

Monday November 18, 2013

4:00 p.m.

Third Biochemistry Faculty Candidate

Room 200, Strong

Divergent Evolution of a Bifunctional *de novo* Protein

Modern proteins are extremely specific, efficient machines. A key question in the study of the origin of life is how these machines evolved. One hypothesis is that early proteins were highly promiscuous and inefficient. As proteins gained efficiency in one activity, they lost their ability to perform secondary activities. We cannot study ancient proteins directly, but one way to study the concept is to use *de novo* proteins that do not come from nature. We previously created and screened a library of *de novo* four-helix bundle proteins for the ability to rescue growth of knockout *E. coli* strains that cannot normally grow on minimal media and found a number of proteins that rescue a single strain. One was found to rescue two different strains: $\Delta ilvA$ and Δfes . Using multiple rounds of directed mutagenesis, here we report the creation of two new proteins, one that rescues each cell line, in both cases significantly faster than the original protein. Both evolved proteins lost their secondary activity, despite no selection against this. These results support the hypothesis that as proteins evolve for one function, promiscuous functions are negatively affected.