# Protein engineering group at VTT Biotechnology

http://www.vtt.fi/bel/bio/prot/index.htm

**Goals**

•to characterize and engineer industrially important enzymes and other proteins. Protein engineering is based either on rational design or directed evolution.

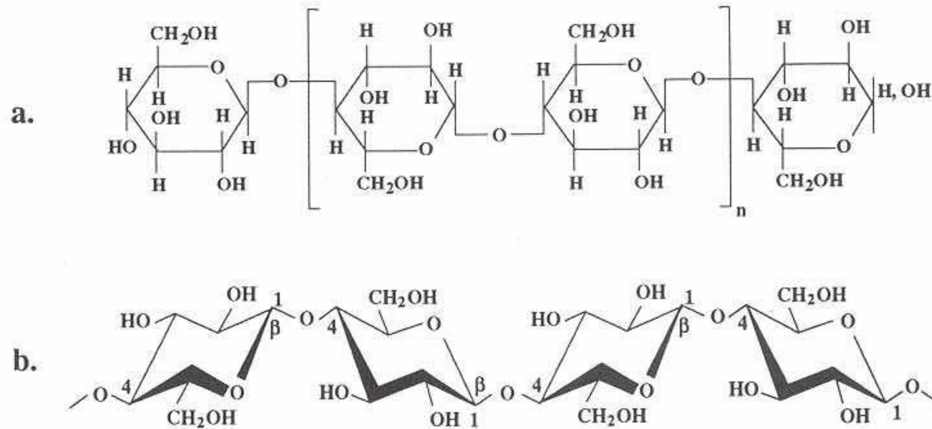
**Target proteins**

•polysaccharide degrading enzymes: cellulases and chitinases
•oxidases, antibodies and hydrophobins

**The work involves**

•protein expression (*E.coli*, *S. cerevisiae*, *P. pastoris*, *T. reesei*, insect cells) and purification
•mutagenesis and design of mutant libraries (based e.g. on structural information)
•setting-up the HTS screen and screening with the robotic work station
•protein characterization (e.g. enzymology, binding studies, unfolding, AFM)

# Cellulose and cellulases




## Cellulose

- Major structural polysaccharide in wood cell walls
- Linear ß-1,4-glucose polymer, repeating unit is cellobiose
- Glucan chains arrange into microfibrils, which form cellulose fibres
- Fibre (surfaces) contain crystalline and amorphous regions
- Surrounded by hemicelluloses and lignin in wood

## Cellulase applications

•textile industry
•detergents
•pulp and paper
•production of bioethanol



VTT

# The action of cellobiohydrolases on insoluble substrates

**Cellobiohydrolases**

- cleave mainly cellobiose units from the chain ends in a processive manner
- have a two-domain architecture

**Catalytic core domain (GH families 6 and 7)**

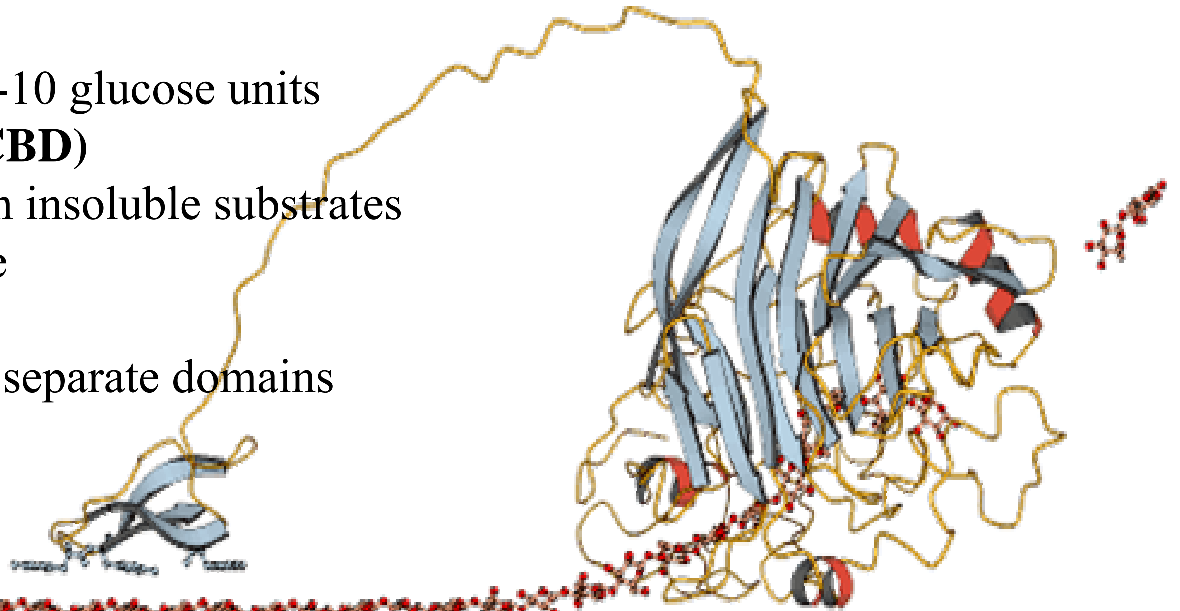
- active site situated in a tunnel going through the catalytic domain
- tunnel contains subsites for 4-10 glucose units

