Molecular mechanism investigation on the neutralization of cadmium toxicity by transferrin†

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Paper Summary

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Table S1 Data derived from 3D fluorescence spectra of TF and TF–$CdCl_2$ systems

Peak	TF		TF- $CdCl_2$	
	Peak position $\lambda_{ex}/\lambda_{em}$ (nm/nm)	Intensity	Peak position $\lambda_{ex}/\lambda_{em}$ (nm/nm)	Intensity
1	275/320	7575	275/320	7172
2	235/315	1784	235/315	1924

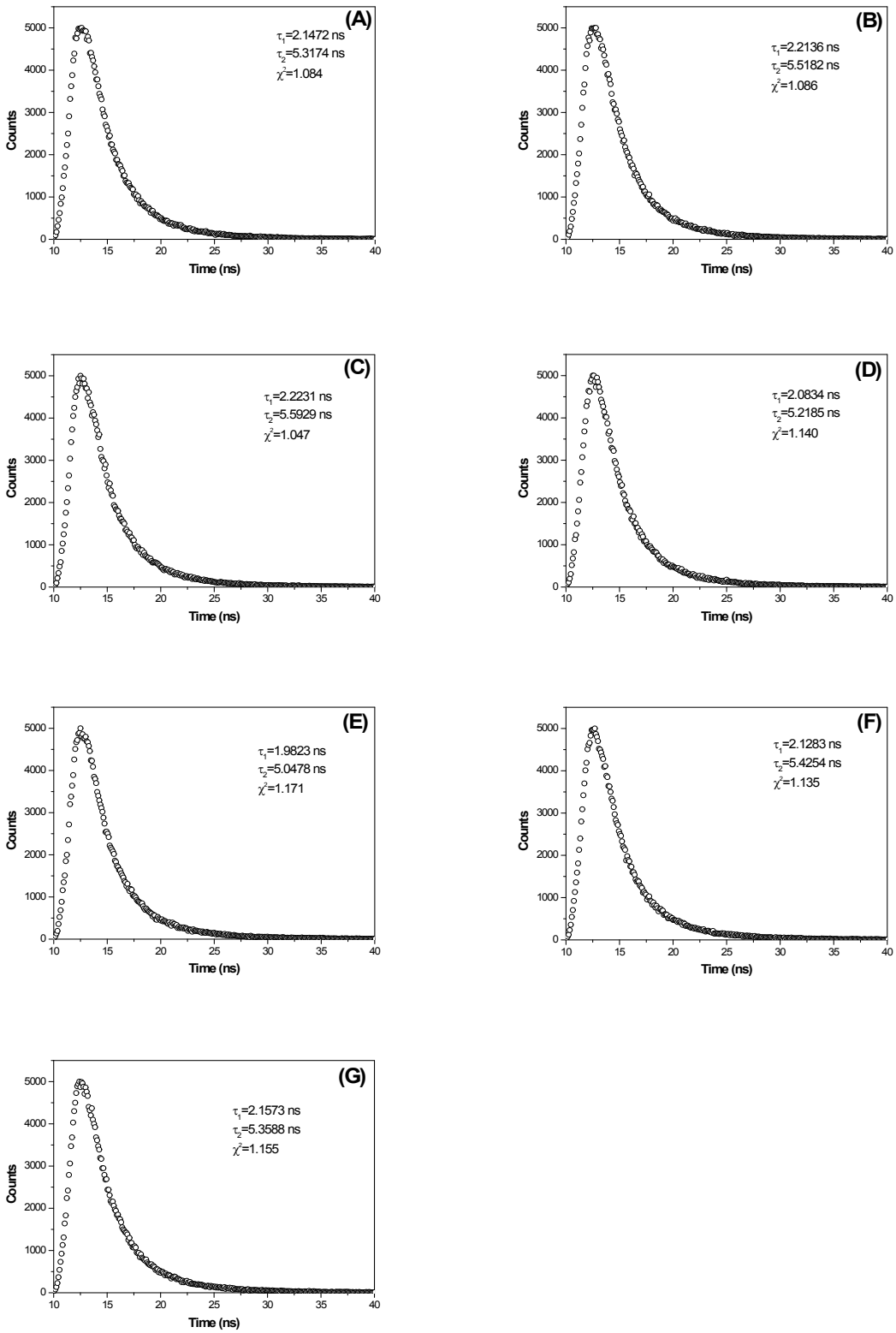


Fig.S1 Time-resolved fluorescence decay profiles of TF in the absence and presence of $CdCl_2$. Conditions: c(TF)= 5 μM; c($CdCl_2$) (mM) (A-G): 0, 0.05, 0.1, 0.5, 1, 5, 10.

