アカデミックプログラム［B講演］ | 06. 分析化学：口頭B講演

2025年3月28日(金) 9:00 ~ 11:30 [C]C402(第2学舎 2号館 [4階] C402)

[[C]C402-3am] 06. 分析化学

座長：西野 智昭、藤枝 俊宣

日本語
9:00 ~ 9:20
[[C]C402-3am-01]
エピオミクスに向けた生体高分子の1分子トンネルシーケンシング
○大城 敬人[1]、小本 祐貴[1]、谷口 正輝[1] (1. 大阪大学)

日本語
9:20 ~ 9:40
[[C]C402-3am-02]
電極間ナノギャップを分析場に用いる単分子分析法の開発
○西野 智昭[1]、高橋 泰星[1] (1. 科学大)

英語
9:40 ~ 10:00
[[C]C402-3am-03]
鋳型分子の単分子分散と蛍光分析を可能とする混合自己組織化単分子膜上での原子層堆積
○小野 赳[1]、細見 拓郎[1]、斉藤 光[2]、正井 宏[1]、池内 みどり[2]、Jiangyang Liu[1]、田中 航[1]、高橋 綱己[1]、金井 真樹[1]、寺尾 潤[1]、柳田 剛[1,2] (1. 東大、2. 九大)

10:00 ~ 10:10
休憩

英語
10:10 ~ 10:30
[[C]C402-3am-04]
ALDで形成した分子インプリンティングによる選択的なVOC捕捉
○松尾 秀明[1]、細見 拓郎[1]、劉 江洋[1]、田中 航[1]、高橋 綱己[1]、柳田 剛[1,2,3] (1. 東大院工、2. 九大先導研、3. 阪大産研)

英語
10:30 ~ 10:50
[[C]C402-3am-05]
Metal oxide adhesion layers with lower wettability for robust and sensitive cellulose nanofiber-based QCM sensors
○JING ZENG[1], Wataru Tanaka[1], Haruka Honda[1], Jiangyang Liu[1], Takuro Hosomi[1], Tsunaki Takahashi[1], Takeshi Yanagida[1,2] (1. Graduate School of Enginering, The University of Tokyo, 2. Institute for Materials Chemistry and Engineering, Kyushu University)

日本語
10:50 ~ 11:10
[[C]C402-3am-06]
薄膜金電極を用いた電気化学アプタマーセンサの物性評価とリアルタイム測定
○寺井 健人[1,2]、孫 健[1,2]、Kevin Plaxco[2]、藤枝 俊宣[1] (1. 東京科学大学、2. カリフォルニア大学サンタバーバラ校)

英語

11:10 ~ 11:30

[[C]C402-3am-07]

Design and Fabrication of Inkjet-Printed Microelectrodes for Electrochemical Sensors of Extracellular Components

○Eduardus Ariasena[1], Toshinori Fujie[1] (1. Institute of Science Tokyo)

エピオミクスに向けた生体高分子の 1 分子トンネルシーケンシング

（阪大 [1]）大城 敬人 [1]、小本 祐貴 [1]、谷口 正輝 [1]
Single-Molecule Tunneling Sequencing of Biomacromolecules for Epiomics ([1]*Osaka University*,) ○Takahito Ohshiro[1], Yuki Komoto[1], Masateru Taniguchi[1]

Peptides, RNA modifications, and amino acid modifications (including phosphorylation) play vital roles in biological processes. This study proposes a single-molecule quantum sequencer using tunneling currents to identify molecular modifications and sequences. By analyzing the signals generated as biomolecules pass through a nanogap electrode, we successfully distinguished peptides, RNA modifications such as pseudouridine, methylpseudouridine, and uridine, and amino acid modifications with high accuracy. The method demonstrated over 80% accuracy for complex mixtures, highlighting its potential in epitranscriptomics and biomolecular research.
Keywords : Peptide ; Single-Molecule Detection; Tunnel-Current; Amino acid

ペプチドや RNA 修飾、アミノ酸修飾（リン酸化を含む）は、生体内で重要な役割を果たす分子であり、それらの修飾状態や配列の詳細な解明は、生命現象の理解や疾患の診断・治療において不可欠である。本研究では、これら生体高分子の修飾状態や配列を 1 分子レベルで識別するための新たな技術として、トンネル電流を指標とした 1 分子量子シーケンサーの開発とその応用を提案する。1 分子量子シーケンサーは、ナノギャップ電極間を通過する一本鎖の生体高分子において、各モノマーや修飾の違いをトンネル電流シグナルの差異によって識別する技術である。従来、我々はこの技術を用いてアミノ酸やペプチドの識別に成功してきたが、本研究では対象を RNA 修飾やアミノ酸修飾にも拡張し、さらにその応用可能性を検討した。

