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A LABORATORY MANUAL

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# CALCIUM TECHNIQUES

A LABORATORY MANUAL

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*Front cover artwork:* Representative  $\text{Ca}^{2+}$  signals generated in zebrafish embryos during (*top*) cytokinesis of the third cell division cycle; (*center*) in the slow muscles, which are developing in the trunk; and (*bottom*) in the developing head (see Chapter 10). Images courtesy of Sarah E. Webb and Andrew L. Miller, The Hong Kong University of Science and Technology.

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# Contents

Preface xv

## SECTION 1. FLUORESCENCE

### CHAPTER 1

---

#### INTRODUCTION

Fluorescence Microscopy 1  
*Michael J. Sanderson, Ian Smith, Ian Parker, and Martin D. Bootman*

### CHAPTER 2

---

#### INTRODUCTION

Ca<sup>2+</sup>-Sensitive Fluorescent Dyes and Intracellular Ca<sup>2+</sup> Imaging 25  
*Martin D. Bootman, Katja Rietdorf, Tony Collins, Simon Walker, and Michael Sanderson*

#### PROTOCOLS

- 1 Loading Fluorescent Ca<sup>2+</sup> Indicators into Living Cells 42  
*Martin D. Bootman, Katja Rietdorf, Tony Collins, Simon Walker, and Michael Sanderson*
- 2 Converting Fluorescence Data into Ca<sup>2+</sup> Concentration 46  
*Martin D. Bootman, Katja Rietdorf, Tony Collins, Simon Walker, and Michael Sanderson*

### CHAPTER 3

---

#### INTRODUCTION

Properties and Use of Genetically Encoded FRET Sensors for Cytosolic and Organellar Ca<sup>2+</sup> Measurements 51  
*J. Genevieve Park and Amy E. Palmer*

#### PROTOCOLS

- 1 Verifying the Function and Localization of Genetically Encoded Ca<sup>2+</sup> Sensors and Converting FRET Ratios to Ca<sup>2+</sup> Concentrations 55  
*J. Genevieve Park and Amy E. Palmer*
- 2 Measuring the In Situ  $K_d$  of a Genetically Encoded Ca<sup>2+</sup> Sensor 65  
*J. Genevieve Park and Amy E. Palmer*

v

## CHAPTER 4

---

### INTRODUCTION

- Photolysis of Caged Compounds: Studying  $\text{Ca}^{2+}$  Signaling and Activation of  $\text{Ca}^{2+}$ -Dependent Ion Channels 75  
*Janos Almassy and David I. Yule*

### PROTOCOLS

- 1 Studying the Activation of Epithelial Ion Channels Using Global Whole-Field Photolysis 79  
*Janos Almassy and David I. Yule*
- 2 Investigating Ion Channel Distribution Using a Combination of Spatially Limited Photolysis,  $\text{Ca}^{2+}$  Imaging, and Patch-Clamp Recording 85  
*Janos Almassy and David I. Yule*
- 3 Analyzing  $\text{Ca}^{2+}$  Dynamics in Intact Epithelial Cells Using Spatially Limited Flash Photolysis 90  
*Janos Almassy and David I. Yule*

## CHAPTER 5

---

### INTRODUCTION

- Electroporation Loading and Flash Photolysis to Investigate Intra- and Intercellular  $\text{Ca}^{2+}$  Signaling 93  
*Elke Decrock, Marijke De Bock, Nan Wang, Mélissa Bol, Ashish K. Gadicherla, and Luc Leybaert*

### PROTOCOLS

- 1 Electroporation Loading of Membrane-Impermeable Molecules to Investigate Intra- and Intercellular  $\text{Ca}^{2+}$  Signaling 104  
*Elke Decrock, Marijke De Bock, Nan Wang, Mélissa Bol, Ashish K. Gadicherla, and Luc Leybaert*
- 2 Flash Photolysis of Caged  $\text{IP}_3$  to Trigger Intercellular  $\text{Ca}^{2+}$  Waves 109  
*Elke Decrock, Marijke De Bock, Nan Wang, Mélissa Bol, Ashish K. Gadicherla, and Luc Leybaert*

