Supplementary Information (SI) for Analyst.
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Supplementary Information

Determination of metal-biomolecule interactions by relative mobility shift partial filling affinity capillary electrophoresis

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1. Optimization of experimental conditions

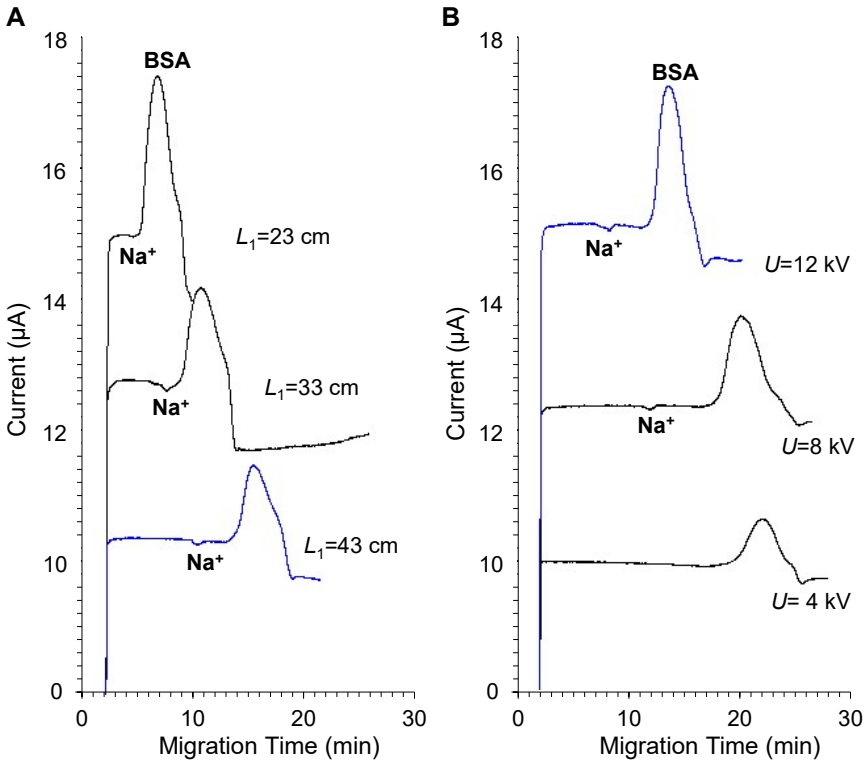

Fig. S1. Results of experimental optimization (N=2). (A) CR-EGs of Na^+ and BSA with different lengths of front-end capillaries. (B) CR-EGs of Na^+ and BSA with different separation voltages. BGE solution: 5 mM ammonium acetate solution (pH 7.5). Concentration of BSA: 60 μM. Concentration of NaCl: 200 μM. Separation pressure: 689.5 Pa. Back-end capillary (i.d. 75 μm, o.d. 365 μm) is 7 cm long. Cartridge temperature is 25℃.

Table S1 The results of the quantitative analysis methodology validation (NaCl solution, N=3).

Concentration /μM	Ions	Intra-day		Inter-day		Inter-batch	
		Precision (RSD%)	Accuracy (RE%)	Precision (RSD%)	Accuracy (RE%)	Precision (RSD%)	Accuracy (RE%)
200	Na^+	2.6	105.8	4.0	101.5	5.4	105.7
	Cl^-	1.3	100.3	3.6	102.3	6.5	102.0
400	Na^+	1.5	100.6	0.9	101.5	1.2	100.8
	Cl^-	4.6	105.3	4.8	100.9	9.7	107.0
600	Na^+	0.8	96.0	1.6	94.0	0.3	95.8
	Cl^-	1.1	106.4	5.6	97.1	4.0	90.8

2. Precision evaluation of relative mobility ($R_{e,M^{n+}}$)

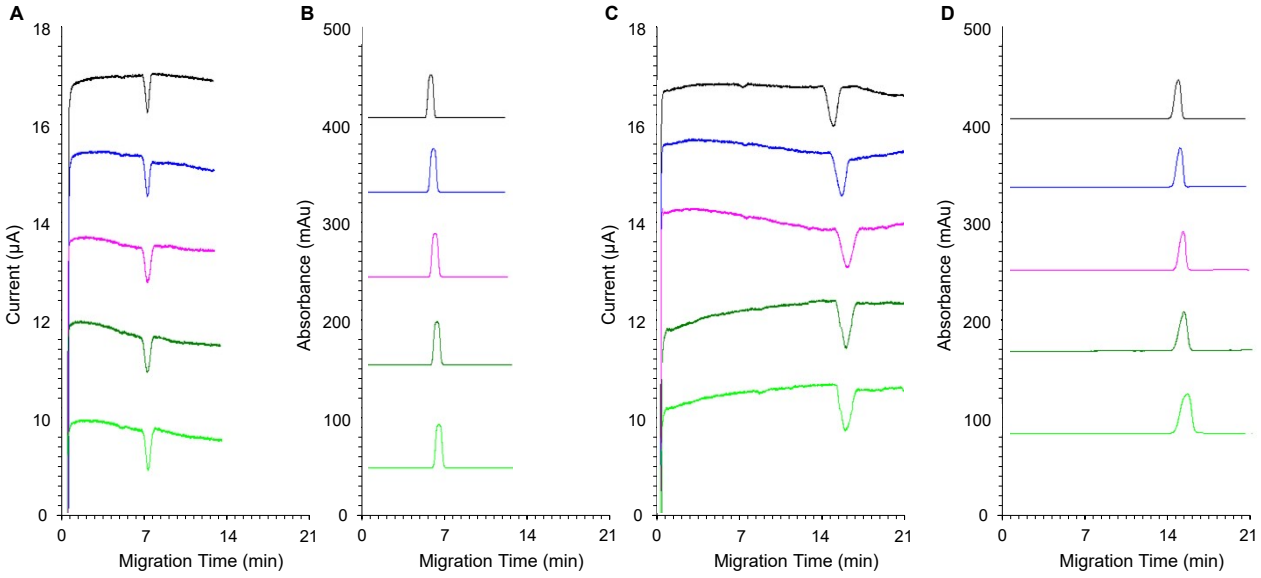

Fig. S2. The repeatability tests of five consecutive injections of 10% DMSO solution (V/V). (A) Current signal peaks of H_2O and (B) UV absorption peak of DMSO with separation pressure (689.5 Pa). (C) Current signal peaks of H_2O and (D) UV absorption peak of DMSO without separation pressure. BGE solution: 5 mM ammonium acetate solution (pH 7.5). Concentrations of metal salts: 200 μM. Beckman ProteomeLab P/ACE MDQ CE system. Injection pressure: 6895 Pa. Separation voltage: 8 kV. Capillaries: the front-end capillary (200 μm i.d., 365 μm o.d.) is 43 cm long, the back-end capillary (75 μm i.d., 365 μm o.d.) is 7 cm long. Cartridge temperature: 25°C.

