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# Aptamer-modified nanopipettes – towards nanoscale chemical mapping

Scanning probe technologies have enabled surface profiling at unprecedented spatial resolutions, providing insight into the structure-property relationships of materials and biological systems [1-3]. But what if we could extend these techniques beyond topography – to mapping chemical dynamics with the same level of precision?

This capability would be particularly valuable for studying the complex communication between brain cells or neurons, driven by the electrically triggered release of neurotransmitters at nanoscale synapses [4]. While there are tools to monitor electrical signaling at high resolution *in vitro (e.g.*, complementary metal oxide semiconductor microelectrode arrays), there are limited analytical techniques that can probe the chemical activity at the resolution at which these interactions occur [5].

One promising approach to fill this technological gap, is adapting nanopipettes – used in scanning ion conductance microscopy (SICM) with nanoscale positional accuracy – into chemical biosensors (Fig. 1). This strategy could facilitate simultaneous topographic and chemical mapping at the nanoscale, providing new opportunities to investigate the complex chemical landscape of biological processes such as neuronal communication.

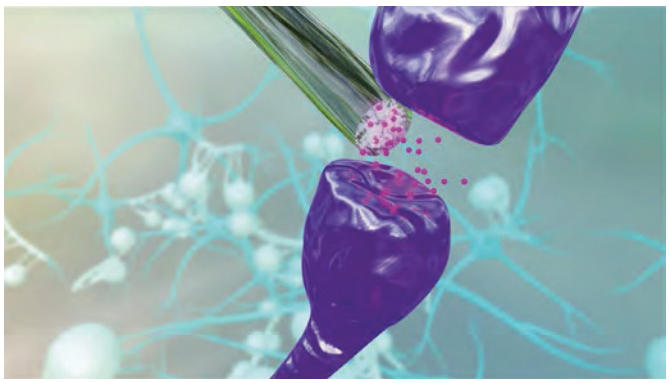

**Fig. 1: Artistic rendering of aptamer-modified nanopipettes as nanoscale probes capable of approaching the spatial scale of synapses, where neuronal communication occurs. When integrated with SICM, these sensors may enable mapping of neurotransmitter dynamics with unprecedented spatial resolution. Figure credit: Hanna Grothe and Kevin Roost.**

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## Chemical biosensing at the nanoscale

A bare quartz nanopipette can be transformed into a chemical biosensor by functionalizing the inner surface with aptamers, artificially engineered, single-stranded oligonucleotides that bind selectively to target molecules (Fig. 2a). Aptamers are generated through an iterative *in vitro* selection process called systematic evolution of ligands by exponential enrichment (SELEX) [6, 7]. The SELEX conditions can be tailored to the intended application. For example, to ensure selectivity against structurally similar interferents, candidate sequences can be exposed to these molecules during counter-SELEX, ensuring only highly specific aptamers for the target analyte are selected [8].

