# Supporting Information for:

# Different molecular recognition by three domains of the full-length GRB2 to SOS1 proline-rich motifs and EGFR phosphorylated sites

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17. **Supplementary Figure S17.** Overlays of 2D $^{1}H$-$^{15}N$ HSQC spectra from multipoint titrations of $^{15}N$-labelled GRB2 with the EGFR phosphorylated peptide, in the presence of S4 PRM.
18. **Supplementary Table S1** Dissociation constants derived from ITC analysis for the full length GRB2 and isolated SH3 domains against SOS1 PRMs.

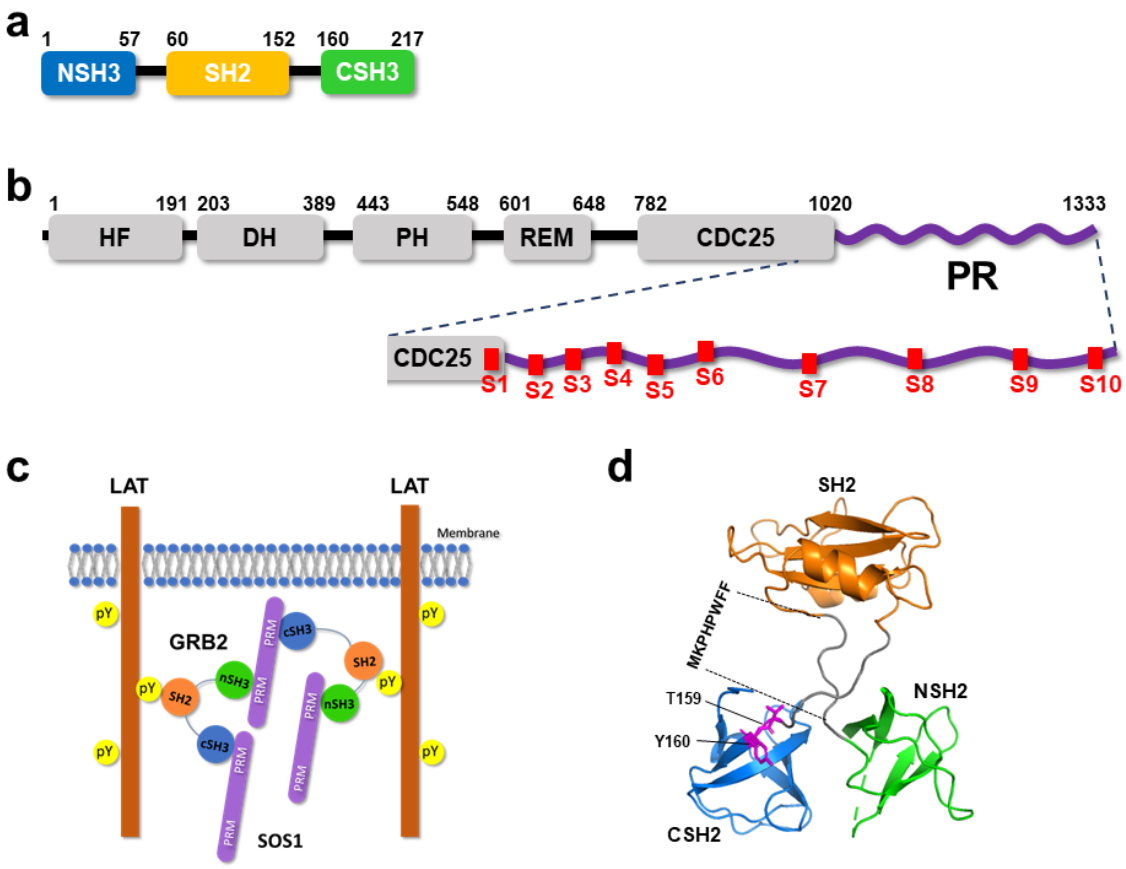



**Figure S1. Domain and three dimensional (3D) structures of GRB2 and SOS1, and schematic illustration of LLPS.** (a) the domain structure of GRB2. (b) the domain structure of SOS1 with the positions of proline-rich motifs, SOS1 S1-S10, indicated by red boxes. (c) Schematic illustration of the considered multivalent interactions among GRB2, SOS1 and LAT in liquid-liquid phase separation (LLPS), proposed in the previous reports. (d) The crystal structure of GRB2.

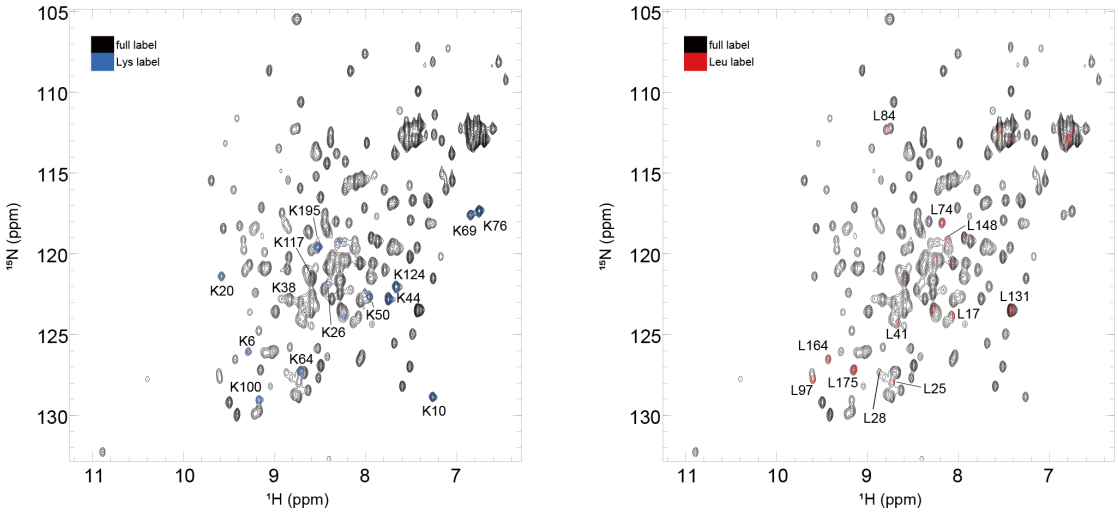



**Figure S2. Overlays of 2D $^{1}$H-$^{15}$N HSQC spectra of $^{15}$N-uniformly and amino-acid-selectively labelled samples.** The spectra of lysine- (left) and leucine- (right) selectively labelled GRB2 were overlaid onto those of the $^{15}$N-uniformly labelled samples.

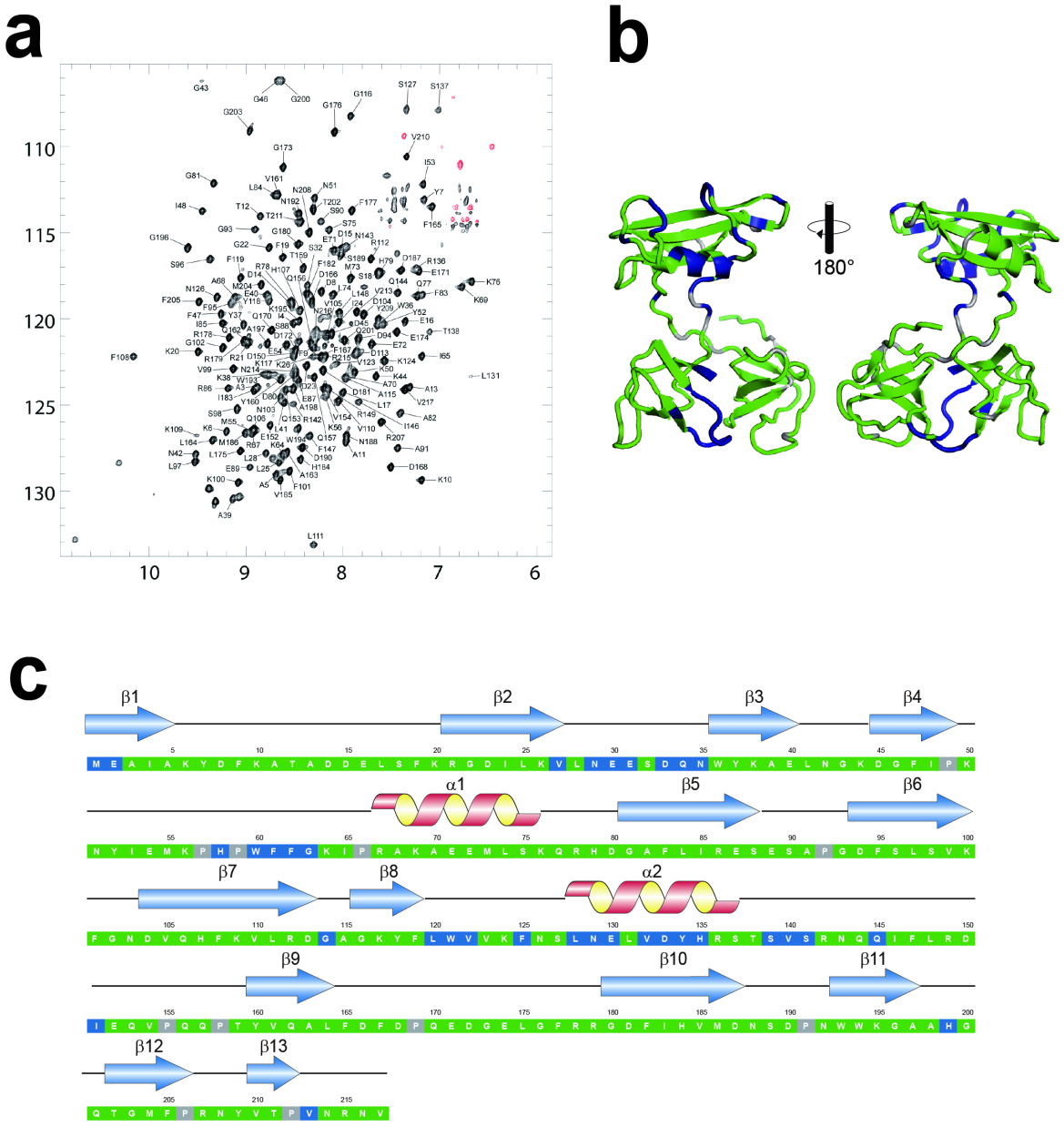



**Figure S3. Backbone resonance assignments of GRB2.** (a) 2D $^{1}$H-$^{15}$N HSQC spectrum and backbone resonance assignments of GRB2. Positive and negative signals (aliased on the $^{15}$N axis) are color-coded in black and red, respectively. (b) The backbone resonance assignments are mapped onto the crystal structure of GRB2. Assigned and unassigned residues are colour-coded in green and blue, respectively. (c) The backbone resonance assignments aligned with

secondary structures. Assigned, unassigned, and proline residues are colour-coded in green, blue, and grey, respectively.

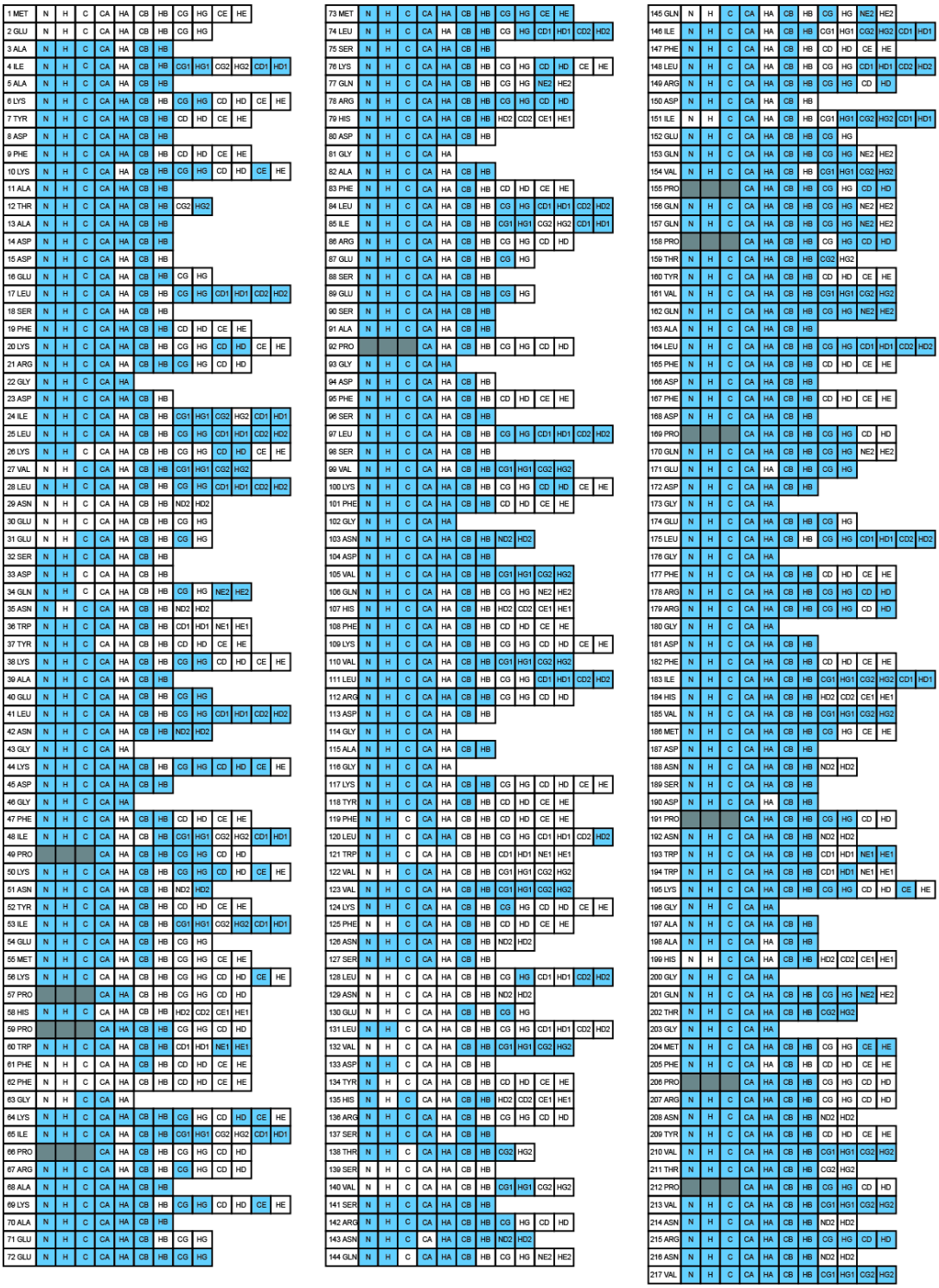


**Figure S4. Table summarising the backbone and side-chain resonance assignments of GRB2.** Blue and white columns indicate assigned and unassigned atoms, respectively.

