UNIVERSITÀ DEGLI STUDI DI GENOVA

AREA RICERCA, TRASFERIMENTO TECNOLOGICO E TERZA MISSIONE

SERVIZIO PER IL TRASFERIMENTO TECNOLOGICO E DELLE CONOSCENZE

SETTORE VALORIZZAZIONE DELLA RICERCA, TRASFERIMENTO TECNOLOGICO E RAPPORTI CON LE IMPRESE

IL RETTORE

Vista la Legge 9 maggio 1989, n. 168 - Istituzione del Ministero dell'Università e della ricerca scientifica e tecnologica e ss.mm.ii;

Visto lo Statuto dell'Università degli Studi di Genova;

Visto il Regolamento Generale di Ateneo;

Visto il Regolamento di Ateneo per l'Amministrazione, la Finanza e la Contabilità;

VISTA la legge 7 agosto 1990, n. 241 recante "Nuove norme in materia di procedimento amministrativo e di diritto di accesso ai documenti amministrativi" pubblicata sulla Gazzetta Ufficiale n. 192 del 18/08/1990 e s.m.i.;

VISTO il Decreto del Presidente della Repubblica 28 dicembre 2000, n. 445 (Disposizioni legislative in materia di documentazione amministrativa) e s.m.i.;

VISTO il Decreto Direttoriale MUR n. 341 del 15/03/2022 di emanazione di un Avviso pubblico per la presentazione di Proposte di intervento per la creazione di "Partenariati estesi alle università, ai centri di ricerca, alle aziende per il finanziamento di progetti di ricerca di base" nell'ambito del Piano Nazionale di Ripresa e Resilienza, Missione 4 "Istruzione e ricerca" – Componente 2 "Dalla ricerca all'impresa" – Investimento 1.3, finanziato dall'Unione europea – NextGenerationEU";

VISTO il Decreto Direttoriale MUR n. 1553 dell'11/10/2022 di concessione del finanziamento del progetto Codice identificativo PE00000006, Acronimo MNESYS, Titolo "*A multiscale integrated approach to the study of the nervous system in health and disease*", registrato alla Corte dei Conti il 23/11/2022 al n. 2948 e relativi allegati;

CONSIDERATO che l'Università degli Studi di Genova è leader dello Spoke 6, dal titolo "*Neurodegeneration, trauma and stroke*";

CONSIDERATO che gli Spoke possono emanare - nell'ambito dei limiti e con le modalità previste dall'Avviso - "bandi a cascata" finalizzati alla concessione di finanziamenti a soggetti esterni per attività coerenti con il progetto approvato;

VISTA la delibera della seduta del 27 settembre 2023 con cui il Consiglio di Amministrazione dell'Università degli Studi di Genova ha approvato l'emanazione del bando a cascata per organismi di ricerca nell'ambito del Progetto MNESYS - "*A multiscale integrated approach to the study of the nervous system in health and disease* - PNRR M4C2 per lo Spoke 6;

VISTO il Decreto del Direttore Generale n. 5418 del 14 novembre 2023 di nomina del Responsabile

del Procedimento;

VISTO il Decreto del Rettore n. 5439 del 14 novembre 2023 e il Decreto Rettorale n. 5474 del 15 novembre 2023 di emanazione del Bando a cascata per il finanziamento di proposte di intervento per le attività di ricerca svolte da Organismi di Ricerca nell'ambito del programma di ricerca PE MNESYS "*A multiscale integrated approach to the study of the nervous system in health and disease*", per lo Spoke 6 dal titolo "*Neurodegeneration, trauma and stroke*", nell'ambito del PNRR, Missione 4, Componente 2, Investimento 1.3 – finanziato dall'Unione europea – NextGenerationEU (CUP D33C22001340002);

CONSIDERATO che alla data di scadenza per la presentazione delle proposte progettuali, fissata entro e non oltre il giorno 14 dicembre 2023, per la **Tematica P – "In vitro and in silico studies on the role and pharmacological targetting of ion channels and endolysosomes associated with neurodegeneration and neuroexcitability defects, integrating next generation electrophysiology and AI approaches"** era pervenuta a mezzo PEC all'indirizzo air3@pec.unige.it la seguente proposta:

PROPONENTE: Consiglio Nazionale delle Ricerche
TITOLO PROPOSTA: RODEND – ROle of Neuronal Endolysosomal ion transport in brain function and Neurodegenerative Diseases

TENUTO CONTO che la Responsabile del procedimento, Ing. Patrizia Cepollina, ha ritenuto ricevibile, ammissibile e conforme la proposta sopra citata;

CONSIDERATO che nel Bando è previsto che la valutazione di merito tecnico-scientifico dei progetti pervenuti sia affidata ad una Commissione composta da almeno tre esperti esterni al Partenariato, indipendenti e competenti dell'Area tematica dello Spoke;

VISTO il Decreto Rettorale n. 6114 del 20 dicembre 2023 con cui è stato emanato l'Avviso di manifestazione di interesse per la costituzione di un albo di esperti indipendenti a supporto della valutazione di merito dei progetti PNRR presentati sui bandi a cascata del progetto MNESYS – A multiscale integrated approach to the study of the nervous system in health and disease;

VISTO l'Estratto del Verbale della Riunione del 12 febbraio 2024 del Comitato Scientifico del programma di ricerca MNESYS "*A multiscale integrated approach to the study of the nervous system in health and disease*" che ha approvato la "Rosa di Candidati" per le Commissioni di Valutazione dei Bandi a cascata sul Programma MNESYS;