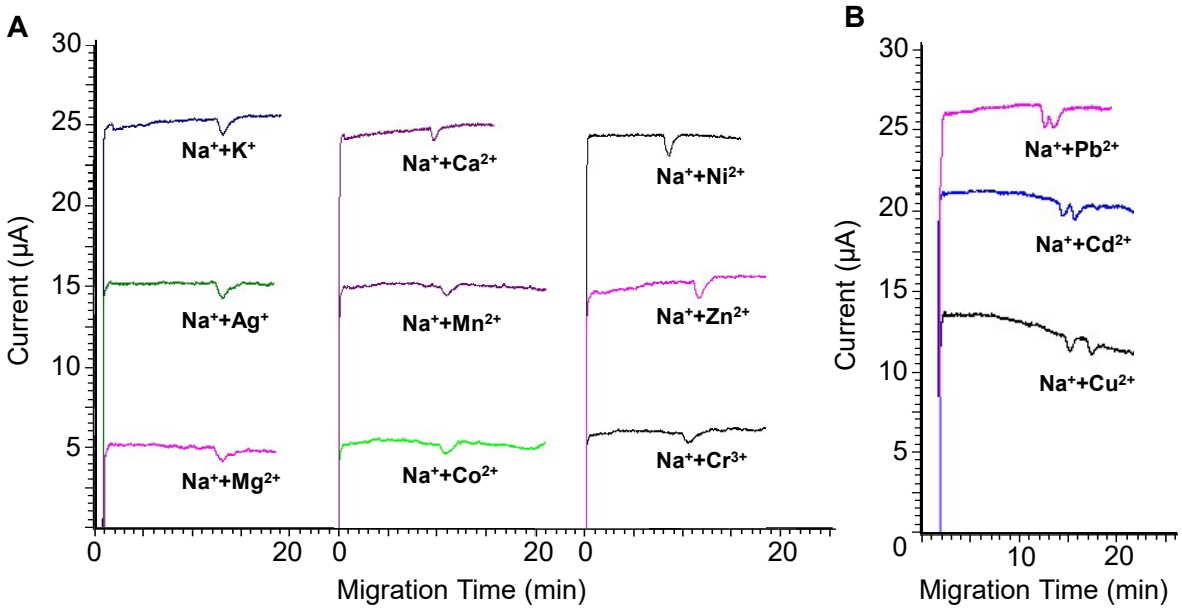

Fig. S3. Results of the precision evaluation of relative mobility. (A) Current signal peaks of K^+, Ag^+, Mg^{2+}, Ca^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+} and Cr^{3+} overlap with the peak of Na^+. (B) Current signal peaks of Cu^{2+}, Cd^{2+} and Pb^{2+} have significant mobility difference with that of Na^+. BGE solution: 5 mM ammonium acetate solution (pH 7.5). Concentrations of metal salts: 200 μM. Beckman ProteomeLab P/ACE MDQ CE system. Injection pressure: 6895 Pa. Separation voltage: 8 kV. Separation pressure: 689.5 Pa. Capillaries: the front-end capillary (200 μm i.d., 365 μm o.d.) is 43 cm long, the back-end capillary (75 μm i.d., 365 μm o.d.) is 7 cm long. Cartridge temperature: 25°C.

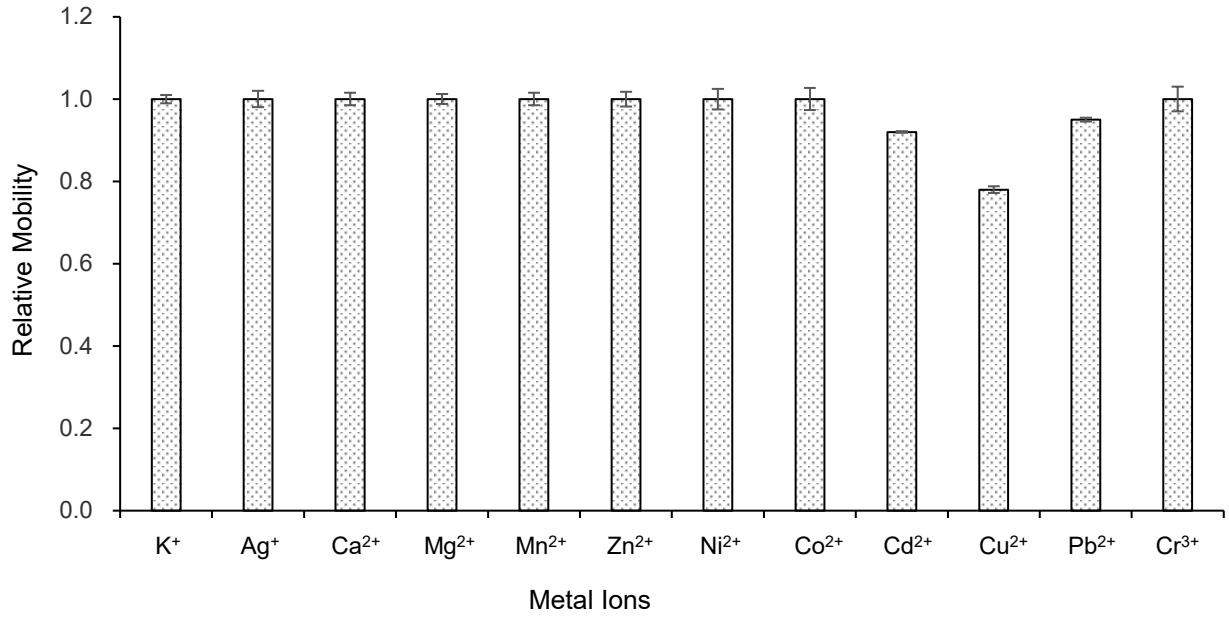

Fig. S4. Results of the precision evaluation of relative mobility of twelve metal ions (N=5). Concentrations of metal salts: 200 μM. Beckman ProteomeLab P/ACE MDQ CE system. Injection pressure: 6895 Pa. Separation voltage: 8 kV. Separation pressure: 689.5 Pa. Capillaries: the front-end capillary (200 μm i.d., 365 μm o.d.) is 43 cm long, the back-end capillary (75 μm i.d., 365 μm o.d.) is 7 cm long. Cartridge temperature: 25°C.

