TruSense 2025

# TEAM RESULTS DOCUMENT

# TruSense


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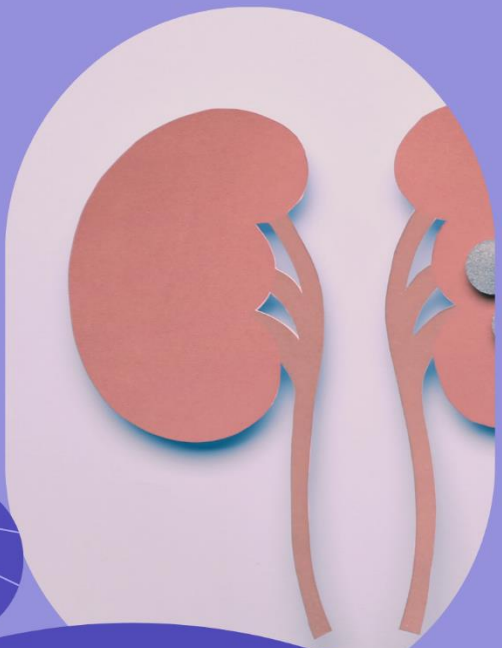


**08/08/2025**

# Contents

## 1.Abstract: Summary for the SensUs website

TruSense introduces a coin-sized wearable patch that painlessly samples interstitial fluid with hollow microneedles. A tri-enzyme cascade converts creatinine to $H_2O_2$, while a Prussian-Blue/MWCNT/ferrocene multilayer electrode amplifies the electrochemical signal for ultrasensitive read-out. The patch encloses a low-power potentiostat and Bluetooth module, streaming live data to a smartphone and operating for two weeks per disposable patch, giving chronic kidney disease patients and high-risk individuals real-time trends and early warnings of AKI progression. Originality comes from three pillars: a non-invasive sampling interface, a synergistic signal-boosting electrode architecture, and fully integrated miniaturized electronics.

## 2.AP award: Biosensor developed for the Eindhoven Testing Event

### 2.1. Molecular recognition

To specifically recognize creatinine, we employ a well-established enzymatic cascade.[1-4] This tri-enzyme system utilizes Creatinine amidohydrolase (CA), Creatinase (CI), and Sarcosine oxidase (SOx) to collaboratively convert creatinine into hydrogen peroxide ($H_2O_2$). This reaction stoichiometrically links the concentration of creatinine to the production of $H_2O_2$, allowing for its quantitative detection.

$$\text{Creatinine} + H_2O \xrightarrow{CA} \text{Creatine}$$

$$\text{Creatine} + H_2O \xrightarrow{CI} \text{Urea} + \text{Sarcosine}$$

$$\text{Sarcosine} + O_2 + H_2O \xrightarrow{SO_x} \text{Glycine} + HCHO + H_2O_2$$

### 2.2. Physical transduction

The hydrogen peroxide produced by the enzymatic reaction is detected electrochemically. We employ Cyclic Voltammetry (CV), a technique where a triangular potential waveform is applied to the electrode, to measure the faradaic current resulting from the electron transfer during the oxidation or reduction of $H_2O_2$. To achieve high sensitivity, we constructed a multi-layered electrode. A foundational layer of Prussian Blue acts as a catalyst for $H_2O_2$ reduction, which is then amplified by an overlying layer of Multi-walled Carbon Nanotubes (MWCNT). Upon this, a biocompatible hydrogel of Chitosan, BSA, and Ferrocene provides a stable matrix for the final layer: the glutaraldehyde cross-linked enzymes. (Fig 1) This synergistic design ensures both highly sensitive detection and robust operational stability.

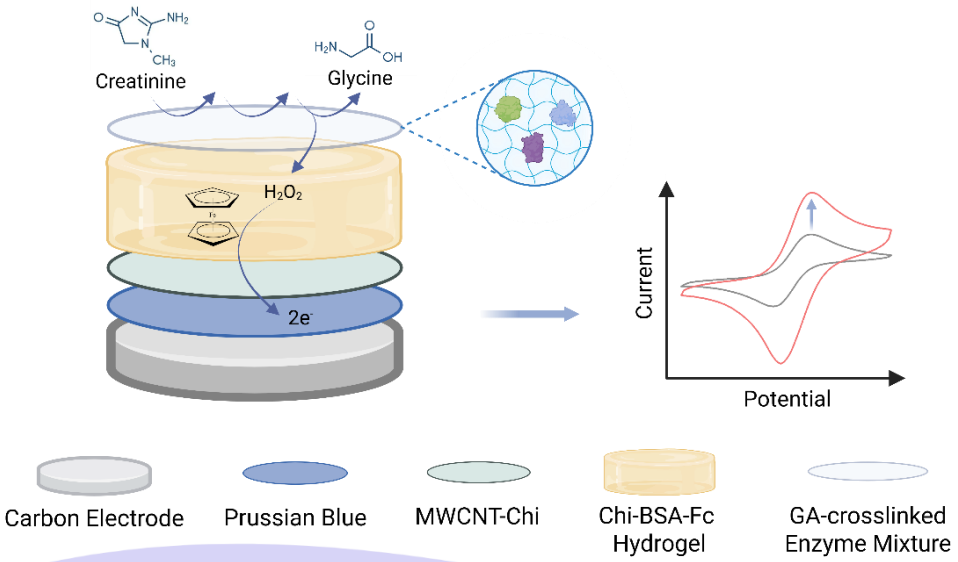



Fig 1. The physical transduction process

## 2.3. Cartridge technology

The microfluidic cartridge comprises a base, a spacer, and a lid. The base seats the electrode; the spacer confines flow to the sensing area and connects to a waste reservoir. The lid contains a 0.8 mm sample port that tapers to a 0.5 mm channel (20°), a vent, and a bridge channel leading to the waste chamber. COMSOL simulations (Fig. 2a) show that the chamber fills in ≈3 s and is cleared by an air plug in ≈4 s, validating the design. In use (Fig. 2b), a pipette introduces the sample, which passes over the electrode, is then pushed into the waste reservoir by injected air, and the cartridge is immediately ready for the next measurement.

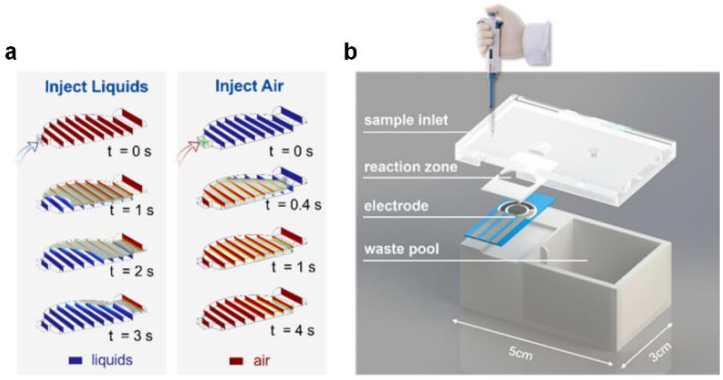



Fig 2 The design and simulation of microfluidic cartridge

## 2.4. Reader instrument and user interaction

In practical use, we employ a microneedle array to access ISF, featuring a square base with three 1mm-high, right-triangular pyramidal microneedles that function as the working, counter, and reference electrodes. The instrument itself is a compact electrochemical workstation built around an MSP430FR2433 microcontroller and a Bluetooth module, all arranged on an 11.6 mm-diameter circular PCB. This circuitry is housed in a custom handheld shell, enabling portable operation and wireless transmission of data to a mobile device. For the user interface, we developed a Progressive Web Application (PWA) that uses the Web Bluetooth API to connect with the hardware for real-time data display. The microneedle sensor, BLE potentiostat, and PWA app integrate into a handheld platform for minimally invasive, real-time creatinine monitoring with wireless data management.

