Curriculum Vitae

Name
Karen N. Allen

Current Position
Professor of Chemistry
Professor of Material Science and Engineering
Boston University

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drkallen@bu.edu

Education & Professional Experiences

Education

Tufts University, Medford, Massachusetts
B.S. Biology, *cum laude*, 1984

Brandeis University, Waltham, Massachusetts
Ph.D. Biochemistry, 1989
Advisor: Robert H. Abeles

Professional Experience

Massachusetts Institute of Technology, Cambridge, Massachusetts
Petsko/Ringe laboratories
July 1989 - December 1989:
Post-Doctoral Associate

Brandeis University, Waltham, Massachusetts
Petsko/Ringe laboratories
January 1990-September 1993
American Cancer Society Post-Doctoral Fellow

Boston University School of Medicine, Boston, Massachusetts
September 1993 – September 2000
Assistant Professor of Physiology and Biophysics

September 2000 – August 2008:
Associate Professor of Physiology and Biophysics

Boston University, Boston, Massachusetts
September 2008- 2009
Associate Professor of Chemistry

July 2019- July 2025
Chair, Department of Chemistry

September 2009- present
Professor of Chemistry

September 2014- present
Professor of Materials Science and Engineering

Research Interests
Prof. Allen's research has focused on the elucidation of enzyme specificity and mechanism and the understanding of how Nature has evolved new chemistries from existing protein scaffolds. The Allen lab utilizes macromolecular X-ray crystallography and Cryo-EM supported by small-angle X-ray scattering, molecular modeling, and kinetics combined with bioinformatics to plumb the physicochemical properties of binding sites with an eye toward therapeutic ligand discovery. Within this context, her laboratory has plumbed the basis of enzyme-mediated phosphoryl transfer, phosphoglycosyl transfer and decarboxylation reactions. In addition, Prof. Allen has sought to provide new tools for the exploration of protein structure and function by the invention and implementation of lanthanide binding tags and methodologies to investigate membrane embedded enzymes.

Selected Publications
Lahiri, S.D., Zhang, G., Dunaway-Mariano, D., **Allen K.N.** (2003) "The Pentacovalent Phosphorus Intermediate of a Phosphoryl Transfer Reaction", *Science* 229, 2067-2071.
https://www.science.org/doi/10.1126/science.1082710

Ho, M.-C., Menetret, J.F. Tsurutu, H. and **Allen, K.N.** (2009) "The Origin of the Electrostatic Perturbation in Acetoacetate Decarboxylase". *Nature 459,* 393-397.
https://www.nature.com/articles/nature07938

Pandya C., Brown, S., Pieper, U., Sali, A., Dunaway-Mariano, D., Babbitt P.C., Xia Y., **Allen K.N.** (2013) Consequences of domain insertion on sequence-structure divergence in a superfold. *Proc. Nat. Acad. Sci.* 110, E3381-7.
https://www.pnas.org/doi/10.1073/pnas.1305519110

Ray L.C., Das, D., Entova, S., Lukose, V., Lynch, A.J., Imperiali, B., **Allen K.N**. (2018) Membrane association of monotopic phosphoglycosyl transferase underpins function. *Nat Chem Biol.* 14, 538-541.
https://www.nature.com/articles/s41589-018-0054-z

Durand, T., Dodge, G.J., Siuda, R.P., Higinbotham, H.R., Arbour, C.A., Ghosh, S., **Allen, K.N.**, Imperiali, B. (2024) Proteome-wide bioinformatic annotation and functional validation of the monotopic phosphoglycosyl transferase superfamily. *Proc. Nat. Acad. Sci.* 121, e2417572121.
https://doi.org/10.1073/pnas.241757212

Lecture Title
Redesign of Microbial Redox Enzymes for Deployment in Biosensors and Therapeutics

Abstract
Microbes represent a vast and largely untapped reservoir of biological components that can be used to monitor molecular signals and act on analytes. Through evolution, microorganisms have developed molecular systems capable of detecting and responding to an extraordinary range of chemical stimuli. To harness this resource, we have developed a platform to discover and engineer microbial redox enzymes that respond to specific analytes. Candidate proteins have been optimized and tested as therapeutics and integrated into electronic sensing devices with nanomolar sensitivity.
One such redox enzyme, *Pseudomonas putida* nicotine oxidoreductase (NicA2) catalyzes the oxidation of S-nicotine to N-methyl-myosmine. Taking advantage of its unique evolutionary adaptation, we have refined the inherent catalytic function and structural features of NicA2 to develop tools for nicotine biosensor development and biotherapeutics for nicotine addiction

and overdose. The X-ray crystal structure of the NicA2/S-nicotine complex refined to 2.6 Å resolution reveals a substrate-binding domain with a unique composition of the aromatic cage flanking the flavin isoalloxazine ring. NicA2 is specific for S-nicotine with an apparent K_m of 44 nM but with a very slow catalytic rate (k_{cat} of 6.6×10^{-3} s^{-1}), yielding a "seemingly efficient" enzyme with $k_{cat}/K_m = 1.5 \times 10^5$ $M^{-1}s^{-1}$. An engineered variant, NicA2-J1, was shown to be effective in treating nicotine addiction in advanced animal models. Computational studies identified residues that enhance oxygen reactivity when modified, informing ongoing therapeutic development.

To optimize NicA2 catalytic efficiency for biosensor applications, the origin of the slow k_{cat} was investigated. Stopped-flow and single-turnover kinetics revealed the rate-limiting step occurs in the half-reaction with oxygen. We leveraged CycN, the natural cytochrome electron acceptor for NicA2, as the mediator for the sensor. Co-immobilization of NicA2 and CycN on the electrode produced dramatic improvements in current response with a linear range of 20–2000 nM, matching the concentrations observed in biological fluids. This finding highlights a general strategy for use of natural mediators in biosensor development.

Using a genomic screen, a steroid-responsive genomic island in *Pimelobacter simplex* containing multiple redox enzymes was identified. Purification and biochemical validation of one enzyme, 3-ketosteroid Δ1-dehydrogenase KSDH4, revealed broad substrate specificity toward steroids such as progesterone and cortisol with minimal activity toward 3-ketosteroids including aldosterone and 4-cholesten-3-one. Computational modeling of steroid binding in the active site combined with bioinformatics identified residues predicted to control substrate specificity. Site-directed mutagenesis produced a double variant (G49T/A356Q) with high specificity for testosterone ($k_{cat}/K_m = 3.3 \times 10^5$ $M^{-1}s^{-1}$). A reduction in binding site volume of more than 80 $Å^3$ in the G49T/A356Q variant is consistent with steric occlusion of the active site, shifting PsKSDH4 specificity toward testosterone.

Overall, these advances in identifying and engineering microbial sensing systems establish a foundation for a new generation of tools for molecular analysis and therapeutic intervention.