Supplementary Information for

The use of Ion Mobility Mass Spectrometry to assist Protein Design: a Case Study on Zinc Finger Fold versus Coiled Coil Interactions

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When a protein has n ligand-binding sites, the reaction is then:

$$P + nL \Leftrightarrow PL_n$$ ***Equation 1***

When a protein has one ligand-binding site, the reaction above will take form:

$$P + L \Leftrightarrow PL$$ ***Equation 2***

In this case, the dissociation constant K_d is expressed as follows:

$$K_d = \frac{[P][L]}{[PL]}$$ ***Equation 3***

where [P] is the concentration of the unbound state of the peptide, [L] is the concentration of free ligand and [PL] is the concentration of bound ligand.

Initial concentrations of peptide [P_i] and added metal salt (i.e. ligand) [L_i] are always known. The degree (*i.e.* velocity *v*) of binding can be defined as the average number of bound ligand units per target unit and is expressed as:

$$v = \frac{[PL]}{[P] + [PL]}$$ ***Equation 4***

Therefore the sum in the denominator of the Equation 4 is the total concentration of the peptide added initially:

$$[\mathrm{P_i}] = [\mathrm{P}] + [\mathrm{PL}]$$ ***Equation 5***

The concentration of the free ligand is given by the difference between initial (added) and bound ligand concentration:

$$[\mathrm{L}] = [\mathrm{L_i}] - [\mathrm{PL}]$$ ***Equation 6***

When the normalised values obtained from Equation 4 are plotted against those in Equation 6, and the Hill curve is fitted to the data points, the point on this curve at 0.5 binding (or 50% target saturation) gives the dissociation constant in ligand concentration units. The Hill curve is described by the following sigmoidal equation:

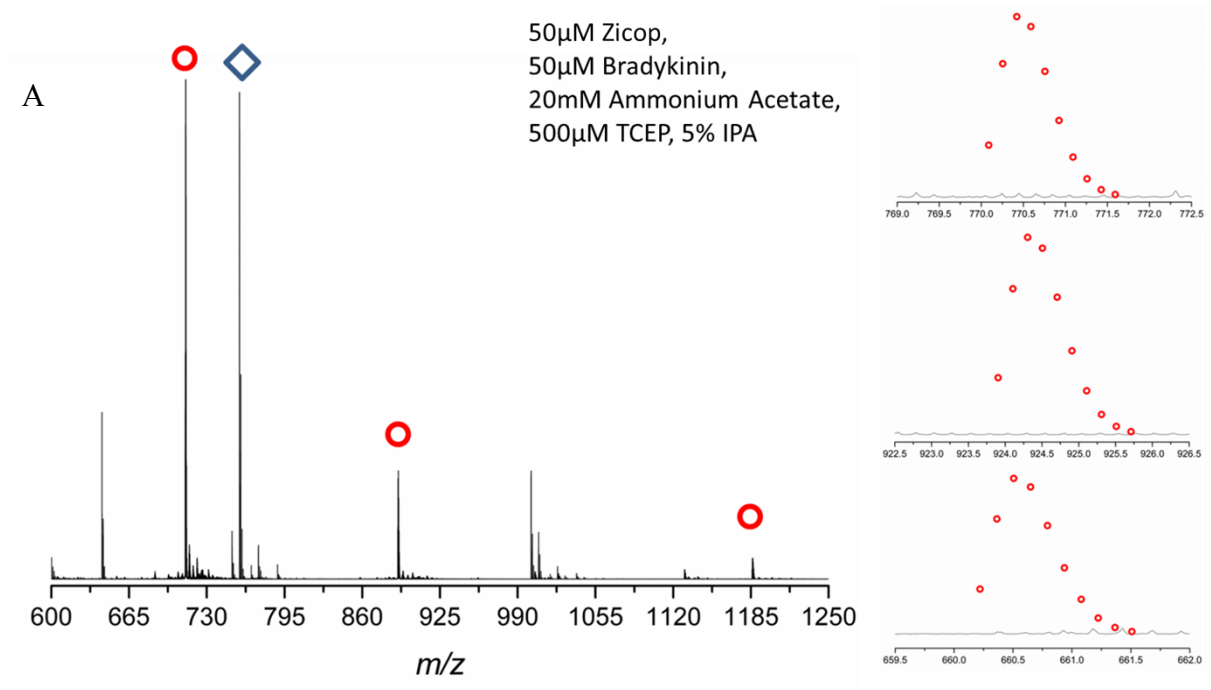
$$v = \frac{V_{\max}[L]^n}{K_d^n + [L]^n}$$ ***Equation 7***

