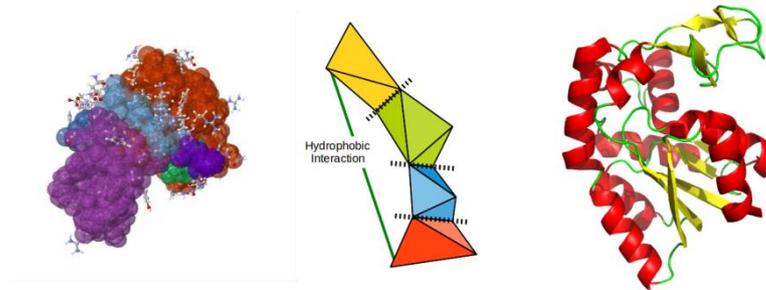


A Combined Molecular Dynamics, Rigidity Analysis Approach for Studying Protein Complexes

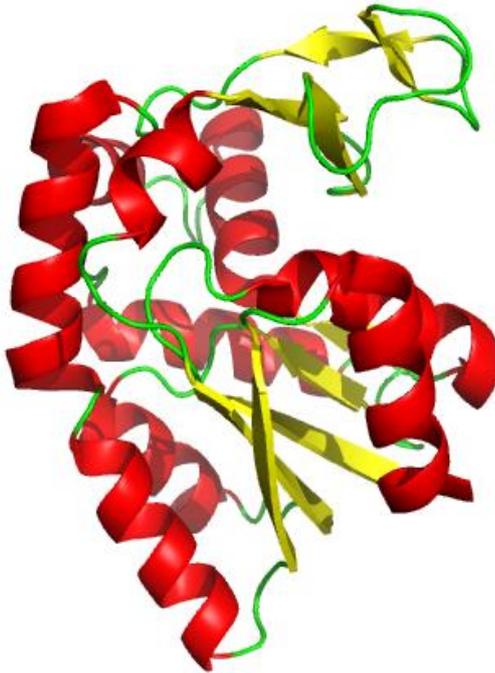


Brian Orndorff
Dr. Filip Jagodzinski

SOURCE
Ellensburg Washington, 15 May 2014

Motivation

Proteins are non-static structures



Barnase
"Closed" Conformation



Barnase
"Open" Conformation

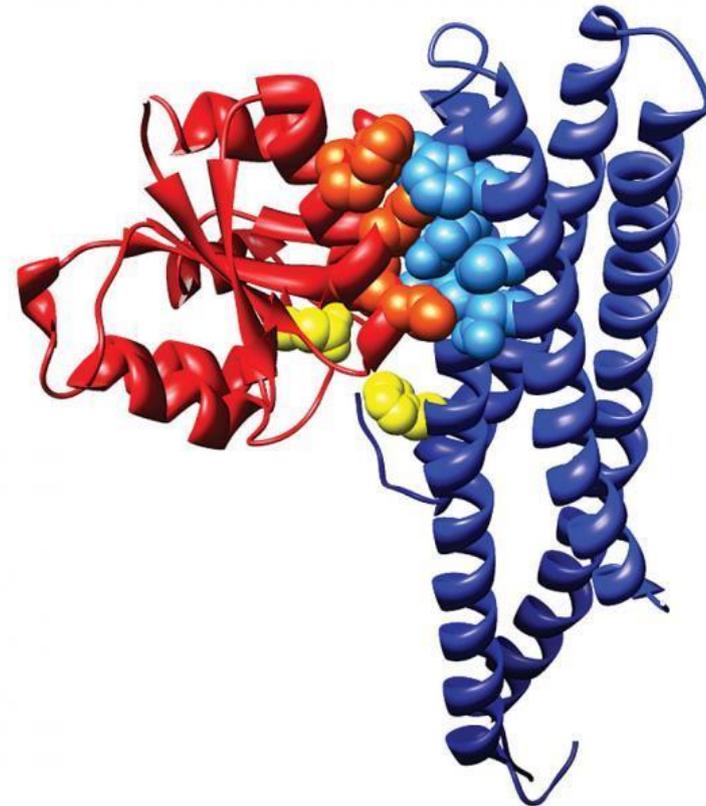
Motivation

Proteins also can exist as complexes, that are composed of two or more associated polypeptide chains.

Motivation

Proteins also can exist as complexes, that are composed of two or more associated polypeptide chains.

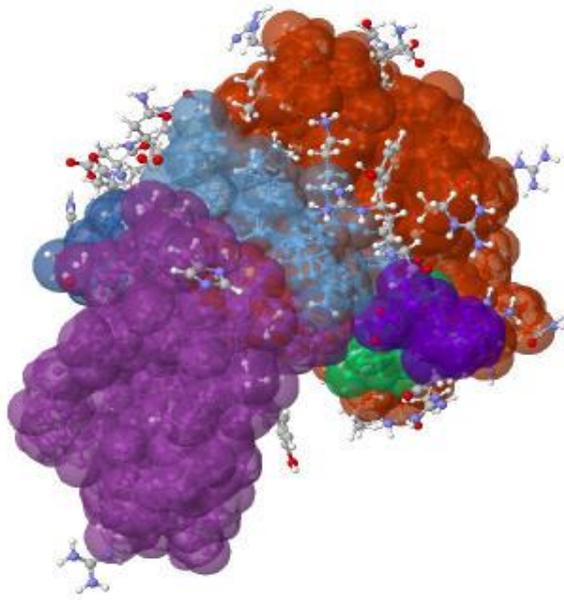
The membrane-bound sensor histidine kinase (shown in blue) and its response regulator (shown in red) share contact residues (shown as orange and blue spheres).



Our Main Objective: Investigate if (and how) polypeptides in a complex affect each other's flexibilities?

