Team Results Document
SECRETUM

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SensUs

SensUs 2024
Acute Kidney Injury

1. Abstract (max. 200 words)

Our team, SECRETUM, would like to introduce the rather novel creatinine sensing and monitoring method employing deoxyribonucleic acid (DNA) aptamers as a sensor at a molecular level. Aptamer-based biosensors usually require the consumption of a predetermined DNA molecule amount for each measurement. Here, we demonstrate that an aptamer-based biosensor can potentially be reused if both the aptamer and its semi-complementary DNA are immobilized on the electrode surface. We have theoretically substantiated how our developed method can be used to create an aptamer-based biosensor for a wide range of different small molecules.

The medical technology market offers significant growth potential, especially in diagnostics. Our solution meets the growing demand for continuous and accurate creatinine level monitoring, could potentially in part solve the healthcare problem of a paramount significance, which is the growth in renal disease rates. Market expansion supports our business case.

2. Biosensor (max. 2 A4)

1) Molecular recognition.

Recognition and specific binding of the creatinine to the electrode surface are performed using DNA aptamers. These aptamers, 30 to 60 single-stranded DNA molecules, bind to the analyte via hydrogen bonds and form hairpins. The aptamers are immobilized on the surface of a gold electrode. The sequence of the aptamer was derived by introducing single-nucleotide mutations into a commercially known creatinine-binding aptamer obtained through SELEX procedure [1]. The thermodynamic characteristics of the mutant aptamer were modeled using NuPack, the dynamics of its binding to creatinine using GROMACS, and the calculation of binding energy using AutoDock Vina.

The necessity of introducing mutations into creatinine-DNA-binding aptamers arises because commercial aptamers have high thermodynamic stability and, accordingly, do not change their conformation upon binding to the analyte molecule. Consequently, the registration of binding requires the introduction of additional molecules into the system, which will compete with the analyte for binding to the aptamer and the registration of the signal will be made by their concentration. Hereinafter, for simplicity, we will refer to the molecule that competes with the analyte for binding to the aptamer as "antimer" to symbolize that usually the competing molecule is ssDNA partially complementary to some region of the aptamer (figure 1 (a)) [2]. However, this approach makes it impossible to reuse the biosensor for commercial purposes, because it requires changing of the antimer after every measurement, thus increasing the cost of the final product.

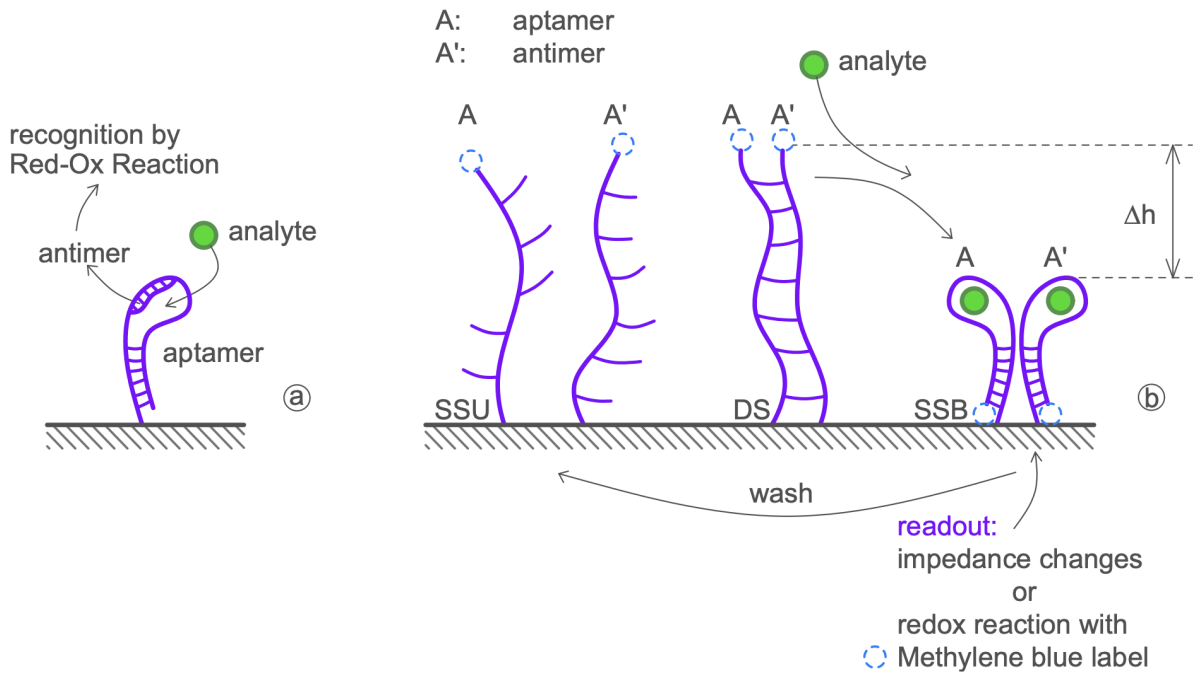

Fig. 1 - Comparison of the Classical and Our Approach to Using Aptamers

a) The classic scheme involves DNA aptamers where the analyte competes with the anti-aptamer, leading to the chemical degradation of the latter.

b) In the alternative scheme, changes in the dielectric layer thickness are recorded, and both DNA molecules remain on the electrode.

We formulated a hypothesis that instead of dissolving the antimer and discarding it after each measurement, we could use it in an immobilized form. This approach would significantly reduce the costs of consumables and the labor intensity required for maintaining the biosensor. To implement this, the following condition must be met: $\Delta Gssu > \Delta Gds > \Delta Gssb$, where ΔG denotes the total Gibbs energy of system state, and the indices SSU, DS, SSB represent the Single-Strand Unbinded, Double - Strand, and Single - Strand Binded states respectively (figure 1 (b)).

