2D BioPAD

Supple Graphene Bio-Platform for point-of-care early detection and monitoring of Alzheimer's Disease

D1.2 System Architecture, Version 1

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Executive Summary

This report, developed within the 2D-BioPAD project funded by the European Union's Horizon Europe Framework Programme for Research and Innovation 2021-2027, outlines the activities and findings related to Task 1.3: System Architecture Co-design under Work Package 1 (WP1). The primary objective of WP1 is to identify and address the needs and challenges for early point-of-care (PoC) diagnostics for Alzheimer's Disease (AD), focusing on creating reliable, cost-effective, safe, and ethical solutions using next-generation 2D-material-based PoC in vitro diagnostic (IVD) systems.

Through iterative co-design processes, we aim to transform requirements identified in Task 1.1 (User-Centred Requirements, Needs, and Challenges for PoC AD Diagnostics) and Task 1.2 (Safety and Ethics by Design) into detailed functional and non-functional technical specifications. Task 1.1 involved mapping and analysing the AD diagnostic landscape in Europe, focusing on clinical needs, technological solutions, and socio-economic perspectives. This was achieved through desk research, interviews, and surveys to establish user-centred requirements for the 2D-BioPAD system. Task 1.2 addressed safety and ethical considerations, complementing the findings of Task 1.1. Insights from the Industrial Advisory Board ensured that the design principles met stringent safety and ethical standards.

Task 1.3 focuses on transforming clinical and user-centred requirements into a robust system architecture for the 2D-BioPAD project. The co-design approach ensures that the final design addresses both functional and non-functional aspects, paving the way for reliable, efficient, and ethical early diagnosis of AD. The next steps involve implementing and refining this architecture through continued research and development activities.

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Table 1: Terms and Definitions

Abbreviation	Definition	Abbreviation	Definition
Aβ	Amyloid Beta	IPR	Intellectual Property Rights
AD	Alzheimer's Disease	IVD	In-Vitro Diagnostics
ADNI	Alzheimer's Disease Neuroimaging Initiative	IVDR	In-Vitro Diagnostics Regulation
AI	Artificial Intelligence	KER	Key Exploitable Result
APOE	Apolipoprotein E gene	LFA	Lateral-flow biosensor assays
"ATN"	Research framework which covers amyloid abnormalities ('A'), tau protein changes ('T'), and evidence of neurodegeneration ('N'), irrespective of clinical phenotypes	LMICs	Low-and middle-income countries
BBBM	Blood-based Biomarker	LOD	Limit of Detection
BRU	Brain Research Unit at UEF	MCI	Mild Cognitive Impairment
CIS	Clinical Information System	MDR	Medical Device Regulation
CSF	Cerebrospinal fluid	MNPs	Magnetic Nanoparticles
D	Deliverable	MRI	Magnetic Resonance Imaging
DFT	Density-functional theory	NACC	National Alzheimer's Coordinating Center
DMP	Data Management Plan	NFL	Neurofilament Light
DNA	Deoxyribonucleic acid	NIA-AA	National Institute on Aging and Alzheimer's Association
DNS	Digital Neuro Signature	NPs	Nanoparticles
EC	European Commission	NTA-tau	N-terminal containing tau fragments
ECR	Ethical Consideration Roadmap	PCR	Polymerase chain reaction
EDC	Electronic Data Capture	PDB	Protein Data Bank
ELISA	Enzyme-linked immunosorbent assay	PET	Positron emission tomography
ePADs	Electrochemical paper-based analytical devices	PhD	Philosophy Doctorate
ESC	Ethics Steering Committee	PoC	Point-of-Care
EU	European Union	Post-Doc	Post Doctoral
FAIR	Findable, Accessible, Interoperable and Re-usable	PPIE	Patient and Public Involvement and Engagement
FDG	Fluorodeoxyglucose	RNA	Ribonucleic acid
FG	Fluorographene	QA	Quality Assurance
GAAIN	Global Alzheimer's Association Interactive Network	QC	Quality Control
GCP	Good Clinical Practice	QoL	Quality of Life
GDPR	General Data Protection Regulation	RWE	Real World Evidence
GFAP	Glial Fibrillary Acidic Protein	SELEX	Systematic Evolution of Ligands by Exponential Enrichment
GFET	Graphene field effect transistor	SCI	Subjective Cognitive Impairment
GGC	Greenlight Guru Clinical	SOP	Standard operating procedure
GMP	Good Manufacturing Practice	sTREM2	Soluble triggering receptor expressed on myeloid cells 2
GP	General practitioner	tau	Tau protein
HCPs	Healthcare Professionals/Practitioners	TDP-43	TAR DNA-binding protein 43
HICs	High-income countries	WMA	World Medical Association
hPSCreg	Human Pluripotent Stem Cell Registry	WP	Work Package

1. Introduction

Alzheimer's Disease (AD) is the most common form of dementia,[1] affecting over 1 in 9 people aged 65 and older. By 2050, it is expected to impact about 18.8 million people in Europe,[2] creating a significant financial burden on healthcare, long-term care, and hospice services (Figure 1). In 2021, the cost of AD care in the US alone was over $355 billion, excluding ~$257 billion in unpaid caregiving. AD also imposes a substantial emotional and psychological burden on caregivers, leading to high levels of stress, depression, and anxiety. The incidence and mortality rates of AD have increased by 145.2% from 2000 to 2019, and this trend is expected to worsen due to population aging. Early diagnosis of Alzheimer's Disease (AD), particularly in the stages of Subjective Cognitive Impairment (SCI) and Mild Cognitive Impairment (MCI), is crucial due to the lack of effective disease-modifying treatments. Early and accurate diagnosis improves treatment outcomes, reduces emotional and social burdens, enhances quality of life, and offers significant cost savings. The absence of widely available and effective disease-modifying drugs for AD makes early diagnosis critical, especially at stages like SCI and MCI.[3] Early and accurate diagnosis offers significant benefits such as better treatment outcomes, reduced emotional and social burden, improved quality of life, and substantial cost savings.[4] This is increasingly important with the emergence of promising novel treatments that require extensive early-stage screening and frequent monitoring to minimise adverse effects.[5]

Current diagnostic techniques for AD include brain MRI, PET, FDG-PET, detection of cerebrospinal fluid biomarkers, such as amyloid and tau proteins, and neuropsychological assessments. However, these methods are often expensive, invasive, and typically used only after significant symptoms appear, though AD-related pathologies can be detected earlier. Neuropsychological tests can identify mild cognitive changes but fail to distinguish the neuropathological changes underlying dementia progression, making early treatment challenging. Additional challenges include diagnostic uncertainty, delays, costs for patients, families, and health systems, and increased cognitive impairment risk following COVID-19.[6] Screening can also cause unnecessary anxiety and overtreatment.**Chyba! Záložka není definována.** The National Institute on Aging and Alzheimer's Association (NIA-AA) "ATN" research framework (covering amyloid abnormalities, tau protein changes, and neurodegeneration) aids in detecting AD pathology but is debated for clinical practice use due to its inability to diagnose the clinical syndrome of AD.[7] Suggestions for evolving these guidelines include expanding them with additional biomarkers for mechanisms like neuroimmune dysregulation and synaptic dysfunction.[8] Research focuses on new AD diagnostic methods, particularly affordable, easy-to-use, fast, and reliable IVD through biosensing technologies for early detection and monitoring. While promising, these biosensors are still under intense research and their clinical deployment remains uncertain.

[1] *https://www.alz.org/media/documents/alzheimers-facts-and-figures.pdf*
[2] *https://www.alzheimer-europe.org/dementia/prevalence-dementia-europe*
[3] A Kumar, et al., A review on Alzheimer's disease pathophysiology and its management: an update. Pharmacological reports 67.2 (2015): 195-203
[4] https://www.alz.org/alzheimers-dementia/diagnosis/why-get-checked
[5] Gustavsson, E., et al. Novel drug candidates targeting AD: ethical challenges with identifying the relevant patient population. J. of Medical Ethics, 47(9), 608-614, 2021.
[6] MN Gordon, et al. Impact of COVID-19 on the Onset and Progression of Alzheimer's Disease and Related Dementias: A Roadmap for Future Research. Alzheimer's & Dementia, 18(5), 1038-1046, 2022.
[7] Jack Jr, C. R., Bennett, D. A., Blennow, K., Carrillo, M. C., Dunn, B., Haeberlein, S. B., ... & Silverberg, N. (2018). NIA-AA research framework: toward a biological definition of Alzheimer's disease. Alzheimer's & Dementia, 14(4), 535-562.
[8] Hampel, H., Cummings, J., Blennow, K., Gao, P., Jack Jr, C. R., & Vergallo, A. (2021). Developing the ATX (N) classification for use across the Alzheimer disease continuum. Nature Reviews Neurology, 17(9), 580-589.

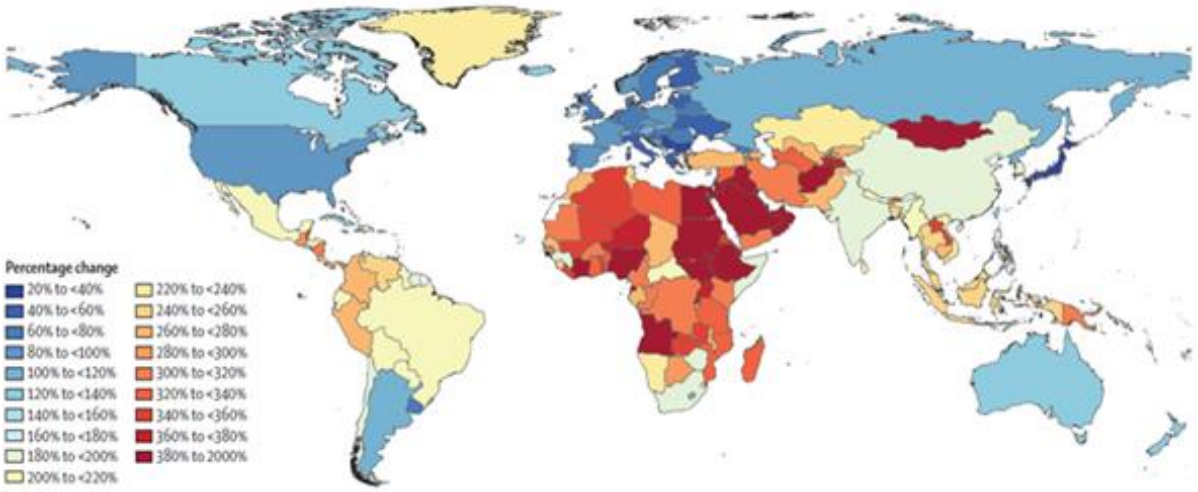

Figure 1. 2019-2050 Percentage change in global prevalence in all-age number of individuals with dementia

The 2D-BioPAD project aims to develop a cost-effective, non-invasive point-of-care/self-testing tool for early and accurate prognosis of AD, focusing on early stages like SCI and MCI. Work Package 1 (WP1) of 2D-BioPAD, titled "Requirements & System Architecture," plays a critical role in identifying and addressing the needs and challenges for early PoC diagnostics for AD. WP1 involved analyzing current needs and solutions, creating design guidelines, and co-designing the system's requirements and architecture. This report elaborates on Task 1.3, which focuses on co-designing the system's requirements and architecture based on the results of previous tasks (Figure 2). Task 1.1 involved a desk research phase, validated and refined through 26 semi-structured interviews with technology providers, healthcare professionals, patients, caregivers, decision makers, and members of the 2D-BioPAD consortium and its advisory board. This was followed by a broader online survey, which received 99 responses from 197 participants, targeting a diverse European audience, including primary and specialised healthcare professionals, patients, caregivers, decision makers, and biomarker experts. The survey captured insights into the needs, concerns, and barriers to the acceptance of AD and PoC IVD tools, leading to several key insights for the design and implementation of the 2D-BioPAD system. Task 1.2, instead, addressed safety and ethical considerations, complementing the findings of Task 1.1. To address ethical considerations, the 2D-BioPAD project, created an Ethical Consideration Roadmap (ECR). The ECR outlines ethics management principles, actions, and responsibilities to ensure compliance with ethical standards. The ECR, based on the European Commission's guide "EU Grants: How to complete your ethics Self-Assessment Version 2.0" and the ethical principles of the UK Statistics Authority, serves as a strategic document detailing the procedures for the 2D-BioPAD consortium. It will be updated as needed throughout the project's lifecycle to address any new ethical issues that arise. The complete ECR is publicly available on the 2D-BioPAD website. Co-defining/co-design workshops, involving interdisciplinary teams of experts and stakeholders, will be central. The first workshop, held in Barcelona on April 17, 2024, marked a significant milestone in refining the functional and non-functional requirements, system architecture, and case study designs for 2D-BioPAD. The iterative nature of these workshops will ensure that the design evolves based on continuous feedback and preliminary results, enhancing the system's effectiveness and user acceptance.