Motivation

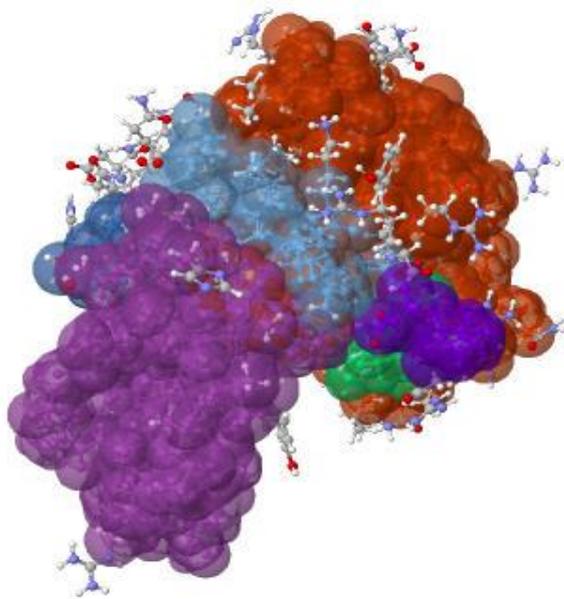
Rigidity analysis of a single
Protein crystal structure can
be misleading



Rigid cluster decomposition
of protein 1LZ1

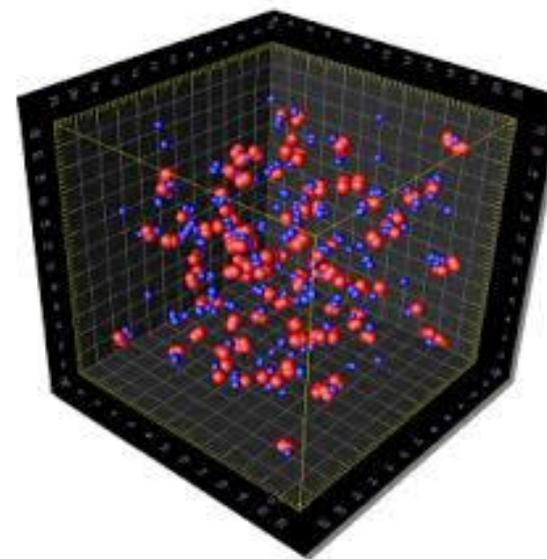
Motivation

Rigidity analysis of a single Protein crystal structure can be misleading



Rigid cluster decomposition of protein 1LZ1

Full-scale Molecular Dynamics simulation runs are computationally intensive



Microsecond (and longer) MD simulations can require thousands of CPUs and/or thousands of wall-clock hours

Our Proposal

Step 1: Use short runs of Molecular Dynamics to generate an ensemble of protein complex conformations

Our Proposal

Step 1: Use short runs of Molecular Dynamics to generate an ensemble of protein complex conformations

Step 2: Analyze the rigidity of a subset of the MD generated conformations

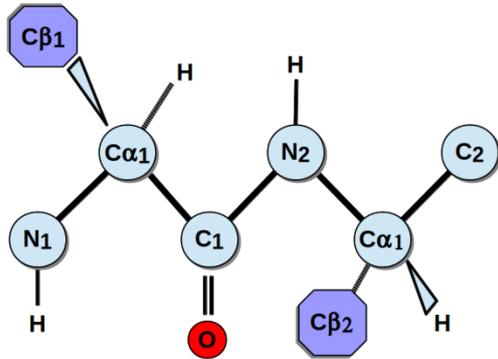
Our Proposal

Step 1: Use short runs of Molecular Dynamics to generate an ensemble of protein complex conformations

Step 2: Analyze the rigidity of a subset of the MD generated conformations

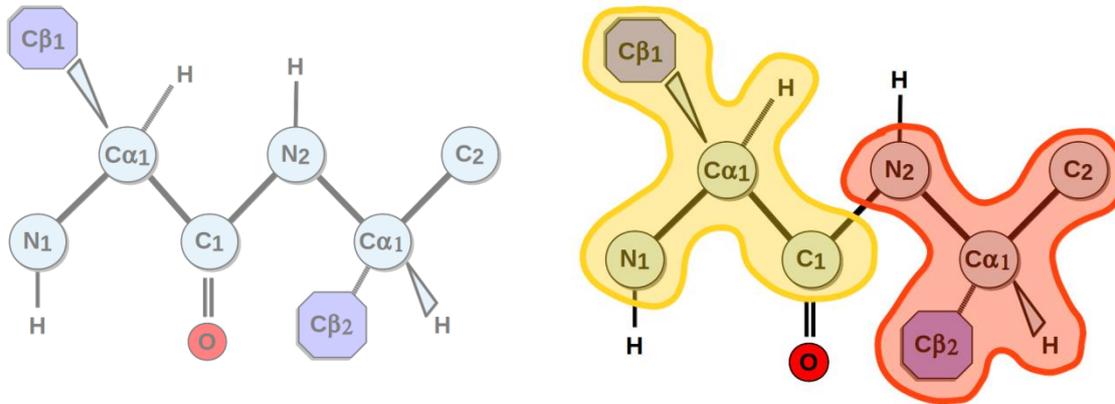
Step 3: Inspect how the rigidity properties of each conformation in the ensemble of states varies, to infer if (and how) the polypeptides in a complex effect each other's rigidity.

Rigidity Analysis



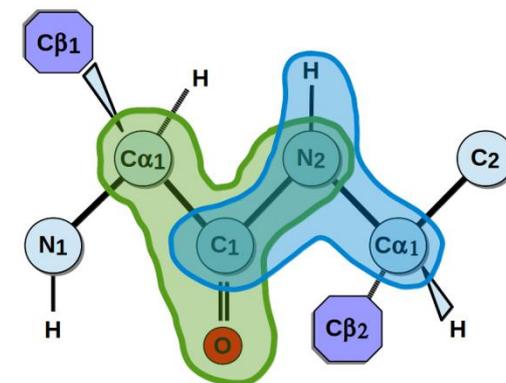
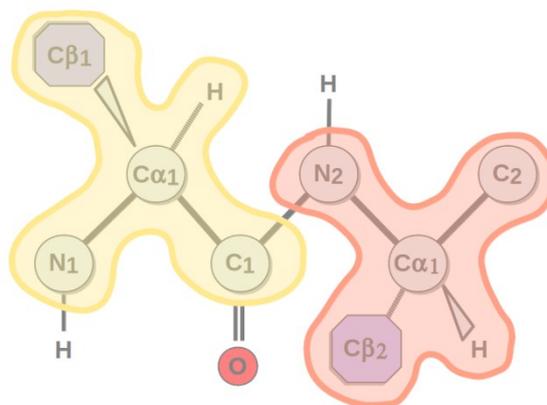
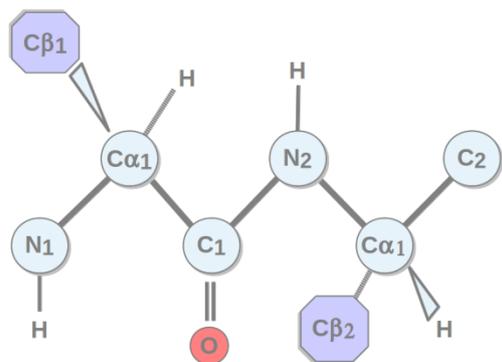
Schematic

