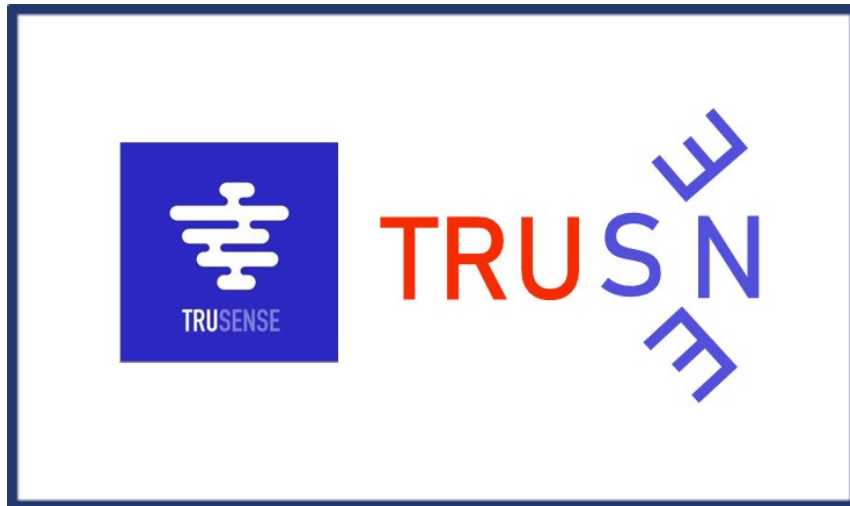


Team Results Document

TruSense

1



University:

Zhejiang University

Team members:

Hongli LUO

Xiaoxiao MENG

Hengshuai BAO

Fengzhou LI

Cheng QUAN

Yuzi WANG

Ci SONG

Yangyang MENG

Geyue YOU

Jinsong WU

Keyan LU

Mingyu SUO

Yuxuan ZHU

Hanyao WANG

Supervisor:

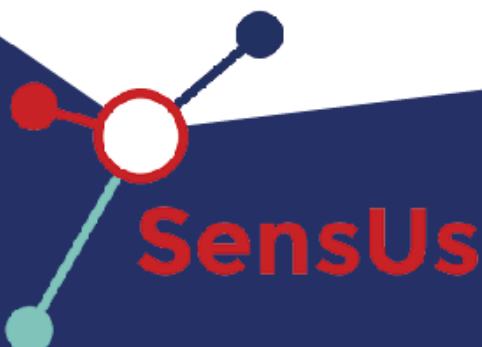
Yong WANG

Coaches:

Lei YAN

Haonan SHENG

12/08/2023



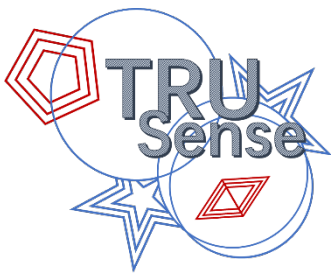


Table of Contents

1 Abstract.....	4
2 Biosensor system and assay	5
2.1 The principle of Localized Surface Plasmon Resonance (LSPR).....	5
2.2 VHH and phage display	5
2.3 Molecular recognition and assay reagents	5
2.4 PDMS microfluidic chip	6
2.5 Backup Plan	6
2.5.1 Gold electrode surface modification	6
2.5.2 Reader instrument and Graphical User Interface (GUI).....	6
3 Technological feasibility.....	7
3.1 antibody preparation	7
3.1.1 phage display screening.....	7
3.1.2 expression and purification of single domain antibody	7
3.2 molecular recognition	7
3.3 microfluidic and support flame	8
*3.4 Backup Plan Result	8
3.4.1 Cyclic Voltammetry (CV) and Electrochemical Impedance Spectroscopy (EIS) results....	8
3.4.2 The standard curve of GFAP concentration	8
4 Originality.....	9
Team captain:.....	9
Supervisor:.....	9
5 Translation potential	10
5.1 Business model canvas	10
5.2 Stakeholder desirability.....	错误!未定义书签。
5.3 Business Feasibility	错误!未定义书签。
5.4 Financial viability	错误!未定义书签。
5.5 A translation prediction model based on Artificial Intelligence.....	错误!未定义书签。
6 Team and support.....	15
Team members	15
People who have given their support	15
7 Final Remarks	16
8 References	16
9 Appendix.....	17

1 Abstract

No one likes to be told they have a traumatic brain injury, but it's even scarier when you're told you may or may not have one. "Well, the MRI showed nothing, so **give it a few years and let's see what happens.**"

The uncertainty of mild traumatic brain injury (TBI) diagnosis motivated our TruSense team to develop a quantitative detection solution. For 8 months, we pursued an innovative combination of localized surface plasmon resonance (**LSPR**) and variable domain of heavy chain antibody fragments (**VHHs**). LSPR enables rapid, accurate optical detection, while VHHs provide customized mass production and robust target affinity. This cross-disciplinary synergy aims to uncover mild TBI biomarkers. Our **premise was pragmatic, cost-effective innovations** for real-world use. We simultaneously optimized the detection method and antibody. Critique from experts helped reorient our focus toward practical applications. This experience has been invaluable, despite challenges, in spurring our growth. We eagerly anticipate sharing our ideas and bringing impactful inspirations to the SensUs platform. Most importantly, we hope our work helps provide diagnostic certainty for those facing the TBI uncertainty.

2 Biosensor system and assay

2.1 The principle of Localized Surface Plasmon Resonance (LSPR)

Localized Surface Plasmon Resonance (LSPR) is an optical phenomenon. When the photons in the vertically incident part are matched with the vibrations of the electron cloud on the surface of the noble metal nanoparticles, the two resonate. The energy of the photon is transferred to the electron, and the macroscopic manifestation is the weakening of the light intensity and the appearance of an absorption peak. In addition, this phenomenon is quite sensitive to the properties of the nanoparticle surface, and when the antibody on the nanoparticle surface binds to the antigen, the wavelength of the light that produces LSPR also changes. Therefore, we can reflect the concentration of the detected substance by detecting the movement of the absorption peak.

Figure 1: AuNP coated with streptavidin and functionalized with sdAb

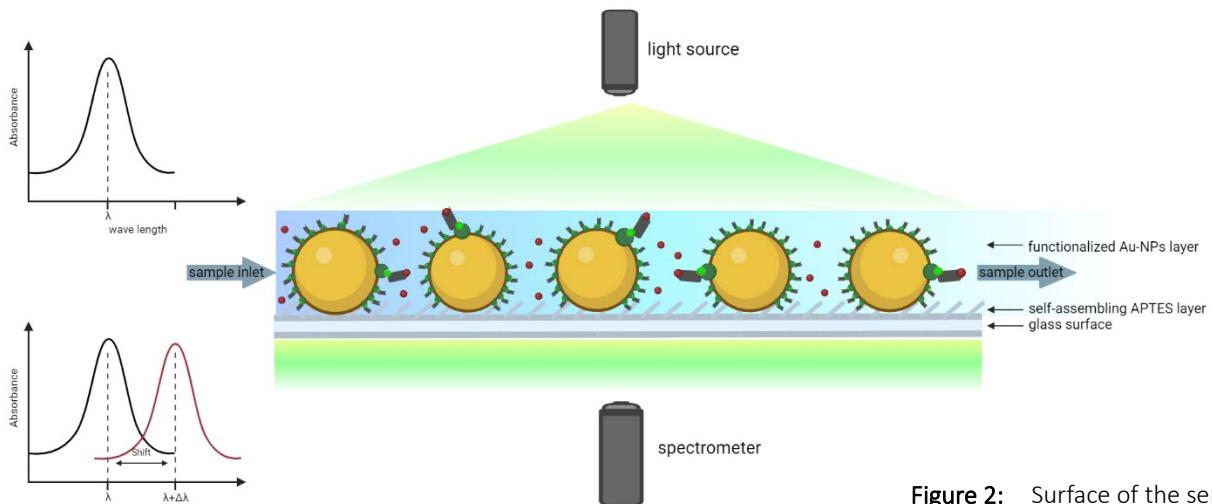
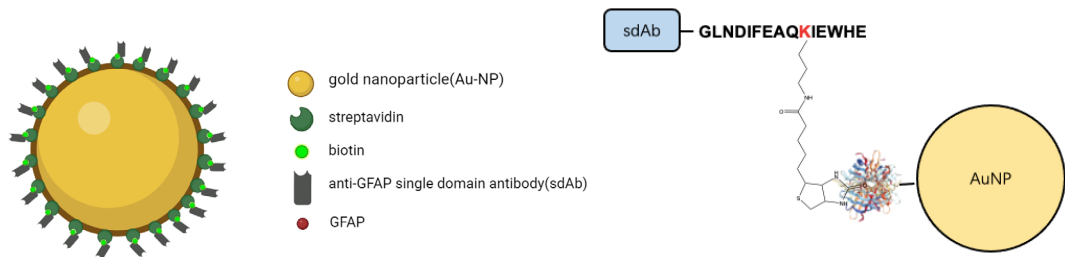


