Experimental investigation on the use of non-focusing nucleic acid probes for sequence-specific detection under isotachophoresis

The seminar will be given in English

Point-of-care biomedical diagnostic techniques must not only be rapid and highly sensitive, but they must also be highly specific: they must be able to detect the target of interest amid all other DNA and RNA sequences present in the sample, usually at much higher total concentration than the target of interest. The challenge of achieving specificity becomes more demanding with increasing sequence similarity between the target of interest and other sequences present in the system. Our group has previously developed a rapid and highly sensitive assay for detection of nucleic acids, using isotachophoresis (ITP) with non-focusing peptide nucleic acid (PNA) probes, achieving 100 fM sensitivity in under 15 minutes; however, the specificity of this assay has not been examined to date.

In this seminar, I will present my work characterizing the specificity of this ITP assay in the presence of total cellular RNA extracts, as well in the presence of synthetic DNA with 1-5 nucleotide differences from the probe sequence. In addition to using PNAs as probes, we also use other synthetic DNA analogs, which have a lower per-base binding energy but a higher solubility (and thus greater experimental versatility) than PNA. We find that it is possible to achieve single-nucleotide specificity with PNAs in a limited-species DNA system by working at 55°C; however, specificity is considerably reduced when working with total RNA extracts. We demonstrate that the concentration of non-specific targets present can be a more important factor in determining the specificity of an assay than the sequence similarity between the targets of interest and the other nucleic acids present, and provide guidelines for future design of such assays.