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Final Report: Reprogramming Proteins and Enzymes for Transition Metal Catalysis

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W911NF-14-1-0334

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER
611102

6. AUTHORS

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAMES AND ADDRESSES
University of Chicago
5801 South Ellis Avenue

Chicago, IL 60637 -5418

8. PERFORMING ORGANIZATION REPORT NUMBER

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U.S. Army Research Office
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14. ABSTRACT

15. SUBJECT TERMS

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a. REPORT	b. ABSTRACT	c. THIS PAGE			Jared Lewis
UU	UU	UU	UU		19b. TELEPHONE NUMBER 812-855-9043

RPPR Final Report
as of 08-May-2019

Agency Code:

Proposal Number: 62247LS **Agreement Number: W911NF-14-1-0334**
INVESTIGATOR(S):

Name: Jared Lewis
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Principal: Y

Organization: **University of Chicago**
Address: 5801 South Ellis Avenue, Chicago, IL 606375418
Country: USA
DUNS Number: 005421136 EIN: 362177139
Report Date: 30-Sep-2017 Date Received: 24-Feb-2019
Final Report for Period Beginning 01-Jul-2014 and Ending 30-Jun-2017
Title: Reprogramming Proteins and Enzymes for Transition Metal Catalysis
Begin Performance Period: 01-Jul-2014 **End Performance Period:** 30-Jun-2017
Report Term: 0-Other
Submitted By: Michael Ludwig Email: io-ura@uchicago.edu
Phone: (773) 702-8669

STEM Degrees: 5 **STEM Participants:** 1

Major Goals: Overview (from the original proposal)

Controlling the selectivity of chemical reactions and conducting these reactions in biological systems, where they could have a transformative impact, stand as key challenges in synthetic chemistry. Artificial metalloenzymes (ArMs) could achieve these goals by combining the reactivity of synthetic metal catalysts and the adaptability and efficiency of enzymes. This proposal describes the development of ArMs generated from protein or enzyme scaffolds and metal catalysts bearing reactive anchoring groups. Ensconcing such cofactors within structurally defined yet genetically mutable scaffolds will provide exquisite control of their reactivity. The impact of this control on catalysis will be studied, and ArMs will be evolved to promote challenging chemical transformations. Ultimately, these ArMs could enable chemistry typically conducted in the confines of flasks and reactors to operate in cells and living organisms.

Objectives (Statement of the problem studied)

i. Establish efficient syntheses of dirhodium tetracarboxylate and manganese terpy cofactors, and develop robust methods for covalent modification of proteins and enzymes with these cofactors to generate ArMs.

ii. Evolve the proposed dirhodium and manganese terpy ArMs to enable site-specific C-H insertion reactions.

iii. Develop cofactor activation approaches that exploit reactive oxygen species (ROS) and the reactivity of natural enzymes to enable in vivo ArM formation and catalysis

During the final funding period, our study on the evolution of dirhodium ArM evolution was completed. This involved refining the previously reported evolution protocol, and developing a second protocol for evolution of immobilized ArMs. A study on the effects of scaffold dynamics on ArM selectivity was also completed and accepted for publication.

Accomplishments: A. Artificial Metalloenzyme (ArM) Evolution

The previous progress report described the development of an evolution protocol suitable for dirhodium ArM evolution. This was used to evolve a variant (3-VRVH) that catalyzes a model cyclopropanation reaction with high enantioselectivity (Fig. 1). During the final funding period, we set out to demonstrate that an ArM could also be engineered to provide the opposite product enantiomer. A starting point for this effort (variant F413Z) was identified

by varying the residue within the POP active site to which the dirhodium cofactor was linked (Fig. 2A). Combinatorial codon mutagenesis was then used to localize random mutations to the ArM active site with the goal of optimizing the low enantioselectivity obtained (40:70 e.r.). This approach led to variant 1-RFY, which catalyzes cyclopropanation to give the opposite product enantiomer with 10:90 e.r. (Fig. 3). Notably, this evolution was achieved using a modified version of our previously reported protocol in which ArM variants are immobilized (Fig. 2B). This approach significantly simplifies screening, and led to further exploration of ArM immobilization. This work, along with results from the previous report, was published in Nature Chemistry.

