Real time high pressure optical spectroscopy in biochemical studies: Pressure perturbation of pH and enzymatic activity

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PBI Pressure BioSciences Inc.

PBI Pressure BioSciences Inc.

- Developing expertize in high pressure equipment and applications
- Engaging academic collaborators to explore new applications
- Looking for industrial partners for commercialization efforts

EQUIPMENT: Compact laboratory scale pressure generators, specialized appliances and analytical tools.

Pressure range: 0 – 100,000 psi; Temperature range: -20°C to +100°C; Sample volume: 1 µL to ~2 L

APPLICATIONS:

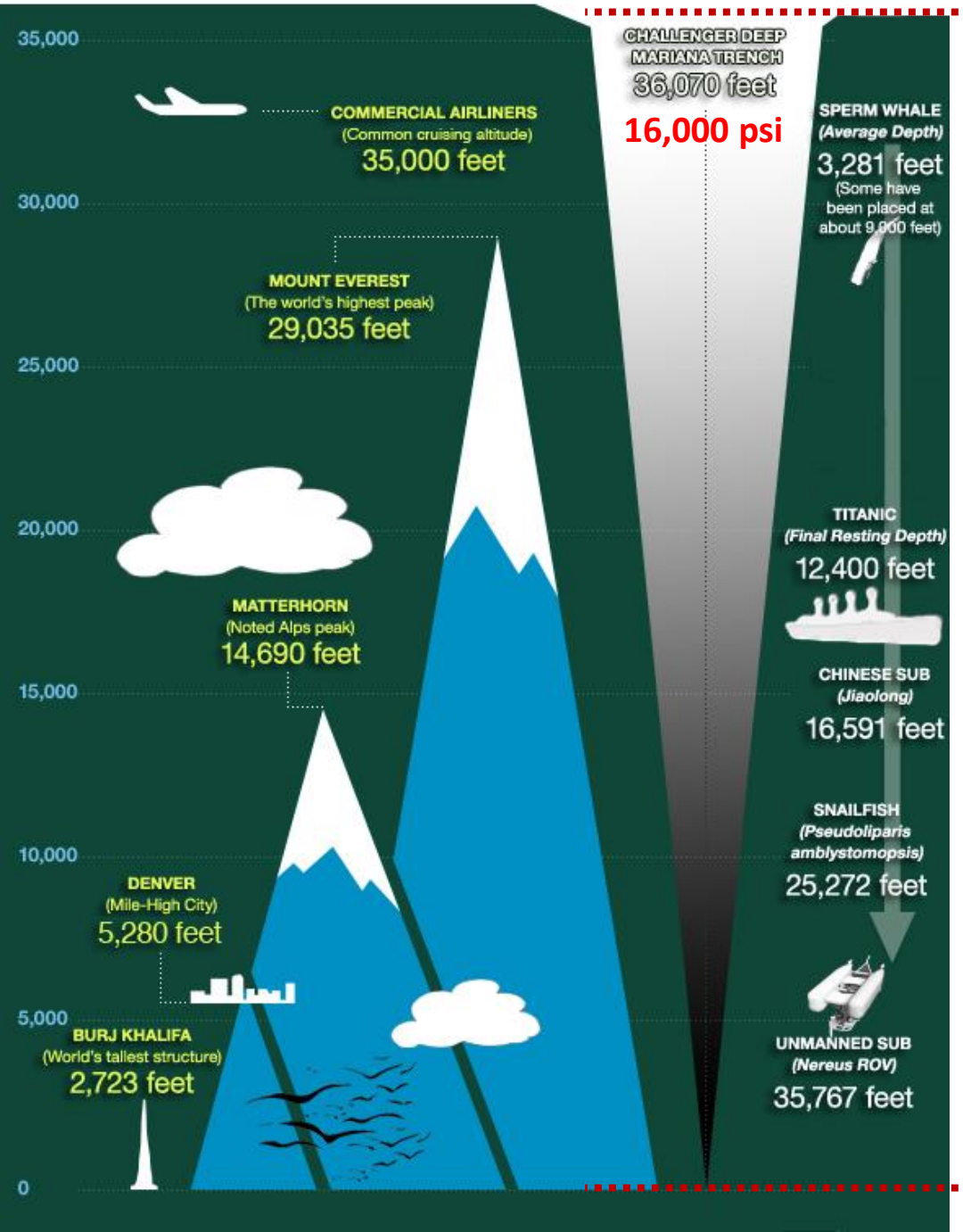
PCT (Hydrostatic Pressure): Biological sample preparation, Biophysical characterization, Biopharmaceutical QC and Biomanufacturing;

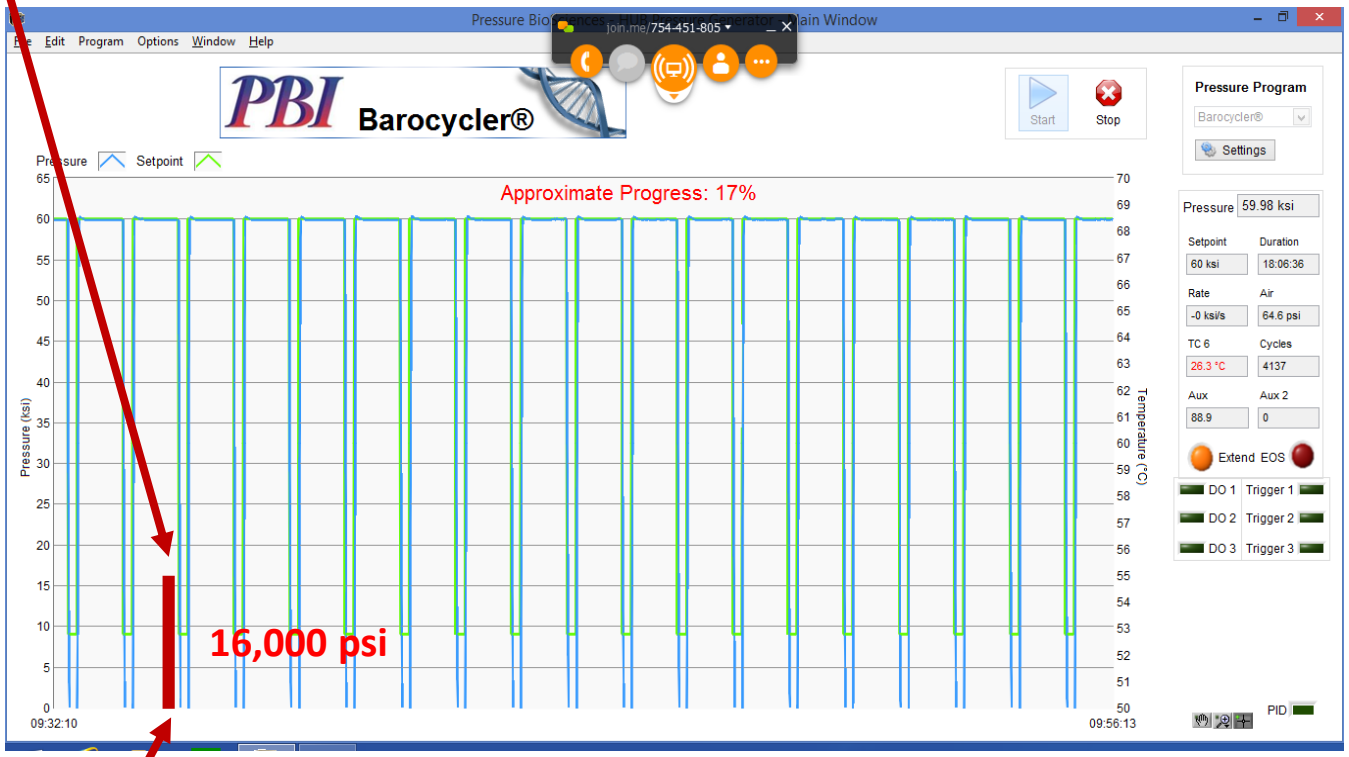
UST (Hydrodynamic Shearing): Biological Sample Prep., Drug Delivery, Food safety and Nanomaterials

PBI's MISSION:

To lower the HIGH PRESSURE equipment barriers faced by scientists and enable new discoveries.

