A template-based approach to the modification of binding properties of globular proteins II: Rationale and proposed approach.

We present a rationale and proposed approach to the modification and development of bind sites using their respective cognate ligands as template. This is in support of a plausible “instructive” role for the ligand and therefore its involvement in determination of the structure and properties of bind sites. We emphasize the relationship between substrate and active site as an example of the relationship between ligand and bind sites, respectively. This is based on the assumption that there are shared features between all ligand:bind site complexes. Therefore, principles that apply to a specific complex can be applied, in general, to other protein-based complexes. We define ligand-associated probability bias as the difference between the probability of finding activity-determining conformations (ADCs) in the presence- and absence of ligands. For cognate ligands, the given bias is in favor of these ADCs. Thus, bind sites are more likely to assume ADCs when their cognate ligands are present. We relate such probability bias to structural reorganization, disorganization, and preorganization events. We then propose a means of deriving an [apparent] preorganized bind site structure by way of reorganization events that occur with cognate ligand. Finally, we propose a means of deriving an [actual] preorganized bind site structure by way of reorganization events that occur with cognate ligand, albeit during the folding process. The assumption is that the role of the ligand in derivation of such [actual] preorganized bind site structures is an instructive role, and is in support of the Haurowitz-Pauling hypothesis.

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DRAFT

A TEMPLATE-BASED APPROACH TO THE MODIFICATION OF BINDING PROPERTIES OF GLOBULAR PROTEINS II: *Rationale and proposed approach.*

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Abstract

We present a rationale and proposed approach to the modification and development of bind sites using their respective cognate ligands as template. This is in support of a plausible "instructive" role for the ligand and therefore its involvement in determination of the structure and properties of bind sites. We emphasize the relationship between substrate and active site as an example of the relationship between ligand and bind sites, respectively. This is based on the assumption that there are shared features between all ligand:bind site complexes. Therefore, principles that apply to a specific complex can be applied, in general, to other protein-based complexes. We define ligand-associated probability bias as the difference between the probability of finding activity-determining conformations (ADCs) in the presence- and absence of ligands. For cognate ligands, the given bias is in favor of these ADCs. Thus, bind sites are more likely to assume ADCs when their cognate ligands are present. We relate such probability bias to structural reorganization, disorganization, and preorganization events. We then propose a means of deriving an [apparent] preorganized bind site structure by way of reorganization events that occur with cognate ligand. Finally, we propose a means of deriving an [actual] preorganized bind site structure by way of reorganization events that occur with cognate ligand, albeit during the folding process. The assumption is that the role of the ligand in derivation of such [actual] preorganized bind site structures is an instructive role, and is in support of the Haurowitz-Pauling hypothesis.

Keywords: Bind sites; active sites; cognate ligands; substrates; conformational selection; induced fit; conformational transitions; reorganization; disorganization; preorganization.

Ligand-bind site interactions and complementarity

Consider that all forms of molecular activity known to occur in biological systems and involving two separate entities [substrate-enzyme, antigen-antibody (epitope-paratope), allosteric modulator-allosteric site, receptor ligand-receptor, and other peptide-peptide interactions] must be preceded by

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physical interactions between involved entities, in a binding process. A requirement for such binding is that the involved surfaces of these entities complement one another: either by docking of the ligand within a bind site groove in the form of **structural complementarity;** or optimal pairing of bind site dipoles with charged groups on ligand in the form of **electrostatic complementarity**[1]; or a combination of both types of complementarity. Irrespective of which, we suppose that an equilibrium involving ligand:peptide binding occurs whenever a free polypeptide, **[P]**, and a cognate binding ligand, [L] are introduced.

$$[\mathbf{P}] \; + \; [\mathrm{L}] \leftrightarrow [\mathbf{P}\mathrm{L}]$$

Although seemingly obvious, we shall however mention that formation of the ligand:peptide complex, **[PL]**, would occur in an absence of external manipulation. This is especially important for experimental protocols involving mixing of ligand and peptide. The reasons for such occurrence are not considered in this paper.

A very important point of mention is that, although variations in binding phenomena may exist for different types of ligand:bind site complexes, we suppose that general themes do exist for the mechanics of these processes. Thus, at least in terms of these shared features, we can apply principles conceived for a given category of ligand:bind site complex formation to other categories. For example, those physical and chemical principles that govern formation of an enzyme:substrate complex should also apply to formation of an antigen:antibody complex and vice versa. Our choice of focus for this paper is substrate:enzyme complexes and catalytic activities that may derive from them. We present an argument in support of a potential "instructive" role for the ligand (substrate) and therefore its involvement in determination of bind site structure and properties.

Probability of finding a given bind site conformation on a physiologically folded peptide[2] under equilibrium conditions and in an absence of ligand.

Two models, induced and selection models, are of importance here. Although they differ from the stated hypothesis in that they concern physiologically folded peptides –peptides in their native states; they can be applied here since they concern ligand influences in determination of those conformations that predominate a complexed bind site. Thus, they are of relevance for this interest. We shall begin by

[2] Peptides and proteins are applied here as interchangeable terms.

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reconciling both models into a single probability-based model, so as to avoid having to produce separate arguments for each.

At a time, the prevailing explanation for enzyme:substrate complementarity was that posited by Emil Fischer, the key-lock model which holds that a binding ligand is structurally complementary to a predominant conformational state of the local region on the peptide onto which it binds –active site. Thus the lock and key model implies a [near] static bind site conformation and a predominance of structural complementarity[2]. However, experimental data were in support of a more dynamic bind site, as opposed to the static, pre-existing active site proposed by the key-lock model.

Of the models proposed to explain the dynamic nature of bind sites, two dominated discourse: induced-fit and conformation selection models. Since there are multiple implications for either model, multiple formulations can be emphasized for each. Here we emphasize two such formulations. **First formulations** for both induced-fit and conformational selection models may be considered to contemplate relationships between set(s) of conformations that occur in the presence and absence of ligand[3-6]. **Second formulations** for both models may be considered consequences of their respective initial formulations, and also intended to contemplate relationships between set(s) of conformational transitions that occur in the presence and absence of ligand

First formulation for the **induced-fit model** alludes to the notion that some bind site *conformations –of special interest are* **activity-determining conformations (ADCs)**– *t*hat occur with ligand-peptide interactions would otherwise not have occurred in an absence of the ligand[7,8]. In other words, the probability, P^o, of finding bind sites that assume these ADCs, in an absence of the ligand is: $P^o = 0$. A second formulation holds that the ligand first binds to the bind site, followed by assumption of ADCs by bind site. Thus the given conformational transition, involving transitions into these ADCs will not occur in an absence of ligand.

First formulation for the **conformation selection model** alludes to the notion that bind site **activity-determining conformations** that occur with ligand-peptide interactions are among the repertoire of conformations that pre-exist ligand peptide interactions and therefore would have occurred even in an absence of the ligand[8]; with the ligand merely affecting the stability of these **ADCs**. This effect of ligand is believed to affect the conformation equilibrium of the peptide ensemble. In other words, the probability, P^o, of finding bind sites that assume these ADCs, in an absence of the ligand molecule is: $P^o > 0$. A second formulation of this model holds that the bind site first assumes **ADCs**, followed by ligand binding. Thus the given conformational transitions involving transitions into **ADCs** will occur in an absence of ligand.

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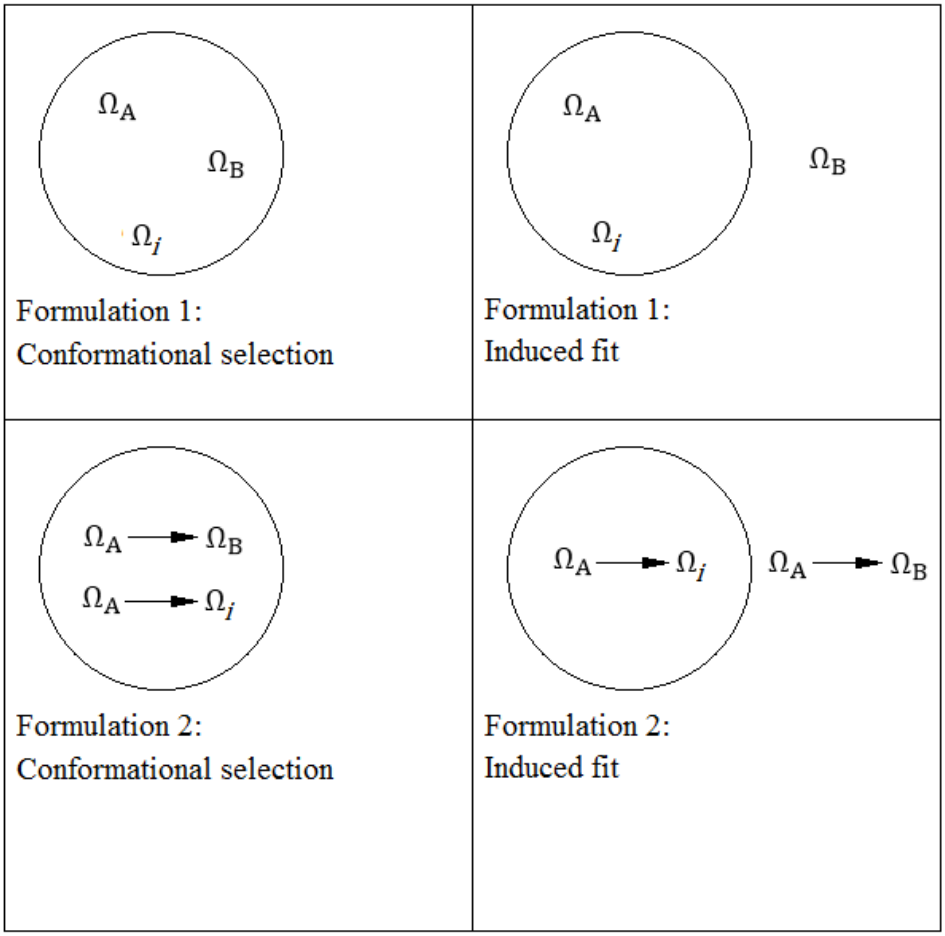

Figure 1: Illustration of each formulation for induced fit and conformation selection models. All conformations are members of the set of conformations for formulation 1 for conformation selection model. Except conformation B, all conformations are members of the set of conformations. All conformational transitions are members of the set of conformational transitions for formulation 2 for conformation selection model. Except conformational transitions from conformation A to conformation B, all other conformational transitions from conformation A are members of the set of conformational transitions.

