# SUPPORTING INFORMATION

# Deciphering Conformational Changes Associated with the Maturation of Thrombin Anion Binding Exosite I

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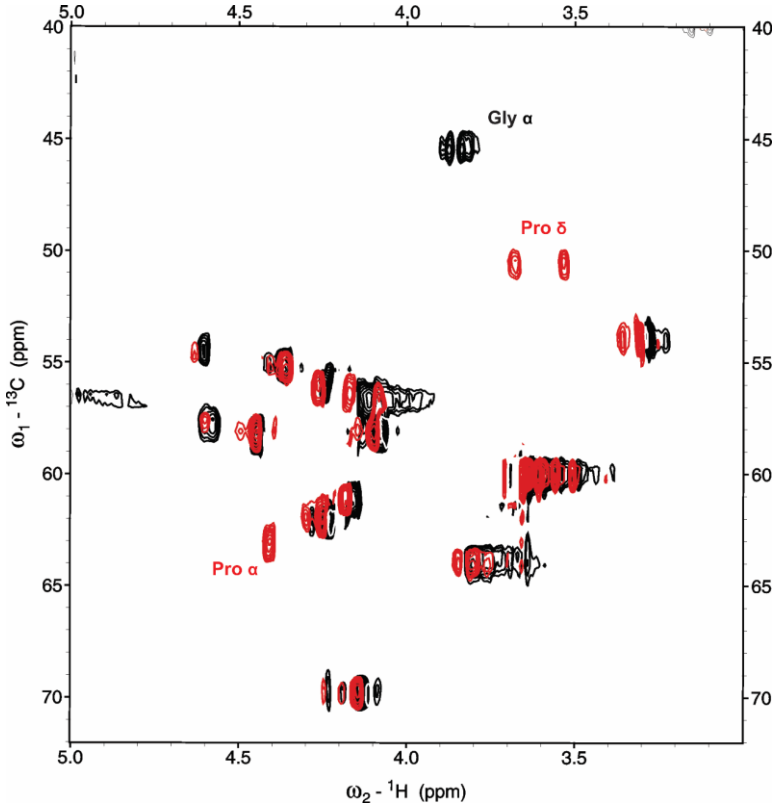


**Figure S1:** An overlay of the 2D $^{13}C$-$^{1}H$ natural abundance HSQC NMR spectra of 1 mM PAR3 (44-56) and 1mM PAR3G (44-56). All NMR samples were in 25mM $H_3PO_4$, 150 mM NaCl, 0.2 mM EDTA and 10 % $D_2O$ (pH 6.5). The PAR3 residues are in red whereas the PAR3G are in black. The expected cross peaks for the unique residues Pro51 versus Gly51 are labeled.

