

High-field asymmetric waveform ion mobility spectrometry and native mass spectrometry

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High-Field Asymmetric Waveform Ion Mobility Spectrometry and Native Mass Spectrometry: Analysis of Intact Protein Assemblies and Protein Complexes

Oliver J. Hale, Eva Illes-Toth, Todd H. Mize, and Helen J. Cooper*



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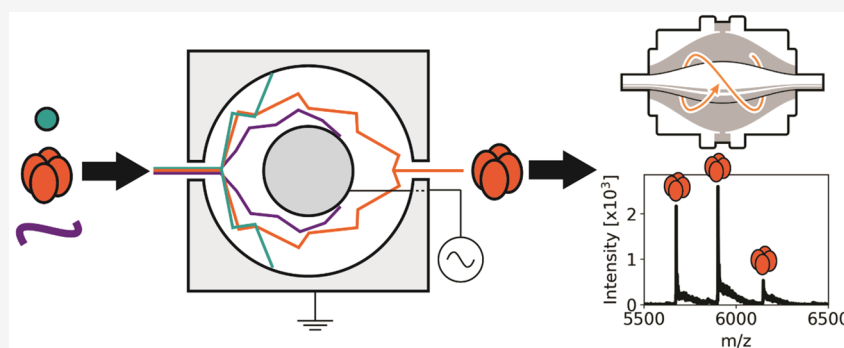
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ABSTRACT: High-field asymmetric waveform ion mobility spectrometry (FAIMS) enables the separation of ions on the basis of their differential mobility in an asymmetric oscillating electric field. We, and others, have previously demonstrated the benefits of FAIMS for the analysis of peptides and denatured proteins. To date, FAIMS has not been integrated with native mass spectrometry of folded proteins and protein complexes, largely due to concerns over the heating effects associated with the high electric fields employed. Here, we demonstrate the newly introduced cylindrical FAIMS Pro device coupled with an Orbitrap Eclipse enables analysis of intact protein assemblies up to 147 kDa. No evidence for dissociation was detected suggesting that any field heating is insufficient to disrupt the noncovalent interactions governing these assemblies. Moreover, the FAIMS device was integrated into native liquid extraction surface analysis (LESA) MS of protein assemblies directly from thin tissue sections. Intact tetrameric hemoglobin (64 kDa) and trimeric reactive intermediate deiminase A (RidA, 43 kDa) were detected. Improvements in signal-to-noise of between 1.5X and 12X were observed for these protein assemblies on integration of FAIMS.

In native mass spectrometry (MS), noncovalent interactions that were present in solution-phase are maintained in the gas-phase, enabling structural information to be inferred.¹ Ion mobility spectrometry (IMS) has played an important role in establishing native mass spectrometry as a powerful tool in the analysis of protein complexes.^{2–6} In most cases, IMS-MS offers the ability to derive collision cross sections (CCSs), delivering insight into tertiary and quaternary structure, in addition to providing information regarding stoichiometry in the case of protein assemblies or complexes. The structural information gleaned from native MS is complementary to that obtained from established biophysical techniques such as X-ray crystallography.⁴ Classical drift tube IMS (DTIMS),^{7–9} and the commercially available traveling wave ion mobility spectrometry (TWIMS)^{5,10} and trapped ion mobility spectrometry (TIMS)^{11,12} all offer the ability to measure CCS. The latter has been evaluated for native MS analysis using ubiquitin.¹³

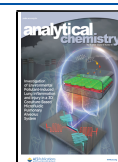
IMS also offers improvements in signal-to-noise (S/N) by enabling ions of differing mobility to be separated from one

another prior to mass analysis. High-field asymmetric waveform ion mobility spectrometry (FAIMS)¹⁴ has shown particular promise in this regard. FAIMS separates gas-phase ions on the basis of their differential mobilities in high and low electric fields. The ions are passed by a carrier gas between parallel electrodes to which an asymmetric waveform is applied. The amplitude of the waveform is the dispersion voltage (DV) (giving rise to the dispersion field (DF)). As a consequence of their differential mobility, ions stray from their original trajectory, an occurrence which is corrected for by applying a dc compensation voltage (CV). By tuning the CV, it is possible to selectively transmit ions through the FAIMS

