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Current Opinion in
Electrochemistry

Review Article

On the intersection of electrochemistry and mass spectrometry

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Abstract

The application of nanopipettes, developed first as a tool for electrochemistry and electrophysiology, as tools for mass spectrometry is considered. Recent examples of advances in electrospray ionization and sampling for mass spectrometry with nanopipettes is discussed. These examples show a scientific intersection that is ripe for further development.

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Current Opinion in Electrochemistry 2019, **13**:140–146

This review comes from a themed issue on **Physical and Nanoelectrochemistry**

Edited by **Michael V. Mirkin**

For a complete overview see the Issue and the Editorial

Available online 13 December 2018

https://doi.org/10.1016/j.coelec.2018.12.001

Keywords

Mass spectrometry, Nanofluidics, Microfluidics, Sampling, Ionization, Imaging.

Introduction

Charge transfer (ionization), either in the form of an ion (often a proton) or an electron, is a necessary step in the analysis of nominally neutral molecules by mass spectrometry. With that in mind, mass spectrometry could be considered as ‘electrochemistry in the gas phase’ (with the caveat that a majority of mass spectrometrists and electrochemists would disagree with this!). Presently, a number of interesting reports are making use of an overlap in the two fields.

The intersection of electrochemistry with mass spectrometry

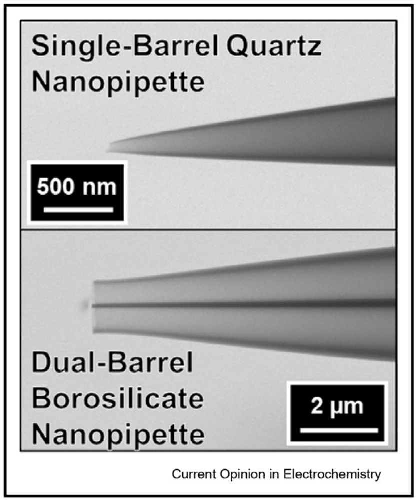
Some history is illustrative of the challenges that are present in working at this intersection. The fundamental steps of two of the most prevalent ionization processes for large molecules, matrix-assisted laser desorption/ionization and electrospray ionization (ESI), remain a matter of significant debate in the mass spectrometry community. For instance, ion formation from small droplets produced in ESI has remained an area of intense interest. Within the mass spectrometry community, the significance of attention to electrochemical processes has also been an area of active debate. As a particularly interesting example, almost 20 years ago, authorities on ESI fundamentals participated in a lively and provocative conversation on the importance of electrochemical processes in ESI that was reported as a Special Feature in *The Journal of Mass Spectrometry.* [1] The contributors to this debate did not arrive at a clear consensus in this manner, but Nobel Laureate John Fenn summarized the debate as follows: “The laudable goal of this Special Feature is to arrive at some consensus on these mechanisms by way of a debate among several investigators who have shown a strong interest in identifying and elucidating them. The participants, each of whom has made substantial contributions to an understanding of the processes that govern ESI behavior, have reached something of an impasse on one of its aspects, namely the role and relative importance of electrochemical processes. This literal ‘elder’ in the ESI congregation has been invited to act as a sort of arbitrator in the hope that their differences might be at least reconciled, if not resolved.” [1] Fenn (who coincidentally was trained as a graduate student in measurement of electromotive force values in electrolyte solutions [2]) summarized the debate between participants as, “In sum, it seems fair to say that in the overall process of electrospray ionization, electrochemical processes do indeed play what might be called an enabling physical role in making possible the electrostatic dispersion of liquids into small charged droplets. However, under the most commonly used operating conditions, only rarely do those processes seem to have much effect on the identity and relative abundances of the ions in an ESI mass spectrum. Even so, in those infrequent cases the consequences of those electrochemical reactions can be of great importance and significance.” [1] For most electrochemists, it is almost unthinkable that electrochemical processes would not be critical and, indeed, most interesting. This view has been held by a number of groups (mass spectrometrists and electrochemists) historically and more recently as well.