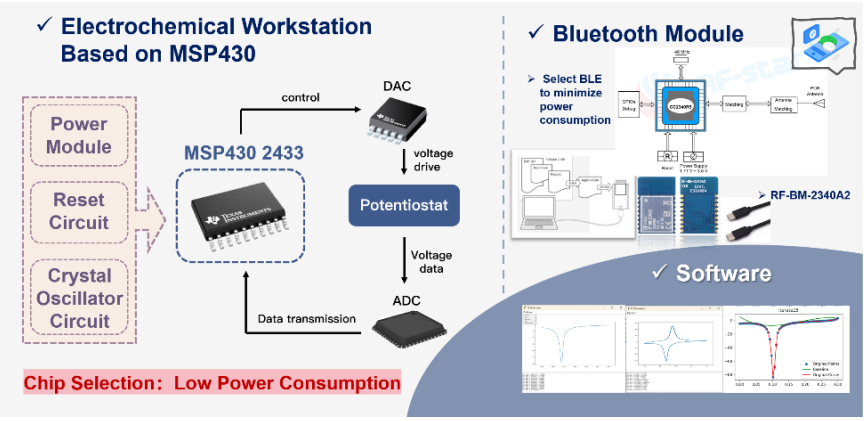



Fig 3 The schematic diagrams of reader instrument and user interaction

## 3.IN award: Biosensor innovation

### 3.1. Wearable sensor

#### 3.1.1. Technological novelty of wearable sensor

Our biosensor is a compact wearable patch encased in a 3D-printed shell, as shown in the overall design (Fig 4). It interacts painlessly with the skin via an array of 1mm hollow microneedles (Fig S1) that penetrate the epidermis to continuously sample interstitial fluid. Inside the hollow microneedle, carbon paste (as working and counting electrode) and Ag/AgCl wire (as reference electrode) is filled[5, 6]. The biorecognition layer is immobilized on one of the carbon paste electrode to catalyze creatinine into hydrogen peroxide. An integrated, wireless electrochemical workstation then measures the resulting reduction current via Cyclic Voltammetry and transmits the data via Bluetooth to a mobile app, providing a real-time display of the user's creatinine concentration. This continuous cycle enables the dynamic tracking of creatinine levels over extended periods.

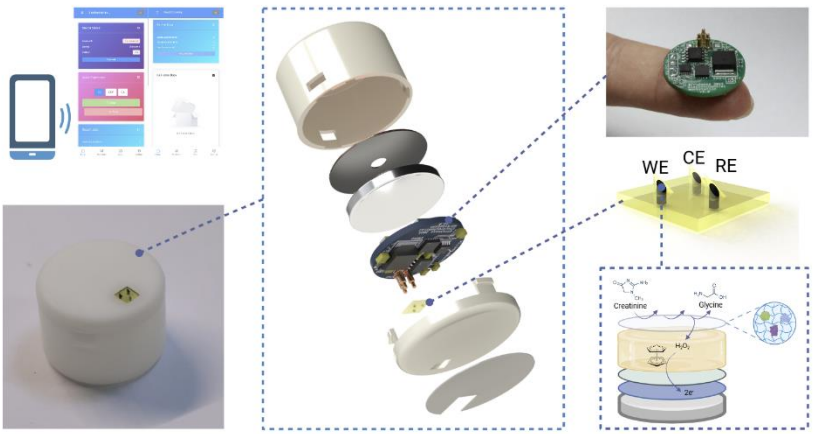



Fig 4. Overall design of our biosensor: exploded view (center); software interface (top-left); product photo (bottom-left); mini electrochemical workstation (top-right); microneedle model (middle-right); biorecognition element (bottom-right).

Our design's novelty lies in integrating **three key advancements**: a non-invasive, sample-free interface enabled by hollow microneedles; an enhanced electrochemical system using synergistic PB and Ferrocene for superior sensitivity; and a highly integrated device architecture that houses all low-power electronics and a battery within a single, compact 3D-printed shell. Future work will leverage DNA origami to further optimize performance, representing a significant step toward continuous and personalized health monitoring.

#### 3.1.2. Technical feasibility of wearable sensor

To prove the efficiency of our biorecognition strategy, we confirmed the signal amplification from MWCNTs over a bare electrode (Fig S2) and established the essential roles of ferrocene and Prussian blue for a better signal (Fig 5a). With the enzyme layers immobilized, the sensor demonstrated a progressive increase in the CV peak value with rising analyte concentration, yielding a linear calibration curve (Fig 5b-c). Furthermore, the unmodified electrode exhibited no response to varying concentrations of creatinine (Fig 5d). The sensor also exhibited a good linear response in the HPLM serum buffer(Fig 5e-f).

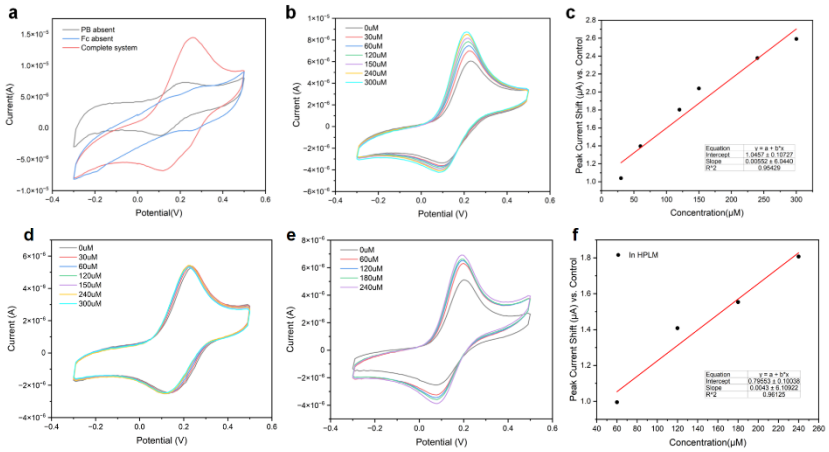



Fig 5. Electrochemical performance of the biosensor. (a) Validation of key signal-enhancing components. (b, c) Linear response and calibration curve in PBS . (d) Specificity control using an unmodified electrode. (e, f) Maintained linear response in HPLM.

To enhance the catalytic efficiency of a multi-enzyme cascade, we investigated two distinct assembly strategies: direct enzyme fusion via protein linkers [7-9] and spatial organization on a DNA origami scaffold [10, 11]. Molecular dynamics (MD) simulations and atomic force microscopy (AFM) results demonstrate the significant potential of these strategies for application in biosensing platforms. (Fig 6)

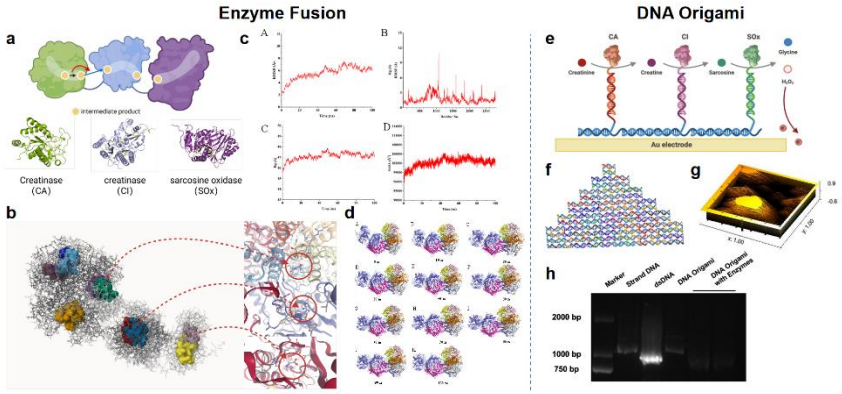



Fig 6. Two strategies for multi-enzyme cascade assembly. (a-d) The enzyme fusion approach, where MD simulations predict a stable conformation with proximate active sites. (e-h) The DNA origami strategy, with fabrication confirmed by AFM and gel electrophoresis.

