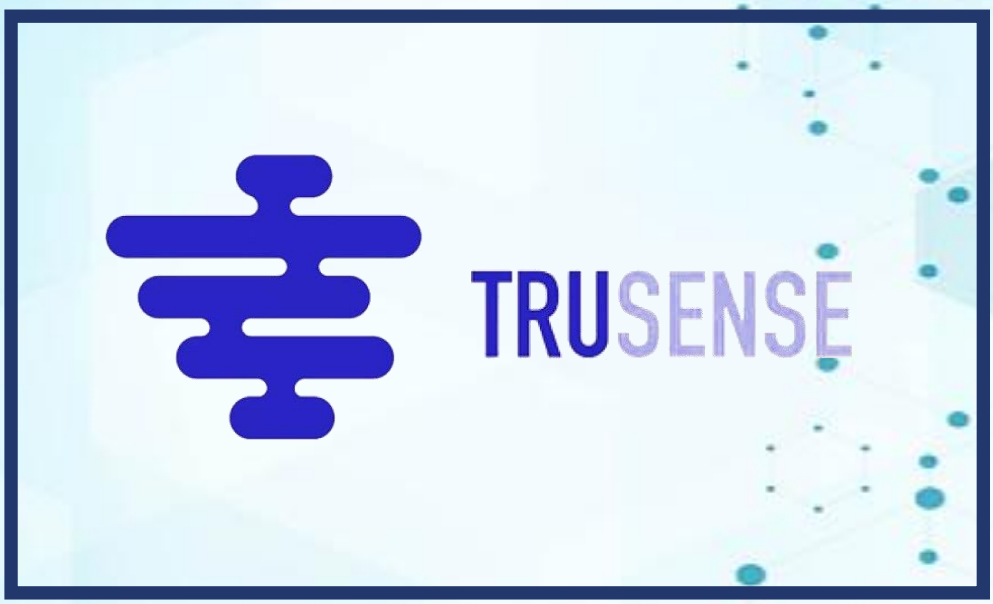
Team Results Document

TruSense



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ZHEJIANG UNIVERSITY

SensUs

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SUMMARY

TruSense is from Zhejiang University, China, which mainly consists of devoted undergra duates to develop a point-of-care impedime tric sensors for inflammation detection espec ially sepsis. Despite the pandemic of the coronavirus, our endeavor yielded TruSensor, a biosensor that integrates molecular biolog y, electrochemistry, engineering and comput er science technologies. We designed two kinds of chips. The first one based on mole cular imprinting technology has been experi mentally verified to be feasible. The second chip based on the immune response, altho ugh not yet completed, has its core technol ogy proven in concept. By dripping a drop of blood onto the chip and gently inserting it into the portable device we developed, interleukin-6 blood concentration can be measured easily and promptly, which provides useful guidance for the diagnosis of inflammation, especially sepsis. Along with the device, we have also designed a user-friendly Android app, enabling real-time update of testing results from the device as well as offering visualized statistical results. To put the biosensor into practical use, we have comprehensively analyzed China's medical market and developed a business model which shows that there is a promising future for our biosensor.

BIOSENSOR SYSTEM AND ASSAY

Molecular recognition and assay reagents

Molecular imprinted polymer

We develop a chip based on molecular imprinted polymer (MIP) for interleukin-6 screen printed electrodes. MIP is a promising technology in the biosensor area attributes to its high selectivity, excellent stability, and amenability to a variety of physical and chemical. Compared to typical biorecognition, MIP is considered artificial recognition without high cost and instability.

In our architecture, monomer is crucial to form MIP. Conducting monomers are used to wrap the templates which are targeting molecules as well by forming hydrogen bond or other inter-molecular forces. Utilizing electro-polymerization and then eliminating the templates, a polymer with the identified target is synthesized.

Because the polymer is electrically conductive and has many caves to specifically bind targeting molecules, it can not only bind target material but also induce a change in resistance. By calculating the degree of shift, the concentration of the target substance can be measured.

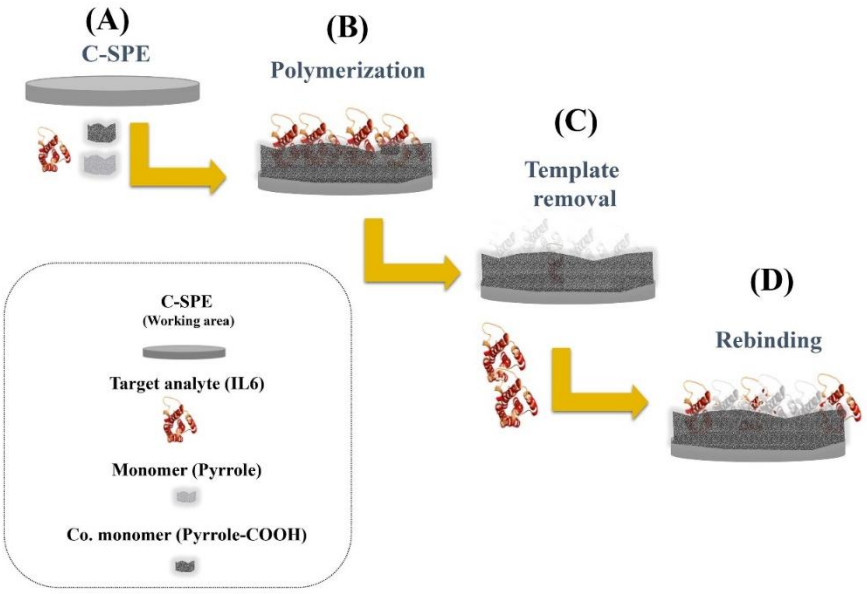


Fig.1. Mechanism of MIP synthesis targeting IL-6

In addition to MIP, we also designed single-chain antibody fragment (scFv) attached to interdigital electrodes to grab IL-6 in plasma. Compared with natural antibodies, single-chain antibodies are more stable, have stronger affinity, and are more conducive to prokaryotic expression. We linked the binding regions of the heavy and light chain moieties of native antibodies from rat (PDB:4CNI) and camel (PDB:4O9H) with a [GGGGS]4 linker to obtain two single-chain antibodies named TS1 and TS2.

