the homologies between NLRC4 oligomerization and prion replication may help to better understand their underlying mechanisms. It remains unknown whether the prion-like mechanism is conserved in other immune NLR sensors for oligomerization. However, one apparent advantage of this mechanism is that a single PAMP or a host-derived danger molecule is sufficient for inducing formation of a fibril assembly, which in principle contains endless ASC because of its self-perpetuation property (98) and thus generates an all-or-none response (99). The requirement of ligand for initiation of this activity could serve to minimize inadvertent activation of an NLR protein through intermolecular collisions.

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INNATE IMMUNITY
Cryo-EM structure of the activated NAIP2-NLRC4 inflammasome reveals nucleated polymerization
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The NLR family apoptosis inhibitory proteins (NAIPs) bind conserved bacterial ligands, such as the bacterial rod protein PrgJ, and recruit NLR family CARD-containing protein 4 (NLRC4) as the inflammasome adapter to activate innate immunity. We found that the PrgJ-NAIP2-NLRC4 inflammasome is assembled into multisubunit disk-like structures through a unidirectional adenosine triphosphatase polymerization, primed with a single PrgJ-activated NAIP2 per disk. Cryo-electron microscopy (cryo-EM) reconstruction at subnanometer resolution revealed a ~90° hinge rotation accompanying NLRC4 activation. Unlike in the related heptameric Apaf-1 apoptosome, in which each subunit needs to be conformationally activated by its ligand before assembly, a single PrgJ-activated NAIP2 initiates NLRC4 polymerization in a domino-like reaction to promote the disk assembly. These insights reveal the mechanism of signal amplification in NAIP-NLRC4 inflammasomes.

The nucleotide-binding domain (NBD) and leucine-rich repeat (LRR)-containing protein (NLR) family participates in the formation of inflammasomes that activate caspase-1 for cell death induction and cytokine maturation. NLR family apoptosis inhibitory proteins (NAIPs) are so far the only NLR family members with specifically defined ligands (1–4). NAIP2 detects the inner rod protein of the bacterial type III secretion system, including Salmonella typhimurium PrgJ, whereas NAIP5 and NAIP6 detect bacterial flagellin such as Salmonella typhimurium FliC. Thus, NAIP-NLRC4 inflammasomes perform effector functions against intracellular bacteria (7, 8), play protective roles in mouse models of colitis-associated colorectal cancer (9, 10), and serve as a potential strategy in tumor immunotherapy (11). Mutations in NLRC4 also induce auto-inflammatory diseases in humans (10, 12–14). We assembled the FliC-activated NAIP5-NLRC4 complex with the PrgJ-activated NAIP2-NLRC4 complex with the use of CARD-deleted NLRC4 (NLRC4ΔC) to avoid potential CARD-mediated aggregation (Fig. 1A). Either full-length NAIP2 or N-terminal baculovirus inhibitor of apoptosis protein repeat

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(BIR) domain–deleted NAIP2 (NAIP2ΔBIR) was used. During purification, sucrose gradient fractions showed variable molar ratios between NAIP2 and NLRC4Δ (Fig. 1B). The fraction with the highest amount of NLRC4Δ relative to NAIP2, by approximately one order of magnitude, contained mostly single, complete disk-like particles (Fig. 1C and fig. S1A). Similarly, overstoichiometry of NLRC4Δ to NAIP5 was observed in the reconstituted FliC-NAIIP5-NLRC4Δ complex (Fig. 1D), also with mostly complete disks under electron microscopy (fig. S1B). In contrast, our earlier PrgJ-NAIP2-NLRC4Δ preparations exhibited much lower NLRC4Δ/NAIP2 molar ratios (fig. S1C), with mostly incomplete disks (fig. S1D). Labeling of NAIP2 with 5-nm Ni-NTA gold particles in the PrgJ-NAIP2-BIR–His-NLRC4Δ inflammasome revealed similarly labeled complexes (Fig. 1E). These data, together with the previous report that NAIP5 did not oligomerize in the presence of flagellin (15), demonstrated that a single NAIP exists in each complex, whether in a full disk or a partially assembled disk (Fig. 1F).