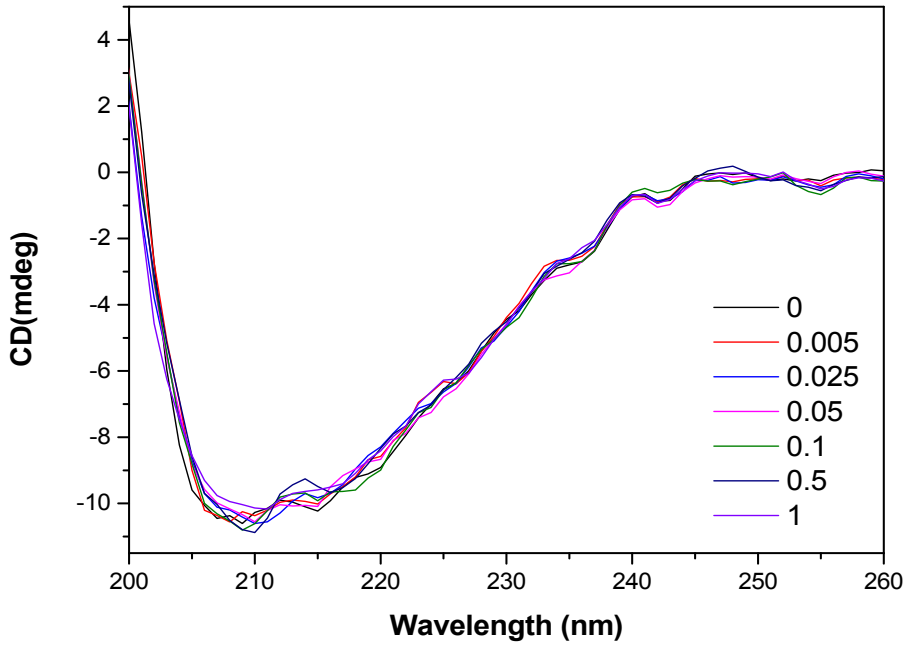
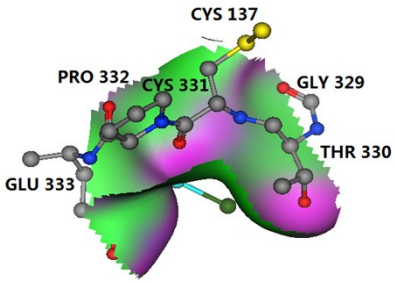
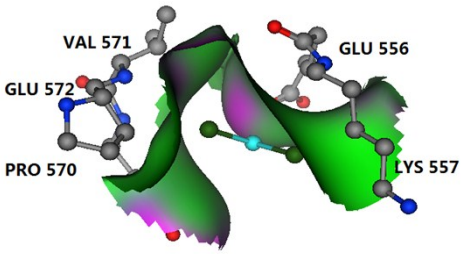


Fig.S2 CD spectra of TF in the absence and presence of $CdCl_2$. Conditions: pH=7.4, c(TF) =2 μM, c($CdCl_2$) (mM) a-g: 0,0.005,0.025,0.05,0.1,0.5,1.

