



# **NMR spectroscopy and modelling group**

## **VTT Biotechnology**

Hannu Maaheimo

# **VTT NMR spectroscopy and modelling group**

**(Molecular structure group)**

**Hannu Maaheimo**

**Maija-Liisa Mattinen**

**Nana Munck**

**Kirsi Tappura (currently at VTT Information technology)**

**Kimmo Pääkkönen**

**Katri Ylönen**

**Paula Jouhten**

# Research fields of the group

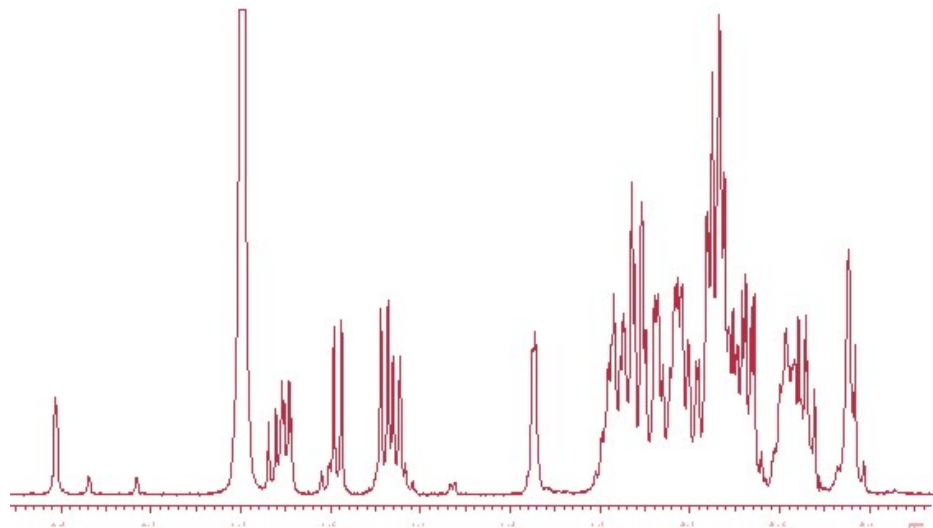
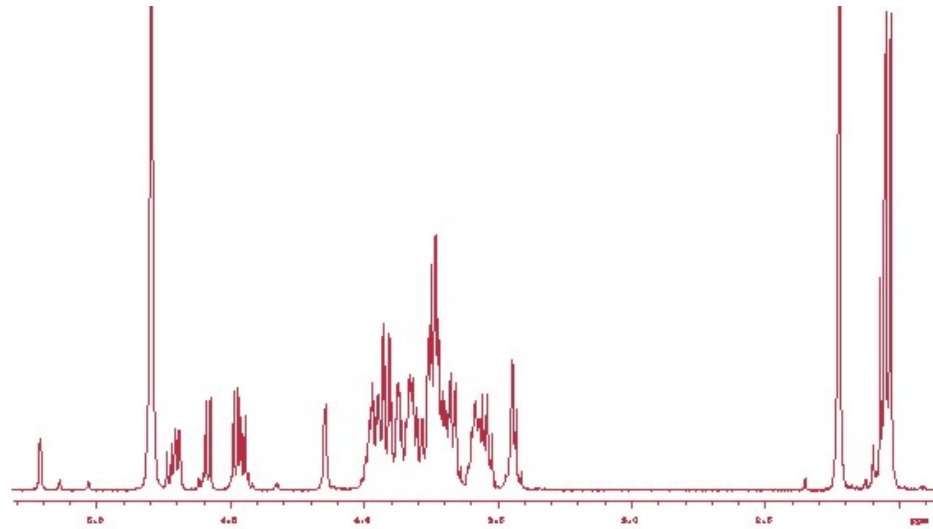
- **oligo- and polysaccharide structures**
- **protein-ligand interactions**
  - **methods based on ligand detection (STD NMR, tr NOE ...)**
  - **extensive molecular modelling**
  - **methods based on protein detection**
- **cellular metabolism**
  - **experimental metabolic flux analysis from  $^{13}\text{C}$  tracer experiments**
  - **metabolite concentrations/profiles (metabolomics)**
  - **tight connection to VTT's metabolic engineering and part of the systems biology platform**

