Lab Protein Synthesis Transcription And Translation

The Swartz lab has put much effort into understanding the underlying principles of E. coli-based cell-free protein synthesis (CFPS), and the technology has developed into a scalable, affordable platform for producing a wide range of protein targets. Key breakthroughs have included activating central metabolism, stabilization of critical amino acids, controlling the redox environment to produce proteins containing disulfide bonds, and using scale-up technologies to produce proteins at milligram quantities. My work has sought to expand this CFPS technology for producing valuable and complex eukaryotic protein targets by manipulating and optimizing the folding of these proteins in the heterologous CFPS environment. Furthermore, I have sought to apply these advances to specific applications of interest. By modifying a key molecular chaperone native to the eukaryotic endoplasmic reticulum (ER), the Hsp70-family chaperone, BiP, soluble production was increased in CFPS reactions for specific proteins normally secreted through this organelle, namely those from the immunoglobulin superfamily which includes antibodies, T-cell receptors, and many membrane receptors. First, the functional properties of BiP were compared to that of the E. coli Hsp70, DnaK. A fusion protein was then constructed between BiP and the ribosome-binding portion of the E. coli protein, trigger factor, to localize BiP to translating ribosomes. This replicated the native function of BiP, which provides co-translational folding assistance to nascent polypeptides. After verifying its bioactivity, this fusion protein was utilized in CFPS reactions to indicate that the chaperone functions of BiP are specific to proteins normally secreted through the eukaryotic ER, whereas DnaK demonstrates a more general chaperone mechanism. Since the discovery that somatic cells could be reprogrammed back to a pluripotent state through the viral expression of a specific set of transcription factors, there has been great interest in reprogramming using a safer and more clinically relevant protein-based approach. Production of these transcription factor proteins was greatly increased by fusing them to the C-terminus of the solubility partner, IF2 domain 1 (IF2D1). While the fusions provided marginal benefit in molar yields using a CFPS approach, in vivo E. coli expression produced the transcription factors in soluble form. The fusion proteins could be purified in milligram quantities from liter shake-flask cultures, whereas essentially no soluble protein accumulated without the fusion partner. The transcription factor fusions bound specifically to their consensus DNA sequences and partially activated some of their downstream gene targets. Another application utilizing CFPS technology is an enhanced luciferase mutant from the marine copepod, Gaussia princeps (GLuc). GLuc is both the smallest and brightest known luciferase, and previous work from our lab demonstrated that this protein could be produced at higher volumetric yields and specific activities in CFPS compared to conventional protein expression systems. By mutating key residues in the Gaussia luciferase sequence, the luminescence half-life was shown to increase over ten-fold while maintaining the initial specific activity of the wild-type. This improved mutant provides a valuable imaging agent to use in fusions and bioconjugates with other proteins such as those that recognize cell surface markers on cancer cells. In a final application, influenza vaccines were produced using CFPS by isolating specific fragments of the protein hemagglutinin (HA), a viral surface protein. Specific mutations as well as a C-terminal trimerization domain were critical for producing this protein fragment in both its monomeric and native trimeric forms. By using the CFPS platform to incorporate non-natural amino acids (nnAAs) with alkyne functional groups, the HA proteins were covalently 'clicked' to virus-like particles (VLPs) that had surface

"G protein-coupled receptors (GPCRs) represent a large family of membrane receptors involved in important physiological functions through regulation of myriad downstream signalling pathways. Many pathways work via heterotrimeric G proteins. In addition to the important role played by G[alpha] subunits, recent work has described non canonical roles for G[beta][gamma] subunits in downstream signalling of GPCRs in organelles such as the nucleus, Golgi apparatus and mitochondria. Our lab has used tandem-affinity purification (TAP) to identify cytosolic and nuclear G[beta][gamma]-interacting proteins. Nonetheless, TAP and other methods to study protein interactions require them to be stable or require long incubation periods, missing transient and/or weak interactions. Furthermore, techniques that provide adequate temporal information often focus on one pathway at a time, requiring a large array of assays. The use of APEX2, a proximity-dependent labeling technique, provides both spatial and temporal information in endogenous protein expression level, allowing for more in-depth characterization of protein networks over time. This thesis describes the validation of APEX2-fused constructs, optimization of samples for LC-MS and subsequent proteins identified from our screens. Comparisons between TAP and APEX2 screens demonstrate various DNA/RNA binding proteins in HEK 293 cells, supporting the growing body of evidence for G[beta][gamma] in regulating transcription and suggests a novel role in protein synthesis. Proteins identified from different organelles also demonstrate the expanding roles of G[beta][gamma], particularly in the nucleus. This work contributes to previously generated LC-MS data and provides a tool to study G[beta][gamma] signaling. Further experiments using fractionated samples will allow us to identify nuclear and cytoplasmic specific interactors, and stimulated conditions will enable us to observe proteome differences with receptor activation"--