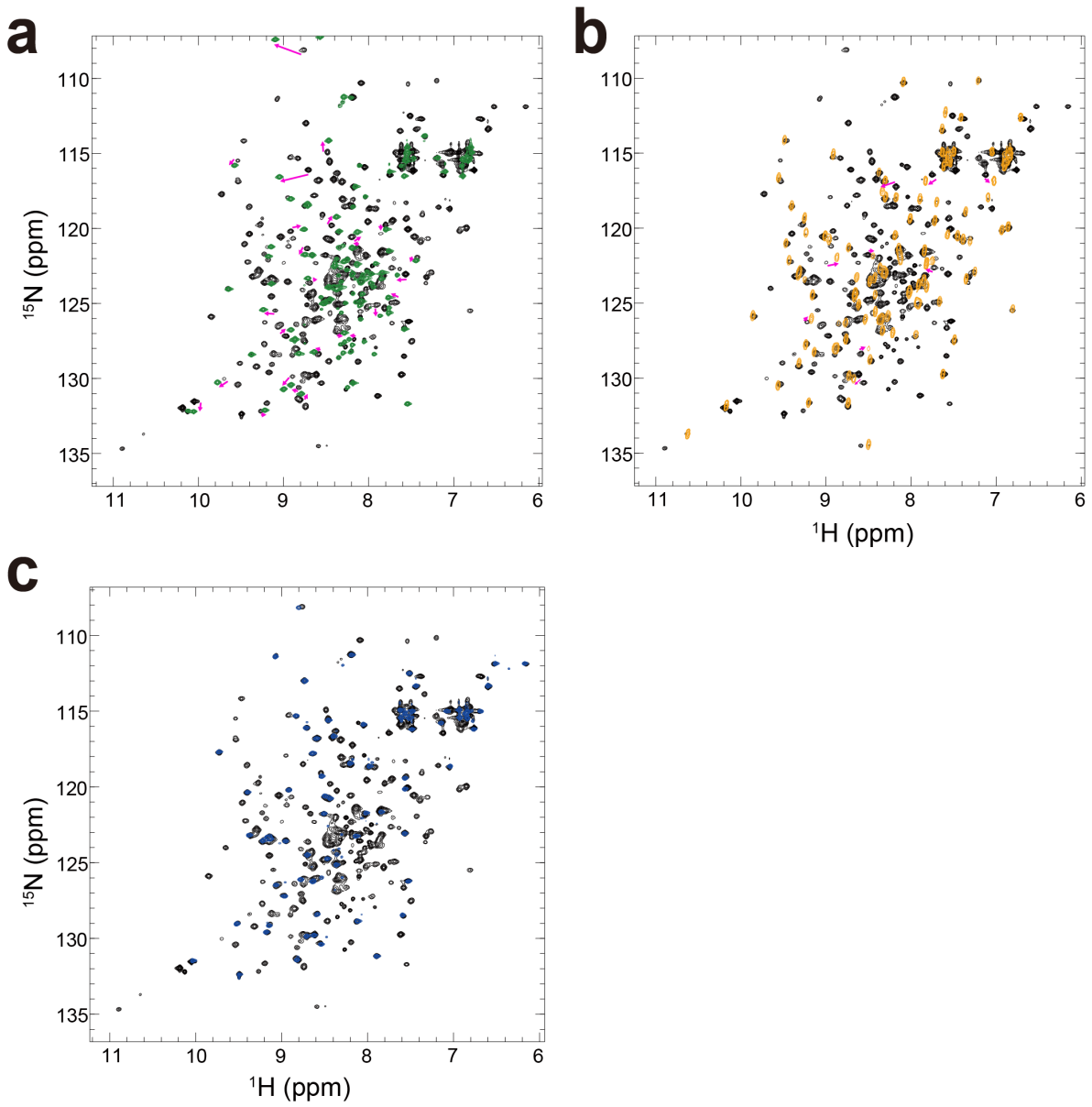



**Figure S5. Overlays of 2D $^{1}$H-$^{15}$N HSQC spectra of the full-length GRB2 and each isolated domain.** The black spectra indicate the full-length GRB2, and green, orange, and blue represent isolated NSH3, SH2, CSH3, respectively. The red arrows indicate representative peaks that significant chemical shift changes were observed.

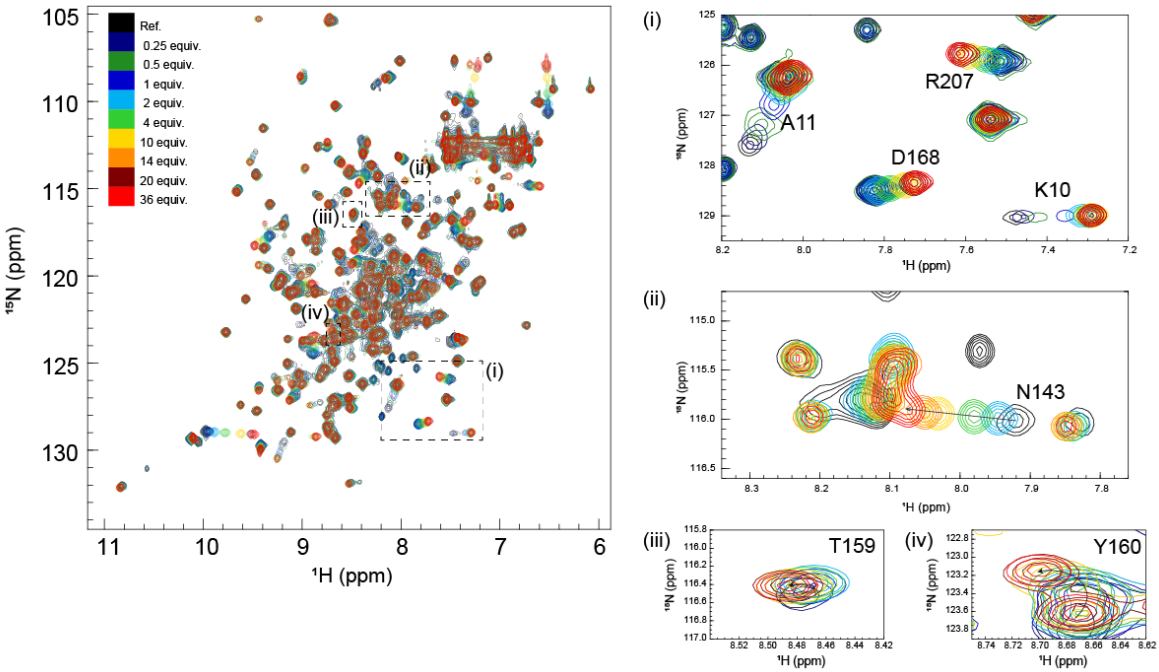



**Figure S6. Overlays of 2D $^{1}$H-$^{15}$N HSQC spectra from multipoint titrations of $^{15}$N-labelled GRB2 with SOS-S5 PRM (DSPPAOPPRQPT).** The peptide concentration was increased stepwise (the protein: peptide molar ratio of 1:0.25, 1:0.5, 1:1, 1:2, 1:4, 1:10, 1:14, 1:20, and 1:36). In this figure, the colour codes of $^{1}$H-$^{15}$N correlation cross-peaks at each titration point, showing the molar ratio of GRB2: SOS-S5, are as follows: black (1:0); dark blue (1:0.25); dark green (1:0.5); blue (1:1); cyan (1:2); green (1:4); yellow (1:10); orange (1:14); dark red (1:20), red (1:36). Cross peaks that showed large chemical shift changes were annotated.

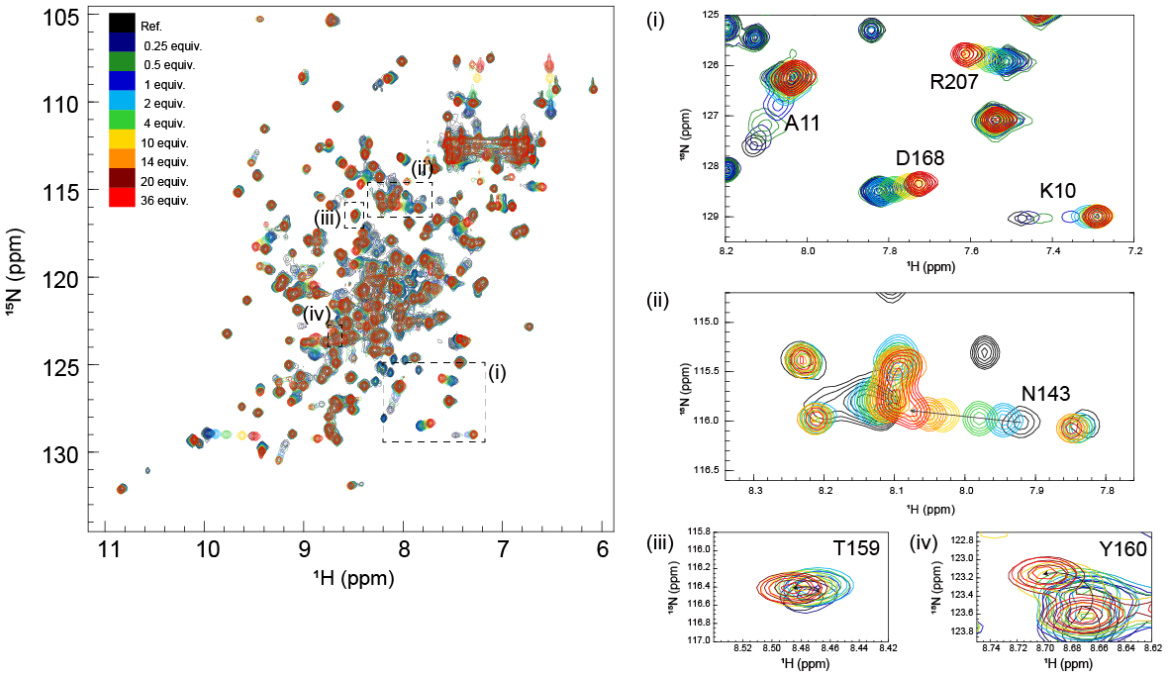



**Figure S7. Overlays of 2D $^{1}$H-$^{15}$N HSQC spectra from multipoint titrations of $^{15}$N-labelled GRB2 with SOS-S9 PRM (IAGPPVPPRQST).** The peptide concentration was increased stepwise (the protein: peptide molar ratio of 1:0.25, 1:0.5, 1:1, 1:2, 1:4, 1:10, 1:14, 1:20, and 1:36). In this figure, the colour codes of $^{1}$H-$^{15}$N correlation cross-peaks at each titration point, showing the molar ratio of GRB2: SOS-S9, are as follows: black (1:0); dark blue (1:0.25); dark green (1:0.5); blue (1:1); cyan (1:2); green (1:4); yellow (1:10); orange (1:14); dark red (1:20), red (1:36). Cross peaks that showed large chemical shift changes were annotated.

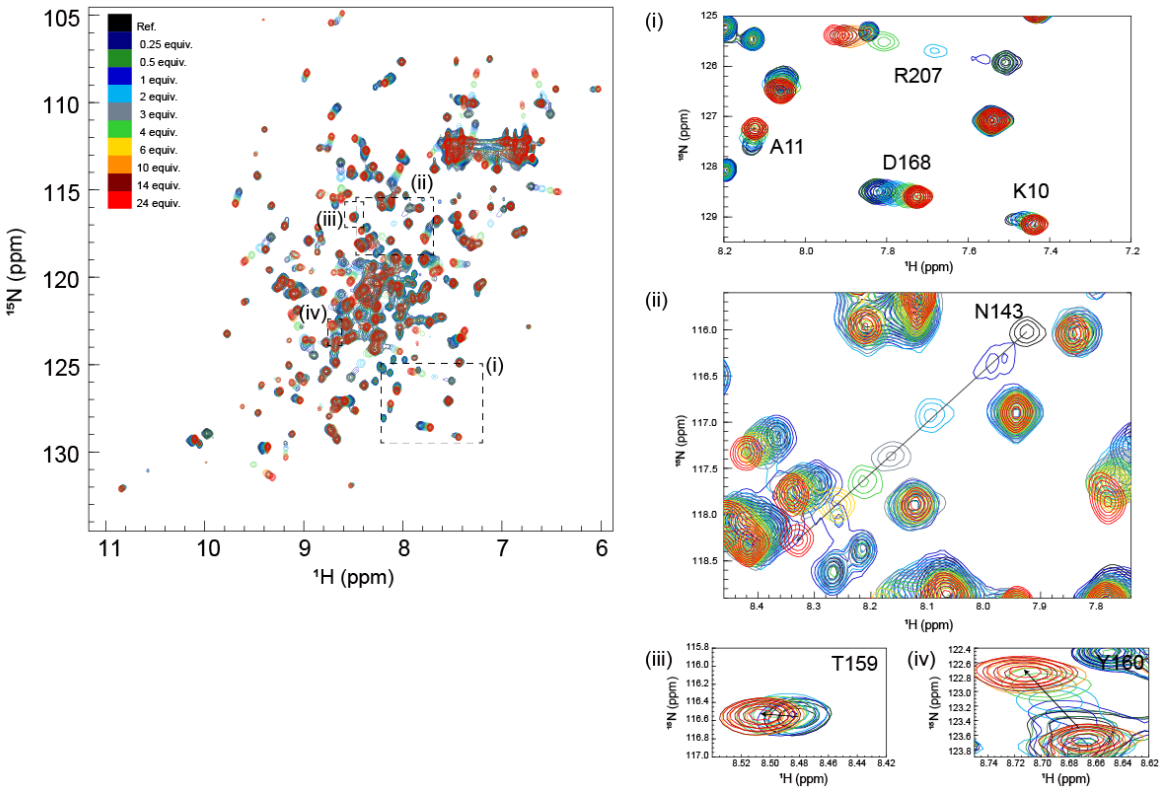



**Figure S8. Overlays of 2D $^{1}$H-$^{15}$N HSQC spectra from multipoint titrations of $^{15}$N-labelled GRB2 with SOS-S10 PRM (PKLPPKTYKREH).** The peptide concentration was increased stepwise (the protein: peptide molar ratio of 1:0.25, 1:0.5, 1:1, 1:2, 1:3, 1:4, 1:6, 1:10, 1:14, and 1:24). In this figure, the colour codes of $^{1}$H-$^{15}$N correlation cross-peaks at each titration point, showing the molar ratio of GRB2: SOS-S10, are as follows: black (1:0); dark blue (1:0.25); dark green (1:0.5); blue (1:1); cyan (1:2); grey (1:3), green (1:4); yellow (1:6); orange (1:10); dark red (1:14), red (1:24). Cross peaks that showed large chemical shift changes were annotated.

