Probing coiled-coil assembly by paramagnetic NMR spectroscopy

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Supporting Information

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Part 1. Mass spectra

LC-MS spectra of all the purified peptides are shown in Figure A1. On the spectra from top to bottom, there are UV (ultraviolet-visible) spectra, ESI (electrospray ionization) spectra, and the mass spectra with number of (MW+2)/2 and (MW+3)/3 peaks (MW stands for molecular weight). All the UV and ESI spectra show each purified peptide comprises a single compound while the mass spectra identify the peptide mass precisely (Figure A 1-3).

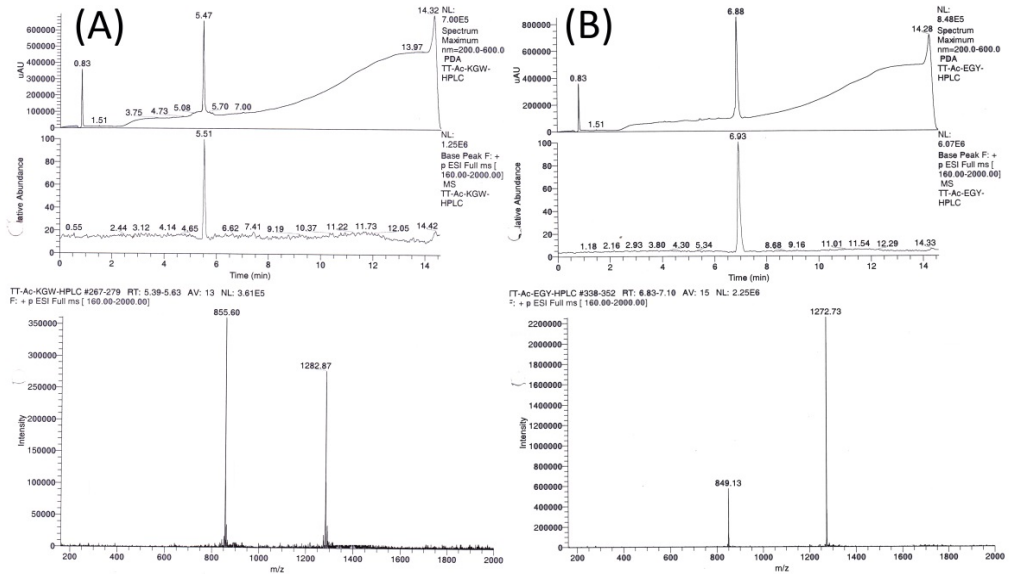

Figure S1. *LC-MS spectra of (A) purified Coil-K_W and (B) purified Coil- E_Y.*

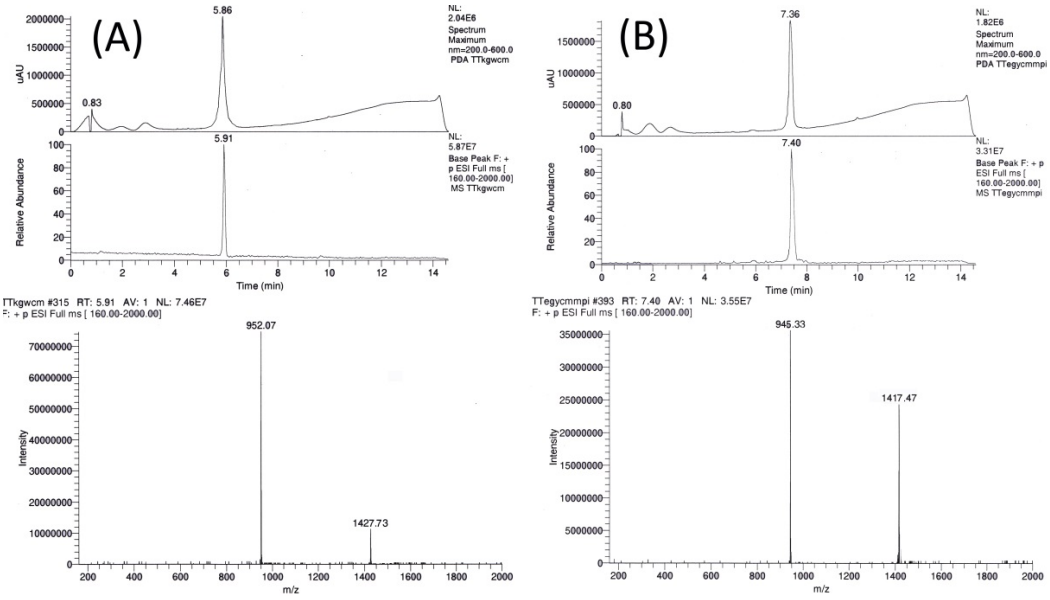

Figure S2. LC-MS spectra of (A) purified Coil-K_W* and (B) purified Coil-E_Y*.

