

Biophysical Methods for Therapeutic Protein Analysis

Biophysical methods probe protein structure, dynamics, and interactions through a molecule's physical properties or behavior. Many characteristics lend themselves to biophysical analysis, but for therapeutic proteins the "big three" applications are aggregation, stability, and affinity or binding. Although no single biophysical method is perfect, their accessibility and low cost allow for the application of two or more orthogonal methods to achieve the desired result.

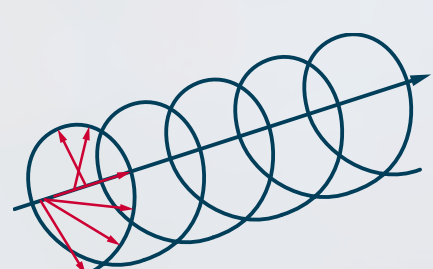
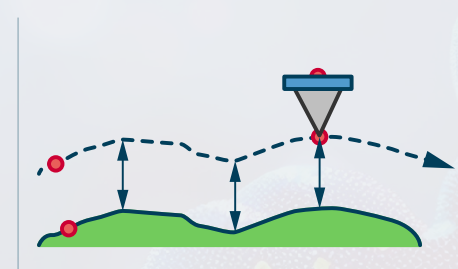
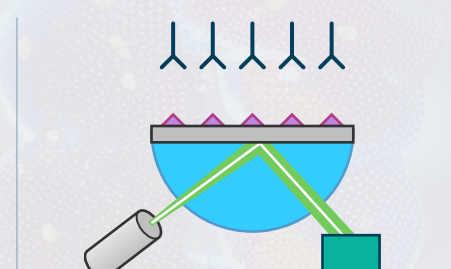
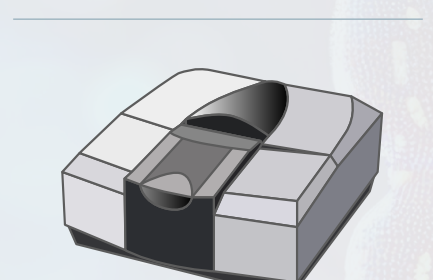
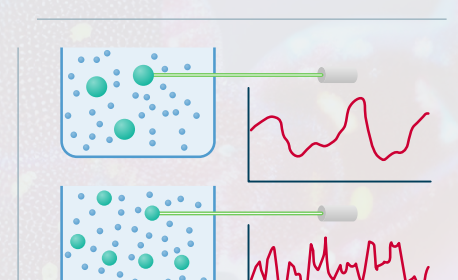
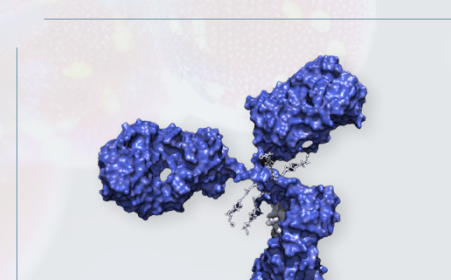
Why Are Biophysical Methods Important?

Biophysical methods (BPMs) relate biological activity to a molecule's physical attributes. Specifically, they:

- Provide biological insights from quantifiable physical properties
- Inform on the molecular-level structure, properties, dynamics, function, biological activity, and interactions of biological compounds through the molecule's physical attributes or behavior
- Are extremely diverse, encompassing microscopy, spectroscopy, electrophysiology, crystallography, chromatography, and molecular modeling¹
- Are simpler overall, with low sample preparation and material requirements, less waste, fewer reagents and related disposal costs, and the potential for greater speed and throughput²

Example: Viral Vectors

BPMs provide value throughout a molecule's lifecycle through screening, lead optimization, affinity determination, kinetic and thermodynamic analysis, and further through development and beyond to post-marketing stages. Below we list the methods most often used for a variety of applications.

 <p>Stability characterization Dynamic or static light scattering, back-reflection, nano differential scanning fluorimetry, circular dichroism³; ultraviolet absorbance, fluorescence⁴, plus AI-based interpretation</p>	 <p>Measuring biological forces Microbead-based traction force microscopy, micropillar-based TFM, atomic force microscopy, magnetic tweezers, optical tweezers, molecular force probes, and micropipette aspiration⁵</p>	 <p>Binding or affinity Spectral shift⁶, microscale thermophoresis⁷, temperature-related intensity change⁸, SPR⁸</p>
 <p>Secondary/tertiary structure determinations Differential scanning calorimetry, circular dichroism, Fourier transform infrared spectroscopy, nuclear magnetic resonance⁹</p>	 <p>Aggregation Size exclusion chromatography, multi-angle light scattering, analytical ultracentrifugation, dynamic light scattering, membrane microscopy⁹</p>	 <p>Molecules Therapeutic proteins and derivatives (antibody-drug conjugates, bispecific antibodies, etc.), proteolysis-targeting chimeras¹⁰, gene therapy¹¹</p>

Biophysical Methods for Protein Structural Analysis Strengths and Weaknesses

Biophysical methods are rapid, label-free, automatable, sample-sparing, high-throughput, require minimal sample preparation, generate little to no waste, do not involve expensive reagents, and are capable of time-course experimentation from a single sample.

While all biophysical methods have limitations their benefits, accessibility, short assay duration, and ease of use afford investigators with opportunities for employing multiple, orthogonal methods.

Biophysical Structure Method	Limitation
Circular dichroism	Low-concentration proteins
Mid-infrared spectroscopy	Low sensitivity
Fluorescence spectroscopy	Requires chromogenic groups
Ultraviolet-visible absorption	Requires chromogenic groups
Nuclear magnetic resonance	Limited to low molecular weight proteins
X-ray diffraction	Requires crystallization
Small-angle neutron diffraction	Difficulty extrapolating to solution
Cryo-electron microscopy	Expensive, resource-intensive
Raman spectroscopy	Method-specific requirements and weaknesses
Mass spectroscopy	Dynamic range problems with biological samples
Laser light scattering	Non-standardized instrumentation, readouts
Differential scanning calorimetry	Destructive method, no direct structural info
Surface plasmon resonance	Requires ligand immobilization (binding study)

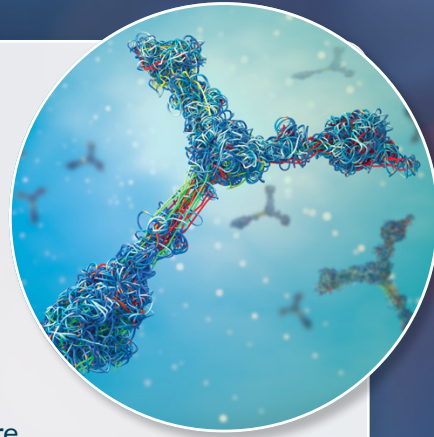
Example: PROTACs

Proteolysis-targeting chimeras (PROTACs) are small, bifunctional molecules that bind an E3-ubiquitin ligase and a target protein at the same time, causing ubiquitination and proteasome destruction of the target protein.¹²

Unlike conventional therapeutic proteins PROTACs are catalytic: after dissociating from its target a PROTAC can bind to additional unaffected targets, and therefore may be administered sub-stoichiometrically, which reduces side effects and broadens the molecule's therapeutic index.

As with many advanced therapies, the analytical requirements for PROTACs are high but current methods fall short. SPR, for example, which requires substrate immobilization, struggles with PROTAC complexes.

When combined, the biophysical methods spectral shift and temperature-related intensity change (TRIC), a fluorescence-based method based on microscale thermophoresis, can detect more true binders and require less assay development.¹³



References

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