The wearable creatinine sensor combines all functions in a palm-sized, three-layer stack: a battery, an 11.6 mm four-layer electrochemical/BLE PCB, and a swap pable microneedle array joined by pogo pins inside a skin-taped 3D-printed shell. Deep-sleep firmware lets the MSP430 wake for only millisecond readings, so one battery powers the unit for weeks, while the fully integrated analog front end and radio deliver low-noise, real-time data without cables. Results are streamed to any phone via a browser-based PWA that pairs over Web-Bluetooth and plots live trends—no app installation required.

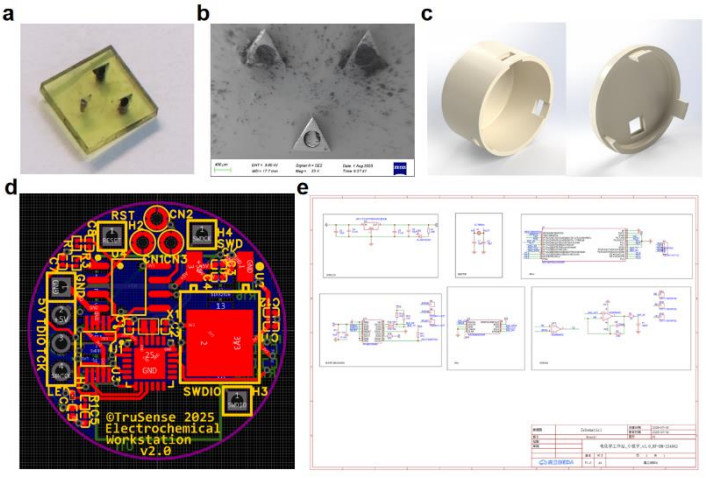



Fig 7. Design of the instrument:(a-b) Photograph and SEM image of the microneedle.(c) 3D model of the shell. (d-e) Circuit schematic and PCB layout

## 3.2. Reliability of sensor output

### 3.2.1. Technological novelty of reliability concept

The dominant sources of variability and drift in our wearable biosensor are multifaceted, spanning biological, chemical, and instrumental domains. Biologically, the primary challenges are biofouling and the inherent instability of the enzyme. Instrumentally, the sensor must also contend with potential drift from temperature fluctuations, power supply instability, mechanical stress, and intermittent wireless connectivity, all of which can corrupt the integrity of the measurement signal.

**System reliability is delivered through a concerted blend of mechanical, firmware, and electronic safeguards. Mechanically**, Ecoflex silicone gaskets seal every housing seam for sweat- and water-proof protection. **Firmware-level** safeguards, including watchdog, brown-out, and CRC routines, allow the MCU to self-recover from faults, ensuring uninterrupted operation. **The electronic signal chain**, built around an ultra-stable DAC8563 and an FRAM-based MSP430FR2433, features nanoamp-standby current and low drift, preserving calibration despite temperature swings and battery aging. Finally, a robust wireless link is supplied by a CC2340R5 BLE 5.3 module, which uses automatic retransmission and +8 dBm output power to maintain a stable on-body connection.

**To ensure measurement accuracy,** a critical prerequisite for subsequent analysis, we implemented a baseline subtraction method. This procedure isolates the true Faradaic current—generated by the electrochemical reaction of interest—from the non-Faradaic background current. The baseline, identified as the segment of the CV curve with a relatively small and constant slope, is computationally removed to yield accurate peak current values.

**To enhance enzyme stability**, we developed a protective hydrogel matrix containing chitosan (for structural and antimicrobial support) and BSA (as a sacrificial protein and crowding agent). [12] This design improves the sensor's operational lifespan. Additionally, to compensate for the inevitable decay in enzyme activity, we integrated a decay function into our calibration model. This corrects for signal drift, extending the sensor's reliable operational lifetime even as enzyme activity diminishes.

### 3.2.2. Technical feasibility of reliability concept

As illustrated in the Fig 8, both the on-board AD and DA modules were evaluated and found to operate with high accuracy. Cyclic-voltammetry scans obtained with our self-developed electrochemical workstation for creatinine sensing match those recorded with a commercial workstation, exhibiting identical peak potentials and current magnitudes. In addition, deliberate disturbances—such as shifting the electrodes during

measurement—did not degrade signal stability, further confirming the robustness and usability of the system.

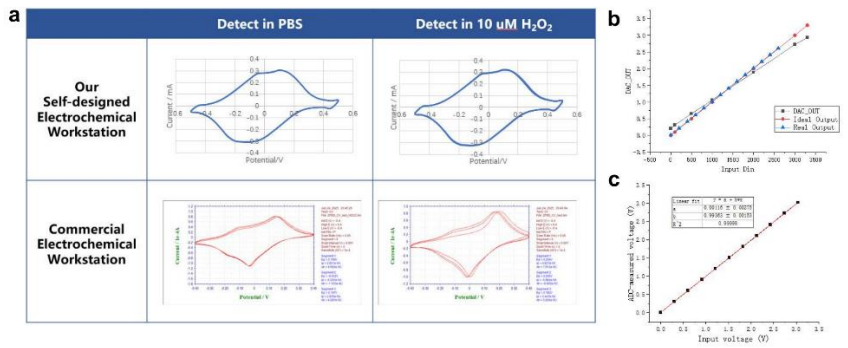



Fig 8. Characterization of hardware accuracy :(a) Comparison of CV test results between a commercial electrochemical workstation and our self-designed circuit. (b) Accuracy test of the DA module. (c) Accuracy test of the AD module.

Then we verified the stability of the enzyme within the hydrogel matrix over consecutive measurements (Fig 9b). For all experiments, we extracted data from the 10th CV cycle of each measurement, followed by automated baseline subtraction using our custom algorithm (Fig 9a). The resulting baseline-corrected peak current was then used for plotting all calibration curves (Fig 5c, 5f). To address long-term performance, we characterized signal attenuation over 50 repeated measurements in a single solution (Fig 9c). It was observed that the electrochemical signal decreased steadily and continuously, eventually stabilizing at approximately 70% of its initial value. Meanwhile, the characteristic shape of the voltammogram remained unchanged, indicating the underlying electrochemical process was stable. The predictable signal decay was then fitted to a mathematical decay function (Fig 9d):

$$y = \left(2.66e^{-\frac{x}{31.39}} + 6.33\right) * 10^{-6}$$

Applying this function to calibrate the raw data computationally corrected for signal drift. This process significantly improved the linearity ($R^2$) of the final calibration curves in both PBS and HPLM serum medium (Fig 9e-f).

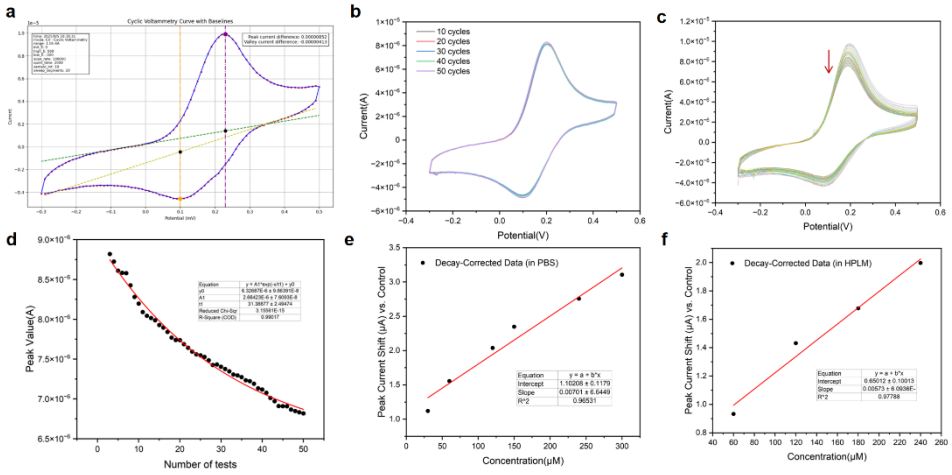



Fig 9. Stability Analysis and Signal Correction for Long-Term Reliability. (a) Baseline correction. (b) Cycle stability. (c) Long-term decay. (d) Decay curve fitting. (e, f) Corrected calibration in PBS and HPLM.

