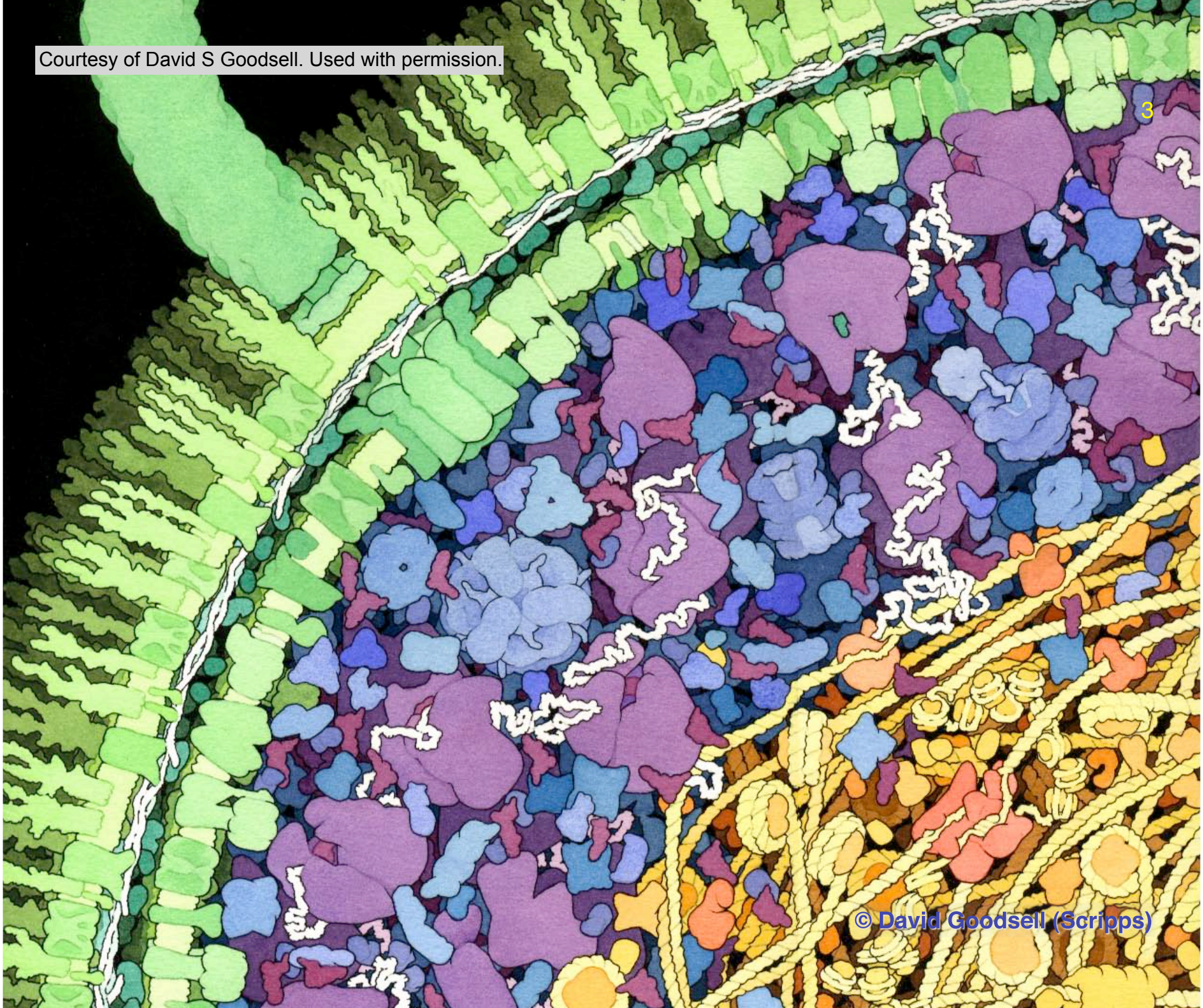
Module 2 overview

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
4. Protein expression	4. Prepare expression system
SPRING BREAK	
5. Review & gene analysis	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

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


Courtesy of David S Goodsell. Used with permission.
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© David Goodsell (Scripps)

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

Photos removed due to copyright restrictions.
Three laboratory devices:
* Blender
* French press
* Sonicator
Image of cells undergoing lysis

