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High-Precision, Gas-Phase Hydrogen/Deuterium Exchange Kinetics by Mass Spectrometry Enabled by Exchange Standards

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Abstract

Mass spectrometry (MS) has become a primary tool for identifying and quantifying biological molecules. In combination with other orthogonal techniques, such as gas-phase hydrogen/deuterium exchange (gHDX), MS is also capable of probing the structure of ions. However, gHDX kinetics can depend strongly on many factors, including laboratory temperature, instrumental conditions, and instrument platform selection. These effects can lead to high variability with gHDX measurements, which has hindered the broader adoption of gHDX for structural MS. Here we introduce an approach for standardizing gHDX measurements using co-sampled standards. Quantifying the exchange kinetics for analytes relative to the exchange kinetics of the standards results in greater accuracy and precision than the underlying absolute measurements. The standardization was found to be effective for several types of analytes including small molecules and intact proteins. A subset of analytes showed deviations in their standardized exchange profiles that are attributed to field heating and the concomitant conformational isomerization. Inclusion of helium during the gHDX process for collisional cooling helps mitigate such variations in exchange kinetics related to ion heating. We anticipate that the outcomes of this research will enable the broader use of gHDX in MS-based workflows for molecular identification and isomer differentiation.

INTRODUCTION

Mass spectrometry (MS) is one of the most widely used techniques for analyzing biological molecules. Since MS by itself is blind to stereochemistry and cannot readily resolve structural isomers, new MS-coupled techniques are needed to thoroughly characterize complex molecules. Tools complementary to MS such as ion mobility spectrometry (IMS), where structural isomers can be resolved by their mobility in an inert gas, or ion spectroscopy to probe gas-phase structure, have emerged for this purpose^{1–5}. The strength of IMS and ion spectroscopy is that they measure intrinsic properties of the analyte, which enables the construction of a database of properties that are platform independent and can subsequently be used for identification. For example, raw IMS data are converted to

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collision cross sections (CCS), a platform independent property of a specific molecule and the gas used for the measurement^{6–11}.

Gas-phase hydrogen/deuterium exchange mass spectrometry (gHDX-MS) has emerged as an orthogonal MS-coupled technique with broad applications in a wide range of biological analytes^{12–18}. The desolvated analyte ion is incubated with a deuterated exchange reagent (e.g. deuterated ammonia, ND₃), to enable exchange of the labile, heteroatom-bound hydrogens and the resulting incorporation of deuterium is monitored by the shift in the mass envelope. Unlike solution-phase HDX, no significant exchange occurs after the gHDX reaction and therefore there is no post-exchange loss of deuterium ('back-exchange'). The kinetics and extent of exchange can reveal information about the structure of the molecule¹⁹ and resolve otherwise indistinguishable isomeric species²⁰. gHDX can be performed in a Traveling Wave Ion Guide (TWIG) on a fast time scale (μ s - ms)^{21,22}, and has shown great promise for resolving isomeric compounds, particularly isomeric carbohydrates^{16,20}. Unlike CCS and spectroscopic measurements, the kinetics from gHDX are highly platform-dependent and easily biased by sampling conditions even within the same instrument^{20,21}.

Here we introduce an approach for minimizing variability and ensure reproducible gHDX measurements that relies on exchange standards that are sampled under the same experimental conditions as the analyte. The deuterium incorporation of the analyte is reported relative to the deuterium incorporation of the exchange standards. This approach shares some similarities with the use of a derivatized oligosaccharide to standardize the extent of deuterium incorporation during in-source HDX of metal-adducted carbohydrates²³ and peptides as exchange reporters to check for consistent labeling conditions^{17,20}, but to date, no gas-phase HDX standards have been established for experiments performed over a wide range of time scales or for analytes with a wide range of exchange rates. Here we develop and evaluate a robust internal exchange reporter that can be used as a standard for gHDX across the timescales achievable in a TWIG. We identify a set of three compounds that provide a robust exchange standard that, when co-sampled along with an analyte, effectively account for offsets in exchange kinetics. We also observed that the exchange standards enabled reproducibility for gHDX not only with deuterated ammonia (ND₃), but showed promise with other deuterating reagents including deuterated methanol (MeOD) and deuterium oxide (D₂O). Quantifying the exchange kinetics for analytes relative to the exchange kinetics for standards results in greater accuracy and precision than the underlying absolute measurements, which in turn increases the utility of gHDX for molecular identification or structural analysis.