Figure 2: Surface of the sensor

2.2 VHH and phage display

Unlike conventional antibodies, about 50% of antibodies in Camelidae lack light chains, yet retain comparable affinity. These heavy-chain only antibodies contain antigen-binding variable regions called VHH nanobodies. VHHs offer several advantages including efficient microbial expression, small size (14-17kDa), and high solubility.

Phage display is a common technique to produce novel small antibodies. It utilizes phage displaying different antibodies on their coats to testing binding affinity. After multiple rounds of enrichment, the highest affinity antibodies dominate the phage pool. In our experimental design, phage display enables rapid optimization by mixing and modifying antibody fragments from various sources, allowing us to rapidly identify the variant with strongest binding affinity and easiest large-scale expression.

2.3 Molecular recognition and assay reagents

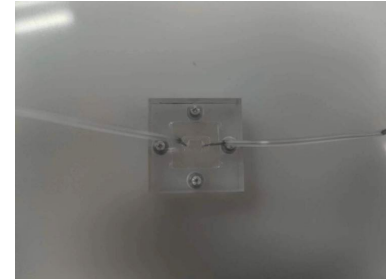
We obtained single domain antibodies (B7) with Avi-tags through prokaryotic induced expression and later biotinylated them. As illustrated in Figure 1, streptavidin-coated gold nanoparticles (AuNPs) were then functionalized with the biotinylated single domain antibodies through biotin-streptavidin interaction.

As shown in Figure 2, the glass surface of the sensor was first activated with ammonia and hydrogen peroxide to generate hydroxyl groups. These were then converted to amino groups by reaction with APTES, giving the surface a positive charge for immobilization of negatively charged AuNPs.

To enable specific detection of GFAP without interference from other proteins in patient blood plasma, the sensor was pre-treated with BSA before each test.

2.4 PDMS microfluidic chip

To enable uniform and sufficient sample flow across the sensor chip, we designed a custom microfluidic channel shown in Figure 3 and S1. Polydimethylsiloxane (PDMS) was selected as the channel material due to its ease of fabrication, flexibility, and durability. Additionally, we designed a fixture to securely seal the channels and prevent leakage, thus protecting the gold nanoparticle layer on the ITO glass sheets.



2.5 Backup Plan

In addition to the optical detection of GFAP, we also developed electrochemical methods as an alternative approach. We modified screen-printed gold electrodes to improve detection performance, while using computer programming methods to automate the detection and data processing procedures to quickly obtain results.

2.5.1 Gold electrode surface modification

The electrode modification mainly focuses on two aspects: the combination of GFAP antibodies and blocking non-specific binding. We used TCEP to reduce disulfide bonds within the antibodies[1], allowing the exposed thiol groups to bind to the gold surface via Au-S bonds. Similarly, we modified the screen-printed electrode surface with cysteine (Cys) groups[2]. The zwitterionic cysteine utilizes its hydrophilic property to prevent non-specific binding of molecules other than the target.

We prepared a 0.01M cysteine and 0.1mM TCEP solution and incubated the electrode surface in this solution for 2 hours. We then washed the electrode with PBS solution to remove any residual solution. To characterize the electrode surface, we dropped 100 μ L of a mixed 0.1mM potassium ferrocyanide/ferricyanide electrolyte solution onto the electrode. We used cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) method[2][3] to characterize the electrode surface before and after modification. Additionally, we incubated 50ul of 50ug/ml BSA solution on the electrode for 1 hour, then washed it off, and characterized the electrode again using cyclic voltammetry with the ferro/ferricyanide electrolyte.

2.5.2 Reader instrument and Graphical User Interface (GUI)

We utilized a circuit development board to interface the screen-printed electrode with a PC. Electrochemical impedance spectroscopy (EIS) was implemented for electrochemical data measurement and processing. We integrated the reading of electrochemical workstation data and the final fitting calculation of charge transfer resistance using programming methods, thereby achieving the acquisition of antibody concentration information in only one automatic step. An overview of the instrument is shown in **Figure 4**.

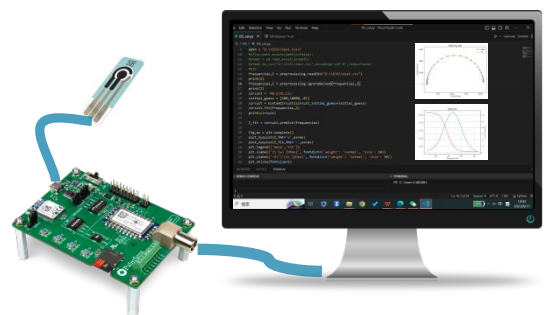


Figure 4: Composition of the electrochemical instrument

3 Technological feasibility

3.1 Antibody preparation

3.1.1 Phage display screening

We construct GFAP-VHH phage display-expression system based on pCANTAB 5E plasmid and camelid heavy chain antibody variable region (VHH) sequence, and use phage to screen high-affinity antibodies (S2 A).

Guided by previous research[4][5][6], we designed and modified eight candidate VHH sequences from camel heavy chain antibodies. We ligated these sequences into the pCANTAB 5B vector via homologous recombination, then transformed the resulting pCANTAB 5B-VHH-E plasmid into the TG1 strain for amplification. We performed three rounds of phage display panning in conjunction with M13KO7 helper phage infection of the bacteria. Panning results showed enrichment of the E9 and B7 phages after round three. Phage ELISA confirmed a gradual increase in antibody affinity over the three panning rounds (Figure 5). SDS-PAGE revealed a ~19kDa band potentially containing the VHH target fragment (S2 B).