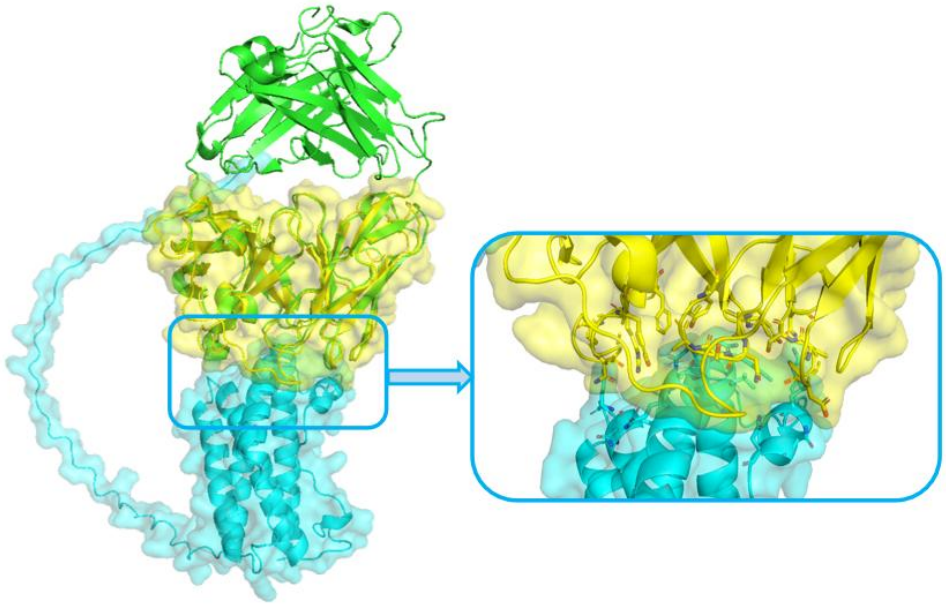


Fig.2.single-chain antibody TS1: Blue, interleukin-6; Green, natural antibody; Yellow, single-chain antibody.

Single chain antibodies were attached to gold interdigitated electrodes. Gold electrode is further modified with the pre-mixture of reduced graphene oxide (rGO) and (3-Aminopropyl) triethoxysilane (APTES), which can generate a large number of -NH2 sites and enhance antibody immobilization. Anti-IL-6 is formed and crosslink ed by APTES. When antigen (IL-6) specifically anchored onto the immobilized antibody, charge transfer resistance variation follows.

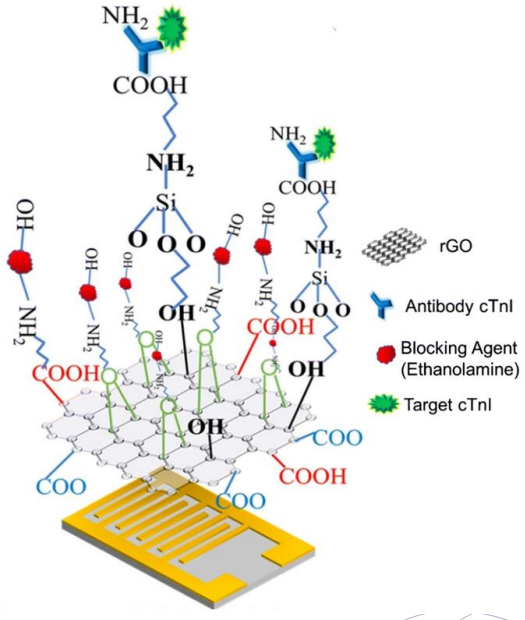


Fig.3.The mechanism of single chain antibody attached to gold interdigitated electrodes

Physical transduction

Whether incubating MIP on screen-printed electrodes or single-chain antibodies on interdigitated electrodes, the physical transduction mechanism is the same for impedimetric sensors. Redox probes exhibit fast kinetics, and normally not interfere with the interfacial properties of the modified electrode. The change in charge transfer resistance for the hexacyanoferrate redox couple associated with a biosensor platform built on screen-printed electrodes for the detection of IL-6 forms the basis of the detection strategy. When antigen (IL-6) specifically anchored onto the immobilized antibody, charge transfer resistance variation follows. EIS measurement is performed to assess this variation. Interleukin-6 has a positive charge, whether it is adsorbed by single-chain antibodies or MIP pores, it will change the charged state of the electrode and thus change the electrical properties of the electrode. In addition, the MIP pores are blocked by IL-6 or antigen-antibody binding, which reduces the Faradaic current on the electrode surface and increases the charge transfer resistance. In this case, the thickness of the insulating layer on the surface of the electrode increases, and the contact area with the solution decreases, resulting in an increase in capacitance and resistance, and an increase in impedance.Therefore, a high concentration of IL-6 in blood plasma induces a high charge transfer resistance, while a low concentration evokes a relatively low resistance, making it possible to detect IL-6 sensitively and rapidly.

Cartridge technology

We purchased adapter headers for dedicated screen-printed electrodes. It measures 36x102x20mm. It is connected to the working electrode, reference electrode and counter electrode. Its volume is smaller than most of the general charging treasures, and it is very easy to carry.

Fig.4. Electrochemical Workstation Compatible Connectors for Screen Printed Electrodes

Reader instrument and user interaction

To provide users with most convenience, we designed an integrated, automated and user-friendly reader instrument. The device is 6.07cm×3.18cm×2cm in dimensions, featured with a switch and a connector for power control and measurement management, respectively. The simple design of user apps allows users to handle measurement safely and easily, offering direct and rapid data acquisition. During testing, users are expected to follow a 2-step simple procedure: collect a drop of plasma onto the cartridge, insert the cartridge into the sensor and push the buttons on the app. Powered by USB cable, OECT detects the chemical signal and converts it into the current signal, which is collected with LMP91000 and processed by an in-house written program in ESP32. The final output is both displayed on the screen as concentrations and medical results. Meanwhile the result is updated to TruSense app on a mobile device through a Bluetooth connection. The TruSense app enables the real-time update of testing results from the device. if the case of temporal connection is in disability, the outputs can also be added into the app manually with ease.

