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 N – "Characterization of structural/conformational features of different amyloid and amyloid-like aggregates and their interaction with hosts using FLIM/STORM in a 2-Photons (2P) analysis and through enzymatic digestion and multiple-fragmentation tecnique in LC-MS triple quadrupole"** era pervenuta a mezzo PEC all'indirizzo air3@pec.unige.it la seguente proposta:

PROPONENTE: Università degli Studi del Molise
TITOLO PROPOSTA: CHAMPS – Characterization of amyloid pattern, structures and interaction with host's cells using innovative multiphoton and LC-MS based technologies

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. 1123 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. 2295 del 10 maggio 2024 con cui è stata approvata la graduatoria di merito per la Tematica N – "Characterization of structural/conformational features of different amyloid and amyloid-like aggregates and their interaction with hosts using FLIM/STORM in a 2-Photons (2P) analysis and through enzymatic digestion and multiple-fragmentation tecnique in LC-MS triple quadrupole", 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 all'Università degli Studi del Molise la comunicazione con prot. n. 41377 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 22 maggio 2024 con prot. n. 44494 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 CHAMPS – Characterization of amyloid pattern, structures and interaction with host's cells using innovative multiphoton and LC-MS based technologies per la **Tematica N – "Characterization of structural/conformational features of different amyloid and amyloid-like aggregates and their interaction with hosts using FLIM/STORM in a 2-Photons (2P) analysis and through enzymatic digestion and multiple-fragmentation tecnique in LC-MS triple quadrupole"** con Soggetto proponente l'Università degli Studi del Molise – come rappresentato negli Allegati B e C alla proposta presentata con domanda di partecipazione prot. n. 74646 del 14 dicembre 2023.

ART. 2

L'entità dell'agevolazione concessa, a fondo perduto, ammonta a 149.933,75 euro complessivi come rappresentati nell'allegato C alla proposta presentata con domanda di partecipazione prot. n. 74646 del 14 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)

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 - Project Title

Characterization of amyloid pattern, structures and interaction with host's cells using innovative multiphoton and LC-MS based technologies (CHAMPS)

- Name of the PIs' host institution for the project : UNIVERSITY OF MOLISE
- Name of the Principal Investigators (PIs): CLAUDIO RUSSO
- 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 (under 40 al 31.12.2023)*	*F/M*
Principal Investigator	*Claudio*	*Russo*	*Medicine and Health Sciences*	*Professor of Biochemistry*		*M*
co-Principal Investigator (PI)	*Alfonso*	*Di Costanzo*	*Medicine and Health Sciences*	*Associate Professor of Neurology*		*M*
Researcher	*Antonella*	*Angiolillo*	*Medicine and Health Sciences*	*Associate Professor of Technical Sciences and Laboratory Medicine*		*F*
Researcher	*Emanuele*	*Foderà*	*Medicine and Health Sciences*	*Technician*		*M*
Technician	*Francesca*	*Fantasma*	*Biosciences*	*Technician*		*F*

Text highlighted in grey should be deleted.
Please respect the following formatting constraints: Times New Roman, Arial or similar, at least font size 11, margins (2.0 cm side and 1.5 cm top and bottom), single line spacing.

ABSTRACT

The project MNESYS is specifically targeted to identify new biomarkers of neuro-pathological conditions and to study the neural bases of neurodegeneration. Our proposal intends to respond to a specific request: to explore using advanced confocal microscopy methods (employing FLIM -Fluorescence lifetime, and STORM-superresolution) in a multiphoton system the structural characterization and cellular localization of pathological protein aggregates or amyloid strains. At the same time, the application of advanced mass spectrometry technologies is required to obtain sequence and structural information on the same samples. In our Medical Department there are LC-MS spectrometry and Confocal facilities with dedicated personnel and equipped with top-level instruments to meet the needs of the call. We have also extensive experience in amyloid analysis mainly in AD and Prions field. In particular, our proposal focuses on three specific workpackages to identify the molecular biochemical characteristics of the proteins/peptides in their aggregated state, collected from patients (brain samples and/or nasal swabs) derived from the most common neurodegenerative disease characterized by amyloidosis (AD, PD, Prions..etc). The overall objective is thus to provide information on the sequences and post-traslational modification (PTMs) present in the aggregates, along with structural information, in. order to clarify whether there are differences between amyloid strains and also between patients with the same pathology but different phenotype (for example Aβ in the early and late onset AD patients). A further objective is to provide information on the interactions with cell membranes of the different amyloid species to get deeper information about pathological mechanisms triggered by specific strains or amyloid aggregates.

RESEARCH PROPOSAL
Sections (a) and (b) should not exceed 4 pages. References do not count towards the page limits.

