APPENDIX 2 – Funding application PN-III-ID-PCE-2021-3

B. Project leader

B1. Important scientific achievements of the project leader (maximum 2 pages)

Prof. Dr. Gabi Drochioiu, PhD, PhD supervisor, Professor in Biochemistry & Toxicology at Al. I. Cuza University of Iasi was born 23.01.1954, in Unţeni, Botoşani County, Romania. (1) There is a link between his previous papers and patents and this project proposal. He has been working since **2003 (**after his first DAAD scholarship at Uni-Konstanz, Germany, under the leadership of Prof. Dr. Michael Przybylski from Analytical Department) in **Proteomics** and **Metabollomics** applied to **neurodegeneration**, such as Alzheimer's disease, being specialized in **mass spectrometry** (ESI-FT-ICR; ESI-Ion Trap; MALDI ToF MS; *Results*: paper in *Ultrason Sonochem*, 29, 93, 2016 (IF **7.491**); *Talanta*, 69, 556, 2006-as first & corresp. author (IF **6.057**); *Biomacromolecules*, 8, 3836, 2007 (IF **6.988**); *Adv Exp Med Biol*.; 1140, 401, 2019 (I.F. 2.622); *Eur J Mass Spectrom*, 13, 331, 2007; *Eur J Mass Spectrom*, 26, 106, 2020); **electrophoresis** (*Electrophoresis* 40, 2747, 2019; I.F. **3.535**); manual & automat **solid phase peptide synthesis** (**SPPS**; Papers in *J Pept Sci*, 17, 512, 2011; IF 1.905; *Int J Pept Res Therap*, 25, 897, 2019; IF 1.931), and characterization of metal-peptide complexes (Papers: *Biophys Chem*, 144, 9, 2009 (I.F. 2.352); *Biopolymers*, 93, 497, 2010; I.F. 2.505); atomic force microscopy (**AFM**), nuclear magnetic resonance (^{1}H $^{\&}$ ^{13}C NMR: *Int J Pept Res Therap*, 25, 303, 2019; *Ultrason Sonochem*, 29, 93, 2016; IF 7.49), **molecular modelling** (*Molecules* 2020, 25, 4536; IF **4.411**), **circular dichroism** spectroscopy (**CD**; *Int J Pept Res Therap*, 25, 897, 2019), Fourier transform infrared (**FTIR**) spectroscopy (*Int J Pept Res Therap*, 25, 303, 2019; *Int J Pept Res Therap*, 25, 897, 2019), with contribution in toxicology as well (*Toxics* 2021, 9, 36; IF **4.146**). His 1997-**Ph.D. Thesis** also refers to amino acid & protein determination, by which he improved analytical methodology. The interest in novel analytical methods can be also seen in patents (A/00057/ 27.01.2015; 114902 B/1999 etc.) and several papers: *Ultrasonics Sonochemistry*, 29, 93–103, 2016; *Analyst*, 125, 939, 2000; *Talanta* (IF 6.057), 56, 425, 2002 (novel protein assay based on his Romanian patent RO90243); *Anal. Bioanal. Chem*. (**IF 4.142**) 372, 744, 2002, *Talanta*, 56, 1163, 2002; *Toxics* 2021, 9, 36; J. Forensic Sci., 66, 1171–1175, 2021; etc. a) **CD** spectroscopy allow us to show metal-, polyphenol-, antioxidizer-, and SDS-induced peptide conformational changes (*Biomacromolecules, Biopolymers, J. Pept. Res., Int. J. Pept. Res. Therap*., 2016 (***Kw***: CD; proteomics). Thus, CD spectra at pH 6.6; 7.4; 8.0; 8.5; 9.0 & 9.2 evidence changings from β-sheet to α-helix with increasing pH, even in the presence of copper ions (unpubl. paper; here, Fig. 2). MS & CD techniques bring evidence for conformational changes dependent on pH at metal ion binding to Aβ and other peptides. Other **CD**

studies: Silver binding to peptides, *J. Pept. Sci*., 17, 512, 2011 (***Kw***: CD; MS; metal binding), or other metal ions (*Eur. J. Mass Spectrom*., 16, 511, 2010 & *Biopolymers*, 93, 497, 2010). Combined techniques, IR, MS & CD studied conformationally-different peptides (*Int. J. Pept. Res. Therap*., 15, 303, 2009). b) By **AFM** & **MS** it was evidenced the oligomerization, aggregation and fibrilation of Aβ-copper complexes and related peptides (*Biopolymers*, 93, 497, 2010; *Biomacromolecules*, 2007 (***Kw***: MS, AFM, metal binding; metallomics). Recently, our team published pH-dependent conformational changes in metal ion binding by all these methods in *Int. J. Pept. Res. Therap*., **2020**, DOI:10.1007/s10989-020-10048-0; c) We extended our research from zinc, copper & silver-Aβ complexes (*Encyclopedia of Metalloproteins*. Eds: Kretsinger et al., Springer, 2013, 2063-2071, (this project); e) Possible role of chloroquine, hydroxychloroquine, clioquinol, NOSH aspirin etc. in Alzheimer's disease will be studied in this project (so far, please see *Med. Hypotheses*, **84**, 262–267, 2015; MS approach of high pH- and copper-induced glutathione oxidation is very significant for this project! First time was published in *Eur. J. Mass Spectrom.*, 19, 71, 2013;

f) Natural compounds with medical effect in *Food Chem.*, 141, 2788, 2013; g) Biostructural approach of living systems, in *BioSystems*, 109, 126, 2012 (We started from molecular to bio-organizational level and advanced to a structural-phenomenological outlook of disease and living organisms);

h) Interaction of β-amyloid(1-40) peptide with pairs of metal ions was studied by MS at various pH values (*Biophys Chem*, 144, 9, 2009). We showed for the first time a pH-dependent competition between heavy metals toward amyloid-β peptides; it happens in AD brains as well);

i) **FTIR** spectroscopy was used as a simple technique to evaluate the proportion of peptide conformers (*Rev. Roum. Chim*., 56, 783, 2011), and this study was highly cited (155 citations; this work published in a national IF journal was cited many times in high ranked journals like *Nat. Methods* (IF 28.467), *Advanced Materials* 2019 & 2015 (twice, IF 25.809); *Angew Chem* (2 times, IF 12.257), *ACS Appl Mat Interf* (three times, IF 8.454); *Acta biomater* (IF 6.638); *J Colloid Interface Sci* (IF 6.361); *Chem Commun* (IF 6.164); *Biomacromolecules* (twice, IF 5.667); *J Mater Chem B* (twice, IF 5.047); *Artif Cells Nanomed Biotechnol* (IF 4.462); *Mol Neurobiol* (IF 4.586); *Structure* (IF 4.576); *ACS Biomat Sci Eng* (IF 4.511); *Front Microbiol* (IF 4.259); *Int J Mol Sci* (IF 4.183); *Colloids and Surfaces B* (IF 3.973); *RSC Advances* (many times; IF 3.049), *Anal Bioanal Chem* (three times, IF 3.286), *Int J Biol Macromol*, (six times, IF 4.784); *Sci Rep* (twice, IF 4.011); *Langmuir* (3.683); *Soft matter* (3.399); *Nanoscale Res Lett* (twice, IF 3.159); *New J Chem* (IF 3.069); *Spectrochim Acta* (twice, IF 2.931); *Mat Chem Phys* (IF 2.791); *J Phys Chem B* (IF 2.923); *PloS One* (twice, IF 2.776); *Int Dairy J* (IF 2.735); *Tetrahedron* (IF 2.389); *ACS omega* (twice, ISI 2.58); *Amino Acids* (IF 2.52); *Pharmac Develop Technol* (IF 2.347) a.s.o.

