

What is Bioanalytical Chemistry?

Bioanalytical Chemistry is an important sub-discipline of analytical chemistry that covers the quantitative measurement of xenobiotics (drugs and their metabolites, biological molecules in unnatural locations or unnatural concentrations) and biotics (macromolecules, proteins, DNA, large molecule drugs, metabolites) in biological systems.

Analytical Chemistry questions:

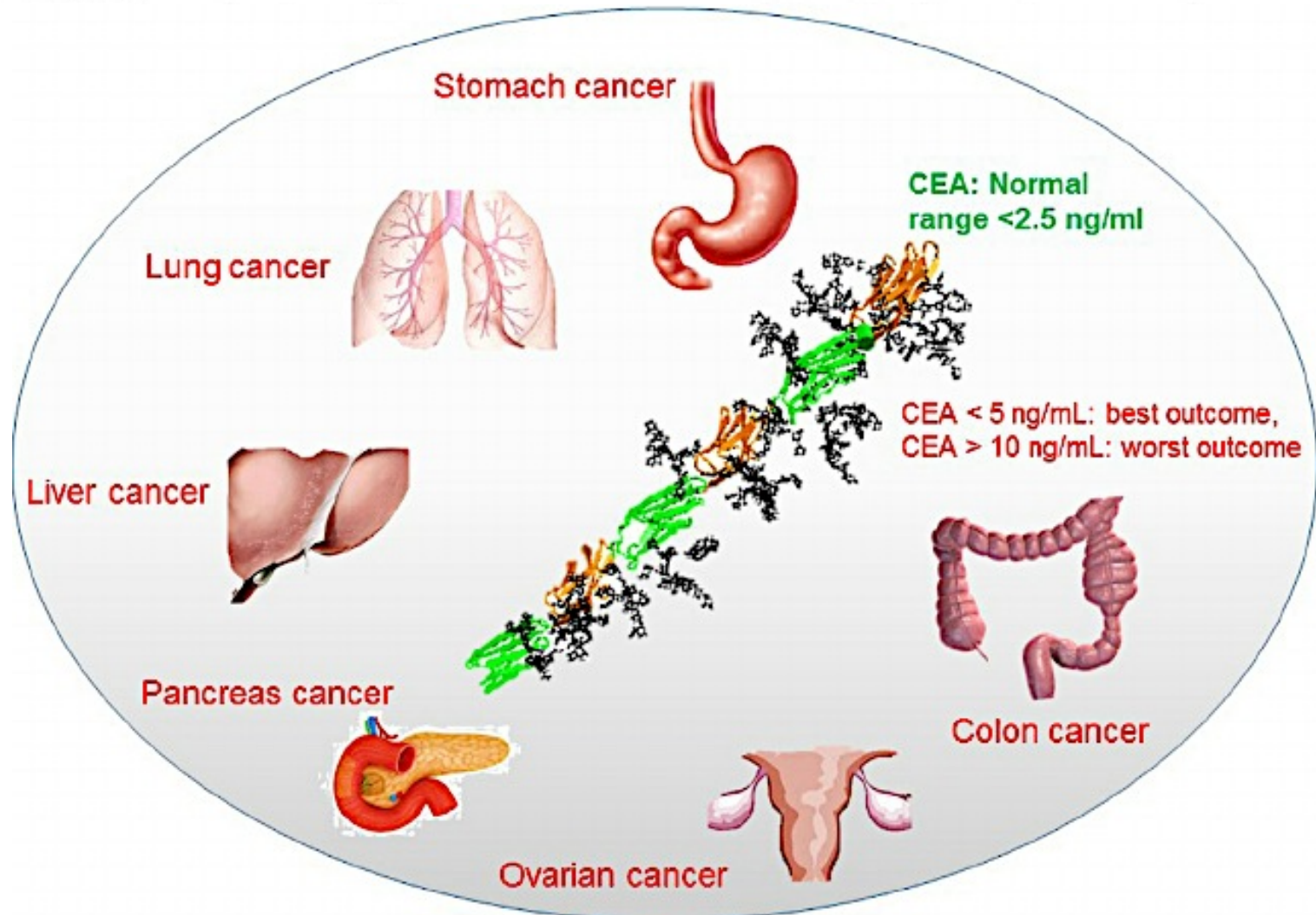
- i) What is there?
- ii) How much?

Bioanalytical Chemistry Specific Questions:

- iii) Where is it?
- iv) What is its activity?
- v) What does it interact with?

