

Supporting Information:

Crystallographic and NMR evaluation of the impact of peptide binding to the second PDZ domain of PTP1E

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Table S1: Global fitting results of 5% RA-GEF2 bound PDZ2 relaxation dispersion data

| Residue | k_{ex} (s ⁻¹) | $\Delta\omega_{\text{CPMG}}$ (ppm) | $[P_A]$ (%) | ^a R_{20} (s ⁻¹) | ^b R_{20} (s ⁻¹) | ^c $\Delta\omega_{\text{titration}}$ (ppm) |
|---------|------------------------------------|------------------------------------|-------------|--|--|--|
| 17 | 320.53±11.79 | 0.97±0.03 | 94.7±0.2 | 9.99±0.11 | 10.55±0.1 | 0.92 |
| 19 | 320.53±11.79 | 2.32±0.20 | 94.7±0.2 | 12.75±0.56 | 13.29±0.42 | 2.16 |
| 20 | 320.53±11.79 | 1.67±0.06 | 94.7±0.2 | 10.38±0.18 | 11.41±0.14 | 1.59 |
| 21 | 320.53±11.79 | 1.15±0.04 | 94.7±0.2 | 10.61±0.17 | 11.3±0.14 | 1.20 |
| 22 | 320.53±11.79 | 0.43±0.12 | 94.7±0.2 | 14.74±0.31 | 14.5±0.21 | 0.28 |
| 23 | 320.53±11.79 | 0.48±0.03 | 94.7±0.2 | 10.79±0.1 | 11.74±0.1 | 0.50 |
| 24 | 320.53±11.79 | 0.60±0.06 | 94.7±0.2 | 12.45±0.25 | 12.66±0.2 | 0.50 |
| 27 | 320.53±11.79 | 1.33±0.14 | 94.7±0.2 | 17.17±0.64 | 20.12±0.53 | 1.27 |
| 31 | 320.53±11.79 | 0.87±0.11 | 94.7±0.2 | 19.32±0.54 | 20.61±0.41 | 0.71 |
| 34 | 320.53±11.79 | 0.77±0.09 | 94.7±0.2 | 15.11±0.29 | 18.21±0.41 | 0.50 |
| 35 | 320.53±11.79 | 1.10±0.05 | 94.7±0.2 | 12.73±0.19 | 13.98±0.18 | 0.92 |
| 40 | 320.53±11.79 | 0.54±0.02 | 94.7±0.2 | 9.59±0.09 | 10.06±0.08 | 0.50 |
| 45 | 320.53±11.79 | 0.80±0.03 | 94.7±0.2 | 10.45±0.12 | 11.19±0.11 | 0.71 |
| 66 | 320.53±11.79 | 0.48±0.03 | 94.7±0.2 | 10.9±0.1 | 11.54±0.1 | 0.43 |
| 67 | 320.53±11.79 | 0.58±0.02 | 94.7±0.2 | 9.82±0.06 | 10.53±0.05 | 0.52 |
| 70 | 320.53±11.79 | 1.07±0.04 | 94.7±0.2 | 11.1±0.14 | 11.69±0.12 | 0.93 |
| 71 | 320.53±11.79 | 0.50±0.03 | 94.7±0.2 | 12.95±0.12 | 13.38±0.1 | 0.36 |
| 72 | 320.53±11.79 | 1.29±0.05 | 94.7±0.2 | 10.91±0.16 | 11.18±0.13 | 1.28 |
| 74 | 320.53±11.79 | 1.06±0.03 | 94.7±0.2 | 10.05±0.08 | 10.69±0.09 | 0.96 |
| 75 | 320.53±11.79 | 0.88±0.02 | 94.7±0.2 | 10.52±0.07 | 11.02±0.07 | 0.81 |
| 76 | 320.53±11.79 | 0.90±0.03 | 94.7±0.2 | 10.05±0.1 | 11.1±0.08 | 0.85 |
| 79 | 320.53±11.79 | 2.52±0.10 | 94.7±0.2 | 11.22±0.21 | 11.81±0.19 | 2.55 |
| 80 | 320.53±11.79 | 0.86±0.03 | 94.7±0.2 | 9.4±0.09 | 9.88±0.09 | 0.82 |
| 81 | 320.53±11.79 | 0.49±0.03 | 94.7±0.2 | 7.78±0.07 | 7.87±0.06 | 0.36 |
| 82 | 320.53±11.79 | 0.59±0.05 | 94.7±0.2 | 14.03±0.22 | 13.72±0.18 | 0.52 |
| 86 | 320.53±11.79 | 0.46±0.03 | 94.7±0.2 | 10.72±0.09 | 11.32±0.08 | 0.35 |

