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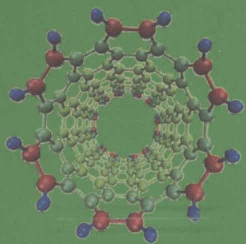
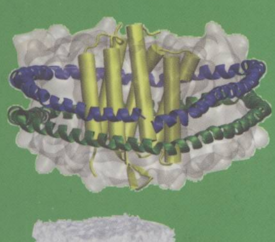


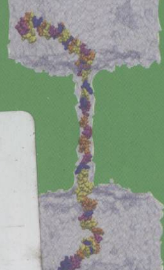
Nanostructure Design

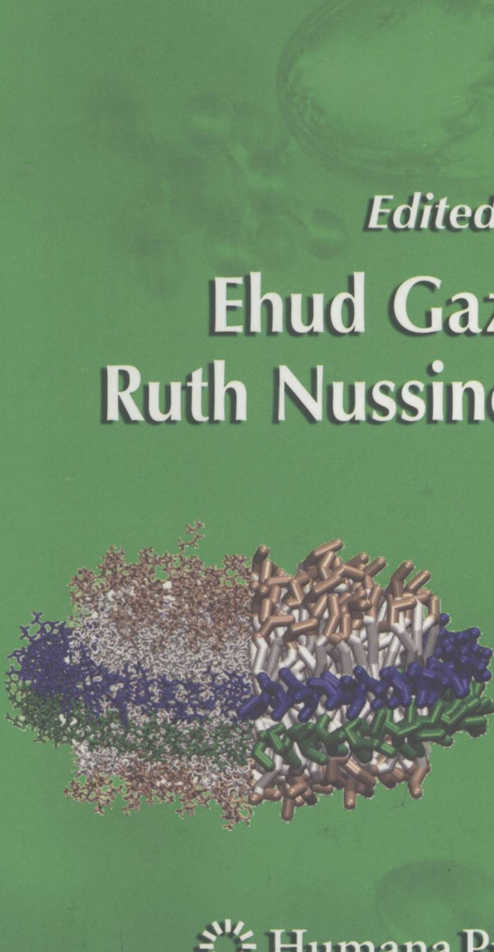
Methods and Protocols

Edited by

Ehud Gazit
Ruth Nussinov







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Nanostructure Design

Methods and Protocols

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ISBN: 978-1-934115-35-0
e-ISBN: 978-1-59745-480-3
ISSN: 1064-3745
e-ISSN: 1940-6029
DOI: 10.1007/978-1-59745-480-3

Library of Congress Control Number: 2008921784

Cover illustration: Provided by Aleksei Aksimentiev et al. (Chapter 11, Figures 4, 9a, 12, 13A)

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Preface

We are delighted to present *Nanostructure Design: Methods and Protocols*. Nanotechnology is one of the fastest growing fields of research of the 21st century and will most likely have a huge impact on many aspects of our life. This book is part of the excellent *Methods in Molecular Biology*™ series as molecular biology offers novel and unique solutions for nanotechnology.

Nanostructure Design: Methods and Protocols is designed to serve as a major reference for theoretical and experimental considerations in the design of biological and bio-inspired building blocks, the physical characterization of the formed structures, and the development of their technological applications. It gives exposure to various biological and bio-inspired building blocks for the design and fabrication of nanostructures. These building blocks include proteins and peptides, nucleic acids, and lipids as well as various hybrid bioorganic molecular systems and conjugated bio-inspired entities. It provides information about the design of the building blocks both by experimental exploration of synthetic chemicals and biological prospects and by theoretical studies of the conformational space; the characterization of the formed nanostructures by various biophysical techniques, including spectroscopy (electromagnetic as well as nuclear magnetic resonance) together with electron and probe microscopy; and the application of bionanostructures in various fields, including biosensors, diagnostics, molecular imaging, and tissue engineering.

The book is divided into two sections; the first is experimental and the second computational. At the beginning of the book, Thomas Scheibel and coworkers describe the use of a natural biological self-assembled system, the spider silk, as an excellent source for the production of nano-ordered materials. Using recombinant DNA technology and bacterial expression, large-scale production of the unique silk-like protein is achieved.

