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## Supplemental Information

# Rapid Quantification of Protein-Ligand Binding via $^{19}$F NMR Lineshape Analysis

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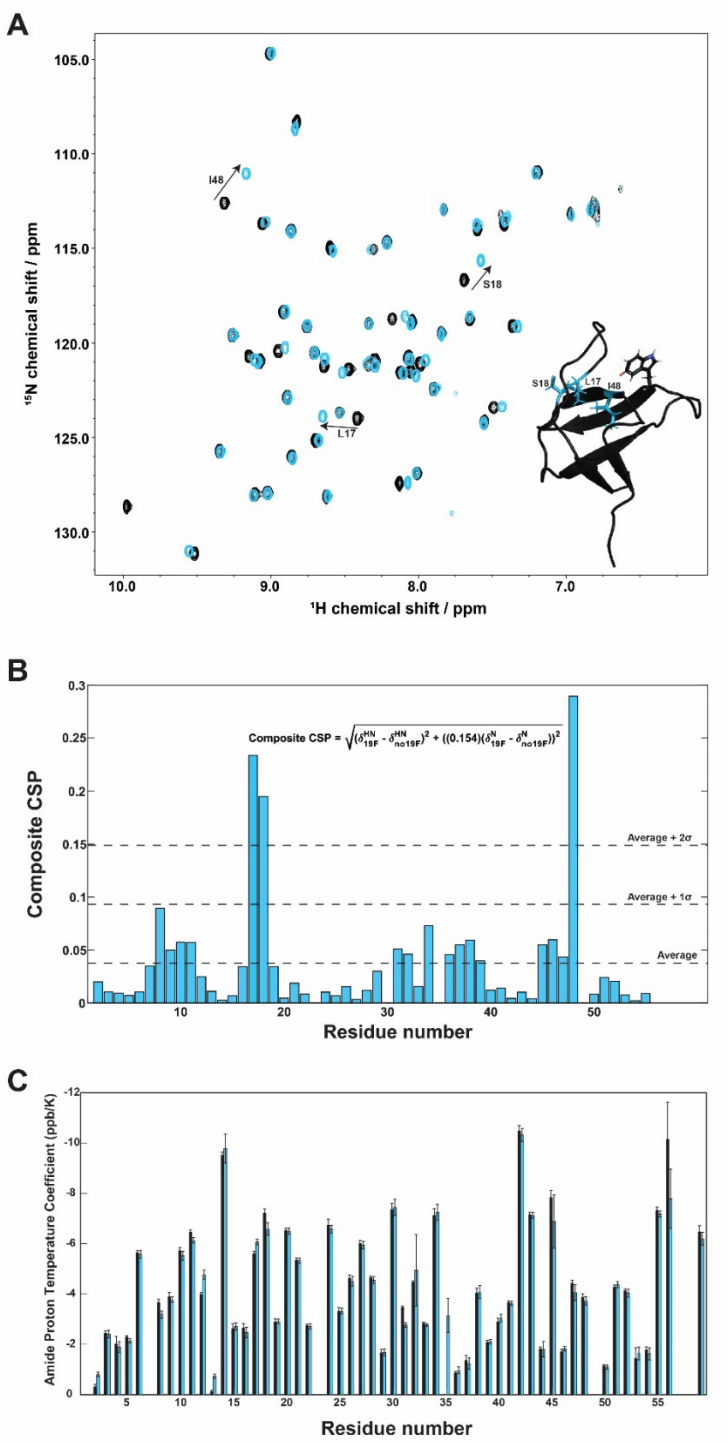


**Figure S1. Effect of $^{19}$F labeling on SH3 T22G. A.** $^{1}$H-$^{15}$N HSQC spectrum of SH3 T22G (black) and SH3 T22G with 5-fluorotryptophan at position 36 (blue) at 45 °C. Residues with average chemical shift perturbations (CSPs) greater than the average CSP plus two standard deviations are highlighted on the spectra as well as the structure in the bottom right (PDB ID: 2A37). The fluorine atom on Trp36 is highlighted in red. **B.** Chemical shift perturbations (CSPs) caused by $^{19}$F labeling. Horizontal lines are given for the average CSP, the average CSP plus one standard deviation, and the average CSP plus 2 standard deviations. **C.** Amide proton temperature coefficients for SH3 T22G (black bars) and $^{19}$F SH3 T22G (blue bars). Uncertainties are reported as 95% confidence intervals of the slope from the linear fit of the amide proton chemical shift against temperature. No bar indicates no data.