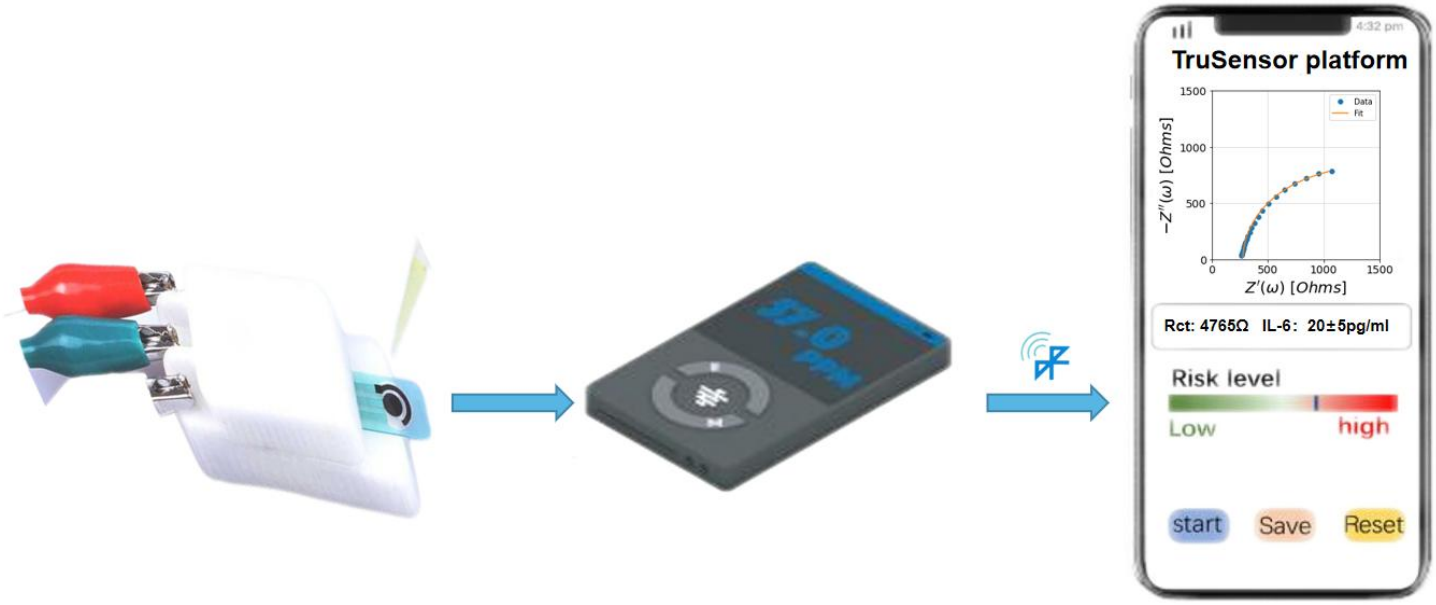


Fig.5. User interface

TECHNOLOGICAL FEASIBILITY

Graphene-modified screen printed

Screen printing electrode is an electrode system formed by screen printing technology, printing conductive ink on the matrix material. It uses a screen printing plate as a mold, has good electrochemical properties and a relatively simple production process, and the size of the electrodes produced can be changed, with the potential for miniaturization. It has several advantages:

1.Low-cost, maintenance-free disposable electrodes
2.A high degree of repeatability between each electrode
3.Compact and powerful

So we chose the screen printed electrode as the electrode carrier for our MIP.

Integration characterization on the electrode

MIP refers to a type of polymer that is synthesized in the presence of template molecules. After removing the molecules, cavities would be left in the polymer matrix with an affinity for the template. The copolymer of pyrrole-2-carboxylic acid and pyrrole is considered to be an important conjugated polymer which exhibits controlled electric conductivity and good stability over a wide pH range.

We choose it as the polymer matrix for molecular recognition of IL-6 molecule.

We designed a basic experiment to verify the reactivity of copolymers to solutions of different IL-6 concentrations.

It is verified by decrease in peak current of CV curve during CV scans in 10 mM [Fe(CN)6]3-/4- solution before and after immersing a MIP electrode in IL-6 solution (Fig. 5), indicating that IL-6 could enter the polymer and block the cavities. Thus, the polymer is potentially capable of specific free IL-6 recognition in plasma sample.

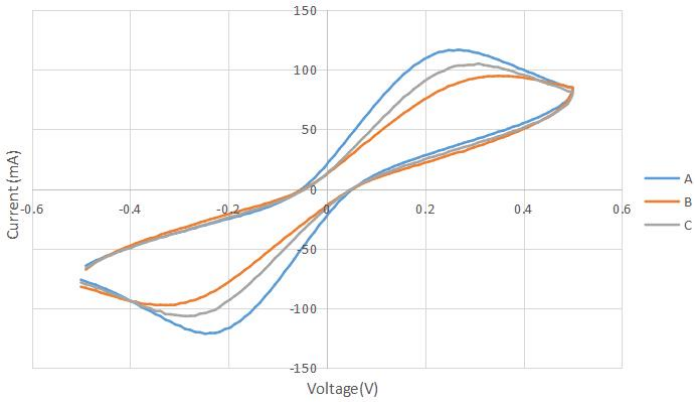


Fig. 6. CV measurements (scan rate of 50 mV/s) .(A) bare C-SPE; (B) NPPy： non-imprinted macterial,without IL-6; (C) MPPy： imprinted sensor,with IL-6

Molecular imprinted polymer (MIP) based on Copolymer of pyrrole-2-carboxylic acid and pyrrole

After immersing the electrodes in different concentrations of IL-6 solution for 2 min, a mixture of potassium ferrocyanide and potassium ferrocyanide is injected into its surface to measure its impedance (Figure x). Obviously obtained from the figure, as the concentration of the solution increases, the Rct decreases. It is shown that IL-6 can enter the cavity of its copolymer and produce an efficient binding to it. Thus, the electrode can specifically select IL-6 and measure its concentration.

