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Supplemental Information

**Kinetic Insights into the Binding between the nSH3 Domain of CrkII and
Proline-Rich Motifs in cAbl**

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Supporting Material

Table S1. The results of CPMG-RD data fitting of individual residues.

Residues	$k_{ex} (s^{-1})$		
	PRM ⁵²⁴	PRM ⁵⁶⁸	PRM ⁷⁵⁸
146	386.7 ± 44.2	706.0 ± 31.3	1132.3 ± 54.5
147	362.3 ± 74.2	790.7 ± 62.7	875.7 ± 168.7
148	401.3 ± 32.9	742.4 ± 24.1	1157.5 ± 42.5
149	311.4 ± 98.9	597.8 ± 22.7	804.2 ± 37.8
150	349.1 ± 44.6	746.3 ± 25.7	888.8 ± 35.9
163	- ^a	- ^a	985.3 ± 126.1
164	- ^a	- ^a	1097.8 ± 94.4
166	412.2 ± 41.3	742.7 ± 35.7	1141.1 ± 88.5
169	385.1 ± 56.2	784.2 ± 36.5	832.8 ± 40.4
181	- ^a	806.3 ± 176.2	- ^a
186	- ^a	- ^a	1128.9 ± 76.9

^a These are not analyzed because either no-dispersion was observed or peaks overlap with other peaks.

Table S2. Individual ^{15}N CPMG-RD data fitting results of binding between nSH3 and PRM⁷⁵⁸ at different temperatures.

Residues	k_{ex} (s^{-1}) ^a				
	15 °C	20 °C	30 °C	35 °C	40 °C
146	370.5 ± 65.8	843.0 ± 82.0	- ^b	2347 ± 390	- ^b
147	- ^b	611.1 ± 45.6	1848 ± 529	- ^b	- ^b
148	397.0 ± 66.9	634.2 ± 28.7	1844 ± 109	- ^b	- ^b
149	465.0 ± 28.2	740.2 ± 46.5	1510 ± 100	2918 ± 113	4434.0 ± 337
150	532.0 ± 50.6	780.9 ± 44.9	1719 ± 85	2912 ± 99	4677.1 ± 313
164	- ^b	615.8 ± 43.1	1697 ± 340	- ^b	- ^b
166	270.4 ± 41.8	811.7 ± 140.8	1760 ± 106	2645 ± 258	4424.6 ± 1087
169	531.6 ± 61.2	740.9 ± 65.2	1600 ± 123	2895 ± 111	4487.5 ± 332
186	314.9 ± 42.9	751.1 ± 30.2	1733 ± 182	2196 ± 352	- ^b
Global fit	393 ± 86.8	681 ± 57.9	1728 ± 76.5	2651 ± 315	4883 ± 80.7

^a Data obtained at 25 °C are shown in Table S1.

^b Data were not analyzed because of either peak overlap or absence or small R_{ex} .

