Supporting information for:

β1 Integrin NPxY Motifs Regulate Kidney Collecting Duct Development and Maintenance by Induced-Fit Interactions with Cytosolic Proteins

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Supporting Figure 1. YY/AA and YY/FF mutants respond differently to FGF-10. Wild type and mutant CD cells were allowed to adhere to collagen I for 1 hour, after which they were treated with FGF-10 for various times. The cells were then lysed and 20 µg of total cell lysates were analyzed by Western blot for levels of pAKT and pERK. Immunoblots of total AKT and ERK are shown to verify equal protein loading. A representative blot from 3 independent experiments is shown. The increases in intensity of phosphorylated proteins were quantified using Image-J software and expressed as fold increase relative to untreated cells.

Supporting Figure 2. The contralateral kidneys of WT and YY/FF mice removed 5 days after UUO injury were subjected to Trichrome Blue staining. There was minimal staining in either phenotype (100X).

Supporting Figure 3. Overlaid spectra comparing the ^{1}H,^{15}N-TROSY spectra of WT β1 TM/CTD (black spectrum) to that of the YY/AA mutant (red spectrum) or the YY/FF mutant (blue spectrum). The ^{1}H,^{15}N-TROSY spectra were collected using a 600MHz Bruker NMR Avance-III spectrometer at 298K, pH 6.5 in DMPC/D7PC bicelles (q=0.3). These spectra show only minor differences between the spectra of the wild type and mutant proteins. The non-overlaid spectra of the WT and mutant proteins are shown in Figure 5 of the paper.

Supporting Figure 4. Far-UV circular dichroism spectrum of the WT integrin β1 TM/CTD. This was acquired at 298K using a JASCO spectropolarimeter (J-810) and a 0.1 cm pathlength sample containing 0.05 mM β1 TM/CTD in sodium phosphate buffer pH 7.2 with 2% DMPC/D7PC bicelles, q=0.3, 298K. The α-helical content of the protein in this sample was calculated from this spectrum to be 84.7% using the K2D2 program (*K2D2: Estimation of Protein Secondary Structure from Circular Dichroism Spectra* Carolina Perez-Iratxeta, Miguel A Andrade-Navarro (2008) *BMC Structural Biology* **8**, p. 25).

Supporting Figure 5. ^{1}H,^{15}N-TROSY spectra illustrating the titration of the U-^{15}N-labeled β1 TM/CTD mutants with unlabeled integrin α2 TM/CTD, which was carried out in DMPC/D7PC bicelles (q 0.3, pH 6.5, 298K). The initial concentration of integrin β1 WT and mutants were 1.0 mM. The integrin α2 TM/CTD was titrated into this sample from a 2.5mM stock solution up through a 2.5 molar excess of α2 relative to β1. The overlaid spectra are: Back (0 mol:mol), orange (0.5), blue (1.0), yellow (1.5) green (2.0) and red (2.5 mol α2:mol β1). Changes in the chemical shifts of peaks were interpreted as reporting on complex formation (see Figure 7B in the paper). **(A)** Titration of the β1 TM/CTD YY/AA mutant. **(B)** Titration of the β1 TM/CTD YY/FF mutant.

Supporting Table 1. Backbone NMR resonance assignments for the membrane distal CTD of WT β1 TM/CTD in DMPC/D7PC bicelles (20%, q=0.3, 298K).