## CHAPTER 6

---

### INTRODUCTION

- Investigating Calcium Signaling by Confocal and Multiphoton Microscopy 113  
*Lars Kaestner and Peter Lipp*

### PROTOCOLS

- 1 Two-Photon Photolysis Combined with a Kilobeam Array Scanner to Probe Calcium Signaling in Cardiomyocytes 116  
*Benjamin Sauer, Martin Oberhofer, Peter Lipp, and Lars Kaestner*
- 2 Multibeam Two-Photon Imaging of Fast  $\text{Ca}^{2+}$  Signals in the Langendorff Mouse Heart 121  
*Karin Hammer, Peter Lipp, and Lars Kaestner*

3	Multichannel Imaging of Cellular Signaling: Interplay of Ca <sup>2+</sup> and Conventional Protein Kinase C	126
	<i>Peter Lipp, Xin Hui, Gregor Reither, and Lars Kaestner</i>	
4	Two-Dimensional Imaging of Fast Intracellular Ca <sup>2+</sup> Release	130
	<i>Qinghai Tian, Lars Kaestner, and Peter Lipp</i>	
5	Confocal FLIM of Genetically Encoded FRET Sensors for Quantitative Ca <sup>2+</sup> Imaging	134
	<i>Benjamin Sauer, Qinghai Tian, Peter Lipp, and Lars Kaestner</i>	

## CHAPTER 7

---

### INTRODUCTION

	Combining Calcium Imaging with Other Optical Techniques	139
	<i>Marco Canepari, Dejan Zecevic, Kaspar E. Vogt, David Ogden, and Michel De Waard</i>	

### PROTOCOLS

1	Combining Ca <sup>2+</sup> and Membrane Potential Imaging in Single Neurons	146
	<i>Marco Canepari, Kaspar E. Vogt, Michel De Waard, and Dejan Zecevic</i>	
2	Combining Ca <sup>2+</sup> Imaging with L-Glutamate Photorelease	150
	<i>Marco Canepari, Michel De Waard, and David Ogden</i>	

## CHAPTER 8

---

### INTRODUCTION

	High-Throughput Analyses of IP <sub>3</sub> Receptor Behavior	155
	<i>Colin W. Taylor, Stephen C. Tovey, and Ana M. Rossi</i>	

### PROTOCOLS

1	High-Throughput Functional Assays of IP <sub>3</sub> -Evoked Ca <sup>2+</sup> Release	159
	<i>Stephen C. Tovey and Colin W. Taylor</i>	
2	High-Throughput Fluorescence Polarization Assay of Ligand Binding to IP <sub>3</sub> Receptors	167
	<i>Ana M. Rossi and Colin W. Taylor</i>	

## SECTION 2. LUMINESCENCE

## CHAPTER 9

---

### INTRODUCTION

	The Use of Aequorin and Its Variants for Ca <sup>2+</sup> Measurements	177
	<i>Veronica Granatiero, Maria Patron, Anna Tosatto, Giulia Merli, and Rosario Rizzuto</i>	

### PROTOCOL

1	Using Targeted Variants of Aequorin to Measure Ca <sup>2+</sup> Levels in Intracellular Organelles	185
	<i>Veronica Granatiero, Maria Patron, Anna Tosatto, Giulia Merli, and Rosario Rizzuto</i>	

## CHAPTER 10

---

### INTRODUCTION

- Introduction of Aequorin into Zebrafish Embryos for Recording  $\text{Ca}^{2+}$  Signaling during the First 48 h of Development 193  
*Sarah E. Webb, Ching Man Chan, and Andrew L. Miller*

### PROTOCOLS

- 1 Microinjecting Holo-Aequorin into Dechorionated and Intact Zebrafish Embryos 197  
*Sarah E. Webb and Andrew L. Miller*
- 2 Reconstitution of Holo-Aequorin with Apoaequorin mRNA and Coelenterazine in Zebrafish Embryos 206  
*Ching Man Chan, Andrew L. Miller, and Sarah E. Webb*

## SECTION 3. RADIOACTIVE TECHNIQUES

## CHAPTER 11

---

### INTRODUCTION

- Measurement of Intracellular  $\text{Ca}^{2+}$  Release in Intact and Permeabilized Cells Using  $^{45}\text{Ca}^{2+}$  211  
*Ludwig Missiaen, Tomas Luyten, Geert Bultynck, Jan B. Parys, and Humbert De Smedt*