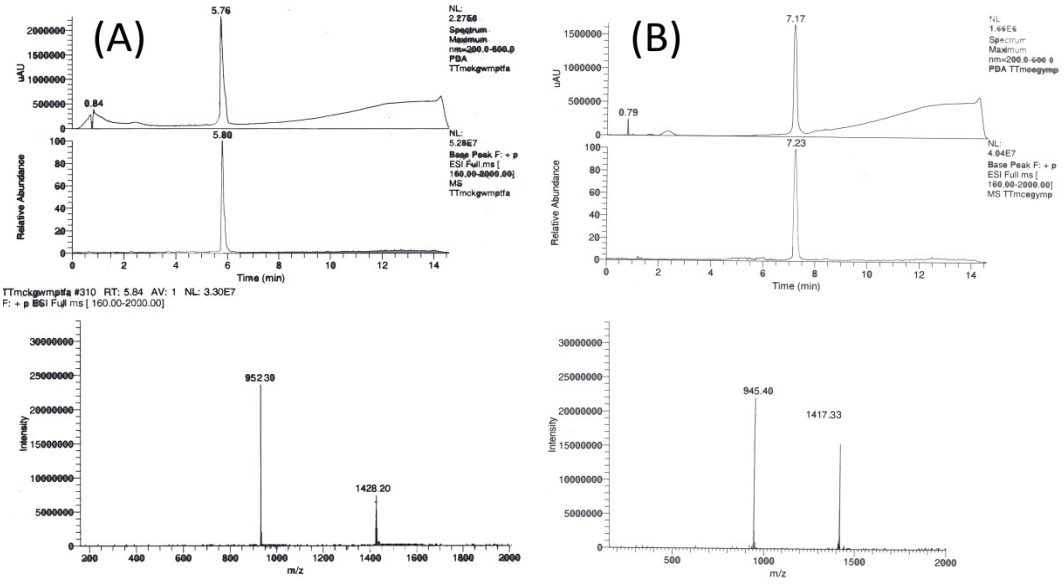

Figure S3. LC-MS spectra of (A) purified Coil-$^{*}K_W$ and (B) purified Coil-$^{*}E_Y$.

Part 2. CD spectra for coil-K and coil-E

Circular dichroism spectroscopy measurements of the original Coil-K ($(KIAALKE)_3$) and Coil-E ($(EIAALEK)_3$) peptides and their equimolar mixture in the absence and presence of 50% TFE. All measurements were performed in 1mm quartz cuvettes at 25 ^{o}C with a total peptide concentration of 200 µM).

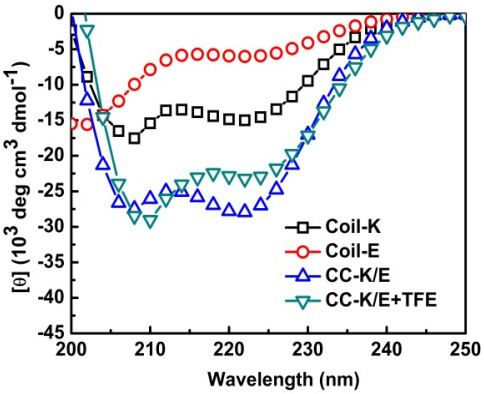

Figure S4. *The secondary structure of the original peptides Coil-K, Coil-E, and a 1:1 mixture of Coil-K and Coil-E in pH=7.4 PBS buffer and in TFE/PBS=1:1 (v/v).*

Table S1. Secondary and quaternary CD spectroscopic data of unmodified peptides Coil-K, Coil-E and their complex CC-K/E.

Peptide [a]	θ_{222}		% α-helix [b]		$\theta_{222}/\theta_{208}$		Coiled-coil [c]
	Benign	50 % TFE	Benign	50 % TFE	Benign	50 % TFE	
Coil-K	-15010	-19788	48	63	0.86	0.92	-
Coil-E	-6016	-17067	19	55	0.60	0.78	-
CC-K/E	-27923	-23234	90	74	1.01	0.82	+

[a] CC-K/E refers to an equimolar concentration of Coil-K and Coil-E peptides. [b] The percentage of α-helicity is calculated from 100 times the ratio between observed $[\theta]_{222}$ to the predicted $[\theta]_{222}$ for an α-helical peptide of n residues. The predicted α-helicity is calculated from the formula: $[\theta]_{222}$=-40000×(1-4.6/n).[1, 2] [c] The signal + signifies a significant decrease in the $[\theta]_{222}/[\theta]_{208}$ ratio from benign to 50% TFE in PBS, indicative of the folded coiled-coil structure. [Total Peptide]=200 µM in pH=7.4 PBS at 25 °C.

