

BIFI 2004
Biology after the Genome:
a Physical View
BOOK OF ABSTRACTS

Instituto de Biocomputación
y Física de Sistemas Complejos (BIFI)

Universidad de Zaragoza (Spain)

February 11, 12, and 13, 2004

Contributions to BIFI 2004 will be reviewed and later published in a special issue of Biophysical Chemistry.

Organizing Committee

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- J.F. Sáenz (Zaragoza)
- J. Sancho (Zaragoza)
- A. Tarancón (Zaragoza)

Program

Wednesday, February 11, 2004

08:30 Registration at the Paraninfo building
10:00 Opening Act

PROTEIN FOLDING AND MACROMOLECULAR INTERACTIONS

Morning Session:

Chairman: **Antonio Rey** (Complutense Madrid and BIFI)
10:45 - 11:40 **J. Onuchic** (San Diego) *Exploring the Protein Folding Funnel Landscape: Theory Meet Experiment.*
11:45 Break (Coffee)
12:20 - 12:45 **G. Favrin** (Lund) *Two-State Folding Over a Weak Free-Energy Barrier.*
13:30 Break (Lunch)

Afternoon Session:

Chairman: **Pierpaolo Bruscolini** (BIFI, Zaragoza)
16:00 - 16:55 **R. Tripiccione** (Ferrara) *What did we Learn in 20 Years of Dedicated Computing for Theoretical Physics.*
17:00 - 17:25 **F. Piazza** (Lausanne) *Freezing Immunoglobulins to See Them Move.*
17:30 Break (Coffee)
Chairman: **Víctor Martín-Mayor** (Complutense Madrid and BIFI)
18:00 - 18:25 **J. Sancho** (BIFI, Zaragoza) *Stability of Native and of Partly Unfolded Proteins: New Concepts and Experiments.*

PHYSICS OF COMPLEXITY

18:30 - 19:25 **W. Kob** (Montpellier) *On the Dynamics of a Rigid Rod in a Disordered Environment.*
21:00 Banquet and Aragon Musical at the Goya Hotel

Thursday, February 12, 2004

PHYSICS OF COMPLEXITY (CONTINUED)

Morning Session:

Chairwoman: **Liliana Arrachea** (Dresden and BIFI)

09:30 - 10:25 **A.P. Young** (Santa Cruz) *Recent Developments in the Theory of Spin Glasses.*

10:30 - 10:55 **G.A. Appignanesi** (Bahía Blanca) *Activated dynamics and timescale separation within the landscape paradigm: signature of complexity, diversity and glassiness.*

11:00 Break (Coffee)

Chairman: **Francisco Guinea** (ICMM, CSIC and BIFI)

11:30 - 12:25 **E. Marinari** (Roma) *Critical Thermodynamics of 2D Ising Spin Glasses.*

12:30 - 12:55 **J.J. Ruiz-Lorenzo** (Badajoz and BIFI) *Phase Transition in Tensionless Surfaces.*

13:00 - 13:25 **V. Shelest** (Novosibirsk) *Optimal Numerization of Symbol Sequences for Spectral Analysis: a Novel Approach to Study Gene Promoters.*

13:30 Break (Lunch)

Afternoon Session:

Chairman: **Ramón Álvarez-Estrada** (Complutense Madrid and BIFI)

16:00 Poster Session

18:30 - 18:55 **P.P. Bruscolini** (BIFI, Zaragoza) *>The Finkelstein model for protein folding: Mean Field Approaches and Numerical Simulations.*

19:00 - 19:25 **V. Martín Mayor** (Complutense Madrid and BIFI) *Supercooled liquids and glasses: on the relationship between high-frequency dynamics and Aging phenomena.*

20:30 Reception at the City Hall by the Mayor of Zaragoza

Friday, February 13, 2004

MOLECULAR DESIGN (FROM THE GENOME TO PROTEINS AND DRUGS) AND DEDICATED COMPUTING

Morning Session:

Chairman: **José María Sancho** (Barcelona)

09:30 - 09:55 **C. Verma** (Singapore) *Protein Stability and Ligand Binding: New Paradigms from In-Silico Experiments.*

10:00 - 10:25 **A. Trovato** (Padova) *Geometrical Model for the Native-State Folds of Proteins.*

10:30 - 11:25 **H.J. Boehm** (Roche) *New Approaches to Structure-Based de Novo Design.*

11:30 Break (Coffee)

Chairman: **José Manuel Sánchez-Ruiz** (Granada and BIFI)

12:00 - 12:55 **M. Amzel** (Johns Hopkins) *Molecular Mechanics/Dynamics Calculations of Biochemical Processes.*

13:00 - 13:55 **E. Freire** (Johns Hopkins and BIFI) *Development of Potent Inhibitors of the SARS Associated Coronavirus Protease 3CL^{pro}*

14:15 Break (Lunch)

CALORIMETRY WORKSHOP - FRIDAY, FEBRUARY 13, 2004

16:30 - 17:10 **A. Velázquez-Campoy** (Zaragoza and BIFI) *Isothermal titration calorimetry of high-affinity interactions.*

17:10 - 17:50 **J.E. Ladbury** (London) *Thermodynamic-structural correlation in biomolecular interactions.*

17:50 - 18:10 Break (Coffee)

18:10 - 18:50 **A. Cooper** (Glasgow) *An alternative view of heat capacity effects on protein folding and interactions.*

18:50 - 19:30 **J.M. Sánchez-Ruiz** (Granada and BIFI) *Differential scanning calorimetry of proteins: studies on barrierless folding, residual structure in denatured states and protein evolution.*

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Part I

Oral Presentations

EXPLORING THE PROTEIN FOLDING FUNNEL LANDSCAPE: THEORY MEET EXPERIMENTS.

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Globally the energy landscape of a folding protein resembles a partially rough funnel. The local roughness of the funnel reflects transient trapping of the protein configurations in local free energy minima. The overall funnel shape of the landscape, superimposed on this roughness, arises because the interactions present in the native structure of natural proteins conflict with each other much less than expected if there were no constraints of evolutionary design to achieve reliable and relatively fast folding. The kinetics of folding is best considered as a progressive organization of an ensemble of partially folded structures through which the protein passes through on its way to the folded structure.

The folding mechanisms for several fast folding proteins can be quantitatively described using an energy landscape theory to set up the correspondence with simulations of protein minimalist models. Using these simulations together with analytical theory, we can learn about good (minimally frustrated) folding sequences and non-folding (frustrated) sequences. An important idea that emerges from the energy landscape theory is that subtle features of the protein landscape can profoundly affect the apparent mechanism of folding. The relationship between various characteristic temperatures in the phase diagrams and landmarks in the folding funnel at fixed temperatures can be used to classify different folding behaviors. Experiments on the dependence of the folding and unfolding times, and the stability of these proteins to denaturant concentration and site-directed mutagenesis, and on the early events of folding allow us to infer the global characteristics of the energy landscape.

In addition to need to minimize energetic frustration, the topology of the native fold also plays a major role in the folding mechanism. Some folding motifs are easier to design than others suggesting the possibility that evolution not only selected sequences with sufficiently small energetic frustration but also selected more easily designable native structures. We have demonstrated for several proteins (such as CI2 and SH3) that they are

sufficiently well designed (i.e., reduced energetic frustration) that much of the heterogeneity observed in their transition state ensemble (TSE) is determined by topology. Topological effects go beyond the structure of the TSE. The overall structure of the on-route and off-route (traps) intermediates for the folding of more complex proteins is also strongly influenced by topology. Utilizing this theoretical framework, simulations of minimalist models and their connections to more computationally-expensive all-atom simulations, we are now in the process of obtaining a quantitative understanding of the folding problem, which allows for a direct comparison to a new generation of folding experiments.

TWO-STATE FOLDING OVER A WEAK FREE-ENERGY
BARRIER

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Many small proteins fold in a two-state manner, the rate-limiting step being the passage of the free-energy barrier separating the unfolded state from the native one. The free-energy barrier is, however, weak or absent for the fastest-folding proteins. Here a simple diffusion picture for such proteins is discussed. It is tested on a model protein that makes a three-helix bundle. Assuming the motion along individual reaction coordinates to be diffusive on timescales beyond the reconfiguration time for a single helix, it is found that the relaxation time can be predicted within a factor of two. It is also shown that melting curves for this protein to a good approximation can be described in terms of a simple two-state system, despite the absence of a clear free-energy barrier.

"WHAT DID WE LEARN IN 20 YEARS OF DEDICATED
COMPUTING FOR THEORETICAL PHYSICS".

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In several areas of physics, numerical simulation and modeling have become key tools to compute accurate physics results. In some cases, such as in Lattice Gauge Theories, computing requirements have been so huge, in the last 15 years or so, that physicists have started to develop their own dedicated and optimized computer systems.

In my talk, I review the main results of this endeavour, trying to point out the basic reasons that have made this effort successful. I then discuss in which ways, and under which conditions, the expertise gained in this field can be applied to other areas of research.

Bongini, L.(1); Fanelli, D.(2); Piazza, F.(3);
De Los Rios, P.(3); Sandin, S.(2);
Skoglund, U.(2)

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(2) Cell and Molecular Biology Department. Karolinska Institutet.
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(3) Laboratoire de Biophysique Statistique - ITP. EPFL. Lausanne.
Switzerland.

The issue of protein dynamics and its implications in the biological function of proteins are arousing greater and greater interest in molecular biology. In Cryo-Electron Tomography experiments one takes several snapshots of a given biological macromolecule. In principle, a large enough collection of snapshots may then be used to calculate its equilibrium configuration in terms of the experimentally accessible degrees of freedom, and hence estimate its potential energy. Consequently, one could analyze the biological functions of biomolecules by directly accessing their dynamics.

In this work, we analyze the results of Cryo-Electron Tomography experiments on monoclonal murine IgG2a antibodies. With the aid of a novel software for image processing, we measure the equilibrium distribution of the angles which describe the configuration of the molecule. This helps us shed some critical light on recent results from X-ray crystallography. We then build a model of the antibody dynamics, which enables us to use the measured angular distribution in order to derive an explicit expression of the IgG potential energy.