VISTO il Decreto del Rettore n. 855 del 20 febbraio 2024 con cui è costituito l'Albo a supporto delle valutazioni dei progetti presentati in risposta al bando pubblico per la selezione di proposte progettuali da finanziare nell'ambito delle attività di ricerca dello Spoke n. 6 di cui al programma di "*A multiscale integrated approach to the study of the nervous system in health and disease*" – MNESYS, a valere sulle risorse del Piano Nazionale di Ripresa e Resilienza (PNRR), Missione 4 "Istruzione e Ricerca", Componente 2 "Dalla ricerca all'impresa", linea di Investimento 1.3 "Creazione di Partenariati Estesi alle università, centri di ricerca, alle aziende per il finanziamento di progetti di ricerca di base";

VISTO il Decreto del Rettore n. 1122 del 5 marzo 2024 con cui è stata nominata la Commissione di valutazione delle proposte pervenute in risposta al bando a cascata di cui al D.R. n. 5439 del 14 novembre 2023, indicato nelle premesse del presente decreto;

ACQUISITO il verbale della Commissione di Valutazione della seduta del 16 aprile 2024 (Prot. n. 37982 del 07/05/2024);

VISTO il Decreto del Rettore n. 2296 del 10 maggio 2024 con cui è stata approvata la graduatoria di merito per la Tematica P – "In vitro and in silico studies on the role and pharmacological targetting of ion channels and endolysosomes associated with neurodegeneration and neuroexcitability defects, integrating next generation electrophysiology and AI approaches", di cui al bando a cascata di cui al Decreto del Rettore n. 5439 del 14 novembre 2023, indicato nelle premesse del presente decreto;

TENUTO CONTO che in data 14 maggio 2024 è stata inviata al Consiglio Nazionale delle Ricerche la comunicazione con prot. n. 41378 in cui si rendevano noti gli esiti della procedura e si richiedeva la documentazione propedeutica all'adozione del provvedimento di ammissione del finanziamento;

VISTO che in data 17 maggio 2024 con prot. n. 42962 la documentazione richiesta è stata ricevuta dall'Università degli Studi di Genova che l'ha ritenuta conforme a quanto previsto nel bando a cascata di cui al Decreto del Rettore n. 5439 del 14 novembre 2023 e il Decreto Rettorale n. 5474 del 15 novembre 2023 , indicato nelle premesse del presente decreto,

DECRETA

ART. 1

L'ammissione a finanziamento del progetto RODEND – ROle of Neuronal Endolysosomal ion transport in brain function and Neurodegenerative Diseases per la **Tematica P – "In vitro and in silico studies on the role and pharmacological targetting of ion channels and endolysosomes associated with neurodegeneration and neuroexcitability defects, integrating next generation electrophysiology and AI approaches"** con Soggetto proponente il Consiglio Nazionale delle Ricerche – come rappresentato negli Allegati B e C alla proposta presentata con domanda di partecipazione prot. n. 73888 del 12 dicembre 2023.

ART. 2

L'entità dell'agevolazione concessa, a fondo perduto, ammonta a 248.552 euro complessivi come rappresentati nell'allegato C alla proposta presentata con domanda di partecipazione prot. n. 73888 del 12 dicembre 2023. L'agevolazione è pari al 100% dei costi di progetto trattandosi di attività di ricerca fondamentale per Organismi di Ricerca. L'agevolazione è concessa a valere sui fondi PNRR - Programma "*A multiscale integrated approach to the study of the nervous system in health and disease*" – MNESYS Codice PE00000006 a valere sulla Missione 4, Componente 2, Investimento 1.3, ai sensi del Decreto di concessione n. 1553 dell'11 ottobre 2022, registrato alla Corte dei Conti il 23/11/2022 n. 2948, iscritto al Bilancio di Ateneo sul progetto UGOV 100009-2022-TF-PNRR-PE_MNESYS_BAC_DINOGMI.

ART. 3

Le attività, come indicate dettagliatamente nell'Allegato B alla domanda di finanziamento, dovranno essere avviate a partire dalla data di sottoscrizione del Contratto e concluse entro e non oltre 12 mesi, affinché siano rendicontate in tempo utile per consentire la chiusura del Programma PE MNESYS, il cui termine è attualmente previsto al 31 ottobre 2025.
Potrà essere valutata e concessa una sola proroga in presenza di ritardi dovuti a circostanze eccezionali e non dipendenti da scelte del Beneficiario esclusivamente nel caso in cui il MUR, a sua volta, proroghi il termine del Programma MNESYS.

ART. 4

Il presente atto sarà pubblicato sul sito UniGe https://unige.it/progetti-finanziati-dal-pnrr e laddove la normativa vigente lo richiede.
Il documento informatico originale sottoscritto con firma digitale sarà conservato presso l'Area Ricerca, Trasferimento Tecnologico e Terza Missione.

ALLEGATI:

Allegato B – Proposta progettuale
Allegato C – Piano economico-finanziario

IL RETTORE
Prof. Federico DELFINO
(documento firmato digitalmente)

ALLEGATO C

STANDARD PERSONNEL COST TABLE				PERSONNEL COST
COST RANGE/LEVEL	**NUMBER OF SUBJECTS**	**HOURLY COST. see note**	**HOURS AMOUNT**	
Low	**0**	29 €		- €
Medium	**4**	33 €	1060	34.980 €
High	**2**	55 €	100	5.500 €
TOTALS	**6**		**1160**	**40.480 €**

HOURLY COST: reference should be made to the Interministerial Decree n. 116 of January 24, 2018

ALLEGATO C

PROJECT BUDGET	PERSONNEL COST	OVERHEAD	Costs for Specialist Consulting Services	License costs directly attributable to the project	Costs for materials and equipment directly attributable to the project	Costs for other types of expenses directly attributable to the project	TOTAL COST
	40.480,00 €	6.072,00 €			163.000,00 €	39.000,00 €	248.552,00 €

