Continuous *In Vivo* Multimarker Biosensing for Healthcare

Background

Modern healthcare remains largely reactive. We treat diseases after symptoms occur, even though biological changes often begin months or years earlier. In contrast, critical engineering systems, such as jet engines, wind turbines, and nuclear reactor cooling systems, are equipped with dense sensor systems to detect any deviations from the norm. Here, preventative maintenance algorithms enable early intervention and prevent catastrophic failure or prolonged downtime. We lack an equivalent for the most complex and valuable system we know: the human body.

Continuous molecular sensing could close that gap. Most diseases show molecular signatures long before the onset of symptoms and early intervention is extremely valuable. For cancers, survival rates can double if diagnosis is made at an early stage; in neurodegenerative diseases, years of brain damage accumulate before symptoms appear; in sepsis, every hour of delayed recognition increases mortality. Yet, most diagnostics today are episodic and lab bound. By continuously monitoring molecular signals in blood and other relevant fluids (such as interstitial fluid or cerebrospinal fluid), we could turn healthcare from delayed, reactive treatment to true prevention. Detecting disease when it is still reversible, guiding therapies at their most effective window, and reducing costs for health care systems worldwide [1].

Continuous biochemical monitoring has long been an aspirational goal, but until recently lacked the necessary enabling technologies. Over the past five years advances in AI driven protein structure prediction revolutionized our ability to design synthetic protein, the fundamental molecular machines of biology. This enables us to design proteins that bind all classes of biomarkers and are stable in relevant biological environments while speeding up the design and selection process by orders of magnitude. That development coincides with advanced semiconductor manufacturing reaching the nanoscale regime and enabling manufacturing of structures that are on the same size scale as proteins. In addition, as we reach the limits of Moore's law the industry is increasingly exploring alternative substrate materials, including those with higher biocompatibility than classic CMOS materials.

The Challenge

Enabling continuous, multimarker biosensing requires a new versatile sensor platform capable of reporting on a wide spectrum of biomarkers, small molecules, peptides, nucleic

acids, and proteins, directly in whole blood. This platform needs to be able to convert the binding or detection mechanism to an electrical signal and interface with semiconductors to enable a seamless readout and the miniaturization and scalability necessary.

Multiple classes of molecular receptors could, in principle, serve this role, including synthetic ligands, nucleic-acid–based constructs, and other engineered materials. Nevertheless, de novo engineered proteins and protein–hybrid architectures that integrate nucleic-acid or synthetic domains currently represent the most promising and versatile route. They have demonstrated high sensitivity and selectivity across a wide range of analytes, from small molecules, DNA fragments to larger proteins and even disordered proteins [2-6]. They can also be designed to function under conditions that often limit traditional aptamers or antibodies, such as stringent Debye screening or complex serum environments [7]. Their hybridization with DNA origami, aptamer modules, or other nanoscale scaffolds can further enhance modularity and expand the accessible design space [8].

The crucial part of a sensor is the signal transduction method and there are several potential modes for molecular sensing constructs:

(1) conformational or conductance-based responses, where ligand-induced structural changes reposition charged groups or redox reporters, generating a measurable output [9];

(2) engineered catalytic activity, where binding activates or modulates a chemical reaction that amplifies the signal [10, 11];

(3) nanopore sensing, where binding modifies the ionic current through a nanopore [12]; and

(4) optical readout, where binding modulates fluorescence, which can be sensed optically [13].

Importantly, inhibition can be as effective as activation and analyte binding may reduce, rather than trigger, signal turnover. Regardless of the transduction mode, the essential requirement is extensibility: once one target (e.g., PSA) can be detected, the same architecture should be adaptable to related biomarkers (e.g., CRP) with minimal redesign.

Program Phases

A challenge to reduce scientific risk and attract private investment would be structured into three stages:

1. **Molecular design and testing:** validate relevant molecular transducers, showing that all previously mentioned classes of biomarkers can be detected via an inherent transduction mechanism.
2. **Device integration:** incorporate engineered transducers into devices and demonstrate reliable detection of a defined biomarker panel in serum or whole blood.
3. **Stability and durability:** Achieve stable operation in whole blood over prolonged time and demonstrate continuous sensing.

Secondary engineering challenges, such as power supply, data transmission, and device replacement, are real but solvable. Solutions exist in related fields with low-power wireless energy transfer, bio-safe rechargeable batteries, and minimally invasive retrieval techniques available. The novelty lies not in any single subsystem, but in their integration with a suitable transducer.