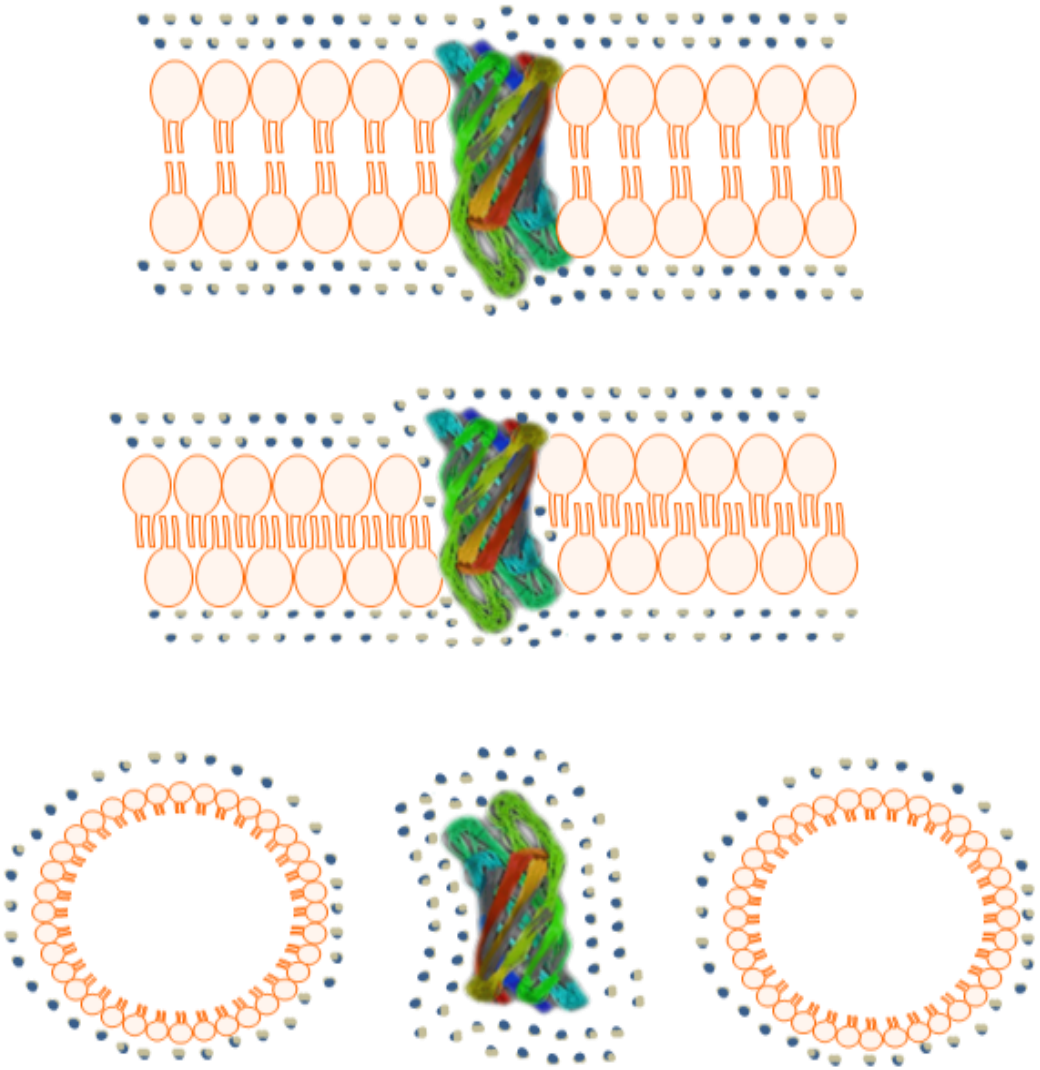
Pressure Cycling Technology (PCT)



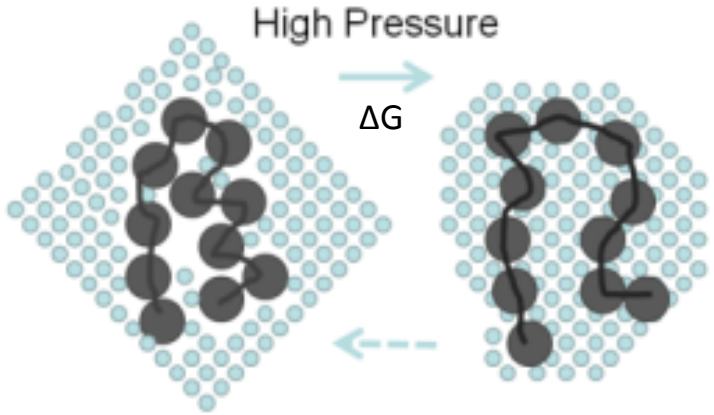


Cycles of hydrostatic pressure between ambient and ultra high levels used for precise control of biomolecular interactions

Lipid bilayers and proteins are destabilized by pressure



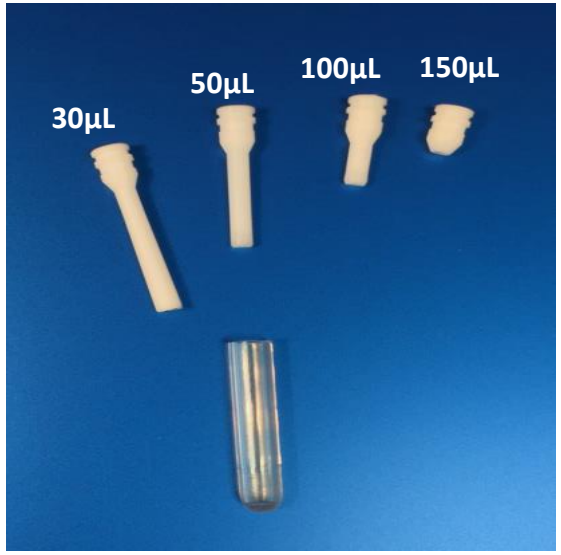
Pressure can selectively alter protein conformation or regulate activity of certain enzymes ***without addition of thermal or chemical energy.***

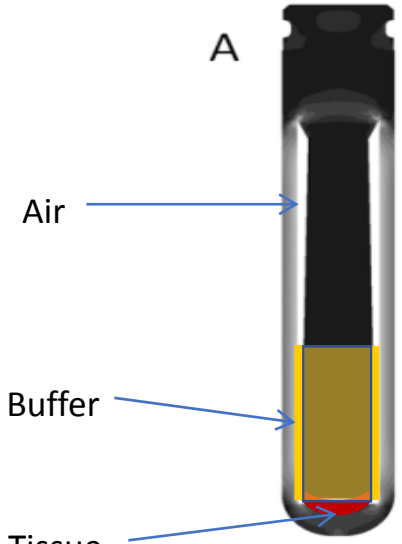


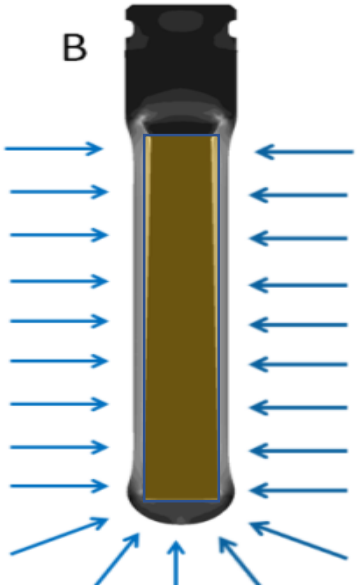
HIGH PRESSURE AFFECTS MACROMOLECULAR INTERACTIONS

- *Pressure causes **phase transitions** in lipid bilayers and partially denatures proteins.*
- *Pressure enhances **activity of several enzymes** used in proteomics and genomics.*
- *Pressure leads to **dissociation** of macromolecular complexes and protein aggregates.*
- *Pressure acts in synergy with **chaotropic reagents***
- *Higher levels of pressure cause irreversible **protein denaturation***

MicroPestle design and mechanism of action
automated tissue homogenization and digestion in a single tube







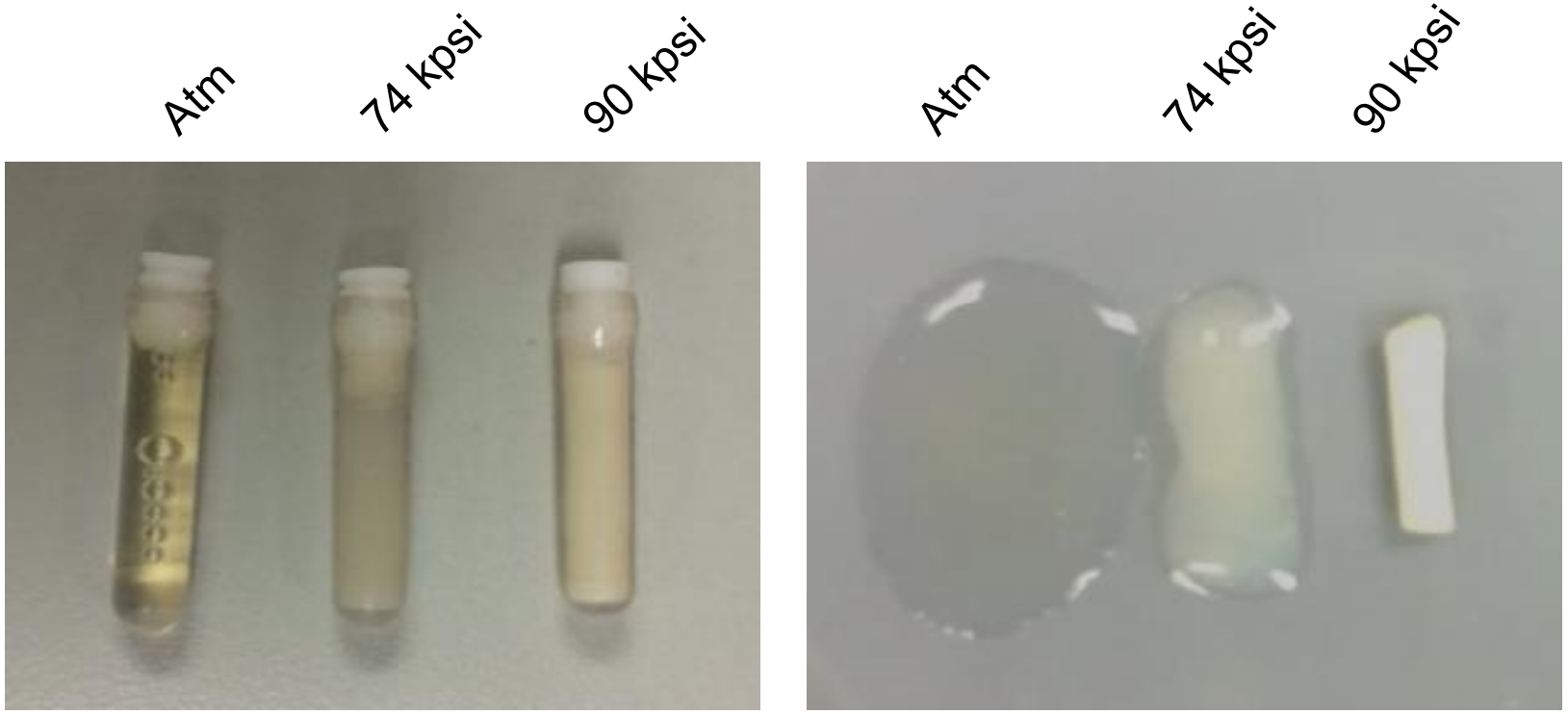
A. At ambient pressure: Tissue at tube bottom (red), 30ul of lysis reagent (yellow) and ~20-30ul of air in the tube (white).

B. Under high hydrostatic pressure: Air is compressed, tube is collapsed and shortened, tissue is homogenized during repeated pressure cycling.

