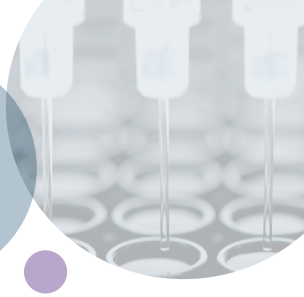
FORTÉBIO

WHITE PAPER



Writing an Equipment Grant Proposal for Biomolecular Interactions Research: Bio-Layer Interferometry (BLI) and Octet Systems

INTRODUCTION

In this white paper, guidelines for preparing an instrumentation grant application will are provided for principal investigators interested in acquiring an Octet® system, the label-free BLI biosensor platform for biomolecular interaction analysis. Many resources are available on the topic of grant writing help and this white paper is designed to specifically help the author in justifying the need for the Octet system as it applies to the institution's research. The structure of the application is modelled after the NIH Shared Instrumentation Grant (S10) program, but the recommendations are broadly applicable for other instrumentation grants.

PROJECT SUMMARY/ABSTRACT

A grant application should start with a project abstract that is an overview summary of the project proposal. The abstract should be short and include:

- The project's goals and objectives
- The applicant's background and qualification as they relate to the stated goals
- An explanation of the process for measuring the success of the project

The proposal's objectives and goals should clearly align with the prospective funding agency's mission. If you imagine yourself as a reviewer reading a grant proposal that does not clearly state the purpose of the grant, you'd most likely not pay much attention to the rest of the document. A grant proposal should therefore clearly articulate the statement of need. The statement of need should describe the problem that the project will attempt to address. Why is this money necessary in your institution? How does the scientific community benefit from the solutions the project aims to provide? For a capital equipment grant proposal, how much does the equipment cost and what differentiates it from other existing technologies? The success of a grant proposal lies in part around the careful communication of these issues in addition to providing a general description of your program.

Instrumentation Plan

The Octet platform includes several models, for more information, visit www.fortebio.com. Give a brief description of the model selected and the rationale behind the selection. Describe the important instrument features needed. It is important to use your own words where possible. Remember, the funding agency is interested in your perspective of the requested equipment and not that of the manufacturer.

The Instrumentation Plan consists of the following sections and includes information critical in highlighting the need, use, and administration of the Octet system.

Justification of Need

The Justification is arguably the most important section, and should explain why the particular technology was chosen and why similar capabilities are not currently available to the researcher(s). Give another description of the Octet system and explain any applicable advantages of this system when compared to other techniques.

Here are some commonly cited advantages for Octet systems:

Speed

- Minimal system start-up time
- Biosensor tips are automatically installed, enabling large multiplex experiments with minimal user intervention
- High-throughput systems that can assay 8–96 samples simultaneously depending on model selected: Octet RED96e system (8 samples), Octet RED384 system (16 samples) or Octet HTX system (96 samples)

Flexibility

- Easy-to-use platform supports multiple applications (screening, affinity characterization, and more)
- Sample plate format allows for re-use of samples hence minimal wastage
- Wide variety of biosensor tips for specific capture and detection methods

Robustness

- No microfluidics
- Easy cleaning and low maintenance

Include preliminary data from the Octet system that shows promise for the research projects (can be shared in the next section). The Appendix section includes additional Octet system benefits and the Downloadable Literature section of the ForteBio website includes application notes and posters that will provide more project-specific benefits of the Octet platforms.

Research Projects

Show the beneficial effects the requested Octet system will have on each major and minor user's research project. In addition, give a detailed structure of the intended usage of the instrument.

Summary Tables

Important summary tables include the list of major and minor users and their expected time allotment (percentage) for using the Octet system.

Administration

Establish the advisory committee that will make decisions on instrument use, training requirements, ongoing service and support of the equipment, etc. The committee should include members who have no conflicting interest in the use of the equipment. If local expertise with the Octet system is not available, including an outside expert on the advisory committee is encouraged.

Technical Expertise

It is important to mention existing researchers currently trained on the Octet system if any. *Note: Any non-manufacturer training and experience can be viewed more favorably over ForteBio-provided training.* A ForteBio Field Application Scientist can be mentioned as an available resource to researchers. For various online resources, visit the ForteBio website (Table 1).

