UNIVERSITY OF POONA

ZOOLOGY DEPARTMENT
Ganeshkhind,
PUNE-411 007 (INDIA).

Telephone No. : 338952
336061
Ext. 20, 42
Gram : UNIPUNA
Fax : (+91) 0212 330087
(+91) 0212 333899
Telex : 0145 7719 UNIP IN

Ref. No. Zool/1993-94/5076

Date : 13/1/94

Dr.N.V.Joshi
Indian Insitute of Science
Bangalore - 560 012

Dear Dr.Joshi,

I could not make it to Bangalore due to flight problem. Enclosed is the reprint which I got from Dr.Barnabas. The data enclosed on regression is the mutation probability for amino acids from figure 9.6 in the text against all the properties. Does it any thing striking? Please also let me know if you can come Pune in middle of February.

Thanking you.

Yours sincerely

(V.Sitaramam)
Co-ordinator
Biotechnology Training Programme
Department Of Zoology

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PUNE-411 007 (INDIA).

Telephone No. : 336061
Ext. 20, 42
Gram : UNIPUNA
Fax : (+91) 0212 330087

Ref. No. Zool./1993-94/7180

15th Feb.1994

Dr.N.V.Joshi
Indian Institute of Science
Bangalore - 560 012

Dear Niranjan,

I was discussing with Dr.John Barnabas regarding the Dayhoff matrix, he strongly felt that the 2 PAM matrix that we are using is based on too little & too old data and we should use the latest information. Such a recent compilation is not available. I told him our difficulty in being able to arrive at such a matrix. He gave the following address who apparently has inherited Dayhoff's work & lab after her. The address is given below. Please write to him to help you to get the data base and latest matrix at the level PAM that you choose. I also suggest that you send the copies of my genetic code paper along with our preprint to interest them.

Please act at your earliest.

Thanking you.

With best regards.

Yours sincerely

(V.Sitaramam)

Address:

Dr.W.C.Barkar
National Biomedical Research Foundation
George Town University
Washington DC 20007

P.S.: The standing question is:

When you are coming to Pune?

UNIVERSITY OF POONA,
PUNE - 411 007, INDIA.
DEPARTMENT OF BIOTECHNOLOGY

Biot./94-95/205

Dr. V.Sitaramam
M.B.,B.S., Ph.D.
Professor in Biotechnology

Phone : + 91 212 335179
Fax : + 91 212 335179

14th Dec. 1994

Dr.Niranjan Joshi
Indian Institute of Science
Bangalore - 560 012

Dear Niranjan,

I thought about MD simulations. Given a chance I would do them. Sooner or later we are going to measure compressibility by osmotic methods on proteins, where β data is available. The lady who should have done this experiment decamped with the salary so there is nobody to do the work yet. I regret (though not fully) for having complicated the problem. You see, when you give solution I begin to understand the problem!

Let me say a few words on biology. I am mailing a review written on fluctuations in proteins separately. My student Zuben got data in miscelles, membrane and protein that voids exists and that they are compressible. He got fairly clear voids like I was telling you. In all these structures as one part of the molecule `freezes' the other part molecules vibrates like crazy as if the total kinetic energy is conserved. So we are considering polymeric structure with some flaws. These flaws are the voids. The trouble is that the different sizes of voids are differentially compressible, like not all animals are equal. What we want is to find out is which are the largest voids that are most compressible and what is their role. We believe that intramolecular energy transfers are mediated by such compressible voids. If these voids are at the end of helices, so much the better as the helix extends into voids on one side. The period of occurrence possibly giving a natural frequency. I can go on like this with several possibilities on how to use and interpret the voids. My least problem is biological application and my most pressing physical problem is to put a number. This is why I wanted to fill the place first with the largest marbles first and then progressively smaller ones. As you can see the number of voids of zero dimension is infinite. Therefore, total voids estimation has less information than a cross section of `marbles' that are available at given time and their placement in space inside the proteins. I hope that this answers your questions in some measure.

With best regards,
Yours sincerely ,

(V.Sitaramam)

UNIVERSITY OF PUNE

DEPARTMENT OF BIOTECHNOLOGY

Ganeshkhind, Pune-411 007.
INDIA
Tel. : (0212) 354952
Gram. : UNIPUNA

Ref. No. : Biotech / .....

July 7-1997

Dear Dr. Jayan

This is a repeat mail because the Comm fellows ruined the dispatch. All the enclosures are not here but the project write up is in toto.

Pl. bring this also with you to Pune

We are looking forward to your visit

Regards

Yours sincerely

Vijay Kumar

Soilvent exclusion in the active site depending on the ligand size: Does it explain the induced fit?

The role of induced fit in the general mechanism of enzyme action is well recognized. Clearly, a competent explanation must reside in energetics. It should be indepedent of any specific reaction mechanism per se or the type oif enzyme. It should be driven by the proximity of the surface of the active site to the substrate. The question ios whether binding energetics in terms of enthalpy alone fully explain the mechanics of indiuced fit or any other factors are invovled, hitherto unrecognized. Conventional explanations including the computational, areb almost exclusivelky restricted to enthalpic contributions(the hydrogen bonds, electrosttic interactions, Van der Waals interactions). Entropy is often thought of as importantand yet a handle on its relevance remains eluisive. It is customary to relegate entropic considerations top arrive at throughputs of activity. The entropic contributions to structural poertyurbations like the induced fit remains notional in the absence of specifife mechanistic insights.

In the simpler case of induced fit, the emzyme structure with the transient state would involve a de crease in the dimensions achieving a better physical dsescriptiona decrease in dimensions achievinga better physical approximation between the enyme and the substarte as a prelude to the transformation of the latter. What needs to be explained is the basis of the wll of teh active site necessarily to close in.. The driving force needs to be identified, not in terms of
substrate binding but in terms of some physical, proximate force arising as necessary condition of the circumstance of the presence a solvated substrate molecule in the active site cleft of the polymeric enzyme.

Such a description, if achieved, has the added advantage of the evolution of catalytic mechanisms as a natural consequence of polymer dynamics. It is attractive to visualize a precatalytic stage of organic evolution of polymers in which one would presume that binding predates catalysis and weak binding predates stronger binding. If an event of catalysis should occur in the face oif weak binding, tethering the molecule by in induced fit could help the evolution of catalysis in the face of even weak binding. Micellar catalysis is a calssic example of orientation effects and proximity, rather than binding, as the basis of catalysis. Such forces could be visualized to be facilitated by an induced fit that requires no more than a polymeric cleft and a ligand in the vicinity.

I present here an argument based on the excluded volume approach, that the induced fit is a natural consequence of exclusion of water molecules between the wall of the active site cleft and the substrate, i.e., entropy driven. The energy
available, not due to enthalpic but due to entropic changes could achivee many things including induced fit and influence
intramolecular dynamics. The solvent water could play an determinant role in contributing to the energetics of the induced fit as well dynamics of intramolecular movements in dissipating the excess free energy thereof.

The model

When a susbtrate molecule enters the active site, a large numbers of perturbations in the active site, some specific (from
a catalytic/binding point of view) and some non-specific, can and do occur. Among the
specific events would be ligand-specific interactions with the active site leading the specific hydrogen bonding, electrostatic and Van der Waal interactions etc. intrinsic to binding. The binding is accompanied by some 'induced fit' in which such intermolecular interactions would play some role. The osmotic pressure contribution by the substrate inside the active site could increase as the induced fit lowers the internal volume. The molecular dynamics occur in a time constant comparable to the time constant associated with the Kcat of the order of 10 -5 s.

Let us consider the active site as a cleft made of a flexible wall offering a concavity. Let the substrate molecule be approximate a large sphere of radius, L. Let the watre moelcules be approximated by a smaller sphere of radius, S. Within the cleft, as the substrate approximates the wall, a depletion potential for water can be visualized struictly sterically.
The joint radius when the substrate interacts with one water molecule would be L+S. Whenever the substrate molecule approaches a wall closer than S, water would be sterically excluded, i.e., the excluded volume for a substrate would be (4/3)p [(L+S)3 - (L)3], i.e., the volume of the large sphere must be subtracted. This excluded volume, Ve, would correspond to [(L+S)3 - (L)3] / (S)3 = ne , i.e., the number of solvent molecules excluded. The energy associated with each such excluded molecule would correspond to kT.