Figure 1

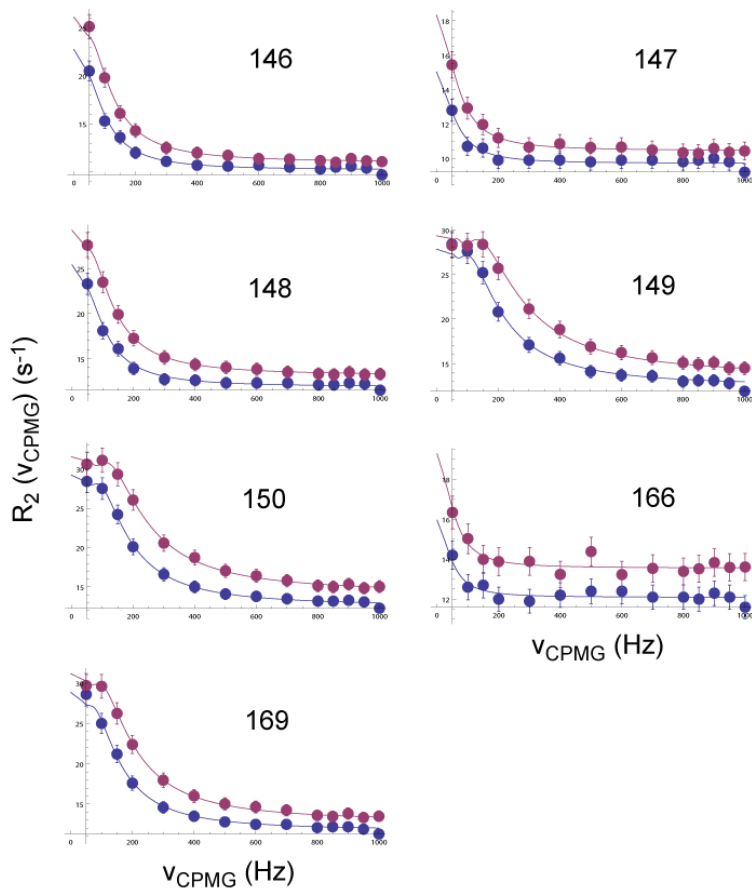


Figure S1a. ^{15}N CPMG-RD profiles of the nSH3 domain, which is mixed with PRM⁵²⁴. Based on the K_d , the population of the nSH3:PRM⁵²⁴ complex is estimated to be ~5 % of the entire population of nSH3 domain. The residue numbers are shown in each figure. The blue and red circles represent the data acquired at 14.1 T and 18.8 T, respectively. The solid lines represent the best fit to the individual residues. The results of individual fit (k_{ex} values) are shown in Table S1.

