Appendix A

^{1}H and ^{13}C assignments for dodecyl-maltoside

A general problem of ^{1}H NMR spectra of oligosaccharides is the large number of signals in a narrow region of the spectrum between 3.5 and 4.2 ppm (Homans, 1990; Hounsell, 1995; van Halbeek, 1996). Signals outside that region are due to the anomeric protons, which can therefore be used a starting point for the assignment of the proton resonances of the sugar. The H1 resonances of dodecyl-maltoside are easily assigned from 1D proton spectra by the difference of the chemical shift and the coupling constant of α- and β-anomeric protons. The overlap of the remaining sugar resonances makes the assignment from two-dimensional proton spectra difficult. The resolution is increased by recording heteronuclear ^{1}H-^{13}C spectra, which are in the present case sensitive enough at natural abundance due to the high dodecyl-maltoside concentrations in the protein detergent complexes. Complete assignment of the resolved resonances for dodecyl-maltoside are derived from a single HMQC-COSY spectrum (Clore, 1988; Norwood, 1990). This experiment is based on a conventional HMQC experiment with a 90° pulse added just before signal acquisition (see Fig. A.1). The resulting spectrum contains the "normal" HMQC cross-peaks as "diagonal" peaks plus additional small cross-peaks between scalar coupled protons. Starting from the unique resonance of the α and β anomeric protons of the two glucose subunits, all protons of both sugar subunits are easily assigned (see Fig. A.2).

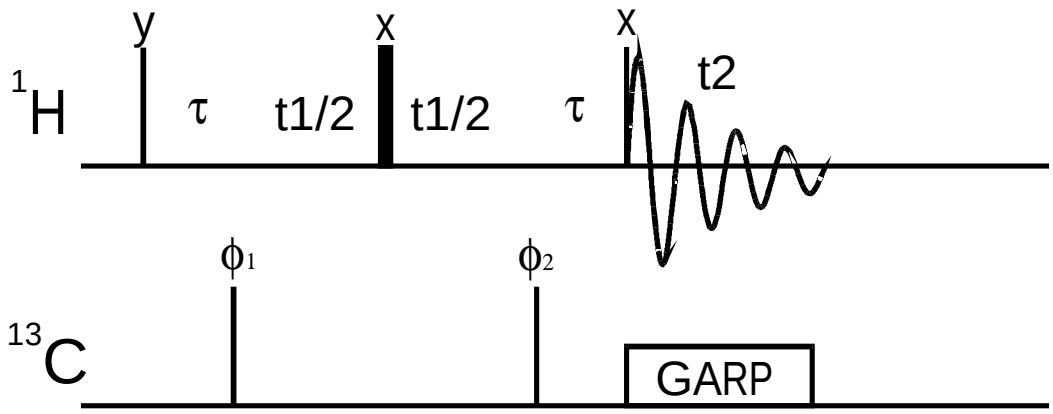

Fig. A.1 Pulse scheme for the 13C-1H HMQC-COSY experiment. The phase cycling is as follows: Φ1 = x, -x; Φ2 = x, x, -x, -x; receiver = x, -x, -x, x. The States-TPPI (Marion,

1989) method is used to obtain a pure phase absorption spectrum. The delay τ is set to 3.6 ms.

	Proton						
	H1	H2	H3	H4	H5	H6	
α-D-Glc (g_α)	5,372	3,642	3,745	3,493	3,767	3,844 3,794	
β-D-Glc (g_β)	4,399	3,395	3,785	3,715	3,510	3,854 3,914	
	H1	H2	H3	H4-H9	H10	H11	H12
Dodecyl (d)	A 3,615 B 3,906	A 1,371 B 1,682	1,376	1,320	1,305	1,325	0,906
	Carbon						
	C1	C2	C3	C4	C5	C6	
α-D-Glc (g_α) β-D-Glc (g_β)	103,1 105,4	74,6 75,7	75,9 79,0	72,0 80,6	75,6 77,5	63,3 63,5	
	C1	C2	C3	C4-C9	C10	C11	C12
Dodecyl (d)	73,0	32,2	28,6	32,6	34,6	25,2	16,5

Tab. A.1 Resonance assignments for dodecyl-maltoside in samples of solubilized bacteriorhodopsin. Referencing of the proton resonances is relative to internal TMS and the carbon resonances are referenced indirectly (Wishart, 1995).

The referencing for the chemical shift is relative to internal TMS. A sample of 0.02 % weight per volume dodecyl-maltoside in D_2O was prepared and the chemical shift of the α-anomeric proton of glucose 1 determined to be at 5.389 ppm relative to the internal TMS proton signal at 0 ppm. The small concentration dependence of the α-anomeric proton signal (Appendix B) leads to a chemical shift of 5.372 at high dodecyl-maltoside concentrations as used in measurements of solubilized bacteriorhodopsin. The reference measurement was done at 45° C, as all of the NOESY measurements for the structure determination of bacteriorhodopsin. A temperature dependence of the α-anomeric proton signal in the range from 16-45°C was not observed. The 13C resonances are indirectly referenced to TMS using the frequency ratios given by (Wishart, 1995).