Figure 2: The outline of 2D-BioPAD Work Package 1

1.1 Needs and Challenges for early PoC diagnostics for AD

The investigation we carried out in the frame of 2D-BioPAD (task 1.1 of WP1) demonstrate that the situation in Europe is highly diverse in terms of clinical needs and processes. AD care practices in Finland, Germany, and Greece (the three countries analysed in task 1.1) differ based on their healthcare systems.

In Finland, elderly patients are referred to public geriatrics clinics for assessments that can take up to a year, while working-age individuals go to neurology clinics via private occupational healthcare, with a six-month diagnostic timeline. Germany's health system sees elderly patients starting with primary care physicians, with about half referred to specialists for further assessment. Despite comprehensive diagnostic capabilities, biomarker-based methods are underutilised due to inadequate reimbursement, leading to diagnostic timelines of up to 1.5 years. In Greece, cognitive issues are usually noticed late, prompting visits to specialised neurologists. Diagnostics involve neuropsychological exams and imaging, with long waiting times at public memory clinics and limited involvement from family doctors and geriatricians. Decision-makers in healthcare are currently responsible for decisions at the hospital or clinic level, such as acquiring lab equipment and determining which biomarkers are covered by public healthcare. However, with the adoption of national dementia strategies and new AD medicines pending EMA approval, decision-making may shift to regional or national levels. This change requires additional data like cost-benefit analyses and health technology assessments, potentially delaying the adoption of new diagnostic systems.[9] Healthcare professionals currently have the flexibility to choose tests and biomarker measurements for patients, but this may change with new medicines and policies. Despite this flexibility, primary healthcare providers often misdiagnose 50-70% of patients with AD symptoms, while specialised healthcare providers misdiagnose 25-30%.[10]

A person-centred approach is essential for providing a personalised and precise patient experience, but healthcare professionals need more knowledge and tools for accurate diagnosis and treatment planning. Telemedicine and digital healthcare have become more challenging, especially post-COVID-19, highlighting the need for better digital device and service usage for biomarker quantification. Patients and caregivers face long waiting times and inconsistent care. In Finland, patients see different healthcare providers at each appointment, complicating care for older patients with multiple conditions. Greece recently adopted a "family doctor" system, but patients often bypass primary care for more detailed examinations from specialists. Lab results are stored in electronic medical records, accessible to varying degrees based on the country. Patients and caregivers need faster, more

[9] Jönsson, L., Wimo, A., Handels, R., Johansson, G., Boada, M., Engelborghs, S., ... & Winblad, B. (2023). The affordability of lecanemab, an amyloid-targeting therapy for Alzheimer's disease: an EADC-EC viewpoint. The Lancet Regional Health–Europe, 29.
[10] Hansson, O., Blennow, K., Zetterberg, H., & Dage, J. (2023). Blood biomarkers for Alzheimer's disease in clinical practice and trials. Nature Aging, 3(5), 506-519.

accurate, and easier-to-understand information about their tests and diagnoses. Clinical information systems and IT support across Europe vary widely, with different standards and systems even within the same country. The European Health Data Space[11] aims to standardise and improve the digital access and use of health data, which would facilitate the integration of new medical devices and reduce the burden on healthcare professionals and IT personnel. Challenges and barriers in diagnosing AD are also identified in task 1.1

A significant issue is the lack of public awareness and knowledge about AD, leading to misconceptions and delays in seeking diagnosis and treatment. This is compounded by healthcare professionals' insufficient training and resources, resulting in frequent misdiagnosis and inadequate patient care. Stigma also plays a critical role, deterring individuals from seeking diagnosis and affecting their quality of life.[12,13,14] Diagnosing AD is complex, particularly in early stages, with a high risk of misdiagnosis[15] and unnecessary treatments.[16] The cost of diagnostics, especially advanced tests like PET scans and MRIs, is prohibitive for many, posing a significant barrier.[17] Recent studies suggest that the use of plasma biomarkers (i.e., p-Tau217) could avoid approximately 57% of PET scans needed for selecting the appropriate treatment option, potentially reducing costs and improving accessibility.[18] The diagnostic process is also time-intensive, with long waits for diagnostic results and follow-ups. Clinical heterogeneity and lack of standardised practices further complicate diagnosis and research, as does the lack of digital interoperability, which hampers data sharing and collaboration. Biases related to sex, gender, and culture lead to disparities in diagnosis and treatment, disproportionately affecting minority groups and women. The aging population exacerbates these challenges,[19] increasing demand for dementia diagnostics and straining unprepared primary healthcare systems. The deployment of new PoC IVDs faces hurdles in clinical validation and regulatory compliance, limiting their use in routine healthcare. Lastly, privacy and confidentiality concerns with digital health records create trust issues and hinder data utilisation for research.[20]

1.2 Plasma biomarkers for early detection and progression monitoring

In 2014, Kiddle et al. reviewed 163 candidate blood biomarkers for AD and replicated 94, but only 9 were associated with AD phenotypes.[21] Despite extensive research, no blood biomarkers have been validated and clinically approved for widespread use. Since 2018, validated AD biomarkers, such as CSF concentrations of Aβ42, the Aβ42/Aβ40 ratio, total tau (t-tau), and p-Tau181, have been introduced into clinical practice.[22] MRI is increasingly used for differential diagnosis, yet these biomarkers are not sufficient alone for confident AD diagnosis or disease progression prediction, and

[11] https://health.ec.europa.eu/ehealth-digital-health-and-care/european-health-data-space_en
[12] An attribute that is deeply discrediting which leaves the bearer "tainted" and "discounted".
[13] Rosin, E. R., et al., (2020). A narrative review of Alzheimer's disease stigma. *Journal of Alzheimer's disease*, *78*(2), 515-528.
[14] Best, R. K., & Arseniev-Koehler, A. (2023). The stigma of diseases: unequal burden, uneven decline. American Sociological Review, 88(5), 938-969.
[15] Boustani, M., et al., (2005). Implementing a screening and diagnosis program for dementia in primary care. Journal of general internal medicine, 20(7), 572-577.
[16] Howard, R., & Schott, J. (2021). When dementia is misdiagnosed. International Journal of Geriatric Psychiatry, 36(6), 799-801.
[17] Depends greatly on the equipment, e.g., 1T vs 3T MRI, etc.
[18] Mattsson-Carlgren, N., Collij, L. E., Stomrud, E., Binette, A. P., Ossenkoppele, R., Smith, R., ... & Hansson, O. (2024). Plasma biomarker strategy for selecting patients with Alzheimer disease for antiamyloid Immunotherapies. JAMA neurology, 81(1), 69-78.
[19] https://www.who.int/news-room/fact-sheets/detail/ageing-and-health
[20] Kahn, S. D., & Terry, S. F. (2024). Who owns (or controls) health data? Scientific Data, 11(1), 156.
[21] Kiddle, S. J., Sattlecker, M., Proitsi, P., Simmons, A., Westman, E., Bazenet, C., ... & Dobson, R. J. (2014). Candidate blood proteome markers of Alzheimer's disease onset and progression: a systematic review and replication study. Journal of Alzheimer's Disease, 38(3), 515-531.
[22] Jack Jr, C. R., Bennett, D. A., Blennow, K., Carrillo, M. C., Dunn, B., Haeberlein, S. B., ... & Silverberg, N. (2018). NIA-AA research framework: toward a biological definition of Alzheimer's disease. Alzheimer's & Dementia, 14(4), 535-562.

should be part of a comprehensive clinical assessment.[23] While PET and CSF biomarkers are common in research, they are not routinely used in clinical care for most patients with cognitive decline due to high costs, limited accessibility, and invasiveness. Blood-based biomarkers are a promising, convenient, cost-effective, and less invasive alternative,[24] with ongoing research refining a specific list of potential blood-based biomarkers (**Chyba! Nenalezen zdroj odkazů.**3).

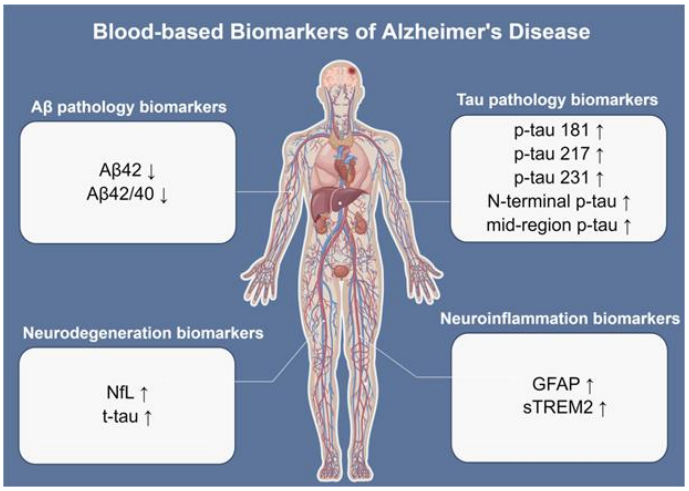

Figure 3. Overview of promising blood-based AD biomarkers[25].

Additionally, the Alzheimer's Association Workgroup (2023-2024) has released a draft of the Revised Criteria for Diagnosis and Staging of Alzheimer's Disease, which includes information on the classification of biomarkers, their intended uses, and biological staging. A list of the AD biomarkers with relative descriptions is reported below.

In the tables below, the complete list of promising biomarkers that have been identified as candidates for the 2D-BioPAD system and relative additional info are reported.

