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Chemistry & Drug Discovery

Supporting Information

Co-administration of Antimicrobial Peptides Enhances Toll-like Receptor 4 Antagonist Activity of a Synthetic Glycolipid

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Cell assays

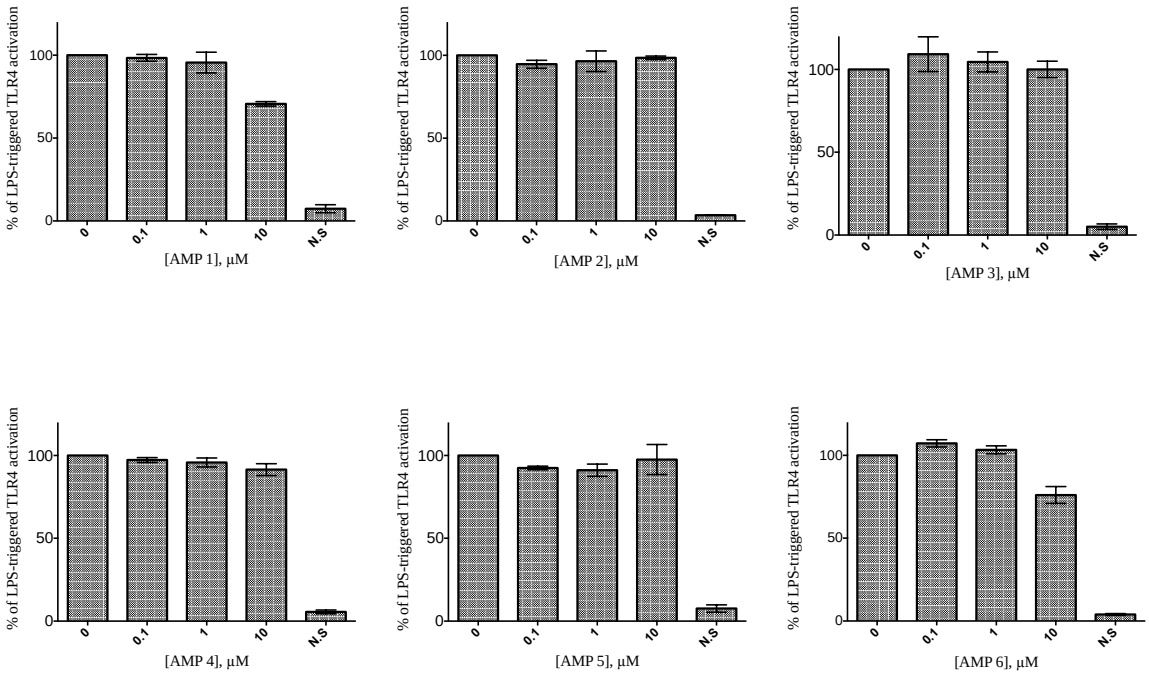

Figure S1. Effect of AMPs 1-6 administration on LPS-stimulated TLR4 signal in HEK-Blue hTLR4 cells. HEK-Blue hTLR4 cells were pre-treated with the indicated concentrations of AMPs 1-6 and stimulated with LPS (100 ng/mL) after 30 minutes. Data were normalized to stimulation with LPS alone. Data represent the mean of percentage ± SEM of at least 3 independent experiments.

