Biomolecular imaging is an essential technology in life science, enabling the detection, quantification, and visualization of biomolecules. As emerging imaging techniques continue to advance, the development of chemical tools, such as molecular probes, remains a key challenge. This study focuses on chemical tools for hyperpolarized nuclear magnetic resonance imaging (MRI) and tissue-clearing fluorescence imaging—two next-generation techniques gaining significant attention. Despite their potential, the limited availability of compatible chemical tools has hindered broader application. To overcome this limitation, this work aims to develop novel molecular probes and chemical methodologies.

(1) Development of molecular probes applicable in vivo for hyperpolarized MRI

Dynamic nuclear polarization (DNP) is a well-developed one of hyperpolarization techniques to dramatically enhance MRI sensitivity. DNP-MRI enables non-invasive molecular imaging in vivo in real-time. However, its application has been greatly limited due to an insufficient number of DNP-MRI molecular probes applicable in vivo. This is because the development of DNP-MRI molecular probes is highly challenging due to the lack of knowledge for designing practical probes. To address this issue, the author and co-workers have developed novel molecular probes by rational molecular design incorporating know-how in various fields such as organic chemistry, biochemistry, computational chemistry, nuclear physics, and oncology. The research group has reported Ala-[1-^{13}C]Gly-d_2-NMe_2 for aminopeptidase N (APN), which is an important biomarker enzyme for cancers and inflammation (*Science Adv.* 2022), and ^{13}C-GSH for glutathione metabolism, and ^{13}C-BCM-5 for dipeptidylpeptidase-4 (DPP-4), which is an important enzyme on diabetes and cancers (*Science Adv.* 2024). These molecular probes were rationally designed to be applicable in vivo by incorporating various knowledge such as organic chemistry, computational chemistry, biochemistry, nuclear physics, and oncology. The developed probes enable to detect each enzymatic activity in tumor region, kidney, and whole body.

(2) Development of chemical methodologies for tissue-clearing fluorescence imaging

Fluorescence imaging is one of the most advanced imaging techniques, providing high sensitivity and high resolution. This technology has contributed significantly to the elucidation of various biological phenomena at the cellular level. However, there is an increasing demand for understanding biological phenomena beyond the cellular level, particularly in vivo. Due to the low permeability of light, visualizing deep tissues using fluorescence imaging remains challenging. To address this issue, tissue-clearing fluorescence imaging has garnered attention. This technique involves rendering biological samples transparent by matching the refractive index of the tissue with the surrounding solution, following fixation and replacement with a specialized solution, enabling the detection of fluorescence signals from deep tissues.

However, since this technique has traditionally been developed in the fields of physics and medicine, chemical insights remain limited. As a result, the selection and development of fluorescent dyes and molecular probes effective for tissue-clearing systems have often relied on trial and error, resulting in highly time-consuming and laborious processes. To address this issue, the author and co-workers have sought to understand the physicochemical properties of fluorescent dyes in tissue-clearing solutions, leading to the successful development of fluorescent dyes and molecular probes optimized for tissue-clearing imaging (*ACS Nano* 2024). Additionally, we have developed a whole-tissue fluorescence labeling method using click chemistry, achieving successful 3D imaging of nascent RNAs in mouse kidney, and hypoxic environments in tumor and brain (*Science Adv*. 2024).