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NMR Studies into the Molecular Dynamics of Human Blood Group B Galactosyltransferase

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Introduction

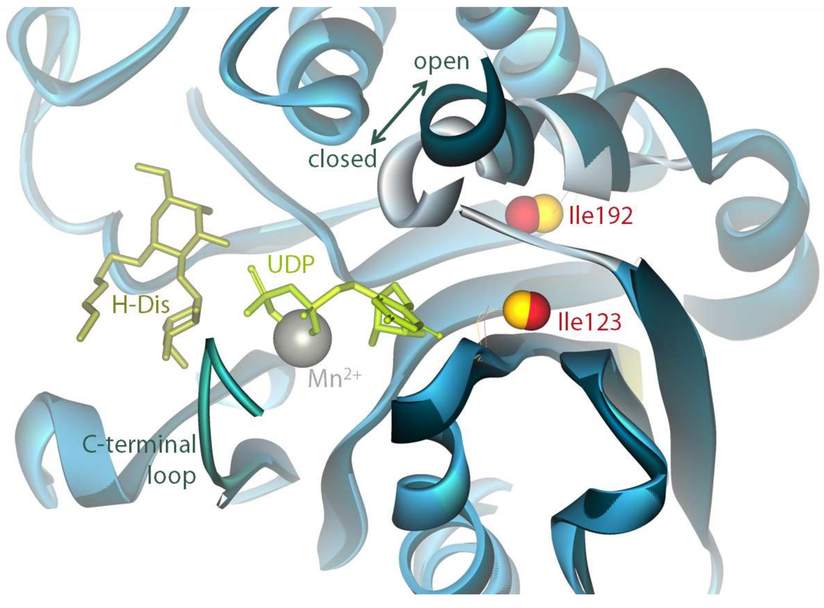


Figure 1 | GTB in the closed and open conformation. Superimposition of GTB in the open conformation (dark blue, pdb:2RIT) and closed conformation (light blue, pdb: 2RJ8). H-Disaccharide-octyl (H-Dis), UDP, and a manganese ion are shown in dark green, green, and grey, respectively. One can see a clear movement of the internal loop, indicated by an arrow. There is also a movement of the methyl group of the isoleucine residue 192 which is located in the internal loop (yellow: open, red: closed) compared to no movement in the methyl group of the isoleucine residue 123. [1]

- **Glycosylation** is one of the most important posttranslational modifications of proteins and is archieved by Glycosyltransferases (GTs).
- **Human blood group ABO Glycosyltransferase (GTA/GTB)** as a model for retaining GTs.
- **Mechanism** for retaining GTs is not well understood.
- Crystal structures reveal two **flexible loops**, which undergo conformational changes upon substrate binding (Figure 1).
- **NMR** provides an ideal tool to study **protein dynamics** during catalysis and binding of ligands.

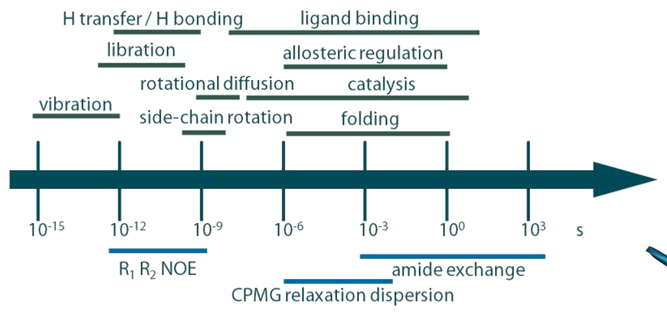
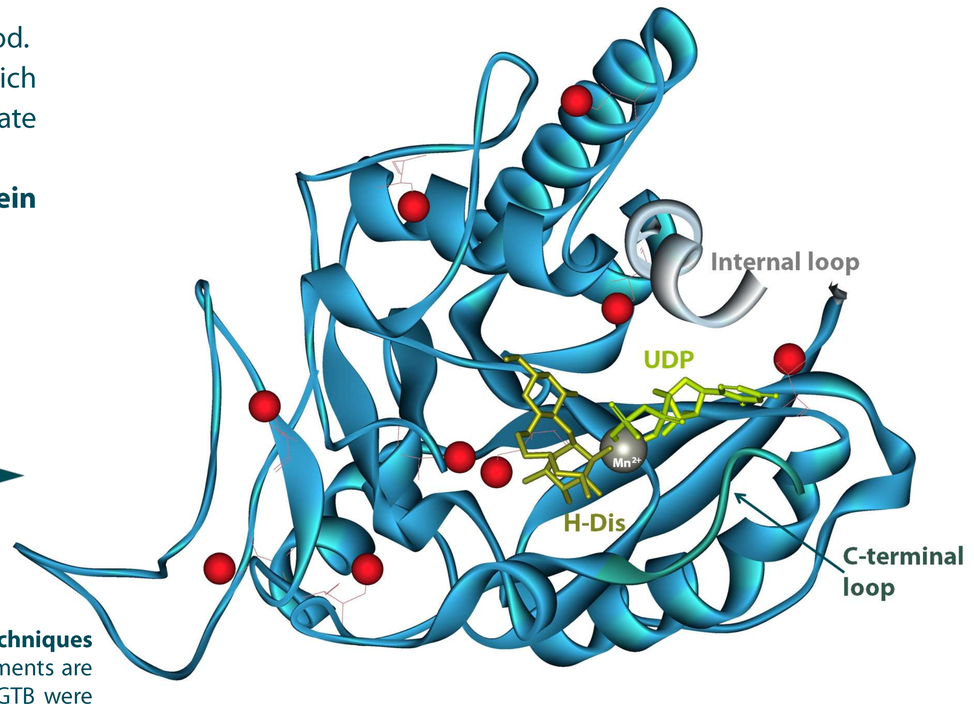