Rigidity Analysis



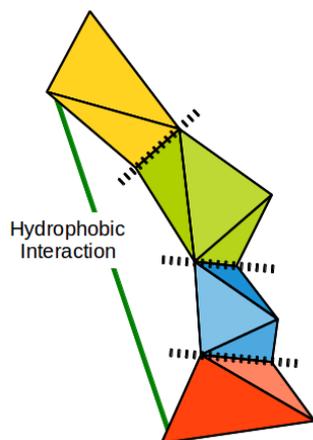
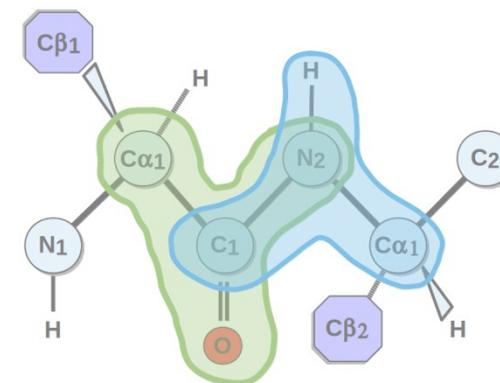
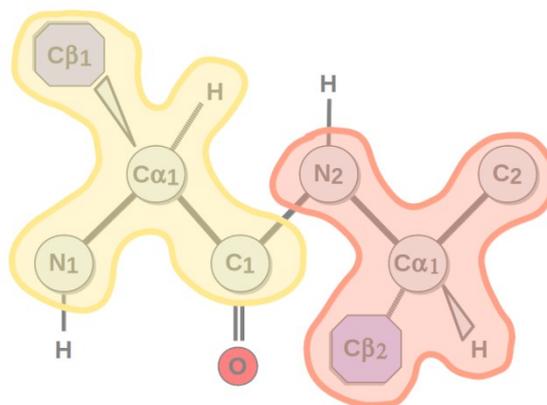
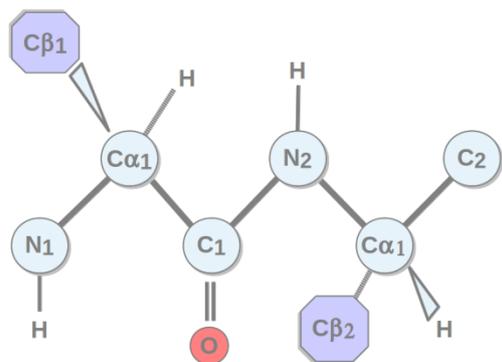
Rigid Bodies

Rigidity Analysis



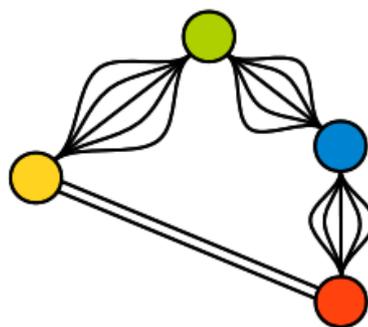
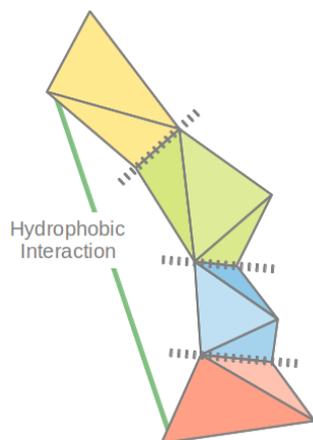
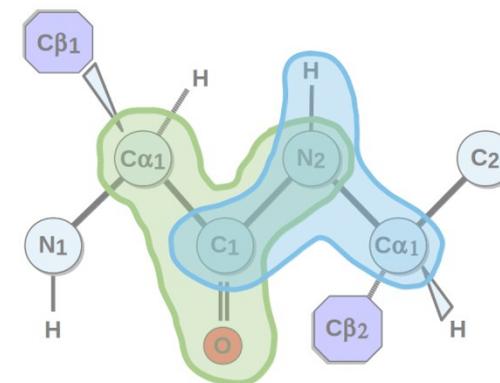
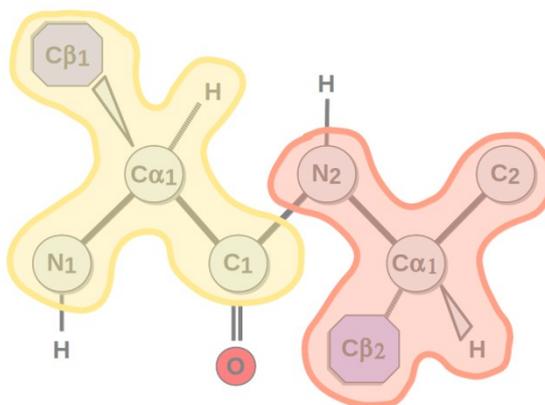
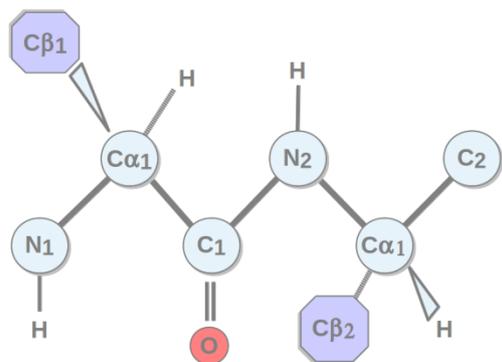
Overlapping Rigid Bodies

Rigidity Analysis



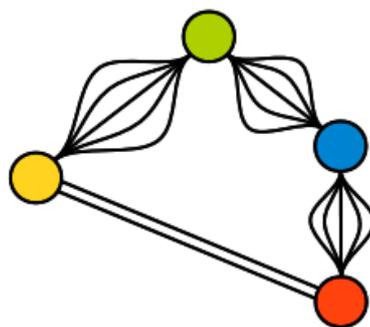
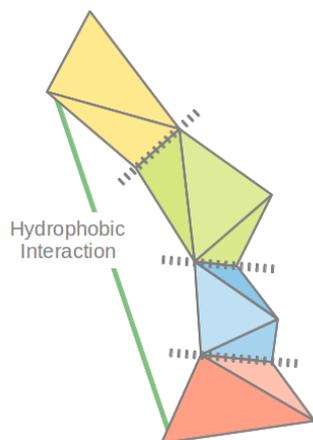
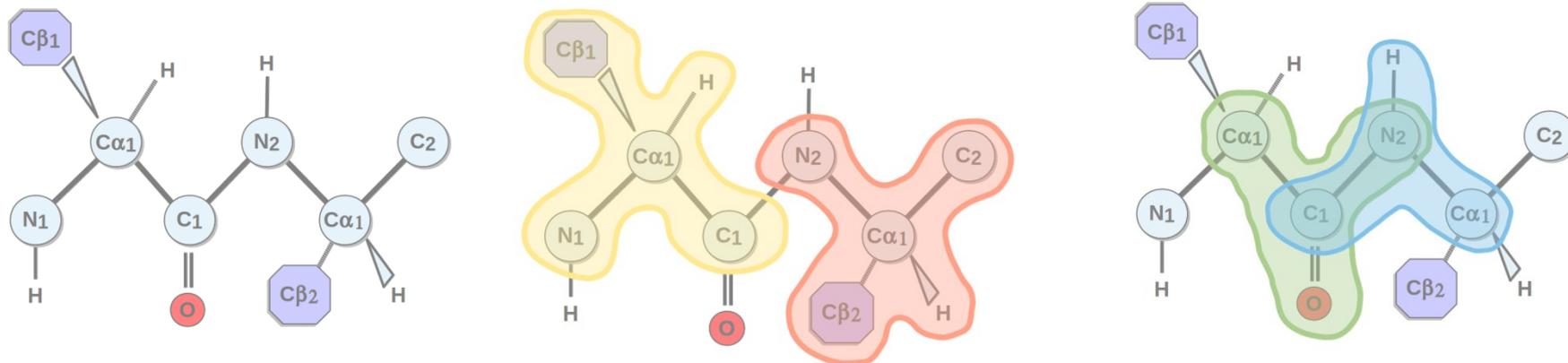
Mechanical
Model

