A rapid biosensor engineering platform by translatable fluorogenic amino acids

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Project Goals: Conditionally fluorescent, or fluorogenic amino acids (FgAAs), can dramatically increase their fluorescence when 'trapped' within macromolecules, for example at the antigen-antibody binding interface. Eventually, we intend to leverage this sensing ability to establish a machine-learning guided and fluorescent in situ sequencing-powered evolution pipeline towards de novo discovery of new binders that will also function immediately as instant biosensors. Toward this goal we begin exploring the molecular rules by which FgAAs can transform protein binders into instant sensors for their targets. We expect these optical biosensors to have immediately useful applications in basic biology research in synergy with the Aim 5 in our renewal grant.

Modification of existing protein binders with fluorogenic probes can effectively transform the binder into an optical biosensor for its target, generating an easily detectable fluorescence change upon target binding. Variety of robust technologies can quickly evolve new and specific protein binders against virtually any target. However, the transformation of protein binders into fluorogenic sensors has been slow and generally limited to small signal changes. This is likely because current approaches rely on low-throughput screens that are not compatible with many probes, which greatly constrains the space. Such approaches are also not compatible with employment of a molecular evolution pipeline because the protein binder sequence cannot easily be fine-tuned once the fluorogenic residue matures, i.e., after the probe conjugation.

Here we present a two-stage biosensor engineering platform. The first, 'transformation', stage relies on the modular, and simple derivatization of protein binders with many new classes of fluorogenic probes. This also enables cost-effective and scalable (>25 mg from 1 L *E. coli* culture) biosensor manufacturing. The multiplexed exploration of hundreds of optimal probe, linker, and position combinations in parallel streamlines biosensor discovery to ~3 weeks from conception. We demonstrate the generalizable applicability of the platform via construction of multiple biosensors to distinct proof-of-principle antigens with ratiometric, nearly instant readouts. The second, 'evolution', stage relies on a new, highly efficient tRNA charging chemistry which enables streamlined engineering of the biosensor toward further optimization of

residues around the mature fluorogenic residue via compensatory mutations. Together, this platform will allow rapid conversion and fine-tuning of a wealth of available protein binders into optical biosensors that can serve as valuable tools for basic research.

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