Measuring the Correlation Times of Molecules Bound to Chiral Molecular Micelles

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Abstract:

NMR spectroscopy was used to determine the correlation times of 1,1'-binaphthyl-2,2'-diyl hydrogen phosphate (BNP) protons when bound to molecular micelles. These molecular micelles contained a dipeptide head group composed of various amino acids. BNP is a chiral molecule and therefore contains both (R) and (S) enantiomers. The chiral analyte interacted with the chiral head group of the polymers by inserting into a chiral pocket. This chiral pocket, depending on which amino acids were contained in the head group, bound the BNP's protons in a way to cause various degrees of anisotropy in the correlation times. This anisotropy was then correlated to the degree to which the polymer could discriminate between the two enantiomers. It was found that large degrees of anisotropy were characteristic of high chiral resolution and vice versa.

Introduction:

Chiral recognition plays an important role in biochemistry and physiology. In many cases, one chiral enantiomer of a drug can lead to the alleviation of the desired symptoms, while the other may cause damage to the body. For example, a racemate mixture of the drug Citalopram works as an anti-depressant with mild side effects, while a dosage of just the (S) enantiomer shows fewer side effects. This result suggests that the (R) enantiomer is the cause of the majority of the side effects.[1] Other examples of biochemical chiral recognition include enzymatic recognition, where an enzyme will only bind one enantiomer.[2] In this research, the binding of a chiral molecule to chiral molecular micelles was investigated. This project was done because mixtures of drug enantiomers have been separated using chiral molecular micelles.[3] The analyte and micelles used in this study will now be introduced.

The molecule 1,1'-binaphthyl-2,2'-diyl hydrogen phosphate, hereby referred to as BNP, has both the (R) and (S) enantiomers. The structures of (R) and (S) BNP are shown below.

[1] McConathy, Jonathan, and Michael J. Owens. "Stereochemistry in Drug Action." *www.psychiatrist.com*. N.p., n.d. Web. 15 July 2010. <www.psychiatrist.com/pcc/pccpdf/v05n02/v05n0202.pdf>.
[2] Garrett, Reginald H., and Charles M. Grisham. *Biochemistry*. 4 ed. New York: Brooks Cole, 2008. Print.
[3] Kingsbury, Stephanie A., Curtis J. Ducommun, Brian M. Zahakaylo, Elizabeth H. Dickinson, and Kevin F. Morris. "NMR characterization of 1,1'-binaphthyl-2,2'-dihydrogen phosphate binding to chiral molecular micelles." *Magnetic Resonance in Chemistry* (2010). Print.

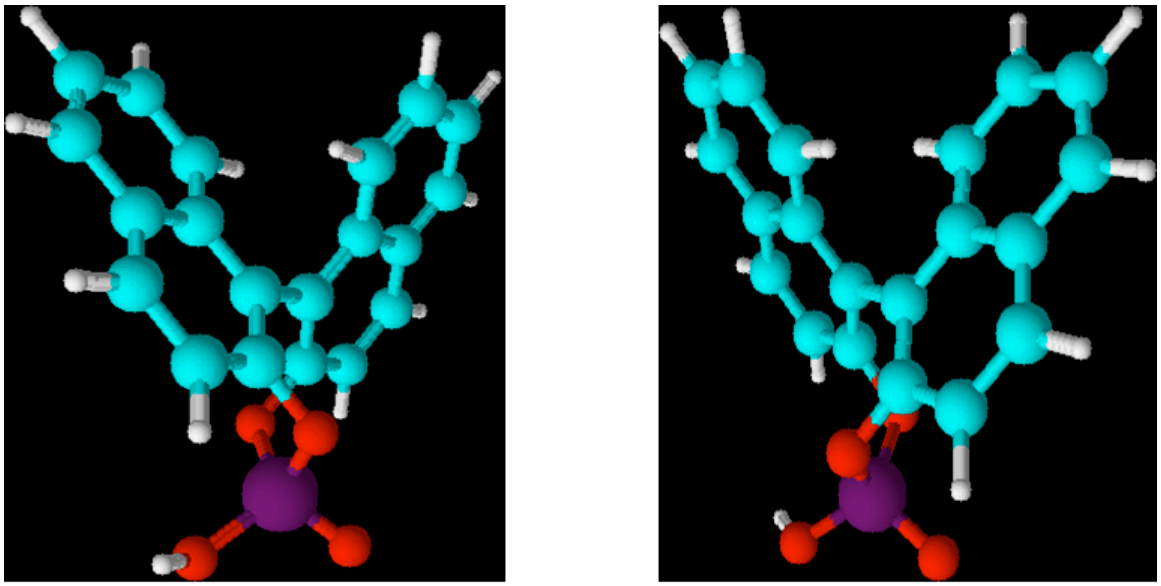

Figure 1. 3D structures of the (R) and (S) enantiomers for BNP.