**Cellulose-binding domain (CBD)**

- Needed for the full activity on insoluble substrates
- Affinity to insoluble substrate

**Inter domain linker peptide**

- Coordination of the action of separate domains



# Structure-function studies on *Trichoderma reesei* cellobiohydrolases Cel7A (CBHI) and Cel6A (CBHII)

**1. Structural studies**

- X-ray crystallography, NMR
- molecular modelling

**2. Protein engineering of the catalytic domain**

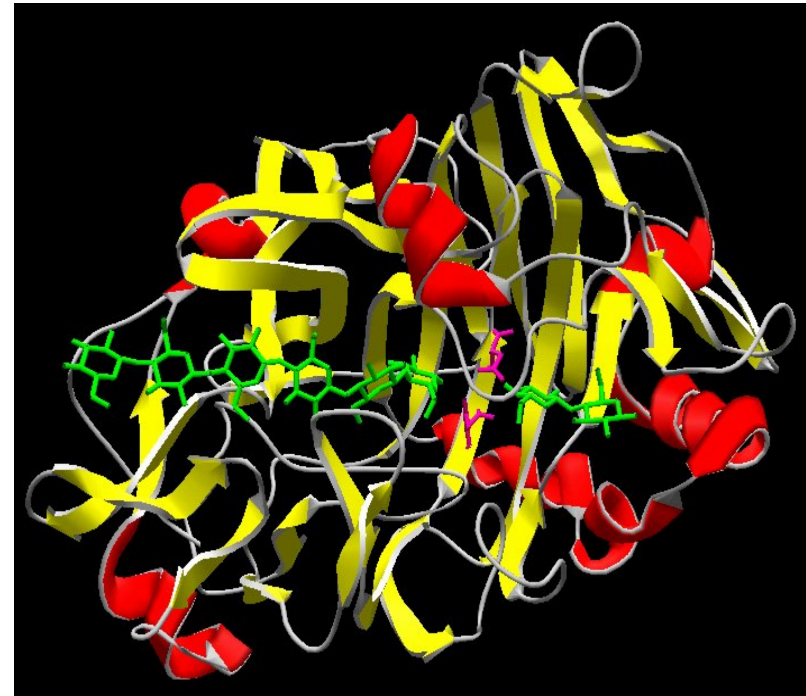
- catalytic mechanism: carboxylic acids and ring distortion
- involvement of tryptophans in the substrate binding
- role of the loops
- pH optimum
- pH stability - thermostability

**3. Protein engineering of the linker peptide**

- linker deletions

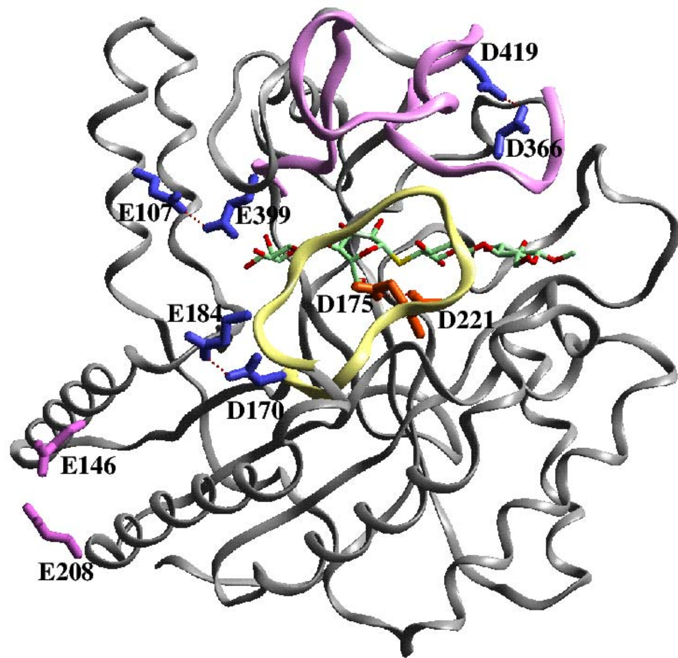
**4. Protein engineering of the cellulose-binding domain (CBD)**

