Supplementary information for

Structural basis for the oligomerization-mediated regulation of NLRP3

inflammasome activation

Authors

Umeharu Ohto^{1,2*}, Yukie Kamitsukasa^{1,2}, Hanako Ishida^{1,2}, Zhikuan Zhang^{1,2}, Karin Murakami¹, Chie Hirama¹, Sakiko Maekawa¹, and Toshiyuki Shimizu^{1*}

Affiliations

1. Graduate School of Pharmaceutical Sciences, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan

2. These authors contributed equally to this work

*Corresponding author:

Umeharu Ohto (U.O.) E-mail: umeji@mol.f.u-tokyo.ac.jp

Toshiyuki Shimizu (T.S.) E-mail: shimizu@mol.f.u-tokyo.ac.jp

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	PYD Linker region	
NLRP3_Human NLRP3_Mouse NLRP3_Bovine NLRP3_Galago NLRP3_Rabbit	CONTRICT NOT CONTRACT. CONTRICT NOT CONTRICT NOT CONTRICT NOT CONTRICT NOT CONTRICT NOT CONTRACT NOT C	119 115 114 117 115
1 NLRP3_Human NLRP3_Mouse NLRP3_Bovine NLRP3_Galago NLRP3_Rabbit	120 130 O 210 220 230 C LEYLSRISICKMKKDYRKKYRKYVRSRFQCIEDRNARLGESVSLNKRYTRLRLIKEHRSQQEREQELLAIGK—TKTCESPVSPIKMELLFDPDDEHSEPVHTVVFQGAAGIGKTILAR LLGYLSRISICKKKKDYCKKYRKYVRSKFQCIKDRNARLGESVMLNKRYTRLRLIKEHRSQQEREQELLAIGRTWAKIODSPVSSVMLELLFDPEDQHSEPVHTVVFQGAAGIGKTILAR LLGYLSRISICKKKKDYCKKYRKYVRSKFQCIKDRNARLGESVMLNKRFTRLRLIKEHRSQQEREQELVAIGR—TKIMDKPMSPIKMELLFDPEDQHSEPVHTVVFQGAAGIGKTILAR LLGYLSRISICKKKKDYCKKYRKYVRSRFQCIEDRNARLGESVMLNKRFTRLRLIKEHRSQQEREQELVAIGR—TKIMDKPMSPIKMELLFDPEDQHSEPVHTVVFQGAAGIGKTILAR LLGYLSICKKKKDYCKKYRKYVRSRFQCIEDRNARLGESVMLNKRFTRLRLIKEHRSQQEREQELVAIGR—TKIMDKPMSPIKMELLFDPEDQHSEPVHTVVFQGAAGIGKTILAR LLGYLSICKKKKDYCKKYRKYVRSRFQCIEDRNARLGESVNLNKRYTRLRLAKEHLSRQEREQELVAIGR—TKIMDKPMSPIKMELLFDPEDQHSEPVHTVVFQGAAGIGKTILAR Walker A	237 233 234 235 233
