Module 1 overview

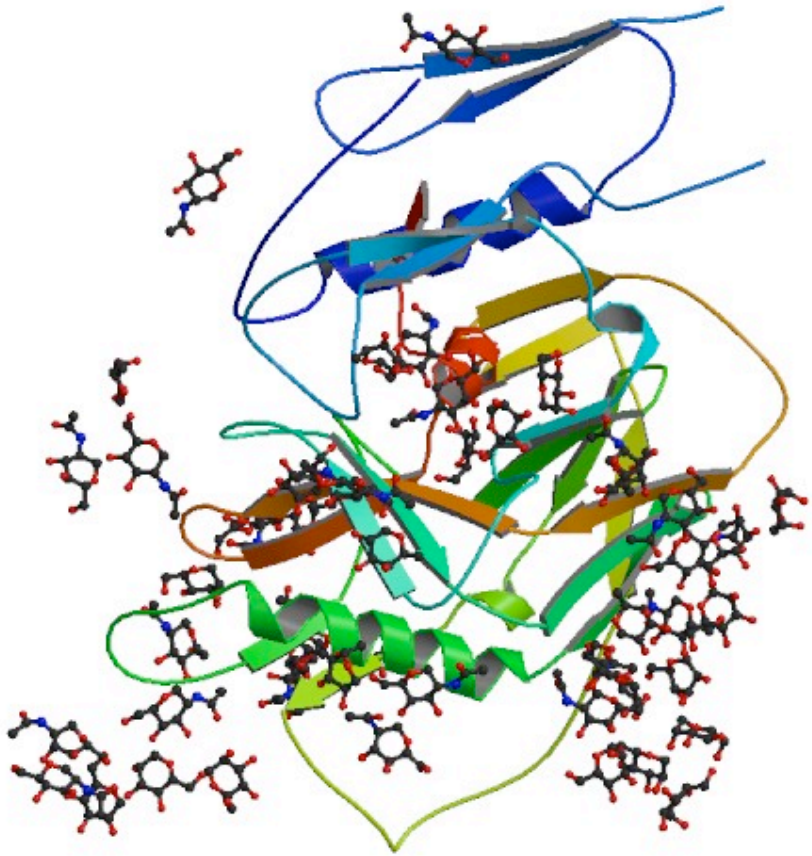
lecture	*lab*
1. Introduction to the module	1. Start-up protein eng.
2. Rational protein design	2. Site-directed mutagenesis
3. Fluorescence and sensors	3. DNA amplification

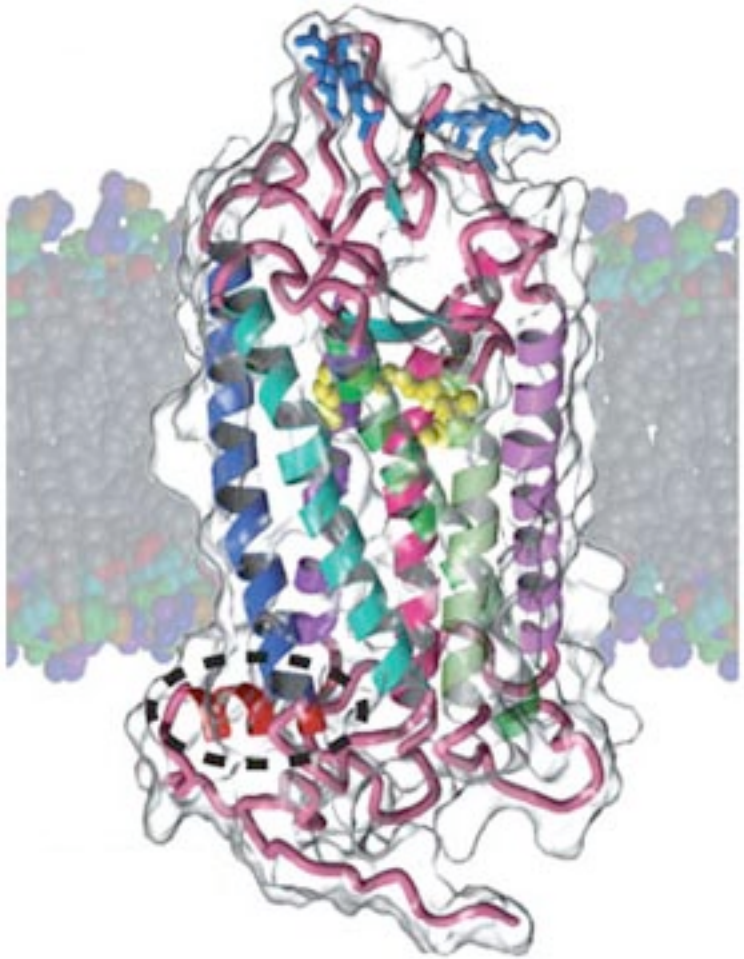
PRESIDENT'S DAY

4. Review & gene analysis	4. Prepare expression system
5. Protein expression	5. Gene analysis & induction
6. Purification and protein analysis	6. Characterize expression
7. Binding & affinity measurements	7. Assay protein behavior
8. High throughput engineering	8. Data analysis

Differences between prokaryotic and eukaryotic proteins sometimes require eukaryotic expression systems.

