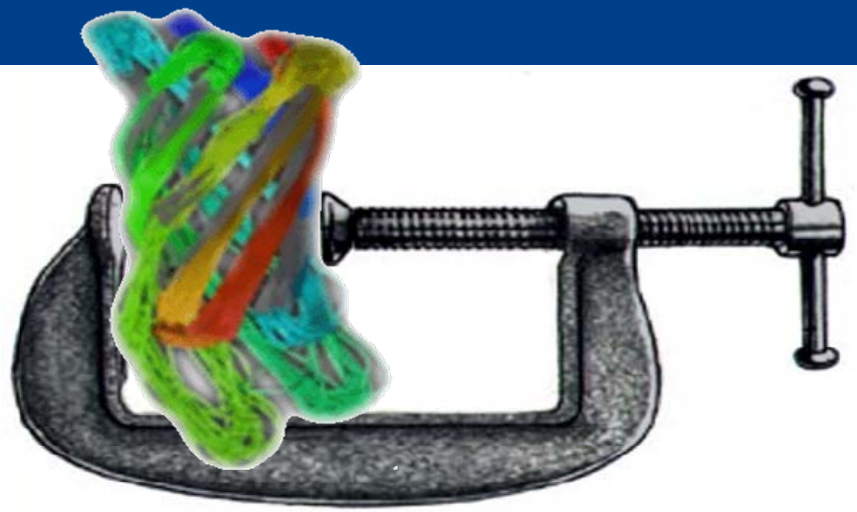

High Pressure in Life Sciences – Trends and Future Opportunities

Alexander Lazarev, PhD
Pressure BioSciences, Inc.

History of High pressure in Life Sciences

- 1623-1662: Blaise Pascal – described fundamental concepts of pressure and vacuum.
- 1895: H. Royer – pressure kills bacteria
- 1899: B.H. Hite *et al*. – pressure preserves milk
- 1914: P.W. Bridgman - pressure coagulates egg white
- 1989: High pressure processing of food products
- 1996: First Pressure Cycling Technology patent is filed
- 2000: First International Conference on HPBB
- 2008: First HPBB Conference held in the USA

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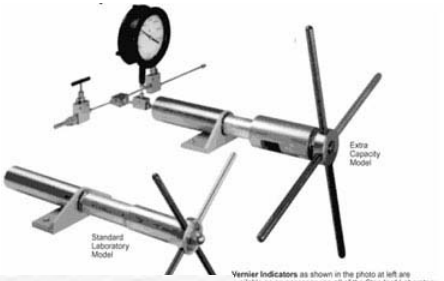
Temperature vs. Pressure

SI50



Vernier Indicators as shown in the photo at left are available as an accessory on all of the Standard Laboratory Models. These indicators provide controlled measurement of column displacement with an accuracy of ± 0.003" movement of stroke.

** If required for GAS application, please consult factory.*

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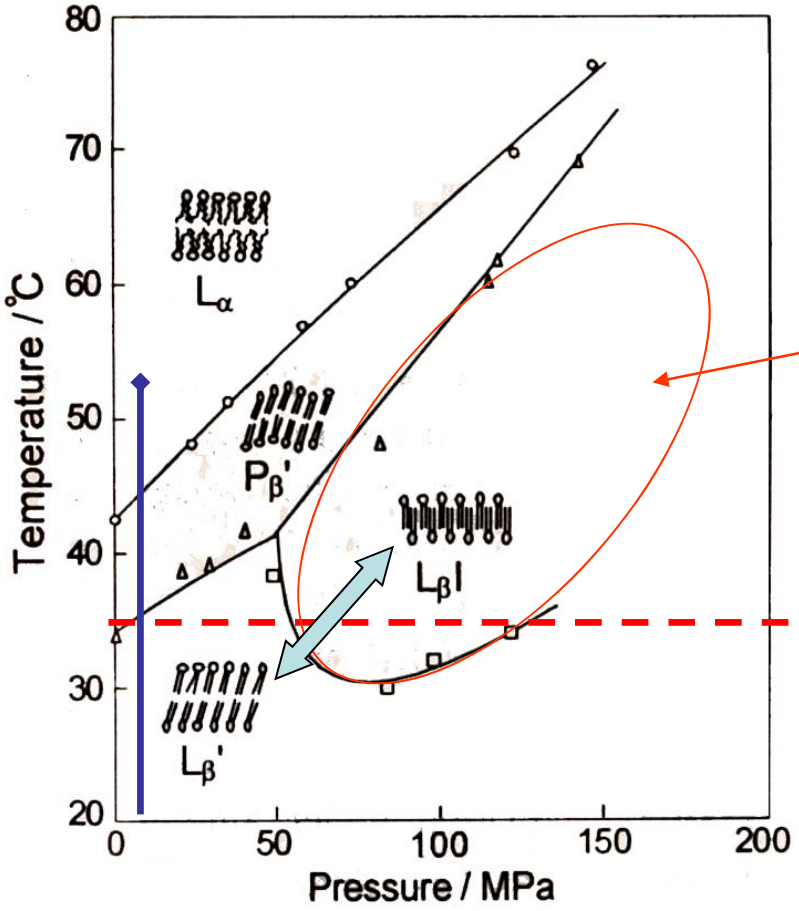
Pressure and Temperature – Two Dimensions in Thermodynamics:



Pressure-induced interdigitation of lipid bilayers in an ester-ester linked HPPC bilayer: HP DSC data.

Interdigitated bilayer

Pressure cycling at 33 ºC

Ichimori H. *et al*., 1999; in: *Advances in High Pressure Bioscience and Biotechnology, Horst Ludwig (Ed.), Proceedings of the Intl. HPBB Conference, Heidelberg, 1998.*

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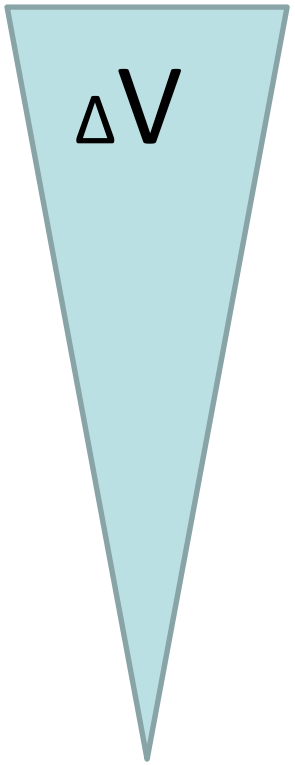
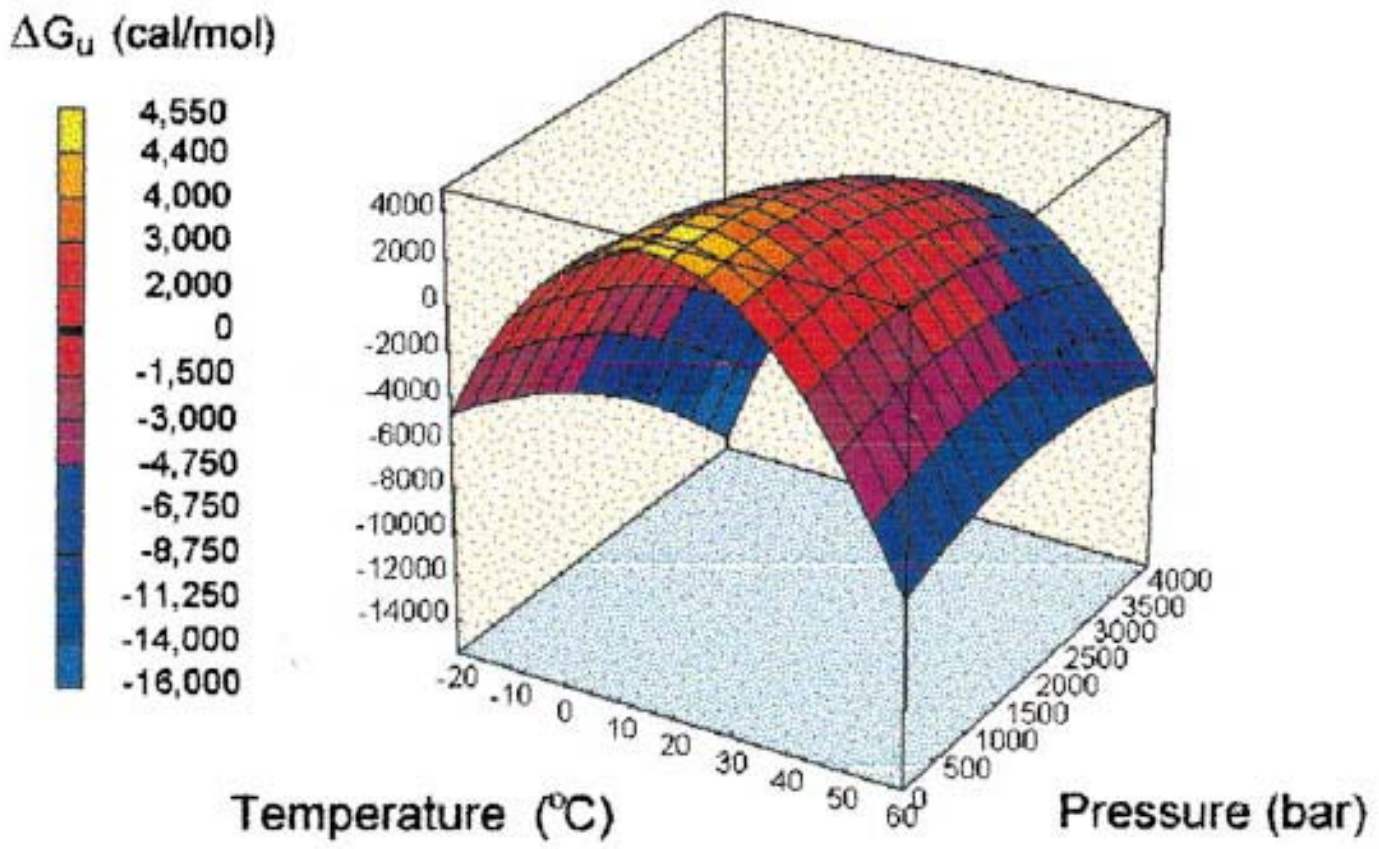
Reaction Activation Volume Drives Pressure Effects

