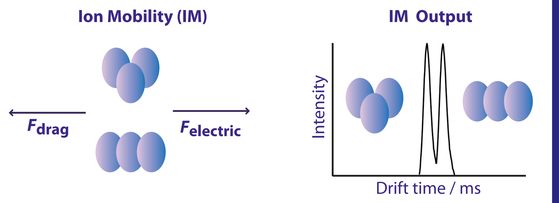
Design and Characterization of a New Ion Mobility Cell for Protein Complexes

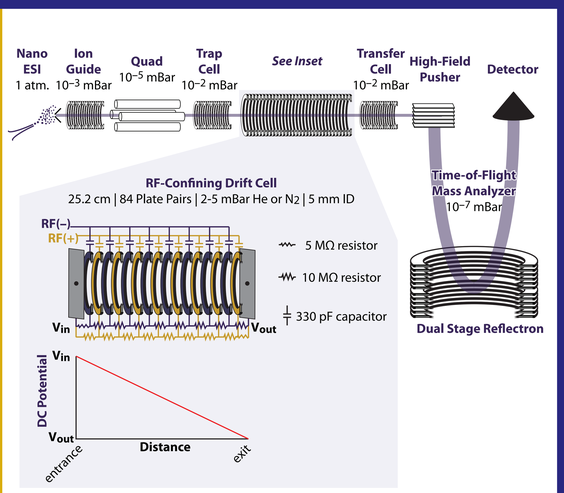
Samuel J. Allen,[1] Samuel T. Marionni,[1] Kevin Giles,[2] Tony Gilbert,[2] Matthew F. Bush[1] | [1]University of Washington | [2]Waters Co.

Ion Mobility

Ion mobility (IM) measurements can provide detailed insights into the structures of protein complexes and other biomolecules. The most widely used IM implementation for proteins uses traveling waves to separate ions based on their mobilities in a gas. Collision cross sections (Ω) from those measurements are determined using calibration with appropriate standards. Here we report a new RF-confining drift tube positioned in a Waters Synapt G2 HDMS, which enables the direct determination of accurate Ω values for *new calibration standards*, *methods validation*, and *other experiments*.



RF-Confining Drift Tubes

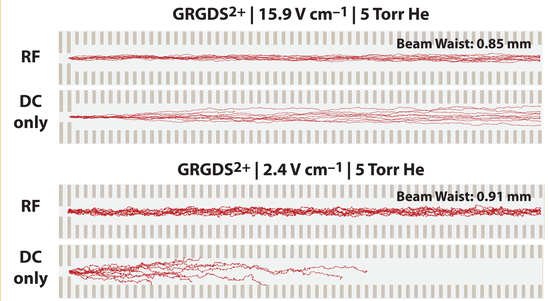


RF-confining drift tubes are stacked-ring ion guides (SRIG) in which alternating RF potentials radially confine ions and superimposed DC potentials establish a uniform electric field along the axis of ion transmission (*Anal. Chem.* **2010**, *82*, 9557-9565). A new RF-confining drift tube has been implemented in place of the traveling-wave ion mobility cell on a Waters Synapt G2 HDMS, which controls all potentials and regulates the gas flow.

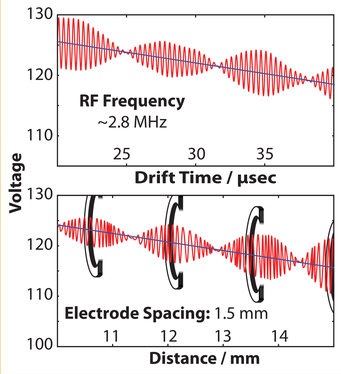
Trajectories

Ion trajectories were simulated using SIMION (Version 8.0) and a hard-sphere approximation for ion-neutral collisions. To reduce computational costs, models used cell lengths and drift voltages that were one-third of those used experimentally. All electrodes have a 2.8 MHz RF component with a 200 V peak-to-peak amplitude. For each simulation, >1000 ions trajectories were run under identical conditions to provide significant population statistics.

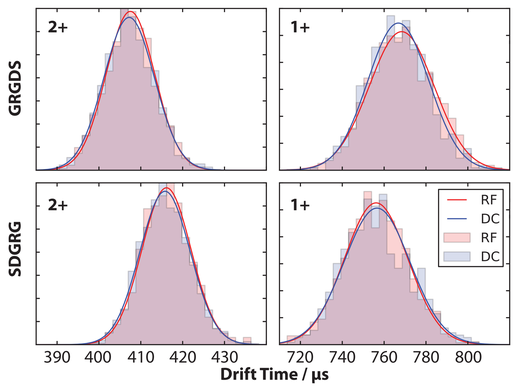
Effects of RF Confinement



RF-confining drift tubes exhibit excellent transmission over a wide range of field energies. Without RF confinement, radial diffusion results in ion losses due to collisions with the ring electrodes or the IM cell exit orifice (2 mm).

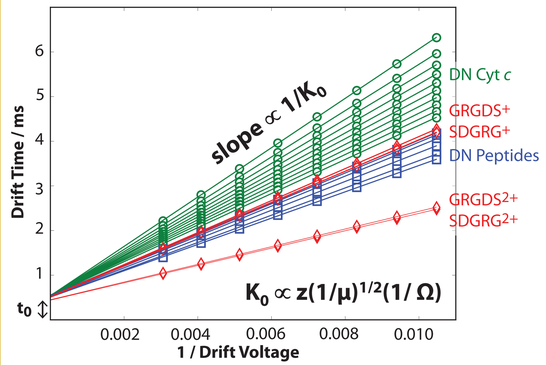


Ion potentials in the RF-confining drift tube include contributions from the RF field (*upper panel*). Modulations in these traces correspond to interference between the phases of adjacent electrodes (*lower panel*). The approximate DC potential is shown in blue as a guide to the eye.