# NMR laboratory in Viikki Biocenter

- **VTT Biotechnology & Institute of Biotechnology, Univ. Helsinki**
- **spectrometers:**
  - **500 MHz Varian Unity Inova**
    - **equiped with Varian nanoprobe with magic angle spinning**
  - **2 x 600 MHz Varian Unity Inova**
    - **cryogenic probe head in use in April 2004**
  - **800 MHz Varian Unity Inova**
    - **cryogenic probe head in June 2004**

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# Our view of a protein - ligand interaction project

- **potentially interesting protein-ligand pair**
- **STD NMR, trNOE experiments (no need for labelling, small protein amounts, even impure protein)**
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  - **back-bone assignment**
  - **binding site from HSQC experiments**
  - **possibly refine the 3D structure (of the binding site) using dipolar coupling information etc.**

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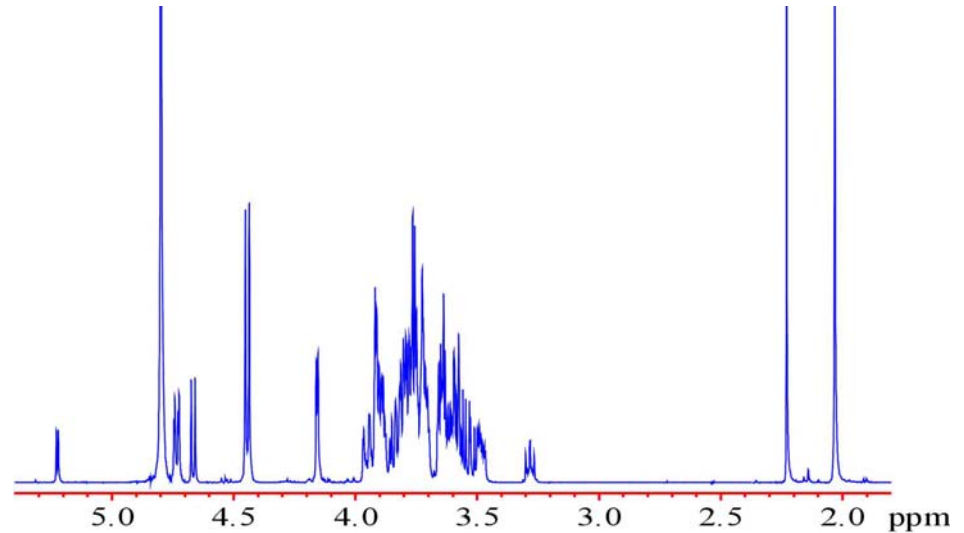
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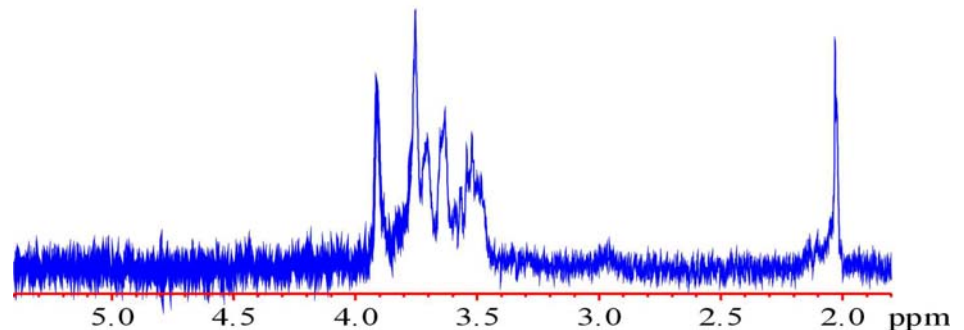
# STD (saturation transfer difference) NMR

