# Designer cellulosomes for future production of biofuels and biomaterials

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## The cellulosome

Many cellulolytic microorganisms produce intricate multi-enzyme complexes called cellulosomes that efficiently degrade cellulose -- the most abundant organic polymer on Earth. The cellulosomes are composed of a conglomerate of subunits, each of which comprises a set of discrete interacting functional modules. A multi-functional integrating subunit (called scaffoldin) is responsible for organizing the cellulolytic subunits into the multi-enzyme complex. This is accomplished by the interaction of two complementary classes of domain, located on the two separate types of interacting subunits, i.e., a cohesin domain on scaffoldin and a dockerin domain on each enzymatic subunit. The high-affinity cohesin-dockerin interaction defines the cellulosome structure (Fig. 1). The scaffoldin subunit also bears a cellulose-binding module (CBM) that mediates attachment of the cellulosome to its substrate.

## The cohesin-dockerin interaction

We have cloned and expressed individual cellulosomal domains and have analyzed their structure-function relationship via biochemical, molecular, and structural studies.

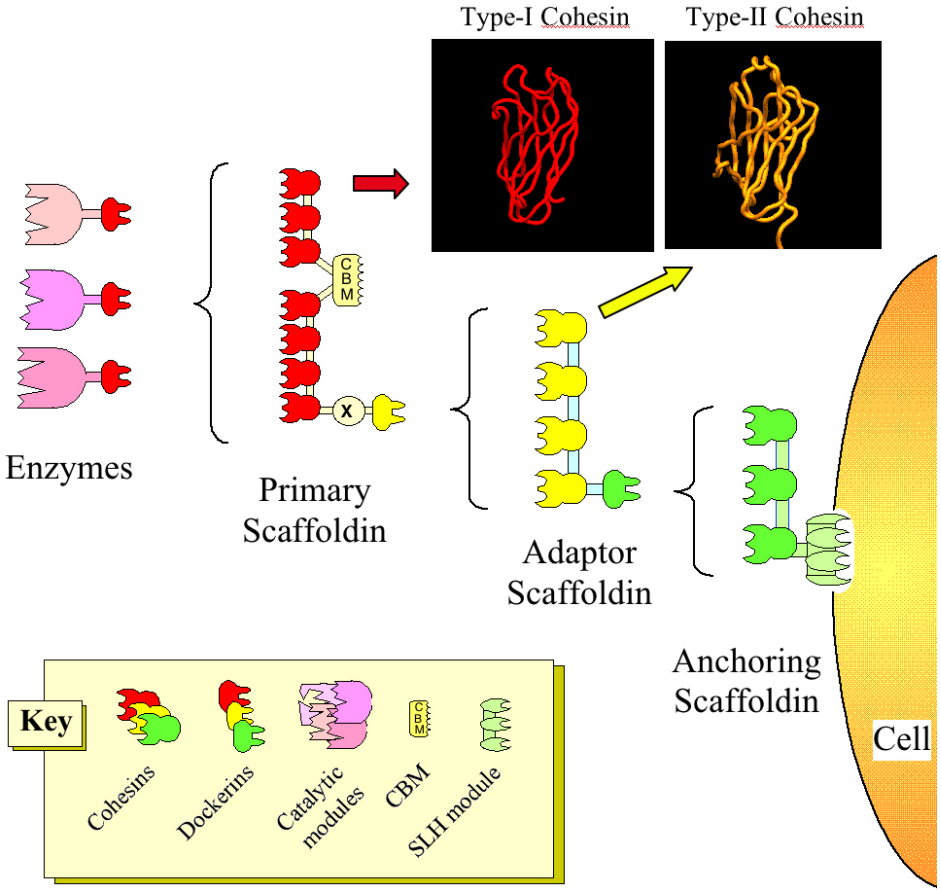


***Fig. 1**. Schematic representation of the supramolecular architecture and disposition on the bacterial cell surface of a representative cellulosome system. Dockerin-containing enzymes are incorporated into a primary scaffoldin via interaction with the resident type-I cohesins. The type-II cohesins of an adaptor scaffoldin interacts with the dockerin of the primary scaffoldin, and its own dockerin binds to the cohesins of an anchoring scaffoldin, whose SLH module serves to attach the entire cellulosome system to the cell surface. The respective specificities of the different cohesin-dockerin pairs are color-coded. A single CBM in the primary scaffoldin targets the complex and the entire cell to the cellulose substrate. Crystal structures for type-I and II were determined in collaborative studies. The figure implies the enormous diversity inherent in the Lego-like strategy of the cellulosome system.*

