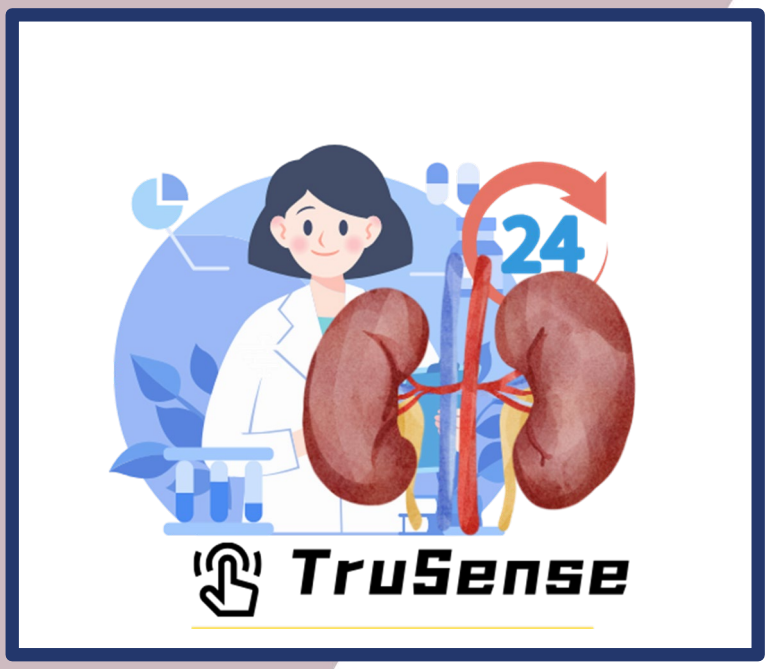
Team Results Document
TruSense



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Contents

1 Summary

Our team developed a **microneedle-based** continuous sensing platform designed to detect biomarkers within interstitial fluid. We created a **miniaturized** electrochemical workstation, integrated with adaptors that connect seamlessly with our **on-needle** biosensing interface. For Acute Kidney Injury (AKI) detection, we employed two strategies: one using aptamers and the other utilizing molecularly imprinted polymers (MIPs). The MIPs, easily crafted through an imprinting process, offer specific binding sites for target molecules, while we optimized anti-creatinine aptamer sequences using computational biology. A key advantage of our sensor is its **real-time, reagent-free** monitoring capability, enabled by a composite electrochemical interrogation method. Additionally, the biosensor is constructed from **biocompatible** materials, making it highly suitable for development into a wearable monitoring device. This technology is particularly crucial for the early detection of AKI, where timely intervention can greatly enhance patient outcomes. Benefited from the integration of MIPs and our innovative microneedle design, our biosensor holds strong potential as a continuous monitoring solution for various disease markers, medications, hormones, and more. Revolutionize Your Health Monitoring with Our Cutting-Edge Microneedle MIP Sensor!

2 Biosensor

2.1 Molecular Recognition

Aptamers are functional nucleic acids that bind specifically to target molecules. Due to their predictable 3D structure, aptamers can be modified to enhance their specificity and selectivity, offering stability and resistance to environmental conditions. Previous studies have identified anti-creatinine aptamer sequences(Ganguly et al., 2023, 2024), leading us to focus on optimizing these structures to improve performance.

Molecularly imprinted polymers (MIPs) are synthetic materials that mimic the binding capabilities of natural antibodies by forming a three-dimensional network around template molecules, thus achieving high specificity(BelBruno, 2019). Compared to other binders, MIPs offer increased durability under various environmental conditions. Since creatinine is non-electroactive and cannot be easily oxidized under standard conditions, we applied a layer of redox-active reporter (RAR) beneath the MIP layer on the screen-printed electrode (SPE) to enable indirect detection.

2.2 Physical Transduction

For the **aptamer-based sensing strategy**, the aptamer is modified with methylene blue (MB) at the 5’ end, serving as a redox-active reporter. Upon binding creatinine, the aptamer undergoes a conformational change, transitioning from a stem-loop structure in its unbound state to an unwound structure (Fig. 1, upper Middle). This conformational shift moves the 5’ end MB away from the electrode, resulting in a loss of electrochemical activity and a reduced peak current of MB, as evidenced by non-faradaic electrochemical impedance spectroscopy (EIS) and voltammertric methods. (Fig. 1, upper Right)

In the MIP strategy, as creatinine is selectively adsorbed onto the MIP layer, the exposure of the RAR to the sample matrix decreases(Wang et al., 2022). Controlled-potential voltammetric techniques, such as differential pulse voltammetry (DPV) or linear sweep voltammetry (LSV), can then be used to measure the oxidation or reduction peak of the RAR.

A decrease in peak current density indicates an increase in creatinine levels(Fig. 1, lower Right).

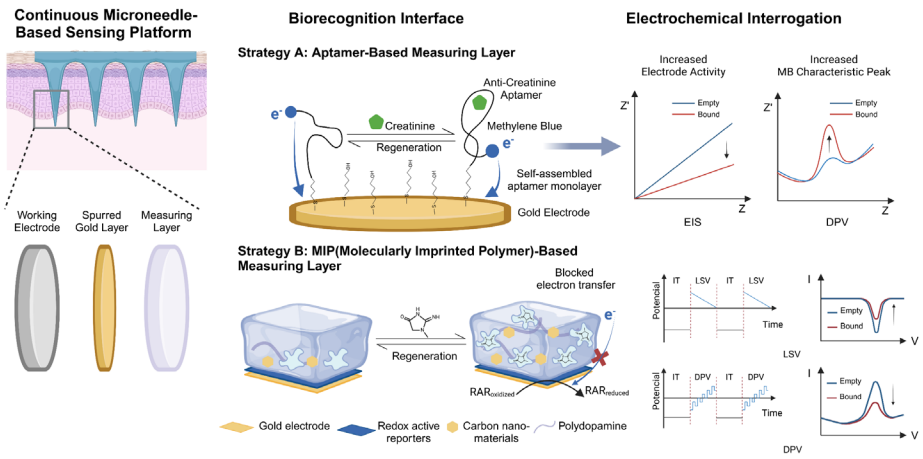


Fig 1. Our continuous sensing platform. Left, Conducted Microneedle-based electrode. Upper Middle, aptamer-based sensing strategy and electrochemical interface. Lower Middle, MIP-based strategy and build-up of MIP electrochemical interface. Upper Right, two electrochemical methods to quantify binding-induced signal. Lower Right, composite electrochemical methods to investigate MIP-mediated binding,

Unlike conventional commercial electrodes, our microneedle electrode is fabricated by 3D printing and metal sputtering (Fig. 2, Left). The microneedles are constructed from a high-temperature-resistant resin, which serves as the substrate. This resin substrate is then modified through metal sputtering: a titanium (Ti) layer is sputtered onto the surface to enhance adhesion and compatibility of the electrode metal with the substrate and on top a gold layer is sputtered onto the working and counter electrodes, while silver (Ag) is sputtered onto the reference electrode(Tehrani et al., 2022; Yang et al., 2022). To further improve the reference electrode's performance, it undergoes chlorination to form an Ag/AgCl electrode.

2.3 Cartridge technology

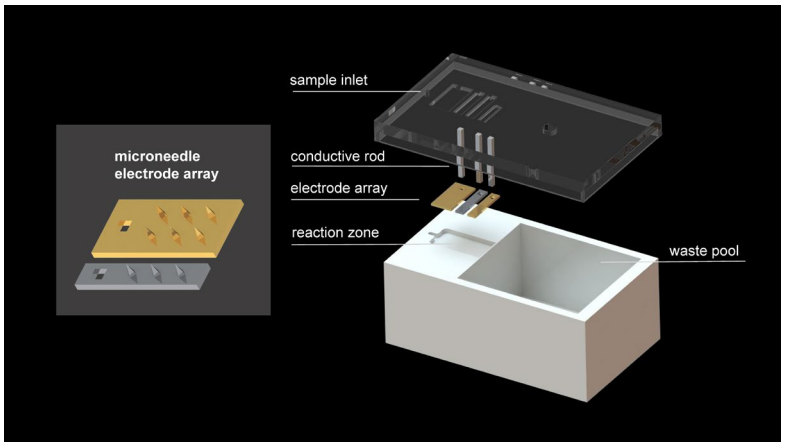


Fig 2. Microneedle electrode array (left) and Microfluidic module (right)

The microfluidic module is designed to streamline the testing and reduce external interference. It consists of three components: the main body, the electrode array, and the cover.