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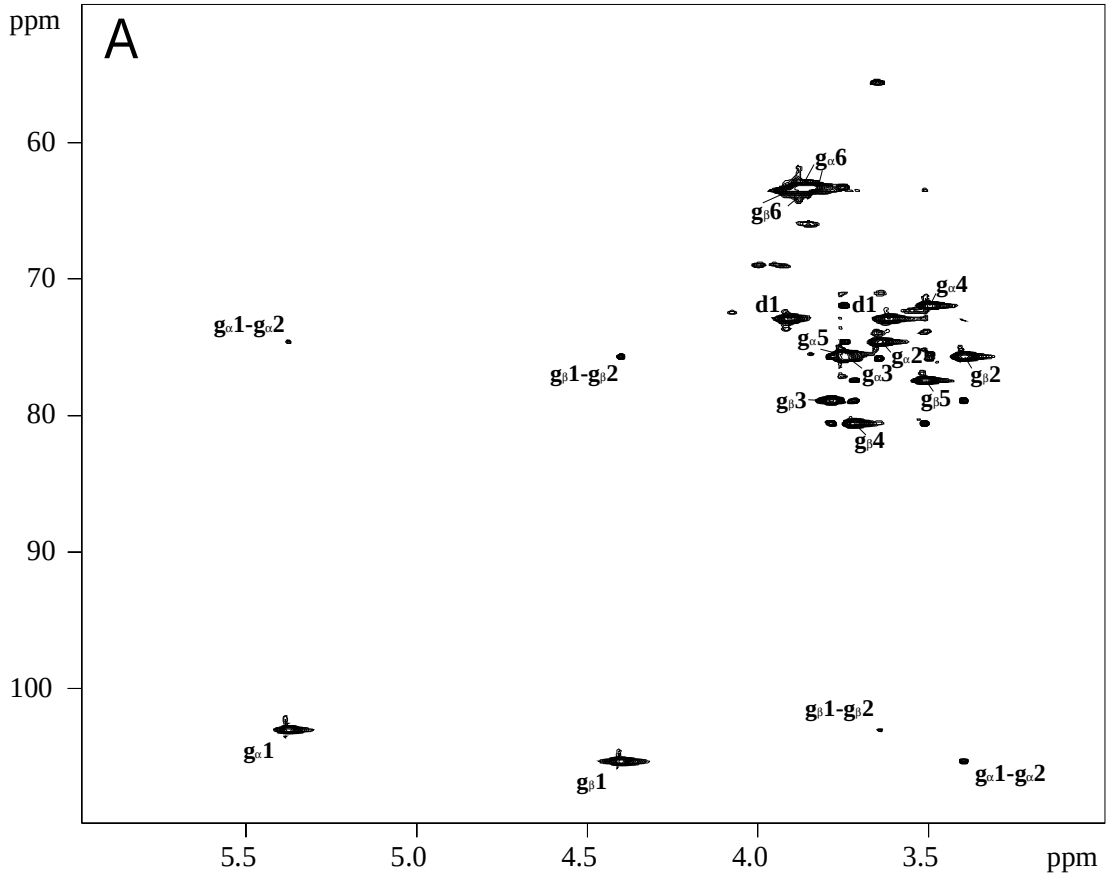
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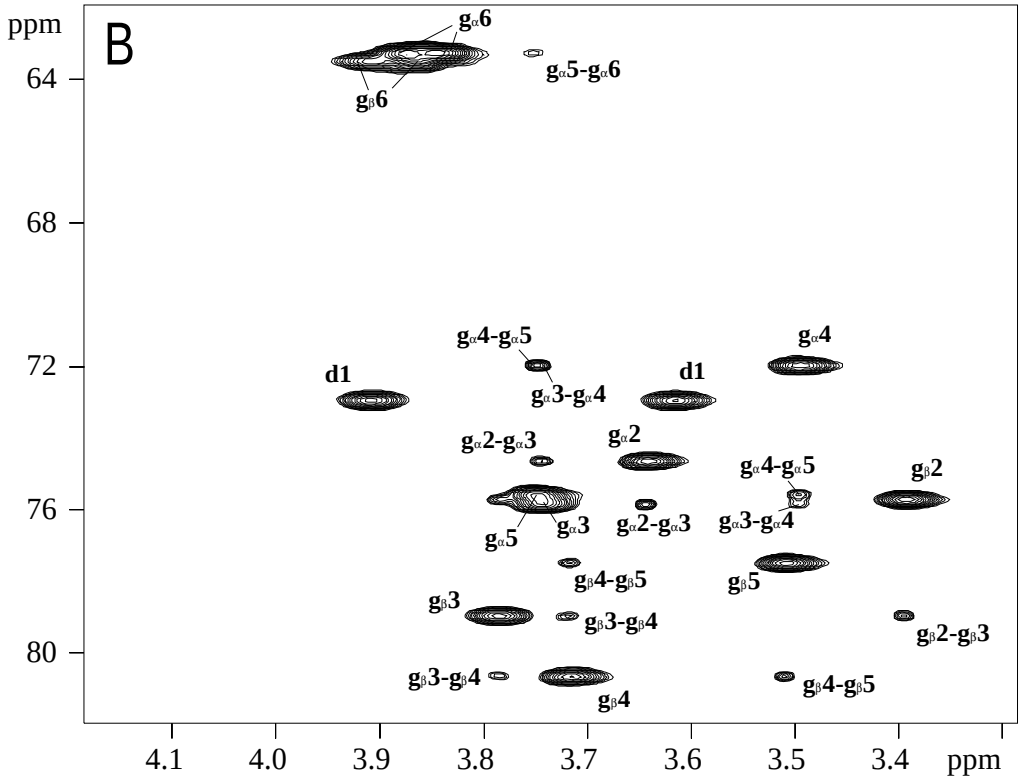
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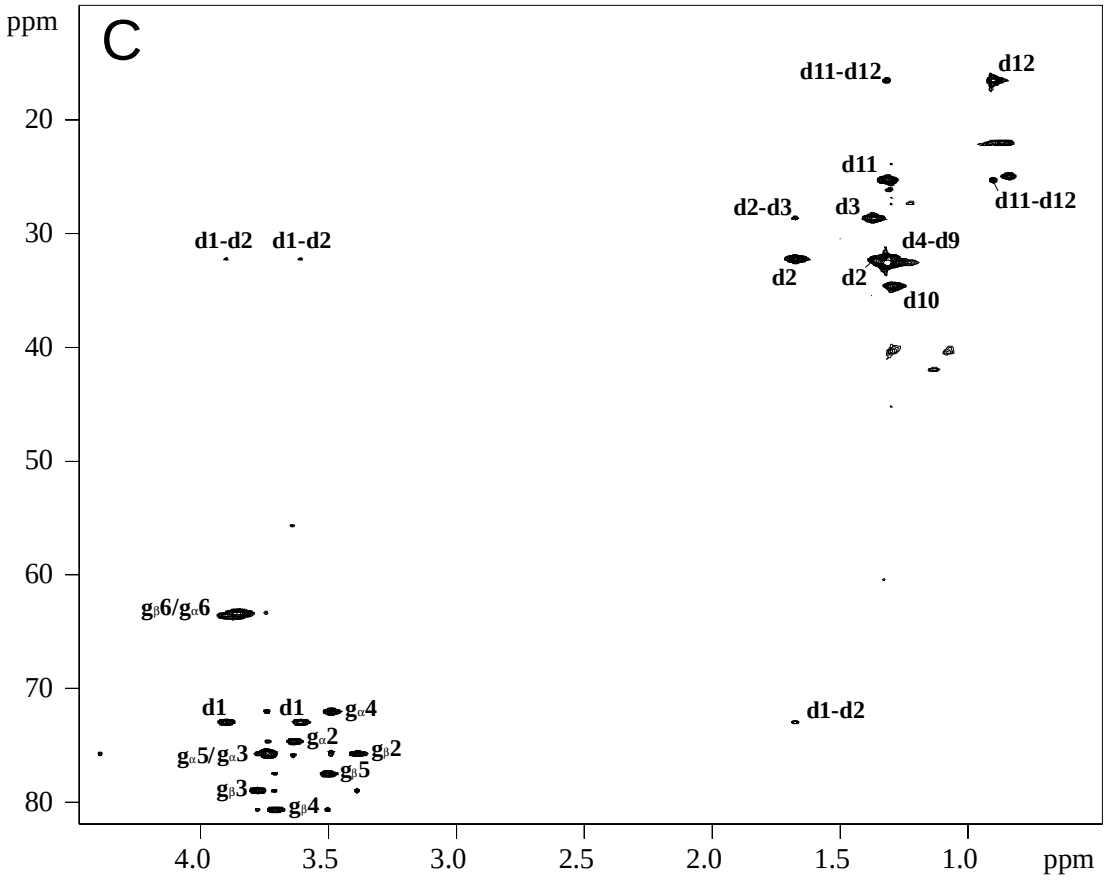

