**V. Cleaning Ion Exchange Columns**

Proper care of the ion exchange columns is necessary for their continued use in experiments. It prevents cross-contamination of proteins from one preparation to another and helps keep the filters clean, such that the columns can be used for longer without repacking. There is a deep cleaning protocol for purification of WRC 3mer, which is sticky and can lead to contamination of your WRC with other WRC components.

**(A) Materials**

\* SourceQ/SourceS column

\* 2M NaCl/50mM NaOH (filtered)

\* 2M NaCl (filtered)

\* 1M NaOH (filtered, PES filter only)

\* DI H20

1. Regular Column Cleaning

1. Connect the column you wish to use for purification to the AKTA system
   1. NOTE: I do this with every column I use, unless I just used it previously. If I used it for something dirty (a dirty purification, anything from insect cells, etc.), I will clean it after I use it.
2. Switch tubing to 2M NaCl/50mM NaOH buffer
   1. NOTE: You should use a flow rate of 4mL/min, otherwise you will run the risk of compacting the column and having to repack it.
3. Run buffer through the column until UV peaks and drops and you see the conductivity plateau
   1. NOTE: Often, the pressure will increase. This is due to the viscosity of the buffer and things coming out of the filter/column
4. After UV has dropped to the baseline, switch tubing to water and immediately do a pump A wash to change the buffer.
5. Let water run through the column until the conductivity drops, then switch to your buffers and proceed as normal

**2. Deep cleaning after 3mer purification**

1. Take the column used for the 3mer purification and flip it, so that buffer enters the bottom of the column and leaves through the top
   1. NOTE: This is to make sure contaminants do not pass through the entire column. It also helps clean the filter at the top of the column, which gets the dirtiest.
2. Change the flow rate to 0.2mL-1mL/min
   1. NOTE: You are aiming for a contact time of at least 1hour to help remove contamination from the column
3. Wash with a least 2CV of 2M NaCl
4. Wash with at least 4CV of 1M NaOH
5. Wash with at least 2CV of 2M NaCl
6. Rinse with at least 4CV of DI H20, followed by 4CV of running buffer