BNP contains six non-equivalent protons of interest located on the naphthyl groups. Each of these protons is represented twice on the molecule, as there are two naphthyl rings per BNP molecule. However, the two BNP rings are equivalent in NMR spectroscopy. The labeling of the protons on the rings can be seen below.

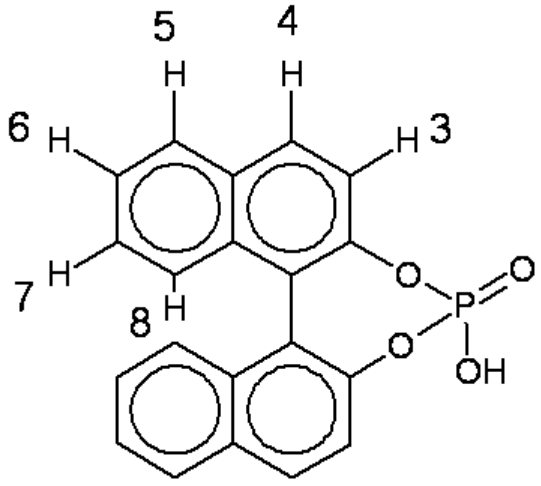

Figure 2. BNP molecule with the six significant protons shown and labeled.

As stated above, the binding of BNP to chiral molecular micelles with dipeptide headgroups was investigated. All of the micelles contained a dipeptide head group. Four different amino acids were used as the head groups; glycine, alanine, valine and leucine. All but the glycine residues contain a chiral center, located at the amino acid α-carbon. The difference between the amino acids is that they contain a varying number of carbons

in the amino acid side chain. A key point to note is that these micelles were not composed of surfactant monomers, but instead a molecular micelle was used. In this case, the polymers were fused together at the end of the hydrocarbon tails to form a chemical bond. These bonds alleviated the need for the aggregation of the monomers to form the micelles in solution and provided a more distinct "pocket" for the BNP to interact with.

The different head groups of the polymers investigated are shown Figure 3. In each case, the R group attached to the amino acids is a hydrocarbon containing 11 carbon atoms. There is also a sodium ion acting as the counter ion to the C-terminus carboxylic acid. Each polymer follows the naming scheme sodium undecyl (X), where X represents the amino acids in the surfactant chain.

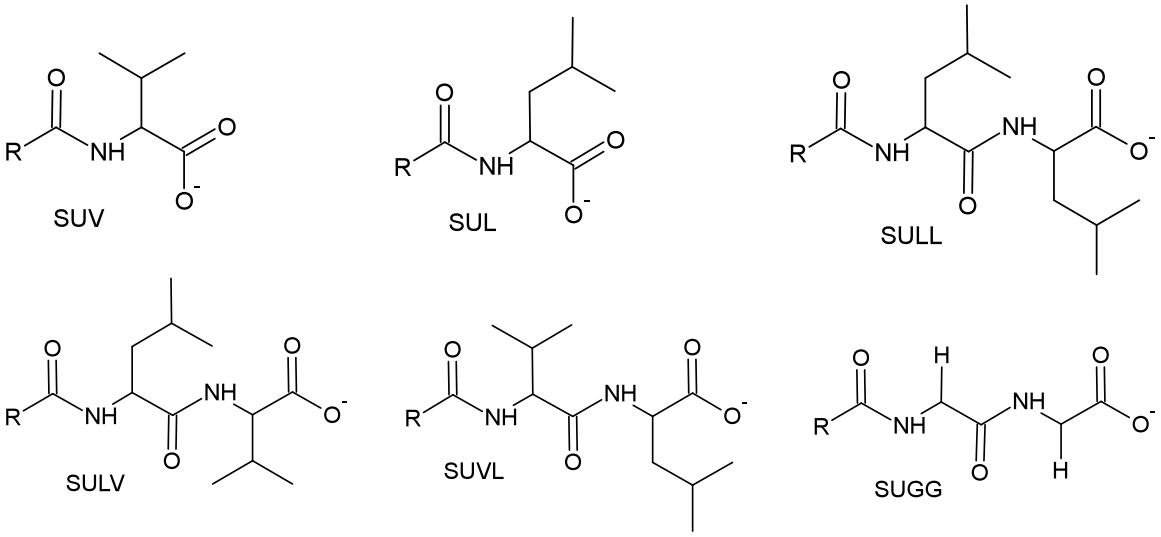

Figure 3. Each of the six polymers studied is shown above, the R group represents the 11 carbon group side chain.

