Determination of protein-ligand affinity by spectral correlation interferometry

A.G. Burenin[1,2], A.V. Betin[1,2], N.G. Massarskaya[1,2], M.P. Nikitin[1,2]

[1]*A.M. Prokhorov General Physics Institute, Russian Academy of Sciences, 38 Vavilov St., Moscow,* AGBurenin@gmail.com

[2]*Moscow Institute of Physics and Technology (State University)*

A method for protein-ligand affinity determination by spectral correlation interferometry (SCI) [1] has been developed. It is a heterogeneous method, therefore protein or ligand needs to be immobilized on the solid phase, which is a surface of a sensor chip. The interaction of biomolecules from solution with those on a surface increases the thickness of biolayer on the chip and, thus, changes the output signal. The SCI measures biolayer thickness with a precision of tens picometers, which provides the detection limit as low as 1/1000 of a protein monolayer. The spectral correlation interferometry was earlier applied for immunoassay development [2], investigation of drugs mechanism of action [3] and properties of polymer brushes [4].

The important quantitative characteristics of biomolecule interaction such as constants of association rate (k_{on}), dissociation rate (k_{off}) and equilibrium dissociation (K_d) can be calculated from the time dependence of the SCI signal. According to the law of mass action, the time dependences of surface coverage θ during binding and dissociation in the case of immobilized protein are described by the equations $\theta = \left(1 - e^{-\left(k_{on}[L] + k_{off}\right)t}\right)$ and $\theta = e^{-k_{off}t}$ respectively, where [L] – free ligand concentration, t – time . The unknown parameters k_{on} and k_{off} can be evaluated by fitting of the experimental data by a theoretical curve.
Seven antibodies to free prostate-specific antigen (fPSA) were chosen as a model, and their affinities have been determined. PSA is an important clinical marker for cancer and high affinity antibodies are of great demand for clinical diagnostics. The calculated K_d are within $1.6 \cdot 10^{-8}$ to $1.9 \cdot 10^{-9}$ M. The difference between these values and those obtained with the surface plasmon resonance (SPR) is ≤ 10 times. Remarkably, that is smaller than the difference of 10 – 100 times between the results obtained with SPR and ELISA [5], both of

them are equally considered as “gold standards”. The deviations can be due to the diverse properties of solid phase surface.

The spectral correlation interferometry has been shown as an effective tool for affinity determination, and has allowed one to choose optimal antibody for further use in medical diagnostics. It should be noted that this affinity measurement method is much less time- and reagent-consuming than the common enzyme linked immunosorbent assay (ELISA)-based methods, first of all because there is no need for calibration curves for antibody concentration determination. Moreover, additional information about kinetic characteristics of interaction is evaluated, which can be very useful for such applications as investigation of biomolecular interaction, clinical diagnostics and quality control in biotechnology. In contrast to the SPR-based methods, the spectral-correlation interferometry is compatible with glass and optic plastic surfaces used in fluorescence immunoassays and many other applications. In addition, it allows much more affordable equipment and sensor chips.

The authors thank Alcor Bio (Russia) for fPSA and antibodies.

1. Nikitin P.I. (2007) Picoscopes, New Label-Free Biosensors, Handbook Biosensors & Biochips. Chapter **27**: 71–13.

2. A.G. Burenin et al. (2013) Detection of Pyrethroids by Spectral Correlation Interferometry, Applied Biochemistry and Microbiology, **49**: 306–311.

3. T.L. Krasnikova et al. (2010) Effect of the C-terminal domain peptide fragment (65–76) of monocytic chemotactic protein-1 (MCP-1) on the interaction between MCP-1 and heparin, Dokl. Biochem. Biophys., **433:** 559–562.

4. A.E. Ivanov et al. (2012) Binding of mucin to water-soluble and surface-grafted boronate-containing polymers, Vysokomol. Soed., Ser. A, **54**: 3–13.

5. L. Heinrich et al. (2010)Comparison of the results obtained by ELISA and surface plasmon resonance for the determination of antibody affinity, J. Immunological Methods, **352**: 13–22