Finanziato dall'Unione europea NextGenerationEU
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MNESYS
Università di Genova

ANNEX B

PE00000006
"A multiscale integrated approach to the study of the nervous system in health and disease"

MNESYS

SPOKE N. 6

Research proposal

Topic addressed by the project
(with reference to Annex 2)

Acronym: RODEND - *Project Title:* ROle of Neuronal Endolysosomal ion transport in brain function and Neurodegenerative Diseases

- Name of the PIs' host institution for the project: **National Research Council (CNR), Institute of Biophysics (IBF)**
- Name of the Principal Investigators (PIs): **Michele Migliore, Michael Pusch**
- Proposal duration in months: **12**

- Name and qualification of the Principal Investigator (PI)
- Name and qualification of the co- Principal Investigator (PI)
- Name and qualification of the components the research team

ROLE IN THE PROJECT	*NAME*	*SURNAME*	*DEPARTMENT*	*QUALIFICATION*	*YOUNG*	*F/ M*
Principal Investigator	*MICHELE*	*MIGLIORE*	*National Research Council (CNR), Institute of Biophysics (IBF)*	*RESEARCH DIRECTOR*	*NO*	*M*
co- Principal Investigator (PI)	*MICHAEL*	*PUSCH*	*National Research Council (CNR), Institute of Biophysics (IBF)*	*RESEARCH DIRECTOR*	*NO*	*M*
Component	*SILVIA*	*VILASI*	*National Research Council (CNR), Institute of Biophysics (IBF)*	*RESEARCHER*	*NO*	*F*
Component	*ROSA*	*PASSANTINO*	*National Research Council (CNR), Institute of Biophysics (IBF)*	*RESEARCHER*	*NO*	*F*
Component	*RAFFAELLA*	*BARBIERI*	*National Research Council (CNR), Institute of Biophysics (IBF)*	*RESEARCHER*	*NO*	*F*
Component	*PAOLA*	*GAVAZZO*	*National Research Council (CNR), Institute of Biophysics (IBF)*	*RESEARCHER*	*NO*	*F*

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ABSTRACT

Ion transport across membranes is essential for neuronal excitability, and synaptic transmission. There is increasing evidence of the involvement of endolysosomes, intracellular vesicular organelles with important roles in cell functions such as protein homeostasis and autophagia, in the pathogenesis of neurodegenerative and metabolic disorders mainly affecting the central nervous system (CNS). A growing number of endolysosomal transport proteins has been identified including cationic TRMPL and TPC channels and anionic CLC transporters. The importance of many of these genes is underscored by their association with developmental and intellectual delay, epilepsy, and neurodegeneration, resulting in severe pathologies or even death. Furthermore, the impact of dysfunctional endolysosomal ion channels and transporters on the electrical activity is not understood.

This project attempts to explore the effect of alterations of endolysosomal ion transporters on the electrical neuronal activity in vitro as well as in silico. To this end, exploiting the experimental expertise available at the Institute of Biophysics, electrical activity of murine cultured neurons and brain slices will be studied using advanced electrophysiological techniques. Experimental data will be integrated in our in-silico brain models obtaining information on how alterations of endolysosomal ion homeostasis impacts electrical activity.

A further focus is to identify pharmacological tools targeting specific endolysosomal ion channels or transporters. Once a more solid understanding of the ion channel/transporter genes involved in neurological diseases is available, their role can be potentially investigated directly in human neurons from patients, using the technology developed within this project.

Data and models will be integrated into the EBRAINS-Italy Research Infrastructure and will be made available to MNESYS partners and the scientific community, following the Open Science and FAIR principles.

RESEARCH PROPOSAL

Section a. State-of-the-art and objectives

-State of the art-

Endolysosomes are intracellular vesicular organelles with important roles in cell functions such as protein homeostasis, clearance of extracellular material, and autophagy.

There is increasing evidence that endolysosomes are involved in the pathogenesis of neurodegenerative diseases, including Alzheimer's disease (AD), Parkinson's disease, Huntington's disease, and amyotrophic lateral sclerosis. Intracellular endolysosomal trafficking regulates important cellular processes and defects affect predominantly neurons, likely because their post-mitotic nature leads to accumulation of damage over years. A characteristic property of endolysosomes is their acidic luminal pH, which decreases from early endosomes towards final stage lysosomes and reaches a pH of 4.5, which is critical for lysosome function.

A growing number of endolysosomal transport proteins has been identified including for example cationic TRMPL and TPC channels and anionic CLC transporters. Modifications in the genes encoding these proteins are at the basis of several neurological conditions like intellectual disability, epilepsy, and neurodegeneration. However, their physiological role including the impact of their function on electrical neuronal activity is still unexplored. Mutations in all brain-expressed endo-lysosomal CLC Cl/H antiporter genes (vCLCs), i.e. CLCN3, CLCN4, CLCN6, and CLCN7 (and its subunit OSTM1), cause neurodegenerative and/or neurodevelopmental disorders (NDD) of varying severity [1-6]. The increasing use of next generation sequencing is expected to reveal more patients with vCLC variants.

Important recent advances regarded the discovery of GOF effects leading to severe disease for CLCN3 and -4 related NDD [5,7], the discovery of potentially dominant negative gating effects of CLCN4 variants [7], and the establishment of protocols able to reveal novel properties of CLC-7 [8] and CLC-6 [6,9]. vCLCs are located to endosomes (CLC-3, -4, -6) and lysosomes (CLC-7), where they are important for ion homeostasis. Mouse models yielded important information on the impact of loss of vCLC, leading to various degrees of lysosomal storage and neurodegenerative phenotypes [1] and revealed that CLC-4 relies on hetero-dimerization with CLC-3 [10].

