

# Intein splicing in the periplasmic space of *Escherichia coli*

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Inteins are protein splicing elements that catalyze their own excision from a precursor protein with the concomitant ligation of the flanking protein sequences. Two peptide bonds are broken and a new peptide bond is formed during the protein splicing process. Inteins thus represent a potentially powerful means of protein manipulation.

In particular, they have been engineered in order to produce cyclic proteins and peptides *in vivo*. The procedure -called SICLOPPS for Split Intein Circular Ligation Of Peptides and Proteins- is based on intein fragmentation and arrangement of the two fragments Int<sub>N</sub> (the N-terminal part) and Int<sub>C</sub> (the C-terminal part), in permuted order Int<sub>C</sub>-extein-Int<sub>N</sub>. The SICLOPPS technology has been applied in the creation of large collections of cyclic peptides *in vivo* (Benkovic et al., 1999).

In the present study, our aim is to evaluate the possibility to achieve protein cyclization by intein splicing in the periplasmic space of *Escherichia coli*. Since this medium is very oxidant, it is possible that the essential cysteine, found in most inteins, would be rapidly inactivated. Moreover, with a permuted intein, the splicing reaction should occur after the exportation for cyclic product to be localized in the periplasmic space.

We chose the well studied Ssp DnaB intein from *Synechocystis* sp. strain PCC6803 and the  $\beta$ -lactamase TEM1 as our cyclization target. The  $\beta$ -lactamase TEM1 was cyclized by joining his native N- and C-termini with a short peptide loop, 12-residues long. A signal peptide was added in front of the construction DnaB<sub>C</sub> –  $\beta$ -lactamase – DnaB<sub>N</sub>. The periplasmic space localization of the protein was demonstrated by SDS-PAGE and by ampicillin resistance phenotype. The successful cyclization was shown by mass spectrometry.

These results could lead to new applications for the SICLOPPS technology. In particular, it becomes possible to create large collection of cyclic peptides in the periplasmic space of *Escherichia coli*.