Amyloid Beta (Aβ) 1-40

Properties	
Concentration in Plasma	Health status unknown: 1-220 pg/ml Assay range: 0.38-280 pg/ml
Method	Simoa Neurology 4-Plex E Advantage Kit
Molecule Size	40 amino acids 4.33 kDa[26]
Requirements	
Transportation	Plasma samples: -
Storage	Plasma samples: -80°C
Sample Volume	100 µl (for current test – plasma)

[23] Dubois, B., von Arnim, C. A., Burnie, N., Bozeat, S., & Cummings, J. (2023). Biomarkers in Alzheimer's disease: role in early and differential diagnosis and recognition of atypical variants. *Alzheimer's Research & Therapy*, *15*(1), 175.
[24] Teunissen, C. E., Verberk, I. M., Thijssen, E. H., Vermunt, L., Hansson, O., Zetterberg, H., ... & Del Campo, M. (2022). Blood-based biomarkers for Alzheimer's disease: towards clinical implementation. The Lancet Neurology, 21(1), 66-77.
[25] Tao, Q. Q., Lin, R. R., & Wu, Z. Y. (2023). Early Diagnosis of Alzheimer's Disease: Moving Toward a Blood-Based Biomarkers Era. Clinical Interventions in Aging, 353-358.
[26] https://pubchem.ncbi.nlm.nih.gov/compound/57339250

Amyloid Beta (Aβ) 1-42

Properties	
Concentration in Plasma	Health status unknown: 0.3-13 pg/ml Assay range: 0.14-100 pg/ml
Method	Simoa Neurology 4-Plex E Advantage Kit
Molecule Size	42 amino acids 4.51 kDa[27]
Requirements	
Transportation	Plasma samples: -
Storage	Plasma samples: -80°C
Sample Volume	100 µl (for current test – plasma)

Tau Protein 181 – pTau

Properties	
Concentration in Plasma	Health status unknown: 1-100 pg/ml Assay range 0.62-1280 pg/ml
Method	Simoa pTau-181 Advantage V2.1 Kit
Molecule Size	48-67 kDa
Requirements	
Transportation	Plasma samples: -
Storage	Plasma samples: -80°C
Sample Volume	100 µl (for current test – plasma)

Tau Protein 217

Properties	
Concentration in Plasma	Not available Expected range based on literature in the range of a few pg/ml
Method	Simoa® ALZpath p-Tau 217 Advantage PLUS
Molecule Size	48-67 kDa
Requirements	
Transportation	Plasma samples: -
Storage	Plasma samples: -80°C
Sample Volume	100 µl (for current test – plasma)

Tau Protein 231

Properties	
Concentration in Plasma	Assay range 0.091-0.837 pg/ml
Method	Simoa® p-Tau 231 Advantage PLUS
Molecule Size	48-67 kDa
Requirements	
Transportation	Plasma samples: -

[27] https://pubchem.ncbi.nlm.nih.gov/compound/57339251

Storage	Plasma samples: -80°C
Sample Volume	100 µl (for current test – plasma)

Neurofilament Light (NFL) chain

Properties	
Concentration in Plasma	Diagnostic service and scientific research: Health status unknown: 3-250 pg/ml Assay range: 0.085-1440 pg/ml Healthy: BMI 25, age dependent[28] (https://doi.org/10.1016/S1474-4422(22)00009-6, serum values) 20-29 y, <7 pg/ml 30-39 y, <9 pg/ml 40-49 y, <11 pg/ml 50-59 y, <15 pg/ml 60-69 y, <19 pg/ml 70-79 y, <23 pg/ml
Method	Simoa NF-light ™ V2 Advantage Kit
Molecule Size	68 kDa
Requirements	
Transportation	Plasma samples: -
Storage	Plasma samples: -80°C
Sample Volume	100 µl (for current test – plasma)

Glial Fibrillary Acidic Protein (GFAP)

Properties	
Concentration in Plasma	Health status unknown: 17-600 pg/ml Assay range: 0.44-20000 pg/ml
Method	Simoa Neurology 4-Plex E Advantage Kit
Molecule Size	432 amino acids 49.88 kDa[29]
Requirements	
Transportation	Plasma samples: -
Storage	Plasma samples: -80°C
Sample Volume	100 µl (for current test – plasma)

TDP-43

Properties	
Concentration in Plasma	Scientific research: Health status unknown: 10-1300 pg/ml Assay range: 2.48-2000 pg/ml
Method	Simoa TDP-43 Advantage Kit
Molecule Size	43 kDa

[28] Benkert, P., et al. (2022). Serum neurofilament light chain for individual prognostication of disease activity in people with multiple sclerosis: a retrospective modelling and validation study. The Lancet Neurology, 21(3), 246-257.
[29] https://pubchm.ncbi.nlm.nih.gov/protein/P14136

Requirements	
Transportation	Plasma samples: -
Storage	Plasma samples: -80°C
Sample Volume	100 µl (for current test – plasma)

Beta-synuclein

Properties	
Concentration in Plasma	Based on literature, expected ranges around a few pg/ml in Plasma
Method	-
Molecule Size	134 aminoacids, 19 kDa
Requirements	
Transportation	Plasma samples: -
Storage	Plasma samples: -80°C
Sample Volume	100 (for current test – plasma)

2. Functional and Non- Functional Technical Specifications

The 2D-BioPAD project aims to create a non-invasive, cost-effective tool for early AD diagnosis to improve treatment outcomes and reduce burdens, addressing the current challenges of long diagnostic timelines and inadequate biomarker diagnostics. In developing effective diagnostic tools for AD, it is crucial to differentiate between functional and non-functional technical specifications. This distinction ensures a comprehensive understanding of the system's requirements and aids in the successful design and implementation of innovative diagnostic solutions.

Functional Specifications define the specific behaviours or functions of the system. These include the following:

1) Early Detection Capability: The system should accurately detect early stages of AD, such as SCI and MCI, using plasma biomarkers like Aβ40, Aβ42, p-Tau181, and p-Tau217.
2) Monitoring Disease Progression: The diagnostic tool should be able to track the progression of AD over time, utilising biomarkers such as NfL and GFAP to provide ongoing assessment of the patient's condition.
3) Integration with Clinical Workflows: The system must seamlessly integrate with existing clinical workflows, allowing healthcare professionals to easily incorporate new diagnostic tests into their routine practice.
4) Data Management: It should facilitate the storage, retrieval, and analysis of patient data, ensuring that healthcare providers can access comprehensive patient histories and biomarker data.
5) User Interface: The system should feature an intuitive and user-friendly interface for both healthcare professionals and patients, ensuring ease of use and accessibility.
6) Accuracy and Reliability: The diagnostic tool must deliver high sensitivity and specificity in detecting AD-related biomarkers, minimising false positives and false negatives.
7) Interoperability: The system should be compatible with various electronic health records (EHR) systems and other medical technologies, facilitating seamless data exchange and integration.
8) Maintainability: It should be easy to maintain and update, with clear protocols for troubleshooting and upgrading the system as new technologies and biomarker discoveries emerge.

Non-Functional Specifications outline the quality attributes of the system, addressing how the system performs its functions. These include the following:

1) Scalability: It should be scalable to accommodate varying patient volumes and adaptable to different healthcare settings, from primary care clinics to specialized diagnostic centers.
2) Security and Privacy: The system must comply with data protection regulations, ensuring the confidentiality and integrity of patient information. Robust encryption and access controls are essential.
3) Regulatory Compliance: It should meet all relevant regulatory standards for IVD, ensuring safety and efficacy in clinical use.

4) Cost-Effectiveness: The diagnostic tool must be affordable, reducing the financial burden on patients and healthcare systems. It should be designed to minimise operational and maintenance costs.
5) Ethical Considerations: The system will consider all the ethical aspects reported in the Ethical Consideration Roadmap (task 1.2 of WP1)

By addressing these functional and non-functional specifications, the 2D-BioPAD project aims to develop a robust, user-centric diagnostic tool that enhances early detection and monitoring of Alzheimer's Disease, ultimately improving patient outcomes and reducing the burden on healthcare systems.

3. System overview

2D-BioPAD aims to deliver a low-cost high-performance AD biomarker detection platform based on the use of aptamers as recognition element, graphene-based material as transductor, magnetic nanoparticles for sample purification, flow control, and signal amplification, for the detection of AD biomarkers (Figure 4).

The 2D-BioPAD detection platform consists of a graphene surface on which an aptamer layer is grafted and will selectively bind to the desired biomarkers. Depending on the detection technique (respectively depicted as A and B in figure 4). Graphene can be used either as an electrochemical electrode or a as a channel of field effect transistors. These components are contained in a disposable cassette with the corresponding connections for the electrodes, presenting the 2D-BioPAD device (3). The 2D-BioPAD cassette/device is then connected to an external device to induce and control the magnetic field along the strip, and a Voltage source / amperemeter for the electronic measurements (4); and finally, a smartphone with a dedicated app for controlling the device and extracting the measurements, offering also the needed visualisation for the end-user (5).

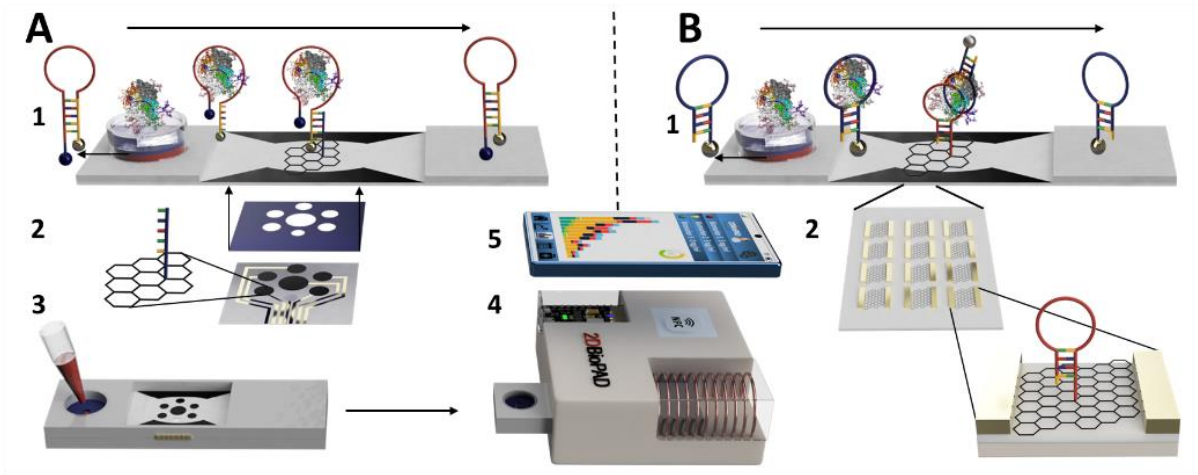

Figure 4. 2D-BioPAD system concept. In architecture A (left) the graphene forms electrochemical working electrodes while in B (right) graphene acts as the channel of a field effect transistor and is connected to two coplanar metallic electrodes (called drain and source). In device A, the graphene is integrated in a paper-based substrate with a sampling pad, detection zone and absorbent pad (A1) and (B1); a passivation layer and an array of graphene functionalised electrodes with single strand DNA (A2), while in device B, the sample is flowing over an array of graphene field effect transistors (B2). Both systems are integrated in a sample handling cartridge including microfluidics (3). A reader (4)

Paper-based lateral-flow biosensor assays (LFA) have become a valuable and popular tool for PoC IVD,[30,31] serving as an alternative to PCR.[32] These biosensors are easy to use, portable, provide real-