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

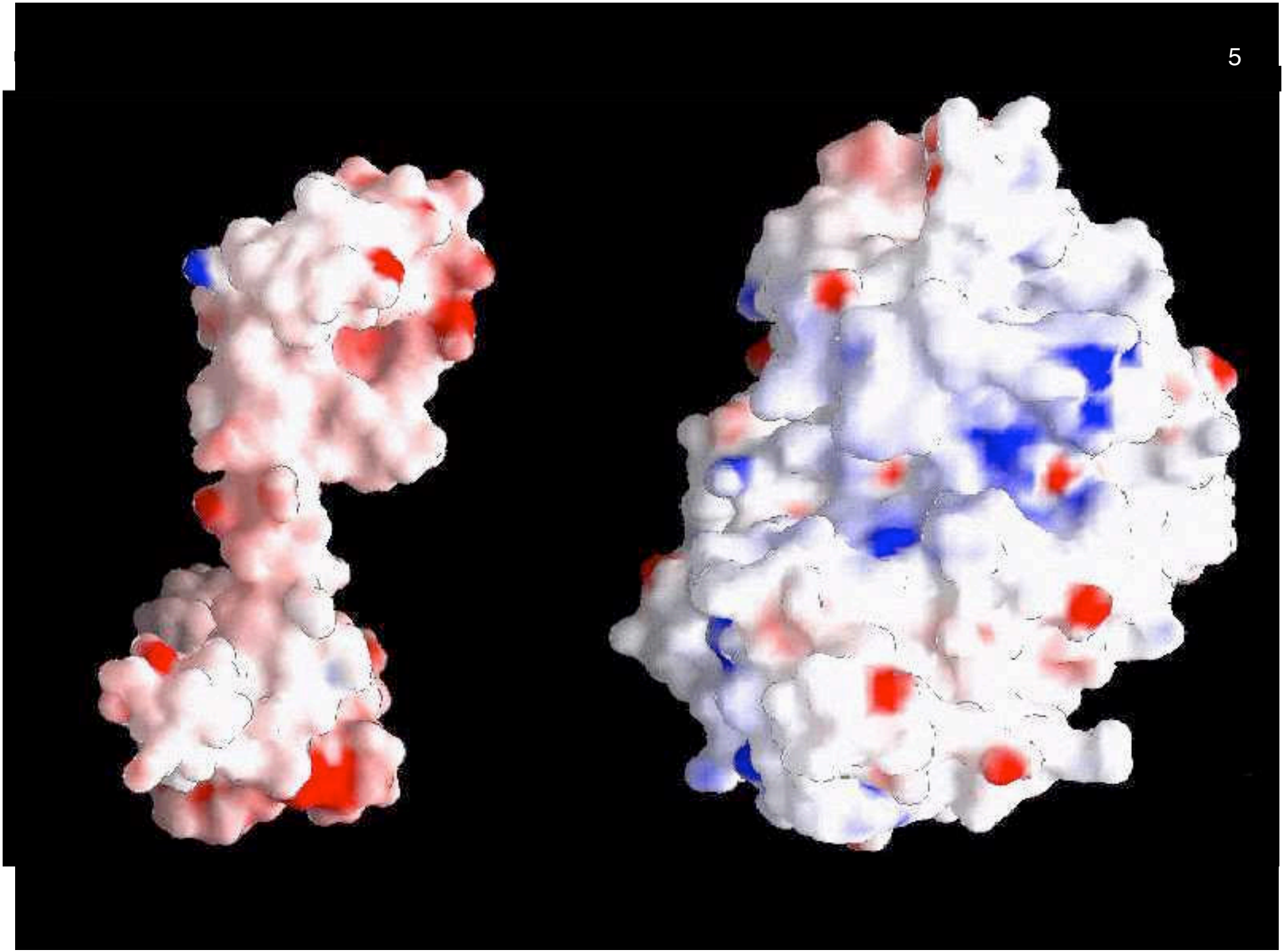
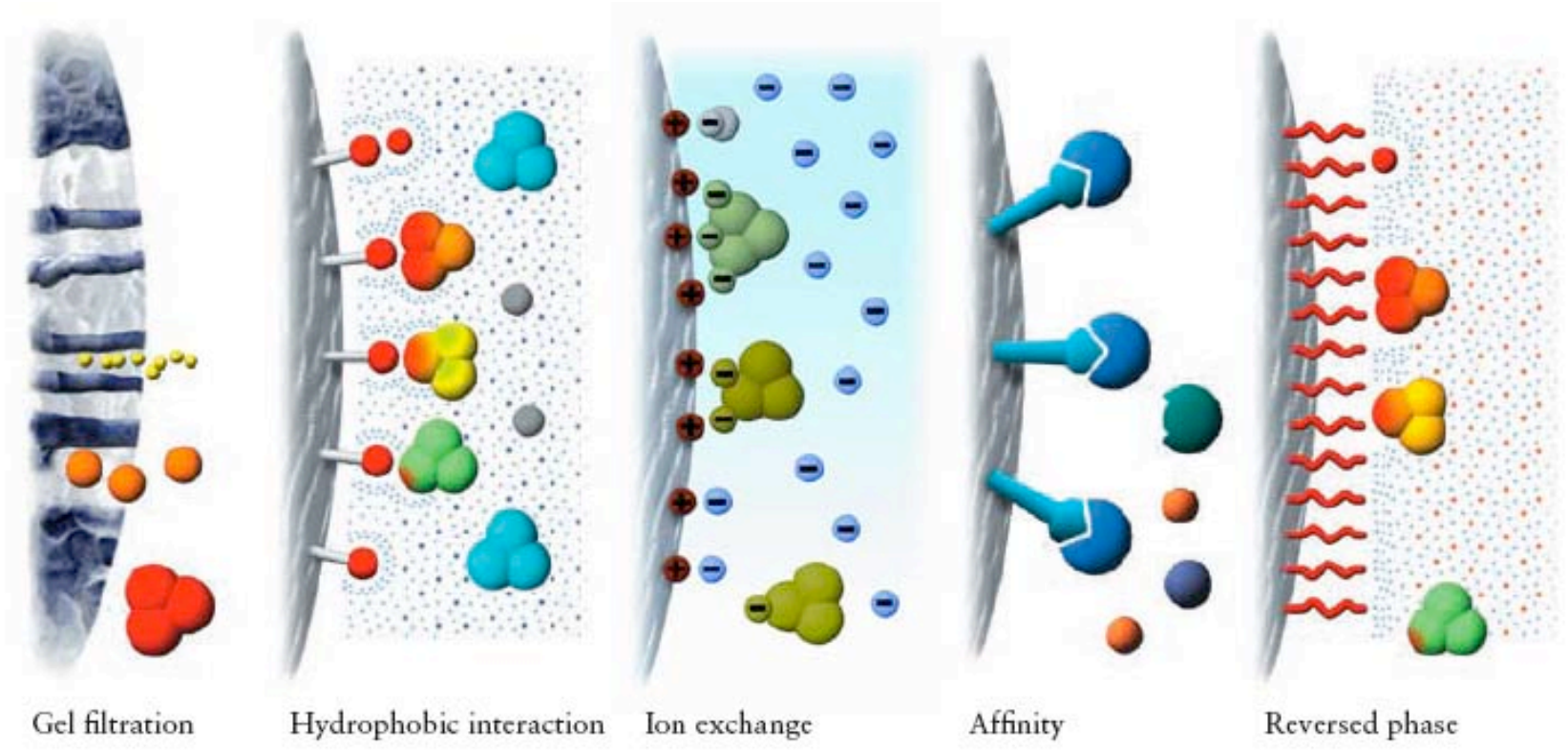