These two proteins exemplify characteristics that frequently call for eukaryotic expression:





www.rcsb.pdb.org

www.rikenresearch.riken.jp

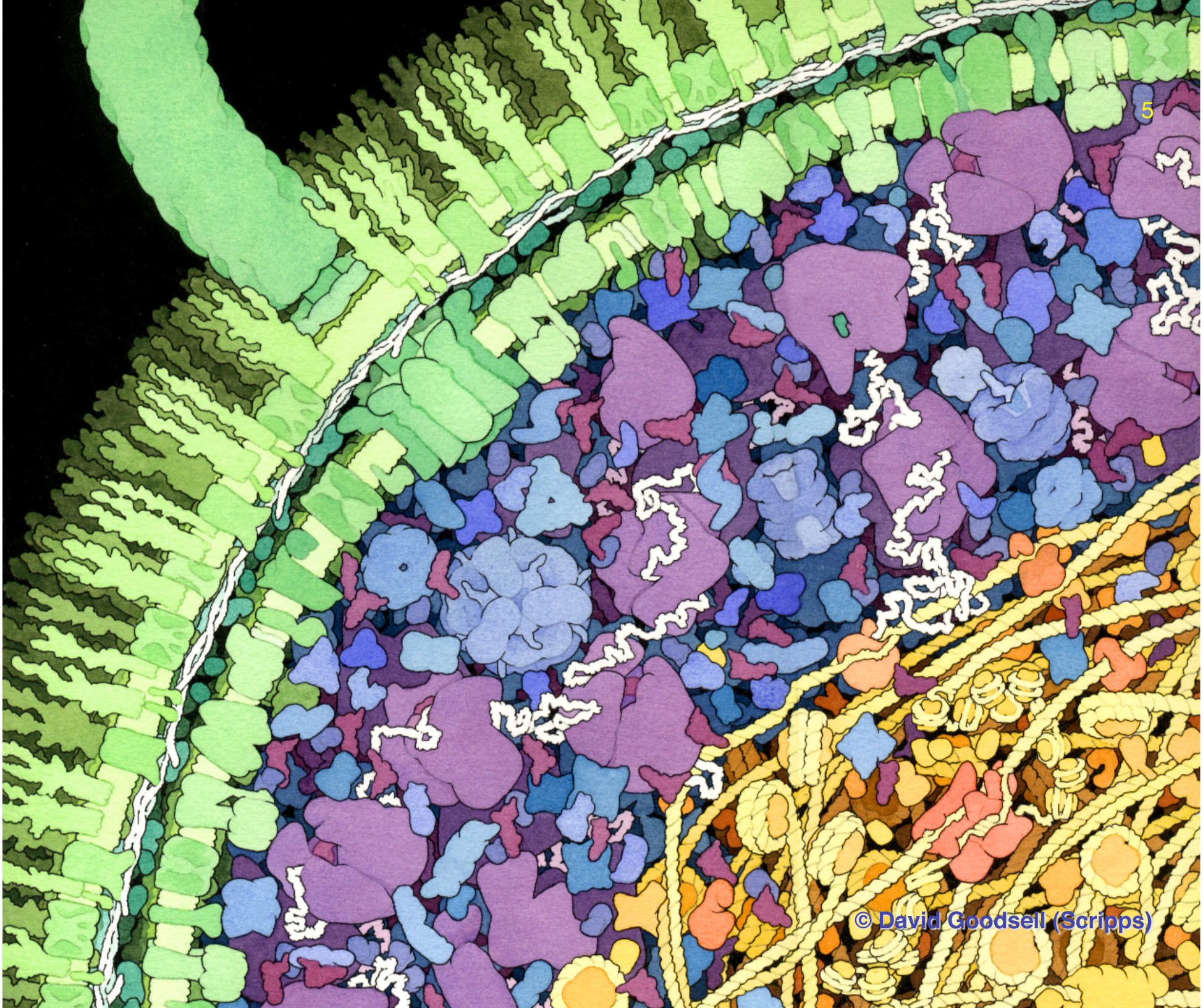
Prokaryotic *vs.* eukaryotic protein expression

property	*prokaryotic*	*higher eukaryotic*
yield/(L culture)	1-100 mg	widely variable
cost/(L medium)	~$5	~$50
introduction of DNA	transformation of competent cells	viral or nonviral transfection
handling	sterile needles, *etc*.	tissue culture hood
cell incubation	shaking incubator	usu. w/CO_2-control
induction	usually IPTG	none, tetracycline
glycosylation, *etc.*	no	yes
notes	best for small, globular proteins	best for complex, eukaryotic proteins

