Analytical Methods Hot Paper

Deutsche Ausgabe: DOI: 10.1002/ange.201603017
Internationale Ausgabe: DOI: 10.1002/anie.201603017

A Disposable Planar Paper-Based Potentiometric Ion-Sensing Platform

Jinbo Hu, Andreas Stein, and Philippe Bühlmann**

Abstract: *Ion-selective electrodes (ISEs) are widely used tools for fast and accurate ion sensing. Herein their design is simplified by embedding a potentiometric cell into paper, complete with an ISE, a reference electrode, and a paper-based microfluidic sample zone that offer the full function of a conventional ISE setup. The disposable planar paper-based ion-sensing platform is suitable for low-cost point-of-care and in-field testing applications. The design is symmetrical and each interfacial potential within the cell is well defined and reproducible, so that the response of the device can be theoretically predicted. For a demonstration of clinical applications, paper-based Cl^- and K^+ sensors are fabricated with highly reproducible and linear responses towards different concentrations of analyte ions in aqueous and biological samples. The single-use devices can be fabricated by a scalable method, do not need any pretreatment prior to use, and only require a sample volume of 20 μL.*

Ion sensing is an important topic in various fields, such as clinical and environmental analysis.[1] As often introduced in general chemistry courses, selective and quantitative ion sensing can be achieved with a potentiometric cell that comprises an ion-selective electrode (ISE), a reference electrode, and a voltmeter as a readout tool. It has been estimated that each year over a billion measurements with ISEs are performed globally in clinical laboratories alone.[1a] Besides detection of analytes with low (for example, K^+, Na^+, Cl^-) and high valence charges (such as heparin),[2] biosensing of proteins[3] and detection of electrically neutral species have been achieved with ISEs.[4]

With the growing demand for point-of-care and in-field testing, paper has recently attracted much attention as a simple, affordable, flexible, and scalable substrate for microfluidic assays.[5] Although paper-based colorimetric sensors offer the advantage of simple data interpretation, detection with electrochemical techniques is insensitive to color interferences and is generally more quantitative.[6] Existing paper-based ion sensors rely on various techniques, including potentiometry,[7] coulometry,[8] chronopotentiometry,[9] and colorimetry.[10] Paper was used in these devices either as a microfluidic sampling tool or as a substrate to mechanically support the sensing components. Strip-type ISEs known as Ektachem slides were available in the 1980s,[11] and were recently adapted with a paper substrate to support carbon nanotubes or conducting polymers as solid contacts.[7b,c] Although miniaturizable, these devices need cumbersome electrode conditioning and individual calibration. A more integrated device, reported by Whitesides et al., utilizes a reusable ISE membrane placed between two disposable wax-imprinted paper substrates.[7d] With different ISE membranes, various clinically relevant ions can be detected. However, the ISE membranes have to be well conditioned, and the devices have to be carefully assembled and calibrated, which may impede their practical use.

With the motivation of developing a simple and pretreatment-free device, we herein report a disposable planar paper-based ion-sensing platform with a potentiometric cell embedded into paper. In contrast to strip-type or other ion sensors that have to be calibrated individually, devices based on this highly integrated platform are suitable for single use and do not require any pretreatment or assembly. By design, each interfacial potential within these cells is well defined, so that their responses can be theoretically predicted, and highly reproducible measurements are achieved. For a demonstration of clinical applications, ion sensors were fabricated and successfully used for Cl^- and K^+ sensing in biological samples with a small sample volume of 20 μL. By using specific sensing membranes, this platform can potentially be adapted for detecting other ions that are currently measured with a conventional ISE setup. The fabrication of these planar devices can be readily scaled up by printing, and their integration into complex paper-based devices for complete analysis is also conceivable.