Image from Rekha, N., and N. Srinivasan. *BMC Structural Biology* 2 (2003): 4.
http://www.biomedcentral.com/1472-6807/3/4

Separation techniques

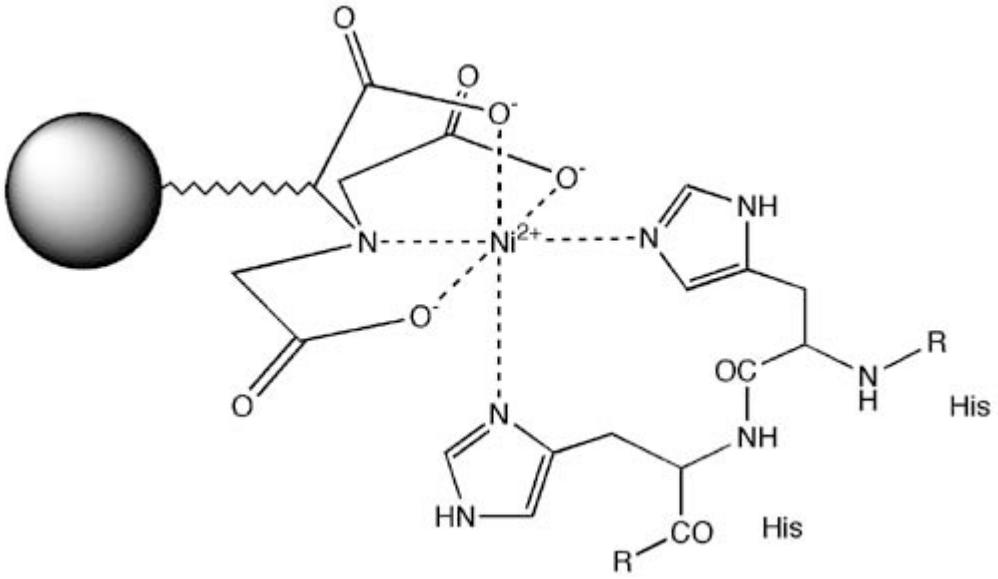


most common, in addition to affinity

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

Source: GE Healthcare *Gel Filtration Principles and Methods* handbook.
http://www.gelifesciences.com/aptrix/upp00919.nsf/Content/LD_153206006-R350?OpenDocument&hometitle=search

Nickel affinity purification with Ni-NTA agarose



Many other tags can be used for protein purification:

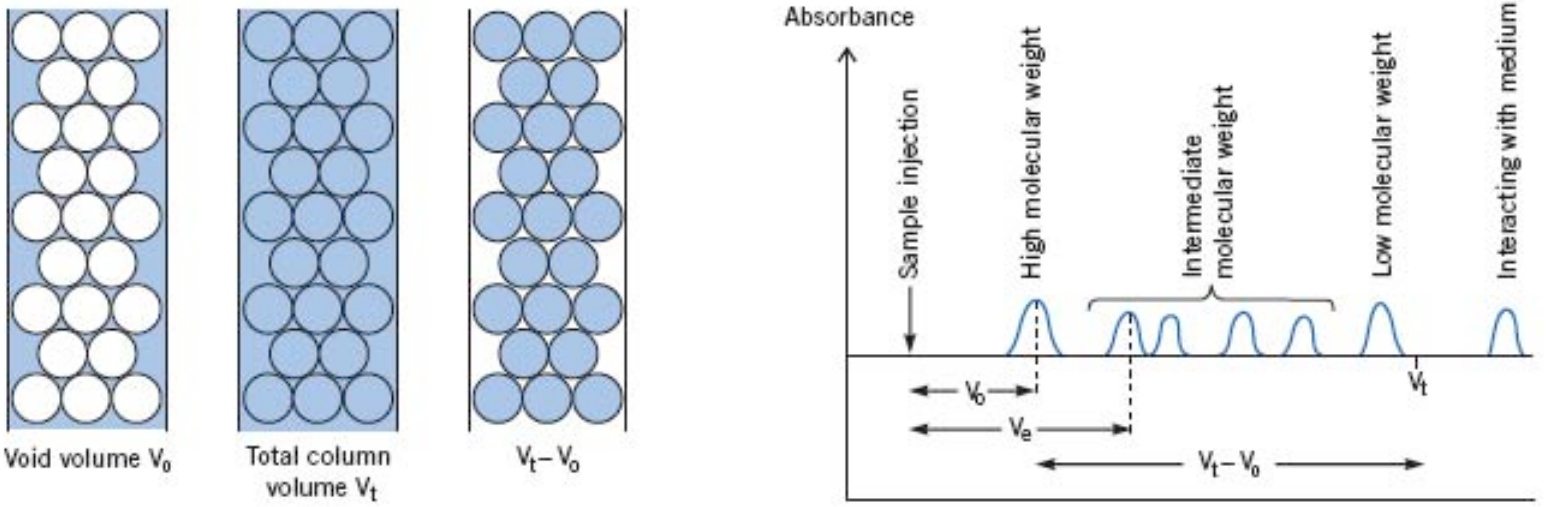
tag	*residues*	*matrix*	*elution condition*
poly-His	~6	Ni-NTA	imidazole, low pH
FLAG	8	anti-FLAG antibody	low pH, 2-5 mM EDTA
c-myc	11	anti-myc antibody	low pH
strep-tag	8	modified streptavidin	2.5 mM desthiobiotin
CBP	26	calmodulin	EGTA, EDTA
GST	211	glutathione	reduced glutathione
MBP	396	amylose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



