Nanopores and Nanofluidics

APPLICATIONS OF SOLID-STATE NANOPORES TO TRANSLATIONAL HYALURONAN ANALYSIS

Adam R. Hall

Virginia Tech-Wake Forest School of Biomedical Engineering and Sciences,
Wake Forest School of Medicine
575 N. Patterson Ave., Ste 530, Winston-Salem, NC 27101, USA
Email arhall@wakehealth.edu

Hyaluronan (or hyaluronic acid, HA) is a biological sugar that is found ubiquitously in mammalian tissues and biofluids. Both the abundance of HA *in vivo* and its varying size (molecular weight) can have important impacts on the critical roles the molecule has in human health and disease. However, current technologies have significant weaknesses in quantitation, resolution, and/or sensitivity that have limited the role of HA in translational diagnostics. To address this gap, our lab has established solid-state nanopores as a platform for robust HA analysis [1]. In this talk, I will discuss the effects of several key experimental conditions on measurement efficacy [2] as well as our development of supporting protocols that enable quantitative isolation of HA from diverse biological matrices. I will then present on extensions of our work towards translational studies as well as a covalently modified HA (heavy chain HA) that has recently emerged as an important regulator of inflammation.

References

[1] F. Rivas, O. K. Zahid, H. L. Reesink, B. T. Peal, A. J. Nixon, P. L. DeAngelis, A. Skardal, E. Rahbar, and A. R. Hall, *Label-Free Analysis of Physiological Hyaluronan Size Distribution with a Solid-State Nanopore Sensor*, Nat Commun **9**, 1037 (2018).

[2] F. Rivas, P. L. DeAngelis, E. Rahbar, and A. R. Hall, *Optimizing the Sensitivity and Resolution of Hyaluronan Analysis with Solid-State Nanopores*, Sci Rep **12**, 4469 (2022).

NANOPORE TRANSPORT BEYONG DNA SEQUENCING

Aleksei Aksimentiev

Department of Physics, University of Illinois at Urbana-Champaign
1100 W. Green Street, Urbana IL, 61801/USA
Email: aksiment@illinois.edu

Nanopore systems are ubiquitous in biology and engineering, with applications ranging from transmembrane transport to power generation and sensing. Modeling and simulations have been integral to the development of the field, providing microscopic interpretation of experimental measurements and exploring transport modalities and phenomena beyond experimental reach. In this lecture, I will describe recent work from our lab directed at increasing realism of nanopore transport simulations, addressing both accuracy of the method and the breadth of systems amenable to it. Specific topics to be covered may include protein sequencing and fingerprinting, DNA molecular motors, artificial water and ion channels, viral genome packaging and transport through the nuclear pore complex. The lecture will highlight recent advances in the methodology of multi-resolution simulations [1-3], which make computational description of to-scale nanopore systems possible.

References

1. James Wilson, Kumar Sarthak, Wei Si, Lyuy Gao, and Aleksei Aksimentiev. Rapid and Accurate Determination of Nanopore Ionic Current Using a Steric Exclusion Model. *ACS Sensors* 4:634–644 (2019).
2. Christopher Maffeo, and Aleksei Aksimentiev. MrDNA: A multi-resolution model for predicting the structure and dynamics of DNA systems. *Nucleic Acids Research* 48: 5135–5146 (2020).
3. Adnan Choudhary, Christopher Maffeo and Aleksei Aksimentiev. Multi-resolution simulation of DNA transport through large synthetic nanostructures. *Physical Chemistry Chemical Physics* 24, 2706 – 2716 (2022).

Nanofluidics in 1D: Ion diffusion and ion transport in small diameter carbon nanotube porins

Aleksandr Noy

Lawrence Livermore National Laboratory and University of California Merced
7000 East Ave., Livermore, CA94550, USA
Email noy1@llnl.gov, anoy@ucmerced.edu

Nanofluidic systems control ion and water transport on a unprecedentedly small scale, which is important for applications ranging from biosensing to precision separations. Living systems, which move ions and small molecules across biological membranes using protein pores, often rely on finely controlled nanoscale confinement effects to achieve efficient and exquisitely selective transport. I will show that carbon nanotube porins—pore channels formed by ultra-short carbon nanotubes assembled in a lipid membrane—can exploit similar physical principles to transport water, protons, and ions with efficiency that rivals and sometimes exceeds that of biological channels [1-3]. I will discuss how molecular confinement, slip flow, and the nature of the pore walls influence the mechanisms of ion diffusion, ion selectivity, electrophoretic transport, and electroosmotic coupling in these nanopores. Overall, carbon nanotube porins represent simple, versatile, and highly controlled biomimetic membrane pores that provide an ideal test bed for development of the next generation of biomimetic channels and pores.

References

1. Zhongwu Li, Yuhao Li, Yun-Chiao Yao, Fikret Aydin, Cheng Zhan, Yunfei Chen, Menachem Elimelech, Tuan Anh Pham, and Aleksandr Noy, ACS Nano **14** (5), 6269 (2020).
2. Yuhao Li, Zhongwu Li, Fikret Aydin, Jana Quan, Xi Chen, Yun-Chiao Yao, Cheng Zhan, Yunfei Chen, Tuan Anh Pham, and Aleksandr Noy, Science Advances **6** (38), eaba9966 (2020).
3. R Tunuguntla, Robert Henley, Y-C Yao, Tuan Anh Pham, Meni Wanunu, and A Noy, Science **357**, *792* (2017).

Using Nanochannels and Nanopores for single-molecule separation and sensing

Amit Meller

Technion - IIT
Haifa / Israel
Email ameller@technion.ac.il
lab site: www.meller-lab.net

SARS-CoV-2 outbreak of the coronavirus disease has underlined the acute need for extremely sensitive, accurate, fast, point-of-care mRNA and proteins quantification sensors. Here I will show how solid-state nanopores can be used to digitally count target mRNA molecules from both biological *and* clinical Covid-19 samples surpassing the accuracy of "gold-standard" RT-qPCR. Moving beyond nucleic acids, I will discuss our on-going efforts towards the use of sub-wavelength depth nanochannels for single protein molecule separation, characterization and quantification using a single particle tracking algorithm. Moreover, we develop unique *plasmonic nanopore* devices for single protein molecules identification based on partial labelling of only two or three amino acids. We show that SDS-denatured protein can be electrically threaded and translocated through sub-5 nm solid-state nanopores given rise to molecular-weight dependent translocation properties. This research opens up new directions ultimately leading towards single-cell proteomics of even rarely expressed proteins.

References:

1.Kooten, X. F. van, Rozevsky, Y., Marom, Y., Sadeh, E. B. & Meller, A. Purely electrical SARS-CoV-2 sensing based on single-molecule counting. Nanoscale 14, 4977–4986 (2022).

2.Alfaro, J. A. et al. The emerging landscape of single-molecule protein sequencing technologies. Nat Methods 18, 604–617 (2021).

3.Zrehen, A., Ohayon, S., Huttner, D. & Meller, A. On-chip protein separation with single-molecule resolution. Sci. Rep. 10, 15313 (2020).

4.Rozevsky, Y. et al. Quantification of mRNA Expression Using Single-Molecule Nanopore Sensing. ACS Nano 14, 13964–13974 (2020).

5.Burck, N. et al. Nanopore Identification of Single Nucleotide Mutations in Circulating Tumor DNA by Multiplexed Ligation. Clin. Chem. 14, 836–10 (2021).

6.Spitzberg, J. D., Zrehen, A., Kooten, X. F. van & Meller, A. Plasmonic-Nanopore Biosensors for Superior Single-Molecule Detection. Adv. Mat. 31, e1900422-18 (2019).

7.Ohayon, S., Girsault, A., Nasser, M., Shen-Orr, S. & Meller, A. Simulation of single-protein nanopore sensing shows feasibility for whole-proteome identification. Plos Comput Biol 15, e1007067 (2019).

TWO-DIMENSIONAL EMPTY SPACE AND ITS UNIQUE PROPERTIES

Andre K Geim

University of Manchester, United Kingdom

I shall provide an overview of our recent work on atomic-scale cavities fabricated by van der Waals assembly of 2D crystals. These ultimately narrow cavities can be viewed as if an individual atomic plane is extracted from a bulk crystal leaving behind a 2D empty space, essentially an angstrom-scale gap connecting two edge dislocations. Gas, liquid, ion and proton transport has been studied using 2D cavities down to one atom in height, revealing interesting and sometimes counterintuitive properties.

The power of single-molecule approaches to biology

Cees Dekker

Kavli Institute of Nanoscience, Delft University of Technology, The Netherlands
c.dekker@tudelft.nl; http://ceesdekkerlab.tudelft.nl

Nanotechnology with its single-molecule techniques offers fantastic opportunities to contribute to biology. I will present some recent examples from my lab where nanotech single-molecule tools are used to unravel the biology of cells down to the single-molecule level as well as build nanoscale structures from the bottom up.

1. Nanopore-based sequential reading of peptides [1]

We recently demonstrated a nanopore-based single-molecule peptide reader capable of reliably detecting single amino-acid substitutions within individual peptides. A peptide is linked to a DNA molecule and sequentially pulled through a biological nanopore by a DNA helicase in single amino-acid steps. Stepping ion-current signals enable discrimination of single-amino-acid substitutions in single reads. Notably, we demonstrated the capability to 'rewind' peptide reads, obtaining indefinitely many independent reads of the same molecule, yielding an undetectably low error rate in single-amino-acid variant identification. These proof-of-concept experiments constitute a promising basis for the development of a single-molecule protein sequencer.

2. A DNA origami turbine powered by nanoscale flow [2]

We recently built an artificial nanoscale turbine. We demonstrate driven rotary motion of a DNA origami turbine which harnesses energy from a water/ion flow generated by a static chemical or electrical potential gradient in a solid-state nanopore. One type of origami nanoturbines consists of a 6-helix DNA bundle that adopts a chiral conformation upon phoretic docking onto the nanopore and subsequently displayed a sustained unidirectional rotary motion of up to 20 revolutions/s. Another type has designed turbine blades. These artificial nano-engines operate autonomously in physiological conditions, converting energy into useful mechanical work.

3. Real-time imaging of DNA loop extrusion by condensin and cohesin SMC complexes [3]

Structural Maintenance of Chromosomes (SMC) proteins like cohesin and condensin spatially organize chromosomes by extruding DNA into large loops. Using single-molecule assays, we provided unambiguous evidence for loop extrusion by directly visualizing the processive extension of DNA loops by SMCs in real-time. In recent extensions of this work, we showed that how this process occurs on supercoiled DNA, how SMCs also can exhibit phase condensation, and that SMC proteins can bypass huge roadblocks of bound proteins on DNA.

References:
[1] H. Brinkerhoff et al, Science 374, 1509 (2021).
[2] X. Shi et al, Nature Physics, in print; X. Shi et al, submitted.
[3] Ganji et al, Science 360, 102 (2018); Kim et al, Nature 579, 438 (2020); B. Pradhan, BioRxiv, 2021; Cell Rep, under review.

Exploring new nanopore candidates from aerolysin like proteins

Chan Cao

Laboratory of Bio and Bio-inspired Nanosystems, Global Health Institute and Institute of Bioengineering, School of Life Sciences
École polytechnique fédérale de Lausanne (EPFL) / Switzerland
Email chan.cao@epfl.ch

Aerolysin-like proteins are a sub-family of β-pore forming toxins that are widely present in all kingdoms of life[1]. Their structure and mechanism of pore formation have been a long-term interest which provides cues for the development of therapeutics in fighting disease. Recently, this family of proteins is also growing attention because of their biotechnological application as nanopore sensors for biological and synthetic molecules sensing and sequencing, especially for single-molecule proteomic analysis[2]. However, in spite of the conserved structural fold, the sequence identity in this family is very low. This complicates their sequence alignment, hindering an understanding of their pore structure and properties, and therefore their further biotechnological applications. In an attempt to further understand the properties of aerolysin-like pores, we created models for the pore structure of three family members, *Clostridium perfringens* epsilon toxin (ETX), *Laetiporus sulphureus* lectin (LSL) and *Bacillus thuringiensis* parasporin-2. Their structures and sensing capabilities for ssDNA have been first assessed by *in silico* methods, and then ETX has been incorporated into a planar lipid membrane for nanopore experiments. Three types of ETX pores have been observed during single-channel recording experiments with only one type of them being able to translocate ssDNA, inducing a bigger depth of current blockade compared to aerolysin nanopore. Our findings open a new venue for improving and diversifying nanopore capabilities for molecular sensing.

References

[1] Matteo Dal Peraro *et al.* Pore-forming toxins: ancient, but never really out of fashion, Nat. Rev. Microbiol. 14, 77-92 (2016).
[2] Chan Cao *et al.* Aerolysin nanopores decode digital information stored in tailored macromolecular analytes, Sci. Adv., 6, eabc2661 (2022).

What Can We Do with Solid-State Nanopores beyond Translocation-Based Sensing and Transport Control?

Chuanhua Duan

Department of Mechanical Engineering, Boston University
110 Cummington Mall, Boston, MA 02215, U.S.A
Email duan@bu.edu

Research on solid state nanopores has attracted great attention over the last two decades because of their great potentials in mimicking protein channels in cell membranes. Although significant progress has been made, past research has mainly utilized solid nanopores for translocation-based single bimolecule/particle sensing and ion/molecule transport control. What can we do with solid-state nanopores beyond these two directions? This question can be answered from two different prospectives. On one hand, single nanopores are the basic constitute of nanoporous membranes. It is therefore possible to use single nanopores to study and understand complicated transport phenomena in nanoporous membranes, paving the way for developing nanoporous membranes with better performance. On the other hand, it is worth noting that most of the past nanopore research is focused on molecule/particle translocating through the nanopore. The opposite scenario, i.e. molecules/particles blocking the nanopore, has not been extensively studied. In this talk, I will present my group's recent efforts on exploring fundamentals and application of solid-state nanopore from these two new aspects.

First, I will present our work on exploring evaporation from single nanopores. We have developed a novel microscope-based optical measurement to measure evaporation rates down to 10 aL/s from single nanopores. I will show that the ultimate evaporation flux from ultrathin silicon nitride nanopores is not limited by liquid transport to the interface and vapor removal from the interface, but by the interfacial evaporation kinetics and shows a strong diameter dependence. I will also show that the kinetically-limited evaporation from graphene nanopores can be much larger than that from silicon nitride nanopores due to edge facilitated evaporation and minimum contaminant accumulation.

Secondly, I will introduce our latest work on studying nanoparticle-blocked nanopore systems. We have found that, when nanoparticles with sizes larger than the diameter of a nanopore are electrophoretically driven towards the nanopore, they can be either electrokinetically trapped near the nanopore or physically block the nanopore based on their surface charge polarity. These two types of nanoparticle blockage modes can respond to various electrical or mechanical stimuli and show stimuli-responsive transport. I will show how we utilize such nanoparticle-blockage-induced stimuli-responsive transport to develop new applications for nanoparticle characterization, nanopore gating as well as bio-sensing.

References

[1] Y. Li, M. Alibakhshi, Y. Zhao and C. Duan, Exploring Ultimate Water Evaporation in Nanoscale Conduits, *Nano Letters*, 17 (8). 4813-4819, (2017).