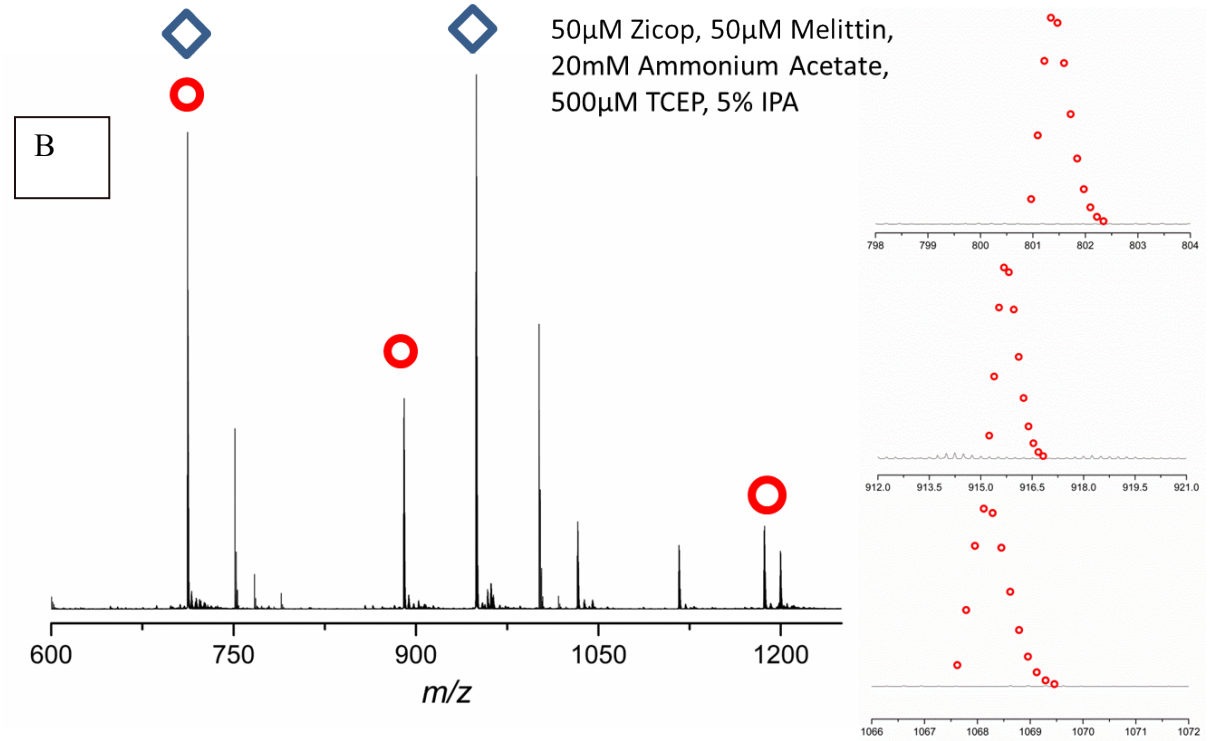
where V_{max} is the highest degree of binding (maximum velocity achieved by the system), n is the number of cooperative sites and K_d is the Michaelis constant that is equal to the ligand concentration at which the binding rate is half of V_{max}. In case of single-site binding (n = 1), no cooperativity is observed, and the curve becomes hyperbolic and is described by the Michaelis-Menten equation:

$$v = \frac{V_{\max}[L]}{K_d + [L]}$$ ***Equation 8***

The linear part of the slope of such curve gives an indication of the range of concentrations at which dissociation occurs.

Control Experiments to verify the specificity of ZiCop binding to the partner peptide.





