enhanced with $^3$H-labeled Leu, due to the longer side chain of Leu when compared to Val. Furthermore, $^3$H-labeled Ala can also be used with this technique. This ESSEM second step-digest 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.

1762-Pos Board B654
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 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-chloromercuri-phenyl)-2,2,5,5-tetramethyl-3-imidazoline-3-1-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.

1763-Pos Board B655
EPR Spectroscopic Studies on the Binding of the Full Length Human KCNE1 Protein with the Voltage Gated Potassium Channel KCNQ1
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KCNQ1 protein has been studied for many years as a channel forming subunit of the hERG potassium channel, but the specific role of KCNE1, its regulatory subunit, remains unclear. It is known that KCNE1 interacts with KCNQ1 and decreases the rate of channel activation, increases conductance, and generates a slowly age gated potassium channels. KCNE1 interacts with KCNQ1 and decreases its activity. KCNE1 is a single transmembrane protein that modulates the activity of voltage-gated potassium channels. KCNE1 interacts with KCNQ1 and decreases its activity. KCNE1 is a single transmembrane protein that modulates the activity of voltage-gated potassium channels.

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) and CAPES (Brazil).

1765-Pos Board B657
Spin Label and SAXS Study of Cetylpalmitate Solid Lipid Nanoparticles Loaded with Dibucaine
Raquel Melo Barbosa$^{1}$, Bruna Renata Casadei$^{1}$, Camila Moraes Gonçalves da Silva$^{1}$, Rosangela Itri$^{2}$, Leandro Barbosa$^{3}$, Elzilda de Paula$^{1}$. 1Biochemistry Department, Institute of Biology, State University of Campinas (UNICAMP), Campinas - SP, Brazil, 2Biophysical Department, Institute of Physics, University of São Paulo (USP), São Paulo - SP, Brazil. Dibucaine (DBC) belongs to the amino amide family of local anesthetics. Unlike other anesthetic compounds it possesses a rigid, butyl substituted, quinoline 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 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.

1766-Pos Board B658
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 (TCSPEC). We examined a wide range of pH values (2 to 9) 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 375 nm. This negative amplitude remains constant

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