Comparison of Biomolecular Interaction Techniques –

SPR vs ITC vs MST vs BLI

The purpose of this white paper is to compare four techniques utilized to quantify biomolecular interactions. It provides a brief introduction to each technique, followed by tables that provide a direct comparison. The four techniques compared in this white paper are Surface Plasmon Resonance (SPR), Isothermal Titration Calorimetry (ITC), Microscale Thermophoresis (MST), and Biolayer Interferometry (BLI).

Surface Plasmon Resonance (SPR)

Surface Plasmon Resonance (SPR) spectroscopy, a technology developed 25 years ago, is now the gold standard technique for the study of biomolecular interactions for a wide variety of analytes – from small molecules in drug discovery to peptides, proteins, viruses and even nano-particles. SPR is a label-free method capable of measuring real-time quantitative binding affinities, kinetics and thermodynamic parameters of interacting molecules. SPR provides the highest quality data, with moderately high throughput, while consuming relatively small quantities of sample.

The information-rich content of an interaction, which is generated in real-time as well as the high sensitivity and accuracy of this method, make SPR an invaluable tool for biological and pharmaceutical R&D, production and QC. SPR is the only technique from those compared that fulfils the requirements of regulatory authorities (FDA, EMA, ICH).[1, 2, 3, 4, 5, 6, 7]

With Reichert's robust and flexible SPR systems it is possible not only to perform standard biomolecular interaction analysis, but also to analyze crude samples and undiluted serum. In addition, Reichert's SPR system can also be used in tandem with liquid chromatography systems and/or mass spectrometers.

As a complement to a robust and flexible instrument hardware that requires little maintenance, **XanTec bioanalytics,** as the leading manufacturer of biosensor chips, offers the broadest range of sensor chips in the marketplace, meeting virtually every experimental requirement.

Isothermal Titration Calorimetry (ITC)

ITC is used for quantitative thermodynamic characterization of a wide variety of biomolecular interactions by directly measuring the heat that is either released or absorbed during a biomolecular binding event. ITC is the only technique that can simultaneously determine all thermodynamic binding parameters in a single experiment. This technique requires no modification of binding partners, with fluorescent tags or through immobilization, but it requires, a significantly larger amount of sample compared with similar techniques. ITC measures the affinity of binding partners in their unmodified state, but kinetics (association and dissociation rate constants) cannot be determined with this low-throughput method. [a]

Biolayer Interferometry (BLI)

BLI uses white light interferometry to quantify biomolecules which are typically adsorbed to the tips of optical fibers. White light travelling through an optical fiber is reflected at the fiber-biomolecular layer interface and at the biomolecular layer-buffer interface. The reflected beams interfere, generating a signal that directly depends on the amount of adsorbed molecules.
Since the measurement is performed using optical fibers instead of using a fluidic system, it is possible to deliver the sample liquids to the sensor by dipping the optical fibers into well plates. Similar to SPR, immobilization of the ligand is to the surface of the tip is required. BLI provides direct binding affinities and rates of association and dissociation as well as concentration measurements. Due to the very limited temperature control of the samples, a thermodynamic characterization is not possible. Given that only substances bound to the sensor surface are detected, the medium surrounding the sensor does not influence the signal and thus no reference channel is needed [c, d].

Microscale Thermophoresis (MST)

MST is performed using thin capillaries in free solution, which is comparable to ITC measurements. When performing an MST experiment, a microscopic temperature gradient is induced by an infrared laser, and the directed movement of molecules is detected by intrinsic fluorescence or in most cases, fluorescent labels of one interactant and quantified. The thermophoretic movement of molecules within the temperature gradient depends on size, charge, hydration shell or conformation that typically changes upon interaction. The thermophoresis signal is plotted against the ligand concentration to obtain a dose-response curve, from which the binding affinity can be deduced. The dynamic affinity range covers the pM to mM range, which is comparable to SPR measurements. MST requires a small amount of sample and the technique is relatively easy to use. [b] However, the requirement to use hydrophobic fluorescent labels adds to the overall experimental process and these labels have been known to cause non-specific binding, thus reducing experimental confidence. Additionally, the MST technique cannot provide kinetic information (association and dissociation rates).

