# Interactions of an artificial zinc finger protein with Cd(II) and Hg(II): competition and metal and DNA binding

Bálint Hajdu, Éva Hunyadi-Gulyás and Béla Gyurcsik*

Section S1. Statistical considerations during ESI-MS measurements

During titrations followed by circular dichroism spectroscopy and fluorimetry, the separate subunits of 1MEY# ZFP could not be distinguished. Therefore, the calculations were performed assuming that the three subunits behave identically and the concentration of 1MEY# binding sites (three times the concentration of 1MEY#) were applied. This approach provided an average metal-binding affinity value for the subunits. During ESI-MS measurements the whole protein could be observed so the following metallated states were assumed:

$$1MEY\# + Zn^{2+} \overset{K'_{Zn_1}}{\rightleftharpoons} Zn_1 1MEY\#$$ (s1)

$$Zn_1 1MEY\# + Zn^{2+} \overset{K'_{Zn_2}}{\rightleftharpoons} Zn_2 1MEY\#$$ (s2)

$$Zn_2 1MEY\# + Zn^{2+} \overset{K'_{Zn_3}}{\rightleftharpoons} Zn_3 1MEY\#$$ (s3)

$$1MEY\# + Cd^{2+} \overset{K'_{Cd_1}}{\rightleftharpoons} Cd_1 1MEY\#$$ (s4)

$$Cd_1 1MEY\# + Cd^{2+} \overset{K'_{Cd_2}}{\rightleftharpoons} Cd_2 1MEY\#$$ (s5)

$$Cd_2 1MEY\# + Cd^{2+} \overset{K'_{Cd_3}}{\rightleftharpoons} Cd_3 1MEY\#$$ (s6)

$$Zn_3 1MEY\# + Cd^{2+} \overset{K'_{Zn_2 Cd_1}}{\rightleftharpoons} Zn_2 Cd_1 1MEY\# + Zn^{2+}$$ (s7)

$$Zn_2 Cd_1 1MEY\# + Cd^{2+} \overset{K'_{Zn_1 Cd_2}}{\rightleftharpoons} Zn_1 Cd_2 1MEY\# + Zn^{2+}$$ (s8)
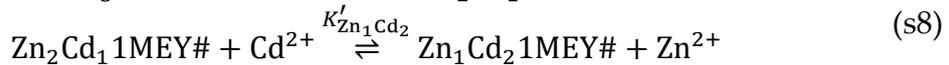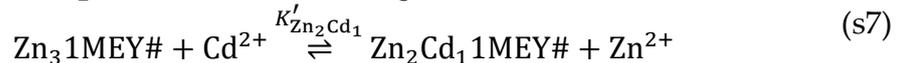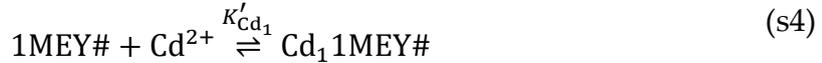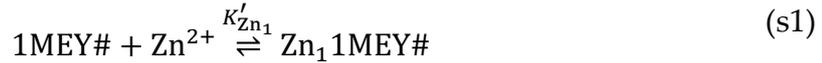
Therefore, statistical considerations were taken into account as described in [1] earlier. The relation between the average determined conditional stability constant $\overline{K}'_M$ and the conditional stepwise stability constants K'_{M_j} can be written as:

$$K'_{M_j} = \frac{(N-j+1)}{j} \overline{K}'_M \cdot x^{(N+1-2j)}$$ (s9)

where j is the number of occupied binding sites, N is the total number of identical binding sites, M is the actual metal ion and x is the 'spreading factor' [2,3]. If the binding sites are identical it can be assumed, that $x = 1$, thus in case of 1MEY# the following equations can be written for the Zn(II)-saturation and the same scheme applies to Cd(II) as well:

$$K'_{Zn_1} = \frac{(3-1+1)}{1} \overline{K}'_{Zn} \cdot 1^{(3+1-2\cdot1)} = 3\overline{K}'_{Zn}$$ (s10)

$$K'_{Zn_2} = \frac{(3-2+1)}{2} \overline{K}'_{Zn} \cdot 1^{(3+1-2\cdot2)} = \overline{K}'_{Zn}$$ (s11)

$$K'_{Zn_3} = \frac{(3-3+1)}{3} \overline{K}'_{Zn} \cdot 1^{(3+1-2\cdot3)} = \frac{\overline{K}'_{Zn}}{3}$$ (s12)