Since the calculation transends any barriers of chemistry or absolute values of the relevant radii, we need only to consider the ratio, L/S = R, such that S=1. Thus,

ne = (R+1)3 - R3
= 3R2 + 3R +1
energy = kT . ne

Dinsmore used such arguments to suggest that vesicles approach closer to the surface of a wall of a cell preferentially only when smaller vesicles are present. Conversely, if the larger sphere approached a planar flerxible surface, it would equally follow that a concave deformation would be preferered among the possible deformations. That is, the wall would rather bend.

Would the active site be in isolation or be in equilibrium with the rest of the protein? The folded globular protein is in a dynamic equilibrium such that the electrostatic forces due to the fixed unscreened charges and the elastic forces that characterize the bends of the condensed polymer are somewhat of the same order of energy level for deformation.
Therefore changes in the active site, if these are significant enough, are likely to affect all aspects of the protein structure and dynamics, most likely seen during binding and catalysis. And yet
the functionally important movements are considered very subtle and virtually undetectable. The current efforts in understanding protein dynamics are restricted to the gross behavioure, e.g., during folding.The large energies associated with entropic changes seen in Table 1 argue for a rigorousd search for movements associated with the protein duirng binding and catalysis.

The question as to why one has not directly detected the FIM becomes more poignant when one considers the recent calculatons by Dinsmore et al. The basic question asked by Dinsmore et al relates to the situation when tehre is a flexible wall bathed by a number of small vesicles. Interestingly when a larger vesicle is introduced into the system, Dinsmore and colleagues have shown that entropy effects would require that the large vesicle moves to the wall, seeking a concavity, excluding the smaller vesicles to the extent of the space displaced by itself. In turn, if the wall is of a flexible polymer, it would tend to wrap itself around the larger vesicle, it being an energetically favoured situation.

The Dinsmore calculation is primarily topological. It has no requirement of actual vesicles nor is it restricted by any kinds of materials. In principle, one can reduce the wall to the active cleft, the smaller vesicle to water molecules and the larger vesicle to the substrate, e.g.,

glucose for hexokiinase. Then the energy associated with the dsiplacement of the water
molecules approximating the glucose molecule to the flexible wall of the active site would be of the order of 30 kcal/mole, indeed a significant amount of energy. Fascinatingly, this also provides an alternative driving force for the induced fit in terms of the wrapping of the active cleft wall around the glucose molecule! The possible weakness in the argument could be in that the specificity of glucose molecule would be less important since it is merely a size effect. On the other hand, this phenomenon could serve to explain solvent effects. One can compute energy where water is replaced by other solvents of increasing molecular mass and glucose in turn by other ligands of varying size (Table 1). We see a significant contribution to the energy as the larger molecule increases and as the solvent molecule becomes smaller. Thus when one investigates the effect of solvents on activity, to probe the role of dielectric or viscosity, it argues in favour of also looking for size effects. For instance, for alcohols and alkanes, the size ad ciscosity and such bulk properties are intimately related.
It is rather difficult to point out a priori which explanation would be satisfactory. It is possible that in different (enzyme) systems, different forces would dominate while all these would make some finite though variable contribution in each and every instance. This makes the question posed by Ken Dill more poignant regarding the rule of additivity: what should we add in energetics? The proximate force for any flux would be a matter of
investigation rather than one of surmise base on strictly macroscopic homogeneous models based on singular causes.

Imagine a one dimensional lattice of 7 units. You have one large molecule of length 3 units, and a small moleucle of length 2 units.

(i) If only the large molecule is placed on the lattice, its leftmost point can be at position 1,2,3,4,5 i.e., it will occupy positions 123, 234, 345, 456, or 567
with equal probability.

(ii) If only the small molecule is placed on the lattice, its leftmost point can be at 1,2,3,4,5,6 - and all positions are equally probable.

