Supplementary Material For: B904751D "A mass spectrometric investigation of the ability of metal complexes to modulate transcription factor activity" by J. Talib et al.

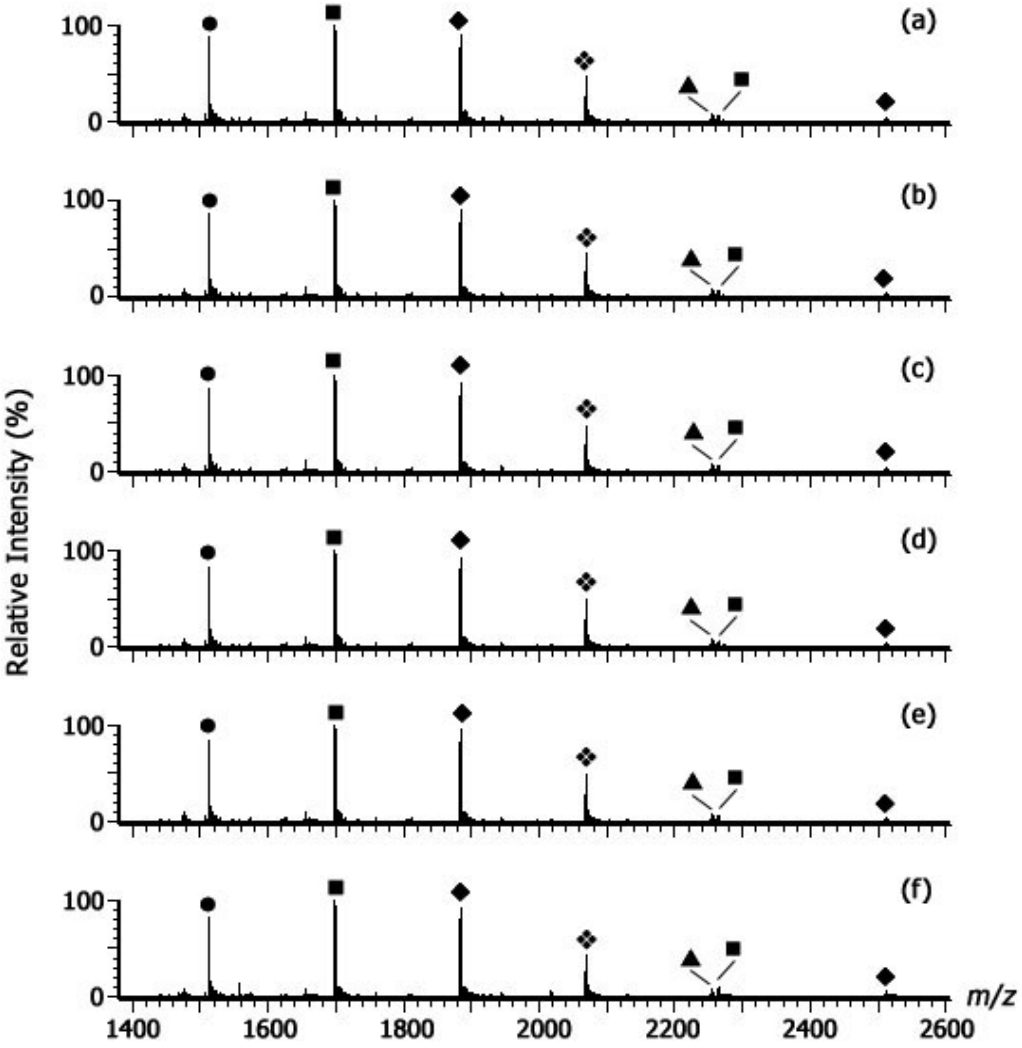
Experimental Details

The ETS domain of transcription factor PU.1 (i.e. residues 158-270 = PU.1-DBD) was purified as previously described,[1] and initially obtained as a 220 μM solution in 10 mM sodium phosphate buffer, pH 7. Prior to mass spectral measurements, aliquots of the protein were diluted to a final volume of 300 μL using 400 mM ammonium acetate, pH 7.2. In order to effect complete removal of sodium phosphate, the resulting protein solution (~ 3.6 μM) was dialysed against two litres of the same solvent at 4˚C (3 changes). $[Ru(phen)_2(dppz)]^{2+}$ and $[Pt(5,6\text{-}Me_2phen)(S,S\text{-dach})]^{2+}$ (the latter also referred to as 56MESS in the literature) were prepared using adaptations of literature procedures.[2,3] Single stranded oligonucleotides were obtained from Geneworks, South Australia, and purified as described previously.[4] The dsDNA molecule P3 was obtained by heating equimolar quantities of the requisite single stranded oligonucleotides (in 400 mM ammonium acetate, pH 7.2) to 20˚C higher than the melting temperature of the duplex for 15 min, and annealing by allowing the solution to cool slowly overnight.

