**Kcat for different peptides for Sirt3**

**1)** **"Substrates and Regulation Mechanisms for the Human Mitochondrial Sirtuins Sirt3 and Sirt5", Steegborn, JMB 2008**

This analysis is based on the results presented in Figure 4a (please see below) in: "Substrates and Regulation Mechanisms for the Human Mitochondrial Sirtuins Sirt3 and Sirt5", Steegborn, JMB 2008.

Based on the figure below, which compares the activities of truncated and full length Sirt3 (using an older version of the FdL assay):



* From the figure, the specific activity of Sirt3 (114-399) is ~ 30 nmol/(min \* mg).
* From the text, the specific activity of Sirt3 (114-399) is ~50 fold more than Sirt3 (102-399). Therefore the specific activity of Sirt3 (102-399) is ~ 0.6 nmol/(min \* mg).
* From the comparative FdL assay (using FdL2 peptide) I just did with in-house truncated Sirt3 and urea Sirt3, taking only the 1 mM NAD dataset for both urea and truncated, the deltaAFU in the 30 min reaction is ~84 and 39 for truncated and urea Sirt3, respectively.
* Using a slope of 180 AFU/uM, the deltaAFU values correspond to 0.47 uM/30 min and 0.22 uM/30 min, or, 0.016 uM/min and .007 uM/min for truncated and urea Sirt3, respectively.
* For the in-house truncated Sirt3, 5 U of which was used in the rxn (50 ul volume), and whose specific activity was measured to be ~0.4 U/ug, expressing the above rate (0.016 uM/min) in the units presented in figure 4a above: = (0.000016 nmols/min \* 0.0125 mg \* uL) \* 50 uL = 0.64 nmol/(min \* mg).
* For the in-house urea Sirt3, 5 U of which was used in the rxn (50 ul volume), and whose specific activity was measured to be ~1 U/ug, expressing the above rate (0.007 uM/min) in the units presented in figure 4a above: = (0.000007 nmols/min \* 0.001 mg \* uL) \* 50 uL = 0.35 nmol/(min \* mg).
* From the HPLC assay using MnSOD (K122) peptide and in-house truncated Sirt3, the average value for # of pmols produced from the 4 data sets (8 mM and 24 mM) is 4306.75 pmol, in a 30 min rxn with 6 U truncated Sirt3 (~0.015 mg).
* Expressing the above pmols produced in units for specific activity in figure 4a above: (4306.75 pmol/30 min \* 0.015 mg) = 9.5 nmol/min\*mg, for truncated Sirt3 deacetylating MnSOD (K122) peptide.

Based on the above analysis:

1. Since the Figure 4a above was from an FdL assay using the p53 (FdL2) peptide, it could be directly comparable to our in-house FdL results comparing truncated and urea Sirt3.
2. From the figure the specific activity of full length is ~ 0.6, whereas our urea treated full length is ~ 0.35 (not surprising since ours is urea treated).
3. From the figure above specific activity of truncated is ~30, whereas our truncated is ~0.64.
4. It is a little surprising that out truncated has similar specific activity as their (non-urea treated) full length Sirt3.
5. However, the above result makes sense if we recall that Jin et all, 2009 JBC also found that the truncated and full length have similar specific activity.
6. Overall, I think our truncated Sirt3 is in good shape.

**2) “Kinetic and Structural Basis for Acyl-Group Selectivity and NAD+ Dependence in Sirtuin-Catalyzed Deacylation”, Feldman 2015 Biochemistry**

For the Denu substrate (AcQTARKacSTGGKAPR-WW-NH2), they report a kcat of 3.3E-1 (+/-) 1E-2 per sec, using 0.2 uM Sirt3.

The concentration of truncated Sirt3 in my assays were ~10 uM.

For my assay using truncated Sirt3 and MnSOD peptide, the product formed over 30 min rxn was 98.95 uM, corresponding to a Vmax of 0.055 uM/sec.

Using their concentration of total enzyme used: 0.2 uM, the Kcat = Vmax/E0 = 0.055/0.2 = 0.275/sec, which is close to their published value.

**3) Urea-treated Sirt3 (102-399) and MnSOD (K122) peptide:**

Based on AU’s HPLC assay datasets below using 5U (3.2 uM) in-house urea treated full length Sirt3 and MnSOD peptide, the Vmax is 0.938 uM/min.

Using 3.2 uM E0, the Kcat for this is ~ 0.005/sec.

|  |  |
| --- | --- |
|   | **Set 1 data, BUFFER** |
| Time | 50 | 100 | 500 | 1000 | 3000 |
| 10 | 3.106 | 4.248 | 8.863 | 9.324 | 7.858738 |
| 20 | 5.012 | 7.369 | 16.138 | 18.386 | 17.33565 |
| 30 | 7.363 | 9.469 | 22.717 | 24.955 | 22.17285 |
| 40 | 8.326 | 12.763 | 26.225 | 30.546 | 29.76995 |
| 80 | 14.296 | 19.794 | 48.811 | 52.948 | 53.47091 |
| 120 | 17.993 | 26.650 | 59.414 | 71.434 | 73.30632 |

|  |  |
| --- | --- |
|   | **Set 3 data, Buffer** |
| Time | 50 | 100 | 500 | 1000 | 3000 |
| 10 | 3.128 | 4.189 | 8.474 | 8.164 | 6.979609 |
| 20 | 4.976 | 7.293 | 14.893 | 15.62 | 16.44558 |
| 30 | 6.882 | 9.525 | 20.826 | 22.737 | 20.99236 |
| 40 | 8.073 | 12.801 | 27.600 | 29.715 | 28.87768 |
| 80 | 13.457 | 21.041 | 44.800 | 51.266 | 51.43471 |
| 120 | 17.005 | 25.171 | 62.967 | 70.116 | 72.87568 |

**4) Kcat for in-house truncated Sirt3 and in-house urea treated full length Sirt3 with FdL2 peptide:**

Truncated Sirt3: Vmax = 0.016 uM/min; E0 = ~7.5 uM, therefore Kcat = ~0.002133 / min

Urea-treated full length: Vmax = .007 uM/min; E0 = ~3.2 uM, therefore Kcat = ~0.0021875 / min