Carcinoembryonic antigen (CEA)

- CEA is a highly glycosylated cell surface glycoprotein involved in intercellular adhesion
- CEA is shed from the cell surface into the circulation and can be detected in serum.



How to Develop a Bioassay:

5 Considerations and 8 Fundamentals

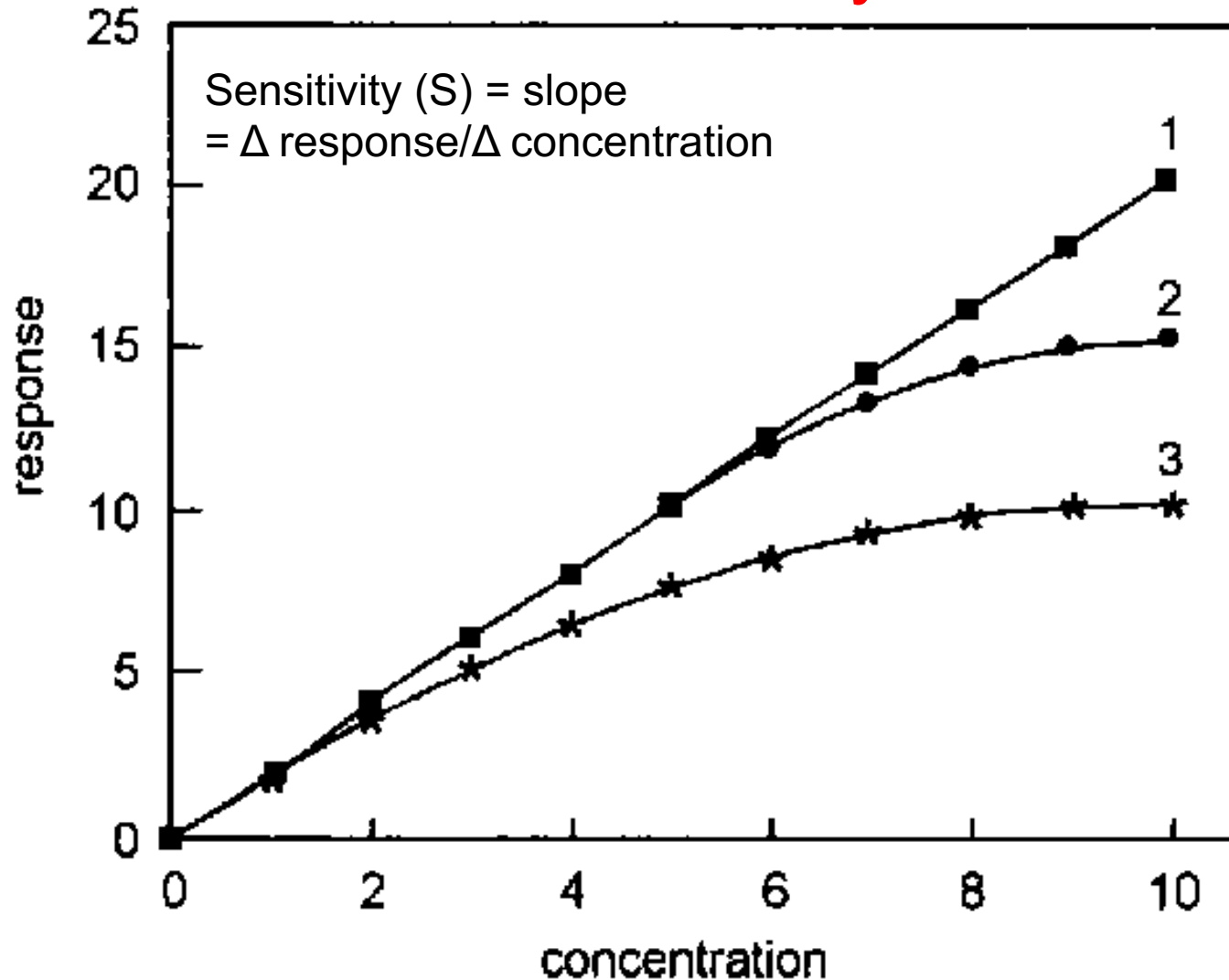
Question to ask	Points to consider
What is the exact molecule to be assayed?	Isoform/splice variant Total or modified (e.g. phosphorylated / acetylated / methylated)? Soluble or membrane-bound?
Parameter to assay?	Amount of molecule present? Biological function?
Source of molecule?	Sample availability Volume of sample Likely concentration of molecule Stability of molecule
Quantitative or semi-quantitative?	Is semi-quantitative measurement of the molecule sufficient, or does the study require rigorous quantitation?
Number of assay points to be run?	10s, 100s, 1000s? Sample-to-data streamlining Automation