We collected cryo–electron microscopy (cryo-EM) data on the PrgJ-NAIP2-NLRC4Δ complex (Fig. 1G and figs. S2 and S3A). Reference-free two-dimensional (2D) classification revealed mostly 11-bladed, but also 12- and 10-bladed inflammasome complexes (fig. S3B), implying conformational flexibility. From the top or bottom view, an inflammasome disk comprises an inner ring and an outer ring (Fig. 1H); 3D classification yielded models with apparent C14, C15, and C12 symmetries (fig. S2). The individual blades did not show observable differences to indicate the single PrgJ-NAIP2 complex in each disk, probably due to similar domain organizations of NAIP2 and NLRC4 (Fig. 1A). Upon imposing the apparent symmetry, the C15 and C12 reconstructions were refined to resolutions of 4.7 Å, 7.5 Å, and 12.5 Å, respectively (Fig. 2, A to F, and fig. S3, C to G). Local resolution estimation of the C15 reconstruction suggests that the inner ring possesses resolutions of 4.0 to 6.0 Å (Fig. 2A and fig. S4), with secondary structural features consistent with a resolution of at least 6.0 Å (Fig. 2, A to D, and fig. S5). Using the crystal structure of NLRC4Δ in the inactive conformation (PDB ID 4KXF) (16), we built and refined an atomic model of the active NLRC4Δ (table S1). All structures have a domed center and a prominent

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**Fig. 1. Preparation and characterization of NAIP-NLRC4Δ complexes.** (A) Domain organizations of *Salmonella typhimurium* PrgJ, mouse NAIP2, mouse NLRC4, and mouse caspase-1. Domain size is drawn approximately to scale; residue numbers are labeled. (B) SDS–polyacrylamide gel electrophoresis (PAGE) of different fractions of the sucrose gradient ultracentrifugation during the purification of the PrgJ-NAIP2-NLRC4Δ complex. Locations of the three component proteins are labeled. The asterisk indicates a contaminating band. (C) A representative negative-stain EM image from fraction 7 in (B). (D) SDS-PAGE of amylose resin elution (lane 1), anti-Flag flow-through (lane 2), and anti-Flag elution (lane 3) fractions during the purification of the coexpressed His-FiIC–Flag-NAIP5–His-MBP-NLRC4Δ complex. An enlarged image of lane 3 is shown. (E) Ni-NTA gold labeling (5 nm) of purified His-Sumo-PrgJ-NAIP2ΔBIR–His-Sumo-NLRC4Δ complex upon removal of the His-Sumo tag. (F) Schematic diagram of partial and complete inflammasome particles that contain variable ratios between NAIP2 (yellow) and NLRC4 (cyan). (G) Representative cryo-EM micrograph of PrgJ-NAIP2-NLRC4Δ particles. (H) An averaged 2D class of the 11-bladed PrgJ-NAIP2-NLRC4Δ inflammasome disk. The dimensions of the image are 43.5 nm × 43.5 nm.
inner hole (Fig. 2, B, E, and F). The inner ring of the disk contains the NBD, helical domain 1 (HD1), and the winged helix domain (WHD); the outer ring comprises helical domain 2 (HD2) and the LRR domain (Fig. 1A and Fig. 2B).

We focus our further discussions on the 11-bladed structure with the highest resolution. The conformation of active NLRC4A in the 11-bladed inflammasome exhibits differences from that of inactive NLRC4A (16). When the NBD-HD1 regions of NLRC4 in the two states are aligned, the WHD-HD2-LRR module needs to rotate 87.5° along an axis at the junction between HD1 and WHD to turn from the inactive state to the active state (Fig. 2, G and H). The pivot point of the long-range hinge motion is where the inactive and active conformations of the α14 helix of WHD intercept (Fig. 2H). The rotation rearranges the intramolecular interactions between WHD and the NBD-HD1 module.

Several missense mutations in human NLRC4 that are associated with auto-inflammatory conditions (12–14)—Thr337 → Ser, Val341 → Ala, and His443 → Pro—localize to this highly dynamic region (fig. S6A).