Of course, there are intersections of electrochemistry and mass spectrometry beyond the fundamental mechanism of ionization in ESI. In recent years, these intersections have advanced significantly. For instance, Donald et al. [3,4] devised mass spectrometry experiments that quite elegantly established an absolute electrochemical scale based on gas phase electron transfer. Likewise, Brown et al. [5,6] and Cheng et al. [7] recently developed clever electrode configurations that allow observation of fleeting intermediates in electrochemical reactions via mass spectrometry. Qiao et al. [8] and Zhong et al. [9,10] reported a number of hybrid electrochemical mass spectrometry platforms, including push—pull sampling devices that could be combined with imaging [11]. Mass spectrometry has also become indispensable in trace analysis and in understanding electrocatalysis and is now frequently applied, especially as differential electrochemical mass spectrometry [12,13]. Many of the developments at this intersection make use of significant advances that have occurred in mass spectrometry in recent years, including ambient ionization techniques and advances in mass analyzers, especially ion traps.

Nanopipettes

Glass or quartz capillaries pulled to a fine tip find important applications in both electrochemistry and mass spectrometry (Figure 1). To an electrochemist, the pulled capillaries can be fashioned into electrochemical platforms such as electrodes for interface between two immiscible electrolyte solutions (ITIES), as pores for resistive-pulse sensing, as probes for scanning electrochemical microscopy, or when filled with a conductive material as a small electrode. Since the 1970s, electrochemists (and electrophysiologists) have used capillaries, typically with microscale tips, in a wide array of experiments. More recently, applications of pulled capillaries have focused on tips with ever-shrinking dimensions, colloquially referred to as nanopipettes (or nanopipets). Nanopipettes used as probes and tools for investigating nanoscale electrochemical phenomenon continue to develop as important tools [14—17].

Figure 1



(top) Single-barrel nanopipette pulled from quartz capillary. (bottom) Dual-barrel nanopipette pulled from borosilicate theta capillary.

To a mass spectrometrist, pulled capillaries present one method to make an emitter for ESI. In fact, capillaries are sold commercially preformed in a variety of shapes and sizes. Typical pipettes used in ESI are tens of microns, with some special commercial tips, available in the microscale regime. Interestingly, there were a number of previous efforts to shrink tip dimensions for ESI emitters, as a number of beneficial properties were likely to emerge at small dimension tips. For instance, higher salt tolerance, ionization efficiencies, and different charge state distributions—all important factors for electrospray ionization — mass spectrometry (ESI—MS) analysis—had been shown [18—21]. Further, a number of mass spectrometry studies that made use of dual-barrel pipettes (which are common in electrochemistry and electrophysiology) showed interesting applications [22—25].

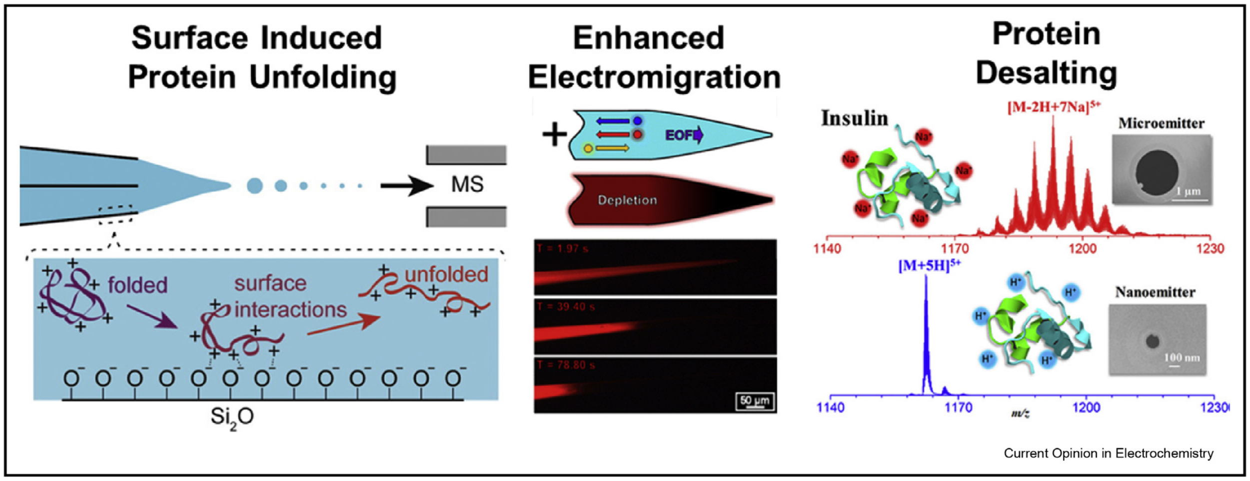
Our research group, having used nanopipettes for electrochemical experiments, reported ESI from nanopipettes with tip dimensions below 100 nm [26]. The results were highly interesting, with significant differences and possible advantages from the perspective of ESI. Furthermore, as demonstrated by other groups and ours, nanopipettes and micropipettes provided a promising platform for local sample collection in mass spectrometric analysis. In this review, we consider selected recent progress in the application of nanopipettes as: (1) nanoscale ESI emitters and (2) probes for local sample collection for mass spectrometry. Work discussed nominally represents an important current intersection between mass spectrometry and electrochemistry, where each community can take advantage of tools developed for initially disparate purposes. The topics are considered with the caveat that this review is not exhaustive in scope but instead highlights recent progress to provide the reader with a current opinion of the state of the field.