## 3.3. Original contributions

By the supervisor

I have supervised the Zhejiang University SensUs team, TruSense, over the past few years, including this year. Early in their work, the students settled on a wearable biosensor concept for continuous creatinine monitoring, with their core design anchored in a microneedle-based sampling system. To meet the competition’s requirements for non-invasive, long-term operation, they resolved to integrate hollow microneedles with a multi-enzyme cascade and electrochemical detection.

The fusion of hollow microneedle sampling, a Prussian Blue/MWCNT/ferrocene electrode, and miniaturized low-power electronics is highly innovative—particularly in its compact, user-friendly form with real-time data transmission. I can confirm the entire technical approach was independently chosen by the team. They reviewed literature to develop enzyme immobilization methods, refined microfluidic and circuit designs, and built the companion app. They proactively sought expert input from various departments and developed every system component—sampling, detection, electronics, and software—on their own, receiving only equipment usage guidance and safety training.

In conclusion, this diligent and independent team has developed a novel wearable biosensor for continuous creatinine monitoring.

Yong Wang

By captains

Our team developed a fully integrated, wearable biosensor for continuous creatinine monitoring in interstitial fluid (ISF). The system features a custom-designed hollow microneedle patch, functionalized in-house with our own sensing inks and integrated with a tri-enzyme cascade in a protective hydrogel. A key innovation was our conception and validation of a synergistic Prussian Blue/ferrocene system for significant signal amplification. To ensure long-term accuracy and reliability, we developed two key software components. First, a custom algorithm performs automated baseline correction on raw cyclic voltammetry data. Second, a mathematical decay function, derived from long-term stability tests, computationally calibrates the signal to compensate for the inevitable loss of enzyme activity. The instrument's electronics, including a custom electrochemical workstation, were engineered in-house for high integration and ultra-low power consumption, requiring four hardware and firmware iterations to achieve a stable, compact design. The system is completed by a custom enclosure and a companion mobile application.

Lingxia Jin Meng Jin

# 4.TP award: Translation potential

## 4.1. Customer interviews

### 4.1.1 Identification of potential customers

We conducted stakeholder interviews at the Nephrology Department of the Fourth Affiliated Hospital, Zhejiang University School of Medicine.

| | | |
|---|---|---|
| Patient1 | Hypertensive Nephropathy, 56 years old, male | Kidney disease patients covering distinct pathological profiles |
| Patient2 | Lupus Nephritis, 32 years old, female | |
| Patient3 | Nephrotic Syndrome, 22 years old, male | |
| Patient4 | Diabetes Mellitus, 48 years old, male | High-risk individuals for kidney disease |
| Pan Hong | Deputy Chief Physician, 35 years old, female | Professional |

### 4.1.2 Interview approach

1. Face-to-face interview: The interview team conducted face-to-face interviews with each potential client accompanied by the doctor.

2. Semi-structured Interview: We prepared a core interview outline before the session, with questions covering how patients discovered their kidney disease, current health status, experiences with medical equipment during treatment, daily medical routines, and personal psychological journeys. We flexibly adjusted the question sequence and follow-up inquiries based on the interview's progress.

3. Use the MOM test technique: We did not put the interview outline and work computer on the table to create a more relaxed communication environment. We communicated as listeners, focusing on interviewees' past experiences and troubles.

### 4.1.3 Results

| | Key points |
|---|---|
| Patient 1 | • **Had no awareness of kidney disease; only became vaguely aware after undergoing a physical examination due to trauma.** A regular jogger who considered himself fit pre-diagnosis, he chalked up occasional fatigue and headaches to aging. A head injury led to a checkup that detected hypertension, prompting the local hospital to advise referral to a higher-level center.<br>• **Delayed further examination due to distance and holiday scheduling difficulties.** Timely follow-up was delayed due to Spring Festival and distance from the recommended tertiary hospital. Over a month later, he was diagnosed with hypertensive nephropathy at Zhejiang University's Fourth Affiliated Hospital. |
| Patient 2 | • **The asymptomatic nature of kidney damage causes hypochondriacal anxiety, and inconvenient hospital visits make it difficult to relieve such anxiety.** Psychological burden: The patient is highly health-conscious and aware of the silent progression of kidney injury. She reported feeling anxious between her bi-monthly follow-ups, especially when experiencing mild physical discomfort. Since frequent hospital visits are inconvenient, she struggles to relieve her health-related anxiety. |
| Patient 3 | • **Symptoms of acute kidney injury can be easily mistaken for other conditions.** Recent kidney status: During his most recent AKI episode, he had been infected with influenza and experienced fever and fatigue. Initially, he believed these were flu symptoms. However, after developing severe diarrhea, he went to the hospital and was found to have an elevated serum creatinine level exceeding 400 μmol/L. |
| Patient 4 | • **Current home devices lack real-time monitoring; simultaneous multi-indicator testing is preferred over single-indicator devices.** The patient stated that he does not monitor his biomarkers regularly, mainly due to a busy work schedule. He typically tests |

| | |
|---|---|
| | at home 1 – 2 times per month, often only when reminded. He is considering purchasing a real-time glucose monitor, but is currently less motivated to purchase a real-time creatinine monitor. However, he said he would be very interested if a device could test both glucose and creatinine simultaneously. |
| Pan Hong | • **Patients with a history of kidney disease need regular check-ups to reduce the risk of AKI.** Regular check-ups can help patients understand their own kidney health and raise alarm if early symptoms occur. The general validity period is one week after the test. **At present, hospital inpatients still lack continuous detection methods** and rely on traditional blood collection methods. Kidney disease inpatients need to monitor the changes of kidney health for a long time, and repeated blood collection is inconvenient and increases the pain of patients during treatment. |

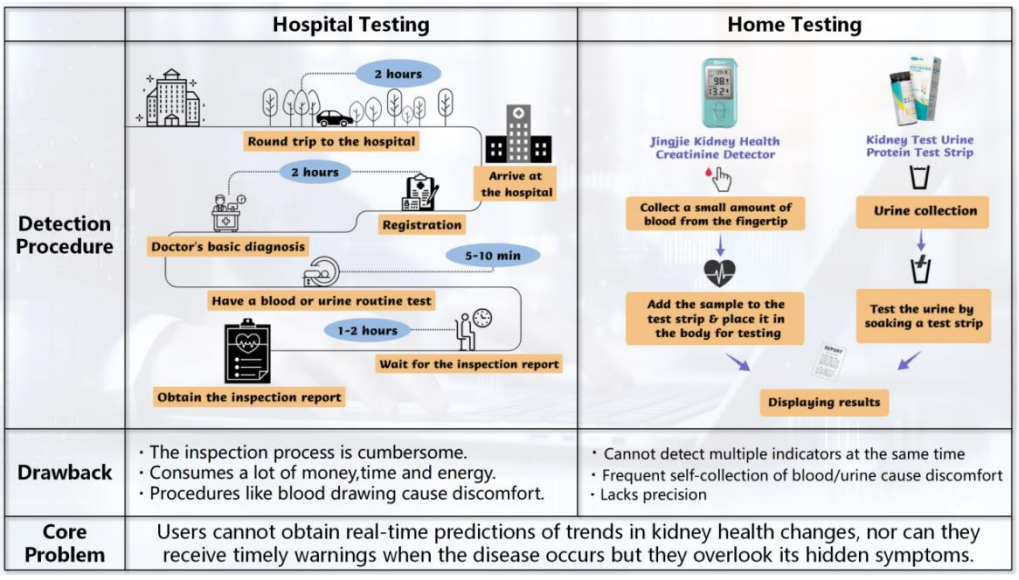
### 4.1.4 Conclusions

1. The subtle early symptoms of kidney disease often lead patients to overlook it or confuse it with other conditions, resulting in psychological stress and delayed diagnosis.

