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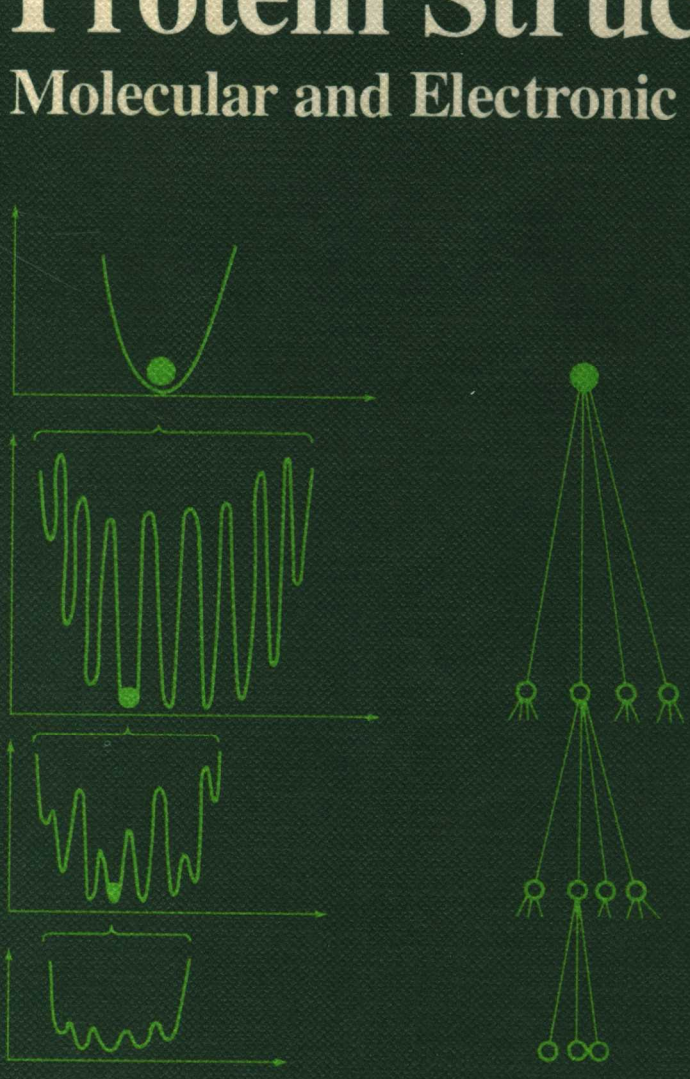
Editors

# Protein Structure

## Molecular and Electronic Reactivity



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V.I. Gol'danskii EDITORS

# Protein Structure

## Molecular and Electronic Reactivity

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# PREFACE

These are the edited proceedings of a conference held April 10-13 1985 in Philadelphia. The title of the conference was "Protein Structure: Molecular and Electronic Reactivity". This conference is the successor to an earlier conference held in Philadelphia on November 3-5, 1977, entitled "Tunneling in Biological Systems".

Britton Chance had proposed holding a similar conference to gauge our progress and had circulated a letter to that effect among interested parties. We are grateful to Professor John Hopfield for suggesting that since the 1977 conference had so carefully laid a solid foundation on tunneling it would be more interesting if the next conference would broaden its horizons and cover a wider perspective in biophysics, yet still remain firmly rooted in the PHYSICS of biomolecules. Professor Hans Frauenfelder made the suggestion that the number of talks be restricted and that time be set aside for frank and probing discussions.

The goals of this conference were to build from the initial ideas that arose in trying to understanding tunneling (electronic and nuclear) in macromoleucles, to explore new static/dynamic models of proteins and possible relation of these ideas to protein function (in the cell!!), and to explore a possible connection between protein physics and molecular machines, not necessarily in the cell.

Here then is the outgrowth of the three seminal suggestions and the assault on our lofty goals. Personally, I feel that we got about two-thirds of the way there towards the original goals. You will note that many of the talks in the conference are of a broad and reflective scope and summarize well our progress in comprehension of macromolecular tunneling since the last conference. A new and exciting perspective is given by the talks wrestling with the question of glass-like aspects of protein structures and dynamics. The physics connection remains

intact, and in this period of tight budgets a new note has been injected: a lively debate will be found on the wisdom of doing biophysics for biophysics sake.