Lecture 6: Protein purification

I. Standard purification methods
   A. Harvesting and lysis
   B. Protein separation techniques

II. Assessing purified proteins
   A. Electrophoresis
   B. Mass spectrometry
   C. Protein sequencing and AA analysis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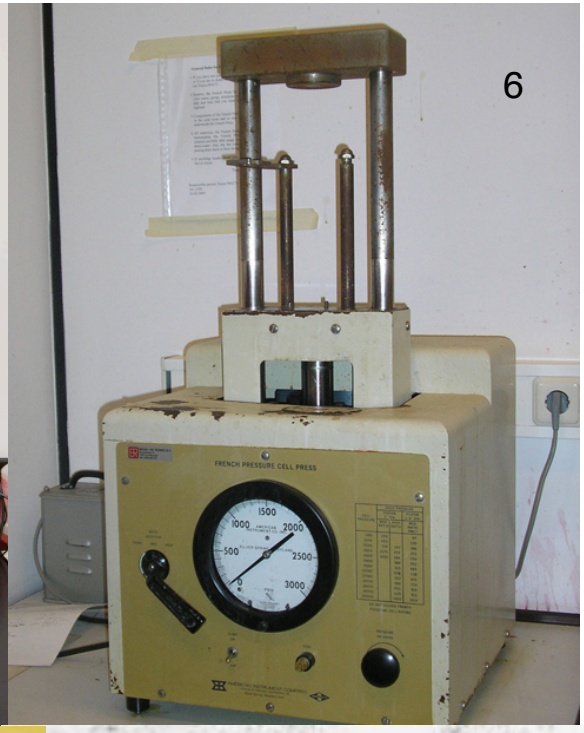
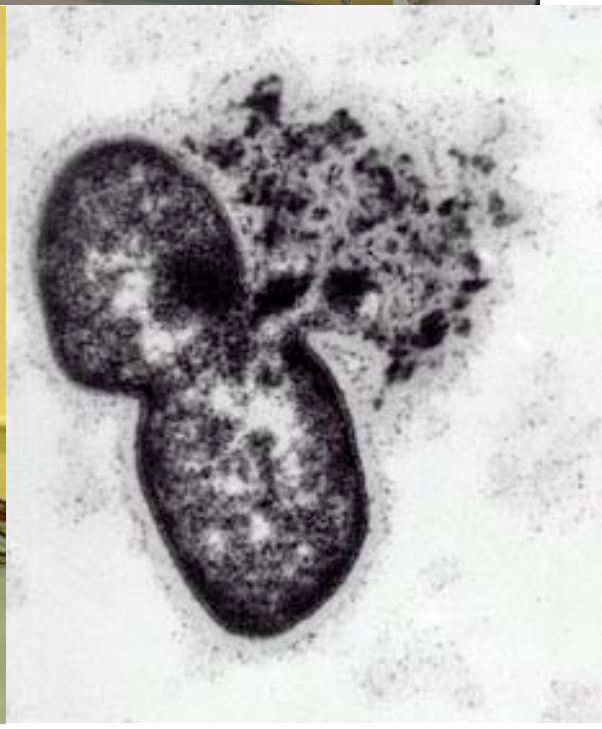
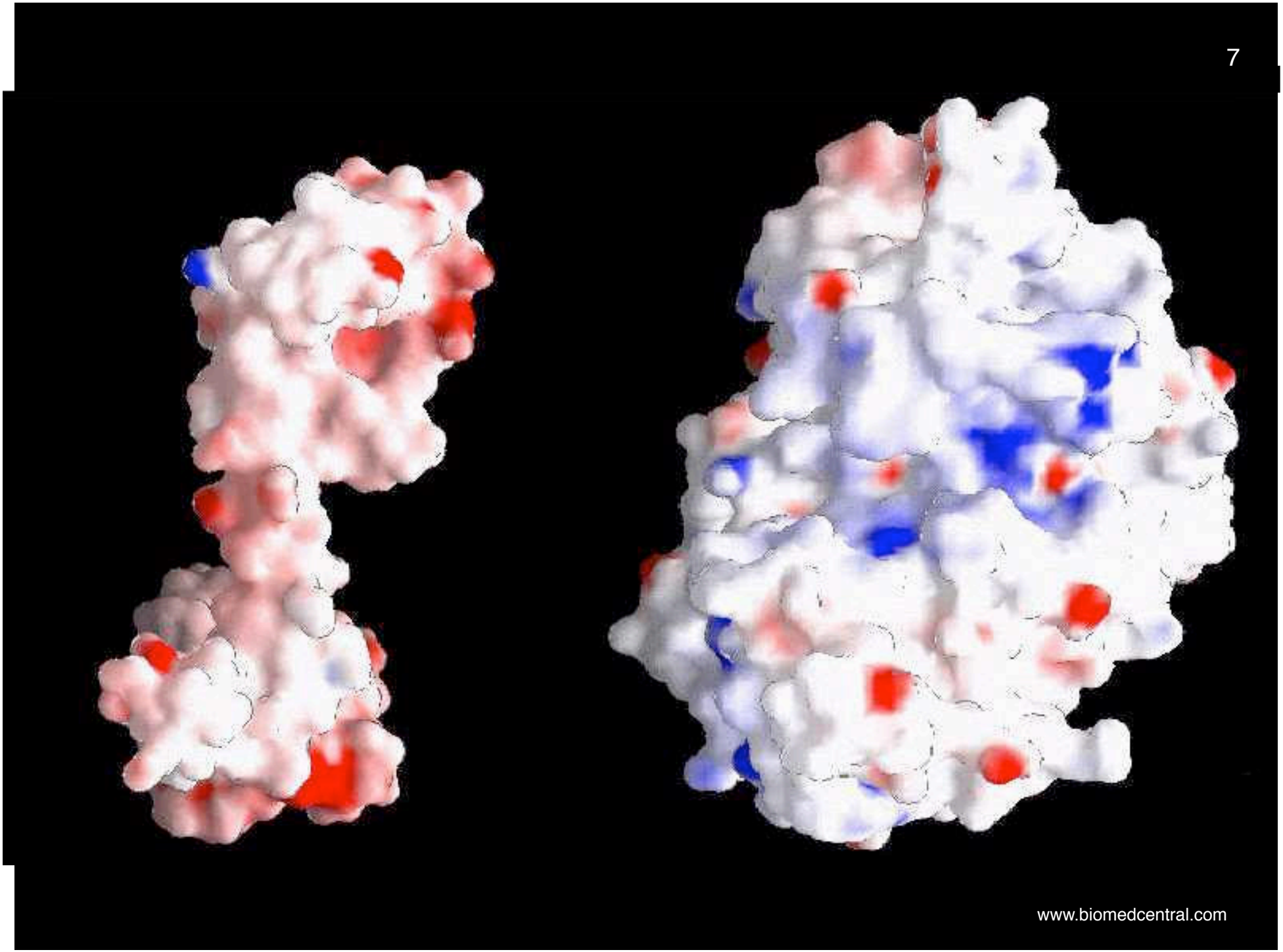
© David Goodsell (Scripps)

Once we've collected the cells, how do we get the proteins out?

