Preface

Understanding Golgi apparatus organization and function continues to be the subject of intense research. Its unique structure and central role in both anterograde and retrograde membrane traffic, along with its conservation across most eukaryotes, place the Golgi apparatus at the center of many studies. Despite knowledge of its existence since its discovery by Camillo Golgi in 1897, the Golgi apparatus continues to be the subject of research that exploits the very latest in cell biological approaches. Collecting protocols in a single volume such as this not only serves multiple purposes in terms of dissemination of new techniques among researchers and sparking ideas but also emphasizes the key role that members of the Golgi community have had in developing these methods and refining the protocols in the first place. These range from *in vitro* reconstitution to whole animal experiments. In this volume, we have sought to bring together examples of state-of-the-art tools that are applied to study Golgi biology. The contributors are truly expert in their arena both in terms of their knowledge of Golgi biology and the techniques described. Many of these techniques are of course equally applicable to many other fields of biology, and this volume provides a snapshot of some of this diversity.

We have organized the volume into three core sections covering Model Systems and Functional Studies, Imaging Studies, and New Frontiers. This organization serves to provide some context for systems that have proven, and continue to prove, immensely valuable to the study of Golgi function across many years.

The section on Model Systems includes examples that span *in vitro* and *in vivo* contexts. *In vitro* reconstitution provides the opportunity to derive functional insight using controlled addition of purified components. The use of model organisms, notably yeast, nematodes, plants, and flies, provides important functional insight in a more physiological context. Bridging these studies are the *in vitro* cell culture models. Here, more advanced modes such as polarized systems and 3D cell cultures are proving of great benefit in providing a link to tissue and organism biology. As it is the case in most cell biology fields, cellular genetics, through the use of RNA interference, is now a common approach to improve our understanding of Golgi organization and function. Many of the specialized functions of the Golgi are also given space in this section—dedicated methods provide clear insight into processes including retrograde traffic through the Golgi (perhaps the true interface of the secretory and endocytic pathways) and in the complex yet tightly controlled glycosylation events. The addition of complex polysaccharides to newly synthesized proteins is of great importance to normal human health and development, and this is evidenced by the complex array of congenital disorders of glycosylation that have been mapped to mutations in genes controlling Golgi function.

The past 20 years have seen an explosion in the application of bioimaging to the study of many processes in cell biology, perhaps none more so than membrane trafficking to and from the Golgi. The juxtanuclear location of the Golgi in many commonly used cell lines provides a clear end point for imaging experiments in the early

secretory pathway or a starting point for post-Golgi trafficking. What are now relatively trivial live-cell imaging experiments based on GFP tagging are augmented by complex protocols enabling controlled trafficking of select components, applications of photobleaching to study and quantify protein dynamics, and more recently the broader adoption of higher resolution light and electron microscopy technologies including superresolution light microscopy and electron tomography. All of these techniques are included in the Imaging Studies section. These technological developments also now enable wider application of high-content screening methods to define the cohort of genes involved in specialist aspects of Golgi function and processes that involve it.

Recent years have seen the introduction of new tools and approaches to this field; these are described in our New Frontiers section. The ordered structure yet complex function of the Golgi provides opportunities for the development of mathematical models to better understand and in future predict key aspects of Golgi function. Nanosurgery methods have been applied to try to define the origin of the Golgi in the absence of a preexisting structure; micropatterning provides opportunities to encourage cells to adopt predefined and more uniform shape, imposing particular intracellular organization. This will likely benefit analysis of those processes for which Golgi orientation and intracellular location are of key importance. We also cannot, of course, understate the importance of the Golgi as an integrator of cellular signals and processes. The core examples we have chosen here develop newly emerging themes of Golgi-dependent signaling pathways and the role of protein phosphorylation in more general Golgi function. This, of course, also links directly to the unique role of the Golgi in mitosis where the ordered disassembly of this single copy organelle is a prerequisite for mitotic entry. The structure, organization, and function of the Golgi are all inherently connected to the cytoskeleton. We focus here on the role of microtubules, yet many of the core concepts can equally be applied to other cytoskeletal networks.

This collection of protocols showcases the intense research activity that is focused on the Golgi itself as well as the importance of this organelle in diverse processes in cell biology. We are very grateful to the contributors for providing these carefully detailed methods, most of which contain the very lab-specific information that is almost impossible to glean without normally visiting the lab concerned. We also would like to acknowledge the help of people at Elsevier, and in particular Sarah Lay and Zoe Kruze.

David Stephens and Franck Perez