3. Formula derivation

In the previous study of PF-ACE methods [1], the receptor molecules are injected into the capillary before the ligand molecules, and the change of migration time of ligand molecules (Δt) is proportional to the amount of receptor molecules (n) when other conditions are fixed, as shown in formula (S1). Since n is proportional to the injection time t_r, by changing t_r of the receptor molecules, the corresponding change of Δt can be used to qualitatively and quantitatively determine the ligand-receptor interaction.

$$\Delta t = \frac{n}{\pi r^2 E K_d \mu_L} \quad \text{(S1)}$$

where r is the radius of capillary, μ_L is the effective mobility of the ligand molecule (with no receptor molecule).

In this research, it is assumed that changes of mobility occur only when M^{n+} interacts with biomolecules. Therefore, the parameter $R_{M^{n+}}$ is introduced to correct the formulas. When there are no biomolecules filled in the capillary, $R_{M^{n+}}$ is a certain value, the ratio of $\Delta t_{e,M^{n+}}$ (the change of migration time of M^{n+}) to $\Delta t_{e,Na^+}$ (the change of migration time of Na^+) is constant as:

$$R_{e,M^{n+}} = \frac{t_{e,Na^+}}{t_{e,M^{n+}}} = \frac{t_{e,Na^+} + \Delta t_{e,Na^+}}{t_{e,M^{n+}} + \Delta t_{e,M^{n+}}} \quad \text{(S2)}$$

$$\Delta t_{e,M^{n+}} = \frac{t_{e,Na^+}}{R_{e,M^{n+}}} - t_{e,M^{n+}} \quad \text{(S3)}$$

After injecting biomolecules, the change of migration time of M^{n+} actually consists of two parts, one of which is the change in migration time caused by the metal-biomolecule interaction Δt, and the other is $\Delta t_{e,M^{n+}}$ as:

$$\Delta t = \Delta t_{M^{n+}} - \Delta t_{e,M^{n+}} \quad \text{(S4)}$$

Because Na^+ is believed to have no interactions with biomolecules in this study, $\Delta t_{Na^+} = \Delta t_{e,Na^+}$, thus formulas (S5)-(S7) can be derived as:

$$\Delta t = \frac{t_{e,Na^+} + \Delta t_{Na^+}}{R_{i,M^{n+}}} - \frac{t_{e,Na^+} + \Delta t_{e,Na^+}}{R_{e,M^{n+}}} = t_{i,Na^+} \times \left(\frac{1}{R_{i,M^{n+}}} - \frac{1}{R_{e,M^{n+}}} \right) \quad \text{(S5)}$$

$$\frac{1}{R_{i,M^{n+}}} - \frac{1}{R_{e,M^{n+}}} = \frac{n}{\pi r^2 E K_d \mu_{M^{n+}} t_{e,Na^+}} \times \frac{1}{t_{i,Na^+}} \quad \text{(S6)}$$

$$R_{e,M^{n+}} \times t_{i,M^{n+}} - t_{i,Na^+} = \frac{n}{\pi r^2 l_e K_d} \quad \text{(S7)}$$

As mentioned above, the injection time is t_r, and the amount of biomolecules is n. Assuming that t_f is the required injection time to make the biomolecules fill the front-end capillary, and the concentration of biomolecules is c_p, then:

$$n = \frac{t_r}{t_f} \times \pi r^2 l_e c_p \quad \text{(S8)}$$

Combining formulas (S7) and (S8), formula (S9) can be obtained as:

$$\Delta t_p = R_{e,M^{n+}} \times t_{i,M^{n+}} - t_{i,Na^+} = \frac{t_{e,Na^+} \times c_p}{t_f \times K_D} \times t_r \quad \text{(S9)}$$

where Δt_p is the corrected value of the migration time change of M^{n+}, $R_{e,M^{n+}}$ is the value of relative mobility of M^{n+} without biomolecule filling, t_{i,Na^+} and $t_{i,M^{n+}}$ are the migration time of Na^+ and M^{n+} with biomolecule filling, respectively, t_{e,Na^+} is the migration time of Na^+ without biomolecule filling, c_p is the concentration of biomolecule, t_f is the required injection time to make the biomolecules fill the capillary and K_D is the equilibrium dissociation constant of metal-biomolecule complex.

The Δt_p-t_r fitting curve can be obtained and the value of equilibrium dissociation constant (K_D) can be calculated according to the slope of the regression equation as formula (S10):