Impact

Today's biosensing landscape is fragmented. Continuous glucose monitors revolutionized diabetes care but largely rely on a natural enzymatic reaction that cannot be generalized to other targets [14]. Antibody- and aptamer-based sensors detect analytes but can fail for large, complex biomarkers and lack robustness in whole blood without modification. Most remaining diagnostic technologies are confined to laboratories, require elaborate preparation, and produce only momentary snapshots. In addition, most approaches tend to focus on single or a small number of biomarkers, yet extensive clinical evidence shows that combinations of markers are needed for sufficient accuracy in diseases like pancreatic cancer, Alzheimer's disease, and acute myocardial infarction [15-18].

Current diagnostics are episodic, condition-specific, and high-effort, demanding patients schedule visits with healthcare professionals and submit samples for each test. A seamless, continuous monitoring platform could instead readily accumulate data to build an AI-driven model of each person's molecular baseline. Thereby detecting subtle deviations before symptoms arise and guiding truly preventive, personalized care.

If successful, this approach could transform healthcare as profoundly as continuous glucose monitoring did for diabetes, extending the principle of early detection and closed-loop management to nearly every major disease. In short, the goal is to create an "engine check light" equivalent for the human body, a continuous molecular warning system that detects problems before failure.

References

[1] Tony Blair Institute for Global Change. *The economic case for Protect Britain: A preventative health care delivery programme.* Rebolledo I; Atkinson I, Bradshaw A, Joshi R (contributors). July 2024.

[2] Wu K. et al., Design of intrinsically disordered region binding proteins. *Science*. 2025.

[3] Cao L. et al., Design of protein-binding proteins from the target structure alone. *Nature.* 2022.

[4] Glasscock C. J. et al., Computational design of sequence-specific DNA-binding proteins. *Nature Structural & Molecular Biology.* 2025.

[5] Guo A. et al., Deep learning–guided design of dynamic proteins. *Science.* 2025.

[6] Lu L. et al., De novo design of drug-binding proteins with predictable binding energy and specificity. *Science*. 2024.

[7] Shen S., Advances in field-effect biosensors towards point-of-use. *Nanotechnology*. 2023.

[8] Zhuo K et al., Toward Precise Manipulation of DNA–Protein Hybrid Nanoarchitectures. *Small* 2019.

[9] Praetorius F. et al., Design of stimulus-responsive two-state hinge proteins. *Science*. 2023.

[10] Suzuki N. et al., Engineered Glucose Oxidase Capable of Quasi-Direct Electron Transfer after a Quick-and-Easy Modification with a Mediator. *Int J Mol Sci.* 2020.

[11] Batchu K. et al., The development and application of an engineered direct electron transfer enzyme for continuous levodopa monitoring. *Npj Biosensing.* 2025.

[12] Ratinho L. et al., Nanopore sensing of protein and peptide conformation for point-of-care applications. *Nat. Commun*. 2025.

[13] Kim H. Et al., Genetically Encoded Biosensors Based on Fluorescent Proteins. *Sensors.* 2021.

[14] Karyakin A., Glucose biosensors for clinical and personal use. *Electrochemistry Communications*. 2021.

[15] Reese KL. et al., Multibiomarker panels in liquid biopsy for early detection of pancreatic cancer – a comprehensive review. *J Exp Clin Cancer Res*. 2024.

[16] Kubota M et al., Plasma biomarkers for early detection of Alzheimer's disease: a cross-sectional study in a Japanese cohort. *Alzheimers Res Ther*. 2025.

[17] Möckel M et al. Development of an optimized multimarker strategy for early risk assessment of patients with acute coronary syndromes. *Clin Chem*. *Acta.* 2008.

[18] Swain AK. Biomarkers of Sepsis: Present and Future Perspectives. *Indian J Crit Care Med*. 2025.

Appendix

A. Detailed challenge design

Milestones

To ensure that the challenge produces the desired breakthrough innovation in biosensing the design of milestones throughout the challenge is important. Below is a list of the main areas that are crucial to advance the field of biosensing and a description of preliminary end-of-challenge milestones.