The paper-based ion-sensing platform was fabricated by integrating a potentiometric cell into a piece of filter paper (Figure 1 a). To define microfluidic channels that contain aqueous solutions, a polyurethane-based hydrophobic barrier was deposited into paper. Polyurethane is used because it is affordable, readily available, inkjet-printable,[12] and it avoids the melting process that is required for wax-printed barriers used in previously reported paper-based devices.[13] As a potentiometric cell, this device contains an ISE with a sensing membrane and a reference electrode with a reference membrane; both of the membranes are embedded into paper. The sensing membrane can be an ionophore-doped ISE membrane, or a hydrophilic high-capacity ion-exchange membrane that is particularly suitable for biological samples.[14] For a demonstration of clinical applications, we used a commercial hydrophilic high-capacity anion-exchange (HHCAE) membrane (fumion FAA-3 ionomer) and a valinomycin-doped ISE membrane for Cl^- and K^+ sensing,

[*] J. Hu, Prof. A. Stein, Prof. P. Bühlmann
Department of Chemistry, University of Minnesota
207 Pleasant St. SE, Minneapolis, MN, 55455 (USA)
E-mail: a-stein@umn.edu
buhlmann@umn.edu

Supporting information and the ORCID identification number(s) for the author(s) of this article can be found under http://dx.doi.org/10.1002/anie.201603017.

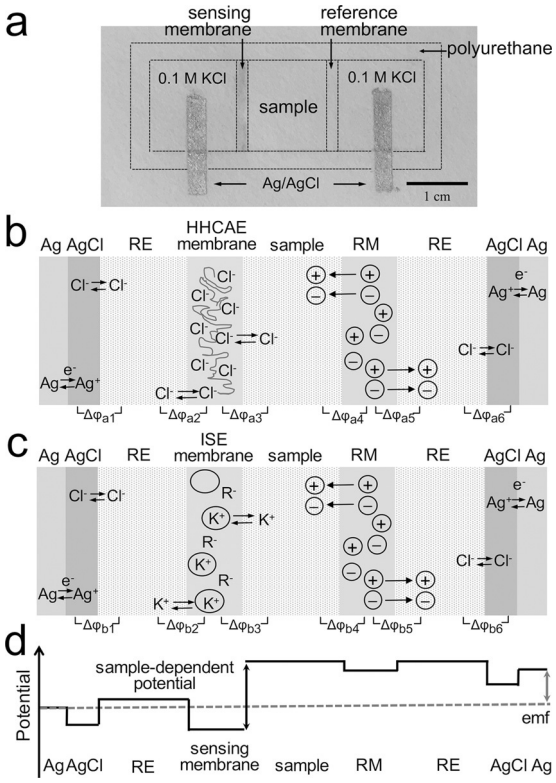

Figure 1. a) Photograph of a paper-based ion-sensing device. b) Schematic representation of all relevant interfaces in a Cl^- sensor with an HHCAE membrane, ionic-liquid-doped reference membrane (RM), and two Ag/AgCl electrodes contacting a 0.1 M KCl reference electrolyte (RE). c) Paper-based K^+ sensor with a sensing membrane doped with an ionophore (shown as an ellipse) and ionic sites (R^-). d) Electrical potential profile across the sensing device. A color version of this figure is available in the Supporting Information (Figure S4).

respectively. The reference membrane was loaded with an ionic liquid that can leach into the adjacent aqueous solutions on a slow but continuous basis, thus providing sample-independent potentials.[15] It avoids direct contact between the sample and reference electrolyte, thus eliminating undesirable liquid-junction potentials that can cause large measuring errors.[16] Analogous to a conventional ISE that contains an AgCl-coated Ag wire as an inner reference coupled with an inner filling solution, this paper-based device utilizes stencil-printed Ag/AgCl electrodes coupled with 0.1M KCl reference electrolyte as inner references for both the ISE and reference electrode.