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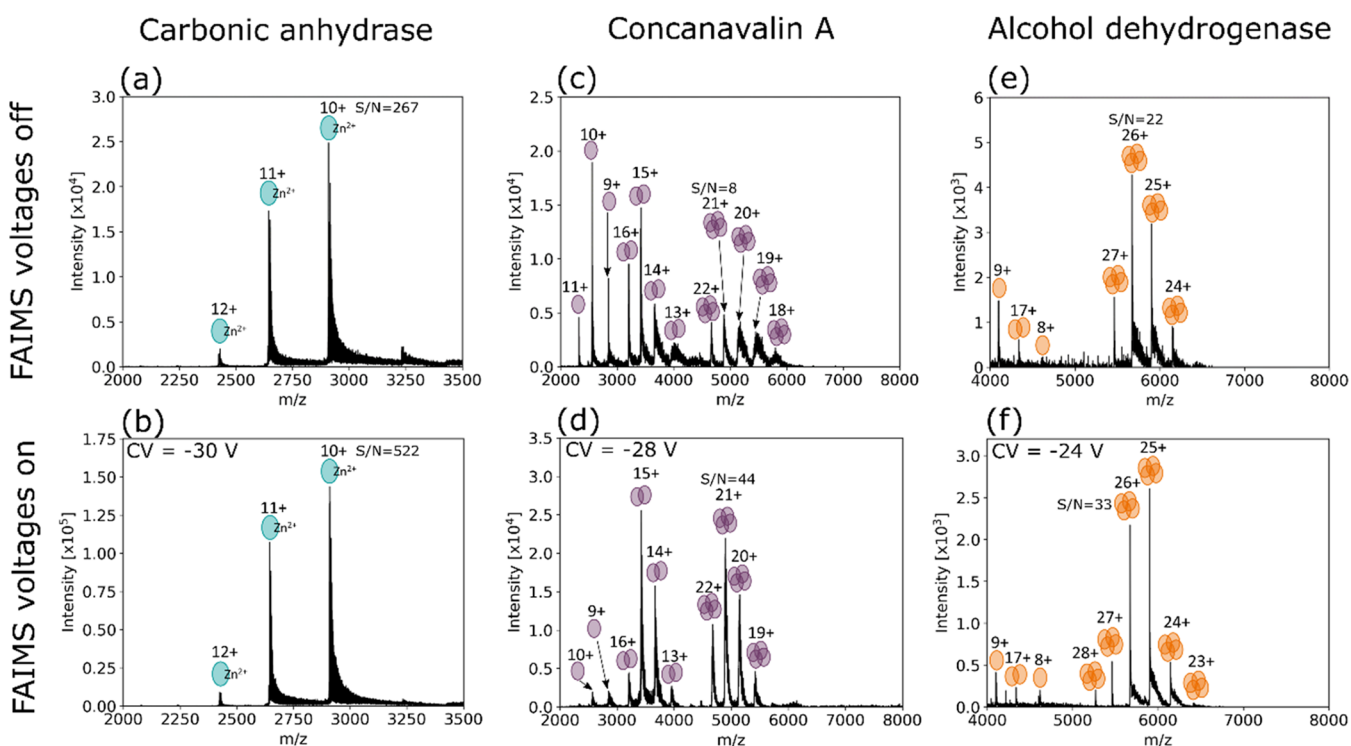


Figure 1. (a) Mass spectrum of carbonic anhydrase acquired with FAIMS voltages off; (b) FAIMS mass spectrum of carbonic anhydrase acquired with a static CV of -30 V; (c) mass spectrum of concanavalin A acquired with FAIMS voltages off; (d) FAIMS mass spectrum of concanavalin A acquired with a static CV of -28 V. Monomer, homodimer, and homotetramer ions were detected both with and without FAIMS; (e) mass spectrum of alcohol dehydrogenase acquired with FAIMS voltages off; (f) FAIMS mass spectrum of alcohol dehydrogenase acquired with a static CV of -24 V. Monomer, homodimer, and homotetramer ions were detected both with and without FAIMS.

device. FAIMS offers a more orthogonal mode of separation to mass spectrometry than other ion mobility techniques.^{15,16}

