

# A Proton Water $T_1$ -NMRD Study of Ganglioside Micelles

Per-Olof Westlund

Department of Chemistry, Umea University, Sweden

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**Abstract** Ganglioside GM1 ( $G_{M1}$ ) micelles have been studied by means of water proton  $T_1$  NMRD experiment. The field dependent spin-lattice relaxation rates were measured for Larmor frequencies ranging from 0.1 to 40 MHz and for two micelle concentrations at three temperatures ( $T=10,15,20^\circ\text{C}$ ). The proton  $T_1$  NMRD-profiles are well described by assuming two proton pools are responsible for the dispersion curves. The proton pools are characterized by an effective correlation time and a proton fraction. The largest correlation time,  $\tau_{c,1} \approx 130 - 160$  ns, is determined by the low field part of the NMRD profile. The second correlation time,  $\tau_{c,2} \approx 12$  ns, is determined by the high field part of the NMRD profile. The radius of the ganglioside micelles has previously been determined as about 54 using fluorescence experiments and with Stoke-Einstein relation the reorientation correlation time becomes  $\tau_R = 120-165$  ns depending on the temperature dependence of the water viscosity. It is thus plausible to identify one pool of water-protons, characterized by the largest effective correlation time, as corresponding to waters residing in the headgroup with an order parameter  $S \neq 0$  and  $\tau_{c,1} \approx \tau_R$  or corresponding to labile protons with a  $\tau_{c,1}$  as the mean life time. The proton NMRD profile reveal a second Lorentzian which also can either be labile and exchanging Ganglioside protons or water molecules residing in the head group with a mean life time as approximately 12 ns. The proton NMRD experiment cannot discriminate between these two cases.

**Keywords** Proton  $T_1$ -NMRD Profiles, Proton Spin-lattice Relaxation, Ganglioside Micelles

## 1 Introduction

GM1 ganglioside are amphiphilic molecules with a hydrophobic part consisting of a sphingosine and a fatty acid. The bulky hydrophilic part is built up by several sugar units. This system is rather well studied and is comprised of large spherical micelles with a stable aggregation number of  $\approx 168 \pm 4$  in the temperature interval  $T_i$   $30^\circ\text{C}$  [1]. Consequently, we may study the information content of the water proton  $T_1$ -NMRD profiles obtained from fast field cycling (FFC)-NMR dispersion experiments. The proton spinlattice

relaxation rate profile is measured at 30 different proton Larmor frequencies ranging from  $\omega_0 = 0.01$  to 40 MHz. The great advantage of this technique is that the relaxation rate is sensitive to molecular motions with correlation times in the range of a few ns to  $\mu\text{s}$  [2],[3]. The aim of this work is to examine what molecular information can be extracted from a relatively simple relaxation model which describes the water proton spin-lattice NMRD profiles very well.

## 2 Materials and methods

The  $G_{M1}$  lipids forms micelles over a broad range of concentrations ( $\geq 2.0 \cdot 10^{-8}\text{M}$ ) mainly because of the bulky head group. The aggregation numbers ( $168 \pm 4$ ) of GM1 micelles have been determined by using fluorescence correlation spectroscopy (FCS) and electronic energy transfer experiments, as well as static/dynamic light scattering. The aggregation number is constant up to  $30^\circ\text{C}$  and it decreases almost linearly from 30 to  $55^\circ\text{C}$ . The hydrodynamic radius was also determined to  $R_H = 5.4\text{nm}$  [1].

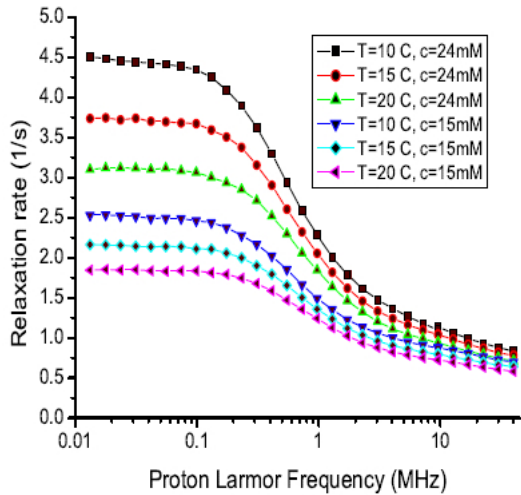
### 2.1 Sample preparation

Two concentrations of GM1 ganglioside, 24 mM and 15 mM were used in preparing the micellar solution according to the procedure described in [1]. The main steps are dissolving, evaporation and hydration. GM1 was dissolved in a chloroformmethanol mixture (2:1, v/v). After evaporation of the organic solvents, sample dried under vacuum for 3 h. The lipid film was then hydrated to the desired concentration by adding a TRIS-HCl buffer (pH 7.4) containing 150 mM NaCl.