Finally, we study the biological functions of a single antibody in solution, based on its Langevin dynamics. In particular, we investigate the dynamical effects in the formation of an antigen-antibody complex. Chiefly, their role in the rate of formation of the encounter complex.

Sancho, J.; Campos, L.A.; Bueno, M.; Cuesta, S.;
Falo, F.; López, J.

Biocomputation & Complex Systems Physics Institute.
Univ. of Zaragoza

The physical basis of native protein stability is still poorly understood. On the other hand, the stability of partly unfolded protein conformations has received little attention. We will show a new approach that allow to quantitate incremental binding energies of residue pairs from experiment, and will use it to determine the contribution to protein stability of a hydrogen bond in a model protein. Besides, we will discuss the concepts of relevant and residual protein stability (as applicable to proteins with equilibrium intermediates) and present mutational studies that demonstrate how well-respected strategies of overall protein stabilisation ($N \leftrightarrow D$) can fail to significantly increase the relevant stability (of the $N \leftrightarrow I$ equilibrium). Finally, a new method of structural determination of equilibrium partly unfolded conformations, based in fi-analysis, will be introduced and exemplified with the structure of an apoflavodoxin thermal intermediate.

ON THE DYNAMICS OF A RIGID ROD IN A DISORDERED ENVIRONMENT

Kob, W.

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We study the relaxation dynamics of a hard rod that moves in a disordered two-dimensional array of frozen scatterers of finite size. Two types of environments are considered: The first one is given by a random configuration of scatterers that has been generated via a Poisson process (thus the problem is a generalization to rods of the standard Lorentz gas) and the second one is a liquid-like structure. Using molecular dynamics computer simulations, we investigate how the translation and orientation dynamics of a rod depends on its length L and the density ρ of the scatterers. We find that with increasing density or/and rod length the dynamics slows down quickly, although no sign for a real dynamical arrest is detected in the (L, ρ) -range investigated. Using kinetic arguments we obtain, for the case of the liquid-like environment, the functional form of how the translational and rotational diffusion constants depend on L and ρ . We find that depending on the value of the product $L^2 \rho$, the dynamics changes qualitatively in that it makes a crossover from a weakly hindered diffusive-like motion to a dynamics that is dominated by the orientational degree of freedom. This coupling between the translational and orientational degrees of freedom leads also to a strong non-Gaussian dynamics that can be observed even at length-scales that are about 30 times larger than the length of the rod. Finally we discuss the applicability of the Stokes-Einstein relation for this system and show that it holds only at sufficiently low density.

RECENT DEVELOPMENTS IN THE THEORY OF SPIN
GLASSES

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After an introduction to spin glasses, recent developments in the theory will be described. A discussion will be given of rival phenomenological theories of the spin glass state, and a comparison between them and results of numerical simulations will be made. Whereas most simulations use discrete "Ising" spins, real spins in many magnetic systems have a vector character, and discussion will be given of whether there is significant difference in behavior between these two types of spin glass.

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Rodriguez Fris, J.A.; Montani, R.A.

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The landscape paradigm has become a widespread picture within the realm of complex systems ranging from glasses to biopolymers. Within this framework, the dynamics can thus be envisioned as a search the system performs on its (potential energy) landscape. This approach rests on the belief that the relaxation behavior depends only on generic features as topography, irrespective of specific detail (a point supported by the fact that a vast amount of very diverse complex systems display a common dynamical behavior) and lies on the validity of a timescale separation scenario (not properly validated yet from first principles).

Within this context, the prevalence of activated dynamics over other kinds of mechanisms will be shown to determine the emergence of complex dynamical behavior. Thus, complexity and diversity are not intrinsic properties of a system but depend on the kind of exploration of the landscape. We shall focus mainly on an ample generic context (complex hierarchical systems which have been used as models of glasses, spin glasses and biopolymers) and a specific one (model glass formers). For the last systems we shall be able to reveal (in mechanistic terms) the microscopic rationale for the occurrence of timescale separation. Furthermore, we shall explore the connections between the two contexts and the relation to a variational principle, and we shall reveal the conditions for the applicability of the landscape approach.

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We compute the exact partition function of 2D Ising spin glasses with binary couplings. In these systems, the ground state is highly degenerate and is separated from the first excited state by a gap of size $4J$. Nevertheless, we find that the low temperature specific heat density scales as $\exp(-2J/T)$, corresponding to an "effective" gap of size $2J$; in addition, the associated length scale grows as $\exp(J/T)$. We justify these scalings via the degeneracy of the low lying excitations and by the way low energy domain walls proliferate in this model.

Cuerno, R. (1); Moro, E.(1);
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(2) Departamento de Fisica. Universidad de Extremadura. Badajoz. Instituto de Biocomputacion y Fisica de los sistemas complejos (BIFI).

The surfaces which grow in Molecular Beam Epitaxy (when the main relaxation mechanism is surface diffusion) can be described assuming no surface tension and can be modeled by the (discrete) bilaplacian model. This model was introduced by Nelson in the context of the two dimensional melting and he predicted two Kosterlitz-Thouless phase transitions. We will describe different analytical approaches based in the Renormalization group and in variational techniques. Finally we will report results from numerical simulations.

Shelest, V.I. (1); Kel, A.E. (2)

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Spectral analysis of DNA sequences is one of the approaches used for detecting latent periodicities. It is well known that the peaks in power spectrum of protein coding regions of genes are related to 3-bp periodicity, the peaks in power spectrum of some complete genomes are related to 11-bp periodicity. The power spectra of long DNA sequences (more than 1000 bp) was shown to be similar to 1/f noise and it is similar to white noise for small sequences (less than 100 bp).

To take the power spectrum of a symbol sequence we must undertake the procedure of numeralization, i.e. to put univocal correspondence between each symbol and some numerical value. It can be done in infinite number of ways, the power spectrum being not invariant with respect to any variant of the numeralization. The usual approach is not to deal with infinite number of variants, considering the symbolic sequence by decomposing it into binary sequences of each symbol and investigating the spectra of mutual correlation functions. We show that it is possible to find such numeralization which gives very specific profiles of power spectra of the initial sequence.

We search for optimal numeralization which maximizes the difference between power spectrum of the sequence and spectrum of white noise. We have analysed a number of sets of regulatory DNA sequences. The obtained results have been applied to improve accuracy of the search for potential transcription factor binding sites and to classify promoter sequences.

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In 1999, Galzitskaya and Finkelstein proposed a simple model to describe the folding of proteins of known native state and identify their folding nucleus. In their analysis, in order to make the exploration of the configuration space easier, they resorted to the so-called "triple-sequence" approximation.

In a recent paper we have proved, for a different but related model, that this kind of approximation is rather defective. For this reason, in this work we test some alternative (mean-field) approximation schemes, applying them to the study of a short beta-hairpin, where a comparison with exact results from an exhaustive enumeration is also possible.

Martín-Mayor, V.

Universidad Complutense de Madrid (Spain) - BIFI

After a short description of the intriguing phenomenon known as "the glass transition" (both from the static and the dynamic point of view), we shall briefly present recent theoretical ideas about the possibility of studying the glass transition by means of inelastic neutron and X-ray scattering experiments in the Terahertz range.

Verma, C.

(1) Structural/Functional Genomics Group, Bioinformatics Institute,
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Computer simulations are used to investigate two features of proteins: ligand entry/exit and ligand binding. Both reveal surprising new insights into the physics of such complex systems and suggest at possible interpretations that depart from the usual paradigms. Ligand binding pathways are mapped and it is found that in agreement with recent interpretations of experimental data emerging from NMR studies, these pathways are characterized by a ruggedness of the energy landscape which leads to a picture that is intuitively more appealing than the traditional two-state paradigm normally invoked for ligand binding. In parallel, a ligand binding study suggests that contrary to the perceived notion that ligand binding induces a tightening of the protein (as would be evidenced by a blue shift in its vibrations), some cases actually lead to an increase in the entropy through a red-shift in its vibrational spectrum.

GEOMETRICAL MODEL FOR THE NATIVE-STATE FOLDS OF
PROTEINS

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Banavar, J.R.(4); Maritan, A. (1,3,5)

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(2) Institute of Physics, NCST. Hanoi. Vietnam

(3) INFN and University of Padova. Italy

(4) Department of Physics. The Pennsylvania State University. USA

(5) SISSA and INFN. Trieste. Italy

We present a simple physical model which demonstrates that the native state folds of proteins can emerge on the basis of considerations of geometry and symmetry. We show that the inherent anisotropy of a chain molecule, the geometrical constraints placed by the hydrogen bonds and sterics, and hydrophobicity are sufficient to yield a free energy landscape with broad minima. These correspond to marginally compact structures comprising the menu of folds that proteins choose from to house their native-states in. Our results provide a general framework for understanding the common characteristics of globular proteins.

Boehm, H.-J.

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The 3D structure of a target protein can be used to design new compounds and chemical libraries with significantly enhanced hit rates. Several examples will be presented to highlight the current status of the field. Recent improvements in scoring function further enhance the hit rates.

Increasingly, physicochemical and pharmacokinetic properties are taken into account in the library design process. The goal is to design compounds that bind to the target protein and have the right "drug-like" properties. Predicted properties include lipophilicity, membrane permeability, solubility, protein binding but also certain aspects of metabolism and toxicity. Combined with novel rapid three-point pharmacophore based searching methods, it is possible to process large virtual libraries.

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The ability to compute intra-and inter-molecular interactions provides the opportunity to gain a deeper understanding of previously intractable problems in biochemistry and biophysics. In this presentation three such problems and their solutions will be presented:

1) estimation of the loss of translational entropy experienced by small molecules when they bind to proteins; 2) estimation of the entropic contribution to reaction rates that result from conformational constraints imposed on the reactants; and 3) the mechanism of unfolding a small protein by the application of a force that separates the N- and C-termini.

For problem 1, a series of small molecules were embedded in a large water bath and long molecular dynamics (MD) runs were used to provide "snapshots" configurations used to calculate the configurational integral. An empirical function was introduced that correlated the entropies estimated by this procedure with known properties of the small molecules.

