

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

1st Semester Internal Report

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Executive Summary

This report summarises the effort and achievements of the 2D-BioPAD consortium during the 1st semester of the project, spanning from the 1st of October 2023 (M1) to the 31st of March 2024 (M6).

In short, the following **main activities** have been carried out:

- A detailed desk research has been conducted which was also supported and refined by the results of 26 semi-structured interviews and 90 online survey responses (out of 197 participants) to draw clear guidelines and requirements for the 2D-BioPAD system in 5 categories: (i) Users' Needs and Challenges, (ii) Clinical Practice, (iii) Biomarkers, (iv) PoC IVD requirements, and (v) Ethics and Safety (WP1).
- The Ethical Consideration Roadmap (ECR) was developed to support the ethics-by-design approach for the design and implementation of the 2D-BioPAD system (WP1).
- Advancements in rGO Electrode Integration and Electrochemical Sensing were made (WP1).
- The binding affinity/interaction of literature aptamers against Aß peptides was evaluated. Different formulation of MNPs were synthesized to be checked for effective conjugation with selected aptamers and preliminary results for aptamers are already running to establish a rigorous protocol for the specific DNA aptamers for AD protein biomarkers (WP2).
- ICN2 produced nanostructured electrochemical three-electrode cells using an environmentally friendly, low-cost printing/stamping technology in a single step. They also successfully modified the electrodes and designed two different strategies for the detection of thrombin as target model (WP3).
- The Clinical pilot study coordination team was established, an outline of the Protocol synopsis was prepared for the retrospective and prospective studies and the mapping of existing clinical and research protocols at all clinical sites was performed. Moreover, UEF started preparing the full clinical study protocol and related documents (WP5).
- The dissemination and communication plan (D6.1) of 2D-BioPAD was defined and implemented, synergies were established with relevant projects, particularly with the Graphene Flagship and a wellestablished innovation and IPR management methodology was deployed, aimed at developing the exploitation and sustainability plan. Moreover, the 2D-BioPAD visual identify along with a basic promotional package were developed, the online channels for communication and dissemination of the project were launched and the project's animated video was produced (WP6).
- Five digital monthly meetings and the Kick-off Meeting took place. The consortium started preparing for the 2nd Semester meeting. The templates for the 1st semester internal financial and activity report were prepared (WP7).
- The first version of the Data Management Plan (D7.2) has been submitted covering in detail the envisioned data expected to be generated or processed during the technical activities of the project, with the exception of the clinical data which will be elaborated in D5.1 and we will be included in the second version of the DMP (D7.3).

Overall, the progress of 2D-BioPAD during the 1st semester is in-line with the planning of the project, as this is described in the Description of the Action (DoA), with all deliverables due so far submitted, with the exception of D1.1, which will be submitted on the 24th of April, after agreed extension. The D1.1 delay had as a result that MS1 "Deep Dive Results, Requirements and Design Principles for 2D-BioPAD Available" will also be delayed by one month and will be achieved by the end of M7. That said, these delays are minor and were decided to secure the quality of comparative analysis among the interview and survey results of D1.1.



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1. Explanation of the work carried out per WP

1.1 Work Package 1 - Requirements & System Architecture (ICN2)

1.1.1 Overall objectives and progress within the semester

Work Package 1 (WP1) of 2D-BioPAD runs from M1 (October, 2023) to M24 (September, 2025) of the project, with a view to meeting the following objectives, as set out in the Description of the Action (DoA):

- **Objective 1.1**. Identify and map the needs and challenges for early point-of-care diagnostics for AD
- **Objective 1.2**. Analyse the needs, challenges, and available solutions for reliable, cost-effective, safe, and ethical early diagnosis of AD in the context of introducing design guidelines for next-generation 2D-material-based PoC IVD systems.
- **Objective 1.3**. Co-design the 2D-BioPAD system requirements and architecture, covering both functional and non-functional aspects towards a reliable, time- and cost-effective point-of-care early diagnosis of AD.

In brief, the activities conducted under WP1 over the 1st semester of the project included:

- A detailed desk research has been conducted to create a knowledge base regarding: (i) biomarkers for early detection of MCI to AD progression, (ii) clinical needs and challenges; (iii) available or forthcoming technological solutions such as 2D-material-based point-of-care (PoC) diagnostic devices; (iv) key actors; and (v) socioeconomic perspectives for clinics and health systems.
- 26 semi-structured interviews and 90 online survey responses (out of the 197 participants) refined the findings of the desk research and allowed to draw clear guidelines and requirements for the 2D-BioPAD system in 5 categories: (i) Users' Needs and Challenges, (ii) Clinical Practice, (iii) Biomarkers, (iv) PoC IVD requirements, and (v) Ethics and Safety.
- Ethical Consideration Roadmap (ECR) Development: i) Prepared an ECR outlining ethical principles and guidelines for the 2D-BioPAD project; ii) Reviewed and commented on by consortium partners, ensuring comprehensive input and buy-in.
- Advancements in rGO Electrode Integration and Electrochemical Sensing: i) Successfully integration of rGO electrodes into LFAs using a scalable method; ii) Optimized transfer process and solutions to prevent sample leakage have been developed; iii) Electrochemical sensing capabilities assessed.

1.1.2 Progress per task

Task 1.1: Point-of-care AD diagnostics' User-centred Requirements, Needs and Challenges (**Q-PLAN** | M1-M6)

In the context of Task 1.1 with a view to providing the necessary intelligence for the design and development of the 2D-BioPAD system and clinical pilot study protocols, a thorough analysis was conducted to establish the knowledge regarding (i) biomarkers for early detection of MCI to AD progression, (ii) clinical needs and challenges; (iii) available or forthcoming technological solutions such as 2D-material-based point-of-care (PoC) diagnostic devices; (iv) key actors; and (v) socioeconomic perspectives for clinics and health systems.



This analysis unfolded in four steps. Desk research has been conducted, using online reports, scientific publications, and expertise from within the consortium to establish an initial knowledge base regarding all required topics, shedding light also on basic technical and clinical aspects related to the 2D-BioPAD activities.

To validate and expand this knowledge base, **26 semi-structured interviews** were carried out with experts, covering technology providers, decision-makers, healthcare professionals, and citizens (patients and caregivers), following a Patient and Public Involvement and Engagement (PPIE) approach, to gather feedback and fine-tune the knowledge base, ensuring its clinical orientation. The core elements of the fine-tuned knowledge gathered from the desk research and the semi-structured interviews was translated into an online survey to gather more detailed views from all involved actors, i.e., decision-makers, healthcare professionals (primary and specialized), biomarker experts, patients and caregivers. The online survey **reached 197 stakeholders with 90 complete responses** in total.

As a final step, the survey results were analysed to extract useful insights concerning five main categories: (i) Users' Needs and Challenges, (ii) Clinical Practice, (iii) Biomarkers, (iv) PoC IVD requirements, and (v) Ethics and Safety.



Figure 1. Summary of main user needs, challenges and PoC IVDs requirements for AD diagnosis and progression monitoring, as extracted from T1.1 and T1.2 activities.

The task was completed successfully during the 1st semester of the project and its detailed outcomes are presented in "D1.1: MCI to AD Biomarker Deep Dive Analysis for Early Diagnosis", which was submitted with an extension on the 24th of April 2024.

Task 1.2: Safety and ethics by design (EVNIA | M1-M6)

An Ethical Consideration Roadmap (ECR) has been prepared by M6 and have the objectives to detail ethics principles that must be applied in the project and guides the 2D-BioPAD project work to be performed in an ethically acceptable manner. Moreover, the ECR describes forthcoming actions, and responsibilities to ensure that the ethics requirements and applicable regulations and guidelines are met and applied within the 2D-BioPAD project.



All consortium partners have had the opportunity to review and comment on the ECR from March 26 to April 4 (project M6). The ECR has been submitted as a part of D1.1 and has been made available on the 2D-BioPAD project website in its full version (link).

The task was completed successfully during the 1st semester of the project and its detailed outcomes are included in "D1.1: MCI to AD Biomarker Deep Dive Analysis for Early Diagnosis", which was submitted with an extension on the 24th of April 2024.

Task 1.3: System Architecture co-design (ICN2 | M4-M12, M22–M24)

In the context of T1.3 activities focused on overcoming current limitations of electrochemical detection in LFAs, by fully integrating rGO electrodes into LFA strips (Figure 2), using CO₂ laser to simultaneously reduce GO and pattern nitrocellulose.



Figure 2: 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.

The suitability of the electrodes for electrochemical sensing has been assessed through CV by allowing 10 μ L of 5 mM [Fe(CN)₆]^{3-/4-}in PBS (10 mM) to flowing into the nitrocellulose, ensuring that everything was sufficiently wetted and that there was no liquid on the top of the rGO electrodes themselves. The system containing [Fe(CN)₆]^{3-/4-} exhibited a quasi-reversible behavior, as shown by the clear separation between the reduction and oxidation peaks, which was above 59 mV (Figure 3). To further evaluate the rGO electrodes on nitrocellulose, the Randles-Sevick equation was applied to calculate the electroactive area, which was determined to be 0.96 ± 0.13 mm².





Figure 3: Electrochemical characterization of the rGO electrodes on nitrocellulose. a) Cyclic voltammograms in 5 mM [Fe(CN)₆]^{3-/4-} (PBS 10 mM, pH 7.4) at different scan rates. b) Oxidation and reduction peak separation at each scan rate.

