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ORIGINAL PAPER

# Dynamic analysis of the atomic vibrations of proteins, as exemplified by the binding of myristic acid to human serum albumin

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**Abstract** Prediction of the biological function of a protein from its three-dimensional structure is an important, still unsolved problem. A new approach to this objective, tried here, is use of crystallographic temperature factors, which contain the same information as IR and Raman spectra but lack their overlap problems. The hypothesis that atomic vibrations are evolutionally optimized for a particular function by adoption of collective modes governed by an attractor has been tested on 19 proteins with the result that strong correlation ($r = 0.98$) was found between the dimension of the attractor and the number of vectors needed to describe the function. The binding of five molecules of myristic acid (MA) to human serum albumin (HSA) at two sites accommodating two or three MA molecules, respectively, gave rise to four conformational changes in distinct regions. Two of these were located at the binding sites but the others occurred in segments far removed from the ligands both in the sequence and spatially. According to the statistical criteria employed, the conformational changes at the ligand-binding sites were not necessarily controlled by an attractor of low order, but the others were governed by one of dimension of 2–3. This was ascribed to entropic compensation. The results were tested using another ligand, an inhibitor of the BCL-2 family of proteins. The HSA underwent the same conformational changes with this ligand as with MA.

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**Abbreviations**

| | |
|---|---|
| HSA | Human serum albumin |
| MA | Myristic acid |
| HSAM | MA bound to HSA |

## Introduction

Classification of proteins as fractal structures enables investigation of these biologically important molecules from a new perspective which illuminates new aspects of their dynamic and functional properties. The indications of the correctness of the assignment of fractal properties to proteins include the fulfillment of statistical criteria and analogies with other natural objects, e.g. contours of coastlines, clouds, crystals, colonies of mold, lightning patterns, and branches of trees, all of which undisputedly belong to the group of fractal objects.

Because important events in the function of proteins occur in an ultra-short time range, methods of high temporal and spatial resolution are needed to identify the mechanisms. Most previous investigations of this kind have used spectral methods, e.g. infrared or Raman spectrometry, but that approach suffers from the inability of the methods available to resolve overlapping spectra. This inherent problem can be circumvented by use of a different measure of the atomic vibrations, the crystallographic temperature factor (or b-factor), which is measured in diffraction experiments with X-ray or cyclotron radiation, but seldom used. The temperature factor is the diameter of

the volume of occupancy of a particular atom and hence a measure of its vibrational amplitude. Because the temperature factor is sensitive to the influence of neighboring atoms, not only those which are connected covalently to the atom under observation but also those that are spatially close, statistical analysis using conventional methods of molecular dynamics can be expected to reveal properties of the local or global assembly of atoms, e.g. the presence or absence of one or more attractors. Because this method sacrifices temporal resolution to assess global properties, it complements, but does not replace, spectrophotometric techniques. So far, a search for attractors has been carried out using nineteen proteins of different functions (Havsteen 1989, 1991, 1997, 1999a, b, 2002, 2004; Isvoran 1997, 2000; Isvoran and Morariu 2000a, b; Isvoran et al. 2000a, b, c, 2001). The latest addition is the binding of myristic acid (MA) to human serum albumin (HSA), reported here. This investigation revealed a new feature of binding interactions, conformational changes without apparent attractor control; these are tentatively ascribed to entropic compensation.

## Methods

The data used in this study were obtained from the Protein Data Bank at Rutgers University under the codes 1bj5 (HSA + M), 1ysx (HSA + inhibitor), and 1bm0 (HSA). They were collected by Curry et al. (1998) and Sugio et al. (1999). The mathematical methods required for evaluation of the properties of a non-linear singularity, for example an attractor, are the saturating order of the correlation integral at increasing imbedding dimension, the correlation length as reflected by the autocorrelation function, the Poincare' projection, the first Lyapunov exponent, the power plot, and the Hurst fractal coefficient. These have been described elsewhere (Havsteen 1989, 1991, 1997, 1999a, b, 2002, 2004; Isvoran 1997, 2000; Isvoran and Morariu 2000a, b; Isvoran et al. 2000a, b, 2001; Ikeguchi and Aihara 1997; Judd 1992, 1994; Anfinrud et al. 1999).