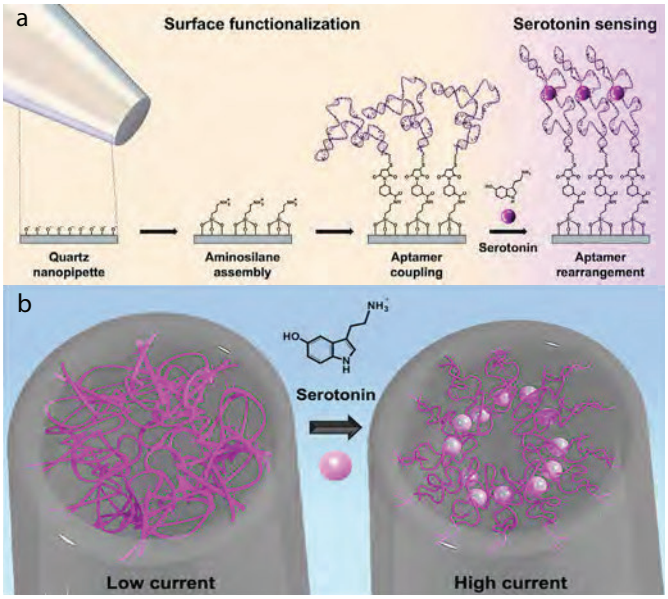


**Fig. 2: a. Schematic of the multi-step surface functionalization process, where the inner wall of quartz nanopipettes are functionalized with aptamers. First, aminosilanes are deposited on the quartz surface through controlled chemical vapor deposition. The exposed amine groups ($NH_3^+$) are then covalently coupled to thiolated serotonin aptamers, which undergo conformational changes upon target binding. b. Artistic rendering of a nanopore modified with aptamers, illustrating their structural rearrangement upon target (serotonin) binding. Both figures reproduced with permission from [9], copyright American Chemical Society.**

An additional layer of selectivity can be built into aptamers by designing them during SELEX, to undergo conformational rearrangements upon target binding [10]. As DNA or RNA-based molecules, aptamers are negatively charged, and structural switching upon target recognition redistributes these charges and associated counterions, generating a change in the electronic signal when integrated with nanopores (Fig. 2b). In conical nanopores, ion current rectification arises from asymmet-

ric ion flow, generating distinct responses under positive and negative voltage biases [11]. This effect depends on parameters such as the nanopore size, conical geometry, and charge density inside the sensitive area within the nanopore [12, 13]. Binding of the target to the aptamers induces charge redistribution within the confined nanopore, altering the measured ionic current in a target-specific manner with high sensitivity [9].

Effective gating of ionic flux requires the aptamer size to match the nanopore dimensions. For serotonin- and dopamine-specific aptamers (~ 5 nm), confinement within a ~ 10 nm pore was necessary [14]. As the structure-switching behavior of aptamers drives the measured signal, characterizing their conformational dynamics is crucial. To this end, we use a range of complementary tools. For example, single-molecule techniques such as Förster resonance energy transfer enable the analysis of aptamer backbone movements in solution [10]. In contrast, methods such as quartz crystal microbalance provide a surface-tethered readout, mimicking the nanopore sensor in an ensemble measurement [9, 14, 15]. Molecular dynamics simulations are used to corroborate experimental findings [16].

Our characterization results indicated that dopamine binding compresses the dopamine-specific aptamer backbone while serotonin recognition elongates the serotonin-specific aptamer (Fig. 3a). For the dopamine aptamer, this structural change decreases the measured ionic current through the nanopore upon dopamine binding (Fig. 3b, left). In contrast, serotonin binding increases the measured ionic current as serotonin levels rise (Fig. 3b, right). Similar divergent behavior has been observed in other biosensing platforms that rely on electrical readouts, such as field-effect transistors [10, 17]. Understanding these aptamer-specific conformational dynamics is crucial for designing nanopipette-based biosensors capable of detecting a wide range of analytes.

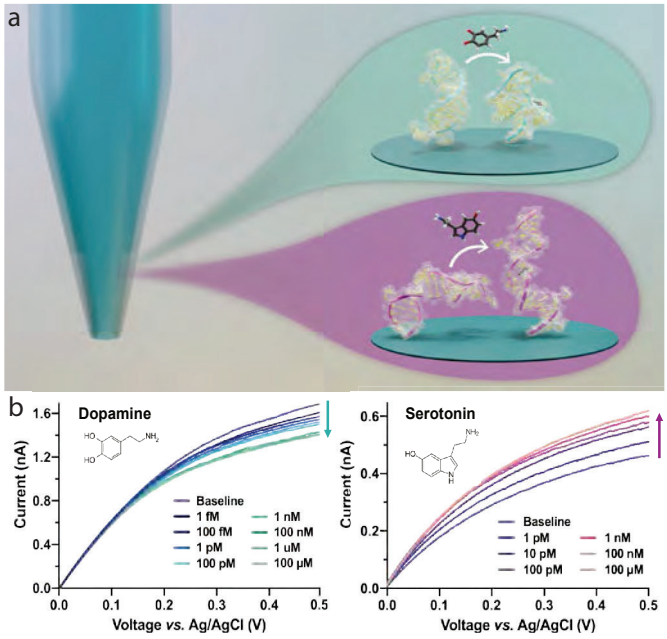


**Fig. 3: a. Illustration of aptamer-modified nanopipettes, highlighting conformational changes in the dopamine (top) and serotonin (bottom) aptamers, as extracted from molecular dynamics simulations. b. Ionic current measurements from dopamine (left) and serotonin (right) aptamer-modified nanopipettes, demonstrating distinct opposite responses upon specific target recognition. Figures reproduced with permission from [5, 14], copyright American Chemical Society.**