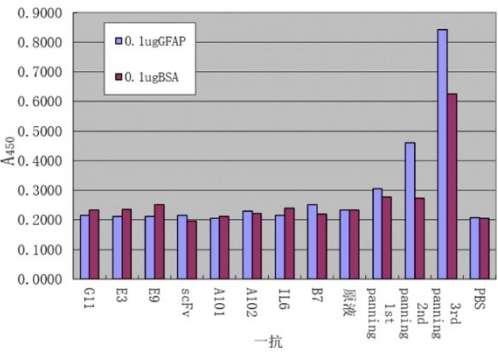
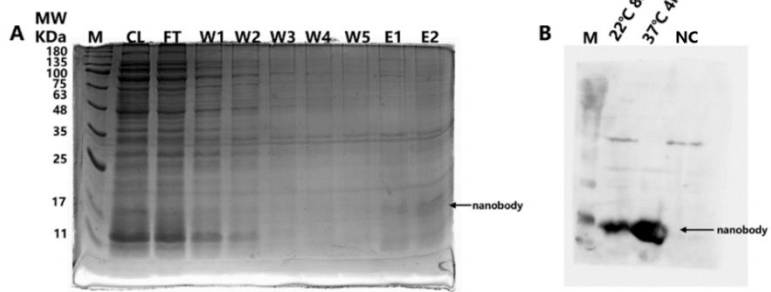


Figure 5: phage ELISA results, PBS refers to phosphate buffer solution with pH=7.4, used for negative control

3.1.2 Expression and purification of single domain antibody

Using the Gibson cloning method, we seamlessly cloned the screened B7 fragments into the pET-His-MCS-Avi vector for expression in shuffle T7 cells. The 10×His-tag on our VHHs enabled purification via Ni-NTA, yielding ~17kDa B7 nanobody fragments (Figure 6 A). The results of western blot showed that the VHH nanobodies induced with IPTG at 22°C for 8h and 37°C for 4h had superior GFAP affinity (Figure 6 B).

Figure 6: A:His-tag VHHs purification effect. M:marker CL: cell lysate FT: flow through W:wash E:elution, B:WB result, M:marker, 22°C8h or 37°C4h: IPTG induction condition, NC: negative control without IPTG induction, .Primary antibody: GFAP, Secondary antibody: anti-GFAP(Mouse), Tertiary antibody: anti-Mouse, HRP.



3.2 molecular recognition

We recombined B7 gene with the pET-His-MCS-Avi vector to express a fusion protein of VHH and Avi peptide. We used western blot to detect the fusion protein's ability to bind biotin (S1), which showed successful binding. To verify that after biotinylation, the fusion protein still specifically binds to GFAP, we performed a sandwich ELISA. The results demonstrated that the fusion protein exhibited stable affinity.

We prepared high concentration AuNPs solution and obtained an absorption peak roughly around 522nm, indicating a probable particle size of ~50nm (Figure. 8A). We verified the hydroxyl activation and APTES binding of the ITO glass surface treatment. Figure 8C shows we observed that the water contact angle of the glass surface after hydroxyl activation is significantly smaller than that of the original ITO glass, indicating that the hydrophilicity of the glass is significantly enhanced after activation. We then measured UV spectra bound to glass sheets of gold nanoparticles, as shown in Figure 8B.

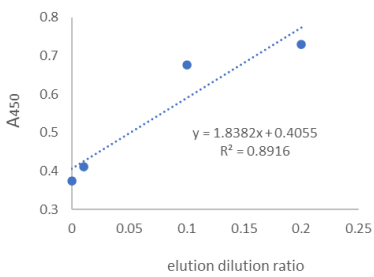


Figure 7: Elution(biotin modification) ELISA testing. Blank control use 5%BSA, n=3. Dilute E2 with 5%BSA. Coating protein: E2 100-fold dilution, E2 10-fold dilution, E2 5-fold dilution. Primary antibody: recombinant GFAP. Secondary ab: anti-GFAP antibody(Mouse). Tertiary antibody: anti-Mouse (HRP, Goat).

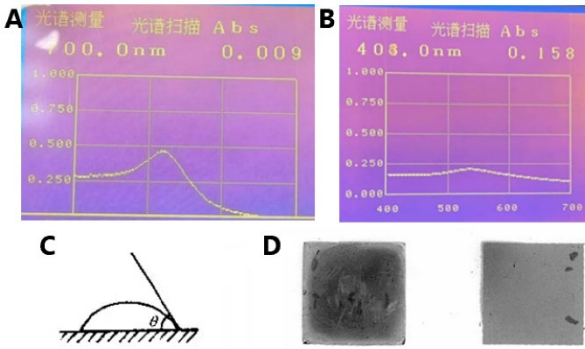


Figure 8: A. The UV spectroscopy of AuNP solution. B. UV spectra bound to glass sheets of gold nanoparticles. C. the contact angle between water and glass. D. Comparison of FITC fluorescence results with or without APTES.

*3.4 Backup Plan Result

3.4.1 Cyclic Voltammetry (CV) and Electrochemical Impedance Spectroscopy (EIS) results

As shown in **F9A**, we characterized three samples to confirm the feasibility of these surface chemical methods. Since the peak current of curve a is significantly smaller than b, it suggests that Cys with considerable ionic activity is bound to the surface of the gold electrode. Meanwhile, due to the high hydrophobicity of GFAP antibodies, the ion exchange rate on the electrode surface is significantly reduced. The peak voltage of curve c is notably greater than that of curve a and b. **F9B** also confirms the effectiveness of the combination between GFAP antibodies, Cys and gold surface. The similar R_{ct} of curve c and d shows valid blocking by the 50 μ g/mL BSA solution for 10min. **F9C** presents the EIS results at different GFAP concentrations, clarifying the R_{ct} increases with higher GFAP concentration.

3.4.2 The standard curve of GFAP concentration

In addition to qualitative analysis, we also aimed to obtain quantitative analytical results. When EIS uses high frequency input, the equivalent circuit can be simplified to:

$$(Z_{Re} - R_{\Omega} - \frac{R_{ct}}{2})^2 + Z_{Im}^2 = (\frac{R_{ct}}{2})^2$$

Based on the above equations, we can obtain a semicircle with $R_{ct}/2$ as the radius(x-axis: Z_{Re} ; y-axis: Z_{Im}). Therefore, after obtaining the frequency and impedance information via the electrochemical workstation, by plotting the real and imaginary parts of the impedance, the radius of the fitted circle in the plot can reflect the charge transfer resistance, which in turn reflects the antibody concentration information. According to above data process, we plot the standard curve(as shown in **Figure 10**).

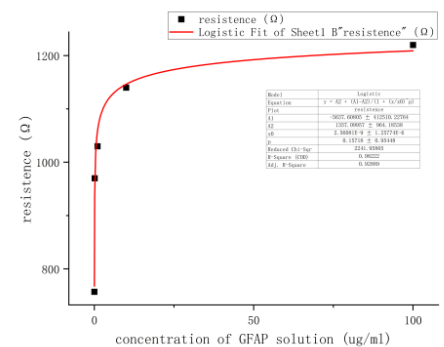


Figure 10: Standard curve for GFAP

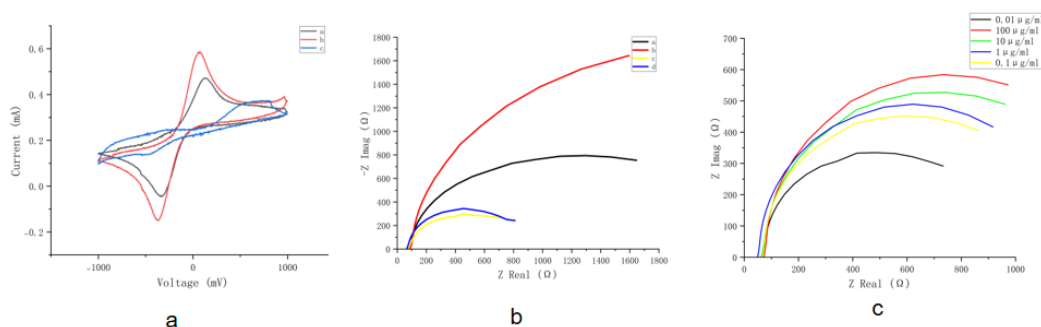


Figure 9: A (a) the CV results of bare gold electrode. (b) the CV results of Cys combined gold electrode. (c) the CV results of GFAP antibodies combined gold electrode. B (a) the EIS results of bare gold electrode. (b) the EIS results of GFAP antibodies combined gold electrode. (c) the EIS results of Cys combined electrode b. (d) the EIS results after immersed electrode c in BSA solution. C EIS results for different GFAP concentrations

We then measured UV spectra bound to glass sheets of gold nanoparticles showed as figure 8B.

