Stepwise and Reversible Assembly of 2Fe– 2S Rhombs to 8Fe–8S Clusters and Their Topological Interconversions

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Graphical Abstract (TOC graphic):



ABSTRACT

Among all enzymatic metallocofactors, those found in nitrogenases, the P- and L-/M-clusters, stand out for their structural complexity. They are assembled by proteins of the Nif gene cluster from Fe_2S_2 rhombs the smallest building blocks in FeS cluster chemistry—through a sequence of reactions constructing a Fe_8S_8 precursor. This fundamental transformation is unknown in chemical synthesis, impeding our understanding of how enzymes selectively build such elaborate inorganic molecules. Here, we report the rational stepwise assembly of $[Fe_8S_8]^{n+}$ (n=2,4,6) clusters from $[Fe_2S_2]^{2+}$ rhombs, within an extensive cyclic synthetic network. We identify a $[Fe_8S_8]^{4+}$ cluster of unique topology, for which we coin the term "interlocked" double-cubane (*ildc*). This topology is not unprecedented in enzymes, as the *ildc* is a molecular analogue of the K-cluster, a proposed biosynthetic precursor to both the P- and M-clusters. Its synthesis, along with the characterization of all related intermediates, offers key insights into the mechanisms governing the assembly of these cofactors, advancing our understanding of both enzymatic and synthetic FeS cluster construction.

INTRODUCTION

Iron-Sulfur (FeS) clusters act as enzymatic metallocofactors in many critical metabolic processes.¹⁻ ³ While *bi-*, *tri-* and *tetra-*metallic FeS cofactors occur frequently, larger ones are rarer, and primarily found in nitrogenases and nitrogenase-like enzymes, as the octa-metallic P- and L-/Mclusters.⁴⁻⁷ To the best of our knowledge, only one other metalloenzyme bearing such a large FeS cofactor has been discovered and structurally characterized, namely the double-cubane cluster protein (DCCP).⁸⁻¹⁰ Since the first compositional and, later on, structural characterizations of these metallocofactors,¹¹⁻¹³ the fundamental understanding of their biosynthesis and their synthetic replication attracted sustained interest.^{6,7,14-19} However, molecular model compounds for such large FeS clusters remain scarce. Notable examples include the fusion of two catechol- or tris(pyrazole)borate- (Tp-) and phosphine- or chloride-supported MFe₃S₄ (M=V,Mo,W) heterocubanes^{20,21} to form edge-bridged double-cubanes²²⁻²⁷ which can be converted to M₂Fe₆S₇ clusters, bearing a $\mu^6(S^{2-})$ ligand, upon treatment with hydrosulfide/-selenide.²⁸⁻³² Additionally, Pcluster models of the type $Fe_8S_{(7-x)}O_x$ (x=0,1)^{17,33-40} can be synthesized through the nonpolar assembly of [Fe(N(SiMe₃)₂)₂] in presence of bulky aryl-thiols, amides, or (in some cases) phosphine ligands and elemental sulfur. Unfortunately, the mechanism of formation for these Fe₈S₇ clusters remains elusive,³⁵⁻³⁷ and well-defined transformations have only been observed to occur in the reverse direction, cleaving the larger clusters to form the more stable Fe₄S₄ assemblies.⁴⁰ The most recent achievement is a report of the first synthetic cluster containing a central μ^6 -carbide unit, [Et₄N][(Tp)₂W₂S₆Fe₆(μ^6 -C)(SPh)₃], constructed by fusion of WFe₃S₃C(SiMe₃) and WFe₃S₃ clusters.⁴¹ A few examples of all-Fe, ligand-bridged, edge-bridged and face-bridged double- and multi-cubanes have been reported by the groups of Holm, Tatsumi and Suess.⁴²⁻⁴⁶ However, these systems maintained the topological integrity of the Fe₄S₄ units composing them, and are supported by phosphines, N-heterocyclic carbenes and tripodal chelating *tris*(thiolates).

Beyond the mere replication of the active site,^{36,38} the sophistication of the P- and M-clusters warrants the exploration of new approaches, aimed not only at reproducing their molecular structure, but also at reflecting and shedding light onto aspects of their biosynthesis, the understanding of which has greatly advanced during recent years:^{6,7} Even though the P- and M-clusters mature separately from one another, an evolutionary link may exist between them. Both

are assembled by proteins encoded in the Nif gene cluster *via* fusion of two Fe₄S₄ (or "Fe₄S₄-like") synthons, forming an Fe₈S₈ precursor.^{6,7,47,48}



Figure 1: Selected crystal structures of enzymes involved in M-cluster maturation. (A) Crystal structure of the NifU-like protein, *Aquifex aeolicus* IscU, with a bound [2Fe–2S]-cluster (PDB code 2Z7E).⁴⁹ A close-up view of the metallocofactor is shown as well. (B) Crystal structure of *Methanotrix thermoacetophila* NifB with bound RS- and K1-clusters (PDB code 6Y1X).⁵⁰ A close-up view of the K1-cluster is shown alongside. (C,D) Crystal structure refinements of *Methanobacterium thermoautotrophicum* NifB with a full complement of FeS cofactors, as proposed by the groups of Ribbe and Hu (C; PDB code 7JMB)⁵¹ and the group of Nicolet (D; PDB code 7BI7).⁵² For each solution of the structure, a close-up view of the K1-/K2-clusters or the K-cluster, respectively, are provided. Images were generated using the ChimeraX program suite.^{53,54}

While the molecular mechanisms underlying the initial cubane fusion, yielding the Fe_8S_8 precursor, remain an active area of investigation, substantial progress has been made in understanding the last steps of the M-cluster maturation:^{6,7,55-60} In a first step, NifS and NifU harness Fe and S to produce Fe₄S₄ clusters via the reductive fusion of two Fe₂S₂ rhombs (Figure 1A and 2A).^{6,7,61-63} Two of these cubane cofactors are subsequently recruited by NifB, sitedifferentiated (Figure 1B and 2B)⁵⁰ and fused to form the Fe₈S₈ precursor. NifB itself is a radical SAM enzyme, which contains three Fe₄S₄ clusters: one is the radical SAM (RS-) cluster, while the other two, referred to as the K1- and K2-clusters, constitute the building blocks for the M-cluster. A crystallographic structure of the enzyme with three bound clusters was only recently reported by Ribbe, Hu and colleagues, and revealed that the K1-/K2-clusters may be coordinatively unsaturated or possibly bound to each other (Figure 1C), forming a double-cubane.⁵¹ While it had been known that these clusters exhibit non-canonical ligands, their exact coordination environment and its dynamics remained a topic of interest.^{64,65} Based on an alternative refinement of the original diffraction data, Nicolet and co-workers proposed another arrangement of the clusters: Rather than existing as two discrete Fe₄S₄ units in close proximity, the K1- and K2-clusters may adopt an interlocked configuration, forming a single Fe₈S₈ cluster, simply termed K-cluster (Figure 1D),⁵² which is topologically related to both the P- and the M-cluster architectures, as summarized in Figure 2B.C. During subsequent stages, NifB functionalizes the K-cluster with a carbide-ligand and a ninth Sulfur atom (derived from SO_3^{2-}), before it is inserted into NifEN. The Iron protein (NifH) then delivers a hetero-metal (Mo or V) and a homocitrate ligand, forming the final MFe₇S₉C M-cluster, which is inserted into NifDK (Figure 2C).^{6,7,55-60} In contrast, the Fe₈S₈ precursor to the P-cluster, which is potentially K-cluster-"like", matures directly in NifDK via the loss of one of its S-atoms (Figure 2D).^{6,7,66-68}