Figure 2 | Timescales of protein dynamics (top) and NMR techniques (bottom). Amide exchange and CPMG relaxation dispersion experiments are used to study the dynamics of GTB. R_1, R_2, NOE experiments with GTB were done by Sophie Weißbach. Adapted and modified from Palmer[2].

Figure 3 | Crystal structure of GTB. Shown is the monomer of GTB (pdb: 2RJ8) H-Disaccharide-octyl (H-Dis), UDP, and a manganese ion are shown in dark green, green, and grey, respectively. The methyl groups of the isoleucine residues are highlighted as red balls and show a very well distribution over the whole protein. In these experiments the methyl groups were selectively labeled by addition of the natural precursor.



Methods & Results

Substrate Binding

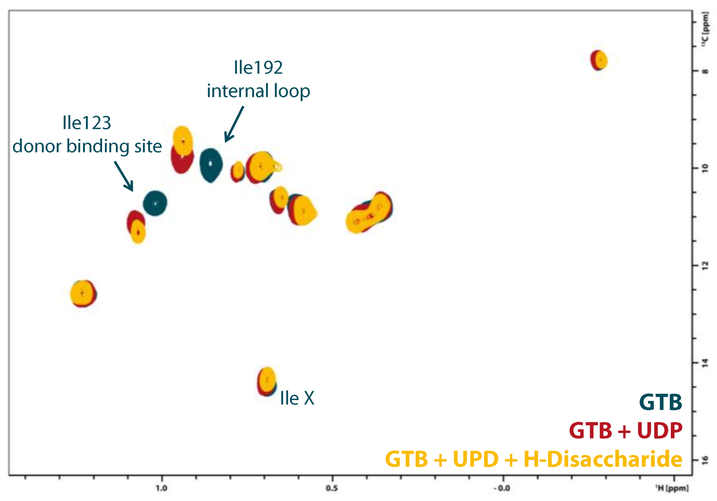


Figure 4 | Methyl-TROSY spectra of the isoleucine residues of GTB (green), with UDP (red) and UDP/H-Disaccharide (yellow).
The spectrum shows a 100 µM sample of uniformly deuterated, [^{1}H,$^{13}C_{\delta1}$]-isoleucine labeled ^{15}N-GTB (green) recorded in 25 mM sodium phosphate, pH 6.7, 5 mM $MgCl_2$, 1 mM b-ME-d_6, 100 µM TSP-d_4, ~100 % D_2O, 298 K (green), with 6.8 mM UDP (red) and additional 380 µM H-Disaccharide (yellow).

- 10 isoleucines are present in GTB, but 11 peaks were detected
- Structural changes of GTB occur upon addition of substrates and are followed by NMR spectra
- Isoleucine in the loop were identified by mutational studies (Ile192)
- Isoleucine in the donor binding site was identified by titration experiments (Ile123)
- Isoleucine samples of GTB are very well suited to study protein dynamics of the whole protein

Amide Exchange

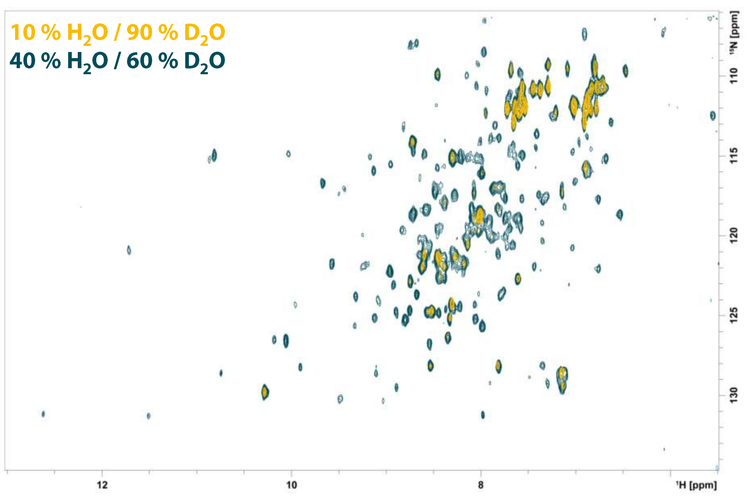


Figure 5 | ^{1}H,^{15}N-TROSY-HSQC spectra of u-^{2}H,^{15}N-GTB.