To date, it has not been possible to derive CCS from FAIMS measurements; however, as mentioned above, FAIMS offers significant advantages in terms of improved S/N. Much of our current interest in FAIMS derives from the improved S/N of protein signals detected following direct sampling of biological substrates, including dried blood spots, bacterial colonies, and tissue.^{17–21} In those works, FAIMS MS was coupled with liquid extraction surface analysis (LESA) using denaturing solvents, i.e., the proteins detected were intact but unfolded. Improvements to S/N have been demonstrated with both FAIMS and TWIMS for desorption electrospray ionization (DESI) imaging of proteins.^{22,23} Kelleher and co-workers have also recently demonstrated the benefits of FAIMS for the analysis of intact (but unfolded) proteins.²⁴

More recently, we have been developing native LESA MS in which folded proteins and protein assemblies are extracted directly from thin tissue sections and dried blood spots.^{25–27} A key benefit of LESA is the reduced (often absent) requirements for sample preparation. In the case of native LESA, the benefits include the potential for integrated structural and spatial information. An obvious question to pose is: Can the benefits of FAIMS be applied to native MS and native LESA MS? Native LESA MS, in particular, is characterized by noisy mass spectra, a consequence of the omission of washing protocols in order to avoid disrupting protein structure and use of aqueous extraction solvents. Improvements in S/N would therefore be most welcome.

In addressing the above question, a key consideration is field heating. That is, FAIMS employs high electric fields meaning

that ions experience collisional heating. This phenomenon was first considered by Purves et al., who calculated that the average increase in temperature experienced by ubiquitin ions was ~ 7 K when the DV was 4.4 kV.²⁸ Later work by Shvartsburg et al., in which they argued that the maximum increase in temperature was more relevant when considering the effects of field heating, found the maximum temperature increase to be ~ 50 K for ubiquitin ions (DV = 4 kV).¹⁶ Field heating is inversely proportional to the square of CCS,²⁹ and has been shown to be enhanced when lighter carrier gases such as He or H₂ are employed.^{30–33} Robinson et al. have shown that field heating effects may be mitigated by cooling the carrier gas.¹⁵

Field heating clearly has the potential to be detrimental to the structural analysis of protein ions by causing unfolding. Nevertheless, Shvartsburg et al. have demonstrated the transmission of noncovalent dimers of bovine serum albumin through a FAIMS device.³⁴ Bovine serum albumin typically exists as a monomer but is known to undergo aggregation under stress conditions. The dimers of BSA observed in that experiment cannot therefore be considered “native”, despite the maintenance of noncovalent interactions. In other work, conformers of monomeric β_2 -microglobulin have been studied by FAIMS MS and correlated with circular dichroism spectroscopy and NMR spectroscopy.³⁵ These studies suggest that FAIMS may be compatible with native MS. To address this issue, here we have considered the FAIMS MS of well-established protein standards including Zn-bound carbonic anhydrase and the homotetramers concanavalin A and alcohol dehydrogenase. We have also coupled FAIMS MS with native LESA of heterotetrameric hemoglobin and homotrimeric