まず、ナノギャップ電極を用い、1 μM の生体高分子溶液をギャップ間距離 0.54-0.58 nm に調整して計測を行った。その結果、分子がギャップを通過する際にトンネル電流の変化が観察され、このシグナルを詳細に解析することで分子識別が可能であることを確認した。ペプチドや RNA 修飾については、機械学習を用いてシグナルの特徴量を学習させ、各分子の識別を行った。その結果、ペプチドの配列再構成や RNA 修飾（シュードウリジン、メチルシュードウリジン、ウリジン等の識別）、さらにアミノ酸修飾（リン酸化アミノ酸の有無）の識別において高い精度を達成した。また、混合溶液中での解析においても、識別精度 80%以上を達成し、修飾や混合比に基づくシグナル再現が可能であることを示した。

これらの成果は、トンネル電流計測が生体高分子の修飾や配列を精度よく識別できる手法であることを示唆しており、エピオミクス研究における応用可能性を大きく拡げるものである。

[1] Ohshiro T. et al., Sci.Rep., (2019), 9, 3886 [2] Ohshiro T. et al., Sci.Rep. (2018), 8,8517. [3] Ohshiro T. et al., Sci. Rep. (2021),11,19304. [4] Ohshiro T. et al., Micromachines (2020), 11, 982.

電極間ナノギャップを分析場に用いる単分子分析法の開発

（東京科学大理 [1]）○西野 智昭 [1]・高橋 泰星 [1]
Single-molecule analysis based on nanogap between metallic electrodes ([1]*School of Science, Institute of Science Tokyo*) Tomoaki Nishino,[1] Taisei Takahashi[1]

Single-molecule junctions are created by accommodating individual molecules in the nanogap between metal electrodes. These junctions enable reliable measurements of electron transport properties of a single molecule, making them valuable tools for exploring novel electronic properties and for developing molecular electronics. The electrical conductance of single molecules is strongly dependent on their electronic structure, which in turn is determined by their chemical composition. This relationship suggests the potential for developing single-molecule analytical methods based on conductance measurements. We have developed single-molecule analysis for various biomolecules, including DNA, glucose, and phosphorylated peptides. However, when large molecules such as proteins are trapped in the nanogap, their conductance decreases significantly due to the increased gap size, making measurements challenging. In this study, we found that single-molecule detection can be achieved by measuring the conductance of proteins captured in the vicinity of the nanogap.
Keywords : Single molecule; molecular junction; DNA; electron transport; electronics

単分子接合は金属電極間のナノギャップを単分子が架橋した微小構造体である．単分子接合により単分子の電気伝導を簡便に再現性よく計測でき，電子物性の探索や分子エレクトロニクスの基礎検討において広く用いられている．一方，単分子の電気伝導度は分子の電子構造に強く依存し電子構造は分子の化学構造に起源をもつ．したがって伝導度計測を分析化学へと展開することによって単分子分析が実現できるものと期待される．当研究室では，これまで DNA[1)]，グルコース [2)]，リン酸化ペプチド [3)]（図 1）などの生体分子に対する単分子検出法を開発した．しかし，タンパク質など分子サイズの大きな試料分子をナノギャップに捕捉すると，ギャップサイズの増大に伴いその伝導度が著しく低下するため計測が困難であった．これに対し，本研究では，ナノギャップ近傍にタンパク質を捕捉し伝導度を計測することによって単分子を検出できることを見出した．

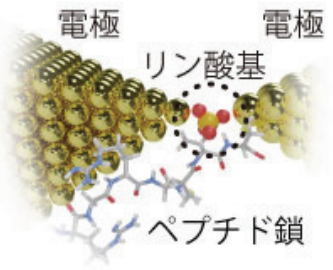


図 1. 電極間ナノギャップにおけるリン酸化ペプチド単分子検出の模式図.

1) One-by-one single-molecule detection of mutated nucleobases by monitoring tunneling current using a DNA tip. P.T. Bui et al., *Chem. Commun.* **2015**, *51*, 1666. 2) Specific single-molecule detection of glucose in a supramolecularly designed tunnel junction. T. Nishino et al. *Chem. Commun.* **2017**, *53*, 5212. 3) Unique Electrical Signature of Phosphate for Specific Single-Molecule Detection of Peptide Phosphorylation. T. Harashima et al. *J. Am. Chem. Soc.* **2022**, *144*, 17449.