Lacto-N-tetraose  
Gal-GlcNAc-Gal-Glc

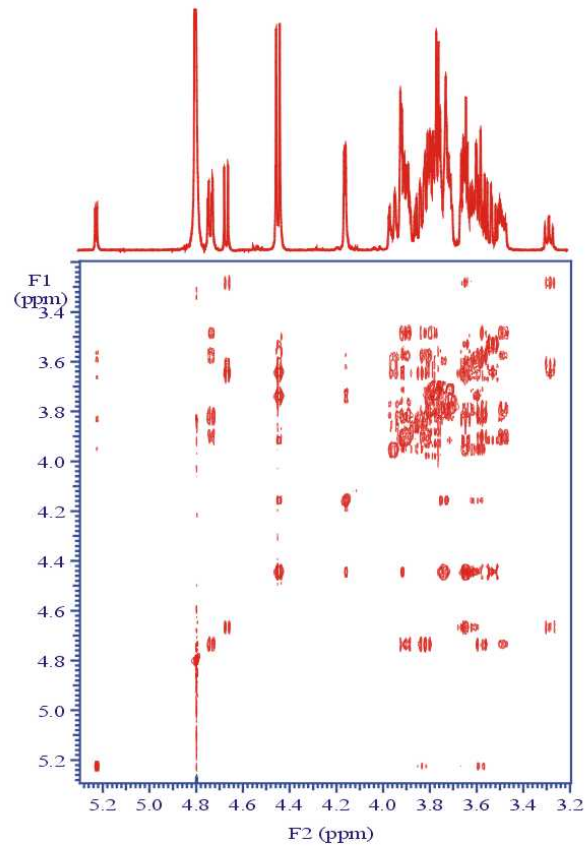
1D spectrum of free saccharide



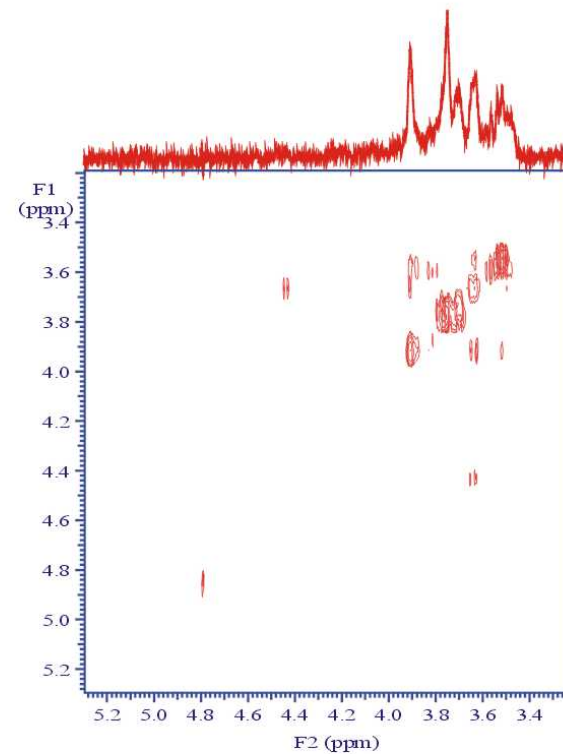
1D STD spectrum of the  
saccharide with *Sambucus  
nigra* agglutinin (SNA)