3.3 microfluidic and support flame

We customized molds to factories that produce PDMS to obtain our microfluidic channels. The channels were fabricated by mixing 1g of uncured PDMS liquid with a curing agent and allowing it to cure. Testing showed the resulting channel has a volume of 20 μ l and is highly sealed. For now, injection into the channel is performed using a syringe. In order to ensure the stability of the optical path, we 3D printed a detachable three-layer shelf, with dimensions of approximately 8cm x 8cm x 18cm. The shelf was sturdy, allowing transportation over long distances without deformation(S4).

4 Originality

Team captain:

Considering the microgram-level detection limit required for GFAP in the blood of mild TBI patients, we selected nanobodies as molecular recognition elements under the premise that antibodies have the most stable and strongest affinity, to ensure sufficient accuracy. Nanobodies basically have affinity comparable to traditional antibodies, while being easier to modify. We innovatively proposed the concept of using the liquid environment of the detection chip to screen nanobodies through phage display, which guaranteed that we could quickly and cost-effectively screen out the most suitable one to be modified on the chip from over 7 nanobodies.

Through three rounds of phage screening, we obtained two nanobodies, one of which was not the one with the highest reported affinity in existing literature. It is apparently less toxic and easy to mass produce, which is a small new discovery of ours. The structure of the nanobody may enveloped the C-terminus and N-terminus, making testing His-tag and E-tag almost impossible. We went through three months of negative results before realizing in a western blot experiment that cell lysates had a strong affinity for GFAP, making us aware that we had long succeeded, and the AlphaFold2 predicted structure had such a fatal error.

The original method of combining nanobodies with gold nanoparticles was through gold-sulfur binding, but we found that one of the original disulfide bonds of the nanobody was crucial for structure and activity. Therefore, we redesigned the protein, changing to a biotin-avidin connection instead, which greatly facilitated the experiment.

Common LSPR technique determines results by detecting the shift of maximum absorption peaks, which leads to higher costs spent on light sources, as it requires a broad spectrum. Professor Fenny ZHANG inspired us that we could use a monochromatic light source to reduce costs. The micro binding of antibodies and antigens causes significant changes in absorbance at the same wavelength. Even if nonlinear, it helps researchers simply delineate intervals, which aligns with the concept of POCT.

I think I will forever remember this team. We designed proposals, defended for funding, sought teachers or self-studied, contacted generous companies willing to help us

with small batch purchases and after-sales service, and made end-to-end efforts and experiments at every step. Each person played an indispensable role and no one quit.

Supervisor:

As the accomplished team captain described, the students demonstrated exceptional creativity, initiative and problem-solving skills throughout this project. Their decision to use nanobodies as molecular recognition elements was well-reasoned, ensuring sufficient detection accuracy while being easier to modify than traditional antibodies. The ingenious idea to screen nanobodies through an on-chip phage display system showcased their resourcefulness in designing novel, cost-effective experimental approaches.

Through successive screening, the students showed diligence in optimizing the nanobody selection for their assay, even discovering new insights compared to existing literature. Their perseverance through initial negative results exemplifies their scientific persistence to eventually achieve success. Redesigning the nanobody connection to avoid disrupting crucial disulfide bonds also highlights the students' adept experimental troubleshooting and redesign capabilities.

In developing their detection methodology, the students praiseworthy incorporated inspiration from experts while also exercising creativity in pioneering original solutions. As noted by the team captain, the students displayed remarkable initiative and self-direction in managing the project end-to-end, from proposals to funding and execution. Their ownership and work ethic were integral to overcoming challenges.

As their supervisor, I validate their statements on displaying exceptional independence, creativity, and problem-solving skills throughout this project. While my background is in computational biology rather than hands-on web-lab experiments, the students' accomplishments and growth through this experience are undeniable. They should take great pride in pioneering ingenious solutions and self-directing their learning process. I applaud their diligence, teamwork, and brilliant innovation on this challenging undertaking. The skills and initiative they demonstrated equip them tremendously for future scientific endeavors.

Yong Wang

罗端

Hongli LUO

鲍恒帅

Hengshuai BAO

5 Translation potential

5.1 Business model canvas

PROBLEM Mild TBI is hard to be diagnosed. The diagnosis process takes too long which delays timely treatment. Too expensive	SOLUTION Portable biocensors Diagnose the severity of TBI with accuracy Quickly display the final result	UNIQUE VALUE PROPOSITION No more extra charges, but tangible convenience. Shorten diagnosis process to minutes without going to hospital	UNFAIR ADVANTAGE A large scale of market in China A dream team fully equipped with knowledge and a good sense of responsibility	CUSTOMER SEGMENTS People suffered impact on the brain Laboratory physicians Clinics Hospitals R&D centers
EXISTING ALTERNATIVES Go to hospital Suffering from continuous sickness	KEY METRICS Key action: TBI detection Success metrics: create a functional biosensor	HIGH-LEVEL CONCEPT A portable biosensor to determine the severity of TBI within minutes with accuracy	CHANNELS "B2B2C" Government policy support Advertising on social media Word of patient's mouth	EARLY ADOPTERS Local community hospitals TBI patients Neural hospitals
COST STRUCTURE Research & Developing cost Material & manufacturing cost Sales & Marketing cost		REVENUE STREAMS Investment and financing Sales of products Government financial support		

5.2 Stakeholder desirability

Traumatic Brain Injury (TBI) is a significant public health concern in China. The country experiences a considerable number of TBI cases each year due to road accidents, falls, and industrial mishaps. In 2019, the numbers of patients with TBI in China exceed those of most other countries, imposing a huge burden on society and families. The number of patients has rose continuously in the past two decades. The population-based mortality rate for TBI in China is estimated at approximately 13 cases per 100,000 people [7]. In this case, quick real-time biosensors assisted with the detection of TBI are in urgent need. Our product can perfectly fill the gap in China's biosensor market and promote social welfare.

Despite the pressing need for improved TBI diagnostics and monitoring, our Customer Journey Map (Appendix 9.1) shows the current healthcare landscape in China faces several challenges: delayed diagnosis, tedious and expensive testing processes, resource constraints, etc.

To find a more accurate method to detect TBI's biomarker GFAP, we adopted optical methods which helps us reach great accuracy within minutes. As an innovative TBI biosensor, Trusensor is designed to address the pain points in TBI management by providing real-time monitoring, data-driven insights, and improved patient outcomes. The successful adoption of TruSense has the potential to make a substantial positive impact on TBI care and management throughout China.

5.2.1 Market Support

The market for traumatic brain injury (TBI) biosensors in China is bolstered by robust policy support from the government. National government health expenditure has been rising since 1991, from 20 billion to 2.07 trillion, and peaked in 2020, with a total expenditure of 2.19 trillion. In January 2022, the

"14th Five-Year Plan" explicitly set forth to accelerate the construction of the metrological service system in the medical and health field.

Besides the strong market support, the TBI biosensors market in China is also facing tremendous demand. Increasing public awareness about TBI and its long-term consequences has led to a higher demand for improved diagnostic and monitoring solutions. Patients and their families seek reliable devices that can aid in early detection and proactive management. In addition, China's aging population is more susceptible to falls and accidents, leading to a higher incidence of TBI. As the elderly population increases, so does the need for efficient TBI diagnosis and management tools.

5.2.2 Value Proposition

In the context of traumatic brain injury (TBI), our biosensor technology brings immense benefits to patients and communities alike. Swift and accurate testing results provide patients with prompt insights into their condition, facilitating early interventions and enhancing the potential for a successful recovery (Appendix 9.2).

The versatility of our biosensor extends beyond TBI applications. Researchers will find our technology to be a valuable tool in their quest for advancements in the field of biosensors. By providing a principle and method applicable to detecting various viruses, we encourage flexible and innovative research, opening new avenues of exploration to tackle healthcare challenges beyond TBI.

