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THERMODYNAMIC PROPERTIES OF PURPLE MEMBRANE

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ABSTRACT We measured the density, expansivity, specific heat at constant pressure, and sound velocity of suspensions of purple membrane from *Halobacterium halobium* and their constituent buffers. From these quantities we calculated the apparent values for the density, expansivity, adiabatic compressibility, isothermal compressibility, specific heat at constant pressure, and specific heat at constant volume for the purple membrane. These results are discussed with respect to previously reported measurements on globular proteins and lipids. Our data suggest a simple additive model in which the protein and lipid molecules expand and compress independently of each other. However, this simple model seems to fail to describe the specific heat data. Our compressibility data suggest that bacteriorhodopsin in native purple membrane binds less water than many globular proteins in neutral aqueous solution, a finding consistent with the lipid surround of bacteriorhodopsin in purple membrane.

INTRODUCTION

Purple membrane functions as a light-driven proton pump in *Halobacterium halobium* (1). The membrane's sole protein constituent is bacteriorhodopsin, and the pump involves the protein in a cycle that is initiated by light. This paper is a report of thermodynamic properties of the native membrane. The data reported in this paper represent the first comprehensive thermodynamic description of any native biological membrane. In addition, our data may be useful in interpreting purple membrane experiments in which temperature and/or pressure are varied.

METHODS AND MATERIALS

Purple membrane samples were obtained from several laboratories (see acknowledgements). A given sample was suspended in phosphate buffer (50 mM) at pH = 6.9, centrifuged at 100,000g for 30 min, and resuspended in fresh buffer by immersion sonication for 30 s while the sample was kept under nitrogen gas. We followed the purification procedure of Braiman and Mathies (2) by repeating the sonication and the centrifugation procedure at least two and usually three times. The final resuspension of the sample in the buffer was done so that a few milliliters of a 1–2% suspension of purified purple membrane was produced. This suspension was degassed under partial vacuum just before use.

For a purple membrane suspension made up only of purple membrane fragments and buffer, the suspension density, ρ , can be expressed in terms of the apparent membrane density, ρ_{mem} , the buffer density, ρ_0 , and the concentration, V_{mem} :

$$\rho = \rho_{\text{mem}} V_{\text{mem}} + (1 - V_{\text{mem}}) \rho_0, \quad (1)$$

where V_{mem} is the volume fraction of membrane in suspension.

Thermal Expansion Measurement

Differentiation of Eq. 1 with respect to temperature (T) yields an equation relating the expansivities of the suspension (α), buffer (α_0), and membrane (α_{mem}):

$$\alpha - \alpha_0 = V_{\text{mem}}(\alpha_{\text{mem}} - \alpha_0), \quad (2)$$

where $\alpha = (-\partial \ln \rho / \partial T)$.

Measurement of the expansivity of the liquids was done in a vibrating quartz pycnometer built by two of us (C. J. Hardy and E. Gratton). We used distilled water and 1% NaCl for calibration. At any temperature between 5° and 30°C, we found that the linear relation, $\Delta\rho = K\Delta P$, between density (ρ) and oscillation period (P) gave the same result as when we used the strictly proper Hooke's Law relation (3), $\Delta(P^2) = K'\Delta\rho$. The constant, $K = (4.12 \pm 0.04) \times 10^{-6}$ g/ml · ns, did not vary over the stated temperature range. All concentrations were measured by a dry weight method that is described later.

To measure the expansivities of the liquids, we used the following technique. After the temperature of the filled pycnometer is allowed to stabilize for 4 h, the temperature, measured as the resistance across a calibrated platinum thermometer, and the period of oscillation for a single liquid, such as water, are first measured at a low temperature (~10°C). The temperature is then reset on the temperature controller to a higher temperature (~20°C) and the system is allowed to reequilibrate for 4 h. The resistance and period are now measured at the higher temperature. These pairs of measurements are repeated several times to signal average the measurement. From these data the slope of the period vs. resistance line, along with its standard error, is calculated.¹ Similarly, the slopes for