Rigidity Analysis



Associated Graph

Rigidity Analysis



A pebble game algorithm is used to identify the rigid regions of the associated graph

The algorithm's results are used to infer the rigid and flexible regions of the protein structure

Data Set

9 protein X-ray structure files were obtained from the Protein Data Bank

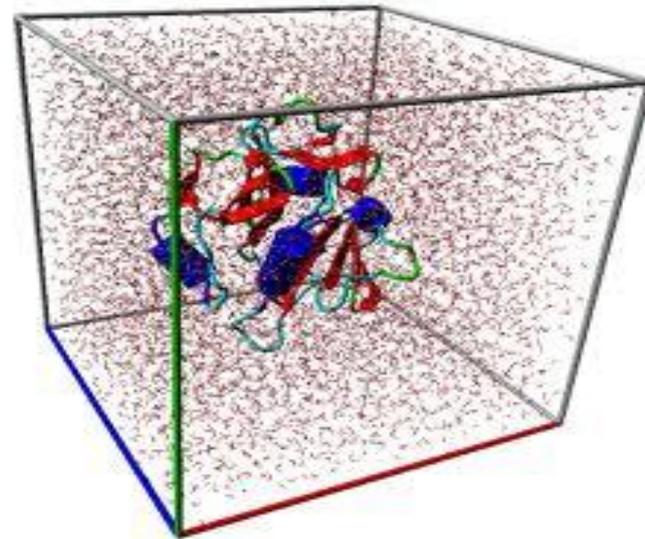
Grouped into 1-chain, 2-chain, and 3-chain sets

PDB	# polymers	# chains	chain length(s)	MD run-time	Rigidity Analysis run-time
1lz1	1	1	130	3 min	5 min
4fd9	1	2	92, 92	2 min	5 min
3vb2	1	2	144, 144	4 min	7 min
4bcz	1	2	110, 110	3 min	6 min
3pth	2	2	82, 15	2 min	5 min
3oy6	2	2	253, 23	6 min	4 min
3vz9	2	2	106, 73	1 min	5 min
4g6t	2	2	128, 82	3 min	4 min
4ext	3	3	96, 23, 204	2 min	8 min

Molecular Dynamics

GROMACS v4.5.5

For each PDB structure, we used 10 conformations randomly selected from a 2 picosecond MD simulation



Rigidity Analysis





KINARI Software

for KINematic And RIGidity analysis of proteins

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KINARI is a suite of tools for calculating and analyzing the rigidity and flexibility of biomolecules. To access the KINARI-Web or KINARI-Mutagen tool, select that application's tab and click on the "Access" button. If you use KINARI for research that you publish, please cite:

- KINARI-Web: Naomi Fox, Filip Jagodzinski, Yang Li, Ileana Streinu. **KINARI-Web: A Server for Protein Rigidity Analysis**, *Nucleic Acids Research*, 39 (Web Server Issue), 2011.
- KINARI-Mutagen: Filip Jagodzinski, Jeanne Hardy, and Ileana Streinu. **Using Rigidity Analysis to Probe Mutation-Induced Structural Changes in Proteins**, *Journal of Bioinformatics and Computational Biology*, Vol. 10, No. 3, 2012.

KINARI-Web
KINARI-Mutagen

Curate a PDB file, analyze its rigidity, and Visualize the Results

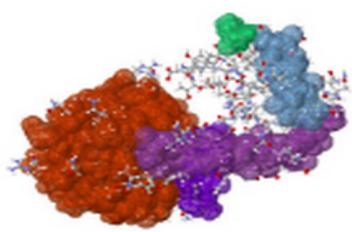
Description

KINARI-Web performs rigidity analysis of a protein, and provides a Jmol-based visualizer to explore the results. The protein can be curated prior to the analysis using our tools. The rigid regions of lysozyme from Bacteriophage T4 are shown on the right.

The curation, rigidity analysis, and visualizer tools in KINARI can also be used as stand-alone applications via the tabs above. Video tutorials and help/documentation are available for each application.

A **Quick-Start** option performs a streamlined curation and analysis using default parameters. For **Advanced Users**, the whole functionality of each individual application is available to retain desired chains or ligands, add or remove stabilizing constraints, add hydrogen atoms, and set modeling options.

Access KINARI-Web



See related **publications**, **case studies**, and **profiling** information. Check the **requirements** for supported browsers and platforms. Submit questions and comments with the **feedback** form.


Funding provided by: NSF DMS-0714934, NSF CCF-1016988, NSF CCF-0728783, and DARPA 23 Mathematical Challenges


<http://kinari.cs.umass.edu>

Rigidity Analysis



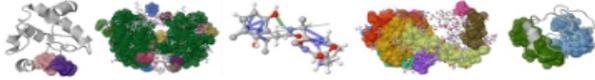
Default Modeling

Largest Rigid Cluster
(LRC) was recorded for
each protein complex
conformation

Rigidity Analysis

 SMITH COLLEGE UMassAmherst Linkage

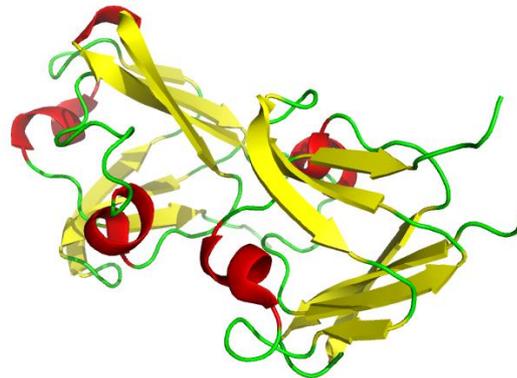
KINARI Software
for KINematic And Rigidity analysis of proteins