Published experimental data show that the rate of intramolecular esterification reactions increases when the reactant groups are constrained by the intervening portions of the molecule. In problem 2, we evaluated the entropic contributions to these rate enhancements by an adiabatic mapping of conformational space. The calculations show that entropic effects are major contributors to the observed rate enhancements.

In problem 3, we analyzed the mechanism of unfolding of a small protein by a force applied between its N- and C-termini. The analysis was carried out by adiabatic mapping using long MD computations to equilibrate intermediate states between the folded protein and the fully extended state.

DEVELOPMENT OF POTENT INHIBITORS OF THE SARS
ASSOCIATED CORONAVIRUS PROTEASE 3CL^{pro}

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SARS (severe acute respiratory syndrome) is caused by a newly discovered coronavirus. A key enzyme for the maturation of this virus and, therefore, a target for drug development is the main protease 3CL^{pro}. We have cloned and expressed in E. Coli the full length 3CL^{pro} from the SARS associated coronavirus. The recombinant protein has been characterized enzymatically using fluorescently-labeled substrates and its structural stability has been analyzed by differential scanning calorimetry. 3CL^{pro} is composed of two domains, a predominantly beta domain with a fold reminiscent of the one found in proteases from the chymotrypsin family and containing the catalytic site, and an alpha helical domain. The two domains interact strongly forming a single cooperative unit. The active site contains a catalytic cysteine and histidine in analogy with other coronavirus proteases. Analysis of the active site cavity reveals the presence of subsites that can be targeted with specific chemical functionalities. This analysis has allowed us to design a first generation of powerful inhibitors of the SARS 3CL^{pro} protease. Isothermal titration microcalorimetric experiments indicate that these inhibitors bind reversible to 3CL^{pro} in an enthalpically favorable fashion, implying that they establish strong interactions with the protease molecule, thus defining an appropriate scaffold for further optimization.

NOTES

Part II

Posters

(ordered by first author's last name)

A NEW WAY OF ACCOUNTING FOR THE ELECTROSTATIC
CONTRIBUTION OF IMPLICIT SOLVENT MODELS

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A generally accepted way of dealing with implicit water in simulations of macromolecules relies on splitting the non-polar part of the solvent-solvent and solvent-solute interactions from the electrostatic part. The former is usually modeled through the solvent accessible area defined by Lee and Richards, this approach showing a considerable success. The electrostatic part, on the other hand, has proved more resistant to be simply described in terms of some natural variable analog to the solvent accessible area. Even the solution of one of the simplest models (namely, treating water as a dielectric continuum and the molecule as a cavity, the border being the molecular or Van der Waals surface) requires to solve the Poisson equation to give an exact result. This is a very heavy computational task and many methods, such as PCM, IC, ASC, FDM, FEM and others, have been designed to circumvent it showing different degrees of success.

Here, we present a new simple way of approximating the free energy function that would come out of the exact solution of the Poisson equation. The new method uses the fact that the one-body terms of this energy seem to be a function of a certain volume of influence defined for each atom, at least for cavities with sizes, charges and shapes typical of those found in proteins. The main advantage of this method is its very low numerical complexity, which makes it suitable for simulation of macromolecules.

'DISORDER UNIVERSALITY': THE CASE OF DNA
DENATURATION

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The aim of this presentation is to show that in mathematical models of natural phenomena in which disorder has a relevant role (as in a large number of biological systems), it is often more important to include the effect of disorder in the correct way than taking special care of the details of the model. This is what we call *disorder universality*. As an example, we study the effect of the genetic sequence of DNA in the denaturation temperature of the molecule. Although the denaturation transition is correctly described by the Peyrard-Bishop model, we will show that a much simpler model, proposed by Chui and Weeks in 1981 in the framework of depinning and wetting transitions, is able to reproduce correctly the dependence of the denaturation temperature with the composition of the molecule, provided the sequence's inhomogeneity is taken correctly into account by the model. This model is analytically solvable and much easier to study using computer simulations than the Peyrard-Bishop model, letting us gain further insight into important aspects as the effects of correlations in the genetic sequence.

MICROSCOPIC THEORY OF VIBRONIC DYNAMICS IN
LINEAR POLYENES

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Photoexcitation in polyatomic molecules leads to the rapid mixing of vibrational and electronic motions, inducing charge redistribution and energy flow in the molecule. This non-adiabatic internal conversion is essential in photochemical processes, in photobiological processes, such as those involved in vision.

We propose a novel approach to calculate dynamical processes at ultrafast time scale in molecules in which vibrational and electronic motions are strongly mixed. The relevant electronic orbitals and their interactions are described by a Hubbard model, while electron-phonon interaction terms account for the bond length dependence of the hopping and dependence of the bond length on the local charge. The latter term plays a crucial role in the non-adiabatic internal conversion process of the molecule.

The time resolved photoelectron spectra are in good qualitative agreement with experiments.

Ref: Phys. Rev. B 67, 134307 (2003)

INFINITE-RANGE QUANTUM RANDOM MAGNETS

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We study the behavior of two archetypal quantum spin-glasses by exact diagonalization techniques, the random Ising model in a transverse and longitudinal fields and the random Heisenberg $S=1/2$ model.

We calculate the critical temperature T_g for the spin-glass to paramagnetic transition. We obtain $T_g \sim 0.13$, in good agreement with previous quantum Monte Carlo and analytical estimates. We provide a detailed picture for the different kind of excitations which intervene in the dynamical response at $T=0$ and analyze their evolution as T increases. We also calculate the specific heat $C_v(T)$. We find that it displays a smooth maximum at $T_M \sim 0.25$, in good qualitative agreement with experiments. We argue that the fact that $T_M > T_g$ is due to a quantum disorder effect.

We also study the spin glass to paramagnet transition of the transverse degrees of freedom in the presence of finite longitudinal field. We estimate the size of the critical region and characterize various crossover regimes. An unexpectedly small energy scale on the disordered side of the critical line is found, and its possible relevance to experiments on metallic glasses is briefly discussed.

Ref: Phys. Rev. Lett. 86, 5172 (2001); Phys. Rev. B 65, 224430 (2002); cond-mat/0302390

SYSTEMATIC SEARCH FOR COMPACT STRUCTURES OF TELOMERIC
NUCLEOSOMES

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Telomeres are structures functionally and structurally distinct from bulk chromatin. They are constituted of highly conserved 5-7 bp tandemly repeated units, organized into nucleosomes with short linkers, whereas the knowledge of the linker histone role in telomeric chromatin is still fragmentary. Experimental evidence suggests the structural organization of telomeric nucleosomes is different from that of the bulk chromatin. This work presents a systematic search of the telomeric nucleosome arrangements. To perform a wider conformational search, we adopted the two-angle Woodcock model of the fibre, but assuming the dinucleosome as the repetitive unit. A low-resolution molecular model was used to evaluate the relative nucleosome packing energy. The model has the advantage of describing the chromatin geometry in terms of a few parameters, which characterize the single nucleosomal structure. In addition, specific sequence-dependent effects could be treated as deviations from the basic model. Structures with non-overlapping nucleosomes were found, reducing the possible telomeric chromatin conformations to two different three-dimensional folds.

CHARACTERIZING THE ENERGETIC LANDSCAPE OF
MODEL PROTEINS

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We study the topology of the energetic landscape of an off-lattice model protein for different sequences.

Recent researches show that the dynamic of a protein between the folding and the glassy temperatures can be summarized as a harmonic vibration around local minima of the potential energy plus an activated process of jump between the basins of attraction of different minima.

Transitions between different basins implies passing through low energy (and thus low order) saddles. The identification of the most thermodynamically relevant minima and first order saddles of the potential allows to define a connectivity graph whose nodes are minima and whose edges are saddles. Connections in this graph are weighted by the activation rate for the jump the corresponding minima. An algorithm is presented to efficiently search for first order saddles. Statistical properties of the connectivity graph give usefull insight on the folding capability of different sequences.

WHAT WORKS (AND WHAT DOESN'T) IN ESTIMATING THE
CHANGE IN FREE ENERGY DUE TO POINT MUTATIONS IN
THE PROTEIN INTERIOR

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We report on a comprehensive thermodynamic and computational analysis of over 200 single amino acid mutations in the protein interior. We show that the main contributions to the change in free energy are due to entropy of the unfolded state (25 % of the total free energy change), and by the change in electrostatics and solvation of the folded state (70 %). The effective contribution of van der Waals (vdW) is consistently found to be less than a tenth of the change of vdW in the folded state. Our analysis suggests that side chain configurational entropy of the folded state might be less important than backbone entropy. A structural parametrization of the folding energetics that includes the electrostatic and solvation energy of the folded state as estimated by CHARMM using a continuum electrostatic approximation minus a conformational entropy term and the standard change of solvent accessible area times an hydrophobic parameter fits well all experimental values within an error bar of 1.5 kcal/mol. For charged or polar mutations, residues are always partially solvated, this approximation seems to be inappropriate since explicit water molecule interactions should be very different between the wild type and the mutants.

A DOUBLE-DELETION METHOD TO QUANTIFY
INCREMENTAL BINDING ENERGIES IN PROTEINS.
EXAMPLE OF A SURFACE HYDROGEN BOND STUDIED BY
EXPERIMENTAL TECHNIQUES AND MOLECULAR
DYNAMICS.

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Although hydrogen bonds are the major determinants of protein structure and function, their specific contribution to protein stability is still unclear. The best method so far devised to estimate the contribution of side-chain interactions to protein stability is double-mutant-cycle analysis, but the interaction energies derived from this method are not identical to incremental binding energies (the net contribution of any two interacting groups to protein stability). Instead, for hydrogen bonds, double-mutant cycle interaction energies represent upper limit values. Here we introduce double-deletion analysis of isolated residue pairs as a means to precisely quantify incremental binding, exemplified by the study of a surface-exposed hydrogen bond in a model protein (the Asp96/Asn128 pair in apoflavodoxin). Substitution of these residues by alanines slightly destabilizes the protein, due to an overall decrease in hydrophobic surface burial. Subtraction of this effect, thanks to the calculation of solvent accessible surfaces of the wild type and the double mutant structures obtained by molecular dynamics, clearly indicates that the hydrogen-bonded groups in fact destabilize the native conformation. Our analysis explains quantitatively that, due to frustration, the hydrogen bond must form nevertheless because, when the two groups get approximated to the rest of the protein upon folding, they become partly desolvated and their binding is favorable.