How to Develop a Bioassay:

5 Considerations and 8 Fundamentals

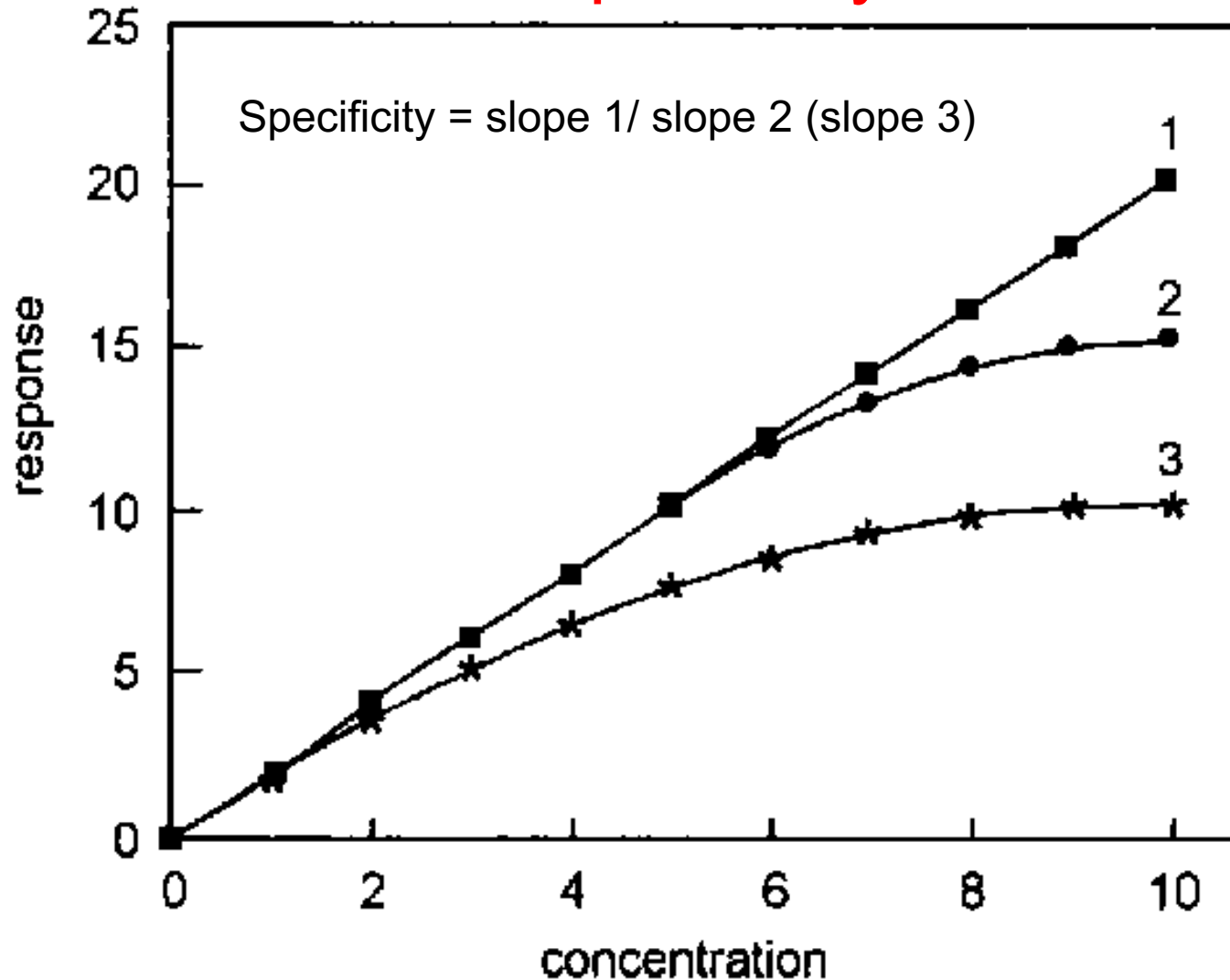
Assay parameter	Key considerations
Specificity	Will the assay detect only the desired molecule?
Sensitivity	Will the assay detect the levels of the molecule in the samples of interest?
Dynamic range	Will the levels of the molecule fall within the dynamic range of the assay?
Interference	Will components in the assay sample interfere with the assay?
Robustness	Can the assay cope with small changes in the assay sample/equipment/operator?
Reproducibility	Does the assay display low inter and intra assay variability?
Accuracy (precision)	Is the assay capable of accurately determining the absolute amount/concentration of the molecule?
Analysis of assay performance	Is it appropriate/desirable to statistically analyse assay performance? Does the assay have sufficient discriminating power?

