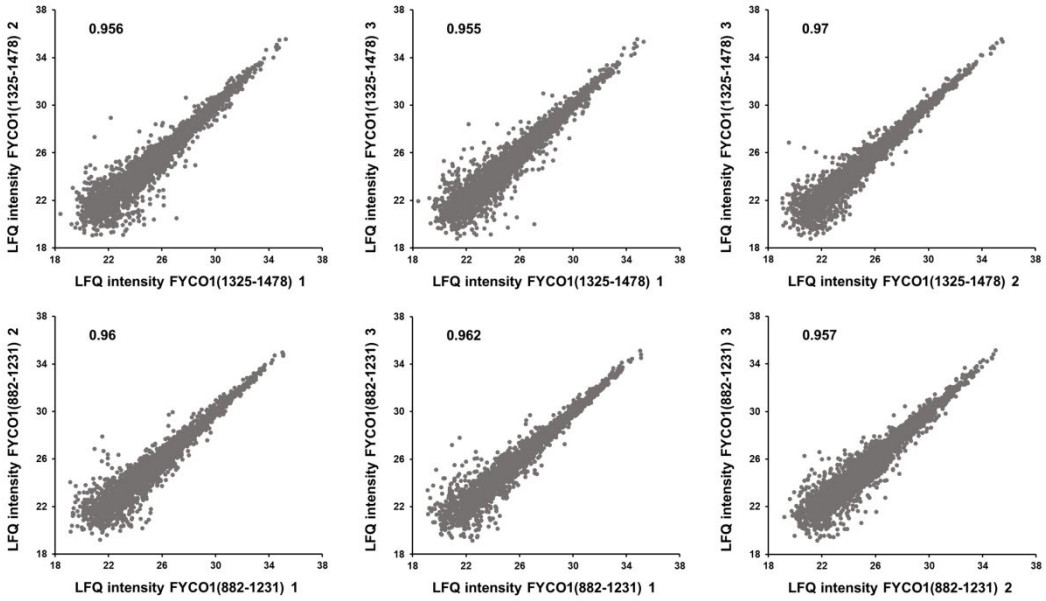


Figure S4. Pearson correlation plots demonstrating the high repeatability mass spectra data between the aliquot samples.

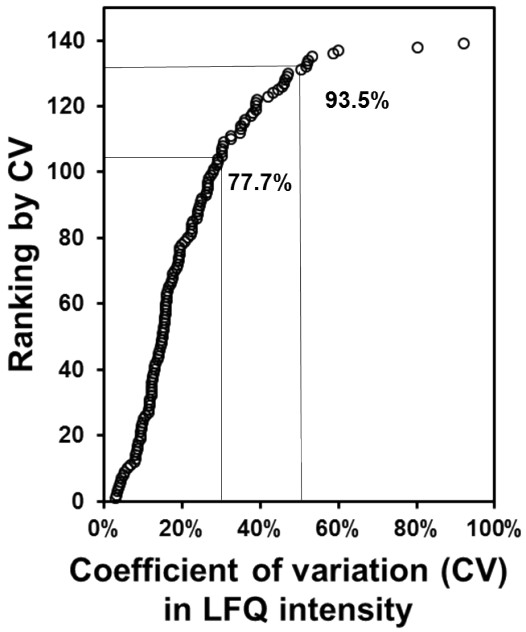


Figure S5. The CV values of the LFQ intensity of the 139 potential interacting proteins of the FYCO1 GOLD domain (amino acids 1325-1478).