Electronic Supporting Information

for

Selectivity of Arsenite Interaction with Zinc Finger Proteins

Linhong Zhao, Siming Chen, Liangyuan Jia, Shi Shu, Pingping Zhu and Yangzhong Liu

Content

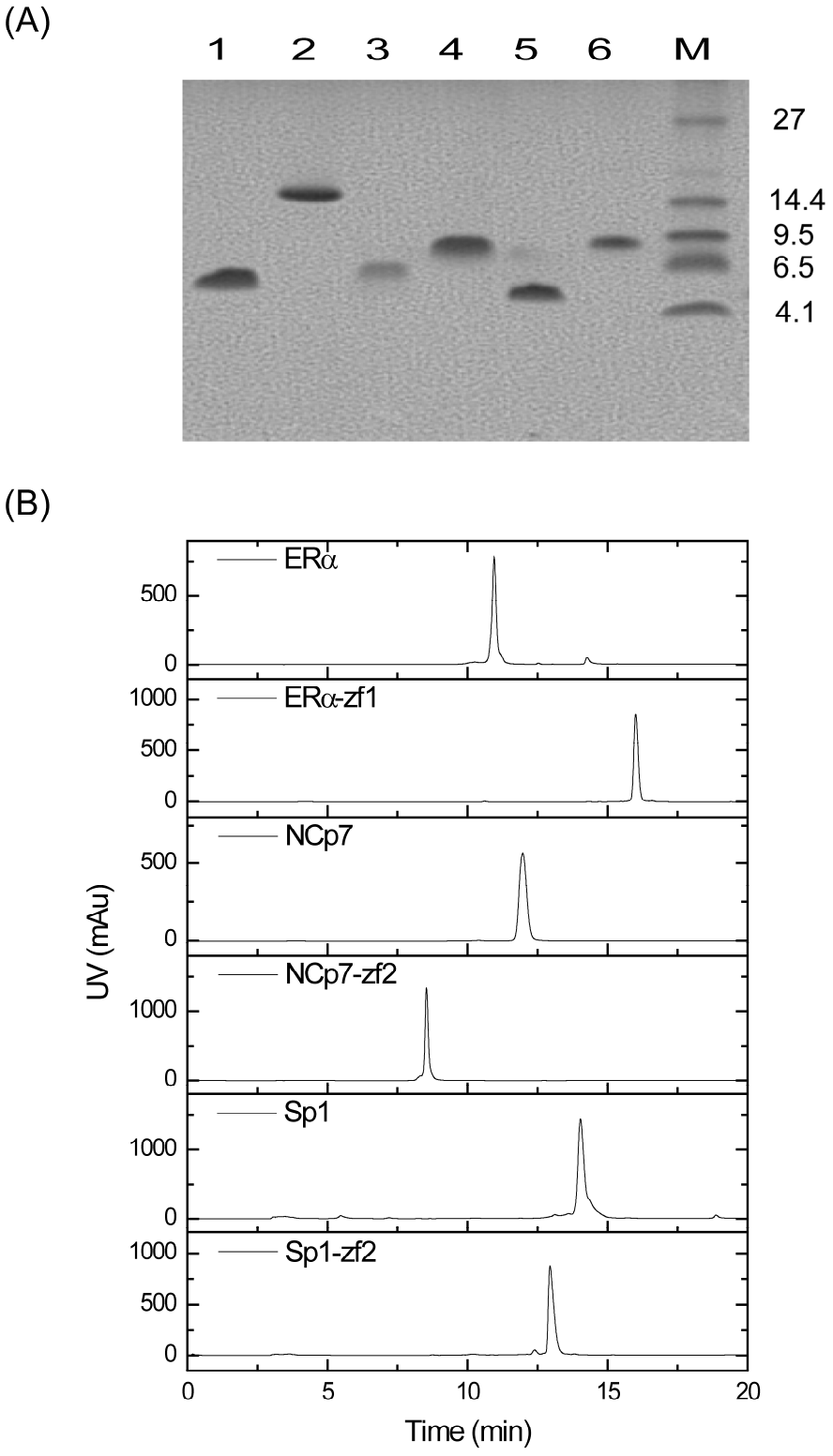

Figure S1. Characterization of the purity of zinc finger proteins. (A) Tricine-SDS-PAGE. Lanes: 1. Sp1-zf2; 2. Sp1; 3. NCp7-zf2; 4. NCp7; 5. ERα-zf1; 6. ERα. 50μM protein samples were loaded in 20 mM phosphate buffer (pH 7.0) containing 1 mM TCEP. (B) HPLC profiles of zinc-finger proteins. HPLC was run on A reverse phase Jupiter C18 column (5μm, 300A) at a flow rate of 1mL/min.