Previous research has shown that there is an interaction between the polymers and BNP and that this interaction takes place in a so called "chiral pocket".[3] What is meant by this term is that the peptides form a pocket with the hydrocarbon chain like the one shown Figure 4, and the BNP inserts into that pocket. There is significant NOESY data to suggest that this is the conformation that the polymer adopts and that BNP is most likely binding within the pocket.[3] With the BNP inserted into the pocket, the protons H3, H4, and H8 are pointed towards the dipeptide head group's N-terminal α-carbon and the protons H5, H6, and H7 point towards the molecular micelle's hydrocarbon chain.

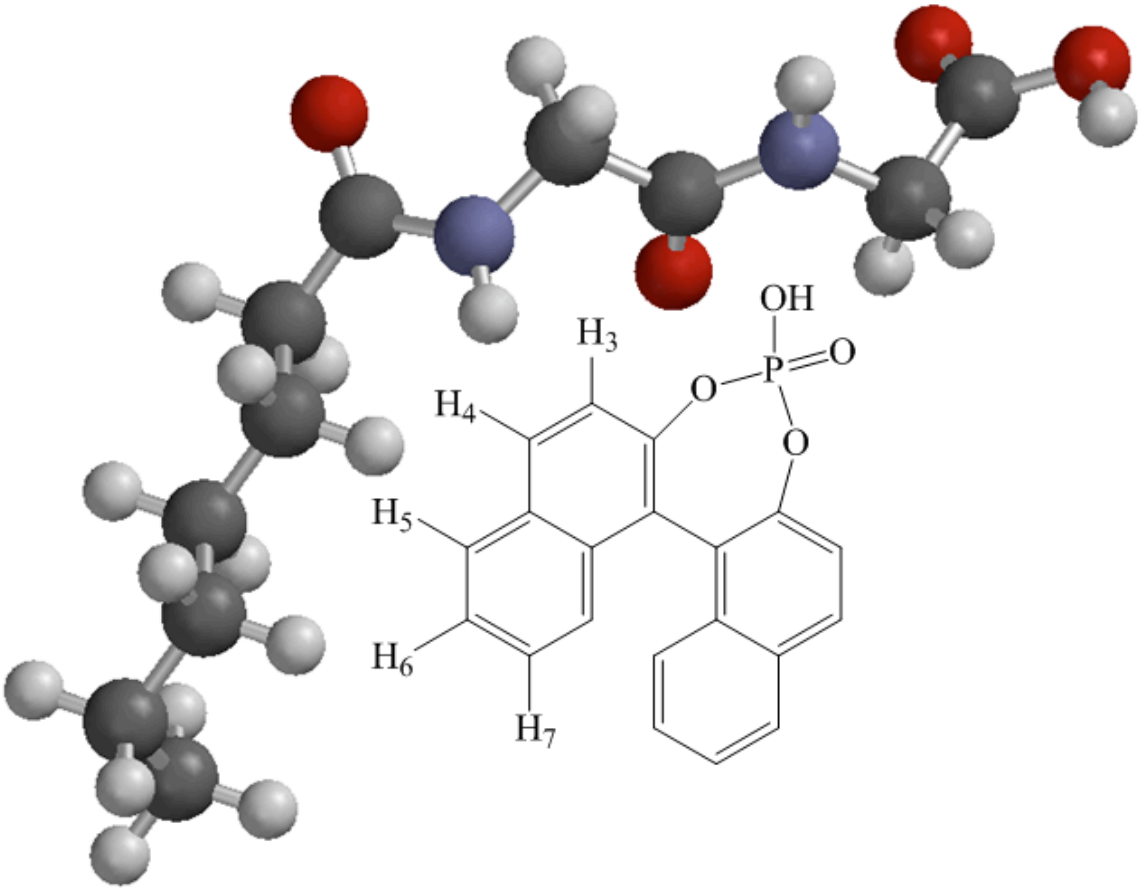

Figure 4. BNP bound to polymer which shows the idea of a chiral pocket being formed.

In order to more fully characterize the chiral recognition of the BNP enantiomers by the molecular micelles, correlations times (τ_c) of each proton were measured. A correlation time is the length of time a molecule spends in one orientation, before tumbling to another. In a liquid, the correlation times of molecules are on the nanosecond time scale. It stands to reason that smaller molecules will tumble much faster than larger molecules. Therefore, smaller molecules will have smaller correlation times. In addition, if a small molecule binds to a large molecule like a molecular micelle, its correlation time should increase.

