

# Supporting Information for “Electrostatic Determinants of Stability in Parallel 3-Stranded Coiled Coils”

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## Materials and Methods

### General

Mass spectrometry of peptides was performed on a Voyager DE-PRO (Applied Biosystems) using using  $\alpha$ -cyano-4-hydroxycinnamic acid as the matrix. Data are the averages of sixteen laser shots. UV-visible absorbance spectra were recorded in 1 cm quartz cells (Hellma, Switzerland) using a Cary-100 spectrophotometer (Varian). Circular dichroism spectrophotometry (CD) was performed on a Jasco 815 spectropolarimeter (Jasco) equipped with Peltier stage. CD spectra were acquired using a 4 nm bandwidth and a scan rate of 2 nmsec<sup>-1</sup>. Per residue molar ellipticities,  $[\theta]_{MRW}$  are calculated for a peptide concentration of 150  $\mu$ M assuming 19 contributing residues.

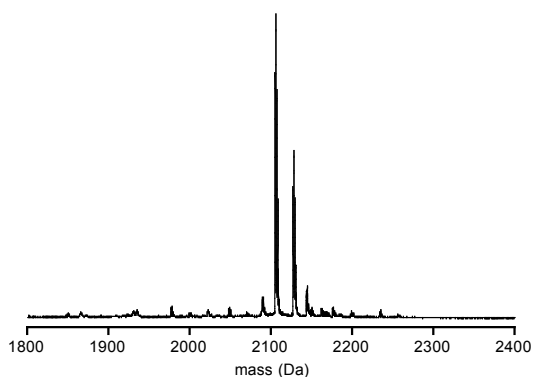
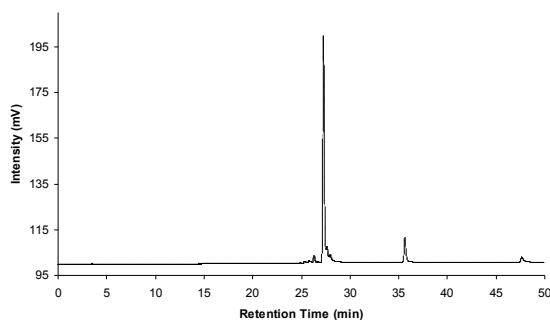
### Peptide synthesis

All reagents were obtained from commercial suppliers and used as received unless otherwise stated. Protected amino acids, N-hydroxybenzotriazolyluronium hexafluorophosphate (HBTU), and 4-methyl benzhydrylamine (MBHA) resin (1% DVB cross-linked polystyrene, 200-400 mesh, 0.2 mmol/g) were purchased from Novabiochem or Advanced Chemtech. 2,2'-Bipyridyl-4-carboxylic acid was prepared according to literature procedures.<sup>1</sup> N,N'-Dimethylformamide (DMF) was dried over freshly activated 4 Å molecular sieves for at least 12 hours before use. Peptides were synthesized on a 50  $\mu$ mol scale using an ACT 396 Omega multiple peptide synthesizer (Advanced Chemtech) using standard Fmoc protocols with eight equivalents of protected amino acid, 7.95 equivalents of HBTU and 16 equivalents of N,N'-diisopropylethylamine (DIEA) per coupling. Amino acid and HBTU solutions were prepared 0.5 M in dry DMF. Coupling times were 30 min, and Fmoc deprotection was effected using 30 % v/v 4-methylpiperidine in DMF for 5 min followed by 15 % v/v 4-methylpiperidine in DMF for 10 min. The resin was washed 5 times with dry DMF between coupling and deprotection cycles.

2,2'-Bipyridyl-4-carboxylic acid was appended to the deprotected N-terminus of the peptide by activation with HBTU (0.95 eq.) and DIEA (4 eq) in DMF over 1 hr. Resin-bound peptides were washed with dry dichloromethane (DCM) and side-chain protecting groups were removed by treatment with trifluoroacetic acid (TFA, 2 x 1 min). Peptides were cleaved from the support by treatment with anhydrous HF (10 mL, 45 min at 0 °C) with 10 % v/v anisole as scavenger. After evaporation of HF, the crude peptide residue was suspended in dry ether, filtered, and washed with dry ether. The solids were resuspended in acetic acid (5% v/v, 100 mL), filtered to remove the resin beads, and the filtrate was lyophilized to dryness. Crude peptides were purified by reverse-phase HPLC (Higgins C18 preparative column) by gradient elution using water and acetonitrile with 0.1% v/v TFA as ion-pairing agent. The effluent stream was monitored by UV-visible absorbance at 230 nm. Peptide purity was assessed by analytical HPLC (Higgins C18 analytical column) and MALDI-TOF mass spectrometry.