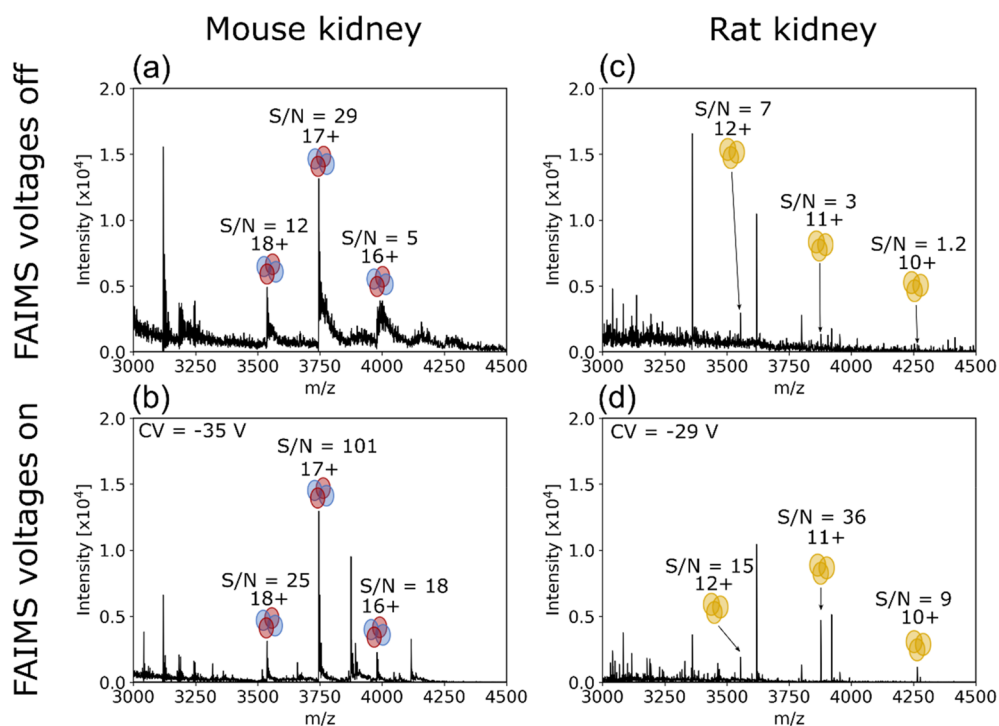


Figure 2. (a) LESA MS spectrum from a location on the mouse kidney section showing hemoglobin tetramer ions (16^+ to 18^+) detected with FAIMS voltages off. (b) With FAIMS voltages on (static CV = -35 V), the S/N for each Hb charge state was increased 2–3-fold with an obvious reduction in baseline signal intensity. Mass spectra are the average of 1 min of acquired data. (c) LESA MS spectrum of a location in the cortex of rat kidney section with FAIMS voltages off. Peaks corresponding to the homotrimeric RidA complex in the 10^+ to 12^+ charge states were detected. (d) LESA FAIMS mass spectrum (CV = -29 V) of an adjacent location in the cortex. S/N was increased and baseline signals reduced for the three charge states with FAIMS voltages on. Spectra are the average of 94 scans with a maximum injection time of 500 ms.

reactive intermediate deiminase A (RidA) from mouse and rat kidney tissue. In all cases, the intact protein complex or protein assemblies were transmitted through the FAIMS device suggesting that field heating does not disrupt noncovalent interactions governing quaternary structure and ligand binding or at least not to the extent that dissociation occurs. The results also demonstrate improvements in S/N for these protein assemblies.

EXPERIMENTAL SECTION

Detailed descriptions of the methods are provided in the [Supporting Information](#). In brief, samples of protein standards carbonic anhydrase (CAH), concanavalin A (ConA), and yeast alcohol dehydrogenase (ADH) were prepared in ammonium acetate solution. Thin tissue sections of mouse and rat kidney were prepared at $10\ \mu\text{m}$ thickness and thaw mounted onto glass slides. Experiments were performed on an Orbitrap Eclipse mass spectrometer (Thermo Fisher, San Jose, CA) equipped with a FAIMS Pro device and the extended mass range option allowing detection of ions up to m/z 8000. FAIMS Pro features optimized gas flows for improved ion transmission and an electrode gap of 1.5 mm, which shortens ion residence time versus previous FAIMS devices.³⁶ Samples of CAH, ConA, and ADH were introduced by nanoESI and thin tissue sections were sampled by liquid extraction surface analysis (LESA), both using the Triversa Nanomate platform (Advion, Ithaca, NY). The dispersion voltage (DV) was fixed at -5 kV (corresponding to a dispersion field of -33.3 kV/cm). Compensation voltage (CV) scans for optimization of transmission of each analyte were performed for the range -60 V to -10 V in steps of 1 V per scan. Subsequent static FAIMS

analyses were performed, in which the optimum CV (chosen for transmission of charge states observed in the absence of FAIMS) was applied. For mass spectra acquired with FAIMS voltages off, the DV and CV were set to 0 V. All other settings were unchanged.

RESULTS AND DISCUSSION

Samples of carbonic anhydrase (CAH), concanavalin A (ConA), and alcohol dehydrogenase (ADH) in ammonium acetate solution were analyzed by direct infusion nESI FAIMS mass spectrometry. As described below, each protein sample exhibited narrow charge state distributions, indicative of retention of intramolecular interactions, and quaternary structure was maintained (as evidenced by m/z values corresponding to protein–ligand complexes or protein assemblies). In each case, the optimum CV for transmission was determined by stepping through the CV range -60 V to -10 V at a rate of 1 V per scan. Extracted ion chromatograms revealed both the range of CVs over which the protein complexes were transmitted and the optimum CV for transmission. Subsequent static FAIMS analysis, employing the optimum CV, were performed.