The question of the effect of crystal defects on the validity of the temperature factors has been examined by Northrup et al. (1980). They found that errors from this source would normally be minor and that they could be eliminated by use of several crystals.

## Results

This project was intended to expand the range of protein varieties the molecular dynamics of which had been studied by our group to assess their possible relationship with biological function. These proteins include enzymes, gas-binding proteins, immunoglobulins, enzyme inhibitors, and prions. Human serum albumin (HSA) was chosen as a representative, physiologically important carrier of many different ligands, a prominent example of which is myristic acid (MA). Up to five molecules of MA can bind to a single molecule of HSA. Three MA molecules, $M_1$, $M_3$, and $M_4$ (in the notation of the crystallographers), bind to the penultimate domain of HSA near the C-terminus and the others, $M_2$ and $M_5$, to the C-terminal domain. Because it was assumed, as in previous studies, that the conformational change induced by the binding of the ligand was contributing to the specificity and affinity of complex formation, the difference between the temperature factors of the free (HSA) and loaded (HSAM) protein was plotted as a function of amino acid residue number (Fig. 1).

Four distinct peaks are seen in the plot; a fifth possible peak near the C-terminus was discounted as a shoulder of the last peak or as an artifact. The locations of the peaks were:

Peak # 1 between residues # 70 and 95,
Peak # 2 between residues # 310 and 335,
Peak # 3 between residues # 520 and 559, and
Peak # 4 between residue # 559 and the C-terminus.

Examination of the location of the five MA molecules revealed that $M_1$, $M_3$, and $M_4$ were bound near peak # 3, whereas $M_2$ and $M_5$ were located near peak # 4. Surprisingly, peaks # 1 and 2 were far removed (40–70 Å) from the ligand-binding sites, both in sequence and spatially.

Because the data, especially on the C-terminal segment of the free HSA, contained a substantial amount of noise, calculation of the integral correlation coefficient was carried out by a parametric method and a non-parametric

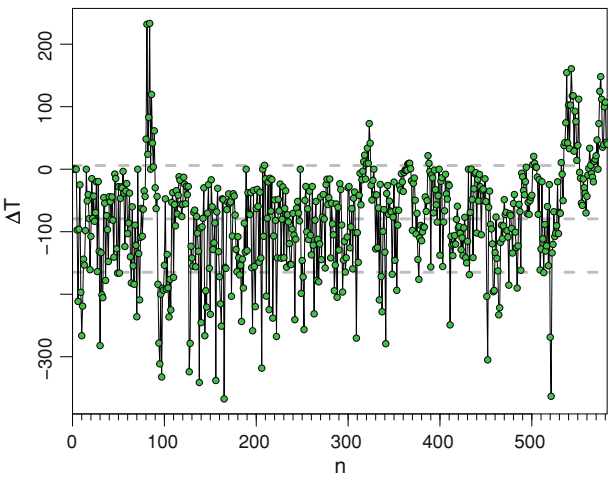


**Fig. 1** Changes in temperature factors along the peptide chain of human serum albumin on binding of five molecules of myristic acid. The peaks are shown as minima here. Peak 1: Res. # 74-94; peak 2: Res. # 310-339; peak 3: Res. # 526-555; peak 4: Res. # 559-582

method to establish whether or not the important criterion for the presence of an attractor, the saturation of this coefficient by an increase in the imbedding phase space dimension, was satisfied. The results are shown in Fig. 2 and Table 1. For peaks 1–3, saturation of the integral correlation coefficient, $\bar{d}$, is plausible, whereas this is not so for the peak near the C-terminus (# 4). The reason may be the great flexibility of the segment. It is notable that the dimensions of the putative attractor at peaks # 1 and 2, which may represent entropic compensation, is 2–3, whereas that at the ligand-binding site for $M_1$, $M_3$, and $M_4$ (peak # 3) is higher (4–5).