It is also hypothetically possible to add a label, such as methylene blue, to the opposite SH group end of the aptamer and antimer. This molecule would undergo a redox reaction when the aptamer bends, thereby changing the potential of the electrode. This approach is likely to have greater sensitivity than the impedance spectrum measurement scheme, but testing this hypothesis will be the subject of further research by our working group.

According to the criterion for spontaneous chemical reactions, if the condition outlined above is met, the system behaves as follows: If the concentration of the analyte in the medium above the electrode is zero, then regardless of the initial conformation, all aptamer molecules will tend to bind to the nearest immobilized antimer molecule and form a duplex, i.e., a DS state. When an analyte molecule comes into proximity, binding to it becomes energetically more favorable than remaining in the duplex state. Consequently, both aptamer and antimer molecules will dissociate and form hairpins that encapsulate the creatinine, transitioning to the SSB state. After the analyte is washed away from the electrode surface, for instance, by a stream of water, the molecules will return through the unstable SSU state back to the duplexes. This approach allows for the detection of analyte presence in the solution through conformational changes of the molecules, rather than changes in the concentration of the antimer.

However, for implementation of such a system it is necessary to select two DNA chains: aptamer and antimer, so that both of them are capable of specific binding to creatinine molecule and to each other, and the energies of these bonds should meet the task set above.

To select such sequences, a commercial aptamer sequence was taken, and as an antimer - a sequence that coincides with it in the region that binds to the analyte and is complementary to it outside this region. These sequences were taken as a source, and using GROMAX and AutoDock Vina, their binding energy with the creatinine molecule was calculated. After that, a library of all possible sequences that can be obtained from them by introducing single-nucleotide mutation in the chain regions that do not affect specific binding to the analyte was compiled, and for each aptamer-antimer pair, the energy of duplex formation and two monomers were calculated using the NuPack package. The pair of sequences, which energy met the balance requirement best was used to experimentally test the hypothesis.

2) Physical transduction.

As shown above, the new method of using aptamers assumes that analyte binding to DNA will lead to conformational changes in the molecule, “bending in half”. Since DNA immobilized on the

gold surface is essentially a dielectric layer between the metal and the ions of the buffer solution, it can be considered as the Stern electric double layer model. This model shows that applied electric potential depends on the thickness of the dielectric layer. Thus, analyte binding to DNA is detected by a peak shift in the impedance-frequency spectrum of the system.

The cartridge is: a gold working electrode, a platinum counter electrode, a working volume into which the analyte and buffer are injected, and leads.

The reader consists of a device for measuring the impedance spectrum, which transmits data to a computer. For users of this device, the spectrogram data is converted into a graph of creatinine concentration in the working container versus time using a pre-calculated calibration curve. According to telemetry data, the graph indicates when the next dose of analyte was introduced into the system.

3. Technological feasibility (max. 2 A4)

1) The calculation of thermodynamic characteristics of DNA oligomers was performed using NuPack software. Conformational changes of two oligomers forming a duplex, also calculation of the energy of binding site between the creatinine molecule and DNA oligomers were performed using AutoDock Vina software.

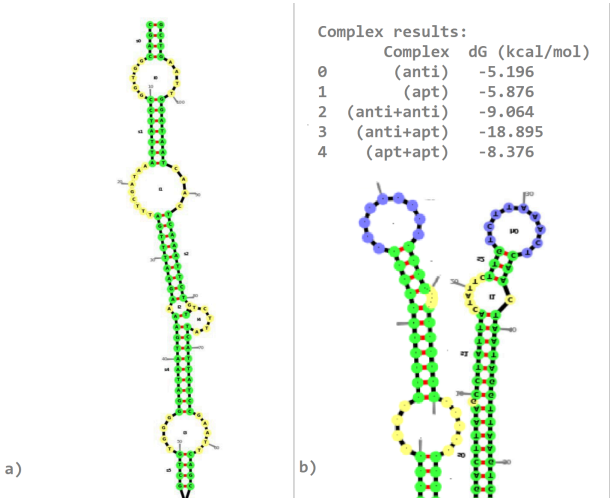

Fig. 2 - Secondary structures calculated using the NuPack package: (a) Dimer (DS) aptamer + antimer, (b) SSU state aptamer + antimer without creatinine.

A table of calculated Gibbs energies for each state is also provided. The probability of transition between states in the quasi-equilibrium approximation can be estimated using the Arrhenius equation and characteristic times of the molecule in each of the states:

$$\frac{1}{\tau} = k \sim e^{\frac{\Delta G}{RT}} \Rightarrow Prob.(SSU \rightarrow DS) \sim e^{\frac{\Delta G_{ssu} - \Delta G_{ds}}{RT}}$$

The probability of transition between states DS -> SSB is calculated similarly, but taking into account the additional Gibbs energy that arises during the formation of hydrogen bonds with the polar residues of the creatinine molecule.

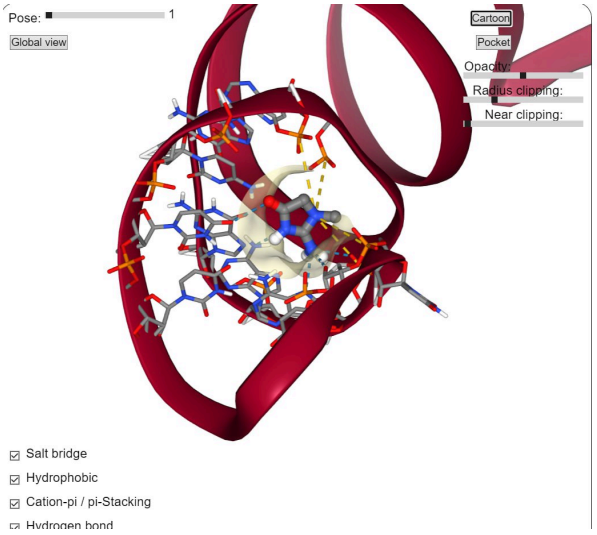

Fig. 3 - The structure of hydrogen bonds between the creatinine molecule and the binding head of DNA oligonucleotides, both aptamer and antimer, calculated using Auto Dock.