Figure 1a,b shows the mass spectra obtained with FAIMS voltages off and at CV = -30 V for CAH. CAH features a Zn^{2+} cofactor under native conditions (see Protein Data Bank entry 1v9e).^{37,38} The Zn^{2+} cofactor remained bound to CAH (approximately 29.1 kDa, $z = 9^+$ to 12^+) when transmitted by FAIMS. The S/N of the 10^+ charge state of the holo-protein improved by ~ 2 -fold. The holo-protein was transmitted over the CV range -50 to -25 V in charge states 9^+ to 12^+ . The apoprotein was not observed at any CV; see **Figure**

S1, Supporting Information. If the protein was unfolded as a result of heating in the FAIMS device, it would likely not be observed under the static conditions optimized for the holo-protein, the so-called FAIMS self-cleaning mechanism; however, it might be observed at another CV. Figure S1c shows the total mass spectrum obtained following a scan of CVs and the apoprotein is not detected.

ConA (PDB entry 1gkb) was detected as the monomer (approximately 25.7 kDa, $z = 9^+$ to 11^+), homodimer (approximately 51.5 kDa, $z = 13^+$ to 16^+) and homotetramer (approximately 103 kDa, $z = 19^+$ to 22^+), the result of a pH-dependent equilibrium.³⁹ The mass spectrum obtained with the FAIMS voltages off is shown in Figure 1c. CV optimization revealed transmission of tetramer charge states 19^+ to 22^+ over the CV range -44 V to -15 V (Figure S2, Supporting Information). The FAIMS mass spectrum shown in Figure 1d was obtained with a static CV of -28 V, providing balanced transmission for the four charge states. At this CV, the S/N of the 21^+ charge state of the tetramer improved by over 5-fold. Monomer units with a narrow charge state distribution were also detected both with and without FAIMS voltages, indicative of their presence in solution rather than a gas-phase dissociation product. At the static CV of -28 V, the monomer ions were poorly transmitted; monomer transmission was optimal at a CV of approximately -36 V.

ADH (PDB entry 4w6z) was detected in its homotetramer conformation (approximately 147 kDa, $z = 23^+$ to 28^+) over the CV range -40 V to -15 V (Figure S3, Supporting Information). Figure 1e shows the mass spectrum obtained with the FAIMS voltages off, and Figure 1f shows the static FAIMS mass spectrum obtained with CV = -24 V (selected for transmission of tetramers over a range of charge states). The S/N of the 26^+ charge state of the tetramer increased by 50% at this CV. Ions with m/z corresponding to the homodimeric conformation ($z = 16^+$ to 18^+) were detected with FAIMS voltages on and off, suggestive of a degree of in-solution or in-source dissociation unrelated to FAIMS voltages.

Subsequent experiments focused on integration of native LESA mass spectrometry with FAIMS. To assess the potential of native LESA FAIMS MS for analyzing protein assemblies directly from tissue, sections of mouse and rat kidney were sampled. The renal pelvis (see Figure S4a, Supporting Information) of the mouse kidney section, which is characterized by abundant vasculature, was sampled using ammonium acetate solution (200 mM) + 5% MeOH. These sampling conditions are known to be suitable for detection of the hemoglobin heterotetramer $(\alpha\beta^{2H})_2$ (PDB entry 3hrw).^{25,27} Intact hemoglobin tetramer $(\alpha\beta^{2H})_2$ (63.6 kDa) ions were observed, i.e., were successfully transmitted by the FAIMS device. (Protein assignment was based on mass measurement.) The CV was optimized for transmission of 17^+ ions of the tetramer (see Figure S5, Supporting Information), and found to be -35 V. The LESA mass spectra with FAIMS voltages off and static FAIMS voltages on (CV = -35 V) are shown in Figure 2a,b, respectively. $(\alpha\beta^{2H})_2$ ions were also detected in charge states 16^+ and 18^+ . S/N was improved by approximately 2–3-fold for each charge state using the static CV while absolute signal intensities remained similar. In addition, heterodimer ions $(\alpha\beta^{2H}, 10^+)$ were detected over the CV range -40 to -16 V and holo-alpha subunit $(\alpha^H, 5^+)$ ions were detected over the CV range -45 to -11 V, both also remaining bound to the heme ligand(s) (see Figure S5, Supporting Information). Heterodimers and heme-

bound monomers are commonly observed in native LESA experiments^{25,40,41} (in the absence of FAIMS) and are the consequence of either physiological or in solution dissociation.^{42,43}

