Methods in Molecular Biology 1824

Springer Protocols

Thomas Mavromoustakos
Tahsin F. Kellici *Editors*

Rational Drug Design

Methods and Protocols

Humana Press

METHODS IN MOLECULAR BIOLOGY

Series Editor
John M. Walker
School of Life and Medical Sciences
University of Hertfordshire
Hatfield, Hertfordshire, AL10 9AB, UK

For further volumes:
http://www.springer.com/series/7651

Rational Drug Design

Methods and Protocols

Edited by

Thomas Mavromoustakos and Tahsin F. Kellici

Division of Organic Chemistry, Department of Chemistry, National and Kapodistrian University of Athens, Panepistimiopolis, Zografou, Athens, Greece

Humana Press

Editors
Thomas Mavromoustakos
Division of Organic Chemistry
Department of Chemistry
National and Kapodistrian
University of Athens
Panepistimiopolis, Zografou
Athens, Greece

Tahsin F. Kellici
Division of Organic Chemistry
Department of Chemistry
National and Kapodistrian
University of Athens
Panepistimiopolis, Zografou
Athens, Greece

ISSN 1064-3745 ISSN 1940-6029 (electronic)
Methods in Molecular Biology
ISBN 978-1-4939-8629-3 ISBN 978-1-4939-8630-9 (eBook)
https://doi.org/10.1007/978-1-4939-8630-9

Library of Congress Control Number: 2018948185

Printed on acid-free paper

This Humana Press imprint is published by the registered company Springer Science+Business Media, LLC part of Springer Nature.
The registered company address is: 233 Spring Street, New York, NY 10013, U.S.A.

Preface

As you set out on the way to Ithaca
hope that the road is a long one,
filled with adventures, filled with discoveries.
The Laestrygonians and the Cyclopes,
Poseidon in his anger: do not fear them, . . .
And if you find her poor, Ithaca didn't deceive you.
As wise as you will have become, with so much experience,
you will understand, by then, these Ithacas; what they mean.

C. P. Cavafy
Translated by Daniel Mendelsohn

What excites us as guest editors in the rational drug design is the decision of beginning a trip toward Ithaca that can lead to the liberation from the thefts that steal our health. This trip that involves Laestrygonians and Cyclops is exciting and adventure. We do not mind if our Ithaca did not fill our expectations. We become mature and happy as we discover new avenues for reaching a new Ithaca. The rational drug design is an Odyssey that never ends and this is the essence of life.

This volume of Methods in Molecular Biology covers several aspects of rational drug design. Such aspects include (a) synthesis of novel bioactive drugs; (b) development and application of new methodologies to tackle problems related to discovery of potent molecules; (c) comprehend on concepts strictly related to the bioactivity, i.e., lipophilicity; (d) development and application of computational methods valuable toward the establishment of new approaches in the Ithaca trip of drug discovery; and (e) the effects of physicochemical and ADMET properties of the designed potential drugs.

Hereby is given an outline of the chapters covered in the volume. The first three chapters are dedicated to the design of peptides and peptidomimetics targeting the amyloid deposits and multiple sclerosis. Chapter 4 offers applications and comprehends on saturation transfer difference (STD) NMR in the mapping of the protein-ligand interface. In the fifth chapter the performance of docking tools is assessed. The use of structural biology in drug design is reviewed in the sixth chapter. Chapter 7 introduces new essential cheminformatic tools in ligand-based drug design. In Chapter 8 a thorough method of bioguided design of trypanosomicidal compounds is explained. The use of hybrid screening protocols is given in Chapter 9. Chapter 10 explains a novel method for the determination of unlabeled compound kinetics using the technique of time-resolved fluorescence resonance energy transfer. The new computational method of dynamic undocking is introduced in Chapter 11. The importance of lipophilicity in drug discovery is explained in Chapter 12. Chapters 13 and 14 explore the polypharmacology and the development of nuclear receptor modulators. An extensible orthogonal protocol that combines structure-based and ligand-based screening tools is introduced in Chapter 15. In Chapter 16 the synthesis of various adamantane derivatives with σ-receptor affinity is described. Examples of supervised molecular dynamics approaches are reviewed in Chapter 17. In Chapter 18 the synergistic action of biomolecular NMR methodologies is explained. Chapter 19 introduces the use of the dynamic and in silico pharmacophore approach in drug discovery. Chapters 20 and 21 deal with the rational design of inhibitors targeting MAGL and methyllysine reader protein spindlin1. The design

of natural product hybrids bearing triple antiplatelet profile is described in Chapter 22. Pharmacophore generation using phase is explained in Chapter 23. The design of inhibitors targeting histone deacetylases by filtering through ADMET, physicochemical and ligand-target flexibility properties is provided in Chapter 24. The occurrence of reactions in NMR tubes that may lead to new drug leads is explored in Chapter 25. The two last chapters deal with the angiotensin II type 1 receptor (AT1R). Chapter 26 provides the application of structure-based methods to develop new antagonists of the receptor, while Chapter 27 explores the importance homology modeling played in the case of AT1R.

