

 **Chemical Engineering**
Spring 2023 Seminar Series

Evolving Enzymes for Biocatalysis and Gene Editing



Monica E. Neugebauer

Postdoctoral Fellow
Broad Institute of MIT and Harvard

February 27, 2023
66-110
4:15-5:15pm
4:00pm Reception

Enzymes catalyze chemical transformations with exquisite selectivity. Through directed evolution, we can reprogram enzymes for applications in biocatalysis and medicine. In the first part, I will discuss my work to discover, characterize, and engineer Fe^{II}/α-ketoglutarate-dependent enzymes that halogenate unactivated C_{sp³}-H bonds. I solved the anaerobic crystal structure of a novel lysine halogenase (BesD), discovered homologs that enable the formation of nine new chlorinated amino acids, and developed enzymatic cascades to produce chlorinated heterocycles, diamines, keto-acids, and peptides. Through structural studies and high-throughput screening, I investigated the mechanistic basis for regioselectivity and catalytic selectivity within this enzyme family and used the resulting insights to engineer hydroxylases to perform halogenation with activity and selectivity comparable to that of native halogenases. In a second story, I developed novel cytosine base editors (CBEs) through directed evolution. Base editors consist of a programmable DNA binding protein, such as catalytically impaired Cas9, fused to a deaminase enzyme, and enable precise nucleotide changes within a target site in the genome. CBEs, which convert C•G base pairs into T•A, are typically larger and have more undesired off-target editing than their adenine base editor (ABE) counterparts. To develop a new class of CBEs that retain the favorable properties of ABEs, I used continuous protein evolution to evolve ABEs to instead perform highly efficient cytosine base editing within therapeutically relevant sites and cell types. These newly evolved base editors overcome several limitations of existing CBEs and demonstrate the power of protein evolution for addressing challenges in biotechnology.

<http://cheme.mit.edu/seminar-series/>