The overall conditional stability constants ($\log \beta'_{M_j}$) can be calculated as:

$$\log \beta'_{M_j} = \sum_{j}^{N} \log K'_{M_j}$$ (s13)

and for the mixed-metal complexes:

$$\log \beta'_{Zn_2Cd_1} = \log K'_{Zn_1} + \log K'_{Zn_2} + \log K'_{Cd_3} \tag{s14}$$

$$\log \beta'_{Zn_1Cd_2} = \log K'_{Zn_1} + \log K'_{Cd_2} + \log K'_{Cd_3} \tag{s15}$$

The same theory can be used for the evaluation of the Hg(II) competition reactions as well, but in that case, additional $\log \beta'_{M_j}$ values must be defined for the excess Hg(II)-binding events. During the simulations N was kept 3.

Table S1: Automated titration script for CLARIOstar Plus plate reader.

```
;Protocol Names:
st1:="Scan"
st2:="Injection"
;Plate ID:
ID1:=""
;Number of readings(number of kinetic cycles):
NumberOfReadings1:=20
;Define the time in seconds after which the plate should be
measured again:
CycleTime:=900
;Define the shaking parameters
ShakingTime:=30
ShakingFrequency:=150
;set target temperature
TargetTemp:=25.0
;================================================================
R_Temp 0.1 ;switch on temperature monitoring
R_Temp TargetTemp ;switch incubator on
wait for temp >= TargetTemp ;wait until target temperature reached
for Reading:=1 to NumberOfReadings1 do begin ;kinetic loop
ID2:="Script"
R_Run"<st1>" ;execute test protocol
R_Run"<st2>" ;execute first injection
;merge horizontal (kinetic):
if Reading>1 then begin
Call "MergeReadings.exe <DataPath> <User> S ID2"
end;
R_Shake 2 ShakeFrequency ShakeTime
end;
R_Run"<st1>" ;execute test protocol
;merge horizontal(kinetic):
Call "MergeReadings.exe <DataPath> <User> S ID2"
;end of script
```
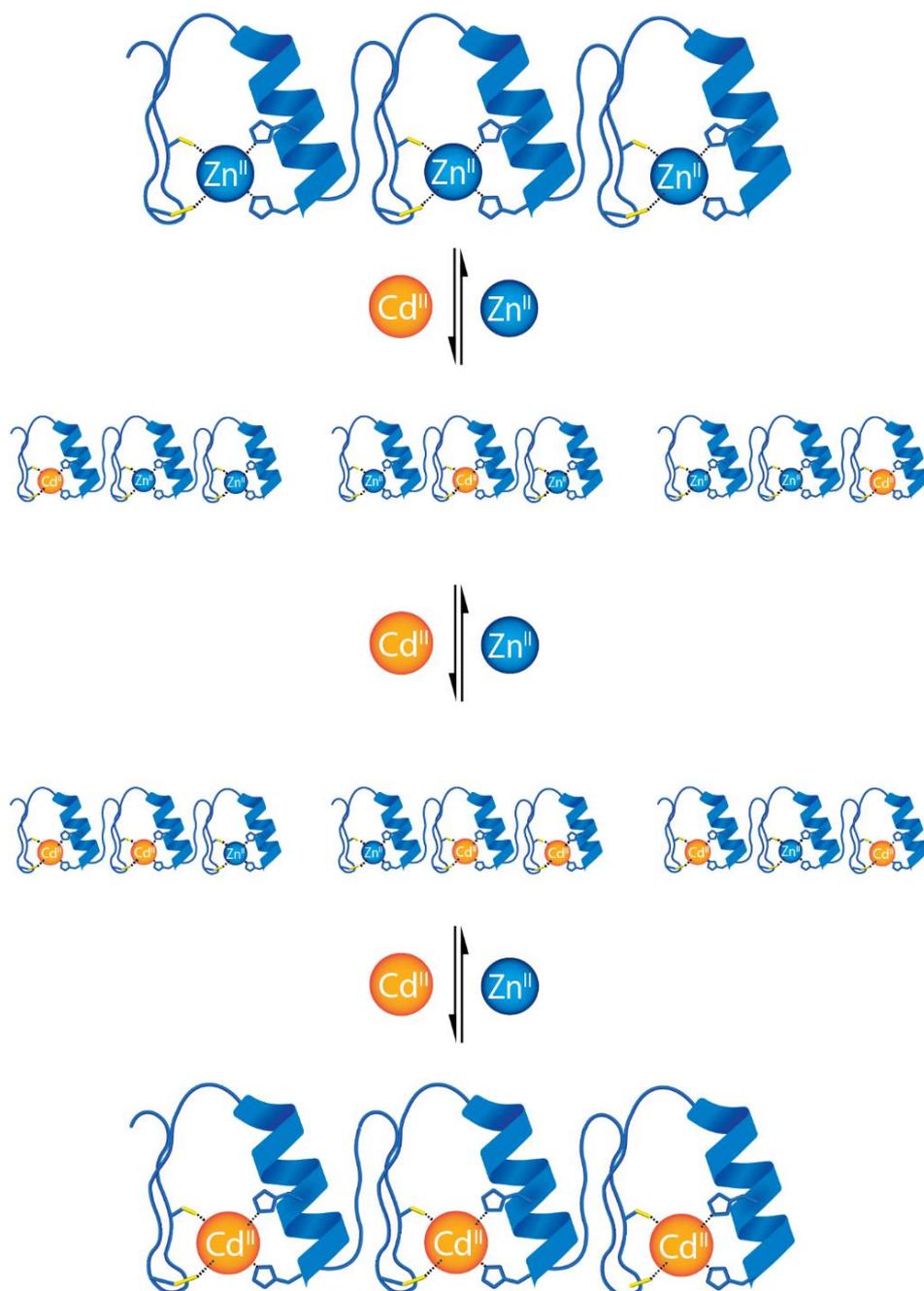
Scheme S1. Schematic representation of the Zn(II)/Cd(II) exchange in the 1MEY# ZFP. In the transition states Cd(II) can replace Zn(II) of any zinc finger subunit with equal probability.