DESIGN AND PRELIMINARY STRUCTURAL AND
THERMODYNAMIC ANALYSIS OF A CHIMERIC SH3
DOMAIN

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SH3 domains interact with proline-rich sequences with moderate affinity. We have used protein-engineering methods to design a chimeric SH3 domain, which includes in its sequence the p41 peptide (APSYSPPPPP) in such a way that it docks into the binding pocket. To do so we started with a circular permutant of the alpha-spectrin SH3 domain (cpSH3), previously designed by us, and extended its sequence from the C-terminus with 13 additional residues, which include a 3-residue linker plus the p41 sequence. We refer to this chimera as cpSH3-p41. We have made a preliminary characterization of the structural and stability properties of cpSH3-p41 in comparison with cpSH3 using differential scanning calorimetry (DSC), circular dichroism (CD) and fluorescence. cpSH3-p41 is more stable than cpSH3 by 7-10 °C and the structural data indicate that the p41 sequence is adopting a well-ordered poly-proline II helix in cpSH3-p41, similarly to what is usually found in the structures of the complexes between proline-rich ligands and SH3 domains. We have started the structure resolution of cpSH3-p41 by heteronuclear NMR. The ¹⁵N-¹H HSQC spectrum of cpSH3-p41 indicates significant chemical shift dispersion, although it also shows double HSQC peaks. This suggests the presence of conformational heterogeneity, probably motivated by some association processes occurring with this chimeric protein. We have also studied the effect of protein concentration by DSC and by dynamic light scattering (DLS).

STIFF POLYMER ADSORPTION: ONSET TO PATTERN
RECOGNITION

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Given a biological molecule that carry a pattern encoded in their sequence distribution, a properly chosen functionality surface might be able to recognize such information and adsorb to it strongly. The understanding of this process is essential to the drug design for specific targets. Despite the complexity of such molecules, much of their characteristic behavior can be easily incorporated in simple models of stiff polymer chains. We present the results of an off-lattice Monte-Carlo numerical study of the adsorption of semiflexible polymers on planar stripped-patterned surfaces. We have found that higher stiffness and increasing chain length enhances the polymer recognition. Our results also indicate that the adsorption transition foregoes the chain-surface recognition, and that chains in the adsorbed state find an optimal stripe width for their stretching.

ANALYSIS AND COMPARISON OF BACTERIAL AND
MAMMALIAN CYTOCHROME P450 BY COMPUTATIONAL
SOLVENT MAPPING

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The computational placement of molecular probes - solvent molecules, such as phenol, isopropanol, acetonitrile, or small natural ligands - can identify favorable binding positions on the surface of a protein. We have applied these methods to two well-characterized bacterial members of the cytochrome P450 (CYP) superfamily, CYP101 and CYP102, and to the available mammalian CYP structures for 2B4, 2C5, and 2C9. Although these proteins have a common structural core, they have very little sequence conservation aside from key residues responsible for binding the heme to the polypeptide chain. For the bacterial proteins in both an unbound and bound conformation, we found the largest consensus sites to be in the ligand-binding channel. The consensus sites for the bound mammalian structures agree with the co-crystallized ligand positions. The unbound mammalian structures also have the largest consensus site in the binding pocket, but the distinction between the best consensus site and false positives is not as clear. We conclude that our method can detect the differences in the active site residues that confer different substrate specificities between bacterial and mammalian P450s. We may also be able to use our methods to identify important residues for future experimental research.

Supported in part by Superfund Basic Research Program at Boston University, NIH grant 5-P42-ES07381.

TOWARDS A NEW THERAPEUTIC TARGET: *Helicobacter pylori*
FLAVODOXIN

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Helicobacter pylori is a gram-negative bacterium related to different digestive diseases. Nowadays, up to 50% of the world human population is chronically infected. Recently, the resistance to antibiotic has increased, so new molecular targets are needed to develop new drugs against the pathogen. The *H. pylori* genome contains a redox protein which is not present in human beings, the flavodoxin, which has been demonstrated to be essential for the survival of the bacterium. Flavodoxins are electron transfer [alpha]/beta proteins. For that purpose they carry a flavin molecule (FMN) non-covalently bound that confers redox properties to the protein. In spite of the structural similarity with other flavodoxins, the *H. pylori* flavodoxin presents a significantly different cofactor binding site. A highly conserved triptophan is replaced by an alanine which leads to the creation of a cavity next to the FMN. We anticipate that small molecules can bind in this cavity and inhibit the redox activity of the FMN. In order to advance towards the design of new inhibitors either rational or irrationally, we study here the apo, holo flavodoxin and the binding of its cofactor. Furthermore, our group has studied the flavodoxin from *Anabaena* for several years, and the homology between them is larger than 60%. Hence, another important fact can be contrasted: the degree to which moderate sequence differences can influence the details of the folding and binding equilibria.

'IN SILICO' ANALYSIS OF THE STRUCTURAL PROPERTIES
OBSERVED IN A HUMAN R-LDL BINDING MODULE.

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Familial Hypercholesterolemia (FH) is an autosomal genetically dominant disorder affecting one in 500 people world-wide, and resulting from any of a number of mutations in the gene encoding the r-LDL. We have focused our attention in the fifth module of the ligand binding domain of this receptor (pdb 1ajj), which adopts a characteristic polypeptide fold in presence of calcium, and it's essential in the binding of both APO E and APO B-100.

Using different Molecular Dynamics techniques, like Nose-Hoover and Langevin dynamics, we have analysed the structural role played by different mutations detected in FH patients. Our results indicate that the original protein conformation is lost or severely distorted in many of the most commonly found pathology-related mutations.

In addition, we have shown that protein stability and functional folding are linked to the presence of a calcium binding site. A fast unfolding, through a sequence of stable states, is observed after an instantaneous removal of the cation, which shows its importance in the stabilization of the protein conformation.

STRUCTURE DETERMINATION OF AMYLOID FIBRILS WITH AB INITIO
CALCULATIONS OF NMR CHEMICAL SHIFT

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The term amyloid fibril refers to a protein that has self-assembled into an insoluble pleated sheet structure. These structures are noncrystalline, formed by peptides and proteins and associated with diseases including Alzheimer's disease and prion diseases. Here we seek to apply quantum mechanical ab initio techniques to the structure determination of transthyretin (TTR) in an amyloid fibril. Specifically, an 11-residue peptide fragment, (105-115), which represents a high level of computational complexity. As a preliminary study, we calculated C, N and H shielding tensors and chemical shifts of Ala-containing peptides in different conformations in order to establish a correlation between shift and local configuration. Consequently, I present here different possible configurations of the peptide and the method used to discriminate them. These first results of our calculations are an initial attempt to refine and elucidate the final structure of TTR (105-115) peptide.

EVOLUTIONARY METHOD FOR THE ASSEMBLY OF
ELEMENTS OF SECONDARY STRUCTURE

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A new Genetic Algorithm (GA)-based method has been developed to approach the protein folding problem. We intent the minimization of the global energy for a series of all- α proteins. Representations of the backbone of the helical segments, that are regarded as rigid bodies, is limited to the positions of the α carbons, which are treated as interaction centers. The distance between contiguous helices is limited in order to keep the connectivity of the peptide chain. Classic GA operators, namely reproduction, crossover and mutation, with a flexible definition of the size of the population have been used.

The performance of the method has been tested using the RMSD between the PDB structure and the predicted one as the fitness function of the evolutionary algorithm. A new strategy, which combines the best individuals from different initial populations run in parallel, has shown to dramatically improve traditional problems in GAs, such as lack of convergence or a doubtful definition of the simulation length.

The energy of each arrangement of the helical segments generated along the simulation is computed as the sum of pair wise interactions between amino acids in different helices. Contact terms obtained by Miyazawa and Jernigan are used in a Lennard Jones-like function. Promising results for various proteins have been obtained. They provide an efficient method for the testing of different contributions of mean field potentials used in protein structure prediction.

CLASSIFICATION OF AMINO ACIDS INDUCED BY
STATISTICAL POTENTIALS.

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We study the classification of amino acids induced by different statistical potential matrices. This continues the project started in a previous paper where the authors establish a fully unsupervised method of cluster decomposition for amino acids. The method uses the subdominant ultrametric associated to the distance between amino acids induced by the potential matrix, then the maximum likelihood principle determines the cluster structure. In this comparative study, by looking at the classifications obtained from diverse statistical potentials, we can get information on how the latter account for the different chemical-physical properties of the amino acids.

ON FOLDING AND FORM: INSIGHTS FROM LATTICE
SIMULATIONS

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We present recent results on the folding kinetics of small (with less than 120 amino acids), single domain proteins obtained in the context of a simple Miyazawa-Jernigan lattice-polymer model and Monte Carlo simulations. Depending on the chain length, two dynamical regimes were identified. We focus on the first regime associated with smaller chains and well modeled by two-state kinetics [PFN Faisca et al., J. Chem. Phys. 112, 8587 (2002)]. We explore the connection between some native state properties and the timescale of folding with particular emphasis on the dependence of the folding time on the relative contact order parameter [PFN Faisca et al., J. Chem. Phys. 116, 7231 (2002)]. We proceed to show that, for a fixed chain length, two different folding mechanisms are at work depending on the relative contact order parameter [PFN Faisca et al., submitted to Phys. Rev. E (2003)].

RELATION BETWEEN PROTEIN STABILITY, EVOLUTION AND
STRUCTURE, AS PROBED BY CARBOXYLIC ACID MUTATIONS

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Native proteins are marginally stable. Low thermodynamic stability may be advantageous, although the accumulation of neutral, destabilizing mutations may have also contributed to it. In any case, once marginal stability has been reached, it appears plausible that mutations at non-constrained positions become fixed in the course of evolution (due to random drift) with frequencies that roughly reflect the mutation effects on stability ("pseudo-equilibrium hypothesis"). We have found that all glutamate->aspartate mutations in wild-type E. coli thioredoxin are destabilizing and that so are most of the aspartate->glutamate mutations. Furthermore, the effect of these mutations on thioredoxin thermodynamic stability shows a robust correlation with the frequencies of occurrence of the involved residues in several-hundred sequence alignments derived from a BLAST search. These results provide direct and quantitative experimental evidence for the pseudo-equilibrium hypothesis and should have general consequences for the interpretation of mutation effects on protein stability, as they suggest that residue environments in proteins may be optimized for stabilizing interactions to a remarkable degree of specificity. We also provide evidence that such stabilizing interactions may be detected in sequence alignments, and discuss the implications of this possibility for the derivation of structural information (on native and denatured states) from comparative sequence analyses.

