Development and Applications of the Automated Benchtop Dynamic Pressure Generator for High Pressure Perturbation of Protein Structure and Protein Dynamics Studies

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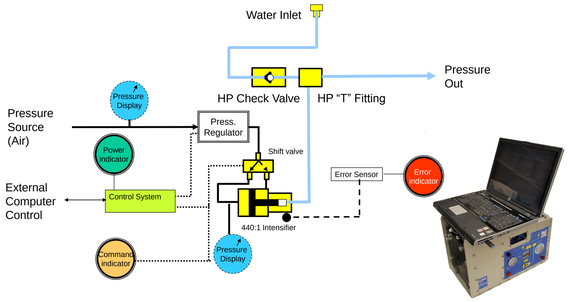
HUB440 Pressure Generator: Flow Diagram



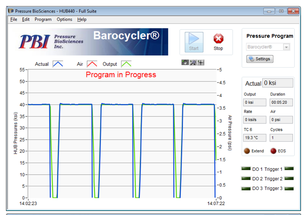
The core of the system is an electronically-controlled air-driven reciprocating pressure intensifier with a fixed ratio of 1:440, single stroke displacement volume of 3.6 mL and a maximum outlet pressure of 4 kBar (~60,000 psi). The intensifier is equipped with the dynamic high pressure seal system with a minimum friction, resulting in the ability to ramp pressure over time with a resolution of several Bar per second in ascending and descending directions. Water is used as a high pressure media due to its low compressibility. Electronic regulator controls the air pressure and direction of air flow to the front or the back of the air cylinder, resulting in a precise extension or retraction of the intensifier piston. Pressure is controlled via manual control panel on the front of the instrument or remotely by the optional USB-powered Data Acquisition and Control interface. Built-in high pressure transducer permits real-time pressure feedback. The entire system is powered by a 24 VDC power supply. High pressure check valves prevent the back flow 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 high pressure type "T" fitting compatible with a wide variety of industry-standard high pressure equipment.

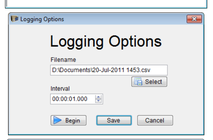
Barocycler ™HUB440 Software Control Interface

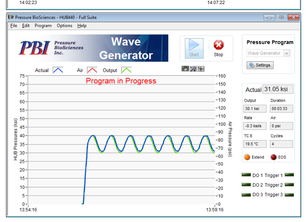
Computer control via optional USB-powered Data Acquisition and Control interface offers flexible programming of pressure and temperature values. Intuitive software also offers multiple channels of data acquisition, including logging of pressure, temperature and outputs from additional sensors. Digital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

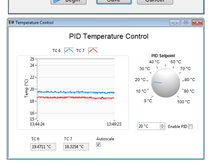
Hardware: National Instruments USB-6211 DAQ Card, Electronically controlled air valves, Windows XP/Vista/7 computer with available USB 2.0 port.

Software: National Instruments LabVIEW 2010 Developer's Suite and National Instruments DAQ-MX drivers were used for development work, run-time executable has been created as a final user-installable program.









Service mode includes channel calibration and

Introduction

Pressure is a significant thermodynamic parameter that is orthogonal to temperature. The work of the 1946 Nobel Prize winner from Harvard University, Percy W. Bridgman, catalyzed our understanding of high pressure thermodynamics with respect to biology, including demonstration of pressure denaturation of proteins. Since, generations of scientists continue to explore high pressure in life science research. High pressure drives water molecules into solvent-inaccessible pockets of proteins, alters the hydration of hydrophobic protein residues and weakens hydrophobic interactions, causing most proteins to undergo reproducible and reversible unfolding typically proportional to the level of applied pressure. High pressure effects on protein conformation, enzyme kinetics, lipid-protein interactions, viability of pathogens and protein expression in living systems have been covered in excellent reviews [1-5].

However, the limitations of material science and engineering have resulted to date in a prominent lack of commercial laboratory equipment suitable to adequately support high pressure research efforts in life sciences. For decades, high pressure research has been driven by do-it-yourself enthusiasts capable of combining fundamental knowledge of biology, physics and chemistry with the need to practice general plumbing and sophisticated mechanical engineering skills. Majority of the high pressure biochemistry and biophysics work had been done using primitive manual equipment and hours of mandatory physical exercise. The goal of this work is to develop user-friendly dynamically-controlled automated pressure generator suitable for unattended pressure perturbation studies of protein conformation, including enzymology, kinetics of protein folding, aggregation, and self-assembly.

