Structural Insight for the Roles of Fas Death Domain Binding to FADD and Oligomerization Degree of the Fas - FADD complex in the Death Inducing Signaling Complex Formation: A Computational Study

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Running Title: Interactions of Fas death domain with FADD

Supporting Information

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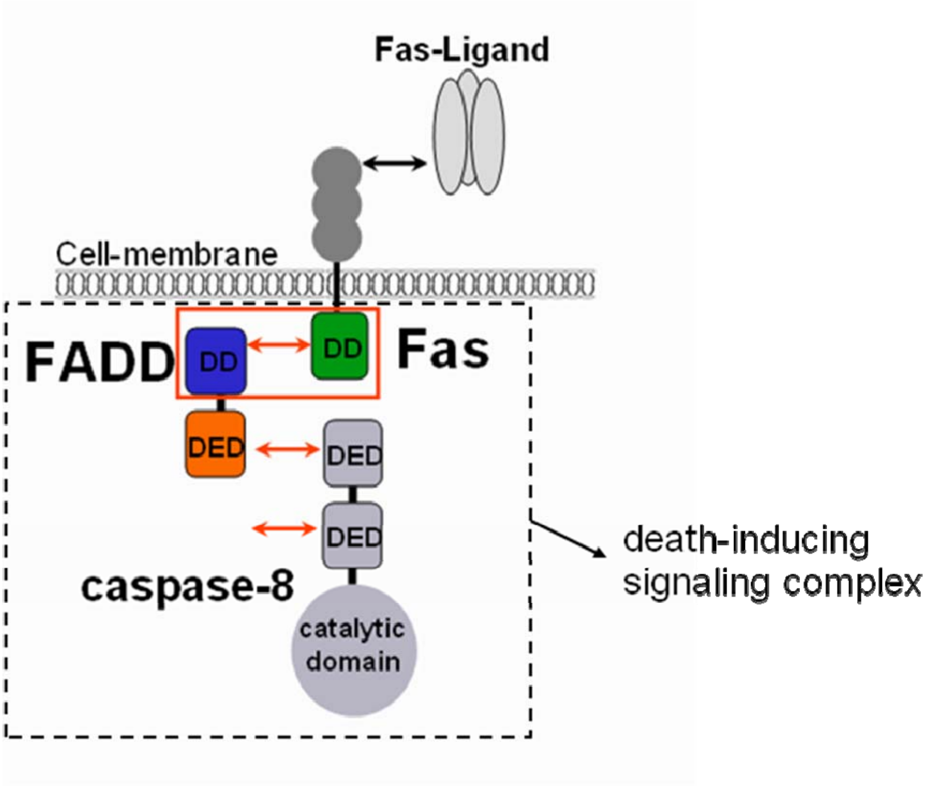

Adapted from "FL Scott *et al. Nature* **457**, 1019-1022, doi:10.1038/nature07606" (ref. 19)

Figure S1. Protein interactions underlying death-inducing signaling complex (DISC) formation. The binding of Fas ligand to Fas receptor recruits of FADD via death domain interactions. FADD in turn recruits caspase-8 via death effector domains (DEDs) of both proteins, leading to activation of the caspase (ref. 19).