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

Amyloidogenic proteins are capable of adopting a number of different three-dimensional amyloid structures, each with distinct molecular repeating structures. Combined with biochemical and pathological processes such as posttranslational modifications (PTMs) or protease cleavages, these differences are known as conformational strains which, in certain conditions, can propagate over multiple passages in animals or cell culture as in the classical prion mechanism (M.L. Cohen et al., 2015; 10.1093/brain/awv006). Different strains of disease-causing proteins such as amyloid-β (Aβ) or α-synuclein lead to different pathologies and localization in the brain and, more generally, the biological properties of amyloids depend critically on their conformations/strains (Russo C. et al., 2000; 10.1038/35014735). However, even non-amyloid proteins like Microtubule Associated Protein Tau (MAPT), in certain conditions may aggregate and propagate in a Prion-like fashion (M. A. Metrick 2nd et al., 2020: 10.1186/s40478-020-0887-z). Therefore a clear-cut correlation between the type of aggregated protein and the clinical phenotype is still obscure and the attempt to characterize each strain/phenotype is complicated technically (purification and sequencing of amyloid peptides is still tricky) by the presence of PTMs (again), and by other confounding factors dependent on: other interacting proteins, cellular localization, interaction with the cell membranes in the site of amyloid formation, the coexistence of multiple aggregates and amyloids in the same patient (such as in a subtype of AD subjects where are detectable α -synuclein deposits, MAPT aggregates, and of course Aβ plaques (O. Bousiges et al., 2022; 10.3390/ijms23126371) altogether.
Parkinson's disease (PD), multiple system atrophy (MSA), dementia with Lewy bodies (DLB) [or Lewy body dementia] are called α-synucleinopathies due to the abnormal accumulation of aggregates of α-synuclein in the brain. However, in 15–20% of patients with AD at autopsy, concomitant DLB pathology can be found, with only a minority of patients having exhibited clear diagnostic features of DLB. However, in patients with AD and diffuse Lewy body pathology, disease duration was shortened (J. Graff-Radford et al., 2017; 10.1001/jamaneurol.2016.4926), indicating that DLB pathology contributes to dementia progression.

Thus, there is a great need for a rapid method to differentiate distinct conformational strains of amyloids in brain tissues, cultured cells, and cell-free in vitro systems either for diagnostic purposes and to better comprehend the mechanic of disease starting and progression.

In this context, a novel assay called real-time quaking induced conversion (RT-QuIC) has shown capability to amplify trace amounts of amyloid aggregates obtained from brain, cerebrospinal fluid, olfactory mucosa, urine skin etc.. from patients with PD, CJD, tauopathies and other conditions with variable diagnostic sensitivity and specificity (C. D'orrù et al., 2014; 10.1056/NEJMoa1315200). As example, using RT-QuIC, α-synuclein aggregates propagate with a prion-like replication mechanism by inducing conversion of recombinant α-synuclein to the misfolded form. Then, the converted α-synuclein initiates amyloid fibril formation which, in turn, enhances the fluorescence of Thioflavin T (ThT) (M. Bongianni et al., 2022;10.1186/s40035-022-00311-3). RT-QuIC, although not effective in all amyloidosis, can give a fairly precise diagnosis for various aggregation pathologies. However, the test is ineffective in providing information on the strains and molecular biochemical characteristics of the aggregates and is therefore relatively little useful in helping to understand the genesis of amyloidosis. At the same time it cannot provide information on the interacting proteins, on the PTMs, on the interactions with host's membranes/cells for each type of amyloid.

Among the aims of MNESYS are: to identify new biomarkers of neural changes over time in pathological conditions, identify the neural bases of neurodegeneration, encourage the development of new biomarkers and pharmacological targets. Our proposal supports these lines of research and aims to identify, in the field of some of the most common neurodegenerative pathologies (PD, AD, tauopathies, CJD...) identified by the spoke 6, the molecular biochemical characteristics of the proteins/peptides in their aggregated state, collected from patients (brain samples and/or nasal swabs).

The primary objective is to provide information on the sequences and PTMs present in the aggregates, together with structural information. The point here is to clarify whether there are differences between amyloid strains (for example in the sequences, pattern and PTMs of α-synuclein in AD or in PD patients) and also between patients with the same pathology but different phenotype (for example Aβ in the early and late onset AD patients). Samples: provided by spoke 6, brain-derived or from nasal swab.

A further objective is to provide information on the interactions with cell membranes of the different amyloid species. From this point of view it is important to remember that plasma membrane tension plays an essential role in numerous cell processes. At the whole cell level, membrane tension is tightly regulated during cell migration, cell spreading and phagocytosis. Membrane tension also regulates subcellular processes such as endocytosis and opening of mechanosensitive ion channels (A. Colon et al., 2018;10.1038/s41557-018-0127-3). These aims will be pursued using innovative technologies: 1-for the characterization of sequences and PTMs of the purified aggregates we will use nano-LC coupled to MS analysis and multiple fragmentation with "Fusion" orbitrap in both bottom-up and top-down configuration, 2-for structural information we will use FLIM and STORM applied to purified aggregates or in cells derived from nasal swabs with antibodies specific for the different protein regions, 3-for the study of interactions with cell membranes we will use the oligomers and aggregates to treat cells and membrane preparations in which are embedded specific dyes capable of giving us structural information on the lipid bilayer using second harmonic analysis and FLIM with two-photon excitation.