B2. The visibility and the impact of the scientific contribution of the project leader

a) 1026 citations (**872** without self-citations); Hirsch index h = **19** of from Web of Science & Scopus, without self-citations h-index : 14; h = **22** from google scolar.

b) link-ul personal din platforma www.brainmap.ro: www.brainmap.ro/gabi-drochioiu;

c) Scopus Author ID: 35582522800, https://www.researchgate.net/profile/Gabi_Drochioiu ORCID: https://orcid.org/0000-0002-8895-8293, Google Scholar https://scholar.google.com/citations?user=Xejm09sAAAAJ&hl=en

d) se vor indica cele mai reprezentative publicaţii (max. 10):

Identification data:	**Drochioiu, G**., Ciobanu, C.I., Bancila, S., Ion, L., Petre, B.A., Andries, C., Gradinaru, R.V., Murariu, M. Ultrasound-based protein determination in maize seeds. Ultrasonics Sonochemistry, 29:93–103 (2016) (IF 7.279; SRI 2.230);
Is she/he the main author?	YES (see Annex 5)
Is it in the project domain?	YES
Number of citations:	14
DOI (Digital Object Identifier)	DOI: 10.1016/j.ultsonch.2015.09.007

Identification data:	Adochitei A., **Drochioiu**, G. Rapid characterization of peptide secondary structure by FT-IR spectroscopy Rev roum Chim, Rev. Roum. Chim 56 (8), 783-791
Is she/he the main author?	YES (see Annex 5)
Is it in the project domain?	YES
Number of citations:	**155**
Identification data:	Surleva, A., **Drochioiu, G**. *A modified ninhydrin micro-assay for the determination of total cyanogens in plants*. Food Chemistry, 141(3):2788–2794 (2013) (**IF 5.399; SRI 2.641**)

Is she/he the main author?	YES (see Annex 5)
Is it in the project domain?	YES (only partly: novel analysis methodology)
Number of citations:	***24***
DOI (Digital Object Identifier)	*DOI: 10.1016/j.foodchem.2013.05.110*
Identification data:	Postu, P. A., Ion, L., Drochioiu, G., Petre, B.A., Glocker, M. O. Mass spectrometric characterization of the zein protein composition in maize flour extracts upon protein separation by SDS-PAGE and 2D Gel electrophoresis. Electrophoresis 40:2747-2758 (2019) (IF 2.754)
Is she/he the main author?	NO (Relevant for his scientific production; see Annex 5)
Is it in the project domain?	YES (Mass spectrometry & Electrophoresis)
Number of citations:	6
DOI (Digital Object Identifier)	DOI: 10.1002/elps.201900108
Identification data:	G Drochioiu, M Manea, M Dragusanu, M Murariu, ES Dragan, BA Petre, Interaction of β-amyloid (1-40) peptide with pairs of metal ions: An electrospray ion trap mass spectrometric model study. Biophys. Chem. 144 (1-2), 9-20 (IF 3.261);
Is she/he the main author?	YES (see Annex 5)
Is it in the project domain?	YES: Relevant for kinetics of compounds disaggregation only
Number of citations:	44
DOI (Digital Object Identifier)	DOI: 10.1016/j.jphotochem.2020.112497
Identification data:	Lupaescu, A.V., Jureschi, M., Ciobanu, C.I., Ion, L., Zbancioc, G., Petre, B.A., Drochioiu, G. FTIR and MS evidence for heavy metal binding to anti-amyloidal NAP-like peptides. Int J Pept Res Therap., 25:303–309 (2019) (IF 1.75);
Is she/he the main author?	YES (see Annex 5)
Is it in the project domain?	YES
Number of citations:	8
DOI (Digital Object Identifier)	DOI: 10.1007/s10989-018-9672-2
Identification data:	D Humelnicu, G Drochioiu, MI Sturza, A Cecal, K Popa,

	Kinetic and thermodynamic aspects of U (VI) and Th (IV) sorption on a zeolitic volcanic tuff. J. radioanal. Nucl. Chem. 270 (3), 637-640
Is she/he the main author?	No
Is it in the project domain?	YES (Hypothesis followed in the project; medical implications)
Number of citations:	74
DOI (Digital Object Identifier)	DOI: 10.1016/j.mehy.2015.01.008
Identification data:	G Drochioiu, NE Damoc, M Przybylski, Novel UV assay for protein determination and the characterization of copper–protein complexes by mass spectrometry. Talanta 69 (3), 556. ISI >6
Is she/he the main author?	YES (see Annex 5)
Is it in the project domain?	YES
Number of citations:	30
DOI (Digital Object Identifier)	DOI: 10.1016/j.ultsonch.2015.09.007
Identification data:	M Murariu, ES Dragan, G Drochioiu, Model peptide-based system used for the investigation of metal ions binding to histidine-containing polypeptides. Biopolymers 93 (6), 497-508
Is she/he the main author?	YES (see Annex 5)
Is it in the project domain?	YES
Number of citations:	28
DOI (Digital Object Identifier)	DOI: 10.1002/bip.21385

Patents*: The patents/utility models with techonogical transfer obtained in other countries from the European Union or in countries member of OCDE will be especially indicated. The presentation format is the following:*

Identification data:	Drochioiu, G. Process for separating floury fractions of high biological or industrial quality RO128468-A2; BOPI nr. 12/2015
Issuing patent bureau	OSIM Bucharest
Is it in the project domain?	YES, it is related to Analytical chem., Mass spectrum. Proteomics

Significant works uploaded on http://teclu.chem.uaic.ro/drochioiu/.

(2) *the management of the research activity* by establishing and/or running some research teams or work groups which had as a result outstanding scientific achievements

Common projects and papers: Przybylski M & Damoc EN Uni-Konstanz, Germany (*Talanta*, 69, 556, 2006); Ilieva DM, Argirova M, Angelova LY, Surleva AR. Univ Chem Technol Metall Sofia, Bulgaria (*Environ Sci Pollut R* 27, 1386, 2020); Glocker MO, Rostock University Germany (*Electrophoresis*, 40, 2747, 2019); Mezo G, Przybylski M. (*Biophys Chem*, 144, 9, 2009); Schlosser G, Przybylski M, Hudecz F, (Eur J Mass Spectrom., 13, 331, 2007) etc.

(3) the most important international prizes[1]: ***Romanian Academy Prize***: The Nicolae Teclu Prize for Chemistry-2013 (*Noxae-cellular components interactions: mass spectrometric, circular dichroism and infrared spectroscopic studies*); Third Prize at the 5th International Olympiad of Chemistry, Sofia, Bulgaria, 1972; DAAD Awards, 2003 and 2006-2007, University of Konstanz, Germany etc.

(4) the status of invited speaker to prestige universities[2]**:** Eőtvos Lorand University of Budapest, Uni-Konstanz, Germany, Harran University, Sanlıurfa, Turkey, University of Chemical Technology and Metallurgy of Sofia, Bulgaria, University of Crete, Greece, University of Vienna, University of Cambridge, UK, Istanbul Medeniyet University, etc.

[1] The prize was awarded by a legal entity from OECD country
[2] Only 500 top universities, according to ARWU Ranking, www.arwu.org

B3. The correspondence between the demostrated experience of the project leader and the proposed theme (max. 1 page).