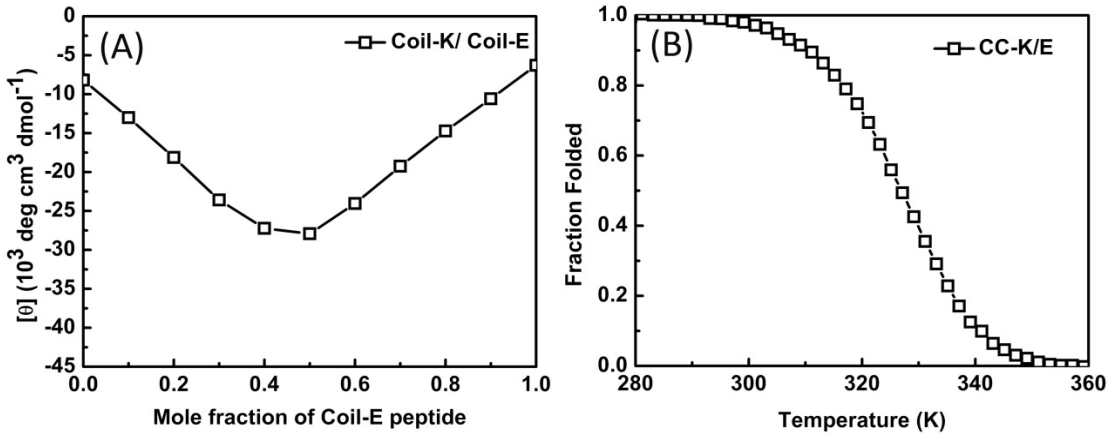

Figure S5. *(A) Job plot of the mean residue molar ellipticity (222 nm) for mixtures of unmodified Coil-K and Coil-E as a function of the mole fraction of the Coil-E peptide. All the measurements were carried out at a total peptide concentration of 200 µM in pH=7.4 PBS saline buffer at 25 °C, in a 1mm quartz cuvette. (B) Thermal unfolding curve monitored at $[\vartheta]_{222}$ during dissociation of the coiled coil complex CC-K/E, as a result of increasing the temperature from 280 K to 360 K in pH=7.4 PBS buffer. [Total peptide]=40 uM, 1cm quartz cuvette with magnet stirring at 900 rpm.*

The Job plot of the signal at $[\theta]_{222\,nm}$ reveals that the minimum occurs when the mole fraction of peptide Coil-E is 0.5, this indicates that Coil-K and Coil-E form a complex with 1:1 binding stoichiometry (Figure S5. (A)). The thermal denaturation curve shows that the unmodified CC-K/E complex dissociates in a two-state transition (Figure S5. (B)).

Part 3. Additional CD spectra for labeled coil-K and coil-E used in this study

The thermal dissociation of all of the coiled coil complexes were completely reversible, see also Figure 2B.

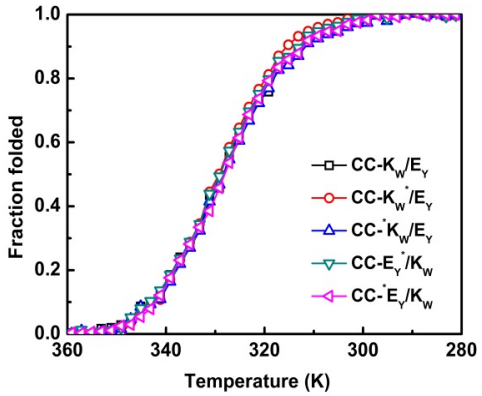

Figure S6. *Thermal folding curves based on the value of ϑ_{222} during formation of the coiled coil complexes as the temperature is decreased from 360 K to 280 K in PBS buffer, pH=7.4. [Total peptide]=40 uM, and 1 cm quartz cuvette with stirring magnet at 900 rpm.*

Table S2. Secondary and quaternary CD spectroscopic data of synthetic peptides used in this study.