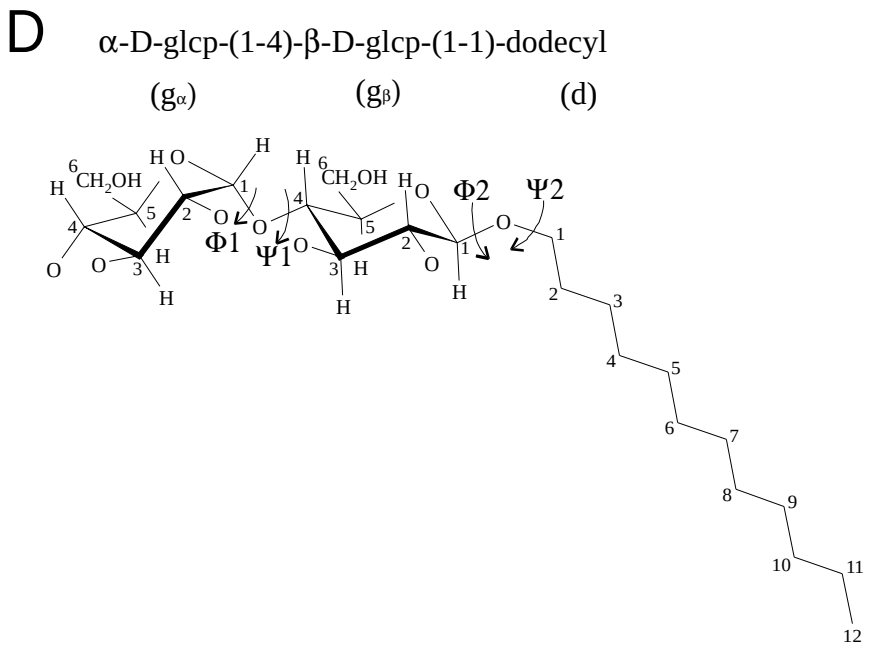

Fig. A.2 HMQC COSY spectrum of dodecyl-maltoside. A) Full spectral range containing signals. The COSY-cross-peaks between H1 and H2 and all HMQC peaks are annotated. B) Spectral region of the sugar peaks (H2-C2 to H6-C6). All COSY cross-peaks are annotated. C) Spectral region of the dodecyl resonances. D) Naming and numbering of the carbon atoms in dodecyl-maltoside.

Appendix B

Concentration dependence of detergent chemical shifts and diffusion coefficients

In the analysis of NMR studies of surfactant systems it is generally assumed that the monomer lifetime in a typical micelle is short on the timescale of the experiment (Söderman, 1994). Micelles are dynamic object which exchange monomers typically on a μs timescale (Zulauf, 1991). Therefore the chemical shift and diffusion coefficients observed follow the equation

$$A_{obs} = p_{free} A_{free} + p_{mic} A_{mic} \qquad \textbf{(B.1)}$$

where A is either the chemical shift δ or the diffusion coefficient D, p_{free} and p_{mic} are the probability to find a molecule in a micelle or as monomer ($p_{free}+p_{mic}=1$).

For practical purposes it is convenient to further analyze the data by treating the micelles as separate phase, which forms above a certain concentration of surfactant molecules, the so-called critical micelle concentration (CMC). It should be kept in mind however, that different methods might lead to slightly different CMCs (Tanford, 1980). Increasing the concentration above the CMC will lead to the formation of more micelles while leaving the monomer concentration approximately unchanged at the CMC value (Israelachvili , 1980). Above the CMC Eq. (B.1) can thus be rewritten as

$$A_{obs} = \frac{CMC}{c_{tot}} A_{free} + \frac{c_{tot} - CMC}{c_{tot}} A_{mic} = A_{mic} + (A_{free} - A_{mic}) \frac{CMC}{c_{tot}} \qquad \textbf{(B.2)}$$

where c_{tot} is the total concentration of surfactant.

The chemical shifts and diffusion coefficients have been monitored by diluting a 1% dodecyl-maltoside solution and a sample of solubilized bacteriorhodopsin with a low salt buffer (10 mM NaH_2PO_4 [in D_2O]). The 1% dodecyl-maltoside solution was diluted in seven steps to a final concentration of 0.002%. The protein-detergent solution was diluted in eight steps to 0.1% of the starting concentration.