### PROTOCOLS

- 1 Measurement of Intracellular  $\text{Ca}^{2+}$  Release in Permeabilized Cells Using  $^{45}\text{Ca}^{2+}$  219  
*Tomas Luyten, Geert Bultynck, Jan B. Parys, Humbert De Smedt, and Ludwig Missiaen*
- 2 Measurement of Intracellular  $\text{Ca}^{2+}$  Release in Intact Cells Using  $^{45}\text{Ca}^{2+}$  225  
*Tomas Luyten, Geert Bultynck, Jan B. Parys, Humbert De Smedt, and Ludwig Missiaen*

## CHAPTER 12

---

### INTRODUCTION

- Measuring  $\text{Ca}^{2+}$  Pump Activity in Overexpression Systems and Cardiac Muscle Preparations 231  
*Tine Holemans, Ilse Vandecaetsbeek, Frank Wuytack, and Peter Vangheluwe*

### PROTOCOLS

- 1 High-Throughput Measurement of the  $\text{Ca}^{2+}$ -Dependent ATPase Activity in COS Microsomes 234  
*Ilse Vandecaetsbeek, Tine Holemans, Frank Wuytack, and Peter Vangheluwe*
- 2 Measuring  $\text{Ca}^{2+}$ -Dependent  $\text{Ca}^{2+}$ -Uptake Activity in the Mouse Heart 245  
*Tine Holemans, Ilse Vandecaetsbeek, Frank Wuytack, and Peter Vangheluwe*



## SECTION 4. ELECTROPHYSIOLOGY

### CHAPTER 13

---

#### INTRODUCTION

- Patch-Clamp Recording of Voltage-Sensitive  $\text{Ca}^{2+}$  Channels 257  
*María A. Gandini, Alejandro Sandoval, and Ricardo Felix*

#### PROTOCOLS

- 1 Whole-Cell Patch-Clamp Recordings of  $\text{Ca}^{2+}$  Currents from Isolated Neonatal Mouse Dorsal Root Ganglion (DRG) Neurons 264  
*María A. Gandini, Alejandro Sandoval, and Ricardo Felix*
- 2 Whole-Cell Patch-Clamp Recording of Recombinant Voltage-Sensitive  $\text{Ca}^{2+}$  Channels Heterologously Expressed in HEK-293 Cells 271  
*María A. Gandini, Alejandro Sandoval, and Ricardo Felix*

### CHAPTER 14

---

#### INTRODUCTION

- Patch-Clamp Measurement of  $I_{\text{CRAC}}$  and ORAI Channel Activity 277  
*Dalia Alansary, Tatiana Kilch, Christian Holzmann, Christine Peinelt, Markus Hoth, and Annette Lis*

#### PROTOCOLS

- 1 Measuring Endogenous  $I_{\text{CRAC}}$  and ORAI Currents with the Patch-Clamp Technique 283  
*Dalia Alansary, Tatiana Kilch, Christian Holzmann, Christine Peinelt, Markus Hoth, and Annette Lis*
- 2 The Minimal Requirements to Use Calcium Imaging to Analyze  $I_{\text{CRAC}}$  291  
*Dalia Alansary, Tatiana Kilch, Christian Holzmann, Christine Peinelt, Markus Hoth, and Annette Lis*

### CHAPTER 15

---

#### INTRODUCTION

- Patch-Clamp Electrophysiology of Intracellular  $\text{Ca}^{2+}$  Channels 297  
*Don-On Daniel Mak, Horia Vais, King-Ho Cheung, and J. Kevin Foskett*

#### PROTOCOLS

- 1 Isolating Nuclei from Cultured Cells for Patch-Clamp Electrophysiology of Intracellular  $\text{Ca}^{2+}$  Channels 308  
*Don-On Daniel Mak, Horia Vais, King-Ho Cheung, and J. Kevin Foskett*
- 2 Nuclear Patch-Clamp Electrophysiology of  $\text{Ca}^{2+}$  Channels 313  
*Don-On Daniel Mak, Horia Vais, King-Ho Cheung, and J. Kevin Foskett*