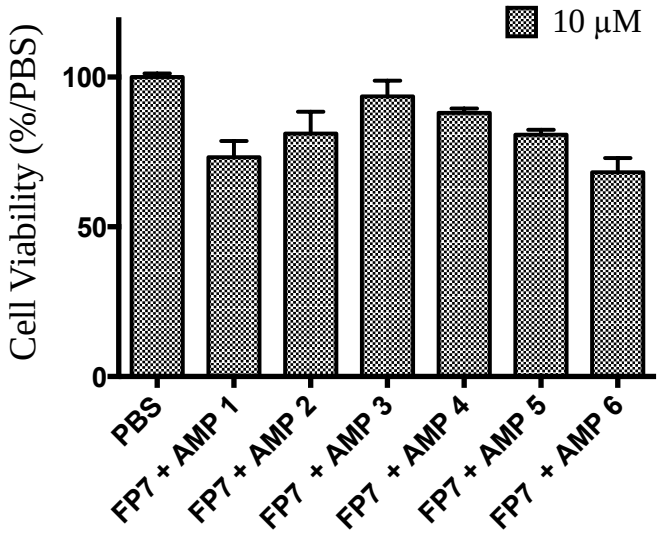

Figure S2. MTT assay of FP7/AMPs co-administrations in HEK-Blue hTLR4 cells. Cells were treated with the six co-administrations used in the other assays; the bars represent the cell viability estimated by using 10 µM of compounds, equivalent to the maximum concentration used previously. Data are normalized with PBS and represent the mean of percentage ± SEM of at least 3 independent experiments.

NMR and TEM experiments

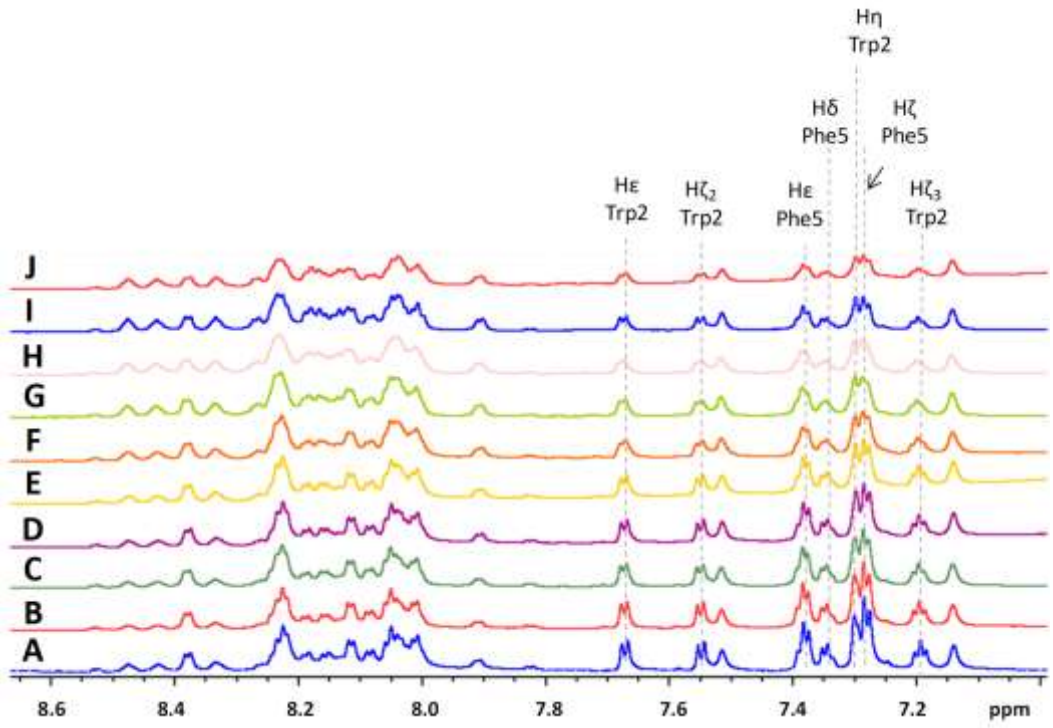

Figure S3. [1]H-NMR of AMP 1 with upon addition of FP. **A:** AMP 1 (300 μM) alone; **B:** AMP 1 (300 μM) with FP7 (10 μM); **C:** AMP 1 (300 μM) with FP7 (20 μM); **D:** AMP 1 (300 μM) with FP7 (30 μM); **E:** AMP 1 (300 μM) with FP7 (40 μM); **F:** AMP 1 (300 μM) with FP7 (60 μM); **G:** AMP 1 (300 μM) with FP7 (80 μM); **H:** AMP 1 (300 μM) with FP7 (140 μM); **I:** AMP 1 (300 μM) with FP7 (200 μM); **J:** AMP 1 (300 μM) with FP7 (300 μM); The samples have 10 % DMSO in PBS 100 mM pH=5.5.

