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New Theoretical Approach for Elucidating the Solution Structure of Peptides from NMR Data

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The early work of this project was carried out in collaboration with Dr. Eva Meirovitch, who also was a Co-PI of the grant; the later work (from January 1996) was done together with my postdocs Dr. Canan Baysal and Dr. Bedamati Das.

In this project we have developed a new computational methodology, based on statistical mechanics considerations, for analyzing experimental structural data of *flexible* peptides and segments of proteins (typically surface loops and chain ends). This methodology is applicable to multidimensional nuclear magnetic resonance (NMR), X-ray crystallography, and potentially fluorescence spectroscopy and other techniques.

NMR is the only physical technique that can generate three dimensional structures of biomolecules in solution. It is well established for globular proteins which reside in a *single* microstate, i.e. a limited region of conformational space around the native structure. Nuclear Overhauser Enhancement (NOE) contacts indicative of structure can also be obtained from more flexible systems (e.g., peptides, carbohydrates, and DNA segments), which are expected to populate significantly *several* microstates in thermodynamic equilibrium. However, in this case the NOEs might become weighted averages of contributions of the individual microstates, which makes the interpretation of the data difficult, because of the need to identify the most stable microstates and calculate their relative populations. Development of reliable analysis techniques in this field is a challenge.

Our methodology is based on an extensive conformational search for low energy minimized structures from which a relatively small set of structures that are *significantly different* is selected; their vicinity is spanned by Monte Carlo (MC) or molecular dynamics (MD) simulations and the free energy is obtained by the local states (LS) method of Meirovitch. Thus, in contrast to other methods, the relative populations are obtained directly from the free energy. This methodology has been applied to Leu-enkephalin H-Tyr-Gly-Gly-Phe-Leu-OH [3-5], to the cyclic hexapeptide *cyclo*-(D-Pro-Phe-Ala-Ser-Phe-Phe) [12,13,18], and to other cyclic peptides (see below).

Within the framework of this methodology several new efficient procedures for confor-

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mational search have been developed (i.e., methods for *global* optimization of the molecule energy or other cost functions with respect to the $3D$ structure of the molecule). SADA (Systematic Alteration of Dihedral Angles) [1] enables one to search for the lowest energy minimized structures. FMCM (Free Energy Monte Carlo Minimization) [2,7] enables the efficient generation of structures with the lowest *harmonic* free energy. For cyclic molecules and loops in proteins we have developed the Local Torsional Deformation (LTD) method [8,9,12,13,16]. Efficiency comparisons between LTD and simulated annealing [10,19] have found the latter method to be significantly inferior to the former. We have also checked the efficiency of the multicanonical simulation method as a conformational search technique [23], and examined the efficiency of several local optimization procedures [17].

Protein and peptide structures are strongly affected by the solvent, which would ideally be described explicitly. However, simulations with explicit solvation models are very time consuming, and it is not feasible to calculate free energy differences between states of a significant structural variance. This is feasible, however, with implicit solvation models using the LS method. Therefore, we have suggested a new way for optimizing implicit solvation models for peptides and proteins. Thus, using a simplified model, the energy of the molecule is $E = E_{\rm FF} + \sum A_i\sigma_i$, where $E_{\rm FF}$ is the force field energy (i.e., the molecule energy without the solvent), A_i is the solvent accessible surface area, and σ_i is the atomic solvation parameter (ASP) of atom i. The ASPs are not obtained, as in other studies, from experimental data of the free energy of transfer from the gas phase to the solvent of interest, but by requiring that E becomes the *global* minimum for the experimental structure. Such an optimization has been carried out for the cyclic hexapeptide in DMSO mentioned above [12,13]. The consistency of these ASPs has been verified by applying the whole methodology to this hexapeptide [18]. To check the transferability of the ASPs, the methodology has been applied to the cyclic pentapeptide *cyclo*(D-Pro^1-Ala^2-Ala^3-Ala^4-Ala^5 [21], and the cyclic heptapeptides *cyclo*(Asn^1-Pro^2-Phe^3-Val^4-Leu^5-Pro^6-Val^7) and *cyclo*(Thr^1-Pro^2-Leu^3-Trp^4-Val^5-Pro^6-Leu^7) [22] in DMSO. Results for proton-proton distances and ^3J coupling constants obtained by our *ab initio* studies have been found to be in a very good agreement with their experimental counterparts. Recently a set of optimized ASPs has been derived for three loops of the protein ribonuclease A 24].

The LS method and the related hypothetical scanning method have been further developed [6,14,15,20,25]. Also, applying the LS method to MD trajectories enabled us to calculate the backbone entropy (hence the relative flexibility) of loops in a Ras protein [11]. This entropy can also be obtained *approximately* (i.e., by ignoring correlations) from the

order parameter measured in dynamical NMR experiments. With our method the extent of approximation in the experimental values can be assessed. Finally, a comprehensive review on the calculation of the entropy and the free energy of macromolecules has been written [15].

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