All reagents used for preparing reaction mixtures containing PU.1-DBD, P3 and either metal complex were themselves dissolved in 400 mM ammonium acetate, pH 7.2. The procedure used for preparing a typical reaction mixture was as follows: 1 μL of 29 μM P3 was added to 8 μL of 3.6 μM PU.1-DBD, which was then allowed to stand for 10 min at room temperature. Subsequently 1 μL of 86.4 μM stock metal complex solution was added, and the reaction mixture allowed to stand for a further 10 min. This gave a final dsDNA concentration of 2.9 μM, and a PU.1-DBD:P3:metal ratio of 1:1:3. ESI mass spectra of solutions were obtained using a Waters Q-ToF Ultima™ ESI mass spectrometer, equipped with a borosilicate capillary for performing nanospray to reduce the volume of sample required for analysis, and a Z-spray probe. The capillary, cone and RF lens 1 energies were 1500, 150 and 70 V, respectively.

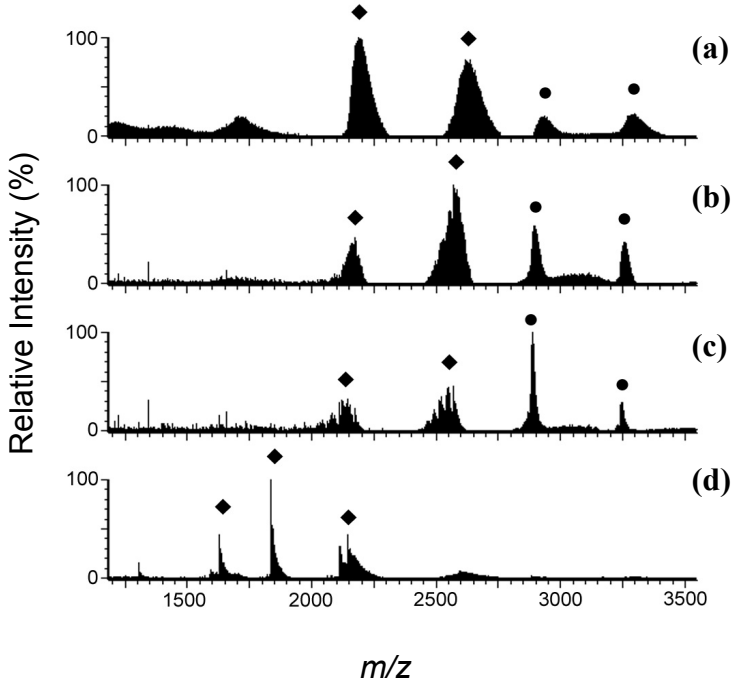
1. C.W. Liew, K.D. Rand, R.J.Y. Simpson, W.Y. Yung, R.E. Mansfield, M. Crossley, M. Proetorius-Ibba, C. Nerlov, F.M. Poulson and J.P. Mackay, *J. Biol. Chem*., 2006, **281**, 28296.
2. C.M. Dupureur and J.K. Barton, *Inorg. Chem*., 1997, **36**, 33.
3. N.J. Wheate, R.I. Taleb, A.M. Krause-Heuer, R.L. Cook, S. Wang, V.J. Higgins and J.R. Aldrich-Wright, *Dalton Trans*., 2007, 5055.
4. C. Kelso, V. Tillott, J.D. Rojas, R.L.A. Furlan, G. Padilla and J.L, Beck, *Arch. Biochem. Biophys*., 2008, **477**, 348.