This book, as can be understood from the analysis of its contents, was made possible through the generous contributions of many scientists, who shared their knowledge, for which we are very grateful. We are also sincerely grateful to the series editor, Professor John Walker, for his help, advice, and patient guidance in preparing this volume.

Athens, Greece

Thomas Mavromoustakos
Tahsin F. Kellici

Contents

Contributors

Antreas Afantitis • *NovaMechanics Ltd., Nicosia, Cyprus*

Busecan Aksoydan • *Department of Biophysics, Computational Biology and Molecular Simulations Laboratory, School of Medicine, Bahcesehir University, Istanbul, Turkey*

Guzmán Ignacio Álvarez Touron • *Laboratorio de Moléculas Bioactivas, CENUR Litoral Norte, Universidad de la República, Paysandú, CP, Uruguay*

Andrew Anighoro • *Department of Life Science Informatics, B-IT, LIMES Program Unit Chemical Biology and Medicinal Chemistry, Rheinische Friedrich-Wilhelms-Universität, Bonn, Germany*

Jürgen Bajorath • *Department of Life Science Informatics, B-IT, LIMES Program Unit Chemical Biology and Medicinal Chemistry, Rheinische Friedrich-Wilhelms-Universität, Bonn, Germany*

Flavio Ballante • *Department of Biochemistry and Molecular Biophysics, Washington University School of Medicine, Saint Louis, MO, USA; Department of Cell and Molecular Biology, Uppsala Biomedicinska Centrum BMC, Uppsala University, Uppsala, Sweden*

Xavier Barril • *Institut de Biomedicina de la Universitat de Barcelona (IBUB) and Facultat de Farmàcia, Universitat de Barcelona, Barcelona, Spain; Catalan Institution for Research and Advanced Studies (ICREA), Passeig Lluís Companys 23, Barcelona, Spain*

Martiniano Bello • *Laboratorio de Modelado Molecular, Bioinformática y Diseño de Fármacos, de la Escuela Superior de Medicina, Instituto Politécnico Nacional, Plan de San Luis y Díaz Mirón s/n, Col. Casco de Santo Tomas, Delegación Miguel Hidalgo, C.P., Ciudad de México, Mexico*

Anirban Bhunia • *Department of Biophysics, Bose Institute, Kolkata, India*

Maria Birkou • *Department of Pharmacy, University of Patras, Patras, Greece*

Jeffrey R. Brender • *Radiation Biology Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA*

Isabella Caligiuri • *Division of Experimental and Clinical Pharmacology, Department of Molecular Biology and Translational Research, National Cancer Institute and Center for Molecular Biomedicine, IRCCS, Pordenone, Italy*

Steven J. Charlton • *School of Life Science, Queen's Medical Centre, University of Nottingham, Nottingham, UK*

Maria V. Chatziathanasiadou • *Section of Organic Chemistry and Biochemistry, Department of Chemistry, University of Ioannina, Ioannina, Greece*

Magda S. Chegkazi • *Institute of Biology, Medicinal Chemistry and Biotechnology, National Hellenic Research Foundation, Athens, Greece; Faculty of Life Sciences and Medicine, Randall Centre for Cell and Molecular Biophysics, King's College London, London, UK*

Evangelia D. Chrysina • *Institute of Biology, Medicinal Chemistry and Biotechnology, National Hellenic Research Foundation, Athens, Greece*

Jose Correa-Basurto • *Laboratorio de Modelado Molecular, Bioinformática y Diseño de Fármacos, de la Escuela Superior de Medicina, Instituto Politécnico Nacional, Plan de San Luis y Díaz Mirón s/n, Col. Casco de Santo Tomas, Delegación Miguel Hidalgo, C.P., Ciudad de México, Mexico*

Ruel Z. B. Desamero • *Department of Chemistry, York College and The Institute for Macromolecular Assemblies, Jamaica, NY, USA; Ph.D. Programs in Chemistry and Biochemistry, The Graduate Center of the City University of New York, New York, NY, USA*