Institutional Commitment / Overall Benefit

It is recommended that the applicant submits letters of support for the requested Octet system from the applicant's institution and any other relevant organization.

APPENDIX

The following sections focus on the requisite knowledge for selection of label-free technologies, specifically the BLI technology used on the Octet platform.

Label-Free Technologies

Analytical techniques are increasingly being adopted earlier on in the drug discovery process to facilitate earlier screening and determination of suitable drug candidates that can be confidently moved to downstream phases of drug development. While techniques such as enzyme-linked immune-sorbent assays (ELISA) and Western blots have traditionally been used and are indeed still quite popular (especially in academia), the current trend is towards the adapta*tion of label-free analytical techniques that do not require intrinsic or external labels such as dyes to monitor binding characteristics*. These systems convert biomolecular interactions into response signals[1] often measured in real time, providing the research community with more detailed analysis of binding mechanisms. This shortens the drug product development cycle by providing information that is not available using techniques such as ELISA. These systems can be used for kinetics characterization, concentration determination and biomolecular interactions screening amongst other things. In kinetics characterization experiments for example, label-free technologies provide information that includes on and off-rates; key determinants in affinity constant derivation and important information which cannot be extracted from end-point analysis techniques such as ELISA. Some commonly used label-free technologies include: Isothermal Titration Calorimetry (ITC), Surface Plasmon Resonance (SPR) and Bio-Layer Interferometry (BLI), each with its unique advantages. With so many options to choose from, the trend is towards techniques that allow researchers to rapidly move the drug candidate through various stages of the drug development process more rapidly while providing accurate data. Of these common label-free techniques,

Resource	Web address	Information provided
Downloadable Literature	www.fortebio.com/literature.html	Application notes, white papers, customer posters and presentations, technical notes, ForteBio posters and presentations, etc.
Customer Knowledgebase	www.fortebio.com/kb	Articles on many common topics that answer technical questions about Octet systems, software, assays, biosensors, BLI technology, etc.
Biopharm Learning Portal	www.fortebio.com/biopharm	Searchable database listing published scientific articles that use ForteBio systems

TABLE 1: ForteBio online customer resources.

ITC has great versatility to provide rich thermodynamics data, but does not provide kinetics-rich information, such as kon and koff values, during an interaction study. SPR and BLI, while not as rich in thermodynamics data extraction, both provide affinity characterization data with additional mechanistic information, such as k_{on} and k_{off} values. Despite this, SPR and BLI are different enough that they could in some cases be used as complementary platforms.

How do I choose the right label-free platform for my research?

With so many technologies to choose from, it can seem like an overwhelming experience to choose the right technology for your research. Real-time, label-free technologies enable researchers to monitor binding interactions in real time without having to label molecules with probes. The ideal platform choice depends on the purpose of the study. When performing an affinity characterization for example, deriving accurate and reproducible data should be the key determinant in choosing a platform. On the other hand, for screening experiments where assay optimization is the goal, or when a large set of samples need to be evaluated; speed, ease of use and flexibility of the platform are the most critical attributes. In addition to these examples, sometimes a researcher may need to perform experiments with samples in crude and less commonly used matrices; in such cases, one needs to consider an instrument with the highest level of robustness to crude systems. Although most SPR technologies provide users with highly accurate and reproducible data, they lack the robustness needed to work with more challenging media types such as samples containing high concentrations of organic solvents such as DMSO or DMF, or with crude non-purified samples that would likely clog their fluidics. By virtue of its design and configuration, BLI on the Octet instruments comes closest to an all-purpose instrument that can provide researchers with data accuracy and reproducibility while broadening the range of sample types (due to its robustness) that can be used at the same time. In addition, Octet instruments can assay up to 96 samples in tandem, which means that they have the potential to remarkably reduce the time to completion of a research project. Moreover, the sample plate configuration provides the kind of flexibility in running experiments so often desired in academic settings where resources may not always be abundant.