## CHAPTER 16

---

### INTRODUCTION

- Bilayer Measurement of Endoplasmic Reticulum  $\text{Ca}^{2+}$  Channels 321  
*Ilya Bezprozvanny*

### PROTOCOLS

- 1 Preparation of Microsomes to Study  $\text{Ca}^{2+}$  Channels 326  
*Ilya Bezprozvanny*
- 2 Reconstitution of Endoplasmic Reticulum  $\text{InsP}_3$  Receptors into Black Lipid Membranes 330  
*Ilya Bezprozvanny*

## CHAPTER 17

---

### INTRODUCTION

- Measurement of Mitochondrial  $\text{Ca}^{2+}$  Transport Mediated by Three Transport Proteins: VDAC1, the  $\text{Na}^+/\text{Ca}^{2+}$  Exchanger, and the  $\text{Ca}^{2+}$  Uniporter 337  
*Danya Ben-Hail, Raz Palty, and Varda Shoshan-Barmatz*

### PROTOCOLS

- 1 Purification of VDAC1 from Rat Liver Mitochondria 343  
*Danya Ben-Hail and Varda Shoshan-Barmatz*
- 2 Reconstitution of Purified VDAC1 into a Lipid Bilayer and Recording of Channel Conductance 349  
*Danya Ben-Hail and Varda Shoshan-Barmatz*
- 3 Assay of  $\text{Ca}^{2+}$  Transport by VDAC1 Reconstituted into Liposomes 355  
*Danya Ben-Hail and Varda Shoshan-Barmatz*
- 4 Assays of Mitochondrial  $\text{Ca}^{2+}$  Transport and  $\text{Ca}^{2+}$  Efflux via the MPTP 359  
*Danya Ben-Hail and Varda Shoshan-Barmatz*
- 5 Mitochondrial  $\text{Na}^+/\text{Ca}^{2+}$  Exchange Assays 362  
*Raz Palty and Varda Shoshan-Barmatz*

## CHAPTER 18

---

### INTRODUCTION

- Calcium-Sensitive Mini- and Microelectrodes 367  
*Roger C. Thomas and Donald M. Bers*

### PROTOCOLS

- 1 How to Make Calcium-Sensitive Minielectrodes 372  
*Roger C. Thomas and Donald M. Bers*
- 2 How to Make Calcium-Sensitive Microelectrodes 376  
*Roger C. Thomas and Donald M. Bers*

## SECTION 5. SPECIAL TISSUES

### CHAPTER 19

---

#### INTRODUCTION

- The *Xenopus* Oocyte: A Single-Cell Model for Studying Ca<sup>2+</sup> Signaling 381  
*Yaping Lin-Moshier and Jonathan S. Marchant*

#### PROTOCOLS

- 1 Nuclear Microinjection to Assess How Heterologously Expressed Proteins Impact Ca<sup>2+</sup> Signals in *Xenopus* Oocytes 388  
*Yaping Lin-Moshier and Jonathan S. Marchant*
- 2 A Rapid Western Blotting Protocol for the *Xenopus* Oocyte 395  
*Yaping Lin-Moshier and Jonathan S. Marchant*

### CHAPTER 20

---

#### PROTOCOL

- Imaging and Manipulating Calcium Transients in Developing *Xenopus* Spinal Neurons 399  
*Nicholas C. Spitzer, Laura N. Borodinsky, and Cory M. Root*

### CHAPTER 21

---

#### INTRODUCTION

- A Systematic Approach for Assessing Ca<sup>2+</sup> Handling in Cardiac Myocytes 411  
*Karin R. Sipido, Niall Macquaide, and Virginie Bito*