- **Main Body**: Contains the reaction zone and a waste pool for storing liquid after testing.
- **Electrode Array**: Placed in the main body, with a conductive rod inserted into a reserved microneedle hole to transmit current to an external sensor.
- **Cover**: Features a sample inlet and a channel that directs the liquid to the detection area, with a vent hole to balance air pressure (Fig. 2, right).

During testing, a pipette injects the sample through the inlet. The sample flows to the detection area, and after detection, air pushes the liquid into the waste area, readying the

module for the next sample. For testing, we also designed a microfluid channel(Appendix VIII)

2.4 Reader instrument and user interaction

The instrument uses an ADI Precision Analog Microcontroller with Chemical Sensor Interface ADuCM355 as its core, a humidity and temperature sensor SHTC3, an ultra-small LDO regulator ADP166 and a Bluetooth module WH-BLE103a to build a small electrochemical workstation. The circuit size is controlled to be 1.6 cm * 2.8 cm. This workstation can implement various electrochemical methods such as amperometry, voltammetry, EIS etc. Plus, we have also designed a supporting mobile applet to facilitate the visual display of data and user-friendliness(Fig 3).

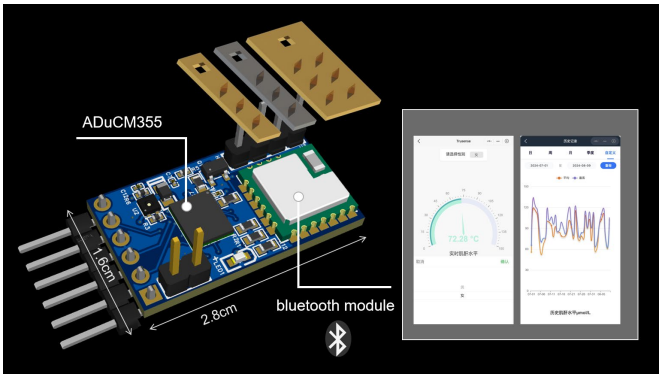


Fig 3. Custom-designed workstation and corresponding user applet

3 Technological feasibilities

3.1 Biorecognition& physical transduction

Aptamer Strategy: Based on previously identified aptamer sequences(see Appendix I& III), we predicted their 3D structures and binding sites. Molecular dynamics simulations and 3D structure prediction tools, such as RoseTTAFold All-Atom (RFAA) and AlphaFold3(methods shown in Appendix II), were employed to refine the binding modes and identify key interaction sites. Guided by these predictions, we modified the aptamer sequences to enhance their binding affinity and stability. Sequence refinements and structural optimizations were conducted, and affinity assessments were followed using Bio-Layer Interferometry (BLI)(see Appendix IV).

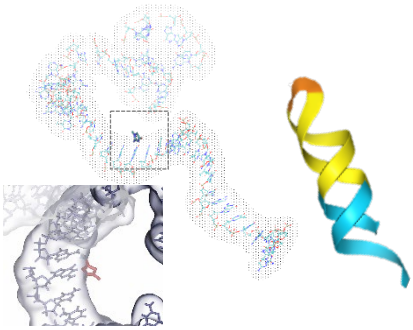


Fig 4. Left, prediction of the aptamer's original sequence binding site to creatinine based on RFAA. Right, tertiary structure prediction of the aptamer's truncated sequence based on AlphaFold3.

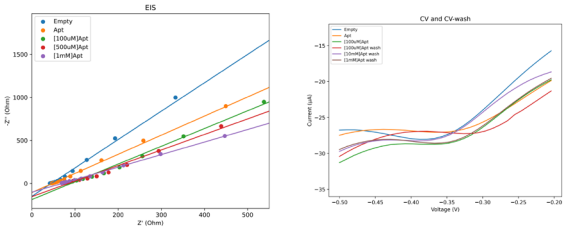


Fig 5. Left, non-Faradic EIS of aptamer modified, and bare gold screen printed electrodes in 0.01 M PBS. Right, CV diagram of creatinine-bound and regenerated aptamer sensors in 0.01 M PBS.

These modifications were validated by non-faradic EIS and cyclic voltammetry(CV) (Fig 5. Left), capabilities of the optimized aptamers. We also validated the regeneration of these electrochemical aptamer sensors (Fig 5. Right) using washing buffers. Finally, the aptamer strategy was not adopted due to time constraints and the challenges associated with testing without using intermediate fluids.

For MIPs fabrication, we have compared several RARs(Prussian blue nanoparticles, PBNPs(Wang et al., 2022) and poly-methylene blue, PMB(Phonklam et al., 2020)) and functional monomers(pyrrole, APBA, dopamine, DA(Li et al., 2022)) and finalized on using PMB as RAR and DA as MIP monomer considering biocompatibility. Before electro-polymerization of PMB and PDA, the electrode is pretreated with carbon nanomaterials(Li et al., 2022) like f-

MWCNT and rGO to enhance active surface area. After polymerization, the template is removed to produce a creatinine-specific biosensor. The effective polymerization of the MIP can be demonstrated by comparing the DPV peaks after the deposition of RARs, after the polymerization of the MIP, and after the template removal(Fig 6).

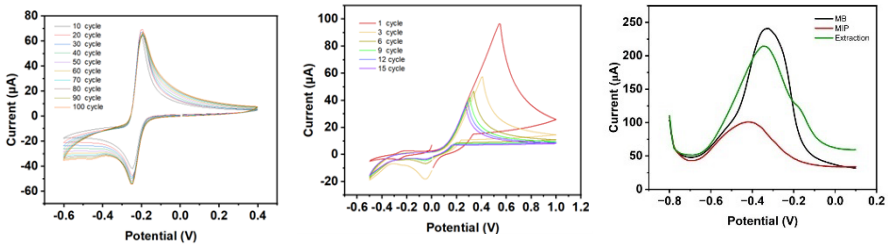


Fig 6. Fabrication of creatinine-specific MIP biosensor. Left, modification with PMB. Middle, electro-deposition with PDA MIP. Right, template removal to create creatinine-specific cavities.

By soaking the sensors in creatinine solutions of different concentrations for 2 minutes and then performing DPV measurements, it can observe a linear increase in DPV peak current with increasing creatinine concentration, indicating the sensor's sensitivity to varying levels of creatinine(Fig 7, left).

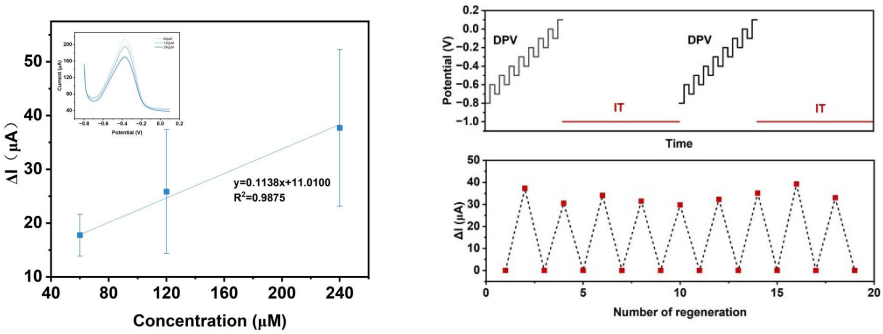


Fig 7. Sensor performance: Left, Inset, DPV diagrams of the developed MIP sensor obtained with 60−240 mM of creatinine in 0.01 M PBS; Out, calibration plot with a linear fit. Right, In situ regeneration of a creatinine-specific MIP sensor in 0.01 M PBS.

These indirect SPE–RAR–MIP sensors can be regenerated in situ by applying a constant potential to the working electrode, which repels the bound target molecules from the MIP layer, achieving prolonged reusability(Fig 7, right). Comparing the DPV responses of MIP to those of non-imprinted polymer (NIP) sensors further demonstrates the specificity of the MIP sensors for creatinine.