The project leader coordinated research projects and supervised Ph.D. theses in the research area of this project proposal. He has also patents and papers as main author. He encouraged young scientists to publish (MS, AFM, SEM, FTIR, etc.) both independently and in group: 1) Dr. Adriana Adochiţei, PhD thesis: Peptides and peptide-metal complexes with implications in biomedical and biological research, defended on 27.08.2012; 2) Dr. Laura Ion-Darie, PhD thesis: Peptide bond compounds and their biomedical applications, defended on 10.11.2015; 3) Dr. Laura Hăbăşescu (married Solomon) PhD thesis: Structural changes and behavior of peptides under the action of metal ions, defended on 09.03.2016; 4) Dr. Claudia Andrieș, PhD thesis: Biologically active compounds involved in disease processes: characterization by mass spectrometry and complementary techniques, defended on 07.12.2016; 5) Dr. Ancuţa Veronica Lupăescu, PhD thesis: Analogues of NAP neuroprotective peptide: synthesis, characterization and interaction with metal ions, defended on 18.12.2019; (2) his original contributions are largely published and were cited in highly ranked journals (Nat. Methods, Advanced Materials, Chem Rev, Angew Chem, Biomacromolecules, etc.). (Please see Scopus Author ID: 35582522800, https://www.researchgate.net/profile/Gabi_Drochioiu ORCID: https://orcid.org/0000-0002-8895-8293, Google Scholar https://scholar.google.com/citations?user=Xejm09sAAAAJ&hl=en (3) the independent research activity of the project leader: main author 59 papers (ISI journals) of a total of 87, many of them on mass spectrometry, proteomics, metallomics etc. International projects (Univ. E.L. Budapest -Top500; US-RO Foundation); DAAD awards; many times as project leader; his activity as PhD supervisor; many citations of his papers in highly ranked journals etc. For instance, a patent application (a 2010 01336/29.07.2016; BOPI nr. 7/2016) for high pH MS measurements of heavy metal: peptide complexes. On replacing ammonium acetate or carbonate with ethanolamine and related compounds as buffers we succeeded to collect mass spectra from pH that excceed the physiological values, even at pH > 10. Because the acquired spectra were complicated, he proposed a simple mathematical solution to get reliable data using „charge state ruler" option (*Talanta*, 69, 556, 2006 (*Kw*: mass spectrometry; metal binding). This inovation allows us to measure complex formation within a large pH gradient (physiological pH 6.6-7.4, yet pH 2 & pH 8.5 can be measured in stomach or mitochondria). Using his MS improvement one can state how metal ions bind to Aβ peptides in various media; the highest interest of scientists in our novel infrared spectroscopic approach, since it was cited 155 times in highly ranked journals. These project-related findings and results of the project leader significantly improved the knowledge in the field of Proteomics, Metallomics & Mass Spectrometry as the number of citations show.

B4. Curriculum Vitae (max. 2 pages)

(Complete CV at www.brainmap.ro/gabi-drochioiu)

Name: **Gabi Drochioiu**; Date/Place of Birth: January 23, 1954, Unţeni, Botosani County, Romania; Citizenship: Romanian. *Addresses: Office*: Chemistry Faculty, Al. I. Cuza University of Iasi, 11 Carol I, Iasi-700506, Romania, Tel. +40-232-201279, Fax. +40-232-201313; e-mail: gabidr@uaic.ro *Home:* Rediu-Iasi, 2 Părului Str., 707405, Romania

a) information about the degrees and diplomas:

1997 – Doctor in Chemistry - Al. I. Cuza University of Iasi, (PhD supervisor: Prof. Dr. Magda-Noela Petrovanu); *Thesis*: Screening the cereal inbred lines by characterizing their amino acids and proteins; *1974-1978* – Faculty of Chemistry from „Gh. Asachi" Polytechnic Institute of Iasi,; *1973* – Graduate from Military High School, Cîmpulung Moldovenesc, Suceava, Romania.

b) *professional experience and jobs.* If the project leader coordinated a team/ group or a research laboratory:

Professor in Biochemistry and Toxicology; *PhD supervisor* in Chemistry since 2007; Researcher & inventor; *Research field*: Proteomics, Metabollomics, Neurochemistry, Mass spectrometry, peptide synthesis, Bioanalytical Chemistry, Bioenergetics, NMR, Circular dichroism spectroscopy, etc.

Leader of the *Biochemistry & Toxicology Group* of Al. I. Cuza University;

Director of Doctoral School "Chemistry and Life and Earth Sciences" (2012-2017);

Team leader of research projects: Two *international* projects as project coordinator (For example, Bilateral Mobility Project Hungary-Romania; projects coordinators: Prof. Dr. Ferenc Hudecz, Rector of University Eotvos Lorand Budapest at that time, and Prof. Dr. Gabi Drochioiu, University Al. I. Cuza Iasi), and seven national ones (of which only two as resposible for the project); project type: partnership, IDEI, a grant from Romanian Academy. *Vice-dean* of the Faculty of Chemistry (2004-2005); *Chancellor* of the Faculty of Chemistry (2005-2008), Manager small comp. (1997-1999).

Brief chronology of employment: *1978-1980* – Chemist, Suceava Cellulose and Paper Factory, Romania; *1980-1982* – Chemist (Biochemistry), the Agricultural Research Station of Suceava, Romania; *1982-1990* – Researcher (Biochemistry), the Agricultural Research Station of Suceava;*1990-1997* - Principal researcher (Biochemistry), Suceava Genebank, Romania; *1997-1999* – Manager, Tao Biochemicals Ltd of Suceava, Romania; *1999 to present* Al. I. Cuza University of Iasi– Lecturer (1999-2002), Assoc. Prof. (2002- 2005), Professor (2005 – 2019); Doctoral School (PhD supervisor 2007-present). University Courses of *Biochemistry*, *Toxicology*, *Nucleic Acids and Proteins*, *Metabolism*, *Forensic Toxicology etc.Member of Societies*: American Chemical Society (ACS Number 2438319); Romanian Society of Chemistry; Romanian Society for Soil Sciences, (1982 – 1997).

Awards: **Romanian Academy Prize** „Nicolae Teclu" for Chemistry-2013; Gold medal Euroinvent 2018; Twice **DAAD** Awards, 3 months, 2003 & 3 months, 2006-2007, University of Konstanz, Germany, under the leadership of Prof. Dr. Michael Przybylski; Third Prize - 5th International Olympiad of Chemistry, Sofia, Bulgaria, 1972; Several prizes for scientific papers from CNCS Bucharest etc.

Visiting professor: Eőtvos Lorand University of Budapest (Top500) & common project, Uni-Konstanz, Germany (Top500) & common papers, University of Chemical Technology and Metallurgy, Harran University, Sanlıurfa, Turkey, University of Crete, Greece etc.

Scientific activity: 7 patents at OSIM Bucharest, 87 papers with impact factor (cumulated impact factor (IF) > 130), numerous papers as open access, 9 books and 3 chapters in other three books (Springer, Trafford Publ. USA & Canada etc.), more than 50 invited talks at the international conferences, over 1000 citations (ISI web of knowledge); Hirsch factor 19 (Scopus), 19 (Web of Science). Papers published in journals like *Ultrasonic Sonochem.* (IF 7.279); *Biomacromolecules* (IF 5.667); *Food Chem*. (IF 5.399); *Talanta* (IF 4.916); *Analyst* (IF 4.019); *Anal Bioanal Chem* (3.286); *J Photoch Photobio A* (IF 3.261); *Environ Sci Pollut R* (IF 2.914); *Electrophoresis* (IF 2.754); *Biometals* (2.455); *Adv Exp Med Biol* (IF 2.126) Citations in *Chem. Rev*., *Nat. Methods*, *Angew*. *Chem., J. Am. Chem. Soc*. etc.

Fundraising by research projects: 1) ***Qualifarin***: ***American-Romanian Foundation***. Team leader **Drochioiu, G.** (10/2013 – 06/2014), 78500 USD (69315 €). **2**) **Hungary**-Romania Bilateral Mobility Project, Code 2/2005; Period: 2006 & 2007, project coordinators: Prof. dr. Ferenc Hudecz, University Eotvos Lorand Budapest & Prof. Dr. **G. Drochioiu**, Al. I. Cuza University of Iasi. 3) PN-III-P4-ID-PCE-2016-0376 by UEFISCDI, Period: 2017-2019, PI **Drochioiu, G.** (185000 €). 4) PN-III-P2-2.1-PED-2016-0869, 2016-2018, PI: **Drochioiu, G.** (103000 €). 5) PN-IIPT-PCCA-2013-4-1149 by UEFISCDI, Period: 2015-2017; Project coordinator: **Drochioiu, G.** (310000 €). 6) PNII Project CNMP Code 4300/2008, Contract 32-173/2008 (2008-2011) PI: **Drochioiu, G.** (476190 €). 7) Partenership PNII Project CNMP Code 2746/2007, Contract No. 31-017/2007 (2007-2010), Project leader: **Drochioiu, G.**

Technical skills and competences: Mass spectrometry, Circular Dichroism, AFM, NMR, and FT-IR investigation of metal complexes of peptides and proteins, HPLC, Electrophoresis, Peptide synthesis, Analytical chemistry & biochemistry, Toxicology, Fluorimetry, Biostructure and biosystems.