$$K_D = \frac{t_{e,Na^+} \times c_p}{t_f \times slope} \quad \text{(S10)}$$

4. Determination of metal-biomolecule interactions

4.1 Metal-protein interactions

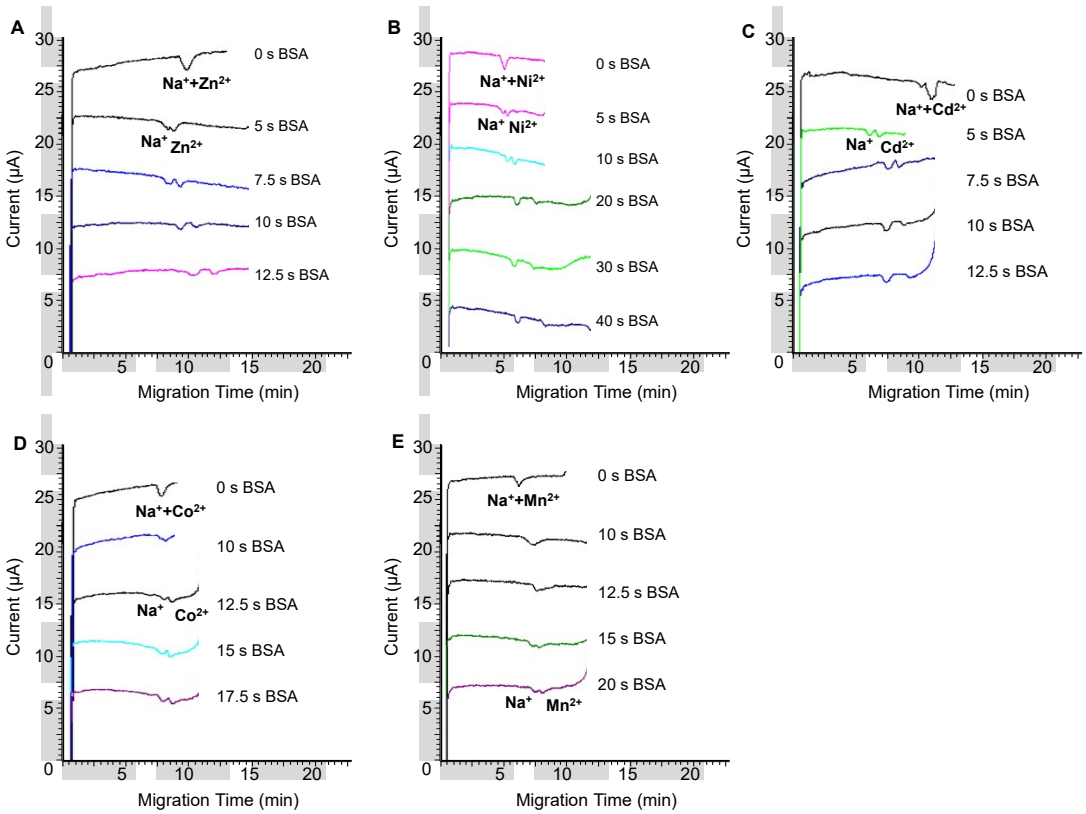

Fig. S5. Positive results of metal-BSA interactions using rmsPF-ACE-IICRD. CR-EGs of (A) Zn^{2+}-BSA interaction, (B) Ni^{2+}-BSA interaction, (C) Cd^{2+}-BSA interaction, (D) Co^{2+}-BSA interaction, (E) Mn^{2+}-BSA interaction. BGE solution: 5 mM ammonium acetate solution (pH 7.5). Concentrations of BSA: 60 μM. Concentrations of NaCl and analyzed metal salts: 200 μM. Beckman ProteomeLab P/ACE MDQ CE system. Injection pressure: 6895 Pa. Injection time of metal ion mixture solution: 10 s. Injection time gradients of BSA are shown in figures. Separation voltage: 8 kV. Separation pressure: 689.5 Pa. Capillaries: the front-end capillary (200 μm i.d., 365 μm o.d.) is 43 cm long, the back-end capillary (75 μm i.d., 365 μm o.d.) is 7 cm long. Cartridge temperature: 25°C.

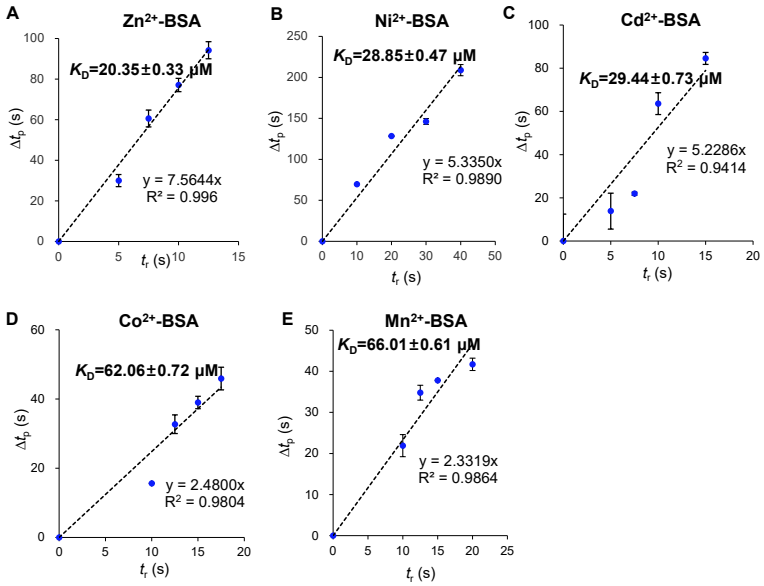

Fig. S6. Fitting curves of Δt_p (s) against t_r (s) for metal-BSA interactions (N=3). (A) Zn^{2+}-BSA interaction, (B) Ni^{2+}-BSA interaction, (C) Cd^{2+}-BSA interaction, (D) Co^{2+}-BSA interaction, (E) Mn^{2+}-BSA interaction.