Sensitivity



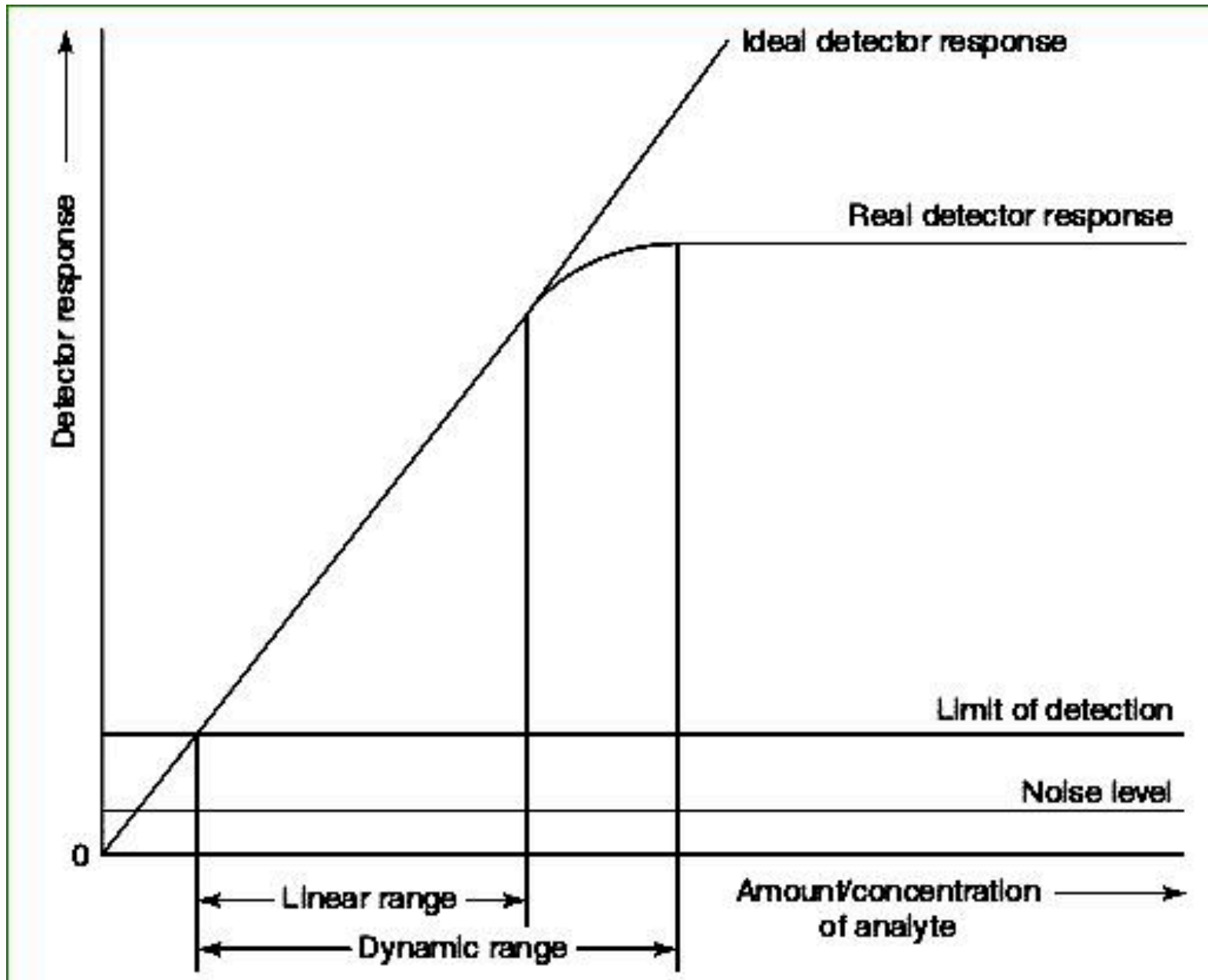
Method 1 is highly sensitive for a compound, method 2 – average, method 3 - low sensitive for the compound.

