

## TRAF2 Docking with Related Proteins *in Silico* Studies

(TRAF2 / Protein-protein docking / Hex)

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**Abstract.** Using the protein-protein docking program, this study investigates the relationship between TRAF2 and its related proteins and the diversity within the 3D structures of TRAF2s. TRAF2 exists in monomer, trimer, and hexamer forms and it can combine with a number of proteins. Through comparative analysis we found that TRAF2(122), TRAF2(22), TRAF2(21740), TRAF2(2), TRAF2-(22ABC), and TRAF2(Phyre) perform very close homology in docking with the same group of ligands, though these TRAF2s come from different sources.

The TRAF2-related proteins of cluster 1 change docking values strongly from top to bottom. The TRAF2-related proteins of clusters 2 and 3 have acceptable variation of the docking values. In consideration of the amino acid percentage, TRAF2-related proteins of cluster 2 represent appropriate docking values.

### Introduction

Tumour necrosis factor receptor-associated factor 2 (TRAF2) is one of the seven TRAF family proteins. TRAFs represent a group of structurally similar adaptor proteins characterized by having ring finger domains, multiple Zn<sup>2+</sup> fingers, and a conserved COOH-terminal TRAF domain (TD) (Ye et al., 1999). TRAF2 achieves diverse functions, combining and linking distinct adaptor proteins. To detect the condition and relationship of these adaptor proteins linking with different polymer TRAF2, we experimentally investigated docking between TRAF2 and its related proteins *in silico* by Hex6.3.

Hex is a primarily docking program to demonstrate the potential for performing fast 3D superposition using the SPF correlation approach (Ritchie et al., 2008). By using program Hex6.3, the automated comparative protein-protein docking is performed (Ritchie, 2008).

TRAF2 exists in monomer, trimer, and hexamer forms, and TRAF3 forms heterotrimers with TRAF2 and modulates its ability to mediate NF- $\kappa$ B activation (He et al., 2004). The cytoplasmic domain of LMP1 binds the signalling adaptor TRAF2 with lower avidity than the cytoplasmic domain of CD40, and TRAF2 is needed for CD40-mediated degradation of TRAFs 2 and 3 (Graham et al., 2009). TRAF3 is a negative modulator of LMP1-mediated NF- $\kappa$ B activation. TRAF3 associates with LMP-1 most avidly and can compete with TRAF1 and TRAF2 for binding to LMP-1. CD40 binds to TRAF2, TRAF3 and TRAF6 to control B-cell differentiation. CD40 engagement also results in its recruitment, together with TRAF2 and TRAF3, to membrane microdomains (Ely et al., 2007). A20 inhibits the E3 ligase activities of TRAF6, TRAF2, and cIAP1 by antagonizing interactions with the E2 ubiquitin-conjugating enzymes Ubc13 and UbcH5c (Shembade et al., 2010). In regulating B-cell survival and responsiveness, cIAP1/cIAP2 define a fundamental role that requires direct

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Abbreviations: A20 –  $\alpha$ -induced protein 3, aa – amino acid, AP1 – Jun proto-oncogene, BMP – protein decapentaplegic, CD30 – tumour necrosis factor receptor superfamily (member 8), CD40 – TNF receptor superfamily member 5, cIAP1 (or 2) – baculoviral IAP repeat-containing protein 1 (or 2), CYLD – turban tumour syndrome, E2 – dihydrolipoamide branched chain transacylase E2, FLIP – CASP8 and FADD-like apoptosis regulator, IKK $\beta$  – inhibitor of  $\kappa$ B kinase  $\beta$ , IL-15 $\alpha$  – interleukin 15 $\alpha$ , JNK – c-Jun N-terminal kinase, LMP1 – PDZ and LIM domain 7 (enigma), MAPK – mitogen-activated protein kinase, MAVS 1a – mitochondrial antiviral signalling protein, MMP-9 – matrix metalloproteinase 9, N-TRADD – N-terminal TNFRSF1A associated via death domain, NEMO – NF- $\kappa$ B essential modulator, NF- $\kappa$ B – nuclear factor of  $\kappa$  light polypeptide gene enhancer in B cells 1, NIK(s) – Nck-interacting kinase(s), p38 – aminoacyl tRNA synthetase complex-interacting multifunctional protein 2, PKN1 – protein kinase N1, Rap2 – Ras-related protein Rap2a, RIP1 – rieske iron-sulphur polypeptide 1, Smad4 – SMAD family member 4, RIG-I – retinoic acid inducible gene I, Smurf2 – SMAD-specific E3 ubiquitin protein ligase 2, SPF – spherical polar fourier, TAB2 – TAK1-associated binding protein 2, TAB3 – TAK1-binding protein 3, TAK1 – TGF- $\beta$  activated kinase 1, TANK – TRAF family member-associated NF $\kappa$ B activator, TNF- $\alpha$  – tumour necrosis factor  $\alpha$ , TNFR1 – tumour necrosis factor receptor superfamily member 1A, TNIK – TRAF2/Nck-interacting kinase, TRAF2 (or 3, 4, 5, 6) – TNF receptor-associated factor 2 (or 3, 4, 5, 6), TRAIL – tumour necrosis factor superfamily member 10, UbcH5c – ubiquitin conjugating enzyme, Ubc13 – ubiquitin carrier protein 13.

binding to TRAF2 (Gardam et al., 2011). The activation of MMP-9 also involves upstream recruitment of TRAF2 and cIAP2 proteins (Li et al., 2009). Tumour necrosis factor receptor 2 stimulation leads to degradation of TRAF2 (Wicovsky et al., 2009).

