



COLUMN MAKING WORKSHOP



LABORATORY 6
COMPLETE SERIES

AMGEN[®] Biotech Experience

Scientific Discovery for the Classroom
San Francisco Bay Area

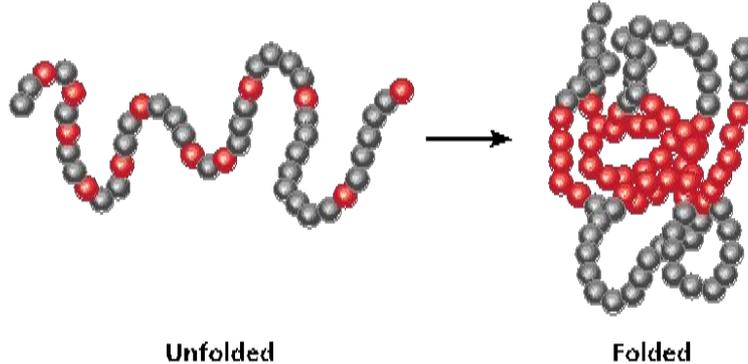
AGENDA

- **Introduction**
- **Review of Basic Column Care**
- **Build Column Set**
- **Buffer Recipes**
- **How to Fix Slow Columns**

PURIFICATION METHODS

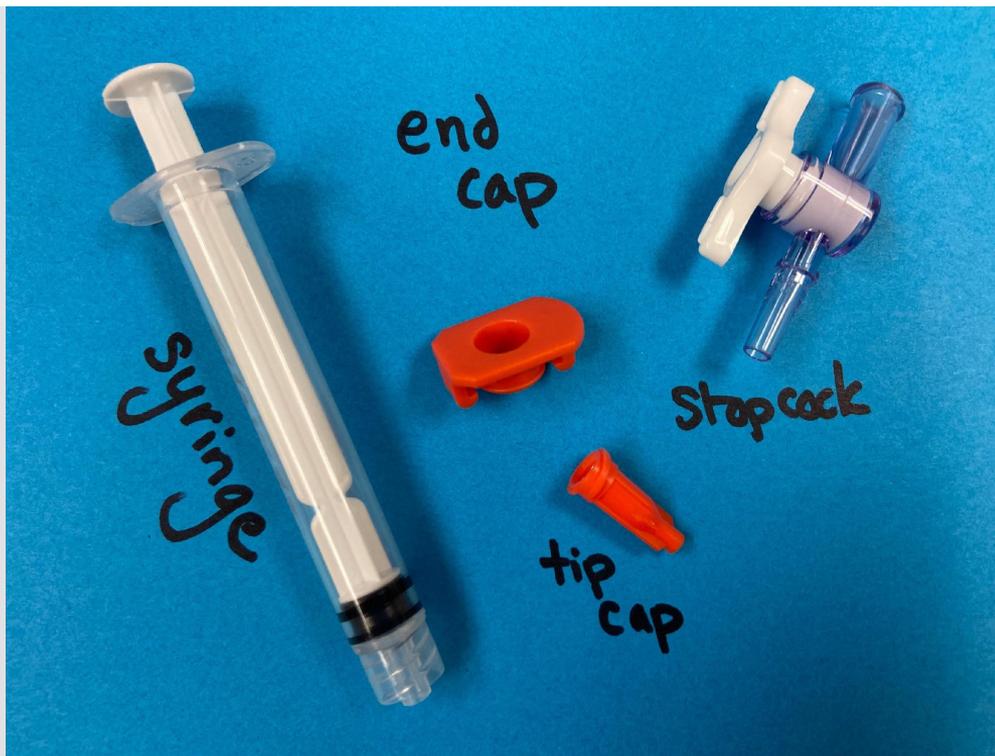
- **Manipulate amino acids in the protein structure**
 - **Hydrophobic regions point inward**
 - “fears water” – e.g. oil, wax, fats
 - **Hydrophilic regions point outward**
 - “loves water” – e.g. salt, sugar
 - **Use buffers with different salt concentrations to make rfp change shape so amino acids will grab onto or let go of resin in column**

CHANGES IN BUFFER CHANGE PROTEIN CONFIGURATION



**Salt concentration
of buffers can
make rfp flip inside
out!**

COLUMN PARTS



COLUMN REMINDERS

- **Columns drip SLOWLY, can't force it**
- **Make sure the tip cap and end cap are removed to allow solution to flow**
- **Do not pipette directly into resin – run buffer down side**
- **Do not let air touch resin - keep liquid meniscus above resin by ~2mm**
- **Wait until each solution has drained to ~2mm above resin before adding another solution**

COLUMN BUILDING TIPS

- **Pour one test column before pouring resin into all columns**
- **Don't lay the resin tube on its side (exposes resin to air)**
- **Use syringe plunger to get column "started" if it's not flowing**
 - minimal pressure required
 - only push in a tiny bit (don't create a vacuum)
 - all caps off
 - can also use your thumb to create pressure if you're worried you'll put too much pressure on
- **End caps will not "snap" into place, as long as they are on the column it's ok!**
- **Be a helicopter column parent for the first few weeks...check caps regularly**

COLUMN STORAGE

- **Add 1mL CEB (column equilibration buffer)**
- **Drain to 1-2mm solution above resin**
- **Add another 2mL CEB and leave in column**
- **Cap column on both ends**
- **Store upright in light tight container**
- **If columns are not used regularly, periodically replace CEB to avoid contamination.**

BUFFER CONCENTRATIONS

- **Column Equilibration Buffer (CEB) – 2 M for column storage**
- **Binding Buffer (BB) – 4 M**
 - rfp changes shape, exposes hydrophobic regions, binds to resin
- **Wash Buffer (WB) – 1.3 M**
 - Washes away less hydrophobic molecules
- **Elution Buffer (EB) – 10 mM**
 - Rfp changes shape, disrupts hydrophobic attraction, rfp releases from resin

CHAPTER 6: VIDEO RESOURCES

- [Preparing a Column](#)
- [Unclogging a Column](#)
- [Overview of Lab 6](#)