"A Subject Collection from Cold Spring Harbor Perspectives in Biology."

Memory occupies a fundamental place in philosophy, playing a central role not only in the history of philosophy but also in philosophy of mind, epistemology, and ethics. Yet the philosophy of memory has only recently emerged as an area of study and research in its own right. The Routledge Handbook of Philosophy of Memory is an outstanding reference source on the key topics, problems, and debates in this exciting area, and is the first philosophical collection of its kind. The forty-eight chapters are written by an international team of contributors, and divided into nine parts: The nature of memory The metaphysics of memory Memory, mind, and meaning Memory and the self Memory and time The social dimension of memory The epistemology of memory Memory and morality History of philosophy of memory. Within these sections, central topics and problems are examined, including: truth, consciousness, imagination, emotion, self-knowledge, narrative, personal identity, time, collective and social memory, internalism and externalism, and the ethics of memory. The final part examines figures in the history of philosophy, including Aristotle, Augustine, Freud, Bergson, Wittgenstein, and Heidegger, as well as perspectives on memory in Indian and Chinese philosophy. Essential reading for students and researchers in philosophy, particularly philosophy of mind and psychology, the Handbook will also be of interest to those in related fields, such as psychology and anthropology.

Evolution since Coding

A Personal Account of the Discovery of the Structure of DNA

Molecular Biology of the Cell

Biological Regulation and Development

Cradles, Halos, Barrels, and Wings

Cell-free Protein Synthesis

A version of the OpenStax text

Diagnostic Molecular Biology describes the fundamentals of molecular biology in a clear, concise manner to aid in the comprehension of this complex subject. Each technique described in this book is explained within its conceptual framework to enhance understanding. The targeted approach covers the principles of molecular biology including the basic knowledge of nucleic acids, proteins, and genomes as well as the basic techniques and instrumentations that are often used in the field of molecular biology with detailed procedures and explanations. This book also covers the applications of the principles and techniques currently employed in the clinical laboratory. • Provides an understanding of which techniques are used in diagnosis at the molecular level • Explains the basic principles of molecular biology and their application in the clinical diagnosis of diseases • Places protocols in context with practical applications

The glomerulopathies are the diseases affecting the filtering units of the kidneys. In Glomerulopathies: Cell Biology and Immunology, the author details how recent advances in cell biology and immunology are able to advance our understanding such that we should be able to ameliorate or even arrest the course of these diseases. In addition to discussing the fundamental concepts, the book covers recent advances pertinent to the pharmacological and clinical management of glomerulonephritides and diabetic nephropathy. The book uses a blend of basic science and clinical and experimental findings in a way that is suitable both for trainees in medical science and professional nephrologists.

RNA and Protein Synthesis is a compendium of articles dealing with the assay, characterization, isolation, or purification of various organelles, enzymes, nucleic acids, translational factors, and other components or reactions involved in protein synthesis. One paper describes the preparatory scale methods for the reversed-phase chromatography systems for transfer ribonucleic acids. Another paper discusses the determination of adenosine- and aminoacyl adenosine-terminated sRNA chains by ion-exclusion chromatography. One paper notes that the problems involved in preparing acetylaminoacyl-tRNA are similar to those found in peptidyl-tRNA synthesis, in particular, to the lability of the ester bond between the amino acid and the tRNA. Another paper explains a new method that will attach fluorescent dyes to cytidine residues in tRNA; it also notes the possible use of N-hydroxysuccinimide esters of dansylglycine and N-methylanthranilic acid in the described method. One paper explains the use of membrane filtration in the determination of apparent association constants for ribosomal protein-RNS complex formation. This collection is valuable to bio-chemists, cellular biologists, micro-biologists, developmental biologists, and investigators working with enzymes.

