Video Article

Assembly, Loading, and Alignment of an Analytical Ultracentrifuge Sample Cell

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Abstract

The analytical ultracentrifuge (AUC) is a powerful biophysical tool that allows us to record macromolecular sedimentation profiles during high speed centrifugation. When properly planned and executed, an AUC sedimentation velocity or sedimentation equilibrium experiment can reveal a great deal about a protein in regards to size and shape, sample purity, sedimentation coefficient, oligomerization states and protein-protein interactions.

This technique, however, requires a rigorous level of technical attention. Sample cells hold a sectored center piece sandwiched between two window assemblies. They are sealed with a torque pressure of around 120-140 in/lbs. Reference buffer and sample are loaded into the centerpiece sectors and then after sealing, the cells are precisely aligned into a titanium rotor so that the optical detection systems scan both sample and reference buffer in the same radial path midline through each centerpiece sector while rotating at speeds of up to 60, 000 rpm and under very high vacuum

Not only is proper sample cell assembly critical, sample cell components are very expensive and must be properly cared for to ensure they are in optimum working condition in order to avoid leaks and breakage during experiments. Handle windows carefully, for even the slightest crack or scratch can lead to breakage in the centrifuge. The contact between centerpiece and windows must be as tight as possible; i.e. no Newton s rings should be visible after torque pressure is applied. Dust, lint, scratches and oils on either the windows or the centerpiece all compromise this contact and can very easily lead to leaking of solutions from one sector to another or leaking out of the centerpiece all together. Not only are precious samples lost, leaking of solutions during an experiment will cause an imbalance of pressure in the cell that often leads to broken windows and centerpieces. In addition, plug gaskets and housing plugs must be securely in place to avoid solutions being pulled out of the centerpiece sector through the loading holes by the high vacuum in the centrifuge chamber. Window liners and gaskets must be free of breaks and cracks that could cause movement resulting in broken windows.

This video will demonstrate our procedures of sample cell assembly, torque, loading and rotor alignment to help minimize component damage, solution leaking and breakage during the perfect AUC experiment.

Video Link

The video component of this article can be found at https://www.jove.com/video/1530/

Protocol

Sample Cell Assembly and Torque

1. We begin sample cell construction by putting together 2 window assemblies.
   Place a window gasket into the window holder. Position the window liner (or it's sometimes called a window cushion) inside the window holder so that the opening of the liner is opposite the keyway of the holder. At a slight angle, place the window in the holder aligning the scribe mark on the window with the key way of the window holder. Press down gently at the very edges on both sides of the window.
2. To ensure proper sealing and accurate torque of the sample cell, we lightly coat the housing gasket and the screw ring with Spinkote lubricant. Spread a very small amount of Spinkote between your thumb and forefinger. Coat the screw ring threads with a thin, invisible film of Spinkote. Likewise, coat the housing gasket. Wipe off any visible lubricant.
3. Begin sample cell assembly by sliding one window assembly, with the window facing up towards you, into the cell housing by aligning the keyway with the housing key. Align the centerpiece keyway with the housing key and let it gently fall on top of the window assembly inside the cell.
4. Never use any sort of tool to push the centerpiece into the cell housing. This could cause permanent damage to the CP resulting in leak during experiments. Turn the second window assembly so that the window is facing towards the centerpiece, away from you, align keyway

with housing key and slide it down on top of the centerpiece. Place a housing gasket on top, then, a screw ring so the word "Out" is visible. Using your fingers and an alignment tool, hand tighten the screw ring.
If the CP doesn't slide easily into the housing barrel, first align it, then, place the second window assembly on top of it. By applying gentle downward pressure on the window assembly, slide both into the cell housing at the same time. This way, we avoid pressing directly on the CP.
Now is a good time to look inside the cell for dust, lint and fingerprints. You will also notice Newton's Rings that indicate there is still air between the CP and the windows. These will disappear after applying torque.

5. With the screw ring up and the word "out" visible, place the sample cell all the way down inside the cell torque collet of the torque stand. Hold cell in place by applying constant pressure on the torque stand handle. In one continuous motion, tighten the screw ring to between 120-140 in-lbs. If using an adjustable micrometer torque wrench, set it to between 120-140 in/lbs and tighten the screw ring until wrench "clicks" indicating the set torque has been reached. Release the torque stand handle and carefully remove the sample cell without touching the windows.

Loading and Sealing the Sample Cell

6. For sedimentation velocity experiments, we routinely load a volume of 400ul into each sector of the 12mm centerpiece using a 200ul pipette. Position the sample cell with the loading holes on top and the word "out" on the screw ring visible. Slide the pipette tip half way down into the LEFT centerpiece sector through the loading holes and slowly dispense 200ul of reference solution. Repeat once to total 400ul. Using the same pipette tip and the same technique, carefully and slowly fill the RIGHT centerpiece sector with 400ul of sample solution.
Volumes of 150-180 ul are used for sedimentation equilibrium experiments but the same care and technique should be followed.
It is imperative to match the volumes of reference and sample solutions as closely as possible. Fitting the pipette tip firmly onto the pipette, slowly loading and dispensing solutions, avoiding loading bubbles and using the same pipette tip for both solutions all aid in keeping the volumes as equal as possible.
7. Seal the cell by placing 2 new red housing plug gaskets snugly into each loading hole making sure they are positioned between the center piece and the aluminum housing. Cover each with a housing plug. Hand tighten with a small flat head screwdriver.

Sample Cell Alignment in Rotor

8. Before loading the rotor, make sure the counterbalance is no more than .5g LIGHTER than the sample cell in the opposite position in case that sample cell leaks. Start out with the balance at zero. First weigh the sample cell that will be in position opposite the CB. Tare the balance to subtract the sample cell weight, then, weigh the CB. Add or subtract weight from the attachment hole of the counterbalance. Make sure weights do not protrude from the top. Reweigh to ensure that the counterbalance final weight is within .5g lighter than the opposite sample cell.
9. Using a 4-place rotor, place the counterbalance into position 4 so that the white arrow is visible and pointing in the direction of the centrifugal force. Make sure it is all the way down inside the rotor. Gently fasten the set screw, found on top of the counterbalance just across from the white arrow, until the counterbalance is firmly in place, but still able to be aligned. Position the balanced sample cell with the housing plugs facing the center of the rotor and the word "out" on the screw ring visible.
10. Looking from underneath the rotor, align the inside scribe mark (the mark closest to the center of the rotor) with the scribe mark on the counterbalance using an alignment tool. Tighten the set screw to lock aligned counterbalance in place. In the same way, align the sample cell scribe mark with the inside scribe mark at position 2 on the rotor.

For more information see https://sedfitsedphat.nibib.nih.gov/default.aspx

Discussion

The proper cell assembly, sample insertion, and cell alignment in the rotor is critical for high quality AUC experiments. This is true, in particular, for experiments that require a very detailed analysis of the acquired data on the macromolecular sedimentation profiles, for example, when studying protein interactions and determining trace aggregate amounts. Often the quality of the experimental setup is limiting for the accuracy and precision of the results from the AUC study. For details on the practical design and further execution of AUC experiments, see References 1 – 3. Workshops covering the practical setup, theory, and data analysis of AUC experiments are being held twice a year at our laboratory at the National Institutes of Health [4].

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