To ensure calibration-free operation, each interfacial potential within the cell has to be well defined and highly reproducible.[17] For this device (Figure 1b), the interfacial potential at the interface between the Ag/AgCl electrode and reference electrolyte ($\Delta\varphi_{a1}$ and $\Delta\varphi_{a6}$) is defined by the redox reaction $AgCl(s) + e^- \leftrightarrow Ag(s) + Cl^-(aq)$, which is fixed by using a 0.1M KCl reference electrolyte. At the sample/reference membrane ($\Delta\varphi_{a4}$) and reference membrane/reference electrolyte interfaces ($\Delta\varphi_{a5}$), the interfacial potentials are governed by partitioning of the ionic liquid between the membrane and aqueous phases, making them sample-independent. For the sensing membrane, the distribution of the primary ion (Cl^- or K^+) between the membrane and adjacent aqueous solutions determines the two interfacial potentials ($\Delta\varphi_{a2/b2}$ and $\Delta\varphi_{a3/b3}$); therefore, their concentration dependence can be quantitatively predicted by the Nernst equation.[1a,d] Since $\Delta\varphi_{a2/b2}$ is controlled by 0.1M KCl reference electrolyte, $\Delta\varphi_{a3/b3}$ is the only sample-dependent potential within the cell. The measured electromotive force (emf) is the sum of all interfacial potentials of the cell (Figure 1d), which are well-defined and, therefore, reproducible and predictable. Consequently, the response of the device can be theoretically predicted, and is, in principle, calibration-free.

The structural features of the device were characterized by scanning electron microscopy (SEM). As shown by a top view (Figure 2a,b), the surface of the cellulose fibers is fully coated by the sensing components (e.g., Ag/AgCl electrode and reference membrane). The sample/sensing membrane interface is located in the cross-section of the paper, into which the membrane is embedded. As the SEM images of the cross-section show (Figure 2c,d), the voids between the cellulose fibers are filled by the sensing membrane, forming a homogenous sensing network and providing good contact between the sample and sensing components.

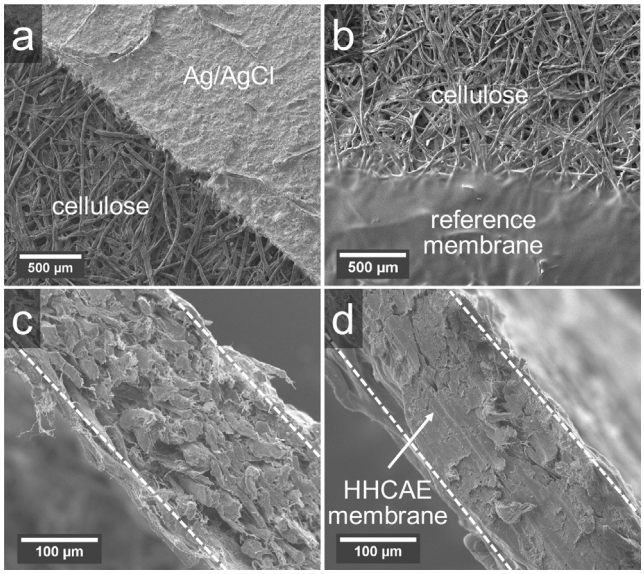

Figure 2. SEM images of the device: a) Top view showing the Ag/AgCl electrode, b) top view showing the reference membrane, c) cross-sectional view of the paper not infiltrated with a sensing membrane, d) cross-sectional view of the paper infiltrated with an HHCAE membrane.

Figure 3 shows a device placed on a piece of a polyvinyl chloride sheet as a mechanical support, with the two Ag/AgCl electrodes connected to a voltmeter using alligator clips. For a measurement, 20 µL of the 0.1M KCl reference electrolyte is applied to each of the areas close to the Ag/AgCl electrodes, and 20 µL of the sample is applied to the sample zone. In principle, the sample volume could be further reduced by patterning smaller sensing areas into paper using inkjet printing. Due to the single-use nature of the device, at least

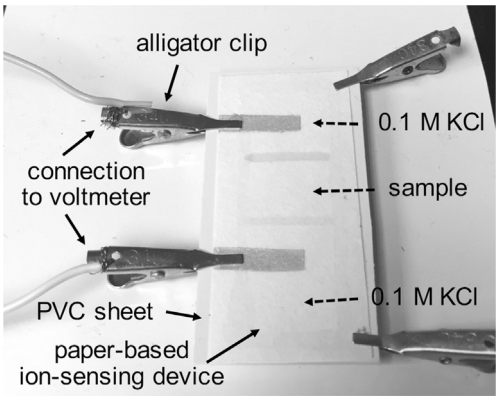

Figure 3. Photograph of a paper-based ion-sensing platform, with two alligator clips on the left for the emf measurements and two clips on the right to balance the device. A color version of this figure is available in Figure S5.

