ENHANCED CHARACTERIZATION WORKFLOW SAVES TIME AND RESOURCES

COMBINING THE POWER OF FIDA™ AND BIACORE™ SPR TECHNOLOGIES

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1. Introduction

An efficient workflow for detailed understanding of protein characteristics is essential for advancing drug discovery and protein research. Structural integrity, aggregation behavior, binding kinetics and affinity all play critical roles in determining the efficacy and reliability of therapeutic candidates and biological reagents. In this context, we present a streamlined workflow that combines FIDA™ and Biacore™ SPR technologies to deliver high-confidence data for protein characterization, quantitation and interaction analysis. To do this, we use a case study on Bruton's tyrosine kinase (BTK) and human serum albumin (HSA).

2. Functional characterization

BTK is a pharmacologically important but assay-sensitive target known for its complexity in producing consistent kinetic results. In this study, Biacore SPR measurements of a freeze – thawed biotinylated BTK sample produced atypical sensorgrams with low R_{max} values, despite stable and even capture on the sensor surface suggesting potential reagent degradation (fig.1). Subsequent analysis with FIDA revealed severe aggregation and absence of a monomeric peak, confirming loss of structural integrity and explaining the irregular binding response (fig.2). This example highlights the importance (and benefits) of thorough reagent quality assessment prior to initiating SPR experiments, especially when working with challenging and assay-sensitive proteins like BTK, where structural integrity directly impacts binding reliability.

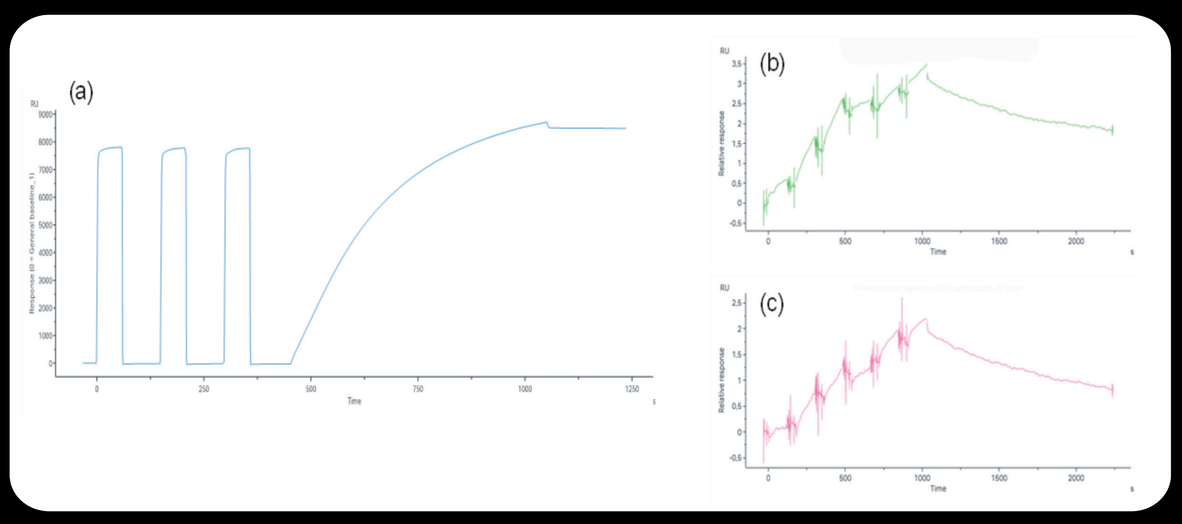

Fig 1. (a) Stable capture response of biotinylated BTK on Sensor Chip SA. Biacore SCK sensorgrams of Fenebrutinib (b) and Vecabrutinib (c) binding to BTK, showing very low Rmax and non-canonical curve shapes.

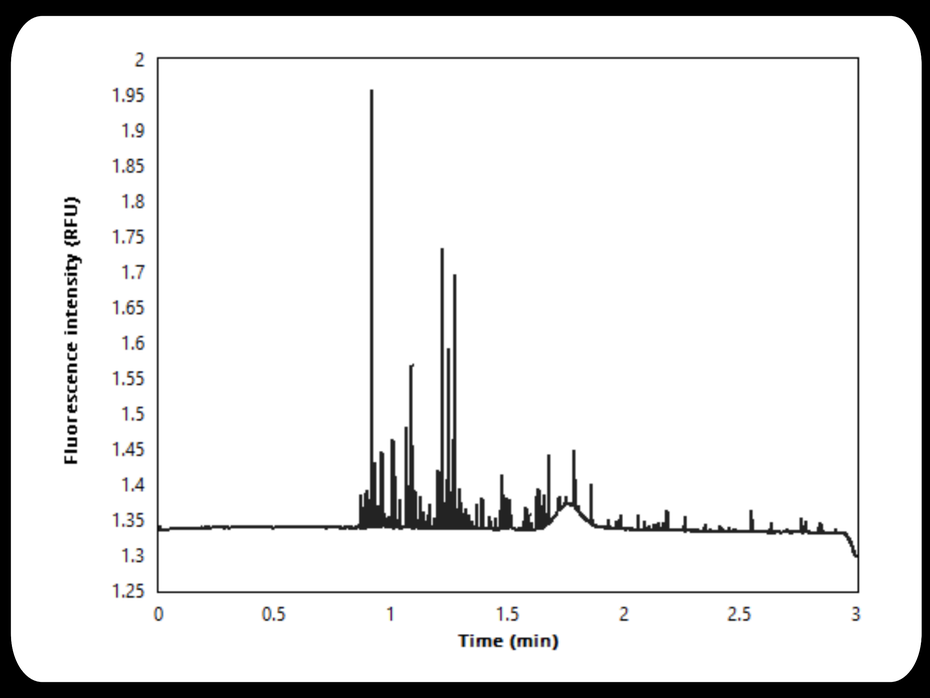

Fig 2.: FIDA taylorgram of freeze-thawed BTK showing severe aggregation and no monomer peak.

3. HSA-LMW binding studies

Another case study further demonstrated how combining FIDA and Biacore SPR provides a reliable workflow for assessing protein integrity and binding behavior with small molecules. HSA was used as a model to show how structural compromises or solubility issues can affect kinetic and affinity data. FIDA analysis of native HSA displays a monodisperse, well-folded protein as seen by the Hydrodynamic radius and Polydispersity Index (fig.3a). Heat-treatment of HSA is on the other hand impacting the structural integrity of HSA which is easily identified in a FIDA analysis looking at the same parameters (fig.3b). Correspondingly, Biacore measurements revealed a 10-fold reduction in binding affinity to warfarin (fig.4), consistent with the structural damage detected by FIDA. Together, these findings highlight how FIDA serves as a rapid, in-solution quality control step that de-risks and strengthens SPR-based interaction analyses by confirming both protein integrity and compound solubility before binding experiments.

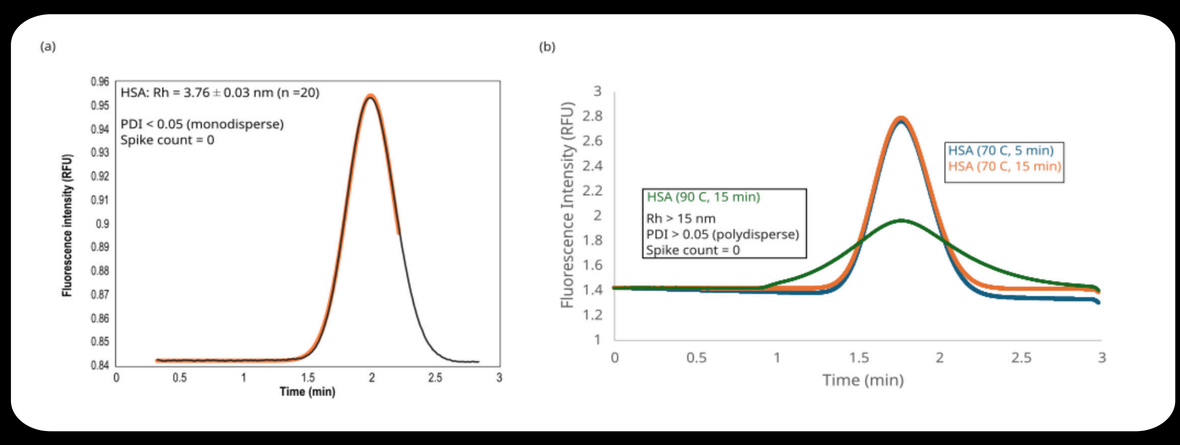

Fig. 3: (a) Native HSA quality check: Representative taylorgram; Rh = 3.76 ± 0.03 nm (n = 20), PDI < 0.05 (b) Overlay of heat-treated HSA FIDA™ results, heat-treated HSA showing broadened, polydisperse profile; Rh > 15 nm.

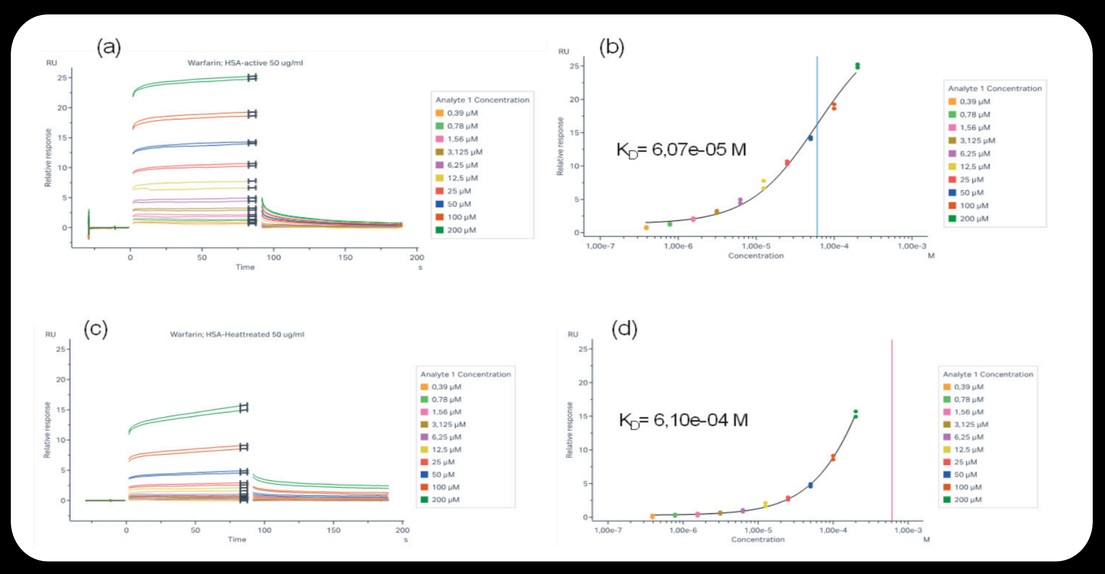

Fig. 4: Sensorgram and affinity plots for Warfarin binding to native HSA (a, b) and heat-treated HSA (c, d) reveal a 10-fold reduction in binding strength, indicating structural and functional compromise in the heat-treated form.

4. Conclusion

By integrating FIDA and Biacore SPR into a unified workflow, researchers can directly connect reagent integrity and solubility with binding kinetics and affinity, providing a more comprehensive view of protein behavior. The BTK and HSA case studies illustrate how this complementary approach allows structural and functional properties to be evaluated in parallel, revealing how aggregation, unfolding, or compound solubility can influence binding outcomes. Together, these technologies improve the reliability of interaction analyses and support a deeper mechanistic understanding of protein function.

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