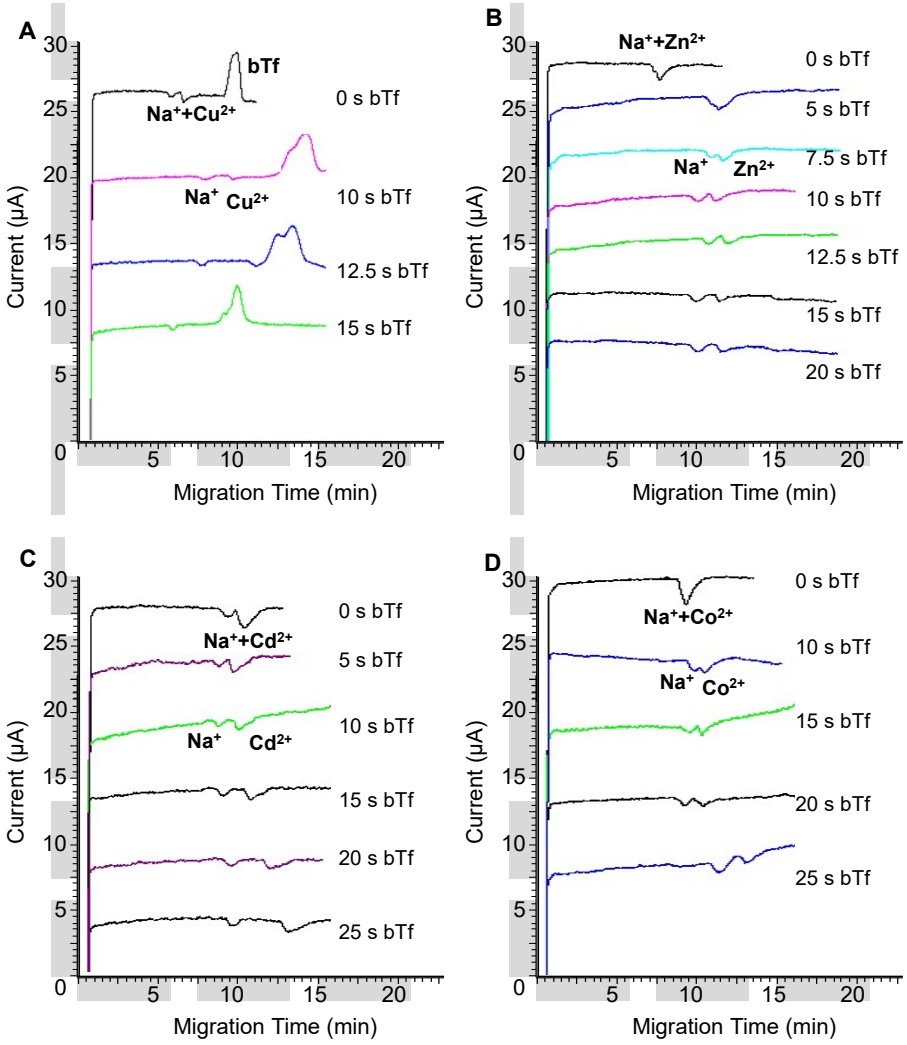

Fig. S7. Positive results of metal-bTf interactions using rmsPF-ACE-IICRD. CR-EGs of (A) Cu^{2+}-bTf interaction, (B) Zn^{2+}-bTf interaction, (C) Cd^{2+}-bTf interaction, (D) Co^{2+}-bTf interaction. BGE solution: 5 mM ammonium acetate solution (pH 7.5). Concentrations of bTf: 60 μM. Concentrations of NaCl and analyzed metal salts: 200 μM. Beckman ProteomeLab P/ACE MDQ CE system. Injection pressure: 6895 Pa. Injection time of metal ion mixture solution: 10 s. Injection time gradients of bTf are shown in figures. Separation voltage: 8 kV. Separation pressure: 689.5 Pa. Capillaries: the front-end capillary (200 μm i.d., 365 μm o.d.) is 43 cm long, the back-end capillary (75 μm i.d., 365 μm o.d.) is 7 cm long. Cartridge temperature: 25℃.

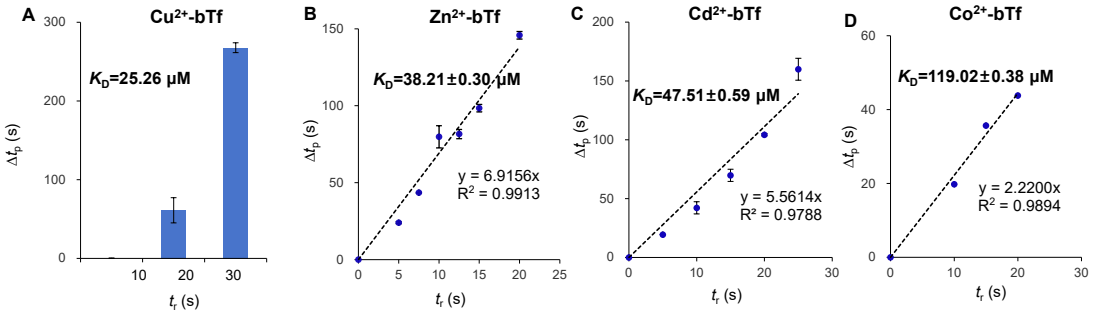

Fig. S8. Fitting curves of Δt_p (s) aganist t_r (s) for metal-bTf interactions (N=3). (A) Cu^{2+}-bTf interaction, (B) Zn^{2+}-bTf interaction, (C) Cd^{2+}-bTf interaction, (D) Co^{2+}-bTf interaction.

4.2 Metal-enzyme interactions

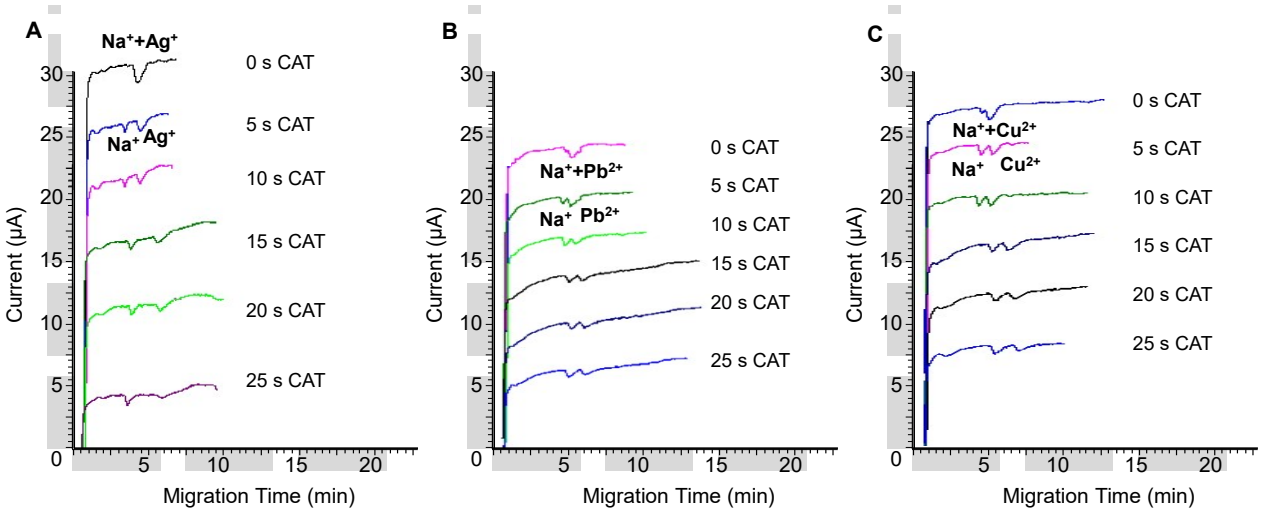

Fig. S9. Positive results of metal-CAT interactions using rmsPF-ACE-IICRD. CR-EGs of (A) Ag^{+}-CAT interaction, (B) Pb^{2+}-CAT interaction, (C) Cu^{2+}-CAT interaction. BGE solution: 5 mM ammonium acetate solution (pH 7.5). Concentrations of CAT: 60 μM. Concentrations of NaCl and analyzed metal salts: 200 μM. Beckman ProteomeLab P/ACE MDQ CE system. Injection pressure: 6895 Pa. Injection time of metal ion mixture solution: 10 s. Injection time gradients of CAT are shown in figures. Separation voltage: 8 kV. Separation pressure: 689.5 Pa. Capillaries: the front-end capillary (200 μm i.d., 365 μm o.d.) is 43 cm long, the back-end capillary (75 μm i.d., 365 μm o.d.) is 7 cm long. Cartridge temperature: 25°C.

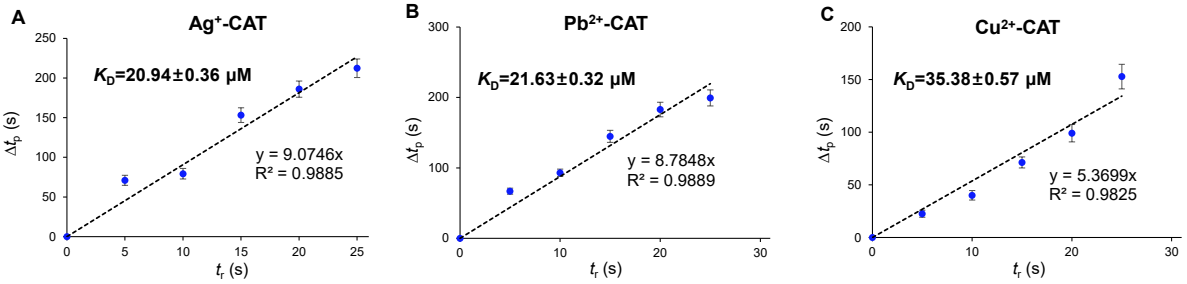

Fig. S10. Fitting curves of Δt_p (s) agtainst t_r (s) for metal-CAT interactions (N=3). (A) Ag^{+}-CAT interaction, (B) Pb^{2+}-CAT interaction, (C) Cu^{2+}-CAT interaction.