Proteins associated with TRAF2 are  $\beta$ -arrestin 1 (Kawamata et al., 2007) and Smurf2 (Carpentier et al., 2008). Through TRAF-binding sites TRAF2 binds the CD30 (Duckett and Thompson, 1997). MAVS 1a does not share sequence homology with the known proteins; instead it contains a putative TRAF2-binding motif and interacts with TRAF2 and RIP1 (Lad et al., 2008). TANK is a TRAF-binding protein that can inhibit the binding of TRAFs to receptor tails and can also inhibit NF- $\kappa$ B activation by these receptors (Pomerantz and Baltimore, 1999).

The N-terminal domain (N-TRADD) promotes the recruitment of TRAF2 to TNFR1 by binding to the C terminus of TRAF2, leading to the activation of JNK/AP1 and NF- $\kappa$ B (Tsao et al., 2000). CYLD exhibits deubiquitinating activity and acts as a negative regulator of NF- $\kappa$ B and c-Jun N-terminal kinase (JNK) signalling through its interaction with NEMO and TRAF2 (Courtois, 2008). The cell cycle arrests at a stage where there is high degradation of the FLIP-TRAF2 complex (Zhang et al., 2004). TNF- $\alpha$ -induced activation of JNK leads to interaction with TRAF2 (Dai et al., 2010). A novel IL-15  $\alpha/\gamma$ /TRAF2 complex that triggers NF- $\kappa$ B activation, TNFR1, TRADD, TRAF2, NIK, and IKK $\beta$  induces the activity of NF- $\kappa$ B reporter (Giron-Michel et al., 2003; Sethi et al., 2008). TRAIL can also activate JNK through the adaptor molecules, TRAF2 and receptor-interacting protein (RIP) (Mahalingam et al., 2009).

The interaction between TRAF2 and Smad4 may play a role in the cross-talk between TNF- $\alpha$  and BMP signalling pathways (Shimada et al., 2009). Smad4 may play a role in the cross-talk between TNF- $\alpha$  and BMP signalling pathways (Shimada et al., 2009). Expression of a transgene encoding Smad7 in mouse skin suppressed inflammation and NF- $\kappa$ B nuclear translocation substantially and disrupted the formation of endogenous TRAF2-TAK1-TAB2 and TRAF2-TAK1-TAB3 complexes (Hong et al., 2007). TRAF2 and TAK1 are essential for RIG-I-mediated activation of p38 MAPK (Mikkelsen et al., 2009). PKN1 has the TRAF2-binding consensus sequences PXQX (S/T) at amino acid residues 580-584 (PIQES), and P580AQ582A mutant is not co-immunoprecipitated with TRAF2 (Gotoh et al., 2004). Misshapen/NIKs-related kinase and closely related TRAF2/Nck-interacting kinase are proteins that specifically bind to activated Rap2 and are thus hypothesized to relay its downstream signal transduction (Hussain et al., 2010).

## Material and Methods

### *Crystal Pdb preparation*

The related crystal Pdb comes from the Protein Database Bank (<http://www.ebi.ac.uk/pdbe/>). The Pdb ID

and included chains are described as follows. A20, 3dkb, includes chains A, C, F; A20(CF) contains chains C and F; A20(A) contains chain A.  $\beta$ -arrestin-1, 2iv8. CD30, 1d01. CD40, 1czz; chain A, TRAF2(21740); chains D and E, CD40(21740); chain E, CD40(S21740). cFLIP, 3h13. CYLD, 2vhf. IL-15, 2z3q, includes chains A and C; IL15C contains chain C. JNK1, 3o17; chains A and B, JNK1; chain A, JNK1(S). LMP1, 2q3g. MAVS 1a, 2vgq. MMP-9, 116j. NF- $\kappa$ B, 1nfi; P56 includes chains A and C; p50 includes chains B and D. N-TRADD, 1f3v. PKN1, 1cxz. Rap2, 3rap, combines with GTP; 1kao, combines with GDP. RIG-I, 2rmj. Smad4, 1mr1. Smurf2, 2jqz. TAB2, 2dae. TANK, 1kzz; chain A, fragment of TRAF3; chain B, TANK. TNFR1, 1ich. TNFR2, 3a1q; chains RSTUVW, TNFR2; chain V, TNFR2(S); chains ABCDEF, TNF- $\alpha$ ; chain A, TNF $\alpha$ (S). TNIK, 2x7f. TRADD, 1f2h. TRAF1, 3m0d; chains A and B, TRAF2(122); chain C, TRAF1. TRAF3-ring, 2ecy. TRAF4-zinc 102-164, 2yuc. TRAF4-zinc 190-248, 2eod. TRAF6, 11b6; chain A, TRAF6(40); chain B, CD40(F640). TRAF6, 3hct; chain A, TRAF6(Ubc); chain B, Ubc13. TRAIL, 1d2q, includes chains A and B; chain A, TRAIL(S). TRAF2, 1ca9; chains ABC, TRAF2(22ABC); chain A, TRAF2(22). TRAF2, 1ca4; chain A, TRAF2(2); chains ABC, TRAF2(2ABC); chains ABCDEF, TRAF2.

