BIOGRAPHICAL SKETCH

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NAME: Aaron Tyler Smith

eRA COMMONS USER NAME (credential, e.g., agency login): atsmith

POSITION TITLE: Associate Professor of Chemistry and Biochemistry

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Boston University, Boston, MA	B.A.	05/2007	Chemistry
University of Wisconsin-Madison, Madison, WI	M.S.	05/2009	Chemistry
University of Wisconsin-Madison, Madison, WI	Ph.D.	05/2012	Chemistry
Northwestern University	Postdoctoral	07/2016	Chemistry/Biochemistry

A. Personal Statement

I am an Associate Professor in the Department of Chemistry and Biochemistry at the University of Maryland, Baltimore County (UMBC). I have been working in the field of bioinorganic chemistry for over 15 years. I first entered the field by conducting synthetic inorganic chemistry aimed at modeling the enzyme active sites of soluble methane monooxygenase (sMMO) and phenylalanine hydroxylase (PAH). My graduate work focused on Cys(thiolate)-ligated hemoproteins involved in transcriptional regulation and amino acid metabolism, whereas my postdoctoral work focused on the structural, biochemical, and functional characterization of both soluble and membrane-bound metallotransporters. Thus, my overall training has endowed me with a broad knowledge of, and rigor of research in, inorganic chemistry, biochemistry, structural biology, and molecular biology necessary to mentor students and to oversee research projects relating to these areas. My current research program blends spectroscopy, structural biology, biophysics, and enzymology to elucidate the mechanism of ferrous iron homeostasis in pathogenic prokaryotes, and the mechanism (including the regulation by iron) of post-translational arginylation in eukaryotes. These systems are indispensable for the establishment of infection within human hosts, and the regulation of normal plant and metazoan developments, respectively. My laboratory has developed a proven track record of being able to study complex membrane and soluble metalloproteins such as those of the Feo system and (very recently) metal-sensing two-component systems (TCSs) (see **Contributions to Science** below), including proposing a connection between the Feo system and the Bqs system

In addition to researching the structure-function relationships of iron-containing metalloproteins, I have been committed to the training, mentoring, and promotion of inclusive and supportive scientific research environments, and I have a history of mentoring students and ensuring their transition into strong careers in the biomedical research workforce. My trainees have learned sought-after techniques from molecular biology and protein expression and purification (both soluble and membrane) to structural biology, spectroscopy, and anoxic work. These skills have made my trainees well-positioned to move forward into the biomedical work force via competitive graduate, postdoctoral, or industrial positions upon receiving their degrees or completing their postdoctoral training. At UMBC, I have previously mentored 20 undergraduates (10 M, 9 F, 1 they/them; 16 of which are URMs), 2 master's student (2 M), 3 Ph.D. students (2 F, 1 M), 1 F visiting international scholar, and 1 F postdoctoral scholar (URM). Supported by current NIH, NSF, and HHMI funding, I am mentoring and training individuals in structural biology and bioinorganic chemistry research at the undergraduate level (3 M, 3 of which are URMs), the post-baccalaureate level (1 F who is a URM), the predoctoral level (2 F of which 1 is a URM, 2 M of which 1 is a URM and an HHMI Gilliam and NSF GRF awardee, 1 they/them), and 1 post-doctoral scholar (1 M). I support these trainees through mentoring and facilitating their participations in activities to ensure their transition into the biomedical workforce, such as participation in local, national, and international conferences,

publishing in primary literature, and helping develop scientific speaking and writing skills. In addition, as a member of the LGBTQ+ community, I have worked hard to shape my laboratory into an inclusive, safe, productive, and inviting location for LGBTQ+ scientists. I am a member of the UMBC LGBTQ+ faculty-staff association (FSA), and am SafeZone-trained, a program aimed at creating a safe and affirming atmosphere for members of the LGBTQ+ community and their allies. I recently completed a year-long culturally-aware mentoring program, supported by HHMI. Additionally, since 2022 I have been the PD of my Department's NIH-funded T32 Chemistry-Biology Interface (CBI) program, where I oversee and mentor *ca*. 40 students across three Departments and two campuses (UMBC and UMB).