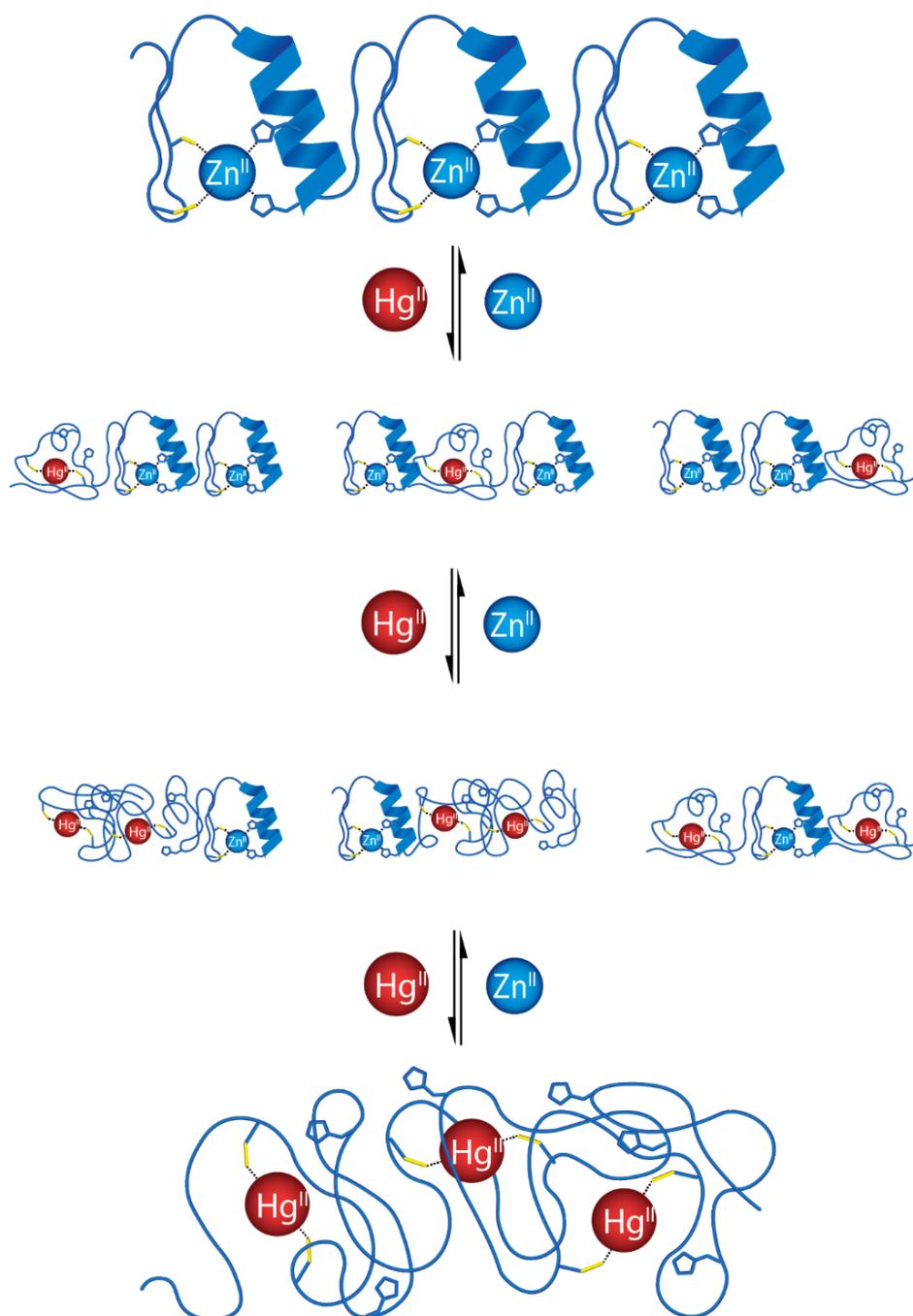
Scheme S2. Schematic representation of the Zn(II)/Hg(II) exchange in the 1MEY# ZFP. In the transition states Hg(II) can replace Zn(II) of any zinc finger subunit with equal probability.
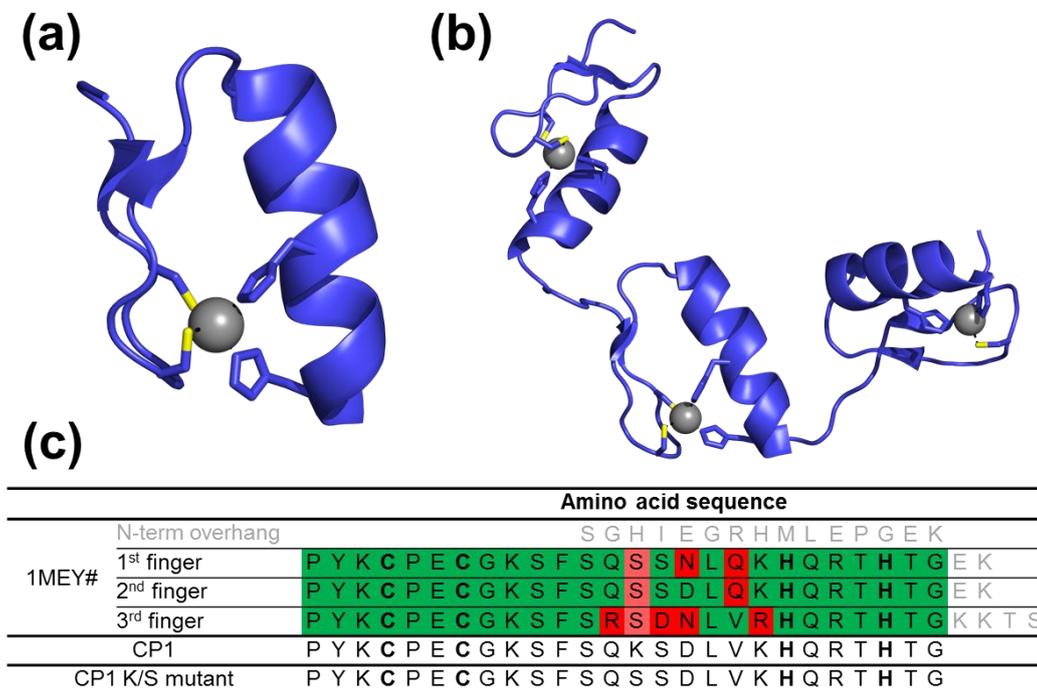
Figure S1. Cartoon representation of the crystal structure of **(a)** the 1st ZF subunit of the 1MEY ZFP; **(b)** the whole 1MEY ZFP. ZFP: blue, Zn(II): grey sphere, cysteine thiolates: yellow (PyMOL representation of 1MEY PDB [4]). **(c)** Alignment of the amino acid sequence of 1MEY# ZFP (constructed from 1MEY ZFP [4]) with the 26 amino acid long consensus Cys2His2 model peptide CP1 and CP1 K/S mutant established and investigated by Berg et al. [5]. The identical amino acids of 1MEY# compared to CP1 are marked with green, while the ones differing both compared to CP1 and CP1 K/S mutant marked with red. The amino acids differing only compared to CP1 are marked with light red.