Serdar Durdagi • *Department of Biophysics, Computational Biology and Molecular Simulations Laboratory, School of Medicine, Bahcesehir University, Istanbul, Turkey*

Youness Elkhou • *Department of Chemistry, York College and The Institute for Macromolecular Assemblies, Jamaica, NY, USA; Ph.D. Programs in Chemistry and Biochemistry, The Graduate Center of the City University of New York, New York, NY, USA*

Leif A. Eriksson • *Department of Chemistry and Molecular Biology, University of Gothenburg, Gothenburg, Sweden*

Ismail Erol • *Department of Biophysics, Computational Biology and Molecular Simulations Laboratory, School of Medicine, Bahcesehir University, Istanbul, Turkey*

Anirban Ghosh • *Department of Biophysics, Bose Institute, Kolkata, India*

Constantinos Giaginis • *Department of Food Science and Nutrition, School of Environment, University of the Aegean, Myrina, Lemnos, Greece*

Carlotta Granchi • *Department of Pharmacy, University of Pisa, Pisa, Italy*

Isik Kantarcioglu • *Department of Biophysics, Computational Biology and Molecular Simulations Laboratory, School of Medicine, Bahcesehir University, Istanbul, Turkey*

Tahsin F. Kellici • *Division of Organic Chemistry, Department of Chemistry, National and Kapodistrian University of Athens, Panepistimiopolis, Zografou, Athens, Greece*

Antonios Kolocouris • *Department of Pharmacy, National and Kapodistrian University of Athens, Panepistimiopolis, Zografou, Athens, Greece*

Athina Konstantinidi • *Department of Pharmacy, National and Kapodistrian University of Athens, Panepistimiopolis, Zografou, Athens, Greece*

Janarthanan Krishnamoorthy • *Department of Biosciences, Jimma University, Jimma, ET, USA*

Panagiotis Lagarias • *Department of Pharmacy, National and Kapodistrian University of Athens, Panepistimiopolis, Zografou, Athens, Greece*

George Lambrinidis • *Department of Pharmacy, National and Kapodistrian University of Athens, Panepistimiopolis, Zografou, Athens, Greece*

Thierry Langer • *Faculty of Life Sciences, Department of Pharmaceutical Chemistry, University of Vienna, Vienna, Austria*

Chiara Luise • *Department of Pharmaceutical Chemistry, Martin-Luther University of Halle-Wittenberg, Halle/Saale, Germany*

Marco Macchia • *Department of Pharmacy, University of Pisa, Pisa, Italy*

Maciej Majewski • *Institut de Biomedicina de la Universitat de Barcelona (IBUB) and Facultat de Farmàcia, Universitat de Barcelona, Barcelona, Spain*

Michael Mamais • *Institute of Biology, Medicinal Chemistry and Biotechnology, National Hellenic Research Foundation, Athens, Greece*

Konstantinos D. Marousis • *Department of Pharmacy, University of Patras, Patras, Greece*

Adriano Martinelli • *Department of Pharmacy, University of Pisa, Pisa, Italy*

Marlet Martínez-Archundia • *Laboratorio de Modelado Molecular, Bioinformática y Diseño de Fármacos, de la Escuela Superior de Medicina, Instituto Politécnico Nacional, Plan de San Luis y Díaz Mirón s/n, Col. Casco de Santo Tomas, Delegación Miguel Hidalgo, C.P., Ciudad de México, Mexico*

