edited by
Jaime Castillo-León
Luigi Sasso
Winnie E. Svendsen

SELF-ASSEMBLED PEPTIDE NANOSTRUCTURES

ADVANCES AND APPLICATIONS IN NANOBIOTECHNOLOGY

edited by
Jaime Castillo-León
Luigi Sasso
Winnie E. Svendsen

SELF-ASSEMBLED PEPTIDE NANOSTRUCTURES

ADVANCES AND APPLICATIONS IN NANOBIOTECHNOLOGY

PAN STANFORD PUBLISHING

Published by

Pan Stanford Publishing Pte. Ltd.
Penthouse Level, Suntec Tower 3
8 Temasek Boulevard
Singapore 038988

Email: editorial@panstanford.com
Web: www.panstanford.com

British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library.

Self-Assembled Peptide Nanostructures: Advances and Applications in Nanobiotechnology

ISBN 978-981-4316-94-1 (Hardcover)
ISBN 978-981-4364-47-8 (eBook)

Printed in the USA

SELF-ASSEMBLED PEPTIDE NANOSTRUCTURES

Preface

In the search for new biomaterials, self-assembled peptide structures appear very promising for applications in fields such as biosensing, electronics, diagnostics, drug delivery, tissue repair, and clean-room fabrication. These building blocks are able to self-assemble into 1D, 2D, or 3D nanostructures in a very simple way without the use of specialized machinery or extreme temperature or pressure conditions. There are a number of peptides that are able to self-organize into nanostructures. These can be found in nature or synthesized in the lab, which offers researchers a wide range of candidate compounds to choose from according to their requirements.

During the past 25 years, researchers from various groups around the world have concentrated on finding, characterizing, manipulating, and applying this fantastic biomaterial in different fields. As a result of these efforts, a group of researchers from different countries joined forces under a European funded project entitled Bioengineered Nanomaterials for Research and Applications (BeNatural). The goal of this project was to use nature as a model for new nanotechnology-based processes by applying fundamental knowledge from the structure and assembly of biological self-assembled nanostructures to new innovative ways of designing and assembling man-made nanodevices.

The idea of this book can be traced to Jaime's presentation at the 1st Nano Today Conference in Singapore in September 2009. At the conference, Jaime presented the latest results of the BeNatural project. After the presentation, Pan Stanford Publishing invited us to write a book on self-assembled peptides and their use in nanotechnology. We managed to bring together some of the world's top researchers and, thus, began working on the book. Thanks to the

efforts by and good collaboration of all the authors, this book has become a reality.

The book begins with a nice discussion by Prof. Carl Henrik Görbitz from Oslo University (Norway) on the basis of the self-assembly process. Prof. Görbitz starts with nucleation and discusses the nature of intermolecular interactions between peptides and the role of solvent molecules in the self-assembly process. In Chapter 2, Profs. Anna Mitraki and Emmanoulis Kasotakis from Crete University (Greece) review the mechanisms and principles that govern peptide self-assembly and discuss how these mechanisms allow the rational design of peptide building blocks for specific functions. In Chapter 3, Prof. Rein Ulijn from the University of Strathcylde (UK) continues the overview of various strategies used to produce molecular materials based on peptides and their derivatives. Prof. Ulijn discusses how to enhance the control of the self-assembly process based on changes in temperature, pH, and light. Next, Prof. Yong-beon Lim from Yonsei University (South Korea) classifies self-assembling peptide nanostructures on the basis of their function, chemical structure, nanostructural properties, and applications (Chapter 4). In Chapter 5, we review different techniques available for the controlled manipulation of peptide nanostructures in order to characterize them or integrate them with transducers for specific applications.

