Supplementary Material (ESI) for Dalton Transactions

Structural features of the Zn^{2+} complex with the single repeat region of of "Prion Related Protein" (PrP-rel-2) of Zebrafish zPrP (63-70) fragment

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SUPPLEMENTARY INFORMATION

Peptide synthesis and purification – Synthesis of zebrafish PrP-rel-2(63-70) fragment was performed on a solid-phase using Fmoc (Fmoc = 9-fluorenylmethoxycarbonyl) strategy with continous-flow methodology (9050 Plus Millipore Peptide Synthesizer) on a polystyrene/polyethylene glycol copolymer resin (TentaGel R RAM Resin, substitution 0.18 mmol/g) (1). Attachment of the first amino acid to the resin and next coupling steps were realized using diisopropylcarbodiimide (DIPCI) as a coupling reagent with 1-hydroxybenzotriazole (HOBt) as an additive. Removal of Fmoc protecting group during peptide synthesis was achieved by means of 20% piperidine solution in DMF / NMP (1:1, v/v) with addition of 1% Triton X-100 (1). The peptides was cleaved from the resin and deprotected by treatment with the mixture containing trifluoroacetic acid, phenol, triisopropylsilane and water (88:5:2:5, v/v), for 2h at room temperature (1). The resulting crude peptide was purified by reversed-phase high-performance liquid chromatography (RP-HPLC) using a C8 semi-preparative Kromasil column (25 × 250mm, 7μm). The purity of the peptide was confirmed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) and analytical RP-HPLC using a C4 Kromasil column (4.6 × 250mm, 5μm) and 30 min. linear gradient of 0-80% acetonitrile in 0.1% aqueous trifluoroacetic acid as a mobile phase. The peptide was first dissolved in H_2O or D_2O. The pH values were measured at room temperature and they were adjusted at desired values with either DCl or NaOD solutions. The desired concentration of copper ions was achieved by using a stock solution of zinc(II) cloride (Sigma Chemical Co.) either in water or deuterium oxide. TSP-d_4, 3-(trimethylsilyl)-[2,2,3,3-d_4] propansulphonate, sodium salt, was used as internal reference standard.

NMR experiments: NMR experiments were carried out at 14.1 T or at 9.4 T at controlled temperature (± 0.1 K) on a Bruker Avance 600 MHz or Bruker AMX 400 MHz spectrometer equipped with a Silicon Graphics workstation. Suppression of residual water signal was achieved either by presaturation or by excitation sculpting, (2) using a selective square pulse on water 2 ms long. Proton resonance assignment was obtained by COSY, TOCSY, NOESY and ROESY experiments. The diffusion coefficients were measured at 298 K by a PFG longitudinal eddy-current delay (LED) pulse sequence with bipolar gradients incorporating spoil gradients during both longitudinal storage periods (3-5). The gradient strength was incremented (with an initial value of

0.86 G cm-1 and a step size of 2.65 G cm-1 for 2 ms), while the separations of the field gradients and the total echo time were kept constant. A series of 16 spectra, with a number of scans ranging from 16 to 32, was recorded in 2D mode for each measurement, with a recycle time of 10 s between scans. The diffusion values were calculated by regression analysis of the signal decay leading to errors not larger than ± 2-5%. The strength of the B_0 field gradient was calibrated by measuring the self-diffusion coefficient of the residual HDO signal in a 100% D_2O sample at 298 K (6-8).

Structure determination and molecular dynamics simulations: For the Zn(II)-zPrP63-74, the intensities of NOESY cross-peaks, referenced to cross-peaks related to proton pairs at fixed distances, were converted into proton-proton distance constraints. These constraints were used to build a pseudopotential energy for a restrained simulated annealing (SA) calculation in torsional angle space. In particular, we performed the calculation with the program DYANA (9), with 300 random starting structures of the complex, and 10000 steps of SA. Since only one molecule can be given as input in the program, the peptide was linked to Zn(II) through a long chain of linkers, *i.e.*, residues made by atoms without van der Waals radius, which could freely rotate around their bonds, without causing steric repulsions, and thus enable one to sample a large number of relative positions of the ligand with respect to the metal ion before the minimization step.

References:

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Figures

Figure S1. ^{1}H-^{13}C HSQC spectra of zPrP63-70 in absence (black contours) and in presence (green contours) of 2.0 Zn(II) eqs. T=298K, pH 7.5