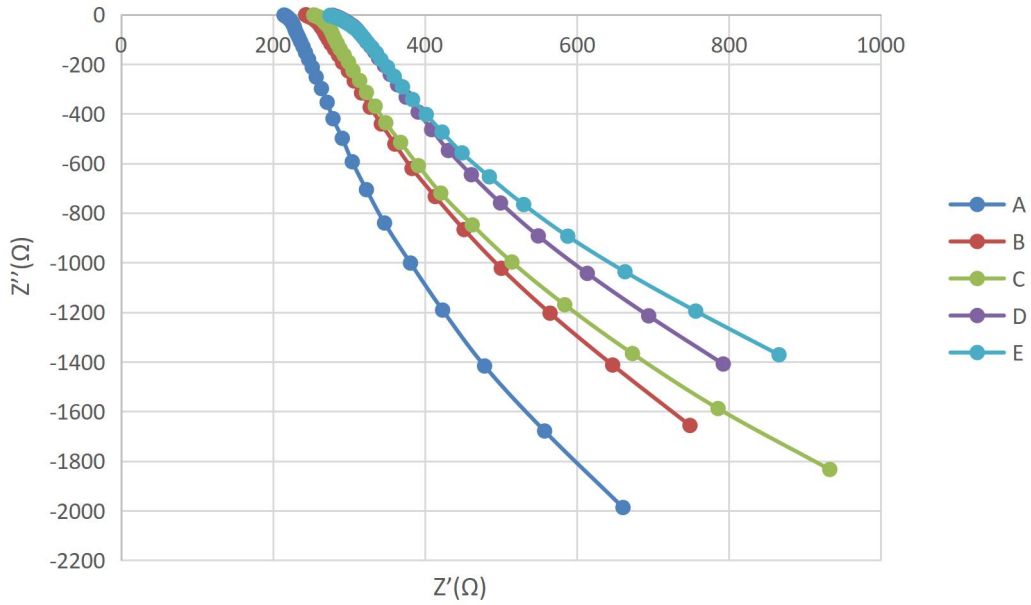


Fig. 7. EIS measurement (scan from 50000 Hz to 10Hz) (A) barely MIPs; (B) 0.02 μg • mL-1 IL-6 reacted with MIPs; (C) 2 μg • mL-1 IL-6 reacted with MIPs; (D) 20 μg • mL-1 IL-6 reacted with MIPs; (E) 2000 μg • mL-1 IL-6 reacted with MIPs;

Single-chain antibody Synthesis Feasibility

The single-chain antibodies specific for IL-6 were engineered to be expressed as fusion proteins with 6xHis tag in prokaryotic expression system (Fig. 7). Optimized sequences corresponding to antibodies were ligated into PET30a vector and the recombinant plasmid was transformed in E. coli BL21 cells. Further, single-chain antibodies were purified by immobilized metal affinity chromatography (IMAC) and then dialyzed. (Figure B) TS1 was found to form inclusion body protein so that extra steps of solubilization and refolding were carried out for renaturation while the majority of TS2 was present in the supernatant of the bacterial lysate.

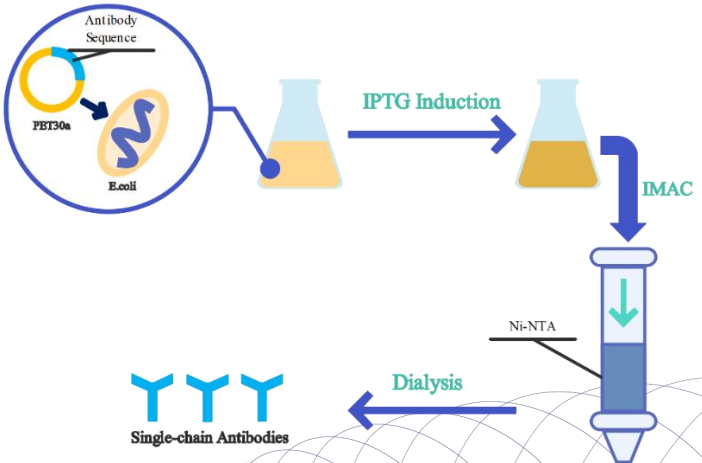


Fig. 8. Process of Single-chain antibody Synthesis

Single-chain antibody Affinity Feasibility

In our sandwich ELISA, the tested IL6 is sandwiched between two antibodies. The capture antibody directly immobilized onto the ELISA plate, binding with the antigen. Single chain fragment variable (scFv), as the secondary antibody, is then added, followed by adding anti-His antibody derived form mouse cell, finally HRP-conjugated anti-Mouse antibody. The addition of TMB initiates the reaction, and the product formed is analyzed by measuring the absorbance at a wavelength of 450nm as the signal. The main advantage of sandwich ELISA over other types is reduced background signal, high sensitivity, and applicability for detecting antigens, especially small ones in biological fluids. We choose the method to detect whether the scFvs(including TS1 and TS2) work specifically with IL6.

Results of TS1 and TS2 ELISA(Figure 9 and 10) showed that both can bind with IL6 from 25pg/ml to 1000pg/ml and the binding ability has specificity and accuracy.