# Identification of the STD signals through 2D experiments

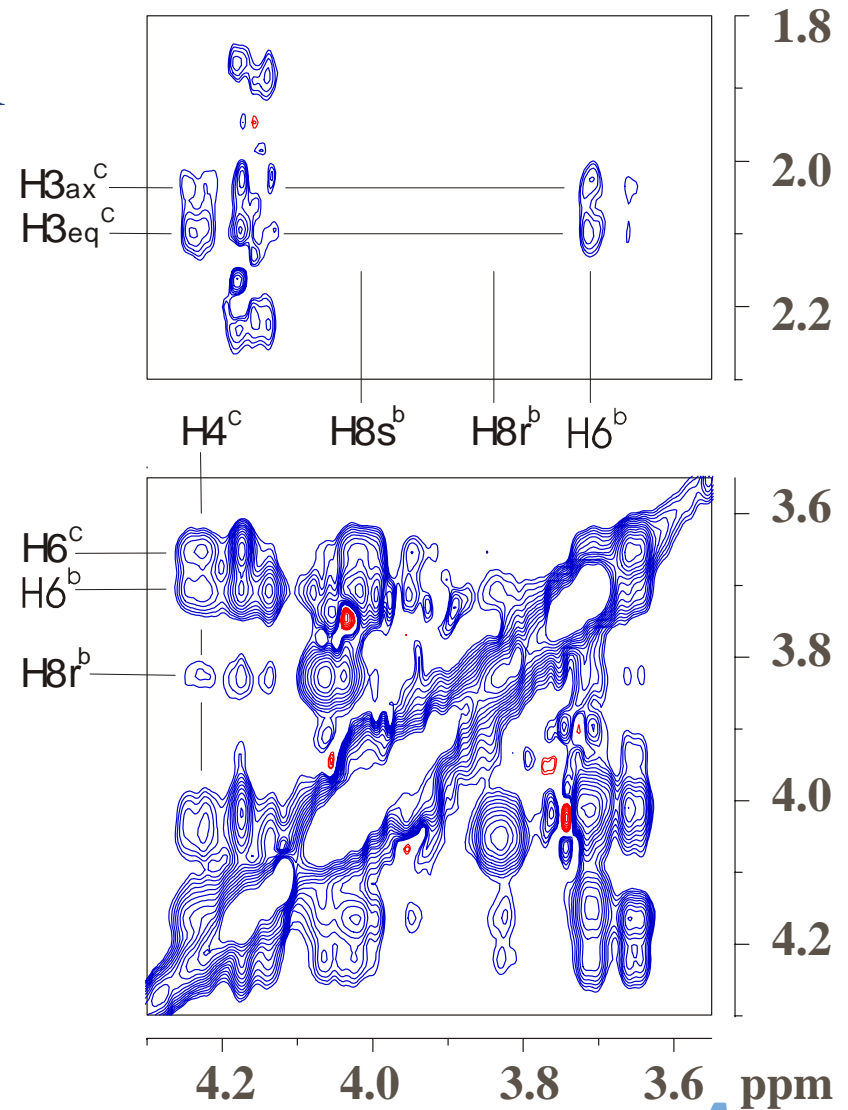
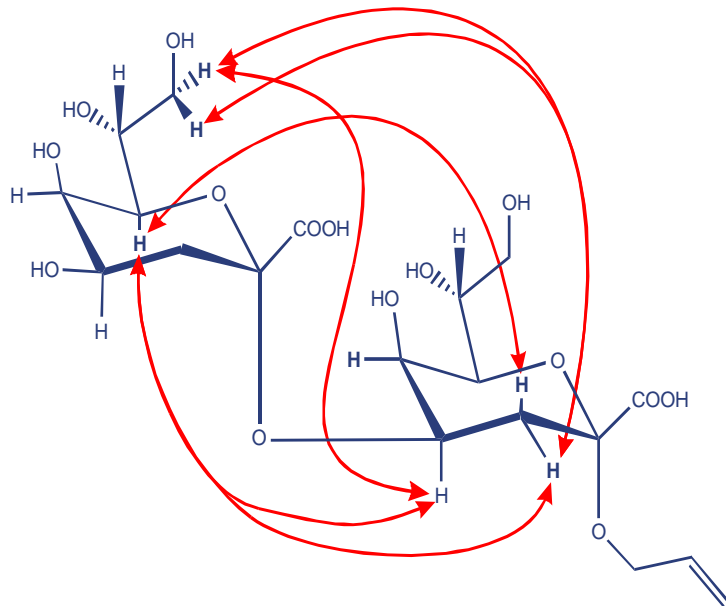


