Electronic Supplementary Material (ESI) for Molecular BioSystems

Supplementary Material

Zhou et al, Molecular Biosystems.

Index

Supplementary Methods

Peptide characterization by LC-MS

PepE was characterized by LC-MS using an ion trap LCQ Deca XP mass spectrometer (ThermoFisher) equipped with an Opton ESI source operated at 5 kV and 300 °C, coupled to the LC part composed of a Surveyor complete HPLC system equipped with a Biobasic 50x1 mm ID C18 column (ThermoFisher). Spectra were acquired in the positive modality between m/z 200 and 2000 for both peptides. 20 ng for both peptides were analyzed at a flow rate of 0.25 mL/min and applying a gradient between 10% and 70% solvent B (CH_3CN, 0.05% TFA) over solvent A (H_2O, 0.08% TFA) in 10 minutes. The peptide was also analyzed by HPLC using a 50x2 ID monolithic column (Phenomenex) operated at 0.6 mL/min, monitoring the eluate by UV at 214 nm and applying a gradient from 10% to 70% in 15 minutes.

CD analysis

CD analysis was performed using a JASCO J-710 spectropolarimeter, equipped with a Peltier system for changing the temperature in a controlled way and quartz cuvettes 110-QS with 1.0 mm path length. Experiments were performed in 10 mM phosphate, pH = 8.0 at a concentration of 0.20 mg/mL. Spectra were collected within the wavelength range 250-190 nm at a scan rate of 20 nm/min, with a data pitch of 0.2 nm, a bandwidth of 1 nm and a response of 4 sec.

Supplementary Results

Peptide characterization by LC-MS

The TIC chromatogram of the peptide PepE is reported in the Supplementary Figure S1A (Upper Panel) and shows that the compound is essentially >99%. Also the molecular weight was consistent with that expected (Exper.: 1994.2 amu/ $Expected_{Average}$ 1994.2 amu, lower panel). Similar results were obtained with the scrambled control peptide (Figure S1B), showing the TIC chromatogram (upper panel) and the ESI mass spectrum (lower panel). The molecular weight was consistent with that expected (Exper.: 1993.7 amu/$Expected_{Average}$ 1994.2 amu, lower panel). HPLC analyses of the same products (chromatogram traces at 214 nm inserted within upper panels) showed they were > 95% pure.

CD analysis

A CD analysis was performed on both PepE and the scrambled peptide to monitor the occurrence of racemization during peptide chain assembly and/or cleavage from the resin. As shown in the supplementary Figure S2, both molecules show strong absorbance bands in the negative region of the spectrum, as expected for all-L polypeptides (about 205 nm for PepE and about 210 nm for the scrambled peptide). The less intense bands exhibited by peptides at about 230 nm suggest that both adopt slightly bent conformations. Though the occurrence of single residue racemization reactions cannot be excluded by these analyses, it is reasonable to assume that compounds were optically highly homogeneous.