PATENTS ISSUED AND PENDING

Classic Bridgman experiment: “Cooking” an egg white at room temperature

PERCY WILLIAMS BRIDGMAN (1882-1961)
THE NOBEL PRIZE IN PHYSICS, 1946



30 min

20 min

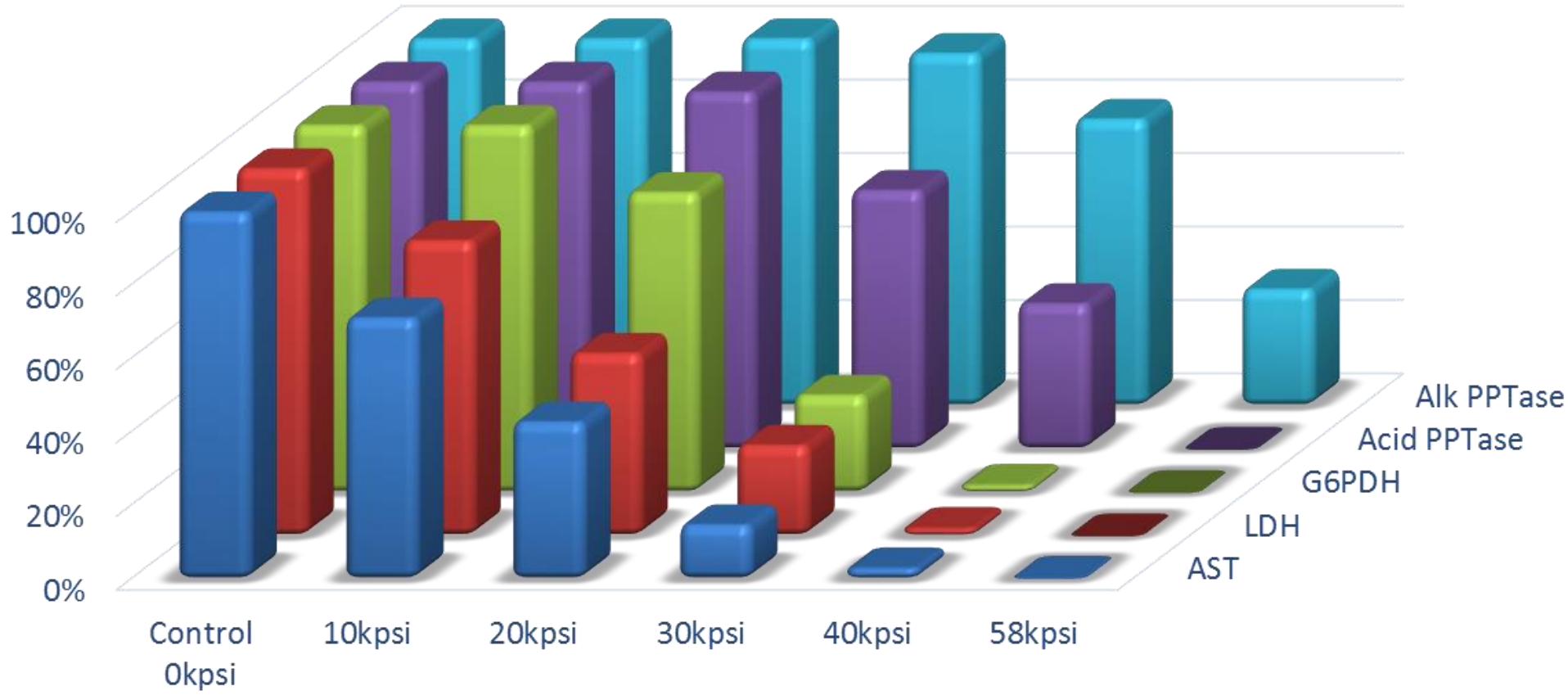
10 min

30 minute incubation at 23°C

time course at 90 kpsi

VERY HIGH PRESSURE LEADS TO PROTEIN DENATURATION

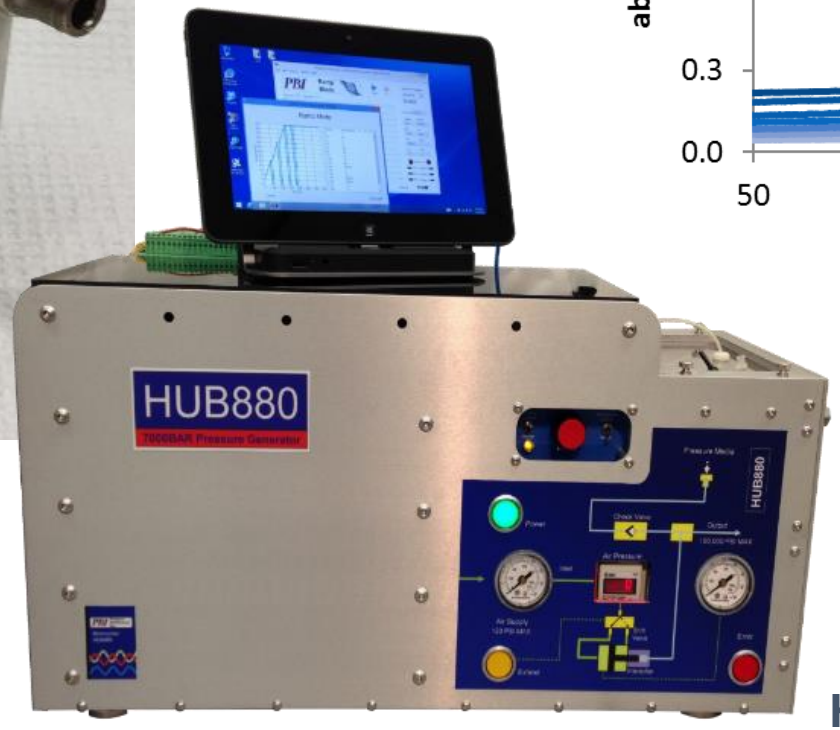
Pressure control of enzymatic activity



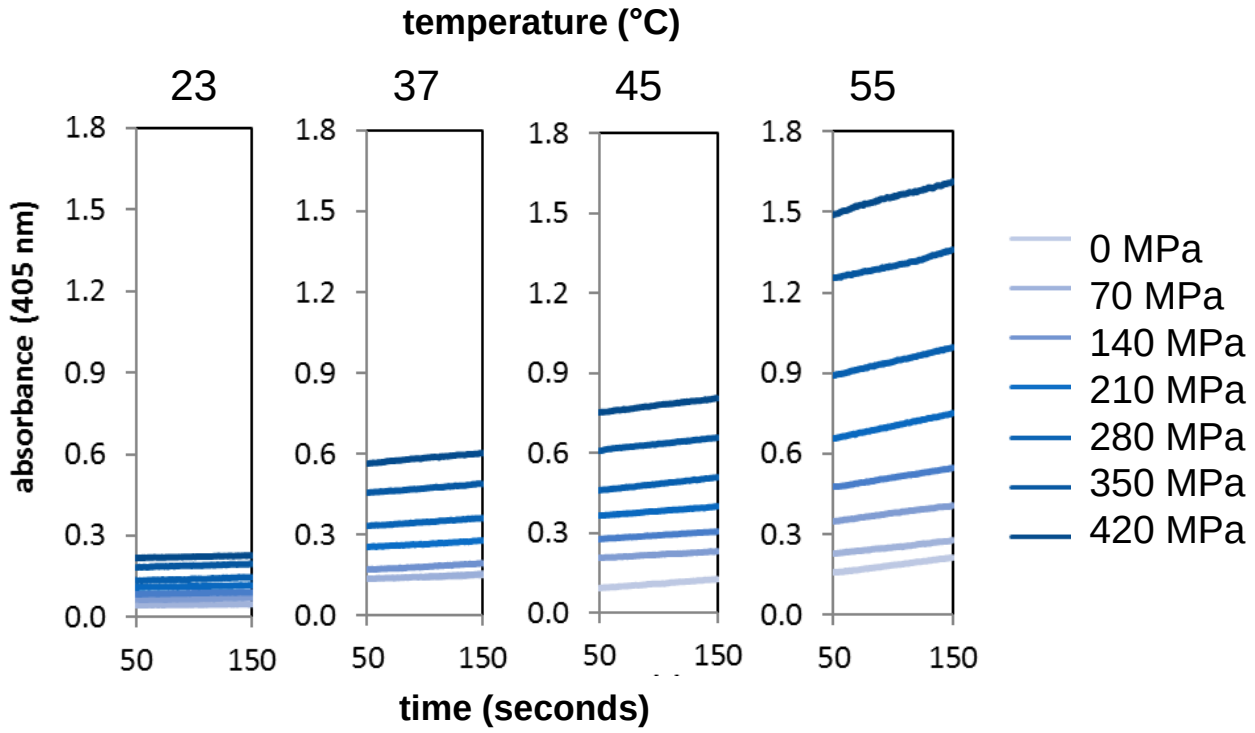
Yeast lysate samples treated at the indicated pressure at 4°C for 10 min.
Activity of each enzyme is shown as percent of control (ambient) value.