Among the downstream effects of lysosomal dysfunction, defects in the metabolism of essential compounds for the maintenance of human health, like vitamin B12 (cobalamin, Cbl) are of particular importance. Specifically, a role of endolysosomes in the association of vitamin B12 deficiency with neurological disorders has been recently proposed. Dysfunction of lysosomal biogenesis or inhibition of lysosomal proteolysis causes vitamin B12 deficiency [11,12]. Moreover, modifications in genes encoding lysosomal Cbl transporters cause inability to release Cbl from lysosomes and consequently intracellular accumulation of methylmalonic acid (MMA) and homocysteine (Hcy) [13,14], both common biomarkers of genetic disorders associated with vitamin B12 metabolism and resulting in severe neurological manifestations [15,16]. Dysregulation of lysosomal acidification results in intralysosomal Cbl accumulation and impaired transport of Cbl to cytoplasm and mitochondria, and is now regarded as a prognostic marker for various neurodegenerative conditions, including AD [17,18]. Furthermore, elevation of lysosomal Cbl levels correlated with the accumulation of lysosomal Aβ peptide associated with AD [19].

It is thus crucial to understand how the dysfunction of endolysosomal ion transporters leading to neurodegeneration phenomena is mediated by alterations in the release and accumulation of vitamin B12. Clarifying these aspects has significant implications for identifying new diagnostic biomarkers and for implementing innovative therapeutic strategies in neurological conditions.

-Objectives-

This project attempts to explore what are the effects of alterations of endolysosomal ion transporters and of vitamin B12 metabolism on the electrical neuronal activity in vitro as well as in silico, leveraging the long-standing experience of our group in the computational modelling of electrical activity of mammalian neural structures. To this end, exploiting the experimental expertise available at the Institute of Biophysics, electrical activity of murine cultured neurons as well as brain slices will be studied using advanced electrophysiological

techniques. The data will be integrated in the in-silico pipeline of the EBRAINS-Italy Research Infrastructure to build brain models, to obtain specific information on how alterations of endolysosomal ion homeostasis impacts electrical activity, with implications for a series of neurodevelopmental and neurodegenerative diseases as well as well as metabolic disorders affecting the CNS. In fact, our group has a long-standing experience in the in silico modelling of electrical activity of mammalian neural structures [20,21], with particular emphasis on the role of plasma membrane localized ion channels and receptors [22,23], and their possible alterations in neurodegenerative diseases [24,25]. A further focus of the project is the development and use of pharmacological tools targeting specific endolysosomal ion channels or transporters, either inhibiting or stimulating activity. In fact, recent developments in the field of structural biology and artificial intelligence make it possible to predict the interactions between proteins and organic molecules and to predict possible consequences of a mutation.
The project regards aspects that integrate very well into MNESYS WP6.3 "Mechanisms of neuronal cell degeneration and drug dependent reversal" and adds an important topic that is currently not present in MNESYS. Data and models will be integrated into the EBRAINS-Italy platform and will be made available to MNESYS partners and the scientific community, following the Open Science and FAIR principles.

Section b. Methodology

The project is formulated into work packages, hereafter WP, each requiring more tasks, hereafter T, with milestones (M)/deliverables (D) as represented in the Gantt Chart (section d):

<u>WP1) Experimental setup of an electrophysiological facility for studying electrical activity from in vitro differentiated neurons as well as brain slices.</u>
An experimental setup for electrophysiological recordings of neuronal circuits in cultures of in vitro differentiated neurons (from pluripotent stem cells) and mouse brain slices is planned to be implemented within the first 6 months.
This will consist of a patch-clamp setup in an upright microscope with anti-vibration table, micro-manipulators, electrical stimulation device in conditions of controlled temperature and CO2 concentration (hardware and software). Included is also a vibratome for brain slice preparation and an optimization tool for confocal reconstruction and morphometric analysis of neurons.

<u>WP2) Evaluation of alterations of endolysosomal ion transporters on the electrical neuronal activity</u>

Task 2.1 Preparation of SH-SY5Y neuroblastoma cell lines as in vitro cell model
As a first step, in pilot experiments, endolysosomal transport will be altered at the genetic level and pharmacologically in an acute manner in SH-SY5Y neuroblastoma cell lines and patch-clamp experiments will provide data to correlate these alterations to changes in the electrophysiological properties and in synaptic transmission.
Several distinct pathogenic mutations leading to neurodegeneration have been identified to date in the genes encoding CLC-3, CLC-4, CLC-6 and CLC-7 These include mutations introducing an early stop codon (frameshift and nonsense mutations) or missense mutations [26, 27]. We will produce SH-SY5Y model cell lines expressing the most common pathological mutations in CLC transporters.

Task 2.2 Exploratory study for the use of induced pluripotent stem cells (iPSCs)
In parallel, we will plan the generation of induced pluripotent stem cells (iPSCs) from patients with inherited disorder of vitamin B12 to obtain derived-neurons as cell model to study alterations of vitamin B12 metabolism and neuronal electrophysiological activity. In particular, we will explore the possibility of reprogramming [28] skin fibroblasts derived from patients of the Clinical and Research Unit of Metabolic Diseases at the Ospedale Pediatrico Bambino Gesù – Roma, with a multicistronic lentiviral vector encoding OCT4, SOX2, KLF4 and MYC that can be differentiated to neuronal cells [29].

Task 2.3 Electrophysiological experiments
Electrophysiological experiments will be carried out in the whole-cell configuration of the patch clamp technique. In SH-SY5Y cells, voltage-clamp recording will be used to characterize quantitative and qualitative alterations of voltage-gated (K, Na, Ca) and ligand-gated (GABA, glutamate) membrane ion channels. In brains-sliced and in networks of iPSC-derived neurons, current-clamp recordings will be performed. The following excitability parameters will be assessed: resting membrane potential, input resistance, threshold of current injection for AP firing (rheobase), and AP firing frequency as a function of current injection. During recording, Lucifer yellow diffuses into the neuron and allows to into visualize the neuronal processes. Recordings of brain-slices and differentiated neurons will be fed into the EBRAINS-Italy Research Infrastructure and will be made available to MNESYS partners and the scientific community.

