Analyzing Protein Flexibility
An Introduction to Combinatorial Rigidity Methods and Their Applications

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Proteins Are Dynamic Molecular Machines

Maltose-binding protein engulfing ligands

Proteins are ...

Proline  Tryptophan  Alanine
Proteins are ...
Protein Secondary Structure
Protein Secondary Structure
Proteins are dynamic structures
Problem: We cannot observe proteins on the atomic level or their motions directly, but we'd like to understand how they flex or bend.
B-factors from X-Ray Crystallography Data
Molecular Dynamics (MD) Simulations

Molecular Dynamics provides the means to solve the equations of particle motion based on approximate (Lennard-Jones) potential.
Molecular Dynamics Simulation - Demonstration

GROMACS 4: Algorithms for Highly Efficient, Load-Balanced, and Scalable Molecular Simulation
Hess, B., Kutzner, C., van der Spoel, D. and Lindahl, E.
Tutorial Outline

- Demonstration of Rigidity Analysis
- Introduction to Rigidity Theory in 2-dimensions
- Introduction to Rigidity Theory in 3-dimensions
- Break
- Molecular Modeling and Rigidity Analysis using KINARI
- Potential Applications and ongoing research using Rigidity Analysis
- Concluding Remarks
Check on Molecular Dynamics Simulation
Molecular Dynamics Simulations – Good for?

- **Local Motions (0.01 to 5 Å, 10^{-15} to 10^{-1} s)**
  - Atomic Fluctuations
  - Sidechain Motions
  - Loop Motions

- **Rigid Body Motions (1 to 10Å, 10^{-9} to 1s)**
  - Helix Motions
  - Domain Motions
  - Subunit Motions

- **Large-Scale Motions (> 5Å, 10^{-7} to 10^{4} s)**
  - Helix coil transitions
  - Dissociation/Associations
  - Folding and unfolding
Part 1:
Demonstrations of Using Rigidity Analysis
A PDB file ...

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Atoms are Sequentially numbered
All (resolved) atoms of a single amino acid are grouped together
Atomic coordinates
Horse heart cytochrome c (PDB file 1HRC) is a 105 residue heme protein found loosely associated with the inner membrane of mitochondria.

What features of KINARI-Web can we use to determine/investigate its rigidity?

Where are the rigid regions? Hinges? Clusters?
Cytochrome C – Rigidity Results

Default curation and modeling options display the largest rigid clusters as highlighted surfaces in a bar-and-stick model.

The same protein can be viewed as a cartoon.

A hinge between two bodies is easily seen when the protein is displayed using a bar-and-stick representation.

KINARI-Web: A Server for Protein Rigidity Analysis  
Naomi Fox, Filip Jagodzinski, Yang Li, Ileana Streinu  
HIV-1 Protease

Can we use rigidity analysis to infer the effect of the ligand on the protease's rigidity?
When the ligand is retained in the molecular model of HIV-1 protease (left), the flaps of the dimer are held rigidly in place. When the ligand is removed (right) the flaps are flexible.

**KINARI-Web: A Server for Protein Rigidity Analysis**  
Naomi Fox, Filip Jagodzinski, Yang Li, Ileana Streinu  
Lysozyme is part of the immune response system. It helps to break apart bacterial cellular walls, thus killing bacteria.
Lysozyme from bacteriophage T4 – Rigidity Results

Default Modeling

Addition of two hydrogen bonds causes the protein to rigidify

By removing hydrophobic interactions, their effect can be easily seen

KINARI-Web: A Server for Protein Rigidity Analysis  
Naomi Fox, Filip Jagodzinski, Yang Li, Ileana Streinu  
Part 2: Comparative overview of tools for studying protein flexibility
Scales of protein motion

Large domain, collective motions

Necessary for important protein functions such as Enzyme catalysis, Signal transduction, and Protein-protein interactions

The enzyme hexokinase changes conformation when glucose binds to it.