Figure 2: Scheme illustrating the stages of P- and M-cluster maturation *via* a K-cluster intermediate. (A-C) Stages of the M-cluster synthesis catalyzed by the enzymes of the Nif gene cluster: (A) Conversion of Fe and S into Fe₂S₂ clusters in NifU and NifS and their reductive conversion into Fe₄S₄ clusters. (B) First steps of biochemical M-cluster maturation: Conversion of site-differentiated Fe₄S₄ clusters into K1- and K2-clusters and their fusion into an interlocked Fe₈S₈ cluster, termed K-cluster, in NifB. (C) Last steps of M-cluster maturation, encompassing installation of the carbide and sulfide ligands in NifB, as well as the Mo ion. (D) A hypothetically simple route from a K- or K-"like"-cluster precursor to the P^N-cluster *via* $S(\mu^2)$ removal. This report replicates the stages (A) and (B), while (C) and (D) summarize the (potential) fate of the K-cluster towards its conversion to the M- and P-clusters, illustrating its central role. Note that we adopted the topology of the K-cluster proposed by Nicolet and coworkers.⁵²

Inspired by this synthetic pathway, we describe here a cyclic and reversible chemical network of FeS cluster interconversions, covering a very fundamental, yet unknown chemical transformation, namely the stepwise assembly of synthetic $[Fe_2S_2]^{2+}$ clusters—being the simplest FeS building blocks—to $[Fe_8S_8]^{n+}$ (*n*=2,4,6) clusters. Among these, the $[Fe_8S_8]^{4+}$ cluster exhibits the same FeS topology as that proposed for the K-cluster by Nicolet and co-workers (Figure 1D),⁵² which we herein coin an "interlocked" double-cubane. Its ultimate and penultimate precursors are themselves Fe_8S_8 clusters, namely a ligand-bridged $[Fe_8S_8]^{6+}$, or an edge-bridged $[Fe_8S_8]^{2+}$ double-

cubane, respectively. Both rather resemble the structure that was proposed by Ribbe, Hu and colleagues for the K1-/K2-clusters (Figure 1C).⁵¹ Based on the characterization of these clusters' electronic, vibrational and ⁵⁷Fe nuclear spectroscopic signatures, we propose that they could aid the identification of such intermediates in NifB, and NifDK, respectively, while also providing fundamental insight into the factors governing their formation and interconversion.

The initial goal of our synthetic strategy was to achieve site differentiation, as this was proposed to play a key role in inducing cluster conversion.^{50,64} In enzymes, Fe₄S₄-site-differentiation occurs as the regiospecific substitution of a cysteine ligand, typically by histidine,⁶⁹ driven by an asymmetric cofactor binding pocket. Efforts to replicate this behavior, using rigid multidentate thiolate ligands^{46,70,71} or bulky NHCs^{72,73} have been explored. However, these strategies are not transposable to $[Fe_4S_4(RS)_4]^{n-}$ clusters bearing simple monodentate thiolate ligands due to the latters' high symmetry, which prevents precise control over the stoichiometry of ligand substitutions. Leveraging our group's expertise with the $K_n[Fe_4S_4(DmpS)_4]$ (*n*=0,1,2,3,4) model system,⁷⁴⁻⁷⁶ and specifically the oxidation-state dependent determination of the cluster's Fe-S covalency values (α^2) using X-ray absorption spectroscopy, we rationalized an original strategy to promote ligand exchange, rooted in the cluster's electronic structure (Figure 3A,B):⁷⁴ High-valent cubanes (i.e. those in the [Fe₄S₄]^{3+/4+} oxidation states) are characterized by exceptionally large Fe-S(thiolate) bond covalencies (>50%). Thus, when the singly occupied $\psi^*(\text{Fe-S}^R)$ orbitals (SOMOs) are sufficiently polarized toward S (covalency being >50%), the binding of a less covalent ligand to Fe can trigger homolytic cleavage of the Fe-S(thiolate) bond. This process reduces the Fe₄S₄ core by one electron, while the thiolate is oxidized to a radical species (Figure 3A). Because of the core's reduction, the remaining Fe-S(thiolate) bonds will be again more covalent than before (α^2 closer to 50%), and dimerization of the thiolate radical renders the reaction irreversible. This ensures that only a specific number of thiolate ligands are exchanged per cluster, allowing control of the substitution stoichiometry. Such mechanisms are disfavored in reduced cubanes (i.e. those in the $[Fe_4S_4]^{0/1+}$ oxidation states), which exhibit low Fe–S(thiolate) bond covalencies (<50%), making them prone to (heterolytic) ligand exchange mechanisms because their ψ (Fe-L/S^R) orbitals are predominantly localized on the ligand or thiolate (Figure 3B).

RESULTS

The synthetic considerations outlined above were successfully implemented to construct the synthetic cycle shown in Figure 3C, where repeated site-differentiation and redox chemistry enabled FeS cluster conversion. Technical aspects of those syntheses as well as additional synthetic efforts and a discussion of the basic characterization data for all shown compounds are described in the *Methods* section (*vide infra*). Detailed synthetic procedures are compiled in the *Supporting Information* file.

Figure 3C highlights the central role of the canonical $[Fe_4S_4]^{2+}$ complex, $K_2[Fe_4S_4(DmpS)_4]$, which constitutes the junction, connecting the [2Fe-2S]-to-[4Fe-4S], [4Fe-4S]-site-differentiating, as well as [4Fe-4S]-to-[8Fe-8S] cluster interconversion cycles (highlighted in *grey*, *green* and *brown* boxes, respectively).





this work: stepwise site-differentiation and redox reactions to construct Fe_8S_8 complexes derived from four Fe_2S_2 rhombs



each ligand substitution. (B) Electronic origin of redox-neutral ligand loss from a low-valent Fe₄S₄ complex *via* heterolytic bond cleavage. The overall cluster charge (*n*) increases by 1, if L is anionic, and remains the same, if L is neutral. Both behaviours (those shown in panels A and B, respectively) are ascribed to specific $[Fe_4S_4]^{x+}$ oxidation states based on our study of the corresponding Fe-L^t (where L^t is an arbitrary terminal ligand) bond covalencies (α^2).⁷⁴ (C) Synthetic pathway from a $[Fe_2S_2]^{2+}$ complex *via* canonical and site-differentiated $[Fe_4S_4]^{n+}$ (*n*=1,2,3) complexes to edge-bridged and ligand-bridged $[Fe_8S_8]^{2+/6+}$ double cubanes, as well as an interlocked $[Fe_8S_8]^{4+}$ cluster, topologically paralleling the proposed K-cluster's architecture, and related to the P- and M-cluster cofactors of nitrogenase. In all clusters, R refers to the 2,6-dimesitylphenyl residue. Refer to the *Supporting Information* for the exact synthetic conditions of each step. Technicalities and Characterization details are summarized in the *Methods* section.