Minos-Timotheos Matsoukas • *Department of Pharmacy, University of Patras, Patras, Greece*
Thomas Mavromoustakos • *Division of Organic Chemistry, Department of Chemistry, National and Kapodistrian University of Athens, Panepistimiopolis, Zografou, Athens, Greece*
Georgia Melagraki • *NovaMechanics Ltd., Nicosia, Cyprus*
Daniel Merk • *Institute of Pharmaceutical Chemistry, Goethe University Frankfurt, Frankfurt, Germany*
Emmanuel Mikros • *Department of Pharmacy, National and Kapodistrian University of Athens, Panepistimiopolis Zografou, Athens, Greece; "Athena" Research and Innovation Center, Athens, Greece*
Filippo Minutolo • *Department of Pharmacy, University of Pisa, Pisa, Italy*
Stefano Moro • *Molecular Modeling Section (MMS), Department of Pharmaceutical and Pharmacological Sciences, University of Padova, Padova, Italy*
Vassilios Myrianthopoulos • *Department of Pharmacy, National and Kapodistrian University of Athens, Panepistimiopolis Zografou, Athens, Greece; "Athena" Research and Innovation Center, Athens, Greece*
Spyridon Nikolakopoulos • *NovaMechanics Ltd., Nicosia, Cyprus*
Dimitrios Ntountaniotis • *Department of Chemistry, Laboratory of Organic Chemistry, National and Kapodistrian University of Athens, Athens, Greece*
Alessandro Padova • *Computer-Aided Drug Design Group, Fondazione Ri.MED, Palermo, Italy*
Ioannis Papanastasiou • *School of Health Sciences, Department of Pharmacy, Division of Pharmaceutical Chemistry, National and Kapodistrian University of Athens, Panepistimiopolis—Zografou, Athens, Greece*
Ugo Perricone • *Computer-Aided Drug Design Group, Fondazione Ri.MED, Palermo, Italy*
Adam A. Profit • *Department of Chemistry, York College and The Institute for Macromolecular Assemblies, Jamaica, NY, USA; Ph.D. Programs in Chemistry and Biochemistry, The Graduate Center of the City University of New York, New York, NY, USA*
Flavio Rizzolio • *Division of Experimental and Clinical Pharmacology, Department of Molecular Biology and Translational Research, National Cancer Institute and Center for Molecular Biomedicine, IRCCS, Pordenone, Italy; Department of Molecular Science and Nanosystems, Ca' Foscari Università di Venezia, Venezia-Mestre, Italy*
Dina Robaa • *Department of Pharmaceutical Chemistry, Martin-Luther University of Halle-Wittenberg, Halle/Saale, Germany*
Sergio Ruiz-Carmona • *Institut de Biomedicina de la Universitat de Barcelona (IBUB) and Facultat de Farmàcia, Universitat de Barcelona, Barcelona, Spain*
Davide Sabbadin • *Syngenta Crop Protection AG, Stein, Switzerland*
Patricia Saenz-Méndez • *Department of Chemistry and Molecular Biology, University of Gothenburg, Gothenburg, Sweden; Computational Chemistry and Biology Group, Facultad de Química, UdelaR, Montevideo, Uruguay*
Veronica Salmaso • *Molecular Modeling Section (MMS), Department of Pharmaceutical and Pharmacological Sciences, University of Padova, Padova, Italy*
Simone Schierle • *Institute of Pharmaceutical Chemistry, Goethe University Frankfurt, Frankfurt, Germany*

Thomas Seidel • *Faculty of Life Sciences, Department of Pharmaceutical Chemistry, University of Vienna, Vienna, Austria*
Anastasia I. Sotiropoulou • *Institute of Biology, Medicinal Chemistry and Biotechnology, National Hellenic Research Foundation, Athens, Greece*
Georgios A. Spyroulias • *Department of Pharmacy, University of Patras, Patras, Greece*
Mattia Sturlese • *Molecular Modeling Section (MMS), Department of Pharmaceutical and Pharmacological Sciences, University of Padova, Padova, Italy*
Evgenios K. Stylos • *Section of Organic Chemistry and Biochemistry, Department of Chemistry, University of Ioannina, Ioannina, Greece; Biotechnology Laboratory, Department of Biological Applications and Technology, University of Ioannina, Ioannina, Greece*
David A. Sykes • *School of Life Science, Queen's Medical Centre, University of Nottingham, Nottingham, UK*
Anna Tsantili-Kakoulidou • *Department of Pharmaceutical Chemistry, Faculty of Pharmacy, National and Kapodistrian University of Athens, Panepistimiopolis, Zografou, Athens, Greece*
Alexandros D. Tselepis • *Atherothrombosis Research Centre/Laboratory of Biochemistry, Department of Chemistry, University of Ioannina, Ioannina, Greece*
Theodore Tselios • *Department of Chemistry, University of Patras, Patras, Greece*
Antonis Tsiailanis • *Section of Organic Chemistry and Biochemistry, Department of Chemistry, University of Ioannina, Ioannina, Greece*
Aikaterini C. Tsika • *Department of Pharmacy, University of Patras, Patras, Greece*
Fotios Tsopelas • *Laboratory of Inorganic and Analytical Chemistry, School of Chemical Engineering, National Technical University of Athens, Athens, Greece*
Maria Tsoumani • *Atherothrombosis Research Centre/Laboratory of Biochemistry, Department of Chemistry, University of Ioannina, Ioannina, Greece*
Andreas Tsoumanis • *NovaMechanics Ltd., Nicosia, Cyprus*
Tiziano Tuccinardi • *Department of Pharmacy, University of Pisa, Pisa, Italy*
Andreas G. Tzakos • *Section of Organic Chemistry and Biochemistry, Department of Chemistry, University of Ioannina, Ioannina, Greece*
Haralambos Tzoupis • *Department of Chemistry, University of Patras, Patras, Greece*
Dimitra-Danai Varsou • *NovaMechanics Ltd., Nicosia, Cyprus*
Jayson Vedad • *Department of Chemistry, York College and The Institute for Macromolecular Assemblies, Jamaica, NY, USA; Ph.D. Programs in Chemistry and Biochemistry, The Graduate Center of the City University of New York, New York, NY, USA*
Eleni Vrontaki • *Department of Pharmacy, National and Kapodistrian University of Athens, Panepistimiopolis Zografou, Athens, Greece*
Marcus Wieder • *Faculty of Life Sciences, Department of Pharmaceutical Chemistry, University of Vienna, Vienna, Austria; Faculty of Chemistry, Department of Computational Biological Chemistry, University of Vienna, Vienna, Austria*

