enhanced with ^{2}H-labeled Leu, due to the longer side chain of Leu when compared to Val. Furthermore, ^{2}H-labeled Ala can also be used with this technique. This ESSEM secondary structural approach can be used with different deuterated amino acids and provide pertinent qualitative structural information on membrane proteins in a short period of time with small amounts of sample.

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EPR and Molecular Dynamics Study of Barstar-Barnase Interaction

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Using spin labeling for studying protein macromolecules may reveal their significant dynamical and structural properties. EPR spectrum contains information about protein dynamics and internal motions, but, unfortunately, these are extremely versatile, and spectrum present only 'digest' of it. There is is an attractive way of joining Molecular Dynamics (MD) simulation with EPR. MD provides detailed system dynamics, and number of attempts has been made to calculate spectra directly from trajectory data. Still there is no currently reliable algorithm developed to date for this purpose.

In this work, the study of complex formation between RNAse Barnase (Bn) and its specific inhibitor Barstar (Bs), is presented. High affinity of Bs to Bn, this protein pair is promising for creating large superstructures with controllable properties. Mutant C40A barstar labeled by C82 with 4-(2-chloromercuriphenyl)-2,2,5,5-tetramethyl-3-imidazoline-D3-1-oxyl, as well as its complex with Bn, was previously studied by X-band EPR to obtain correlation times for macromolecule Brownian diffusion and order parameters for internal dynamics.

We built a model of labeled Bs, and BsBn complex, and ran a number of full-atom MD simulations. Both MD and EPR revealed two motional states of the spin label, one highly ordered, and another flexible in free Barstar. Detailed analysis of calculated data showed that difference between these two states was solely due to internal dynamics of the protein. Corresponding azimuthal order parameters calculated from MD trajectories well coincided with experimental ones, obtained from EPR spectra. It was found that formation of BsBn complex leads to complete disappearance of disordered state. Experimental evidence (provided by spin labeling) of key features observed in MD trajectories provide a validation of used parameters and protocols therefore.

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EPR Spectroscopic Studies on the Binding of the Full Length Human KCNE1 Protein with the Voltage Gated Potassium Channel KCNQ1

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KCNE1 is a single transmembrane protein that modulates the activity of voltage gated potassium channels. KCNE1 interacts with KCNQ1 and decreases the rate of channel activation, increases conductance, and generates a slowly activating K^{+} current critical for cardiac repolarization. Mutations on either KCNE1 or KCNQ1 genes in E1/Q1 complex can lead to cardiac long QT syndrome. Despite the biological significance of the Q1/E1 interaction, its exact nature is not fully understood yet. In this study, we have used different electron paramagnetic resonance (EPR) spectroscopic techniques to provide the direct evidence of binding activities of KCNQ1 with KCNE1. A CW-EPR titration experiment was conducted on different sites in the C-terminus of KCNQ1 with the addition of WT-KCNE1 to determine the binding mechanism of Q1 and E1. The CW EPR line shape analysis indicates that the motion of the spin label on Q1 decreases with the addition of WT-KCNE1 and saturates at molar ratio of 1:3 (Q1:E1). Double electron-electron resonance (DEER) Spectroscopy was used to measure distances between the spin labels attached on E1 and Q1 separately in Q1/E1 complex. A DEER distance of 33 Å was obtained between the spin labels at 318th position on Q1 and 106th position on E1 that provides the direct evidence of formation of a Q1 and E1 complex. These EPR techniques are very useful for determining the structural model of the Q1/E1 complex.