[2] Y. Li, H. Chen, S. Xiao, M. Alibakhshi, A. Lo, M. Lu, and C. Duan, Ultrafast Diameter-Dependent Water Evaporation in Nanopores, *ACS Nano*, 13 (3). 3363-3372, (2019).

[3] S. Xiao, K. Meng, Q. Xie, L. Zhai, Z. Xu, H. Wang and C. Duan, Edge Enhanced Ultrafast Water Evaporation from Graphene Nanopores, *Cell Reports Physics Science*, accepted.

[4] R. Yazbeck, M.A. Alibakhshi, J. Von Schoppe, K. L. Ekinci and C. Duan, Characterization and Manipulation of Single Nanoparticles Using Nanopore-Based Electrokinetic Tweezer, *Nanoscale*, 11 (47), 22924-22931, (2019).

[5] Y. Xu, R. Yazbeck, and C. Duan, Anomalous Mechanosensitive Ion Transport in Nanoparticle-Nanopore-Integrated Confinements, *Journal of Chemical Physics*, 154 (22), 224702, (2021).

[6] R. Yazbeck, Y. Xu, T. Porter and C. Duan, Nanoparticle-Blockage-Enabled Rapid and Reversible Nanopore Gating with Tunable Memory, PNAS, in revision.

NOISE-DRIVEN TRANSPORT IN A VISCOSITY GRADIENT

Derek Stein

Brown University, Department of Physics and School of Engineering
182 Hope Street, Providence, RI /USA
Email DEREK_STEIN@BROWN.EDU

Gradients of voltage, pressure, temperature, or salinity can transport objects in micro- and nanofluidic systems by well-known mechanisms. I will describe the discovery of an electrokinetic transport effect driven by a viscosity gradient: An imposed liquid viscosity gradient causes an ionic current to flow inside a glass nanofluidic channel [1]. Measurements of the current and numerical simulations reveal that the counterions in the electric double layers near the nanochannel surfaces drift in the direction of decreasing viscosity. The measurements are well described by a simple model in which the counterion drift speed equals the gradient of an ion's local diffusivity. Drift in a viscosity gradient, which we call "viscophoresis", is a consequence of multiplicative (state-dependent) noise, where the magnitude of the thermal fluctuations experienced by a particle depends on its position. Viscophoresis is also observed in the motion of fluorescent nanoparticles, and I will briefly discuss biological settings where it may play an underappreciated role.

References

[1] Wiener, Benjamin, and Derek Stein. "Electrokinetic Current Driven by a Viscosity Gradient." *arXiv preprint arXiv:1807.09106* (2018).

ION PERMEATION IN NARROW CARBON NANOTUBES: PUTTING THE PUZZLE TOGETHER VIA COMBINED AB INITIO AND MEAN-FIELD MODELING

Vadim Neklyudov and Viatcheslav Freger*

Wolfson Department of Chemical Engineering, Technion – Israel Institute of Technology
Haifa 32000, ISRAEL
Email vfreger@technion.ac.il

Narrow carbon nanotubes (CNTs) are unique mimics of water channels in biological membranes, yet the physics behind their selectivity, especially, relative roles of water and ion interactions within CNT and with surrounding matrix, is still unclear. Here we report ab initio investigation of water and ion transfer from solution into CNTs of diameters 0.68 nm and wider 0.8 nm tubes, common in experimental studies. We first focus on the effect of the medium surrounding CNT, defined by its dielectric constant ε. The transfer energies computed for $1 < \varepsilon < \text{inf}$ permit a transparent breakdown of transfer energy to three main contributions: binding to CNT, intra-CNT hydration, and dielectric energy [1]. The dielectric energy is small for water but very significant for ions and scales linearly with $1/\varepsilon$, reminiscent of the Born equation, with the slope of the order 100 kJ/mol for all ions and CNTs. It may easily turn ion transfer from preferential to strong exclusion, as observed for potassium. In contrast, chloride appear to be strongly excluded for all ε. Simulations also demonstrate that, while water arranges in a single file in (5,5) tubes, it is strongly distorted in (6,6) tubes, both for water without and with some (but not all) ions.

Subsequently, we incorporate thermodynamic quantities computed ab initio in a mean-field model, adding to the picture proton and hydroxide inherently present in water and a few other ions. We first consider transfer of free ions, to which ions pair formation is subsequently added as a proxy of ion-ion interactions [2]. Experimentally observed affinity of CNTs to hydroxide does not show up in computed quantities for single ions, yet it is revealed as an exceptionally favorable transfer of KOH pairs. Nevertheless, we conclude that free ions, coexisting with more abundant, but less mobile ion pairs control the ion transport. The model successfully explains most observed effects of salt and ion type, concentration and pH on conductivity, ion transport numbers, ion permeation, activation energies, and current rectification. The proposed approach may be extended to other sub-nanometer nanochannels, which may advance our understanding and help design novel desalination and osmotic materials and devices.

References

[1] V Neklyudov, V Freger, *J. Phys. Chem. Lett*. 12, (2020)
[2] V Neklyudov, V Freger, *Nanoscale,* in press

Scanning Ion Conductance Microscopy and Spectroscopy

Georg E. Fantner, Aleksandra Radenovic , Samuel M. Leitao, Vytautas Navikas, Barney Drake

Institute for Bioengineering, École Polytechnique Fédéral de Lausanne
EPFL-STI-IBI /Switzerland
Email georg.fantner@epfl.ch

Scanning ion conductance microscopy (SICM) has been around for decades [1], yet it has not received as much attention as other forms of scanning probe microscopy. Recently, this true non-contact technique has kindled renewed interest among biophysicists and biologists because it is ideally suited for label-free imaging of fragile cell surfaces where it achieves exquisite resolution down to the nanometer regime without distorting the cell membrane [2,3]. SICM uses a glass nanopipette as a scanning probe and measures the current through the glass nanopore as a proximity detection of the sample surface. The challenge to harness this technique for time resolved 3D nanocharacterization of living cells lies in the relatively slow imaging speed of SICM. In this presentation I will show how we apply what we have learned from high-speed AFM to the field of SICM. By reengineering the SICM microscope from the ground up, we were able to reduce the image acquisition time for SICM images from tens of minutes down to 0.5s while extending the imaging duration to days [4,5].

SICM, however, is much more versatile than just an imaging tool. I will also discuss our recent results using SICM as a single molecule characterization tool. We term this method scanning ion conductance spectroscopy (SICS). Using capillaries with exceptionally small nanopores [6], we are able to detect and manipulate single molecules in a repeatable and high throughout manner.

References (American Physical Society style)

[1] P. Hansma *et al.*, Science (80-.). **243**, 641 (1989).
[2] P. Novak *et al.*, Nat. Methods **6**, 279 (2009).
[3] Y. E. Korchev *et al.*, Biophys. J. **73**, 653 (1997).
[4] V. Navikas *et al.*, Nat. Commun. **12**, 1 (2021).
[5] S. M. Leitao *et al.*, ACS Nano **15**, 17613 (2021).
[6] L. J. Steinbock *et al.*, ACS Nano **7**, 11255 (2013).

Selective Permeation under Low-Dimensional Confinement

Hyung Gyu Park

Director, Center for Low-Dimensional Transport Physics
Head, Nanoscience for Energy Technology & Sustainability
Prof., Department of Mechanical Engineering, Division of Environmental Science & Engineering
PI, Center for Epitaxial van der Waals Quantum Solids, Institute for Basic Science (IBS)
Pohang University of Science and Technology (POSTECH)
Pohang, Gyeongbuk 37673 / Republic of Korea
Email parkhg@postech.ac.kr; iduserpark@gmail.com

In view of process intensification that membrane technology promises to bring in, it is crucial for membrane materials to attain great selectivity, enhanced permeation, and in-operando durability. To this end, understanding of transport phenomena at low dimensions could help renew our insight for membrane pore design. This talk presents selective transport phenomena across 0D-, 1D- and 2D-confined space that atomically thin orifices [1], nanotubes [2] and 2D material lamellae [3] provide respectively. As the pore dimension increases, permeation tends to decrease from ultimate permeation to fast transport to unimpeded diffusion, whereas selectivity can be engineered on its own. Hence, proper design of the pores and the membrane architecture can collaborate with process operation to further tailor the selectivity-permeance characteristic. Thus-obtained knowledge could lay the cornerstone of advancing membrane transport properties toward process intensification.

References

[1] Kemal Celebi, Jakob Buchheim, Roman M. Wyss, Amirhossein Droudian, Patrick Gasser, Ivan Shorubalko, Jong-Il Kye, Changho Lee, Hyung Gyu Park, *Science* (2014)
[2] Jason K. Holt, Hyung Gyu Park, Yinmin Wang, Michael Stadermann, Alex B. Artyukhin, Costas P. Grigoropoulos, Aleksandr Noy and Olgica Bakajin, *Science* (2006)
[3] Mengmeng Deng, Kijeong Kwac, Meng Li, Yousung Jung, Hyung Gyu Park, *Nano Letters* (2017)

MICROSCOPIC BIODETECTION ANALYSIS IN MoS2 MULTI-LAYER NANOPORE TRANSISTORS

Jean-Pierre Leburton

University of Illinois
208 N. Wright Street, Urbana, Il 61801 /USA
Email: jleburto@illinois.edu

We report on various resistive effects involved in the electronic detection of single biomolecules in a nanopore of a MoS_2 nanoribbon. Our approach based on all-atom molecular dynamics simulation coupled with Boltzmann transport formalism accounts for self-consistent interaction among ions, charge carriers around the pore rim and biomolecules, and provides a comprehensive picture of the effects of electrolyte concentration, pore size, nanoribbon geometry, but also the doping polarity of the nanoribbon on the electrical sensitivity of the nanopore in detecting biomolecules [1]. Furthermore, we show that vertically stacked monolayer MoS_2-hBN nanopore FETs in a multi-sensing electronic scheme exhibit improved sensing robustness and noise reduction in detecting biomolecules such as DNA and proteins. Our model indicates naturally occurring conformational motion quenching of the bio-molecule penetrating the multi-layer membrane. The synchronization of electronic sensing current signatures across the successive MoS_2 probes achieved by time-lagged cross-correlation (TLCC) enhances the signal-to-noise ratio notably in the lower frequency spectrum, enabling the identification of homopolymers [2].

References

[1] Mingye Xiong, Michael Graf, Nagendra Athreya, Aleksandra Radenovic and Jean-Pierre Leburton *"Microscopic Detection Analysis of Single Molecules in MoS_2 Membrane Nanopores" ACS Nano 14(11) pp16131-16139* (2020).

[2] Nagendra Athreya, Mingye Xiong, Rajat Charkraborty, Henry T. Crawford-Eng and Jean-Pierre Leburton *"A Multi-Probe MoS_2-hBN Nanopore Bio-transistor" Nanoscale Horizon (in review (2022*

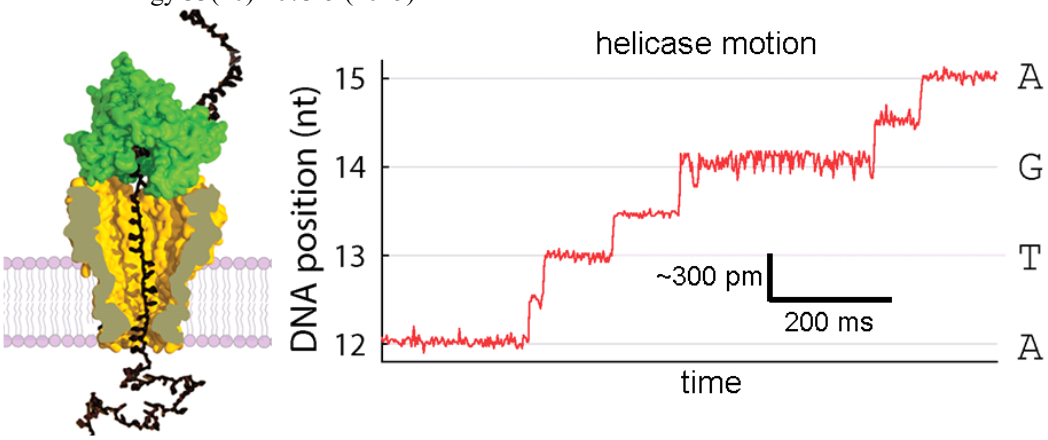
Single-Molecule Biophysics Using Protein Nanopores

Jens H. Gundlach

Department of Physics, University of Washington, Seattle, Washington, USA
Email: gundlach@uw.edu

My group has been at the nexus of developing nanopore sequencing[1] and establishing nanopores as a new tool for single-molecule biophysics[2]. Much of our work is based on the engineered protein pore MspA. Here, I will show the stunning capabilities of using nanopores to observe enzyme mechanics in real-time as these enzymes move along DNA or RNA. We easily achieve ten times better position and time resolution than optical tweezers, while simultaneously measuring the exact nucleotide sequence in the enzyme. I will show hereto unseen detail in the motion of helicases, DNA and RNA polymerases, reverse transcriptases, etc. Besides establishing decisive kinetic enzyme models, we find (surprisingly) that the kinetics of most of these enzymes depends strongly on the template nucleic acid sequence. Of particular contemporary interest are the data we collected with the SARS-CoV-2 helicase nsp13.

1. "Reading DNA at single-nucleotide resolution with a mutant MspA nanopore and phi29 DNA polymerase," E.A. Manrao *et al.*, Nature Biotechnology 30(4):349-53 (2012).
2. "Subangstrom single-molecule measurements of motor proteins using a nanopore," I.M. Derrington *et al.*, Nature Biotechnology 33(10):1073-5 (2015).



MEASURING BIOPOLYMERS AT THE SINGLE MOLECULE LIMIT: FROM SEQUENCING DNA TO IDENTIFYING PROTEINS WITH NANOMETER-SCALE PORES

John J. Kasianowicz

University of South Florida, Dept. of Physics, Tampa (USA)
& the Freiburg Institute for Advanced Studies (Germany)
Email jjkemail@mac.com

Biological nanometer-scale protein pores are the major basis of nerve and muscle activity and macromolecular transport across cell membranes. With the ultimate goal of providing low-cost measurements for health care applications, we have been studying the physical properties of bio-nanopores [1,2] and adapting them for the detection, characterization, and identification of molecules in aqueous solution [3-5]. In the absence of the target molecules to be detected, an applied voltage drives ions through a nanopore embedded in an electrically insulating lipid bilayer membrane. When a single molecule enters the pore, its physical and chemical properties control the degree by which it reduces the nanopore's ionic current and the molecule's residence time in the pore. Our work led to two novel DNA sequencing methods [6-8], the ability to discriminate between individual polymers based on their size [9,10], a method to identify subtly different species of metallo-nanoparticles [11], a single-molecule implementation of Eigen's temperature-jump method [12], and a technique to detect proteins in aqueous solution [13,14].

In collaboration with Abdelghani Oukhaled (Cergy, Université Paris), we recently demonstrated that a bio-nanopore can discriminate between proteins of similar mass [15]. If this method, which is designed to replace the mass spectrometry part of the clinical protein discrimination workflow, is implemented in chip-based devices, it might prove useful at point-of-care facilities.

We will also discuss our preliminary results that compare experimentally measurable properties of bio-nanopores with predictions based on computer molecular dynamics simulations (a collaboration with Jan C. Behrends and Tobi Ensslen at Universität Freiburg).