Spatiotemporal Characterization of G Protein [beta] and [gamma] Subunit Interactors Via APEX2 Labeling

Campbell Biology

Lactic Acid Bacteria

RNA and Protein Synthesis

Biology for AP ® Courses

HSP60 Family of Chaperonins

This text offers a fresh, distinctive approach to the teaching of molecular biology that reflects the challenge of teaching a subject that is in many ways unrecognizable from the molecular biology of the 20th century - a discipline in which our understanding has advanced immeasurably, but about which many questions remain to be answered. With a focus on key principles, this text emphasizes the commonalities that exist between the three kingdoms of life, giving students an accurate depiction of our current understanding of the nature of molecular biology and the differences that underpin biological diversity.

Transcriptional regulation controls the basic processes of life. Its complex, dynamic, and hierarchical networks control the momentary availability of messenger RNAs for protein synthesis. Transcriptional regulation is key to cell division, development, tissue differen- ation, and cancer as discussed in Chapters 1 and 2. We have witnessed rapid, major developments at the intersection of computational biology, experimental technology, and statistics. A decade ago, researches were struggling with notoriously challenging predictions of isolated binding sites from low-throughput experiments. Now we can accurately predict cis-regulatory modules, conserved cl- ters of binding sites (Chapters 13 and 15), partly based on high-throughput ch- matin immunoprecipitation experiments in which tens of millions of DNA segments are sequenced by massively parallel, next-generation sequencers (ChIP-seq, Chapters 9, 10, and 11). These spectacular developments have allowed for the genome-wide mappings of tens of thousands of transcription factor binding sites in yeast, bacteria, mammals, insects, worms, and plants. Please also note the no less spectacular failures in many laboratories around the world.

Recombinant Protein Expression, Part A, Volume 659 in the Methods in Enzymology series, highlights new advances in the field with this new volume presenting interesting chapters on Multiplexed analysis protein: Protein interactions of polypeptides translated in Leishmania cell-free system, MultiBac system and its applications, performance and recent, Production of antibodies in Shuffle, Designing hybrid-promoter architectures by engineering cis-acting DNA sites to enhance transcription in yeast, Designing hybrid-promoter architectures by engineering cis-acting DNA sites to deregulate transcription in yeast, Antibody or protein-based vaccine production in plants, Cell-free protein synthesis, Plant-based expression of biologic drugs, and much more. Additional sections cover the Use of native mass spectrometry to guide detergent-based rescue of non-native oligomerization by recombinant proteins, Advancing overexpression and purification of recombinant proteins by pilot optimization through tandem affinity-buffer exchange chromatography online with native mass spectrometry, Method for High-Efficiency Fed-batch cultures of recombinant Escherichia coli, Method to transfer Chinese hamster ovary (CHO) shake flask experiments to the ambr® 250, and Expression of recombinant antibodies in Leishmania tarentolae. Provides the authority and expertise of leading contributors from an international board of authors Presents the latest release in the Methods in Enzymology serial Updated release includes the latest information on Recombinant Protein Expression