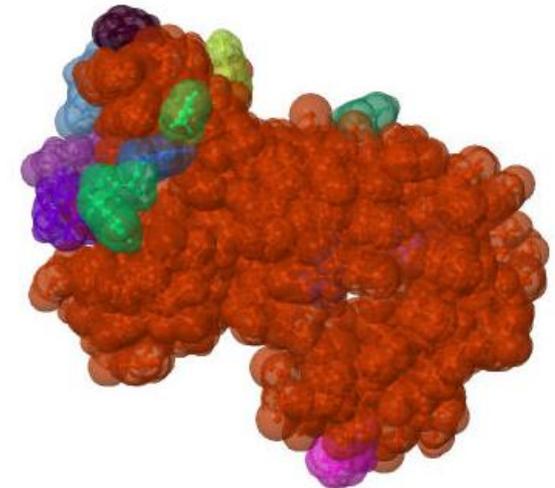
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Default Modeling

Largest Rigid Cluster (LRC) was recorded for each protein complex conformation



Input protein structure



Calculated Rigid Clusters
(same color atoms belong to the same rigid cluster)

Overall Approach

Input

Atomic Coordinates: PDB Format

Atom	Element	Amino Acid		Chain name		Sequence Number			Coordinates			(etc.)
		Element	Residue	Chain	ID	X	Y	Z				
ATOM 1	N	ASP	L	1		4.060	7.307	5.186	...			
ATOM 2	CA	ASP	L	1		4.042	7.776	6.553	...			
ATOM 3	C	ASP	L	1		2.668	8.426	6.644	...			
ATOM 4	O	ASP	L	1		1.987	8.438	5.606	...			
ATOM 5	CB	ASP	L	1		5.090	8.827	6.797	...			
ATOM 6	CG	ASP	L	1		6.338	8.761	5.929	...			
ATOM 7	OD1	ASP	L	1		6.576	9.758	5.241	...			
ATOM 8	OD2	ASP	L	1		7.065	7.759	5.948	...			

Element position within amino acid

PDB File

Overall Approach

Generate 10 (only 3 are shown here) conformations using a short MD run

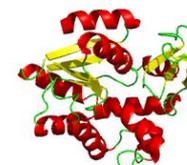
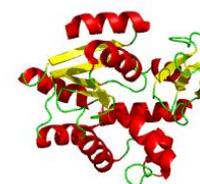
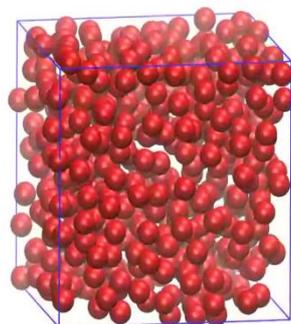
Input

Atomic Coordinates: PDB Format

Atom	Element	Amino Acid		Chain name		Sequence Number			Coordinates			(etc.)
		1	2	3	4	5	6	7	8	9	10	
1	N	ASP	L	1				4.060	7.307	5.186	...	
2	CA	ASP	L	1				4.042	7.776	6.553	...	
3	C	ASP	L	1				2.668	8.426	6.644	...	
4	O	ASP	L	1				1.987	8.438	5.606	...	
5	CB	ASP	L	1				5.090	8.827	6.797	...	
6	CG	ASP	L	1				6.338	8.761	5.929	...	
7	OD1	ASP	L	1				6.576	9.758	5.241	...	
8	OD2	ASP	L	1				7.065	7.759	5.948	...	

Element position within amino acid

PDB File



Overall Approach

Generate 10 (only 3 are shown here) conformations using a short MD run

Analyze Rigidity of each structure

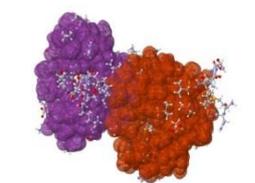
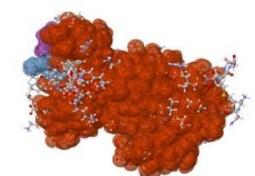
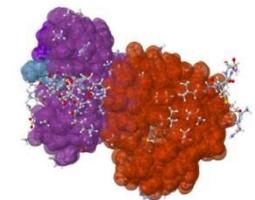
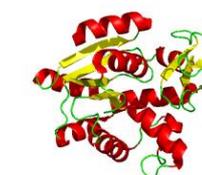
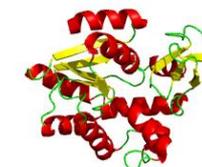
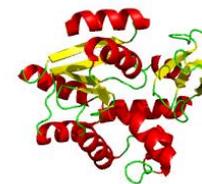
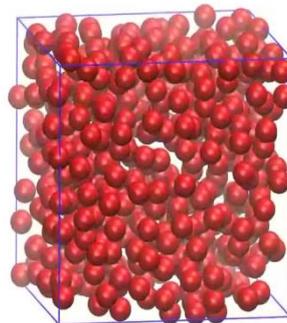
Input

Atomic Coordinates: PDB Format

Atom	Amino Acid		Chain name		-----Coordinates-----			(etc.)
	Element	Sequence Number			X	Y	Z	
ATOM 1	N	ASP L 1	1		4.060	7.307	5.186	...
ATOM 2	CA	ASP L 1	1		4.042	7.776	6.553	...
ATOM 3	C	ASP L 1	1		2.668	8.426	6.644	...
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ATOM 5	CB	ASP L 1	1		5.090	8.827	6.797	...
ATOM 6	CG	ASP L 1	1		6.338	8.761	5.929	...
ATOM 7	OD1	ASP L 1	1		6.576	9.758	5.241	...
ATOM 8	OD2	ASP L 1	1		7.065	7.759	5.948	...

Element position within amino acid

PDB File



PDB	# polymers	# chains	chain length(s)	MD run-time	Rigidity Analysis run-time
1lz1	1	1	130	3 min	5 min
4fd9	1	2	92, 92	2 min	5 min
3vb2	1	2	144, 144	4 min	7 min
4bcz	1	2	110, 110	3 min	6 min
3pth	2	2	82, 15	2 min	5 min
3oy6	2	2	253, 23	6 min	4 min
3vz9	2	2	106, 73	1 min	5 min
4g6t	2	2	128, 82	3 min	4 min
4ext	3	3	96, 23, 204	2 min	8 min

PDB	# polymers	# chains	chain length(s)	MD run-time	Rigidity Analysis run-time
1lz1	1	1	130	3 min	5 min
4fd9	1	2	92, 92	2 min	5 min
3vb2	1	2	144, 144	4 min	7 min
4bcz	1	2	110, 110	3 min	6 min
3pth	2	2	82, 15	2 min	5 min
3oy6	2	2	253, 23	6 min	4 min
3vz9	2	2	106, 73	1 min	5 min
4g6t	2	2	128, 82	3 min	4 min
4ext	3	3	96, 23, 204	2 min	8 min

In each case, the run-time of our combined MD, rigidity analysis approach of our 9 proteins took at most 11 minutes.