MATERIALS AND METHODS

Reagents

Deuterated-ammonia (ND₃ 99%) and deuterated-water (D₂O 99.99%) were purchased from Cambridge Isotope Labs (Tewksbury, MA, USA). Deuterated-methanol (MeOD 99.5%) was purchased from Sigma Aldrich (St. Louis, MO, USA). Diisopropanolamine (DIPA), myoglobin from equine skeletal muscle, and arginine (Arg) were purchased from Sigma Aldrich. Tris, bis-tris methane (BTM) and bis-tris propane (BTP) were purchased from Fischer Scientific (Fair Lawn, NJ, USA). P1 peptide (HHHHHHIHKIK) with c-terminal

amidation was obtained from Anaspec (San Jose, CA). Galacto-N-biose (GalNb) was purchased from Carbosynth (Compton, UK).

Mass Spectrometry

Instrumental modifications to the Waters Synapt HDMS Q-TOF mass spectrometer to enable infusion of ND₃ have been described previously^{17,20,21}. Mixtures of helium (He) and ND₃ were achieved by using two flow controllers (Aalborg, Orangeburg, NY) and mixing the gasses just prior to the gas inlet to the transfer cell with a stainless-steel tee mixer. Analytes were resuspended in Optima LC-MS grade water from Fischer Scientific (Hampton, NH, USA) and diluted to a working concentration of 10–200 µM in aqueous 0.1% formic acid. Myoglobin from equine skeletal muscle was prepared for MS analysis as previously described¹¹. P1 peptide was prepared at a concentration of 1.5 µM in 99% isopropanol with 0.1% formic acid. Exchange standards: Tris (200 µM), BTM (20 µM), and BTP (20 µM) were infused through the secondary (lock mass) inlet at 10 µL/min in aqueous 0.1% formic acid; this solution will be referred as “TrisMix”. A series of acquisitions were collected using a transfer collision energy of 5 V, wave heights of 4, 8, 12 V and multiple traveling wave velocities (8 – 512 m/s) to sample a temporal- range of 0.12 to 9.9 ms (see Fig. S1). Unless specified all other MS settings were: source temperature 100 °C, capillary voltage 2.5 kV; sampling cone voltage 30 V; extraction cone voltage 3.3 V; trap CE 5 V; trap bias 8 V; IMS TW velocity 100 m/s; IMS TW height 5 V; RF confining voltage 350 V; transfer TW height 8 V. The IMS TWIG was supplied with a constant flow of nitrogen at 5 mL/min to ensure a higher pressure relative to the transfer TWIG and prevent any bleed of ND₃ from the transfer TWIG into the IMS TWIG. Each exchange standard was co-sampled with GalNb, myoglobin, P1 peptide, arginine, and DIPA under various gHDX conditions. All samples were collected in triplicate. Despite the relatively high concentrations infused in the TrisMix we did not observe any degradation of signal or fouling of the ion source even after 12 hours of data collection. No other precautions beyond flushing the lines with 0.1% formic acid, methanol, and water along with daily cleaning of the outer source cone were needed. Data was batch converted using scripts in UniDec²⁴ and deuterium uptake was analyzed using HX-Express v2²⁵.

To perform gHDX-MS experiments using deuterated water or methanol we constructed a “reagent oven” (Figure S1). It consisted of a fine leak valve, (part number: 315002 MDC Vacuum Products, Haywood, CA), a temperature controller, (part number: 4314K2 McMaster-Carr, Chicago, IL), J-type thermocouple, (part number: 9251T91 McMaster-Carr) and a 0.5” diam. X 2” long 250 W cartridge heater, (part number: 35025K315 McMaster-Carr) (see Figure S2). The center of the chamber holds a glass ampule containing 300 µL of the liquid exchange reagent (D₂O or MeOD). Attached to the lid was an adjustable fine leak valve that allowed exchange reagent vapor to enter the mass spectrometer. The cartridge heater and thermocouple were wired to a temperature controller which heated the aluminum assembly to a specified and regulated steady temperature (± 1 °C, to a maximum of 300 °C). The leak valve was manually adjusted to achieve the desired readout pressure in the IMS cell (0.20, 0.10, 0.02 torr), and equilibrated for at least 15 minutes before collecting measurements. For off exchange reactions, the TrisMix and the arginine samples were

prepared in 99% D₂O with 0.1% formic acid, and the reagent chamber was loaded with 300 μ L of Optima grade methanol (Fisher Scientific).

