Comprehensive characterization of vesicle formation with a self-assembling amphiphilic peptide
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ABSTRACT SUMMARY
Previously we reported on the capacity of an amphiphilic peptide SA2 (Ac-AAVVLLLWEE) to self-assemble into spherical nanostructures under physiological conditions. Here, we characterize the self-assembly of SA2 comprehensively using various analytical techniques, demonstrating that peptide self-assembly is driven by beta-sheet formation and colloidal stability is maintained by the negative charges present at the C-terminal end. Such peptide vesicles may have applications as nanocarrier systems for drug or vaccine delivery.

INTRODUCTION
Amphiphilic peptides (Aps) have gained increasing attention as versatile molecules to generate diverse supramolecular structures, such as hydrogels, nanofibres, micelles and vesicles. SA2 (Ac-AAVVLLLWEE) is such an amphiphilic peptide developed in our group that assembles into vesicles of approx. 70 nm in size with a net negative surface charge. However, the mechanisms by which these amphiphilic peptides formed vesicles remained unclear. The aim of this study was to gain more information about the morphology, stability and behavior of these peptide nanoparticles with respect to parameters such as pH, temperature, and ionic strength. To characterize these peptide nanoparticles, several complementary methods were applied, including AFM, DLS, CD, fluorescence spectrometry and atomistic and coarse grain computer simulations.

RESULTS AND DISCUSSION
Dynamic light scattering revealed that peptide self-assembly gives spherical nanostructures of approximately 70 nm in size (Rg=35 nm). By determining the radius of gyration Rg, using static light scattering the shape factor (ρ) which equals Rg/Rh was determined to be close to 1, suggesting a vesicular nature of the peptide nanostructures. AFM images at different peptide concentrations were in agreement with these findings (Figure1). An increase in the fluorescence properties of nile red and pyrene at the CAC point (10µM) demonstrated that hydrophobic forces were involved in self-assembly. On the other hand, circular dichroism and solid state NMR results revealed that β-sheets were the predominant secondary structure in the peptide.

EXPERIMENTAL METHODS
Self-assembled peptide particles were prepared by dispersion of peptide powder in phosphate buffer saline using bath sonication. The final dispersion was incubated for overnight at 25°C for the peptide particles to reach equilibrium. The accurate concentration of peptide in the solution was measured by UV-spectrophotometry using a tryptophan extinction coefficient of 5500M⁻¹cm⁻¹. The critical aggregation concentration was determined by 4 methods: Light scattering, Nile red assay, Pyrene assay and Tryptophan anisotropy. We monitored separately the fluorescence of 2.5 µM nile red (Ex 550 nm /Em635 nm) and 0.4 µM pyrene (Ex 300-360nm /Em390 nm) in a series of peptide concentrations (0.1-500 µg/ml) in PBS (pH=7.4). Furthermore, Rayleigh scattering was determined at 650 nm. In addition, anisotropy of tryptophan residue in the peptide was measured. Concentration dependency and stability as a function of time, temperature and pH were analyzed by dynamic light scattering.

Static light scattering and AFM imaging were performed to get more information on the morphology of formed nanoparticles.

Circular dichroism, Solid state NMR and computer simulation (atomistic MD) were conducted to determine the secondary structure of peptides in aggregate and monomeric state.
vesicles. Thus hydrophobic forces and intermolecular hydrogen bonding simultaneously strengthen the vesicle structure. Furthermore, peptide vesicles were stable in time (more than 9 days) and at elevated temperatures (up to 70 °C) (Figure2).

Figure1. Atomic force microscopy of SA2 particles 2mg/ml immobilized on Poly-L-lysine coated mica.

Figure2. Influence of temperature on Z-average of SA2 particles.

Atomistic simulation was initiated with several conformation (extended, α-helix and PPII) which interestingly Extended and PPII conformation ended up to β-sheet conformation and most α-helixes after 200 ns were unfolded (Figure3). Stability of these nanoparticles were also analyzed at different pH values. DLS results showed that acidic pH (less than 6) induced particles aggregation through neutralizing the negative surface charges.

AFM image of this precipitate showed a cluster of particles which preserved their spherical shape (results not shown).

Figure 3. Snapshot Before(A) and after 200ns(B) simulation of 60 peptides, starting from a fully extended peptide conformation.

Furthermore by increasing the pH above 6, again particles reverted into the colloidal state.

CONCLUSION
De novo designed short amphiphilic peptides (SA2) are able to form spherical nanovesicles which are remarkably stable and resist temperatures up to 70 °C. Supramolecular assembly is driven by beta-sheet formation as was demonstrated by ssNMR and molecular simulation. Colloidal stability is maintained as long as the nanovesicles have a negative surface charge, enabling low pH-triggered destabilization of such nanovesicles. These features hold promise for pharmaceutical applications such as drug and vaccine delivery.

REFERENCES

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