3.2 Microneedle based electrochemical platform

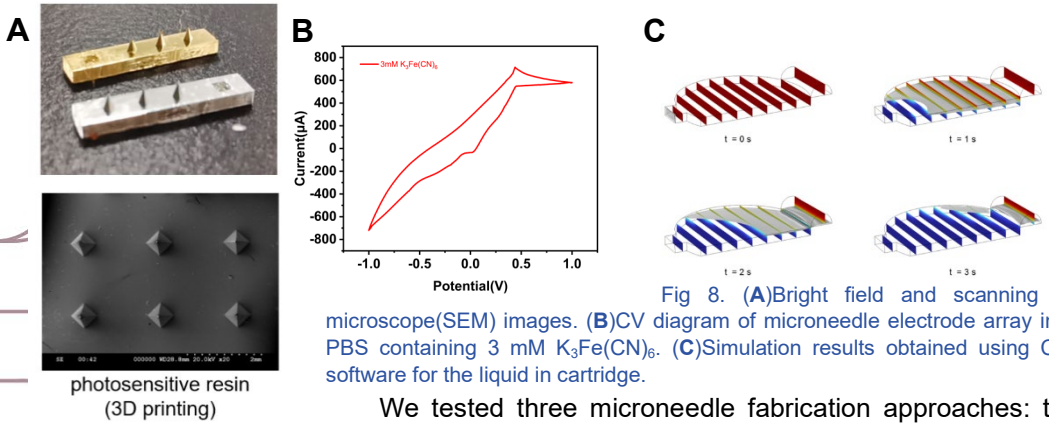


Fig 8. (**A**)Bright field and scanning electron microscope(SEM) images. (**B**)CV diagram of microneedle electrode array in 0.01 M PBS containing 3 mM $K_3Fe(CN)_6$. (**C**)Simulation results obtained using COSMOL software for the liquid in cartridge.

We tested three microneedle fabrication approaches: thermal polymerization micro-needles based on PMMA, photocurable microneedles based on NIPAM and NVP materials, and 3D printing using photosensitive resin and end up with the last method(see comparison in Appendix V). The detailed dimension

information can be found in Appendix V as well.

3.3 Cartridge technology

The main body and interlayer were made from FUTURE R4600 resin, which allows for fine detail printing and clear edges, facilitating the embedding of the interlayer into the main body. The cover was made from the same brand's transparent photosensitive resin, whose transparency is advantageous for observing liquid flow during testing.

To verify that the microfluidic module could be filled with liquid, simulations were conducted using COMSOL software. The water contact angle on the two resin materials was measured(see in Appendix VI) and had been inputted into the COMSOL software along with parameters for flow rate and pressure to validate that the liquid sample could fully occupy the chamber when injected into an air-filled system. Fig 8 C shows the process of a 100uL liquid sample flowing into the test chamber over 3 seconds. Initially, the chamber is filled with air, represented in red, while the blue represents the liquid sample. The bottom surface is where the electrodes are located. It can be observed that the sample fully occupies the test chamber and makes sufficient contact with the electrodes.

3.4 Reader Instrument

We designed and fabricated the workstation that is remarkably compact, with a size comparable to a fingertip, as illustrated in Fig 9. This miniaturization is a key feature of our sensor system, allowing for easy integration into wearable devices without sacrificing functionality. Detailed circuit see Appendix VII.

Fig 9.Finished appearance of the electrochemical workstation circuit

4 Originality

BY THE SURPERVISOR

I am immensely proud of the innovative strides made by the team, both in the biological and instrumentational aspects of the project. The creativity and technical acumen demonstrated by the team have led to an advanced approach in biosensing technology.

The TruSense team has made significant strides in creatinine detection through a novel combination of biology and engineering. By employing both Molecularly Imprinted Polymers (MIPs) and aptamers, we have developed a highly sensitive and specific biosensor. The innovative use of reverse voltage to sustain MIP-based detection addresses key challenges in sensor longevity and regeneration. Furthermore, our computational biology-enhanced aptamer design optimizes target recognition. On the instrumentation front, a miniaturized potentiostat with advanced electrochemical capabilities and Bluetooth connectivity has been developed. This device, integrated with a 3D-printed microneedle array, enables painless and accurate interstitial fluid sampling. This groundbreaking approach lays the foundation for a comfortable, wearable, and high-performance continuous monitoring system.

In conclusion, the team has excelled in achieving originality by not only pushing the boundaries of current biosensor technologies but also by designing with future applications in mind. The integration of sustainable and wearable technology into this year’s project

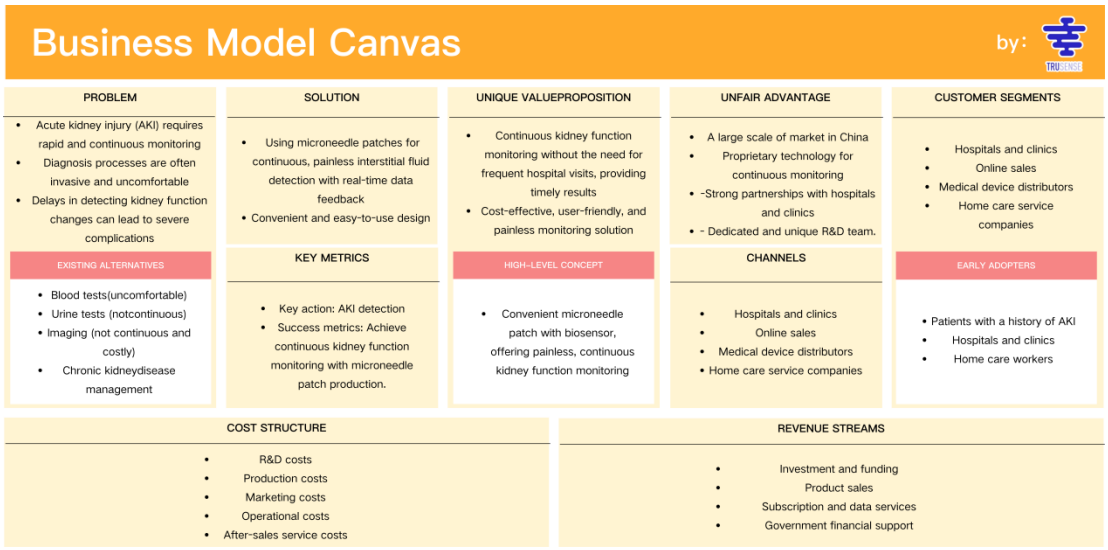
underscores the potential for wide-scale adoption and commercial viability, making this a standout project in the field of biosensing.

BY THE CAPITAINS

This year's TruSense project has achieved tremendous innovation from the biological side to the instrument side, and I think this is inseparable from the dedication and contribution of every member of the team. On the biological side, we explored two detection methods, one is MIP and the other is aptamer. In terms of MIP, we have achieved sustainable detection of creatinine molecules by applying a reverse voltage; In terms of aptamers, we directly innovate in principle, design target-induced aptamer switches, and stably detect creatinine molecules through the different affinities between sequences and use computational biology methods to assist our sequence design. On the instrument side, in order to make the sensor more portable to wear, we independently designed a small potentiostat, which is only 2.8cmx1.6cm in size and has a wealth of electrochemical detection methods and Bluetooth transmission functions. And where the biological side and the instrument side are combined, we designed the microneedle sequence and performed metal sputtering as an electrode, which can painlessly penetrate the skin to obtain interstitial fluid for detection. The design is full of innovation and fully considering the goal of sustainability testing, and more importantly, we not only focus on the design of the runner during this competition, but also consider the wearability of this sensor in the future, so that it has a wider market value.

5 Translation Potential

5.1 business model canvas



5.2 stakeholder desirability

5.2.1 Disease Background

Acute kidney injury (AKI), formerly known as acute renal failure (ARF), is a clinical syndrome characterized by a sudden decline in kidney function due to various causes. Its pathogenesis is complex, contributing to high incidence and mortality rates, along with significant adverse effects. Research indicates that AKI patients face a 2.67-fold increased risk of developing chronic kidney disease (CKD), a 4.81-fold increased risk of progressing to uremia, a 38% higher risk of cardiovascular events, and a 1.80 times higher risk of death [1]. Over five years, AKI prolongs hospitalization rates by 32.4%, contributing to a global annual death toll exceeding two million cases [2]. This condition imposes substantial medical and financial burdens on patients' families and society, posing a critical global public health challenge.

5.2.2 Market Background

Currently, kidney testing options include hospital-based urine, blood, or imaging examinations, as well as home-based self-tests using kits for urinary albumin/serum creatinine and blood uric acid. Hospital visits are cumbersome, involving travel, registration, testing, and result waits, which can be exhausting for patients. Pre-test requirements such as fasting further add to inconvenience and costs. While home testing kits offer convenience, they lack real-time renal function monitoring.

There's a critical need in the market for a quick, portable, highly accurate, dynamic, and cost-effective point-of-care renal function testing device. Trusensor aims to address these needs effectively. The comparison table below illustrates Trusensor's advantages over other detection methods.

