POST-TRANSCRIPTIONAL ASSOCIATION OF PROTEINS TO STUDY SPATIAL ORGANISATION WITHIN MULTI-ENZYME COMPLEXES

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To catalyse chemical reactions, nature has evolved enzymatic cascades. These multistep reactions in living cells are often performed by multi-enzyme complexes to maintain high local concentrations of intermediates to enhance reaction rates, called substrate channelling. Based on the fact that natural proteins or nucleic acid-protein interactions can be used as scaffolds for the construction of functional multi-enzyme complexes, artificial scaffolds have been developed for the construction of multi-enzyme complexes which carry out multi-step enzymatic catalysis processes[1]. However, none of them have systematically investigated the spatial organisation of the enzymes and its effect on the product(s) released.
We are investigating this question using the Molecular Welding Tool[2] consisting of two small proteins, Jo and In, which spontaneously form an intramolecular isopeptide bond and, incidentally, provide an original means of orienting enzymes[3,4].

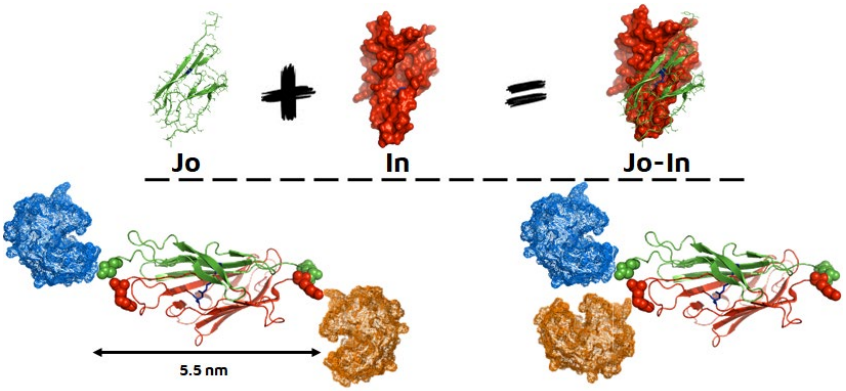

Figure 1 – Proteins Jo and In form spontaneously a covalent bond, allowing the association of enzymes in a tailored spatial orientation

We exemplified our strategy inspired by two different type of multi-enzymatic organizations; the plant cell wall degrading enzymes complexes known as cellulosome and a heterologous pathway introduced into Escherichia coli to produce limonene from glucose. A large array of complementary technics including Small Angle X-rays Scattering or immunofluorescence labelling allows us to correlate distance and/or spatial orientation with specific enzymatic activity of plant cell wall acting hydrolases. We aim to apply the same strategy to increase limonene production in vivo.
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