Since the predicted bond energy between the oligonucleotide and creatine is 6.2 kcal/mol, the energy balance condition is satisfied: in the absence of creatinine in the medium, the DS state is energetically more favorable than the SSU state by 9.9 kcal/mol, while in the presence of creatine, the SSB state is more favorable than DS by 5.1 kcal/mol. The sequence shown in this illustration was the most successful, and then the synthesis of oligonucleotides was ordered according to the sequence to test this method.

2) According to physical estimates the diffusion coefficient of the creatinine molecule in water is estimated using the Einstein-Smoluchowski relation at 3.5e-10 m^2\s, and the time required for the diffusion of the creatinine molecule from the center of the cuvette to the electrode is about 3.2 seconds, which is the limiting stage of the system's reaction for creatinine to appear in the medium. About 10e10 molecules of the aptamer-antimer duplex can be immobilized on the surface of an electrode with a diameter of 4 mm, accordingly the upper limit of the working range of the electrode is about 1000 ng\ml with a working volume of 200 µl.

   As shown above, the behavior of the electrode and the double electrical layer (DES) when creatinine enters the medium is similar to a flat capacitor, in which the distance between the plates has decreased by half (since the DNA molecules separating the electrode from the solution have folded in half). Let us imagine that N molecules are immobilized on the electrode, and creatinine has bound with q*N of them (q < 1). Then the new capacity of the DES will be equal to C0*(1+q), respectively, the capacity of the DES depends on the concentration of creatinine in the medium linearly and can be used for its indirect measurement. At the same time, due to the large number of DNA molecules on the electrode, the accuracy of the measurements and, accordingly, the sensitivity limit of the biosensor are limited not strongly by the electrode but by the accuracy of the device used to measure the capacity of the DES - the impedancemeter.

3) In this work, 5 electrodes with aptamers on the surface were created, and then these electrodes were tested for changes in impedance when creatinine entered the measuring cuvette at a final concentration of 20 ng/ml. The averaged graph of the dependence of the phase shift on frequency is given below:

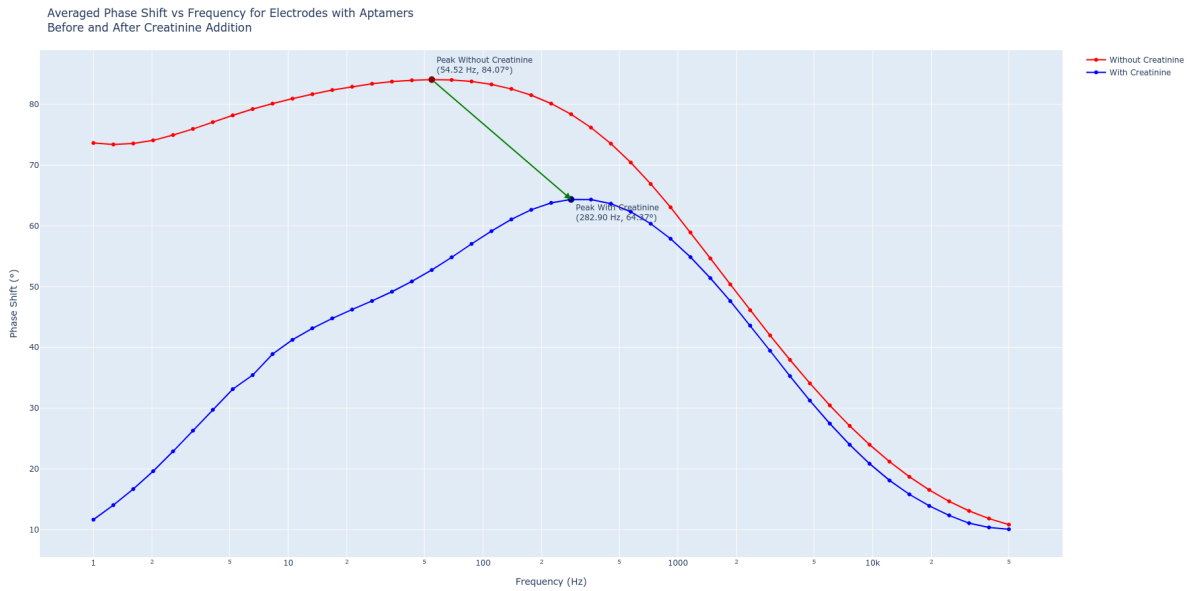

Fig. 4 - Changes in the phase-frequency characteristics of the electrode when creatinine is added to the medium at a final concentration of 20 ng/ml