## Nanopores tackle nonspecific binding

Aptamer-modified nanopipettes overcome key challenges in small-molecule biosensing, particularly for selective detection in biological environments. A major bottleneck in complex milieus is the non-specific binding of interferents, which can cause false positives, sensor biofouling, and signal degradation [18]. Nanopipettes mitigate this issue by confining aptamer-target interactions inside the ~ 10 nm nanopore pre-filled with aptamers, naturally excluding larger interfering molecules. This strategy has led to the development of highly selective serotonin and dopamine sensors that have been translated to *in vitro* and *ex vivo* settings.

For example, serotonin aptamer-modified nanopipettes enabled direct quantification of serotonin release from human induced pluripotent stem cell-derived neurons [19]. The sensors detected nanomolar changes in serotonin levels following treatment with an antidepressant that modulated neurotransmitter release. Similarly, dopamine aptamer-modified nanopipettes enabled real-time tracking of endogenous dopamine release upon electrical stimulation in the striatum, highlighting their potential for tissue implantation [20].

Nanopipettes are among the smallest implantable small-molecule biosensors available, operating label-free and directly in complex biofluids such as neurobasal medium and serum, without requiring sample pre-treatment or dilution. Their nanoscale dimensions and compatibility with live tissue make them a promising tool for monitoring neurochemical flux at localized positions when coupled to SICM in the future.

## Remaining challenges for chemical mapping

Despite significant progress in developing and validating aptamer-modified nanopipettes, several challenges must be overcome to improve their performance and facilitate integration with SICM. These limitations primarily relate to sensor fabrication and reproducibility, real-time operation, and localization within complex biosystems.

One key hurdle is ensuring the reproducibility of the silanization process, the first step in the sequential surface chemistry inside the nanopipette (Fig. 2a), which is critical for sensor performance. We have made significant progress in optimizing silanization protocols by carefully controlling environmental factors to achieve consistent results across different nanopipettes and experimental conditions. However, further improvements are needed to enhance reliability and scalability.

Another fabrication challenge is precise control over the nanopore size, which is essential for adapting the sensor to different aptamers. The ~ 10 nm pore size was optimized for aptamers ~ 5 nm in their folded conformation. However, expanding the range of aptamer-modified nanopipettes to diverse targets re-

quires refining nanopore dimensions to accommodate aptamers of varying sizes. While interface nanopores enable size-tunable pores [21], they are static and cannot be placed in specific locations or interfaced with SICM, limiting their applicability.

Beyond fabrication, an important operational challenge is resetting the nanopipette after analyte binding inside the nanopore. To enable real-time quantification of unknown concentrations, the sensor must first be calibrated with known analyte amounts and then reset to its original state, ensuring free, unbound aptamers are available for subsequent measurements. For charged molecules like serotonin and dopamine, applying an electric field by sweeping voltages can effectively reset the sensor. However, this method is ineffective for neutral molecules, which do not respond to electric fields in the same way.

Beyond resetting, controlling the temporal dynamics of the aptamer-modified nanopipettes is crucial for enabling fast measurements. This capability is particularly important for applications requiring high-speed detection and continuous monitoring, such as capturing neurotransmitter fluctuations from neurons at millisecond resolution. Achieving rapid response times without compromising sensor stability remains a key technical challenge.

