MS+M_22

MASS SPECTROMETRY
ELECTRON MICROSCOPY
SCANNING PROBE MICROSCOPY

Frontiers in Native Mass Spectrometry and Single-Molecule Imaging

14–18 August 2022

Wadham College

University of Oxford

Program Panel with Local(∗) Organisers:

Stephan Rauschenbach∗ (contact: stephan.rauschenbach@chem.ox.ac.uk)
Josh Coon (University of Wisconsin–Madison)
Brandon Ruotolo (University of Michigan)
Michael Westphall (University of Wisconsin–Madison)
Joseph Gault (Vertex Pharmaceuticals)
Tim Esser∗ (University of Oxford)
Lindsay Baker (University of Oxford)
Tanmay Bharat (University of Oxford)

Contact msplusm_22@chem.ox.ac.uk

PRESENTING

Before your talk, please setup and test your computer with the presentation system. Please talk to the conference desk or just come to the presentation system in the breaks before your session.

We offer to connect your computer to the presentation system via HDMI, or USB-C (Apple).

If you do not want to use your own, we have a presentation computer. We can upload your presentation if you bring it to us before your session at the conference desk or send it to us: msplusm_22@chem.ox.ac.uk

HOLYWELL MUSIC ROOM

The lecture venue is a historic building which implies some special rules regarding usage.

Entering and leaving: At the beginning and end of each session we will use the main entrance and enter the college via Wadham's side gate, which we will open for the time (see map below).

For getting in and out during the lectures, a side door will be opened which leads you directly into college, so you can go to the conference desk, the coffee area, or your room. **Please only use this passage for that purpose, as it is not made for many people to move through.**

Respecting the room: The historic room was not made for modern conferencing. We will set up the projection on the stage, which also contains valuable musical instruments. This means there are also some cables on the floor. **Please take extra care when moving around for your own safety. Most importantly any food or drink is not permitted in the rooms.**

POSTER

Posters walls will be in the JCR café, which is the area where we have coffee breaks and gather between the talks. The conference desk has materials to attach them to the boards.

The **main poster session** is on Wednesday after lunch and we ask the presenters to be at their posters for questions and discussion during this time.

There is a print shop just next to Wadham, which prints posters on A0.
https://www.oxford-print-centre.co.uk/page/banners-poster-plan-prints.php
They offer to print one poster for 35 GBP.

We recommend portrait format, but we are also able to accommodate landscape posters.

REIMBURSEMENT

Invited speaker please come to the conference desk to submit details for reimbursement. We will also send you forms and instructions shortly and you can do it online.

CONFERENCE BADGE

We produced badges and did not want to use plastic sleeves and ribbons. Thus, please bring your favorite ribbon – we are sure our badges will fit.

COVID-19

The UK has no mandatory Covid19 precautions in place. However, we are aware the Covid is still present and not harmless.

We advise you to test yourself shortly before the meeting. If the test is positive and you cannot join, please let us know and we will try to reimburse your fees in full.

Therefore, we recommend to wear mask to protect yourself. We will have some at the conference desk.

ACCOMODATION

If you have accommodation booked in college, you will receive the keys at the conference desk. All accommodation is in Wadham college's main site. See the map above for the location of your staircase (SC).

There is always someone at the college lodge who is able to help you. The college gate is closed at night. If you arrive late, you will need to ring the bell at the lodge to be let in.

CONFERENCE OUTING

After the Tuesday's session, we go to the Cherwell boat house for a punt (weather permitting). This very 'Oxford' past time is both challenging and relaxing. Up the Cherwell River is the pub "Victoria Arms", which can be reached within 30-60 minutes, depending on punting proficiency.

We advise to familiarise yourself with the technique:
http://cherwellboathouse.co.uk/punting/how-to-punt/

Punts take up to 6 people, we recommend groups of 4 or 5. Walking to the boathouse will take 20-30 minutes through the park or you can use a hire car (001, royal car) for a 5-minute, 5 pounds trip there. We will help you get on the boats and explain a bit how it works.

MEALS

On Sunday, we have a **Welcome Cafe** in the JCR cafe with some snacks. After the opening lecture there will be drinks and snacks in the JCR bar.

Breakfast will be served in Hall every morning starting 8am.

There will be coffee and biscuits available during the **coffee breaks** in the morning and afternoon.

Lunch will be served in Hall during the lunch breaks.

Dinner

Sunday
After the opening lectures we can meet at the Wadham bar where we offer some snacks or you explore the city.

Monday
After the Thermo Fisher Special Event a special dinner takes place on Monday evening. (requires a separate invitation)

Tuesday
The **Conference Dinner** is on Tuesday evening after the conference outing for all participants. It starts with predinner Drinks in the Cloister Garden from 6.30pm. Dinner starts at 7.15pm in the Wadham Hall. Please be on time.

While there is no dress code to this event, it is tradition in Oxford to dress smart when dining in Hall.

We did not make further arrangements other than the above for **dinner**, assuming that colleagues want to explore the city and its plenty of Restaurants and Pubs with new and old friends.

Here some suggestions for areas of the city where you find dining easily (see also city map next page):

City Centre: Plenty of options for casual dining in the Covered Market, High Street, Cornmarket Street, George Street, and the Westgate Centre

Jerico: Along Walton Street. Food and pubs not far from the Centre.

Cowley: Beyond Magdalen Bridge here you find many small restaurants and shops and the pubs of the students.

For pubs and restaurants without reservation we recommend to go in small groups (<6). Otherwise, a reservation is advisable, which can often be done quickly online or by calling.

TRANSPORTATION

Oxford has a bus system (https://www.oxfordbus.co.uk) to reach the suburbs – you will rarely need it. Tickets can be bought in the bus.

In addition to 'black cabs', there are very reliable hire cares which you need to book via phone or online (app):
Royal Cars https://royal-cars.com
001 Taxi https://www.001taxis.com

Wadham lodge has a fast order button for these companies. 10 minutes after pushing you cab is usually there.

AIRPORT TRANSFER

The 'Airline' of the Oxford Bus Company (https://www.theairlineoxford.co.uk/oxford-to-heathrow-bus/) offers a fast, frequent, and reliable airport transfer for GBP 35 for both ways Oxford- Heathrow. It also connects Gatwick.

Alternatively, also the hire car firms offer airport transfer for around GBP 80-100.

TRAVEL ADVICE

On Saturday 13 August as well as on Thursday 18 August there are strikes planned on the UK rail transportation system, which might affect trains to London as well as the London tube network. Please inform yourself (national rail, BBC) and make arrangements accordingly.

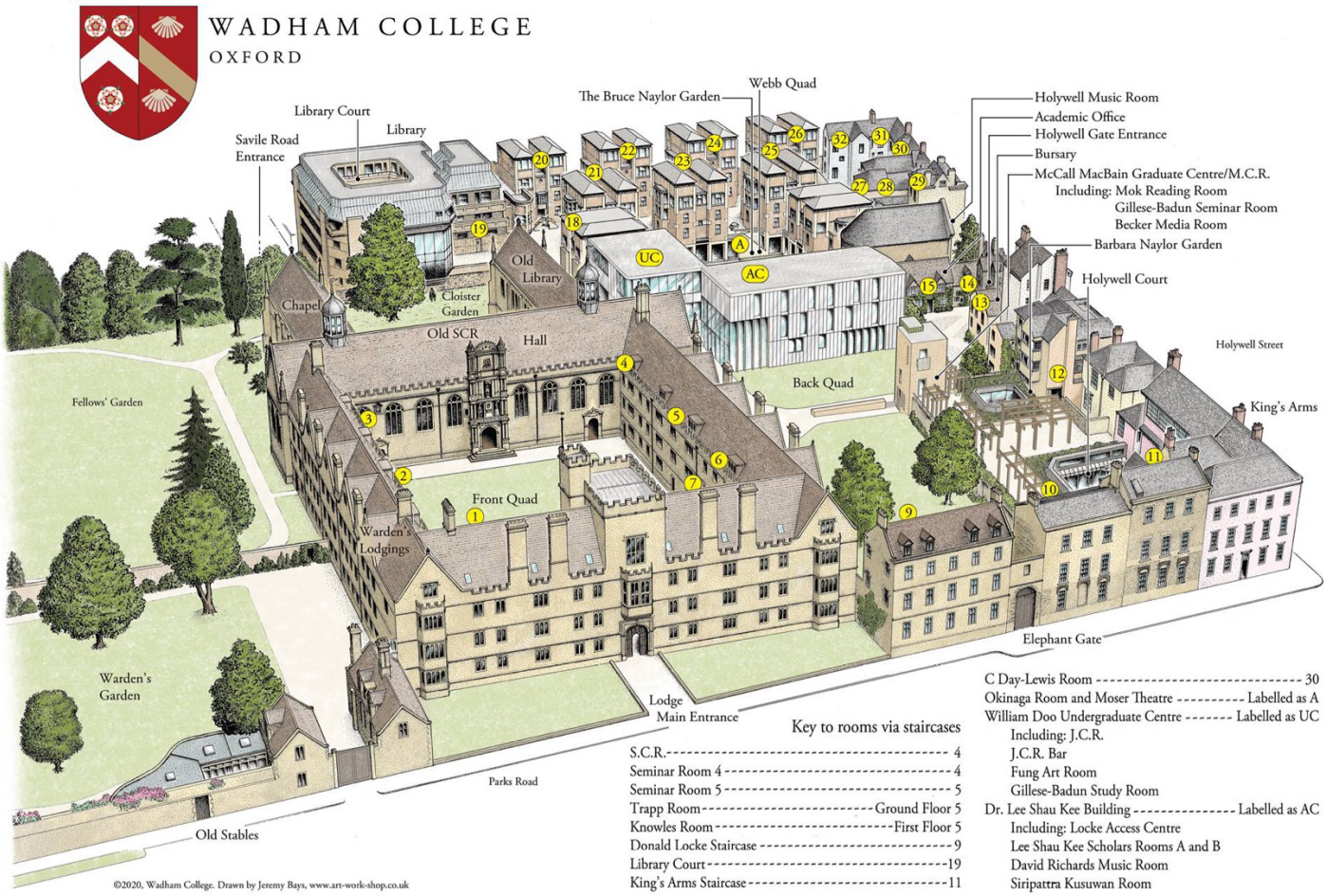
This should not affect the Heathrow-Oxford airport transfer. If there are no trains running, the Oxford tube (bus line) is a viable alternative, running from Victoria station in London (and many other stops) to Oxford 24/7.

Oxford Tube stops in London. In Oxford use *Queens Lane* to get off close to Wadham College.

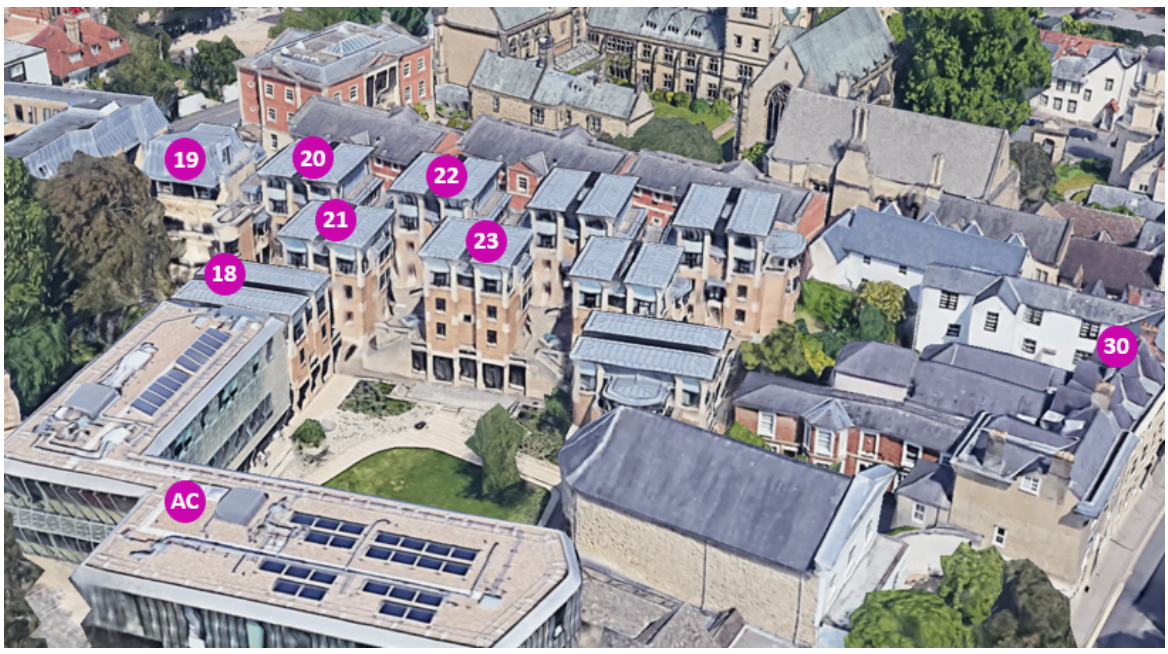
LOCATIONS

Wadham College:	accommodation, talks, breakfast and lunch
Holywell Music Room:	talk on Sunday, Monday, Wednesday
Oxford Martin School:	talks on Tuesday
Cherwell Boathouse:	punting (Tuesday afternoon, 15–20 min walk through university park)

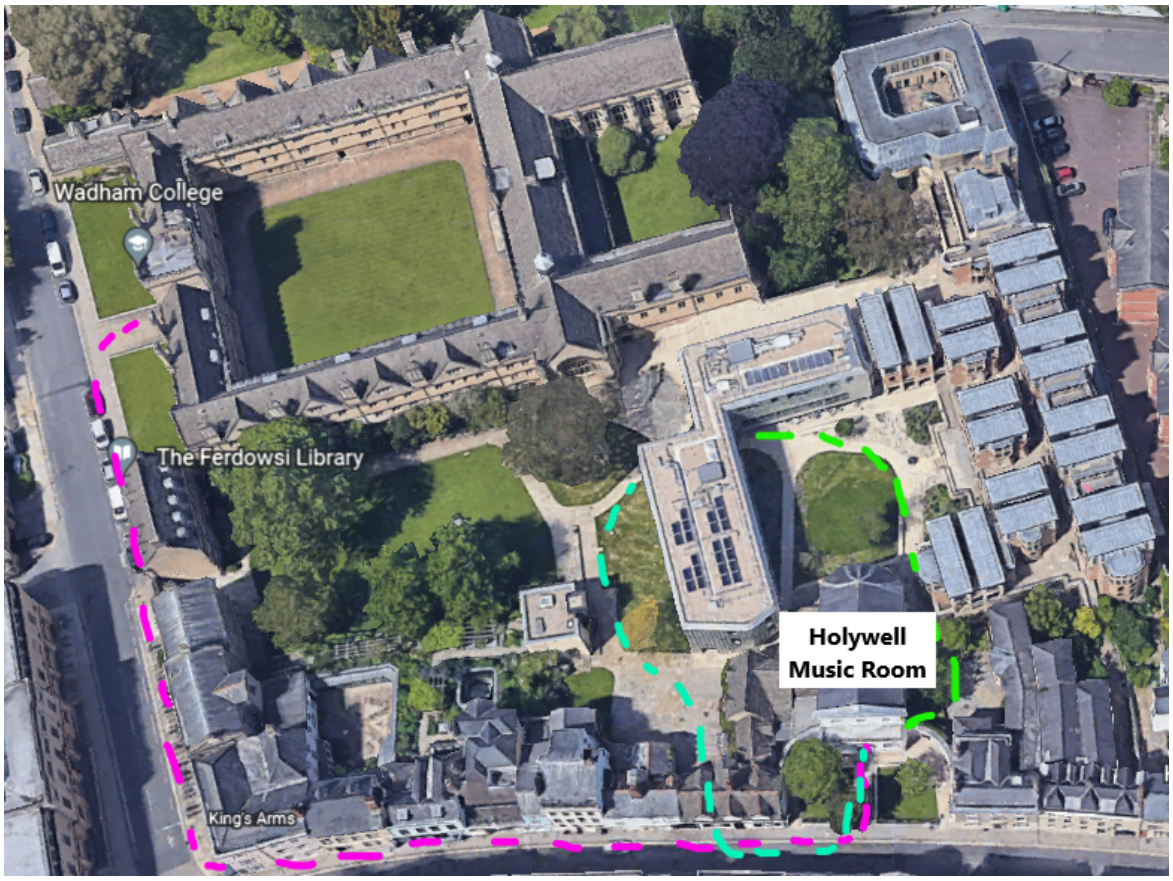
Link for the above map with points of interest as indicated:
https://goo.gl/maps/9J6SrQKPKk4GAumY9



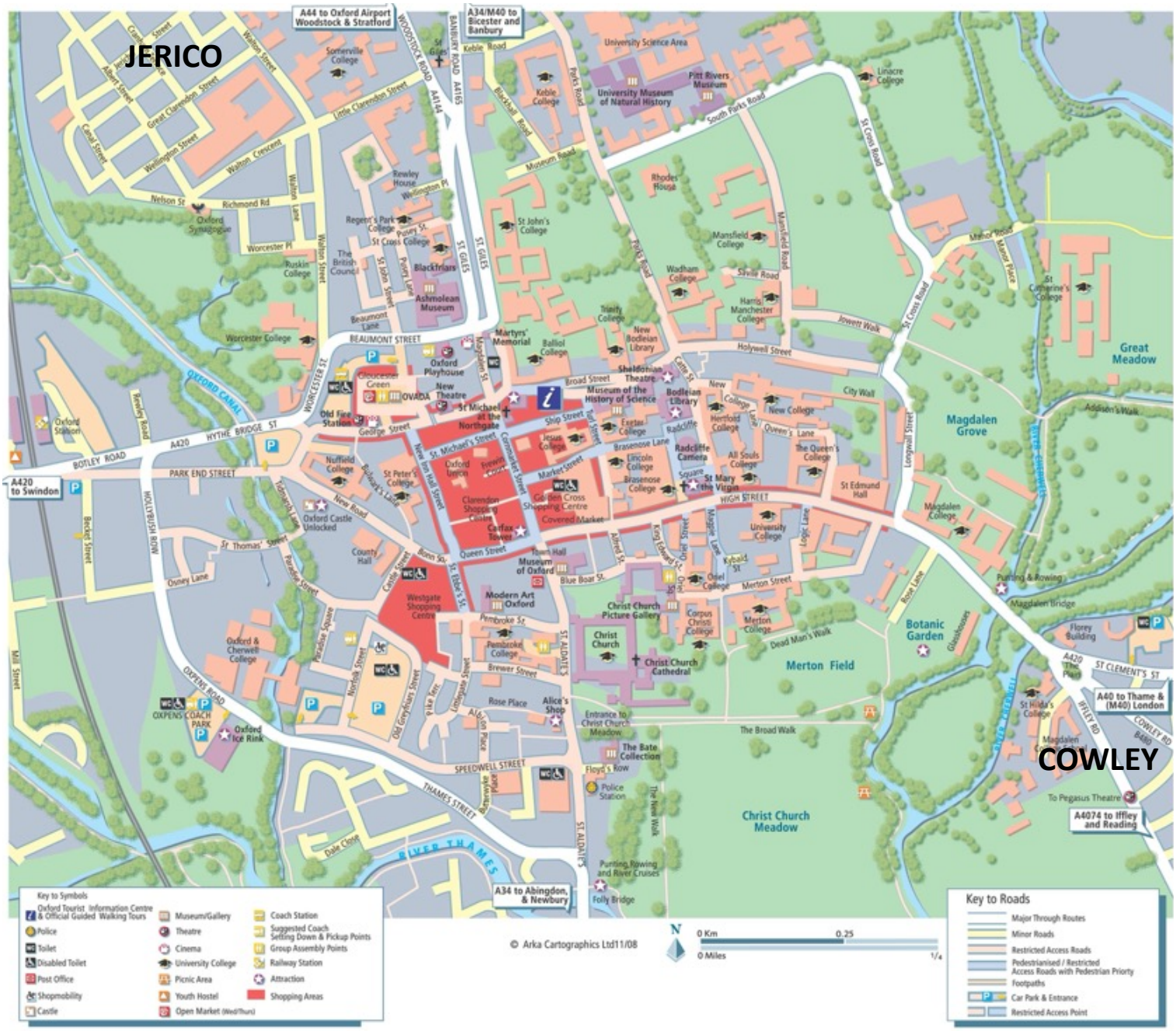
Map of College: The **Lodge** is the main entrance point.
Talks will be in the **Holywell Music Room.**
Conference Desk, Poster and Coffee break in the **Access Centre.**
Breakfast and lunch will be in **Hall.**



Map of Staircases: where the accommodation is.



Ways from **Access Centre** to **Holywell Music Room.** Use main path (center), unless



OXFORD

Areas with shops and restaurants.