Source: GE Healthcare *Gel Filtration Principles and Methods* handbook.
http://www.gelifesciences.com/aptrix/upp00919.nsf/Content/LD_153206006-R350?OpenDocument&hometitle=search

Quantification of purified proteins

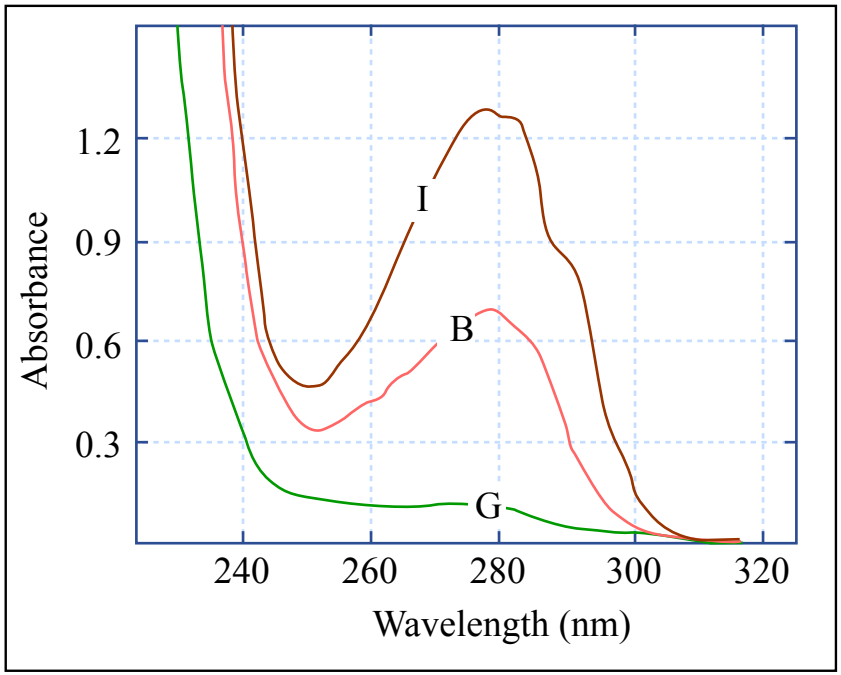


Image by MIT OpenCourseWare.

use Beer-Lambert law:

$$A_{280} = \varepsilon_{280} cl$$

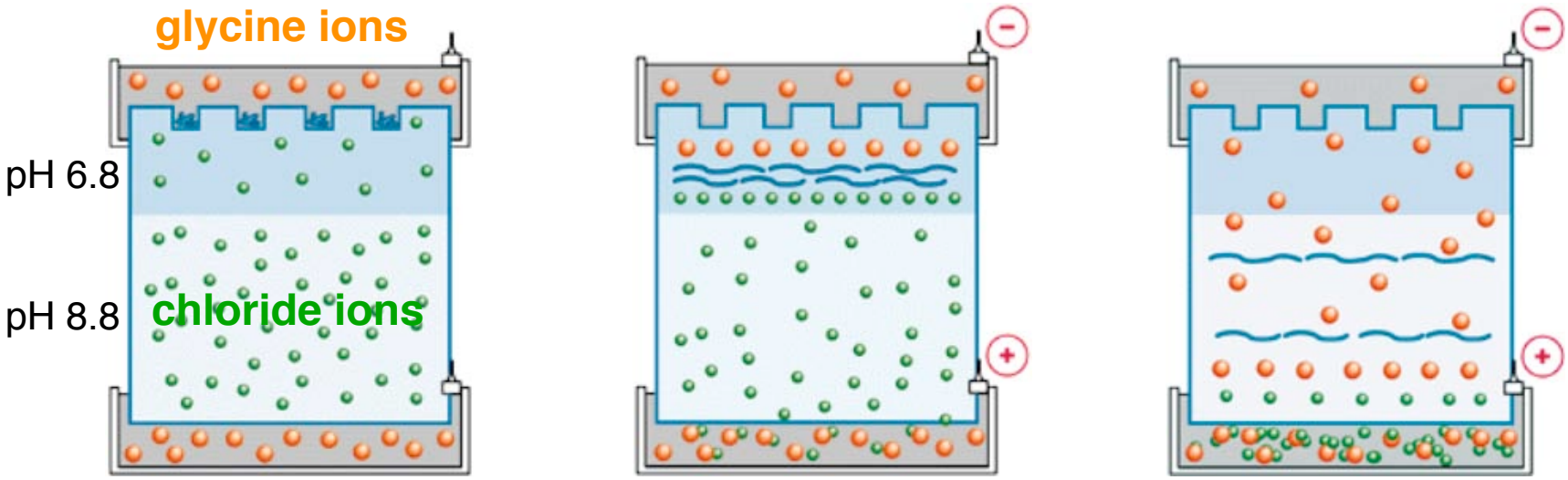
ε_{280} is the extinction coefficient; it can be determined rigorously, or estimated:

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

Assessing proteins for identity and purity

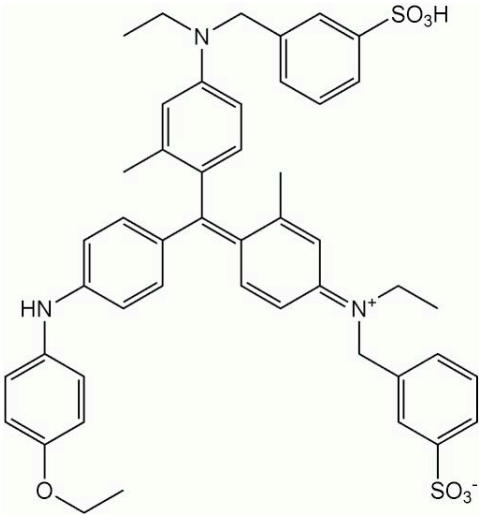
Most standard technique is sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE):