Sources of funding: NIH NHGRI, NIST Office of Law Enforcement Standards, NSF, Marie Skłodowska-Curie Senior Fellowship, and the Agence Nationale de la Recherche.

References

[1] Nablo, B.J., *et al.* Sizing the *Bacillus anthracis* PA63 channel with nonelectrolyte poly(ethylene glycols). *Biophys. J.* **95**, 1157 (2008).
[2] Michelman-Ribeiro, A., K.A. Rubinson, V. Silin, & J.J. Kasianowicz. Solution structures of *Bacillus anthracis* Protective Antigen proteins using small angle neutron scattering and Protective Antigen 63 ion channel formation kinetics. *Toxins* **13**, 888 (2021).
[3] Bezrukov, S.M., I. Vodyanoy, R. Brutyan, & J.J. Kasianowicz. Dynamics and free energy of polymers partitioning into a nanoscale pore. *Macromolecules* **29**, 8517 (1996).
[4] Kasianowicz, J.J. & S.M. Bezrukov. Protonation dynamics of the alpha-toxin ion channel from spectral analysis of pH-dependent current fluctuations. *Biophysical Journal* **69**, 94 (1995).

[5] Kasianowicz, J.J., J.W.F. Robertson, E.R. Chan, J.E. Reiner, & V.M. Stanford. Nanoscopic porous sensors. *Ann. Rev. Analyt. Chem.* **1**, 737 (2008).
[6] Kasianowicz, J.J., E. Brandin, D. Branton, & D.W Deamer. Characterization of individual polynucleotide molecules using a membrane channel. *Proc. Natl. Acad. Sci. (USA)* **93**, 13770 (1996).
[7] Kumar, S. *et al.* PEG-labeled nucleotides and nanopore detection for single molecule DNA sequencing by synthesis. *Scientific Reports* **2**, 684 (2012).
[8] Reiner, J.E., A. Balijepalli, J.W.F. Robertson, B.S. Drown, D.L. Burden, & J.J. Kasianowicz. The effects of diffusion on an exonuclease/nanopore-based DNA sequencing engine. *J. Chem. Phys.* **137**, 214903 (2012).
[9] Robertson, J.W.F., C.G. Rodrigues, V.M. Stanford, K.A. Rubinson, O.V. Krasilnikov, & J.J. Kasianowicz. Single-molecule "mass spectrometry" in solution using a solitary nanopore. *Proc. Natl. Acad. Sci. (USA)* **104**, 8207 (2007).
[10] Reiner, J.E., J.J. Kasianowicz, B.J. Nablo, & J.W.F. Robertson. Theory for polymer analysis using nanopore-based single-molecule mass spectrometry. *Proc. Natl. Acad. Sci. (USA)* **107**, 12080 (2010).
[11] Ettedgui, J., J.J. Kasianowicz, & A. Balijepalli. Single molecule discrimination of heteropolytungstates and their isomers in solution with a manometer-scale pore. *J. Am. Chem. Soc.* **138**, 7228–7231 (2016).
[12] Reiner, J.E., J.W.F. Robertson, D.L. Burden, L.K. Burden, A. Balijepalli, & J.J. Kasianowicz. Temperature sculpting in yoctoliter volumes. *J. Am. Chem. Soc.* **135**, 130214090109003 (2013).
[13] Kasianowicz, J.J., S.E. Henrickson, H.H. Weetall, & B. Robertson. Simultaneous multianalyte detection with a nanometer-scale pore. *Analyt. Chem* **73**, 2268 (2001).
[14] Halverson, K. M. *et al.* Anthrax biosensor, protective antigen ion channel asymmetric blockade. *Journal of Biological Chemistry* **280**, 34056–34062 (2005).
[15] Bakshloo, M.A., J.J. Kasianowicz, M. Pastoriza-Gallego, J. Mathé, R. Daniel, F. Piguet, & A. Oukhaled. Nanopore-based protein identification. *J. Am. Chem. Soc.* **144**, 2716 (2022).

Ion transport through atomically thin crystals

Marcelo Lozada-Hidalgo

The University of Manchester
Schuster Building, Oxford Road, Manchester M13 9PL /UK
Email marcelo.lozadahidalgo@manchester.ac.uk

The basal plane of graphene is impermeable to all atoms and molecules - even for helium, the smallest - at ambient conditions [1]. Nevertheless, it is permeable protons at ambient conditions [2]. This talk will provide an overview of our investigation of permeation of protons and other small ions through new 2D materials [3-5], including the unexpectedly fast ion exchange properties of atomically thin clays and micas [6].

References

[1] Bunch, J. S. *et al.* Impermeable atomic membranes from graphene sheets. *Nano Lett.* (2008)
[2] Hu, S. *et al.* Proton transport through one-atom-thick crystals. *Nature* (2014).
[3] Mogg, L. *et al.* Atomically-thin micas as proton conduting membranes. *Nat. Nano* (2019).
[4] Mogg. L. *et al.* Perfect proton selectivity in ion transport through two-dimensional crystals. *Nat. Commun.* (2019).
[5] Griffin, E. *et al.* Proton and Li-Ion Permeation through Graphene with Eight-Atom-Ring Defects. *ACS Nano* (2020).
[6] Y.-C. Zhou et al, Ion exchange in atomically thin clays and micas. Nat. Materials (2021).

Sculpting of 2D Materials:
From Pores and Nanoporous Membranes to Sequencing and Water desalination

Marija Drndic
Department of Physics and Astronomy
University of Pennsylvania, drndic@physics.upenn.edu

Introducing atomic-scale holes in 2D materials changes their electrical and optical properties. When 2D materials are suspended, vacancies make the membranes permeable to ions and molecules in liquid or gas phases, allowing transport studies at atomic scales. Angstrom-size holes allow the passage of water molecules but block the larger hydrated salt ions and can effectively desalinate water. Raman peak shifts combined with TEM, provide a comprehensive approach to characterize the holes and transport through them. When molecules are driven through 2D nanopores in solution, they can perturb the ion current flow through the pore, from which molecule's physical and chemical properties can be inferred. DNA other biomolecules can be detected in this way. Thanks to advanced materials, device designs and custom electronics, the temporal and spatial resolution for their detection has been rapidly improving.

References:

[1] Zehui Xia, Chih-Yuan Lin, Marija Drndić, Proteomics 22 (5-6), 2022.

[2] Nasim Hassani, Mahdi Ghorbani-Asl, Boya Radha, Marija Drndić, Arkady V. Krasheninnikov, Mehdi Neek-Amal, J. Phys. Chem. C, 2021.
[3] Marija Drndić, Nature Review Physics 3, 606, 2021.
[4] Joshua Chen, Adrian Balan, Paul Masih Das, Jothi Priyanka Thiruraman, Marija Drndić, Ultramicroscopy, 113249, 2021.
[5] Yung-Chien Chou, Joshua Chen, Chih-Yuan Lin, Marija Drndić, Journal of Chemical Physics 154, 105012, 2021.
[6]] Jothi Priyanka Thiruraman, Paul Masih Das and Marija Drndić, ACS Nano 14, 11831, 2020.

Aerolysin pores for molecular sensing

Matteo Dal Peraro

Institute of Bioengineering, School of Life Sciences, Ecole Polytechnique Fédérale de Lausanne, EPFL, Lausanne 1015, Switzerland

Email: matteo.dalperaro@eplf.ch

Evolution has found countless ways to transport material across cells and cellular compartments separated by membranes, producing channels and pores that enable a regulated passage of molecules in and out of cells. As in several other occasions, we have borrowed from the natural properties of these biological systems to push technology forward and have been able to hijack these nanoscale proteinaceous pores to learn about the physical and chemical features of molecules passing through them [1]. Today, a large repertoire of biological pores is exploited for molecular sensing with the aim of characterizing molecules relevant for the advancement of medicine and technology. Aerolysin, a bacterial pore-forming toxin that my lab has been studying for more than a decade, is a promising system in this context. After having revealed its structure and pore-forming mechanism using integrative structural biology methods [2,3], we characterised the conduction properties of this pore and understood its ability to sense molecular entities such as DNA and peptides [4,5]. Exploiting this fundamental knowledge we could then design and engineer mutant pores that showed enhanced single-molecule sensing properties for applications as diverse as the detection of protein post-translational modifications for disease diagnosis and the reading of informational polymers for future data storage solutions [6].

References

1. SF Mayer, C Cao, M Dal Peraro, Biological nanopores for single-molecule sensing, *iScience*, 104145, 2022
2. Degiacomi MT, Iacovache I, Pernot L, Chami M, Kudryashev M, Stahlberg H, van der Goot FG, Dal Peraro M, Molecular assembly of the aerolysin pore reveals a swirling membrane-insertion mechanism. *Nature Chemical Biology*, 9 (10), 623, 2013.
3. I Iacovache, S De Carlo, N Cirauqui, M Dal Peraro, FG Van Der Goot, B Zuber, Cryo-EM structure of aerolysin variants reveals a novel protein fold and the pore-formation process, *Nature Communications*, 7, 12062, 2016
4. C Cao, MY Li, N Cirauqui, YQ Wang, M Dal Peraro, H Tian, YT Long, Mapping the sensing spots of aerolysin for single oligonucleotides analysis, *Nature Communications,* 9(1), 2823, 2018
5. C Cao, N Cirauqui, MJ Marcaida, E Buglakova, A Duperrex, A Radenovic, M Dal Peraro, Single- molecule sensing of peptides and nucleic acids by engineered aerolysin nanopores, *Nature Communications,* 10(1):1-11, 2019
6. C Cao, L Krapp, A Al Ouahabi, N Konig, N Cirauqui, A Radenovic, J-F Lutz, M Dal Peraro Aerolysin nanopores decode digital information stored in tailored macromolecular analytes, *Science Advances*, 6(50), eabc2661, 2020

Inching closer to translation: The evolution of nanopores from genomics to proteomics

Meni Wanunu, Department of Physics, Northeastern University, Boston USA

Nanopores have gained a lot of attention recently for their ability to sequence nucleic acids. Recently, however, a surge of interest in the use of nanopores for analyzing proteins has been witnessed. I will talk about two approaches that our lab has taken in order to characterize proteins. First, I will describe a method for full-length single-file protein translocation and discrimination using a biological pore. Second, I will describe a method for probing conformational states of a protein and its electrical unfolding. Time permitting, I will also discuss other ongoing nanopore-related projects currently pursued in my lab.

Monitoring Conformational Dynamics of Single Proteins with Plasmonic Double Nanoholes

Michael Mayer

Adolphe Merkle Institute, University of Fribourg
Chemin des Verdiers 4, Ch-1700 Fribourg / Switzerland
Email: michael.mayer@unifr.ch

This talk will present our ongoing efforts to assess the potential of nanoplasmonic optical tweezers for interrogating the conformational dynamics of single unmodified proteins in aqueous solution. Specifically, we employ double nanohole (DNH) structures to trap single enzyme proteins [1,2] for minutes to hours. While the protein resides in the trap, we monitor changes in transmission through the DNH in response to exposing the protein to substrate, product, or inhibitor molecules. We show that experiments with trapped enzymes that are known to undergo significant conformational changes during their catalytic cycle, exhibit multiple transmission levels.[3] Increasing concentration of substrate molecules increases the frequency of transitions between these levels in a dose-dependent manner, while the presence of different inhibitors reduces the frequency of transitions by favoring specific transmission levels (Fig. 1). Step-fitting the transmission recordings makes it possible to follow the rate of transition between levels, revealing individual enzymatic cycles, single molecule turnover frequencies, as well as heretofore unknown enzymatic sub-cycles during catalysis.[3] The talk will conclude with an outlook of applying this approach to additional unmodified enzymes, motor proteins, and transporters as well as a discussion of its current limitations and possible improvements.

References

[1] Juan, M. L.; Gordon, R.; Pang, Y.; Eftekhari, F.; Quidant, R. **2009**, *Nat. Phys.* 5, 915–919
[2] Pang, Y.; Gordon, R. **2012**, *Nano Lett.* 12, 402–406.
[3] Ying, C.; Karakaci, E.; Bermudez-Urena, E.; Ianiro, A.; Foster, C.; Awasthi, S.; Guha, A.; Bryan, L.; List, J.; Balog, S.; Acuna, G.P.; Gordon, R.; Mayer, M. **2021**, *arXiv Prepr,* arXiv2107.06407.
[4] Booth, L. S.; Browne, E. V.; Mauranyapin, N. P.; Madsen, L. S.; Barfoot, S.; Mark, A.; Bowen, W. P. **2022**, *Scientific Reports*, 12:1995

Single-molecule probing by rectification in a nanogap

Aleksandar Ž. Tomović, Miloš S. Dražić, Ivana V. Djurišić, Vladimir P. Jovanović and Radomir Zikic*

Institute for Multidisciplinary Research, University of Belgrade,
Kneza Višeslava 1, 11000 Belgrade, Serbia

Towards efficient single-molecule detection, here in this talk we propose simultaneous measurement of rectification and amplitude of tunneling current during electrical probing of molecule in a nanogap. Also, we propose application of nitrogen-terminated graphene/or CNT nanogaps, due to theirs inherent outstanding features. With DFT and Non-Equilibrium Green's Functions formalism, we show that tunneling current through various molecules including ssDNA, TATP or small organics placed in those nanogaps, exhibit unique rectification behavior under square pulses of alternating biases. The rectification arises by on-off switching of electronic transport through the molecule's HOMO or LUMO level, sustained by partial charging of the probed molecule, which is generated by asymmetric hybridization of the molecule's level with Bloch states from one of the electrodes. This effect is strongly influenced by interaction between the molecule and the nitrogen-induced dipole moment located at the N-C interface of the electrode ends, an effect that mimics local gating. For example, in gas phase we found that TATP is detectable (triacetone triperoxide is a potent and hard to detect explosive made from commonly available chemicals and is a terrorist's weapon of choice in the last two decades). In liquid phase, we found that effects of the environment (neighboring nucleotides, water molecules and counter ions) do not mask rectification of ssDNA during its translocation through the nanogap, offering the possibility for high-throughput and precise ssDNA sequencing by rectification.

Nanofluidics with ultrathin nanopores

Sanjin Marion
imec
Kapeldreef 75, 3001 Leuven / Belgium
Email: sanjin.marion@imec.be

Nanopores in solid state membranes are a tool able to probe nanofluidic phenomena or can act as a single molecular sensor. They also have diverse applications in filtration, desalination, or osmotic power generation. Many of these applications involve chemical, or hydrostatic pressure differences which act on both the supporting membrane and analyte, and can influence the ion transport through the pore. Although all of these diverse applications are done in an aqueous environment, little is known about fluid flow and its coupling with ion transport properties.

I will demonstrate an approach using hydraulic pressure coupled with alternating current which is used to probe small differences in ion transport characteristics of ultrathin nanopores. Through hydraulic pressure differences between the sides of the membrane we are able to induce two separate phenomena. First, due to a low hydraulic resistance at the mouth of the ultrathin pore, advective ion transport dominates diffusive, causing nonlinear coupling of ion transport with the applied pressure. This coupling can be leveraged to increase nanopore properties like ion selectivity, and can produce strong pressure dependent effects even without external driving forces. Secondly, we demonstrate that blistering of the membrane under pressure induces enlargement of the pore diameter, and is a direct measure of the strain at the pore. This allows controlled application of in-situ strain on nanopores in 2D materials like MoS2 or hBN, opening up pathways for probing ionic hydration layers and artificial mechanosensitive sensors.