Inner city, Jerico, Cowley

PROGRAM

SUNDAY 14 afternoon

TIME	TOPICS
1400	EM+holography
	native mass spec and IMS
	molecular SPM
1415	soft landing
1430	
1445	
1500	**Welcome Coffee** *JCR cafe*
1515	
1530	
1545	
1600	
1615	
1630	
1645	
	Wadham Holywell Music Room
1700	**Organisers** *Opening Adress*
1715	chair: S. Rauschenbach **Carol Robinson** *University of Oxford* Promises and pitfalls of native mass spectrometry
1730	
1745	
1800	**Yifan Cheng** *Univ. of Califomia San Fransisco* Structural studies of endogenous proteins in HEK cells by combination of single particle cryo-EM and mass spectrometry
1815	
1830	
1845	Get together *Wadham bar*
1900	
1915	
1930	

MONDAY 15 morning

TIME	*Wadham Holywell Music Room*
0900	chair: I. Liko **Leonhard Grill** *University Graz* Imaging and Manipulation of Single Molecules: Switches, Wires and Motors
0915	
0930	
0945	**Grant Johnson** *PNNL* Harnessing the potential of atomically precise materials using preparative mass spectrometry
1000	
1015	
1030	**coffee + poster** *JCR cafe*
1045	
1100	chair: W. Struwe **Michal Sharon** *Weizmann Institute* Native Mass Spectrometry Under "Close-To-Life" Conditions
1115	
1130	**Cagla Sahin** *Karolinska Institutet* Divergent assembly mechanisms
1145	**Victor Yin** *Utrecht University* Weighing in on SARS-CoV-2...
1200	**Albert Heck** *Utrecht University* Sizing, counting and chasing individual particles by native mass spectrometry
1215	
1230	**Lunch** *Hall*
1245	
1300	**Coffee** *JCR cafe*
1315	
1330	chair: L. Baker **Alex Noble** *New York Structural Biol.Center* Current approaches and prospects in combining MS and cryo-EM/ET
1345	

MONDAY 15 afternoon

TIME	*Wadham Holywell Music Room*
1400	**Tim Grant** *University of Wisconsin-Madison* New methodologies for imaging and processing cryo-EM samples
1415	
1430	(online) **Julia Laskin** *Purdue University*
1445	
1500	**coffee + poster**
1515	
1530	chair J. Benesh **Tim Esser** *U of Oxford / Thermo Fisher* Mass-selective and ice-free cryo-EM protein sample prep. via native ESIBD
1545	
1600	**Josh Coon** *University of Wisconsin–Madison* 3D structure determination of protein complexes using matrix-landing mass spectrometry
1615	
1630	**Henry Benner** *IonDX* Mobility-Selected Ions on EM Grid
1645	**Elizabeth Hecht** *Genetech* Ion mobility, nMS and Cryo-EM
1700	**break with refreshments** JCR cafe
1715	
1730	**THERMO FISHER** *special event* (on invite from Thermo Fisher) *light refreshment talks* *Talk 1 (AB): 45+10* *Talk 2 (AK): 20+10*
1745	
1800	
1815	
1830	
1845	
1900	Refreshments
1915	Thermo Special Dinner *old library*
1930	

TUESDAY 16 morning

TIME	*Oxford Martin School*
0900	
0915	chair: F. Baier (online) **Xu Wu** *MPI for Solid State Research* Non-averaged Imaging of Biomolecules
0930	
0945	**Florian Albrecht** *IBM Research Europe* Selectivity in single-molecule rxns
1000	**Andreas Walz** *TU Muenchen* *A wide range of (bio)organic* species soft-landed using preparative mass spectrometry
1015	
1030	**coffee**
1045	
1100	chair: C. Lutomski **Vicky Wysocki** *Ohio State University* Native MS Tools for Characterization of Protein and Nucleoprotein Complexes
1115	
1130	**Brian Chait** *The Rockefeller University* Native mass spectrometry for screening and probing dynamic macromolecular structures
1145	
1200	**Frank Sobott** *University of Leeds* Molecular footprints of protein aggregation
1215	
1230	**Lunch** *Hall*
1245	
1300	**Coffee** *JCR cafe*
1315	chair: A. Botman **Christopher Russo** *MRC Laboratory* The potential of electron cryomicroscopy in situ: identifying molecules in cells
1330	
1345	**Lindsay Baker** *University of Oxford* Identifying single mol. from cro-ET
1400	**Ulrich Lorenz** *EPFL* Microsecond Time-Resolved Cryo-Electron Microscopy
1415	
1430	

TUESDAY 16 afternoon

TIME	
	... continues from before
1430	EXCURISON INSTRUCTIONS
1445	EXCURSION **walk to boathouse** 30
1500	
1515	**distibution into boats** 30
1530	**punt towards Victoria Arms** 60
1545	
1600	**Break at pub**
1615	
1630	
1645	
1700	**punt back** 60
1715	
1730	
1745	
1800	
1815	**return boat and walk back** 30
1830	
1845	PREDINNER DRINKS (starts 1815)
1900	
1915	CONFERENCE DINNER
1930	*... until 10pm*

WEDNESDAY 17 morning

TIME	*Wadham Holywell Music Room*
0900	chair: B. de la Torre **Jiang Ying** (online) *Bejing University* *Submolecular Insights into* Interfacial Water by Hydrogen-Sensitive SPM
0915	
0930	**Kelvin Anggara** *MPI Stuttgart* Visualizing Glycans and Glycoconjugates One-at-a-time
0945	
1000	(online) **Yeliang Wang** *Beijing Institute of Technology* *Selective adsorption and interplay* of functional molecules on 2D materials
1015	
1030	**coffee + poster**
1045	
1100	chair: D. Wu **Perdita Barran** *University of Manchester* tba
1115	
1130	**Tarick El-Baba** *University of Oxford*
1245	
1200	**Brandon Ruotolo** *University of Michigan* *High-throughput Collision Induced* Unfolding as a Screening Technology for High-Resolution Structural Biology
1215	
1230	**Lunch** *Hall*
1245	
1300	
1315	**Poster Session** *JCR cafe*
1330	
1345	
1400	
1415	
1430	

WEDNESDAY 17 afternoon

TIME	*Wadham Holywell Music Room*
1400	chair: A. Bahm **Tatiana Latychevskaia** *University of Zurich* Low-energy electron holography and coherent diffraction imaging
1415	
1430	**Hannah Ochner** *MPI for Solid State Research* Amplitude and Phase Reconstruction of Low Energy Electron Holograms of Individual Proteins
1445	
1500	**Pilar Cossio** *Flatiron Institute* Free-energy profiles from cryoEM particles
1515	
1530	**coffee + poster**
1545	
1600	chair: J. Gault **Peng Wang** *Warwick Univeristy* Cryo-EPt SPA
1615	**Stefania Moro** *University of Warwick* High-resolution imaging for sub-molecular scale characterisation of conjugated polymers
1630	
1645	**Johannes Seibel** MPI for Solid State Research Visualization of D- and L-glycan
1700	**Alexander Makarov** *Thermo Fisher Scientific* Integration of Orbitrap Mass Spectrometry and Novel In-Vacuo Techniques: Abundant Marriage Opportunities
1715	
1730	Closing Remarks
1745	
1800	curry and pub
1815	
1830	
1845	
1900	
1915	
1930	

SUNDAY, 14 August

15:00-17:00 Welcome Coffee

Session 1
Holywell Music Room
Chair: Stephan Rauschenbach

17:00-17:15 Stephan Rauschenbach, Josh Coon, Brandon Ruotolo
Welcome address by the organisers

17:15-18:00 Carol Robinson
University of Oxford, Kavli Institute for Nanoscience Discovery
From GroEL folding to receptor signalling across native membranes – The promises and pitfalls of native mass spectrometry

18:00-18:45 Yifan Cheng
University of California, San Fransisco
Structural studies of endogenous proteins in HEK cells by combination of single particle cryo-EM and mass spectrometry

18:45 Get Together - Wadham Bar

MONDAY, 15th August

Session 1
Wadham Holywell Music Room
Chair: I. Liko

09:00-09:45 Leonhard Grill
University of Graz
Imaging and Manipulation of Single Molecules: Switches, Wires and Motors

09:45-10:30 Grant Johnson
Pacific Northwest National Laboratory
Harnessing the potential of atomically precise materials using preparative mass spectrometry

10:30-11:00 Coffee + Poster – JCR Café

Session 2
Wadham Holywell Music Room
Chair: W. Struwe

11:00-11:30 Michal Sharon
Indiana University
Native Mass Spectrometry Under "Close-To-Life" Conditions

11:30-11:45 Cagla Sahin
Karolinska Institutet
Divergent assembly mechanisms of RNA-binding proteins result in distinct architectures during liquid-liquid phase separation

11:45-12:00 Victor Yin
Utrecht University
Weighing in on SARS-CoV-2 / Antibody Interactions using Single Particle Mass Measurements

12:00-12:30 Albert Heck
Utrecht University
Sizing, counting and chasing individual particles by native mass spectrometry

12:30-13:15 Lunch – Hall

13:15-14:00 Poster Session 1 – JCR Café

Session 3
Wadham Holywell Music Room
Chair: L. Baker

14:00-14:30 Alex Noble
New York Structural Biology Center
Current approaches and prospects in combining MS and cryo-EM/ET

14:30-15:00 Tim Grant
University of Wisconsin-Madison
New methodologies for imaging and processing cryo-EM samples

15:00-15:30 Julia Laskin
Purdue University
tbd

15:30-16:00 Coffee + Poster

Session 4
Wadham Holywell Music Room
Chair: J. Benesch

16:00-16:30 Tim Esser
University of Oxford
Mass-selective and ice-free cryo-EM protein sample preparation via native electrospray ion-beam deposition

16:30-17:00 Josh Coon
University of Wisconsin–Madison
Matrix-Landing Mass Spectrometry and Imaging of Native Protein Complexes

17:00-17:15 Henry Benner
IonDX
Considerations for Soft Landing of Mobility-Selected Ions onto EM Grids

17:15-17:30 Elizabeth Hecht
Genentech
Interfacing ion mobility, native mass spectrometry, and Cryo-EM

Thermo Fisher Special Event

17:45-19:15	Presentations - *tbc*
19:15-19:30	Drinks Reception - *tbc*
19:30	Special Dinner - *Old Library*

TUESDAY, 16th August

Session 1
Oxford Martin School
Chair: F. Baier

09:15-09:45 Xu Wu (online)
Max Planck Institute for Solid State Research
Non-averaged Imaging of Biomolecules

09:45-10:00 Florian Albrecht
IBM Research Europe
Selectivity in single-molecule reactions by tip-induced redox chemistry

10:00-10:30 Andreas Walz
Technical University of Munich
A wide range of (bio)organic species soft-landed using preparative mass spectrometry

10:30-11:00 Coffee

Session 2
Oxford Martin School
Chair: C. Lutomski

11:00-11:30 Vicky Wysocki
Ohio State University
Native MS Tools for Characterization of Protein and Nucleoprotein Complexes

11:30-12:00 Brian Chait
The Rockefeller University
Native mass spectrometry for screening and probing dynamic macromolecular structures

12:00-12:30 Frank Sobott
University of Leeds
Molecular footprints of protein aggregation

12:30-13:15 Lunch – Hall

Session 3
Oxford Martin School
Chair: A. Botman

13:15-13:45 Christopher Russo
MRC Laboratory of Molecular Biology
The potential of electron cryomicroscopy in situ: identifying molecules in cells

13:45-14:00 Lindsay Baker
University of Oxford
Identifying single molecules in heterogeneous data from cryoET

14:00-14:30 Ulrich Lorenz
Ecole Polytechnique Fédérale de Lausanne
Microsecond Time-Resolved Cryo-Electron Microscopy

EXCURSION

14:30-14:45 Excursion Instructions

14:45-18:15 Excursion - Cherwell Boathouse

CONFERENCE DINNER

18:30-19:15 Pre-dinner Drinks - Cloister Garden

19:15-22:00 Conference Dinner – Wadham Hall

WEDNESDAY, 17th August

Session 1

Wadham Holywell Music Rom

Chair: B. de la Torre

09:00-09:30 Jiang Ying (online)
Peking University
Submolecular Insights into Interfacial Water by Hydrogen-Sensitive Scanning Probe Microscopy

09:30-10:00 Kelvin Anggara
Max Planck Institute for Solid State Research
Visualizing Glycans and Glycoconjugates One-at-a-time

10:00-10:30 Yeliang Wang *(online)*
Beijing Institute of Technology
Selective adsorption and interplay of functional molecules on 2D materials

10:30-11:00 Coffee

Session 2

Wadham Holywell Music Room

Chair: D. Wu

11:00-11:30 Perdita Barran
University of Manchester
tba

11:30-12:00 David Clemmer
Indiana University
Towards Characterization of Oxygenated Hemoglobin Conformations Using IMS-IMS on a Cyclic Instrument

12:00-12:30 Brandon Ruotolo
University of Michigan
High-throughput Collision Induced Unfolding as a Screening Technology for High-Resolution Structural Biology

12:30-13:15 Lunch – Hall

13:15-14:00 Poster Session 2 – JCR Café

Session 3

Wadham Holywell Music Room

Chair: A. Bahm

14:00-14:30 Tatiana Latychevskaia
University of Zurich
Low-energy electron holography and coherent diffraction imaging

14:30-15:00 Hannah Ochner
Max Planck Institute for Solid State Research
Amplitude and Phase Reconstruction of Low Energy Electron Holograms of Individual Proteins

15:00-15:30 Pilar Cossio
Flatiron Institute
Free-energy profiles from cryoEM particles

15:30-16:00 Coffee + Poster

Session 4

Wadham Holywell Music Room

Chair: J. Gault

16:00-16:15 Peng Wang
Warwick University / Nanjing University
Cryogenic Electron Ptychographic Single Particle Analysis (Cryo-EPt SPA)

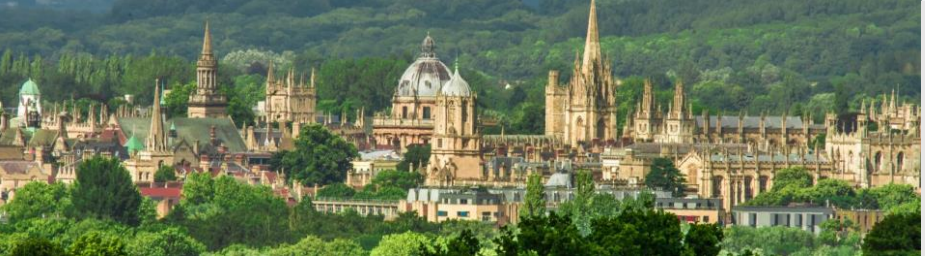
16:15-16:45 Stefania Moro
University of Warwick
High-resolution imaging for sub-molecular scale characterisation of conjugated polymers

16:45-17:00 Johannes Seibel
Max Planck Institute for Solid State Research
Visualization of D- and L-glycan assemblies and their chirality by high-resolution scanning tunneling microscopy

17:00-17:30 Alexander Makarov
Thermo Fisher Scientific
Integration of Orbitrap Mass Spectrometry and Novel In-Vacuo Techniques: Abundant Marriage Opportunities

17:30-17:45 Stephan Rauschenbach, Josh Coon, Brandon Ruotolo
Closing Remarks

TALKS

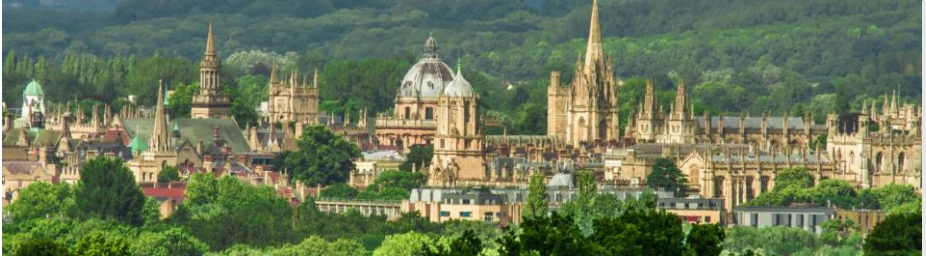


From GroEL folding to receptor signalling across native membranes – The promises and pitfalls of native mass spectrometry

Carol V. Robinson[1]

[1]Kavli Institute for Nanoscience Discovery, University of Oxford, Oxford, UK

Given that gas phase protein complexes were never expected to stay together yet alone retain structure, the fact the we are now bringing together this field with microscopy is testament to the tremendous progress that has been made. In this lecture I will chart this evolution highlighting steps that helped move the field from initial scepticism to final acceptance. Challenges remain however as we move away from recombinant proteins to more native-like environments. With this transition we need to overcome nature's heterogeneity, to sequence proteins within complexes and to capture ligand binding in many different scenarios. Starting with personal milestones of folding within the GroEL cavity and ending with receptor signalling across native membranes I will at each stage present the challenges that were faced and the steps we took to try to overcome them.



Structural studies of endogenous proteins in HEK cells by combination of single particle cryo-EM and mass spectrometry

Wooyong Choi,[1] Hao Wu,[1] and Yifan Cheng[1,2]

[1] Department of Biochemistry and Biophysics, University of California San Francisco, CA 94158, USA

[2] Howard Hughes Medical Institute, University of California San Francisco, CA 94158, U.S.A

In the era of modern single particle cryogenic electron microscopy (cryo-EM), the capability of structural biology has been extended beyond determining atomic structures from well-behaved conformational and compositional homogeneous samples purified either from recombinant overexpression systems or natural sources. The power of computational classifications of compositional and conformational heterogenous particles in a single particle cryo-EM sample enables structural studies of macromolecular complexes extracted from native environment, such as within cells or from cells of different animal tissues. Correlated to this transition, protein production by tagging endogenous macromolecular complexes in HEK has the potential to enable affinity purification of endogenous macromolecular complexes for structural studies by single particle cryo-EM. Combining single particle cryo-EM with mass spectrometry will further enable capturing interaction partners, regulators, or substrates, etc. during critical cellular process.

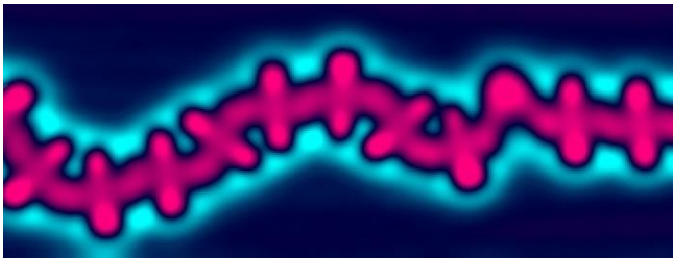
The CRISPR/Cas9 technology provides an ideal tool kit to enable tagging endogenous proteins. Using a self-developed plasmid homology directed repair (HDR) donor, we knocked-in affinity tags to six different genes with different endogenous expression levels in HEK293 cells. We will highlight the endogenous human glyceraldehyde 3-phosphate dehydrogenase (GAPDH). With an affinity tag, we purified native endogenous GAPDH from cytosol and nuclear of cells without and with oxidative stress. Atomic structures of these endogenous GAPDH from different cellular compartments reveal an increase of catalytic inactive subunits in nuclear GAPDH caused by a prolonged oxidative stress. Together, we were able to trace important biological events, including catalytic breakdown of GAPDH and its nuclear translocation, under oxidative stress. We envision a new frontier in structural biology by combining single particle cryo-EM studies with mass spectrometry analysis of endogenous protein complexes from native cellular environment.

Imaging and Manipulation of Single Molecules: Switches, Wires and Motors

Leonhard Grill

Dept. of Physical Chemistry, University of Graz, Austria, (www.nanograz.com)

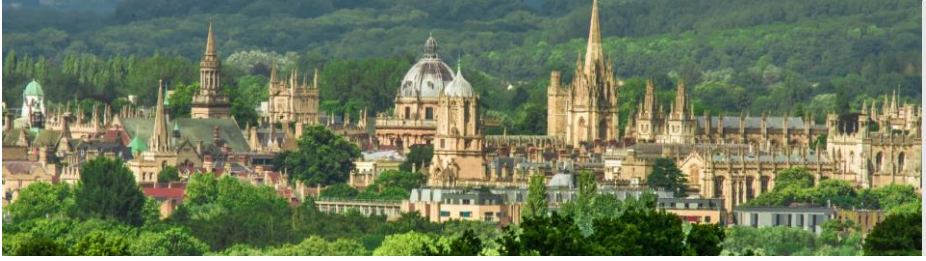
Controlling and understanding molecular functions is key in nanoscience as it gives insight into basic physical and chemical processes. In this presentation, various examples of functional molecules, ranging from molecular wires to switches and motors will be discussed. Special emphasis will be given on what can be done by scanning tunneling microscopy and spectroscopy in terms of characterization and manipulation of single molecules at surfaces.



By using on-surface polymerization [1], molecular wires can be fabricated from specifically designed molecular building blocks and their conductance can be measured at the single-molecule level [2]. This can also be done in a hierarchical manner that connects linear polymers – grown in a first step – to two-dimensional networks by using inequivalent halogen substituents [3]. Incorporation of donor and acceptor units into the building blocks leads to flexible wires (see Figure) [4] that maintain their specific electronic signatures encoded in the building blocks.

We are also interested in molecular switches that are based for instance on isomerization [5] or proton transfer (tautomerization) [6]. Surprisingly, the atomic-scale surroundings were found to strongly affect the switching properties in an unexpected way, leading for instance to periodic switching patterns. Moreover, molecular 'Feringa' motors were observed to translate laterally on a metal surface, triggered by a combination of heat and light [7]. It turns out that UV light does not change the *number* of moving molecules, but their *distance* of displacement. A much faster and more precise type of motion has been recently observed for molecules that move easily and very far when they are oriented along the atomic rows of the Ag(111) surface [8]. The high precision of motion could be used to even transfer them between separate STM tips that are controlled independently from each other.

[1] L. Grill et al., *Nature Chem. 12, 115 (2020)*; [2] L. Lafferentz et al., *Science 323, 1193 (2009)*; [3] L. Lafferentz et al., *Nature Chem. 4, 215 (2012);* [4] C. Nacci et al., *Nature Comm. 6, 7397 (2015);* [5] C. Dri et al., *Nature Nanotech. 3, 649 (2008*; [6] T. Kumagai et al., *Nature Chem. 6, 41 (2014)*; [7] A. Saywell et al., *ACS Nano 10, 10945 (2016)*; [8] D. Civita et al., *Science 370, 957 (2020)*



Harnessing the potential of atomically precise materials using preparative mass spectrometry

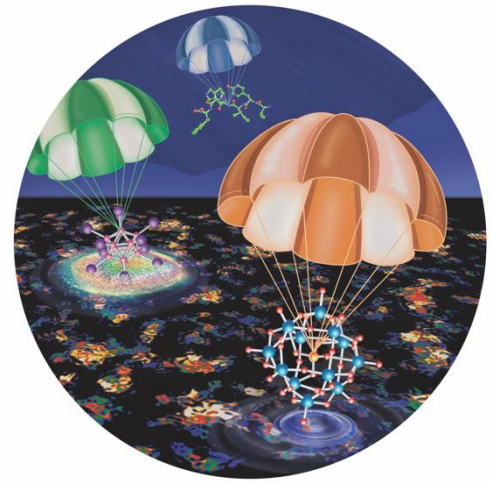
Grant E. Johnson,[1] Venkateshkumar Prabhakaran,[1] Jonas Warneke,[2] Andrei Federov,[3] and Julia Laskin[4]

[1] Physical and Computational Sciences Directorate, Pacific Northwest National Laboratory, Richland, Washington 99354, United States

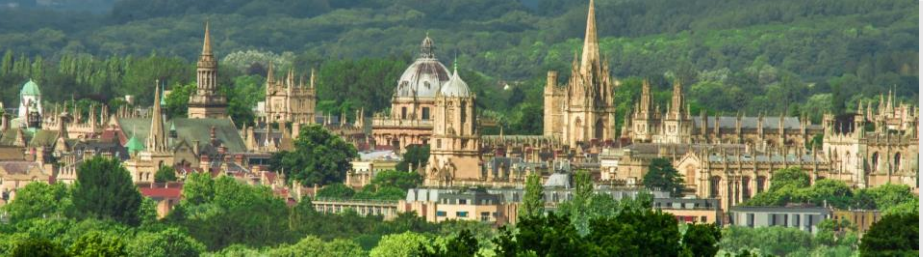
[2] Wilhelm-Ostwald-Institut für Physikalische und Theoretische Chemie, Universität Leipzig, 04103 Leipzig, Germany

[3] G.W. Woodruff School of Mechanical Engineering and Parker H. Petit Institute for Bioengineering and Bioscience, Georgia Institute of Technology, Atlanta, Georgia 30332, United States

[4] Department of Chemistry, Purdue University, West Lafayette, Indiana 47906, United States



Challenges in efficient energy generation and storage, green manufacturing, and quantum computing may be addressed through a fundamental molecular-level understanding of material properties and processes enabled by unconventional mass spectrometry techniques. Novel materials not obtainable by conventional synthesis approaches may be prepared in the gas phase and delivered to surfaces using ion soft landing. A wide range of polyatomic ions with precisely defined composition and ionic charge may be immobilized on different supports with predetermined coverage and kinetic energy, thereby circumventing the sample heterogeneity, contamination, and aggregation that often confound experimental characterization and theoretical modeling of materials. Novel in situ spectroscopic and electrochemical characterization techniques provide unprecedented insight into the role of ion-surface and ion-ion interactions in determining the emergent behaviour of meso- and macroscopic materials prepared by bottom-up assembly of atomically precise species. In this presentation, I will illustrate how ion soft landing is being employed to understand the underlying phenomena that may be exploited to improve the performance of advanced materials for fuel cell catalysts, supercapacitors, and molecular qubit arrays. Well-defined materials prepared by ion soft landing, combined with state-of-the-art characterization techniques and high-level theoretical modeling, are providing transformative insight into how materials may be designed and controlled at the atomic-level to address a range of energy-related challenges.



