Self-assembling hexapeptide-polymer conjugates to be used as drug carriers

Federica Novelli, Serena De Santis, Cesare Giordano, Valerio Viali, Mattia Titubante, Giancarlo Masci, Anita Scipioni

Department of Chemistry, Sapienza University of Rome, P.le A. Moro, 00185-Rome, Italy

email: *anita.scipioni@uniroma1.it*

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Well-designed hybrid functional biomaterials based on peptide-polymer conjugates are able to self-assemble into nanostructures useful for drug delivery since they allow a careful tuning of their self-assembling properties at nanometer scale. [1]. Among the strategies developed for peptide engineering, those characterized by peptides with regularly alternating enantiomeric sequences are particularly attractive, since they are able to self-assemble in stacks directed and stabilized by hydrogen bonds [2]. When the peptide and polymer are suitably chosen in order to achieve the proper balance of hydrophobic/hydrophilic regions, these conjugates are able to self-assemble in water in stable core-shell morphology nanoparticles with greater resistance to phagocytosis and, consequently, with enhanced circulation half-life.
Herein, the self-assembling properties of the conjugates Cbz-(L-Ala-D-Val)$_3$-NH-(CH$_2$-CH$_2$-O)$_{45}$-CH$_3$ (Pep$_6$-PEG, Cbz = carbobenzyloxy) and CH$_3$-(O-CH$_2$-CH$_2$)$_{45}$-C$_2$H$_2$N$_3$-(D-Leu-L-Trp)$_3$-NH$_2$ (PEG-Pep$_6$Trp) are investigated. The amphiphilic conjugates are characterized by the same length of the peptide moiety and PEG molecular weight but different amino acid sequence. Pep$_6$-PEG was obtained by end-linking the linear hexapeptides Cbz-(L-Ala-D-Val)$_3$-OH, obtained by solid phase peptide synthesis, to an amine-end functionalized poly (ethylene glycol) chain whilst PEG-Pep$_6$Trp was prepared through a highly efficient solid-phase synthesis by Cu(I) catalyzed azide/alkyne Huisgen 1,3-dipolar cycloaddition (CuAAC "Click" conjugation). The self-assembling behaviour of both conjugates was assessed by NMR, CD and fluorescence spectroscopies, DLS, and SEM microscopy. According to previous results, spectroscopic evidence suggests that the self-assembly process is ruled by the hydrophobic interactions and hydrogen bond network of peptide moiety; in the case of PEG-Pep$_6$Trp self-assembly, NPs are further stabilized by stacking interactions among indole groups. SEM micrographs (Figures 1A and B), where peculiar donought morphologies are apparent, and DLS data indicate that the conjugates self-assemble in NPs with a vesicular structure. Based on such findings, a structural model (Figure 1C) of NPs is proposed where two hybrid molecules self-assemble in head-to head (Pep$_6$-PEG) or tail-to-tail (PEG-Pep$_6$Trp) association with the peptide moiety forming the hydrophobic core of the aggregates whilst PEG in the outer shell confers stability to the system. The ability of the NPs to act as efficient drug delivery systems was investigated using curcumin as a model of hydrophobic drugs to evaluate the drug loading (DL) and pharmacokinetics. In spite of the similar structure of the aggregates, a good DL of 9 wt% and a sustained drug release over 72 hours was shown only for PEG-Pep$_6$Trp NPs, proving the importance of the hydrophobic peptide sequence to tune the DL and releasing ability of such kind of drug carriers.

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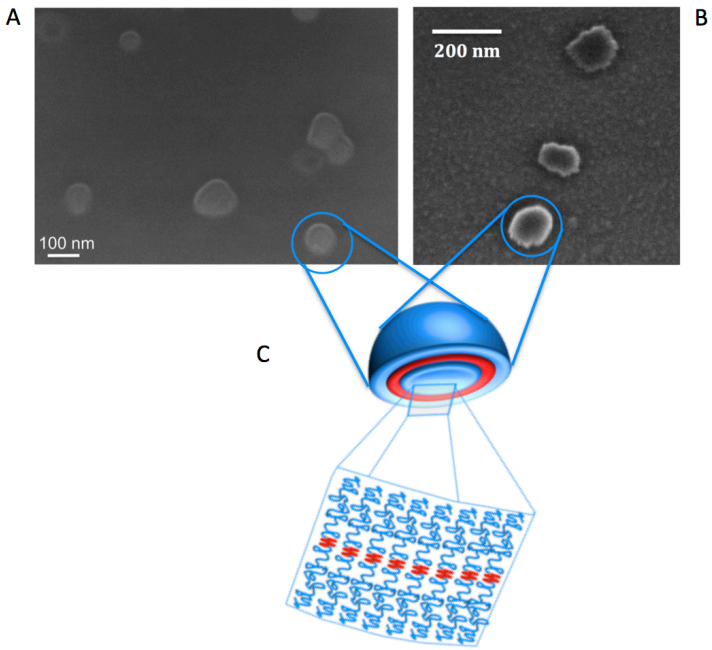

Figure 1 SEM micrographs of the hybrid conjugates Pep$_6$-PEG (A) and PEG-Pep$_6$Trp (B) with the peculiar donought morphologies; (C) Model of the vesicular structure of the aggregates obtained for self-assembly of Pep$_6$-PEG and PEG-Pep$_6$Trp in water.