Figure 4: Structures and mechanistic conversion of $[Fe_8S_8]^{n+}$ (*n*=2,4,6) clusters. Solid-state molecular structures of *ebdc* (A), *lbdc* (B) and *ildc* (C) in crystals of K₄[Fe₈S₈(DmpS)₆]·3.5(C₇H₈),

 $[Fe_8S_8(DmpS)_6]$ · $3(C_7H_8)$ and $[Fe_8S_8(DmpS)_4]$, respectively. Displacement ellipsoids are displayed at the 50% probability level for *ebdc* and *ildc*, and at 30% for *lbdc*, and are only shown for the Fe, S and K atoms. Co-crystallized solvent molecules and hydrogen atoms have been omitted for clarity. (D) Proposed mechanism for the interconversions of the three Fe₈S₈ clusters (*ebdc*, *lbdc* and *ildc*).

As anticipated from the Fe-S(thiolate) bond covalency considerations (Figure 3A), [4Fe–4S]-sitedifferentiation is enabled by cluster oxidation (Figure 3A): The redox congeners of $K_2[Fe_4S_4(DmpS)_4]$ in the $[Fe_4S_4]^{3+}$ and $[Fe_4S_4]^{4+}$ oxidation states readily undergo homolytic Fe-S(thiolate) bond cleavage to exchange their DmpS⁻ ligands for the less covalent 1,2,4,5tetramethylimidazole (Im*) ligand. Thereby, the cluster core is reduced by one electron per Im* ligand, until the $[Fe_4S_4]^{2+}$ oxidation state is reached. This enabled the syntheses of $K[Fe_4S_4(DmpS)_3(Im^*)]$,⁷⁷ $[Fe_4S_4(DmpS)_3(Im^*)]$ and $[Fe_4S_4(DmpS)_2(Im^*)_2]$, whereby the latter two are interconvertible depending on the stoichiometry of Im*, owing to the $[Fe_4S_4]^{3+}$ oxidation state of $[Fe_4S_4(DmpS)_3(Im^*)]$. Conversely, because the $[Fe_4S_4]^{2+}$ -(DmpS) bond is more covalent than the $[Fe_4S_4]^{2+}$ -(Im*) bond, Im* ligands are easily substituted from $[Fe_4S_4(DmpS)_3(Im^*)]^-$ or $[Fe_4S_4(DmpS)_2(Im^*)_2]$ by DmpSK, re-forming $K_2[Fe_4S_4(DmpS)_4]$.

[2Fe-2S]-to-[4Fe-4S] cluster conversion chemistry occurs upon reaching the all-ferric oxidation state:⁷⁸ [Fe₄S₄(DmpS)₄] readily coordinates four pyridine ligands, resulting in cubane scission, to form 2 equivalents of $[Fe_2S_2(DmpS)_2(py)_2]$. Pyridine ligands can be removed reversibly using $B(C_6F_5)_3$, leading to the fusion of the two $[Fe_2S_2]^{2+}$ rhombs and re-forming $[Fe_4S_4(DmpS)_4]$, while the reduction of $[Fe_2S_2(DmpS)_2(py)_2]$ reconstitutes $K_2[Fe_4S_4(DmpS)_4]$ through the fusion of two transient $[Fe_2S_2]^{1+}$ synthons.

In contrast to the [2Fe–2S]-to-[4Fe–4S] and [4Fe–4S]-site-differentiation cycles, [4Fe–4S]-to-[8Fe–8S] conversion chemistry is initiated reductively: Addition of one electron and one K⁺ ion to K[Fe₄S₄(DmpS)₃(Im^{*})] yields the edge-bridged [Fe₈S₈]²⁺ double-cubane K₄[Fe₈S₈(DmpS)₆] (*ebdc*; Figure 4A) *via* fusion of two site-differentiated [Fe₄S₄]¹⁺ cubanes. This result is counterintuitive, considering that the Im^{*} substitution of one of the thiolate ligands shifts the cluster's redox potential anodically, which, in turn, suggests an overall better stabilization of lower oxidation states. However, ligand-field considerations offer a different perspective regarding the reactivity of the cluster: Reduction affords less covalent Fe₄S₄-ligand bonds,⁷⁵ enabling the facile heterolytic dissociation of the least covalent ligand (arguably Im^{*}) and generating a formally coordinatively unsaturated Fe center. The self-coordination of this intermediate *via* two $\mu^4(S^{2-})$ ligands, affording an *ebdc*, results in a more stable ligand field for the cluster, in which net covalency is maximized (i.e. α^2 approaching 50%), compared to the one in which Im* ligation is maintained.

ebdc can be reversibly oxidized by up to four electrons. Its fully oxidized congener, however, does not possess an edge-bridged architecture. Instead, the four-electron oxidation is accompanied by what we hypothesize to be a sliding motion of the two Fe₄S₄ subclusters, until they symmetrically face each other. Concomitantly, two of the $\mu^1(\text{DmpS}^-)$ ligands convert to $\mu^2(\text{DmpS}^-)$ ligands, vielding the ligand-bridged $[Fe_8S_8]^{6+}$ double-cubane $[Fe_8S_8(DmpS)_6]$ (*lbdc*; Figure 4B,D). Though *lbdc* is formally "homoleptic", the inequivalent DmpS⁻ ligands (bridging and terminal) render it site-differentiated. We thus found that the primary coordination sphere is destabilized enough to induce spontaneous cluster conversion: Metastable *lbdc* slowly converts to the "interlocked" [Fe₈S₈]⁴⁺ double-cubane, [Fe₈S₈(DmpS)₄] (*ildc*; Figure 4C), likely through a transition state initiated by a tilting motion of *lbdc*'s two ligand-bridged Fe₄S₄ subclusters, whereby the μ^2 (DmpS⁻) ligands act as hinges. Simultaneously, two $\mu^3(S^{2-})$ ligands rearrange themselves to one $\mu^2(S^{2-})$ and one $\mu^6(S^{2-})$ ligand, while two of the formerly $\mu^1(DmpS^-)$ -ligands undergo reductive elimination (Figure 4D). Given the precedence of covalency-driven homolytic Fe-S(thiolate) bond cleavage at $[Fe_4S_4]^{3+/4+}$ clusters in this work, it seems reasonable to assume that the reductive elimination of (DmpS)₂ from *lbdc*—formally composed of two [Fe₄S₄]³⁺ clusters—occurs homolytically as well. Though *ildc* appears coordinatively saturated and electrochemically robust (Figure S71), it readily converts back to Fe₄S₄ complexes in presence of competing ligands: 4 equivalents of Im* "unlock" and cleave the double-cubane, yielding 2 equivalents of [Fe₄S₄(DmpS)₂(Im*)₂], substitution of which by DmpSK regenerates K₂[Fe₄S₄(DmpS)₄] and closes the [4Fe–4S]-to-[8Fe–8S] cycle (Figure 3C).

