

Engineering, biophysical and structural characterization of new Jo/In complexes for the design of artificial cellulosomes

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Abstract

Lignocellulose is found in plant cell-walls and offers great potential for a wide range of applications. Its degradation, however, requires a pre-treatment step due to the insolubility of the compounds and the crystalline structure of cellulose. In nature, anaerobic bacteria and fungi have demonstrated their ability to use lignocellulose by producing cellulosomes, which are self-assembling extracellular multi-enzyme complexes specialized in lignocellulose decomposition. Although the prevalence of cellulosomes is rare, they show great diversity among species. Their characteristics are currently studied for numerous potential biotechnological applications, mainly large-scale biomass conversion. The efficiency of cellulosomes relies on the synergistic action of their enzymes, due to their spatial proximity. Artificial cellulosomes have been designed using the cohesin-dockerin modules, essential for cellulosome assembly. However, enzyme attachment is not covalent and is highly flexible, complicating studies aiming to understand spatial organization and degradation. Overall, cellulosome assembly and mechanism of action remain poorly understood. In this project, we set out to fix the enzymatic spatial distance using a covalent system, in order to reveal the spatial organization effect on enzymatic degradation of lignocellulose. This covalent system is called the Jo-In complex and is able to achieve spontaneous covalent bonding. It composes the Domain 2 of RrgA protein from *S. pneumoniae* and could be used to achieve functional enzymatic assemblies.