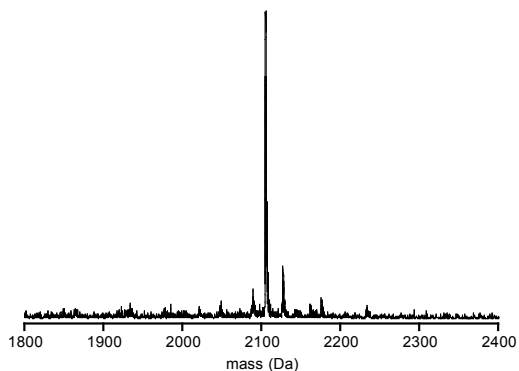
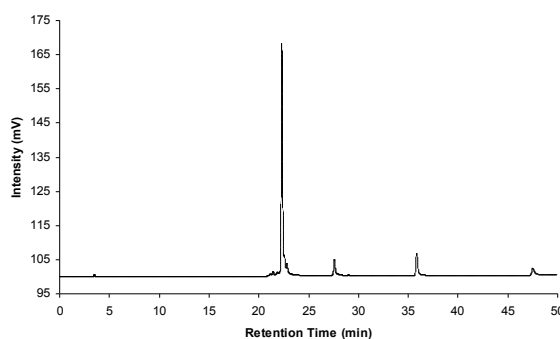
*KeEc'*

Observed: 2105.82; 2127.84  
Calculated: 2106.46 (MH<sup>+</sup>); 2128.46 (MNa<sup>+</sup>)



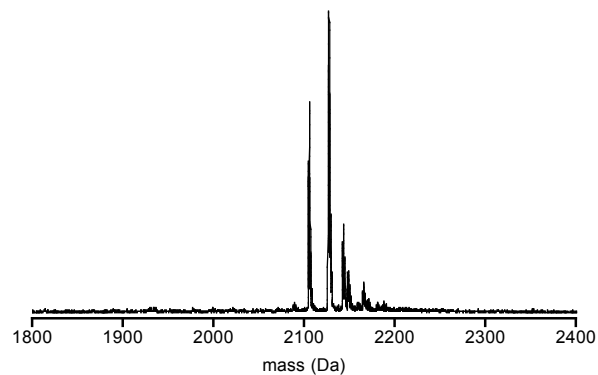
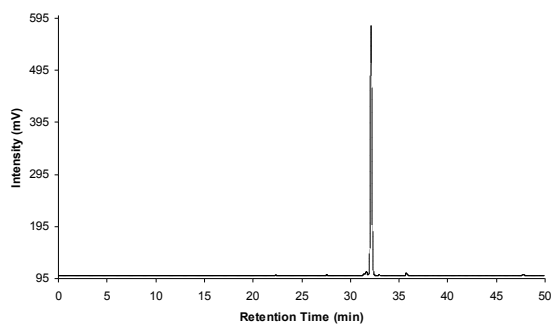
*KeEg'*

Observed: 2105.04; 2127.01  
Calculated: 2106.46 (MH<sup>+</sup>); 2128.46 (MNa<sup>+</sup>)



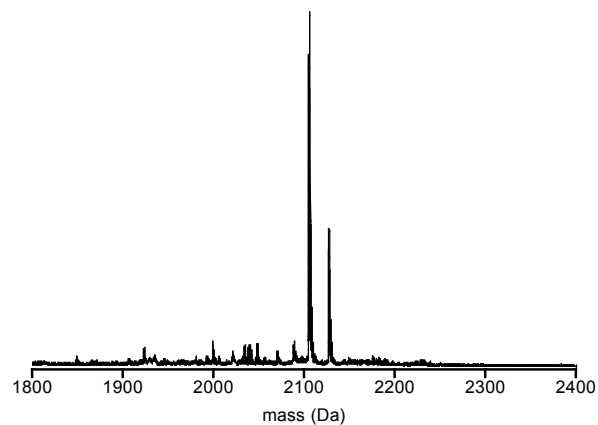
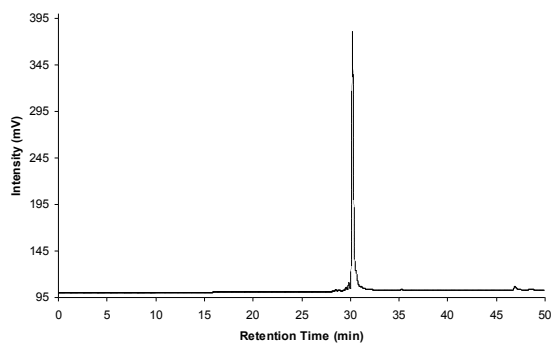
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Observed: 2105.00; 2126.97  
Calculated: 2106.46 (MH<sup>+</sup>); 2128.46 (MNa<sup>+</sup>)