CHAOTIC DYNAMICS IN A GENE NETWORK MODEL

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A gene network is constructed by making links between pairs of genes when they are known to interact with each other. The connectivity distribution $P(k)$ (the probability that a gene has k links) reveals a power law distribution (Scale Free network). For generating numerically scale-free networks we use the method developed by Barabasi and Albert.

The interaction between linked nodes (interacting genes) is modeled using the Michaelis-Menten model:

$$\delta g_i / \delta t = -g_i + \gamma (\Phi(\sum_j h W_{ij} g_j)) / (\Phi(\sum_j h W_{ij} g_j) + 1)$$

where W_{ij} can be 0 (no interaction), 1 (activatory int.) and -1 (inhibitory int.). $\Phi(z)$ is defined as $\Phi(z)=z$ if z is positive and $\Phi(z)=0$ if z is negative and the parameter h accounts for the degree of nonlinearity.

After the network is generated we assign the character of the interaction: inhibitory with a probability p and activatory with $(1-p)$. Then we integrate the model with random initial conditions and compute the largest Lyapunov exponent. This process is repeated for 1000 iterations. With this scheme we calculate for a fixed value of p and h the fraction of chaotic, periodic and steady networks.

We obtain the complete chaotic diagram in the whole (p,h) -space and analyze the structure of the dynamical islands (or motifs) in order to derive common topological characteristics for the formation of these motifs. Our conclusions are then directed to explain the robustness observed in real biological networks and hint at new connections between graph theory, nonlinear dynamics and biological processes.

A NEW IMPLICIT SOLVENT MODEL FOR
LIGAND-RECEPTOR ELECTROSTATIC BINDING FREE
ENERGY CALCULATIONS

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A correct prediction of ligand-receptor complexes structure demands an accurate quantification of every energetic contribution involved in the interaction. As binding takes place in an aqueous medium, the task is complex, particularly when accounting for the electrostatic component of the binding free energy. Water effects on electrostatic interactions are twofold: first, it effectively screens charge to charge interactions of the atoms in the system (ligand and protein); second, it directly interacts with these same charges solvating them. Among the theoretical approaches employed to implicitly model these physical effects, the numerical solution of the Poisson-Boltzmann Equation (PBE) has been shown to provide the best agreement with both explicit water simulations and experimental data. However, this approach cannot be applied to fast docking algorithms or virtual screening applications due to its computational inefficiency. The Generalized Born approach (GB) has recently emerged as a practical solution for this drawback although its applicability is still being studied. Departing from the implicit solvent model previously established by Hassan et al. (J Phys Chem B 2000, 104, 6478), which stems from GB models and polar liquids theory, we have developed, implemented and validated a new approach to compute the electrostatics binding free energy of a ligand-receptor interaction. The model provides good agreement with the PBE along with a very high computational efficiency.

ASYMPTOTIC AGING IN STRUCTURAL GLASSES

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Using a non-local Monte Carlo algorithm, we study the aging of a fragile glass, being able to follow it up to equilibrium well below the Mode-Coupling temperature and up to unprecedentedly large waiting times at lower temperatures. We show that the fluctuation-dissipation ratio is independent of the dynamics chosen and is compatible with a phase transition, and that the scaling behaviour of the aging part of the correlation supports the full-aging scenario.

FLUCTUATIONS OF EPIDEMICS NEAR A CRITICAL POINT

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We analyze some properties of the outbreaks of a disease in a population whose percentage of immunized individuals is close to the threshold needed to prevent large scale epidemics. We consider the susceptible-infected-recovered (SIR) model[1], following the analyses in[2,3]. We study the finite size effects in small populations near the critical point, and extend our results to a recent model of meningitis epidemics[2].

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CONFORMATIONAL STUDY OF EUKARYOTIC INITIATION FACTOR 4E
BINDING PROTEIN BP1.

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Eukaryotic initiation factor eIF4E is one of the components of the translational machinery. eIF4E binds to the 5'-5' m⁷GpppG cap-structure at the 5'-end of the messenger RNA and to the initiation factor eIF4G. eIF4G is a scaffold protein which binds poly(A)-binding protein, eIF4E, eIF4A and eIF3. eIF3 in turn binds the 40S ribosomal subunit during initiation of translation. Binding of the protein eIF4G to the dorsal site of eIF4E therefore allows recruitment of several other factors to the mRNA. eIF4E-binding proteins (4E-BPs) bind to eIF4E and inhibit the eIF4E-eIF4G interaction. 4E-BP1 protein is mostly unfolded in solution but adopts more helical form in complex with eIF4E protein. 4E-BP1 folding in the presence of trifluoroethanol and 1,1,1,3,3,3-hexafluoro-2-propanol was studied by CD and isothermal titration calorimetry. Presence of the isodichroic point showed that all residues are either in helical or random-coil conformation. The data obtained allowed us to calculate the thermodynamic parameters of BP-1 folding. We have also obtained that folding of the BP1 protein in the presence of TFE is not a continuous process but, perhaps, goes through number of discrete steps. The dependence of the helicity on temperature was determined allowing us to suggest that 4E-BP1 protein is very thermostable, probably due to small amount of secondary structure. High temperatures induce a transition in BP-1 resulting in a partially folded form.

STABILITY AND STRUCTURE OF THE ENZYME I OF THE
PHOSPHOTRANSFERASE SYSTEM IN STREPTOMYCES COELICOLOR

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The phosphorylation phosphotransferase system (PTS), only found in bacteria, is responsible, for the detection, migration towards and concurrent metabolic sugar phosphorylation and uptake. The PTS catalyses the transfer of an activated phosphoryl group from phosphoenolpyruvate (PEP) to the imported carbohydrate. This catalysis occurs through a cascade of five proteins from PEP via phosphointermediates of the general phosphotransferases enzyme I (EI) and the histidine-containing phosphocarrier (HPr) to substrate-specific enzyme II permeases (the so-called IIABCsug). These proteins are also involved in regulation of sugar uptake at the level of gene expression or directly influence the catalytic activities of other proteins and antibiotic regulation. In an attempt to fully characterize structurally the whole PTS, we are now focusing on the structure and stability of EI.

In *Streptomyces coelicolor*, a Gram-positive bacteria with a high GC content, the EI protein is a dimer formed by 576-residues-long monomers. We have carried out spectroscopic analysis by fluorescence, circular dichroism and FTIR trying to get insight into its structure and stability. From these studies we have estimated that the protein is composed by a mixture of β -sheet and α -helix. The thermal CD denaturation studies and fluorescence measurements, at different protein concentrations, allow an estimation of the dissociation constant of 1 mM. Thermal and chemical denaturation studies are also presented.

AGING IN SPIN GLASSES IN THREE, FOUR AND INFINITE DIMENSIONS.

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The SUE machine is used to extend by a factor of 1000 the time-scale of previous studies of the aging, out-of-equilibrium dynamics of the Edwards-Anderson model with binary couplings on large lattices ($L=60$). The correlation function, $C(t+t_w, t_w)$, being t_w the time elapsed under a quench from high-temperature, follows nicely an slightly-modified power law for $t > t_w$. Very tiny (logarithmic), yet clearly detectable deviations from the full-aging t/t_w scaling can be observed. Furthermore, the $t < t_w$ data shows clear indications of the presence of more than one time-sector in the aging dynamics. Similar results are found in four-dimensions, but a rather different behaviour is obtained in the infinite-dimensional $z=6$ Viana-Bray model. Most surprisingly, our results in infinite dimensions seem incompatible with dynamical ultrametricity. A detailed study of the link correlation function is presented, suggesting that its aging-properties are the same as for the spin correlation-function.

DEPENDENCE OF THE CONCENTRATION OF PEA LECTIN
ON THE EXPRESSION LEVEL OF THE PEA LECTIN NPTII
GENE IN TRANSGENIC OILSEED RAPE.

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Plant lectins have shown themselves as transgenic resistance factors against various insect pests. Among them, pea lectin had been selected as a potential candidate for use against pollen beetle, a serious pest of *Brassica napus*. To evaluate a potential use of pea lectin, transgenic plants of oilseed rape were produced in which the pea lectin gene (NPTII) under control of the pollen-specific promoter *Sta44-4* was introduced. Significant reduction in larval weight and in larval survival rate was observed in beetles feeding on transgenic plants. These plants were analysed by PCR and RT-PCR for the presence and expression level of the NPTII gene. Plants tested on larvae were also analysed for the pea lectin concentration using western blotting. The concentration of pea lectin was determined using the Quantity One Quantitation Bio-Rad Software. Then the concentration of total soluble protein was measured and the concentration of pea lectin for every sample was calculated in percents. It ranged up to 1.8% of total soluble protein. The data on the expression level of the NPTII gene and the concentration of pea lectin were compared and the graphs were plotted. A stable correlation between the expression level of pea lectin gene and the level of pea lectin concentration was observed. These data also were processed statistically using a polynomial regression.

FOLDING SIMULATIONS OF SMALL PROTEINS

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Understanding how a protein folds is a long-standing challenge in modern science. Computer simulations have been carried out to understand the folding mechanism. However, direct folding simulations have been mainly focused on simple models, such as lattice models or models where only native interactions are included (Go-type models). Also, investigations with an all-atom potential have been restricted to unfolding simulations. Here we use an atomistic potential whose parameters are optimised simultaneously for proteins of various structures: protein A (alpha structure), HP-36 (alpha), 1fsd (alpha/beta) and betanova (beta). Extensive Monte Carlo simulations starting from non-native conformations were performed for each protein. In all cases, collapse occurs at a very early stage. We observe that all proteins fold into their native-like conformations at appropriate temperatures. At high temperatures native-like conformations rarely appear, and at low temperatures some runs are trapped in local minima with non-native conformations. We observe that the folding mechanism is controlled by both kinetic and thermodynamic factors: The way a protein folds into its native structure, is determined by the convergence point of early folding trajectories relative to the native state. Our results provide the unifying principle behind those in the literature, and yield new insights into the folding mechanism.