NLRP3_Human NLRP3_Mouse NLRP3_Bovine NLRP3_Galago NLRP3_Rabbit	240 250 260 270 280 290 300 310 320 330 340 350 KMMLDWASGTLYQDRFDYLFYIHCREVSLVTORSLGDLIMSCCPDPNPPIHKIVRKPSRILFLMDGFDELQGAFDEHIGPLCTDWQKAERGDILLSSLIRKKLLPEASLLITTRPVALEK KIMLDWALGKLFKDKFDYLFFIHCREVSLGTORSLGDLIASCCPGPNPPIGKIVSKPSRILFLMDGFDELQGAFDEHIGEVCTDWQKAVRGDILLSSLIRKKLLPEASLLITTRPVALEK KIMLDWASGKLYQDKFDYLFYIHCREVSLGTORSLGDLIASCCPGPNPPIGKIVSKPSRILFLMDGFDELQGAFDEHTEALCTNWRKVERGDILLSSLIRKKLLPEASLLITTRPVALEK KIMLDWASGKLYQDKFDYLFYIHCREVSLVTERSLRDLMVSCCPDPNPPIGKIVSKPSRILFLMDGFDELQGAFDEHTEALCTNWRKVERGDILLSSLIRKKLLPEASLLITTRPVALEK KIMLDWASGKLYQDKFDYLFYIHCREVSLVTERSLRDLMVSCCPDPNPPIGKIVSRPSRILFLMDGFDELQGAFDEHTGALCTDWQKAERGDILLSSLIRKKLLPEASLLITTRPVALEK KIMLDWASGQLFQDRFDYLFYIHCREMSLGMHRSLVDLIAGCCSDPSPPVSKMLHRPSRVIFLMDGFDELQGAFDESGALCSDWRVAVRGDVLLGSLIRKKLLPEASLLITTRPVALEK KIMLDWASGQLFQDRFDYLFYIHCREMSLGMHRSLVDLIAGCCSDPSPPVSKMLHRPSRVIFLMDGFDELQGAFDESGALCSDWRVAVRGDVLLGSLIRKKLLPEASLLITTRPVALEK KIMLDWASGQLFQDRFDYLFYIHCREMSLGMHRSLVDLIAGCCSDPSPVSKMLHRPSRVIFLMDGFDELQGAFDESGALCSDWRVAVRGDVLLGSLIRKKLLPEASLLITTRPVALEK KIMLDWASGQLFQDRFDYLFYIHCREMSLGMHRSLVDLIAGCCSDPSPVSKMLHRPSRVIFLMDGFDELGGAFDESGALCSDWRVAVRGDVLLGSLIRKKLLPEASLLITTRPVALEK KIMLDWASGQLFQDRFDYLFYIHCREMSLGMHRSLVDLIAGCCSDPSPVSKMLHRPSRVIFLMGFDELGGAFDESGALCSDWRVAVRGDVLLGSLIRKKLLPEASLLITTRPVALEK KIMLDWASGQLFQDRFDYLFYIHCREMSLGMHRSLVDLIAGCCSDPSPVSKMLHRPSRVIFLMGFDELGGAFDESGALCSDWRVAVRGDVLLGSLIRKKLLPEASLLITTRPVALEK KIMLDWASGULFQDRFDYLFYIHCREMSLGMHRSLVDLIAGCCSDPSPVSKMLHRPSRVIFLMGFDELGGAFDESGALCSDWRVAVRGDVLLGSLIRKKLLPEASLLITTRPVALEK KIMLDWASGULFQDRFDYLFYIHCREMSLGMHRSLVDLIAGCSDPSPVSKMLHRPSRVIFLMGFDELGGAFDESGALCSDWRVAVRGDVLLGSLIRKKLLPEASLLITTRPVALEK KIMLDWASGULFQDRFDYLFYIHCREMSLGMHRSLVDLIAGCSDPSPVSKMLHRPSRVIFLGAGFDENGAFDENGT	357 353 354 355 353