In Fig. 2 the data were fitted to exponential functions. For Figs 2a and 2b:

$$d = \bar{d}(1 - e^{-an}),$$

where $d$ is the correlation coefficient, $\bar{d}$ the dimension of the putative attractor, and a is a constant. For Fig. 2c:

$$d = \bar{d}e^{-an}$$

The reasons for the choice of these fitting equations were:

1. The fit to these simple equations was satisfactory, because the correlation coefficients, $r$, of the corresponding logarithmic plots were 0.82, 0.88, and 0.72, respectively.
2. The progress of the fitting of a model to the point group in the phase space with an increase in the dimension of the attractor tested, i.e. $n = 1, 2, 3, 4$, etc., corresponding to the attractor shapes point, line, sphere, rotation ellipsoid, (toroid?), etc. was assumed to follow a power function:

$$d = \bar{d}\, a^{-cn}$$

where c is a constant. The reason for this choice was the apparent ubiquity of the power relationship in natural phenomena, e.g. phase transition and membrane filtration (Havsteen 1984, 1993). The second criterion for the presence of one or more attractors in the HSA–ligand complex is the damping of the autocorrelation coefficient, $\Psi$, by an increase in the distance of interaction, $n$. The result, which is shown in Fig. 3, does not contradict the hypothesis of the presence of attractors. In Fig. 3, the data can be fitted with an exponential or a power function almost equally well ($s_D = 0.092$ and 0.107, respectively).

The third criterion for the test of the hypothesis is the pattern of the Poincare' plot of successive pairs of temperature factors that may be regarded as a cross-section of the putative attractor. The pattern in Fig. 4 is compatible with the presence of a weak attractor.

The fourth test of the attractor hypothesis is the positivity of the first Lyapunov exponent $\lambda$, which characterizes the convergence of two arbitrarily chosen, almost parallel

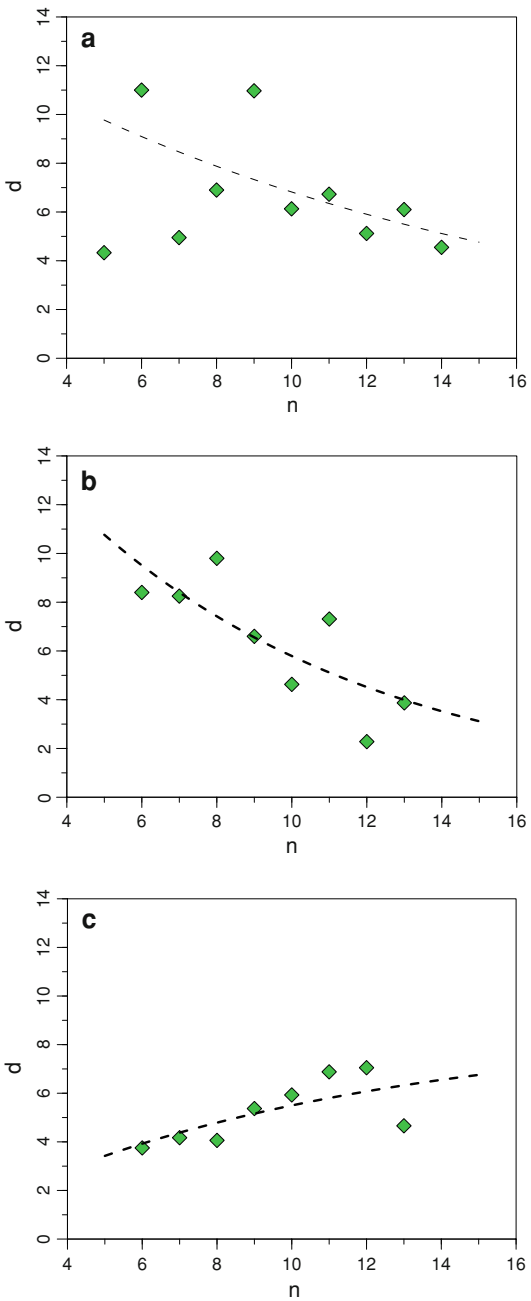


**Fig. 2** Dependence of the parametric correlation integral $d$ of the putative attractors associated with multiple ligand binding on the number of phase space coordinates. **a** Peak # 1. **b** Peak # 2. **c** Peak # 3

