**Methods:**

**1. Recombinant expression and purification of human Sirt3 (residues 118-399):**

A fragment of the human Sirt3 gene (corresponding to residues 118-399) was cloned into a pET-21b (+) vector (Novagen, Genscript USA), resulting in an N-terminal 6x His tag and a TEV protease cleavage site upstream of the Sirt3 gene. Proteins were expressed in *E. coli* Rosetta 2 (DE3) cells (Novagen). Cells were cultured at 37 °C, 250 rpm, to an OD 600 nm of 0.3 and growth was continued at 30 °C to an OD 600 nm of 0.6. Sirt3 (118-399) overexpression was induced by equilibrating the culture to 18 °C and addition of 0.3 mM isopropylthiogalactopyranoside (IPTG). Post induction, the cell growth was continued at 18 °C for 24 hrs. Cells were harvested by centrifugation, resuspended in lysis buffer (25 mM HEPES-NaOH, 500 mM NaCl, 5 mM 2-mercaptoethanol, 5 mM imidazole, 5 mM MgCl2, 5 mM adenosine triphosphate, 5% glycerol, pH 7.5) supplemented with 0.1 mM phenylmethylsulfonylfluoride (PMSF) and 1 mg/ml lysozyme. The homogenized cells were lysed by stirring gently at 4 °C for 30 min, following which they were sonicated. The cell lysate was centrifuged at 14,000 × *g* at 4 °C for 25 min and the supernatant was loaded on to a 5 ml His-trap column (GE Healthcare), attached to an AKTA pure FPLC system (GE Healthcare), pre-equilibrated with equilibration buffer (lysis buffer with 20 mM imidazole, pH 7.5). The column was washed with 10 column volumes of wash buffer (lysis buffer with 75 mM imidazole, pH 7.5) and Sirt3 (118-399) was eluted using a linear gradient of wash buffer and elution buffer (lysis buffer with 500 mM imidazole, pH 7.5). The eluted protein fractions were pooled and dialyzed into lysis buffer, overnight, at 4 °C. The dialyzed sample was loaded on to the His-trap column and subjected to a second round of purification using the above method. Eluted fractions were checked by SDS-PAGE and pure fractions were pooled together and dialyzed overnight at 4 °C into dialysis buffer (25 mM Tris-HCl, 100 mM NaCl, 5 mM dithiothreitol, 10% glycerol, pH 7.5). Purified Sirt3 (118-399) aliquots were stored in – 80 °C till further use.

**2. Dynamic light scattering (DLS):**

Data were collected at a regulated temperature of 25 °C using a Dynapro Nanostar (model WDPN-08, Wyatt Technology, Alliance Protein Laboratories, Inc., USA) using a 1 µl quartz scattering cell. Prior to use, the samples were centrifuged at 10,000 × *g* for 10 minutes. Typically 25 ten-second data accumulations were recorded and averaged to improve signal/noise. The resulting data were analyzed with the Dynamics version 7.1.8.93 software provided by the manufacturer. Mean (*z*-average) sizes are based on the cumulants method. Size distributions were calculated using the Dynals analysis method, with the resolution set at the default value. Weight fractions were estimated using the Rayleigh spheres model. The instrument calibration was absolute, based on units of time and distance (with distance measured by the wavelength of the light source). Since the sample was in buffer containing 10% glycerol, the viscosity and refractive index of the buffer were assumed to be equivalent to those for 10% glycerol within the 1-2% precision of this technique.

**3. Size exclusion chromatography (SEC):**

A superdex-200 column (1 × 30 cm, GE Healthcare, Alliance Protein Laboratories, Inc., USA) was equilibrated with 0.1 M Na phosphate, 0.2 M arginine, pH 6.8 at a flow rate of 0.5 ml/min or with 0.1 M Na phosphate, 0.5 M NaCl, pH 7.2 at a flow rate of 0.2 ml/min. A 0.1 ml aliquot of 4 mg/ml Sirt3 (118-399) sample was injected into the column. The elution was monitored with the absorbance at 280 nm.