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Review

Computational Mechanistic Insights into Non-Cubane Iron-Sulfur Cluster-Dependent Enzymes with [2Fe–2S] and [4Fe–4S] Cores: A Review

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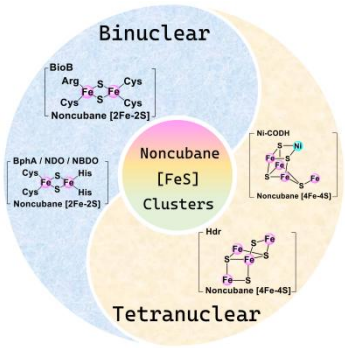
Abstract: Iron-sulfur ([FeS]) clusters are among the most ancient and functionally diverse cofactors in biology, mediating processes ranging from electron transfer to substrate activation and catalysis. Within this broad class, non-cubane [FeS] clusters exhibit outstanding structural flexibility and chemical versatility. This review summarizes recent computational advances in elucidating the mechanisms of non-cubane [FeS] cluster-dependent enzymes, focusing on systems containing [2Fe–2S] and distorted [4Fe–4S] clusters. Representative enzymes discussed include biotin synthase (BioB), Rieske dioxygenases (NDO, BphA, NBDO), heterodisulfide reductase (Hdr), and carbon monoxide dehydrogenases (CODHs), that perform diverse chemical transformations such as sulfur insertion, aromatic cis-dihydroxylation, S–S bond cleavage, and CO_2/CO interconversion. Special emphasis is placed on how quantum chemical cluster modelling and QM/MM simulations have provided insights into transient intermediates, rate-determining steps, and the roles of metal nuclearity and heterometal incorporation in tuning catalytic reactivity.



Key words: computational chemistry, non-cubane [FeS] cluster, metalloenzyme, reaction mechanism.

1. Introduction

Iron-sulfur ([FeS]) clusters are among the most ancient bioinorganic cofactors and have played significant roles in the evolution of cellular life [1,2]. They are essential components of a wide range of proteins and metalloenzymes, including radical *S*-adenosylmethionine (RS) enzymes [3], Rieske dioxygenase (RDOs) [4], carbon monoxide dehydrogenases (CODHs) [5], nitrogenases [6], and hydrogenases [7,8]. [FeS] clusters serve diverse functions such as electron transfer [9], catalysis [3], structural stability [10], sensor [11], and sulfur donation [12]. These versatile functional roles are closely linked to the structural diversity of [FeS] clusters. While the cubane-type [4Fe-4S] cluster has been extensively studied [13], non-cubane [FeS] clusters have attracted increasing attention for their notable conformational flexibility and high chemical reactivity.

[FeS] clusters are composed of iron and sulfur atoms and are generally denoted as "[*m*Fe-*q*S]^n", where *m*, the nuclearity, represents the number of iron atoms (ranging from 2 to 18), *q* denotes the number of bridging sulfide ligands (typically 1 to 30), and *n* indicates the overall cluster charge [14]. The variability in nuclearity (m) underpins the remarkable structural diversity of [FeS] clusters, which in turn dictates their functional and reactive properties. At low nuclearity (e.g., *m* = 2), [FeS] clusters commonly adopt nonlinear configurations in biological systems [15], such as the rhomboidal geometry of [2Fe-2S] clusters [16]. As the nuclearity increases, more complex architectures emerge, including cuboidal (*m* = 3) [17], cubane (*m* = 4) [18], prismatic or basket (*m* = 6) [19], monocapped prismatic (*m* = 7) [20], rhombic

dodecahedral (m = 8) [21], and edge-bridged tetracubane (m = 16) clusters [21]. Among these, the cubane-type [4Fe-4S] cluster (Figure 1.A) is the most extensively studied due to its central role in electron transfer reactions [22]. In this structure, three iron ions are typically ligated by cysteine residues from the protein, while the fourth iron serves as a binding site for an additional cysteine or other cofactors [22].

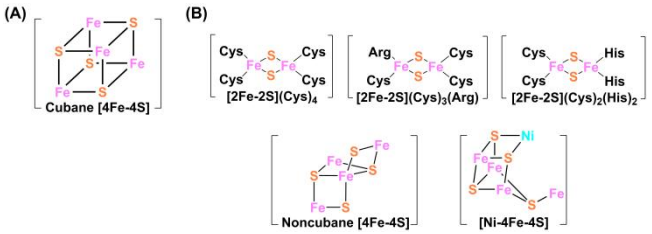
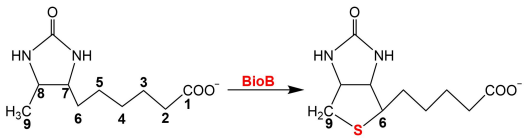
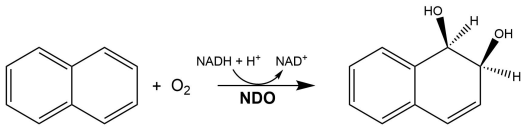
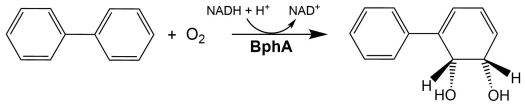
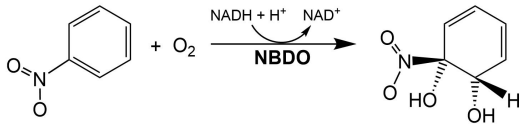
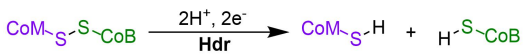
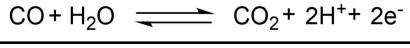


Figure 1. Structural diversity of iron–sulfur ([FeS]) clusters. (A) The canonical cubanetype [4Fe-4S] cluster. (B) Two representative non-cubane [FeS] clusters with nuclearities of 2 or 4.

Beyond the canonical cubane-type cluster, growing attention has been directed toward non-cubane [FeS] clusters in living organisms. This expanding class of [FeS]-dependent enzymes continues to reveal novel chemical properties and biochemical functions. Recent studies have shown that non-cubane [FeS] clusters possess diverse coordination environments and pronounced structural plasticity, enabling them to participate in functions beyond electron transfer [23-26]. Due to the vast diversity of [FeS]-containing proteins and their wide-ranging biological roles, a comprehensive rationalization remains challenging. Therefore, this review focuses primarily on non-cubane [FeS] clusters with nuclearity of 2 or 4, the two most common forms (Figure 1.B) [27].

In binuclear rhomboidal configurations, the [2Fe-2S] core ligated by four conserved cysteine residues, $[2Fe\text{-}2S](Cys)_4$, is the predominant motif, commonly found in plant-type ferredoxins (Fd) involved in electron transport chain [28]. An unusual [2Fe–2S] configuration has also been identified in the RS enzyme superfamily [22], where the cluster features three cysteine ligands and one conserved arginine, forming $[2Fe\text{-}2S](Cys)_3(Arg)$ [29]. This unique cluster acts as a sulfur donor during reaction [22]. Another distinct coordination pattern, $[2Fe\text{-}2S](Cys)_2(His)_2$, defines the Rieske-type dioxygenases, which catalyze the dihydroxylation of recalcitrant aromatic compounds [30] and hold great promise for bioremediation applications [31].

Table 1. Reactions catalyzed by non-cubane [FeS] cluster-containing enzymes.

Nuclearity	Enzyme	Reaction	[FeS] Function
2	BioB		Sulfur donor
	NDO		Electron transfer
	BphA		Electron transfer
	NBDO		Electron transfer
4	Hdr		Electron transfer
	CODHs		Substrate binding/activation Electron transfer