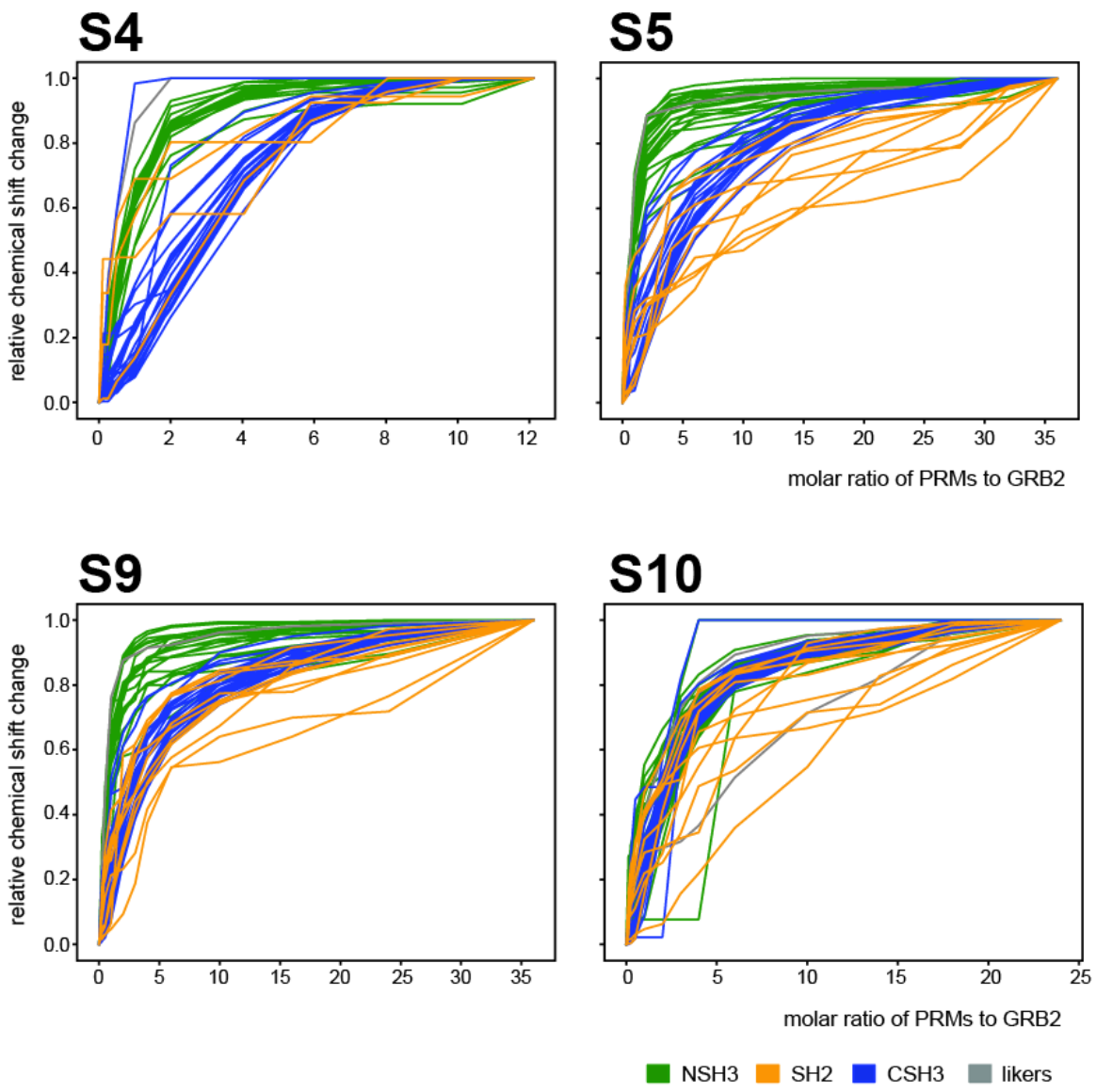



**Figure S9. Plots of relative change in chemical shift perturbation (CSP) of backbone $^{1}H^{N}$ and $^{15}N$ nuclei of GRB2 upon the titration with S4, S5, S9, and S10 PRMs.** A line represents the relative CSP beyond 0.06 ppm for a residue. Lines are colour-coded for each domain and linker.

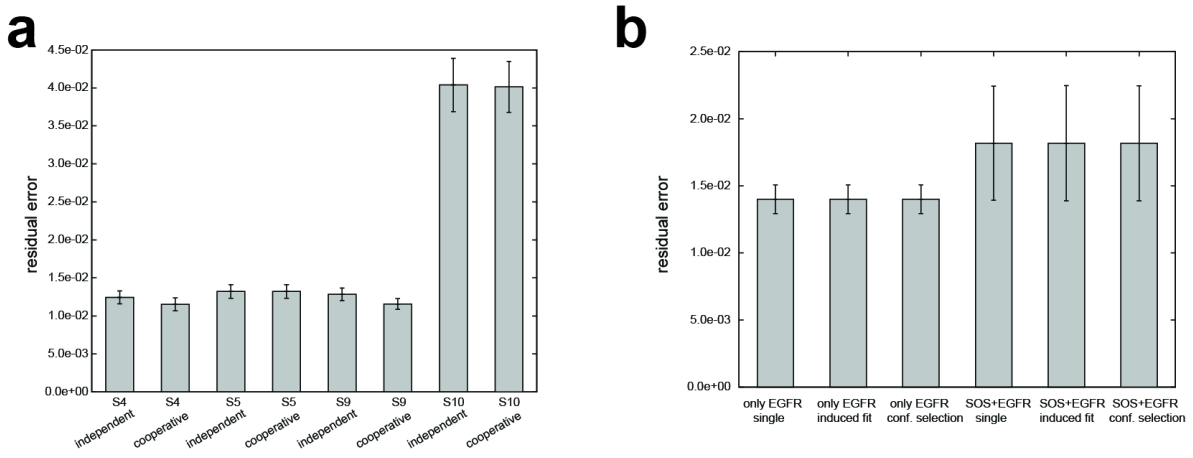
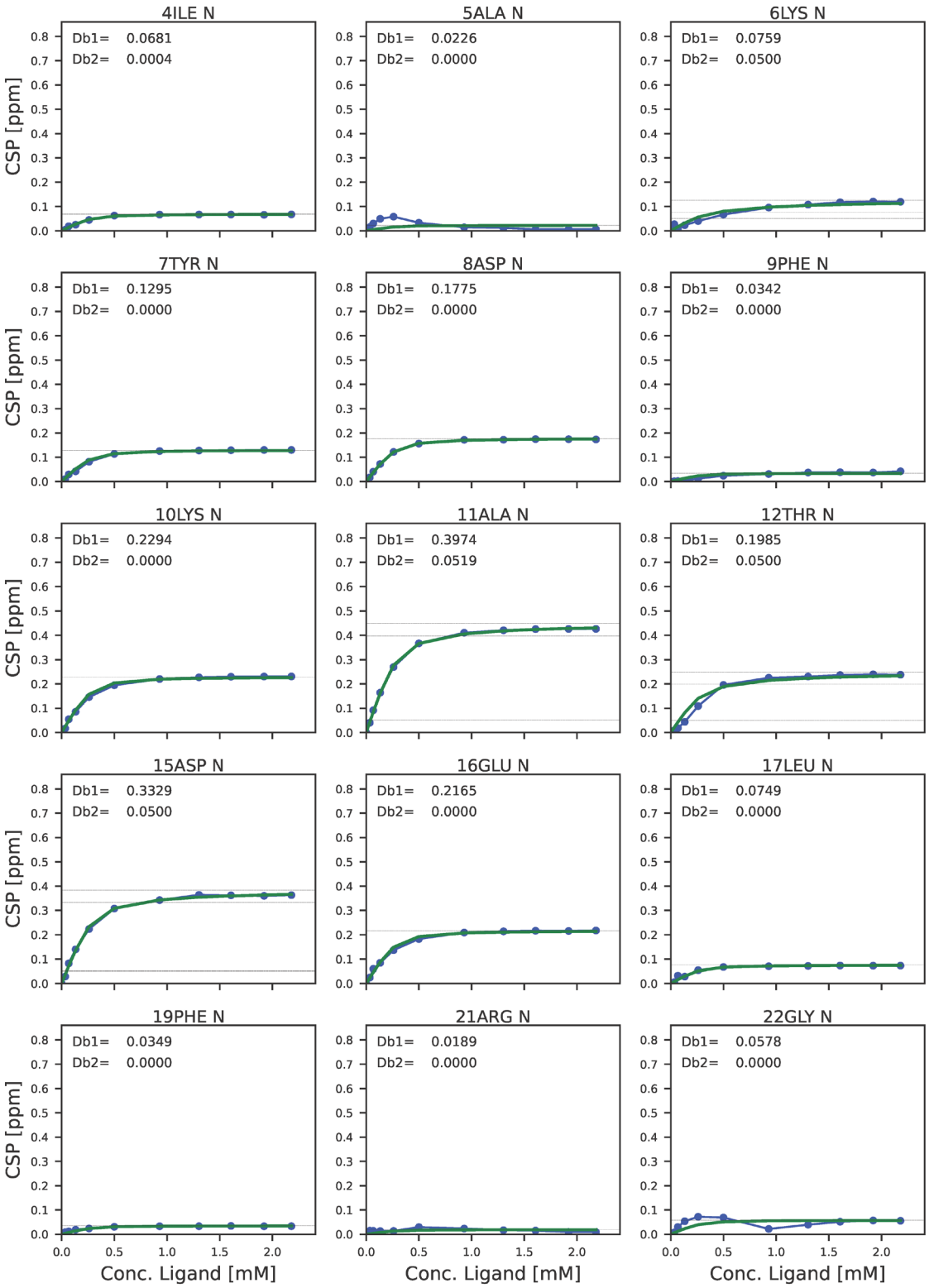



