13b An Engineered Chimeric Enzyme for Use in Drug Sensing, Discovery and Development

David W. Wood and Georgios Skretas

This work describes the development of multi-domain allosteric biosensor proteins based on rational protein engineering. These proteins, expressed under appropriate conditions, allow the detection of various hormones and hormone-like compounds through easily detectable and selectable phenotypes in *Escherichia coli*. To construct the sensor, the ligand-binding domain of the human estrogen receptor (ER) was genetically fused to the sensitive reporter enzyme thymidylate synthase (TS). Ligand binding by the receptor ligand-binding domain activates the TS enzyme, such that *E. coli* TS knockout cells expressing this fusion exhibit estrogen-dependent growth phenotypes. This biosensor has been successful in reliably identifying a large variety of estrogenic compounds, in some cases at sub-nanomolar concentrations. The identified compounds include synthetic and plant-derived estrogens as well as androgens with very weak ER binding ability. It was further observed that levels of cell growth correlated well with ligand-binding affinity. Surprisingly, this simple biosensor is also able to recognize the pharmacological properties of test compounds, as it can reliably discriminate between known estrogen agonists and antagonists and detect subtype-selective estrogen analogues. Interestingly, this estrogen-sensing protein could be trivially converted to a sensor for thyroid hormone by simple domain swapping of ER with the ligand-binding domain of the human thyroid hormone receptor. This system constitutes a facile technique for detection of novel ligands with potential medical applications. Finally, because the hormone-dependent phenotypes are also selectable, it offers an easy tool for evolving new compounds with hormone-mimicking properties, as well as novel receptors with engineered binding characteristics.