Electronic Supplementary Material (ESI) for Molecular BioSystems.

Electronic Supplementary Information

Structural and functional insights into CARDs of zebrafish (*Danio rerio*) NOD1 and NOD2, and their interaction with adaptor protein RIP2

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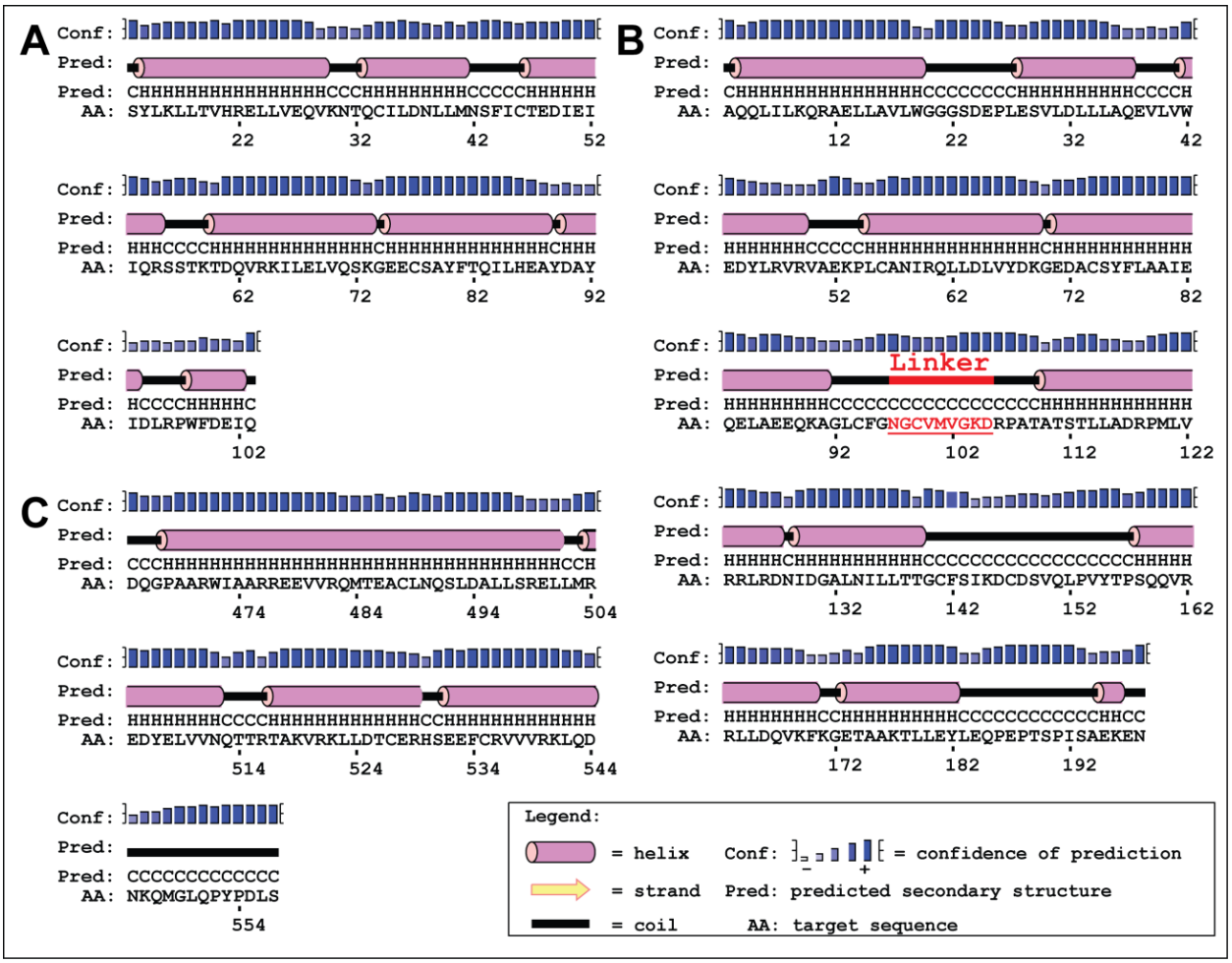

Fig. S1

Predicted secondary structure of zNOD1, zNOD2 and RIP2-CARD domains by PSIPRED; the secondary structure components were depicted in legend box.