B. POP Structure and Bioconjugation

Studies on the peptidase activity of Pfu POP suggest that its unique two-domain architecture regulates substrate access and specificity. As outlined above and in previous reports, we have established that Pfu POP also serves as an efficient host for asymmetric cyclopropanation upon active-site modification with a dirhodium cofactor. To understand how Pfu POP controls both peptidase and dirhodium catalysis, we determined the crystal structures of this enzyme and its S477C mutant (noted in the previous report) and used these structures as starting points for MD simulations of both the apo structures and systems containing a covalently linked peptidase inhibitor or a dirhodium catalyst. Key aspects of previously reported peptidase kinetics (Fig. 4) and cyclopropanation selectivity (Fig. 5) can be rationalized in the context of inter-domain opening and closing of the Pfu POP host. This finding constitutes a remarkable example in which the conformational dynamics of a supramolecular host affect two different catalytic activities and suggests that Pfu POP could serve as a host for a wide range of non-native catalysts. A manuscript describing the importance of POP dynamics for both peptidase and ArM activity was posted to ChemRxiv. A manuscript describing only the peptidase activity was recently accepted for publication in Biochemistry, and the model outlining the importance of POP dynamics for ArM activity was included in a review recently accepted for publication in Accounts of Chemical Research.

Training Opportunities: As noted elsewhere, this award funded research conducted by several graduate students and two postdocs. These researchers received extensive training in molecular biology, bioconjugation methods, biocatalysis, and protein engineering. They learned valuable presentation skills in weekly group and subgroup meetings and other regular departmental seminars. As a result, they are now applying their training in a range of exciting industrial and academic labs.

Graduate students:
Landon Durak: Scientist, Takeda Oncology
Hao Yang: Senior Scientist, Merck Process
Chen Zhang: Associate Scientists, Provivi Inc.
Ken Ellis-Guardiola: postdoc, UCLA
Yifan Gu: Research Investigator, Incyte Corporation
Alan Swartz (deceased)
Natalie Chan (current graduate student)

Postdocs:
Hyun June Park: Senior Researcher, CJ CheilJedang (South Korea)
Ketaki Belsare: postdoc UCSF

Results Dissemination: 1. Yang, H.; Swartz, A. M.; Srivastava, P.; Ellis-Guardiola, K.; Park, H. J.; Upp, D.; Belsare, K.; Lee, G.; Gu, Y.; Zhang, C.; Moellering, R. E.; Lewis, J. C.* Evolving Artificial Metalloenzyme Selectivity via Random Mutagenesis. Nat. Chem. 2018, 10, 318-324.
2. Lewis, J. C. Beyond the Second Coordination Sphere: Engineering Dirhodium Artificial Metalloenzymes to Enable Protein Control of Transition Metal Catalysis. Accounts of Chemical Research, 2019, accepted.
3. Ellis-Guardiola, K.; Rui, H.; Beckner, R.;# Srivastava, P.; Sukumar, N.*; Roux, B.*; Lewis, J. C.* Crystal Structure and Conformational Dynamics of Pyrococcus furiosus Prolyl Oligopeptidase. Biochemistry, 2019, accepted.

Over the course of this award, the funded research was presented at a number of lectures:

2017
21st Annual Green Chemistry and Engineering Conference, 6/13
NC State University, 4/21
Northwestern University (guest lecture for Advances in Biotechnology course), 4/19
Harvard University, 3/9
Bioinorganic Chemistry GRS discussion leader (Metals in Biology GRC), 1/26-29

