



# Probing the Dynamics of Scapharca Dimeric Hemoglobin with Normal Mode Analysis



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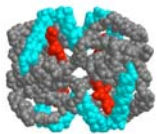
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## Abstract

Proteins are essential components of all cellular processes. Hemoglobin is one of the most well studied proteins and is the standard model for allosteric regulation. Allostery is an important, yet poorly understood, component of biochemistry. Scapharca dimeric hemoglobin is one of the simplest models to study as its protein structure contains only two ligand binding sites. Using eINémo, an elastic rod modeling program, a normal mode analysis (NMA) was performed to predict the dynamics of Scapharca dimeric hemoglobin upon ligation. The data from mode 20, and other low frequency modes, correlates with previous crystallographic data. This indicates that NMA may be a useful tool for predicting conformational and explaining allosteric regulation at the molecular level.

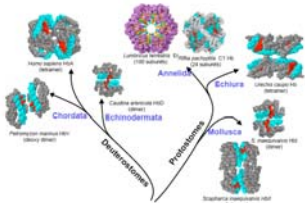
## Hemoglobin: The model of allostery



(Shaanan, 1983)

- Traditional physiological role of hemoglobin is oxygen transport
- Human hemoglobin (HbA) is a tetrameric protein with two different subunits, alpha and beta, and contains four heme prosthetic groups
- Upon binding oxygen, HbA undergoes a structural change from the T (tense) state to the R (relaxed) state
- To efficiently bind oxygen, HbA changes its conformational state upon ligand binding from T to R
- HbA is an allosteric protein; the binding of a ligand (oxygen) at one heme group affects the binding properties of the other heme groups<sup>1</sup>

## Hemoglobin Phylogenic tree

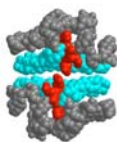


(Royer, et al., 2006)

- Hemoglobins are the most widespread oxygen-carrying molecules found in all five kingdoms of life
- All hemoglobin proteins share a common tertiary structure
- Amino Acid residues are conventionally designated by the homologous helical (A-H) or corner (AB-GH) in the sperm whale myoglobin
- Tertiary structural similarities suggest that all hemoglobins are evolutionarily related
- The different assemblies of vertebrate and invertebrate hemoglobins strongly argue for the evolutionarily independent acquisition of cooperativity<sup>2</sup>

The tetrameric and dimeric hemoglobin proteins are shown with the heme groups colored red, the E and F helices in cyan and the rest of the main chain in gray. The Lumbricus hemoglobin subunits are colored magenta and the linker regions blue and gold.

## Scapharca dimeric hemoglobin (HbI)



(Royer, et al., 2006)

- Scapharca dimeric hemoglobin (HbI) is one of the simplest models for allosteric regulation
- Upon ligand binding, small subunit rotations occur
- Communication between the subunits occurs at the EF helical interface
- R state binds oxygen an estimated 300 times more than the T state<sup>3</sup>

## Normal Mode Analysis (NMA)

- Normal Mode Theory is based on the harmonic approximation of the potential energy function around an energy minimum
- Describes the collective motions of coupled atoms
- Appropriate for studying fast motions in molecules
- Time scales of motions are less than the residence time in an energy minimum<sup>3</sup>
- NMA can be used to predict protein motions that have relevant biological functions
- Lowest frequency modes correspond to large domain motions - biological functions
- Useful when structural dynamics data does not exist
- Over 1,700 proteins have had their motions accurately predicted by NMA

$$\text{Harmonic potential energy function: } E = E_0 + \sum_i \frac{\partial E}{\partial x_i} \Delta x_i + \frac{1}{2} \sum_{i,j} \frac{\partial^2 E}{\partial x_i \partial x_j} \Delta x_i \Delta x_j$$

## eINémo : An Elastic Rod Model Program

- eINémo is a web interface to an elastic rod model program
- Protein structures are modeled as elastic rods with nodes at the alpha carbons of amino acids
- NMA is then performed on the model to compute the normal modes of the protein structure using a simplified Hookean potential
- Using the starting structure as a reference, the state of energy minimization is not necessary<sup>4,5</sup>