### *Homology modelling Pdb preparation*

Homology Modelling builds TRAF2 by the Protein Homology/analogy Recognition Engine (phyre). The website is located at <http://www.sbg.bio.ic.ac.uk/~phyre/>; the TRAF2 is at TRAF2(phyre). Another homology modelling of TRAF2 comes from the Bio-Molecular Modelling Laboratory (Cancer Research UK, London, UK) (3D-JIGSAW). The website is located at <http://bmm.cancerresearchuk.org/~3djigsaw/>, the TRAF2 is at TRAF2(JIG).

### *Pdb checking and protein sequence analysis*

Protein amino acid sequences were obtained from <http://www.ebi.ac.uk/uniprot/>. Pdb checking was done by SYBYL-x 1.1 for Windows; this software can also count the amino acid number from the Pdb file of the protein and draw the image of the protein.

### *Docking with Hex6.3*

TRAF2s were treated as receptors, while other related proteins were treated as ligands; docking was done between one receptor and one ligand, respectively. Based on the energy minimization and 3D shape appropriate matching, the Hex6.3 docking control was set up as follows. Correlation Type: Shape + Electrostatics, FFT Mode: 3D, Grid Dimension: 0.6, Solutions: 500, Receptor Range: 45, Step Size: 7.5, Ligand Range: 45, Step Size: 7.5, Twist Range: 360, Step Size: 5.5, Distance Range: 40, Scan Step: 0.8, SubSteps: 2.

## Results

Docking between each TRAF2 of different source and TRAF2-related proteins is requested to be performed by shape and electrostatics, respectively. The minimum electrostatics value was selected to demonstrate the docking within the receptor and ligand. Each TRAF2 of Pdb file interacted with every selected TRAF2-related protein. There are nine kinds of Pdb files of TRAF2s, known as TRAF2(122), TRAF2(22), TRAF2(21740), TRAF2(2), TRAF2(2ABC), TRAF2(22ABC), TRAF2(Phyre), TRAF2, TRAF2(JIG); these characteristics of the TRAF2s are described in Pdb preparation. TRAF2-related proteins were prepared from 1 to 46. Docking values are shown in Fig. 1.

Amino acid percentage was calculated from the amino acid number in protein Pdb file and the amino acid number of the entire protein sequence. The results are illustrated in Fig. 2 and Fig. 3. The number of amino acids in protein Pdb file was counted by software SYBYL-x 1.1 for Windows. The entire protein sequence was taken from the Protein Database Bank.

TRAF2s' 3D images were drawn by SYBYL-x 1.1 for Windows. Different source Pdb files displayed subtle variations in protein conformation. Although the TRAF2s possess the same or very close primary sequence, the 3D constructs show a strong distinction, which induces the diversity of docking values between TRAF2 protein groups.

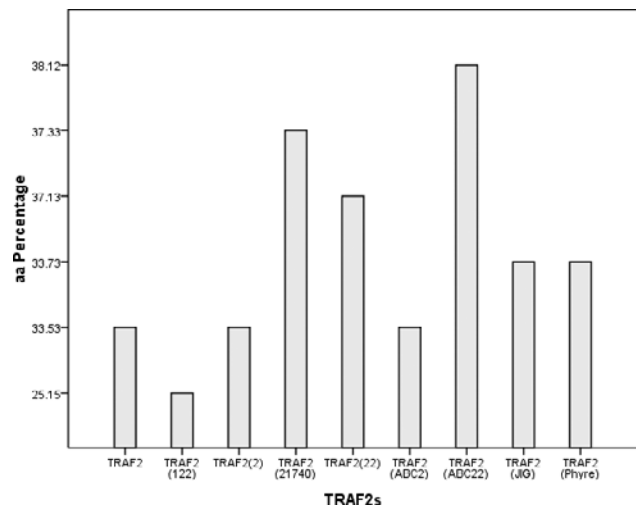


Fig. 2. Amino acid (aa) percentage of TRAF2. The aa percentage comes from the amino acid number in TRAF2 Pdb file and the aa number in the entire TRAF2 sequence. The aa number of TRAF2 Pdb file was counted by SYBYL-x 1.1 for Windows. The aa number in the entire TRAF2 sequence was obtained from the Protein Database Bank.

## Discussion

The docking values demonstrate two aspects in TRAF2s and TRAF2-related proteins. The trend curve within TRAF2s spreads partial homoplasy in feature. That derives combining capability between these TRAF2s.

