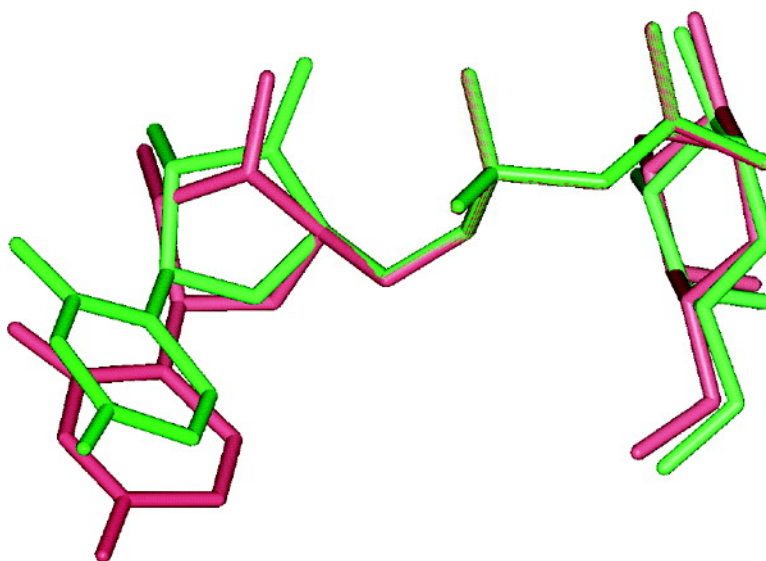Communication

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Refinement of the Conformation of UDP−Galactose Bound to Galactosyltransferase Using the STD NMR Intensity-Restrained CORCEMA Optimization

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The galactosyltransferase family of enzymes transfers galactose (Gal) from UDP−galactose to an acceptor substrate. For example, β1,4-galactosyltransferase-I (β4 GalT1) transfers galactose to *N*-acetylglucosamine (GlcNAc).[1] Recently, Ramakrishnan et al.[1] reported the structure of β4 GalT1 cocrystallized with UDP−Gal and $MnCl_2$. We wanted to determine similarities and/or differences between solution and solid-state structures. Therefore, we refined the conformation of UDP−galactose within the binding pocket of β4 GalT1 using a hybrid refinement procedure[2] that employs complete relaxation and conformational exchange matrix (CORCEMA) calculations and simulated annealing optimization of torsion angles using STD NMR intensities as experimental constraints. The refined solution NMR structure of the complex is compared with the crystal structure. This work provides the first experimental example where the STD data has been utilized to refine the conformation of a ligand bound to a receptor protein. Our work significantly extends the utility of the STD NMR method beyond its current application in compound library screening[3,4] and qualitative ligand epitope mapping.[5−8]

A computer program called CORCEMA-ST (ST, saturation transfer) was developed for use in MatLab for the quantitative analysis of STD NMR data and to refine the bound conformation of ligands within the binding pocket of the macromolecular receptor.[2] In this approach, the global minimum for the bound ligand conformation is obtained by a refinement of the torsion angles of any starting structure for the bound ligand using STD NMR intensities as experimental constraints and the NOE *R*-factor[9] as the energy function to be minimized (see Supporting Information for details). The simplified expression for the observable magnetization $\mathbf{I}(t)$ in a STD experiment for a long delay between each scan is given by[10]

$$\mathbf{I}(t) = \mathbf{I_0} + [1 - \exp\{-\mathbf{D}t\}]\mathbf{D}^{-1}\mathbf{Q} \qquad (1)$$

where the bold letters stand for matrixes and have been defined in the Supporting Information. The *t* is the time period for which the protein proton(s) experience rf irradiation.

