Development of *N*-oxide-based Probes for Photoacoustic Imaging of Hypoxia

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Hypoxia occurs when limited oxygen supply impairs physiological functions and is a pathological hallmark of many diseases including cancer and ischemia. Thus, detection of hypoxia can guide treatment planning and serve as a powerful predictor of patient prognosis. Unfortunately, current methods suffer from invasiveness, poor resolution and/or low specificity. To address these limitations, we present Hypoxia Probe 1 (HyP-1), the first hypoxia-responsive agent for photoacoustic imaging. This emerging modality converts safe, non-ionizing light to ultrasound waves, enabling acquisition of high-resolution 3D images in deep tissue. HyP-1 features a novel and generalizable *N*-oxide trigger that is reduced in the absence of oxygen by heme proteins such as CYP450 enzymes. Reduction of HyP-1 produces a spectrally distinct product, facilitating identification via photoacoustic imaging. HyP-1 exhibits excellent selectivity for hypoxic activation *in vitro*, in living cells and in multiple disease models *in vivo*. HyP-1 is also compatible with NIR ratiometric fluorescence imaging, establishing its versatility as a multimodal imaging agent. In addition to HyP-1, we have synthesized a panel of red-shifted analogs compatible with ratiometric photoacoustic imaging in an effort to minimize signal variations and increase reliable hypoxia detection in deep tissue.