A brief practical guide to using Schepartz AU

Jade Qiu

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Beckman has detailed instructions, regarding both experimental setup and theoretical considerations, on their website http://www.beckmancoulter.com/resourcecenter/literature/BioLit/BioLitList.asp?ProductCategoryID=ANA. For users wish to learn about AU in depth, it's recommended that they both check out the website, as well as subscribe to RASMB (Reversible Associations in Structural and Molecular Biology) at http://www.bbri.org/RASMB/rasmb.html.

1. Sample preparation

The buffer for AU sample should contain a minimum of 100 mM salt to prevent non-ideality. The salt concentration should increase substantially for highly charged peptides, especially when at high loading concentrations. For typical sedimentation equilibrium experiments, 120 µL each of the same peptide at three different concentrations need to be prepared for every cell. Each peptide solution is blanked against a 125 µL buffer. The concentrations used for each peptide are decided by two primary factors: the range of concentrations of interests, and the extinction coefficient (absorbance) of the peptide at a particular wavelength. For systems where association events occur, loading concentrations far below or above Kd should be avoided if one wishes to accurately determine the association constant. Within the ideal concentration range, the peptide concentration should be such that the absorbance of the sample is between 0.1 and 0.7 with a 1 cm path length at the chosen wavelength. Typical wavelengths used are 280 nm, 230 nm, and occasionally 257 nm if only Phe residue is present in the peptide. Since each system is unique, it may be necessary to carry out an initial trial run and then optimize the conditions accordingly. It is usually not necessary to dialyze the peptide sample against the buffer, unless there is small molecule entities that absorb at the wavelength monitored.

2. Starting up

Assemble cells and load samples as described in the Beckman handbook. Note the highest concentration sample should be loaded into the channel closest to the center of the rotor, with progressing lower concentrations away from the rotor center. The counterbalance should always stay in rotor position 4, and be balanced against position 2 cell to within 0.01 g. Since an exact match is rather uncommon, the position 2 cell should always be the slightly heavier one, so that a potential leak won't overly exaggerate the imbalance. If all positions are used for the run, then position 1 and position 3 cells should be balanced against each other, too.

Start the setup by turning on the AU instrument with the red power switch on the right side panel. Open the chamber door, load the rotor, and install the optical assembly before close the door and promptly press the 'vacuum' button. It takes a good ten minutes for the pressure to drop, while the rotor tends to be overheated if the instrument is on without vacuum, so it's crucial to start the vacuum pump as soon as possible. Turn on the computer, or if the computer is already on, make sure to restart it. The program controlling the AU instrument is named 'ProteomeLab', which has a shortcut on the desktop. Double click it to start up, and make sure the flashing lightening bolts appear while loading. Otherwise, the program is not loaded properly. Close it and try again. If this is your first time ever using the instrument, now is a good time to set up your data folder. Go to 'My Computer', select 'C', 'xladata', and create a folder for yourself within.

3. Beckman software for sedimentation equilibrium setup

To start a new equilibrium run, click on 'file' and select 'new'. A new control window pops up. First of all, look for the 'speed' entry at the top middle of the window. Make sure it is set to '3000' (meaning 3000 rpm). The main body of the window contains choices for the three cells. For each cell, click 'wavelength', then 'absorbance', enter the range of wavelength for monitoring in the 'Wmin' and 'Wmax' boxes, finally click 'detail' and change 'wavelength step size' to '1', for 'data folder' browse to your data folder (under 'xladata'), before click 'OK'. The 'comment' box can be used for

bookkeeping purpose. Enter information like peptide name, concentrations, buffer, etc. here and it will show up in the header of the SE files later. Click 'options' in the upper left corner of the window, and in the popup window, make sure the 'radial calibration' choice is checked, while all other boxes are unchecked. Click 'start single scan' in the upper right corner of the window. This will start a wavelength scan, with both the status window and monitor window popping up to show the current status of the instrument. The purpose of the wavelength scan is to make sure that the setup is correct and samples display expected UV/Vis spectra. This helps with early detection of possible problems, as each scan of the actual equilibrium run takes hours to finish. Two most common problems at this stage are inverted signal (negative absorbance) or much lower signal / absorbance than anticipated. A negative absorbance spectrum arises because either the samples are loaded into the buffer channels, or the cell is inserted into the rotor upside down. The remedy is to stop the run, take out the rotor, retrieve the cell, flip it over and insert into rotor again with the holes on the side still facing the center of the rotor. Then start the equilibrium run again from the beginning. A much lower absorbance spectrum typically indicates peptide crashing out of the solution. In the case one needs to find a better buffer to avoid peptide non-specific aggregation.

While the wavelength scan is running, one can setup the equilibrium method by clicking the 'method' button in the upper left corner of the main window. A new window shows up, where the columns are, from left to right, speed in rpm, accelerate/decelerate rate, delay time, temperature, and scan number. For small peptides, a good initial set of conditions are scans at 42000 rpm every three hours for 24 hr, then scans at 50000 rpm every three hours for 18 hr, and finally scans at 60000 rpm every three hours for 18 hr. An additional deceleration phase, where the speed is decreased stepwise from 60000 to 50000, then 40000 and finally 30000 rpm, is added at the end to protect the cells in the event of a leak. 'Speed in rpm' is the speed at which the specific scan is carried out; 'accelerate/decelerate rate' should be 400, except during the deceleration phase when it should be 100; 'delay time' is always zero right after speed up or change of speed, and is specified to desired value to control how often the scans occur; 'temperature' is usually held constant throughout the run; and 'scan number' must be '1' for the specified scan to occur, therefore it is set to 1 even during the deceleration phase even though no useful

data is obtained there. After filling the table with the scan schedule, click 'OK' to save and exit. If the wavelength scan turns out to be OK, one can then proceed to start up the equilibrium run.

In the main window, for each cell position, click 'equilibrium' then 'absorbance', and enter in the 'W1' box the monitoring wavelength. Click 'detail', then specify 2nd and 3rd wavelengths for monitoring if necessary. Note that this will increase the experimental time dramatically. Change 'radial step size' to 0.001, and 'replicate' to 20. Make sure 'step' mode and '6 channel' centerpiece are selected, as well as the right data folder is selected. **Do not change any other settings**. Click 'OK'. Click 'options' in the upper left corner of the main window, and **make sure the 'radial calibration' option is unchecked**. Also check the 'stop XLI after last scan' option', especially if the run is going to finish in the middle of the night, or during the weekend. Now you are all set to go. Click the 'start method scan' button in the upper right corner of the main window when you are ready to start! Stay around for the rotor to come up to speed and the laser start to firing. Then come back in a couple of hours to see if your first scan is OK. If no problems are detected, then your run is likely to be worry free. However, it is a good practice to check on your run at least once per day, and more diligently if there is a thunder storm. The special power circuit that the AU is on can get cut off for short periods of time during a storm, which will stop the rotor and crash your run, unfortunately. When that happens, the only solution is to restart an equilibrium experiment at the speed where the previous run was stopped. Any data collected before the crash is not affected.