5.3 Business Feasibility

5.3.1 Support

TruSense is financed by SREP (Student Research and Entrepreneurship Project), a special fund for basic scientific research operations of central universities. Additionally, our team has entered the Zhejiang University business incubator Meta-space, so that we have access to the full range of scientific research and early entrepreneurship resources provided by the School of Innovation & Entrepreneurship of Zhejiang University.

An important part of the project is validation, both in clinical and business models. Clinical trials and internal testing of the product will be completed with Shaw Hospital. To make our business transformation model more scientific and feasible, we have professional marketing directors of life technology companies as guidance and basic-level hospitals for field research.

5.3.2 Marketing Strategies

We have formulated a dual-pronged revenue approach encompassing device sales and subscription services. Drawing insights from extensive discussions with frontline medical practitioners and entrepreneurial experts specializing in biological instrumentation, we have meticulously charted the trajectory of biosensor development over the forthcoming five-year span.

Phase I (Initialization, 2024): In the first year, we will collaborate closely with the Affiliated Hospital of Zhejiang University. This strategic partnership will enable us to offer patients a pre-sales free trial with an enduring commitment to lifelong post-sales maintenance, thereby fortifying our customer base. Also, we intend to glean precise market needs and requirements for further improvement.

Phase II (Development: 2025-2026): Over the ensuing biennium, our focal point will shift toward addressing the exigent requirements of underserved township health stations, constrained by limited resources for expansive diagnostic equipment. Additionally, we will cater to individual patients necessitating remote monitoring within the confines of their homes. To facilitate this, we shall establish a mobile application platform interlinked with the biosensors, logging monitoring data and delivering responsive feedback.

Phase III (Growth, 2027-2028): In the subsequent two-year period, our endeavors will encompass governmental support and potential partnerships. The envisioned utility extends to scenarios such as the portable deployment of our devices in disaster-stricken regions. Moreover, with an extensive repository of monitoring data securely ensconced in the cloud, we aspire to construct an intricate model that serves as a touchstone for both scientific inquiry and clinical diagnoses. This robust foundation ensures a symbiotic relationship with the vanguard scientific community, bolstering our enduring presence in the field.

5.3.3 Strategic Planning

To excel within the TBI point-of-care market, a comprehensive strategy has been crafted, delineated across three progressive stages. These stages form the bedrock of our steadfast dedication to both the advancement of our products and their effective promotion.

First Stage: A strategic partnership with the Affiliated Hospital of Zhejiang University will be the cornerstone of our initial phase. This collaborative venture will facilitate rigorous clinical trials, affording us invaluable insights for the perpetual refinement of product functionalities in alignment with diverse market exigencies.

Second Stage: The strategic fusion of online and offline marketing paradigms will define the contours of our second phase. We intend to meticulously improve products and strategies, guided by market research data. During this juncture, we will try to achieve strategic alliances with medical device manufacturers, concomitantly enabling a gradual reduction in production costs.

Third Stage: The third and culminating stage will be a zealous dedication to product optimization informed by user feedback. A pervasive ethos of technological iteration and evolution shall pervade all facets of production and sales, culminating in the dual growth in economic prosperity and societal value.

In consonance with our multifaceted approach, three principal retail conduits shall serve as the vanguard of our sales strategy: e-commerce platforms, specialized medical equipment stores, and pharmacies. The symbiotic interplay of online and offline sales mechanisms shall facilitate the expansion of our sales trajectory, while our commitment to tailored services shall underpin a superlative customer experience.

5.4 Financial viability

The incidence rate of Traumatic Brain Injury (TBI) is approximately 790 cases per 1 million individuals in China. This projection leads us to a rough estimate of 1,106,000 TBI cases annually in China. Our overarching objective is to supply the testing equipment to all community hospitals and concurrently provide related services to the patients.

China comprises approximately 970,000 community hospitals. Among these, around two-thirds of hospitals within relatively affluent regions represent our potential clientele. Assuming a hypothetical scenario in which we accomplish 90% of our target market penetration within a span of 5 years, while also considering a conservative compound annual growth rate of 70% (a judicious rate for a POCT startup), we can extrapolate the projected tester sales as follows.

YEAR	year 1	year 2	year 3	year 4	year 5
Sales	42355	50826	86404	146887	249708

Next, we delve into the net profit derived from the sale of a singular testing device. Recognizing that the initial production expenses tend to be elevated, resulting in relatively diminished primitive product profitability, we must factor in the evolution of costs as production scales. As an initial cost of approximately \$150 per device, production costs will decline over time, culminating in a net profit ranging

from \$10 to \$20 per unit. This progression translates to a cumulative gross profit increase from \$423,550 in year 1 to \$4,994,160 by year 5 (Appendix 9.3).

Beyond the machinery sales, we anticipate generating revenue from auxiliary services and accompanying consumables. These supplementary services could contribute approximately 10% of the overall net profit. Additionally, we need \$100,000 annually for human resources, accounting for a team of 5 employees.

During the initial triennial period, a substantial proportion of the foundation will be spent on fixed asset investment. It includes a \$300,000 expenditure for production machinery and an additional \$50,000 for rental costs. Considering \$500,000 as the required startup capital and accounting for prevailing interest rates, financial costs amount to \$15,000 annually. It will be directed toward sales, promotional efforts, product development, and streamlined management practices to yield substantial returns. The net profit trajectory from year 1 to year 5 increases from \$300,905 to \$5,328,576 (Appendix 9.4). As for the estimated production cost of \$140 per unit, we have set the selling price of sensors at \$150 each. Through a comprehensive evaluation of the prevalence of TBI patients and the overall population in China, we envisage a targeted net profit of 1.08 million within the initial five-year period. Additionally, we have remarkable cash flow management. (Appendix 9.5)

5.5 A translation prediction model based on Artificial Intelligence

5.5.1 Abstract

Deep learning (DL) represents a cutting-edge pathway in the realm of artificial intelligence (AI). This technique allows for the extraction of inherent patterns and representations from sample data. When novel data is input, the improved model generated through learning empowers the computer to provide desired predictive outcomes. The insights gained during this learning process are valuable for interpreting diverse data types. Hence, our TruSense team will employ deep learning methodologies, meticulously considering the influencing factors of market dynamics and customer behavior on both local and global scales. This approach aims to achieve a more precise and comprehensive prediction of our product's potential.

5.5.2 Datasets Selection

Evaluating the commercial viability of a product involves a complex interplay of factors, spanning political, economic, cultural, legal, regional, and societal dimensions. In the quest to develop a predictive model for the commercial potential of Trusense biosensors, we have compiled datasets encompassing a comprehensive analysis of market dynamics and customer behaviors. (See Appendix 9.6)

5.5.3 Result

Owing to the scarcity of medical and market data about Traumatic Brain Injuries (TBI), we adopted an alternative approach for data collection. We relied on the annual count of articles and patents published on the Web of Science website to gauge relevant trends. The keyword "Biosensor" was chosen to gauge global biosensor development trends, while "Traumatic Brain Injury" was selected to capture a partial reflection of public awareness and demand for biosensing products catering to this ailment.

In addition to multiple input factors, specifying the label is imperative for relevant deep-learning research. To evaluate the market potential of instantaneous TBI brain injury products, we utilized literature search outcomes with the keywords "sensor, brain injury" as the label. With

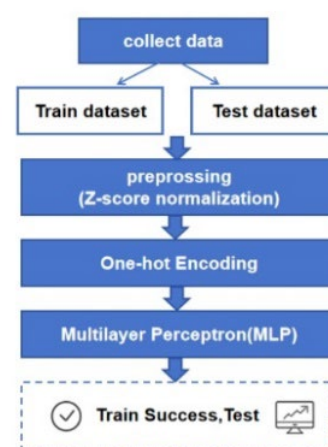


Figure 11: Flowchart for the process

these steps, our data collection phase reached completion. We chose the period from 1985 to 2020 for our research scope.

