Reversible peptide particle formation using a mini amino acid sequence

Thomas B. Schuster[a], Dirk de Bruyn Ouboter[a], Enrica Bordignon[b], Gunnar Jeschke[b], Wolfgang Meier*[a]

Supporting information

Materials and methods

Gel permeation chromatography (GPC)

Measurements of gramicidin A (gA) and Ac-X_3-gT (c = 1 mg/mL) were performed on a Shimadzu Prominence HPLC, using an Agilent PLgel column (5 µm, 10^3 Å, 2 x 250 mm) and isocratic elution with THF/10% H_2O. Stabilizer-free solvent improved detection at the tryptophan adsorption maximum of 280 nm.

Tensiometer

Surface tension was measured on a Sigma 703D Tensiometer (KSV Inst., Finland) with a platinum Wilhelmy plate, pre-cleaned with ethanol and water, followed by flame annealing. Solutions were prepared 24 h prior to measurements, which were performed at room temperature.

UV-VIS

A Perkin Elmer Lambda 35 UV/VIS Spectrometer, equipped with a Peltier temperature control element and a 1 mm cuvette, was used to detect (280 nm) the onset of scattering due to particle formation.

Atomic force microscopy (AFM)

Measurements were carried out using a 5100 Agilent system (formerly PicoLE Molecular Imaging) equipped with a multi-purpose scanner. Images were acquired using a silicon cantilever (type-NCHR PointProbe® Plus, force constant 42 N/m) as indicated by the manufacturer for acoustic mode images. Samples were prepared by exposing aqueous peptide solution to a freshly cleaved mica surface for 2 min, removing the supernatant solution, and drying.

Results

Dimersiation probed by GPC

Fig. S1 illustrates GPC elution profiles of Ac-X_3-gT and gramicidin in THF and EtOH, whereby a clear separation of monomer and dimer was visible as two peaks for gramicidin only. The amphiphilic peptide elution profile was characterized by a slightly broader peak and no indication of distinct dimerisation.

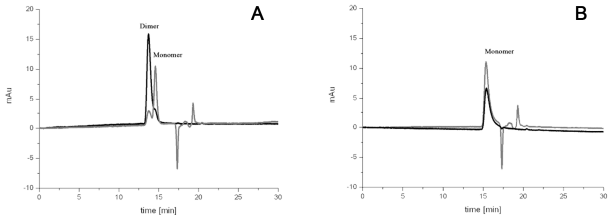

Fig. S1 A) GPC elution profiles of gramicidin mono and dimer, and B) Ac-X_3-gT monomers in different solvents (THF black line, EtOH gray line)

Peptide purity and characterisation

Preparative HPLC profiles with the collected fractions for K_3-gT are presented in Fig. S2.

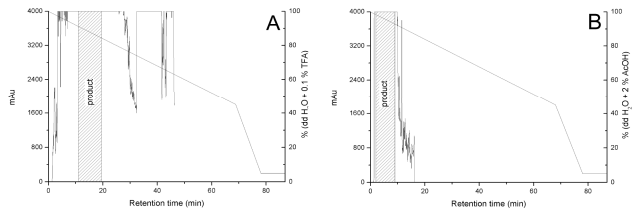

Fig. S2 Preparative HPLC elution profile of K_3-gT A) first run with ACN/dd H_2O 0.1 % TFA B) second run using ACN/dd H_2O 2 % AcOH

Verification of mass and purity of the collected fractions was analysed by MALDI-Tof-MS and HPLC. Examples are illustrated in Fig. S3.