Native Mass Spectrometry Under "Close-To-Life" Conditions

Gili Ben-Nissan and Michal Sharon

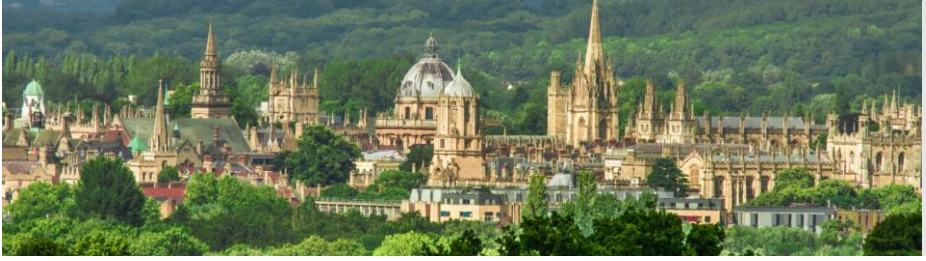
Indiana University

A grand goal of structural biology is to understand the mechanics of the molecular machines that coordinate the functions of the cell. This challenging task has traditionally been approached in a reductionist manner, in which cellular molecular components are fractionated and purified before being studied individually. Recent years have witnessed a revolution in the field of structural biology, stemming from the growing awareness to the importance of performing structural studies under more physiologically relevant conditions, ones that retain the effects of the intracellular environment on protein structure, stability, interactions, function, and dynamics. This notion has already given rise to cryo-electron tomography techniques and in-cell nuclear magnetic resonance spectroscopy. Yet, conformational variability, heterogeneity, fast dynamics and asymmetric structures can be a challenge for these methods.

To propel the field of cellular structural biology forward complementary methods are required. One such approach, the focus of my talk, is direct-MS that enables analysis under "close-to-life" conditions. Direct-MS is based on a conceptual frameshift in the field by turning the inherent limited dynamic range of MS (wherein low-abundance proteins are masked by highly abundant ones), typically considered a weakness, into an advantage. The method enables the biased detection of a highly produced target protein, while disregarding the lower-abundant endogenous proteins. Thus, signal suppression allows one to overcome the need for prior protein purification.

I will demonstrate that direct-MS acquisitions enable the immediate, high-resolution assessment of a wide range of structural features: sequence variations, assembly states, folding conditions, PTMs, overall structure, stability and the association of ligands of the generated proteins. The applicability of the method to analyzing drug uptake and target engagement in human cells will be discussed as well as its development towards full organ analysis.

Direct-MS enables structural studies under "close-to-life" conditions, preserving as much as possible the natural environment and biological diversity, features that are often lost during biochemical purifications.



Divergent assembly mechanisms of RNA-binding proteins result in distinct architectures during liquid-liquid phase separation

Cagla Sahin[1,2,*], Aikaterini Motso[1,#], Xinyu Gu[3,4,#], Hannes Feyrer[5], Dilraj Lama[1], Tina Arndt[6], Anna Rising[6,7], Erik. G. Marklund[8], Nicholas P. Schafer[2,3], Katja Petzold[5], Kaare Teilum[2], Peter G. Wolynes[3,4], and Michael Landreh[1,*]

[1] Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, Solna, Sweden.
[2] Structural Biology and NMR laboratory and the Linderstrøm-Lang Centre for Protein Science, Department of Biology, University of Copenhagen, Copenhagen, Denmark
[3] Center for Theoretical Biological Physics, Rice University, Houston, TX 77005
[4] Department of Chemistry, Rice University, Houston, TX 77005
[5] Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Solna, Sweden.
[6] Department of Biosciences and Nutrition, Karolinska Institutet, Huddinge, Sweden.
[7] Department of Anatomy, Physiology and Biochemistry, Swedish University of Agricultural Sciences, Uppsala, Sweden.
[8] Department of Chemistry - BMC, Uppsala University, Uppsala, Sweden

The ability of some RNA-binding proteins to undergo liquid-liquid phase separation (LLPS) into liquid droplets is a major regulator of the cellular stress response. Heterogeneous ribonucleoproteins (hRNPs) found in stress granules contain RNA recognition motifs (RRMs) and low-complexity domains (LCDs) that mediate LLPS but also predispose hRNPs to toxic aggregation. Structural information about the droplet states of proteins is challenging to obtain due to the low stability and heterogeneous interactions of hRNPs. Here, we turned to protein engineering, native mass spectrometry, and molecular dynamics (MD) simulations to investigate the structural basis for hRNP self-assembly. Through fusion to an LLPS-compatible spider silk domain (NT*), we engineered natively soluble forms of the hRNPs FUS, TDP-43, and hCPEB3 that are implicated in neurodegeneration, cancer, and memory storage. We then followed pH-controlled self-assembly using ion mobility mass spectrometry (IM-MS), which enables us to monitor conformational changes in a wide range of conditions, from denaturing to native. We find that NT*-FUS monomers undergo a pronounced unfolded-to-globular transition, whereas NT*-TDP-43 oligomerizes into partially disordered dimers and trimers during LLPS. NT*-hCPEB3, on the other hand, remains fully disordered with a strong preference for fibrillar aggregation over LLPS. Using the insights from IM-MS to inform MD simulations, we obtain atomistic models of FUS and TDP-43 assembly intermediates. In these models, FUS has the least β-sheet content, hCPEB3 has the most, and the β-sheet content of TDP-43 is intermediate between that of the other two proteins. Our results show that despite their superficial similarity, all three hRNPs adopt distinct configurations in their assembled states, resulting in different orientations of bound RNAs. We speculate that these specific assembly architectures serve to regulate RNA processing and translation in specific ways depending on biological context.

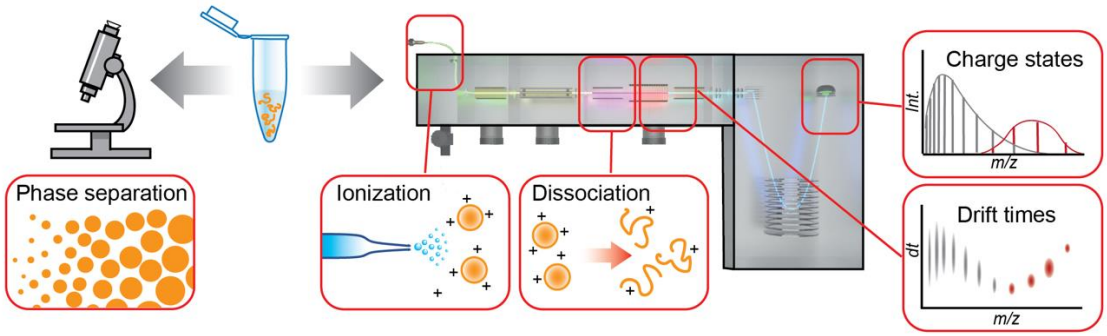


Figure 1: Microscopy and ion mobility mass spectrometry combined to study LLPS assembly. Brightfield microscopy is used to visualize and confirm formation of liquid droplets, whereas structural information of the assembly process is obtained by CCS calculations and charge state distributions. The mass spectrometer image is adapted and modified from Landreh *et al.*, ChemComm 2015.

Weighing in on SARS-CoV-2 / Antibody Interactions using Single Particle Mass Measurements

Victor Yin,[1] Szu-Hsueh Lai,[1] Tom G. Caniels,[2] Philip J. M. Brouwer,[2] Mitch Brinkkemper,[2] Yoann Aldon,[2] Hejun Liu,[3] Meng Yuan,[3] Ian A. Wilson,[3] Rogier W. Sanders,[2] Marit J. van Gils,[2] and Albert J. R. Heck[1]

[1] University Utrecht, Utrecht, the Netherlands

[2] Amsterdam University Medical Centers, Amsterdam, the Netherlands

[3] The Scripps Research Institute, La Jolla, United State

Native mass spectrometry provides information on protein assemblies that can be difficult to ascertain using other experimental approaches. However, native MS typically struggles in cases where high levels of micro-heterogeneity (e.g. glycosylation) hampers charge state assignment. Mass photometry (MP) and Orbitrap charge-detection MS (CD-MS) are two recently developed mass analysis techniques with sufficient sensitivity to detect individual ions/molecular particles. These methods solve the charge inference problem and enable measurement of challenging, heterogeneous assemblies. Here, we demonstrate and compare the unique capabilities of these two methods to probe the binding mechanisms of neutralizing antibodies to the SARS-CoV-2 spike protein [1]. Structural studies using methods such as XRD or EM typically employ simplified constructs such as Fab fragments and/or monomeric receptor binding domains to alleviate the inherent complexity of these interactions. However, these may not fully recapitulate the binding behaviour of the true antibodies and/or trimeric spike protein. Using single particle mass methods, we accurately measure the mass of the fully glycosylated S protein trimer. We subsequently determine its binding behaviour against a panel of anti-SARS-CoV-2 mAbs. Surprisingly, we observe that (1) mAbs display a wide range of binding stoichiometries not predicted a priori from symmetry arguments, (2) the binding behaviour of full IgGs can differ greatly from its corresponding Fabs, and (3) sub-stoichiometric IgG is sufficient to block ACE2 binding. Our divergent results suggest that monomeric constructs paint an incomplete picture of the complex, multimeric interactions that can occur between mAbs and viral spike proteins.

[1] Yin et al., ACS Central Science 2021. Front Cover Article.doi:10.1021/acscentsci.1c00804

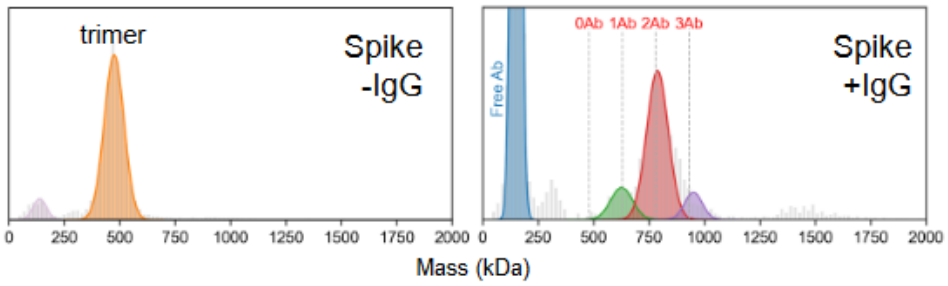
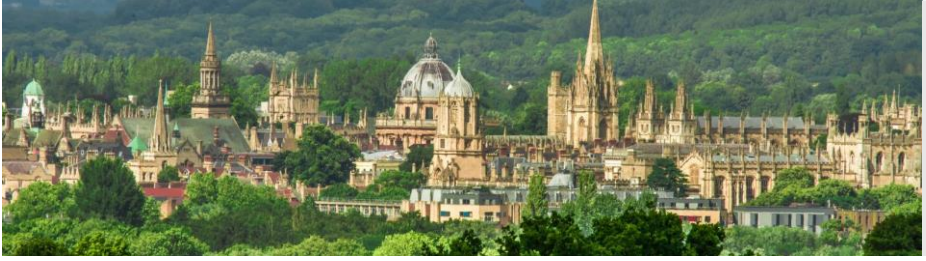


Figure 1. Single particle mass measurements reveal binding stoichiometries of an anti-SARS-CoV-2 IgG to the intact S trimer.



Sizing, counting and chasing individual particles by native mass spectrometry

Albert J. R. Heck[1,2]

[1] Biomolecular Mass Spectrometry and Proteomics, Bijvoet Center for Biomolecular Research and Utrecht Institute for Pharmaceutical Sciences, University of Utrecht, Padualaan 8, Utrecht 3584 CH, the Netherlands

[2] Netherlands Proteomic Center, Padualaan 8, Utrecht 3584 CH, the Netherlands

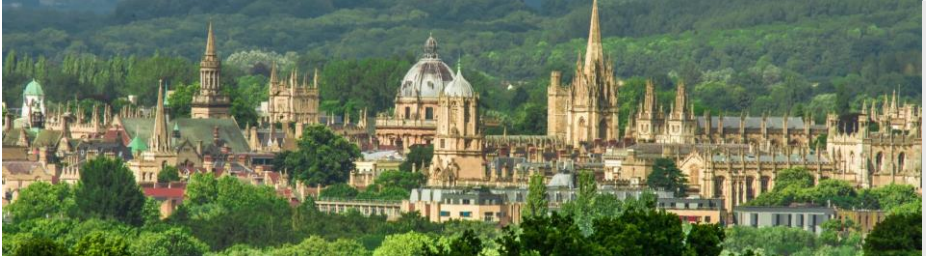
High-resolution native mass spectrometry enables the mass analysis of intact proteins and protein assemblies, ranging from antibodies to naïve viruses. In this lecture advances in the field will be highlighted, including extending the attainable mass range, the sensitivity of detection, the mass resolving power and the proteoform specificity. Cumulatively, these advances have initiated many new applications, not only in the detailed characterization of advanced biopharmaceuticals, but also in characterization of the plasma (glyco)proteome and the mass analysis of very large virus-like gene delivery vehicles.

This lecture will focus on the analysis and characterization of adeno-associated viruses (AAV), being either empty or partly packed with a transgene. By using high-resolution native mass spectrometry, we followed the assembly of these particles and revealed that this assembly is divergent and stochastic.

Modern mass analyzers, including Orbitraps, are sufficiently sensitive to detect single molecules by charge detection mass spectrometry, enabling the analysis by native MS of very large heterogeneous assemblies like immunoglobulins, nanocontainers, SARS-Cov2 spike protein-antibody immune-complexes, viruses, and virus-based gene-delivery vectors such as AAVs, and allow for the latter to measure the integrity of genome packing. These advances illustrate the broad impact of native MS in various disciplines of the life sciences.

References:

1. High-Resolution Native Mass Spectrometry. Tamara S, den Boer MA, Heck AJR. ***Chem Rev***. 2021 Aug 20. doi: 10.1021/acs.chemrev.1c00212
2. Adeno-associated virus capsid assembly is divergent and stochastic. Wörner TP, Bennett A, Habka S, Snijder J, Friese O, Powers T, Agbandje-McKenna M, Heck AJR. ***Nat Commun***. 12 (2021) 1642. doi: 10.1038/s41467-021-21935-5.
3. Mass Spectrometry-Based Structural Virology. Wörner TP, Shamorkina TM, Snijder J, Heck AJR. ***Anal Chem***. 93 (2021) 620-640. doi: 10.1021/acs.analchem.0c04339.
4. Resolving heterogeneous macromolecular assemblies by Orbitrap-based single-particle charge detection mass spectrometry. Wörner TP, Snijder J, Bennett A, Agbandje-McKenna M, Makarov AA, Heck AJR. ***Nat Methods*** 17 (2020) 395-398. doi: 10.1038/s41592-020-0770-7.
5. Frequency chasing of individual megadalton ions in an Orbitrap analyser improves precision of analysis in single-molecule mass spectrometry. Wörner TP, Aizikov K, Snijder J, Fort KL, Makarov AA, Heck AJR. ***Nat Chem.*** 2022 May;14(5):515-522. doi: 10.1038/s41557-022-00897-1.



Current approaches and prospects in combining MS and cryo-EM/ET

Alex Noble[1]

[1] NYSBC

I will discuss the current approaches in the literature for combining mass spec and cryo-EM/ET. Combinations that enable biological analyses will be highlighted. Future prospects with regards to methods and hardware developments will be discussed, focusing on how mass spec may help disentangle single particle cryo-EM data and in-situ cellular cryo-ET.

New methodologies for imaging and processing cryo-EM samples

Tim Grant,[1,2] Gan Li,[1,2]

[1] Department of Biochemistry, University of Wisconsin-Madison, Madison, WI, USA.
[2] Morgridge Institute for Research, Madison, WI, USA.

I will discuss a new imaging method we are developing, "Defocus Sweep Imaging", which, via computational microscope control, allows the microscope defocus to be changed as movies are recorded. This allows high resolution close-to-focus information to be recorded at the beginning of an exposure when radiation damage is low, and lower resolution far-from-focus data to be recorded at the end of the exposure when the sample is highly damaged. I will also discuss some recent developments in the software package cisTEM.

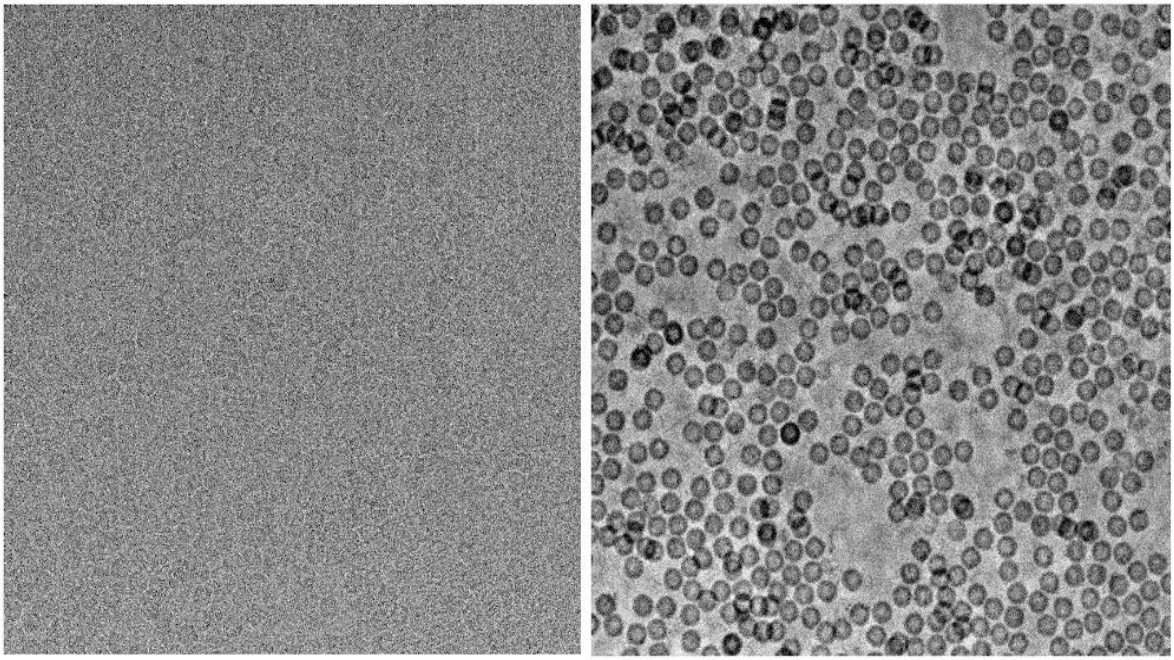
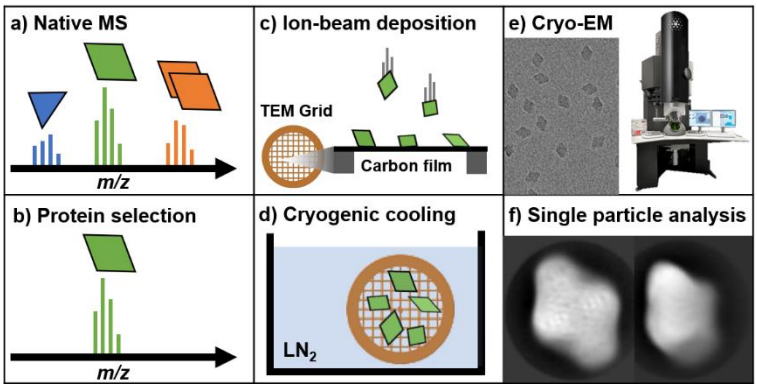


Figure 1. Comparison image of apoferritin taken at 4 µM defocus (left) and with defocus sweep imaging starting at 4 µM defocus (right)

Mass-selective and ice-free cryo-EM protein sample preparation via native electrospray ion-beam deposition

Tim Esser,[1,*] Stephan Rauschenbach[1,2]

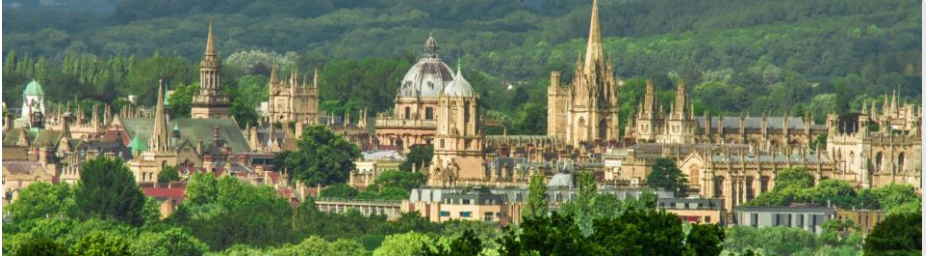
[1] Department of Chemistry, University of Oxford, Oxford OX1 3TF, UK
[2] Max Planck Institute for Solid State Research, Heisenbergstrasse 1, Stuttgart, DE-70569, Germany
[*] Current address: Thermo Fisher Scientific, 1 Boundary Park, Hemel Hempstead, HP2 7GE, UK



Despite tremendous advances in sample preparation and classification algorithms for electron cryomicroscopy (cryo-EM) and single-particle analysis (SPA), sample heterogeneity remains a major challenge and can prevent access to high-resolution structures. In addition, optimization of preparation conditions for a given sample can be time consuming. We have recently developed a native mass spectrometer with ion-beam deposition capability for the mass-selective preparation of samples of large native protein complexes.[1] Based on this setup, we demonstrate that native electrospray ion-beam deposition (native ES-IBD) is an alternative, reliable approach for preparation of extremely high-purity cryo-EM samples.[2] In native ES-IBD, folded protein ions are generated by native electrospray ionization, separated from other proteins, contaminants, aggregates, and fragments, gently deposited on cryo-EM grids, frozen in liquid nitrogen, and subsequently imaged by cryo-EM (see Fig. 1). We demonstrate homogeneous coverage of ice-free cryo-EM grids with mass-selected protein complexes. SPA reveals that the complexes remain folded and assembled, but variations in secondary and tertiary structure are currently limiting information in 2D classes and 3D EM density maps. We identify and discuss challenges that need to be addressed to obtain a resolution comparable to that of the established cryo-EM workflow. Our results show the potential of native ES-IBD to increase the scope and throughput of cryo-EM for protein structure determination and provide an essential, direct link between gas-phase and solution-phase protein structures.