peptide[a]	**$[\theta]_{222}$**		**% α-helix**[b]		**$[\theta]_{222}/[\theta]_{208}$**		**Coiled-coil**[c]
	Benign	50% TFE	Benign	50% TFE	Benign	50% TFE	
Coil-K$_W$	-13925	-19699	43	60	0.70	0.81	-
Coil-K$_W$*	-15337	-19805	46	61	0.80	0.82	-
Coil-*K$_W$	-15411	-20057	47	61	0.81	0.82	-
Coil-E$_Y$	-4748	-16080	14	50	0.53	0.78	-
Coil-E$_Y$*	-8180	-17727	25	54	0.61	0.78	-
Coil-*E$_Y$	-10119	-18886	31	58	0.70	0.80	-
CC-K$_W$/E$_Y$	-28747	-23909	90	74	1.02	0.83	+
CC-K$_W$*/E$_Y$	-31892	-24251	99	75	1.04	0.78	+
CC-*K$_W$/E$_Y$	-31818	-260565	99	80	1.02	0.84	+
CC-K$_W$/E$_Y$*	-33297	-267780	100	82	1.04	0.81	+
CC-K$_W$/*E$_Y$	-31395	-25326	98	78	1.07	0.84	+

[a] CC-K/E refers to an equimolar concentration of the Coil-K and Coil-E peptides. [b] The percentage of α-helicity is calculated from 100 times the ratio between observed $[\vartheta]_{222}$ to the predicted $[\vartheta]_{222}$ for an α-helical peptide of n residues. The predicted α-helicity is reckoned from formula: $[\vartheta]_{222}$=-4oooo×(1-4.6/n). [1, 2] *[c] The signal + signifies a significant decrease in the $[\vartheta]_{222}/[\vartheta]_{208}$ ratio from benign to 50% TFE in PBS, indicative of the folded coiled-coil structure and vice versa. [Total Peptide]=200μM in pH=7.4 PBS at 25 °C.*

Table S3. Binding constants of E and K coiled coil complex from CD spectroscopy

Coiled-coil Complex	T_m(℃) [b]	ΔG_u (kcal mol^{-1}) [c]	K_d (M) [d]
CC-K/E[a]	58	9.6	7×10^{-8}
CC-K_W/E_Y	57	10.8	7.7×10^{-8}
CC-K_W^*/E_Y	56	11.6	6.4×10^{-8}
CC-$^*K_W$/E_Y	57	11.0	7.5×10^{-8}
CC-K_W/E_Y^*	56	11.8	6.5×10^{-8}
CC-K_W/$^*E_Y$	57	10.7	7.6×10^{-8}

[a] data taken from literature.[3, 4] [b] T_m = melting temperature, at which half of the peptide is in the unfolded form. [c] Gibbs free energy of unfolding at 25°C. [d] K_d = the dissociation constant.

Part 4. Hyperchem simulations

Hyperchem release 8.0 package was used to simulate the peptide conformation and to determine the distance between MTSL and the aromatic amino acids in Coil-E and Coil-K.

For this, Coil-K, Coil-E or their 1:1 complex were placed in a periodic box containing water molecules and the system was equilibrated at 300 K. The peptide can move in a constant-density environment which is similar to being in a liquid. The size of the box was set as a cube with W=H=D= 56.104 Å, and the minimum distance between solvent and solute atoms (atoms from peptides) is 2.3 Å.[88]

Molecular Mechanics simulations were based on a classical Newtonian calculation. Here, atoms were treated as Newtonian particles interacting through a potential energy function, which depend on bond lengths, bond angles, torsion angles, and non-bonded interactions (including van der Waals forces, electrostatic interactions, and hydrogen bonds). In these calculations, the forces on atoms are functions of the atomic position.

Furthermore, the AMBER force field which is typically used for developing proteins and nucleic acids was used to develop an all-atom model. The simulations were performed in standard way, with temperature at 300 K and 30 ps run time.

Figures beneath show the details of the peptide conformation zoomed in to an atomic level after simulation. Coil-is shown in red, and Coil-E in blue. For the highlighted Trp and Tyr residues and the MTSL label, violet dots indicate carbon atoms, light blue dots indicate hydrogen atoms, yellow dots indicate nitrogen atoms, green dots indicate oxygen atom and orange dots indicate sulfur atoms (Figures S7 – S10).

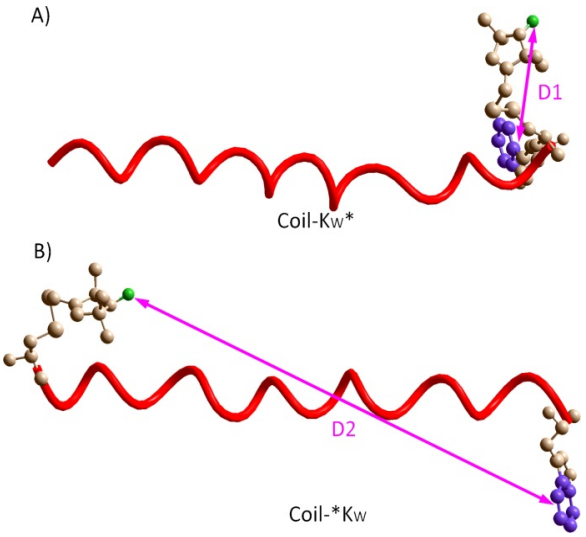

Figure S7. Structures of peptide Coil-K_W* (A) and Coil- *K_W (B). In (A) D1 indicates the distance between the nitroxyl group of the MTSL label and the aromatic ring of Trp in peptide Coil-KW*. The average length of D1 is 6.617 Å. In (B) the average distance, D2, between the nitroxyl group of the MTSL label and W is 36.701 Å.