LESA sampling of the rat kidney tissue was performed in the cortex region (see Figure S4b, Supporting Information) using an extraction solvent comprising ammonium acetate solution (10 mM) containing 0.125% C8E4 detergent. This combination of sampling conditions and location has previously been shown to result in the detection of ions corresponding to the homotrimer reactive intermediate deiminase A (RidA).²⁶ The homotrimer ions of RidA (42.6 kDa, PDB entry 1qah) were successfully transmitted through the FAIMS device and observed in charge states 9^+ through 12^+ (protein assignment was based on mass measurement). Extracted ion chromatograms for charge states 10^+ , 11^+ , and 12^+ are shown in Figure S6, Supporting Information. The optimum CVs for transmission of these charge states were CV = -24 V, -27 V, and -32 V, respectively. A CV of -29 V was selected for subsequent LESA static FAIMS analysis to enable transmission of multiple charge states. The LESA mass spectra obtained from the rat kidney cortex with FAIMS voltages off and on (CV = -29 V) are shown in Figure 2c,d. For the 12^+ charge state, S/N improved ~ 2 -fold with the FAIMS on and the absolute signal intensities were similar. For the 11^+ and 10^+ charge states, S/N improved between 7- and 12-fold with concomitant increases in absolute signal intensities.

For all the species described above, both the protein standards and the endogenous proteins sampled directly from tissue, the detection of the intact protein complexes in their native states, i.e., their established oligomeric forms in the case of the protein assemblies (homotetrameric ADH and ConA, heterotetrameric Hb, and homotrimeric RidA) and Zn-bound CAH, confirm that the FAIMS Pro is suitable for integration in native mass spectrometry. Any FAIMS-associated field heating is insufficient to cause dissociation of the protein complexes. The ions considered here are much larger than those previously considered in field heating experiments and, with the exception of CAH, have higher charge states. For ADH and ConA, monomer and dimer subunits were observed both with and without FAIMS indicating that they are present in solution rather than a consequence of heating in the gas-phase. For CAH, the holo-protein was observed exclusively. A scan of CV values designed to address the possibility of self-cleaning revealed no evidence for the apoprotein.

CONCLUSIONS

Intact protein complexes can be selectively transmitted by FAIMS prior to mass analysis, as evidenced by detection of protein assemblies up to 147 kDa. Improvements in S/N following integration of FAIMS were observed for all proteins studied here. Transmission through the FAIMS device suggests that any field heating induced by the device is insufficient to disrupt noncovalent interactions governing quaternary structure and ligand binding. Additionally, this is the first demonstration of native LESA FAIMS-MS for intact protein complexes sampled directly from tissue. Hemoglobin tetramer ions remained assembled and were separated from dimer and monomer units on the basis of their differential mobility. Trimeric RidA ions also remained intact. For complex samples analyzed with minimal sample preparation, such as thin tissue sections, FAIMS now presents an opportunity for improving

native protein ion signals by reduction of chemical noise, an advantage previously shown for denaturing LESA MS.^{18,20,21}

■ ASSOCIATED CONTENT

SI Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.analchem.0c00649>.

Experimental details; Figure S1, expanded m/z range showing the 10^+ ion of holo-CAH obtained with the FAIMS voltages off, under static FAIMS conditions (CV = -30 V), and following a CV sweep from -60 V to -10 V; Figure S2, extracted ion chromatograms for ConA tetramers; Figure S3, extracted ion chromatograms for ADH tetramers; Figure S4, photographs of mouse and rat kidney sections; Figure S5, optimization of CV for transmission of hemoglobin ions; and Figure S6, optimization of CV for transmission of rat Rida ions (PDF)

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Notes

The authors declare no competing financial interest. Supplementary data supporting this research is openly available from the University of Birmingham data archive at DOI: 10.25500/edata.bham.00000476.

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