The structural biology of protein-nucleic acid interactions is in some ways a mature field and in others in its infancy. High-resolution structures of protein-DNA complexes have been studied since the mid 1980s and a vast array of such structures has now been determined, but surprising and novel structures still appear quite frequently. High-resolution structures of protein-RNA complexes were relatively rare until the last decade. Propelled by advances in technology as well as the realization of RNA's importance to biology, the number of example structures has ballooned in recent years. New insights are now being gained from comparative studies only recently made possible due to the size of the database, as well as from careful biochemical and biophysical studies. As a result of the explosion of research in this area, it is no longer possible to write a comprehensive review. Instead, current review articles tend to focus on particular subtopics of interest. This makes it difficult for newcomers to the field to attain a solid understanding of the basics. One goal of this book is therefore to provide in-depth discussions of the fundamental principles of protein-nucleic acid interactions as well as to illustrate those fundamentals with up-to-date and fascinating examples for those who already possess some familiarity with the field. The book also aims to bridge the gap between the DNA- and the RNA- views of nucleic acid - protein recognition, which are often treated as separate fields. However, this is a false dichotomy because protein - DNA and protein - RNA interactions share many general principles. This book therefore includes relevant examples from both sides, and frames discussions of the fundamentals in terms that are relevant to both. The monograph approaches the study of protein-nucleic acid interactions in two distinctive ways. First, DNA-protein and RNA-protein interactions are presented together. Second, the first half of the book develops the principles of protein-nucleic acid recognition, whereas the second half applies these to more specialized topics. Both halves are illustrated with important real life examples. The first half of the book develops fundamental principles necessary to understand function. An introductory chapter by the editors reviews the basics of nucleic acid structure. Jen-Jacobsen and Jacobsen discuss how solvent interactions play an important role in recognition, illustrated with extensive thermodynamic data on restriction enzymes. Marmorstein and Hong introduce the zoology of the DNA binding domains found in transcription factors, and describe the combinational recognition strategies used by many multiprotein eukaryotic complexes. Two chapters discuss indirect readout of DNA sequence in detail: Berman and Lawson explain the basic principles and illustrate them with in-depth studies of CAP, while in their chapter on DNA bending and compaction Johnson, Stella and Heiss highlight the intrinsic connections between DNA bending and indirect readout. Horvath lays out the fundamentals of protein recognition of single stranded DNA and single stranded RNA, and describes how they apply in a detailed analysis of telomere end binding proteins. Nucleic acids adopt more complex structures - Lilley describes the conformational properties of helical junctions, and how proteins recognize and cleave them. Because RNA readily folds due to the stabilizing role of its 2'-hydroxyl groups, Li discusses how proteins recognize different RNA folds, which include duplex RNA. With the fundamentals laid out, discussion turns to more specialized examples taken from important aspects of nucleic acid metabolism. Schroeder discusses how proteins chaperone RNA by rearranging its structure into a functional form. Berger and Dong discuss how topoisomerases alter the topology of DNA and relieve the superhelical tension introduced by other processes such as replication and transcription. Dyda and Hickman show how DNA transposes mediate genetic mobility and Van Duyne discusses how site-specific recombinases "cut" and "paste" DNA. Horton presents a comprehensive review of the structural families and chemical mechanisms of DNA nucleases, whereas Li in her discussion of RNA-protein recognition also covers RNA nucleases. Lastly, FerrÚ-D'AmarÚ shows how proteins recognize and modify RNA transcripts at specific sites. The book also emphasises the impact of structural biology on understanding how proteins interact with nucleic acids and it is intended for advanced students and established scientists wishing to broaden their horizons.

Glomerulopathies:Cell Biol/Imm

A Practical Approach

The Web of Life

Science Strategies to Increase Student Learning and Motivation in Biology and Life Science Grades 7 Through 12

Cell-Free Synthetic Biology

Biology

This manual introduces the reader to basic methods used in the isolation, cloning and analysis of genetic material. The protocols include RT-PCR amplification, gene cloning, hybridization analysis and sequencing of nucleic acids, PCR-based site-specific mutagenesis, analysis of protein DNA-specific interaction, cell-free protein synthesis and product electrophoretic and immunological analysis. Each protocol includes short background information, a detailed description of the necessary materials, step-by-step procedures, a troubleshooting guide and useful practical hints.

Life is produced by the interplay of water and biomolecules. This book deals with the physicochemical aspects of such life phenomena produced by water and biomolecules, and addresses topics including "Protein Dynamics and Functions", "Protein and DNA Folding", and "Protein Amyloidosis". All sections have been written by internationally recognized front-line researchers. The idea for this book was born at the 5th International Symposium "Water and Biomolecules", held in Nara city, Japan, in 2008.