* GRAPHEAL did not provide input about the design of the GFETs.

1.1.3 Role of partners

Task		Partners' main role
T1.1	•	Q-PLAN : As the Task leader, Q-PLAN was responsible for the coordination of the Task, as well as the coordination and preparation of D1.1 with partners' input. Q-PLAN performed the desk research with supporting material from all partners and organized the process for the Semi-structured Interviews. Q-PLAN conducted 3 interviews and analysed the results from all 26 after their completion. Q-PLAN also supported the design and promotion of the Online survey. Finally, Q-PLAN led the documentation of D1.1 with close collaboration with EVNIA.
	•	UP-CATRIN : Performed 1 semi-structured interview (technology provider) and promotion of the online survey. Provided technical information about the use of graphene for biosensing applications, with focus on AD.
	•	ICN2 : Performed 1 semi-structured interview with a technical provider and promoted the online survey to relevant stakeholders in Spain. Provided technical information about the use of graphene for biosensing.
	•	GRAPHEAL : Provided technical information about the use of graphene for biosensing.
	•	AUTH : Performed 2 semi-structured interviews. Provided technical information about the use of magnetic nanoparticles for biosensing applications in AD.
	٠	NOVA : Provided technical information about the use of aptamers for the targeting specific analytes in biosensing applications, with examples related to AD.
	•	UEF : Participated in development of interview guides and questionnaires, carried out 5 semi-structured interviews with biomarker experts, HCPs, and citizens
		(patients/caregivers) using a PPIE approach; shared online survey; provided technical information about AD biomarkers and their current status in Finland.
	•	GAADRD : (i) Performed 5 semi-structured Interviews with HCPs, Expert, and Patients, Shared the online Survey to a large amount of GAADRD contacts; (ii) Communicated the project to social and scientific public, shared the newsletter to the official Alzheimer Europe webpage; (iii) Provided technical information about AD biomarkers and their current status in Greece.



Task	Partners' main role
	 EVNIA: (i) Performed two semi-structured interviews, (ii) Prepared and conducted the Online Survey, (iii) Prepared the Online Survey Report, (iv) Reviewed D1.1 Report. ZI: Performed 6 semi-structured interviews with biomarker experts, HCPs, and citizens (patients/caregivers) using a PPIE approach; shared online survey; provided technical information about AD biomarkers and their current status in Germany. CeADAR: Carried out an interview with an HCP decision maker. Provided technical information about the use of AI in material sciences and aptamer selection.
T1.2	 EVNIA: As the Task leader, EVNIA has prepared the ECR as guidance on how to apply ethics in the 2D-BioPAD project. UP-CATRIN: Identified and suggested two possible experts for the SIAB. Q-PLAN: Collaborated with EVNIA and UEF to introduce ethics and safety elements in the semi-structured interviews and the online survey under T1.1. Reviewed and provided feedback and input in the ECR. ICN2: Reviewed and provided feedback and input in the ECR. GRAPHEAL: Reviewed and provided feedback and input in the ECR. UEF: Collaborated with EVNIA and Q-PLAN to introduce ethics and safety elements in the semi-structured interviews and the online survey under T1.1. GAADRD: Reviewed and provided feedback and input in the ECR. ZI: Reviewed and provided feedback and input in the ECR. ICN2 and UEE performed the guality review of D1 1
T1.3	 ICN2: As the Task leader, ICN2 started to produce electrochemical lateral flow by making use of graphene-based materials. First prototypes available UP-CATRIN: Informed partners on the possibilities on tailored functionalized graphenes that UP-CATRIN can offer. Q-PLAN, EVNIA: Supported with providing input from T1.1 and T1.2 to align on requirements for the design of the 2D-BioPAD system. GRAPHEAL: - AUTH: prepared three different morphologies of magnetice/Au nanohybrids (dupbells, core-shell, in network) and provided samples to ICN2 (Feb 2024) for further evaluation in biomarker binding and quantitative analysis. NOVA: Provided relevant information for integration of aptamers in the assays. UEF, GAADRD, ZI: Provided technical information about AD biomarkers. CeADAR: Literature review in AI models for aptamer affinity prediction and aptamer generative AI.

1.1.4 Deviations

The deliverable D1.1 deadline was extended (by 3 weeks) due to the need to analyse and compare in more detail the results from the semi-structured interviews and online survey.



1.2 Work package 2 – Biomarkers binding and quantitative analysis (AUTH)

1.2.1 Overall objectives and progress within the semester

Work Package 2 (WP2) of 2D-BioPAD runs from M4 (January, 2024) to M30 (March, 2026) of the project, with a view to meeting the following objectives, as set out in the Description of the Action (DoA):

- **Objective 2.1.** Identification, synthesis, functionalisation, and optimisation of DNA aptamers for AD protein biomarkers.
- **Objective 2.2.** Identification, synthesis, characterisation, and evaluation of MNPs as carriers and enablers.
- **Objective 2.3.** Conjugation of aptamers, MNPs, and Biomarkers with AD protein biomarkers.
- **Objective 2.4.** Evaluation and validation of individual and conjugated components.

In brief, the activities conducted under WP2 over the 1st semester of the project included:

- Task 2.1 initiated and is still running to identify DNA aptamers for specific AD protein biomarkers. Three aptamers from the literature have been evaluated for targeting Aβ1-40 and Aβ1-42 biomarkers without positive results, leading to the initiation of a SELEX process for new aptamers.
- Task 2.3 has also initiated its activities, within which different formulation of MNPs have already been provided (synthesis and characterisation) to be checked for effective conjugation with selected aptamers of Task 2.1.
- Task 2.4 is currently underway with reference specimens to provide a rigorous framework for future experimental sequences.

1.2.2 Progress per task

Task 2.1: Identification, synthesis, and evaluation of Aptamers for AD protein biomarkers (**NOVA** | M4-M24)

NOVA has evaluated the binding affinity/interaction of literature aptamers (i.e., RNV95, T-SO508 and Aβ7-92-1H1) against Aß peptides. The interaction was observed by standard techniques of Surface Plasmon Resonance (SLR), Bio-Layer Interferometry (BLI), and Electrophoretic Mobility Shift Assays (EMSA).

During the literature exploration, NOVA has also identified reported aptamers against Tau proteins phosphorylated at specific sites, GFAP and NFL.

After thorough investigation, it was found that aptamers RNV95 and TSO508 were not able to recognize neither $A\beta_{40}$ nor $A\beta_{42}$ peptides (HFIP treated peptides). Accordingly, $A\beta$ 7-92-1H1 was only found to have affinity to $A\beta$ 40 instead of $A\beta$ 42, as originally mentioned in the literature.

Since stability and change in the form/structure is a big concern for these peptides, NOVA is performing additional experiments on A β -Apt aptamer (i.e., A β 7-92-1H1) before coming to the final interaction report.



Since the performance of existing aptamers has been found inefficient, NOVA has initiated a new selection process for new aptamers for the A β biomarkers. The aptamer selection process (SELEX) is underway for both A β_{40} and A β_{42} peptides.

To support the selection process, CeADAR performed a detailed comparative analysis of various advanced computational methodologies used in aptamer binding research, including MLPD¹, RaptGen², AptaNet³, APIPred⁴, AptaBERT⁵, and AptaTrans⁶, to facilitate understanding of the essential computational strategies for aptamer research. These methods ranged from experimental techniques combined with machine learning to sophisticated transformer—based models designed for predicting and generating high—affinity aptamer sequences.

Each methodology was assessed on its description, key features, and inherent limitations, which informed their functionality ratings and suitability for specific research scenarios. This analysis aimed to enhance the selection process and development of high–affinity aptamer candidates for targeting AD biomarkers.

Additionally, we checked existing databases such as Apta-Index, <u>Aptagen</u> and the <u>UTexas</u> Aptamer database to support our research efforts. Following the guideline outlined by Heredia et al., 2021⁷, we performed a sample data analysis protocol which was completed. Next step is to specifically examine how this is applied to the specific biomarkers of 2D-BioPAD interest.

Key findings from this exploration will support the aptamer selection process to maximise its effect and accelerate the identification of appropriate aptamers.

Task 2.2: Optimization and functionalisation of aptamers (**NOVA** | M13-M24)

This task did not start within this reporting period of the project thus no actions have been taken yet.

Task 2.3: Synthesis and characterisation of magnetic nanoparticles as carriers and enablers (AUTH | M4-M24)

In Task 2.3 of WP2, AUTh focused on the synthesis and characterization of magnetic nanoparticles (MNPs) for biomarker binding and quantitative analysis. The implementation thus far has centred on the synthesis of Au-Magnetite (Au/Fe₃O₄) MNPs of various sizes, with a keen eye on structural, morphological, and magnetic characterization through techniques such as TEM, XRD, and VSM. To date, AUTh has successfully synthesized and structurally characterized three distinct NP systems.