(iii) If both the molecules are placed on the lattice, the following distinct positions are possible

L denotes large, S denotes small and X denotes an empty site (void!!)
Since L is three units in length, three consecutive Ls would be there. Similarly, two S will always be consecutive. A wall, denoted by W is present at both the ends.

(1) WLLLSSXXW
(2) WLLLXSSXW (3) WLLLXXSSW
(4) WXLLLSSXW (5) WXLLLXSSW
(6) WXXLLLSSW (7) WSSLLLXXW
(8) WXSSLLLXW (9) WSSXLLLXW
(10) WXXSSLLLW (11) WXSSXLLLW (12) WSSXXLLLW

You can verify that these are the ONLY possibilities. All of them are equally probable.

Now, if you examine where the leftmost end of the large molecule can be located, the answer is EXACTLY as in (i), namely, it can be at 1,2,3,4, or 5.

HOWEVER, these five outcomes are NO LONGER equally likely!

In fact, the end-positions have a HIGHER PROBABILITY then the middle positions.

If we call the region beyond the end position as walls, what this seems to show is that the large molecules are 'attracted' towards the wall!!!

This is (my understanding of) the 'depletion potential' which has attracted the large molecules towards the 'walls'.

P.S. - You may wish to look for the preferences in the location of X, the 'voids'.

You will find that the relative probability of finding a void atpositions 1,2,3,4,5,6,7, is

void:	6:2:2:4:2:2:6
large:	3:2:2:2:3
small	3:2:1:1:2:3

In other words, voids also seem to be attracted towards the walls. However, I prefer to refrain from exploring this further - at least in this mail I should not make snide remarks.

------ XXXX ----- XXXXX -----

Dear Prof. Sitaramam

> What happens if there are no voids?

In fact, the minimal model does not distinguish between voids, and still smaller molecules of length/width one unit - the symbol X can stand for either a void or a 'molecule'. The results would be the same.

> What if void is simply a vanishinglky small particle in size?

The depletion potential will be seen as decreasing linearly from the two walls. These two lines will meet at a minimum in the center if the total length is small. They will meet a flat line if the total length is large enough. i.e. something like

```
X               X
 X             X
  X           X
   XXXXXXXXXXXXX
```

I can send an exact expression if you wish. May be you would prefer that I do it after you return.

> I would like to calculate if you care to send em the equation with one
> illustration to see the 3D map. Will that be okay?

What equation? If you mean the 'hexokinase' kind of calculation, it is no longer three dimensionl, but only two dimensional, depending on the

ratio of the radii of the two spheres, as explained towards the end of themail.
May be we can discuss after you return.

From sitaram@unipune.ernet.in Tue Jan 25 17:16:11 2000
Date: Wed, 20 Oct 1999 17:46:43 +0530 (IST)
From: "Dr. V. Sitaramam Faculty member" <sitaram@unipune.ernet.in>
To: "Prof. N.V.Joshi" <cesnj@ces.iisc.ernet.in>
Subject: Re: first objection (you would have expected it any way!)

> Dear Prof. Sitaramam
> While I will get back with more worthwhile (hopefully!) comments soon, I
> must point out the problem right at the beginning!
>
I must right away say that I agree with what you said. This is what I meant by telling you on phone later that I overinterpreted induced fit. The induced fit of Koshland has nothing to do with what I am writing. I should simply say that when a substrate is bound inthe active site, often this results in tightening of the active site around the substrate. This would contribute to the description we refer to also as induced fit.
My point is merely to say that there exists a general mechanism that allows the polymer to embrace the substrate. In this process the complex would be non-specific. But if there are any specific contact points, line opposing charges etc. that can complex or cordinate, these would automatically be chosen in defining and augmenting this interaction which would otherwise be non-specific.

What am I finally saying?
Enzymes do tend to wrap around susbtrates, so often seen that it rarely surprises any one anymore. IS there a singular reasosn that it should do so? A unique explanation for this wrapping behaviour has not been accounted for in any meaningul way. Induced fit, which matches the enzyme with the transitional state also would be contributed to by this wrapping behaviour. Yet an explanation seems missing.
How is that?