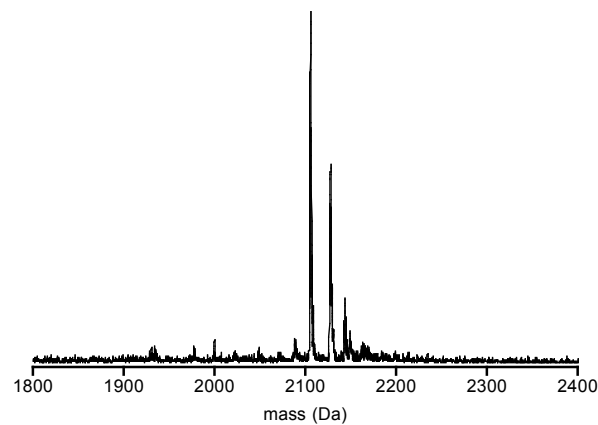
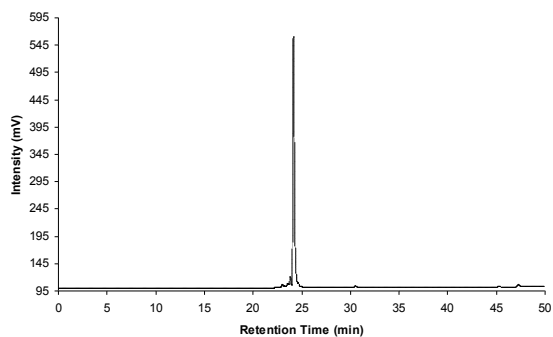
*EeKc'*

Observed: 2105.13; 2127.07  
Calculated: 2106.46 (MH<sup>+</sup>); 2128.46 (MNa<sup>+</sup>)

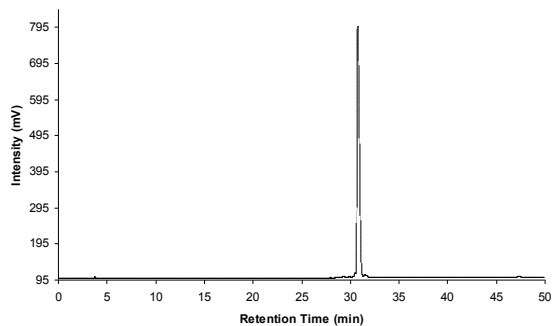


*EeKg'*

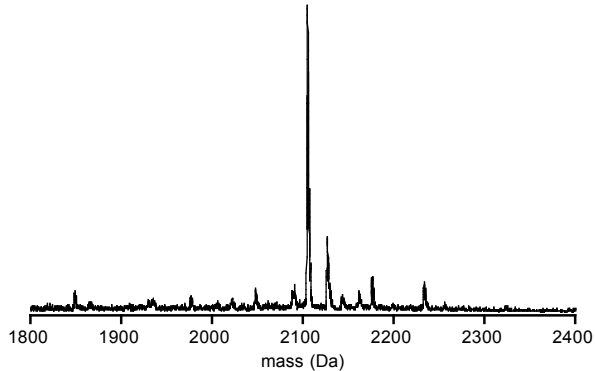
Observed: 2105.09; 2127.20  
Calculated: 2106.46 (MH<sup>+</sup>); 2128.46 (MNa<sup>+</sup>)



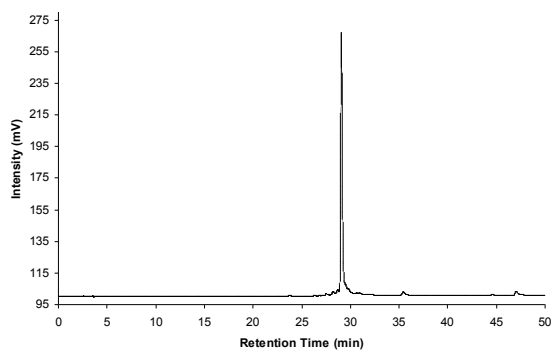
*EbKg'*



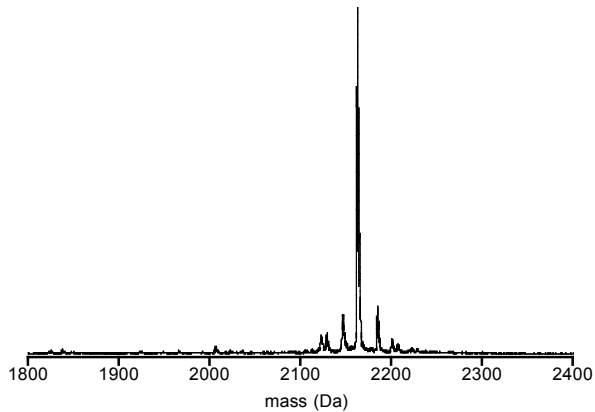
Observed: 2104.72; 2126.92  
Calculated: 2106.46 (MH<sup>+</sup>); 2128.46 (MNa<sup>+</sup>)