¹ 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

² Iwano, N., Adachi, T., Aoki, K. et al. Generative aptamer discovery using RaptGen. Nat Comput Sci 2, 378–386 (2022). https://doi.org/10.1038/s43588-022-00249-6

³ Emami, N., Ferdousi, R. AptaNet as a deep learning approach for aptamer–protein interaction prediction. *Sci Rep* 11, 6074 (2021). https://doi.org/10.1038/s41598-021-85629-0

⁴ Fang, Z., Wu, Z., Wu, X., Chen, S., Wang, X., Umrao, S., & Dwivedy, A. APIPred: An XGBoost-Based method for predicting aptamer– protein interactions. *J. Chem. Inf. Model.* 64, 7 (2023). https://doi.org/10.1021/acs.jcim.3c00713

⁵ Morsch, F., Umasankar, I. L., Sanz Moreta, L., Latawa, P., Lange, D. B., Wengel, et al. AptaBERT: Predicting aptamer binding interactions. *bioRxiv*, 2023-11 (2023). https://doi.org/10.1101/2023.11.24.568626

⁶ 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). https://doi.org/10.1186/s12859-023-05577-6

⁷ Heredia FL, Roche-Lima A, Parés-Matos EI (2021) A novel artificial intelligence-based approach for identification of deoxynucleotide aptamers. *PLoS Comput Biol* 17(8): e1009247. https://doi.org/10.1371/journal.pcbi.1009247



- Firstly, we achieved Au-Magnetite (Au/Fe₃O₄) MNPs through a coprecipitation method, utilizing phytic acid as a solid template for subsequent citrate reduction of Au nanoparticles.
- Secondly, we developed Fe₃O₄@Au core-shell MNPs employing a rapid and efficient coprecipitation method. This approach yielded Fe₃O₄-Au NPs with enhanced properties.
- Thirdly, AUTh pursued the synthesis of dumbbell-like Au-Fe₃O₄ nanoparticles. Here, AUTh utilized the Turkevich method⁸ to create reference gold nanoparticles, which served as seed materials in a two-step synthesis process. Iron pentacarbonyl (Fe(CO)₅) decomposition over the surface of Au nanoparticles, followed by oxidation under air, facilitated the creation of these unique structures.



Figure 4: XRD Analysis of Synthesized MNPs. The analysis depicts diffraction peaks of the prepared magnetic nanoparticles (MNPs). The peaks observed correspond to the main diffraction patterns of gold (Au) and magnetite (Fe₃O₄), represented in gold and black colours respectively, highlighting the distinct crystalline structures present in the synthesized samples.

The XRD analysis of the synthesized systems showcases distinct diffraction peaks corresponding to Fe_3O_4 and Au, affirming the successful synthesis of our desired nanoparticles. Overall, the progress in WP2 demonstrates significant strides in the synthesis and characterization of magnetic nanoparticles, laying a solid foundation for further biomarker binding and quantitative analysis studies.

Task2.4:Immobilization,FunctionalizationandEvaluationofConjugatedMNPs/Aptamers/Biomarkers (AUTH | M18-M30)

Task 2.4 initiated actions at M4, **prior to its official kick-off (M18)**, when WP2 activities started. AUTH firstly reviewed suggested protocols for aptamer conjugation on gold MNPs while also receiving information from our colleagues from T2.3. We have decided to begin experimenting on thiolo (SH)-functionalized aptamers (maybe the most referenced of the techniques for conjugating aptamers on gold), while also another

⁸ Dong, Jiaqi, et al. "Synthesis of precision gold nanoparticles using Turkevich method." KONA Powder and Particle Journal 37 (2020): 224-232.



approach: polyadenylated (pA)-tail aptamers are also to be studied as a cost-effective and probably more promising functionalization technique. Thus, on close communication with partners from NOVA and ICN2, AUTH begun experimentation with SH- and pA-aptamer targeting thrombin: a widely referenced aptamer.

To date, AUTH has experimented around two different conjugation protocols on pure gold nanoparticles: a) salt-aging and b) freezing-directed conjugation. This ability has been verified with both spectroscopic and fluorometric techniques.

In addition to experimental work, AUTH has also advised on the efforts of the other tasks of WP2. First, we have provided feedback to Task 2.3 regarding conjugation efficiency and thus assisted on the planning of future experiments. Also, AUTH has provided information to Tasks 2.1 and 2.2 regarding the handling of amyloid beta species and provision of peptides from vendors. Also, sensitivity values of interest for clinical evaluations were shared, in order to increase signal to noise ratio.

Task	Partners' main role
T2.1	 NOVA: As the task leader, NOVA was responsible for Identification, synthesis, functionalization, and optimization of DNA aptamers for AD protein biomarkers. They are checking which aptamers provide adequate conjugation with selected AD protein biomarkers. AUTH: Organized monthly meetings between partners to discuss Task 2.1 progress and next steps. CeADAR: (i) Literature review and model proposals for application when a dataset is received from the WP partners; (ii) detailed comparative analysis of various advanced computational methodologies used in aptamer binding research, to minimize the reservoir of potential biomarkers.
T2.2	• No action as this task will start in M13.
T2.3	 AUTH: As the task leader, worked (and will continue working) on MNPs synthesis and characterization to provide adequate functionalization with selected aptamers and provide source material for initial tests in WP3. Also, as the WP leader, AUTh organized regular meetings for WP2 while concurrently working on Task 2.3 to provide suitable NPs to be effectively conjugated with selected aptamers (Task 2.4) as outlined in Task 2.1. NOVA: Provided (and will continue providing) the necessary feedback and testing of conjugated MNPs/aptamers with respect to sole aptamers.
T2.4	• AUTH: Initiated reference experiments to validate a rigorous protocol to quantify MNPs/aptamers conjugation.

1.2.3 Role of partners

1.2.4 Deviations

There was no deviation.



1.3 Work package 3 – Graphene-based platform design and implementation (UP-CATRIN)

1.3.1 Overall objectives and progress within the semester

Work Package 3 (WP3) of 2D-BioPAD runs from M7 (April, 2024) to M36 (September, 2026) of the project, with a view to meeting the following objectives, as set out in the Description of the Action (DoA):

- **Objective 3.1.** Identify and develop optimum graphene designs for conjugation of the biorecognition units (ssDNA), preserving conductivity, electrochemical activity and active structure of the ssDNA.
- **Objective 3.2.** Identify and develop optimum Janus graphene designs for non-covalent, but highly robust modification of the graphene gate on the FET sensor.
- **Objective 3.3.** Fabricate and evaluate electrochemical and GFET sensor devices, integrating effectively the conductive graphene electrodes with the functionalized graphene derivatives for high selectivity.

Even though WP3 hasn't officially started yet, preliminary actions have been taken by consortium partners. In brief, the **activities conducted under WP3 over the 1**st **semester** of the project included:

1.3.2 Progress per task

Task 3.1: Functionalized graphene synthesis and ssDNA conjugation (UP-CATRIN | M7-M30)This task did not start within this reporting period of the project thus no actions have been taken yet.

Task 3.2: Functionalized Janus Fluorographene synthesis and ssDNA conjugation (**UP-CATRIN** | M7-M30)

This task did not start within this reporting period of the project thus no actions have been taken yet.

Task 3.3: Fabrication and Testing of the Graphene-based Electrochemical biosensor (**ICN2** | M13-M36)

Even though T3.3 starts in M13, ICN2 has been proactive and initiated activities early on. More specifically, ICN2 produced nanostructured electrochemical three-electrode cells using an environmentally friendly, low-cost printing/stamping technology in a single step.

ICN2 successfully modified the electrodes with oligonucleotides terminally modified with a thiol group by using thiol chemistry.

ICN2 also designed two different strategies for the detection of thrombin as target model. In **strategy #1**, a self-complementary motif has been introduced into the thrombin aptamer to create an aptamer beacon (AB), that in the absence of the target will be in its non-binding state. In **strategy #2** the thrombin aptamer has been splitted in two portion that will assemble just in the presence of the target (i.e. Thrombin).





Figure 5: a) Design of rGO@AuNPs electrodes. b) Image of rGO@AuNPs electrodes. c) SEM micrographs of rGO@AuNPs film. d) Cyclic Voltammetry of rGO@AuNPs electrodes performed in 3 mM in $[Fe(CN)_6]^{4-}/ [Fe(CN)_6]^{3-}$ solution in KCl 0.1 M at 25 mVs⁻¹.

Task 3.4: Fabrication and Testing of the Graphene-based FET biosensor (**GRAPHEAL**| M13-M36) *This task did not start within this reporting period of the project thus no actions have been taken yet.*

Task 3.5: AI-based optimisation graphene/ssDNA – MNPs/Aptamer/Biomarker optimisation (**CeADAR** | M18-M36)

This task did not start within this reporting period of the project thus no actions have been taken yet.

1.3.3 Role of partners

Task	Partners' main role
T3.1	No action as this task will start in M7.
T3.2	• No action as this task will start in M7.
Т3.3	 ICN2: First prototype of the electrochemical biosensor introduced, targeting Thrombin.
T3.4	• No action as this task will start in M13.
T3.5	No action as this task will start in M18.

1.3.4 Deviations

Not applicable.



1.4 Work package 4 – Device development and system integration (GRAPHEAL)

1.4.1 Overall objectives and progress within the semester

Work Package 4 (WP4) of 2D-BioPAD runs from M10 (July, 2024) to M42 (March, 2027) of the project, with a view to meeting the following objectives, as set out in the Description of the Action (DoA):

- **Objective 4.1.** Apply advanced microfluidics for effective handling of biological fluids on the device towards reliability identifying simultaneously multiple biomarkers
- **Objective 4.2.** Deliver the required firmware to support a digitalised PoC IVD including an Intelligent Decision Support module for more effective data processing and diagnostics
- **Objective 4.3.** Develop a mobile app-based user interface that will allow interoperable and secure connection with the PoC IVD device and the Cloud, while also offering user-friendly guidance and visualisation of the extracted results
- **Objective 4.4.** Integrate, test, and re-fined the 2D-BioPAD PoC IVD system

Within this reporting period of the project, WP4 hadn't started yet.