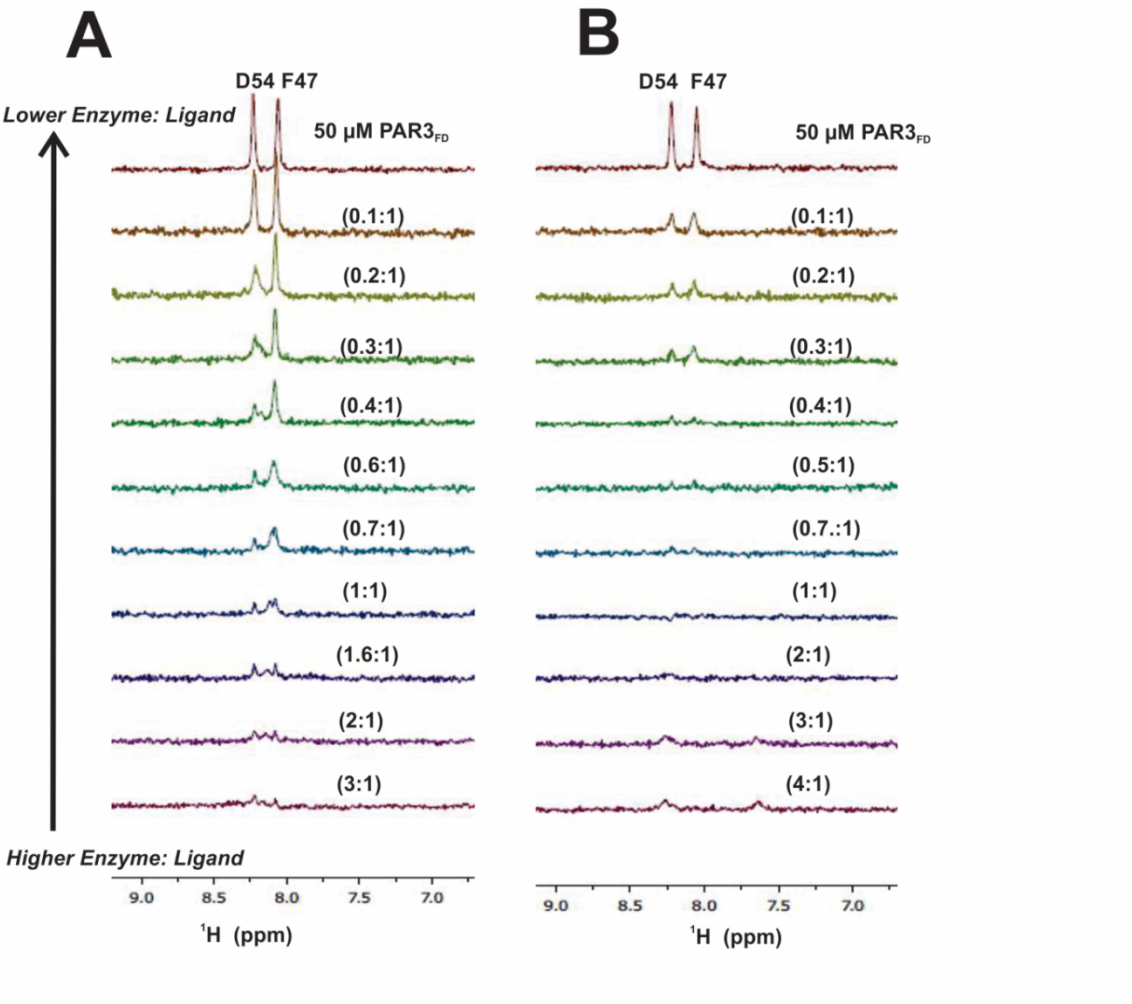


**Figure S2: 1D $^{15}$N-HSQC NMR titrations of PAR3$_{FD}$ (44-56) in the presence of ProT and PPACK- IIa** All NMR samples were in 25mM $H_3PO_4$, 150 mM NaCl, 0.2 mM EDTA and 10 % $D_2O$ (pH 6.5). **(A)** For the PAR3$_{FD}$ binding studies with ProT, starting complexes included 50 μM PAR3$_{FD}$ (44-56, $^{15}$N-F47, $^{15}$N-D54) in 137 μM ProT. The serial dilutions resulted in ProT to PAR3 ratios that spanned from 3:1 to 0.1:1. **(B)** For PPACK- IIa, starting complexes included 50 μM PAR3$_{FD}$ (44-56, $^{15}$N-F47, $^{15}$N-D54) in 210 μM PPACK- IIa. The serial dilutions resulted in PPACK- IIa to PAR3$_{FD}$ ratios that spanned from 4:1 to 0.1:1. Representative data sets are shown.

