Urinary samples of 14 individuals (Table 1) were lyophilized, and protein pellets were resuspended in 1 mL of distilled water for 2D-DIGE analyses. Protein concentration was determined using the 2D Quant Kit (GE Healthcare, Uppsala, Sweden). Inorganic salts from urine samples were removed using PD 10 Columns (GE Healthcare). Following extraction, any other interfering components were removed using 2D Clean-Up Kit (GE Healthcare).
Electron-transfer dissociation (ETD) is a newly developed fragmentation method, which is related to electron capture dissociation (ECD) in that labile PTMs are preserved while the backbone of the peptide is fragmented to yield c and z product ions. Often, peptides with charge states of 3+ or higher are required for effective fragmentation. Small peptides with predominantly 2+ charge states have been shown to exhibit poorer fragmentation efficiency with ETD or ECD. Large proteolytic peptides (e.g., Lys-C digestion) inherently carry additional charges to yield peptide charge states of 3+ or higher. Thus, ETD should be well-suited to the large peptides. In addition, because of the ability of ETD fragmentation to retain labile PTMs, the deglycosylation step, often required to determine peptide backbone sequence in glycopeptide identification (for N- and O-linked glycopeptides), may be unnecessary.
Microfluidic High-Resolution Free-Flow Isoelectric Focusing

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A microfluidic free-flow isoelectric focusing glass chip for separation of proteins is described. Free-flow isoelectric focusing is demonstrated with a set of fluorescent standards covering a wide range of isoelectric points from pH 3 to 10 as well as the protein HSA. With respect to an earlier developed device, an improved microfluidic FFE chip was developed. The improvements included the usage of multiple sheath flows and the introduction of preseparated ampholytes. Preseparated ampholytes are commonly used in large-scale conventional free-flow isoelectric focusing instruments but have not been used in micromachined devices yet. Furthermore, the channel depth was further decreased. These adaptations led to a higher separation resolution and peak capacity, which were not achieved with previously published free-flow isoelectric focusing chips. An almost linear pH gradient ranging from pH 2.5 to 11.5 between 1.2 and 2 mm wide was generated. Seven isoelectric focusing markers were successfully and clearly separated within a residence time of 2.5 s and an electrical field of 20 V mm⁻¹. Experiments with pI markers proved that the device is fully capable of separating analytes with a minimum difference in isoelectric point of Δ(pI) = 0.4. Furthermore, the results indicate that even a better resolution can be achieved. The theoretical minimum difference in isoelectric point is Δ(pI) = 0.23 resulting in a peak capacity of 29 peaks within 1.8 mm. This is an 8-fold increase in peak capacity to previously published results. The focusing of pI markers led to an increase in concentration by factor 20 and higher. Further improvement in terms of resolution seems possible, for which we envisage that the influence of electroosmotic flow has to be further reduced. The performance of the microfluidic free-flow isoelectric focusing device will enable new applications, as this device might be used in clinical analysis where often low sample volumes are available and fast separation times are essential.
Figure 1. μ-FFIEF chip layout and working principle: (a) no voltage applied; no separation (b) voltage applied, IEF of three components.

1. bottom chip plate; 2. top chip plate; 3. microfluidic channel; 4. separation chamber; 5. outlets; 6. low-pH sheath flow inlet; 7. high-pH sheath flow inlet; 8. ampholytes 1 inlet; 9. ampholytes 2 + sample inlet; 10. ampholytes 3 inlet; 11. electrode compartment; 12. conductive membrane; 13. not separated sample; 14. focused sample; and 15. collected sample.

20 mm x 20 mm;
2.2 mm thickness
Figure 2. pH gradient formation using preseparated ampholytes: (a–c) show a cross section of the separation chamber at different positions x and the corresponding pH gradient. With the increasing residence time, the pH gradient is further developing. (1, anodic electrolyte; 2, cathodic electrolyte; 3, membrane; 4, acidic sheath flow; 5, basic sheath flow; 6, prolyto1; 7, prolyto2 + sample; 8, prolyto3; 9, fully focused sample component).
Probing the Unfolding and Refolding Processes of Carbonic Anhydrase 2 Using Electrospray Ionization Mass Spectrometry Combined with pH Jump

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A novel method for proving the time course of the unfolding and refolding processes of metalloprotein bovine carbonic anhydrase 2 (CA2) is demonstrated using electrospray ionization mass spectrometry (ESI MS) combined with pH jumps between 3.6 and 4.4. The shift in mass accompanied by the release or coordination of a zinc ion and the change in the charge state distribution were measured to evaluate the folding process. The time course of the ESI mass spectra revealed the existence of four types of ions in the experimental system, i.e., lower charged apo-CA2 and holo-CA2 ions and higher charged apo-CA2 and holo-CA2 ions. The deconvolution spectrum of the ion peak ensemble for each type of ion was processed and time course plots of the relative intensities of the four ions were prepared in order to analyze the folding processes. These analyses revealed the coexistence of two folding states of the lower and higher charged apo-CA2 under the condition of pH 3.6. The lower and higher charged apoproteins spontaneously refolded to the lower charged holoprotein by a pH jump from 3.6 to 4.4 without the addition of an extra zinc ion. The higher charged holoprotein observed during both the unfolding and refolding processes was considered to be an intermediate of the change in folding. The present study indicates that ESI MS combined with pH jump would be a powerful method to probe the unfolding and refolding of proteins. This method simultaneously measures mass spectra and analyzes the folding processes as a function of time using deconvolution spectra constructed by selecting a suitable m/z range for the analysis from the peaks of charge state distributions.