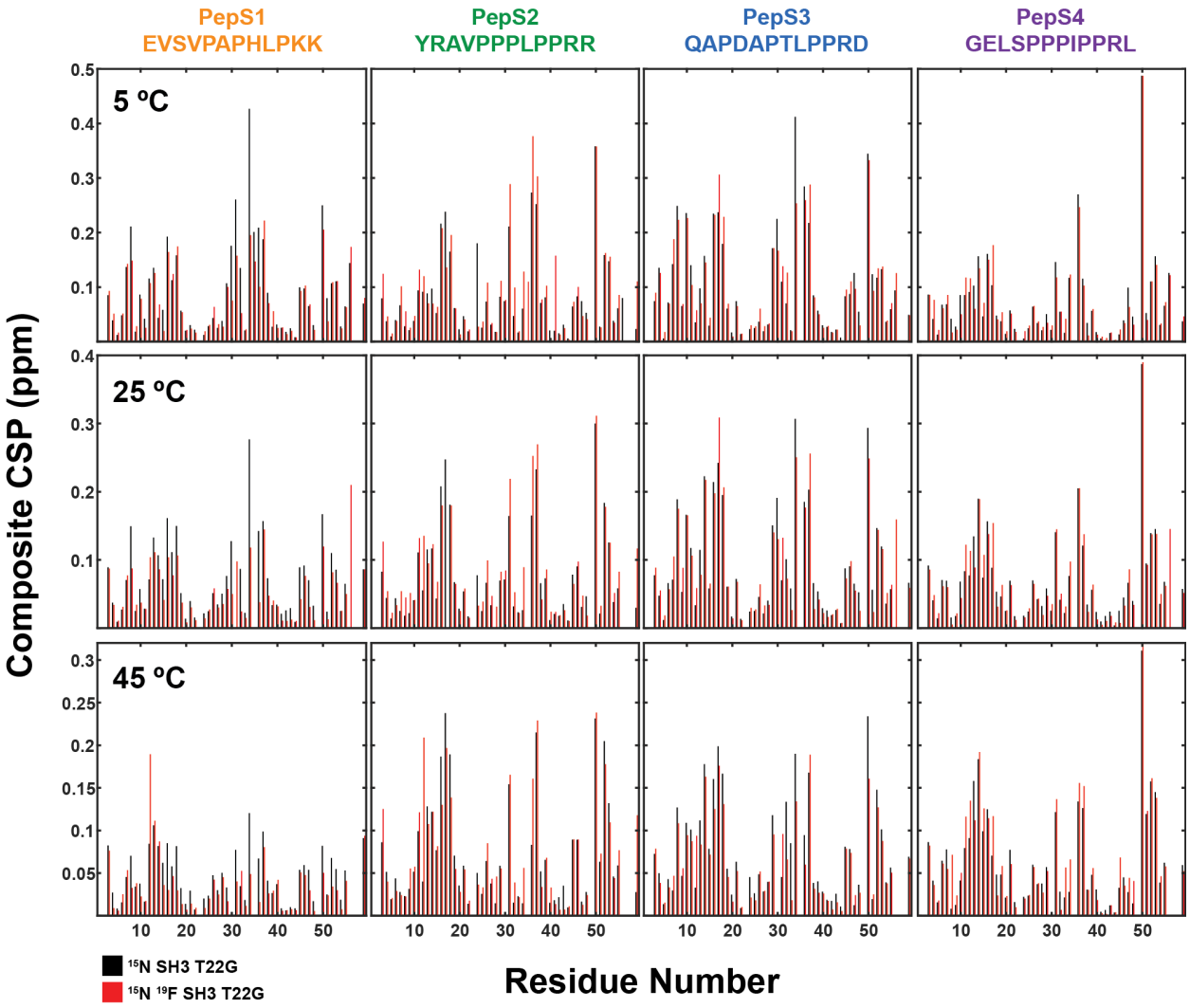


**Figure S2. Chemical shift perturbations caused by peptide binding to SH3 T22G with and without fluorine labeling.** Composite chemical shift perturbations at three temperatures caused by binding of peptides to SH3 T22G with fluorine (red bars) and without fluorine (black bars). Composite chemical shift perturbations were determined using the equation: $Composite\ CSP = \sqrt{(\delta^{HN}_{Bound} - \delta^{HN}_{Free})^2 + ((0.154)(\delta^{N}_{Bound} - \delta^{N}_{Free})^2}$. No bar indicates no data. The peptide sequences are shown above each column. The data in the first row were acquired at 5 °C, those in the middle row at 25 °C, and those in the bottom row at 45 °C.

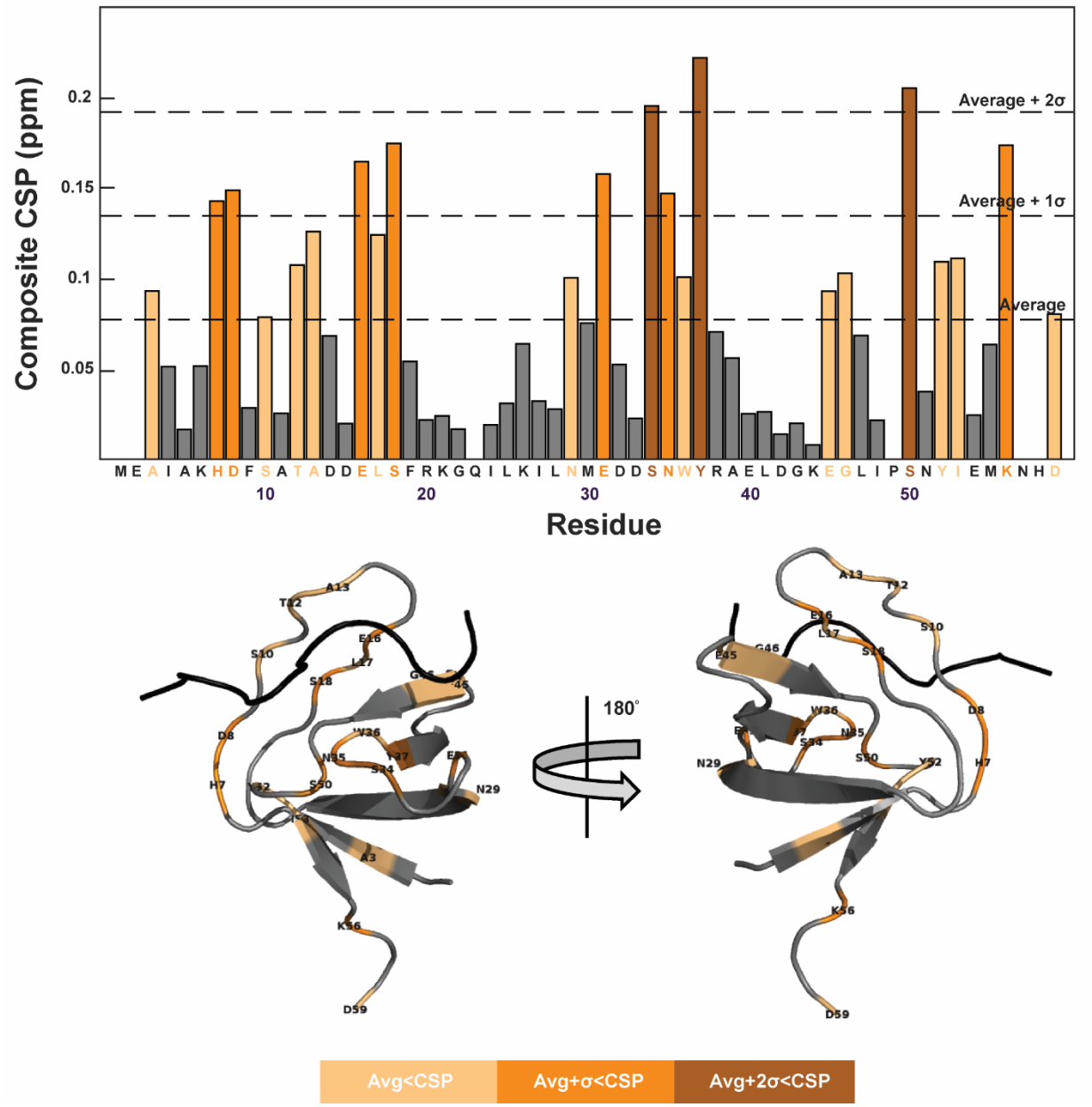


**Figure S3.** Chemical shift perturbations caused by binding of PepS1 to SH3 T22G at 5 °C. Details are provided in the caption to Figure S2. Residues with CSPs greater than the average are colored and labeled on the structure.