¹Here we provide justification for the use of straight line fits to period vs. temperature data sets: A priori the plots do not need to be linear. However, in developing a differential method for measuring the mean thermal expansivity of liquids, we noticed that plots of temperature (measured in ohms with a platinum thermometer) vs. period (of oscilla-

the buffer solution and for the purple membrane suspension are determined. $\partial\rho_0/\partial T$ is then determined by comparing the buffer slope with the water slope, as follows:

$$K \left[\frac{\Delta P (\text{buffer})}{\Delta T} - \frac{\Delta P (\text{water})}{\Delta T} \right] = \frac{\Delta\rho}{\Delta T} (\text{buffer}) - \frac{\Delta\rho}{\Delta T} (\text{water}).$$

$\Delta\rho$ (water) can be obtained from standard tables, so $\Delta\rho$ (buffer) is determined. Similarly, the suspension's $\partial\rho/\partial T$ can be determined by comparing the suspension slope with the buffer slope or water slope.

The volume fraction of purple membrane in the suspension is determined by the weight fraction and pycnometrically measured values for the apparent density of the purple membrane (ρ_{mem}) and the suspension density (ρ_{sus}) as follows:

$$V_{\text{mem}} = \frac{\rho_{\text{sus}}}{\rho_{\text{mem}}} \left[\frac{C_1 - C_2}{1 - C_2} \right], \quad (3)$$

where

$$C_1 = \frac{\text{grams (membrane + buffer salts)}}{\text{grams suspension}}$$

$$C_2 = \frac{\text{grams (buffer salts)}}{\text{grams (buffer solution)}}.$$

Thermostated water flow through a fanned radiator gave a pycnometer temperature stability of $>0.02^\circ\text{K}$. Typical temperature excursions during any particular period measurement were $<0.01^\circ\text{K}$.

To check our differential expansivity measurement, we also measured the apparent specific volume of purple membrane at 15° and 25°C . The measurements were performed with a Mettler-Paar densimeter (Mettler Instrument Corp., Hightstown, NJ) in which a reference pycnometer is always filled with double-distilled water. Temperature fluctuations, which can be a large source of error in this type of measurement, are effectively filtered out of the data coming from the sample pycnometer, resulting in greater accuracy than be obtained from the sample pycnometer alone. The calibration and sample fluids were always introduced into the densimeter, and their periods measured, in the following order: N_2 gas, double-distilled water, buffer, purple membrane suspension, buffer, double-distilled water, and N_2 gas. N_2 gas and double-distilled water were the calibration fluids that were used to determine the instrumental constants that relate the period of oscillation of the sample pycnometer to the density of the sample (3). After obtaining the density data for the buffer and the purple membrane suspension, the weight fraction of the sample that is attributed to purple membrane was determined by a dry weight method: two weighed samples (each of buffer and suspension) were dried at 40°C , slowly under oil pump vacuum, until the dried samples had constant weight.

The measurements of the apparent specific volumes were made at 15° and 25°C at two concentrations (~ 0.025 and 0.015 , expressed as weight

tion of the pycnometer) invariably gave straight lines with correlation coefficients very close to -1.000 . As a measure of the deviation of those plots from perfect linearity, we chose to use a number derived from the correlation coefficient, r , but more sensitive than r to deviations from perfect linearity: $\theta = (1 - |r|)^{1/2}$. θ describes the relative error of the slope derived from standard linear regression. Typical values for θ obtained for temperature scans of purple membrane suspensions, buffer solutions, salt solutions, and distilled water are all $\sim 4 \times 10^{-3}$. These values for θ , each of which is derived from a single curve, are of the same order of magnitude as the relative standard errors of the slopes that were calculated from several values of the slope and that we reported in this paper (Table I). This suggests that most, if not all, of θ results from random deviations from a linear relation and not from systematic artifact that would result from attempting to fit nonlinear data to a straight line.

fractions). The latter sample was prepared by diluting a portion of the former sample with buffer.²

We also checked if we could detect an effect of the two-dimensional lattice structure of native purple membrane on the apparent expansivity. To do this, we bleached a 50 mg sample of purple membrane by suspending it in 1 l of an aqueous solution that was composed of 0.1 M NaCl and 1 M NH_2OH . 15 mM Tris buffer was added until the suspension had a pH of 9.5. The suspension was then irradiated with light from a projector and incubated at 40°C for 4 h. The suspension was then centrifuged, and the pellet was washed four times in the 50 mM phosphate buffer. Previous work has shown that bleaching purple membrane disrupts the two-dimensional periodicity of the membrane (4).