## Methods for studying protein kinematics

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<th>In Vitro</th>
<th>In Silico</th>
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<td>Fluorescence</td>
<td>ENM and NMA</td>
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<tr>
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<td>Cryo-EM</td>
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X-ray crystallography

Step 1:
Crystallize the protein in the native state

Step 2:
Get diffraction patterns
X-ray crystallography

Step 3: Determine electron density map

Step 4: Fitting and refinement

Image from Wikimedia courtesy Thomas Splettstoesser
X-ray crystallography

B-factors – describe the displacement from mean coordinate

“Missing” atoms – position could not be determined

>70,000 X-ray data files in PDB
NMR Spectroscopy

Computes coordinates from set of distance constraints

Multiple conformations produced

~9,000 NMR-resolved structures in PDB

PDB File 1BVE, HIV-1 protease
Cryo-electron microscopy

Rapidly freeze protein in solution and view under electron microscope

< 400 structures in PDB
Molecular Dynamics

Model all the interatomic interactions in a system and simulate with fs time step.

Can only simulate fast timescales

Elastic Network Models and Normal Mode Analysis

Build an elastic network model of the protein

Determine the normal modes of the model

Part 3:
Introduction to Rigidity Theory in 2 and 3 Dimensions
A framework $G(p)$ is **rigid** if it has no continuous deformation.

Which frameworks are rigid and which are not?
Is this rigid?
Exercise: Counting Degrees of Freedom

A 2D framework with n points and no edges has 2n degrees of freedom (DOF).
Exercise: Counting Degrees of Freedom in 2D

Adding a bar reduces the number of degrees of freedom by 1.

Framework has 7 DOF
Exercise: Counting Degrees of Freedom in 2D

Adding a second bar again reduces the number of degrees of freedom by 1.

Framework has 6 DOF
Exercise: Counting Degrees of Freedom in 2D

Adding a third bar again reduces the number of degrees of freedom by 1.
Framework has 5 DOF
Exercise: Counting Degrees of Freedom in 2D

Adding a fourth bar again reduces the number of degrees of freedom by 1.

Framework has 4 DOF
Exercise: Counting Degrees of Freedom in 2D

Adding a fifth bar again reduces the number of degrees of freedom by 1.
Framework has 3 DOF
Exercise: Counting degrees of freedom in 2D

7 DOF

6 DOF

5 DOF

4 DOF

3 DOF

3 DOF
The Maxwell-Laman theorem

A framework $G(p)$ is generically minimally rigid iff

- For every subset of vertices, $|E'| \leq 2|V'| - 3$
- $|E| = 2|V| - 3$

$|V| = n = 4$
$|E| = m = 4$
$|E| < 2n-3$

$|V| = n = 4$
$|E| = m = 5$
$|E| = 2n-3$
Rigid components

Is this graph rigid?

- $n = 6$
- $m = 9$
- $2n - 3 = 9$
Rigid Components

Rigid components are maximal sets of vertices which are rigid to each other.
## 2D Bar-and-Joint Rigidity

### Rigid components

<table>
<thead>
<tr>
<th>Rigid component</th>
<th>Description</th>
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<tbody>
<tr>
<td>Rigid zcomponent</td>
<td>Maximal set of vertices that are rigid to each other.</td>
</tr>
<tr>
<td>(2,3)-component</td>
<td>Maximal set of vertices containing a (2,3)-tight graph.</td>
</tr>
</tbody>
</table>

Component is rigid if it contains a spanning Laman subgraph.

Not a component.

---

Component is rigid if it contains a spanning Laman subgraph.
Can we extend Laman to 3D?

Will $3n-6$ edge counts work for 3D?

Why $3n-6$?

For each vertex:

Translations along $x,y,z$ axes.

For the entire framework:

Rigid translations along $x,y,z$ axes

Rigid rotations around $x,y,z$ axes
Can we extend Laman to 3d?

Can we rigidify with $3(4) - 6 = 6$ edges?

$n = 4, m=4$
8 DOF

$n = 4, m=5$
7 DOF

$K_4$ is rigid
6 DOF
Exercise: Counting Degrees of Freedom in 3D

A 3D framework with $n$ points and no edges has $3n$ DOF.

Framework has $3(4) = 12$ DOF.
Exercise: Counting Degrees of Freedom in 3D

11 DOF  10 DOF  9 DOF  8 DOF  7 DOF  6 DOF
Exercise: Counting Degrees of Freedom in 3D

- 14 dofs
- 13 dofs
- 12 dofs
- 11 dofs
- 10 dofs
- 9 dofs
- 8 dofs
- 7 dofs
- 6 dofs
- 6 dofs
Counterexample to (3,6)-counts

The double banana

- Edges satisfy (3,6)-counts, but framework is Not rigid

(3,6)-tight

3(8)-6=18=m

Not rigid
The triple banana

For the entire framework:
n = 12, m=27
3(12)-6=36-6=30
Flexible with 9 dofs
3 non-trivial dofs.