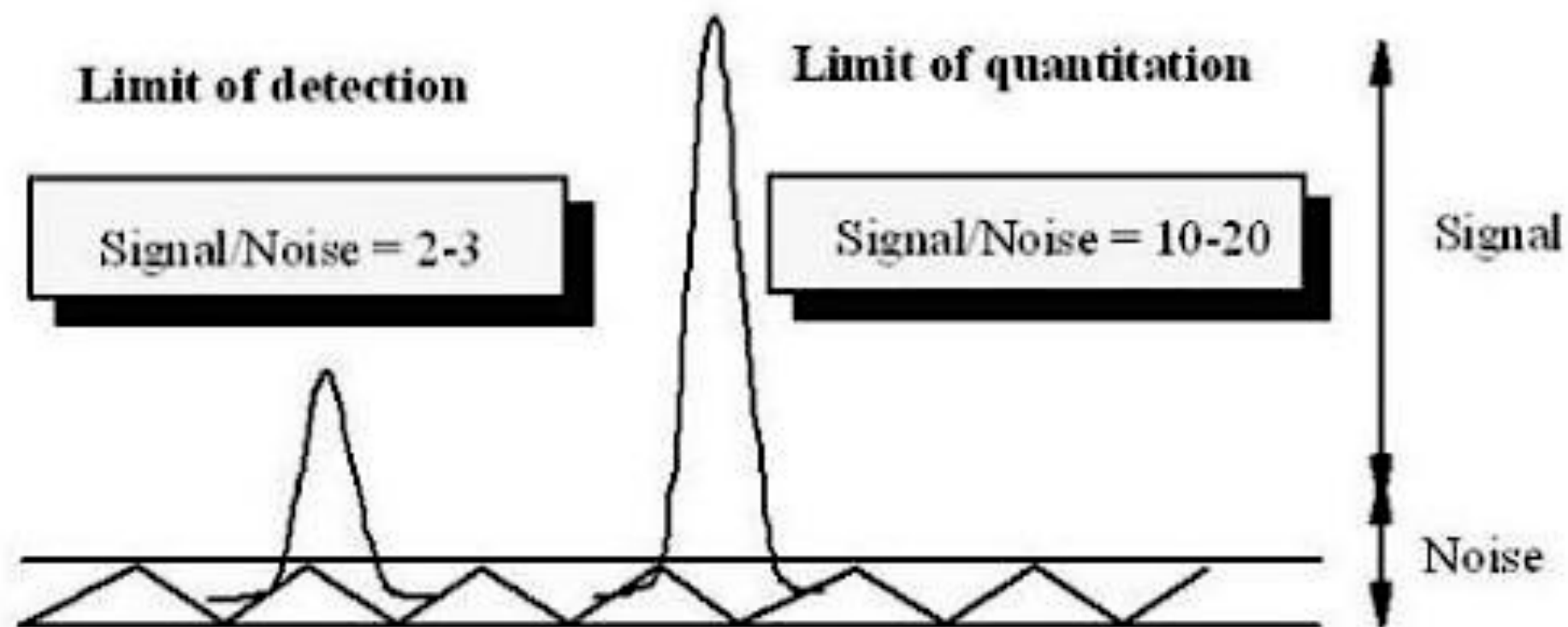
Specificity



A method is highly specific for compound 1, average for 2, low for 3.

Calibration or Standard Curve





$$\text{LOD} = \bar{x}_{\text{Blank}} + 3 * \text{SD}_{\text{Blank}}$$

$$\text{LOQ} = \bar{x}_{\text{Blank}} + 10 * \text{SD}_{\text{Blank}}$$

Analysis of Biopolymers: Identification and Quantitation

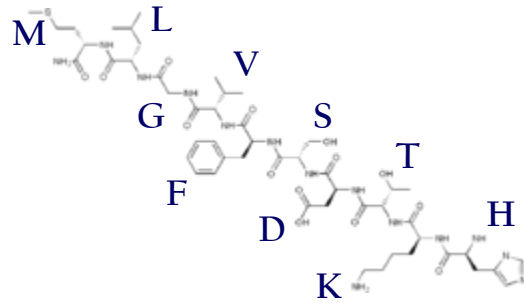
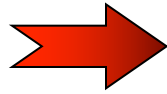
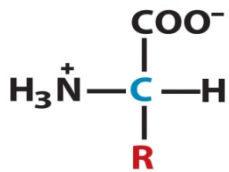
Properties of biopolymers that can be used for their analysis