For the CORCEMA calculations, we have used the crystal structure of β4 GalT1/UDP−Gal complex (PDB entry 1O0R).[1] The crystal structure consisted of two copies (A and B chains) of the protein−ligand complex. After the addition of hydrogens to the crystallographic structure using QUANTA, a bad contact (~1.39 Å) between H3 and H5 protons of ribose residue was observed in both A and B chains. Further, the O5−C5−C4 bond angle in the ribose (139.5°) showed a significant deviation from the normal tetrahedral angle.[11] To fix this bad contact and the abnormal bond

Table 1. Comparison of Experimental and Calculated Percentage Fractional STD Intensities for UDP-Gal/GalT1 Complex[a]

| | | STDs from CORCEMA-ST method | | |
proton	experimental STDs	crystal structure	energy-minimized crystal structure	SICO structure
H5 U	3.1[b]	1.1351	1.0779	1.3702
H6 U	0.96	1.5968	1.1041	1.2716
H1 R	6.4	7.5426	5.4127	5.6467
H2/H3 R	2.6	15.066	11.5059	3.7414
H4 R	2.3	3.0819	1.5681	1.3812
H5/H5′ R	1.2	4.638	1.2678	0.9081
H1 G	0.73	1.068	0.9051	0.8214
H2 G	1.1	1.6626	1.1797	1.2586
H3 G	0.99	1.4312	0.8771	1.0954
H4 G	1.1	1.3076	0.9046	1.0485
H5 G	0.76	0.4261	0.3198	0.3903
R-factor		1.8486	1.2386	0.332

[a] Protein correlation time = 24.12 ns; K_d = 1.6 × 10^{-6} M; time delay = 2.3 s; *t* = 2 s; order parameter = 0.8; ligand correlation time = 0.5 ns; Rholeak = 0.2 s^{-1}; τm = 10 ps; [L] = 0.8 mM; [E] = 14.5 × 10^{-6} M. [b] H5U has a systematic error (see Supporting Information).

angle observed in the crystal structure, we performed an energy minimization of the complex (using Insight-II) by including the residues within the binding pocket. In the energy-minimized structure, the O5−C5−C4 bond angle in ribose was 112.6 and the bond distance between H3 and H5 protons of ribose was 2.59 Å. Thus, we have used this energy-minimized structure as the starting structure for the complex in CORCEMA optimization. In the uncomplexed state, the protein structure was defined by the coordinates for the free enzyme.[12] The free ligand was assumed to have the same torsion angles as in free UDP−glucose, which is very similar.[13] The experimental STD NMR data recorded on UDP−Gal at 500 MHz at a ligand/protein ratio of 55:1, 293 K, and a saturation time of 2 s using the procedures described before[14] were utilized in these calculations. Table 1 shows the experimental and predicted STD NMR intensities for UDP−Gal. The NOE *R*-factors for different structures are shown at the bottom. The crystal structure results in a large NOE *R*-factor (1.84), indicating poor fit with the experimental data. In particular, the STDs for H2/H3R, H5/H5′R, and H5U show rather significant deviations. The energy-minimized crystal structure has a slightly improved *R*-factor (1.23), but still with significant deviations for H2/H3R and H5U. However, optimization of the energy-minimized crystal structure using STD NMR intensity-restrained CORCEMA optimization (SICO) resulted in a substantial improvement in the NOE *R*-factor (0.33) and in the overall fit between calculated and experimental data. For the optimization, we fixed the torsion angles across bonds linking the phosphate groups since they are known to be coordinated with the metal ion in the crystal structure.[1] The SICO procedure

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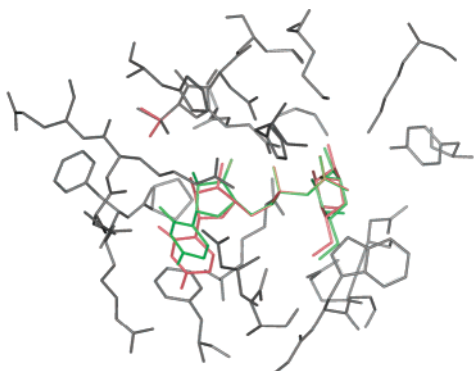‡ University of Luebeck.