Bio-Layer Interferometry (BLI) Principles

Bio-Layer Interferometry (BLI) is an optical analytical technique that utilizes the changing interference pattern of white light shown over a reflective biosensor surface with an immobilized ligand and an interacting analyte in solution. The binding between the ligand and the analyte produces an increase in optical thickness on the tip of the biosensor that can be measured as a wavelength shift from the reference surface (Figure 1) and is a direct measure of the change in thickness of the biological layer as a result of the binding between the pre-immobilized molecule on the biosensor surface and the sample in solution[2]. The flagship instrument for BLI, the Octet system, records the difference in λmax (nm) between the interference pattern from the reference surface and that of the sample surface as a function of time. In this regard, it is uniquely different from Surface Plasmon Resonance (SPR) in that sample or solvent viscosity changes upon binding are not major factors; rather the optical thickness changes that can result from the binding or dissociation of two interacting partner molecules is the key determinant in signal response. As a result, refractive index changes typically associated with SPR that can sometimes be a hindrance in the operation of the technique play a minimal role in BLI. BLI is thus more robust to matrix changes and to cruder matrix than SPR.

The Octet Advantage

With numerous potential new biological molecules under discovery either as biomarkers, therapeutic molecules or for the understanding of the numerous biological pathways that aid in designing the right drugs/molecules to fight disease, researchers prefer analytical techniques that can help speed the

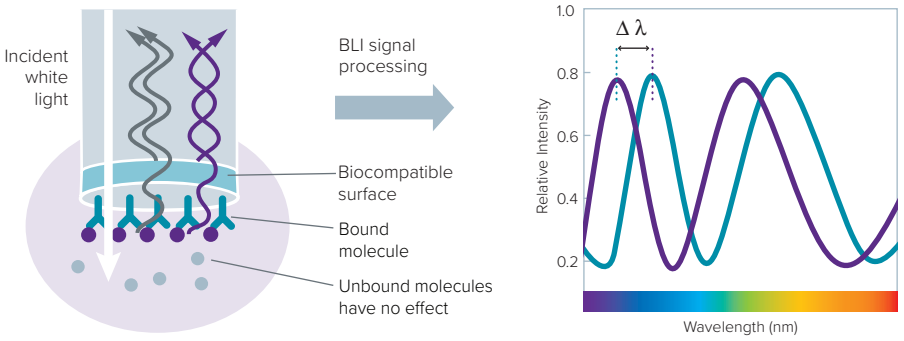


Figure 1: Relative intensity of the light reflection pattern from the two surfaces on the biosensor. BLI systems measure the difference in reflected light's wavelength (Δλ) between the two surfaces.

discovery process. Octet instruments accord researchers the flexibility to perform experiments more rapidly than comparative label-free technologies by virtue of their "Dip and Read™" format where samples are placed in 96 or 384-well sample plates. The instrument can speed research on binding behaviors of interacting molecules as there is ample space on the sample plate for any given experiment. In addition, the system's experimental workflow involves the simple movement of biosensors from the biosensor tray to a sample plate, with the ability to assay as many as 96 sample conditions in tandem. This format can allow a user to perform full factorial design of experiments (DoEs) where relationships between key reagent input factors that can affect the functional behavior of biological molecules can be understood. For example, since protein function depends on well-conformed structures that may be influenced by buffer conditions such as pH and ionic strength, and since the protein's specific binding behaviors may be confounded by the presence of hydrophobic moieties that may introduce non-specific binding patches completely unrelated to the specific binding functions of the protein, it may be necessary to understand the relationship between the key input variables in the assay. Understanding the interactional relationships between key input variables and how they affect the properties of any given pair of binding molecules could take an exorbitant amount of time when using analytical platforms devoid of high-throughput sample analysis features under equivalent experimental conditions. The Octet platform therefore offers an unparalleled advantage in this regard.

Running a BLI Experiment on the Octet System

Several key applications can be performed on Octet systems. These can be generally categorized under:

- Affinity characterization of different binding pairs
- Screening of binding between different pairs of molecules in quick yes/no experiments
- Assay development and optimization experiments where different input variables are monitored for their effect on the functional properties of binding pair molecules
- Protein concentration determination
- Determination of binding thermodynamic parameters such as ΔG, ΔH and ΔS

To run an assay on Octet instruments, simply select the appropriate biosensor surface for the immobilization of the ligand based on the ligand properties. Octet system consumable kits include a variety of biosensor surfaces developed to allow ease of capture/immobilization of ligands (potential ligands include proteins, nucleic acids, antibodies, viruses and bacterial and mammalian cells amongst others). Biosensors include:

- Streptavidin-coated biosensors for immobilization of biotinylated ligands
- Amine-reactive biosensors that use EDC/NHS chemistries to covalently couple ligands through their primary amino groups
- Capture-based biosensors such as anti-His, anti-GST, anti-human capture or anti-mouse capture; all of which can be useful for the immobilization of both purified and non-purified ligands
- Amino propyl silane biosensors which can be used to capture hydrophobic ligands or can be derivatized to create desired novel chemistries amongst others.

