

Supporting Material

A Structural View of Negative Regulation of Toll-like Receptor-Mediated Inflammatory Pathway

Emine Guven-Maiorov,^{1,2} Ozlem Keskin,^{1,2,*} Attila GURSOY,^{2,3} and Ruth Nussinov^{4,5,*}

¹Department of Chemical and Biological Engineering; ²Center for Computational Biology and Bioinformatics; and ³Department of Computer Engineering, Koc University, Istanbul, Turkey; ⁴Cancer and Inflammation Program, Leidos Biomedical Research, Inc. Frederick National Laboratory for Cancer Research, National Cancer Institute, Frederick, MD 21702, USA and ⁵Sackler Institute of Molecular Medicine, Department of Human Genetics and Molecular Medicine, Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel

Table S1: Sequence identities, coverage, and normalized Z-scores between the query proteins and the template structures. The normalized Z-score greater than 1 implies a good alignment (1)

Query protein	Template protein	PDB_ID of template	Sequence identity of query and template	Coverage	Normalized Z-score
BCAP	PdTIR from <i>Paracoccus denitrificans</i>	3h16A	0.22	0.93	1.23
BCAP	TIR domain of Mal/TIRAP	3ub2A	0.19	0.83	1.19
BCAP	<i>Brucella</i> protein TcpB TIR domain	4lqcA	0.21	0.86	1.13
BCAP	TIR domain from the <i>Arabidopsis Thaliana</i> disease resistance protein RRS1	4c6sA	0.22	0.95	1.06
SIGIRR	TIR domain of human IL-1RAPL	1t3gA	0.32	0.96	2.72
ST2L	TIR domain of human IL-1RAPL	1t3gA	0.35	0.89	2.53
ST2L	Homology model of human Toll-like receptor 5 fitted into an electron microscopy single particle reconstruction	3j0aA	0.22	0.87	3.5
ST2L	TIR domain of human TLR1	1fyvA	0.23	0.87	2.63
TLR4	TIR domain of human TLR1	1fyvA	0,39	0,97	2,76
TLR4	Homology model of human Toll-like receptor 5 fitted into an electron microscopy single particle reconstruction	3j0aA	0.29	0.99	3.68
TLR4	TLR2 TIR domain	1fyxA	0.4	0.97	2.77

Table S2: Details of interactions, namely the target and template structures, interaction energies obtained by PRISM and the references that show the physical interactions of the given protein pairs.

PPI	Protein1	Protein2	Template Interface	Interface Residues on Protein1	Interface Residues on Protein2
BCAP-TLR4	Model	Model	3dahAB	H44, L46, G47, P48, E49, A50, S51, S53, A54, L57, Q76, H77, F78, K80, P81, A82, L83, P85, L86, L87, Q88, R89	W686, E690, V692, K693, N694, E696, E697, G698, R709, D798, V800, L801, R803, H804, W807, R811
BCAP-Mal	Model	4lqdA	3urrAB	E20, E21, Q24, Y25, Q27, T28, L29, L31, S32, Q35, I41, L42,	L152, K158, M161, L162, L165, E167, P188, P189, E190, R192, F193, M194