Chapter 1

Molecular Dynamics Simulations on the Bioactive Molecule of hIAPP22–29 (NFGAILSS) and Rational Drug Design

Panagiotis Lagarias, Youness Elkhou, Jayson Vedad, Athina Konstantinidi, Adam A. Profit, Tahsin F. Kellici, Antonios Kolocouris, Ruel Z. B. Desamero, and Thomas Mavromoustakos

Abstract

This chapter includes information about the structure in equilibrium of the bioactive molecule hIAPP22–29 (NFGAILSS). The experimental structure was derived using X-ray and its 2D NOESY NMR experiments in d_6-DMSO and d-HFIP solvents. This molecule contains eight of the ten amino acids of the 20–29 region of the human islet amyloid polypeptide (hIAPP) often referred as the "amyloidogenic core." Amyloid deposits are well-known to cause as many as 20 pathological neurodegenerative disorders such as Alzheimer, Parkinson, Huntington, and Creutzfeldt-Jakob. The experimental structure was relaxed using molecular dynamics (MD) in simulation boxes consisting in DMSO and HFIP; the latter not provided by the applied software. The calculations were performed in GPUs and supercomputers, and some basic scripting is described for reference. The simulations confirmed the inter- and intramolecular forces that led to an "amyloidogenic core" observed from NOE experiments. The results showed that in DMSO and HFIP environment, Phe is not in spatial proximity with Leu or Ile, and this is consistent with an amyloidogenic core. However, in an amphipathic environment such as the model lipid bilayers, this communication is possible and may influence peptide amyloidogenic properties. The knowledge gained through this study may contribute to the rational drug design of novel peptides or organic molecules acting by modifying preventing amyloidogenic properties of the hIAPP peptide.

Key words hIAPP22–29, Aggregation of proteins, Molecular dynamics, NMR, Amyloids

1 Introduction

The aggregation of proteins into structures known as amyloids is observed in many neurodegenerative diseases, including Alzheimer disease. Amyloids are composed of pairs of tightly interacting, stranded, and repetitive intermolecular β-sheets, which form the cross-β-sheet structure. This structure enables amyloids to grow by recruitment of the same protein, and its repetition can transform a weak biological activity into a potent one through cooperativity and avidity. Amyloids therefore have the potential to self-replicate and

Thomas Mavromoustakos and Tahsin F. Kellici (eds.), *Rational Drug Design: Methods and Protocols*, Methods in Molecular Biology, vol. 1824, https://doi.org/10.1007/978-1-4939-8630-9_1,

can adapt to the environment, yielding cell-to-cell transmissibility, prion infectivity, and toxicity [1].

Due to the enormous conformational space of the full-length protein and the limitations of computational algorithms and capacity, the aggregation as an example of protein folding has not been understood yet. Therefore, the folding and aggregation mechanism of full-length proteins is studied using short fragments as models with key amino acid sequences which have been reported to form fibrils and cause toxicity in vitro. For this reason, the 20–29 region of the human islet amyloid polypeptide (hIAPP) is often referred as the amyloidogenic core. Amyloid deposits are well known to cause as many as 20 pathological neurodegenerative disorders such as Alzheimer, Parkinson, Huntington, and Creutzfeldt-Jakob [2–4]. The shorter peptide hIAPP22–29 (NFGAILSS) is still capable of aggregating into amyloid fibrils [5, 6] (Fig. 1a).