Synergistic Activities: *Journal reviewer* for: (1) Biomacromolecules, (2) International Journal of Environmental Analytical Chemistry, (3) Romanian Biotechnological Letters, (4) Science of the Total Environment, (5) Journal of Food Science, (6) Food and Chemical Toxicology, etc. *oreign Languages*: English (good); French and German (acceptable).

C. Funding application (*max. 11 pages*)

C1. Motivation of the proposed theme in the current scientific context. Originality and degree of innovation

Scientific motivation: Using analytical modern methods we intend to find those peptides and derivatives able to interact with amyloid-β peptides in Alzheimer brains and to improve the knowledge on the neurochemistry of degenerative pathologies. Experiments based on analytical methods like mass spectrometric (MS), infrared (IR), UV-vis and microscopic techniques revealed significant changes in the structure of peptides, including their aggregation and fibrillation, associated with metal binding [1-4]. In addition, new experimental data will be published or patented, related to complex process of neurodegeneration and the mechanism of amyloid occurence, metal ions binding, and amyloid plaque formation as well as the conditions in which amyloids, fibrils and aggregates can be degraded and removed. We enhance both the investigating methods (larger range of pH, time-developing phenomena) and the research conception, which starts from the molecular mechanisms of (neuro)degeneration to reach a structural-phenomenologic outkook of aging and age-related diseases [5,6]. Furthermore, a new mass spectrometric (MS) method (our patent application) will be applied here to the investigation of peptide complexes, namely metal ions-peptides-antiaggregants, as well as to the pH- and time-dependent formation of amyloid [7]. Defeating Alzheimer's disease (AD) and other dementias is a priority for European science and society [8]. The double hit of dementia and Covid-19 pandemic has raised great concerns for people affected by AD [9]. The prevalence of AD continues to increase worldwide, becoming a great healthcare challenge of the twenty-first century [10]. Data from literature suggest that AD and Covid-19 may adopt a common solution. In addition, they have in common the relation with heavy metals (e.g. Zn), abnormal proteolytic activities, pH, the effect of quinoline derivatives, anti-inflammatory drugs etc. Hydroxychloroquine can also inhibit replication of SARS-CoV-2 in vitro [11]. Some observational studies have suggested benefits of hydroxychloroquine for the treatment of Covid-19, whereas other treatment reports have described mixed results [12]. Very little is known about how pH- and time-dependent metal ion binding to specific Aβ sequences influences the structure, aggregation, fibrillation and plaque formation *in vitro* and in AD brains. In this project, we aimed to address this knowledge gap and determine the relative contribution of metal binding of one or several Aβ residues in to the regulation of its biophysical and functional properties. Towards this goal, we use a combination of MS, CD, AFM, NMR, FTIR, electrophoresis, DLS, RP-HPLC and other techniques. NMR studies should demonstrate if aggregation is mediated by a local increase in β−sheet propensity of Aβ domain. Our findings will underscore multifactorial role of

hypoxia-induced acidity, metal binding, and Aβ aggregation and highlight the importance of further studies to elucidate their role in AD and in regulating Aβ function in prevention and disease.

Scientific context. AD is characterized by an intracranial amyloid aggregates (neural plaques of $A\beta_{1-42}$, $A\beta_{1-40}$; cholesterol; metal ions like Cu, Fe, Zn etc), which are dependent upon the production β-amyloid peptides by proteolysis of their integral membrane Aβ precursor protein [13,14]. Aluminum and amyloid-β are co-located in a senile plaque-like structure [15]. The interaction of Aβ peptides with ions of copper, zinc, iron, aluminium, manganese or mercury may contribute to neuronal damage [16-18]. Such proteolytic Aβ peptides are degraded and removed, but they may accumulate in AD brains, mainly in presynaptic terminals of neurons to form insoluble plaques and thus preventing the transmission of nervous impulse [19]. Aβ plaques contribute to neuronal loss and ultimate failure of cognitive function [20]. Once amyloid-ß begins to accumulate, it then promotes the build-up of tau [21]. Peptide aggregates lead to the death of affected hippocampal neurons [22]. Hence, blocking the production of Aβ peptides and aggregates is considered of interest to pathogenic disease therapy [23]. AD is not curable, but treatment is available to slow down the disease progression and reduce the cognitive impairment and behavioral problems, but do not stop the progression of neurodegeneration [24]. Healthy diet, lifestyle improvement and nutraceuticals targeting of oxidative stress, inflammation, abnormal mitochondrial dynamics and the mitochondrial interaction with abnormal disease-related proteins and assessment of impact of environmental contaminants can be a promising approach in the treatment of neurodegenerative diseases.

Delirium caused by ***hypoxia***, a prominent clinical feature of Covid-19, could complicate the presentation of AD. Hypoxia facilitates AD pathogenesis by up-regulating BACE1 gene expression [25,26]. Hypoxia also affects oxidative metabolism and accelerates reactive oxygen species (ROS) generation [27]. Moreover, hypoxia inhibits the mitochondrial function by impairing calcium homeostasis, diminishing Na^+/K^+-ATPase activity, and declining membrane resting potential, leading to inhibited energy production [26,28,29]. Furthermore, enhanced glycolysis related to increased phosphofructokinase-1 and pyruvate kinase activity under hypoxic conditions is associated with increasing lactate levels [30]. This increases β-amyloid production and impairs β-amyloid clearance due to dysfunction of the blood–brain barrier from vascular damage [31]. Prolonged hypoxia stimulates hypoxia-inducible factors, which alters gene expression, inactivates anabolism, and inhibits mitochondrial aerobic metabolism by inhibiting pyruvate dehydrogenase, resulting in lactate production [26,32]. In AD, amyloid-β peptide (Aβ) and ROS impair mitochondrial function. Neurons switch to glycolysis to maintain ATP production and to produce molecules involved in antioxidant

metabolism in an attempt to survive. Aging seems to affect both the production and clearance of lactate in otherwise normal functioning brain [33].

AD is characterized by an intracranial amyloid aggregates (neural plaques of $A\beta_{1-42}$, $A\beta_{1-40}$; cholesterol; metal ions like Cu, Fe, Zn etc), which are dependent upon the production β-amyloid peptides by proteolysis of their integral membrane Aβ precursor protein [34,35]. Aluminum and amyloid-β are co-located in a senile plaque-like structure [36]. The interaction of Aβ peptides with ions of copper, zinc, iron, aluminium, manganese or mercury may contribute to neuronal damage [37-39]. Such proteolytic Aβ peptides are degraded and removed, but they may accumulate in AD brains, mainly in presynaptic terminals of neurons to form insoluble plaques and thus preventing the transmission of nervous impulse [40]. Aβ plaques contribute to neuronal loss and ultimate failure of cognitive function [41]. Once amyloid-ß begins to accumulate, it then promotes the build-up of tau [42]. Peptide aggregates lead to the death of affected hippocampal neurons [43]. Hence, blocking the production of Aβ peptides and aggregates is considered of interest to pathogenic disease therapy [44]. AD is not curable, but treatment is available to slow down the disease progression and reduce the cognitive impairment and behavioral problems, but do not stop the progression of neurodegeneration [45]. Healthy diet, lifestyle improvement and nutraceuticals targeting of oxidative stress, inflammation, abnormal mitochondrial dynamics and the mitochondrial interaction with abnormal disease-related proteins and assessment of impact of environmental contaminants can be a promising approach in the treatment of neurodegenerative diseases.