NMR spectroscopy can be used to measure two different relaxation times, T1 and T2, through well established techniques.[4] These values can then be used to calculated the correlation times using the following equation; [5]

$$Tc = -0.240395 + 0.394030(T1/T2) - 0.030491(T1/T2)^2 + 0.001324(T1/T2)^3 + 0.000021(T1/T2)^4.$$

[4] Atkins, Peter, and Julio De Paula. *Atkin's Physical Chemistry*. 8th ed. Oxford : Oxford University Press, 2006. Print.

[5] Carper, W. Robert, and Charles E. Keller. "Direction Determination of NMR Correlation Times from Spin-Lattice and Spin-Spin Relaxation Times." *J. Phys. Chem* 101.18 (1997): 3246-3250. Print.

The relaxation of molecules depends on the correlation times because unlike most types of spectroscopy, where the difference between two energy levels is large, the difference between energy levels in NMR spectroscopy is small and molecules require energy to transition from an excited to a ground state. This energy comes when the molecules interacts with a magnetic field oscillating at frequencies determined by the correlation time.

Using these correlation times, we hoped to gain insight into why some polymers had high α values and other had low α values. The α value of a polymer relates to how well it can distinguish between or separate enantiomers of BNP in chromatography. If a polymer posses a high α value, then its ability to recognize two enantiomers is better, or it does a better job separating the BNP (R) and (S) enantiomers. If a polymer posses a low α value, or a value close to 1.00, then the polymer does not recognize differences in enantiomers as well, and does not separate them.

Results and Discussion:

A tabulation of the correlation times for each of the polymers that was studied is shown in Table 1. The table also lists the Δ(ΔG) values, binding constants (K) and the α values of each of the polymers. The Δ(ΔG) values were found by using the following equation;

$$\Delta(\Delta G) = \left| \Delta G(S) - \Delta G(R) \right|$$

ΔG is the free energy of binding of each enantiomer to the respective molecular micelle. A large Δ(ΔG) value leads to a large α value. The α values were take from the literature.[3] The K values reported are the average of the association constants for the (R) and (S) enantiomers. The higher the K value, the stronger the BNP interacts with the molecular micelle.

		H3	H4	H5	H6	H7	H8
SULV	T_c (ns)	1.915 ± 0.057	3.104 ± 0.086	3.384 ± 0.165	2.542 ± 0.136	2.542 ± 0.136	3.063 ± 0.272
	α = 1.066 Δ(ΔG) = 0.48 kJ/mol K = 114.00						
SUGG (S)	T_c (ns)	1.735 ± 0.070	2.407 ± 0.073	2.562 ± 0.077	2.226 ± 0.051	2.226 + 0.051	2.727 ± 0.178
	α = 1.000 Δ(ΔG) = 0.00 kJ/mol K = 249.21						
SULL	T_c (ns)	1.738 ± 0.044	2.142 ± 0.074	2.304 ± 0.094	2.026 ± 0.107	1.832 ± 0.062	2.180 ± 0.055
	α =1.042 Δ(ΔG) = 0.47 kJ/mol K = 46.00						
SUVL (S)	T_c (ns)	1.584 ± 0.059	1.784 ± 0.050	2.014 ± 0.172	1.696 ± 0.059	1.534 ± 0.032	1.783 ± 0.048
	α = 1.000 Δ(ΔG) = 0.00 kJ/mol K = 73.00						
SULG (S)	T_c (ns)	1.956 ± 0.119	2.975 ± 0.150	3.553 ± 0.164	2.449 ± 0.088	2.449 ± 0.088	2.851 ± 0.124
	α = 1.096 Δ(ΔG) = 0.71 kJ/mol K = 67.00						
SUGL (S)	T_c (ns)	1.917 ± 0.046	3.023 ± 0.134	2.532 ± 0.153	2.214 ± 0.086	2.214 ± 0.086	2.460 ± 0.104
	α = 1.047 Δ(ΔG) = N/A K = 58.88						

Table 1. Tabling listing the correlation times of each polymer's protons and the Δ(ΔG) values, binding constants (K) and the α values.

Two plots of the correlation times for the six BNP protons are shown below. Figure 5 shows data for three polymers with correlation times that vary over a small range of values, while Figure 6 shows data for three different polymers where the correlations times vary over a larger range.