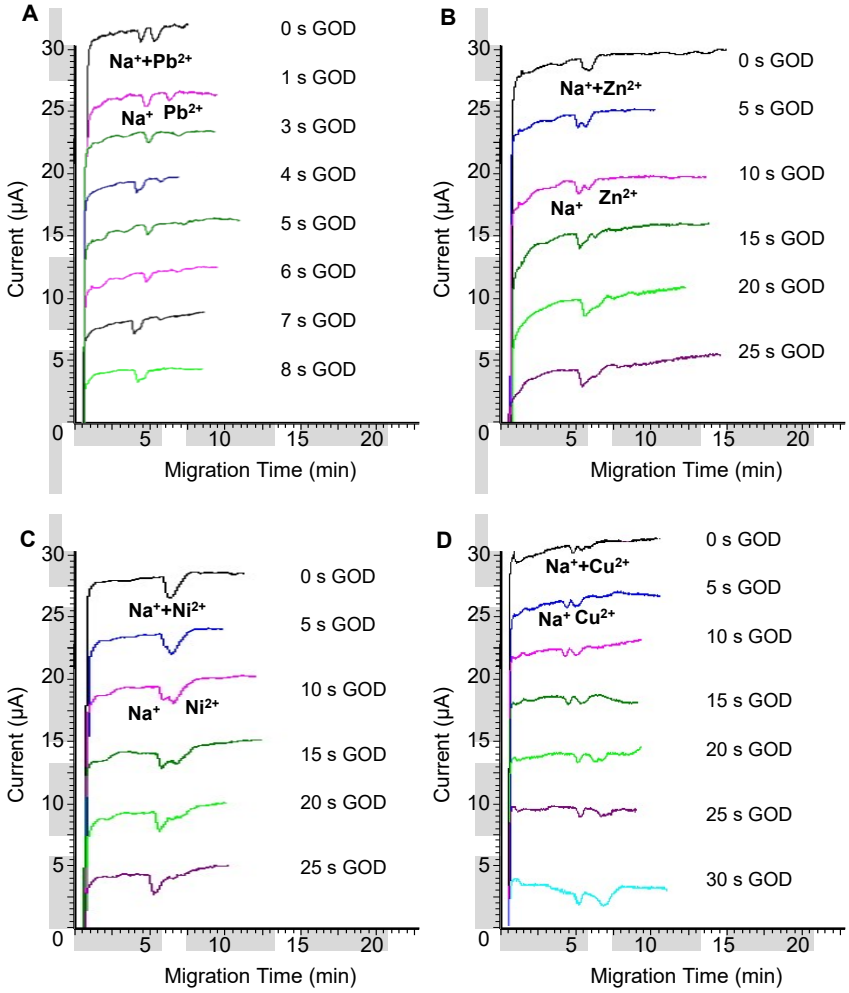

Fig. S11. Positive results of metal-GOD interactions using rmsPF-ACE-IICRD. CR-EGs of (A) Pb^{2+}-GOD interaction, (B) Zn^{2+}-GOD interaction, (C) Ni^{2+}-GOD interaction, (D) Cu^{2+}-GOD interaction. BGE solution: 5 mM ammonium acetate solution (pH 7.5). Concentrations of GOD: 60 μM. Concentrations of NaCl and analyzed metal salts: 200 μM. Beckman ProteomeLab P/ACE MDQ CE system. Injection pressure: 6895 Pa. Injection time of metal ion mixture solution: 10 s. Injection time gradients of GOD are shown in figures. Separation voltage: 8 kV. Separation pressure: 689.5 Pa. Capillaries: the front-end capillary (200 μm i.d., 365 μm o.d.) is 43 cm long, the back-end capillary (75 μm i.d., 365 μm o.d.) is 7 cm long. Cartridge temperature: 25℃.

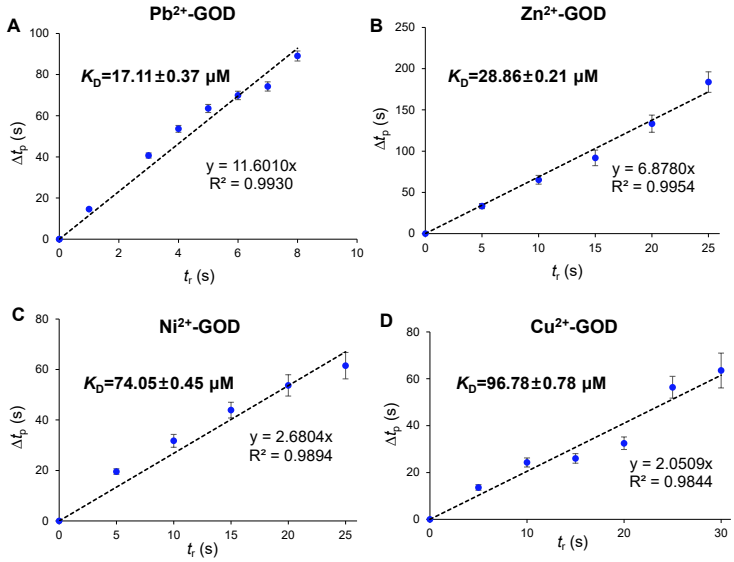

Fig. S12. Fitting curves of Δt_p (s) aganist t_r (s) for metal-GOD interactions (N=3). (A) Pb^{2+}-GOD interaction, (B) Zn^{2+}-GOD interaction, (C) Ni^{2+}-GOD interaction, (D) Cu^{2+}-GOD interaction.

