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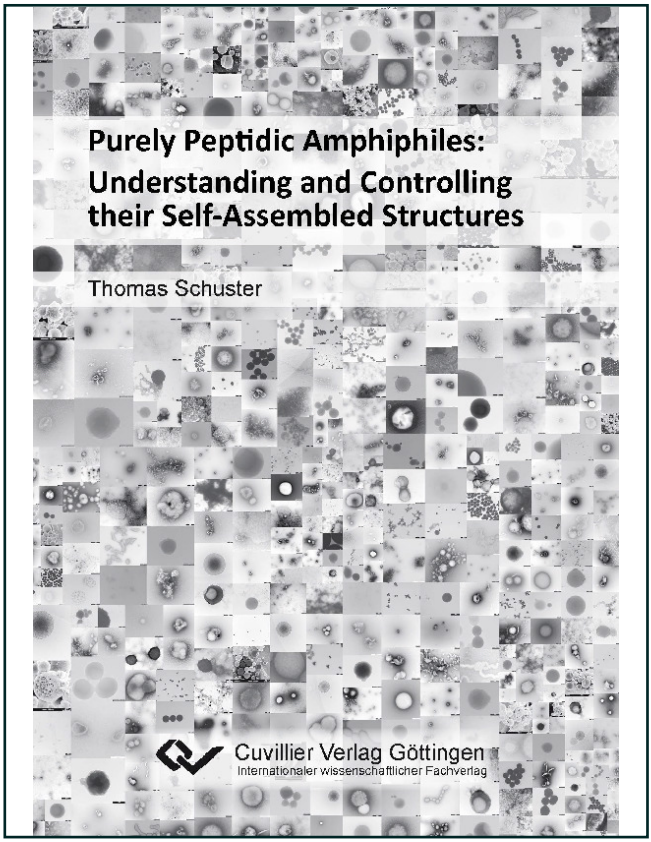
Purely Peptidic Amphiphiles: Understanding and Controlling their Self-Assembled Structures



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Summary of the thesis

Part 1. Introduction

This first chapter will provide a brief overview of the self-organization of amphiphilic molecules into supramolecular aggregates. The latter exist in as many sizes and morphologies as there are factors to influence them. Especially defined amino acid sequences offer numerous functionalities and are able to respond to environmental stimuli. Furthermore they consist of biodegradable material and, therefore, accumulation in the human body might be avoided in potential medical applications. Additionally, advantages and drawbacks among different synthesis methods, *e.g.* polymerization, bioengineering techniques or solid-phase peptide synthesis, are discussed.

Part 2. Reversible peptide particle formation using a mini amino acid sequence

The self-assembly of amphiphilic peptides with a specific amino acid sequence into micelles and spherical peptide nanoparticles has been investigated. The undecamer peptide that was used features a repetitive L-tryptophan and D-leucine [LW-DL] motif representing the hydrophobic block – which is a truncated version of gramicidin A (gA), named gT. The N-terminally attached hydrophilic section was either lysine (K) or acetylated lysine (X) and was optionally terminated with cysteine for post-functionalization of the thiol group. Aggregation into micelles and a minor fraction of peptide particles was observed for peptides containing charged lysine. Charge shielding with anionic counter ions followed the trends in the Hofmeister series and shifted the equilibrium towards the larger peptide aggregates.

Similarly, it was demonstrated that the corresponding uncharged peptide assembled into micelles and subsequently into peptide particles, termed 'peptide beads'. Furthermore formation of the peptide beads was studied as a function of temperature and solvent composition. We hypothesized that the peptide beads consisted of micelles – a structure described as multicompartment micelles (MCM).