We do not know whether NLRC4 conformational transition is accompanied by exchange of adenosine diphosphate (ADP) in the inactive state to adenosine triphosphate (ATP) in the active state, as observed for apoptosome assembly by

![Cryo-EM structure determination and conformational activation of NLRC4.](image)

Fig. 2. Cryo-EM structure determination and conformational activation of NLRC4. (A) Cryo-EM map of the C11 Prg-J-NAIP2-NLRC4A complex colored with local resolution calculated by ResMap using two separately refined half maps. (B) Superimposed ribbon diagram and transparent surface of the C11 NLRC4A structure. (C) Cryo-EM density superimposed with one NLRC4A subunit. (D) A close up view of the structure of the NBD of NLRC4A superimposed with the cryo-EM density. (E) Cryo-EM maps and fitted NLRC4A models for the C12 reconstruction at 12.5 Å resolution (E) and the C12 reconstruction at 7.5 Å resolution (F). (G) The WHD-HD2-LRR domain of NLRC4 swings 87.5° to transit from the inactive conformation (left, PDB ID 4KXF) to the active conformation (right). NBD and HD1 are shown in superimposed ribbon diagram and transparent surface, and the WHD-HD2-LRR module is shown in ribbon diagram. (H) Superimposed inactive (colored) and active (gray, except for α14 helix, which is in dark blue) conformations of NLRC4A. The α14 helices in the two conformations are labeled to show the relative rotations and the rotational pivot point.
the related adenosine triphosphatase (ATPase) Apa-f1 (17, 18). Lack of nucleotide density in the cryo-EM map, local conformational changes, a modified Walker B motif, and absence of a conserved Arg in the sensor I motif (19) may all suggest alternative mechanisms (fig. S6, B and C). Consistently, the NLRC4 Walker A motif mutant Lys520 → Arg induced cell killing almost as effectively as did the wild type when coexpressed with NAIP5, flagellin, and caspase-1 (2). To facilitate analysis on NAIP2-NLRC4 interactions, we generated a homology model of NAIP2ABIR based on the NLRC4 structure (20) (Fig. 3A). By replacing one of the NLRC4 molecules in the initially fitted Cγ structure, we generated a NAIP2-NLRC4 inflammasome model with one NAIP2 and 10 NLRC4 molecules, in which a single PrgJ-activated NAIP2 initiates NLRC4 activation and polymerization in a domino-like reaction (Fig. 3B). This mechanism differs from other oligomeric ATPases such as apoptosome proteins Apaf-1, CED-4, and DArk, which need other oligomeric ATPases such as apoptosome complex formation of an inflammasome disk (Fig. 3D). Consistently, the NAIP2-A surface interacts with the NLRC4-B surface to initiate the directional formation of an inflammasome disk (Fig. 3D). The NAIP2-B surface is largely incompatible with either NLRC4-A (Fig. S7A and B) or NAIP2-A (Fig. S7C and D). We thus named NAIP2-A the nucleating surface.

Interaction analysis revealed that residues Lys613, Tyr615, Arg631, Arg634, Pro635, Tyr636, and Gln778 of NAIP2-A and residues Pro22, Asn195, Glu222, Asp235, Ile237, Leu238, Leu239, Met346, and Asp366 of NLRC4-B bury a large surface area at the interface (Fig. 3E). To test this interface, we used the His-Flag-NAIP5–His-MBP-NLRC4A inflammasome system because of the availability of differentially tagged constructs for coexpression in 293T cells and because of the sequence similarity between NAIP2 and NAIP5 (Fig. 3F). For NAIP5-A, we mutated Arg646 and Arg649, which correspond to Arg221 and Arg218 of NAIP2-A, to Asp (mutation RR2D). For NLRC4-B, we mutated the three acidic residues, Gln222, Asp235, and Asp237 to Ala (mutation EDD2A). Whereas the wild-type constructs showed robust copurification of NAIP5 and FliC by NLRC4, mutations on either NAIP5-A or NLRC4-B reduced the amount of copurified NAIP5 and FliC (Fig. 3F).