				P129, E130, V133, A134	
BCAP-MyD88	Model	4eo7A	3flrAB	A54, L57, S58, L61, S62, R64, R89, A90, F91, H92, P94, H95	S209, E210, E213, K214, F235, K238, F239, K241, S242, L243, S244
BCAP-TRAM	Model	2m1wA	3bdvAB	F91, H92, P93, P94, R96, V97, V98, L111, D116, A118, H119, W120, K138, A139, E142, D143	E87, D88, T90, D91, L94, R95, V96, N98, L99, Q101, D102, D103, I111, E114, L176, N177, E197, E198, F202
BCAP-TRIF	Model	2m1xA	3dhxAB	P17, D18, E20, E21, W22, Q24, Y25, Q27, T28, L31, S32, Q35, Q39, K40, I41, T43, R45	R463, L464, L466, H467, N470, M473, M474, S475, L477, G481, P483, D502, T503, S505, L506, S508
SIGIRR-TLR4	Model	Model	3imoBD	E209, L233, S234, R235, A236, C238, S239, S241, F242, R243, E244, P268, A269, A272, L275, Q278	D685, N689, K693, N694, E697, V699, V800, L801, R803, H804, W807, R808, R811
SIGIRR-Mal	Model	4fz5A	1unhBE	T260, R274, R277, Q278, R280, H281, V283, T284, L285, L286, L287, R289, D298, F299	L152, P155, W156, K158, Y159, L162, T166, A185, A186, P188, P189, E190, R192
SIGIRR-MyD88	Model	4domA	3f13AB	A208, E209, P210, S211, A212, S234, R235, A236, C238, S239, F242, R243, E244, A272, L275	R218, P245, G246, A247, H248, Q249, K250, R251, L252, I253, I267, F270, I271, T272, L295, P296
SIGIRR-TRAM	Model	2m1wA	1pzmAB	Q264, R265, R266, D267, H270, L273, R274, R277, L285, L286, L287, R289, P290, G291	E87, D88, D89, T90, D91, E92, R95, F112, A113, E114, M115, H117, H121, L122, Q123, N177
SIGIRR-TRIF	Model	2m1xA	1ntcAB	W237, C238, S241, F242, E244, C247, L250, E251, P271, A272, L275, L276, Q278, H279, R280, H281	A402, E429, N459, F460, D461, R463, L464, L466, H467, Q468, N470, S500, D502, S505, L506
ST2L-TLR4	Model	Model	2bq1EF	L376, Y377, R385, Y387, E398, H402, D497, E410, N411, G414, T416, L417, C418, I419, G421, R422, D423, M424, L425, P426, K440	S681, Q682, E684, D685, W686, N689, E690, L691, K693, N694, Q738, H739, I768, L770, Q771, K772, V773, E774, L777, W796, D798, S799, V800, L801, R803, W807
ST2L-Mal	Model	3ub2A	3urrAB	R385, Y387, S389, E398, H399, H402, Q403, I404, D407, E410, N411, I419, G421, R422, D515, N519, K520, R521, S522	L152, Q153, P155, K158, Y159, L162, L165, T166, P188, P189, E190, R192, F193, M194
ST2L-MyD88	Model	4eo7A	2oh1CD	R396, E481, M482, E483, A484, L485, S486, E487, E494, Q497, T509, I510, K511, R513, E514, H516, I517, K526	L228, Q229, K231, D234, T237, L241, K262, E263, F264, P265, S266, I267, R269, F270
ST2L-TRAM	Model	2m1wA	3sf8AB	F458, E461, Q462, E463, V464, H467, M490, L491, Q492, A495, L496, S499, L503	D91, L94, R95, N98, L99, P175, L176, N177, P179, L180, P181, R184, E197, E198
ST2L-TRIF	Model	2m1xA	3grzAB	P384, R385, Y387, K388, S389, A394, S395, E398, V401, H402, R422, L425, P426	A402, V433, H434, S458, N459, F460, D461, R463, L466, H467, N470, Q471, Q498, S500, D502, L506
TRAF6-p62	2k0bX	3hctA	3jygAB	E65, S66, E69, C70, P71, L74, H87, V107	P387, A390, P392, R393, I395, E396, N421, Y422
p62-CYLD	1q02A	1whmA	2yvzAB	A390, D391, L394, I395, L398, L402, E409, G410, G411, T414, L417,	S223, S225, P229, L230, N300, I303, P304, E305, S306, S307, G308, P309

				Q418, T419	
TRAF6-CYLD	1whlA	3hcuA	2ekyBD	P62, P63, E65, S66, P71, H87, K104, C105, P106, V107, D108, N109	V127, P130, R147, Q185, L186, F187, Q188, S207, G206, P209, S210
TRAF6-A20	3hcsA	3zjgB	1f5qAB	Q54, D57, P71, I72, C73, L74, M75, C93, D100, A101, G102, K104, C105, P106, V107, D108, N109, P135	K20, R24, T42, R45, Y46, Q241, Y244, D331, N334, P336, K337, E338, I339,
A20 dimer	2vfjB	2vfjC	3dkbCF	P7, L12, S13, N14, M15, R16, V19, R22, E23, D119, D344, L348, H351	P7, L12, S13, N14, M15, R16, V19, R22, E23, D119, D344, L348, H351, E352
TRAF3-DUBA	3tmp	1flIA	2a6aAB	H470, R505, R506, L508, G509, D510, F537, V538, A539, T541, V542, N545, G546	M191, D192, T195, Q198, W202, K205, Y301, T303, G304, T305, S306