Task 2.4 Correlation between endolysosomal alteration and defect in Vitamin B12 metabolism
Moreover, considering the crucial role of endolysosomal transport system in metabolism of vitamin B12, the electrophysiological records will be correlated to the accumulation of MMA and Hcy, markers of defects in B12 metabolism, that will be quantified in conditioned culture medium by ELISA.

WP3) Pharmacological approach
The wide mutational spectrum of CLC transporters also includes nonsense mutations leading to the absence of functional protein or to the production of truncated proteins with possible toxic activity. For these mutants, the enhancement of ribosomal read-through of premature stop codons with restoring production of full-length protein could represent a therapeutic strategy.
In order to understand whether the read-through of premature stop codons could result in restoring the protein production and activity in pathological disorders caused by CLC nonsense mutations, we will evaluate the ability of some oxadiazoles to act as translational read-through-inducing drugs (TRIDs) in cell models of CLC with nonsense mutations [30].
Specifically, the tasks correlated to WP3 can be schematized as follows:

Task 3.1 Pilot study on the use of TRIDS for read-through of premature stop codons
We will carry out a pilot study to understand the ability of TRIDS to promote the specific read-through of premature stop codons expression plasmids by coding fusion proteins between the green fluorescent protein (GFP) and the CLC proteins carrying nonsense mutations. These will be used to transfect mammalian cell lines and the expression of the fusion proteins will be evaluated after treatment with TRIDS by fluorescence microscopy and western-blot.

Task 3.2 Study of the effect of TRIDS on neuronal activity
We will evaluate by electrophysiological experiments the ability of TRIDS to restore the neuronal activity in SH-SY5Y model cell lines expressing the most common non-sense pathological mutations in CLC transporters and assessed in WP2.

WP4) Computational models
The aim is to implement detailed computational models that will reproduce and help explaining the effects of alteration in lysosome functionality on neuronal systems. The in-silico models will encompass synaptic plasticity models and will be based on electrophysiological data provided by WP2. A series of simulations will be conducted to assess both synaptic plasticity and spiking patterns in response to various stimulation protocols, under control and pathological conditions.

WP5) Results dissemination.

This work package aim is the communication of the obtained results in terms of both scientific publications and dissemination toward different communities.

As mentioned, electrophysiological data will be fed into the EBRAINS-Italy Research Infrastructure and will be made available to MNESYS partners and the scientific community.

We will organize periodic remote meetings open to all the researchers involved in the project, and we expect to expand and strengthen the scientific network within the MNESYS researchers' community by the development of this project. Research results will be published in refereed journals and presented at major international conferences. The project will define and implement a comprehensive strategy to communicate and disseminate its results to relevant stakeholders. Different target groups will be reached by different means. Activities will include the use of specific dissemination platforms (like https://www.outreach.cnr.it/). We will develop a format for outreach activities that explains how fundamental research may be translated into tangible benefit for the citizens and patients. This communication strategy will match with the objectives of patient's organization (such as "cblC onlus Association") that will contribute to developing formats of communication events.

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Section c. Available instrumentations and resources

The following is the list of facilities available at the Institute of Biophysics, CNR, Palermo:

EBRAINS-Italy Supercomputing and data-storage resources

Cell culture room: STEVIL_VBH Laminar flow hood; NIKON TS100 Eclipse Microscope; THERMO Forma Steri-cycle Incubator

Microscopy: NIKON Eclipse Ti fluorescence Microscope; JPK AFM NanoWizard 3, coupled with NIKON Eclipse Ti Optical Microscope and Temperature controlled Petri Dish Heater; NIKON Eclipse 80i fluorescence Microscope

Spectroscopy: JASCO J-815 Circular Dichroism; SHIMADZU UV-VIS Spectrophotometer; JASCO FP6500 Spectro fluorimeter; THERMO Ascent FL Fluoroscan HAMAMATSU C9413 FCS Unit; Spectrofluorimeter CARY Agilent Eclypse, equipped with thermostated control, microplane reader and polarization module

Static and Dynamic Light scattering: BROOKHAVEN SM200 Spectrometers BROOKHAVEN BI9000 Correlators BROOKHAVEN BI-DNDC Differential refractometer Homemade CCD Low angle scattering system