Beyond these results, our synthetic explorations lead to the adventitious discovery of two other species, which are worthy of note, namely the edge-bridged $[Fe_{12}S_{12}]^0$ triple cubane, $K_6[Fe_{12}S_{12}(DmpS)_6]$ (*ebtc*), and $[Fe_{24}S_{24}(DmpS)_{10}]$; the latter being—to the best of our knowledge—the largest structurally characterized molecular FeS cluster (refer to the *Methods* section and the *Supporting Information* for additional details).



Figure 5: UV-vis electronic absorption, ⁵⁷Fe NRVS PVDOS and ⁵⁷Fe Mössbauer spectra of the 8Fe-**8S clusters.** (A) UV-vis electronic absorption spectra of $1 \cdot 10^{-4}$ M toluene solutions of *lbdc (magenta), ildc* (vellow) and ebdc (blue), are shown as solid lines. For comparison, the spectra of the canonical and sitedifferentiated Fe_4S_4 and Fe_2S_2 complexes in the relevant oxidation states are also shown, as *dotted lines*. They include $K_3[Fe_4S_4(DmpS)_4]$ (blue). $K_2[Fe_4S_4(DmpS)_4],$ $K[Fe_4S_4(DmpS)_3(Im^*)]$ and $[Fe_4S_4(DmpS)_2(Im^*)_2]$ (*yellow*), K[Fe_4S_4(DmpS)_4] and [Fe_4S_4(DmpS)_3(Im^*)] (*magenta*), as well as $[Fe_4S_4(DmpS)_4]$ and $[Fe_2S_2(DmpS)_2(py)_2]$ (cyan). (B) ⁵⁷Fe NRVS PVDOS spectra of *lbdc*, *ildc* and *ebdc*, respectively (solid coloured lines). For comparison, the spectra of the corresponding canonical cubanes in their respective oxidation states are shown alongside, as *dotted coloured lines*. All spectra were recorded on powdered ⁵⁷Fe enriched (>95%) samples between 30 and 50 K. (C-E) 80 K Mössbauer spectra (vertical bars) recorded on a powder sample of the ebdc (C), lbdc (D) and ildc (E) complexes. No external magnetic field was applied in C and E, whereas a 0.06 T external magnetic field was applied along the γ -rays' direction in D. Simulations are overlaid as thick grey solid lines and components are displayed above the

spectra as colored thin solid lines. The nuclear parameters are the following: (C) Doublet 1 (*light blue*): $\delta = 0.57 \text{ mm s}^{-1}$, $\Delta E_Q = 1.82 \text{ mm s}^{-1}$; Doublet 2 (*medium blue*): $\delta = 0.55 \text{ mm s}^{-1}$, $\Delta E_Q = 1.43 \text{ mm s}^{-1}$; Doublet 3 (*blue*): $\delta = 0.53 \text{ mm s}^{-1}$, $\Delta E_Q = 1.14 \text{ mm s}^{-1}$; Doublet 4 (*dark blue*): $\delta = 0.47 \text{ mm s}^{-1}$, $\Delta E_Q = 0.59 \text{ mm s}^{-1}$; Common linewidth: $\Gamma_{\text{fwhm}} = 0.50 \text{ mm s}^{-1}$. (D) Doublet 1 (*violet*): $\delta = 0.48 \text{ mm s}^{-1}$, $\Delta E_Q = 0.81 \text{ mm s}^{-1}$; Doublet 2 (*magenta*): $\delta = 0.31 \text{ mm s}^{-1}$, $\Delta E_Q = 0.92 \text{ mm s}^{-1}$; Common linewidth: $\Gamma_{\text{fwhm}} = 0.39 \text{ mm s}^{-1}$. (E) Doublet 1 (*brown*): $\delta = 0.34 \text{ mm s}^{-1}$, $\Delta E_Q = 0.89 \text{ mm s}^{-1}$; Doublet 2 (*vellow*): $\delta = 0.68 \text{ mm s}^{-1}$, $\Delta E_Q = 2.57 \text{ mm s}^{-1}$; Common linewidth: $\Gamma_{\text{fwhm}} = 0.43 \text{ mm s}^{-1}$.

We hypothesized that the change in the topology of the Fe₈S₈ clusters would be reflected in their spectroscopic properties. To this end, the electronic, vibrational and ⁵⁷Fe nuclear spectroscopic signatures of *ebdc*, *lbdc* and *ildc* were compared by UV-vis electronic absorption, ⁵⁷Fe NRVS and ⁵⁷Fe Mössbauer spectroscopy, respectively (Figure 5). While *ebdc* and *lbdc* exhibit UV-vis electronic absorption spectra with features at similar energies as their $[Fe_4S_4]^{1+}/[Fe_4S_4]^{3+}$ redox congeners, *ildc* is characterized by electronic transitions that are atypical for an FeS cluster possessing equal numbers of Fe^{II} and Fe^{III} ions, and thus, an average oxidation state of Fe^{2.5}: The intense peak at 467 nm falls within the range of the maxima observed for the more oxidized, Fe^{2.75} containing clusters,⁷⁵ and the shoulder at 631 nm is equally unusual (Figure 5A). Similarly, the ⁵⁷Fe PVDOS spectra of *ebdc* and *lbdc* appear close to those of the canonical $[Fe_4S_4]^{1+/3+}$ complexes, respectively, but, once again, *ildc* shows distinct vibrational bands, which differ from the modes observed for its [Fe₄S₄]²⁺ counterpart (Figure 5B).⁷⁹ The most notable differences are evident in the region of the spectrum associated with FeS bending and twisting (or "breathing") motions, around 80-220 cm⁻¹. This illustrates that, while *ebdc* and *lbdc* are formally Fe_8S_8 clusters, their vibrational (and electronic) behavior closely resembles that of cubanes because they retain intact Fe₄S₄ building blocks. Conversely, the FeS skeleton of *ildc* is topologically rearranged, rendering its properties distinct from those of Fe₄S₄ complexes. A mode at ca. 200 cm⁻¹ is of further interest, because FeS cubanes usually display minimal intensity in this region.⁸⁰ Similar vibrations have however been observed for the M-cluster, which possesses a $\mu^6(C^{4-})$ ligand and an associated cluster breathing mode around 180-190 cm^{-1.81} The $\mu^6(S^{2-})$ ligand in *ildc* may induce breathing modes at comparable energies, as they were shown to depend more on the cluster's topological architecture than on the nature of the interstitial atom.⁸¹

