# SLH anchoring modules in *Clostridium thermocellum* surface


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## Introduction:

*Clostridium thermocellum* is an anaerobic, thermophilic, cellulolytic, and ethanogenic bacterium capable of directly converting cellulosic substrate into ethanol. In this bacterium, degradation of the cellulosic materials is carried out by a large extracellular cellulase system called the cellulosome.

The ability of the cellulosome to attach to the cell surface is a result of the existence of S-layer homology (SLH) modules as part of associated scaffoldin anchoring proteins. The interaction between the cell wall (peptidoglycan) and the S-layer proteins is very strong, although not covalent. Thus, the high level of expression of these proteins, together with their efficient binding to the cell wall, makes this system very attractive for studying cell surface anchoring. Despite the vital role of the SLH domain in the cellulosome system, it is still one of the few components of this system for which no three dimensional structure is available.

Extensive search in the *C. thermocellum* genome revealed several (~20) SLH containing orfs, each composed of 2-3 sub-domains. Some are recognized as cellulosome anchoring proteins and others are novel, One of them is a part of a huge multi-domain surface protein, termed anaK. This 0.8 MDa protein comprises a signal peptide, multiple domains possibly involved in protein-protein interactions and a C-terminal S-layer homology (SLH) domain.

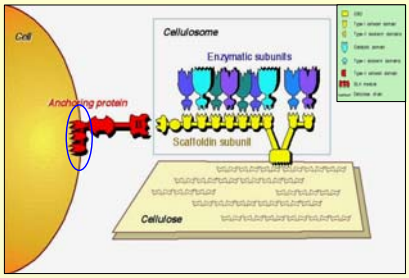


**Figure 1: Schematic view of the molecular disposition of the cellulosome and one of the associated anchoring proteins on the cell surface of *C. thermocellum*.** SLH is indicated with blue circle.

## Goals:

- **To characterize the putative SLH domains from *C. thermocellum*.**
  - **To clone several SLH modules.**
  - **To crystallize the S-layer homology (SLH) modules.**
  - **To examine the SLH ability to bind to cell surface components.**

## Results:

### Bioinformatic analysis

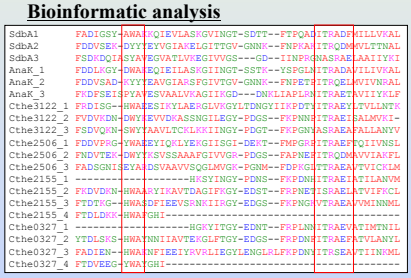


**Figure 2: Multiple sequence alignment (MSA) of SLH modules that were selected for cloning** [Produced by using ClustalW]. SdbA (cthe1307) was taken as reference.

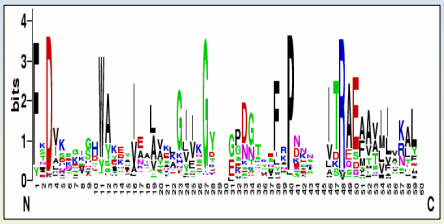


**Figure 3: Consensus sequence of SLH sub-domain** (60 amino acids). [Produced by using weblogo].

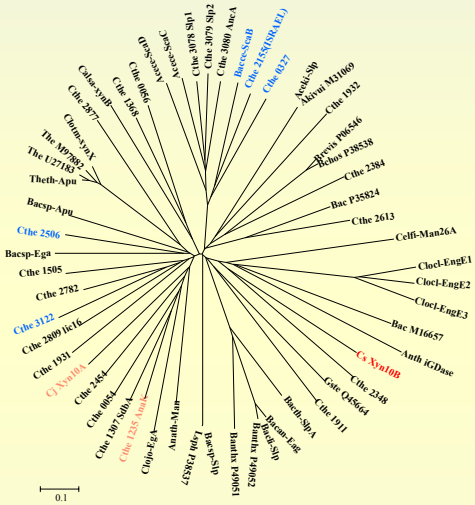


**Figure 4: Phylogenetic analysis of SLH modules sequences.** Blue: new clones. Red: clones that were previously expressed in our lab. (see fig. 6).

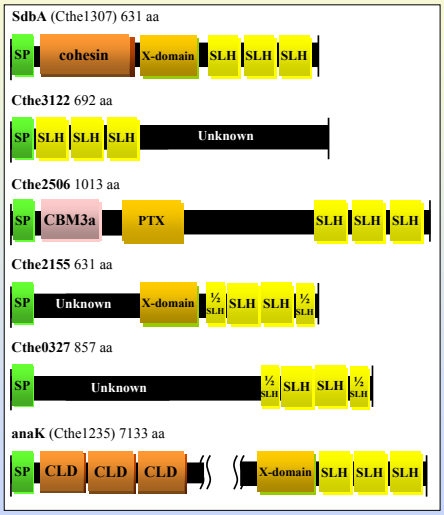


**Figure 5: Graphical view of SLH containing orfs from *C. thermocellum* that were selected for cloning.** (see fig. 2).

## Cloning and expression:


**Figure 6: Schematic representation of the vector used for the SLH cloning.**

We finally succeeded in cloning and expressing several soluble SLH domains from *C. thermocellum*. (fig.7)
The recombinant proteins were purified by Nickel affinity purification followed by Gel filtration procedures.

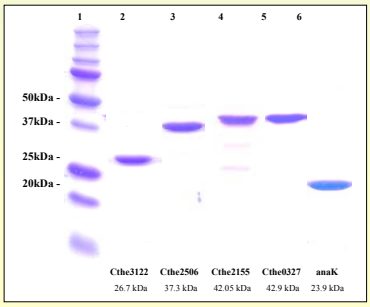


**Figure 7: 12.5% SDS gel of the purified proteins.**
Lane 1: marker. Lanes 2-6: purified proteins.

## Crystallization:

After purification, Crystallization experiments were done (Micro batch and Hanging drop).
Few crystals were obtained, Optimization steps are in process.


**Figure 8: Crystals of Cthe3122 SLH that were obtained from microbatch experiment.**
(a)10% PEG 3000, 0.1M Na/K phosphate buffer pH=6.2
(b) 15% Ethanol, 0.1M imidazole pH=8.0, 0.2M $MgCl_2$

## Future plans:

- **To proceed in crystallization experiments and optimization steps.**
- To express and crystallize Seleno-L-Methionine labelled SLH modules.
- To obtain more "crystallizable" proteins by changing surface residues characterized by high conformational entropy (e.g Lysine and Glutamate) to Alanines (in process with anaK SLH).
- **To pursue parallel NMR approach in order to solve the 3D structure of SLH** – ( in process with anaK SLH. collaboration with Steven Smith. Queen's University, Ontario, Canada).
- **To perform binding assays of SLH modules:** to peptidoglycan (from *E. coli* and *C. thermocellum*), and to *C. thermocellum* cells.