Table 1 Comparison of detection methods

Detection method	Advantage	Disadvantage	Number of Chinese listed products
Hospital check-up	①High accuracy ②Professional medical services	①Complicated process & high labor cost ②Static detection ③Limitations to patients（more light diet, fasting blood）	——

Urinary protein kit	①Lower price ②Rapid reporting of results	①Prone to errors (contamination, overtime) ②User unfriendly (elderly group, menstruating women) ③Low accuracy ④Static detection ⑤no professional medical services	15
Serum creatinine kit	①High accuracy ②Lower price ③Rapid reporting of results	①Prone to errors (contamination, overtime) ②User unfriendly (elderly group, menstruating women) ③Low accuracy ④Static detection ⑤no professional medical services	306
Serum uric acid kit/tester	①Lower price ②Rapid reporting of results	①no reference significance(synergy index) ②Static detection	343
Kidney dynamic detection biosensor (Trusensor)	**①High accuracy, fast and portable ②Dynamic monitoring, risk early warning ③Raw material recycling, controllable cost ④Professional medical services of online doctors**	①Higher instrument cost ②Patients may reject the microneedle design	**0**

5.2.3 Target users

1. Postoperative Patients:

Postoperative patients are in a critical phase of physical recovery, where monitoring renal function is crucial. Anesthetics and contrast media used during procedures can impact renal function, especially in those with pre-existing renal conditions. Close monitoring post-surgery is essential to promptly detect and manage potential issues. Renal dysfunction post-surgery can lead to serious complications like AKI or CKD progression. The Trusense biosensor offers real-time, continuous monitoring of renal function indices, enabling timely interventions to reduce complications and enhance recovery success rates compared to traditional hospital monitoring instruments.

2. Patients with Chronic Diseases:

Impaired renal function is a common complication of chronic diseases. Treatment for conditions such as diabetes, hypertension, and others may stress the kidneys or affect their function. Many chronic disease patients also have concurrent renal insufficiency. Common diseases potentially affecting kidney function include diabetes, hypertension, high cholesterol, rheumatic diseases, chronic inflammatory diseases, autoimmune diseases, cardiovascular diseases, chronic pain, osteoporosis, gout, and cancer. Continuous monitoring of renal function is necessary due to long-term medication needs. The Trusense biosensor provides convenient, continuous renal function monitoring and data uploads to an app, facilitating timely adjustments to treatment plans and improving patient outcomes and quality of life.

3. Home Users:

Renal function is closely linked to age, with elderly users at higher risk of kidney disease. The TruSense biosensor detects changes in creatinine concentration in skin interstitial fluid swiftly, allowing for early detection of abnormal renal function in middle-aged and elderly family members or those with nephropathy history. This capability guides prompt medical interventions when necessary.

5.3 Business Feasibility

5.3.1 Medical Compliance

The project has already received funding support from the Zhejiang University Jiang Zhi Modern Biotechnology and Pharmaceutical Technology Research Award Scholarship and has preliminarily obtained some licensing qualifications for operation. Our medical devices fall under Class II medical devices. According to relevant Chinese laws and regulations, conducting live sales of Class II medical devices on the platform requires meeting a series of conditions and preparing the necessary qualifications.

Firstly, the company must have a business license, and its business scope should include the sale of Class II medical devices. Additionally, a medical device operation license, a medical device online sales record certificate, and a medical device production license are required. A trademark registration certificate and brand authorization are also necessary. Once these qualifications are prepared, one can apply to open an online store and live streaming channels, and pay a deposit for medical devices, amounting to 100,000 yuan.

5.3.2 Marketing Strategy

The TruSense Kidney Injury Detector plans to promote and improve the product through three phases: development, growth, and maturity.

Development Phase(2024-2025):

In the product development stage, the TruSense team has improved the accuracy and stability of the creatinine biosensor through repeated experiments and tests to reach ideal values. During this phase, we plan to conduct joint clinical trials with the Second Affiliated Hospital of Zhejiang University School of Medicine and engage in technical cooperation with related hospital laboratories to enhance product performance. Additionally, the team's business potential transformation group will conduct preliminary market research to understand the existing detection methods of creatinine in medical diagnostics and predict the demand for this product scientifically, laying the foundation for subsequent product pricing and sales promotion.

Growth Phase(2026-2027):

In the product growth stage, the team plans to adopt multi-stage and multi-dimensional promotion strategies to enhance brand and product awareness and conduct real-time market surveys to make timely adjustments based on market information. During this phase, we will cooperate with OEM factories to achieve industrial production of orders to improve production efficiency. Furthermore, we will establish a good customer feedback mechanism to iteratively upgrade sensor performance and optimize the companion app system periodically.

Maturity Phase(2028-2029):

In the product maturity stage, we will further research and improve the detailed issues encountered by customers during use and enhance product performance. Based on the core concept of personalized medicine, the team plans to independently upgrade and build a biosensor analysis platform for detecting different sample concentrations, designing biosensors for representative diseases and drugs with a broad audience.

5.3.3 Self-Built E-Commerce Platform

To further optimize product marketing, our team will rely on the existing technical platform to establish an online product sales website and integrate popular e-commerce platforms. By adopting an online sales model, we will extend product reach, breaking traditional space and time constraints, allowing for the sale of detection instruments, consumables, and personalized health services nationwide.

Tmall and JD.com Flagship Stores:

We will establish flagship stores on popular e-commerce platforms like Tmall and JD.com. In the initial stages of the online store, we will offer free trials to obtain real user evaluations and suggestions, collect user needs, and optimize product design. By promoting trial samples, we will enable potential users to experience our products, achieving conversion from trial users to regular users, fostering a stable user base.

Live-streaming Platforms:

Douyin and Kuaishou are two famous live-streaming e-commerce platforms in China. Among them, Kuaishou primarily targets middle-aged and elderly groups, which aligns more with our product's promotional needs. As mentioned in section 5.3.1, after undergoing the relevant qualification review and paying the deposit, we can collaborate with live streamers to promote our products through live-streaming sales, reaching a wide range of middle-aged and elderly audiences.

5.4 Financial Viability

Based on market research, there are approximately 120 million chronic kidney disease (CKD) patients in China, with about 1 million new acute kidney injury (AKI) cases annually. Our target market includes: (1) patients with kidney diseases such as CKD and AKI; (2) individuals at risk for kidney diseases, including postoperative patients, elderly individuals, and those with a family history of related genetic conditions; (3) health-conscious individuals.

Assuming we achieve a 50% market penetration over five years and considering a conservative annual compound growth rate of 50% (reasonable for a POCT startup), we can estimate the following sales volumes for the kidney function testing device.

Year	Year 1	Year 2	Year 3	Year 4	Year 5
Sales Volume	10,000	15,000	22,500	33,750	50,625

Our revenue will be calculated in RMB, and for ease of comparison, we will convert it to USD at an exchange rate of 7 RMB to 1 USD.

Next, we delve into the net profit derived from the sale of a single kidney function testing device. Recognizing that initial production expenses are elevated, resulting in relatively diminished initial product profitability, we must factor in the evolution of costs as production scales. With an initial cost of approximately $28.57 (200 RMB) per device, the profit margin will increase over time, from an initial 30% to 50% by the fifth year. This is due to economies of scale, improved management of upstream and downstream processes, and enhanced supply chain positioning.

Year	Sales Volume (units)	Sales Revenue (USD)	Cost (USD)	Gross Profit (USD)	Gross Margin
Year 1	10,000	408,157	285,714	122,443	30%
Year 2	15,000	663,257	428,571	234,686	35%
Year 3	22,500	1,045,875	642,857	403,018	40%
Year 4	33,750	1,648,527	964,286	684,241	45%
Year 5	50,625	2,705,357	1,446,429	1,258,929	50%

In addition to the sale of the testing device, we anticipate generating revenue from disposable testing patches. Our kidney function testing device can be used continuously for 7-

14 days per test; postoperative patients require continuous testing, at-risk individuals and CKD patients are recommended to test every 3-6 months, and healthy individuals are recommended to test once a year. The cost of each testing patch is $1.43 (10 RMB), and the profit margin will increase from an initial 50% to 60% by the fifth year. This is due to increased production scale, improved production efficiency, and increased product awareness.

Year	Patch Sales Volume (units)	Sales Revenue (USD)	Cost (USD)	Gross Profit (USD)	Gross Margin
Year 1	50,000	142,857	71,429	71,429	50%
Year 2	75,000	241,071	107,143	133,929	55%
Year 3	112,500	401,786	160,714	241,071	60%
Year 4	168,750	669,643	241,071	428,571	65%
Year 5	253,125	1,084,821	361,607	723,214	70%

Overall, through the sale of kidney function testing devices and disposable testing patches, we expect significant net profit growth over five years. We will also spend $300,000 annually on human resources, covering the salaries of 10 employees.