Generalizability of the platform:

A big value of the proposed biosensing platform is its ability to sense all classes of biomarkers and adjust the biomarkers sensed readily as new biomarkers are discovered or depending on patients need. This contrasts with the current fragmentation of the field where different sensing modalities and products are often used to sense different biomarkers. To guarantee that we can sense a variety of targets and can add additional ones, the platform needs to be able to sense 8 predetermined biomarkers (encompassing small molecules, DNA/RNA fragments, and large proteins/peptides) with the same platform design. This doesn't mean that the molecular recognition structure needs to be equivalent but the basic principle of sensing and crucially thereby transduction should be the same. This ensures that we can reuse the bulk of the device and employ the same strategies to mitigate biofouling and foreign body responses for all biomarkers while having to only redesign the protein structure when addressing new targets.

Sensing capabilities:

The most important part of any biosensor is its ability to sense the target. In this challenge, the biosensor needs to achieve a physiologically relevant limit of detection and measurement range in a whole blood equivalent. This is different for each target with the suggested targets covering a relatively wide range as can be seen in the table shown in the next section. In addition, the sensor needs to achieve a response time which ensures it can resolve the typical timescale of change for biomarkers within our body. We set the target response time for the sensor to be <5 minutes for small molecules and <30 min for proteins and cell-free DNA/RNA . Lastly, the footprint of the sensor is a metric that is important as it will have a bearing on the size of the sensor and is also a way to influence the achievable limit of detection. Increasing the number of sensing elements on the sensor by designing a larger array in parallel decreases the achievable limit of detection at the cost of taking up more space. Our assumption is that the sensing element won't be the major driver of

overall sensor size (the battery and antenna will most likely play a larger role) and therefore the aim of the challenge is to achieve the required sensing capabilities at a minimal footprint, with the footprint being one metric to compare performer teams to each other.

Stability:

To be able to use the sensing mechanism for an *in vivo* sensor the mechanism needs to effectively sense for a prolonged time in whole blood or an equivalent environment. This capability is demonstrated by maintaining the stability of the sensing mechanism in whole blood equivalent for 7 days. Stability here is defined as less than 20% drift relative to absolute values (determined by gold standard tests such as Mass Spectrometry, quantitative polymerase chain reaction and Enzyme-Linked Immunosorbent Assays for small molecule, cell-free DNA/RNA and large protein/peptides respectively).

Scalability of production:

Lastly, to ensure the sensor can be mass produced at a reasonable cost it is important that the protein production is scalable. This is indicated through requiring a preliminary ≥100 mg/L expression yield with <5-step purification for the de novo protein components at the end of the challenge.

All milestones will be assessed by an independent performer/lab rather than relying on self-reporting. Below is a summary of the milestones discussed in bullet points:

Challenge Objectives

All milestones assessed by independent lab/performer.

Generalizability of the platform:

- Sensing of 8 predetermined biomarkers (small molecule, DNA/RNA fragment, and large protein/peptide) with the same platform.

Sensing capabilities:

- Achieving a physiologically relevant LOD and measurement range in whole blood equivalent dependent on the biomarker (see next slide).
- Response time of <5 minutes for small molecules and <30 min for proteins and cell-free DNA/RNA at a minimal footprint.

Stability:

- Maintaining stability in whole blood equivalent for 7 days, defined as <20% drift relative to absolute values (determined by gold standard tests MS, qPCR and ELISA for small molecule, cell-free DNA/RNA, and large protein/peptides respectively).

Scalability of production:

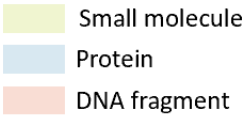
- Achieving ≥100 mg/L expression yield with <5-step purification for de novo protein components.

List of proteins and required sensitivity

The proteins and analytes listed here were selected because they span clinically important pathways (inflammation, stress, metabolism, organ injury, therapeutic drug monitoring) and cover different molecular sizes, concentrations, and sensing challenges. They span high-abundance proteins such as CRP in the nanomolar–micromolar range, as well as ultralow-abundance cytokines, neuronal markers in the picomolar–femtomolar range, and small molecules ranging from micromolar to millimolar. This diversity provides a representative test set for demonstrating the breadth of the platform without locking the program into any single clinical indication. Importantly, these analytes are suggestions rather than fixed requirements and can be adapted based on expert and jury input. However, it will be important to finalize the target panel before the challenge begins, so benchmarking can proceed with clear, consistent definitions across all teams. Lastly, since the challenge aims to create an extensible biosensor, we should be able to readily add additional biomarkers for sensing.