The ^{1}H, ^{15}N-TROSY-HSQC shows an overlay of the spectra with H_2O/D_2O of 10 %/90 % (yellow) and 40 %/60 % (green). The spectra were recorded with ~460 µM (yellow) and ~300 µM (green) of u^{2}H,^{15}N-GTB in 25 mM sodium phosphate, pH 6.7, 5 mM $MgCl_2$, 1mM 2-mercaptoethanol-d_6, 100 µM TSP-d_4 at 298 K with a 500 MHz spectrometer.

- NMR spectra of GTB in deuterated buffer show only 5 weak signals after an incubation of 5 h, these signals are gone after 1 day (data not shown).
- With the exchange to 40 % of H_2O buffer, all peaks can be detected.
- The fast exchange suggests a very flexible protein with motions faster than milliseconds.

CPMG Relaxation Dispersion

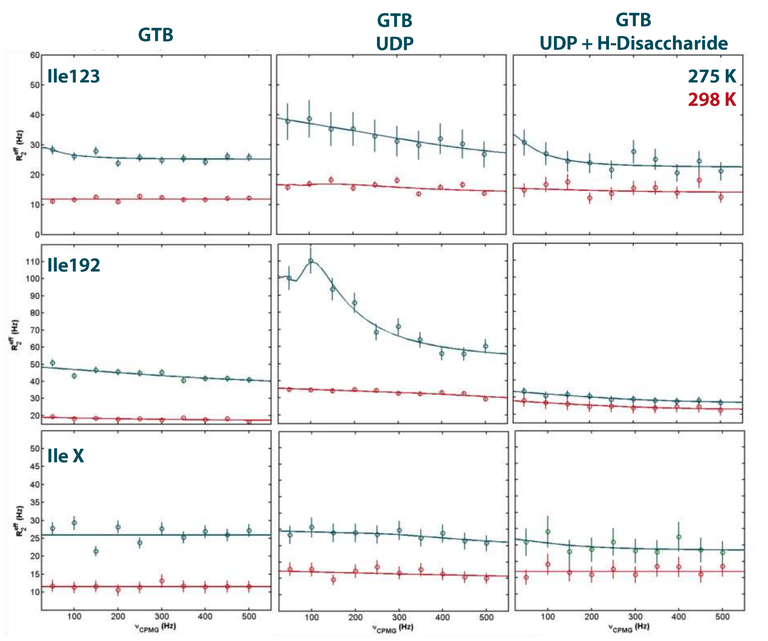


Figure 6 | Methyl relaxation dispersion studies of GTB and of GTB in complex with the natural substrates.
Relaxation dispersion profiles of 3 methyl groups of isoleucine residues of GTB at different Temperatures (275 K green, 298 K red) without, with 9.45 mM UDP, and additional 380 µM H-Dis-O-^{13}C-metyl (from left to right, respectively). Data was measured in duplicates and the lines are best fits to a two-state exchange model using GUARDD. [3]

- Internal loop undergoes significant changes in terms of dynamics upon binding of UDP and H-Disaccharide
- No major changes in dynamics on the rest of the protein upon binding of substrates
- This suggest a µs to ms movement of the internal loop only in the presence of UDP
- Otherwise the movement is expected to be faster than µs

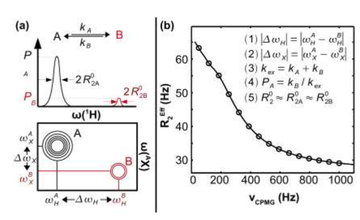


Figure 7 | Example for a two-state relaxation dispersion profile[3]
(a) A single NMR probe alternates between two states A and B, although the "minor" state B is not detected the exchange has a quantitative effect on the measured relaxation dispersion curve (b) of signal A. The insert in (b) shows the parameters required for a two-state exchange model.

Conclusion & Outlook

NMR is ideally suited to study dynamics of proteins over a wide range from picoseconds to seconds. Here we are measuring protein dynamics based on methyl TROSY experiments with selectively labeled methyl groups of the isoleucine residues of GTB. These experiments are very sensitive, therefore, only a small amount of protein is required.
Amide exchange experiments suggest internal movement faster than milliseconds. Relaxation dispersion experiments show movements faster than microseconds. Upon binding of the donor substrate, only the dynamics of the internal loop changes into the timescale of micro- to milliseconds. This could be an indication for the presence of a semi-closed conformation shown previously in the crystal structures.[1] These experiments give further insight into protein dynamics of GTB during substrate binding and catalysis and further our understanding of how these GTs function.

Acknowledgement:
We thank Monica Palcic for help and support in any respect and especially for highly stimulating discussions and joint seminars.
We thank the DFG for financial support.

[1] Alfaro, J. A. et al., *J. Biol. Chem., 283(15)*, 10097-108 (2008)
[2] Palmer, A. G. et al., *Chem. Rev.*, 104, 3623-3640(2004)
[3] Kleckner, I. R., & Foster, M. P., J Biomol NMR, 52(1), 11-22(2012) / http://google.de/p/guardd