- Protein Dissociation
- Protein Denaturation
- Hydrophobic hydration
- Water dissociation
- Dissociation of COOH and NH_2
- Hydrogen bond formation



Protein “Phase Diagram”

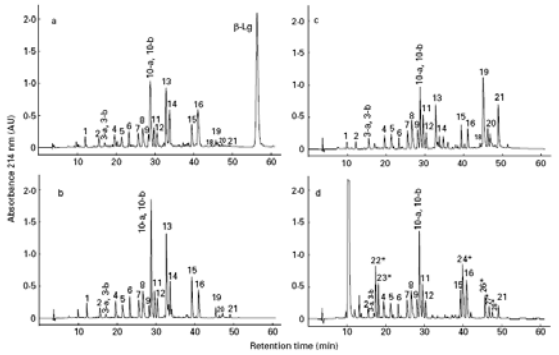
Pressure protects proteins from thermal denaturation



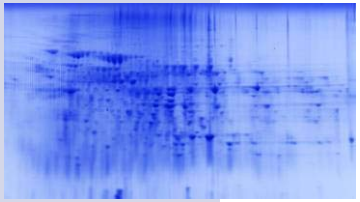
B.B. Boonyaratanakornkit et al. / Biochimica et Biophysica Acta 1595 (2002) 235-249