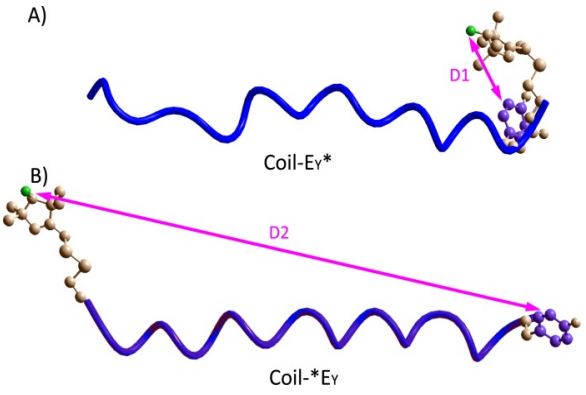

Figure S8. Structure of peptide Coil-E_Y* (A) and Coil- *E_Y (B). (A) The average distance D1 = 13.0357 Å. (B) The average distance D2 = 40.0889 Å.

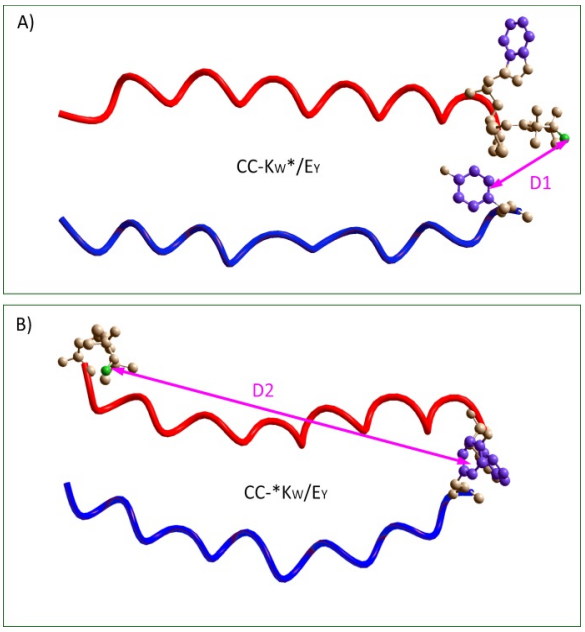

Figure S9. Structure of the CC-K/E complex with Coil-K contains the MTSL label. (A) The average distance D1 = 8.9315 Å in the CC-K_W^*/E_Y complex. (B) The average distance D2 = 37.2628 Å for the CC-$^*K_W/E_Y$ complex.

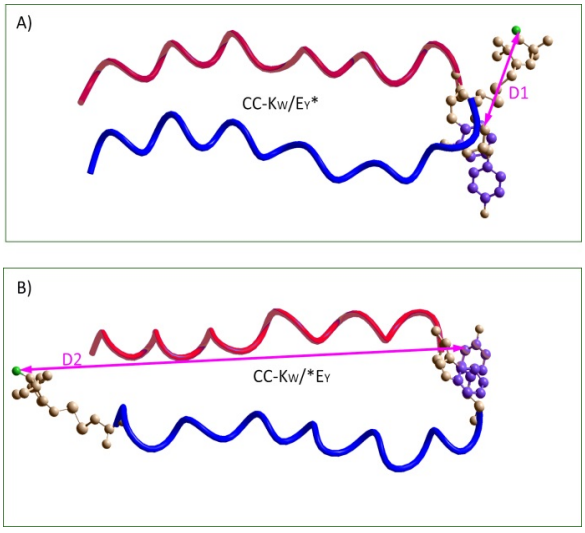

Figure S10. Structure of the CC-K/E complex where Coil-E incorporates the MTSL label. (A) The average distance D1 = 8.9315 Å for the CC- K_W/E_Y^* complex. (B) The average distance D2 = 38.7715 Å for the CC-$K_W/^*E_Y$ complex.

