Development of Automated Benchtop Dynamic Pressure Generator Platform for Biological Sample Preparation, High Pressure Perturbation of Protein Structure, and other Applications of Hydrostatic Pressure.

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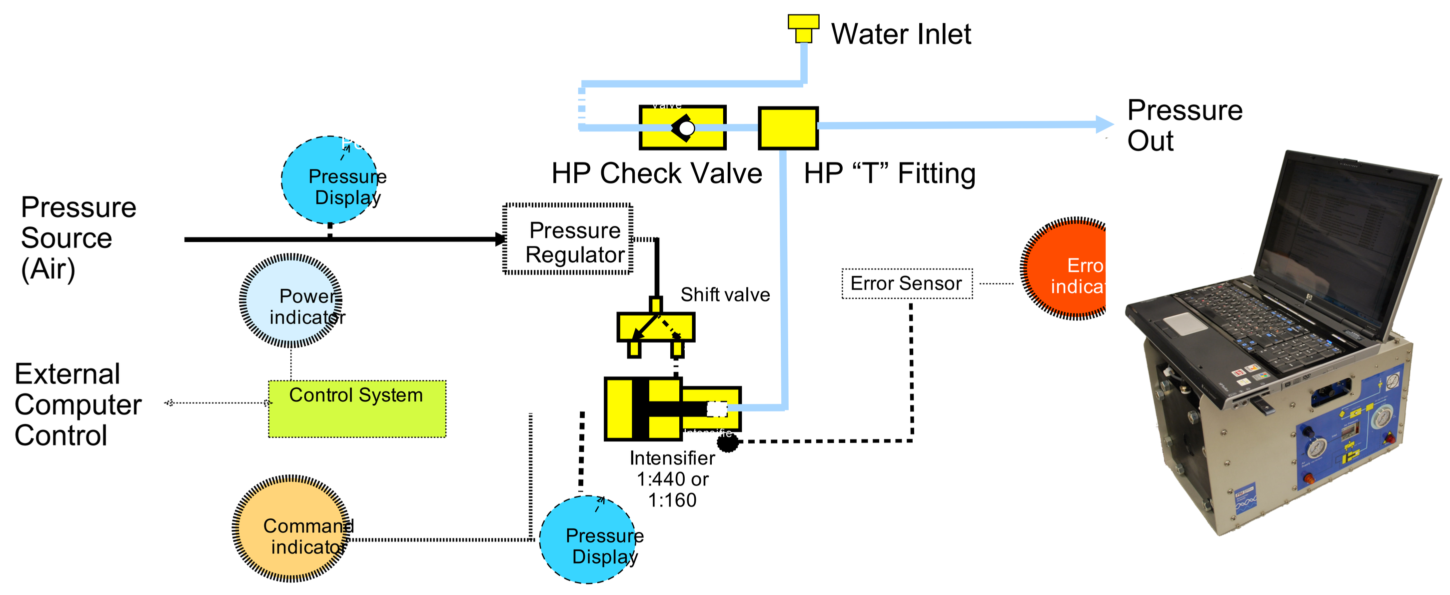
Abstract

While high pressure research is gaining recognition in life sciences, there is a prominent lack of user-friendly laboratory equipment to adequately support these research efforts.
We present the design of two new pressure generators and examples of their applications for pressure-perturbation spectroscopy [1, 2], chemical synthesis [3] and pressure-enhanced sample preparation methods. The cores of these instruments contain air-driven intensifiers with piston cross-section area ratios of 1:160 or 1:440, offering accurate pressure control within the range of 20 to 1,380 bar (2 - 138 MPa) or 50 to 4,000 bar (5 - 400 MPa), respectively, and real-time feedback using proportional–integral–derivative (PID) algorithms to maintain pressure with a resolution of ±1 bar (0.1 MPa). Computer control offers programming of pressure values and valve events over time as well as logging of pressure, temperature and outputs from additional sensors. Pressure jump kit containing rapid valves and accumulator reservoirs permits ascending and descending pressure jump experiments with amplitude of up to 1.3 kilobar on a sub-millisecond time scale.
Adoption of user-friendly high pressure tools by the broad biochemistry and biophysics research community is promising to accelerate high pressure research and lead to better understanding of fundamental pressure effects on biological systems.

