Electronic Supplementary Information

The resolution of two clinical agents, bleomycin and desferrioxamine B, from a *Streptomyces verticillus* fermentation mixture using multi-dimensional immobilised metal ion affinity chromatography

J. Gu and R. Codd

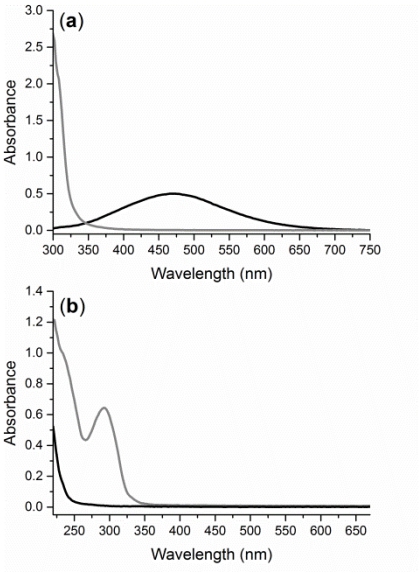

Fig. S1 Electronic absorption spectra from a solution of DFOB (black) or BLM (gray) at (**a**) 0.313 mM in the presence of excess Fe(III); or (**b**) 0.078 mM as free ligands.

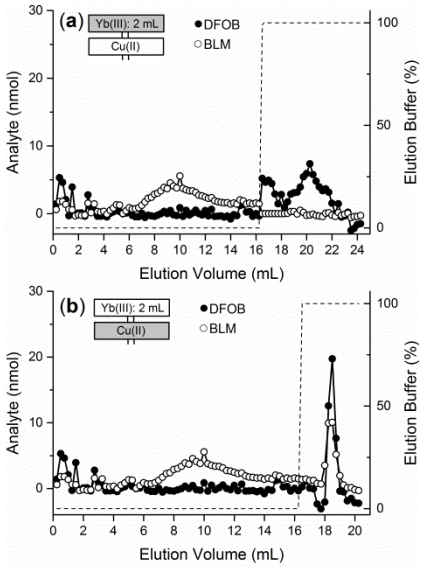

Fig. S2. Resolution profile of DFOB and BLM upon processing an equimolar solution of DFOB and BLM (156 nmol:156 nmol) on two columns configured in series, which contained Yb(III)-charged IDA resin (2 mL, upper) and Cu(II)-charged IDA resin (1 mL, lower).

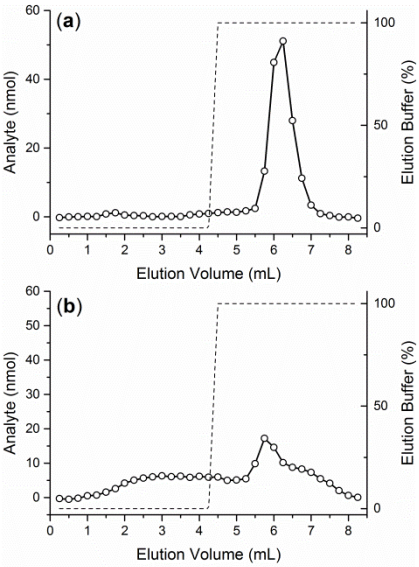

Fig. S3 Binding profile of 1 mL of Cu(II)-charged (**a**) IDA or (**b**) NTA resin towards BLM (156 nmol).

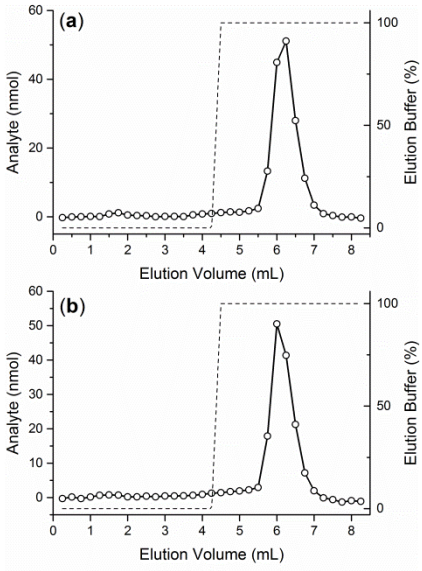

Fig. S4 Binding profile of 1 mL of Cu(II)-charged IDA resin towards BLM (156 nmol) at (**a**) 5 min or (**b**) 3 h incubation time before elution.