THE LONG RETURN FROM DNA TO DMC.

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The question on the mechanism of gene expression appear any years later after the discovery of DNA double helix . The most prevalence have got idea about local opening of DNA helix in area its complexing with RNA (formation R-loop) or at the interaction with DNA unwinding proteins. Area of helix opening was considered as sites of initiations of transcriptions and replication. However physical mechanism of DNA opening was not explained. It is known that triplexes DNA-RNA have the temperature of melting much above physiological for the majority of organisms. Earlier we proposed the model of DNA-membrane contacts (DMC), including the mechanism of R-loop formation, but process of their formation occur in lipid environment of membrane. Later by technique of microcalorimetry we have shown that melting temperature of such hybrids in the complex with lipids decrease before room temperature and below. In this work we propose a mechanism of DMC formation on the base of last own and literature data of study of system: DNA, liposomes and extract of *Xenopus laevis* oocytes. Here we discuss the preference of mode of regulation of gene expression by formation of DMC. Also we consider possible participation of DMC in the nuclear envelope assembly with pores and in the formation of nucleoid and chromosome structures.

PROTEIN TERTIARY STRUCTURE PREDICTION BASED ON
FRAGMENT ASSEMBLY AND PARAMETER OPTIMIZATION

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We propose a novel method for ab initio prediction of protein tertiary structures based on the fragment assembly and global optimization. Fifteen residue long fragment libraries are constructed using the secondary structure prediction method PREDICT, and fragments in these libraries are assembled to generate full-length chains of a query protein. Tertiary structures of 50 to 100 conformations are obtained by minimizing an energy function for proteins, using the conformational space annealing method that enables one to sample diverse low-lying local minima of the energy.

Then in order to enhance the performance of the prediction method, we optimize the linear parameters of the energy function, so that the native-like conformations are

energetically more favorable than the non-native ones for proteins with known structures. We test the feasibility of the parameter optimization procedure by applying it to the training set consisting of three proteins: The 10-55 residue fragment of staphylococcal protein A (PDB ID 1bdd), betanova, and 1fsd.

ON THE RELATIONSHIP BETWEEN SEQUENCE CHANGES
AND STRUCTURAL DEFORMATIONS IN PROTEIN CORES

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Natural proteins cluster in a finite and relatively small number of superfamilies. Structural genomics projects are being pursued to map this structural space so that most proteins in sequenced genomes can be found within a so-called structural modeling distance. In principle, homology modeling tools can then be used to extrapolate the structure of a query sequence from a template structure found within modeling distance. In practice, homology modeling tools have shown over the years limited ability to model the structural adaptations required to fit a mutated sequence. Here, we study this problem by investigating how structural cores respond to sequence changes in protein families. We first present a new multiple structural alignment algorithm and then use this tool to align a representative number of highly populated families. We then apply a Principal Components Analysis (PCA) method to relate structural and sequence spaces. We show that the main structural deformations in the core span a low dimensional space, and that clear correspondences can be established between these principal deformations and specific core mutations. Structures seem to respond by collective deformations around the mutated regions, and not by local readjustment of the mutated residue. The implications in homology modeling and protein design are discussed.

SHANNON ENTROPY AND LMC COMPLEXITY: A
STRAIGHTFORWARD APPROACH.

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A new approach to the LMC complexity, an indicator of complexity based on a probabilistic description, is proposed. The Taylor expansion of the Shannon entropy around its maximal value gives us a new insight for interpreting the LMC complexity for a general non equilibrium system. This insight allows us to establish the time evolution of this indicator in a near-equilibrium situation. One of the advantages of the LMC complexity is that its calculation does not require a considerable computational effort in many cases of physical and biological interest.

DISSECTION OF THE ROLE OF PROLINES IN AN
SH3-PEPTIDE INTERACTION USING A SINGLE-CHAIN
CHIMERIC SH3-PEPTIDE FUSION PROTEIN.

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In a recent work (1) we designed a single-chain chimeric protein by connecting a circular permutant of the α -spectrin SH3 domain (2) to the proline-rich decapeptide APSYSPPPPP (3). The engineered protein, named cpSH3-p41, mimics in its tertiary fold the typical interactions found in complexes between SH3 and proline-rich peptides. In this work we have made six mutants of cpSH3-p41 by replacing each one of the six prolines of the p41 sequence for alanine. Using differential scanning calorimetry we have measured the changes in the thermodynamic parameters of unfolding produced by the mutations. We have also investigated the conformation of the peptide sequence in each of the mutant chimeras using circular dichroism spectroscopy. The results allow us to estimate the importance of each proline in the interactions between the p41 peptide and the putative SH3 binding site.

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TOWARDS A NEW INTERACTION ENZYME:COENZYME

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During the photosynthesis, the flavoenzyme ferredoxin-NADP⁺ reductase (FNR) catalyses the reduction of NADP⁺ to NADPH, being this reaction highly specific for NADP⁺/H versus NAD⁺/H. The aim of the current project is to study the biochemical and structural basis for this coenzyme specificity in FNR. Starting from numerous experiments previously reported on that topic, we have pointed several aminoacid residues in FNR sequence that could have an important role in specific recognition of NADP⁺ versus NAD⁺. Selected positions were T155, A160, S223, Y235, L263, R264 and G265 in the enzyme sequence. These residues are thought to be involved in pyrophosphate binding of NADP⁺ and so might be also involved in determining such specificity. Site directed mutagenesis of these residues by other residues conserved in NAD⁺/H dependent reductases in similar positions, could enable us to create a quimera FNR with affinity for NAD⁺ instead for NADP⁺.

Poster number 7

NOTES

IDENTIFICATION OF MEMBRANE-ACTIVE REGIONS OF THE HIV-1
ENVELOPE GLYCOPROTEIN GP41 USING A 15-MER GP41-PEPTIDE
SCAN

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The identification of membrane-active regions of the ectodomain of the HIV-1 envelope glycoprotein gp41 has been made by determining the effect on membrane integrity of a 15-mer gp41-derived peptide library. By monitoring the effect of this peptide library on membrane leakage on model membranes, we have identified three delimited regions on the gp41 ectodomain with membrane-interacting capabilities: region 1, which would roughly correspond to the polar sequence which follows the fusion domain and extends to the N-terminal heptad repeat region, region 2, which would correspond to the immunodominant loop, and region 3, which would correspond to the pre-transmembrane region of gp41. The identification of these three regions supports their direct role in membrane fusion as well as it facilitates the future development of HIV-1 entry inhibitors.

LOCATION OF THE MISSENSE MUTATION E37K AT THE
STRUCTURAL (BETA-ALFA)⁸ TIM BARREL MODEL OF
HMG-CoA LYASE

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3-Hydroxy-3-methylglutaril-CoA (HMG-CoA) lyase (HL) is a member of the HMG-CoA lyase family proteins, which catalyzes the cleavage of HMG-CoA in acetyl-CoA and acetoacetate. HL deficiency causes 3-Hydroxy-3-methylglutaric aciduria an autosomal recessive genetic disorder presenting in neonates or infants. Symptoms look like Reye Syndrome.

To date 25 different allelic variants of HL gene in 39 patients have been reported. Our group have described the new mutation G109A affecting the same nucleotide that the very common Mediterranean mutation (G109T) does. However, there are no coincidences in the mechanism of enzymatic inactivation. The nonsense mutation G109T produces a stop codon and a skipping in exon 2, while the missense mutation G109A causes the change E37 K. Substitution of the acid residue glutamate 37 by the basic lysine suppose an important polarity change. Comparative studies shows that the residue E37 is highly conserved in HMG-CoA lyase proteins, as well as many proteins of the HMG-CoA lyase family. Studies precedent in other Claisen-condensing enzyme reactions, such as malate synthase and citrate synthase, have suggested multiple roles for conserved acidic residues in catalysis or cation ligation.

Recently our group have proposed a 3-D model for human HL, containing a (beta-alfa)⁸ (TIM) barrel structure. Trying to understand better the effects of mutation E37K, we have located this change on the beta-sheet 1 of the HL model.

SYNCHRONIZATION OF COUPLED OSCILLATORS IN
SCALE-FREE NETWORKS

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Recent advances in the analysis and modeling of complex systems have provided a promising approach to understanding basic organizational principles. Complexity manifests itself through self-organization, synchronization, the emergence of order, etc. At the same, it has been recently shown that many real systems exhibit a complex interconnection pattern among the elements that form the system. In this work, we study the synchronization of coupled oscillators on the underlying topology of scale-free networks. In particular, we assume that each network's component is an oscillator and that each interacts with the others following the Kuramoto model. We then study the onset of global phase synchronization by constructing the phase diagram of the system's dynamics. Our results show that the synchronizability of oscillators coupled as dictated by the topology of scale-free networks is easily achieved which in turn may help understand why this network architecture is so ubiquitous in Nature. Finally, we report on the fact that recent findings about persistence in the evolution of cellular motifs might be explained in terms of their improved fitness for synchronization. Our conclusions are then twofold. On one hand, we suggest that non-linear mechanisms and cooperation may be key ingredients for the understanding of biological systems. On the other hand, taking into account the real topology of the systems under analysis may provide answers to open problems.

DUAL PICTURE OF A SCALE-FREE NETWORK: METABOLIC PATHWAYS

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The scale-free distributions have recently captured the interest of many research groups working on different fields as biology, economy, or sociology. Several experimental studies have shown that real complex networks are following a power-law distribution like $P(k) \sim k^{-\gamma}$. From the theoretical side, Barabasi model [1,2] successfully identified major mechanisms involved in the power-law scaling. However, most real scale-free networks have two complementary representations embedded. In particular the metabolic networks, which could be understood as a first network with the nodes being enzymes(or reactions) and as a second network considering the substrates(or products) as nodes. In order to study these kind of networks, here we have carried out a theoretical study on scale-free networks, where a line graph transformation is applied on a power-law distribution. Our results indicate that a power-law distribution as $P(k) \sim k^{(-\gamma+1)}$ is found for the transformed graph together a peak for the less connected nodes. In the present paper we show a parametrization of this behaviour and discuss its possible application on metabolic pathways from KEGG database [3] and other large networks.