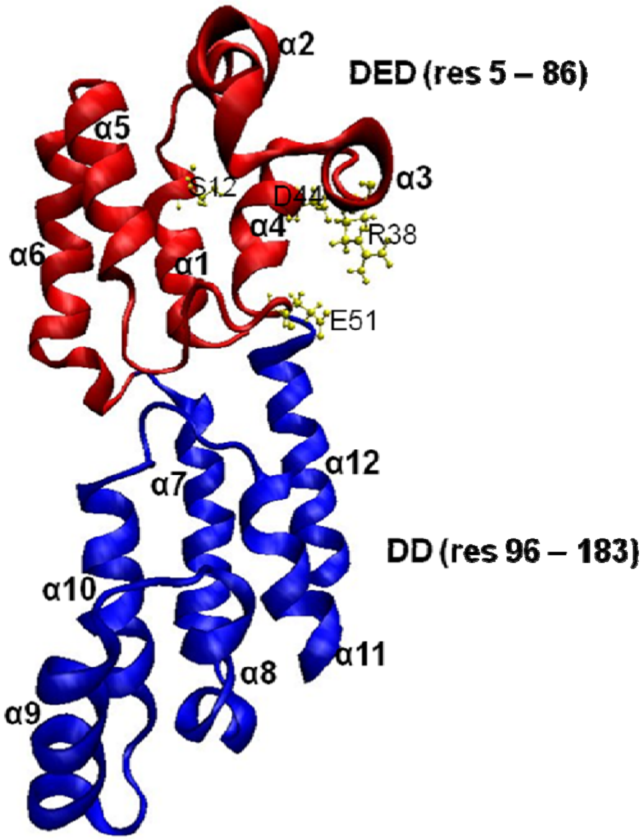

Figure S2. Structure of FADD (PDBID: 2GF5). Both FADD DD and FADD DED domains adopt a six α-helical bundle structure that is characteristic of a structural family of “death motifs” (ref. 23). Red: FADD death effector domain (FAD DED); blue: FADD death domain (FADD DD); residues represented as CPK are residues S12, R38, D44, and E51 in FADD DED domain that are important for FADD binding to procaspase-8; the α1 and α4 helices of the FADD DED interact with the death effector domain 2 of procaspase-8 (ref. 23).

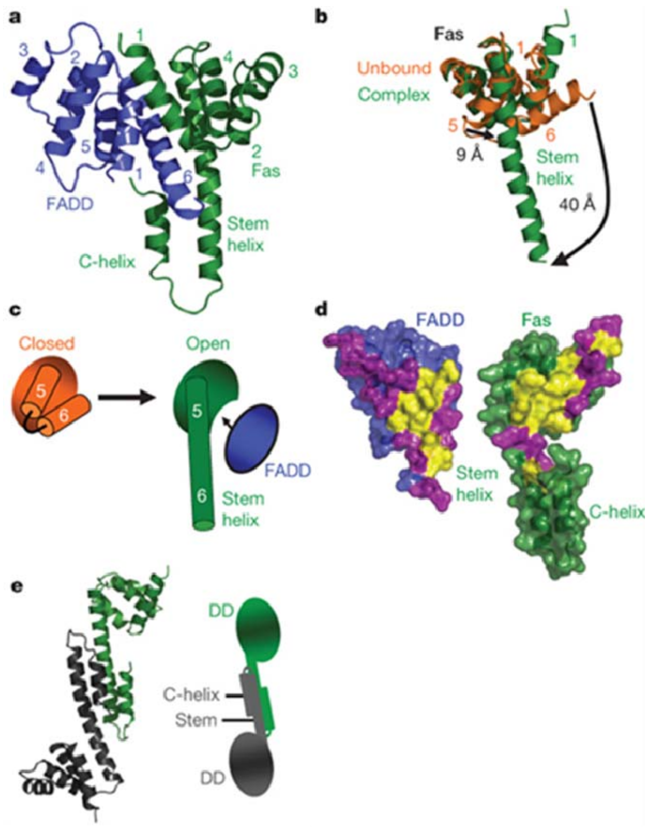

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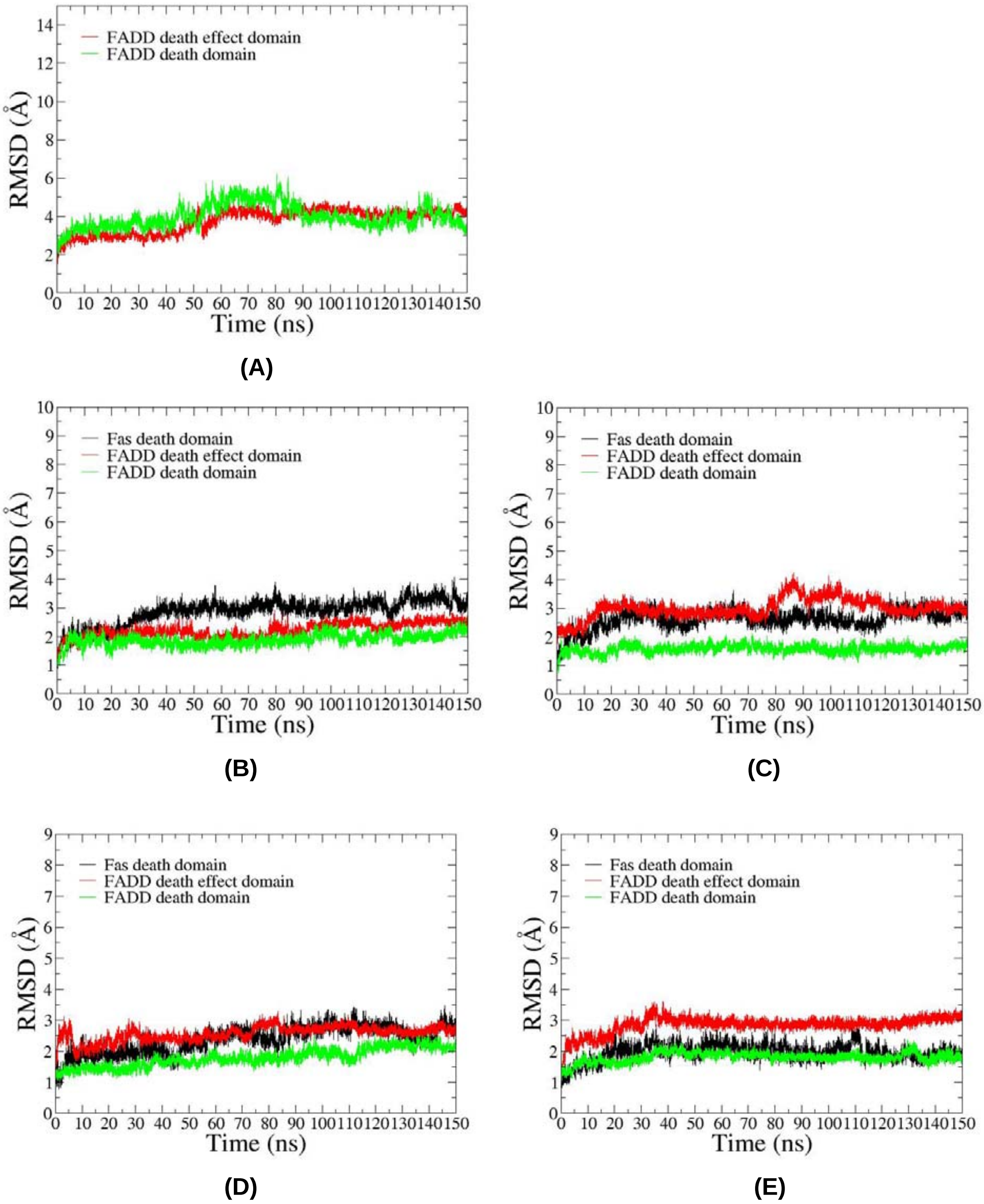
Figure S3. Fas–FADD DD complex: Fas–FADD and Fas–Fas interactions are dependent on Fas opening (ref. 19). (a) Primary Fas–FADD death domain complex (blue: FADD; green: Fas DD). For Fas DD, only helices one to four approximately adopt a death-domain-like fold, whereas helix α6 shifts and merges with helix α5 to form a relatively long helix called stem helix , and a new helix named C-helix at the C-terminal of Fas DD is formed. (b) Comparison of the structure of unbound Fas death domain (closed form in orange (PDBID: 1DDF)) and Fas in the Fas–FADD complex (green color). (c) Cartoon illustration of Fas DD opening when Fas binds to FADD. (d) Primary Fas–FADD interface. Surface representation shows complementary hydrophobic patches (yellow) on FADD and Fas surrounded by polar residues (magenta) and the hydrophobic interface on Fas becomes exposed upon Fas opening. (e) Fas dimer unit. (ref. 19).

Root mean square deviation (RMSD) of Fas DD and FADD for a single FADD and FADDs in the Fas DD-FADD complexes



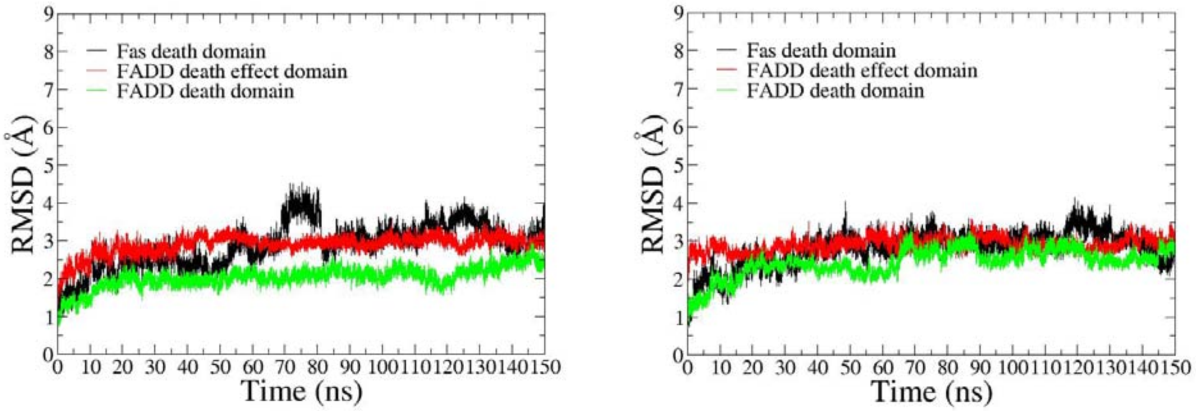
(A)