The following chapters focus on the applications of self-assembled peptides in bionanotechnology. In Chapter 6, Prof. Charlotte Hauser and collaborators from the Institute of Bioengineering and Nanotechnology (Singapore) show the results of their work with ultra-small peptide hydrogels for their use in regenerative medicine, biomineralization, molecular imaging, nanofabrication, and delivery of bioactive therapeutics. Next, Prof. Junbai Li and his team from the Beijing National Laboratory at the Chinese Academy of Sciences, the School of Chemistry at the Qufu Normal University (China) and the Max Planck institute of Colloids and Interfaces (Germany) show the potential applications of peptide and protein micro- and nanostructures. They focus on the use of peptide self-assembled fibers, tubes, and particles in biosensors, biocatalysis, and bioimaging, among others. The book ends with the contribution by Prof. Amalia Aggeli's team at the University of

Leeds (UK), in which they present results of their work using self-assembled peptide nanostructures in tissue engineering discussing their biocompatibility and usefulness as scaffolds.

We hope that this book will motivate the interest of a broad audience of students and researchers in biomaterials so that they consider self-assembled peptides an alternative in their research. Here they can find the advantages and challenges when working with these materials as well as inspiring works using these building blocks. We want to acknowledge all the authors for their contributions as well as the people at Pan Stanford Publishing for their help during the publishing process.

Jaime Castillo-León
Luigi Sasso
Winnie E. Svendsen

Contents

Chapter 1

Theoretical Basis of Biological Self-Assembly

Carl Henrik Görbitz
Department of Chemistry, University of Oslo, P.O. Box 1033 Blindern
N-0315 Oslo, Norway
c.h.gorbitz@kjemi.uio.no

The term self-assembly describes processes where a system of disordered pre-existing components forms a more organized structure or pattern as the result of specific, local interactions among the components themselves, without external direction. When the constitutive components are molecules, the process is called molecular self-assembly, or biological self-assembly if the molecules in question have particular biological relevance. This chapter discusses the basis of the self-assembly process, starting with nucleation, with discussion of the nature of intermolecular interactions between peptides and the role of solvent molecules in the process leading to various types of three-dimensional crystal-packing patterns.

1.1 Introduction

Biological self-assembly may lead to a diverse array of arrangements ranging from zero-dimensional lipid micelles and one-dimensional

Self-Assembled Peptide Nanostructures: Advances and Applications in Nanobiotechnology
Edited by Jaime Castillo-León, Luigi Sasso, and Winnie E. Svendsen

ISBN 978-981-4316-94-1 (Hardcover), 978-981-4364-47-8 (eBook)
www.panstanford.com

DNA double helices to two-dimensional lipid bilayers and three-dimensional solid state structures. Although this chapter is mainly focused on three-dimensional structures typically studied by X-ray crystallography, the dimensionality of the packing pattern is always an important issue, as some crystal structures contain very obvious molecular dimers (0D), while other have chains (1D), layers (2D) or packing arrangements with interactions of comparable strength in all directions (3D). The outcome of a crystallization of a particular compound is far from random, but can be rationalized from an analysis of the nature and properties of the molecular building blocks. Taking it one step further, any deliberate effort to design a specific type of structure, such as peptide nanotubes, is greatly aided by a fundamental knowledge of the forces that trigger the spontaneous organization of these structures.

1.2 Formation of a Crystal

Crystallization of an organic molecule is a complex series of events involving molecular recognition. Ever so often, obtaining crystals suitable for diffraction experiments constitutes the bottleneck in a planned investigation of a new compound or material. Adding to traditional techniques applied by organic chemists, a series of specialized crystallization techniques have been developed for macromolecules [1], many of which may also be applied to smaller peptides. The key feature of these methods is to control the speed of the nucleation process.

