

Ligand-Protein Target Screening from Cell Matrices Using Reactive Desorption Electrospray Ionization-Mass Spectrometry via a Native Denatured Exchange Approach

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Supplemental Methods

Calculation of ligand-target protein binding metrics

The holo/apo ratio is defined as the relative abundance of the bound versus unbound proteins. Consequently, we calculate this metric by dividing the summed intensities of the complex ions at all charge states (denoted by [PL]) by the sum of the intensities of unbound protein ions (denoted by [P]) of all charge states based on the deconvoluted spectra.

$$R = \frac{[PL]_{eq}}{[P]_{eq}} = \frac{\sum_n Ab(PL^{n+})}{\sum_n Ab(P^{n+})}$$

Subsequently, K_d (dissociation constant) is further acquired by a direct ESI-MS deduced by the Klassen group using the equation shown below. $[P]_0$ and $[L]_0$ refer to the initial concentration of protein and ligand prepared as solution

$$K_a = \frac{1}{K_d} = \frac{R}{[L]_0 - \frac{R}{1+R}[P]_0}$$

Table S1. Holo/apo ratios and the corresponding K_d values calculated based on a direct ESI-MS model for the complexes formed between RNase A and nine ligand candidates.

	CTP	CDP	CMP	ADP	AMP	UMP	ChA	Cytidine	Dox
Holo/apo	1.317	0.343	0.157	0.790	0.283	0.050	0.724	0.000	0.000
$K_d/\mu\text{M}$	21.740	101.726	237.491	39.460	125.754	780.952	43.648	∞	∞

$[P]_0 = 20 \mu\text{M}$, $[L]_0 = 40 \mu\text{M}$.

Table S2. The values of K_d measured for the complexes formed between target proteins and candidate ligands by MST. The original data is partially recorded in ref[13].

Complexes	K_d
Ubiquitin-NAG3	NA (no binding)
Ubiquitin-CDP	NA (no binding)
RNase A-NAG3	NA (no binding)
Lysozyme-NAG3	$28.50 \pm 5.37 \mu\text{M}$
RNase A-CTP	$0.52 \pm 0.02 \mu\text{M}$

Figure S1. Total ion chromatogram (TIC) of (A) pure lysozyme, (B) lysozyme spiked in HCT116 cell lysate, (C) RNase A and (D) RNase A spiked in cell lysate separated by size exclusion chromatography (SEC). The final concentration of spiked target protein in each fraction is estimated as 20 μ M.

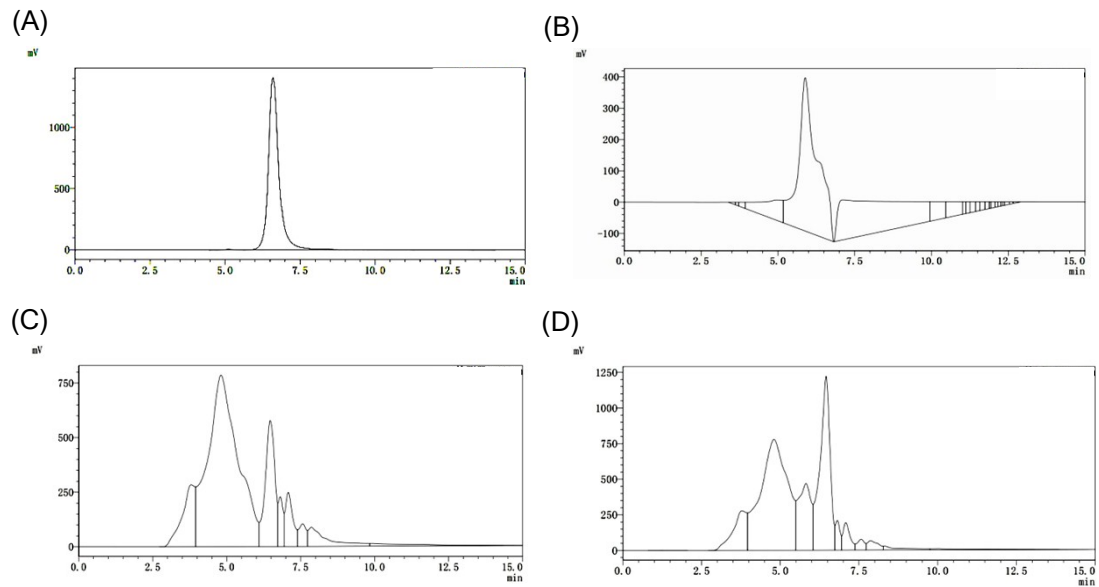


Figure S2. MS spectra of formed protein complex of (A) lysozyme-ligand mix; and (B) lysozyme-ligand mix, detected under various DESI spray solvent compositions.