Part 5. Summary of signal intensities associated with distance

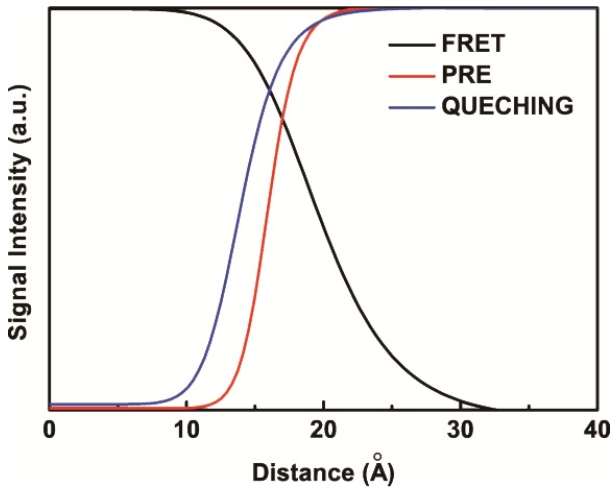

Figure S7. *The distance dependent signal intensity of FRET, PRE or fluorescence quenching. The black line indicates the response distance between the FRET pair (donor and acceptor) for fluorescence resonance energy transfer; the red line indicates the response distance between the aromatic fluorophore and the MTSL nitroxyl radical in paramagnetic 1H-NMR measurements; the blue line indicates the response distance between the fluorophore and the MTSL nitroxyl radical in fluorescence quenching measurements.*