Effect of Cone Voltage on the Appearance of ESI Mass Spectra of Solutions Containing $[Ru(phen)_2(dppz)]^{2+}$ and the DNA duplex P3.



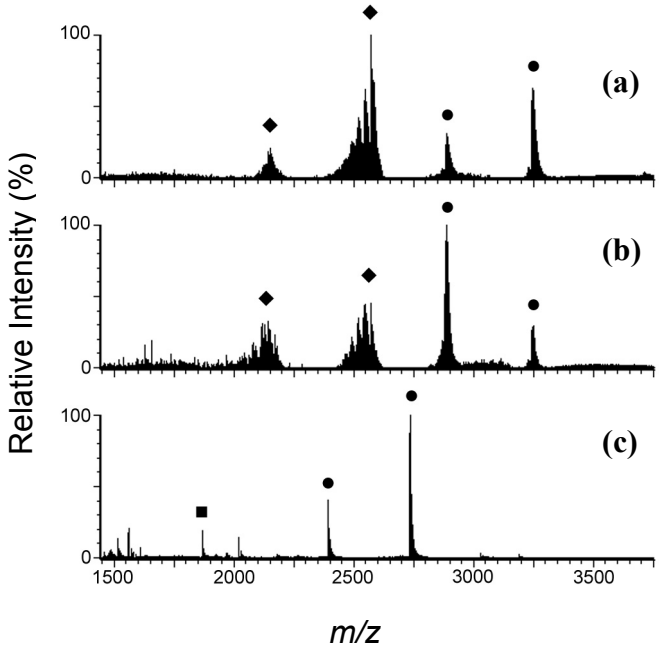
Effect of varying cone voltage on the appearance of the negative ion nanoESI mass spectrum of a solution containing a 10:1 ratio of $[Ru(phen)_2(dppz)]^{2+}$ and P3. (a) Cone voltage = 200 V; (b) Cone voltage = 170 V; (c) Cone voltage = 150 V; (d) Cone voltage = 130 V; (e) Cone voltage = 100 V; (f) Cone voltage = 70 V. ● Free P3; ■ P3 + 1M; ◆ P3 + 2M; ❖ P3 + 3M where M = $[Ru(phen)_2(dppz)]^{2+}$.

Effect of NH_4OAc Concentration on the Appearance of ESI Mass Spectra of Solutions Containing PU.1-DBD and the DNA molecule P2 (5′-TTGGTTTCACTTCCTTTTATT-3′/5′-AATAAAAGGAAGTGAAACCAA-3′).



Positive ion nanoESI mass spectra of reaction mixtures containing equimolar amounts of PU.1-DBD and P2 in: (a) 100 mM, (b) 250 mM, (c) 400 mM and (d) 1000 mM NH_4OAc, pH 7.2. ◆ Free P2; ● P2 + PU.1-DBD.

Effect of Changing DNA on the Quality of ESI Mass Spectra of Solutions Containing PU.1-DBD and duplex DNA.



Positive ion nanoESI mass spectra of solutions containing a 1:1 ratio of PU.1-DBD and: (a) P1, (b) P2 and (c) P3. ◆ dsDNA ; ●PU.1-DBD/DNA complex ; ■ PU.1-DBD.

P1 = 5′-CTGGTTTCACTTCCTCTCGCG-3′/5′-GCGGAGAGGAAGTGAAACCAG-3′
P2 = 5′-TTGGTTTCACTTCCTTTTATT-3′/5′-AATAAAAGGAAGTGAAACCAA-3′
P3 = 5′-CACTTCCGCT-3′/5′-AGCGGAAGTG-3′