To further understand the asystematic electronic and vibrational properties of *ildc*, compared to *ebdc* and *lbdc*, the valence topology of the three Fe_8S_8 clusters was rationalized by ⁵⁷Fe Mössbauer spectroscopy (Figure 5C-E): *ebdc* and *lbdc* exhibit zero-field 80 K powder spectra with two main absorption lines (Figure 5C,D), while *ildc* shows four (Figure 5E). Regardless of the simulation

model (details are summarized in the *Methods* section), the average isomer shift of the spectra, δ_{avg} , coincides well with the formal average Fe oxidation state.⁷⁵ However, while the simulations of the spectra of *ebdc* and *lbdc* suggest delocalized Fe valences (δ_i between 0.46 and 0.72 mm s⁻¹ for *ebdc* and between 0.31 and 0.48 mm s⁻¹ for *lbdc*), which is typical for Fe₄S₄ complexes, the unique simulation of the *ildc*'s spectrum, shown by the brown and yellow doublets in Figure 5E, presents 0.34 mm s⁻¹ and 0.68 mm s⁻¹ isomer shifts, respectively. These values are substantiated by the analysis performed on the 5.7 K spectra recorded with a 0.06 to 7 T magnetic field applied along the γ -rays' direction (Figure 6), evidencing a diamagnetic ground state with localized ferric and ferrous sites.⁸²⁻⁸⁴ Therefore, we establish an unusual valence trapping in *ildc*, for which a bond-valence sum analysis of the crystallographic structure^{85,86} suggests that [Fe3,Fe4] are in the +II and Fe1 and Fe2 in the +III oxidation states (Table S10).



Figure 6. 5.7 K Mössbauer spectra of *ildc.* Experimental data (*black vertical bars*) were recorded on a powder sample of [Fe₈S₈(DmpS)₄] using a 0.06 (A), 2 (B), 4 (C) and 7 T (D) external magnetic field applied parallel to the γ -beam. The simulated spectra are overlaid as *thick grey solid lines*. They were obtained considering two equally contributing diamagnetic sites, their contributions being displayed above the spectra as colored thin solid lines. Nuclear parameters: Site 1 (*brown*): $\delta = 0.36(1) \text{ mm s}^{-1}$, $\Delta E_Q = -0.89(3) \text{ mm s}^{-1}$, $\eta = 0.8(1)$; Site 2 (*yellow*): $\delta = 0.70(1) \text{ mm s}^{-1}$, $\Delta E_Q = -2.63(3) \text{ mm s}^{-1}$, $\eta = 0.5(1)$; Common linewidth: $\Gamma_{\text{fwhm}} = 0.34 \text{ mm s}^{-1}$ at 0.06, 2 and 4 T, $\Gamma_{\text{fwhm}} = 0.36 \text{ mm s}^{-1}$ at 7 T.

DISCUSSION

The sequence of reactions required to traverse the entire network of FeS cluster conversions shown in Figure 3C-arguably the most extensive and systematic network reported to date-offers valuable insights into potential mechanisms at stake in the biogenesis of the nitrogenase P- and Mcluster cofactors. All three octa-Fe clusters (ebdc, lbdc and ildc) can serve as molecular models for the Fe₈S₈ K-cluster, the structure of which remains a topic of discussion (vide supra), and together, may represent molecular snapshots of the [4Fe-4S]-to-[8Fe-8S] fusion occurring in NifB.^{6,7} We envision that our analysis of the clusters' essential spectroscopic fingerprints (Figure 5) will aid in confirming, respectively disproving their involvement in the corresponding cofactor maturation processes. For this, particularly the trapped valences in *ildc* should represent a useful spectroscopic feature. However, we would like to point out that among the three Fe₈S₈ complexes, *ildc* is distinct because it exhibits the identical FeS topology as that proposed most recently by Nicolet and colleagues.⁵² Incidentally, the latter is also the one most closely related to that of the P- and M-clusters. Our results show that such a topology can be systematically assembled through stepwise synthesis, beginning from an $[Fe_2S_2]^{2+}$ complex as the simplest FeS cluster building block. This involves approximately nine fundamental chemical steps, the exact number of which depends on whether they occur in a concerted or stepwise manner:

- (1) Reduction of two $[Fe_2S_2]^{2+}$ clusters to $[Fe_2S_2]^{1+}$ clusters;
- (2) Fusion of two $[Fe_2S_2]^{1+}$ clusters to form a canonical $[Fe_4S_4]^{2+}$ cluster;
- (3) 1-Electron oxidation to a $[Fe_4S_4]^{3+}$ cluster;
- (4) Reductive site-differentiation of the canonical cluster by a labile non-canonical ligand, regenerating a [Fe₄S₄]²⁺ cluster;
- (5) Reduction, leading to the loss of the labile non-canonical ligand, yielding a coordinatively unsaturated [Fe₄S₄]¹⁺ cubane;
- (6) Fusion of two coordinatively unsaturated cubanes to form an edge-bridged double-cubane;
- (7) Oxidation of the edge-bridged double-cubane;
- (8) Rearrangement of the cluster architecture into a ligand-bridged double-cubane through a sliding motion;

(9) "Interlocking" of the two Fe₄S₄ subclusters by mobilization of the $\mu^3(S^{2-})$ ligands and a tilting motion along the hinge provided by the two bridging ligands, concomitant with reductive elimination of disulfide.

The cluster conversions summarized above are exclusively driven by alternating redox- and sitedifferentiation reactions. This alternation repeatedly destabilizes the FeS cluster's primary coordination sphere, sequentially forcing it to relax into a new, more stable structure, while also increasing its size. In a broader context, this leads us to stress that two key factors govern FeS cluster conversion chemistry: (i) the FeS cluster's ligands, and (ii) its oxidation state. In enzymes, these are controlled by the residues accessible in the cofactor binding pocket and the local electrochemical potential. We also rationalized that the cluster oxidation state, and the associated ligand covalencies control whether Fe₄S₄ site-differentiation occurs homolytically or heterolytically: Polarized bonds (α^2 being <50% or >50%) are prone to cleave either homolytically, if α^2 is >50%, or heterolytically, if α^2 is <50% (with respect to the ligand). In between, highly covalent bonds ($\alpha^2 \approx 50\%$) are stable, rendering [Fe₄S₄]²⁺ complexes the thermodynamic sink of FeS cluster conversion chemistry. This was exactly reflected in the reactivity observed in this work, where we demonstrated homolytic Fe-ligand bond cleavage for $[Fe_4S_4]^{3+}$ and $[Fe_4S_4]^{4+}$ complexes *versus* heterolytic bond cleavage in [Fe₄S₄]¹⁺, as well as that observed in previous studies, where we reported the aggregation of [Fe₄S₄]⁰ clusters *via* (heterolytic) loss of DmpS⁻ ligands.⁷⁶ Given that the reduction potential of the DmpS⁻ ligand (*ca.* -1.2 to -1.4 V vs. NHE; Figure S58) closely matches that of cysteinate (-1.38 to -1.45 V vs. NHE),⁸⁷ it appears plausible that similar considerations could govern the reactivity of natural Fe₄S₄ cofactors.

