07/10/16

Testing HDX sample for solubility in quench buffer

This protocol is used to assess the solubility/stability of HDX samples in quench buffer as past measurements have shown that not all proteins /protein-ligand complexes are stable under quench conditions.

Buffers and reagents:

1. HDX sample to be tested
2. Quench Buffer (400 mM KH_2PO_4 pH 2.2)
3. 5X SDS sample Buffer

Procedure:

All steps are carried out on ice or at 4°C

1. Prepare 100µl of HDX sample ($\frac{1}{10}$ of the concentration to be used in HDX-MS)
2. Mix 20µl of your HDX sample with 5µl of 5X SDS sample buffer and keep on ice. This will be the input sample.
3. Thoroughly mix the remaining HDX sample 1:1 (v/v) with quench buffer by pipetting and incubate on ice for 5 minutes.
4. Mix 20µl of the quenched HDX sample with 5µl of 5X SDS sample buffer and keep on ice. This will be the quenched sample.
5. Centrifuge the remaining HDX sample at full speed in a tabletop centrifuge for 5 minutes.
6. Carefully check the centrifuged test tube and inspect if precipitation occurred which should be visible in form of a pellet.
7. Draw off 20µl of the supernatant and mix with 5µl of 5X SDS sample buffer. This will be the after centrifugation sample.
8. In case a pellet is visible, carefully draw off the remaining supernatant without disrupting the pellet and proceed with step 9. If no pellet is visible proceed with SDS-PAGE.
9. Prepare a 1X dilution of the 5X SDS sample buffer and use 25µl to take up the pellet. This will be the pellet sample.

SDS-PAGE:

1. Boil all SDS-PAGE samples for 5 minutes at 98°C and spin down in a tabletop centrifuge at full speed for an additional 5 minutes
2. Load the samples onto a SDS gel and proceed with standard SDS-PAGE procedure