	WT (pH 6.5)	WT(pH 4.0)	CA	CB	CO
T777	(7.96,114.39)	(7.969,114.036)	61.902	69.203	175.314
G778	(8.30, 111.50)	(8.328,110.953)	45.029		174.154
E779	(7.91,120.13)	(8.018,119.823)	55.441	29.103	175.703
N780	(8.36, 120.94)	(8.348,121.339)	51.05	38.458	
P781			63.159		177.196
I782	(7.92,120.23)	(7.976,119.658)	61.466	37.679	176.194
Y783	(7.77,123.26)	(7.795,122.639)	57.359	37.965	175.615
K784	(7.88,123.7)	(7.919,123.218)	55.915	32.452	176.213
S785	(8.08,117.77)	(8.101,117.361)	58.084	63.153	174.227
A786	(8.23,126.86)	(8.26,124.944)	52.29	18.647	177.706
V787	(7.98,119.70)	(8.012,119.249)	62.081	32.014	176.425
T788	(8.10,118.85)	(8.127,118.346)	61.801	69.278	174.376
T789	(8.02,118.40)	(8.049,118.053)	61.743	69.333	174.203
V790	(8.06,124.30)	(8.081,123.799)	61.938	32.129	175.723
V791	(8.14,125.60)	(8.161,124.983)	61.774		175.297
N792	(8.43,124.72)	(8.445,124.24)	50.818	32.085	
P793			63.056	38.37	176.728
K794	(8.08, 121.19)	(8.106,120.668)	56.027		176.266
Y795	(8.01,122.18)	(8.031,121.635)	57.483	31.914	175.705
E796	(8.16,124.33)	(8.129,123.473)	55.5	37.971	176.188
G797	(7.64,110.37)	(7.639,109.858)	45.008	29.024	172.914
K798	(7.65,126.18)	(7.712,125.51)	56.909	32.847	

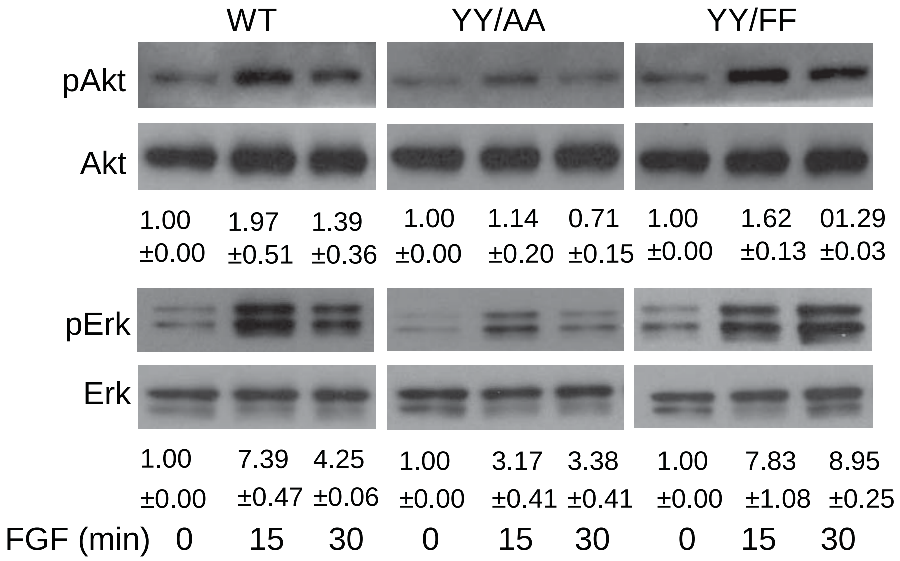
Supporting Table 2. Backbone NMR resonance assignments for the membrane distal CTD of YY/FF β1 TM/CTD in DMPC/D7PC bicelles (20%, q=0.3, 298K).