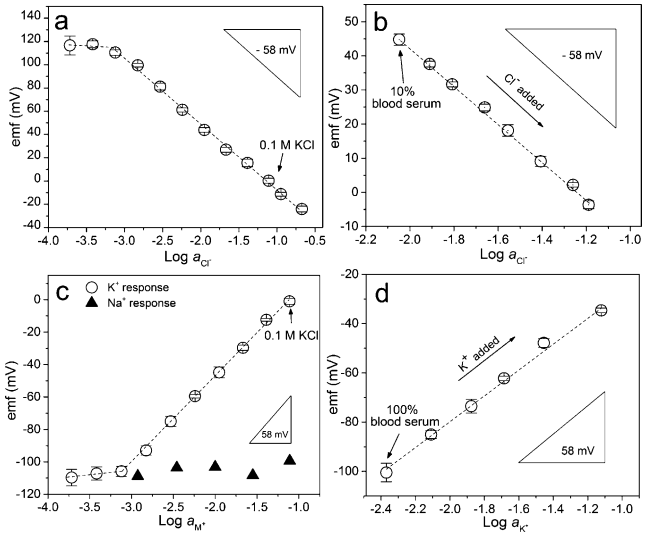

Figure 4. Paper-based ion sensors: a) Cl^- response of Cl^- sensors to KCl solutions, b) Cl^- response of Cl^- sensors to tenfold-diluted blood serum samples, c) response of K^+ sensors to K^+ and Na^+ (aqueous samples), d) response of K^+ sensors to K^+ in a background of undiluted blood serum. The parameter a_x is the activity of the given ionic species in solution. Each data point for the primary ion (Cl^- and K^+) is from three individual devices. A magnified version of this figure is available in Figure S6.

ten individual devices are needed for a calibration curve with ten different concentrations.

When an HHCAE membrane is used for Cl^- detection with samples of aqueous solutions (Figure 4a), these devices exhibit highly reproducible responses with an $E°$ value of -63.6 ± 2.0 mV and the theoretically predicted (Nernstian) slope of -56.6 ± 1.0 mV decade^{-1} in the range from $10^{-0.7}$ to $10^{-3.1}$ M, which covers the clinically relevant range. This reproducibility of the $E°$ value and slope are similar as for conventional ISEs with HHCAE-membrane-infiltrated papers as sensing membranes ($E° = 10.3 \pm 2.2$ mV and slope $= -57.4 \pm 0.5$ mV decade^{-1}, see Figure S1 in the Supporting Information). When a 0.1 M KCl solution is used as both the sample and reference electrolyte, a symmetrical potentiometric cell is formed, and the theoretical emf value is 0 mV.[18] As Figure 4a shows, the measured emf value is 0.3 ± 2.1 mV (number of samples, $n = 3$), correlating well with the predicted value. Although the HHCAE membrane exhibits limited selectivity towards various hydrophilic anions, it is chosen specifically for clinical Cl^- sensing (98–107 mM in blood serum) because its use decreases interference from lipophilic ions (such as Br^- or SCN^-)[19] and biofouling caused by lipids,[14] and also resists Donnan failure at high ion concentrations because of its high ion-exchange capacity.[20] As the HHCAE membrane was obtained with Br^- as counterions, Cl^- substitution was required before device fabrication to ensure an accurate response (see Figure S2).

