FixEL: a new method for visualizing ligand dynamics in the brain by reframing the PFA fixation chemistry.

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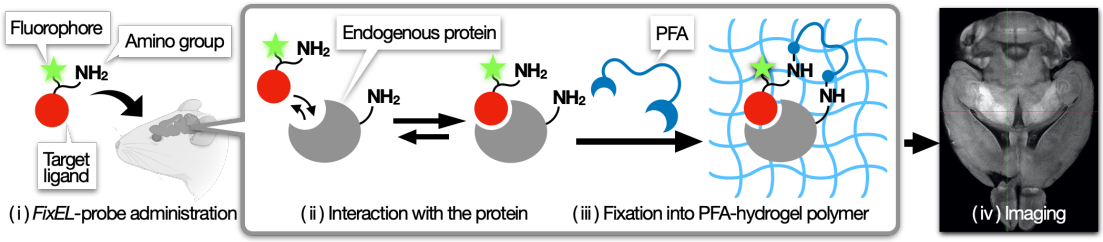
Keywords: *Protein-ligand interaction, Endogenous glutamate receptor, Endogenous dopamine receptor, Nanobody, 3D imaging.*

Various small molecules have been used as functional probes for pharmaceuticals and medical diagnostics. However, there are still limited methods to accurately evaluate the spatial distribution and diffusion/excretion dynamics of small molecules in tissues for elucidating their functions. In this study, we developed a novel chemical biology method termed "***Fix*ation-driven chemical crosslinking of *e*xogenous *l*igands** (***FixEL***)", which enables the visualization of the small molecule distributions in complex tissues[1]. In the *FixEL* method, we employ a designer *FixEL* probe, which is a small molecule ligand modified with a nucleophilic functional group that can react with PFA polymer. After the probe is administered to the animal, a hydrogel-like three-dimensional structure consisting of protein and PFA is formed in the tissue by PFA-fixation treatment. Simultaneously, the nucleophilic functional groups on the probe rapidly form covalent bonds with the PFA polymer, and the 3D distribution of the probe based on the protein-ligand interaction is immobilized on the hydrogel-tissue complex. This chemistry allows us to capture and analyze a snapshot of the small molecule dynamics based on the protein-ligand interactions in tissues. Indeed, we succeeded by *FixEL* in evaluation of the diffusion/excretion kinetics of small molecule ligands for the mGlu1 receptor in mouse brain. Moreover, we further applied the *FixEL* method to other glutamate receptors (AMPARs) and dopamine receptors (DRD2s). In addition to small molecules, clear 3D imaging of nanobodies distributed throughout the brain was also achieved by *FixEL* method with high spatial resolution.



1) bioRxiv 2021.12.21.473647