KNAUER Refraction Index Detector

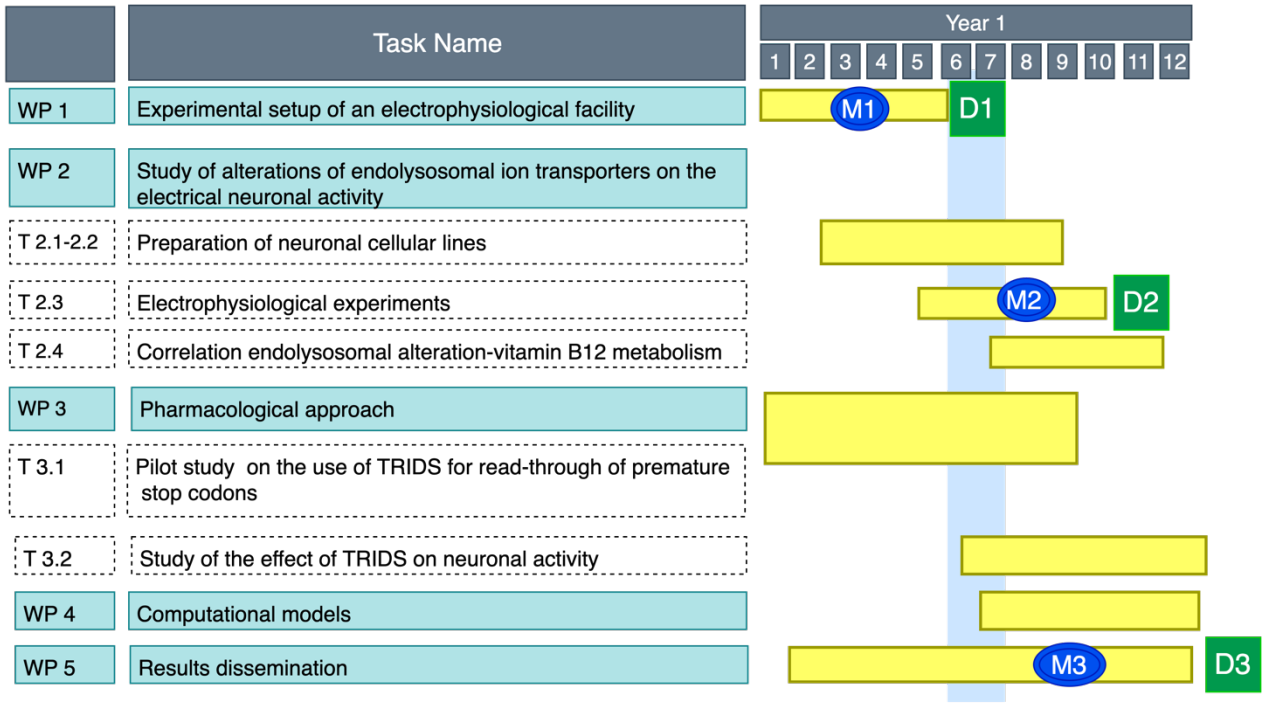
Rheology facilities: TA INSTRUMENTS AR1000 Rheometer TA INSTRUMENTS AR G2 Rheometer
Calorimetry: TA INSTRUMENTS Nano DSC TA INSTRUMENTS Nano ITC HART 707 Microcalorimeter

Chromatography: SHIMADZU gc2010 fid Gas Chromatography; SHIMADZU gc2010 gcms 910 Gas Chromatography; GE-AKTA Pure HPLC; SHIMADZU LC 20AT-SPD HPLC; GE F9-R Fraction Collector; Thermo Scientific Vanquish Core HPLC, equipped with DAD, FLD, RI Thermo Scientific detectors and Postnova Analytics PN3621 MALS detector.

Lab system for Tangential Flow Filtration (3 series), equipped with MasterFlex Easy-Load Pump heads and refrigerated cabinet.

Culture chamber FALC Instruments FCCE 500 with thermostated control and light exposure cycles.

Section d. GANTT diagram



Milestones: **M1** Setup of an elecrophysiological facility
M2 Voltage-clamp recording in neuronal cell models
M3 Communication of research results

Deliverables: **D1** Facility for ectrophysiological experiments
D2 Information on the role of endolysosomes in neuronal electrical activity
D3 Data available in EBRAINS-Italy Research Infrastructure; scientific pubblications

PI Curriculum vitae (max. 2 pages)

PERSONAL INFORMATION

Family name, First name: Migliore, Michele
Researcher unique identifier(s) ORCID: http://orcid.org/0000-0002-7584-6292
Date of birth: 21/03/1957
Nationality: Italian
URL for web site: http://www.pa.ibf.cnr.it/personale/migliore/index.php

- **EDUCATION**

1980 University of Palermo; Palermo (Italy) D. Phil. (Physics, Summa cum Laude)

- **CURRENT POSITION(S)**

2018 –present Research Director, National Research Council (CNR), Institute of Biophysics (IBF), Palermo (Italy).
2020 – present Visiting Professor of Computational Neuroscience, University of Rome "La Sapienza", Department of Neurobiology, Rome, Italy

- **PREVIOUS POSITIONS**

1998-2020 Senior Research Scientist, CNR IBF, Palermo (Italy).
1983- 1998 Research Scientist, Institute for Interdisciplinary Applications of Physics, CNR, Palermo (Italy).
1994-1995 Visiting Professor, Physics Dept. Palermo, Theory and Applications of artificial neural networks.
2004-2020 Visiting Research Scientist, Yale University School of Medicine, Dept. of Neuroscience, New Haven, USA
2001-2015 Visiting Professor of Cybernetics, University of Palermo, Dept. of Mathematics and Informatics.
2002-2003 Visiting Professor, Informatics, University of Palermo, Dept. of Natural Sciences, Palermo, Italy.
2009-2010 Research Fellow, Krasnow Inst. of Advanced Study, George Mason University (Fairfax, USA).

- **FELLOWSHIPS AND AWARDS**

1987 NATO Advanced Fellowship in Biophysics, IBM Research Centre (Kingston, NY, USA)
1997 NATO Senior Fellowship in Biotechnologies and Molecular Biology, Baylor College of Medicine (Houston, USA)
2007 DEISA (Distributed European Infrastructure for Supercomputing Applications) award for project LASIMOLF (Large scale realistic Simulations of the OLFactory bulb)
2012 PRACE (Partnership for Advanced Computing in Europe) award for project LSS-BULB, Large Scale Simulations of the olfactory BULB
2014 PRACE award for project 3DBULB
2015 PRACE award for project SMOLER, Synaptic Mechanisms underlying Odor LEarning and Recognition
2016 PRACE award for project LACEHIP, LArge scale CEllular model of the HIPpocampus
2019 PRACE award for project BEMM Brain functions Emerging from a data-driven Multi scale realistic Model of the hippocampus CA1 circuitry