[30] Parolo, C. et al., (2020). Tutorial: design and fabrication of nanoparticle-based lateral-flow immunoassays. Nature Protocols 15(12), 3788-3816.
[31] Sena-Torralba, A., et al. Toward Next Generation Lateral Flow Assays: Integration of Nanomaterials. Chemical Reviews, 122(18): 14881–14910, 2022
[32] Merkoçi, A., et al. COVID-19 biosensing technologies. Biosensors and Bioelectronics, 178: 113046, 2021

time analysis in a single step, and are inexpensive to manufacture with a good shelf life.[33] However, they have limitations such as low sensitivity, binary results (positive/negative), difficulty handling complex matrices like blood or serum, and a limited number of detectable biomarkers simultaneously, which restrict their use for conditions like AD. Electrochemical paper-based analytical devices (ePADs), first introduced by Dungchai et al. in 2009,[34] offer sensitive, portable, disposable, and cost-effective solutions by generating an electrochemical signal directly related to the analyte amount. Electrochemical biosensors work by converting a biochemical signal into an electrical signal (current, potential, conductance, or impedance) through a redox reaction, which is then measured by the transducer to quantify the result. These advancements make ePADs a promising alternative for more complex and sensitive diagnostic applications.[35] Research has focused on improving ePAD functionality, especially electrode technology, to enhance electrical signals and simplify functionalisation.[36]

Aptamers are short DNA or RNA strands which contain complementary sequence along their chain thus leading to 3D folded structure that can mimic antibodies, known for their high specificity and affinity. Aptamers are cheaper, easier to produce, more stable, and non-toxic compared to antibodies, making them ideal for bio-analysis and therapeutics. [37] They can target various analytes and have been integrated into nanomaterials, enhancing their performance and commercial potential in low-cost diagnostic systems.[38] Identified using a process called “systematic evolution of ligands by exponential enrichment (SELEX)”, aptamers face challenges like lengthy development times and low success rates,[39,40] but advancements in selection methods are improving outcomes.[41,42] Since the first aptamers targeting Aβ peptides were reported in 2002,[43] many studies have used them to target Alzheimer's disease (AD) biomarkers.[44] Electrochemical aptasensors, in particular, have shown promise in detecting AD biomarkers such as Aβ40, Aβ42, and tau protein isoforms like p-Tau231, often outperforming traditional ELISA methods.[45,46,47,48]. However, creating aptasensors that can simultaneously detect multiple biomarkers remains a challenge.

[33] Miku, E. (2021). Recent advancements in electrochemical biosensors for Alzheimer’s disease biomarkers detection. Current Medicinal Chemistry, 28(20), 4049-4073.
[34] Dungchai, W., Chailapakul, O., & Henry, C. S. (2009). Electrochemical detection for paper-based microfluidics. Analytical chemistry, 81(14), 5821-5826.
[35] Solhi, E., Hasanzadeh, M., & Babaie, P. (2020). Electrochemical paper-based analytical devices (ePADs) toward biosensing: recent advances and challenges in bioanalysis. Analytical methods, 12(11), 1398-1414.
[36] Bhattacharya, G., et al., (2022). Disposable paper-based biosensors: Optimizing the electrochemical properties of laser-induced graphene. ACS Applied Materials & Interfaces, 14(27), 31109-31120.
[37] Shui, B., et al., (2018). Biosensors for Alzheimer's disease biomarker detection: a review. Biochimie, 147, 13-24.
[38] Kim, Y. S., Raston, N. H. A., & Gu, M. B. (2016). Aptamer-based nanobiosensors. Biosensors and Bioelectronics, 76, 2-19.
[39] Tuerk, C., & Gold, L. (1990). Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. science, 249(4968), 505-510.
[40] Ellington, A. D., & Szostak, J. W. (1990). In vitro selection of RNA molecules that bind specific ligands. nature, 346(6287), 818-822.
[41] Chen, Z., et al., (2021). Artificial intelligence in aptamer–target binding prediction. International journal of molecular sciences, 22(7), 3605.
[42] Mikuła, E., & Malecka-Baturo, K. (2023). An Overview of the Latest Developments in the Electrochemical Aptasensing of Neurodegenerative Diseases. Coatings, 13(2), 235.
[43] Ylera, F., Lurz, R., Erdmann, V. A., & Fürste, J. P. (2002). Selection of RNA aptamers to the Alzheimer's disease amyloid peptide. Biochemical and biophysical research communications, 290(5), 1583-1588.
[44] Murakami, K., Izuo, N., & Bitan, G. (2022). Aptamers targeting amyloidogenic proteins and their emerging role in neurodegenerative diseases. Journal of Biological Chemistry, 298(1).
[45] Khang, A., Idegwu, N., & Lee, J. H. (2023). A cost-effective aptasensor capable of early diagnosis and monitoring of Alzheimer's disease with the rapid analysis of beta-amyloid peptide 1–40. Sensors & Diagnostics, 2(2), 409-417.
[46] Negahdary, M., Veloso, W. B., Bacil, R. P., Buoro, R. M., Gutz, I. G. R., Paixao, T. R. L. C., ... & Angnes, L. (2023). Aptasensing of beta-amyloid (Aβ (1– 42)) by a 3D-printed platform integrated with leaf-shaped gold nanodendrites. Sensors and Actuators B: Chemical, 393, 134130.
[47] Jia, Z., Maghaydah, Y., Zdanys, K., Kuchel, G. A., Diniz, B. S., & Liu, C. (2023). CRISPR-Powered Aptasensor for Diagnostics of Alzheimer’s Disease. ACS sensors, 9(1), 398-405.
[48] Phan, L. M. T., & Cho, S. (2022). Fluorescent aptasensor and colorimetric aptablot for p-Tau231 detection: Toward early diagnosis of Alzheimer’s disease. Biomedicines, 10(1), 93.

Graphene, a one-atom-thick layer of carbon atoms arranged in a hexagonal lattice, is the thinnest, lightest, strongest, and most flexible material known.[49] It is optically transparent and heat conductive and selectively permeable., but it is its excellent electronic properties that are the most remarkable. These properties make graphene highly suitable for various electronic applications, including life sciences and biosensing. Graphene's unique structure and properties enable it to provide a simple, low-cost, stable, and modular platform for real-time electronic-based biosensing applications. Its excellent conductivity, large surface area, and ability to immobilise different molecules (due to its hydrophobic and aromatic structure) make it an effective transducer, converting chemical information into measurable signals.[50]

Among nanomaterials, Magnetic Nanoparticles (MNPs) are extensively utilised in life science due to their high surface-to-volume ratio, superparamagnetic nature, excellent biocompatibility, low toxicity, and site-specific targeting ability. They are cost-effective and sustainable to manufacture, enhancing their appeal for various biomedical uses.[51] When integrated with biosensors, MNPs significantly enhance sensitivity and reliability.[52] They are crucial in sample purification, minimising non-specific signals, and regulating flow during various stages of bioassays, such as bioreceptor incubation, purification, recognition, and signal acquisition.[53,54] Magnetically responsive nanoparticles can target and interact with specific proteins through appropriate surface functionalisation or conjugation with probes like antibodies or aptamers. They can be precisely controlled by an external magnetic field, facilitating the quantification of bound targets by supporting fine-tuned separation or reading out magnetic signals, with minimal interference from biological samples.[55] Various nanoparticles, including gold and silver NPs, quantum dots, graphene oxide NPs, Prussian Blue NPs, carbon nanostructures, and various forms of MNPs, have been employed in biosensing techniques for AD.[56,57] These nanomaterials have been used to detect blood biomarkers like Aβ40, Aβ42, and p-Tau with good selectivity, specificity, fast response, and low limits of detection (LOD), across different stages of AD. [58,59]

[49] Novoselov, K. S., et al., (2004). Electric field effect in atomically thin carbon films. Science, 306(5696), 666-669.
[50] Peña-Bahamonde, J., et al., (2018). Recent advances in graphene-based biosensor technology with applications in life sciences. Journal of nanobiotechnology, 16(1), 75.
[51] Chavan, N., Dharmaraj, D., Sarap, S., & Surve, C. (2022). Magnetic nanoparticles–A new era in nanotechnology. Journal of Drug Delivery Science and Technology, 77, 103899.
[52] Le, T. D., Suttikhana, I., & Ashaolu, T. J. (2023). State of the art on the separation and purification of proteins by magnetic nanoparticles. Journal of Nanobiotechnology, 21(1), 363.
[53] Esmaeili, E., Ghiass, M. A., Vossoughi, M., & Soleimani, M. (2017). Hybrid Magnetic-DNA Directed Immobilisation Approach for Efficient Protein Capture and Detection on Microfluidic Platforms. Scientific reports, 7(1), 194.
[54] Zhu, N., et al., (2004). DNA Hybridization at Magnetic Nanoparticles with Electrochemical Stripping Detection. Electroanalysis, 16(23), 1925-1930
[55] Cao, B., Wang, K., Xu, H., Qin, Q., Yang, J., Zheng, W., ... & Cui, D. (2020). Development of magnetic sensor technologies for point-of-care testing: Fundamentals, methodologies and applications. Sensors and Actuators A: Physical, 312, 112130.
[56] Hou, F., et al., (2023). The application of nanoparticles in point-of-care testing (POCT) immunoassays. Analytical Methods.
[57] Xianyu, Y., Wang, Q., & Chen, Y. (2018). Magnetic particles-enabled biosensors for point-of-care testing. Trends Analytical Chemistry, 106, 213-224.
[58] Devi, R., et al., (2020). Au/$NiFe_2O_4$ nanoparticle-decorated graphene oxide nanosheets for electrochemical immunosensing of amyloid beta peptide. Nanoscale Advances, 2(1), 239-248.
[59] Chiu, M. J., et al., (2020). Nanoparticle-based immunomagnetic assay of plasma biomarkers for differentiating dementia and prodromal states of Alzheimer's disease–A cross-validation study. Nanomedicine: Nanotechnology, Biology and Medicine, 28, 102182.

4. 2D-BioPAD Device Components

4.1 Aptamers as recognition element

The aptamer selection process (SELEX) will be carried out by NOVATECH against AD specific biomarker proteins viz. Aß40, Aß42, NF-L, ptau-217, and GFAP. different biomarker targets. The SELEX process will be then followed by characterisation Aptamers are short single-stranded DNA (ssDNA) and hence are capable to form unique 3D structures which bind to different biomarker targets. The SELEX process will be then followed by characterisation of selected aptamers by standard techniques of biomolecular interaction such as surface plasmon resonance (SPR), Bio-layer interferometry (BLI), etc. Aptamers thus capable to bind specific targets will work as recognition element in the different sensors systems mentioned ahead. In comparison to traditional antibodies, aptamers here, will provide increased flexibility in terms of handling & modification thus enhancing integration capability in sensors.

To support the selection process, CeADAR will perform a detailed comparative analysis of various advanced computational methodologies used in aptamer binding research, including MLPD[60], RaptGen[61], AptaNet[62], APIPred[63], AptaBERT[64], and AptaTrans[65], to facilitate understanding of the essential computational strategies for aptamer research. Such methodologies range from experimental techniques combined with machine learning to sophisticated transformer—based models designed for predicting and generating high—affinity aptamer sequences. Each methodology will be assessed on its description, key features, and inherent limitations, which informed their functionality ratings and suitability for specific research scenarios. This analysis aims to enhance the selection process and development of high—affinity aptamer candidates for targeting AD biomarkers. Key findings from this exploration will support the aptamer selection process to maximise its effect and accelerate the identification of appropriate aptamers.

4.2 Magnetic Nanoparticles for sample purification, flow control, and signal amplification

AUTH will synthesise tailor made MNPs to conjugate with aptamers. In this framework biphasic (Magnetite/Au) MNPs, with variable size composition, morphology, will be tested,[66,67,68] characterised and adequately functionalised before and after the conjugation with DNA.[69] Au component will serve as the conjugation probe with selected aptamers, while magnetite component as a natural magnet to enhance aptamer concentration under external magnetic field guidance, thus designate flow control and signal amplification upon request.