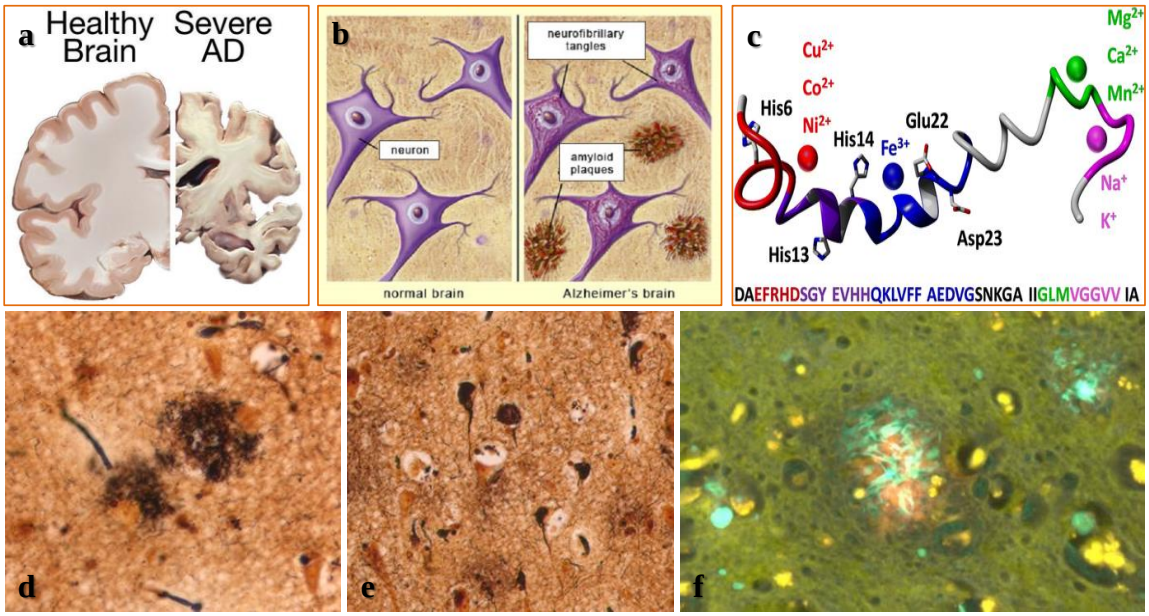

Fig. 1. Amyloid plaques in Alzheimer's brains (Lermyte et al. 2019)

Inflammation actually contributes to the AD progress [46]. The molecular mechanisms of neurodegeneration are still elusive [47]. So far, the so-called age-related pathologies were investigated from the molecular point of view only, without keeping in mind the whole complexity of living organisms (life style, sleep quality, cybernetic feed-back, time-dependent evolution, cellular biostructures, chemical environment: metals, peptides, proteins, glucose, oxygen, NADH, glutathione etc. in the same place) [48]. However, we have recently been looking for evidence of such complexity using new mass spectrometry procedures advanced by us [49]. We proved the pH- and time-dependent formation of complex multi-component systems that were then confirmed by CD and AFM [50-52]. We take into consideration at the same time 1) Aß peptides and fragments, including various mutant ones, 2) various metal ions, such as Cu, Zn, Fe, Hg, Al, Pb, Mn and 3) antioxidants like polyphenols, resveratrol or curcumin, and metal chelating agents such as clioquinol, 8-hydroxyquinoline derivatives, antiaggregants like NAP peptide to create *in vitro* models and investigate them under laboratory conditions. Their degradation conditions, using an adequate mathematical instrument and new analytical confirmatory methods will be studied. In vitro γ-secretase cleavage of the APP correlates to a subset of presenilin complexes and is inhibited by zinc [53,54]. Indeed, misfolded proteins could be as a therapeutic target in AD [55]. However, older people are more exposed to viral infections and die more easily from comorbidities [56]. A new perspective appeared on inflammation, related to the increase of cellular acidity and, respectively, to a stronger binding of metal ions to peptides [57]. In fact, recent data suggest that AD and Covid-19 may adopt a common solution. Besides, the investigation of Covid-19 may accelerate AD research. The irreversible AD is not fatal in the elderly if there are no comorbidities. Aggregation of the amyloid peptides (Aβ), a known contributor in AD pathogenesis is triggered by several metal ions through occupational exposure and disrupted metal ion homeostasis. Lifestyle, bad diet, comorbidities affect health and aggravate AD, so that the prevention and the study of the molecular mechanisms of AB aggregation are essential. The hypothesis we start from is: hypoxia-induced decrease of brain tissue pH leads to the activation of β- and γ-secretases, simultaneously with α-secretase inactivation, which results in production in excess of amyloid-β peptides from APP, together with stronger binding of metal ions and peptide aggregation.

Thioflavin T assay, light scattering (DLS), and AFM showed that micromolar concentrations of dopamine, L-dopa, norepinephrine, and epinephrine inhibit *in vitro* formation of Aβ fibrils [58]. It has become increasingly evident that the soluble form of the peptide is neurotoxic, not the amyloidogenic species. DLS and transmission electron microscopy (TEM) evidenced the interactions between metal ions and Aβ peptide, which is of greatest impact on modulating Aβ aggregation [59].

Scientific, technological, socio-economic aspects. In general, amyloid is an extracellular, proteinaceous deposit exhibiting β-sheet structure.[41-43] Thus, several degenerative conditions, including AD, light-chain amyloidosis and the spongiform encephalopathies, are associated with the deposition in tissue of such proteinaceous aggregates known as amyloid fibrils or plaques [60]. However, amyloid formation and deposition, oligomerization, and higher order aggregation, and the structure adopted by these assemblies, as well as their functional relationship with cell biology are still underscored.[42] Significant advances made so far in amyloid structure, dynamics and cell biology were directed to elucidate these issues and their relationship with senile dementia. Moreover, the intrinsic β-sheet structure and stability of an in vitro Aβ aggregate depends much on the aggregation conditions.[45] Amyloid plaques may include heavy metal or aluminum ions, sugars, or the 25,387 Da-serum amyloid P-component.[46] Although, there is ***no cure*** in AD or the other neurodegenerative pathologies, but some symptomatic treatments to improve the patients life quality, little is known about the biological and biochemical mechanisms of amyloid formation, protein fibrillation and neuron death.

(2) Difficulties in addressing the AD topic. Current literature on AD and other neurodegenerations contains many limitative research directions (drawbacks): (1) AD is thought as a age-related disease and not as a comorbidity; (2) While AD neurons are irreversible destroyed, scientists try to find simple relationships **(a)** between the neurodegeneration progress and the presence of individual promotors, such as heavy metals, Aβ(25-35) peptide, lack of sleep, some other diseases like diabetes melitus, obesity, Covid-19 or **(b)** between AD regression and the role of some antioxidants like resveratrol, curcumin, or chelators like clioquinol; (3) the instruments and methods are still not adequate to reveal the biological mechanism of neurodegeneration, since the investigations are limited to narrow range of pH, simple molecular systems formed, for example, by two compounds manifested under limited concentration and pH conditions, while the living cells provide very complex systems and conditions, whereas the external environment acts in real time, and very different from the lab conditions. The modern medicine is a emergency one, and not a preventive therapeutics; this results in less research on understanding time-dependent disease evolution and healing.

The project team's previous expertise in mass spectrometry [51,61-66], various spectroscopic techniques like NMR [67], CD [50,68], fluorescence [50], Fourier transform infrared [50,64,66], or atomic force microscopy (AFM) [52], applied to analogue synthetic peptides (SPPS) [61,66,69] and their complexes metal ions [50-52] makes this project feasible.

(3) Limitations of current approaches. Accumulation of amyloid results in neuron damaging by senile plaques, and finally to senility. Therefore, understanding the effect of antiaggregants, antioxidants, or chelatants is less usefull for healing, although it can bring important information on

how aggregation occurs, the composition of amyloid plaques, or the role of heavy metals and antioxidants. Findings like „copper ions can induce AD symptoms in only a week", „insomnia is related to AD", „amyloid plaques contain heavy metals", „low pH induce amyloid aggregation", „high pH induce α-helix formation" etc., are not taken together all the time to explain neurodegeneration. Besides, new techniques such as high pH mass spectrometry, the relation between non-histidine peptides and heavy metals and other new data are not fully considered so far.