- role of three aromatic amino acids on the flat surface
- pH behaviour
- fusion proteins



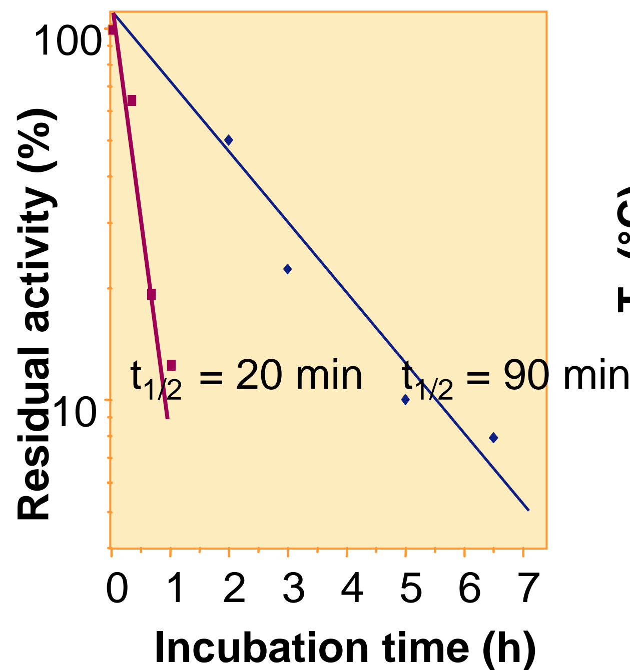
The 3-D structure of *T.reesei* Cel7A in complex with a cellulose chain. The catalytic carboxylic acid side-chains are shown in pink.

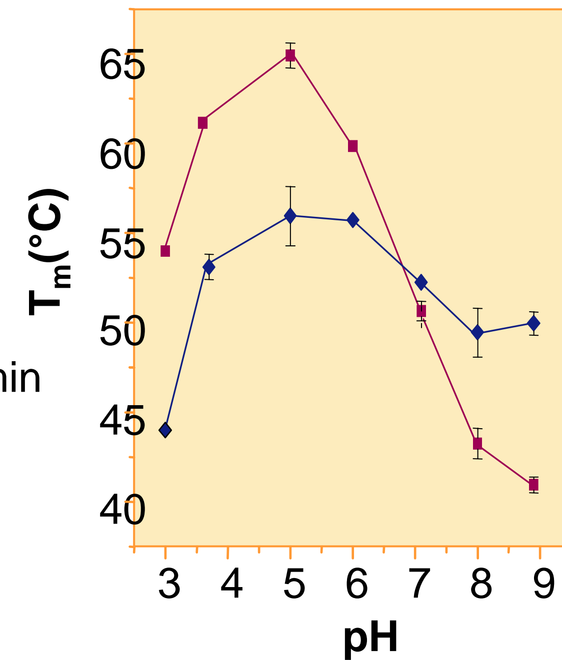
# Improving the alkaline stability of *Trichoderma reesei* cellobiohydrolase Cel6A by carboxylic acid pair engineering




**Results on the Cel6A acid pair mutant:**

- The unfolding temperature at pH 7.7 could be increased by 5 °C, and the half-life at 44 °C, pH 7.7 by 4-fold.
- The Cel6A mutant can also function better (longer) on crystalline cellulose



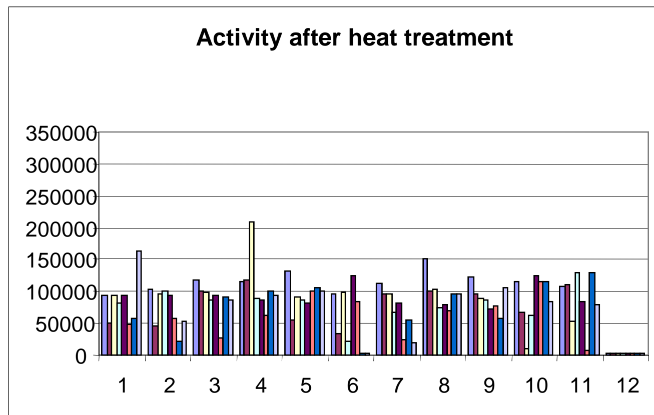
# The robotic work station at VTT used for high-throughput screening (HTS)