1.2.1 *Self-Assembly Prior to Nucleation*

For most compounds an undersaturated solution is dominated by individual molecules (monomers), but occasionally dimers or larger aggregates may prevail [2, 3]. Among small molecules the archetypical formation of carboxylic acid dimers is well-known, but even an amino acid like glycine may form a small number of dimers in solution [4]. In organic solvents, where zwitterionic peptides are virtually insoluble, cyclic, or *N*- and *C*-blocked peptides are particularly prone to satisfy their hydrogen bonding requirements

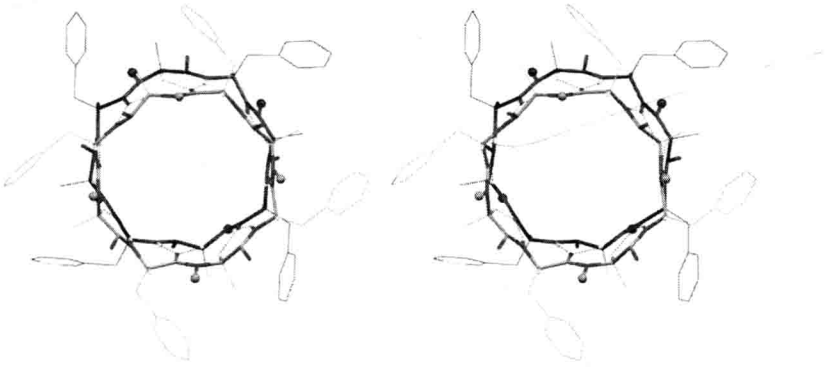

Figure 1.1. Stereo drawing of a dimer of cyclo(Phe-D-*N*-MeAla)$_4$ [5]. C atoms of the lower molecule are depicted in a darker grey tone, side chains are shown in wireframe style. The *N*-methyl groups of D-Ala residues appear as small spheres. See also Color Insert.

through formation of dimers. The structure of cyclo(Phe-D-*N*-MeAla)$_4$[1] shown in Fig. 1.1 [5] is a typical example of such a molecule.

1.2.2 *Nucleation*

Classical nucleation theory tells that the initial nucleus, defined as the minimum amount of a new phase capable of independent existence, is formed from a small, compact group (20–100) of molecules already displaying the packing arrangement of the mature, macroscopic crystal. Due to the obvious shortcomings of classical theory, most importantly the failure to predict nucleation rates with any degree of accuracy, a more advanced two-step mechanism has recently been proposed [6, 7]. According to this model, the first step is the formation of a liquid-like cluster of solute molecules. While suspended in solution, this cluster is subsequently reorganized into the ordered structure in the second step. The latter is assumed to be rate-determining, based on the observation that nucleation from solution takes more time for complex molecules

[1] In this chapter the L-form is assumed for chiral amino acids unless stated otherwise.

with a high degree of conformational freedom, as they may need more time to arrange themselves into a regular crystalline lattice [8].

1.2.3 *Crystal Growth and Crystal Habit*

After formation of the initial nucleus, molecules (potentially as dimers or even larger growth units) attach to the surface of the growing crystal. Models for non-uniform lateral growth (stepwise mechanism) and uniform normal growth from the melt have been applied to solutions of proteins and viruses [9], but details on the growth mechanism of crystals of small organic molecules are still largely lacking. Among amino acids the polymorphism (see below) of glycine has been studied [10], and a molecular dynamics simulation of α-glycine crystal growth was recently reported [11]. The observed shape of the crystals of a particular compound, the habit, gives indirect information on the attachment energy. If two opposing faces are strongly favored, needle-shaped crystals are obtained, a common observation for peptides forming nanotubular structures. The habit may be affected by the solvent or by selected additives, providing ways to control the resulting crystal shape.

1.2.4 *Polymorphism*

True polymorphs (in distinction to so-called pseudopolymorphs which differ with respects to the type of solvent included and thus have different overall formulas) are different crystal forms of the same compound [12]. Occasionally, two or more crystal forms may be observed in the same experimental setup, but more frequently the crystallization outcome can be controlled by the choice of crystallization method, the solvents used, temperature, or other external factors. It follows that the crystals formed initially may not belong to the thermodynamically most stable form, but to another form as the result of kinetic factors. Such a metastable form can be stable indefinitely in solution, but can also gradually convert to and replaced by the more stable polymorph. Although polymorphism is widespread among organic molecules, it appears to be very rare for peptides. In fact, Gly-Gly [13, 14] and cyclo(D-Ala-L-Ala) [15, 16]