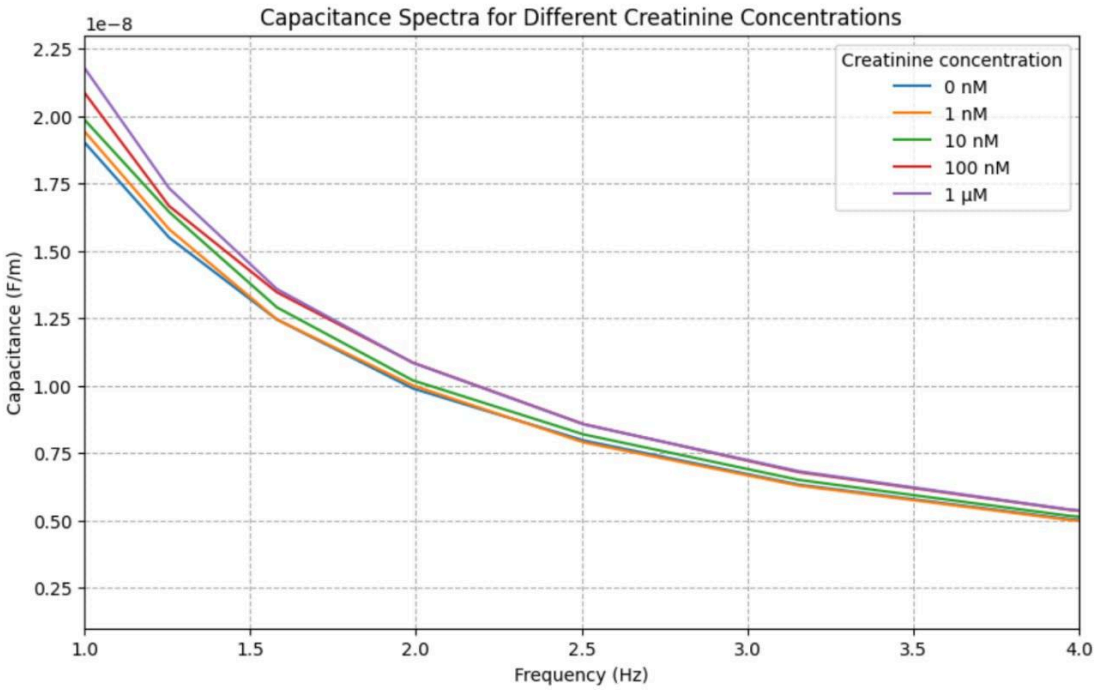

Fig. 5 - Capacitance Spectrum for Different Creatinine Concentrations for Non-Aptamer-Coated Electrodes

First, the behavior of aptamer-coated electrodes cannot be explained solely by the creatinine-gold reaction, as the spectra of coated and uncoated electrodes differ.

Second, the behavior of aptamer-coated electrodes is not exactly as we expected, although there is some trend. Later, we will conduct statistical tests to verify the statistical significance of the obtained results and develop a more comprehensive calibration curve.

Thus, we estimate the deviation of the measurement setup to be approximately E-11 F/m.

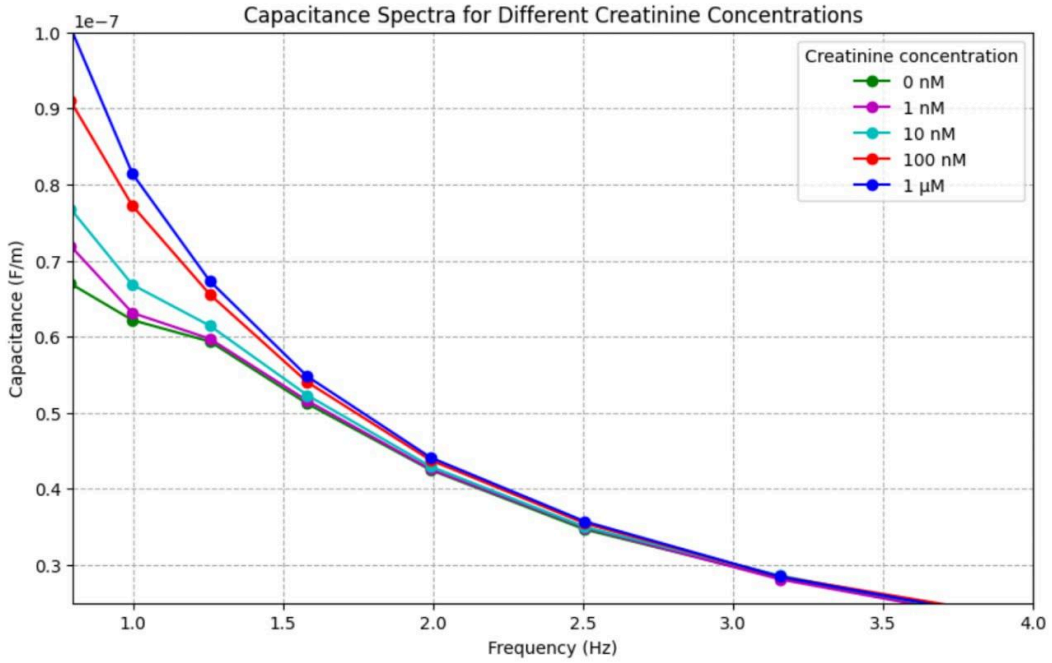

Fig. 6 - Capacitance spectrum for Different Creatinine Concentrations Aptamer-Coated Electrodes

4) Thus, the electrode react to the appearance of creatinine, but since this method is new and aptamers are used in this way for the first time, it is necessary to create not 5, but many more such electrodes, and test them in order to draw a conclusion about their reactivity based on statistical data with a high level of reliability, which is not feasible within the framework of the competition due to time limit. At the time of creating this document, we are conducting multiple experiments to demonstrate this with high statistical significance.

5) Portability and usability for everyday tasks: the design we developed is easily scalable, since the electrode is a 10 by 10 millimeter glass plate that fits inside the capillary. At the moment, the hugest element of the setup is the device for measuring the impedance-frequency spectrum, but its size can also be reduced to a chip with a processor no larger than a wristwatch, for example, by creating the architecture of a meter with automatic conversion of spectrum data into terms of creatinine concentration based on an FPGA board.

   The main advantage of the design is, according to our opinion, its simple maintenance. To wash away creatinine from the surface of the electrode, microscopic volumes of distilled water are sufficient, which can be refilled into the cartridge, for example, once a day. The DNA aptamers themselves degrade (according to calculations) for about two weeks - this is the time for one cartridge to be used.