2.The existing kidney health testing programs all have defects, which can not bring sustainable prediction of kidney health status, and allow users to get timely warning without ignoring the hidden symptoms of kidney injury.

3.For long-term hospitalized patients with kidney disease, hospitals still lack continuous detection methods and rely on traditional blood collection approaches, which is inconvenient to operate and adds to the patients' suffering during treatment.

4.Potential customers demonstrate strong demand for multifunctional creatinine testing devices. Among high-risk groups prone to kidney damage, many also suffer from other underlying conditions.



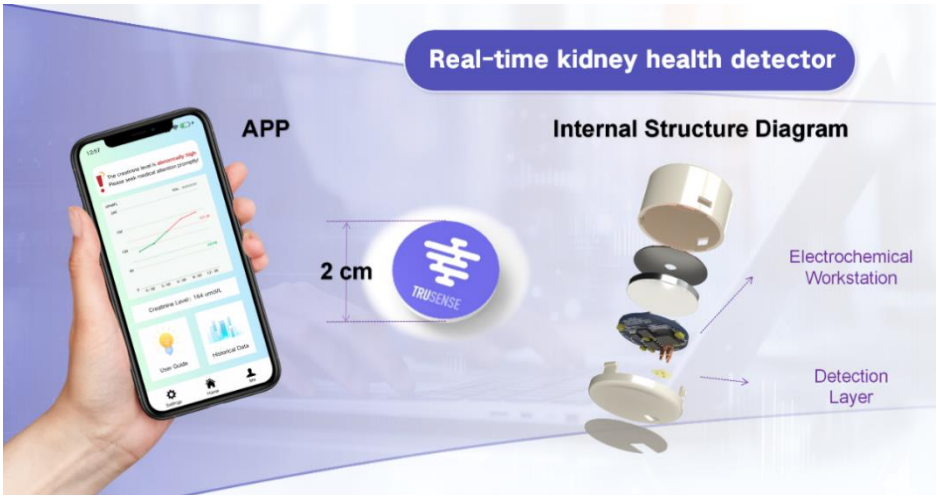

## 4.2 Design of validation study

### 4.2.1 Selection of the problem

How to enable users to conveniently monitor their real-time kidney health, get reliable trend predictions and receive timely accurate warnings of kidney damage when unaware of a kidney disease onset.

### 4.2.2 Design of the solution

Our device is designed to be coin-sized—compact, lightweight, and easy to wear. Simply attach the sensor to your abdomen or upper arm, then use the mobile app to monitor real-time kidney function and predict health trends. When detecting kidney damage, the system triggers urgent alerts via pop-up notifications or SMS, urging immediate medical attention. The patch lasts two weeks, requiring only partial replacement without needing to replace the main device—a user-friendly solution with cost-effective advantages. The secure, waterproof design ensures stable performance during daily activities like exercise or showers. We've also developed an upgraded model for combined monitoring of blood sugar, uric acid, and other indicators, catering to diverse user needs.




This product is ideal for chronic kidney disease patients to manage their condition comprehensively and provides early warning for acute kidney injury (AKI).It is also ideal for high-risk populations such as individuals with diabetes, hypertension, or advanced age, enabling effective monitoring of both chronic and acute renal conditions. The device is versatile enough to be worn comfortably in daily life or clinic settings. The companion app supports data sharing with family members, allowing caregivers to monitor the user's kidney health—especially helpful for users who may have difficulty operating smartphones. Additionally, users can access their personalized kidney health profiles through the app to facilitate telemedicine consultations and AI-assisted diagnostic services.

Added value of the new solution：provide kidney health prediction and injury alerts for timelier detection, thereby significantly increasing awareness of kidney disease and mitigating the risks associated with delayed or missed diagnosis; enhance the usability of kidney function testing devices; reduce user anxiety such as hypochondria; and offer affordable, accessible pricing.

### 4.2.3 Validation study design

Critical aspects:

1.Need to verify users' real demand for real-time kidney injury detection, prediction and warning, and whether the product's multiple daily detections can technically deliver effective results.

2.Despite being small, microneedle wear and long-term use may affect experience; verify if it is more convenient than self-collection devices or hospital checks.

3.Kidney disease's insidiousness may cause anxiety in high-risk groups; verify if the device's real-time alerts alleviate this.

| Stage | Validation Plan | Goals |
|---|---|---|
| I | Produce a complete product promotional video to popularize kidney disease knowledge and introduce product functions and usage. Collect user feedback via questionnaires to obtain data like video view rate, kidney health attention, product demand rate, and price acceptance. | -Low-fidelity verification of market demand.<br>- Completed. |
| II | Produce 100 detectors. Recruit 100 kidney disease susceptible volunteers (covering all ages, genders, disease backgrounds) to wear them for 4 - 6 months. Data is uploaded to the cloud via APP, analyzed daily by backend staff, with prediction and warning results returned to volunteers. After the trial, interview volunteers and statistically gather data on: demand rate for kidney injury prediction and warning function, satisfaction with convenience, acceptance of microneedles and long - term wear, anxiety relief, experience comparison with other home devices and hospital check - ups, dissatisfaction, and acceptable price range. | -Medium-fidelity verification of market demand.<br>- Uncompleted. |
| III | Have 100 kidney disease-susceptible volunteers across ages, genders and disease backgrounds wear Sensor II for 4-6 months. Market demand feedback collection is the same as in Phase 2; to verify the solution's feasibility, collect indicators like product damage or detachment rate, accuracy of kidney injury prediction, and warning effectiveness (the product warned before volunteers noticed). | - High-fidelity verification of market demand and feasibility of the solution.<br>- Uncompleted. |
| **Preliminary verification results:** Within one week after the product promotional video was released on Bilibili (a video platform with extensive influence in China), the video received 2,129 views, reflecting the public's attention to the needs of kidney health prediction and warning. Among the 84 questionnaires collected, 72.6% of the respondents showed that they were relatively concerned or very concerned about their kidney health. They also pointed out that the current diagnosis and treatment methods have high time and money costs, and the inspection methods are relatively uncomfortable. 66.7% of the respondents said they were very or relatively willing to buy the device for themselves or their families. | | |
| Stage I: Concept stage and no physical model<br>Stage II: The sensor enables real - time creatinine detection and Bluetooth data transmission, but the APP - side prediction and warning system is unfinished.<br>Stage III: Sensors and the APP have been basically developed, but mass production has not been achieved. | | |

# 5.Team and support

## 5.1. Contributions of the team members

Supervisor & Captains:

**Yong Wang** (team supervisor): Provided profession guidance on advanced biochemical strategies, including enzyme fusion and DNA origami, while being deeply committed to our academic and personal development.

**Lingxia Jin** (team captain): Oversaw the project's overall progress and led the design and validation of the core biorecognition strategy.

**Meng Jin** (team captain): Coordinated the overall instrument development, including the design and testing of the custom electrochemical workstation.

Translation group

**Saibin Yang:** Managed the team's budget and organized participation in competition.

**Pinghe Zhu:** Developed the project's business model and authored the business plan.

**Chenxi Zhao:** Led the user interviews and the development of the financial plan.

**Yuhan Zhang:** Designed all team visual branding.

Instrument group

**Yixin Gao:** Contributed to all stages of the workstation design, from schematics to system integration, and designed the 3D-printed enclosure.