The concentration of dodecyl-maltoside in the bacteriorhodopsin/dodecyl-maltoside sample used for the above measurement is unknown. The sample was prepared by detergent exchange of bacteriorhodopsin solubilized in Triton X-100 subsequent

concentration (Patzelt, 1997). The dodecyl-maltoside concentration was therefore estimated by comparison of the integrals of the dodecyl-maltoside signals in both samples, an approach, which can be used because the sample volume was kept constant for all measurements. The precision of this measurement was estimated to be on the order of 1% by comparing the signals within either the dodecyl-maltoside or the dodecyl-maltoside/bacteriorhodopsin dilution series, which scale corresponding to the known dilution factor. However, the accuracy of the approach might be influenced by the presence of dodecyl-maltoside molecules which do not contribute to the signal because they are immobilized in large detergent aggregates or on the sample wall and it can not be assumed a priori that the effect is the same in the pure dodecyl-maltoside solution and the dodecyl-maltoside/bacteriorhodopsin mixture. The similarity of the critical micelle concentrations found for both the free dodecyl-maltoside micelles and the bacteriorhodopsin/dodecyl-maltoside micelles might be seen as justification that the dodecyl-maltoside concentration is in the bacteriorhodopsin/dodecyl-maltoside system is deduced correctly.

Although, the chemical shift changes of the proton resonances in dodecyl-maltoside are smaller than 0.1 ppm for all resolved signals, these small changes can be monitored very accurately, and show a concentration dependence, that is very well described by Eq. (B.2) above the critical micelle concentration (Fig. B.1). The critical micelle concentration has been evaluated by nonlinear least-square fitting of the relative chemical shift change δ_{free}-δ_{mic} to Eq. (B.2) and are summarized in Tab. B.1.

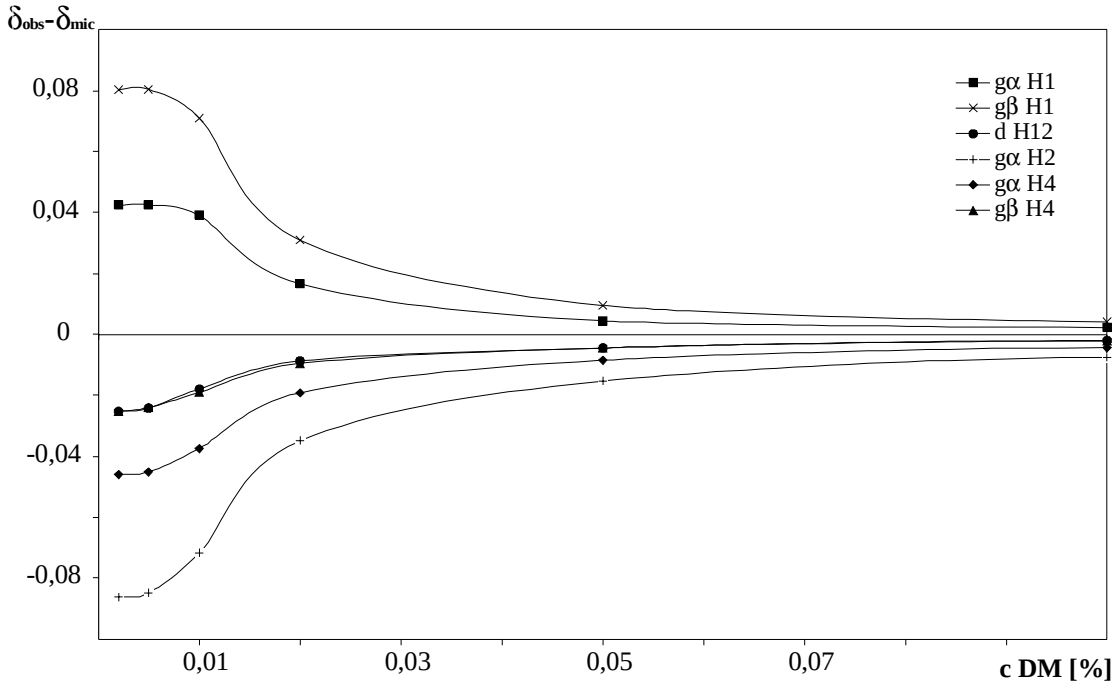

Fig. B.1 Concentration dependence of the proton chemical shifts in dependence of the total dodecyl-maltoside concentration. A line to guide the eye connects the measured points.

	g_α H1	g_β H1	d H2A	d H12	g_β H2	g_α H4	g_β H4	ave.
CMC_{DM}	170	166	164	141	164	161	151	160
[μm]	±8	±8	±8	±14	±4	±8	±14	±10
δ_{free}-δ_{mic}	0,043	0,080	-0.038	-0.021	-0.083	-0,049	-0,027	
$CMC_{DM/BR}$	123	114	151	141	157	151	164	143
[μm]	±12	±14	±12	±22	±6	±12	±20	±18
δ_{free}-δ_{mic}	0,043	0,080	-0,047	-0,025	-0,085	-0,046	-0,025	

Tab. B.1 Critical micelle concentrations determined from monitoring the concentration dependence of the relative chemical shift changes of resolved dodecyl-maltoside proton resonances. The values for the critical micelle conentration have been obtained by a least-squares fit to Eq. (B.2). The error estimates are derived from the χ^2-values of the fit.

The CMC for the bacteriorhodopsin/dodecyl-maltoside micelles is slightly lower compared to the free micelles. The difference is however relatively small, indicating that the affinity of the dodecyl-maltoside monomers for the free and the protein micelle are approximately the same.

The largest relative chemical shift changes occur for the protons, which are close to the hydrophobic-hydrophilic interface (g_β H1 and g_β H2), where the largest sterical interactions between monomers are expected.