1.4.2 Progress per task

Task 4.1: Advanced Microfluidics for Identifying Multiple Biomarkers (GRAPHEAL | M19-M36) *This task did not start within this reporting period of the project thus no actions have been taken yet.*

Task 4.2: Intelligent decision support module & User Interfaces (GRAPHEAL | M10-M36)This task did not start within this reporting period of the project thus no actions have been taken yet.

Task 4.3: Casing prototyping and assembly (GRAPHEAL | M25-M36)

This task did not start within this reporting period of the project thus no actions have been taken yet.

Task 4.4: Integration, lab testing, and fine-tuning (GRAPHEAL | M18-M42)

This task did not start within this reporting period of the project thus no actions have been taken yet.

1.4.3 Role of partners

Task	Partners' main role
T4.1	 No action as this task will start in M19.
T4.2	 No action as this task will start in M10.
T4.3	No action as this task will start in M25.





1.4.4 Deviations

Not applicable.



1.5 Work package 5 – Clinical Pilot Studies Design, Deployment, Evaluation & and Validation (UEF)

1.5.1 Overall objectives and progress within the semester

Work Package 5 (WP5) of 2D-BioPAD runs from M4 (January, 2024) to M48 (September, 2027) of the project, with a view to meeting the following objectives, as set out in the Description of the Action (DoA):

- **Objective 5.1.** Identification, analysis, and preparation of the 2D-BioPAD clinical pilot studies.
- **Objective 5.2.** Deployment and close monitoring of the 2D-BioPAD device at the clinical centres.
- **Objective 5.3.** Evaluation and validation of the 2D-BioPAD device's performance under several clinical use cases.
- **Objective 5.4.** Perform a cross-pilot comparative assessment.
- **Objective 5.5.** Consolidate lessons learnt, best practices and recommendations for policies, regulations, and standardisation.

In brief, the **activities conducted under WP5 over the 1**st **semester** of the project included:

- Clinical pilot study coordination team established, including PIs and core team members from each clinical site;
- Protocol synopsis prepared for retrospective and prospective studies, and shared with all Consortium members for feedback;
- Mapping of existing clinical and research protocols at all clinical sites;
- Started clinical site activities for facilitating the deployment of the clinical studies;
- Started preparing the full clinical study protocol and related documents.

1.5.2 Progress per task

Task 5.1: Pilot Studies' Deployment & Evaluation Design (**UEF** | M1-M12, M22-M24)

During this reporting period of the project, focus has been on preparatory activities for the 2D-BioPAD clinical pilot study including both retrospective and prospective parts. The clinical pilot study coordination team was established, including PIs and core team members from each clinical site. An online meeting of the coordination team was organized to jointly plan specific Task 5.1 activities. The team prepared a protocol synopsis for the retrospective and prospective studies, which was shared with all Consortium partners for feedback. A detailed mapping of existing clinical and research protocols at all clinical sites was conducted to facilitate decisions on specific study design aspects, including list of clinical, cognitive and other biomarker assessments. Work on drafting the full clinical study protocol and related documents is ongoing. Other site-specific preparatory activities have also been initiated.

Task 5.2: Retrospective pilot study deployment and technical validation (**UEF** | M25-M36)

This task did not start within this reporting period of the project thus no actions have been taken yet.



Task 5.3: Prospective pilot study deployment and clinical validation (**GAADRD** | M30-M48) This task did not start within this reporting period of the project thus no actions have been taken yet.

Task 5.4: Cross-regional pilot studies evaluation and validation (**ZI** | M40-M48)

This task did not start within this reporting period of the project thus no actions have been taken yet.

1.5.3 Role of partners

Task	Partners' main role
T5.1	 UEF: As the Task leader, UEF was responsible for the coordination of the Task, as well as the coordination and preparation of the clinical study protocol (synopsis, full protocol and associated documents) with partners' input, organizing clinical coordination team meetings, and developing the template for clinical site mapping; conducted clinical site preparations. UP-CATRIN: - Q-PLAN: Reviewed all documents circulated by the Task leader. Interaction in terms of timeline and expectations as per the GA. Alignments with T1.1 findings. ICN2: read study design and protocols defined by the task leader and provided input if necessary. GRAPHEAL: - AUTH purchased a fully automated benchtop immunological analyzer for biomarker determination: β-Amyloid 1-40, β-Amyloid 1-42, pTau 181, pTau 217, NfL, ApoE4 and Pan-ApoE and informed all partners in BCN meeting. NOVA: Read study design and protocols defined by the task leader and provided input. GAADRD: Completed the shared excel file with the current clinical practice of GAADRD, provided key input on all clinical study design aspects and protocol and documents; conducted clinical site preparations. EVNIA: Provided input on ethical aspects related to the clinical study protocol and documentation. ZI: Completed the shared excel file with the current clinical practice of ZI, provided key input on all clinical study design aspects of ZI, provided key input on all clinical study design aspects and protocol and documentation.
TE 3	CeADAR: No activities yet.
15.2	No action as this task will start in M25.
T5.3	No action as this task will start in M30.
T5.4	 No action as this task will start in M40.

1.5.4 Deviations

There was no deviation.

1.6.1 Overall objectives and progress within the semester

Work Package 6 (WP6) of 2D-BioPAD runs from M1 (October, 2023) to M48 (September, 2027) of the project, with a view to meeting the following objectives, as set out in the Description of the Action (DoA):

- **Objective 6.1.** Create awareness, communicate, and disseminate value propositions, activities and results.
- **Objective 6.2.** Analyse 2D-BioPAD's market and conclude on commercially viable and sustainable business models.
- **Objective 6.3.** Elaborate a regulatory and business plan to guide sustainable post-project commercial exploitation.
- **Objective 6.4.** Set up continuous communication pathways and synergies with complementary initiatives, including support to the Graphene Flagship Initiative.
- **Objective 6.5.** Define and apply 2D-BioPAD's Innovation and IPR Management Strategy throughout the project.

In brief, the **activities conducted under WP6** over the 1st semester of the project included:

- **Development of 2D-BioPAD visual identify along with a basic promotional package** (i.e. logo, colour palette, .doc and .ppt templates, banner, leaflet and poster) to be used by project partners while interacting with stakeholders in the framework of their communication and dissemination activities.
- Launch of online channels for communication and dissemination, including the project's website and social media pages within Facebook, Twitter, LinkedIn and YouTube.
- Production of the project animated video.
- Deployment of dissemination and communication activities, including:
 - Regular update of the project's **website and social media pages** with content about the progress of the project, participation in events and conferences, etc.
 - Development of content and publication of the **first biannual newsletter** on M6 and **one (1) press release** in M1.
 - Presentation of the 2D-BioPAD activities in **14 events**
 - Participation in 1 joint activity with Graphene Flagship Initiative.

In order to raise awareness about the project as well as foster participation in its activities we also completed the below mentioned activities:

Elaboration of the project's "Dissemination and Communication Plan" (DCP) to organize and guide the
partners' activities throughout the course of the project. The documentation outlining the
dissemination and communication strategy, along with related KPIs and milestones for the 1st period,
can be found in D6.1, titled "Dissemination and Communication Plan and Activities, Version 1". This
document serves to facilitate the implementation and monitoring of the strategy, along partners in



planning and executing their respective activities with clearly defined KPIs to measure their progress and results.

- Establishment of synergy with one (1) relevant project (i.e., MUNASET) at European level, that can feed and synergise with the different activities planned throughout the course of 2D-BioPAD, and identification of two (2) more.
- Definition and implementation of 2D-BioPAD Innovation and IPR Management Strategy with a view to ensuring a smooth pathway for the post-project exploitation of the project's results, and thus, safeguarding their long-term sustainability. The IPR Management Strategy is included in D6.4 "Exploitation and Sustainability Plan –Version 1", designing potential exploitation plans per Key Exploitable Result (KER).
- Active engagement and interaction with the GrapheneEU CSA project and the Graphene Flagship Initiative stakeholders, with key partners involved in all working groups, with highlight the coorganisation of the Graphene Week 2024 in Prague in October 2024.