[1] P. Fremdling, T. K. Esser, B. Saha, A. Makarov, K. Fort, M. Reinhardt-Szyba, J. Gault, and S. Rauschenbach, 'A preparative mass spectrometer to deposit intact large native protein complexes', *arXiv:2203.04671 [q-bio]*, vol. preprint, 2022.

[2] T. K. Esser, J. Boehning, P. Fremdling, M. T. Agasid, A. Costin, K. Fort, A. Konijnenberg, A. Bahm, A. Makarov, C. V. Robinson, J. L. P. Benesch, L. Baker, T. A. M. Bharat, J. Gault, and S. Rauschenbach, 'Mass-selective and ice-free cryo-EM protein sample preparation via native electrospray ion-beam deposition', *PNAS Nexus*, accepted.

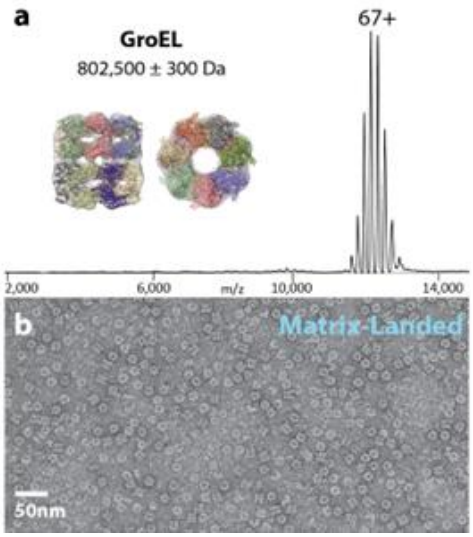


Matrix-Landing Mass Spectrometry and Imaging of Native Protein Complexes

Austin Z. Salome,[1] Kenneth W. Lee,[1] Michael S. Westphall,[1] Timothy Grant,[2] Joshua J. Coon[1]

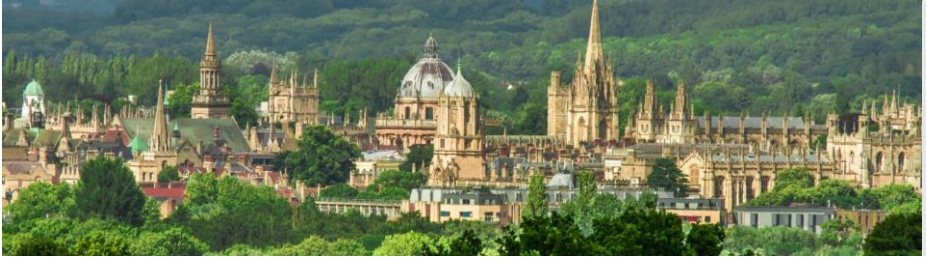
[1] University of Wisconsin-Madison, Madison, WI, United States

Native MS has proven valuable for understanding the structural organization of proteins and macromolecular complexes. Recently we have shown that the use of glycerol as a landing matrix allows these macromolecular complexes to retain their condensed-phase configurations when desolvated and placed in vacuum. This was achieved by modifying a Thermo Fisher Q Exactive UHMR Hybrid Quadrupole-Orbitrap mass spectrometer, mass range m/z 350 – 80,000, to deposit charged analyte particles onto the surface of a TEM grid. For these experiments the HCD cell was removed, and the instrument was modified to accept a direct insertion probe holding a single grid. The grid surface was cleaned in advance by air-glow discharge and the glycerol matrix was applied before loading into the insertion probe. After ion exposure the grid was recovered and negatively stained in 1% uranyl acetate followed by imaging on a FEI Tecnai 120kV TEM equipped with an AMT camera.



Structural integrity of GroEL is preserved after ionization, transit through the mass spectrometer, and landing in a matrix of PPG. (a) Mass spectrum of GroEL, estimated mass, and 3D overlay of GroEL crystal structure with matrix-landed wire-mesh reconstructed model. (b) Negative stain TEM grid of matrix-landed ions preserved in a thin layer of PPG.

While glycerol protected deposited particles and allowed for TEM imaging, the triol had a variety of unfavourable characteristics including (1) clumping of deposited particles on the TEM grid, (2) high variability in density of particles between matrix landings and (3) poor staining of the TEM grid after deposition leading to under stained particles. To remove and/or mitigate these drawbacks, the following chemical matrices were investigated: Triethanolamine (TEA), poly(ethylene) glycol (PEG), poly(propylene) glycol (PPG), TritonX-100, and diglycerol. Initially, a matrix-protein (GroEL) mixture was pipetted onto a TEM grid and placed under vacuum to access vacuum protection capabilities. PPG, TritonX-100, and diglycerol, which resulted in low particle clumping and strong contrast of imaged particles, were then further investigated through matrix-landing using the modified mass spectrometer followed by TEM imaging. PPG showed promising results with minimal clumping of deposited GroEL, favourable staining, and high particle density. Using this new landing matrix, the 20S Proteasome core and β-Galactosidase were also matrix-landed at high densities. Reconstructions of each protein complex showed excellent agreement with pipetted standards and reported crystal structures. The matrix-landing of each of these protein complexes shows the benefits of using PPG as a matrix while also further confirming that non-covalent protein-protein complexes can transverse the mass spectrometry and remain largely, if not fully, intact.



Considerations for Soft Landing of Mobility-Selected Ions onto EM Grids

W. Henry Benner,[1] Ben Aguilar[1] and Ananya Dubey Kelsoe[1]

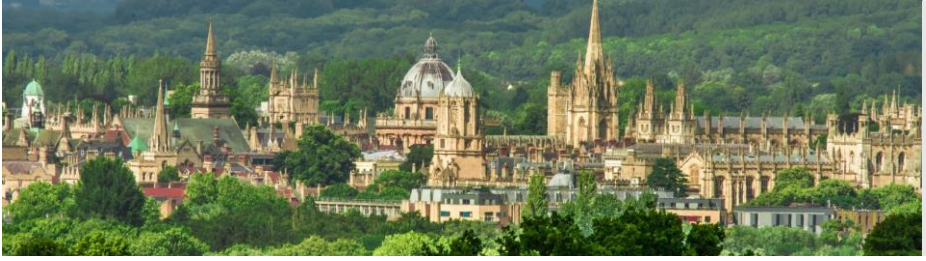
[1] IonDX, Inc., Monterey, CA

We will present the operating characteristics of IMgenius™, a bench top ion-mobility spectrometer that could be adapted for soft landing experiments. The combined features of the ion source, the simplicity of the spectrometer and the method for detecting deposited ions show promise for improving the quality of EM images by eliminating the need for staining or suspending molecules in ice. It will provide a way to study single conformers, a range of conformers and noncovalent complexes. The rate of ion deposition and the time for deposition, as needed to obtain appropriately loaded samples is an additional feature.

Lowly-charged analyte ions are generated by electrospraying nanoparticles or proteins into an atmosphere of bipolar air ions which, after interaction, reduce the highly-charged electrospray droplets to lowly-charged droplets. After the droplets evaporate, lowly-charged nanoparticles or ions become available for study. This charge-reducing technique is borrowed from the field of aerosol science and dates to the days of Boltzmann. Protein ions generated in this fashion never pass through a high charge state and as a result are not coulombically distorted and remain near to their native conformation.

The lowly-charged ions are subsequently carried by atmospheric pressure air into the body of the spectrometer where nanoparticles or protein ions having a predetermined electrical mobility are guided onto an electrode connected to a femtoammeter. The electrical current produced by the particles or ions is useful for monitoring the deposition rate. Nanoparticles and ions generated and deposited in this way were used extensively during our investigation of femtosecond single molecule X-ray diffraction experiments conducted at FLASH (Hamburg, DE) and at LCLS (Stanford Linear Accelerator Center). We will describe our plan to extend the deposition process using this spectrometer to include depositing protein ions onto small pieces of electrically conducting materials, including EM grids.

We will present images of mobility selected nano-particles and Lsr-F to show how mobility selection reduces the heterogeneity of nanoparticles. We will show how mobility selection can be used to clean up a protein sample that prior to mobility selection contained aggregates and after mobility selection revealed mostly monomers. We will report the operating parameters of the spectrometer and quantify the calculated kinetic energy of ions that land onto the detector ring or ultimately onto an EM grid. Lowly-charged ions travelling through atmospheric pressure air while under the influence of the electric field in this spectrometer, have low velocities at the time they strike the detector electrode and therefore are expected to retain their structure after they are deposited onto an EM grid.



Interfacing ion mobility, native mass spectrometry, and Cryo-EM

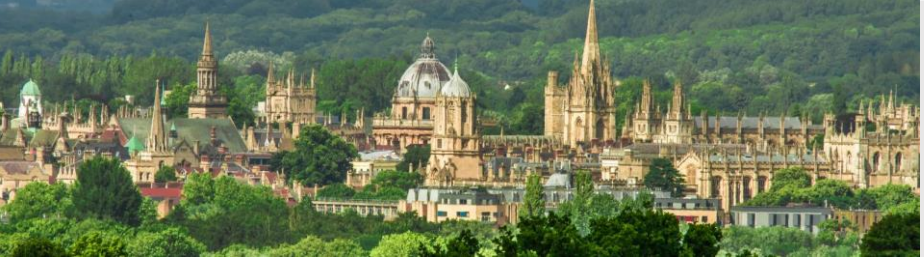
Elizabeth Hecht,[1*] Henry Benner[2*], Ben Aguilar[2], Matthew Johnson[1], Alberto Estevez[1], Dimitry Tegunov[1], Mike Reichelt[1], Ananya Dubey Kelsoe[1], Claudio Ciferri[1], Wayne Fairbrother[1], Chris Arthur[1], Alexis Rohou[1]

[1] Genentech, Inc. South San Francisco, CA USA

[2] IonDX, Inc. Monterey, CA USA

**These authors contributed equally to this work*

The design of a new soft landing device is demonstrated for the deposition of molecules onto EM grids. Scanning electron microscope, negative stain, cryo-EM, and mass spectrometry data support that the gross structure of proteins landed is well preserved and that improved contrast is observed in images. New results highlighting the effects of chilled landing will be particularly discussed. Correlations are drawn between measurements of particles in images, mobility-derived hydrodynamic radii, and predictions from mass spectrometry measurements.



Selective adsorption and interplay of functional molecules on 2D materials

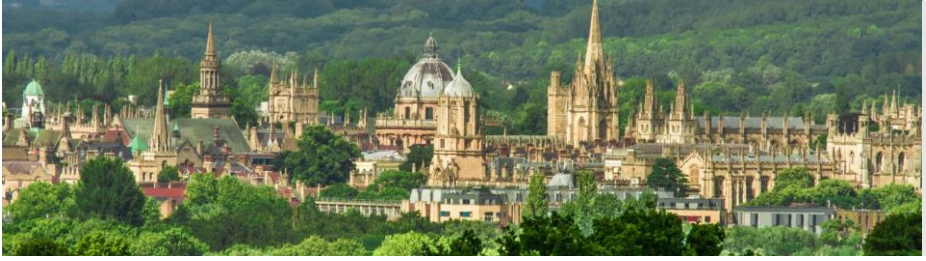
Yeliang WANG (王业亮)

School of Integrated Circuits and Electronics, Beijing Institute of Technology, Beijing 100081, China

The novel properties of graphene honeycomb structure have spurred tremendous interest in investigating other two-dimensional (2D) layered structures beyond graphene for nanodevices. In this talk, I will mentioned the fabrication and properties of several 2D materials such as silicene, antimonene, magnetic VSe_2 monolayer, semiconducting $PtSe_2$ monolayer, superconductor transition-metal-trichalcogenide $HfTe_3$, and charge density wave $NbSe_2$ superstructure, as well as quantum properties for nanoelectronics and valleytronics will also be introduced. In addition, selective adsorption and interplay of funtional molecules on 2D materials will be also presented. The precise structural configurations at atomic-resolution of these materials will also be introduced, based on the measurements by several advanced techniques like LEED, STM/STS and STEM.

References:

Nano Lett. 13, 685 (2013); **Nano Lett.** 13, 4671 (2013); **Adv. Mater.** 26,4820 (2014); **Nano Lett.** 15, 4013 (2015). **Adv. Mater.** 28,5013 (2016); **Adv. Mater.** 29,1605407 (2017); **Nat. Mater.** 16, 717 (2017); **Nano Lett.** 18, 2213 (2018); **Science Bulletin** 63, 419 (2018). **Nano Lett.** 19, 6323 (2019); **Nat. Commun.** 11, 659 (2020); **Nat. Commun.**12, 1978 (2021); **Nat. Commun.** 12, 58 (2021); **ACS Nano** 15, 16589 (2021); **InfoMat** 4, e12274 (2022); **ACS Nano** 16, 1332 (2022); **Nano Letters** 22, 1190 (2022); **Nat. Commun.** 13, 1843 (2022); **Nat. Mater.**, accepted (2022).



Selectivity in single-molecule reactions by tip-induced redox chemistry

Florian Albrecht[1], Shadi Fatayer[1,2], Iago Pozo[3], Ivano Tavernelli[1], Jascha Repp[4], Diego Peña[3], Leo Gross[1]

[1] IBM Research Europe – Zurich, Rüschlikon, Switzerland

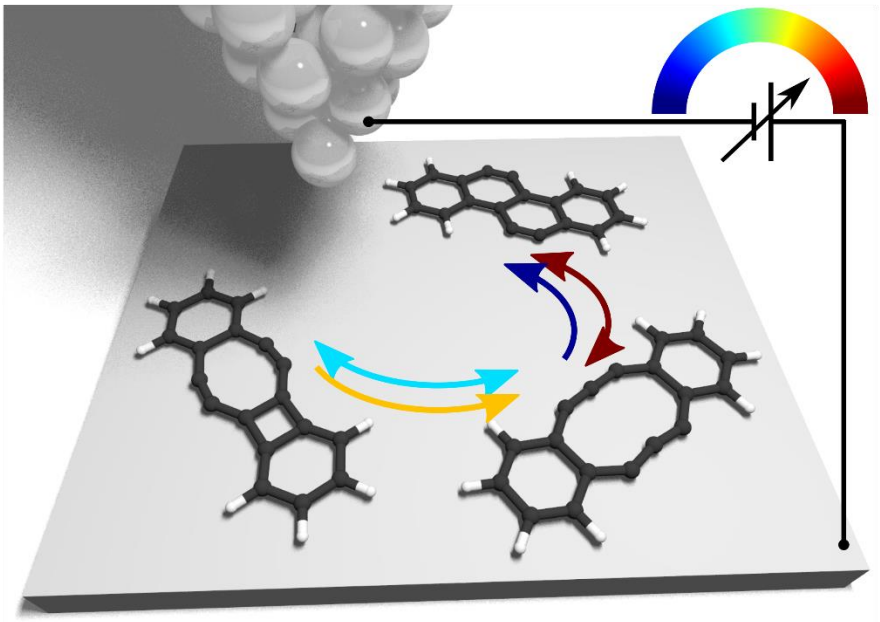
[2] Physical Science and Engineering Division, King Abdullah University of Science and Technology (KAUST), Thuwal, Saudi Arabia

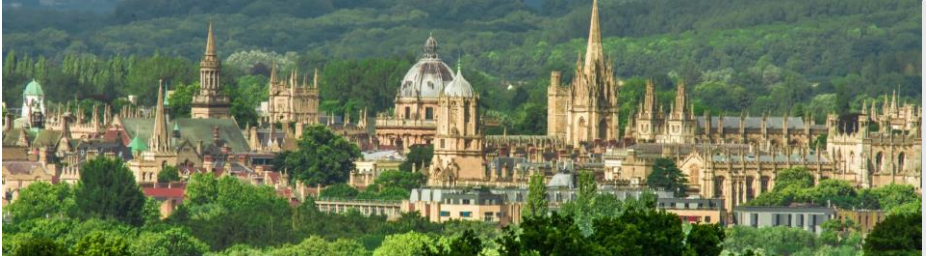
[3] Centro Singular de Investigación en Química Biolóxica e MateriaisMoleculares (CiQUS) and Departamento de Química Orgµnica, Universidade de Santiago de Compostela, Santiago de Compostela, Spain

[4] Institute of Experimental and Applied Physics, University of Regensburg, Regensburg, Germany

Since the first experiments by S.-W. Hla in 2000 [1], tip-induced on-surface synthesis is an active field. Not only in on-surface chemistry, selective control over the outcome of a reaction is a major quest. Here, we activate a molecule adsorbed on ultrathin insulating films by dehalogenation and perform selective constitutional isomerization reactions in a low temperature UHV combined STM and AFM. The selectivity is controlled by the polarity and amplitude of applied voltage pulses. The insulating films stabilize the isomers in different charge states and allow for their characterization. The importance of molecular charge state on the reaction is supported by DFT-derived isomerization energy landscape.

[1] S.-W. Hla et al., Phys. Rev. Lett. 85, 2777 (2000).





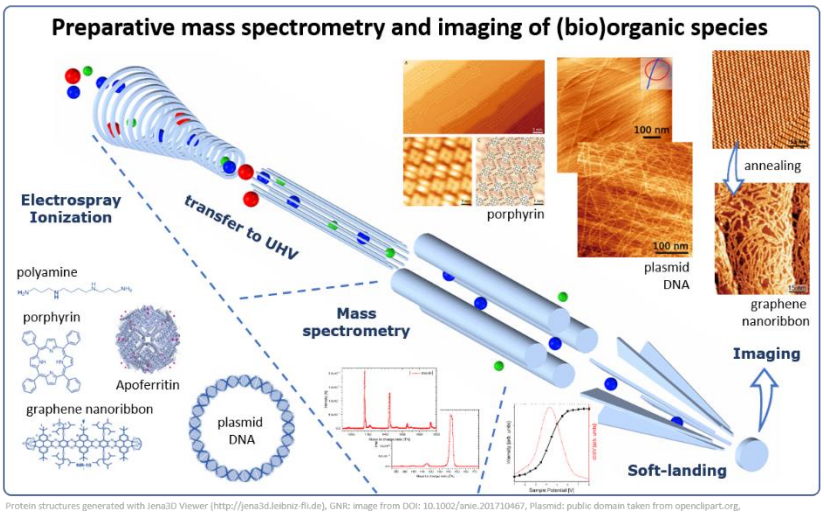
A wide range of (bio)organic species soft-landed using preparative mass spectrometry

Andreas Walz,[1] Dennis Meier,[1] Benedikt Schoof,[1] Karolina Stoiber,[1] Wei Ran,[1] Peter Knecht,[1] Annette Huettig,[1] Joachim Reichert,[1] Anthoula Papageorgiou,[1] Hartmut Schlichting,[1] Johannes Barth[1]

[1] Department of Physics E20, Technical University of Munich, Germany

A home-built prototype[1] for conducting highly versatile and efficient preparative mass spectrometry is presented. The system enables soft-landing of mass selected ions in ultra-high vacuum (UHV) revealing encouraging performance data and deposition results. Non-volatile or fragile molecules can be deposited with controlled landing energy and dose in an atomically clean manner. Exemplary, species from a test panel comprising small organic molecules, proteins, DNA, graphene nanoribbons[2] and artificial helices (foldamer)[3] covering a mass range from 200 Da up to 2 MDa are presented with an m/z up to about 10.000 Th.

Innovative and miniature ion guides and a digital quadrupole mass filter employ square wave signals with adjustable frequency. This allows for high ion transmission, low gas loads, low kinetic energies and a virtually unlimited mass range. Samples are characterized by a variable temperature UHV-STM. Future investigations will be supported by a recently introduced vacuum suitcase accessing other analytical techniques (LT-STM/AFM, XPS, etc.).



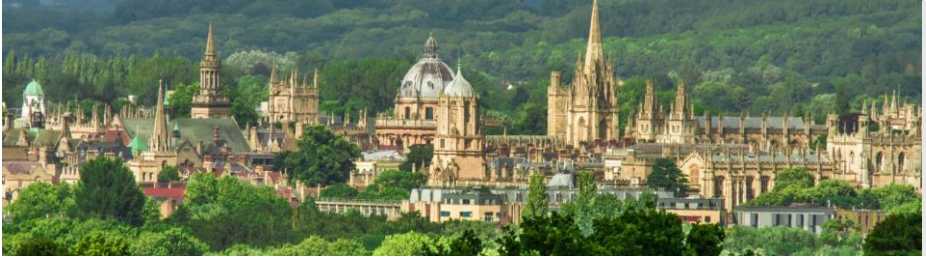
Experimental flow from ion generation to deposition and subsequent imaging by VT-STM in UHV. Depiction of selected test objects.

References:

[1] A. Walz et al., Anal. Chem., 2022, 94, 7767-7778.

[2] W. Ran et al., Angew. Chem. Int. Ed., 2022, 61, e202111816.