Diffusion coefficient were determined by the use of a stimulated echo sequence with a longitudinal eddy current (LED) interval (Gibbs and Johnson Jr., 1991) added before the signal acquisition to allow eddy currents to decay and give unperturbed high resolution spectra. During the diffusion delay and the LED delay, spoil gradients along x and y were applied. Weak presaturation was applied to suppress the signal of the HDO resonance. The gradient amplitude was increased in 32 steps to a maximum value of 61 G/cm. To avoid inaccuracies for the gradient strength arising from finite rise and fall times of rectangular gradients, the leading and falling edge of the gradient were given the shape of a 5% truncated Gaussian. The time of the Gaussian part was set to be 100μs, corresponding to the typical rise and fall times for rectangular gradient pulses on the gradients system. The integral under a 5 % truncated Gaussian pulse is exactly half of the integral of a rectangular pulse of the same length. The duration of the gradient pulses was either 2,1ms or 3,1ms corresponding to a rectangular gradient of 2 ms or 3 ms duration. The time between the two diffusion gradients, which were applied with a separation of 50ms for measurements of the water diffusion and 200-300ms for measurements of the dodecyl-maltoside diffusion. The correction of the usual Stejskal/Tanner equation induced by the shapes of the raising and falling edge of the gradients is negligible under these conditions (Price and Kuchel, 1991). The self-diffusion coefficient, D, was obtained by fitting

$$I = I_0 \exp[-D(\gamma G\delta)^2\left(\Delta - \frac{\delta}{3}\right) \qquad \textbf{(B.3)}$$

to the integral over the signal intensities I in the Fourier transformed spectra (γ is the gyromagnetic ratio of the proton, δ is the duration of the gradient pulses and Δ is the separation of the starting edge of the gradients). The gradient strength G was calibrated by the measurement of the known diffusion coefficient of the residual protons in heavy water, which is equal to 1.872 10^9 m^2s^{-1} at 25° C (Mills, 1973). All measurements were done at 25°C. For each concentration, the diffusion of dodecyl-maltoside and HDO were determined in two different experiments. The HDO diffusion coefficient, which is for the low concentration data equal to the value of bulk water serves as an additional internal reference to check the accuracy of the measurement. The most crucial point in for the accuracy of the data is the use of a constant sample volume positioned always at the same position inside of the gradients

coil (Lapham, 1997). This is due to the nonlinearity of the gradient in the active sample volume. The nonlinearity has a negligible effect on the accuracy of a typical diffusion experiment in the case of a constant sample volume (Håkanson, 1997). However, we observed an increase of the apparent diffusion coefficient by 10%, if we reduce the sample volume by 25%, keeping all other conditions unchanged. This compares to a relative difference of 1% in the measured diffusion coefficient of HDO in different samples.

The experimentally determined diffusion coefficients for dodecyl-maltoside in dodecyl-maltoside and dodecyl-maltoside/bacteriorhodopsin micelles vs. the inverse of the concentration are shown in Fig. B.2, the values are listed in Tab. B.2. This plot allows a graphical determination of the critical micelle concentrations. The values obtained are the same within the accuracy of the data as those determined from the chemical shift changes.

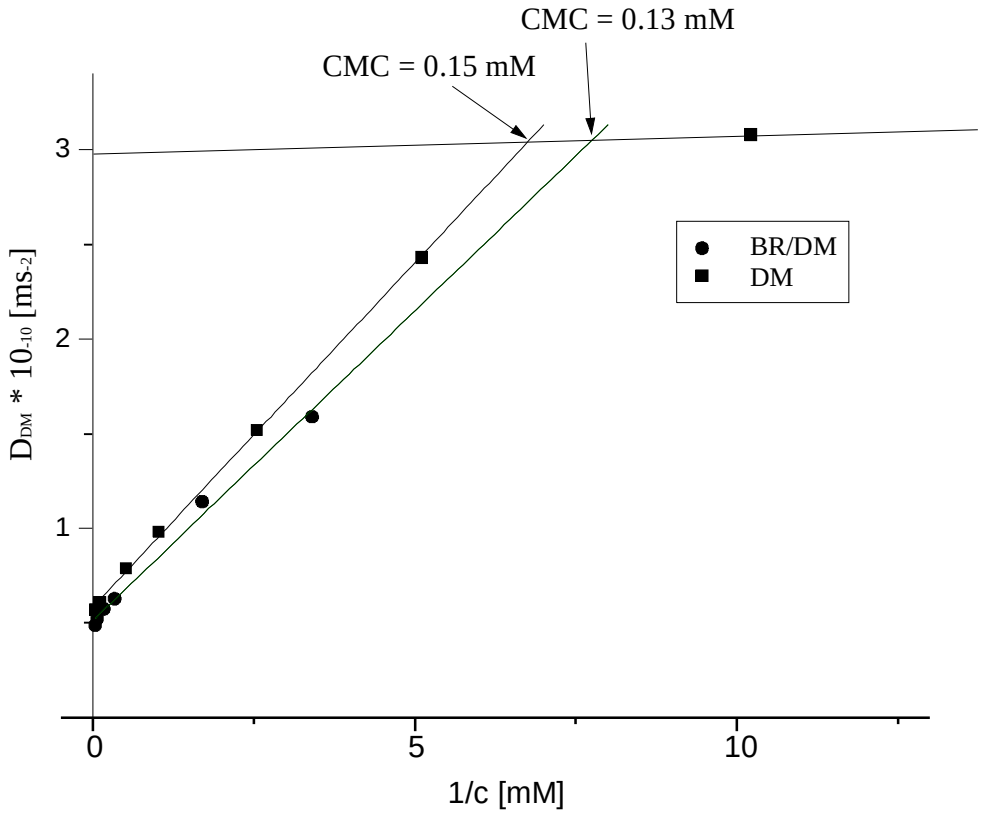

Fig. B.2 Diffusion coefficient of dodecyl-maltoside plotted versus the inverse of the total dodecyl-maltoside concentration. The two lines with the larger slope are linear least square fits to the data points at concentrations higher than the critical micelle concentrations (CMC). The line with the smaller slope connects the two values determined below the CMC, one of which is outside the range of the plot (see Tab. B.2)

free detergent micelles								
DM conc. [%]	1	0.5	0.1	0.05	0.02	0.01	0.005	0.002
D_{DM} [$10^{-11}m^2s^{-1}$]	5.71	6.06	7.88	9.81	15.2	24.3	30.8	32.6
protein-detergent mixed micelles								
DM conc. [%]	15	2.5	1.5	0.75	0.3	0.15	0.03	0.015
D_{DM} [$10^{-11}m^2s^{-1}$]	2.06	4.56	4.88	5.22	5.74	6.27	11.5	16.7

Tab. B.2 Concentration dependence of the diffusion coefficient of dodecyl-maltoside in dependence of concentration. The dodecyl-maltoside concentration in the mixed micelles has been estimated by integration of the NMR signals (see text).