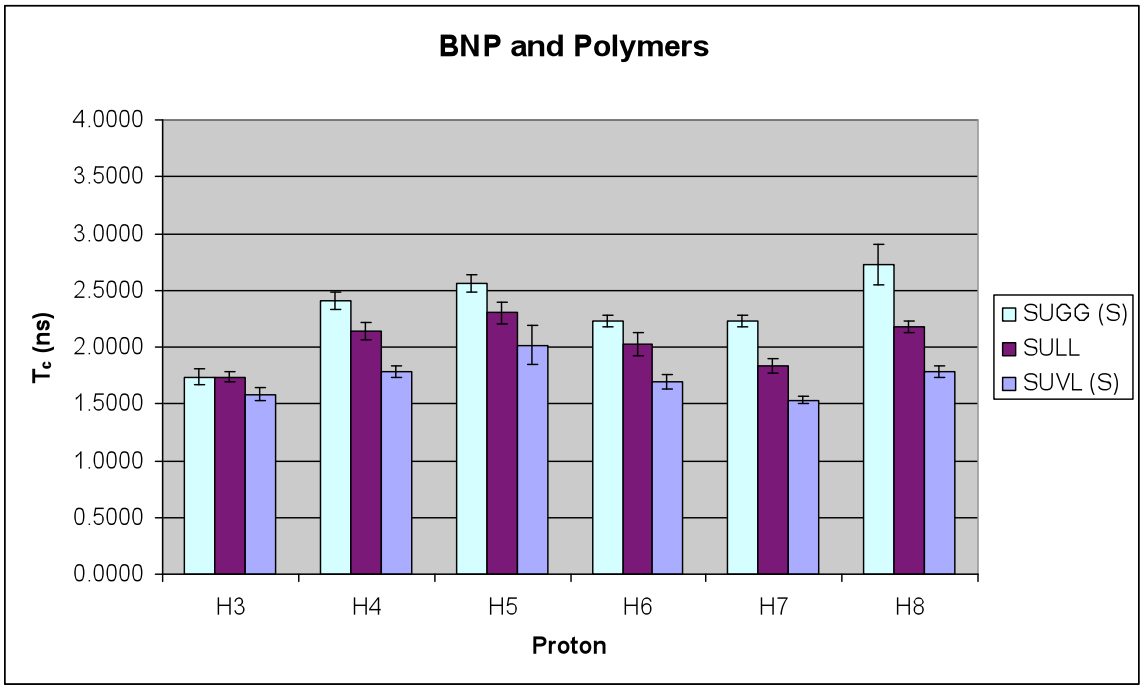

Figure 5. Graph showing the trend of polymers with a small range of correlation times.

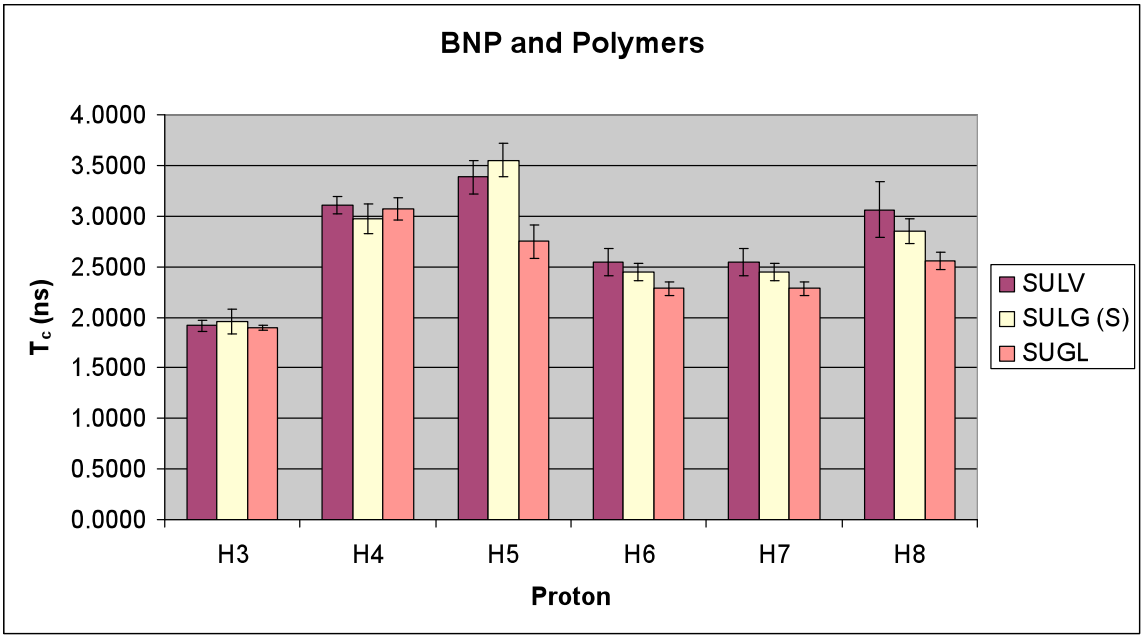

Figure 6. Graph showing the trend of polymers with a large range of correlation times.