Determinants of "inclusiveness" within the equilibrium sets of conformations and conformational transitions

1. Let us suppose that there exists a peptide ensemble with all members of the ensemble being the given polypeptide of interest. Let us also suppose that the state of the surrounding medium wherein this occurs is such that effects of ligand on local or global peptide conformations are negligible. Such an insignificant effect may derive from low ligand concentration or a complete absence of ligand. We shall call this the **reference condition**, and

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designate all parameters pertaining to this condition with a superscript (example: $P^o, \Delta E^o$, etc.). Irrespective of condition under consideration, we suppose a constant temperature for the milieu. Of the polypeptide, we are most interested in the bind/active site of which we suppose there exist only one of such bind site per peptide molecule. Thus, we can refer to the ensemble as a bind site ensemble.

2. We suppose there exists a set of conformations, members of which bind sites of ensemble map into (**Figure 2A**). A reasonable assumption is that two or more bind site(s) can map to the same conformation inasmuch as they may map to different conformations. In addition to the set of conformations, we suppose there also exists a set of conformational transitions, members of which bind sites of ensemble map into (**Figure 2B**). As was stated for bind site mapping to conformations, we assume that two or more bind sites can map to the same conformational transition. Of interest are equilibrium sets of conformations and conformational transitions.

3. Let us now suppose that we allow the bind site ensemble approach and reach conformational and conformational transition equilibria. We are interested in the probability of finding a bind site, within the given ensemble, that assumes (or maps into) a given conformation $\boldsymbol{B}$ at equilibrium.

4. Let us now apply the formulations for induced fit and conformational selection models in terms of conformation B at equilibrium. If, at equilibrium, $P_B^o = 0$ is the probability of finding a bind site that assumes a given conformation *B*, then the given conformation *B* can be said to ***not*** be included within the set of equilibrium conformations for the bind site ensemble. If at equilibrium, $P_B^o > 0$ is the probability of finding a bind site that assumes the given conformation *B*, then the given conformation $\boldsymbol{B}$ can be said to be included within the set of equilibrium conformations for the bind site ensemble. We infer from second formulations that the probability, P_B^o of finding a given conformation B within a set of conformations is proportional to the probability, P_{AB}^o, of conformational transition involving transition from an initial conformation $\boldsymbol{A}$, within the set of conformations, and transition into conformation $\boldsymbol{B}$ as opposed to a conformation $\boldsymbol{Z}$ within the set of conformations. Thus, by determination of P_{AB}^o we can determine P_B^o and therefore whether or not conformation $\boldsymbol{B}$ is included within the set of conformations at equilibrium.

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5. The question that remains to be answered is: what factors determine whether or not a given conformation is included within equilibrium set of conformations?

6. Consider that transition from a conformation, let us call it conformation A, to a conformation, let us call this conformation *B* would depend on the number of other conformations that can also be assumed from an initial conformation *A*. Let the total number of conformations that can be assumed by direct transition from conformation A be $\boldsymbol{n}$. In addition, we suppose that all transition events are equally likely and thus have equal a priori probabilities. The probability P_{AB}^{o}, of transition from conformation *A* to conformation *B*:

$$P_{AB}^{o} = {}^{1}/_{n}$$

Also, note that the sum over all probabilities must equal unity:

$$\sum_{i}^{n} P_{Ai}^{o} = \sum_{i}^{n} \left({}^{1}/_{n}\right)_{i} = 1$$

Where, $\sum_{i}^{n} P_{Ai}^{o}$ is the sum of the terms of the sequence, and the terms of the given sequence are arranged in descending order. Thus, the first and last terms of the given sequence are the maximum and minimum probability values, respectively.

$$\sum_{i}^{n} P_{Ai}^{o} = \cdots + P_{Ai}^{o} + \cdots$$

The probability, P_{AB}^{o}, can also be expressed as:

$$P_{AB}^{o} = \frac{P_{AB}^{o}}{\sum_{i}^{n} P_{Ai}^{o}} = \frac{{}^{1}/_{n}}{\sum_{i}^{n}\left({}^{1}/_{n}\right)_{i}}$$

Where, $\frac{P_{AB}^{o}}{\sum_{i}^{n} P_{Ai}^{o}}$ is a general expression and thus applies irrespective of condition; and $\frac{{}^{1}/_{n}}{\sum_{i}^{n}\left({}^{1}/_{n}\right)_{i}}$ is a specific expression that only applies to the a priori probability condition.

7. In addition, as n increases indefinitely, the probability, P_{AB}^{o}, approaches zero.

$$P_{AB}^{o} = \lim_{n\to\infty} \frac{{}^{1}/_{n}}{\sum_{i}^{n}\left({}^{1}/_{n}\right)_{i}} \cong 0$$

In other words, for a priori probability conditions, there is zero probability of finding a given conformation B within the set of conformations ($P_{B}^{o} = 0$) when a substantially large number of conformations occur within the set.

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8. Consider that in order to transition from a given conformation A to conformation B, those interactions that characterize conformation A but not conformation B must either no longer exist or their influence on conformational transitions be negligible. Also, consider that those interactions that characterize conformation B must be formed. In other words, there should be a change in enthalpy of interactions, $\Delta\Delta H^o_{AB}$, for the given transition.

$$\Delta\Delta H^o_{AB} = \Delta E^o_{AB} - T\Delta\Delta S^o_{AB}$$

Where,

$$\Delta E^o_{AB} = E^o_B - E^o_A$$
$$\Delta\Delta H^o_{AB} = \Delta H^o_B - \Delta H^o_A$$
$$\Delta\Delta S^o_{AB} = \Delta S^o_B - \Delta S^o_A$$

Note that the entropy term is a function of the number of alternate spatial arrangements for the given conformation. For our purposes, we suppose that these values are significantly diminished such that:

$$\Delta\Delta H^o_{AB} \cong \Delta E^o_{AB}$$

9. Using Boltzmann's proportionality between probabilities and energy values, we offer that the probability of finding a given conformational transition (CT), within the equilibrium set of CTs for the bind site ensemble, with energy ΔE^o_{AB} is proportional to $exp - \left(\frac{\Delta E^o_{AB}}{kT}\right)$. Here ΔE^o_{AB} is the conformational transition energy (CTE) from conformation A to conformation B; k is Boltzmann's constant; and T is the absolute temperature in Kelvins.

$$P^o_{AB} \propto exp - \left(\frac{\Delta E^o_{AB}}{kT}\right)$$

10. Ideally, we would want a one-to-one correspondence between a given conformational transition and a single CTE value for the given transition. That is, all CTs occupy energy states (**Figure 2A**), and there are no two or more CTs that occupy the same energy state. Thus, allowing the CTE to be unique to the given CT. However, there is no evidence against the possibility that two or more different CTs may have the same CTE.

11. To attempt at approximating a one-to-one correspondence, we can define each conformational transition in terms of a range of CTEs as opposed to a single CTE term. The assumption here is that the likelihood of finding any two CTs that assume the same range of

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CTEs, for defined limits, would be less than if these were single CT terms. Such energy variations of a single CT may be correlated with molecular fluctuations affecting conformations involved in transition. Such thermodynamic fluctuations are noted to occur for peptide structures, thus resulting in multiple spatial arrangements of minimal variations from a most-consistent structure. Also, each of these minimally variant structural arrangements may have different energy values, thus complicating the concept of a single conformational energy value for a given peptide conformation. Thus, a variety of CTE values may result from differences between these conformational energies. To account for all such variations, we take the integral over the CTE minimum and maximum values. The probability, P^o_{AB} of finding a given conformation with CTE, is proportional to $\int_{a_B}^{b_B} exp - \left(\frac{\Delta E^o_{AB}}{kT}\right) \mathrm{d}\Delta E^o_{AB}$.

$$P^o_{AB} \propto \int_{a_B}^{b_B} exp - \left(\frac{\Delta E^o_{AB}}{kT}\right) \mathrm{d}\Delta E^o_{AB}$$

Where,

$$a_B = \overline{\Delta E}^o_{AB} - md\Delta E^o_{AB}$$

$$b_B = \overline{\Delta E}^o_{AB} + md\Delta E^o_{AB}$$

Where, $\overline{\Delta E}^o_{AB}$ is the average CTE for transition from conformation *A* to conformation *B*; and $\boldsymbol{m}$ is an integer.

12. We can express the a priori probabilities in terms of Boltzmann probability.

$$P^o_{AB} = {}^1\!/_n = \frac{1}{n} \frac{\left(\int_{a_B}^{b_B} exp - \left(\frac{\Delta E^o_{AB}}{kT}\right) \mathrm{d}\Delta E^o_{AB}\right)}{\left(\int_{a_B}^{b_B} exp - \left(\frac{\Delta E^o_{AB}}{kT}\right) \mathrm{d}\Delta E^o_{AB}\right)}$$

$$= \frac{\int_{a_B}^{b_B} exp - \left(\frac{\Delta E^o_{AB}}{kT}\right) \mathrm{d}\Delta E^o_{AB}}{n \int_{a_B}^{b_B} exp - \left(\frac{\Delta E^o_{AB}}{kT}\right) \mathrm{d}\Delta E^o_{AB}}$$

Since all conformations have equal probabilities,

$$\int_{a_B}^{b_B} exp - \left(\frac{\Delta E^o_{AB}}{kT}\right) \mathrm{d}\Delta E^o_{AB} = \int_{a_i}^{b_i} exp - \left(\frac{\Delta E^o_{Ai}}{kT}\right) \mathrm{d}\Delta E^o_{Ai}$$

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$$P_{AB}^{o} = \frac{\int_{a_B}^{b_B} exp - \left(\frac{\Delta E_{AB}^{o}}{kT}\right) \mathrm{d}\Delta E_{AB}^{o}}{n \int_{a_i}^{b_i} exp - \left(\frac{\Delta E_{Ai}^{o}}{kT}\right) \mathrm{d}\Delta E_{Ai}^{o}}$$

$$= \frac{\int_{a_B}^{b_B} exp - \left(\frac{\Delta E_{AB}^{o}}{kT}\right) \mathrm{d}\Delta E_{AB}^{o}}{\sum_{i}^{n} \int_{a_i}^{b_i} exp - \left(\frac{\Delta E_{Ai}^{o}}{kT}\right) \mathrm{d}\Delta E_{Ai}^{o}}$$

Where,

$a_i = \overline{\Delta E}_{Ai}^{o} - md\Delta E_{Ai}^{o}$

$b_B = \overline{\Delta E}_{Ai}^{o} + md\Delta E_{Ai}^{o}$

Where, $\overline{\Delta E}_{Ai}^{o}$ is the average CTE for transition from conformation *A* to a conformation whose probability is at the i^{th} position of the pre-arranged sequence of conformational transition probabilities; and m is an integer. The following applies for the a priori probability condition and thus can be expressed as:

$$P_{AB}^{o} = \frac{1/n}{\sum_{i}^{n}(1/n)_i}$$

$$= \frac{\int_{a_B}^{b_B} exp - \left(\frac{\Delta E_{AB}^{o}}{kT}\right) \mathrm{d}\Delta E_{AB}^{o}}{\sum_{i}^{n} \int_{a_i}^{b_i} exp - \left(\frac{\Delta E_{Ai}^{o}}{kT}\right) \mathrm{d}\Delta E_{Ai}^{o}}$$

The noted formula can also be applied to conditions for which probabilities are unequal, thus it can be applied as a general expression:

$$P_{AB}^{o} = \frac{P_{AB}^{o}}{\sum_{i}^{n} P_{Ai}^{o}}$$

$$= \frac{\int_{a_B}^{b_B} exp - \left(\frac{\Delta E_{AB}^{o}}{kT}\right) \mathrm{d}\Delta E_{AB}^{o}}{\sum_{i}^{n} \int_{a_i}^{b_i} exp - \left(\frac{\Delta E_{Ai}^{o}}{kT}\right) \mathrm{d}\Delta E_{Ai}^{o}}$$

As ΔE_{AB}^{o} increases indefinitely, the probability approaches zero.