WP1-LC-MS analysis of amyloid aggregates.

Proteomic analysis of brain samples or amyloid aggregates has provided with important information on the composition of plaques and on the protein complement of extra and intra cellular aggregates in various neurodegenerative pathologies with amyloid deposits (A. Montero-Calle et al., 2023; 10.1007/s00018-023-04791-y; E. Drummond et al., 2017; 10.1007/s00401-017-1691-0). However, top-down approaches to obtain information on the sequences of whole peptides/proteins in the aggregates are rare, and to our knowledge there are no applications on preparations obtained from nasal swabs using LC-MS in amyloid patients.

Our approach will use top-down analysis on intact aggregates purified form brains of from nasal swab, via mass spectrometry (MS) on an Orbitrap Lumos analyzer (ThermoFisher)which has the potential to capture nearly all of the relevant information encoded in each protein, including primary sequence information, combinatorial patterns of post-translational modifications (PTMs), and protein gas-phase structure. At the same time a classical bottom-up approach in which peptides are cleaved with different enzymes and fragments analyzed in classical LC-ESI.

WP2-FLIM and STORM on amyloid aggregates to get structural information

Super-resolution microscopy enables tremendously detailed studies of cellular processes. By using STORM and STED microscopy, it has been shown that γ-secretase is present both at the post- and presynaptic sides of synapses, while $A\beta_{42}$is only present on the presynaptic side 10.1186/s40478-016-0296-5, and also that presenilin 1 (the active component of γ-secretase) is detectable in plasma membranes in ratio 1:1 with nicastrin (A.A. Escamilla-Ayala et al., 2020; 10.7554/eLife.56679). To date, the main approach to image nanoscopic structures in tissues relies on transmission electron microscopy, a time-consuming technique which requires ultrathin tissue sections (50-70 nm) with stringent sample preparation and limits immune-targeting diversity and 3D acquisition. Conversely, FLIM and STORM offers the advantages of optical fluorescence microscopy with respect to sample preparation, vast observation fields, multiple molecular labelling and 3D acquisition, with image acquisition and reconstruction taking only a few minutes.

Fluorescence Lifetime Imaging Microscopy (FLIM) is not only a powerful tool to distinguish different amyloid structures, but also to monitor the dynamic process of amyloid remodeling by the cellular environment. FLIM is a versatile technique that can be applied to compare the fibrilization process of different amyloid proteins, environmental stimuli, or genetic backgrounds in vitro and in vivo in a non-invasive manner (M.L. Pigazzini

et al. 2020: 10.3791/61004). The lifetime of the fluorophores is in fact independent of the quantity of the fluorophore itself, but instead is strictly dependent on the surroundings: for this reason FLIM focuses on the study of the interactions between fluorescent proteins or with the surrounding microenvironment or, as in our case, to analyze the proximity between specific epitopes and thus to obtain structural information. The possibility of having specific antibodies for various regions of the proteins that cause cerebral amyloidosis already available in our laboratory is clearly an advantage (see methods).

WP3-Multiphoton studies on cell membranes challenged with amyloid

Our laboratory has a significant experience with fluorescent probes that specifically targets the plasma membrane of cells and reports membrane tension changes through their FLIM changes. The probe spontaneously inserts into the plasma membrane of cells and is only fluorescent when inserted in a lipid membrane. Treatment with various aggregated peptides or amyloid strains can induce variations in FLIM directly correlated with changes in tension on the membrane proportional to its fluidity and variations in osmotic pressure, A second dye is a membrane dye that responds to Second Harmonic signal upon two photon excitation. Also in this case the challenge with specific amyloid could induce local variations in the SHG signal directly correlated to membrane integrity, fluidity and correct lipid partitioning. Beside, a recent study highlighted the possibility that the "core" of amyloid plaques in brain generate a SH signal at 810nm (R. Cunha et al., 2021; 10.1039/d1an00074h) as well. In this part of the project therefore, the information will be related to the possible difference in interactions with cell membranes between different types of amyloid, strains and a potential correlation with mechanisms of cellular toxicity.

Altogether our aim is to provide a new methodological approach to get information about sequences, structures and "environmental" interaction of different amyloid strains to deepen or knowledge about amyloid formation, for diagnostic and therapeutic purposes. Although dedicated to the most common amyloid proteins detected in AD, PD and CJD, our approach can be applied virtually to any kind of amyloid or aggregated protein provided by spoke 6 in the future.