**Houlin Wang:** Managed all PCB layout and fabrication submissions and contributed to circuit design and testing.

**Bohao Su:** Independently led the development of the Bluetooth communication module.

**Ziqi Huang:** Independently developed the user application.

Biology group

**Zhangying Wang:** Actively explored modification strategies for the tri-enzyme system and successfully validated a hydrogel-based approach.

**Yiyang Zhao:** Managed the enzyme fusion strategy and contributed to the MD simulations and protein expression.

**Muyan Yang:** Characterized and conjugated the DNA origami with enzymes.

**Boming Pan:** Led the initial construction and assembly of the DNA origami.

**Canyu Ma:** Contributed to strategic discussions and contacted SensUs.

## 5.2. People who have given support

**Professor Fan Yang**: Provided the laboratory space, equipment, and reagents that were essential for the successful completion of our project.

**Professor Qingjun Liu:** As an expert in wearable biosensors, Professor Liu provided invaluable guidance on our hardware design, helping us overcome development challenges and sharing his expertise in biorecognition element modification.

**Dr. Hong Pan:** As our medical consultant, Dr. Pan provided critical frontline insights into kidney disease diagnosis, facilitated patient and doctor interviews, and informed our product development and business model.

**Professor Gang Zheng:** As our entrepreneurship advisor, Professor Zheng guided the refinement of our business model, plan, and financials. He also connected us with investors for frontline advice, significantly shaping our commercialization strategy.

**Professor Zhen Gu & Ziqi Gao (Coach):** Provided critical assistance with the design and fabrication of our microneedle patch.

## 5.3. Sponsors and partners

浙江大学创新创业学院 School of Innovation and Entrepreneurship, ZJU

浙江大学教育基金会 Zhejiang University Education Foundation

竺可楨學院 CHU KOCHEN HONORS COLLEGE ZHEJIANG UNIVERSITY

浙江大学 医学院 SCHOOL OF MEDICINE ZHEJIANG UNIVERSITY

TRUSENSE

## 6.Final remarks

We are very excited to connect with other teams during the competition in Eindhoven. Through the SensUs platform, we aim to learn from and share with teams from across the world, exploring topics such as technical innovation, industrial translation, and sustainable development.

The competition is not the end, but a new beginning. In the future, we hope to improve our product design so that TruSense sensors can move from lab-based research to real-world application, truly benefiting the public. To achieve this, we will continue to enhance the sensor's performance in real-world conditions, improve the detection and prediction accuracy of the supporting app, and produce small batches of sensors for testing and validation. Based on real-world use and user feedback, we will iterate the product further.

## 7.References


[1] Yadav, S., Kumar, A., & Pundir, C. S. (2011). Amperometric creatinine biosensor based on covalently coimmobilized enzymes onto carboxylated multiwalled carbon nanotubes/polyaniline composite film. *Analytical Biochemistry*, *419*(2), 277–283. https://doi.org/10.1016/j.ab.2011.07.032

[2] Kumar, P., Jaiwal, R., & Pundir, C. S. (2017). An improved amperometric creatinine biosensor based on nanoparticles of creatininase, creatinase and sarcosine oxidase. *Analytical Biochemistry*, *537*, 41–49. https://doi.org/10.1016/j.ab.2017.08.022

[3] Yadav, S., Devi, R., Kumar, A., & Pundir, C. S. (2011). Tri-enzyme functionalized ZnO-NPs/CHIT/c-MWCNT/PANI composite film for amperometric determination of creatinine. *Biosensors and Bioelectronics*, *28*(1), 64–70. https://doi.org/10.1016/j.bios.2011.06.044

[4] Liu, Y., Luo, X., Dong, Y., Hui, M., Xu, L., Li, H., Lv, J., Yang, L., & Cui, Y. (2022). Uric acid and creatinine biosensors with enhanced room-temperature storage stability by a multilayer enzyme matrix. *Analytica Chimica Acta*, *1227*, 340264. https://doi.org/10.1016/j.aca.2022.340264

[5] Mishra, R. K., Goud, K. Y., Li, Z., Moonla, C., Mohamed, M. A., Tehrani, F., Teymourian, H., & Wang, J. (2020). Continuous Opioid Monitoring along with Nerve Agents on a Wearable Microneedle Sensor Array. *Journal of the American Chemical Society*, *142*(13), 5991–5995. https://doi.org/10.1021/jacs.0c01883

[6] Teymourian, H., Moonla, C., Tehrani, F., Vargas, E., Aghavali, R., Barfidokht, A., Tangkuaram, T., Mercier, P. P., Dassau, E., & Wang, J. (2020). Microneedle-Based Detection of Ketone Bodies along with Glucose and Lactate: Toward Real-Time Continuous Interstitial

Fluid Monitoring of Diabetic Ketosis and Ketoacidosis. *Analytical Chemistry*, *92*(2), 2291–2300. https://doi.org/10.1021/acs.analchem.9b05109

[7] Hatlem D, Trunk T, Linke D, Leo JC. Catching a SPY: Using the SpyCatcher-SpyTag and Related Systems for Labeling and Localizing Bacterial Proteins. Int J Mol Sci. 2019;20(9):2129. Published 2019 Apr 30. https://doi.org/10.3390/ijms20092129

[8] Eva Chovancova and others, 'CAVER 3.0: A Tool for the Analysis of Transport Pathways in Dynamic Protein Structures', PLOS Computational Biology, 8.10 (2012), p. e1002708, https://doi.org/10.1371/journal.pcbi.1002708.

[9] Zhenyu Yang and others, AlphaFold2 and Its Applications in the Fields of Biology and Medicine, Signal Transduction and Targeted Therapy, 8.1 (2023), pp. 1 – 14, https://doi.org/10.1038/s41392-023-01381-z

[10] Rothemund, P. W. K. (2006). Folding DNA to create nanoscale shapes and patterns. *Nature*, *440*(7082), 297–302. https://doi.org/10.1038/nature04586

[11] Klein, W. P., Thomsen, R. P., Turner, K. B., Walper, S. A., Vranish, J., Kjems, J., Ancona, M. G., & Medintz, I. L. (2019). Enhanced Catalysis from Multienzyme Cascades Assembled on a DNA Origami Triangle. *ACS Nano*, *13*(12), 13677–13689. https://doi.org/10.1021/acsnano.9b05746

[12] Fatoni, A., Numnuam, A., Kanatharana, P., Limbut, W., Thammakhet, C., & Thavarungkul, P. (2013). A highly stable oxygen-independent glucose biosensor based on a chitosan-albumin cryogel incorporated with carbon nanotubes and ferrocene. *Sensors and Actuators B: Chemical*, *185*, 725–734. https://doi.org/10.1016/j.snb.2013.05.056

## 8.Appendix

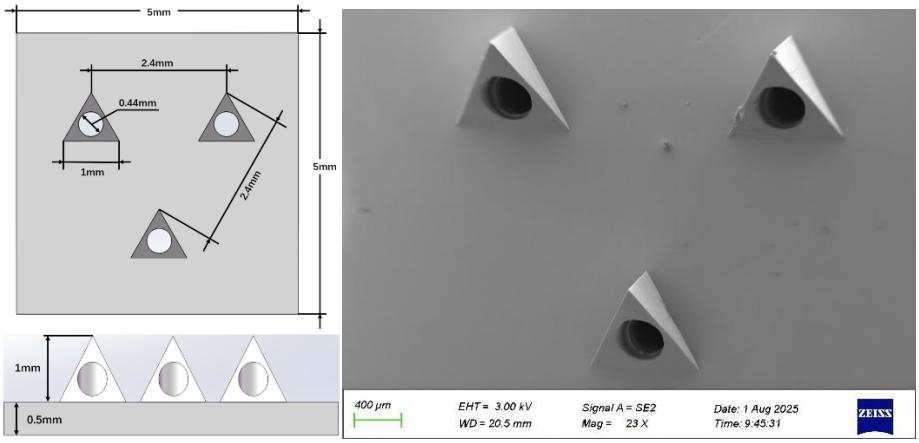



Fig S1. Microneedle dimensions (left) and a scanning electron microscopy (SEM) image of the microneedle patch before filling (right).