### 2.2 Proton spin relaxation measurements

Water  $^1\text{H}$  longitudinal relaxation rates,  $R_1^{exp}(\omega)$  of figure 1, was measured on a 1T Stelar FFC2000 fast-field-cycling instrument with polarization at 25 MHz and detection at 16.29 MHz. Relaxation takes place at different proton Larmor frequencies ranging from 0.01 to 40 MHz. The switching time is 3 ms, with a  $90^\circ$  pulse length of  $7.8 \mu\text{s}$ . The polarization and recovery times were set to  $4 T_1$  and the number of accumulated transients was 4 for all samples. Sample temperatures were 10, 15 and  $20 \pm 0.1^\circ\text{C}$ , maintained using the

temperature unit of the Stellar spectrometer. Set temperatures are given by the spectrometer with an accuracy of about  $\pm 1$  degree. The temperature is maintained, however within  $\pm 0.1$  degree. In Figure 1, the six experimental NMRD profiles are displayed.



**Figure 1.** The experimentally measured proton  $R_1^{exp}(\omega)$  NMRD curves at three temperatures and two concentrations. The temperatures are 10C, 15C and 20C and the concentration of micelles with a constant aggregation number of 168 are  $24/168 = 0.143$  mM and  $0.089$  mM, respectively.

### 3 The water proton $R_1$ -NMRD relaxation model

The experimentally determined relaxation rates,  $R_1^{exp}(\omega)$  at different Larmor frequencies  $\omega$  (Figure.1) were analysed using a simple theoretical model where the corresponding theoretical relaxation rate,  $R_1^T(\omega_0)$  is expressed in terms of (four) relaxation contributions referring to waters/protons in different micro environments. The "bulk" is ordinary water whereas surface water (S) is water at molecular surfaces and with a retardation of its reorientation by a factor of 4-5 relative to bulk. The field dependent relaxation of water is denoted  $\beta$ -water and their relaxation contributions characterized by a residence time  $\tau_{wi}$ . In an exchange system the total relaxation rate  $R_1^T(\omega_0)$  is given as a sum of intrinsic relaxation rates weighted by their fraction of water protons. Protons of the ganglioside molecules only represent 2-3% of all water protons. The theoretical relaxation model has been thoroughly discussed and analysed for tissues [4] and protein solutions by Halle and coworkers [5, 6, 7]. This model has been customized for the micellar solution of interest here, with the relaxation model expressed by the following:

$$R_1^T(\omega_0) = \overbrace{f_{Bulk}R_{1Bulk} + f_S R_{1,S}}^{in \alpha} + \frac{f_{\beta 1} R_{1\beta 1}}{1 + R_{1\beta 1} \cdot \tau_{w1}} + \frac{f_{\beta 2} R_{1\beta 2}}{1 + R_{1\beta 2} \cdot \tau_{w2}}. \quad (1)$$

The field dependence of the relaxation rate is due to so called  $\beta$  water molecules residing in the bulky micelle head group or by labile proton exchanging with water and characterized by relatively long residence times or an effective correlation time larger than  $\approx 1$ ns ( $1/\omega$ ).

In order to reproduce the experimental NMRD profiles, with a reliable pure fit using Levenberg-Marquardt method, introduction of two types of  $\beta$  water pools was sufficient. However, this is of course a great simplification and one may expect a distribution of water residence times or mean life times of labile protons but it was not permissible to extract more proton pools because of the information content of the experimental NMRD profiles.

Intrinsic relaxation rates of the  $\beta$  waters are denoted  $R_{1,\beta 1}$  and  $R_{1,\beta 2}$  and the water fractions satisfy the relation:  $f_{Bulk} = 1 - f_S - f_{\beta 1} - f_{\beta 2}$  where  $f_\alpha = f_{Bulk} + f_S$ . Fast chemical exchange conditions mean that  $R_{1\beta i} \cdot \tau_{wi} \ll 1$ . In the high field limit of the NMRD profile relaxation rates display the characteristic temperature dependence as expected in the extreme narrowing regime. That is, the highest temperatures give shortest correlation times and the lowest relaxation rates.