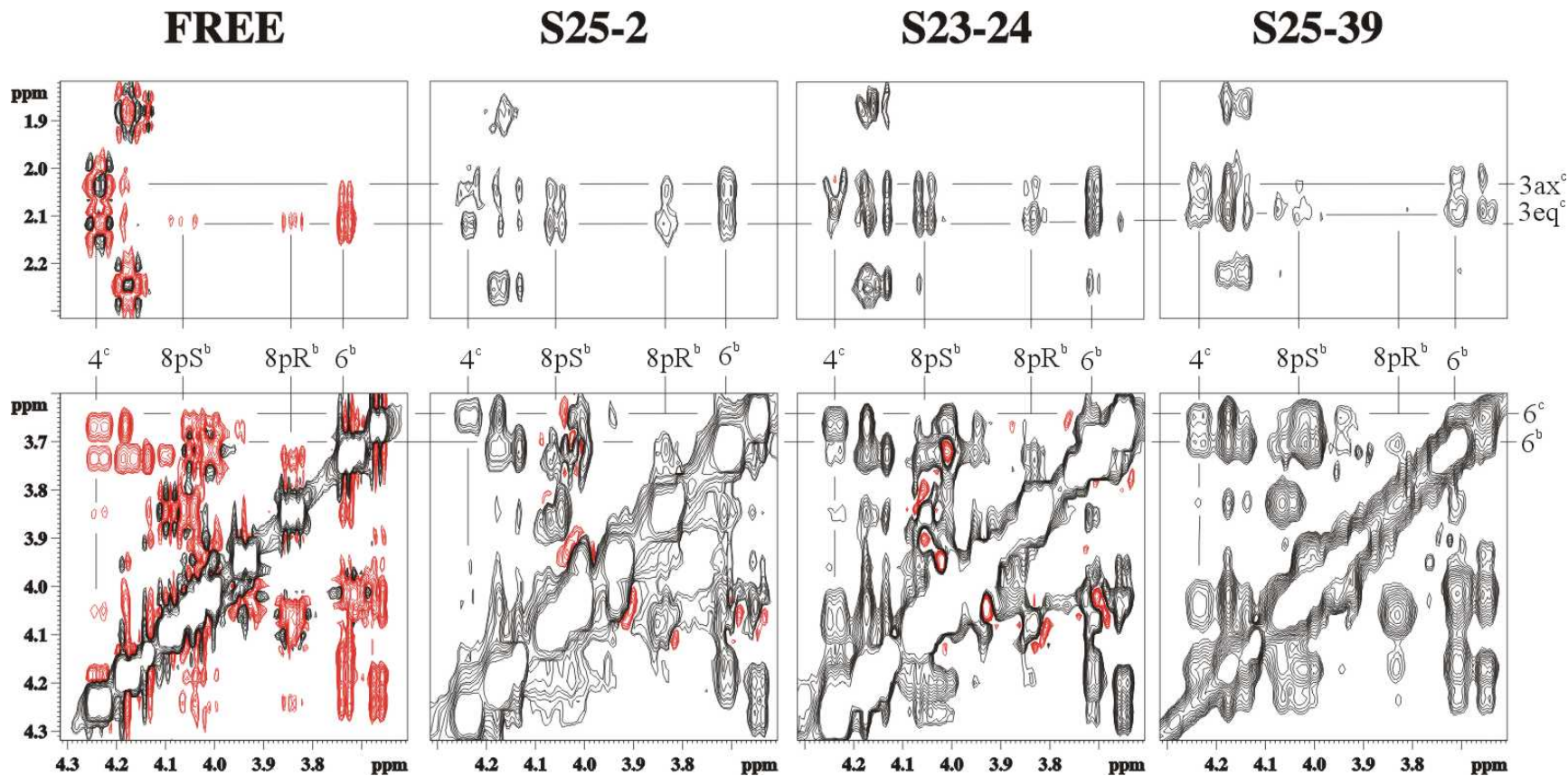
TOCSY



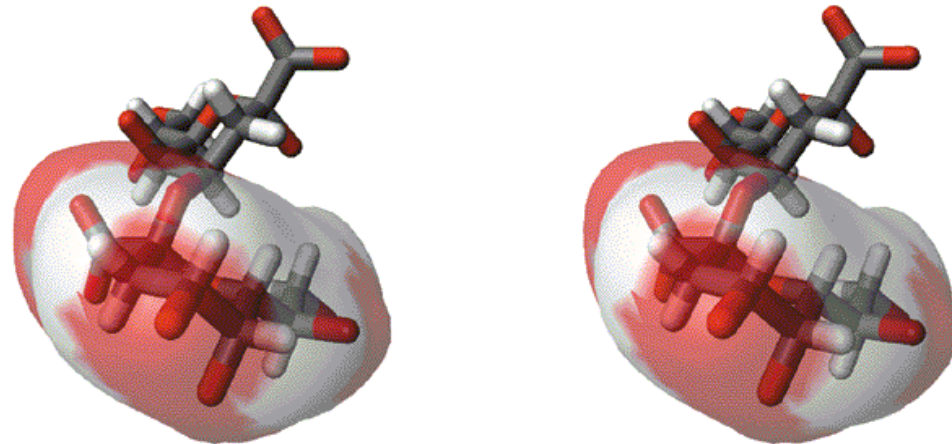
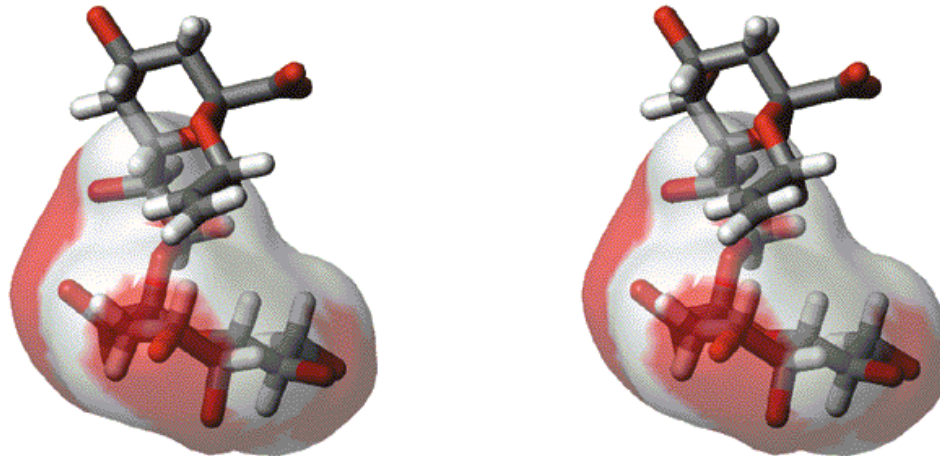
STD-TOCSY

