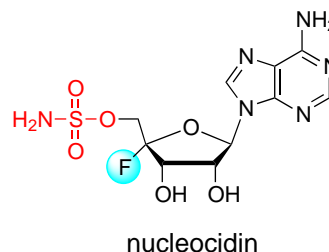


BCHM 421/422 – 2019/2020

Project Outline:

Nucleocidin is a rare example of a natural product that contains a fluorine atom. Only six fluorinated natural products are known, including nucleocidin. Nucleocidin is unique as a derivative of the common nucleoside adenosine, with fluorine incorporated at C4' of the ribose ring. Remarkably, the 5'-OH of ribose is modified with a second rarely seen substituent in natural product chemistry, a sulfamate group. Nucleocidin is a potent inhibitor of ribosomal



peptide synthesis and is highly active against trypanosomes, the parasite that causes sleeping sickness. The Zechel lab has recently identified the biosynthetic genes encoding nucleocidin in the producing bacterium *Streptomyces calvus*. However, the role of each gene remains undefined. Of particular interest is the gene encoding the enzyme that catalyzes the stereospecific incorporation of fluorine into nucleocidin. This project will involve characterizing the function of the individual biosynthetic genes through gene disruption and in vitro biochemical analysis of the encoded enzymes.

Supervisor: David Zechel

Project Title: Biosynthesis of a Fluorinated Natural Product in *Streptomyces calvus*

Keywords (3-5):

1. Enzyme catalysis
2. Biosynthesis
3. Natural product
4. Streptomyces
5. Halogenase

Project Goals:

To determine the biosynthetic pathway for nucleocidin in *S. calvus* by assigning functions to enzymes within this pathway.

Experimental Approaches:

- Gene deletion experiments either in *S. calvus* or the cloned nucleocidin pathway.
- Expression and purification of nucleocidin enzymes for study in vitro.
- Enzyme assay development with purified enzymes.
- Monitoring nucleocidin production in *S. calvus* or a heterologous system using ¹⁹F-NMR spectroscopy and UPLC-mass spectrometry.

References:

- (1) Zhu, X. M.; Hackl, S.; Thaker, M. N.; Kalan, L.; Weber, C.; Urgast, D. S.; Krupp, E. M.; Brewer, A.; Vanner, S.; Szawiola, A.; et al. Biosynthesis of the Fluorinated Natural Product Nucleocidin in *Streptomyces calvus* Is Dependent on the *bldA*-Specified Leu-tRNA(UUA) Molecule. *ChemBioChem* **2015**, *16* (17), 2498–2506.