Preface

"While early ideas about the impact of nanotechnology on healthcare focused on fanciful ideas involving small submarines and cancer-zapping robots, current advances have been enabled by advances in imaging, control over materials and an increased understanding of how biology works at the nanoscale" (Tim Harper, CEO Cientifica).

This book is dedicated to showcase the most recent advances that have been made in utilizing the enormous potential of nanotechnology for probing, imaging, and manipulating life on a cellular and subcellular level. All chapters were written by leading experts in their particular fields. Daniel Moyano and Vincent Rotello describe a novel "chemical nose" approach, i.e., nanoparticle-based sensor arrays for the differentiation of biomolecules through pattern recognition that utilizes functionalized gold nanoparticles as receptors and Green Fluorescent Protein as transducer. This new strategy allows the identification of cellular signatures in early stages of cancer without previous knowledge of specific receptors or ligands. Sunaina Surana and Yamuna Krishnan demonstrate the utility of an externally introduced, pH-triggered DNA nanomachine inside the multicellular eukaryote *Caenorhabditis elegans*. This nanomachine uses FRET to effectively map spatiotemporal pH changes associated with endocytosis in coelomocytes of wild type as well as mutant worms. Syed K. Sohaebuddin and Liping Tang describe a method which allows the assessment of lysosomal membrane integrity upon exposure to various nanoparticles. The electron microscopic visualization of 1–2 nm gold nanoparticles, which are used as nano markers, allows Valeriy Lukyanenko and Vadim Salnikov to determine the precise localization of a variety of nano-objects within a cell. The same author also describes a saponin-based method for membrane permeabilization allowing the delivery of particles up to 20 nm in size to the perinuclear and perimitochondrial space of cardiomyocytes.

Howard Gendelman's laboratory provides in two chapters protocols for the isolation of subcellular compartments containing sequestered nanoparticles. Indriati Pfeiffer and Michael Zäch describe the use of nanostructured SiO_2 surfaces prepared by the colloidal lithography technique to scrutinize the formation of suspended lipid bilayers from a solution of nano liposomes. These authors employ atomic force microscopy (AFM) and quartz crystal microbalance with dissipation monitoring (QCM-D) to characterize nanostructure fabrication and lipid bilayer assembly on the nanostructured surface. QCM-D is also being utilized by Rickard Frost and Sofia Svedham to monitor the interaction of nanoparticles with lipid membranes in real time. The authors demonstrate how the outcome of such analysis provides information on the adsorption process (importantly kinetics and adsorbed amounts) as well as on the integrity of both the nanoparticles and the lipid membrane upon interaction. A protocol for studying the interactions of nanoparticles with proteins is provided by Lennart Treuel and Marcelina Malissek. These authors describe a procedure to study the adsorption of proteins onto nanoparticle surfaces based on circular dichroism (CD) spectroscopy. Jerry Chang and Sandra Rosenthal describe the principles, methodologies, and experimental protocols for quantum dot-based single-molecule imaging.