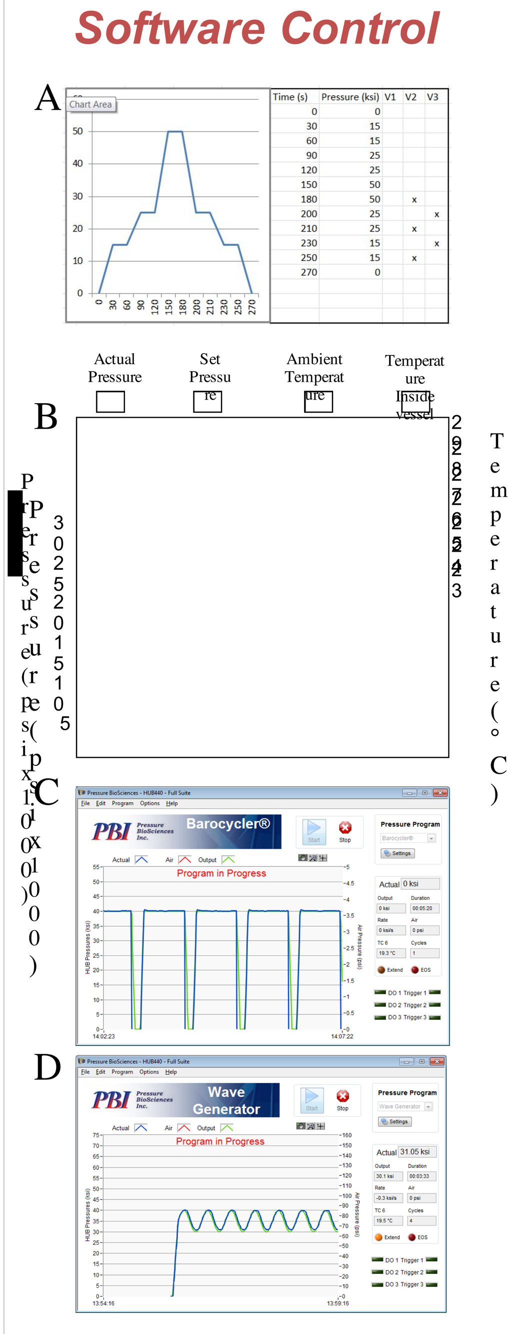
HUB160 and HUB440 Pressure Generators: Flow Diagram and Specifications



The core of the system is an electronically-controlled air-driven reciprocating pressure intensifier equipped with a dynamic high pressure seal system with minimum friction, resulting in the ability to ramp pressure over time with a resolution of several bar per second. Water is used as the high pressure medium due to its low compressibility. An electronic regulator controls the air pressure and the direction of air flow to the front or back of the air cylinder, resulting in precise extension or retraction of the intensifier piston. Pressure is controlled either via a manual control panel on the front of the instrument, or remotely by the USB-powered Data Acquisition and Control interface. Built-in high pressure transducer permits real-time pressure feedback. The system is powered by a 24 VDC power supply. High pressure check valves prevent the backflow of water and enable rapid refill of the intensifier with water for multi-stroke operation. Open frame packaging of the instrument provides easy access to all serviceable components and enables rapid customization. High pressure outlet is equipped with the standard high pressure type "T" fitting compatible with a wide variety of industry-standard high pressure equipment.