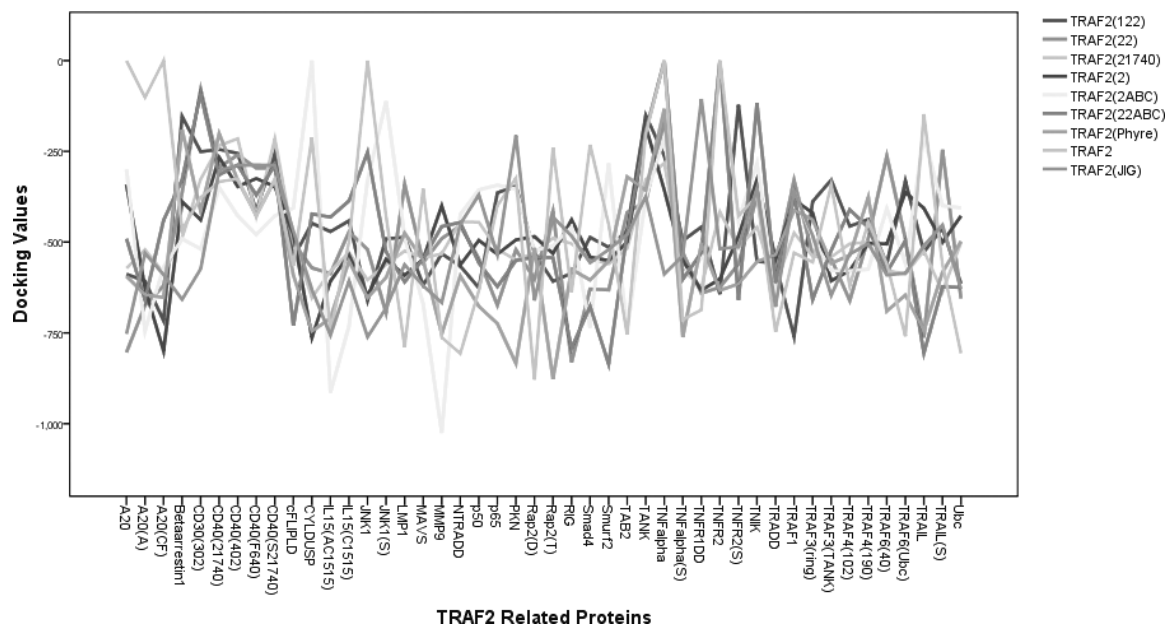


Fig. 1. The docking value between the receptor and the ligand. The colour lines represent TRAF2(122), TRAF2(22), TRAF2(21740), TRAF2(2), TRAF2(2ABC), TRAF2(22ABC), TRAF2(Phyre), TRAF2, TRAF2(JIG), respectively. Forty-six kinds of proteins as ligands were 1 – A20, 2 – A20(CF), 3 – A20(A), 4 –  $\beta$ -arrestin 1, 5 – CD30(302), 6 – CD40(402), 7 – CD40(F640), 8 – CD40(S21740), 9 – CD40(21740), 10 – cFLIPLD, 11 – CYLD, 12 – IL15(C1515), 13 – IL15(AC1515), 14 – JNK1, 15 – JNK1(S), 16 – LMP1, 17 – MAVS, 18 – MMP9, 19 – NTRADD, 20 – p50, 21 – p65, 22 – PKN, 23 – Rap2(D), 24 – Rap2(T), 25 – RIG, 26 – Smad4, 27 – Smurf2, 28 – TAB2, 29 – TANK, 30 – TNF- $\alpha$ , 31 – TNF- $\alpha$ (S), 32 – TNFR1, 33 – TNFR2, 34 – TNFR2(S), 35 – TNK, 36 – TRADD, 37 – TRAF1, 38 – TRAF3(ring), 39 – TRAF3(TANK), 40 – TRAF6(40), 41 – TRAF6(Ubc), 42 – TRAF4(102), 43 – TRAF4(190), 44 – TRAIL, 45 – TRAIL(S), 46 – Ubc.