[3] D. Meier et al., Structural adaptations of electrosprayed aromatic oligoamide foldamers on Ag(111), 2022, submitted



Native MS Tools for Characterization of Protein and Nucleoprotein Complexes

Vicki Wysocki[1]

[1] Department of Chemistry and Biochemistry and Resource for Native Mass Spectrometry Guided Structural Biology, Columbus, OH, USA

Characterization of the overall topology and inter-subunit contacts of protein complexes, and their assembly/disassembly and unfolding pathways, is critical because protein complexes regulate key biological processes, including processes important in understanding and controlling disease. Tools to address structural biology problems continue to improve. Native mass spectrometry (nMS) and associated technologies such as ion mobility are becoming an increasingly important component of the structural biology toolbox. When the mass spectrometry approach is used early or mid-course in a structural characterization project, it can provide answers quickly using small sample amounts and samples that are not fully purified. Integration of sample preparation/purification with effective dissociation methods (e.g., surface-induced dissociation, SID), ion mobility, and computational approaches provide a MS workflow that can be enabling in biochemical, synthetic biology, and systems biology approaches. Native MS can determine whether the complex of interest exists in a single or in multiple oligomeric states and can provide characterization of topology/intersubunit connectivity, and other structural features. Examples will be presented to illustrate the development of surface-induced dissociation as a mass spectrometry activation method for the characterization of protein and nucleoprotein complexes. Coupling of SID to electron capture charge reduction and complementarity with other structural tools will be illustrated. Progress made and planned for the NIH-funded nMS-Guided Structural Biology Center will be briefly discussed.

1) Snyder, Harvey, Wysocki, V. H., Chemical Reviews,2022,122, 8, 7442–7487.

2)Karch, Snyder, Harvey, Wysocki Annu. Rev. Biophys. 2022. 51,157–79.

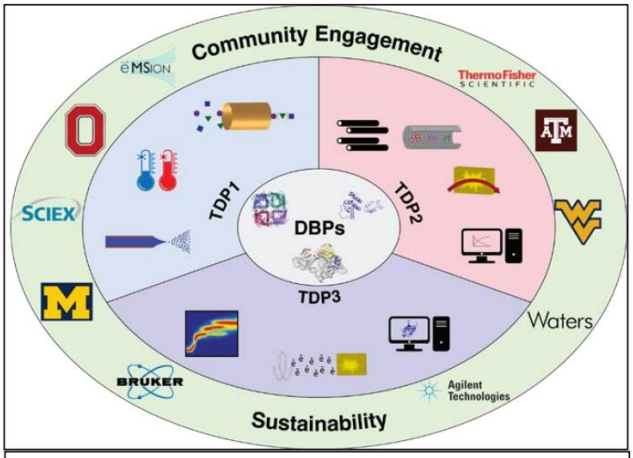


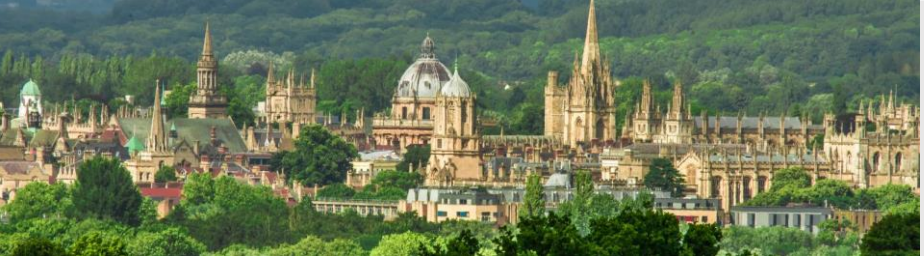
Figure 1. Overview of the integration of driving biomedical projects, technology development, and community engagement proposed for continuation of the nMS-guided structural biology center (OSU, WVU, TAMU, U Michigan).

Native mass spectrometry for screening and probing dynamic macromolecular structures

Brian T Chait and Paul Dominic B Olinares

The Rockefeller University, New York

Recent advances in single-particle cryogenic electron microscopy (cryo-EM) have enabled the structural determination of numerous protein assemblies at high resolution, yielding unprecedented insights into their function. However, despite its extraordinary capabilities, cryo-EM remains time-consuming and resource intensive. It is therefore beneficial to have a means for rapidly assessing and optimizing the quality of samples prior to lengthy cryo-EM analyses. To do this, we have developed a native mass spectrometry (nMS) platform that provides rapid feedback on sample quality and highly streamlined biochemical screening. Because nMS enables accurate mass analysis of protein complexes, it is well suited to routine evaluation of the composition, integrity, and homogeneity of samples prior to their plunge-freezing on EM grids. We demonstrate the utility of our nMS-based platform for facilitating cryo-EM studies using structural characterizations of bacterial transcription complexes, with examples that include near atomic resolution structures of reaction intermediates of a bacterial RNA polymerase at work. In cases where it has not been feasible to obtain cryo-EM structures of intermediates, we have explored an nMS-based methodology that monitors the mass changes in multiprotein assemblies as they undergo or perform specific chemical reactions. The method involves continuous, real-time sampling of the reaction as it is being directly electrosprayed into the mass spectrometer. Here, we demonstrate the utility of the method for dissecting the peptidase and ATPase activities of a multi-functional membrane protein transporter, capturing relevant intermediates and uncovering reaction trajectories that revealed key details on reaction stoichiometry, sequence of events, and together with cryo-EM structures ultimately provide a model for the transport cycle.



Molecular footprints of protein aggregation

Frank Sobott

School of Molecular and Cellular Biology, Faculty of Biological Sciences, University of Leeds, Leeds, UK.

Astbury Centre for Structural and Molecular Biology, Faculty of Biological Sciences, University of Leeds, Leeds, UK.

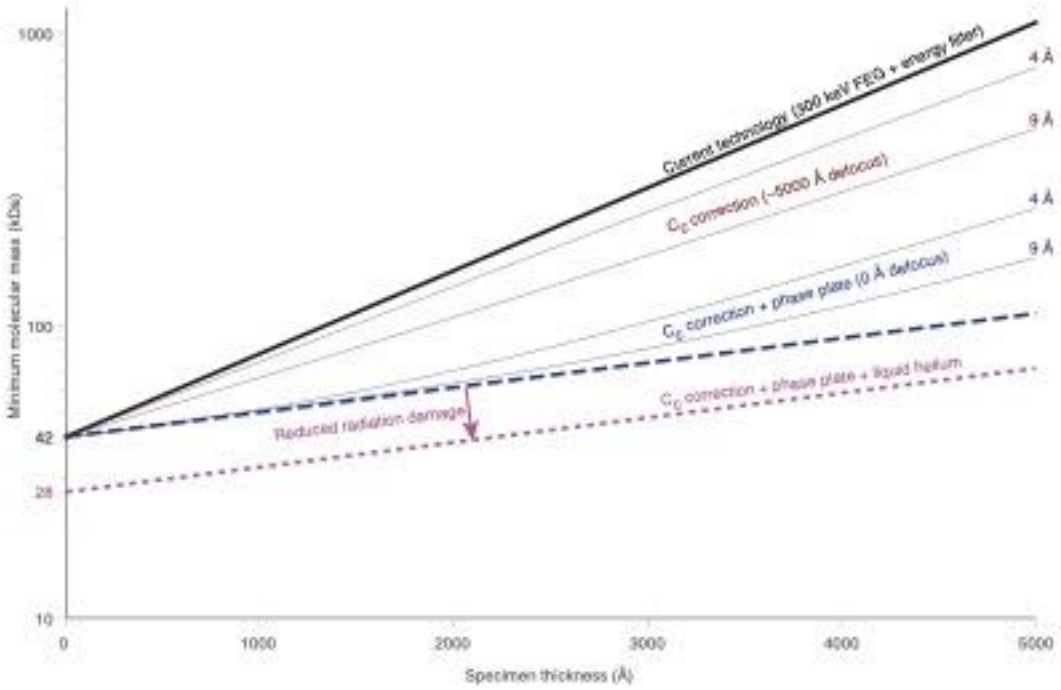
In this contribution we will discuss a structural MS toolbox which comprises a comprehensive set of methods to characterize stoichiometry, global size and shape of amyloid-related proteins such as α-synuclein and IAPP, implicated in Parkinson's disease and Type 2 diabetes, respectively, as well as their oligomerization/aggregation and interactions. We are developing methods which address aspects of dynamic and heterogeneous protein conformations and assemblies, both in vitro and in vivo, using an integrated structural approach based on "native" mass spectrometry and top-down structure-sensitive fragmentation, limited digestion, ion mobility, H/D exchange, crosslinking and surface mapping techniques (e.g. covalent labelling by Fast Photochemical Oxidation of Proteins) in combination with electron microscopy and other biophysical approaches. Together with computational modelling, this will allow us to significantly improve our fundamental understanding of key species which are implicated in debilitating neurodegenerative diseases.

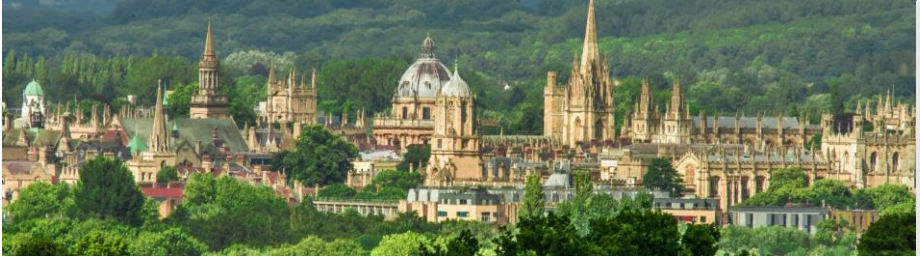
The potential of electron cryomicroscopy in situ: identifying molecules in cells

Christopher J. Russo,[1] Joshua L. Dickerson,[1] Katerina Naydenova[1]

[1] MRC Laboratory of Molecular Biology, Francis Crick Avenue, Cambridge CB2 0QH UK

Electron cryomicroscopy (cryoEM) of biological specimens preserved by vitrification in water ice has made great strides in the last decade. The atomic structure of most biological macromolecules can, at least in principle, be determined by direct imaging using bright field phase contrast. Major technological advances – in electron imaging hardware, data analysis software, and cryogenic specimen preparation technology – continue at pace and contribute to the exponential growth in the number of atomic structures determined by cryoEM. It is now likely, that within a few years we will have structures for hundreds of thousands of unique protein and nucleic acid molecular complexes. But the answers to many important questions in biology would become obvious if we could identify these structures precisely inside cells with quantifiable error. In the context of an abundance of known structures, it is appropriate to now consider the current state of electron cryomicroscopy for frozen specimens prepared directly from cells, and try to understand what technology can be brought to bear on this goal, both now and in the foreseeable future.

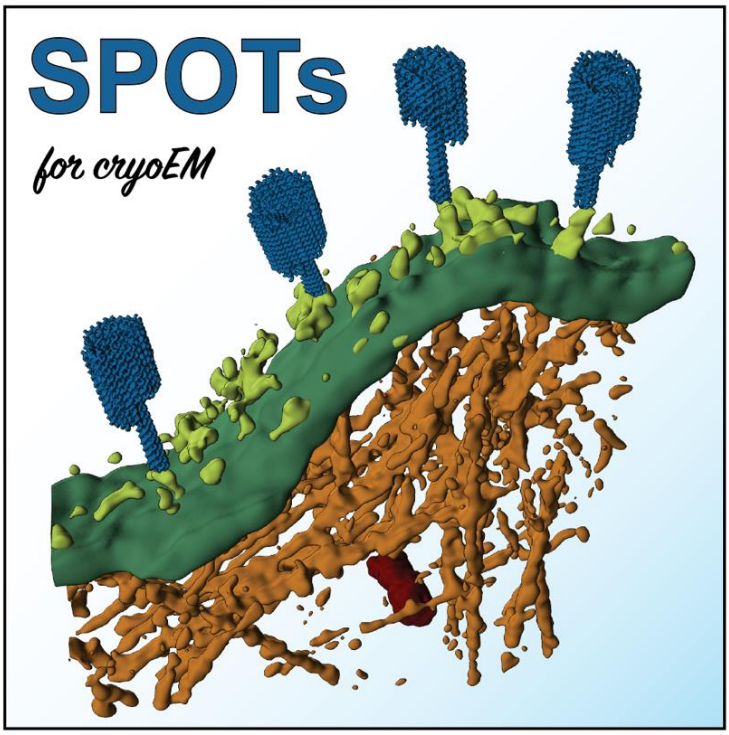




Identifying single molecules in heterogeneous data from cryoET

Lindsay Baker

Department of Biochemistry, University of Oxford, Oxford, OX1 3QZ, UK



Electron cryotomography (cryoET) can reveal molecular details about native biological samples such as membranes, viruses, and cells. However, due to sample crowding and low signal-to-noise ratios, individual molecules within tomograms are challenging to identify. We have developed a tagging strategy for cryoET that precisely identifies individual proteins, using DNA origami methods to produce 'molecular signposts' that target molecules of interest. This talk will describe development and applications of these tags, as well as limitations and future possibilities for expanding their use.

Microsecond Time-Resolved Cryo-Electron Microscopy

Jonathan M. Voss, Oliver F. Harder, Gabriele Bongiovanni, Pavel K. Olshin, Marcel Drabbels, and Ulrich J. Lorenz

Ecole Polytechnique Fédérale de Lausanne (EPFL), Laboratory of Molecular Nanodynamics, CH-1015 Lausanne, Switzerland

While cryo-electron microscopy (cryo-EM) is rapidly becoming the dominant method in structural biology, its time resolution is currently insufficient to directly observe proteins in action, leaving our understanding of these nanoscale machines fundamentally incomplete. Here, we demonstrate a novel approach to time-resolved cryo-EM that affords microsecond time resolution. Our method is illustrated in Fig. 1. It involves melting a cryo sample *in situ* with a laser beam (a), which allows dynamics of the embedded particles to occur in liquid once a suitable stimulus is provided, for example by releasing a caged compound (b). While the dynamics occur, the heating laser is switched off at a well-defined point in time (c), causing the sample to rapidly recool, so that it vitrifies, and the particles are trapped in their transient configurations (d), in which they can subsequently be imaged (e). We demonstrate that our approach affords a time resolution of 5 µs or better. Moreover, near-atomic resolution reconstructions show that the revitrification process does not damage the protein structure. Finally, we present initial results on a microsecond time-resolved pH jump experiment, demonstrating that our method is suitable to observe atomic-scale dynamics with microsecond time resolution. It thus promises to fundamentally advance what we can learn about the function of proteins.

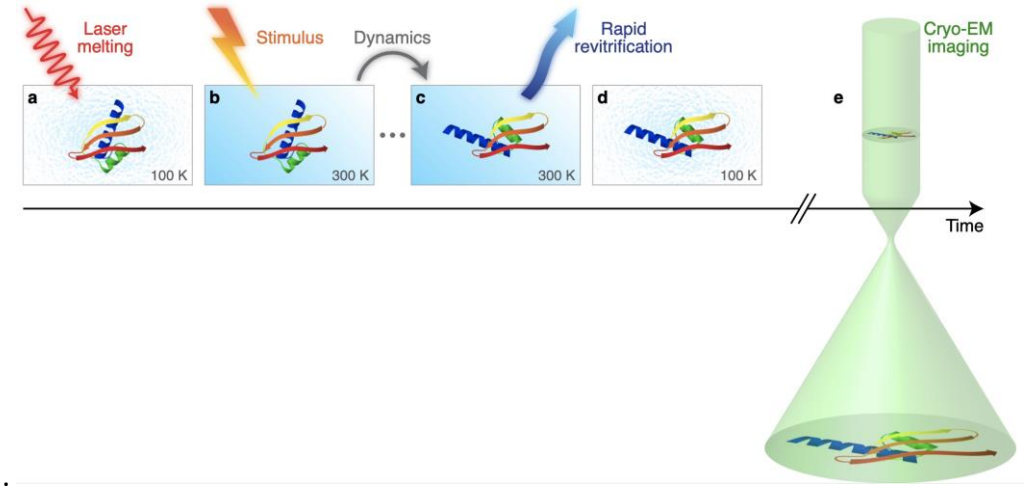
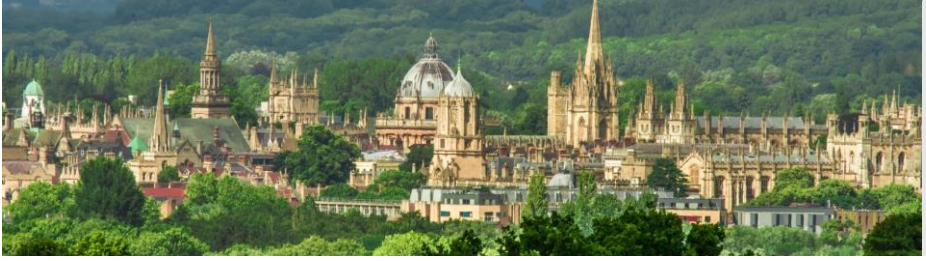


Figure 1. Experimental concept for microsecond time-resolved cryo-EM.

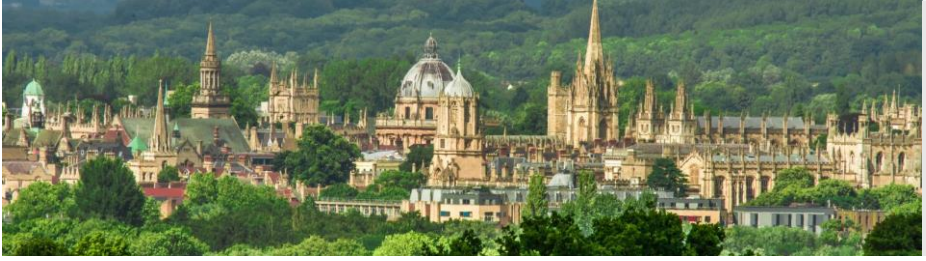


Submolecular Insights into Interfacial Water by Hydrogen-Sensitive Scanning Probe Microscopy

Ying Jiang

International Center for Quantum Materials, School of Physics, Peking University, Beijing, 100871, People's Republic of China

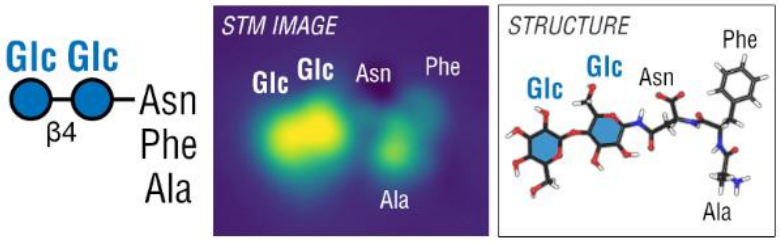
Water-solid interfaces have attracted extensive attention due to the crucial roles in a wide range of chemical and physical processes, such as ice nucleation and growth, dissolution, corrosion, heterogeneous catalysis and electrochemistry. To understand these processes, enormous efforts have been made towards the molecular-level understanding of the structure and dynamics of water on various solid surfaces. Using scanning probe microscopy (SPM), many remarkable structures of hydrogen bonding (H-bonding) networks have been directly visualized, which significantly advances our understanding of the delicate competition between water–water and water–solid interactions. Moreover, the detailed dynamics of water molecules, such as diffusion, clustering, dissociation, intermolecular and intramolecular proton transfer, have been investigated in a well-controlled manner by tip manipulation. However, resolving the submolecular structure of surface water has remained a great challenge for a long time due to the small size and light mass of protons. Discerning the position of hydrogen in water is not only crucial for the accurate determination of the structure of H-bonding networks, but also is indispensable in probing the proton transfer dynamics and the quantum nature of protons. In this talk, I will introduce the recent development of high-resolution scanning tunneling microscopy/spectroscopy (STM/S) and qPlus-based atomic force microscopy (AFM), which allows the access to the degree of freedom of protons in both real and energy space. I will then showcase its applications in probing the structures, dynamics and nuclear quantum effects (NQEs) of surface water and ion hydrates at the submolecular level.



Visualizing Glycans and Glycoconjugates One-at-a-time

Kelvin Anggara[1]

[1] Max Planck Institute for Solid State Research, Stuttgart, Germany



STM image and structure of a single glycopeptide

Glycans (a.k.a carbohydates) are the most abundant biomolecules on Earth – playing key roles in intercellular signaling and energy storage. Among all classes of biomolecules, structural elucidation of glycans remains a difficult problem that hinders efforts to understand glycan properties. Ensemble-averaged methods to elucidate glycan structures are met with difficulties due to the high flexibility and complexity of glycans. Here we bypass these limitations by using scanning tunneling microscopy (STM) to image single glycan molecules on surface, landed using electrospray ion-beam deposition (ES-IBD).

Imaging a single glycan molecule directly reveals how its constituent monosaccharide subunits connect to one another i.e. its primary structure [1]. Imaging multiple structures of a glycan reveals its shapes/conformations i.e. its secondary structure [2] and its flexibility at the single linkage level [3]. Our approach permits the structures and mechanical properties of glycans to be determined for any molecule that can be electrosprayed. We have recently extended our methods to reveal the structures of glycoconjugates (i.e. glycans covalently attached to peptides, lipids, or proteins) one-molecule-at-a-time, starting from simple glycopeptides and glycolipids to complex glycosaminoglycans and glycoproteins.

[1] X. Wu, M. Delbianco, K. Anggara, T. Michnowicz, A. Pardo-Vargas, P. Bharate, S. Sen, M. Pristl, S. Rauschenbach, U. Schlickum, S. Abb, P. H. Seeberger, and K. Kern, Nature 582, 375 (2020).

[2] K. Anggara, Y. Zhu, M. Delbianco, S. Rauschenbach, S. Abb, P. H. Seeberger, and K. Kern, J. Am. Chem. Soc. 142, 21420 (2020).

[3] K. Anggara, Y. Zhu, G. Fittolani, Y. Yu, T. Tyrikos-Ergas, M. Delbianco, S. Rauschenbach, S. Abb, P. H. Seeberger, and K. Kern, Proc. Natl. Acad. Sci. 113, e2102168118 (2021).