Gating of Nanopores with Large Polarizable Ions and Organic Solvents

Jake W. Polster,[1] Wilfred S. Russell,[1] Fikret Audin,[2] Pedro de Souza,[3] Martin Z. Bazant,[3,4] John T. Fourkas,[5] Narayana R. Aluru,[6] Anh Pham,[2] **Zuzanna S. Siwy[7,8]**

[1]*Department of Chemistry, University of California, Irvine, CA 92697, USA*

[2]*Quantum Simulations Group and Laboratory for Energy Applications for the Future, Lawrence Livermore National Laboratory, Livermore, California 94551, USA*

[3]*Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA*

[4]*Department of Mathematics, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA*

[5]*Department of Chemistry and Biochemistry, University of Maryland, College Park, Maryland, USA*

[6]*Department of Mechanical Science and Engineering, University of Illinois at Urbana-Champaign, Urbana, Illinois, USA*

[7]*Department of Physics and Astronomy,* [8]*Department of Biomedical Engineering, University of California, Irvine, CA 92697, USA*
E-mail: zsiwy@uci.edu

Solid-state nanopores have become the basis for biological sensors as well as preparation of nanopores with transport properties inspired by functionality of biological channels. Not surprisingly, therefore, the majority of experiments with solid state nanopores have been performed in aqueous media. My talk will be divided into two parts. In the first part I will show how ion specific effects can be induced in nanopores lined with hydrophobic groups.[1] Synergistic experimental and modeling work has revealed that large polarizable ions such as iodide and bromide accumulate at the hydrophobic walls, leading to pore wetting when external electric field is applied. The same nanopores are closed for any transport in chloride salts. The second part of the talk will show tuning transport properties of nanopores by the choice of a solvent. We have discovered that nanopores that are negatively charged in aqueous media acquire effective positive surface charge when in contact with electrolyte solutions in aprotic solvents, propylene carbonate and acetonitrile.[2] The effective positive charge stems from the long-range, bilayer-like structure of the solvent, revealed by the Vibrational Sum Frequency Generation Spectroscopy. We hypothesize that the highly robust organization of the solvent at the interface dictates partition of anions and cations to the surface, and consequently the effective surface charge. Consequences of the positive surface charge for electrokinetic phenomena will be presented. Description of solid/liquid interfaces discussed in this talk necessitates considering ion specific effects and solvent structure that cannot be captured by the classical electrical double layer model.

1. Polster, J.W., Acar, E.T., Aydin, F.; Cheng, Z.; Pham, T.A., Siwy Z.S. Gating of Hydrophobic Nanopores with Large Anions, *ACS Nano* **14**, 4306-4315 (2020).
2. Polster, J.W., Souna, A.J., Motevaselian, M.H., Lucas, R.A., Tran, J.D., Siwy, Z.S., Aluru, N.R., Fourkas, J.T. The electrical double layer revisited, *Natural Sciences* **2**, e20210099 (2022).

Stimuli-Responsive Coacervates as Universal Carriers for Intracellular Delivery of Macromolecular Therapeutics

Ali Miserez

Center for Sustainable Materials (SusMat), School of Materials Science and Engineering and School of Biological Sciences, Nanyang Technological University (NTU), Singapore.
Email: ali.miserez@ntu.edu.sg

Abstract

Macromolecular therapeutics (peptides, proteins, mRNAs, plasmid DNAs, etc…) hold vast therapeutic potential across human disease states by providing opportunities to address targets that have proven refractory to traditional approaches. However, a critical impediment for the successful application of these modalities is their inability to cross cellular membranes, preventing access to intracellular targets. Current approaches to solve this key issue are based on nanoscale carriers to deliver the payloads, which however have several drawbacks including a tendency to get entrapped in endosomal compartments, poor biodistribution, and in some cases dose-limiting toxicity. Bypassing endosomal entrapment for direct cytosolic payload delivery is an attractive alternative approach but current methods suffer from their own pitfalls. For example, the carriers are typically limited to delivery of a particular therapeutic modality or to relatively low molecular weight (MW) cargos. Furthermore, many approaches involve laborious synthetic procedures and/or encapsulation processes using organic solvents that can decrease bioactivity of the therapeutic cargo.
In this talk, I will present a unifying delivery strategy of macromolecular therapeutics recently developed by our team that is cargo-agnostic, does not cross the cell membrane through classic endocytosis, and non-cytotoxic[1]. This new method exploits Liquid-Liquid Phase Separation (LLPS) of engineered peptides[2,3] self-assembling into therapeutic-carrying coacervate microdroplets that are capable to release their cargo in the cytosol. These peptide microdroplet carriers benefit from several unique advantages that set them apart from other approaches[1]:

(1) A remarkable wide range of therapeutics can be quickly recruited in the droplets, from short therapeutic anti-cancer stapled peptides to very large enzymes (430 kDa) to mRNAs;
(2) The recruitment process is rapid and carried out under aqueous environments, thus preserving bioactivity of the therapeutics[4]. Furthermore, the recruitment efficiency is above 90% in all tested macromolecular therapeutics tested so far;
(3) The coacervates readily cross the cellular membrane, bypassing classical endocytosis pathways to enter in the cytosol[5];

(4) The side-chains of the peptides are conjugated with a redox-responsive moiety, which triggers disassembly of the droplets in the reducing environment of the cell, leading to efficient payload release;
(5) Finally, we have demonstrated that the bioactivity of the released therapeutics is retained in the cell and that mRNAs exhibit high transfection efficiency.

Together, this platform thus represents a general and robust strategy for the intracellular delivery of a range of macromolecular modalities with promising potential for the treatment of a spectrum of human diseases such as cancers, metabolic diseases, or genetic disorders**.** Furthermore, these peptide coacervates could also be used as novel carriers for next-generation mRNA-based therapeutics.

References

1. Sun, Y. *et al.* Phase-separating peptides for direct cytosolic delivery and redox-activated release of macromolecular therapeutics. ***Nature Chemistry*** **14**, 274-283, (2022).
2. Gabryelczyk, B. *et al.* Hydrogen Bond Guidance and Aromatic Stacking Drive Liquid-Liquid Phase Separation of Intrinsically Disordered Histidine-Rich Peptides. ***Nature Communications 10*:5465**, (2019).
3. Sun, Y., Lim, Z. W., Guo, Q., Yu, J. & Miserez, A. Liquid–liquid phase separation of proteins and peptides derived from biological materials: Discovery, protein engineering, and emerging applications. ***MRS Bulletin 45***, 1039-1047, (2020).
4. Lim, Z. W., Ping, Y. & Miserez, A. Glucose-Responsive Peptide Coacervates with High Encapsulation Efficiency for Controlled Release of Insulin. ***Bioconjugate Chemistry 29***, 2176-2180, (2018).
5. Lim, Z. W., Varma, V., Ramanujan, R. V. & Miserez, A. Magnetically Responsive Peptide Coacervates for Dual Hyperthermia and Chemotherapy Treatments of Liver Cancer. ***Acta Biomaterialia 110***, 221-230, (2020).

RNA-DNA nanotechnology identifies native RNA with nanopore sensing

Ulrich F. Keyser

Cavendish Laboratory, University of Cambridge
JJ Thomson Ave, Cambridge, CB3 0HE, United Kingdom
Email ufk20@cam.ac.uk

Abstract body

DNA nanotechnology is transformative for experiments that require molecular control over the shape of nanometer-sized objects. In combination with nanopores DNA self-assembly allows for novel experiments that reveal the physics of ions, and polymers on the single molecule level.

Nanopore sensing, best known for DNA sequencing, translates the three-dimensional structure of molecules into ionic current signals. Designed DNA molecules enable multiplexed protein sensing with an all-electrical approach [1]. Here, I will discuss our recent developments to detect and localise structures as accurately as possible along DNA molecules approaching super-resolution microscopy [2]. Based on our high-resolution measurements, I will show how to use the fundamental understanding for the identification of miRNA, RNA viruses and their variants [3], and RNA isoforms without reverse transcription or amplification [4]. In the future, our technology will enable to identify and quantify RNA structural elements and offer a strategy for the mapping of RNA binding proteins.

References (American Physical Society style)

1] N. A. W. Bell and U. F. Keyser. Digitally encoded DNA nanostructures for multiplexed, single-molecule protein sensing with nanopores. Nature Nanotechnology, 11:645-651 (2016). http://dx.doi.org/10.1038/nnano.2016.50

[2] K. Chen, I. Jou, N. Ermann, M. Muthukumar, U. F. Keyser, and N. A. W. Bell. Dynamics of driven polymer transport through a nanopore. Nature Physics, 17, 1043-1049 (2021).

[3] F. Bošković, J. Zhu, R. Tivony, A. Ohmann, K. Chen, M. Alawami, M. Djordjevic, N. Ermann, J. Pereira Dias, M. Fairhead, M. Howarth, S. Baker, and U. F. Keyser. Simultaneous identification of viruses and SARS-CoV-2 variants with programmable DNA nanobait. medRxiv 2021.11.05.21265890v1, (2021).

[4] F. Bošković and U. F. Keyser. Nanopore microscope identifies RNA isoforms with structural colors. bioRxiv 10.1101/2021.10.16.464631v1, (2021).

Is water just a substrate?

Vasily Artemov

École Polytechnique Fédérale de Lausanne (EPFL)
CH-1015 Lausanne/Switzerland

Email vasily.artemov@epfl.ch

Abstract
Water is the most important but also the most underestimated biological liquid. I will present the latest understanding of the structure and dynamics of water, based on state-of-the-art dielectric, terahertz, and infrared spectroscopic data. I will discuss the details of short sub-picosecond dynamics in water and will show its importance for understanding the aqueous biological and artificial systems. The particular reference will be given to aqueous solutions and the properties of water at nanoconfinement. The recent theoretical models will be discussed along with their application in electrochemical energy storage, nanofiltration, and biological systems.

Acknowledgment
I would like to acknowledge my co-authors and colleagues Alexander Ryzhov, Svetlana Ponomarenko, Keith Stevenson, Henni Ouerdane, Pavel Kapralov, Martin Dressel, Artem Pronin, and Ece Uykur, who helped to convert the ideas into reality. Special thanks to Aleksandra Radenovic and the Laboratory of Nanoscale biology at EPFL, with whom I am happy to work on this and related topics.

References
[1] V. Artemov, The Electrodynamics of Water and Ice, *Springer: Ser. in Chem. Phys.* 124 (2021).
[2] S. Ponomarenko, A. Ryzhov, A. Radenovic, K. J. Stevenson, V. Artemov, Confinement-controlled Water Engenders High Energy Density Electrochemical-double-layer Capacitance, *arXiv:2204.10127* (2022).
[3] V. Artemov, E. Uykur, S. Roh, A. Pronin, H. Ouerdane, and M. Dressel, Revealing excess protons in the infrared spectrum of liquid water, *Sci. Rep.*, 10, 11320 (2020).
[4] V. Artemov, E. Uykur, P. O. Kapralov, A. Kiselev, K. Stevenson, H. Ouerdane, M. Dressel, Anomalously High Proton Conduction of Interfacial Water, *J. Phys. Chem. Lett.*, 11, 3623 (2020).
[5] V. Artemov, A. Ryzhov, H. Ouerdane, K. J. Stevenson, Ionization difference between weak and strong electrolytes: the role of protonic quantum effects, *ChemRxiv* (2021).

ELUCIDATING TRANSPORT DYNAMICS IN SOLID-STATE NANOPORES

Martin Charron, Lucas Philipp, Liqun He, Philipp Karau, Kyle Briggs and Vincent Tabard-Cossa

Department of Physics, University of Ottawa
150 Louis-Pasteur Ottawa, K1N 6N5, Ontario, Canada
Email vincent@tcossalab.net

Many emerging applications in diverse areas such as proteomics, clinical diagnostics and molecular information storage make use of chimeric or nanostructured polymers and DNA nanotechnology (nanostructures self-assembled via specific base pairing of DNA) to fingerprint proteins, detect disease biomarkers, or encode digital information. Understanding the details of the process by which these polymers are captured and traverse solid-state nanopores however remains a challenging task due to the complex nature of the non-equilibrium translocation dynamics which occur on multiple timescales and that are dictated by forces over which experimental control is often limited. This unfortunately also makes verification of theoretical concepts difficult.

In this work, we present experimental solid-state nanopore data of linear DNA fragments and DNA nanostructures for a wide range of conditions to elucidate the dynamics of polymer capture and translocation and to highlight how the relevant forces and transport processes can change. More precisely, using asymmetric salt concentration conditions and DNA polymers patterned with 3 helix bundle (3HB) sub-structures, we report the scaling of various transport metrics on voltage and polymer length and use this data to corroborate the predictions of tension propagation theory and verify the impact of the electric field gradient on pre-stretching approaching polymers [1]. Furthermore, we present data relating the dependence of translocation time on pore size, in which longer translocation times for larger pores are counterintuitively observed, which we argue further support tension propagation theory. Finally, we show how statistics of folded translocations of linear and nanostructured DNA can give insights into polymer-pore interactions, and polymer conformations at the onset of translocation. In particular, we discuss how the passage of 3HB DNA nanostructures can be dictated by its defect density [2]. These results are used to inform several biosensing and digital data storage applications.

References

[1] Charron, M.; Philipp, L.; He, L.; Tabard-Cossa, V., Elucidating the Dynamics of Polymer Transport through Nanopores Using Asymmetric Salt Concentrations, ChemRxiv (2022). doi.org/10.26434/chemrxiv-2021-8wm8v

[2] Charron, M.; He, L.; Karau, P.; Briggs, K.; Tabard-Cossa, V., Folded Passage of DNA Assemblies through Solid-State Nanopores Gives Insight on Structural Rigidity, in preparation (2022).

TOOLS TO ACCELERATE SOLID-STATE NANOPORE RESEARCH

Vincent Tabard-Cossa[1,2], Matthew Waugh[1,2], Dmytro Lomovtsev[1,2], Gengyang Mu[1,2], Mathieu Gibeault[2] and Kyle Briggs[1,2]

[1]Department of Physics, University of Ottawa, Ottawa, Ontario, Canada; [2]Northern Nanopore Instruments, Ottawa, Ontario, Canada

Email vincent@tcossalab.net

Solid-state nanopores are promising sensors for electrical detection of a range of single copies of biological molecules. There are many potential applications, including sequencing, proteomics, clinical diagnostics, molecular information storage, and more. However, the pace of discovery and translation into practical applications has been hampered by the difficulty of fabricating precisely sized, low-noise, and stable solid-state nanopores, which in turn limits the rate of high-quality data generation. To address this challenge, we invented the controlled breakdown (CBD) nanopore fabrication method,[1] and developed a small benchtop tool and associated disposables which automates the CBD method. We made it freely available to the community,[2] and more recently are providing a turn-key solution through Northern Nanopore Instruments to further facilitate the dissemination of this technology.