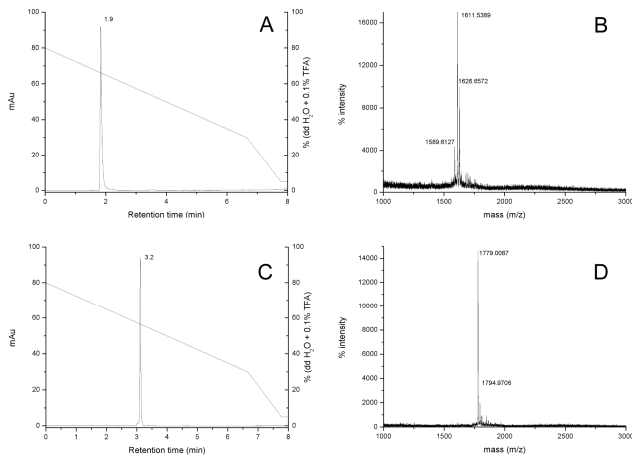

Fig. S3 Analysis of C-K_3-gT A) HPLC profile B) MALDI-ToF-MS and AcC-X_3-gT C) HPLC profile D) MALDI-ToF-MS

Table S1 presents a summary of the measured mass and purity of the purified compounds.

Table S1 Verification of mass and purity of the amphiphilic peptides used.

Code	Mass g/mol	Mass (MALDI-ToF-MS)	Purity (Area %, analyt. HPLC)
K_3-gT	1485,9	1486.7	> 99 %
C-K_3-gT	1589,9	1588.6	97 %
Ac-X_3-gT	1653,9	1677.3 ($M+Na^+$)	98 %
AcC-X_3-gT	1757,9	1779.0 ($M+Na^+$)	96 %
AcC(sl)-X_3-gT	1947,0	1948.5	97 %

Behaviour of C-K_3-gT as surfactant

Amphiphilic peptides behave like surfactants. It therefore was possible to determine a critical micelle concentration (cmc), as illustrated in Fig. S4).

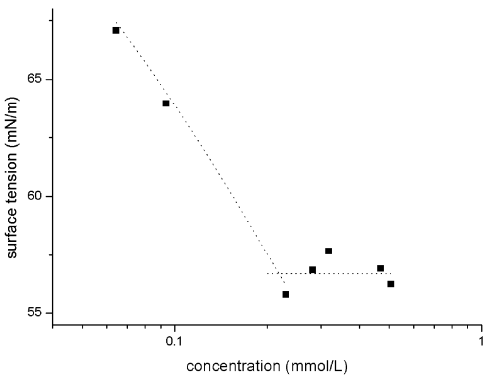

Fig. S4 Cmc of C-K_3-gT.

Self-assembly of C-K_3-gT into micelles

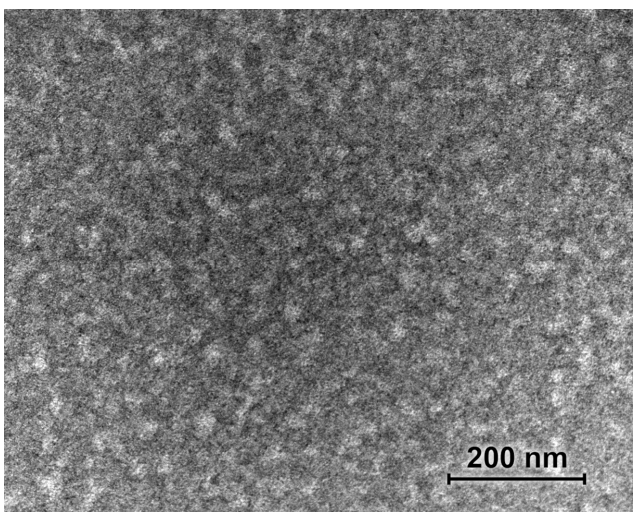

Fig. S5 Representative TEM image of C-K_3-gT micelles. The minor peptide bead fraction, which is not present in this micrograph, can be seen in Fig. 2 (blank)

Peptide bead formation due to charge shielding

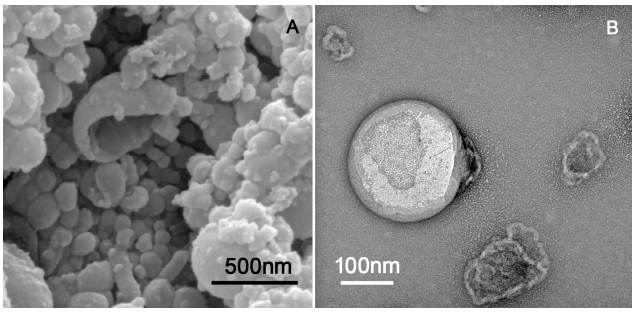

Fig. S6 A) SEM of C-K_3-gT with NaH_2PO_4 (0,025mol/L) B) TEM of AcC-K_3-gT with NaSCN (0.1mol/L)

Self-assembly of Ac-X_3-gT

Self-assembled spherical structures - so-called peptide beads - from Ac-X_3-gT were imaged using SEM, TEM, and AFM (Fig. S7).