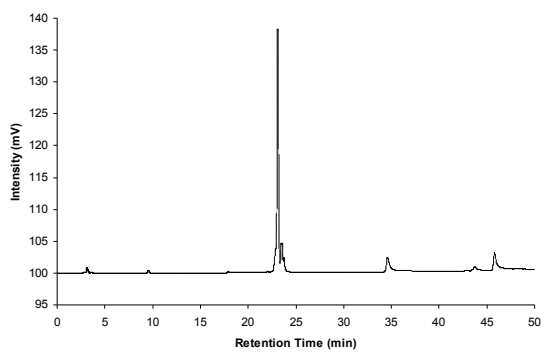
*EeRc'*



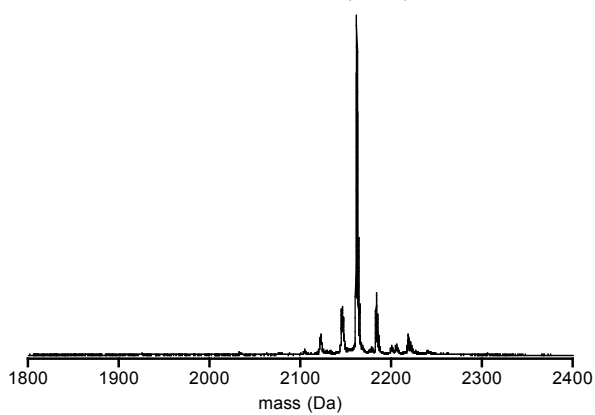
Observed mass: 2162.72  
Calculated mass: 2162.49 (MH<sup>+</sup>)



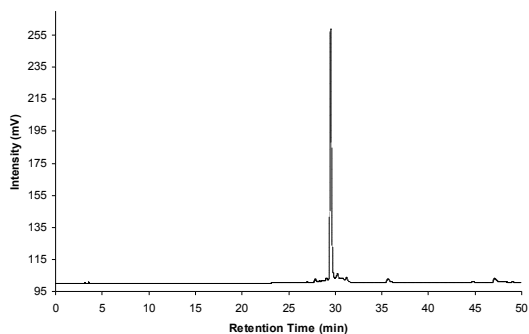
*EeRg'*



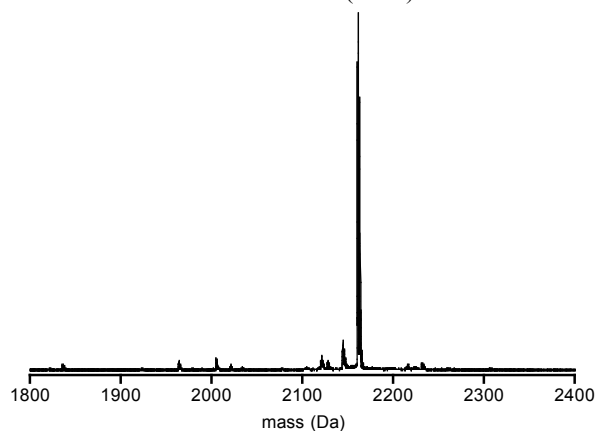
Observed mass: 2161.74  
Calculated mass: 2162.49 (MH<sup>+</sup>)



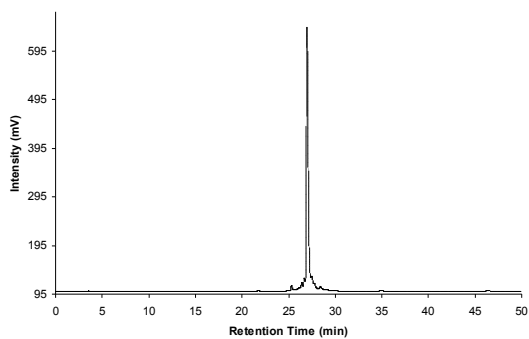
*EbRg'*



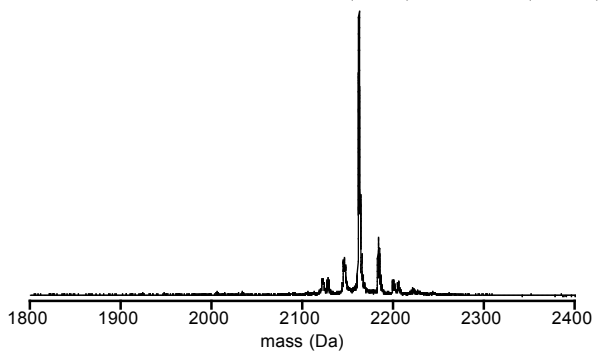
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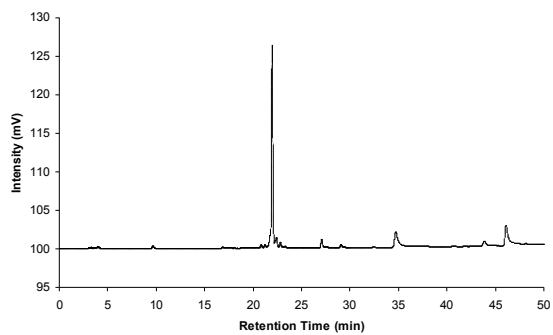
*ReEc'*