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Appendix C

Size of the detergent and protein/detergent micelles

The self-diffusion coefficient of a macromolecule in solution is proportional to the temperature and the inverse of the frictional coefficient. The frictional coefficient in turn depends on the volume and hydration of the macromolecule. The exact functional form of the dependency, the Stokes-Einstein equation, is derived on a macroscopic model of Brownian motion for the macromolecule. The model breaks down for molecules with molecular weights smaller tan about 2000 Dalton, which diffuse faster than spheres of equal size. These molecules do not experience the solvent as a continuum and move most likely by a "jump and wait" mechanism for a significant fraction of their net movement, taking advantage of cavities in the liquid (Teller, 1979).

Macromolecular volumes in solution do not correspond generally to volumes of dry molecules. The proper thermodynamic property governing self-diffusion is the partial volume, which is the volume change of a solution upon addition of the molecule at constant temperature, pressure and composition of all other compounds of the solution. Partial volumes include all volume changes derived from hydration, solute binding and electrostriction. Partial specific volumes are calculated from the partial volume by multiplication with Avogadro's number and division by the molecular mass. For pure substances, the partial specific volume is equal to the inverse of the density.

Partial specific volumes can be measured directly by monitoring the density of solutions as a function of the weight concentration of the solute. Because large quantities of substance are needed to perform these measurements, approximate methods are often used. For proteins and carbohydrates, the volume can be estimated from amino acid or monosaccharide composition (Perkins, 1986). The estimate is based on average partial specific volumes of the monomers, which have been determined by different methods. In cases where the overall shape of the molecule is known, the volume can be computed directly. The frictional properties of spheres and ellipsoids of revolution have been calculated analytically (Perrin, 1934; Perrin, 1936).

More complex molecules can be treated by approximating the shape by a collection of spherical point sources of friction (de la Torre and Bloomfield, 1981; de la Torre, 1994; Teller, 1979). The main problem of these methods is the correct treatment of the hydration water. This can be approximated by adding one layer of water molecules to the surface of the macromolecule (Teller, 1979; Venable and Pastor, 1988).

The translational diffusion coefficient D at infinite dilution is related to the hydrodynamic radius R_h of the micelles by the Stokes-Einstein equation

$$D_0 = \frac{k_B T}{6\pi\eta f R_h} \qquad \textbf{(C.1)}$$

where k_B is the Boltzmann constant, T is the absolute Temperature and η the viscosity of the solvent. The dimensionless parameter f is equal to 1 for spherical particles and takes values >1 for other shapes. For ellipsoid particles, the Perrin equations relate f to the axial ratio of prolate or oblate ellipsoids (Cantor, 1980). The hydrated volume of a macromolecular solute is (Cantor, 1980)

$$V_h = \frac{4\pi}{3} R_h^3 = \frac{M}{N_A}\left(v_{mic} + \delta v_{wat}\right) \qquad \textbf{(C.2)}$$

where N_A is Avogadro's number, M is the molecular weight of the solute, δ is the hydration of the solute in g of water per g of solute and v_{mic} and v_{wat} are the partial specific volumes of the solute and the solvent. The partial specific volume of a pure substance is equal to the inverse density, thus $v_{wat}=1/\rho_{D2O}$.

Eq. (C.1) relates the diffusion coefficient to the particle size at infinite dilution. For dilute solutions, the diffusion coefficient D reduces linearly with the volume fraction Φ of the solute.

$$D = D_0(1 - k\Phi) \qquad \textbf{(C.3)}$$

The reason for this reduction is the obstruction effect discussed above for the water diffusion. The proportionality constant k is suggested to be equal to 1.7 for hard spheres with a screened coulomb interaction (Ohtsuki and Okano, 1982). In detergent system it is impossible to get the diffusion at infinite dilution directly, because the aggregate size might be a function of concentration. The monitored diffusion coefficient is influences by obstruction effects as well as aggregate size changes.

The measured diffusion coefficient of dodecyl-maltoside was corrected for the contribution of molecules in monomeric form using Eq. (B.2). The resulting values for the diffusion coefficient and the hydrodynamic radius of the micelles are summarized in Tab. C.1. The values which are close to the CMC (0.0081/0.0073 % w/V) are strongly influenced by small variations in the values of the CMC and the monomer diffusion coefficient. So the observed apparent decay of the hydrodynamic radius at values slightly above the CMC should not be overinterpreted. It should be kept in mind, that the concept of a two-phase model is an approximation and that the micelle size and size distribution might depend on the concentration as well. For higher concentrations, the interaction between the micelles will influence the measured diffusion coefficient (Eq. C.3). In this case the measured diffusion coefficient is not equal to the value at infinite dilution and the calculated radius does not correspond any more to the Stokes radius (Eq. C.1). From Eq. (C.3) we expect a decrease of ~ 1.4 % for a solution containing 1% (w/V) dodecyl-maltoside.

free detergent micelles								
DM conc. [%]	1	0.5	0.1	0.05	0.02	0.01		
D_{mic} [$10^{-11}m^2s^{-1}$]	5.52	5.68	6.02	6.11	5.85	5.03		
R_h [nm]	3.59	3.49	3.29	3.24	3.39	3.94		
protein-detergent mixed micelles								
DM conc. [%]	15	2.5	1.5	0.75	0.3	0.15	0.03	0.015
D_{mic} [$10^{-11}m^2s^{-1}$]	2.12	4.62	4.88	5.07	5.31	5.31	5.95	5.34
R_h [nm]	9.39	4.30	4.07	3.92	3.74	3.74	3.34	3.72

Tab. C.1 Concentration dependence of the diffusion coefficient of dodecyl-maltoside in dependence of concentration. The dodecyl-maltoside concentration in the mixed micelles has been estimated by integration of the NMR signals (see text). The diffusion coefficient of the micelles has been calculated using Eq. (B.2)from the observed diffusion coefficient listed in Tab. B.2 with the average CMC of Tab. B.1 and a monomer diffusion constant of 32.6 10^{-11} m^2 s^{-1}.