The spectral density, describing water proton spin relaxation, has been separated into two parts assuming the presence of two distinctively different modulation time scales. One is a fast (local) anisotropic reorientational motion of water which thus introduce a partial average of the fluctuating dipole-dipole coupling. Consequently, an order parameter  $S$ , ( $0;S;1$ ) is introduced for the partial averaged interaction which is characterized by a correlation time in the ns time regime. The effective correlation time combines the micelle reorientation correlation time ( $\tau_R$ ) and the water residence time ( $\tau_{w,i}$  ( $i=1,2$ )).

$$\tau_{c,i} = \frac{\tau_R \cdot \tau_{w,i}}{\tau_R + \tau_{w,i}}. \quad (2)$$

This type of model was introduced in the 1980's and sometimes named "the two step model"[8] or "the model free approach"[9] because no detail diffusion or molecular dynamic description is introduced to describe the effect of the stochastic modulations[10]. The spectral densities for the  $\beta$  water pools ( $i=1,2$ ) reads:

$$J_i(n\omega) = (1 - S_i^2)\tau_f + S_i^2\tau_{c,i} \frac{1}{1 + (\tau_{c,i}n\omega)^2} \quad (3)$$

Field dependent relaxation is due to both the intra- and inter- molecular spin dipole-dipole relaxation mechanisms.

$$R_{1\beta,i} = S_i^2\tau_{c,i}\beta_{Intra} \left\{ \frac{0.2}{1 + (\omega\tau_{c,i})^2} + \frac{0.8}{1 + 4(\omega\tau_{c,i})^2} \right\} + W_D \left\{ 0.1 + \frac{0.3}{1 + (\omega\tau_{c,i})^2} + \frac{0.6}{1 + 4(\omega\tau_{c,i})^2} \right\} \quad (4)$$

The weight factor  $W_D$  takes the value in the range 0.3 (protein)-0.6(bulk) thus relating the inter-molecular coupling constant to the intra-molecular dipole dipole coupling [3]. This assumption means that  $\beta_{Inter} = \beta_{Intra}W_D$  and that both intra- and inter- molecular spin dipole-dipole couplings have the same type of spectral density. This simplification assumes that water reorientation is dominant. The intra-molecular spin dipole dipole interaction constant is given by:

$$\beta_{Intra} \equiv \frac{3}{2} \left( \frac{\mu_0}{4\pi} \hbar \gamma_H^2 r_{HH}^{-3} \right)^2 \quad (5)$$

Here,  $\beta_{Intra} = 5.492036 \cdot 10^{10}$  where  $\mu_0$ ,  $\hbar$ ,  $\gamma_H$ , and  $r_{HH}(=1.58)$  refer to vacuum permeability, Plancks

constant/ $2\pi$ , magnetogyric ratio of a proton, and the intra molecular distance between the water protons, respectively.

The relaxation rate  $R_{1Bulk}$  refers to waters which are unperturbed by the micelle and thus similar to bulk water. For the milleQ water used as solvent for the micelles proton relaxation rates were measured at three temperatures. We obtained the following proton spin-lattice relaxation rates:  $R_{1Bulk}(1/s) = 0.37 \pm 0.02(T = 20^\circ C)$ ;  $0.42 \pm 0.02(T = 150^\circ C)$ ;  $0.50 \pm 0.02(T = 100^\circ C)$ ; which corresponds to correlation times  $\tau_f$  ranging from 4.2ps to 5.7 ps using  $W_D=0.6$ .