(**4**) the elements of **originality and innovation** of the proposed objectives in the context of the current state of the art in the field and in relation to the previous projects developed by the project leader.

C2. Objectives, methodology and work plan

(1) the **concrete objectives** of the project;

The **specific project objective** is to study the time- and pH-dependent formation and complex interactions of amyloid peptides with both aggregating and anti-aggregating agents using our patented high pH mass spectrometric (MS) and other modern techniques (NMR, CD, FTIR, AFM, SEM, DLS etc), under various concentration & solvent conditions (*outcome*: a new patent, three highly ranked papers, invited talks at international meetings). Another project **objective** is to prove the effectiveness of mass spectrometric measurements and to confirm the formation or dissolution of amyloid complexes at various pH values by other instrumental techniques than MS, such as infrared, fluorescence or UV-vis spectroscopy, atomic force microscopy, scanning electron microscopy, dynamic light scaterring etc (Project *outcome*: international conferences, papers, improved MS techniques). Our research will increase understanding of the pathways involved in protein aggregation, and to highlight the possible molecular mechanisms of cellular toxicity (*outcome*: published papers in *Biomacromolecules*, *J. Neurochem.*, *Curr. Alzheimer Res.* or *Alzheimer Res. Ther*). These may lead to approaches toward rational therapeutics. Another **specific objective** is the transfer of knowledge generated in basic research in neurochemistry, biochemistry, and mass spectrometry on peptide synthesis and their adducts toward the lab-validated technology (Project *outcome*: a new patent). Besides, PhD students will be integrated in the project and trained with the instruments, reagent and protocols usage. They will write their theses, make oral and poster presentations, and will become co-authors of papers and patents (another project outcome).

The **elements** of **originality and innovation** consist of new approach in a larger range of pH of chemical and biological complexity of time-developing phenomena as well as a new mass spectrometric technique, a new mathematical model for MS spectra, confirmation of MS data by UV-vis, NMR, fluorescence and FTIR spectroscopy, and presentation of new biomedical ideas.

C3. Impact. The expected impact of the project will be discussed in a broader context of the scientific domain, by focusing on: (1) the potential to significantly advance the knowledge of the field, by introducing new concepts or approaches and by opening new areas or research directions (if applicable); (2) the potential impact of the project and/or of the applied research directions explored in the project (if applicable) on the scientific, social, economic or cultural environment.

The project has (1) **the potential** to significantly influence the scientific field through its new **concepts** (biological phenomena develop in a chemical environment and are influenced by external factors) or **approaches** (new high pH-MS measurement; role of GSH, NAP, and metals at variations of pH, time), and by **opening** of **research directions** (structural-phenomenologic outlook); (2) **the potential impact in the scientific environment** (more papers & oral presentations by our biochemistry group will result in many citations)**, social** (will press to change the point from healing to prevention)**, economic** (neurodegeneration means social costs; synthetic drugs; families forced to take care of old relatives) and **cultural medium** (pleading for healthy life style; people should read books instead stay in noise and stress). **The applicative directions to be explored within the project** are related to the possible patent, which will allow MS instruments to operate in a large range of pH and heavy metal concentrations**. Outcome**: The new experimental data will be published in high impact journals-at least 3 papers, patented or presented orally at international conferences. They will afford a better understanding of the complex process of neurodegeneration as well as the mechanism of amyloid occurence, metal ions binding, and amyloid plaque formation. Furthermore, the conditions in which amyloids, fibrils and aggregates can be degraded and removed will be studied. PhD students and master students will use experimental data under the experienced researchers control.

The **research methodology** will be described **in detail**, by mentioning **key milestones of the research**, when possible. The following aspects will be particularly underlined in this section: (1) the choice of the investigation methodologies and tools and their use to address the problem proposed in the context of the state-of-the art in the field; (2) the design of the work plan and the timelines proposed in relation to the project objectives; (3) the potential risks that may arise during the project implementation and the approaches proposed to mitigate them.

We obtained very original and novel results, that were already published and much cited, in showing by mass spectrometry and other techniques (see Bibliography) that amyloid peptides are formed, accumulated and complexed with heavy metals and other compounds which inhibit their proteolysis as

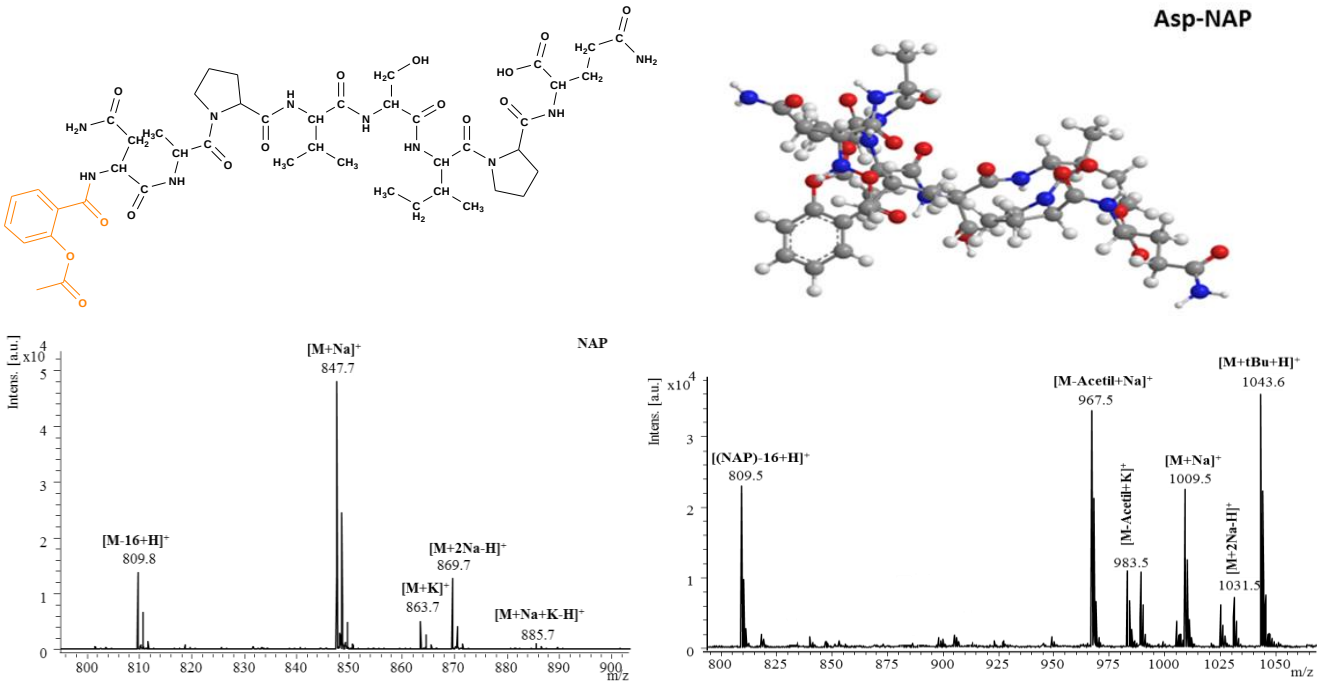

Fig. 2 Overall structure and the MALDI ToF MS spectrum of NAP peptide (left) and its conjugate with acetylsalicylic acid (right; Asp-NAPVSIPQ-COOH).

a function of environmental pH and other physiological conditions (inflammation, intoxication, stress). Thus, we increased much the pH range of measurements by replacing ammonia buffer by ethanolamine derivatives, and using the option "charge state ruler" of the Bruker soft to assign the peak complexity of each molecular ion. On increasing pH, we showed for the first time that more copper was extracted from insoluble phosphate to form up to 1: 6 molar peptide-copper adducts.[23] Furthermore, the peptides became oxidized with increasing copper concentrations or pH.[22] Similarly, the new mass spectrometric method, was able to evidence a pH-dependent competition between various metal ions when bound to amyloid-β peptides.[24] Fig. 1 shows that glutathione (GSH) which protects cells from oxidative stress, removes toxic compound and heavy metals, but is low concentrated in AD, can remove copper only on increasing pH values.

