known whether Nppb and Sst are themselves involved in this process).

Mechanical itch is mediated by a different set of sensory neurons, called low-threshold mechanoreceptors (LTMRs)[6]. These neurons are also involved in a form of pathological itch known as alloknesis[7,8], which arises in response to light mechanical stimuli in skin that has become sensitized — for instance, in skin disorders such as atopic dermatitis or by a chemical itch triggered by histamine.

Hill and colleagues have now uncovered an LTMR-independent mechanism that also mediates mechanical itch. They set out to examine the possible role of Piezo1 in itch, because this protein is known to be expressed in sensory neurons[9], but its role in these cells was unknown. The authors analysed expression of the *Piezo1* gene in mouse sensory neurons, and found that its expression was high in *Mrgprd*-expressing neurons and in neurons expressing the *Nppb* and *Sst* genes. Both populations have roles in mediating chemical itch[3,5]. Corroborating these findings, the researchers demonstrated *in vitro* that sensory neurons that are excited by a Piezo1-activating drug, Yoda1, are typically also excited by β-alanine or histamine (Fig. 1).

Next, Hill and colleagues examined itch responses in mice genetically engineered so that *Piezo1* was deleted in *Sst*-expressing cells. The authors tested mechanical itch in these mice by measuring scratching elicited by a light touch with a nylon filament. They observed a dramatic decrease in mechanical itch in mice lacking *Piezo1* compared with mice in which *Piezo1* was expressed as normal.

The group then investigated mice in which *Piezo1* was mutated to show increased activity. These animals not only exhibited greater mechanical itch than controls, but also showed increased alloknesis and histamine-evoked scratching. Injection of Yoda1 in the mice produced scratching behaviours but not inflammation or pain sensitization, supporting the researchers' hypothesis that Yoda1 directly activates Piezo1 in sensory neurons to produce itch.

To further investigate the role of Piezo1 in chronic itch conditions, Hill and colleagues turned to a mouse model of atopic dermatitis. In this model, topical treatment with a vitamin D analogue induces chronic itch, resulting in increased scratching, alloknesis and skin inflammation. Deletion of the *Piezo1* gene in these mice significantly reduced alloknesis and resulted in less time spent scratching.

In addition, the authors demonstrated that treatment of sensory neurons with histamine *in vitro* did not alter the expression of Piezo1 ion channels, but instead made them more sensitive to mechanical stimulation, suggesting that histamine induces alloknesis in part by modifying the Piezo1 channel. It is likely that other pathways, such as those involving LTMRs, also contribute to histamine-induced alloknesis.

These data provide strong evidence not only that Piezo1 drives mechanical itch, but also that it has a role in the development of alloknesis. When Hill and colleagues applied a Piezo1 inhibitor to the mice with induced atopic dermatitis, they observed a significant decrease in alloknesis, highlighting the therapeutic potential of targeting Piezo1 to treat some forms of itch sensitization.

Taken together, Hill and colleagues' work clearly demonstrates that Piezo1 in *Nppb*-expressig neurons is involved in mediating mechanical itch. Future research should investigate how the newly discovered Piezo1 pathway can be integrated into previous models of mechanical itch and alloknesis, such as those mediated by LTMRs. The study also provides the first evidence, to our knowledge, that the neurons that contribute to chemical itch can also initiate mechanical itch, suggesting that the same sensory neuron can produce both the sensitization underlying alloknesis and the sensation of alloknesis itself.

Looking at the results from a therapeutic perspective, the mechanisms that regulate mechanical itch and alloknesis in the authors' mouse models might differ from those that underlie human alloknesis. Mutations in the human *PIEZO1* gene have been reported that facilitate the channel's function[10] — it will be interesting to see whether people carrying these mutations experience heightened mechanical itch. Finally, the demonstration that Piezo1 has a role in mediating itch provides a potential therapeutic target for the treatment of alloknesis.