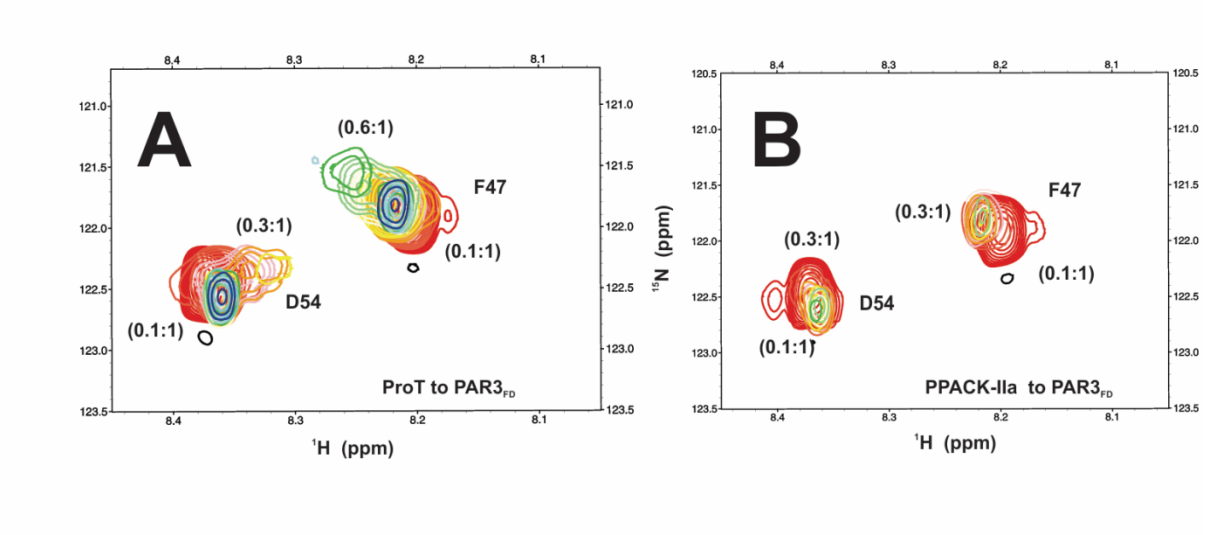


**Figure S3:** 2D $^{1}H$-$^{15}N$ HSQC NMR titrations of $PAR3_{FD}$ (44-56) in the presence of ProT and PPACK-IIa. All NMR samples were in 25mM $H_3PO_4$, 150 mM NaCl, 0.2 mM EDTA and 10 % $D_2O$ (pH 6.5). **(A)** For the $PAR3_{FD}$ binding studies with ProT, starting complexes included 50 μM $PAR3_{FD}$ (44-56, $^{15}N$-F47, $^{15}N$-D54) in 130 μM ProT. The serial dilutions resulted in ProT to $PAR3_{FD}$ ratios that spanned from 3:1 to 0.1:1. **(B)** For PPACK -IIa, starting complexes included 50 μM $PAR3_{FD}$ (44-56, $^{15}N$-F47, $^{15}N$-D54) in 210 μM PPACK-IIa. The serial dilutions resulted in PPACK-IIa to $PAR3_{FD}$ ratios that spanned from 4:1 to 0.1:1. Representative data sets are shown. Colors for the HSQC crosspeaks span from blue (highest protein-peptide ratio) to red (free peptide).