The performance of paper-based Cl^- sensors was also tested in biological samples. A series of blood serum samples with different Cl^- concentrations was prepared by adding 0.8 M KCl aqueous solutions into tenfold diluted blood serum samples with a certified Cl^- concentration (99 mM for undiluted blood serum). As shown in Figure 4b, reproducible and Nernstian responses are obtained in diluted blood serum samples with a slope of -55.7 ± 1.0 mV decade^{-1} and an $E°$ value of -68.8 ± 1.6 mV. The resistance of the devices is 1.2 ± 0.8 MΩ ($n = 27$), demonstrating that they are compatible with affordable low-impedance voltmeters as a readout tool.

Besides functioning with HHCAE membranes for Cl^- sensing, the paper-based ion-sensing platform is also compatible with ISE membranes exhibiting high selectivity. This is essential for K^+ sensing in blood, since the K^+ level (3.5–5.1 mM) in blood serum is much lower than that of the interfering ion Na^+ (135–145 mM).[19] In this study, a valinomycin-doped K^+-ISE membrane was used as the sensing membrane, with 20 wt % of an inert electrolyte (ETH 500) as a membrane additive (see Figure S3). The response curve of the resulting devices with samples of KCl solutions is shown in Figure 4c, where highly reproducible responses are obtained in a clinically relevant range from $10^{-1.0}$ to $10^{-3.1}$ M, with a linear slope of 53.3 ± 0.7 mV decade^{-1} and an $E°$ value of 59.6 ± 1.6 mV. Interestingly, the experimentally observed lower detection limit is $10^{-3.1}$ M, which is higher than for a conventional valinomycin-based K^+-ISE. Similar behavior was reported previously, where paper was used as a sampling tool for Ag^+ detection.[7a] The reason for the different detection limit is not yet known, but it is likely that the slightly anionic surface of cellulose contributes to this behavior.[21] When a 0.1 M KCl solution was used as sample, the observed emf value was -0.9 ± 1.9 mV ($n = 3$), matching well with the predicted value (0 mV). Compared to devices with HHCAE membranes, the cell resistance increases to 4.4 ± 0.8 MΩ ($n = 21$) with the K^+-ISE membranes, but is still sufficiently low to be compatible with low-cost voltmeters.

The selectivity of the K^+-sensing device was evaluated by testing its response with NaCl solutions. As shown in Figure 4c, no significant Na^+ response is recorded even at high Na^+ concentrations (0.1 M). Finally, K^+ calibrations were performed with a series of samples of undiluted blood serum containing various K^+ concentrations with a high Na^+ background (certified as 140 mM). It can be seen in Figure 4d

that these devices exhibit reproducible responses in undiluted blood serum with a well-retained linear slope of 53.6 ± 1.8 mV decade^{-1} and an $E°$ value of 27.6 ± 3.2 mV, demonstrating their high sensitivity and selectivity in biological media. By using different ISE membranes, sensors for other clinically relevant ions can also be fabricated in the same way.

According to the U.S. Code of Federal Regulations, in clinical laboratories the acceptable measuring error is $\pm 5\%$ for Cl^- and ± 0.5 mM for K^+,[22] which corresponds to a variation in emf value of approximately 1.1 mV and 2.6 mV, respectively. In this study, the obtained $E°$ variations of paper-based ion sensors in blood serum samples are ± 1.6 mV (Cl^-) and ± 3.2 mV (K^+), which are close to the requirement for calibration-free ion sensing. Improvement of the $E°$ value reproducibility for practical uses may be achieved by detailed sensor optimization (for example through selection of the ionic liquid and mass production using inkjet printing). Further improvements of the $E°$ reproducibility (to reach, for example, ± 0.2 mV)[18b] may be achieved with highly reproducible all-solid-state ISEs and reference electrodes coupled with robust redox buffers,[16,23] which are under development.