RESULTS AND DISCUSSION

We first sought to identify suitable exchange standards that effectively sample the full range of time scales ($\sim 0.1 - 10$ ms) achievable in a TWIG using ND₃. Through a screening of various amino acids, peptides, and common small molecule reagents we identified three compounds: tris, bis-tris methane (BTM) and bis-tris propane (BTP) that ionized well, and exchanged on relatively fast, medium, and slow timescales, respectively (Fig. 1). To test how gHDX of these compounds are sensitive to exchange conditions, the flow of ND₃ infused into the transfer TWIG was adjusted from 0.5 to 4 mL/min, which span the lower and upper limits imposed by the mass flow controller and vacuum system, respectively. All three compounds responded as expected, exhibiting faster exchange with higher pressures of ND₃¹⁷. At high pressures, Tris was exchanging so quickly that even by the early time points its mass was shifted by 6.03 ± 0.03 Da, the expected maximum based on the six labile protons in the molecule (Fig. 1A). BTM displayed a gradual exchange profile starting at much lower levels of deuteration (Fig. 1B). Only under the highest ND₃ pressure at the longest time point was it fully deuterated (6.01 ± 0.02 Da; corresponding to the number of labile protons). BTP had the slowest exchange kinetics, with no exchange at the shorter time points (Fig. 1C). Even at the highest pressure and longest time point BTP was not fully deuterated (7.69 ± 0.02 Da, significantly less than the nine exchangeable protons).

We next examined whether the exchange kinetics of the three compounds were influenced by the potentials applied to ion optics prior to the trap TWIG and any concomitant activation. Tris and BTM showed no difference in exchange kinetics when pre-activated by elevated cone voltage or collisional activation in the trap TWIG, prior to gHDX (Fig S1A). For BTP, under very gentle source conditions (cone V = 20) with no subsequent activation, a slight increase in exchange kinetics was observed for the early time points. With higher activation, either through elevation of the cone voltage (to 30 V or higher) or the trap collision energy (CE) led to indistinguishable gHDX kinetics. Raising the trap TWIG bias from 8 V (default) to 22 V, shown to cause ion activation²⁶, had little influence on the gHDX kinetics and only a very subtle increase in the exchange of Tris was observed with the elevated trap bias (Fig. S2B). Adjustment of the TW height in the transfer cell led to no changes in exchange kinetics for Tris or BTM. Only when the TW height was lowered to 4V did BTP show slightly slower gHDX at the longest time points (Fig. S2C). Lowering the RF confining voltage from 350 (default) to 100 V led to both Tris and BTM having slightly less exchange at the higher voltage (Fig. S2D). Lastly, we examined the influence on the TW velocity setting in the IMS cell as the speed of the ions exiting from the IMS cell into the transfer TWIG will potentially alter their residence time. Only at IMS TW velocities below 100 m/s is there an increase in exchange for all compounds sampled (Fig. S2E). Overall, this systematic test identifies which MS parameters influence gHDX in the transfer TWIG and the minimal level of pre-activation that is necessary for robust exchange profiles for the TrisMix standards.