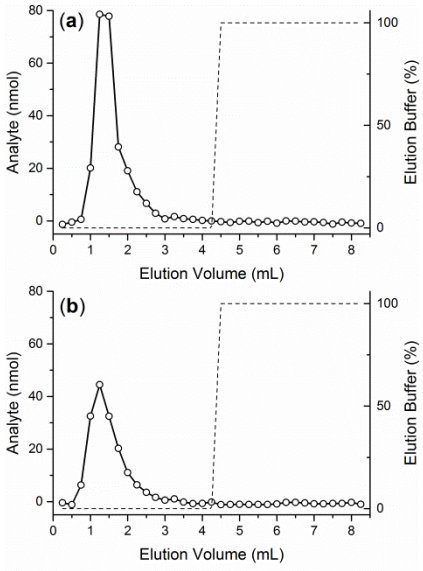

Fig. S5 Binding profile of 1 mL of In(III)-charged (**a**) IDA or (**b**) NTA resin towards BLM (156 nmol).

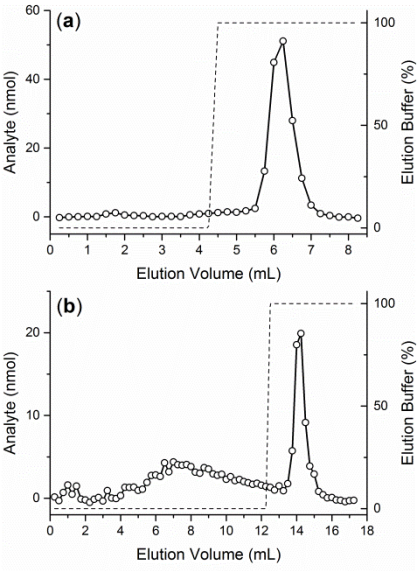

Fig. S6 Binding profile of 1 mL of Cu(II)-charged IDA resin towards BLM (156 nmol) upon washing the resin prior to elution with high-pH BB in volumes of (**a**) 4 mL or (**b**) 12 mL.

Table S1. Binding capacity of Yb(III)-COM and Ni(II)-IDA resin formats towards DFOB

	Yb(III)-COM resin (1 mL)			Ni(II)-IDA resin (1 mL)	
DFOB (µmol)	Unbound Fraction (%)	Bound Fraction (%)		Unbound Fraction (%)	Bound Fraction (%)
3	0.087	99.913		5.0127	94.987
3.5	0.276	99.724		4.8912	95.109
4	0.342	99.658		10.272	89.728
5	1.081	98.919		16.516	83.484
6	2.336	97.664		20.008	79.992
8	5.544	94.456		36.637	63.363
10	9.523	90.477		44.417	55.583

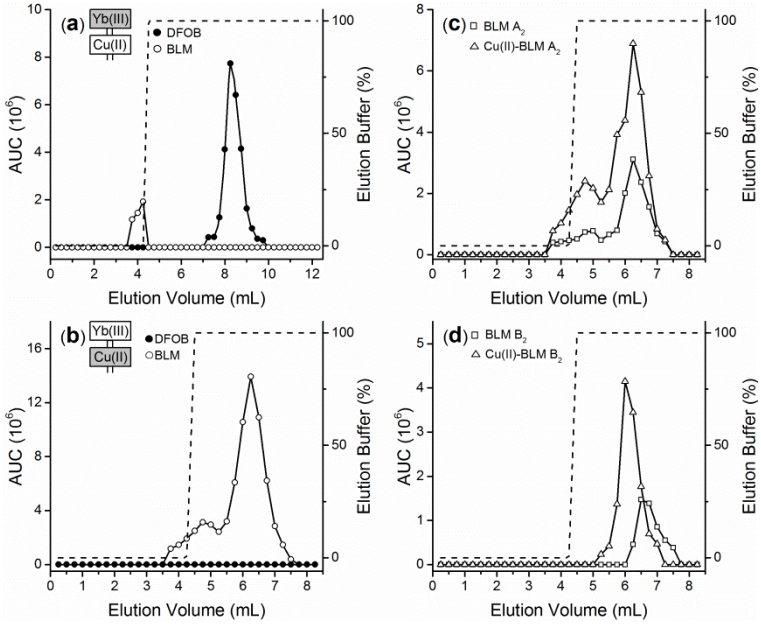

Fig. S7 The resolution profile of DFOB (closed circles) and BLM (open circles) upon processing an aliquot (250 µL) of bacteriological medium that was processed by XAD-2 chromatography prior to the addition of exogenous BLM (140 nmol) and DFOB (140 nmol) on two columns configured in series, which contained 1 mL of IMAC resin charged with Yb(III) (upper column, COM resin) or Cu(II) (lower column, IDA resin). Analyte concentrations were measured as area under the curve (AUC) from TIC detection in the fractions eluted with high-pH binding buffer from the two-column assembly, and from the individual columns with the system-relevant low-pH elution buffer after disassembly (Yb(III), (**a**); Cu(II), (**b**)). The gradient is shown as the broken line. The distribution of BLM A_2 (open square) and Cu(II)-BLM A_2 (open triangle) or BLM B_2 (open square) and Cu(II)-BLM B_2 (open triangle) is shown in (**c**) or (**d**), respectively.

