**Commentary on DLS results for Enzo Sirt3 (E-Sirt3) and truncated Sirt3 (T-Sirt3):**

**E-Sirt3:**

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* The main peak by intensity, which represents 78.1% of the total scattering intensity, is the second peak, which has a mean radius (the intensity-weighted average across the peak) of 33.6 nm. That radius corresponds to a molar mass of 13 MDa, based on globular protein standards.
* The polydispersity index of this main peak is moderately high at 22.4%, indicating this single peak may include contributions from multiple different species.
* The first peak has a mean radius of 5.51 nm, which corresponds to an estimated molar mass of 180 kDa, and a moderately low polydispersity of 12.5%.
* Although this peak represents only 18.6% of the dynamic scattering intensity, it is estimated to represent 22.4% of the total on a weight basis.
* In addition to the main peak two peaks at larger radii were detected, at mean radii of 444 nm and 27.7 μm.
* it is not possible to make meaningful fraction by weight estimates for species larger than ~1 μm because (1) the scattering from particles significantly larger in diameter than the wavelength of the incident light is very dependent on the detailed shape of the particle (due to internal reflections), and (2) nearly all the scattered light is emitted in the forward direction, with only a tiny fraction at the 90° angle observed here.

**T-Sirt3:**

* The scattering intensity for this sample (shown below) is dominated by the third peak, a broad peak at 122 nm (97.7% of the total intensity).
* Although the first peak at 3.75 nm (74 kDa estimated molar mass) is only 0.6% of the total intensity (and thus only marginally above detection threshold), it is estimated to represent 63.8% of the total on a weight basis.
* A minor peak was also resolved at 14.3 nm (1.7% of intensity).
* The expected size for the monomeric Sirt3 (based on MW ~ 33 kDa) would be ~2.5 nm, but it was not observed.

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**Commentary on truncated Sirt3 vs full length Sirt3:**

1. **Crystal Structures of Human SIRT3 Displaying Substrate-induced Conformational Changes**

**Jin et al., JBC 2009**

* The author’s mention that recombinant SIRT3 (102–399) did not crystallize readily. During the course of their studies, they designed several truncations of SIRT3. SIRT3-(118–399) expressed abundant soluble protein in E. coli and could be purified to high purity.
* In addition, SIRT3 (118–399) and SIRT3 (102–399) had similar deacetylation activity and responded similarly to a panel of SIRT3 modulators (data not shown). Therefore, SIRT3-(118–399) was selected for crystallization work.
* The two enzymes have similar Km values for the peptide substrate (33.0 uM for SIRT3 (102–399) and 28.7 uM for SIRT3 (118–399)) and for NAD (600 uM for SIRT3 (102–399) and 598 uM for SIRT3 (118–399)).
* They also displayed very similar activity for their deacetylase function (0.0037 for SIRT3-(102–399) and 0.0033 for SIRT3-(118–399) in the unit of percentage of conversion of acetylated peptide substrate to deacetylated product over time and enzyme concentration.
* This is in disagreement with a recent report that SIRT3 (114–399) has a ~50-fold-higher specific activity than SIRT3 (102–399).
* The cause of the discrepancy of activity between SIRT3 (118 –399) and the published SIRT3 (114 –399) is not clear.



1. **Substrates and Regulation Mechanisms for the Human Mitochondrial Sirtuins Sirt3 and Sirt5**

**Steegborn, JMB 2008**

* Another mechanism for the regulation of Sirt3 activity appears to be proteolytic processing: Removal of the N-terminal signaling sequence through proteolytic cleavage at position 101 occurs during translocation into the matrix and activates the inactive full-length precursor.
* A second cleavage might occur at position 110 and the influence of further truncations of Sirt3 to its activity in the peptide-based assay, was tested.
* Indeed, an N-terminally shortened construct (residues 114–399) displayed a ∼50-fold-higher specific activity than residues 102–399 in the peptide-based deacetylation assay.
* Removing the C-terminal part of Sirt3 resulted in a protein (residues 114–380) with further increased specific activity in the peptide assay, but only ∼2-fold compared to Sirt3 (114–399) and thus ∼100-fold compared to Sirt3 (102–399).