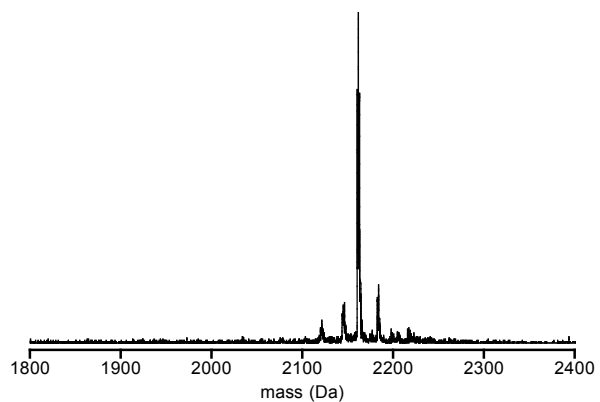
Observed mass: 2161.83; 2183.72  
Calculated mass: 2162.49 (MH<sup>+</sup>); 2184.49 (MNa<sup>+</sup>)



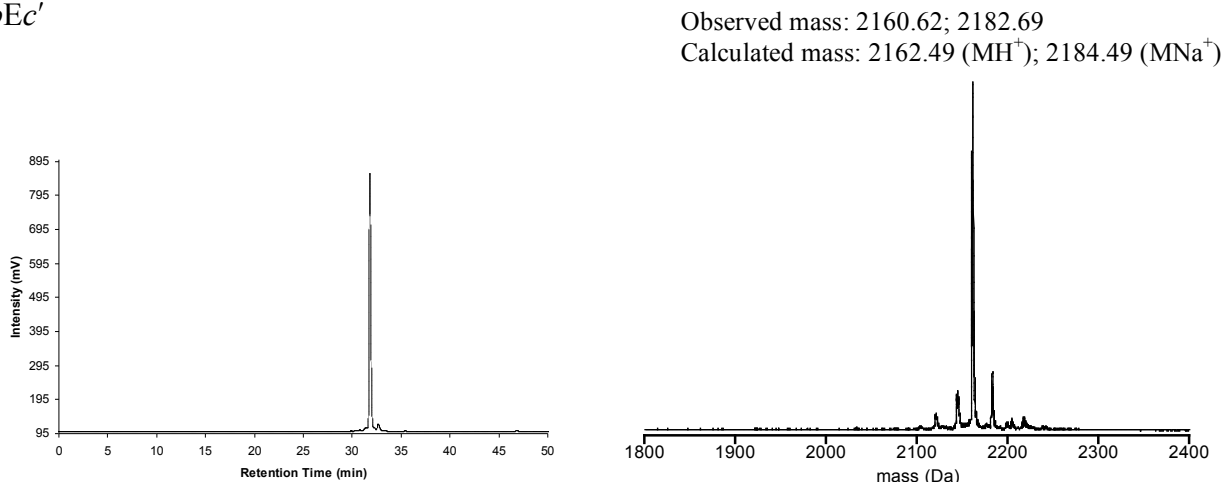
*ReEg'*



Observed mass: 2160.95; 2182.93  
Calculated mass: 2162.49 (MH<sup>+</sup>); 2184.49 (MNa<sup>+</sup>)



RbEc'

