

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of it

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE 5 Aug 03	3. REPORT TYPE AND DATES COVERED Final report 1 May 96-30 Apr 99
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4. TITLE AND SUBTITLE Signal Transduction by Designed Metal-Binding Proteins	5. FUNDING NUMBERS N00014-95-1-0913
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9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) Office of Naval Research 800 N. Quincy St. Arlington, VA 22217-5000	10. SPONSORING / MONITORING AGENCY REPORT NUMBER
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11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Distribution Unlimited

20030829 045

13. ABSTRACT (Maximum 200 words)

The objective of this research is to develop designed metal-binding proteins to report upon the presence of metal ions in solution. We use small, robust protein frameworks as scaffolds on which to design novel metal-ion binding sites. The metal binding site design is computational, to allow an exhaustive sampling of all possible sites. The sites are designed with defined geometries and a variety of primary ligands to metal, which allow a range of different metal-ion binding specificities and affinities to be arrayed. The aim is to couple the metal-binding event to a change in fluorescence of an appropriate probe. For practical applications, reagent-less systems are preferred, but in the development stages, extrinsic reporter probes are also investigated.

14. SUBJECT TERMS metal binding rubredoxin protein design IgG	15. NUMBER OF PAGES 3
16. PRICE CODE	

17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT UL
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FINAL REPORT

Grant #: N00014-95-1-0913

PRINCIPAL INVESTIGATOR: Prof. Lynne Regan

INSTITUTION: Yale University

GRANT TITLE: Signal Transduction by Designed Metal-Binding Proteins

AWARD PERIOD: 1 May 1996 - 30 Apr 1999

OBJECTIVE: To develop designed metal-binding proteins to report upon the presence of metal ions in solution

APPROACH: We use small, robust protein frameworks as scaffolds on which to design novel metal-ion binding sites. The metal binding site design is computational, to allow an exhaustive sampling of all possible sites. The sites are designed with defined geometries and a variety of primary ligands to metal, which allow a range of different metal-ion binding specificities and affinities to be arrayed. The aim is to couple the metal-binding event to a change in fluorescence of an appropriate probe. For practical applications, reagent-less systems are preferred, but in the development stages, extrinsic reporter probes are also investigated.

ACCOMPLISHMENTS: We have designed a number of variants of the B1 domain of IgG-binding protein G that bind metal ions. The sites are at different positions in the protein and are formed by different combinations of primary ligands to metal (His and Cys combinations). The designed sites bind metals with a range of affinities, from sub-nanomolar to micromolar. They show a range of metal-ion binding specificities, with 1000-fold higher affinity for Zn versus Co at tetrahedral sites. We have shown that for a number of these sites there are changes in intrinsic Trp fluorescence upon metal-ion binding. The wavelength of Trp fluorescence emission is not ideal for practical sensing purposes (too short). We have therefore also investigated the effect of metal-ion binding upon the fluorescence of a hydrophobic dye, ANS. ANS is thought to interact with exposed hydrophobic patches on a protein. Several of our designed metal-ion binding proteins show differences in both the intensity and wavelength maximum of ANS fluorescence upon interaction with metal ions. Presumably, metal-ion binding induces conformational changes in the proteins, which can be detected by changes in their interaction with ANS. The observation of changes in wavelength maximum of emission in response to metal ion binding is particularly exciting, because it allows for ratiometric sensing, with its many attendant advantages.

In collaboration with Prof. David Walt, Tufts University, also supported by the ONR, we have immobilized these proteins in gels at the ends of fiber-optic cables, in the system that the Walt group has developed. The immobilized proteins plus ANS are able to detect the presence of low concentrations of Zn ions in beakers of test solution; there is no such response from control solutions.

CONCLUSIONS: The strategy holds great potential for the development of sensors for metal ions in the range of concentrations that is of interest to the ONR.

SIGNIFICANCE: Demonstration of how rational design of metal-ion binding sites can be linked with signal transduction system.

PATENT INFORMATION: None

AWARD INFORMATION: Herbert Dickerman Award, Wadsworth Center, New York, for "Exceptional Creativity in Research"

PUBLICATIONS AND ABSTRACTS:

"'Morphs' (MRFs): metal-reversible folding domains for differential IgG binding" Marino S.F., Shechner D., Regan L. *Chem Biol.* 2001 8:1221-9.

"Secondary ligands enhance affinity at a designed metal-binding site" Marino S.F., Regan L. *Chem Biol.* 1999 6:649-55.

"The *de novo* design of a rubredoxin-like Fe site" Farinas E., Regan L. *Protein Sci.* 1998 7:1939-46.