(B)

(C)

(D)

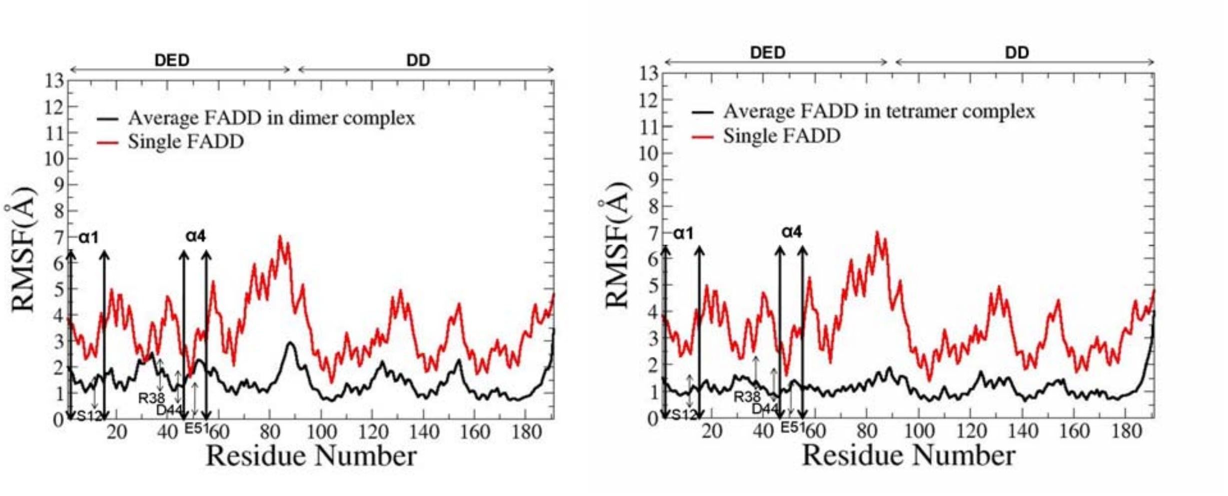
(E)



(F) **(G)**

Figure S4. Root mean squared deviation (RMSD) of Fas DD protein core (res 225–318) and FADD over the 150ns MD simulation. (A) Single FADD; (B) Fas DD-FADD complex 1 in Fas DD-FADD dimer complex; (C) Fas DD-FADD complex 2 in Fas DD-FADD dimer complex; (D) Fas DD-FADD complex 1 in Fas DD-FADD tetramer complex; (E) Fas DD-FADD complex 2 in Fas DD-FADD tetramer complex; (F) Fas DD-FADD complex 3 in Fas DD-FADD tetramer complex; (G) Fas DD-FADD complex 4 in Fas DD-FADD tetramer complex.