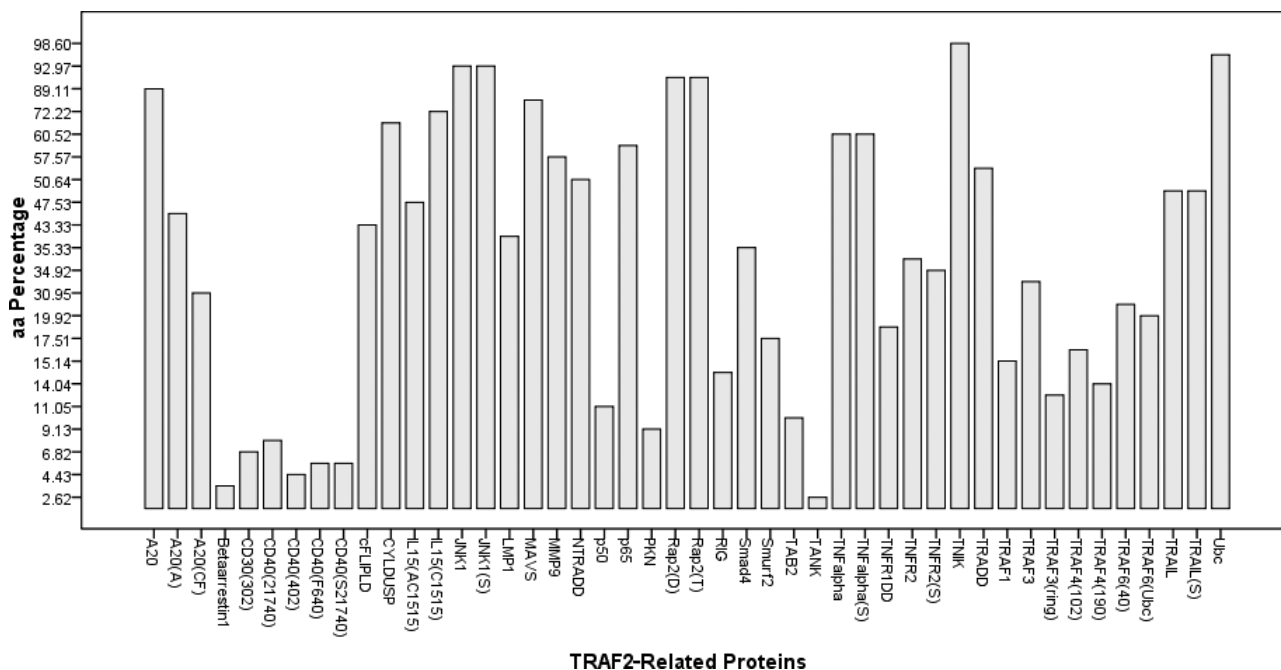


Fig. 3. Amino acid percentage of TRAF2 related-proteins. The aa percentage comes from the aa number in TRAF2-related protein Pdb file and the aa number in the related entire protein sequence. The aa number of TRAF2-related protein Pdb file was counted by SYBYL-x 1.1 for Windows. The aa number in the entire TRAF2-related protein sequence comes from the Protein Database Bank.

Analysis using TwoStep cluster by SPSS for TRAF2s is shown in Fig. 4. By Bonferroni adjustment applied, two figure results represent a very close situation. TRAF2(JIG), TRAF2, and TRAF2(2ABC) perform obvious inconsistency in docking with the same group of ligands. Other TRAF2s such as TRAF2(122), TRAF2(22), TRAF2(21740), TRAF2(2), TRAF2-(22ABC), TRAF2(Phyre) perform very close homoousia in docking with the same group of ligands.

The TRAF2 possesses six monomers (Fig. 6, Fig. 7) and has the maximum amino acid number in Pdb. In the 3D structure, the combining capability to ligand has been restrained; the same condition is adapted to describe

the restraint in TRAF2(2ABC), which possesses three monomers in 3D structure (Fig. 7, Fig. 8). For TRAF2-(JIG), its conformation affects the docking value. TRAF2(122), TRAF2(22), TRAF2(21740), TRAF2(2), TRAF2(22ABC), and TRAF2(Phyre) possess appropriate conformation in docking with their ligand.

TRAF2-related proteins were divided to three clusters by TwoStep cluster analysis. The cluster membership is revealed in Fig. 5, Table 1. Cluster 1 includes A20, A20(CF), CYLDUSP, JNK1, JNK1(S), TNFR2, and TRAIL. They change the docking values strongly from top to bottom. Their amino acid number is located in the upper part of the whole group of TRAF2-related

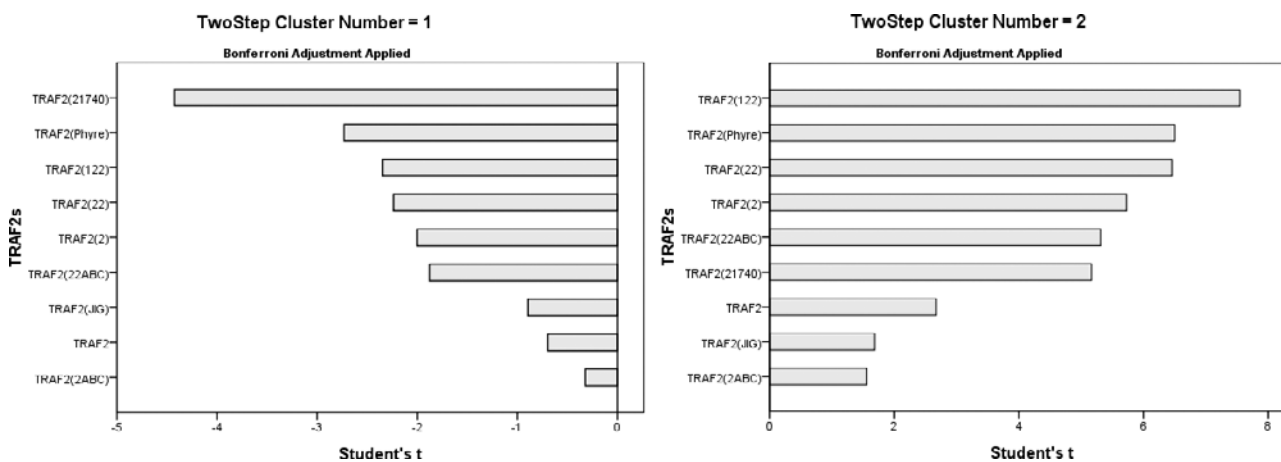


Fig. 4. TwoStep cluster analysis from SPSS17.0 for TRAF2s. Two groups derived from the above figure as group 1: TRAF2(122), TRAF2(22), TRAF2(21740), TRAF2(2), TRAF2(22ABC), TRAF2(Phyre), group 2: TRAF2(2ABC), TRAF2, TRAF2(JIG).