Figures 5 and 6 show two levels or degrees of anisotropy that arise from the six polymers. In Figure 6, it can be seen that certain protons are greatly slowed when compared to others. For example with SULG, proton 5 has a correlation time around 3.5 ns, while proton 3 has a correlation time around 2.0 ns. The data in Figure 5 shows a smaller anisotropy in the correlation times because the smallest and largest correlation times differ by only about 0.50 ns. Although the shortest and longest protons in each data

set do t-test to be significantly different at the 95% confidence interval, it appears that many of the proton correlation times are relatively close to one another. In fact, many of the protons would not be considered to be statistically different. On the 95% confidence level, the data sets in Figure 5 generally produced only two statistically different protons.

These data also show that a larger range in correlation times is a characteristic of overall longer correlation times. For example, the polymers SULV, SULG and SUGL had overall longer correlation times and their correlation times varied over the widest range. Conversely, SUGG, SULL and SUVL had smaller overall correlation times and their correlation times varied over a narrower range. A graph showing this effect is shown in Figure 7.

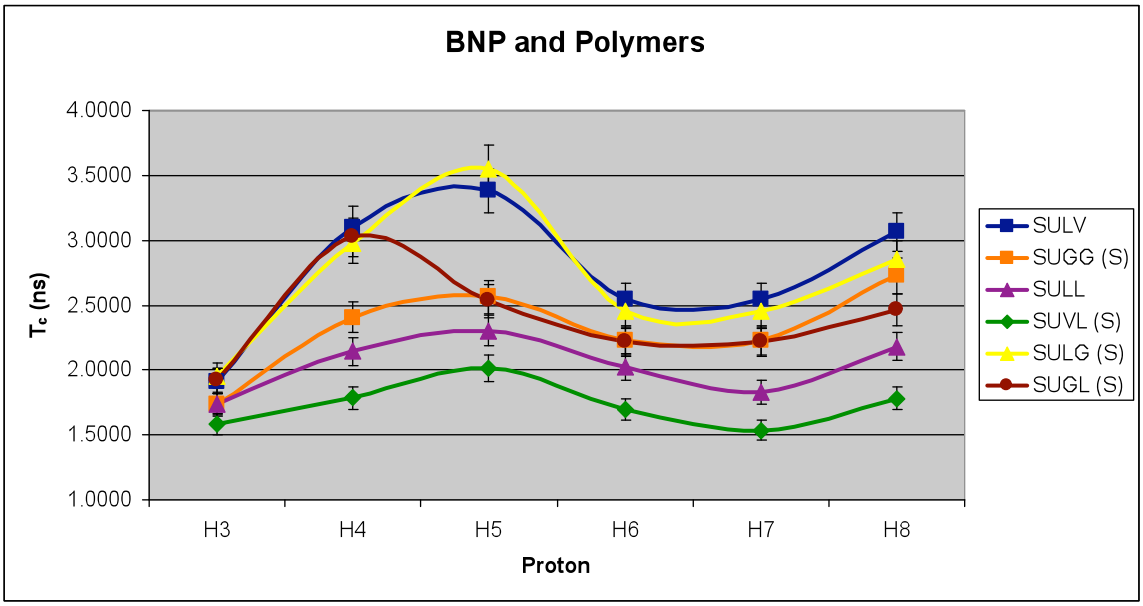

Figure 7. Graph show the correlation times of each proton with each polymer. A set value of 5% was set for the error in each proton, this is the average amount of error incurred amongst all data sets.

This effect however, is not directly related to the binding affinity of the BNP to the polymers. In other words, the binding constant does not correlate with the range of correlation times. For example, SULL has a small BNP binding constant (46) but a large range of BNP correlation times, while SUVL has a larger BNP binding constant (73) but a smaller range of BNP correlation times. An explanation of this effect would be due to the size of the pocket that is formed by the different amino acids. Another factor would be steric hindrance between the BNP and the amino acid side chains within the chiral pocket which would not allow for longer correlation times, despite a high binding constant.