- 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

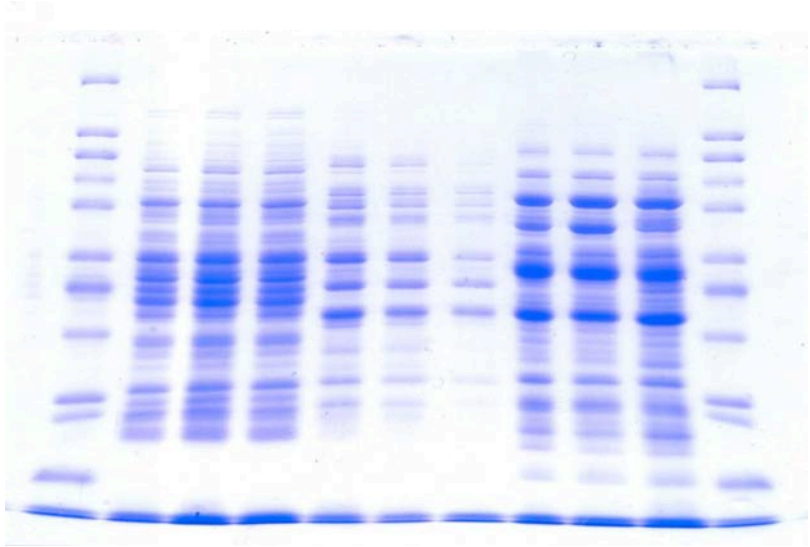


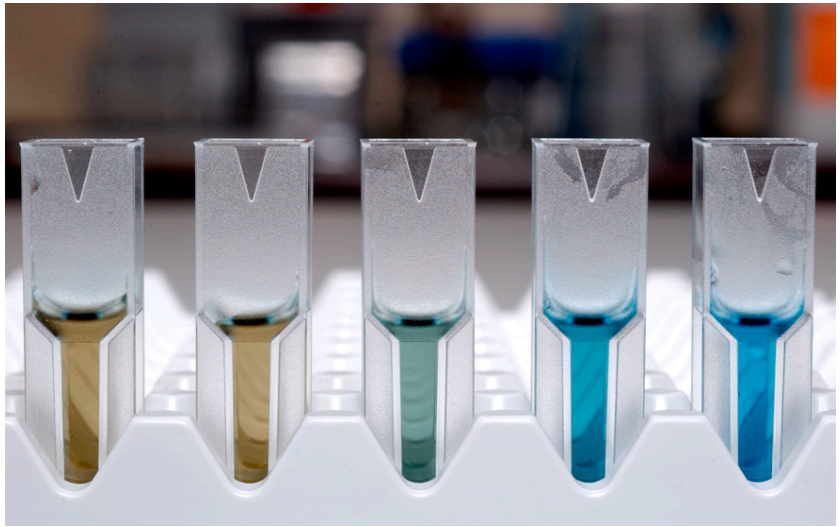
Source: "Multiphasic Buffer Systems" (http://nationaldiagnostics.com/article_info.php/articles_id/10).

