Based on the current information from 2bind, Ascendex, Dalton and in-house results regarding c-NAD, we have the following options:

a) 2bind:

1)      They could try to make the 3 mM c-NAD solution using only HDAC, using whatever remaining “recovered” c-NAD they have left

2)      Using the above 3 mM-HDAC solution, they could estimate the Kd

3)      They could then add 5% DMSO (final) to the above solution, and if no insolubility, they could repeat the binding with < 3 mM c-NAD-5% DMSO-HDAC solution to see if changes occur

4)      If the Kd for NAD does not change upon refitting in the 3 mM range, then this range will be used for comparison to c-NAD binding

5)      If after point 3) they see insolubility, then based on lowest amount of DMSO required for HKL solubility, they can add the required % of DMSO and get binding data in presence of HKL (Ascendex can help with this).

6)      After testing in-house (if 3 mM c-NAD in 5% DMSO-HDAC is soluble), we could send them the 3 mM stock solution prepared in-house.

b) Ascendex:

1)      Based on their initial response, they would need ~50 mg c-NAD and ~ 100 ml HDAC buffer to do the solubility studies.

2)      Spoke to them again and told them that we don’t have that much c-NAD. They said that they would need to modify the procedure to accommodate a lower amount (~ 10 mg), but they are not sure if it would be possible. They will discuss and confirm.

3)      We can ask them to determine the lowest % DMSO required for HKL solubility in HDAC buffer so as to use that % for c-NAD/HKL co-binding MST studies.

c) Dalton:

1)      They haven’t yet sent us their revised proposal for maximum solubility determination.

2)      Based on their price for just the solubility studies (customized to the points we have specified), we can decide if it would be more economical to ask them to do it or Ascendex (since Dalton said they would need ~ 5 mg for $1400)

d) In-house:

1)      3 mM solubility of c-NAD in water and HDAC buffer has been tested in-house. The solutions have been frozen.

2)      It would be important to reproduce the (in)solubility of 3 mM c-NAD in 5% DMSO-HDAC buffer, in house. It is possible that either Thomas or us may have been doing something wrong, so we need to verify just this concentration point, in house. In case we see that 3 mM is indeed soluble in 5% DMSO-HDAC buffer, then we will note the exact steps we took and let Thomas know. If we do end up making the 3 mM solution, then we will freeze it and send it to Thomas.

3)      Since the microbalance has sensitivity issues, to test 3 mM solubility in 5% DMSO-HDAC buffer, we would need 1 mg c-NAD.

4)      The combined expertise of XG, AU and SM would be used to do the 3 mM solubility experiment.