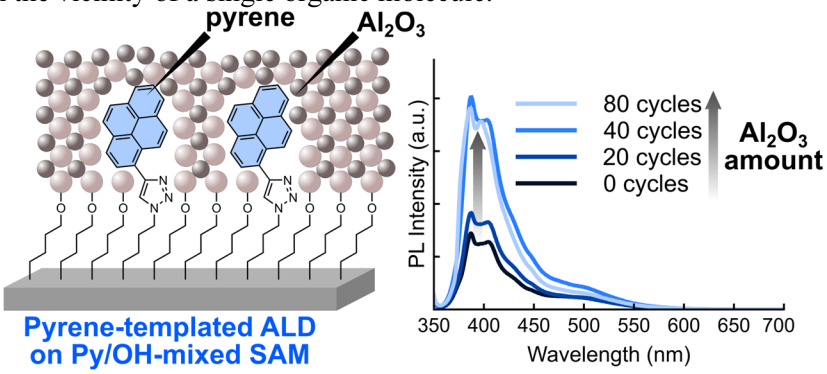
Molecular-templated ALD on Mixed SAM to Achieve Unimolecular Dispersion and In-process Fluorescent Monitoring of Template Molecules

([1]*The University of Tokyo*, [2]*Kyushu University*) ○Takeshi Ono,[1] Takuro Hosomi,[1] Hikaru Saito,[2] Hiroshi Masai,[1] Midori Ikeuchi,[2] Jiangyang Liu,[1] Wataru Tanaka,[1] Tsunaki Takahashi,[1] Masaki Kanai,[1] Jun Terao,[1] Takeshi Yanagida[1,2]

Keywords: Self-Assembled Monolayer; Atomic Layer Deposition; Organic-Inorganic Hybrid Material; Pyrene; Fluorescence

Atomic layer deposition (ALD) in the presence of organic template molecules on substrates (molecular-templated ALD) is a promising technique for designing a molecular selectivity with thermally robust metal oxides. However, self-aggregations of template molecules impair the selectivity of the resulting molecules, which is difficult to prevent or observe. Here, we propose a rational method for unimolecularly dispersing template molecules and revealing their states in the molecular-templated ALD process. Pyrene templates were dispersed into OH-terminated self-assembled monolayers (SAMs), enabling their fluorescence to monitor the microenvironments around the templates.

Mixed SAMs where pyrene templates were dispersed in the OH-terminus allowed the pyrene molecules to be 1) covalently immobilized, 2) isolated from other pyrene molecules, and 3) surrounded by ALD-reactive OH groups.[1] Systematic spectroscopic studies of pyrene probes revealed the successful ALD of metal oxides surrounding pyrene templates without their undesired aggregations. Furthermore, when Al_2O_3 was deposited, pyrene fluorescence showed enhanced intensity, lifetime, and quantum yield. These improvements in fluorescence are attributed to the suppression of non-radiative decay, indicating the growth of Al_2O_3 in the vicinity of a single organic molecule.[2]



1) T. Ono, S. Mitamura, T. Hosomi, H. Saito, M. Ikeuchi, J. Liu, K. Nagashima, T. Takahashi, W. Tanaka, M. Kanai, and T. Yanagida, *ACS Appl. Mater. Interfaces* **2023**, *15* (22), 27099. 2) T. Ono, T. Hosomi, H. Saito, H. Masai, M. Ikeuchi, J. Liu, W. Tanaka, T. Takahashi, M. Kanai, J. Terao, T. Yanagida, *Adv. Mater. Technol.* **2024**, 2401639.

