Sample preparation and use of DSC

Sample preparation:

1.) Dialyze concentrated sample versus buffer you want to measure denaturation in
    a. This buffer will be used to diluted sample and in actual DSC as reference buffer
2.) After dialysis, spin dialysate in microfuge for 10 minutes to remove any insoluble matter.
3.) Quantitate protein concentration by diluting into 6M Gnd•HCl and measuring A_{280}
    a. Sample:
       X μl protein
       (200 – X) μl buffer
       800 μl 7.5 M Gnd•HCl, 25 mM NaPhos pH6.5
    b. Blank:
       200 μl buffer
       800 μl 7.5 M Gnd•HCl, 25 mM NaPhos pH6.5
    c. Extinction coefficient=(#W) * 5640+(#Y) * 1280 (M^{-1} cm^{-1})
4.) Aim for protein concentration in the 20 μM range. Cell loading is easiest if you have 2 ml of protein. You will need to change protein concentration depending on the protein in order to get good signal to noise and reversibility.
5.) Filter 50 ml dialysis buffer with a 0.22 mm cutoff filter
6.) Degas 3 ml buffer for 5 -10 minutes with stirring

DSC Preparation:

1.) Turn on DSC by switch in the back (usually keep on)
2.) Open VP DSC program
    a. Program should begin setting temperature to 25 ºC, if you want to start at a lower temperature, change the thermostat temperature, under Thermostat/PlotControl tab.
3.) Check the LED on front of DSC
    a. Should be yellow on the right and red on the right
4.) Create a folder
    a. My computer->C drive-> VPDSC-> data -> your name -> data mmddyy
5.) Set up run parameters, the only things you are likely to change are:
    a. Starting temp: low temp
    b. Final temp: high temp
    c. Scan rate: 90ºC/hr (good to start with 1.5ºC/min
    d. Prescan thermostat time: 15 minutes
    e. #scans: set high so it will keep going
    f. filling range (audible fill indicator): 20 – 40ºC

g. postscan thermostat: 0 min
h. feed back mode/gain: none for proteins (broad transitions), mid to high for lipids(sharper transitions)
i. Filtering period(time period over which data samples are averaged): 10-20 sec for proteins, 1-5 sec. for lipids
j. thermostat/calib rate: nothing
k. setup/maintenance tab: data file path: click on it and find your file data folder
l. constants: nothing

Running sample:

First you will run buffer vs. buffer. You need at least 3 scans to establish thermal history and reproducibility of the baseline scan.

1.) Rinse cells with ~15ml buffer (doesn't have to be degassed)
2.) Put loading funnel into first cell
3.) Fill syringe with 2.0 ml of degassed buffer. Remove bubbles by flipping syringe upside down and knocking
4.) Slowly load 0.65 ml of buffer to cell
5.) Rapidly plunge barrel down 100 ul, slowly pull barrel up 100 ul. Repeat 10x
6.) Move assemply to 2 nd cell and repeat steps 3 and 4
7.) Remove excess liquid by using the volume adjustment needle and syringe. Insert needle until you can't push down any further. Suck out excess liquid. Repeat 2x
8.) Put cap on DSC. Screw metal part in first. Then, tighten white part until pressure gets to ~ 25 p.s.i.
9.) Hit start on software

Loading Sample:

If your 2nd and 3rd scans are within 0.005, you canload sample for the fourth scan.

The DSC cycle has three phases:

1. Cooling to start temperature
2. Prescan thermostat at start temp
3. Upscanning to final temp (data collection)

You want to load during the cooling phase. The lower the temperature the better, but you need to do it before phase 2. There is an audible beep when the instrument has reach the range you set up in your experiment.

Cleaning DSC:

1. Put pressure port protector in place.
2. Use vacuum pump or Thermovac to wash cells (see picture below).
3. Use contrad detergent mixed with water, microwave for 2 minutes
4. Run 150 ml through sample cell and 50 ml through reference cell
5. Run 500 ml dH_20 through each
6. Leave cells with dH_20 in them and put cap back on (no need to pressurize)