Biological applications of real-time High Pressure Spectroscopy (HPS)

High pressure optical cell



HUB880



Lysyl endopeptidase conversion of N-(p-tosyl)-Gly-Pro-Lys p-nitroanilide

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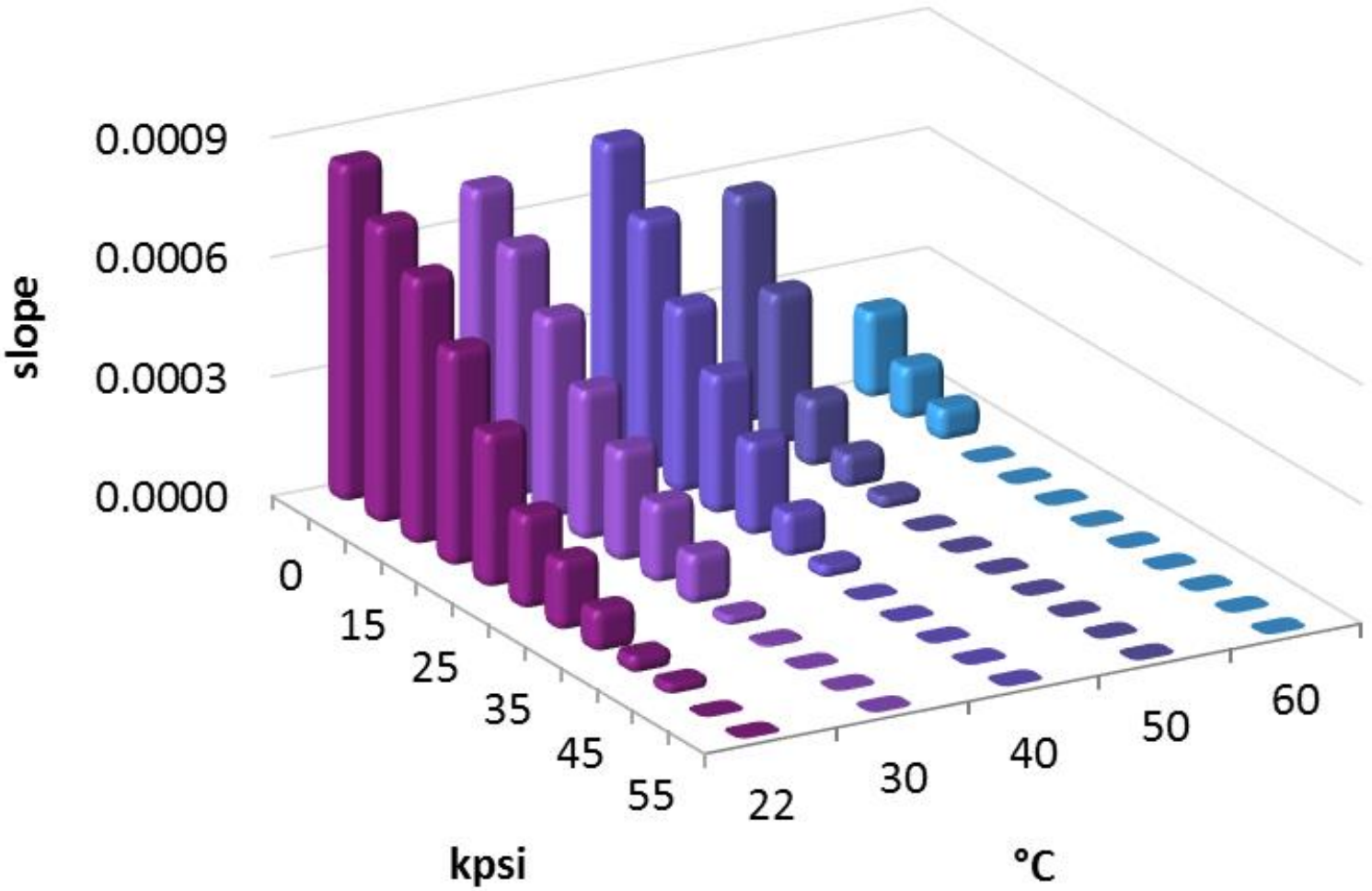
High pressure processing (HPP) in food processing

- HPP extends shelf life of foods while retaining their natural color and texture.
- Slows browning of fruits and vegetables (e.g. guacamole) by inactivating polyphenol oxidases and peroxidases.
- Inactivation of pathogens such as *Vibrio parahaemolyticus* (e.g. “safe” raw oyster)
- Deshells crustaceans without cooking.

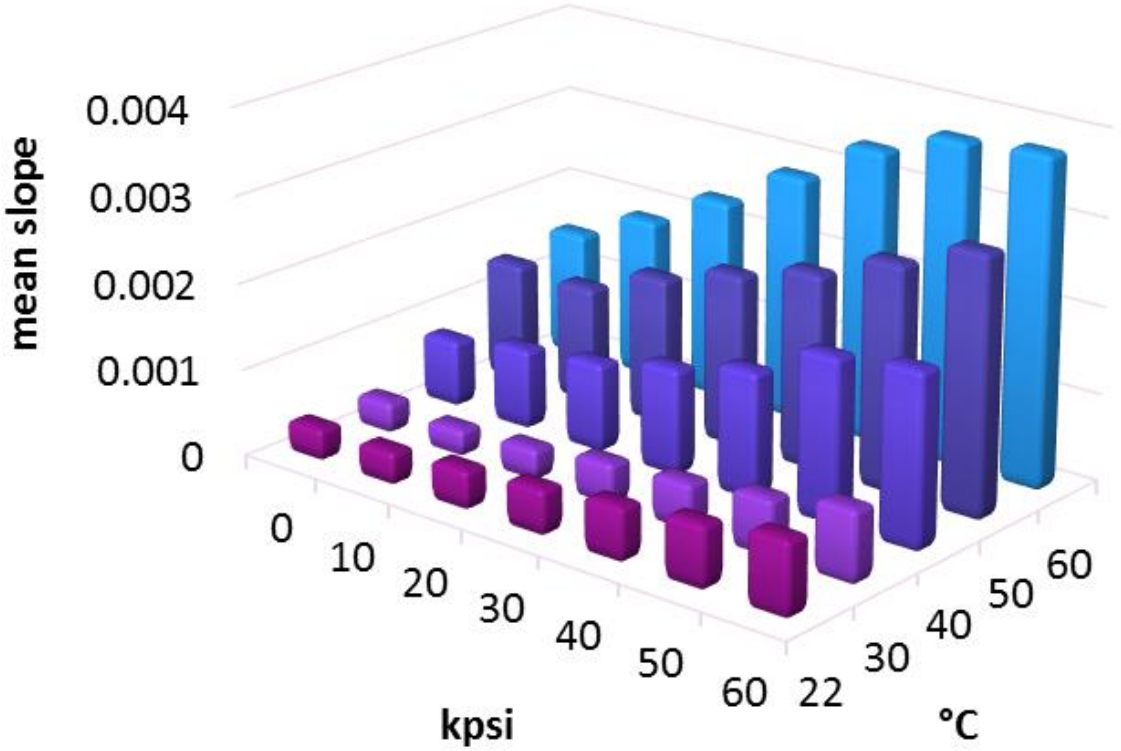
Peroxidase conversion of substrate at high pressure and temperature in the optical cell



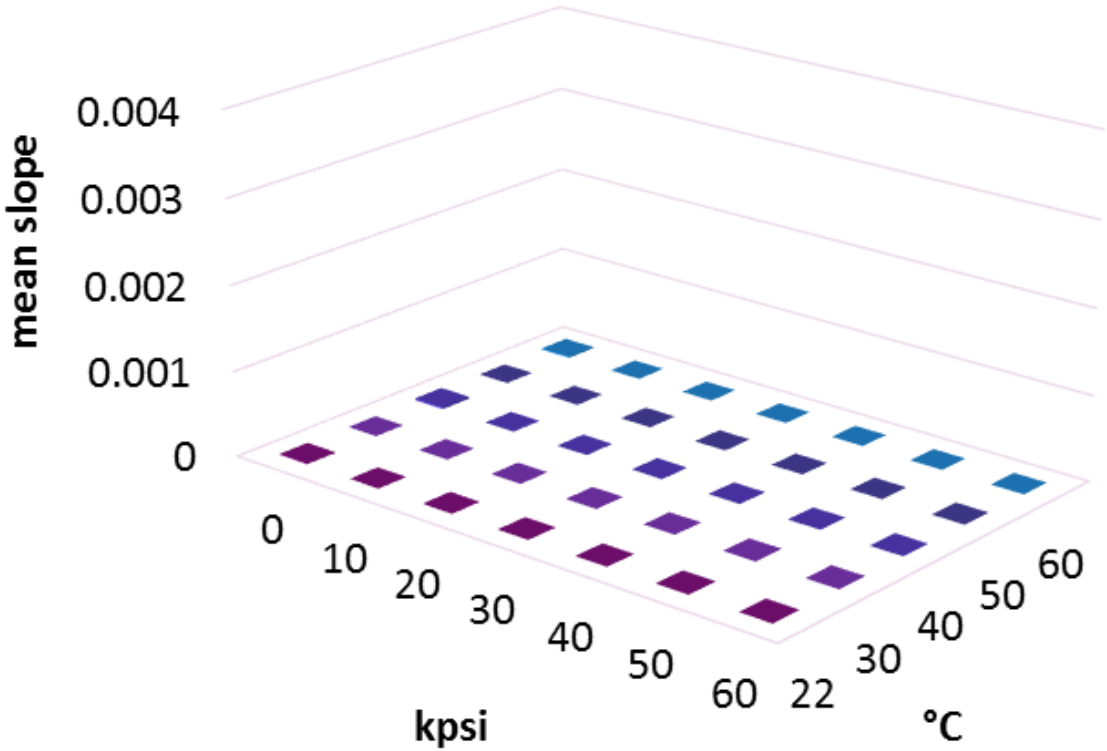
Real time kinetics showing the rate of horseradish peroxidase conversion of synthetic substrate tetramethylbenzidine (TMB) showing the inactivation of the enzyme at high pressure and lower temperatures. The rate of substrate conversion was measured over 100 seconds at each pressure interval