4. Originality (max. 1 A4)

(1) A piece written by the team:

Our biosensor represents a novel approach to creatinine detection using a customly engineered DNA aptamer. The biosensor implements several unique features that form the core novelty of this product:

1. Customly Engineered DNA Aptamers: A pool of DNA aptamers with targeted mutations to tune their binding activity towards creatinine was engineered. Generally, the commercially available aptamers are under very high thermodynamic stability constraints so that they cannot change conformation after analyte binding. In this regard, single-nucleotide mutations will be introduced to such aptamers to make them amenable to conformational changes, hence making the biosensor more sensitive and adaptable.
2. Antimer Concept: This is where the real innovation in our biosensor comes into play with the utilization of "antimers," which are exogenous molecules that will bind with the aptamer and thus provide competition for the analyte. Monitoring these rates of binding allows for a more specific detection of analytes through changes in conformation rather than concentration alone. The introduction of antimers that are complementary to specific regions of the aptamer and differ outside those regions enhances the specificity and accuracy of the biosensor.
3. Reusability of the biosensor: Our approach, in comparison to traditional methods, where the antimer needs to be replaced every time after measurement, can have an immobilized antimer for reusability. This eliminates the cost and labor associated with the maintenance of biosensors. We have shown that by carefully selecting DNA sequences for both the aptamer and antimer, we can achieve the necessary energy balance to ensure effective and reusable biosensor operation.
4. A Novel Physical Transduction Mechanism: Our biosensor relies on a new physical transduction mechanism, where changes in the thickness of the dielectric layer result in analyte binding. Such changes can be detected using impedance spectroscopy, an electrochemical technique that generates high-resolution data about the presence of the analyte.

The team has been instrumental in the conception of these innovations: designing and testing mutant aptamers, developing the concept of antimer competition, and optimizing the physical transduction mechanism. We utilized advanced modeling tools such as NuPack, GROMACS, and AutoDock Vina to validate our designs and ensure their effectiveness.

Mikhail Vinogradov Anna Smirnova

(2) A piece written independently by the team's supervisor.

I am writing on behalf of the SECRETUM team participating in SensUs2024 competition under my supervision. The team approached me with an innovative concept that is based on two customized DNA aptamers for creatinine monitoring. Since commercially available creatinine aptamers are rigid and only show small conformational changes after analyte binding, the team introduced single-nucleotide mutations to change the properties of the aptamer, making it better suitable for a monitoring aptamer-based creatinine sensor. The concept offers an innovative reusable approach by immobilizing antimers – which can also capture the target molecule – together with the aptamer on the electrode. Simultaneously, this approach reduces the cost, as the complementary DNA remains on the sensor's surface after adding the analyte solution.

Throughout the project, two of my PhD students, Senyao Wang and Sebastian Freko, helped the team conceptualize experiments and supervised their lab work. The initial idea was presented by the students and tailored in several meetings. The students showed a great initiative and motivation in modeling potential aptamer/antimer sequences for their sensor concept and independently conducted their simulations regarding aptamer-antimer binding.

Senyao and Sebastian guided the students through the fabrication of the electrodes, which included the use of standard cleanroom technologies, such as photolithography. The students then successfully functionalized the fabricated electrodes with their self-designed aptamers and antimers. Finally, they characterized the electrodes using impedance spectroscopy and surface plasmon resonance (SPR), demonstrating their ability to apply theoretical knowledge to practical challenges. The team showed great motivation as they followed another strategy supervised by my colleague Prof. Hayden. Due to the late registration the team was limited in time for the final development and testing of their sensor concept.

Kind regards,

Bernhard Wolfrum

5. Translation potential (max. 5 A4)

Understanding the nature of the problem

As part of the competition, our team thoroughly examined the current methods of diagnosing, treating, and monitoring renal failure. To clearly demonstrate each stage of the process, we developed a customer journey model (see Figure 7). Additionally, we delved into the financial aspects of renal failure management.

Expenses Forecast

Months 1-2: Diagnosis and Start of the Treatment

At this stage, both the patient and the insurance company cover the costs associated with diagnosis. Once the diagnosis is confirmed, therapy follows. This phase is primarily funded by the insurance company. Regular monitoring of creatinine levels is conducted.

Months 3-4: Preparation for Transplantation and Prevention of Complications

The patient, insurance company, or medical funds cover the costs for evaluating the need for transplantation, donor search, as well as examinations and tests. Therapy is adjusted, which also requires financing. Special attention is given to preventing complications.

Months 5-6: Operative Preparation and Possible Transplantation

During the preoperative period, both the patient and the insurance company cover the costs of surgeon consultations, examinations. If transplantation proceeds, the expenses for the surgical procedure and hospitalization are also borne by both parties. Conversely, if the surgery does not occur, the patient will continue dialysis treatment, with the costs shared in this case too.

Months 7-8: Postoperative Monitoring and Adaptation

After the surgery, either the patient or their insurance company is responsible for covering the costs of regular doctor visits, immunosuppressants, and tests. Additionally, the patient must also bear the expenses associated with adapting to a new lifestyle. If the transplantation has not occurred, the patient continues to require costly dialysis sessions.

Months 9-12: Long-Term Monitoring and Maintenance

The long-term monitoring and maintenance phase involves ongoing examinations and therapy adjustments, covered by both the patient and their insurance company. For patients remaining on dialysis, regular treatment and state monitoring continue to be paid for in the same way. Additionally, patients may discuss new treatment options with their doctor and consider participating in clinical trials. These activities could incur additional costs.

PATIENT JOURNEY

FRANK male, 57 y.o, 92 kg weight, 175 cm height, Married, two adult children, retired engineer, higher technical education, middle class.
Medical history: Diagnosed with type 2 diabetes 10 years ago, hypertension for the past 15 years, overweight with a BMI of about 30, poor diet, high salt and fatty food intake, low physical activity.
Habits: used to smoke (quit 5 years ago), moderate alcohol consumption.
Family history: father was diagnosed with heart failure, mother - diabetes.
Living conditions: lives in an apartment building in a residential area, access to medical services is within walking distance, wife works, children live separately, but regularly visit their parents.
Emotional state: feels anxious due to deteriorating health, especially when he began to notice swelling and frequent fatigue, feels depressed, but despite his fears, he is determined to follow the recommendations of doctors, as he wants to maintain the quality of life.

Goals: More accessible treatment for the patient, providing emotional and physical support at every stage, and additional measures to improve the patient's condition.