NLRP3_Human NLRP3_Mouse NLRP3_Bovine NLRP3_Galago NLRP3_Rabbit	360 370 380 400 410 420 430 440 450 460 470 LOHLLDHPRHVEILGESEAKRKEYFFKYSDEAQARAAFSLIQENEVLFTNCFIPLVCHIVCTGLKQQMESGKSLAQT-SKTTAVYVFFLSSLLQRGGSQEGHGLCAHLWGLCSLAAD LOHLLGARHVEILGESEAKRKEYFFKYSDELQAREAFRLIQENEVLFTNCFIPLVCHIVCTGLKQQMESGKSLAQT-SKTTAVYVFFLSSLLQSGGGSQEHNSATLWGLCSLAAD LOHLLGARHVEILGESEARRKEYFFKYSDEQQAREAFRLIQENEVLFTNCFIPLVCHIVCTGLKQQMESGKSLAQT-SKTTAVYTFFLSSLLQSGGSQEHNSATLWGLCSLAAD LOHLLGARHVEILGESEARRKEYFFKYSDETQAREAFRLIQENEVLFTNCFIPLVCHIVCTGLKQQMESGKSLAQT-SKTTTAVYTFFLSSLLQSGGSQEHNSATLWGLCSLAAD LOHLLGARHVEVLGESEARRKEYFFKYFSDETQAREAFRLIQENEVLFTNCFIPLVCHIVCTGLKQQMESGKSLAQT-SKTTTAVYTFFLSSLLQSGGSQEHNSATLWGLCSLAAD LOHLLDRPRHVEVLGFSEARRKEYFFKYFLEEAQAREAFSLLQENEVLLSNCCIPLVCHIVCTGLKQQMQGGKHRAKTTTSKTTAVYTFFLSSLLQSRGGSQEORLSGHLRGLCSLAAD PhhCW + HD2	475 471 472 473 473
NLRP3_Human NLRP3_Mouse NLRP3_Bovine NLRP3_Galago NLRP3_Rabbit	480 490 500 510 520 530 540 550 560 570 580 G1WNQK1LFEESDLRNHGLQKADVSAFLRMNLFQKEVDCEKFYSF1HMTFQEFFAAMYYLLEEEKEGRTNVPGSRLKLPSRDVTVLLENYGKFEKGYL1FVVRFLFGLVNQ G1WNQK1LFEECDLRKHGLQKADVSAFLRMNLFQKEVDCEKFYSF1HMTFQEFFAAMYYLLEEEAEGETVRKGPGGCSDLLNRDVKVLLENYGKFEKGYL1FVVRFLFGLINQ G1WNQK1LFQECDLRNHGLQKADVSAFLRMNLFQKEVDCEKFYSF1HMTFQEFFAAMYYLLEEDNHGEMRNVTGRSKLPNRDVKVLLENYGKFEKGYL1FVVRFLFGLINQ G1WNQK1LFDESDLRKHGLQKVDVSSFLRMNLFQKEVDCEKFYSF1HMTFQEFFAAMYYLLEEEEVEEEERMRNVLGRSSKLPNRDVKVLLENYGKFEKGYL1FVVRFLFGLVNQ G1WNQK1LFDESDLRKHGLQKADVSAFLRMNLFQKEVDCEKFYSF1HMTFQEFFAAMYYLLEEEEVEEEERMRNVLGRSSKLPNRDVKVLLENYGKFEKGYL1FVVRFLFGLVNQ G1WNQK1LFEERDLRRHGLQEAEVSAFLRMSLFQKEVDCEAFYSFVHLTFQEFFAAMYYLEEEEEKEEEEGAGTGRSPPARGLDLPHRDVRVLLENYGKFEKGYL1FVVRFLFGLVNQ	586 584 584 588 593