During the initial three-year period, a substantial portion of the foundation will be spent on fixed asset investment. This includes $42,857 (300,000 RMB) for production machinery and an additional $7,143 (50,000 RMB) for rental costs. Considering $71,429 (500,000 RMB) as the required startup capital and accounting for prevailing interest rates, financial costs amount to approximately $2,143 (15,000 RMB) annually. These funds will be directed toward sales, promotional efforts, product development, and streamlined management practices to yield substantial returns.

The net profit trajectory increases from $122,443 in the first year to $1,258,929 in the fifth year. Through a comprehensive evaluation of kidney disease patients and the overall population in China, we expect to achieve significant profit growth within the initial five-year period and maintain excellent cash flow management.

We will raise funds through various means such as competitions, employee stock ownership, interest-free startup loans, bank loans, venture capital, and school support, with an estimated total of $464,185 (3,250,000 RMB). The estimated distribution of funds includes: office rental costs, advertising expenses, employee salaries, utility costs, research and development expenses, travel expenses, and other expenses (such as office supplies), with a reserve of working capital for contingencies.

6 Team and support

6.1 Contributions of the Team Members

Supervisor & Captains:

Yong Wang is the team supervisor. He is very responsible, in addition to giving us professional guidance, he is also a guide in our life and learning.
Cheng Quan is the team captain of TruSense 2024. He has excellent professional ability, organizes the overall experimental progress, and is mainly responsible for the content related to the biology group.
Mingyu Suo is the team captain of TruSense 2024. She managed the teamwork and was responsible for communicating with the SensUs Organization. She is also responsible for driving the overall progress of the instrument group.

Translation Group:

Yangyang Meng is in charge of the Translation group, and she also managed the team finance.
Liangxuan Du excelled in making business plan and giving high quality pitches.
Hanyang Zou is responsible for Art production and commercial promotion.
Yinuo Ruan makse contributions in building business model and managed the team finance.

Instrument Group:

Xueming Zhang has devoted a lot of time to the research and development of the potentiostat and the subsequent software construction.
Meng Jin contributed a lot of wonderful ideas, and she did both the cartridge and the software simulation.
Canyu Ma completes a complete experiment with microneedling**.**
Yuxuan Gao completed the code for the applet.
Biology Group:
Xinyue Tu is fully committed to the team's experiments, and she is mainly responsible for aptamer-related issues.
Lingxia Jin is also responsible for MIP, and she has a brilliant scientific literacy that delivers great results every time.
Ziyi Xu is responsible for the aptamer sequence prediction part and invested a lot of time and effort.
Saibin Yang is responsible for the experimental operations of the biogroup.
Ziqi Huang looked up a lot of information on aptamers.

6.2 Acknowledgement to:

Special thanks to Professor YANG Fan for providing us with lab area and all the equipment and regents needed.

7 Final remarks

We are keenly interested in connecting with other teams during the competition in Eindhoven. We believe that through the whole SensUs organization, the collaboration and knowledge exchange are key to advancing innovation in this field. We're excited to learn from the diverse approaches taken by other teams and share our own insights. Looking ahead, we are committed to enhancing the performance of our sensor. One of our goals is to explore and integrate some of the antifouling strategies after exchanging ideas with other professionals. We see this as an invaluable opportunity to improve our biosensor's reliability and effectiveness, and we are eager to incorporate these new techniques into our ongoing research and development.

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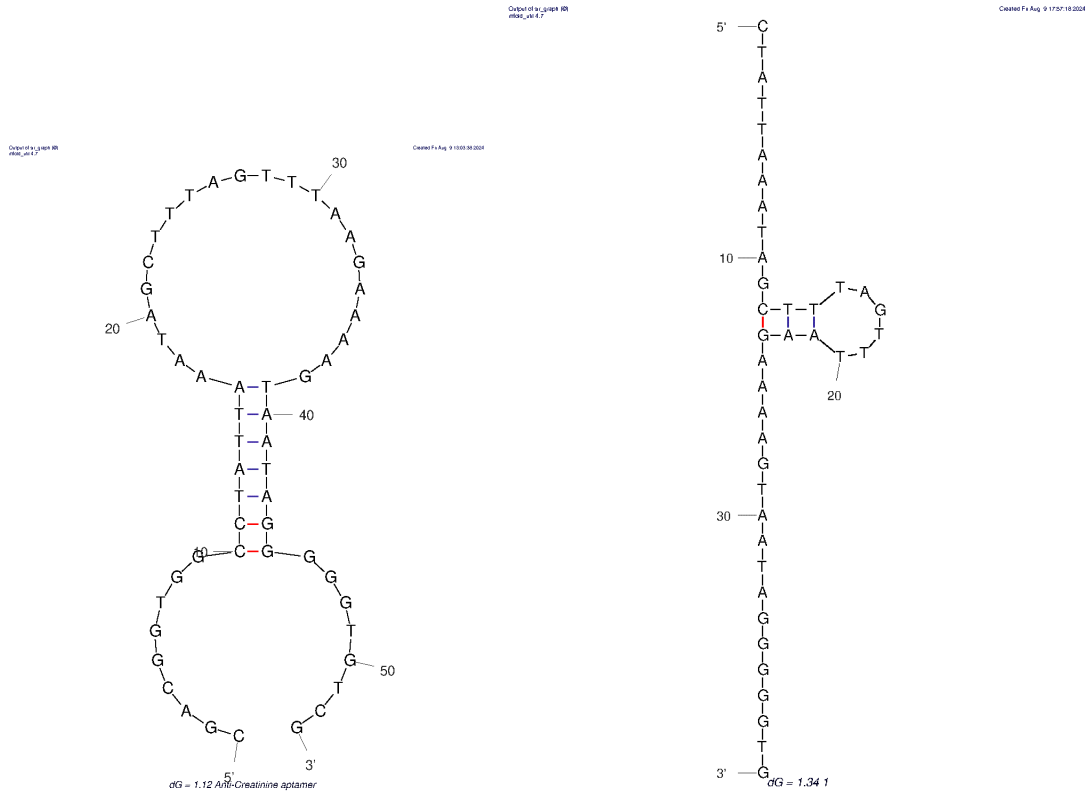
9 Appendix

Appendix I Aptamer sequences we used and their secondary structures

Sequences we used in our project

Name	Sequence(5’-3’)
Original Sequences	5'-CGACGGTGGCCTATTAAATAGCTTTAGTTTAAGAAAAGTAATAGGGGGTGTCG-3'
Truncated Sequences by TruSense	5‘-CTATTAAATAGCTTTAGTTTAAGAAAAGTAATAGGGGGTG-3’

Predicted secondary structures of original sequence(Left) and truncated sequence(Right) by The UNAFold Web Server



Utilizing RoseTTAFold All-Atom (RFAA) for Chemical Understanding of DNA Aptamer Sequence Features

1. Data Preparation: Compile or generate sequence data for DNA aptamers, encompassing the complete DNA sequence along with potential variations or mutations.
2. Model Training: Employ the RFAA tool to model the DNA aptamer sequences. Input the DNA sequences into the RFAA system and utilize its algorithms to predict and analyze the structural and functional characteristics of the DNA aptamers.
3. Feature Extraction: The output from RFAA allows for the extraction of various chemical and physical properties of the DNA aptamers. For instance, understanding the interactions between DNA aptamers and proteins or other molecules can be achieved by analyzing the distance-dependent scoring functions between atoms.

AlphaFold3: Accuracy and Efficiency in Predicting DNA Aptamer Affinity

1. High-Precision Prediction: AlphaFold 3 demonstrates higher accuracy than previous specialized tools, comparing favorably in protein-ligand interactions against current state-of-the-art docking tools, in protein-nucleic acid interactions against nucleic acid-specific predictors, and in antibody-antigen predictions against AlphaFold-Multimer v.2.
2. Enhanced Architecture: AlphaFold 3 incorporates a significantly updated diffusion-based architecture, capable of predicting the joint structure of complexes including proteins, nucleic acids, small molecules, ions, and modified residues.
3. Handling Complex Chemical Structures: The model enhances the efficiency of learning data by reducing the complexity of multiple sequence alignment (MSA) processing, employing a simpler pairformer module, and directly predicting raw atomic coordinates.
4. Generative Diffusion Approach: AlphaFold 3 employs a generative diffusion model to handle atomic coordinates, requiring the network to learn protein structures across various length scales, emphasizing the understanding of local stereochemistry at low noise levels and the overall structure of the system at high noise levels.
5. Credibility Metrics: AlphaFold 3 has developed credibility metrics for predicting atomic and pairwise errors in the final structure, aiding in the assessment of prediction accuracy.
6. Technical Challenges: Although AlphaFold 3 has made significant strides in predictive accuracy, there are still technical challenges, such as the potential for hallucinations in generative models, where the model might invent plausible structures in unstructured regions.