Several studies reported a β-strand formation of key amino acid sequences that may play an important role in the aggregation process. For example, Chakraborty et al. studied the amyloidogenic structure of hIAPP peptide (19–27) [7]. They illustrated a four-residue turn spanning (22–25) adopting preferentially helix-coil and extended β-hairpin. Residues 17, 22, and 23 are found to play an important role in amyloid formation. Mo et al. investigated the structural diversity of the soluble peptide trimmer of hIAPP20–29 by MD simulations. The amorphous trimmer in room temperature contains a central structural amino acid residue part FGAIL (23–27) capable of forming interpeptide β-sheets and antiparallel β-strands with higher propensity than parallel β-strands [8].

Cao et al. performed MD simulations for the NFGAILSS motif of hIAPP associated with the type 2 diabetes. They found that the stability of an IAPP22–28 oligomer was not only related with its size but also with its morphology. The driving forces to form and stabilize the oligomers are the hydrophobic effects and backbone H-bond interactions. The simulations indicate that IAPP22–28 peptides tend to form an antiparallel strand orientation within the sheet [9]. Crystal structures of IAPP amyloidogenic segments revealed a novel packing of out-of-register β-sheets [9, 10]. Comprehensive studies have been performed to examine the effect of electron donating and withdrawing groups along with heteroaromatic surrogates at position 23 (F) of the hIAPP22–29 to interrogate how π-electron distribution affects amyloid formation [11–13].

In an attempt to understand the first principles underlying the possibility of this peptide to form closed structures, NFGAILSS was synthesized, and its conformational properties are studied in the well-dissolved d_6-DMSO and *d*-HFIP using NMR spectroscopy and in silico using molecular dynamics (MD) in the former environments and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) bilayers. These bilayers simulate the cellular membranes involved in the formation of amyloids. This is a contribution to the