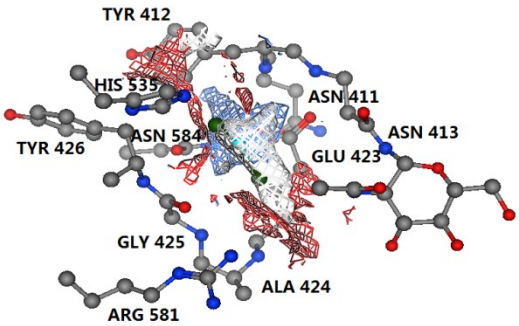
H3



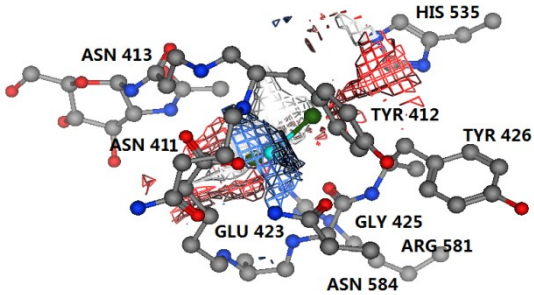
H4



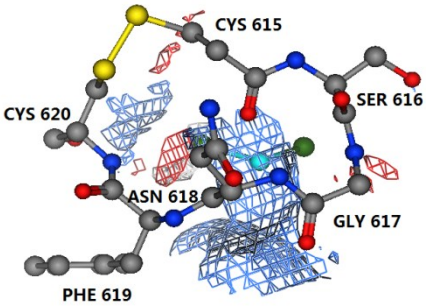
E3



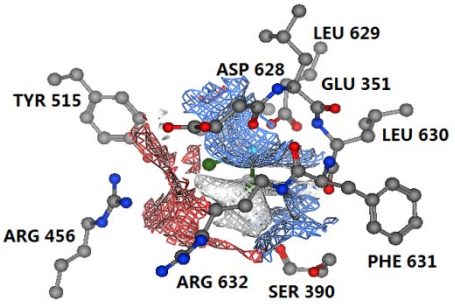
E4



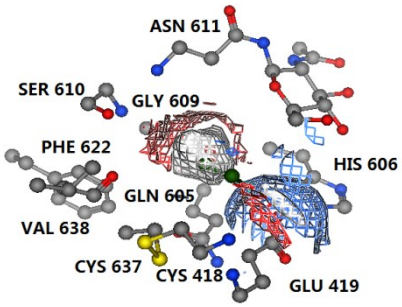
E5



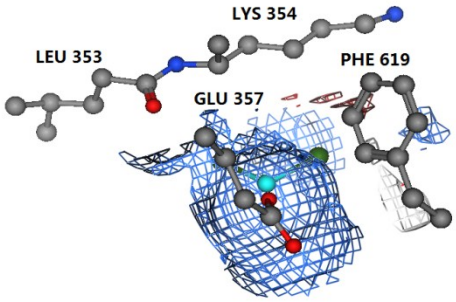
E6



E7



E8



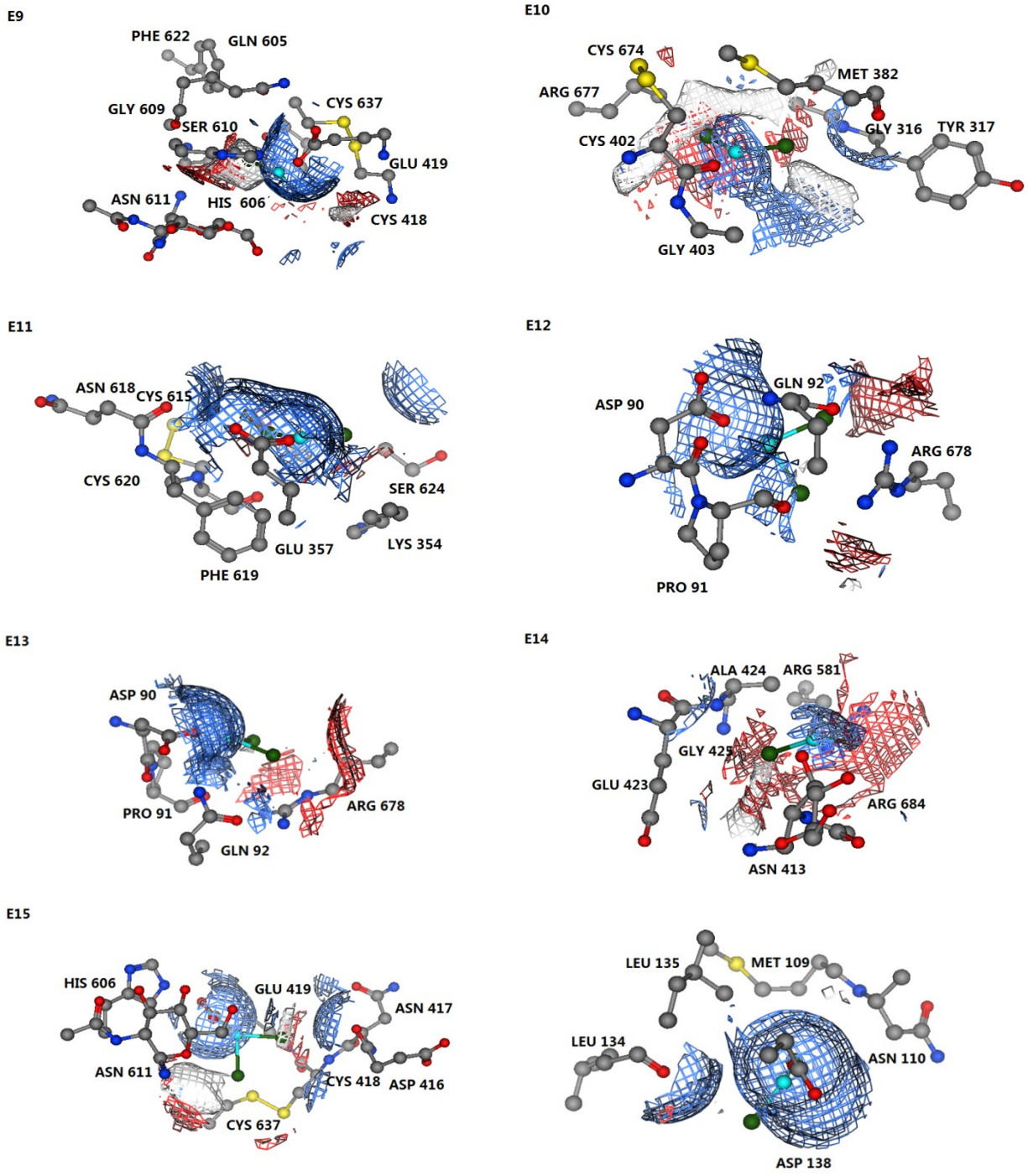


Fig.S3 (H3-H4): Gaussian contact maps superimposed with the ligand $CdCl_2$ and the receptor TF, hydrophobic preference is indicated in green, hydrogen-bonding in pink and mild polar in blue. (E3-E16): Electrostatic maps superimposed with the ligand $CdCl_2$ and the receptor TF, positive (charge region) preference is indicated in blue, negative in red and neutral in white.

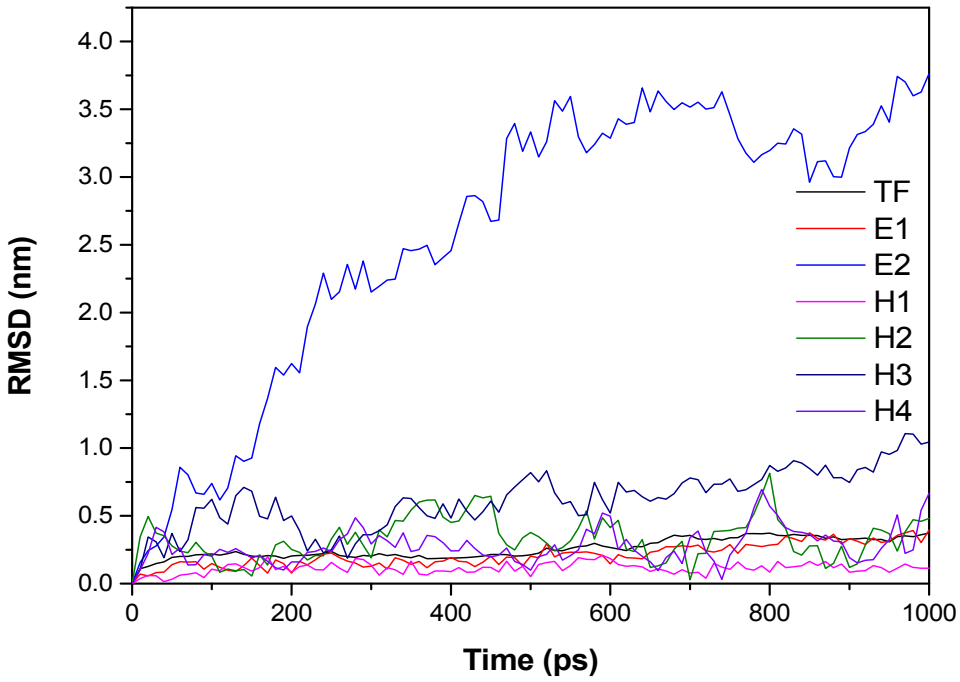


Fig. S4. Time dependence of RMSD values of TF and TF-$CdCl_2$ complexes during MD simulation.