The results (Figure 1A–C and S1) for these compounds, which will be collectively referred to as ‘TrisMix’, suggest that they have excellent properties as standards for gHDX. To evaluate this possibility, we measured deuterium incorporation for diisopropanolamine (DIPA) co-sampled with TrisMix using the same reaction times and pressures of ND₃. Figure 1D shows that DIPA is fully deuterated after the longest reaction time (+4.03 ± 0.02 Da, consistent with four labile protons) and that the exchange rate depends on the pressure of ND₃ (Fig. 1D). In order to compare the results for DIPA with the those for the TrisMix standards, the deuterium exchange of DIPA (EX_{DIPA}, Fig. 1D) was plotted as a function of the sum of the deuterium exchange by each of the TrisMix standards (EX_{TrisMix}, Fig. 1E). To visualize the standardized HDX data the DIPA exchange (y axis) is plotted relative to the co-sampled TrisMix exchange (x axis), rather than time (Fig. 1F). This plot shows that the relationship between EX_{DIPA} and EX_{TrisMix} is very strong, despite the wide range of reaction time and pressure of ND₃ used for the underlying measurements. Therefore the “exchange profile” of DIPA relative to TrisMix (“DIPA_(ΣTrisMix)”) illustrates the potential of using exchange standards to standardize gHDX measurements by controlling for many factors that affect the absolute rates of gHDX. Furthermore, summing the deuterium incorporation by each of the three compounds in TrisMix enables a single quantity to capture a wide temporal range of exchange kinetics. For example, the BTP alone would not be able to report on exchange conditions below 0.25 ms, whereas Tris alone would not be able to accurately report on the exchange conditions longer than 1 ms.

Having a promising panel of exchange reporters, we next tested this approach to standardize gHDX measurements for a variety of other analytes including proteins, peptides, carbohydrates, and amino acids. All data collected were co-sampled with TrisMix at two flowrates of ND₃. Myoglobin, a small protein, was present in the spectra as several charge states, all of which showed different levels of deuterium exchange (Fig. 2A). The charge-state dependence on exchange has been observed on several protein and peptide systems using gHDX on a similar platform¹⁷. Just as with the small compounds, the offset of the ND₃ pressure had a stark effect on the exchange kinetics of myoglobin. Standardization of the data to the TrisMix effectively corrects the offset and the resulting gHDX profiles are consistent for each charge state observed (Fig. 2A, right panel).

Application of the standardization approach to other classes of analytes revealed some limitations. Galacto-N-biose (GalNb), a disaccharide, also displayed gradual exchange that was dependent on the ND₃ pressure (Fig. 2B). When standardized relative to the TrisMix, only the portion of the exchange profile corresponding to the shorter time points was consistent for the two conditions. At longer exchange times there is more exchange with the higher ND₃ flow. A similar discrepancy was observed when examining the exchange of a peptide (P1: HHHHHHIKIK) (Fig. 2C). After standardization, the later portion of the exchange profiles are very consistent, but the region corresponding to the short time points deviate. Most interestingly, when the same study was performed using an amino acid, arginine, the standardized gHDX profiles of the two conditions were not consistent (Fig. 2D).

Protonated arginine was investigated at additional sampling conditions to examine what was causing the changes in gHDX kinetics. Pre-activation through either elevated cone voltage,

trap CE, or trap bias visibly altered the exchange kinetics of Arg (Fig. S2A–C). The transfer TWIG wave height also offset the kinetics more than what was observed for any of the tris compounds (Fig. S2D). Since arginine is known to adopt multiple conformers with a difference in the site of protonation^{27–31}, it is likely that the anomalous changes in gHDX kinetics at different conditions are attributed to ion activation shifting the conformational equilibrium. Field heating within the TWIG has been shown to drastically change the effective ion temperature, especially for smaller analytes, resulting in conformational isomerization²⁶. The field heating was most prominent at relatively low TWIG pressures, similar to what was used here for gHDX³². Furthermore, the sites of protonation (‘protomers’) are known to alter gHDX kinetics, and the barriers to proton migration are usually lower than dissociation^{33–36}.

The arginine dataset illustrates an example where the internal TrisMix standard does not get offset by the sampling conditions (e.g. ND₃ pressure) in the same way as the analyte. With some analytes, likely those with multiple protonation sites that are close in energy, the altered ND₃ pressure may lead to structural perturbations that alter gHDX kinetics, while the co-sampled TrisMix is structurally unaffected, ultimately resulting in a change to the standardized gHDX profile. While this may be a limitation for standardizing gHDX profiles, it also presents a useful tool for discerning changes in ion conformation. Including the standard can provide a way to resolve whether a change in gHDX kinetics was due to systematic changes in exchange conditions (transit time, ND₃ pressure), or a perturbation in the analyte ion’s conformational state.