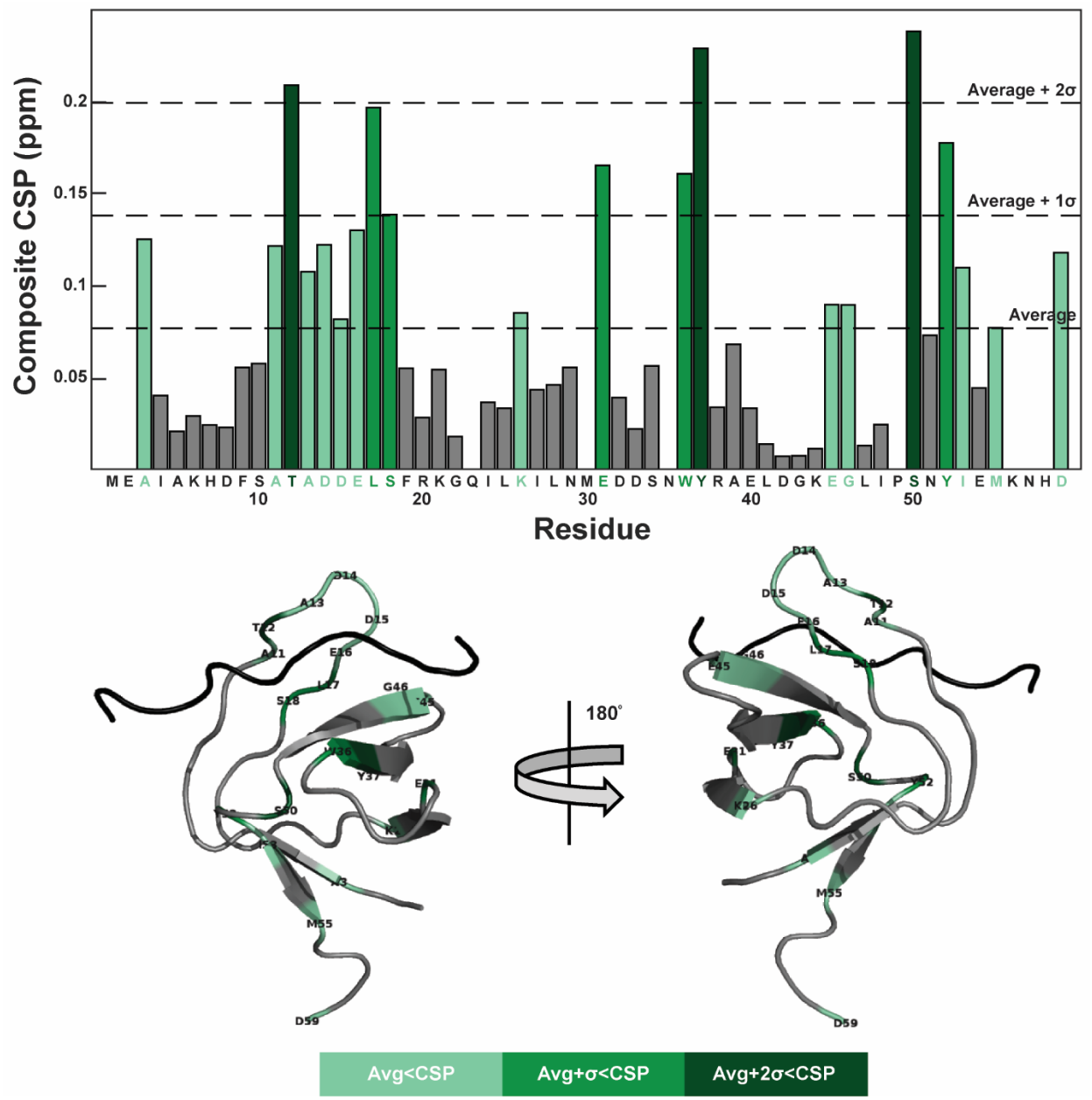


**Figure S4.** Chemical shift perturbations caused by binding of PepS2 to SH3 T22G at 45 °C. Details are provided in the caption to Figure S2. Residues with CSPs greater than the average are colored and labeled on the structure.

