

**Crystal structure of afadin PDZ domain–nectin-3 complex shows the structural
plasticity of the ligand-binding site**

Supporting information

Supplementary experimental method

Mouse afadin PDZ domain (AFPDZ) (1003-1096) was cloned, produced and purified with the same procedures as AFPDZ-nec3C (Materials and Methods in manuscript). The purified AFPDZ and AFPDZ-nec3C were concentrated to 360 μ M, and 3.6 ml of samples were loaded to HiLoad Superdex 75 HR 26/60 column (GE healthcare) equilibrated with 20 mM Tris-HCl (pH 7.5 at 4 °C) and 150 mM NaCl.

Supplementary Fig. S1 Chromatograms of AFPDZ and AFPDZ–nec3C by size-exclusion chromatography. The molecular masses of proteins were estimated using a Superdex 75 HR 26/60 column. The dimer and the monomer were detected for AFPDZ, while AFPDZ-nec3C formed dimer. Due to the difference of extinction coefficients, chromatograms were recorded in the range of 0.2 or 0.5, therefore the normalized peaks are shown in the fixed range of absorbance.

Supplementary Fig. S2 Two-dimensional representation of the interactions between afadin PDZ domain residues (orange) (molecule E) and nectin-3 peptide (purple) (molecule A). Hydrogen bonds are shown as dashed lines and hydrophobic interactions as arcs with radial spokes. The main-chains of Val(0) and Trp(-2) of nectin-3 peptide formed β -sheet with Leu1020 and Ile1022 of AFPDZ, and the side-chain of Glu(-3) interacted with Lys1038. The side-chains of Tyr(-1) and Trp(-2) made hydrophobic contacts with Ser1021 and Gln1071, respectively. The figure was prepared using LIGPLOT.¹

Supplementary Fig. S3 Model for tethering nectins by afadin dimer. At the adherens junctions, both afadin and nectins are clustered. Afadin associates to C-terminus of nectin *cis*-dimer. The dimerized AFPDZ tethers nectin C-terminus from the neighboring or identical nectin *cis*-dimer at both sides of the dimer.

Supplementary reference

1. Wallace A C, Laskowski R A TJM (1995) LIGPLOT: a program to generate schematic diagrams of protein-ligand interactions. *Protein Eng.* 8:127–134.

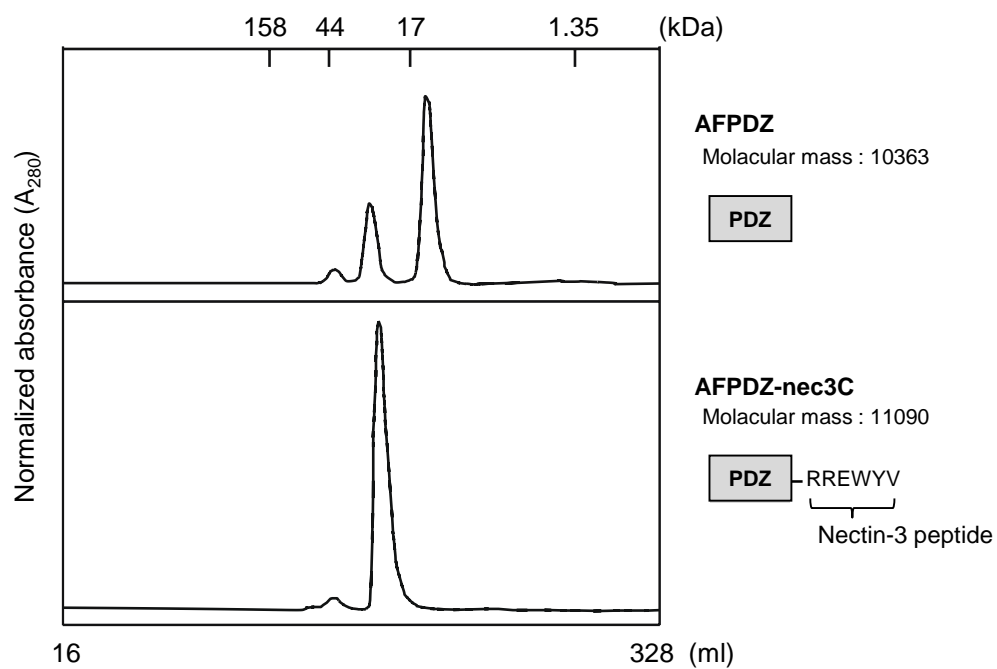
Supporting information Table I. Sequence similarity among C-terminus of nectins