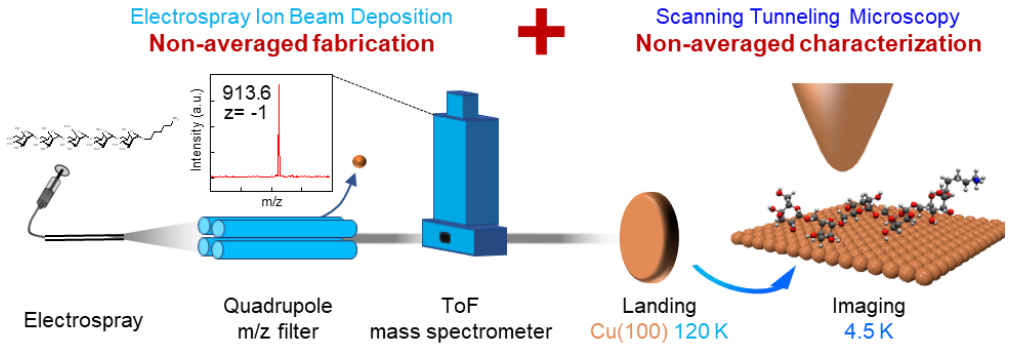
Non-averaged Imaging of Biomolecules

Xu Wu,[1] Kelvin Anggara,[1] Stephan Rauschenbach,[2] Klaus Kern[1]

[1] Max Planck Institute for Solid State Research, Stuttgart, Germany

[2] Department of Chemistry, University of Oxford, Oxford, UK

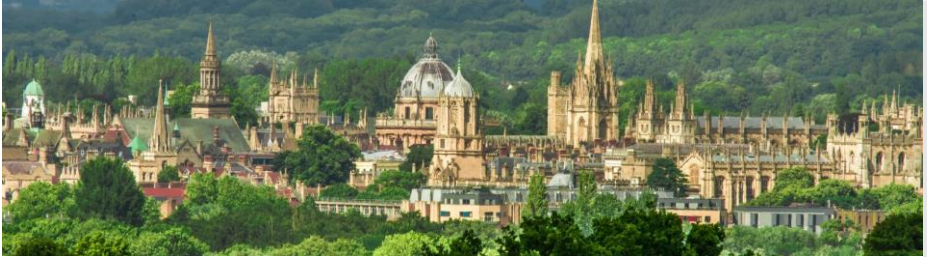
Imaging biomolecules guides the understanding of their diverse structures and functions. Real space imaging at sub-nanometer resolution by cryo-electron microscopy has provided key insights into proteins and their assemblies. Direct molecular imaging of intrinsic disordered biomolecules, such as glycans, the predominant biopolymers on earth with a plethora of structural and biological functions, is currently not possible. Inherent glycan complexity and backbone flexibility require single molecule approaches for real space imaging, instead of the indirect visualizing used currently. Here, we show direct imaging of single glycan molecules using the combination of mass-selective, soft-landing electrospray ion-beam deposition (ES-IBD) and low temperature scanning tunneling microscopy (STM).[1] Sub-nanometer resolution allows for the visualization of glycan connectivity and discrimination between regio-isomers.[2,3] With this technique, other flexible biomolecules, such as glycopeptides, glycolipids, can be imaged as well, and moreover, the protonation of biomolecules can be investigated in atomic scale. Direct single molecule imaging is an important step towards a better understanding of the structure of biomolecules.



The schematic of the combination with ES-IBD and STM

References:

1. Rauschenbach, S.; Ternes, M.; Harnau, L.; Kern, K. Annu. Rev. Anal. Chem. 2016, 9, 473.
2. X. Wu, et al. Nature, 2020, 582, 375.
3. Kelvin Anggara, et al. J. Am. Chem. Soc. 2020, 142, 21420.



High-throughput Collision Induced Unfolding as a Screening Technology for High-Resolution Structural Biology

Brandon T. Ruotolo

University of Michigan, Department of Chemistry

Medicines of the future will rely heavily upon our ability to quickly assess the structures and stabilities of complex macromolecular machines, as well as the influence of large libraries of conformationally-selective small molecule binders and protein-based biotherapeutics. Such endeavours are nearly insurmountable with current tools. In this presentation, I discuss recent developments surrounding collision induced unfolding (CIU) methods that aim to bridge this technology gap. CIU uses native ion mobility-mass spectrometry (IM-MS) to measure the stability and unfolding pathways of gas-phase proteins, without the need for covalent labels or tagging, and consuming 10-100 times less sample than almost any other label-free technology. Recent developments in high-throughput CIU screening methods, their ability to track alterations in target structure over a wide array of proteoforms, the ability of CIU to inform high-resolution structural biology campaigns (*e.g.,* using CryoEM), and software developments that seek to enhance CIU information content, will all be discussed.

Low-energy electron holography and coherent diffraction imaging

Tatiana Latychevskaia,[1]

[1] University of Zurich, Switzerland

[2] Paul Scherrer Institute, Villigen, Switzerland

Principles of in-line, or Gabor-type, holography will be introduced and holography with low energy (30 – 300 eV) electrons will be presented in detail, including: dedicated electron microscope arrangement [1], sample preparation, holograms acquisition and reconstruction. Low-energy electron holography of individual macromolecules, proteins [2, 3], adsorbates on graphene [4, 5], and other samples will be presented. The current challenges in experiments, data analysis and the resolution limits will be overviewed.

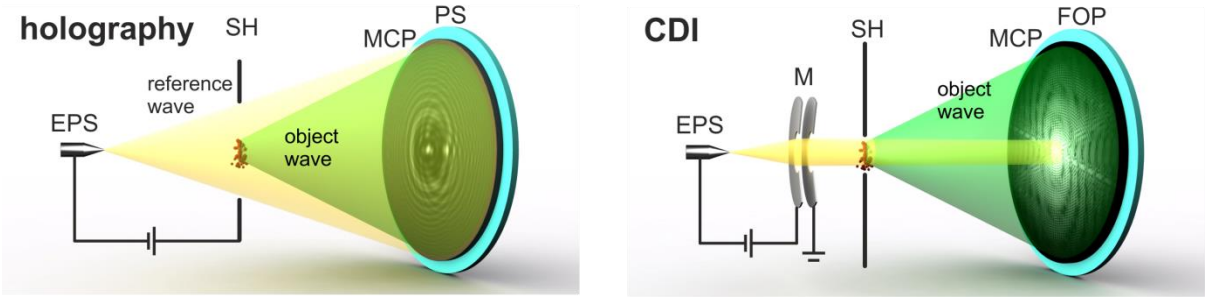


Figure. Coherent low-energy electron microscopes for holography and coherent diffraction imaging acquisition. EPS: electron point source; SH: sample holder; MCP: micro-channel plate; PS: phosphorous screen; M: microlens (M); FOP: fiber optic plate. Adapted from [6].

1. H.-W. Fink, W. Stocker, and H. Schmid, "Holography with low-energy electrons," Phys. Rev. Lett. **65**, 1204–1206 (1990).
2. T. Latychevskaia, J.-N. Longchamp, C. Escher, and H.-W. Fink, "Coherent diffraction and holographic imaging of individual biomolecules using low-energy electrons," in *Advancing Methods for Biomolecular Crystallography*(Springer, 2013), pp. 331–342.
3. J.-N. Longchamp, S. Rauschenbach, S. Abb, C. Escher, T. Latychevskaia, K. Kern, and H.-W. Fink, "Imaging proteins at the single-molecule level," PNAS **114**, 1474–1479 (2017).
4. T. Latychevskaia, F. Wicki, J.-N. Longchamp, C. Escher, and H.-W. Fink, "Direct observation of individual charges and their dynamics on graphene by low-energy electron holography," Nano Lett. **16**, 5469–5474 (2016).
5. M. Lorenzo, C. Escher, T. Latychevskaia, and H. W. Fink, "Metal adsorption and nucleation on free-standing graphene by low-energy electron point source microscopy," Nano Lett. **18**, 3421–3427 (2018).
6. T. Latychevskaia, "Phase retrieval methods applied to coherent imaging," Advances in Imaging and Electron Physics **218**, 1–62 (2021).

Amplitude and Phase Reconstruction of Low Energy Electron Holograms of individual Proteins

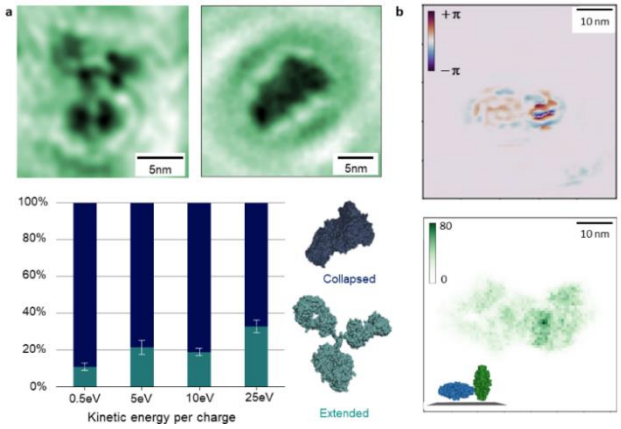
Hannah Ochner,[1] Sven Szilagyi,[1] Moritz Edte,[1] Sabine Abb,[1] Joseph Gault,[2] Carol V. Robinson,[2] Luigi Malavolti,[1] Stephan Rauschenbach,[1,2] Klaus Kern,[1,3]

[1] Max Planck Institute for Solid State Research, Heisenbergstr. 1, 70569 Stuttgart, Germany

[2] Department of Chemistry, University of Oxford, 12 Mansfield Road, Oxford OX1 3TA, UK

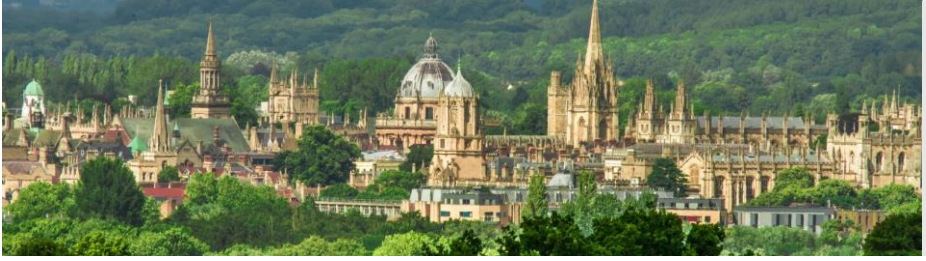
[3] Institut de Physique, École Polytechnique Fédérale de Lausanne, 1015 Lausanne, Switzerland

Low-energy electron holography (LEEH) allows for the single-molecule imaging of individual proteins and hence can serve as a promising complementary method to established biomolecular imaging techniques. Since holography is not a real-space imaging method, the numerical reconstruction of the object from the measured hologram, in which the information resulting from the interaction of the incident electron beam with the molecule is encoded in the form of an interference pattern, is a central part of the method. Biological matter in general has both absorbing and phase-shifting characteristics, hence structural information about the imaged molecules can be extracted from both amplitude and phase reconstructions. A one-step amplitude reconstruction yields information about the size and shape of the imaged objects and can thus be used for imaging conformational variability and to track the influence of the sample preparation process, as will be discussed using the example of IgG antibodies.



a *Top: Amplitude reconstruction of an IgG antibody in an extended Y-shaped conformation (left) and of an IgG antibody in a collapsed gas phase-related conformation (right). Bottom: Percentage of extended and collapsed structures observed on the surface depending on landing energy.* ***b*** *Phase reconstruction (top) and projected density (bottom) of two β-Galactosidase molecules with different orientations with respect to the graphene substrate (see inset). Regions of higher projected density correlate with regions of higher phase shift.*

Phase retrieval, on the other hand, requires an iterative reconstruction method. The resulting phase distributions map the number of scatterers in the electron path, which points to a sensitivity of phase imaging to molecular thickness and to changes in local potential.

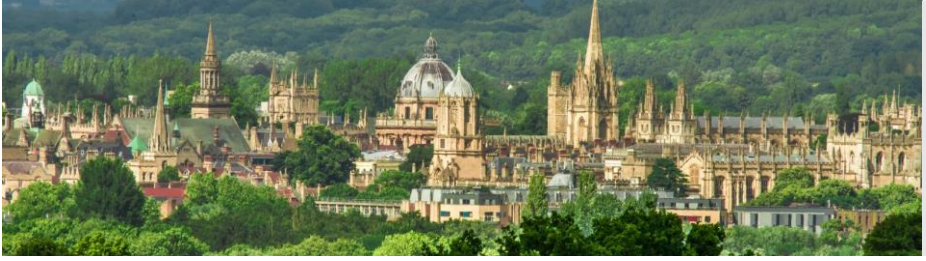


Free-energy profiles from cryoEM particles

Pilar Cossio,[1]

[1] Center for Computational Mathematics, Flatiron Institute, USA

Cryo-electron microscopy (cryo-EM) is an experimental technique that measures single-particle projections of biomolecules. Although single-particle cryo-EM is widely used for 3D reconstruction, it has the potential to provide information about a biomolecule's conformational variability, which leads to the underlying free-energy landscape of the system. However, cryo-EM images are challenging to analyze due to their low signal-to-noise ratio. To address these issues, we developed the cryo-BIFE method. This method uses a path collective variable together with a Bayesian approach to infer free-energy profiles and their uncertainties from cryo-EM raw images. We apply the method over a diverse set of synthetic and real systems, finding that the signal-to-noise ratio and pose estimate as key determinants to extracting accurate profiles.



Cryogenic Electron Ptychographic Single Particle Analysis (Cryo-EPt SPA)

Xudong Pei[1], Liqi Zhou[1], Chen Huang[2], Mark Boyce[3], Judy S. Kim[2,4], Emanuela Liberti[2], Takeo Sasaki[5], Peijun Zhang[3,6], David I. Stuart[3], Angus I. Kirkland[2,4,7] and Peng Wang[1,8]

[1] College of Engineering and Applied Sciences and Collaborative Innovation Center of Advanced Microstructures, Nanjing University, Nanjing 210093, China
[2] The Rosalind Franklin Institute, Harwell Science and Innovation Campus, Didcot, OX11 0FA, UK
[3] Division of Structural Biology, Welcome Trust Centre for Human Genetics, University of Oxford, Oxford, OX3 7BN, UK
[4] Department of Materials, University of Oxford, Parks Road, Oxford OX1 3PH, UK
[5] JEOL Ltd., 3-1-2 Musashino, Akishima, Tokyo 196-8558 Japan.
[6] Electron Bio-Imaging Centre, Diamond Light Source, Harwell Science and Innovation Campus, Didcot OX11 0DE, UK
[7] Electron Physical Sciences Imaging Centre, Diamond Light Source Ltd., Harwell Science and Innovation Campus, Didcot, OX11 0DE, UK
[8] Department of Physics, University of Warwick, Coventry CV4 7AL, UK

Cryo-electron microscopy (cryo-EM) is a powerful method for the high-resolution three-dimensional structural characterization of a wide range of biological samples in a close-to-native, frozen-hydrated state. Cryo-electron ptychography (Cryo-EPty) [1] as shown in Fig. 1a is an alternative technique based on scanning ptychographic diffractive imaging [2]. Ptychography uses a defocused probe to scan over a specimen with highly overlapping probe positions. As ptychography utilizes the full diffraction pattern, it is dose-efficient particularly when data is recorded using direct electron detectors, which record high signal-to-noise at low electron dose [1]. Here, we show a new 3D SPA technique based upon cryo-EPty SPA and experimentally demonstrate that it can restore 3D information from a single sample. Experimental cryo-EPty SPA datasets (Fig. 1b) [8], the ptychographic phase of rotavirus double-layered particles (DLPs) were reconstructed at a dose of 22.7 e/A^2. The particle-picking procedures that have been developed for cryo-EM SPA can be directly applied to the phase, giving a positionally coordinated stack of particle phases, as shown in Fig. 1b. A 3D density map of rotavirus DLPs (Fig. 1 c) can be reconstructed with 300 particles from the stack of particle phases [1]. We expect that cryo-EPty combined with SPA has great potential to yield high-resolution 3D reconstructions of biological samples[3].

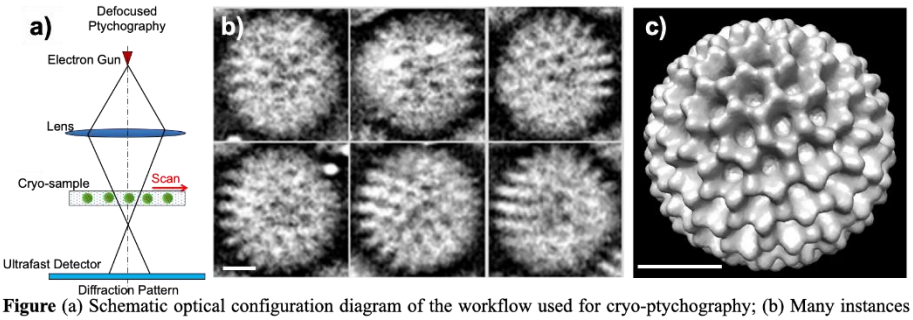
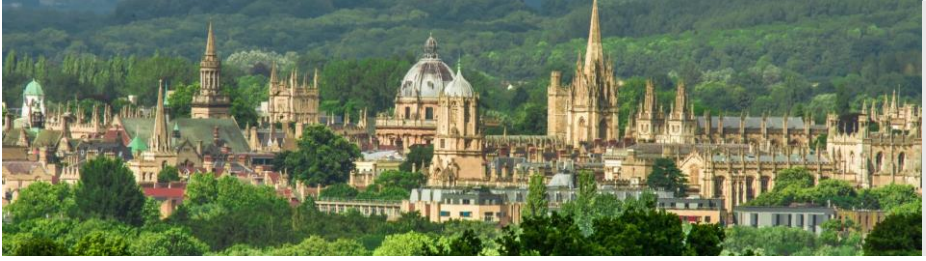


Figure (a) Schematic optical configuration diagram of the workflow used for cryo-ptychography; (b) Many instances of the viral particles for single particle analysis can be extracted from reconstructed ptychographic phases, scale bars: 20 nm. c) 3D map corresponding to the particle instances, scale bars: 25 nm

References:

[1] L Zhou, et al., Nature Communications **11** (2020), p. 2773.
[2] JM Rodenburg, Advances in Imaging and Electron Physics **150** (2008), p. 87.

[3] The authors acknowledge funding from University of Warwick Research Development Fund (RDF) 2021-22 Science Development Award.



High-resolution imaging for sub-molecular scale characterisation of conjugated polymers

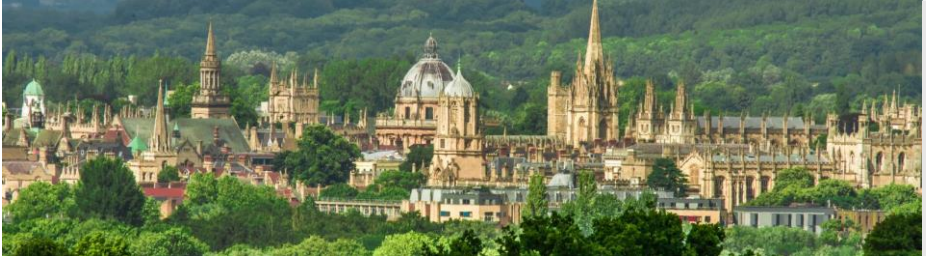
Stefania Moro,[1,2] Michael Sommer,[3] Iain McCulloch,[4,5] Hugo Bronstein,[6] Giovanni Costantini[1,2]

[1] Department of Chemistry, University of Warwick, Coventry, UK
[2] Department of Chemistry, University of Birmingham, Birmingham, UK
[3] Department of Chemistry, Technische Universität Chemnitz, Chemnitz, Germany
[4] King Abdullah University of Science and Technology (KAUST), Physical Science and Engineering Division, Thuwal, Saudi Arabia
[5] Department of Chemistry, Chemistry Research Laboratory, University of Oxford, Oxford, UK
[6] Department of Chemistry, University of Cambridge, Cambridge, UK

Conjugated polymers (CPs) are promising materials for modern organic electronics, combining structural and electronic adaptability to deliver efficient and low-cost devices.[1] The molecular-scale characterisation of conjugated polymers is, however, still unsatisfactory, as analytical techniques that can provide high-resolution information on polymers are scarce, are averaging ensemble methods and are often limited by the strong tendency towards aggregation of CPs.[2] In this context, we report here an innovative approach to study CP, *i.e.* the combination of ultrahigh vacuum (UHV) electrospray deposition (ESD) with low-temperature scanning tunnelling microscopy (LT-STM).[3] This method allows for intact deposition of thermally fragile macromolecules and their characterisation molecule-by-molecule at the ultimate spatial resolution of UHV-STM, revealing the self-assembly, length distribution, sequence, and exact chemical structure of the materials.

In this talk I will demonstrate the power and versatility of ESD-STM for a variety of different systems, where the information obtained from our images has been used to investigate fundamental problems in the microscale behaviour of CPs, as to directly compare the efficiency of different polymerisation techniques, to understand the effect of different side chains on the assembly and packing of materials, and to fully characterise CPs where conventional techniques as nuclear magnetic resonance or gel chromatography cannot be fully trusted. These results show how ESD-STM can push beyond the conventional analytical limits and grants access to materials that could not have been characterised before with high levels of detail.

[1]Polymer 2020, 207, 122874; [2]Sci. Rep. 2017, 7, 1078; Prog. Polym. Sci. 2018, 83, 135-201; [3]Sci. Adv. 2018, 4, 6, 9543



Visualization of D- and L-glycan assemblies and their chirality by high-resolution scanning tunnelling microscopy

Johannes Seibel,[1,6] Giulio Fittolani,[2,5] Hossein Mirhosseini,[3] Martina Delbianco,[2] Kelvin Anggara,[1] Xu Wu,[1] Stephan Rauschenbach,[4] Peter H. Seeberger,[2,5] Thomas D. Kühne,[3] Uta Schlickum,[6,1] and Klaus Kern[1,7]

[1] Max Planck Institute for Solid State Research, Stuttgart, Germany

[2] Max Planck Institute for Colloids and Interfaces, Potsdam, Germany

[3] Dynamics of Condensed Matter and Center for Sustainable Systems Design, Chair of Theoretical Chemistry, University of Paderborn, D-33098 Paderborn, Germany

[4] Department of Chemistry, University of Oxford, Oxford, UK

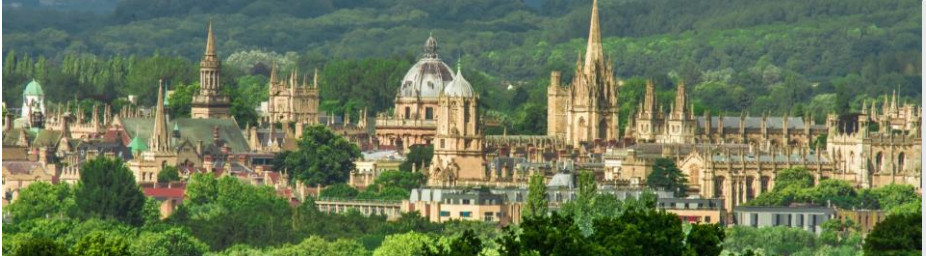
[5] Institute for Chemistry and Biochemistry, Free University Berlin, Berlin, Germany

[6] Institute of Applied Physics, Technische Universität Braunschweig, Braunschweig, Germany

[7] Institut de Physique, École Polytechnique Fédérale de Lausanne, Lausanne, Switzerland

The main molecular building blocks of life appear predominantly in one handedness. Among these, carbohydrates are the most abundant biomolecules and play important roles in areas ranging from sustainable and environmentally friendly materials to cellular processes and cell recognition. However, characterization of cellulose materials at the single glycan level remains elusive. Only recently, mass-selective, soft-landing electrospray ionization deposition of glycan molecules and their subsequent characterization by scanning tunneling microscopy has emerged as characterization tool for glycan molecules with real-space information down to the single glycan level.1 Here, we use STM imaging to visualize and differentiate D- and L-cellohexaose on the single molecule level. By modifying the STM tip with a CO molecule and using constant-height scanning, the improved resolution allows the differentiation between D- and L-cellohexaose on the single glycan level. Further, the deposition of enantiopure D- or L-cellohexaose reveals their assembly in mirror-symmetric chiral patterns and the deposition of a racemic mixture revealed conglomerate formation, which is, to the best of our knowledge, the first example of a crystallization-based separation of racemic carbohydrates.