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Figure 1. ESI mass spectra of CA2 measured at (a) pH 4.4 and (b) pH 3.6. The measurements were performed under 0.2% acetic acid containing aqueous ammonia for pH adjustment. The deconvolution spectra are shown in the right side.
Electrospray Characteristic Curves: In Pursuit of Improved Performance in the Nanoflow Regime

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Depending on its coordinates in the parameter space, an electrospray can manifest in one of several known regimes—stable, quasi-stable, transitional chaotic, and nonaxial—that ultimately impact measurement sensitivity and precision. An electrospray operating in the cone-jet regime provides relatively large and stable spray current, as well as smaller initial droplets, that are prerequisites for higher sensitivity and quality mass spectrometric analyses. However, the dynamic conditions encountered, for example, in gradient elution-based liquid separations create difficulties for continuous operation in this regime. We present a preliminary study aimed at providing the basis for stabilizing the electrospray in the cone-jet regime. On the basis of spray current measurements obtained using solvent conditions typically found in liquid chromatography–mass spectrometry, an improved description of the cone-jet stability island is provided by including transitions to and from the recently described astable regime. Additionally, the experimental conditions in which the astable regime marks the transition between pulsating and cone-jet regimes are further clarified.

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**Figure 1.** Characteristic curves measured for 15% B solution at 30, 50, 100, and 200 nL/min reflect the regime sequence an electrospray undergoes with increasing applied voltage (A). General trends for 15% B solution at flow rates between 20 and 100 nL/min, partially illustrating the cone-jet stability island (B). General trends indicating the breakdown line and the breakdown region for 5% B solution (C).
Recent advances in nanotechnology have produced a variety of nanoparticles ranging from semiconductor quantum dots (QDs), magnetic nanoparticles (MNPs), metallic nanoparticles, to polymeric nanoparticles. Their unique electronic, magnetic, and optical properties have enabled a broad spectrum of biomedical applications such as ultrasensitive detection, medical imaging, and specific therapeutics. MNPs made from iron oxide, in particular, have attracted extensive interest and have already been used in clinical studies owing to their capability of deep-tissue imaging, non-immunogenesis, and low toxicity. In this Research Highlight article, we attempt to highlight the recent breakthroughs in MNP synthesis based on a non-hydrolytic approach, nanoparticle (NP) surface engineering, their unique structural and magnetic properties, and current applications in ultrasensitive detection and imaging with a special focus on innovative bioassays. We will also discuss our perspectives on future research directions.
Fig. 2 Comparison of amphiphilic polymer encapsulated hydrophobic MNPs (left) and dextran-coated CLIO particles (right). The amphiphilic polymer coating is generally thin and can be made from biocompatible polymers; in contrast, the crosslinked dextran coating layer increases the particle hydrodynamic radius significantly. (Images adopted from ref. 37 and ref. 41, and re-plotted.)
Fig. 3 Schematic illustration of DNA and protein screening applications of the magneto-optical sandwich assay. A pair of molecular probes recognizing distinct domains of target molecules is linked to an optical barcode (multicolor) and a magnetic bead (brown) for DNA (top panel) and protein (bottom panel) biomolecular target analysis. If the target is present, it brings the two beads together. After magnetic separation, the optical barcodes can be read with a spectrometer or flow cytometer at the single bead level for identification of the targets.
Add magnetic chromatography Dynabeads® to sample for adsorption of proteins/peptides to beads.

Wash to remove unwanted components by magnetic separation.

Desorption by changing buffer conditions followed by magnetic separation.

Fraction 1: Further fractionation is optional.
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Suction blister fluid as potential body fluid for biomarker proteins

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Early diagnosis is important for effective disease management. Measurement of biomarkers present at the local level of the skin could be advantageous in facilitating the diagnostic process. The analysis of the proteome of suction blister fluid, representative for the interstitial fluid of the skin, is therefore a desirable first step in the search for potential biomarkers involved in biological pathways of particular diseases. Here, we describe a global analysis of the suction blister fluid proteome as potential body fluid for biomarker proteins. The suction blister fluid proteome was compared with a serum proteome analyzed using identical protocols. By using stringent criteria allowing less than 1% false positive identifications, we were able to detect, using identical experimental conditions and amount of starting material, 401 proteins in suction blister fluid and 240 proteins in serum. As a major result of our analysis we construct a prejudiced list of 34 proteins, relatively highly and uniquely detected in suction blister fluid as compared to serum, with established and putative characteristics as biomarkers. We conclude that suction blister fluid might potentially serve as a good alternative biomarker body fluid for diseases that involve the skin.

Keywords:
Biomarker / Depletion / Serum / Suction blister fluid