Nanopipette electrospray ionization (ESI) emitters

A number of groups have recently taken advantage of the unique properties that nanopipettes provide as ESI emitters. In particular, effects of surface charge, adsorption, ion transference, and electric field are altered for nanoscale relative to microscale ESI emitters.

In an example, Williams et al. have carried out a number of studies that revealed enhanced interactions of surface charges at the capillary wall for nanoscale emitters (Figure 2, left) [27—29]. In these reports, time dependence in signal from nanoscale ESI emitters attributed to surface adsorption processes was described. The nature of the protein being electrosprayed (e.g., folded/

Figure 2



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Recent developments with nanopipette ESI emitters. (left) Enhanced surface interactions in nanopipette ESI emitters reported by Donald et al. Surface interactions were reported to affect protein structure on emission and even to suppress proteins from emission. (middle) Depletion of ions under electroosmotic flow (EOF) in nanopipette ESI emitters reported by Yuill and Baker. (right) Salt adduct formation in ESI emitters reported by Xu and coworkers. Mass spectra of 10 ppm insulin (acetonitrile/water [50:50 vol %]) solution containing 0.1 mM NaCl obtained with ~1.2 µm microemitters (top right) and ~120 nm nanoemitters (bottom right). Images used with approval from the American Chemical Society and from Springer-Nature.

unfolded, stability, etc.) was found to influence the mass spectrometry signal observed differently for nanoscale vs. microscale ESI emitters. It was postulated that the adsorption at the glass interface and the high surface-to-volume ratio of nanoscale ESI emitters selectively depleted specific proteins (based on folding or charge) from solution at the tip of the pipette, which then altered distributions observed in spectra recorded. In this model, eventually, the adsorption to the glass surface was saturated, which resulted in increased similarity in spectra recorded at microscale or nanoscale ESI emitters. Similar desalting effects as described below were also reported in these studies.

Our research group combined fluorescence microscopy and electrospray to optically monitor ion concentration polarization effects for anions and cations in nanopipette ESI (Figure 2, middle) [30]. In particular, low electrolyte conditions were considered, which were found to exacerbate depletion/enrichment effects in small emitters. At low electrolyte concentration (500 µM), concentration polarization was found to be more significant than that at high concentration (50 mM). Furthermore, depletion was found for both self-aspirated and pressure-driven flow, which suggests that electromigration processes are important considerations regardless of fluid delivery mechanism.

