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Combining Orthogonal tRNA/synthatase Pair and Amber Codon Suppression to Genetically Encode Oxidative Damage in High Density Lipoproteins

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Combining orthogonal tRNA/synthatase pair and amber codon suppression to genetically encode oxidative damage in high density lipoproteins

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Abstract

Apolipoprotein A-I (apoA-I) is the main protein constituent of high density lipoprotein (HDL - the "good cholesterol"). Oxidatively damaged apoA-I has been isolated from circulating plasma and atherosclerosis plaque with the amino acid residue tryptophan 72 (W_{72}) of apoA-I identified as a primary oxidation site. ApoA-I designed to include specific oxidized amino acids can be used to further investigate the role of site-specific oxidative damage in atherosclerosis. Genetic encoding of oxidized amino acids through orthogonal tRNA/aminoacyl-tRNA synthetase (aaRS) pairs offers a reliable method for producing site-specific oxidized proteins. Our project involves the generation of *Saccharomyces* tryptophan-RS mutants for recognition of oxidized tryptophan (ox-W) but not naturally occurring tryptophan. To study the role of oxidative damage on HDL function we need oxidized proteins that mimic the oxidatively damaged protein observed *in vivo*. Thus, our goal was to produce site-specific oxidized apoA-I (ox-W_{72}) for incorporation into reconstituted nascent HDL. The two aims we hope to achieve within this project are 1) to provide targeted mutations that increase the specificity and affinity of aaRS towards 2-hydroxy-W-apoA-I, as well as 2) express and confirm the presence of ox-W_{72} in modified apoA-I.