

## **The Structure of the Plakin Domain of Plectin Reveals an Extended Rod-like Shape.**

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## **SUPPLEMENTAL TABLES**

**Table S1. Relative orientations of adjacent repeats of the plakin domain of plectin<sup>a</sup>.**

| Repeats | Fragment       | PDB entry | Monomer <sup>b</sup> | Tilt (x) | Roll (y) | Twist (z) |
|---------|----------------|-----------|----------------------|----------|----------|-----------|
| SR3-SR4 | SR3-SR4        | 3PDY      | A                    | -1.5°    | 13.3°    | 73.4°     |
|         | SR3-SR4        | 3PDY      | B                    | -0.2°    | 18.1°    | 65.3°     |
| SR4-SR5 | SR4-SR5-SH3    | 3PE0      | A                    | -14.1°   | 8.4°     | 37.0°     |
|         | SR4-SR5-SH3    | 3PE0      | B                    | -14.2°   | 10.1°    | 37.8°     |
| SR5-SR6 | SR5-SR6-ΔSH3-A | 5J1H      | A                    | -16.5°   | -9.9°    | 10.6°     |
|         | SR5-SR6-ΔSH3-A | 5J1H      | B                    | -21.7°   | -14.0°   | 14.3°     |
|         | SR5-SR6-ΔSH3-B | 5J1F      | A                    | -23.3°   | -19.6°   | 13.9°     |
|         | SR5-SR6-ΔSH3-B | 5J1F      | B                    | -21.7°   | -3.3°    | 12.6°     |
| SR7-SR8 | SR7-SR8        | 5J1G      | A                    | -8.5°    | 8.7°     | 49.4°     |
|         | SR7-SR8        | 5J1G      | B                    | -9.7°    | 8.2°     | 50.0°     |
|         | SR7-SR9        | 5J1I      | A                    | -8.6°    | 10.8°    | 49.6°     |
|         | SR7-SR9        | 5J1I      | B                    | -10.8°   | 10.0°    | 51.3°     |
| SR8-SR9 | SR7-SR9        | 5J1I      | A                    | -4.2°    | -8.5°    | 68.4°     |
|         | SR7-SR9        | 5J1I      | B                    | -4.7°    | -7.3°    | 70.5°     |

<sup>a</sup> The simultaneous rotations around the three Cartesian axes that relate each pair of repeats was calculated as follows. The center of mass of the N-terminal repeat was placed at the origin and the longitudinal axis of the repeat was aligned to the z axis with the C-terminus pointing towards the positive end. The orientation matrix to superimpose the N-terminal repeat onto the C-terminal repeat was calculated with LSQKAB and it was decomposed into three simultaneous rotations around x, y, and z.

<sup>b</sup> Independent molecules in the asymmetric unit of the crystal.

**Table S2. Relative orientations of adjacent repeats of the SR15-SR17 region of α-spectrin<sup>a</sup>.**

| Repeats   | PDB entry | Monomer | Tilt (x) | Roll (y) | Twist (z) |
|-----------|-----------|---------|----------|----------|-----------|
| SR15-SR16 | 1U5P      | A       | -5.4°    | 2.6°     | 50.5°     |
|           | 1U4Q      | A       | -4.6°    | 0.7°     | 46.5°     |
|           | 1U4Q      | B       | -8.0°    | -3.4°    | 32.4      |
| SR16-SR17 | 1CUN      | A       | -19.9°   | -4.5°    | 30.2°     |
|           | 1CUN      | B       | -24.3°   | -2.8°    | 26.3°     |
|           | 1CUN      | C       | -23.5°   | -2.5°    | 26.2°     |
|           | 1U4Q      | A       | -9.2°    | -1.2°    | 35.0°     |
|           | 1U4Q      | B       | 0.0°     | 1.5°     | 40.3°     |

<sup>a</sup> The relatively large conformational variability of the SR16-SR17 pair is mainly associated to large differences in the tilt angle. The angles that describe the relative orientation of the SR15-SR16 region, which has smaller conformational variations, are shown for comparison.