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THE PROTECTIVE EFFECT OF AMBROSIA MARITIMA EXTRACTS ON
SOME HEPATIC BIOCHEMICAL DISORDERS, HEMATOLOGICAL AND
HORMONE CHANGES IN GAMMA IRRADIATED RATS

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The present work aimed at studying the effect of *Ambrosia maritima* extract on the Hemoglobin concentration, red blood cells counts, white blood cells counts and Heinz bodies percentages Triiodothyronine thyroxine testosterone liver protein DNA and RNA before and after of gamma radiation. Whole body gamma irradiation of rats at six Gray single dose caused a significant decrease in the hemoglobin concentrations and red blood cells counts accompanied by a highly significant decrease of white blood cells level as recorded on seven days after irradiation as compared with the control group. On the other hand, there was a significant increase in Heinz bodies percentages. Data obtained in this study revealed that whole body gamma irradiation induced significant increase in triiodothyronine while thyroxine and testosterone showed statistical significant decrease as compared with the control group. However, liver protein, DNA and RNA were significantly reduced after seven days of irradiation when compared with control group. Pretreatment with *Ambrosia maritima* extract is shown to minimize the radiation induced biochemical disorders in the experimental animals. Data obtained suggest that *Ambrosia maritima* extract administrations confer protection against damage infected by radiation when given before to exposure to irradiation and may have more pronounced ant-inflammatory effects of radiation injury.

TREATMENT OF SOME BIOCHEMICAL DISORDERS BY NIGELLA
SATIVA EXTRACT AGAINST LIVER DAMAGE INDUCED BY
THIOACETAMIDE IN ALBINO RATS

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It has been reported that *Nigella sativa* possess antinematode actions. Besides, it produced a hepatoprotective effect on some models of liver toxicity. Therefore, the aim of this work was to study the hepatoprotective effects of the *Nigella sativa* extract against thioacetamide induced hepatic damage. Thioacetamide at a dose of two hundred milligram per kilogram produced liver damage in rats as manifested by the significant rise in serum levels of alkaline phosphatase aspartate amino transferase and alanine amino transferase compared with control values. On the other hand, pretreatment of rats with *Nigella sativa* seeds ameliorated the alkaline phosphatase aspartate amino transferase and alanine amino transferase values to approximately control value. There was a significant increase in the mean values of total lipids and triglycerides of the thioacetamide treated rats compared to the normal controls. However, the liver protein, DNA and RNA of the thioacetamide groups showed significant decrease compared with control group. Where as, *Nigella sativa* seeds extract ameliorates the liver protein values to approximately control value. It could be concluded that *Nigella sativa* extract might act as the drugs of hepatoprotective and antioxidant agent.

THERMODYNAMIC DISECTION OF THE BINDING
ENERGETICS OF PROLINE-RICH PEPTIDES TO ABL-SH3
DOMAIN. INSIGHTS FOR RATIONAL LIGAND DESIGN.

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SH3 domains are implicated in the regulation of kinase activity of tyrosine kinases by mediating specific but transient protein-protein interactions via recognition of proline-rich sequences in their partners. High affinity ligands of SH3 domains with the ability to block and modulate these interactions are emerging as promising therapeutic agents for the treatment of cancer, viral infections, and other diseases where SH3 domain are involved. The development of specific and high affinity ligands has proven a difficult task due, in part, to the lack of understanding of the origins of the binding affinity. We present a complete thermodynamic analysis of the binding energetics of the p41 proline-rich decapeptide (APSYSPPPPP) to the SH3 domain of the c-Abl oncogene. The calorimetric analysis have revealed a thermodynamic signature (very favorable enthalpic contributions opposed by an unfavorable binding entropy) inconsistent with the highly hydrophobic nature of the ligand and the Abl-SH3 binding site. In the light of the structural and thermodynamic analysis, we conclude that the establishment of a complex hydrogen bond network mediated by buried water molecules at the binding interface is responsible for the observed thermodynamic behaviour. The complexity of the interaction between proline-rich ligands and the Abl-SH3 domain has been confirmed by the surprising effects on the binding energetics of conservative substitutions at solvent-exposed positions in the ligand.

CURVATURE AND FLEXIBILITY IN PROMOTER SEQUENCES
ANALYSIS

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Static and dynamic curvatures are determinant in biologically important processes, such as transcription, nucleosome positioning and gene silencing.

An analysis of promoter sequences of eukaryota and prokaryota in terms of their curvature and flexibility properties is presented. Sequence dependent curvatures are evaluated by integrating local deviations from the canonical B-DNA of the different dinucleotide steps. To evaluate local flexibilities normalized dinucleotide melting temperatures, as previously proved by us, has been used (*Biophys. J.*, **79** (2000) 601-613; *J. Mol. Biol.*, **286** (1999) 1293-1301). Other different scales available in literature are used for comparison.

These DNA intrinsic properties have been averaged over statistically significant sets of promoter sequences, for both prokaryota and eukaryota. In the investigated promoter sets, a dominant signal in correspondence to origin transcription site for the average profile of both curvature and flexibility was found even when different scales were used. From these results, we can make the hypothesis that there is an average signal along the promoters that could be recognized by binding proteins, to activate the biological processes in which they are involved.

Anabaena PCC 7119 APOFLAVODOXIN FOLDING ANALYSIS
BY FLUORESCENT DYES.

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Flavoproteins are a wide family of redox proteins which cofactor is a flavin. *Anabaena* PCC 7119 apoflavodoxin is a flavoprotein without the cofactor that has been studied as a protein folding model. Our aim is to complete these studies performing a labelling system for the protein with fluorescent (IAEDANS,IAF) dyes. Such labelled apoflavodoxin samples will be used to evaluate the changes in intramolecular distances between specific groups of the protein in its different folding stages. Intramolecular distances will be measured by using FRET (Fluorescent Resonance Energy Transfer). Mutants of *Anabaena* PCC 7119 flavodoxin have been developed placing some cysteins for dye labelling at strategic places on the apoflavodoxin surface. Some of these mutants have been labelled with the fluorescent dyes, and their stability and folding were followed by fluorescence and by FRET.

INTERACTION OF VISCOTOXIN A3 ON MODEL BIOMEMBRANE
SYSTEMS BY NMR

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Viscotoxin A3 (VA3) is a protein isolated from *Viscum album*, which belongs to the thionin's family. VA3 is a polypeptide comprising 46 amino acid residues stabilized by three disulphide bridges having a positive net charge. It has been described that VA3 has cytotoxic activities against human myeloid, leukaemia and sarcoma cells, these activities seem to depend on its ability to interact with plasma membranes. We have studied by NMR the VA3 structure in solution at two different concentrations and we have found evidences which suggest that it might form oligomers like other members of the thionin's family. Because of the strong dipole-dipole broadening between neighboring hydrogen nuclei, ¹H NMR in membrane containing systems is intrinsically poor and leads to increased spectral line widths. In this work we also report the first study of VA3 by using HR-MAS NMR spectroscopy in the presence of model biomembranes. We have applied HR-MAS on model biomembrane systems consisting of perdeuterated DMPC/DMPA and in the presence of VA3 to study the protein-lipid interactions that should help us to understand the structure-function relationship of VA3 and membranes; in this way, we have been able of observing the probable membrane interacting residues of VA3.

THE EFFICIENCY OF DIFFERENT SALTS TO SCREEN CHARGE
INTERACTIONS IN PROTEINS: A HOFMEISTER EFFECT?

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Understanding the screening by salts of charge-charge interactions in proteins is important for at least two reasons: a) screening by intracellular salt concentration may modulate the stability and interactions of proteins in vivo; b) the in vitro experimental estimation of the contributions from charge-charge interactions to molecular processes involving proteins is generally carried out on the basis of the salt effect on process energetics, under the assumption that these interactions are screened out by moderate salt concentrations. Here, we explore experimentally the extent to which the screening efficiency depends on the nature of the salt. To this end, we have carried out an energetic characterization of the effect of NaCl (a non-denaturing salt), guanidinium chloride (a denaturing salt) and guanidinium thiocyanate (a stronger denaturant) on the stability of the wild-type form and a T14K variant of *E. coli* thioredoxin. Our results suggest that the efficiency of different salts to screen charge-charge interactions correlates with their denaturing strength and with the position of the constituent ions in the Hofmeister rankings. This result appears consistent with the plausible relation of the Hofmeister rankings with the extent of solute accumulation/exclusion from protein surfaces.

REVELATION OF EXPRESSING REGION IN THE PROCESSED
CERULOPLASMIN GENE IN HUMAN GENOME BY
BIOCOMPUTATIONAL AND BIOCHEMICAL METHODS

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A key protein of iron metabolism is ceruloplasmin (Cp), a multi-copper ferroxidase that converts toxic ferrous iron to its non-toxic ferric form. Iron homeostasis is important for mitochondria as its disorder leads to a mitochondria-mediated apoptosis. No mitochondrial copper ferroxidase has been yet identified. In this study the search for a potential mitochondrial ferroxidase was carried out. Cp-like proteins were scanned for a signal peptide of mitochondrial protein import (SPMPI) by MitoProt II 1.0a4. The sequence of 40 aa length with the high probability of import to mitochondria was found in human Cp processed gene mapped in chromosome 8. Cp pseudogene sequence is translated in 328 aa sequence with predicted SPMPI at N-terminus. Upstream the sequence, the potential promoter, including the consensuses of TATA-, CAAT-boxes and capping site, was predicted. The aa sequence of Cp pseudogene has one copper oxidase motif H-X-H. Cp pseudogene codes for potential mitochondrial oxidase with 86% homology to Cp. Cp pseudo mRNA in HepG2 and HuTu 80 cells was verified by RT-PCR analysis. The transcripts of Cp pseudogene were found in both human cell lines. HuTu 80 cells had no transcripts of Cp mRNA or GPI-Cp mRNA. 30 kDa Cp protein was detected in mitochondrial fractions of HuTu 80 cells by Western blot analysis. These findings make us think that Cp pseudogene can play a role in the maintenance of iron homeostasis in mitochondria. Supported by RFBR (01-04-49597, 03-04-06937, 36).