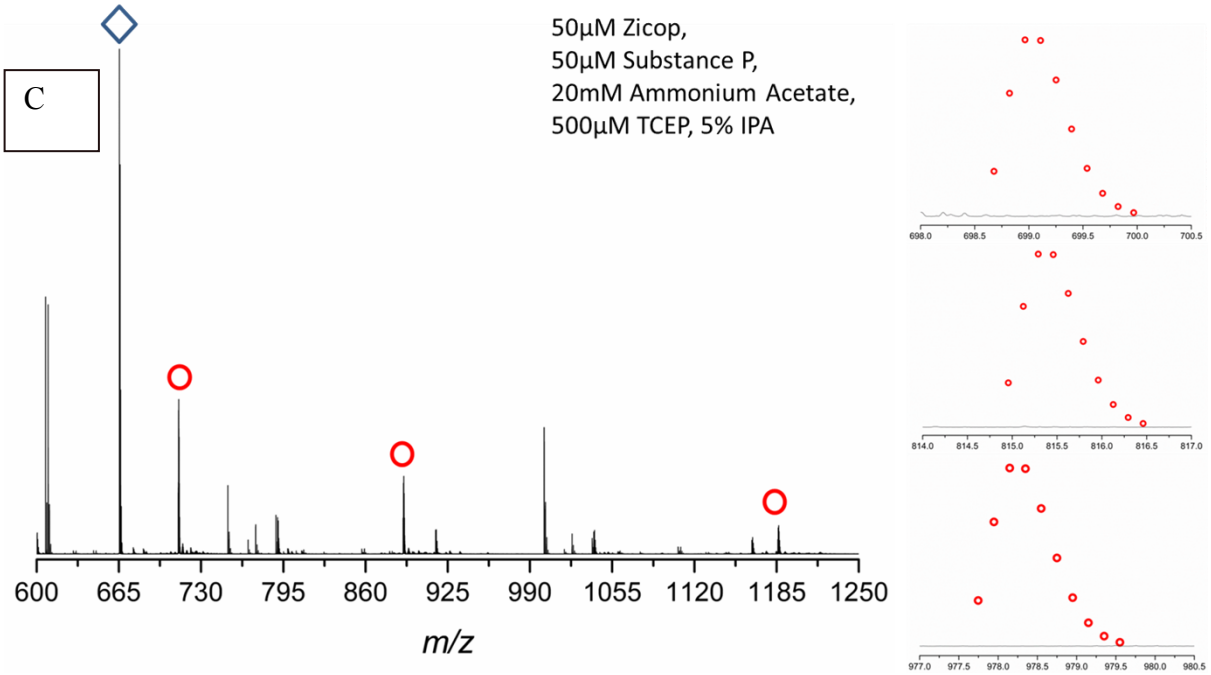


Figure S1. *Zicop Incubated with Bradykinin 1-7 (756.85 Da) (A), Melittin (2846.4 Da) (B) and Substance P (1329.8 Da) (C). In each spectrum the circle corresponds to a charge state of ZiCop, and the square to assigned species of the incubated control peptide. The inserts are regions of the mass spectrum where any bound complex would be located and clearly indicate the lack of any such complex. The predicted isotopic distribution of the putative complexes is added for completion.*

Metal ions and ZiCop – quantifying metal ion affinity by spectroscopic measurements