1.6.2 Progress per task

Task 6.1: Dissemination and communication strategy, plan, and activities (Q-PLAN | M1-M48)

- **Design of a visually appealing graphical identity for 2D-BioPAD**, including a logo with a standard colour palette, as well as templates to be used for presentations and project deliverables.
- **Development of well-tailored** <u>promotional material</u>, including the <u>leaflet</u> of the project, a <u>poster</u>, and a <u>roll up banner</u> that are being used in the framework of creating awareness about the project and its results during relevant events.
- Establishment of an active social media presence by setting up and animating social media, including an X (Twitter) account, a LinkedIn page, a Facebook page and a YouTube channel, to support awareness raising and engagement in project activities, with 446 followers so far.
- Search, identification, and engagement of relevant stakeholders through social media, attracting and enhancing the number of followers of the project's respective social media channels.
- Preparing the project's <u>promotional video</u>. It provides an overview of the project, including vital information, and serves as a great way to highlight the mission and vision. Up to date, the video has been **viewed by 1.687 individuals**, across all project channels.
- Design, launch, and continuous update of the <u>2D-BioPAD website</u>, to serve as the central channel for informing stakeholders on the activities and opportunities offered within the frame of the project as well as development and fine-tuning of its content. The website has already achieved **1.800 unique visitors**.
- **"Dissemination and Communication Plan"** (DCP) guidelines have been developed to better guide the partners' respective activities throughout the course of the project in alignment with its DCP.
- One (1) issue of 2D-BioPAD's <u>newsletter</u> has been published and distributed among various stakeholders.
- One (1) press releases in M1 summarising the kick-off meeting
- **Participation** in **17 external events,** as presented in the table which follows:

No	Partner	Date	Title/Type of activity
1	UP	05.02.2024	Graphene Flagship Initiative kick-off meeting
2	GAADRD	21.10.2023	Speech on "Dealing with Dementia today and in the future. Pharmacological interventions and behavioural disorders" at the one-day conference "Let's Talk about Dementia" at Aradipou Town Hall in Larnaca

Table 1: Participation in external events



No	Partner	Date	Title/Type of activity
			District, Greece, under the auspices of the Honorable Minister of Health Dr. Popi Kanaris
3	GAADRD	06.11.2023	Interview with the journalist Ms Arvanitidis on ERT3 TV station, Thessaloniki, Greece
4	GAADRD	16.11.2023	Speech on "New data in the prevention, diagnosis and treatment of Alzheimer's disease" during a training course at the Healthcare Centre of Thermi, Greece
5	GAADRD	14-16.12.2023	Lecture on "Personalized scanning" in Parkinson's Disease, Dementia, and Alzheimer's Disease at the 3rd Panhellenic Conference of Personalized Medicine at Zappeion Megaron, Athens, Greece
6	GAADRD	16.12.2023	Lecture on "New data on the prevention, diagnosis and treatment of Alzheimer's Disease" upon invitation by the Municipality of Koufalia in Koufalia, Greece
7	GAADRD	16.12.2023	Speech on "Alzheimer's disease: therapeutic developments and prospects" at the Annual Panhellenic Medical Congress, Thessaloniki, Greece
8	GAADRD	21.12.2023	Episode: "Latest advances in the diagnosis of Alzheimer's disease" on the broadcast "We start together" of DION TV station, Thessaloniki, Greece
9	GAADRD	12.01.2024	Lecture on "New data on the prevention, diagnosis and treatment of Alzheimer's Disease" upon invitation by the Municipality of Koufalia in Agios Athanasios, Greece
10	GAADRD	15.01.2024	Episode on "Dementia: The youngest patient is 12 years old - Gene therapy in experimental stage" on the broadcast "Mera Me Chroma" of ERT3 TV station, Thessaloniki, Greece
11	GAADRD	22.01.2024	Broadcast on the radio station "One", 90.4
12	GAADRD	03.02.2024	Lecture on "The function of our brain and its strengthening" at the one-day conference "Education and modern reality" under the auspices of the Directorate of Secondary Education of Eastern Thessaloniki and the Metropolis of Nea Krini and Kalamaria, Greece
13	GAADRD	12.02.2024	Lecture on "Modern diagnostic and treatment procedure in Alzheimer's Disease" upon invitation from the Parents' School at the Open University of Katerini, Greece
14	GAADRD	28.02.2024	Speech on "New approaches in the prevention, diagnosis and treatment of Alzheimer's disease" upon invitation by the Greek Association of Alzheimer's Disease and Related Disorders of Edessa, Greece
15	GAADRD	29.02.2024	Lecture on "New data for the prevention, diagnosis and treatment of Alzheimer's disease" upon invitation by the Municipality of Thermi, Greece
16	GAADRD	31.03.2024	Speech on "Alzheimer's Dementia" at the Municipal Library of Kavala, Greece, upon invitation by the Association of Political Pensioners of Kavala Prefecture
17	AUTh	12.10.2023	Lecture "Biomarkers and Alzheimer's disease

Task 6.2: Innovation management, exploitation, and sustainability (**Q-PLAN** | M1-M48)

Task 6.2 aims to manage the knowledge that will emerge from the 2D-BioPAD activities and plan for their protection (where relevant) and exploitation beyond the end of the grant.

Within the first reporting period of the project, the Exploitation and Sustainability Plan, Version 1 (D6.4) was elaborated and submitted in M6.

The report sheds light on the objectives of IP management in the framework of 2D-BioPAD, presenting an overview of key concepts and terms towards creating better awareness amongst the partners of the



consortium. At the same time, it lays down the main components of our strategy and methodology in this respect before ultimately, describing the results of our work, in terms of Background as well as Key Exploitable Results (KERs) identified so far by the partners. The outline of 2D-BioPAD's KERs is presented in this report together with the partners' preliminary plans and actions for post-project exploitation. In particular, specific exploitation plans were crafted per partner and each identified KER, including target groups that stand to benefit from their use, key exploitation routes, necessary protection measures, as well as actions required for advancing the exploitation readiness and potential of each KER by the end of the project and beyond.

Task 6.3: Business modelling and planning (**Q-PLAN** | M13-M48)

This task did not start within this reporting period of the project thus no actions have been taken yet.

Task 6.4: Regulatory Acceptability Activities, Plan, and Policy Recommendations (**EVNIA** | M1-M6, M37-M48)

A Regulatory Affairs Plan Template has been prepared in M2 and has the objectives to:

- Connect design input (URS) and design outputs (SRS) with device classification and risk assessment.
- Connect design outputs and classification with clinical requirements.
- Provide guidance on regulatory requirements and standards to be marketed in Europe.
- Provide a timeline of regulatory and clinical activities, towards device approval in EU, under the EU MDR.

Policy Workshops will be carried out in the 2D-BioPAD project process. No workshops are planned at present.

The Regulatory Affairs Plan will be finalised based on design inputs provided during the 2D-BioPAD project activities and workshop discussions (project M48).

Task 6.5: Networking & joint activities with relevant initiatives (**Q-PLAN** | M1-M48)

To support networking and joint activities with relevant initiatives, Q-PLAN created the excel file "<u>2D-BioPAD</u> <u>Synergies List</u>" to collect the partners' suggestions of potential candidates for synergies. The document will remain on the repository throughout the duration of the project, so partners can indicate their suggestions, as well as the synergetic actions they performed with relevant initiatives.

Q-PLAN along with UP-CATRIN established a communication pathway with the Graphene Flagship Initiative (GFI) and participated representing 2D-BioPAD: (i) in the GFI's meetings on the 24/11/2023, 12/12/2023; (ii) in the common Kick-off meeting on the 05/02/2024; (iii) in the D&C WG meeting on the 14/03/2024; (iv) in the Graphene Flagship Workshop on Research Data Management on the 12/03/2024; (v) in the Roadmap WG meeting on the 27/03/2024. 2D-BioPAD representatives have been appointed in all WGs, actively engaging and supporting GF activities.

Q-PLAN and UP Initiated communication with the sister project MUNASET and held an introductory meeting with them on the 27th of February, where Q-PLAN invited them to participate online in the 2D-BioPAD 2nd Project Meeting in Barcelona, presenting their project. The MUNASET partners participated in the 2nd project



meeting, presenting their project and engaging in meaningful discussions with the 2D-BioPAD partners, about more synergies in the future.

Q-PLAN has also already initiated discussions with Alzheimer Europe, the European Brain Council and the project COMFORTAGE, whereas the European Academy of Neurology has supported the diffusion of the online survey through their website.

1.6.3 Role of partners

Task	Partners' main role
T6.1	 Q-PLAN: As the Task leader, Q-PLAN was responsible for:(i) the design, implementation monitoring and fine-tuning of the Dissemination and Communication Plan (DCP); (ii) Developed the project's logo and visual identity; (iii) Developed the project's website; (v) Created LinkedIn, X (Twitter), Facebook accounts, and YouTube channel; (vi) Created the project's promotional video; (vii) Creating social media posts for the project; (viii) Released one (1) newsletter per six-monthly frequency; (ix) Issued one (1) press release; (x) Prepared and submitted the Deliverables D6.1; (xi) Monitoring of project activities and collection of input from partners regarding their activities and results. ICN2 shared all the promotional materials, the newsletter and all the news on its communication channel. AUTH and UP-CATRIN participated in the common Graphene Flagship Initiative (GFI) Kick-off meeting on the 05/02/2024 and discussed the possibility to integrate 2D-BioPAD activities with other relevant projects (i.e. MUNASET). GAADRD: had a very strong presence in external events, having participated in 19. All partners were responsible for performing individual dissemination and communication activities and reporting them in the dissemination reporting excel of the project (here). All partners also provided their feedback on dissemination materials and provided materials to be included in the newsletter.
T6.2	 Q-PLAN: As the Task leader, Q-PLAN (i) created templates to collect input from partners regarding their plans to exploit the project's assets; (ii) supported partners in providing their input; and (iii) elaborated the "Exploitation and Sustainability Plan", Version 1 (D6.4). All partners provided their input regarding their plans to exploit the project's assets. Task Leaders provided more details regarding the KERs' description and exploitation pathways.
T6.3	 No action as this task will start in M13.
т6.4	 EVNIA: As the Task leader, EVNIA prepared the Regulatory Plan Template (M2). All partners provided feedback for the Regulatory Plan Template.
т6.5	 Q-PLAN: As the Task leader, Q-PLAN (i) Created an excel file to collect the partners' suggestions of potential candidates for synergies; (ii) established a communication pathway with the Graphene Flagship Initiative and with the MUNASET project, (iii) participated in the GFI's meetings and working groups; (iv) invited MUNASET to introduce themselves online during the 2nd project meeting of 2D-BioPAD. Appointed as representative for the GF D&C, Innovation/Business, and Roadmapping WGs.



