

## ELECTRONIC SUPPLEMENTARY INFORMATION

Hydrophobic, organically-modified silica gels enhance the secondary structure of encapsulated apomyoglobin

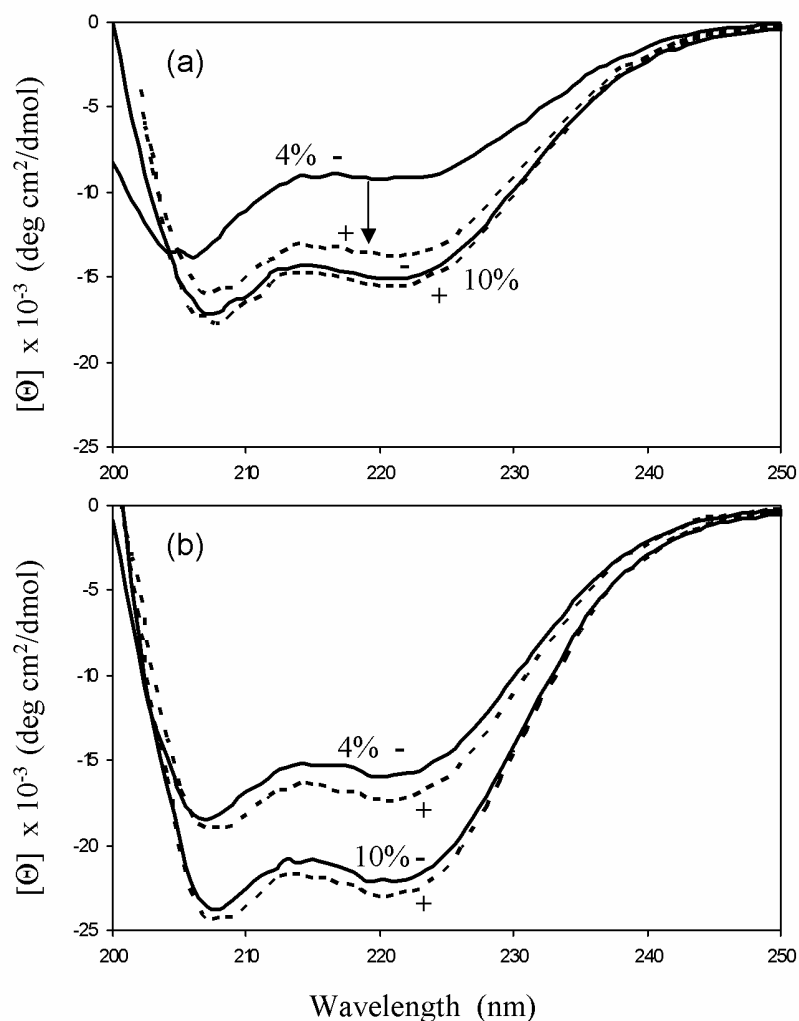
### ***Materials and Methods:***

Apomyoglobin was prepared by 2-butanone extraction of myoglobin from horse heart (Sigma-Aldrich, M-1882) with subsequent dialysis against 5.0 mM potassium acetate buffer, pH 5, containing 1.0 mM dithiothreitol and 0.50 mM EDTA. Following clarification with a 0.22  $\mu\text{m}$  syringe filter, the apomyoglobin stock concentration was determined by CD using a molar ellipticity of  $-19,000 \text{ deg cm}^2/\text{dmol}$  at 222 nm. After prolonged storage at 5  $^\circ\text{C}$ , the structural integrity of apomyoglobin was confirmed by reappearance of the Soret band at 408 nm upon heme addition. Dried chicken egg white lysozyme (Sigma-Aldrich, L-6876) was reconstituted in 2 mM potassium phosphate, pH 7, and the stock concentration was calculated by CD using a molar ellipticity value of  $-10,000 \text{ deg cm}^2/\text{dmol}$  at 222 nm. Human serum albumin (Sigma-Aldrich, A-8763) was also reconstituted in 2 mM potassium phosphate, pH 7, and the stock concentration was determined by UV-Vis absorption using the formula  $A_{280}^{1\%} = 5.8$ . The final concentration of protein in each glass ranged from 0.30 - 0.55 mg/ml, based on the total volume of the glass.