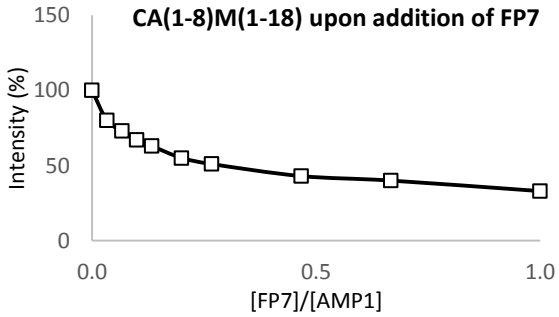

Figure S4. Intensity (%) of the **Hε- Trp2** of AMP 1 as a function of the [FP7]/[AMP 1] molar ratio.

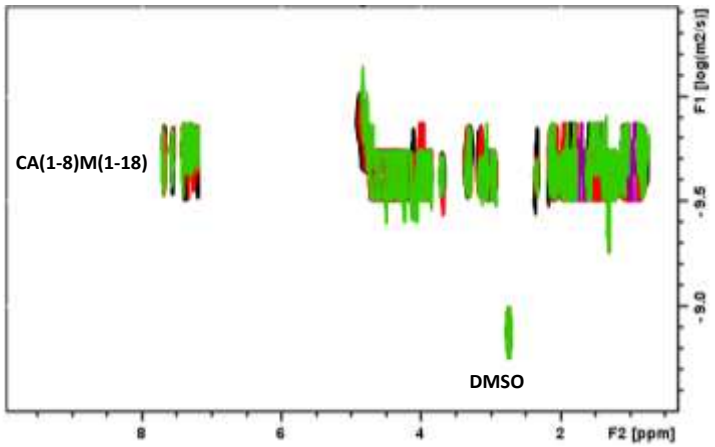

Figure S5. DOSY spectrum. **Black:** DOSY spectrum of AMP 1 (300 µM) **Red:** DOSY spectrum AMP 1 (300 µM) with FP7 (80 µM). **Green:** DOSY spectrum AMP 1 (300 µM) with FP7 (200 µM).

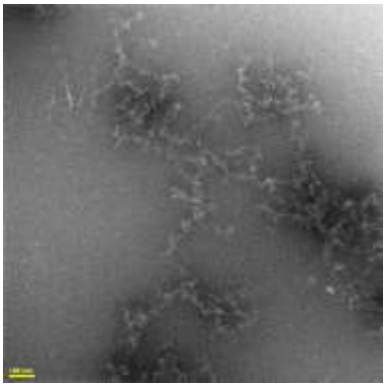

Figure S6. Transmission Electron Microscopy. Negative Staining Analysis of AMP 1 peptide at 2.5 mg/ml. nominal magnification of 30,000 X (0.36nm/pixel).