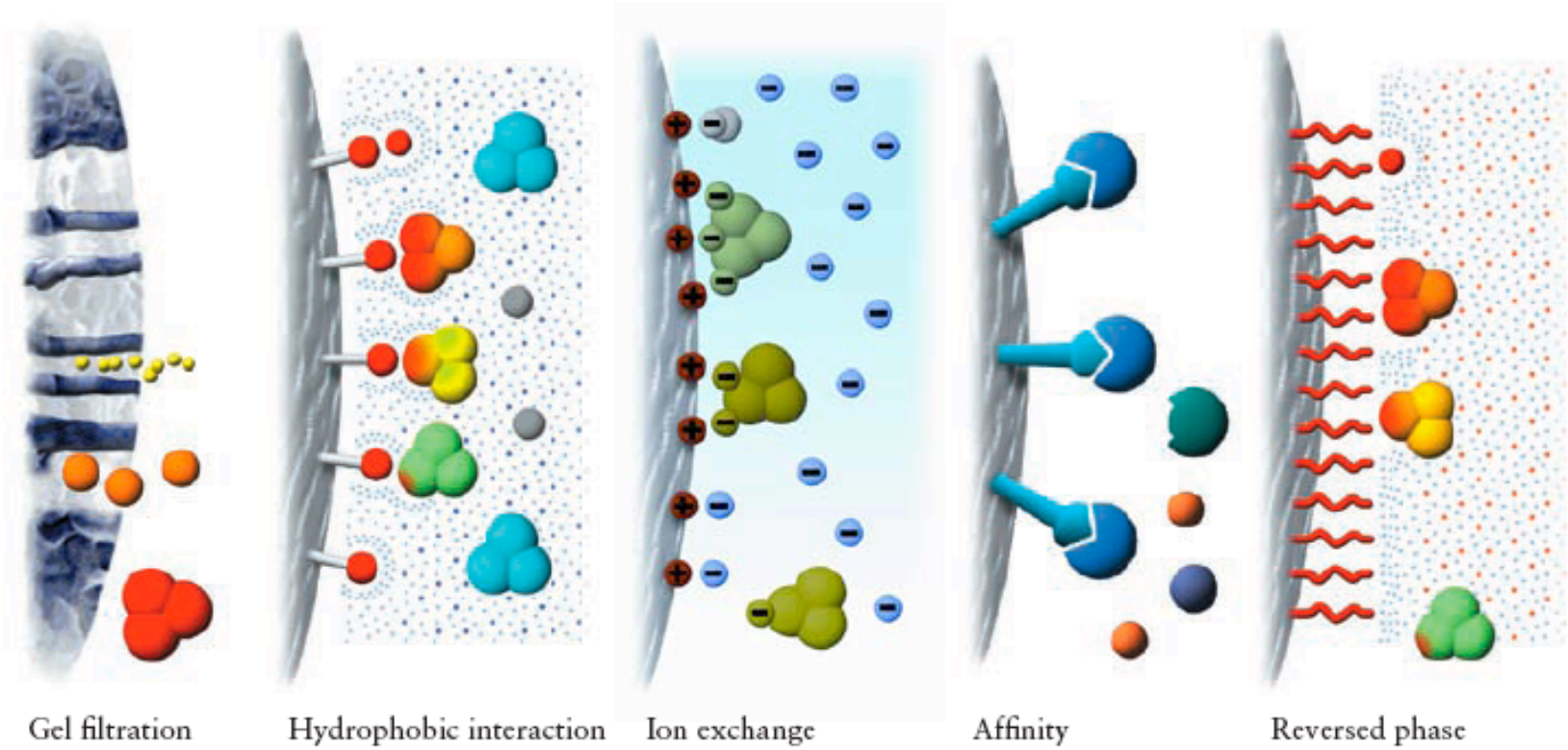




clockwise from top left:
lh6.ggpht.com
www.biomembranes.nl
bioinfo.bact.wisc.edu
matcmadison.edu



