Multiscale Chemical Approaches to Map Oxidative Stress

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Radical species are an unavoidable consequence of respiration and the environment, and are tightly buffered by small molecule antioxidants and redo detoxifying enzymes. Oxidative stress emerges when an imbalance develops between the levels of reactive oxygen species and the cell's ability to readily eliminate the reactive intermediates or to repair the resulting damage. Aberrant oxidative signaling is perhaps one of the most important factors contributing to aging, neurodegeneration, heart disease, diabetes, and cancer. In order to induce a phenotypic change, oxidative stress must induce biochemical alterations to the genome, proteome and/or metabolome. Crystallographic analysis revealed that DJ-1 harbors a stabile sulfinic acid, and this oxidative modification is required for the suppression of mitochondrial oxidative stress. We have now shown that this sulfinic acid can react with nitrosothiols to form a thiosulfonate linkage, which can then be reduced by cellular thiols. This provides a potential mechanism for DJ-1 function, which will be further explored with this award. We extended this approach to develop biotin-linked sulfinates for the direct detection and enrichment of endogenous nitrosated proteins. In preliminary experiments, this method led to the identification of hundreds endogenous nitrosated proteins, and establishes a robust new platform to functionally interrogate the dynamics of S-nitrosation. In addition, we describe a new methodology for the selective enrichment of sulfinic acids based on orthogonal alkylation reagents, and propose to identify novel functional sulfinates in the proteome. Finally, we present a new class of ratiometric fluorescent probes for live-cell imaging and ^{19}F-NMR of protein sulfenation *in vivo*. Despite the central role of oxidative stress in human health, our ability to study the precise mechanisms of such modifications is hampered by a lack of selective chemical and analytical methods. In this proposal, we present a series of innovative chemical approaches to study oxidative damage across experimental scales, from live-cell imaging to in vivo imaging, in addition to proteome-wide annotation of oxidative post-translational modifications. Furthermore, we present a likely mechanism for the Parkinson's disease-linked redox chaperone DJ-1, and present new mechanism-based probes to functionally annotate and profile S-nitrosation (R-SNO), S-sulfenation (R-SOH), and S-sulfination (R-SO_2H).