4.3 Metal-DNA interactions

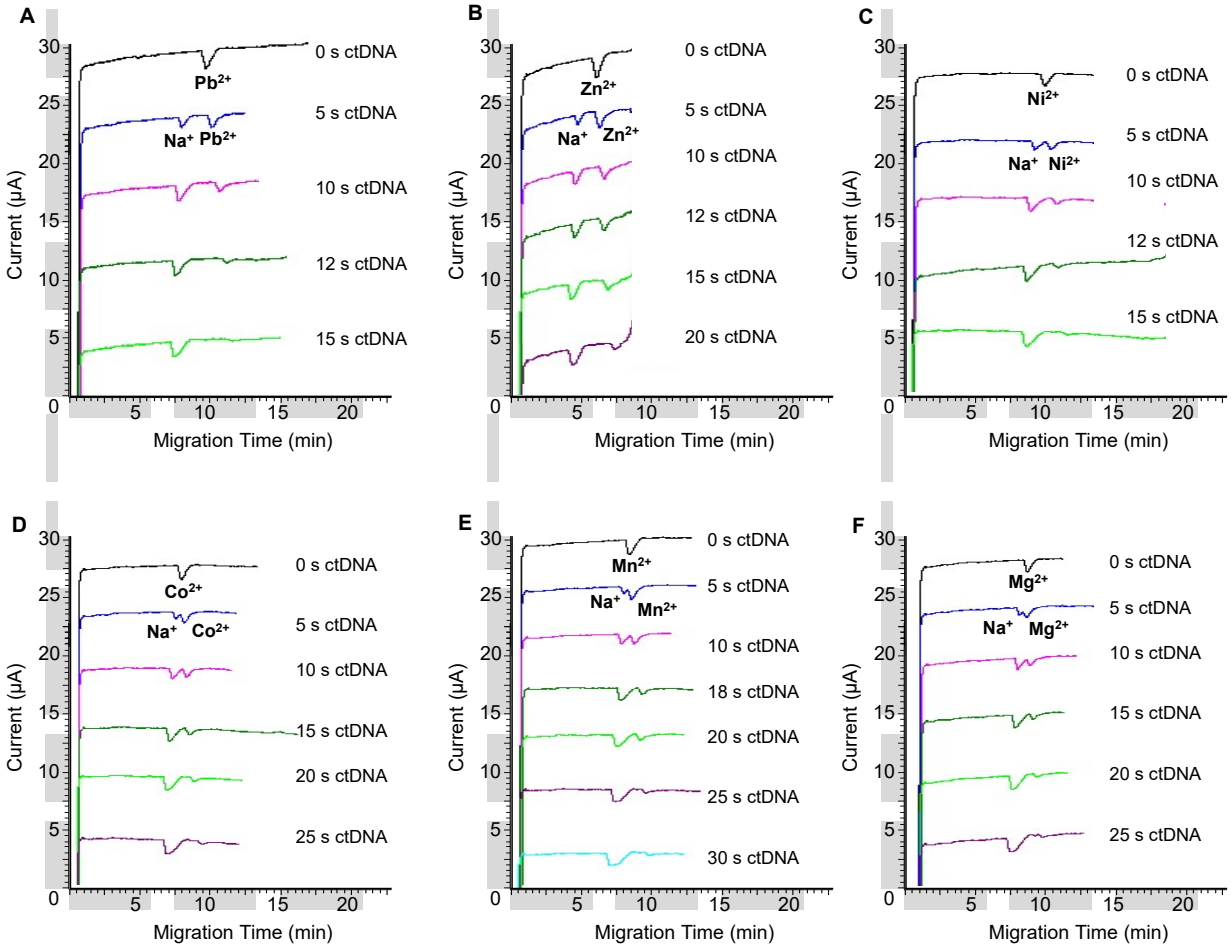

Fig. S13. Positive results of metal-ctDNA interactions using rmsPF-ACE-IICRD. CR-EGs of (A) Pb^{2+}-ctDNA interaction, (B) Zn^{2+}-ctDNA interaction, (C) Ni^{2+}-ctDNA interaction, (D) Co^{2+}-ctDNA interaction, (E) Mn^{2+}-ctDNA interaction, (F) Mg^{2+}-ctDNA interaction. BGE solution: 5 mM ammonium acetate solution (pH 7.5). Concentrations of ctDNA: 60 μM. Concentrations of analyzed metal salts: 200 μM. Beckman ProteomeLab P/ACE MDQ CE system. Injection pressure: 6895 Pa. Injection time of metal ion mixture solution: 10 s. Injection time gradients of ctDNA are shown in figures. Separation voltage: 8 kV. Separation pressure: 689.5 Pa. Capillaries: the front-end capillary (200 μm i.d., 365 μm o.d.) is 43 cm long, the back-end capillary (75 μm i.d., 365 μm o.d.) is 7 cm long. Cartridge temperature: 25℃.

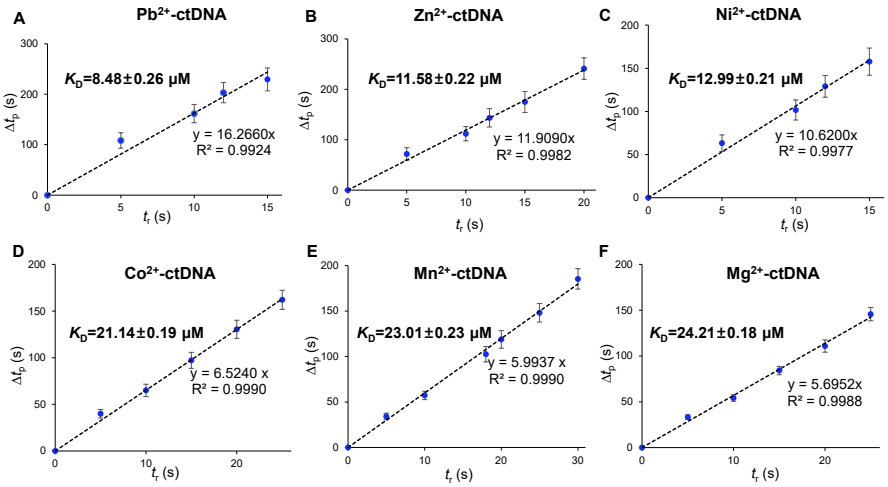

Fig. S14. Fitting curves of Δt_p (s) aganist t_r (s) for metal-ctDNA interactions (N=3). (A) Pb^{2+}-ctDNA interaction, (B) Zn^{2+}-ctDNA interaction, (C) Ni^{2+}-ctDNA interaction, (D) Co^{2+}-ctDNA interaction, (E) Mn^{2+}-ctDNA interaction, (F) Mg^{2+}-ctDNA interaction.

