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Protein flexibility of dimers: Do symmetric motions play a role in allosteric interactions?

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Abstract

It is well-understood in biochemistry that the functioning of a protein depends both on having basic stable forms (tertiary structure) and having some residual flexibility supported within that structure. The modeling of protein flexibility and rigidity in terms imported from physics and engineering has been developed within the theory of rigid frameworks and is available via fast combinatorial algorithms in programs such as [1], and is described in papers such as [3, 8, 9].

Recent theoretical work on rigidity of frameworks has modified this analysis to include the basic symmetry of some structures and predict motions which preserve this symmetry. In particular, a framework which would normally count to be combinatorially minimally rigid in generic realizations has been shown to become flexible when realized with 2-fold rotational symmetry in 3-space [7].

Protein dimers, formed by two copies of a protein are a good case study for the possible impact of this added flexibility, due to 2-fold rotational symmetry, as they generally self-assemble with a 2-fold rotational axis, for reasons of minimal energy [2]. What is the significance of this for the behavior of dimers, such as tryptophan repressor? Does the pathway of a symmetry preserving motion better support the allostery, so that when one tryptophan binds (or leaves) the entire protein is pushed along to make the same change at the second binding site? We will explore this case study, describe some algorithms, and suggest further areas of work.

Introduction

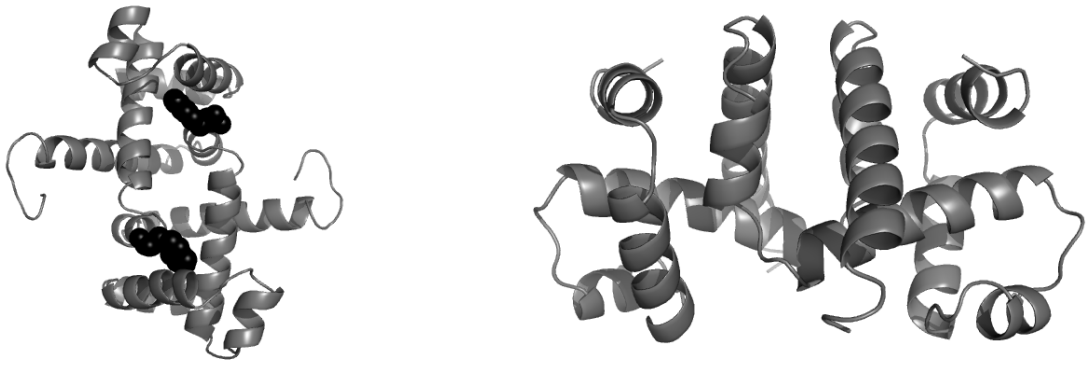
The functioning of a protein depends on having basic stable forms (tertiary structure) and also having some residual flexibility supported by that structure. Being too flexible (without enough shape) causes disease (e.g. cystic fibrosis), as does being too rigid (e.g mad cow disease) to the point of not being recycled. From the theory of rigid frameworks, fast combinatorial algorithms for predicting flexibility and rigidity from a single snap shot (PDB file) have been developed and verified. These programs are described in papers such as [3, 8, 9] and an on-line version is available at [1].

We extend these analyses to account for the surprising impact of some symmetries (but not others) on the flexibility / rigidity of structures [7]. In particular, a framework or molecule which would normally count to be combinatorially minimally rigid in generic realizations becomes flexible when realized with 2-fold rotational symmetry in 3-space [7]. Protein dimers, formed by two copies of a protein are a good case study for the possible impact of this added flexibility, due to 2-fold rotational symmetry, as they generally self-assemble with a 2-fold rotational axis, for reasons of minimal energy [2]. What is the significance of this for the behavior of dimers, such as tryptophan repressor? There are several possibilities:

(a) the pathway of a symmetry preserving motion may better support the allostery, so that when one tryptophan binds (or leaves) the entire protein is pushed along to make the same change at the second binding site;

(b) while the dimer may not be flexible, without breaking some hydrogen bonds, the initial breaking or binding at one site may be pushed along towards recovering symmetry through breaking the symmetrically placed bonds or through affording a symmetric binding.

2. Generic flexibility of molecular structure



(a) (b)

Figure 1: Tryptophan Represor (DOI:10.2210/pdb3wrp/pdb) is a dimer that exists in two shapes: with tryptophan bound (a) and with no tryptophan bound (b). Black spheres in (a) represent tryptophan. We show two different orientations indicating 2-fold rotational symmetry.

