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Biocomplexity Faculty Search Committee
c/o Prof. Rob de Ruyter van Steveninck
Department of Physics
Indiana University
Swain Hall West 117

Bloomington, IN 47405-7105

Application for a junior faculty position

Dear Search Committee,

I am a Postdoctoral Fellow at the University of Texas Medical Branch at Galveston (Dr. Wayne Bolen's Lab, Experimental Biophysical Chemistry) and at the University of Houston (Dr. Montgomery Pettitt's Lab, Solution Theory). I am very much interested in a faculty position in Biocomplexity as advertised in *naturejobs*.

My current and previous work is primarily in the areas of Biophysical Chemistry and Solution Physical Chemistry of biochemical compounds and their behavior in reaction networks. My *long-term goal* is to quantify the non-ideal environment of the cytoplasm with respect to equilibrium and kinetic processes. The *purpose* is to bridge the gap between the actual thermodynamics and kinetics occurring in the cytoplasm, and that which is assumed from extrapolations of dilute solution results. Currently, I am performing experiments on crowded solutions of biochemical compounds and I have developed a statistical thermodynamic theory of solution that is able to exactly describe the mutual influence of biomolecules in highly concentrated solution. The results of this research are certain to have an important impact on the solution of biochemical, medical and medicinal problems. A statement of research plans is attached to this application.

I participated in delivery and planning of lab courses, theoretical courses and lectures for Biology, Chemistry and Physics students. I was also involved in the supervision of graduate and undergraduate students. Upon popular request by students at UTMB, I am currently giving an informal course on statistical thermodynamics of biological compounds. Please find attached a more detailed statement of teaching merits.

Three letters of reference will be sent to you separately.

I am looking forward to hearing from you.

Sincerely,

(Jörg Rösgen)

Molecular crowding in the cytoplasm

Description of Research Plans
Jörg Rösgen
December 13, 2004

SUMMARY / ABSTRACT

The complexity in the number and kinds of known interconnecting reactions and equilibria taking place in the highly crowded volume of the cell grows rapidly each year. Traditionally, reaction rate and equilibrium constants of these processes are evaluated in terms of concentrations of the reacting species. Within the highly crowded and thermodynamically non-ideal intracellular conditions of the cell, however, concentrations are not the relevant information needed [1]. Under such crowded conditions, *chemical activities* are required in place of concentrations [2]. Estimates of the difference between chemical activities and concentrations of proteins in the crowded environment of the cell are of several orders of magnitude [3]. So, one readily sees that if biology is to make the transition from dilute solution extracellular *in vitro* studies, to quantitative biology within the intracellular environment, a *shift in paradigm* is necessary: A shift from the thermodynamically ideal conditions currently used to quantitatively study biochemistry, to the thermodynamically non-ideal conditions existing in the cell. To bridge this gap, my *objective* is to develop means to evaluate chemical activities of macromolecules and small molecules in solutions resembling the complex intracellular environment. The *long-term goal* and ultimate objective is to evaluate and understand the actual rates and equilibria in the confines of the cell itself.

My *approach* brings together experiment, statistical thermodynamic theory and computer simulation. I have made significant headway in understanding the physical-chemical origins of the relationship between chemical activity and concentration in solutions of naturally occurring osmolytes [4, 5]. On the experimental side, this project requires an unprecedented collection of multi-component activity coefficient data obtained in a non-standard fashion. It also requires the development of instrumentation appropriate for the advancement of solution physical chemistry to solution conditions approximating those of the interior of cells.

If we ever want to fully understand the complex molecular processes, which constitute life on the cellular level, the problem of physical non-ideality of biological systems must be pursued and solved. The combination of statistical thermodynamics and experimental solution thermodynamic methods I am pursuing provides the framework for achieving this goal.

Research involving such fundamental issues of non-ideality is essential for advancement of quantitative biology and is supportable through NSF and NIH.

SPECIFIC AIMS

The conceptual leap from dilute solution to the crowded intercellular environment will be made by the primary research activities outlined below. Pursuit of these activities will allow for discovery of fundamental issues of molecular interactions and identification of important problems in hypothesis driven research projects in the fields of Biochemistry and Medicine, as demonstrated in my current and previous research [6-8].