Sound Velocity Measurement

Sound velocity measurements were performed on a purple membrane suspension to determine the apparent adiabatic compressibility of the membrane. Our acoustic resonator apparatus is described in detail elsewhere (5). The suspension adiabatic compressibility, β^S , can be found from the suspension density ρ and sound velocity c in the suspension through the relationship $\beta^S = 1/\rho c^2$. In this equation S refers to the entropy of the system, which remains constant during compression. Likewise the buffer compressibility β_0^S is obtained from $\beta_0^S = 1/\rho_0 c_0^2$. The apparent adiabatic compressibility, β_{mem}^S , of the purple membrane relative to the solvent is then determined from the relation, $\beta^S/\beta_0^S = 1 - V_{\text{mem}}(1 - \beta_{\text{mem}}^S/\beta_0^S)$, which is analogous to Eq. 2. For protein solutions, β^S/β_0^S is linear with protein volume fraction V_{pro} to at least 0.02. Our measurements were performed at a V_{mem} value of 0.007, which is well within the linear region for proteins (6).

Specific Heat Measurement

The apparent specific heat of the purple membrane was determined in a DASM-IM scanning microcalorimeter (Mashpriborintorg, Moscow, Union of Soviet Socialist Republics) (7). In one type of scan both cells (the volume was ~ 1 cc) of the calorimeter were filled with buffer, and in the other type one cell was filled with membrane suspension and the other with buffer. A scan rate of $1^\circ\text{K}/\text{min}$ was used. Frequent measurements were made during each scan of the time, the temperature, and the voltages across each cell heater of known resistance. From these data the heat capacities of the cells and the contents were evaluated. The volumes of the cells and the heat capacities of the empty cells were known from earlier calibration experiments, the volumes having been obtained from scans with both cells filled with water. These data, together with the densities of the buffer and the suspension and the weight fraction of the membrane in the suspension, determined as described above, permitted evaluation of the apparent specific heat at constant pressure of the membrane, C_p^{mem} , according to the equation

$$C_p^{\text{sus}} = (1 - X_{\text{mem}}) C_p^{\text{buf}} + X_{\text{mem}} C_p^{\text{mem}},$$

where C_p^{sus} is the specific heat of the suspension, C_p^{buf} is that of the buffer, and X_{mem} is the weight fraction of the membrane in the suspension.

RESULTS

Thermal Expansivity

Table I gives the expansivity data from both the slope and the specific volume methods. Note that the values for the apparent specific volume near 15°C , as determined from suspensions with different concentrations of purple mem-

²The reader is referred to reference 3 for further details concerning the calculations and error analysis for apparent specific volumes from liquid density data and dry weight data.

TABLE I
EXPANSIVITY DATA

A. Slope data				
– Slope	Concentrations			
ns/Ω	(as in Eq. 3)			
water: 826.8 ± 1.2	$V_{\text{mem}} = 0.0216$			
buffer: 837.2 ± 0.2	$C_2 = (7.988 \pm 0.001) \times 10^{-3}$			
suspension: 843.5 ± 1.4	$C_1 = (3.3280 \pm 0.003) \times 10^{-2}$			
$\alpha_{\text{mem}} = (6.05 \pm 0.82) \times 10^{-4}/^{\circ}\text{K}$				
B. Apparent specific volume data				
Weight fraction	T	ρ	ρ_0	Apparent specific volume
	$^{\circ}\text{C}$	g/ml	g/ml	ml/g
0.02511 (native)	15.43	1.00904	1.00496	0.8350
	25.29	1.00679	1.00280	0.8402
0.01458 (native)	5.10	1.00882	1.00603	0.8055
	15.23	1.00769	1.00496	0.8101
0.01582 (bleached)	15.43	1.00766	1.00497	0.8268
	25.29	1.00545	1.00281	0.8315
$\alpha_{\text{mem}} = 6.04 \times 10^{-4}/^{\circ}\text{K}$				
$\alpha_{\text{bleached mem}} = 5.75 \times 10^{-4}/^{\circ}\text{K}$				