Papainase summary

A. papainase digestion of BAPNA



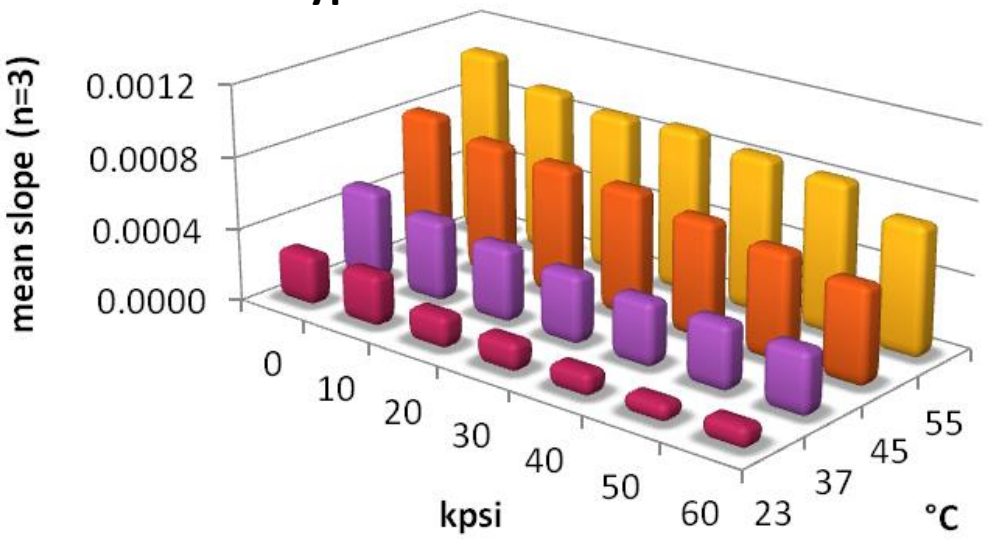
B. BAPNA negative control



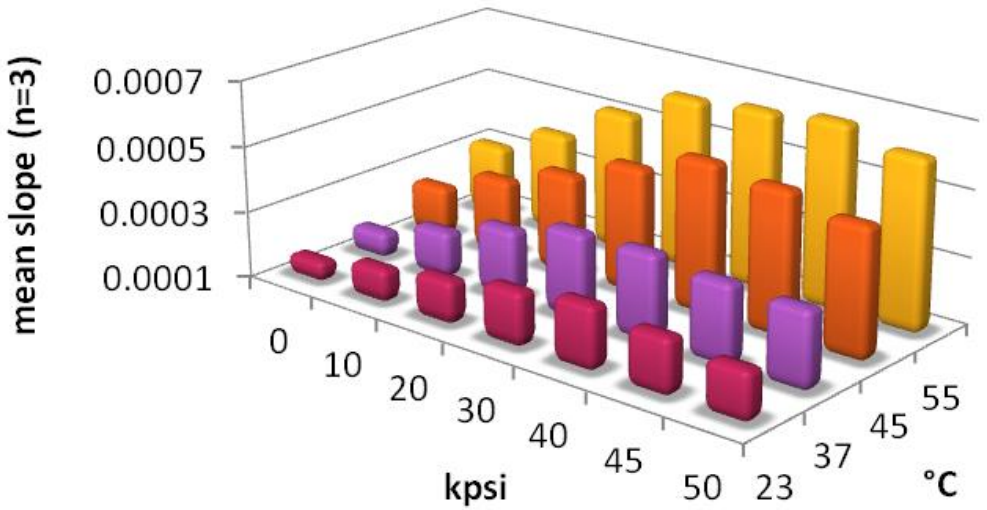
Papainase (a cysteine endopeptidase from *Carica papaya*) digestion of the synthetic substrate BAPNA. Digestion rates (slopes) were measured over 0-60 kpsi at temperatures ranging from 22-60°C.

Synergistic effects of pressure and temperature on digestion rates of three proteases

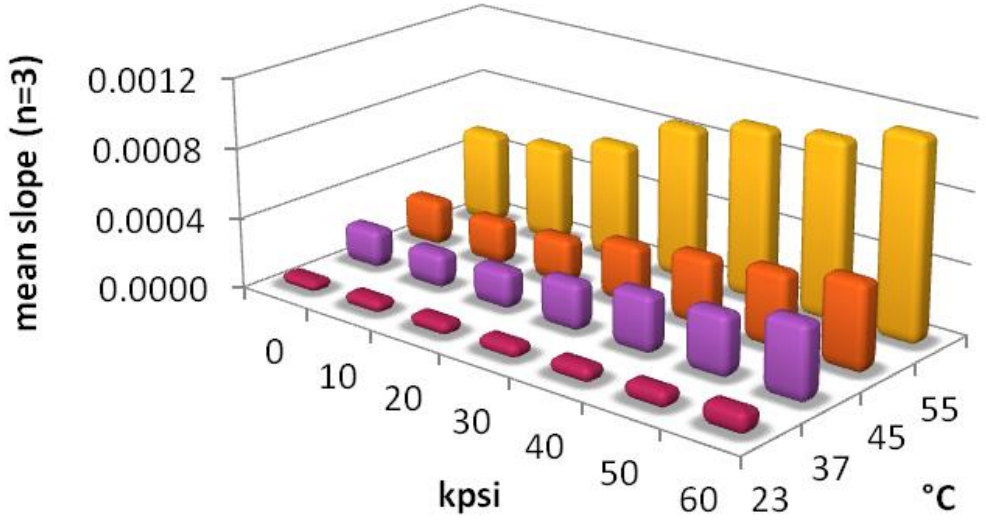
A. trypsin



B. chymotrypsin



C. Lys-C

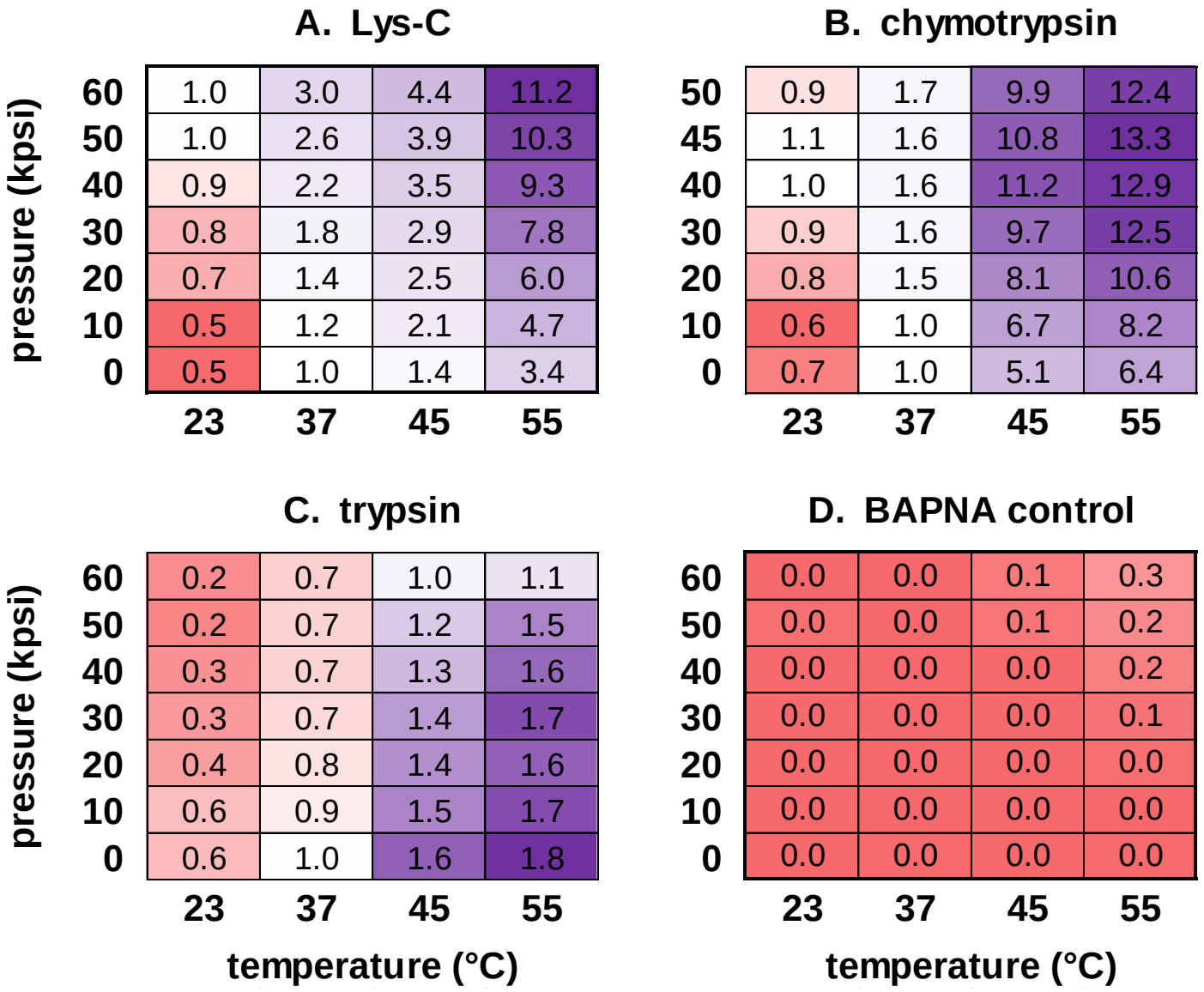


(A) Trypsin digestion of BAPNA substrate at pressure ranging from 0-60 kpsi and temperatures ranging from 23-55°C.

(B) Chymotrypsin conversion of AAPP-pNa substrate.

(C) Lysyl endopeptidase (Lys-C) conversion GPL-pNA substrate.