# Tr-NOESY spectrum of S25-39 / Kdo2-4Kdo-allyl complex





# The major binding epitopes of Kdo $\alpha$ 2-4Kdo (A) and Kdo $\alpha$ 2-8Kdo (B)

**A****B**

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- **structural models based on the known structures of homologues**
- **extended molecular dynamics (MD) simulations with explicit water using MD simulation package GROMACS**
- **a novel soft-core potential energy function used in addition to the conventional force field**
  - the aim is to make the search problem of the conformational space more tractable by reducing the height of the barriers surrounding the energy minima without losing the main equilibrium properties of the original force field
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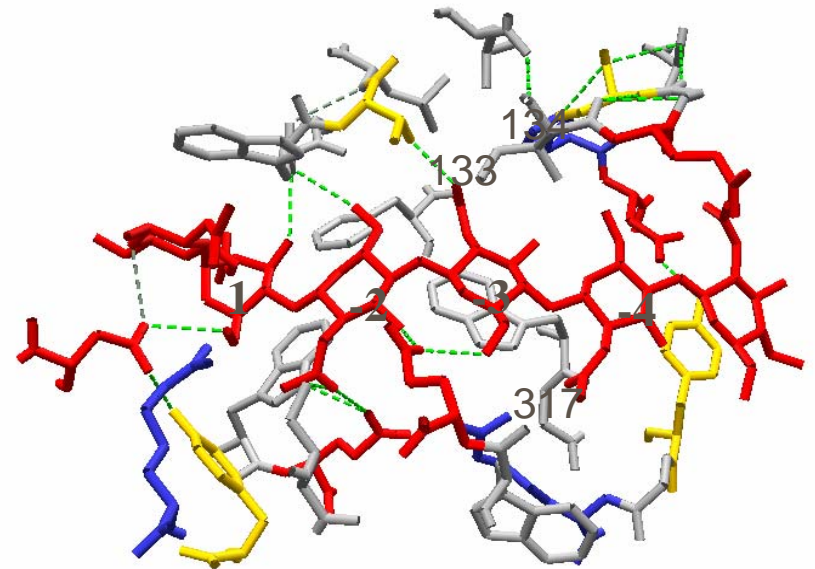
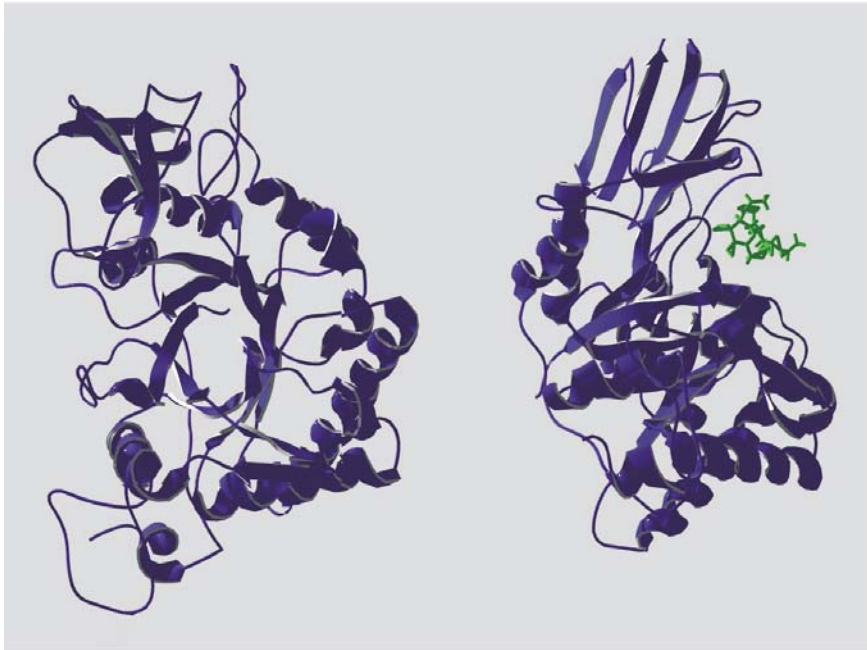
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## Protein-oligosaccharide interaction mechanisms in a fungal chitinase

