Case Study

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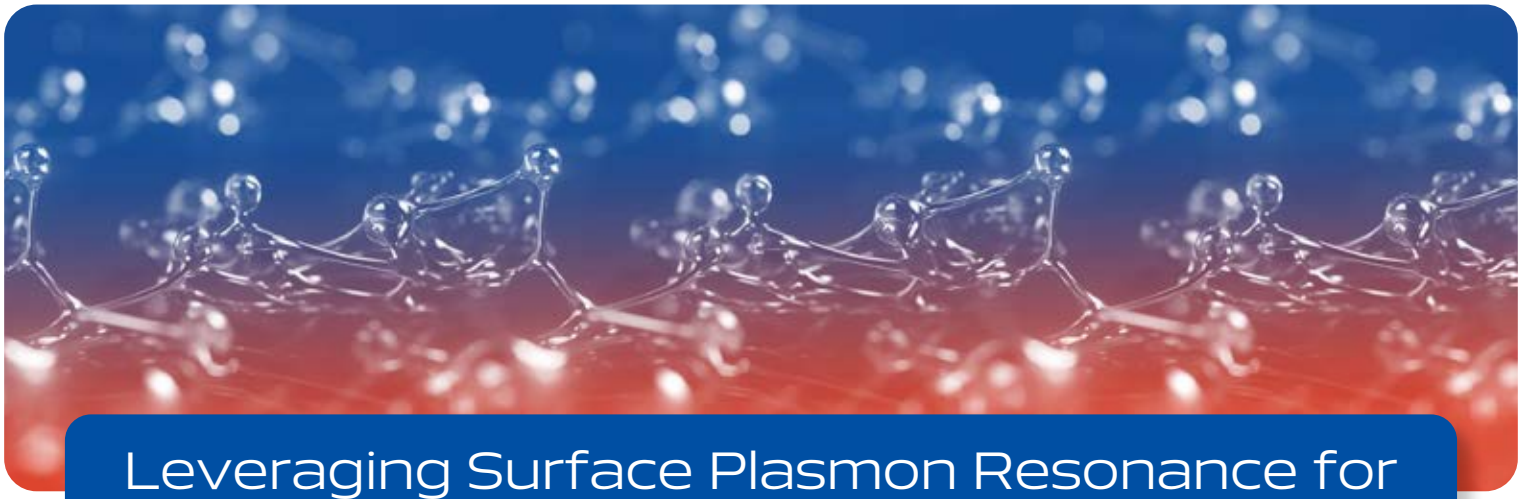


Leveraging Surface Plasmon Resonance for Characterizing Ternary Complexes

Overview

Surface Plasmon Resonance (SPR) is a cutting-edge, label-free technology used to study biomolecular interactions in real-time. It detects changes in the refractive index near a sensor surface when molecules bind to an immobilized partner, allowing researchers to analyze the kinetics and affinity of interactions between biomolecules, from small ligands to large complexes. SPR is particularly well-suited to detect binding interactions at any site on a protein, making it an invaluable tool in the development of heterobifunctional degraders, such as Proteolysis Targeting Chimeras (PROTACs), for therapeutic use.

PROTACs are bifunctional molecules designed to induce the targeted protein degradation by binding to both a target protein and an E3 ligase, forming a ternary complex (Figure 1). The ternary complex promotes the ubiquitination and subsequent degradation of the target protein by the proteasome.

Interaction	Ligand	Analyte
	His - Target	PROTAC
	Biotin - E3 Ligase	PROTAC
	Biotin- E3 Ligase	PROTAC:His-Target

Figure 1: PROTAC interactions- binary (target protein and PROTAC; PROTAC and E3 ligase) and ternary (target protein and PROTAC and E3 ligase ternary complex).

Challenge

Analyzing complex molecular structures, such as ternary complexes, is inherently difficult due to the intricate interactions between multiple binding partners, which complicates the study of their kinetics and binding affinity. Conventional techniques like Fluorescence Polarization (FP), Time-Resolved Fluorescence Resonance Energy Transfer (TR-FRET) and Amplified Luminescent Proximity Homogeneous Assay (AlphaLISA) can study ternary complex formation but cannot provide label-free kinetic analysis for both binary and ternary complexes. Isothermal Titration Calorimetry (ITC) provides thermodynamic data but is limited by low throughput, high sample requirements, and lacks kinetic insights.

SPR overcomes these challenges by enabling label-free, real-time measurement of binding kinetics and affinity for both binary and ternary complexes, making it ideal tool for optimizing PROTAC kinetics and augmenting targeted protein degradation.

Aragen's Solution

At Aragen, we utilized SPR to characterize the stability and efficacy of a PROTAC-target-E3 ligase ternary complex. The experimental strategy is outlined below:

- **Binary Complex Formation:** Determine the binding affinity (K_D) for two binary complexes, PROTAC-target protein and PROTAC-E3 ligase.
- **Ternary Complex Formation:** Immobilize biotinylated E3 ligase on a sensor chip, then titrate a mix of PROTAC and target protein (PROTAC:target protein) at varying concentrations to form the ternary complex.
- **Stability Evaluation:** Calculate the cooperativity factor (**α**), a ratio of dissociation constants (K_D) obtained from the binary ($K_D$binary) and ternary ($K_D$ternary) complexes to measure the stability of ternary complex between the E3 ligase, PROTAC molecule and the target protein.

$$\alpha = \frac{K_D^{binary}}{K_D^{ternary}}$$

where ,

α >1 positively cooperative

α <1 negatively cooperative

α =1 non-cooperative

Outcomes

- **Binding Kinetics of Binary Complexes:** We performed multicycle kinetics to measure the binding affinity of the binary complexes using Biacore T200 Evaluation Software, with data double-referenced and fitted to a 1:1 binding model. The binding affinities for the PROTAC-target protein (K_D = 136.4 nM) and PROTAC-E3 ligase (K_D = 118.5 nM) complexes are shown in Figures 2 and 3, respectively.

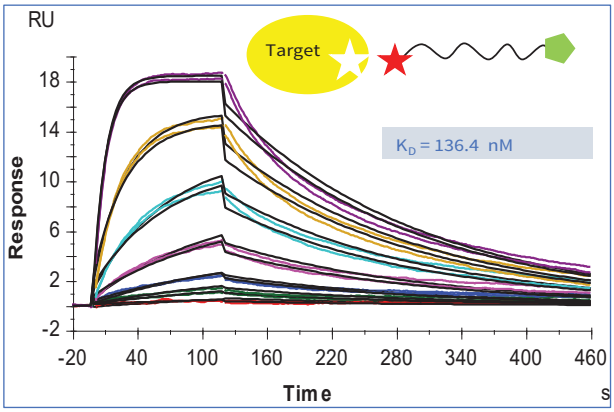


Figure 2: Multicycle binding kinetics of PROTAC for target protein.

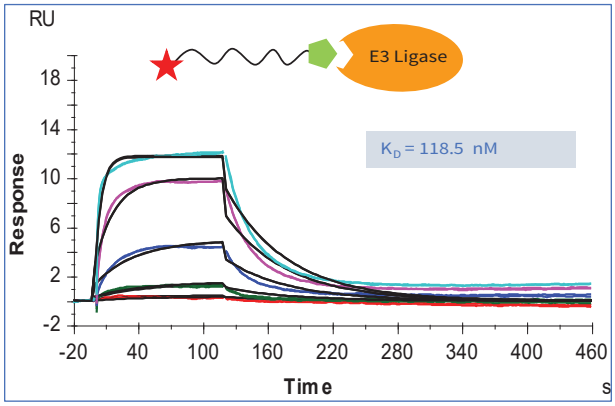
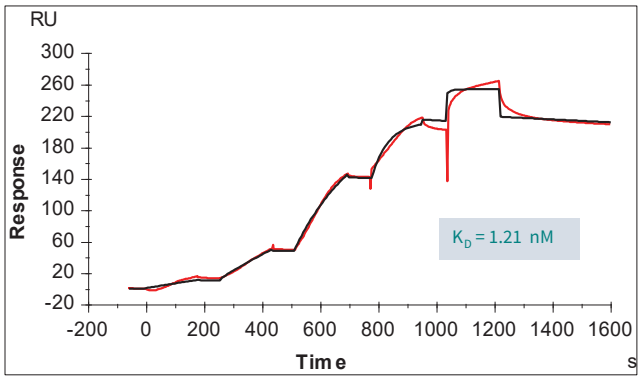
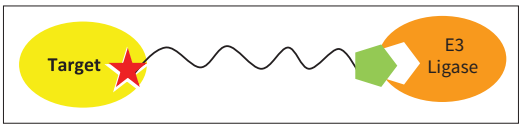


Figure 3: Multicycle binding kinetics of PROTAC for E3 Ligase.

- **Binding Kinetics of Ternary Complex:** Single-cycle kinetics was performed to study the ternary complex formation, and a significantly potent K_D value of 1.21 nM (Figure 4) was obtained, indicating a stable PROTAC-based ternary complex.
- **Cooperativity and Stability:** The cooperativity factor was calculated to be **α** = 97.93, indicating positive cooperativity. This result suggests that the ternary complex, formed by the E3 ligase, PROTAC molecule, and target protein, is highly stable, with stronger interactions between the binding partners.





$K_D^{Ternary}$ (nM)	1.210
K_D^{Binary} (nM)	118.5
α	97.93

Figure 4: Binding Kinetics of PROTAC:target protein with E3 ligase

SPR has proven to be an effective technique for studying the binding affinity of PROTAC interactions and optimizing their efficiency in targeted protein degradation. It offers:

- Label-free kinetic monitoring of binding affinities with minimal analyte volume requirements.
- Accurate determination of binding affinity (K_D) for both binary and ternary complexes.
- A reliable method to assess the stability and cooperativity of ternary complexes, which is essential for optimizing PROTAC design.

These insights underscore the applicability of SPR technology in optimizing PROTAC design, ensuring better stability, selectivity, and efficiency in targeted protein degradation.

Why Aragen?

With nearly a decade of SPR expertise, we accelerate drug development by providing trusted insights that optimize drug candidate profiling and efficacy. We offer:

- **Extensive Expertise:** Skilled in various SPR-based binding studies, including receptor-cytokine affinity, antibody screening and validation, small molecule screening and drug competition assays.
- **High-Quality Data:** Accurate, reproducible binding and kinetic data for informed drug development decisions.
- **Customized Solutions:** Tailored SPR services, including drug competition assays and quality control, to meet your precise R&D needs.
- **Comprehensive Support:** End-to-end SPR services from early research to later development phases, enhancing insights into molecular interactions, stability, and selectivity.