REPORT	DOCI	JMENTA	TION	PAGE
--------	------	--------	------	------

5

:

Form Approved - OMB No. 0704-0188

Public reporting burden for this collection of in	formation is estimated to average 1 h	our per response, including the time		schola, searching existing data	
sources, gathering and maintaining the data needed, an	d completing and reviewing the collec	tion of information. Send comments	regarding this bure	den estimate or any other aspect of it	
1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND Final report	1 May 04	6 20 Amm 00	
	5 Aug 03	rimai report	I Hay 90	5-30 APF 99	
4. TITLE AND SUBTITLE	• • • • • •		5. FUNDING N	NUMBERS	
Signal Transduction	hy Decigned Matel	Dinding Ductoins	10001/		
Signal Hansudelion	by Designed Metal-	binding Proteins	N00014-9	95-1-0913	
]		
6. AUTHOR(S)					
Lynne Regan, Ph.D.					
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)				8. PERFORMING ORGANIZATION REPORT NUMBER	
Professor Lynne Rega					
Dept. of Molecular B	iophysics and Bioc	hemistry			
Yale University					
PO Box 208114					
New Haven, CT 06520-	8114				
9. SPONSORING / MONITORING AGEN	CY NAME(S) AND ADDRESS(ES)	· ·		RING / MONITORING REPORT NUMBER	
Office of Naval Resea					
800 N. Quincy St.	~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~			2	
	5000		1		
Arlington, VA 22217-	0000				
			1		
11. SUPPLEMENTARY NOTES				· · ·	
		•	•		
				N	
				1	
				P	
128. DISTRIBUTION / AVAILABILITY S	TATEMENT				
		200	Z002	0 0/5	
12a. DISTRIBUTION / AVAILABILITY S		200	3082	9 045	
		200	3082	9 045	
		200	3082	29 045	
		200	3082	29 045	
Distribution Unlimi	ited	200	3082	29 045	
	ited	200	3082	29 045	
Distribution Unlimi	lted		I		
Distribution Unlimi 13. ABSTRACT (Maximum 200 words) The objective of th	ited	o develop design	ed metal		
Distribution Unlimi 13. ABSTRACT (Maximum 200 words) The objective of the binding proteins to	ited nis research is t o report upon the	o develop design presence of met	ed metal al ions	 in	
Distribution Unlimi 13. ABSTRACT (Maximum 200 words) The objective of the binding proteins to solution. We use st	ited his research is t o report upon the mall, robust prot	o develop design presence of met ein frameworks a	ed metal al ions s scaffo	 in lds	
Distribution Unlimi 13. ABSTRACT (Maximum 200 words) The objective of the binding proteins to	ited his research is t o report upon the mall, robust prot	o develop design presence of met ein frameworks a	ed metal al ions s scaffo	 in lds	
Distribution Unlimi 13. ABSTRACT (Maximum 200 words) The objective of the binding proteins to solution. We use st	ited nis research is t p report upon the mall, robust prot novel metal-ion	o develop design presence of met ein frameworks a binding sites. T	ed metal al ions s scaffo he metal	- in lds	
Distribution Unlimi 13. ABSTRACT (Maximum 200 words) The objective of the binding proteins to solution. We use solution. We use solution which to designed binding site designed	ited nis research is t o report upon the nall, robust prot novel metal-ion n is computationa	o develop design presence of met ein frameworks a binding sites. T l, to allow an e	ed metal al ions s scaffo he metal xhaustiv	- in lds e	
Distribution Unlimi 13. ABSTRACT (Maximum 200 words) The objective of the binding proteins to solution. We use so on which to designe binding site designe sampling of all pose	ited his research is t o report upon the mall, robust prot novel metal-ion h is computationa ssible sites. The	o develop design presence of met ein frameworks a binding sites. T l, to allow an e sites are desig	ed metal al ions s scaffo he metal xhaustiv ned with	- in lds e	
Distribution Unlimit 13. ABSTRACT (Maximum 200 words) The objective of the binding proteins to solution. We use so on which to designe binding site designed binding of all posed defined geometries	ited nis research is t o report upon the mall, robust prot novel metal-ion n is computationa ssible sites. The and a variety of	o develop design presence of met ein frameworks a binding sites. T l, to allow an e sites are desig primary ligands	ed metal al ions s scaffo he metal xhaustiv ned with	- in lds e	
Distribution Unlimit 13. ABSTRACT (Maximum 200 words) The objective of the binding proteins to solution. We use solution. We use solution which to designe binding site designed binding site designed binding of all posed defined geometries which allow a ranged	nis research is t preport upon the mall, robust prot novel metal-ion n is computationa ssible sites. The and a variety of e of different me	o develop design presence of met ein frameworks a binding sites. T l, to allow an e sites are desig primary ligands tal-ion binding	ed metal al ions s scaffo he metal xhaustiv ned with to meta	- in lds e	
Distribution Unlimit 13. ABSTRACT (Maximum 200 words) The objective of the binding proteins to solution. We use so on which to designe binding site designed binding of all posed defined geometries	nis research is t preport upon the mall, robust prot novel metal-ion n is computationa ssible sites. The and a variety of e of different me	o develop design presence of met ein frameworks a binding sites. T l, to allow an e sites are desig primary ligands tal-ion binding	ed metal al ions s scaffo he metal xhaustiv ned with to meta	- in lds e	