Subsequently, the collected data underwent preprocessing, involving feature transformation into a standardized range of -1 to 1. To expand feature dimensions, we employed one-hot encoding. The neural network model was configured as a classic Multi-Layer Perceptron (MLP) with three Linear layers. After multiple rounds of training, we honed the model's performance. Using this refined model for predicting the 2021 development potential yielded a value closely aligned with actual data, corroborating the model's strong predictive capabilities (Figure 12).

14

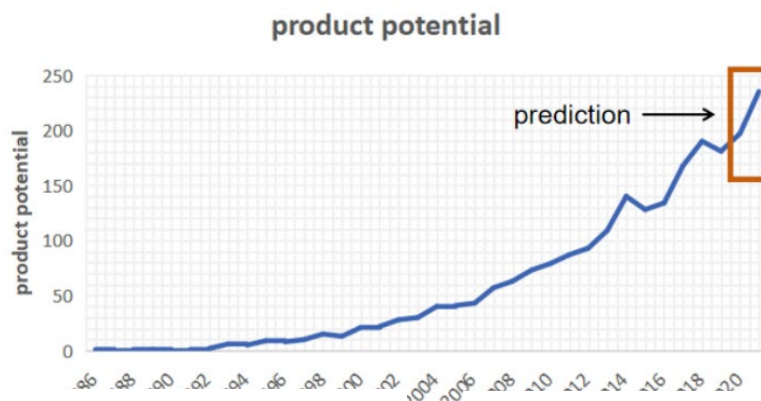


Figure 12: product potential in year 1986-2020, and our prediction for year 2021

6 Team and support

Team members

Hongli LUO is the captain of team, responsible for team schedule making, external communication, as well as leading the biopart in designing, panning and inducing nanobody.	Xiaoxiao MENG assumes the role of co-captain within the team, primarily entrusted with the leadership of the translation potential pitch. She took the lead in developing our business plan and actively engaged in innovation and entrepreneurship competitions. She has meticulously devised a sophisticated model underpinned by artificial intelligence to prognosticate commercial translational prospects.
Hengshuai BAO is the leader of principle and equipment group, co-captain of team, showing great responsibility in proposing, realizing the team's backup plan, the electrochemical solution. He at the same time tried various ways for the optical chip chemical modification	Jinsong WU is part of the equipment group, playing a supporting role in chip fabrication. He spent plenty of time in lab and communicating with companies for the design of fluidic channels and fabrication of instrument structures.
Hanyao WANG is a member of the biological group, he helped with the phage screening and promotes practical methods for purification process of anti-GFAP single domain antibody.	Yuzi WANG is part of the bio group and worked hard on the plasmid design. Her lab work is especially amazing in that she successfully conducted gene experiments despite unexpected difficulties.
Fengzhou Li is one of the members of equipment group, mainly responsible for proposing protocols and related experiments for optical detection, also responsible for communicating with teachers in the optical laboratory. Other than that, his is good in telling stories.	Cheng QUAN is a member of the biological group. He greatly contributed to the phage display, probed building methods of biological affinity molecules and attempted to establish a robust conjunction between our nanobody and detecting elements.
Yuxuan ZHU is a member of business team. He was mainly in charge of the building business model and analyzing financial viability.	Yangyang MENG is a member of business group, responsible for the economic theory in AI modeling and involved in TRD completion, her active mind helps a lot in the intersection of business and research.
Geyue YOU is part of the business group. She contributed to the final result and fueled entrepreneurship sessions. Her impressing pitch won the second price in Tsingshan Entrepreneurship Cup. Team player, innovation enthusiast!	Song CI is part of the the business subunit who helped with the market data collection and responsible for the design and production of PPT.
Mingyu SUO is part of the equipment group, also the core member in AI deep learning modeling program. Also, she did great work in making the product appearance 3D models and wrote codes for the electrochemistry part process.	
Keyan LU is one of the members of equipment group, as well as the team's financial reimbursement and worked a lot in itinerary planning. She, almost individually, applied for and passed a project defense of 40,000 RMB for team.	

People who have given their support

Fenny ZHANG kindly gave us her advice on the cost of different alternative optical methods, and one of her student taught us how to prepare AuNPs.

Fan YANG, Zhijian YAN and Zihang YE friendly provided assays and bio protocols in 413 labs.

Qingjun LIU's student provided suggestions on electronic methods.

Ye ZHAO offered information about how to have a good entrepreneurship pitch into details.

Zekai ZHENG deeply explained the principle of a physics model related to AuNPs to us.

7 Final Remarks

- Thanks to supervisor **Yong WANG**, who supported us without hesitation with funding and administrative affairs in the college. His trust and help allowed the team to have more possibilities.
- Thanks to **Zhejiang University** and the College of Life Sciences at ZJU for supporting the team's external exchange projects. Thanks to **Lab 413** and ZJU's Institute of Innovation and Entrepreneurship for providing us with a beautiful, clean laboratory, allowing the team to gain experience in innovation and entrepreneurship competitions, and giving us the opportunity to obtain funding support through presentations.
- Thanks to **Palmsens**, especially **Ardy** developer. We accidentally broke three chemstations in a row. They generously sponsored the team with a very useful development board at a great price, shipping it to China in just 5 days. Ardy kindly answered our questions in emails and was willing to provide suggestions on repairing the damaged workstations.
- Thanks to the **Shanghai branch of Hytest** for their antibody support. The shipping speed was quite impressive. The goods were well packaged. Interestingly, the enthusiastic and friendly Ms. Ma was in charge of liaising with us again in her second year. And thanks to micronit for the helpful suggestions in the partner session!
- Finally, sincere thank you to **SensUs organization** for the platform that allowed TruSense to continue for another year. The three feedback moments and two entrepreneurship trainings all pushed the team to progress.

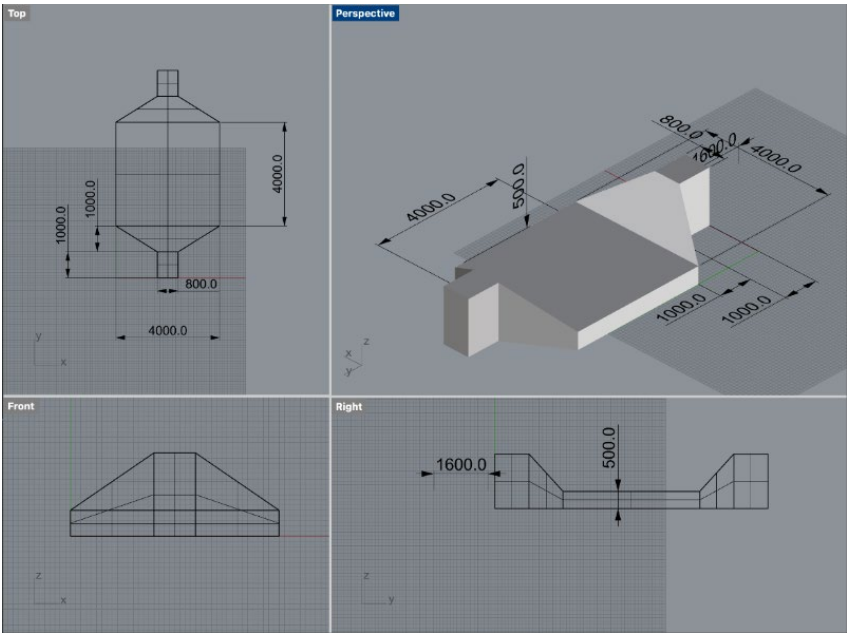
Next year's TruSense will preserve this year's technical solutions and original documents, actively improving them into products, applying for patents in China, and participating in business promotion competitions. We look forward to the moment when the team accumulates enough technology to become a startup team.