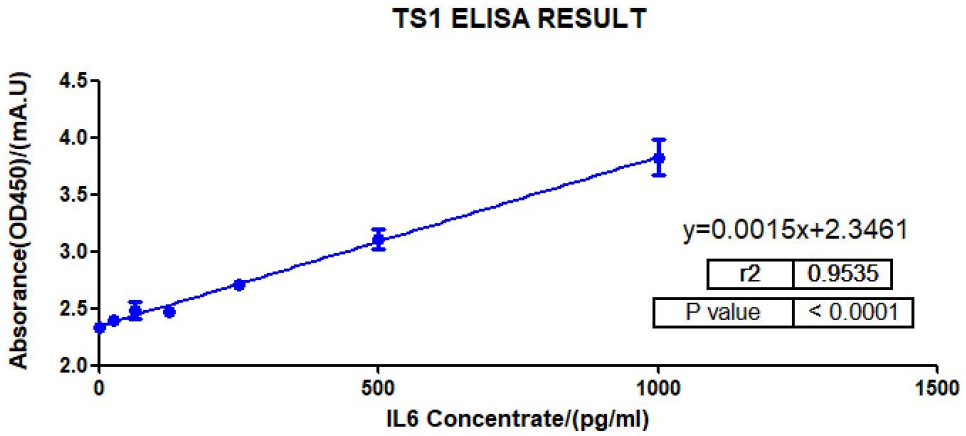


Fig. 10. TS1 ELISA result. Each point contains 3 data. The graph was drawn with GraphPad Prism.

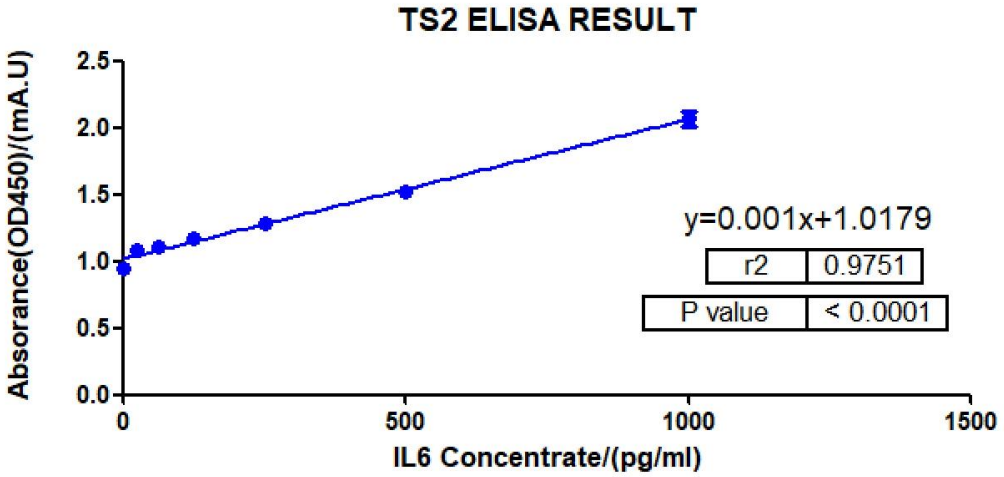


Fig. 11. TS2 ELISA result. Each point contains 3 data. The graph was drawn with GraphPad Prism.

Overall feasibility

The biosensor meets the need of real-time measurement of IL-6 concentration in the plasma sample, with a high accuracy (Fig. 6). The incubation time of IL-6 solution on the electrode and the time it takes for the electrode to be measured in Fe_{2+}/Fe_{3+} solution are only 2 min. Therefore, the total measurement time is expected to be controlled within 5 minutes. The accuracy of the measurements is also supported by a series of experiments.To summarize, we conclude that our biosensor would be a qualified and promising POCT device.

ORIGINALITY

Written by the team

It has been an amazing year working together with each other in Team TruSense. We have recruited twelve members of different personalities and talents - most are sophomores and juniors from various majors, ranging from engineering, biology, to finance, etc. We are divided into three groups with respective functions. Namely, the group of principle, the group of instruments, and the group of commercial potential. We have clear divisions on labor and responsibility, thus creating a collaborative and pleasant atmosphere when working together – we make plans together and achieve goals together.

Thanks to all the efforts, we have eventually explored two different schemes, trying to use MIT and scFv to grab IL-6. It was a pleasant process adopting different schemes while inventing the impedimetric biosensor. We are proud of its excellent performance and hope that it can receive good results in this year's competition. It is a pity that we are cannot make it to the Netherlands and participate in the competition offline this year due to the COVID-19 pandemic, but we are still thrilled to meet with all the friends from different countries during the Innovation Day. Looking forward to seeing you soon!

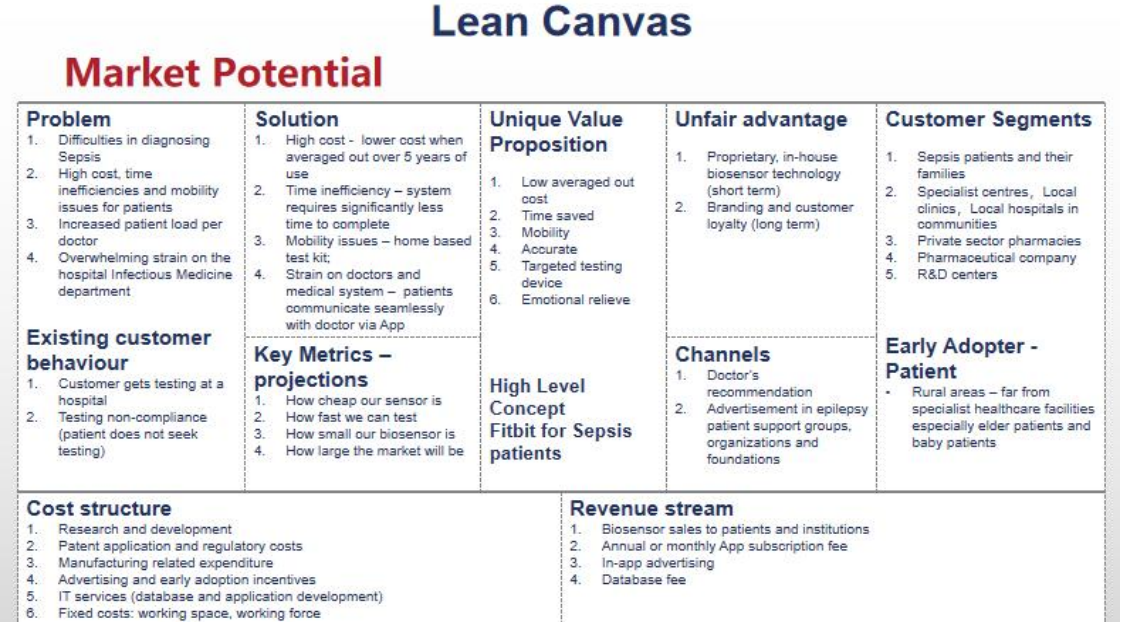
Written by the supervisor

2022 has witnessed the diligence and perspiration of team TruSense. With pandemics still tiding in and out in China, it was easy for all of us during the early stage of sensor development. But the action of our team showed sheer tenacity, we continued to model biosensor structure and run bio-probe simulations even when lockdown fell onto our city. Through trial and error, we explored several possible designs like GFET, QCM, IDE, etc., and the final decision impedimetric biosensors.