Proof of Concept

- Group 1: Range of LRC as % of Max(LRC) is < 30
- Group 2: Range of LRC as % of Max(LRC) is > 30 but < 60
- Group 3: Range of LRC as % of Max(LRC) is greater than 60

PDB ID	Range of size of LRC	Range of LRC as % of Max(LRC)	Group
1lz1	1005	0.67	3
3pth	163	0.41	2
3vb2	827	0.82	3
3vz9	379	0.22	1
4bcz	1408	0.79	3
4fd9	1055	0.55	2
3oy6	228	0.08	1
4g6t	362	0.18	1
4ext	1132	0.41	2

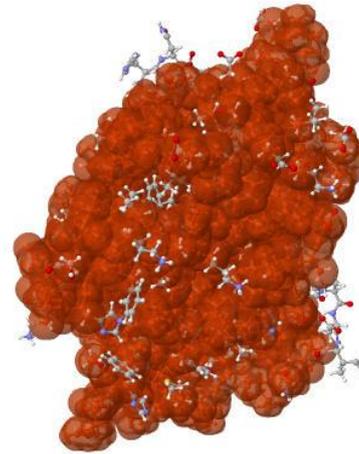
Proof of Concept

In some structures, the rigidity varied **little** across the 10 studied conformations

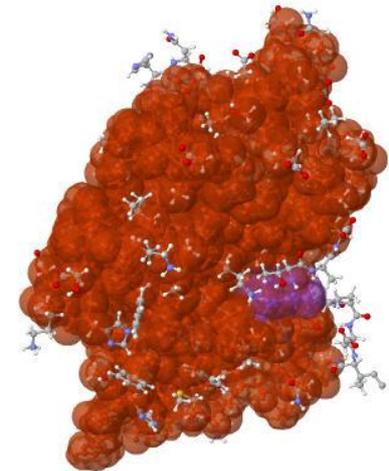
Proof of Concept

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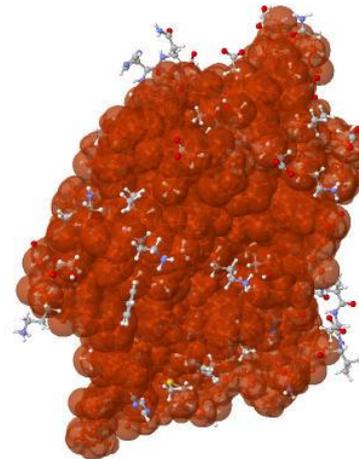
3VZ9 LRC variance : 0.22



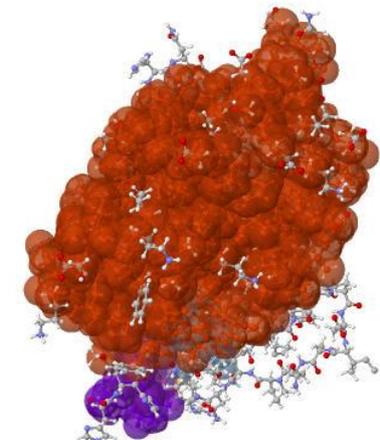
3VZ9 Struct. 3



3VZ9 Struct. 2



3VZ9 Struct. 4



3VZ9 Struct. 5

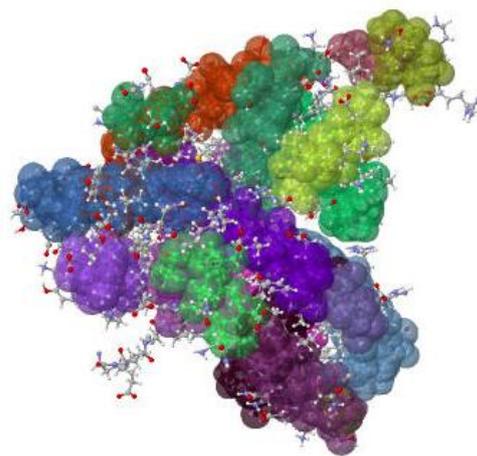
Proof of Concept

In other structures, the rigidity varied **greatly** across the 10 studied conformations

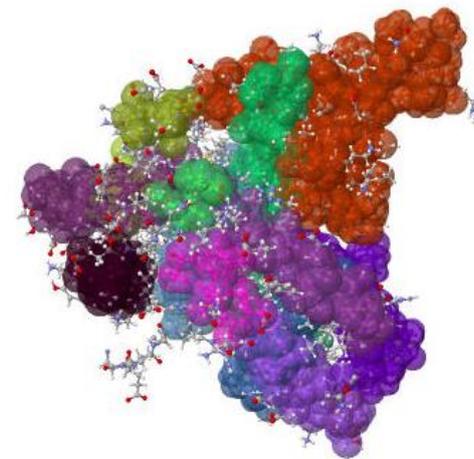
Proof of Concept

In other structures, the rigidity varied **greatly** across the 10 studied conformations