	YYFF (pH 6.5)	YYFF (pH 4.0)	CA	CB	CO
T777	(7.96,114.38)	(7.878,113.757)	62.242	69.68	175.25
G778	(8.30,111.50)	(8.271,110.842)	45.364		173.947
E779	(7.92,120.19)	(7.947,119.496)	55.687	29.33	175.462
N780	(8.36,120.93)	(8.265,121.31)	51.463	38.989	
P781			63.669	32.107	176.948
I782	(7.92,120.19)	(7.923,119.586)	61.985	38.286	176.028
F783	(7.87,123.23)	(7.795,121.87)	57.614	39.433	175.475
K784	(7.93,123.75)	(7.924,122.604)	56.529	33.084	176.256
S785	(8.10,117.91)	(8.065,117.161)	58.547	63.815	174.252
A786	(8.26,126.93)	(8.207,126.303)	52.7	19.229	177.608
V787	(8.00,119.84)	(7.94,118.859)	62.56	32.589	176.335
T788	(8.12,118.99)	(8.039,117.865)	62.154	69.649	174.407
T789	(8.03,118.47)	(7.965,117.672)	62.123	69.72	174.106
V790	(8.07,124.41)	(7.984,123.314)	62.31	32.691	175.672
V791	(8.16,125.74)	(8.061,124.365)	62.192	32.671	175.236
N792	(8.44,124.78)	(8.34,124.037)	51.089	38.895	
P793			63.517		176.86
K794	(8.08,121.11)	(8.046,120.294)	56.556	32.488	176.465
F795	(8.08,122.14)	(7.99,120.832)	57.58	39.345	175.526
E796	(8.19,123.96)	(8.104,109.967)	55.842	29.247	175.962
G797	(7.81,110.62)	(7.791,109.979)	45.342		173.013
K798	(7.64,126.40)	(7.7,125.143)	57.025	33.544	

Supporting Table 3. Backbone NMR resonance assignments for the membrane distal CTD of β1 TM/CTD YY/AA in DMPC/D7PC bicelles (20%, q=0.3, 298K).

	YYAA (pH 6.5)	YYAA(pH 4.0)	CA	CB	CO
T777	(7.97,114.47)	(7.908,114.006)	62.239	69.639	175.294
G778	(8.30, 111.61)	(8.263,111.094)	45.346		173.957
E779	(7.93, 120.26)	(7.955,119.77)	55.672	29.352	175.476
N780	(8.36,120.95)	(8.295,121.148)	51.295	38.833	
P781			63.453	32.015	176.889
I782	(7.96,121.09)	(7.938,120.58)	61.188	38.376	176.015
A783	(8.05,128.42)	(8.01,127.335)	52.414	19.18	177.574
K784	(7.93,123.75)	(8.098,121.142)	56.438	32.955	176.62
S785	(8.11,117.46)	(8.083,117.042)	58.254	63.858	174.119
A786	(8.22,126.98)	(8.188,125.899)	52.495	19.258	177.539
V787	(8.00,119.84)	(7.944,119.452)	62.333	32.656	176.33
T788	(8.12,118.99)	(8.087,118.524)	61.923	69.736	174.351
T789	(8.04,118.57)	(8.002,118.026)	61.873	69.81	174.064
V790	(8.09,124.60)	(8.039,123.974)	62.204	32.708	175.709
V791	(8.17,126.04)	(8.105,124.643)	62.17	32.671	175.312
N792	(8.47,124.76)	(8.401,124.168)	50.905	38.8	
P793			63.548		177.009
K794	(8.24,121.00)	(8.048,120.455)	56.337	32.55	176.475
A795	(8.06,126.19)	(7.956,124.46)	52.431	19.137	176.937
E796	(8.19, 123.96)	(8.094,119.845)	56.088	29.24	176.523
G797	(8.31, 111.24)	(8.244,110.688)	45.35		173.188
K798	(7.66, 126.52)	(7.697,124.735)	56.999	33.436	