Task	Partners' main role
	• UP-CATRIN : As the coordinator, it represents the project in the GF Governance and Coordination bodies, including the Project Managers Network. Co-chair for the Graphene Week 2024.
	 ICN2: Participation at Graphene Flagship Innovation Working Groups meeting. GRAPHEAL: Appointed as representative for the GF Standardisation WG AUTH: participated in the common kick-off meeting of Graphene Flagship Initiative (05/02/2024) and discussed potential synergies with relevant projects on
	 biomedicine and nanomaterials. EVNIA: Was appointed as representative for the GF Standardisation WG. All partners provided suggestions of potential candidates for synergies.

1.6.4 Deviations

There was no deviation.



1.7 Work package WP7 – Project management & Coordination (UP-CATRIN)

1.7.1 Overall objectives and progress within the semester

Work Package 7 (WP7) of 2D-BioPAD runs from M1 (October, 2023) to M48 (September, 2027) of the project, with a view to meeting the following objectives, as set out in the Description of the Action (DoA):

- **Objective 7.1.** Safeguard effective collaboration, fulfilment of the project's goals and delivery of highquality results.
- **Objective 7.2.** Set up and manage the operation of the Industrial Advisory Board of 2D-BioPAD.
- **Objective 7.3.** Ensure sound and FAIR management of the data collected, processed and/or generated by the project.
- **Objective 7.4.** Prepare and run project meetings as well as produce progress (periodic and final) reports.

All the above objectives were targeted throughout the first semester of the project, as **Work Package 7 (WP7)** runs since M1. All the objectives were successfully met until now, and all the WP7 deliverables (D7.1-Management and Quality Plan & D7.2- Data Management Plan, Version 1) were submitted.

During the 1st semester, the main activities that took place under this WP include:

- **Coordination and quality management**, to safeguard effective coordination and collaboration among project partners and monitor progress.
- Establishment of the Scientific and Industrial Advisory Board (SIAB) with 7 experts.
- Documentation of the initial version of the Data Management Plan (DMP) covering 34 datasets.
- Organisation of the 1st project meeting as well as 5 digital meetings amongst project partners to coordinate, exchange ideas and plan the activities of the project.
- Distribution of the prefinancing payment was completed on time.

The following part offers a more detailed description of the activities performed under the framework of WP7.

1.7.2 Progress per task

Task 7.1: Coordination and quality management (UP-CATRIN | M1-M48)

In task 7.1, Deliverable 7.1 "Management and Quality Plan" was elaborated and distributed to all project partners and finally submitted at the end of December 2023. defines the overall project management principles and procedures applied to 2D-BioPAD and the quality assurance (QA) provisions for safeguarding high-quality project outcomes. It describes the roles and responsibilities of each project participant, with emphasis on work breakdown and management, progress reporting, financial monitoring, payment processes, risk identification and change management.



More details with respect to management and coordination in the framework of **2D-BioPAD** are included in "D7.1- Management and Quality Plan", which was submitted on M3.

Within the same task, a common repository was created on the Microsoft Teams platform, which is hosted by UP-CATRIN. This repository allows sharing files and instant communication between partners, document editing by several authors in parallel, and hosting meetings. Finally, an extension to the due date for the delivery of D1.1 was requested to allow the elaboration of the survey analysis.

To facilitate the project management, an Action List (.xlsx format) was created and was continuously updated during the 1st semester, with to the focus on monitoring the status of all key project activities under each Task and Work Package.

Furthermore, to facilitate the monitoring of the project's progress and the activities performed, UP-CATRIN and Q-PLAN administered and organised **monthly updating calls** (see T7.4). The latter was also served by several ad-hoc **communications and discussions that were taking place** among project partners, either via e-mail, or through digital tools and applications to coordinate, align on and organise the activities of the project, as well as to deliver quality results on time. To facilitate this type of communication within the consortium, a **dedicated mailing list** was developed: <u>2d-biopad@qplan-intl.gr</u>, as well as a relevant excel sheet including the main contact persons per each partner.

Task 7.2: 2D-BioPAD Industrial Advisory Board (GAADRD | M1-M48)

Within this reporting period of the project, GAADRD was responsible for the formulation of the Scientific and Industrial Advisory Board (SIAB) of the project. In particular, starting in M1, GAADRD collected 15 candidates for the SIAB, proposed by all partners based on their expertise on the different aspects of this project. From the initial 9 candidates, we have already onboard 7 members who have already been invited to attend the 2nd project meeting. The SIAB members are selected based on their expertise in Clinical settings, Nanotechnology, Policy, and Ethics. We intend to add two more members covering the industrial aspect as well.

No.	Name	Organisation	Position	Type of Organisation	Country
1	Charlotte Teunissen	Amsterdam UMC	Professor	Academia	The Netherlands
2	Graham Armitage	EIT Health Ireland	Interim MD	Academia	UK
3	Fabiana Arduini	University of Rome / SENSE4MED	Professor / Founder	Academia / Industry	Italy
4	Puerto Morales	Institute of Material Science in Madrid	Professor	Academia	Spain
5	Oliviero Gobbo	Trinity College Dublin	Senior Research Fellow	Academia	Ireland
6	Mercè Boada	Ace Alzheimer Center Barcelona	Professor	Academia	Spain
7	Oliver Smith	Daedalus Futures	Founder and Director	Industry	UK

Table 2: Composition of the 2D-BioPAD SIAB



Task 7.3: Data Management (Q-PLAN | M1-M48)

In the frame of Task 7.3, Deliverable 7.2 "Data Management Plan, Version 1" was elaborated and distributed to all project partners and finally submitted at the end of January 2024. It sets out the overall methodological principles pertaining to the management of the data that will be collected, generated and/or re-used in the framework of 2D-BioPAD, safeguarding sound and ethical data management along the entire duration of the project. Moreover, it provides a first, yet still meaningful overview of 2D-BioPAD's data (34 datasets in Version 1), as identified in this early stage of the project, along with information on the methodology pertaining to their management as well as to making them Findable, Accessible, Interoperable and Re-usable (FAIR).

The initial version of the DMP is the first of the three versions of 2D-BioPAD's DMP to be produced in the course of the project and will serve as a living document (D7.2 DMP – Version 1 delivered in M4 will be updated to D7.3 DMP-Version 2 in M24 and ultimately fixed as D7.4 DMP- Version 3 in M48). Along these lines, the DMP will be updated and further elaborated during the project to reflect an accurate, up-to-date and ultimately comprehensive plan for managing the data that will be collected, generated and/or re-used by the project across their entire life cycle, both during and after the completion of 2D-BioPAD.

Following the guidelines for open data, the 2D-BioPAD Zenodo Community was created.

All the information on data management, as well as the identification and description of the different data collected, processed and/ or generated in the context of **2D-BioPAD** is provided in "Data Management Plan, Version 1", which was submitted on M4.

Task 7.4: Project Meetings and Reporting (**UP-CATRIN** | M1-M48)

In the frame of Task 7.4, the **kick-off meeting** of 2D-BioPAD took place digitally on the 10th – 11th of October 2023 in Thessaloniki, Greece and was organised by the Aristotle University of Thessaloniki (AUTh), with the participation of representatives of all project partners. The PC presented the project, including overall aim and objectives, foreseen activities and results, the role of partners, expected outcomes, and work structure breakdown. Each Work Package (WP) leader, presented the overall objectives, team members, tasks, time plan, foreseen deliverables, milestones, and means of WP communication & expected results of their WP. The Task leaders presented the objectives, approach to follow, KPIs, interdependencies with other tasks, time plan & expected outcomes of their Tasks. An Action Plan for the first semester was defined, including activities per partner, important deadlines & responsible partner(s). The project kick-off meeting minutes were prepared by the PMO (Q-PLAN) and distributed to all project partners, providing an overview of the meeting and its different sessions, emphasising the critical decisions made.





Figure 6: The 1st Project Meeting

In addition, **digital monthly meetings** took place in November and December 2023, as well as January, February and March 2024 to follow up the actions foreseen in the Action Plan of the first semester. After each meeting, the updated version of the action plan and the meeting minutes were distributed to all partners.

The second project meeting of the 2D-BioPAD project will take place on the 17th and 18th of April in Barcelona, with ICN2 as the host.

Finally, **internal reporting templates** were prepared, for both financial and activity reporting, and distributed to all partners. Each partner is responsible for submitting internal financial and activity reports to the project coordinator every six months, based on those templates. Based on all partners' internal financial and activity reports, the project coordinator will elaborate the respective "1st Semester Internal Report" for the whole project, as well as the following reports to come. At the end of the 1st Semester, all partners will be asked to report their activities and financials.