Section b. Methodology

WP1-LC-MS analysis of amyloid aggregates

Top-down .and Bottom Up The Orbitrap Fusion (Thermo Fisher Scientific, San Jose, CA) is equipped with High precursor capacity ETD implemented with a dual pressure linear ion trap (A-QLT). To enable high capacity ETD, instrument control code was modified to allow transfer of precursor ions directly from the ion routing multipole to the center section of the HPC for storage using a DC potential well of approximately 4 volts, omitting relocation of precursor ions to the back section prior to the ETD reaction. Amyloid aggregates are either provided by spoke 6 or purified from nasal swab and extracted using acetonitrile/water/formic acid and are resuspended at approximately 10 picomole per microliter in 49.9:49.9:0.2 acetonitrile/water/formic acid, infused via syringe pump into the mass spectrometer at five microliters per minute through a 500 microliter syringe, and ionized with electrospray ionization (ESI) at +3.5 kV with respect to ground. Each type of amyloid will have a specific setting that should be verified experimentally. For a classical 18kDa protein, MS/MS scans are performed in the Orbitrap with unthresholded transient acquisition at a resolving power of 120,000 (full width at half maximum) at 200 m/z with a range of 200–2000 Th. Precursor ions are isolated with the mass selecting quadrupole with an isolation width of 10 m/z, and automatic gain control (AGC) targets values ranging from 100,000 to 1,000,000 charges as indicated. Transient averaging began after data acquisition is started so that scans with 1–100 transients averaged could be analyzed. Bottom up approaches are used routinely in our proteomic facility

Data Analysis

MS/MS m/z spectra are deconvoluted with Proteome discoverer (Thermo Fisher Scientific) using default parameters and a S/N threshold of 2 specific feature are present to generate matched fragments using a 10 ppm

tolerance. ETD spectra are matched with c-, z-, and y- type ions, and EThcD spectra are also matched with those fragment types in addition to b- type ions.

WP2-FLIM and STORM on amyloid aggregates to get structural information

For super resolution imaging, the cavity of a clean single depression slide (Paul Marienfeld, Lauda-Königshofen, Germany) is filled with 50 µl of switching buffer (Abbelight, Paris, France), and covered by a coverslip, sealed with a two-component glue. The device is placed on the stage of an inverted motorized microscope NIKON ECLIPSE Ti-E AX MP (Nikon Instruments Europe, Amsterdam, The Netherlands) equipped with a CFI SR APO TIRF 100X ON1.49 objective, a Perfect Focus System and a total internal reflection fluorescence module (NIKON) as well as with a N-STORM module dedicated and coupled to a single-photon sensitive camera ORCA flas4.0 (Hamamatsu Japan. Acquisitions are performed at fixed 25°C in a dark heating chamber (Okolab NA, Pozzuoli, Italy). Phase contrast is first used for orientation and focus adjustment. The TIRF angle is then adjusted for each channel to reduce the background excitation. The region of interest is defined using the 647 and 532 nm laser line, and the 405 nm laser line is used to assess the autofluorescence signal. Prior to STORM imaging, a multichannel conventional fluorescence microscopy image is acquired for subsequent comparison with STORM image (snapshot). The excitation power of either 647 or 532 nm laser line is then strongly increased (~50 to 100 mW before the objective lens) to induce fluorophore blinking and preform STORM imaging. The em-gain of the camera is set to high amplification (300) to optimize the signal-to-noise ratio. Images are acquired with an integration time of 30 ms per frame.

NIS software (Nikon Instruments Europe, Amsterdam, The Netherlands) is used for real time localization and reconstruction. Spatial coordinates of each localized molecule are retrieved in real-time in two or three dimensions. The blinking spot is detected using 6x6 pixels region in a 180-250 intensity threshold range. In order to ensure optimal molecule density all along the acquisition process, an automatic feedback control on the 405 laser power is used. The total acquisition time points for each sequence are adapted to the observed structure and to the labelling density (5,000 to 20 000 frames). NIS software (Nikon Instruments Europe, Amsterdam, The Netherlands) is used for image processing and visualization and for measurements.

FLIM is done on the same samples using a Picoquant FLIM apparatus (PMA Hybrid) coupled to the same AX MP microscope, equipped with 520/35 and 600/50 filters (H560lpxr) or, alternatively 482/35 and 550/49 (H488lpxr). Acquisition is driven using the NIS software and MP settings (Coherent Chamelon II) at different wavelength (for most purposes the optimal range is around 900-976 nm) in 3D acquisition and analysis using Symphotime 64 software to obtain τ lifetimes. Different lifetimes can be accurately determined in FLIM or FLIM FRET configuration to get information about the proximity of fluorofores. In our conditions we will use specific antibodies (some of them are custom made) specifically addressed to amyloid regions. Here a brief and not exhaustive list of antibodies couples already available in our lab: Aβ peptides -R3660 (N-terminus-custom), pE3 (pyroglutamate 3-40/42, Nterminus), 4G8, 6E10 intermediate regions, Aβ40 or Aβ42 specific (C-terminus); Prions -3F4 (Aa109-112), 6H4, and PRNP (internal regions); Synuclein- Syn211 (epitopes Aa121-125), α-Synuclein (Aa117-131), KM51, LB509 (Aa115-122).; MAPT, AT8, AT270, Tau pThr181, M1