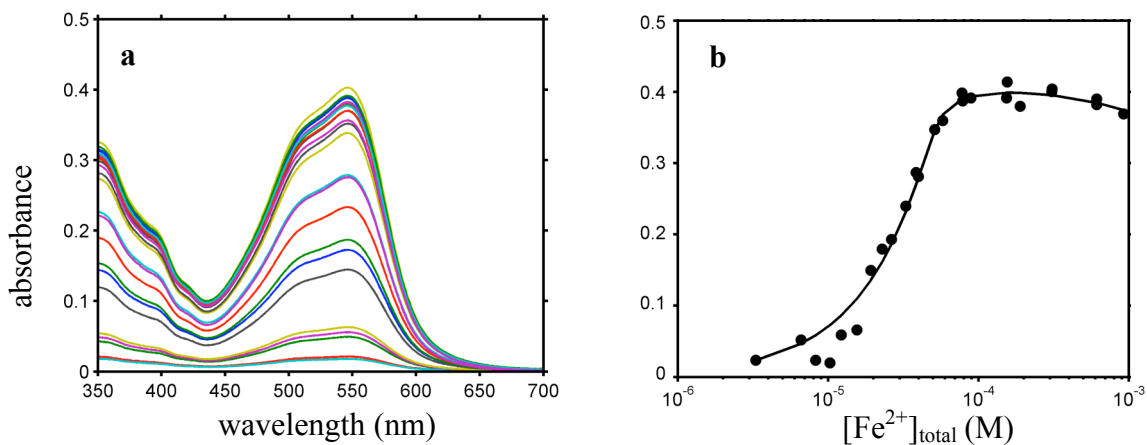


### Iron(II) complexes

Solutions of lyophilized peptides (1–5 mM, 250  $\mu$ L) were prepared in ddH<sub>2</sub>O resulting in solutions of pH  $\sim$ 3. The peptide solutions were degassed (3 cycles of freeze-pump-thaw) and transferred to a nitrogen glove box. Excess solid ferrous ammonium sulfate (2 – 3 mg) was added under N<sub>2</sub>, and the red complex allowed to form over 30 min. Excess iron salts were removed by gel filtration (PD 10, Pharmacia) using acetate buffer (pH 6.5, 5 mM except where stated otherwise). The resulting solutions were stable in air indefinitely. The concentrations of the solutions were determined from the absorbance at 303 nm ( $\epsilon_{303} = 36,000 \text{ M}^{-1} \text{ cm}^{-1}$ ).

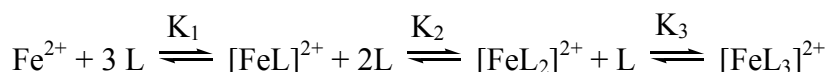
### Titration of 2,2'-bipyridyl-5-glycinamide with iron(II)

Spectra were recorded in sodium acetate buffer (20 mM, pH 6.0) using 1 cm path length quartz cells (Hellma).



**Figure S1.** Titration of 2,2'-bipyridyl-5-glycinamide with iron(II). (a) Raw data, (b) first principal component fitted to 5-component equilibrium.

2,2'-Bipyridyl-5-glycinamide was 300  $\mu\text{M}$ , and concentrations of  $\text{Fe}^{2+}$  (as ferrous ammonium sulfate) ranged from 2.5  $\mu\text{M}$  to 9.2 mM. Solutions of ferrous ammonium sulfate in water were prepared fresh for each sample and added to the 2,2'-bipyridyl-5-glycinamide within 30 s of dissolution. Singular value decomposition of the matrix of spectra shown in Figure S1(a) yielded 3 principal components. The dominant vector  $\mathbf{u}_1$  was identified with the complex  $[\text{Fe}(\text{bpy-gly-NH}_2)_3]^{2+}$ . The corresponding orthogonal vector  $\mathbf{v}_1$  is shown in Figure S1(b) together with the fit to the 5-component equilibrium



where L is 2,2'-bipyridyl-5-glycinamide. Mass balance is satisfied by the following relationships:

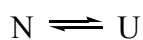
$$[\text{Fe}^{2+}]_{\text{total}} = [\text{Fe}^{2+}](1 + K_1[\text{L}] + K_1K_2[\text{L}]^2 + K_1K_2K_3[\text{L}]^3)$$
$$[\text{L}]_{\text{total}} = [\text{L}] + K_1[\text{Fe}^{2+}][\text{L}] + 2K_1K_2[\text{Fe}^{2+}][\text{L}]^2 + 3K_1K_2K_3[\text{Fe}^{2+}][\text{L}]^3$$

SVD and data fitting were performed using Matlab (MathWorks).

### Urea unfolding studies

CD spectra from 350–210 nm were recorded at 16 urea concentration increments of approximately 0.375 M from 0 to 6 M with an equilibration time of 20 mins at each step. Peptide concentrations were 150  $\mu\text{M}$  (50  $\mu\text{M}$  in trimer). Thermodynamic parameters were extracted from those data sets that showed sigmoidal cooperative unfolding. Singular value decomposition of the matrix of spectra  $\mathbf{D}$  (wavelength vs. ellipticity vs. urea concentration) into orthogonal basis vectors  $\mathbf{U}$ , a diagonal matrix of singular values  $\mathbf{S}$ , and a unitary row matrix of weighting coefficients  $\mathbf{V}$ , indicated that for cooperative unfolders > 97% of the data could be synthesized from two principal components, consistent with a two-state unfolding process. The two principal vectors of the matrix product  $\mathbf{SV}^T$  (the reduced data) were fitted to a model function as follows:

For the unfolding equilibrium



The equilibrium constant at any denaturant concentration  $c$  is given by

$$K(c) = [U]/[N]$$

The CD signal, and the ellipticities of the fully folded and fully unfolded states are thus related according to

$$\theta = (\theta_N + \theta_U K(c)) / (1 + K(c))$$

where

$$K(c) = \exp(-\Delta G^\circ(c)/RT)$$

According to Schellman's model<sup>2</sup> for urea denaturation

$$\Delta G^\circ(c) = \Delta G^\circ(\text{H}_2\text{O})(1 - c/c_m)$$

Where  $\Delta G^\circ(\text{H}_2\text{O})$  is the unfolding free energy at zero denaturant concentration, and  $c_m$  is the denaturant concentration at which  $\Delta G^\circ(c)$  is zero.