In Chapter 2, by Anna Mitraki and coworkers in collaboration with Mark van Raaij, yet another fascinating biological system is explored for technological uses. The authors, inspired by biological fibrillar assemblies, studied a small trimerization motif from phage T4 fibritin. Hybrid proteins that are based on this motif are correctly folded nanorods that can withstand extreme conditions.

In Chapter 3, Maxim Ryadnov, Derek Woolfson, and David Papapostolou study yet another important self-assembly biological motif, the leucine zipper. Using this motif, the authors demonstrate the ability to form well-ordered fibrillar structures. In Chapter 4, Joseph Slocik and Rajesh Naik describe methodologies that exploit peptides for the synthesis of bimorphic nanostructures. Another demonstration of the use of peptides for self-assembled structures is described in Chapter 5 by Radhika P. Nagarkar and Joel P. Schneider. The authors use these peptides for the formation of hydrogel materials that may have many applications in diverse fields, including tissue engineering and regeneration.

In the last chapter of the book's experimental section (Chapter 6), Yingfu Li and coworkers describe a protocol for the preparation of a gold nanoparticle combined with a DNA scaffold on which nanospecies can be assembled in a periodical manner. This demonstrates the combination of biomolecules with inorganic nanoparticles for technological applications.

In Part II, on the computational approach, Bruce A. Shapiro and coauthors describe in Chapter 7 recent developments in applications of single-stranded RNA in the design of nanostructures. RNA nanobiology presents a relatively new approach for the development of RNA-based nanoparticles.

In Chapter 8, Idit Buch and coworkers describe self-assembly of fused homo-oligomers to create nanotubes. The authors present a protocol of fusing homo-oligomer proteins with a given three-dimensional structure to create new building blocks and provide examples of two nanotubes in atomistic model details.

The authors of Chapter 9, Joan-Emma Shea and colleagues, present a thorough discussion of the theoretical foundation of an enhanced sampling protocol to study self-assembly of peptides, with an example of a peptide cut from the Alzheimer Aβ protein. The self-assembly of Aβ peptides led to amyloid fibril formation. Thorough and efficient sampling is crucial for computational design of self-assembled systems.

In Chapter 10, Maarten G. Wolf, Jeroen van Gestel, and Simon W. de Leeuw also model amyloid fibril formation. The fibrillogenic properties of many proteins can be understood and thus predicted by taking the relevant free energies into account in an appropriate way. Their chapter gives an overview of existing simulation techniques that operate at a molecular level of detail.

Klaus Schulten and his coworkers provide an overview in Chapter 11 of the impressive array of computational methods and tools they have developed that should allow dramatic improvement of computer modeling in biotechnology. These include silicon bionanodevices, carbon nanotube-biomolecular systems, lipoprotein assemblies, and protein engineering of gas-binding proteins, such as hydrogenases.

In the final chapter (Chapter 12), Ugur Emekli and coauthors discuss the lessons that can be learned from highly connected β-rich structures for structural interface design. Identification of features that prevent polymerization of these proteins into fibrils should be useful as they can be incorporated in interface design.

Biology has already shown the merit of a nanostructure formation process; it is the essence of molecular recognition and self-assembly events in the organization of all biological systems. Biology offers a unique level of specificity and affinity that allows the fine tuning of nanoscale design and engineering. While much progress has been made, challenges are still ahead. We hope that *Nanostructure Design: Methods and Protocols*, which is based on biology and uses its principles and its vehicles toward design, will be useful for newcomers and experienced nanobiologists. It can also help scientists from other fields, such as chemistry and computer science, who would like to explore the prospects of nanobiotechnology.

Ehud Gazit
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Contents

I

Experimental Approach

1

Molecular Design of Performance Proteins With Repetitive Sequences

Recombinant Flagelliform Spider Silk as Basis for Biomaterials

Charlotte Vendrely, Christian Ackerschott, Lin Römer, and Thomas Scheibel

Summary

Most performance proteins responsible for the mechanical stability of cells and organisms reveal highly repetitive sequences. Mimicking such performance proteins is of high interest for the design of nanostructured biomaterials. In this article, flagelliform silk is exemplary introduced to describe a general principle for designing genes of repetitive performance proteins for recombinant expression in *Escherichia coli*. In the first step, repeating amino acid sequence motifs are reversely transcripted into DNA cassettes, which can in a second step be seamlessly ligated, yielding a designed gene. Recombinant expression thereof leads to proteins mimicking the natural ones. The recombinant proteins can be assembled into nanostructured materials in a controlled manner, allowing their use in several applications.