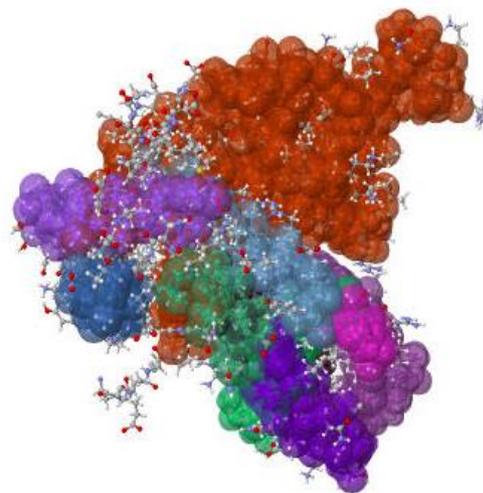
3VB2 LRC variance : 0.82



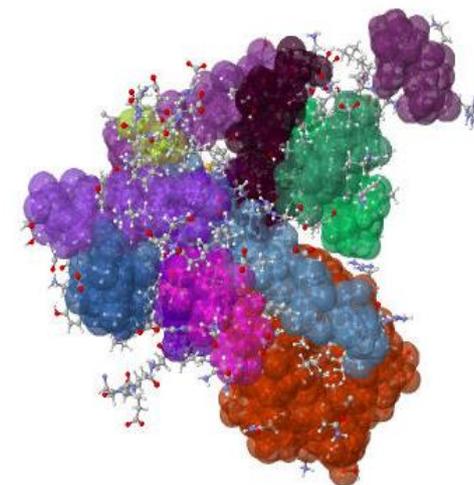
3VB2 Struct. 0



3VB2 Struct. 2



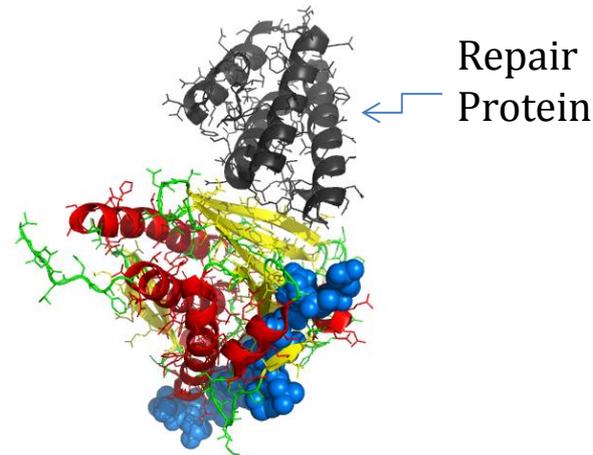
3VB2 Struct. 1



3VB2 Struct. 6

Case Study 1

- Detailed visual analysis of 4EXT

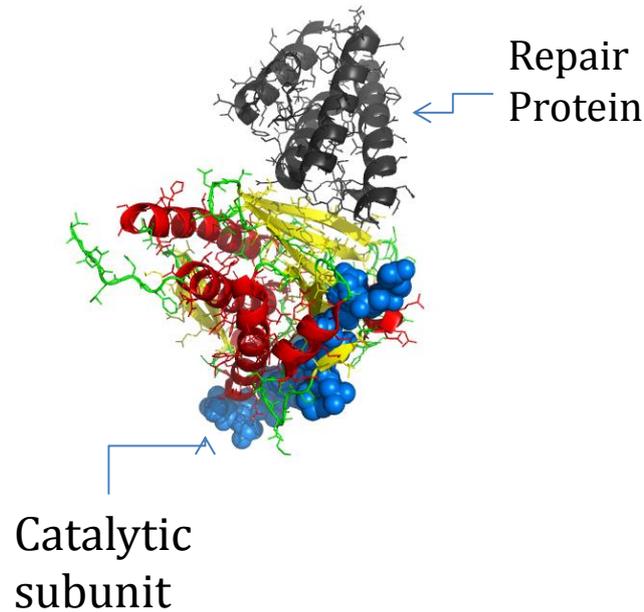


DNA repair protein RVA1 (black) is thought to play a scaffold role in which it recruits DNA polymerase to help repair damaged nucleic acid bases

Lin W, Xin H, Zhang Y, Wu X, Yuan F, Wang Z (Dec 1999). "The human REV1 gene codes for a DNA template-dependent dCMP transferase". *Nucleic Acids Res* 27 (22): 4468–75. doi:10.1093/nar/27.22.4468. PMC 148731. PMID 10536157.

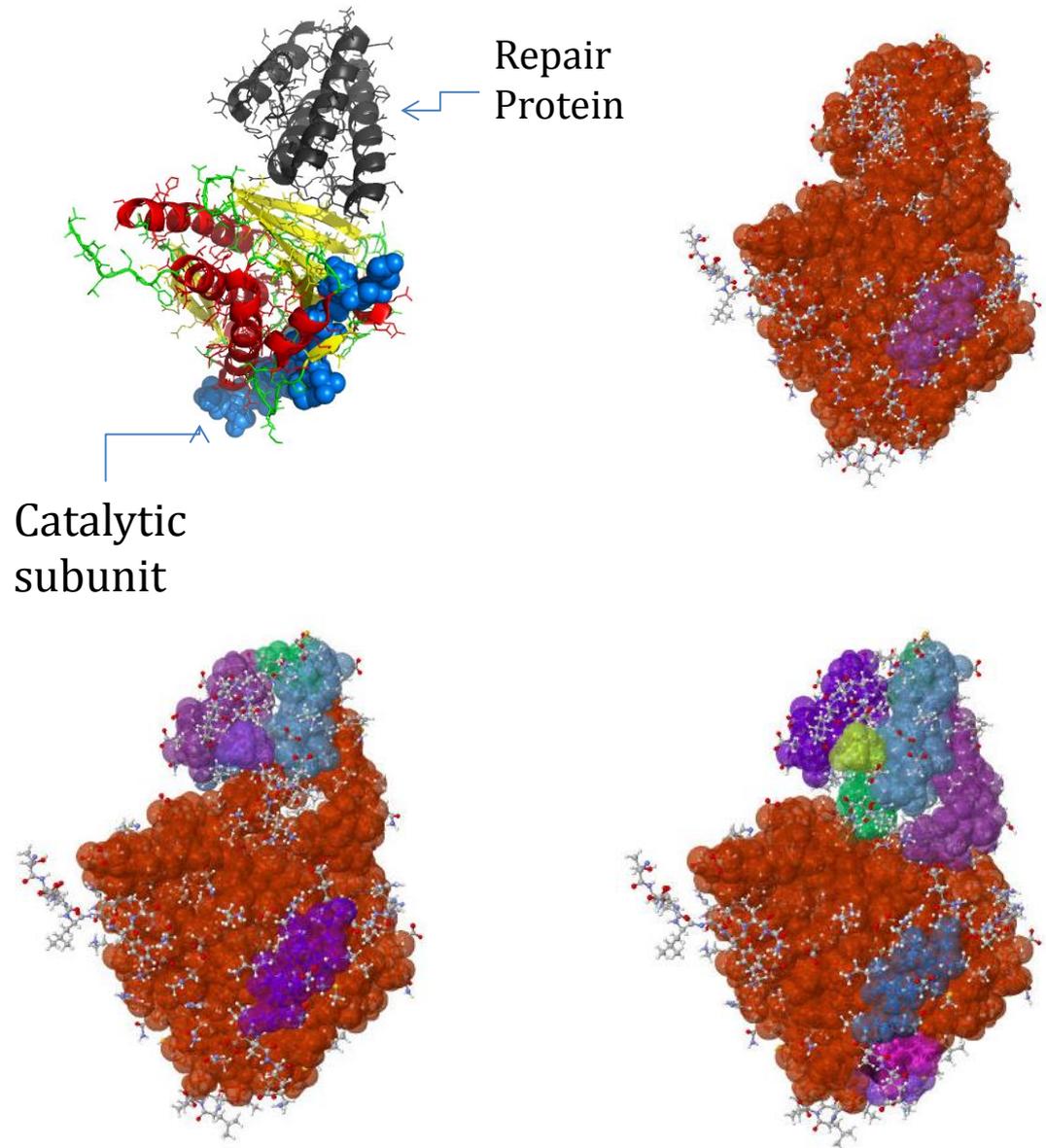
Case Study 1

- Detailed visual analysis of 4EXT
- Catalytic subunit (blue) is closely associated with the repair protein (black)