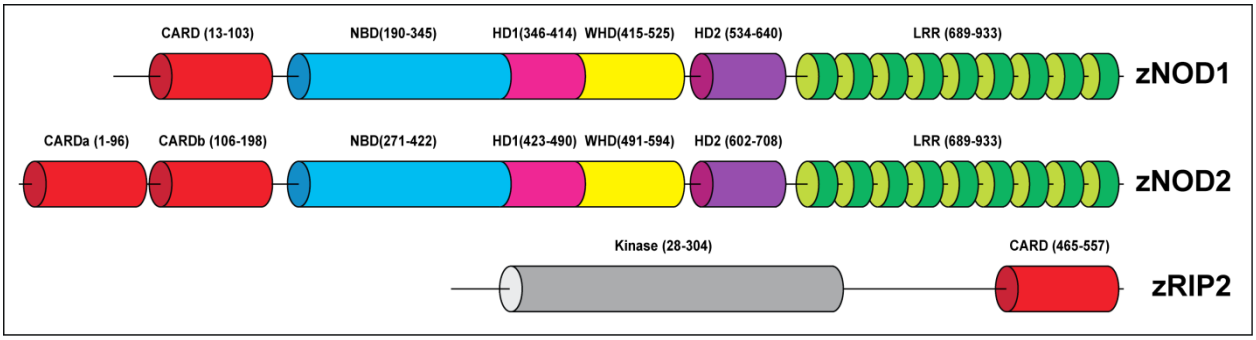

Fig S2

Figure illustrates domain organization of zNOD1, zNOD2 and zRIP2 protein. Different colors indicate different domains and sub-domains.