Separation techniques

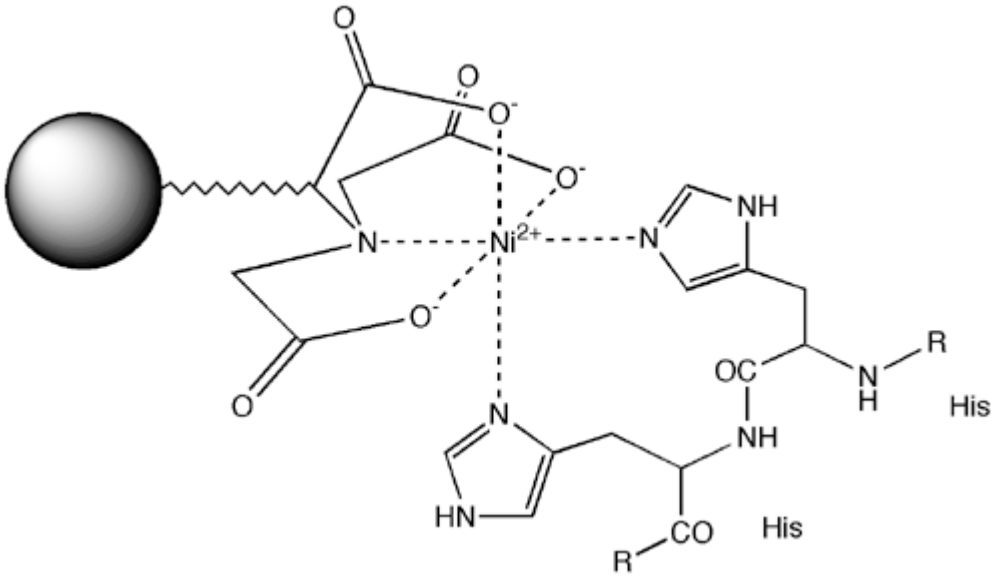


most common, in addition to affinity

***e.g.* Ni-NTA**

GE Healthcare (2007) *Recombinant Protein Purification Handbook*

Nickel affinity purification with Ni-NTA agarose



Many other tags can be used for protein purification:

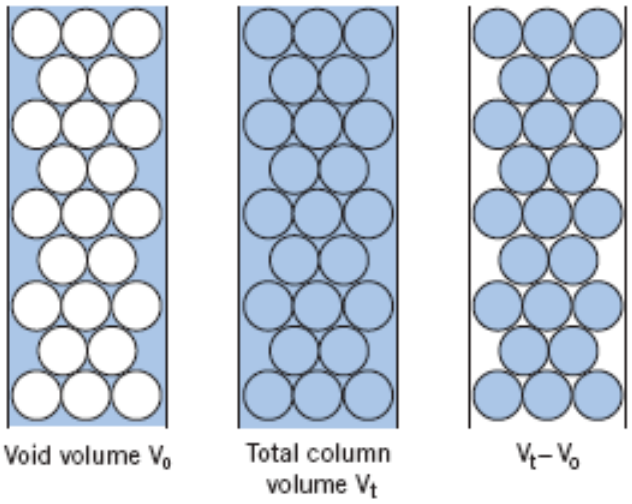
tag	*residues*	*matrix*	*elution condition*
poly-His	~6	Ni-NTA	imidazole, low pH
FLAG	8	anti-FLAG antibody	low pH, 2-5 mM EDTA
c-myc	11	anti-myc antibody	low pH
strep-tag	8	modified streptavidin	2.5 mM desthiobiotin
CBP	26	calmodulin	EGTA, EDTA
GST	211	glutathione	reduced glutathione
MBP	396	amylose	10 mM maltose

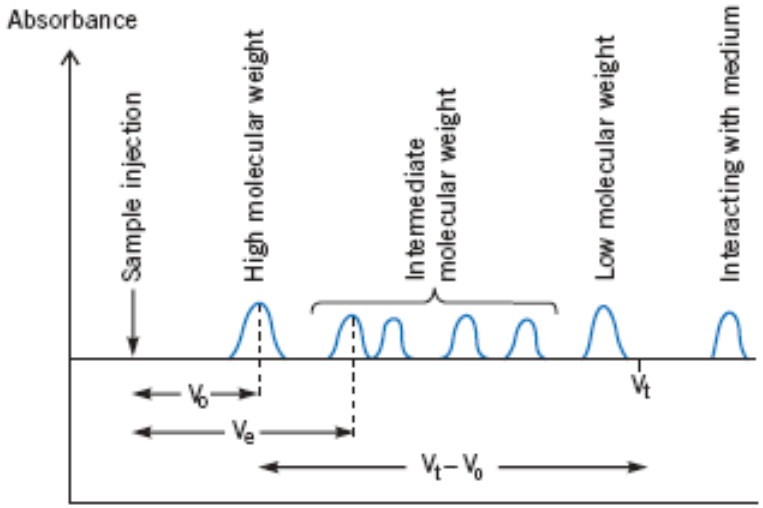
Tags may be chosen because they

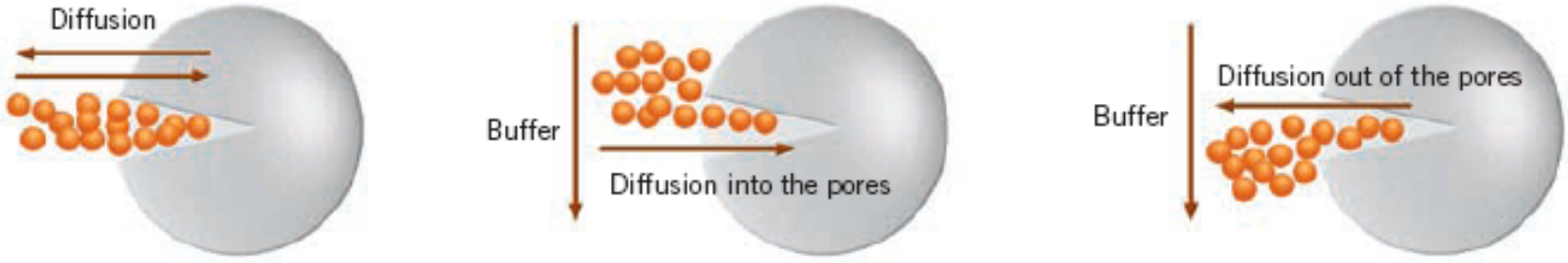
- interfere minimally with protein structure/function
- improve recombinant protein expression or solubility
- offer most convenient purification methods

All tags may be cleaved from expressed proteins using specific proteases, if desired.