Ongoing and recently completed projects that I would like to highlight, include:

NIH T32 GM066706 **Aaron T. Smith (co-PD)** **Project Dates: 06/19—07/25**
"Graduate Training at the Chemistry Biology Interface"

NIH R35 GM133497 **Aaron T. Smith (PI)** **Project Dates: 08/19—06/29**
"Deciphering the Mechanism of Pathogenic Ferrous Iron Acquisition and the Role of Iron in the Eukaryotic Arginine Transferases"

NSF CAREER 1844624 **Aaron T. Smith (PI)** **Project Dates: 04/19—04/25**
"Structure, Mechanism, and Selectivity of Microbial Ferrous Iron Transport"

HHMI GT15765 **Aaron T. Smith (mentor and DEI lead)** **Project Dates: 08/22—07/25**
"Biophysical Characterization of the *Pseudomonas aeruginosa* (*Pa*) BqsR and BqsS Two-Component System"

B. Positions, Scientific Appointments, and Honors

Positions and Employment

2022—present	Associate Professor of Chemistry and Biochemistry, UMBC, Dept. of Chemistry and Biochemistry and & Joint UMBC/UMB Biochemistry program (GPILS)
2022—present	Program director (PD) of UMBC-UMB T32 CBI program
2020—2022	Co-program director (PD) of the UMBC-UMB T32 CBI program
2016—2022	Assistant Professor of Chemistry and Biochemistry, UMBC, Dept. of Chemistry and Biochemistry and & Joint UMBC/UMB Biochemistry program (GPILS)
2013—2016	Ruth L. Kirschstein NRSA Postdoctoral Fellow, Northwestern University, Dept. of Molecular Biosciences
2012—2013	Postdoctoral Scholar, Northwestern University, Dept. of Molecular Biosciences
2007—2012	Teaching assistant, University of Wisconsin-Madison, Dept. of Chemistry

Honors and Awards (last 10 years)

2023	Carl S. Weber Excellence in Teaching Award (UMBC)
2022	HHMI Gilliam Fellowship Mentor (UMBC)
2022	Beckman Foundation Scholar Faculty Mentor (UMBC)
2022	Early Career Faculty Excellence Award (UMBC)
2019	NSF Career Award (UMBC)
2019	American Heart Association Career Development Award (UMBC)
2018	Career Center Impact Recognition (UMBC)
2017	Strategic Award for Research Transitions (UMBC)
2017	Summer Faculty Fellowship (UMBC)
2015	Interdisciplinary Biological Sciences (IBiS) Postdoctoral Travel Award (NU)

Other Experience and Professional Memberships (last 10 years)

2025	Member NIH Maximizing Investigators' Research (MRAB) study section, *ad hoc*
2024	Member NIH Macromolecular Structure/Function–A (MSFA) study section, *ad hoc*
2023—present	National High-Throughput Crystallization Center (HWI, University of Buffalo) Advisory Board Member
2020—2021	AAAS Science & Technology Policy Fellowship Committee Member

2020—present	Proposal reviewer for SSRL Bio XAS/Single Crystal XAS Beamtime Requests
2020—present	American Chemical Society (ACS) Division of Biological Chemistry (DBC) Executive Committee Alternate Councilor
2020	Member NSF Chemistry of Life Processes (CLP) division Iron Proteins and Enzymes Review Panel
2019—present	Member, American Society for Biochemistry and Molecular Biology
2019—present	Governing Board Member of the UMB-UMBC Joint Biochemistry & Molecular Biology Program
2019-2022	Guest Researcher, National Institute of Standards and Technology (NIST)
2018-present	Laboratory Member of the University of Maryland, School of Pharmacy Metallotherapeutics Research Center
2016-present	Member, American Heart Association
2016—present	Member, Society of Biological Inorganic Chemistry
2012—present	Member, American Chemical Society