**Figure S10. Residual errors after the bootstrap calculations.** Residual errors after the bootstrap calculations with the CSPs of the S4, S5, S9, and S10 PRMs titration experiments for two-independent and two-cooperative binding models. residual errors after the bootstrap calculations for two-independent and two-cooperative binding models (right)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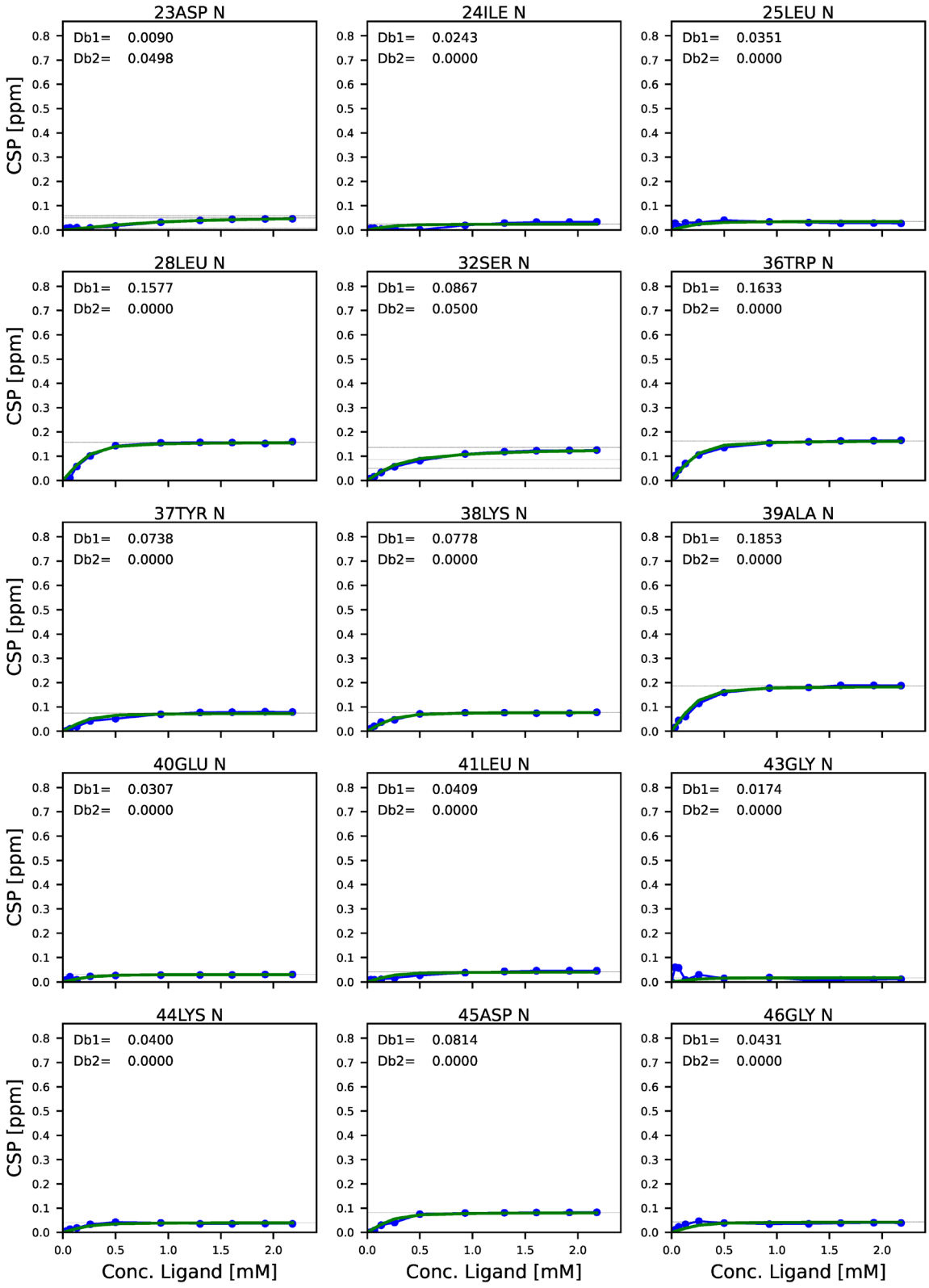
Conc. Ligand [mM]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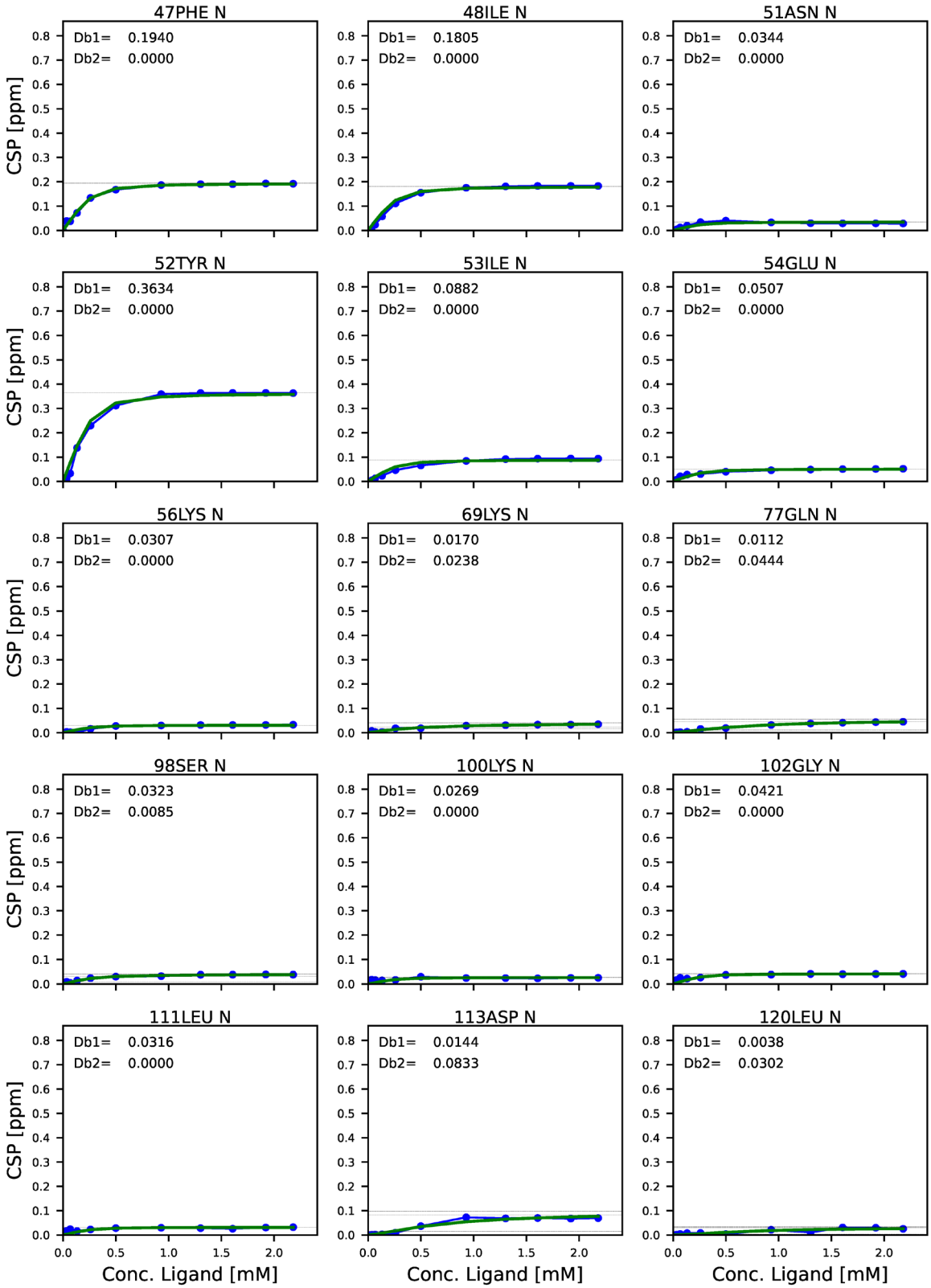
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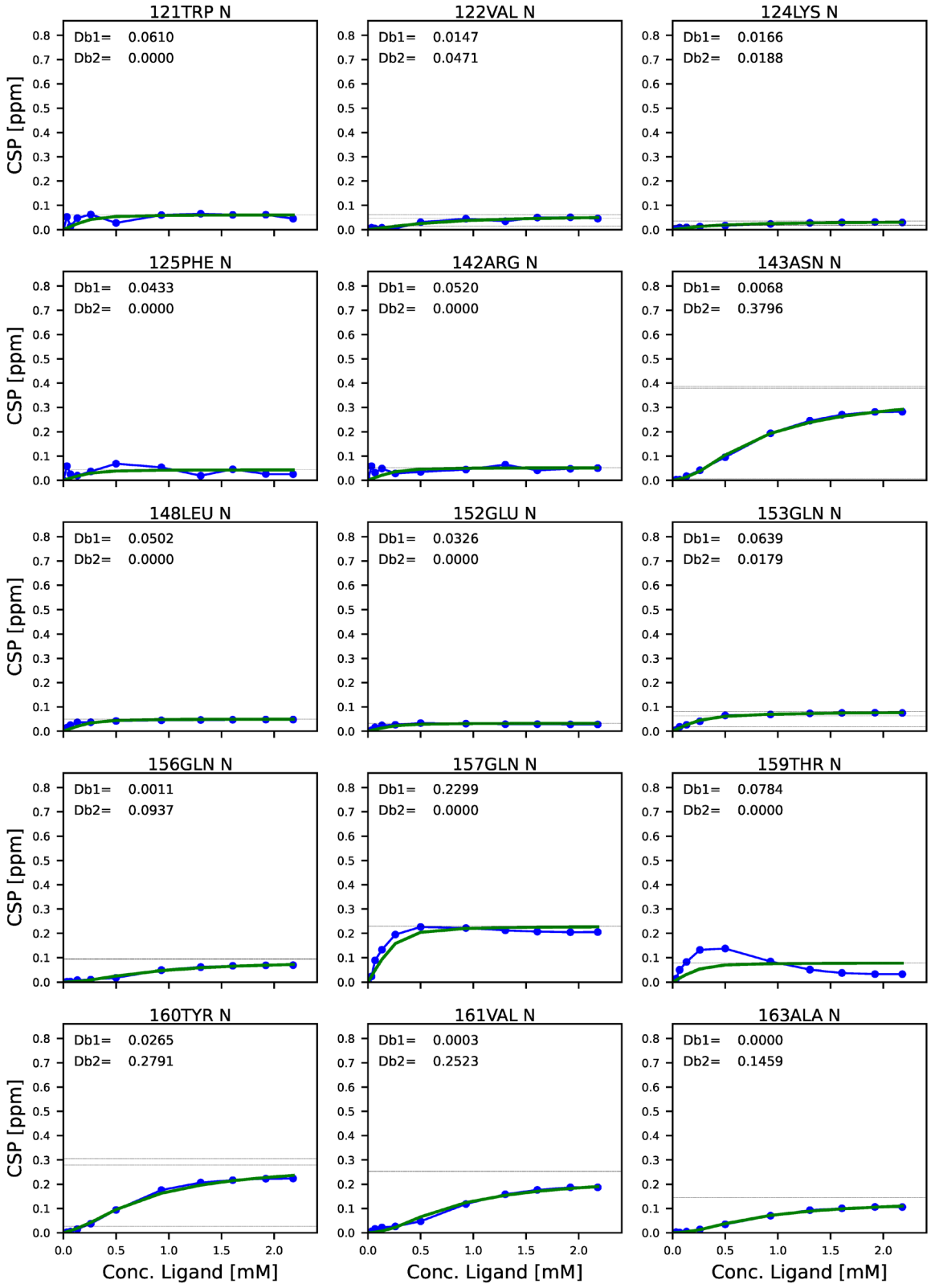
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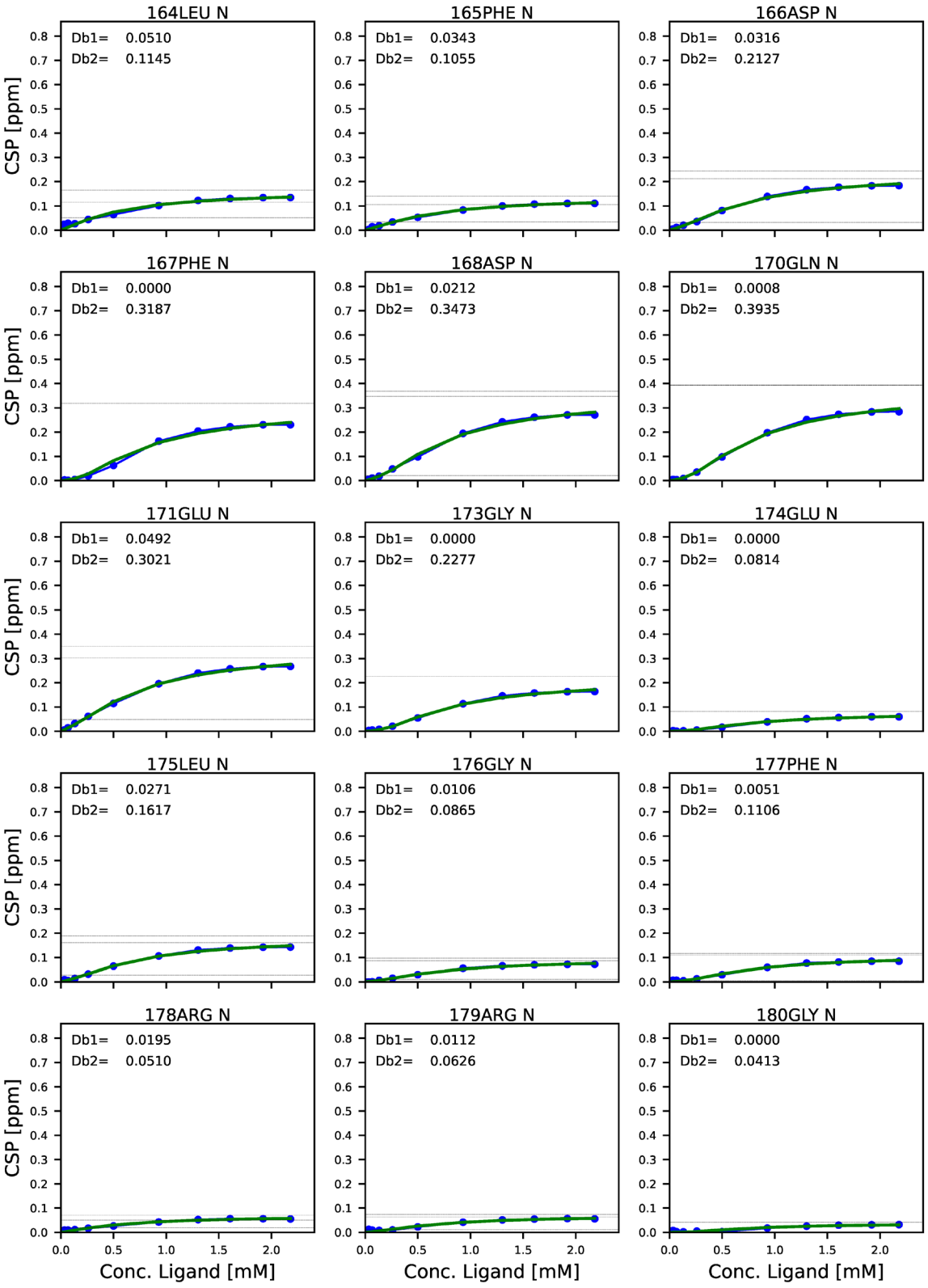
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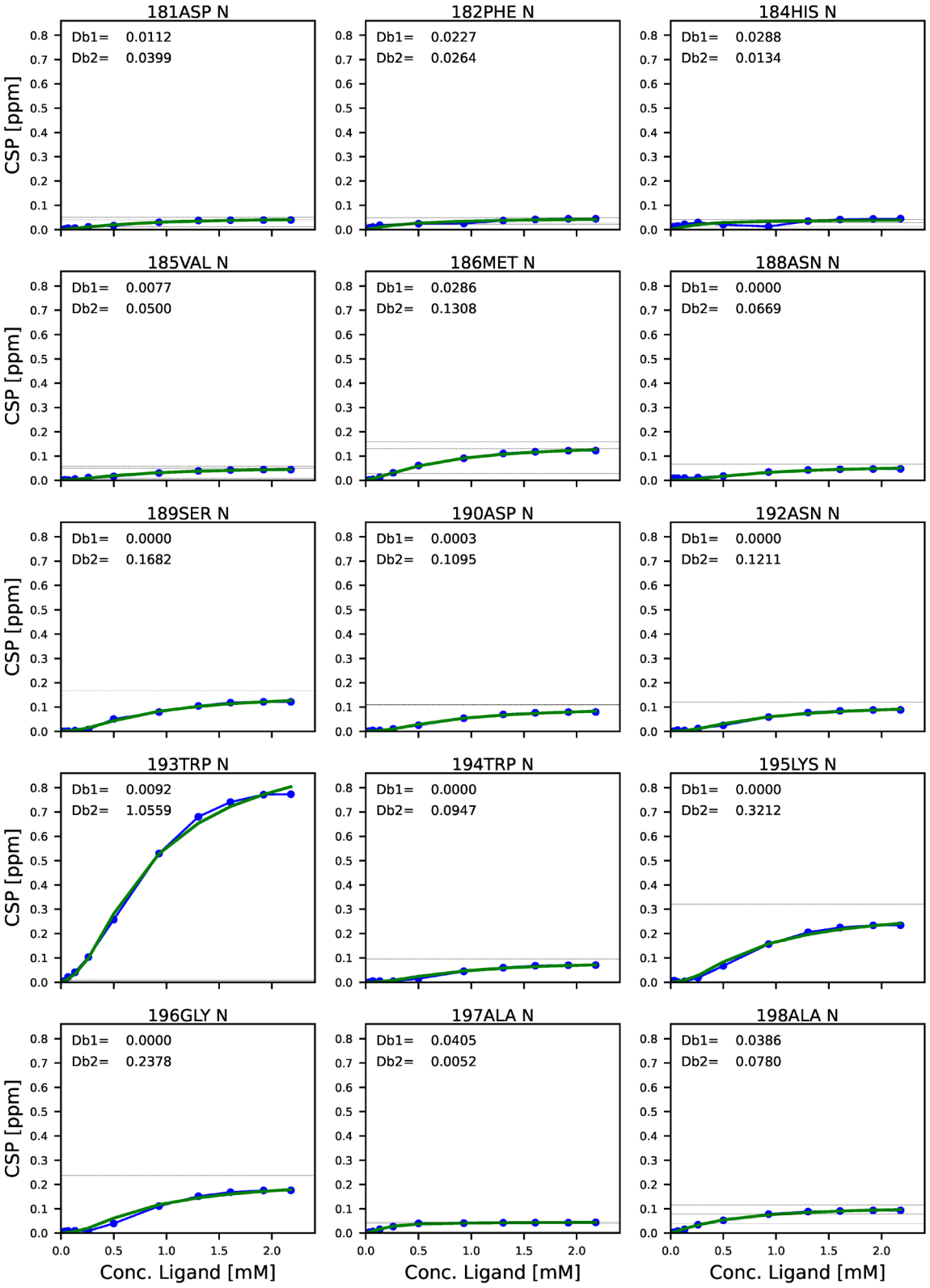
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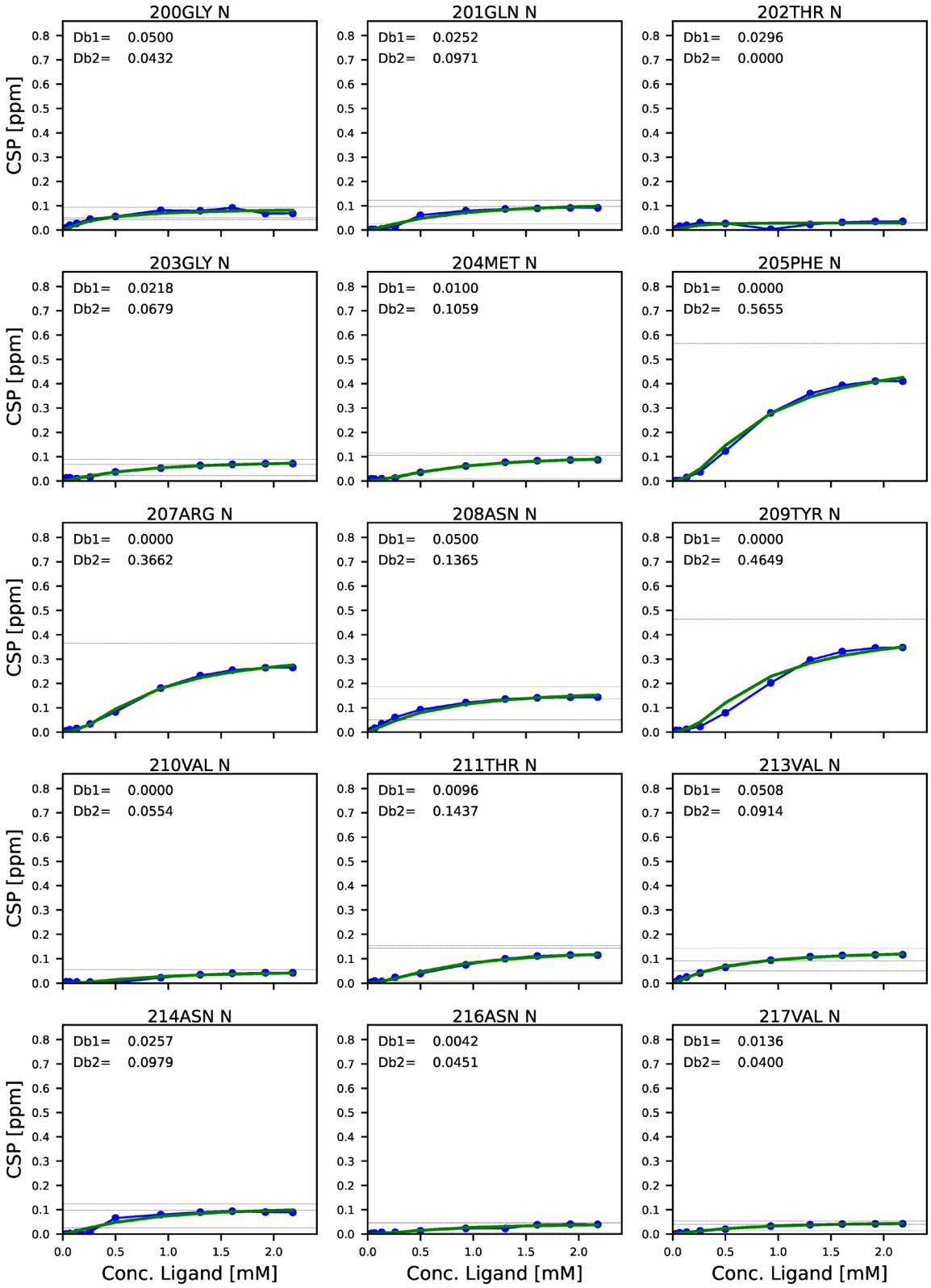
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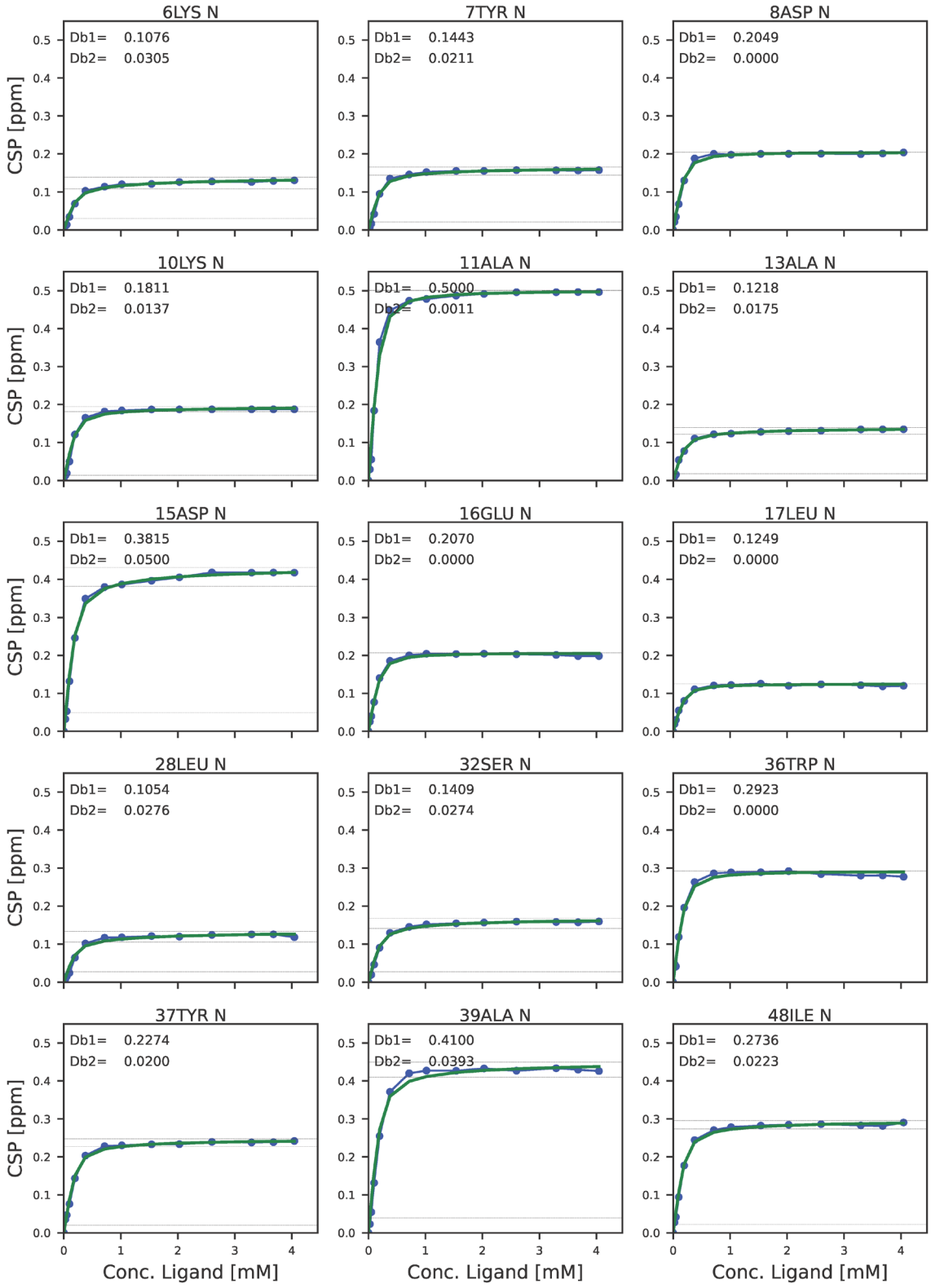
**Figure S11. Chemical shift perturbation profile mapped on each residue, against the concentration of ligand with SOS S4 PRM.** The blue dots and lines indicate CSP values at each titration point, while the green lines represent the theoretical curves calculated based on the two-site independent binding model.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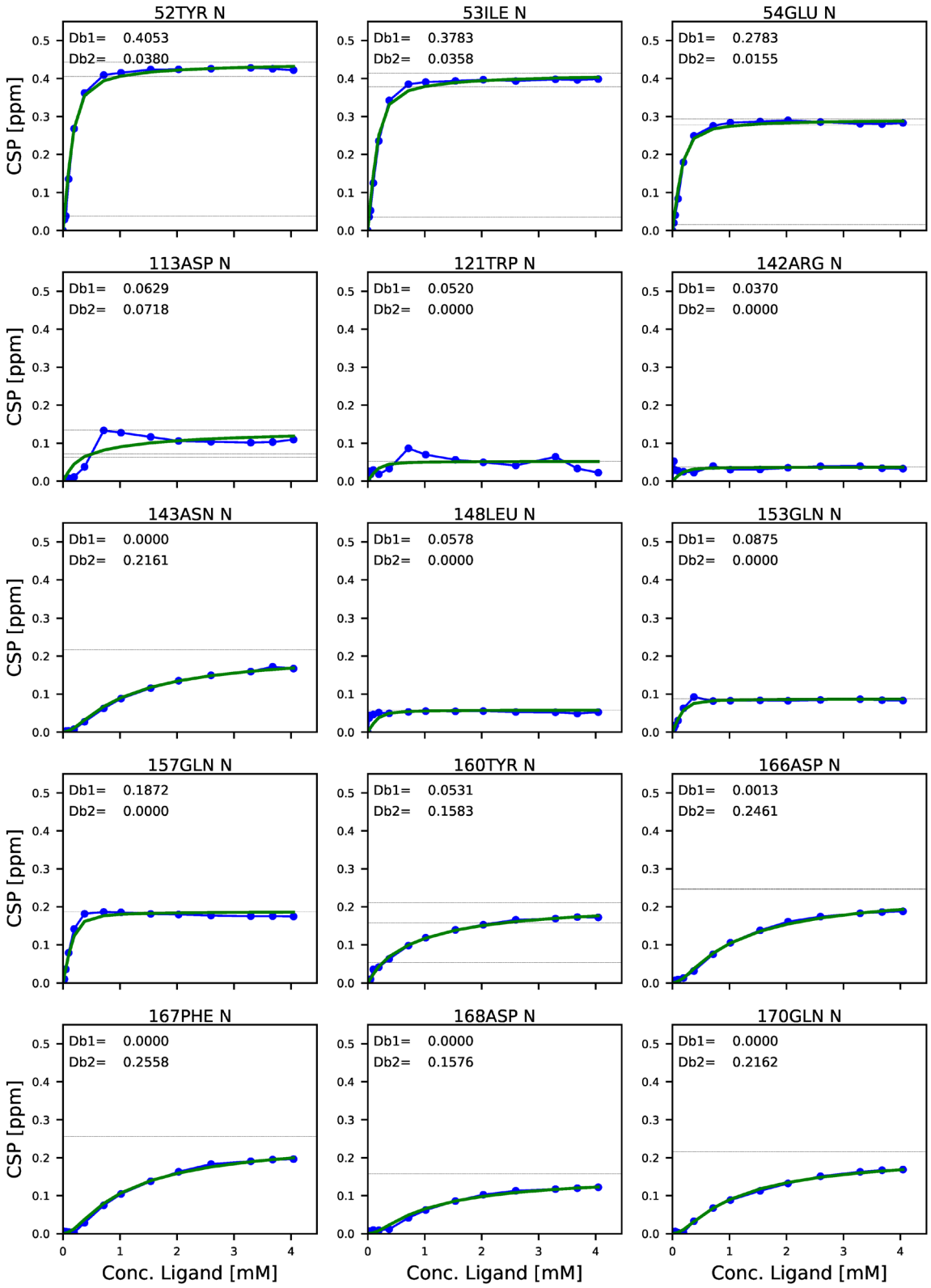
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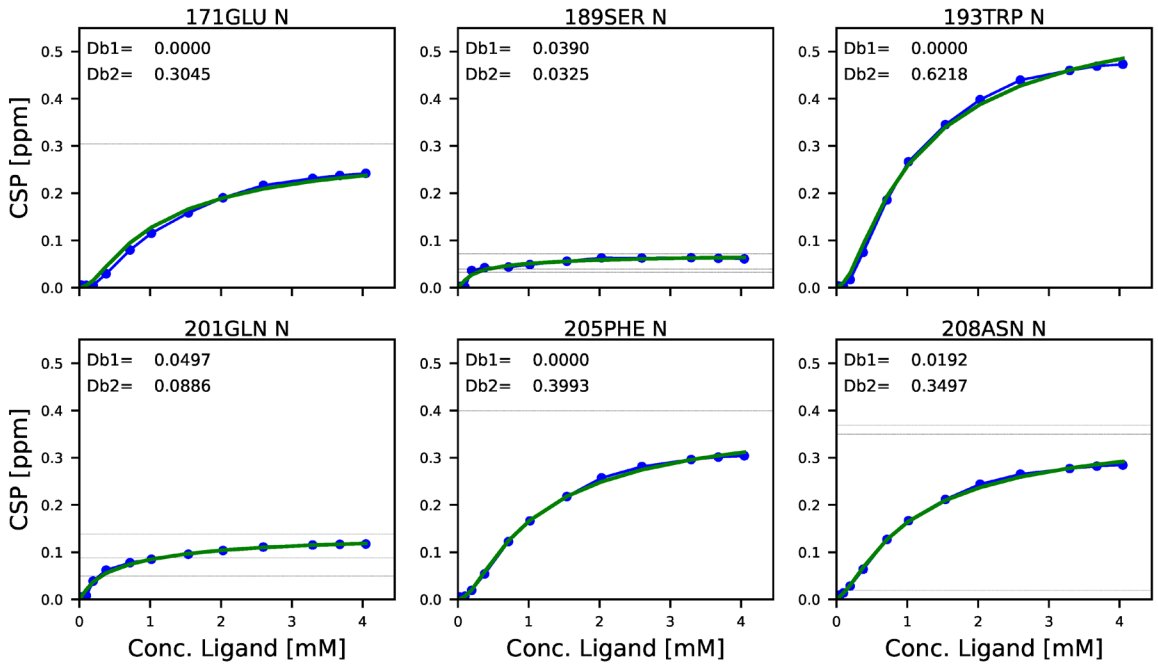
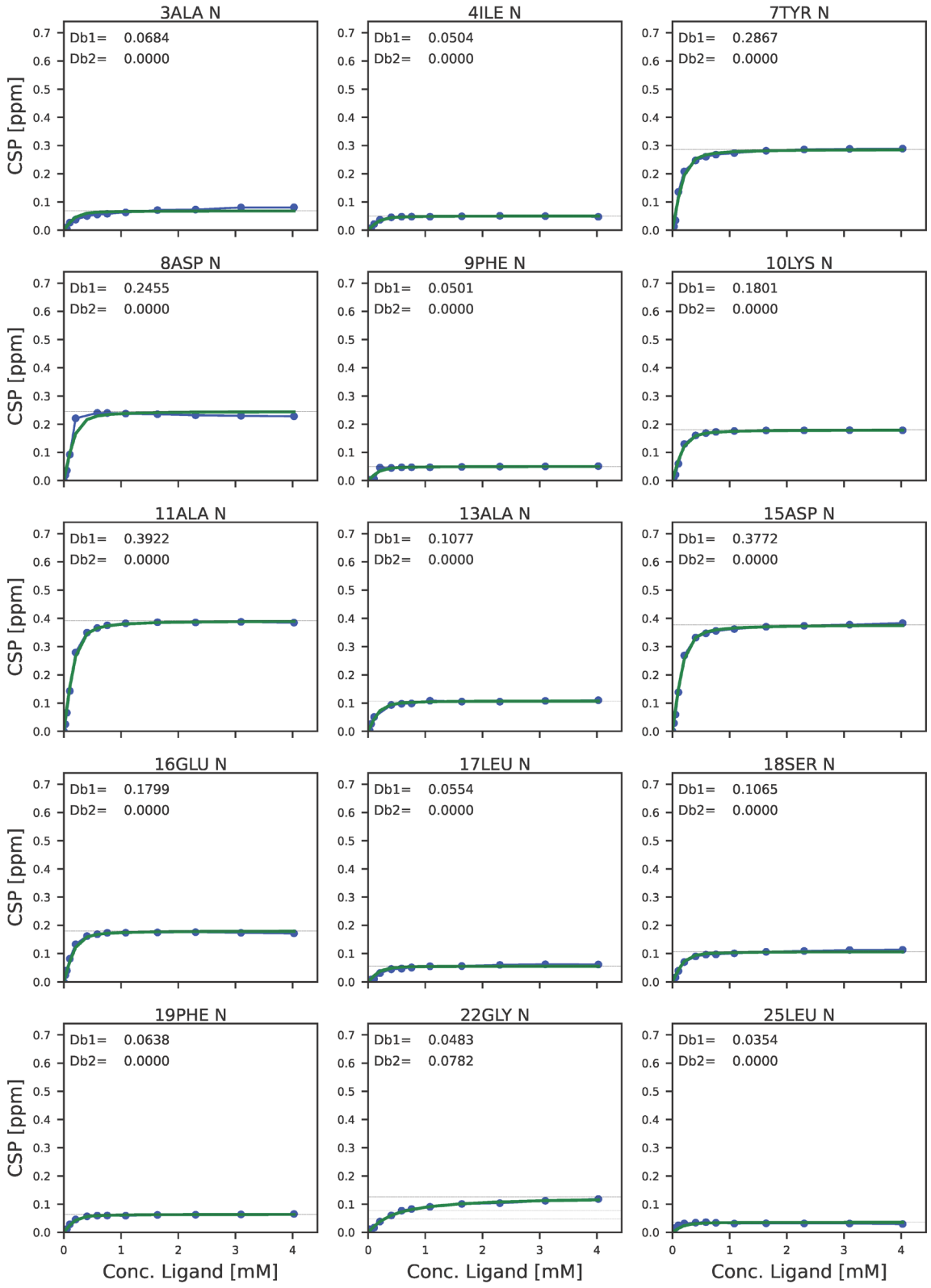