**Table 1** Properties of the putative attractors

| Peak | Parametric $\bar{d}$ | Non-parametric | Ligands | Hurst coeff. |
|---|---|---|---|---|
| 1 | 3.3 ± 0.4 | 3 ± 1 | None | 1.57 ± 0.04 |
| 2 | 2.0 ± 0.5 | 3 ± 1 | None | 1.55 ± 0.03 |
| 3 | 5 ± 1 | 4.0 ± 0.1 | $M_1$, $M_3$, $M_4$ | 1.75 ± 0.03 |
| 4 | n.s.[a] | n.s. | $M_2$, $M_5$ | 1.31 ± 0.05 |

[a] Not saturated

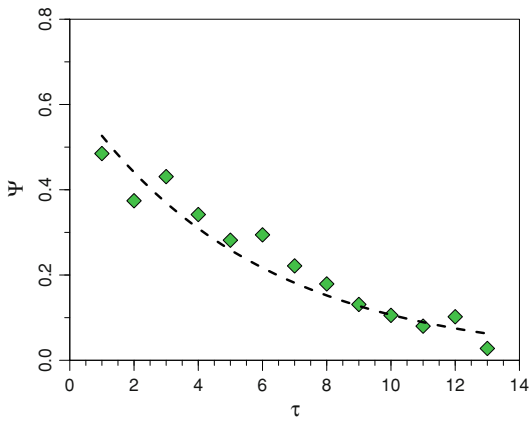


**Fig. 3** The dependence of the autocorrelation function, $\Psi$, on the distance of interaction, $\tau$

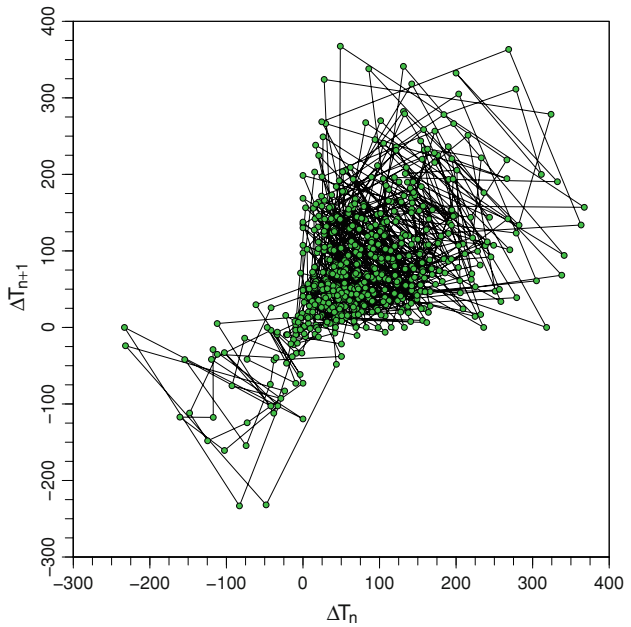


**Fig. 4** Poincare' plot of successive pairs of temperature factors ($\Delta T$ values) on binding of myristic acid to human serum albumin. The plot may be regarded as a cross-section of the putative attractor

trajectories approaching the attractor. The curve drawn in Fig. 5 shows that this condition is fulfilled.

An additional criterion for the presence of fractality, which is a property of attractors, is the Hurst coefficient, *i/f*, which is listed in Table 1 for each of the peaks. The result lends support to the hypothesis, because the characteristic value for random movement, 1.50, for all of the four peaks is clearly different when experimental errors are considered.