- Size “SMS CIA CREST”
- Mass
- Shape
- Charge
- Isoelectric point
- Affinity interactions
- Chemical Reactivity
- Electron Structure

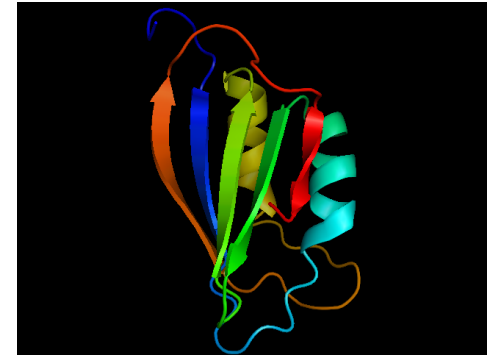
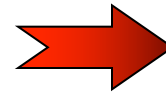


Proteins

☀ Amino Acids



Peptides



Proteins

☀ Proteins are definitely absolutely and totally the most important and coolest biological molecule!!

☀ Analytical Questions:

i) Which amino acids?

ii) In what order?

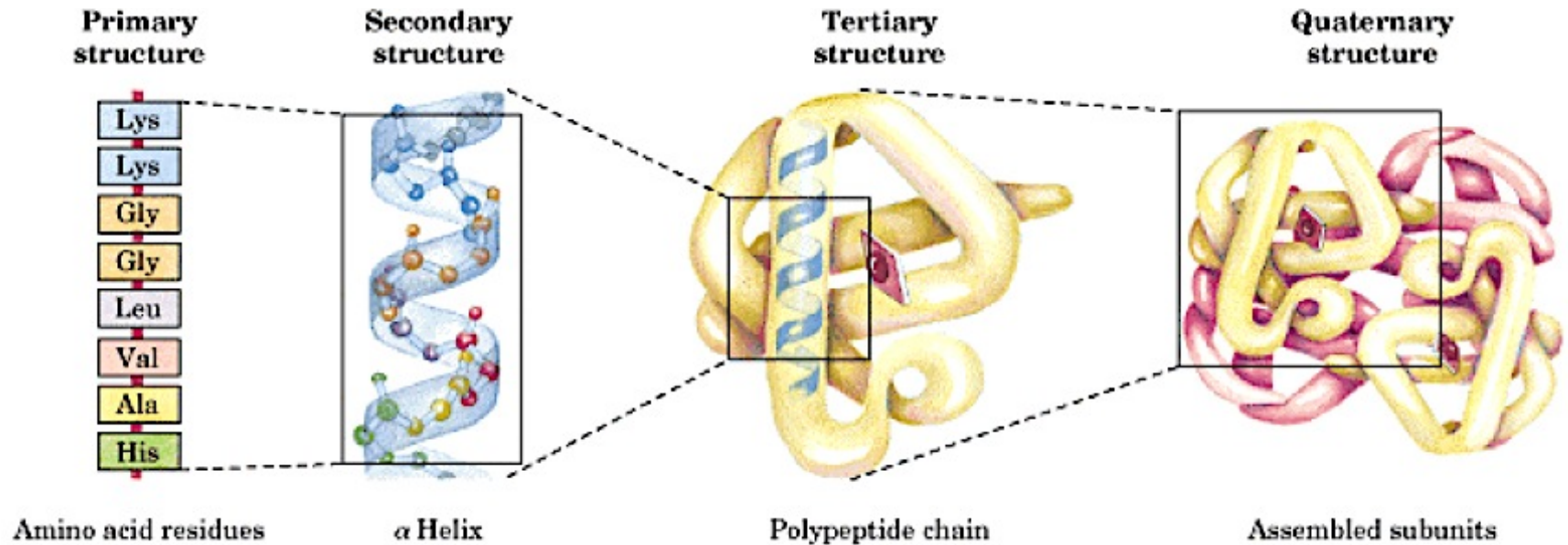
iii) Structure?

iv) Biological Function?

v) Interactions?

vi) Quantitation / Localization?