^a Values at 500 MHz. ^b Values at 600 MHz. ^c The experimental $\Delta\omega_{\text{titration}}$ values were calculated as the difference between apo and RA-GEF2 saturated PDZ2.

Table S2: Global fitting results of 5% APC bound PDZ2 relaxation dispersion data

| Residue | k_{ex} (s ⁻¹) | $\Delta\omega_{\text{CPMG}}$ (ppm) | $[P_A]$ (%) | ^a R_{20} (s ⁻¹) | ^b R_{20} (s ⁻¹) | ^c $\Delta\omega_{\text{titration}}$ (ppm) |
|---------|------------------------------------|------------------------------------|-------------|--|--|--|
| 11 | 534 ±17 | 0.452 ±0.02 | 94.5 ±0.3 | 10.05 ±0.06 | 10.49 ±0.07 | 0.54 |
| 17 | 534 ±17 | 1.146 ±0.037 | 94.5 ±0.3 | 9.88 ±0.09 | 10.41 ±0.09 | 1.10 |
| 19 | 534 ±17 | 2.184 ±0.143 | 94.5 ±0.3 | 12.23 ±0.49 | 13.86 ±0.48 | 2.26 |

| | | | | | | |
|----|---------|--------------|-----------|-------------|-------------|------|
| 20 | 534 ±17 | 1.255 ±0.04 | 94.5 ±0.3 | 10.44 ±0.11 | 11.43 ±0.13 | 1.19 |
| 23 | 534 ±17 | 0.624 ±0.023 | 94.5 ±0.3 | 11.38 ±0.07 | 11.78 ±0.08 | 0.57 |
| 24 | 534 ±17 | 1.591 ±0.076 | 94.5 ±0.3 | 12.5 ±0.27 | 12.05 ±0.32 | 1.53 |
| 27 | 534 ±17 | 1.585 ±0.124 | 94.5 ±0.3 | 16.88 ±0.54 | 18.98 ±0.59 | 1.49 |
| 28 | 534 ±17 | 0.589 ±0.065 | 94.5 ±0.3 | 16.67 ±0.25 | 18.34 ±0.33 | 0.52 |
| 31 | 534 ±17 | 0.925 ±0.077 | 94.5 ±0.3 | 19.23 ±0.37 | 21.18 ±0.42 | 0.88 |
| 35 | 534 ±17 | 0.904 ±0.032 | 94.5 ±0.3 | 13.18 ±0.11 | 14.3 ±0.13 | 0.82 |
| 45 | 534 ±17 | 0.767 ±0.024 | 94.5 ±0.3 | 10.68 ±0.09 | 11.28 ±0.1 | 0.72 |
| 54 | 534 ±17 | 0.478 ±0.019 | 94.5 ±0.3 | 10.96 ±0.06 | 11.24 ±0.06 | 0.44 |
| 66 | 534 ±17 | 0.495 ±0.024 | 94.5 ±0.3 | 11.35 ±0.08 | 11.57 ±0.08 | 0.48 |
| 70 | 534 ±17 | 1.03 ±0.031 | 94.5 ±0.3 | 11.25 ±0.09 | 11.71 ±0.1 | 0.96 |
| 74 | 534 ±17 | 0.822 ±0.023 | 94.5 ±0.3 | 10.34 ±0.06 | 10.8 ±0.06 | 0.79 |
| 76 | 534 ±17 | 1.293 ±0.042 | 94.5 ±0.3 | 10.33 ±0.1 | 10.82 ±0.11 | 1.28 |
| 78 | 534 ±17 | 0.551 ±0.02 | 94.5 ±0.3 | 10.58 ±0.07 | 11.11 ±0.08 | 0.49 |
| 79 | 534 ±17 | 2.604 ±0.142 | 94.5 ±0.3 | 10.91 ±0.25 | 11.73 ±0.24 | 2.72 |
| 81 | 534 ±17 | 0.534 ±0.016 | 94.5 ±0.3 | 7.55 ±0.05 | 8.11 ±0.05 | 0.50 |

^a Values at 500 MHz. ^b Values at 600 MHz. ^c The experimental $\Delta\omega_{\text{titration}}$ values were calculated as the difference between apo and APC saturated PDZ2.

