

# VU Learning HUB

## BIO601 Quiz File

Chapter-wise Solved MCQs

[vulearninghub.xyz](http://vulearninghub.xyz)

## Lesson 1: Introduction to Proteomics and the Proteome

**Q1. Who coined the term "proteome" in 1994 to refer to the entire set of proteins expressed by a genome, cell, tissue, or organism?**

- A) John Fenn
- B) Marc Wilkins
- C) Koichi Tanaka
- D) Kurt Wuthrich

**Q2. Why is the human proteome estimated to contain over 1 million distinct proteoforms despite the genome containing only about 20,000 protein-coding genes?**

- A) Because cell division duplicates protein genes continually
- B) Because of alternative splicing and post-translational modifications
- C) Because mRNA abundance directly multiplies protein translation tenfold
- D) Because genomic DNA changes in every cell type

**Q3. Which branch of proteomics specifically compares protein abundance between different samples, such as healthy versus diseased tissue?**

- A) Structural proteomics
- B) Functional proteomics
- C) Expression proteomics
- D) Clinical proteomics

**Q4. What is the estimated dynamic range of protein concentration in human plasma?**

- A)  $10^3$  to  $10^4$  orders of magnitude
- B)  $10^6$  orders of magnitude
- C)  $10^8$  orders of magnitude
- D)  $10^{12}$  orders of magnitude

### Answer Key

Q1: B -- Marc Wilkins coined the term "proteome" in 1994 to describe the complete protein set expressed by a genome, cell, or organism.

Q2: B -- Alternative splicing of mRNA transcripts and post-translational modifications expand about 20,000 genes into over 1 million proteoforms.

Q3: C -- Expression proteomics focuses on measuring and comparing protein abundance across different biological conditions or tissues.

Q4: D -- The dynamic range of protein concentration in human plasma spans  $10^{12}$  orders of magnitude, making analytical detection challenging.

## Lesson 2: Protein Separation I -- Gel Electrophoresis

**Q1. What is the primary role of sodium dodecyl sulfate (SDS) in SDS-PAGE?**

- A) It forms a pH gradient along the gel strip
- B) It denatures proteins and confers a uniform negative charge for size-based separation
- C) It digests proteins into small peptide fragments
- D) It acts as a fluorescent dye for spot detection

**Q2. What occurs when a protein reaches its isoelectric point (pI) during Isoelectric Focusing (IEF)?**

- A) Its net charge becomes zero and it stops migrating in the pH gradient
- B) It becomes positively charged and accelerates toward the anode
- C) It is cleaved into b- and y-ions by electrical voltage
- D) It binds irreversibly to cyanine dyes

**Q3. In Two-Dimensional Gel Electrophoresis (2-DE), by what property are proteins separated in the first and second dimensions, respectively?**

- A) Molecular mass in the first dimension, and pI in the second dimension
- B) Hydrophobicity in the first dimension, and charge in the second dimension
- C) Charge/pI in the first dimension (IEF), and molecular mass in the second dimension (SDS-PAGE)
- D) Affinity binding in the first dimension, and solubility in the second dimension

**Q4. How does Difference Gel Electrophoresis (DIGE) minimize gel-to-gel variability in 2-DE quantitative comparisons?**

- A) By replacing polyacrylamide gels with agarose matrices
- B) By multiplexing samples labeled with distinct cyanine dyes (Cy2, Cy3, Cy5) on the same gel with an internal standard
- C) By eliminating the first-dimension charge separation step
- D) By staining gels exclusively with silver stain

### Answer Key

Q1: B -- SDS is an anionic detergent that denatures proteins and coats them with negative charge so migration depends strictly on mass.

Q2: A -- At its pI, a protein carries zero net charge in the pH gradient and halts migration during isoelectric focusing.

Q3: C -- 2-DE combines charge/pI separation in the first dimension (IEF) with molecular mass separation in the second dimension (SDS-PAGE).

Q4: B -- DIGE uses distinct fluorescent cyanine dyes to co-run multiple samples and an internal standard on a single gel.