SECRETUM

JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP-DEC
Frank begins to notice symptoms: fatigue, swelling, and changes in urination. He feels anxious and worried, and decides to make an appointment with a general practitioner. He visits the general practitioner, who orders blood and urine tests for him. The test results indicate elevated levels of creatinine and urea. Frank's anxiety increases. The general practitioner suggests using a biosensor for continuous monitoring of creatinine levels, and Frank starts using it. The general practitioner refers him to a nephrologist.	After the consultation, the nephrologist, utilizing data from the biosensor, confirms the diagnosis: chronic kidney disease. The doctor prescribes medication and provides dietary recommendations. Frank begins taking the prescribed medication and changes his lifestyle. Emotions of hope and anxiety constantly alternate. The biosensor allows for monitoring of creatinine levels and assessing the effectiveness of the therapy.	There is progress in the medication treatment. The patient observes this from the biosensor data and confirms his assumptions with expert medical opinions. Frank feels some relief and has also started attending seminars and consultations on diet and self-care. For precautionary reasons, Frank is advised about the need for dialysis. The biosensor continues to monitor creatinine levels, assisting the doctor in making decisions.	Frank attends his first dialysis sessions, feeling fear and anxiety about the procedure. The biosensor helps optimize treatment by monitoring creatinine levels before and after dialysis. Frank is adjusting to the new routine and dealing with side effects.	Monitoring the effectiveness of dialysis. Frank regularly undergoes tests and adjusts his routine. He experiences some fatigue from the entire treatment process. The doctor assesses potential improvements and deteriorations. If dialysis proves ineffective, discussions about the need for a kidney transplant begin. The search for a donor starts, along with preparation for the surgery. The biosensor assists in evaluating the need for transplantation.	Preoperative preparation is underway: donor search, comprehensive examinations, and consultations with the surgeon. Frank is experiencing significant anxiety before the surgery. If a transplant is not needed, the focus shifts to stabilizing his condition. The current treatment regimen is maintained, and medications are adjusted as necessary. Frank consults with a psychologist to help manage his stress. The biosensor continues to monitor his condition, aiding in reducing stress related to the treatment.	Operation (if applicable) and postoperative care. Frank is experiencing fear and hoping for an improvement in his quality of life. Rehabilitation begins, and measures are taken to prevent transplant rejection. The biosensor assists in preventing complications. If a transplant is not performed, dialysis continues, and his condition is monitored.	Postoperative monitoring (if applicable). Frank regularly visits the doctor and monitors his immunosuppressant levels. He develops a strong hope for the successful acceptance of the kidney. He adapts to his new lifestyle, strictly controlling his diet and activities. The biosensor continues to monitor creatinine levels and adjust the treatment.	Long-term monitoring after transplantation (if applicable). Frank periodically undergoes check-ups with his doctor and adjusts his therapy. The biosensor aids in long-term monitoring of his condition. If the kidney transplant was not performed, supportive dialysis continues. Frank constantly adapts to living with a chronic illness. He discusses new treatment methods and examinations with his doctor. The biosensor provides data to support informed decision-making.
First symptoms and initial diagnosis	Confirmation of diagnosis and initiation of treatment	Active treatment and preparation for dialysis	Initiation of dialysis (if required) and continuation of treatment	Monitoring and evaluating progress	Preparation for transplantation or stabilization of the condition	Surgery (transplantation) or continued treatment	Postoperative observation or continuation of dialysis	Long-term monitoring and maintenance

The goal of establishing an accurate diagnosis has been achieved!
The goal of initiating therapy has been achieved!
The goal of continuous monitoring of creatinine levels has been achieved!

The goal of assessing the need for transplantation and finding a donor has been achieved!
The goal of adjusting current treatment has been achieved!
The goal of preventing complications has been achieved!

The goal of adapting to a new lifestyle has been achieved!
The goal of optimizing treatment has been achieved!

The goal of establishing long-term monitoring has been achieved!
The goal of adapting to a chronic illness has been achieved!
The goal of planning for the future has been achieved!
The goal of using the biosensor for long-term monitoring has been achieved!

	1st month (JAN)	2nd month (FEB)	3rd month (MAR)	4th month (APR)	5th month (MAY)	6th month (JUN)	7th month (JUL)	8th month (AUG)	9-12th month (SEP-DEC)
EMOTIONS	Anxiety and worry	A mixture of hope and anxiety	Some relief if treatment is effective, anxiety and uncertainty	Anxiety and fear before the procedure	Some fatigue from treatment	Pre-operative anxiety	Fear and hope for an improved quality of life	Hope for successful kidney engraftment	Gradual adaptation to life with a chronic illness
CONTACT POINTS	Therapist, laboratory	Nephrologist, medical center	Nephrologist, laboratory technician, nutritionist, nurse	Nurse, dialysis center doctor, laboratory, nephrologist	Dialysis center doctor, nephrologist	Surgeon, nephrologist, transplant center, nutritionist, psychologist, support group	Surgeon, medical staff, nephrologist, dialysis center doctor	Nephrologist, surgeon	Nephrologist, surgeon
FINANCIAL ASPECTS	The cost of consultation and tests, paid either by the patient or the insurance company.	Cost of consultation and additional tests, medication costs	Potential training costs	Cost of dialysis, possible compensation from insurance company	Ongoing costs for dialysis and testing	Constant costs for medications and dialysis, costs for a psychologist	Costs of surgery and aftercare	Costs for immunosuppressants and monitoring	Constant costs for medications and examinations

Fig. 7 - Patient Journey

Stakeholders

Stakeholders in the treatment process include medical institutions such as hospitals, clinics, and dialysis centers, which receive payments for their services. Insurance companies cover the majority of medical expenses. Additional costs, such as donor search and participation in clinical trials, may be covered by government and private funds. Following this analysis, efforts were made to understand the patient and their troubles, leading to the proposal of solutions to certain issues. The resulting value proposition canvas is presented on Figure 8.