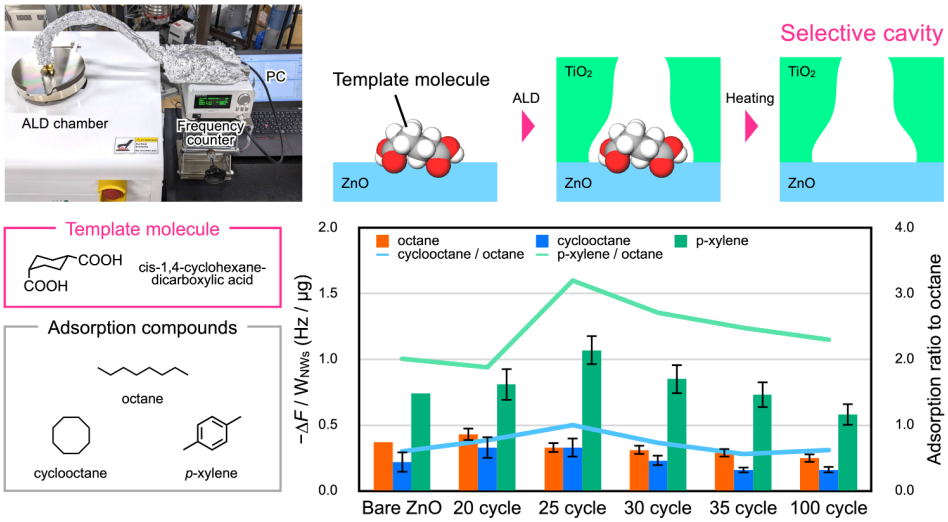
Selective VOC Capture by ALD-Formed Molecular Imprinting

([1]*Graduate School of Engineering, University of Tokyo*, [2]*Institute for Materials Chemistry and Engineering, Kyushu University*, [3]*SANKEN, Osaka University*) ○Hideaki Matsuo,[1] Takuro Hosomi,[1] Jiangyang Liu,[1] Wataru Tanaka,[1] Tsunaki Takahashi,[1] Takeshi Yanagida[1,2,3]

Keywords: VOC Sensing, Atomic Layer Deposition, Molecular Imprinting, Quartz Crystal Microbalance

The detection of volatile organic compounds (VOCs) is expected to be applied in a wide range of fields. Although various VOC sensor materials have been developed, a sensor comparable to the sense of smell has not yet been put to practical use. In recent years, molecular imprinting technology, which can impart molecular selectivity to robust inorganic materials, has been attracting attention.[1–3] We have previously developed an apparatus that uses atomic layer deposition (ALD) to carry out the adsorption process of organic molecules and the subsequent deposition process of metal oxides in situ. By monitoring these processes using a quartz crystal microbalance (QCM), the deposition behavior of metal oxides in the presence of organic molecules was clarified.[4]

In this study, we investigated conditions such as molecular species and the concentration of template molecule and evaluated the adsorption amount of template before and after ALD using FT-IR. As a result, by using a molecular species that can withstand the ALD process and be desorbed after metal oxide deposition as a template, a space reflecting the shape of the target molecule was formed, enabling selective VOC capture. The results of the molecular adsorption evaluation suggested that not only the adhesion layer but also van der Waals interaction with the deposited layer may contribute to the increase in the adsorption amount.



1) C. P. Canlas *et al.*, *Nature Chem.* **2012**, *4*, 1030–1036. 2) P. Ruff *et al.*, *Microporous Mesoporous Mater.* **2016**, *235*, 160–169. 3) N. Sobel and C. Hess, *Angew. Chem. Int. Ed.* **2015**, *54*, 15014–15021. 4) H. Matsuo *et al.*, *ACS Appl. Nano Mater.* **2024**, *7*, 24498–24507.

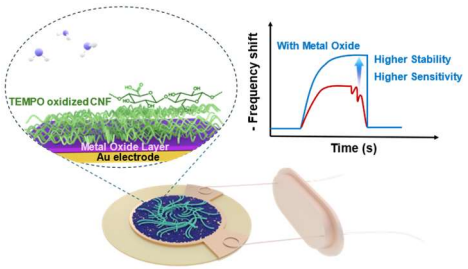
Metal oxide adhesion layers with lower wettability for robust and sensitive cellulose nanofiber-based QCM sensors

([1]*Graduate School of Engineering, The University of Tokyo*, [2]*Institue for materials Chemistry and Engineering, Kyushu University*) ○ Jing ZENG,[1] Wataru Tanaka,[1] Haruka Honda,[1] Jiangyang Liu,[1] Takuro Hosomi,[1] Tsunaki Takahashi,[1] Takeshi Yanagida[1,2]

Keywords: Humidity sensor; Cellulose Nanofiber; Metal oxide; Quartz crystal microbalance; Wettability

The Quartz crystal microbalance (QCM) device is an effective methodology for sensing analytes in gas or liquid phases, relying on linear resonance frequency decrease due to mass adsorption. Since the electrode materials lack molecular selectivity, surface modification with sensing materials like polymers, metal oxides, and carbon materials is commonly used. Recently, cellulose nanofibers (CNF) have gained attention as sensing materials due to their biocompatibility, eco-friendliness, and functional hydroxyl groups. For coating CNF on QCM sensors, an anchoring agent is necessary. A conventional method utilizes coating cationic polymers like polyethyleneimine (PEI) to improve CNF attachment on QCM. [1] However, we found that the sensor showed instability under high humidity conditions, which may be attributed to viscoelasticity changes of the PEI thin film after absorption of water.[2]