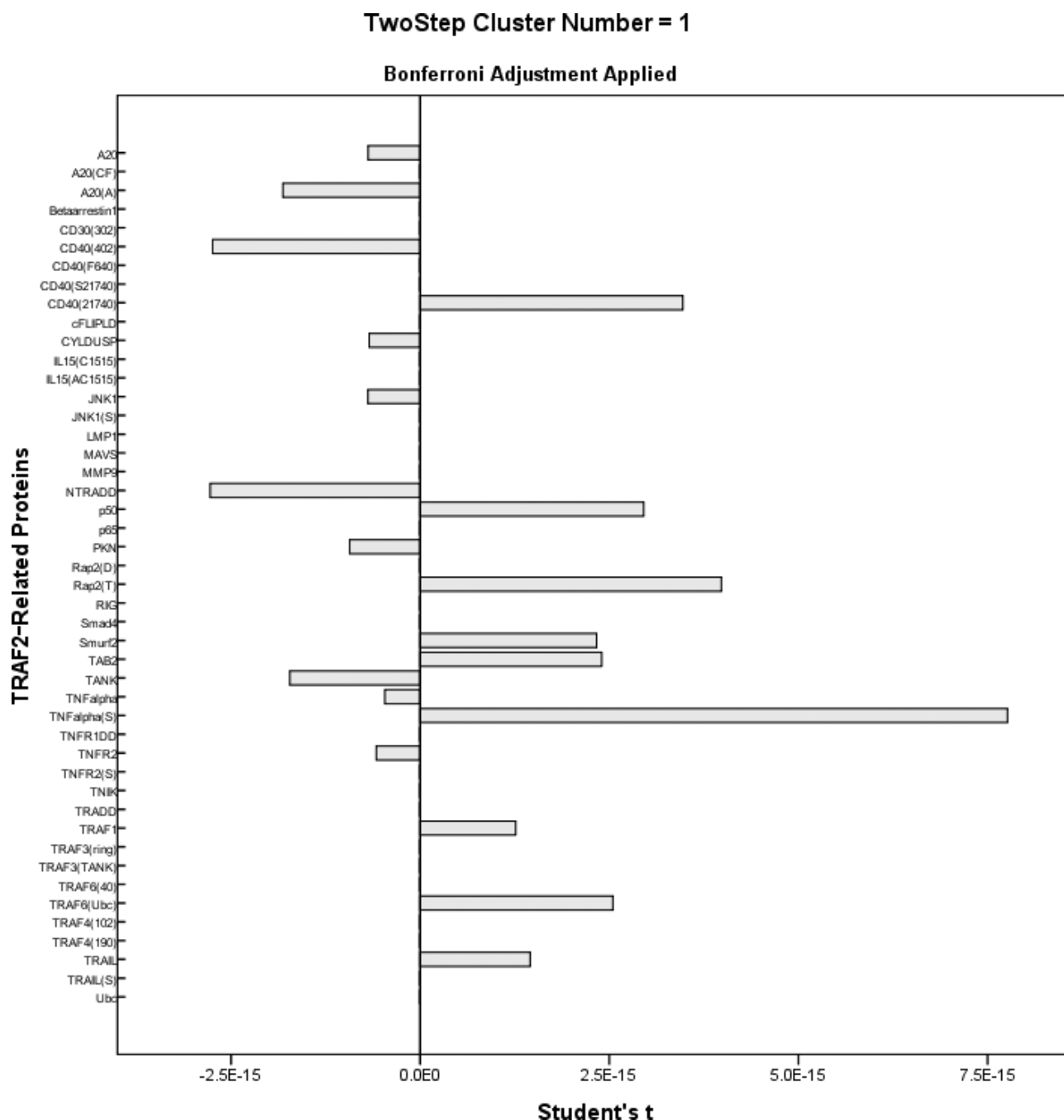


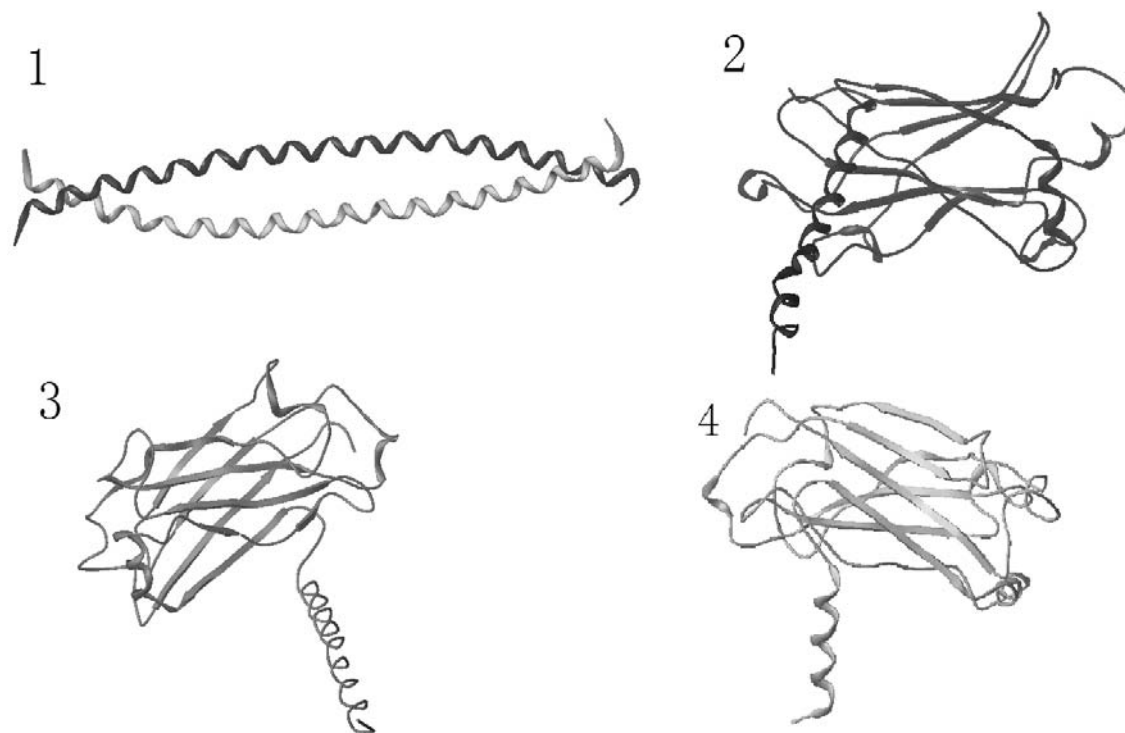
Fig. 5. TwoStep cluster analysis from SPSS17.0 for TRAF2-related proteins. Three clusters are shown in the figure, summarizing proteins in Table 1 to describe the name of the proteins and correlative clusters.

proteins. Interestingly, distribution in cluster 3 displays opposite amino acid number changes, the members of this cluster have a lower amino acid number, and all of them are located in the lower part of the whole group of TRAF2-related proteins. Combined with Fig. 1 for analysis, the membership of cluster 2 shows smooth variation