Case Study 1

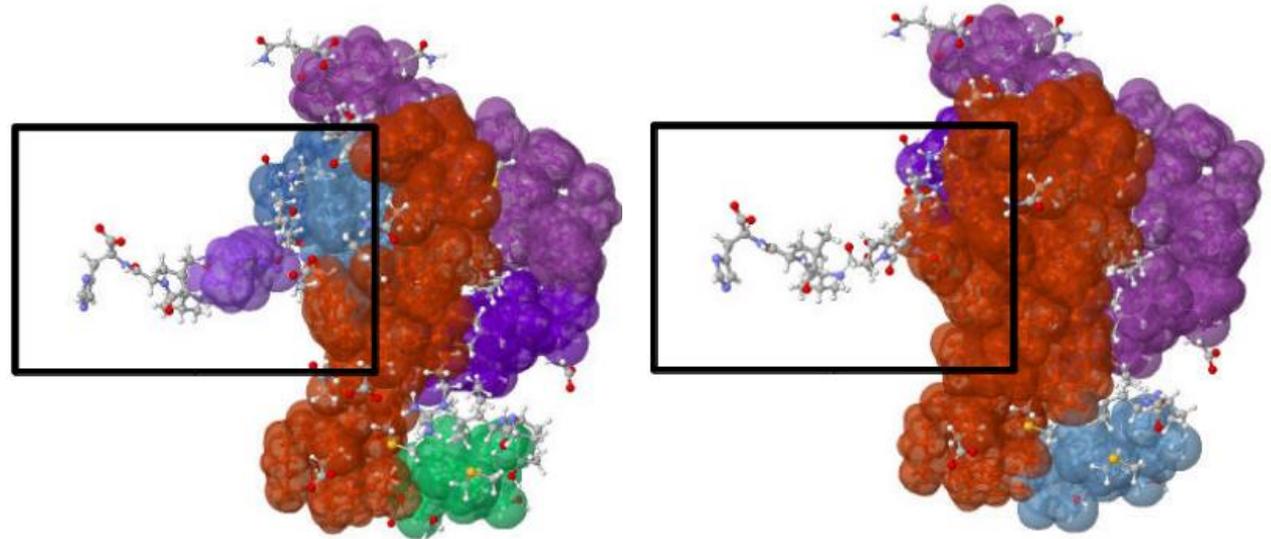
- Detailed visual analysis of 4EXT
- Catalytic subunit (blue) is closely associated with the repair protein (black)
- The combined MD, rigidity analysis approach provides previously unavailable information



Case Study 2

- Detailed visual analysis of 3PTH

The La family of proteins is characterized by a nuclear phosphoprotein that recognizes newly synthesized RNA. Structure 3PTH contains a synthetic construct (yet unpublished) coupled with LARP4B.



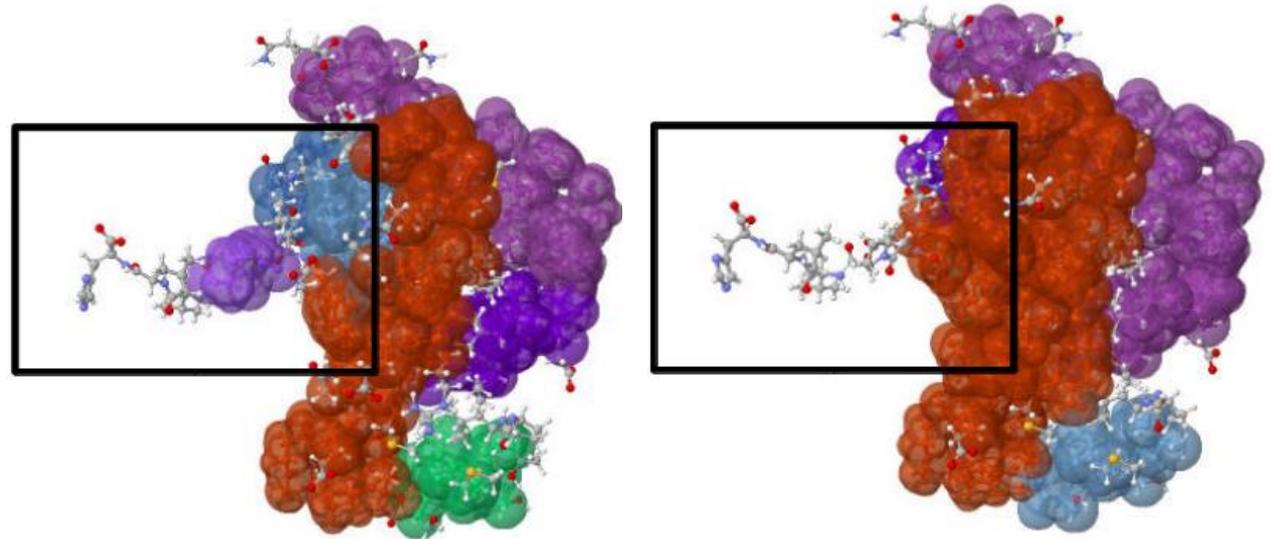
3PTH Struct. 1

3PTH Struct. 2

Dong G, Chakshusmathi G, Wolin SL, Reinisch KM. Structure of the La motif: a winged helix domain mediates RNA binding via a conserved aromatic patch. *EMBO J.* 2004 Mar 10;23(5):1000-7. Epub 2004 Feb 19.

Case Study 2

- Detailed visual analysis of 3PTH
- Ligand has tenuous effect on the protein's rigidity
- Again, the combined MD, rigidity analysis approach provides previously unavailable information



3PTH Struct. 1

3PTH Struct. 2

Conclusion

Our combined MD, rigidity analysis approach provides information that neither method (rigidity analysis of single PDB structure, or full-length MD run) alone can provide about the rigidity and flexibility of the studied protein complex

Conclusion

Our combined MD, rigidity analysis approach provides information that neither method (rigidity analysis of single PDB structure, or full-length MD run) alone can provide about the rigidity and flexibility of the studied protein complex

Future Work

Reproduce results for 20, 30, etc. MD generated conformations of each structure, to determine an optimal run-time of MD simulation

Measure complex flexibility using a metric other than Size of Largest Rigid Cluster

Incorporate the entire combined MD, rigidity analysis method into a freely-available online tool

Thank you

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