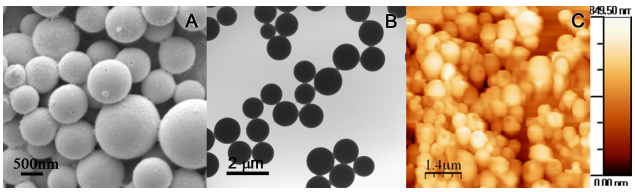

Fig. S7 A) SEM of Ac-X_3-gT peptide beads, B) TEM stained with uranyl acetate and C) AFM.

Cw EPR measurements in differnent ethanol/water mixtures

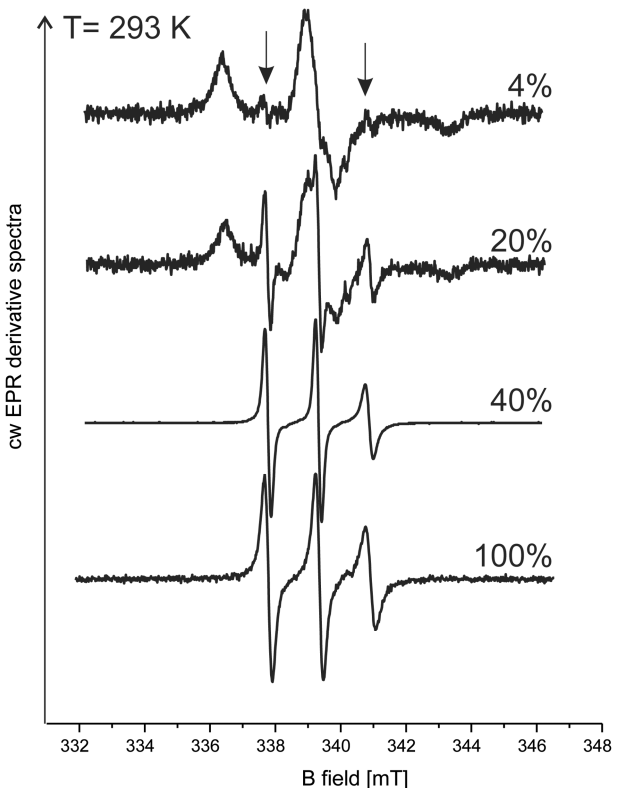

Fig. S8 cw EPR spectra of AcC-X_3-gT / AcC(sl)-X_3-gT mixtures depending of ethanol concentration. Arrows mark the characteristic features of the mobile species, which are getting dominant with increasing ethanol concentration.

Correlation functions

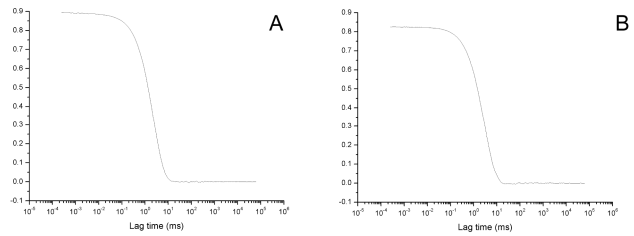

Fig. S9 Correlation functions of AcC-X_3-gT/AcC(sl)-X_3-gT mixture in A) 40 wt % ethanol and B) 4 wt % ethanol

Equilibrium of Ac-X_3-gT in ethanol mixtures

Equilibrium between micelles and peptide beads arises in ethanol/water mixtures. Converting peptide solutions at 4 wt % ethanol to 40 wt % ethanol and vice versa (using dialysis) always produces the same characteristic DLS signals, proving the reversibility of the system. A diagram of the experiment and the corresponding results are presented in Fig. S7.