NLRP3_Human NLRP3_Mouse NLRP3_Bovine NLRP3_Galago NLRP3_Rabbit	590 600 610 620 630 640 650 660 670 680 700 ERTSYLEKKLSCK ISQ0 IRLELLKWIEVKAKAKKL0 IOPSOLELFYCLYEMQEEDFVQRAMDYFPK IE INLSTRMDHWSSFC IENCHRVESLSLGFLHNMPKEEEEEKEGRHLDM ERTSYLEKKLSCK ISQ0VRLELLKWIEVKAKAKKL0 IOPSOLELFYCLYEMQEEDFVQRAMDYFPK IE INLSTRMDHVSSFC IENCHRVKTLSLGFHNSPKEEEEERRGGRHLDM ERTSYLEKKLSCK ISQ0 IRLELLKWIEVKAKAKKL0 IOPSOLELFYCLYEMQEEDFVQRAMSHFPK IE INLSTRMDHVSSFC IENCHRVKTLSLGFHNSPKEEEEERRGVRHSHM ERTSYLEKKLSCK ISQ0 IRLELLKWIEVKAKAKKL0 IOPSOLELFYCLYEMQEEDFVQRAMSHFPK IE INLSTRMDHVSSFC IANCHSVESLSLGFLHNTPKEEEEEEEVRHSHM ERTSYLEKKLSCK ISQ0 IRLELLKWIEVKAKAKKL0 IOPSOLELFYCLYEMQEEDFVQRAMSHFPK IE INLSTRMDHVSSFC IANCHSVESLSLGFLHNTPKEEEEEEEGRHLDM ERASYLEKKLSCK ISQ0 IRLELLKWIEVKAKAKKL0 IOPSOLELFYCLYEMQEEDFVQRAMSHFPK IE INLSTRMDHVSSFC IANCHSVESLSLGFLHNTPKEEEEEEEGRHLDM ERASYLEKKLSCK ISQ0 IRLELLKWIEVKAKAKKL0 IOPSOLELFYCLYEMQEEAFVREAMGHFPR IE INLSTRMDHVSSFC IANCHSVESLSLGFLHNTPKEEEEEEEEEEEGRHLDM	703 701 700 705 713
NLRP3_Human NLRP3_Mouse NLRP3_Bovine NLRP3_Galago NLRP3_Rabbit	710 720 740 750 760 770 780 790 800 810 820 VQCVLPSSSHAACSHGLVNSHL TSSFCRGLFSVLSTSQSLTELDLSDNSLGDPGMRVLCETLQHPGCNIRRLWLGRCGLSHGCCFDISLVLSSNQKLVELDLSDNALGDFGIRLLCVGLK VQCVFPD-THVACSSRLVNOCLTSSFCRGLFSSLSTNRSLTELDLSDNTLGDPGMRVLCEALQHPGCNIQRLWLGRCGLSHQCCFDISSVLSSQKLVELDLSDNALGDFGIRLLCVGLK DRSVLSD-FEVAYSQGLVN-YLTSSICRGIFSVLSTNQSLTELDLSDNTLGDPGMRVLCETLQOPGCNIRRLWLGRCGLSHQCCFDISSVLSSQKLVELDLSDNALGDFGIRLLCVGLK VQRVLPG-PHAACFHRLVNCYLTSSFCRGLFSVLSTNQSLTELDLSDNTLGDSGMKVLCEALQHPSCNIQRLWLGRCGLSHQCCFDISVLSSQKLVELDLSDNALGDFGIRLLCVGLK VGCPNRQGPHAACSWRLV <mark>NSCLTSS</mark> FCRGLFSVLSTNQSLTELDLSDNSLGDPGMRVLCEALQHPGCNIRRLWLGRCGLSHQCCFDISLVLSSQKLVELDLSDNALGDFGIRLLCVGLK	823 820 818 824 833