There are three groups of research activities comprising my current research and the research planned for the future. In short, they are

1) Quantify experimentally, how biomolecules react to crowding

a) *Measurement of activity coefficients of biomolecules at room temperature*

This project involves the quantification of the non-ideality of organic cosolutes (osmolytes) in mixed aqueous solutions both with macromolecule present and absent. In the low concentration regime commercially available instruments can be used for the osmometric measurements. A necessary step in this project, however, is the construction of a high throughput isopiestic distillation apparatus (I am currently prototyping this instrument).

b) *Determination of the temperature dependence of chemical activities*

The temperature dependence of the chemical potentials will be determined calorimetrically through the heats of dilution of the solutes of interest and their partial molar heat capacities.

2) Quantify how biochemical reactions respond to crowding

Spectroscopic and calorimetric measurement of the dependence of macromolecular reactions on solution non-ideality

My Phase Diagram Method [9] allows for a straightforward determination of the dependence of equilibrium constants (i.e. ratios of activity coefficients) and kinetic constants on the presence of elevated concentrations of cosolutes and macromolecules. Conformational transitions, ligand binding and enzymatic reactions will be investigated as the first application and implementation of theory and experiment.

3) Further develop the theoretical foundation for the experimental findings

a) *Solution theory*

In my current theory of solution [4, 5] crowded two-component systems are described in terms of excluded volume, apparent solute-solvent and solute-solute association as well as long range interactions. I will extend this approach to multi-component systems and macromolecular equilibria.

b) *Computer simulation*

Computer simulations will be performed in order to learn what are the effects of specific kinds of interaction on both the structure and energetics of solutions of biochemical compounds. The Kirkwood-Buff theory [10] links the simulation results to my solution theory by providing a direct connection between the structure and the thermodynamics of solutions.

SCIENTIFIC CONTEXT

Activity Coefficients and Non-ideal Solutions in Biophysics

From the very beginning of solution physical chemistry there has been a good understanding of the properties and characteristics of dilute solutions, the so-called ideal solution limit [11-13]. The concentration dependent deviation from this ideal behavior is described by the activity coefficients of the solutions components [14]. Experimental investigation of the activity coefficients of biological substances gained early attention [15, 16]. It was noted, that the conditions operative in biological systems might give rise to as much as a 100,000-fold deviation from ideal behavior, which should lead to a stabilization of the native state *in vivo* [3]. However, urea was recently found to be a significantly more effective denaturant *in vivo* than *in vitro* in at least the limited number of cases studied thus far [17, 18]. Also, in comparison with their efficacy in stabilizing proteins *in vitro*, protecting osmolytes seem to be unexpectedly more effective *in vivo*. For instance, as little as 75mM of the osmolyte TMAO can protect a mutant p53 protein from misfolding *in vivo* [19], and also the cystic fibrosis related CFTR ΔF508 can be brought to a functional state *in vivo* by the osmolyte TMAO [20].

Organic cosolvents (osmolytes) play a prominent role in the biology of nearly all taxa [21]. Such osmolytes are known to be accumulated in *archea* as well as higher plants and animals (including humans) to modulate the behavior of the biological macromolecules in vivo [22]. The influence of these cosolvents on the molecular transitions of proteins seems to follow a linear relationship [23, 24] the origin of which is poorly understood [25], and is usually described in terms of preferential interactions with macromolecules [26, 27]. Although recently the (de)stabilization has been qualitatively described in terms of the osmophobic effect [28], it seems, that it is difficult to deal with the observed phenomena quantitatively if activity coefficients are not taken into account explicitly [29]. This was recognized early [30], but for a long time the amount of research in this field has been very modest [31, 32], possibly due to the difficulty of performing the required experiments with high enough throughput to make timely advances [33]. In addition, until recently [4, 5] the intricacy of data evaluation in the absence of a practicable and rigorous thermodynamic solution theory of activity coefficients has resulted in progress that is piecemeal with little effort to develop a unified understanding of biological processes within the context of the intracellular environment.

In proceeding towards the quantification of complex biological solutions we are faced with a twofold task. 1) *We need to understand the physical and biological consequences of the highly crowded context on biomolecules* and 2) *we need an efficient strategy for quantifying coupled reactions and reaction networks* between multiple kinds and species of small molecules as well as macromolecules.