Analyte	**Typical Physiological Value (Approx. Required Measurement Range)**	**Required LOD (M)**	**Rationale / Clinical Use**
C-reactive protein	<8 nM (8 nM – 0.4 uM)	1 nM	Universal inflammation marker; conserved acute-phase response.
Interleukin-6	<0.1 pM (0.1 pM – 50 pM)	0.1 pM	Early immune activation; precedes C-reactive protein rise.
Lactate	0.5 mM – 2 mM (2 mM – 20 mM)	0.1 mM	Rapid indicator of perfusion/metabolic stress; universal chemistry.
Cortisol / Corticosterone	80 nM – 800 nM (80 nM – 800 nM)	1 nM	Tracks stress and diurnal recovery; cortisol (human), corticosterone (rodent).
Cell-free DNA	1.5 pM – 15 pM (1.5 pM – 75 pM)	1 pM	Universal damage / tumour marker; shared across species.
Methotrexate	0 µM (0.05 µM – 10 µM)	10 nM	Narrow therapeutic window; critical for dose safety; oncology cross-species use.
Creatinine	40 µM – 120 µM (40 µM – 400 µM)	1 µM	Key kidney-function marker.
Neurofilament light chain (NfL)	0.015 pM – 3 pM (0.01 pM – 3 pM)	0.01 pM	Sensitive neuronal injury marker; translational for trauma studies.

Legend:



Schedule and timeline

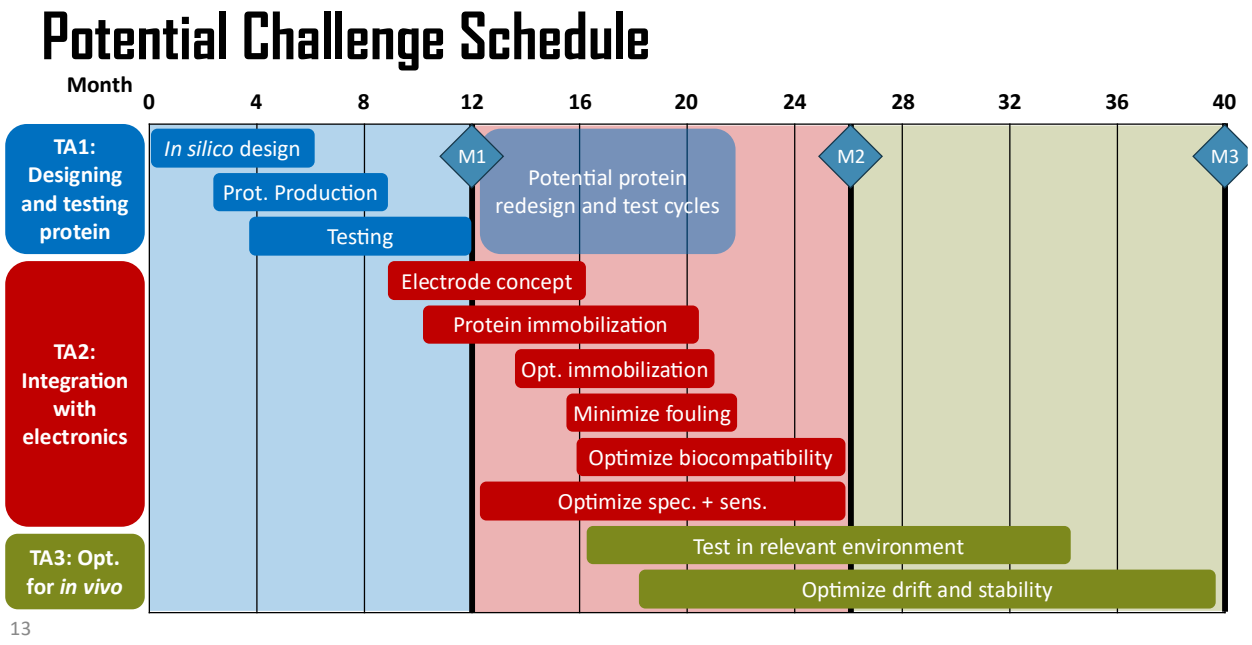
The challenge is divided into three separate stages with approximately half of the teams eliminated after each stage. It is currently designed to last 40 months and has a preliminary budget of approx. €36m. The three stages, including their respective milestones at the end of each stage, are as follows:

Stage 1, designing the proteins (6-8 teams, ca. €1-1.5m each team, 12 months): Produce and test protein transducer structures that can sense at least 6 out of the 8 predetermined target biomarkers. The 6 biomarkers need to encompass at least one out of each biomarker class. This is intended to demonstrate that the proposed transduction mechanism is functional. As such the demonstration needs to be on the final protein structure, i.e. any potentially required anchoring group or redox active groups or tags need to be part of the protein structure. The final and tested protein structure needs to be ready to be incorporated into a device without further changes.