Which are the rigid components?
### 2D vs 3D generic rigidity

<table>
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<tr>
<th>2d</th>
<th>3d</th>
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<tbody>
<tr>
<td>G contains a spanning (2,3)-tight subgraph $\leftrightarrow$ G is generically rigid.</td>
<td>Sometimes, when G contains a spanning (3,6)-tight subgraph, G is generically rigid.</td>
</tr>
<tr>
<td>Rigid components ALWAYS induce spanning (2,3)-tight subgraphs.</td>
<td>Sometimes, rigid components induce spanning (3,6)-tight subgraphs.</td>
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</table>

Is there a class of frameworks for which:

G contains a spanning (3,6)-tight subgraph $\leftrightarrow$

G is generically rigid in 3D.
Bar and Joint and Proteins

Bars

- bonds
- bond-bending constraints

Joints:

- Atoms

Protein backbone
A body-bar-hinge framework is generically minimally rigid iff:
- its associated (multi) graph has exactly 6n-6 edges
- and every subgraph has at most 6n-6 edges

Tay 84, 89; White and Whiteley 87; Katoh and Tanigawa 09
Educational site and demos
http://linkage.cs.umass.edu/pg/pg.html
Part 4: Molecular Modeling and Rigidity Analysis using KINARI
Covalent bonds impose constraints

- Fix bond length
- Fix angle between bonds
- Peptide and double bonds fix dihedral angle
Modeling Molecules for Rigidity

- An atom and its covalent-bonded neighbors form a rigid body.
- Rotatable covalent bonds act as hinges.

![Diagram showing rigid and flexible molecules](image-url)
Determining Maximal Rigid Bodies

- We know how to determine trivial bodies.
- Would like to find maximal bodies.
- A body is maximal if there are no other atoms which are rigidly attached to it.

![Proline](image)

*proline*
A body or rigid cluster is a maximal set of atoms and all bonds and interactions that hold the atoms rigidly together.
Steps of Protein Rigidity Analysis

1. **Build molecule**
   - Import PDB file

2. **Model mechanics**
   - Assign structural properties

3. **Build graph**
   - Represent molecule as a network

4. **Run pebble game**
   - Analyze connectivity

5. **Edge contraction**
   - Simplify graph

6. **Determine flexibility rigid clusters**
   - Identify stable structures
Includes atoms and coordinates from X-ray crystallography or NMR experiments

Usually, does not include hydrogen atoms
Important interactions for holding a protein's 3D shape

- Covalent bonds
- Hydrogen bonds
- Hydrophobic interactions

Main chain and side chains connected by covalent bonds.

Secondary structures held by backbone hydrogen bonds.

3D folded shape held together by hydrogen bonds and hydrophobic interactions.
Covalent bonds – modeled as hinges, remove 5 degrees of freedom.

Hydrogen bonds – include only strong hydrogen bonds. Model as hinges.

Hydrophobic interactions – weaker interactions. Model with 2 'bars' removing 2 degrees of freedom.
Differences in Systems

ASU-FIRST bodies do not overlap at hinges
Output From KINARI

- Body-bar-hinge file (xml file)
  - Set of bodies – lists of atoms
  - Set of bars – pairs of bodies, pairs of atoms
  - Set of hinges – pairs of bodies which share a hinge along the axis between pair of atoms
Part 5: Applications of Protein Rigidity Analysis
Applications

- Dilution Analysis (Hespenheide, *et al*)
- Redundancy Analysis (Fox, Streinu)
- FRODA Geometric Simulation (Wells, *et al*)
- Probabilistic Roadmaps (Thomas, *et al*)
- Thermophile stability (Gohlke, Radestock)
- Flexibility of RNA (Fulle, Gohlke)
- KINARI Mutagen (Jagodzinski, Streinu)
Observing unfolding pathway

**Motivation:** Contributions of noncovalent interactions:

- Responsible for holding a protein’s 3D shape.
- Break and form during normal fluctuations