Specialized Peripheral High Pressure Components

Ultra-fast pressure-jump valves

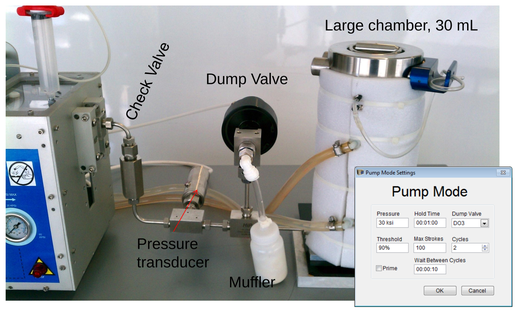
High pressure chambers

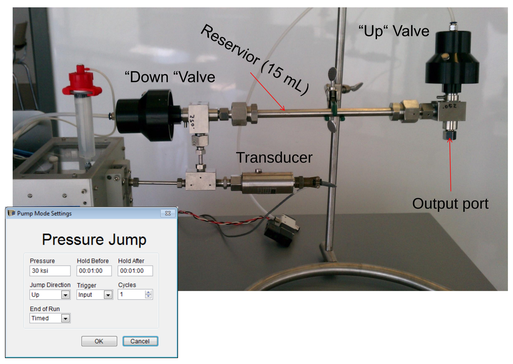
High pressure check valves

High pressure tubing adapters

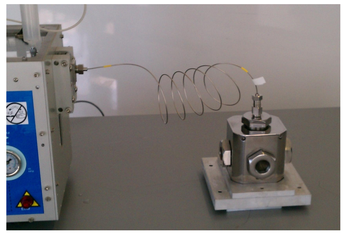
Caps, connectors and tees

Examples of Operating Modes and Configurations





High Pressure Applications for Optical Spectroscopy



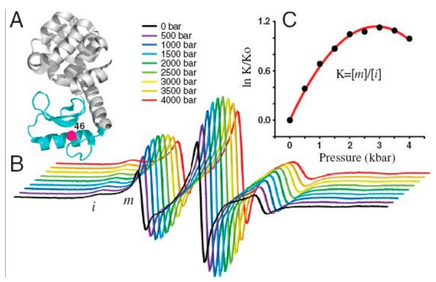
Standard HP fittings allow connection to the ISS Instruments high pressure cell (pictured) which enables UV-Vis absorbance and fluorescence measurements up to 4 kBar. PBI is currently developing user-friendly optical cells of smaller sample volumes that will provide full isolation between sample and pressure media.

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

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.

McCoy J., Hubbell W. L., 2011 [6]

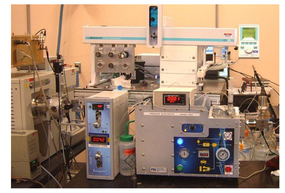
$$\ln\frac{K(P)}{K(0)} = -\frac{\Delta\bar{V}^{o}}{RT}(P) + \frac{\Delta\bar{\beta}_{T}}{2RT}(P)^{2}$$



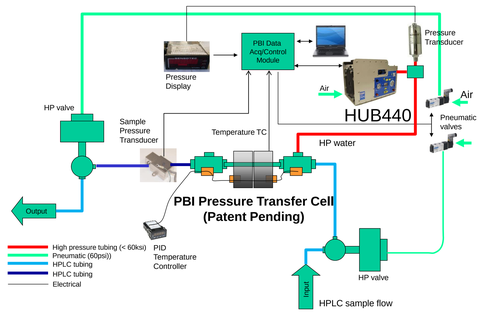
(A) Ribbon diagram of T4 lysozyme mutant T4L46R1 (PDB ID code 3LZM) showing the location of residue L46. (B) The pressure dependence of the EPR spectra normalized to the same number of spins. (C) The equilibrium constant determined from fits to the spectra is plotted as indicated *vs.* pressure (dots); the solid line is a fit to theoretical equation shown above.

LC-MS proteomics: On-line tryptic digestion in a flow-through pressure chamber

The HUB440 pressure generator has been used to pressurize the 200 µL flow-through Pressure Transfer Cell (PTC) for pressure-enhanced tryptic digestion of protein samples directly prior to UHPLC separation and analysis by FT-ICR mass spectrometer.



Hyung *et al*. Poster at the 59th ASMS Conference, Denver, CO, June 5-9, 2011 [7]



Conclusions

We have developed an automated high pressure generator capable of unattended dynamic pressure control up to 4 Kbar. The flexible software control interface has also been designed. This system enables rapid integration with a wide variety of analytical instruments such as spectroscopy and chromatography equipment.

Possible applications for the new system relevant to protein research include high pressure perturbation of protein conformation, thermodynamics of protein folding, enzyme and chemical reaction kinetics, analysis of protein aggregation and amyloid self-assembly, optimization of protein crystallization, studies of protein-lipid interactions and phase transitions of lipid bilayer, compressibility measurements, deep sea biology, vaccine development and pathogen inactivation.

The new instrument is expected to lower the barrier for adoption of high pressure research in many life sciences laboratories leading to the broader awareness of the biophysics and biochemistry research community to the benefits of high pressure bioscience.

References

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Please request the live demonstration of this device at the Pressure BioSciences booth at Protein Society 2011