Gel filtration (size exclusion chromatography) principle

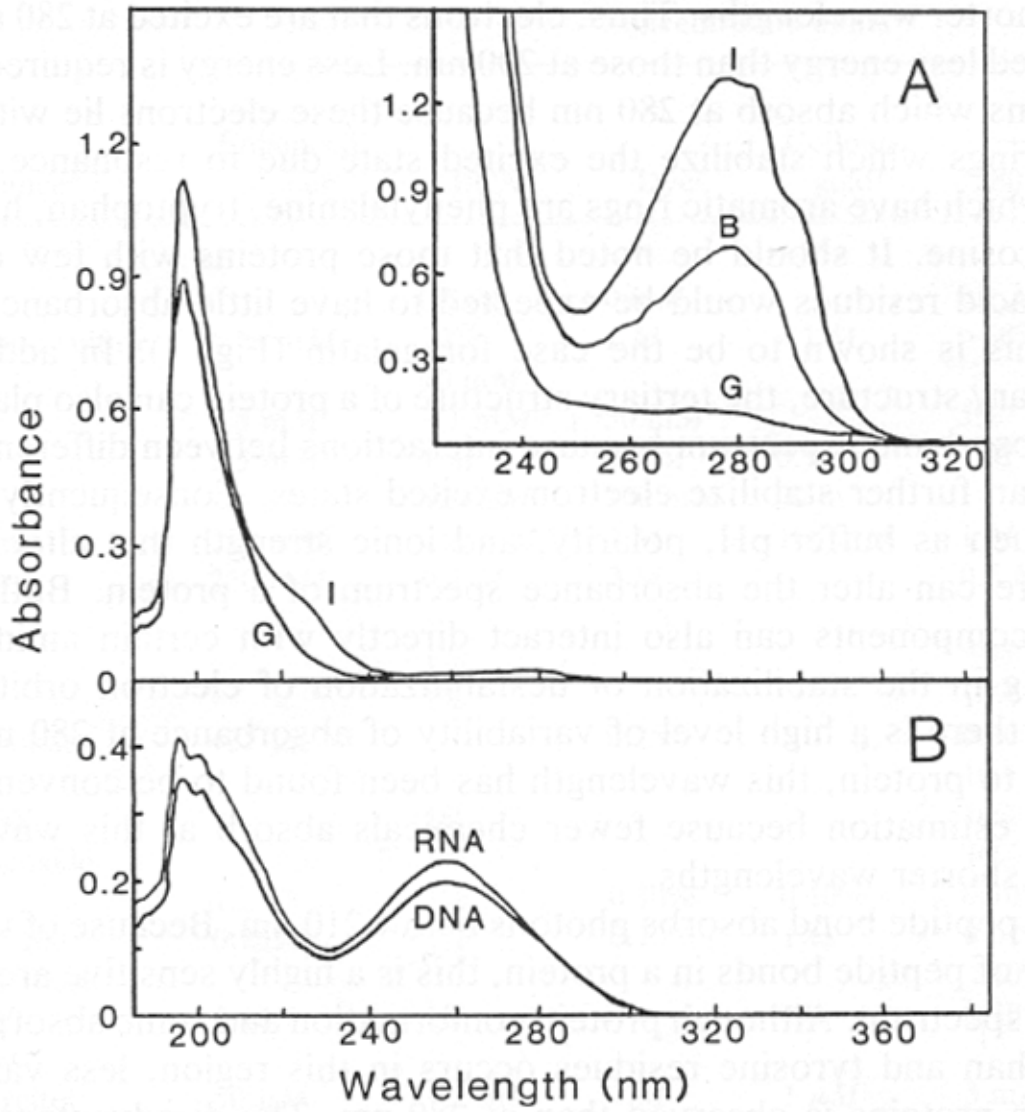






GE Healthcare (2007) *Gel Filtration Principles & Methods*

Quantification of purified proteins



use Beer-Lambert law:

$$A_{280} = \varepsilon_{280} c l$$

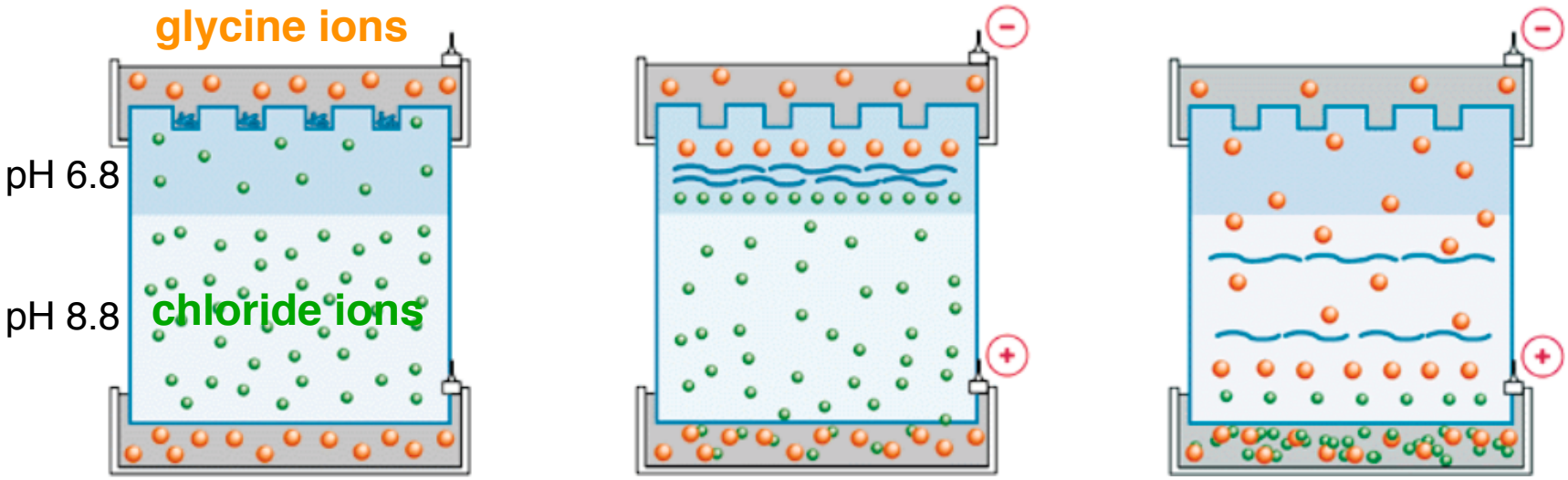
ε_{280} is the extinction coefficient; it can be determined rigorously, or estimated:

$$\varepsilon_{280} \sim n_W \times 5500 + n_Y \times 1490 + n_C \times 125$$

Assessing proteins for identity and purity

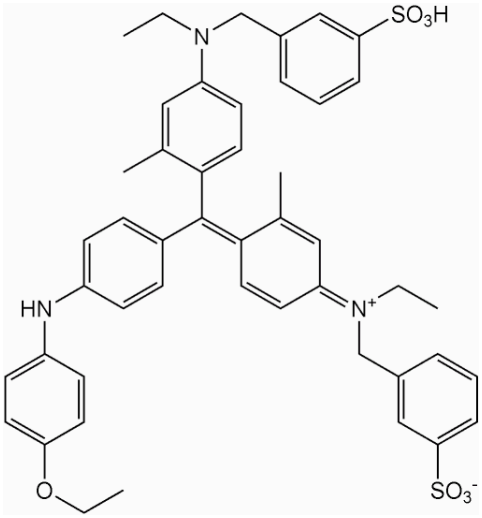
Most standard technique is sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE):

- basis is the tendency of proteins to unfold in SDS and bind a fixed amount SDS per protein (1.4 g/g)
- negative charge of SDS overwhelms protein charges
- proteins have same charge to mass ratio, but are differentially retarded by the separation gel
- stacking layer “focuses” proteins before separation layer

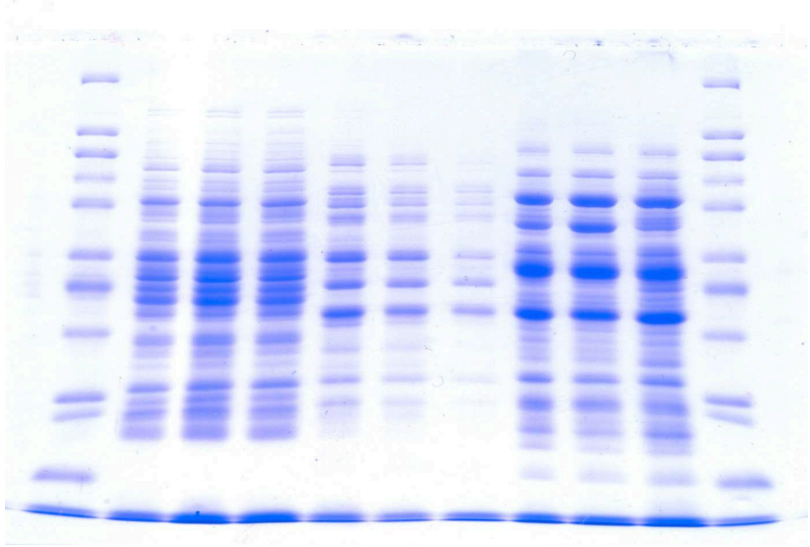


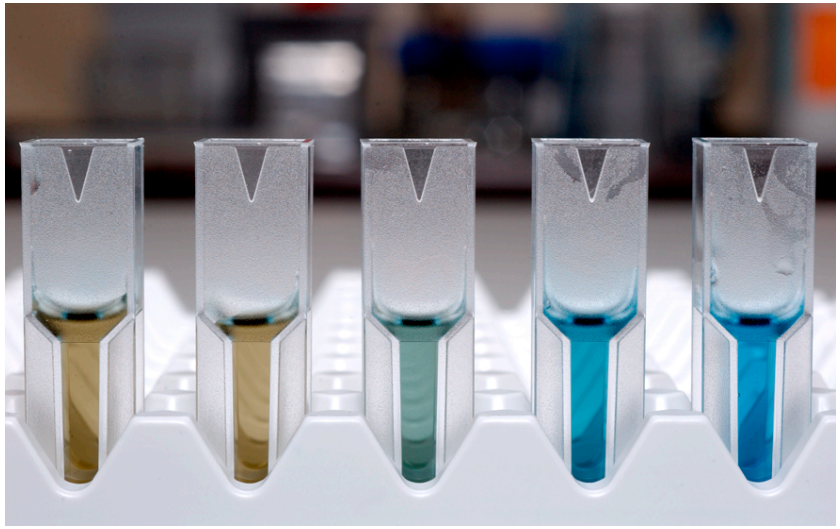
http://nationaldiagnostics.com/article_info.php/articles_id/10

Coomassie brilliant blue staining



- binds proteins primarily via aromatic residues and arginine
- undergoes absorbance shift from 465 nm (brownish) to 595 nm (blue)
- basis for Bradford Assay; can be used to quantify proteins over ~3 kD