Hu et al. [31] demonstrated effects of nanopipette ESI emitters on protein–metal adducts (e.g., Na^+, Ca^{2+}, etc.) formation (Figure 2, right), finding that a number of possible factors diminish adduct formation. Acidification of the solution due to electrostatic enrichment (similar to the case for enrichment/depletion described above), coupled with high proton transport (relative to other cations), was purported to reduce non-proton cation concentrations at the tip of the nanopipette emitter. Because presumably droplet formation occurs from this area of solution in the pipette, initial droplets formed have less chance to form metal adducts. In addition, as is generally expected to be the case for nanopipette ESI emitters, the initial droplets formed from small diameter tips are expected to undergo fewer droplet fissions during ESI. As each fission event is accompanied by solvent evaporation, fewer fission events result in less salt enrichment when ionized proteins are finally formed and, in turn, produces fewer salt adducts.

These studies provide examples of the considerations in use of nanoscale emitters. Generally, beneficial properties are observed, particularly with respect to desalting. Desalting with nanopipette ESI provides significant advantages in preserving solution-state structures, simplifying analysis, and preventing suppression of ionization by salt. Desalting is also a key advantage for that nanopipette ESI emitters hold for small-volume samples that can be difficult to manipulate, such as those found in local sampling studies (described further below). Work reported has also demonstrated that the desalting properties of nanopipette emitters allow realization of applications in solutions of relevance to biological settings. There are still subtleties in applying nanopipette ESI emitters that remain to be resolved, but these small tips certainly provide an interesting tool that has distinct influence on spectra observed.

Nanopipette sampling for mass spectrometry

Nanopipettes essentially provide a nanopore on a stick, meaning that the small diameter pores can be moved to regions of interest where sample can be manipulated (e.g., reagents added, solutions withdrawn, etc.). Nanopipetting has been used in a number of electrochemical applications for dispensation of material [16,32]. Microscale positioning of nanopipettes can be attained easily with stepper motors or manual manipulators commonly used in electrophysiology. Nanoscale control over the positioning of nanopipettes can be attained with piezoelectric control of position and feedback-based imaging such as scanning ion conductance microscopy (SICM) or shear-force microscopy. Translating these tools to mass spectrometry applications is not necessarily trivial as the potentials applied (kV vs. mV) and flow rates to support ESI are often not well matched between electrochemical and mass spectrometry applications.

However, mass spectrometry imaging (MSI) and, in particular ambient, ionization techniques have exploded in popularity [33–35]. In addition, sensitivity and range in mass spectrometry measurements has increased recently, which has afforded drive to small scale analysis, with single cell mass spectrometry becoming an increasingly important area of work.

There have been several different platforms developed that use nanoscale pipettes to enable mass spectrometry measurements. Three general approaches are highlighted here, which provide a general flavor of what can be achieved.

Our laboratory has reported a series of experiments with nanoscale (and microscale) pipettes that allowed analysis by both matrix-assisted laser desorption/ionization and electrospray ionization [36–38]. In one of the more versatile versions of these experiments, we used a dual-barrel pipette (Figure 3, left) in a 'collect-react-analyze' strategy for electrospray ionization [37]. In these experiments, an electrode and aqueous solution were loaded into one barrel of the emitter. The second barrel contained the inert, water-immiscible liquid, perfluorodecalin. When the probe was brought to a surface of interest, pressure-assisted withdrawal was used to collect material into the perfluorodecalin-filled barrel. Then, the assembly was mounted at the inlet of a mass spectrometer. Application of a potential to the aqueous barrel electrifies solution at the tip of both barrels and results in electrospray that mixes the two solutions. The advantage of this method is that reactive species (acidic conditions or phenyl boronic acid were demonstrated) could be placed in the aqueous barrel and upon electrospray mixing of the solution resulted in chemical reactions (hydrolysis for acid or complexation for phenyl boronic acid) between analytes of interest that had been aspirated into the other barrel. With this strategy, single cell and biofilm samples could be collected from an interface and chemically modified for enhanced analysis.

Laskin et al. have developed nano-desorption electrospray ionization (nano-DESI) as a versatile tool for ambient sample collection from surfaces [35,39–41]. Nano-DESI differs from traditional DESI in that a liquid junction is formed at the surface by a capillary, a second capillary in contact with the drop then transports fluid to the mass spectrometer inlet where it is introduced by ESI for analysis. By mounting the nano-DESI apparatus on a shear-force probe, the nano-DESI capillaries could be translated over a surface with feedback (Figure 3, top right). This allowed topographical imaging with collection of concomitant mass spectra. Nano-DESI with shear force was used to image bacterial colonies directly on agar plates, providing information related to the chemical secretions from colonies. Laskin's group has also reported SICM approach to surfaces for positioning with small pipettes [42].