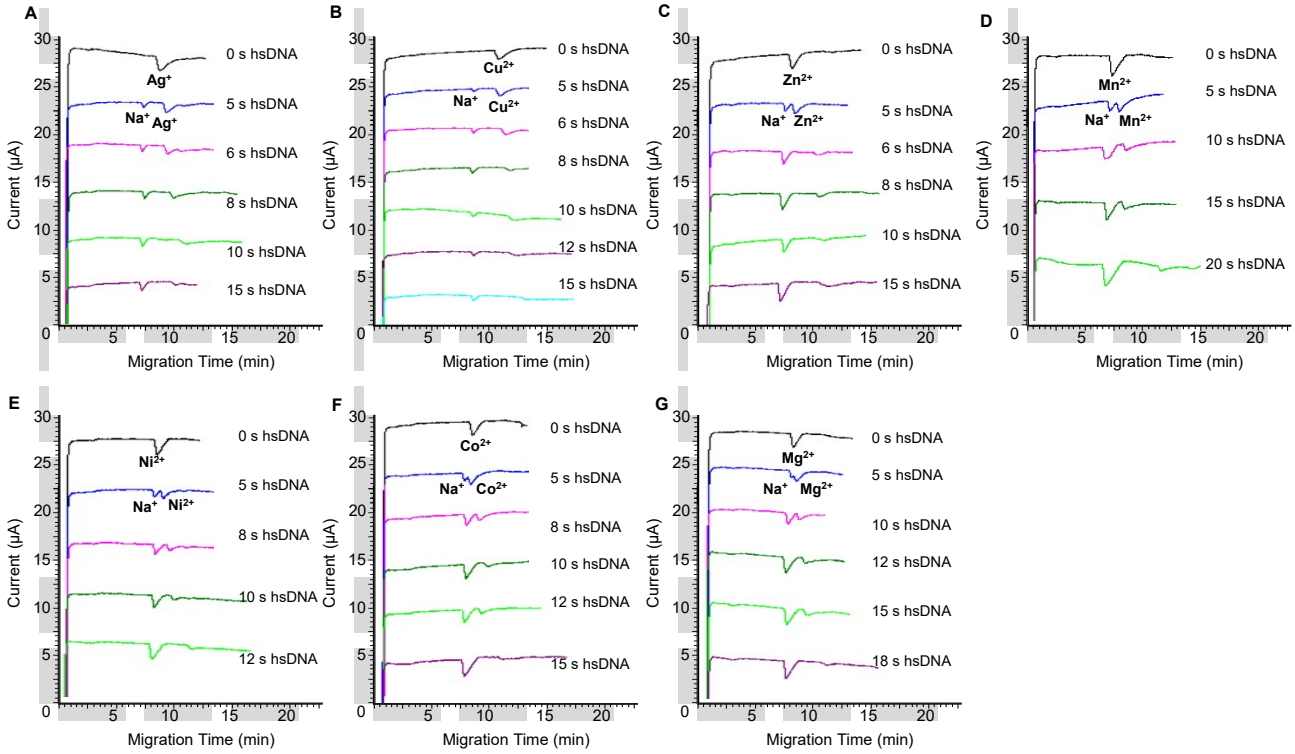

Fig. S15. Positive results of metal-hsDNA interactions using rmsPF-ACE-IICRD. CR-EGs of (A) Ag^{+}-hsDNA interaction, (B) Cu^{2+}-hsDNA interaction, (C) Zn^{2+}-hsDNA interaction, (D) Mn^{2+}-hsDNA interaction, (E) Ni^{2+}-hsDNA interaction, (F) Co^{2+}-hsDNA interaction, (G) Mg^{2+}-hsDNA interaction. BGE solution: 5 mM ammonium acetate solution (pH 7.5). Concentrations of hsDNA: 60 μM. Concentrations of analyzed metal salts: 200 μM. Beckman ProteomeLab P/ACE MDQ CE system. Injection pressure: 6895 Pa. Injection time of metal ion mixture solution: 10 s. Injection time gradients of hsDNA are shown in figures. Separation voltage: 8 kV. Separation pressure: 689.5 Pa. Capillaries: the front-end capillary (200 μm i.d., 365 μm o.d.) is 43 cm long, the back-end capillary (75 μm i.d., 365 μm o.d.) is 7 cm long. Cartridge temperature: 25°C.

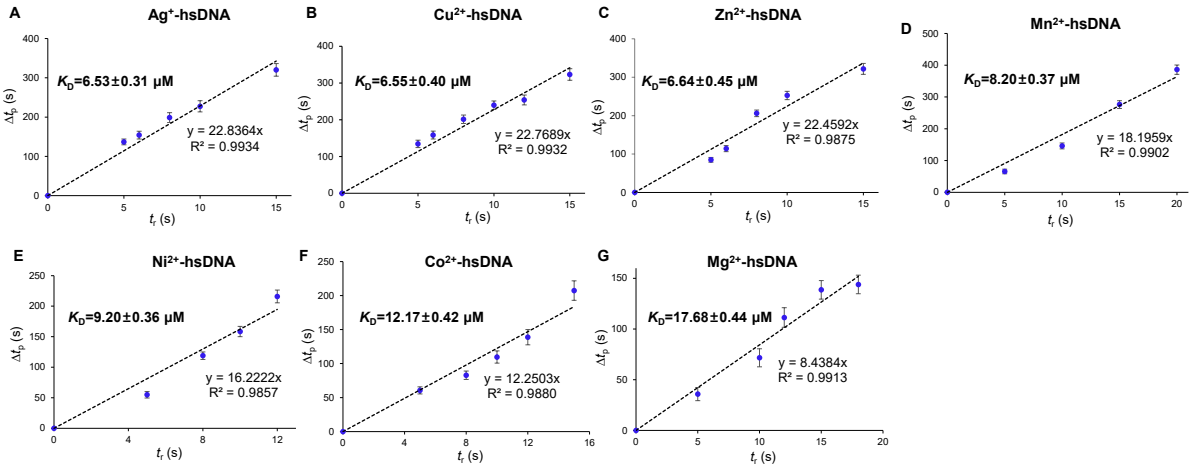

Fig. S16. Fitting curves of Δt_p (s) aganist t_r (s) for metal-hsDNA interactions (N=3). (A) Ag^{+}-hsDNA interaction, (B) Cu^{2+}-hsDNA interaction, (C) Zn^{2+}-hsDNA interaction, (D) Mn^{2+}-hsDNA interaction, (E) Ni^{2+}-hsDNA interaction, (F) Co^{2+}-hsDNA interaction, (G) Mg^{2+}-hsDNA interaction.

References

(1) V. Šolínová, L. Žáková, J. Jiráček, V. Kašička. Pressure assisted partial filling affinity capillary electrophoresis employed for determination of binding constants of human insulin hexamer complexes with serotonin, dopamine, arginine, and phenol. Anal. Chim. Acta. 1052 (2019) 170–178.