Staff

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In both basic and applied science it is often required to uncover the mechanism of protein-protein and protein-ligand interactions. In the Biomolecular Interaction Laboratory we study and characterize the interaction of biomolecules using Biacore-3000 instrument, MicroCal iTC200, Monolith NT.115 and NT.LabelFree equipments.

Biacore-3000 equipment based on the principles of surface plasmon resonance. To carry out such studies relatively low amount of the binding partners are needed. One of the binding partners is immobilized on the surface of a sensor chip by covalent coupling or other affinity type interactions (streptavidin-biotin, antigen-antibody, etc.) then the solution containing the other partner is injected over the surface and the interaction is detected and quantified. The equipment is suitable for multi-channel analyses at wide temperature (4-40°C) and flow rate (1-2 µl/min to 50-100 µl/min) range. This method is also suitable to detect relatively weak (K_a~10^4) interactions.

MicroCal iTC200 equipment measures heat changes during isothermal titration calorimetry (ITC). The ITC-based MicroCal iTC200 is suitable for the simultaneous determination of binding parameters such as association constant (K_a), the binding stoichiometry (n, mol/mol binding), enthalpy (ΔH) and entropy change (ΔS) of the interaction. The equipment directly measures sub-millimolar to nanomolar binding constants (K_a ~10^2 to 10^9 M^{-1}) and also suitable to measure nanomolar to picomolar binding constants using competitive binding technique (K_a ~10^9 to 10^{12} M^{-1}). Experiments require only 200 µl of sample and as little as 5-10 µg of protein in the sample cell. It is a true “in-solution” technique: no immobilization or labelling required, there are no buffer restrictions and even turbid solutions can also be handled easily.

Monolith NT.115 and **NT.LabelFree** devices use the phenomenon of microscale thermophoresis (MST) which is a highly sensitive technique based on the Soret effect that enables the quantitative analysis of molecular interactions in solution at the microliter scale. MST measures the strength of the interaction between two molecules by detecting variations in fluorescence signal as a result of an IR-laser induced temperature change. One interacting partner must contain intrinsic fluorophores (preferably tryptophan, or tyrosine) or alternatively may be labelled with an extrinsic fluorophore or, another option is to use fusion proteins with fluorescence tags (GFP, YFP, mCherry, etc.). The concentration of fluorescent molecule should be in the same range or lower than the expected K_d. The ligand is diluted directly at various concentration in the solution of the fluorescence target (at constant concentration). The highest concentration of ligand is about 20 fold above the expected K_d. An important requirement is that the dilution buffer should not vary in composition during serial dilution. This technique requires relatively low volume of samples for the measurement (~20 µl). Binding constants can be determined for biomolecular interactions like protein-protein, protein-DNA and protein-small molecule associations. The range of the variation in the fluorescence signal correlates with the binding of a ligand to the fluorescent target. Two MST instruments are available in our laboratory: Monolith NT.115 requires a fluorescently labelled molecule in the analysed system, while Monolith NT.LabelFree utilizes the intrinsic tryptophan fluorescence of native proteins.

Prometheus NT.48 device was installed in our laboratory, which utilizes the technique based on the nano differential scanning fluorimetry (nanoDSF) in analysing states and stability of proteins. This device complements the interaction studies as the correct folding and the aggregation state of proteins should be determined before analyses. NanoDSF monitors the fluorescence change of the tyrosine/tryptophan in the structure of the proteins during thermal unfolding. These amino acid residues have a fluorescence intensity maximum at 330 nm in hydrophobic environment. During unfolding tryptophan/tyrosine residues become hydrated and the fluorescence intensity maximum is shifted to 350 nm, therefore the ratio of the tryptophan/tyrosine fluorescence at 330 and 350 nm changes. This way protein denaturation curves as a function of increasing temperature is determined from which important stability parameters are derived. The thermal stability of a given protein is typically described by the onset of domain unfolding (T_{onset}) and thermal unfolding transition midpoint T_m (°C), at which half of the protein population is unfolded. T_m can be calculated from the changes in tryptophan fluorescence intensity, or from the ratio of tryptophan emission at 330 and 350 nm, which describes the shift of tryptophan emission upon unfolding. NanoDSF is the method of choice for easy, rapid and accurate analysis of protein folding and stability, with applications in protein engineering, formulation development and quality control.

The above techniques complement each other too, since kinetic data are generally available from SPR and MST measurements, while thermodynamic data are more reliably obtained by ITC technique. In addition, ITC is suitable to determine interactions as weak as 10^2 M^{-1}, while the limit is 10^4 M^{-1} in SPR experiments.