#### PROTOCOLS

- 1 Basic Methods for Monitoring Intracellular Ca<sup>2+</sup> in Cardiac Myocytes Using Fluo-3 414  
*Virginie Bito, Karin R. Sipido, and Niall Macquaide*
- 2 Characterizing the Trigger for Sarcoplasmic Reticulum Ca<sup>2+</sup> Release in Cardiac Myocytes 420  
*Virginie Bito, Niall Macquaide, and Karin R. Sipido*
- 3 Measuring Sarcoplasmic Reticulum Ca<sup>2+</sup> Content, Fractional Release, and Ca<sup>+</sup> Buffering in Cardiac Myocytes 425  
*Niall Macquaide, Virginie Bito, and Karin R. Sipido*
- 4 Measuring Ca<sup>2+</sup> Sparks in Cardiac Myocytes 430  
*Niall Macquaide, Virginie Bito, and Karin R. Sipido*
- 5 Assessing Ca<sup>2+</sup>-Removal Pathways in Cardiac Myocytes 438  
*Virginie Bito, Karin R. Sipido, and Niall Macquaide*

## CHAPTER 22

---

### INTRODUCTION

- Monitoring  $\text{Ca}^{2+}$  Signaling in Yeast 445  
*Renata Tisi, Enzo Martegani, and Rogelio L. Brandão*

### PROTOCOLS

- 1 Monitoring Yeast Intracellular  $\text{Ca}^{2+}$  Levels Using an In Vivo Bioluminescence Assay 456  
*Renata Tisi, Enzo Martegani, and Rogelio L. Brandão*
- 2 Total Cellular  $\text{Ca}^{2+}$  Measurements in Yeast Using Flame Photometry 460  
*Renata Tisi, Enzo Martegani, and Rogelio L. Brandão*
- 3 Measurement of Calcium Uptake in Yeast Using  $^{45}\text{Ca}$  463  
*Renata Tisi, Enzo Martegani, and Rogelio L. Brandão*

## CHAPTER 23

---

### INTRODUCTION

- $\text{Ca}^{2+}$  Imaging in Plants Using Genetically Encoded Yellow Cameleon  $\text{Ca}^{2+}$  Indicators 465  
*Smrutisanjita Behera, Melanie Krebs, Giovanna Loro, Karin Schumacher, Alex Costa, and Jörg Kudla*

### PROTOCOLS

- 1 Live Cell Imaging of Cytoplasmic  $\text{Ca}^{2+}$  Dynamics in *Arabidopsis* Guard Cells 469  
*Smrutisanjita Behera and Jörg Kudla*
- 2 High-Resolution Imaging of Cytoplasmic  $\text{Ca}^{2+}$  Dynamics in *Arabidopsis* Roots 474  
*Smrutisanjita Behera and Jörg Kudla*
- 3 Live Cell Imaging of Cytoplasmic and Nuclear  $\text{Ca}^{2+}$  Dynamics in *Arabidopsis* Roots 479  
*Melanie Krebs and Karin Schumacher*
- 4 Imaging of Mitochondrial and Nuclear  $\text{Ca}^{2+}$  Dynamics in *Arabidopsis* Roots 484  
*Giovanna Loro and Alex Costa*

## SECTION 6. NAD(P)-DERIVED MESSENGERS

## CHAPTER 24

---

### INTRODUCTION

- Cyclic ADP-Ribose: Endogenous Content, Enzymology, and  $\text{Ca}^{2+}$  Release 489  
*Andreas H. Guse, Tanja Kirchberger, and Santina Bruzzone*

## PROTOCOLS

- 1 Cycling Assay for Determining Intracellular Cyclic ADP-Ribose Levels 493  
*Santina Bruzzone and Andreas H. Guse*
- 2 Measuring CD38 (ADP-Ribosyl Cyclase/Cyclic ADP-Ribose Hydrolase) Activity by Reverse-Phase HPLC 498  
*Tanja Kirchberger and Andreas H. Guse*
- 3 Measuring Ca<sup>2+</sup> Release Evoked by Cyclic ADP-Ribose 503  
*Andreas H. Guse*

## CHAPTER 25

---

### INTRODUCTION

- Methods in Nicotinic Acid Adenine Dinucleotide Phosphate Research 509  
*Antony Galione, Kai-Ting Chuang, Tim M. Funnell, Lianne C. Davis, Anthony J. Morgan, Margarida Ruas, John Parrington, and Grant C. Churchill*