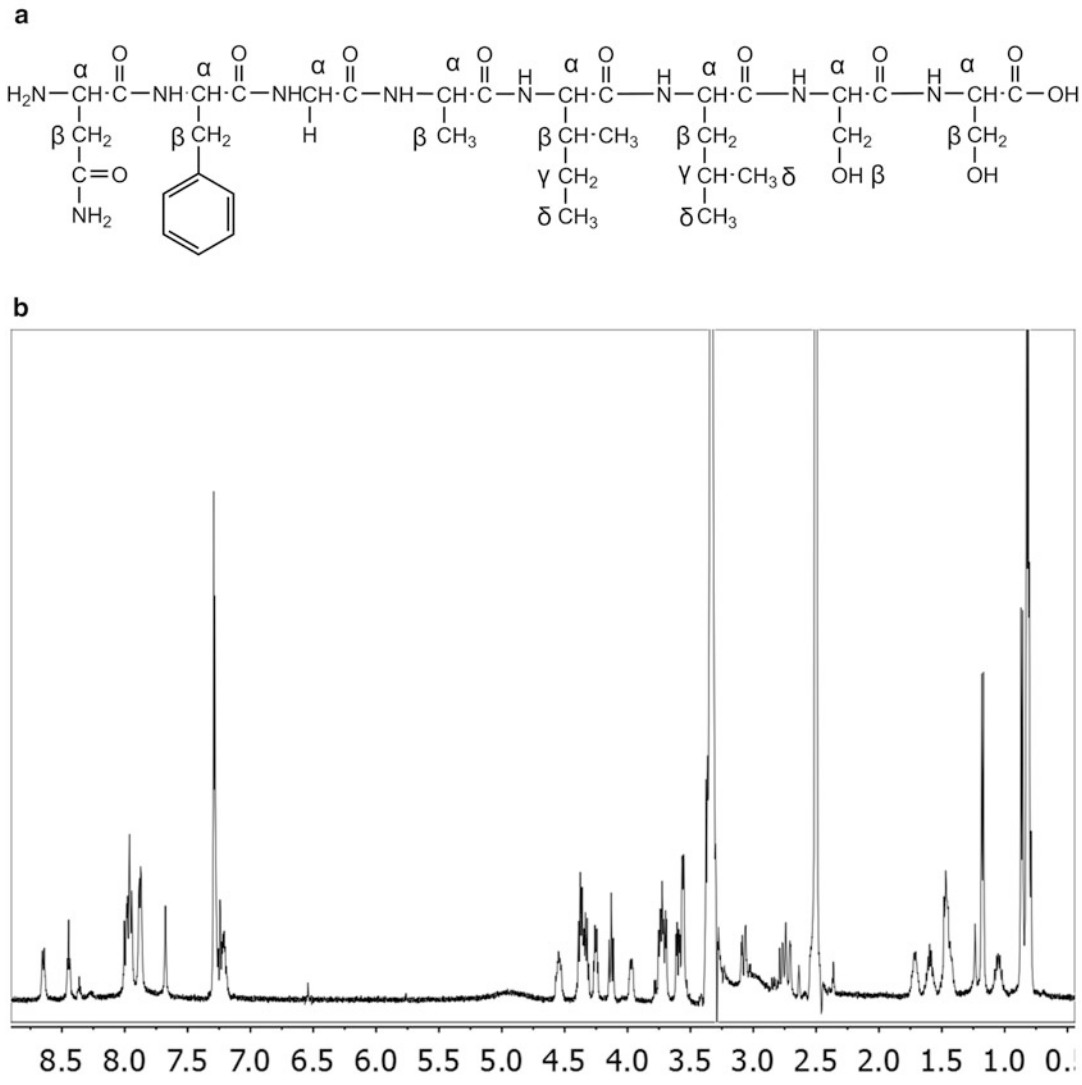

Fig. 1 (**a**) Structure of NFGAILSS; (**b**) 1H NMR spectrum of NFGAILSS run at DMSO-d_6 and using 500 MHz Agilent spectrometer at 25 °C. Through the combination of 2D TOCSY experiment (not shown), all the observed peaks are identified, and the peptide was structurally elucidated. This is an important step before proceeding to conformational studies using 2D NOESY experiments and in silico MD calculations [1]

understanding of intra- and intermolecular forces that govern the peptide NGFAILSS. NMR experiments cannot be directly utilized in lipid bilayers for hIAPP due to the rapid aggregation of the peptide, and studies are restrained in SDS micelles or solid state [14–16].

The understanding of these intra- and intermolecular forces is the driving force for our laboratories to design and synthesize novel peptide derivatives that might lack the ability of aggregation. In this chapter, the in silico MD methodologies will be described giving details for the procedure in ascending complexity systems.

In particular information of the MD simulation on the systems are as follows: (a) NFGAILSS simulated in implicit environment; (b) NFGAILSS simulated in DMSO a solvent existing in Desmond; (c) NFGAILSS simulated in the not common HFIP and not existing in Desmond; and (d) NFGAILSS in POPC bilayers.

2 Materials

For the current tutorial, we have chosen a variety of different versions in order to show that even the older versions seem quite exceptional for several specific purposes. More specifically:

Desmond Maestro, version 2011 or later (for building of the NFGAILSS oligopeptide).

Desmond Maestro, version 2013-1 or later (for energy minimization, system setup, equilibration, molecular dynamics production, and trajectory analysis and visualization).

Access at a high-performance computer cluster (HPC) is needed (for the Subheading 3.2.2 part of the tutorial).

3 Methods

The major emphasis in the chapter is given in the computational analysis using MD simulations. NMR spectroscopy was used as a complementary experimental technique to provide an initial low energy structure to start the in silico MD simulation.

3.1 NMR Spectroscopy

1. 2 mM peptide is dissolved in d_6-DMSO or d-HFIP (0.002 g peptide in 1 mL DMSO-d_6 or HFIP-d).
2. Tetramethylsilane (TMS) is added as a reference, and the sample is pipetted into a high-precision NMR tube.
3. Spectra are obtained using a 500 MHz Agilent NMR spectrometer using pulse sequence regular parameters stored in the library of the NMR spectrometer.
4. ROESY experiments are run at different mixing times (0.2–2 s) in order to ensure NOE buildups.
5. 2D TOCSY is also run at various spin locks in order to assure the best experimental conditions for observing all the expected correlations.

2D TOCSY experiment was sufficient to assign unequivocally all proton resonances appeared in the ^{1}H NMR spectrum (Fig. 1b). 2D NOESY and ROESY spectra did not provide any additional information in respect to the conformational properties of the molecule. Special care was given in the clustering of the phenyl ring of phenylalanine with the alkyl chains of leucine and isoleucine. No medium- or long-range ROEs are observed between

phenylalanine ring and isoleucine or leucine amino acids. As no ROEs have been observed between the other amino acids, it is evident that the peptide adopts an opened conformation.

3.2 MD Simulations

The following steps are applied for the conformational analysis:

1. Building the 3D structure of NFGAILSS. The building of NFGAILSS peptide is achieved using the Maestro program. A table opens, and "grow" is selected and "from fragments" the small rectangle box, named "aminoacids." (Note: The procedure, for more recent Desmond releases, is identical, *see* **Notes 1** and **2**.
2. The various amino acids are presented under the fragments small rectangle box. The amino acid (NFGAILSS) consisting the peptide is chosen for building the peptide. Once all the amino acids have been selected and checked that the fragment matches with the (NFGAILSS), hydrogen atoms are added to complete the structure.
3. Then, the selected application tab located on top of the screen is chosen and scrolled down to macromodel and apply minimization. The "Minimization" tab opens and the parameters are used (Table 1, *see* **Notes 3** and **4**).
4. In the (Potential) tab, the parameters are used (Table 2).