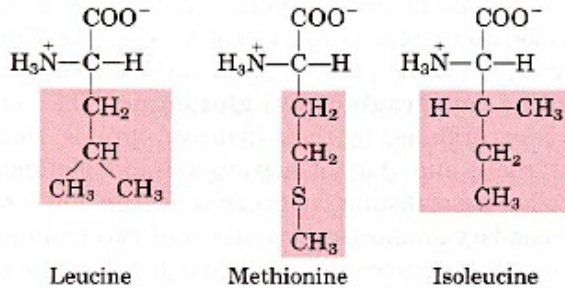
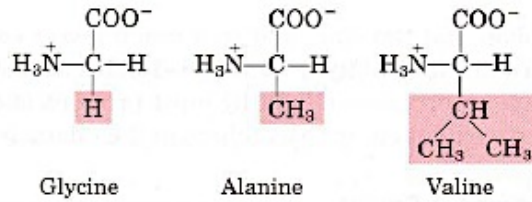
Four Levels of Protein Structuring



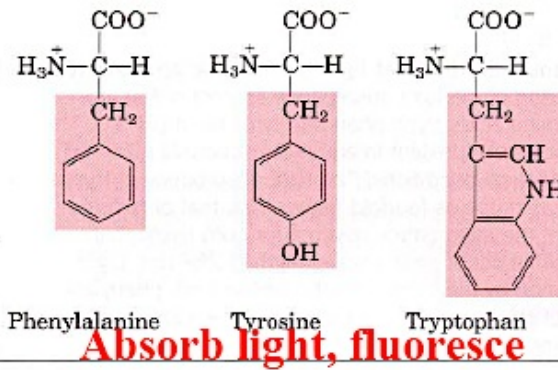
- Primary structure is the amino acid sequence
- Secondary structure is repeating geometry (α -helix and β -strand)
- Tertiary structure is overall spatial arrangement
- Quaternary structure is arrangement of several (folded) polypeptides

hydrophobic

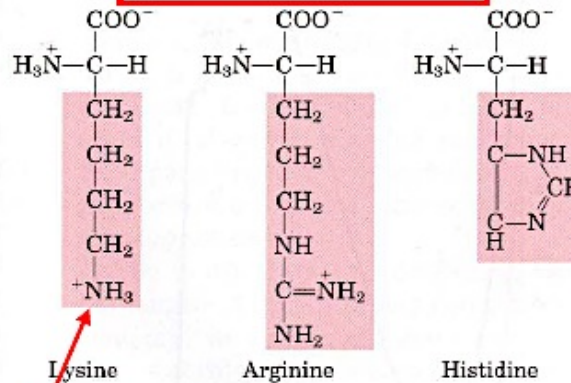
Nonpolar, aliphatic R groups



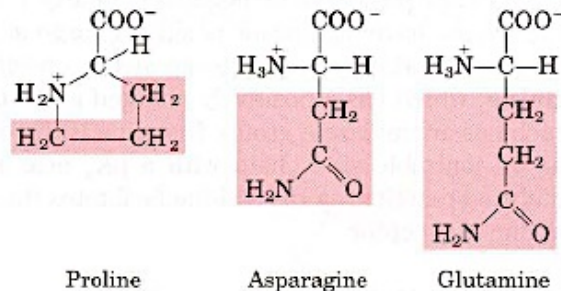
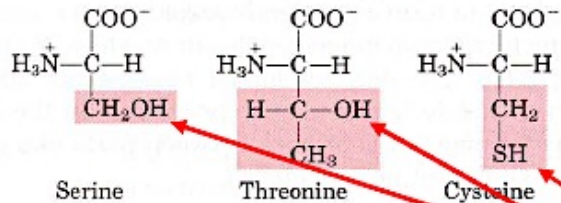
Aromatic R groups