2016
University of Minnesota, 11/17
Scripps Research Institute, 11/4
Scripps Institute of Oceanography, 11/3
Tufts University, 10/18
University of Wisconsin, 10/11
University of Rochester, 10/7
University of Michigan, 9/20
Aachen-Osaka Catalysis Symposium (Aachen, Germany), 8/5
Organic Reactions and Processes GRC, 7/17-7/21
Biocatalysis GRC, 7/10-7/15
Abbvie, 6/17
Princeton University, 5/3
University of Pennsylvania, 5/2
Stanford University, 4/13
Gilead Sciences, 4/12
Loyola University Chicago, 3/24
UC Berkeley, 2/9
UCSF, 2/8
Emory University, 1/27
UC Irvine, 1/14
Caltech, 1/13

2015
Pacifichem (Biocatalysis and Cooperative Catalysis sessions), Honolulu, HI, 12/18 and 12/19
Yale University, 10/29
University of Illinois, Urbana/Champaign, 10/26
ACS National Meeting (The Role of the Outer Coordination Sphere on the Activity of Enzymes and Molecular Catalysts Symposium), Boston, MA, 8/16
Chicago Organic Symposium, 7/11
Canadian Chemistry Conference (C-H Functionalization Symposium), 6/15
Hope College, 4/17
Calvin College, 4/16
ACS National Meeting (ACS Chemical Biology Lectureship Symposium), Denver, CO, 3/24
Metals in Biology GRC, Stiefel Lecture, 1/28

2014
Novartis Institutes for Biomedical Research, Cambridge, MA, 10/17
Iowa St. University, 10/10
University of Iowa, 10/9
Merck Research Laboratories, Rahway, NJ, 8/21

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Honors and Awards: Awards received by the PI during the lifetime of this award include:
2016 Dreyfus Teacher-Scholar Award
2015 Ed Stiefel Young Investigator Award (given by the Metals in Biology GRC)
2014 NSF CAREER Award

Protocol Activity Status:

Technology Transfer: Nothing to Report

PARTICIPANTS:

Participant Type: Graduate Student (research assistant)
Participant: Landon Durak
Person Months Worked: 4.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Graduate Student (research assistant)
Participant: Hao Yang
Person Months Worked: 4.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Graduate Student (research assistant)
Participant: Chen Zhang
Person Months Worked: 6.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Graduate Student (research assistant)
Participant: Yifan Gu
Person Months Worked: 12.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Graduate Student (research assistant)
Participant: Ken Ellis-Guardiola
Person Months Worked: 12.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:

National Academy Member: N
Other Collaborators:

Participant Type: Graduate Student (research assistant)
Participant: Alan Swartz
Person Months Worked: 6.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Postdoctoral (scholar, fellow or other postdoctoral position)
Participant: Ketaki Belsare
Person Months Worked: 4.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Postdoctoral (scholar, fellow or other postdoctoral position)
Participant: Hyun June Park
Person Months Worked: 12.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

BOOKS:

Publication Type: Book Peer Reviewed: Y **Publication Status:** 0-Other
Publication Identifier Type: DOI Publication Identifier: 10.1002/9783527804085
Book Edition: Volume: Publication Year: 2018 Date Received: 24-Feb-2019
Publication Location: Weinheim, Germany
Publisher: Wiley-VCH Verlag GmbH & Co. KGaA
Book Title: Artificial Metalloenzymes and MetalloDNAzymes in Catalysis
Authors: Diéguez Montserrat, Bäckvall Jan-E?, Pàmies Oscar
Editor:
Acknowledged Federal Support: Y

DISSERTATIONS:

RPPR Final Report
as of 08-May-2019

Publication Type: Thesis or Dissertation
Institution: University of Chicago
Date Received: 24-Feb-2019 Completion Date: 2/1/15 9:28PM
Title: DESIGN, CONSTRUCTION, AND DIRECTED EVOLUTION OF ARTIFICIAL METALLOENZYMES
Authors: Zhang, Chen
Acknowledged Federal Support: **N**

Publication Type: Thesis or Dissertation
Institution: University of Chicago
Date Received: 24-Feb-2019 Completion Date: 3/1/16 10:04PM
Title: ENGINEERING ARTIFICIAL METALLOENZYME FOR SELECTIVE CATALYSIS
Authors: Yang, Yao
Acknowledged Federal Support: **N**