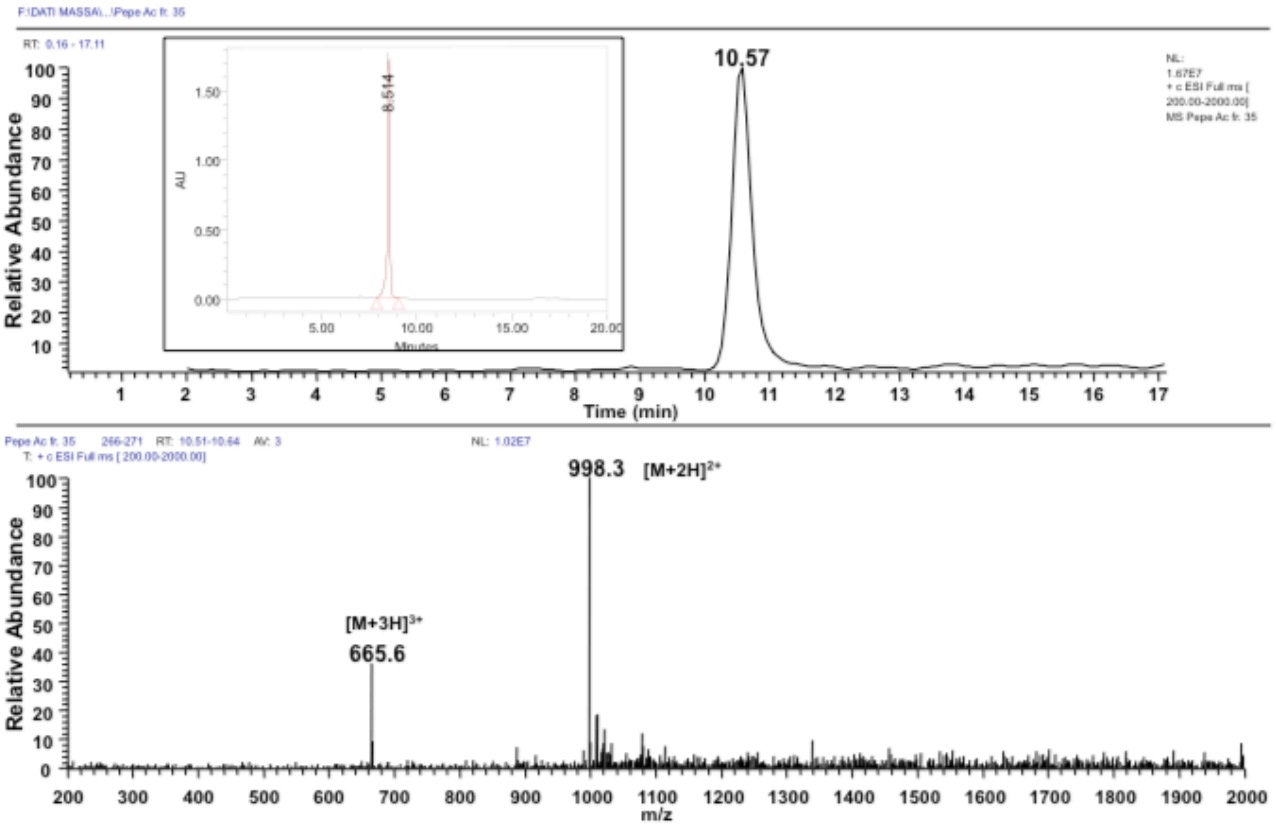

Figure S1A

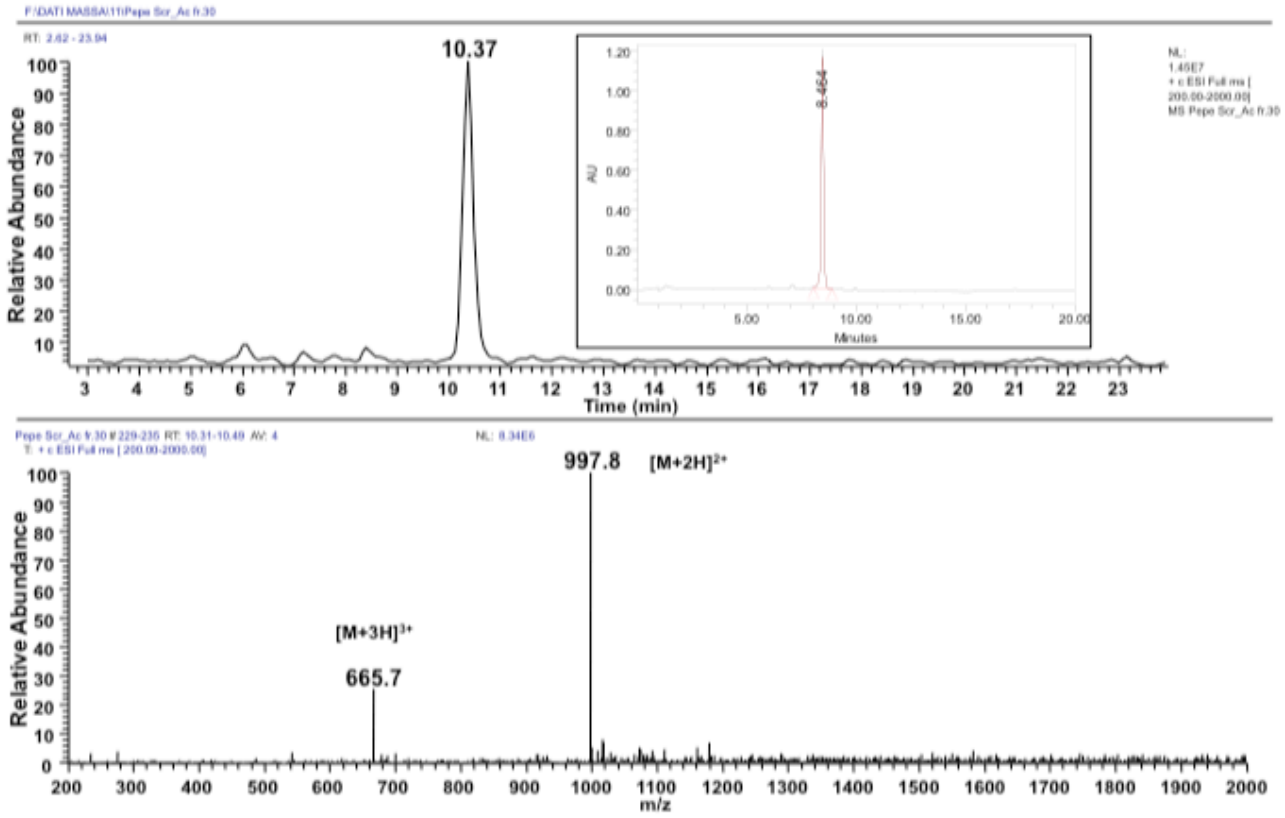