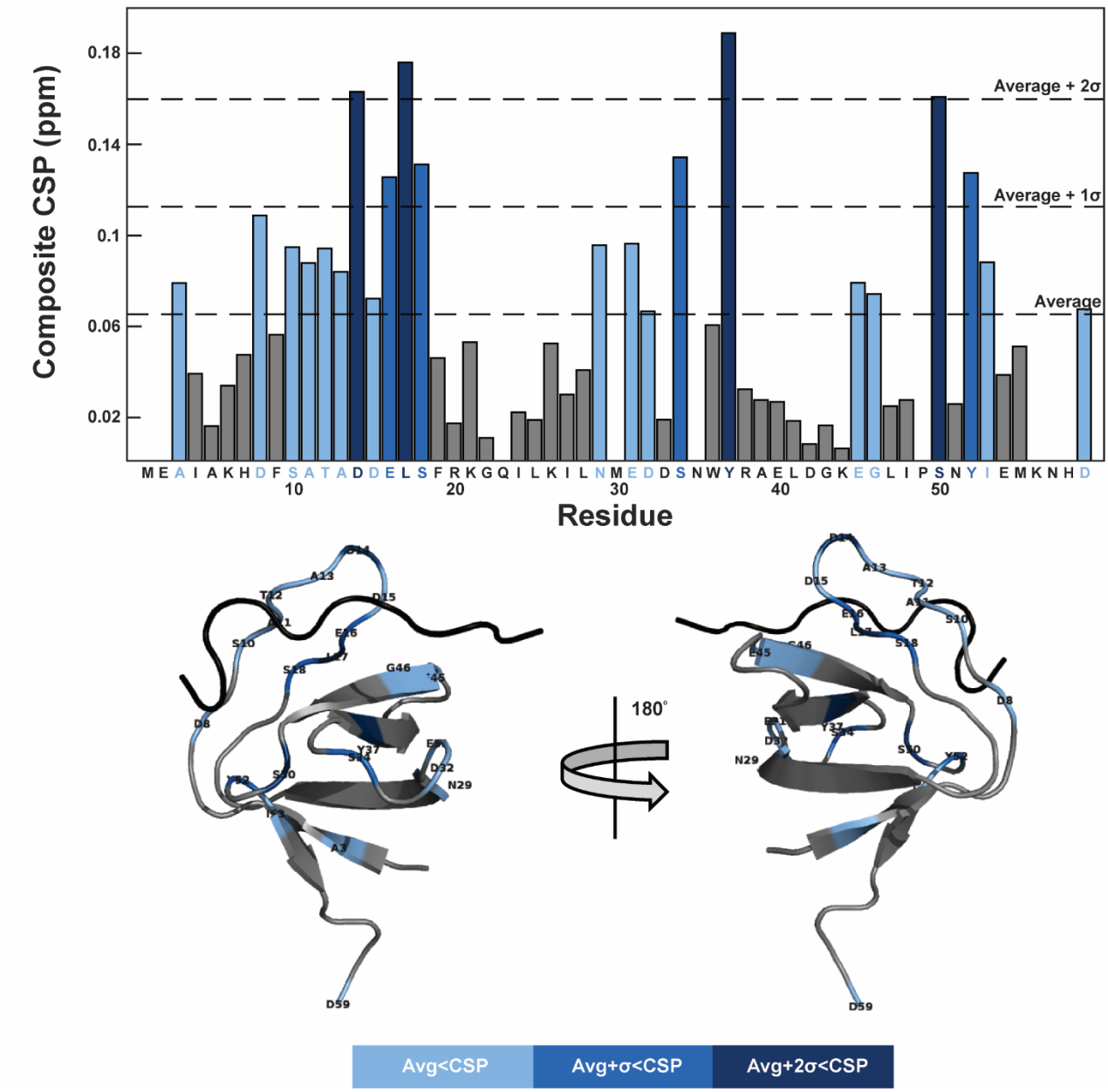


**Figure S5.** Chemical shift perturbations caused by binding of PepS3 to SH3 T22G at 45 °C. Details are provided in the caption to Figure S2. Residues with CSPs greater than the average are colored and labeled on the structure.

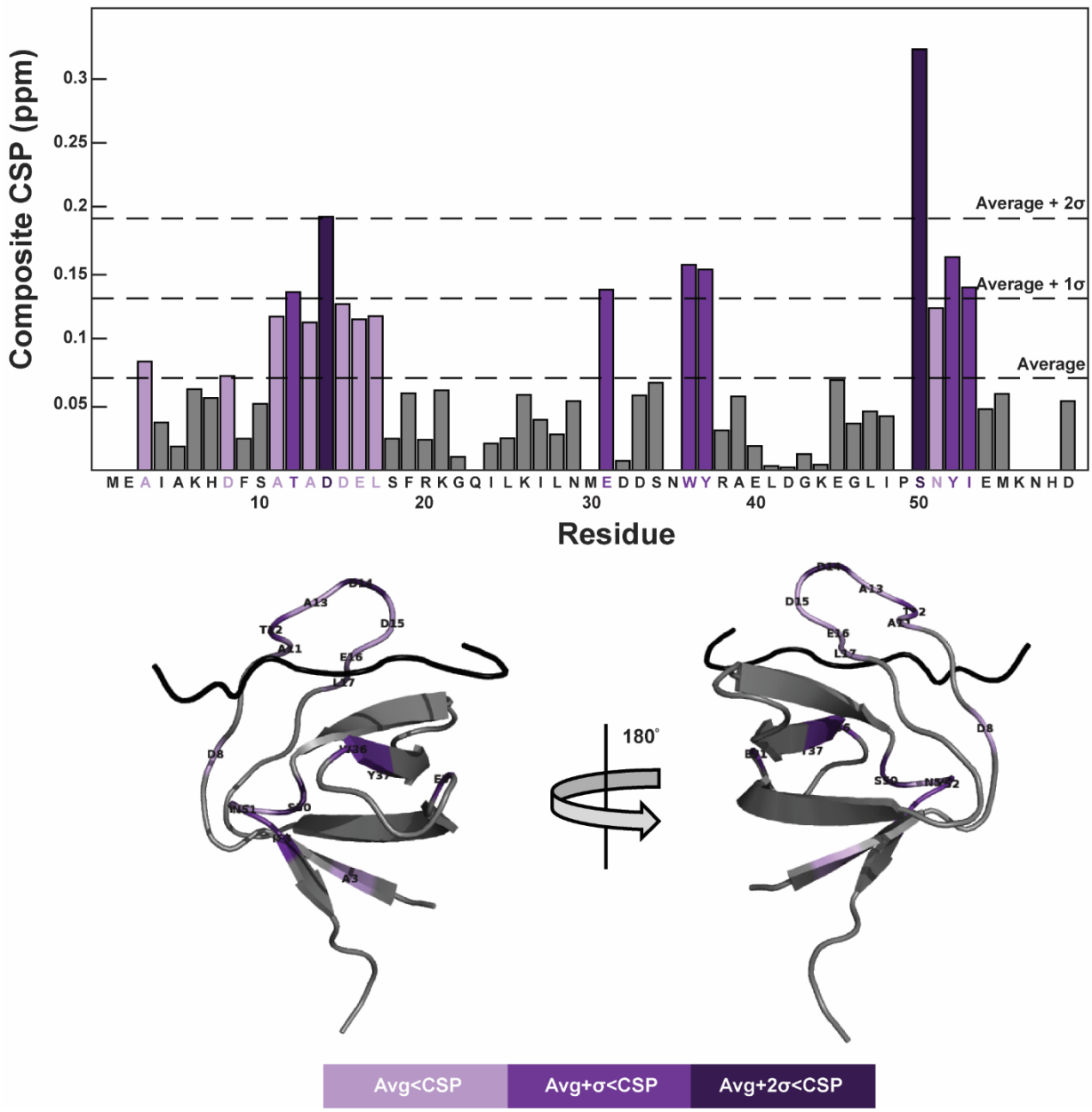


**Figure S6.** Chemical shift perturbations caused by binding of PepS4 to SH3 T22G at 45 °C. Details are provided in the caption to Figure S2. Residues with CSPs greater than the average are colored and labeled on the structure.

