KLVFF peptide functionalization of graphene oxide as suitable Aβ biomarker detection platform

Rita Turnaturi[a], Giuseppina Sabatino[a], Giuseppe Di Natale[a], Giuseppe Nocito[b], Angela Scala[c], Viviana Scuderi[d], Simona Filice[d], Antonino Mazzaglia[b], Silvia Scalese[d], Giuseppe Pappalardo[a].

[a]CNR-IC Catania, Italy [b]CNR-ISMN URT Messina, Italy
[c]Dip ChiBioFarAm, Università di Messina, Messina, Italy [d]CNR-IMM Catania, Italy

Background
Accurate diagnosis of the preclinical stages of Alzheimer's disease (AD), by means of biomarker early detection, is considered crucial for therapeutic advancements.
Current AD diagnosis relies on the combinatorial use of biomarkers detection, derived from cerebrospinal fluid (CSF), with advanced molecular imaging and cognitive assessment tools [1].
However, the invasiveness and the difficult access to routine diagnosis, due to high costs of these tools, represent some challenges that need to be overcome.
Low-cost and non-invasive alternatives, such as plasma, saliva, or urine biomarkers, could be a valid substitute in AD screening and diagnosis.

Aim
Nanomaterial-based biosensors development for recognition of AD biomarkers, such as amyloid beta Aβ(1–40) and the more toxic form Aβ (1–42), could pave the way for early diagnosis of AD [2].

KLVFF

In this scenario, KLVFF (H₂N-Lys-Leu-Val-Phe-Phe-COOH, Aβ (16–20)), a peptide fragment deputed to interact with the homologous region of full-length Aβ(1–40) or Aβ (1–42), was selected as molecular recognition element of the selected Aβ biomarkers [3].

Graphene oxide (GO) was chosen as nanomaterials-based sensing layer

PLATFORM FOR ELECTRICAL BIO-SENSORS

GO surface decorated with amphiphilic cyclodextrin SC16OH nanovesicles entangling KLVFF conjugates

MW-SPPS and characterization of Ada-PEG₄-KLVFF (Ada-Pep) and Fc-PEG₄-KLVFF (Fc-Pep)

Nanohybrid preparation

The amphiphilic cyclodextrin/graphene oxide/KLVFF nanohybrids (Ada-Pep and the Fc-Pep) were prepared following the thin film hydration method [4] and characterized from Scanning Electron Microscopy and Dynamic Light Scattering analyses

SC16OH/GO/Ada-Pep
SC16OH/GO/Fc-Pep

Synthesized peptide conjugates were characterized by HPLC, mass and ¹H-NMR spectroscopy.

SC16OH/GO/Ada-PEG₄-KLVFF and SC16OH/GO/Fc-PEG₄-KLVFF analisys

Ada-Pep nanohybrid did not show electrical conductivity

Mean hydrodynamic diameter (D_H) from DLS analysis

SEM imaging

Cyclic voltammetry (CV) from SPGE (10 mV/s)

CV from modified SPGE (10 mV/s)

Mean hydrodynamic diameter (D_H) from DLS analysis

The Fc-Pep inclusion onto SC16OH was confirmed from CV.

The SC16OH/GO/Fc-Pep modified screen printed gold electrode showed 30 % current increase.

GO surface covalently functionalized with NH₂-PEG₄-KLVFF

MW-SPPS of NH₂-PEG₄-KLVFF

NH₂-PEG₄-KLVFF

GO functionalization with NH₂-PEG₄-KLVFF by means EDC/NHS protocol

Peptide NH₂-PEG₄-KLVFF was covalently conjugated with GO by EDC/NHS coupling protocol.

SEM imaging

In the GO ATR-FTIR spectrum (black) are clearly distinguishable the bands at 1) 1710 cm⁻¹ (C=O vibration of the –COOH), 2) 1626 cm⁻¹ (aromatic C=C stretching). 3) 1069 cm⁻¹ (epoxy vibrations). The spectrum of peptide conjugate NH₂-PEG₄-KLVFF (red) shows a strong Amide I band (at 1632.23 cm⁻¹) and the Amide II band (at 1537.47 cm⁻¹). PEG characteristic bands found in the spectra NH₂-PEG₄-KLVFF are that due to CH₂ twisting and CH₂ wagging (1276.07 and 1399.49 cm⁻¹, respectively) and C-C and C-O stretching at 835.68 cm⁻¹.

In the ATR-FTIR spectrum of GO-PEG₄-KLVFF (blue) the band at 1710 cm⁻¹ disappears whereas it tuns appreciable the band at 1648-1560 cm⁻¹ (amide group).

ATR-FTIR

XPS analysis

Bond type	GO		NH₂-PEG₄-KLVFF		GO-PEG₄-KLVFF	
	BE (eV)	%	BE (eV)	%	BE (eV)	%
−NC(=O)	400,380	*	399,359	8,5	400,390	43,1
−C−NR₂	398,298	*	397,984	91,5	398,491	56,9

N1s XPS spectra of NH₂-PEG₄-KLVFF reports two main peaks related to -N-(C=O)- and -C-NR₂. The former contribute is at higher binding energy with respect to the latter.
When the peptide is grafted to GO layers, the -N-(C=O)- contribution increases because of the formation of amide bonding between carboxyl groups of GO and amine groups of NH₂-PEG₄-KLVFF.

Conclusions
- Ada-Pep nanohybrid did not show electrical conductivity, probably for an extensive shielding operated by cyclodextrins.
- The inclusion of Fc-Pep onto SC16OH amphiphilic cyclodextrin was confirmed from cyclic voltammetry and the modified screen printed gold electrode showed 30 % current increase.
- β-amyloid detection with SC16OH/GO/Fc-Pep modified SPGE is in validation.
- Preliminary results with Fc-Pep (control without cyclodextrin) indicated the electrochemical sensing of β-amyloid.
- Preliminary investigations show the nanoconjugate GO-(PEG)₄-KLVFF potential as a multifunctional platform for biomedical applications as biosensing layers for Alzheimer's biomarker detection.

References
[1] Zhou, Y., Liu, L., Hao, Y., & Xu, M. Detection of Aβ Monomers and Oligomers: Early Diagnosis of Alzheimer's Disease. *Chemistry, an Asian journal* **2016**, *11*, 805–817.
[2] Chen, G. F., Xu, T. H., Yan, Y., Zhou, Y. R., Jiang, Y., Melcher, K., & Xu, H. E. Amyloid beta: structure, biology and structure-based therapeutic development. *Acta pharmacologica Sinica* **2017**, *38*, 1205–1235.
[3] Tjernberg, L. O., Näslund, J., Lindqvist, F., Johansson, J., Karlström, A. R., Thyberg, J., Terenius, L., & Nordstedt, C. Arrest of beta-amyloid fibril formation by a pentapeptide ligand. *The Journal of biological chemistry* **1996**, *271*, 8545–8548.
[4] Mazzaglia, A., Di Natale, G., Tosto. R., Scala, A., Sortino, G., Piperno, A., Casaletto, M. P., Riminucci, A., Giuffrida, M.L., Mineo, P.G., Villari, V., Micali, N., Pappalardo, G. KLVFF oligopeptide-decorated amphiphilic cyclodextrin nanomagnets for selective amyloid beta recognition and fishing *Journal of Colloid and Interface Science* **2022**, *613*, 814–826.