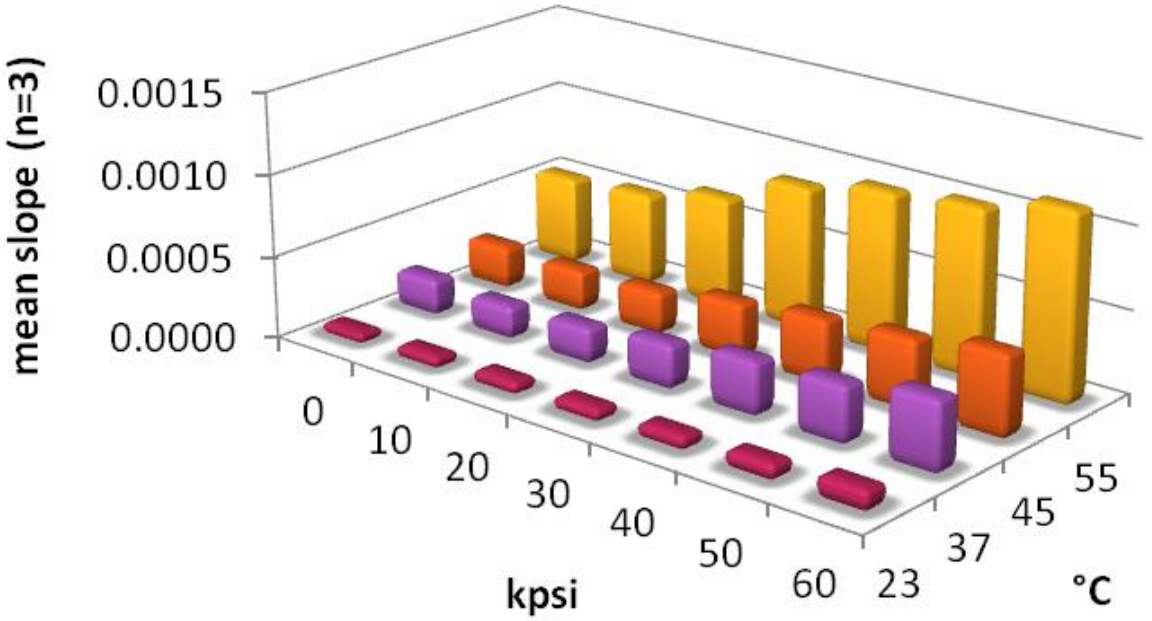
Heat maps showing relative rates of substrate conversion by three enzymes



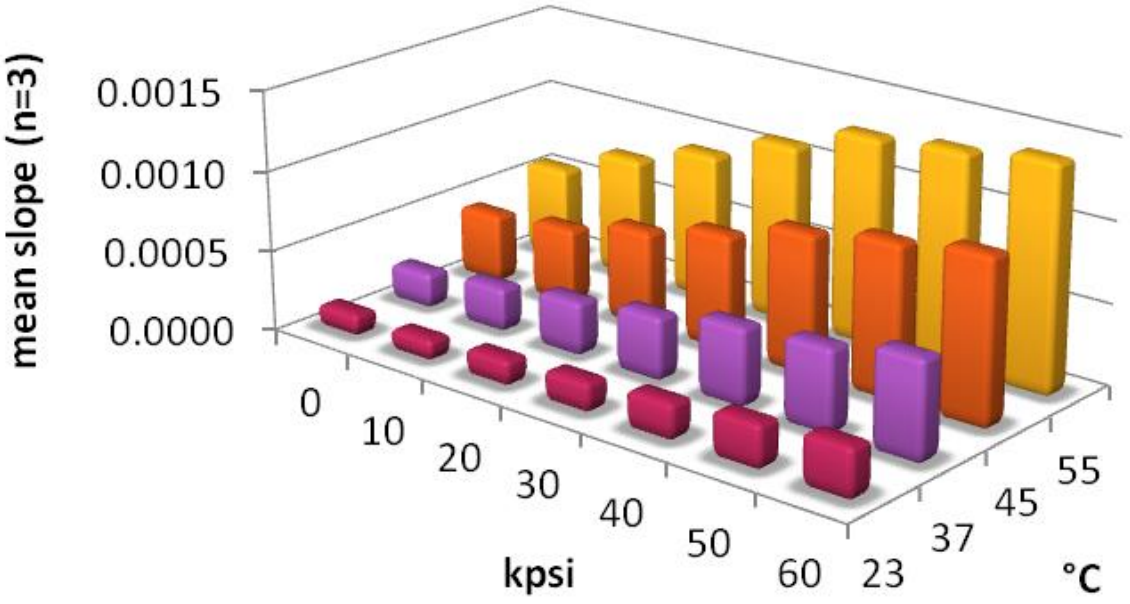
Relative rates of substrate conversion (expressed relative to the rate of conversion for each enzyme at atmospheric pressure at 37°C) for **(A)** Lys-C, **(B)** chymotrypsin, and **(C)** trypsin. A synergy of high pressure and temperature was observed for chymotrypsin and Lys-C. Note the opposite trend observed for trypsin. **(D)** BAPNA control showing minimal conversion to pNA in the absence of trypsin.

Influence of solvent, pressure and temperature on enzyme digestion rate

(A) 100 mM AmBiC

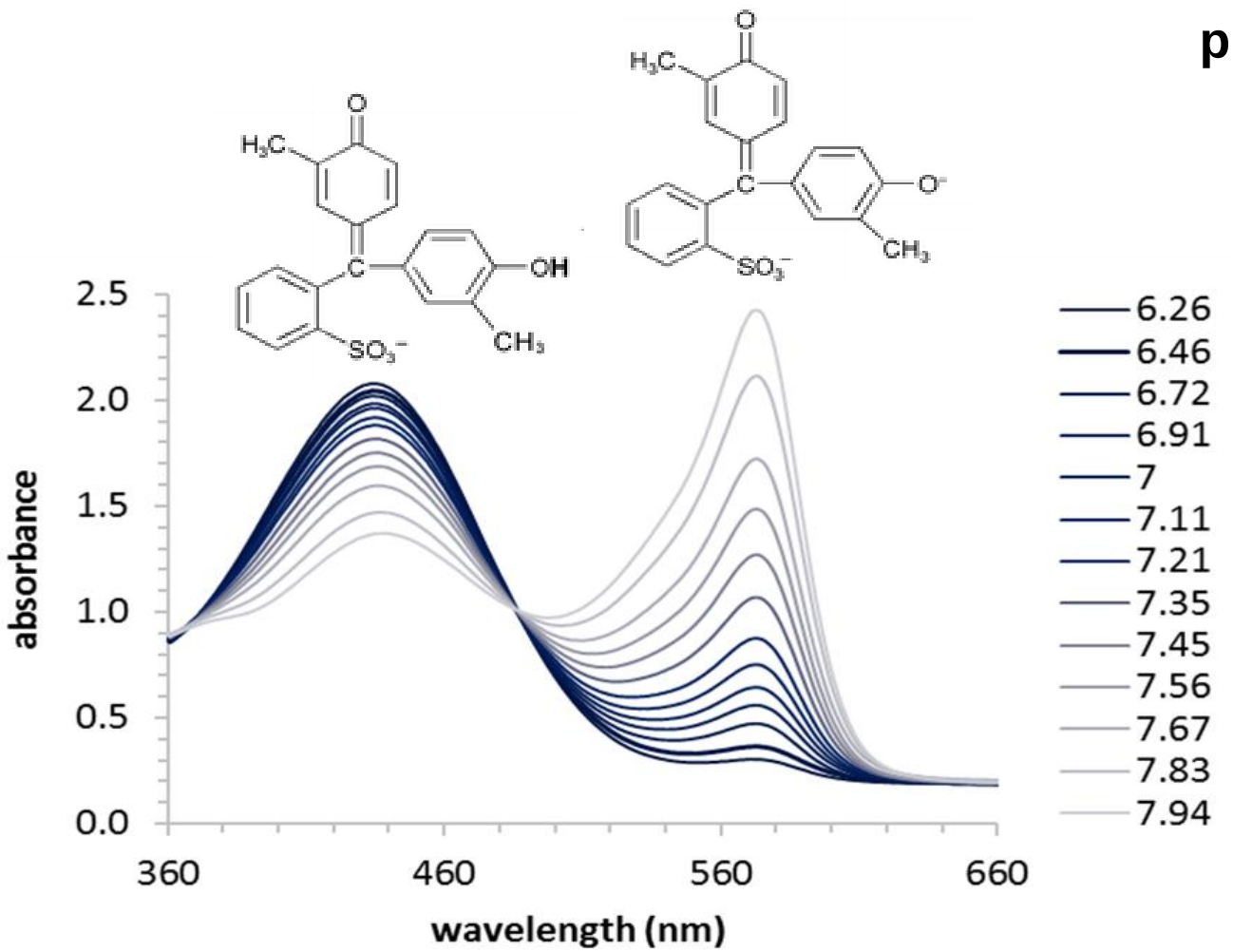


(B) 100 mM AmBiC, 10% acetonitrile.



Lys-C digestion of AAPP-pNa substrate in **(A)** 100 mM AmBiC or **(B)** 100 mM AmBiC containing 10% acetonitrile. Digestion rates reported as mean slope derived from triplicate digests.

Use of Cresol red for real time measurement of pH in a high pressure optical cell