Furthermore, we suggest that the *ildc*, or a topologically similar cluster, could represent a key intermediate at which the biosynthetic pathway of the P- and M-cluster cofactors diverge. Considering the uncertainties surrounding whether the K1-/K2-clusters fuse to an interlocked topology before or after the C-atom transfer from SAM,⁵⁶ and whether the 8th sulfur of the P- cluster is lost prior to, during, or following cluster interlocking,^{66,67} it is plausible that an interlocked topology of fused Fe₄S₄ synthons serves as a precursor in both biosynthetic pathways, aligning with the fact that they are thought to be evolutionarily related.^{47,48}

CONCLUSION

Altogether, this work introduced an original approach to control FeS cluster conversion chemistry through alternating redox- and site-differentiation reactions. Based on this, we replicated the initial steps of M-cluster maturation, starting from a Fe₂S₂ cluster, and ultimately arriving at a mimic for the Fe₈S₈ K-cluster, within a reversible synthetic cycle. The isolation and characterization of all stable intermediate products allowed rationalizing the conditions driving each of the required (electro-)chemical steps and provides a framework to better understand and identify similar intermediates in the biogenesis of the P- and M-clusters.

DATA AVAILABILITY

The *Supporting Information* file (in PDF format) contains all synthetic procedures, experimental details, and characterization data relevant to this work. This includes UV-vis electronic absorption spectra, ¹H and ¹³C NMR spectra, cyclic voltammograms, elemental analyses, singlecrystal X-ray diffraction structures and further discussions on some of the spectroscopic and synthetic results. All raw data as it is shown in the figures of the main manuscript text and/or the *Supporting Information* file will be provided by the authors upon request. The *Supporting Information* file can be found online free of charge. All molecular structures derived from single-crystal X-ray diffraction analyses are deposited as crystallographic information files (.cif) in the Cambridge Structural Database (CSD) under the accession numbers 2389108-2389127 and 2389327-2389329. All data were analyzed using standard software plugins whenever appropriate, as described in the Methods section or the *Supporting Information* file. No custom code was written.

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Author Contributions

L.G. conducted the experiments with support from M.L.W. and H.S.; L.G. and G.B. analyzed data and visualized it. M.W., M.C., H.W., Y.Y. and S.P.C. contributed to spectroscopic data collection and analysis. L.G. and V.M. wrote the article with support from all authors; L.G. and V.M. designed and conceptualized the research; and V.M. supervised the project.

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Competing Interests

The authors declare no competing interests.

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METHODS

General Considerations

Detailed synthetic procedures as well as description of the instrumentation and additional characterization data for all discussed compounds are provided in the *Supporting Information* file to this article. This also includes elemental analyses, ¹H and ¹³C NMR spectra, cyclic voltammograms, ⁵⁷Fe Mössbauer spectra, as well as details on crystallography and ⁵⁷Fe NRVS spectroscopy. The following sections, however, summarize and discuss the key technical aspects of the syntheses presented in the main text of this work, together with a brief discussion of the compounds' basic spectroscopic properties. Unless otherwise stated, all these syntheses were carried out using dried and degassed toluene as solvent, under an Ar atmosphere, inside a glovebox.

Syntheses and Characterizations

The [2Fe-2S] Cluster

Upon dissolution of all-ferric [Fe₄S₄(DmpS)₄] in neat pyridine, single-crystalline di-ferric $[Fe_2S_2(DmpS)_2(py)_2]$ can be isolated in 77% yield from a pyridine solution layered with pentane (Figure 3C and S1). It has a characteristic ¹H NMR spectrum (Figure S27), accounting for all signals of the DmpS⁻ and pyridine ligands, and a UV-vis electronic absorption spectrum in line with those of similar synthetic or native $[Fe_2S_2]^{2+}$ clusters.⁸⁸⁻⁹¹ Furthermore, ⁵⁷Fe Mössbauer spectroscopy conducted at 5.3 K and 0.06 T as well as at 5.3 K and 7 T evidences nuclear parameters supporting the fact that it contains two high-spin ferric sites, which are antiferromagnetically coupled to adopt a S=0 spin ground state: δ =0.31 mm s⁻¹, ΔE_0 =0.85 mm s⁻¹ ¹ and η =1.00 (Figure S96). The 0.31 mm s⁻¹ isomer shift value is similar to that observed for ferric sites in biological 2Fe-2S clusters presenting a mixed Cys/His coordination as in IscR,92 MitoNEET⁹³ and Apd1 mutants.⁹⁴ In analogy to what has been reported by Tatsumi for [Fe₄S₄(N(TMS)₂)₄]/[Fe₂S₂(N(TMS)₂)(py)₂],⁷⁸ this reaction can be quantitatively reversed by treatment of [Fe₂S₂(DmpS)₂(py)₂] with stoichiometric amounts of B(C₆F₅)₃ (Figure S7A, S30 and S31). However, in contrast to Tatsumi's system, the Fe₄S₄ cluster scission does not proceed cleanly if pyridine is not used as a neat reagent in great excess (Figures S7B, S28 and S29). $[Fe_2S_2(DmpS)_2(py)_2]$ can be reduced over 1 equivalent of potassium graphite (KC₈), yielding the [Fe₄S₄]²⁺ cubane complex, K₂[Fe₄S₄(DmpS)₄]⁷⁵ (Figure 3C and S1A), as the product of fusion

between two transient $[Fe_2S_2]^{1+}$ rhombs. This behavior is reflected in the cyclic voltammogram of $[Fe_2S_2(DmpS)_2(py)_2]$ recorded in 1,2-difluorobenzene (*o*-DFB), wherein a cathodic current at – 1.21 V vs. Fc/Fc⁺ indicates an irreversible chemical step (Figure S59).

The [4Fe-4S] clusters

One-electron oxidation of $K_2[Fe_4S_4(DmpS)_4]$ with 1 equivalent of $[FeCp_2]PF_6$ followed by treatment with 1 equivalent of 1,2,4,5-tetramethylimidazole (Im*) results in the formation of the 3:1 site-differentiated cluster $K[Fe_4S_4(DmpS)_3(Im^*)]$ (Figure 3C). The K⁺ ion can be sequestered from the complex into 18-crown-6 ether or [2.2.2]-cryptand, yielding well-separated cation-anion pairs. The oxidation of $[Fe_4S_4(DmpS)_3(Im^*)]^-$ preserves the $[Fe_4S_4]^{2+/3+}$ architecture, forming $[Fe_4S_4(DmpS)_3(Im^*)]$, which can be further site-differentiated to the $[Fe_4S_4(DmpS)_2(Im^*)_2]$ cluster by treatment with an additional equivalent of Im* (Figure 3C). Alternatively, $[Fe_4S_4(DmpS)_2(Im^*)_2]$ can be synthesized from all-ferric $[Fe_4S_4(DmpS)_4]$, upon treatment with 2 equivalents of Im* (Figure 3C). Treatment of $[Fe_4S_4(DmpS)_2(Im^*)_2]$ with stoichiometric amounts of DmpSK causes substitution of the Im* ligands, restoring the canonical $[Fe_4S_4]^{2+}$ cubane, $K_2[Fe_4S_4(DmpS)_4]$, *via* $K[Fe_4S_4(DmpS)_3(Im^*)]$ as intermediate (Figure S57).