Surface waters, characterized as  $\alpha$  waters, are interacting only weakly with the micelle and thus are weakly perturbed with a retarded reorientational motion, in comparison with bulk water the correlation time is  $\approx (4 - to - 6) \times \tau_f$ . This relaxation contribution is therefore expected to be field independent and expressed as  $R_{1,S} \equiv \frac{1}{T_{1S}(\tau_R)}$ , thus assuming fast exchange conditions where  $T_{1S}(\tau_S) \gg \tau_M$ . These relaxation rates can thus be combined to an averaged relaxation rate:  $\langle R_{1,\alpha} \rangle = f_{Bulk}R_{1,Bulk} + f_S R_{1,S}$ . There is also a field-independent relaxation contribution from the  $\beta$  waters due to the fast anisotropic motion which is given by the expression:  $(1 - S_i^2)\tau_f\beta_{Intra}(1 + W_D)$ . These contributions are added to form the  $\alpha$  relaxation term which is used as a fitting parameter and defined as:

$$\alpha = \langle R_{1\alpha} \rangle + [f_{\beta 1}(1 - S_1^2) + f_{\beta 2}(1 - S_2^2)]\tau_f\beta_{Intra}(1 + W_D). \quad (6)$$

Consequently, the relaxation contribution  $\alpha$  is expected to have a value larger than 0.5 in all fittings given in table 1. We also give the averaged water retardation reorientational correlation time which thus indicate the relaxation contribution due to surface water and local fast anisotropic motion summarized as a qualitative retardation correlation time  $\langle \tau_\alpha \rangle$ :

$$\langle \tau_\alpha \rangle = \alpha / \beta_{Intra} \quad (7)$$

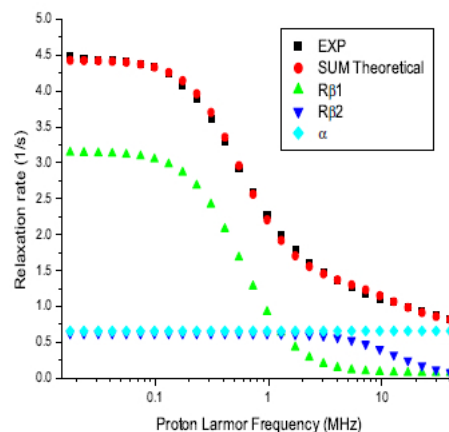
The fraction of perturbed water hydrating the micelle head group is given by  $f_{\beta i} = \frac{N_{w,i} \cdot [Gan]}{N_g \times [H_2O]}$  where  $[Gan]$  is the molar concentration of ganglioside molecules,  $[Micelle] = \frac{[Gan]}{N_g}$  is the micelle concentration with an aggregation number  $N_g = 168[1]$ . In the analysis of the NMRD profiles the fitting parameters are the fraction of the  $\beta$  pools multiplied by a characteristic order parameter:  $S_1^2 \cdot f_{\beta 1}$  and  $S_2^2 \cdot f_{\beta 2}$  and the effective correlation times. In summary, the relaxation model has the following five fitting parameters:  $\tau_{c,1}$ ,  $\tau_{c,2}$ ,  $(S_1^2 \cdot f_{\beta 1})$ ,  $(S_2^2 \cdot f_{\beta 2})$  and  $\alpha$ . In figure 2 the different relaxation contributions are shown for a typical  $R_1^{exp}$ -NMRD profile ( $T=10^\circ C$ ,  $c=24mM$ ).

## 4 Results and Discussion

One aim of the present study is to determine the information content in water spin-lattice NMRD profiles when applied to a rather well characterized micellar system. The aggregation number and the radius of the spherical ganglioside micelles are known to be temperature independent for  $T \leq 30$  C[1]. The hydration on the other hand is not well known and here the proton water  $R_1$ -NMRD experiment provide some new information about the hydration of the micellar head group.

The theoretical expression (Eq.(1) for relaxation model is simplified as:

$$R_1^T(\omega) = \alpha + f_{\beta 1} \cdot R_{1\beta,1} + f_{\beta 2} \cdot R_{1\beta,2} \quad (8)$$



**Figure 2.** The measured proton  $R^{exp}(\omega)$  NMRD profile for  $T=10$  C and the concentration 24mM. The theoretical NMRD is composed by  $R_{1\beta,1}(0) = S_1^2 f_{\beta 1} \beta_{Intra} \tau_{c,1} (1 + W_D)$  and  $R_{1\beta,2} = S_2^2 f_{\beta 2} \beta_{Intra} \tau_{c,2} (1 + W_D)$  and  $\alpha$ . The theoretical predictions are shown in red points whereas the experimental values are in black.