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





http://www.eiroforum.org/media/gallery/embl.php

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

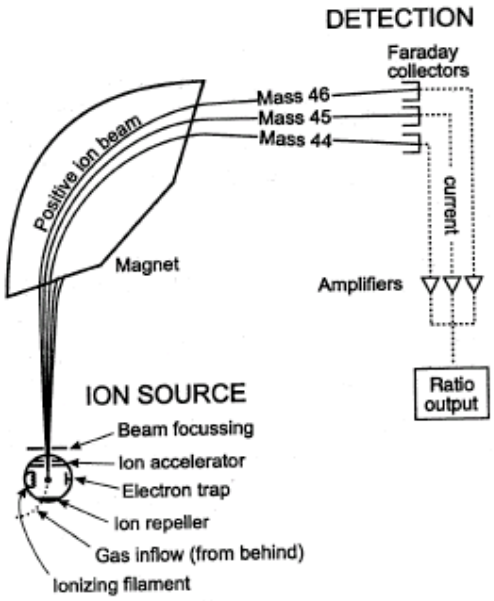
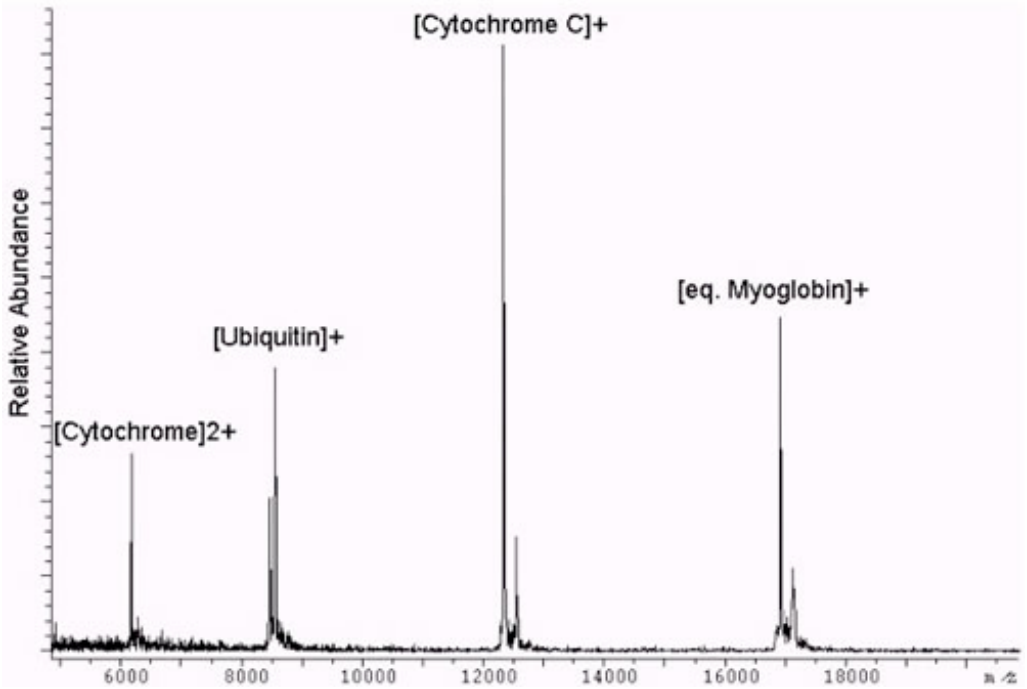
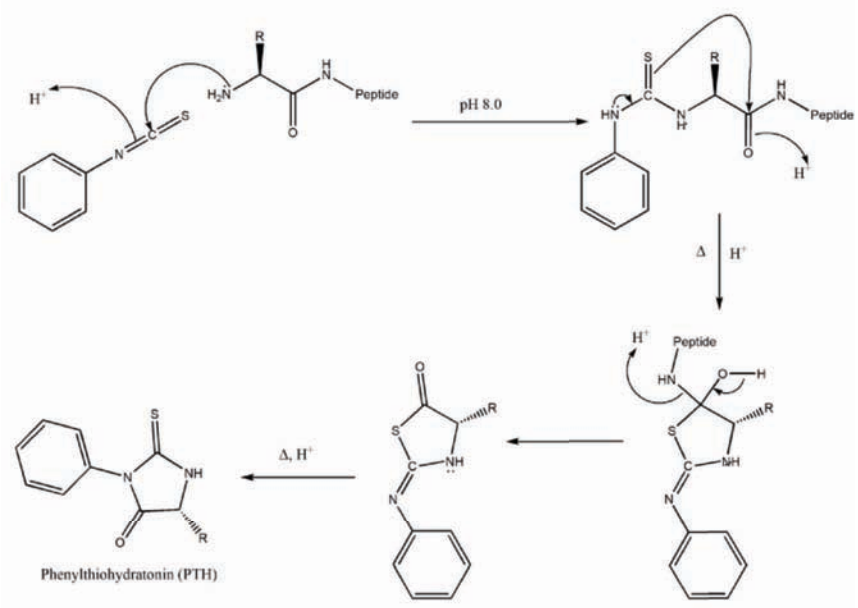