8 References

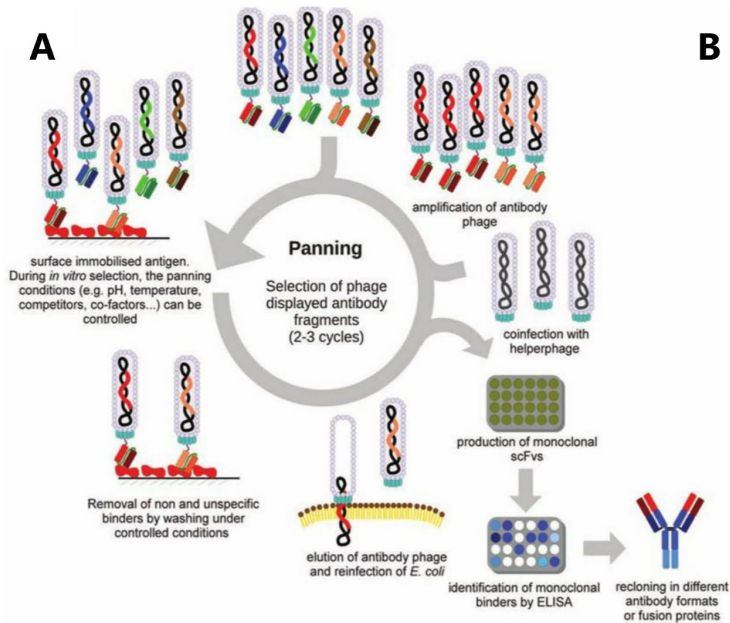
- [1] Sharma H, Mutharasan R. (2013). Half antibody fragments improve biosensor sensitivity without loss of selectivity. *Anal Chem.* Feb 19;85(4):2472-7. <https://pubs.acs.org/doi/10.1021/ac3035426>
- [2] Lixin Cao, Peisheng Yanb, Kening Sun, et al. (2008). Tailor-made gold brush nanoelectrode ensembles modified with l-cysteine for the detection of daunorubicine. *Electrochimica Acta* 53, 8144–8148. <https://doi.org/10.1021/ac3035426>
- [3] Sunil K. Arya, Tze Sian Pui, Chee Chung Wong, et al. (2013). Effects of the Electrode Size and Modification Protocol on a LabelFree Electrochemical Biosensor. *Langmuir* 29: 6770–6777. <https://doi.org/10.1021/la401109r>
- [4] Li, T., Vandesquille, M., Bay, S., Dhenain, M., Delatour, B., & Lafaye, P. (2017). Selection of similar single domain antibodies from two immune VHH libraries obtained from two alpacas by using different selection methods. *Immunology Letters*, 188, 89–95. <https://doi.org/10.1016/j.imlet.2017.07.001>
- [5] Perruchini, C., Pecorari, F., Bourgeois, J.-P., Duyckaerts, C., Rougeon, F., & Lafaye, P. (2009). Llama VHH antibody fragments against GFAP: better diffusion in fixed tissues than classical monoclonal antibodies. *Acta Neuropathologica*, 118(5), 685–695. <https://doi.org/10.1007/s00401-009-0572-6>
- [6] Mesgari-Shadi, A., & Sarrafzadeh, M. H. (2017). Osmotic conditions could promote scFv antibody production in the Escherichia coli HB2151. *BioImpacts: BI*, 7(3), 199–206. <https://doi.org/10.15171/bi.2017.23>
- [7] Ji-Yao Jiang et al. (2019). Traumatic brain injury in China. *Lancet Neurol* 18: 286–95.

9 Appendix

S1 design of the fluidic(3D model)

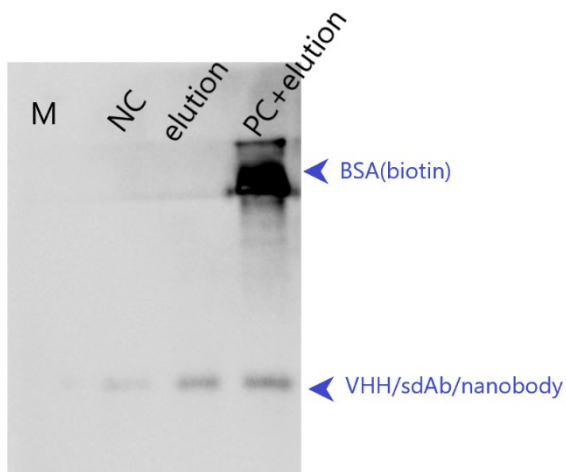


S2 Phage display feasibility



S 2: A.scheme of phage-display screening (Schirrmann et al. Molecules 2011) **B.** SDS results, the red box part shows the nanobody.

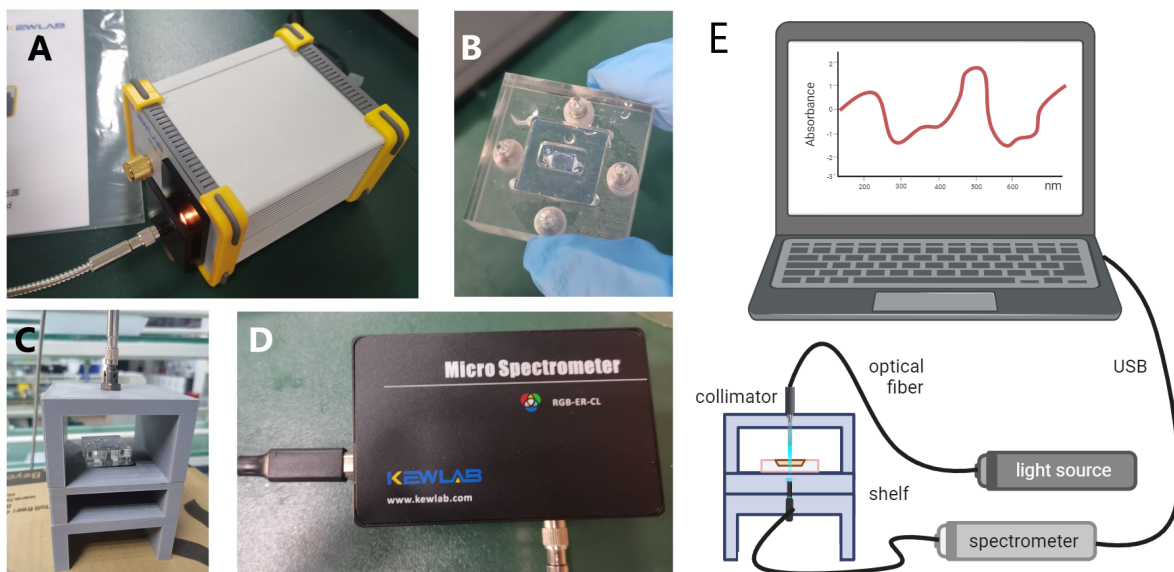
S3 Biotin modification WB results



S3: Biotin modification western blot. M: marker, NC: negative control, elution: E2 in purification modified with biotin, PC: BSA modified with biotin. Primary antibody: streptavidin (HRP).