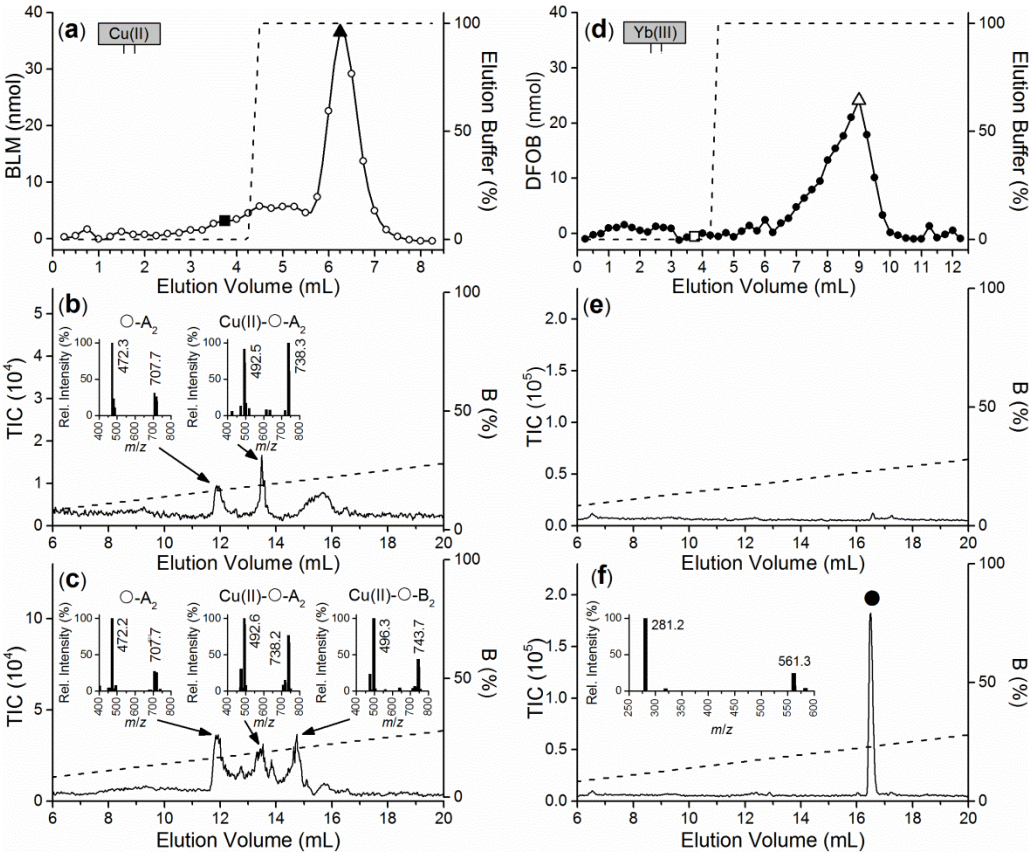

Fig. S8 Binding profile of BLM (156 nmol) on a 1-mL volume of Cu(II)-IDA resin (**a**). Fractions denoted with a solid square (F15) or solid triangle (F25) were analysed using LC-MS, as shown in panels (**b**) or (**c**), respectively. The insets in (**b**) show isotope patterns from the signals at t_R 11.92 min or t_R 13.49 min, consistent with BLM A_2 or Cu(II)-BLM A_2, respectively. The insets in (**c**) show isotope patterns from the signals at t_R 11.92 min, t_R 13.53 min or t_R 14.74 min, consistent with BLM A_2, Cu(II)-BLM A_2 or Cu(II)-BLM A_2, respectively. Binding profile of DFOB (156 nmol) on a 1-mL volume of Yb(III)-COM resin (**d**). Fractions denoted with an open square (F15) or open triangle (F36) were analysed using LC-MS, as shown in panels (**e**) or (**f**), respectively. The inset in (**f**) shows an isotope pattern from the signal at t_R 16.49 min consistent with DFOB, eluted as free ligand.