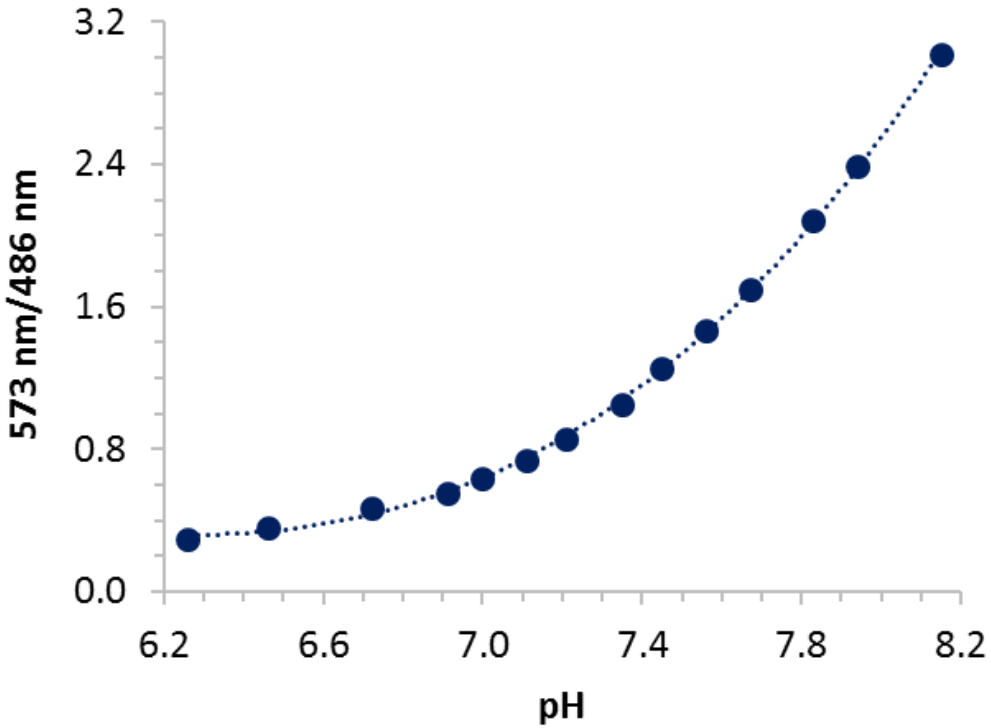
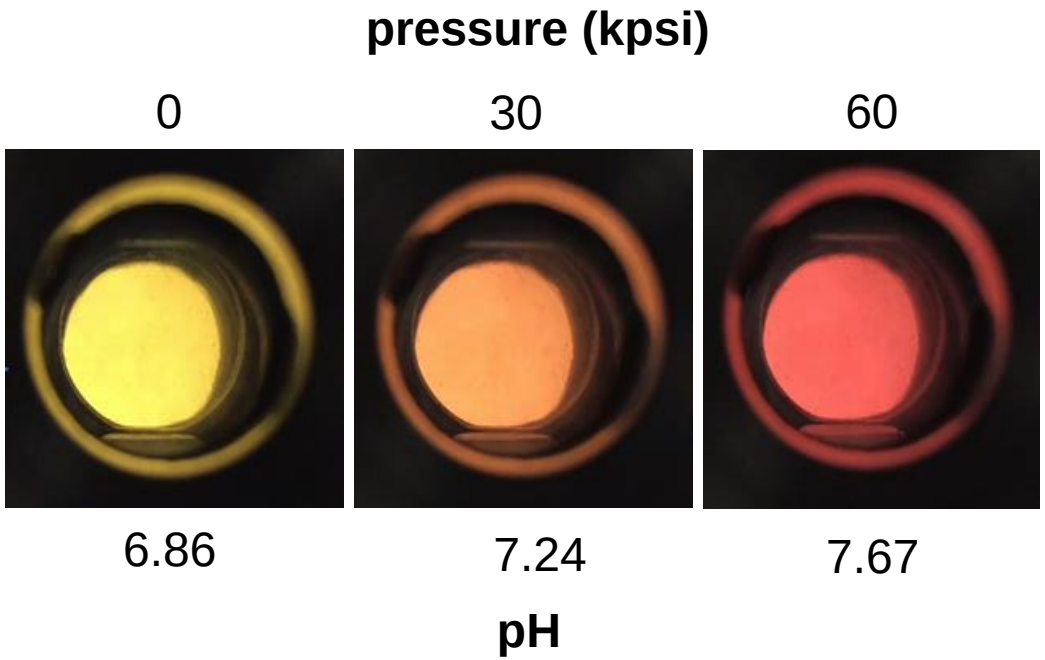
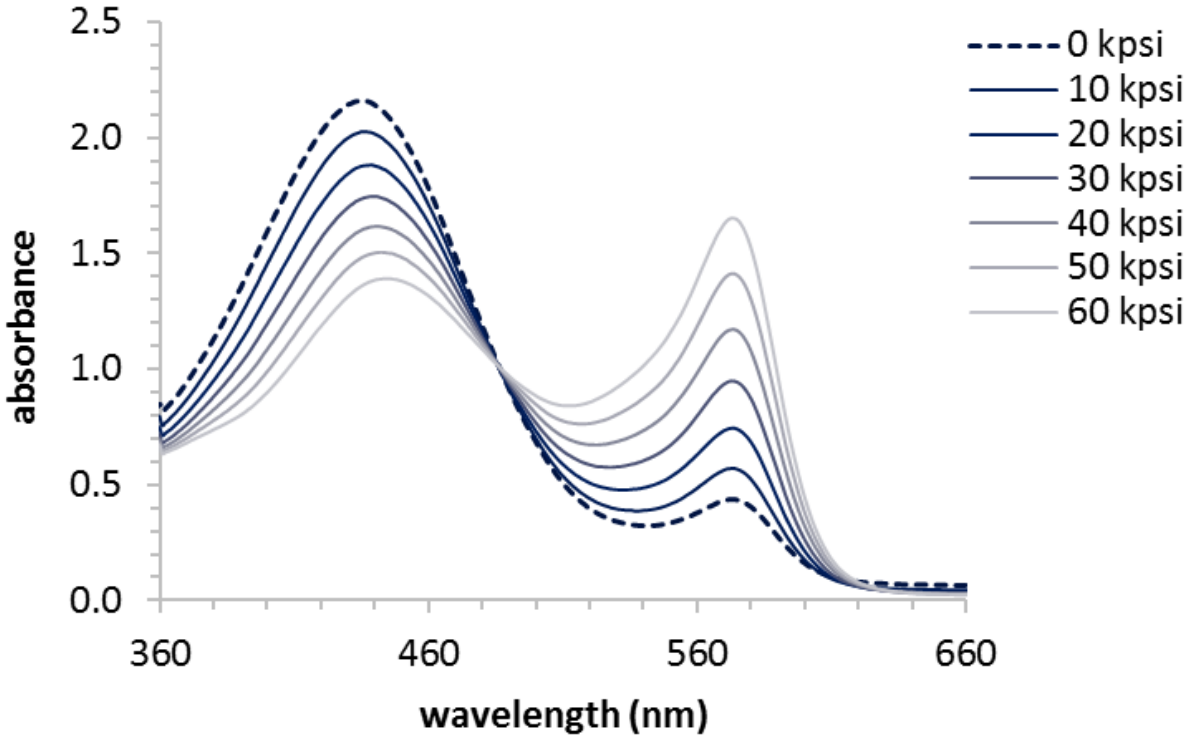


Figure 4. ***(A)*** *pH calibration curve showing changes in cresol red spectra in Tris solutions ranging from pH 6.2 to 8.0 performed at atmospheric pressure in the optical cell. Protonated and deprotonated forms of the dye are correlated to peaks at 434 and 573 nm, respectively.* ***(B)***

Real time measurement of pH in a high pressure optical cell

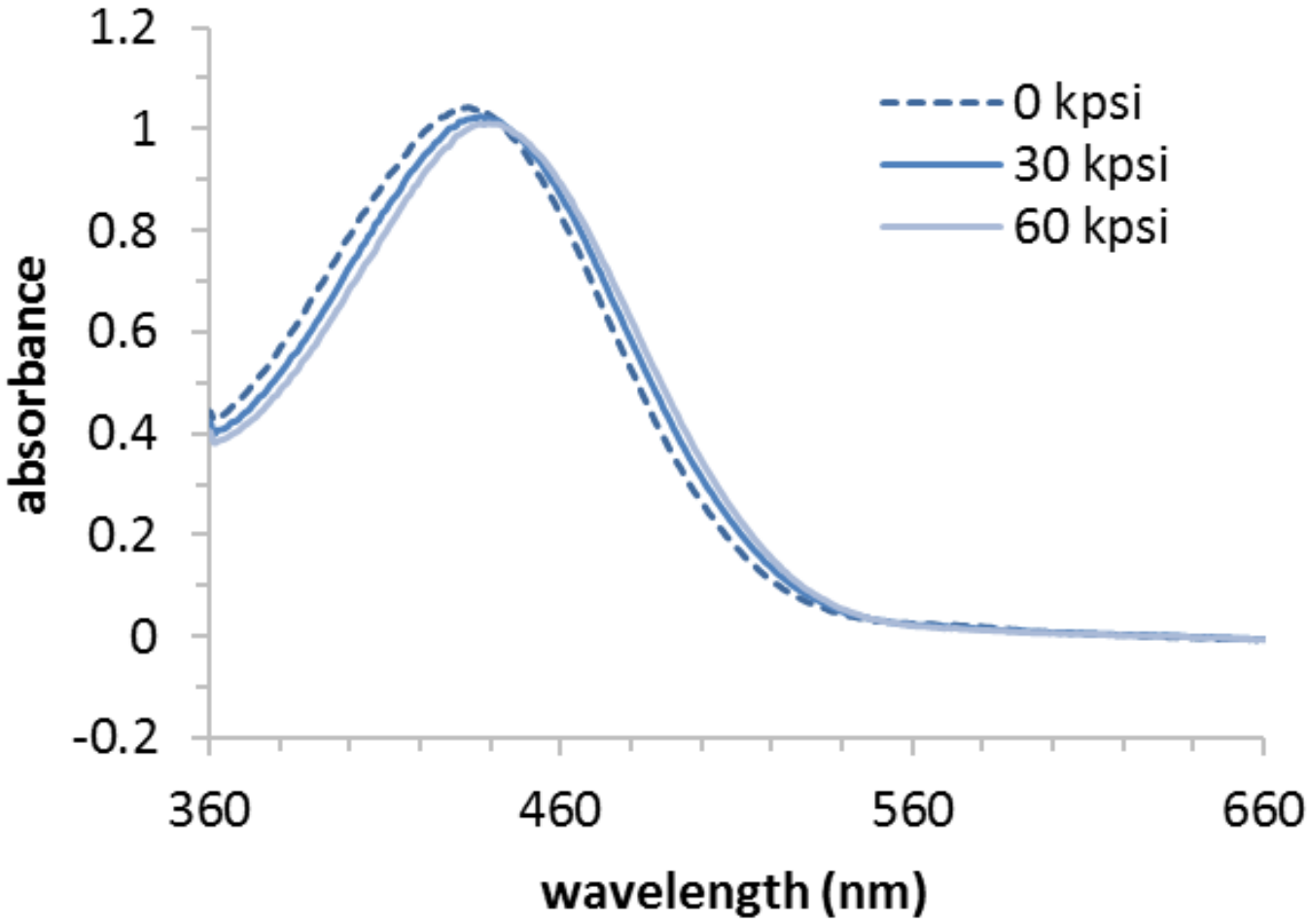


Visualization of color transition through the sapphire windows of the optical cell. Buffer was 100 mM Tris-HCl pH 6.86 photographed over a programmed 0-60 kpsi pressure gradient using the HUB880 pressure generator.