## Lesson 3: Protein Separation II -- Chromatography

**Q1. In size-exclusion (gel filtration) chromatography, which molecules elute from the column first?**

- A) Smallest molecules, because they pass through internal pore volumes
- B) Positively charged molecules, because they bind cation exchange resins
- C) Hydrophobic peptides, because they interact with C18 stationary phases
- D) Largest molecules, because they are excluded from the stationary phase pores

**Q2. Why is nano-LC (operating at flow rates of 200-500 nL/min) widely adopted for LC-MS proteomics?**

- A) It increases column heating to accelerate solvent flow
- B) Low flow rates significantly boost electrospray ionization efficiency and analytical sensitivity
- C) It allows direct separation of intact cells without lysis
- D) It eliminates the need for liquid mobile phase gradients

**Q3. Which two orthogonal separation techniques are integrated into Multidimensional Protein Identification Technology (MudPIT)?**

- A) Anion exchange and size exclusion chromatography

- B) Strong cation exchange (SCX) and reversed-phase chromatography (RPC)
- C) Immunoaffinity enrichment and gel filtration
- D) Hydrophobic interaction and metal chelate chromatography

**Q4. What is a primary limitation associated with gel-free shotgun (bottom-up) proteomics workflows?**

- A) Inability to separate membrane or hydrophobic proteins
- B) The protein inference problem when assigning shared peptides to specific parent proteins
- C) Lower overall proteome coverage compared to 2-DE gels
- D) Excessive labor requirements for spot picking

**Answer Key**

Q1: D -- Large molecules cannot enter gel pores in size-exclusion chromatography and thus elute first in the void volume.

Q2: B -- Low nanoliter flow rates in nano-LC enhance electrospray ionization efficiency, allowing attomole-level peptide sensitivity.

Q3: B -- MudPIT combines strong cation exchange (charge separation) and reversed-phase LC (hydrophobicity separation) online.

Q4: B -- Bottom-up digestion creates shared peptides, leading to ambiguity when assigning peptides back to specific parent proteoforms.

## Lesson 4: Mass Spectrometry Fundamentals

**Q1. Which two soft-ionization developments earned the 2002 Nobel Prize in Chemistry for making proteomics possible?**

- A) Electron ionization (EI) and chemical ionization (CI)
- B) Electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI)
- C) Fast atom bombardment (FAB) and secondary ion mass spectrometry (SIMS)
- D) Inductively coupled plasma (ICP) and thermal ionization

**Q2. What key feature of Electrospray Ionization (ESI) allows large peptides and proteins to be analyzed within standard mass analyzer ranges?**

- A) It forms exclusively singly charged ions
- B) It generates multiply charged gas-phase ions, reducing their  $m/z$  values
- C) It completely fragments peptides prior to mass analysis
- D) It relies on solid matrix co-crystallization

**Q3. Which mass analyzers measure ion oscillation frequencies to deliver ultra-high resolution and mass accuracy?**

- A) Linear ion traps and 3-D ion traps
- B) Single quadrupole filters
- C) Orbitrap and Fourier-transform ion cyclotron resonance (FT-ICR) analyzers
- D) Time-of-flight (TOF) drift tubes

**Q4. What is the calculated  $m/z$  ratio of a peptide with a monoisotopic mass of 1500 Da carrying 2 protons (proton mass = 1.0073 Da)?**

- A) About 1502.0
- B) About 751.0
- C) About 501.0

D) About 3000.0

### Answer Key

Q1: B -- ESI (developed by John Fenn) and MALDI (developed by Koichi Tanaka) won the 2002 Nobel Prize in Chemistry for soft protein ionization.

Q2: B -- ESI produces multiply charged ions, bringing the mass-to-charge ratio ( $m/z$ ) of large biomolecules into the measurable range of mass analyzers.

Q3: C -- Orbitrap and FT-ICR instruments determine mass by measuring ion oscillation frequencies, providing highest resolution and accuracy.

Q4: B -- The  $m/z$  value is calculated as  $(1500 + 2 \times 1.0073) / 2 =$  about 751.0.

## Lesson 5: Peptide Mass Fingerprinting and Tandem MS

### Q1. Why is trypsin the standard cleavage protease in bottom-up proteomics?

- A) It cleaves non-specifically to generate single amino acids
- B) It cleaves specifically after Lys and Arg (unless followed by Pro), producing ideal 7-20 AA peptides with basic C-termini
- C) It cleaves only at C-terminal methionine residues
- D) It functions without requiring protein denaturation

### Q2. What is a key requirement and limitation of Peptide Mass Fingerprinting (PMF)?

- A) It requires a pure single protein and a fully sequenced database, failing on complex mixtures or unexpected PTMs
- B) It requires MS/MS fragmentation spectra for every peptide
- C) It can only analyze unpurified cell lysates
- D) It relies exclusively on electron transfer dissociation

### Q3. In collision-induced dissociation (CID/HCD) of peptides, which fragment ion series correspond to N-terminal and C-terminal fragments, respectively?

- A) a-ions and x-ions
- B) b-ions (N-terminal) and y-ions (C-terminal)
- C) c-ions and z-ions
- D) Precursor ions and reporter ions

### Q4. Which pair of amino acid residues are isobaric (both having a residue mass of 113 Da) and cannot be distinguished by mass alone?