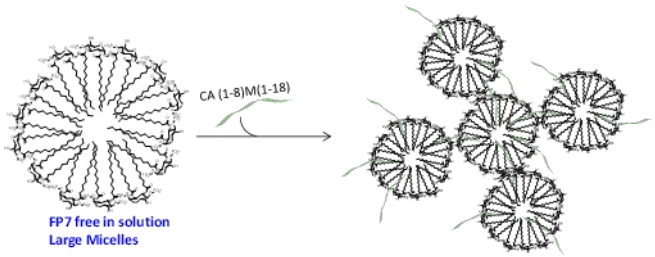

Figure S7. The peptide acts as linker between different FP7 aggregates

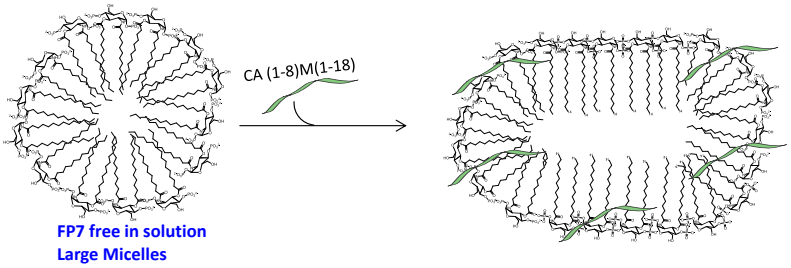

Figure S8. The peptide acts as linker between different FP7 aggregates

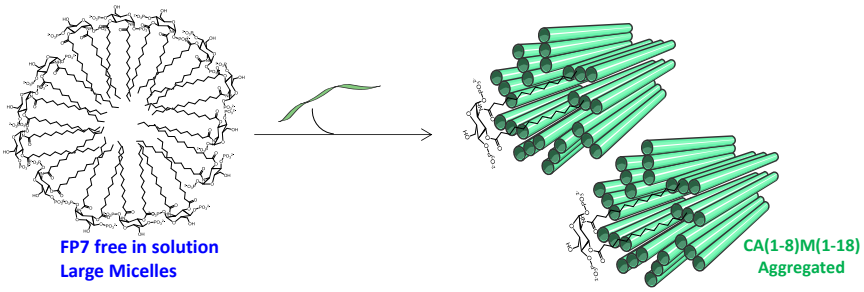

Figure S9. The aggregated peptide and the formed supramolecular aggregate displays the behaviour of a large molecule.

