KINARI-Web Case Study of 2LZM
Adding and Removing Interactions

Filip Jagodzinski

February 23, 2011

KINARI-Web [3] can be used to add or remove bonds from a protein's molecular model to determine how each chemical constraint helps to maintain a biomolecule's rigid clusters. We demonstrate the use of KINARI-Web by performing performed several rigidity analyses of Bacteriophage T4 Lysozyme, 2LZM. To investigate the roles of hydrogen bonds and hydrophobic interactions in T4 lysozyme, we have performed rigidity analysis with (a) all chemical constraints, (b) without hydrophobic interactions, and (c) without hydrogen bonds. In a final investigation, we have added two constraints between neighboring α-helices in 2LZM to determine how their inclusion affects the protein's rigidity. The sizes of the largest rigid clusters for the four rigidity experiments for 2LZM are shown in Table 1, and the visualizer results are shown in Fig. 1. When all calculated chemical interactions are included in the protein's molecular model, the largest rigid clusters contains 830 atoms (Fig. 1(a)).

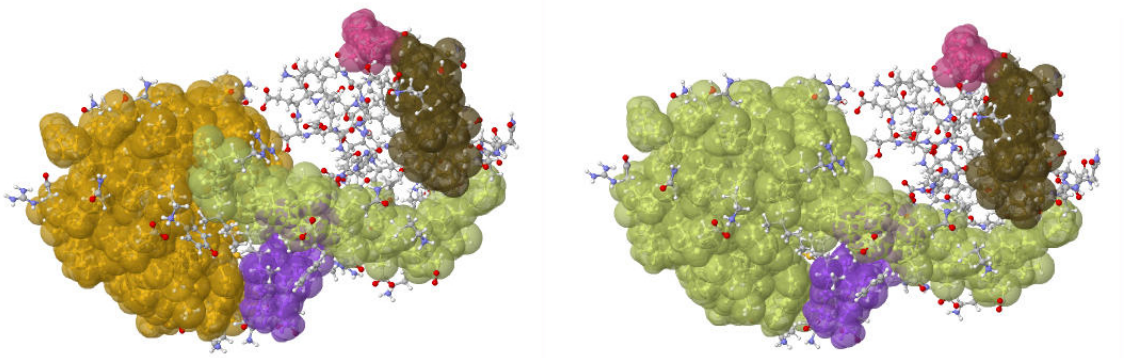
Table 1: Rigidity analysis of Bacteriophage T4 Lysozyme, 2LZM, was performed with all interactions, without hydrogen bonds, without hydrophobic interactions, and with 2 user-added constraints. Results can be used to determine how each interaction affects a protein's rigidity.

Constraints Removed/Added	**Largest Rigid Body**
All Constraints Included	830 atoms
2 Bonds Between Neighboring α-helices added	1,012 atoms
Hydrophobic Interactions Removed	222 atoms
Hydrogen Bonds Removed	29 atoms

The option to add constraints can be used to probe whether the rigidity properties of a specific part of a protein are affected by the introduction of ligands or water molecules, or because of a mutation to the protein's amino acid sequence which would make a specific interaction more or less likely. Many mutation studies have been performed on Bacteriophage T4 Lysozyme to infer the role that different residues have on the stability of the protein [2, 1]. When two constraints are added between the lime and purple α-helices in Fig. 1(a) (between residues 103 and 116, and 100 and 75), the rigidity of the protein changes significantly; the largest cluster increases from 830 to 1,012 atoms (Fig. 1(b)).