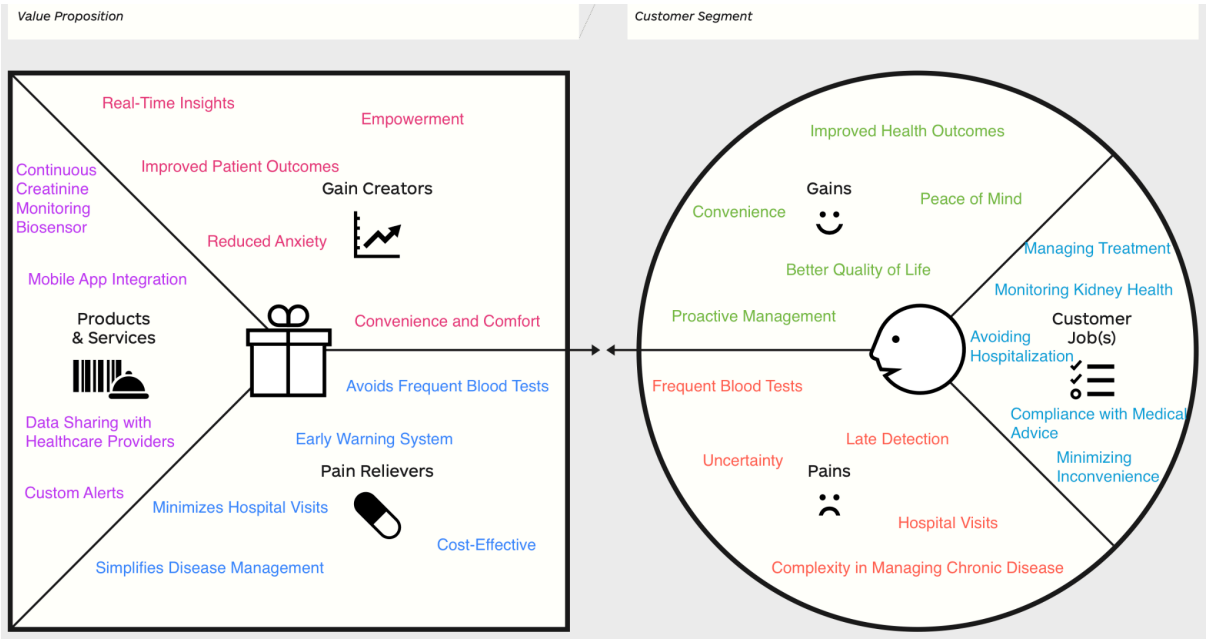

Fig. 8 - Value Proposition Canvas - Customer Segment

Customer Journey Variation: To validate the customer journey, we employed various data collection methods. We engaged with nephrologists and general practitioners in Russia and Germany to gather detailed insights. Discussions were also held with other teams participating in the competition to broaden our understanding. Additionally, we interacted with engineers and scientists, including our advisors and assistants, who are involved in the development of biosensors.

Understanding the scale of the problem

Overall On Medical Technology Market in Germany: In 2023, the medical technology market in Germany was valued at approximately $37 Bn, making Germany one of the largest markets for medical technologies in Europe. The market is projected to grow at a compound annual growth rate (CAGR) of about 32.5% from 2023 to 2029 [3].

Biosensor and Diagnostics Market in Germany: The Germany Biosensors Market was valued at $1057.7 Mn in 2023 and is predicted to grow at a CAGR of 6.2% from 2023 to 2030. The key drivers of the market include increasing burden of chronic diseases, technological advancements, and growing demand for Point-of-Care (POC) testing. The prominent players of the Germany Biosensors

Market are Lifesensors, Siemens Healthcare, B. Braun Melsungen AG, Meridian Bioscience, and Biosensors International [4].

Number of patients with kidney diseases: Chronic kidney disease prevalence is about 10%, awareness of CKD is generally low, hindering early diagnosis and treatment [5]. AKI cases increased almost sevenfold from 11,964 in 2000 to 77,719 in 2019. After adjusting for demographics, the highest incidence—6300.5 per million person/years—was observed in individuals over 79 years old [6].

Dialysis market: Germany's dialysis market is projected to grow from $1.395 Bn in 2022 to $2.581 Bn by 2030, registering a CAGR of 8% during the forecast period of 2022-2030. One of the main reasons propelling is the expanding elderly population [7].

Growing Demand for Innovations: There is an increasing demand for more accurate and accessible monitoring methods. New technologies that provide more effective patient monitoring have significant potential for integration into medical practice in Germany [8, 9].

Government support: Germany has several government programs and initiatives supporting the development of medical technologies and innovative solutions [10].

Business model

With this understanding of the market dynamics and growth potential, we can now transition to discussing our business model (Figure 9). Our approach is designed to leverage these market opportunities, addressing key needs identified in the analysis and aligning with the broader trends in healthcare innovation.