WP3-The study of cell membrane Second harmonic generation using AP3 dye (Funakoshi, Japan) and FLIM signals usingd the dye Dye FliptR as a fluorescent probe (Spirochrome, Switzerland) are routinely used in our lab in the MP setting. FliptR staining of cell membrane allows reliable imaging of membrane tension in living cells since, the intrinsic dependence of FliptR response on lipid composition generates information about the correlation between increasing lifetimes with increasing membrane tension. This response is consistent with tension-induced lipid phase separation into more ordered domains with more planarized probes and less ordered domains with more deplanarized probes. Amyloid strains will be challenged on FliptR loaded membranes to ascertain lipid changes and osmotic modification at different concentrations and timepoint. Ap3 is a non-fluorescent, photostable SHG-imaging dye used to membrane structure and function in multimodal imaging by no interference of signals from other fluorescent molecules. In this case AP3 loaded cells will be treatred with different strains of amyloid to measure SHG modifications and to correlate these changes with FLIM measurement of specific epitopes labelled with fluorescent antibodies in a correlative analysis.

Section c. Available instrumentations and resources

1- Orbitrap Fusion™ Mass Spectrometer (Thermo Scientific™) is a triple analyzer system: combining the speed of Quadrupole, the selectivity of Linear Ion Trap, as well as the high resolution of Orbitrap, enables an high degree of parallelization and, in turn, high throughput analyses of challenging samples. The system is coupled to an Ultra-Performance Liquid Chromatography (UltiMate™ 3000 RSLCnano System, Thermo Scientific™).

2-NIKON AX MP is a Eclipse Ti multiphoton (Chameleon II) confocal microscopes, equipped with a high-speed resonant scanner with 2K resolution, N-STORM, TIRF modules as well as FLIM (Picoquant) detectors. Is the first instrument of this type (all in one) settled in Europe by Nikon.

3-lOur ab fully equipped for routine preparations of RNA and protein analysis, amyloid purifications and separations from brain tissues, or from other samples.

Section d. GANTT diagram

cronoprogramma	Voce di costo	Mese1	Mese2	Mese3	Mese4	Mese5	Mese6	Mese7	Mese8	Mese9	Mese10	Mese11	Mese12
WP1-LC-MS analysis of amyloid aggregates	Personale; Materiale												
WP2-FLIM and STORM on amyloid aggregates to get structural information	Personale; Materiale												
WP3-Multiphoton studies on cell membranes challenged with amyloid	Personale; Materiale												

Curriculum vitae (max. 2 pages)

Finanziato dall'Unione europea NextGenerationEU
Ministero dell'Università e della Ricerca
Italiadomani PIANO NAZIONALE DI RIPRESA E RESILIENZA
MNESYS
Università di Genova

PERSONAL INFORMATION

Claudio Russo

University of Molise
Department of Medicine and Health Sciences
Via F. De Sanctis snc, 86100 Campobasso, Italy
+39-0874 404897 +39-3204794775

claudio.russo@unimol.it

Male | *03/12/1966* | Italy

ORCiD:https://orcid.org/0000-0002-2520-2958
Scopus: https://www.scopus.com/authid/detail.uri?authorId=7101896360

Metrics overview: **69** Documents; **3403** Citations by **2356** documents; **32** h-index (SCOPUS)

WORK EXPERIENCE

2015- to date	Full Professor of Biochemistry, Dept. of Medicine and Health Sciences, School of Medicine, University of Molise
2005- 2015	Associate Professor of Pharmacology, Dept. of Medicine and Health Sciences, School of Medicine, University of Molise.
2001- 2005	Research Associate at Dept. of Oncology, Biology and Genetics, School of Medicine, University of Genova.
1997- 2001	Ph.D. in Neurophysiology and Neuropharmacology at Dept. of Oncology, Biology and Genetics, School of Medicine, University of Genova.
1995- 1998	Research Associate at Neuropatology Dept. Case Western Reserve University, Cleveland, U.S.A.
1993-- 1994	National Research Council Fellowship. Dept. of Pharmacology at University of Genova.