Here we present advances toward the next generation of scientific tools and protocols to streamline and accelerate solid-state nanopore research. We present advances which enable parallel nanopore sensing at high bandwidth, including fully automated tools and workflows for rapid fabrication of many solid-state nanopores in parallel, utilizing multi-channel millifluidic flow cells and multi-membrane chips. Together these reduce experimental time and cost while increasing throughput. Furthermore, we present advanced, yet easy-to-use software tools to analyze time series of ionic current nanopore data. This tool simplifies data post-processing and automates most common nanopore analysis tasks without requiring any programming knowledge by the user, facilitating collaboration and reproducibility. Together, these tools are expected to democratize the use of solid-state nanopores and allow experts and non-experts alike to accelerate the pace of their research toward a host of applications including the fundamentals of ionic and polymer transport through nanofluidic channels, characterization of proteins, design of biomimetic nuclear pore complexes, and development of different bioassay schemes for diagnostic purposes.

References

[1] Kwok et al. Nanopore Fabrication by Controlled Dielectric Breakdown, PLoS ONE 9(3): e92880 (2014)

[2] Waugh et al. Solid-State Nanopore Fabrication by Automated Controlled Breakdown, Nature Protocols 15(1) 122-134, (2020)

Prolonging the Nanopore Electro-Osmotic trap time for protein dynamics detection

Chenyu Wen[1,2], Eva Bertosin[2], Pierre Stömmer[3], Hendrik Dietz[3], Cees Dekker[2], Sonja Schmid[1]

1 NanoDynamicsLab, Laboratory of Biophysics, Wageningen University, Stippeneng 4, Wageningen, 6708 WE, The Netherlands.
2 Department of Bionanoscience, Kavli Institute of Nanoscience, Delft University of Technology, Van der Maasweg 9, Delft, 2629 HZ, The Netherlands.
3 Physik Department, Technische Universität München, Garching near Munich, 85748, Germany
Email: chenyu.wen@wur.nl

Protein dynamics detection is of great interest to a wide array of researchers in the life science [1]. The Nanopore Electro-Osmotic trap (NEOtrap) is an emerging single-molecule label-free technology for protein dynamics detection [2]. In the NEOtrap system, target proteins are trapped by the electroosmotic flow generated from the docking of a DNA origami sphere onto a nanopore. By analyzing ionic current traces during the trapping, which monitor volume and shape changes, various protein-specific features can be accessed, including unique morphological and dynamic information [3]. However, so far, large proteins could easily be trapped but the trapping time of small proteins was limited, e.g. by thermal fluctuations of the origami sphere, which sets a lower size limit of detection for this technology. In order to prolong the trapping time, we functionalized the surface of the DNA sphere with cholesterol molecules, which act as lipid anchors to lock the DNA sphere to on the lipid-coated surface of nanopore. The stabilized DNA sphere indeed significantly prolongs the trapping time of proteins by an order of magnitude, allowing to stably trap and monitor proteins with smaller molecular weights, down to at least 29 kDa (Carbonic Anhydrase). This stable docking likely is explained by elimination of a possible escape pathway between the nanopore and DNA sphere. We aim to clarify the trapping time and its relation to the size of nanopore and the orientation of the docked DNA sphere. The improvements presented here extend the detection ability of NEOtrap towards smaller proteins, which will promote the wider applications of NEOtrap in various biological and medical scenarios.

References

[1] S. Schmid, and C. Dekker, Nanopores: a versatile tool to study protein dynamics. *Essays in Biochemistry* **65**, 93–107, (2020).
[2] S. Schmid, and C. Dekker, The NEOtrap – en route with a new single-molecule technique. *iScience* **24**(10), 103007, (2021).
[3] S. Schmid, P. Stömmer, H. Dietz, and C. Dekker, Nanopore electro-osmotic trap for the label-free study of single proteins and their conformations. *Nat. Nanotechnol.* **16**, 1244–1250 (2021).

Single-particle Methods for Quantitative Assessment of Geometrical Parameters of Small Viruses

Kun Li[1,2,3], Arjav Shah[1,2], Patrick Doyle[1,2], Slaven Garaj[1,3]

1. Singapore-MIT Alliance for Research and Technology Centre, CREATE Tower, Singapore

2. Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts, 02139, USA

3. Department of Physics, National University of Singapore, Singapore

Email kun.li@smart.mit.edu

The geometrical structure of biological entities – including eukaryotic cells, bacteria, viruses, bacteriophage and macromolecules – are important to understand as their activity, function and heterogeneity[1–3] are critical for many future technologies, such as sustainable energy harvesting[4] , DNA-based information storage[5], nanotechnology medicine[6] and imaging applications[7]. Therefore, precise, high-throughput methods of characterization have garnered significant interest to measure their geometrical features. For large structures, e.g. bacteria and large viruses, the task is manageable. For structures having dimensions below 100 nm, the precise measurements become complicated and new high-throughput methodology is needed. In this work, we explored and benchmarked two emerging single-particle methods, nanopore tomography (NT) and helium ion microscope (HIM) imaging, to measure bio-structures having dimension below 10 nm. Our model system is filamentous *fd* bacteriophage (*fd-wt*) and its modifications with different mechanical properties (*fd-Y21M*). It is a good model system – long filament makes it easy to find, but lateral dimension is hard to determine. NT methodology was established to detect viruses with current modelling. The results of NT and HIM are compared to our measurements of *fd* diameters with transmission electron microscopy (TEM) and atomic force microscopy (AFM), and also compared to an ensemble of methods in the literature, including transient electrical birefringence (TEB)[8], x-ray diffraction (XRD)[9], neutron scattering (NS)[10], nuclear magnetic resonance (NMR)[11]. The measured *fd* diameters of NT [6.85±0.49 nm (*fd-wt*), 7.18±0.48 nm (*fd-Y21M*)] and HIM [8.69±0.55 nm (*fd-wt*), 6.38±1.09 nm (*fd-Y21M*)] showed as good accuracy as that of TEM [7.21±1.36 nm (*fd-wt*), 7.83±0.86 nm (*fd-Y21M*)] and AFM[8.00±0.39 nm (*fd-wt*), 10.23±0.83 nm

(*fd-Y21M*)]. NT has very large throughput that 4568 individual virions were measured within 45 minutes operating time, but measurements rely on an accurate theoretical model to convert current blockade signals into geometric dimensions, which has to be well understood. NT could be used for fingerprinting viruses and pathogens by establishing a library of NT signals versus geometry. HIM has relatively high throughput and low operating time due to the greater depth of field than TEM and faster scanning speed than AFM, and simple sample preparation that does not require negative staining or gold coating. Given the continued importance of viruses, our work aims to develop the use of HIM and NT for fingerprinting and exploring heterogeneity of small viruses.

References

[1] J. E. Jones, V. Le Sage, and S. S. Lakdawala, *Viral and Host Heterogeneity and Their Effects on the Viral Life Cycle*, Nat. Rev. Microbiol. **19**, 272 (2021).

[2] M. G. Mateu, editor , *Structure and Physics of Viruses: An Integrated Textbook*, 2013th ed. (Springer, Dordrecht, Netherlands, 2013).

[3] K. F. Edwards, G. F. Steward, and C. R. Schvarcz, *Making Sense of Virus Size and the Tradeoffs Shaping Viral Fitness*, Ecol. Lett. **24**, 363 (2021).

[4] Y. J. Lee, H. Yi, W.-J. Kim, K. Kang, D. S. Yun, M. S. Strano, G. Ceder, and A. M. Belcher, *Fabricating Genetically Engineered High-Power Lithium-Ion Batteries Using Multiple Virus Genes*, Science **324**, 1051 (2009).

[5] G. M. Church, Y. Gao, and S. Kosuri, *Next-Generation Digital Information Storage in DNA*, Science **337**, 1628 (2012).

[6] J. Judd, M. L. Ho, A. Tiwari, E. J. Gomez, C. Dempsey, K. Van Vliet, O. A. Igoshin, J. J. Silberg, M. Agbandje-McKenna, and J. Suh, *Tunable Protease-Activatable Virus Nanonodes*, ACS Nano **8**, 4740 (2014).

[7] X. Huang, B. D. Stein, H. Cheng, A. Malyutin, I. B. Tsvetkova, D. V. Baxter, N. B. Remmes, J. Verchot, C. Kao, L. M. Bronstein, and B. Dragnea, *Magnetic Virus-like Nanoparticles in N. Benthamiana Plants: A New Paradigm for Environmental and Agronomic Biotechnological Research*, ACS Nano **5**, 4037 (2011).

[8] J. Newman, H. L. Swinney, and L. A. Day, *Hydrodynamic Properties and Structure of Fd Virus*, J. Mol. Biol. **116**, 593 (1977).

[9] D. A. Marvin, R. L. Wiseman, and E. J. Wachtel, *Filamentous Bacterial Viruses. XI. Molecular Architecture of the Class II (Pf1, Xf) Virion*, J. Mol. Biol. **82**, 121 (1974).

[10] J. Torbet, *Neutron Scattering Study of the Solution Structure of Bacteriophages Pf1 and Fd*, FEBS Lett. **108**, 61 (1979).

[11] A. C. Zeri, M. F. Mesleh, A. A. Nevzorov, and S. J. Opella, *Structure of the Coat Protein in Fd Filamentous Bacteriophage Particles Determined by Solid-State NMR Spectroscopy*, Proc. Natl. Acad. Sci. U. S. A. **100**, 6458 (2003).

ELUCIDATING TRANSPORT DYNAMICS IN SOLID-STATE NANOPORES

Martin Charron, Lucas Philipp, Liqun He, Philipp Karau, Kyle Briggs and Vincent Tabard-Cossa

Department of Physics, University of Ottawa
150 Louis-Pasteur Ottawa, K1N 6N5, Ontario, Canada
Email vincent@tcossalab.net

Many emerging applications in diverse areas such as proteomics, clinical diagnostics and molecular information storage make use of chimeric or nanostructured polymers and DNA nanotechnology (nanostructures self-assembled via specific base pairing of DNA) to fingerprint proteins, detect disease biomarkers, or encode digital information. Understanding the details of the process by which these polymers are captured and traverse solid-state nanopores however remains a challenging task due to the complex nature of the non-equilibrium translocation dynamics which occur on multiple timescales and that are dictated by forces over which experimental control is often limited. This unfortunately also makes verification of theoretical concepts difficult.

In this work, we present experimental solid-state nanopore data of linear DNA fragments and DNA nanostructures for a wide range of conditions to elucidate the dynamics of polymer capture and translocation and to highlight how the relevant forces and transport processes can change. More precisely, using asymmetric salt concentration conditions and DNA polymers patterned with 3 helix bundle (3HB) sub-structures, we report the scaling of various transport metrics on voltage and polymer length and use this data to corroborate the predictions of tension propagation theory and verify the impact of the electric field gradient on pre-stretching approaching polymers [1]. Furthermore, we present data relating the dependence of translocation time on pore size, in which longer translocation times for larger pores are counterintuitively observed, which we argue further support tension propagation theory. Finally, we show how statistics of folded translocations of linear and nanostructured DNA can give insights into polymer-pore interactions, and polymer conformations at the onset of translocation. In particular, we discuss how the passage of 3HB DNA nanostructures can be dictated by its defect density [2]. These results are used to inform several biosensing and digital data storage applications.

References

[1] Charron, M.; Philipp, L.; He, L.; Tabard-Cossa, V., Elucidating the Dynamics of Polymer Transport through Nanopores Using Asymmetric Salt Concentrations, ChemRxiv (2022). doi.org/10.26434/chemrxiv-2021-8wm8v

[2] Charron, M.; He, L.; Karau, P.; Briggs, K.; Tabard-Cossa, V., Folded Passage of DNA Assemblies through Solid-State Nanopores Gives Insight on Structural Rigidity, in preparation (2022).

Liquid-activated quantum emission from native hBN defects for nanofluidic sensing

Nathan Ronceray[1,2], Yi You[3,4], Evgenii Glushkov[1], Martina Lihter[1], Benjamin Rehl[2], Tzu-Heng Chen[1], Gwang-Hyeon Nam[3,4], Kenji Watanabe[5], Takashi Taniguchi[6], Sylvie Roke[2], Ashok Keerthi[3,4], Jean Comtet[7], Boya Radha[3,4] and Aleksandra Radenovic[1]

[1] Laboratory of Nanoscale Biology, Institute of Bioengineering (IBI), School of Engineering (STI), Ecole Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland.
[2] Laboratory for fundamental BioPhotonics, IBI, STI, EPFL, Lausanne, Switzerland
[3] Department of Physics and Astronomy, School of Natural Sciences, University of Manchester, United Kingdom
[4] National Graphene Institute, University of Manchester, Manchester, United Kingdom
[5] Research Center for Functional Materials, National Institute for Materials Science, Namiki, Tsukuba, Japan
[6] International Center for Materials Nanoarchitectonics, National Institute for Materials Science, Namiki, Tsukuba, Japan
[7] Soft Matter Science and Engineering Laboratory, ESPCI Paris, CNRS UMR 7615, France

nathan.ronceray@epfl.ch, aleksandra.radenovic@epfl.ch

Nanostructures made of two-dimensional (2D) materials have become the flagship of nanofluidic discoveries in recent years[1, 2] . By confining liquids down to a few atomic layers, anomalies in molecular transport[3–5] and structure[6, 7] have been revealed. Currently, only indirect and ensemble averaged techniques have been able to operate in such extreme confinements, as even the smallest molecular fluorophores are too bulky to penetrate state-of-the-art single-digit nanofluidic systems[8]. This strong limitation calls for the development of novel optical approaches allowing for the direct molecular imaging of liquids confined at the nanoscale. Here, we show that native defects present at the surface of hexagonal boron nitride[9] (hBN) - a widely used 2D material - can serve as probes for molecular sensing in liquid, without compromising the atomic smoothness of their host material. We first demonstrate that native surface defects are readily activated through interactions with organic solvents and confirm their quantum emission properties. Vibrational spectra of the emitters suggest that their activation occurs through the chemisorption of carbon-bearing liquid molecules onto native hBN defects. The correlated activation of neighboring defects reveals single-molecule dynamics at the interface, while defect emission spectra offer a direct readout of the local dielectric properties of the liquid medium. We then harvest these effects in atomically smooth slit-shaped van der Waals channels, revealing molecular dynamics and increasing dielectric order under nanometre-scale confinement. Liquid-activated native defects in pristine hBN bridge the gap between solid-state nanophotonics and nanofluidics and open up new avenues for nanoscale sensing and optofluidics.

References

1. Faucher, S. et al. *J. Phys. Chem. C* **123**, 21309–21326 (2019).
2. Bocquet, L. *Nat. Mater.* **19**, 254–256 (2020).
3. Majumder, M., Chopra, N., Andrews, R. & Hinds, B. J. *Nature* **438**, 44–44 (2005).
4. Radha, B. et al. *Nature* **538**, 222–225 (2016).
5. Mouterde, T. et al. *Nature* **567**, 87–90 (2019).
6. Agrawal, K. V., Shimizu, S., Drahushuk, L. W., Kilcoyne, D. & Strano, M. S. *Nat. Nanotechnol.* **12**, 267–273 (2017).
7. Fumagalli, L. et al. *Science* **360**, 1339–1342 (2018).
8. Grimm, J. B. & Lavis, L. D. *Nat. Methods* **19**, 149–158 (2022).
9. Wong, D. et al. *Nat. Nanotechnol.* **10**, 949–953 (2015)

Preprint : https://arxiv.org/abs/2204.06287

Nanopore-based single-molecule protein identification

Sylvia Liang [1,3] , Wenxu Zhang [1,3] and Xiaohua Huang [1,2,*]
[1] Materials Science and Engineering Program and [2] Department of Bioengineering, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0412, USA
[3] These authors contribute equally to this work.