For a general body-bar framework with a multi-graph $G = (B, E)$, we have the necessary counts for rigidity of a subset E^* of bars (edges) which will be a basis for the row space of the corresponding rigidity matrix, whose $6|B|$ columns are used to track possible (infinitesimal) motions, allowing for the trivial infinitesimal motions (a vector space of dimension 6 generated by three independent infinitesimal translations and three independent rotations about the origin). The necessary counts are then: each body has 6 degrees of freedom, a rigid structure has a residue of 6 degrees of freedom overall, and each bar removes one degree of freedom. If there are sufficient independent bars, then we must have $|E^*| = 6|B| - 6$, and not have placed too many bars inside some subset. Together these become necessary and sufficient conditions.

Theorem 1 (Tay's Theorem) *A body-bar framework with a multigraph $G = (B, E)$ is infinitesimally rigid (and rigid) for generic selections of the lines of the bars if and only if there is a subset of bars E^* such that $|E^*| = 6|B| - 6$, and $|E'| \leq 6|B'| - 6$ for all subgraphs induced by subsets E' of E^*.*

Algorithmically, this condition looks like we must check all possible subsets (an exponential process). However, these counts on a multigraph define independent sets in a matroid, and the counts lead to a greedy algorithm called the pebble game, which is quadratic in the number of edges [5, 8].

Recent results have confirmed that these same counts (and the corresponding pebble game algorithms) also characterize rigid molecular structures in which the atoms (and their covalent bonds) become the bodies, and the shared bonds become a special set of hinges (5 constraints on the motions between the two atoms). The result is called the Molecular Theorem (confirming the 20 year old molecular conjecture) [4]

These algorithmic methods are implemented for basic predictions of flexibility and rigidity of a protein, from a single geometric snap-shot of the protein (e.g. a PDB file), in the web based server FIRST at flexweb.asu.edu developed and supported by Mike Thorpe.

Dimers and symmetry in proteins

Dimers are common among allosteric proteins such as tryptophan repressor. In this dimer there are a pair of sites, a half-turn apart, at which tryptophan binds - causing an overall shape change (still with half-turn symmetry) so that the modified dimer shape fits over the DNA sequence which codes for tryptophan production - that is it suppresses the production when bound (when there is a lot of tryptophan around). When there is little tryptophan around, one - then both - bound tryptophan leave and the dimer no longer stays over the DNA and production starts up. This is called allostery - shape change at a distance (the two sites are not close). Given the energy pressure to maintain half-turn symmetry, it is possible that when one has unbound, this energy pathway moves the pair back to a new symmetric form giving a force to unbind the second copy, since there is not enough tryptophan

around to make rebinding the first one likely. Conversely, one binding applies pressure to resume symmetry either by changing the shape to invite a second binding, or by completing the symmetric shape change through the binding of a second tryptophan. These are at least suggestive mechanisms for transferring (transmitting) shape change between sites that are distant, but are within a dynamic combined symmetry shape.

4. C_2 flexibility

We now consider the direct impact of half-turn (C_2) symmetry in a structure. With a symmetric framework (body-bar or molecular) we have an additional orbit rigidity matrix whose columns track possible symmetric motions [7]. In this symmetry analysis, we have 6 degrees of freedom for each orbit of atoms (pair of vertices related by the half-turn), and each orbit of constraints (pair of edges related by the half-turn). The modified counts begin with 6 degrees of freedom for each orbit of bodies (atoms) since the motions of one atom will tell you the motions of the symmetric atom. Each orbit of (two) bars restricts the motion of the combined structure by one as well. However, only a 2-space of the possible symmetric motions are trivial, generated by a half-turn around the symmetry axis, and a translation along the axis. Writing $|E_o|$ for the number of edge orbits and $|B_o|$ for the number of body orbits (atoms), the result is:

Theorem 2 (2-Fold Rotational Rigidity [7, 6]) *A body-bar framework in 3-space which is generic within the realizations with 2-fold rotational symmetry has only trivial symmetric motions only if there is a subset of bars E_o^* such that*

$$|E_o^*| = 6|B_o| - 2 \text{ and } |E_o'| \leq 6|B_o'| - 2 \text{ for all subsets } E_o' \text{ of } E_o^*.$$

While this result is only a necessary condition, failure to satisfy these counts is a guarantee that there is a symmetric motion (both infinitesimal and finite at a generic set of edge lengths). We *conjecture* that this condition is also sufficient, but that is not needed here. For the earlier general theory of Tay, the counts were equivalent to the existence of 6 spanning trees. Here, the counts are equivalent to a decomposition into 2 spanning trees and 4 spanning map graphs (a forest containing all the vertices, and with one extra edge added to each tree in the forest). We note that this form applies when no vertices are fixed (on the C_2-axis) and no edges are fixed (centered on and perpendicular to the C_2-axis), assumptions which are appropriate for dimers.