Positively charged R groups

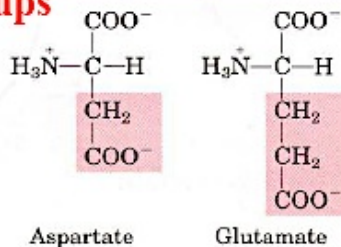


Polar, uncharged R groups



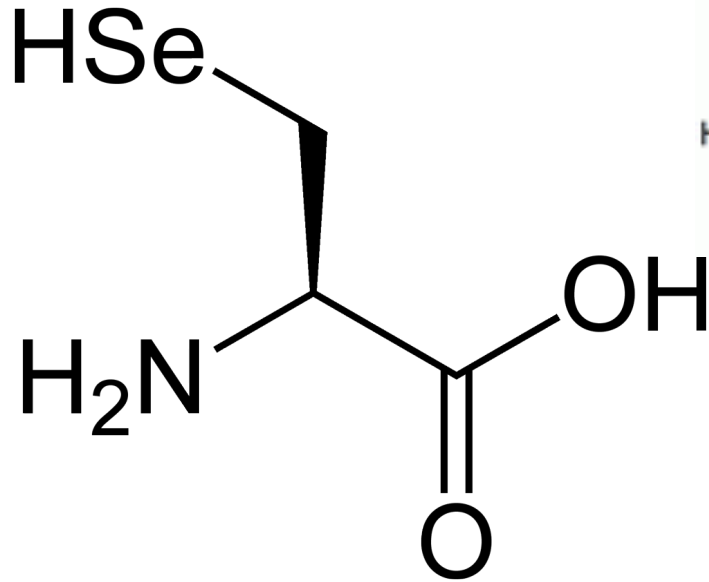
Reactive groups in R groups

Negatively charged R groups

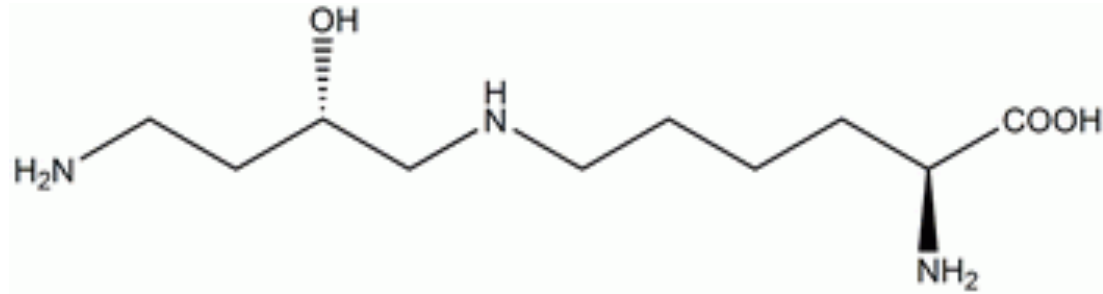


20 Amino Acids

Non-standard amino acids



Selenocysteine is an amino acid that is present in several enzymes (for example glutathione peroxidases, tetraiodothyronine 5' deiodinases, thioredoxin reductases, formate dehydrogenases, glycine reductases and some hydrogenases).



Hypusine is a non-standard amino acid found in all eukaryotes and in some archaea, but not in bacteria. The only known protein containing hypusine is eukaryotic translation initiation factor 5A (eIF5A) and a similar protein found in archaeobacteria

pKa Values of Amino Acids Define the Charge of Protein

Properties and Conventions Associated with the Standard Amino Acids

Amino acid	Abbreviated names		M_r	pK _a values		pK _R (R group)	pI	Hydropathy index*	Occurrence in proteins (%)
				pK ₁ (-COOH)	pK ₂ (-NH ₃ ⁺)				
Nonpolar, aliphatic R groups									
Glycine	Gly	G	75	2.34	9.60		5.97	-0.4	7.2
Alanine	Ala	A	89	2.34	9.69		6.01	1.8	7.8
Valine	Val	V	117	2.32	9.62		5.97	4.2	6.6
Leucine	Leu	L	131	2.36	9.60		5.98	3.8	9.1
Isoleucine	Ile	I	131	2.36	9.68		6.02	4.5	5.3
Methionine	Met	M	149	2.28	9.21		5.74	1.9	2.3
Aromatic R groups									
Phenylalanine	Phe	F	165	1.83	9.13		5.48	2.8	3.9
Tyrosine	Tyr	Y	181	2.20	9.11	10.07	5.66	-1.3	3.2
Tryptophan	Trp	W	204	2.38	9.39		5.89	-0.9	1.4
Polar, uncharged R groups									
Serine	Ser	S	105	2.21	9.15		5.68	-0.8	6.8
Proline	Pro	P	115	1.99	10.96		6.48	1.6	5.2
Threonine	Thr	T	119	2.11	9.62		5.87	-0.7	5.9
Cysteine	Cys	C	121	1.96	10.28	8.18	5.07	2.5	1.9
Asparagine	Asn	N	132	2.02	8.80		5.41	-3.5	4.3
Glutamine	Gln	Q	146	2.17	9.13		5.65	-3.5	4.2
Positively charged R groups									
Lysine	Lys	K	146	2.18	8.95	10.53	9.74	-3.9	5.9
Histidine	His	H	155	1.82	9.17	6.00	7.59	-3.2	2.3
Arginine	Arg	R	174	2.17	9.04	12.48	10.76	-4.5	5.1
Negatively charged R groups									
Aspartate	Asp	D	133	1.88	9.60	3.65	2.77	-3.5	5.3
Glutamate	Glu	E	147	2.19	9.67	4.25	3.22	-3.5	6.3
Average			120						

Important for terminal amino acids only