brane, differ by >1%. This error is mostly caused by the error in the weight fraction determination, which is ~1%. However, this concentration error contributes little to the error in the expansivity because the exact same weight fraction is used in the calculation of the apparent specific volumes at the higher and lower temperatures. The error in the expansivity is almost entirely a result of systematic and random errors in the period data from the pycnometer. The apparent expansivity of purple membrane from the most concentrated sample is $6.29 \times 10^{-4}/^{\circ}\text{K}$. The value we obtain from the diluted sample is $5.62 \times 10^{-4}/^{\circ}\text{K}$, with a weighted average of $6.04 \times 10^{-4}/^{\circ}\text{K}$, which agrees with our differential (slope) measurement value of $(6.05 \pm 0.82) \times 10^{-4}/^{\circ}\text{K}$. We obtained an expansivity of $5.75 \times 10^{-4}/^{\circ}\text{K}$, for bleached membrane. For comparison, Table II gives the values for the expansivity of some elements, proteins, and compounds.

Adiabatic Compressibility

A value for $\beta_{\text{mem}}^S/\beta_{\text{water}}^S$ of 0.506 ± 0.034 was obtained for the purple membrane at 25°C. Within the uncertainty, the value was constant over the frequency range 600 KHz–10 MHz. Using a value for β_{water}^S of 4.55×10^{-5} atm (8), for the membrane we obtain $\beta_{\text{mem}}^S = (2.30 \pm 0.15) \times 10^{-5}/\text{atm}$. For comparison, Table III gives β^S values of other materials.

Apparent Specific Heat at Constant Pressure

The values obtained for the apparent specific heat of the purple membrane are 0.484, 0.504, and 0.500 cal/ $^{\circ}\text{K} \cdot \text{g}$

TABLE II
THERMAL EXPANSIVITY OF SELECTED MATERIALS

Material	$-\frac{\partial \ln \rho}{\partial T}$	Reference
	$^{\circ}\text{K}^{-1}$	
Benzene	1.25×10^{-3}	(19)
DMPC	8.8×10^{-4}	(9)
Olive oil	7.19×10^{-4}	(17)
Purple membrane (10°–20°C)	6.05×10^{-4}	(This work)
BSA	4.99×10^{-4}	(18)
Human apotransferrin	4.53×10^{-4}	(10)
Water (10°–20°C)	1.5×10^{-4}	(17)
Copper	5.02×10^{-5}	(17)
Iron	3.56×10^{-5}	(17)
Diamond	3.43×10^{-6}	(19)

at 15, 25, and 35°C, respectively, having an estimated uncertainty of ± 0.045 cal/ $^{\circ}\text{K} \cdot \text{g}$.

DISCUSSION

A simple model that describes some of our results is one in which the apparent membrane density is expressed as a volume-weighted average of the protein density and the lipid density:

$$\rho_{\text{mem}} = \rho_{\text{pro}} V_p + \rho_{\text{lipid}} (1 - V_p), \quad (4)$$

where V_p is the volume fraction of bacteriorhodopsin in the membrane. Differentiation with respect to temperature or pressure gives:

$$\frac{\partial \ln \rho_{\text{mem}}}{\partial X} = V_p \left(\frac{\partial \ln \rho_{\text{pro}}}{\partial X} \right) + (1 - V_p) \left(\frac{\partial \ln \rho_{\text{lipid}}}{\partial X} \right), \quad (5)$$

where X is either the temperature or the pressure.

Consider Eq. 5 as a model for thermal expansion, i.e., with temperature as the variable of differentiation. We used published ditetradecanoyl phosphatidylcholine data at 20°C as typical lipid data (9), i.e., $\rho_{\text{lipid}} = 1.06$ g/ml and $\partial \rho_{\text{lipid}}/\partial T = -9.3 \times 10^{-4}$ g/ml $^{\circ}\text{K}$. A value of $\partial \rho_{\text{pro}}/\partial T = -6.5 \times 10^{-4}$ g/ml $^{\circ}\text{K}$ was chosen as a typical value for

