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PNNL-31947

Structural and Dynamical Design Principles for Iron-Sulfur Clusters in Sequence Defined Polymers

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U.S. DEPARTMENT OF ENERGY

Prepared for the U.S. Department of Energy
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Overview

This integrated synthetic and computational effort uses sequence defined polymers such as peptoids, and triazines, capable of mimicking a flexible protein environment to control the positioning of side chains around redox cofactors. This strategy will allow modulation of redox properties through both direct and indirect mechanisms. To this end the aim is defined to test the underlying hypothesis: *that principles and critical functionalities governing the binding and tuning of naturally occurring electron carrier co-factors can be identified and used to predictably design and synthesize maquettes based on non-natural polymers resulting in new modular biomimetic scaffolds for Electron Transfer Materials*. This will be achieved through design and synthesis of ligands that can stabilize iron-sulfur clusters and tune the redox potential based on sequence.

Introduction

Electron transfer (ET) is one key mechanism in nature that is crucial for the conversion of energy sources, such as light, to usable forms for chemical transformations, as well as in catalysis of oxidation/reduction reactions. The biological structures that function in a manner similar to electronic wires are mainly constructed of insulating organic polymers, which make up about 80% of a cell. Most of this electronic conduction system is made of metalloproteins that participate in metabolic redox reactions and mediation of biological ET reactions.

These proteins behave as central energy-conserving redox hubs, serving as conduits between multiple redox donors and acceptors. The redox potential is the major contributor in controlling the ET rates and thus regulating the ET processes in bioenergetics. To maximize the efficiency of ET processes, tuning of the redox potential is essential. The local structure around the redox centers and the exquisite organization in biological systems act in concert to facilitate efficient ET and energy conversion; establishing a paradigm that serve as an inspiration for bioengineering or biomimetic approaches.

The engineering of natural proteins relies, in part, on identifying the key structural and energetic terms selected to carry out each specific enzymatic mechanism. It is generally recognized that ET performance is enabled by a highly evolved three-dimensional environment around the active site. Precisely positioned functional groups control different aspects of the ET process. Yet, significant gaps remain in our understanding of this environment and how it controls the precise transfer of electrons. For instance, this environment is predicted to structurally stabilize the redox site; tune the redox potentials and provide basic sites for proton delivery. While there are cases where the scaffold is known to serve specific functions, the mechanistic details are not generally understood, providing a significant gap in our knowledge, and crippling our ability to introduce these principles into other systems. The properties of the scaffold may also be further controlled by conformational dynamics occurring on diverse length and time scales, the

measurement of which is difficult, representing another gap limiting our understanding of the role of the scaffold.[1]

One promising framework is the use of non-natural sequence defined polymers. In particular N-substituted glycine oligomers (peptoids) are an attractive choice, as side chain chemistry and sequence can be precisely tuned with synthetic ease. Moreover, due to the shift of the side-chain position, backbone amide H-bond donors are removed in peptoids compared to peptides, which simplifies intermolecular interactions and thereby design. Thus, peptoid (secondary) structures depend predominantly on design rules specifying the selection of (chiral) side chains. [2] Due to the structural similarities with amino acids, computational approaches for structure prediction can be adopted in a facile manner. [3,4] Another sequence defined polymer that can be combined in a seamless manner with peptoids is the triazine framework developed recently at PNNL. [5] Triazines feature a more complex backbone that introduces hydrogen bonding and π-π-interactions as design elements, as well as a large chemical side chain library. Our proposed synthetic and computational effort will use sequence defined polymers, such as peptoids and triazines, capable of mimicking a flexible protein environment to control the positioning of side chains around redox cofactors. We hypothesize that this strategy will allow modulation of redox properties, resulting in novel biomimetic electron carriers with tunable chemical environment.

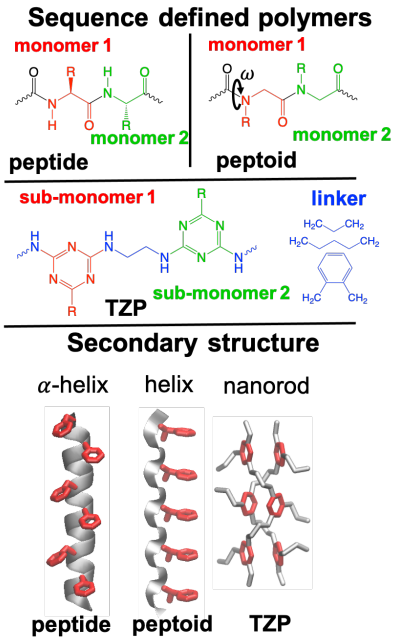

Fig 1. Comparison of peptide, peptoid, and TZP backbone chemistry and known secondary structure elements highlighting aromatic sidechains for a synthetic α-helical conducting peptide and a prototypical peptoid helix, with backbone elements in red, and a dimer of TZP trimers.

Results

One important observation is the preferential tetrahedral coordination of the iron in [4Fe-4S], which predetermines the optimal position of ligating moieties (**Fig. 2**). Chemistry-centered efforts, inspired by FeS-containing proteins, have resulted in a well-established protocol for the synthesis of FeS emulating naturally occurring compositions. This afforded the ability to both mimic the local binding motif and modulate the preferred oxidation state and reduction potential. Control of the reduction potential is achieved through the chemical diversity of ligands. Ideally, up to four different ligands can be placed around the [4Fe-4S], but site selectivity, or control over the arrangement of multiple ligands with a predefined order, is challenging. In general, the control of second coordination sphere effects is difficult.

In contrast, the selection of natural amino acids as building blocks introduced another constraint for the optimization of [4Fe-4S] binding. The challenge presented by geometric restriction due to iron tetrahedrality is often solved with a common motif in which usually three ligands are found in a so-called consensus sequence. The preferred spacing in the consensus sequence, with two cysteines separated by two residues, is unique to cysteine and not found for other amino acids with this high occurrence. In consensus sequence the rare and only redox active amino acid can be protected by forming a disulfide bond. Therefore, the spacing are not only found in FeS or single ion binding motifs.

Analysis of available crystal structures reveals a structural design in which the FeS resides in an unstructured loop coordinated with three ligands. The coordination sphere is completed with the fourth ligand, which is often part of the secondary structure element.

Inspired by the idea to combine a flexible backbone that could allow a predetermined arrangement of ligands with the available chemical diversity, we demonstrated the feasibility of binding [4Fe-4S] to these polymers via mercapto ligands from the side chains. Experimental results demonstrate that the ligand exchange reaction between [Fe4-S4](tert-butyl-thiol)$_4$, peptoids, and TZP as a synthetic route is possible. Cyclic voltammetry measurements in DMSO show the stability of [4Fe-4S] and ligand-dependent shifts (**Fig. 2**). Modeling shows that, unlike many model [4Fe-4S]-containing complexes, these polymers can also provide -NH hydrogen-bond donors to the cluster S atoms, mimicking natural protein amide NH groups found to directly interact with [4Fe-4S].

Simulations of short polymers with up to 12 monomer units with varying side chains demonstrate that both peptoids and TZPs can adopt unstructured conformations with side chain spacing that would allow for optimal ligating of [4Fe-4S] given the geometric constraints (**Fig. 2**). Both peptoids and TZPs offer an almost unlimited library of side chains to choose from and show secondary structural elements (see **Fig. 1**) that can be tuned by the sequence of sidechains and/or linkers.

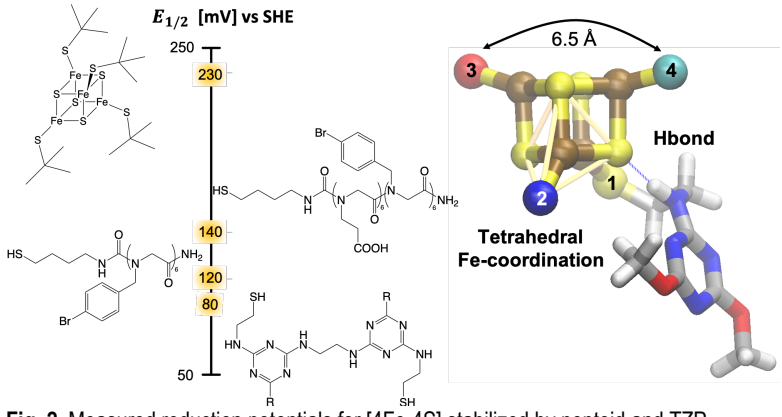

Fig. 2. Measured reduction potentials for [4Fe-4S] stabilized by peptoid and TZP ligands. Hydrogen bond formed by TZP side chains to bridge S, similar to the HiPIP protein. [4Fe-4S] with tetrahedral coordination defined ligand sites.

These synthetic sequence-defined polymers meet the prerequisites for designing sequences that can bind and stabilize [4Fe-4S] and fold into structures that can be arranged into sequences of modules by noncovalent assembly or covalent linking. The high density and alignment of aromatic residues for the peptoid helix with aromatic side chains, as well as the TZP nanorod with aromatic backbone elements (see Fig. 2), also make these polymers ideal candidates to be explored as molecular wires between redox cluster elements.

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