It is my honor to supervise such a dynamic, intelligent group of students. Though it's my first year in this position, I have already seen the great potential of TruSense and SensUs community and I firmly believe that TruSense will continue to make progress in the following years.

TRANSLATION POTENTIAL

Business model canvas



Stakeholder desirability

More than 48.9 million people are diagnosed with sepsis around the world every year. The disease has a high mortality rate-around 11 million a year-because many victims ignore early warning signs and only seek medical help once the disease has advanced. It means one among five people who get this disease died.

It is not convenient for some patients to get tested at hospitals, especially those who are living far away from hospitals, who have poor mobility such as the old and disabled, and who have no free time to go to hospitals. The current devices are too large or too expensive for individuals to have one at home or for communities to have one at their medical center. Our testing device for sepsis that can both produce accurate results and is of medium size and price so that either individuals or community medical centers would be able to buy and keep. Citizens will be able to quickly and conveniently have tests at home or at medical centers in the community, instead of going far to the hospitals. 4 major stakeholders are involved in the diagnosis and medication procedure: patients, hospitals, pharmaceuticals corporation, medical centers in communities.

Our TruSense biosensor, a point-of-care device that can monitor the blood concentration of IL6 in sepsis patients. With only 200μL of blood on the testing chip and easy operation, users can read their blood concentration and record their health status on our app, which is connected to the device via Bluetooth. Based on users' profiles of the blood concentration-efficacy, doctors could optimize the treatments efficiently, which drastically cut down the relevant costs of the patients and the workload of doctors.

Market support

Chinese medical devices are in a golden age.The scale of the domestic medical device market has grown rapidly, and the growth rate is higher than that of the pharmaceutical, pharmaceutical distribution, and medical service markets.

The ratio of medicine to equipment in China is much lower than that of developed countries. In 2016, China's drug-device ratio was 1:0.23. With the rapid development of China's medical device market, the drug-device ratio will reach 1:0.35 in 2020, but there is a significant gap with the 1:1 device ratio in some developed countries.

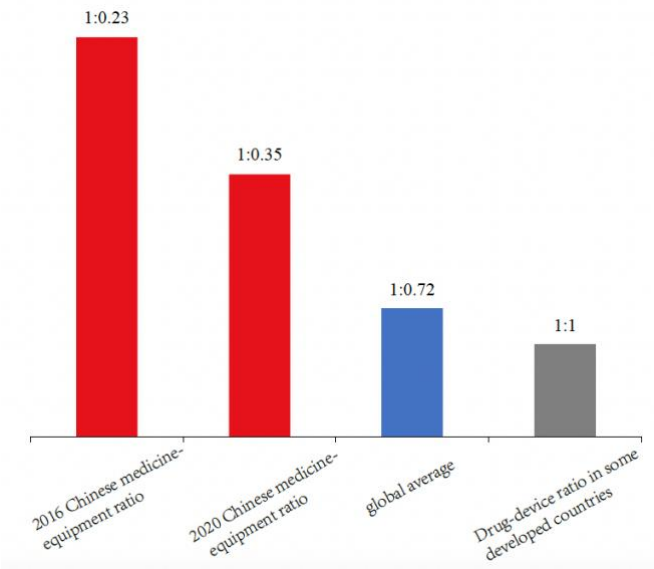


Fig. 12. Chinese and world medicine-equipment market

China's Special Market Position

In 2020, there were 620 million medical treatments in community health service stations in China, with an average annual medical treatment of 5248 per station.

According to State Statistical Bureau, at the end of 2021 there were 1.031 million medical and health institutions in China, including 37000 hospitals, 12000 public hospitals and 25000 private hospitals; There were 977000 primary medical and health institutions, including 35000 township health centers, 36000 community health service centers, 307000 outpatient departments, and 599000 village clinics.

Although the incidence and mortality of sepsis vary greatly among different regions, the situation in China still cannot be underestimated. According to official statistics the disease killed over 830,000 people every year in China. Additionally, there are about 4.86 million new cases of sepsis are reported every year. In that case, there is an increased demand for sepsis medical equipment in China.

Value Proposition

Our sensor is designed to provide convenience for customers in the following fields:

Time saved: Among all the indicators of inflammation, IL-6 is significantly characterized with its feature of fast detection. Apart from choosing this indicator as the testing target, our sensor is also able to reach the outcome in a reasonably fast speed. Compared to the devices in hospitals, we saved time from the waiting process after haemospasia (hospitals would not operate the device for a single person. The blood samples will not be sent for testing until there are enough samples collected), the time to test other indicators (this sensor is for testing IL-6 only), and the time to commute to the hospital (accessibility).

Targeted testing device: This sensor is designed only to test the indicator of IL-6. It fills in the blank space in the current medical service market, as there are rarely any devices designed for one single indicator only, but can save time, cost, and the mobility of the device (such design would successfully reduce the weight of the machine, giving it more mobility to be carried, delivered, placed in local hospitals, and even at home).