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60 Bashir, A., Yang, Q., Wang, J. et al. Machine learning guided aptamer refinement and discovery. *Nat Commun* 12, 2366 (2021). https://doi.org/10.1038/s41467-021-22555-9

61 Iwano, N., Adachi, T., Aoki, K. et al. Generative aptamer discovery using RaptGen. *Nat Comput Sci* 2, 378–386 (2022). 6

62 Emami, N., Ferdousi, R. AptaNet as a deep learning approach for aptamer–protein interaction prediction. *Sci Rep* 11, 6074 (2021).

63 Fang, Z. et al., APIPred: An XGBoost-Based method for predicting aptamer–protein interactions. *J. Chem. Inf. Model*. 64, 7 (2023).

64 Morsch, F. et al. AptaBERT: Predicting aptamer binding interactions. *bioRxiv*, 2023-11 (2023).

65 Shin, I., Kang, K., Kim, J. et al. AptaTrans: a deep neural network for predicting aptamer-protein interaction using pretrained encoders. *BMC Bioinformatics* 24, 447 (2023).

66 Miyazaki et al. Gold conjugated-magnetite nanoparticles for magnetic concentration towards reproducibility and repeatability of SERS measurements. Colloids Surf. A Physicochem. 671 (2023): 131661.

67 Zhou, H., et al. "Ultrasensitive DNA monitoring by Au–Fe3O4 nanocomplex." *Sensors and Actuators B: Chemical* 163.1 (2012): 224-232.

68 Garanina, A. S., et al. Bifunctional magnetite–gold nanoparticles for magneto-mechanical actuation and cancer cell destruction. *Magnetochemistry* 8.12 (2022): 185.

69 Gamboa J. et al., Aptamers for the Delivery of Plant-Based Compounds: A Review Pharmaceutics **2024**, 16, 541.

The conjugation with aptamers will be achieved by using well-established cross-linking methodologies[18], aiming to (i) provide an adaptable and scalable solution that can easily be adapted to different biomarkers; and (ii) target a 1 (MNP): 1 (aptamer) ratio to be achieved, further increasing the selectivity and specificity capability per biomarker. Conjugation of thiolo-modified aptamers on MNPs will be evaluated with alternative protocols to validate enhanced signal and performance i.e. overnight freezing[70], amino-modified aptamers with EDC/NHS chemistry on carboxyl-modified NPs,[71] or APTES/glutaraldehyde chemistry on OH-modified NPs.[72,73]

To evaluate the conjugation efficiency, FAM-modified fluorescent aptamers will be employed. After conjugation, aptamers will be detached from the MNPs and quantified with fluorometry. In addition, the conjugation of aptamers on MNPs will be assessed by determining the electrophoretic mobility on agarose electrophoresis and with spectrophotometric analysis of the MNPs. Aptamer-modified MNPs are expected to retain or even increase their absorbance, or present slight upshifts at their absorbance maxima, while non-modified MNPs typically aggregate and appear greyish after chemical treatment.

4.3 Electrodes

4.3.1 Graphene-based Electrodes

Graphene derivatives are highly promising for chemical and biochemical sensors, particularly in electrochemical[74,75] and field-effect transistor (FET) devices.[76,77] When used as a functionalised gate electrode in electrochemical sensors or as a single layer in FETs, graphene exhibits high electronic mobility and acts as a zero-bandgap semiconductor with excellent transconductance. Their attributes include high transconductance, stability, mechanical flexibility, biocompatibility, and chemical inertness, making them ideal for high-quality biosensors.[10,78] Key to advanced graphene biosensors is the tailored, reproducible chemical functionalisation of the graphene surface. This functionalisation is crucial for the effective and selective recognition of target analytes, enhancing signal generation, selectivity, and sensitivity. Fluorographene (FG) chemistry (Figure 5), pioneered by UP-CATRIN in 2010[79], allow for up to 15% functionalisation, [80] enhancing electron transfer between the biorecognition site and the electrodes, improving the signal-to-noise ratio, and boosting the selectivity

[70] B. Liu and J. Liu Freezing Directed Construction of Bio/Nano Interfaces: Reagentless Conjugation, Denser Spherical Nucleic Acids, and Better Nanoflares, J. . Am. Chem. Soc. 2017, 139, 9471−9474

[71] F. Odeh et al., Aptamers Chemistry: Chemical Modifications and Conjugation Strategies, Molecules **2020**, 25, 3.

[72] Gunda N. S. K. et al., Optimization and characterization of biomolecule immobilization onsilicon substrates using (3-aminopropyl)triethoxysilane (APTES) and glutaraldehyde linker Applied Surface Science 305 (2014) 522–530

[73] Sypabekova M. et al., Review: 3-Aminopropyltriethoxysilane (APTES) Deposition Methods on Oxide Surfaces in Solution and Vapor Phases for Biosensing Applications, Biosensors **2023**, 13, 36.

[74] Wang, M, et al. A Wearable Electrochemical Biosensor for the Monitoring of Metabolites and Nutrients. Nat. Biomed. Eng 2022, 1–11.

[75] Lee, H., et al. A Graphene-Based Electrochemical Device with Thermoresponsive Microneedles for Diabetes Monitoring and Therapy. Nature Nanotech 2016, 11 (6), 566–572.

[76] Xue, M., et al. Integrated Biosensor Platform Based on Graphene Transistor Arrays for Real-Time High-Accuracy Ion Sensing. Nat Comm 2022, 13 (1), 5064.

[77] Goldsmith, B. R., et al. Digital Biosensing by Foundry-Fabricated Graphene Sensors. Sci Rep 2019, 9 (1), 434.

[78] Georgakilas, V., et al. Noncovalent Functionalization of Graphene and Graphene Oxide for Energy Materials, Biosensing, Catalytic, and Biomedical Applications. Chem. Rev. 2016, 116 (9), 5464–5519.

[79] Zbořil, R., et al. Graphene Fluoride: A Stable Stoichiometric Graphene Derivative and Its Chemical Conversion to Graphene. Small 2010, 6 (24), 2885–2891.

[80] Bakandritsos, A., et al. Cyanographene and Graphene Acid: Emerging Derivatives Enabling High-Yield and Selective Functionalization of Graphene. ACS Nano, 11 (3), 2982–2991, 2017.

and sensitivity of the devices.[81] Furthermore, graphene derivatives can also be integrated with other materials to tackle similar challenges.[82].

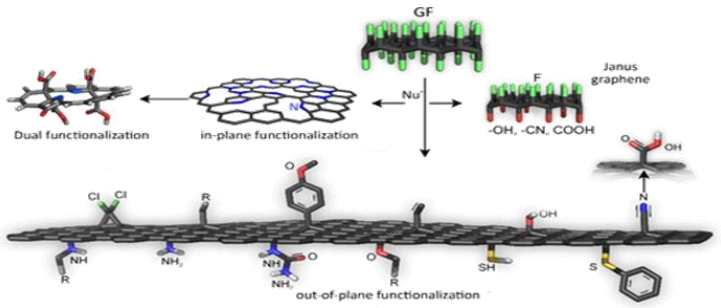

Figure 5. Fluorographene chemistry

Part of the electrode design will include covalently functionalised graphene sheets with small organic molecules. These molecules will bear terminal handles/functional chemical groups for the conjugation of the recognition aptamers. The current design strategy of molecular handles includes three options: i) carboxylic groups, ii) amino groups and iii) thiol groups. It also includes combinations thereof. For example, the combination of thiol groups and amino groups will allow for example the rapid click-conjugation of cyanobenzothiazole-modified aptamers (Figure 6). In another example, the combination of the thiol groups and carboxylic groups will greatly improve the dispersibility of the graphene derivative to promote handling processing and integration with the other components of the sensors.

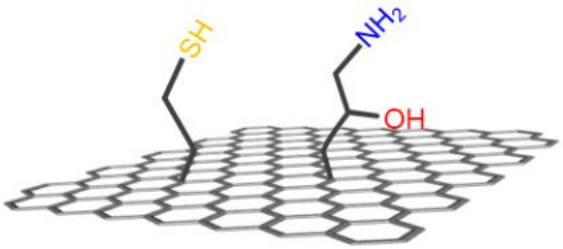

Figure 6. Amino- and thiol-, doubly functionalised graphene derivatives for providing handles for aptamer conjugation (the analyte recognition units) and for endowing the material high dispersibility in solvents for its proper integration with the rest of the device components.

On the other hand, ICN2 has developed a technology that utilises an infrared laser to enable the simultaneous and precise fabrication of highly exfoliated reduced graphene oxide (rGO) decorated in situ with gold nanoparticles (AuNPs) by instantaneous laser-induced co-reduction of graphene oxide (GO) and gold cations (Au^{3+}). A solvent-free solution of GO and Au^{3+} is filtered on a vacuum-filtered membrane to form a film, which is then reduced to conductive rGO with a CO_2 laser and patterned with AuNPs in the form of electrodes. These strips are then transferred to a flexible support such as PET or nitrocellulose. This method reduces production time and complexity, by using a one-step process, the overall cost of manufacturing is reduced, being this is beneficial for large-scale production

[81] Flauzino, J. M. R., et al. Label-Free and Reagentless Electrochemical Genosensor Based on Graphene Acid for Meat Adulteration Detection. Biosensors and Bioelectronic, 195, 113628, 2022.
[82] Palley, B. F., et al., (2023). Electrochemical Biosensors Composed of Polyethylenimine (PEI) and Graphene Derivatives for Rapid Detection of Alzheimer's Disease. In Electrochemical Society Meeting Abstracts 244 (No. 63, pp. 3006-3006).

where cost efficiency is an important factor. Furthermore, this process is also environmentally friendly. In addition, the use of an infrared laser enables precise fabrication and control over the deposition and reduction process. This method, in fact, enables the fabrication of highly exfoliated rGO electrodes, with a larger surface area and improved electrochemical properties, leading to higher sensitivity and performance in sensor applications. Finally, the simultaneous and precise decoration of the rGO electrodes with AuNPs during fabrication ensures uniform distribution and controlled coverage of the nanoparticles. This increases the sensitivity and selectivity of the electrodes for the detection of target analytes. The ability to transfer the fabricated electrodes to flexible substrates such as nitrocellulose increases the versatility and application potential of the sensor devices. As evidence of this, this method has been used for the fabrication of electrochemical lateral flow assays (LFAs).

Conventionally, the strategy for fusing electrochemistry and LFAs has focused on the overlay of SPEs on nitrocellulose substrates during the preparation of LFAs (Figure 7). However, this approach has significant limitations in terms of scalability. The full integration of rGO electrodes into LFA strips is a solution to these limitations. The use of the CO_2 laser makes it possible to simultaneously reduce GO and pattern nitrocellulose, exposing the backside to create junctions that are impermeable to sample leakage. The rGO and nitrocellulose can then be placed side by side and placed in a roll-to-roll system using a wax printer. The pressure applied facilitates the transfer of rGO to the nitrocellulose.