	HUB440	HUB160
Intensifier Ratio	440:1	160:1
Maximum Pressure	4 kbar (60,000psi)	1.4 kbar (20,000psi)
Pressure Transducer Span	5 kbar (72,500psi)	1.4 kbar (20,000psi)
Required Air Pressure	9 bar (130psi)	9 bar (130psi)
Intensifier Displacement	Approx 3 mL	Approx 9.5 mL

Software Control



Computer control via optional USB-powered Data Acquisition and Control interface offers flexible programming of pressure values (Figure A). Intuitive software also offers multiple channels of data acquisition, including logging of pressure, temperature and outputs from additional sensors (Figure B). Digital trigger inputs and outputs offer several options for integration with external equipment such as optical and magnetic resonance spectrometers and HPLC components.

Hardware: National Instruments USB-6211 DAQ Card, Electronically controlled air valves, Microsoft Windows XP/Vista/7 computer with available USB 2.0 port.

Software: 32-bit and 64-bit software versions are available with standard functionality: Basic pressure control, large vessel pumping, Wave Generator, Barocycler and Pressure Jump (Figures C and D). Custom software development is possible using LabVIEW 2010.

Automated Calibration (Calibration Wizard) allows the user to quickly and automatically calculate the calibration values (Slope and Y-Intercept) for the HUB channels (air pressure and intensifier pressure output) as well as auxiliary data acquisition channels. Calibration curves can contain between 2 and 100 calibration points. Default calibration values supplied with the software correspond to the average calibration parameters necessary to accommodate the PBI pressure transducer supplied with the HUB Pressure Generator.

High Pressure EPR using Site-Directed Spin Labeling (SDSL)

The Barocycler HUB440 is used to pressurize fused silica capillary EPR cell on a Bruker EleXsys 580 EPR spectrometer fitted with the high sensitivity cavity. SDSL-EPR allows direct determination of pressure-dependent equilibrium constants for protein conformational equilibria [4].

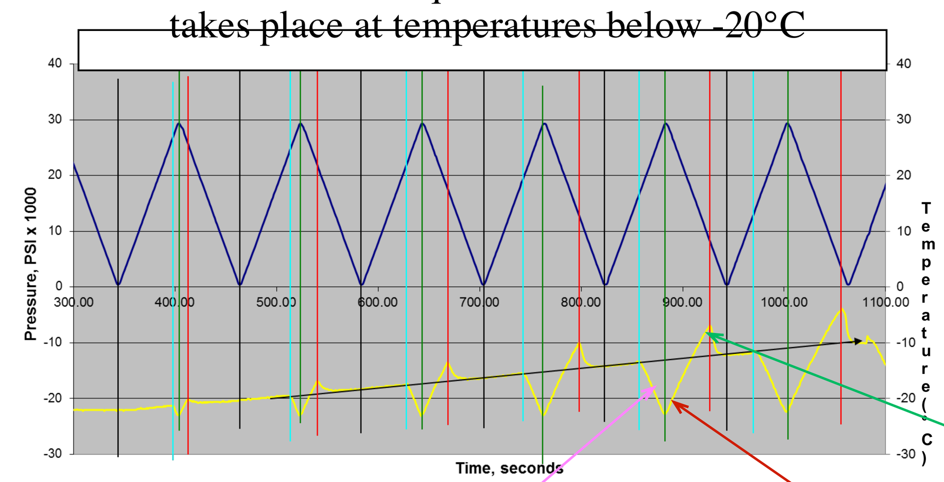
(a) Ribbon diagram of T4 lysozyme mutant T4L46R1 (PDB ID code 3LZM) showing the location of residue L46.

(b) The pressure dependence of the EPR spectra normalized to the same number of spins.

(c) The equilibrium constant determined from fits to the spectra is plotted as indicated *vs.* pressure (dots); the solid line is a fit to the theoretical equation shown above.

Pressure-Induced Phase Transition Monitoring: Melting and Freezing of Water

Pressure induced Liquid ↔ Solid transition that takes place at temperatures below -20°C



1. Increasing pressure leads to melting of ice. The heat of fusion is extracted from the water, resulting in detectable cooling.