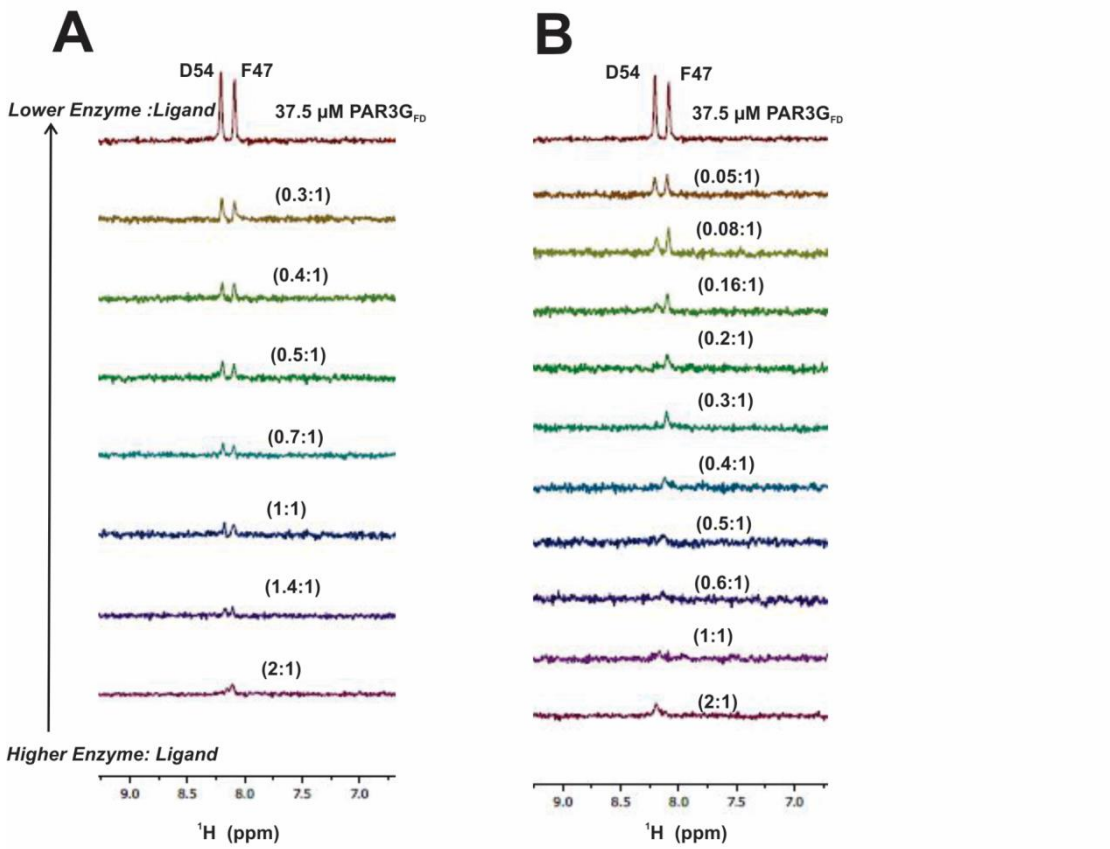


**Figure S4: 1D $^{15}$N-HSQC NMR titrations of PAR3G$_{FD}$ (44-56) in the presence of ProT and PPACK- IIa** All NMR samples were in 25mM $H_3PO_4$, 150 mM NaCl, 0.2 mM EDTA and 10 % $D_2O$ (pH 6.5). **(A)** For the PAR3G$_{FD}$ binding studies with ProT, starting complexes included 37.5 µM PAR3G$_{FD}$ (44-56, $^{15}$N-F47, $^{15}$N-D54) in 70 µM ProT. The serial dilutions resulted in ProT to PAR3G ratios that spanned from 2:1 to 0.3:1. **(B)** For PPACK- IIa, starting complexes included 37.5 µM PAR3G$_{FD}$ (44-56, $^{15}$N-F47, $^{15}$N-D54) in 70 µM PPACK- IIa. The serial dilutions resulted in PPACK- IIa to PAR3G$_{FD}$ ratios that spanned from 2:1 to 0.05:1. Representative data sets are shown.

