
Preface

From the relative obscurity of an allosteric activator of a bacterial cellulose synthase, dimeric ($3' \rightarrow 5'$) GMP (cyclic di-GMP or c-di-GMP) has emerged as one of the most common, important, and truly universal bacterial second messengers. Cyclic di-GMP plays key roles in lifestyle changes of many bacteria, including transition from the planktonic to the sessile lifestyle, which aids in the establishment of multicellular biofilm communities, and from the virulent state in acute infections to the less virulent but more resilient state characteristic of chronic infectious diseases. C-di-GMP has also been shown to regulate motility, the cell cycle, and differentiation and to participate in interkingdom signaling, with c-di-GMP being recognized by mammalian immune systems as a uniquely bacterial molecule. Modulating c-di-GMP signaling pathways is based on c-di-GMP levels, with the second messenger being enzymatically modulated by diguanylate cyclases (DCG), proteins containing a GGDEF domain, and phosphodiesterases (PDE) containing either an EAL or HD-GYP domain. Additionally, riboswitches regulate gene expression in response to cyclic di-GMP concentrations in many but not all bacteria.

This volume of the *Methods in Molecular Biology* series provides a collection of protocols for many of the common experimental approaches used in the burgeoning field of c-di-GMP-dependent signaling to synthesize, detect, quantitate, and modulate the levels of c-di-GMP present in cells. Additionally, procedures to detect and evaluate the interaction of c-di-GMP with proteins and bacterial response to varying c-di-GMP levels including virulence, swarming, and matrix production are included. Additionally, some less common but up-and-coming approaches focusing on the inhibition of c-di-GMP signaling are included.

This book is divided into eight major parts, reflecting the breath of techniques used in the field of c-di-GMP. The chapters are as follows: synthesis of c-di-GMP, detection and quantitation of c-di-GMP, visualizing c-di-GMP levels using biosensors, indirect detection of c-di-GMP levels, modulation of c-di-GMP levels and bacterial responses, measuring c-di-GMP modulating activities, c-di-GMP binding proteins, and targeting c-di-GMP signaling. The methods chapters are preceded by a review on the discovery of the intracellular signaling molecule c-di-GMP. Presented methods are diverse and range from thin layer chromatography (TLC) and mass spectrometry to fluorescence-activated cell sorting (FACS), footprinting, pulldown assays, and isothermal titration calorimetry to methods aiming at inhibiting c-di-GMP-dependent signaling and virulence models.

All chapters are written in the same format as that used in the *Methods in Molecular Biology*TM series. Each chapter opens with a description of the basic theory behind the method being described. The Materials section lists all the chemicals, reagents, buffers, and other materials necessary for carrying out the protocol. Since the principal goal of the book is to provide experimentalists with a full account of the practical steps necessary for carrying out each protocol successfully, the Methods section contains detailed step-by-step descriptions of every protocol that should result in the successful completion of each method. The Notes section complements the Methods section by indicating how best to deal with any problem or difficulty that might arise when using a given technique. Considering the contribution of c-di-GMP to biofilm formation, with the human pathogen *Pseudomonas aeruginosa* being a paradigm organism for the study of biofilm communities, the book is most detailed for *P. aeruginosa* but includes also protocols for other model

species such as *Escherichia coli*, *Salmonella enterica* serovar Typhimurium, *Xanthomonas campestris*, and *Myxococcus xanthus*.

Together, I hope that this volume will be an essential part of many laboratory libraries. However, I hope that this book is more often on the bench top than in the book shelf and inspire researchers to step out of their comfort zone and try their hands on new approaches.

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