Overcoming Universal Restrictions on Metal Selectivity By Protein Design

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Supplementary Method

Mathematical calculation of metal-free and metal-bound $(AB)_2$ in competitive conditions with Cu^{II}. Fractions of metal-free and metal-bound $(AB)_2$ species were calculated using K_d values of $1Cu^{II}$-$(AB)_2$, $2Ni^{II}$-$(AB)_2$, and $2Co^{II}$-$(AB)_2$ as a function of metal concentration. All mathematical models of the $(AB)_2$ fractions were derived from equations for equilibrium dissociation constants. Fractions of individual $(AB)_2$ species are represented as

$$F_{2M^{II}} = \frac{[2M^{II}\text{-}(AB)_2]}{[(AB)_2]_{tot}},\ F_{M^{II}} = \frac{[M^{II}\text{-}(AB)_2]}{[(AB)_2]_{tot}},\ F_{Cu^{II}} = \frac{[Cu^{II}\text{-}(AB)_2]}{[(AB)_2]_{tot}} \quad - (1)$$

where $[(AB)_2]_{tot}$ indicates the total amount of metal-free and metal-bound $(AB)_2$ species and M^{II} indicates Co^{II} or Ni^{II}. Using the definition of K_d, $[(AB)_2]_{tot}$ can be expressed as follows:

$$[(AB)_2]_{tot} = [(AB)_2] + [Cu^{II}\text{-}(AB)_2] + [M^{II}\text{-}(AB)_2] + [2M^{II}\text{-}(AB)_2] \quad - (2)$$

$$= [(AB)_2] + \frac{[(AB)_2][Cu^{II}]}{K_{d,Cu^{II}}} + \frac{[(AB)_2][M^{II}]}{K_{d1,M^{II}}} + \frac{[(AB)_2][M^{II}]^2}{K_{d1,M^{II}}K_{d2,M^{II}}} \quad - (3)$$

$$[2M^{II}\text{-}(AB)_2] = \frac{[(AB)_2][M^{II}]^2}{K_{d1,M^{II}}K_{d2,M^{II}}} \quad - (4)$$

Incorporating (3) and (4) into (1) yields:

$$\therefore F_{2M^{II}} = \frac{[2M^{II}\text{-}AB_2]}{[(AB)_2]_{tot}} = \frac{\frac{[M^{II}]^2}{K_{d1,M^{II}}K_{d2,M^{II}}}}{1+\frac{[Cu^{II}]}{K_{d,Cu^{II}}}+\frac{[M^{II}]}{K_{d1,M^{II}}}+\frac{[M^{II}]^2}{K_{d1,M^{II}}K_{d2,M^{II}}}} \quad - (5)$$

Fractions of $[(AB)_2]$, $[M^{II}$-$(AB)_2]$, and $[Cu^{II}$-$(AB)_2]$ are derived in the same manner.

$$[M^{II}\text{-}(AB)_2] = \frac{[(AB)_2][M^{II}]}{K_{d1,M^{II}}} \quad - (6)$$

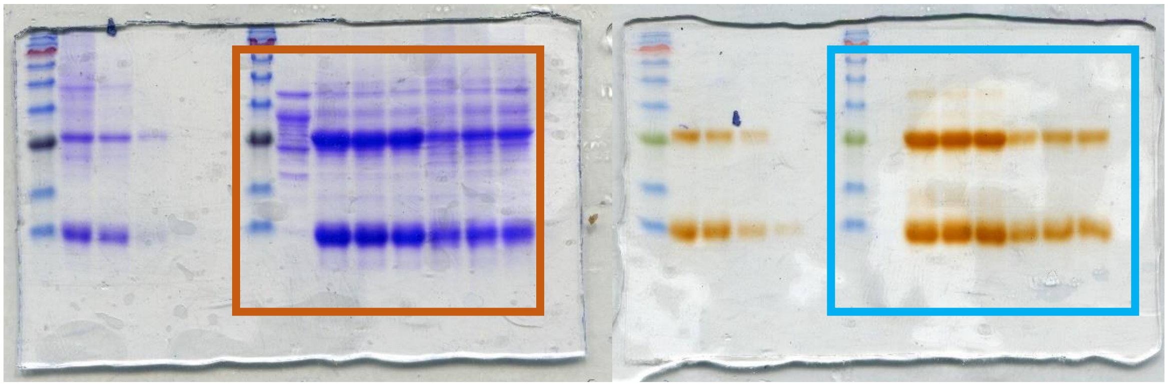
$$\therefore F_{M^{II}} = \frac{[M^{II}\text{-}(AB)_2]}{[(AB)_2]_{tot}} = \frac{\frac{[M^{II}]}{K_{d1,M^{II}}}}{1+\frac{[Cu^{II}]}{K_{d,Cu^{II}}}+\frac{[M^{II}]}{K_{d1,M^{II}}}+\frac{[M^{II}]^2}{K_{d1,M^{II}}K_{d2,M^{II}}}} \quad - (7)$$

$$[Cu^{II}\text{-}(AB)_2] = \frac{[(AB)_2][Cu^{II}]}{K_{d,Cu^{II}}} \quad - (8)$$