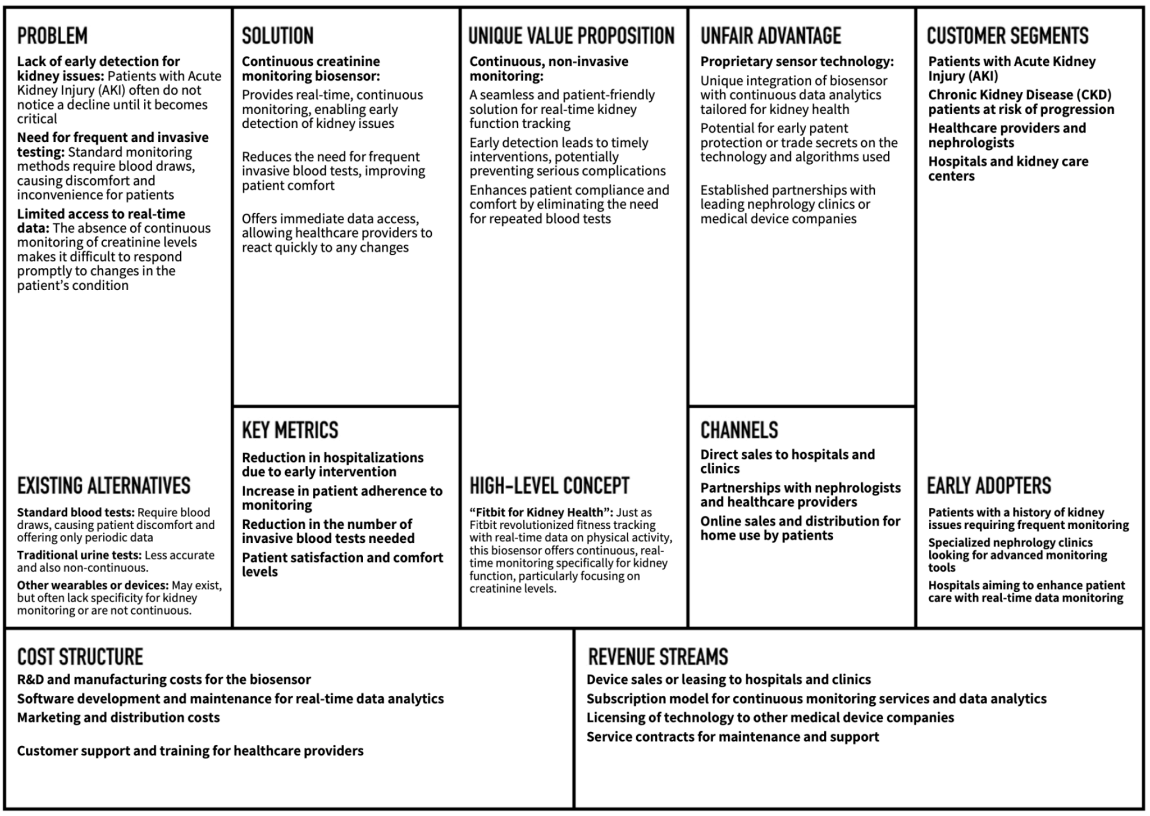

Fig. 9 - Business model

Here's a positioning statement that brings everything together:

Our continuous creatinine biosensor helps patients with chronic kidney disease or at risk of acute kidney injury and healthcare providers who want to manage kidney health proactively by providing real-time data and early warnings, enabling better treatment decisions and reducing the need for frequent blood tests and hospital visits.

Assessing the market potential

Assessing the market potential: To assess the attractiveness of the developed solution, we used the market opportunity evaluation tools developed by Gruber and Tal (2013). The attractiveness of an opportunity depends on the market potential, and the challenges of realizing that potential.

Compelling reason to buy: Our solution addresses the growing demand for accurate and accessible diagnostic and monitoring tools in medical technology. Given the increasing number of patients with chronic kidney diseases, our solution is highly valuable to the target customers.

Market volume: As previously noted, the medical technology market is quite large and continues to expand. The dialysis market too shows significant growth, underscoring the potential for our solution in this segment.

Assessment of Challenges

Implementation obstacles: Regulatory processes required for certification and approval of medical solutions in Germany may lead to lengthy approval times.

Time to revenue: Regulatory processes also delay the start of revenue generation. However, we believe that the potential profit from successful market entry in a rapidly growing sector compensates for this waiting period.

External risks: Changes in regulatory requirements, economic fluctuations, and competition from other companies operating in similar market segments.

Validation of the Problem and Solution

Based on the conducted analysis, the problems and needs of the customer segment have been confirmed. Our biosensor-based solution effectively addresses the need for accurate and accessible methods for health monitoring.

The Business Model includes premises about target markets, distribution channels, key partners, and revenue sources, which will be refined with further research and testing.

6. Team and support (max. 1 A4)

In this chapter, we would like to express our deepest gratitude to the people, who supported the team in its endeavor. In the beginning, however, there was just a team of highly motivated, dexterous students. These students from the Technical University of Munich, except for one who pursues a chemistry degree at the Ludwig Maximilian University, have and always will reify the project's core. Members, their roles, and contributions are highlighted in the following passages:

Mikhail Vinogradov, Anna Smirnova were elected as captains of the team. As team captains, Mikhail and Anna have been playing pivotal roles in successfully executing our project. They have managed all organizational tasks, including coordinating with event organizers, scientific supervisors, and securing financial support. Their efforts also extended to maintaining our social media presence and handling public relations.

Aleksei Guzman, Anna Deputatova, Anna Kogan, Kamil Zakharov, Konstantin Stepovoi, Valeriia Danyliuk comprise the biology and chemistry department. This department focuses on the respective sides of the project, including the design and implementation of biochemical assays, ensuring the biosensor's sensitivity and specificity, and, of course, the tedious research of all the available materials on the topic.

Damir Safin, Valentin Safronov, Varvara Kondratyeva represent the engineering and programming department. The team is responsible for developing and integrating hardware and software components. This includes the design of electronic circuits and the establishment of the interface for data analysis.

Next, we would like to thank those, who took their time, helped us, supporting us not only financially, but "scientifically" by providing valuable advice, giving a consultation, analyzing the sketches with the team, and much more. These exceptional scientists and professors have influenced the project and the team's work most and guided us on the right path. We are extremely grateful to:
Prof. Dr. Bernhard Wolfrum and his doctoral students, Senyao Wang and Sebastian Freko, for giving us access to their laboratory and equipment, ensuring the smooth transition of the team members to the experimental phase of our project. Moreover, for the time spent with us, considering and assessing our ideas and results.

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Finally, TUM School of Computation, Information and Technology, and TUM School of Natural Sciences for their financial support and trust in the team.

7. Final Remarks (max. ½ A4)

We acknowledge that the software packages we are currently using may not provide the most accurate results and that there are other programs that could perform the simulations more effectively and precisely. However, at present, we lack both the computational power and time resources to utilize them. We plan to address this, most certainly, in the future.

Since only 15-25 nucleotides in the middle of the aptamer are responsible for specific binding to the analyte molecule, and the mutations were introduced into nucleotides outside of this region, this method is technically and hypothetically applicable not only to creatinine but to any relatively small molecule. Of course, calculations will need to be performed anew for each specific case, but these will be within the framework of a well-established protocol.

8. References (no page limitation)

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