Stage 2, integrating the protein structure into the device (3-4 teams, ca. €2-3m each, 14 months): Integrate the protein transducer into a device, enabling us to sense binding events and perform measurements. At the end of the stage reach the required limit of detection for 6 biomarkers out of the 8, where the 6 biomarkers chosen need to encompass at least one out of each biomarker class. Achieve this with <15% drift in whole blood equivalent over 6h measurement time.

Stage 3, optimizing for *in vivo* operation (1-2 teams, ca. €5m each, 14 months): Show the ability to measure all 8 biomarkers at the required limit of detection and within the required response time. Perform these measurements over a period of 7 days in whole blood equivalent with less than 20% drift. In addition, demonstrate scalability of the protein production process by achieving a protein yield of at least 100 mg/L with less than 5 steps of purification required. See the previous section for a detailed list of the end of challenge milestones

Below is a preliminary potential schedule for the challenge:



B. Transition strategy

A major difficulty with bringing an implantable biosensor to the market is the timeline until a product is approved. In order to get regulatory approval animal and human trials are usually required and typical approval times in the US and the EU are on the order of 5 years. This means that any company aiming to enter this market needs to be able to survive an extended period of little expected revenue before being able to launch a product for the human market.

The challenge described here sets out the milestones needed to show that the technology is viable as a platform for highly multiplexed, in vivo biosensing rather than requiring a finished product. So even after successfully completing the challenge, further development will be needed to optimize the sensor and integrate it into a biocompatible, implantable device with data transmission capability.

To make sure that a company can survive long enough to enter the human market, we propose to initially focus on simpler applications. One is monitoring early preclinical models for drug testing, such as organoids and other lab-on-chips systems. Traditionally, such early preclinical systems are characterized using optical imaging techniques and destructive analysis methods. There is a need for a biosensor that can continuously monitor multiple targets and provide additional information about the development of the preclinical model by showing temporal trends. This additional data can help pharma

companies understand their new drug candidate's mechanism of action and effectiveness earlier and importantly identify potential failure mechanisms such as drug toxicity.

A second application is animal model monitoring as a service for the pharma industry. Here a biosensor can give information about the level of a new drug in the blood of an animal model at the same time as monitoring important biomarkers correlated to treatment effectiveness.

For a biosensing company, the strategy of focusing on preclinical systems and models opens a much faster path towards revenue and allows for optimization of the sensor technology in a relevant environment without immediately facing the same technical integration challenges and stringent regulations of operating in humans. In addition, any path towards regulatory approval in human patients will have to provide relevant testing data in preclinical models before entering a human trial and preliminary data can help speed up that process.

C. Current Biosensing Landscape

The current landscape is shaped by misaligned incentives across the major players. Academic groups generate much of the foundational innovation but are typically rewarded for novelty and publication rather than for translation. Consequently, many sensing technologies emerge as elegant laboratory demonstrations yet lack the manufacturability, robustness, generalizability, or selectivity needed for real-world deployment. At the same time, even if a sensing technology is feasible, academics rarely have the resources, industrial partnerships, or regulatory pathways required to advance an invention from proof-of-concept to application. Large medical-device companies such as Abbott, Dexcom, and Medtronic remain commercially focused on glucose and a limited set of small-molecule analytes, producing fragmented per-target solutions with long development cycles rather than pursuing a generalizable sensing platform. Existing affinity-based approaches (such as antibodies and aptamers) have not overcome some of their core limitations: narrow target coverage, reliance on external tags or complex assay formats, and insufficient stability for long-term *in vivo* use. As a result, progress has remained incremental and largely confined to single-biomarker improvements in processed samples, rather than enabling the broad, multiplexed, continuous *in vivo* monitoring that would meaningfully change clinical practice.