Ben Zhong Tang and his colleagues describe the fabrication of fluorescent silica nanoparticles (FSNPs) containing aggregation-induced emission (AIE) luminogens. By employing surfactant-free sol–gel reaction the authors are able to generate FSNPs with uniform size and high surface charge and colloidal stability. Simon C.W. Richardson group applies single cell imaging technology for studying the intracellular trafficking of both biological and synthetic macromolecules and they demonstrate the possibility of temporally dissecting novel and default trafficking of both macromolecular "drugs" and macromolecular drug delivery systems. Irene Canton and Giuseppe Battaglia describe a polymersomes-mediated delivery of fluorescent probes for targeted and long-term imaging in live cell microscopy. Junghae Suh and colleagues explain in their chapter one of the most complicated aspects of real-time particle tracking, i.e., the mean square displacement (MSD) calculation, in a simple manner designed for the novice particle tracker. By providing comprehensive instructions needed to perform particle tracking experiments, their chapter will enable researchers to gain new insight into the intracellular dynamics of nanocarriers, potentially leading to the development of more effective and intelligent therapeutic delivery vectors. Mi-Sook and Song Her provide a direct method for quantifying cellular transduction of PTD in vitro and in vivo using bioluminescence imaging. Their methodology exploits noninvasive techniques to create an environment suitable for the real-time imaging of PTD transduction and appears therefore as a promising tool for studying the mechanism of PTD transduction and the in vivo application of new therapeutic candidates. Achim Göpferich group describes a procedure for monitoring the intracellular route of polyplexes based on the use of labeling PEI and pLL with a reduction-sensitive fluorescent dye. Katye M. Fichter and Tania Q. Vu describe the use of single nanoparticle quantum dot (QD) probes to quantitatively investigate the complex endocytic trafficking pathways that receptors undergo following ligand activation. The use of cell-penetrating peptides (CPPs) to facilitate the cellular internalization of quantum dots (QDs) is described by Yue-Wern Huang and colleagues. Their approach is based on simple noncovalent interactions between CPPs and QDs. Lo and Wang describe the use of peptide-based carriers for the intracellular delivery of biologically active proteins as well as methods for the qualitative and quantitative evaluation of their delivery efficiency. Jan van Hest's laboratory presents a novel strategy for the preparation of gold nanoparticles exhibiting a stimuli-responsive behavior, which is based on the use of a ligand consisting of only a single repeat of the elastin-based pentapeptide VPGVG. The authors provide protocols for the solid-phase peptide synthesis of thiol-terminated VPGVG ligand and for the preparation of gold nanoparticles covered with the pentapeptide through a ligand-exchange reaction. Jae Sam Lee and Ching-Hsuam Tung have developed an improved CPP-based cellular delivery vector, named lipo-oligoarginine peptide (LOAP), by conjugating an oligoarginine peptide with a fatty acid moiety. The prepared LOAPs were further stabilized by introducing different combinations of D-Arg residues into the peptide backbone, and were systematically evaluated for their membrane penetrating properties and metabolic stabilities in cells. Andrea Alessandrini and Paolo Facci describe the use of electrochemical scanning tunneling microscopy (ECSTM) and spectroscopy (ECSTS) for studying the electron transport through single redox molecules with the aim of understanding the transport mechanisms ruling the flow of electrons via a single molecule placed in a nanometer-sized gap between two electrodes, while elucidating the role of the redox density of states brought about by the molecule. Yamuna Krishnan's group has constructed an icosahedron from DNA using a modular self-assembly strategy. They describe a method to determine the functionality of DNA polyhedra as nanocapsules by encapsulating different cargo such as gold nanoparticles and functional biomolecules like FITC dextran from

solution within DNA icosahedra. The use of polymer-gold nanorods assemblies for the delivery of plasmid DNA into mammalian cells is described by Kaushal Rege's laboratory. Puiyan Lee and Kenneth K.Y. Wong describe a technique for the synthesis of a novel lipophilic nano carrier for the incorporation of hydrophobic and toxic potent cancer drugs, such as gold (III) porphyrin. Tamer Elbayoumi's laboratory provides protocols for preparing mitochondria-targeted nanoemulsions loaded with tocopherol and Cyclosporine A which are able to protect cardiac muscle mitochondria from doxorubicin-induced oxidative stress. Achim Weber and colleagues describe the production of uniform protein-binding biofunctional fluorescent spherical silica core-shell nanoparticles. The authors characterize their novel nanoparticle system including its surface functionalization via microelectrophoresis, dynamic light scattering (DLS) and a colorimetric detection of the amount of nanoparticle-attached protein via a bicinchoninic acid (BCA) assay. Such fluorescently spiked nanoparticle cores with biofunctional shells for molecular recognition reactions may be used as imaging tools or reporter systems. Neskovic and her colleagues describe the assessment of genotoxic properties of purified single wall carbon nanotubes (SWCNT), multiwall carbon nanotubes (MWCNT), and amide functionalized purified SWCNT using cultured human lymphocytes and human fibroblasts. Dusica Maysinger's group has developed a suitable fractionation method for field flow fractionation, an analytical technique that allows the separation of nano and microparticles over a wide size range. The authors present asymmetrical flow field-flow fractionation (AF4) conditions that have proven their reliability for the analysis of quantum dots and other nanoparticles in the 5–50 nm size range. Maxwell B. Zeigler and Daniel T. Chiu give detailed steps necessary to perform laser surgery upon single adherent mammalian cells, where individual organelles are extracted from the cells by optical tweezers and the cells are monitored post-surgery to check their viability. Yaron R. Silberberg and Andrew E. Pelling describe a method to quantify the intracellular mechanical response to an extracellular mechanical perturbation, specifically the displacement of mitochondria. A combined fluorescent-atomic force microscope (AFM) was used to simultaneously produce well-defined nanomechanical stimulation to a living cell while optically recording the real-time displacement of fluorescently labeled mitochondria.

We are extremely grateful to all authors for having spent parts of their valuable time to contribute to this book. It is our hopes that together we have succeeded in providing an essential source of know-how and at the same time a source of inspiration to all investigators who are as fascinated as we are about the potential of applying nanotechnology to all areas of biomedical sciences. Last but not least we would like to thank John Walker, the series editor of "Methods in Molecular Biology" for having invited us to assemble this book and above all for his unlimited guidance and help throughout the whole process.

Glendale, AZ, USA

Volkmar Weissig
Tamer Elbayoumi
Mark Olsen