Procedure

Screening and analysis of fragments using Biacore systems

Successful use of surface plasmon resonance (SPR) biosensors in fragment-based drug discovery (FBDD) requires specialized methods for assay development, primary screening, confirmation testing, and data analysis; tools which are built into current generation Biacore™ systems.

This reference guide covers the important practical aspects and considerations of setting up a successful FBDD campaign.

Plastics and glassware

When handling dimethylsulfoxide (DMSO), it is important to ensure that all vessels will withstand contact with the solvent. All sample handling equipment (tips, plates, tubes and glass bottles) should be compatible for use with organic solvents.

Plastics

Certain plastics, such as polycarbonate and polysulfone, have been observed to release contaminants into DMSO, which may bind to proteins such as human serum albumin (HSA). Therefore, it is recommended that Cytiva polypropylene vials be used when working with this solvent. Recommended polypropylene microplates from Greiner™ are article numbers 650201 (96-well) and 781280 (384-deep well).

Glassware

To avoid contamination issues rinse glassware with 50 mM NaOH and filtered deionized water before use.

Instrument preparation

Each Biacore system will have its own specific maintenance and cleaning procedures. The description that follows is generally applicable to all Biacore systems. More detailed operational information can be found in the relevant instrument manuals.

Routine instrument preparation

Whenever the buffer source is changed on the instrument, the ***Prime*** procedure should be run to ensure equilibration of the fluidics with the new buffer system.

Maintenance

Desorb, plus ***Desorb and Sanitize*** should be run according to instrument guidelines. ***Superclean*** for small molecule assays can be run as required.

Buffers

The SPR signal is sensitive to all molecules that pass over the sensor chip. Therefore, it will be affected by even small differences in salt concentration, additives, and components with a high refractive index, such as DMSO in sample and running buffers. Conditions should be carefully optimized for each individual interaction and all components should be compatible. Chosen buffers should be tested during assay development to ensure a stable, robust, and reproducible biological system.

Buffer

Phosphate buffered saline (PBS) at 10 to 20 mM with 0.05% of P20 is recommended as a buffer for most low-molecular weight (LMW) analyte assays (Cytiva: PBS-P+ 10×, product code 28995084). Organic buffers, such as HEPES have been observed to interfere with small molecules binding to human serum albumin, phosphates and kinases. TRIS has been shown to work well for kinase targets [8].

Ionic strength

Use ionic strength at or close to physiological levels (150 mM monovalent ions) to reduce nonspecific binding of compounds to the sensor surface.

Detergent

Including a detergent in the buffer can reduce nonspecific adsorption of proteins to the autosampler tubing and the IFC channels and should not be excluded without good reason (e.g., in the case of detergent-sensitive targets). P20 generally works well for small-molecule work, but other detergents, such as Brij™ have also been used.

The choice of detergent is often determined by compatibility with the target. For example,

GPCR's have been shown to work well with 0.1% n-Dodecyl-β-D-Maltopyranoside (DDM).

Solvent

Many small molecules require organic solvents for solubility. DMSO is the most used. A concentration of 2% to 5% is recommended, depending on the target tolerance of the protein and the solubility requirements of the compounds. DMSO is hygroscopic and the use of fresh, high-quality DMSO is preferred.

Additional additives

Include additives necessary for the activity of the target, such as cofactors and reducing agents (TCEP or DTT). Biacore systems are compatible with most biological buffer systems.

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Filtering
Running buffer, should be made fresh and filtered through a 0.22 µm membrane DMSO-resistant filter, (e.g., nylon) to prevent DMSO leaching filter and plastic components into the buffer. Note that buffer stocks made by Cytiva do not need to be filtered.

Degassing
Several Biacore systems are equipped with an in-line buffer degasser, which is sufficient for degassing most buffer systems.

Surface preparation

Since the SPR response is proportional to the mass of bound complex, FBDD requires high immobilization levels of the target on the surface. Given this, direct attachment is most common, but capture assays are becoming routine due to instrument sensitivity. Different immobilization levels should be tested during assay development to ensure surface stability, target activity, and dynamic range of the response in the screen.