Image: public domain (USGS)



Source: http://www.kcl.ac.uk/research/facilities/mspec/instr/maldi-tof-introa.html

N-terminal sequencing (Edman degradation)

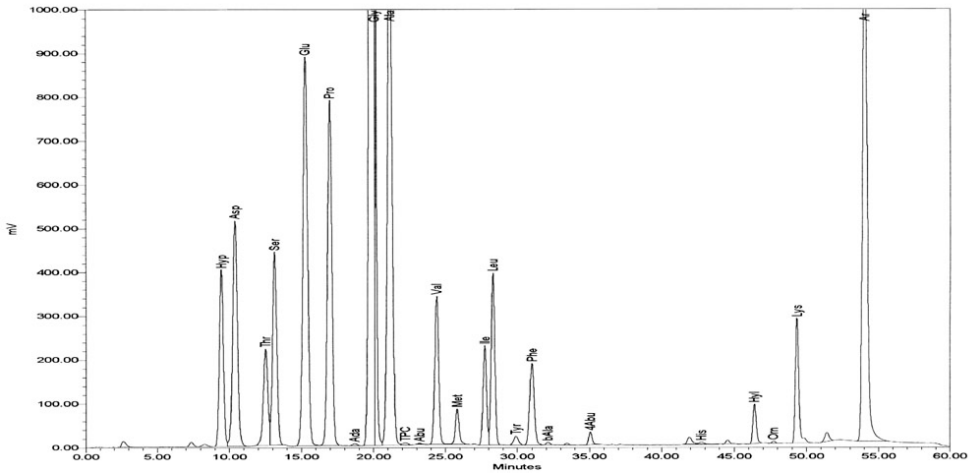


- products identified by chromatography or electrophoresis
- typically ~5 cycles practical for routine N-term. sequencing

en.wikipedia.org/wiki/Edman_degradation
public domain image

Amino acid analysis

- HCl digestion to digest peptide bonds
- HPLC to quantify AA components



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