The partial specific volume of dodecyl-maltoside and the molecular weight and shape of the micelles at a concentration of 1% (w/V) have been determined by Timmins et al. (Timmins, 1988). From the measured diffusion coefficient we can therefore directly determine the hydration of the micelles (Eq. C.2). The neutron diffraction data have been performed at a temperature of 16° C. To exclude a temperature dependence of the size of the micelles, we monitored the diffusion coefficient in dependence of the temperature between 16° and 45° C. The term $T/\eta D$ remains constant up to a temperature of 30°C. Therefore, the data of Timmins can therefore be compared

directly with our measurements at 25°C. Above 30°C, an accurate determination of the diffusion coefficient was not possible, because convection is induced in the sample by the inhomogeneous sample heating in the probe-head.

Timmins et al. determined a molecular weight of dodecyl-maltoside micelles at 1% (w/V) concentration of 66 kDa, and a partial specific volume of 0.837 cm^3g^{-1}. The shape of the micelle is a prolate ellipsoid with half axis of 26.8 x 28.5 x 28.5 Å. The shape factor f (1,00046) for this geometry is negligible. The viscosity and the density of D_2O at 25° are 1.097 cP and 1.104 gcm^{-3} respectively (Kell, 1972). The measured diffusion coefficient for the 1% dodecyl-maltoside solution is 5,5 10^{-11} m^2s^{-1}. From Eq. (C.1) and (C.2), the hydration δ is determined to be 0.88 g water per g micelle. This corresponds to 2904 water molecules per micelle. The average number of water molecules per monomer is 22.

In the simulation of methyl-maltoside in water (Brady and Schmidt, 1993) 35-38 molecules of water hydrogen bonded to the sugar. The above result indicates that this number of bound water molecules per sugar reduces by approximately 40% upon micellation. The level of hydration of biomolecules is approximately proportional to the solvent accessible surface (Teller, 1979; Venable and Pastor, 1988). Therefore we might conclude that the solvent accessible surface of the maltoside head-group in dodecyl-maltoside micelles is about 60% compared to free maltose.

The self-diffusion coefficient of the water in the sample depends on the concentration of the solute. Proteins or micelles have much larger volumes than the water molecules and appear to the water as impermeable stationary particles. The influence on the bulk water diffusion is caused by two different mechanisms: (1) A purely geometrical argumentation leads to the so-called obstruction effect. The water diffusion is hindered by the presence of inaccessible volume, which is occupied by the macromolecule. (2) The direct hydration of the macromolecule will lead to a slowing down of the bound water. The exchange rate of bound water is much faster than the NMR time scale and the hydration water will lead to a decrease in the diffusion coefficient of the bulk water.

A theoretical description for the dependence of the water self-diffusion coefficient on protein concentration was derived by Wang (Wang, 1954) and a correction to the

model was added by Clark et al. (Clark, 1982). It is important to note, that monitoring the water self-diffusion coefficient can give some complementary information on the shape of a protein, which is not obtainable form the protein diffusion coefficient (Callaghan and Lelievre, 1985).

However, in the concentration region monitored in our experiments doesn't change significantly. For the pure dodecyl-maltoside sample, which was monitored up to a concentration of 1% w/v dodecyl-maltoside, the water diffusion is the same as for bulk water. For the highest protein concentration it reduces to 80% of the value of bulk water. This change is too small to allow a quantitative analysis in terms of form factors.

The diffusion coefficient dodecyl-maltoside in the bacteriorhodopsin/dodecyl-maltoside system at 1% dodecyl-maltoside concentration is equal to 5.0 10^{-11} m^2s^{-1}. If we assume that only one type of aggregate is present in the solution, this leads to an increase of the hydrodynamic radius and the molecular weight by approximately 10%, where any changes in the partial specific volume and the hydration are neglected. Any protein free detergent micelles will increase the estimated size of the protein detergent complex. The analysis is further complicated by the presence of small amounts of remaining Triton X-100 and lipids of the native membrane. Nevertheless, the estimates for the hydrodynamic radius for the bacteriorhodopsin/dodecyl-maltoside micelle in Tab. C.1 compare very well to the apparent Stokes radius of 3.7±0.3 nm for bacteriorhodopsin solubilized in dodecyl-maltoside measured by size-exclusion HPLC (Seigneuret, 1991).

A direct accurate measurement of the protein diffusion in $^1H/^2H$ labelled samples was impossible due to poor signal-to-noise ratio. In a ^{15}N or ^{13}C enriched protein-sample such measurements would be possible with the methods described in chapter 2.

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Appendix D

Estimated proton relaxation times

For liquid state NMR experiments, the rotational reorientation of the macromolecule is of special importance, since it leads to fluctuations of the direct dipolar interaction of the magnetic spins, which is the main source of spin relaxation. Having precise information of the rotational motion of a molecule allows the derivation of precise structural data from cross-relaxation rates (NOEs). Rotational correlation times for proteins are most precisely determined from heteronuclear relaxation studies of amide nitrogens (Kay, 1998; Kay, 1989; Palmer, 1993). The amide relaxation is governed by the interaction with the directly attached amide proton. The ratio of longitudinal relaxation time T_1 to transverse relaxation time T_2 depends in the limit of slow reorientation and the absence of fast internal motion only on the overall tumbling of the molecule and the bond length. For small globular proteins, a model of isotropic rotational motion is often appropriate. In a more general case, a rigid molecule will tumble with different correlation times around three perpendicular main axes. This leads to a dependence of the T_1/T_2 ratio on the relative orientation of the line connecting the ^{15}N and ^{1}H nuclei to the molecular axis, which are defined by the rotational diffusion tensor. In favorable cases, the T_1/T_2 ratio can provide direct structural constraints (Tjandra, 1997). If the overall rotational motion of the molecule is known, the relaxation data provide information of internal motions, which are faster than the overall tumbling of the molecule.