Minimizing ion isomerization by infusing helium

Since the extent of ion heating is influenced by the pressure and the identity of the neutral gas in the TWIG^{37,38} we sought to test whether a mixture of gasses could be used for gHDX to minimize unintended activation and isomerization. The addition of helium, which has a small mass and low polarizability, increases the total pressure and contributes to improved ion thermalization²⁶ during the gHDX reaction. The gas plumbing was modified to include two mass flow controllers whose outputs (helium and ND₃, respectively) were mixed immediately before entering the transfer TWIG. As a first test, the exchange profiles of the TrisMix were monitored using a flow of either 1 mL/min ND₃, or 2 mL/min of a 50/50 mix of ND₃/He. This results in an elevated TWIG pressure, but nearly an equivalent partial pressure of ND₃. In all cases there was remarkable consistency in the exchange kinetics for both conditions, indicating that additional pressure from the He does not influence the exchange of the TrisMix (Fig. S2F). To test whether helium would contribute to improved gHDX standardization, GalNb was sampled using a flow ratio of He:ND₃ at 3:1, 1:1, or pure ND₃. The effect of the different partial pressures of ND₃ is evident on both the exchange of the TrisMix and the GalNb (Fig. 3A, B). The standardized GalNb gHDX profiles over a wide range of time scales are now far more consistent between conditions where helium is included, which show only a slight deviation at the very longest time point (Fig. 3C). Therefore, the standardization approach seems to benefit from including helium in the gHDX reaction, likely through limiting the degree of conformational isomerization from higher levels of heating at lower TWIG pressures.

Standardization of results from experiments using other exchange reagents

To test if the standardization approach would work with other deuterium exchange reagents we utilized a custom reagent oven to infuse stable pressures up to 0.20 torr of either D₂O or MeOD into the Waters Synapt HDMS (Fig. S3). These studies were conducted inside the IMS TWIG rather than the transfer region as it can tolerate much higher pressures and poses less risk to contamination of the TOF region. The wave velocities were varied as described for transfer TWIG experiments to measure gHDX kinetics within the IMS cell. Surprisingly, we found that neither Tris, nor BTM exhibited any exchange with either MeOD or D₂O, even for the longest times and highest pressures. BTP, the compound that showed the slowest exchange in ND₃, was the only one that gradually took up deuterium taking up 5.71 $\pm$ 0.11 Da with MeOD and 1.88 $\pm$ 0.03 Da in D₂O at 0.2 torr and at the longest time point tested (Fig. S4A, B). The lack of exchange with Tris and BTM is likely related to the difference in the intrinsic exchange mechanism with ND₃ vs. MeOD/D₂O and the gas-phase basicity of these compounds compared to the exchange reagents^{15,39}.

Among the other analytes tested only arginine showed any observable deuterium uptake with MeOD and D₂O, exchanging up to 3.11 $\pm$ 0.04 and 0.49 $\pm$ 0.05 Da, respectively. Despite arginine's caveats associated with its multiple conformations in the gas phase, we explored how effective a standardization strategy is for MeOD and D₂O using only BTP as the reference standard. Three different pressures of the exchange reagent were achieved by offsetting the leak valve to a pressure of 0.20, 0.10, and 0.02 torr. For MeOD, both BTP and arginine respond as expected, higher pressures of the reagent lead to faster exchange kinetics (Fig. 4A). Standardizing to the BTP exchange 'Arg_(BTP)' reveals that for the short time points all three pressures line up well. For the 0.02 and 0.10 torr data, the remainder of the plot is also entirely consistent, but the data at the highest pressure, 0.20 torr, deviates for the later time points. While imperfect, the comparisons indicate that using just BTP as an exchange standard for MeOD is promising.

When using D₂O, the BTP responded as expected to the offsets in D₂O pressure, showing faster exchange at higher pressures (Fig. 4B). Arginine also showed slower exchange at 0.02 torr, but surprisingly had similar exchange kinetics at both 0.10 and 0.20 torr. When the arginine exchange was standardized to the BTP, only a portion of the data could be effectively standardized. The data between the 0.1 and 0.02 torr overlay well, except for the extreme longest time point (highlighted portion in the zoomed view), whereas the 0.20 torr data diverges. Overall, the comparisons inform us that the standardization strategy only works in a TWIG well when using lower pressures of D₂O as the exchange reagent.