As described below, I successfully pursued these problems and have the expertise to solve them. My vision is to push this research forward to enable quantitative descriptions of thermodynamic and kinetic behavior in the cytosol within my lifetime.

CURRENT RESEARCH AND RESEARCH PERSPECTIVE

Solution Non-Ideality

Recently, I developed an analytic statistical thermodynamic theory that provides the physical chemical basis of the concentration dependence of activity coefficients [4, 5]. In conjunction with the laboratories of Wayne Bolen (Univ. Texas Med. Branch) and Montgomery Pettitt (Univ. Houston) a series of additional papers is in preparation, that describe the derivation of activity coefficients from the (semi) grand canonical partition function and the relationship between activity and osmolyte function. The semigrand partition function successfully describes the osmotic- and activity coefficients of a large variety of different compounds from the ideal dilution limit up to the solubility limit, and it provides a basis for interpretation of activity vs. concentration in terms of interactions in solution, molecular crowding and aggregation. It also describes and provides insight into phase transitions, such as micelle formation and protein crystallization. This approach applies to compounds as different as salts, sugars, amino acids, peptides and lipids. Success in describing such a large array of systems and phenomena of fundamental importance to biology with one partition function demonstrates the versatility of the approach and its capacity for exploring a wide range of biochemical questions.

Successful as this approach is, it can only be considered as a good beginning in that it demonstrates proof of principle for a large and encompassing research project. This project will involve experiments *(Aim 1a,b.)* on different classes of biological molecules (Proteins, nucleic acids, lipids, polysaccharides, smaller organic molecules and salts) and different kinds of interactions (specific ligation/association, non-specific solvation, electrostatic interaction). In the statistical thermodynamic theory *(Aim 3a.)* short-range interactions can be accounted for without regard to the kind of species studied. While long-range electrostatic interactions in solution and their impact on biological molecules are becoming better understood [34-38], the Debye-Hückel theory of dilute electrolyte solutions [39, 40] is clearly the best understood. It represents the first successful activity coefficient model and was readily applied also to proteins [41]. Electrostatics will be an important part of the research, especially in view of the large poly-ions in biology, most notably DNA and RNA. It is significant that activity coefficients of 1:1 salts are accurately accounted for by means of the Debye-Hückel theory embedded into the current statistical mechanical model [4].

(Aim 3b.) Thermodynamically, the major gross overall contribution to the non-ideality and the volume of binary aqueous solutions of small molecules involves the phenomenon of packing [4, 5]. Computer simulation offers the opportunity to investigate the effects of pure volume exclusion and packing with favorable kinds of interactions switched off. As a starting point, I am currently working on the complete thermodynamic characterization of multi-component hard sphere mixtures as a reference model system for such packing effects.

Reaction Networks

In vivo, macromolecules participate in complex reaction networks. Even *in vitro*, a single protein can be shown to change between dozens of thermodynamically distinguishable states. Such states can be revealed by perturbing the protein (addition of a specific ligand or a cosolute; a change in pH, temperature, pressure; electric fields or mechanic forces on the single molecule level). As a consequence, the exhaustive characterization of a single protein can become quite complicated due to the large number of available states. Therefore, a robust method of exploring reaction schemes is needed, one that yields the number of different states and stoichiometries.

Recently, I developed such a method based on phase diagrams [9]. It enables a systematic screening of a protein system for protein states and gives estimates of how they are related energetically. The method was illustrated using the moderate to low affinity calcium binding protein Annexin I [7, 42]. After the reaction scheme is established, global analysis of all experimental raw data yields precise information on the energetics of the total reaction scheme. For the most powerful technique of investigating protein energetics, differential scanning calorimetry, a general analytic solution required for performing such a global analysis was recently published [42, 43].

(Aim 2.) In the proposed project, this method will be extended to non-ideal conditions. It is important to be able to measure biochemical reactions under such conditions, because non-ideality can influence the activity coefficients of different states of the same molecule differently, thereby shifting equilibria. Understanding activity coefficients of macromolecules and smaller biochemical compounds alone is an important goal itself, but only the implementation of chemical activity formulation into chemical reactions will provide for the first time the basis for understanding reactions and molecular events within the complex crowded environment of the living cell.

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Presentation of teaching merits

Teaching is an integral part of scientific research. Good teaching provides the students with knowledge, tools and methods required for a successful career. Teaching enables the students to participate and help effectively in current research. Teaching is also an important, but frequently unappreciated route of publication.