Figure S1 continued

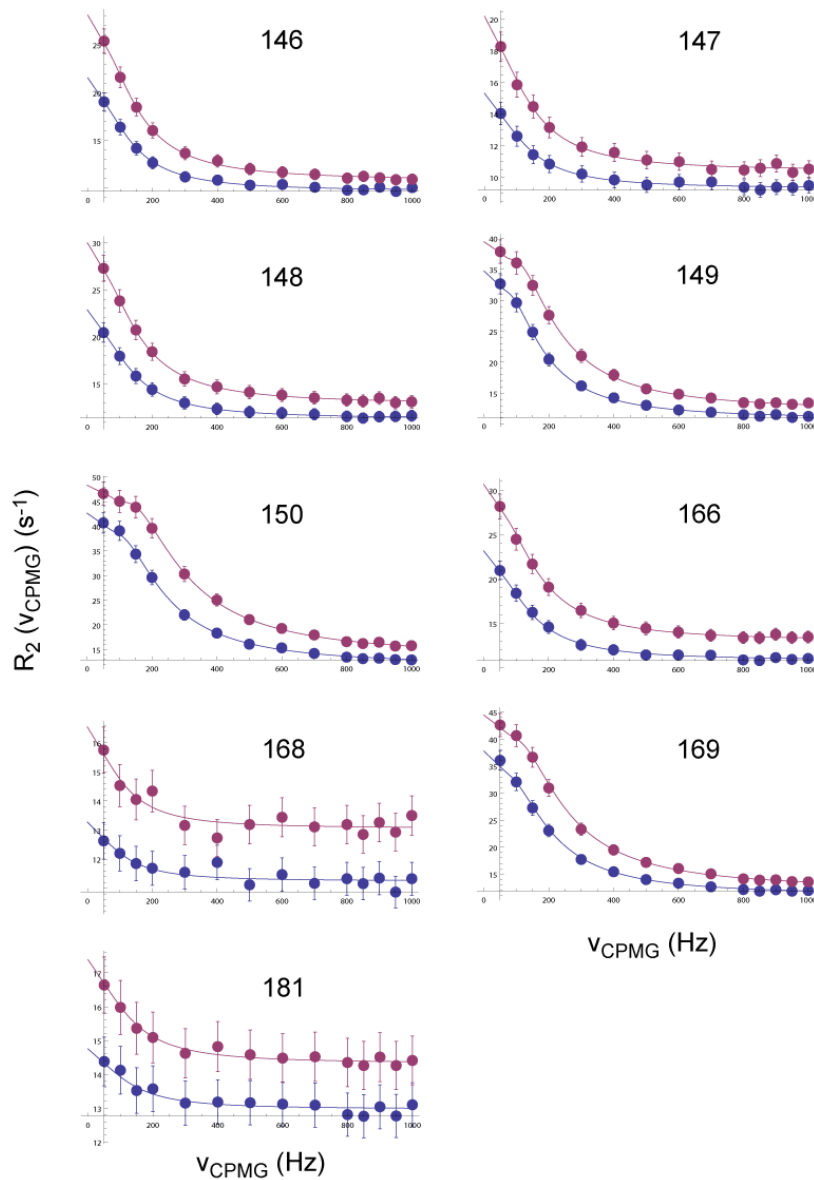


Figure S1b. ^{15}N CPMG-RD profiles of the nSH3 domain, which is mixed with PRM⁵⁶⁸. Based on the K_d , the population of the nSH3:PRM⁵⁶⁸ complex is estimated to be ~5 % of the entire population of nSH3 domain. The residue numbers are shown in each figure. The blue and red circles represent the data acquired at 14.1 T and 18.8 T, respectively. The solid lines represent the best fit to the individual residues. The results of individual fit (k_{ex} values) are shown in Table S1.

Figure S1 continued

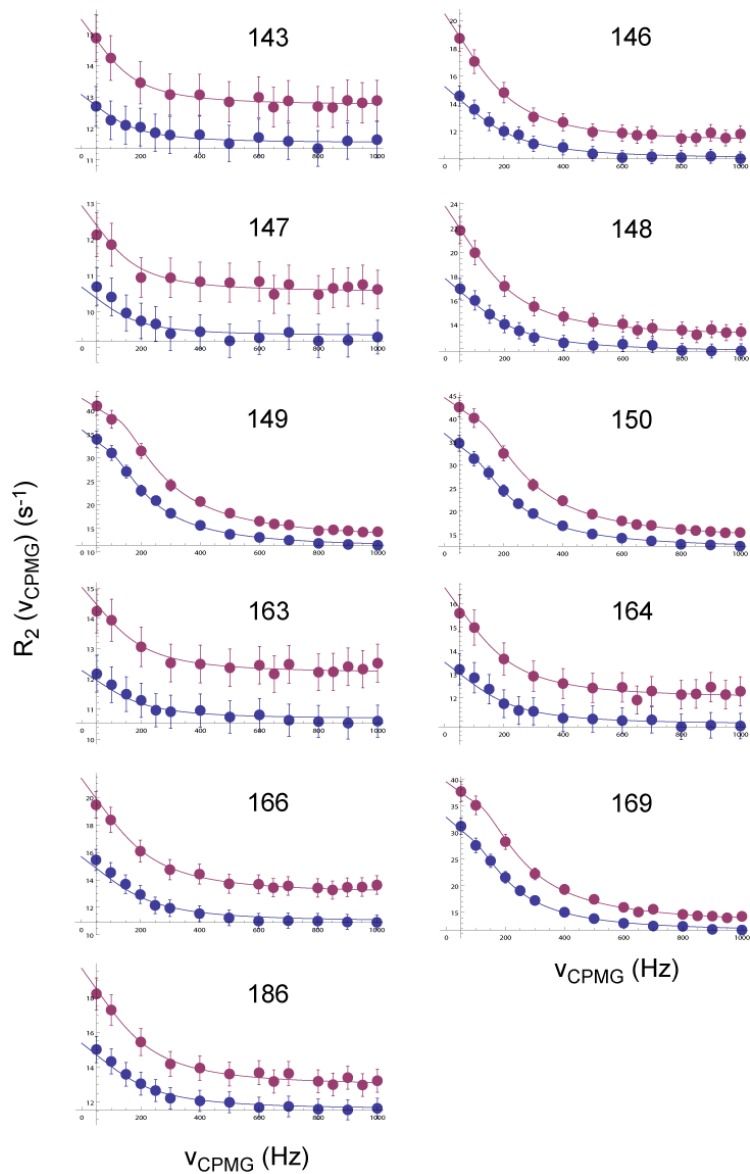


Figure S1c. ^{15}N CPMG-RD profiles of the nSH3 domain, which is mixed with PRM⁷⁵⁸. Based on the Kd, the population of the nSH3:PRM⁷⁵⁸ complex is estimated to be ~5 % of the entire population of nSH3 domain. The residue numbers are shown in each figure. The blue and red circles represent the data acquired at 14.1 T and 18.8 T, respectively. The solid lines represent the best fit to the individual residues. The results of individual fit (k_{ex} values) are shown in Table S1.

