These tasks can be addressed by the person most familiar with each, assuming that person is free to do so. You can discuss amongst yourselves as needed for allocation of tasks. I assume you will have time during HPLC runs, e.g., to work on these.

Please review all below and let me know if some are already finished, where they can be found. Can post figs in dropbox and updates to this doc on wiki. Unless otherwise noted, assume the modulator in question is HKL. Update as tasks finish.

**RC: Updated materials should go in dropbox**

Note the figs and tables need to prepared in pub quality format with consistent annotations, etc, if not already done.

**General question: What is part a and b refer to?**

**RC: If the figure says “2 double reciprocal” that means they are parts a,b**

**Figures**

**1) 2 double reciprocal mixed with respect to NAM w, wo 200 HKL; MnSOD; parts a,b**

**Delayed as Praba via RC**

**2) 2 double reciprocal mixed with respect to NAM w, wo 200 HKL; FdL; parts a,b**

**By XG**

**Annotate x coord of intersection of lines as before. Also, add the following inset for MnSOD**: double exponential times series fit for two data points in double reciprocal, same y axis scale and tick marks for both. Do this for both w, wo HKL (pts a,b). Two options for the choice of two data points: a) 0 and 200uM NAM, 100uM NAD (a vertical box can be drawn around the 0 and 200uM NAM). Color code the time series lines in colors corresponding to the colors of the double reciprocal lines for 0 and 200uM NAM in this case. b) 200uM NAM, 100 and 3000uM NAD. No vertical box in this case – separately zoom in on the two points for two insets. For FdL, choose any two experiments for inset because they are relatively similar in time series. Present single exp fit in latter case. Scale should be the same for both w,wo HKL and inset should focus on the first 30 mins

XG: What do two data points refer to?

Do you mean: two initial rates? The insets are the "uM vs. Time" curves separately?

RC: Two initial rates that would be plotted in the figures. Yes.

In this figure, it includes 2 double reciprocal plots 1/v vs. 1/[NAD+]

(1) at different NAM without HKL

(2) at different NAM with HKL

Then, choose two data points (initial rate point), zoom in and have two insets.

The inset should be prepared using single exponential for time series fit (uM vs. Time).

RC: Yes. Two data points for each a,b.

**3) 2 double reciprocal initial rate dose response, 0 and 200 HKL in each plot (for two nam – 0,5mM); MnSOD**

**Delayed**

**4) 2 double reciprocal initial rate dose response, 0 and 200 HKL in each plot (for two nam – 0,5mM); FdL**

**By XG**

**5) 2 Dixon (mnsod only, for two nad – 3000,15000).** Include all the annotations we have used for Dixons including plateau, etc

**Delayed**

**All above should of course include both data points and model predicted curves**

**RC will edit the eqns in the figs as needed. Provide RC with an editable version of these figs.**

**6)** **Dose response of HKL – a-c) for MnSOD in the three formats we studied – 30 min, 50uM NAD; 10 mins, 100uM NAD; 10 mins, 100uM NAD/100uM NAM. At unsat NAD. Leave c) empty for now**

Alternatively, may superimpose all three for mnsod color coded on same axes w legend – do both

**Question: This task is MnSOD related, should this be delayed as well?**

**RC: No**

**Are you referring a,b and c as follows?**

1. **30 min, 50uM NAD**
2. **10 mins, 100uM NAD**
3. **10 mins, 100uM NAD/100uM NAM (leave this blank?)**

**By AU and XG.**

**RC: Do that and also make the alternate superimposed version which would be just a) with all three. In that version b) can be unsat peptide dose response.**

**7)** **Dose response of HKL – for FdL. At unsat NAD**

**By XG but need to double check for the data.**

**8) Dose response of DHP for FdL (see also below)**

**If you have unsat peptide dose response available as well, please provide those but they are lower priority at this time.**

**Provide dose responses on both linear and log scales**

**Note: may need to add unsat peptide substrate dose response to show that we have rigorously evaluated whether they are activators. Start w above**

**Question: Is unsat pep exp for both FdL and MnSOD or just for FdL or just MnSOD?**

**RC: If you have it in both cases, provide it. Otherwise, it’s not essential.**

F**ig captions mentioning HKL and new concs (instead of DHP and old concs as in biorxiv) for above figs. Some of above like 3-5 were prepared for MnSOD in past (no captions). Also, some of above were (like part of 6) already prepared for patent, can use those as a starting point.**

**9) MST overlays with two curves (e.g., w, wo HKL) on same axes:** so far, many such figures were presented one on top of the other. Will advise on whether they needed be plotted on same axes – don’t revise yet. Example captions can be added to a couple of these plots in the ppt that you posted to wiki. Will review and then revisions can be made based on those examples.

**By SM**

See ppt posted by XG/SM on wiki

-remove slides 1-3

-Remove sirt3 plus nad from last fig in ppt.
Keep other nad complexes

those are where the measurements were made.