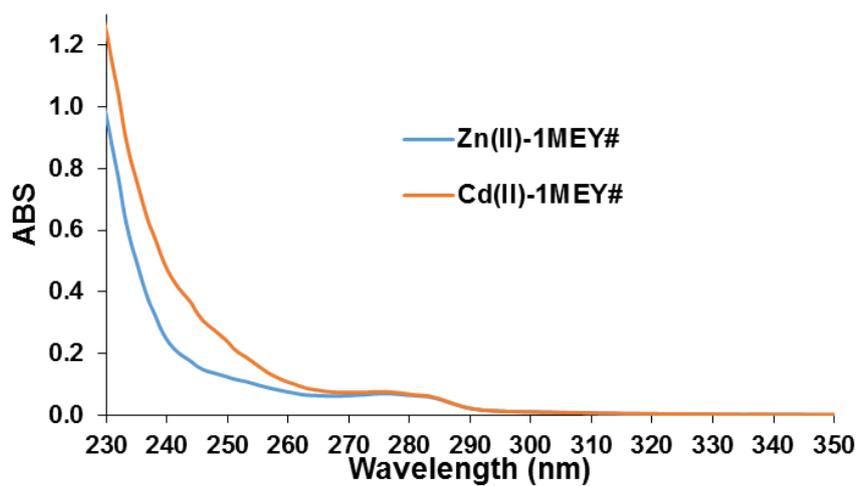
Figure S2: UV-Vis absorption spectra of 1MEY# in either Zn(II) (blue), or Cd(II) (orange) saturated form. $c_{1MEY\#}$ = 13.5 μM in 10 mM HEPES 50 mM NaClO$_4$ (pH 7.4); l = 1 cm.
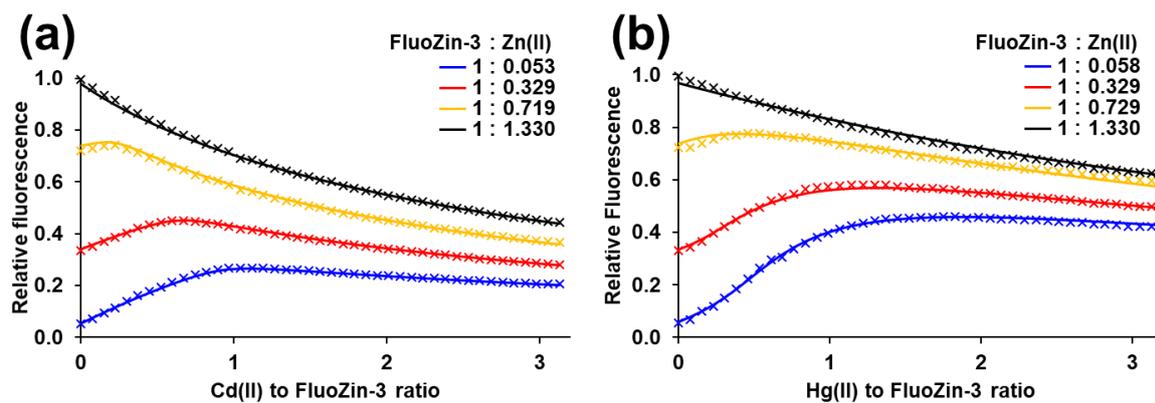
Figure S3 Measured (separate symbols) and calculated (full lines) relative fluorescence values of Zn(II)–FluoZin-3 systems in the presence of increasing amount of **(a)** Cd(ClO$_4$)$_2$; **(b)** Hg(ClO$_4$)$_2$. 200 µl samples were loaded into the plate wells and titrated with 3 µl aliquots of titrant at 25 °C. $c_{\text{FluoZin-3}}$ = 3.98 µM, 10 mM HEPES, 150 mM NaClO$_4$ (pH 7.40). The calculations were performed by PSEQUAD program [6].
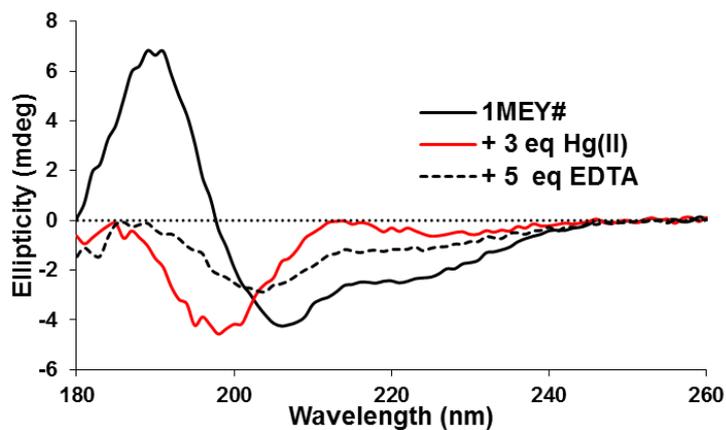
