

***In vivo* monitoring of drug-target interactions through the use of semisynthetic biosensors**

Drug-target interactions have been exhaustively studied through *in vitro* analysis. These studies provide a simplified environment for the evaluation of a particular protein, network, or signaling pathway. The artificial nature of the environment and the lack of other cellular components present *in vivo* often restricts the observation to certain cellular processes or may produce misleading results. Due to the complexity of living systems and the interconnected networks of metabolites, proteins, lipids, glycans, ions, and biopolymers, the use of *in vivo* systems to study protein interactions, specifically drug-target interactions, is preferred. *In vivo* analyses can provide information on off-target and whole organism effects that cannot be observed *in vitro*.¹

The ability to monitor discrete proteins in their native habitat has been furthered by the use of genetically encoded tags, such as green fluorescent protein and its variants. Even though fluorescent microscopy and flow cytometry can be used to visualize and quantify the protein, respectively, the tag has the potential to perturb protein structure and thereby alter protein expression, function, and localization. Recently, the development of non-native, non-perturbing bioorthogonal chemical reporters has combined the simplicity, specificity, and versatility of previous methods with the additional advantage of *in vivo* applicability.¹ Ligand binding with synthetic probes has enhanced the study of protein structure and function in cells and organisms. Use of the ligand-directed tosyl (LDT) technique has the ability to non-genetically label the target and effectively re-open the active site to endogenous substrates. This occurs via a one step reaction that activates an SN2 nucleophilic substitution reaction by the ligand acting as a good leaving group.² Therefore, *drug-target interactions in vivo can be monitored by semisynthetic biosensors constructed by non-genetic labeling of endogenous proteins*.

The cytosolic metalloenzyme carbonic anhydrase II (CAII) was employed for determination of the ability of LDT chemistry to selectively label a protein as assessed via fluorescence analysis.^{3,4} Purified CAII and CAII present in simple and complex mixtures as well as mice, displayed the specificity of this technique in labeling a protein of interest and its applicability *in vivo*. Enzymatic analysis confirmed that labeled CAII maintained normal functional levels when compared to native CAII.⁴ The study of FK506 binding protein, FKBP12, and the galectin Congerin II demonstrated the versatility of LDT chemistry for various proteins with an assortment of functions.^{4,5,6}

The ability to specifically and non-genetically label an endogenous protein with minimal structural perturbation has the power to revolutionize *in vivo* protein studies. The capacity to more accurately study protein function, localization, and interactions *in vivo* will provide an improved understanding of therapeutic targets, thereby aiding in the development of inhibitors and advancing the progress of biomarkers. The relevance of LDT chemistry for complex proteins needs to be assessed in order to ascertain the extent this technique can be used in drug and inhibitor screening assays and to further realize the potential of *in vivo* protein modification therapy.

References:

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