**Figure S12. Chemical shift perturbation profile mapped on each residue, against the concentration of ligand with SOS S5 PRM.** The blue dots and lines indicate CSP values at each titration point, while the green lines represent the theoretical curves calculated based on the two-site independent binding model.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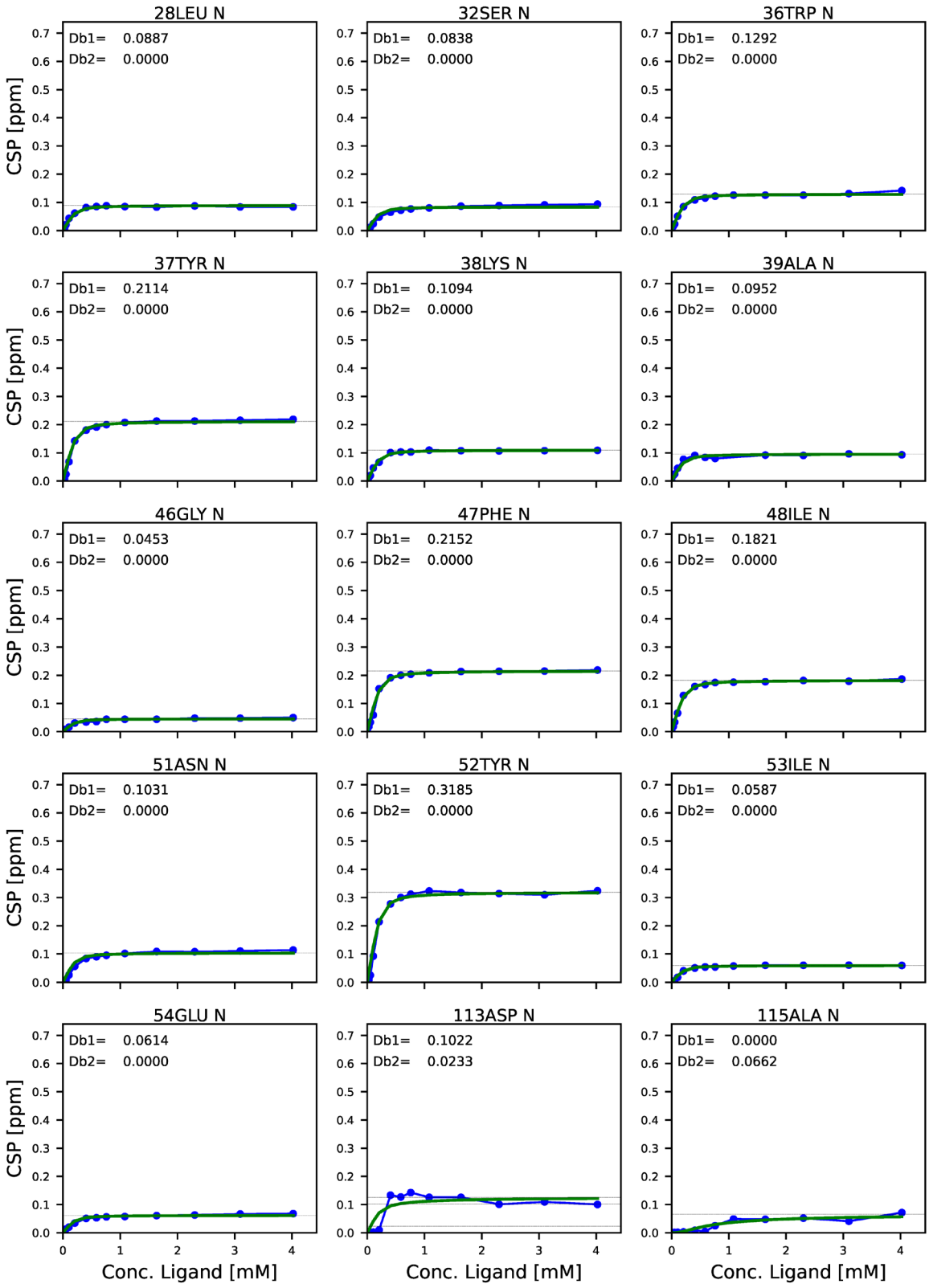
Conc. Ligand [mM]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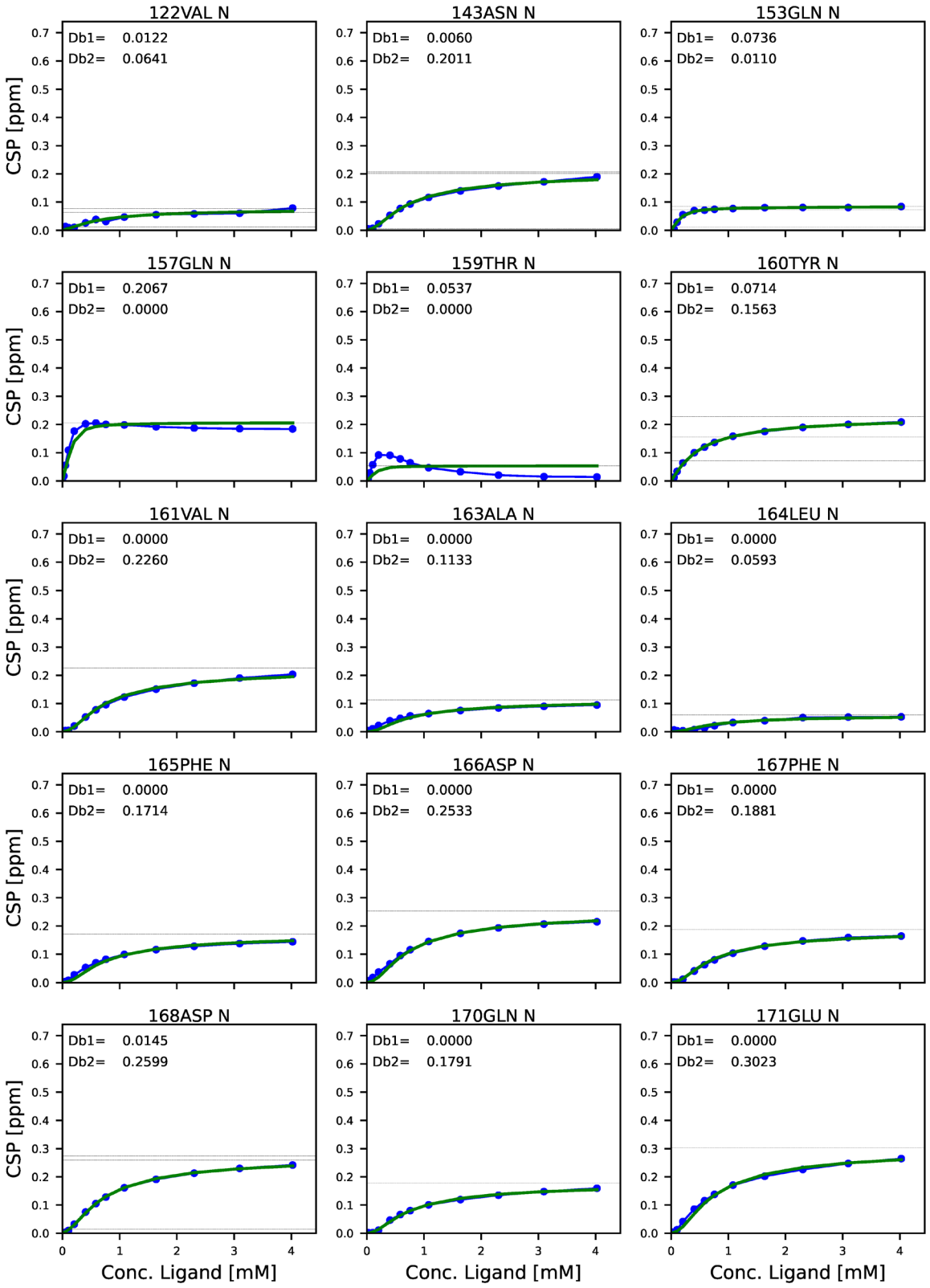
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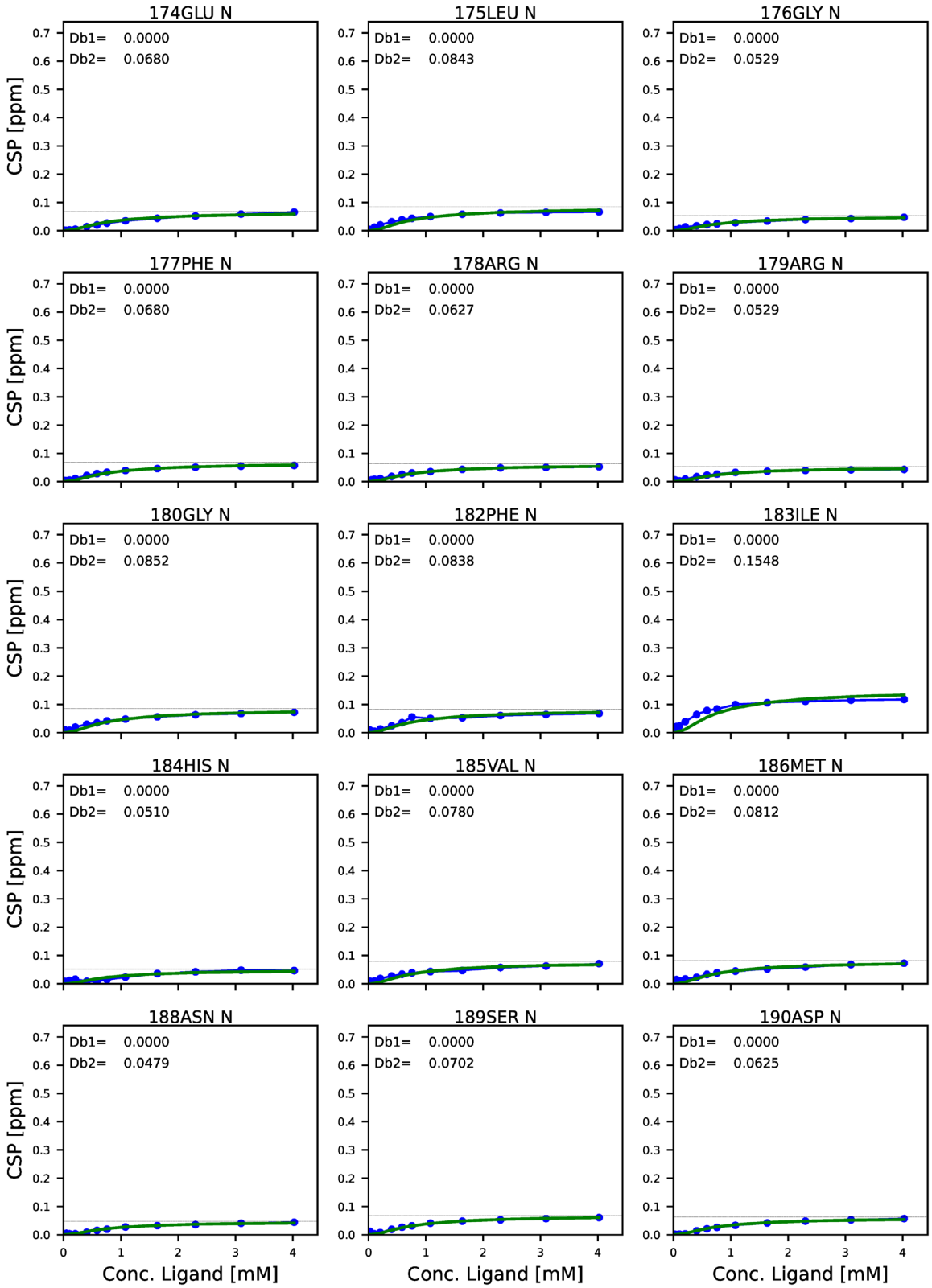
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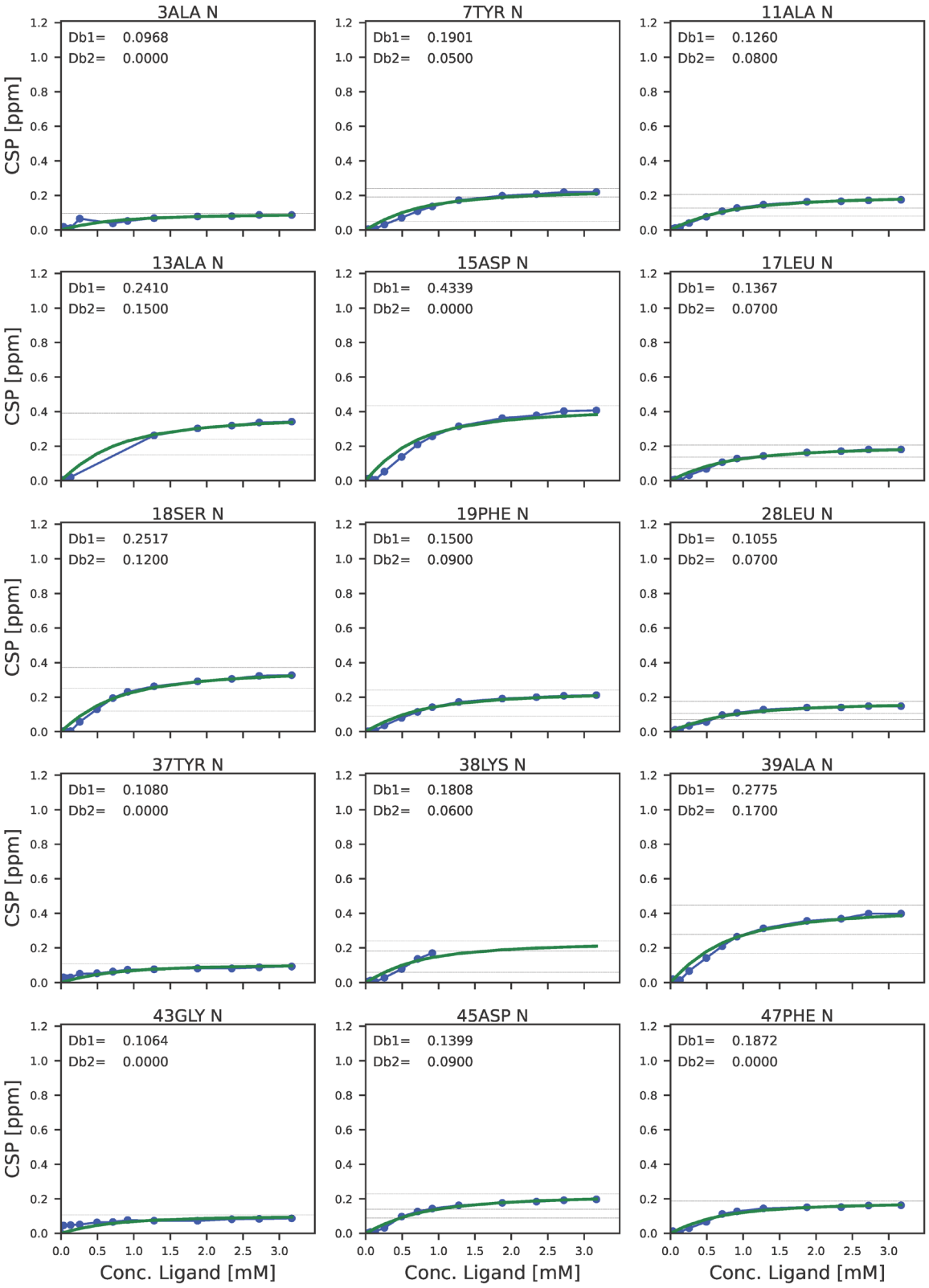
Conc. Ligand [mM]

