In the Molecular Interactions Facility we perform two types of analytical ultracentrifugation experiments:

- On the one hand we perform "sedimentation velocity" experiments, which provide information on the number of species present in the sample, their size, shape and their sedimentation coefficient. If the polydispersity, referring to the number of species, is low, it is easy to determine wether they are monomers, dimers, etc., since the size is calculated more accurately the fewer species are present in the sample and the purer the sample.
- On the other hand, we carry out "sedimentation equilibrium" experiments. In these we obtained the molecular weight of the molecule or complex under study. For this the sample must contain no more than two species (e.g. monomer and dimer) and be very pure. Remember that what you get is the average mass of everything in the sample, so if there is only one type of complex in the sample then the mass will match that of that complex, but if there are other species present, then it will be the average of all of theml.

  In both experiments the maximum number of samples per experiment is seven.

Normally, before performing a sedimentation equilibrium, the presence of species is studied by sedimentation velocity at different protein concentrations. If at the speed a single complex is observed, then we proceed to obtain its mass by equilibrium in those same conditions. Each experiment allows you to analyze 7 samples at a time and the data analysis is done in the Facility.

As regards the requirements to be met by the samples, the following should be taken into account:

- The concentrations can range from 0.1 mg/mL to 1-2 mg/mL and the volume required for each sample is 400 microlitres, in the case of sedimentation velocity, and 100 microlitres, in the case of sedimentation equilibrium. In addition, for each condition to be tested, it is necessary to parallel 420 microlitres of the buffer in which each concentration or sample goes. More than the concentration, the limiting factor is the absorbance of the sample, which must be between 0.2 and 1.3 u.a., (with an optical step of 1 cm) to be able to analyze it later.
- the buffer must not contain beta-mercaptoethanol or DTT, as it has absorbance between 230 and 260 nm. If necessary for the stability of the sample we recommend reducing its concentration to 0.2 mM and if possible, replace it with TCEP, which is more stable
- Also, if it is absolutely necessary for the buffer to carry glycerol, we recommend a maximum of 5%.