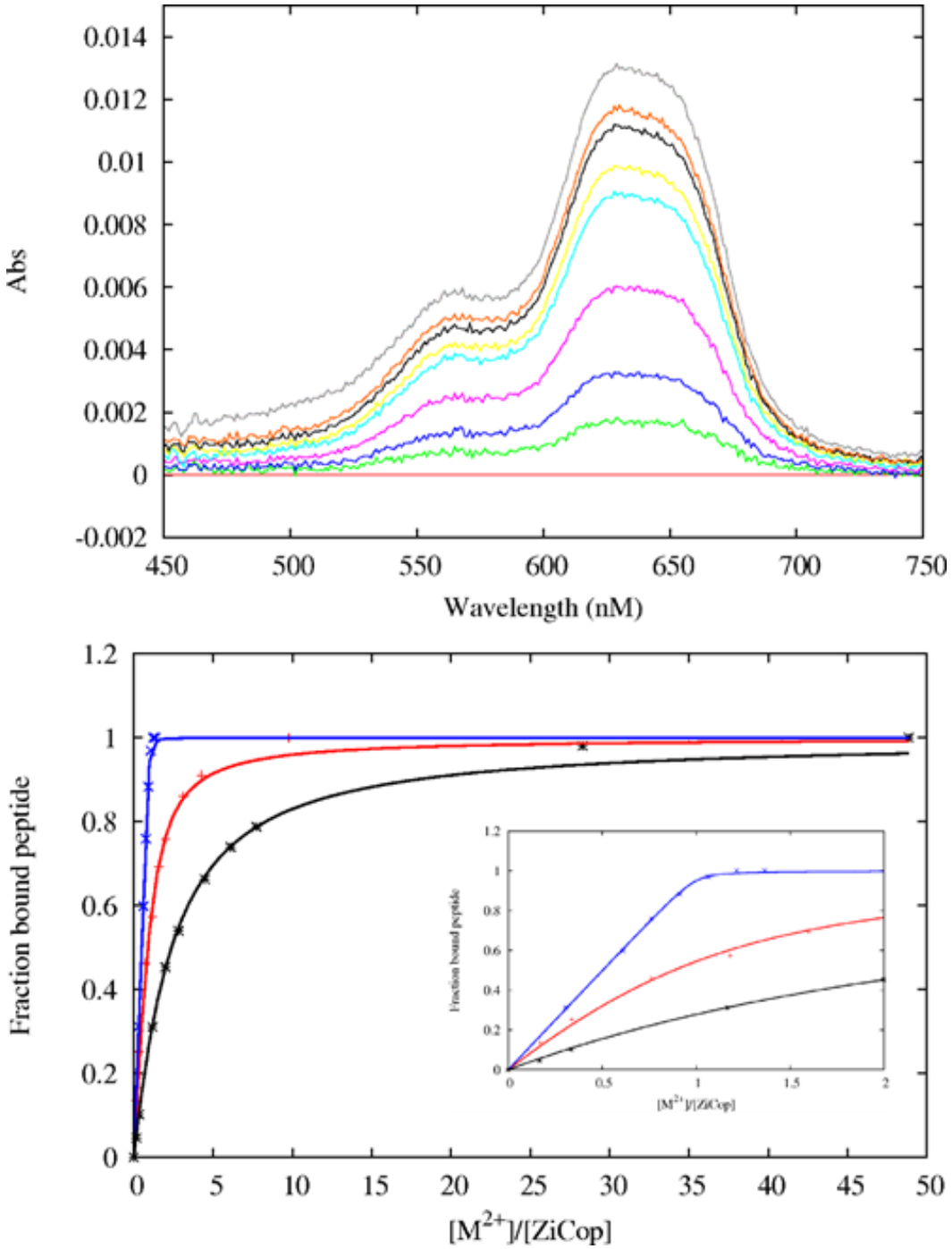


Figure S 2. *Metal-binding properties of ZiCop as monitored by UV-visible spectroscopy. (A) The spectra obtained when cobalt was added to ZiCop (peptide conc. 19.57 µM, cobalt concentration ranging from 1 to 190 µM). (B) Titrations followed at 630 nm plotted as a function of added cobalt. Red – cobalt binding to 19.6 µM ZiCop (Kd ≈ 7.5 µM), Blue – zinc displacing 278 µM cobalt from 14.5 µM ZiCop (Kd ≈ 40 nM), black – cobalt binding to ZiCop in the presence of 21.2 µM partner peptide (Kd ≈ 28 µM). The inset shows the binding properties at lower metal concentrations more clearly. All spectra recorded at pH 7.2.*

Peptide-metal and peptide-peptide – qualitative definition of complex stability by CID

The dissociation events of the two homo-dimers – 2ZiCop and 2Pp, are shown in Figure S3 A and B respectively and are discussed in the main text. The dissociation product of the dimerised zinc-bound ZiCop – 2(Zn-ZiCop), shown in Figure S2 C – is largely peptide apo-form, with a small population of holo-ZiCop. Thus we can infer that a large proportion of the precursor ion was not involved in specific association between peptide and metal. Additionally, the 2(Zn-ZiCop) ion has the same dissociation energy as the 2ZiCop ion, yet again confirming the non-specific nature of the 2(Zn-ZiCop) aggregate whereby the metal ion is not coordinated tetrahedrally.

Indeed, stabilisation of coiled coils by metal ions has been explored as an independent target design by a number of groups, whereby Cys and/or His on the first coil and the same residues on the second (and sometimes third) coil in the bundle are held together by transition metal ions[1; 2]. The K_ds of such associations are one to two orders of magnitude higher[2; 3] (*i.e.* weaker affinity) than those reported for designed zinc finger folds measured by analogous spectroscopic methods[4; 5]. Therefore, such metal-sandwiched complexes are innately weaker than highly-structured zinc finger folds, and the observed effect here is possibly due to this type of associations.