If we started with a body-bar framework which has exactly $|E| = 6|B| - 6$, then dividing $|E|, |B|$ by 2 (to count the orbits), we have

$$|E_o| = 6|B_o| - 3 < 6|B_o| - 2$$

Such a structure, previously predicted to be minimally rigid, becomes flexible with a motion preserving the rotational symmetry [7]!

Example 1 *Consider a ring of six atoms with six bonds ($6 \times 5 = 30$ bars). As a generic body-bar framework, we have the required count $|E| = 30 = 6 \times 6 - 6 = 6|B| - 6$. All subsets also satisfy the required inequalities, so that this structure is infinitesimally rigid (and rigid) in almost all configurations, including the cyclohexane ring with 3-fold symmetry (the chair configuration).*

However, realized with 2-fold rotational symmetry, the boat configuration of cyclohexane has the orbit count $|E_0| = 15 < 16 = 6 \times 3 - 2 = 6|B_o| - 2$. It becomes flexible, moving along a path of configurations all of which have the 2-fold rotational symmetry.

This example confirms that we need to test this added criterion in addition to the previous criterion for body-bar frameworks without symmetry. The following example also shows that a body-bar framework (actually molecular) can be full rank for this 2-fold symmetry criterion, but fail the basic test for generic rigidity - and therefore have a (possibly symmetry breaking) finite flex.

Example 2 *Consider two 4-fold rings, sharing a 2-fold axis, connected by four bars. Without symmetry, each of the 4-fold molecular rings counts as $|E| = 4 \times 5 \geq 4 \times 6 - 6 = 6|B| - 6$, which is overbraced by 2. With the symmetry, each of the rings counts as $|E_o| = 2 \times 5 = 2 \times 6 - 2 = 6|B_o| - 2$ which still predicts no symmetric motions. With the four attaching connections between the rings (with 2-fold symmetry), we have: $|E_o| = 4 \times 5 + 2 = 4 \times 6 - 2 = 6|B_o| - 2$. This still does not have any symmetric motions.*

However, counted without symmetry, we have to select only 18 *bars from each ring, to make the subset* E^**. This leaves an overall count of*

$$|E^*| = 18 + 18 + 4 < 8 \times 6 - 6 = 6|B| - 6.$$

The 'generic' attachment between the two rings leaves two degrees of non-trivial finite freedom starting with translations perpendicular to the axis.

We conclude that to test a structure, such as a dimer, for rigidity we must use both of the criteria, as they are distinct.

5. Algorithms for predicting C_2 flexibility

While this is still an area of ongoing research, we can describe an efficient algorithm which is at least necessary for having rigidity. We describe it in terms of a dimer, in which each atom is a body and each molecular bond becomes 5 bars.

Algorithm 3 *Given a body-bar multi-graph* $G = (B, E)$ *with* 2*-fold rotational symmetry, apply the following sequence of steps:*

I *Apply the* $6|B| - 6$ *pebble game to the entire copy in the dimer (see [8, 5] for details on the pebble game). If this step returns a maximal set of edges* E^* *with* $|E^*| < 6|B| - 6$*, then the dimer is flexible.*

For efficiency, apply it to one protein (storing that), then copy the entire set of pebble placements to the second protein, and proceed with the pebble game on the bridging edges.

II *Start from the* $6|B| - 6$ *on one copy of the protein (which represents all orbits of vertices and a subset of orbits of edges). With this pebbling preserved, test only the edge orbits between the two proteins, using the* $6|B_o| - 2$ *pebble game. If this produces a set* E_o^* *of edges with* $|E_o^*| < 6|B_o| - 2$ *then the dimer is flexible.*

We note that the first part of [I] above is based on the observation that the orbit matrix on the set of edges which lie between the selected representatives of the orbits is identical to the rigidity matrix on these edges. Only the edges which wrap from a selected vertex representative to the second copy of the other selected representative require the modified count!

Of course, we might anticipate that a fluctuating dimer pair which is initially predicted to be flexible would move along this flexible path and stabilize with an additional hydrogen bond. This would, overall, have additional bonds for the overall $6|B| - 6$ count, since $|E| = 2|E_o| = 2(6|B_o| - 2) > 6|B| - 6$. That is, the molecule would be overall redundantly rigid - a property which is currently conjectured to be the rigidity form of saying a molecule is 'stable'.

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