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Inorganic chemistry

Iron in a molecular cube reduces nitrogen

Daniël L. J. Broere

Enzymes use molecular clusters containing iron and sulfur atoms to bind and 'fix' nitrogen gas into a bioavailable form. A synthetic cluster that binds and reduces nitrogen molecules casts light on the mechanism of fixation. **See p.86**

Nitrogen is crucial for all known life, but it is predominantly found in its most unreactive form, gaseous dinitrogen (N_2). The challenging process of nitrogen fixation, in which dinitrogen is chemically reduced to make bioavailable forms of nitrogen, is achieved in nature only by lightning, and by a group of bacteria and archaea called diazotrophs[1]. These microorganisms use clusters consisting of two fused cube-shaped molecules containing iron atoms to enable fixation, but the exact mode of binding and reduction of dinitrogen is still unclear. Ohki *et al.*[2] report on page 86 that iron atoms located at the corner of a synthetic cube-shaped molecule can bind and reduce dinitrogen, providing a fresh clue about the molecular machinery that nature uses for nitrogen fixation.

It is well established that diazotrophs use enzymes called nitrogenases to reduce dinitrogen into ammonia (NH_3), the key building block for the biosynthesis of molecules that make up proteins, DNA and many vitamins. Like several other enzymes found in nature, nitrogenases contain cube-shaped molecules, known as clusters, made from iron and sulfur atoms. Such iron–sulfur clusters are generally used to transport and store electrons in enzymes. However, unlike other enzymes, nitrogenases also contain a cluster that looks as if two iron–sulfur cubes have been fused together around a single carbon atom (Fig. 1a). The difficult tasks of binding dinitrogen and reducing it to ammonia take place at this cluster.

Decades of biochemical analysis — mainly on fixation by the most active variant of the cluster, which contains a molybdenum atom

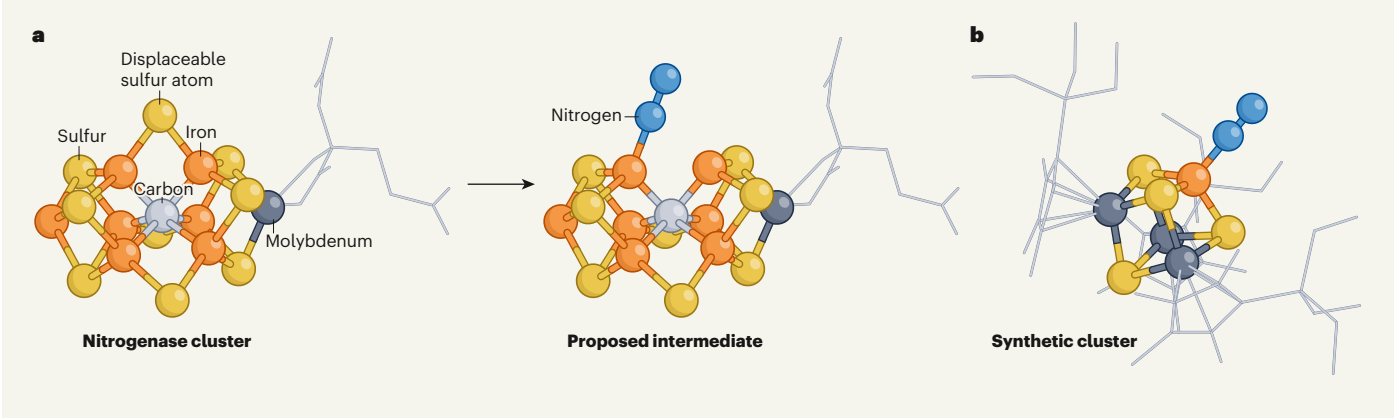

Figure 1 | A synthetic model of the active site of nitrogenase enzymes. **a**, Nitrogenase enzymes 'fix' unreactive dinitrogen (N_2) by converting it into ammonia, a bioavailable form of nitrogen. The active site responsible for fixation contains a molecular cluster that looks as though two cubes of iron and sulfur atoms have fused around a single carbon atom. The most active variant of the cluster also contains a molybdenum atom. Studies suggest that, during nitrogen fixation, a sulfur atom is displaced, exposing an iron atom at which dinitrogen binds end-on. The line structure represents the ligand molecule bound to the molybdenum atom. **b**, Ohki *et al.*[2] have prepared a synthetic cluster that models the proposed binding site of dinitrogen in the nitrogenase active site. The bulky Cp^{XL} ligands cause the dinitrogen molecule to bind end-on to the single iron in the cluster, analogous to the proposed binding in **a**. Moreover, the cluster chemically reduces the dinitrogen molecule sufficiently to enable its conversion to an ammonia analogue (not shown). (Adapted from Fig. 1 of ref. 2.)