Proteins are the workhorses of living systems. Complete cataloging and accurate quantification of this physiologically important class of molecules are essential for proteome-scale elucidation of their functions, and the development of precise protein-based diagnosis and treatment of human diseases. However, a physical method for identification and counting of single protein molecules has remained elusive. Here, we describe a nanopore-based method for identification and digital counting of single protein molecules. The average human proteins contain many lysine residues and are relatively long. Our strategy is to use the pattern of lysine residues along the primary sequence of a protein as a fingerprint to identify the protein by pattern matching to a reference proteome database. The lysine patterns of single protein molecules are determined by measuring the current blockage of ionic current flow through a nanopore by the fully denatured linear polypeptides whose lysine residues that have been labeled with a chemical moiety. We investigated the feasibility of this method by developing an algorithm to calculate the probability of matching the simulated profiles to the references profiles of human proteome. We found that full-length proteins can be identified with >95% accuracy and up to 98% of protein fragments can be identified for fragments with 8 or more labeled lysine measurements in their signal profiles.

Activated carbon nanofluidics: from blue energy to ionic memory

T. Emmerich, K. S Vasu, P. Robin, A. Niguès, A. Siria, L. Bocquet

EPFL
Rte Cantonale 1015, Lausanne /Switzerland
theo.emmerich@epfl.ch

Nanofluidics is the study of fluids confined at the (sub)-nanoscale. From an experimental standpoint, a first and challenging step is to fabricate channels with such extreme dimensions. In that context 2D materials represent attractive building blocks to realize nanoconduits thanks to their versatility in geometry and surface properties. Here, we first display the fabrication of activated carbon nanochannels made by electron-beam induced etching and van der Waals assembly of bidimensional graphite crystals [1]. Using both voltage and pressure drop experiments, we then show that activated carbon exhibit a unique combination of high surface charge and small but non-negligible hydrodynamic slippage. Such favorable surface properties result in a strongly enhanced ionic transport and in particular osmotic currents coming from salinity gradients with single pore power densities exceeding hundreds of thousands of watts per meter-square. Finally, we show that activated carbon nanochannels can behave as ion-based memories with minute to hour long timescales [2]. This effect is related to the accumulation or depletion of ions caused by entrance effects and adsorption/desorption of ionic carriers on the channel walls. It directly echoes the building of memory in living organisms related to ionic accumulations at specific locations of neurons' membranes. Relying on this advanced response we implement an Hebbian learning algorithm using our nanofluidic channel. Our work paves the way to the development of neuromorphic nanofluidic machines and the study of ions as information carrier.

References

[1] Theo Emmerich, Kalangi S. Vasu, Antoine Niguès, Ashok Keerthi, Boya Radha, Alessandro Siria & Lydéric Bocquet. *Enhanced Nanofluidic transport in activated carbon nanoconduits. Nature Materials* (2022).

[2] P. Robin†, T. Emmerich†, A. Ismail†, A. Niguès, Y.You, G-H. Nam, A. Keerthi, A. Siria, A.K. Geim, B. Radha, L. Bocquet. *Long-term memory and synapse-like dynamics of ionic carriers in two dimensional nanofluidic channels. Under review* (2022).

†Equal contribution

Is water just a substrate?

Vasily Artemov

École Polytechnique Fédérale de Lausanne (EPFL)
CH-1015 Lausanne/Switzerland

Email vasily.artemov@epfl.ch

Abstract
Water is the most important but also the most underestimated biological liquid. I will present the latest understanding of the structure and dynamics of water, based on state-of-the-art dielectric, terahertz, and infrared spectroscopic data. I will discuss the details of short sub-picosecond dynamics in water and will show its importance for understanding the aqueous biological and artificial systems. The particular reference will be given to aqueous solutions and the properties of water at nanoconfinement. The recent theoretical models will be discussed along with their application in electrochemical energy storage, nanofiltration, and biological systems.

Acknowledgment
I would like to acknowledge my co-authors and colleagues Alexander Ryzhov, Svetlana Ponomarenko, Keith Stevenson, Henni Ouerdane, Pavel Kapralov, Martin Dressel, Artem Pronin, and Ece Uykur, who helped to convert the ideas into reality. Special thanks to Aleksandra Radenovic and the Laboratory of Nanoscale biology at EPFL, with whom I am happy to work on this and related topics.

References
[1] V. Artemov, The Electrodynamics of Water and Ice, *Springer: Ser. in Chem. Phys.* 124 (2021).
[2] S. Ponomarenko, A. Ryzhov, A. Radenovic, K. J. Stevenson, V. Artemov, Confinement-controlled Water Engenders High Energy Density Electrochemical-double-layer Capacitance, *arXiv:2204.10127* (2022).
[3] V. Artemov, E. Uykur, S. Roh, A. Pronin, H. Ouerdane, and M. Dressel, Revealing excess protons in the infrared spectrum of liquid water, *Sci. Rep.*, 10, 11320 (2020).
[4] V. Artemov, E. Uykur, P. O. Kapralov, A. Kiselev, K. Stevenson, H. Ouerdane, M. Dressel, Anomalously High Proton Conduction of Interfacial Water, *J. Phys. Chem. Lett.*, 11, 3623 (2020).
[5] V. Artemov, A. Ryzhov, H. Ouerdane, K. J. Stevenson, Ionization difference between weak and strong electrolytes: the role of protonic quantum effects, *ChemRxiv* (2021).

Building DNA nanoturbines on nanopores

Xin Shi,[1] Anna-Katharina Pumm,[2] Jonas Isensee,[3] Wenxuan Zhao,[1] Daniel Verschueren,[1, †] Alejandro Martin-Gonzalez,[1] Christopher Maffeo,[5] Aleksei Aksimentiev,[5] Ramin Golestanian,[3,4] Hendrik Dietz,[2] Cees Dekker[1,*]

[1] Department of Bionanoscience, Kavli Institute of Nanoscience Delft, Delft University of Technology, Delft, The Netherlands.
[2] Department of Physics, Technical University of Munich, Garching 85748, Germany
[3] Max Planck Institute for Dynamics and Self-Organization, Göttingen 37077, Germany
[4] Rudolf Peierls Centre for Theoretical Physics, University of Oxford, OX1 3PU, Oxford, UK
[5] University of Illinois Urbana-champaign, Loomis Laboratory of Physics, 1110 West Green Street, Urbana, IL
† Current address: The SW7 Group, 2 Allen Street, W8 6BH, London, UK
* Corresponding authors. Emails: c.dekker@tudelft.nl

Our societies have flourished because of macroscale machinery powered by engines and motors. And we are not alone in our reliance on active machines: all life itself depends on energy-consuming nanoscale machines, as work at the nanoscale is being done by millions of sophisticated molecular motors. However, until today, designing and building active energy-consuming machines at the nanoscale has remained challenging.

In this talk, I will be presenting our latest results on designing and building nanoscale DNA turbines: DNA nanostructures on nanopores that can autonomously convert transmembrane electrochemical potentials into rotary motion, similar to natural rotary motor proteins such as FoF1-ATP synthase and bacterial flagella motors. We have successfully designed and built two generations of such nanoturbines: a self-organized DNA active rotor, and a designed chiral-shaped DNA turbine. We observe sustained unidirectional the rotary motion of these nanoturbines at the single molecule level as we apply a voltage or salt gradient across the nanopore. These exciting results lay the groundwork, both theoretically and experimentally, for further studies and development of autonomous nanomachines that leverage autonomous, unidirectional rotational motion.

Simulations to complement experiments for quantitative assessment of geometrical parameters of filamentous *fd* bacteriophage

Arjav Shah[1,3], Kun Li[2,3], Slaven Garaj[2,3], Patrick S Doyle[1,3]
1. Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts, 02139, USA
2. Department of Physics, National University of Singapore, Singapore
3. *Singapore-MIT Alliance for Research and Technology Centre, CREATE Tower, Singapore*
Email:aushah@mit.edu

An understanding of the morphology of viruses is critical to understanding their function in a physiological environment, and for modern, bio-inspired applications such as batteries and medicine, etc [1, 2]. Several imaging techniques have been used to study single particles. These include transmission electron microscopy (TEM), atomic force microscopy (AFM), and nuclear magnetic resonance (NMR) among many others. However, there is a need for a rapid and high-throughput method. Nanopore tomography (NT) is one such emerging single-particle technique with the ability to measure the geometrical features of individual viruses [3]. Here, this methodology is applied to measure the diameter of the wild and mutant types of *fd*, a long, filamentous bacteriophage translocating through a solid-state nanopore at high salt concentrations.

The diameter of the translocating virus is subject to the interpretation of current blockades reported from experiments through robust theoretical and simulation approaches. In principle, the diameter can be estimated from the conductance theory that defines the change in the current based on geometrical specifications of the pore and the virus. However, it neglects any surface charge dependence and variance in bulk conductivity and access resistance due to the presence of the virus. In such cases, carefully designed numerical simulations can be a valuable tool. Steady-state, continuum simulations are performed in COMSOL Multiphysics to estimate the phage diameter from experimentally reported changes in current due to the presence of the virus. The calculated diameters [6.85±0.49 nm (*fd-wt*), 7.18±0.48 nm (*fd-Y21M*)] match closely with the measured values using imaging techniques such as helium ion microscopy (HIM), AFM, and TEM. Furthermore, the robustness and sensitivity of these estimates to variations in surface charge densities of pore and virus as well as pore thickness are investigated.

References:

1. Lee, Y.J., Yi, H., Kim, W.J., Kang, K., Yun, D.S., Strano, M.S., Ceder, G. and Belcher, A.M., 2009. Fabricating genetically engineered high-power lithium-ion batteries using multiple virus genes. Science, 324(5930), pp.1051-1055.
2. Wen, A.M. and Steinmetz, N.F., 2016. Design of virus-based nanomaterials for medicine, biotechnology, and energy. Chemical Society Reviews, 45(15), pp.4074-4126.
3. Darvish, A., Lee, J.S., Peng, B., Saharia, J., VenkatKalyana Sundaram, R., Goyal, G., Bandara, N., Ahn, C.W., Kim, J., Dutta, P. and Chaiken, I., 2019. Mechanical characterization of HIV-1 with a solid-state nanopore sensor. Electrophoresis, 40(5), pp.776-783.

Second-harmonic imaging of passive ions transport through lipid membranes

Maksim Eremchev, D. Roesel, S. Roke

Laboratory for Fundamental BioPhotonics (LBP), Institute of Bioengineering (IBI), School of Engineering (STI), École Polytechnique Fédérale de Lausanne (EPFL),
CH-1015 Lausanne, Switzerland
Email maksim.eremchev@epfl.ch

In biology, release of Ca^{2+} ions in the cytosol is essential to trigger or control many cell functions. Calcium signalling acutely depends on lipid membrane permeability to Ca^{2+}. For proper understanding of membrane permeability to Ca^{2+}, both membrane hydration and the structure of the hydrophobic core must be considered. In this work, we revisit the hypothesis that lipid membranes are impermeable to Ca^{2+} by second harmonic (SH) imaging the water structure at the interface of GUVs in contact with $CaCl_2$ solution. Varying the hydrophobic core of the bilayer membranes, different types of behavior are observed in the high throughput wide-field SH images. Ca^{2+} translocation is observed through mono-unsaturated (DOPC:DOPA) membranes and reduced upon adding cholesterol. Translocation occurs at different rates for different locations showing that a non-equilibrium membrane restructuring is needed. The complete inhibition of translocation is observed for branched (DPhPC:DPhPA) and poly-unsaturated (SLPC:SLPA) lipid membranes. The latter are found in cells such as neurons, whose function critically depends on impermeability of their membrane to Ca^{2+}. Translocated ions stay bound to the membrane which makes them invisible to conventional methods used to determine permeability. Our findings suggest that hydrophobic structure of lipids play a much more sophisticated regulating role than previously thought and that the membrane itself can play a role as a Ca^{2+} reservoir.

Manipulation of Interfacial hBN Emitters using Electrochemical Bias and AFM

Eveline Mayner

LBEN EPFL
Lausanne, Switzerland
eveline.mayner@epfl.ch

Point defects in the crystal lattice of 2D materials can significantly alter their optical, electrical, and quantum properties and the manipulation of these defects could allow the realization of nanoscale sensors and quantum information processing devices [1–3]. Hexagonal boron nitride (hBN) is a 2D material, as well as a wide-band gap semiconductor, meaning that it is generally transparent but defects in the lattice structure create intra-band gap energy levels and result in localized emission of visible light, known as colour centres [4]. Spectral single molecule localization microscopy (sSMLM) utilizes the blinking nature of these emitters to localize with nanometre resolution and determine emitter density, dynamics, intensity, and emission spectrum with a wide-field microscope [5] While the nature of these defects is not well understood, they are significantly more active in carbon bearing liquids and scanning tunnelling microscopy (STM) has shown they exist with multiple charge states [6,7]. To investigate the interfacial dynamics of these charged sites in situ, we will employ an electrochemical cell adapted to our widefield spectral SMLM and confocal microscopes, applying an out-of-plane, and later in-plane, electrochemical bias. Various processes including plasma, focused ion beam, and atomic force microscopy have been employed to engineer defects beyond hBN's native population [8–10]. With an integrated AFM-sSMLM microscope we will also quantify and localize emitters while deterministically disrupting the crystal lattice by contact with a hard AFM cantilever.

References

[1] J. Feng, H. Deschout, S. Caneva, S. Hofmann, I. Lončarić, P. Lazić, and A. Radenovic, Nano Letters **18**, 1739 (2018).
[2] A. Sajid, K. S. Thygesen, J. R. Reimers, and M. J. Ford, Communications Physics **3**, (2020).
[3] T. Vogl, G. Campbell, B. C. Buchler, Y. Lu, and P. K. Lam, (2017).
[4] M. Abdi, J. P. Chou, A. Gali, and M. B. Plenio, ACS Photonics **5**, 1967 (2018).
[5] J. Comtet, E. Glushkov, V. Navikas, J. Feng, V. Babenko, S. Hofmann, K. Watanabe, T. Taniguchi, and A. Radenovic, Nano Letters **19**, 2516 (2019).
[6] D. Wong, J. Velasco, L. Ju, J. Lee, S. Kahn, H. Z. Tsai, C. Germany, T. Taniguchi, K. Watanabe, A. Zettl, F. Wang, and M. F. Crommie, Nature Nanotechnology **10**, 949 (2015).
[7] N. Ronceray, Y. You, E. Glushkov, M. Lihter, B. Rehl, T.-H. Chen, G.-H. Nam, K. Watanabe, T. Taniguchi, S. Roke, A. Keerthi, J. Comtet, B. Radha, and A. Radenovic, (2022).
[8] Y. S. Na, J. H. Kim, S. Kang, J. H. Jeong, S. Park, D. H. Kim, K. Ihm, K. Watanabe, T. Taniguchi, Y. K. Kwon, Y. D. Kim, and G. H. Lee, 2D Materials **8**, (2021).
[9] E. Glushkov, M. Macha, E. Räth, V. Navikas, N. Ronceray, C. Y. Cheon, A. Ahmed, A. Avsar, K. Watanabe, T. Taniguchi, I. Shorubalko, A. Kis, G. Fantner, and A. Radenovic, ACS Nano **16**, 3695 (2022).
[10] X. Xu, Z. O. Martin, D. Sychev, A. S. Lagutchev, Y. P. Chen, T. Taniguchi, K. Watanabe, V. M. Shalaev, and A. Boltasseva, Nano Letters **21**, 8182 (2021).