EDUCATION AND TRAINING

2001	Ph.D. in Neurophysiology and Neuropharmacology at Dept. of Oncology, Biology and Genetics, School of Medicine, University of Genova.
Nov 1993	Graduated in Pharmacy, University of Genova, 105/110
Nov 1992	Graduated in Chemistry and Pharmaceutical Technologies, University of Genova, 104/110

Grants

2022 **Principal investigator** spoke3 in D34Health, Digital Driven Diagnostics, Prognostics and Therapeutics for Sustainable Health Care. PNRR project

2019	**Principal Investigator** "Diagnosi precoce della malattia di Alzheimer per ottimizzare la terapia farmacologica ed il percorso assistenziale" Progetti scientifici di cui all'art. 1 comma 34 e 34 bis, L. 23.12.1996, n. 662 per l'attuazione degli obiettivi di carattere prioritario e di rilievo nazionale.
2012-2015	**Principal Investigator** Progetti di Carattere Prioritario e di Rilievo Nazionale, Regione Molise: D15I13000430001, D15I13000430001
2004-2006	**Principal Investigator** Alzheimer Association IIRG-02-3976,
2004-2006	**Principal Investigator**: European Community contract N° LSHM-CT-2003-503330/ APOPIS,

Professional Experiences	Director of the Inter-Universities Consortium for Engineering and Medicine
2019-to date	Director of CERFU (Center for Research and Training in Pharmacoutilization) at University of Molise
2016-to date	
2018-to date	Scientific Director of the Regional Center for Pharmacovigilance (Molise)

Selected Publications in Peer-reviewed Journals;

[1] Medoro, A., et al. Journal of Alzheimer's Disease 2019, 68, 931. doi: 10.3233/JAD-181284
[2] Medoro, A., et al. Journal of Alzheimer's Disease 2018, 61, 1. doi: 10.3233/JAD-170628
[3] Penna, I., et al. Oncotarget 2017, 8, 8189. doi: 10.18632/oncotarget.14138
[4] Cristofano, A., et al. PLoS ONE 2016, 11. doi: 10.1371/journal.pone.0155694
[5] Porcile, C., et al. Journal of Cellular Physiology 2014, 229, 1444. doi: 10.1002/jcp.24582
[6] Nizzari, M., et al. Journal of Toxicology 2012, 2012. doi: 10.1155/2012/187297
[7] Nizzari, M., et al. Journal of Alzheimer's Disease 2012, 29, 211. doi: 10.3233/JAD-2011-101590
[8] Penna, I., et al. International Journal of Molecular Sciences 2011, 12, 5461. doi: 10.3390/ijms12095461
[9] Massone, S., et al. Neurobiology of Disease 2011, 41, 308. doi: 10.1016/j.nbd.2010.09.019
[10] Massone, S., et al. Journal of Cell Biology 2011, 193, 851. doi: 10.1083/jcb.201011053
[11] Errico, F., et al. Neurobiology of Aging 2011, 32, 2061. doi: 10.1016/j.neurobiolaging.2009.12.007
[12] Venezia, V., et al. Neurodegenerative Diseases 2007, 4, 101. doi: 10.1159/000101834
[13] Nizzari, M., et al. Journal of Biological Chemistry 2007, 282, 13833. doi: 10.1074/jbc.M610146200
[14] Russo, C., et al. Brain Research Reviews 2005, 48, 257. doi: 10.1016/j.brainresrev.2004.12.016
[15] Piccini, A., et al. Journal of Biological Chemistry 2005, 280, 34186. doi: 10.1074/jbc.M501694200
[16] Venezia, V., et al. Journal of Neurochemistry 2004, 90, 1359. doi: 10.1111/j.1471-4159.2004.02618.x
[17] Russo, C., et al. Journal of Neurochemistry 2002, 82, 1480. doi: 10.1046/j.1471-4159.2002.01107.x
[18] Russo, C., et al. Journal of Biological Chemistry 2002, 277, 35282. doi: 10.1074/jbc.M110785200
[19] Russo, C., et al. Nature 2001, 411, 655. doi: 10.1038/35079684
[20] Russo, C., et al. Neurobiology of Aging 2001, 22, 343. doi: 10.1016/S0197-4580(01)00212-3
[21] Russo, C., et al. Neurobiology of Disease 2001, 8, 540. doi: 10.1006/nbdi.2001.0419
[22] Russo, C., et al. Neurobiology of Disease 2001, 8, 173. doi: 10.1006/nbdi.2000.0357
[23] Gambetti, P., et al. Journal of Alzheimer's Disease 2001, 3, 87. doi: 10.3233/JAD-2001-3113
[24] Russo, C., et al. Nature 2000, 405, 531. doi: 10.1038/35014735
[25] Russo, C., et al. Proceedings of the National Academy of Sciences of the United States of America 1998, 95, 15598. doi: 10.1073/pnas.95.26.15598
[26] Teller, J. K., et al. Nature Medicine 1996, 2, 93. doi: 10.1038/nm0196-93

Campobasso, 14/12/2023

According to law 679/2016 of the Regulation of the European Parliament of 27th April 2016, I hereby express my consent to process and use my data provided in this CV

PERSONAL INFORMATION

Alfonso Di Costanzo

Affiliation
University of Molise
Department of Medicine and Health Sciences "Vincenzo Tiberio" (DiMeS)
Via Francesco De Sanctis 1, 86100 Campobasso