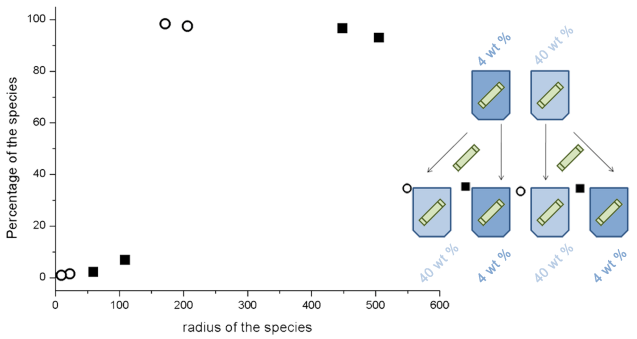

Fig. S10 Reversibility of the system: Two different starting points (4 wt % and 40 wt %) yield the same DLS features at 4 wt % (■) and at 40 wt % (O) final ethanol content.

Temperature-depentent self-assembly of Ac-X_3-gT

The self-assembly process was initiated by solvent exchange from 100 w % to 20 wt % ethanol. A reversible, temperature-dependent peptide bead formation was monitored at that ethanol concentration. Temperature-dependent UV absorption measurements tracked bead formation as absorption increased due to scattering. As Fig. S11 shows, peptid beads begin to assemble below 25 °C at 20 wt % ethanol. This process was reversible. The cooling (black line) - heating (red line) cycle exhibits a slight histeresis due to the experimental setup.

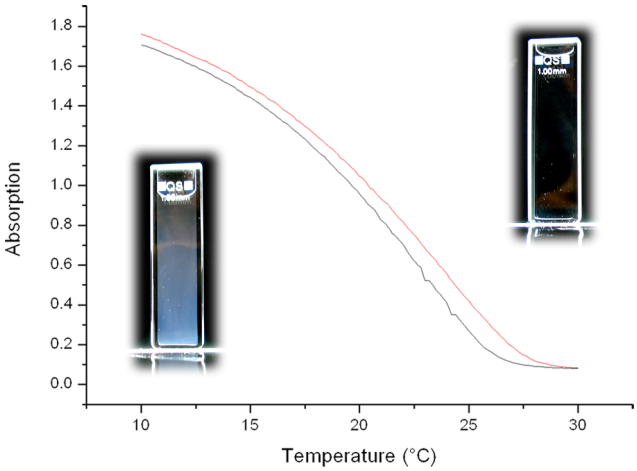

Fig. S11 UV measurement of temperature-dependent bead formation (heating (red line), cooling (black line)) using Ac-X_3-gT at 20 wt % EtOH. Photographs were taken at 4 °C (left) and RT (22 °C, right).

Size dependeny of peptide beads in dd H_2O upon initial peptide concentration

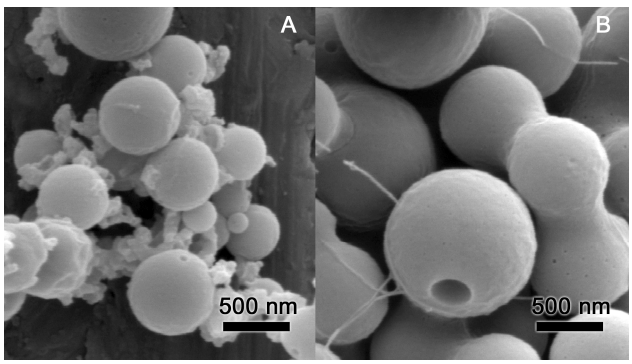

Fig. S12 SEM micrographs of Ac-X_3-gT at A) 1.0 mol/L B) 1.5 mol/L

Notes

[a] *Department of Chemistry, University of Basel, Klingelbergstrasse 80, CH- 4056 Basel, Switzerland; E-mail: wolfgang.meier@unibas.ch*

[b] *Laboratory for Physical Chemistry, ETH Zürich, Wolfgang-Pauli-Str. 10, CH- 8093 Zürich, Switzerland*