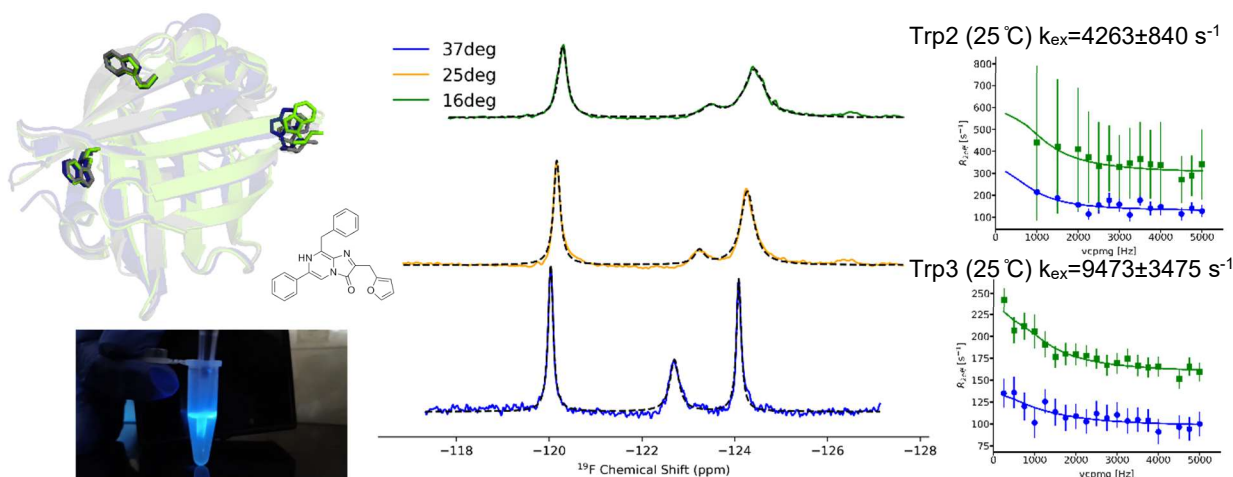


Inferring functional relevance from aromatic sidechain dynamics in ^{19}F labelled bioluminescent Nanoluciferase

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Nanoluciferase (Nluc), the brightest known bioluminescent reporter and a workhorse of imaging experiments, generates blue light (~ 470 nm) by catalyzing the oxidation of its substrate furimazine in the presence of oxygen.^{1,2} Redesigning Nluc and its substrate to achieve red-shifted emission better suited to biological applications remains a highly sought-after goal.³ X-ray crystallographic snapshots of apo Nluc and Nluc bound to product and substrate analogs have revealed inactive "closed" and active "open" conformations,⁴ but the solution-state dynamics underlying this conformational switch remain unknown. Here, using ^{19}F NMR spectroscopy of 5F-Trp-labeled Nluc, we detect and quantify μs – ms conformational exchange of three native tryptophan sidechains (W10, W132, and W161) and discuss the putative functional relevance of each residue to substrate gating and catalytic activity.



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