of the docking values. The membership of cluster 1 changes the docking values strongly from top to bottom. Cluster 3 includes members such as  $\beta$ -arrestin 1, CD30(302), CD40(402), CD40(F640), CD40(S21740), CD40(21740), TANK, TNF- $\alpha$ , TNIK, and TRAF1, and within docking they change the docking values slightly.

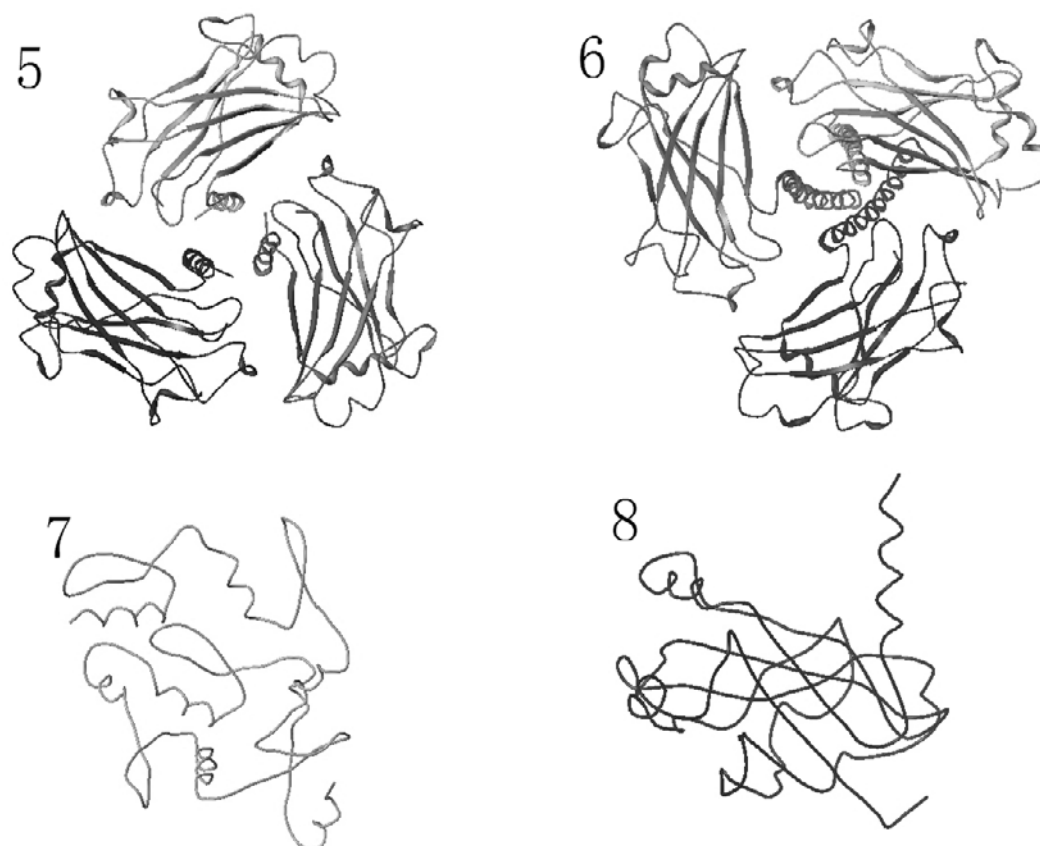
TRAF2s have variant 3D structure. Most of them do not affect the docking process. By shape, only complex polymer molecules demonstrate unstable protein-protein docking values. Otherwise, the shape is similar, but TRAF2(JIG) represents variable docking values.