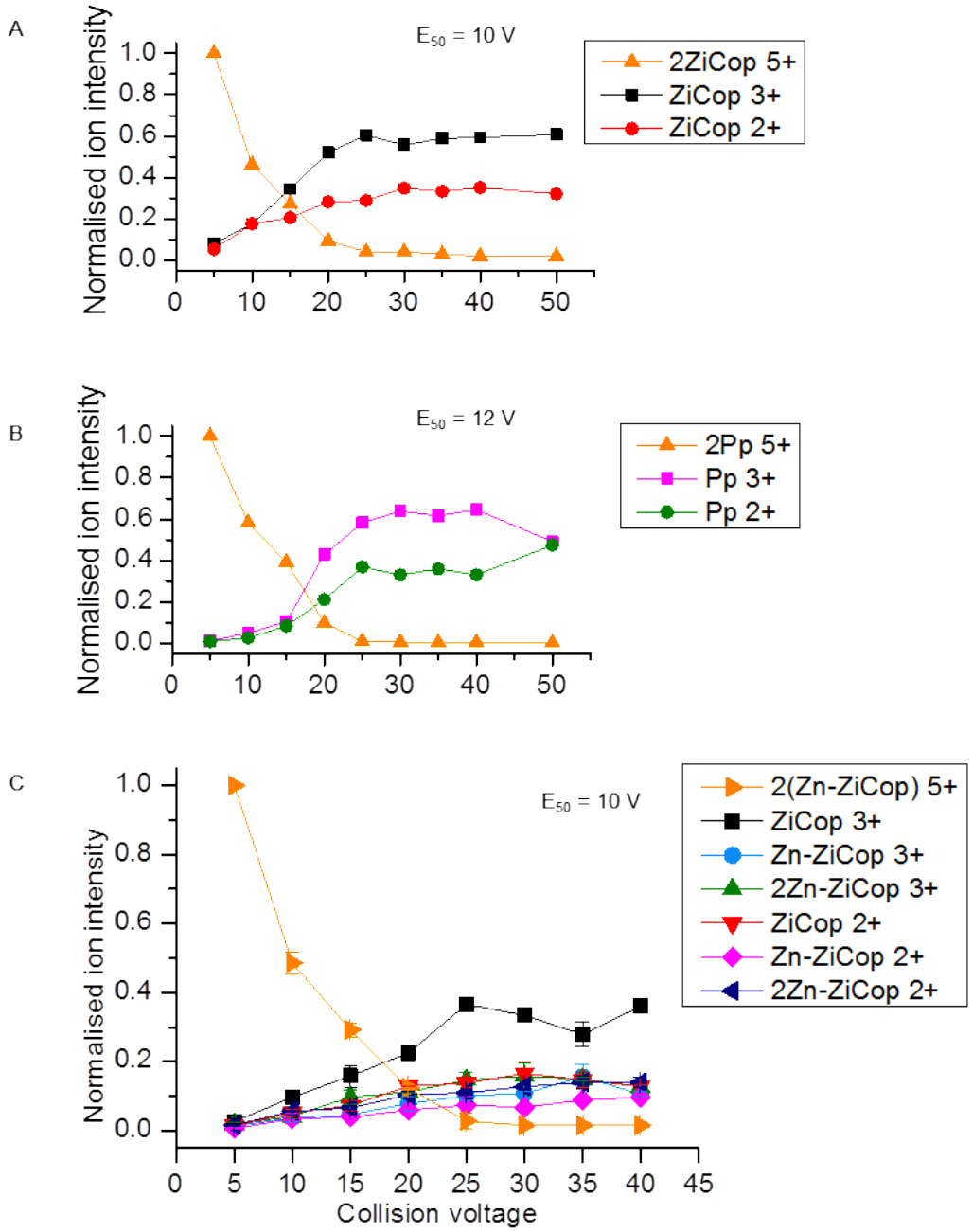


Figure S 3. *Stability of non-covalent complexes as a function of dissociation energy. Fragmentation results of the following +5 charge state complexes are shown: A –2ZiCop; B – 2Pp; C – 2(Zn-ZiCop). Stability of complexes is calculated from the intensities of the precursor and product ions during CID. The orange curves show dissociation of the precursor ion, all other curves are dissociation products, and the E_{50} values for the precursors are shown for each plot. Each data point is the mean (± standard error) of the equilibrium concentrations from three mass spectra.*

References

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