Finally, a challenging aspect of interfacing nanopipettes in complex environments, such as brain tissue, is precisely determining the location of the nanopore. As the nanoscale tip cannot be directly visualized, accurately pinpointing its exact placement remains difficult. Coupling nanopipettes with SICM offers a potential solution by enabling positional tracking, but simultaneously monitoring ionic currents for both topographical mapping and chemical sensing presents technical hurdles that must be addressed to ensure accurate and reliable measurements.

Overcoming these challenges will unlock the full potential of aptamer-modified nanopipettes for chemical mapping. Advancing high-speed measurements and real-time spatial tracking will be key for seamless SICM integration and broader translational use [22]. These innovations will pave the way for nanoscale biosensing technologies that enable precise interactions with biological systems, opening new avenues for studying intricate molecular processes in complex environments.

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**Sandra Hernández Escobar, M. Sc.**

Sandra Hernández Escobar completed her bachelor's degree in materials science engineering at UPM in Madrid, Spain, before pursuing a master's in life science engineering at EPFL in Lausanne, Switzerland. Throughout both her bachelor and master's studies, she focused on biomaterials and bioelectronics. Her passion for these fields led her to join the Laboratory of Chemical Nanotechnology (CHEMINA), led by Prof. Nako Nakatsuka, for her master's thesis. There, she worked on scaling up nanopipette fabrication techniques. In 2025, Sandra began her PhD at CHEMINA under the supervision of Prof. Nakatsuka. Her research interests include implantable biosensors, nanotechnology for life sciences, and flexible electronics.

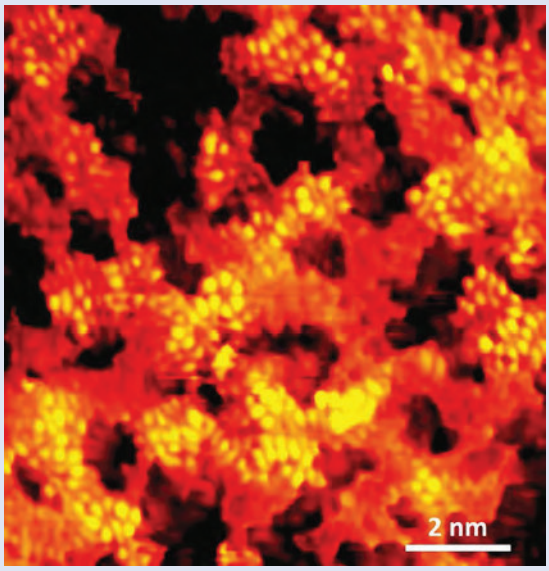
**The art of SPM**

Artist: **Lars Mohrhusen (Carl von Ossietzky Universität Oldenburg), Jeppe V. Lauritsen (Aarhus University)**

SPM technique: **Scanning tunneling microscope (STM)**

Investigated System: **Carbonaceous contaminants on a Au(111) surface (after a NAP treatment, scanned in UHV).**

Titel: „***Carbon disorder***"




**Prof. Nako Nakatsuka**

Prof. Nako Nakatsuka leads the Laboratory of Chemical Nanotechnology (CHEMINA) at the Neuro-X Institute, EPFL, since January 2024. Her research lies at the intersection of chemistry, engineering, and neuroscience, focusing on developing innovative technologies for human health. Born and raised in Tokyo, Japan, she moved to the U.S. for her bachelor's degree in chemistry at Fordham University (Bronx, NY). She then earned her Ph.D. at UCLA (Los Angeles, CA), where she developed transistor-based biosensors for neurochemical detection in the brain. After receiving the ETH Zürich Postdoctoral Fellowship, she moved to Switzerland and later became a senior scientist at the Laboratory of Biosensors and Bioelectronics. During this time, she pioneered aptamer-modified nanopipettes with the long-term vision of probing synapses at the nanoscale. For this work, she was named an MIT Under 35 Pioneer (2021), received the iCanX Young Scientist award (2022), the ACS Nano Lectureship Award (2023), and the C&EN Talented 12 Award (2024). Beyond research, Prof. Nakatsuka is passionate about science communication. She illustrated the children's chemistry book "A is for Atom: ABCs for Aspiring Chemists" to inspire future generations of scientists.