IonDX Insider

CORPORATE UPDATE

IonDX Appoints Life Sciences Industry Leader Luis Carbonell as Chief Executive Officer

Mr. Carbonell is a seasoned life sciences industry executive with a broad range of experiences encompassing research tools, clinical diagnostics, and pharmaceuticals. He has navigated small companies through their earliest stages (pre-incorporation and seed funding), as well as led large international expansion projects for multi-billion-dollar corporations.

"I am honored to join such a talented team. IonDX has developed amazing applications for ion mobility that are poised to make a significant impact in the life sciences field, and I am excited to become a part of it.", said Mr. Luis Carbonell.

"IonDX has been a small R&D company and with Mr. Carbonell's leadership we will become a full-fledged scientific instrument company. Just watch." said Dr. Henry Benner, Founder of IonDX.

Visit www.iondx.com for full article.

IonDX receives patent for innovative technology featured in our current product portfolio.

Ion Mobility spectrometer with Center rod

Patent Issued 10/24/2023

US Patent No 11,796,505

IonDX continues to engage in rich collaborative pipeline

Collaboration has been a key focus of IonDX's efforts to engage with scientists and educate the life sciences industry on their innovative technologies.

Recently published jounal articles and presentations at conferences have highlighted IonDX's impact in the industry.

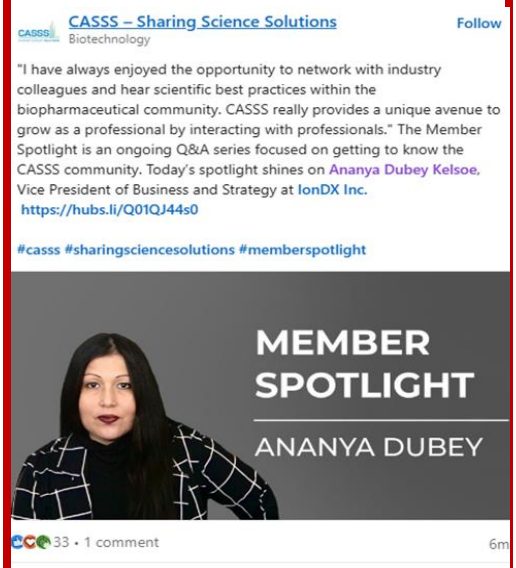
Recently, Justin W. Torpey, Ph.D, Principal Scientist at Scribe Therapeutics, Inc shared this comment on the recent collaboration: *"The collaboration with Scribe and IonDX has given us tremendous insight into the folded nature of many of our sgRNA molecules under different conditions. We learned that small changes in the sequence can produce various degrees of structural heterogeneity and stability, including varying degrees of higher-order structure."*

For more information on IonDX's technology and collaborations, please contact Ananya Dubey at adubey@iondx.com

IonDX attends 2023 CASSS Higher Order Structure Conference in Chicago

2023 CASSS HOS booth

IonDX's Vice President of Business & Strategy, Ananya Dubey, was recently interviewed by CASSS for their Member Spotlight feature!



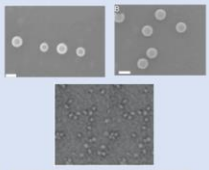
[Member Spotlight: Ananya Dubey (casss.org)]

IonDX is now a registered supplier on **scientist.com**

IonDX's SLD Provides Controlled Sample Prep

2007: before soft-landing garnered attention

Electrosprayed polystyrene latex particles before (left) and after mobility-selection (middle).



Mobility-selected Lsr-F shows greatly improved images of individual molecules

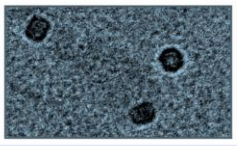
Bogan, ML, et al., J. Aerosol Sci., 38, 1119-1128, 2007

2022: initial results with GFP

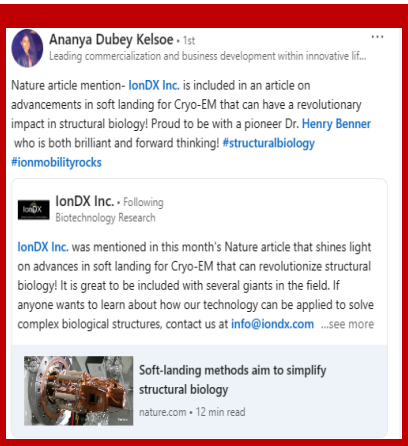
Green fluorescent protein (GFP) is naturally fluorescent and loses its fluorescence when damaged

GFP remained fluorescent after 0.1 meV impacts

2023: Success! GroEL survived soft landing



Soft-landed GroEL shows barrel structure at 75,000 X



IMgenius™ solves the complexity problem

IonDX's patented ion mobility spectrometer

IMgenius characterizes proteins, RNA and nucleic acids

SLD solves the sample prep problem

IonDX's patented soft landing device (SLD)

SLD sample prep improves cryo-EM image quality

A New Soft-Landing Method for Preparing Cryo-EM Samples Avoids the Plunge-Freezing Step

IonDX

Genentech
A Member of the Roche Group

W. Henry Benner[1], Ben Aguilar[1], Matt Johnson[2], Dimitry Tegunov[2], Mike Reichelt[2], Chris Arthur[2], Wayne Fairbrother[2], Ananya Dubey Kelsoe[1], Alexis Rohou[2] and Elizabeth Hecht[2]
1) IonDX, Monterey, CA; 2) Genentech, San Francisco, CA

Motivation

The growing acceptance of cryo-EM for determining high resolution structures of proteins stems from hardware and software advancements and because the method does not necessitate the growth of protein crystals. However, sample preparation remains largely unchanged since the technique was first developed in the 1980's using grid wetting, blotting and plunge freezing steps that often cause problems. Improvements are needed to prepare samples more efficiently and reproducibly (Earl).