We show here the structure and MS spectra of NAP peptide and its conjugate with salicylic acid.

Here, we will carry out a lab research on the formation of peptide complexes with metal ions, other peptides such as NAP, polyalanine, and Aβ(25-35) peptide, antiaggregants like clioquinol, resveratrol, curcumin, or other organic compounds (salicylic acid, NOSH-aspirin, etc). The pH is going to increase slowly in batch system or in time to follow the binding process to peptides of the other components in solution. Furthermore, the complex formation in gas-phase, will be followed by UV-vis, electrophoresis, fluorimetry in solution, and by SEM, AFM, FTIR in solid. Confirmation is necessary because some authors still do not agree our results obtained only by high pH mass spectrometry,

considering that they are too complex to be well deciphred, others are afraid to use alkaline solution in measurement, and others cannot consider high pH measurements since they are far from the physiological range. However, our findings clearly showed that ethanolamine, diethanolamine or triethanol amine are very fluid, do not clog the electrospray syringe, whereas pH 8.5 and over is still physiologic. So far, we demonstrated that pH is even more important than amino acid sequence of a peptide for copper or other metal ions binding, contrary to the literature.

The relationship between environmental factors, such as the presence of heavy metals and aggregating and anti-aggregating agents, pH, and the formation of amyloids and their degradation will be followed and described in the view of a biological outlook. Results will be disseminated as high impact papers, oral presentation at international conferences.

Amyloid-β staining: first with Congo red and thioflavin S as suggested by Mold et al. [36] will be used. The chemical structures of the monomer and aggregates are characterized by ^{1}H & ^{13}C NMR spectra and by dynamic light scattering (DLS).

Table 1. The work schedule, describing the activities in relation with the proposed objectives.

Year - Objective	Associated Activities	Team member - activity	Results	Time schedule/ member
2022 State-of-art of pH-dependent heavy metals-induced aggregation associated with AD and the synthesis and characterization of β-amyloid peptides and aggregates	1.1.Updating information on MS, CD, NMR, FTIR, DLS, AFM, etc. and peptide aggregation-*permanent* 1.2. Working methodology and data processing methods 1.3. Chemical syntheses of specific Aβ fragments and purification by RP-HPLC 1.4. Characterization of new compounds/ complexes by MS, CD, FTIR, NMR, AFM, SEM etc. 1.5. Documentation stages & conferences[a)] 1.6. Purchase equipment (Fluorimeter; 2D electrophoresis device), materials, reagents, consumables, glassware	-Whole team– 1.1, 1.2, 1.3, 1.4, 1.8 -Drochioiu– 1.1-1.8 -Experienced researchers: 1.2-1.5 -PostDocs: 1.5, 1.6 -PhD students: 1.1, 1.3, 1.4, 1.5 -Students: 1.1, 1.3, 1.5 -Technicians: 1.6, 1.8	Report, Submitting 1st manuscript/review, International Conferences	12 months/ Drochioiu 10 months/ Experienced researchers 6 months/ PhD students and PostDocs

	necessary to conduct the proposed activities 1.7. Creation Web site of the project; 1.8. Reports			
2023 Study of multiple metal ion-amyloid-β peptide complexes at various pH and concentrations by MS, CD, NMR, FTIR, fluorescence spectroscopy and AFM & SEM microscopy	2.1. Purchase equipment (only what is necessary), materials, reagents, consumables, glassware necessary to conduct the proposed activities 2.2. Peptide synthesis including β-amyloid peptides and other compounds is needed 2.3. Study of chelating/ anti-aggregating agents 2.4. Characterization of β-amyloid peptides and their complexes- HPLC, MS, UV-Vis, FTIR, AFM, DLS 2.5. Study of interaction between β-amyloid peptide metal ion-chelating agents / anti-aggregants 2.6. Dissemination of results[b)] 2.7. Editing & submitting scientific manuscript 2.8. Update project web page & Report	- Drochioiu - 2.1 - 2.8 -Experienced researchers: 2.2-2.7 -PostDocs: 2.2-2.6 -PhD students: 2.2-2.6 -Students: 2.2, 2.3, 2.4, 2.6 -Technicians: 2.1, 2.4, 2.5	Report, Submitting 2nd manuscript, Participation to national and / or international events (PostDocs & PhD students: oral presentation at internat. conf.; Students: poster at national conf.)	12 months/ Drochioiu 10 months/ Experienced researchers 9 months/PhD students and PostDocs
2024 Disaggregation process of β-amyloid aggregates and fibrils; effect of amino acid residue, antiaggregating agents like clioquinol,	3.1. Study of complex systems involved in neuro-degeneration by the improved methods MS, FT-IR, NMR, AFM, DLS 3.2. Conferences in the country and abroad 3.3. Dissemination and patent application of the new method of MS	-Drochioiu - 3.1-3.5 -Experienced researchers: 3.1-3.3 -Gradinaru: 3.1, 3.3 -PostDocs: 3.1 -	- Submitting 3rd manuscript, -Patent application -Participation to national and / or international events -Final Report	6 months/ Drochioiu 1 months/ Experienced researchers 1 months/PhD

resveratrol, HCQ, CaT, NAP-like peptides etc.	investigation 3.4. Update project web page; 3.5. Final report of the project	3.3 -PhD students: 3.1-3.3		students and PostDocs

[a)] For comparative methods (nanoESI Q-TOF II instrument, Waters MS Technologies, Uni-Konstanz, EL Budapest etc). [b)] 4th International Conference on Neurology and Brain Disorders, Paris, France, April 19-21, 2021; Appl. Experim Neuropsychology Conf., Rome (Sep 16-17, 2021); FENS Regional Meeting, August 25-27, 2021, Krakow, Poland; FENS Forum, Paris, France, July 9-13, 2022; XX International Congress of Neuropathology, 21-24.09.2022, Berlin, Germany etc.

Description of the potential risks. No risks was envisaged with the implementation of this project activities, since our group has previously recognized results, a patent application, experienced senior researchers and trained PhD students. Besides, the infrastructure is suitable and the tasks correct assigned to each team and each team member.

The **Gantt Diagram** with planned research activities during the project life-span.

Stage/ Activity	**Year 1.** State-of-art of heavy metals-induced aggregation associated with the neurodegenerative diseases & the synthesis and characterization of mutant peptides and β-amyloid complexes											
Month	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
1.1.												
1.2.												
1.3.												
1.4.												
1.5.												
1.6												
1.7												
1.8												
Stage/ Activity	**Year 2.** Investigation of metal ion-amyloid-β peptide complexes under various pH and concentration conditions by mass spectrometry, circular dichroism, NMR, FTIR, fluorescence spectroscopy and AFM & SEM microscopy											
Month	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
2.1.												
2.2.												

2.3.												
2.4.												
2.5.												
2.6.												
2.7.												
2.8.												
Stage/ Activity	**Year 3.** Investigation of the degradation of β-amyloid aggregates and fibrils by using of antiaggregation agents such as clioquinol, resveratrol, NAP-like peptides etc.											
Month	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
3.1.												
3.2.												
3.3.												
3.4.												
3.5.												

Ethical aspects. We promote obtaining knowledge, truth, and avoidance of error in research. Activities are respect ethical norms. No animals or human being will be hurt, since the experiments are *in vitro*. All team members agree the prohibition against fabricating, falsifying, or misrepresenting research data. The trust, accountability, mutual respect, and fairness are essential values to our collaborative work. The guidelines for authorship, copyright and patenting policies, data sharing policies, and confidentiality rules in peer review will be strictly followed. The authors will retain copyright on all data obtained, according to their contribution in project and work. Any potential conflicts of interest will be mentioned and the source of funding will be acknowledged in any patent, manuscript, oral presentation or poster. People which will not significantly contributed to research but made solutions, measurements, calculations, suggestions and are not coauthors in a paper will be acknowledged. All experiments will be done according to the law, and public health and safety and no data will issue to danger people, animal or environment. The health and safety of staff and students during the experiments are envisaged according to lab rules. All data are of public, scientific and health interest.

