

METHODS IN MOLECULAR BIOLOGY™

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Unnatural Amino Acids

Methods and Protocols

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Preface

Even though nonproteinogenic amino acids are present in nature, they are usually defined as unnatural or nonnatural, meaning that they are other than the 22 natural L- α -amino acids (L- α -AAs) constituting natural peptides and proteins. The definition usually adopted is not specific for defined structural classes since among the group there are L- α -AAs differing in their chemical structure, α -AAs of D-configuration, non- α -AAs of both absolute configurations. Besides their structural diversity, interest in these compounds is due to a number of reasons such as

their occurrence in nature

their biological properties

their biosynthetic pathway (often related to posttranslational modification of natural AAs) and the enzymes acting on them

the chemical and enzymatic methods for their production

the analytical aspects

their use as probes

their incorporation into peptides and proteins (and the related biotechnological uses).

The volume of *Methods in Molecular Biology* series entitled “Unnatural Amino Acids” with the well-established format of detailed experimental procedures in the step-by-step protocols approach (an introductory overview, a list of the materials and reagents needed to complete the experiment, followed by a detailed procedure supported with a helpful notes section offering tips and tricks of the trade as well as troubleshooting advices) addresses these topics, assembling the material into five sections (parts). Part I (*Synthesis of Unnatural Amino Acids*) deals with methods where enzyme catalysis is used to produce nonnatural amino acids. Attention is focused mainly on the obtainment of those enzymes and also on the practical aspects of their application in biotransformations (target compounds are required for the study and preparation of drug candidates of increasing interest and complexity). The second section (Part II, *Applications of Unnatural Amino Acids*) deals with aspects concerning the presence of unnatural AAs in peptides with antibiotic properties. This includes the application of unnatural amino acids to the *de novo* design of selective antibiotic peptides, the site-specific incorporation of unnatural amino acids, and the related conformational changes observed in proteins, as well as the use of unnatural amino acids to probe structure–activity relationships in antimicrobial peptides.

Part III, *Use of Unnatural Amino Acids in Protein Synthesis*, deals with applications of specific techniques allowing unnatural amino acids to be genetically incorporated into proteins both in yeast and mammalian cells. In both cases, orthogonal tRNA/aminoacyl-tRNA synthetase pair is used taking advantage of suitable techniques and allowing high incorporation of the nonnatural AA. Most aspects concerning AAs of D-configuration are grouped in

Parts IV and V (12 chapters), namely, *Analysis and Applications*, and *Enzymes Active on D-Amino Acids*. The analytical studies address two main aspects:

- the determination of D-AAAs in food – where their presence is associated with bio-availability and impairing of food nutritional value, thus becoming indicators of food quality
- their determination in biological fluids – e.g., investigations of different neurological and psychiatric disorders correlating the appearance/susceptibility to these disorders with the concentration of D-serine in brain.

the importance of enzymes acting on (D-specific oxidases, proteases) or producing (racemases, isomerases) D-AAAs is also emphasized in chapters dedicated to the study of these specific biocatalysts.

We believe that the future will likely see many more researchers focusing on the investigation and utilization of unnatural amino acids: these molecules promise of becoming an argument with enormous potential. Comprehensive but convenient, this manual, giving detailed prescription in *Molecular Biology*, will contribute to drive the attention to the many fields of growing scientific interest in nonnatural amino acids.

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