- **SUPERVISION OF GRADUATE STUDENTS AND POSTDOCTORAL**

1997 – 2023 approximately 20 Master students (University of Palermo and University of Rome La Sappienza), 5 PhD students (University of Palermo, and Univ. of Camerino), 20 postdocs

- **ORGANISATION OF SCIENTIFIC MEETINGS (if applicable)**

2017 First annual Summit of the CIRPROT EU Project
2018 HBP School: The Brain Simulation Platform, Palermo, Italy, Sep. 2018

- **INSTITUTIONAL RESPONSIBILITIES**
  - Scientific Coordinator of the EBRAINS-Italy Research Infrastructure (PNRR)

- Member of the Scientific Infrastructure Board, Human Brain Project, EU
- Member of the High-Level Support Team Steering Committee, Human Brain Project, EU
- Director's Delegate, CNR IBF, Palermo Section (Italy), 2015 – 2017
- Leader of Tasks/WPs for EU, FET Flagship Human Brain Project SGA3 (2020-2023) SGA2 (2018-2020)
- Leader of Tasks/WPs for the project Flag -ERA JTC 2019, Multiscale Modelling of Impaired Learning in Alzheimer's Disease and Innovative Treatments (MILEDI) (2020-2023)
- Leader of Tasks/WPs for the project JPCOFUND Synaptic circuit protection in AD and HD: BDNF/ TrkB and Arc signalling as rescue factors (CIRCPROT) (2016-2019)
- National Coordinator: Research project *Development, implementation and applications of new parallel architectures and concurrent algorithms for Transputers networks*, 1990.
- National Coordinator: Research project *Development, implementation and modeling of neuronal networks*, 1992.
- National Coordinator: Research project *Neuronal networks: biophysical modeling and applications*, within the CNR Strategic Project on Neural Networks, 1993.
- Member of Scientific Board, National Research Council - Institute for Interdisciplinary Applications of Physics, 1984-1988, 1988-1992, 1992-1996, 1996-1998.
- Committee Member, National Research Council, Palermo Research Area, 1994-1997.

- **REVIEWING ACTIVITIES**

- Editorial Board Member, International Journals: *Computational and Mathematical Methods in Medicine*, Hindawi Publishing Corporation; *Computational Intelligence and Neuroscience*, Hindawi
- Associate Editor: *PLoS Computational Biology, Frontiers in Cellular Neuroscience.*
- Reviewer of proposals submitted to EU FP2,3,4, Biotechnology and Biological Sciences Research Council (UK), and Medical Research Council (UK); 1996- present.
- Reviewer for many international journals, such as PNAS, J.Neurosci., J.Neurophysiol., Neural Comp., Eur. J. Neurosci., Biol. Cybern., J. Physiol., Neuroinf., PLOS journals, Frontiers Journals, and others.

- **MEMBERSHIPS OF SCIENTIFIC SOCIETIES**

1998 - present Member of the Italian Society of Pure and Applied Biophysics
1995 – present Member of the Society for Neuroscience
2000 – present Member of the Organizazion for Computational Neuroscience

- **MAJOR COLLABORATIONS**

- E. Clementi, IBM Research Center, Poughkeepsie, NY, USA. (1983);
- A. Geiger, Institut for Physikalischke Chemie, Aachen, Germany. (1984);
- K. Heinzinger, Max Planck Institut fur Chemie, Mainz, Germany. (1985);
- J.J. Hopfield, Institute for Theoretical Physics, Santa Barbara, CA, USA. (1986);
- Clementi e G. Corongiu IBM Research Center, Kingston, NY, USA. (1988);
- D. Johnston Baylor, College of Medicine, Houston, TX, USA (1992-1996);
- G. Buzsaki, Rutgers University, Newark, NJ, USA (1998).;
- G. Einevoll, Norwegian University of Life Sciences, Aas, Norway (2009-2011);;
- G. Shepherd Jr, Feinberg School of Medicine, Northwestern University, Chicago, USA (2010-2012);
- G.A. Ascoli, Krasnow Institute of Advanced Study, George Mason Univ., Fairfax, USA (2004-2011);
- M. Shah, University College London, Department of Pharmacology, UK;
- M. Taglialatela and A. Marasco, University of Naples "Federico II"
- E.Cherubini, European Brain Research Institute, Roma (Italy), collaboration with Enrico Cherubini;
- G. Shepherd and M. Hines, Yale University, Department of Neuroscience, New Haven, USA;
- H. Marie CNRS, Institut de Pharmacologie Moléculaire et Cellulaire, Valbonne (FR);
- V. Lessmann Otto-von-Guericke-Universität, Institute for Physiology, Magdeburg;
- EPFL, Blue Brain Project, Lausanne, CH;
- S. Kali, Hungarian Academy of Sciences, Institute of Experimental Medicine.

Appendix: All current grants and on-going and submitted grant applications of the PI (Funding ID)

Mandatory information (does not count towards page limits)

Current grants (Please indicate "No funding" when applicable):

Project Title	*Funding source*	*Amount (Euros)*	*Period*	*Role of the PI*	*Relation to current proposal*
EBRAINS-Italy	PNRR	7,871,275 eur (out of a total of 22,370,240 eur)	Nov 2022-Oct 2025	Scientific Coordinator	Common scientific and technological interests
HIPPOCOMP	PRIN	113,000 eur	Oct 2023 – Set 2025	Responsible of Research Unit	None
EBRAINS 2.0	EU	350,000 eur	Jan 2024 – Dec2026	PI	Common scientific and technological interests
EIDD	FISA-MUR	4,000,000 eur	Jan 2024 – Dec 2027	PI	None

CoPI Curriculum vitae (max. 2 pages)