In conclusion, a disposable and low-cost paper-based ion-sensing platform was developed with a potentiometric cell embedded into paper. These devices are simple to use, do not need any pretreatment ("conditioning"), and only require a low sample volume of 20 μL. They are compatible with HHCAE and ionophore-doped ISE membranes to detect clinically relevant ions in biological samples with high sensitivity and reproducibility, and could be potentially adapted to detect other charged analytes by changing the sensing membrane.

Acknowledgements

This work was supported by the University of Minnesota Initiative for Renewable Energy and the Environment. J.H. thanks the Krogh family for a Lester C. and Joan M. Krogh Fellowship.

Keywords: analytical methods · clinical analysis · ion-selective electrodes · paper-based sensors · potentiometry

How to cite: *Angew. Chem. Int. Ed.* **2016**, *55*, 7544–7547
Angew. Chem. **2016**, *128*, 7670–7673

[1] a) E. Bakker, P. Bühlmann, E. Pretsch, *Chem. Rev.* **1997**, *97*, 3083–3132; b) E. Bakker, E. Pretsch, *Angew. Chem. Int. Ed.* **2007**, *46*, 5660–5668; *Angew. Chem.* **2007**, *119*, 5758–5767; c) S. Amemiya in *Handbook of Electrochemistry* (Ed.: C. G. Zoski), Elsevier, Amsterdam, **2007**; d) J. Bobacka, A. Ivaska, A. Lewenstam, *Chem. Rev.* **2008**, *108*, 329–351.

[2] S. C. Ma, V. C. Yang, M. E. Meyerhoff, *Anal. Chem.* **1992**, *64*, 694–697.

[3] K. Y. Chumbimuni-Torres, Z. Dai, N. Rubinova, Y. Xiang, E. Pretsch, J. Wang, E. Bakker, *J. Am. Chem. Soc.* **2006**, *128*, 13676–13677.

[4] R.-N. Liang, D.-A. Song, R.-M. Zhang, W. Qin, *Angew. Chem. Int. Ed.* **2010**, *49*, 2556–2559; *Angew. Chem.* **2010**, *122*, 2610–2613.

[5] a) A. W. Martinez, S. T. Phillips, M. J. Butte, G. M. Whitesides, *Angew. Chem. Int. Ed.* **2007**, *46*, 1318–1320; *Angew. Chem.* **2007**, *119*, 1340–1342; b) A. W. Martinez, S. T. Phillips, G. M. Whitesides, E. Carrilho, *Anal. Chem.* **2010**, *82*, 3–10; c) D. M. Cate, J. A. Adkins, J. Mettakoonpitak, C. S. Henry, *Anal. Chem.* **2015**, *87*, 19–41; d) K. Yamada, T. G. Henares, K. Suzuki, D. Citterio, *Angew. Chem. Int. Ed.* **2015**, *54*, 5294–5310; *Angew. Chem.* **2015**, *127*, 5384–5401; e) A. A. Kumar, J. W. Hennek, B. S. Smith, S. Kumar, P. Beattie, S. Jain, J. P. Rolland, T. P. Stossel, C. Chunda-Liyoka, G. M. Whitesides, *Angew. Chem. Int. Ed.* **2015**, *54*, 5836–5853; *Angew. Chem.* **2015**, *127*, 5932–5951.

[6] a) W. Dungchai, O. Chailapakul, C. S. Henry, *Anal. Chem.* **2009**, *81*, 5821–5826; b) E. J. Maxwell, A. D. Mazzeo, G. M. Whitesides, *MRS Bull.* **2013**, *38*, 309–314; c) J. Adkins, K. Boehle, C. Henry, *Electrophoresis* **2015**, *36*, 1811–1824; d) J.-M. Oh, K.-F. Chow, *Anal. Methods* **2015**, *7*, 7951–7960; e) J. Mettakoonpitak, K. Boehle, S. Nantaphol, P. Teengam, J. A. Adkins, M. Srisa-Art, C. S. Henry, *Electroanalysis* **2016**, DOI: 10.1002/elan.201501143.