and the quality of the fitting is expressed as

$$\chi^2 = \frac{1}{N} \sum_j \frac{[R^{exp}(\omega_j) - R_1^T(\omega_j)]^2}{\sigma_j^2} \quad (9)$$

where  $j$  runs over ca 30 Larmor frequencies from 0.01 MHz to 40 MHz. From a single  $T_1$  NMRD experiment that deviation  $\sigma_i^2$  of a relaxation rate  $R_1^{exp}(\omega_j)$  for each field is given as a result of a fitting procedure using about 1000 FID amplitudes. However, this deviation was much too small compared to the relaxation deviation obtained from three independent  $T_1$  NMRD experiments. Actually, the  $\sigma$ -vector of a single NMRD experiment has to be multiplied with 10 before included in Eq.(9). Then the  $\chi^2$  value is about 1.0(0.99-0.95) in all performed fittings. Consequently, one cannot motivate to include a third Lorentzian representing an additional pool of protons. The results of the NMRD profile fitting using a Levenberg-Marquardt(L-M) method were obtained using four parameters. The maximum relaxation rate at low field was determined experimentally which equals the sum of  $\alpha$  and two  $\beta$ -relaxation contributions ( $(S^2 f_{\beta 1}, S^2 f_{\beta 2})$ ) thus making the  $\beta$  fractions dependent. The fitting were performed for three values of the factor  $W_D$  (cf Eq.(4) determine the amount of inter-molecular proton dipole dipole relaxation present. Clearly the  $W_D$  factors introduce very small changes in the extracted fractions and correlation times. The dispersion at low field determine  $\tau_{c,1}$  and the end of the dispersion at high fields determine the second correlation time  $\tau_{c,2}$ . All parameters with their variations obtained from the L-M fitting procedure are summarized in Table 1.

### 4.1 Reorientation correlation times of the micelles

The reorientation of the spherical ganglioside micelles is expected to be well described using the Stoke-Einstein-Debye(SED) theory, which predicts that the reorientation correlation time,  $\tau_{SED}$ , is proportional to the water viscosity,  $\eta_{H_2O}$  and the volume of the micelle:

$$\tau_{SED} = \frac{4\pi}{3} \frac{\eta_{H_2O} R_{SED}^3}{kT} \quad (10)$$

**Table 1.** The model parameters obtained from a non-linear least-square fit by Levenberg-Marquardt method using 4 fitting parameters with  $W_D$  constant at 0.1, 0.3 and 0.4 and three temperatures and two micelle concentrations.

