**Covalent Probes for Activity-Based Photoacoustic Tumor Imaging and Histidine-Selective Bioconjugation**

Photoacoustic (PA) imaging is an emerging modality that combines the contrast of optical imaging with the spatial resolution of ultrasound. Activatable PA contrast agents have been developed for tumor imaging, but most rely on single-factor activation and remain susceptible to interference from endogenous chromophores such as hemoglobin. To address these challenges, we developed NOx-JS013, an activity-based PA imaging probe that combines hypoxia-responsive activation with covalent targeting of neutral cholesterol ester hydrolase 1 (NCEH1). NCEH1 is a serine hydrolase that is highly expressed and active in aggressive cancers, making it a promising marker of metastatic progression. Competitive activity-based protein profiling (ABPP) showed that NOx-JS013 labels active NCEH1 in live cells with high selectivity over other serine hydrolases. Our data suggest that NOx-JS013 binds NCEH1 first, and the *N*-oxide is reduced only afterward under hypoxia, so the PA signal comes mostly from probe that is already bound to its target. In mouse models, NOx-JS013 visualized aggressive PC3 prostate tumors but was barely detected in non-aggressive LNCaP tumors.

I will then discuss efforts to expand the residue scope of covalent probes. Chemoselective covalent modification of histidine remains limited by its moderate nucleophilicity and competition from other nucleophiles. I will present the development of a histidine-selective bioconjugation reaction.