How Compressible is a Protein?

Compressibility is a measure of the physical state of a protein and has been shown to change during the unfolding of various proteins including mitochondrial protein cytochrome c. For this reason, when researchers from Argonne, HASYLAB, and Northeastern University developed a new method for determining protein compressibility, they used cytochrome *c* as a model. Their method involves the use of the inelastic x-ray scattering (IXS) and nuclear resonance vibrational spectroscopy (NRVS) techniques, available on XSD beamline 3-ID end stations C and D at the APS, respectively. This method provides a global measurement of protein compressibility at ambient pressure and gives a more native state result than other methods due to its use of the natural iron-containing heme group associated with the protein. The method is accurate in that it is in agreement with molecular dynamics simulations and will be widely applicable to other materials where compressibility is an important measure of structure or function.

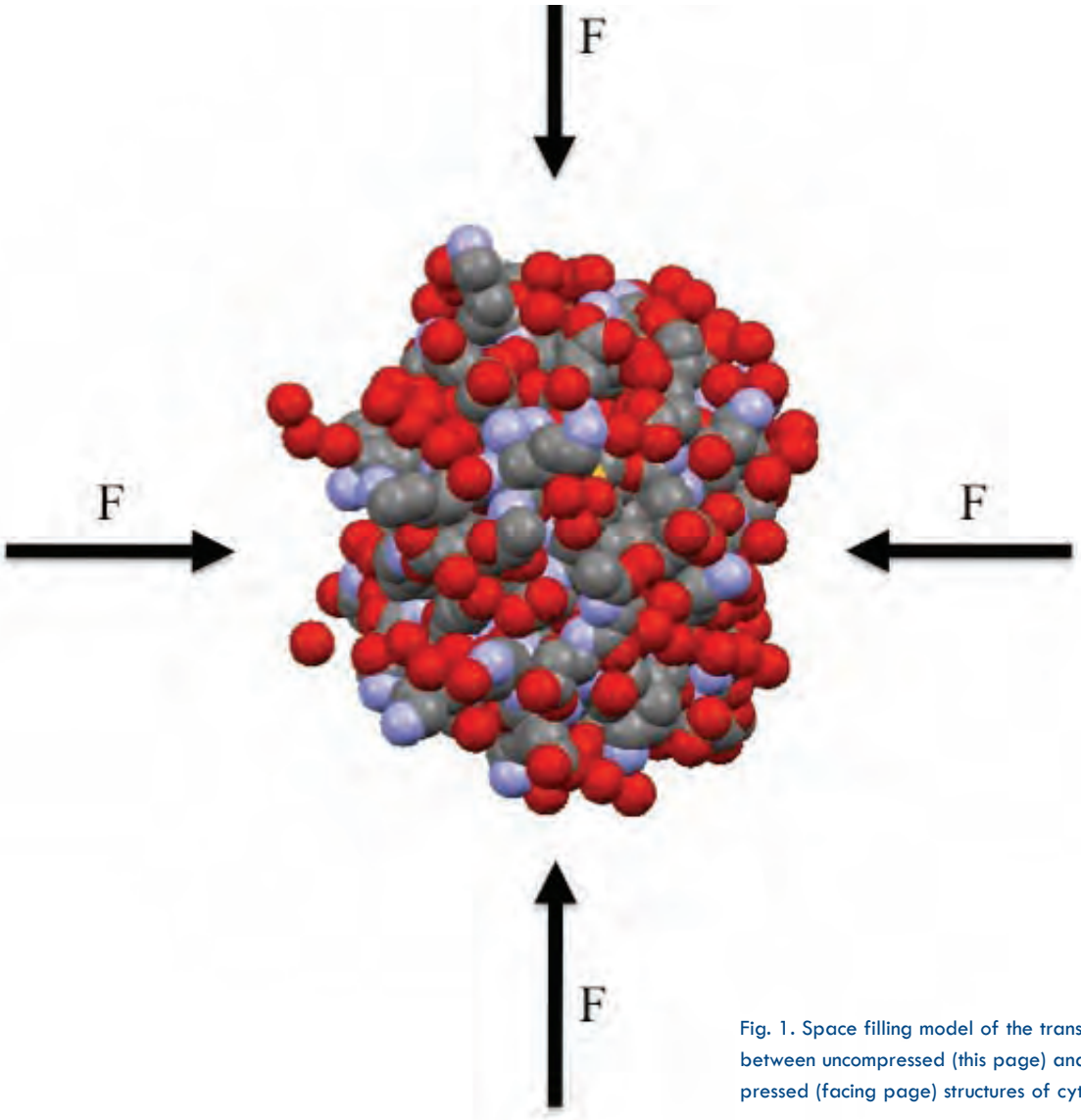

Fig. 1. Space filling model of the transition between uncompressed (this page) and compressed (facing page) structures of cytochrome c.