### PROTOCOLS

- 1 Preparation and Use of Sea Urchin Egg Homogenates for Studying NAADP-Mediated Ca<sup>2+</sup> Release 512  
*Antony Galione, Kai-Ting Chuang, Tim M. Funnell, Lianne C. Davis, Anthony J. Morgan, Margarida Ruas, John Parrington, and Grant C. Churchill*
- 2 Synthesis of NAADP-AM as a Membrane-Permeant NAADP Analog 517  
*Antony Galione, Kai-Ting Chuang, Tim M. Funnell, Lianne C. Davis, Anthony J. Morgan, Margarida Ruas, John Parrington, and Grant C. Churchill*
- 3 Measurement of Luminal pH of Acidic Stores as a Readout for NAADP Action 521  
*Antony Galione, Kai-Ting Chuang, Tim M. Funnell, Lianne C. Davis, Anthony J. Morgan, Margarida Ruas, John Parrington, and Grant C. Churchill*
- 4 Synthesis of Caged NAADP 525  
*Antony Galione, Kai-Ting Chuang, Tim M. Funnell, Lianne C. Davis, Anthony J. Morgan, Margarida Ruas, John Parrington, and Grant C. Churchill*
- 5 Synthesis of [<sup>32</sup>P]NAADP for Radioreceptor Binding Assay 529  
*Antony Galione, Kai-Ting Chuang, Tim M. Funnell, Lianne C. Davis, Anthony J. Morgan, Margarida Ruas, John Parrington, and Grant C. Churchill*

## SECTION 7. MEASURING AND MODELING CA<sup>2+</sup> DYNAMICS

## CHAPTER 26

---

### INTRODUCTION

- Measuring Ca<sup>2+</sup>-Binding Kinetics of Proteins 533  
*Guido C. Faas and Istvan Mody*

### PROTOCOLS

- 1 Measuring the Steady-State Properties of Ca<sup>2+</sup> Indicators with a Set of Calibrated [Ca<sup>2+</sup>] Solutions 536  
*Guido C. Faas and Istvan Mody*

2	Measuring the Rate Constants of Ca <sup>2+</sup> Indicators <i>Guido C. Faas and Istvan Mody</i>	541
3	Collecting Data to Determine the Ca <sup>2+</sup> -Binding Properties of DM-Nitrophen and Proteins <i>Guido C. Faas and Istvan Mody</i>	546

---

**CHAPTER 27**

---

**INTRODUCTION**

	Translating Intracellular Calcium Signaling into Models <i>Rüdiger Thul</i>	553
--	--	-----

**PROTOCOLS**

1	Exploring Oscillations in a Point Model of the Intracellular Ca <sup>2+</sup> Concentration <i>Rüdiger Thul</i>	562
2	Time to Blip—Stochastic Simulation of Single Channel Opening <i>Rüdiger Thul</i>	566

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**APPENDIX 1**

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	General Safety and Hazardous Material Information	571
	Index	577

**General Safety and Hazardous Material Information**

This manual should be used by laboratory personnel with experience in laboratory and chemical safety or students under the supervision of such trained personnel. The procedures, chemicals, and equipment referenced in this manual are hazardous and can cause serious injury unless performed, handled, and used with care and in a manner consistent with safe laboratory practices. Students and researchers using the procedures in this manual do so at their own risk. It is essential for your safety that you consult the appropriate Material Safety Data Sheets, the manufacturers' manuals accompanying equipment, and your institution's Environmental Health and Safety Office, as well as the General Safety and Disposal Cautions in this appendix for proper handling of hazardous materials in this manual. Cold Spring Harbor Laboratory makes no representations or warranties with respect to the material set forth in this manual and has no liability in connection with the use of these materials.

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Appropriate sources for obtaining safety information and general guidelines for laboratory safety are provided in General Safety and Hazardous Material Information (Appendix 1 of this manual).