All the site-differentiated cubane clusters present ¹H NMR (and ¹³C NMR, where applicable) spectra, accounting for the protons on all ligands (Figures S32-S38 and S41-S45). Additionally, all Fe₄S₄ complexes, save for ^[2.2.2]K[Fe₄S₄(DmpS)₃(Im*)], have been structurally characterized in the solid state by single-crystal X-ray diffraction analyses (Figures S76-S79). Between $[Fe_4S_4(DmpS)_4]^{2-}$, $[Fe_4S_4(DmpS)_3(Im^*)]^-$ and $[Fe_4S_4(DmpS)_2(Im^*)_2]$, sequential Im* substitution, decreases the net extinction of the UV-vis electronic absorption spectrum as it concomitantly becomes broader and absorbs over a wider energy range (Figure S15A). Thereby, however, the energy position of the extinction maxima appears to remain constant. Between $[Fe_4S_4(DmpS)_3(Im^*)]$, The UV-vis electronic absorption spectra are similar, but the main extinction maximum redshifts from 469 nm in $[Fe_4S_4(DmpS)_3(Im^*)]$ to 489 nm in $[Fe_4S_4(DmpS)_3(Im^*)]$. Furthermore, cyclic voltammetry evidences that each Im* substitution of one of the thiolate ligands shifts the cluster's redox potential by *ca.* +450 mV (Figure S60-S67), both for the $[Fe_4S_4]^{2+/3+}$ and the $[Fe_4S_4]^{1+/2+}$ redox couples. In analogy to our previous work, the voltammograms appear different in presence and absence of K⁺ cations in the electrolyte, as we demonstrate, for example for $[Fe_4S_4(DmpS)_3(Im^*)]^{0/-}$ (Figure S63).

Among all $[Fe_4S_4]^{n+}$ oxidation states, the all-ferric one appeared to hold a special place, because it exhibited a broad array of reactivity: As detailed in the *Supporting Information*, a variety of conditions involving the presence of neutral, weak N-donor molecules (besides Im*) leads to reductive site-differentiation, cluster scission, FeS cluster aggregation, or even cluster disruption *via* Fe-removal from the cubane core (Figure S4).

The [8Fe-8S] clusters

Reduction of K[Fe₄S₄(DmpS)₃(Im^{*})] over 1 equivalent of KC₈ results in the formation of the edgebridged [Fe₈S₈]²⁺ double-cubane, K₄[Fe₈S₈(DmpS)₆] (*ebdc*, Figure 3C and 4A) in which the [Fe4S4]¹⁺ complex preferably undergoes self-fusion rather than maintaining its 3:1 sitedifferentiated structure with a bound Im* ligand. *ebdc* is characterized by a poorly resolved ¹H NMR spectrum, both in THF-d8, and in C₆D₆, suggesting a dynamic behavior in solution as well as strong paramagnetism and its UV-vis electronic absorption spectrum shows a nearly featureless rise in extinction at lower wavelength, with two discernible shoulders at 405 and 336 nm (Figure 5A). In the solid state, *ebdc* crystallizes in the space group $P\overline{1}$, containing two cubanes linked *via* one of their respective edges. Both are coordinated by three DmpS⁻ ligands, yet they display distinct coordination environments in the second sphere: One of the cubanes maintains close contacts with three K^+ ions (K2, K3, K4), whereas the other interacts closely with only one K^+ ion (K1) and shares another (K2) with the first cubane. The cyclic voltammogram of ebdc recorded in a 0.1 M solution of ["Bu₄N][PF₆] in *o*-DFB exhibits a large number of quasi-reversible redox waves between -1.88 V and -0.11 V vs. Fc/Fc⁺ (Figure S68A). The near perfect overlap of two consecutive scans of the voltammogram at all the investigated scan rates (from 10 mV s⁻¹ to 1 V s^{-1}) suggests that all observed processes are chemically reversible. By analogy with what we had described for the canonical Fe₄S₄ complexes,⁷⁵ the scan rate dependency of the anodic peak potentials of the four oxidative events (Figure S68B) is associated with the kinetics of K⁺ ion decoordination from the structure. In addition, and similar to the behavior of other K⁺-ion containing FeS clusters reported by us,^{75,76} the voltammogram of *ebdc* appears different if recorded in a 0.1 M K[BArF₂₄] solution in THF as supporting electrolyte (Figure S69).

Stirring of a toluene solution of *ebdc* over 4 equivalents of $[FeCp_2]PF_6$, followed by fast crystallization of the product from the concentrated mother liquor by layering with pentane, leads to the formation of single-crystals of the ligand-bridged double-cubane, $[Fe_8S_8(DmpS)_6]$ (*lbdc*,

Figure 4B). In the solid state, *lbdc* crystallizes in the space group $P2_1/c$, exhibiting two $[Fe_4S_4]^{3+}$ cubanes bridged via two μ^2 (DmpS⁻) ligands (Figure 4B). The four-electron oxidation is reversible, as demonstrated by treating *lbdc* with 4.1 equivalents of KC₈, which results in the regeneration of ebdc (Figure 3C). In C₆D₆ solution, the lbdc's bridging ligands are clearly distinguishable by their ¹H NMR spectroscopic signature which differs from that of the terminal $\mu^{1}(\text{DmpS}^{-})$ ligands (Figure S51). This strongly suggests that the ligand-bridged structure is preserved in solution, with the inequivalence of the two sets of signals arising from distinct magnetic environments around the bridging and terminal ligands.⁹⁵ Obtaining such a ligand-bridged structure is only possible in toluene, or other non-coordinating solvents. Dissolution of *lbdc* in THF immediately leads to the scission of the double-cubane and the formation of 3:1 site-differentiated [Fe₄S₄(DmpS)₃(THF)₃], in direct analogy with the reactivity observed with the all-ferric cubane [Fe₄S₄(DmpS)₄]⁷⁷ (Figure S84). *Ibdc* is further characterized by an electronic absorption spectrum with a large molar extinction coefficient of its maximum (46.9 \cdot 10³ at 471 nm; Figure 5A), which has a similar energy as that observed for *lbdc's* canonical and 3:1 site-differentiated redox congeners.⁷⁵ The cyclic voltammogram of *lbdc*, recorded in *o*-DFB, is very close to that of the canonical all-ferric cubane, [Fe₄S₄(DmpS)₄], showing two quasi-reversible features at -1.00 V and -0.18 V as well as a broad current response between them, at -0.52 V, and an irreversible reduction at -2.34 V vs. Fc/Fc⁺ (Figure S70). Notably, our attempts to generate Fe_8S_8 clusters in oxidation states between $[Fe_8S_8]^{2+}$ (that of *ebdc*) and $[Fe_8S_8]^{6+}$ (that of *lbdc*) by treating *ebdc* with <4 equivalents of oxidant were unsuccessful, as we repeatedly crystallized either one or the other (or a canonical Fe₄S₄) complex from the reaction mixture. This indicates the occurrence of redox disproportionation and ligandscrambling equilibria.