- A) Lysine and Arginine
- B) Leucine and Isoleucine
- C) Glycine and Alanine
- D) Aspartic acid and Glutamic acid

### Answer Key

Q1: B -- Trypsin cleaves after Lys and Arg, generating peptides of 7-20 amino acids with basic C-termini that favor MS fragmentation.

Q2: A -- PMF compares single-MS peptide mass lists against database digests, requiring pure proteins and sequenced genomes.

Q3: B -- Peptide bond cleavage during CID/HCD generates N-terminal b-ions and C-terminal y-ions.

Q4: B -- Leucine and Isoleucine share identical chemical formulas and an exact residue mass of 113 Da.

## Lesson 6: Protein Identification and Sequence Databases

### Q1. How is UniProtKB/Swiss-Prot distinguished from UniProtKB/TrEMBL?

- A) Swiss-Prot contains automatically translated unreviewed sequences, while TrEMBL is manually curated
- B) Swiss-Prot is manually curated and reviewed with functional annotation, while TrEMBL contains automatically annotated unreviewed translations
- C) Swiss-Prot contains only plant proteomes, while TrEMBL contains human proteomes
- D) Swiss-Prot is an MS raw spectrum database, while TrEMBL stores DNA sequences

### Q2. Which scoring algorithms are utilized by Mascot and SEQUEST database search engines, respectively?

- A) Mascot uses cross-correlation (XCorr); SEQUEST uses MOWSE score
- B) Mascot uses a probability-based MOWSE score; SEQUEST uses cross-correlation (XCorr)
- C) Mascot uses AlphaFold confidence; SEQUEST uses E-values
- D) Mascot uses spectral counts; SEQUEST uses peak area ratios

### Q3. How is the False Discovery Rate (FDR) estimated and controlled in database searching?

- A) By searching experimental spectra against a target-decoy database (real vs. reversed/shuffled sequences) to set a threshold (typically 1% FDR)
- B) By comparing peak intensities of synthetic isotope standards
- C) By excluding all peptides containing methionine oxidation
- D) By re-sequencing peptides using Edman degradation

### Q4. What strategy is used to resolve the protein inference problem when peptides are shared among multiple candidate proteins?

- A) Reporting every possible protein match without grouping
- B) Applying the parsimony principle to group proteins and report the minimal set explaining observed peptides
- C) Discarding all shared peptides from the analysis
- D) Increasing precursor mass tolerance to 1000 ppm

### Answer Key

Q1: B -- UniProtKB/Swiss-Prot provides manually reviewed functional annotations, whereas TrEMBL holds unreviewed computational translations.

Q2: B -- Mascot scores peptide matches using a MOWSE probability algorithm, whereas SEQUEST uses cross-correlation (XCorr).

Q3: A -- The target-decoy strategy searches decoy (reversed) sequences alongside target sequences to maintain a standard 1% FDR cutoff.

Q4: B -- Parsimony grouping identifies the smallest set of protein sequences necessary to account for all identified peptides.

## Lesson 7: Quantitative Proteomics

### Q1. Which quantitative proteomics approach incorporates stable isotopes ( $^{13}\text{C}/^{15}\text{N}$ -labeled amino acids) metabolically during cell growth?

- A) iTRAQ chemical labeling
- B) TMT isobaric tagging
- C) SILAC (Stable Isotope Labeling by Amino Acids in Cell Culture)

D) Label-free spectral counting

**Q2. What technical limitation affects multiplexed isobaric chemical tagging (iTRAQ/TMT), and how is it mitigated?**

- A) High precursor mass drift; mitigated by increasing spray voltage
- B) Ratio compression caused by co-isolated interfering precursor ions; mitigated by MS3-based quantitation (SPS-MS3)
- C) Inability to pool samples; mitigated by running separate LC columns
- D) Degradation of reporter ions; mitigated by trypsin digestion

**Q3. Which targeted mass spectrometry technique on a triple quadrupole instrument monitors specific precursor-to-fragment transitions as the gold standard for biomarker verification?**

- A) Selected Reaction Monitoring / Multiple Reaction Monitoring (SRM/MRM)
- B) Peptide Mass Fingerprinting (PMF)
- C) Data-Independent Acquisition (DIA/SWATH)
- D) Isoelectric Focusing (IEF)

**Q4. What are the two primary metrics used to perform label-free quantitation (LFQ)?**

- A) Reporter ion heights and isobaric tag ratios
- B) Spectral counting and precursor ion chromatographic peak intensity integration
- C) Gel spot volume and silver stain brightness
- D) Retention time shifts and buffer pH changes

### Answer Key

Q1: C -- SILAC metabolically incorporates heavy  $^{13}\text{C}/^{15}\text{N}$  lysine and arginine into living cell cultures for accurate MS1 quantification.