## Preface

No other intracellular messenger rivals  $\text{Ca}^{2+}$  for complexity. It has long been established that cells use a variety of sources, buffers, and transporters to generate  $\text{Ca}^{2+}$  signals that span considerable temporal and spatial ranges. Some of the fastest  $\text{Ca}^{2+}$  signals occur within neurons, and underlie synaptic communication. These microsecond events provide nanoscopic  $\text{Ca}^{2+}$  transients that trigger the release of neurotransmitter-containing vesicles. At the other end of the temporal and spatial scale are signals such as the  $\text{Ca}^{2+}$  waves that persist for many tens of seconds and propagate within, and between, cells of various tissues. The rhythmic peristaltic contraction of gut and airway smooth muscle is driven by such repetitive, propagating  $\text{Ca}^{2+}$  waves. Given that cellular  $\text{Ca}^{2+}$  signals are hugely diverse, it is not surprising that cells express a wide range of effector molecules to interpret  $\text{Ca}^{2+}$  changes. Typically, these effector molecules bind  $\text{Ca}^{2+}$  ions directly, or via an intermediate/adaptor protein, and then allosterically regulate the activity of other proteins. The ability of an effector molecule to sense and respond to  $\text{Ca}^{2+}$  depends on factors such as its affinity and location. By mixing different  $\text{Ca}^{2+}$  sources with specific effectors, cells are able to utilize  $\text{Ca}^{2+}$  in a manner that uniquely suits their physiological functions.

The rich diversity of intra- and intercellular  $\text{Ca}^{2+}$  signaling mechanisms requires that a range of technical approaches are required to understand how cells generate and interpret particular  $\text{Ca}^{2+}$  transients. Consequently, over the past decades, researchers within the  $\text{Ca}^{2+}$  signaling field have been hugely inventive in driving perpetual methodological and technical advances to develop ever more sensitive and sophisticated means of detecting  $\text{Ca}^{2+}$  signals and their significance. This volume seeks to provide a resource for both novice and expert  $\text{Ca}^{2+}$  signaling researchers who are seeking to develop, or refine, the techniques they use. Several chapters within this volume are dedicated to  $\text{Ca}^{2+}$ -sensitive fluorescent reporters, and the different fluorescence imaging modalities (e.g., wide-field, confocal, FRET, two-photon) that are used in conjunction with these reporters. The reason for this emphasis is obvious: since the first introduction of Quin2 by Roger Tsien and colleagues in the early 1980s, the  $\text{Ca}^{2+}$  signaling field has been substantially propelled by findings obtained using fluorescence imaging. Indeed, fluorescent reporters enable visualization of  $\text{Ca}^{2+}$  signals in real time within subcellular domains, and the multiplexing of  $\text{Ca}^{2+}$  recordings with physiological readouts. However, whereas fluorescence is by far the most commonly used method for characterizing  $\text{Ca}^{2+}$  signals, it was predated by a number of techniques—electrophysiology of channels in situ or in planar lipid bilayers, radioactive  $^{45}\text{Ca}^{2+}$  flux assays, and the use of  $\text{Ca}^{2+}$ -sensitive photoproteins—that are still vital in answering specific questions. Correspondingly, these topics are presented in detailed chapters. The majority of techniques described in this volume were developed to monitor  $\text{Ca}^{2+}$  signaling in mammalian cells. Nevertheless, they can be equally applied to other eukaryotic cells, and in some cases, prokaryotic cells too. A number of chapters have been devoted to measuring  $\text{Ca}^{2+}$  in specific cell types—oocytes, zebrafish embryos, neurons, cardiac myocytes, plants, and yeast—that require particular technical approaches. Predominant plasma membrane and intracellular  $\text{Ca}^{2+}$  transport mechanisms are also described within this volume, as are techniques for characterizing the relatively new  $\text{Ca}^{2+}$ -release pathways involving nicotinic acid adenine dinucleotide phosphate (NAADP) and cyclic adenosine diphosphate ribose (cADPR), methods for modulating intra- and intercellular  $\text{Ca}^{2+}$  signals using photolytic uncaging or electroporation, techniques for assessing  $\text{Ca}^{2+}$ -binding kinetics, and insights into developing mathematical models of  $\text{Ca}^{2+}$  signaling from empirical data.

From the outset, our aim was to compile a resource that was historically reflective while being forward-looking, and technically detailed but also readable. We hope these goals have been achieved. For some, the experimental protocols should encompass complete solutions for implementing new techniques. For others, the protocols may provide suggestions or ideas that the reader can

incorporate and adapt to extend their technical abilities. In either case, we hope for yet more fascinating insights into  $\text{Ca}^{2+}$  signaling.

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