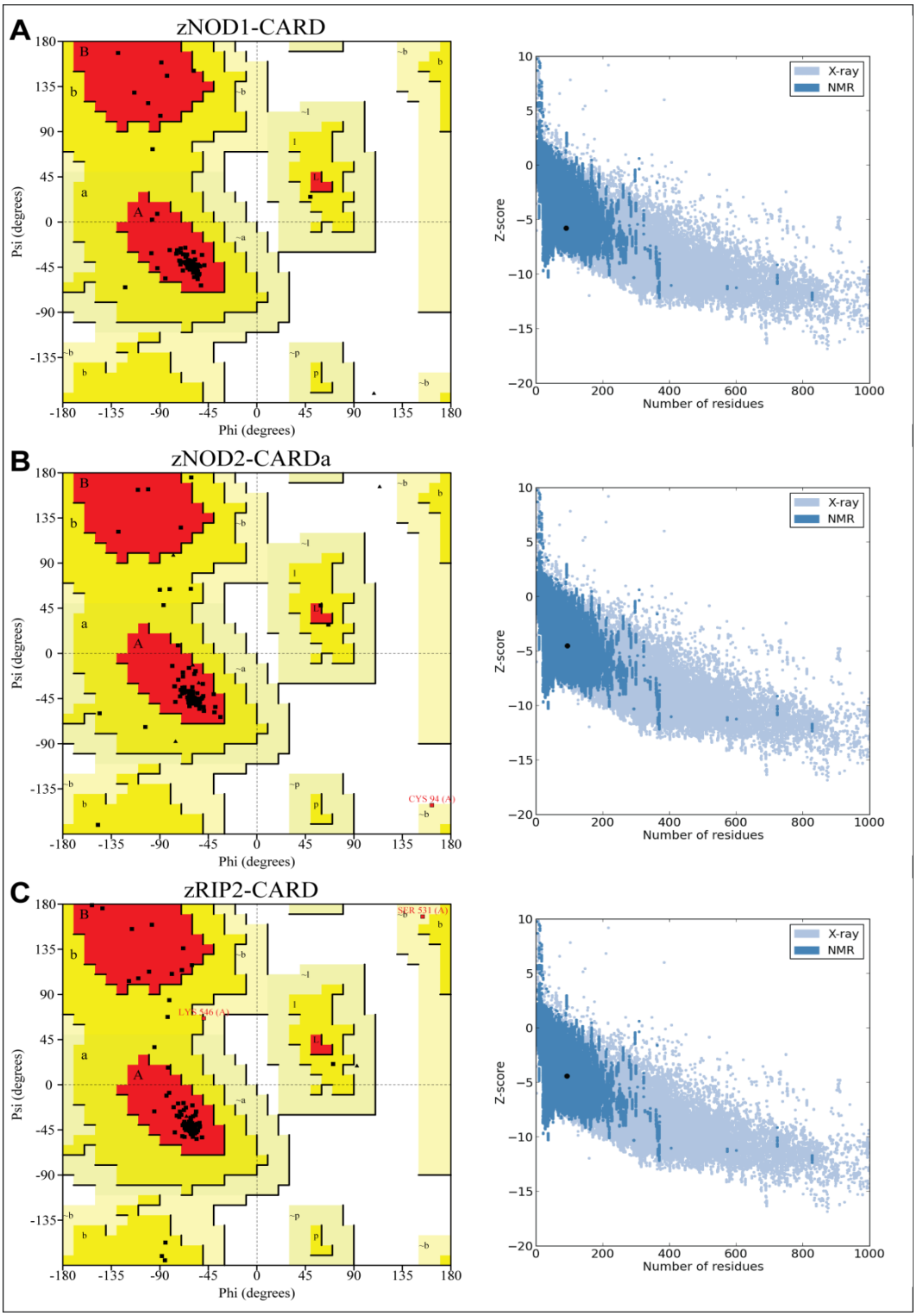

Fig. S3

Model validation reports of zNOD1-CARD (a), zNOD2-CARDa (b) and zRIP2-CARD (c) models by POCHECK-Ramachandran plot and ProSA web.

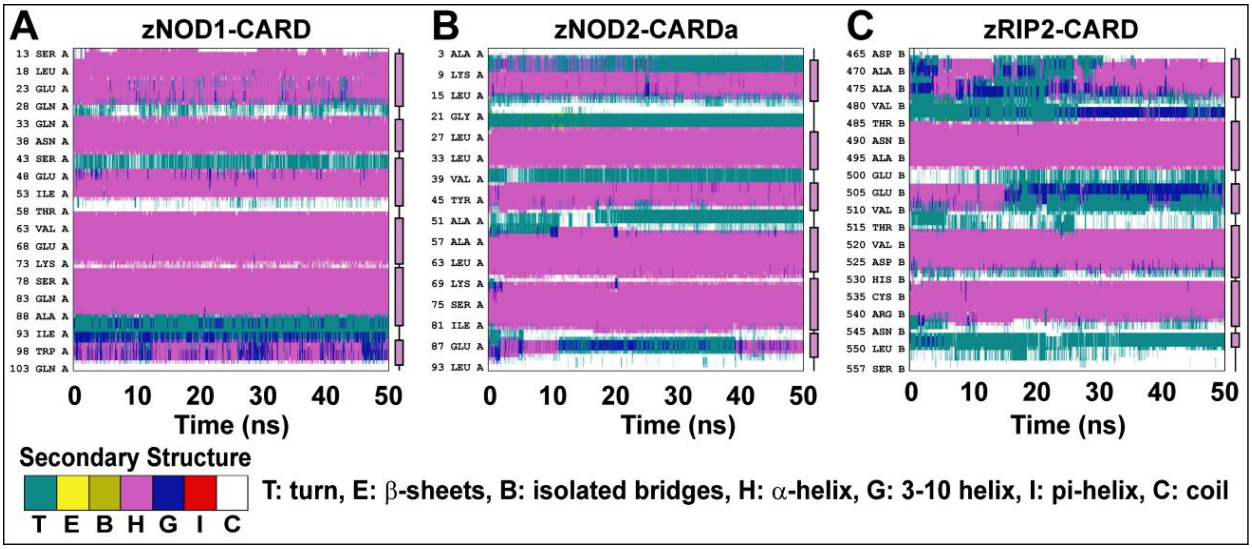

Fig. S4

Secondary structures assessment of three different CARD models (a) zNOD1-CARD, (b) zNOD2-CARDa and (c) zRIP2-CARD as a function of simulation time. Color segments given at the bottom of the figure represents the different secondary structural properties and helical contents of initial models were represented nearby.

