13a Protein Switches Created by Non-Homologous Recombination

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We have developed a directed evolution approach for creating protein switches involving the *in vitro* recombination of two non-homologous genes with the prerequisite input and output functions. We have recombined the genes coding for TEM1 b-lactamase (BLA) and the *E. coli* maltose binding protein (MBP) to create a family of MBP-BLA hybrids in which maltose is either a positive or negative effector of b-lactam hydrolysis. Some of these MBP-BLA switches were effectively 'on-off' in nature, with maltose altering catalytic activity by as much as 600-fold. One MBP-BLA switch was identified that could be positively allosterically regulated by maltose and negatively allosterically regulated by Zn^{2+}, which is surprising considering that neither BLA nor MBP bind Zn^{2+}. The origin of this effect has implications for how novel protein function evolves. The ability of the MBP-BLA switches to confer an effector-dependent growth/no growth phenotype to *E. coli* cells was exploited to rapidly identify, from a library of 4×10^6 variants, MBP-BLA switch variants that respond to sucrose as the effector. The transplantation of these mutations into wildtype MBP converted MBP into a 'sucrose-binding protein,' illustrating the switches potential as a tool to rapidly identify novel ligand-binding proteins.