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NLRP3 is its own gatekeeper: a group hug of NLRP3 monomers controls inflammation

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Abstract

A recent study by Hochheiser *et al.* describes the CryoEM structure of autoinhibited NLRP3 decamer that assembles via LRR interactions and is further stabilized by small molecule NLRP3 specific inhibitor CRID3 binding into a cleft within the NACHT domain. The study provides a springboard for the development of novel NLRP3-based therapies.

Main text

Two decades of investigations into inflammasomes brought enormous advances in our understanding of how these sensors of cell well-being are activated. Inflammasomes are a group of multiprotein complexes with an ultimate task in activating inflammatory caspases, such as caspase-1. Caspase-1 drives inflammasome response by activating proinflammatory cytokines IL-1β and IL-18 and gasdermin D, a pore-forming executioner of pyroptosis. While inflammasomes guard against infection, they also leave a pathological mark in a number of maladies. Antagonizing the action of IL-1 receptor signaling is an approved therapy for cryopyrin associated periodic syndromes (CAPS), rare inflammatory diseases that arise due to mutations in the gene encoding NLRP3 (nucleotide binding domain (NBD)-, leucine-rich-repeat (LRR)- and pyrin (PYD) domain-containing protein 3). NLRP3 is one of the most enigmatic inflammasome sensors and a suspected felon in various conditions from neurodegenerative to cardiovascular diseases and aging. In contrast to some other inflammasome sensors such as NAIP/NLRC4, where mechanisms of activation are structurally well defined, the disobedient nature of the NLRP3 molecule and missing information on direct activating ligands limited our knowledge of how NLRP3 is regulated and what is going on during the activation process. In accordance to other members of the NLR family, including the structural data establishing the mechanism of NAIP/NLRC4 activation, NLRP3 was proposed to undergo a structural rearrangement in the NACHT domain upon activation and to form an oligomer that could, via an appropriate orientation of PYD domains, act as a seed for ASC (apoptosis-associated speck-like protein containing a CARD) recruitment via homotypic $NLRP3^{PYD}$ and ASC^{PYD} interactions. ASC polymerization in turn leads to recruitment of pro-caspase-1 and its self-activation (reviewed in [1,2]).

Now, Hochheiser et al. [3] determined the cryo-electron microscopy (cryoEM) structure of full-length human NLRP3, revealing that the inactive form of NLRP3 is in fact not a monomer, but a decamer (Figure 1). Size-exclusion chromatography revealed that NLRP3 exists in two fractions, a very large molecular weight fraction that is able to hydrolyze ATP, and a decamer-sized fraction. Neither of those fractions were able to seed ASC polymerization. The decamer was not capable of ATP uptake, suggesting that it represents an autoinhibited state that, once formed, could not be disrupted by the kinase NEK7, which is a known LRR-targeting NLRP3 ligand [4]. The autoinhibited state was further stabilized by addition of the small molecule inhibitor CRID3 (MCC950), leading to structural resolution of 3.8-4.2 Å (Figure 1). The decamer is in fact assembled as a pentamer of dimers, where ten intertwined LRR domains are placed in equatorial plane and five NACHT domains extend toward each of the poles (Figure 1A, B). The majority of NLRP3 domains are well defined; however, only two PYDs were defined in the interior of an autoinhibited decamer. Disruption of intermolecular interactions at interfaces A and C (Figure 1A, left) increased NLRP3 basal activity, demonstrating that decamer formation is important for suppressing NLRP3's constitutive activity.

An equally fascinating study by Andreeva et al. [5] showed that similar oligomers form at negatively charged membranes in mammalian cells. Purification of mouse NLRP3 in the presence of dATP yielded dodecamers (hexamers of dimers) and enabled structure determination at 4.2 Å (Figure 1B). Density inside the oligomer suggests that PYD domains are trapped inside the oligomeric cage. Dodecamers are also unable to seed ASC polymerization, demonstrating that these structures are inactive in inflammasome formation. However, dodecamer-disrupting mutations did not support disassembly of trans-Golgi network upon nigericin treatment that has been previously identified as a signal downstream of various NLRP3 activators [6]. Moreover, dodecamer-disrupting mutants failed to support inflammasome activation, suggesting observed NLRP3 cages are on-pathway oligomers.

