

# Towards engineering allosteric regulation into a circularly permuted TEM-1 beta-lactamase

*Valéry Mathieu<sup>1</sup>, Jacques Fastrez, Patrice Soumillon*

<sup>1</sup>*Université catholique de Louvain-la-Neuve, Institut des Sciences de la Vie, Laboratoire d'Ingénierie des Protéines et des Peptides, Croix du Sud 4-5 boîte 3, B-1348 Louvain-la-Neuve - Belgium*

Enzymes are often regulated by the binding of a ligand far from the active site in a so called “allosteric site”. Engineering of artificial allosteric regulation applied to enzymes could open new routes for the development of soluble biosensors for analytical biochemistry or as diagnostic tools. Indeed, if an enzyme activity can be modulated by the presence of a ligand in solution, then measuring the specific activity allows its correlation to the ligand.

We have chosen TEM-1 beta-lactamase as a scaffold for ligand binding. The production of beta-lactamases is the most common mechanism of bacterial resistance to antibiotics such as penicillins or cephalosporins, the enzymes being able to catalyze the hydrolysis of the beta-lactam ring of these compounds. The TEM-1 beta-lactamase (class A beta-lactamase) is composed of two domains, alpha and alpha/beta linked by two hinges [1]. These two hinges are supposed to be important for the enzyme activity because they could position correctly the two domains, and consequently the active site residues lying at the interface. The enzyme on which we are working is a circular permutant of the wild type TEM-1 in which one of the two hinges has been broken and the N- and C-terminal extremities have been linked by a little peptide (we called it TEM-1cp) [3].

In a directed evolution approach we wish to create an allosteric binding site for varied ligands such as metal ions, anions, proteins or small organic molecules. The objective is to regulate the enzymatic activity by modifying the positioning of the domains. Here we describe the construction of the library in which the allosteric binding site will be composed of three degenerated surface loops in TEM-1cp. The selection method using the “phage-display” technique is also described.

First, we have randomly mutated six residues in the remaining hinge. To do this we have replaced the wild type coding sequence by a degenerated cassette. By this technique we have created a preliminary library of about  $1,6 \cdot 10^7$  mutants in E.coli TG1 bacteria. The percentage of active clones has been estimated to about 0,6 % (in vivo selection on agar plates containing 20mg/l of ampicillin). Some of these mutants have been sequenced but showed no consensus sequence. Then, the “phage-display” selection method has been tested on small size libraries. It allowed us to find one beta-lactamase mutant which is inhibited by Nickel ions. Finally, a PCR strategy has been developed to recover the two other degenerated loops from previously constructed libraries in TEM-1 [2]. These loops are structurally contiguous to the mutated hinge. The construction of a combinatorial library should allow to engineer more complex allosteric sites and is still on-going.

1. Jelsch C., Mourey L., Masson JM., Samama JP., 1993, *Proteins: Struct. Funct. Genet.*, **16**, 364-83

2. Mathonet P., Deherve J., Soumillon P., Fastrez J., 2006, *Prot. Sci.*, **15**(10), 2323-34.

3. Osuna J., Pérez-Blancas A. & Soberon X., 2002, *Prot. Engng.*, **15**, 463-70.