**Table S3. Oligonucleotides used for making plectin and desmoplakin constructs.**

| Direction | Name                  | Sequence  |
|-----------|-----------------------|---|
| Forward   | Ple-U543              | 5' TGAGAATTCCATATGGAGCTGGAGGACTCCACTC 3'<br>NdeI E L E D S T<br>543                         |
| Forward   | Ple-U750 <sup>a</sup> | 5' TGAGAATTCCATATGGCCTACGCTCAGTTCTTCTCAGATGTG 3'<br>NdeI A Y A Q F F S D V<br>750           |
| Forward   | Ple-U1004             | 5' TGAGAATTCCATATGCAGGAAGAGTCTCGCTGC 3'<br>NdeI Q E E S R C<br>1004                         |
| Reverse   | Ple-L1006             | 5' TGAGAATTCGGATCCCTACTACTCTTCTGTGCACCCGTGTC 3'<br>BamHI * * E E Q A G Q E<br>1006          |
| Reverse   | Ple-L1233             | 5' TGAGAATTCGGATCCCTAGCCAGTTGCTCGAGCTC 3'<br>BamHI * G L Q E L E<br>1233                    |
| Reverse   | Ple-L1372             | 5' TGAGAATTCGGATCCCTACTCCATGCGCCGAGAG 3'<br>BamHI * E M R R L T<br>1372                     |
| Reverse   | Ple-L818-Link5        | 5' ACCGCTCCCTGATCCGACGGCCTTGGCCCGCTTGGC 3'<br>G S G S G V A K A R K A<br>linker 818         |
| Forward   | Ple-U889-Link5        | 5' GGATCAGGGAGCGGTAACCAGGAGGCCAGGAGGCC 3'<br>G S G S G N Q E A Q E A<br>linker 889          |
| Reverse   | Ple-L818-Link3        | 5' CTGGTCCCTGATCCGACGGCCTTGGCCCGCTTGGC 3'<br>Q N G S G V A K A R K A<br>889 linker 818      |
| Forward   | Ple-U889-Link3        | 5' GCCGTCGGATCAGGGAACCAGGAGGCCAGGAGGCC 3'<br>A V G S G N Q E A Q E A<br>818 linker 889      |
| Forward   | DSP-U180              | 5' TGAGAATTCATATGTGGGATGAGTTCACCAAACATGTCACC 3'<br>NdeI W D E F T K H V T<br>180            |
| Forward   | DSP-U660              | 5' TGAGAATTCATATGGAAAATGACAAGCAAGAAACATGGATGCTG 3'<br>NdeI E N D K Q E T W M L<br>660       |
| Reverse   | DSP-L1025             | 5' GAATTCGGATCCCTACTATTCCAAACTCTTCAGCATCTCACTTAAG 3'<br>BamHI * * E L S K L M E S L<br>1025 |

<sup>a</sup> F752, which packs against the SR4 in the SR4-SR5 structure (PDB entry 3PE0), was substituted by Ala in the SR5-SR6-ΔSH3 constructs.

**Table S4. Combinations of oligonucleotides used to amplify the cDNA fragments of plectin and desmoplakin.**

| Protein     | Construct   | Oligonucleotides |                |
|-------------|---|------------------|----------------|
|             |   | Forward          | Reverse        |
| Plectin     | SR3-SR6 (543-1006)                                    | Ple-U543         | Ple-L1006      |
| Plectin     | SR7-SR8 (1004-1233)                                   | Ple-U1004        | Ple-L1233      |
| Plectin     | SR7-SR9 (1004-1372)                                   | Ple-U1004        | Ple-L1372      |
| Plectin     | SR3-SR9 (543-1372)                                    | Ple-U543         | Ple-L1372      |
| Plectin     | SR5-SR6- $\Delta$ SH3-A, intermediate I <sup>a</sup>  | Ple-U750         | Ple-L818-Link5 |
| Plectin     | SR5-SR6- $\Delta$ SH3-A, intermediate II <sup>a</sup> | Ple-U889-Link5   | Ple-L1006      |
| Plectin     | SR5-SR6- $\Delta$ SH3-A (750-818-GSGSG-889-1006)      | Ple-U750         | Ple-L1006      |
| Plectin     | SR5-SR6- $\Delta$ SH3-B, intermediate I <sup>a</sup>  | Ple-U750         | Ple-L818-Link3 |
| Plectin     | SR5-SR6- $\Delta$ SH3-B, intermediate II <sup>a</sup> | Ple-U889-Link3   | Ple-L1006      |
| Plectin     | SR5-SR6- $\Delta$ SH3-B (750-818-GSG-889-1006)        | Ple-U750         | Ple-L1006      |
| Desmoplakin | SR7-SR9 (660-1025)                                    | DSP-U660         | DSP-L1025      |
| Desmoplakin | SR3-SR9 (180-1025)                                    | DSP-U180         | DSP-L1025      |