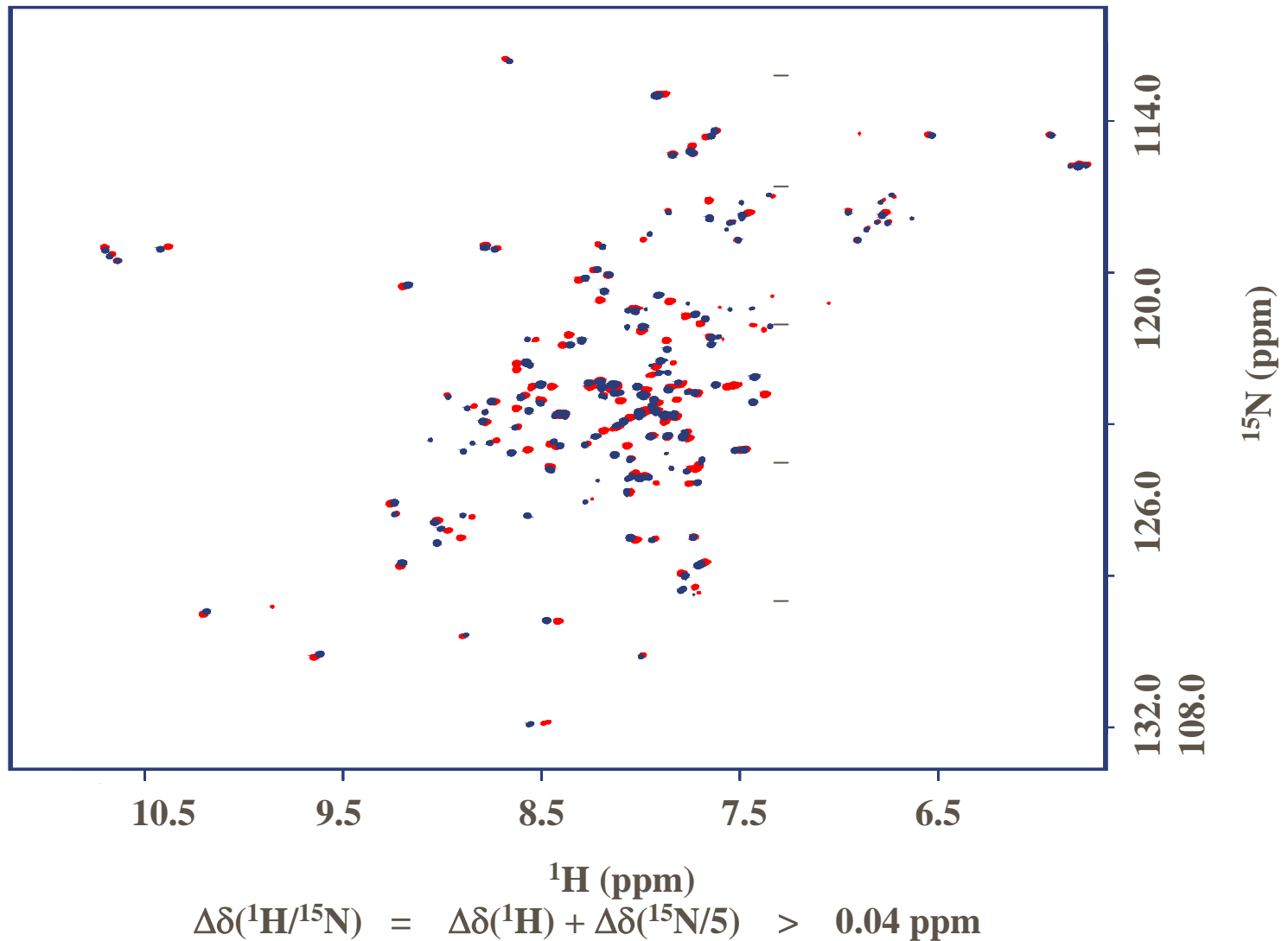


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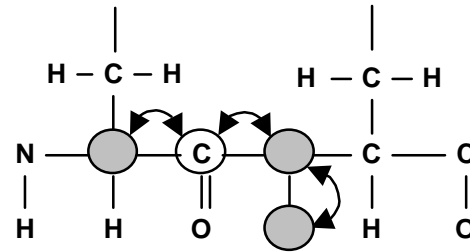
# $^1\text{H}$ - $^{15}\text{N}$ -HSQC spectrum

in the absence (black contours) and presence (red contours) of the ligand



# Back-bone assignment

- experiments needed:  
15N-HSQC, HNCA (trocy),  
HN(CO)CA, HNCACB,  
HN(CO)CACB,HNCO

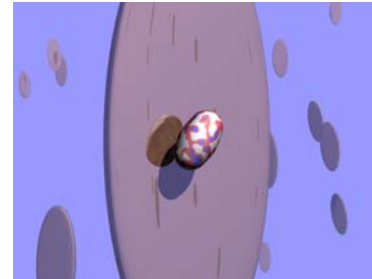


