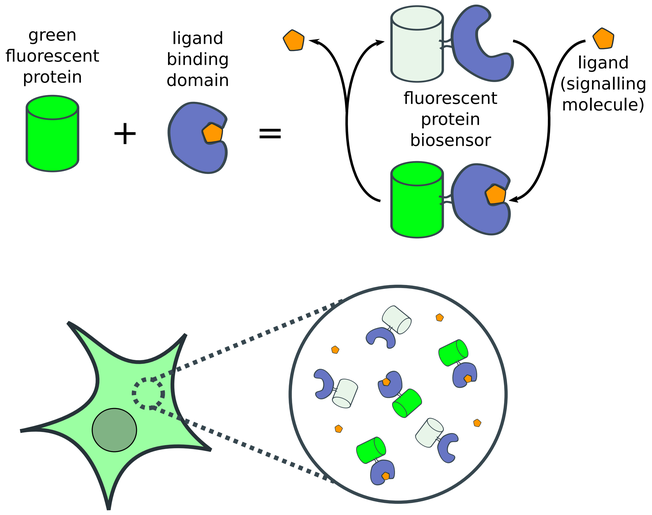
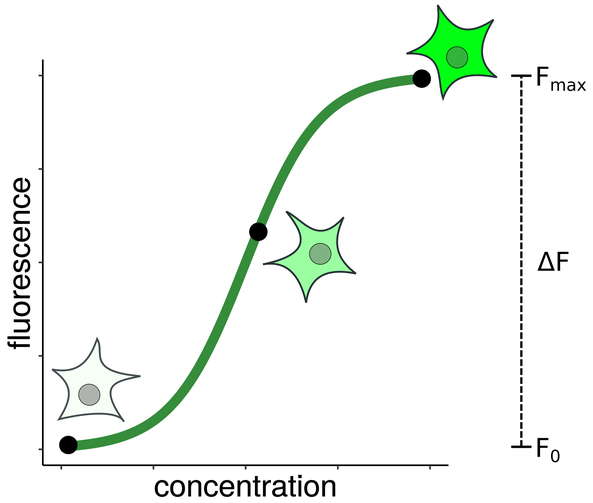
Development of metabolite biosensors using massively parallel assays of domain-insertion variant libraries

John Koberstein, Melissa Stewart, Emma Krnacik, Michael Cohen and Richard Goodman

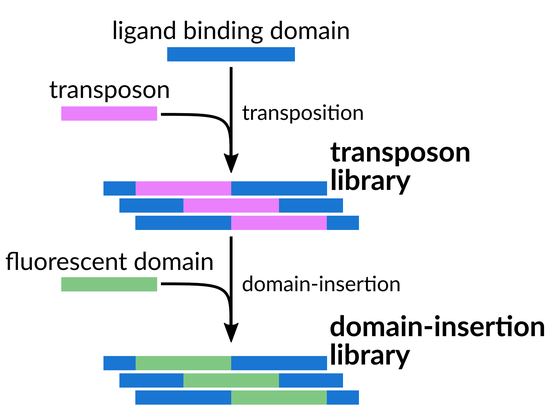
Biosensors combine a ligand-binding domain with a fluorescent protein to measure the concentration of signalling molecules in live cells.