Task	Partners' main role
T7.1	 UP-CATRIN: As the Task leader, reviewed and submitted deliverable D7.1: "Management and Quality Plan". Moreover, as PC, they made the final review of deliverables D6.1, D6.4 and D7.2 and submitted them. Finally, they asked for a one- month extension for the submission of D1.1. Q-PLAN, as the PMO, prepared the deliverable D7.1: "Management and Quality Plan" and developed a set of 8 administrative forms to be used during the project implementation. All partners supported the effective and efficient organisation of project activities by participating and contributing to the discussions held to this end. All partners reviewed and provided feedback for D7.1.
T7.2	 GAADRD: As the Task leader, was responsible for organising and monitoring the selection process for the SIAB members of the project, for contacting them and securing their participation. They set-up and finalized the SIAB, by gathering proposals from all partners, contacting the candidates, and collecting all the necessary data. UP-CATRIN, Q-PLAN, GRAPHEAL, NOVA EVNIA: Identified and suggested possible experts for the SIAB.

1.7.3 Role of partners



Task	Partners' main role
	 ICN2: Identified and suggested possible experts for the SIAB. Among them <u>Fabiana</u> <u>Arduini</u> that accepted the invitation.
	 AUTH: Identified and suggested possible experts for the SIAB. Among them <u>Oliviero</u> <u>Gobbo</u> and <u>M.P. Morales</u> who accepted the invitation.
	 UEF, GAADRD, ZI: Identified and suggested possible biomarker and clinical experts for the SIAB. Among them <u>Charlotte Teunissen</u> who accepted the invitation.
	• CeADAR : Identified and suggested possible experts for the SIAB. Among them <u>Merce</u> <u>Boada</u> , <u>Oliver Stone</u> and <u>Graham Armitage</u> who accepted the invitation.
T7.3	 Q-PLAN: As the Task leader, prepared the deliverable D7.2: "Data Management Plan, Version 1", developed guidelines for partners to identify and report datasets to be collected during the project and crated the project's Zenodo community. UP-CATRIN & EVNIA performed the quality review of D7.2. All partners provided input/feedback on D7.2 DMP Version 1.
T7.4	 UP-CATRIN: As the Task leader and the leader of WP7, (i) assisted the organisation of the kick-off project meeting, and organised 5 digital monthly meetings to follow up on the progress of project activities; (ii) prepared the financial internal reporting templates to be completed by all partners; (iii) prepared for the 2nd Project Meeting that will take place in Barcelona on the 17th -18th of April 2024. Q-PLAN: (i) supported the organization of the kick-off project meeting, as well as the 5 digital monthly meetings; (ii) prepared the activity reporting templates to be completed by all partners; and (iii) supported the preparation of the 2nd Project Meeting. ICN2: As the host of the 2nd Project Meeting, made preparations to welcome the other partners in Barcelona. AUTH: Hosted the Kick-off meeting of the project in Thessaloniki in October 2023. All partners participated in the monthly digital meetings and the Kick-off Meeting in Thessaloniki in October 2023.

1.7.4 Deviations

No deviations encountered under the activities of WP7.



2. Progress against targets and impact

Туре	WP#/ Task#	Indicator of success	Overall Target	Lead Partner	Progress By M6	Status	Comment
01	T2.1	Protein Biomarkers for AD analysed	>=7	NOVA	2	Ongoing	Binding Evaluation for literature aptamers is complete. We are now doing selection of aptamers against Aβ40 and Aβ42 peptides.
	T4.4	System production cost – Device	< 50 €	GRAPHEAL, ICN2	0	Not started	-
	T4.4	System production cost – Strip	<5€	GRAPHEAL, ICN2	0	Ongoing	-
	T4.2	Fast Digitised Testing Results	5-10 min	GRAPHEAL	0	Not started	-
	T4.4	Testing - Screening Reliability	> 85%	GRAPHEAL	0	Not started	-
02	T3.3	Electrochemical	~10 Nm	ICN2	0	Not started	We started to detect DNA sequences in low nM range in order to test our rGO@AuNPs electrodes
	T3.3	GFET Sensitivity	~ 100 atoM	GRAPHEAL	0	Not started	-
	T4.1	Simultaneous biomarker detections	<=5	GRAPHEAL	0	Ongoing	Demonstrated devices up to 2 channels
	T3.1	Functionalisation Degree	10-15%	UP	0	Not started	-
03.	T1.2	Safety and Ethics Guidelines report	1	EVNIA	1	Completed	ECR available - Self-assessments will be made from month 7 to 48
	T1.1	Semi structured interviews	>= 20	Q-PLAN	26	Completed	-
	T1.1 & T5.4	Online Surveys	2	EVNIA	1	Ongoing	The first part is completed. The second is on track for next deployment
	T1.1 & T5.4	Online Surveys' Participants	150	EVNIA	197	Ongoing	-
	T5.1	Training workshops	3 (one per clinical centre)	UEF	0	Not started	Could count training to academic partners. e.g. ICN2 training



Туре	WP#/ Task#	Indicator of success	Overall Target	Lead Partner	Progress By M6	Status	Comment
	T5.1	Training workshops' participants	-	UEF	0	Not started	-
04	T5.3	Clinical Centres involved	3	GAADRD	3	Ongoing	-
	T5.2	Retrospective Study Samples	>=60 (app. 20 per clinical centres)	UEF	0	Not started	-
	T5.3	Feasibility study HCPs	>=6	GAADRD	0	Not started	-
	T5.3	Feasibility study Patients	>=30	GAADRD	0	Not started	-
	T5.3	Prospective study HCPs	>=12	GAADRD	0	Not started	-
	T5.3	Prospective study Patients	>=300	GAADRD	0	Not started	-
05	T6.4	Policy Workshops	2	EVNIA	0	Not started	Co-organise with MUNASET Check if possible to organise within Final Conference
	T6.4	Policy Workshops' participants	>25	EVNIA	0	Not started	-
	T6.4	Policy Briefs	1 (regulation) + 1 (standardisation)	EVNIA	0	Not started	Check with ENVIA + GRAPHEAL if they can handle one each
	T6.4	Regulatory Acceptance Plan	1	EVNIA	0	Ongoing	The plan template is provided. And data from URS activities will be fed in.
06	T6.5	Synergies Accomplished	>= 5	Q-PLAN	4	Ongoing	GFI, MUNASET, EBC, EAN
	T6.5	Joint Events with other initiatives	>= 2	Q-PLAN	0	Ongoing	Policy workshops could be the two + Scientific workshops during the GF2024 + Workshop with MUNASET
	T6.3	Sustainable business models defined	>= 2	Q-PLAN	0	Not started	-
	T6.3	Sustainable business plan defined	1	Q-PLAN	0	Not started	-
	T6.2	Innovation and IPR management strategy	1	Q-PLAN	1	Completed	-
Other	T2.1	DNA aptamers per target synthesized and evaluated for their binding properties and specificity of chosen aptamers	up to 5 per biomarker total 25	NOVA	0	Ongoing	-
	T2.4	LOD	<= 0.25 fM	NOVA	0	Not started	-



Туре	WP#/ Task#	Indicator of success	Overall Target	Lead Partner	Progress By M6	Status	Comment
	T5.1	Protocols for the 2 clinical studies	for the 2 clinical studies	UEF	0	Ongoing	-
KPI	T4.1	AD Biomarkers Identification and Quantification	5	GRAPHEAL	0	Not started	-
	T5.4	Diagnostic/Screening Cost Reduction	Up to €2.500 per patient per examination	ZI	0	Not started	-
	T5.4	Reduced Hospitalization Time	Over 10%	ZI	0	Not started	-
	T4.4	Reduced Blood Testing Time	90-95%	GRAPHEAL	0	Not started	-
	T5.4	Increase in societal trust and acceptance	>75% acceptance from endusers engaged	ZI	0	Not started	-
	T5.3	Provision of evidence-based performance results on realworld problems	Real-world testing at 3 clinics	UEF	3	Ongoing	-
	T5.4	AD literacy and better health management	>300 people	ZI	0	Not started	WP6 & WP1 as well
	T6.1	Misconception phenomena addressed	>1000 people	Q-PLAN	0	Not started	Count workshops, surveys, policy, articles etc.
Diss.Ind.	T6.1	Project workshops and events	>= 6	Q-PLAN	0	Not started	-
	T6.1	Stakeholders participated in project events	> 1000	Q-PLAN	223	Ongoing	-
	T6.1	External events/conferences attended	> 20	Q-PLAN	17	Ongoing	See Table 1
	T6.5	Synergies with initiatives & networks	10 joint actions	Q-PLAN	0	Ongoing	Workshops with MUNASET, Graphene Week, etc
	T6.1	Publications at international journals	>= 4	Q-PLAN	0	Ongoing	-
	T6.1	Publications at international conferences	>= 12	Q-PLAN	0	Ongoing	-
	T6.1	Followers on social media	> 1,000	Q-PLAN	446	Ongoing	-
	T6.1	Views of the promotional video	> 1,000	Q-PLAN	1687	Completed	Including views across all online channels