Appendix III The tertiary structure of original and truncated aptamers

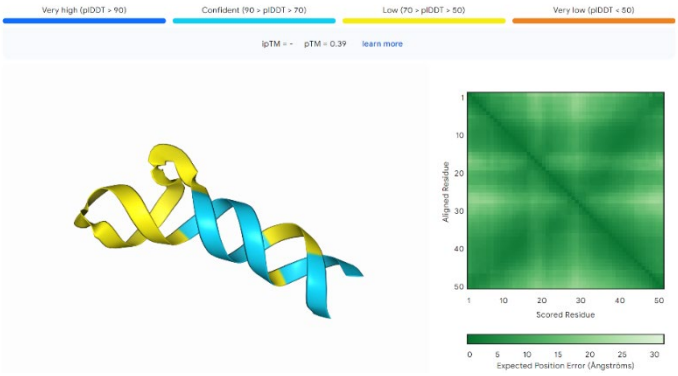


Fig. Tertiary structure prediction of the aptamer's original sequence based on AlphaFold3
5'-CGACGGTGGCCTATTAAATAGCTTTAGTTTAAGAAAAGTAATAGGGGGTGTCG-3'

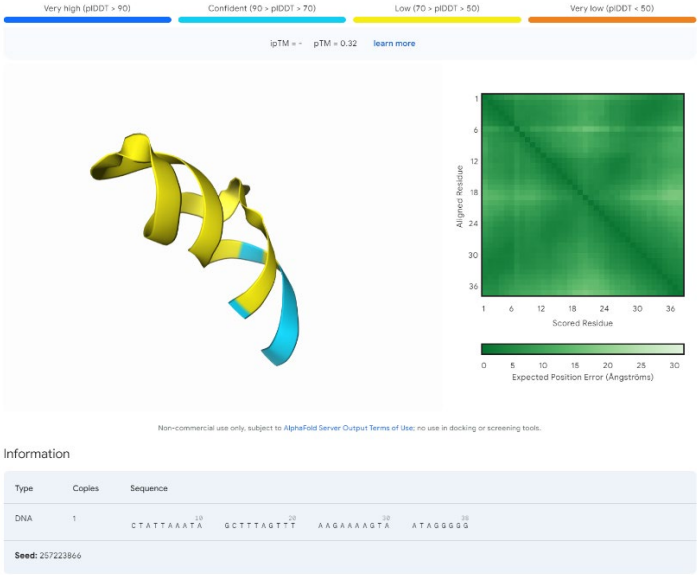
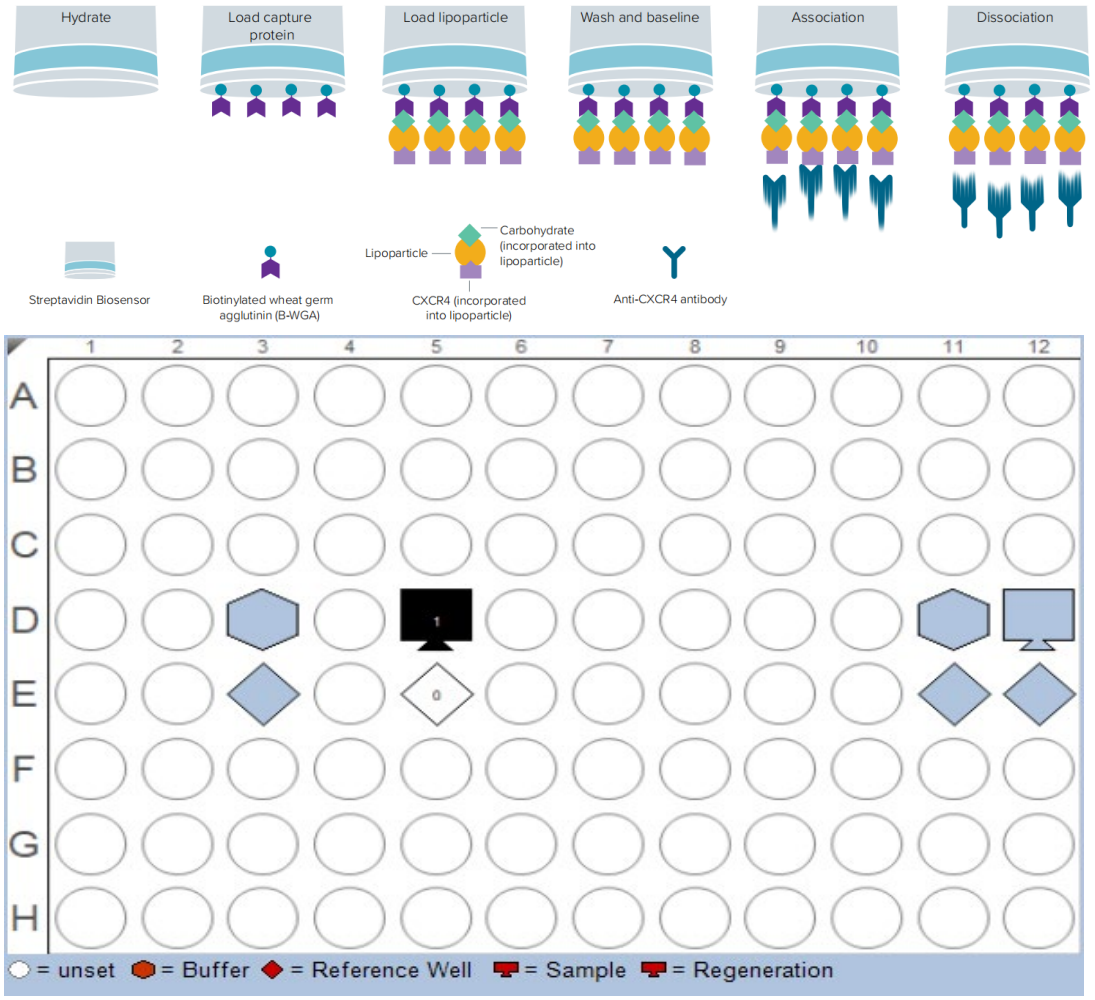
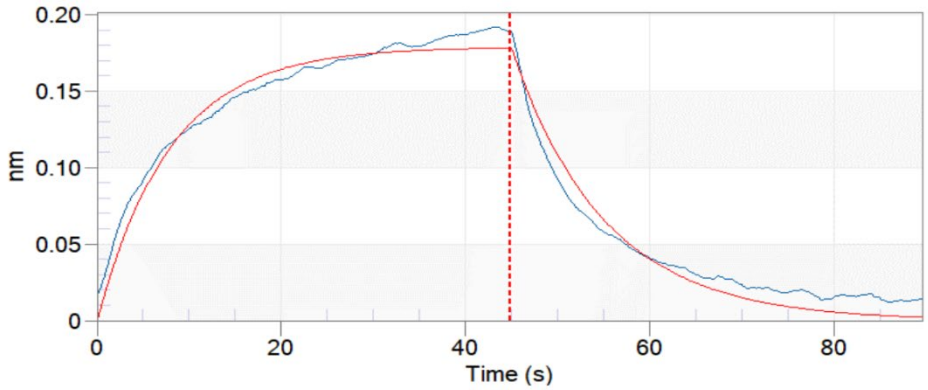


Fig. Tertiary structure prediction of the aptamer's truncated sequence based on AlphaFold3
5‘-CTATTAAATAGCTTTAGTTTAAGAAAAGTAATAGGGGGTG-3’

