SE Sample Submission Guideline for Beckman Coulter ProteomeLab XL-I/A absorbance optics system, Analytical Ultracentrifugation (AUC) in IBC402

I. Sample Submission

- 1. Apply an account on the BCF reservation system.
- 2. File sample submission form on-line under instrument "Analytical Ultracentrifugation BCF".
- 3. After confirming the charges and experiment time through reservation system, samples and buffers may be submitted to Miss Wang Szuhuan.
 - Tel: 27855696 x4024; e-mail: <u>szuhuan@gate.sinica.edu.tw</u>
- 4. BCF may not compensate for your sample loss or data loss under any circumstances (hardware or software failure, operator error, or others). All experimental results are for research only.
- 5. Data analysis is the responsibility of users.

II. Sample Preparation

- 1. Use fresh prepared samples, samples having stored under frozen condition, gone through freeze/thaw cycle or filtered by concentration treatments may result in aggregation. Gel filtration is recommended for buffer exchange.
- 2. Only samples having good SV results are allowed to run SE experiments.
- 3. Perform a serial dilution to 3 different sample concentrations, i.e. serial dilution with a ratio of 1 to 2. A range of starting concentrations with absorbance form 0.1-1.0 is employed, so that the absorbencies of samples are about 1.0, 0.33 and 0.11. Provide us at least 150µl for each concentration and additional 1.5mL buffer for reference and rinse.
- 4. It is helpful to know the extinction coefficients of your sample at different wavelengths, which can be used to convert all absorbencies to the same concentration scale using Beer-Lambert's Law to permits an even wider range of concentrations to be used.

III. Experimental Setting

- 1. We will run your samples with 3 rotor speeds using SEDFIT tool "Estimate Equilibrium Rotor Speeds" with meniscus position at 6.8 cm. Slowest speed runs first, then at progressively higher speed to minimize the time to reach equilibrium.
- 2. We use 6-channel centerpiece and load 100, 110 and 120uL of sample in the inner, middle and outer channel with highest concentration in the outer channel.
- 3. A test run of 3000 rpm will be performed to check sample condition and cell assembly.
- 4. Although we ensure that the cell are assembled as carefully and professionally as we can, there might be slight chance of cell leakage due to the imperfection of cell components. BCF will not compensate for sample loss and will not charge setup fee for the experiment.
- 5. Depends on the experimental setting, an SE experiment takes about 72-120 hours.

IV. End of Experiment

- 1. Cells are disassembled and cleaned by BCF staffs.
- 2. The sample will not be recovered.
- 3. Raw data will be sent via reservation system.

V. Acknowledgement

Please acknowledge us if research supported and/or data generated by this instrument results in publications. For example, "We acknowledge AUC SE data collected by [*operator*] in the Biophysics Core Facility funded by Academia Sinica Core Facility and Innovative Instrument Project (AS-CFII-108-111)."