1Wu, X.; Delbianco, M.; Anggara, K.; Michnowicz, T.; Pardo-Vargas, A.; Bharate, P.; Sen, S.; Pristl, M.; Rauschenbach, S.; Schlickum, U.; Abb, S.; Seeberger, P. H.; Kern, K. Imaging Single Glycans. Nature 2020, 582, 375



Integration of Orbitrap Mass Spectrometry and Novel In-Vacuo Techniques: Abundant Marriage Opportunities

Alexander Makarov,[1] Kyle Fort,[1] Tobias Woerner[1]

[1] Thermo Fisher Scientific, Bremen, Germany

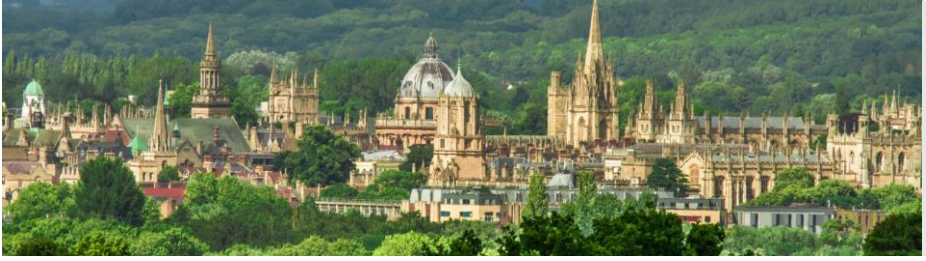
The widespread adoption and use of the Orbitrap analyser for mass spectrometry has been driven by its characteristic ability to acquire high-resolution, high mass accuracy data for qualitative and quantitative analyses. This presentation provides an overview of several directions of recent research, which are all united by a common theme of development of instrumentation integrating this analyser with emerging analytical techniques operating under high vacuum conditions.

A first example of such integrated instrumentation brings Orbitrap-based analysis to secondary ion mass spectrometry imaging for life-sciences applications, including sub-cellular 3D imaging of metabolites, imaging of bacteria and biofilms. Data acquired for intact lipids, drugs and metabolites demonstrate the unique advantages and modes of operation which could be also combined with electron microscopy.

It has also been shown that Orbitrap analysis can be effectively combined with different soft-landing techniques. A particularly interesting example is the combination with an array of nanoelectromechanical systems (NEMS) where each individual device in the array measures the mass of just one molecule at a time, based on time-correlated frequency jumps of oscillating nanocantilevers. This approach provides an independent channel of direct mass measurement without the influence of charge state and could be used for analysis of substances in the megadalton mass range. Moreover, experimental imaging data of the soft-landed protein complexes show the mass spectrometer is capable of depositing these ions in a tight spatial area, allowing the mass spectrometer to function as a universal quality control and preparative tool. This capability could be easily extended to any technique utilizing soft landing of ions and, thanks to the recent extensions of the Orbitrap mass range, limitations on ion mass are practically eliminated.

In conclusion, future trends and outlook for instrumentation integrating the Orbitrap analyser with complementary analytical technologies are discussed.

POSTERS



Towards Characterization of Oxygenated Hemoglobin Conformations Using IMS-IMS on a Cyclic Instrument

Edie M. Sharon,[1] Lucas W. Henderson,[1] David E. Clemmer[1]

[1] Indiana University, Bloomington, Indiana

Ion mobility spectrometry-mass spectrometry (IMS-MS) is a valuable tool for studying protein structure. The recent development of a cyclic IMS instrument has increased the resolution of these measurements by lengthening the path of the separation. We first present results that demonstrate structural features in the hemoglobin tetramer that cannot be observed in shorter separations. The additional dimension of variable temperature electrospray ionization (vT-ESI) reveals differences in the temperature dependencies of these structures which indicates that they represent separate conformations in solution. The cyclic IMS instrument also allows for tandem IMS or "slicing" experiments where a portion of the mobility spectrum is isolated and subjected to additional separation. The results of slicing experiments for hemoglobin reveal some conformations whose temperature dependencies vary similarly to structures in the original spectra as well as others that vary differently, suggesting that additional conformations from solution are detected. Finally, we present preliminary results from multi-pass and slicing experiments performed on the oxygenated hemoglobin tetramer. Hemoglobin is known to undergo a structural change upon oxygen binding which gives rise to allosteric activity. Initial results suggest that similar structures are present in the oxygenated sample, but their abundances as a function of temperature vary differently which indicates that oxygen binding has stabilized some conformations and destabilized others. This work demonstrates the utility of cyclic IMS for adding to the current understanding of the structural heterogeneity of hemoglobin as it transitions between oxygenated and deoxygenated states.

The use of integrated mass spectrometry approaches to investigate hit compounds for pyroptosis inhibition

Emma Norgate,[1] Jonathan Hopper,[1] Idlir Liko,[1] Kleitos Sokratous,[1] Agni Gavriilidou,[1] Antonio Biasutto,[1] Srinath Krishnamurthy,[1] Karolina Gherbi,[1] Laurence Towner,[1] Vivi Mignone,[1] Lewis Pennicott,[1] Abena Amponsah,[1] Tatiana Silva,[1] Luigi De Colibus,[1] Katharina Dürr,[1] Ali Jazayeri.[1]

[1] OMass Therapeutics, Oxford, UK

The ability of native mass spectrometry to preserve weak non-covalent interactions enables detection of initial chemical hits from either drug-like compound libraries or fragment screens. Gasdermin D (GSDMD) was recently identified as the key executioner of pyroptosis, a pro-inflammatory form of cell-death and a pivotal innate immune response to a wide range of pathogen-associated and sterile-induced inflammation. A broad range of immunological and inflammatory diseases have already been linked to this key pathway, as well as numerous rare genetic disorders[1]. Despite growing interest, progressible compounds which possess the necessary specificity and functional potency have failed to be identified thus far.

We have employed integrated mass spectrometry approaches to drive hit-finding through automated native MS workflows, allowing hundreds of thousands of drug-like molecules and fragments to be screened against GSDMD. We were able to further characterise the potential mechanism of inhibition using native MS to monitor the recruitment of GSDMD and subsequent processing by caspase-1; a key step in this inflammatory pathway. HDX techniques coupled with molecular dynamics simulations predict distinct binding pockets which provide more informed chemistry designs which are currently being used to probe the SAR of these leads.

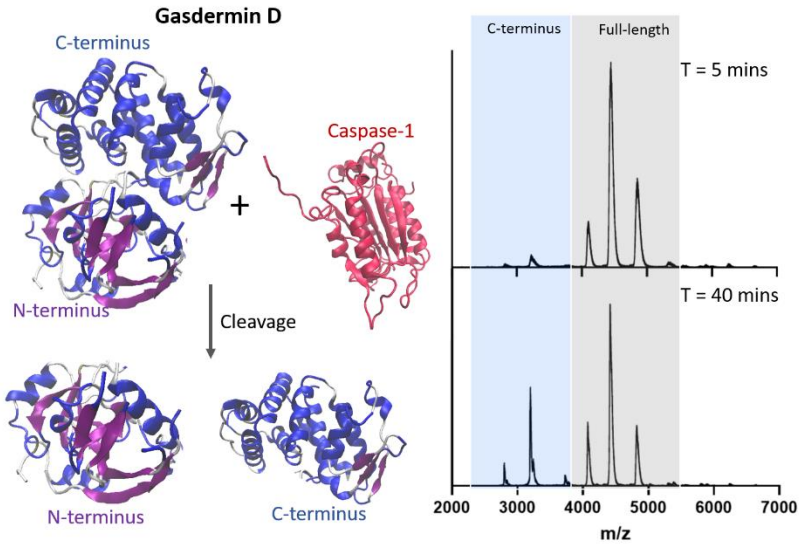


Figure 1: Gasdermin is activated by proteolytic processing by caspase. Novel hits which inhibit this process can be monitored using native MS.

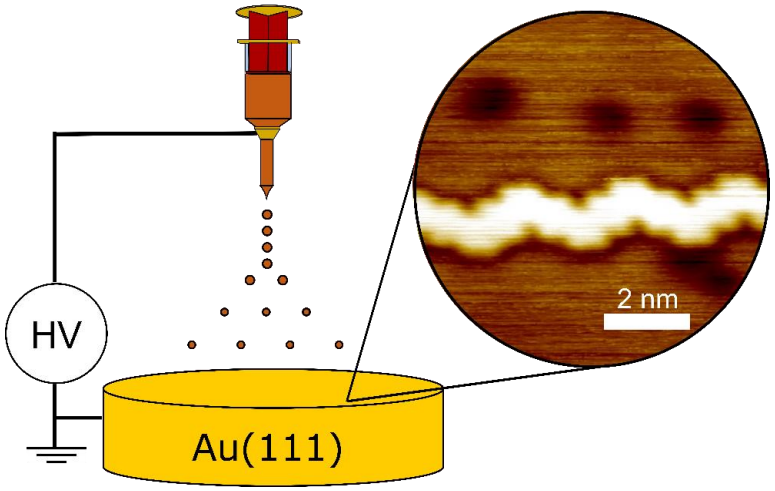
1. Liu, X., Xia, S., Zhang, Z., Wu, H., Lieberman, J., Channelling inflammation: gasdermins in physiology and disease, Nature Reviews Drug Discovery, 20, 2021

On-surface GNR fabrication via electrospray deposition of monomers and polymers from solution

Felix Baier,[1] Mamun Sarker,[2] Christoph Dobner,[1] Alexander Sinitskii,[2] Axel Enders[1]

[1] Universität Bayreuth, Bayreuth, Germany

[2] University of Nebraska - Lincoln, Lincoln, USA



Strategies for depositing large organic molecules such as proteins, DNA or graphene nanoribbons (GNRs) are urgently needed because the conventional method of thermal evaporation is impossible due to the size of the molecules. GNRs prepared in solution are of particular interest because they are longer compared to those synthesized on the surface and they can be produced in large quantities. Since GNRs form crystallites, they cannot be brought onto the surface by direct contact printing and characterized using STM. Therefore, a new electrospray setup was developed for the deposition of GNRs, large polymers and other molecules from a solution. The electrospray setup consists of a heatable stainless steel capillary to which a high voltage in the range -8 to 8 kV, with respect to the sample can be applied. The assembly is placed in a glovebox which ensures the cleanliness of the samples. The characterization of the deposits was done with STM under ultra high vacuum after sample transfer. For example, the deposition of TPTP monomers from solution onto Au(111) brought results, which were comparable to those by on-surface synthesis archives in UHV. Larger polymers were also deposited and completely cyclized on the surface after deposition, forming promising GNRs that have not been studied before. This new approach might open the door for the on-surface characterization of a large range of nano-objects formed in solution that can not be thermally evaporated or transferred onto the surface otherwise. Furthermore, it is possible to produce devices by electrospray deposition (ESD) from suitable GNRs that have been previously characterized with STM.

Figure 1: Schematic representation of the deposition process of the dissolved molecules on an Au(111) single crystal. The subsequent investigation is performed with the STM.

Enhanced declustering and charge-stripping enables mass determination of AAVs in Tof MS

Jakub Ujma, Kevin Giles, Malcolm Anderson, Keith Richardson

Waters Corporation, Wilmslow, United Kingdom

Adeno Associated Virus (AAV) capsids are the most attractive candidate for gene therapy vectors. These ball-like particles consist of 60 subunits of three viral proteins (VP1, VP2 and VP3) assembled in a variety of stoichiometries (expected VP ratio of [5:5:50]),[1] corresponding to an average mass of ~3.7 MDa. "Full" capsids encapsulate ~4.7 kb single-stranded DNA. Precise determination of capsid mass remains challenging due to the high level of adduction and heterogeneity in the VP ratio which yields congested distributions of overlapping charge state peaks. Herein, we demonstrate a method of enhanced declustering using a modified StepWave™ ion guide[2] as well as extensive charge stripping using an electron capture dissociation (ECD) device,[3] to enable determination of AAV mass distribution (Figure 1).

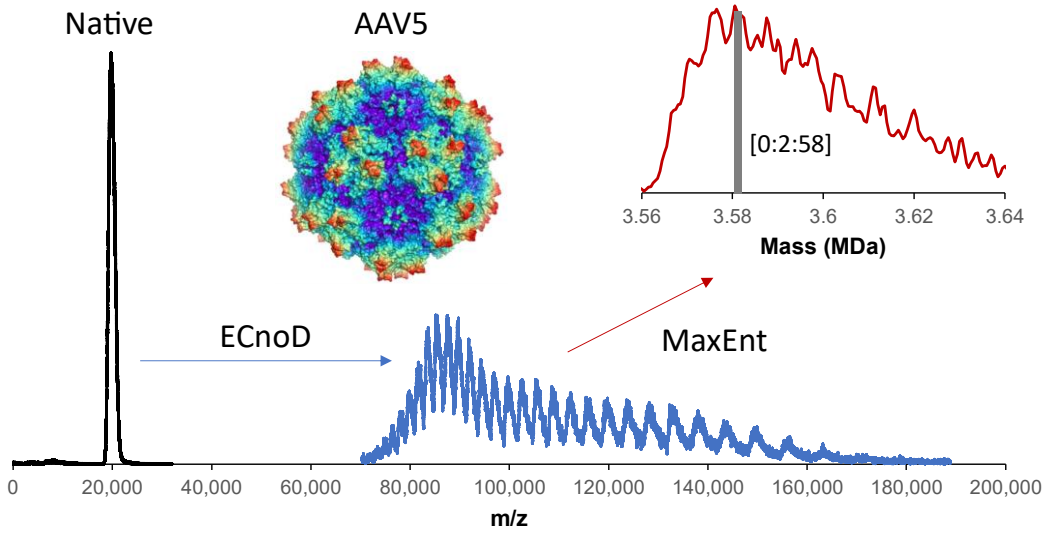
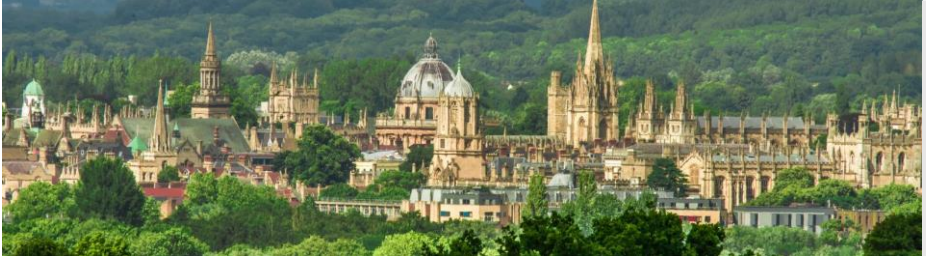


Figure 1. ToF-MS data of empty AAV5 capsids[4] obtained on a SELECT SERIES™ CYCLIC™ IMS instrument. Significant overlap of charge state peaks in the native spectrum (black) precludes the determination of mass. Passing the AAV5 ions through ECD cell results in "Electron Capture no Dissociation" (ECnoD) process and a significant m/z shift (m/z ~20,000 to ~90,000) yielding distinct peaks with increasing m/z spacing, characteristic of a charge state distribution (blue). Resolved charge state peaks enable the use of MaxEnt algorithm to deconvolve the mass distribution (red).

References:

[1] Johnson et al. Journal of Virology, 1971, 8, 860–863, [2] Sokratous et al. 68th ASMS "Reboot" Proceedings, 2020, 303789, [3] Beckmann et al. 69th ASMS Conference Proceedings, 2021, 303977, [4] AAV5 picture adapted from: Large et al. Viruses, 2021, 13, 1336., Stepwave, SELECT SERIES and Cyclic are trademarks of Waters Corporation



Harnessing native MS combined with cryo-EM and other biophysical techniques to develop new therapeutics for challenging membrane protein targets

Katharina L. Duerr,[1] Fernando Almeida[1], Reda Assal[1], Elvira Diamantopoulou[1], Agni Gavriilidou[1], Jon Hopper[1], Ali Jazayeri[1], Parth Kapoor[1], Srinath Krishnamurthy[1], Idlir Liko[1], Christine Oswald[1], Kundan Sharma[1], Ben Tehan[1], Shahid Rehan[1] & Chi Tung Wong[1,2]

[1] OMass Therapeutics Ltd., Oxford, United Kingdom

[2] Present address: GlaxoSmithKline Pharmaceuticals Ltd., Stevenage, United Kingdom

Historically, small molecule drug discovery has focused on targets that operate in relative isolation e.g. enzymes. However, many important molecular targets – Solute Carriers, GPCRs, Inflammasome – operate within a membrane or an intracellular complex. To drug these targets, we need to interrogate their full spectrum of physical interactions within the native ecosystem. Today, researchers are forced to make trade-offs:
In cell-based systems, researchers know that biology is observed with high fidelity. However, there is a disconnect between what is measured and the drug's action resulting in false leads and missed opportunities. In cell-free approaches, confounding factors have been stripped away giving precise data on how tightly or how quickly a drug binds to its target. However, the target protein no longer faithfully represents its living counterpart and endogenous biomolecules are absent. OMass' platform retains biological relevance at high resolution, delivering cell-system fidelity with cell-free precision. This approach has the following advantages:

- Faithful to the native ecosystem. We use proprietary biochemistry to preserve downstream partners, lipid interactions and endogenous regulatory molecules.
- Biology distilled to its essential elements. We use our patent protected native mass spectrometry (MS) technology to measure the physical interactions that drive biology.
- The full spectrum of pharmacology. We leverage custom chemistry enabled for MS to interrogate binding and multiple discrete biological outcomes rationally and systematically.

Here, we present the screening strategy and preliminary data for three programs in our current drug discovery pipeline. These are the potassium chloride transporter KCC2 (a target for Rett syndrome), the melanocortin 2 receptor MC2R (a target for the treatment of congenital adrenal hyperplasia) and the solute carrier SLC15A4/TASL complex (a target for lupus erythematosus and other inflammatory diseases).

Flexible ion guides for integrating mass spectrometry and microscopy platforms

Mazdak Taghioskoui[1]

[1] Trace Matters Scientific, Somerville, MA, United States

Simultaneous use of mass spectrometry and microscopy techniques is gaining popularity for characterizing biological samples due to the complementary structural and functional information they provide regarding proteins and protein complexes. A seamless integration of a mass spectrometer with upstream and downstream microscopy stations will provide a streamlined platform for multimodal protein analysis, and will allow for both upstream in situ sampling/ionization as well as downstream soft landing of ions for microscopy. However, constructing such a streamlined platform requires that significant technical challenges related to transporting ions between the instruments are addressed.

We will present a novel flexible ion guiding technology called sequentially-packed ion (SPion) transfer device that allows for connecting a mass spectrometer to an upstream microscopy station for in situ ionization/sampling or a downstream microscopy station for soft landing of ions. SPion employs radio frequency stacked-ring or multipole ion guides that are flexibly connected to each other and extends a mass spectrometer's internal ion optics. SPion allows for lossless transport of ions for several meters. The lossless ion transfer can be achieved via differential pressure between the two ends of SPion, or alternatively, via a novel ion transport mechanism by ion bunching. The benchmarking results of SPion coupled to a TSQ Altis triple quadrupole instrument will be presented, and some novel design concepts will be discussed.

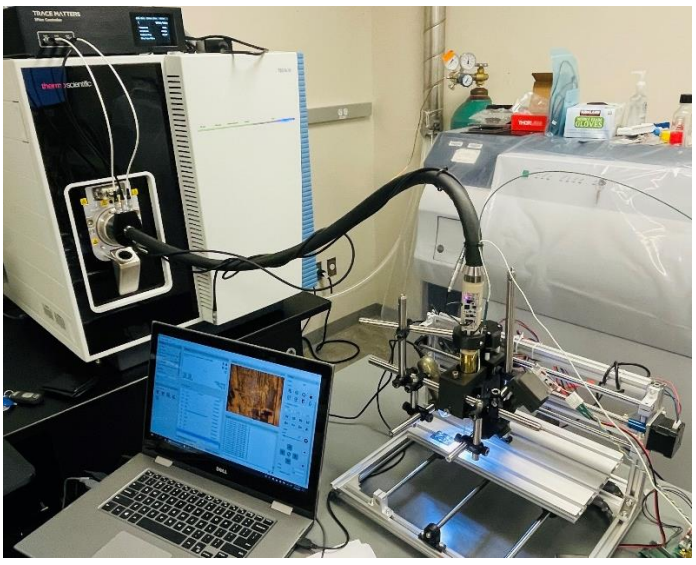


Figure 1. An exemplary implementation of SPion with a mass spectrometer and an imaging station.

Acknowledgment: The proof-of-principle demonstration results were obtained in collaboration with Thermo Fisher and Brigham and Women's Hospital of Harvard Medical School with partial support from NIH and NASA.