Whereas future research will need to clarify the structural and functional differences observed in the described studies [3,5], both studies indicate there are likely two major regulatory levels that suppress NLRP3 activation: 1. LRR oligomer formation that sequesters PYD domains thus disabling ASC recruitment and 2. stabilization of the NACHT domain in an inactive conformation (Figure 1B). This two-level mechanism of self-regulation suggests that posttranslational modifications targeting various domains of NLRP3 could be acting on one or the other level and variants lacking the canonical LRR that cannot form the autoinhibited decamer are not constitutively active because they are still restricted at the second level (reviewed in [2,3,5]). These observations open new speculations on the action of most CAPS-related NLRP3 mutants. While reported NLRP3 SNPs are scattered throughout the molecule, only one disease-causing mutation (Y861C) is located in the LRR and likely disrupts dimer/oligomer formation. Other pathogenic mutations are located in the NACHT domain, suggesting that these mutations disrupt NBD integrity, nucleotide binding, and hydrolysis or NACHT interdomain interactions [3]. With the structure of the inactive NLRP3 oligomer resolved, further mechanistic research is needed to determine the steps releasing inhibitory interactions. The LRR oligomer could be disrupted by NEK7 binding to LRR [4] and K^+ efflux was shown to involve linker-FISNA region to induce an open state (reviewed in [5]). Processes such as posttranslational modifications [2] leading to a rearrangement of the NACHT domain into the active conformation, however, remain to be addressed in light of these novel findings.

Several studies in 2019 demonstrated that NLRP3 is a direct target of the NLRP3 inflammasome selective inhibitor CRID3 [7-9]. Hochheiser et al. [3] now reveal that CRID3 stabilizes the NACHT domain and NLRP3 in an inactive conformation by connecting five domains (NBD, HD1, WHD, HD2, trLRR) (Figure 1A). This is further supported by the crystal structure of a CRID3 analogue bound to the NACHT domain of NLRP3 [10] and a bioluminescence resonance energy transfer study of conformational changes in the NLRP3 molecule upon activator and CRID3 treatment [8]. By sequestering NACHT-trLRR together, CRID3 prevents the characteristic conformational changes needed for ATP binding and formation of the active NACHT domain-mediated oligomer. It is thus possible to effectively inhibit inflammasome activation by locking the NACHT domain despite not

suppressing upstream events such as trans-Golgi network disassembly [5]. Furthermore, presented structural information on the inhibitor binding site and action can help explain why CRID3 is unable to restrict inflammation due to some CAPS-related mutations [7-9]. This insight is crucial for structure-based design of novel inhibitors and hence development of effective personalized therapy.

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Declaration of interests

I.H.B. has no competing interests to declare.