Nanopore-Based Scanning Probe Technology for Controlled-Translocation of DNA

S.M.Leitao*, V.Navikas*, H. Miljkovic, S. Marion, Pistoletti Blanchet G., Chen K., Mayer S., Keyser U., Kuhn A., Fantner G. and Radenovic A

École Polytechnique Fédérale de Lausanne (EPFL)
Laboratory of Nanoscale Biology, Bioengineering Department IBI-STI EPFL
Email helena.miljkovic@epfl.ch

Uncontrolled dynamics of the translocation of the analyte is a key problem in solid state nanopore technology [1]. Here, we show the detection of complex topological variations in DNA by using a nanopore-based scanning probe approach. We use a glass nanopore as a scanning probe to translocate and map out molecules, tethered on a glass surface, at a constant velocity, independent of the applied bias, salt concentration and pH. Controlled-translocations with our approach increased the SNR two orders of magnitude (i.e. 100X) compared with free-translocations [2]. This improvement was achieved by decreasing the velocity of the scanning probe to achieve a constant motion and averaging multiple consecutive readings of the same molecule. We applied our method to molecular rulers, DNA gaps, Hairpins, DNA-dCas9 complexes with correlative fluorescence imaging. This method enabled high-throughput data acquisition above 100'000 readings per experiment and scanning rate of 4 readings/s. Our scanning probe approach is a promising platform for the development of diverse nanopore-based probes and redefining advanced solid-state and biological nanopore systems.

References

[1] Xue, L., Yamazaki, H., Ren, R. *et al.* Solid-state nanopore sensors. *Nat Rev Mater* **5,** 931–951 (2020).

[2] Bell, N.A.W., Chen, K., Ghosal, S. *et al.* Asymmetric dynamics of DNA entering and exiting a strongly confining nanopore. *Nat Commun* **8,** 380 (2017).

Label-free Identification of Neurodegenerative Protein Inclusions Using Deep Learning

Khalid Ibrahim[1,3*], Kristin Grußmayer[2], Hilal Lashuel[3] & Aleksandra Radenovic[1]

[1] Laboratory of Nanoscale Biology, École Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland
[2] Department of Bionanoscience and Kavli Institute of Nanoscience Delft, Delft University of Technology, Delft, Netherlands
[3] Laboratory of Molecular and Chemical Biology of Neurodegeneration, École Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland.
** khalid.ibrahim@epfl.ch*

Huntington's disease (HD) is a progressive neurodegenerative disease caused by a mutation in the Huntingtin (Htt) protein, which causes HD when its polyglutamine (polyQ) length is larger than 35 [1]. Mutant Htt has been observed to form intranuclear and cellular inclusions in HD post-mortem brain tissues [2]. The first exon of Htt (Httex1) is thought to be an important fragment to be studied, as it is a key component of intracellular protein inclusions and recapitulates the key features of HD human pathology [3]. Studies of Htt inclusions frequently use large fluorescent tags to visualize and monitor Htt expression. Despite these tags being extremely useful tools that have elucidated our current understanding of HD neuropathology, they also have their limitations, as fluorescently-tagged proteins have been shown to sometimes exhibit altered or destroyed structures and cellular functionalities [4]. It has been shown that labeling Httex1 with GFP induces a different structural organization, proteome composition and stiffness [5, 6]. It is therefore crucial to develop label-free techniques to study neurodegenerative protein inclusions to better recapitulate their true nature. In recent years, the concept of artificial staining has emerged, which uses deep learning to predict fluorescence signals from label-free signals [7, 8]. Here, we employ this concept to identify label-free Httex1 inclusions from quantitative phase images by training a convolutional neural network to do so. We have developed pixel-classification and pixel-regression models and validated them on different constructs of Httex1. Using such models, we can then analyze the properties of label-free inclusions – including their morphology, propensity and rate of aggregate growth. This proof-of-concept paves the way for similar techniques which can be of great aid in label-freely identifying other neurodegenerative inclusions.

References

[1] G. P. Bates, R. Dorsey, J. F. Gusella, M. R. Hayden, C. Kay, B. R. Leavitt, M. Nance, C. A. Ross, R. I. Scahill, R. Wetzel, E. J. Wild and S. J. Tabrizi, "Huntington disease," Nature Reviews Disease Primers (2015).

[2] M. DiFiglia, E. Sapp, K. O. Chase, S. W. Davies, G. P. Bates, J. P. Vonsattel and N. Aronin, "Aggregation of Huntingtin in neuronal intranuclear inclusions and dystrophic neurites in brain," Science (1997).

[3] L. Mangiarini, K. Sathasivam, M. Seller, B. Cozens, A. Harper, C. Hetherington, M. Lawton, Y. Trottier, H. Lehrach, S. W. Davies and G. P. Bates, "Exon 1 of the HD gene with an expanded CAG repeat is sufficient to cause a progressive neurological phenotype in transgenic mice," Cell (1996).

[4] M. T. Swulius and G. J. Jensen, "The helical mreb cytoskeleton in Escherichia coli MC1000/pLE7 is an artifact of the N-terminal yellow fluorescent protein tag," Journal of Bacteriology (2012).

[5] N. Riguet, A.-L. Mahul-Mellier, N. Maharjan, J. Burtscher, M. Croisier, G. Knott, J. Hastings, A. Patin, V. Reiterer, H. Farhan, S. Nasarov and H. A. Lashuel, "Nuclear and cytoplasmic huntingtin inclusions exhibit distinct biochemical composition, interactome and ultrastructural properties," Nature Communications (2021).

[6] F. J. Bäuerlein, I. Saha, A. Mishra, M. Kalemanov, A. Martínez-Sánchez, R. Klein, I. Dudanova, M. S. Hipp, F. U. Hartl, W. Baumeister and R. Fernández-Busnadiego, "In situ architecture and cellular interactions of polyQ inclusions," Cell (2017).

[7] C. Ounkomol, S. Seshamani, M. M. Maleckar, F. Collman and G. R. Johnson, "Label-free prediction of three-dimensional fluorescence images from transmitted-light microscopy," Nature Methods (2018).

[8] E. M. Christiansen, S. J. Yang, D. M. Ando, A. Javaherian, G. Skibinski, S. Lipnick, E. Mount, A. O'Neil, K. Shah, A. K. Lee, P. Goyal, W. Fedus, R. Poplin, A. Esteva and S. Finkbeiner, "In silico labeling: predicting fluorescent labels in unlabeled images," Cell (2018).

High-throughput nanopore fabrication and classification using FIB irradiation and automated pore edge analysis

Michal Macha[1], Sanjin Marion[1,2], Mukesh Tripathi[4], Mukeshchand Thakur[1], Martina Lihter[1], Andras Kis[4], Alex Smolyanitsky[3], and Aleksandra Radenovic[1]

[1]Laboratory of Nanoscale Biology, Institute of Bioengineering, Ecole Polytechnique Federale de Lausanne (EPFL), Lausanne, Switzerland
[2]imec, Kapeldreef 75, B-3001 Leuven, Belgium
[3]National Institute of Standards and Technology, Applied Chemicals and Materials Division, 325 Broadway, Boulder, CO 80305, USA
[4]Laboratory of Nanoscale Electronics and Structures, Electrical Engineering Institute and Institute of Materials Science

***Email**: michal.macha@epfl.ch*

Large-area nanopore drilling is a major bottleneck in state-of-the-art nanoporous 2D membrane fabrication protocols. In addition, high-quality structural and statistical descriptions of as-fabricated porous membranes are key to predicting the corresponding membrane-wide permeation properties[1,2]. In this work, we investigate Xe-ion focused ion beam as a tool for scalable, large-area nanopore fabrication on atomically thin, free-standing molybdenum disulphide. The presented irradiation protocol enables designing ultrathin membranes with tunable porosity and pore dimension, along with spatial uniformity across large-area substrates. Fabricated nanoporous membranes were characterized using scanning transmission electron microscopy imaging and the observed nanopore geometries were analyzed through a pore-edge detection script. We further demonstrated that the obtained structural and statistical data can be readily passed on to computational and analytical tools to predict the permeation properties at both individual pore and membrane-wide scales. As an example, membranes featuring angstrom-scale pores were investigated in terms of their emerging water and ion flow properties through extensive all-atom molecular dynamics simulations. We believe that the combination of experimental and analytical approaches presented here should yield accurate physics-based property estimates and thus potentially enable a true function-by-design approach to fabrication for applications such as osmotic power generation, desalination/filtration, as well as their strain-tunable versions.

References

1. Chen, J., Balan, A., Masih Das, P., Thiruraman, J. P. & Drndić, M. Computer vision AC-STEM automated image analysis for 2D nanopore applications. *Ultramicroscopy* **231**, 113249 (2021).
2. Thiruraman, J. P. *et al.* Angstrom-Size Defect Creation and Ionic Transport through Pores in Single-Layer MoS2. *Nano Letters* **18**, 1651–1659 (2018).

Dielectric properties of liquids confined in atomically thin nanochannels

M.H. Souilamas, R. Wang, Q. Yang, A. Esfandiar, A.K. Geim and L. Fumagalli

University of Manchester, National Graphene Institute
Oxford Rd, Manchester M13 9PL
Email Mordjann.souilamas@postgrad.manchester.ac.uk

New advances in 2D heterostructure technology has allowed the controlled fabrication of arrays of nanochannels with thicknesses varying from a single atomic layer to tens of nanometers[1]. Fumagalli et al[2] were able to measure the out-of-plane dielectric constant, $\varepsilon_{\perp}$, of water for the first time under strong confinement in these nanochannels by *in situ* dielectric characterization based atomic force microscopy (scanning dielectric microscopy)[3]. This was the first time that this fundamental property was directly measured in such extreme confinement, despite its huge impact on a myriad of phenomena, including van der Waals and electrostatic interactions between surfaces, ion solvation and transport, and the functioning of biomolecules. By building on that work, we are continuing the study of water confined in nanochannels of different materials such as graphene, hBN and mica and investigate their impact on water's dielectric properties. We are also studying the impact of confinement on the dielectric properties of electrolytic solutions and other solvents.

References

[1] B. Radha et al. Molecular transport through capillaries with atomic-scale precision. *Nature* 538, 222 (2016).

[2] L. Fumagalli et al. Anomalously low dielectric constant of confined water. *Science* 360, 1339-1342 (2018).

[3] L. Fumagalli et al. Label-free identification of single dielectric nanoparticles and viruses with ultraweak polarization forces. *Nature Mater.*11, 808-816 (2012).

On Durability and Stability of 2D Nanofluidic Devices for Long-term Single-Molecule Sensing

Mukeshchand Thakur,[1,*] Nianduo Cai,[1] Miao Zhang,[1] Yunfei Teng,[1,3,4] Andrey Chernev,[1] Mukesh Tripathi,[2] Yanfei Zhao,[2] Michal Macha,[1] Farida Elharouni,[1] Martina Lihter,[1] Liping Wen,[3,4] Andras Kis,[2] and Aleksandra Radenovic[1,*]

[1]Laboratory of Nanoscale Biology, Institute of Bioengineering, School of Engineering, EPFL, 1015, Lausanne, Switzerland

[2]Laboratory of Nanoscale Electronics and Structure, Institute of Electrical Engineering and Institute of Materials Science and Engineering, School of Engineering, EPFL, 1015, Lausanne, Switzerland

[3]CAS Key Laboratory of Bio-inspired Materials and Interfacial Science, Technical Institute of Physics and Chemistry, Chinese Academy of Sciences, 100190, Beijing, China

[4]School of Future Technology, University of Chinese Academy of Sciences, 100049, Beijing, China

*Email: mukeshchand.thakur@epfl.ch, aleksandra.radenovic@epfl.ch

Solid-state nanopores hold immense potential in various fields such as biosensing, osmotic power generation, and information storage. Atomically thin two-dimensional membranes such as MoS_2 can be beneficial for single-molecule nanopore-based sensing of biopolymers such as DNA. [1,2] Recent advances on 2D nanopores have mainly focused on the manufacturing and scalability of the MoS_2 nanopore devices. [3] However, there still remains a bottleneck to control the nanopore size of such atomically thin pores. Here, we evaluate major factors responsible for the instability of the monolayer MoS_2 nanopores. We identify chemical dissolution and detachment of monolayers from their underlying substrates as the major reasons for the instability of MoS_2 nanopores. Using an oxygen-free buffer environment and surface-modification of the substrate rendering them hydrophobic, improved the nanopore stability and increased their shelf-life. Understanding nanopore growth and stability can provide insights into controlling the pore size, shape, long-term measurements with high signal-to-noise ratio and engineering of durable nanopore devices.

References

[1] J. Feng, K. Liu, R. D. Bulushev, S. Khlybov, D. Dumcenco, A. Kis, and A. Radenovic, *Identification of Single Nucleotides in MoS2 Nanopores*, Nature Nanotech **10**, 12 (2015).

[2] M. Graf, M. Lihter, M. Thakur, V. Georgiou, J. Topolancik, B. R. Ilic, K. Liu, J. Feng, Y. Astier, and A. Radenovic, *Fabrication and Practical Applications of Molybdenum Disulfide Nanopores*, Nat Protoc **14**, 4 (2019).
[3] M. Thakur, M. Macha, A. Chernev, M. Graf, M. Lihter, J. Deen, M. Tripathi, A. Kis, and A. Radenovic, *Wafer-Scale Fabrication of Nanopore Devices for Single-Molecule DNA Biosensing Using MoS2*, Small Methods **4**, 2000072 (2020).

2D MoS_2 Nanopore Devices for Energy Harvesting

Nianduo Cai[1], Yunfei Teng[1], Andrey Chernev[1], Mukeshchand Thakur[1], Aleksandra Radenovic[1]*

[1]Laboratory of Nanoscale Biology, Institute of Bioengineering, School of Engineering, EPFL

Lausanne, 1015 / Switzerland
Email: nianduo.cai@epfl.ch
**Correspondence: aleksandra.radenovic@epfl.ch*

Two-dimensional (2D) material MoS_2-based nanopore [1] has provided a superior platform for various applications from biosensing to energy harvesting. The atomic thickness of monolayer MoS_2 offers high temporal and spatial resolution for biosensing applications; meanwhile, atomic thickness also provides high osmotic conductance and thus high-power density when used for osmotic energy generation. Single MoS_2 nanopore power generator has been demonstrated [2] and theoretically extrapolating the single-pore value to a multi-pore system, a world-record high power density (up to 1 MW m^{-2}) can be expected, but no experimental study on upscaling MoS_2 osmotic power generation has been done yet. Here, scaling potentiality of MoS_2 nanopore arrays for osmotic power generation is studied with 4 by 4 arrays and by precisely controlling the porosity on a "single-nanopore" level. By defining the pore size and the number of pores with the help of transmission electron microscope, the upscaling effect is observed as generated power increases when the pore amount is increased from single pore to multiple pores, meanwhile the pore area difference is maintained within ~1%. In addition, with the same number of pores, generated power increases with the pore size when it is below 10 nm. The inspiring outcomes from these studies suggest MoS_2 nanopores are promising candidates for developing renewable energy source with osmotic power.