PCT Sample Preparation Applications

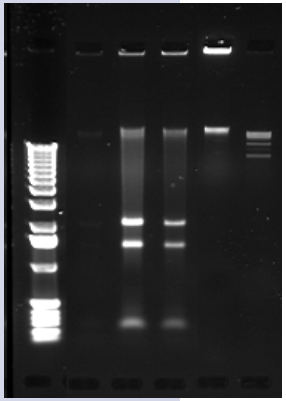
ACCELERATED ENZYMATIC DIGESTION



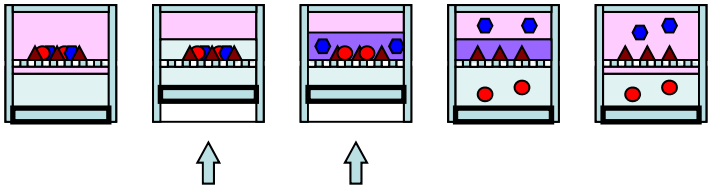
CELL LYSIS



PATHOGEN INACTIVATION



EXTRACTION AND FRACTIONATION DNA, RNA, LIPIDS AND PROTEINS



PATHOLOGY SAMPLE PREPARATION

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New directions

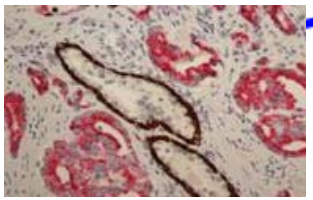
- Enzymatic activity
- Clinical applications
- Automation and miniaturization
- Chemical synthesis
- Differential lysis
- Real-time analytical tools:

  Enzymology

  Structural Biology

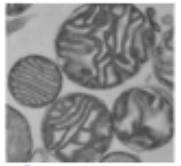
  Material science

Differential Lysis for Organelle Isolation

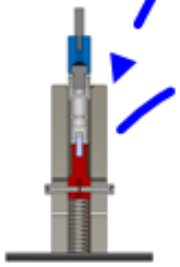


Fresh Tissue Sample

PULSE
Tube
FT-500



Functional
Mitochondria



PCT
Shredder

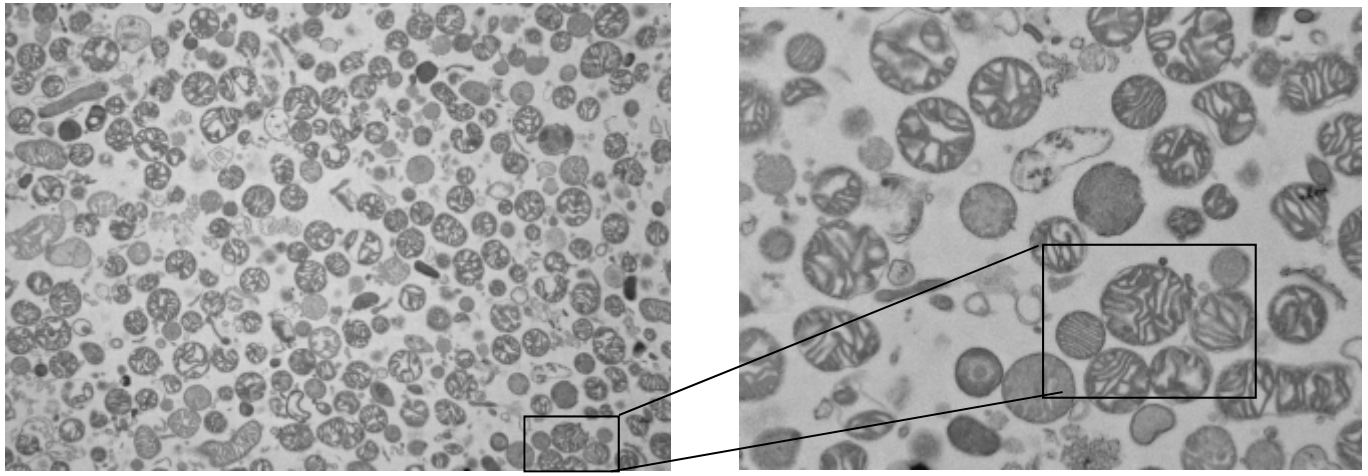
PST SPS

Differential
Centrifugation

The PCT Shredder and/or PCT SPS Replace Manual Methods such as Scissors and Potter-Elvehjem Homogenizers.

Mitochondrial Morphology

Mitochondria isolated from rat kidney
by pressure cycling at 10,000 psi (5 cycles)
exhibit normal morphology consistent with intact mitochondria.