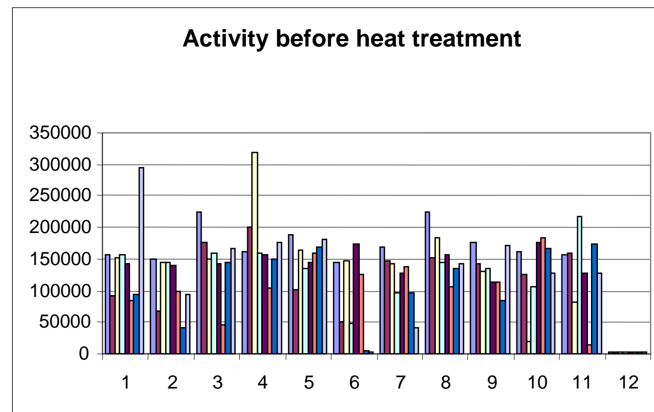
1. Genetix Qpix colony picker
2. Two humidity controlled incubators (not integrated)
3. Beckman ORCA robotic arm on a 3m track
4. Plate carousel for storage of 90 MTPs
5. Beckman Biomek and Multimek liquid handling stations
6. Barcode reader
7. Multichannel dispensers
8. Beckman Lid removing station
9. Wallac Victor$^2$ V reader (absorbance, luminescence, fluorescence)
10. Sami NT and ORCA NT scheduling software
11. LabVantage Database for HTS data storage and analysis

Throughput: about 10.000 samples / day

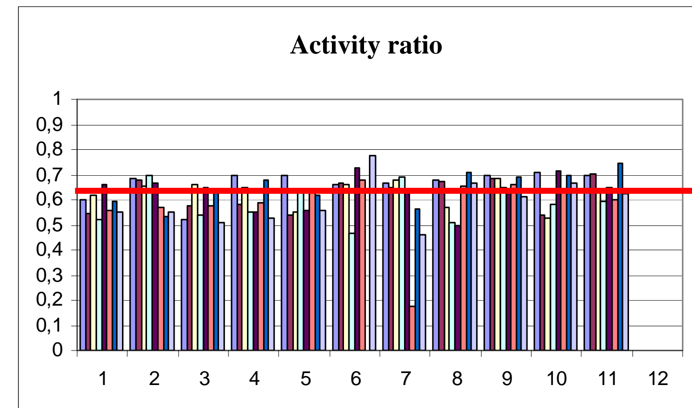
# Directed evolution of a fungal hydrolase: Screening of a random mutant library for improved thermostability

**Screen** : Enzymatic activity before and after a heat inactivation treatment
**Calculate**: [Activity after /activity before heat treatment] and compare to the wt data
**Pick positive clones** (cherries), repeat the assay

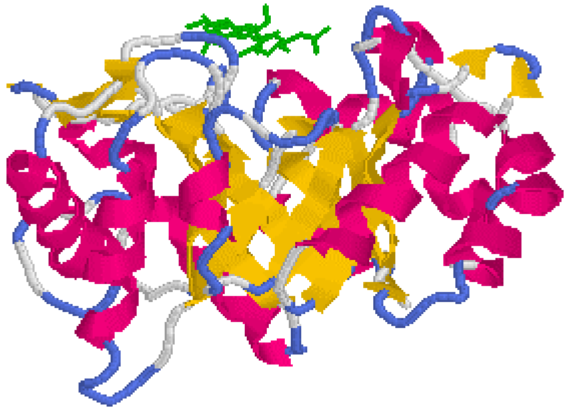


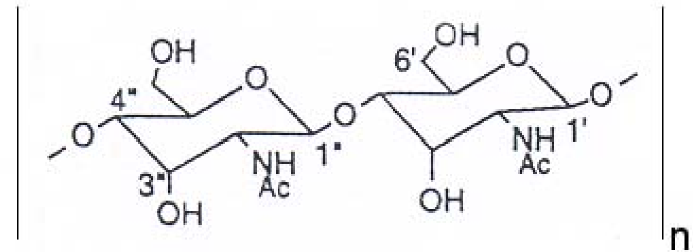




Results from screening one microtiter plate (96 wells):