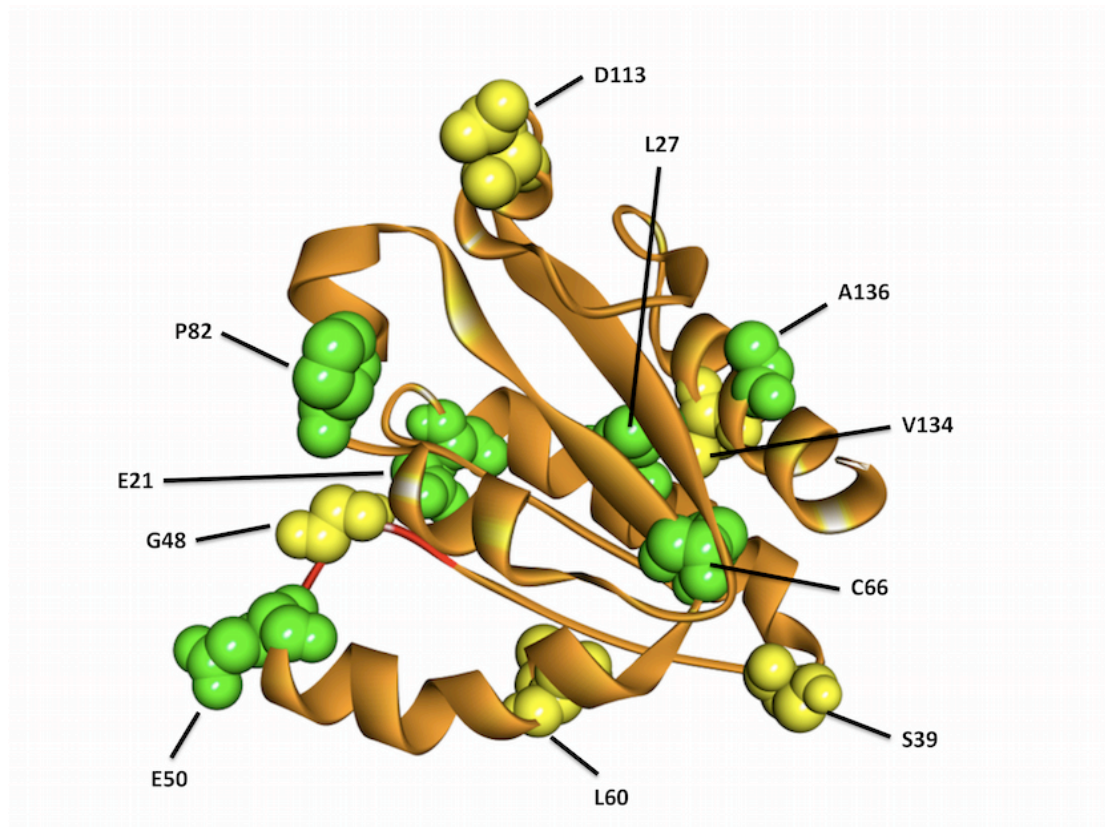


Figure S1: Clinically observed BCAP mutations that fall onto interfaces of BCAP interactions with other TIR domain-containing proteins. The mutations do not cluster at a particular location on 3D structure. The green-labeled residues abolish the interactions when they get mutated, but the yellow-labeled ones do not.

