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Electronic Supplementary Material

A Novel 3D-printed Centrifugal Ultrafiltration Method Reveals in vivo Glycation of Human Serum Albumin Decreases its Binding Affinity for Zinc

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Isolation of Human Serum Albumin from Plasma. Plasma was obtained from a donor with type 1 diabetic (T1D) via venipuncture. Isolated plasma from various patients with diabetes (Type 2) was ordered and obtained from BioIVT (Hicksville, NY) and stored at -20°C. The day of separation, plasma was thawed at room temperature and 550 mL of isolated plasma was diluted to 1,100 mL with phosphate buffered saline (PBS, 1.44 g Na_2HPO_4, 0.2 g KCl, 8 g NaCl, 0.24 g KH_2PO_4, at pH 7.4) in a 15 mL tube. PureProteome™ albumin magnetic beads (Millipore, Burlington, MA) were placed into another 15 µL tube from the stock vial totaling a volume of 3 mL. These magnetic beads were washed three times with 10 mL PBS by placing the 15 mL tube into a magnet and decanting the excess solution. A 550 µL aliquot of equal parts plasma (275 µL) and PBS (275 µL) was added to the tube containing the washed magnetic beads and this was incubated at room temperature on an orbital shaker (260 rpm) for one hour. The 15 mL tube containing plasma, PBS and magnetic beads was put into the magnet and the excess liquid was decanted off. The sample was then washed three times with 10 mL PBS. Human serum albumin (HSA) was isolated from the beads via the addition of 3 mL of glycine buffer (0.1 M glycine, pH 3.0), and subsequently incubated at room temperature for 1 minute. The tube containing glycine buffer, HSA, and magnetic beads was put into the magnet. The solution was removed and put into another 15 mL tube containing 50 uL of 40 mM TRIS buffer (pH 8.0) to correct for the acidic pH. This process was repeated two additional times. All washes were combined, totaling a final volume of approximately 9-10 mL. This solution was added to an Amicon Ultra-15 ultrafiltration centrifugal filter unit (10 kDa MWCO, Millipore, Burlington, MA) and diluted up to 15 mL with DDI H_2O. This filter unit was put in a centrifuge for 17 minutes at 3060*g*. Following each centrifugation step, the filtrate was discarded. This step was repeated six additional times, for a total of seven washes. After the final wash, the HSA solution above the membrane was transferred into a 50 mL tube. This solution was lyophilized and stored at -20°C.

Separation of enriched glycated HSA using Boronate Affinity Chromatography. Wash buffer (50 mM HEPES, 0.5 M NaCl, pH 8.0-8.5) and Elution buffer (100 mM sorbitol, 50 mM HEPES, 0.5 M NaCl, pH 8.0-8.5) were created the day of experimentation. A gravity-flow column (Takarta, Mountain View, CA) was put in the upright position and glycoprotein enrichment resin (Takarta, Mountain View, CA) was added. An HSA/Wash Buffer solution was added to the column and incubated at room temperature (25°C) for 20 minutes on an orbital shaker (260 rpm). Subsequently, the column was washed four times with wash buffer. The Elution Buffer was added, and the enriched glycated HSA (gHSA) was eluted off the column and collected. The enriched gHSA was lyophilized and stored at -20°C until further experimentation.

Fabrication and 3D-Printing Process of Centrifuge-Enabled Ultrafiltration Device. A "stacked-printing" method was employed, as previously described by *Castiaux et al*. This allowed for membranes to be integrated directly into the device without the use of any sacrificial support material, in addition to allowing material changes along the Z-axis. The printer was programmed to print without any bed of sacrificial support material by accessing the Stratasys Parameter Manager software and changing values under the title of *carpet height*, *carpet protector Z*, and *improve support thick of pedestal* to 0 mm. The first layer of the model was printed from a hard plastic (1 mm, VeroClear) containing four holes to allow fluid across. Then, without removing this model from the build-tray, the height of the build tray (z-axis) was lowered by exactly the height of the model, 1.0mm). Then, the next layers were printed directly on top of this model. By printing without a bed of support-material, successive layers fuse together to create one solid device. The next three layers were made from a rubber material (0.1 mm, Tango) in a ring-like shape to hold both membranes in place. These were printed in the order of Tango, polycarbonate membrane (0.1 mm), Tango, Dialysis membrane (12 kD), and Tango. Lastly, the top layer was made from VeroClear (15 mm) containing four holes at the bottom and a hollow cup-like structure to hold a liquid sample. In all cases, the build tray height was corrected for and lowered based on the height of the previous material printed.

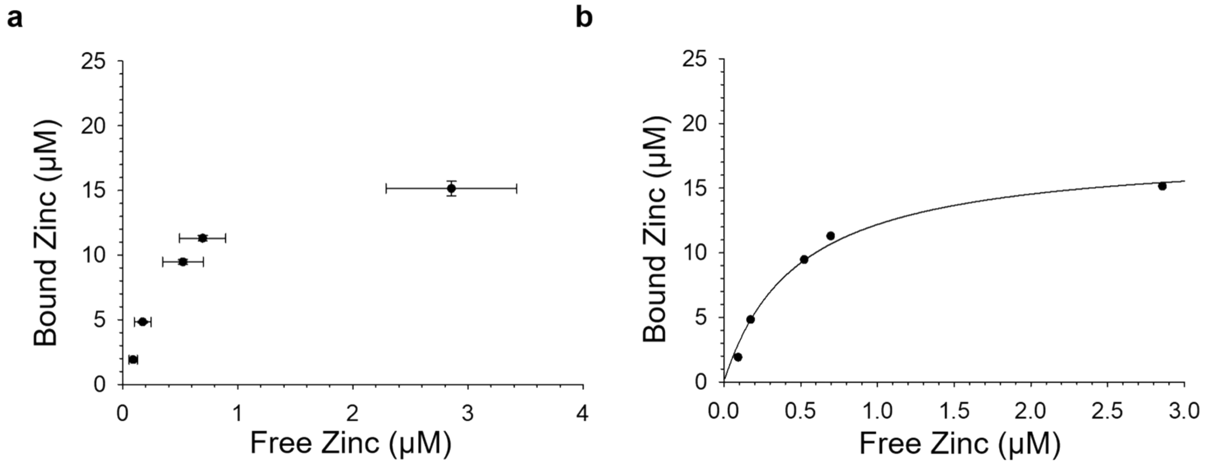

Figure S1. Zn^{2+} Binding to Enriched gHSA. In (a), the binding data was graphed (n=5-6, error=s.d.), and in (b) the data was analyzed using non-linear regression software (SigmaPlot 13.0) enabling a binding constant (K_d = 4.8 (± 0.8) × 10^{-7} M) and stoichiometry (B_{max} = 18.0 (± 1.1) μM, *n* = 1.2 ± 0.1) to be determined.