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$$P_{AB}^{o} = \lim_{\Delta E_{AB}^{o} \to \infty} \frac{\int_{a_B}^{b_B} exp - \left(\frac{\Delta E_{AB}^{o}}{kT}\right) \mathrm{d}\Delta E_{AB}^{o}}{\sum_{i}^{n} \int_{a_i}^{b_i} exp - \left(\frac{\Delta E_{Ai}^{o}}{kT}\right) \mathrm{d}\Delta E_{Ai}^{o}} \cong 0$$

Also, as n increases indefinitely, the probability approaches zero.

$$P_{AB}^{o} = \lim_{n \to \infty} \frac{\int_{a_B}^{b_B} exp - \left(\frac{\Delta E_{AB}^{o}}{kT}\right) \mathrm{d}\Delta E_{AB}^{o}}{\sum_{i}^{n} \int_{a_i}^{b_i} exp - \left(\frac{\Delta E_{Ai}^{o}}{kT}\right) \mathrm{d}\Delta E_{Ai}^{o}} \cong 0$$

In other words, irrespective of condition, there is zero probability of finding a given conformation B within the set of conformations ($P_{B}^{o} = 0$) if CE of conformation B far exceeds that of conformation A; and/or if a substantially large number of conformations occur within the set.

13. If $P_{AB}^{o} \cong 0$ is interpreted as $P_{AB}^{o} \neq 0$, then, based on this interpretation, all possible conformations can occur within the set of conformations. However, it is important to also note that such apparent all-inclusiveness may be relevant for some inconsequential considerations, but for all practical purposes can be deemed irrelevant. Thus, although in principle, the number of members within the set of conformations may increase indefinitely, the number of relevant conformations within a set would remain constant. For example, let us again suppose a total of n conformations occur within a set of conformations at equilibrium. As previously stated, the sum total of all probabilities, irrespective of number of conformations must equal 1.

$$\lim_{n \to \infty} \sum_{i}^{n} P_{Ai}^{o} = \cdots + P_{Aw}^{o} + \cdots = 1$$

From the above limit, we can express the sum total of probabilities for the first $\boldsymbol{w}$ conformations of the corresponding sequence as:

$$\sum_{i}^{w} P_{Ai}^{o} = \cdots + P_{Aw}^{o}\,, \qquad w \leq n$$

If

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$$\sum_{i}^{w} P_{Ai}^{o} \cong 1$$

Then the sum total of probabilities of conformations within the given set at equilibrium is,

$$\lim_{n\to\infty} \sum_{i}^{n} P_{Ai}^{o} \cong \sum_{i}^{w} P_{Ai}^{o}$$

Thus, whereas $\lim_{n\to\infty} \sum_{i}^{n} P_{Ai}^{o}$ can be considered the sum probabilities of all conformations within the set of conformations, at equilibrium, $\sum_{i}^{w} P_{Ai}^{o}$ can be considered the sum probabilities of relevant conformations within the set at equilibrium; where irrelevant conformations are those with probability values that approach zero. Thus,

$$\lim_{n\to\infty} \sum_{i}^{n} P_{Ai}^{o} = \sum_{i}^{w} P_{Ai}^{o} = 1$$

Thus, we can substitute w for the summation index n in the equation.

$$P_{AB}^{o} = \frac{\int_{a_B}^{b_B} exp - \left(\frac{\Delta E_{AB}^{o}}{kT}\right) \mathrm{d}\Delta E_{AB}^{o}}{\sum_{i}^{w} \int_{a_i}^{b_i} exp - \left(\frac{\Delta E_{Ai}^{o}}{kT}\right) \mathrm{d}\Delta E_{Ai}^{o}}$$

Considering the above relationships, we can therefore express P_{AB}^{o} as a function of ΔE_{AB}^{o} and w.

$$P_{AB}^{o} = p(\Delta E_{AB}^{o}, w)$$

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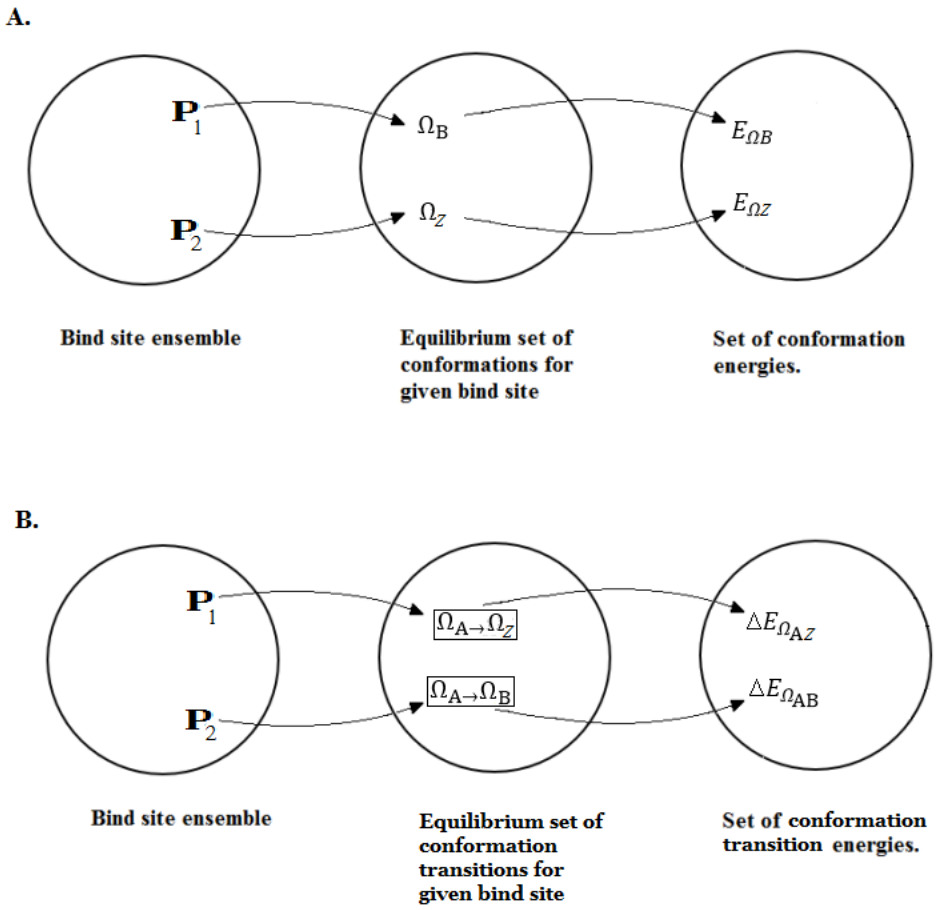

Figure 2A: Relationships between bind site ensemble, equilibrium set of conformations, and set of conformation energies (CE). At any given moment, each bind site of the ensemble maps to a conformation within the equilibrium set of conformations. Two or more bind sites can map to the same conformation, but a given bind site can only map to one conformation. In turn, each conformation maps to a single conformation energy within the set of conformation energies. **Figure 2B**: An illustration of relationships between bind site ensemble, equilibrium set of conformational transitions, and set of conformational transition energies (CTE). Bind sites within ensemble map to conformational transitions within the equilibrium set of conformational transitions. In turn, each conformational transition maps to a single CTE within the set of CTEs.

Probability of finding a given bind site conformation on a physiologically folded peptide under equilibrium conditions and in the presence of ligand.

For the following, we shall apply the term *bind sites* as a general term for description of the non-substrate component of a binding complex. Bind sites as used here, refer to both enzyme active site and solvent hydration cage structures. Thus, when applied, the reader should consider such generalization.

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On effects of substrate binding on the active site structure: Preorganization and reorganization of active site dipole orientations and conformations.

14. Here we emphasize the effect of substrate binding to the active site of an enzyme. To understand this, we shall first revisit the relationship between binding and enzyme catalysis event.

15. Natural enzymes are known to catalyze reactions which would otherwise be too slow to support life. The rate accelerations by natural enzymes (as compared to uncatalyzed reactions) can reach $10^6 - 10^{17}$.[9,10] Both experimentation and computer simulations have been employed in the study of enzyme catalyzed reactions. Of particular interest is elucidating the means by which natural enzymes achieve large k_{cat} values. These studies follow from Pauling's proposed role of enzymes in catalysis: In binding and stabilization of the substrate transition state (**TS-substrate**), thereby decreasing the activation energy for conversion from substrate ground state (**GS-substrate**)- to **TS-substrate**[11]. It is this reduction in activation energy that yields an increase in k_{cat}. For these studies, enzyme catalyzed reactions were compared to reactions occurring in aqueous solution and in an absence of enzymes –uncatalyzed reaction.

16. It was posited that a reasonable measure for comparison is the activation energy: where $\Delta g^{\ddagger}_{cat}$ is the activation energy for the enzyme catalyzed reaction; and $\Delta g^{\ddagger}_{cage}$ is the activation energy for the uncatalyzed reaction[12,13]. Also compared were solvation free energies, ΔG_{sol} of substrates in solvent and active site. The activation energy and solvation free energy are related in that the reduction in activation energy for the enzyme catalyzed reaction is believed to ultimately derive from the solvation free energy, ΔG_{sol}.