Figure 1. Best-fit superposition of CORCEMA-ST-optimized structure (red) with the energy-minimized starting structure. Protein residues within the binding pocket, black; ligand residues, green. The hydrogens were omitted for clarity. The stereoview is shown in the Supporting Information.

included the "simultaneous" refinement of six torsion angles (galactose (ϕ, ψ, and ω) and ribose (α, β, and γ)) in the ligand (Figure S1, Supporting Information), the χ_1 angle for V253 side chain, and the protein correlation time. Refinement of the six ligand torsion angles only for the energy-minimized structure while fixing the V253 side-chain orientation as in the crystal structure resulted in a slightly larger R-factor (0.42) because of deviations in STDs for the H2/H3R protons. This is not surprising since in the crystal structure the methyl group (pro-R) of V253 is very close ($\sim$2 Å) to the H2 proton of the ribose residue, thus resulting in a significantly larger STD value for the ribose H2/H3 protons compared to that of the experiment. Optimization of V253 side chain alone for the energy-minimized crystal structure yielded an R-factor of 0.44, whereas a simultaneous optimization of the V253 side chain together with the six ligand torsion angles resulted in a significantly lowered R-factor of 0.33. The optimized χ_1 value of $\sim$180° (trans) is energetically equally possible as the gauche+ orientation in the crystal structure, but the distance from the ribose H2/H3 is larger. It thus appears that in solution the trans-rotamer for V253 side chain is favored. Interestingly, even though the optimization of the V253 side chain alone for either the X-ray structure or its energy-minimized structure resulted in an improvement in the R-factor: the value is more than 0.44, suggesting that the ligand torsion angles also needed an optimization.

These calculations show that the crystal structure is not entirely compatible with the STD NMR data in solution, whereas the CORCEMA refined structure is significantly more compatible with an R-factor of 0.33. The optimized correlation time of 24.12 ns that we determined for the protein is a very reasonable value for a 38 kDa protein.[15] This value was used for predicting STDs for all structures in Table 1. The UDP–Gal and V253 torsion angles are shown in Table S1 (Supporting Information) for the various structures. There are some differences (especially for V253 χ_1, ribose γ, Gal φ, and Gal side chain) between the CORCEMA-ST structure and the energy-minimized crystal structure. Figure 1 shows a comparison of the energy-minimized structure and the CORCEMS-ST structure for the ligand and the V253 residue, along with other protein residues in the binding pocket. Subsequent energy minimization of the CORCEMA-ST-optimized structure did not produce any significant changes in the torsion angles, indicating the structure to be energetically favorable. The stacking interaction

between the aromatic rings of the uracil and Phe226 as well as the hydrogen bonds between the protein and the ligand observed in the crystal structure is also preserved in the CORCEMA-optimized structure (e.g., between uracil and Arg189, O3 of ribose and V253 amide nitrogen, etc). A simulation of the loop movement of residues 345–365 and change in Trp314 side-chain orientation observed in the crystal structure of the complex did not produce any significant changes in STDs generated from a saturation of the methyls at 0 ppm. This is because the STDs and CORCEMA-ST calculations are sensitive to only those conformational changes in the ligand or protein that affect significantly the saturation transfer from the saturated protein protons (E2′) to the bound ligand (L′) by either direct (E2′ → L′) or indirect (E2′ → E1′ → L′) pathways.[10] That is not the case with the loop residues and Trp314 for saturation of methyls at 0 ppm.

In conclusion, using high-quality STD NMR data on UDP–Gal weakly binding to β4 GalT1, we demonstrated that it is possible to refine the crystal structure (or any docked structure that serves as the starting structure) to obtain a global-minimum conformation for the bound ligand at that position. Further, if the resulting R-factor is low (e.g., 0.35 or less), the global-minimum conformation is compatible with experimental STD NMR data. This method does not require an explicit knowledge of the bound-ligand conformation from transferred NOE but relies only on STD intensities as constraints. However, the calculations can be augmented, if necessary, by inclusion of intraligand distance constraints (from tr-NOE), ligand-to-saturated protein residue distances from the initial slopes of STD intensity build up,[10] torsion angle, and intraligand relaxation rates as additional constraints. Thus, the CORCEMA-ST program together with the SICO methodology presented here is likely to be useful as a tool in structure-based drug design programs where the early stages may involve the determination of the bound conformation (within the protein binding pocket) of promising lead compounds that bind weakly (K_d in the millimolar to micromolar range) to proteins of pharmaceutical interest.

Supporting Information Available: Details on the STD NMR measurements on UDP–Gal/β4 GalT1, CORCEMA-ST calculations, and stereoview of Figure 1. This material is available free of charge via the Internet at http://pubs.acs.org.

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