Nectin-1: SFISKKEWYV

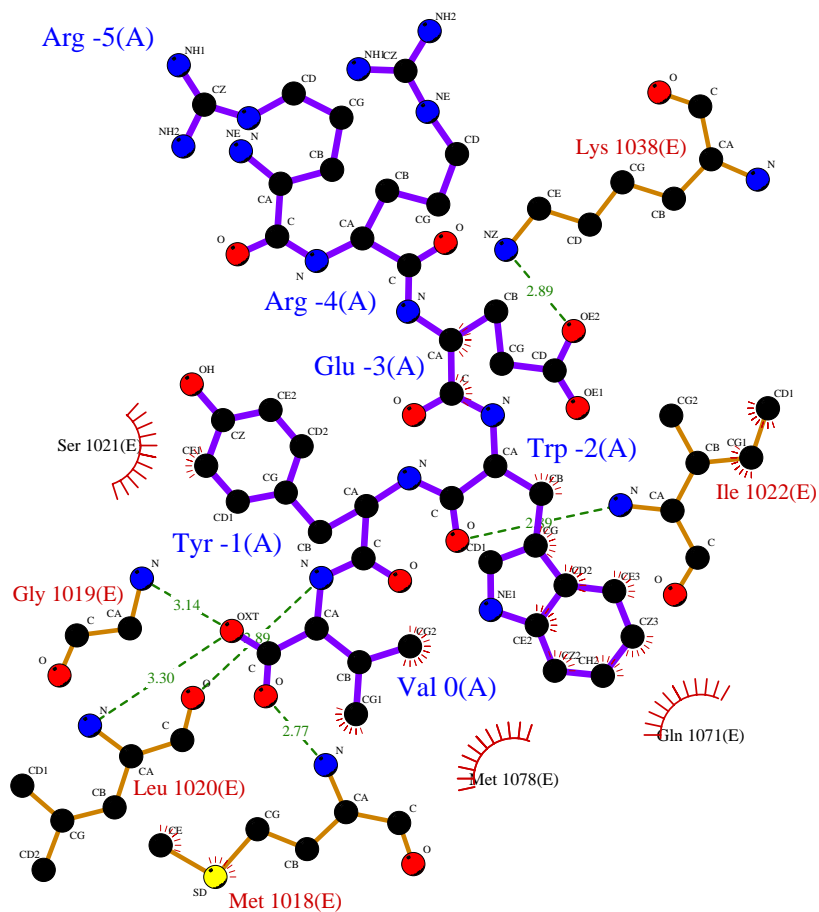
Nectin-2: SLISRRAVYV

Nectin-3: SVISRREWYV

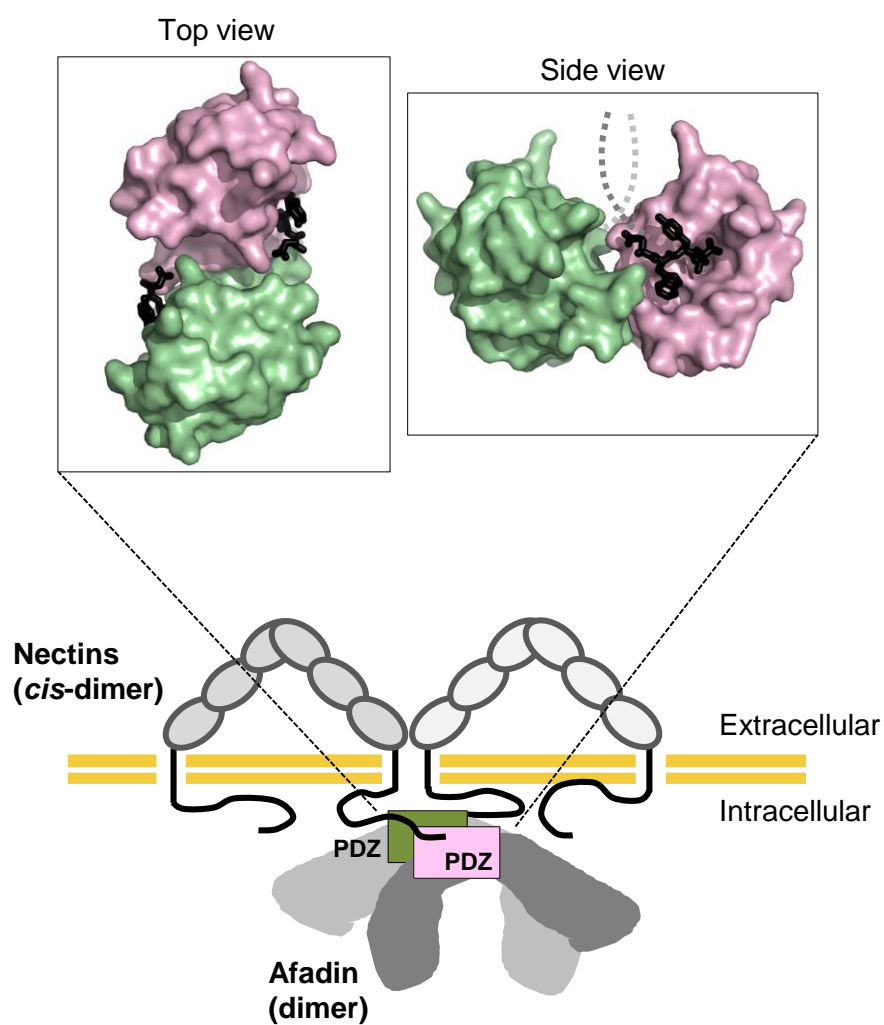
Underlines indicate the conserved charged region.



Supporting Information Fig. S1



Supporting Information Fig. S2



Supporting Information Fig. S3