THE SKIPPING OF EXON 2 AND EXON 2 PLUS 3 OF
HMG-CoA LYASE (HL) GENE PRODUCE THE LOSS OF BETA
SHEETS 1 AND 2 AT THE RECENTLY PROPOSED
(BETA-ALFA)⁸ TIM BARREL MODEL OF HL

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HMG-CoA lyase (HL) deficiency is a rare autosomal recessive genetic disorder that affects ketogenesis and L-leucine catabolism. Two allelic variants have been found predominant G122A very frequent in Saudi Arabia and G109T, also called Mediterranean mutation, is very common in Spain. G109T is a nonsense mutation that causes a multiple aberrant splicing with three mRNA species: one of the expected size that contains the premature stop codon TAA, another with a deletion of 84 bp corresponding to the whole of exon 2 and a third with a deletion of 192 bp corresponding to skipping of the whole exons 2 and 3. Recently our group proposed a 3-D model for human HL containing a (beta-alfa)⁸ (TIM) barrel structure. The model is supported by the similarity with analogous TIM barrel structure of functionally related proteins, by the localization of catalytic amino acids at the active site, and by the coincidence between the shape of the substrate (HMG-CoA) and the predicted inner cavity. In this work, we have studied the effect of the deletions of exon 2 and exon 2 plus 3 on the HL proposed model. As a result, the exon 2 skipping produced the loss of beta-sheet 1, and the skipping of exons 2 and 3 caused the disappearance of alfa helix 1 and beta-sheets 1 and 2, respectively. The important role of beta-sheets on the stability of proteins could explain the lack of activity of these mutants.

ELASTICITY OF SEMIFLEXIBLE POLYMERS WITH AND WITHOUT
SELF-INTERACTIONS

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In this work, a new interpolation formula for the stretching behaviour of semi-flexible polymers is introduced. Taking explicitly into account the discrete nature of the chain, a crossover force between worm-like chain and freely jointed chain behaviour is found. Comparisons with some recent experiments and Monte Carlo and Molecular Dynamics simulations are also given.

EVALUATION OF THE ALGOCOR STRUCTURE-BASED POTENTIAL FOR
LIGAND DOCKING

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New structure-based potentials (AlgoCor) have been developed to reproduce experimentally determined protein-ligand three-dimensional structures. In contrast to known knowledge-based potentials, that estimate binding affinities, AlgoCor as itself developed for the reliable description of the pair-wise contributions to the binding affinity: the energy of a protein-ligand complex and the hydrophobic term, defining ligand docking locations. AlgoCor does not contain fitting parameters and was not calibrated to the known binding affinities. Physical basis of the potentials is analyzed. We then propose a novel method to score binding affinities using the AlgoCor and additional non pair-wise entropic terms.

Four different methods for extracting structure-based potentials were considered. To test the accuracy of the potentials we have performed rigid docking of ligands from a set of 130 known PDB complexes and analyzed root-mean square deviations (RMSD) of the docked positions regarding the experimentally determined poses.

An optimal atom types were obtained as a result of regrouping of 49 atom types with account of interaction properties and on basis of docking results. We get the best value of the averaged (through the test set) RMSD for 18 atom types: 6 carbon, 4 oxygen, 5 nitrogen, 1 sulfur, 1 phosphorus and 1 halogens atom types. Further reducing of the atom types increases the RMSD. Also we show that splitting of elements (C,N,O,P,S) of the periodic system up to the optimal atom types essentially improves docking accuracy.

UNDERSTANDING THE STRUCTURAL CHARACTERISTICS
OF COMPSTATIN BY CONFORMATIONAL SPACE
ANNEALING

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Complement is implicated in various diseases including auto-immune diseases and other clinical conditions such as stroke, heart attack and burn injuries. The complement activation is essential for the development of normal inflammatory responses against foreign pathogens. However, inappropriate complement activation leads to host cell damage. In particular, the third component of complement (C3) plays a central role in activation, and its activation generates the toxic peptide called C3a. Thus, there is a clear need for specific complement inhibitors with therapeutic application.

The 13-residue (ICVVQDWGHRCT) cyclic peptide, compstatin, that binds to C3 and inhibits complement activation is a promising candidate for drug development due to its activity, low toxicity and structural simplicity. It has a disulfide bridge and a type I β -turn spanning residues Gln5-Asp6-Trp7-Gly8. It has been shown that the four residues of the β -turn, disulfide bridge and Val3 are essential for inhibitory activity.

To investigate the structural characteristics of compstatin, we use the conformational space annealing (CSA) method, a powerful global optimization method, and all-atom force field with generalized Born solvation model. The CSA method enables one to sample diverse low-lying local minima of a given function. In this work, we obtain the important features of the structural characteristics of compstatin including the global minimum-energy conformation and many other local minima.

STABILITY OF BACTERIAL LUCIFERASES IN ORGANIC
SOLVENTS

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A comparative study was performed regarding the catalytic activity and stability of two luciferases of luminous bacteria *Ph.leiognathi* and *V. harveyi* in the presence of a number of organic solvents. Luciferases - flavin-dependent monooxygenases catalyze the oxidation reaction of the long-chain aliphatic aldehyde and reduced flavinmononucleotide involving molecular oxygen to a respective fatty acid emitting light quanta in the visible spectrum. It is shown that at small concentrations of organic solvents the maximum reaction rate and quantum yield increases; at high concentration the enzyme is inactivated following the threshold pattern. The degree of reaction rate activation and inhibition depends on kinds of enzymes, aldehydes chain length and the nature of the organic solvent and its concentration in the media. Solvents are characterized by a wide set of parameters, such as viscosity, hydrophobicity, dielectric constant, capacity of organic solvents to form hydrogen bonds and etc. Effect of microenvironment change on kinetic parameters of enzyme-induced bioluminescence reaction was studied and analysed using kinetic graphical methods, light-emission spectra studies, cluster analysis. Combined differences in protein structure and nature of organic solvents are suggested to explain the differences in luciferases stability observed in the present study. This project was supported by RF MINOBR-Grant PD02-1.4-315-P and CRDF-Grant Y1-B-02-17.

EFFECTS OF STATIC AND DYNAMIC DISORDER ON THE
PERFORMANCE OF NEURAL AUTOMATA

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We shall report on recent analytical and numerical results concerning stochastic neural automata that exhibit the following (biologically inspired) features:

1. The neurons and the synapses evolve stochastically on the same time scale but subject to different thermal noise, as if they were in contact with respective baths at different temperature.
2. The connectivity between neurons may be varied from full connection and high random dilution to the case of a network that has the small-world property.
3. The synaptic kinetics simulates the repeated scanning of all the stored patterns.

Though these features may sometimes apparently result in additional disorder, there is a range of parameter values for which the model exhibits extraordinary computational performance and some of the qualitative behavior that has been reported to occur in natural systems.

More specifically, the model shows very efficient and robust associative memory and jumping between the attractors that correspond to the stored patterns. In particular, we shall demonstrate that a power-law topology, which is known to characterize many natural, including neural systems, is advantageous compared to the corresponding diluted network, and hubs, the few most highly connected nodes in a scale-free architecture, play a fundamental role in making the retrieval of information more robust and efficient. Our findings suggest paths for a convenient design of artificial systems, and they are fully consistent with two main observations, namely, that memory is a global dynamic phenomenon, and that oscillations are essential to cortex functions.

We acknowledge financial support from MCyT-FEDER (project BFM2001- 2841 and a Ramon y Cajal contract) and from the UGR-MADOC agreement.

STABILITY AND CATALYTIC EFFICIENCY OF BACTERIAL
BIOLUMINESCENCE

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The kinetics of the bacterial luciferases from luminous bacteria *Photobacterium leiognathi* and *Vibrio harveyi* in organic systems were investigated. A number of organic solvents of different physicochemical characteristics were used. Quantum yield of bacterial bioluminescence reaction in vitro by adding of a number of organic solvents in the reaction medium increases. The degree of activation depends on properties of a solvent. The effect of increasing concentration of co-solvents on the stability of luciferases was studied. However, thermal stability of luciferases for these cases decrease. Enzymes did achieve thermal and solvent stabilization. Effects of microenvironmental change on kinetic parameters of steady-state and non-steady-state enzyme-induced bioluminescent reactions are investigated. The thermal stability of luciferases, the spectra of absorption and spectra of bioluminescence are studied. Combined differences in protein structure and nature of organic solvents are suggested to explain the differences in stability and catalytic efficiency observed in the present investigation. This project was supported by RF MINOBR - (Grant PD 02-1.4-315) and CRDF - (Grant Y1-B-02-17).

STRUCTURAL AND THERMODYNAMIC ESCAPE
MECHANISM FROM DRUG RESISTANT MUTATIONS OF
HIV-1 PROTEASE

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The efficacy of HIV-1 protease inhibition therapies is often compromised by the appearance of mutations in the protease molecule that lower the binding affinity of inhibitors while still maintaining a viable catalytic activity and substrate affinity. The structures of two inhibitors bound to the wild type HIV-1 protease and resistant mutant V82F/I84V have been solved by x-ray crystallography at 2.0Å resolution. KNI-764, a second generation inhibitor currently under development, maintains significant potency against this mutation by entropically compensating enthalpic losses. KNI-577 differs from KNI-764 by a single functional group critical to the inhibitor response to the protease mutation. The presence of two asymmetric functional groups linked by rotatable bonds to the inhibitor scaffold, allows KNI-764 to adapt to the mutated binding site cavity more readily than KNI-577 with a single asymmetric group. As a result, KNI-764 is able to gain binding entropy by a twofold mechanism: it gains solvation entropy by burying itself deeper within the binding pocket, and, it gains conformational entropy as it loses some interactions with the protease. Supported by grant GM57144 from the National Institutes of Health.

Part III

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