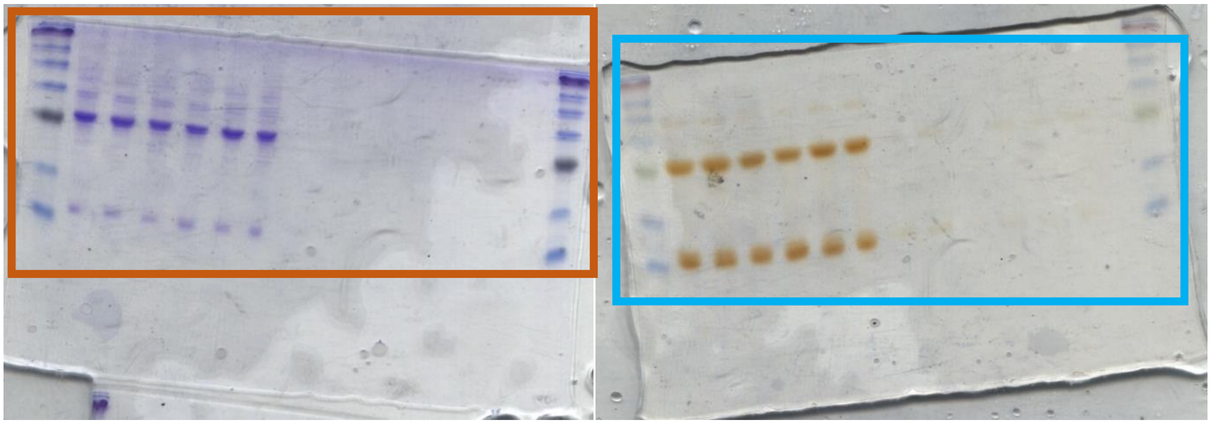
$$\therefore F_{Cu^{II}} = \frac{[Cu^{II}\text{-}(AB)_2]}{[(AB)_2]_{tot}} = \frac{\frac{[Cu^{II}]}{K_{d,Cu^{II}}}}{1+\frac{[Cu^{II}]}{K_{d,Cu^{II}}}+\frac{[M^{II}]}{K_{d1,M^{II}}}+\frac{[M^{II}]^2}{K_{d1,M^{II}}K_{d2,M^{II}}}} \quad - (8)$$

$$\therefore F_{(AB)_2} = 1\text{-}F_{2M^{II}} - F_{M^{II}}\text{-}F_{Cu^{II}} = \frac{1}{1+\frac{[Cu^{II}]}{K_{d,Cu^{II}}}+\frac{[M^{II}]}{K_{d1,M^{II}}}+\frac{[M^{II}]^2}{K_{d1,M^{II}}K_{d2,M^{II}}}} \quad - (9)$$

M^{II} is the concentration of Co^{II} or Ni^{II}. The concentrations of M^{II} and Cu^{II} were considered as buffered species to calculate the fractions of metal-free and metal-bound $(AB)_2$ species. Since the fractions of $[M^{II}\text{-}(AB)_2]$ were negligible in actual calculations using K_d values, they were not included in Fig. 2a. Additionally, because there was no experimental evidence for the formation of heterometallic species (e.g. $Cu^{II}+M^{II}\text{-}(AB)_2$) in ESI-MS (Extended Data Fig. 3d), the fractions of the heterometallic $(AB)_2$ complexes were excluded in the equilibrium.



Supplementary Figure 1 | Uncropped gel images of Figures 4b (brown) and 4c (cyan).



Supplementary Figure 2 | Uncropped gel images of Extended Data Figures 7a (brown) and 7b (cyan).

Supplementary Table 1 | Crystallization conditions for reported PDB structures.