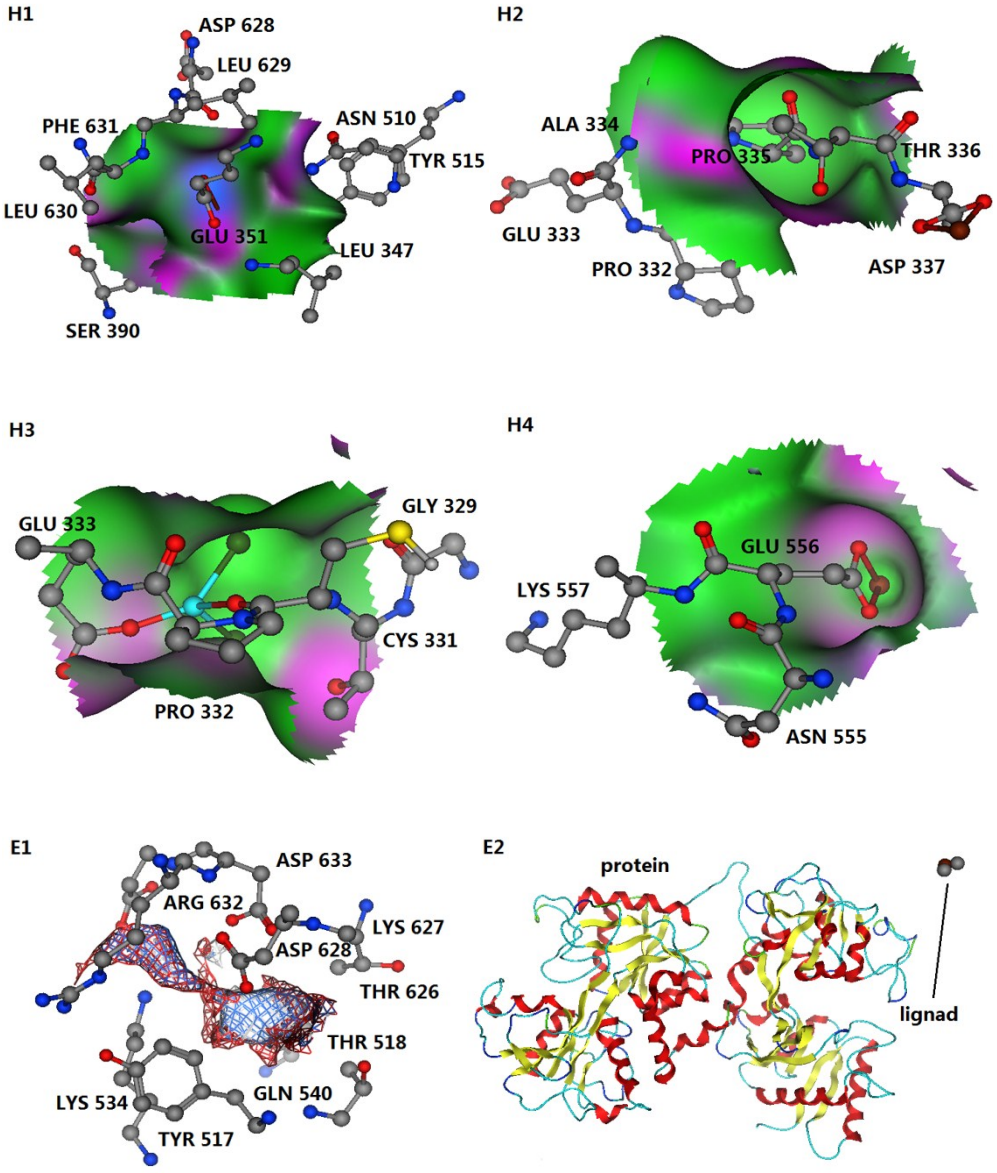


Fig.S5 (H1-H4): Gaussian contact maps superimposed with the ligand $CdCl_2$ and the receptor TF after MD simulation, hydrophobic preference is indicated in green, hydrogen-bonding in pink and mild polar in blue. E1: Electrostatic maps superimposed with the ligand $CdCl_2$ and the receptor TF after MD simulation, positive (charge region) preference is indicated in blue, negative in red and neutral in white. E2: Docking pose of $CdCl_2$ bound to TF after MD simulation. TF is shown in cartoon mode and $CdCl_2$ is represented as spheres.

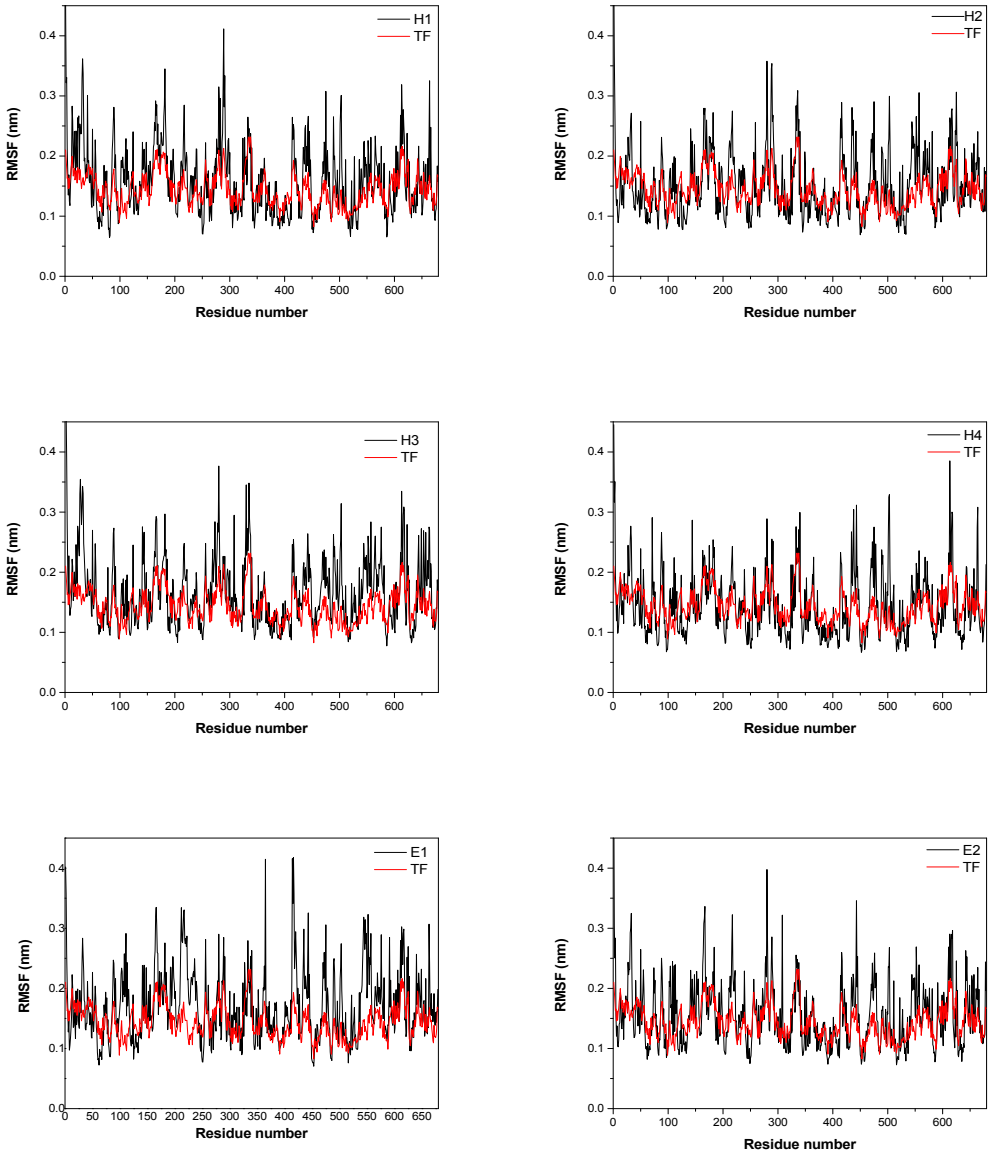


Fig.S6 RMSF values of TF and TF-$CdCl_2$ complexes were plotted against residue numbers.