Given initial estimates of  $\theta_N$ ,  $\theta_U$ ,  $c_m$  and  $\Delta G^\circ(\text{H}_2\text{O})$ , model isotherms of  $\theta$  vs.  $c$  can be constructed. For two-state unfolding  $c_m$  and  $\Delta G^\circ(\text{H}_2\text{O})$  are identical for the isotherms of the fully folded and fully unfolded species. The first two principal components of the matrix product  $\mathbf{S}\mathbf{V}^T$  can be thus be simultaneously iteratively fitted to two  $\theta$  vectors to yield values of  $c_m$  and  $\Delta G^\circ(\text{H}_2\text{O})$ .

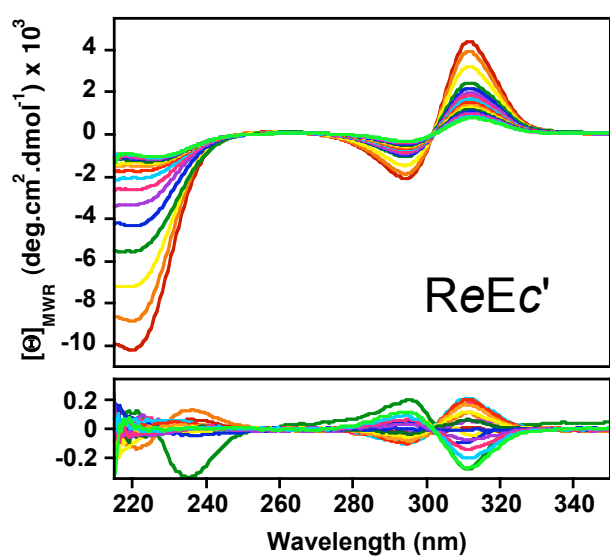
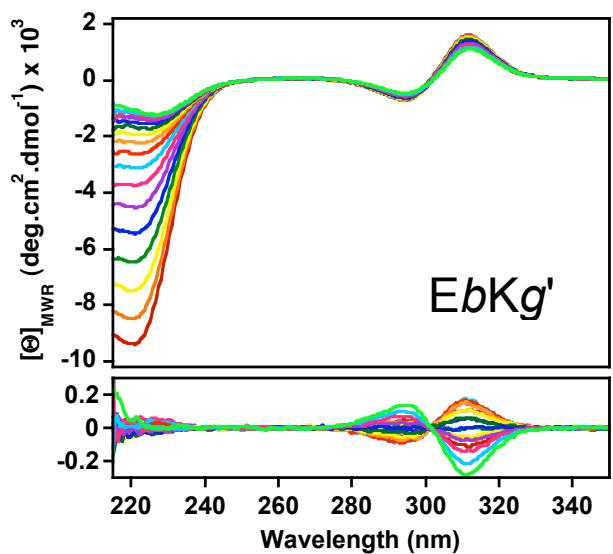
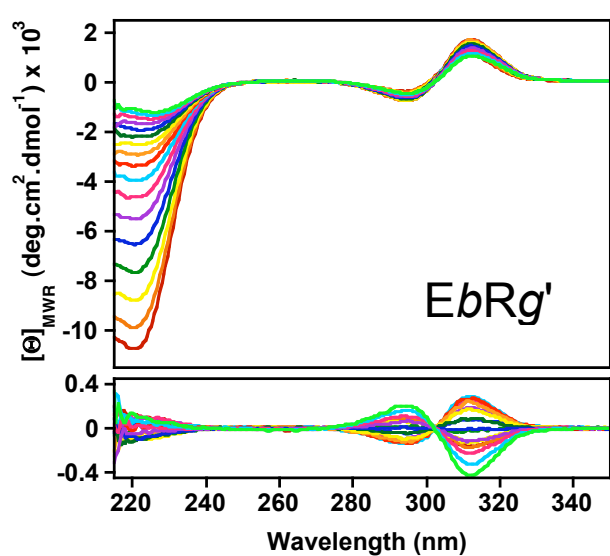
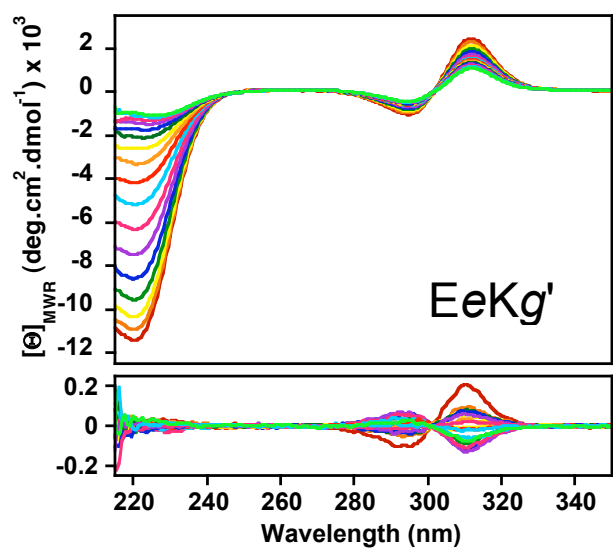
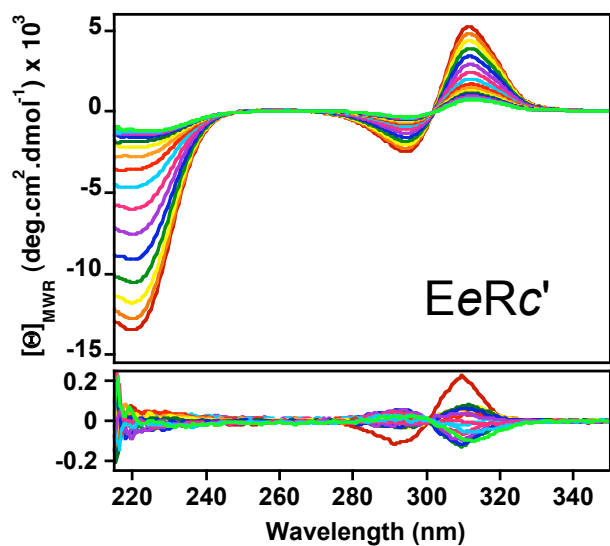
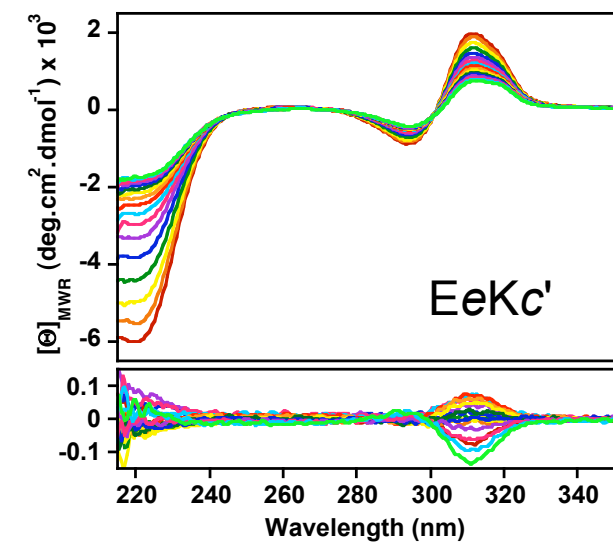
As an additional safeguard the mole fractions of N and U can be calculated from the fitted values of  $c_m$  and  $\Delta G^\circ(\text{H}_2\text{O})$  for all values of  $c$ . Basis spectra corresponding to the folded and unfolded species can then be extracted from the CD data matrix:

$$\mathbf{U}_{\text{calc}} = \mathbf{D}/\mathbf{F}^T$$

where  $\mathbf{F}$  is the 2-column matrix of mole fractions. The extracted spectra should be physically reasonable. The raw data  $\mathbf{D}$  and the residuals from  $\mathbf{D} - \mathbf{U}_{\text{calc}}\mathbf{F}^T$  for the six sequences that show cooperative unfolding are shown below.

SVD and data fitting were performed using Matlab (MathWorks).





## Database searches

A list of coiled-coil structures was compiled from the SOCKETS database of coiled-coil structures<sup>3</sup> concatenated with protein databank entries post 2001 containing the keyword “coiled-coil”. The resulting list contains 897 entries. A list of 158 EeRc' sequences was compiled from two ScanProsite<sup>4</sup> searches using the 9-residue search strings [FILMV]-{PC}-R-[FILMV]-{PC}(2)-{PCRK}-[FILMV]-{PC} for arginine at the *c* position of a heptad (2574 entries) and [FILMV]-{PC}(2)-[FILMV]-E-{PC}(2)-[FILMV]-{PC} for glutamate at the *e* position (2773 entries). The EeRc' list comprises 158 common sequences.

## Molecular modeling

Molecular modeling studies were performed using InsightII and Discover3 (Accelrys). Unconstrained minimization and molecular dynamics were performed using the esff forcefield<sup>5</sup> and a continuum dielectric of 80.

## References

1. Krohnke, F. *Synthesis* 1976, 1-25.
2. Schellman, J. A. *Biopolymers* 1987, **17**, 1305-1322.
3. Walshaw, J.; Woolfson, D. N. *J. Mol. Biol.* 2001, **307**, 1427-1450.
4. De Castro, E.; Sigrist, C. J. A.; Gattiker, A.; Bulliard, V.; Langendijk-Genevaux, P. S.; Gasteiger, E.; Bairoch, A.; Hulo, N. *Nucleic Acids Res.* 2006, **34** (Web Server issue), W362-W365.
5. Shi, S. H.; Yan, L.; Yang, Y.; Fisher-Shaulsky, J.; Thacher, T. *J. Comp. Chem.* 2003, **24**, 1059-1076.