Q2: B -- Co-isolated precursor ions cause ratio compression in TMT/iTRAQ, which can be overcome using synchronous precursor selection MS3 (SPS-MS3).

Q3: A -- SRM/MRM selectively filters target precursor and fragment transition pairs on triple quadrupoles for precise, sensitive validation.

Q4: B -- Label-free quantitation evaluates protein abundance via MS/MS spectral counts or integrated MS1 peptide peak areas.

## Lesson 8: Post-Translational Modifications

**Q1. Which affinity enrichment methods selectively capture phosphopeptides prior to MS analysis?**

- A) Immobilized Metal Affinity Chromatography (IMAC) and Titanium Dioxide ( $\text{TiO}_2$ )
- B) Lectin affinity columns and hydrazide beads
- C) C18 reversed-phase resins and cation exchange
- D) Anti-ubiquitin antibody beads and protein A resins

**Q2. Why is Electron Transfer Dissociation (ETD) advantageous over CID for analyzing phosphopeptides?**

- A) ETD induces a dominant 98 Da neutral loss of phosphoric acid
- B) ETD breaks the peptide backbone while preserving labile phosphate groups, allowing precise site localization
- C) ETD removes all post-translational modifications completely
- D) ETD works without mass analyzers

**Q3. Following tryptic digestion, what characteristic modification remnant is left on modified lysine residues by ubiquitination?**

- A) A neutral loss of 98 Da
- B) A Gly-Gly (diglycine) remnant producing a mass shift of +114.04 Da
- C) An acetyl group producing a mass shift of +42.01 Da
- D) A phosphate group producing a mass shift of +79.97 Da

**Q4. What is the characteristic mass shift imparted to a peptide by phosphorylation?**

- A) +15.99 Da (oxidation)
- B) +42.01 Da (acetylation)
- C) +79.97 Da (phosphorylation)
- D) +114.04 Da (ubiquitination remnant)

### Answer Key

Q1: A -- IMAC (utilizing Fe<sup>3+</sup>/Ga<sup>3+</sup>) and TiO<sub>2</sub> selectively bind negatively charged phosphate groups to enrich sub-stoichiometric phosphopeptides.

Q2: B -- ETD fragment cleavage preserves non-covalent and labile PTMs on amino acid side chains, enabling exact site localization.

Q3: B -- Tryptic cleavage of ubiquitin leaves a signature Gly-Gly remnant attached to lysine residues (+114.04 Da).

Q4: C -- Covalent addition of a phosphate group (HPO<sub>3</sub>) increases a peptide's monoisotopic mass by 79.97 Da.

## Lesson 9: Protein Structure, Interactions, and Applications

**Q1. Which structural proteomics method resolves near-atomic 3-D structures of large protein complexes in solution without requiring crystals?**

- A) X-ray crystallography
- B) Cryo-Electron Microscopy (cryo-EM)
- C) Nuclear Magnetic Resonance (NMR) spectroscopy
- D) Hydrogen-deuterium exchange (HDX-MS)

**Q2. What artificial intelligence system achieved breakthrough accuracy in predicting 3-D protein structures directly from primary amino acid sequences?**

- A) Mascot
- B) Cytoscape
- C) AlphaFold
- D) STRING

**Q3. What are the three standard phases of a biomarker discovery and translation pipeline?**

- A) (1) Top-down, (2) Middle-down, (3) Bottom-up
- B) (1) Discovery (TMT/DIA), (2) Verification (SRM/MRM), (3) Clinical validation (ELISA/antibody assay)
- C) (1) IEF, (2) SDS-PAGE, (3) Spot picking
- D) (1) Y2H, (2) AP-MS, (3) Microarray

**Q4. What is the main objective of the international Human Proteome Project (HPP)?**

- A) To sequence the human genome using next-generation sequencing
- B) To map every human protein and identify remaining "missing proteins" lacking MS evidence
- C) To synthesize artificial proteomes for cell-free expression

D) To eliminate the need for mass spectrometry in clinical diagnostics

### Answer Key

Q1: B -- Cryo-EM captures macromolecular complexes in near-native states at near-atomic resolution without crystallization.

Q2: C -- DeepMind's AlphaFold AI system accurately predicts 3D protein structures from linear amino acid sequences.

Q3: B -- Biomarker translation moves from unbiased discovery (TMT/DIA) to targeted verification (SRM/MRM) and large-scale clinical validation (ELISA).

Q4: B -- The HPP coordinates global research to map all human proteins and confirm currently unannotated "missing proteins".

# End of Quiz

BIO601 — 36 solved MCQs  
Prepared by VU Learning HUB  
[vulearninghub.xyz](http://vulearninghub.xyz)