Mathew Sup. Fig_1



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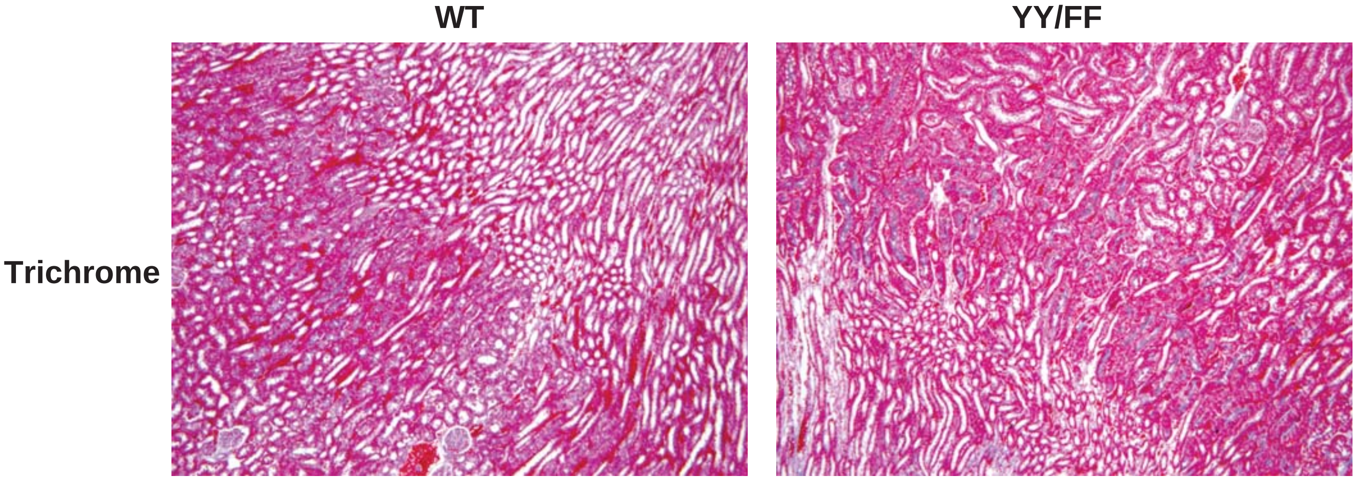