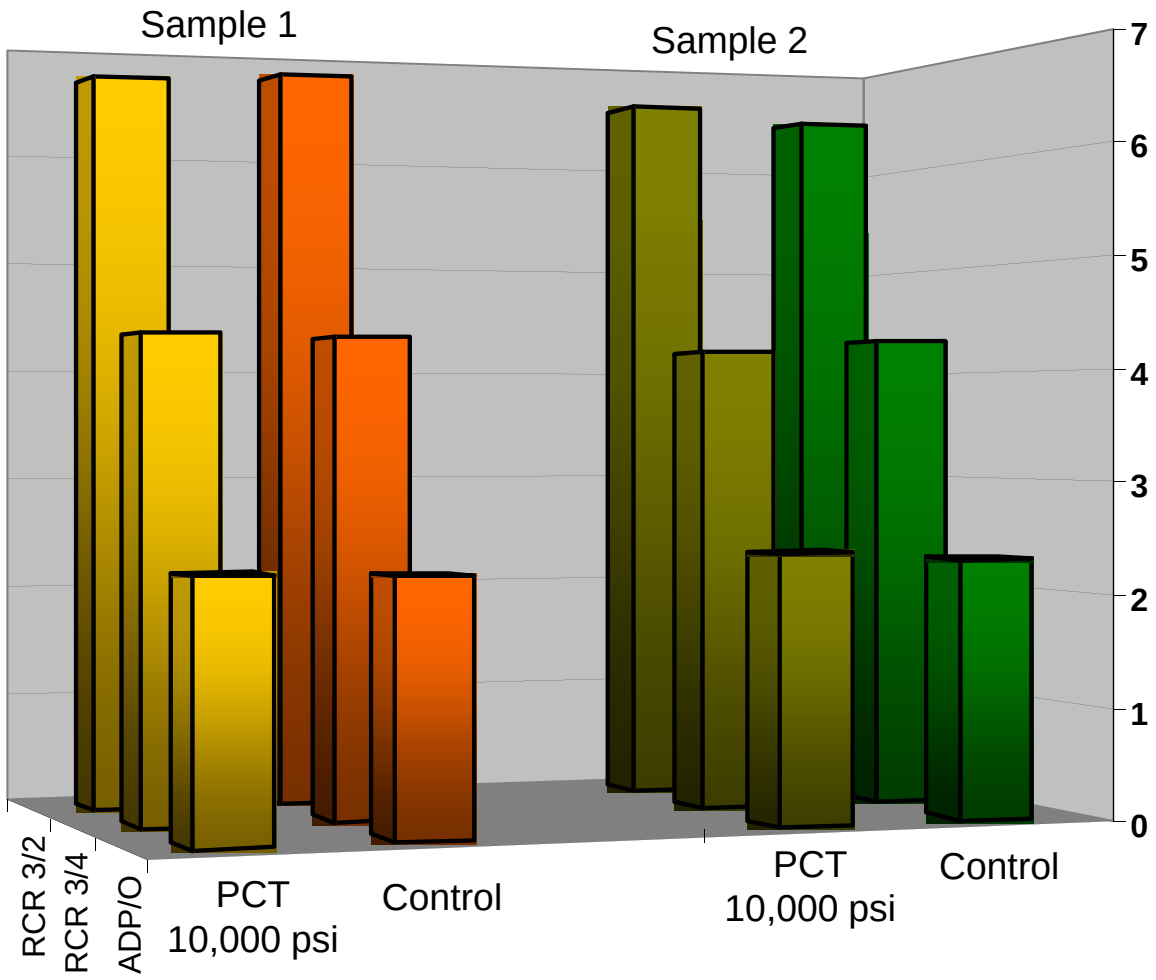


3000x 8000x

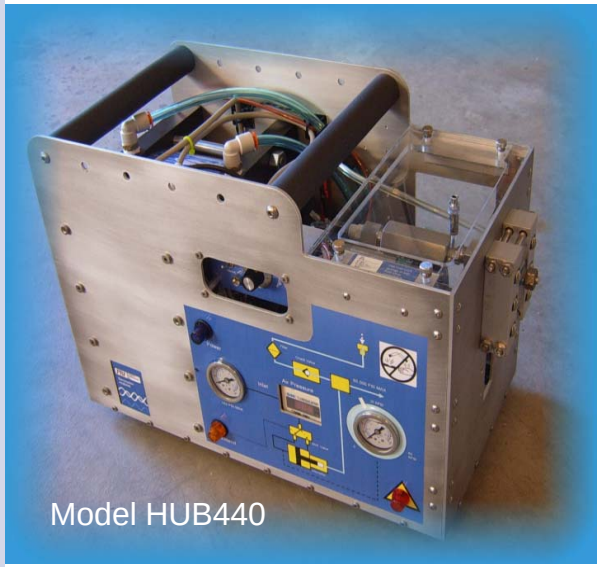
This work is funded by the NIGMS SBIR Phase II grant GM079059

Mitochondrial Function

Mitochondria from Rat Skeletal Muscle Are Not Damaged By Exposure to 10,000 psi for 5 cycles at 4˚C



High Pressure Control Systems for EPR and NMR spectroscopy



Real-time measurements of pressure-induced conformational changes are critical for understanding protein structure and function.

Applications include mechanistic enzymology, structural biology and drug development.

