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NMR Investigation into the Dynamics of the Human Blood Group A and B Glycosyltransferases

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Human Blood Group ABO Glycosyltransferases

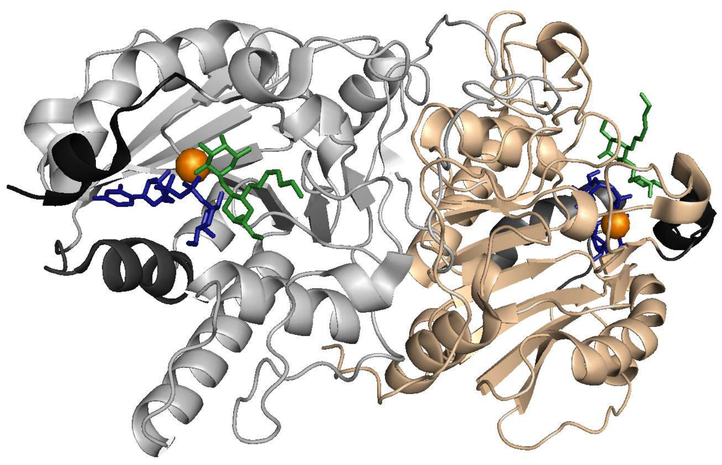

Figure 1: 70 kDa Homodimer of chimeric GTA in complex with UDP-Gal (blue), H-Disaccharide (green) and Mn^{2+} (orange). Loop regions shown in black (pdb: 2RJ7)[1].

- The **Human Blood Group ABO Glycosyltransferases** α-1,3-N-acetylgalactosaminyltransferase **(GTA)** and α-1,3-galactosyltransferase **(GTB)** are responsible for the last step of the A- and B-Antigen synthesis, respectively.
- GTA and GTB differ by only 4 amino acids which are crucial for donor specificity and differences in kinetics.
- They follow the retaining mechanism which is up to date not well understood[2].
- It is known from crystal structures that complex conformational transitions upon substrate binding take place[1].
- NMR spectroscopy is a perfect tool to further analyze the dynamic behavior of GTA and GTB and the influence of substrate binding in different timescales.
- Different labeling schemes are applied to achieve that task.

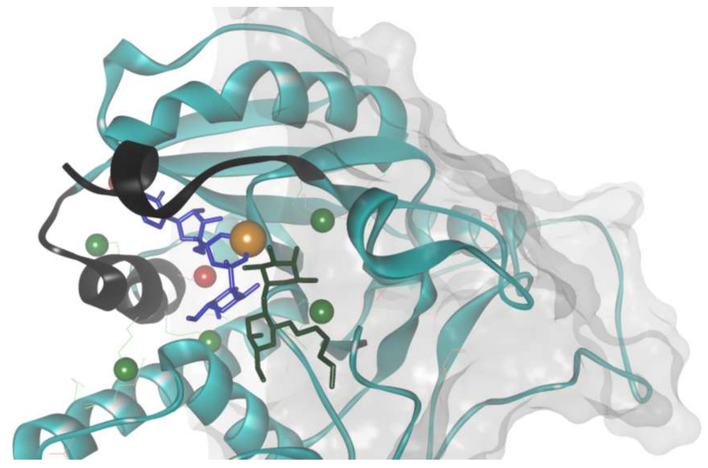

Figure 2: Active site of with bound substrates (UDP-Gal (blue), H-Disaccharide (dark green)) and Mn^{2+} ion (orange)). Flexible internal and C-terminal loop shown in black and Ile and Met residues are shown in red and green, respectively (pdb: 2RJ7)[1].

Dynamic Studies of GTA and GTB

Side Chain Dynamics in the Micro- to Millisecond Time Scale – Relaxation Dispersion Experiments with $Ile^{\delta 1}$-GTA

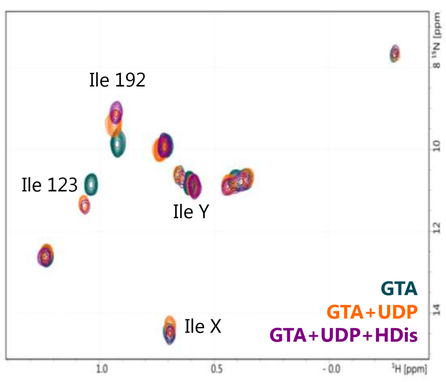

Figure 3: Methyl TROSY spectrum of $Ile^{\delta 1}$-[^{1}H,^{13}C],^{15}N GTA in an otherwise deuterated background. 75 µM protein in 25 mM sodium phosphate buffer, 5 mM $MgCl_2$, 1 mM 2-ME-d_6 was measured at a 500 MHz spectrometer with cryogenic probe. GTA was saturated with 6 mM UDP donor substrate (orange) and 1 mM H-Disaccharide (purple).