2. As pressure decreases, the ice begins to solidify. The heat of fusion is transferred to the water, resulting in detectable warming.

3. Solidification is completed. The ice cools down to ambient temperature and then continues to warm.

A triangular pressure wave cycled between 0 and 2kbar (30,000psi) was generated by the HUB440 system. The temperature was monitored by a thin metal-clad type K thermocouple inserted into the pressure chamber. Pressure Chamber was chilled to -20°C with the methanol-dry ice bath and allowed to gradually warm up to -10°C during data collection. All data were collected using the HUB440 DAC and plotted in Microsoft Excel. Prolonged phase transitions from Ice I to liquid (turquoise) and back (red) is clearly observed by inflection of temperature profile.

Pressure Jump Setup for Rapid Pressure Perturbation Kinetics Experiments

"Up" Valve

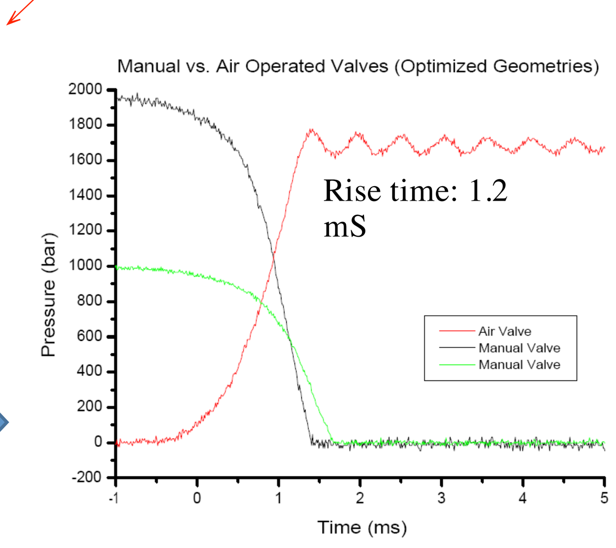
Reservoir (15 mL)

"Down "Valve

Output port

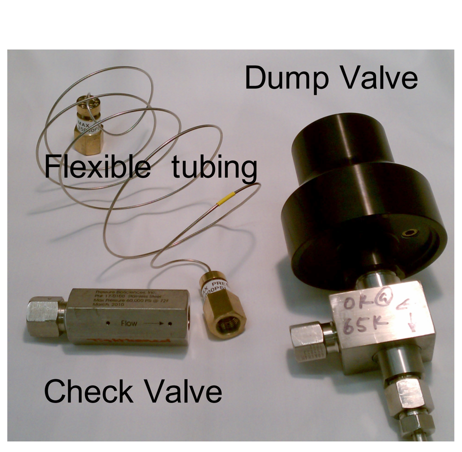
Transducer

Pressure Jump Data recorded with the high speed piezoelectric transducer at Dr. Hubbell's laboratory at UCLA.