17. It was also proposed that the free energy of solvation, ΔG_{sol} of a cognate **TS-substrate** when in an active site environment is approximately equal to that for the [bulk] solvent environment. However, unlike within the bulk solvent where the free energy of solvation, ΔG_{sol} derives, in its entirety, from water molecules interacting (via hydrogen bonds) with **TS-substrate**; that for an enzyme active site derives from interactions between **TS-substrate**s and both induced and permanent dipoles of residues within the active site. It should also be noted that hydrogen bonding involving water molecules may also contribute to ΔG_{sol} for **TS-substrate** in an active site environment[14]. However, such contributions pale in comparison to that for the bulk solvent.

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18. The electrostatic nature of these interactions would require that stabilization of TS-substrate involve stabilization of charge(s) on TS-substrate[14]. Maximal stabilization should require optimization of these interactions. In turn, such optimization should require proper alignment of bind site dipoles in relation to charges on TS-substrate. Thus, if initial interactions are non-optimal, then realignment of key dipoles on bind site structure must occur so as to affect [near] optimal interactions[14]. This would require that free energy be invested in the realignment process. The given process of dipole realignment is termed **reorganization**, which we shall properly define later. Thus, the solvation free energy, ΔG_{sol}, can be partitioned into: the free energy of [electrostatic] interactions between TS substrate and bind site, $\Delta G_{\mu Q}$; and free energy invested in realignment of bind site dipole-dipole interactions, $\Delta G_{\mu\mu}$. As previously stated, such rearrangements facilitate formation of a dipole structure that favors [near] maximum stabilization of TS-substrate[15].

$$\Delta G_{sol} = \Delta G_{\mu Q} + \Delta G_{\mu\mu}$$

19. At this juncture we shall apply designations which are intended to help differentiate enzyme catalyzed reactions from uncatalyzed reactions. It is important to note that the cited works do not use these designations. However, we believe that in order to diminish ambiguities that may arise, these parameters should be specified. Henceforth, the solvation free energy for solvent and active site would be designated $\Delta G_{sol_{uncat}}$ and $\Delta G_{sol_{cat}}$, respectively. Also, the free energy of [electrostatic] interaction between TS-substrate and solvent molecules shall be designated , $\Delta G_{\mu Q_{uncat}}$, the free energy of [electrostatic] interaction between TS-substrate and active site shall be designated, $\Delta G_{\mu Q_{cat}}$; the free energy invested in rearrangement of solvent dipole-dipole interactions shall be designated, $\Delta G_{\mu\mu_{uncat}}$; and the free energy invested in rearrangement of active site dipole-dipole interactions shall be designated, $\Delta G_{\mu\mu_{cat}}$. Thus, the above equation can be written for the uncatalyzed reaction:

$$\Delta G_{sol_{uncat}} = \Delta G_{\mu Q_{uncat}} + \Delta G_{\mu\mu_{uncat}}$$

And for the catalyzed reaction:

$$\Delta G_{sol_{cat}} = \Delta G_{\mu Q_{cat}} + \Delta G_{\mu\mu_{cat}}$$

In addition, the effective activation energy, $\Delta\Delta g^{\ddagger}_{cage}$ can be expressed as the difference between activation energy and the free energy of interactions between the TS-substrate and hydration cage structure.

$$\Delta\Delta g^{\ddagger}_{cage} = \Delta g^{\ddagger}_{cage} - \Delta G_{\mu Q_{uncat}}$$

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Similarly, when in active site the effective activation energy, $\Delta\Delta g^{\ddagger}_{cat}$ can be expressed as the difference in activation energy and the free energy of interactions between the substrate transition state and active site residues.

$$\Delta\Delta g^{\ddagger}_{cat} = \Delta g^{\ddagger}_{cat} - \Delta G_{\mu Q\,cat}$$

20. Both catalyzed and uncatalyzed reactions (involving fully formed hydration cage structures) were shown to have approximately equal $\Delta G_{\mu Q}$ values[16]. That is,

$$\Delta G_{\mu Q\,cat} \cong \Delta G_{\mu Q\,uncat}$$

Also, the free energy of solvation, ΔG_{sol} for the TS-substrate in enzyme active site is approximately equal to that when in [bulk] solvent. That is:

$$\Delta G_{sol\,cat} \cong \Delta G_{sol\,uncat}$$

Thus, differences between reaction rates of the catalyzed and uncatalyzed reactions are most likely as a result of differences in the energy required to realign dipoles for water molecules of solvent and amino acid residues of active site in relation to the charged TS-substrate[15,17]. In other words, the difference between $\Delta\Delta g^{\ddagger}_{cat}$ and $\Delta\Delta g^{\ddagger}_{cage}$ most likely results from differences in $\Delta G_{\mu\mu}$ for enzyme active site and solvent[16]. Rewriting the above equations in terms of $\Delta G_{\mu\mu}$

$$\Delta\Delta g^{\ddagger}_{cat} = \Delta g^{\ddagger}_{cat} - \Delta G_{sol\,cat} + \Delta G_{\mu\mu\,cat}$$

And

$$\Delta\Delta g^{\ddagger}_{cage} = \Delta g^{\ddagger}_{cage} - \Delta G_{sol\,uncat} + \Delta G_{\mu\mu\,uncat}$$

Warshel proposed that, $\Delta G_{\mu\mu}$ for uncatalyzed reactions is greater than that for catalyzed reactions[15]. That is,

$$\Delta G_{\mu\mu\,uncat} > \Delta G_{\mu\mu\,cat}$$

The greater $\Delta G_{\mu\mu}$ for uncatalyzed reactions represents a greater amount of free energy invested in formation of the hydration cage structure[16]. Recall that $\Delta G_{\mu\mu}$ reflects the energy invested in the process termed reorganization.

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21. **Reorganization** involves changes in bind site dipole-dipole interactions and are required to achieve [near] optimal alignment of involved residues, in the case of active site, or water molecules in the case of formation of hydration cage. As previously stated, such realignment results in a dipole organization that [almost] maximally stabilizes TS-substrate[15].

A reasonable inference that can be drawn from these proposals and findings is that the greater $\Delta G_{\mu\mu}$ for reorganization of a hydration cage, $\Delta G_{\mu\mu_{uncat}}$, as compared to that for reorganization of the active site, $\Delta G_{\mu\mu_{cat}}$, must derive from the greater extent of disorganization of the hydration cage as compared to the active site structure.

Here **disorganization** refers to the extent to which the initial dipole-dipole orientations differ from the fully reorganized orientations. With dipole alignments that deviate from the maximally stabilizing orientations having a greater extent of disorganization than those which show less deviation.

The noted inference is motivated by the position that the active sites of enzymes are believed to have a preorganized structure that already affects partial stabilization of TS-substrate, and thus requires little reorganization. Thus, explaining the lower values of $\Delta G_{\mu\mu}$ for enzyme-based– than for solvent-based- reactions[15]. In other words, the active site structure undergoes less disorganization than the bulk solvent.

Structural reorganization and disorganization

22. In addition to induced dipoles, permanent dipoles (e.g., charges on active site residues), have been proposed to also be involved in stabilization of substrate transition state[14]. An example of transition state stabilization by permanent dipoles (hydrogen bonds) is the means by which the hydration cage structure stabilizes the charged transition state substrate when in an absence of catalysts. For these uncatalyzed reactions, the optimally stabilizing bind site, solvent cage structure, must be organized either from an initial poorly stabilizing structure[18] or an amorphous, and thus highly disorganized structure (figure 3B). The noted process can be considered the structural component of reorganization for the solvent-based reaction, whereas the reverse is considered the structural component of disorganization for the solvent-based reaction (figure 3B). Such considerations are based on the notion that, in principle, alignment of these [permanent] dipoles may involve structural changes as the aligning units assume orientations that approximate optimal interactions with TS-substrates.

23. Two relevant questions are of interest: 1) whether proper alignment of permanent dipoles in relation to substrate transition state require changes to active site structure? and 2) what is the lower limit for the extent of structural change that can be considered significant.

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Regarding the first question, consider that for dipole-dipole reorganization events involving induced dipoles, mere electron dispersal may occur for a fixed active site structure. However, for reorganization events involving permanent dipoles –that is to alter the orientation of the given permanent dipole relative to the substrate– bond rotations and thus changes to molecular configurations must occur. Again, consider the changes in orientation of water molecules that must occur in order to form the hydration cage.

In principle, the extent to which active site structures are altered can range from such small single bond rotations –with changes in side chain dihedral angles– to more widespread and thus profound changes. Of these changes, it is a reasonable stance to consider that widespread changes have a significant impact on catalytic rates. Based on the same line of reasoning, one may consider that diminishingly smaller alterations would yield negligible change in catalytic rates. Thus, regarding the second question, the lower limit of structural changes may be erroneously considered to involve extensive displacement of involved segment. However, significant alterations in catalytic rates have been shown to be associated with insignificant changes in active site structure. For example, Mesecar and Koshland (1997) showed that affecting isocitrate dehydrogenase structure such that the resultant changes in amino acid positions are less than an Angstrom can result in significant orders of magnitude change in catalytic rates[19].

24. Thus, in principle, the reorganization event may also comprise significant conformational (structural) changes. We consider such conformational changes as the structural component of reorganization for active site. We also consider the extent of disorganization of these permanent dipoles, and thus bind site structures, prior to reorganization as a determinant of the degree of change in both structural features that must occur to yield the given transition state stabilizing structure. This can be considered the structural component of disorganization for active site. Also, a structural component of preorganization would exist for the active site (figure 4), and should explain the difference between the extent of structural reorganization that must occur for active site and bulk solvent.

25. Based on such explicit distinction between induced and permanent dipole contributions, it should hold that a fraction of the free energy of reorganization, $\Delta G_{\mu\mu_{cat}}$, may be invested in such structural changes.

$$\Delta G_{\mu\mu_{cat}} = x\Delta G_{\mu\mu_{cat}} + (1-x)\Delta G_{\mu\mu_{cat}}$$

Where,

$x\Delta G_{\mu\mu_{cat}}$ = Fraction of $\Delta G_{\mu\mu_{cat}}$ that affects structural reorganization

$(1-x)\Delta G_{\mu\mu_{cat}}$ = Fraction of $\Delta G_{\mu\mu_{cat}}$ that affects nonstructural reorganization

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Structural reorganization and ligand-associated probability bias.

26. Suppose an initial condition of which all bind sites of the ensemble are saturated with substrates and that the substrate is at just the threshold concentration to yield such saturated bind sites.