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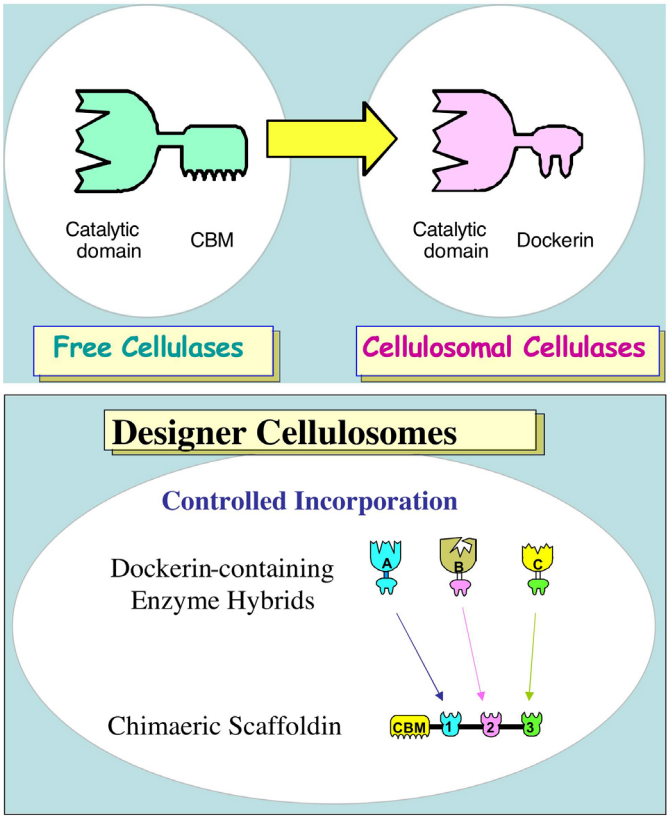


***Fig. 2.*** *"Designer cellulosome"— an engineered system, designed to convert a free cellulase system into a cellulosomal one, by replacing the enzymes' CBM with a dockerin module. Since the different enzymes are appended with dockerins of divergent specificity, we can control their incorporation into precise positions of the artificial chimaeric scaffoldin by virtue of their selective interaction with matching cohesins.*

One approach has been to determine the crystal structures of the important domains. In an alternative, complementary bioinformatics-based approach, we have analyzed the cohesin-dockerin interaction by site-directed mutagenesis and "progressive gene swapping". The native interaction itself was found to be one of the strongest protein-protein interactions in nature. By comparing the cohesin-dockerin interaction between two different dockerin species, we were able to identify the important residues on both the cohesin and dockerin modules that contribute to interspecies specificity.

**Designer cellulosomes -- nanosomes**

Designer cellulosomes comprise recombinant chimaeric scaffoldin constructs and selected dockerin-containing enzyme hybrids as a conceptual platform for promoting synergistic action among enzyme components (Fig. 2). This approach enables the precise incorporation of complementary dockerin-containing components into a designer cellulosome by simply mixing them in solution together with the chimeric scaffoldin, thus controlling the composition and architecture of the resultant complexes. This approach will eventually be appropriate for general use as a molecular Lego for application in biotechnology and nanotechnology.

**Conversion of cellulosic biomass to biofuels**

The depletion of fossil fuels has raised the world need for cheap and clean alternative energy sources. One promising approach is the conversion of cellulose to bioethanol. Thus, by reducing the amount of agricultural waste, we can create an environmentally friendly fuel. In our lab we try to pursue this objective by harnessing the unique, thermostable cellulose-degrading systems from *Clostridium thermocellum* and from other bacteria. For this purpose, selected cellulosomal components (the CBM, cohesins, dockerins, catalytic modules and linker segment) are reassembled into desired interacting nanostructures using recombinant genetic techniques.

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