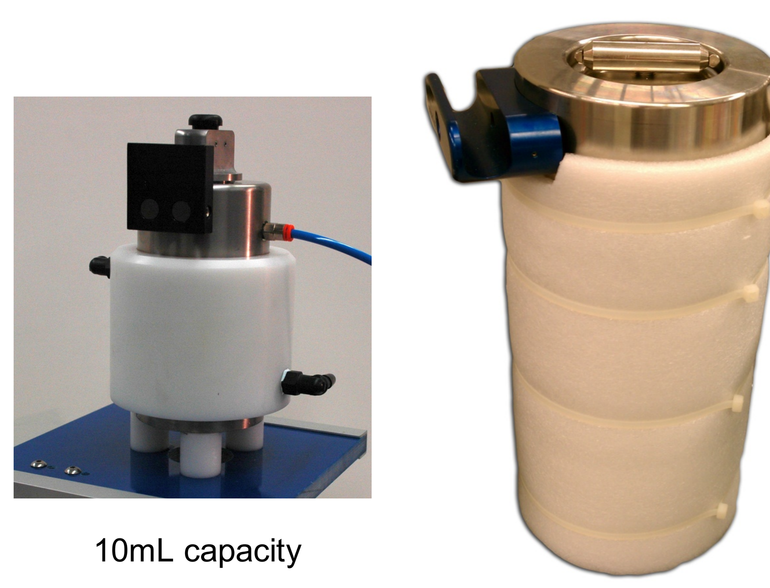


Specialized Peripheral High Pressure Components

- Ultra-fast pressure-jump valves
- High pressure chambers
- High pressure check valves
- High pressure tubing adapters
- Caps, connectors and tees
- Valves
- EPR cells



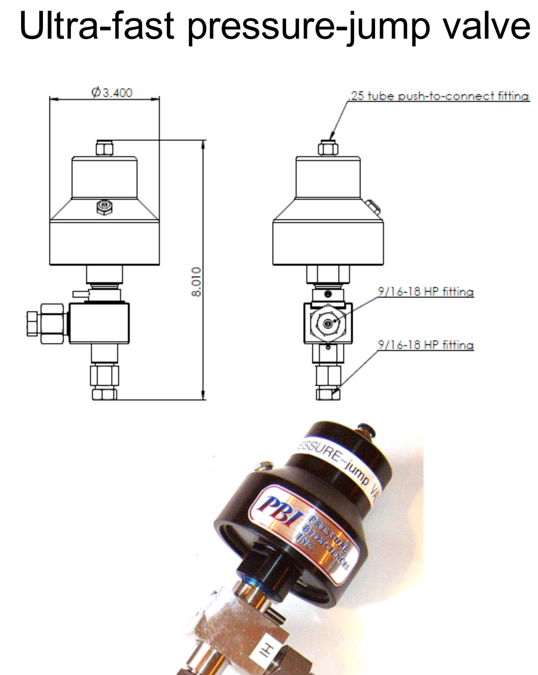
Large and small pressure chambers



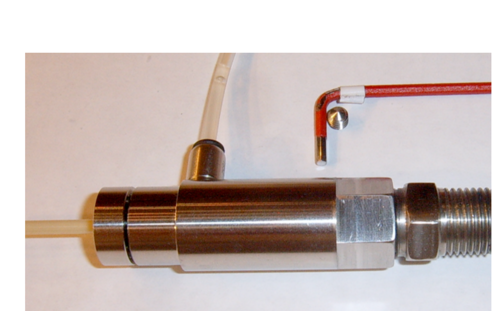
10mL capacity

30mL capacity

Ultra-fast pressure-jump valve



Electron paramagnetic resonance cell



Optical Cells

Standard HP fittings allow connection to the high pressure cell from ISS Instruments (pictured above) which enables UV-Vis absorbance and fluorescence measurements up to 4 kilobar.

References

[1] J. McCoy, W. L. Hubbell. High-pressure EPR reveals conformational equilibria and volumetric properties of spin-labeled proteins. *Proc Natl Acad Sci USA. 2011*, **108**(4):1331-6.
[2] Ando N., Barstow B. High Hydrostatic Pressure Effects on Proteins: Fluorescence Studies. In: *Encyclopedia of Analytical Chemistry*, Online ©2006–2012 John Wiley & Sons, Ltd.
[3] Tomin A, Lazarev A, Bere MP, Redjeb H, Török B. Selective reduction of ketones using water as a hydrogen source under high hydrostatic pressure. *Org Biomol Chem.* 2012, **10**, 7321-7326.
[4] J. McCoy, W. L. Hubbell. High-pressure EPR reveals conformational equilibria and volumetric properties of spin-labeled proteins. (2011) *Proc Natl Acad Sci U S A.* 108(4):1331-6
[5] S.-K. Hyung *et al.* Development of a 20 kpsi Enzymatic Digester for High Throughput Proteomic Analysis and Its Application to Membrane Proteomics. Poster at the 59th ASMS Conference, Denver, CO, June 5-9, 2011.