Sample $W_D=0.1$	$\tau_{c,1}$ (ns)	$\tau_{c,2}$ (ns)	$\alpha(1/s)(< \tau_\alpha \pm 0.4 >)$ (ps)	$S_1^2 f_{\beta 1}(\times 10^3)$	$S_2^2 f_{\beta 2}(\times 10^3)$
T=10 $\pm$ 1 C,c=24mM	176.4 $\pm$ 4.0	13.5 $\pm$ 1.4	0.79 $\pm$ 0.02( $\downarrow$ 14.4 $\downarrow$ )	0.303 $\pm$ 0.005	1.01 $\pm$ 0.04
T=15 $\pm$ 1 C,c=24mM	151.9 $\pm$ 3.7	10.3 $\pm$ 1.2	0.73 $\pm$ 0.02( $\downarrow$ 13.3 $\downarrow$ )	0.287 $\pm$ 0.005	1.02 $\pm$ 0.04
T=20 $\pm$ 1 C,c=24mM	141.9 $\pm$ 3.9	12.2 $\pm$ 1.7	0.68 $\pm$ 0.02( $\downarrow$ 12.4 $\downarrow$ )	0.240 $\pm$ 0.005	0.79 $\pm$ 0.06
T=10 $\pm$ 1 C,c=15mM	151.9 $\pm$ 4.4	10.5 $\pm$ 1.7	0.67 $\pm$ 0.02( $\downarrow$ 12.2 $\downarrow$ )	0.177 $\pm$ 0.005	0.63 $\pm$ 0.05
T=15 $\pm$ 1 C,c=15mM	131.5 $\pm$ 4.0	9.4 $\pm$ 1.6	0.61 $\pm$ 0.02( $\downarrow$ 11.2 $\downarrow$ )	0.170 $\pm$ 0.005	0.58 $\pm$ 0.05
T=20 $\pm$ 1 C,c=15mM	122.6 $\pm$ 3.8	10.4 $\pm$ 1.7	0.56 $\pm$ 0.02( $\downarrow$ 10.2 $\downarrow$ )	0.147 $\pm$ 0.005	0.50 $\pm$ 0.04
Sample $W_D=0.3$	$\tau_{c,1}$ (ns)	$\tau_{c,2}$ (ns)	$\alpha(1/s)(< \tau_\alpha >)$ (ps)	$S_1^2 f_{\beta 1}(\times 10^3)$	$S_2^2 f_{\beta 2}(\times 10^3)$
T=10 $\pm$ 1 C,c=24mM	178.2 $\pm$ 4.0	13.5 $\pm$ 1.4	0.74 $\pm$ 0.02( $\downarrow$ 13.3 $\downarrow$ )	0.303 $\pm$ 0.005	1.01 $\pm$ 0.04
T=15 $\pm$ 1 C,c=24mM	153.6 $\pm$ 3.7	10.4 $\pm$ 1.2	0.68 $\pm$ 0.02( $\downarrow$ 12.4 $\downarrow$ )	0.289 $\pm$ 0.005	1.03 $\pm$ 0.04
T=20 $\pm$ 1 C,c=24mM	143.3 $\pm$ 3.9	12.1 $\pm$ 1.7	0.65 $\pm$ 0.02( $\downarrow$ 11.8 $\downarrow$ )	0.241 $\pm$ 0.004	0.80 $\pm$ 0.06
T=10 $\pm$ 1 C,c=15mM	153.5 $\pm$ 4.5	10.4 $\pm$ 1.7	0.65 $\pm$ 0.02( $\downarrow$ 11.8 $\downarrow$ )	0.177 $\pm$ 0.005	0.64 $\pm$ 0.04
T=15 $\pm$ 1 C,c=15mM	132.9 $\pm$ 3.0	9.4 $\pm$ 1.7	0.59 $\pm$ 0.02( $\downarrow$ 10.8 $\downarrow$ )	0.170 $\pm$ 0.005	0.58 $\pm$ 0.04
T=20 $\pm$ 1 C,c=15mM	123.7 $\pm$ 3.8	10.4 $\pm$ 1.7	0.54 $\pm$ 0.02( $\downarrow$ 9.8 $\downarrow$ )	0.150 $\pm$ 0.005	0.50 $\pm$ 0.04
Sample $W_D=0.4$	$\tau_{c,1}$ (ns)	$\tau_{c,2}$ (ns)	$\alpha < \tau_\alpha >$ (ps)	$S_1^2 f_{\beta 1}(\times 10^3)$	$S_2^2 f_{\beta 2}(\times 10^3)$
T=10 $\pm$ 1 C,c=24mM	179.0 $\pm$ 4.0	13.6 $\pm$ 1.4	0.72 $\pm$ 0.02( $\downarrow$ 13.0 $\downarrow$ )	0.300 $\pm$ 0.005	1.02 $\pm$ 0.04
T=15 $\pm$ 1 C,c=24mM	154.3 $\pm$ 3.7	10.4 $\pm$ 1.2	0.67 $\pm$ 0.02( $\downarrow$ 12.2 $\downarrow$ )	0.288 $\pm$ 0.005	1.04 $\pm$ 0.04
T=20 $\pm$ 1 C,c=24mM	143.8 $\pm$ 3.9	12.1 $\pm$ 1.7	0.63 $\pm$ 0.02( $\downarrow$ 11.5 $\downarrow$ )	0.243 $\pm$ 0.004	0.89 $\pm$ 0.06
T=10 $\pm$ 1 C,c=15mM	154.2 $\pm$ 4.5	10.5 $\pm$ 1.7	0.64 $\pm$ 0.02( $\downarrow$ 11.6 $\downarrow$ )	0.197 $\pm$ 0.005	0.64 $\pm$ 0.04
T=15 $\pm$ 1 C,c=15mM	133.6 $\pm$ 4.1	9.4 $\pm$ 1.7	0.58 $\pm$ 0.02( $\downarrow$ 10.6 $\downarrow$ )	0.171 $\pm$ 0.005	0.59 $\pm$ 0.04
T=20 $\pm$ 1 C,c=15mM	124.2 $\pm$ 3.8	10.4 $\pm$ 1.7	0.54 $\pm$ 0.02( $\downarrow$ 9.8 $\downarrow$ )	0.149 $\pm$ 0.005	0.51 $\pm$ 0.04

For ganglioside micelles with a radius of 54, the reorientation correlation time,  $\tau_{SED} \approx 125 - 165 ns$  depending on the temperature dependence of the water viscosity ( $1.307 \cdot 10^{-3}$  (kg/sm), T=10C- $1.02 \cdot 10^{-3}$ , T=20C). The effective correlation time  $\tau_{c,1}$  may thus be identified as the reorientation correlation time of the micelle which also is consistent with the reorientation correlation time determined by other methods[1].