Publication Type: Thesis or Dissertation
Institution: University of Chicago
Date Received: 24-Feb-2019 Completion Date: 2/1/17 5:07PM
Title: PROLYL OLIGOPEPTIDASE-BASED ARTIFICIAL METALLOENZYMES FOR SELECTIVE CATALYSIS
Authors: Ellis-Guardiola, Ken
Acknowledged Federal Support: **N**

Publication Type: Thesis or Dissertation
Institution: University of Chicago
Date Received: 24-Feb-2019 Completion Date: 8/1/15 4:09PM
Title: ORGANOMETALLIC AND CHEMOENZYMATIC APPROACHES TO ARENE ARYLATION VIA C-H BOND CLEAVAGE
Authors: Durak, Landon
Acknowledged Federal Support: **N**

Publication Type: Thesis or Dissertation
Institution: University of Chicago
Date Received: 24-Feb-2019 Completion Date: 2/1/18 5:10PM
Title: NEW APPROACHES IN ARTIFICIAL (METALLO)ENZYMES
Authors: Gu, Yifan
Acknowledged Federal Support: **N**

Introduction

Overview (from the original proposal)

Controlling the selectivity of chemical reactions and conducting these reactions in biological systems, where they could have a transformative impact, stand as key challenges in synthetic chemistry. Artificial metalloenzymes (ArMs) could achieve these goals by combining the reactivity of synthetic metal catalysts and the adaptability and efficiency of enzymes. This proposal describes the development of ArMs generated from protein or enzyme scaffolds and metal catalysts bearing reactive anchoring groups. Ensconcing such cofactors within structurally defined yet genetically mutable scaffolds will provide exquisite control of their reactivity. The impact of this control on catalysis will be studied, and ArMs will be evolved to promote challenging chemical transformations. Ultimately, these ArMs could enable chemistry typically conducted in the confines of flasks and reactors to operate in cells and living organisms.

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i. Establish efficient syntheses of dirhodium tetracarboxylate and manganese terpy cofactors, and develop robust methods for covalent modification of proteins and enzymes with these cofactors to generate ArMs.

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During the final funding period, our study on the evolution of dirhodium ArM evolution was completed. This involved refining the previously reported evolution protocol, and developing a second protocol for evolution of immobilized ArMs. A study on the effects of scaffold dynamics on ArM selectivity was also completed and accepted for publication.

List of Appendixes, Illustrations, and Tables (see "Upload" section)

Figure 1. A) Model cyclopropanation reaction used for ArM evolution. B). ArM formation via SPAAC. C). Structure of dirhodium cofactor **1**.

Figure 2. Combinatorial codon mutagenesis sites and protocol. A) Ribbon model of POP variant 0-ZA4 showing the location of residue 477 (green sphere), residue 413 (blue sphere), and residues selected for combinatorial codon mutagenesis (remaining spheres). Mutations in 1-RFY (Q98R, G99F, and P239Y) are shown as red, orange, and yellow spheres, respectively. B) Scaffold immobilization and ArM formation on Ni-NTA resin in 96-well plates using libraries of scaffold variants generated via error prone PCR.

Figure 3. Complementary selectivity of evolved ArMs.

Figure 4. Conceptual summary of updated model for effects of POP structure and dynamics on peptidase activity (S = substrate).

Figure 5. A model for the effects of conformational dynamics on POP bioconjugation and catalysis. A) bioconjugation can readily occur with the open conformation (shaded, left), while exclusion of water from the closed conformation (shaded, right) allows for selective reaction of carbene precursor **2** with styrene to give cyclopropane **3**. B) The closed form of the ArM is favored by both halide and Rh binding within the active site.

3. Summary of the Most Important Results

A. Artificial Metalloenzyme (ArM) Evolution

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4. Planned activities

Results form this award formed the basis of follow-up proposal that has been awarded. Progress on that work will be documented accordingly.