PERSONAL INFORMATION

Family name, First name: Pusch, Michael
Researcher unique identifier: ORCID https://orcid.org/0000-0002-8644-8847
Date of birth: 02/08/1963
Nationality: German
URL for web site:http://users.ge.ibf.cnr.it/pusch/

- **EDUCATION**

1990	PhD in Physics, University of Göttingen / Max Planck Institute for Biophysical Chemistry, Germany, supervisor: Walter Stühmer
1987	Master in Physics, University of Göttingen / Max Planck Institute for Biophysical Chemistry, Germany, supervisor: Erwin Neher

- **CURRENT POSITION**

Since 2007	Research Director ("Dirigente di Ricerca") at the Institute of Biophysics of Italy's National Research Council in Genoa, Italy (IBF)

- **PREVIOUS POSITIONS**

2013 – 2017	Director of IBF
2002 – 2007	Senior Research scientist ("Primo Ricercatore") at IBF
1997 – 2002	Research scientist ("Ricercatore") at IBF niversity/ Institution/ Country
1991 – 1997	Post-doctoral fellow, Center for Molecular Neurobiology, University of Hamburg, Germany. Supervisor: Thomas Jentsch
1990 – 1991	Post-doctoral fellow, Istituto di Cibernetica e Biofisica, CNR and University of Genova, Italy. Supervisor: Franco Conti.

- **FELLOWSHIPS AND AWARDS**

1996	Bernard-Katz-Lecturer, Prize awarded by Alexander von Humboldt-Stiftung

- **SUPERVISION OF GRADUATE STUDENTS AND POSTDOCTORAL FELLOWS (if applicable)**

1997 – 2023	4 master students (University of Genoa), 8 PhD students (University of Genoa, SISSA Trieste), 24 postdocs

- **ORGANISATION OF SCIENTIFIC MEETINGS (if applicable)**

2017	Co-organization of the Erice school "Channels and transporters", Erice, Italy
2014	Co-organization of the Erice school "Channels and transporters", Erice, Italy
2012	Co-organization of the meeting of the Italian Society for Pure and Applied Biophysics (SIBPA) in Ferrara, Italy
2003	Co-organization of the 2003 meeting of the Society of General Physiologists, "The biology

of chloride", Woods Hole, USA

- **INSTITUTIONAL RESPONSIBILITIES**

2013 – 2017 Director of the Institute of Biophysics, Italy (with head quarter in Genoa and sections in Milan, Palermo, Pisa and Trento)
2012 – 2013 Local Director ("Delegato") of the Genoa head office of the Institute of Biophysics (responsibility for administration and human resources)

- **REVIEWING ACTIVITIES**

Since 2023 Member of reviewing committee of the Italian Cystic Fibrosis Foundation
2007 Review panel member of Deutsche Forschungsgesellschaft for SFB in Würzburg, Germany
1987 – 2023 Reviewer for numerous journals including Nature, Science, PNAS, Nature Structural and Molecular Biology, Embo Journal, Nature Communications, Science Advance, Journal of Clinical Investigation, and many others
2001 – 2023 Reviewing of grant applications for the following granting agencies:
Israel Science foundation, Deutsche Mucoviscidose Gesellschaft, DFG, US National Science Foundation, CNISM (consorzio interuniversitario struttura della materia), The Wellcome Trust, European Research Council (ERC), BBSRC, Danish Medical Research Council, Österreichische Nationalbank Anniversary Fund, Cottrell College Science Award, Cystic Fibrosis Trust, Sparks Childrens medical research foundation

- **MEMBERSHIPS OF SCIENTIFIC SOCIETIES (if applicable)**

Since 1986 Member of the German Physiological Society
Since 1987 Member of the American Biophysical Society
Since 1986 Member of the of the Society of General Physiologists
Since 2002 Member of the German Physiological Society
Since 2002 Member of the Italian Society of Pure and Applied Biophysics

- **MAJOR COLLABORATIONS**

Dr. Vera Kalscheuer, Max-Planck Institut, Berlin, Germany
Dr. Emma Palmer, Royal North Shore Hospital, Sydney, Australia
Prof. Tobias Stauber, Hamburg Medical University, Germany
Dr. Pontus Gourdon, University of Copenhagen, Denmark
Prof. Raùl Estévez, University of Barcelona, Spain
Prof. Fernando Morales, University of San José, Costa Rica
Prof. Guiscard Seebohm, University of Münster, Germany
Prof. Jean-Francois Desaphy, Università di Bari, Italy
Prof. Antonella Liantonio, Università di Bari, Italy
Prof. Rossana Morabito, University of Messina, Italy
Prof. Silvia Dossena, Paracelsus University, Salzburg, Austria
Dr. Vanessa Checcehtto, University of Padova, Italy

Appendix: All current grants and on-going and submitted grant applications of the PI (Funding ID)

Mandatory information (does not count towards page limits)

Current grants (Please indicate "No funding" when applicable):

Project Title	*Funding source*	*Amount (Euros)*	*Period*	*Role of the PI*	*Relation to current proposal*
Functional properties, localization and physiopathology of putative glucose transporter SLC45A1 involved in epilepsy and intellectual disability	Telethon-Italy and Cariplo Foundation	165000	2023-2024	PI	No overlap
Mechanisms and disease models of neurodevelopmental disorders involving CLC anion transporters	Telethon-Italy	76000	2023- May 2024	PI	Partial scientific overlap, but no temporal overlap
Digital Driven Diagnostics, prognostics and therapeutics for sustainable Health care	Italian Ministry of Research	459000	2022-2025	Co-PI	No overlap
The muscle chloride channel CLC-1 as a drug target - a combined structural and functional approach	Italian Ministry of Foreign Affairs	50000	submitted	Co-PI	No overlap