The results were tested by examination of the binding to domain 3 of the HSA of a ligand other than myristic acid, an inhibitor of the BLC-2 family of proteins, 4-((2-[(2, 4-dimethylphenyl)sulfanyl]ethyl)amino)-*N*-[(4′-fluoro-1,

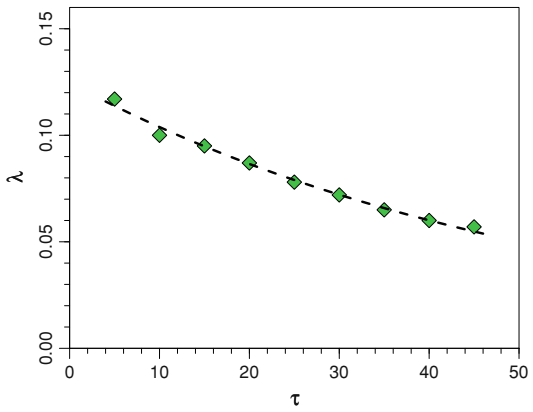


**Fig. 5** Plot to test the positivity of the first Lyapunov exponent, $\lambda_1$, of the putative attractor associated with ligand binding. The exponent characterizes the convergence of two, arbitrarily chosen, almost parallel trajectories approaching the attractor. $\lambda = 1/\tau\ [\ln(P_\tau/P_0)/\ln 2]$

1′-biphenyl-4-yl)carbonyl]-3-nitrobenzenesulfonamide, which shares some structural features with myristic acid but lacks the negative charge of the carboxyl group. The result was that the HSA, upon binding of this inhibitor, underwent the same four conformational changes in the same positions as those with HSAM and to approximately the same extent (not shown) (Oltersdorf et al. 2005).

## Discussion

Protein structure and dynamics are controlled by numerous non-linear interactions, which are expected to yield fractal properties. Many observations unanimously support their actual existence (Havsteen 1989, 1991, 1997, 1999a, b, 2002, 2004; Isvoran 1997, 2000; Isvoran and Morariu 2000a, b; Isvoran et al. 2000a, b, c, 2001). The question whether or not this fractality, especially attractors, plays a role in the function of proteins by restraining the available modes of vibration and hence the accessible structural forms, was approached by collection of typical examples of correlation between the dimension of the attractor in the protein and the number of vectors needed to describe the protein function.

The variety of proteins which have been investigated so far to test the hypothesis, is seen in Table 2. Although it contains only 19 proteins, a pattern appears to emerge, which is shown in Fig. 6. I included human serum albumin in the selection of proteins to be tested to ascertain whether binding of several ligands would reveal an example that did not fit into the relationship indicated in Fig. 5. The choice of the binding of myristic acid to HSA led to several surprises, although the evidence did point to the presence of several attractors of the expected magnitude in the HSA–MA complex. However, the attractors that were the better

**Table 2** Correlation between attractor dimension and the number of functional vectors

| Protein | Attractor $\bar{d}$ | Vectors |
|---|---|---|
| Myoglobin | 1.46 ± 0.03 | 1 |
| Chymotrypsin | 1.9 ± 0.2 | 2 |
| Lysozyme | 2 ± 0.2 | 2 |
| $PrP_c \rightarrow PrP_{Sc}$ | 2.7 ± 0.2 | 3 |
| $PrP_c$ C-terminal | 5.3 ± 0.3 | |
| $PrP_{Sc}$ C-terminal | 3.9 ± 0.3 | |
| Cyt $c_{inner} \leftrightarrow$cyt ox $\theta$-transfer | 3.08 ± 0.08 | 3 |
| Cyt $c_{outer} \leftrightarrow$cyt ox $\theta$-transfer | 4.2 ± 0.2 | 4 |
| mATPase $\alpha$-chain ADP-binding | 3.0 ± 0.3 | 3 |
| mATPase $\beta$-chain ATP-synthesis | 4.48 ± 0.07 | |
| mATPase $\beta$-chain ADP-binding | 5.03 ± 0.03 | |
| mATPase $\alpha$-chain ATP-synthesis | 6.2 ± 0.2 | 6 |
| Abenzyme H-chain esterolysis | 3.0 ± 0.3 | 3 |
| Abenzyme L-chain esterolysis | 7.5 ± 0.3 | |
| Antidigoxin Ig L-chain | 4 | |
| Antidigoxin Ig H-chain | 5 | |
| Deoxyhemiglobin tetramer | 3.5 | |
| HIV-1 protease dimer | 4.3 | |
| HSA + M, peak 1 | 3.2 ± 0.3 | 3 |
| HSA + M, peak 2 | 2.7 ± 0.2 | 3 |