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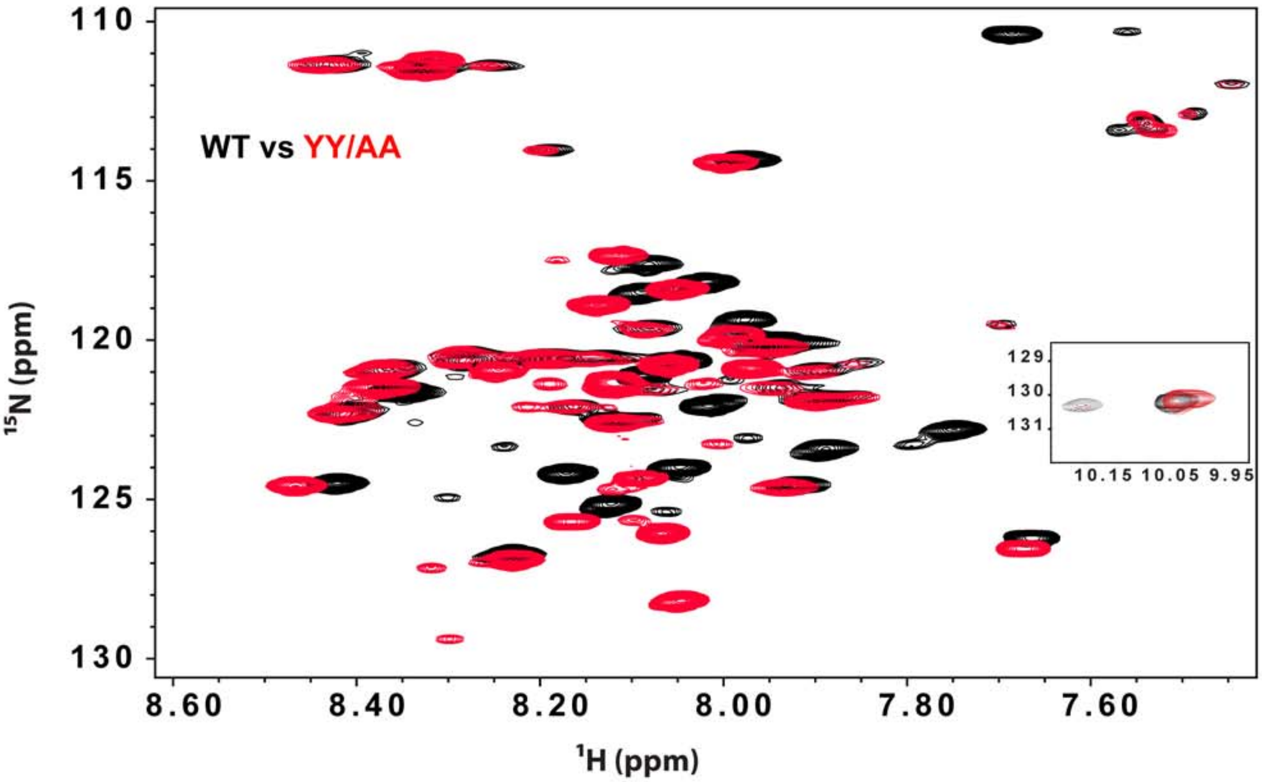
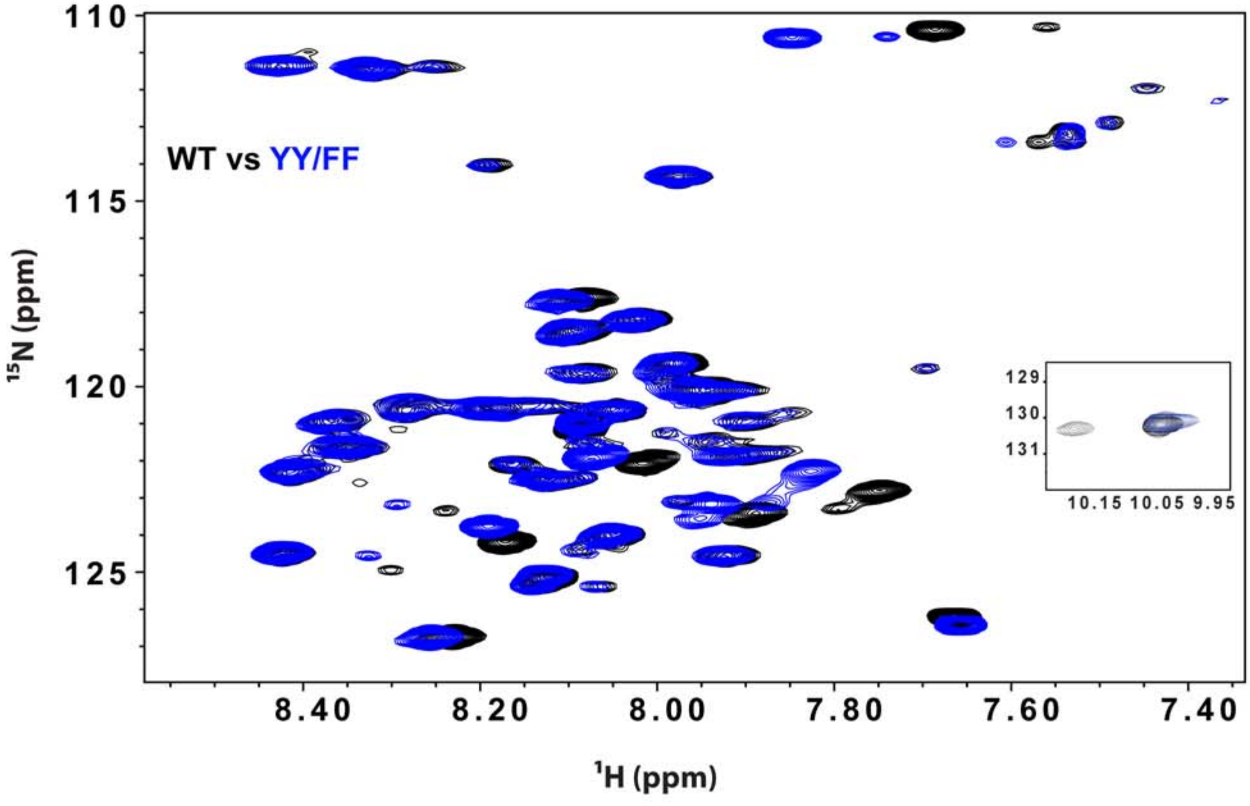