– have provided valuable insights into how the cluster proceeds through 11 different proposed intermediates[3]. However, the instability of several of these intermediates has prohibited their structural characterization. Insights into how and where dinitrogen binds to the cluster are especially sought after. A crystal structure of the cluster with a bound dinitrogen molecule was reported[4] in 2020, but there is still debate about the interpretation of these data[5]. That said, a growing collection of studies indicates that one of the sulfur atoms that bridges the two cubes in the cluster is displaced, opening up a site on an iron atom at which dinitrogen binds[3,4,6,7] (Fig. 1a).

The challenges associated with isolating short-lived intermediates have motivated researchers studying synthetic inorganic chemistry to make structural and functional models of nitrogenases[8–10]. Such model systems can provide support for the existence of proposed enzyme intermediates through comparison of their spectroscopic features. But binding a dinitrogen molecule to synthetic, cube-shaped iron–sulfur clusters is challenging, because it requires a binding site to be generated on an iron atom – which then attracts sulfur atoms of other clusters, leading to aggregation.

The long-standing task of isolating a synthetic iron–sulfur cluster with a bound dinitrogen molecule was finally overcome last year[11]. However, no chemical reaction of the bound molecule was reported. This is probably because each atom of dinitrogen binds to a different cluster – a bridging binding mode that lowers the reactivity of the molecule.

Ohki *et al.* take things to the next level. They report cube-shaped metal–sulfur clusters containing a corner iron atom that binds dinitrogen in a 'terminal' mode. In this mode, only one of the two atoms in dinitrogen is bound to the iron atom (Fig. 1b), thereby activating the other nitrogen atom so it can undergo chemical reactions.

The key to this achievement lies in the attachment of large organic molecules, known as Cp^{XL} ligands, to the molybdenum atoms that are located on three corners of the cube-shaped cluster. The bulkiness of these Cp^{XL} ligands forces them into a conformation in which they fold around the space in which the dinitrogen molecule binds to the iron atom. This prevents another cluster from binding to the other nitrogen atom in the molecule, thereby enforcing the terminal binding mode. Ohki *et al.* also show that dinitrogen binds in the less-reactive bridging binding mode when smaller Cp ligands bind to the molybdenum atoms.

Unlike nitrogenase, the authors' synthetic clusters do not convert dinitrogen into ammonia catalytically. Instead, Ohki *et al.* demonstrate that their clusters catalyse the conversion of dinitrogen to an ammonia analogue in which silicon atoms are attached to the nitrogen atom. This reaction proceeds through a different mechanism from that of nitrogen fixation, however, and is known to be catalysed efficiently by molybdenum-based catalysts[12].

The synthetic cluster contains three molybdenum atoms, and, to mediate the catalytic conversion of dinitrogen, the cluster is subjected to reaction conditions that could make it fall apart. Therefore, the possibility that this reaction is catalysed by an unidentified species other than the cluster itself cannot be excluded; this is acknowledged by the authors. However, Ohki and co-workers demonstrate that they can selectively attach two silicon fragments to a dinitrogen molecule that is bound to the iron atom in their synthetic cluster – the first and most difficult step in the proposed cluster-catalysed reaction. This is a breakthrough, because it is the first demonstration that iron atoms in metal–sulfur clusters can bind to dinitrogen at one of its nitrogen atoms and simultaneously reduce the dinitrogen sufficiently to enable chemical modification of the other nitrogen atom.

The chemical reactivity of Ohki and colleagues' synthetic cube-shaped clusters provides a valuable clue to how the molecular cluster in the active site of nitrogenase might bind to and reduce dinitrogen. However, chemists in this field are not out of a job yet, because there are still plenty of questions to be answered. Perhaps the most pressing is, what is the role of the carbon atom at the centre of the cluster? The lessons in cluster design outlined by the authors will surely aid the development of synthetic models that mimic this aspect of nitrogenase's fixation system, too.

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