Conclusion

A comparison of common techniques for biological interaction analysis reveals that SPR is the most versatile and information-rich analytical method. The tables on the following page further illustrate and highlight the differences between the techniques. The high precision and repeatability of analyte injections compared to MST, BLI and ITC as well as the detailed information about specificity and activity of interacting analytes through the entire analysis and in real time, makes SPR the only accepted method by regulatory authorities. "When ligand binding is part of the functional activity of a biosimilar or therapeutic product, FDA and EMA guidelines require this property be quantified[1,2]. Binding assays are essential to defining immunological properties and biological activity of monoclonal antibodies[3,4,5,6,7]." [e]

	SPR (Reichert systems)	ITC	MST	BLI
Kinetics	Yes	No	No	Yes
Affinity	Yes	Yes	Yes	Yes
Thermodynamics	Yes	Yes	Yes	Limited
Stoichiometry	Yes	Yes	Yes	Yes
Concentration – analyte	Yes	No	No	Yes
Affinity range	pM - mM	nM - µM	pM – mM	pM – mM
Precision of read-out	High	Medium	Low	Medium
Sensitivity	High	Low	Medium	High
Temperature range	10°C below ambient – 70°C	2-80°C	20-45°C	Ambient + 4°C – 40°C
Sample consumption	Low	High	Very low	Low
Sample types*	Maximum flexibility	Few	Intermediate	Maximum flexibility
Buffers	No restrictions for aqueous solutions Concentrated Acids/Bases Most organic solvents	Aqueous solutions with small amounts of organic solvents	No restrictions for aqueous solutions (incl. 8M urea, 4M MgCl2 etc.)	Aqueous solutions with small amounts of organic solvents
Maintenance	Operator or service technician	Service technician	NA	Service technician
Signal read-out (change of...)	Refractive index	Temperature	Fluorescence intensity	Wavelength shift
Benefits	Highest information content Reference method for FDA/ICH/EMA Max experimental flexibility	No immobilization No labeling	Low sample consumption	Highest throughput
Disadvantages	Immobilization of one binding partner required. Trained personnel is required for high quality data	High sample Consumption Limited applicability – reactions with measureable temperature change.	Cannot discern 2nd binding site or non-specific binding Labeling with hydro-phobic fluorophores required, which can alter binding profile. High CV of fluorescence data.	Immobilization of one binding partner required.

See table on following page for detail list of sample types

Sample Types

	SPR (Reichert systems)	ITC	MST	BLI
Fragments	√			√
Small Molecules	√	√	Limited	√
Peptides	√	√		√
Proteins	√	√	√	√
Cell Culture Supernatants	√		√	√
Serum	√		√	√
Cell Lysates	√		√	√
Viruses	√			√
Bacteria	√			√
Whole Cell Kinetics	√			

References

a) A Wei, Chin-Chuan, et al. „Isothermal Titration Calorimetry and Macromolecular Visualization for the Interaction of Lysozyme and Its Inhibitors." Journal of Chemical Education 92.9 (2015): 1552-1556.
b) Jerabek-Willemsen, Moran, et al. „MicroScale Thermophoresis: Interaction analysis and beyond." Journal of Molecular Structure 1077 (2014): 101-113.
c) Fortebio, Interactions, 2008, Vol. 1 Issue 1
d) Fortebio, Interactions, 2009, Vol. 2 Issue 1
e) http://www.sgs.com/en/news/2014/02/surface-plasmon-resonance-molecular-interactions-and-ligand-binding-analysis

1. ICH Topic Q 6 B Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products (1999).
2. FDA Draft Guidance for Industry, Quality Considerations in Demonstrating Biosimilarity to a Reference Protein Product (2012).
3. FDA Point to consider in the manufacturing and testing of monoclonal antibody products for human use (1997).
4. FDA Guidance for Industry Monoclonal Antibodies Used as Reagents in Drug Manufacturing (2001).
5. The European Pharmacopoeia - Monoclonal antibodies for human use (current edition).
6. Guideline on development, production, characterisation and specification for monoclonal antibodies and related products, EMEA/CHMP/BWP/157653/2007.
7. Guideline on similar biological medicinal products containing monoclonal antibodies – non-clinical and clinical issues, EMA/CHMP/BMWP/403543/2010.

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