Supplementary Information

for

Crystal Structure of the F27G AIM2 PYD Mutant and Similarities of its Self-association to DED/DED Interactions

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This document contains the following supplementary information:

Supplementary Table 1

Supplementary Table 2

Supplementary References

Supplementary Table 1. Crystallographic statistics

Diffraction data	
Space group	$P2_12_12_1$
Cell dimensions	
a, *b*, *c* (Å)	29.94, 37.95, 76.95
Resolution (Å)	38 - 1.82
R_{sym} *	0.057 (0.679)
I / σ_I *	24.9 (7.4)
Completeness (%) *	90.6 (50.81)
Redundancy *	10.6 (9.2)
Refinement	
Resolution (Å)	38 - 1.82
No. reflections	7,497
R_{work} / R_{free}	0.18 / 0.24
No. atoms	829
Protein	738
Water	91
Average B factor (Å^2)	19.6
Protein (Å^2)	18.7
Water (Å^2)	26.8
R.M.S. Deviations	
Bond lengths (Å) / angles (°)	0.006 / 0.84
Ramachandran plot	
Favored (%)	99
Allowed (%)	1

For crystallization, the SUMO-AIM2^{PYD} F27G fusion protein was incubated overnight with Ulp1 at a molar ratio of 1/1000 of the fusion protein while dialyzing against the gel filtration buffer at 4°C. The free His-SUMO was removed by another incubating the sample with Ni-NTA resin. The flow-through was collected and concentrated to load on a Superdex200 10/300 GL column. Fractions corresponding to the cleaved AIM2^{PYD} F27G were pooled and concentrated to 18mg/mL for crystallization. AIM2^{PYD} F27G was crystallized by hanging-drop vapor diffusion in a 48-well VDX tray (Hampton) at 16°C by mixing equal volumes of the protein sample and a mother liquor containing 0.2 M $CaCl_2$, 0.1 M Sodium Acetate at pH 4.7, and 23 % PEG3350. During optimization, trypsin was added to a mass ratio of 1/50 of AIM2^{PYD} F27G for *in situ* proteolysis. The best diffracting crystals were shaped like rigid, single needles with a long dimension of > 150 µm and short dimensions of < 5 µm. X-ray diffraction data were collected at the NSLS X29 beamline of Brookhaven National Laboratory and processed with the HKL2000 program suite [1]. Because of the low sequence identity of AIM2^{PYD} to any known PYD structures, we generated a structural composite by superimposing PDB models of NLRP10 (2DO9), ASC (1UCP), NLRP7 (2KM6), NLRP3 (3QF2) and NLRP1 (1PN5), and selecting common secondary structural elements with high sequence similarity and low root-mean-square deviations. We trimmed the composite to a poly-Ala model and searched for the molecular replacement solution in the program MOLREP [2]. Model building and refinement at 1.87 Å was carried out using Coot [3] and PHENIX [4]

*Values in parentheses are for the highest-resolution shell.

Supplementary Table 2. Top 20 hits from structural homology search using DALI [5]

DALI Hits	Protein domains	PDB IDs	Z-scores
1	MBP-hAIM2 PYD	3VD8	17.8
2	hASC PYD	1UCP	13.5
3	hNLRP3 PYD	3QF2	13.5
4	hASC2 PYD	2HM2	13.2
5	hASC PYD	2KN6	12.8
6	hNLRP4 PYD	4EWI	11.9
7	hMNDA PYD	2DBQ	11.8
8	hNLRP7 PYD	2KM6	11.8
9	vFLIP MC159 DED2	2F1S	11.3
10	mNLRP10 PYD	2DO9	11.2
11	hNLRP12 PYD	2L6A	10.8
12	mPEA15 DED	2LS7	10.8
13	chPEA-15 DED	4IZ7	10.6
14	vFLIP MC159 DED2	2BBZ	10.3
15	hFADD DD	3EZQ	10.1
16	mP205 PYD	2YU0	9.2
17	cCED4 CARD	3LQQ	9.0
18	cCED9 CARD	2A5V	8.9
19	hApaf-1 CARD	3YGS	8.5
20	hTNFR1 DD	1ICH	8.5

h: human; m: mouse; c: C. elegans; ch: Chinese hamster; v: viral

Supplementary References

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