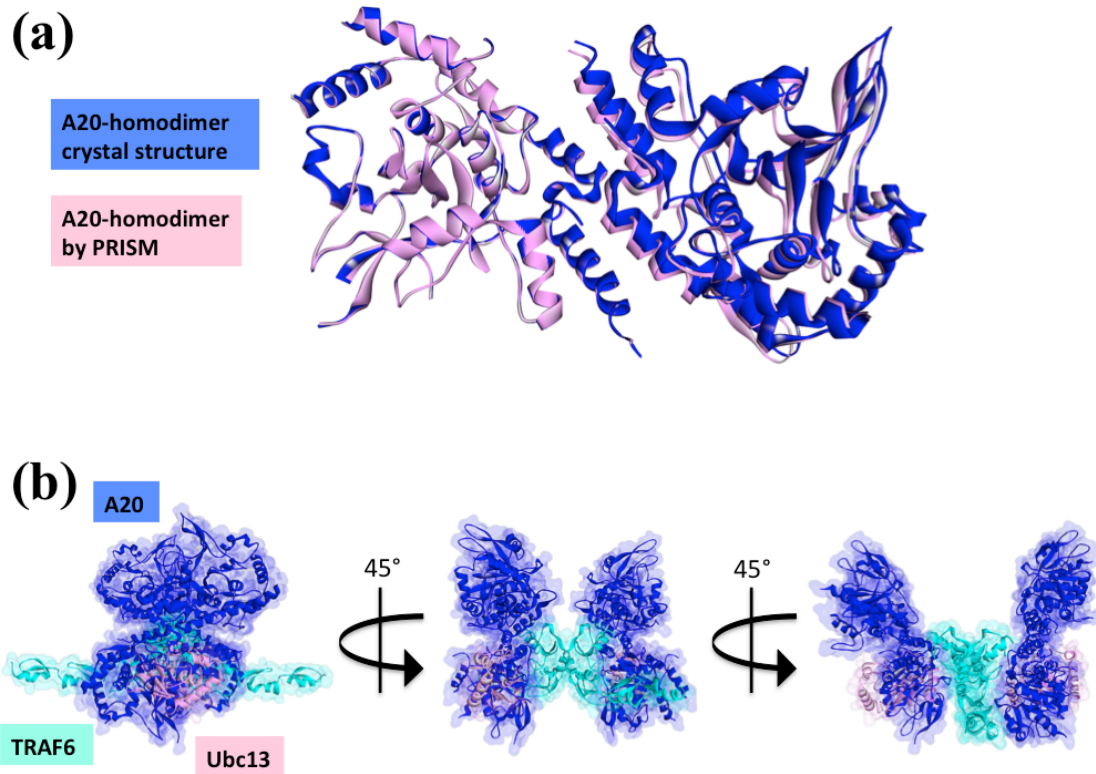


Figure S2: A20 needs to dimerize in order to execute its function. **(a)** We obtained A20-dimer structure that is very similar to the ones that are observed in asymmetric units of two pdb entries (3dkb_CF and 2vfj_BC), which were suggested to be biological. **(b)** Structure of TRAF6 interaction complex with A20-homodimer. This interaction interferes with TRAF6-Ubc13 interaction.

Table S3: Nonsense or frame-shift mutations on negative regulators. Although these mutations do not fall onto the interface residues, they lead to loss of the whole domain of the proteins that are covered in the PPI complexes modeled in this study.

Cancer Study	Mutation	Mutation Type	Protein	Disrupted PPI
Stomach (TCGA pub)	G9*	Nonsense	BCAP	BCAP-TLR4, BCAP-Mal, BCAP-MyD88, BCAP-TRAM, BCAP-TRIF
Prostate (Broad/Cornell 2012)	P2fs	FS del	SIGIRR	SIGIRR-TLR4, SIGIRR-Mal, SIGIRR-MyD88, SIGIRR-TRAM, SIGIRR-TRIF
Bladder (TCGA)	Q111*	Nonsense	SIGIRR	SIGIRR-TLR4, SIGIRR-Mal, SIGIRR-MyD88, SIGIRR-TRAM, SIGIRR-TRIF
Esophagus (Broad)	E30*	Nonsense	ST2	ST2-TLR4, ST2-Mal, ST2-MyD88, ST2-TRAM, ST2-TRIF
Lung adeno (TCGA pub)	Y50fs	FS del	ST2	ST2-TLR4, ST2-Mal, ST2-MyD88, ST2-TRAM, ST2-TRIF
Lung adeno (TCGA pub)	S125*	Nonsense	ST2	ST2-TLR4, ST2-Mal, ST2-MyD88, ST2-TRAM, ST2-TRIF
Stomach (TCGA pub)	G228fs	FS ins	ST2	ST2-TLR4, ST2-Mal, ST2-MyD88, ST2-TRAM, ST2-TRIF
NCI-60	A248fs	FS del	ST2	ST2-TLR4, ST2-Mal, ST2-MyD88, ST2-TRAM, ST2-TRIF
chRCC (TCGA)	M285fs	FS ins	ST2	ST2-TLR4, ST2-Mal, ST2-MyD88, ST2-TRAM, ST2-TRIF
Stomach (Pfizer UHK)	L350fs	FS del	ST2	ST2-TLR4, ST2-Mal, ST2-MyD88, ST2-TRAM, ST2-TRIF
Melanoma (Broad)	W361*	Nonsense	ST2	ST2-TLR4, ST2-Mal, ST2-MyD88, ST2-TRAM, ST2-TRIF
Colorectal (TCGA pub)	G47*	Nonsense	CYLD	CYLD-p62, CYLD-TRAF6
Colorectal (TCGA)	G47*	Nonsense	CYLD	CYLD-p62
CCELE	S159fs	FS del	CYLD	CYLD-p62
Head & neck (TCGA)	G179fs	FS del	CYLD	CYLD-p62
Head & neck (TCGA pub)	G179fs	FS del	CYLD	CYLD-p62
NCI-60	E245*	Nonsense	CYLD	CYLD-p62

REFERENCES

1. Roy, A., A. Kucukural, and Y. Zhang. 2010. I-TASSER: a unified platform for automated protein structure and function prediction. *Nature protocols* 5:725-738.