**Figure S13. Chemical shift perturbation profile mapped on each residue, against the concentration of ligand with SOS S9 PRM.** The blue dots and lines indicate CSP values at each titration point, while the green lines represent the theoretical curves calculated based on the two-site independent binding model.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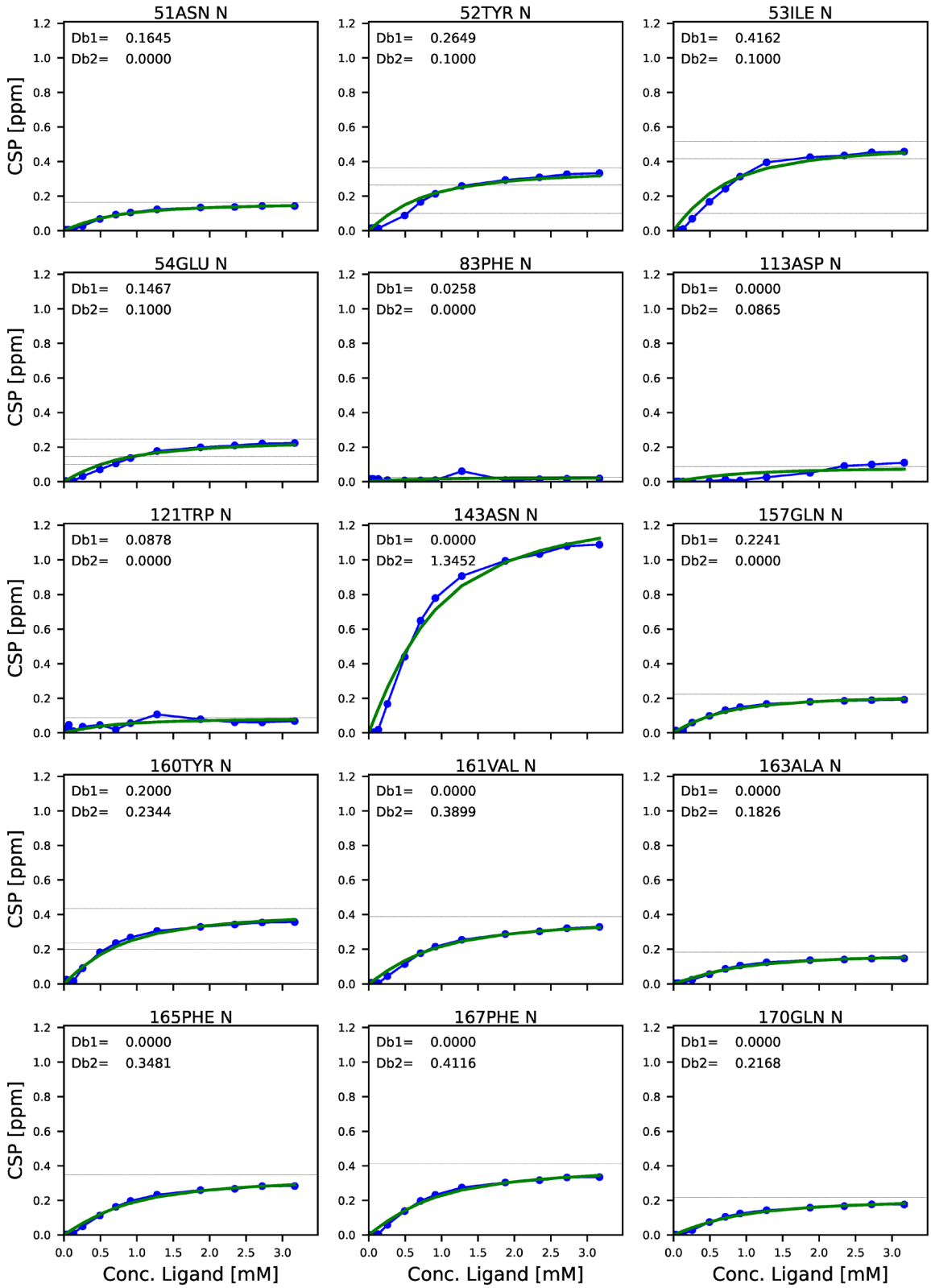
Conc. Ligand [mM]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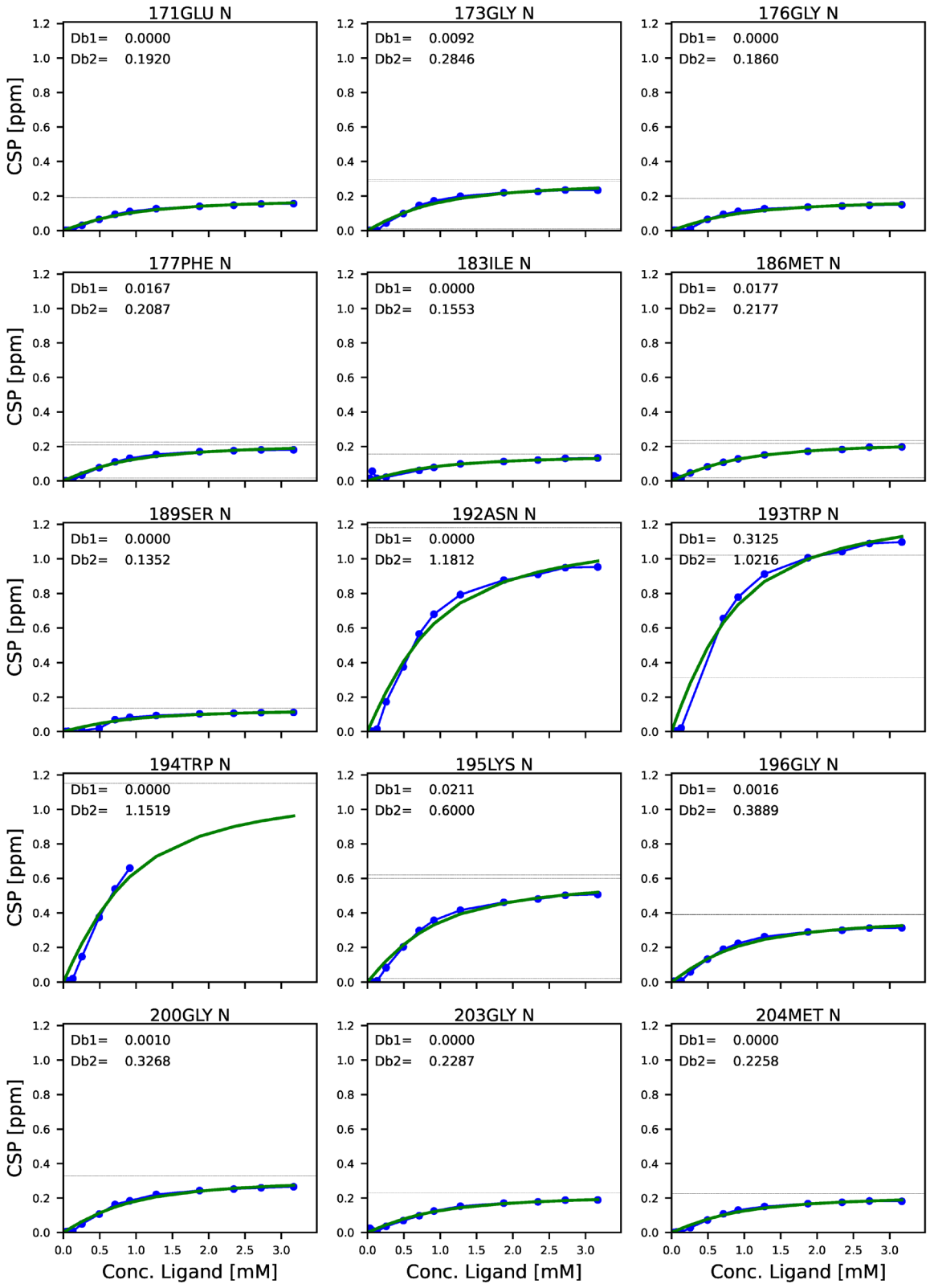
Conc. Ligand [mM]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Conc. Ligand [mM]