Part 3. Highly ordered composite peptide – gold nanoparticles (Au-NP)

Peptide beads using AcC-X_3-gT and its analogues – including Ac-X_3-gT-C, a new peptide with the cysteine residue at the C instead of the N-terminus – have been further studied. Ac-X_3-gT-C peptide beads within the formation process were imaged by scanning electron microscopy (SEM), which demonstrated the aggregated micelles within the beads and agrees with the multi-compartment micelles (MCM) as hypothesized. Furthermore, we succeeded in creating highly ordered Ac-X_3-gT-C – Au-NP composites, which allowed us to visualize the inner structure of the nanoparticles. The presented composite materials are expected to exhibit exceptional electronic and optical properties.

Part 4. Molecular thin films produced by short amphiphilic peptides

The amphiphilic peptides – K_3-gT, C-K_3-gT and AcC-X_3-gT – have been studied with regard to the interaction within a lipid monolayer but also in pure peptide layers at the air-water interface. Experiments on the Langmuir trough allow the separate investigation of different parameters such as the choice of buffer subphase or its concentration. Thus, the intrinsic parameters for a peptide as well as the environmental conditions necessary to form a stable film have been determined. Results indicate the formation of a unimolecular flat film. Peptide layers have been successfully transferred onto a solid support or self-

assembled peptide monolayers (SAMs) have been created equally well via immersion and spin-coating. Preliminary mineralization experiments confirmed the successful creation of calcium phosphate crystals. Therefore, the created homogenous monomolecular interfaces are potentially useful for mineralization.

Part 5. From fibers to micelles using point mutated amphiphilic peptides

A peptide library has been synthesized to correlate the primary sequence, its secondary structure and the resulting self-assembly. The peptide design includes three parts: (a) a charged lysine part, (b) an acetylated lysine part and (c) a constant hydrophobic rod-like helix, based on gramicidin A. By stepwise replacement of free lysine (K) with acetylated lysine (X) we generated a library of a total of ten peptides Ac-X_8-gA and K_mX_{8-m}-gA (m ranging from 0 to 8). By using point mutations, we adjusted the degree of acetylation (DA) and thus the overall amphiphilicity of the peptides, which led to a change in the secondary structure in the aqueous environment from a β-sheet to an α-helix. This transition generated a significant change in the morphology of the self-assembled structures from fibers to micelles. Two different regions where observed with the conformation of the hydrophilic part of the peptide: one region, a β-sheet-like secondary structure, inducing fiber formation (high DA), the other an α-helical-like secondary structure generating micelle formation (moderate and low DA). The micellar structures depended on the degree of acetylation, which influenced their critical micelle concentration (cmc). In conclusion, our results demonstrate the control of self-assembled morphologies by point mutation of a peptide's primary sequence. This study is precisely important because it presents a first step towards molecular switches based on acetylation of a peptide inspired by the example of

phosphorylation of proteins or enzymes, to convert them into the active or inactive state by structural changes.

Part 6. Exploiting dimerization of amphiphilic peptides to form vesicles

This chapter focuses on essential parameters to successfully manage membrane formation from a purely peptidic system. Literature already provides fundamental rules for supramolecular aggregation such as amphiphilic design or a hydrophilic to hydrophobic ratio of about 1:3. However, smaller molecules of a defined nature need additional interaction to create stable vesicular structures. A crucial step in the formation of peptide membranes appears to be dimerization which originates from the introduction of intermolecular interactions, such as H-bonds or $\pi-\pi$ stacking of aromatic rings. The formation of a stabilized membrane subunit, *i.e.* dimers in the lateral or perpendicular directions (relative to the membrane) produced stable, purely peptidic vesicles and may well apply to other peptidic systems. These novel peptidic systems offer hydrophilic and hydrophobic compartments to encapsulate and integrate different drugs or payloads and could be used for gene delivery, since the design includes charged moieties.

Part 7. General conclusions and outlook

In this last chapter the achievements of the presented work are discussed and summarized. Furthermore, lines of research are suggested, *e.g.* the development of medical applications and the mimicking of lipid membranes, which, from the present point of view, appear to be the most promising and should be the focus of subsequent experiments.