<sup>a</sup> Intermediate fragments that were used as templates in a second round of PCR to create the final construct.

**Table S5. Yields of purified recombinant fragments of plectin and desmoplakin**

| Protein     | Fragment                | Yield (mg) <sup>a</sup> |
|-------------|-------------------------|-------------------------|
| Plectin     | SR5-SR6- $\Delta$ SH3-A | 80                      |
|             | SR5-SR6- $\Delta$ SH3-B | 46                      |
|             | SR3-SR6                 | 55                      |
|             | SR7-SR8                 | 36                      |
|             | SR7-SR9                 | 20                      |
|             | SR3-SR9                 | 14                      |
| Desmoplakin | SR7-SR9                 | 12                      |
|             | SR3-SR9                 | 1                       |

<sup>a</sup> Representative yields expressed as mg of purified protein per liter of *E. coli* culture

**Table S6. Detailed data collection statistics of the plectin SR7-SR9 dataset after applying anisotropic resolution limits.**

| Resolution limit (Å) | Unique reflections | Average multiplicity | Completeness (%) | Mean $I/\sigma(I)$ | $R_{\text{meas}}$ | CC 1/2 |
|----------------------|--------------------|----------------------|------------------|--------------------|-------------------|--------|
| 12.53                | 295                | 6.6                  | 96.4             | 64.89              | 0.021             | 1.000  |
| 8.86                 | 541                | 7.1                  | 99.8             | 59.92              | 0.025             | 1.000  |
| 7.23                 | 682                | 7.3                  | 99.9             | 44.54              | 0.030             | 1.000  |
| 6.26                 | 814                | 7.5                  | 99.9             | 29.54              | 0.063             | 0.999  |
| 5.60                 | 920                | 7.6                  | 100              | 19.87              | 0.100             | 0.998  |
| 5.11                 | 989                | 7.7                  | 99.3             | 17.61              | 0.083             | 1.000  |
| 4.73                 | 1074               | 7.7                  | 99.1             | 19.39              | 0.065             | 1.000  |
| 4.43                 | 1080               | 7.7                  | 92.3             | 19.09              | 0.094             | 0.999  |
| 4.18                 | 1044               | 7.7                  | 84.9             | 15.84              | 0.137             | 0.997  |
| 3.96                 | 1059               | 7.7                  | 81.1             | 11.82              | 0.212             | 0.993  |
| 3.78                 | 1050               | 7.7                  | 75.5             | 8.88               | 0.315             | 0.985  |
| 3.62                 | 968                | 7.7                  | 66.6             | 6.40               | 0.448             | 0.964  |
| 3.47                 | 767                | 7.7                  | 52.3             | 5.60               | 0.517             | 0.959  |
| 3.35                 | 689                | 7.6                  | 42.9             | 4.50               | 0.703             | 0.927  |
| 3.23                 | 561                | 7.6                  | 35.2             | 3.99               | 0.767             | 0.915  |
| 3.13                 | 456                | 7.5                  | 27.7             | 3.98               | 0.763             | 0.920  |
| 3.04                 | 363                | 7.4                  | 20.7             | 4.57               | 0.620             | 0.938  |
| 2.95                 | 304                | 7.2                  | 17.4             | 4.01               | 0.707             | 0.929  |
| 2.87                 | 263                | 7.0                  | 14.2             | 3.27               | 0.820             | 0.752  |
| 2.80                 | 208                | 6.2                  | 11.4             | 1.73               | 1.384             | 0.555  |
| Total                | 14129              | 7.5                  | 55.7             | 17.23              | 0.083             | 1.000  |