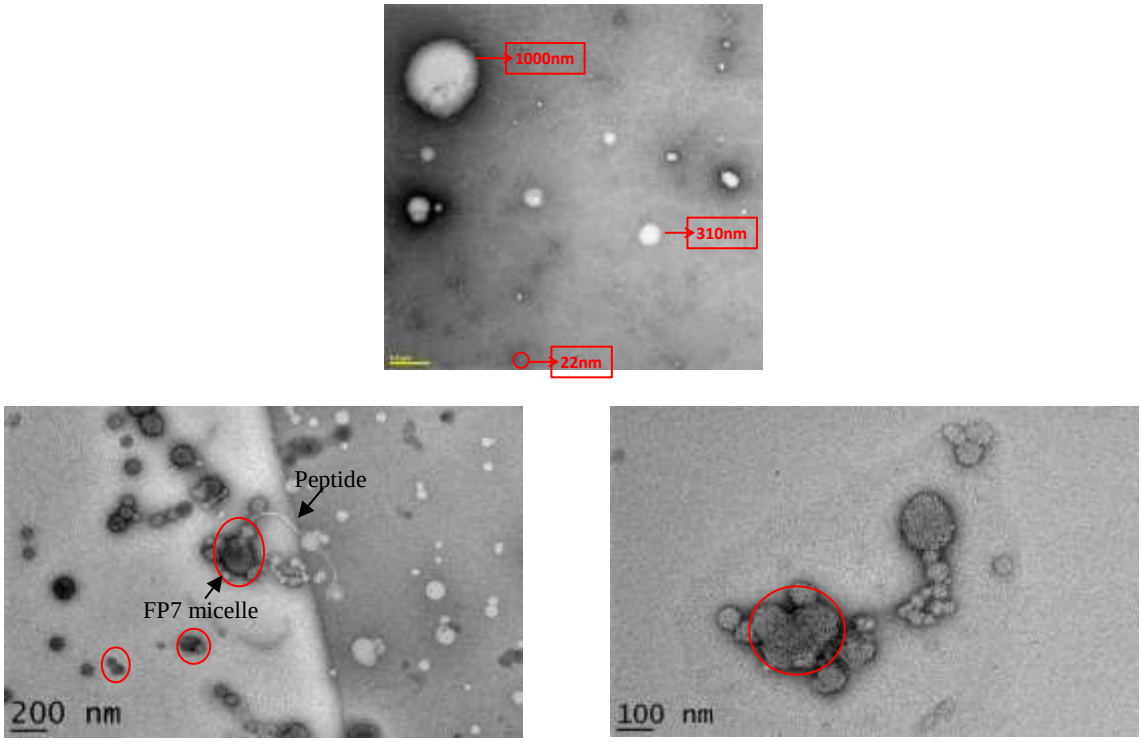

Figure S10. Transmission Electron Microscopy - Negative Staining Analysis. Top: FP7 Lipid at 2.5 mg/ml. Nominal magnification of 10,000 X (1.1nm/pixel). **Bottom: FP7** Lipid (320 μM) with **AMP 1** (80 μM) in H_2O and DMSO 10 %, nominal magnification of 20000 X (0.36 nm/pixel)

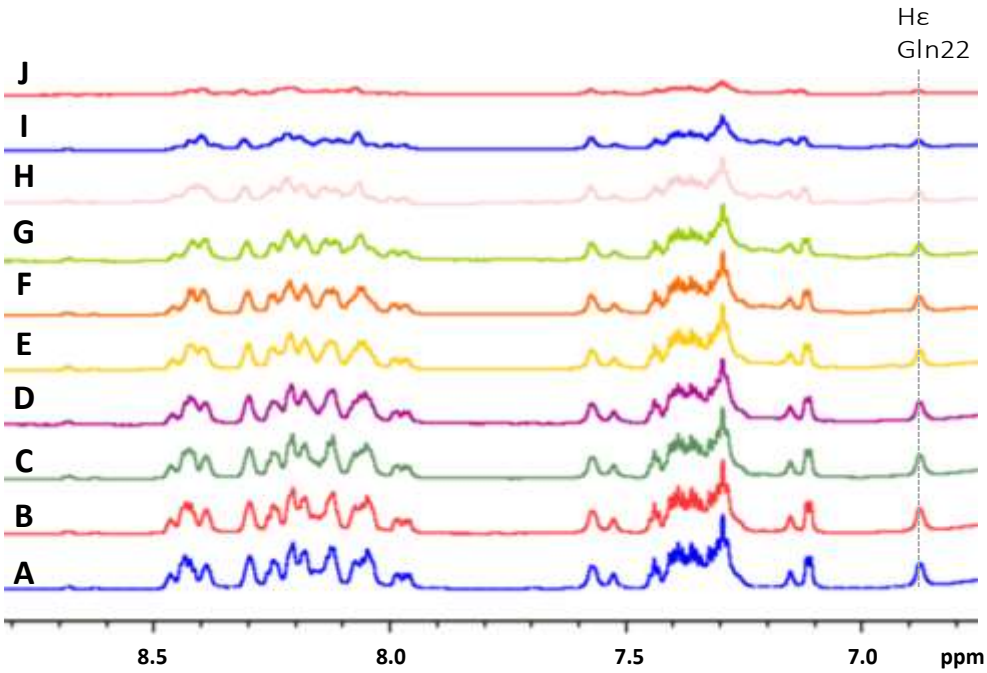

Figure S11. [1]H-NMR of AMP 6 with upon addition of FP. **A:** AMP 6 (300 μM) alone; **B:** AMP 6 (300 μM) with FP7 (10 μM); **C:** AMP 6 (300 μM) with FP7 (30 μM); **D:** AMP 6 (300 μM) with FP7 (80 μM); **E:** AMP 6 (300 μM) with FP7 (140 μM); **F:** AMP 6 (300 μM) with FP7 (200 μM); **G:** AMP 6 (300 μM) with FP7 (300 μM); **H:** AMP 6 (300 μM) with FP7 (400 μM); **I:** AMP 6 (300 μM) with FP7 (600 μM); **J:** AMP 6 (300 μM) with FP7 (900 μM); The samples have 10 % DMSO in PBS 100 mM pH=5.5.