In view of the emergence of a growing number of new interdisciplinary research activities, also teaching should go beyond the established borders between departments – based on the needs and interests of the involved students and the long-term visions in science. I am ready to respond to these needs and I have a vision, viz. to quantify the cytoplasm with respect to equilibrium and kinetic processes.

Teaching experience

I have teaching experience in several different scientific fields ranging from Plant Physiology to Mathematics with a main focus on Physical Chemistry and Biophysics. In the following I will give details on the teaching mentioned in my *curriculum vitae*. The courses are ordered chronologically. Since I had mostly either stipends or research positions, I did not have to teach, with the exception of the Plant Physiology course. All other courses I taught voluntarily.

Lab course, Plant Physiology, WWU Münster

This long established three-week lab course was for advanced Biology students. My task was to supervise experiments and perform short oral exams.

Lab course, Biophysics, WWU Münster

This three-week course was mainly for advanced Biology students, but it was also open to students from other departments. My responsibilities included supervision of experiments (two to three students at a time), supporting students in seminar talk preparation and presentation, and oral and written exams. I also had to modify several experiments, because the knowledge originally required for performing the experiments turned out to be inadequate for Biology students with a typical background in Physics and Mathematics.

Lab course, Physical Chemistry, WWU Münster

This course was for advanced Chemistry students. My responsibility was to plan, prepare and supervise three-week research projects. Approximately 20 additional people at the institute participated in organizing such projects. The projects were added to a menu from which students (two to three in a group) could select according to their interests.

Theoretical course and lecture, Mathematics for Chemists (Analysis), WWU Münster

This weekly four credit hour course was for 1st and 2nd year Chemistry students. My responsibility was to teach exercise classes (20 to 30 students per class), to occasionally deliver the lecture as a substitute lecturer and to participate as a team member in planning, overseeing and grading of the final written exam.

Lecture, Molecular Biophysics I, UTMB Galveston

On invitation of Dr. Wayne Bolen I contributed and delivered a chapter on transient state kinetics of protein conformational transitions to this three credit hour lecture for graduate students in the Biophysical, Structural & Computational Biology program.

Theoretical course, Biological Applications in Statistical Physics, UTMB Galveston

A group of graduate students asked me to develop and deliver a course on statistical mechanics of biomolecules in addition to their regular curriculum. Prior to the beginning of the course I met with the students to present a syllabus and to adjust it to their special needs and interests. Currently, we are in the sixteenth week of this two-hour weekly course. The course includes lecture sections and exercise sections. In the exercises the students solve tasks either on paper/blackboard or using the computer programs "Mathematica" and "Gnuplot".

Experience as a supervisor to students

I have been informally involved in supervising PhD students at WWU Münster and at UTMB Galveston, as well as a master student in Münster. The supervision ranged from designing and supporting subprojects to major parts of PhD projects.

Kristian Boehm, Master thesis 2002, Research group Prof. Hinz, WWU Münster.
This thesis was on the simultaneous measurement of expansion coefficient and heat capacity using my extension to the N-DSC II microcalorimeter. I did the supervision for most of the time. After I left to Galveston, I kept contact through extensive e-mailing.

Yvonne Guttzeit, PhD thesis 2002, Research group Prof. Hinz, WWU Münster.
This thesis dealt with the volumetric behaviour of proteins as a function of temperature and pH. I designed the PhD project and supervised in the start-up phase.

Tea Tarielashvili, PhD thesis 2004, Research groups Prof. Hinz, WWU Münster and Prof. Mrevlishvili, Tbilisi State University.
This thesis was on the thermodynamic behaviour of protein oligomers and of DNA in isolation and in specifically aggregated mixtures (bacteriophages). I supervised most of the start-up phase in Münster. For the final PhD exam and defence in Tbilisi I wrote an evaluation on the part of the thesis done in Münster.

Allan Chris Ferreon, PhD thesis 2004, Research group Prof. Bolen, UTMB Galveston.
This thesis was on the thermodynamics of the B domain of protein G as a function of chemical denaturants. After my arrival in Prof. Bolen's research group Allan Ferreon became excited about my approach to the physics of macromolecules and he asked me to supervise a research project that constituted the second half of his thesis.