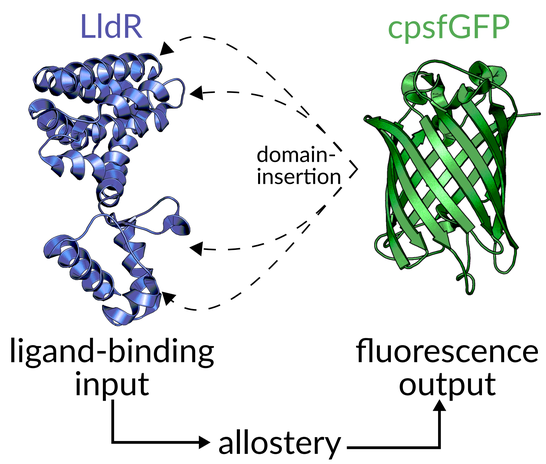




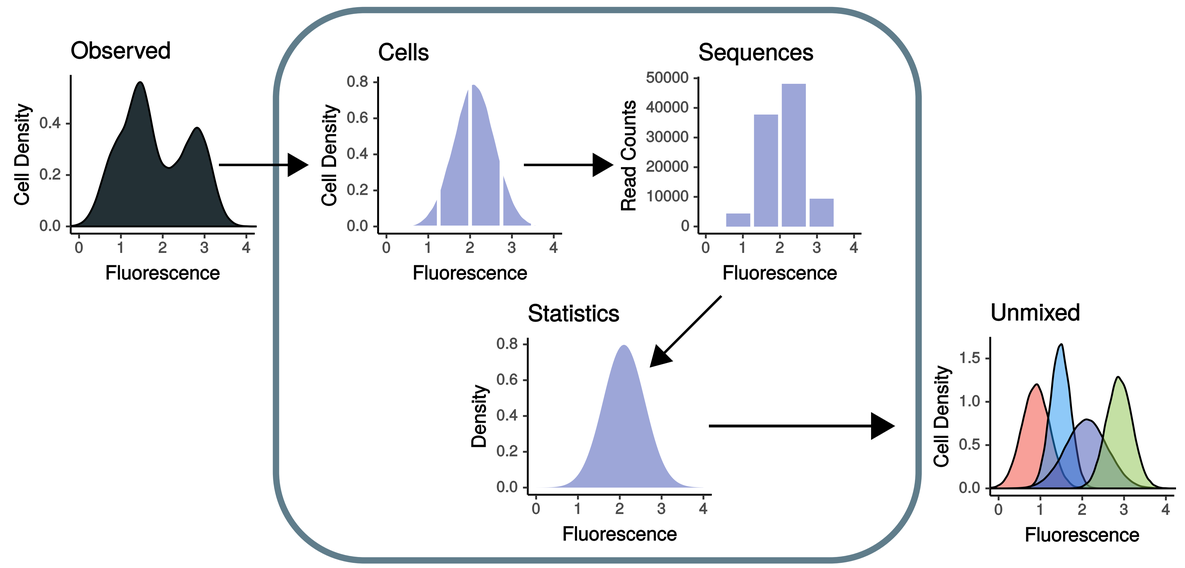
Design of a novel biosensor requires identification of the optimal site to insert the fluorescent protein into the ligand-binding domain.

This process can be accelerated through a transposon mediated domain-insertion library strategy that generates all possible insertion combinations.[1]



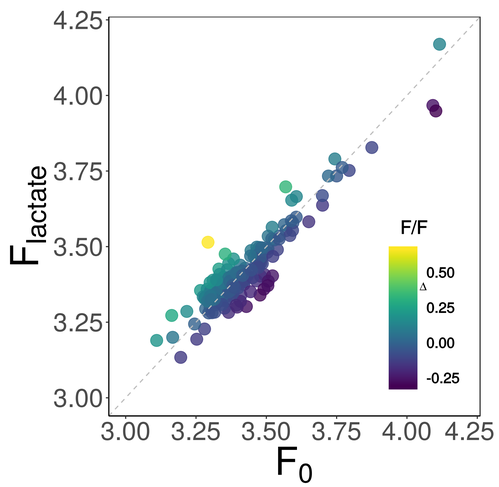


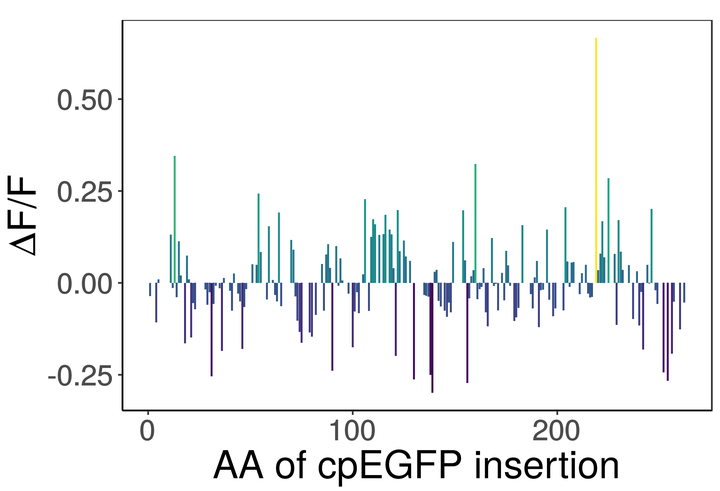
Cell sorting, sequencing and statistics (sort-seq) enables the quantification of fluorescence intensity for many biosensor variants in parallel.[2]



sort-seq

Sort-seq assay applied to lactate biosensor identifies insertion site with max ΔF/F from library of 221 variants.





References

1. Nadler D, Morgan S, Flamholz A, Kortright K, Savage D. Rapid construction of metabolite biosensors using domain-insertion profiling. *Nat Commun*. **2016**; 7:12266.
2. Peterman N, Levine E. Sort-seq under the hood: implications of design choices on large-scale characterization of sequence-function relations. *BMC Genomics*. **2016**; 17:206.