Services provided, conditions and charges

Access to measurements includes service charge and the conditions and time for the services are required to set in advance with the staff of the laboratory. The Biomolecular Interaction Laboratory provides the following services:

- quantitative characterization of protein-protein and protein-ligand interactions using purified components: determination of the association and dissociation rate constants as well as the association/dissociation constants for the interaction
- characterization of antigen-antibody interaction including epitope mapping
- study of the binding of possible drug molecules to the target protein and /or to receptor
- characterization of protein-nucleic acid and protein-carbohydrate interactions
- separation of protein(s) that bind to an immobilized partner from cell lysate or partially purified samples via a sample recovering system: binding proteins are recovered in a form suitable for analyses by mass spectrometry or by immunological methods.
- determination of parameters for protein unfolding and characterization of aggregation states of proteins.

The following charges are applied for the services

Work	Unit	Price (HUF)
Biacore-3000		
Equipment usage time	hour	10 000
Immobilization	1 chip/4 surfaces	15 000
Data evaluation	1 concentration series	18 000
MicroCal ITC200		
Equipment usage time	hour	10 000
Data evaluation	1 ligand titration with 1 interacting partner	5 000
Monolith NT.115 and NT.LabelFree		
Equipment usage time	hour	10 000
Standard Capillaries	16 pieces per measurement	2 600
Premium Capillaries	16 pieces per measurement	11 500
Capillaries Monolith NT.LabelFree	16 pieces per measurement	7 800
Premium Capillaries Monolith NT.LabelFree	16 pieces per measurement	13 500
Protein labelling	per protein	35 000
Data evaluation	hour	5 000
Prometheus NT.48		
Equipment usage time	hour	10 000
Standard Capillaries	3 pieces of per sample	750
High Sensitivity Capillaries	3 pieces of per sample	2 700
Data evaluation	hour	5 000

Recent publications in which the techniques of Biomolecular Interaction Laboratory have been applied:

Kónya Z, **Bécsi B**, Kiss A, Horváth D, Raics M, E. Kövér KE, Lontay B, **Erdődi F**. (2019) Inhibition of protein phosphatase-1 and -2A by ellagitannins: structure-inhibitory potency relationships and influences on cellular systems, Journal of Enzyme Inhibition and Medicinal Chemistry, 34:1, 500-509, DOI: 10.1080/14756366.2018.1557653

Kónya, Z, **Bécsi, B,** Kiss, A, Tamás, I, Lontay, B, Szilágyi, L, Kövér, K.E, **Erdődi, F.** (2018) Aralkyl selenoglycosides and related selenosugars in acetylated form activate protein phosphatase-1 and -2A. Bioorg Med Chem 26: (8) 1875-1884.

Kis-Bicskei N, **Bécsi B**, **Erdődi F**, Robinson RC, Bugyi B, Huber T, Nyitrai M, Talián, GC. Tropomyosins Regulate the Severing Activity of Gelsolin in Isoform-Dependent and Independent Manners, Biophysical Journal (2018), https://doi.org/10.1016/j.bpj.2017.11.3812

Ergülen E, **Bécsi B,** Csomós K I, Fésüs L, Kanchan**.** Identification of DNAJA1 as a novel interacting partner and a substrate of human transglutaminase 2.*Biochem J. 2016; 473(21):3889-3901.*

Sipos A, Iván J, **Bécsi B,** Darula Z, Tamás I, Horváth D, Medzihradszky KF, **Erdődi F,** Lontay B. Myosin phosphatase and RhoA-activated kinase modulate arginine methylation by the regulation of protein arginine methyltransferase 5 in hepatocellular carcinoma cells. *Sci Rep. 2017; 7:40590.*

Bátori R, **Bécsi B,** Nagy D, Kónya Z, Hegedűs C, Bordán Z, Verin A, Lontay B, **Erdődi F.** Interplay of myosin phosphatase and protein phosphatase-2A in the regulation of endothelial nitric-oxide synthase phosphorylation and nitric oxide production. *Sci Rep. 2017; 7:44698.*

Kun M, Szuber N, Katona É, Pénzes K, Bonnefoy A, **Bécsi B, Erdődi F,** Rivard GE, Muszbek L. Severe bleeding diatheses in an elderly patient with combined type autoantibody against factor XIII A subunit; novel approach to the diagnosis and classification of anti-factor XIII antibodies. *Haemophilia. 2017 doi: 10.1111/hae.13205.*

Horváth D, Tamás I, Sipos A, Darula Z, **Bécsi B,** Nagy D, Iván J, **Erdődi F,** Lontay B. Myosin phosphatase and RhoA-activated kinase modulate neurotransmitter release by regulating SNAP-25 of SNARE complex. *PLoS One. 2017; 12(5):e0177046.*

Kengyel, A., **Bécsi, B.**, Kónya, Z., Sellers, J., **Erdődi, F**., Nyitrai, M. Ankyrin domain of myosin 16 influences motor function and decreases protein phosphatase catalytic activity. *Eur. Biophys. J* 44 (4), 207-218., 2015.