NLRP3_Human NLRP3_Mouse NLRP3_Bovine NLRP3_Galago NLRP3_Rabbit	830 840 850 860 870 880 890 900 910 920 930 940 HLLCNLKKLWLVSCCLTSACCODLASVLSTSHSLTRLYVGENALGDSGVAILCEKAKNPOCNLOKLGLVNSGLTSVCCSALSSVLSTNONLTHLYLRGNTLGDKGIKLLCEGLLHPDCKL HLLCNLOKLWLVSCCLTSACCODLALVLSSNHSLTRLYIGENALGDSGV0VLCEKMKDPOCNLOKLGLVNSGLTSICCSALTSVLKTNONFTHLYLRGNALGDTGLRLLCEGLLHPDCKL HLFCNLKKLWLVSCCLTSASCEDLASVLSTNHSLTRLYIGENALGDSGV0ILCEKVKNPHCNLOKLGLVNSGLTSGCCPALSSVLSTNONLTHLYLGONALGDMGVKLLCEGLLHPNCKL HLFCNLKKLWLVSCCLTSACCODLASVLSTNHSLTRLYIGENTLGDSGV0ILCEKVKNPHCNLOKLGLVNSGLSSVCCTALSSVLSTNONLTHLYLGGNALGDMGVKLLCEGLLHPNCKL HLFCNLKKLWLVSCCLTSMCCODLASVLSPSRSLTRLYVGENTLGDSGV0ILCEKVKNPHCNLOKLGLVNSGLSSVCCTALSSVLSTNONLTHLYLGGNALGDMGVKLLCEGLLHPNCKL HLFCNLKKLWLVSCCLTSMCCODLASVLSTNHCLTRLYIGENTLGDYGVGLLCEKVKHPOCNLOKLGLVNSGLTSACCAALSAALSTSRSLTHLYLGGNALGDAGVKLLCEGLLHPSCRL	943 940 938 944 953
NLRP3_Human NLRP3_Mouse NLRP3_Bovine NLRP3_Galago NLRP3_Rabbit	950 960 970 ● 980 990 1000 1010 1020 1030 NEK7 interface 0VLELDNCNLTSHCCWDLSTLLTSSQSLRKLSLGNNDLGDLGVMMFCEVLKQQSCLLONLGLSEMYFNYETKSALETLQEEKPELTVVFEPSW 1036 0MLELDNCSLTSHCCWDLSTLLTSNQSLRKLCLGNNDLGDLCVVTLCEVLKQQGCLLQSLQLGEMYLNRETKRALETLQEEKPELTVVFEPSW 1031 0VLELDNCSLTSHCCWDLSTLLTSNQSLRKLCLGNNDLGDLGVMMLCEVLRQQSCLLQSLQLGEMYFNYETKRALETLQEEKPELTVVFEPSW 1031 0VLELENCSLTSHCCWDLSTLLTSNQRLRKLSLGNNDLGDLGVMMLCEVLRQQSCLLQSLQLCEMYFNYETKRALETLQEEKPELTVVFEPSW 1037 0MLELDSCSLTSHCCWNLSTILTSNQRLRKLSLGNNDLGDLGVMMLCEVLRQQSCLLQSLQLCEMYFNYETKRALETLREEKPALTVVFEPSW 1037 0MLELDSCSLTSHCCWNLSTILTSNQRLRKLSLGNNDLGDLGVMMLCEVLRQQSCLLQSLQLCEMYFNYETKRALETLREEKPALTVVFEPSW 1037 0MLELDSCSLTSHCCWNLSTILTCSRSLRKLSLGNNDLGDLGVMMLCEVLRQPDCPLQRLQLCEMYFNYETHALETLREEKPALTVVFEPSW 1046 ADP interface	face rface