The probability of finding a bind site that undergoes conformation transition from conformation ***A*** to conformation ***B*** increases with either: increasing conformational energy (CE) of conformation ***A*** for a fixed CE of conformation ***B*** (figure 5A); decreasing CE of conformation ***B*** for a fixed CE of conformation ***A*** (figure 5B); or both a reduction of CE for conformation ***B*** and an increase in CE of conformation ***A*** (figure 5C).

The probability of finding a bind site that undergoes conformation transition from conformation ***A*** to conformation ***Z*** decreases with either: an increase in CE of conformation ***Z*** for a fixed CE of conformation ***A*** (figure 6A); or reduction of CE of conformation ***A*** for a fixed CE of conformation ***Z*** (figure 6B), or both an increase in CE for conformation ***Z*** and a reduction in CE of conformation ***A*** (figure 6C).

In essence, the probability of finding a bind site that undergoes conformation transition from conformation ***A*** to conformation ***B*** increases with reduction in CTE for transition from conformation ***A*** to conformation ***B***. Also, the probability of finding a bind site that undergoes conformation transition from conformation ***A*** to conformation ***Z*** decreases with an increase in conformation transition energy for transition from conformation ***A*** to conformation ***Z***.

A.

FULL EXTENT OF DISORGANIZATION OF BIND SITE STRUCTURE

FULLY-REORGANIZED BIND SITE STRUCTURE

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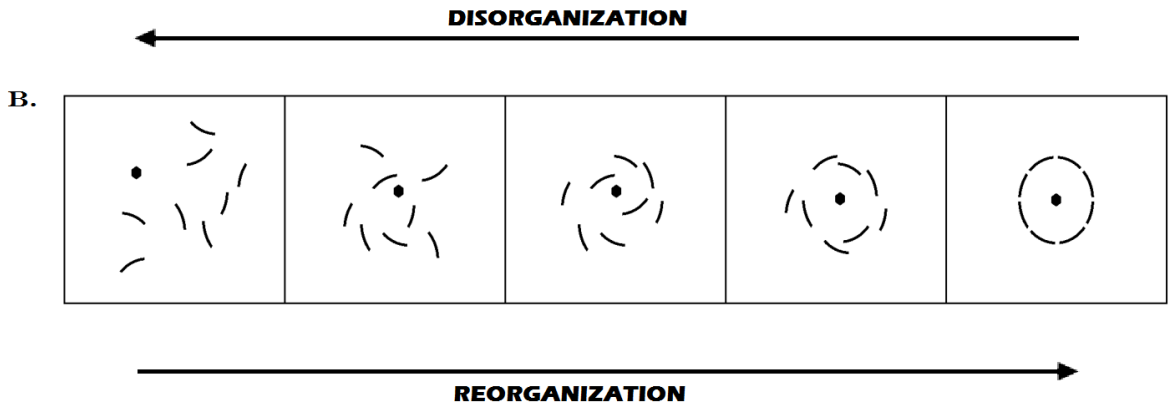
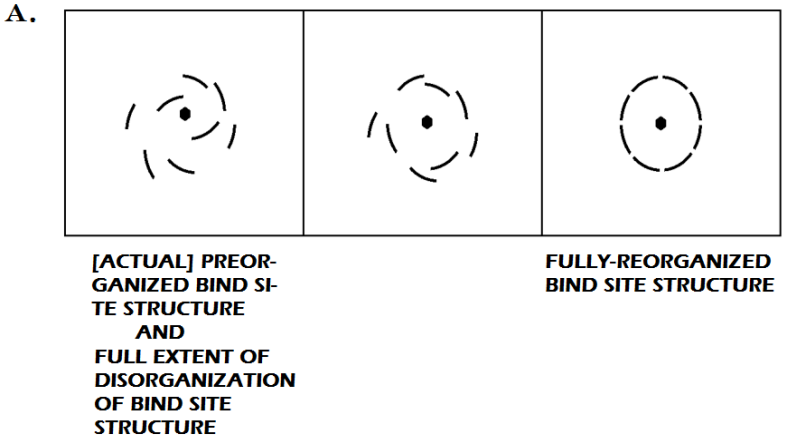


Figure 3A: Depicts the range of structures of a bind site. At one extreme is a very disorganized structure termed the full-extent of disorganization of bind site structure. At the other extreme is the most reorganized structure termed the fully-reorganized bind site structure. **Figure 3B**: Illustrates the direction of reorganization and disorganization. For reorganization, the bind site structure is organized from the full-extent of the disorganized structure to the fully-reorganized bind site structure. Disorganization involves a reversal of the noted direction for reorganization. That is, fully-reorganized bind site structure, the bind site is transformed to the full-extent of the disorganized structure.



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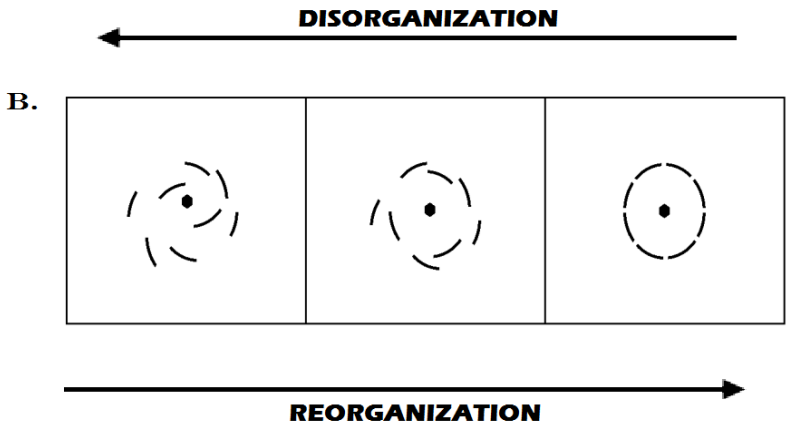

Figure 4A: Depicts the range of structures of a preorganized bind site. Compare the full-extent of disorganization of bind site structure to that of figure 3. Note that the extent of disorganization is less than that of figure 3. In other words, the most disorganized structure has been preorganized so as to prevent the extent of disorganization noted for figure 3. **Figure 4B**: Illustrates the direction of reorganization and disorganization. For reorganization, the bind site structure is organized from a preorganized structure to the fully-reorganized bind site structure. Disorganization involves a reversal of the noted direction for reorganization. That is from the reorganized bind site structure to the preorganized structure.

If significant increase in CE of conformation ***Z*** such that the probability of finding a bind site that assumes conformation ***Z*** when at equilibrium approaches zero, then the total number of conformations within set – the upper limit for summation index of probabilities– can therefore be considered to be decreased. Thus, an increase in CE of conformation *Z* is also proportional to an increase in the probability of finding a bind site that undergoes conformation transition from conformation ***A*** to conformation ***B***.

A combination of these effects can affect a probability bias mostly in favor of a given conformation or number of conformations. Thus, some conformational probabilities may require that some CEs be increased, whereas others are decreased or stay unchanged

27. Let us now suppose that the reorganized structures is defined by conformation ***B*** and only by conformation ***B***. In other words, the reorganized structure occurs if and only if

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conformation ***B*** is assumed. **Thus,** conformation transition from conformation ***A*** to conformation ***B*** can be considered a structural reorganization event.

Thus, in a similar way as formation of appropriate [electrostatic] interactions between TS-substrate and active site environment can affect a reduction in activation energy for the given change in substrate state, so too can these same interactions affect reduction in conformation transition energy as the actives site undergoes reorganization. Thus, we can define the effective CTE when cognate ligand is present and bind site is saturated with cognate ligand as: as the difference between ΔE^{o}_{AB} and $x\Delta G_{\mu\mu_{cat}}$ for the peptide molecule.

$$\Delta E_{AB} = \Delta E^{o}_{AB} - x\Delta G_{\mu\mu_{cat}}$$

Thus in addition to decreasing the activation energy $\Delta g^{\ddagger}_{cat}$ for conversion from GS-substrate to TS-substrate, such binding interactions may also decrease the conformational transition energy required for transition from conformation A to conformation B.

We can determine the probability, P_{AB} of conformation transition involving transition from an initial conformation ***A,*** within the set of conformations, and transition into conformation ***B*** as opposed to a conformation ***Z*** within the set of conformations.

$$P_{AB} = \frac{P_{AB}}{\sum_i^n P_{Ai}} = \frac{\int_{a_B}^{b_B} exp - \left(\frac{\Delta E_{AB}}{kT}\right) \mathrm{d}\Delta E_{AB}}{\sum_i^n \int_{a_i}^{b_i} exp - \left(\frac{\Delta E_{AB}}{kT}\right) \mathrm{d}\Delta E_{Ai}}$$

Considering the above relationships, we can therefore express P_{AB} as a function of ΔE_{AB} and w

$$P_{AB} = p(\Delta E_{AB}, w)$$

28. We define a measure of conformational transition probability bias as the extent of ligand effect on the probability of finding a bind site, within the ensemble, that assumes a given conformation transition.

$$Probability\ bias = P_{AB} - P^{o}_{AB}$$

If $P_{AB} - P^{o}_{AB} > 0$, then a greater probability of finding a bind site that assumes the given conformation transition when in the presence- than in an absence of ligand. If $P_{AB} - P^{o}_{AB} < 0$, then a lower probability of finding a bind site that assumes the given conformation transition

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when in the presence- than in an absence of ligand. If $P_{AB} - P_{AB}^{o} = 0$, then there is no difference in probability of finding a bind site that assumes the given conformation transition when in the presence- than in an absence of ligand.

A view of reorganized and rigidified bind site structures as [apparent] preorganized structures.

29. During formation of the active site: substrate complex, the active site can be viewed as initiating from a less organized structure –limits of which we consider the full extent of disorganization. It evolves to a more organized structure–limits of which we consider the fully reorganized structure. A reversal of this process should also occur for loss of complex (figures). Also, CT cycles of active site are between those conformations that define the full extent of disorganization for active site and those that define fully-reorganized structures.

30. As compared to the bulk solvent, the active site undergoes a lesser extent of disorganization (figure). As was previously stated, this is due to the preorganized structure of the active site. Warshel posited that formation of the preorganized structure occurs during the folding process[13]. Henceforth, we shall designate the preorganized structure that derives from this early preorganization event as an [actual] preorganized structure. Also, the [actual] preorganized structure is maintained throughout the life-time of the active site when in native state. As compared to active sites, water molecules of the solvent (uncatalyzed reaction) cannot maintain such preorganized structures. Hence the greater extent of disorganization than their active site counterparts.