If I could fault anything, it would be in the over-reluctance of the participants to speculate on the "What if...." questions concerning molecular control and microengineering. Dr. Barrett's call for far-out ideas went unanswered. Perhaps the next time around we can prod the heavy hitters into going out onto a limb a bit more. In any event, in the course of editing these talks we have been informed, enlightened, entertained and made to think about the purpose of our life's labors. We hope you, the reader, will be similarly intrigued by the proceedings.

A note about the concluding talk by Brit Chance. We suspect that Brit got a bit concerned about the unremitting physical level of the talks and decided in his after-dinner speech at the banquet to give a "sweet dessert" about how physical techniques can lead to real clinical insight. We left the talk in the proceedings to remind the reader, as Professor Chance did the participants, of the very real problems that are calling for attention and the contributions we can make...a fresh wind into the ivory tower.

Two people made absolutely vital contributions to the success of the conference. Dr. Barrett of the Office of Naval Research provided critical funding and psychological support when things looked quite dark. Judy Flanagan, Associate Director of Communications at the University City Science Center was constantly in the thick of things searching for support, writing letters, organizing the actual proceedings, paying the bills, etc. etc. We suspect we aged Judy a bit in the process, but it was good for building her character. Further, Judy's coworkers Sarah, Suzanne, Rita, Chilton and Sandy helped make the actual proceedings a smoothly functioning machine. My valued colleague and friend Dr. Ephraim Buhks helped greatly in the editing of the charge transport section of the proceedings.

The creation of the actual manuscript is the result of two people: Dot somehow typed up preliminary drafts of the garbled tape recordings, and Lorraine Nelson, a TEX-nician of the first rank typed/typeset all the manuscripts into the computer. A note: Professor George Feher is the only author of equal TEX-nical abilities to Lorraine. Let us hope the talks from the next conference will be

uniformly submitted in TEX.

Funding for the conference came from a number of sources:

Naval Air Systems Command

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Advanced Technology Center of Southeastern Pennsylvania

University Science Center

International Union of Pure and Applied Biophysics

We are most grateful for the generous support. Welcome to the written proceedings of a stimulating conference that we predict will over the years have some impact on how biophysics evolves.

Bob Austin

# CONTENTS

**Session VI: Applications**

## Welcome & Introduction

Dr. Terence Barrett, (NAVAIR)

Well, before getting down to the scientific presentations, I'd just like to take some time to very quickly explain the reason why the Naval Air Systems Command (NAVAIR) is interested in partially funding this conference. The reason is that we have problems in the field of biotechnoloy for materials applications, and those problems are in protein engineering.

We're considering using proteins in various ways which are sometimes unrelated to the *in vivo* functioning but derive from that functioning. We're also interested in using the results of the wild and wonderful field of biotechnology to obtain non-medical products.

Now, you can make a very good living from a small amount of insulin, for example, in the biomedical field. But small amounts are insufficient for economic survival in the commodity chemical field. There is no way, at the present time, to obtain bulk chemical production from biological processes. So we are interested in using protein engineering in some way to increase the yield from these biological processes.

We also see problems, which we hope you will address, if you are interested, in the separation of product from byproducts and microbes, and also in the ability of enzymes to function in various solvents.

We see problems particularly in the field of bioenergetics. For example, we don't understand how, if you have a high energy phosphate bond of only 0.46 e.v. usable for work purposes, how that energy can be transduced into an enzyme. Now there are various Ansätze for solving this vibrational energy transfer problem. And if you object to this approach, well, the reply is: If not these – what? Because one has to understand how you transduce that energy amount in order to obtain engineering control of enzymatic reactions. And you must also understand how you transduce that energy amount in order to have a rigorous science of bioenergetics.

So this endeavor comes under the general rubric of *molecular control*[*] or the

* *Molecular Control*, a joint NAVAIR (Code AIR-310P) and NASA (Code EB) booklet.

obtainment of controlled energy transfer to molecular systems for useful purposes. We're looking forward to entropy engines and the understanding of how this energy transfer will enable us to *energy harvest*. Not only is NAVAIR interested in this, but so is NASA.

One would, as I say, hope for molecular control and e.m. field control of enzymes to speed up the reactions to get the yield we want. Now this is not easy to obtain, because this involves us in considering both classical and quantum mechanical resonance energy transfer for laser specific chemistry. The conceptual framework is there for classical energy transfer. It is not easy, and the conceptual framework has not been completed for quantum mechanical resonance energy transfer.