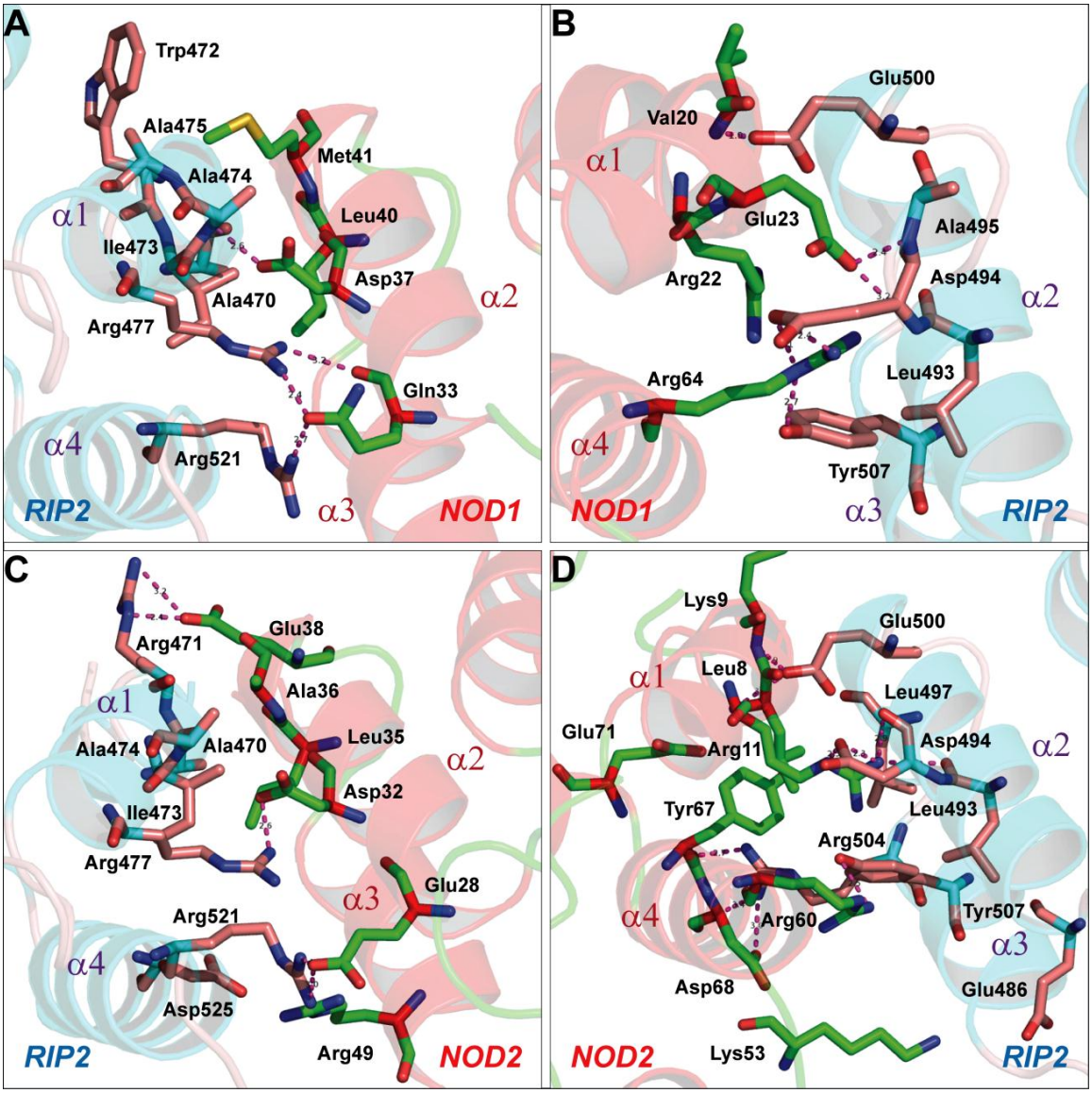

Fig. S5

The figure represents the residual interaction of NOD and RIP2 CARD-CARD complexes. zNOD1-RIP2 Complex-I (a), Complex-II (b), zNOD2-RIP2 Complex I (c) and Complex II (d).

Table S1

Atomic compositions and properties of different simulation systems

Simulation system	**Distance between protein surface and box edge (Å)**	**Number of water molecule**	**Number of Na^+**	**Number of Cl^-**	**Total number of atoms**
zNOD1-CARD (96 aa)	10	4504	20	14	15055
zNOD2-CARDa (94 aa)	10	7374	31	22	23659
zRIP2-CARD (93 aa)	10	14207	41	41	44228
zNOD1-CARD (96 aa) + zRIP2-CARD (93 aa)					
Complex I	10	17208	56	50	54764
Complex II	10	22932	71	65	71966
zNOD2-CARDa (94 aa) + zRIP2-CARD (93 aa)					
Complex I	11	16235	56	47	51817
Complex II	10	23431	75	66	73443