The classic personal account of Watson and Crick's groundbreaking discovery of the structure of DNA, now with an introduction by Sylvia Nasar, author of A Beautiful Mind. By identifying the structure of DNA, the molecule of life, Francis Crick and James Watson revolutionized biochemistry and won themselves a Nobel Prize. At the time, Watson was only twenty-four, a young scientist hungry to make his mark. His uncompromisingly honest account of the heady days of their thrilling sprint against other world-class researchers to solve one of science's greatest mysteries gives a dazzlingly clear picture of a world of brilliant scientists with great gifts, very human ambitions, and bitter rivalries. With humility unspoiled by false modesty, Watson relates his and Crick's desperate efforts to beat Linus Pauling to the Holy Grail of life sciences, the identification of the basic building block of life. Never has a scientist been so truthful in capturing in words the flavor of his work.

A practical and self-contained introduction to methods of researching the structure and function of the ribosome in light of the increasing recognition of the potential capability of RNA molecules to act as molecular catalysts. Also describes protein synthesis and cell-free synthesizing systems. Annotation copyrighted by Book News, Inc., Portland, OR

Diagnostic Molecular Biology

Atp-Deprived Mammalian Cells

The New Science of Experiment Planning

Their Roles in Protein Synthesis and Stress Protection

The Double Helix

Research Awards Index

For nearly 30 years, Principles of Medical Biochemistry has integrated medical biochemistry with molecular genetics, cell biology, and genetics to provide complete yet concise coverage that links biochemistry with clinical medicine. The 4th Edition of this award-winning text by Drs. Gerhard Meisenberg and William H. Simmons has been fully updated with new clinical examples, expanded coverage of recent changes in the field, and many new case studies online. A highly visual format helps readers retain complex information, and USMLE-style questions (in print and online) assist with exam preparation. Just the right amount of detail on biochemistry, cell biology, and genetics – in one easy-to-digest textbook. Full-color illustrations and tables throughout help students master challenging concepts more easily. Online case studies serve as a self-assessment and review tool before exams. Online access includes nearly 150 USMLE-style questions in addition to the questions that are in the book. Glossary of technical terms. Clinical Boxes and Clinical Content demonstrate the integration of basic sciences and clinical applications, helping readers make connections between the two. New clinical examples have been added throughout the text.

The motivation for us to conceive this series of volumes on regulation was mainly our belief that it would be fun, and at the same time productive, to approach the subject in a way that differs from that of other treatises. We thought it might be interesting and instructive for both author and reader-to examine a particular area of investigation in a framework of many different problems. Cutting across the traditional boundaries that have separated the subjects in past volumes on regulation is not an easy thing to do-not because it is difficult to think of what interesting topics should replace the old ones, but because it is difficult to find authors who are willing to write about areas outside those pursued in their own laboratories. Anyone who takes on the task of reviewing a broad area of interest must weave together its various parts by picking up the threads from many different laboratories, and attempt to produce a fabric with a meaningful design. Finding persons who are likely to succeed in such a task was the most difficult part of our job. In the first volume of this treatise, most of the chapters dealt with the mechanisms of The second volume involved a somewhat regulation of gene expression in microorganisms. broader area, spanning the prokaryotic-eukaryotic border. Topics ranged from phage mor phogenesis to the role of gradients in development. The last volume-Volume 3A-con cerned hormones, as does this volume-Volume 3B.

The Eureka! Science, Corporation presents information on protein synthesis as part of I Can Do That!, which offers science facts for children. In protein synthesis, ribosomes use a messenger-RNA to determine which amino acid belongs where. A specific group of amino acids is then joined together to form a protein.

Engineering the Next Revolution in Neuroscience presents a framework for accelerating discovery in neuroscience. Deriving principles directly from detailed case studies, the authors show how maps of research findings will enable researchers to see what their field has accomplished and where the unexplored territories still reside.

Heat Shock Proteins and Cytoprotection

Physical Chemistry of Life Phenomena

Cell-Free Protein Expression

Computational Biology of Transcription Factor Binding

Bioengineering and Industrial Applications

Energy Research Abstracts

This book introduces readers to basic studies on and applied techniques involving lactic acid bacteria, including their bioengineering and industrial applications. It summarizes recent biotechnological advances in lactic acid bacteria for food and health, and provides detailed information on the applications of these bacteria in fermented foods. Accordingly, it offers a valuable resource for researchers and graduate students in the fields of food microbiology, bioengineering, fermentation engineering, food science, nutrition and health.