ΔpH = +0.85 ± 0.05 pH units.

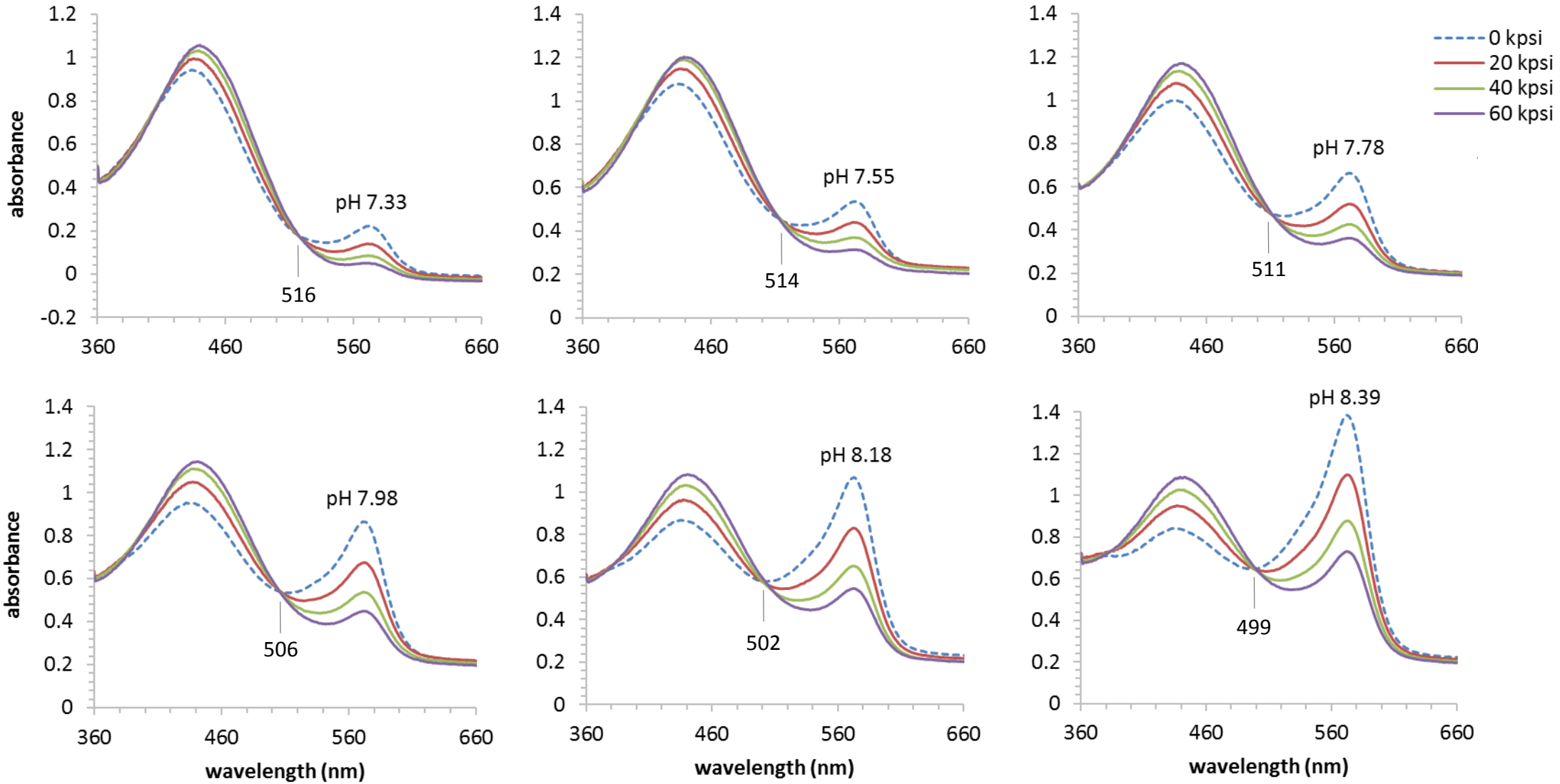
Pressure insensitivity of cresol red pH indicator



Visible spectra of cresol red in H_2O at atmospheric (dashed line) or high pressure.

Peak height was normalized to compensate for the decreased activation volume at high pressure.

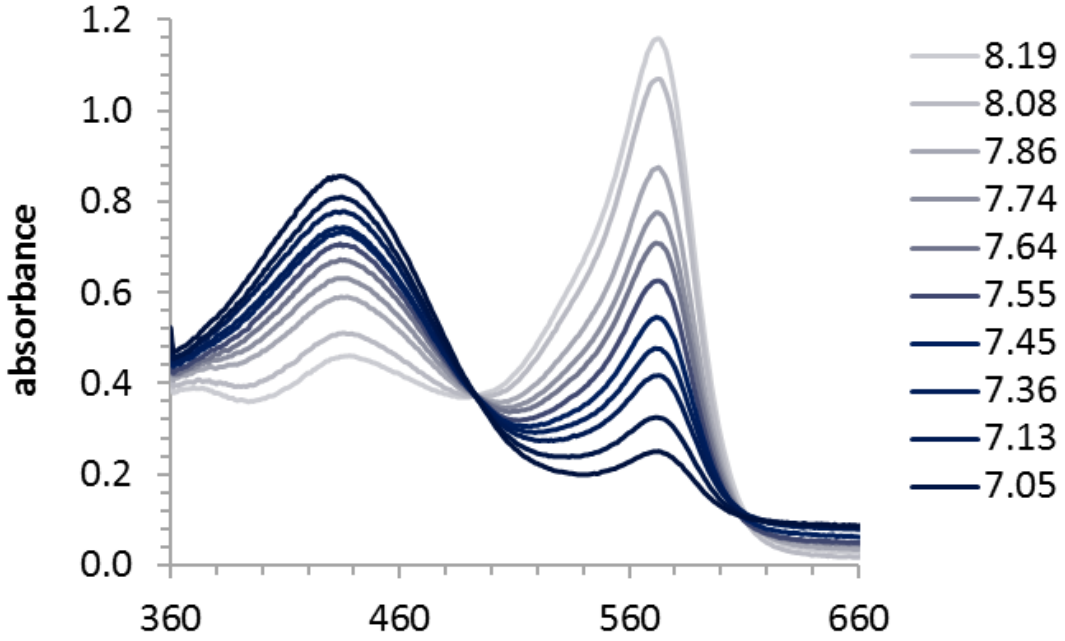
Decreased pH of potassium phosphate buffers at high pressure



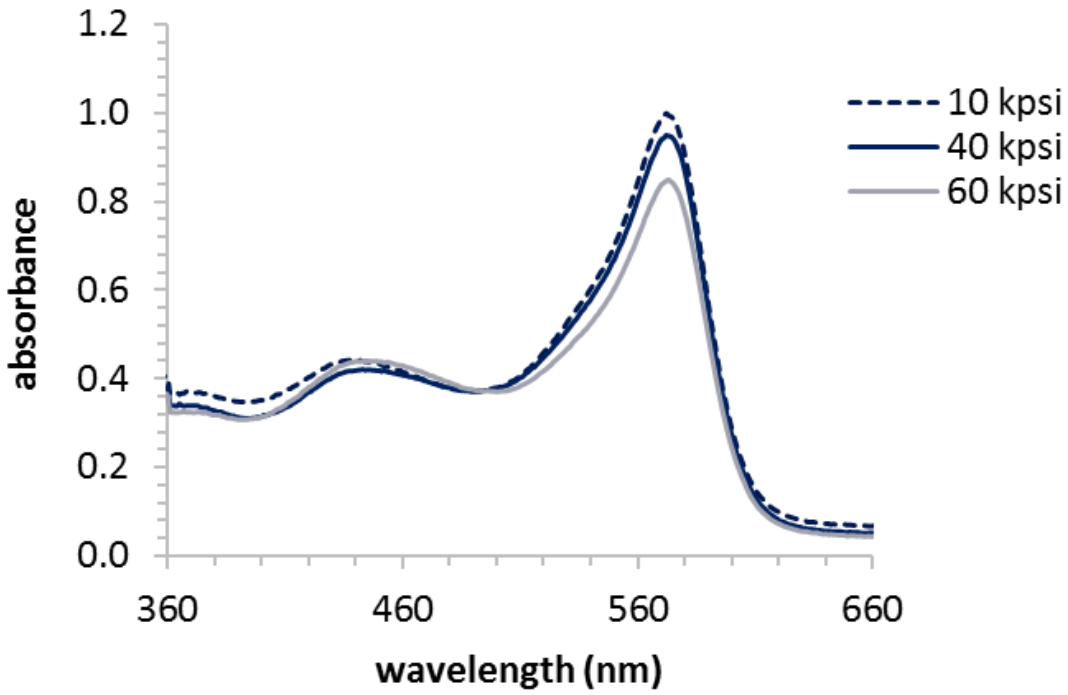
ΔpH = -0.7 pH units

Ammonium bicarbonate exhibits minimal pH change at high pressure

A. calibration curve



B. AmBiC at high pressure



(A) pH calibration curve showing changes in cresol red spectra in ammonium bicarbonate buffers ranging from 7.0 to 8.2 performed at atmospheric pressure in the optical cell. **(B)** 100 mM ammonium bicarbonate pH 8.02 at 10, 40, and 60 kpsi.

SUMMARY

- Since many buffers are pressure sensitive, buffer selection will be of critical importance in high pressure enzymology.
- Here, the pH of Tris, phosphate, and bicarbonate buffer buffers were monitored real time under pressure using the pH indicator dye cresol red.
- The pH of Tris buffer was shown to increased 0.85 pH units at 60,000 psi.
- The pH of phosphate buffer decreased 0.7 pH units at 60,000 psi
- The pH of ammonium bicarbonate decreased only 0.2 pH units at 60,000 psi.
- Knowledge of the pressure sensitivity of these buffers is essential to experimental optimization.

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