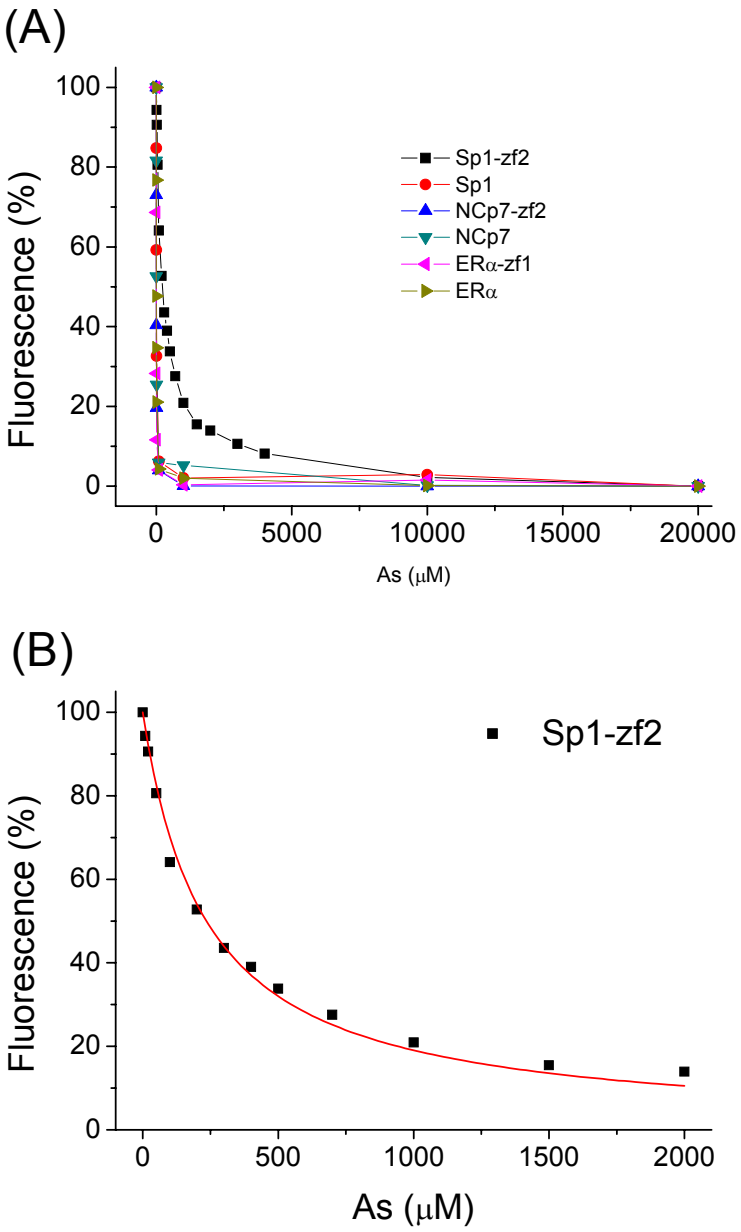

Figure S2. (A) Fluorescence of zinc finger proteins titrated by arsenite from 0 to 20 mM. (B) A representative fitting result from the titration of Sp1-zf1. The curve was obtained by nonlinear least-squares fitting of the titration data (■) with the equation $Y= 100*K_d/(K_d+X)$.