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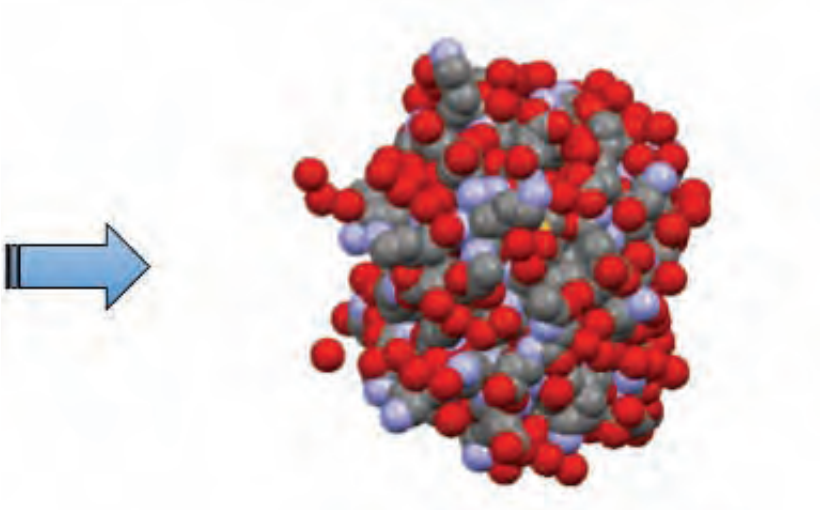
The role of protein unfolding has only recently come to light as an important factor in the way cells regulate their life and death decisions. Conventional thinking subscribes to the idea that the amino acids in a protein interact in a complex manner to fold the polypeptide chain into a final, stable configuration and that this is the three-dimensional structure that carries out the business of that protein. Now, however, evidence suggests that proteins can adopt different folded or partially unfolded states that are active in various roles. The mitochondrial protein



cytochrome *c*, for example, appears to play a role in both electron transport, which generates energy for a living cell, and also in apoptosis, a cell's decision to undergo programmed cell death. These two apparently opposing functions may be mediated by differently folded or partially unfolded states of cytochrome *c* within a cell. Furthermore, in different species, small variations in the structure of cytochrome *c*, seem to be responsible for whether programmed cell death occurs. This suggests that differently folded states may have different activities in cells. Since cytochrome *c*'s compressibility has been shown to change during unfolding, the research team decided it would be a good model for their new compressibility measurement technique.

The strength of the method comes from using both NRVS and IXS to make the compressibility measurements. NRVS reveals the complete vibrational spectrum of a probe nucleus, in this case the targeted nucleus is the heme iron, ^{57}Fe. The vibrations of other atoms in the molecule are ignored by this method. Measurements using the low-energy part of the NRVS spectrum correspond to collective motions from which the Debye sound velocity is determined. Information from the IXS spectrum is used to determine the longitudinal sound velocity of the protein. Together, the Debye sound velocity and the longitudinal sound velocity are used to calculate compressibility of the protein by a series of related equations. From this, changes in the protein's volume and the motion of atoms in the protein can be determined.

Using these techniques, the researchers were able to measure the adiabatic compressibility of hydrated horse heart Fe (III) cytochrome *c* (Fig. 1). Their results show that this method agrees very well with dynamic simulations of compressibility proving the usefulness of the method and its sensitivity. Now that they have shown the method works with proteins, the team will move on to other materials to demonstrate the almost limitless potential of their method. — *Sandy Field*

See: Bogdan M. Leu[1]*, Ahmet Alatas[1], Harald Sinn[2], E. Ercan Alp[1], Ayman H. Said[1], Hasan Yavaş[1], Jiyong Zhao[1], J. Timothy Sage[3], and Wolfgang Sturhahn[1], "Protein elasticity probed with two synchrotron-based techniques," J. Chem. Phys. **132**, 085103 (2010). DOI:10.1063/1.3332585
Author affiliations: [1]Argonne National Laboratory, [2]HASYLAB, [3]Northeastern University
Correspondence: *leu@aps.anl.gov

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