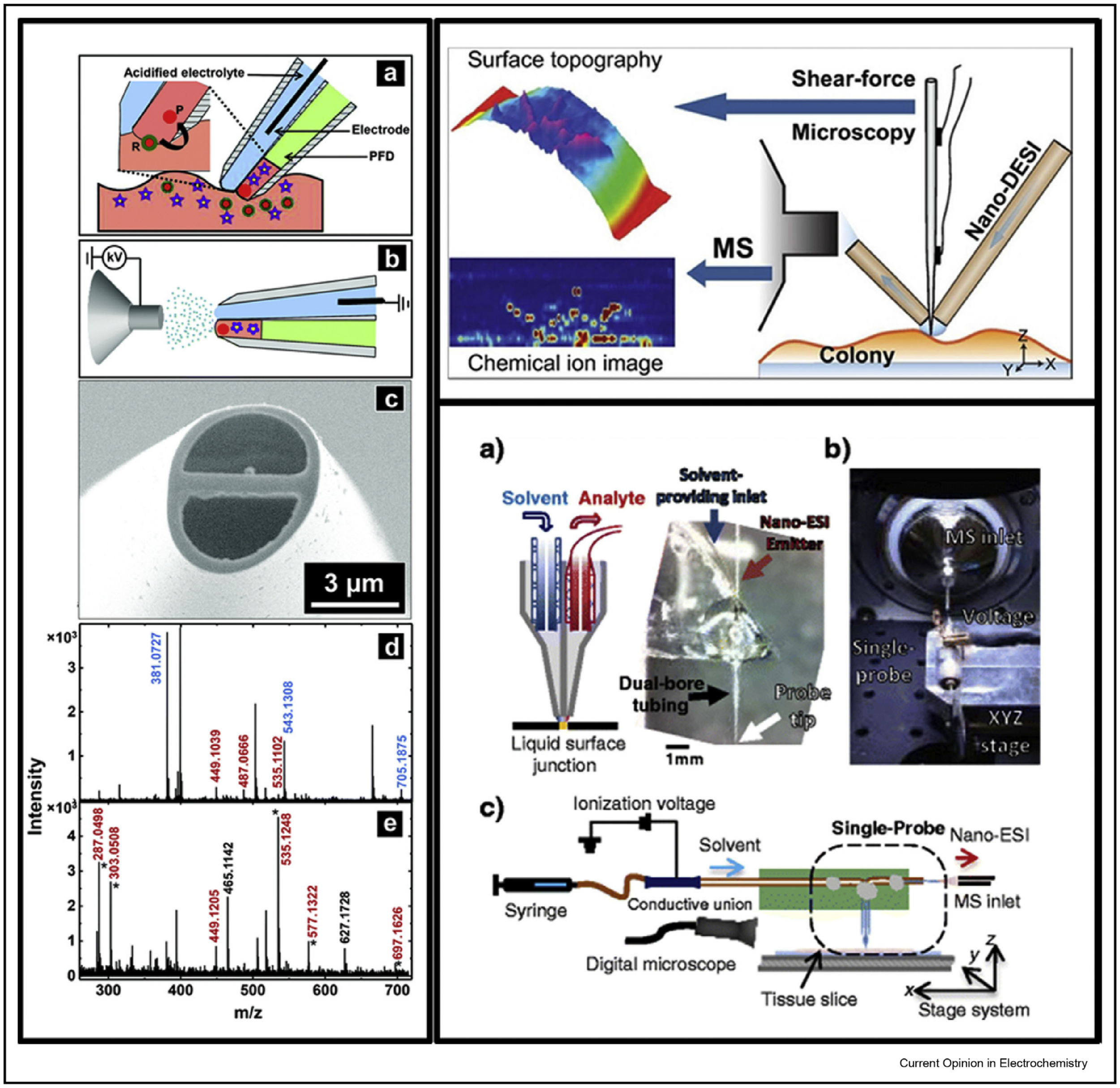
Yang et al. have reported another application of micropipettes/nanopipettes in local sample collection which is coined 'the single probe' (Figure 3, bottom right) [43–49]. This approach is essentially a dual-barrel pipette operating in a push–pull mode. Typical single probes used are micron in scale, as fluidic resistances for nanoscale pipettes becomes increasingly difficult to operate in a reproducible, controlled manner. The appeal of the single probe approach developed is the simplicity and flexibility of the approach for collecting high spatially resolved (microscale) mass spectrometry data. Diverse samples have been analyzed by the single probe, which include brain tissue, plant cells, and single cells.