TABLE III
ADIABATIC COMPRESSIBILITY OF SELECTED MATERIALS

Material	$\beta = \frac{\partial \ln \rho}{\partial P}$	References
	atm^{-1}	
Pentane (20°C)	1.58×10^{-4}	(8, 17)
Ether (20°C)	1.49×10^{-4}	(8, 17)
Acetone (20°C)	8.86×10^{-5}	(8, 17)
Water (20°C)	4.61×10^{-5}	(8, 17)
DMPC	4.34×10^{-5}	(9, 11)
Purple Membrane (25°C)	2.30×10^{-5}	(This work)
BSA	1.05×10^{-5}	(12)
Lysozyme	4.67×10^{-6}	(12)
Mercury (20°C)	3.55×10^{-6}	(8, 17)

proteins (10). To find plausible values for V_p and ρ_{pro} , we solved Eq. 4, subject to the constraints that ρ_{mem} is equivalent to our measured value of 1.22 g/ml, that the purple membrane is 75% protein by weight (1), and that $\rho_{lipid} = 1.06$ g/ml. As a result, Eq. 4 gives values for $\rho_{pro} = 1.28$ g/ml and for $V_p = 0.71$. Inserting these values into Eq. 5 gives a predicted value of $\alpha_{mem} = 6.15 \times 10^{-4}/^\circ\text{K}$, which agrees well with our measured value of $(6.05 \pm 0.82) \times 10^{-4}/^\circ\text{K}$.

Now consider Eq. 5 as a model for adiabatic compression. We use $(\partial\rho_{lipid}/\partial P_S) = 4.60 \times 10^{-5}$ g/ml \cdot atm (11), and 0.15 β_{water} for the adiabatic compressibility of bacteriorhodopsin, treating bacteriorhodopsin as a typical globular protein (12). Eq. 5 now yields $\beta_{mem}^S = 1.74 \times 10^{-5}/\text{atm}$ for the membrane, which compares to our measured value of $(2.30 \pm 0.15) \times 10^{-5}/\text{atm}$.

This deviation between the model and the measurement is expected. The value for the compressibility of protein that we used in our model is typical for charged, hydrated globular proteins. The apparent adiabatic compressibility of globular proteins has been shown to be greatly influenced by the bound water (13). Conditions that release the bound water (e.g., appropriate pH changes) yield higher apparent compressibilities for globular proteins than conditions that tend to bind water to charged groups on the protein. The fact that our measured compressibility is greater than the simply modeled value is thus expected because of the absence of bound water on those surfaces of the bacteriorhodopsin molecules that are in contact with the lipid molecules.

The fact that the simple additive models that we have constructed give predictions that agree so much with our data suggests that the lipid and protein components of the purple membrane act independently in thermal expansion and adiabatic compression. Other evidence that supports the simple additive model for thermal expansion is that, within the errors of our expansivity measurements, native and bleached membrane have the same expansivity. The existence of the 2-D lattice did not measurably affect α_{mem} .

Wilkinson and Nagle (14) reported an average value of ~ 0.50 cal/ $^\circ\text{K} \cdot \text{g}$ for the apparent specific heats of lipids in bilayer suspensions at temperatures 20° below and above the main transition temperature. Assuming additivity in specific heats for the protein-lipid mixture gives a value of ~ 0.50 cal/ $^\circ\text{K} \cdot \text{g}$ for the bacteriorhodopsin in the membrane, a value that is 50% larger than the average value for globular proteins given by Privalov and Khechinashvili (15).

Presently, we do not understand why this apparent specific heat for the purple membrane is unusually large. We can speculate that differences in the structure or amount of bound water between bacteriorhodopsin and the globular proteins discussed in reference 15 may account for the large apparent specific heat of the membrane. Further-

more, it is not completely clear that our data do not agree with the data in reference 15 because we do not know the error in our specific heat measurement. We only estimated it from the dispersion in our three measurements.

The determination of the apparent ratio of the specific heat at constant pressure to the specific heat at constant volume can, a priori, be accomplished via either of two calculations using the data that have been presented in the Results section of this paper. One can calculate the apparent values for the density, adiabatic compressibility, specific heat at constant pressure, and expansivity of the purple membrane, and use these values in the following equation to calculate the apparent isothermal compressibility of the membrane:

$$\beta_{mem}^T = \beta_{mem}^S = + \frac{T\alpha_{mem}^2}{C_p^{mem} \rho_{mem}}. \quad (6)$$

One then just calculate:

$$\gamma_{mem} = \frac{\beta_{mem}^T}{\beta_{mem}^S} = \frac{C_p^{mem}}{C_v^{mem}}. \quad (7)$$