Following its inception in the 1950s, cell-free protein synthesis made a tremendous impact on the basic life sciences. The use of cell-free systems was key to understanding molecular mechanisms underlying one of the most complicated processes found in nature: protein translation. Since this time, aggressive cutting-edge research and stiff commerica

Every year, an estimated 1.7 million Americans sustain brain injury. Long-term disabilities impact nearly half of moderate brain injury survivors and nearly 50,000 of these cases result in death. Brain Neurotrauma: Molecular, Neuropsychological, and Rehabilitation Aspects provides a comprehensive and up-to-date account on the latest developments in the area of neurotrauma, including brain injury pathophysiology, biomarker research, experimental models of CNS injury, diagnostic methods, and neurotherapeutic interventions as well as neurorehabilitation strategies in the field of neurotraum research. The book includes several sections on neurotrauma mechanisms, biomarker discovery, neurocognitive/neurobehavioral deficits, and neurorehabilitation and treatment approaches. It also contains a section devoted to models of mild CNS injury, including blast and sport-related injuries. Over the last decade, the field of neurotrauma has witnessed significant advances, especially at the molecular, cellular, and behavioral levels. This progress is largely due to the introduction of novel techniques, as well as the development of new animal models of central nervous system (CNS) injury. This book, with its diverse coherent content, gives you insight into the diverse and heterogeneous aspects of CNS pathology and/or rehabilitation needs.

NIH: An Accounting of Research in Its Laboratories and Clinics contains collected accounts of the Intramural Research Program, as they happened in the laboratories and clinics, in various installations of the National Institutes of Health across the U.S.A. One paper discusses the etiology of schizophrenia which notes that, based on evidence and expanded adoption studies by Ketty, Rosenthal, and Wender, genetic factors actually contribute to the development of the disease. In developing countries, schizophrenia follows a more benign course. Some papers describe bacteriology, mycology, viral hepatitis, basic immunology, clinical immunology, and the development of enzymology. Researchers studying proteins elucidate on the synthesis and folding of protein chains, protein conformation and dynamics, the semisynthesis and protein function, as well as on sequence analysis and collagen research. Other papers describe the breaking of the genetic code, the progress made from the genetic code to beta thalassemia, to investigations of genetic diseases (such as galactosemia, gout, Lesch-Nyhan disease, mucopolysaccharide storage disease, and sickle cell disease). One paper notes the contribution of the intramural clinical research program of the National Cancer Institute to cancer therapy with emphasis in cancer chemotherapy. Professors in pharmacology, practitioners of general medicine, specialists or researchers dealing with microchemistry, toxicology, drug therapy, or oncology will find the collection valuable.

The Routledge Handbook of Philosophy of Memory

Molecular Biology

Protein-Nucleic Acid Interactions

Protein synthesis

Bio 181

Recombinant Protein Expression: Prokaryotic hosts and cell-free systems

With its detailed description of membrane protein expression, high-throughput and genomic-scale expression studies, both on the analytical and the preparative scale, this book covers the latest advances in the field. The step-by-step protocols and practical examples given for each method constitute practical advice for beginners and experts alike.

