UNIVERSITY OF SASKATCHEWAN

PROTEIN CHARACTERIZATION AND CRYSTALLIZATION FACILITY
COLLEGE OF MEDICINE – UNIVERSITY OF SASKATCHEWAN
HEALTH SCIENCES CENTER 3D40

Nano-ITC

ANYONE USING THIS EQUIPMENT SHOULD BE PROPERLY TRAINED BY PCCF SUPERVISOR BEFORE INDEPENDENT OPERATION !!!

Nano-ITC is built from four components:

1. COMPUTER: access to nano-ITC software, analyzing software, cleaning station.
2. CLEANING STATION: used to clean reaction cell with solvents before and after experiments. There is no ON/OFF switch, DO NOT disconnect from POWER
3. DEGASSING STATION: used to degas buffers and water (ON/OFF switch on the back)
4. Nano-ITC machine (ON/OFF switch on the back)

BEFORE EXPERIMENT:

1. It is advisable to load water in the reaction cell and reference cell 24 hours before experiments to stabilize system.
2. Make sure there is enough cleaning solutions in 500 ml bottles and 1 L of mili-Q water available.
3. Turn on computer and start run-ITC software. It will ask for homing. Confirm. Adjust temperature if needed (25 C by default)
4. After homing is done, remove black burette from nano-ITC.
5. Buffers should be filtered and degassed (vacuum degas). You will need at least 50 mL of buffer and 50 mL of water for water-to-water titrations. Check buffer compositions on PCCF website. Buffers should be matched between reaction cell and injection syringe.
6. Clean reaction cell using BEFORE protocol programmed on cleaning station.
7. Fill reference cell with degassed water (400 uL) using loading syringe and long needle. DO NOT drop needle in the cell to avoid damaging gold layer of the cell. HINT: Use your fingers to keep needle sliding down slowly.
8. Put ‘dummy’ needle inside the reference cell slowly.
9. Fill reaction cell with 350 uL of water (after cleaning) and remove this water. Repeat 3x to wash cell. Load 350 uL of degassed water.
10. Wash injection syringe with water and load water into syringe.
11. Injection syringe and needle are easy to bend. Any bend will affect your baseline. Be careful as replacement of the syringe is your PI responsibility.
12. After securing syringe, lower burette in nano-ITC (using both hands) and secure by twisting clockwise.

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TITRATIONS:

1. Above steps can be followed to load your reactant and ligands into cell and syringe. Wash the cell with degassed buffer before injecting your sample.
2. To start spinning needle: set up correct speed and click run button. It will take time to stabilize the system when needle is spinning.
3. When running experiments, put concentration in the run info so it will be transferred into analyzing software.
4. Set-up number and volume, and time between injections. You can save this for later use or make a note of your settings.
5. Check auto calibrate at medium variations and time out of 1800 s. It will not start injecting unless baseline is reached. If the baseline is not reached, usually it indicates dirty cell or bend needle. Contact PCCF supervisor.
6. Click start run and observe baseline. Data is saved automatically to the file of your choice. Be organized with file names as you may end up with many of them.

BETWEEN EXPERIMENTS:

1. When the run is complete you may remove burette out of the instrument using both hands. Make sure the motor is stopped.
2. Insert cleaning needle in the reaction cell and wash cell using BETWEEN protocol on cleaning station
3. Wash injection syringe with water and ethanol.
4. If you are done for the day but would like to continue next day, put fresh degassed water inside reaction cell.

FINISHING THE WORK

1. Wash reaction cell using AFTER protocol on cleaning station.
2. Wash injection syringe
3. If the set of experiments is done, perform water to water titrations to check if the cell is clean and needle is not bend.
4. Remove water from reaction cell and reference cell
5. Take buffers, water, samples back to your lab. Do not store anything around ITC as it will be discarded.
6. Put everything in its place and clean the area.