Fig. S1. Sequence alignment of NLRP3

Sequence alignment of human, mouse, rabbit, and galago NLRP3 were calculated using Clustal Omega. The Walker A, Walker B, Sensor I, PhhCW motifs are indicated. The interface residues for ADP, MCC950, NEK7 (PDB 6NPY), the "face-to-face" interface, and the "back-to-back" interface are highlighted in yellow, magenta, orange, blue, and pink, respectively. The known phosphorylation and ubiquitination sites are shown in red and green, respectively.

Human NLRP3∆P hexamer



Fig. S2. Cryo-EM analysis of the NLRP3 oligomer

Data processing workflow of the human NLRP3AP hexamer (top) and the mouse NLRP3 dodecamer (bottom). Representative motion-corrected micrograph, 2D class averages, 3D class-averages, goldstandard FSC curves of the final 3D reconstruction (resolution cut-off at FSC = 0.143), and the final 3D map (colored according to the local resolution) are shown. 2D class averages were calculated using the refined particles that were used for the final reconstruction. 3D classes selected for the following analyses are indicated with red dotted boxes.

a Human NLRP3∆P hexamer

b





Fig. S3. Cryo-EM density maps of the NLRP3 oligomer

Representative cryo-EM density maps of the human NLRP3 Δ P hexamer (**a**) and the mouse NLRP3 dodecamer (**b**). The map levels are indicated in each panel.

a NLRP3ΔP (hexamer)



b



Fig. S4. Structural comparison of inactive form of NLRP3

a, Structural comparison between the protomer in the NLRP3∆P hexamer (this study) and NLRP3∆P-NEK7 complex (PDB 6NPY) ¹.

b, Structural comparison between the protomers in the human NLRP3∆P hexamer and in the mouse NLRP3 dodecamer.

c, Structural comparison of the "back-to-back" interfaces in the human NLRP3∆P hexamer and in the mouse NLRP3 dodecamer.



Mouse NLRP3 (full-length) (dodecamer)





The electrostatic surface potentials of the "head-to-tail" dimer and protomer in the human NLRP3∆P hexamer (top) and the "face-to-face" dimer and protomer in the mouse NLRP3 dodecamer (bottom) are shown. The residues in the polybasic region are highlighted in the right panels.



Fig. S6. Phosphorylation sites of NLRP3

Known phosphorylation sites of NLRP3 are mapped onto the protomer structure of human NLRP3 Δ P hexamer (**a**, **b**), NLRP3-NEK7 complex (PDB 6NPY) ¹ (**c**), and the hypothetical activated form of NLRP3 (**d**). The phosphorylation sites are indicated with red spheres. The "face-to-face" interface (**b**), "back-to-back" interface (**b**), NEK7-binding interface (**c**), and NACHT-NACHT interface between adjacent protomers in the hypothetical activated oligomer of NLRP3 (**d**) are shown in blue, pink, orange, and green semitransparent surfaces, respectively.



Fig. S7. NLRP3 dodecamer model containing PYD.

a, Cryo-EM map of the full-length mouse NLRP3 dodecamer (left) and surface representation of PYD monomer (PDB 2NAQ) (right). Each protomer is shown in a different color. The position of the N-terminus of each protomer in the current model (D131) is shown in the green sphere.

b, NLRP3 dodecamer model containing PYD. The PYDs (white surface representations) and the following linker regions (dashed lines) are not resolved in the cryo-EM map but tentatively positioned for the visualization purpose.

Table S1. Statistics for data collection and structural refinement

	Human NLRP3∆P	Mouse NLRP3		
Data	hexamer	dodecamer		
EMDB ID	EMD-32119	EMD-32120		
PDB ID	7VTP	7VTQ		
Data collection and processing				
Microscope / Voltage (kV)	Titan Krios G4, 300			
Detector	K3 (CDS mode)	K3 (CDS mode)		
Magnification	105,000			
Pixel size (Å)	0.83			
Total dose $(e^{-}/Å^2)$ / Frames per movie	68 / 64	68 / 64		
Total movie stacks	5,563	6,243		
Final particle images (no.)	105,139	73,930		
Symmetry imposed	D3	D6		
Map resolution (Å)	3.23	3.55		
FSC threshold	0.143	0.143		
Map sharpening B factor (Å ²)	-84.9	-138.3		
Refinement				
Software	COOT, Chimera, Phenix			
Model resolution (Å)	2 2 / 2 5	25/41		
FSC threshold=0.143 / 0.50	5.27 5.5	5.5 / 4.1		
Model composition				
Protein chains	6	12		
Residues	4,770	9,468		
Average B-factors (Å ²)	109.1	62.5		
R.m.s deviations	-			
Bond lengths (Å)	0.003	0.003		
Bond angles (°)	0.64	0.64		
Validation				
Molprobity score	1.73	2.03		
Clash score	11.46	13.91		
Poor rotamers (%)	0.09	0.05		
Ramachandran plot				
Favored (%)	97.1	94.4		
Allowed (%)	2.7	5.6		
Outliers (%)	0.2	0.0		