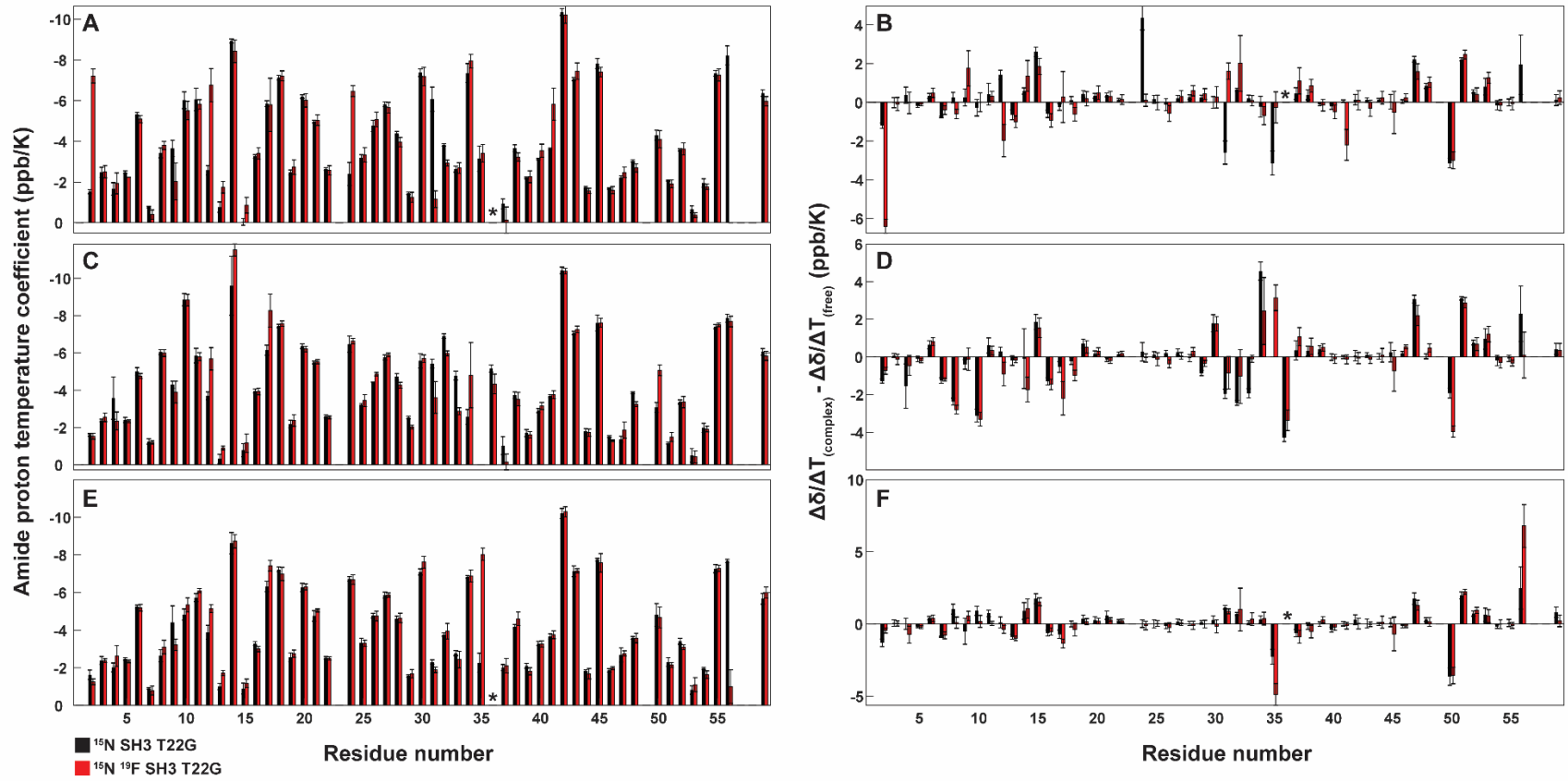


**Figure S7.** Amide proton temperature coefficients for the peptide bound states of SH3 T22G. Amide proton temperature coefficients for the binding of PepS2 (**A**), PepS3 (**C**), and PepS4 (**E**) to $^{15}$N enriched SH3 T22G with (red bars) and without (black bars) fluorine. Uncertainties reported represent 95% confidence interval from linear fit of chemical shift vs. temperature. Plots of the difference in amide proton chemical shift ($\left(\frac{\Delta\delta}{\Delta T}\right)_{complex} - \left(\frac{\Delta\delta}{\Delta T}\right)_{free}$) between the free and PepS2- (**B**), PepS3- (**D**), and PepS4- (**F**) bound states for SH3 T22G with (red bars) and without (black bars) fluorine are also shown. A temperature coefficient was determined for Trp 36 for the PepS2- and PepS4-bound states; however, the value was positive. Uncertainties determined by propagation of uncertainties in amide proton temperature coefficients in the free and bound states.

