Explicit modeling of binding partners to investigate the intrinsic dynamics and modulation of protein oligomerization

Sandhya P. Tiwari[a] , Bhaskar Dasgupta[b]

[a] Institute of Protein Research, Osaka University, 3-2 Yamadaoka, Suita-Shi, Osaka 565-0871, Japan

[b] Research Center for Advanced Science and Technology, University of Tokyo, 4-6-1 Komaba, Meguro-ku, Tokyo 153-8904, Japan

Motivation

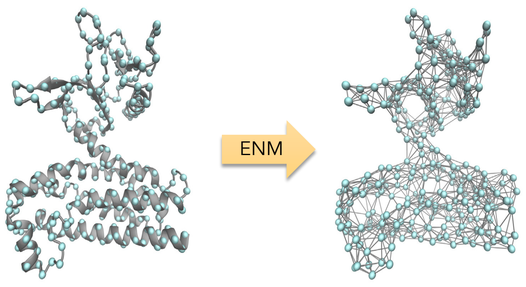
Protein-protein interactions are important building blocks for understanding complex cellular processes

PPIs emerge as a complicated network of interactions as part of the "interactome"

Targets for drug therapy, rational design of large macromolecular assemblies

Regulation of PPI often involve modulation of intrinsic flexibility of proteins

Elastic Network Models



Introduced by Tirion, ENMs are a simple way to describe the intrinsic flexibility of proteins. ENMs are well suited for larger assemblies via coarse-graining. Wide variety of applications, including comparative analysis of dynamics.

Fuglebakk, E., et al. Biochim Biophys Acta General Subjects (2015), 1850(5), 911-922

Formalism

For N number of atoms, the total potential energy is defined as the sum of individual Hookean oscillators between all pairs of atoms (*i* and *j*):

$$U = (\frac{K}{2}) \sum_{i,j}^{N} c_{i,j}(d_{i,j} - d_{i,j}{}^{(0)})2$$

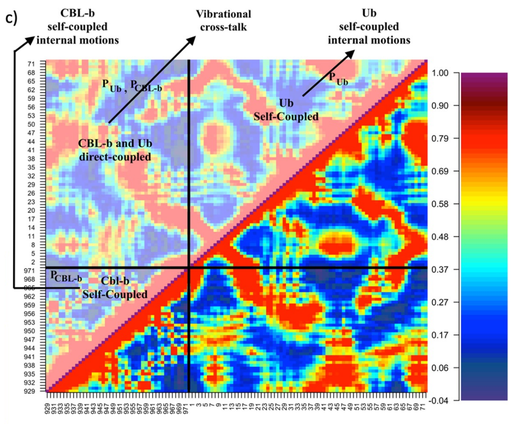
where *K* is a constant, c_{ij} is the force constant, d_{ij} is the pairwise distance and d_{ij} is the pairwise equilibrium distance in the structure's native conformation.

The partial derivatives of the potential constitute a 3*N* x 3*N* Hessian

Hessian can be diagonalized to retrieve eigenvalues ~ frequencies, eigenvectors ~ normal modes

For the purposes of reducing the size of the Hessian, coarse-graining can be implemented e.g. C-alpha atoms only.

Modelling coupling using ENMs



- Correlation matrix from the normal modes of AB heterodimer can be split into Ub and Cb parts.
- Remaining rectangular part of the matrix includes directly coupled motions.
- To obtain vibrational signatures from self- and directly coupled submatrices, we use their projection matrices

Dasgupta, B & Tiwari, S.P.
Biophysical Reviews 2022, **(14)**:1379–1392

Implicit vs. Explicit consideration of coupling

AB Heterodimer	
Without separating A and B:	**By separating A and B:**
self-couped motion Of A Of B	Motion of A with implicit B Motion of B with implicit A
Directly-coupled motion Between A and B	

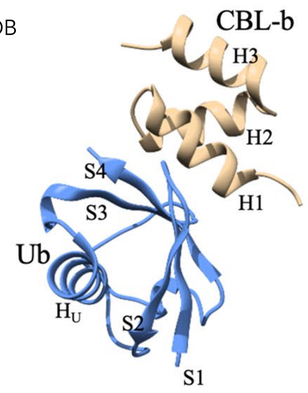
Explicit consideration / Implicit consideration

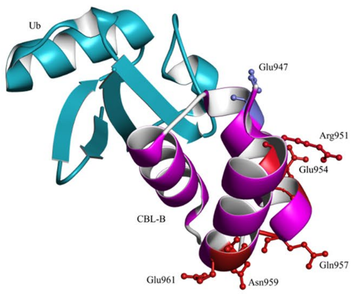
Future plans

- *Comparisons across self-coupled motions and direct motions*
- *Validation against more detailed dynamics modelling*
- *Scaling up analysis to large structural dataset*