Appendix IV Fast affinity determination by single point measurement

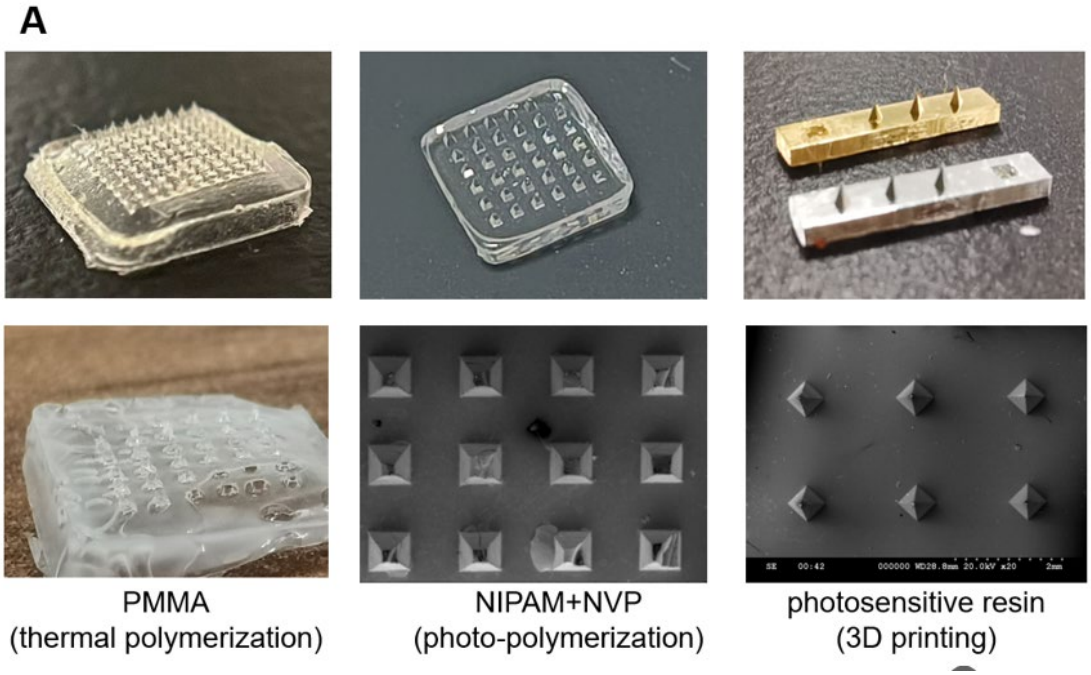




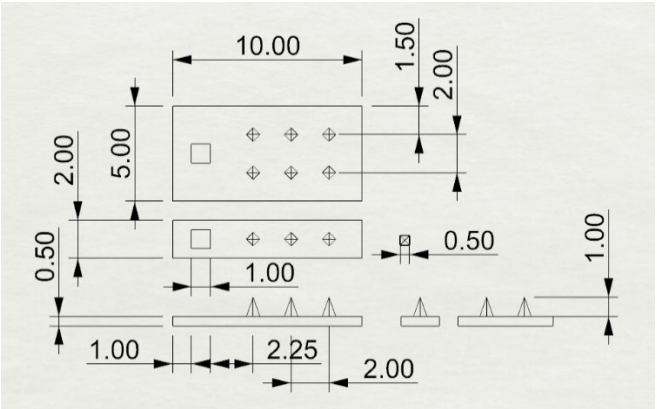
KD (M)	KD Error	kon(1/Ms)	kon Error	kdis(1/s)	kdis Error	RMax	RMax Error	kobs(1/s)	Req	Req/Rmax(%)	Full X^2	Full R^2
3.77E-03	1.07E-03	2.63E+01	7.41E+00	9.92E-02	3.24E-03	0.8524	0.2105	1.26E-01	0.1788	21	0.0069	0.9827

Fig. Aptamer BLI Affinity Assay Results (3.77E-03 M)

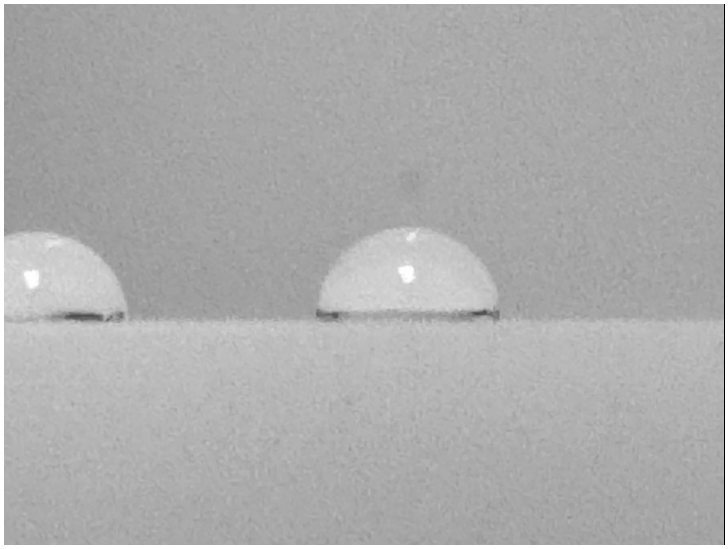
Appendix V Comparison between different fabrication methods and fabrication dimensions



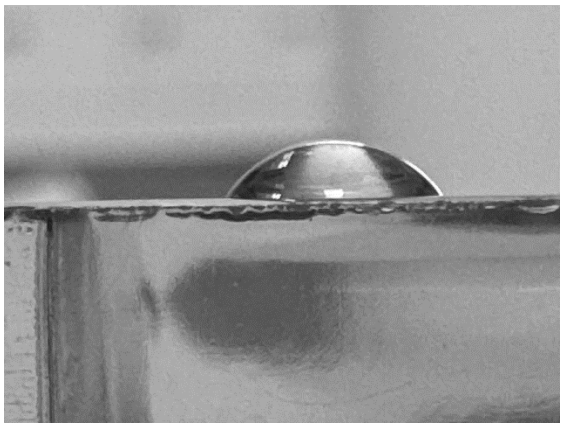
We tested three microneedle fabrication approaches: thermal polymerization micro-needles based on PMMA (Polymethyl Methacrylate), photo-polymerization micro-needles based on NIPAM (N-Isopropylacrylamide) and NVP (N-Vinyl-2-pyrrolidone) materials, and 3D printing using photosensitive resin. The respective test results are summarized below. Comparatively, the thermal polymerization method requires higher fabrication environment demands, exhibits significant experimental variability, and yields micro-needles with poor strength and morphology. Micro-needles made from NIPAM & NVP showed tip damage under electron microscopy, whereas 3D printed micro-needles demonstrated superior electron microscopy results (SEM) with well-defined tip shapes, higher precision, and greater mechanical strength. They are less susceptible to environmental factors such as temperature and humidity, making them more suitable for in vivo biosensing applications. Consequently, we adopted the 3D printing approach and proceeded with surface metal electrode modification.



To fabricate the microfluidic module, SolidWorks 2021 was utilized for modeling, and 3D printing technology was employed to manufacture the three components: the main body, the interlayer, and the cover. WeNext Technology Co., Ltd.'s materials were utilized for these components.