Table S2 RMSF values of the residues located in the binding site

Site	Residue number	RMSF (bound/unbound)
H1	LEU 347	0.1054/0.1243
	GLU 351	0.1054/0.1223
	SER 390	0.0972/0.1118
	ASN 510	0.1145/0.1106
	TYR 515	0.0923/0.101
	ASP 628	0.1142/0.1127
	LEU 629	0.1039/0.1045
	LEU 630	0.1126/0.1167
	PHE 631	0.0972/0.132
H2	PRO 332	0.2005/0.2042
	GLU 333	0.2837/0.2226
	ALA 334	0.2423/0.2312
	PRO 335	0.2902/0.2317
	THR 336	0.3088/0.2149
	ASP 337	0.2308/0.2293
H3	GLY 329	0.2611/0.1945
	CYS 331	0.2282/0.1949
	PRO 332	0.2725/0.2042
	GLU 333	0.2355/0.2226
H4	ASN 555	0.1978/0.1651

	GLU 556	0.1543/0.1556
	LYS 557	0.2357/0.1719
	TYR 517	0.1036/0.0989
	THR 518	0.112/0.1088
	LYS 534	0.1226/0.1229
	GLN 540	0.1939/0.1355
E1	THR 626	0.187/0.133
	LYS 627	0.2198/0.1159
	ASP 628	0.0965/0.1127
	ARG 632	0.1344/0.1241
	ASP 633	0.1191/0.1309

MD simulations were performed to further evaluate the structural stability of the docking complexes. We performed molecular dynamic simulation of site H1-H4 and site E1-E2 since verifications of these sites are sufficient to support the main conclusions drawn from molecular docking studies and dialysis experiments; that is, $CdCl_2$ could form complexes with TF through preferentially binding to higher binding affinity sites (H1-H4) with no release of Fe and no interface to the Fe binding site. Subsequently, $CdCl_2$ binds to lower binding affinity sites (E1-E16) of TF and interacts with the key residues around the Fe binding sites (E1 and E2), resulting in the release of Fe content.

The stability was checked by the analysis of root mean square deviation (RMSD) and root mean square fluctuation (RMSF) of the protein and the complexes. RMSD

values of the protein backbone with and without the ligand bound were plotted and shown in Fig. S4. For site H1-H4 and E1, RMSD is overall stable and overlapping for all five systems. These complexes remain in a stable binding position with relatively low RMSD fluctuations during the MD simulations, confirming the possibility of the binding poses obtained from docking studies. As shown in Fig.S5, compared with results obtained from docking studies in Fig.6 and Fig.S3, ligand positions in the binding site were almost unchanged with only some slight changes of the relevant residues after MD simulation. However, for site E2, the RMSD plot of the complex is much higher than that of free TF. As shown in Fig.S5, the docking pose after MD simulation showed that the ligand is located at the outside of the protein molecule. Although site E2 might be an impossible site for $CdCl_2$ binding TF, the confirmation of site H1-H4 and E1 could also draw the main conclusion mentioned earlier in the previous paragraph. To investigate the flexibility of residues in the binding sites, the RMSF plots of all amino acid residues of free TF and TF-$CdCl_2$ complexes were calculated and shown in Fig.S6. The RMSF of free TF provides a baseline for comparison of the fluctuations with ligand-bound complexes. The RMSF values of the individual residue in binding site H1-H4 and E1 are listed in Table S2. When compared with free TF, the RMSF values of all the residues in Table S2 have relatively low fluctuations, suggesting that these residues locating in binding site are more rigid due to the TF-$CdCl_2$ complex formation; that is, the ligand specifically binds to these sites in TF.