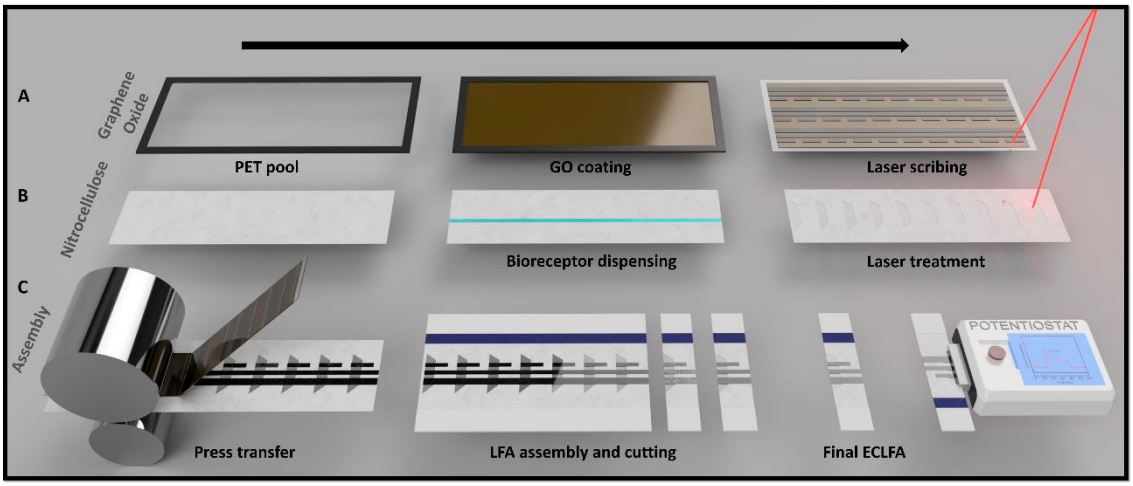

Figure 7. Sketch of the fabrication of electrochemical LFA strips. A) GO is dried in an acrylonitrile butadiene styrene (ABS) frame and reduced with a laser. B) Simultaneously, bioreceptors are dispensed on the nitrocellulose. After drying, the laser is used to remove parts of the nitrocellulose membrane and expose the underlying plastic, which will serve as a support for the connection to the potentiostat. C) Transfer of rGO to the nitrocellulose membrane, assembly of all LFA pads, cutting into strips and connection to a potentiostat.

4.4 Bio-sensing

4.4.1 Electrochemical sensing

Following a proprietary SELEX procedure by **NOVA** two cases will be explored: (i) for non-literature aptamers: in vitro structure-switching-enabled selection will be carried out; (ii) for literature aptamers structure-switching aptamers will be functionalised in strand displacement reactions (Figure 8). To do that, a self-complementary motif will be introduced into the aptamer to create an aptamer beacon (**AB**), that in the absence of the target will be in its non-binding state. The presence of the target destabilises

the non-binding state and stabilises the binding conformation, bringing to the displacement of the introduced self-complementary portion (Intramolecular strand displacement).

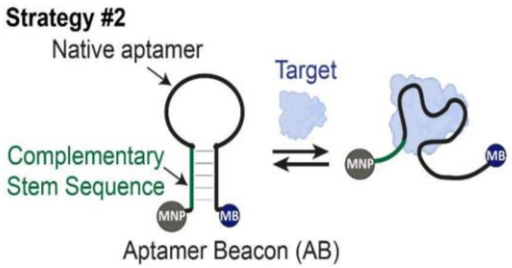

Figure 8. Design of structure-switching aptamers

AB will be then doubly modified at the two edges with MNP to one end to allow sample purification, flow control, and signal amplification, and with a redox tag (i.e., Methylene Blue - MB) to the other end to allow the transduction of the binding events as described below.

A graphene-based platform described previously will be employed to transduce the conformational changes induced by the binding event between aptamers and their target biomolecules into detectable electrochemical signals (Figure9). To do that, DNA strands complementary to the displaced portion of the AB will be immobilised on graphene-based electrodes.

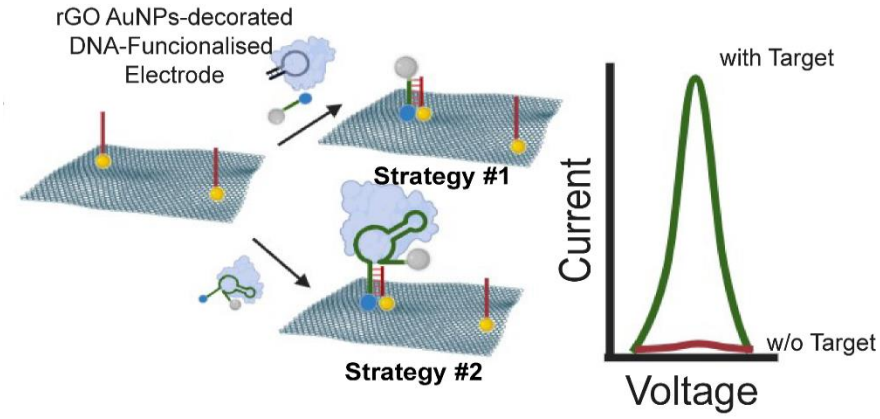

Figure 9. Transduction Mechanisms for the proposed detection strategy

Different chemistry will be exploited to attach the DNA on graphene-derivatives and nanocomposites (i.e., exploiting the chemistry of the carboxylic or hydroxyl groups present in rGO or decorating the surface with π-π anchor molecules or gold nanoparticles). Specifically, the electrodes will be modified with a DNA capture strand that is complementary to the portion of the aptamer that will be displaced following the conformational change of the aptamer in the presence of the target. As a result, a signal increase will be achieved only in the presence of the target, due to the hybridisation event between the capture strands immobilised on the electrodes and the DNA strands tagged with MB. Different electrochemical techniques will be investigated (i.e., square wave voltammetry and impedance).

4.4.2 GFET sensing

Compared to Electrochemical sensors, GFET sensing is based on a different physical phenomena. At the difference of previous technology there is in GFET no measurable current flowing through the solution to be tested. Instead, the current is flowing within the plane of a single layered graphene and,

because this current is circulating on the very top surface of the material (which has no volume because it is single atom thick) it is strongly affected by the binding of biomarkers on the graphene surface. To be effective this phenomena need to be implemented in devices based on Field Effect

4.4.3 Graphene Field Effect Transistors

A graphene field effect transistor (GFET) consists of a graphene channel side contacted by two metal electrodes, with extra electrode (gate) that is used to apply a static electric field that enable modulate the electronic response of the channel (Figure 0**Chyba! Nenalezen zdroj odkazů.**). The exposed graphene surface is functionalised with receptor molecules (e.g., antibodies, aptamers). When a target analyte binds to these receptors, it alters the electronic charge distribution, changing the static electric field seen by the charge travelling across the channel which in turns affect the channel conductance. The high charge-to-current conversion of high mobility graphene. This change in conductivity provides a measurable signal, allowing both qualitative (binary detection) and quantitative analysis of the analyte concentration.

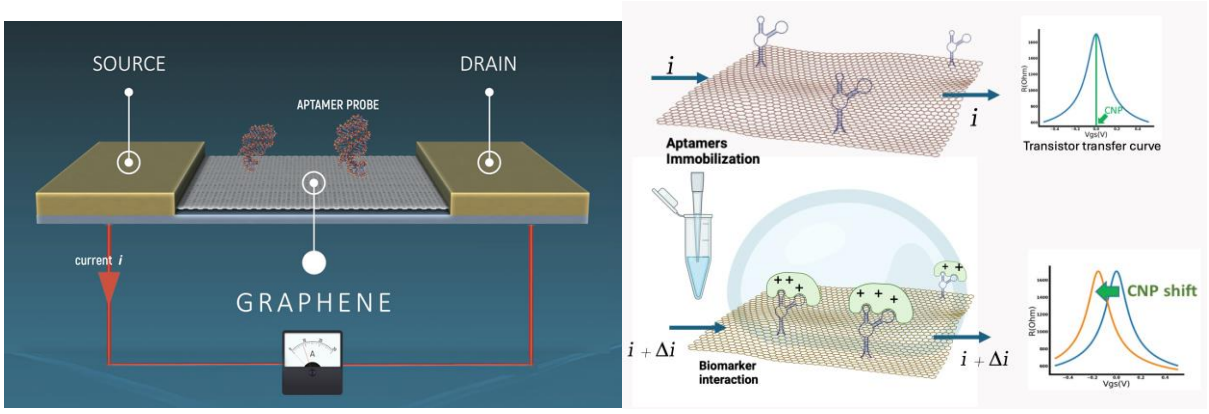

Figure 10. Left: Structure of a graphene field effect transistor (GFET), a current is applied between the two sides contacted electrode and flow inside the graphene base plane this current is affected by the binding of the aptamer to the target protein which are chosen in the set AD biomarkers. This binding can also be characterized by a shift of the charge neutrality point of the GFET (CNP Shift, as seen in right figure).

4.5 Microfluidics

Microfluidics are a key part for the assay design and functionality as they are determining both the diagnostics ergonomics and quality through their effect on the sample preparation. Depending on the 2D-BioPAD system concept (A & B, see figure 7), microfluidics will use respectively the capillary and chromatographic and filtration properties of paper for sample preparation & handling (System A) or the microfluiding channeling of precut laminated plastic hydrophilic channels for system B.

In System A, the paper-based sensing device leverages the intrinsic passive microfluidic properties of paper to facilitate the movement of liquids. This natural characteristic of paper allows it to wick and transport fluids such as buffers and electrolytes efficiently without requiring any external pumps or power sources. When a sample is applied to the paper, the capillary action inherent to the paper's fibrous structure ensures that the liquid spreads evenly across the substrate, effectively "wetting" the paper with the necessary reagents for the electrochemical measurements.

This passive microfluidic action is crucial for the initial stages of the assay, where it ensures that the paper is adequately saturated with the buffer solution and electrolyte. This saturation is essential for maintaining the proper ionic environment necessary for accurate electrochemical measurements. By relying on the passive flow, the device simplifies the operation, making it user-friendly and suitable for point-of-care applications where simplicity and reliability are paramount.

In addition to the passive microfluidic mechanism, the device incorporates active control of the flow using magnetic fields to manipulate aptamers conjugated with magnetic nanoparticles. This magnetic control plays a critical role in enhancing the overall assay performance. During the incubation phase, the external magnetic field ensures that the magnetic nanoparticles, which are functionalised with aptamers, remain concentrated in the vicinity of the target biomarkers. This focused interaction increases the likelihood of binding events, thereby improving the capture efficiency of the target molecules.

Once the incubation is complete, the magnetic field is used to transport the nanoparticles to the detection zone where the graphene electrodes are located. This precise movement ensures that the captured biomarkers are effectively brought into contact with the functionalised electrodes, enabling highly specific and sensitive electrochemical detection. The use of magnetic manipulation allows for greater control over the assay conditions, ensuring that the target molecules are optimally positioned for detection.

Following the detection phase, the magnetic nanoparticles are guided to the absorbent pad. This step is essential for the cleaning process, as it helps to remove unbound and non-specifically bound molecules from the detection area. By directing the nanoparticles to the absorbent pad, the device minimises background signals and reduces non-specific binding, thereby enhancing the accuracy and reliability of the sensor.

In system B which involves GFETs, microfluidics is achieved through hydrophilic channels obtained by stacking, laser-etched laminated PET foils (Figure 11). The precise design of such channel depends on the number of multiplex sensors to be integrated. A pre-processing unit need to be integrated upstream in order to precondition complex matrix such as full blood samples. We will incorporate cellular trapping units or micro-trenches along the flow path to capture blood cells, allowing purified plasma to flow past. These microfluidic techniques offer advantages such as requiring small sample volumes (often just a few microliters), providing rapid, and enabling planar integration with the biosensing unit that are placed downstream on the same laminated foil. Many of these methods can achieve high purity plasma separation with minimal cell lysis, making them suitable for our diagnostic application.