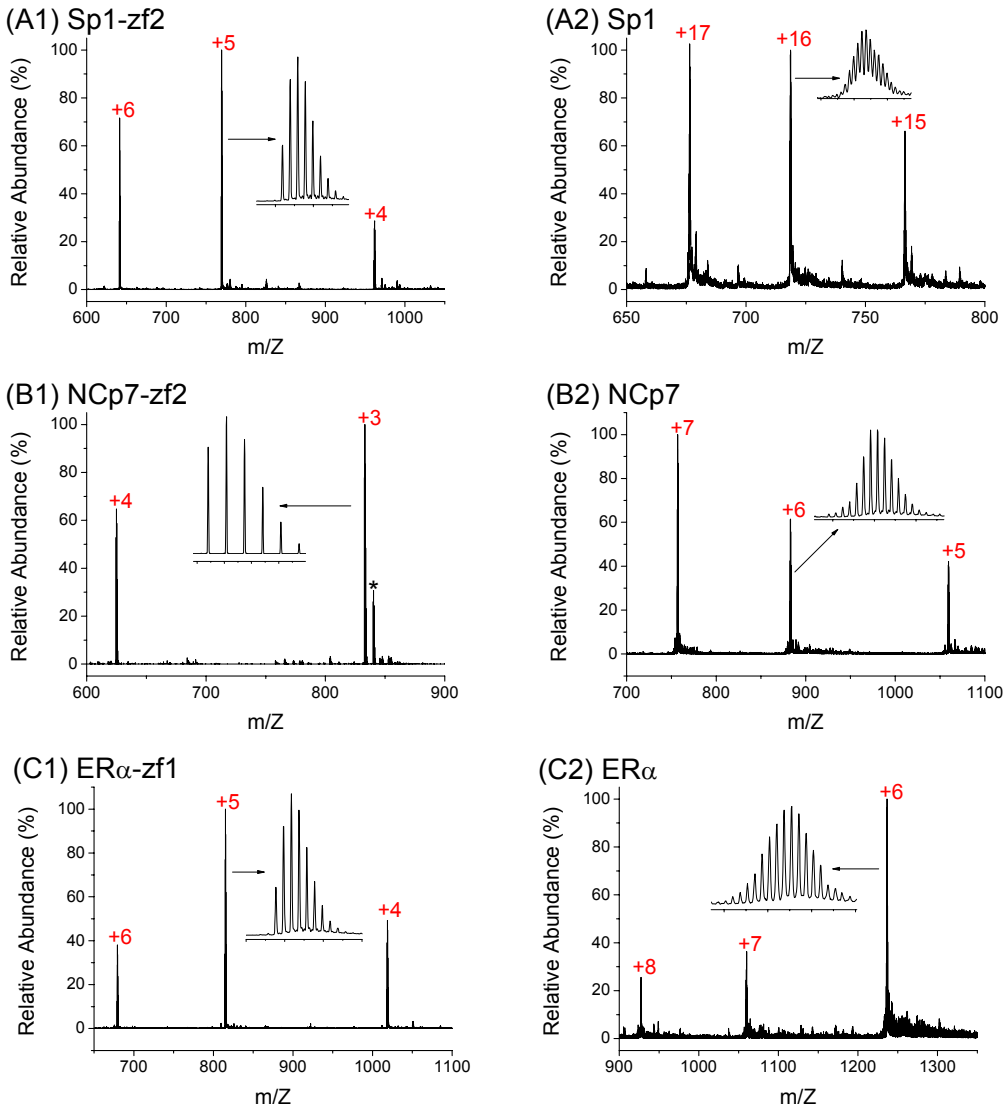

Figure S3. ESI-MS mass spectra of zinc finger proteins. Spectra were recorded on 100 μM proteins in ultrapure water in the presence of 1 mM TCEP. (A1) Sp1-zf2; (A2) Sp1; (B1) NCp7-zf2; (B2) NCp7; (C1) ERα-zf1; (C2) ERα. The assignments and molecular weights are listed in Table S1.

Table S1. The analysis of ESI-MS peaks detected in Figure 4S.