Simplified Potential

$$E_p = \sum_{i,j} c(d_{ij} - d_{ij}^0)^2$$

$$E_s = 0 \quad R_s = 8 - 13$$

Collectivity

$$K_s = \frac{1}{N} \exp \left( - \sum_{i,j} \alpha \Delta A_{ij}^2 \log \alpha \Delta A_{ij}^2 \right)$$

Displacement of alpha carbon atoms

$$r_i(t) = \frac{1}{\sqrt{m_i}} \sum_k C_{ik} a_{ik} \cos(\omega_k t + \phi_k)$$

Overlap

$$I_s = \frac{\left| \sum_{i,j} a_{ij} \Delta r_i \right|}{\left| \sum_{i,j} a_{ij}^2 \sum \Delta r_i^2 \right|^{1/2}}$$

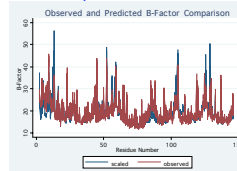


(Tama and Sanejouand, 2001)

## NMA Results

- Protein Data Base (PDB) files submitted to eINémo : 3SDH (HbI in R state) and 4SDH (HbI in T state)
- The first 100 modes were calculated for HbI
- Thermal (B) factor correlation was identified
- The R.M.S.D. values were compared with the crystallographic data
- Mode 20 showed the most relevant motions seen in crystallographic

## B-factor prediction



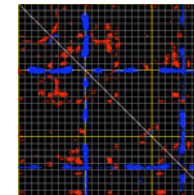
Correlation between B factor computed by NMA and observed B factor from crystallographic data R = 0.674 for 290 alpha carbon atoms

## NMA Results

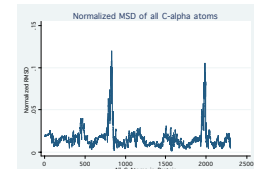
Properties of Mode (k) 20

Frequency	Overlap	Collectivity	Amplitude
$\omega_k = 2.48$	$I_k = 0.426$	$K_s = 0.3429$	$C_k = -215.4607$

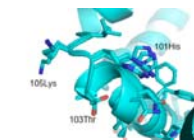
Distance fluctuation map\*



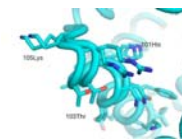
R.M.S.D. values for alpha carbons



F Helix (R and T states)



Crystallographic structures



NMA transition prediction

- Largest R.M.S.D. values occur within the F Helix (residues 88-104)
- Greatest residue fluctuations appear at residues 90-106 and at the neighboring residues in each subunit
- Distance fluctuation map shows symmetry between subunits
- Shift in the F-helix residues (alpha carbons) in the NMA correspond to the shifts from the crystallographic structures

## Conclusions

- NMA is useful tool for studying motions in proteins that correspond to biological functions
- Structural transitions in HbI have been predicted using NMA and agree with crystallographic data
- NMA can predict structural transitions from ligation events (Transition to energy minimum which corresponds to R state)

## References

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- 2) Royer, W.E., Jr., et al., *Allosteric hemoglobin assembly: diversity and similarity*. *J Biol Chem*, 2005, **280**(30): p. 27477-80
- 3) Atkins, P.W. and J. De Paula, *Physical chemistry*, 7th ed. 2002, New York: W.H. Freeman, xx, 1139 p.
- 4) Valade, H., et al., *Dynamical properties of the MsL of Escherichia coli: a normal mode analysis*. *J Mol Biol*, 2003, **332**(3): p. 657-74
- 5) Suhre, K. and Y.H. Sanejouand, *eINémo: a normal mode web server for protein movement analysis and the generation of templates for molecular replacement*. *Nucleic Acids Res*, 2004, **32**(Web Server issue): p. W510-4.
- 6) Royer, W.E., Jr., *High-resolution crystallographic analysis of a co-operative dimeric hemoglobin*. *J Mol Biol*, 1994, **235**(2): p. 657-81

\* This correlation matrix displays the maximum distance fluctuations of all pairs of alpha carbons and between the two extreme conformations computed for k=20. Distance increases are plotted in blue and decreases in red for the strongest 10% of the residue pair changes. Gray lines are drawn every 10 residues and yellow lines every 100 (counting from the upper left corner).