Towards a high-performance genetically-encoded fluorescent biosensor for pyruvate

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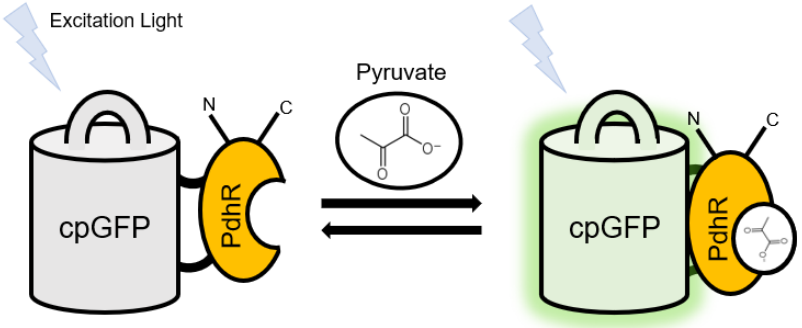
It is known that neurological diseases are closely related to metabolic abnormalities, but these mechanisms have yet to be fully understood. Genetically-encoded single-fluorescent protein (FP) based biosensors have enabled investigation of these mechanisms through their versatility and high spatial and temporal resolution. A molecule of particular interest is pyruvate, an important metabolite at the intersection of several metabolic pathways. Although a couple of single-FP based biosensors for pyruvate have already been reported, there is still a demand for one that is affinity-tuned and that generates a large pyruvate-dependent fluorescence response.[1,2]

A single-FP based biosensor is composed of an FP inserted into a target binding domain such that target binding induces a confrontational change and subsequent fluorescence change.[3] To construct a prototype pyruvate biosensor (**Figure**), we inserted circularly permuted green FP (cpGFP) into a DNA-binding domain-deleted form of the pyruvate-binding bacterial protein PdhR at 14 different sites using the Alphafold software to predict the structure of cpGFP-inserted PdhR. Of 14 variants, one variant designated Pyron0.1 showed a substantial pyruvate-dependent fluorescence change ($\Delta F/F = 0.16$). To improve the fluorescence response upon pyruvate binding, we optimized the length and sequence of linkers that connect cpGFP and PdhR and then performed directed protein evolution. In this work, we present the latest progress of the directed evolution.



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