Examining the dynamics of Ubiquitin-CBL-b complex

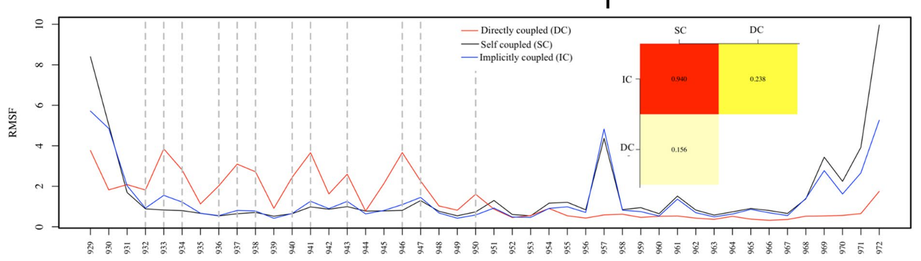
PDB 2OOB



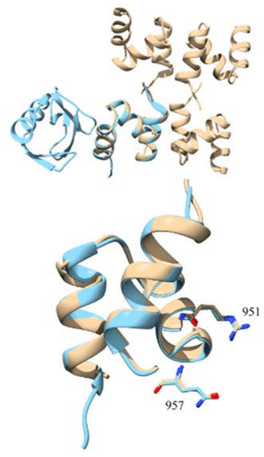


The residues that show high RMSF in the SC profile are indicated as red sticks. Glu957 of CBL-b is close to the binding interface.

Ub-CBL-b coupled motions

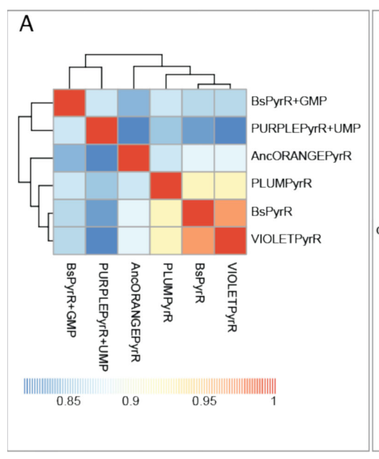


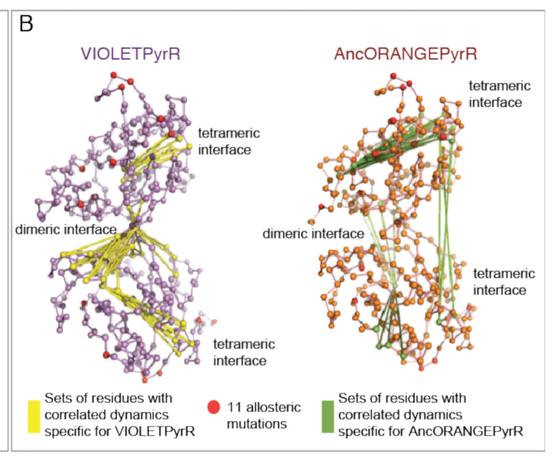
Root-mean-square fluctuations (RMSFs) averaged over all residues for Cb are compared for directly coupled, self-coupled, and implicitly coupled motion.



Here, implicit modeling was able to capture the high fluctutation of Arg951 and Glu957, corresponding to residues involved in the hypothetical oligomer model of Ub (above).

Modeling larger complexes, datasets – PyrR proteins





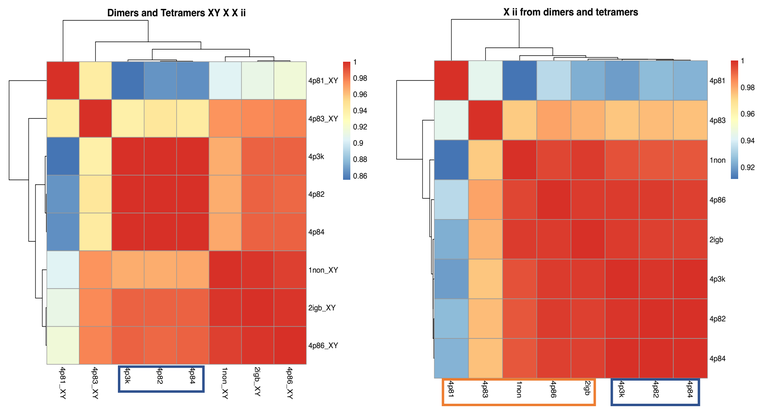
Correlation differences

The sets of residues with correlation differences shown here have a cluster size of more than three (corresponding to patches on the difference map) and fall within the correlation difference threshold of 0.1.