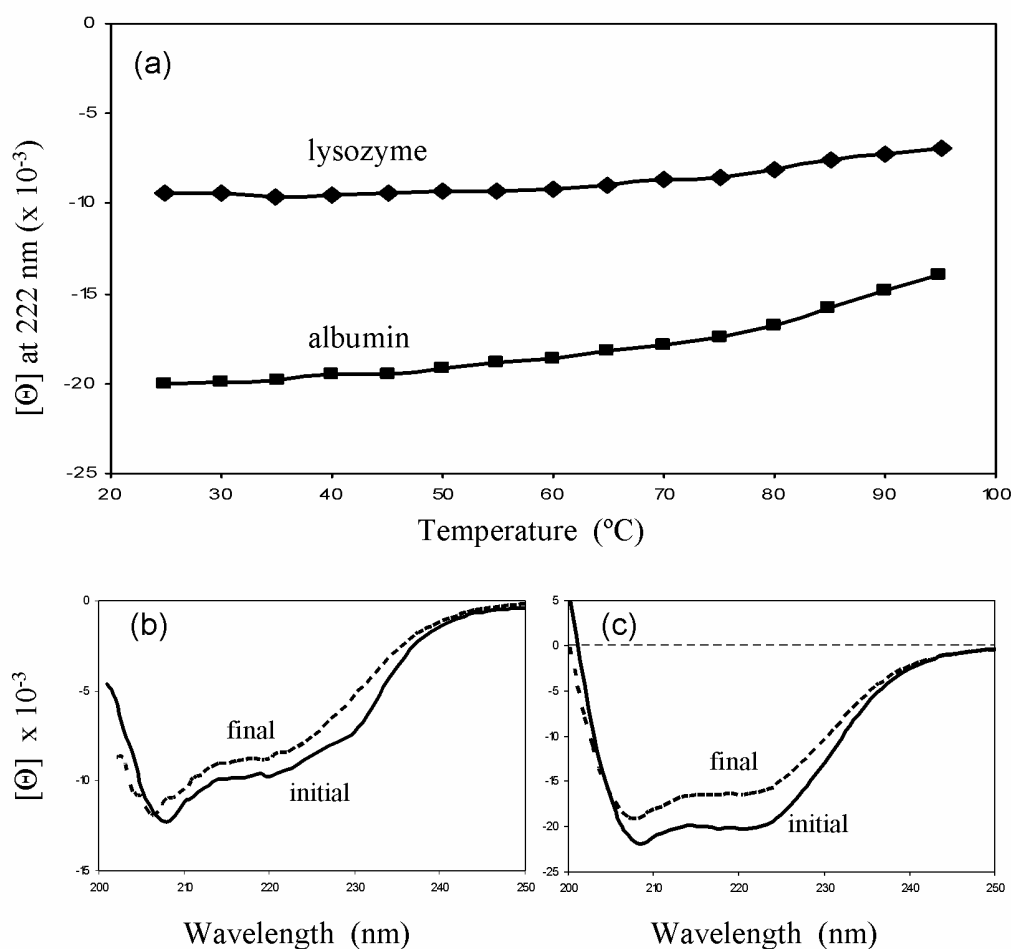
Proteins were encapsulated in sol-gel glasses prepared from tetramethoxysilane (Aldrich, #341436) following the protocol of Eggers and Valentine.<sup>4</sup> Modified sol-gel glasses were supplemented with either trimethoxy(propyl)silane (Aldrich, #34,403-6), or (3,3,3-trifluoropropyl)trimethoxysilane (Gelest, #SIT8372.0). Molar content of the modified silane was calculated based on the reported density, molecular weight, and purity provided by the manufacturer. The sol was formed by mixing each reagent with tetramethoxysilane and by sonicating the silane in 1-mL aliquots with 214  $\mu\text{L}$  of water and 30  $\mu\text{L}$  of 0.040 N HCl.

Sonication was performed in contact with an ice-water bath for 20 minutes (Branson 1510 ultrasonic cleaner). Typically, a 3.10 mL volume of sol was combined with 4.65 mL of protein in 2-5 mM potassium phosphate, gently mixed, and immediately transferred to a plastic cassette with 1 mm spacing (Invitrogen, #NC2010). Samples were allowed to set for 5-10 minutes at room temperature until gelation was confirmed by eye. Following gelation, the cassette was covered with parafilm and moved to a refrigerator. Solid glasses were layered with distilled water after aging for 12-24 hours and stored at 5 °C for 2-3 weeks before use. No leaching of protein from the glasses was observed. After the wet-aging period, glass sheets were removed from the cassette housing and cut with a razor blade into small rectangular wafers for CD analysis. No shrinkage was observed with glasses containing apoMb, whereas lysozyme and albumin glasses were observed to shrink to 90% of their original length in each dimension. In the case of apomyoglobin, most glass sheets were found to stick partially to both sides of the cassette, making a portion of each sheet unusable for CD analysis. Typically, each new glass wafer was immersed in 3 mL of the desired equilibration solution for 1-3 days at room temperature before analysis.

Spectra were obtained on an Aviv Model 215 circular dichroism spectrometer equipped with a peltier-type thermoelectric cell holder. Glass samples were placed in a 2 mm path quartz cuvette filled with the corresponding equilibration buffer during CD analysis. All wavelength spectra were collected three times at 25 °C and averaged prior to subtraction of the background and conversion to molar ellipticity. In the cases of lysozyme and albumin, shrinkage of the glass was considered in the calculation of molar ellipticity. Spectra obtained in the presence of guanidinium chloride were truncated at 215 nm due to high sample absorbance. Thermal stability curves were recorded in 5 degree steps with a 30-second delay at each temperature before recording the ellipticity at 222 nm.



**Fig. 3** Response of glass-encapsulated apomyoglobin to high concentrations of potassium phosphate at pH 7.0. (a) Propyl-modified glasses made with two concentrations of propyltrimethoxysilane, 4% and 10%, were incubated with 10 mM potassium phosphate (solid line, -), or 1.0 M potassium phosphate (dashed line, +). (b) Trifluoropropyl-modified glasses made with two concentrations of trifluoropropyltrimethoxysilane, 4% and 10%, were incubated with 10 mM potassium phosphate (solid line, -), or 1.0 M potassium phosphate (dashed line, +).



**Fig. 4** Thermal stability of chicken egg white lysozyme and human serum albumin in 8% trifluoropropyl-modified silica glasses. (a) Thermal denaturation of lysozyme (triangles) and albumin (squares), as monitored by the CD signal at 222 nm. The reversibility of thermal denaturation was tested by repeating the wavelength scan after returning each glass sample to the initial temperature of 25 °C. The initial spectra, before heating, and the final spectra, after heating and cooling, are indicated on the graphs for (b) lysozyme, and for (c) albumin. Both samples were immersed in 10 mM potassium phosphate, pH 7.0.