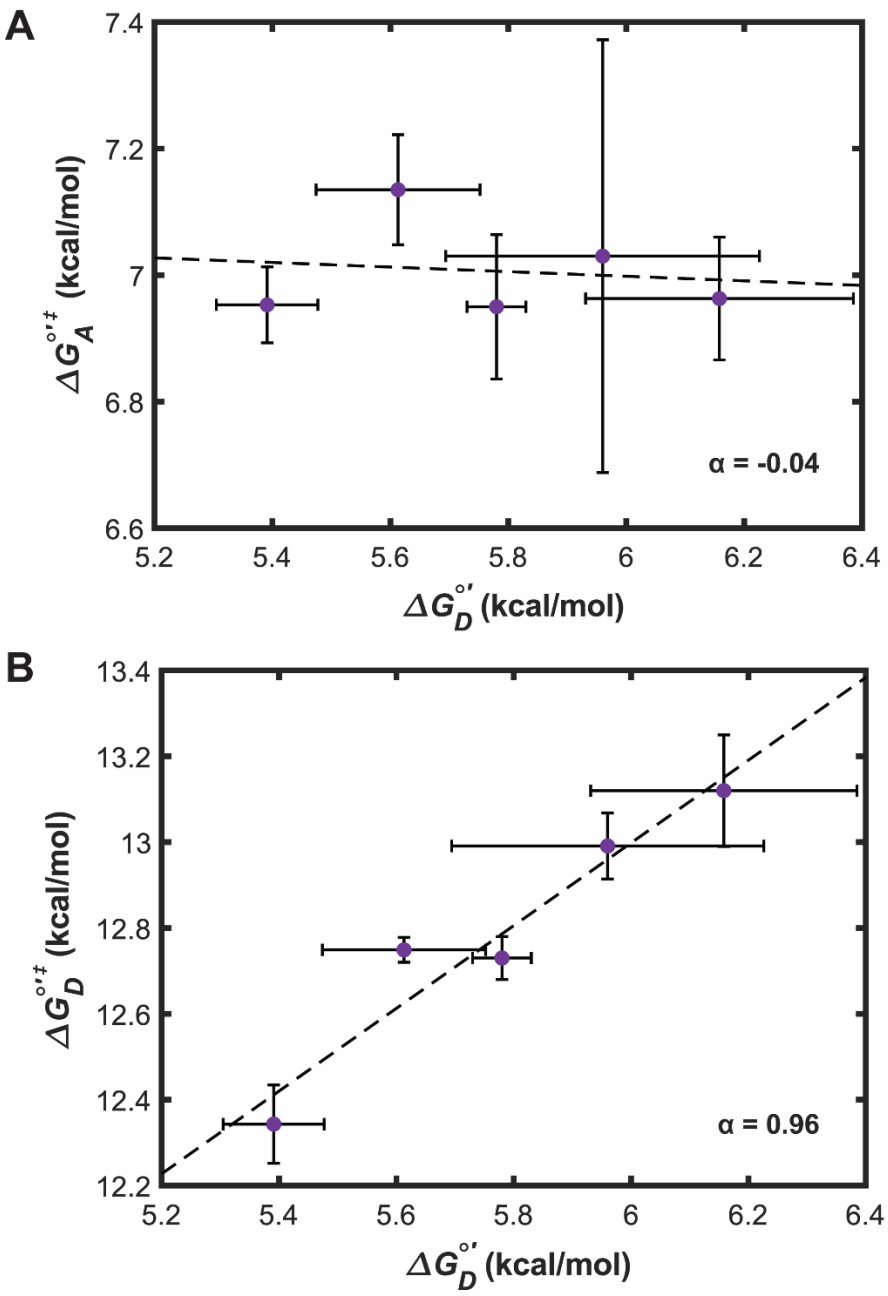


**Figure S8.** Linear free energy relationship between equilibrium free energy ($\Delta G_D^{\circ\prime}$) and activation free energy of the association ($\Delta G_A^{\circ\prime\ddagger}$, A) and activation free energy of dissociation ($\Delta G_D^{\circ\prime\ddagger}$, B). Lines represent linear least-squares fits. Leffler values ($\alpha$), which correspond to the slope, are given.

**Table S1. Residues perturbed by $^{19}F$ labeling of SH3 T22G.**

| Temperature (°C) | Average < CSP < Average + σ | Average + σ < CSP < Average + 2σ | Average + 2σ < CSP |
|---|---|---|---|
| **5** | H7, F9, S10, A11, N29, E31, Y37, R38, A39, G46, L47 | D8, S34 | L17, S18, I48 |
| **10** | H7, F9, S10, A11, N29, E31, S34, Y37, R38, A39, G46, L47 | D8 | L17, S18, I48 |
| **15** | H7, F9, S10, A11, N29, E31, S34, W36, Y37, R38, A39, E45, G46, L47 | D8 | L17, S18, I48 |
| **20** | H7, F9, S10, A11, K21, E31, S34, W36, Y37, R38, A39, E45, G46, L47 | D8 | L17, S18, I48 |
| **25** | H7, F9, S10, A11, E16, E31, S34, W36, Y37, R38, A39, G46, L47 | D8 | L17, S18, I48 |
| **30** | H7, F9, S10, A11, E31, S34, W36, Y37, R38, A39, G46, L47 | D8 | L17, S18, I48 |
| **35** | D8, F9, S10, A11, E31, D32, S34, W36, Y37, R38, A39, E45, G46 | | L17, S18, I48 |
| **40** | D8, F9, S10, A11, E31, S34, W36, Y37, R38, A39, G46, L47 | | L17, S18, I48 |
| **45** | D8, F9, S10, A11, E31, D32, S34, W36, Y37, R38, A39, E45, G46, L47 | | L17, S18, I48 |

Abbreviations: CSP, chemical shift perturbation; σ, standard deviation

**Table S2. Residues perturbed by binding of PepS1 to SH3 T22G with and without fluorine.**

| | $^{15}$N SH3 T22G | | | $^{15}$N $^{19}$F SH3 T22G | | |
|---|---|---|---|---|---|---|
| **Temperature (°C)** | Average < CSP < Average + σ | Average + σ < CSP < Average + 2σ | Average + 2σ < CSP | Average < CSP < Average + σ | Average + σ < CSP < Average + 2σ | Average + 2σ < CSP |
| **5** | H7, T12, A13, L17, S18, N29, D32, E45, G46, Y52, I53, K56 | D8, E16, M31, N35, W36, Y37, S50 | E31, S34 | A3, S10, T12, A13, L17, N29, W36, E45, G46, Y52, I53, D59 | H7, D8, E16, S18, E31, N35, K56 | S34, Y37, S50 |
| **10** | H7, T12, A13, L17, S18, N29, D32, R38, E45, G46, Y52, I53, K56 | D8, E16, M30, E31, N35, W36, Y37, S50 | S34 | A3, T12, A13, L17, N29, M30, W36, R38, E45, G46, Y52, I53, D59 | H7, D8, E16, S18, E31, N35 | S34, Y37, S50, K56 |
| **15** | H7, T12, A13, L17, S18, N29, D32, E45, G46, Y52, I53 | D8, E16, M30, E31, N35, W36, Y37, S50, K56 | S34 | A3, H7, T12, D14, L17, N29, M30, N35, E45, G46, L47, Y52, I53, D59 | D8, A13, E16, S18, E31, S34, S50 | Y37, K56 |
| **20** | A3, H7, T12, A13, D14, L17, N29, D32, R38, E45, G46, Y52, I53, D59 | D8, E16, S18, M30, E31, W36, Y37, S50, K56 | S34 | A3, H7, D8, T12, D14, L17, N29, E45, G46, Y52, I53, D59 | A13, E16, S18, E31, S34, S50 | Y37, K56 |
| **25** | A3, T12, D14, D15, L17, N29, D32, R38, E45, G46, Y52, I53, D59 | D8, A13, E16, S18, M30, W36, Y37, S50 | S34 | A3, H7, D8, D14, L17, K26, N29, G46, Y52, I53, D59 | T12, A13, E16, S18, E31, S34, S50 | Y37, K56 |
| **30** | A3, T12, D14, D15, L17, E45, G46, L47, Y52, I53, D59 | D8, A13, E16, S18, M30, E31, W36, Y37, S50 | S34 | A3, H7, D8, D14, E16, L17, K26, N29, E31, G46, Y52, I53 | T12, A13, S18, S34, Y37, S50, D59 | K56 |
| **35** | A3, T12, D14, D15, L17, E45, G46, L47, Y52, I53 | D8, A13, E16, S18, M30, E31, W36, Y37, S50, D59 | S34 | A32, H7, D8, E16, L17, S18, K26, N29, E31, G46, Y52, I53, M55 | A13, D14, S34, Y37, S50, D59 | T12, K56 |
| **40** | A3, T12, D14, D15, L17, W36, E45, G46, L47, Y52, I53, M55 | D8, A13, E16, S18, E31, Y37, S50, D59 | S34 | H7, D8, E16, L17, S18, K26, L28, E31, D32, S34, E40, G46, S50, Y52, M55 | A3, A13, D14, Y37, D59 | T12 |
| **45** | D8, D15, L17, K36, L28, W36, E45, G46, L47, Y52, I53, M55 | A3, T12, D14, E16, S18, E31, Y37, S50, D59 | S34 | H7, F9, L17, K26, L28, E31, D32, S34, E40, E45, G46, S50, M55 | A3, D14, Y37, D59 | T12, A13 |

Abbreviations: CSP, chemical shift perturbation; σ, standard deviation

**Table S3. Residues perturbed by binding of PepS2 to SH3 T22G with and without fluorine.**

| | $^{15}$N SH3 T22G | | | $^{15}$N $^{19}$F SH3 T22G | | |
|---|---|---|---|---|---|---|
| **Temperature (°C)** | Average < CSP < Average + σ | Average + σ < CSP < Average + 2σ | Average + 2σ < CSP | Average < CSP < Average + σ | Average + σ < CSP < Average + 2σ | Average + 2σ < CSP |
| **5** | A11, T12, A13, D14, N29, A39, G46, I53 | E16, S18, I24, E31, Y52 | L17, W36, Y37, S50 | A3, H7, A11, T12, LA7, K26, N29, D32, S34, N35, A39, L41, G46, Y52, I53 | E16, S18 | E31, W36, Y37, S50 |
| **10** | A11, T12, A13, D14, I24, N29, A39, G46, I53, K56 | E16, S28, E31, Y52 | L17, W36, Y37, S50 | A3, A11, T12, D14, L17, K26, N29, D32, S34, A39, L41, G46, Y52, I53, D59 | E16, S18 | E31, W36, Y37, S50 |
| **15** | A11, A13, D14, I24, M30, G46, I53, K56 | E16, S18, E31, W36, Y52 | L17, Y37, S50 | A3, A11, T12, D14, K26, N29, S34, A39, G46, I53, M55, D59 | E16, L17, S18, Y52 | E31, W36, Y37, S50 |
| **20** | A3, A11, A13, D14, I24, E45, G46, I53, K56 | E16, S18, E31, W36, Y52 | L17, Y37, S50 | A3, A11, T12, D14, K26, N29, S34, A39, G46, I53, M55, D59 | E16, S18, E31, Y52 | L17, W36, Y37, S50 |
| **25** | A3, A11, A13, D14, I24, E45, G46, I53 | E16, S18, E31, W36, Y52 | L17, Y37, S50 | A3, A11, T12, A13, D14, K26, M30, S34, A39, G46, I53, D59 | E16, S18, E31, Y52 | W36, Y37, S50 |
| **30** | A3, A11, A13, D14, E45, G46, I53 | S18, E31, W36, Y52 | E16, L17, Y37, S50 | A3, A11, T12, A13, D14, K26, M30, S34, A39, G46, I53, M55, D59 | E16, S18, E31, Y52 | L17, W36, Y37, S50 |
| **35** | A3, A11, A13, D14, W36, E45, G46, I53 | S18, E31, Y52 | E16, L17, Y37, S50 | A3, A11, A13, D14, K26, D32, E45, G46, I53, M55, D59 | T12, E16, S18, E31, Y52 | L17, W36, Y37, S50 |
| **40** | A3, A11, A13, D14, W36, E45, G46, I53 | E16, S18, E31, Y52 | L17, Y37, S50 | A3, A11, A13, D14, K26, G46, I53, M55, D59 | T12, E16, S18, E31, W36, Y52 | L17, Y37, S50 |
| **45** | A3, A11, A13, D14, D15, W36, E45, G46, I53 | E16, S18, E31 | L17, Y37, S50, Y52 | A3, A11, A13, D14, D15, E16, K26, E45, G46, I53, M55, D59 | L17, S18, E31, W36, Y52 | T12, Y37, S50 |