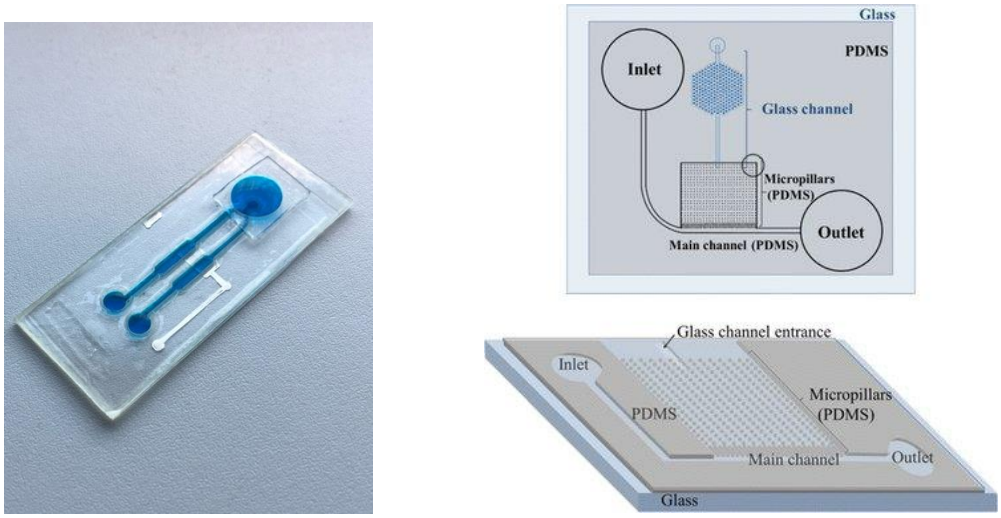

Figure 11. Left: Prototype from Grapheal showing hydrophilic microchannel guiding fluids on graphene GFET sensors Right figure: top and side views of the microfluidic device incorporating rugged structures withing the channel (such as micropillars arrays) that separate red blood cells from the blood samples. (Park et al. 2016) taken from "Capillary flow-driven blood plasma separation and on-chip analyte detection in microfluidic devices" by Snaha maria et al. Microfluidics and Nanofluidics 21(4) 2017, DOI: 10.1007/s10404-017-1907-6.

4.6 Processor, (s) Wireless communications and Casing

A common data flow approach (Figure 12) based on system compliant with both technologies will be chosen, even if in the first version the electronic board component may differ significantly between System A and System B.

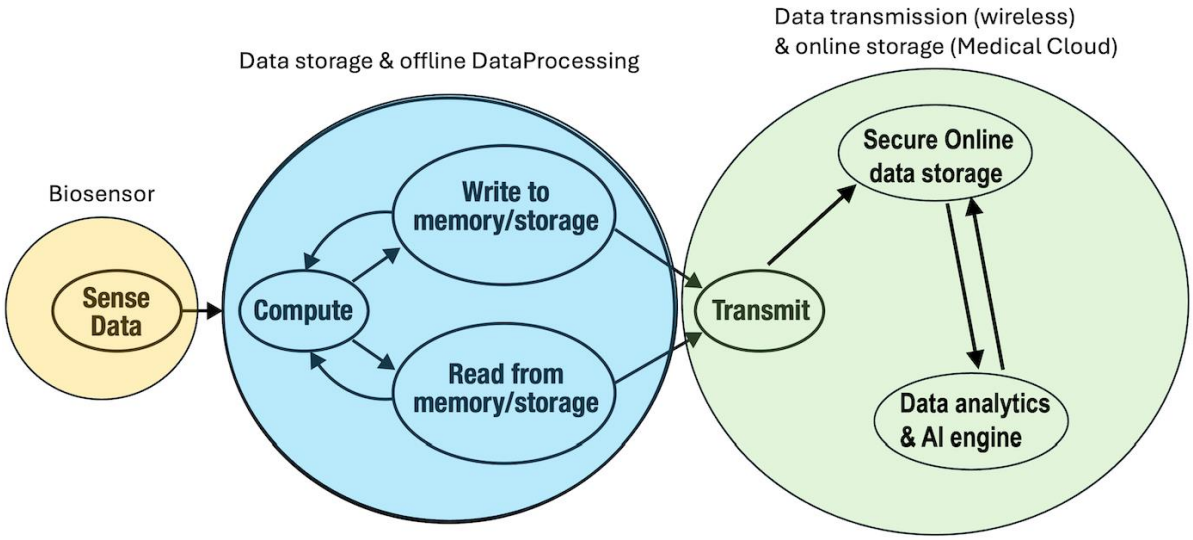

Figure 12: Principle for the Data flow and analytics in an in-vitro digital diagnostic device.

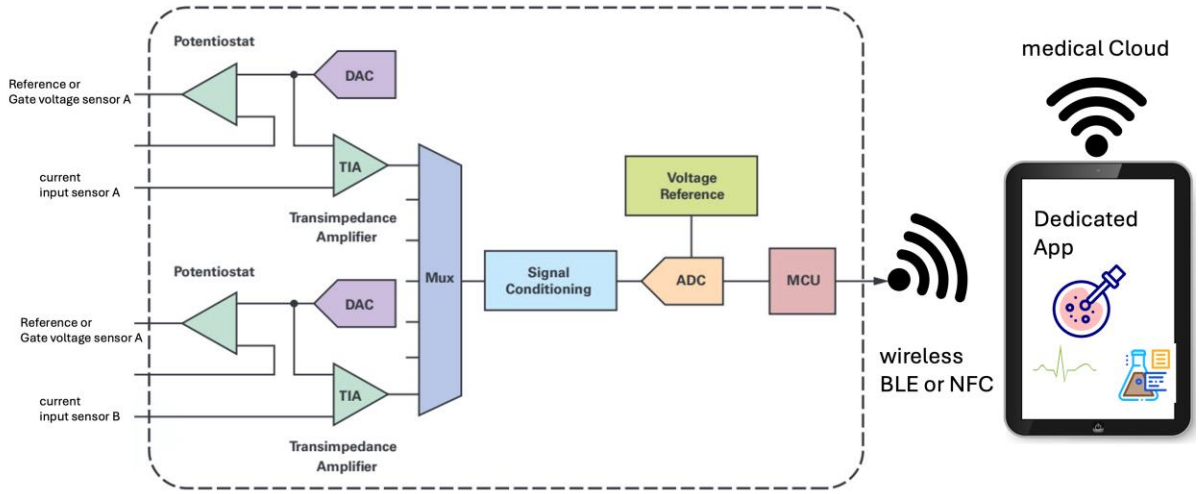

Figure 13: Analytical diagram for the analog/digital hardware principle.

We are designing an in vitro diagnostic device based on electronic sensor with wireless communication to a tablet (Figure 13). There are key considerations for the processor, wireless communications, and casing.

4.6.1 Processor, (s) Wireless communication and Casing for System A (potentiostat and nanoparticles)

The system will be controlled by an Arduino BLE 33 microcontroller, which includes a Bluetooth module for configuring the magnetic device via a mobile app. The Arduino's digital pins will activate and deactivate the various coils as needed. Due to the high current required to generate the magnetic fields, power transistors from the IRF family will be used. These transistors cannot be directly operated by the Arduino's logic pins, so they are interfaced through an NDS7002 transistor.

In series with each coil, a shunt resistor with a very low value is placed to sense the current flowing through the coil. This information is fed back to the microcontroller, which uses a digital-to-analog converter (DAC) to define the voltage across the coil, ensuring the desired current and magnetic field are achieved. Figure 14 shows the general schematic as well as diagrams for controlling each coil's operation and measuring the current in them.

The casing will initially be prototyped using a 3D printer and/or resin printing to test different iterations until the final design is achieved. This process will depend on the controllers and the size of the PCB mentioned earlier. Once the final design is reached, the components can be machined to ensure the device is robust.

The robust casing will serve multiple purposes: housing all the components of the device, ensuring protection and durability, and facilitating the control of magnetic nanoparticles throughout the assay. Additionally, the casing will ensure effective wireless communication with the app and the precise measurement of biomarkers at the electrode.

The system will utilize two integrated circuits (ICs): an NFC-enabled potentiostat (SIC4341) and an NFC-analog sensor interface (SIC4340). Both ICs operate without batteries and connect to smartphones using NFC technology for biomarker detection.

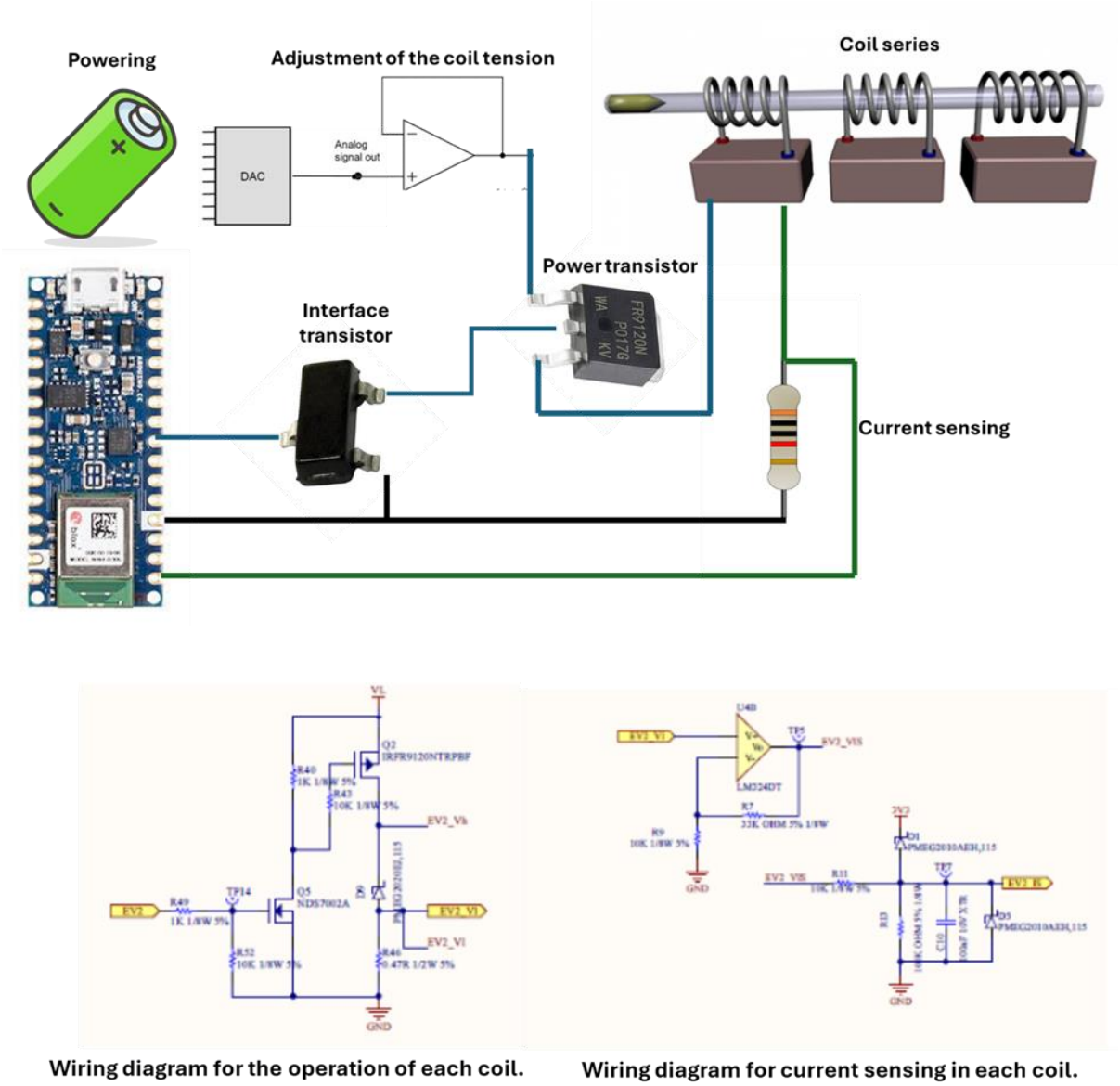

Figure 14. General schematic and detailed diagrams for controlling the operation of each coil and measuring the current.