The Swartz lab has put much effort into understanding the underlying principles of E. coli-based cell-free protein synthesis (CFPS), and the technology has developed into a scalable, affordable platform for producing a wide range of protein targets. Key breakthroughs have included activating central metabolism, stabilization of critical amino acids, controlling the redox environment to produce proteins containing disulfide bonds, and using scale-up technologies to produce proteins at milligram quantities. My work has sought to expand this CFPS technology for producing valuable and complex eukaryotic protein targets by manipulating and optimizing the folding of these proteins in the heterologous CFPS environment. Furthermore, I have sought to apply these advances to specific applications of interest. By modifying a key molecular chaperone native to the eukaryotic endoplasmic reticulum (ER), the Hsp70-family chaperone, BiP, soluble production was increased in CFPS reactions for specific proteins normally secreted through this organelle, namely those from the immunoglobulin superfamily which includes antibodies, T-cell receptors, and many membrane receptors. First, the functional properties of BiP were compared to that of the E. coli Hsp70, DnaK. A fusion protein was then constructed between BiP and the ribosome-binding portion of the E. coli protein, trigger factor, to localize BiP to translating ribosomes. This replicated the native function of BiP, which provides co-translational folding assistance to nascent polypeptides. After verifying its bioactivity, this fusion protein was utilized in CFPS reactions to indicate that the chaperone functions of BiP are specific to proteins normally secreted through the eukaryotic ER, whereas DnaK demonstrates a more general chaperone mechanism. Since the discovery that somatic cells could be reprogrammed back to a pluripotent state through the viral expression of a specific set of transcription factors, there has been great interest in reprogramming using a safer and more clinically relevant protein-based approach. Production of these transcription factor proteins was greatly increased by fusing them to the C-terminus of the solubility partner, IF2 domain 1 (IF2D1). While the fusions provided marginal benefit in molar yields using a CFPS approach, in vivo E. coli expression produced the transcription factors in soluble form. The fusion proteins could be purified in milligram quantities from liter shake-flask cultures, whereas essentially no soluble protein accumulated without the fusion partner. The transcription factor fusions bound specifically to their consensus DNA sequences and partially activated some of their downstream gene targets. Another application utilizing CFPS technology is an enhanced luciferase mutant from the marine copepod, Gaussia princeps (GLuc). GLuc is both the smallest and brightest known luciferase, and previous work from our lab demonstrated that this protein could be produced at higher volumetric yields and specific activities in CFPS compared to conventional protein expression systems. By mutating key residues in the Gaussia luciferase sequence, the luminescence half-life was shown to increase over ten-fold while maintaining the initial specific activity of the wild-type. This improved mutant provides a valuable imaging agent to use in fusions and bioconjugates with other proteins such as those that recognize cell surface markers on cancer cells. In a final application, influenza vaccines were produced using CFPS by isolating specific fragments of the protein hemagglutinin (HA), a viral surface protein. Specific mutations as well as a C-terminal trimerization domain were critical for producing this protein fragment in both its monomeric and native trimeric forms. By using the CFPS platform to incorporate non-natural amino acids (nnAAs) with alkyne functional groups, the HA proteins were covalently 'clicked' to virus-like particles (VLPs) that had surface exposed nnAAs with azide functionality. The VLPs provide an immunogenic delivery platform that efficiently traffics to lymph nodes and allows for co-attachment of other adjuvants in addition to the primary HA antigen. This vaccine platform was characterized and tested in mouse models and compared to both a standard influenza vaccine as well as free HA protein fragments. In summary, CFPS is a valuable and robust method of protein production for a variety of targets. My thesis has sought to use this platform as a means to better understand folding pathways of complex, eukaryotic proteins and improve production of these proteins. To this end, CFPS has been shown to be a valuable method for elucidating and manipulating chaperone function, producing difficult proteins with enhanced function, and as a platform to produce novel vaccines.

Biology for AP® courses covers the scope and sequence requirements of a typical two-semester Advanced Placement® biology course. The text provides comprehensive coverage of foundational research and core biology concepts through an evolutionary lens. Biology for AP® Courses was designed to meet and exceed the requirements of the College Board's AP® Biology framework while allowing significant flexibility for instructors. Each section of the book includes an introduction based on the AP® curriculum and includes rich features that engage students in scientific practice and AP® test preparation; it also highlights careers and research opportunities in biological sciences.