Abbreviations: CSP, chemical shift perturbation; σ, standard deviation

**Table S4. Residues perturbed by binding of PepS3 to SH3 T22G with and without fluorine.**

| | $^{15}$N SH3 T22G | | | $^{15}$N $^{19}$F SH3 T22G | | |
|---|---|---|---|---|---|---|
| **Temperature (°C)** | Average < CSP < Average + σ | Average + σ < CSP < Average + 2σ | Average + 2σ < CSP | Average < CSP < Average + σ | Average + σ < CSP < Average + 2σ | Average + 2σ < CSP |
| **5** | I4, H7, A11, S18, N29, E31, L47, N51, Y52, I53 | D8, S10, E16, L17, M30, W36, Y37 | S34, S50 | I4, H7, D14, N29, M30, E31, D32, G46, Y52, I53, K56 | D8, S10, E16, S18, S34, W36 | L17, Y37, S50 |
| **10** | H7, A11, S18, N29, L47, N51, Y52, I53 | D8, S10, D14, E16, L17, M30, Y37 | S34, W36, S50 | H7, A11, N29, M30, D32, G46, Y52, I53, K56 | D8, S10, D14, E16, S18, W36 | L17, S34, Y37, S50 |
| **15** | H7, A11, A13, N29, Y52, I53, K56 | D8, S10, D14, E16, L17, S18, M30, W36, Y37 | S34, S50 | H7, A11, N29, M30, D32, G46, Y52, I53, K56 | D8, S10, D14, E16, S18, W36 | L17, S34, Y37, S50 |
| **20** | A11, N29, Y52, I53, K56 | D8, S10, D14, E16, L17, S18, M30, W36, Y37 | S34, S50 | H7, A11, N29, M30, E45, G46, Y52, I53, K56 | D8, S10, D14, E16, S18, W36 | L17, S34, Y37, S50 |
| **25** | S10, A11, A13, N29, D32, Y52, I53 | D8, D14, E16, L17, S18, M30, W36, Y37 | S34, S50 | H7, S10, A11, N29, M30, E31, G46, Y52, I53, K56 | D8, D14, E16, S18, W36 | L17, S34, Y37, S50 |
| **30** | S10, A11, A13, N29, D32, W36, E45, Y52, I53 | D8, D14, E16, S18, M30, Y37 | L17, S34, S50 | A3, H7, F9, S10, A11, N29, M30, E31, W36, G46, Y52, I53, K56 | D8, D14, E16, S18, S34 | L17, Y37, S50 |
| **35** | S10, A11, A13, N29, S32, W36, E45, G46, Y52, I53 | D8, D14, E16, S18, M30, Y37 | L17, S34, S50 | A3, S10, A11, A13, N29, M30, E31, W36, E45, G46, Y52, I53, K56 | D8, D14, E16, S18, S34 | L17, Y37, S50 |
| **40** | S10, A11, A13, N29, D32, D33, W36, E45, G46, I53 | D8, D14, E16, S18, Y37, Y52 | L17, S34, S50 | A3, S10, A11, T12, A13, N29, E31, W36, E45, G46, I53 | D8, D14, E16, S18, S34, Y52 | L17, Y37, S50 |
| **45** | D8, S10, A11, A13, D15, N29, D33, W36, E45, G46, I53 | D14, E16, S18, D32 S34, Y37, Y52 | L17, S50 | A3, D8, S10, A11, T12, A13, D15, N29, E31, D32, E45, G46, I53, D59 | E16, S18, S34, Y52 | D14, L17, Y37, S50 |

Abbreviations: CSP, chemical shift perturbation; σ, standard deviation

**Table S5. Residues perturbed by binding of PepS4 to SH3 T22G with and without fluorine.**

| | $^{15}$N SH3 T22G | | | $^{15}$N $^{19}$F SH3 T22G | | |
|---|---|---|---|---|---|---|
| **Temperature (°C)** | Average < CSP < Average + σ | Average + σ < CSP < Average + 2σ | Average + 2σ < CSP | Average < CSP < Average + σ | Average + σ < CSP < Average + 2σ | Average + 2σ < CSP |
| **5** | A3, S10, A11, T12, A13, L17, E31, S34, Y37, L47, Y52, K56 | D14, E16, I53 | W36, S50 | A3, I4, H7, A11, T12, D14, E31, S34, Y52, I53, M55, K56 | E16, L17 | W36, S50 |
| **10** | A3, K6, S10, A11, T12, A13, L17, S34, Y37, L47, Y52, K56 | D14, E16, E31, I53 | W36, S50 | A3, I4, H7, A11, T12, D15, E31, S34, Y37, L47, Y52, I53, K56 | D14, E16, L17 | W36, S50 |
| **15** | A3, S10, A11, T12, A13, L17, S34, Y37, L47, N51, Y52 | D14, E16, E31, I53, K56 | W36, S50 | A3, A11, T12, A13, D15, E31, S34, Y37, Y52, K56 | D14, E16, L17, I53 | W36, S50 |
| **20** | A3, S10, A11, T12, A13, L17, S34, Y37, L47, N51, Y52 | D14, E16, E31, I53, K56 | W36, S50 | A3, A11, T12, A13, D15, S34, Y37, N51, Y52, K56 | D14, E16, L17, E31, I53 | W36, S50 |
| **25** | A3, K6, H7, A11, T12, A13, D15, L17, K26, S34, Y37, N51 | D14, E16, E31, Y52, I53 | W36, S50 | A3, A11, T12, A13, D15, E16, S34, L47, N51, Y52, I53 | D14, L17, E31, K56 | W36, S50 |
| **30** | A3, K6, H7, A11, T12, D15, L17, K21, K26, S34, Y37, N51 | A13, E16, E31, W36, Y52, I53 | D14, S50 | A3, A11, T12, A13, D15, E16, S34, N51, I53, K56 | D14, L17, E31, W36, Y37, Y52 | S50 |
| **35** | A3, H7, A11, T12, D15, L17, K21, K26, Y37, N51 | A13, E16, E31, W36, Y52, I53 | D14, S50 | A3, A11, T12, A13, D15, E16, L17, D32, S34, N51, K56 | D14, E31, W36, Y37, Y52, I53 | S50 |
| **40** | A3, H7, T12, D15, L17, K21, K26, Y37, N51 | A13, E16, E31, W36, Y52, I53 | D14, S50 | A3, A11, T12, A13, D15, E16, L17, D33, S34, N51, I53 | D14, E31, W36, Y37, Y52 | S50 |
| **45** | A3, K6, H7, A11, T12, D15, L17, K21, E31, N51 | A13, E16, W36, Y37, Y52, I53 | D14, S50 | A3, D8, A11, A13, D15, E16, L17, N51 | T12, E31, W36, Y37, Y52, I53 | D14, S50 |

Abbreviations: CSP, chemical shift perturbation; σ, standard deviation