Alternatively, one has, for a given purple membrane suspension

$$\beta^T = \beta^S + \frac{T\alpha^2}{C_p\rho}. \quad (8)$$

Similarly, one has, for the buffer:

$$\beta_0^T = \beta_0^S + \frac{T\alpha_0^2}{C_p^0\rho_0}. \quad (9)$$

Subtracting these two equations yields:

$$\beta^T - \beta_0^T = (\beta^S - \beta_0^S) + T[(\alpha^2/C_p\rho) - (\alpha_0^2/C_p^0\rho_0)]. \quad (10)$$

We have measured all the quantities on the right side of Eq. 10. We can therefore calculate the apparent isothermal compressibility of the membrane via its defining equation:

$$\beta^T - \beta_0^T = V_{mem} (\beta_{mem}^T - \beta_0^T),$$

where V_{mem} = volume fraction. Now one proceeds as before to calculate the ratio of specific heats:

$$\gamma_{mem} = \beta_{mem}^T/\beta_{mem}^S.$$

Note that these two ways of performing the calculation give different results, because neither the density, the expansivity, nor the specific heat at constant pressure enters into Eq. 8 linearly. We believe that the latter type of calculation (i.e., Eq. 10) is the correct one because the equation of the form of Eq. 8 is true only for a well-defined thermodynamic system in equilibrium (16). Purple membrane fragments suspended in an aqueous buffer are not well-defined systems because of their interactions with the solvent. In particular, they do not have well-defined masses

or volumes, but only apparent densities. In contrast, the suspension and buffer are well-defined systems.

For these reasons, we performed the second type of calculation for the purple membrane suspensions and buffers. Because the specific heat, expansivity, and sound velocity measurements were all performed on suspensions that had different purple membrane concentrations (but contained the same buffer), it was necessary to normalize all the V_{mem} values to the same concentration, which can be chosen arbitrarily. We chose to use the weight fraction of 0.02701 for convenience. For this concentration, we found the following values, expressed in cgs units:

$$\begin{aligned}\beta^S - \beta_o^S &= - (5.05 \pm .08) \times 10^{-13} \\ \alpha_o &= (2.183 \pm .017) \times 10^{-4}/^\circ\text{K} \\ \alpha &= (2.43 \pm .02) \times 10^{-4}/^\circ\text{K} \\ C_p^c &= (4.14 \pm .02) \times 10^7 \\ C_p &= (4.09 \pm .02) \times 10^7 \\ \beta^T - \beta_o^T &= - (4.20 \pm .09) \times 10^{-13} \\ \beta_o^T &= (4.53 \pm .05) \times 10^{-11}.\end{aligned}$$

We finally arrive at

$$\beta_{\text{mem}}^T = (2.67 \pm 0.18) \times 10^{-5}/\text{atm}.$$

This yields an apparent value of:

$$\gamma_{\text{mem}} = \frac{\beta_{\text{mem}}^T}{\beta_{\text{mem}}^S} = 1.16 \pm 0.08.$$

Hence, the apparent value for the specific heat at constant volume is

$$C_v^{\text{mem}} = \frac{C_p^{\text{mem}}}{\gamma_{\text{mem}}} = \frac{.504}{1.16} = 0.434 \text{ cal/g} \cdot ^\circ\text{K}.$$

The apparent values for thermodynamic properties of the purple membrane at 25°C are summarized below

$$\begin{aligned}\rho_{\text{mem}} &= 1.22 \pm .01 \text{ g/ml} \\ \alpha_{\text{mem}} &= (6.05 \pm 0.82) \times 10^{-4}/^\circ\text{K} \\ \beta_{\text{mem}}^S &= (2.30 \pm .15) \times 10^{-5}/\text{atm} \\ \beta_{\text{mem}}^T &= (2.67 \pm .18) \times 10^{-5}/\text{atm} \\ C_p^{\text{mem}} &= (0.50 \pm .05) \text{ cal/g} \cdot ^\circ\text{K} \\ C_p^{\text{mem}} &= (0.43 \pm .06) \text{ cal/g} \cdot ^\circ\text{K}.\end{aligned}$$

In conclusion, we wish to stress that the thermodynamic quantities that we have reported are apparent values, i.e., there may be contributions from the bound water in addition to contributions that are intrinsic to the lipid and protein molecules. We did not specifically study the contributions of the bound water to any of our reported quantities, and we stress the importance of understanding such

contributions before attempting to make hypotheses concerning the contributions from the protein and lipid molecules.

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