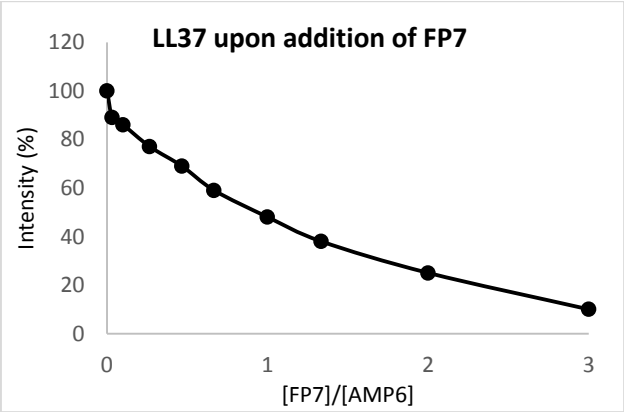

Figure S12. Intensity (%) of the **Hε-Gln22** of AMP 6 as a function of [FP7]/[AMP 6].

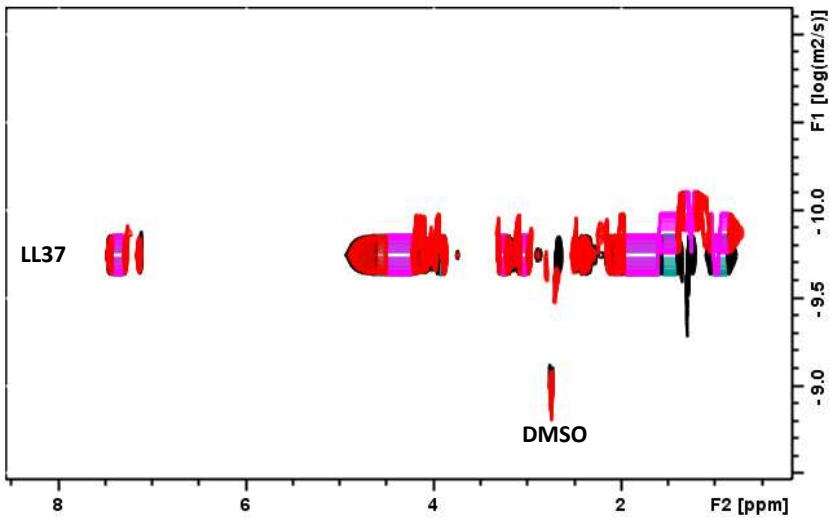

Figure S13. DOSY spectra for AMP6. **Black:** DOSY spectrum of AMP 6 (300 μM) **Red:** DOSY spectrum AMP 6 (300 μM) with FP7 (200 μM).

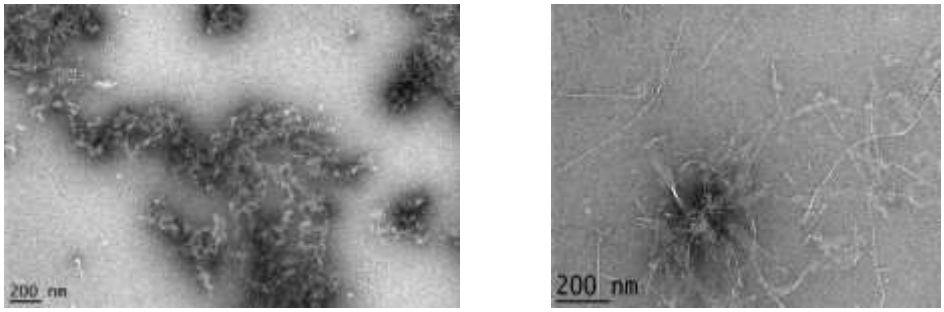

Figure S14. Transmission Electron Microscopy. Negative Staining Analysis. Left: AMP 6 peptide at 45mg/ml. nominal magnification of 30,000 X (0.36nm/pixel). **Right: FP7** (588 μM) with **AMP 6** (400 μM) in H_2O and DMSO 10 %, nominal magnification of 20000 X (0.36 nm /pixel).