To address this issue, we deposited various metal oxide films (NiO, TiO_2, ZnO, WO_3) as robust adhesion layers to stabilize CNF films on QCM sensors. These layers significantly enhanced CNF adhesion strength and ensured sensing stability under high humidity conditions (>90%). We found a negative correlation between the QCM sensitivity and the water wettability of the metal oxide surfaces, which was attributed to the highly center-concentrated CNF microstructures observed on the hydrophobic metal oxide surfaces. This trend was further confirmed by modifying the hydrophobicity of NiO adhesion surfaces. Thus, this strategy using robust metal oxide adhesion layers will be a foundation for further developments of various CNF-based QCM gas sensors.



1) Hu, W.; Chen, S.; Liu, L.; Ding, B. *Sens. Actuators B Chem*. **2011**, 157 (2), 554–559.
2) Yao, Y.; Huang, X.; Chen, Q. *Nanomaterials (Basel)*. **2020**, *10* (11).

薄膜金電極を用いた電気化学アプタマーセンサの物性評価とリアルタイム測定

(東京科学大生命理工 [1]・カリフォルニア大サンタバーバラ校 [2]) ○寺井 健人 [1,2]・孫 健 [1,2]・Kevin Plaxco [2]・藤枝 俊宣 [1]

Physical Properties and Real-time Measurements of Electrochemical Aptamer-Based Sensors Using Thin-Film Gold Electrodes ([1]*School of Science and Technology, Institute of Science Tokyo*, [2]*Institute for Collaborative Biotechnologies, University of California, Santa Barbara*) ○Kento Terai[1,2], Kon Son[1,2], Kevin Plaxco[2], Toshinori Fujie[1]

Electrochemical Aptamer-Based (EAB) sensors are biosensors that utilize the DNA conformational change caused by the specific binding of methylene blue (MB)-modified DNA aptamer to target molecules[1]. Since EAB sensors can monitor target molecules *in vivo* in real-time, they are being applied to implantable sensors using gold wires as electrode substrates[2]. In this study, we envisioned to fabricate thin-film gold electrodes that can be applied to biological tissues such as skin, and evaluate their electrochemical properties for the development of wearable EAB sensors. Specifically, we evaluated the usefulness of various thin-film gold electrodes for EAB sensors by comparing the electrochemical properties of gold electrodes fabricated by sputter, thermal evaporation (therm. evap.) or ink-jet printing (two types of Au ink, Au-J ink: gold nano-ink and Au-JB ink gold nano-ink with binder particles) with those of conventional gold wire electrodes. Stability evaluation by Square Wave Voltammetry (SWV) showed that current baseline was 1-2 µA lower for wire, therm. evap., and Au-JB ink than for sputter and Au-J ink. SWV showed that therm. evap. and Au-JB ink are thin-film gold electrodes that prevent charge accumulation due to overcurrent, compared to sputter and Au-J ink (**Fig. 1**), suggesting the suitability for long-term use in the EAB sensor[3].
Keywords : Electrochemical Aptamer-Based (EAB) sensors, DNA aptamer, Inkjet printing, Real-time monitoring, Phenylalanine

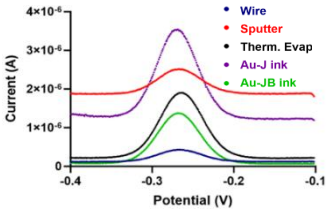


Figure 1. SWV of different thin-film gold electrodes near MB redox potential.

電気化学アプタマー (Electrochemical Aptamer-Based, EAB) センサは、メチレンブルー (Methylene Blue, MB) を修飾した DNA アプタマーとターゲット分子の特異的な結合により生じるアプタマーの構造変化を利用したバイオセンサである[1]。EAB センサは、リアルタイムで生体内分子を捕捉可能なため、金線電極を基材とする埋め込み型センサへの応用が進められている[2]。本研究では、ウェアラブル EAB センサの開発に向けて、生体組織に貼付可能な薄膜状金電極を作製し、電気化学特性およびセンシング能を評価した。従来の金線電極に対して、スパッタや蒸着により製膜した金電極、ならびに、インクジェット印刷にて作製した 2 種類の金電極を評価した。方形波ボルタンメトリー (Square Wave Voltammetry, SWV) による安定性評価では、金線、蒸着、Au-JB ink において、スパッタ、Au-J ink よりベースラインが 1-2 µA 低下した。SWV の結果より、蒸着と Au-JB ink は、スパッタや Au-J ink と比べて、過電流による電荷蓄積を抑制できており (**Fig. 1**)、EAB センサにおける長期的な使用[3]に適することが示唆された。