Figure S2

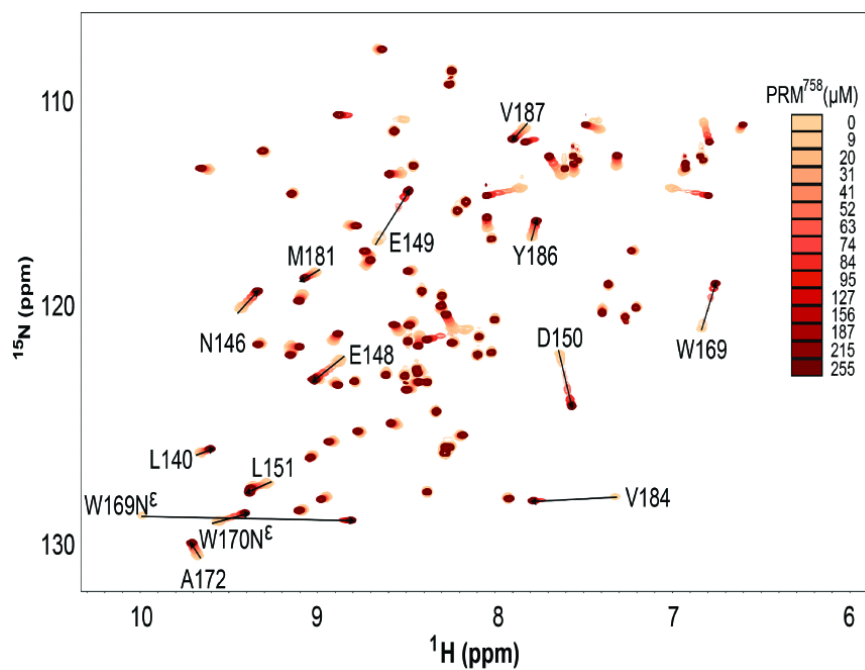


Figure S2. The NMR titration result showing the binding between the nSH3 domain and PRM^{758} . The gradual changes of peaks indicate that the binding process occurs in the intermediate – fast NMR timescales.