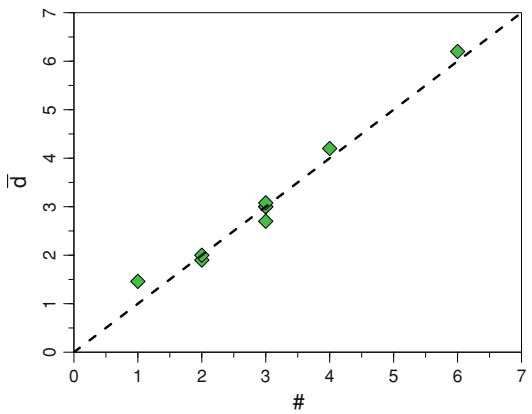


**Fig. 6** Correlation between the attractor dimension and the number of vectors needed to describe the protein function. Correlation coefficient $r = 0.98$. *Abcissa*, number of vectors; *ordinate*, attractor dimension. Because the ATPase contains three catalytic subunits per molecule, the point at $x = 6$ is a triple point

defined (peaks # 1 and 2) were not located at the ligand-binding sites but at a substantial distance from these, both in space and in the amino acid sequence. Hence, the two attractors were tentatively attributed to entropic compensation effects. The complexity of the data at the ligand-binding sites were tentatively ascribed to their, in this connection, unfortunate proximity to the C-terminus of the HSA, which is so flexible that there are large experimental errors in the associated temperature factors. Yet the linear correlation shown in Fig. 5 between the attractor dimension and the number of vectors needed to describe the protein function, has a high correlation coefficient. This piece of evidence seems to lend considerable support to the hypothesis of the role of attractors as guides of protein function.

Because HSA underwent the same four conformational changes with MA and with the BCL-2 inhibitor and in both the same positions and to the same extent, the charge neutralization between amino groups in the binding sites and the carboxyl group of MA cannot be part of an allosteric regulation. Therefore, the alternative interpretation, entropic compensation, is probably preferable. Besides, the conformational changes do not open new binding sites for the ligand, although they probably increase the affinity between the carrier and the ligand.

Compared with NMR studies, analysis of the temperature factors offers higher spatial resolution and does not rely on nuclear species, which are especially suited to NMR studies. Hence, it could serve as a useful supplement to traditional methods.

The structure of proteins is, to a substantial extent, determined by non-linear interactions, which confer fractal properties upon these biologically important macromolecules. In such molecules, the possibility of formation of stable, dynamic structures, called attractors, exists. An attractor may be regarded as a global solution to the equation of motion of the contributing atoms and as a soliton, like a tsunami. If one or more attractors are present in a protein, then it or they would restrict the atomic vibrations in the respective domains or globally, thus guiding conformational changes in the protein accompanying its biological function into an obligatory path and protecting the reproducibility of the function. If this is the case, then attractors play an important role in protein function, e.g. enzyme catalysis, electron or gas transport, and ion translocation. Unfortunately, definitive evidence of the existence of an attractor cannot be obtained directly, but only indirectly with the aid of a series of statistical methods. Hence, the only means of obtaining convincing data in support of the existence of attractors in proteins, and their probable function, is to gather a substantial number of examples that indicate the correctness of the hypothesis. Nineteen such examples have shown a distinct correlation ($r = 0.98$) between the dimension of the putative attractor and the number of vectors needed to describe the protein function, thus yielding plausible evidence of the hypothesis.

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