In the absence of ^{15}N relaxation data, the overall motion of a protein might be estimated from proton relaxation data. However, the relaxation properties of the protons depend on all proton proton distances, which makes a detailed analysis very difficult because of a mixture of dynamic and structural information in the data. For the system bacteriorhodopsin in dodecyl-maltoside micelles, the situation is complicated by the fact, that the resonances of the detergent dominate the proton NMR spectrum. Detergent peak intensities are typically 100 times larger than those of protein signals, which makes it impossible to observe any protein resonance, that is close to a detergent peak. Even for peaks far away from the spectral region of the detergent signals, large baseline artifacts complicate a precise determination of protein

peak volumes. Proton relaxation data for the protein could therefore not be determined accurately to estimate the overall motion of the protein.

In the last paragraph we get however an estimate of the size of the protein detergent micelle, which allows to calculate the approximate rotational diffusion. The rotational correlation time of a hydrodynamic particle according to Stokes is given by

$$\tau_C = \frac{V\eta}{k_B T} = \frac{4\pi R_h{}^3 \eta}{3k_B T} \qquad \textbf{(D.1)}$$

Most NOESY measurements were done at 45°C, where the viscosity of D_2O is 0.79 cP. The rotational correlation time for dodecyl-maltoside micelles at this temperature can be estimated to be equal to 30ns (R_h=3.5 nm). The lower limit of the correlation time for the protein/detergent micelle is 38ns (R_h=3.7nm), but a value of 65 ns (R_h=4.4nm) is consistent with the experimental data as well. A radius of 4.4nm for the protein detergent micelle is obtained, if we assume that a detergent molecule has only 50% probability to be part of a protein/detergent micelle and a fast exchange of monomers between protein/detergent and pure detergent micelles. The overall diffusion coefficient is than determined by the average of the diffusion coefficients of the protein/detergent and the detergent micelles ($D=p_{DM}*D_{DM}+p_{BR/DM}*D_{BR/DM}$). For p=0.5, D_{DM}=5.5 10^{-11} m^2s^{-1} and D=5 10^{-11} m^2s^{-1}, the diffusion coefficient of the protein/detergent micelle is 4.5 10^{-11} m^2s^{-1} and R_h=4.4nm. This analysis neglects interactions between the free detergent micelle and the bacteriorhodopsin/detergent micelle, since it assumes the same diffusion coefficient for the free micelle as in the pure detergent system.

The main contribution to the relaxation in the protein is the dipolar interaction of the protons. The relaxation of one proton can be approximated by summing the contributions of the pairwise dipolar interactions with all other protons in the sample. This leads to the following equation for the transverse relaxation:

$$R_2^i = \frac{1}{T_2^i} = \left(\frac{\mu_0}{4\pi}\right)^2 \gamma^4 \eta^2 \frac{1}{20} \tau_c \sum_{j \neq i} \frac{1}{r_{ij}^6} \left[4 + \frac{1}{1+(\omega_i - \omega_j)^2 \tau_c{}^2} + \frac{3}{1+\omega_i{}^2 \tau_c{}^2} + \frac{6}{1+\omega_j{}^2 \tau_c{}^2} + \frac{6}{1+(\omega_i + \omega_j)^2 \tau_c{}^2}\right] \qquad \textbf{(D.2)}$$

For simplicity, it is assumed that the molecule is tumbling isotropically, all internal motions and cross-correlation effects can be neglected. (For a discussion of methyl group relaxation see (Tropp, 1980), for cross-correlated relaxation see (Goldman, 1988; Werbelow and Grant, 1977). In the spin diffusion limit $\omega\tau_c >> 1$, the equation can be approximated by

$$R_2^i = \frac{1}{T_2^i} = \left(\frac{\mu_0}{4\pi}\right)^2 \gamma^4 \eta^2 \frac{1}{4} \tau_c \sum_{j \neq i} \frac{1}{r_{ij}^6} \approx 112 \tau' \sum_{j \neq i} \frac{1}{r_{ij}'^6} \quad , \; \tau' = \tau * 10^9, r' = r * 10^{10} \qquad \textbf{(D.3)}$$

where the τ' is the correlation time in ns and r'_{ij} is the distance between the relaxing spin i and neighbor spins j in Å.

The expected linewidths for protons in a Tryptophan or Lysine sidechain for different rotational correlation times are listed in Tab. D.1. The exact geometry corresponds to Trp86 and Lys216 of a bacteriorhodopsin structure file.

	τ_{rot}=38ns		τ_{rot}=65ns	
	T_2 [ms]	**LW [Hz]**	**T_2 [ms]**	**LW [Hz]**
Tryptophan				
Hα	8.21	39	4.80	66
Hβ1	2.66	120	1.56	205
Hβ2	2.77	115	1.62	196
Hδ1	12.56	25	7.35	43
Hε3	11.48	27	6.89	46
Hζ3	11.08	29	6.48	49
Hη2	14.03	23	8.20	39
Hζ2	27.56	12	16.11	20
Lysine				
Hα	9.91	32	5.79	55
Hβ1	2.13	150	1.24	256
Hβ2	2.20	144	1.29	247
Hγ1	2.13	149	1.25	256
Hγ2	2.02	158	1.18	270
Hδ1	1.67	191	0.97	327
Hδ2	2.33	137	1.36	234
Hε1	2.20	145	1.29	248
Hε2	2.09	152	1.22	260
Methylene	3.11	102	1.82	175

Tab. D.1 Calculation of proton relaxation times assuming dipolar relaxation and isotropic rotational tumbling (Eq. (D.2)). The geometry of the protons is taken from Trp 86 and Lys 216 of a bacteriorhodopsin coordinate file.

The most important point is the large linewidth of > 100 Hz of methylene protons. Signals are only observable, if the maximal signal intensity exceeds the signal-to-

noise ratio. The maximal signal intensity in turn is directly proportional to the transverse relaxation rate of the resonance. From the estimated rates it is conceivable, that signals of methylene protons are the first to disappear under the noise floor, especially in cases where several neighboring methylene groups are influencing each other.

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