0039 08744041 0039 3389468552

alfonso.dicostanzo@unimol.it

www2.unimol.it

Sex M | *Date of birth* 29/07/1957 | *Nationality* Italian

CURRENT POSITION

University	EPR	Enterprise
☐ Full professor	☐ Research Director and 1st level Technologist / First Researcher and 2nd level Technologist / Principal Investigator	☐ Management Level
☒ Associate Professor	☐ Level III Researcher and Technologist	☐ Mid-Management Level
☐ Researcher and Technologist of IV, V, VI and VII level / Technical collaborator	☐ Researcher and Technologist of IV, V, VI and VII level / Technical collaborator	☐ Employee / worker level

WORK EXPERIENCE

from 2023

Responsible for the welfare program "Center for Cognitive Disorders and Dementia (CDCD)/ Molise Regional Health Agency (ASReM) Network Outpatient Service" (equated to U.O.C.);

Public Health Company of Molise Region (ASReM)

- *Clinical assistance*

from 2022

Member of the Permanent Table on Dementia Ministry of Health

Ministry of Health

- *Research and documents production*

from 2022

Responsible for the welfare program "Screening and treatment of metabolic disorders in patients with neurodegenerative diseases" (equated to U.O.S.V.D.)

Public Health Company of Molise Region (ASReM)

- *Clinical assistance*

from 2021

President of the degree course in Physiotherapy
University of Molise

- *Didactic and training coordination*

from 2018

Member of Institutional Review Board of the University of Molise.
University of Molise

- *Research assistance*

from 2013

Director of Centre for Research and Training in Medicine for Aging (CeRMI)
University of Molise

- *Clinical research*

from 2005

Associate professor of Neurology
Department of Medicine and Health Science "V. Tiberio", University of Molise
Research and teaching

from 2017 to 2021

Management Committee (MC) member to COST Action CA16112 "Personalized Nutrition in aging society: redox control of major age-related diseases – NutRedOx".
Founded by European Union

- *Promote and connect research initiatives across Europe*

EDUCATION AND TRAINING

1986

Residency in Neurology (50/50 cum laude)

Replace with EQF (or other) level if relevant

University of Naples Federico II, 1st School of Medicine

1982

License for professional medical practice

Replace with EQF (or other) level if relevant

University of Naples Federico II, 1st School of Medicine

1982

Degree in Medicine and Surgery (110/110 cum laude)

Replace with EQF (or other) level if relevant

University of Naples Federico II, 1st School of Medicine

MERITS

Editorial activity

Reviewer for several international journals, including Journal of Neurology, Neurosurgery and Psychiatry; Journal of Neurological Sciences; European Radiology; Neuroradiology; Neuromuscular Disorders; European Journal of Paediatric Neurology.

Invited presentations

12th International Conference on Dementia and Alzheimer diseases, September 03, 2021, Webinar
"Altered Blood Levels of Anti-Gal Antibodies in Alzheimer's Disease: A New Clue to Pathogenesis?"
Nutrition and Ageing Meeting-NutRedOx Cost Action 16112, Lisbona October 03, 2019
"Effects of Lippia citriodora leaves extract on lipid and oxidative blood profile of volunteers with hypercholesterolemia: a preliminary study"
7th Munich Metabolomics Meeting "Recent advancements in Metabolomics - from technology to applied clinical research" Virtual Edition November 13-14, 2020.
"Potential blood biomarkers in Alzheimer's disease: a case-control pilot study"

Grants

Principal investigator of PRIN -PNRR project " Understanding the link between comorbidities and Alzheimer's disease: focus on peripheral inflammation" (100,000 euros), Associated investigator of PRIN project " Lipidomic characterization of cell membranes and body fluids of patients with Alzheimer's disease and interaction with the phosphorylation status of Amyloid Precursor Protein" (75,000 euros), of PRIN project "Targeting tyr682 residue on the amyloid precursor protein for the development of diagnostic and therapeutic strategies in Alzheimer's disease" (100,000 euros), of POR – FESR -FSE project "Plug & Imaging Plus" (104,000 euros) and of Gatekeeper project "Radiolytx-DOPA: Parkinson's disease treatment decision support system" (40,000 euros)

PERSONAL SKILLS

Mother tongue(s): Italian

Other language(s): English and French

ADDITIONAL INFORMATION

Publications

Bibliometric
total number of citations: 2635 (Google Scholar) and 1571 (Scopus)
H index: 30 (Google Scholar) and 23 (Scopus)
Average IF/paper: 2.6