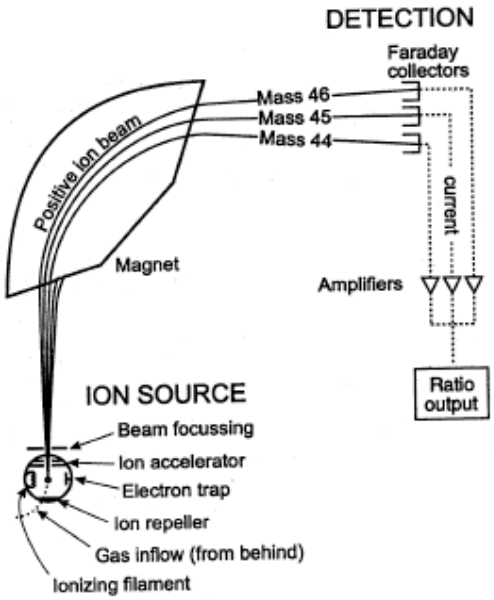


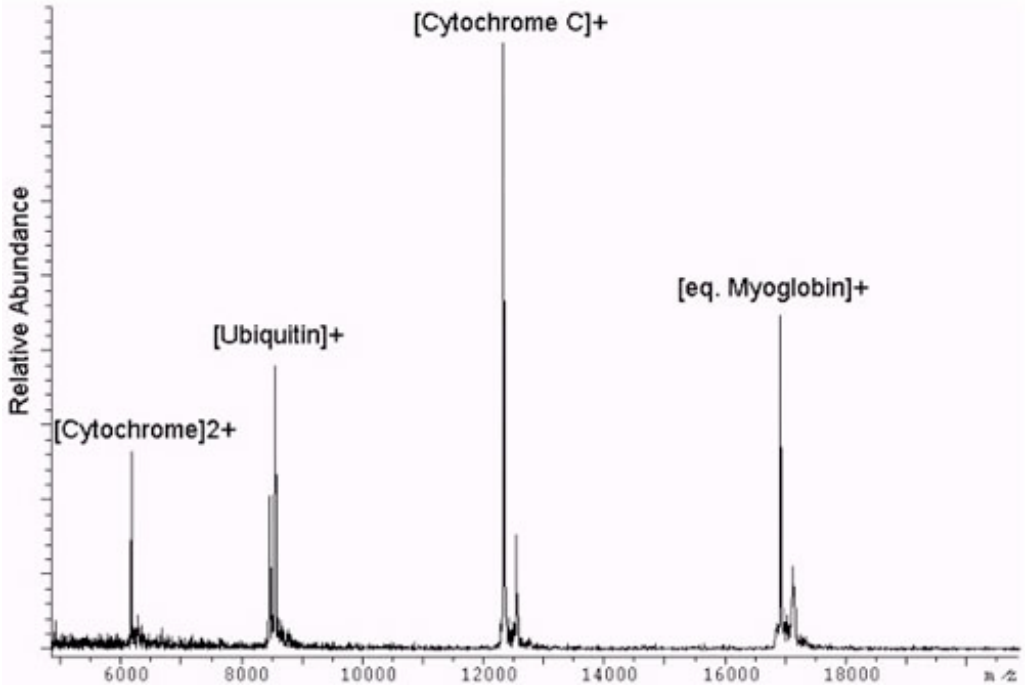


www.eiroforum.org/press/media_embl.html

SDS-PAGE gives an approximate MW and purity estimate, but how can we be sure the protein we've purified is the correct one?

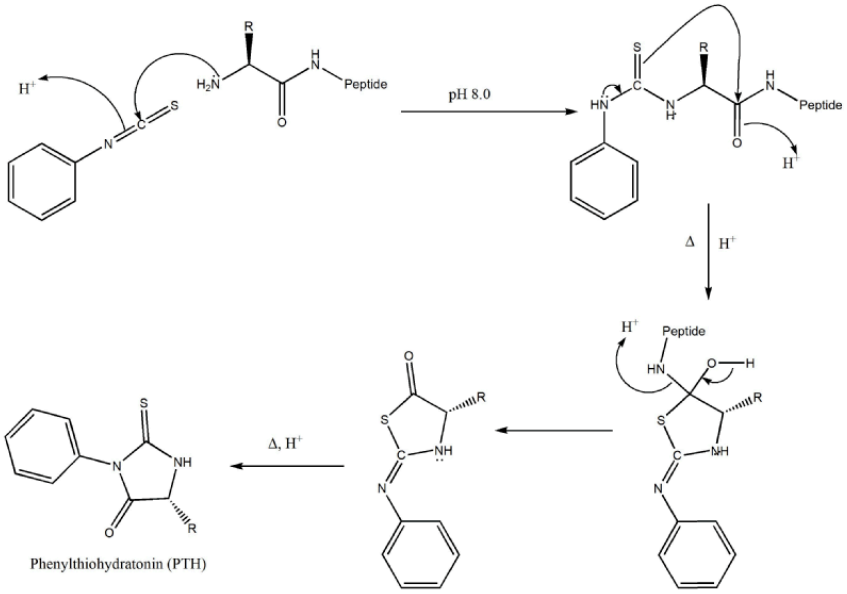
- activity assay if one is available
- knowledge of exact mass (mass spectrometry)
- N-term. sequencing and AA analysis, if necessary





en.wikipedia.org/wiki/Mass_spectrometry
www.kcl.ac.uk/ms-facility/images/maldispec2.jpg

N-terminal sequencing (Edman degradation)



- products identified by chromatography or electrophoresis
- typically ~5 cycles practical for routine N-term. sequencing

en.wikipedia.org/wiki/Edman_degradation