Arrival times were simulated using a model of the RF-confining drift tube (red) and an equivalent drift tube using only DC potentials (blue). Arrival times obtained using the two models are indistinguishable, suggesting that the ***RF component has a negligible net effect on the drift times*** in these experiments.

Experimental Data



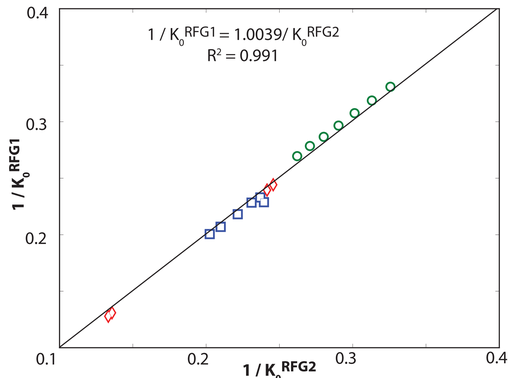
Drift times for denatured peptides (DN Peptides), inverse peptides (GRGDS and SDGRG), and denatured cytochrome *c* (DN Cyt *c*) were measured at 8 drift voltages ranging from 95 - 327 V in 2 Torr of helium gas.

Ω Measured Using the New RF-Confining Drift Tube

	m / Da	z	$\Omega_{Lit.}$ / nm^2	$\Omega_{Exp.}$ / nm^2
GRGDS	490	1	1.32	1.31
		2	1.39	1.42
SDGRG	490	1	1.30	1.28
		2	1.42	1.44
Angiotensin fragment 1-7	898	2	2.26	2.22
RASG-1	1000	2	2.25	2.22
Angiotensin II	1046	2	2.45	2.44
Bradykinin	1060	2	2.37	2.34
Angiotensin I	1296	3	3.28	3.21
Renin substrate	1758	3	3.80	3.75
Enolase T35	1872	3	3.80	3.79
		14	25.2	24.0
		15	26.0	24.8
		16	26.7	25.4
cytochrome *c*	12 k	17	27.4	26.0
		18	28.0	26.6
		19	28.7	27.1
		20	29.2	27.6

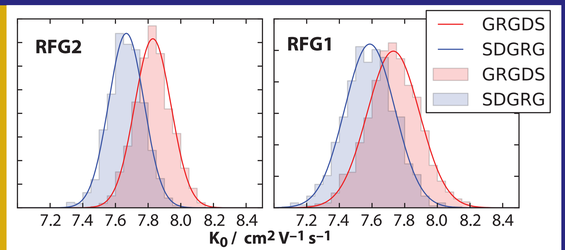
Bush, M. F.; Hall, Z.; Giles, K.; Hoyes, J.; Robinson, C. V.; Ruotolo, B. T. *Anal. Chem.* **2010**, *82*, 9557-9565.

Validation

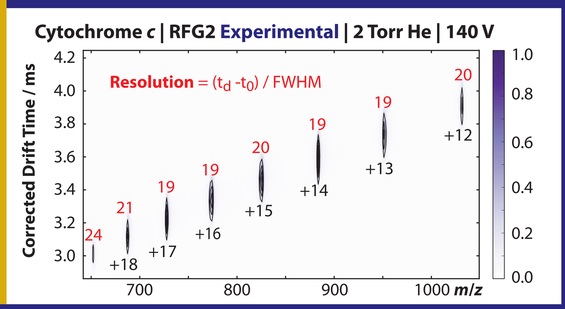


Reciprocal reduced mobilities measured using the prototype 2nd generation RF-confining drift tube (RFG2, this work) are similar to those measured previously using the 1st generation device (RFG1, *Anal. Chem.* **2010**, *82*, 9557-9565). The small differences in measured mobilities are under investigation.

Resolution



Simulated arrival time distributions for GRGDS^{2+} and SDGRG^{2+} are plotted as a function of reduced mobility (K_0). These results suggest that RFG2 will exhibit a 43 % increase in resolution relative to RFG1 (R ≈ 15 typical in most experiments). The increase is similar to that suggested using approximations for analogous DC-only drift tubes. The centroids of the reduced mobilities from the RFG2 and RFG1 simulations are not identical, a result that is under investigation.



Conclusions

A new RF-confining drift tube enables the direct determination of accurate Ω values for new calibration standards, method validation, and other experiments. The new design is expected to result in a ~40 % increase in resolution relative to that of the existing RF-confining drift tube (RFG1), although preliminary data exhibits more modest improvements. Computational models constructed using the ion simulation program SIMION provide insights into the transport properties of ions that experience both DC and RF fields. These results suggest that the drift times measured using RF-confining drift tubes are independent of the RF field.

Future

- Install the new RF-confining drift tube on a Synapt G2 HDMS equipped with a 32k *m/z* quadrupole generator.
- Experimentally characterize the new drift tube using higher pressures and drift voltages.
- Characterize the differences between the 1st and 2nd generation RF-confining drift tubes, and analogous devices without RF confinement, for less-mobile ions.
- Develop new calibration standards and calibration protocols for biomolecules that have a wider range of masses and mobilities.

Thanks

UNIVERSITY of WASHINGTON Research Royalty Fund

Waters THE SCIENCE OF WHAT'S POSSIBLE.™

http://depts.washington.edu/bushlab