## 4.2 The $\alpha$ relaxation contribution

The field independent relaxation contribution denoted  $\alpha$  and defined in Eq.(6) represent bulk water, weakly perturbed water at the micelle water interface as well as  $\beta$ -waters. Qualitatively, the retardation correlation times range between 10-13 ps. This relaxation contribution can be analysed at higher fields where it may display frequency dependence. Such studies may thus result in detail information about weakly perturbed interfacial water.

## 4.3 The hydration of the Ganglioside micelle or proton exchange with labile non-water protons

The bulk water proton spin-lattice relaxation observed as the NMRD profile is due to the influence of the micelle. One mechanism is bulk(B) water exchanging with long-lived(II) water residing in the micellar head group:  $H_2O)_B \rightleftharpoons H_2O(l)$  where the II-waters are characterized by an order parameter S and a distribution of different residence times ( $\tau_{w,i}$ ) in the ns time regime. It is expected, from a NMRD/MD study of water residing in a protein, where no exchange between labile non-water protons is present, that the NMRD profile becomes rather stretched (rather broad distribution of residence times) with three or more Lorentzian needed in order to describe the NMRD profile[11]

The second mechanism is bulk water exchanging with intermediate II-waters which are in material exchange with labile non-water protons:  $H_2O)_B \rightleftharpoons H_2O(l) \rightleftharpoons H - X$ .

Now  $\tau_{w,i}$  is referring to the life-time of the non-water proton in  $H - X$  The correlation time characterize the modulation of the dipole-dipole interaction according to the EMOR model[4]. Thus for  $\tau_{w,i} \leq \tau_R$  in eq.(2)  $\tau_c$  is determined by  $\tau_{w,i}$  which is characterizing mean life time of labile protons. However, distribution of different life times of labile  $H - X$  is expected to be more narrow thus resulting in a less stretched NMRD profile. The fraction of protons for the Lorentzian describing the NMRD profile refer to the number of II-water in material exchange with labile protons( one-to-one relation).

The fraction of water molecules is given by  $S_i^2 f_{\beta i} = N_{w,i}[Micelle]/55.56$  ( $i = 1, 2$ ) per micelle This number is estimated for the two concentrations and a consistent number  $N_{w,i}$  is obtained as 110-120 water molecules at T= 10 C and about 90-100 at T=20 C. The effective correlation time  $\tau_c = \tau_R$  The number thus corresponds to slightly less than one water for each ganglioside lipid. For the second pool of water,  $N_{w,2}$ , with  $\tau_c(12ns) \leq \tau_R$ , is about 400 at 10 C and 300 at 20 C.

These estimated values of  $N_w$  represent a lower limit. The unknowing values of the order parameters may be estimated using NMR relaxation measurement of heavy water in ordered ganglioside lamellar phase samples or very large vesicles, where the reorientation is in the static regime. One may observe a quadrupole splitting of a powder spectra and the order parameter can be estimated from line shape analysis[12]-[15].

## 5 Conclusions

Using a relatively simple relaxation model our analysis of water proton  $T_1$ - NMRD profiles of the ganglioside micelles give the micelle reorientation correlation time  $\tau_R$ . However the two fractions of protons may be interpreted in terms of two cases: as exchangeable labile protons or "buried" waters. In the case of waters the fraction is given times the order parameter squared. The absolute value of water hydrating the micelle was not possible to without knowing the water order parameter. Temperature dependence of the fractions or  $N_w$

may indicate as observable difference between the two cases where the number of labile protons is expected to be constant in the temperature interval studied. However, data is not sufficient to give the relative importance of the two cases. However, if we consider the simplicity of the proton  $T_1$  NMRD dispersion profile, it indicates that it is due to labile protons with effective correlation time determined by the mean life time of the labile protons and fractions giving the number of intermediate waters exchanging with  $H - X$  protons (  $600(T=20C)$ - $800(T=10C)$ ).

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