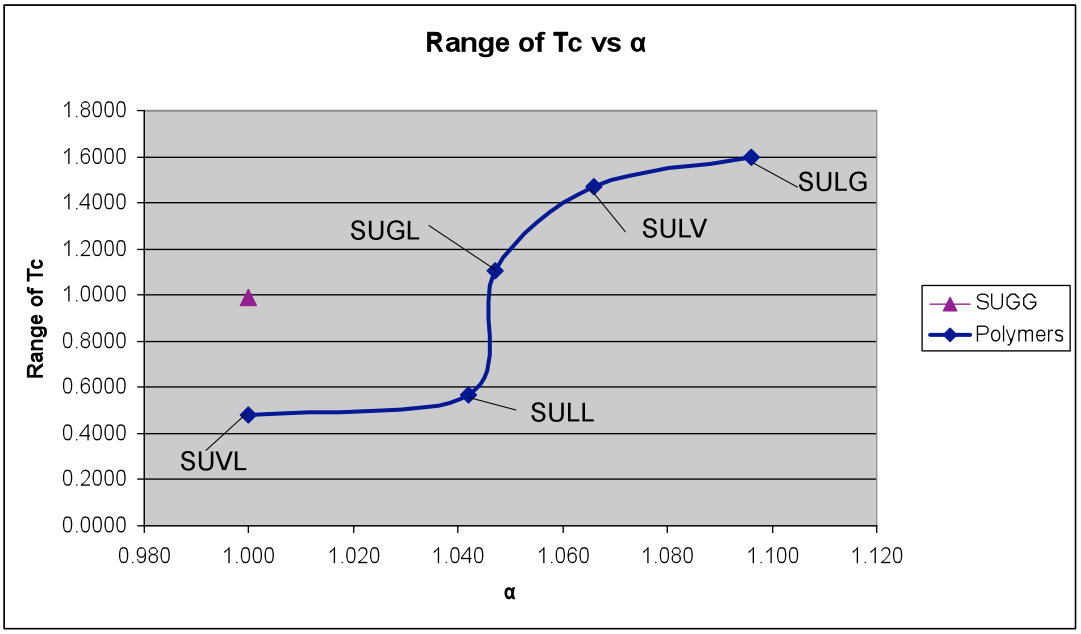

Figure 8. Graph showing the trend of increasing selectivity relating to and increased range in correlation times.

Figure 8 shows a plot of the α values for each polymer against the range in correlations times. This plot shows that a wide range of correlation times is characteristic of large α value and high chiral selectivity and vice versa. In other words, as the α value or selectivity increases for a given polymer, so does the range in correlation times for the bound BNP. This result is explained as follows. A polymer that exibits a high chiral selectivity must posses some form of chrial recognition. It appears from this graph that this recognition is most pronounced when there are large differences in the BNP's protons' correlation times. In cases were the range in times is small, the selectivity is also low. The protons on the BNP are all moving at a comparable rate, so this makes it hard for the polymer to detect differences in chirality and suggests that all protons on BNP are in similar environments in the bond state. However, when the protons are moving at much different rates, then the polymer must be interacting with the BNP in such a way to promote this effect. When the BNP binds to these polymers, regardless of how favorable this interaction is, the protons are experiencing varying amounts of motional freedom. These different interactions, allowing for various freedom of motion of the protons promotes chiral recognition. If the polymer has a way of sensing differences in these protons then the polymer, according to the above data, will also be more capable of sensing different enantiomers.

Earlier work with chiral recognition showed that as the chiral selectivity increased, the fluorescence anisotropy changed as well.[6,7] In fluorescence anisotropy, light that is emitted from an excited molecule is detected in both the parallel and perpendicular directions. A molecule that is moving isotropically has a small

[6] McCarroll, Matthew E., Feresteh Haddadian Billiot, and Isiah M. Warner. "Fluorescence Anisotropy as a Measure of Chiral Recognition." *J. Am. Chem. Soc* 123.13 (2001): 3173-3174. Print.
[7] Kimaru, Irene W., Yafei Xu, and Matthew E. McCarroll. "Characterization of Chiral Interactions Using Fluorescence Anisotropy." *Analytical Chemistry* 78.24 (2006): 8485-8490. Print.

fluorescence anisotropy while a molecule moving in an anisotropic fashion has a large fluorescence anisotropy. Furthermore, large fluorescence anisotropy correlates linearly with high chiral selectivity.[7] These results are consistent with our measurements because a large anisotropy in correlation times was found to be characteristic of high chiral selectivity and a small anisotropy in correlations times was consistent with low chiral selectivity.