Protein	Molecular Formula	m/Z (charge)	Mw: obsd./cald.
Sp1-zf2	$C_{165}H_{257}N_{55}O_{46}S_3$	641.49 (+6) 769.59 (+5) 961.73 (+4)	3842.93/3842.87
Sp1	$C_{498}H_{786}N_{166}O_{133}S_8$	676.47 (+17) 718.68 (+16) 766.52 (+15)	11482.90/11482.78
NCp7-zf2	$C_{100}H_{162}N_{34}O_{33}S_4$	625.03 (+4) 833.04 (+3)	2496.12/2496.10
	$C_{100}H_{161}N_{34}O_{33}S_4Na$	840.37 (+3)	2518.10/2818.08
NCp7	$C_{217}H_{357}N_{77}O_{64}S_7$	757.07 (+7) 883.07 (+6) 1059.48 (+5)	5292.46/5292.52
ERα-zf1	$C_{180}H_{261}N_{49}O_{52}S_4$	679.48 (+6) 815.17 (+5) 1018.72 (+4)	4070.87/4070.82
ERα	$C_{309}H_{474}N_{100}O_{96}S_9$	927.66 (+8) 1060.04 (+7) 1236.54 (+6)	7413.28/7413.28

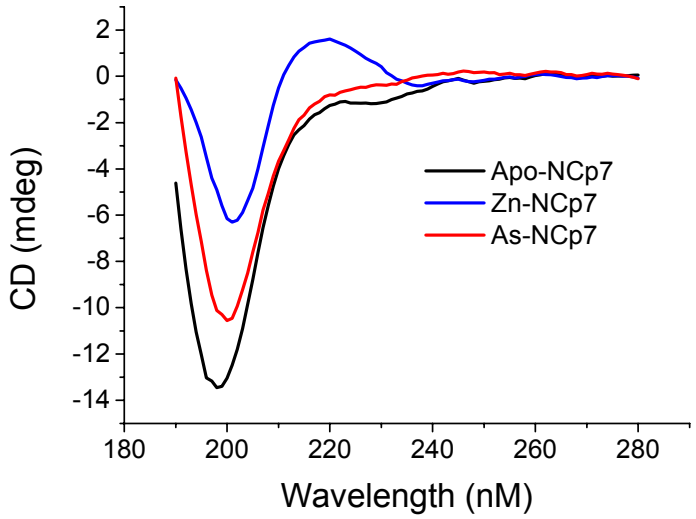

Figure S4. Far-UV CD spectra of apo-NCp7 (black), Zn-NCp7 (blue) and As-bound product with 10 molar equivalent of $NaAsO_2$ (red). 25 μM protein was used in 20 mM phosphate buffer, pH 7.0, 20 mM NaCl, and 0.2 mM TCEP.

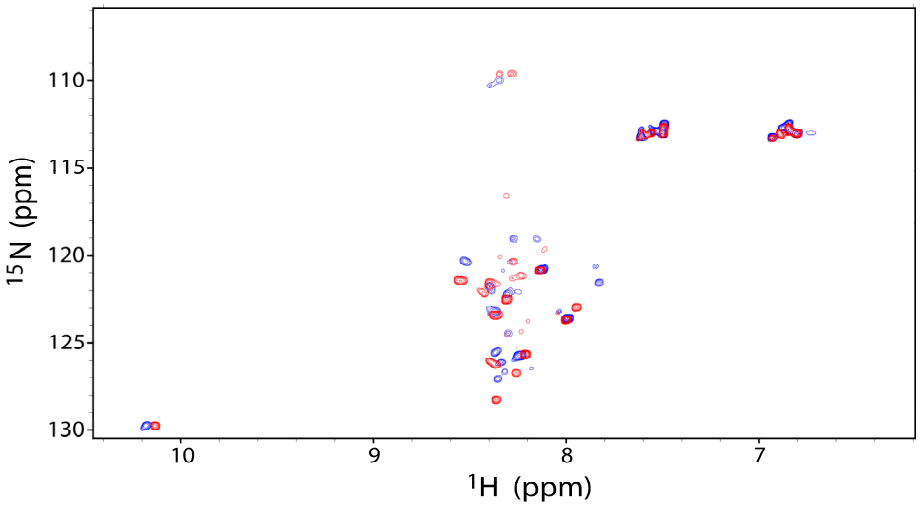

Figure S5. Overlay of 2D ^{1}H-^{15}N HSQC NMR spectra of apo-NCp7 (red) and As-coordinated NCp7 (blue) at 298K and pH 7.0 in 50 mM phosphate buffer, 100 mM NaCl and 2 mM TCEP. 1 mM ^{15}N labeled NCp7 was used in the experiment, and $NaAsO_2$ was 10 times excess for complete coordination of the protein. No protein aggregation was formed with the addition of arsenite, and the identical NMR condition was used for the two samples.