Approach

We evaluated a sample preparation method that deposits gas-phase protein ions directly onto cryo-EM grids using soft-landing conditions – an approach that allows ions to land onto a surface gently so that the molecular conformation of the ion is not altered during the landing process.

Method Summary

1) A protein in 100 mM ammonium acetate is electrosprayed and converted to singly-charged gas-phase ions using our charge-reducing electrospray ion source.
2) The singly-charged ions are passed through a differential electric mobility analyzer for the purpose of selecting a subset of the molecular ions which have a narrow range of conformation as determined from the electric mobility of the selected ions
3) The humidity of the ion-laden gas stream is adjusted as needed to control ion hydration
4) The selected ions are focused to land at low velocity onto a temperature-controlled cryo-EM grid
5) The flux of deposited ions is monitored via a deposited ion current to control the surface density of the deposited ions
6) The loaded grid is preserved in liquid nitrogen for subsequent analysis by cryo-EM

Advantages of the Approach

1) The method is compatible with all buffer solutions and deposits molecules randomly onto grids,
2) Atmospheric pressure ion mobility is readily implemented into the work flow and provides an additional purification step through mobility-selection of a narrow size distribution of molecules, thus removing multimers and enriching the target,
3) The deposition rate can be monitored in order to control loading density,
4) Image noise is reduced and contrast is improved because molecules are not embedded in a film of vitreous ice and instead hydrated and frozen molecules are imaged on thin carbon films.

References

Earl, LA, Curr. Opinion Struct. Biol., Oct., 46: 71-78, 2017.
Kaufman, SL., et al., Anal. Chem., 68, 1895-1904, 1996.
Kaufman, SL, J. Aerosol Sci., 29,537-552, 1998.

·Contact: Henry Benner, hbenner@iondx.com

Fig. 1

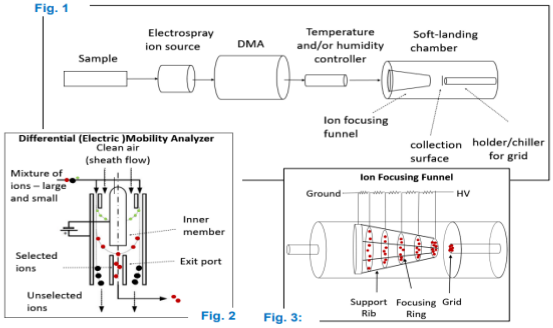


Fig. 2

Fig. 3:

System Description

Fig. 1 illustrates the soft-landing technique and the equipment needed to generate, select and deposit mobility-selected ions onto a cryo-EM grid. Fig. 2 describes the operation of a differential mobility analyzer (DMA, Kaufman) in which larger (black), medium (red) and smaller (green) circles represent singly-charged ions with extended, normal or compact conformations. A predetermined voltage applied to the inner member of the DMA guides the red ions to be selected and pass through the DMA to the ion focusing funnel. Fig. 3 illustrates the operation of the ion focusing funnel – as ions travel through progressively smaller rings, the electric field increases and focuses ions onto a grid mounted onto the end of a chilled pedestal.

Initial Results

Fig. 4 shows a typical result for the first steps of preparing a sample and checking it's purity. Green Fluorescent Protein (GFP) at 0.05 µg/mL in 100 mM NH4OAc was electrosprayed and charge-reduced to generate lowly-charged ions. Voltage applied to the inner member of the DMA was ramped from 0 to 1 kV while detecting a GFP ion current on the holder for the grid. The mobility spectrum in Fig. 4 correspond to M^+, $2M^+$, $3M^+$, $4M^+$ ions of GFP. The DMA was then set to transmit only $4M^+$ ions. The ion focusing funnel was set at 4 kV to load a grid efficiently with tetramers at room temperature. Fig. 5 shows a fluorescent signal (green glow) of GFP deposited onto a copper cryo-EM grid. GFP is easily deformed and loses its fluorescence after its gross conformation is altered. The detected fluorescence indicates the GFP conformation was not damaged after deposition.