Table S3. Residues for which RDCs values were used in Q-factor calculations.

| Apo PDZ2 | RA-GEF2 bound | APC bound |
|----------|---------------|-----------|
| 7 | 6 | 7 |
| 8 | 7 | 8 |
| 10 | 8 | 10 |
| 11 | 9 | 11 |
| 12 | 10 | 12 |
| 15 | 11 | 14 |
| 16 | 12 | 16 |
| 17 | 14 | 17 |
| 22 | 15 | 19 |
| 23 | 16 | 22 |
| 24 | 17 | 23 |
| 26 | 19 | 24 |
| 27 | 21 | 25 |
| 31 | 23 | 26 |
| 33 | 24 | 27 |
| 34 | 26 | 28 |
| 36 | 27 | 29 |
| 37 | 28 | 30 |
| 38 | 29 | 31 |
| 39 | 30 | 33 |
| 47 | 31 | 34 |

| | | |
|----|----|----|
| 48 | 34 | 35 |
| 49 | 37 | 37 |
| 50 | 38 | 38 |
| 51 | 40 | 39 |
| 56 | 41 | 43 |
| 57 | 43 | 44 |
| 58 | 44 | 45 |
| 59 | 45 | 46 |
| 62 | 47 | 47 |
| 63 | 48 | 48 |
| 66 | 49 | 49 |
| 67 | 51 | 50 |
| 69 | 52 | 51 |
| 70 | 54 | 52 |
| 71 | 55 | 54 |
| 72 | 56 | 55 |
| 73 | 57 | 56 |
| 74 | 59 | 58 |
| 76 | 61 | 61 |
| 78 | 62 | 62 |
| 79 | 63 | 63 |
| 80 | 64 | 64 |
| 81 | 65 | 65 |
| 85 | 66 | 66 |
| 87 | 67 | 67 |
| 88 | 69 | 69 |
| 89 | 70 | 70 |
| 90 | 71 | 71 |
| 93 | 72 | 72 |
| | 74 | 73 |
| | 76 | 74 |
| | 77 | 76 |
| | 79 | 77 |
| | 80 | 78 |
| | 81 | 79 |
| | 82 | 80 |
| | 86 | 81 |
| | 87 | 85 |
| | 88 | 87 |
| | 89 | 88 |
| | 90 | 89 |
| | 91 | 90 |

Figure S1. Crystal packing within the asymmetric unit for apo PDZ2. (A) top view of the asymmetric unit. The six monomers are colored red, green and blue, and three-blade propeller organization is found. (B) side view of the asymmetric unit. Two layers of three-blade propellers are visible. The figure was prepared using PyMOL.

Figure S2. Involvement of residue 30-33 in crystal packing. (A) Apo PDZ2 crystal packing. An arbitrary asymmetric unit (colored as red) which contains six apo PDZ2 monomers is selected and six surrounding asymmetric units are also displayed. The fragment 30-33 is shown as stick model and colored the same for the fragment belonging to the same asymmetric unit. (B) RA-GEF2 bound PDZ2 crystal packing. Six molecules belonging to six different adjacent asymmetric units are shown. For clarity, fragment 30-33 was shown as stick and colored differently (red or green) for neighboring molecules. The figure was prepared using PyMOL.

Figure S3. Determination of K_D for PDZ2-APC peptide interaction by NMR titration. ^1H - ^{15}N HSQC spectra were acquired at increasing APC:PDZ ratios until no further peak shifts were observed. Only data for the backbone amide at Ile20 are shown, but K_D values for the other reporters are within 10%. The ordinate axis contains the reduced chemical shift difference ($\Delta\delta$) between free and APC-bound PDZ as determined from HSQC spectra. The data were then fit to the standard quadratic equation for binding. The apparent irregularities in the fitted/theoretical line are due to changes in PDZ2 concentration during the titration.