So, in order to solve our problems we are interested in developing the idea of molecular engines, that is: entropy and internal energy enzymes, which will bring in its wake a fundamental understanding of how proteins function *in vivo*. The transfer of energy in an entropy engine fashion is *not* represented in the case, say, of rhodopsin in which you have a one-shot transfer of energy. Unless you pump in energy using respiratory enzyme sources, the rhodopsin will not function again. But one might take bacteriorhodopsin, say, as a representative case of an entropy engine, which is able to regenerate itself for a second cycle and do useful work, using the external photonic energy source for both these processes.

All this boils down to some very fundamental problems and although an approach is beginning to be developed and understood in classical mechanics – namely, that one must, in order to achieve these goals, circumnavigate chaotic events to reach quasi-periodicity – we don't understand at the present time how you achieve resonance for energy storage in quantum mechanical systems.

So, in summary, the reason why we decided to partially fund this conference is that we have a number of problems and in this very fast review, I hope have indicated some of them. In the next three days, if any of these strike your interest, well, please come up and we'll have a discussion about it.

# Debye-Waller Factor in Solid State and Biological Samples

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## Abstract

The Debye-Waller factor is discussed as it relates to X-Ray diffraction and to EXAFS.( Extended X-Ray Absorption Fine Structure). The two measure different quantities and are complementary. Biological metalloproteins in solution have a temperature dependence of the EXAFS Debye-Waller factor at the metal site which is similar to that of crystallized small molecules. Expressions for the free energy and entropy contributions of the vibrations that cause the Debye-Waller factor are presented. EXAFS measurements permit one to estimate these contributions. In the metalloprotein hemerythrin that serves to transport and store dioxygen, it is found that a large part of the entropy change as $O_2$ is released is localized at the active site and is accounted for by changes in the vibrational modes.

## I. INTRODUCTION

There are two Debye-Waller factors (DWF) involved in structure determinations. In discussing these DWFs the assumption will be made initially that the harmonic approximation is appropriate. Anharmonic effects are important in biological macromolecules, and the same is also true in solid state samples. However, in the presentation of the physics of the DWF it is convenient to assume the harmonic approximation. The corrections to the harmonic approximations are known [1].

In diffraction from a primitive lattice the intensity of the diffraction line is decreased by a DWF given by [5]

$$I = I_0 \exp[-(\vec{G} \cdot \vec{u})^2],$$

where $I_0$ is the intensity if no displacement from the lattice point is present,

$$| \vec{G} | = \frac{4\pi}{\lambda} \sin\theta$$

is the reciprocal lattice vector which diffracts the incident wave of wavelength $\lambda$ through an angle $2\theta$, and $\vec{u}$ is the rms displacement of the diffracting atom from the lattice point due to vibrations.

In EXAFS a DWF also occurs [1], but this factor has an essential difference from that in diffraction. It is usually written as

$$\exp[-2k^2\sigma^2].$$

Here $k = 2\pi/\lambda_e$ is the wave number of the excited photoelectron, while $\sigma$ is the rms deviation about the average distance between the center atom and a neighboring atom. It is important to note that $\sigma$ is the *relative* displacement between two atoms, while $| \vec{u} |$ is the displacement of a *single* atom from the lattice site.

In comparing EXAFS and diffraction, it should be noted that besides the difference in the DWF, EXAFS is a short-range probe within $\sim 5\mathring{A}$ of the center atom [1], while diffraction is a long-range probe requiring long-range order [5].

## II. COMPARISON OF $\sigma$ AND $u$

To illustrate the difference between *sigma* and $u$, consider (as shown in Fig. 1) an imidazole ring which vibrates by rotating about the bottom $N$ atom fixed at a lattice point. The imidazole ring remains rigid in this vibration. In this case $u$ is nonzero for the four other atoms in the ring and is proportional to their distance to the $N$ atom. However, $\sigma$ remains between atoms to allow a molecule to pass through the ring, it is clear that $\sigma$ is the pertinent quantity, not $u$.