-report the Kds (w std errors If available) directly in the figure itself (next to the respective curves)

-Need to prep analogous MST comparative figs for c-NAD – both substrates, with and without sat HKL on same axesAcmnsod 0 and sat HKL titrated c nad in same fig. Same for acp53 w and wo amc (two versions of fig. Total three figs

-make a fig w deac peptide titrated w oaadpr (no HKL) if we have it. We should also have deac peptide titrated w nad (no hkl) in same fig. Also, include deac peptide plus oaadpr plus NAM in same fig [then consider two squares as a,b]

-for ac mnsod keep the fig in the ppt w wo nam w titrated hkl.

-for carba nad figs and acmnsod w wo nam w titrated hkl, each interaction studied can be represented visually by a square face excerpted from the cube fig in biorxiv. The squares are in the attached ppt. Take these excerpts from the cube and place them alongside the superimposed mst fig as parts a,b of each of the two above figs. (You can see that these squares apply to the E.AcPr plus titrated cNAD w wo HKL mst fig and the E.AcPr plus titrated HKL w wo NAM mst fig respectively.)

-highlight in bold those sides of square that are being measured in that mst fig
-Present one example fig of T-SIRT3 MST – AcPr and titrated NAD, w wo HKL on same axes to show effect of different construct

Sudipto could potentially make the mst figs

Guan to provide std errors on mst fittings? Or 2bind? They to decide

RC: Guan would ideally provide std errors on the Kds. But the first draft can omit it if it takes longer. I would like to see a draft version of a couple of these asap along with captions to make sure they are on right track.

**Following Fig data are not yet available but the format is suggested when available:**

**10) OAADPR inhibition and 2xe0:** Here, pairs of time series plots can be shown for comparison in each case (two figures with parts a,b in each case). Captions should be prepared. For the 2xe0 case, the initial rates calculated according to two different methods (one being an omit 0 method with single exponential) should be reported as well. The two fittings and associated rates can be shown directly in the figures.

**By AU/XG**

**Tables**

**Question on table 1 and 2: Are these delayed as it has MnSOD?**

**RC: MnSOD table is delayed for now.**

**1) Table(s) of time series fittings: all the time series fitted parameters and std errors of those parameters for double exp, MnSOD only.** Organize in one table. Along with table header

**2) Tables of eq 1 and mixed inh fittings (MnSOD and FdL, respectively) including all results reported by Prism.** Along with Table headers. Omit 0 min from MnSOD for now, single exp for FdL. Already presented but need in consistent format with headers (can revise old headers from biorxiv or patent).

Add e0 (Bradford) for respective batches to the eq 1 and mixed fitting tables and also mention e0 for the t sirt3 batch used for mst in the mst caption  If multiple measurements were made, provide mean and standard errors.

**By XG: Table 2 FdL (assuming MnSOD is delayed)**

**3) Solubility data on dhps and HKL (as in patent)**

**By AU/SM**

**Methods**

**1) MST method para (including description of labeling procedure)**

**2)** **c-NAD synthesis**

Did we use custom synthesis for DHP-2? If so, provide that too.

**3) Will advise shortly on time series fitting methods – don’t start yet**

**4)** **T-SIRT3 purification (in case we choose to present any data on it)**

**5) SEC, DLS (on wiki)**

**By SM, 1, 2, 4 and 5. By XG- DHP2 symthesis write up.**

**Add these to the most complete methods writeup you already have available (e.g., from the patent)**

**False positive testing of labeled assays – add’l figures and methods**

**By XG 1, 2, 3, and 4 (whichever is available, need to double check)**

1) **DHP and HKL** **dose response for FdL substrate via hplc vs the labeled FdL assay – two parts of same fig**, **with caption**. Assuming we did this.

Need:

-Dose response hkl fdl assay, amc. If not done, at least need a bar chart comparing the conditions that were done w labeled and unlabeled assays to show no false positive.

-Dose response dhp 2 w fdl assay, amc -- any unsat conds. If not done, at least need a bar chart comparing the conditions that were done w labeled and unlabeled assays to show false positive

2) **autofluorescence scans - for both dhps and HKL checking for overlap between wavelength ranges – two parts of same fig, with caption**
3) **Discussion of** **any other controls xg did including those w custom synthesized peptides and possibly sirtainty (overlapping wavelength range).** Do not show any results from above just summarize in words. Indicate that some subtracting background autofluorescence at time 0 is not sufficient. (Change in fluorescence upon product formation cannot be accurately quantified in such cases.)
Hence such labeled assays cannot be used with compounds having overlapping wavelength autofluorescence.

**4) Describe above protocols in methods in addl para(s) under methods for fdl** (revise biorxiv or patent methods)
**Miscellaneous**

-write small para introducing the different substrates studied along with their physiological relevance (assign to lab members) -- Alok

**Question: How many substrates should be included? Only those which we are using in the lab?**

**RC: Yes only those. Can be brief. Make sure all required info about them are in methods as well.**

-write small para on how SEC and DLS were used in enzyme characterization, including summary of results – Sudipto (based on commentary 2-7-17.doc)