Туре	WP#/ Task#	Indicator of success	Overall Target	Lead Partner	Progress By M6	Status	Comment
	T6.1	Number of newsletters released	8	Q-PLAN	1	Ongoing	-
	T6.1	Promotional material distributed	> 300	Q-PLAN	0	Ongoing	-
	T6.1	Stakeholders reached in overall	3.000	Q-PLAN	2.290	Ongoing	-
	T6.1	Unique visits to the website	> 5,000	Q-PLAN	1.800	Ongoing	Check if we can measure visits to GFI website.
Event	T6.1	Final conference	1	Q-PLAN	0	Not started	We will seek to organise it as a satellite event at a larger international event.
	T6.1	Participants in final conference	-	Q-PLAN	0	Not started	-
	T1.2 & T1.3	Series of co-creative exercises for materialising the 2D-BioPAD functional and non-functional requirements (co-designing the 2D-BioPAD device)	(at least 3 workshops)	ICN2	2	Ongoing	During the 2nd meeting in presence of SIAB members: -ICN2 will perform the first co- creative exercise. T1.3 Co- creative exercise: "Toward next generation lateral flow assays: Background and recent advances.", and - EVNIA will deploy thevT1.2 Workshop: "Ethical Consideration Roadmap – Application of Ethics in the 2D- BioPAD Project"
	T1.3	Participants in Series of co-creative exercises	-	ICN2	0	Not started	Complementary entry as it is part of KPI#42 - Stakeholders participated in project events
	T5.4	Validation workshops	2	ZI	0	Not started	Will they engage the SIAB? Could do a validation policy workshop?
	T5.4	Participants in Validation workshops	-	ZI	0	Not started	-
GFI	T6.4	Scientific publications (# publications)	>=16	Q-PLAN	0	Not started	-



Туре	WP#/ Task#	Indicator of success	Overall Target	Lead Partner	Progress By M6	Status	Comment
	T6.4	Professional publications (# publications)	-	Q-PLAN	3	Ongoing	UP-CATRIN article and GAADRD articles in newspapers
	T6.4	Open access results (# results (additional description of type of result is requested))	-	Q-PLAN	1	Ongoing	1 st dataset released on Zenodo
	T6.4	Awards (# Awards and type of award)	-	Q-PLAN	0	Not started	-
	T6.4	Invited talks (# talks and event, date, place)	-	Q-PLAN	16	Ongoing	See Table 1
	T6.4	PhDs recruited (# PhDs)	-	Q-PLAN	0	Ongoing	-
	T6.4	Postdocs recruited (# Postdocs)	-	Q-PLAN	2	Ongoing	2 recruited from UP-CATRIN (Dr Sharad Sachan and MSc. Vishnu Gupta)
	T6.4	Joint project proposals (# proposals and project partners involved)	-	Q-PLAN	1	Ongoing	-
	T6.4	Patent Applications (# applications)	-	Q-PLAN	0	Not started	-
	T6.4	Contribution to GrapheneEU CSA organised actions (# contributions, including list of actions)	-	Q-PLAN	1	Ongoing	Presentation in the Kick-off meeting



Туре	WP#/ Task#	Indicator of success	Overall Target	Lead Partner	Progress By M6	Status	Comment
	T6.4	Attendance to GrapheneEU CSA organised actions (# attendees, including which events)	-	Q-PLAN	16	Ongoing	We need to capture how many people have participated in GFI events - 5 in the Kick-off meeting - 4 in the Data management - 1 in CSA board meetings (x3 until March 2024) - 1 Participation in Graphene week organization meetings: x 4 until 5th of April 2024. - 1 in the roadmapping / innovation group - 1 in the D&C group - 2 in the Standardisation Group
	T6.4	Joint workshops organized (# workshops and projects involved)	-	Q-PLAN	0	Not started	-
	T6.4	Early career researcher collaboration (Estimated # of activities, including examples of collaboration)	-	Q-PLAN	0	Not started	-



3. Deliverables scheduled to be submitted within the reporting period

Del. No.	Deliverable name	Lead partner	Nature ⁹	Dissem. level ¹⁰	Due Date ¹¹	Calendar Month	Delivered (Yes/No)	Actual Delivery Date	Remarks
D1.1	MCI to AD Biomarker Deep Dive Analysis for Early Diagnosis	Q-PLAN	R	PU	6	Mar-24	Yes	24/04/2024	Extension requested (see section 1.1.4)
D6.1	Dissemination and Communication Plan and Activities, Version 1	Q-PLAN	R	SEN	3	Dec-23	Yes	20/12/2023	-
D6.4	Exploitation and Sustainability Plan, Version 1	Q-PLAN	R	SEN	6	Mar-24	Yes	28/03/2024	-
D7.1	Management and Quality Plan	UP	R	SEN	3	Dec-23	Yes	20/12/2023	-
D7.2	Data Management Plan, Version 1	Q-PLAN	R	PU	4	Jan-24	Yes	31/12/2024	-

⁹ Nature of deliverable: R = Report, O = Other.

¹⁰ Dissemination level: PU = Public, SEN = Sensitive.

¹¹ Month 1 marking the start date of the project and all delivery dates being relative to this start date.



4. Milestones scheduled to be achieved within the reporting period

Milestone	Milestone Name	WP	Lead	Due	Means of	Achieved	Actual date	Remarks
No.		No	Beneficiary	Date ¹²	Verification	(Yes/No)	achieved	
1	Deep Dive Results, Requirements and Design Principles for 2D-BioPAD Available	1	Q-PLAN	6	D1.1 Available	No	24/04/2024	Achieved based on the extension of D1.1 (see section 1.1.4)

¹² Month 1 marking the start date of the project and all delivery dates being relative to this start date.



5. Future planning

The **main activities foreseen for the following semester** under each WP are listed below.

- Planned activities under WP1 (ICN2)
 - ✓ Optimization of the electrochemical lateral flow strip production.
 - ✓ Preparation of ethical self-assessments per relevant project task will be done from M7 to M48 by WP task leaders to describe and document how ethics is applied in specific project activities.
 - ✓ Work on creating publications based on the results of D1.1, focusing on elaborating more on the survey results and including any additional responses that will be received until the end of May 2024.
 - Elaboration of D1.2 "System Architecture, Version 1" by M9. ToC to be available by the end of May (M8) with clear sections to all technical partners (especially ICN2 and GRAPHEAL).

• Planned activities under WP2 (AUTH)

- ✓ Regular meetings on WP2 will continue occurring in 15-30 days intervals to discuss findings and potentially redefine methodological approach.
- ✓ Discussion with clinical partners and SIAB members is scheduled by the end of May 2024 to validate aptamer selection (and provision of biomarkers).
- ✓ MNPs characterization is underway. MNPs to be examined on operation on graphene substrate (ICN2).
- ✓ MNPs/aptamer conjugation validation and operational controls.
- ✓ Fine-tuning the synthesis parameters to exert precise control over the size of the nanoparticles, both in their core and shell dimensions.
- ✓ Conduct further structural and magnetic characterization of the samples. This comprehensive analysis will provide deeper insights into the composition, morphology, and magnetic behavior of the synthesized nanoparticles.
- Elaboration of D2.1 "Conjugated MNPs/Aptamers Design, Synthesis, and Selection, Version 1" by M12. A ToC should be available by the end of July 2024 (M10).

• Planned activities under WP3 (UP-CATRIN)

- ✓ Graphene derivatives will be synthesized with functionalities appropriate to conjugate aptamers.
- ✓ Different variants of the thrombin aptamer as model aptamer will be tested for the electrochemical detection of thrombin based on the use of graphene related materials.
- ✓ Align with T1.3 to include needed information to D1.2.

• Planned activities under WP4 (GRAPHEAL)

✓ Design of microfluidics for sample fluid management.



- ✓ Integration with digital environment and optimization of ergonomics.
- ✓ Align with T1.3 to include needed information to D1.2.

• Planned activities under WP5 (UEF)

- ✓ Completing protocol for the clinical pilot studies and related documents for ethical approval.
- ✓ Submit protocol to "local" ethical committees in the 3 clinical centres for approval by the end of June 2024 (M9) or beginning of July 2024 (M10).
- ✓ Elaboration of D5.1 "Clinical Pilot Studies Initiation Package and Ethics check" by M12. The approved protocol should be included. There is relevant Milestone "Ethics Check".

• Planned activities under WP6 (Q-PLAN)

- ✓ Continuous implementation and monitoring of the communication and dissemination strategy, including:
 - o Regular updates of SMAs and website of the project with news and events
 - Release of 2nd newsletter in September 2024
 - Monitoring of stakeholder engagement in dissemination activities
 - Monitoring of relevant KPIs
- ✓ Organising discussions with sister project MUNASET and exploring ways for meaningful synergies through joint activities. Joint tech workshop to be organised in June 2024. Joint biomed workshop to be organised during the Graphene Week 2024.
- ✓ Continuous mapping of relevant initiatives for the creation of new synergies. Establishment of synergies with other GFI projects (e.g., GRAPHERGIA) or other Horizon Europe projects (e.g., COMFORTage).
- ✓ Creation of "Dissemination articles" with explanations of important scientific terms used by 2D-BioPAD, utilising the work that has been already done in D1.1.

• Planned activities under WP7 (UP-CATRIN)

Within the following reporting period of the project, the PC and PMO:

- ✓ will perform all the required project-management activities, including coordination of partners, quality assurance, identification and analysis of risks, and any other day-to-day administrative activities.
- ✓ will periodically update and further elaborate on the 2D-BioPAD Data Management Plan.
- ✓ will host monthly digital progress meetings and prepare the third project meeting.



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

GA 101120706

Partners

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