Affordable price: As our sensor has reduced the number of indicators to be tested, the cost of the device is significantly lower than that in hospitals. In consequence, both the price of the sensor itself and the expense for a test of IL-6 have greatly decreased than that in hospitals. Accurate outcome: Though the device may look smaller than the ones used in hospitals, we promise that this will not affect the accuracy of the outcome. Our sensor is still able to get

accurate outcome. **Accessibility:** Our sensor is planned to be sold to individuals and local hospitals in communities, which greatly increased people's accessibility to IL-6 tests. Many hospitals, instead of hospitals only in developed areas or of high rankings, would all be able to provide IL-6 testing. Mobility: The sensor will be much lighter than that in hospitals.Therefore, it will have greater mobility to be delivered and placed in local hospitals in communities, and even to be placed at home. **Emotional relieve:** Our sensor would also be able to provide emotional relieve for the customers for letting them know that it is no difficult to get tested for sepsis, and they will be able to do an early testing whenever they want.

Business Feasibility

Our business feasibility is mainly ensured by three critical factors: powerful supports, appropriate marketing strategy and wise promotion strategy.

Support

TruSense biosensor has gained supports both technically and financially. There are currently four scientific research institutions expressing the willingness to provide technical back-up for our team in clinical experiments, including the Sir Run Run Shaw Hospital (affiliated to the School of Medicines, Zhejiang University (ZJU)), the First Affiliated Hospital of ZJU School of Medicine, DeCell, and Innobridge. We have also won funds from cooperating enterprises who would sponsor our subsequent innovation and entrepreneurship, including SIEMENS AG and other suppliers of medical devices, the 'Zhejiang News-Ali' Geek Program, and the Engineering Biology Center of the ZJU Haining International Campus. In addition, we have also consulted several local hospitals and interviewed relevant doctors, thus receiving credible guidance of the current sepsis-testing market and analysis of potential improvements of medical devices.

Marketing Strategy

The target customers of our biosensor mainly involve five sections: infants, infirmaries, old people's homes, distress areas, and people with the need of self-testing.

Our marketing strategy has a multi-dimensional customer positioning. There will be enterprise customers like agents (e.g.: manufacturing enterprises and agents of medical devices) and medical institutions (e.g.: medical institution centers and medical Research and Development (R&D) Platform); and there will also be customers on the individual or governmental level, including the sepsis patient community which creates new retail demands and the government users who are responsible for providing large amounts of data of public health surveys for governmental policies.

The diverse customer positioning would, therefore, bring profits and income from four directions, including the SEM/Bidding-flow revenue, the right of representation for agents, the patent technology authorization, and the technical guidance or solutions that we can provide.

Promotion Strategy

The promotion strategy is closely related to the feature of "adaptability" of our biosensor. During the process of R&D, our team has adopted adaptable sensor assemblies, allowing our biosensor to be adopted to other inflammatory research fields. Therefore, the core concept of our promotion strategy is to establish a "TruSense Ecosystem" in the field of healthcare. The blueprint goes as follows:

During the first phase of development, we plan to bring the "TruSensor" biosensor into reality, and will then contact with medical institution platforms for internal tests in order to update the core components of our first-generation products.

In the second phase, we will expand the online and offline market at the same time. We plan to introduce our product to the WeChat mall applet and increase the number of users by 35% in three years. We will also connect with e-commerce platforms in the hope of seeing a stable increase in both the monthly sales and the repurchase rate. Meanwhile, we plan to rope in

offline customers, mainly agents and medical institutions, to focus on the industrial process and work for breakthrough. We are planning to raise the threshold of agents for better resources and larger markets, and establish an offline supply channel for a larger scale and greater influence in the Jiangsu-Zhejiang-Shanghai District (where our team is in).

In the third phase, we are planning to establish a Medical Assistance Sharing Platform in five years. We are going to create a data-sharing chain in the field of healthcare. When relevant data becomes available on the platform, it will, hopefully, be able to guide the analysis of health conditions for patients; updated medical cases will also refresh the database. Furthermore, apart from the offline blood drug testing, there will also be an online auxiliary monitoring system on the platform which provides a more reliable result when working together with the offline tests, thus expanding a larger market both online and offline.

Financial Viability

In terms of financial support, we estimate the first round of financing at $200,000, which will be used for sales, promotions, product development and management. We priced the sensor and test chip at $59 and $2, respectively, based on estimated production costs for the sensor ($20 each) and chip ($1 each). According to the number of inflammatory patients and the market share of POCT in China, our target population can reach 19.8 million. We forecast market coverage to reach 0.5%, 0.6%, 0.9%, 1.2% and 1.5% in the next 5 years, respectively. We are confident that we will achieve a projected net profit of $820.98 million in the fifth year.

	year	2022	2023	2024	2025	2026
revenues	Sensor		584100	1051380	1401840	1752300
	Test strip		99000	2772000	5148000	8119000
	Financing	200000	0	0	0	0
Costs	COGs	0	202950	370260	554400	726660
	G&A	21100	71600	127446	218328	329043
	R&D	473000	68310	382338	654984	987130
	Sales&Marketing	106600	34155	191169	327492	493565
Net profit		-400700	306085	2752167	4794636	7334902

Table. 1. five year cash flow

TEAM AND SUPPORT

Contributions of the Team Members

Supervisor & coach & captains

Prof. Liquan Huang is the team supervisor. He is very responsible and have offered us valuable guidance.

Yusen Wang is the team coach, who offered major experimental supports, especially the skills of construction of MIP, impedimetric sensors. He also provided us with insightful suggestions to build a better biosensing system and learn about the physical basis of biosensing system.

Lei Yan is the captain of TruSense2022. He coordinated the work within the team, designed single-chain antibodies, participated in some biological experiments, and provided software support for the team.

Lou Xiang is the captain of TruSense2022. He is responsible for team organization and coodinating arrangement of events with SensUs.

Translation group

Xinran Zhao played an important role in the business transformation team. She determined the team's business strategy, analyzed stakeholder wishes and financial viability.

Xiaoxiao Meng was mainly responsible for forming partnership, doing market research and making business plans. Positive and passionate about her work, she is someone who can be relied upon.

Yuhe Sun is responsible for planning part of the business potential, translation, and presentation.

Principle group

Bangjie Huang spent much time in the expression and purification of single-chain antibodies and optimizing their production. He also gave suggestions about affinity measurement of scFvs independently designed the experimental process.