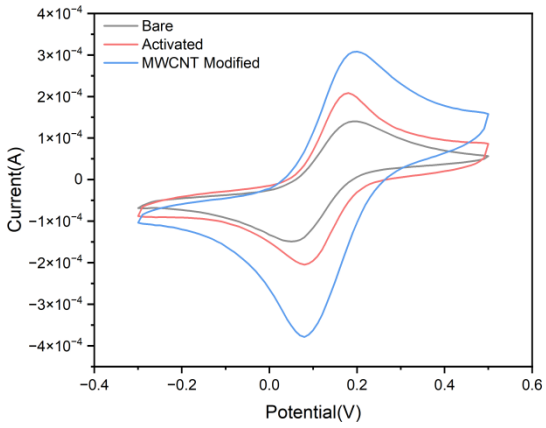



Fig S2. MWCNT can significantly amplify the signal. Bare means bare carbon electrode; Activated means apply CV in H2SO4 and NaOH solution to activate the electrode; MWCNT modified means drop MWCNT-Chi mixture on the surface of electrode.

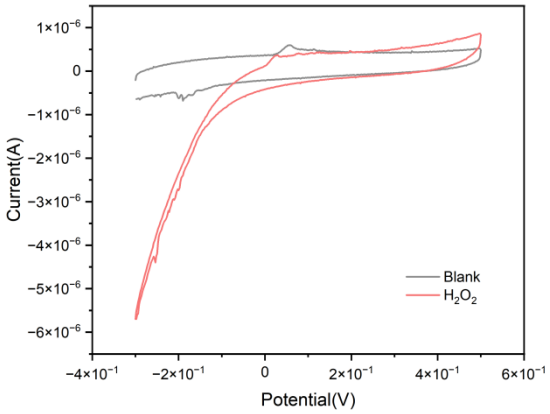



Fig S3. CVs demonstrating the difficulty of directly detecting hydrogen peroxide ($H_2O_2$) on an unmodified (bare) carbon electrode, which fails to produce a well-defined, easily analyzable peak.

Enzyme Fusion Strategy

1. Enzyme Sequence

CA：

HHHHHHSSGLVPRGSHMGAMVTTLSGLEQGPSGDMTEEDSATHIKFSKRDEDGREALGATMEL
RDSSGKITSIVMSDKHVKDFLYVPGKYTFVETAAPDGYEVATPIFEVTNDGEQVTVDGEATGEGD
AHTGSSGGGGGMSSKSVFVGELTWKEYEARVAAGDCVLMLPVGALEQHGHHMHCMNVDVLP
TAKCRVAERGIALVMGPLQYGYSKQKSGGGNHFPGETTLSLDGATLTTGYQDIRILEARGHARRV
LMNGHYENSMIFVEIGDLALERLAYQGIDFKVVVLSVVDVFDKQAPVIQLQYPGEFLGWSDIEHG
QVFETSLLAMYLPDLVLDDRVVDHDPATFPPYDVFPVDPARTPAPGTLSASKTARSREKGIILVEV
CVQGIAADIREEFFPT

CI：

HHHHHHSSGLVPRGSHMRGVPHIWVMDAYRKYGGGGSMQQITDLERTKILQNQGGEKVKPTF
SEKEMTRRNTRLEYMAKAGIDAVMFTSYHNINYYSFDLYTSFNRSYALVVTQDKHVTVSANIDAG

MPWRRSFDENIVTYDWDKRNFLYAVKVLNEGSSFSGRLGVENDHMTLDLRRQVDALPNTELV
DYQSAVMGHRMKSFDEEDILINKGARAIDIGAAVVEIAREGVPEYEVALHTGEAMVREIARTYPH
AELRDTWIWFGQSINTDGAHINWATSRKLQRGDILSNCFPMIAGYYTALETRLFLEEVSDHLEWE
LINCKVRRGLELLKPGARCMIDAAELNEIRYREHDLLANRTFGYGHSGFVLSHYYGREAGILERDE
IDTEVLEPGMVVSMEPIMMIPEGEPGAGGYREHDILVISENIGENTIKFFPGEPHINIKKGGGGSKL
GDIEFIKVNK

SOx：

HHHHHHSSGLVPRGSHMADLLPEHPEFLWANPEPKKSYDVVIVGGGGHGGLATAYYLANKHG
TINVAVLEEVKGAGMNARNMTLSNYLVDESAGYYEKSLLDKPSDPLFFSQGQNLLVKAARVRESL
VYAEKNAAVETLTPEQKVECPINIIDGDIYDKITLDEVRIVPDVHKHAAWAFARKANEMGVDIIQN
ECVTGFLGKDGGKVKTRTGITHGIHAGVKALAGAHGSSLVELAAGFELIPQSHPLQALAPLPDGA
KVVLVVGAGAGMSGYAGKVDLELVGGMAGGSYIDGFMEEQMAQAALEFYKVRDAHVLTVRRHI
VGDTMADSPISIKTPQIGNLIVDLDGDGLVVDKGFTPGAGFTLAHTMAEEPAAAVLTHVLERFETG
HLILEDGRAAALVGGGGSMKPLRGAAVFISLQQKHQPDYPPDLYGAIDQNGTYQNVRTGEDGP
KSGGSSKGYKRLFNESGPYAGVIPQNASTKLSDSNTVISVPQDIAPYETNGKHTITYNEPK

2. linker

Initially, the three enzymes were connected using designed and screened amino acid linkers. However, due to the tendency of protein expression to form inclusion bodies, the SpyCatcher/Tag and SnoopCatcher/Tag systems were introduced to assemble the CA, CI, and SOx monomers. Specifically, the designs are as follows: CA-linker-SpyCatcher, SpyTag-linker-CI-linker-SnoopCatcher, and SnoopTag-linker-SOx. These three individually expressed monomers can self-assemble into a composite chain, which, upon recruitment of an additional five CA monomers and one CI monomer, forms the complete structure.

3. Protein complex structure prediction

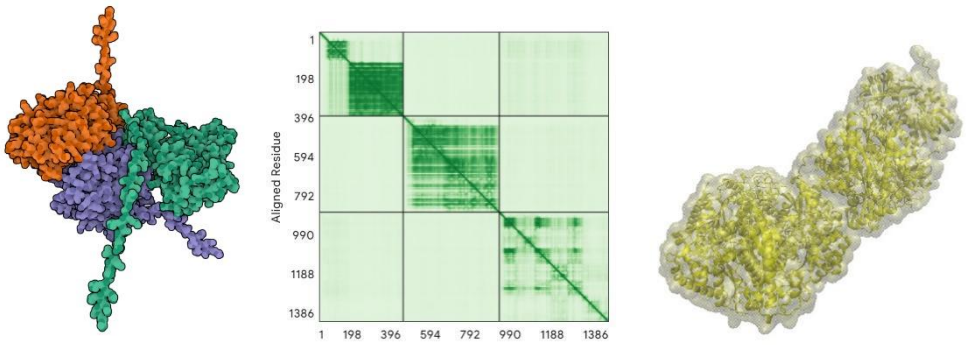
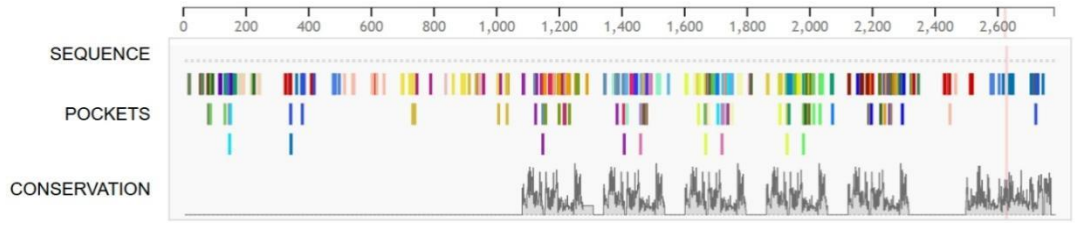



Fig S4. Left panel: A complex chain consisting of only one CA monomer, one CI monomer, and one SOx monomer linked together; Right panel: The complete complex structure formed by the recruitment of 5 CA monomers and 1 CI monomer by the complex chain. The results are clear and the conformation is stable.