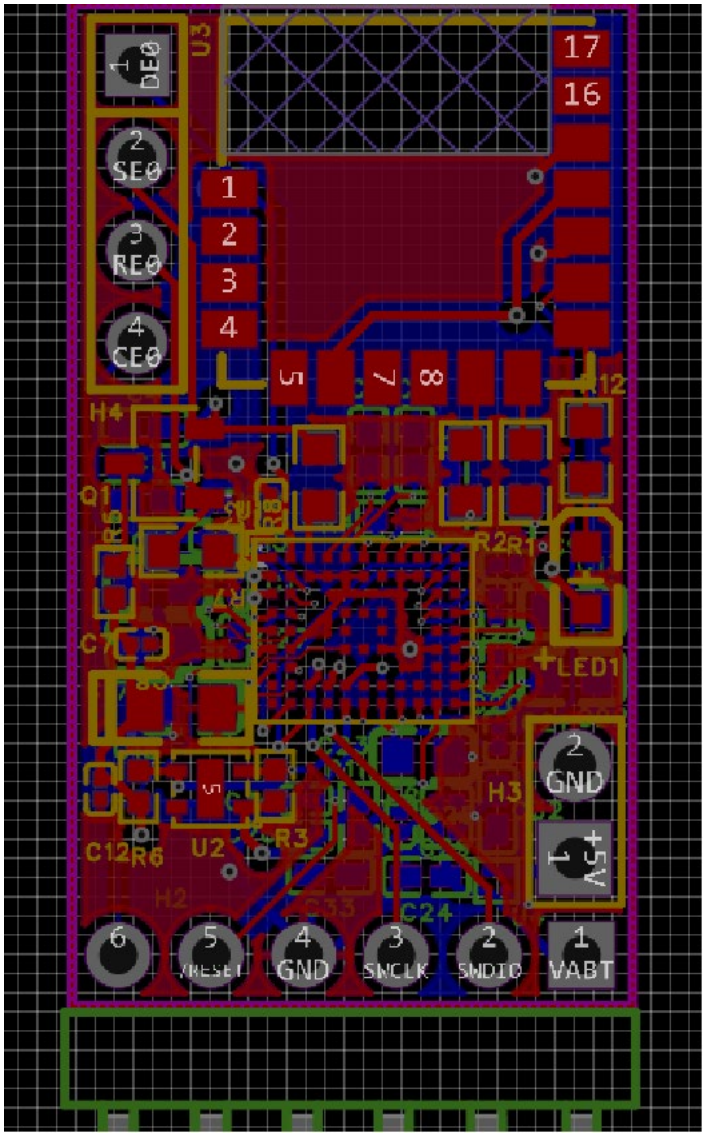


Water contact angle on FUTURE R4600 Rasin

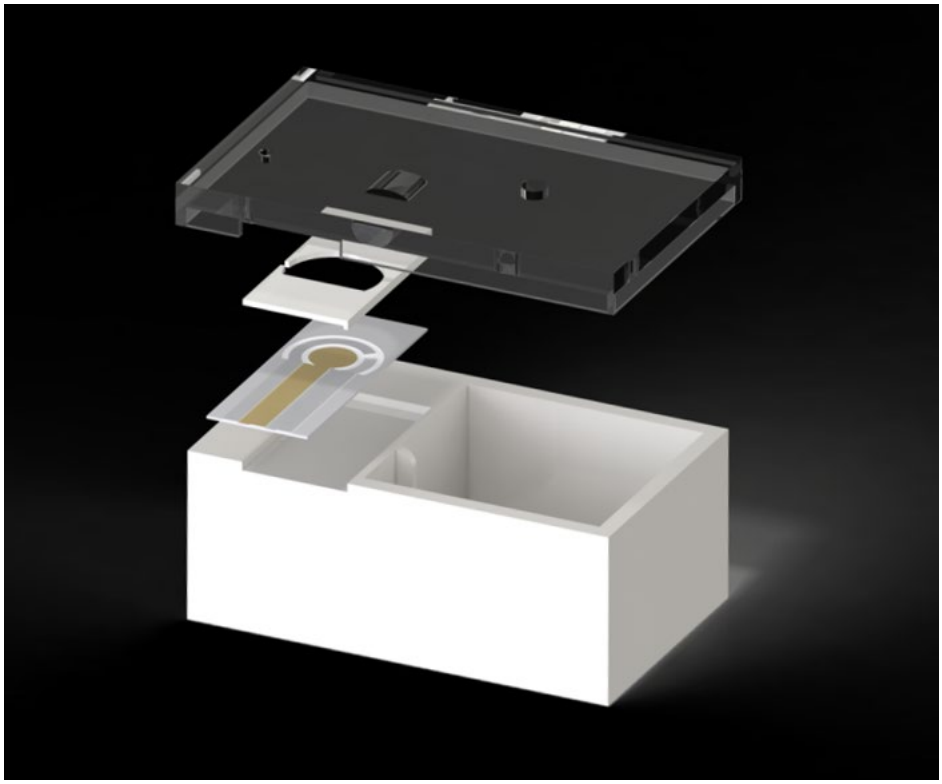


Water contact angle on translucent photosensitive rasin

Appendix VII Detailed Circuit board

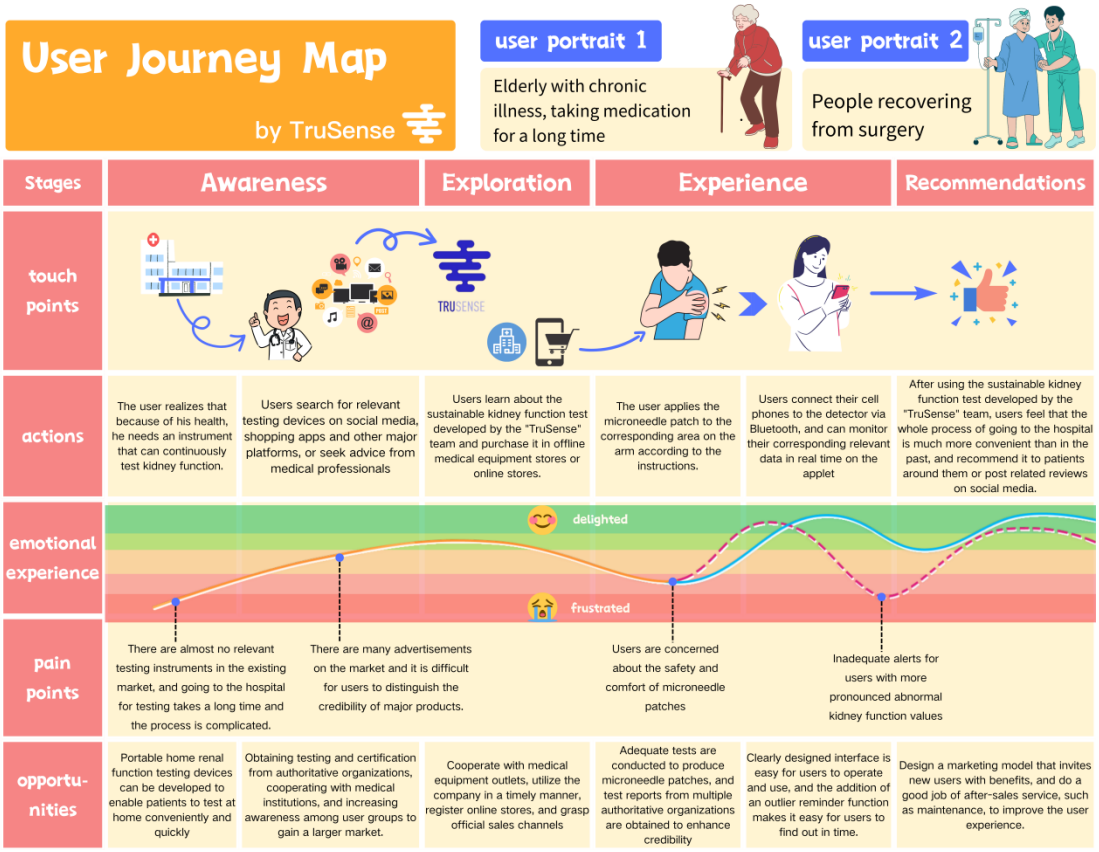


Appendix VIII Design of microfluidic channel suitable for commercial SPCE



3D exploded view for commercial screen-printed electrode cartridge systems: the module is divided into three parts: the main body, the interlayer, and the cover. The main body includes a designated position for placing the electrode. After placing the electrode, the interlayer is embedded into the main body above the electrode, ensuring that the test liquid sample flows exclusively over the electrode detection area. The larger cavity on the right side serves as the waste liquid storage area. Finally, the cover is placed on top. The left side of the cover features a sample inlet with a radius of 0.8 mm, narrowing to a 0.5 mm inlet channel at an angle of 20 degrees. The central recess serves as the channel through which the liquid flows from the detection chamber into the waste liquid area. The hole on the right side is used to balance the air pressure inside and outside the chamber.

Appendix IX translational appendix



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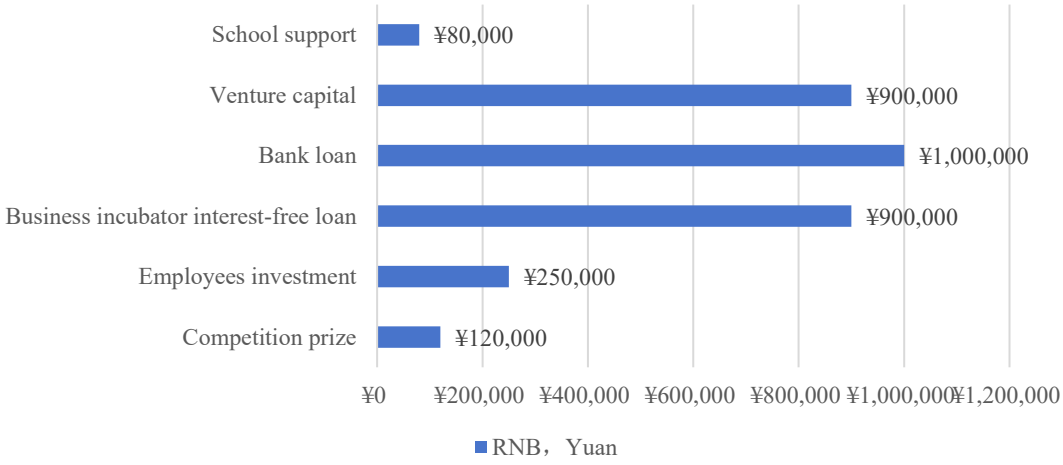
Account of Sources of Funds Statement



Account of Applications of Funds Statement