Direct immobilization

Biacore sensor chips
Usually, the Biacore Sensor Chip CM5 is employed for direct immobilization. In cases where sufficient levels or activities of target protein are unobtainable, the Biacore Sensor Chip CM7, which is designed primarily for use with LMW analytes, is recommended.

Amine coupling
Direct immobilization via amine coupling consists of preparing the protein in a low salt buffer of at least 1 pH unit below the target's isoelectric point. In some cases, prolonged exposure to the coupling buffer can impact the activity of the target. Several strategies may be used to good effect: reducing exposure time; using the ligand dilute feature; enhancing protein stability by immobilizing in the presence of an inhibitor; or amine coupling during neutral-pH capture of a polyhistidine-tagged protein target using the Biacore Sensor Chip NTA [8]. *See* Application note 29007929 for more information.

Capture

When the protein target is not compatible with or rapidly loses activity following direct coupling, capture through affinity tags is the next most common strategy for immobilization. Two significant benefits are that capture can be performed at neutral pH and will create homogenous surfaces. Biotin and poly-histidine are the most commonly used tags. Some tags, such as those derived from antibodies, are not appropriate for FBDD.

Biotin
Limited biotinylation or use of an AviTag™ have been used to good effect in FBDD programs to capture sufficiently high target levels on streptavidin or neutravidin surfaces.

Polyhistidine (his) tags
As with biotin tags it is possible to capture his-tagged proteins at high densities. Unlike capture with streptavidin, the target protein can be removed from the surface of the Biacore Sensor Chip NTA using a pulse of EDTA. In this way the target protein is always fresh and will not decay. For an example of a successful FBDD program using polyhistidine tagged proteins being captured by Ni^{2+} -NTA, *see* Application Note 28979418.

Assay setup

Running a fragment screen requires balancing throughput with data quality. Shortcuts on injection quality, data collection rates, control compound injections, blank injections, solvent corrections and wash procedures will invariably impact the data quality resulting in difficult to interpret data and increases in false positives and negatives. The following advice will help maximize data quality, optimize throughput and minimize maintenance related issues associated with problematic or sticky LMW compounds. The recommendations below are included by default in current LMW and fragment methods within Biacore 8K/Biacore 8K+ v3.0, Biacore T200 v2.0, and Biacore 4000 V1.1 software.

Start-up cycles
Start-up cycles should always be included to ensure the instrument equilibrates fully before compound injections start. It is recommended to run at least 3 start-up injections of running buffer in order to allow the baseline to stabilize sufficiently before compound injections begin.

Flow rates
Flow rates ≥ 30 µL/min are recommended to minimize dispersion, maximize data quality, and reduce artifacts.

Solvent corrections
Eight correction points are recommended to compensate for buffer/DMSO mismatches, with the solvent correction repeated routinely throughout the assay. For Biacore 8K series instruments, a four-point correction curve is recommended.

Blank Injections
Include buffer blanks for use in double reference subtraction during post experiment data analysis. This allows for the subtraction of systematic, time dependent artifacts in the data set. These should be prepared in the same format as the screened compounds for greater comparability, instead of drawing directly from the buffer bottles. Generally, it is advisable to subtract the closest blank to minimize changes over the time course of the assay. Current software allows for subtracting the closest blank, choosing preceding or following blanks, or using averaged blanks.

Extra washes
The extra wash command using a 50% DMSO solution should be run after each compound injection to minimize the risk of compound carryover across injections. The additional cleaning is *critical* to both data quality and long-term maintenance of the instrument. Omission of the extra wash, whilst time saving in the short term, can lead to false positives, false negatives, and the need for more frequent instrument cleanings.

Controls

Positive controls
Run routinely throughout the assay to allow for correction of surface decay by normalization of responses in the screen in post run analysis. This will also give better comparability of data across multiple experiments, sensor chips, or surfaces.

Negative controls
Include to aid in the setting of threshold values in post experiment analysis. These should be prepared in the same format as the screened compounds for greater comparability, instead of drawing directly from the buffer bottles.

References for SPR in FBDD

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