- requires double labelled protein ( $^{13}\text{C}$  and  $^{15}\text{N}$ )
- large protein => also  $^2\text{H}$  labelling needed
- auto-assignment programs => 40-90%

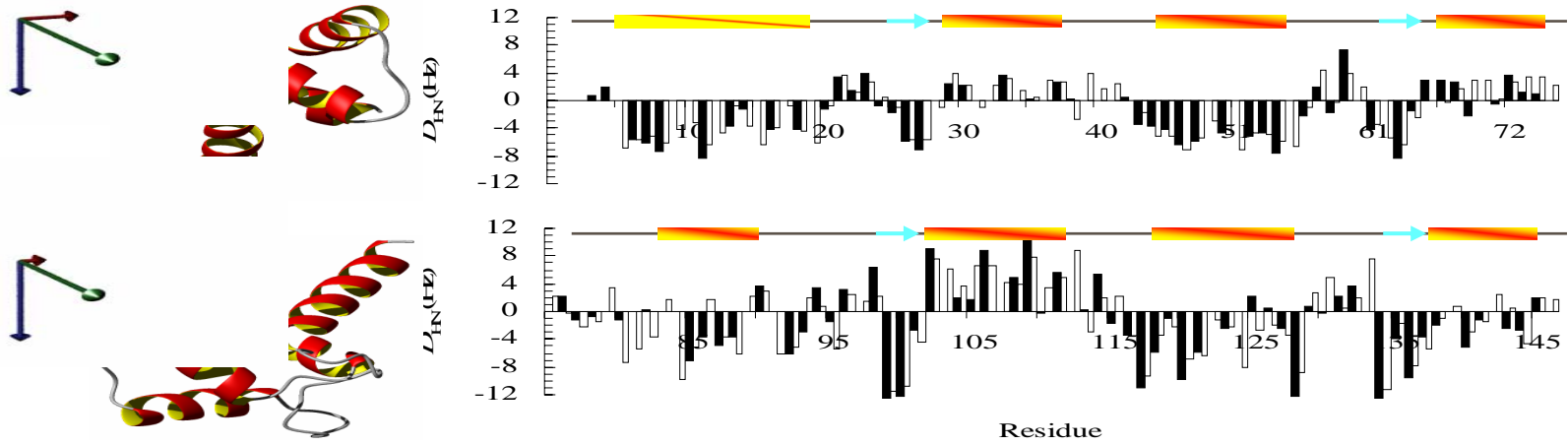
- binding site identification
- conformation
- dynamics

# Residual dipolar couplings

## Protein alignment for dipolar coupling detection



## Domain orientations



First we have to buy  
an NMR spectrometer