C. Contributions to Science

1. Spectroscopically and functionally characterized novel roles of heme in soluble and membrane proteins

My graduate work focused on elucidating the role of heme in diverse biological processes such as transcriptional regulation, microRNA processing, and sulfur amino acid metabolism. In each of these processes, key proteins bind a heme *b* cofactor with a Cys(thiolate) ligand that is required for regulatory function. In each study, I identified key spectroscopic signatures associated with the unique ligation motifs of these regulatory proteins that are distinct from the larger cytochrome P450 and chloroperoxidase families. These spectroscopic studies helped develop a framework by which Cys(thiolate)-ligated hemoproteins involved in regulatory processes might be easily distinguished, classified, and characterized. Furthermore, my graduate studies probed the role of heme *b* in these diverse processes by examining: the effect of metalloporphyrin substitution and second-sphere interactions on activity of the human enzyme cystathionine β-synthase (CBS); the result of site-directed mutagenesis on the transcriptional regulator of CO metabolism (RcoM) from *Burkholderia xenovorans*; and the impact of changes in heme iron oxidation and coordination states on the eukaryotic microRNA processing partner protein DiGeorge Critical Region 8 (DGCR8).

This previous training has recently been expanded into the study of unicellular ferric reductases, which are either flavin-dependent (soluble) or heme *b*-dependent (membrane) enzymes. These reductases shuttle electrons to ferric (Fe^{3+})-chelated small molecules and/or siderophores, causing reductive dissociation of the now ferrous (Fe^{2+}) ion from these otherwise thermodynamically-stable molecules. The liberated Fe^{2+} is then commonly assimilated into iron-based metalloproteins after being imported into the cytosol most frequently via the Feo system (*vide infra*). My lab is currently investigating the heme-based membrane ferric reductases in order to understand their contribution to ferrous iron acquisition and their connection to pathogenesis. ***Key publications include***:

a. Barr, I.; Smith, A.T.; Chen, Y.; Senturia, R.; Burstyn, J.N.; Guo, F. Ferric, not ferrous, heme activates RNA-binding protein DGCR8 for primary microRNA processing. *Proc. Natl. Acad. Sci. U.S.A.* **2012**. *109*, 1919-1924. PMC3277547

b. Smith, A.T.; Su, Y.; Stevens, D. J.; Majtan, T.; Kraus, J.P.; Burstyn, J.N. The effect of the disease-causing R266K mutation on the heme and PLP environments of the human enzyme Cystathionine β-Synthase. *Biochemistry* **2012**, *51*, 6360-6370. PMC3569099.

c. Smith, A. T.; Pazicni, S.; Marvin, K. A.; Stevens, D. J.; Freeman, K. M.; Burstyn, J. N. Functional divergence of heme-thiolate proteins: a classification based on spectroscopic attributes. *Chem. Rev*. **2015**. *115*, 2532-2558. PMID 25763468

d. Cain, T. J. and Smith, A. T. Ferric iron reductases and their contribution to unicellular ferrous iron uptake. *J. Inorg. Biochem.* **2021**, *218*, 11407, 1-9. PMC8035299

2. Characterized molecular mechanisms of membrane protein-based metal transport

My postdoctoral work focused on the structural and biochemical characterization of metal transport in the P_{1B}-ATPases. This superfamily of membrane proteins is responsible for helping maintain homeostasis of nearly every biologically-relevant transition metal, and mutations in genes encoding for these enzymes result in growth and developmental abnormalities in bacteria and plants as well as diseases in humans. Based on a historic

classification scheme, I undertook a large bioinformatics analysis and discovered two new subfamilies of the P_{1B}-ATPases that may be involved in Fe and Zn transport. I also identified several putative metal-binding domains in a large number of established P_{1B}-ATPase subfamilies. In one case (*Cupriavidus metallidurans* CzcP), I was able to clone, express, purify, and determine a structure of one of these new metal-binding domains. Coupled with biochemical data on the intact transporter and several variant proteins, my work pointed to a new regulatory mechanism in these enzymes and suggested that the metal-binding domains in these pumps evolved independently from different metal-binding proteins. I have also used homology modeling, site-directed mutagenesis, and spectroscopic measurements to show how key residues in the transmembrane domain of CzcP contribute to metal selectivity.