4. Substrate channel prediction

The overall prediction scores are stable with high confidence. The substrate channels of CA and CI show no displacement, which is further confirmed in the subsequent analysis of substrate binding sites.




| Rank ↑ | Score | Probability | # of residues | Avg conservation |
|---|---|---|---|---|
| 1 | 17.16 | 0.795 | 29 | 0 |
| 2 | 14.78 | 0.742 | 32 | 0.2 |
| 3 | 12.97 | 0.691 | 30 | 0.577 |
| 4 | 11.91 | 0.656 | 30 | 0.327 |
| 5 | 8.89 | 0.523 | 20 | 0.579 |
| 6 | 5.87 | 0.307 | 11 | 1.558 |
| 7 | 5.48 | 0.275 | 19 | 0 |
| 8 | 5.33 | 0.264 | 17 | 0.721 |
| 9 | 4.56 | 0.209 | 19 | 0 |
| 10 | 4.27 | 0.189 | 14 | 0.252 |

Fig S5. Prediction results and prediction scores of the fusion enzyme protein complex.

5. Substrate–active site docking prediction

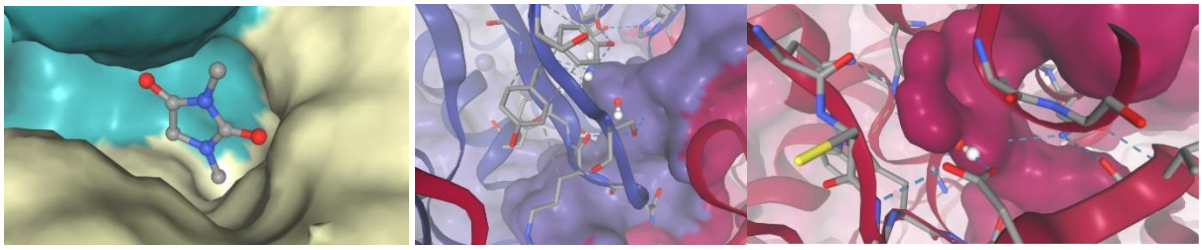


Fig S6. Docking site prediction between CA hexamer and creatinine , CI dimer and creatine, SOx and sarcosine.

6. Stability analysis through Molecular Dynamics Simulations: (100ns)

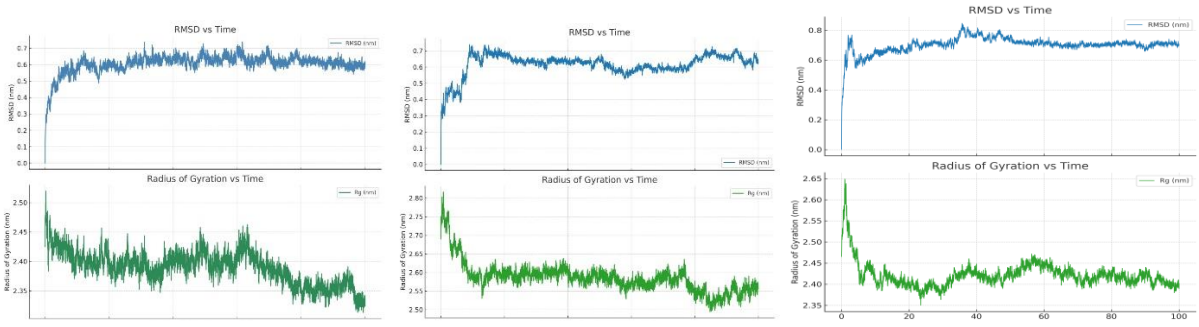



Fig S7. RMSD and Rg results of modified sequence of CA monomer, CI monomer and Sox monomer.

The RMSD remained around 0.7 Å, with no significant perturbation observed.

7. Stability of the CA–CI–SOx assembly chains：

The radius of gyration (Rg) shows a certain degree of shift in the later stages, indicating good short-term stability, but some conformational changes occur at the N- and C-termini over time. Therefore, after the assembly of the scaffold chain, it is necessary to rapidly recruit CA and CI monomers to complete the formation of the functional enzyme complex.

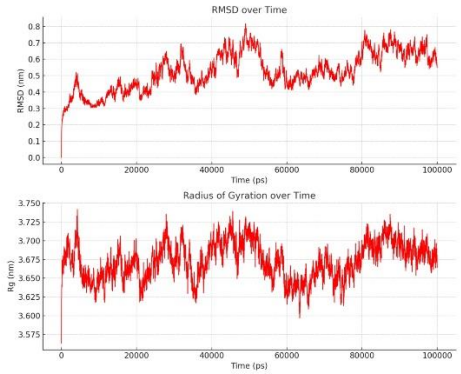
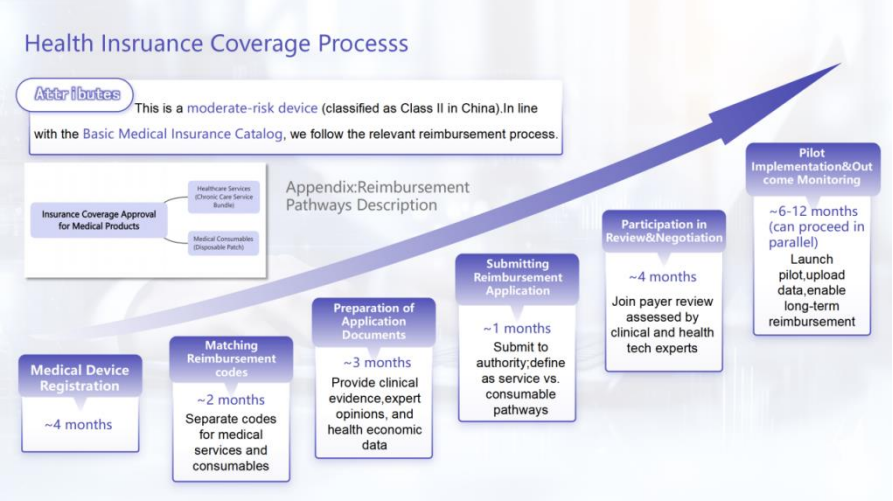



Fig S7. RMSD and Rg results of the complete complex structure formed by the recruitment of 5 CA monomers and 1 CI monomer by the complex chain

Product Launch and Pricing

User purchase intention is influenced to some extent by product pricing. How to set the price, and whether it can be made more accessible through measures like reducing raw material costs or having the equipment covered by the social medical security system, are questions requiring verification.

Studies have shown that including this product in China's social medical security system is feasible.





Availability of public attention data in validation study

Questionnaire:https://pan.baidu.com/s/1XPwqi1BaztzXMgmsmCLKKQ?pwd=inhj

Promotional video:https://www.bilibili.com/video/BV1p8hJzpEx5/?spm_id_from=333.337.search-card.all.click