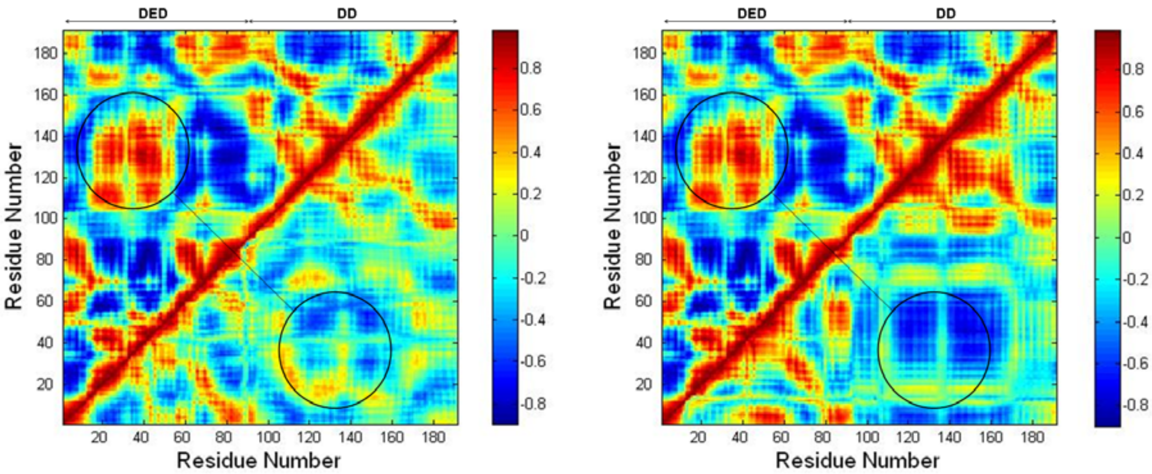
The root mean squared fluctuation (RMSF) comparison of FADD in single FADD and FADDs in the Fas DD-FADD complexes



(A) **(B)**

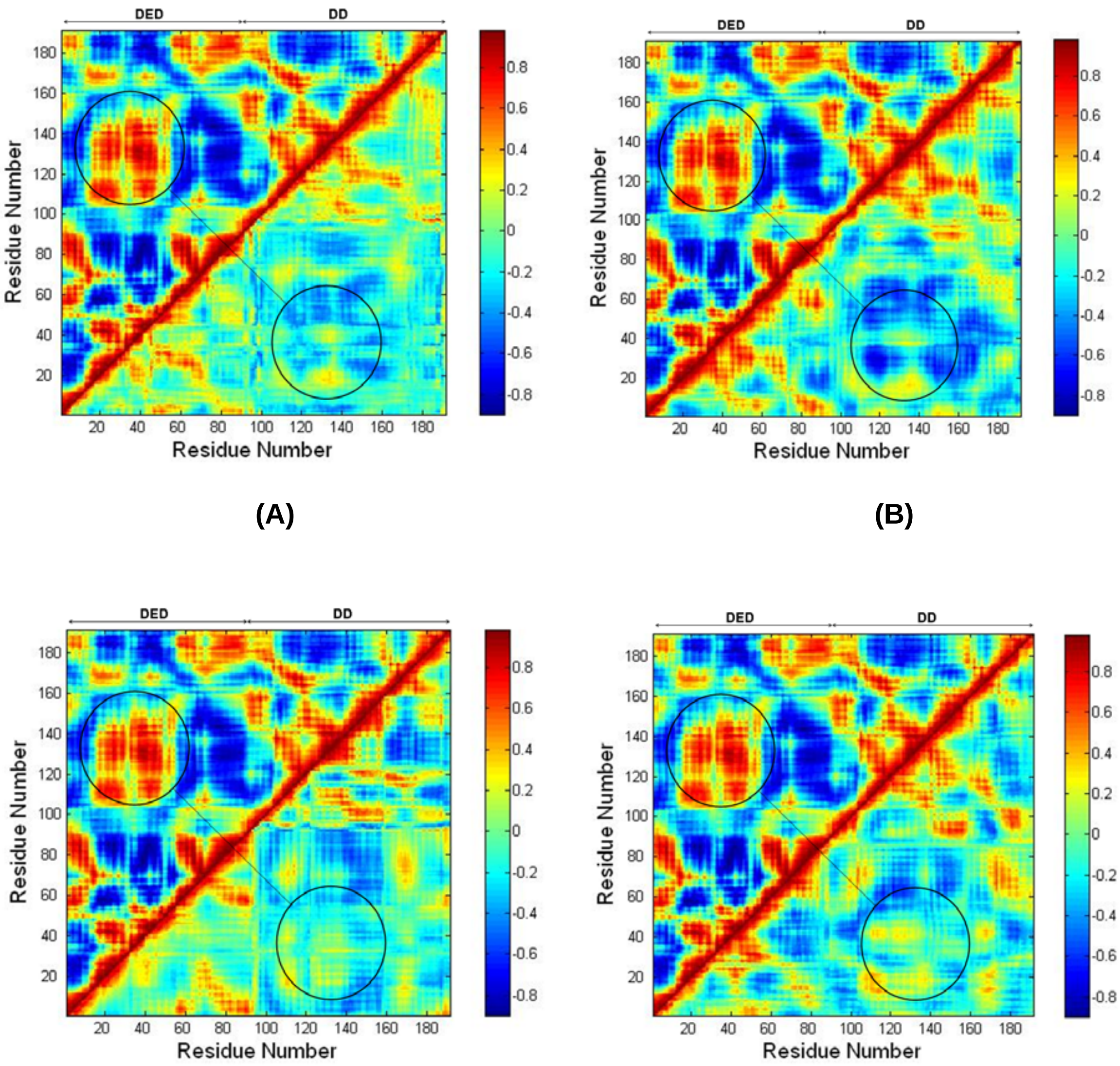
Figure S5. (A) RMSF of single FADD compared to the average RMSF of the two FADDs in Fas DD-FADD dimer complex; (B) RMSF of single FADD compared to the average RMSF of the four FADDs in Fas DD-FADD tetramer complex.