**Our contribution:** Classify each interaction in terms of its contribution to the rigidity.

Cluster Redundancy Score

\[
\Phi(i) = \frac{\sum_{j \in R(i)} w_j}{\sum_{k \in N(i)} w_k}
\]

Redundancy score of cluster \(i\):
- Redundant interactions in cluster \(i\)
- Weight of interaction \(j\)
- Sum of weights of all redundant interactions
- Sum of weights of all interactions
- Noncovalent interactions in cluster \(i\)

Example: 1HRC

<table>
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<tr>
<th>Cluster</th>
<th>Atoms</th>
<th>H-Bonds redund / all</th>
<th>HPh-inter. redund / all</th>
<th>Redun. Score</th>
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<td>1 / 5</td>
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\[
\frac{5 \times 0 + 2 \times 6}{5 \times 23 + 2 \times 10} = \frac{12}{135} \approx 0.089
\]

Survey Results

Almost all hydrogen bonds were critical. The majority of hydrophobic interactions were not critical.

<table>
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<th>PDB</th>
<th>Cluster</th>
<th>Atoms</th>
<th>H-Bonds</th>
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</table>
Use rigid clusters to generate motion for Barnase (110 residue)

The enzymatic protein has three very similar conformations (open, closed, and occluded), most significant differences in residues 14-24 (M-20 loop)/Mobile loop in closed (red) and occluded (blue) positions.

PRM With Rigidity Analysis

- Extension of Probabilistic Roadmap algorithm
- Models protein backbone as a linkage, and samples different conformations
- Uses rigidity analysis to restrict conformers sampled

Do mesophile/thermophile homologs have corresponding states during unfolding?

Rigidity analysis for RNA

- Different parameterization required for calculating hydrophobics
- Found correspondence between B-values and flexibility index

Crambin is a 46 amino acid plant protein, whose crystals diffract to ultra-high resolution.

Can we use Rigidity Analysis to infer critical residues of crambin?

The insight: “mutate” different residues to generate *in-silico* mutants, and perform rigidity analysis on them, to infer which residues, IF mutated, would destabilize the protein, and hence are important.
To simulate a substitution to a glycine, all hydrogen bonds and hydrophobic interactions for a residue are removed from the molecular model; this effectively removes the side chain from the molecular model because it cannot contribute stabilizing interactions.

Wild-type α-defensin 1 (PDB File 2PM1) has two hydrogen bonds (light green bars) and two hydrophobic interactions (blue bars) among residues 3, 5, and 13.

Mutating residue 5 removes hydrogen bonds between residue 5 and 13 and hydrophobic interactions between residue 5 and 3.

Mutating residue 3 removes hydrophobic interactions that it forms with residue 5.

Filip Jagodzinski, Jeanne Hardy, Ileana Streinu, Using Rigidity Analysis To Probe Mutation-Induced Structural Changes in Proteins, CSBW 2011.
KINARI Mutagen – Rigidity Analysis of Mutants

We can compare the rigidity results of mutants to infer which mutations disrupt the protein's rigidity, and hence which residues are critical.

The while type of the crambin contains one large rigid cluster (purple).

Mutating residue 4 affects the rigid bodies of crambin.

Mutating Residue 40 causes the largest rigid body to break down, but not as much as when residue 4 is mutated.

Filip Jagodzinski, Jeanne Hardy, Ileana Streinu, Using Rigidity Analysis To Probe Mutation-Induced Structural Changes in Proteins, CSBW 2011.
Rigidity results for different mutants can be compared to infer which residue(s) is (are) critical.

**Distribution of Rigid Bodies, By Residue**

Filip Jagodzinski, Jeanne Hardy, Ileana Streinu, Using Rigidity Analysis To Probe Mutation-Induced Structural Changes in Proteins, CSBW 2011.
KINARI Mutagen – Correlating results to Web-Lab Experiments and Other Metrics

Largest Rigid Cluster and SASA vs Excised Residue

Residue on Which Excision Was Performed

Filip Jagodzinski, Jeanne Hardy, Ileana Streinu, Using Rigidity Analysis To Probe Mutation-Induced Structural Changes in Proteins, CSBW 2011.
Residues 3, 4, 40, and 41 (among others), have been found to be identical among Crambin and two homologous plant toxins viscotoxin A3 and α1 purothioni.

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