Figure 1.

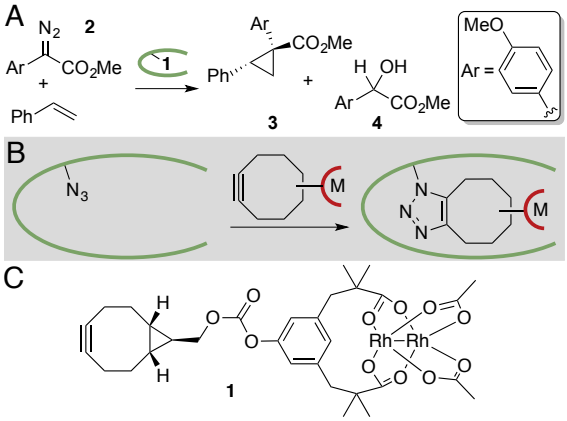


Figure 2.

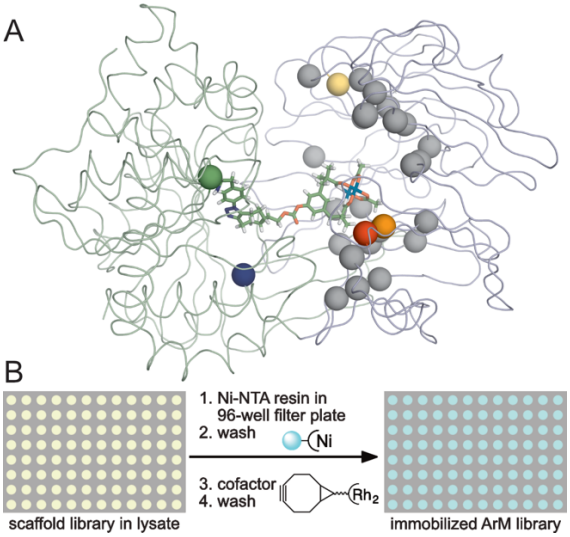


Figure 3.

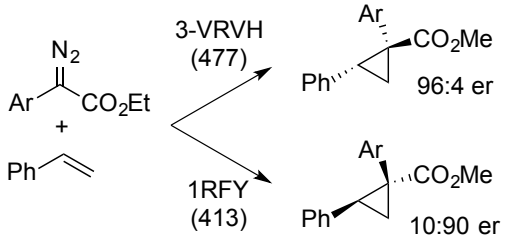


Figure 4.