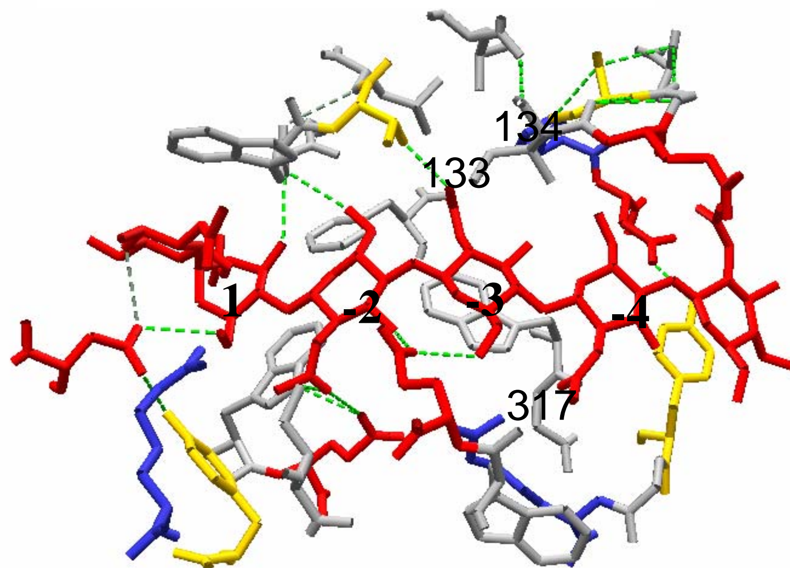


# Changing the substrate specificity of a fungal chitinase by saturation mutagenesis





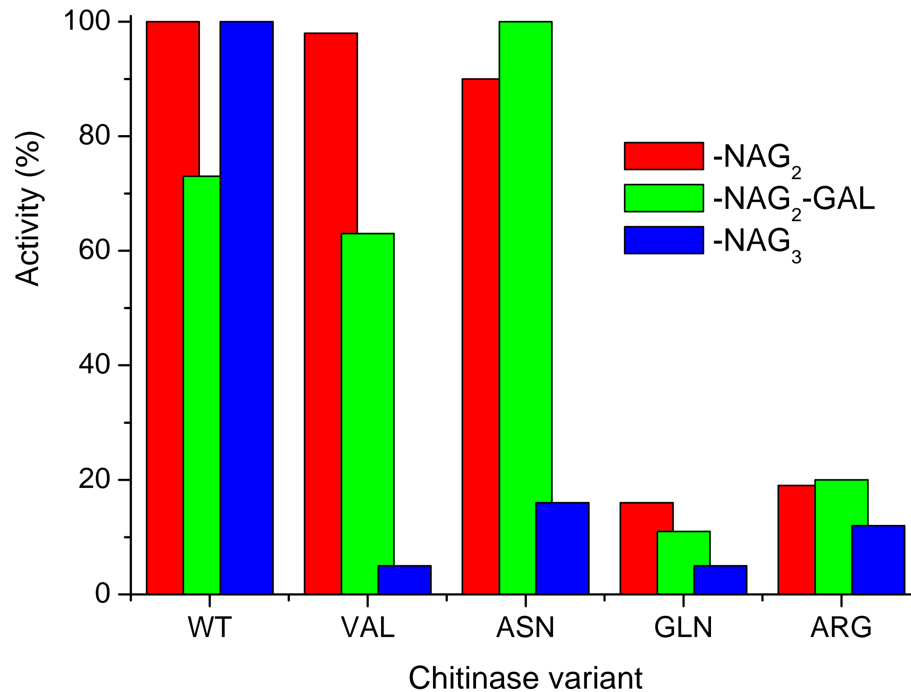

**Substrate:** β–1,4 linked N-acetylglucosamine




Mutagenesis study

1. Catalytic amino acids
2. Subsites -3 and -4

# Kinetics of the selected chitinase mutants on three different substrates




**Results:**

The substrate-binding specificity of the chitinase could be changed



VTT

# Collaborators

**Crystallography**

- Alwyn Jones, Jerry Ståhlberg et al., BMC, Uppsala, Sweden
- Juha Rouvinen, University of Joensuu, Finland

**Modelling, NMR**

- Gerd Wohlfahrt, OrionPharma Ltd., Finland
- Torbjörn Drakenberg (VTT); Nana Munck and Hannu Maaheimo, VTT Biotechnology, Finland

**Enzymology**

- Marc Claeyssens et al., RUG, Ghent, Belgium
- Michael Sinnott et al., UMIST, Manchester, UK

**Substrates and inhibitors**

- Andrea Vasella, ETH, Zurich, Switzerland
- Sylvain Cottaz and Hugues Driques, CERMAV-CNRS, Grenoble, France
- Ossi Renkonen et al., University of Helsinki, Finland
- Biokemis Ltd., St. Petersburg, Russia

**Genes and proteins**

- Jari Vehmaaperä, Roal Ltd., Finland
- Tahia Benitez et al., University of Sevilla, Spain