Part 6. Full range 1H-NMR spectra of peptides

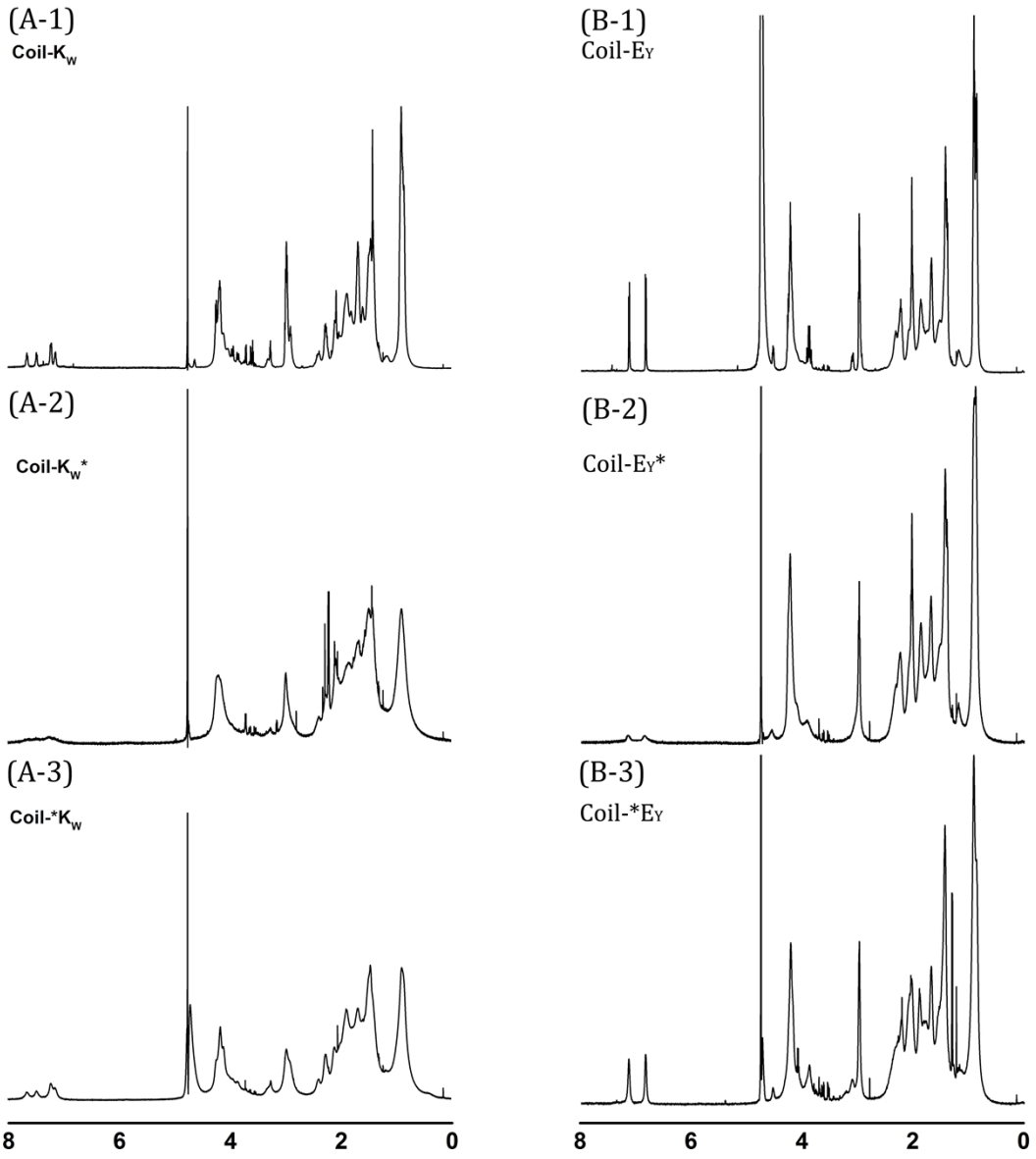

Figure S8. *600 MHz 1H-NMR spectra of peptide before and after spin labeling (0-8 ppm). [Total peptide]= 0.8 mM, PBS, pH=7.4.*

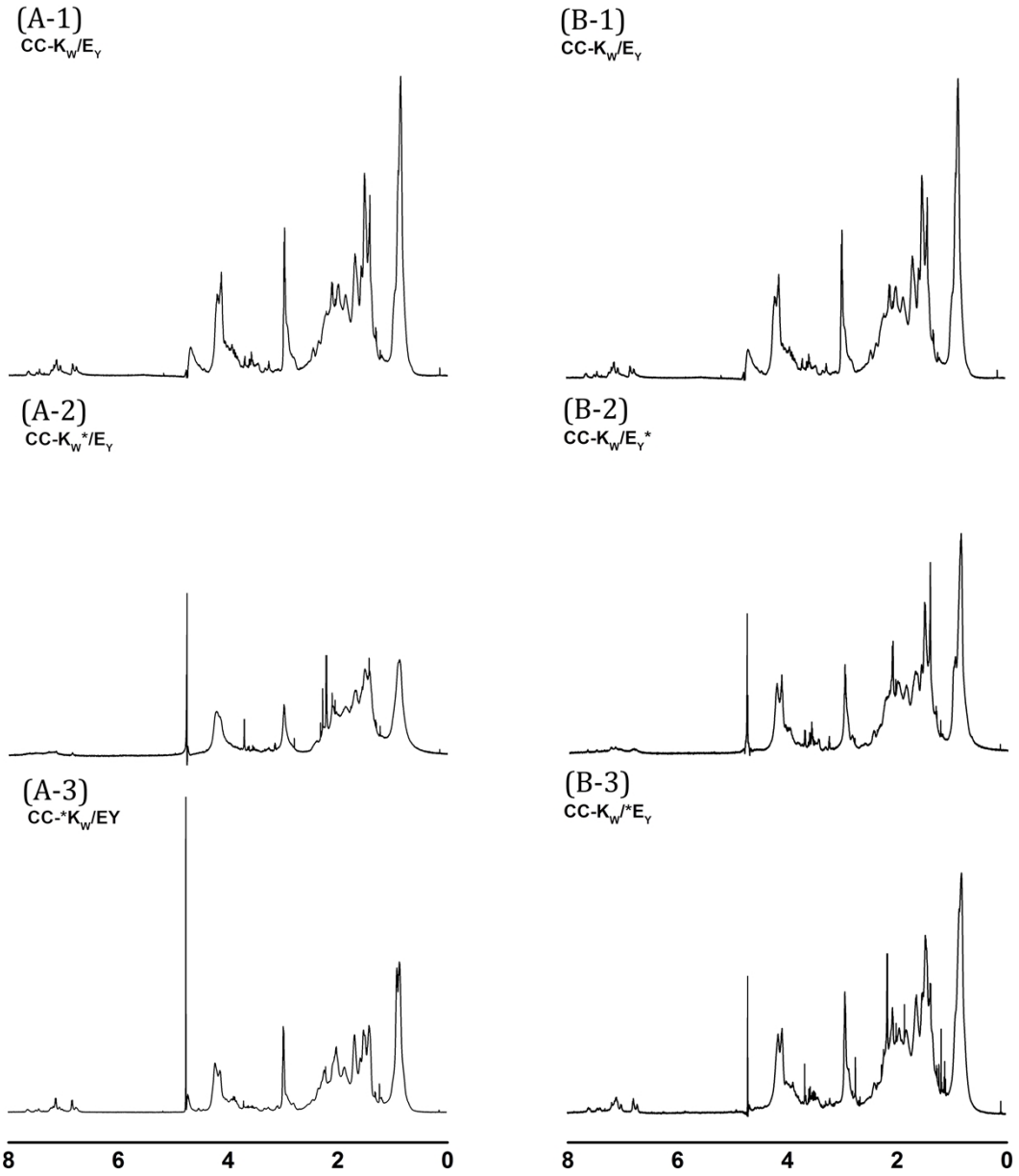

Figure S9. *600 MHz 1H-NMR spectra of peptide coiled coil complex (0-8 ppm). [Total peptide]= 0.8 mM, PBS, pH=7.4.*