List **relevant** publications

Tortora F, Rendina A, Angiolillo A, Di Costanzo A, Aniello F, Donizetti A, Febbraio F, Vitale E. CD33 rs2455069 SNP: Correlation with Alzheimer's Disease and Hypothesis of Functional Role. Int J Mol Sci. 2022 Mar 26;23(7):3629.
Briganti S, Truglio M, Angiolillo A, Lombardo S, Leccese D, Camera E, Picardo M, Di Costanzo A. Application of Sebum Lipidomics to Biomarkers Discovery in Neurodegenerative Diseases. Metabolites. 2021; 11(12):819.
Angiolillo A, Gandaglia A, Arcaro A, Carpi A, Gentile F, Naso F, Di Costanzo A. Altered Blood Levels of Anti-Gal Antibodies in Alzheimer's Disease: A New Clue to Pathogenesis? Life (Basel). 2021;11(6):538.
Li J, Antonecchia E, Camerlenghi M, Chiaravalloti A, Chu Q, Di Costanzo A, Li Z, Wan L, Zhang X, D'Ascenzo N, Schillaci O, Xie Q. Correlation of [(18)F]florbetaben textural features and age of onset of Alzheimer's disease: a principal components analysis approach. EJNMMI Res. 2021 Apr 21;11(1):40.
Fiorilli G, Quinzi F, Buonsenso A, Casazza G, Manni L, Parisi A, Di Costanzo A, Calcagno G, Soligo M, di Cagno A. A Single Session of Whole-Body Electromyostimulation Increases Muscle Strength, Endurance and proNGF in Early Parkinson Patients. Int J Environ Res Public Health. 2021;18(10):5499.
Di Costanzo A, Paris D, Melck D, Angiolillo A, Corso G, Maniscalco M, Motta A. Blood biomarkers indicate that the preclinical stages of Alzheimer's disease present overlapping molecular features. Sci Rep. 2020 Sep 24;10(1):15612.
Iuliano E, di Cagno A, Cristofano A, Angiolillo A, D'Aversa R, Ciccotelli S, Corbi G, Fiorilli G, Calcagno G, Di Costanzo A; EPD Study Group. Physical exercise for prevention of dementia (EPD) study: background, design and methods. BMC Public Health. 2019 May 29;19(1):659.
Italiani P, Puxeddu I, Napoletano S, Scala E, Melillo D, Manocchio S, Angiolillo A, Migliorini P, Boraschi D, Vitale E, Di Costanzo A. Circulating levels of IL-1 family cytokines and receptors in Alzheimer's disease: new markers of disease progression? J Neuroinflammation. 2018 Dec 12;15(1):342.

Corso G, Cristofano A, Sapere N, la Marca G, Angiolillo A, Vitale M, Fratangelo R, Lombardi T, Porcile C, Intrieri M, Di Costanzo A. Serum Amino Acid Profiles in Normal Subjects and in Patients with or at Risk of Alzheimer Dementia. Dement Geriatr Cogn Dis Extra. 2017;7(1):143-159.
Palmieri G, Cocca E, Gogliettino M, Valentino R, Ruvo M, Cristofano G, Angiolillo A, Balestrieri M, Rossi M, Di Costanzo A. Low Erythrocyte Levels of Proteasome and Acyl-Peptide Hydrolase (APEH) Activities in Alzheimer's Disease: A Sign of Defective Proteostasis? J Alzheimers Dis. 2017;60(3):1097-1106.

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Alfonso Di Costanzo

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*
Investigation of common biochemical pathways, centered around the Amyloid precursor protein (APP), in Alzheimer's Disease and breast cancer models: possible role of Purine Nucleoside Phosphorylase (PNP).	MUR "PRIN 2022"	89661€	2023-2025	Co-PI	None
D3 4 Health	PNRR	3930000€	2022-2025	PI spoke	None

Digital Driven Diagnostics, prognostics and therapeutics for sustainable Health care					
Fast, **R**eliable, **E**conomic and **E**thic **Mo**dels to Study **De**livery of Nucleic Acid-based Therapeutics FREEMODE RNA	PNRR-Submitted	740000€	2024	PI	None

TABELLA COSTI PERSONALE STANDARD				COSTO DEL PERSONALE
FASCIA DI COSTO /LIVELLO	**NUMERO SOGGETTI**	**COSTO ORARIO vedi nota**	**MONTE ORE**	
Basso				- €
Medio	**2**	48 €	700	33.600 €
Alto	**1**	73 €	125	9.125 €
TOTALI	**3**		**825**	**42.725 €**
COSTO ORARIO:	si deve far riferimento al Decreto Interministeriale n. 116 del 24/1/2018			

ALLEGATO C

BUDGET DI PROGETTO	COSTO DEL PERSONALE	OVERHEAD	Costi per servizi di Consulenza Specialistica	Costi per licenze direttamente imputabili al progetto	Costi per materiali e attrezzature direttamente imputabili al progetto	Costi per altre tipologie di spese direttamente imputabili al progetto	COSTO TOTALE
	42.725,00 €	6.408,75 €	12.000,00 €	0,00 €	73.500,00 €	15.300,00 €	149.933,75 €