Once the biosensor choice is made, samples are aliquoted onto either 96- or 384-well non-protein adsorbing sample plates that ensure the integrity of the samples is consistent throughout the assay. The assay sequence and step times are then designed using an easy to navigate experimental setup wizard in the software. The assay steps are designed to instruct the instrument to pick up biosensors from the biosensor tray (installed inside the instrument) and sequentially dip them into the various sample columns on the sample plate (see an example of a plate design

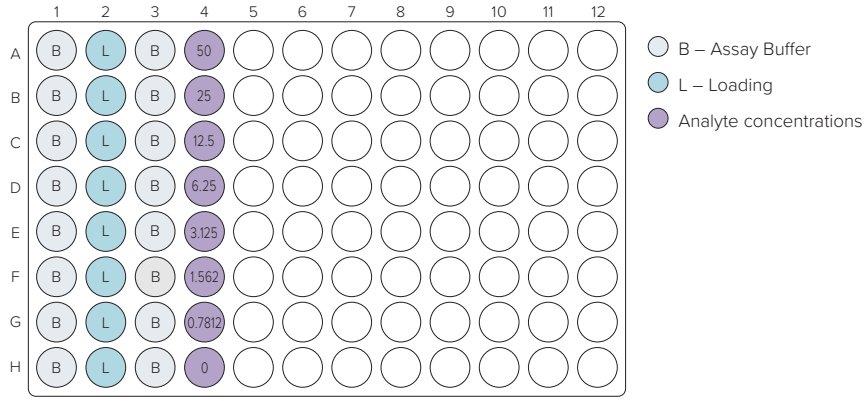


Figure 2: An example of a sample plate configuration for an affinity characterization experiment. The "Dip and Read" format allows the user to assay multiple analyte concentrations (purple) in parallel.

in Figure 2). The instruments are configured to assay 2–96 samples depending on the instrument type with some systems allowing for an unprecedented time savings when performing high throughput analysis.

As depicted in column 4 of the sample plate in Figure 2, the sample plate layout and the Octet instrument's workflow allows for the dose response analysis (Figure 3) for different concentrations of an analyte simultaneously, hence ensuring the direct comparison of sample response signals under similar conditions while rapidly speeding the analysis process. The data in Figure 3 can then be subjected to Langmuir fit-model equations in the analysis software to extract on- and off-rate values, and to calculate dissociation constants (K_D).

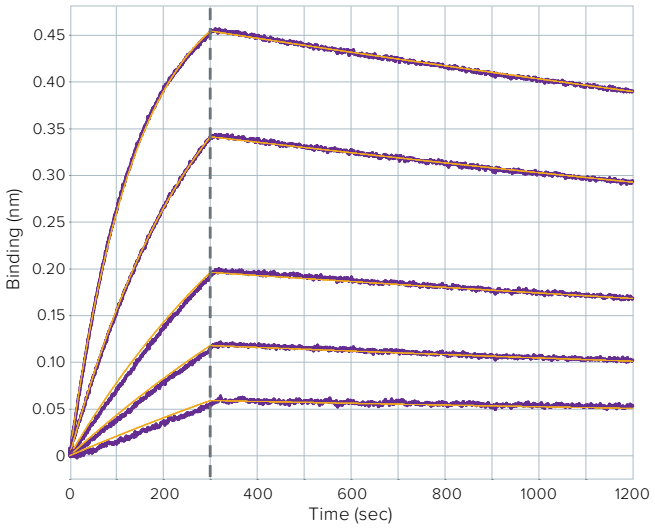


Figure 3: Real-time affinity characterization data showing response signals for a binding pair on the Octet RED96e system, an 8-channel BLI platform.

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