References (American Physical Society style)

[1] M. Graf, M. Lihter, M. Thakur, V. Georgiou, J. Topolancik, B. R. Ilic, K. Liu, J. Feng, Y. Astier, and A. Radenovic, *Fabrication and Practical Applications of Molybdenum Disulfide Nanopores*, Nat Protoc **14**, 1130 (2019).

[2] J. Feng, M. Graf, K. Liu, D. Ovchinnikov, D. Dumcenco, M. Heiranian, V. Nandigana, N. R. Aluru, A. Kis, and A. Radenovic, *Single-Layer MoS 2 Nanopores as Nanopower Generators*, Nature **536**, 197 (2016).

Towards Protein Fingerprinting Using Biological Nanopores

Simon Finn Mayer, Tzu-Heng Chen, Verena Rukes, Lucien Krapp, Chan Cao, Matteo Dal Peraro, Aleksandra Radenovic
Institute of Bioengineering, School of Engineering, School of Life Science
EPFL 1015, Lausanne, Switzerland
simon.mayer@epfl.ch, tzu-heng.chen@epfl.ch

Biological nanopores are successfully used for DNA sequencing today [1], a technological leap that took only 25 years from concept to commercialization[2]. The next significant advancement in the field is the sequencing of proteins, a far greater challenge than DNA sequencing due to several hurdles: Unlike DNA, proteins are not homogeneously charged, and instead of 4 bases, 20 amino acids have to be read out. Furthermore, the protein backbone is much more flexible than DNA, making the signal highly dependent on entropy which changes during translocation [3]. Thus, the signal contrast needs to be enhanced to achieve protein sequencing. To move towards a solution for these problems, we labeled the cysteines and lysines of proteins to fingerprint them in biological nanopores; our label is large, rendering it visible and providing negative charges, which aids translocation and unfolding. Reading only two amino acids is a step toward fingerprinting. Reading just two amino acids is far from sequencing but an important step, nonetheless.

References

[1] Garalde et al., Highly parallel direct RNA sequencing on an array of nanopores, nature methods (2018).

[2] Deamer et al., Three decades of nanopore sequencing, Nat Biotechnol. (2016).

[3] Oldach., The scramble for protein nanopore sequencing, ASBMB today. (2022)
Muthukumar., Macromolecular Mechanisms of Protein Translocation, Protein Pept Lett. (2014)

Using aptamer-functionalized interface nanopores for amino acid-specific peptide sensing

T. Schlotter, T. Kloter, J. Hengsteler, N. Nakatsuka, J. Vörös, T. Zambelli
Laboratory for Biosensors and Bioelectronics, ETH Zurich
Gloriastrasse 35, 8045 Zürich
Email: schlotter@biomed.ee.ethz.ch

In recent years nanopore (NP) technology has emerged as a powerful sequencing platform that enables differentiation of the four nucleobases in individual nucleic acids. Single-molecule protein sequencing has made progress over the last years [1], but is not achieved yet due to the daunting challenge of distinguishing not four, but 20 amino acids. One approach to overcome this challenge is to use engineered NPs that allow for amino acid-specific sensing and eventually put them in series.

In earlier works we showed that NPs can be integrated into hollow Atomic Force Microscope (AFM) cantilevers to *e.g.* sense inside cells [2] and that the same setup can be used to create interface nanopores between a soft polymeric substrate and the rigid AFM cantilever [3]. Here we extend this setup in order to allow for amino acid-specific peptide sensing. We harness DNA aptamers, single-stranded oligonucleotides specifically designed to recognize analytes of interest, to address nanopore selectivity while slowing translocation times through molecular interactions. We couple such selective recognition elements to a dynamic interface nanopore (INP) system that enables tuning of the nanopore size to nanoscale resolutions [3]. Adjustment of the dynamic pore by altering the force applied by a cantilever to a soft polymer interface enables optimized aptamer-specific stochastic sensing. As proof-of-concept, a recently isolated and validated DNA aptamer for phenylalanine (Phe) is integrated into the INP system [4]. Measuring translocations of peptides that do not contain Phe yields only short current spikes (Figure 1b) while peptides with same residue number, charge, and similar molecular weight yield specific, sequence-like signals. Correlation analysis seems to validate that current trace correlates to the sequence of the peptide of interest.

Our current attempts extend the interface nanopore system to manufacturing of serial nanopores and protein-tailored nanostructures, as well as improved data processing methodologies.

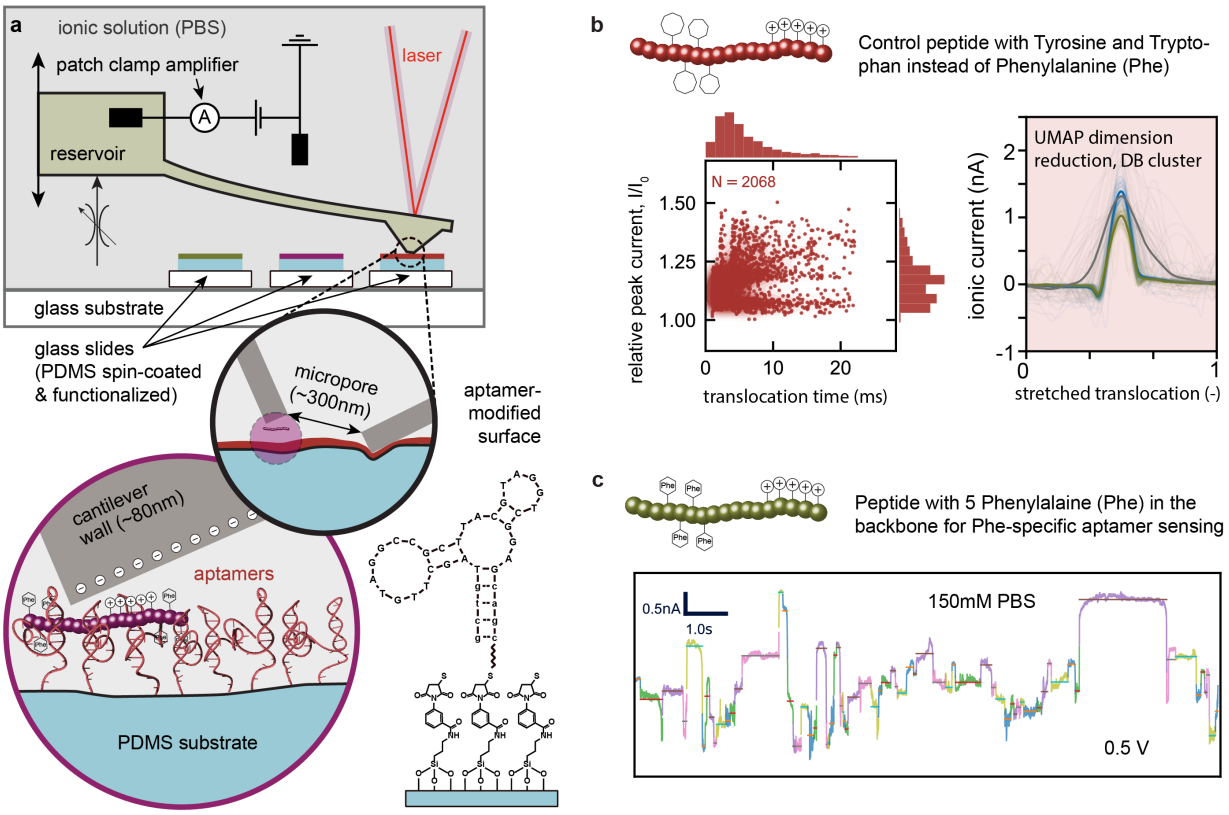


Figure 1: Translocations of specific and control peptide through force-controlled interface nanopore. a, Schematic of the force-controlled interface nanopore. **b,** translocation events of a control peptide that has 5 positive charges at one end and no phenylalanine (Phe) in the backbone. Left figure shows the translocation density plot. The right figure shows the mean shape of three different clusters of translocations that were identified using a UMAP dimension reduction with a subsequent density based (DB) clustering algorithm. **c,** time series current measurement of a PBS solution containing a specific peptide with 5 positive charges at one and 4 phenylalanine amino acids in the backbone for amino acid-specific aptamer sensing. The current trace shows color-coded current levels identified in the signal.

References

[1] H. Brinkerhoff, A. S. W. Kang, J. Liu, A. Aksimentiev, and C. Dekker, *Multiple Rereads of Single Proteins at Single-Amino Acid Resolution Using Nanopores*, Science (80-.). **374**, 1509 (2021).

[2] M. Aramesh, C. Forró, L. Dorwling-Carter, I. Lüchtefeld, T. Schlotter, S. J. Ihle, I. Shorubalko, V. Hosseini, D. Momotenko, T. Zambelli, E. Klotzsch, and J. Vörös, *Localized Detection of Ions and Biomolecules with a Force-Controlled Scanning Nanopore Microscope*, Nat. Nanotechnol. **14**, 791 (2019).

[3] T. Schlotter, S. Weaver, C. Forró, D. Momotenko, J. J. Vörös, T. Zambelli, and M. Aramesh, *Force-Controlled Formation of Dynamic Nanopores for Single-Biomolecule Sensing and Single-Cell Secretomics*, ACS Nano **14**, 12993 (2020).

[4] K. M. Cheung, K. A. Yang, N. Nakatsuka, C. Zhao, M. Ye, M. E. Jung, H. Yang, P. S. Weiss, M. N. Stojanović, and A. M. Andrews, *Phenylalanine Monitoring via Aptamer-Field-Effect Transistor Sensors*, ACS Sensors **4**, 3308 (2019).

Defect engineering of 2D material for biosensing applications

Wayne Yang, Yuta Shimoda, Eveline Simone Mayner, Michal Macha, Nathan Ronceray, Aleksandra Radenovic

École Polytechnique Fédérale de Lausanne (EPFL)
Switzerland
Email wayne.yang@epfl.ch

2D materials offer huge potential as substrates to build devices for biosensing applications but are plagued by unwanted interactions such as binding/sticking. Controlling such interactions will be critical for the continued exploration of 2D materials in biosensing. We report ongoing work where we engineer and tune the surface interactions of hexagonal boron nitride (hBN) to direct the motion and diffusion of DNA. Using fluorescence microscopy techniques, we explore the nanoscopic interactions of DNA with different 2D materials [1]. We show that pristine hBN flakes exhibit the lowest surface interactions and DNA bind preferentially to the edges and regions of high defect density of the hBN flake. We tap into a recently reported Xenon Focused Ion Beam (FIB) technique to engineer edges and defects on hBN flakes. Our technique harnesses a Xenon-FIB to lightly irradiate the desired regions of the hBN flake followed by subsequent etching in water which allows for a much cleaner hBN surface [2]. We are able to enhance DNA binding and affinity at defined locations by inducing defects using FIB. By creating long tracks of defects, we induce diffusion along our created tracks, thereby allowing us to direct motion of the DNA molecules. We envision future devices where such engineered interactions are able to direct biomolecules to sensing regions (such as a nanopore) on 2D material based devices thereby increasing the rate of analyte capture and sensitivity of single molecule sensing devices.

References

[1] Feng, J., Deschout, H., Caneva, S., Hofmann, S., Lončarić, I., Lazić, P., & Radenovic, A. (2018). Imaging of optically active defects with nanometer resolution. Nano letters, 18(3), 1739-1744.

[2] Glushkov, E., Macha, M., Rath, E., Navikas, V., Ronceray, N., Cheon, C. Y., ... & Radenovic, A. (2022). Engineering optically active defects in hexagonal boron nitride using focused ion beam and water. *ACS nano*, *16*(3), 3695-3703.

A promising nanopore technology to detect human viral infections

Montrucchio G.[1], Porro A.[1], Rosano R.[1], Marchini E.[2], Fantinati A.[2], Carli S.[3], Rizzo R.[2], Trapella C.[2], Thei F.[1]

[1]*Elements srl*
Viale Europa 596, Cesena (FC), 47521, Italy
Email fthei@elements-ic.com

[2]*Department of Chemical, Pharmaceutical and Agricultural Sciences, University of Ferrara*
Via Luigi Borsari 46, Ferrara (FE), 44121, Italy
Email anna.fantinati@unife.it ; roberta.rizzo@unife.it ; claudio.trapella@unife.it

[3]*Department of Environmental and Prevention Sciences, University of Ferrara*
Via Luigi Borsari 46, Ferrara (FE), 44121, Italy
Email stefano.carli@unife.it

Viruses are ubiquitous in the environment. While many impart no deleterious effects on their hosts, several are major pathogens. The risk for pathogenicity and the fact that many viruses can rapidly mutate highlights the need for suitable, rapid diagnostic measures. Currently, nucleic-acid detection and immunoassay methods are the most popular means for quickly identifying viral infection [1]. Despite these systems gaining attention after the spread of SARS-CoV-2, the analytical sensitivity of these assays is still arguable [2]. Moreover, the current gold standard RT-PCR, still has some limitations such as the need for an equipped lab and the long turnaround time for results [3]. Here, we propose a highly innovative system for the rapid detection of SARS-CoV-2 based on a nanopore technology combined with the resistive pulse sensing technique. In particular, the system consists of scalable, cost-effective nanopore chips and a portable high-precision current measuring instrument.
Taking advantage of the SARS-CoV-2-Spike-ACE2 cryo-EM structure we have isolated a small (20 aa long) ACE2 peptide that we linked to gold nanoparticles of different sizes in order to make the virus detection specific for SARS-CoV-2. In our preliminary data, we show that our polyimide nanopores are suitable platforms to detect particles similar in size to SARS-CoV-2 whole virions. Also, we interchanged different types of nanoparticles like, commercial gold nanoparticles, self-made nanoparticles, and polystyrene nanoparticles suspended in different types of electrolyte solutions and concentrations. However, this approach still needs some tuning, especially for what concerns the reproducibility of the nanopore size and geometry as the translocation of the nanoparticles is inconsistent among the nanopores units. This system is a promising tool not only to develop novel diagnostics on SARS-Cov-2 but also to diagnose other human viral infections.

References

[1] Cassedy A., Parle-McDermott A., O'Kennedy R , *Virus Detection: A Review of the Current and Emerging Molecular and Immunological Methods, Frontiers in Molecular Biosciences vol.8* (2021).

[2] Wang, Xueliang et al. *"Analytical sensitivity comparison of 14 conventional and three rapid RT-PCR assays for SARS-CoV-2 detection." Journal of virological methods* vol. 293 (2021)

[3] Vandenberg, O., Martiny, D., Rochas, O. et al. *Considerations for diagnostic COVID-19 tests. Nat Rev Microbiol* 19, 171–183 (2021).