Evolution since Coding: Cradles, Halos, Barrels, and Wings describes genesis of metabolism, transcription, translation, cell structure, eukaryotic complexity, LUCA (the last universal common (cellular) ancestor), the great divergence of archaea and bacteria, LECA (the last eukaryotic common ancestor), extinction, and cancer in very simple ways. The work (almost) "synthesizes life from scratch" (since coding) and describes the tools for readers to check the author's work. As a result, readers understand living systems and their evolution in a conceptual way and are empowered to utilize powerful but accessible tools in computer-based biology. The work serves as foundational reading for a variety of researchers, academics, and students in life sciences, for example in evolution/evolutionary biology, biochemistry, genetics/molecular genetics, molecular biology, cell biology, and microbiology, as well as disciplines beyond biological science. Its approachable style makes the book accessible for introductory students and educated laypersons. Evolution since Coding is suitable to supplement college courses that mix computers, evolution, and biology from freshman to senior level. Provides a simple, hands-on, conceptual route to understanding ancient evolution and the diversification of life on earth Offers a conceptual understanding of biology, evolution, protein structure, RNA synthesis systems, protein synthesis systems, signaling systems, genesis of the three domains, and cell structures Approaches ancient evolution via code-breaking protein and RNA sequences and motifs

Ribosomes and Protein Synthesis

Basic Cloning Procedures

Recombinant Protein Expression: Eukaryotic hosts

Principles of Medical Biochemistry E-Book

Water and Biomolecules

Research Grants Index

On the first day of school, have you ever thought of your classrooms as newly opened boxes of crayons? I do. Like pencil-sticks of colored wax, the students each have different names, individual characteristics, and various levels of brightness. I set a goal each year to promote not only creativity but to draw out of my students' reasons about why science is so important. As science educators, we not only need to illustrate the importance of knowing facts and terminology; but, also be able to frame those concepts in such a way that students are motivated to want to study and understand biology. When I began teaching, I never thought that I would have the multitude of experiences I have now. I have taught in schools ranging from city to rural, public to private, and large to small; not to mention classes ranging from general science to advanced biology. Through these diverse experiences, I have developed a number of strategies that have enhanced student achievement and science appreciation. In this book, I will share with you these experiences and techniques, showing you how to enhance teaching skills, increase student drive, create mental connections, better manage your class time, use proper technology, practice forms of differentiation, and incorporate the NGSS. In addition, this text allows me to share my most treasured philosophies, experiences, and teaching strategies and how they can be applied to biology/life science classrooms.

Recombinant Protein Expression, Part B, Volume 660 in the Methods in Enzymology series, highlights new advances in the field with this new volume presenting interesting chapters on Multiplexed analysis protein: Protein interactions of polypeptides translated in Leishmania cell-free system, MultiBac system and its applications, performance and recent, Production of antibodies in Shuffle, Designing hybrid-promoter architectures by engineering cis-acting DNA sites to enhance transcription in yeast, Designing hybrid-promoter architectures by engineering cis-acting DNA sites to deregulate transcription in yeast, Antibody or protein-based vaccine production in plants, Cell-free protein synthesis, Plant-based expression of biologic drugs, and much more. Additional sections cover the Use of native mass spectrometry to guide detergent-based rescue of non-native oligomerization by recombinant proteins, Advancing overexpression and purification of recombinant proteins by pilot optimization through tandem affinity-buffer exchange chromatography online with native mass spectrometry, Method for High-Efficiency Fed-batch cultures of recombinant Escherichia coli, Method to transfer Chinese hamster ovary (CHO) shake flask experiments to the ambr® 250, and Expression of recombinant antibodies in Leishmania tarentolae. Provides the authority and expertise of leading contributors from an international board of authors Presents the latest release in the Methods in Enzymology serial Updated release includes the latest information on Recombinant Protein Expression

Cell-free synthetic biology is in the spotlight as a powerful and rapid approach to characterize and engineer natural biological systems. The open nature of cell-free platforms brings an unprecedented level of control and freedom for design compared to in vivo systems. This versatile engineering toolkit is used for debugging biological networks, constructing artificial cells, screening protein library, prototyping genetic circuits, developing new drugs, producing metabolites, and synthesizing complex proteins including therapeutic proteins, toxic proteins, and novel proteins containing non-standard (unnatural) amino acids. The book consists of a series of reviews, protocols, benchmarks, and research articles describing the current development and applications of cell-free synthetic biology in diverse areas.

NIH: An Account of Research in Its Laboratories and Clinics

Federation Proceedings

Hormone Action

Anatomy & Physiology

Methods and Protocols

Engineering the Next Revolution in Neuroscience