Figure S1B

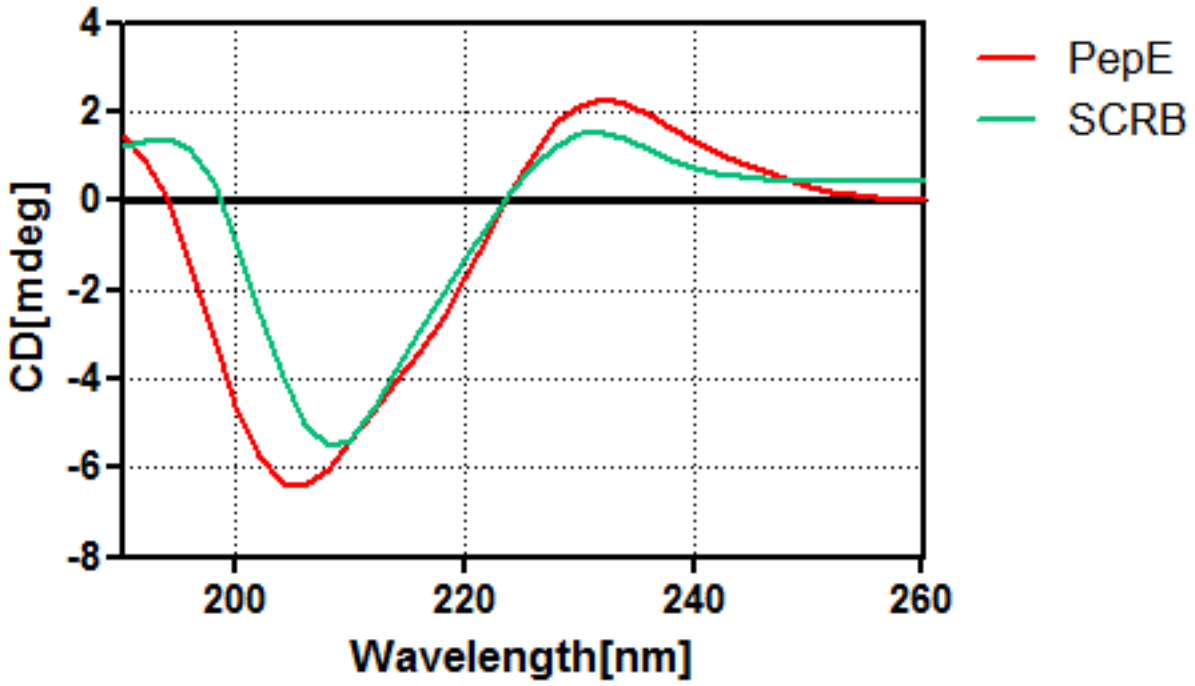

Figure S2