Conclusions:

The correlation times of six BNP protons were measured using two well established NMR experiments, to determine the T1 and T2 relaxation times. When the BNP was bond to molecular micelles in solution, this binding significantly increased the correlation times of the BNP protons when compared to BNP in free solution. The correlation times of BNP in free solution are all equal to about 0.2 ns. When the BNP was bound to the micelles the correlation times increase to a minimum of 1.5 ns and a maximum of 3.5 ns. The range in correlation times of the protons was found to be related to the chiral selectivity of the polymer to which the BNP was bond to. The polymer SULG for example has an α value of 1.096 and a range in correlation times of over 1.5 ns, while the polymer SUVL has an α value of 1.000 and a range in correlation times of only 0.5 ns. This correlation was then compared to earlier work done with fluorescence anisotropy and was found to have the same trend; a larger α value for a polymer also showed a larger range in correlation times of the BNP's protons.

Experimental Section:

Sample Preparation

Each sample was prepared by making a dissolving in 1 mL of pH 10 boric acid buffer, 10 mg of polymer and 2 mg of BNP. This gave a polymer concentration of approximately 25 mM. The buffer was made by dissolving 0.9534 g of sodium tetraborate decahydrate into 25 mL of D_2O, NaOD was used to adjust the pH to 10.00. For diffusion and binding map experiments, the sample was then transferred to a standard NMR tube and capped. For relaxation experiments, the samples were first degassed and then transferred to a controlled environment NMR tube under nitrogen. The degassing process required that the sample be placed into a vacuum safe flask that contained a valve. This was then placed on a Schlenk line. The flask was placed into a liquid nitrogen filled Dewar until the liquid was solid. The valve to the vacuum line was opened and the sample was evacuated for five minutes. After this time had elapsed, the valve was closed and the sample was removed from the Dewar and allowed to thaw. The above process was then repeated twice more for a total of three times. The sample was then taken off the vacuum line, valve closed, and put into a glove bag. The bag was then filled with gaseous nitrogen and then sealed. The sample was then transferred to the controlled environment NMR tube and sealed.

Spectroscopy Experiments

The experiments were done on a Bruker Avance 300 MHz Spectrometer, running Topspin v1.3. Diffusion and binding map (NOESY) experiments were performed using typical experimental procedures for each respectively. The diffusion experiments used

the following gradients; 5, 7, 9, 11, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 40, 45, 50, and 60 % for the 19 experiments respectively. The relaxation experiments were done using an Inversion Recovery (T1) and a Carr-Purcell-Meiboom-Gill Experiment (T2) experiments. The file name for the T1 experiment was "INVREC" and the parameters are such; D1 (relaxation delay) = 3 s, D2 (tau delay) 0.03, 0.045, 0.064, 0.10, 0.12, 0.14, 0.24, 0.30, 0.40, 0.60, 0.75, 1.00, and 2.00 s for 13 experiment respectively and a PL9 of 50 dB for the water suppression. Each experiment ran for 160 scans. The file name for the T2 experiment was "cpmgH2O" and the parameters are as follows; D1 (relaxation delay) = 3 s, D20 = 0.0015 s (echo time of 0.0030 s) and VCLIST file name of "kay 2009". Within this file, the following number of echos were applied; 2, 4, 6, 8, 10, 12, 16, 20, 24, 26, 28, 30, 34, 36, and 40 which gave total echo times of; 0.006, 0.012, 0.018, 0.024, 0.030, 0.036, 0.048, 0.060, 0.072, 0.078, 0.084, 0.090, 0.102, 0.108, and 0.120 s for the 15 experiments respectively. The water suppression power, PL9, was set to 54 dB. Each experiment ran for 128 scans.

Data Workup:

For both the T1 and T2 experiments, the peak intensities of each proton was individually summed. For the T1 experiment, the $\ln(M_o-M_z)$ vs τ was plotted and a line was fit to the data. Where M_o is the longest τ value's peak intensity and M_z is the value at a given τ. For the T2 experiment, the ln(I) vs t was plotted and line was fit to the data. Where I relates to the sum of the peak intensities and t is the total echo time. For both experiments, 1/slope of the each line was taken and the three trials were averaged to give a T1 and T2 for each proton. These values were then put into the following equation along with the average standard deviation, $Tc = -0.240395+0.394030(T1/T2)-0.030491(T1/T2)^2+0.001324(T1/T2)^3+0.000021(T1/T2)^4$ to give the correlation times with error for each individual proton.