Table S2

Threading of zNOD2-CARDa, CARDb and zRIP2-CARD

Program	zNOD2-CARDa			zRIP2-CARD		
	Template	**% of identity**	**Score**	**Template**	**% of identity**	**Score**
Coma	1dgn	11	2.1e-12	1dgn	25	1.1e-10
	2b1w	13	1.1e-08	2b1w	25	1.8e-08
	2kn6	13	1.4e-08	2kn6	21	2.9e-08
Prc	1dgn	28	2.4e-10	1dgn	20	1.5e-18
	3ygs	16	0.00022	1cy5	15	1.5e-12
	2p1h	20	0.00038	3ygs	24	9e-10
Compass	1dgn	30	1.76e-34	2dbd	28	2.57e-36
	2dbd	28	1.08e-31	1dgn	22	1.53e-33
	3ygs	21	2.17e-21	3crd	19	9.49e-24
ffas	1dgn	29	-34.8	1dgn	21	-33.6
	2nz7	25	-30	2nsn	26	-30.5
	2nsn	25	-29.7	2nz7	26	-30.4
hhsearch	1dgn	32	99.1	4e9m	26	99.8
	4e9m	26	99.0	2nz7	26	99.8
	2nz7	25	98.7	1dgn	22	99.7
Phyre	2b1w	28	0.077	2b1w	26	5.1e-10
	2dbd	27	0.12	1dgn	23	1.7e-09
	1dgn	32	0.51	2dbd	27	6.7e-09
Sparks-x	1dgn	27	8.94	2nz7	25	9.88
	2nz7	22	7.38	1dgn	24	8.73
	3kat	19	6.76	1z6t	14	7.68
Modlink+	1dgn	30	92.8	1dgn	22	89.3
	3crd	23	58.5	3gys	24	46.1
	1cy5	17	52.4	1cy5	16	42.7
mGenthreader	1dgn	31	0.006	2nz7	26	0.0008
	2dbd	26	0.008	1dgn	25	0.0008
	2nz7	27	0.008	2dbd	26	0.0008
pDomThreader	1dgn	-	6.090	1dgn	-	7.661
	3ygs	-	4.248	1cy5	-	5.403
	1cy5	-	3.964	3ygs	-	3.178

Template characteristics:

1dgn A: ICEBERG (*Homo sapiens*);

1cy5 A, 2kn6 A, 3ygs A, 2p1h A: APAF1 CARD (*Homo sapiens*);

2nz7 A, 2dbd A, 2nsn A, 2b1w A, 4e9m A: NOD1 CARD (*Homo sapiens*);

3crd A: RAIDD CRAD (*Homo sapiens*);

3kat A: NLRP1 CARD (*Homo sapiens*).

Table S3

Interaction analysis (H-bond and hydrophobic contacts) of both the CARD-CARD complexes of (a) zNOD1-RIP2 and (b) zNOD2-RIP2 before molecular dynamics

Interaction type	**Complex I**			**Complex II**		
(a)	**zNOD1-CARD**	**zRIP2-CARD**	**length (Å)**	**zNOD1-CARD**	**zRIP2-CARD**	**length (Å)**
Hydrogen bond	Asp37:OD1	Arg477:HH11	2.71	Arg64:NH1	Asp494:OD1	2.64
	Asp37:OD2	Arg477:NH2	3.45	Arg64:NH2	Leu493:O	2.79
	Gln33:O	Arg477:NH1	3.09	Arg64:NH2	Asp494:O	3.20
	Gln33:OE1	Arg477:NH2	2.77	Glu68:OE1	Tyr507:OH	2.61
	Gln33:OE1	Arg521:NH1	2.73	Gln71:O	Arg504:NH1	2.80
	Asp37:OD1	Arg477:CD	3.16	Val20:O	Ser498:CA	3.41
Hydrophobic	Leu40	Ile473	5.33	Arg22	Leu497	5.41
	Leu40	Ala470	4.20			
	Met41	Ala474	4.05			
(b)	**zNOD2-CARD/a**	**zRIP2-CARD**	**length (Å)**	**zNOD2-CARD/a**	**zRIP2-CARD**	**length (Å)**
Hydrogen bond	Glu28:OE1	Arg521:NH2	2.72	Arg11:NH2	Asp494:OD1	2.82
	Glu28:OE2	Arg477:NH2	3.31	Lys53:NZ	Glu486:OE2	3.40
	Glu28:OE2	Arg521:NH1	2.71	Asp68:OD1	Arg504:NH2	2.82
	Glu38:OE2	Arg471:NH2	2.78	Arg11:NE	Asp494:OD1	2.74
	Arg49:NE	Asp525:OD2	3.31	Arg11:NH1	Tyr507:OH	2.89
	Asp32:OD1	Arg477:CD	3.14	Arg11:NH2	Leu493:O	2.78
				Arg11:NH2	Asp494:O	2.74
				Tyr67:O	Arg504:NH2	2.74
				Asp68:O	Arg504:NH1	2.75
				Leu8:CA	Glu500:OE2	3.38
				Lys9:CA	Ser498:O	3.64
				Asp68:OD1	Arg504:CD	3.31
Hydrophobic	Leu35	Ile473	4.69	Leu8	Leu493	4.45
	Ala36	Ala474	4.01			
	Leu35	Ala470	4.02			