Distribution Unlimit 13. ABSTRACT (Maximum 200 words) The objective of the binding proteins to solution. We use solution. We use solution which to designe binding site designed binding site designed binding of all posed defined geometries which allow a ranged	ited his research is t o report upon the mall, robust prot novel metal-ion h is computationa ssible sites. The and a variety of e of different me affinities to be	o develop design presence of met ein frameworks a binding sites. T l, to allow an e sites are desig primary ligands tal-ion binding arrayed. The aim	ed metal al ions s scaffo he metal xhaustiv ned with to meta is to	- in lds e l,	
Distribution Unlimit 13. ABSTRACT (Maximum 200 words) The objective of the binding proteins to solution. We use so on which to designe binding site designed binding of all pose defined geometries which allow a range specificities and a couple the metal-bin	ited nis research is t o report upon the nall, robust prot novel metal-ion n is computationa ssible sites. The and a variety of e of different me affinities to be inding event to a	o develop design presence of met ein frameworks a binding sites. T l, to allow an e sites are desig primary ligands tal-ion binding arrayed. The aim change in fluor	ed metal al ions s scaffo he metal xhaustiv ned with to meta is to escence	- in lds e l, of	
Distribution Unlimit 13. ABSTRACT (Maximum 200 words) The objective of the binding proteins to solution. We use so on which to designe binding site designed binding of all pose defined geometries which allow a range specificities and a couple the metal-bit an appropriate prob	nis research is t o report upon the mall, robust prot novel metal-ion n is computationa ssible sites. The and a variety of e of different me affinities to be inding event to a pe. For practical	o develop design presence of met ein frameworks a binding sites. T l, to allow an e sites are desig primary ligands tal-ion binding arrayed. The aim change in fluor applications, r	ed metal al ions s scaffo he metal xhaustiv ned with to meta is to escence eagent-l	- in lds e l, of	
Distribution Unlimit 13. ABSTRACT (Maximum 200 words) The objective of the binding proteins to solution. We use some on which to designe binding site designed binding site designed ampling of all posed defined geometries which allow a ranged specificities and a couple the metal-bind an appropriate profession systems are preferent	nis research is t preport upon the mall, robust prot novel metal-ion n is computationa ssible sites. The and a variety of e of different me affinities to be inding event to a pe. For practical red, but in the d	o develop design presence of met ein frameworks a binding sites. T l, to allow an e sites are desig primary ligands tal-ion binding arrayed. The aim change in fluor applications, r evelopment stage	ed metal al ions s scaffo he metal xhaustiv ned with to meta is to escence eagent-l	- in lds e l, of	
Distribution Unlimit 13. ABSTRACT (Maximum 200 words) The objective of the binding proteins to solution. We use so on which to designe binding site designed binding of all pose defined geometries which allow a range specificities and a couple the metal-bit an appropriate prob	nis research is t preport upon the mall, robust prot novel metal-ion n is computationa ssible sites. The and a variety of e of different me affinities to be inding event to a pe. For practical red, but in the d	o develop design presence of met ein frameworks a binding sites. T l, to allow an e sites are desig primary ligands tal-ion binding arrayed. The aim change in fluor applications, r evelopment stage	ed metal al ions s scaffo he metal xhaustiv ned with to meta is to escence eagent-l	- in lds e l, of	
Distribution Unlimit 13. ABSTRACT (Maximum 200 words) The objective of the binding proteins to solution. We use some on which to designe binding site designed binding site designed ampling of all posed defined geometries which allow a ranged specificities and a couple the metal-bind an appropriate profession systems are preferent	nis research is t preport upon the mall, robust prot novel metal-ion n is computationa ssible sites. The and a variety of e of different me affinities to be inding event to a pe. For practical red, but in the d	o develop design presence of met ein frameworks a binding sites. T l, to allow an e sites are desig primary ligands tal-ion binding arrayed. The aim change in fluor applications, r evelopment stage	ed metal al ions s scaffo he metal xhaustiv ned with to meta is to escence eagent-l s,	- in lds e l, of ess	
Distribution Unlimit 13. ABSTRACT (Maximum 200 words) The objective of the binding proteins to solution. We use so on which to designe binding site designed binding of all pose defined geometries which allow a rangeneric specificities and a couple the metal-bind an appropriate profession systems are preferred	nis research is t preport upon the mall, robust prot novel metal-ion n is computationa ssible sites. The and a variety of e of different me affinities to be inding event to a pe. For practical red, but in the d	o develop design presence of met ein frameworks a binding sites. T l, to allow an e sites are desig primary ligands tal-ion binding arrayed. The aim change in fluor applications, r evelopment stage	ed metal al ions s scaffo he metal xhaustiv ned with to meta is to escence eagent-l s,	- in lds e l, of	