My current independent work in the field of metal homeostasis focuses on ferrous (Fe^{2+}) iron transport mediated by the prokaryotic Feo system, which is necessary for many pathogens to establish infection within human hosts. Advancement on this system has been hampered by the inability to generate large quantities of the membrane-bound, GTP-utilizing FeoB. Recent results from my lab have demonstrated the ability to obtain sufficient quantities of this enzyme to study using biophysical techniques. Importantly, we have demonstrated that FeoB from some organisms may be capable of rapidly hydrolyzing GTP. Using X-ray crystallography, we have identified a conserved hydrophobic motif in FeoA that mediates protein-protein interactions with FeoB to regulate GTP hydrolysis. My lab has postulated a mechanism by which a Met-lined channel within FeoB mediates ferrous iron transport, which is currently under investigation. Finally, we have shown that some FeoCs assembly a [4Fe-4S] cluster that is redox and O_2-sensitive, but the cluster-binding capabilities are not conserved across bacteria. Our current work aims to determine the mechanistic details of prokaryotic Fe^{2+} transport, which will allow for the targeting of this system for therapeutic intervention. ***Key publications include***:

a. Smith, A. T.; Barupala, D.; Stemmler, T. L.; and Rosenzweig, A. C. A new metal binding domain involved in cadmium, cobalt and zinc transport. *Nature Chem. Biol*. **2015**. *11*, 678-684. PMC4543396

b. Sestok, A. E.; Brown, J. B.; Obi, J. O.; O'Sullivan, S. M.[#]; Garcin, E. D.; Deredge, D. J.; and Smith, A. T. A fusion of the *Bacteroides fragilis* ferrous iron import proteins reveals a role for FeoA in stabilizing GTP-bound FeoB. *J. Biol. Chem.* **2022**, *298*, 101808. PMC8980893

c. Lee, M.; Magante, K.[#]; Gómez-Garzón, C.; Payne, S. M.; and Smith, A. T. Structural determinants of *Vibrio cholerae* FeoB nucleotide promiscuity. *J. Biol. Chem.* **2024**, *300*, 107663. PMCID11406355

d. Lee, M.; Armstrong, C.M.; and Smith, A. T. Characterization of intact FeoB in a lipid bilayer using styrene-maleic acid (SMA) copolymers. *BBA—Biomembranes.* **2025**, *1867*, 184404. PMCID in progress.

3. Unraveled regulatory and mechanistic aspects of post-translational arginylation

Independent work in my laboratory has also focused on the role of iron in protein translation and post-translational modifications, and we have placed special attention on understanding the mechanistic and regulatory aspects of eukaryotic post-translational arginylation. Catalyzed by the arginyltransferase 1 (ATE1), this essential post-translational modification is involved in plant development, cardiovascular maturation, neurodegeneration, cellular regeneration, and is even implicated in some forms of cancer. We have recently shown that ATE1s are hitherto unrecognized [Fe-S] cluster-binding proteins. The presence of this cluster functions as a positive effector of arginylation efficacy, which may be utilized to sense small molecules such as O_2 and NO within the cell. This discovery has shifted the paradigm that once assumed that ATE1s were heme-containing proteins. In addition, we have solved the first structure of a eukaryotic ATE1 from *Saccharomyces cerevisiae*. This structure and our [Fe-S] regulatory paradigm now position us to understand how ATE1s recognize substrate polypeptides and whether this process may be targeted, which we are probing through use of site-directed mutagenesis, small-angle X-ray scattering, X-ray crystallography, and mass spectrometry. ***Key publications include***:

a. Van, V. and Smith, A. T. ATE1-mediated post-translational arginylation is an essential regulator of eukaryotic cellular homeostasis. *ACS Chem. Biol.* **2020**, *15*, 3073-3085. PMC7749041

b. Van, V.; Ejimogu, N.-E.[#]; Bui, T. S.[#]; and Smith, A. T. The structure of *Saccharomyces cerevisiae* arginyltransferase 1 (ATE1). *J. Mol. Biol*. **2022**, *434*, 167816. PMC9992452

c. Van, V.; Brown, J. B.; O'Shea, C. R.; Rosenbach, H.; Mohamed, I.[#]; Ejimogu, N.-E.[#]; Bui, T. S.[#]; Szalai, V. A.; Chacón, K. N.; Span, I.; Zhang, F.; and Smith, A. T. Iron-sulfur clusters are involved in post-translational arginylation. *Nature Communications*. **2023**, *14*, 458. PMC9884297

d. Cartwright, M.; Parakra, R.; Oduwole, A.[#]; Zhang, F.; Deredge, D. J.; Smith, A. T. Identification of an intrinsically disordered region (IDR) in arginyltransferase 1 (ATE1). *Biochemistry*. **2024**, *63*, 3236-3249. PMCID In Progress.

4. Determined the SAXS and X-ray crystal structures of soluble and membrane proteins and modeled de novo unknown structures of essential proteins

My lab and I have been involved in solving the X-ray crystal structures and low-resolution SAXS profiles of several soluble and membrane proteins with multiple different functions. These efforts have resulted in the crystal structures of several key enzymes, including the first (and highest resolution) structure of a covalent inhibitor bound to an E3 ubiquitin ligase. My lab has additionally solved the highest-resolution structure of the cytoplasmic form of the human Arg tRNA synthetase. We have solved the crystal structure of the multidrug efflux pump known as AcrB in the presence of cadmium sulfate and choline chloride. We have determined the structure and solution SAXS profile of a novel NFeoAB fusion. We have shown that numerous eukaryotic ATE1s have an intrinsically disordered region (IDR) that facilitates recognition between ATE1 and tRNA. We have been involved in structural determination and molecular modeling of key proteins involved in the sensing of the bacterial SOS signal. Finally, we have been involved in determining the structures of soluble and membrane components of the Fe^{2+} and Ca^{2+}-sensing two-component biofilm regulator BqsR-BqsS from *Pseudomonas aeruginosa*. ***Key publications include***:

a. Kathman, S. G., Span, I.; Smith, A. T.; Xu, Z.; Zhan, J.; Rosenzweig, A. C.; and Statsyuk, A. V. A small molecule that switches a ubiquitin ligase from a processive to a distributive enzymatic mechanism. *J. Am. Chem. Soc.* **2015**. *137*, 12442-12445. PMC4669213
b. Sánchez-Osuna, M.; Cortés, P.; Lee, M.; Smith, A. T.; Barbé, J.; and Erill, I. Non-canonical LexA proteins regulate the SOS response in the Bacteroidetes. *Nucleic Acids Res*. **2021**. *49*, 11050-11066. PMC8565304
c. Brown, J. B.; Lee, M. A.; and Smith, A. T. The NMR structure of *Vibrio cholerae* FeoC reveals conservation of the helix-turn-helix motif but not the cluster-binding domain. *J. Biol. Inorg. Chem.* **2022**. *27*, 485-495. PMC9398973
d. Paredes, A.; Iheacho, C.[#]; and Smith, A. T. Metal messengers: communication in the bacterial world through transition-metal-sensing two-component systems. *Biochemistry*. **2023**, *62*, 2339-2357. PMC10530140.

[#]*Indicates undergraduate author; Underlined indicates URM*

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