Structure[a]	Metal		Beamline	Precipitant
$2Co^{II}$-$(AB)_2$	$CoCl_2$	4 mM	UCSD[b]	PEG1500 25%, NaCl 140 mM, pH 6.6 MES 100 mM
$2Ni^{II}$-$(AB)_2$	$NiCl_2$	4 mM	ALS 5.0.2.	PEG1500 25%, pH 8 EPPS 100 mM
$1Cu^{II}$-$(AB)_2$	$CuCl_2$	4 mM	UCSD[b]	PEG1500 25%, $CaCl_2$ 200 mM, pH 6 MES 100 mM
Co^{II}//Cu^{II}-$(AB)_2$	$CoCl_2$//$CuCl_2$	4 mM//4mM	SSRL 9-2	PEG1500 25%, NH_4Ac 160 mM, pH 8.4 EPPS 100 mM
Cu^{II}//Co^{II} -$(AB)_2$	$CuCl_2$//$CoCl_2$	4 mM//4mM	SSRL 9-2	PEG1500 25%, NaCl 200 mM, pH 8 EPPS 100 mM
Ni^{II}//Cu^{II}-$(AB)_2$	$NiCl_2$//$CuCl_2$	4 mM//4mM	SSRL 9-2	PEG1500 22%, NH_4Ac 160 mM, pH 8 EPPS 100 mM
Cu^{II}//Ni^{II} -$(AB)_2$	$CuCl_2$//$NiCl_2$	4 mM//4mM	SSRL 9-2	PEG1500 25%, NH_4Ac 200 mM, pH 8.4 EPPS 100 mM
$1Co^{II}$-$^{H100A}(AB)_2$	$CoCl_2$	4 mM	SSRL 9-2	PEG1500 25%, $MgCl_2$ 200 mM, EPPS 100 mM, pH 8
$1Ni^{II}$-$^{H100A}(AB)_2$	$NiCl_2$	4 mM	SSRL 9-2	PEG1500 25%, $MgCl_2$ 200 mM, MOPS 100 mM, pH 7

[a]Dimer concentration was 2 mM. [b]Diffraction data was collected on a Bruker APEX II CCD detector equipped with Cu K_α source in UCSD X-ray crystallography facility.

Supplementary Table 2 | Theoretical and observed *m*/*z* values of $(AB)_2$ complexes observed in non-competitive conditions.

Species	Theoretical *m/z*	Observed *m/z*
$(AB)_2$	2275.09	2275.09
$1Co^{II}$-$(AB)_2$	2280.27	2280.27
$2Co^{II}$-$(AB)_2$	2285.45	2285.45
$1Ni^{II}$-$(AB)_2$	2280.25	2280.27
$2Ni^{II}$-$(AB)_2$	2285.40	2285.40
$1Cu^{II}$-$(AB)_2$	2280.70	2280.63
$2Cu^{II}$-$(AB)_2$	2286.29	2286.26

Supplementary Table 3 | Experimental *m/z* values of $(AB)_2$ complexes observed in the competitive conditions with Cu^{II}.

Species	*m/z* (Co^{II}/Cu^{II})	*m/z* (Cu^{II}/Co^{II})	*m/z* (Ni^{II}/Cu^{II})	*m/z* (Cu^{II}/Ni^{II})
$1M^{II}$-$(AB)_2$	2280.50	2280.45	-	-
$2M^{II}$-$(AB)_2$	2285.54	2285.54	2285.45	2285.45
$3M^{II}$-$(AB)_2$[a]	-	-	2290.98	2290.98

[a]$2Ni^{II}$+$1Cu^{II}$-$(AB)_2$ (Theo *m/z* 2290.99)

Supplementary Table 4 | Theoretical and experimental *m*/*z* values of $^{H100A}(AB)_2$ complexes observed in non-competitive conditions.

Species	Theoretical *m/z*	Observed *m/z*
$^{H100A}(AB)_2$	2263.05	2263.05
$1Co^{II}$- $^{H100A}(AB)_2$	2268.22	2268.22
$1Ni^{II}$- $^{H100A}(AB)_2$	2268.19	2268.22
$1Cu^{II}$- $^{H100A}(AB)_2$	2268.64	2268.63
$2Cu^{II}$- $^{H100A}(AB)_2$	2274.24	2274.25
$3Cu^{II}$- $^{H100A}(AB)_2$	2279.83	2279.88

Supplementary Table 5 | Experimental *m*/*z* values of $^{H100A}(AB)_2$ complexes observed in the competitive conditions with Cu^{II}.

Species	*m*/*z* (Co^{II}/Cu^{II})	*m*/*z* (Cu^{II}/Co^{II})	*m*/*z* (Ni^{II}/Cu^{II})	*m*/*z* (Cu^{II}/Ni^{II})
$1M^{II}$-$^{H100A}(AB)_2$	2268.58	2268.58	2268.37	2268.37
$2M^{II}$- $^{H100A}(AB)_2$	2274.02	2274.22	2273.90	2273.90
$3M^{II}$- $^{H100A}(AB)_2$[a]	2279.60	2279.60	2279.44	2279.44