These examples underscore where nanopipettes used routinely in electrochemistry can be put to use in MSI formats. Although typical MSI spatial resolution is 10s–100s of microns in size, the move to ever shrinking scales with nanopipette/micropipette sampling techniques is an appealing route to analysis at the fore front of spatial resolution improvements in MSI.

Nanopipettes as a continuing intersection of electrochemistry and mass spectrometry

The beneficial properties of nanopipette ESI emitters (desalting and high ionization efficiencies) are ideally coupled to the spatial resolution that can be achieved with precision spatial control of nanopipettes. We can expect that techniques such as SICM and similar electrochemical imaging techniques will be well matched in the future to realize single-cell and even subcellular

Figure 3



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(left) Collect-react-analyze strategy for nanopipette sampling at interfaces reported by Saha-Shah et al. (right, top) Shear force feedback control in nano-DESI developed by Laskin et al. (right, bottom) The single-probe nanopipette sampler reported by Yang et al. Images used with permission from the Royal Society of Chemistry, the American Chemical Society, and Springer-Nature.

mass spectrometric analysis, especially as mass spectrometry analyzers improve in sensitivity.

An area that likely needs special attention in these applications is a true determination of droplet size emitted from nanoscale emitters. As the size of nanoscale pipettes is beyond the resolution of standard optical techniques, the exact nature of droplet formed or ion emission from nanopipettes can be inferred but not known. The formation of the Taylor cone at typical microscale pipettes (10s of microns) is well characterized, and equations based on work at these larger scales are frequently used (or more likely misused) for small tips as when the limiting dimension of the emitter is shrunk to nanoscale dimensions, it is reasonable to expect that relevant forces, especially surface tension, will not scale directly with what is known for micron emitters [50,51].

Concluding remarks

The interface and interactions of tools for mass spectrometry and electrochemistry have come a long way since the debate on ESI discussed in the introduction. Further developing an environment that is receptive and welcoming to cross-fertilization is most assuredly of benefit to electrochemistry. As we seek to measure increasingly complex chemical and biochemical systems, it is worth remembering that every molecule has a mass—not everything has a redox potential. Thus, crossing into unfamiliar territory, as many have demonstrated previously, can be especially beneficial. The examples discussed here are a selected slice of recent results that highlight where intersections have been beneficial. There are still a number of advances in electrochemistry with aspects that are intriguing candidates for applications in mass spectrometry that we hope will be explored in the future. Applications in native mass spectrometry, with low electrolyte concentration and often more biologically similar electrolyte composition, present a great opportunity to make use of fundamental knowledge of double-layer and concentration effects commonly found in electrochemistry. Nanoscale thin-layer flow cells, photonic manipulation of ion channels and subsequent chemical reactions, and probing electrocatalysis of species that cannot easily be monitored electrochemically are a few examples. We expect there will be many, many more.

Author contributions

L.A.B. and G.S.J. contributed to the ideas and discussion of this opinion.

Funding sources

The author's laboratory has been supported by NSF CHE-1808133; NIH 5R21EB022297-02; NSF CBET-1803262; and NIH R01NS105888-01 during preparation of this manuscript.

Conflicts of interest

Nothing declared.

Acknowledgments

The authors gratefully acknowledge members of the bakergrp (past and present) and stimulating colleagues who contributed to work described here.

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- •• of outstanding interest

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