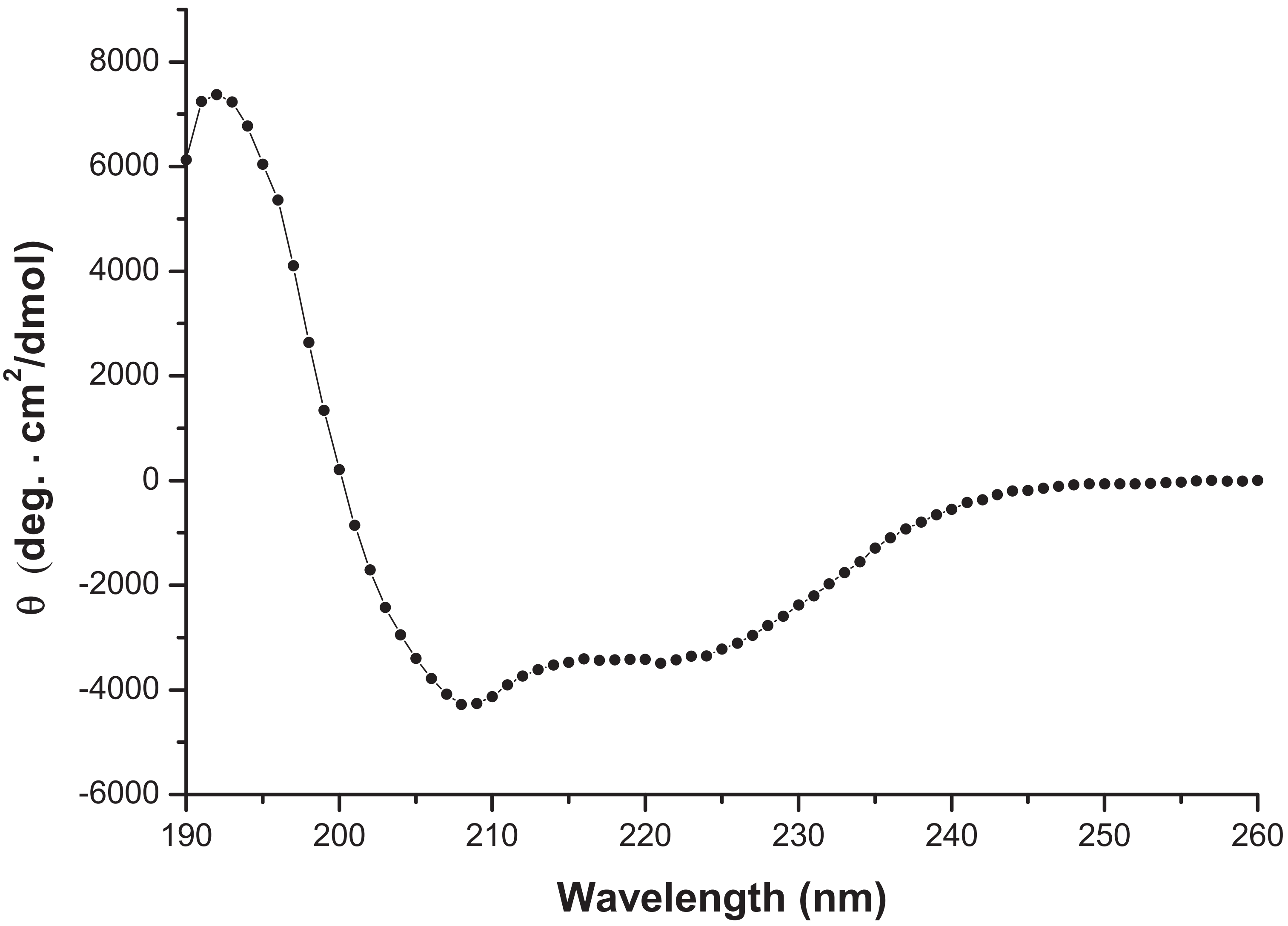
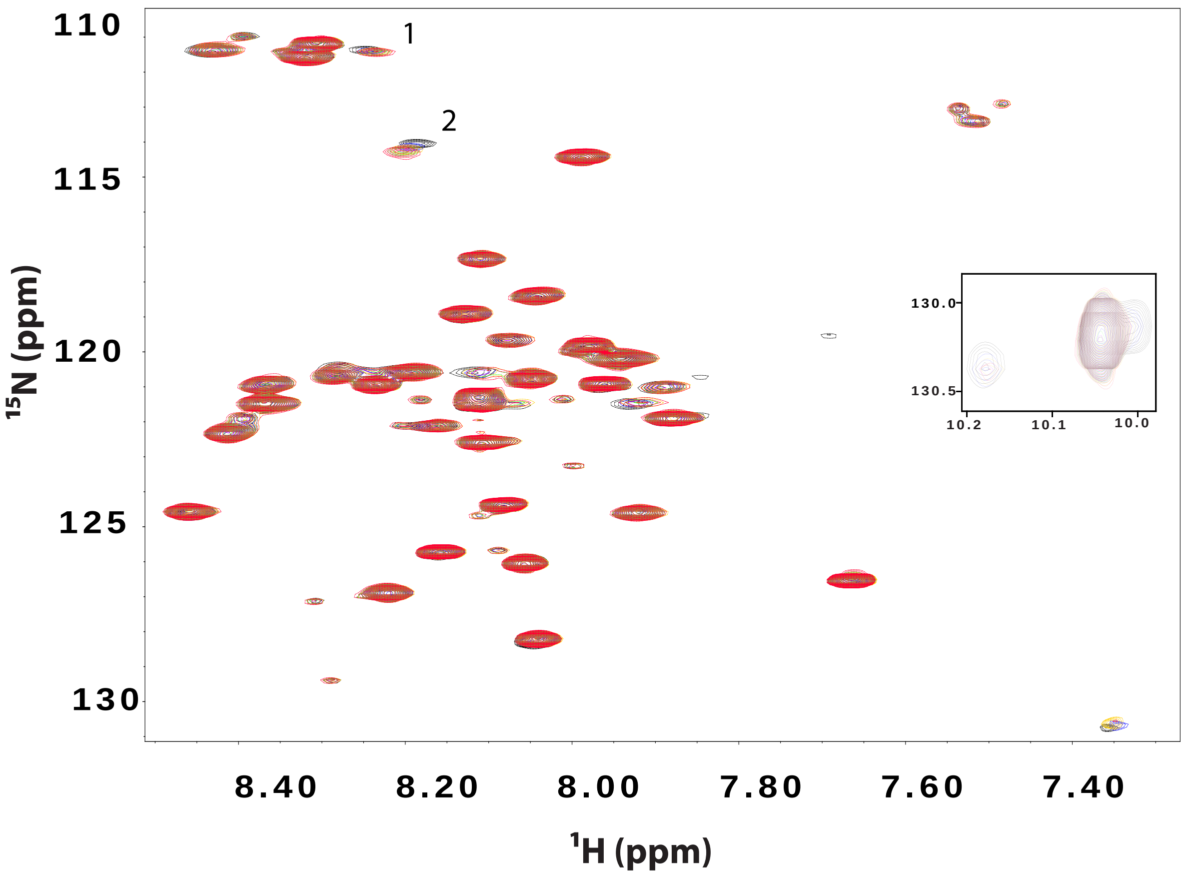






θ (deg. · cm^2/dmol)
8000
6000
4000
2000
0
-2000
-4000
-6000
190
200
210
220
230
240
250
260
Wavelength (nm)

A) YY/AA titration with α2



B) YY/FF titration with α2