Figure S3

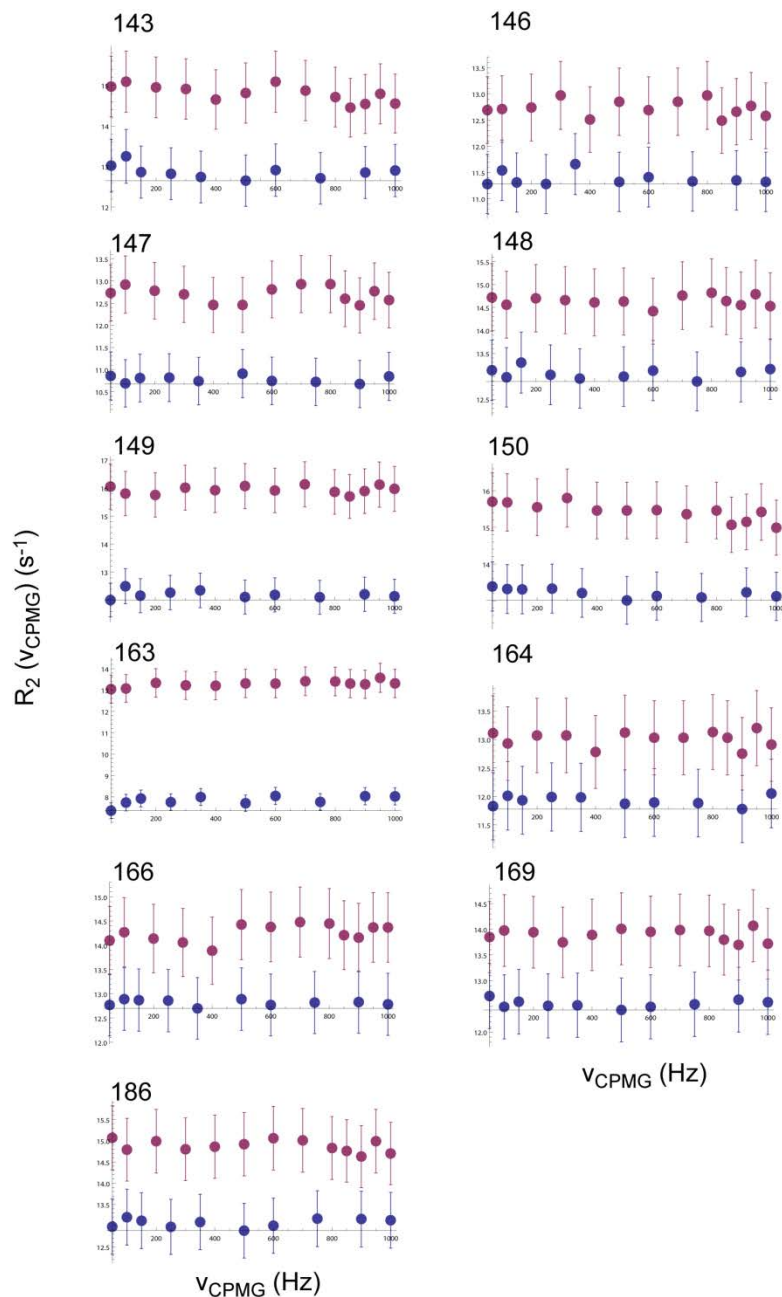


Figure S3. The ^{15}N CPMG-RD profiles of the peaks that were included in the analysis of binding between nSH3 domain and PRMs. The residue numbers are shown on top of each panel. The blue and red circles represent the CPMG-RD data for the free and PRM-saturated nSH3 domains, respectively. For clarity, only the data obtained at 800MHz are shown.

Figure S4

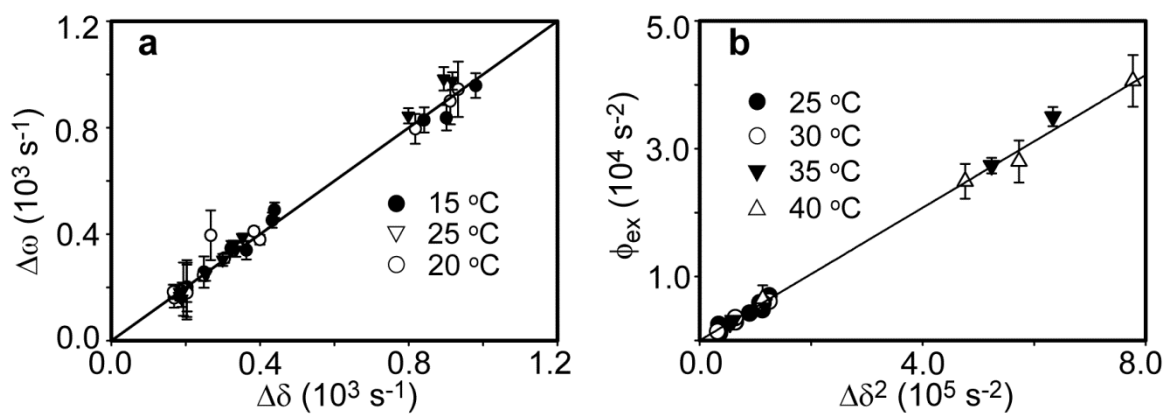


Figure S4. Correlation of $\Delta\delta$ and (a) $\Delta\omega$ or (b) ϕ_{ex} at different temperatures. The diagonal line in (a) is shown to guide the eye and is not a regression curve. The line in (b) is a linear regression line.

Figure S5

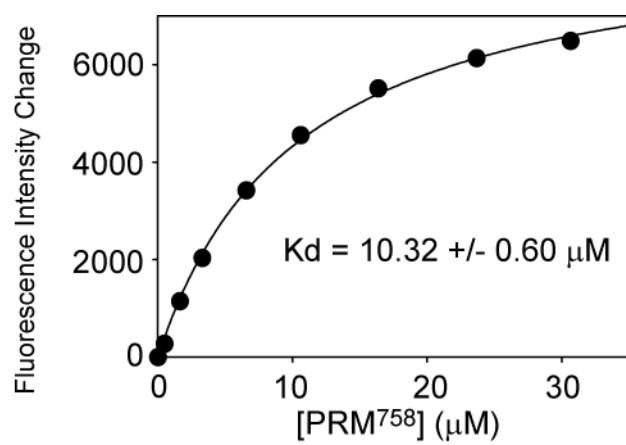


Figure S5. Fluorescence-probed binding isotherm between PRM⁷⁵⁸ and the nSH3 domain in the presence of 1 M NaCl at 298 K.