Title: High Enzyme Stability May Enable Slow Microbial Life in the Subsurface

**Authors: Andrew D. Steen**,<sup>1,2\*</sup> (<u>asteen1@utk.edu</u>), Taylor M. Royalty<sup>1</sup>, Jenna M Schmidt,<sup>2</sup> Karen G. Lloyd<sup>1</sup>

**Institutions:** <sup>1</sup>University of Tennessee, Knoxville, Department of Microbiology; <sup>2</sup>University of Tennessee, Knoxville, Department of Earth and Planetary Sciences

**Project Goals:** To test the hypothesis that the enzymes of subsurface microbes are unusually stable

Abstract Text: Subsurface microbes live extraordinarily slow lives, with timescales of biomass turnover on the order of decades or longer. Because living microbes must have functional enzymes, and enzyme biomass typically comprises the majority of microbial biomass, this implies that enzyme lifetimes must scale with biomass turnover times, and therefore that the enzymes of subsurface microbes are likely to be extraordinarily stable. We present measurements of extracellular enzyme activities from subsurface sediments near Denmark, combined with a simple theoretical model of the minimum feasible turnover time for extracellular enzymes, which suggest the minimum possible turnover time for extracellular peptidases in those sediments is at least 7.5 months. This is orders of magnitude slower than typical enzyme lifetimes. We also present preliminary analyses of the structures of those extracellular enzymes, which bear structural features that confer excess stability. In surface environments, there is little apparent selective pressure on enzymes to be more stable than required to maximize their activity, since biomass is turned over rapidly in those environments. In the subsurface, selective pressure to produce stabler enzymes may lead to novel biochemical mechanisms for enzyme stability that are not present in more frequently-studied, fast-growing microbes.

**Funding Statement:** *Example for grants* - This research was supported by the DOE Office of Science, Office of Biological and Environmental Research (BER), Genomic Science Program (DE-SC0020369).