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Structural Effects of Dibucaine Encapsulation into Solid Lipid Nanoparticles and Nanostructured Lipid Carriers

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Solid Lipid Nanoparticles (SLN) and Nanostructured Lipid Carriers (NLC) are innovative alternatives in nanoencapsulation with a wide potential for controlled drug release. Dibucaine (DBC) is a potent long-acting local anesthetics but it is also toxic to the central nervous system. The purpose of this work was to produce SLN and NLC formulations at pH 7.4 - composed by myristyl myristate (MM) or MM / myglyol® in their lipid matrices, respectively, plus Pluronic F68 - for DBC delivery, aiming a future application for pain control in the skin. The lipid particles were characterized by nanoparticle tracking analysis (NTA) and electron paramagnetic resonance (EPR) with nitroxide spin probes. The size of SLN and NLC loaded with DBC were 175.67 ± 10.69 and 170.67 ± 3.79nm, respectively; no significant changes were detected after DBC addition ($p \leq 0.05$, unpaired t-test). Size distribution, given by the Span value, was kept below 1, as expected for homogeneous dispersed colloids. EPR spectra of nitroxide probes revealed that dibucaine insertion into the nanoparticles below the fusion temperature of MM increased lipid packing ($\eta h+1/h0$ height ratio) while the opposite ($\eta h+1/h0$) occurs at 55oC. Moreover, the partition of DBC into SLN/NLC seems to be a stable process since no hysteresis was observed after a few heating/cooling cycles. In conclusion, we successfully prepared SLN and NLC particles containing DBC which, in turn affects their structural properties. Supported by FAPESP (# 06/00121-9) and CAPES (Brazil).

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Spin Label and SAXS Study of Cetylpalmitate Solid Lipid Nanoparticles Loaded with Dibucaine

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Dibucaine (DBC) belongs to the amino amide family of local anesthetics. Unlike other anesthetic compounds it possesses a rigid, butyl substituted, quinolinic ring that imposes restrictions to its interaction with membranes. Solid Lipid Nanoparticles (SLN) has been attracting attention as a promising drug delivery system because it combines advantages such as versatility, use of safe excipients, a wide potential for the controlled drug release of drugs and good shelf stability. The aim of this study was to develop SLN prepared with cetylpalmitate (CP) as the lipid matrix and poloxamer 188 as a colloidal stabilizer to encapsulate DBC. Nanoparticles were prepared with the high pressure hot homogenization method and characterized by transmission electron microscopy (TEM), small-angle X-ray scattering (SAXS) and electron paramagnetic resonance (EPR). The association constant or partition coefficient was measured (177.02 ± 6.31). TEM images disclosure structures with delineated spherical surfaces and homogenous size distribution (ca. 250-300 nm), in good agreement with photon correlation spectroscopy data; incorporation of DBC did not change the morphology and size of the nanoparticles. SAXS data showed that the lipids in the SLN are organized in a liquid crystal-like structure, with small amounts of water inside it (the same results were observed in the SLN loaded with DBC). EPR spectra of 5-nitroxyl stearic acid incorporated into SLN were compatible with SAXL data and revealed that dibucaine insertion into the nanoparticles changed the lipid packing sensed by the spin label, decreasing its isotropic signal. In conclusion we have shown that DBC can be successfully incorporated into SLN, changing its lipid packing without destabilizing the overall nanoparticle structure, indicating that this is a promising drug delivery system. Supported by FAPESP (# 06/00121-9), CAPES, CNPq.

Fluorescence & Other Luminescence I

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Trp Fluorescence in GB1: Nanosecond Dynamics Strongly Depend on pH While 30Ps Relaxation is Constant

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The fluorescence dynamic Stokes shift(FDSS)of W43 in the model protein GB1, was measured in both picosecond and nanosecond time domains by combining Fluorescence Upconversion with Time Correlated Single Photon Counting (TCSPC). We examined a wide range of pH values (from 8 to 3) where GB1 is known from NMR to be stable. We observe large changes in the nanosecond lifetimes and DAS; Trp lifetime declines with reduced pH, likely due to differences in the Trp charge environment. Stern-Volmer plots revealed changes in Trp exposure. In the picosecond domain, however, a characteristic risetime of 30 ps was seen for GB1 at 375nm. This negative amplitude remains constant