

Introduction to Biophysical Techniques

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Key concepts, common methods, and practical considerations

1. What Are Biophysical Techniques?

Definition	Purpose	Typical Applications
Experimental methods that apply physical principles to study biological molecules and systems.	Quantify structure, dynamics, interactions, thermodynamics, and mechanical properties of biomolecules.	Protein folding, membrane protein analysis, nucleic acid binding, enzyme kinetics, drug target validation.

2. Core Physical Principles Used

Principle	Observable	Typical Instrumentation
Electromagnetism	Absorbance, fluorescence, scattering, conductivity.	UV-Vis spectrophotometer, fluorometer, SPR sensor.
Thermodynamics	Enthalpy (ΔH), entropy (ΔS), binding constants (K_D).	Isothermal titration calorimetry (ITC), differential scanning calorimetry (DSC).
Mechanics	Force, elasticity, viscosity.	Atomic force microscopy (AFM), optical tweezers, magnetic tweezers.
Quantum Mechanics	Electron spin, nuclear spin.	Electron paramagnetic resonance (EPR), nuclear magnetic resonance (NMR).
Wave phenomena	Diffraction, interference.	X-ray crystallography, cryo-electron microscopy (cryo-EM), small-angle X-ray scattering (SAXS).

3. Major Technique Categories

3.1 Spectroscopic Methods

Technique	What It Measures	Strengths	Limitations
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UV-Vis Absorption	Chromophore concentration, conformational changes.	Simple, fast, inexpensive.	Low structural detail.
Fluorescence (steady-state & time-resolved)	Environment of fluorophores, binding, distance (FRET).	High sensitivity, can be done in live cells.	Photobleaching, requires labeling.
Circular Dichroism (CD)	Secondary structure content of proteins, nucleic acids.	Quick secondary structure estimate.	Limited to low-resolution information.
Raman & FTIR	Vibrational modes of chemical bonds, secondary structure.	Minimal sample preparation, can work in aqueous solution.	Weak signal (Raman), water absorption (FTIR).

3.2 Scattering & Diffraction

Technique	Sample State	Information Obtained
X-ray Crystallography	Crystals	Atomic-resolution 3D structure.
Small-Angle X-ray Scattering (SAXS)	Solution	Low-resolution shape, radius of gyration, oligomeric state.
Small-Angle Neutron Scattering (SANS)	Solution	Contrast variation (e.g., deuterium labeling) for multi-component complexes.
Cryo-EM	Vitrified particles	Near-atomic structures for large complexes; does not require crystals.

3.3 Microscopy & Imaging

Technique	Resolution	Typical Use
Atomic Force Microscopy (AFM)	~0.1 nm (lateral)	Surface topography, single-molecule force spectroscopy.
Optical Tweezers	~10 nm	Manipulating and measuring forces on single biomolecules.
Total Internal Reflection Fluorescence (TIRF) Microscopy	~200 nm axial	Single-molecule dynamics near surfaces.