    This ability to maintain a preorganized structure stems from differences in rigidity of the structures involved[17]. It has been proposed that such rigidity may derive, in part, from the large sizes of enzymes, as gauged by the positive correlation between peptide size and heat capacity[10]. In addition, the entirety of intra-peptide interactions may favor significant rigidity. Other sources of rigidity may be extra-peptide interactions, as may occur between peptide and milieu constituents. Of these extra-peptide interactions, those involving cognate ligand is especially significant. There is evidence from literature that binding of cognate ligand can increase the rigidity of peptide structures[20-22].

31. Thus, we deduce from the above analysis that, at least ***in theory***, reorganization events that are followed by *permanent and significant increments in rigidity of the fully reorganized structure*

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should yield an [apparent] preorganization. The "apparent" term is intended to differentiate such seemingly preorganized structures from those that result from "actual" preorganization that occurs during folding. Thus, in an absence of prior knowledge, this initial structure may be considered to have derived from [actual] preorganization.

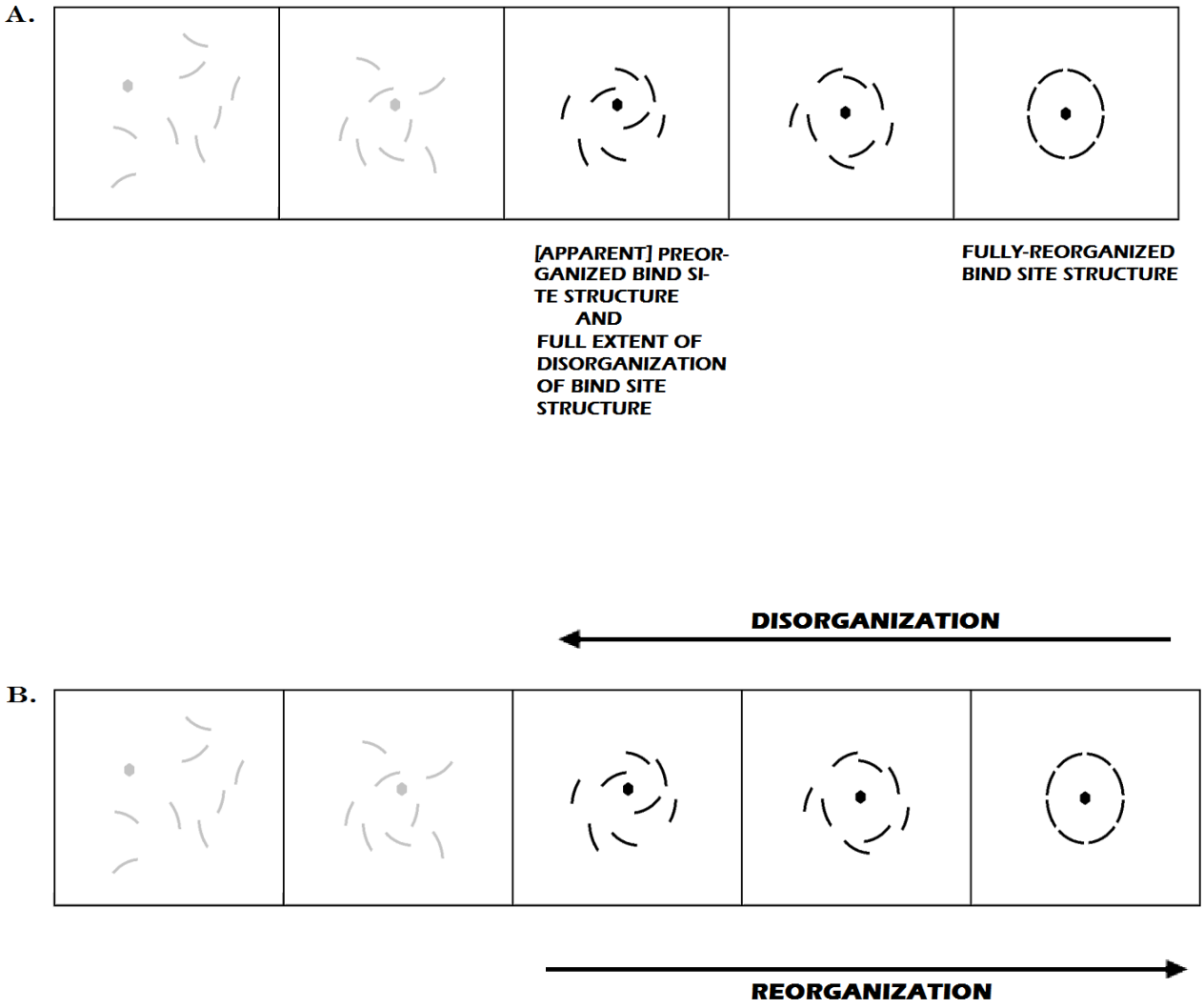

Figure 7A: Depicts the range of structures of a bind site. At one extreme is the full-extent of disorganization of bind site structure. However, note that although the full-extent of disorganized structure was initially identical to that for figure 3 (grey), an [apparent] preorganization results in a less disorganized structure. Figure 7B: Illustrates the direction of reorganization and disorganization. Reorganization from an [apparent] preorganized structure, as compared to that

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occurring from full-extent of disorganized structures as in figure 3, would require less free energy of reorganization.

A view of [actual] preorganized structures as resulting from reorganization events that occur during peptide folding.

32. As previously stated, formation of the [actual] preorganized structure occurs during the folding process. That is, an enzyme that starts out in an unfolded state, for which little to none of its native state features can be appreciated must undergo alignment and realignment of its constituent amino acid residues until the final native product results. In other words, the active site can be viewed as evolving from a less organized- to a more organized structure; where organization reflects an increasing resemblance to an active site structure when complexed with substrate. Thus, formation of [actual] preorganized structure can be considered analogous to reorganization event that yields the fully-reorganized structure. In effect, we may consider [actual] preorganization events as incomplete reorganization events that occurs during the folding process; with a second or main component of reorganization event occurring post-folding – during formation of the ligand:bind site complex.

33. Thus, we not only have reconciled reorganization with the process of formation of [apparent] preorganized structures, we also reconcile reorganization with the process of formation of [actual] preorganized structures.

34. Thus, ***at least in theory***, just as we considered that *reorganization events that are followed by permanent and significant increments in rigidity of the reorganized structure* would yield an [apparent] preorganized structure, this same approach should yield an [actual] preorganized structure, if occurring during the folding process.

35. Structures that typify those of unfolded and/or partially folded intermediates of peptides undergoing either folding or refolding have been noted in the literature: $p21^{Waf1/Cip1/Sdi1}$;[23] poplar apo-plastocyanin[24]; Substrate-binding peptide (peptide I) from DNA polymerase I[25]; and a host of others. A list of these peptides can be found in references [26] and [27]. Upon binding its cognate ligand, these so-called "intrinsically disorganized peptides" have been shown to yield structures with greater extent of order[28].

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36. Using this same approach and a similar role of the cognate ligands we can attempt at affecting the "organization" of unfolded and/or partially folded intermediates of peptides undergoing folding. That is, upon formation of complex with ligand, the folding peptides should assume more organized structures that are reminiscent of their physiologically-active folded counterparts.

37. On this basis, a similar approach to affecting probability bias in favor of conformations that define reorganized structures – and thus in favor of reorganization events – for the folded peptide, should yield similar outcomes if utilized for completely unfolded and/or partially folded peptides. That is, presentation of the cognate substrate at a given concentration range should affect reorganization events of bind sites on completely unfolded and/or partially folded peptide.

38. In theory, we could determine the ligand-derived probability bias, in favor of reorganization event, that would result for such presentation. More importantly is determination of the fold state of peptide undergoing folding that would give the greatest probability bias in favor of reorganization event. In order to achieve this, we shall contemplate changes in key parameters that occur with progression of peptide folded states.

39. Keep in mind that we are interested in determination of the fold state of peptide(s) undergoing folding that would give the greatest probability bias. However, in order to achieve this, we must compare different folded states of peptide.

40. Both for the sake of brevity and since this addresses a template role of ligands in determination of binding properties during folding, we shall only emphasize probabilities of finding bind site conformations when in the presence of ligand.

Probability of finding a given bind site conformation at different folded states of peptide under equilibrium conditions and in the presence of ligand.

41. In the same way we compared catalyzed and uncatalyzed reactions (reference state) for physiologically folded peptides, we can compare the reorganization energies, $\Delta G_{\mu\mu}{}_{fold}$ between different fold states of the folding peptide and for a fixed solvation free energy, $\Delta G_{sol}{}_{fold}$; where $\Delta G_{\mu Q}{}_{fold}$ is the given free energy of interactions between the substrate transition state and peptide at a specific fold state.

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The given equation can be rewritten in terms of $\Delta G_{\mu\mu}{}_{fold}$ as:

$$\Delta G_{\mu\mu}{}_{fold} = \Delta G_{sol}{}_{fold} - \Delta G_{\mu Q}{}_{fold}$$

42. It was proposed that formation of the preorganized active site structure is furnished by the folding energy[16,29]. With the given relationship:

$$\Delta G_{\mu\mu} = \Delta G_{fold}$$

In terms of conventions used here, we can rewrite the above equation as:

$$\Delta G_{\mu\mu}{}_{fold} = \Delta G_{fold}$$

Based on our considerations, the preorganization (early reorganization) energy, $\Delta G_{\mu\mu}{}_{fold}$ would be:

$$\Delta G_{\mu\mu}{}_{fold} = \Delta G_{fold} + \Delta G_{sol}{}_{fold} - \Delta G_{\mu Q}{}_{fold}$$

Where, ΔG_{fold} is the folding energy contribution to the reorganization energy;

and, $\Delta G_{sol}{}_{fold} - \Delta G_{\mu Q}{}_{fold}$, is the contribution from extra-peptide interactions with the substrate. In this sense, the given equality:

$$\Delta G_{\mu\mu}{}_{fold} = \Delta G_{fold}$$

Can be considered a special condition for which $\Delta G_{sol}{}_{fold} - \Delta G_{\mu Q}{}_{fold} = 0$.