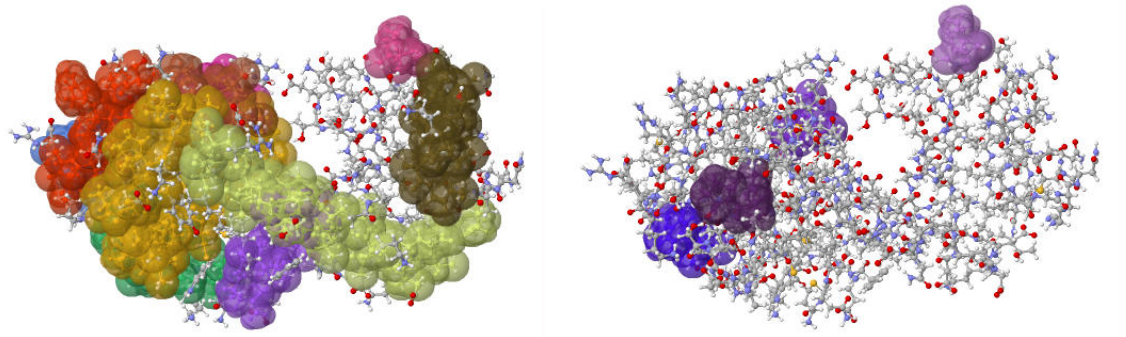
The curation feature can be used to determine how different chemical constraints affect a protein's rigidity. When hydrophobic interactions are excluded from the molecular model of 2LZM, but hydrogen bonds are retained, then the largest rigid clusters is composed of 222 atoms (Fig. 1(c)). When hydrogen bonds are excised from the molecular model, but hydrophobic interactions are

retained, then the largest rigid cluster is composed of only 22 atoms. This analysis suggests that hydrogen bonds play a more important role in stabilizing the protein.



(a) Default modeling

(b) Two constraints added



(c) w/o Hydrophobic interactions

(d) w/o Hydrogen bonds

Figure 1: KINARI-Web can be used to add or remove chemical interactions from the molecular model to help determine how different constraints affect a protein's rigidity. Fig. 1(a) shows the rigid bodies in Bacteriophage T4 Lysozyme, 2LZM, when no constraints are added or removed, and when default modeling and curation options are enabled. Fig. 1(b) shows the effect of adding constraints between α-helices. Fig. 1(c) and 1(d) show the largest rigid bodies when hydrophobic interactions and hydrogen bonds are excluded, respectively.

References

[1] T. Alber, S. Dao-pin, J. A. Nye, D. C. Muchmore, and B. W. Matthews. Temperature-sensitive mutations of bacteriophage t4 lysozyme occur at sites with low mobility and low solvent accessibility in the folded protein. *Biochemistry*, 26:3754–3758, 1987. [DOI:10.1021/bi00387a002].

[2] S. Dao-pin, D. E. Anderson, W. A. Baase, F. W. Dahlquist, and B W. Matthews. Structural and thermodynamic consequences of burying a charged residue within the hydrophobic core of t4 lysozyme. *Biochemistry*, 31:11521–11529, 1991. [DOI:10.1021/bi00113a006].

[3] N. Fox, F. Jagodzinski, Y. Li, and I. Streinu. KINARI-Web server for rigidity analysis of proteins. http://kinari.linkage.cs.umass.edu, 2011.

Curation & Modeling Paramters

Curation Options		Rigidity Analysis Modeling	
Program Version	v0.04-94-gf5c6ab9	Program Version	v0.01-64-g738c6fd
Chains Retained	A (all)	Cluster Representation	KINARI
Waters Retained	None	Single Covalent Bonds	Hinges
Hydrogne Atoms Added	Using Reduce	Double Covalent Bonds	6 Bars
Retain LINK Records	No	Disulfide Bonds	Hinges
Retain CONECT Records	No	Hydrogen Bonds	Hinges
Hydrogen Bonds	Using Hbplus	Resonance Bonds	6 Bars
Interactions Removed	all hydrogen bonds (†)	Hydrophobic Interaction	2 Bars
	all hydrophobics (‡)	Link Records	Hinges
	none ⋆		
Interactions Added	2 Hydrogen Bonds ∗		

Website & Notes

Website	http://kinari.linkage.cs.umass.edu
Date Performed	20 February 2011
Notes	† - all hydrogen bonds removed; Fig. 1(d)
	‡ - all hydrophobic interactions removed; Fig. 1(c)
	⋆ - no interactions removed, Fig. 1(a) resulted
	∗ - hydrogen bonds added between residues 103 & 116, and 100 & 75; Fig. 1(b)