Lastly, we wanted to test whether standardization works equally well for deuterium-to-hydrogen exchange reactions (off exchange). For this we introduced non-deuterated methanol (MeOH) into the IMS cell and infused fully deuterated solutions of BTP and arginine in D₂O. As expected, the exchange profiles appear as the mirror images of the forward exchange data (Fig. S4C, D). Both BTP and arginine exhibit more deuterium loss at higher pressures of MeOH, and the standardization is effective for the data at the two lower pressures. For the higher pressures, this approach only works for the shorter half of the time points, just as seen for the forward exchange reaction.

Potential for platform-independent gHDX

In general, the deuterium uptake during gHDX depends on four factors: 1) exchange conditions, which reflect the exchange time and pressure of exchange reagent; 2) identity of the deuterated exchange reagent; 3) isotopic enrichment, corresponding to the percent deuteration of the exchange reagent; and 4) the structure and conformation of the analyte. Therefore, gHDX measurements are sensitive to several factors beyond simply the identity of the analyte. With regard to ND₃-mediated gHDX, we have determined the conditions necessary to ensure that no conformational perturbations offset the exchange of the TrisMix so that it reliably measures exchange across a wide range of conditions. The ability to standardize gHDX kinetics provides a way to measure a property of an ion that could be developed into a platform-independent framework for molecular identification and isomer differentiation. Fortunately, gHDX data can be collected on the second or sub-second timescale and collecting a wide range of time points across different exchange conditions is readily feasible using existing technology, even on a chromatographic timescale. At present, we rely on only the centroid of the isotopic mass envelope at each time point for our analysis of deuterium uptake. Future studies will likely benefit from also examining the width and shape of the isotopic distribution, as it will contain more information that may be useful for resolving closely related structures. While we show that BTP also appears promising for tracking exchange conditions with other exchange reagents (MeOD, D₂O), which can have distinct mechanisms and very different timescales of exchange, further studies are warranted to establish the best standards and sampling conditions for gHDX standardization.

We predict that a gHDX database approach will be most useful for carbohydrate and glycan analytes which are highly exchangeable with ND₃ and for which the subtle structural differences among isomers can be distinguished²⁰. Once the standardized gHDX profiles are established, we anticipate that results from only a few select time points will be required to distinguish among the potential structural isomers by comparing against the database (illustrated in Fig. S5). The development of the TrisMix gHDX standard is reminiscent of the developments of CCS calibrants in the ion mobility field. Apart from drift tube IMS (DT-IMS), where careful measurement of pressure, temperature and ion optics allow direct calculation of CCS, all other IM-MS platforms rely on calibrants for accurate CCS determination^{10,40}. The development of such calibrants has since paved the way to construction of CCS databases for a variety of analytes including the recently established GlycoMOB database for carbohydrates^{41–44}.

As illustrated for protonated arginine and P1 peptide, gHDX results can depend on the specific *conformational isomers* present in an experiment, which in turn can depend on the isomers present in solution, the ionization process, and the extent of activation the ions experience prior to gHDX. In order to improve the performance of our approach for the identification of structural isomers, thermometer ions may be very useful for characterizing and/or standardizing the levels of heating^{37,38} and ultimately establishing the most general, platform-independent databases. Additionally, peptide probes that can monitor the degree of internal migration of protons ('scrambling'), which is likely governed by similar heating effects as those which influence structural isomerization, might be useful for assessing levels of ion heating⁴⁵. We find that inclusion of helium into the gHDX reaction helps achieve

more consistent standardization. The increased pressure and additional collisional cooling work to minimize heating and lessens the degree to which the conformational landscape is perturbed. We have previously observed that prior ion activation of carbohydrates can influence their gHDX kinetics²⁰. Interestingly, the same ion activation of monosaccharides also offset the observed mobility and thereby the CCS measurements. Therefore, this caveat of conformational perturbation is not limited to gHDX, but to any tool that depends on the conformational state of an ion, as suggested previously for CCS-based measurements³².