43. As previously proposed, reorganization energy can be partitioned into structural and non-structural reorganization energies.

$$\Delta G_{\mu\mu}{}_{fold} = x\Delta G_{\mu\mu}{}_{fold} + (1-x)\Delta G_{\mu\mu}{}_{fold}$$

Thus, the structural reorganization energy is:

$$x\Delta G_{\mu\mu}{}_{fold} = \Delta G_{fold} + \Delta G_{sol}{}_{fold} - \Delta G_{\mu Q}{}_{fold} - (1-x)\Delta G_{\mu\mu}{}_{fold}$$

44. At the earliest stages of folding (unfolded state) when the active site is least organized, the reorganization energy should be greatest. The most likely trend to occur is a decreasing reorganization energy with folding up until a relative minima occurring at the last stages of folding (native state) –when the active site is most organized. Note that the assumption here is that with folding, ΔG_{fold} increases such that it offsets contributions of increasing rigidity that

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should occur with progression to native state. Thus, the effective determinant of reorganization energy is the extent of disorganization, which is greatest for the unfolded protein.

Thus for a constant $\Delta G_{sol_{fold}}$, the given free energy of interactions between the substrate transition state and peptide, $\Delta G_{\mu Q_{fold}}$ increases with progression of folding and up to a relative maximum when peptide assumes its native state.

45. We can extend this to apply to the effective activation energy for enzyme catalysis involving the folding peptide, $\Delta\Delta g^{\ddagger}_{fold}$. Using the equation:

$$\Delta\Delta g^{\ddagger}_{fold} = \Delta g^{\ddagger}_{fold} - \Delta G_{\mu Q_{fold}}$$

Thus $\Delta\Delta g^{\ddagger}_{fold}$ should also increase with folding and up to a relative maximum when at native state. Note that at native state:

$$\Delta\Delta g^{\ddagger}_{fold} = \Delta\Delta g^{\ddagger}_{cat}$$

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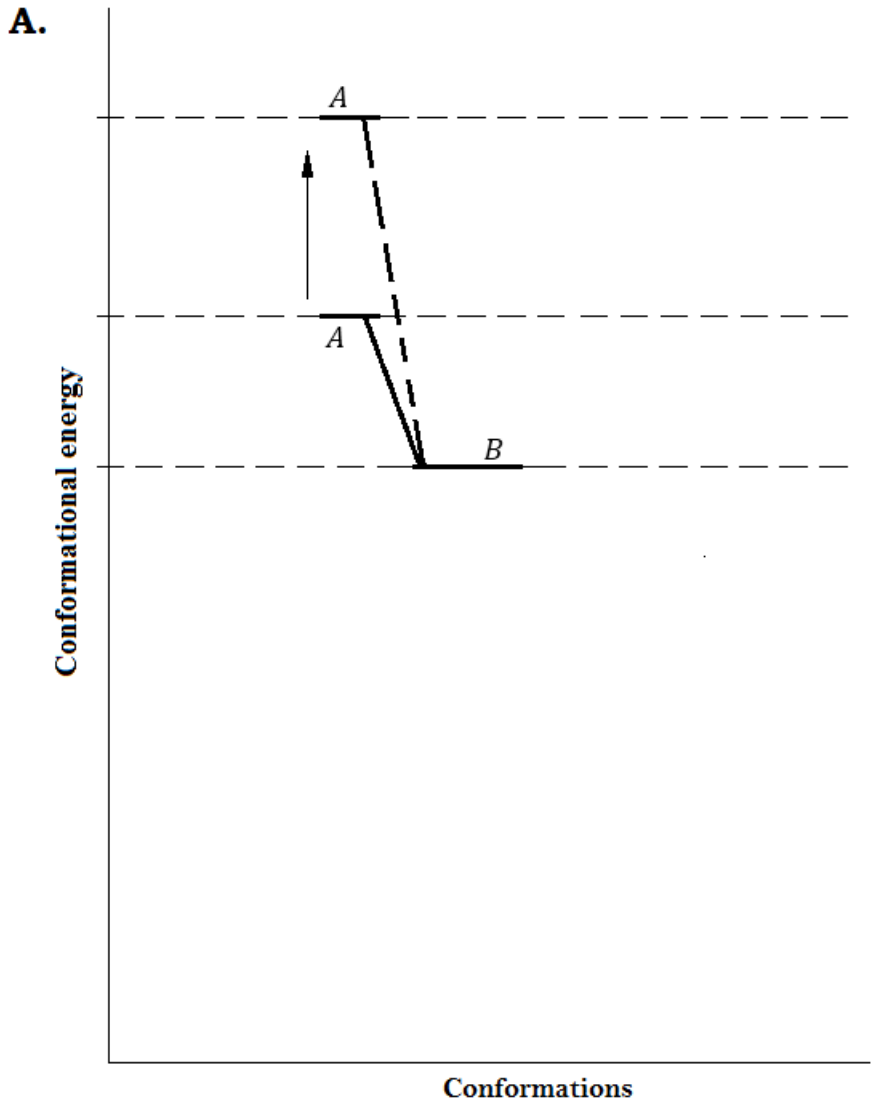

Figure 5A

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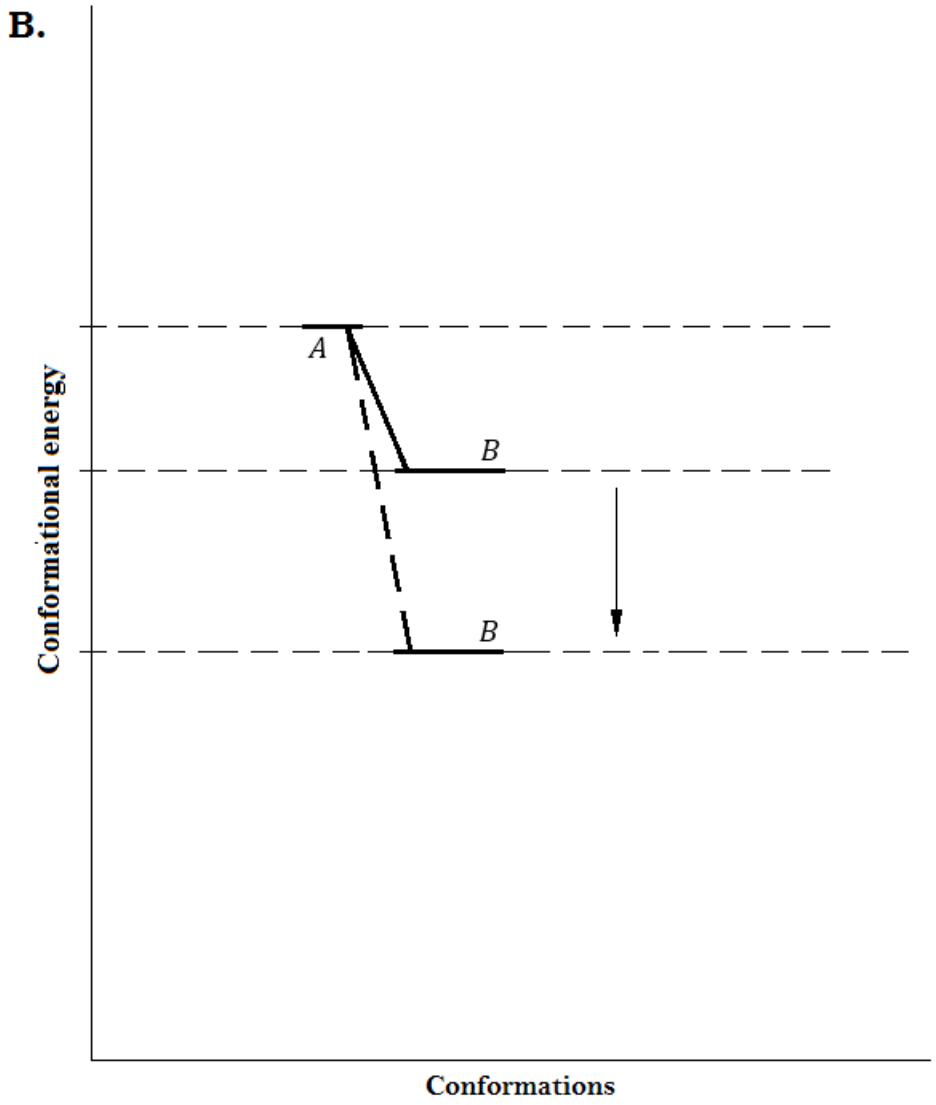

Figure 5B

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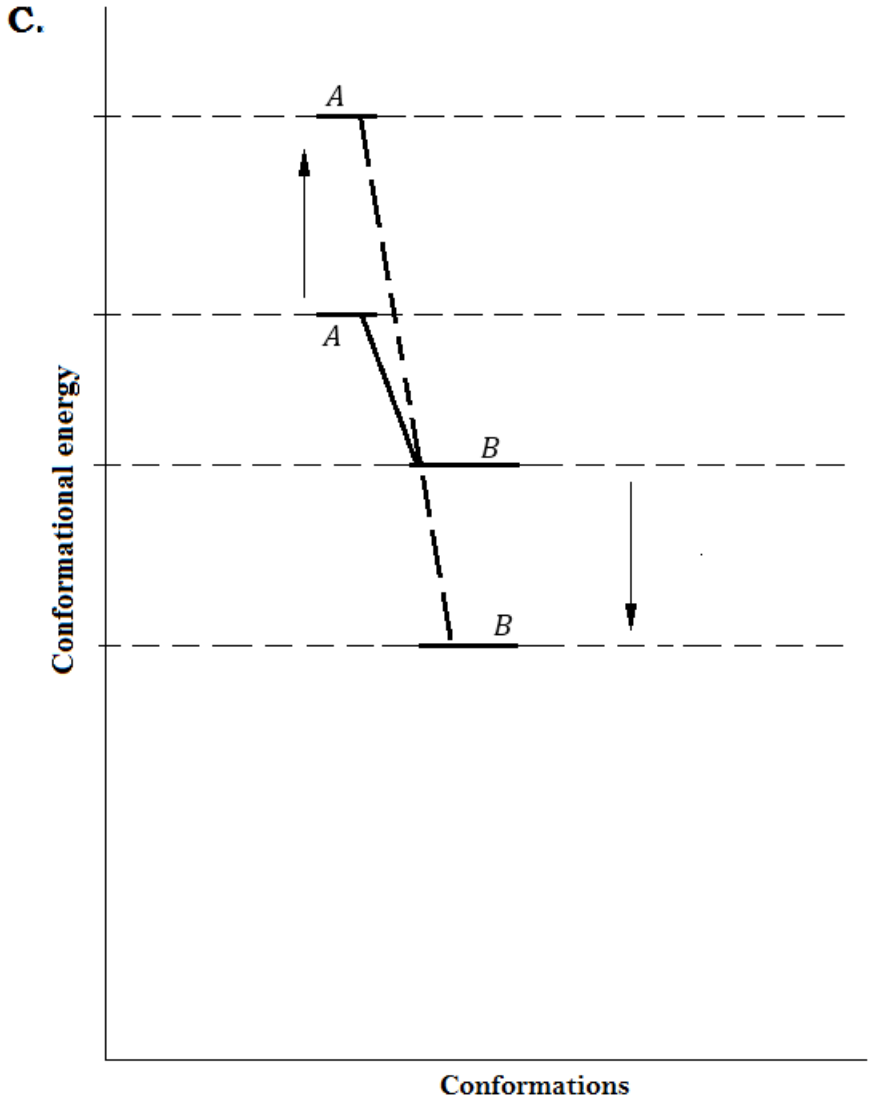