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Applications of Differential lysis

- Sample fractionation by subcellular localization
- Differentiation between cell populations in heterogeneous samples (tissues, stool, environmental samples, etc.)
- Cell biology - targeted impact on certain cell populations
- etc.

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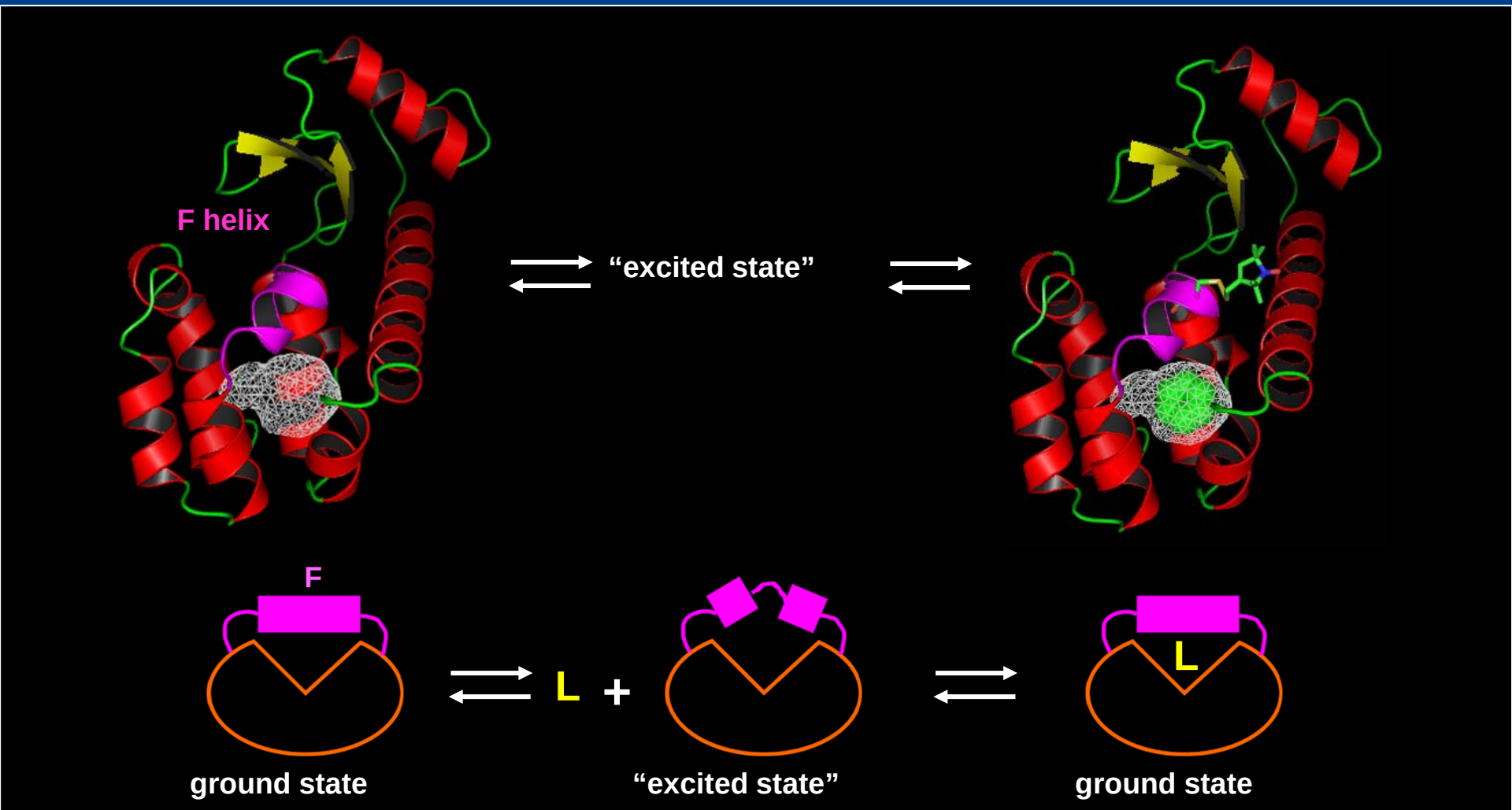
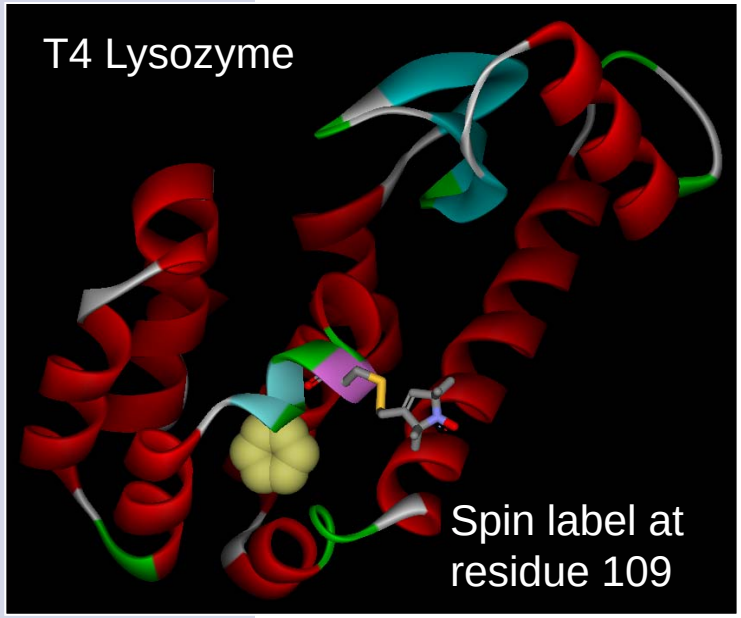
Conformational exchange in a ligand-binding T4Lysozyme mutant (L99A)