Hongli Luo carried out part of the early investigation of interdigital electrode and finished scFv activity test by ELISA.She is in charge of the team's finances and manages the team's funds.

Minqing Yan is meticulous about the lab work. She also discovered and solved p problems in experimental plan.

Haonan Sheng Sheng Haonan proposed the MIP scheme and participated in related electrochemical experiments. He put forward constructive comments at the group meeting many times.

Changfen Xu bridged the principle group and the instrument group, providing Trusense team with creative ideas and lab-work assistance.

Instrument goup

Hengshuai Bao is the main force of the instrument group, and he has made great efforts in the experiment. He sacrificed his summer vacation to provide MIP with a lot of test data, and fumbled to prepare interdigital electrodes linked to single-chain antibodies.

Han Zhang bravely explored the unfamiliar field of electrochemistry, explored the preparation scheme of MIP, and provided a large amount of preliminary data for MIP.

Sponsors

浙江大學
ZHEJIANG UNIVERSITY

FINAL REMARKS

In 2022, TruSense has achieved remarkable progress. We have implemented electrochemical sensors and tried two approaches in molecular recognition mechanisms. Working together with joint effort, our team has vertically integrated the hardware (instrument) and software part with the molecular recognition part. TruSense biosensor uses a self-designed electrochemical workstation and Arduino programming tools, which are cheap and highly accessible. During the year, the MIT has performed well with screen-printing. These improvements have greatly enabled primary technicians like undergraduate team members to quantitively produce reliable devices. The high reproducibility and operability have promoted the translation potential of our biosensor, and the cost of our MVP (Minimal Viable Product) can also be reduced to under €12 and under €1 per test.

We would like to express our sincere gratitude to Yusen Wang, who has offered strong support on the electrochemical workstation and software - we couldn't have invented the biosensor without him. Heartfelt thanks to Professor Fan YANG for sparing no effort on experimental instruments, reagents, and venues. And sincerest gratitude to Professor Yong WANG for his great help on funding and setting principles.

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APPENDIX

China's POCT industry

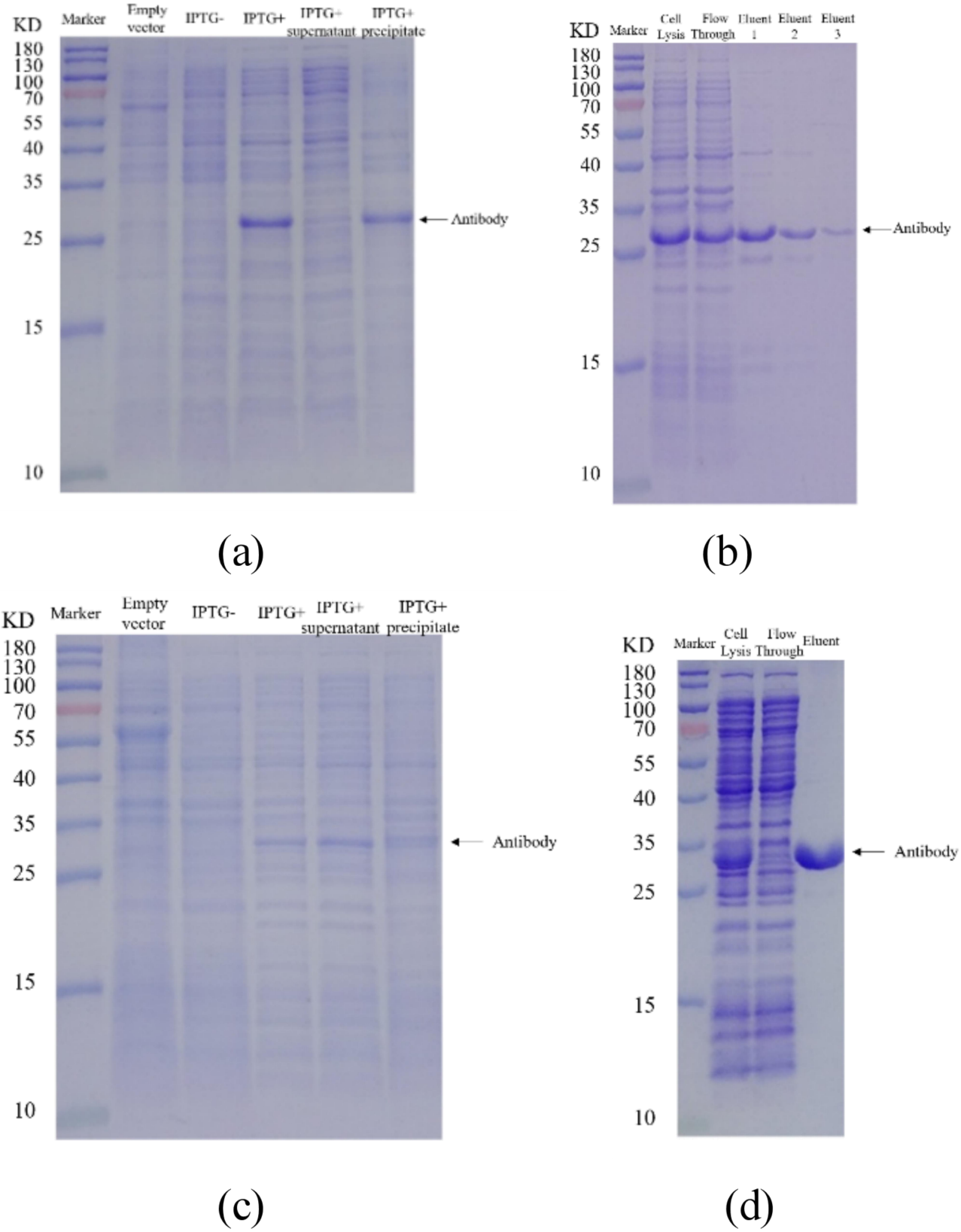

Fig. 9. Expression and purification of single-chain antibody. (a) Expression of TS1. (b) Purification of TS1. (c) Expression of TS2. (d) Purification of TS2.


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