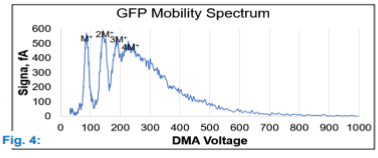


Fig. 4:

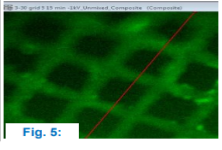


Fig. 5:

Apoferritin Results

Apoferritin (~450,000 Da) was landed for 20 min onto a single layer of graphene supported by a pre-clipped 2000Cu grid cooled to -40 ˚C produced 50-100 particles/um². The proteins were observed within non-crystalline ice (red arrows). In Fig. 6, images of the softly-landed ApoF show a preserved ring structure of 24 protomers and features of a 5-point pentagon structure. Particles were found to be larger ~10% larger than expected, however. Fig. 7 shows a mobility spectrum of ApoF. Only M^+ ions were landed, thus excluding solution-phase monomer species, and electrospray dimers ($2M^+$) (Fig. 7).

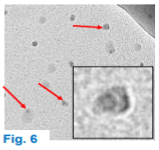


Fig. 6

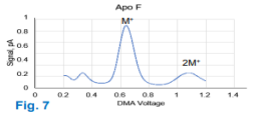


Fig. 7

GroEL Results

GroEL (~812,000 Da, 14-mer) was landed for 20 min onto a single layer of graphene supported by a pre-clipped 2000Cu grid cooled to -20 ˚C produced a distribution of randomly-oriented particles. The proteins were observed within non-crystalline ice. Landed GroEL showed a preserved barrel structure as indicated by observed top and side views. Image classifications indicated the barrel shape was partially denatured, due presumably the relatively high landing temperature of - 20 ˚C. Fig. 9 shows a mobility spectrum of GroEL.

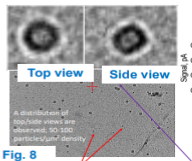


Fig. 8

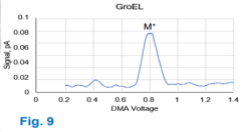
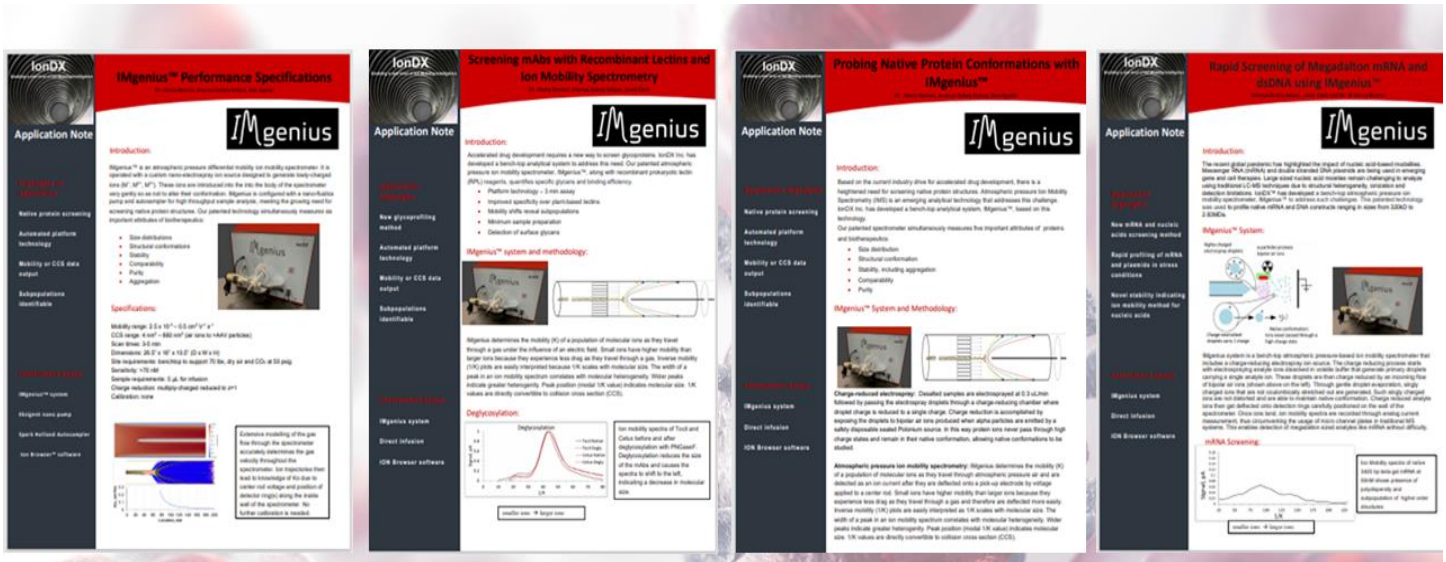


Fig. 9

Summary

The approach described here focuses mobility-selected ions in air at atmospheric pressure to land onto thin carbon films. The landing process is easy to control and can be automated. The kinetic energy of the ions at the time of impact with a thin carbon film is small enough to prevent gross conformation damage and to preserve multimeric noncovalent protein assemblies (Fig. 4); we predict that to preserve the fine structure of proteins at the atomic level, further reduction of the temperature on the grid and EM optimization is needed.



- IMgenius™ Performance Specifications
- Screening mAbs with Recombinant Lectins and Ion Mobility Spectrometry
- Probing Native Protein Conformations with IMgenius™
- Rapid Screening of Megadalton mRNA and dsDNA using IMgenius™

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