**In response to Xg’s Schedule-**

The documents should have provided the duplicate scenario as -footnotes to the original- with all dates as indicated several times. As noted several times the final decision on whether to implement duplicate rather than triplicate in house will be made at a later date.

They should be in final form and requiring no further review unless desired. They will be checked only after wiki posting when convenient.

There was also a question asked about increasing Hplc utilization.

AU, XG: We can use autosampler after hours to increase the HPLC utilization. However, we have to optimize the conditions to see if the samples will be stable for 7-8 hours. For example, the sample vial is open system. The total amount of sample is max. 50 ul. The sample most likely will be evaporated if it stays out for long time. This may change the concentration of samples which create error from sample to sample. We need to do experiment to confirm.

We are still waiting on sudiptos revised schedule within the next day or so.

If both old and new xy are listed it just totally clear which is which. Any old should include reference to the results from old expts.

XG: OK

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**In response to AU’s schedule-**

As noted everything mentioned in my last several emails should be addressed in this version with footnotes as needed, with complete consistency and alignment such that no further edits from me are immediately needed, and posted on the wiki.

AU, XG: OK, posted.

Sudipto's revised schedule was also requested.

Thanks

Raj

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You need to make the notes about the alternate schedule with duplication directly in the schedule docs and post them, as mentioned. Post them in a final form with alternative dates indicated. The notes can be made at the bottom of the schedules as footnotes, and must be very clear about the alternate plan and revised dates under the duplication scenario. It is not convenient to interpret the various scenarios you mentioned below in an email and provide advice at this level of detail.

AU: AU has added footnote as required.

XG: Since the initial rate experiment will be rearranged by doing triplicate or duplicate, it is hard to address it as a footnote. So the changes have been addressed in most updated schedule.

-Looks like 1 Hplc use by xg will continue into end of aug. But alok starts using 2 before that?

RC: It's not clear how your answer addresses this in the original schedule. How can Alok use 2 HPLCs before end of Aug if Guan occupies 1 till the end of August, esp since 3rd HPLC will not be available before 25th.

Appears to be a problem with the original schedule here.

Perhaps this is a minor point, if you think it can be done just indicate that in response.

AU, XG: In the revised AU/XG schedules, there is no conflict of usage of HPLC. AU will use one HPLC for expt. PMC-AU4, which aligned to XG’s.

-AU: Experiment AU2 a, b, are for saturating NAD determination. And c, d is to see if selected condition gives quantifiable product with saturating [NAD] and minimum peptide conc chosen.

RC: I know. And they are all called saturating NAD determination, which is not the case.

Add your other comment below as a footnote.

AU: OK

-xy docs: same story. I don't want the responses to be provided by email if there are changes required. I could not tell  from your email whether you are making changes to render all the tables consistent between XG and AU and address my points. I will assume you are revising them now and will post them when done. I can't review them until then since it is quite time consuming to infer what you meant if they are not clear.

RC: Where are the rest coming from and why diff from schedule?

XG: has been corrected in the new xy doc.

You showed many rows in the xy doc that have no entries for % activation. It is unclear why these rows are there if they are for old experiments.

You said xy doc is for old expts. If they are for old experiments why are there no data?

XG: In x,y doc, the old and new experiments for both XG and AU are listed.

RC: Aus xy table for proposed expts on other hand appears am same as that in schedule.
Again, for his old expt tables, there are many rows that are not yellow. What are these?

AU: The highlight ones are the old experiment conditions. The rest are for new experiments.

RC: Why are there new experiments listed in AUs xy doc whereas Guan's doesn't do this?

Why this inconsistency? We wanted everything aligned.

--You need to make it entirely clear which are old expts and which are new in each doc; you need to make sure these are presented logically and in the simplest possible manner (i.e., don't mix and old new in xy doc without explanation and assume a reader will understand everything); and you need to align everything between XG and AU.--

Raj

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--Why can initial rate Hplc not start before end of sept? Move up?

When is earliest it can go.

XG: Based on current AU schedule, until end of September, all the HPLCs will be occupied by AU. HPLCs will be available only after 9-27-2016.

Before Tecan initial rate or earlier? See next point, which proposes how to deal with this.

XG: Taking account of NEXT POINT, which suggested that AU will use 2 HPLC for duplicate, then XG can have 1 HPLC to start working on DHP initial rate experiment. The earliest date will be August 30, 2016.

--I see au is using 3 Hplc till end of sept. It is ok for him to use 3.

Make a note that if initial rate studies on Hplc were done in duplicate, how things would change in au and xg schedules.  This will likely be the preferred schedule - duplication for initial rates for both honokiol and dhp, followed by triplicate later with cro or in house when time permits (triplicate not scheduled).

You can leave schedule as is for now if it's easier but make these detailed notes that we will likely do duplication and how both schedules would be adjusted in that case.

XG: AU can use 2 HPLC for initial rate and XG can start DHP initial rate experiment (no duplicate) from Aug 30th to October 7th (one set, no repeats). The duplicate of above experiments can be done after AU finishes Honokiol work.

--Looks like 1 Hplc use by xg will continue into end of aug. But alok starts using 2 before that?

AU,XG: This is based on the previous requirement (triplicate for initial rate experiments). If we only need duplicate at this moment, AU will continue use one HPLC for his experiments and   when the new start working, he continues with new HPLC. This way AU’s schedule may be pushed back by extra ~3 days.  XG will use one HPLC for her work and continue DHP initial rate experiment as mentioned above. However, if AU’s work is more priority, XG will wait for 3rd HPLC to be functional, which is expected to start in same week. 3rd HPLC will be functional by 8-25. We will double check with Sherry. Please advise.

--When will u post first expt results and discussion. When will minimum conc need to be chosen
AU, XG: 8.12.2016.
--Present cv % on activity when you get it
OK
--Sm will need to provide a truncated sirt 3 batch after these expts.  Further initial rate expts will need to use truncated sirt3. Add to schedule

OK

--Au2 needs to be revised to indicate that c.d are not saturating peptide determination

AU: Experiment AU2 a, b, are for saturating NAD determination. And c, d is to see if selected condition gives quantifiable product with saturating [NAD] and minimum peptide conc chosen.

This reminds me, please note, when I determined saturating [peptide], I used 2 mM NAD for those reactions. If 2 mM is in fact a saturating concentration then 600 uM peptide will be saturating concentration. In another scenario, if 2 mM NAD is not a saturating concentration then I have to add another reaction to find out what will be the saturating [peptide]. This can be done in one set of the reactions.

--Xy doc - it is unclear what you are showing in this doc vs schedules.

Xgs tables in this doc are not the sane as those in schedule. 3 rows look like they were done before. Where are the rest coming from and why diff glum schedule?

XG: The tables in schedule are the conditions for current experiments. The tables in xy doc are conditions used in the old experiments.

Which of above expts are being repeated in new schedule; the ones you listed don't look like repeats of old.

XG:  The listed concentrations of [NAD+] (375, 750, 1500, and 3000uM) and [FdL2 peptide] (100uM) were the ones used in the old experiments. They are going to be repeated partially (1500uM and 3000uM NAD) in the new experiments.

Aus xy table for proposed expts on other hand appears am same as that in schedule.
Again, for his old expt tables, there are many rows that are not yellow. What are these?
AU: The highlight ones are the old experiment conditions. The rest are for new experiments.
Please align all tables.