If vibrations of two atoms are uncorrelated, then

$$\sigma^2 = u_1^2 + u_2^2, \tag{1}$$

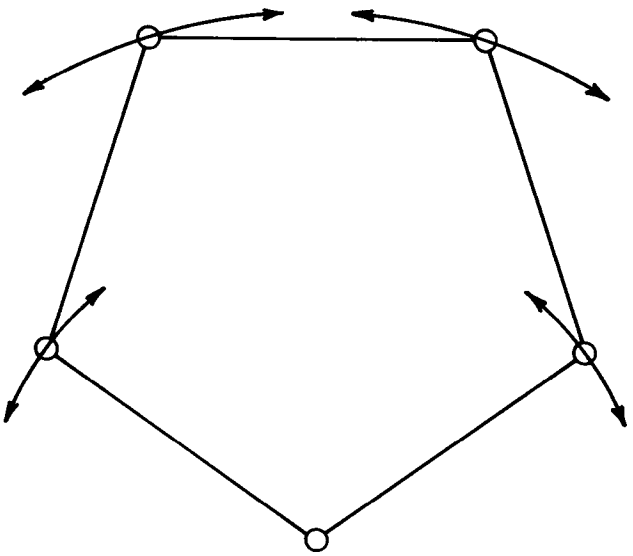

*Figure 1. Illustrating the difference between u and σ for an imidazole ring vibrating by pivoting rigidly about the atom at the lower vertex. The motion of the other atoms is u and is indicated by the arcs with arrows at each end. In this case* $\sigma = 0$.

where the subscripts 1 and 2 refer to the two atoms. However, if the motions of the two atoms are correlated, as in the case of the imidazole ring discussed above, then

$$\sigma^2 \neq u_1^2 + u_2^2. \tag{2}$$

For atoms far apart one expects no correlation and Eq. (1) applies. For neighboring atoms, correlation is expected and relation (2) applies. In general one expects significant correlation effects to extend out to second neighbors, or about 3-4 Å. Thus, one needs to exercise caution in employing (1) to interpret the $u^2$ from diffraction as a measure of the fluctuation in the relative distance between neighboring atoms.

In respect to the fluctuations in the relative distance between atoms, EXAFS and diffraction are complementary. Diffraction determines, the principle if not

always in practice, the $u^2$ for all atoms. Use of (1) to determine the $\sigma^2$ for pairs of atoms is usually valid if the atoms are greater than $\approx 4\mathring{A}$ apart. EXAFS is able to measure $\sigma^2$ directly, but is usually limited to doing so for the nearest neighboring atoms less than about 4 $\mathring{A}$ apart.

## III. EXAMPLES OF $\sigma^2$

Table I gives values of $\sigma^2$ as a function of temperature for solid state samples and biological macromolecules. As can be noted, there are not any large differences in the values of $\sigma^2$ in these two cases. The behavior of $\sigma^2$ as a function of temperature in biological macromolecules follows behavior similar to that in small molecules.

## IV. IMPORTANCE OF $\sigma$ IN UNDERSTANDING BIOLOGICAL FUNCTION

It has recently been emphasized that the kinetics of macromolecules and the diffusion of molecules to the active site are dynamic processes, and vibrations are an important contribution to these processes [6]. As discussed in Sect. II the quantity $\sigma$ is the pertinent vibration variable.

It has been suggested that different configurations or conformations can be frozen out as the macromolecule is cooled, and these different conformations can change the barrier heights and thus tunneling rates by significant amounts [6]. Such a significant change in the potential distribution around the active site would be expected to also affect the vibrational frequencies. Such changes may be detected by measuring $\sigma^2$ as a function of temperature and comparing the low temperature disorder with the expected zero point disorder. The freezing of configurations may also introduce visible hysteresis effects in the temperature dependence of $\sigma^2$.

As shown in the discussion below, EXAFS measurements of Table I indicate that the free energy changes in biological reactions may have a significant con-

Table I. Einstein temperatures and $\Delta\sigma^2$ at room temperature of pairs of atoms for some crystallized small molecules and some forms of hemerythrin (Hr) in solution as obtained by EXAFS. The center atom in all cases except for Ge is Fe. The other atom in the pair is indicated in parentheses. The azidomet- and oxy-Hr have a short μ-oxo bond in addition to more standard O and N bonds in the first shell. These are distinguished in the Table. Listed are the values of $\Delta\sigma^2$ for the first and second coordination shells of atoms and their corresponding Einstein temperature $\theta_E$.