The system includes an Arduino BLE 33 microcontroller interfaced with IRF power transistors through NDS7002 transistors. A shunt resistor in series with each coil allows current sensing, which is fed back to the microcontroller for precise control of the magnetic field.

4.6.2 Processor, (s) Wireless communication and Casing for System B (GFET controller)

Processing Unit:

The device incorporates a microcontroller that handle sensor data acquisition, signal processing, local storage and communication. Key features are integrated and include:

1. Low power consumption to extend battery life and minimize it size and cost

2. Sufficient processing power to handle sensor data, on board analytics and wireless communication (Bluetooth or NFC)

3. High quality (24 bits) Integrated analog-to-digital converter (ADC) coupled to multiplex for interfacing with multiple graphene biosensors targeting different biomarkers

4. Built-in timers and interrupts for precise timing of measurements

5. Adequate memory for storing firmware and temporary data.

Our option is based on an ARM Cortex-M series microcontroller, such as the STM32L4 series, which offers a good balance of performance and power efficiency [1].

Wireless Communications:

For Bluetooth communication with a tablet, we will have

1. Bluetooth Low Energy (BLE) module for energy-efficient wireless communication

2. Compliance with Bluetooth 5.0 or later for improved range and data transfer rates

3. Integrated antenna or provision for an external antenna

4. Support for secure pairing and data encryption to protect sensitive medical information.

We are basing this part on the Nordic nRF52 series, which integrates both the microcontroller and Bluetooth functionality in a single chip.

Casing:

Its main use is to protect the internal components from physical damage and contamination/ medical-grade plastics (e.g., ABS, polycarbonate) will be chosen for the reader casing, with a minimalistic design to avoid unnecessary waste the following

1. Biocompatibility: Use materials that are safe for contact with biological samples and resistant to common disinfectants

2. Protection: Ensure the casing provides adequate protection for internal components against moisture, dust, and physical impact

3. Ergonomics: Design for ease of use, considering factors like grip, and overall form factor

4. Regulatory compliance: Adhere to relevant medical device standards and regulations

5. Aesthetics: Create a professional and reassuring appearance appropriate for a medical device

6. Assembly and serviceability: Design for easy assembly and potential future servicing or battery replacement.

5. User Interfaces & User Guide

5.1 Mobile app and data visualisation

5.1.1 Overview

ICN2 will work on the control of nanoparticles with magnetic properties to enable real-time movement and positioning as well as performing electrochemical measurements. To achieve this, a device capable of interacting with the magnetic properties of the particles will be developed, along with software to control it. This document outlines the expected operation of the software and specifies the functional and non-functional requirements anticipated for the system, while allowing for the possibility of future requirements.

The software will be developed as a smartphone application for both iOS and Android platforms. This application will control the movement of the nanoparticles (and aptamers, in turn) through a magnetic actuator system and measure variations with a sensor to determine the position of the particles.

To control movement, the system can activate, deactivate, or invert the magnetic field multiple times as needed to induce motion. Additionally, it will be necessary to control the intensity, frequency, and duration of the magnetic field. Position control of the particles is a primary objective, so the system must continually locate the particles, save this data, and allow the user to view it.

Our application will be designed for non-technical personnel, presenting a simple and intuitive interface. However, it must also provide researchers with more precise control, necessitating two different interfaces and a mechanism to differentiate users according to their roles.

Finally, the application will connect to the physical device using NFC (Near Field Communication) potentiostat, which operates at short distances for the electrochemical measurements. Data will be stored in a European hub, enabling data exchange.

5.1.2 Functional Requirements

RF-01	The program will be a smartphone application for controlling and tracking magnetic particles.
RF-02	The application will allow electrochemical measurements using NFC potentiostats.
RF-03	The application will have an advanced interface for researcher/administrator personnel.
RF-04	The application will have a basic interface for non-technical personnel (medical staff).
RF-05	The application will communicate with various commercially available NFC chips.
RF-06	The application will be able to identify the detected chip.
RF-07	The application will offer appropriate measurement options and parameters based on the detected chip.
RF-08	The application will distinguish between administrator and clinical staff roles via identification methods (passwords, fingerprints, etc.).
RF-09	Administrator users will be able to redefine the storage hub.

RF-10 Administrator users will manage user information (create, modify, delete).

RF-11 The application will include a user guide.

RF-12 The application will store settings related to specific sensor use.

RF-13 The application will allow modification of parameters related to test control.

RF-14 The application will control the start of the test.

RF-15 The application will store trial data in a European hub.

RF-16 The application will access data from previous tests stored in the European hub.

RF-17 The administrator interface will manually determine test characteristics.

RF-18 The administrator interface will allow the creation of new tests and their storage in the hub.

RF-19 The administrator interface will allow test modifications and updates in the hub.

RF-20 The clinical staff interface will allow selection of the test type to be performed.

RF-21 The application will check the hub for characteristics necessary to perform a specific test.

RF-22 The application will control the position of the magnetic particles during the test.

5.1.3 Non-Functional Requirements

RNF-01 The system will be compatible with Android smartphones, and iOS if the NFC chip libraries are available.

RNF-02 Minimum system requirements will be specified to ensure optimal application performance.

RNF-03 The end-user interface must support multiple measurements for a specific sensor if required by the administrator.

RNF-04 The user interface (UI) will be modern with good usability.

RNF-05 The application will be entirely in English, with the possibility of other languages.

RNF-06 The project will include detailed technical documentation on the application's operation.

RNF-07 For custom development, the source code will be well-commented for proper interpretation by other programmers, especially for functions, variable declarations, queries, etc.

RNF-08 The application will be scalable to allow for future improvements.

RNF-09 Tests will be conducted to ensure the system operates correctly.

RNF-10 A tutorial will be included to guide users through the application upon first use.

RNF-11 A section for contacting administrators for support and feedback will be included.

RNF-12	The application will alert the user if measured values fall outside the range set by the administrator.

5.2 Digital User Guide

The mobile app will feature a comprehensive Digital User Guide designed to assist HCPs in effectively utilising the device. This guide will provide detailed, step-by-step instructions on various aspects of the device's operation, ensuring that users can easily navigate and utilise all functionalities. Key sections of the guide will include: i) Getting Started: Initial setup instructions, including how to power on the device, pair it with the mobile app, and perform the first diagnostic test; ii) Using the Device: Detailed usage instructions, covering how to collect samples, load them into the device, and interpret the results displayed on the app; iii) Maintenance and Care: Guidelines on how to properly clean and maintain the device to ensure optimal performance and longevity; iv) Troubleshooting: Common issues and their resolutions, helping users quickly address and fix minor problems without needing technical support.

5.3 HCP Training

To enhance the usability and ensure proper handling of the device, we will incorporate a training feature within the mobile app. This training feature should be comprehensive and user-friendly, guiding users through the essential steps and best practices for using the device. Below are the preliminary guidelines to be included in this training module:

1. Getting Started with the Device

Unboxing the Device: Instructions on how to safely unbox and set up the device for the first time.

Charging the Device: Detailed steps on how to charge the device, including the type of charger to use and the estimated charging time.

2. Using the Device

Powering On/Off: How to turn the device on and off, including any necessary steps to prepare it for use.

Operating the Device: A step-by-step guide on using the device, including: i) How to load the cassette; ii) How to initiate the testing process; iii) How to interpret the results displayed on the device.

Maintenance Tips: Regular maintenance procedures to keep the device in optimal condition, such as cleaning instructions and software updates.

3. Disposing of the Cassette

Safe Removal: Instructions on how to safely remove the used cassette from the device.

Disposal Guidelines: Proper disposal methods for the cassette, adhering to local regulations on biomedical waste. Include information on:

Disposal containers.

Safety precautions to avoid contamination or injury.

Contact information for local disposal services if needed.

4. Troubleshooting

Common Issues: A list of common issues users might encounter and how to resolve them, such as:

Device not powering on.

Error messages during operation.

Unclear or incomplete test results.

Customer Support: Contact information for customer support for issues that cannot be resolved through troubleshooting.

5. Additional Resources

User Manual: Access to a digital version of the comprehensive user manual within the app.

Video Tutorials: Links to video tutorials for visual and step-by-step guidance on using the device.

FAQs: Frequently asked questions and answers to provide quick help for common concerns.

Implementation Steps

App Integration: Embed the training module within the app, ensuring it is accessible from the main menu.

Interactive Elements: Include interactive elements such as quizzes and checklists to enhance user engagement and retention.

Updates and Feedback: Regularly update the training module based on user feedback and advancements in device features.

By incorporating these guidelines and features, the mobile app will serve as a comprehensive resource, ensuring users can effectively and safely use the device, thereby enhancing overall user experience and device performance.

5.4 User Feedback

To continuously improve the device and the mobile app experience, a dedicated feature for HCPs to provide feedback will be integrated into the app. This feature will allow HCPs to share their experiences, report any issues, and suggest enhancements directly through the app. Feedback submission will be straightforward, with a designated section accessible from the main menu.

6. Conclusions

The development of the 2D-BioPAD device represents a significant advancement in the early diagnosis and monitoring of AD. Our comprehensive approach addresses the urgent needs and challenges faced by HCPs in POC diagnostics for AD. By leveraging plasma biomarkers such as amyloid beta (Aβ) 1-40 and 1-42, tau proteins, neurofilament light chain (NFL), glial fibrillary acidic protein (GFAP), TDP-43, and beta-synuclein, the 2D-BioPAD offers a robust and non-invasive method for early detection and progression monitoring of AD.

The functional and non-functional technical specifications have been meticulously crafted to ensure the device's reliability, efficiency, and ease of use. The system overview and detailed examination of subcomponents, including aptamers, magnetic nanoparticles, electrodes, biosensing technology, microfluidics, processors, wireless communications, and casing, highlight the innovative engineering and design that underpin the 2D-BioPAD's capabilities.

Our mobile app will enhance user experience by providing data visualisation, a digital user guide, and comprehensive training modules for HCPs, ensuring they can effectively utilise the device in clinical settings. Additionally, the app's feedback mechanism will allow for continuous improvement and adaptation to user needs, fostering an interactive and responsive user interface.

In conclusion, the 2D-BioPAD device embodies our commitment to revolutionising AD diagnostics through cutting-edge technology and user-centric design. It aims to empower HCPs with precise, timely, and actionable insights, ultimately improving patient outcomes and advancing the field of neurodegenerative disease diagnostics.

2D BioPAD

Supple Graphene Bio-Platform for point-of-care early detection and monitoring of Alzheimer's Disease

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