Supplementary Material (ESI) for Chemical Communications

Supporting Information for

A Temperature Independent pH (TIP) Buffer for Biomedical Biophysical Applications at Low Temperatures

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1. Procedure for low temperature UV-vis spectroscopy

To obtain low temperature UV-vis spectra, a Cary 3E UV-vis (Varian, Palo Alto, CA) spectrometer was modified with a transparent double-dewar system. A transparent disposable cuvette (Fisher Scientific) was inserted and stabilized in the inner dewar. The thermometer rod was attached at the corner of the cuvette in order to measure the temperature of cuvette directly. Liquid nitrogen was poured through a plastic funnel into the dewar to control the temperature. To avoid condensation outside of the dewar, a fast stream of nitrogen was blown over the window throughout the experiments. The temperature was monitored with a digital thermometer (Digi-sense, Cole Parmer). The pH measurements at room temperature were made using an Accumet AB15 (Fisher Scientific) pH meter. Absorption ratios measured as A_{420}/A_{620} (x) were correlated to apparent pH (y) using the equation $y = 6.25x^2-1.34x-0.07$ ($R^2 = 0.993$) over the room temperature calibrated range of 6.0 to 8.6 pH units. This equation is relevant for 0.1 v/v% 1:1 mol/mol BCG:BTB dye and 50 mM buffer in 50 v/v% glycerol at a path length of 4.3 mm.

2. Protocol for protein preparation

Lyophilized human hemoglobin (Aldrich) was dissolved in 50 mM buffer solutions and centrifuged at 13 $x10^3$ rpm to separate undissolved protein. The supernatant was then oxidized with 0.9 equivalents of potassium ferricyanide. Excess ferricyanide was removed by passing the solution down a PD-10 column (GE Healthcare). Eluted protein was then concentrated using a microcentrifuge membrane (10,000 MWCO, Amicon) and stored at 4 °C until experimentation. The molar extinction coefficient of the Soret transition at 405 nm (179 $mM^{-1}cm^{-1}$) for human met-Hb was used to determine protein concentration.

3. Oxacillin degradation assay conditions

HPLC analysis was performed on a Waters Delta 600 HPLC system equipped with a Vydac reverse-phase C-18 column (5 μm, 4.6 mm). Separations of 200 μL aliquots were carried out at 25 ˚C with an aqueous methanol gradient from 20% to 96% in the presence of 0.1 % TFA while monitoring A_{220} nm as modified from literature protocol (27). Degradation was monitored by loss of peak area associated with oxacillin around T_r = 22.5 min.

4. ESI-MS conditions

Mass spectra were obtained on a Quattro II electrospray mass spectrometer in negative ion mode using 50:50 water/acetonitrile as the mobile phase. Collected HPLC samples were diluted with acetate buffer and brought to pH 7.5 with ammonium hydroxide solution just prior to injection.

5. ESI-MS spectrum of oxacillin hydrolysis product: