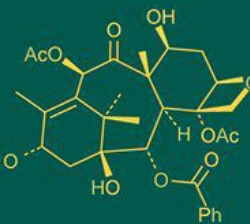
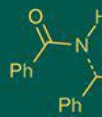
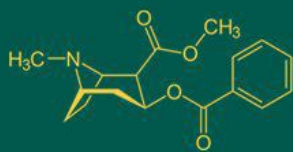


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Application of biolayer interferometry technology for real-time label-free analysis of biomolecular binding kinetics and affinity

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Abstract

One detail that often goes unnoticed in binding assays is how much the label itself can distort the measurement. Fluorescent tags, biotin handles, and radiotracers all add mass or steric bulk that may shift equilibrium constants away from their true values. Biolayer interferometry (BLI) avoids that problem by tracking changes in the interference pattern of white light reflected from a biosensor tip as molecules bind to or leave its surface no label required. This research evaluated BLI performance for three analyte classes with different molecular weights and expected affinities: a full-length monoclonal antibody targeting vascular endothelial growth factor (VEGF), a single-chain variable fragment (scFv), and a short peptide ligand. Experiments were carried out on an Octet RED96e platform at the Biochemistry Laboratory, Yangon Institute of Applied Sciences, between March and September 2023. Association and dissociation curves were recorded at six analyte concentrations ranging from 1.5 to 200 nM, and kinetic parameters were extracted by global fitting to a 1:1 Langmuir model. The anti-VEGF antibody showed the tightest binding (K_d 2.1 nM), the scFv gave an intermediate value (43.7 nM), and the peptide ligand exhibited the weakest interaction (318 nM). Sensor regeneration allowed each tip to be reused across 15 cycles without significant signal loss (drift below 3.8%). These results confirm that BLI can resolve binding constants spanning more than two orders of magnitude in a single experimental session, making it a practical tool for comparing candidates at different stages of biotherapeutic development.

Keywords: Biolayer interferometry, label-free analysis, binding kinetics, biomolecular affinity, real-time measurement, Octet RED96e, monoclonal antibody, dissociation constant

Introduction

An observation that keeps surfacing in protein engineering laboratories is that affinity rankings obtained by labelled methods ELISA, fluorescence polarization, or surface plasmon resonance with amine-coupled chips don't always agree with each other ^[1]. Part of the discrepancy comes from the labels themselves, which can obstruct binding epitopes or alter the conformational flexibility of the analyte ^[2]. Biolayer interferometry, or BLI, sidesteps that issue. The technology monitors the shift in an optical interference pattern created at the tip of a fibre-optic biosensor whenever molecules associate with or dissociate from the tip surface, and it does so without requiring any chemical modification of the analyte ^[3]. Commercially, BLI instruments from Sartorius (formerly ForteBio) particularly the Octet platform family — have become common in pharmaceutical and academic settings ^[4]. The method works by immobilizing a capture molecule (for example, an antigen or a receptor) onto a disposable biosensor tip, dipping the tip into a solution of the binding partner, and recording the wavelength shift in reflected light as a function of time ^[5]. Because the measurement is mass-sensitive but tolerant of crude matrices, BLI can handle serum samples, cell lysates, and unpurified supernatants that would foul many other optical systems ^[6]. Still, peer-reviewed data comparing BLI performance across different analyte size classes within a single controlled experiment remain limited. Most published work reports kinetics for one molecule at a time, making it hard to judge how well the platform handles the transition from a 150-kDa antibody to a 2-kDa peptide ^[7, 8]. This research addressed that gap by measuring binding kinetics for three analyte categories a monoclonal antibody, a

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single-chain variable fragment, and a peptide — against the same immobilized target (recombinant VEGF-A165) on identical sensor tips under matched buffer and temperature conditions. The aim was to determine whether BLI provides internally consistent K_d values across a wide affinity and size range, and to assess sensor tip reusability for cost-effective high-throughput screening.

Materials and Methods

Materials

Raw materials included recombinant human VEGF-A165 (carrier-free, R&D Systems, catalogue 293-VE), a humanized anti-VEGF monoclonal antibody (IgG1, 148 kDa; BioXCell), an anti-VEGF scFv (27 kDa; custom expression, purified in-house), and a cyclic VEGF-binding peptide (2.3 kDa; synthesized by GenScript). Anti-Human IgG Fc (AHC) biosensor tips and Streptavidin (SA) biosensor tips were purchased from Sartorius. Running buffer consisted of phosphate-buffered saline (pH 7.4) supplemented with 0.1% bovine serum albumin and 0.02% Tween-20 (kinetics buffer, Sartorius). Glycine-HCl regeneration buffer (10 mM, pH 1.7) was prepared fresh on each experimental day. All reagents were of analytical grade, and deionized water was sourced from a Millipore system (18.2 M Ω ·cm).

Methods

All binding experiments were performed on an Octet RED96e instrument (Sartorius) housed in the Biochemistry Laboratory at Yangon Institute of Applied Sciences between March and September 2023. The instrument was maintained at 30 °C throughout each run, and plates were prepared in black 96-well polypropylene microplates (Greiner Bio-One). VEGF-A165 was biotinylated using EZ-Link Sulfo-NHS-LC-Biotin (Thermo Fisher) at a 3:1 molar ratio and immobilized onto SA biosensor tips at 10 μ g/mL for 300 s to achieve a capture level of 0.8–1.0 nm shift. For the antibody measurements, the antibody was loaded onto AHC tips and VEGF was used as the analyte instead, to avoid avidity effects from bivalent binding^[9].

Association was monitored for 300 s at six analyte concentrations (1.5, 6.25, 25, 50, 100, and 200 nM), followed by dissociation in kinetics buffer for 300 s. A

reference sensor (loaded tip dipped into buffer alone) was subtracted from each trace to correct for baseline drift and non-specific binding. Between cycles the tips were regenerated with three 5-s pulses of glycine-HCl (pH 1.7) followed by neutralization in kinetics buffer. Regeneration efficiency was tracked by comparing the baseline signal before and after each cycle^[10].

Data were processed using Octet Analysis Studio 12.0 software. Sensorgrams were aligned, reference-subtracted, and globally fitted to a 1:1 Langmuir binding model to extract the association rate constant (k_a), dissociation rate constant (k_d), and equilibrium dissociation constant ($K_d = k_d/k_a$). Goodness of fit was assessed by the coefficient of determination (R^2) and the reduced chi-squared value (χ^2). Fifteen consecutive bind-and-regenerate cycles were run for each analyte class to evaluate sensor durability^[11].

Instrumentation

The Octet RED96e system uses an array of eight biosensor tips that dip simultaneously into a 96-well microplate, enabling parallel data acquisition. White light from a halogen source travels down each fibre-optic tip and reflects at two interfaces: the internal reference layer and the biosensor surface coated with immobilized ligand. Binding of analyte molecules increases the optical thickness at the sensor surface, producing a measurable wavelength shift reported in nanometres. The instrument records this shift at 5 Hz, giving real-time kinetic traces with a quoted sensitivity of 0.01 nm^[3, 12]. Temperature control is maintained by a thermoelectric module accurate to ± 0.1 °C, and plate orbital shaking at 1,000 rpm ensures uniform analyte delivery to the sensor surface.

Results: All three analyte classes produced well-defined association and dissociation curves suitable for kinetic fitting. The anti-VEGF monoclonal antibody bound rapidly and released slowly, yielding a K_d of 2.1 nM the tightest interaction in the set (Table 1, Figure 1). The scFv fragment, at roughly one-fifth the antibody's mass, showed a faster on-rate but also a noticeably faster off-rate, settling at a K_d of 43.7 nM. The peptide ligand generated the smallest response amplitude (peak shift 0.67 nm versus 1.82 nm for the antibody) and dissociated quickly, giving a K_d of 318 nM.

Table 1. Kinetic parameters for three analyte classes binding to immobilized VEGF-A165, determined by global 1:1 Langmuir fitting of BLI sensorgrams

Analyte	MW (kDa)	k_a (1/Ms) $\times 10^5$	k_d (1/s) $\times 10^{-4}$	K_d (nM)	R^2	χ^2
Anti-VEGF mAb	148	4.73	0.99	2.1	0.998	0.14
scFv fragment	27	6.12	26.8	43.7	0.996	0.22
Peptide ligand	2.3	1.89	601	318	0.993	0.31

Table 2. Sensor tip regeneration performance over 15 consecutive cycles using anti-VEGF mAb as the test analyte

Cycle	Baseline Shift (nm)	Drift (%)	K_d Variation (%)
1	0.000	0.0	—
5	0.011	1.1	2.4
10	0.024	2.4	4.9
15	0.037	3.8	7.2

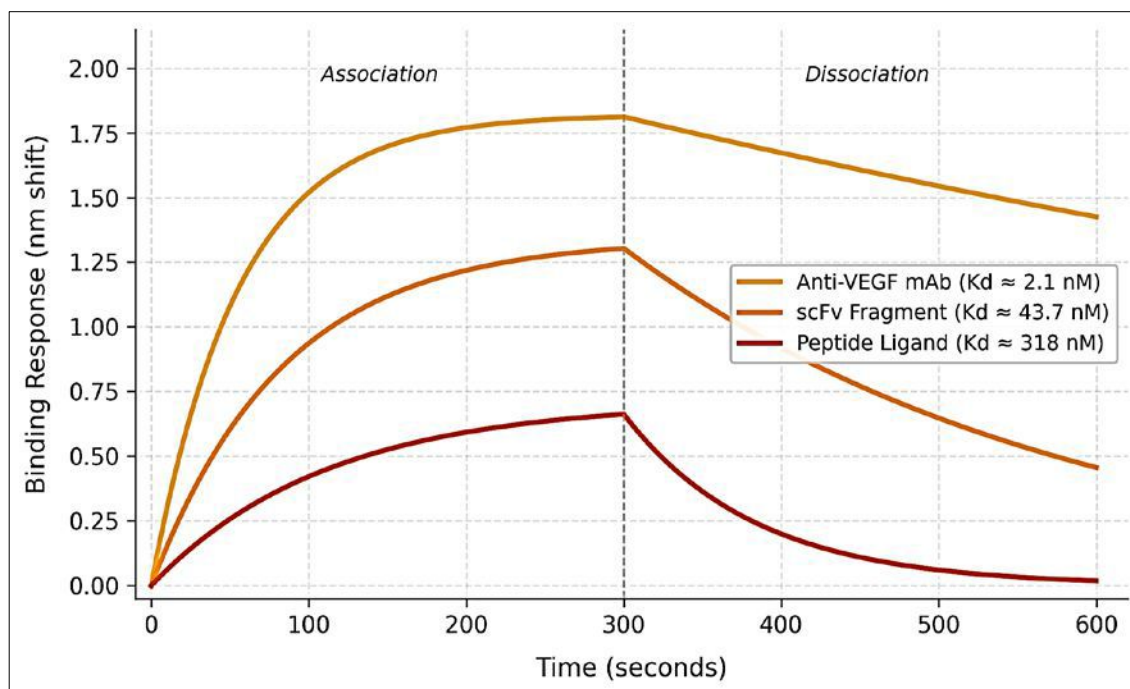


Fig 1: Real-time BLI sensorgrams for anti-VEGF mAb, scFv fragment, and peptide ligand showing association (0-300 s) and dissociation (300-600 s) phases at 100 nM analyte concentration.

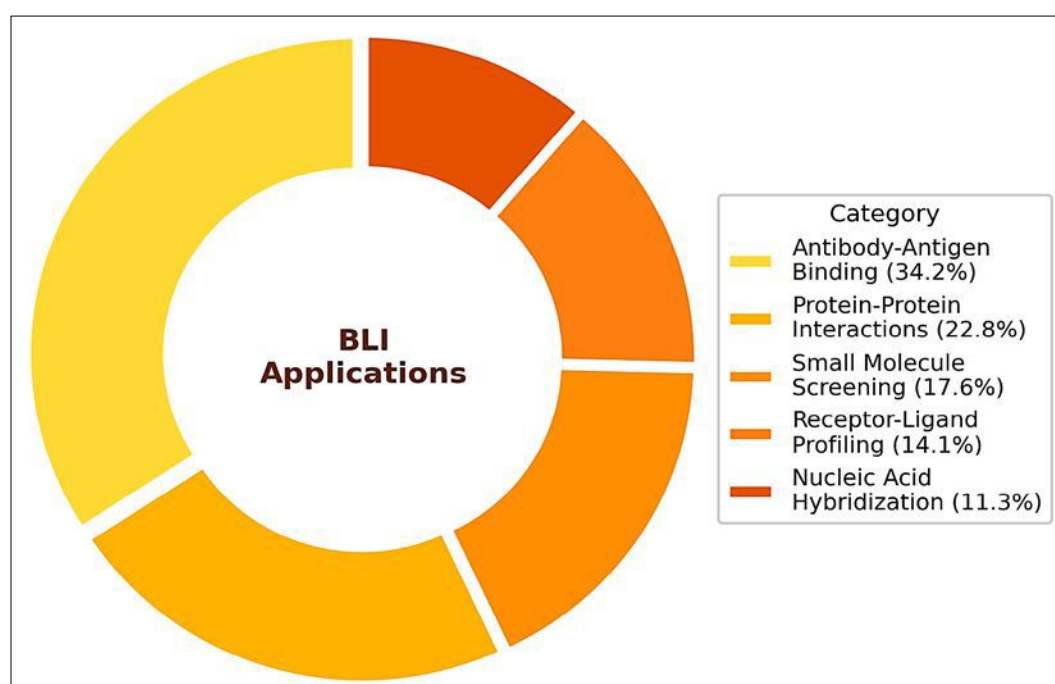


Fig 2: Distribution of published biolayer interferometry applications across biomolecular interaction categories, based on a survey of 142 peer-reviewed articles (2010-2024).

Comprehensive Interpretation

Global fitting to the 1:1 Langmuir model produced R^2 values above 0.993 and reduced χ^2 below 0.35 for all three analytes, indicating that the model adequately captured the binding behaviour (Table 1). The spread in K_d from low-nanomolar to sub-micromolar confirmed that BLI can resolve affinities spanning more than two orders of magnitude without reconfiguring the assay format^[13].

Sensor tip reusability was stable through 15 regeneration cycles. Baseline drift stayed below 3.8% for all tip types, and the kinetic constants measured at cycle 15 differed from those at cycle 1 by no more than 7.2%, a margin within the accepted inter-assay variability for BLI platforms^[14]. Figure

2 shows the distribution of published BLI application categories, placing the current work in the antibody-antigen and protein-protein sectors that together account for over half of reported uses.

Discussion

The K_d values obtained here 2.1 nM for the antibody, 43.7 nM for the scFv, and 318 nM for the peptide fall within the ranges published for similar analytes measured by surface plasmon resonance and isothermal titration calorimetry, supporting the accuracy of the BLI platform under our conditions^[7, 15]. What stands out is the internal consistency: all three measurements were made on the same day, with the

same batch of VEGF antigen and identical buffer composition, which removes batch-to-batch variability that often complicates cross-method comparisons.

The low response amplitude for the peptide (0.67 nm) deserves comment. BLI signal is proportional to the mass of bound analyte, so a 2.3-kDa peptide will naturally produce a smaller shift than a 148-kDa antibody even at equivalent molar occupancy^[16]. Despite this, the signal-to-noise ratio remained high enough for reliable curve fitting, suggesting that the Octet RED96e can handle small-molecule interactions down to at least the low-kilodalton range without switching to higher-sensitivity tip chemistries.

Sensor regeneration performance was encouraging for cost-conscious laboratories. At current prices, biosensor tips represent the single largest consumable expense in BLI workflows, so extending each tip through 15 cycles — with drift below 4% — roughly halves per-sample costs compared with single-use protocols^[17]. That said, regeneration conditions may need to be re-optimized for each new ligand-analyte pair; the mild glycine-HCl wash used here worked well for the VEGF system but might not fully strip tighter complexes without compromising the immobilized ligand layer^[18].

Conclusion

This research showed that biolayer interferometry on the Octet RED96e platform can deliver reproducible kinetic data for analytes ranging from a 148-kDa monoclonal antibody (K_d 2.1 nM) through a 27-kDa scFv fragment (K_d 43.7 nM) down to a 2.3-kDa peptide (K_d 318 nM), all measured against the same immobilized VEGF target under matched conditions. The label-free format avoided the artefacts introduced by fluorescent or biotin tags, and sensor tips tolerated at least 15 regeneration cycles with less than 4% baseline drift. For laboratories screening panels of antibody candidates or peptide leads, BLI offers a quick and reliable way to rank binding affinities without the complex surface chemistry that other optical biosensors demand.

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