Comparison of dynamical cross-correlation maps for the degree of correlated motion of the residues of FADD in single FADD and FADDs in the Fas DD-FADD complexes



(A) **(B)**

Figure S6. Dynamical cross-correlation maps for the degree of correlated motion of the residues of FADD in single FADD and in Fas DD-FADD dimer complex (red: correlation between residues; blue: anticorrelation between residues). (A) top left: single FADD; bottom right: FADD1 in Fas-FADD dimer complex; (B) top left: single FADD; bottom right: FADD2 in Fas-FADD dimer complex. (Circled regions show the degree of correlated motion between res 10-70 in FADD DED and res 100-170 in FADD DD)



(A)

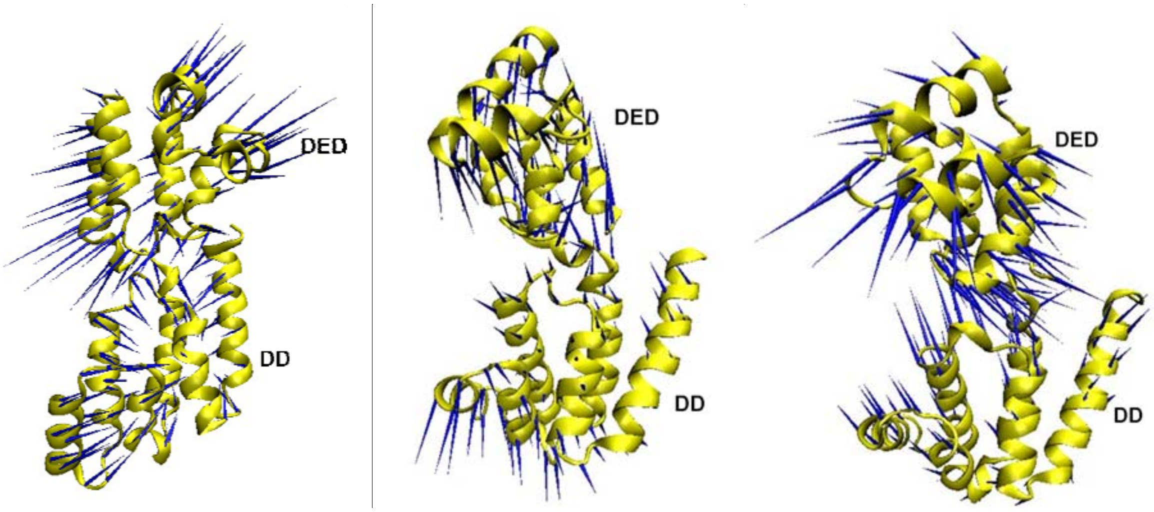
(B)

(C)

(D)

