Laboratory evolution of *de novo* and random proteins

Background: New protein coding genes frequently evolve from existing protein coding genes, via various mechanisms. Recent studies have shown that protein coding genes can also emerge "*de novo*" from genomic regions that did not previously encode any gene. It has been shown that *de novo* genes exist in a continuum, such that the properties of *de novo* genes are a function of their evolutionary age. For example, old *de novo* genes more closely resemble conserved protein coding genes in various aspects such as protein structure and composition. For most conserved proteins, their folding into a defined 3D structure is critical for their function. Therefore *de novo* genes that encode proteins that fold well, are likely to be functional, than genes that encode proteins with poor foldability. Furthermore, a *de novo* gene can gradually evolve such that it encodes proteins with better foldability.

Objective: The student will investigate if proteins encoded by *de novo* genes (*de novo* proteins) fold better than random proteins with same composition. They will also study if *de novo* proteins adapt faster than random proteins towards a folded structure. To this end, they will use a twin-arginine-translocase (TAT) exporter assay. TAT exporter complex exports only folded proteins from the cytoplasm to the periplasm of *E.coli* (host cell). Furthermore, it recognizes its client proteins via a signal sequence. A plasmid system available in our lab (pSalect) allows tagging of *de novo* and random proteins with a TAT-signal sequence in its N-terminus and a beta-lactamase in its C-terminus. Proteins that fold well will carry the beta-lactamase to periplasm where it can neutralize ampicillin. To measure the rate of adaptation towards a folded structure the student will perform directed evolution on protein sequences. Faster adaptation to survival in ampicillin containing media would indicate faster adaptation towards a folded structure.

Methods:

- Gene cloning
- Mutagenesis PCR
- Bacterial cell culture
- Bacterial growth assay

Requirements:

- Interest and basic training in molecular biology lab work
- Interest and basic knowledge on molecular genetics

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Relevant literature:

- Heames B, Schmitz J, Bornberg-Bauer E. *A Continuum of Evolving De Novo Genes Drives Protein-Coding Novelty in Drosophila.* J Mol Evol. 2020 May;88(4):382-398. doi: 10.1007/s00239-020-09939-z.
- Van Oss SB, Carvunis AR. *De novo gene birth*. PLoS Genet. 2019 May 23;15(5):e1008160. doi: 10.1371/journal.pgen.1008160.