[1] S-Barnes *et al.*, *Annu. Rev. Anal. Chem.*, **9**, 163 (2016). [2] Alex M. *et al.*, *ACS Sens.* **7**, 2823 (2022). [3] A. J. Bandodkar *et al., ACS Sens.*, **1**, 464 (2016).

Design and Fabrication of Inkjet-Printed Microelectrodes for Electrochemical Sensors of Extracellular Components

([1]School of Life Science and Technology, Institute of Science Tokyo) ○Eduardus Ariasena,[1] Toshinori Fujie[1]

Keywords: Inkjet Printing; Micro-Physiological System; Electrochemical Sensors; Calcium Ion; Dopamine

Micro-physiological systems (MPS) utilizing cell cultures offer a more ethical, simplified alternative to animal testing for disease modeling and drug development.[1] One key component is the electronic sensing device, whose flexibility and softness have been recently studied due to improved biocompatibility.[2] Electrochemistry is an effective sensing technique in this system, but only a few studies have incorporated it into flexible electronics. Regarding the fabrication process, photolithography has been dominant due to its reliability, but it requires specialized equipment with limited substrate options.[3] In contrast, inkjet printing offers an alternative, maskless microfabrication process, especially for flexible or stretchable substrates.[3, 4] This study developed an inkjet-printed electrochemical microelectrode on a flexible polymeric thin film that can be integrated with bioengineered cells for non-invasive monitoring of extracellular physiological activities. The electrodes are made of gold (Au) nano-ink printed on a 5 µm-thick Kapton film. Polyimide (PI) ink was printed on top of the Au wiring to establish the insulation layer. The fully printed electrodes device was then annealed in 175°C oven for one hour and laser-cut according to the pattern. It was then placed to a 12-well cell dish by first stacking it to a glass substrate (**Fig. 1a**). Cyclic voltammogram (CV) in H_2SO_4 exhibited characteristic Au electrode behavior (**Fig. 1b**). Proof of concept of sensing application is shown by the increase in differential pulse voltammogram (DPV) current peak as dopamine concentration increased (**Fig. 1c**). The Au electrode can also be functionalized by coating it with ion-selective membrane to detect Ca^{2+}, which holds potential for sensing extracellular essential components.

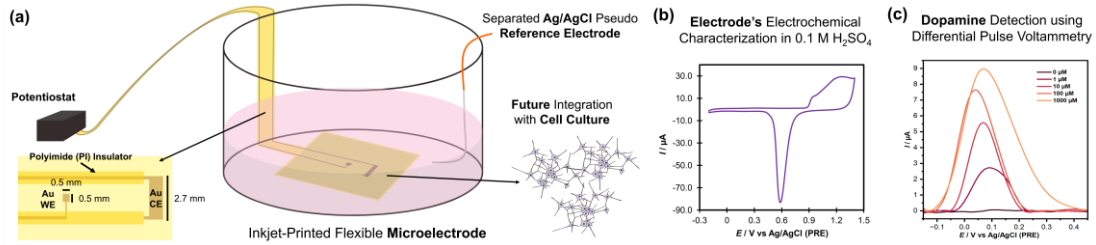


Figure 1. (a) Schematic of the electrodes design and setup. (b) CV of the electrode in H_2SO_4. (c) DPVs of the electrode in different dopamine concentrations.

1) D. E. Ingber, *Nat. Rev. Gen.* **2022**, *23* (8), 467–491. 2) M. C. Lefevre, G. Dijk, A. Kaszas, M. Baca, D. Moreau, R. P. O'Connor, *Npj Flex. Elec.* **2021**, *5* (1). 3) L. D. Garma, L. M. Ferrari, P. Scognamiglio, F. Greco, F. Santoro, *Lab on a Chip* **2019**, *19* (22), 3776–3786. 4) A. Imai, S. Takahashi, S. Furubayashi, Y. Mizuno, M. Sonoda, T. Miyazaki, E. Miyashita, T. Fujie, *Adv. Mater. Tech.* **2023**, *8* (21).