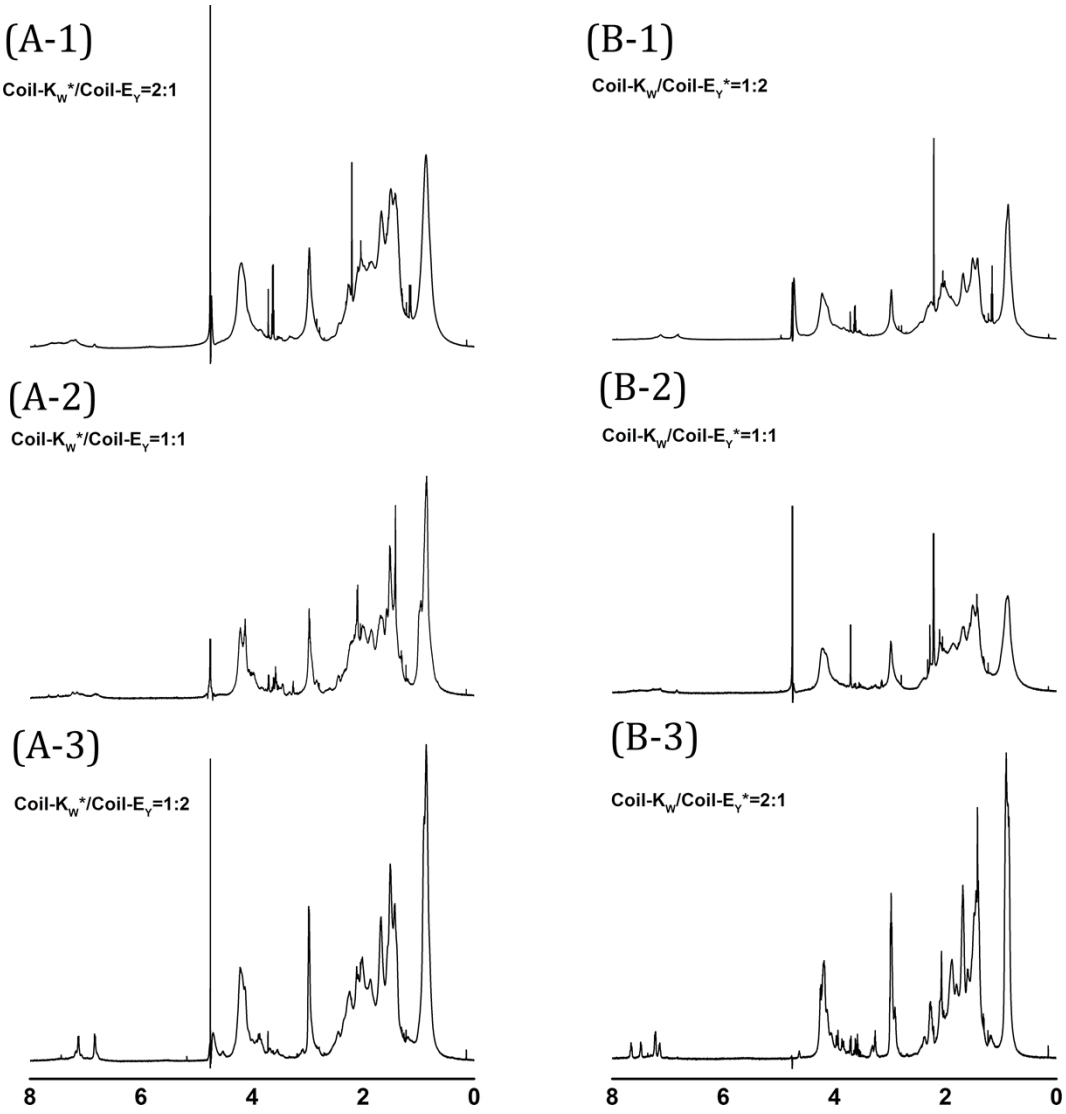

Figure S10. 600 MHz 1H-NMR spectra of peptide complex stoichiometry measurement (0-8 ppm). [Total peptide]= 0.8 mM, PBS, pH=7.4.

References

1. Y. H. Chen, J. T. Yang and K. H. Chau, *Biochemistry*, 1974, **13**, 3350-3359.

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