Electronic Supplementary Material (ESI) for Soft Matter
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β-Relaxation Dominates Protein Stability in Sugar Glass Matrices

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Supplemental

Methods & Materials

All enzymes, reagents, and glassformers were obtained from Sigma.‡ All materials were used as received. The peroxidase (HRP) was type II from horseradish, and the alcohol dehydrogenase (ADH) was from bakers' yeast. The bovine serum albumin (BSA) was fraction V. Dextran of 70k and ficolls of 70k and 400k weight-average MW were used in these studies. These gave no discernible difference in results. Polyvinylpyrrolidone was K = 29 to 32.

Sample Preparation All samples were made in aqueous solution in preparation for freeze-drying. Solutions contained 100 mmole/L CaCl2, 300 g/mL Tween 20, 0.5 % by mass BSA, and 60 nmole/L enzyme for stabilization (HRP or ADH). All solutions contained a combined glassformer concentration of 20 % by mass, except solutions containing polyvinylpyrrolidone (PVP), which were 13 % by mass. Solutes were added to buffer at 20 % by mass. Salt and surfactant were first added to stock solutions of glassformer and plasticizer. After these were mixed well, the BSA and enzyme to be stabilized were added. HRP solutions were made up in 50 mmole/L histadine buffer (pH 6.0), and ADH solutions were made up in 50 mmole/L Tris buffer (pH 7.0). All solutions were made with milliQ water. Each sample was divided into aliquots of approximately 150 µL previous to freeze-drying. The aliquots were dispensed into 1.7 mL microfuge tubes, then placed uncapped into the freeze-dryer for lyophilization. Table 1 gives a typical freeze-drying protocol. After completion of the final drying step, the glassy samples are removed from the freeze-dryer and immediately capped in a dry environment to prevent excessive reabsorption of moisture from the ambient air. We determined that there was typically 0.01 mass fraction residual water. Dried masses were consistent with low molecular weight glassformers (antiplasticizer) not being lost during the freeze-drying. This is noteworthy, because some antiplasticizers, such as DMSO, would, in the pure state, be completely lost to evaporation during the last stage of the freeze-drying protocol.

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‡ Certain commercial equipment, instruments, or materials are identified in this paper in order to specify the experimental procedure adequately. Such identification is not intended to imply recommendation or endorsement by the National Institute of Standards and Technology, nor is it intended to imply that the materials or equipment identified are necessarily the best available for the purpose.

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Enzymatic Assay Assays of enzyme activity were carried out in 96-well plates on a Ceres UV 900 HDI plate reader (Bio-Tek Instruments Inc). Standard colorometric methods were used to assay enzymatic activity of both HRP [1] and ADH [2]. Calibration curves were established with standard solutions of enzyme, obtained by serial dilutions of a known concentration of fresh enzyme. HRP concentrations for calibration curves ranged from 10 nmole/L to 10 pmole/L, and those for ADH calibration ranged from 25 nMole/L to 2.5 pmole/L. The nominal enzyme concentration of the rehydrated, stabilized-enzyme aliquots is 7.6 nmole/L in all cases, giving us three orders of magnitude over which we could reliably measure enzyme activity. In all assays, rates of change of optical density are established by acquiring data for 7 min, with readings at 5 s intervals.

Evaluation of Formulations Values of τ_d were determined by rehydrating freeze dried protein / glass samples after varying times at fixed temperature, and measuring the residual enzymatic activity relative to the enzyme activity of the freshly freeze-dried glass. We observed that enzyme activity (both HRP and ADH) showed an initial exponential decrease with time under heat-stress. We occasionally observe another, slower activity decay after extended heat stress. Similar bimodal activity decay has been seen before and has been linked to structural collapse of the freeze-dried cake [3]; the values of τ_d quoted here are all for the faster initial decay.

Identity of Glassforming Systems The glassformers in which HRP or ADH were sequestered to yield data in Figure 2 are presented graphically below with their relative degradation rates:

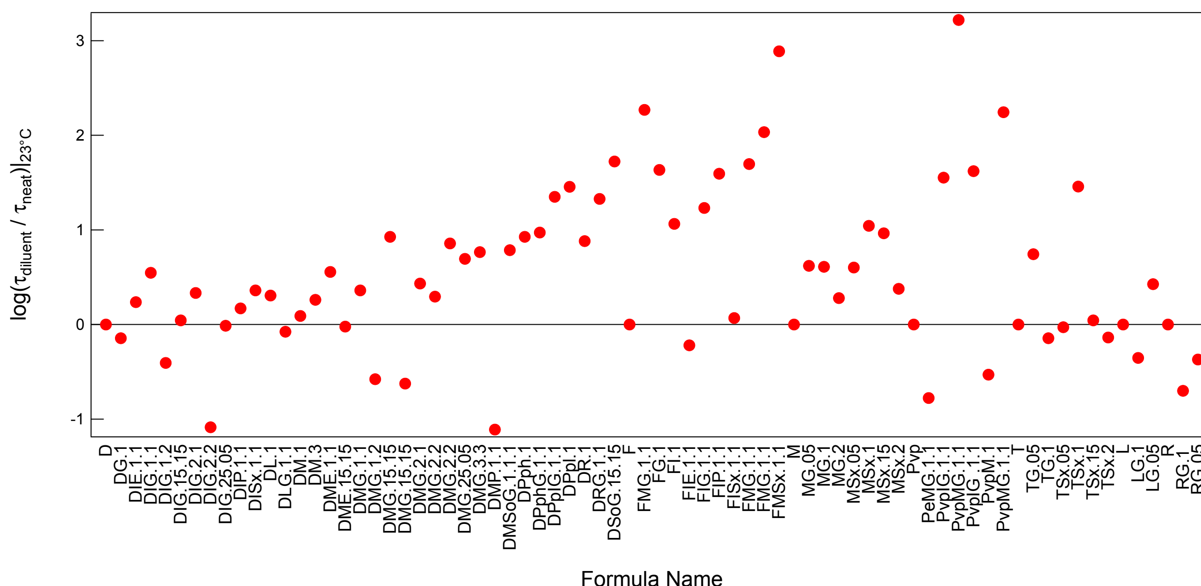

Figure S1: Formulas used in the data of figure 2 in the main paper. On the abscissa are listed formulation names using the convention: XYZ.yy.zz, where X is an abbreviation for the first major component of the glass, Y is an abbreviation for the next most abundant (usually the first plasticizer), etc. yy is the mass fraction of second most abundant glassformer, zz the third, etc. The mass fraction of the most abundant glassformer, xx = 1-yy-zz. On the ordinate are plotted degradation rates of HRP in these plasticized or antiplasticized glasses, relative to degradation rates in the unplasticized glass, which are designated by a single letter on the abscissa. The codes for substances are:

D – dextran

G – Glycerol
I – Inulin
E – ethylene glycol
L – lactose
M – Maltitol
P – Propylene glycol
So – Sorbitol
R – Raffinose
Ppl – Poly(propylene)glycol – 425 MW
Pph - Poly(propylene)glycol – 3000 MW
F – Ficoll
Sx – Dimethyl sulfoxide
Pvp – Poly(vinly)pryllodone

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