Distribution Unlimit 13. ABSTRACT (Maximum 200 words) The objective of the binding proteins to solution. We use some on which to designe binding site designed binding of all pose defined geometries which allow a rangeneric specificities and a couple the metal-bind an appropriate profession systems are preferent extrinsic reporter 14. SUBJECT TERMS	nis research is t preport upon the mall, robust prot novel metal-ion n is computationa ssible sites. The and a variety of e of different me affinities to be inding event to a pe. For practical red, but in the d probes are also	o develop design presence of met ein frameworks a binding sites. T l, to allow an e sites are desig primary ligands tal-ion binding arrayed. The aim change in fluor applications, r evelopment stage	ed metal al ions s scaffo he metal xhaustiv ned with to meta is to escence eagent-l s,	in lds e l, of ess 5. NUMBER OF PAGES 3	
Distribution Unlimit 13. ABSTRACT (Maximum 200 words) The objective of the binding proteins to solution. We use so on which to designe binding site designed binding of all pose defined geometries which allow a rangeneric specificities and a couple the metal-bind an appropriate protection systems are preferent extrinsic reporter 14. SUBJECT TERMS metal binding	nis research is t preport upon the mall, robust prot novel metal-ion n is computationa ssible sites. The and a variety of e of different me affinities to be inding event to a pe. For practical red, but in the d probes are also	o develop design presence of met ein frameworks a binding sites. T l, to allow an e sites are desig primary ligands tal-ion binding arrayed. The aim change in fluor applications, r evelopment stage	ed metal al ions s scaffo he metal xhaustiv ned with to meta is to escence eagent-l s,	in lds e l, of ess 15. NUMBER OF PAGES	
Distribution Unlimit 13. ABSTRACT (Maximum 200 words) The objective of the binding proteins to solution. We use so on which to designe binding site designed binding of all pose defined geometries which allow a rangeneric specificities and a couple the metal-bind an appropriate protection systems are preferent extrinsic reporter 14. SUBJECT TERMS	nis research is t preport upon the mall, robust prot novel metal-ion n is computationa ssible sites. The and a variety of e of different me affinities to be inding event to a pe. For practical red, but in the d probes are also	o develop design presence of met ein frameworks a binding sites. T l, to allow an e sites are desig primary ligands tal-ion binding arrayed. The aim change in fluor applications, r evelopment stage	ed metal al ions s scaffo he metal xhaustiv ned with to meta is to escence eagent-l s,	in lds e l, of ess . NUMBER OF PAGES 3 	
Distribution Unlimit 13. ABSTRACT (Maximum 200 words) The objective of the binding proteins to solution. We use solution. We use solution on which to designe binding site designed binding of all posed defined geometries which allow a ranged specificities and a couple the metal-bid an appropriate protection systems are preferent extrinsic reporter 14. SUBJECT TERMS metal binding protein design	nis research is t preport upon the mall, robust prot novel metal-ion n is computationa ssible sites. The and a variety of e of different me affinities to be inding event to a pe. For practical red, but in the d probes are also rubredoxin IgG	o develop design presence of met ein frameworks a binding sites. T l, to allow an e sites are desig primary ligands tal-ion binding arrayed. The aim change in fluor applications, r evelopment stage	ed metal al ions s scaffo he metal xhaustiv ned with to meta is to escence eagent-l s,	in lds e l, of ess 5. NUMBER OF PAGES 3	
Distribution Unlimit 13. ABSTRACT (Maximum 200 words) The objective of the binding proteins to solution. We use sm on which to designe binding site designed binding of all pose defined geometries which allow a rangeneric specificities and a couple the metal-bind an appropriate protection systems are preferent extrinsic reporter 14. SUBJECT TERMS metal binding protein design	nis research is t preport upon the mall, robust prot novel metal-ion n is computationa ssible sites. The and a variety of e of different me affinities to be inding event to a pe. For practical red, but in the d probes are also	o develop design presence of met ein frameworks a binding sites. T l, to allow an e sites are desig primary ligands tal-ion binding arrayed. The aim change in fluor applications, r evelopment stage investigated.	ed metal al ions s scaffo he metal xhaustiv ned with to meta is to escence eagent-l s,	in lds e l, of ess . NUMBER OF PAGES 3 	

Standard Form 298 (Rev. 2-8 Prescribed by ANSI-Std 239 298-1

FINAL REPORT

Grant #: N00014-95-1-0913

PRINCIPAL INVESTIGATOR: Prof. Lynne Regan

<u>INSTITUTION</u>: Yale University

<u>GRANT TITLE</u>: Signal Transduction by Designed Metal-Binding Proteins

<u>AWARD PERIOD</u>: 1 May 1996 - 30 Apr 1999

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

<u>APPROACH</u>: 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.

<u>CONCLUSIONS</u>: 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.

<u>SIGNIFICANCE</u>: Demonstration of how rational design of metalion binding sites can be linked with signal transduction system.

PATENT INFORMATION: None

<u>AWARD INFORMATION</u>: 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 metalbinding 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.