
Preface

By the end of the 1980s only two microtubule-dependent motors, the plus end-directed kinesin and the minus end-directed cytoplasmic dynein, had been identified. At the time, these two motors seemed almost sufficient to explain directional motility events on polar microtubule tracks in the cell. Nonetheless, shortly after, the tip of the iceberg began to emerge with the identification of proteins containing in their sequences a domain found in kinesin. This domain, called the “motor domain,” conferred on these proteins the essential property of moving on microtubules, using the energy derived from ATP hydrolysis. Since then, the identification of new proteins belonging to the kinesin superfamily of microtubule-dependent motors has gone at such a pace that nowadays more than 200 entries with motor domain sequences are deposited in the database. Kinesin family members are found in all eukaryotic organisms tested. They present a wide range of domain organizations with a motor domain located at different positions in the molecule. Their motility properties are also variable in directionality, velocity, and such other characteristics as bundling activity and processivity. Finally, and most important, they participate in a multitude of cellular functions. Our understanding of many cellular events, such as mitotic spindle assembly and neuronal transport, to cite only two, has progressed substantially in the last few years thanks to the identification of these motors. Kinesin-like proteins (KLP) appear now to be involved in so many different cellular events that it is no longer a surprise to see researchers finding a KLP involved in their favorite cellular process. The general interest in this field is thus growing and many researchers are now faced with the task of identifying and/or characterizing a KLP to understand the process they are studying.

The aim of *Kinesin Protocols* is to provide a set of clear and concise protocols for the identification and characterization of members of the kinesin superfamily of microtubule-dependent motors. These protocols should prove to be especially useful for nonspecialists in the field, but others are actually up-to-date protocols based on recent findings that may turn out to be of interest for specialists as well. As in other books in the *Methods in Molecular Biology* series, a special emphasis has been put on the “Notes” section. There, the reader will find all the little tricks—gathered through experience by the authors—that make the difference between a successful and an unsuccessful experiment.

The protocols presented in *Kinesin Protocols* cover many different aspects of kinesin identification and characterization. The volume opens with a few chapters dealing with various approaches to the identification and purification of kinesins from different sources (Chaps. 1–6). These include methods to express and purify kinesins in different systems. Purified proteins or fragments are used to determine some of the properties intrinsic to members of this family. Methods presented in the following chapters are aimed at characterizing microtubule-enhanced ATPase activity (Chap. 7) and motility properties at different levels (Chaps. 8 and 9). Although most KLPs indeed have the property to move directionally on microtubules, new types of activities have recently been found for some members of the class. Several ways to test their microtubule destabilizing activity are presented in Chap. 10. Some examples of how to address functional studies are presented in the following few chapters (Chaps. 11–17). Although the function of KLPs is generally thought to be the transport of cargoes, it has become clear that they also play an important role in the organization of microtubules in three dimensions. Chapter 18 describes some very new methods to address this aspect of KLP function. Finally, two chapters present technically more demanding protocols for the study of kinesins at the structural level. One chapter describes methods that have been successful in crystallizing kinesin motor domains (Chap. 19) and the other chapter presents protocols to study the structure of the kinesin–microtubule complex (Chap. 20).

Obviously, every reader will select and read first the chapter(s) of interest in *Kinesin Protocols*. Nonetheless, the content of the entire volume is recommended to everyone working in the field as a means to gain a better understanding of how best to handle kinesins experimentally. Some redundancy or overlap among chapters has proved unavoidable because certain basic experimental principles may be used for different purposes. We are confident that our book will both help the reader solve current research problems and stimulate the design of new experimental approaches adapted to the kinesin of interest in their work.

Isabelle Vernos



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Vernos, I. (Ed.)

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