Key Words: Biomaterials; recombinant production; repetitive sequence; spider silk proteins.

1. Introduction

Proteins with repetitive sequences often have specific structural properties and functions in nature. Such proteins comprise transcription factors, developmental proteins *(1)*, or structural biomaterials like elastin *(2)*, collagen *(3)*, and silk *(4)*.

Spider silks, for instance, possess outstanding mechanical properties *(5–7)*, which are highly important for the stability, for a spider's web. Among the diversity of silks produced by an individual spider, major ampullate silk forms the frame of the web and is responsible for its strength. In contrast, flagelliform silk building the capture spiral provides the elasticity necessary for dissipating

From: *Methods in Molecular Biology, vol. 474: Nanostructure Design: Methods and Protocols*
Edited by: E. Gazit and R. Nussinov © Humana Press, Totowa, NJ

the energy of prey flying into the web. Typically, all spider silks are composed of proteins that have a highly repetitive core sequence flanked by short, nonrepetitive sequences at the amino and carboxy termini (**Fig. 1**) *(8,9)*.

Sequence comparison of common spider silk proteins reveals four oligopeptide motifs that are repeated several times in each individual protein: (1) $(GA)_n/(A)_n$, (2) GPGGX/GPGQQ, (3) GGX, and (4) "spacer" sequences that contain charged amino acids *(4,10–14)*. Previously, distinct secondary structure contents (i.e., nanostructures) have been detected for silk proteins, depending on these amino acid sequences. The structural investigation of the motifs has often been performed using either entire silk fibers or short, nonassembled peptides mimicking the described oligopeptide sequences. Methods like Fourier transform infrared (FTIR), X-ray diffraction, and nuclear magnetic resonance (NMR) revealed that oligopeptides with the sequence $(GA)_n/(A)_n$ tend to form α-helices in solution but β-sheet structures in assembled fibers *(15–22)*. Such β-sheets presumably assemble the crystalline domains found within the natural silk fiber *(19,23–25)*.

In contrast, the structures adopted by GPGGX/GPGQQ and GGX repeats remain unclear. Based on X-ray diffraction studies, these regions have been described to resemble amorphous "rubber" *(26,27)*, and NMR studies suggested that they form 3_1-helical structures or can be incorporated into β-sheets *(17,19)*. Flagelliform silk, which is rich in GPGGX and GGX motifs (**Fig. 1**), likely

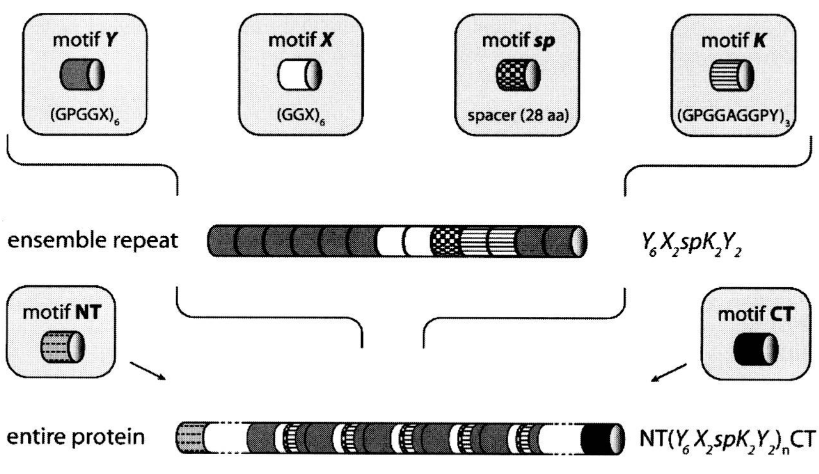

Fig. 1. Repetitive nature of the flagelliform silk protein sequence. The core sequence consists of 11 ensemble repeats that contain four consensus motifs: *Y, X, sp*, and *K*. Sfl, the recombinant protein mimicking the core domain of natural flagelliform protein, is composed of $Y_6X_2spK_2Y_2$. In the natural protein, the repetitive core sequence is flanked by nonrepeated sequences at the amino terminus (NT) and the carboxy terminus (CT).