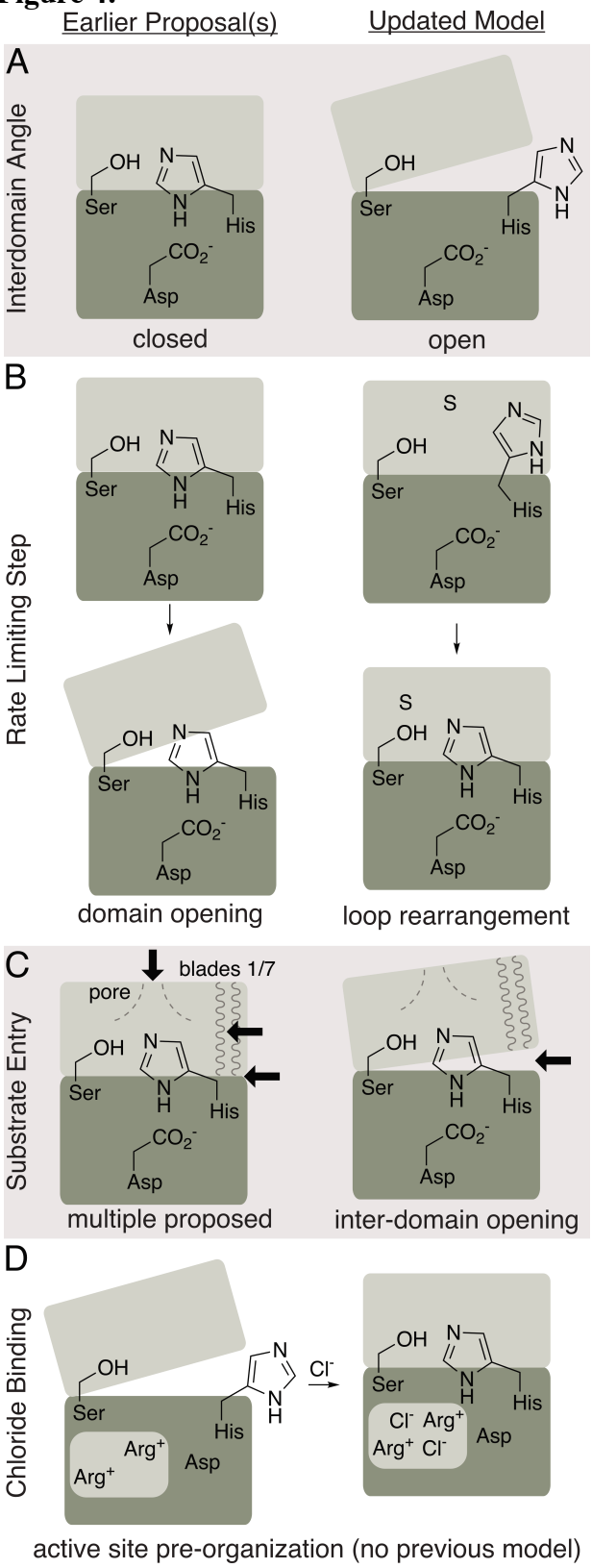
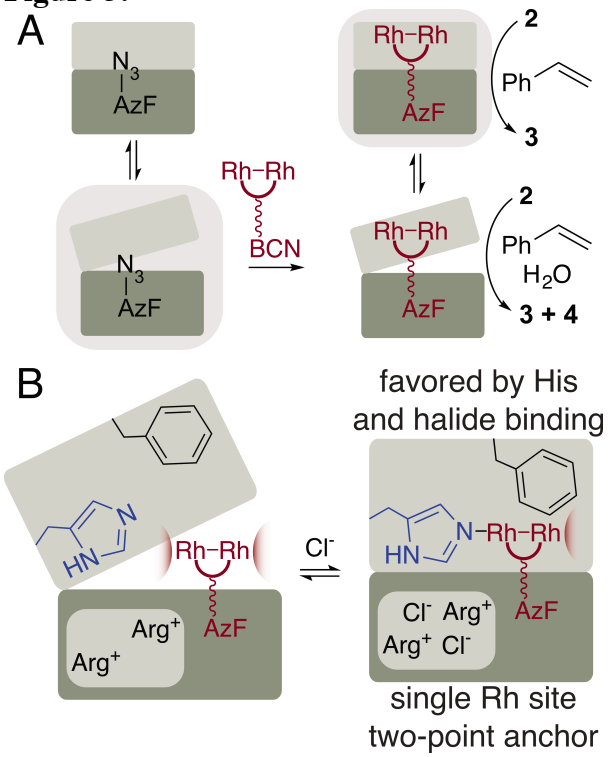

Earlier Proposal(s)
Updated Model
A
Interdomain Angle
OH
N
Ser
N
H
His
CO2-
Asp
closed
open
B
Rate Limiting Step
S
domain opening
loop rearrangement
C
Substrate Entry
blades 1/7
pore
multiple proposed
inter-domain opening
D
Chloride Binding
Cl-
Arg+
active site pre-organization (no previous model)

Figure 5.


A
N3
AzF
Rh–Rh
BCN
Rh–Rh
AzF
2
Ph
3
Rh–Rh
AzF
2
Ph
H2O
3 + 4
B
favored by His
and halide binding
HN
Rh–Rh
Cl-
Arg+
AzF
Arg+
N–Rh–Rh
HN
Cl- Arg+
Arg+ Cl-
AzF
single Rh site
two-point anchor