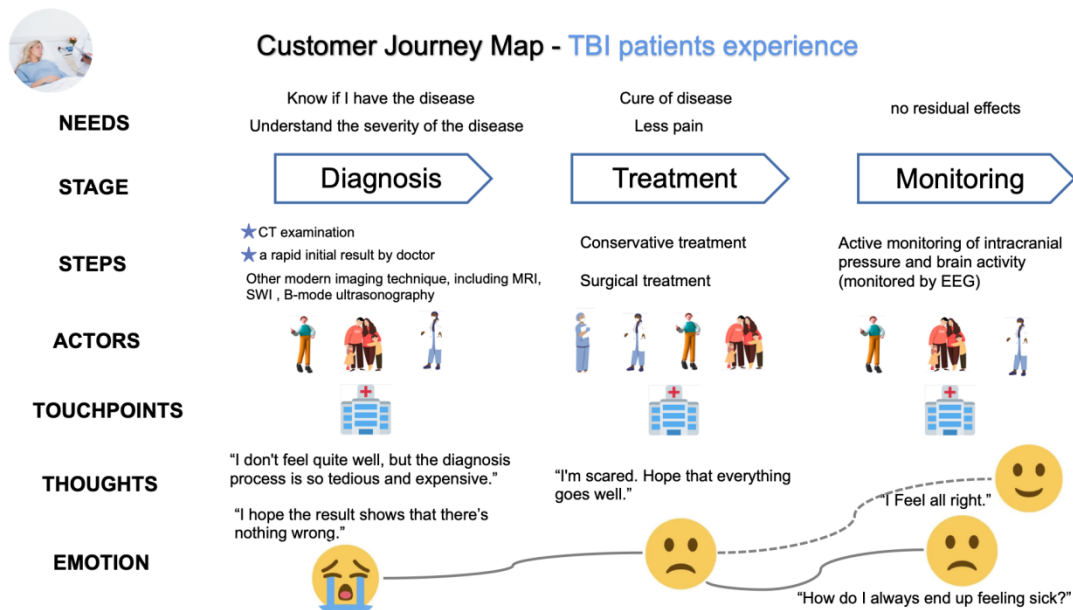
18

S4 The model as well as photo of microfluidic, 3-layer shelf and the whole optical biosensor



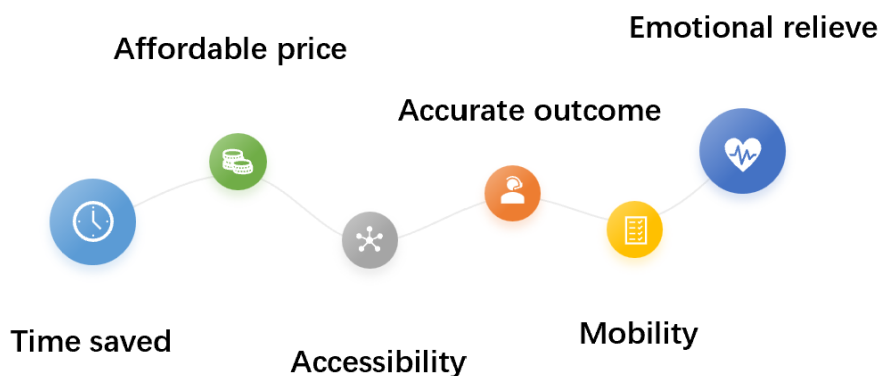
S4: A. light source, B. microfluidic, C. shelf, D. micro spectrometer, E. overview of the whole optical biosensor.

9.1 Customer Journey Map



19

9.2 Value Proposition



9.3 Gross Profits

YEAR	year 1	year 2	year 3	year 4	year 5
selling price	150	150	150	150	150
cost	140	140	130	130	130
Revenue	10	10	20	20	20
Sales	42355	50826	86404	146887	249708
NET PROFIT (per device)	10	10	20	20	20
Total revenues	6353250	7623900	12960600	22033050	37456200
Total direct costs	5929700	7115640	11232520	19095310	32462040

Total gross profits	423550	508260	1728080	2937740	4994160
---------------------	--------	--------	---------	---------	---------

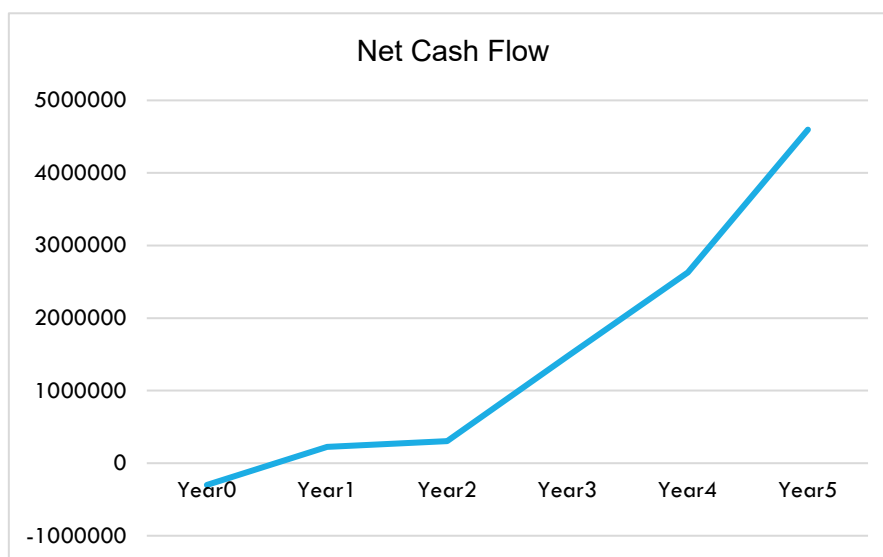
9.4 Net Profit

YEAR	year 1	year 2	year 3	year 4	year 5
Total gross profits	423550	508260	1728080	2937740	4994160
profit from relative service	42355	50826	172808	293774	499416
human resources cost	-100000	-100000	-100000	-100000	-100000
financial cost	-65000	-65000	-65000	-65000	-65000
net profit	300905	394086	1735888	3066514	5328576

20

9.5 Cash Flow

Year	Year0	Year1	Year2	Year3	Year4	Year5
Operating profit	-300000	315905	409086	1750888	3081514	5343576
+ Depreciations	0	60000	60000	60000	60000	60000
- Change in NWCN	0	0	0	0	0	0
Cash Flow from operations	0	375905	469086	1810888	3141514	5403576
- Exceptional income or costs	0		0	0	0	0
- Taxes	0	33267.65	45381.18	219815.44	392796.82	686864.88
Net profit	0	222637.35	303704.82	1471072.56	2628717.18	4596711.12
- Investments	300000	0	0	0	0	0
- Interest on long term debt	0	15000	15000	15000	15000	15000
- Reimbursements	0	0	0	0	0	0
Net Cash Flow	-300000	222637.35	303704.82	1471072.56	2628717.18	4596711.12



9.6 Datasets Selection

The assessment of a product's commercial potential is governed by a myriad of factors encompassing political, economic, cultural, legal, regional, and societal dimensions. To construct a predictive model for Trusense biosensors' commercial potential, we have meticulously curated datasets spanning market dynamics and customer behavior.

Market Dynamics

The market can be divided into two aspects: vertical and horizontal. Vertically, we have gathered market development data from China over the past three decades, including market size, growth trends, competitive analysis, macroeconomic indicators, and household income. Horizontally, we have compared the development of China's biosensor industry with that of various countries around the world. By analyzing market performance and competitors, this approach helps in understanding the market landscape and formulating relevant competitive strategies.

In order to determine the unique advantages of the Trusense sensor, we conducted a comprehensive comparison of the market sizes of biosensors in different countries and different years, along with the quantity of patent documents related to biosensors from various countries worldwide.

Customer Behavior

Through the application of machine learning algorithms, customer behavior data is dissected to comprehend preferences, demands, conversion rates, and more. This process substantiates product and service optimization, consequently elevating commercial conversion potential. In this context, we selectively utilize investigation data from China's internet medical sector, encompassing Chinese medical Q&A, patient clinical data, and the ODC-TBI public dataset, thereby furnishing a comprehensive portrait of operational dynamics and consumption behavior.

Finally, the quantity of research papers related to biosensors published in China is adopted as the outcome. The training and testing datasets are randomly allocated.