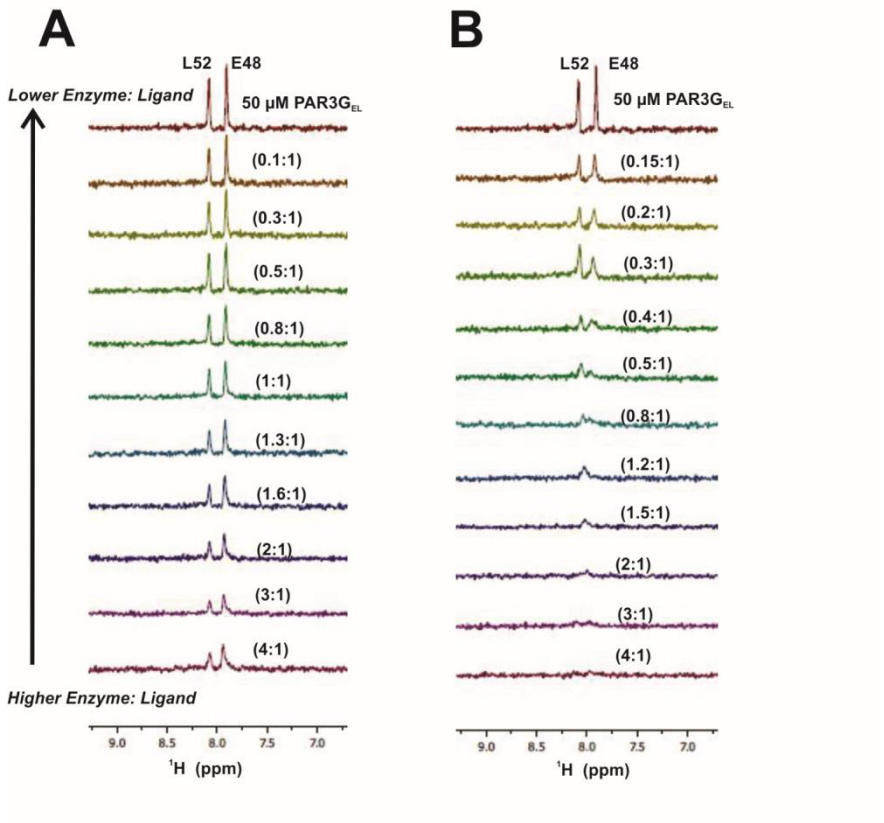


**Figure S5: $^{15}$N HSQC NMR titrations of PAR3G$_{EL}$ (44-56) in the presence of ProT and PPACK- IIa** All NMR samples were in 25mM $H_3PO_4$, 150 mM NaCl, 0.2 mM EDTA and 10 % $D_2O$ (pH 6.5). **(A)** For the PAR3G$_{EL}$ binding studies with ProT, starting complexes included 50 μM PAR3G$_{EL}$ (44-56, $^{15}$N-E48, $^{15}$N-L52) in 180 μM ProT. The serial dilutions resulted in ProT to PAR3G ratios that spanned from 4:1 to 0.1:1. **(B)** For PPACK- IIa, starting complexes included 50 μM PAR3G$_{EL}$ (44-56, $^{15}$N-E48, $^{15}$N-L52) in 211 μM PPACK- IIa. The serial dilutions resulted in PPACK- IIa to PAR3G$_{EL}$ ratios that spanned from 4:1 to 0.1:1. Representative data sets are shown.

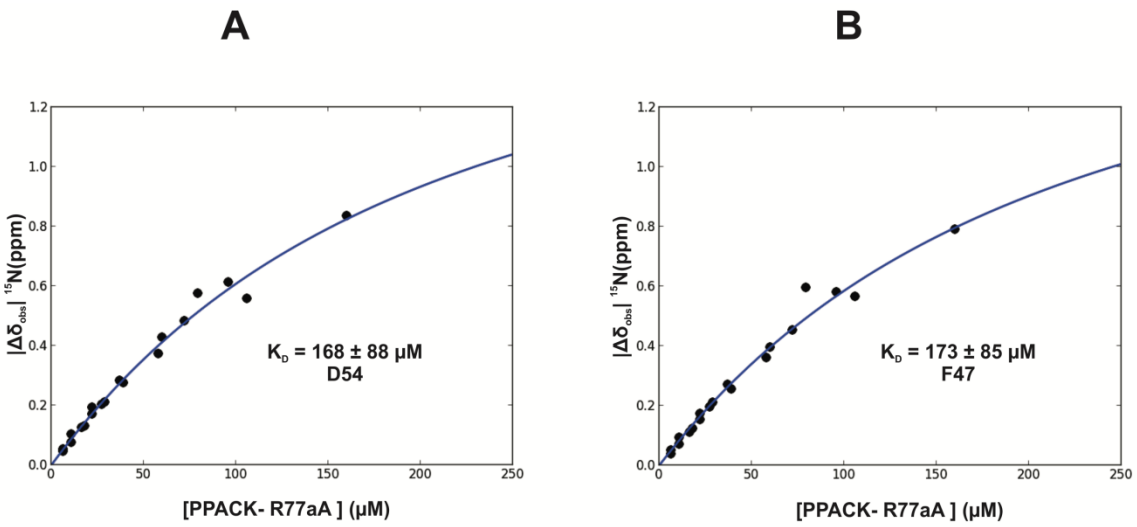


**Figure S6: Determination of Binding Affinity ($K_D$) for $^{15}$N-labeled F47 and D54 of PAR3G$_{FD}$ interacting with PPACK-R77aA IIa** For this NMR titration series, the peptide ligand concentration was kept constant and the ProT and PPACK-R77aA IIa concentrations were serially diluted. As a result, the NMR titrations were measuring the binding of protein to a defined peptide concentration. **(A)** Interactions between PPACK-R77aA IIa and PAR3G $^{15}$N-D54 led to a $K_D$ = 168 ± 88 µM and **(B)** ProT and PAR3G $^{15}$N-F47 led to a $K_D$ = 173 ± 85 µM. NMR titrations were done in duplicate. The reported $K_D$ values were determined using in-house scripts written using Python. The term $|\Delta\delta obs|$ $^{15}$Nppm = $\delta^{15N}_{Bound} - \delta^{15N}_{Free}$ reflects the absolute difference in chemical shift between the bound and free states of the particular $^{15}$N-amide. Error analysis was carried out using a Monte-Carlo approach assuming a 10% error in the serially diluted protein samples. See Materials and Methods for more details.

