

Comprehensive evaluation of the analytical capabilities of a capillary electrophoresis coupled with microscale thermophoresis (CE-MST)

The creation and development of modern methods for characterization of biologically-important compounds and examination of their covalent and non-covalent interactions are the basic purposes of the modern analytical and bioanalytical chemistry. This project will be devoted to developing and evaluating new potential tools that could offer hitherto unknown possibilities in biomolecular research. The key concept will be based on the use of microscale thermophoresis (MST) – relatively new technique devoted to biomolecular studies and developing rapidly from several years, in a totally new format. In principle, thermophoretic analysis is carried out by measuring how concentration of a molecule or molecular complex changes after formation of a microscale temperature gradient. This process is dependent on such factors like molecular size, charge, conformation and solvation, thus it carries important information. In the project, the standard MST instrumentation will be adapted to the miniaturized and automated flow-through format, and further coupled on-line with other instruments such as capillary electrophoresis (CE) and mass spectrometry (MS), connected within a single instrument (CE-MST, CE-MST-MS).

Such combinations of various techniques and the unconventional format of thermophoretic analysis will establish totally new tools that may offer many important benefits. For example, the coupling between capillary electrophoresis and microscale thermophoresis (CE-MST) will provide the following added values: (i) the same molecular process (e.g. interaction between protein and its receptor, or between drug candidate and its target) will be studied by two independent (orthogonal) research techniques virtually without any loss of a sample; (ii) thermophoretic measurement will take place directly after previous separation process, so that the complex and impure samples will be handled despite the presence of structurally-similar molecules, like isomers; (iii) consumption of reagents will be minimized (2-3 orders of magnitude) in comparison to the classical thermophoresis; (iv) the injection of sample to the capillary will occur automatically (not manually as in the traditional thermophoresis) and will be programmable using a software. In addition, in comparison to the classical electrophoresis, thermophoretic measurement will provide the additional analytical information on a given process at the same time, concerning e.g. stability of some molecular complex, its aggregation, conformational changes, etc. Thermophoretic signal may also serve as an additional criterion for identification of unknown molecules. The further integration with mass spectrometry, in turn, will offer additional information on molecular weight, isotopic composition and structure, particularly useful in the potential examination of natural products and newly synthesized molecules, like drug candidates.

To enable an efficient coupling, the MST instrument will be connected with capillary electrophoresis (CE) via a single silica capillary, without any major instrument modification. This new technique will be tested with the selected model molecules by examination of their thermophoretic properties used in affinity studies (determination of binding constant), and analysis of model enzymatic/chemical reactions. A special attention will be paid to critically evaluate both advantages and drawbacks of the proposed approach. It will be done using an RGB additive color model – a special, original algorithm designed and developed by the applicant for that purpose.

The feasibility and functionality of the planned CE-MST interface has already be confirmed experimentally by the applicant in a special preliminary test (recently published results obtained for the prototype CE-MST combination). Therefore, the project has a big chance for successful realization even taking into account its high experimental novelty and complexity.