Figure 5C

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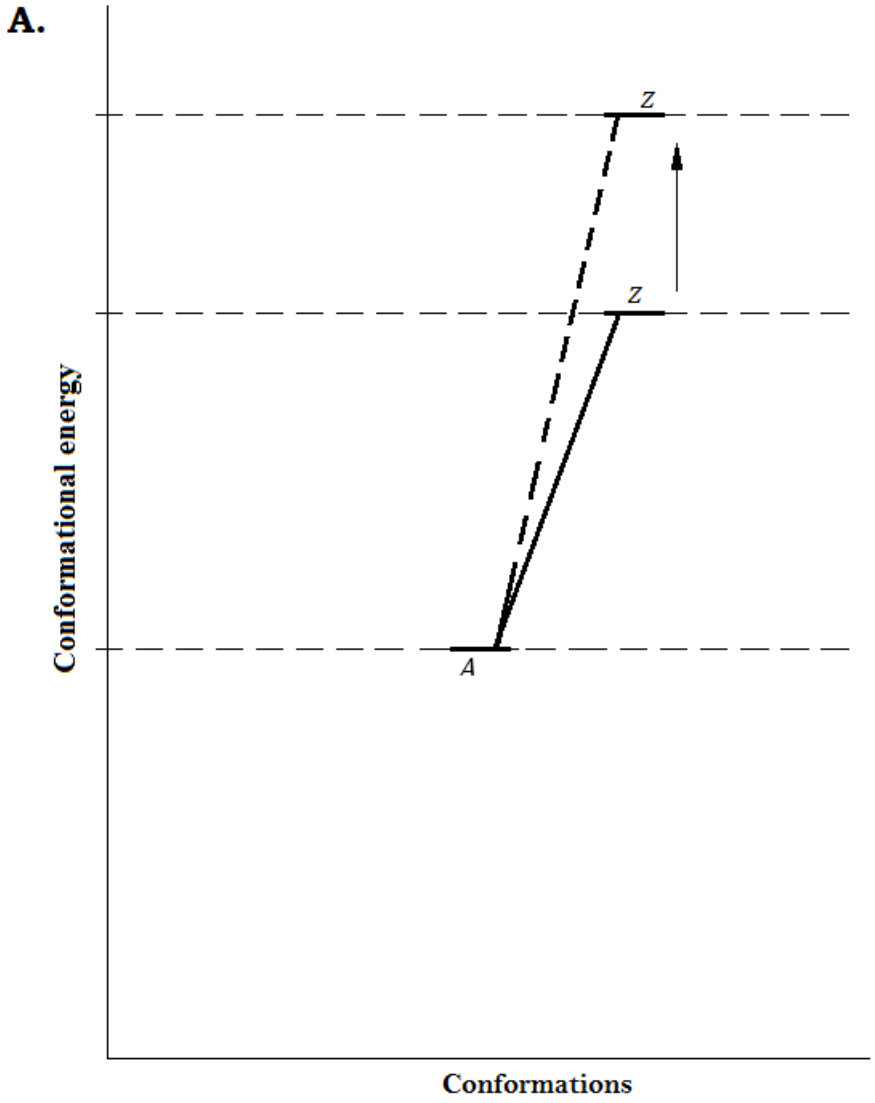

Figure 6A

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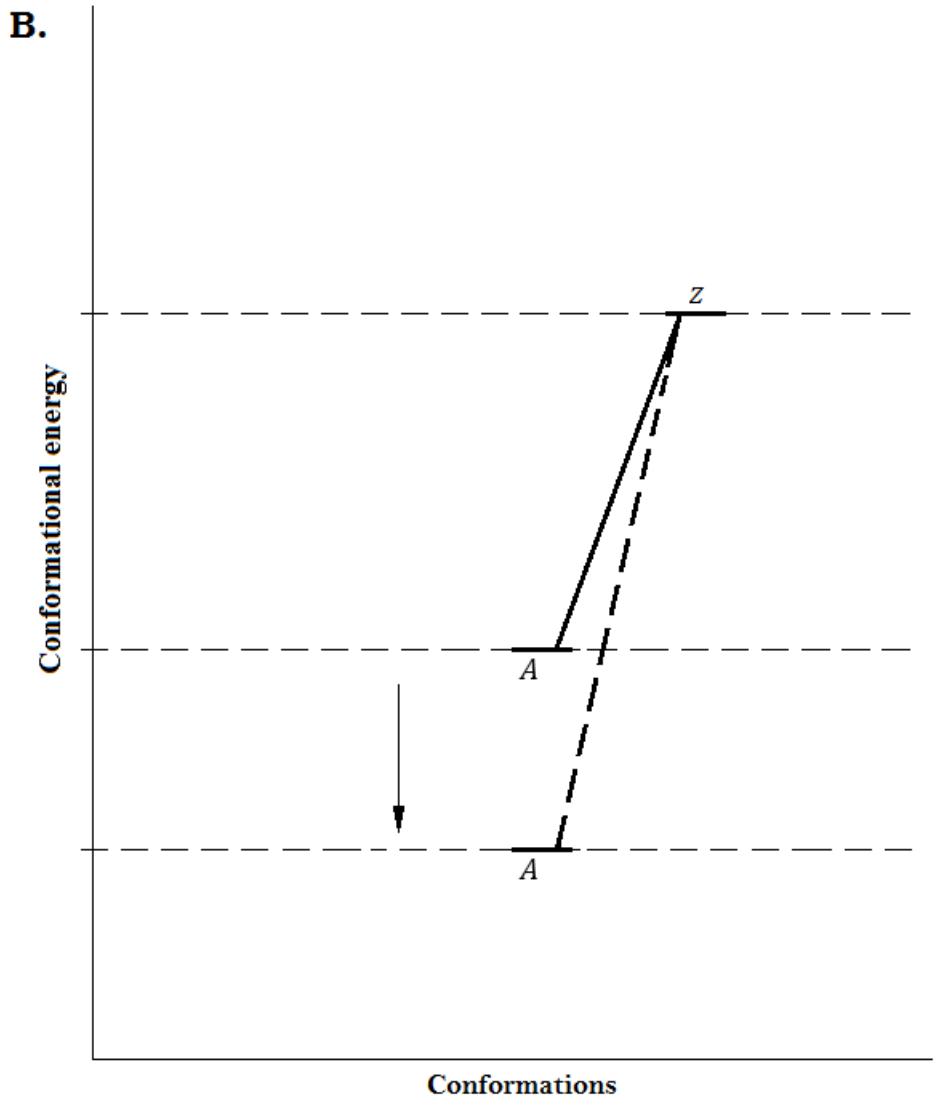

Figure 6B

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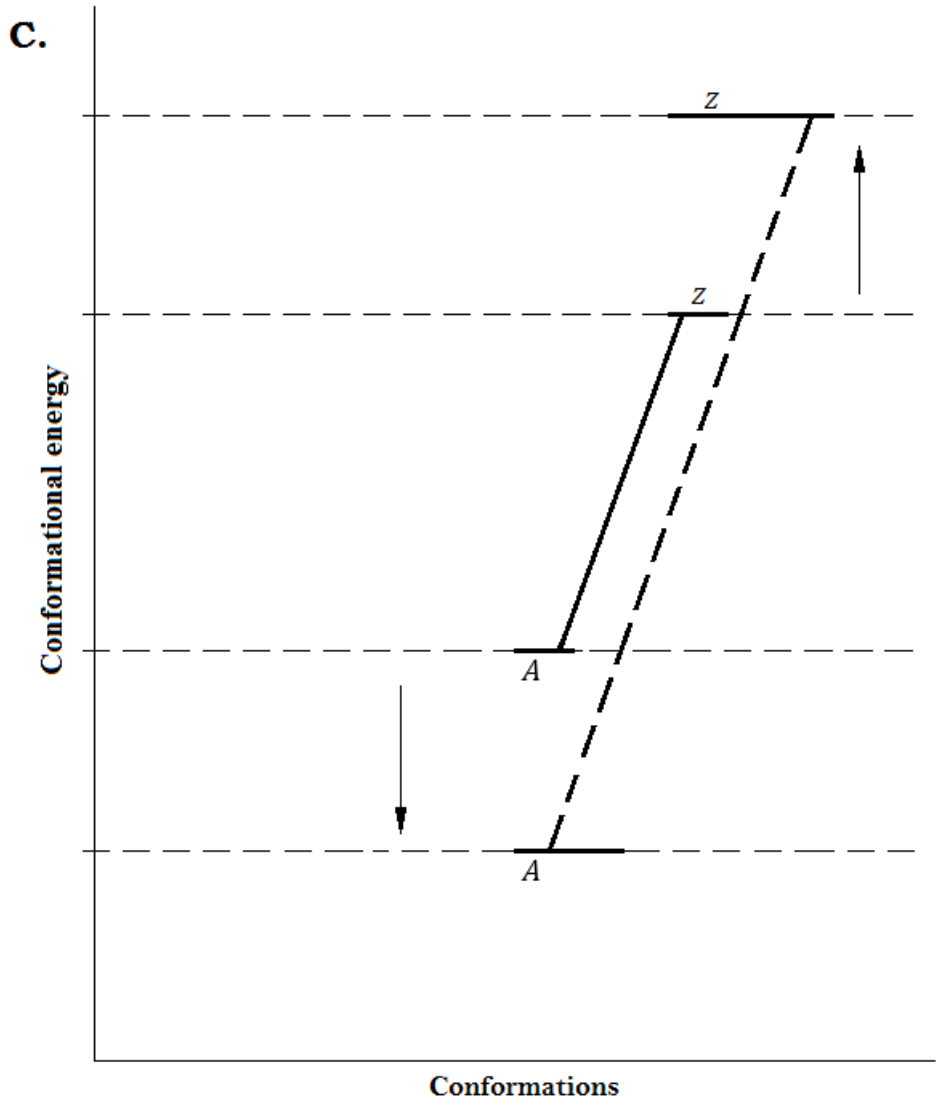

Figure 6C

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