| | $\Delta\sigma^2$ ($10^{-3}$ Å$^2$) | | $\theta_E$ (K) | |
|---|---|---|---|---|
| Sample | First | Second | First | Second |
| *A. Solid State Crystals of Small Molecules* | | | | |
| Ge | 1.6 (Ge) | 6.3 (Ge) | 353 | 207 |
| Fe-gly.[a] | 2.7 (O) | 2.5 (Fe) | 435 | 331 |
| Fe-TIM[b] | 1.0 (N) | -- | 621 | -- |
| FeOFe[c] | -- (O,N) | 1.5 (Fe) | -- | 400 |
| FeOHFe[d] | 2.0 (O,N) | 2.5 (Fe) | 490 | 335 |
| *B. Macromolecules in Solution* | | | | |
| Azimomet-Hr | 2.0 (O,N)<br>0.6 (μ-O) | 3.1 | 490<br>720[e] | 305 |
| Oxy-Hr | 2.6 (O,N)<br>0.6 (μ-O) | 1.0 | 448<br>720[e] | 463 |
| Deoxy-Hr | 4.3 (O,N) | 8.4 | 375 | 217 |

[a] $[Fe_3O\cdot(glycinato)_6(H_2O)_3](ClO_4)_7$.

[b] bis(acetonitrile)(2,3,9,10-tetramethyl-1,4,8,11-tetrazacyclotetradeca-1,3,8,10-tetraene)iron(II)hexafluorophosphate.

[c] $Fe_2O(O_2CCH_3)_2(HB(pz)_3)_2$.

[d] $Fe_2(OH)(O_2CCH_3)_2(HB(pz)_3)_2$.

[e] Obtained from Raman spectroscopy; see, e.g., R.M. Solbrig, L.L. Duff, D.F. Shriver, and I.M. Klotz, J. Inorg. Biochim. 17, 69 (1982).

tribution from changes in vibrational frequencies. The formula that relates free energy changes to vibrational changes between a pair of atoms is

$$F_2 - F_1 = \frac{3k}{2}(\theta_1 - \theta_2) + 3kTln\frac{\Delta\sigma_1^2\theta_1}{\Delta\sigma_2^2\theta_2}. \quad (3)$$

Here subscripts 1 and 2 refer to the states before and after, respectively. The change in mean square amplitude of vibration due to thermal excitation is denoted by $\Delta\sigma^2$, i.e., the increase in mean square vibration from 0 K to temperature T. The Einstein temperature is $\theta$, and $k$ is Boltzmann's constant. EXAFS

degrees of freedom have the same free energy change as the radial distance.

To illustrate the importance of thermal vibrations to the energetics of biological processes, the case of hemerythrin is considered. Hemerythrin is a respiratory protein found in the coelomic fluid of a number of marine invertebrates. The active site of the protein contains two nonheme iron atoms that reversibly bind one molecule of oxygen. The iron in deoxyhemerythrin is in the ferrous state. Formation of oxyhemerythrin results in the oxidation of both iron atoms to an antiferromagnetically coupled ferric state with concomitant reduction of dioxygen to peroxide. Replacement of the bound peroxide by anions such as azide or hydroxide leads to methemerythrins in which the two Fe(III) ions retain their antiferromagnetic coupling but are no longer functional in transporting oxygen.

The energetics of the binding and release of $O_2$ in hemerythrin obtained from various invertebrates has been measured. There is a wide spread in the values, which probably is an indication more of the uncertainty in the measurements than of variations among the invertebrates. However, in order to have values to compare with, the values of enthalpy and entropy change for *P. gouldii* hemerythrin are chosen, the same source of hemerythrin as used in our EXAFS measurements. The experimental enthalpy values $\Delta H$ are $9.2 \pm 0.4$ kcal mole$^{-}$1, obtained from calorimetry, and 12.4 kcal mole$^{-}$1, obtained from measurements of the kinetics of the reaction [8]. Only one experiment value of the entropy change of $\Delta S = 18$ e.u. was found in the literature [8]. The entropy increases when the $O_2$ is released, and it has been suggested that this indicates that deoxyhemerythrin has fewer ligands at the active site. The increase in entropy is thus largely attributed to the extra degrees of freedom of the freed ligand, namely $)_2$.

Recent EXAFS results [9] suggest that the number of ligands at the active site does not decrease as $O_2$ unbinds. The site vacated by $O_2$ is replaced by another oxygen, presumably from a water or OH molecule. The entropy increase can be largely accounted for by changes in vibrational excitations. The total free energy change per atom pair is given by (3), while the vibrational energy change is given by

$$U_2 - U_1 = \frac{3\mu k^2}{\hbar^2}(\theta_2^2 \Delta\sigma_2^2 - \theta_1^2 \Delta\sigma_1^2) + \frac{3}{2}k(\theta_2 - \theta_1), \qquad (4)$$