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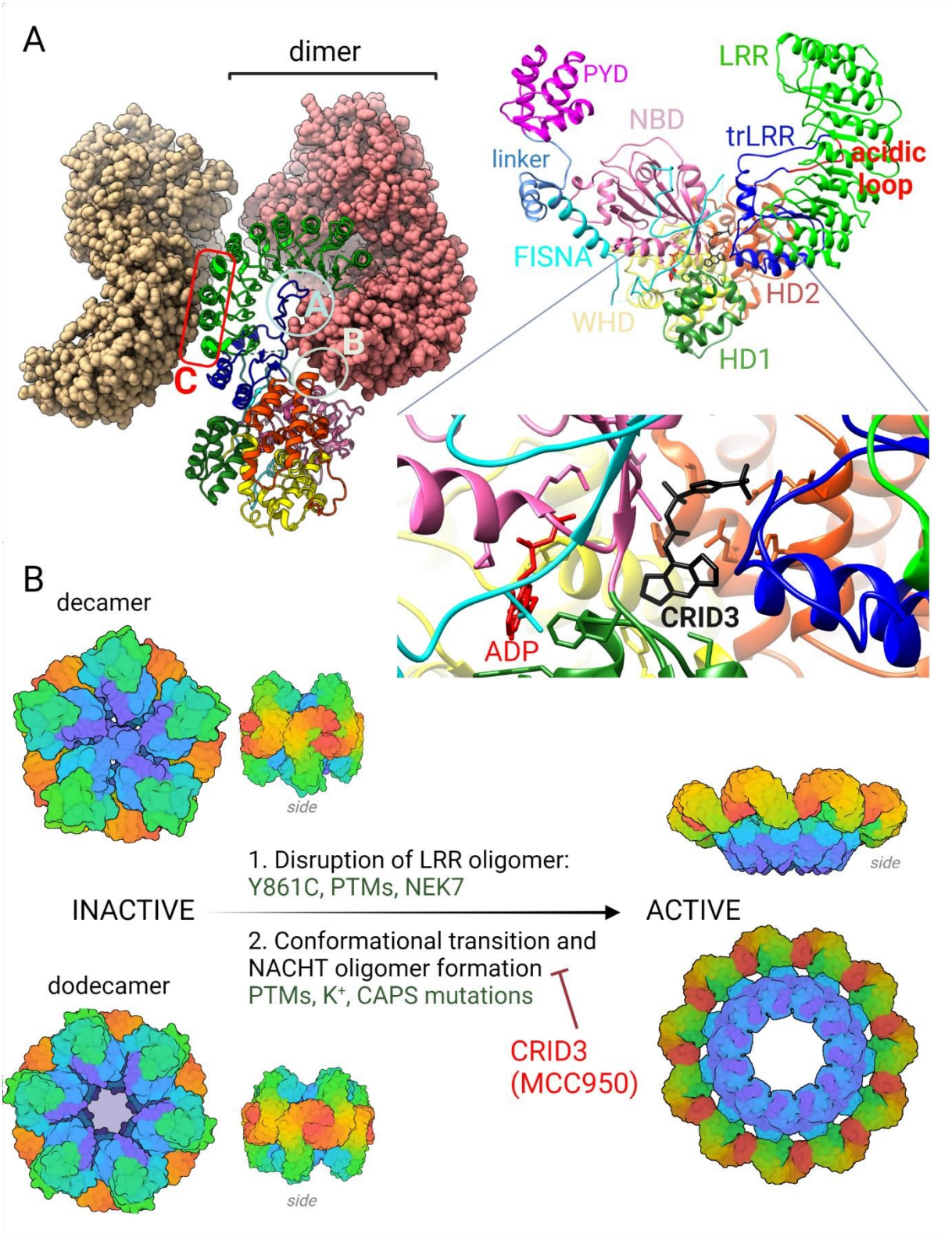
Figure legend

Figure 1: Two-level regulation of NLRP3 by LRR oligomer formation and inhibitor-mediated NACHT domain stabilization.

a) Right: The NLRP3 molecule (PDB ID: 7PZC) is composed of eight domains: PYD (1-94); the fish-specific NACHT-associated domain, FISNA (131-218); NBD (219-372); helical domain 1, HD1 (373-434); winged helical domain, WHD (435-541); helical domain 2, HD2 (542-649); transition LRR, trLRR (650-742) and canonical LRR (743-1036). The NACHT domain (present in NAIP, CIITA, HET-E and TP1) consists of NBD, HD1, WHD and HD2. A unique feature in NLRP3 is the acidic loop (red) in the trLRR, important for dimer formation. Bottom right: CRID3/MCC950 (black) binds into a cleft in the NACHT domain and stabilizes the inactive form by holding NBD, HD1, WHD, HD2 and trLRR together. CRID3 does not compete for binding with ADP (red). Left: There are three major interaction surfaces in the NLRP3 decamer: two opposite molecules dimerize via surfaces A and B. Interface C through 4^{th} and 5^{th} LRR repeat helices supports decamer formation.

b) Schematic representation of the inactive decamer (7PZC [3]) and dodecamer (7LFH [5]). Processes that could lead to destabilization of inactive oligomer and NACHT domain structural transition resulting in active oligomer formation are proposed. Hypothetic active oligomer is modeled on NAIP/NLRC4 oligomer. Created by Biorender.com.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dimer
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HD2
WHD
HD1
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B
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ADP
CRID3
B
decamer
side
1. Disruption of LRR oligomer:
Y861C, PTMs, NEK7
INACTIVE
ACTIVE
side
2. Conformational transition and
NACHT oligomer formation
PTMs, K+, CAPS mutations
CRID3
(MCC950)
dodecamer
side