PREFACE

This volume of *Methods in Enzymology* introduces the applications and methods of nanomedicine, a field that has been emerging in the past decade, in conjunction with advances in nanotechnology and bionanoscience. The chapters cover applications in antiviral, antibacterial, and genetic nanomedicine, immunotherapy, antifibrotics, diagnosis, as well as the methods used to evaluate the toxicity of nanomedicines.

In the section Antiviral Nanomedicines, Roberta Cavalli and colleagues, in Chapter 1, describe the preparation of polymeric nanoparticles based on a β-cyclodextrin-poly(4-acryloylmorpholine) mono-conjugate as carriers of acyclovir, involving the solvent displacement method. They employ fluorescent-labeled nanoparticles for cellular trafficking studies and provide methods for the assessment of the antiviral activity of the nanoparticles. In Chapter 2, Soledad Penadés and coauthors demonstrate that oligomannoside-coated gold nanoparticles (*manno*-GNPs) are able to interfere with HIV high-mannose glycan-mediated processes and present methods for the preparation and characterization of *manno*-GNPs and the experiments using surface plasmon resonance and saturation transfer difference-NMR techniques. Supriya D. Mahajan and colleagues describe the stable incorporation of amprenavir within a transferrin-conjugated quantum dot, the ability of this nanoparticle to transverse an *in vitro* blood–brain barrier model, and its antiviral efficacy in HIV-1-infected monocytes in Chapter 3.

In the section Antibacterial Nanomedicines, Ranjita Misra and Sanjeeb K. Sahoo (Chapter 4) describe the improvement of the entrapment efficiency of doxycycline-loaded poly(lactide-co-glycolide):poly(ε-caprolactone) nanoparticles, evaluate the efficacy of these nanoparticles against *E. coli*, and show that doxycycline-loaded nanoparticles have superior effectiveness compared to native doxycycline, resulting from the sustained release of the antibiotic from the nanoparticles. In Chapter 5, Robert P. Allaker and colleagues write about copper oxide nanoparticles that are embedded into a polyurethane matrix forming thin porous films and demonstrate their activity against methicillin-resistant *Staphylococcus aureus*. This approach may have applications in designer filters, adhesive films instead of sutures, and mechanically supporting structures.

In Chapter 6 of the section Nanomedicines in Immunotherapy, Didier Merlin and coauthors show the development of a technique for specific targeting of nanoparticles to digestive tract regions, including the colon, using a hydrogel based on electrostatic interactions between positive ions

and negative polysaccharides. Thus, nanoparticle degradation in the gastrointestinal tract is reduced, and lower doses of drug can be delivered to the colon to reduce colonic inflammation. Olga Borges and collaborators, in Chapter 7, outline the preparation of chitosan-based particles as an antigen-delivery system for mucosal surfaces, in particular for the hepatitis B surface antigen. They also describe the characterization of the particles in terms of size, morphology, and zeta potential; antigen adsorption onto particles; toxicity of the particles; and particle uptake into lymphoid organs. In Chapter 8, Luis J. Cruz and coauthors discuss the development of targeted nanodelivery systems carrying vaccine components, including antigens and adjuvants, to dendritic cells and provide an overview of antigen-delivery vehicles currently under investigation. They then focus on the use of liposomes, poly(lactic-co-glycolic acid) polymers and gold nanoparticles to obtain safe and efficacious vaccines.

In Chapter 9 of the section Diagnostic Nanomedicine, which deals with the application of nanotechnology to diagnostic medicine, Venkatesan Renugopalakrishnan and colleagues cover the design of unique, single-platform, integrated, multichannel sensors based on carbon nanotube-protein adducts specific to each one of the major analytes of blood. Carbon nanotubes enhance the signals derived from the interaction of the enzymes with the different analytes in blood.

Two chapters focus on the toxicity of nanoparticles in the section Nanomedicine Toxicity. In Chapter 10, Stefaan J. Soenen and colleagues describe the measurement of the potential toxicity of iron oxide nanoparticles, involving acute cytotoxicity, induction of reactive oxygen species, cell-associated iron, cell morphology, cell proliferation, cell functionality, as well as pH-induced or intracellular degradation of the nanoparticles. Willi Jahnen-Dechent and coworkers indicate that we are still far from predicting the toxicological properties of new nanoparticles and summarize some basic concept to assess the toxicity of gold nanoparticles, including death pathways, cell cycle arrest, and oxidative stress in Chapter 11.

In an application of the nanomedicine approach to tissue reconstruction, Tero A.H. Järvinen in Chapter 12 of the section Antifibrotic Nanomedicine, describe the development and use of a targeting system for an antifibrotic biotherapeutic, decorin, based on a vascular homing peptide that specifically recognizes angiogenic blood vessels in regenerating and inflammatory tissues.

The section Genetic Nanomedicine starts with Chapter 13 by Xiaoyuan Chen and collaborators who highlight the basic concepts and applications of nonviral gene delivery vehicles based on low-molecular-weight *N*-alkyl polyethylenimine-stabilized iron oxide nanoparticles. Many cell-penetrating peptides have been applied successfully to mediate the intracellular delivery of nucleic acids. In Chapter 14, Maria C. Pedroso de Lima and coauthors provide a description of the experimental procedures for the preparation of

cell-penetrating peptide-based nucleic acid complexes, their biophysical characterization, and the evaluation of the biological and cytotoxic effects of the complexes. Hideyoshi Harashima and colleagues, in Chapter 15, describe their experience with the concept of "programmed packaging," leading to the production of a multifunctional envelope-type nanodevice (MEND) as a nonviral gene delivery system. They focus on the construction of a tetra-lamellar MEND, which can be utilized to overcome intracellular membrane barriers, since it involves stepwise membrane fusion. They also describe the delivery of a bioactive molecule targeted to the nucleus and mitochondria in living cells. Conchita Tros de Ilarduya and collaborators, in Chapter 16, describe an efficient, nonviral gene transfer system that employs polyethylenimine (PEI 800, 25, 22 kDa), a cationic lipid and cholesterol at different lipid/DNA molar ratios and five different protocols of formulation. Some of these ternary complexes mediate highly efficient gene delivery even in the presence of a high concentration of serum. Huricha Baigude and Tariq M. Rana describe the preparation and evaluation of interfering nanoparticles for the delivery of anti-miRNA or siRNA, using a lipid-functionalized poly-L-lysine dendrimer, in Chapter 17. They outline methods for the measurement of gene silencing, including Northern blot, PCR, and reporter gene assays. In Chapter 18, Nejat Düzgüneş and Conchita Tros de Ilarduya describe the preparation and evaluation of plain and targeted lipoplexes, using targeting ligands, including epidermal growth factor and transferrin. Ligand-associated lipoplexes may be used to target DNA or other nucleic acid drugs to specific cells, particularly cancer cells that overexpress the receptors. They also outline two animal models in which transferrin-lipoplexes have been used for antitumor therapy.

I would like to thank Zoe Kruze and Shaun Gamble of Elsevier London and Priya Kumaraguruparan of Elsevier Chennai for all their help in preparing the volume. I would also like to thank my family for their support during the editing of this volume.

I dedicate this volume to my dear children, Maxine and Avery.

NEJAT DÜZGÜNEŞ