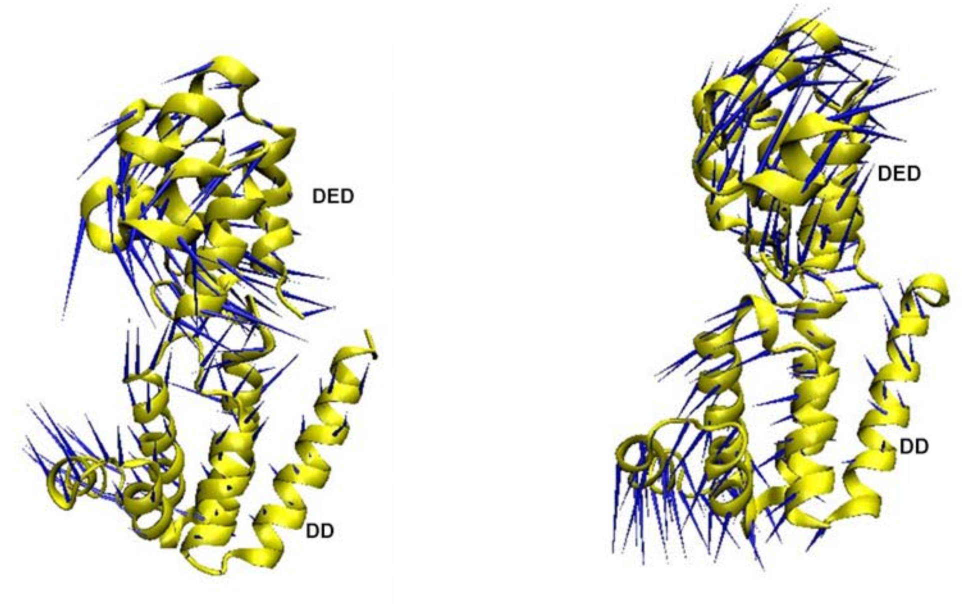
Figure S7. Dynamical cross-correlation maps for the degree of correlated motion of the residues of FADD in single FADD and in Fas DD-FADD tetramer complex (red: correlation between residues; blue: anticorrelation between residues). (A) top left: single FADD; bottom right: FADD1 in Fas-FADD tetramer complex; (B) top left: single FADD; bottom right: FADD2 in Fas-FADD tetramer complex; (C) top left: single FADD; bottom right: FADD3 in Fas-FADD tetramer complex; (D) top left: single FADD; bottom right: FADD4 in Fas-FADD tetramer complex. (Circled regions show the degree of correlated motion between res 10-70 in FADD DED and res 100-170 in FADD DD)

The principle component analyses of FADD in single FADD and FADD in the Fas-FADD complexes

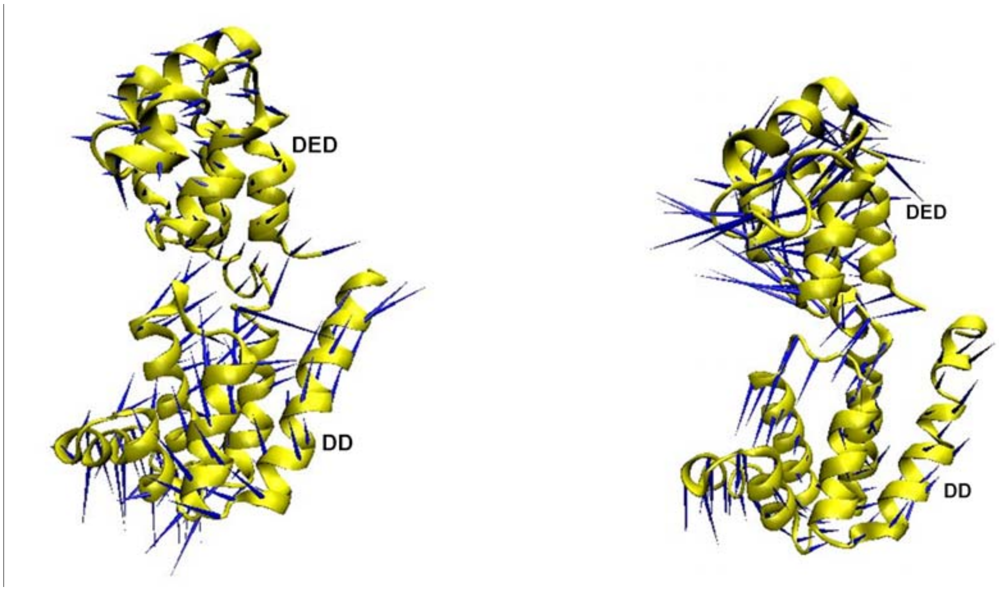


(A) (B) (C)

Figure S8. Porcupine plots of the principal motion of FADDs. (A) Single FADD; (B) FADD1 in dimer Fas DD-FADD complex; (C) FADD2 in dimer Fas DD-FADD complex.



(A) (B)



(C) (D)

Figure S9. Porcupine plot of the principal motion of FADDs in tetramer Fas DD-FADD complex. (A) FADD1 in tetramer complexes; (B) FADD2 in tetramer complexes; (C) FADD3 in tetramer complexes; (D) FADD4 in tetramer complexes.