Figure S4. Distribution of residues used in calculation of Q-factors (shown in Table 4). Residues for which ^1H - ^{15}N RDC values were used for fitting to structural models are shown in blue on PDZ2. RDC sets are for apo PDZ2 (A), RA-GEF2 bound PDZ2 (B), and APC bound PDZ2. Secondary structural units in PDZ2 are indicated.

Figure S5. Internal correlation time (τ_e) changes induced by RA-GEF2 and APC binding with respect to free PDZ2.

Figure S1

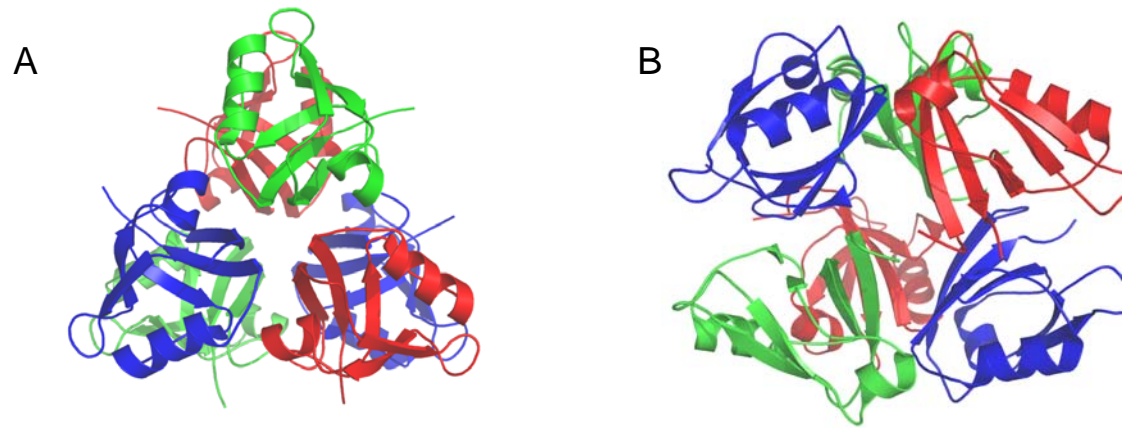


Figure S2

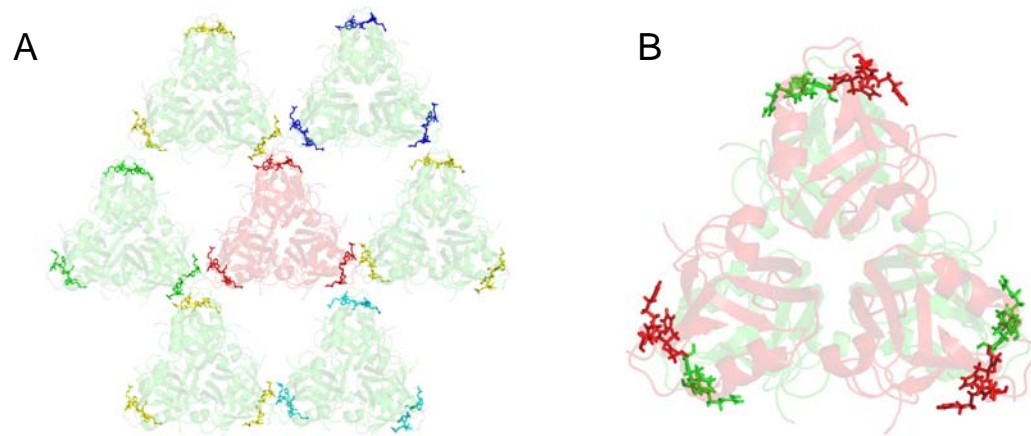


Figure S3

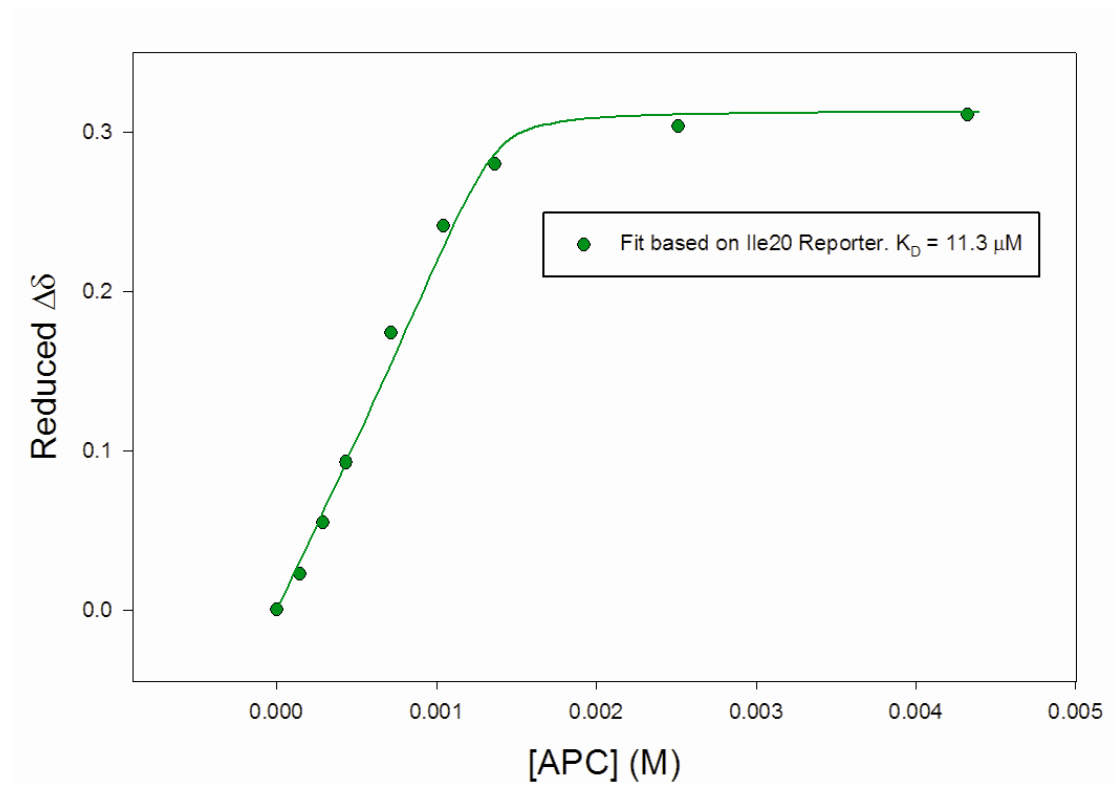


Figure S4

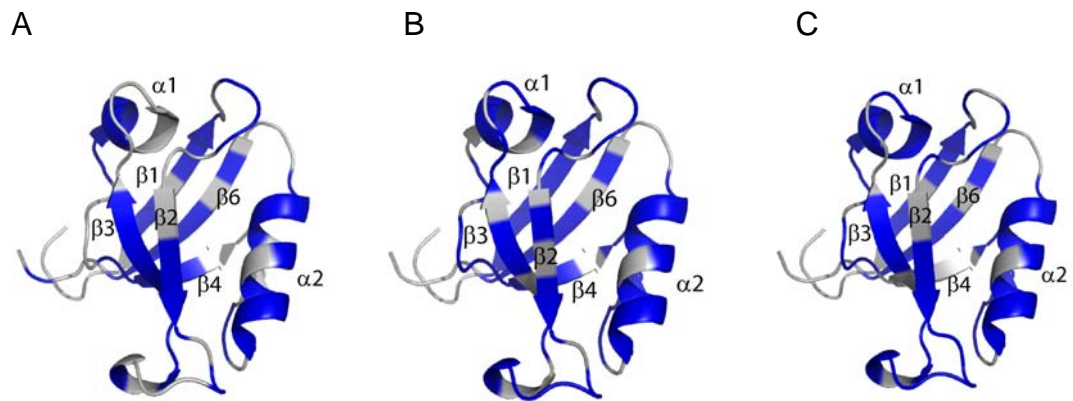


Figure S5