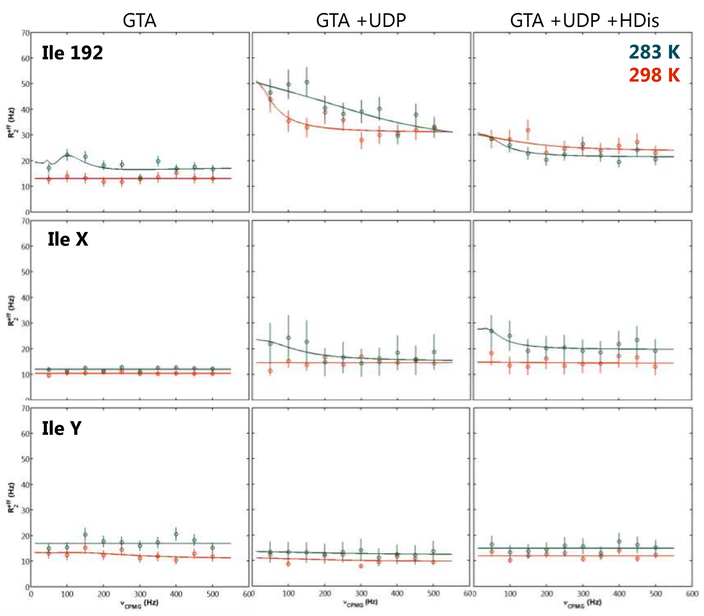

Figure 4: Exemplary relaxation dispersion profiles of Ile residues at 283 K and 298 K with and without the respective substrates. Spectra were recorded with a constant time delay of T = 20 and υ_{CPMG} values ranging from 50 to 500 Hz. $R_{2,eff}$ = (-1/T) $\ln(I_{CPMG}/I_0)$. Data were analyzed with GUARDD[3].

- Methyl groups of Ile are easy to label and lead to well resolved spectra. All 10 Ile in GTA are detectable.
- Ile side chains close to the active site (Ile123) and within the internal loop (Ile192) move mainly upon substrate binding.
- Ile192 also shows dispersion in the presence of UDP. Other Ile signals show only weak or even no dispersion. This indicates that only loop regions show dynamics in µs-ms time scale.
- GTB shows similar behavior (Poster L.L. Grimm).

Relaxation Dispersion Experiments with Met^{ε}-GTB

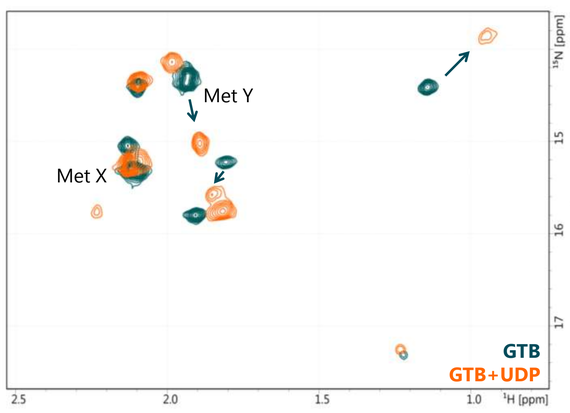

Figure 5: Methyl TROSY spectrum of Met^{ε}-[^{1}H,^{13}C],^{15}N GTB in an otherwise deuterated background. 220 µM GTB in 25 mM sodium phosphate buffer, 5 mM $MgCl_2$, 1 mM 2-ME-d_6 was measured at a 500 MHz spectrometer with cryogenic probe (green). GTB was saturated with 9 mM UDP donor substrate (orange).

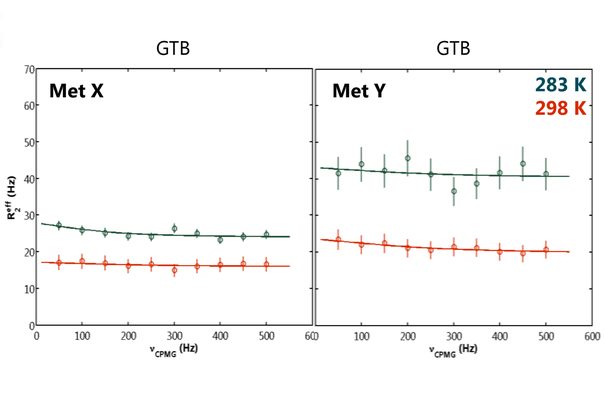

Figure 6: Exemplary relaxation dispersion profiles of methyl groups of Met residues at 283 K and 298 K. Spectra were recorded with a constant time delay of T = 20 and υ_{CPMG} values ranging from 50 to 500 Hz. $R_{2,eff}$ = (-1/T) $\ln(I_{CPMG}/I_0)$. Data analyzed with GUARDD[3].