Figure S4: Circular dichroism spectra of Zn(II)-loaded 1MEY# (full black line), Hg(II)-loaded (red), and metal-free form using 5 eqs of EDTA per 1MEY# (1.7 eqs per binding site) (dashed black) are also presented. $c_{1MEY\#}$ = 16.4 μM in 7.5 mM HEPES (pH = 7.4) buffer. CD1 beamline of the storage ring ASTRID, Aarhus l = 0.2 mm.
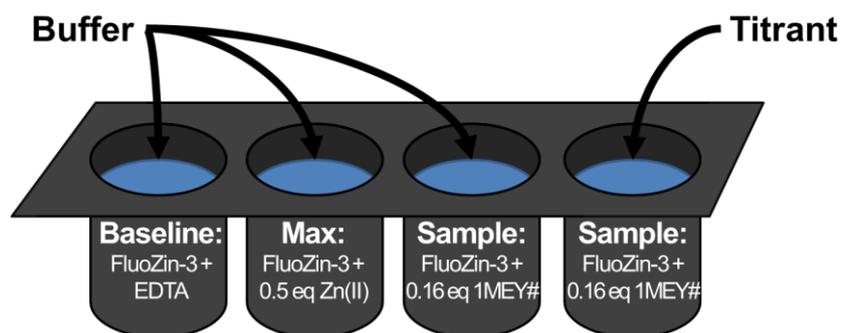
Figure S5: Fluorimetric titration procedure. Baseline fluorescence was determined by applying 10 fold excess EDTA over FluoZin-3. The maximal achievable fluorescence value was determined by applying 0.5 eq Zn(II) to FluoZin-3 ('Max'). The twofold excess of FluoZin-3 was necessary to make sure 100% of Zn(II) is in complex. The sample containing identical amount of Zn(II) to the 'Max' reference well was titrated with the titrant. Dilution effect during titration was determined by the injection of buffer (instead of the titrant) to the reference wells and to an additional sample well.

References

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