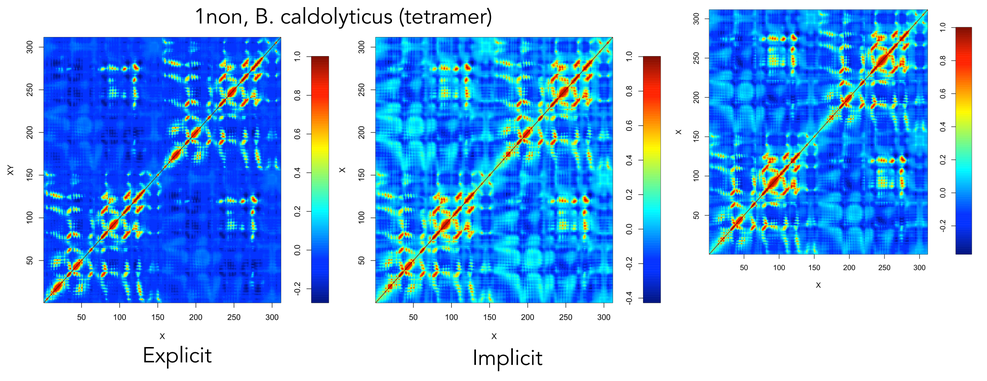
Analysis performed on dimer units only!

Perica et al, Science, 2014,, 346(6216): 1254346.

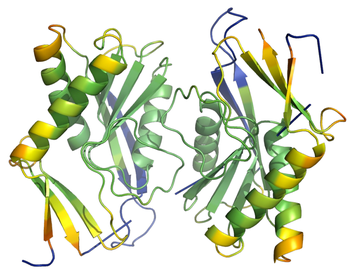
Global similarity (Bhattacharyya coefficient) self-coupled motions vs. implicit



Correlations (tetramers): self-coupled motions with Y vs. without Y

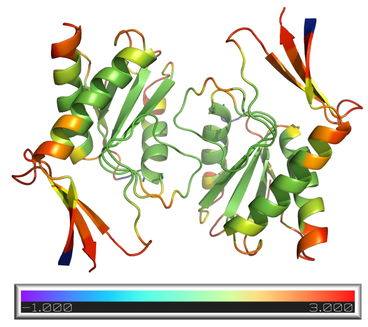


Root mean squared fluctuations



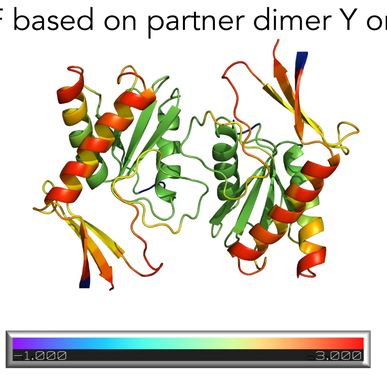
Dimer 4p82 (B. subtilis)

RMSF based on dimer unit X alone



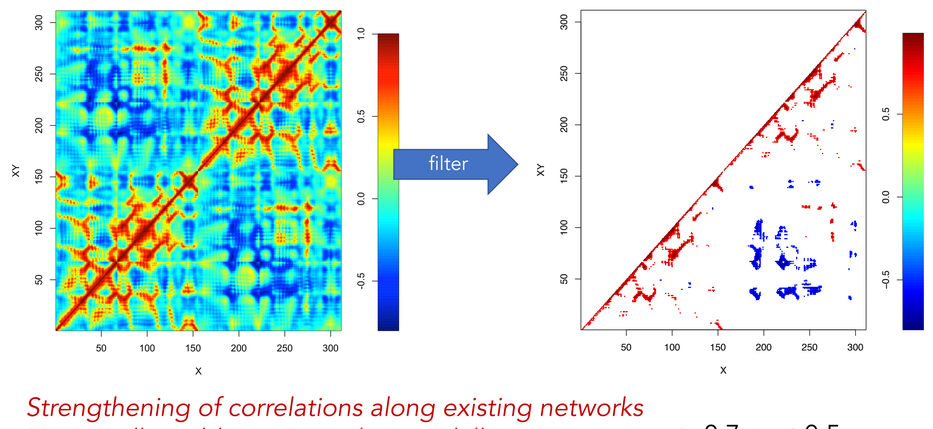
Tetramer 1non (B. caldolyticus)

RMSF based on partner dimer Y on X



Predicts the span of the tetrameric interface in more detail

Correlations from X-Y coupled motions



Strengthening of correlations along existing networks
Not usually visible using implicit modelling

> 0.7 or <-0.5

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