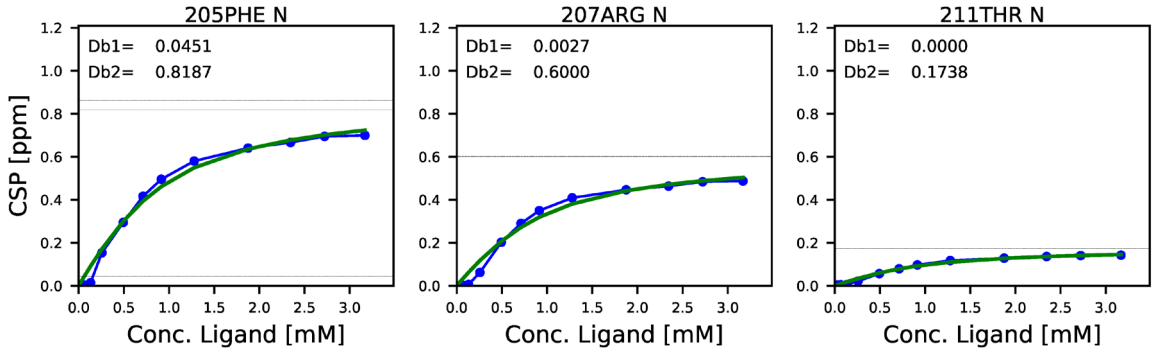


**Figure S14. Chemical shift perturbation profile mapped on each residue, against the concentration of ligand with SOS S10 PRM.** The blue dots and lines indicate CSP values at each titration point, while the green lines represent the theoretical curves calculated based on the two-site independent binding model.