[7] a) J. Szűcs, R. E. Gyurcsányi, *Electroanalysis* **2012**, *24*, 146–152; b) M. Novell, M. Parrilla, G. A. Crespo, F. X. Rius, F. J. Andrade, *Anal. Chem.* **2012**, *84*, 4695–4702; c) S. T. Mensah, Y. Gonzalez, P. Calvo-Marzal, K. Y. Chumbimuni-Torres, *Anal. Chem.* **2014**, *86*, 7269–7273; d) W.-J. Lan, X. U. Zou, M. M. Hamedi, J. Hu, C. Parolo, E. J. Maxwell, P. Bühlmann, G. M. Whitesides, *Anal. Chem.* **2014**, *86*, 9548–9553.

[8] M. Cuartero, G. A. Crespo, E. Bakker, *Anal. Chem.* **2015**, *87*, 1981–1990.

[9] J. Kofler, S. Nau, E. J. W. List-Kratochvil, *J. Mater. Chem. B* **2015**, *3*, 5095–5102.

[10] X. Wang, Y. Qin, M. E. Meyerhoff, *Chem. Commun.* **2015**, *51*, 15176–15179.

[11] B. Walter, *Anal. Chem.* **1983**, *55*, 498A–514A.

[12] A. M. J. van den Berg, P. J. Smith, J. Perelaer, W. Schrof, S. Koltzenburg, U. S. Schubert, *Soft Matter* **2007**, *3*, 238–243.

[13] E. Carrilho, A. W. Martinez, G. M. Whitesides, *Anal. Chem.* **2009**, *81*, 7091–7095.

[14] E. Grygolowicz-Pawlak, G. A. Crespo, M. Ghahraman Afshar, G. Mistlberger, E. Bakker, *Anal. Chem.* **2013**, *85*, 6208–6212.

[15] a) T. Kakiuchi, T. Yoshimatsu, *Bull. Chem. Soc. Jpn.* **2006**, *79*, 1017–1024; b) T. Zhang, C.-Z. Lai, M. A. Fierke, A. Stein, P. Bühlmann, *Anal. Chem.* **2012**, *84*, 7771–7778.

[16] J. Hu, K. T. Ho, X. U. Zou, W. H. Smyrl, A. Stein, P. Bühlmann, *Anal. Chem.* **2015**, *87*, 2981–2987.

[17] a) J. Hu, A. Stein, P. Bühlmann, *TrAC Trends Anal. Chem.* **2016**, *76*, 102–114; b) P. Bühlmann, L. D. Chen in *Supramolecular Chemistry: From Molecules to Nanomaterials*, *Vol. 5* (Ed.: P. A. G. Jonathan W. Steed), Wiley, New York, **2012**.

[18] a) L. F. J. Dürselen, D. Wegmann, K. May, U. Oesch, W. Simon, *Anal. Chem.* **1988**, *60*, 1455–1458; b) G. Rumpf, U. Spichiger-Keller, H. Bühler, W. Simon, *Anal. Sci.* **1992**, *8*, 553–559.

[19] M. G. Scott, J. W. Heusel, V. A. LeGrys, O. Siggaard-Andersen in *Tietz Textbook of Clinical Chemistry*, 3rd ed. (Eds.: C. A. Burtis, E. R. Ashwood), W. B. Saunders Company, Philadelphia, **1999**.

[20] S. Ogawara, J. L. Carey, X. U. Zou, P. Bühlmann, *ACS Sens.* **2016**, *1*, 95–101.

[21] R. Pelton, *TrAC Trends Anal. Chem.* **2009**, *28*, 925–942.

[22] Laboratory Requirements, Code of Federal Regulations, Section 493.931, Title 42, **2003**.

[23] a) J. Hu, X. U. Zou, A. Stein, P. Bühlmann, *Anal. Chem.* **2014**, *86*, 7111–7118; b) X. U. Zou, X. V. Zhen, J. H. Cheong, P. Bühlmann, *Anal. Chem.* **2014**, *86*, 8687–8692.

Received: March 27, 2016
Published online: May 13, 2016