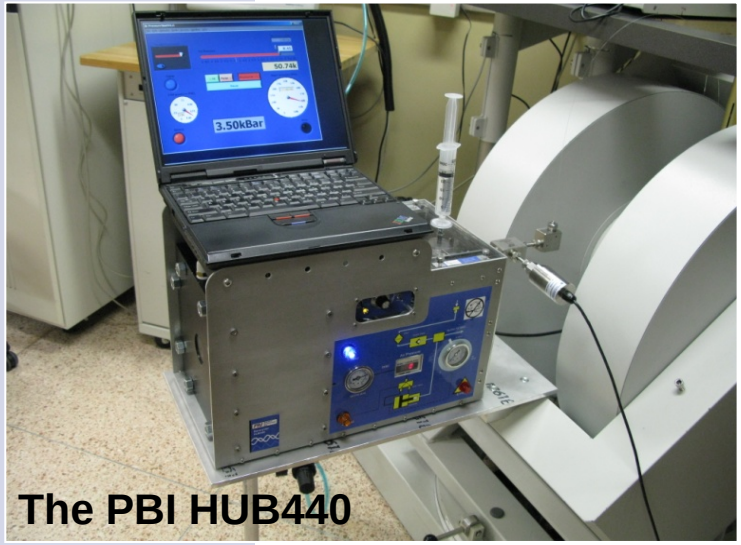


(~3%; NMR)