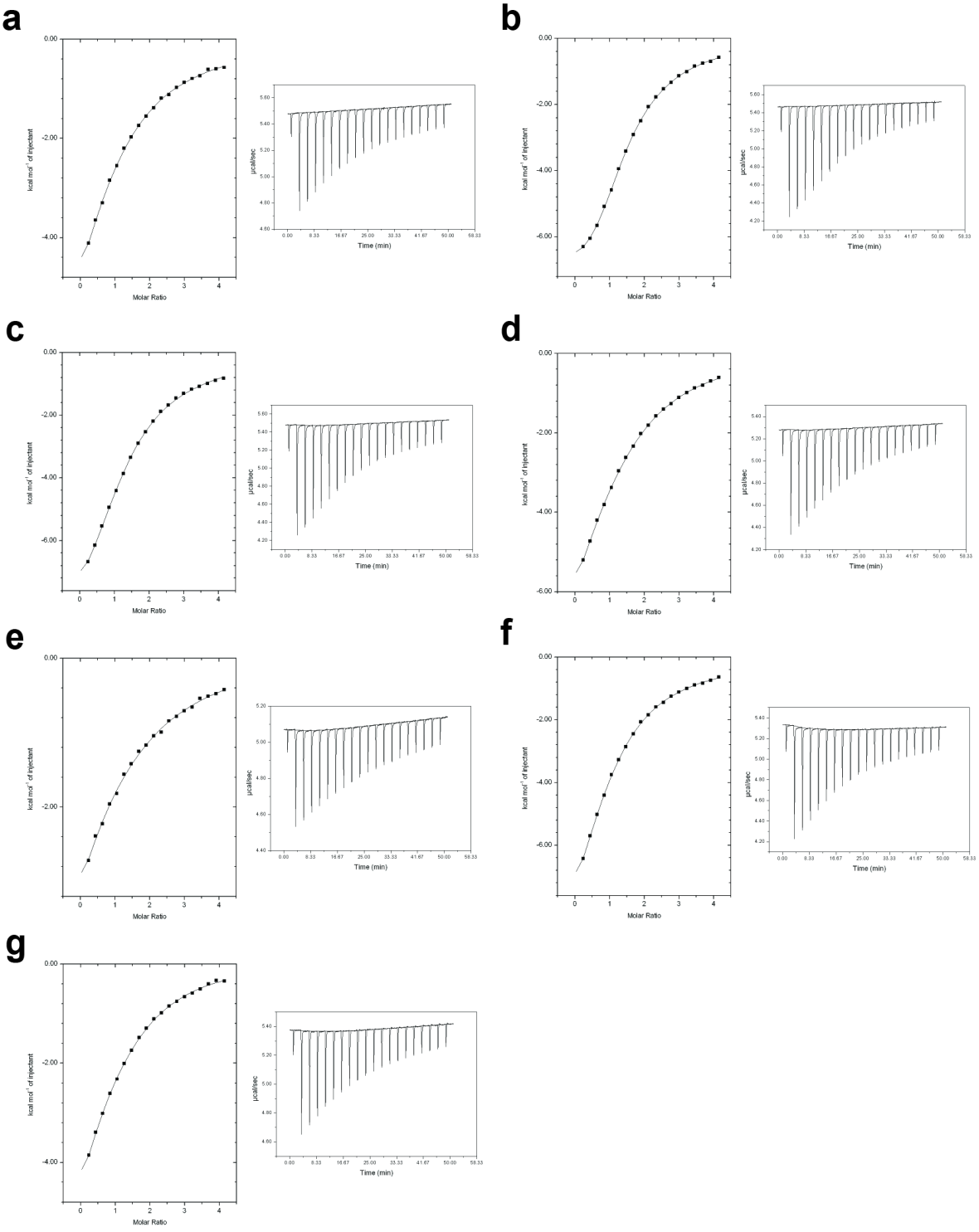


**Figure S15. ITC analysis for the binding of the full-length GRB2 to PRMs.** Figures a-g indicate ITC analysis for S2 (**a**), S4 (**b**), S5 (**c**), S6 (**d**), S8 (**e**) S9 (**f**), and S10 (**g**) PRMs,

respectively. Data with S1, S3, and S7 are not shown here because of no clear thermogram curves. The solid lines show the fit of the data to a "Two Set of Sites" model based on the binding of a ligand to a macromolecule, as incorporated in the Origin software.

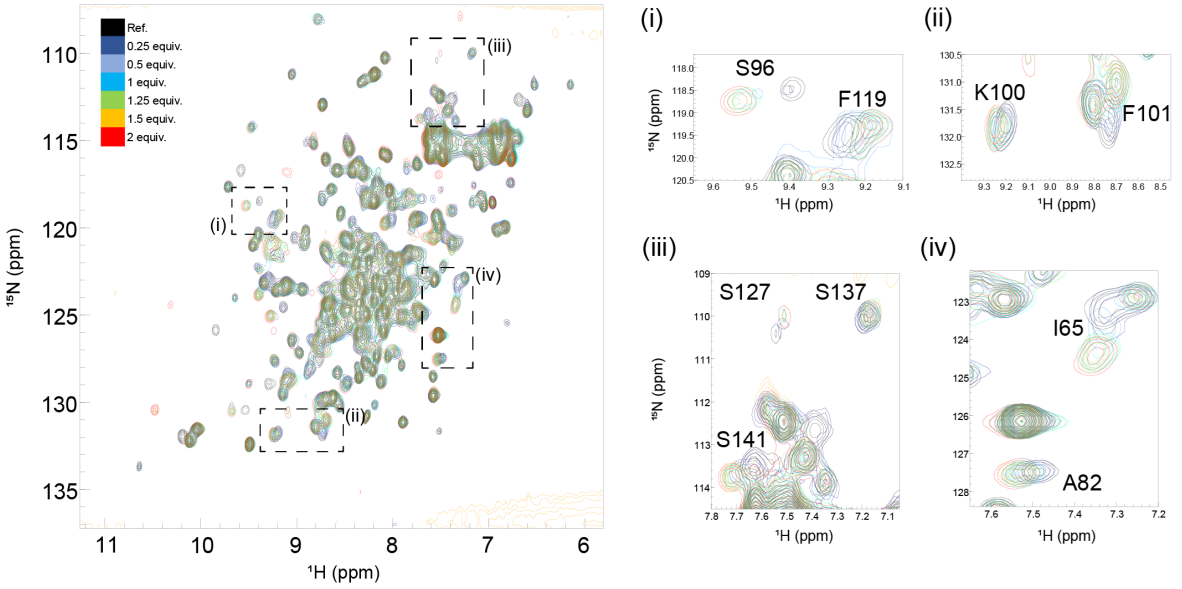


**Figure S16. Overlays of 2D $^{1}$H-$^{15}$N HSQC spectra from multipoint titrations of $^{15}$N-labelled GRB2 exclusively with the EGFR phosphorylated peptide (EpYINSQV).** The peptide concentration was increased stepwise (the protein: peptide molar ratio of 1:0.25, 1:0.5, 1:1, 1:1.25, 1:1.5, and 1:2). In this figure, the colour codes of $^{1}$H-$^{15}$N correlation cross-peaks at each titration point, showing the molar ratio of GRB2: EGFR, are as follows: black (1:0); green (1:0.25); blue (1:0.5); yellow (1:0.75); magenta (1:1); cyan (1:1.25); brown (1:1.5); coral (1:2); red (1:4). Cross peaks that showed large chemical shift changes were annotated.

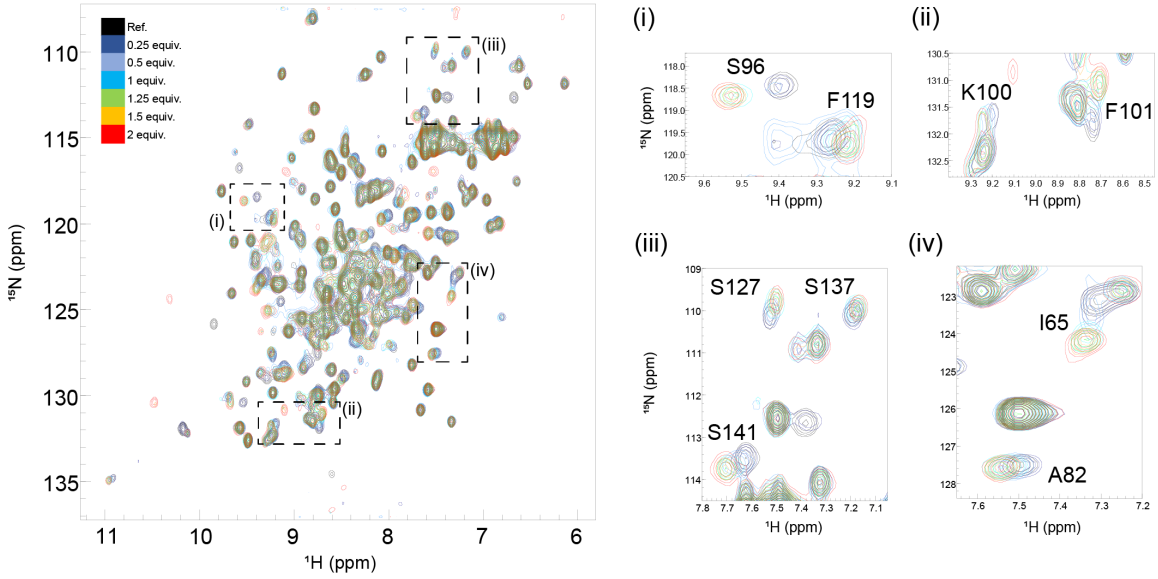


**Figure S17. Overlays of 2D $^{1}$H-$^{15}$N HSQC spectra from multipoint titrations of $^{15}$N-labelled GRB2 with the EGFR phosphorylated peptide (EpYINSQV), in the presence of S4 PRM.** The peptide concentration was increased stepwise (the protein: peptide molar ratio of 1:0.25, 1:0.5, 1:1, 1:1.25, 1:1.5, and 1:2). In this figure, the colour codes of $^{1}$H-$^{15}$N correlation cross-peaks at each titration point, showing the molar ratio of GRB2: EGFR, are as follows: black (1:0); green (1:0.25); blue (1:0.5); yellow (1:0.75); magenta (1:1); cyan (1:1.25); brown (1:1.5); coral (1:2); red (1:4). Cross peaks that showed large chemical shift changes were annotated.

**Table S1. Dissociation constants derived from ITC analysis for the full length GRB3 and isolated SH3 domains against SOS1 PRMs**

| PRM | Sequence | ITC analysis | | | |
|---|---|---|---|---|---|
| | | $K_D$ (μM) from the full length GRB2[a] | | $K_D$ (μM) from isolated SH3[b] | |
| | | $K_{D1}$ | $K_{D2}$ | $K_{D1}$ | $K_{D2}$ |
| S1 | VPPPVPPRRR (1148-1159) | NA | NA | — | — |
| S2 | VPPPVPPRRR (1148-1159) | 65.1 ± 23.7 | NA | — | — |
| S3 | VPPPVPPRRR (1148-1159) | NA | NA | — | — |
| S4 | VPPPVPPRRR (1148-1159) | 5.3 ± 0.4 | 51.3 ± 1.7 | 39 ± 1 | 125 ± 13 |
| S5 | DSPPAOPPRQPT (1177-1188) | 15.9 ± 1.5 | 109.7 ± 6.5 | 56 ± 5 | 1396 ± 87 |
| S6 | DSPPAOPPRQPT (1177-1188) | 40.1 ± 6.5 | 97.3 ± 10.5 | 117 ± 2 | 1718 ± 33 |
| S7 | DSPPAOPPRQPT (1177-1188) | NA | NA | — | — |
| S8 | DSPPAOPPRQPT (1177-1188) | 122.9 ± 18.6 | NA | — | — |
| S9 | IAGPPVPPRQST (1287-1298) | 35.6 ± 4.2 | 640.0 ± 291.1 | 82 ± 1 | 1318 ± 44 |
| S10 | PKLPPKTYKREH (1303-1314) | 71.5 ± 24.2 | 130.8 ± 54.4 | — | — |

[a]The dissociation constants were obtained by globally fitting ITC data to the "Two Set of Sites" model using MicroCal ITC-ORIGIN Analysis Software (Malvern Panalytical). [b]The dissociation constants are published data from ITC in the previous work using isolated SH3 domains{McDonald, 2009 #67}.