C5. *Resources and budget*. (1) the time allocated to the project by each member, according to the work plan. (2) The **existing instruments** at Al. I. Cuza University of Iași, is suitable to fulfill all the project objectives and it can see at www.erris.gov.ro. We use mass spectrometers (MALDI ToF Axima Performance instrument; Shimadzu, Japan; ESI-MS measurements on Esquire 3000+ ion trap mass

spectrometer, Bruker Daltonics, Bremen, Germany; GC-MS Schimadzu QP2010 mass spectrometer;), automated peptide synthesizer (ResPep SL, Intavis, Germany); peptide purification with a BIO-RAD Model 2700Elite RP-HPLC system (Bio-Rad Laboratories GmbH, Germany) using Spherisorb (ODS) C18 and C8 semi-preparative columns; FTIR spectrometers (Shimadzu 8400S FTIR spectrophotometer, Shimadzu, Japan; JASCO 660+ FT-IR spectrophotometer); NMR spectrometer (Bruker Avance III, 500 MHz, Germany); AFM microscope (NT-MDT Solver Pro-M Russia); CD spectra-Jasco-715 spectropolarimeter (Labor and Datentechnik GmbH, Germany); absorption measurements (Biochrom Libra S35 PC UV–visible spectrophotometer, Cambridge, England); We possess an ***old fluorescence spectra - Kontron SFM-25 spectrofluorimeter***; pH-meter (pH 211 Microproc. Hanna Instr.); thermomixer (Compact Eppendorf AG 22331 Hamburg); Hettich Mikro 22R centrifuge (Tuttlingen,Germany); SDS–PAGE gel electrophoresis (Mini-PROTEAN Tetra Cell from Bio-Rad, Germany); gel capture and analysis (G: BOX F3 gel scanner, Syngene, Germany); deionized water (18.2 MΩ·cm) from a Milli-Q system (Millipore, Bedford, MA); etc (http://www.chem.uaic.ro/en/research/advanced_studies_chemistry.html).

Besides, this group includes Dr. Marcela Mihai, so that we have access to instruments of Petru Poni Institute of Iasi. The **research infrastructure** of Al. I. Cuza Univ. of Iasi: https://erris.gov.ro/main/index.php?&ddpN=1277756672&we=91f3e762f11c8934eaf7cc29a4587c15&wf=dGFCall&wtok=bcc5f58c9061a661128aecf2603a5d329b1a611a&wtkps=S7QysqoutjK2UirOTFGyBrKMrJRMExONjQ1Nk1JNgTDN1DzRLNko0TDV0tgsJcnSwCQVpA6orBzMMDS1UsrNTynNSdVLLCjQSywtyVCyrgUA&wchk=977c5d63e9550ada75664ec710d007c954ed7f7c, and that from the Petru Poni Institute of Macromolecular Chemistry of Iasi: (http://www.erris.gov.ro/main/index.php?&ddpN=1693097241&we=d3cdf3482aed0446e2532b946e1769a8&wf=dGFCall&wtok=5d618e6c93003e7198eaada224ff9abf0ae7cc11&wtkps=bY5BCsIwEEWvInOA0lYl+rMX3HgFCcmogWjTTKuIeHfHYneu5v/34TEOG7wES5DEQPZgBS3owaShMaBrF8bElcu5EnbFX6YB1E+3BmUeyrjI3S3+ljvZiFrzFpSiDMfszjwzMzMhG7BSslbXyOX536cOf9o5H9M+fLv+2TaG7PsD&wchk=b85b737d379e00518c2ab43576de5b35759a96f1). In addition, our group have access to Regional Institute of Oncology Iasi through Dr. Brînduşa Alina Petre and to that of the Agricultural Sciences and Veterinary Medicine University (https://erris.gov.ro/CCO-Iasi), due to our large collaboration through our former students. (3) Recently, we bought a HPLC instrument and expect a new spectrofluorimeter. **However**, at the moment we need to by a ***new and modern spectrophotometer***, a new lab **centrifuge** and a FTIR spectrometer for drop analysis of solutions of peptide and their complexes. (4) the budget breakdown per category of expenses/ year. Each member has precise tasks according to his/her skills. Prof. Dr. **Gabi Drochioiu**- team coordinator; mass

spectrometry; complex characterization, writing reports, patent and manuscripts; project management. Experienced researchers (ER): Dr. **Robert Gradinaru**, specialized by postdoc in Uni-Freiburg, Germany, PhD thesis in ***Biology/Microbiology*** at Prof. Dr. Biol. Sandro Ghisla, Uni-Konstanz, Germany – tasks in project: fluorescence & UV-vis spectroscopy, RP-HPLC, manuscript editing, PostDocs coordination. Assoc. prof. dr. Brinduşa Alina Petre, PhD thesis at Prof. Przybylski, Uni-Konstanz, Germany, with papers in the field of project, experienced scientist in peptide synthesis and characterization & project management, supervisor of peptide synthesis and PhD students; Vacant ER(2): specialist in mass spectrometry, ELISA, 2D electrophoresis, peptide synthesis, with experience in universities abroad; Vacant ER(3): scientist specialized in MS, & HPLC instruments; Postdoc Dr. **Catalina Ciobanu**, PhD in organic chemistry at Prof. Dr. Dan Scutaru, Gh. Asachi Technical University of Iasi, specialized postdoctoral in peptide synthesis and NMR spectroscopy, integration of PhD students. Postdoc Dr. **Laura Ion** –PhD thesis in Biochemistry (2015): Compounds with peptide bonds and their biomedical applications, Al. I. Cuza University of Iași. She published 12 research articles of which 3 as main author in prestigious journals (Ultrason. Sonochem., Electrophoresis, Adv. Exp. Med. Biol). Her profesional experience is reflected by 2 projects as a project manager (PN-III-P1-1.1-MC-2017-0822; PN-III-P1-1.1-MC-2017-0560) and 8 projects as a member of the research team (6 national projects and 2 international projects). She has many oral presentations at international meetings. Dr. Laura Ion's expertise covers various experimental techniques: solid phase peptide synthesis, protein gel electrophoresis, HPLC, MALDI-ToF MS, FT-IR etc. PhD students (1) & (2): to prove significant knowledge in peptide synthesis and characterization. PhD student **Mocanu Cosmin Stefan** will be involved also in molecular modeling of peptides and complexes. PhD student **Stefania-Claudia Jitaru**: peptide synthesis and other syntheses, mass spectrometry, FTIR and NMR, as well as AFM studies.

Budget Breakdown (lei)/year

Category of expenses	**2022 (lei)**	**2023 (lei)**	**2024 (lei)**	**Total (lei)**
Personnel	**201600,00**	**201600,00**	**100800,00**	**504000,00**
Logistics	**260000,00**	**100000,00**	**5000,00**	**365000,00**
Travel	**34000,00**	**50000,00**	**20000,00**	**104000,00**
Overhead	**44340,00**	**52740,00**	**18840,00**	**115950,00**
Total	**539940,00**	**404340,00**	**144670,00**	**1088950,00**

Budget Breakdown (EUR)/entire project

Category of expenses	Total budget 2021 - 2023 (EUR)
Personnel	**105000,00**
Logistics	**76041,67**
Travel	**21666,67**
Overhead	**24156,25**
Total	**226864,58**

Salaries are comensurate with time alocated by each researcher to the project (Table x), his/her expertize (from experienced researcher to student or technician), and qualification/skill (doctor degree or not), according to Romanian Law 583/2015. Project member agreed this calculation; project leader will be 100% involved, PhD students will defend their theses during the project life-span, experienced researchers have more other activities, students have vacations etc. **Logistics**: we need no more instruments to fulfill the tasks, but consumables and new spectrophotomer, centrifuge and a FTIR instrument. **Travel**: international conferences and short documentation stages (especially for PhD students).

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