The combination of three compounds instead of a single gHDX standard compound presents additional advantages. Although a single compound would negate the need for three parallel measurements, we were not able to identify any compound that adequately samples deuterium exchange on a wide temporal scale that is unaffected by isomerization effects from field heating. For example, P1 peptide was initially considered as an exchange standard, however its gHDX profile was found to vary highly with ionization conditions making it less suitable for our objectives (data not shown). One additional benefit to using three compounds is that with every condition tested, at least one of the time points will be fully exchanged and can be used to report the quality of the exchange reagent. In our case the ND₃ was rated at 99% deuterated, which is consistent with the mass shift of 6 Da for Tris. However, if the isotopic enrichment had been only 80%, then the decreased maximal deuteration of the Tris would be readily apparent. If data is acquired with suboptimal deuterium enrichment, then the standardized gHDX profiles can be corrected by utilizing the information of the level of deuterium enrichment as reported from the Tris maximal mass shift. For example, if the Tris compound only exchanged 5 Da, we anticipate that it will be possible to re-standardize the data by scaling both the exchange of the standard and the analyte by a factor of 6/5, and thereby recover the same accurate standardized exchange profile.

CONCLUSIONS

The TrisMix exchange standard enables reproducible gHDX-MS measurements for a variety of analytes by correcting for the systematic effects of exchange conditions (Fig. 1). The standardized gHDX profiles reflect a platform-independent measurement that provides a framework for improving the identification of analytes (including structural isomers) using databases of gHDX profiles. For a subset of smaller analytes, the gas-phase conformation is susceptible to perturbation by field heating prior to or during gHDX, which was dampened by including helium in the exchange reaction (Fig. 3). The ability to discern systematic offsets in exchange conditions from changes in conformational states provides a more robust approach for probing the conformational landscapes of ions and a significant step towards enabling gHDX for structural analysis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Example mass spectra of undeuterated (top) and after various exchange times with ND₃ (middle, bottom) for Tris (A), BTM (B), BTP (C) and DIPA (D). Exchange plots for each compound are shown on the right side at three different flow rates of ND₃: 0.5 (blue), 1.5 (purple) and 4.0 mL/min (red) with structures shown. Error bars represent standard deviations from triplicate measurements. E) Sum of each exchange for Tris, BTM, and BTP (ETrisMix) at each ND₃ flow rate. F) The DIPA exchange (y-axis) is shown as a function of total exchange of TrisMix (x-axis) for each flow of ND₃. The DIPA_(ΣTrisMix) profiles appear to be independent of ND₃ flow rate.

Figure 2.

Standardized HDX data of different protonated analytes including myoglobin (A), galacto N-biose (GalNb) (B), P1 peptide (HHHHHHIHKIK) (C), and Arginine (D). Each dataset was collected with the TrisMix at either 1 mL/min (blue) or 2 mL/min (red) flow rate of ND₃. For each analyte the sum of the exchange for Tris, BTM, and BPT (Σ TrisMix) is shown in the left panel, the exchange of the analyte is shown in the middle panel, and the exchange of the analytes relative to the Σ TrisMix are shown in the right panel. For myoglobin three charge states were examined indicated by dashed ($z=11$), dotted ($z=12$), and solid ($z=13$) lines. Highlighted regions for (B) and (C) indicate the portions of the exchange regime that align accurately after standardization.

Figure 3.

Effect of using a mixture of gasses for HDX. HDX data for the TrisMix (A) and galacto N-biose (B) were collected using a helium:ND₃ mixture with flow rates of 1.5:0.5 mL/min (blue), 1:1 mL/min (purple), or pure ND₃ at 2 mL/min (red). C) The exchange plot of galacto N-biose relative to the TrisMix (GalNb($\Sigma\text{TrisMix}$)). The highlighted region indicates the portion the exchange that aligns after standardization. Exchange for individual compounds in the TrisMix are shown in Fig. S2F.

Figure 4.

Application of the TrisMix standard for alternative exchange reagents including deuterated methanol (MeOD) (A) or deuterated water (D₂O) (B) at pressures from 0.02 (blue) to 0.2 torr (red). Exchange plots are shown for BTP (left) and arginine (middle). The exchange data for arginine relative to BTP is shown on the right plot. The boxed region for Arg(BTP) is zoomed in to illustrate the region where standardization is effective (highlighted). Error bars represent the standard deviation from triplicate measurements. Representative mass spectra for BTP and arginine are shown in Fig. S4A–C.