Characterizing the lipid-membrane protein interactions of an evolutionarily deprived in silico designed protein

Mia L. Abramsson,[1] Robin A. Corey,[2] Phillip J. Stansfeld,[3] and Michael Landreh[1]

[1] Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, 171 65, Stockholm, Sweden

[2] Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, UK

[3] School of Life Sciences & Chemistry, University of Warwick, Coventry CV4 7AL, UK

Membrane proteins (MPs) are major targets for drug discovery due to their importance in cellular function and survival. The membrane-spanning regions of MPs are embedded in the lipid bilayer. However, it is becoming increasingly clear that lipids can mediate changes to structure, function, and stability of MPs. Using an in silico designed protein called TMHC4_R [1], which lacks evolutionary constraints, as scaffold, we employ mutational studies to establish first principles for specific lipid recognition and lipid-mediated stabilization. Point mutations are designed with the help of artificial intelligence predictions and coarse-grained molecular dynamics simulations to create or disrupt the lipid interaction of this artificial protein. However, capturing the interactions between MPs and lipids is challenging for most biophysical approaches. Through native mass spectrometry (MS) combined with ion mobility spectroscopy (IM), we can monitor lipid binding and how it affects the oligomeric state and conformational stability of TMHC4_R mutants to identify sequence features that contribute to specific lipid recognition. Using coarse-grained simulations, we found that TMHC4_R interacts with most lipids in a non-specific manner but contains a specific binding site for cardiolipin (CDL) (Figure 1) [2]. Strikingly, native IM-MS revealed that despite being specifically recognized, bound cardiolipin has no pronounced effect on the stability of tetrameric TMHC4_R. These findings form a suitable basis for targeted mutational studies that connect cardiolipin recognition and conformational stabilization. In conclusion, our approach to identify first principles of lipid interactions can open new avenues for studying the interplay between lipids and MPs.

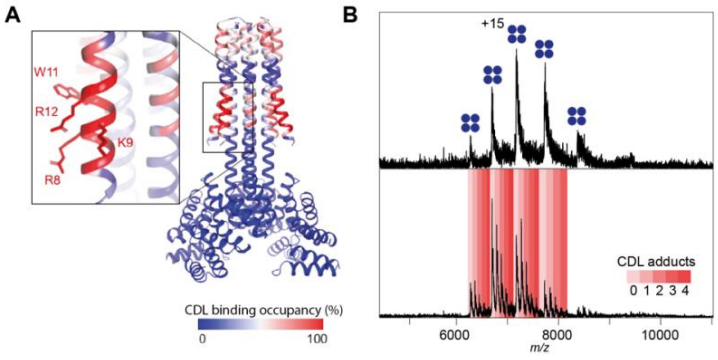
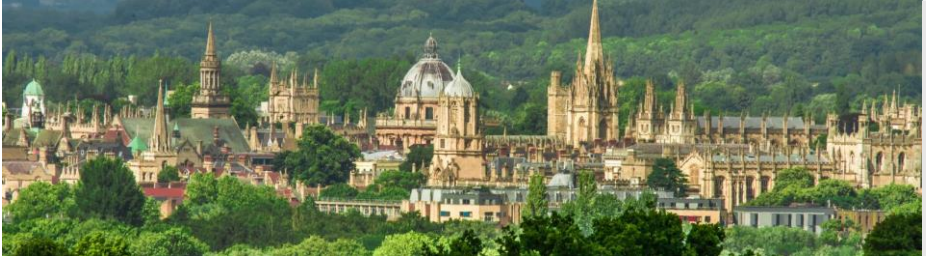


Figure 1. (A) Coarse-grained simulation of TMHC4_R in CDL-containing membranes reveals residues involved in specific CDL interaction. (B) Native MS spectra of tetrameric TMHC4_R without (top) and with (bottom) CDL, shown binding of up to four CDL molecules.

[1] Lu, P, et al. Accurate computational design of multipass transmembrane proteins. Science (2018) 359, 1042–1046. [2] Corey, R. A, et al. Identification and assessment of cardiolipin interactions with E. coli inner membrane proteins. Sci. Adv. (2021) 7, eabh2217.



Low Energy Electron Holography investigation of biomolecules

Moritz Edte,[1] Sven Szilagyi[1], Hannah Ochner[1], Luigi Malavolti[1], Stephan Rauschenbach[1,2], and Klaus Kern[1,3]

[1] Max-Planck-Institute for solid state research, Stuttgart, Germany

[2] University of Oxford, Oxford, United Kingdom

[3] École polytechnique fédérale de Lausanne, Lausanne, Switzerland

At the molecular level, biochemical processes that are essential for any form of life are often tied to the ability of flexible biomolecules to dynamically change their conformation. For rigid molecules, the averaged structure and the structure of individual molecules are expected to be similar, hence state-of-the-art imaging methods such as X-ray crystallography, electron microscopy, and nuclear magnetic resonance spectroscopy, which require averaging over many molecules, yield fruitful results. However, for flexible molecules, structural characterization of individual molecules is required, which we do by low-energy electron holography (LEEH). Since LEEH provides high-contrast images of such molecules at the single molecule level with sub-nanometer resolution, we can use it to investigate the native structure of biomolecules. For this, an ultraclean sample preparation is required which, additionally, preserves a native molecular structure. For this purpose, we use Electrospray Ion Beam Deposition (ES-IBD) which allows us to selectively deposit molecules based on their mass-to-charge ratio. LEEH, in combination with ES-IBD, has been demonstrated to be able to image the conformational flexibility of proteins on the single molecule level. Since LEEH is not limited to proteins, the technique can be applied to other classes of molecules of great biological relevance, e.g. DNA or glycans, which are elusive to state-of-the-art imaging methods. I will present the concept of LEEH and our workflow and how we retrieve structural data on the single molecule level encoded in the holograms of DNA-Origami and individual proteins.

Why interlayer exchange is crucial for temperature-programmed desorption

Tobias Dickbreder,[1] Ralf Bechstein,[1] Angelika Kühnle[1]

[1] Faculty of Chemistry, Physical Chemistry I, Bielefeld University, 33615 Bielefeld, Germany

Understanding the desorption process of molecules from surfaces is fundamental in many areas such as dewetting, weathering and catalysis. Temperature programmed desorption (TPD) is a powerful method to gain mechanistic insights into the kinetics of molecules desorbing from surfaces. Several methods exist in literature for analysing TPD data. These methods have in common that they are based on the Polanyi–Wigner equation. Using the Polanyi-Wigner equation requires proposing a desorption mechanism with a single (dominating) desorption path. In reality, however, several coupled desorption paths are easily possible. In this poster, we present an analysis addressing the influence of exchange between the first and the second adsorbate layer on the desorption process. We show that adding an additional desorption pathway alters the desorption spectrum considerably. As interlayer exchange is expected to be common in real systems, this pathway needs to be considered when analysing TPD data. [1]

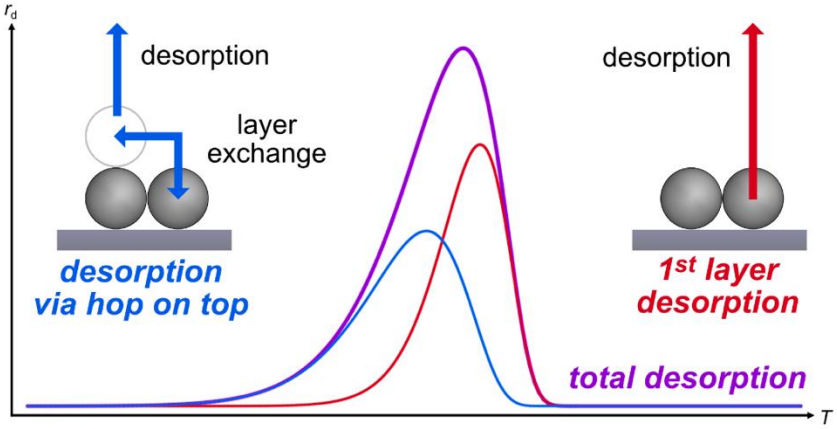
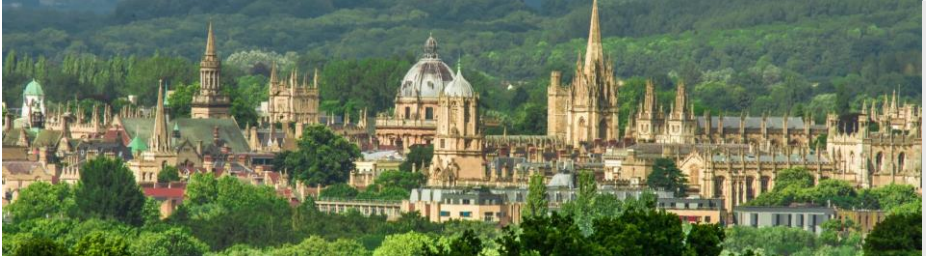


Figure 1: Schematic temperature-programmed desorption curves showing the contributions of direct desorption from the first layer (red curve) and desorption via hop on top (blue curve) to the total desorption rate (violet curve). The desorption mechanism behind direct desorption and desorption via hop on top are shown as insets. [1]

[1] Dickbreder, T.; Bechstein, R.; Kühnle, A. Crucial impact of exchange between layers on temperature programmed desorption. *Physical Chemistry Chemical Physics*. **2021**, *23*, 18314-18321, DOI: 10.1039/d1cp01924d



Impact of the (2x1) Reconstruction of Calcite (10.4) on the Desorption Behaviour of Water and Ethanol

Tobias Dickbreder,[1] Jonas Heggemann,[2] Simon Aeschlimann,[3] Dirk Lautner,[3] Antonia Köhler,[1] Lea Klausfering,[1] Ralf Bechstein,[1] Philipp Rahe[2] and Angelika Kühnle[1]

[1] Faculty of Chemistry, Physical Chemistry I, Bielefeld University, 33615 Bielefeld, Germany

[2] Fachbereich Physik, Universität Osnabrück, Barbarastraße 7, 49076 Osnabrück, Germany

[3] Institute of Physical Chemistry, Johannes Gutenberg University Mainz, Duesbergweg 10-14, 55099 Mainz, Germany

As the most abundant carbonate mineral in the Earth's crust, calcite plays a dominate role in a variety of environmental processes such as carbon sequestration and buffering of the seawater pH value. Upon cleavage, calcite exposes the (10.4) cleavage plane, which exhibits a rectangular unit cell. Interestingly, several experiments have been presented that suggest a (2x1) surface reconstruction both in ultrahigh vacuum and under ambient conditions. However, the existence of the (2x1) reconstruction remained under debate, because clear experimental evidence and a theoretical confirmation were long missing. Recently, a combined atomic force microscopy and density functional theory study provides convincing experimental indication for a (2x1) reconstruction at low temperatures (5 K). [1] Nevertheless, it remained unclear whether the (2x1) reconstruction persists at room temperature and how it effects the surface properties of calcite. Here, we present temperature-programmed desorption (TPD) curves of water and ethanol desorbing from calcite (10.4). Our experiments show a characteristic double-peak structure, which cannot be explained by a simple single-path desorption process. [2] Instead, the data can be excellently described by a kinetic model considering two different adsorption positions, as would be present in the case of a (2x1) reconstruction. This finding applies to both the desorption of water and ethanol, suggesting that the effect is not specific for a given molecule but characteristic for the calcite cleavage plane. Our results thus show that the (2x1) reconstruction is not limited to low temperatures but also exists at room temperature. This work, thus, demonstrates that the presence of the (2x1) reconstruction has significant impact on the interfacial properties of calcite, as illustrated by the desorption behaviour of water and ethanol from calcite. [2]

[1] Heggemann, J.; Ranawat, Y.; Krejčí, O.; Foster, A.; Rahe, P. *The (2x1) reconstruction of the calcite(104) surface*. *to be submitted* **2022**.

[2] Dickbreder, T.; Lautner, D.; Köhler, A.; Klausfering, L., Bechstein, R.; Kühnle, A. Evidence for the (2x1) Reconstruction of Calcite (10.4): Impact on the Desorption Behavior of Water and Ethanol. *to be submitted* **2022**.

Synthesis of metal sulfide nanoribbons on graphene by self-assembly of inorganic ions

Xuejiao Zhang, Kelvin Anggara, Vesna Srot, Xu Wu, Peter A. van Aken, Klaus Kern

Max Planck Institute for Solid State Research, 70569 Stuttgart, Germany

Bottom-up synthesis of nanostructures on surfaces depends on the self-assembly of nanoscale building blocks. The diversity of accessible nanostructures, however, have been limited by the choice of atomic and molecular building blocks that can be evaporated on surfaces. Here, we bypass this limitation by using complex inorganic ions generated from electrospray ionization as building blocks to synthesize nanostructures on surfaces. $HMo_nS_{3n+1}^-$ (n = 3-6) ions were deposited onto freestanding single-layer graphene by Electrospray Ion-Beam Deposition (ESIBD) and imaged by aberration-corrected Scanning Transmission Electron Microscopy (STEM) (Figure 1). The molecules form anisotropic, single-layered, crystalline MoS_2 nanoflakes of sizes smaller than 100 nm^2, which in turn self-assemble into MoS_2 nanoribbons extending as far as 1 µm. The first observation of such nanostructures evidences the potential of this approach to fabricate previously inaccessible nanomaterials on surfaces.

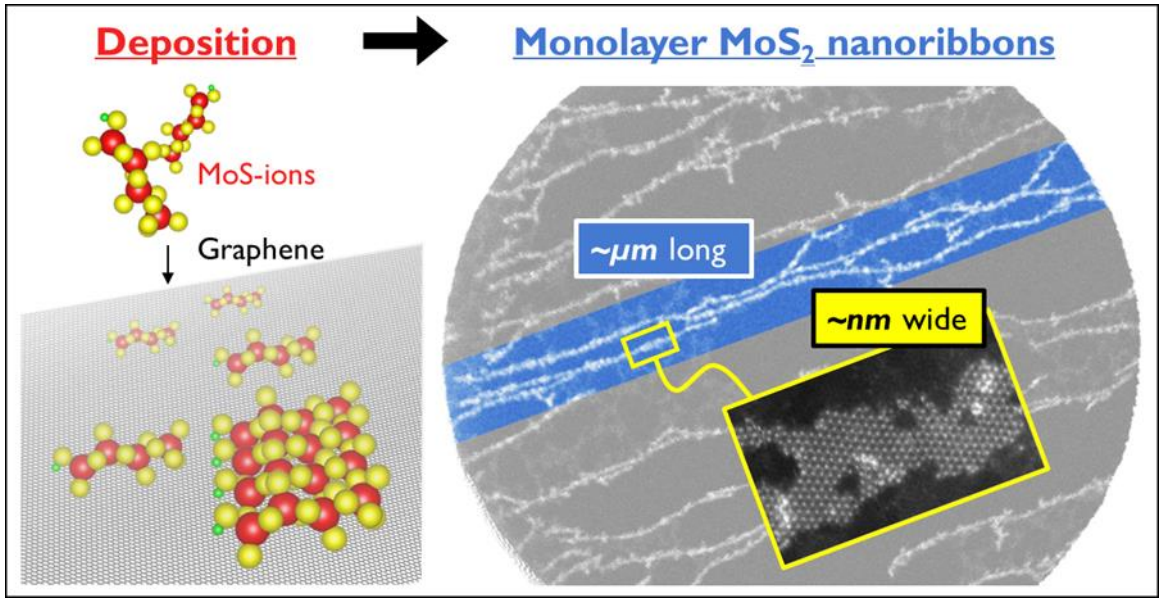


Figure.1 $HMo_nS_{3n+1}^-$ (n = 3-6) ions were deposited onto single layer graphene by ESIBD, and self-assembled into 1 µm long monolayer MoS_2 nanoribbons (4 ± 1 nm wide) as observed by STEM.

Developing a native MS-based screening of MHC peptide complexes

Janine-Denise Kopicki[1,2], Ankur Saikia[3], Stephan Niebling[4], Christian Günther[4], Raghavendra Anjanappa[3], Maria Garcia-Alai[4], Sebastian Springer[3], Charlotte Uetrecht[1,2,5].

[1] Centre for Structural Systems Biology (CSSB), Leibniz Institute of Virology (LIV), Notkestraße 85, 22607, Hamburg, Germany

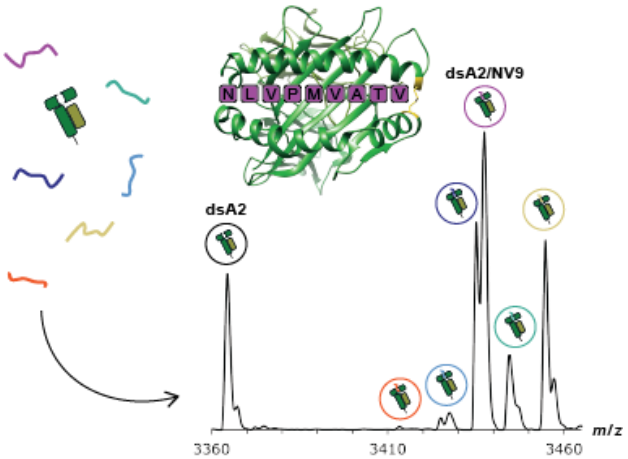
[2] University of Siegen, Am Eichenhang 50, 57076, Siegen, Germany

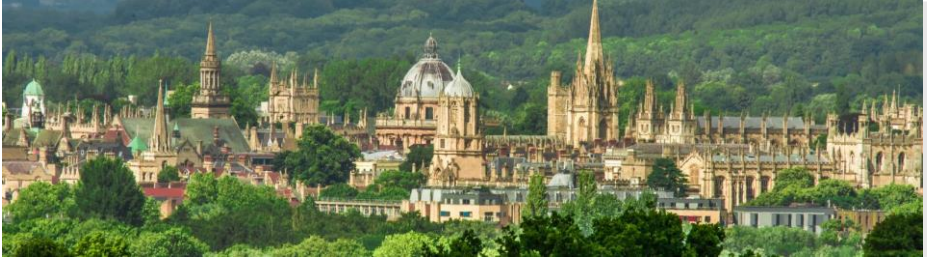
[3] Life Sciences and Chemistry School of Science, Jacobs University Bremen, Campus Ring 1, 28759, Bremen, Germany

[4] Centre for Structural Systems Biology (CSSB), European Molecular Biology Laboratory – Hamburg Outstation, Notkestraße 85, Hamburg, 22607, Germany

[5] European XFEL GmbH, Holzkoppel 4, 22869, Schenefeld, Germany

An essential element of adaptive immunity is selective binding of peptide antigens by major histocompatibility complex (MHC) class I proteins and their presentation to cytotoxic T lymphocytes. Structural knowledge and extensive databases of eluted peptides have informed computational methods that predict tumour epitopes. This approach, however, suffers from the uncertainty created by matching the eluted peptide to one of the 4-6 MHC class I allotypes present in a human being. Hence, to accelerate biological testing, it is desirable to rank candidate peptides by their binding affinity using a direct approach. Yet, to date, simple equilibrium binding assays that support high-throughput screening are not available because they require empty peptide receptive class I molecules, which are conformationally unstable.
Using native mass spectrometry (nMS), we analyse the binding of peptides to an empty disulfide-stabilized HLA-A*02:01 molecule and, due to its unique stability, we determine binding affinities of various peptides. As a complement to computational prediction tools, our method estimates binding strength of even low-affinity peptides to MHC class I complexes quickly and efficiently. Our measurements at higher energies yield a simple test for strong peptide binding. At an acceleration voltage of 50 V, only strongly bound ligands are retained. This value hence serves as a cut-off value in our nMS approach that could easily be employed in an nMS-based screening. Apart from some attempts, there is currently no high-throughput method for the identification of MHC class I binding peptides with immunogenic potential that is elaborated and reliable. Still, such high-throughput screenings are the ultimate key to the development of synthetic peptide vaccines that offer decisive advantages over conventional vaccines, as there is a lesser risk of unwanted host responses, and no possibility of reversion to pathogenic phenotypes, and no limitation for target diseases. Vaccine production is rather easy and detached from the natural source itself, which may be challenging to culture. Taken together, our work provides a valid, sensitive, and rapid method to determine affinities of the MHC class I-peptide complex and the basis to develop a novel high-throughput peptide screen for MHC class I epitopes. Since our technique is based on mass spectrometry, it allows working with very low sample consumption, and it also offers the possibility of simultaneous multi-species analysis. It has huge potential to eliminate binding affinity biases and thus accelerate drug discovery in infectious diseases, autoimmunity, vaccine design, and cancer immunotherapy.





Native Ion Mobility Mass Spectrometry of Alpha-1 Antitrypsin

Sarah Vickers,[1,3] Ibrahim Aldobiyan,[2,3] Aisha Ben-Younis,[1,3] Hannah Britt,[1,3] James Irving,[2,3] David Lomas,[2] Konstantinos Thalassinos[1,3]

[1] Institute of Structural and Molecular Biology University College London, London, U.K.
[2] Centre for Respiratory Biology, University College London, London, U.K.
[3] Department of Biological Sciences, Birkbeck College London, London, U.K.

Alpha-1 antitrypsin (AAT) is a serine protease inhibitor essential to the control of proteolytic pathways. AAT has a complex mechanism for inhibition involving a conformational change, leaving it vulnerable to misfolding. Mutant AAT can misfold and polymerise, leading to two complications: cirrhosis in the liver and emphysema in the lungs. Using ion mobility mass spectrometry (IM-MS) we examine the difference in structure and stability of plasma samples from healthy and diseased patients. We have begun developing a method for analysing ex-vivo liver samples using IMMS.

Wild type and mutant samples of AAT were purified from plasma and polymeric mutant samples were purified from human liver, as described in the literature. Samples were buffer exchanged into ammonium acetate and diluted to 5 uM and introduced to a SELECT SERIES Cyclic IMS QToF (Waters Corp.). Mobility and collision activation (CA) experiments were performed to assess conformational stability and compare the collision cross section (CCS) of both samples.

Differences in glycosylation and gas phase stability were seen between monomeric disease associated mutants and wild type samples of plasma. When studied using CA, both samples traversed multiple intermediates in unfolding, which could be linked to the known reactive intermediate in the polymerisation pathway. Further experiments employed a small molecule that blocks polymerisation by selectively binding to an intermediate structure to assess the effect of the small molecule on conformation and stability.

In our preliminary investigations of liver polymer samples using IM-MS, high order polymers and their multiple conformations were observed for the first time. The structure and stability of liver polymers were compared to heat induced polymers to gain further understanding of the polymerisation pathway. Further experiments will analyse the liver polymers using cyclic ion mobility mass spectrometry to separate these conformations and gain further insight into the formation of the toxic polymers.

A reproducible IMMS method has been developed for the structural characterization of monomeric samples of wild type and mutant AAT which lays the groundwork for future investigations of the serpins using mass spectrometry. The application of this workflow to biologically relevant liver polymer will provide an exciting insight into the structural basis for this disease. Using IMMS to understand AATs' polymerisation pathway opens up avenues in other misfolding diseases, for example, Parkinson's and Alzheimer's.