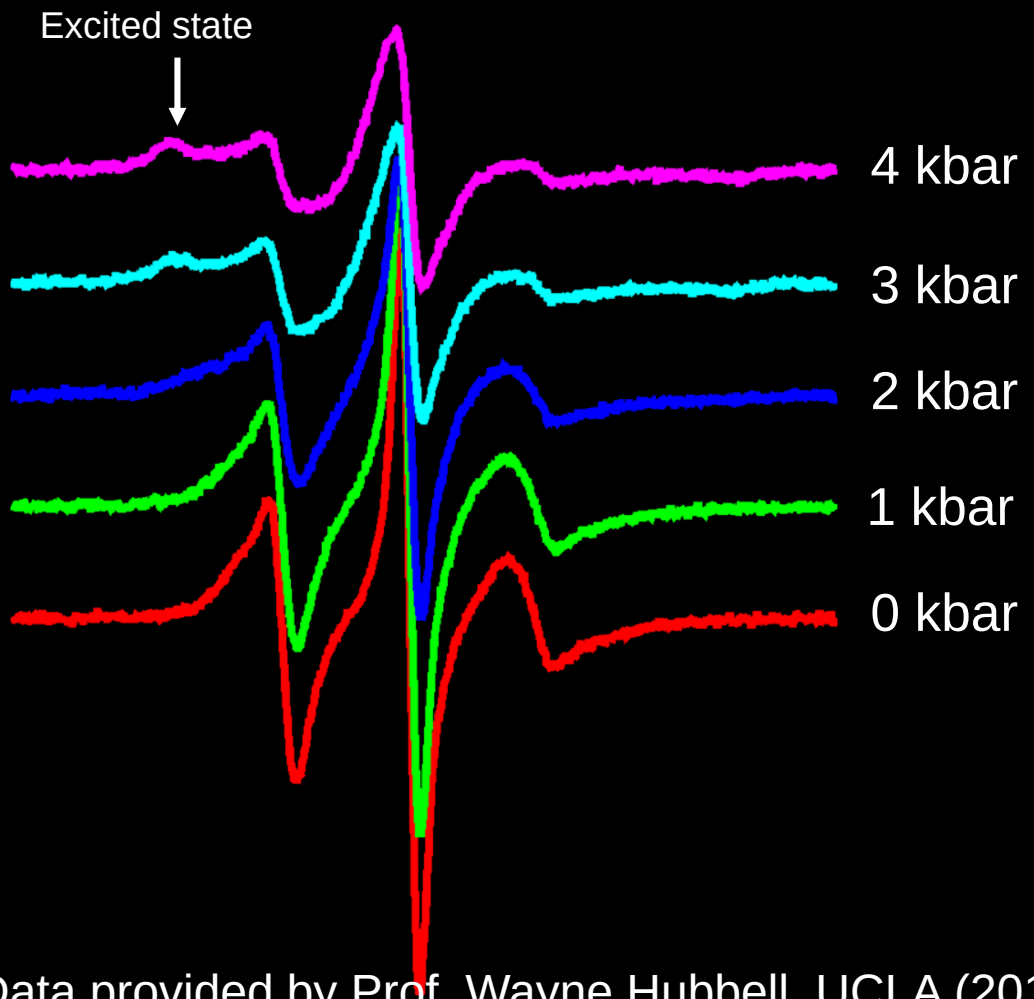
Mulder et al (2001) *Nature*, 8:932

Pressure reveals an “Excited State” of a benzene-binding cavity mutant of T4 Lysozyme





EPR spectra of 109R1 in T4 Lysozyme as a function of pressure generated by HUB440



Data provided by Prof. Wayne Hubbell, UCLA (2010)

Conclusions

•High hydrostatic pressure is a new dimension in biomedical research

•Availability of commercial high pressure equipment drives new research

•High pressure presents attractive opportunities for discovery and innovation

•Widespread adoption of new technologies is impossible without collaborations

THANK YOU!

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Acknowledgements

PBI R&D	Vera Gross Greta Carlson
BWH/HMS	Bruce Kristal Irina Stavrovskaya Sergei Baranov
UCLA	Wayne Hubbell John McCoy
NIH	NIGMS SBIR grant GM079059