Upon prolonged standing of a dilute toluene solution of *lbdc* layered with pentane, single-crystals of the interlocked double-cubane, $[Fe_8S_8(DmpS)_4]$ (*ildc*, Figure 3C and 4C), form as the main product, albeit in low yield (*ca.* 15-40%, depending on the duration of the crystallization process). Relatedly, monitoring a dilute C₆D₆ solution *via* ¹H NMR spectroscopy over the course of 10 days shows that it slowly forms *ildc* as major product, firmly establishing *lbdc* as a metastable intermediate in the pathway to *ildc* (Figures S54-S56 and Note S4). The corresponding ¹H NMR spectrum of *ildc*, recorded in C₆D₆ solution, shows a complex set of signals in the diamagnetic region of the spectrum (Figure S49). This complexity arises from the different coordination-modes of the two sets of DmpS⁻-ligands: Two of these (S8) coordinate in a μ^1 - and two (S6 and S7) in a

 μ^2 -fashion (Figure 4C). Additional splitting of some of the signals suggests that the rotation of the mesitylene groups is sterically hindered, rendering the *endo/exo*-Me protons of the $\mu^2(\text{DmpS}^-)$ ligand inequivalent. In the solid state, *ildc* crystallizes in the space group $P2_1/m$, only half of the molecule present in the asymmetric unit. Unfortunately, our efforts to accelerate and promote the formation of *ildc* with heat or light irradiation have so far been unsuccessful. Alternative synthetic routes were also explored, starting from [Fe₄S₄(DmpS)₄] and [Fe₄S₄(DmpS)₂(Im*)₂], but they also failed (refer to the Supporting Information file for additional details). Notably, however, we observed that, in analogy to the reduction of K[Fe₄S₄(DmpS)₃(Im*)] yielding *ebdc*, the reduction of [Fe₄S₄(DmpS)₂(Im*)₂] over 2 equivalents of KC₈ yields an edge-bridged [Fe₁₂S₁₂]⁰ triplecubane K₆[Fe₁₂S₁₂(DmpS)₆] (*ebtc*; Figure S3). Furthermore, while removal of Im* from $[Fe_4S_4(DmpS)_2(Im^*)_2]$ by B(C₆F₅)₃ does indeed occur, forming Im^{*}·B(C₆F₅)₃ (Figure S86), the resulting putative coordinatively unsaturated [Fe₄S₄(DmpS)₂] cubanes do not fuse and interlock in a well-defined manner. Instead, this reaction led to the identification of $[Fe_{24}S_{24}(DmpS)_{10}]$ among the reaction products, which is-to the best of our knowledge-the largest molecular FeS cluster ever characterized by single-crystal X-ray diffraction (Figures S2 and S87), spanning ca. 2 nm in diameter, and the product of uncontrolled FeS cluster aggregation. Similarly, attempts to generate *lbdc* by treating $[Fe_4S_4(DmpS)_3(Im^*)]^{77}$ with $B(C_6F_5)_3$ (Figure S17) were also unsuccessful. These failed attempts underscore the importance of the formation of *lbdc* from *ebdc* in a controlled fashion, and in absence of coordinating molecules, for its subsequent conversion to *ildc*.

ildc exhibits a UV-vis electronic absorption spectrum, characterized by an intense peak at 467 nm and a shoulder at 631 nm. Its cyclic voltammogram recorded in a 0.1 M solution of ["Bu₄N][PF₆] in DCM exhibits two quasi-reversible currents at -0.58 and -1.19 V vs. Fc/Fc⁺, putatively marking the reductions of the [Fe₈S₈]⁴⁺ core to the [Fe₈S₈]³⁺ and [Fe₈S₈]²⁺ oxidation states, respectively. Despite this apparent robustness at the electrochemical timescale, *ildc's* structural integrity is not maintained upon treatment with a competing ligand: Addition of 4 equivalents of Im* to a toluene solution of *ildc* leads to the formation of [Fe₄S₄(DmpS)₂(Im*)₂] (Figure 3C and S79), resulting from the "unlocking" and scission of the structure.

Simulation of [8Fe-8S] Mössbauer spectra

The low- or zero-field 80 K powder spectra of ebdc, lbdc and ildc are displayed in Figure 5C-E as black vertical bars. Whereas two absorption lines are observed for *ebdc* and *lbdc*, four are detected for *ildc*. Due to the large linewidth and the lack of symmetry of *ebdc* according to its solid-state structure, a simulation is presented in Figure 5C assuming four doublets, sharing the same linewidth and contribution (see the overlaid grey line). The proposed solution is obviously not unique (refer to the Supporting Information, Figures S97 and S98, Note S6 and Table S11, for a discussion). However, all the simulations present the same average isomer shift value, namely 0.53 mm s⁻¹. This high value indicates a major ferrous character and is indeed similar to those observed for [Fe₄S₄]¹⁺ clusters.^{75,96-98} Two doublets in a 1:1 ratio allowed to reproduce the spectrum of *lbdc* (Figure 5D). Those displayed in Figure 5D present isomer shifts that differ by more than 0.1 mm s⁻¹, while a 0.05 mm s⁻¹ difference is determined for the other pairing (Figure S99). Similarly to *ebdc*, whatever the simulation, a 0.39 mm s^{-1} average isomer shift value is deduced. This value indicates a higher oxidation state for this cluster and is consistent with [Fe₄S₄]³⁺ cubanes.^{75,99,100} In contrast, the four distinct lines in the *ildc*'s spectrum can be paired in a single way, forming two equally contributing nested doublets (Figure 5E) with 0.34 mm s⁻¹ and 0.68 mm s⁻¹ isomer shift values, respectively. In contrast, the two intertwined doublets are centered at 0.09 and 0.93 mm s⁻¹, which are unreasonably low and high isomer shift values, respectively, for Fe ions in sulfide-/thiolate-ligated tetrahedral environment. The four 5.7 K spectra reproduced in Figure 6 were simultaneously simulated using a homemade program (see Supporting Information). In analogy to the simulation of the zero-field 80 K spectrum, two diamagnetic Fe sites were considered in a 1:1 ratio. It can be noticed that the line positions are satisfyingly reproduced but not the intensities. We thus suspected the contribution of a close lying paramagnetic excited state. Accordingly, four Fe sites with fictitious 1/2-electronic spin and interacting by pairs were considered to reproduce the spectra recorded at 2, 4 and 7 T. The same anisotropic exchange interaction was assumed in the two pairs in order that the four sites experienced the same spin ladder ($\widehat{S_a} \, \widehat{J_{ab}} \, \widehat{S_b}$ convention). Anisotropy then allowed introducing a ZFS effect in the excited S=1 state. To reduce the number of unknowns, isotropic hyperfine interactions were considered. This hypothesis led to significant improvement of the simulation of the 7 T-spectrum, and only to a marginal one at lower field (see Supporting Information; Figure

S100). Therefore, while this model sustained the hypothesis of magnetic contributions, it may still be too restricted to reproduce all the observed features.

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