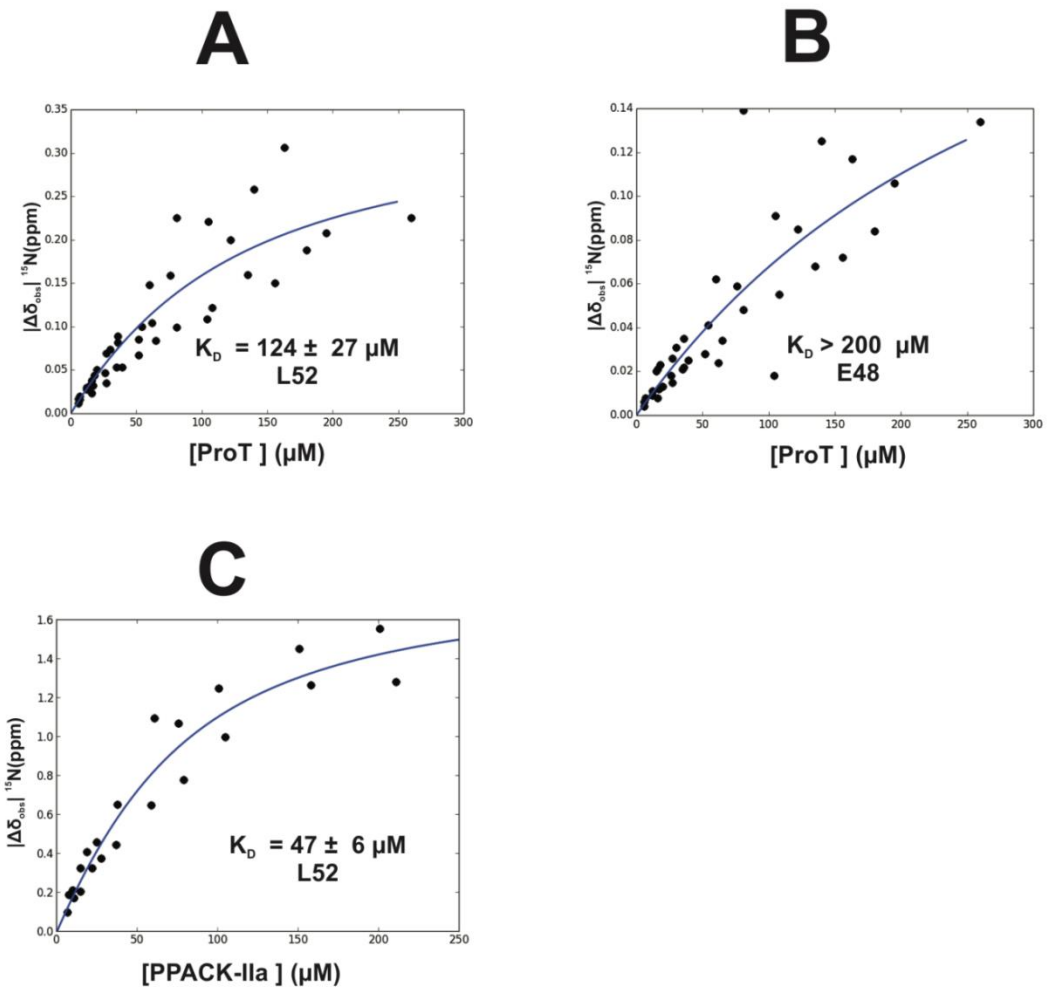


**Figure S7: Determination of Binding Affinity ($K_D$) for $^{15}$N-labeled L52 and E48 of PAR3G$_{FD}$ interacting with Prothrombin and PPACK-IIa** For this NMR titration series, the peptide ligand concentration was kept constant and the ProT and PPACK-IIa concentrations were serially diluted. As a result, the NMR titrations were measuring the binding of protein to a defined peptide concentration. **(A)** Interactions between ProT and PAR3G $^{15}$N-L52 led to a $K_D$ = 124 ± 27 µM, **(B)** ProT and PAR3G $^{15}$N-E48 led to a $K_D$ = > 200 µM, and **(C)** PPACK-IIa and PAR3G $^{15}$N-L52 led to a $K_D$ = 47 ± 6 µM. NMR titrations were done in triplicate. The reported $K_D$ values were determined using in-house scripts written using Python. The term $|\Delta\delta obs|$ $^{15}$Nppm = $\delta^{15N}_{Bound} - \delta^{15N}_{Free}$ reflects the absolute difference in chemical shift between the bound and free states of the particular $^{15}$N-amide. Error analysis was carried out using a Monte-Carlo approach assuming a 10% error in the serially diluted protein samples. See Materials and Methods for more details.