- Only 10 out of 11 Met signals in GTB are unambiguously detectable.
- Five Met are within an 15 Å distance to the active site so UDP binding has a major influence on the Met side chains.
- Without any ligand Met side chains show no or only weak dispersion.

Backbone Dynamics in the Pico- to Nanosecond Time Scale

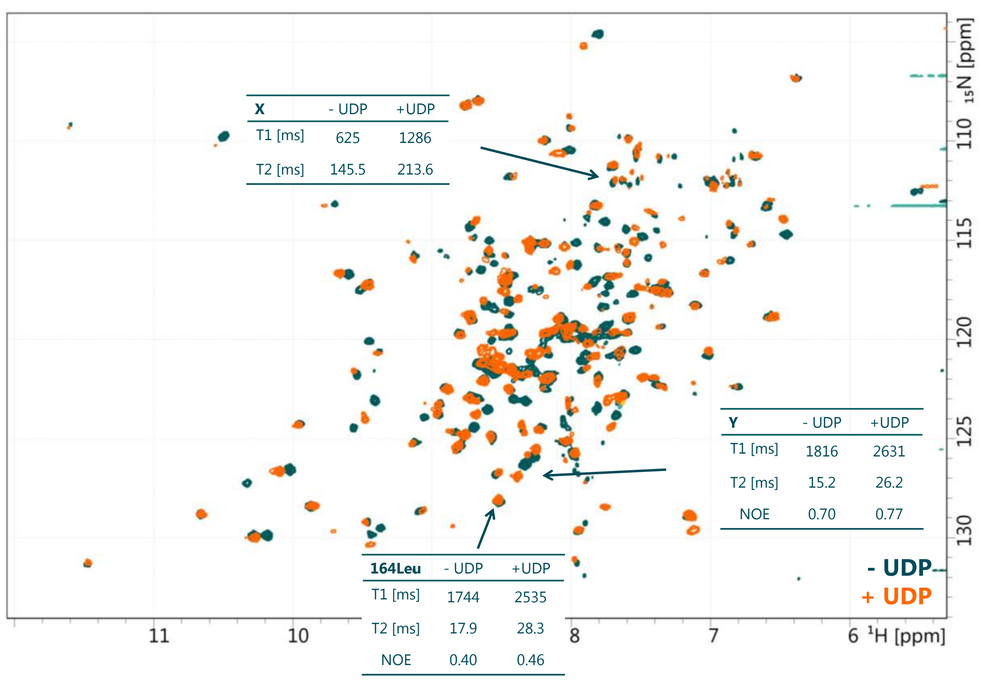

Figure 7: ^{1}H, ^{15}N-TROSY HSQC spectrum of ^{2}H,^{15}N GTA without and with 6,15 mM UDP donor substrate (91% saturation) at 500 MHz and 298 K. Arrows and corresponding tables illustrate ^{15}N T_1, T_2 and ^{15}N{^{1}H} NOE relaxation data and the influence of UDP binding exemplarily.

Table 1: ^{15}N T_1, T_2 and ^{15}N{^{1}H} NOE mean values for the main chain amides of GTA without and with UDP.

	T_1 [ms]	T_2 [ms]	NOE
GTA without UDP	1573,77 ± 369	58,89 ± 56,55	0,66 ± 0,17
GTA with UDP	2587,88 ± 624	71,72 ± 54,47	0,78 ± 0,19
Number of data points	41	28	11

- Broad distribution of relaxation data reveal wide variation in the flexibility within the protein.
- Addition of UDP increases T_1 and T_2 relaxation times. This indicates that the structure gets more rigid upon UDP binding and moves slower.
- Referring to the size of the protein only little peaks can be analyzed unambiguously. For such a large protein (70 kDa) we are at the limit of the detectability for backbone dynamics in the pico- to nanosecond time scale.

Conclusion & Outlook

- **Backbone Dynamics** in the pico- to nanosecond time scale are hard to analyze due to the huge size of the protein.
- The wide range of relaxation times indicates that some parts of GTA are highly flexible and others are rather rigid.
- Methyl labeling of e.g. Ile and Met side chain is a perfect tool to investigate **Side Chain Dynamics.**
- In the micro- to millisecond time scale only the loop region of GTA in the presence of UDP is moving. Other areas probably move in a faster time scale.
- It will be investigated if Met side chains confirm this result.

Acknowledgement

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[1] Alfaro, J.A. et al., *J. Biol. Chem., 283 (15)*, 10097-108 (2010)
[2] Lairson, L.L. et al., *Annu Rev Biochem, 77, 521*-55 (2008)
[3] Foster, M.P. et al., *J Biomol NMR, 52 (1)*, 11-22 (2012)