## Conclusion

The 3D structure of TRAF2s reveals that the shape and chemical potential decide the combining capability in protein-protein docking. Even though the shape of TRAF2(122) is simple, its docking is performed smoothly; most of TRAF2s have complex 3D structures, and



*Fig. 6.* TRAF2 3D structure 1. 1 – TRAF2(122), 2 – TRAF2(22), 3 – TRAF2(21740), 4 – TRAF2(2). Schematic image drawn by SYBYL-x 1.1 for Windows. TRAF2(122) is a fragment of TRAF2; TRAF2(22), TRAF2(21740), TRAF2(2) represent one chain of TRAF2.



*Fig. 7.* TRAF2 3D structure 2. 5 – TRAF2(2ABC), 6 – TRAF2(22ABC), 7 – TRAF2(Phyre), 8 – TRAF2(JIG). Schematic image drawn by SYBYL-x 1.1 for Windows. TRAF2(2ABC), TRAF2(22ABC) are trimers of TRAF2; TRAF2(Phyre) contributed by homology modelling of Phyre; TRAF2(JIG) built by homology modelling of 3D JIGSAW.

Table 1. TRAF2-related protein docking value clusters

Cluster Membership		
1	2	3
A20, A20(CF), CYLDUSP JNK1, JNK1(S), TNFR2, TRAIL	A20(A), cFLIPLD, IL15(C1515), IL15(AC1515), LMP1, MAV S, MMP9, NTRADD, p50, p65, PKN, Rap2(D), Rap2(T), RIG, Smad4, Smurf2, TAB2, TNF $\alpha$ (S), TNFR1DD, TNFR2(S), TRADD, TRAF3(ring), TRAF3(TANK), TRAF6(40), TRAF6(Ubc), TRAF4(102), TRAF4(190), TRAIL(S), Ubc	$\beta$ -arrestin 1, CD30(302), CD40(402), CD40(F640), CD40(S21740), CD40(21740), TANK, TNF $\alpha$ , TNIK, TRAF1

Cluster 1 collects TRAF2-related proteins that change the docking value strongly from top to bottom. Cluster 2 assembles TRAF2-related proteins that display smooth variation of the docking value. Cluster 3 includes TRAF2-related proteins that change the docking value slightly.

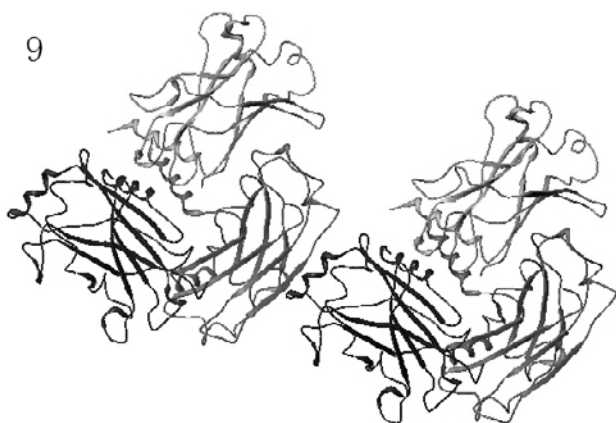


Fig. 8. TRAF2 3D structure 3. 9 – TRAF2. The hexamer of TRAF2. Schematic image drawn by SYBYL-x 1.1 for Windows.

they implement docking successfully. Most complicated 3D structures will reduce the combining capability in protein-protein docking. TRAF2(122), TRAF2(22), TRAF2(21740), TRAF2(2), TRAF2(22ABC), TRAF2-(Phyre) demonstrate steady combining capability with their ligand.

The TRAF2-related proteins of cluster 1 change docking values strongly from top to bottom. The TRAF2-related proteins of clusters 2 and 3 have acceptable variation of the docking values. In consideration of the aa percentage, TRAF2-related proteins of cluster 2 represent appropriate docking values.

#### Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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