For NAIP2 to initiate NLRC4 polymerization, we hypothesized that it must have weak affinity with the inactive NLRC4. Mapping NLRC4-A and NLRC4-B residues onto the inactive NLRC4 conformation showed that NLRC4-B, but not NLRC4-A, is already largely formed, with only a small part of the WHD in clash with an interacting NAIP2-A (Fig. 3G and fig. S7E). The bound NAIP2-A at this site would push the WHD of NLRC4 exactly at α14, the hinge for conformational changes to occur (Fig. 2, G and H). We propose that the active NAIP2-A surface makes an initial encounter with the NLRC4-B surface in the inactive conformation to initiate the activating conformational change (fig. S7F).
Calculation of surface electrosstatics revealed charge complementarity between the mostly basic NLRC4-A surface and the opposing, largely acidic NLRC4-B surface (Fig. 4, A and B); this finding supports unidirectional polymerization nucleated by NLRC4-A. Structural analysis identified interfacial residues including His144, Arg145, His269, Arg285, His286, Arg288, His289, Glu433, and Arg434 of NLRC4-A, and Asn116, Glu122, Asp123, Asp125, Ile127, Leu133, and Asp135 of the opposing NLRC4-B (Fig. 4C). We mutated NLRC4-A residues Arg285 to Asp (mutation RH2D) and Arg286 to Asp (mutation R286D) and tested their interactions using the same 293T cell coexpression system of His-FliC, Flag-NAIP5, and His-MBP-NLRC4A. Both RH2D and R286D mutations reduced the amount of NLRC4A in the inflammasome complex (Fig. 4D). Therefore, like a ligand-activated NAIP2, a newly activated NLRC4 triggers activation of another NLRC4 molecule by inducing conformational changes (fig. S7F). The NBD-NBD interactions between adjacent NLRC4 subunits differ from those in the heptameric apoptosome, with distinct angular relationships that may have explained the existence of more subunits in each NLRC4 disk (21, 22) (fig. S8, A to C).

ASC, like NLRC4, is an inflammasome adapter protein. We showed previously that ASC-dependent inflammasomes activate caspase-1 by ASCCARD-mediated caspase-1CARD polymerization.
As the most abundant energy source in living organisms, ATP is used widely in enzymes to mediate force generation, conformation change, oligomerization, and transport. The ATPase-mediated nucleated polymerization through a domino-like chain reaction identified here adds an important, elegant mechanism to this universal and already complex enzyme family. Nucleated polymerization in NAIP-NLRC4 inflammasomes also presents yet another mode of higher-order oligomerization, which may play a role in facilitating proximity-induced enzyme activation, threshold response, and prion-like propagation in immune signaling (27–30).

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SUPPLEMENTARY MATERIALS
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Materials and Methods
Fig. S1 to S8

METALLIC SUPERCONDUCTIVITY IN A TWO-DIMENSIONAL SUPERCONDUCTOR

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Recently emerging two-dimensional (2D) superconductors in atomically thin layers and at heterogeneous interfaces are attracting growing interest in condensed matter physics. Here, we report that an ion-gated zirconium nitride chloride surface, exhibiting a dome-shaped phase diagram with a maximum critical temperature of 14.8 kelvin, behaves as a superconductor persisting to the 2D limit. The superconducting thickness estimated from the upper critical fields is a 1.8 nanometers, which is thinner than one unit-cell. The majority of the vortex phase diagram down to 2 kelvin is occupied by a metallic state with a finite resistance, owing to the quantum creep of vortices caused by extremely weak pinning and disorder. Our findings highlight the potential of electric-field–induced superconductivity, establishing a new platform for accessing quantum phases in clean 2D superconductors.

Recent technological advances of materials fabrication have led to discoveries of a variety of superconductors at heterogeneous interfaces and in ultrathin films; examples include superconductivity at oxide interfaces (1, 2), electric-double-layer interfaces (3), and mechanically cleaved (4), molecular-beam-epitaxy–grown (5, 6), or chemical-vapor-deposited (7) atomically thin layers. These systems are providing opportunities for searching for superconductivity at higher temperatures, as well as investigating the intrinsic nature of two-dimensional (2D)
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