Table 1
Minimization parameters used to obtain low energy structure of NFGAILSS peptide

Method	Optimal
Maximum iterations	10,000
Converge on	Gradient
Convergence threshold	0.05

Table 2
Applied parameters using the potential tab for NFGAILSS peptide

Force field	OPLS_2005
Solvent	None
Changes from	Force field
Cutoff	Extended
Electrostatic treatment	Dielectric constant
Dielectric constant	46

Table 3
MD parameters used for NFGAILSS peptide

Method	Molecular dynamics
Shake	Bond to hydrogen
Simulation temperature (K)	500.0 (used different temperatures)
Time steps (fs)	1.2
Equilibrium time (ps)	1000
Simulation time (ps)	1000

Table 4
MD parameters used in the (mini) tab for NFGAILSS peptide

Method	Optimal
Maximum iterations	10,000
Convergence on	Energy
Convergence threshold	0.05

5. The following MD parameters are used (Table 3).
6. In the (mini) tab, the following parameters are used (Table 4).
7. In the application tab located on top of the screen, scroll down to macromodel, and then click on dynamics. The dynamic window should pop up.
8. In the monitor tab, the number of desirable conformations to be produced by the dynamics experiment is selected, click on minimize sampled structure, and use the already described parameters. The conformations on the project table are processed to generate the desirable conformers. The clustering is performed at the script tab and scrolled down to cheminformatics and selected conformers for cluster, *see* **Notes 5**–**7**.
9. In the case of using explicit DMSO or HFIP or POPC, the following parameters are used (Table 5).

3.2.1 Desmond-Available Solvents to Run Molecular Dynamics Simulations, Using Either Workstation or High-Performance Computing (HPC)

The most common solvent systems, DMSO, TIP3P, and TIP3P-POPC membrane, are used in this study. The procedure is explained in details.

1. After opening Maestro (Desmond 2013-1 till Desmond 2016-3 Classic Edition GUI), the .mae structure is imported in the structure/directory/path/*.mae. The following utilities are consequentially selected: Protein Preparation Wizard, Assign bond order, Add hydrogens, Remove original hydrogens, Cap termini, and Preprocess button.

Table 5
MD parameters used for NFGAILSS peptide treated with explicit solvents of DMSO or HFIP or POPC

Method	Molecular dynamics
Force field	OPLS_2005
Simulation temperature	300
Time steps (fs)	1.2
Equilibration time (ps)	1200
Simulation time (ps)	50,200
Production class ensemble	NPT
Simulation cutoff	9.0 Angstrom
Recording interval (ps)	1.2
Number of frames	41,833
Thermostat	Nose-Hoover
Barostat	Martyna-Tobias-Klein
Restraints	None
Randomized velocities	None
Equilibration stages	8
Equilibration class ensemble	NVT

2. On the left side of GUI, "also display" → "all hydrogens" is selected, and the structure is checked.
3. The hybridization is selected by clicking on "label all" → atom type (macromodel).
4. Labels are deleted. The preferred tasks are selected as follows: molecular dynamics → system setup → predefined, DMSO; distances, 15.0; force field, OPLS_2005 (or OPLS3 if possible. OPLS3 is not freely available).
5. The box-shape orthorhombic (or cubic) and box size calculation method are used: buffer and press are run. DMSO solvent is detected after some seconds in the Workspace.

3.2.2 System Setup, Using TIP3P

1. Using the same protocol above on stage 5, TIP3P instead of DMSO is pressed.
2. "Setup Membrane" → POPC → (the POPC membrane appears red) and then "adjust membrane" are chosen. The middle mouse button to turn the red membrane barriers to be parallel to our system peptide is used.
3. The "adjust membrane position" and save membrane position → OK. → Run is selected.

As soon as the membrane is presented and the peptide is embedded within the lipid bilayer, the following steps are used:

1. Tasks → molecular dynamics.
2. "Load" is pressed (otherwise, Maestro will not allow you to continue with the next steps).
3. Simulation time used is 50 ns (or 250 ns) or the simulation time you wish.
4. Ensemble class, NPT; temperature, 300 K.
5. Advanced options → output → record velocities.
6. Advanced options → miscellaneous → and "Randomize Velocities" is unclicked, and then "apply" or "OK" is pressed.
7. The reversed triangle next to the cogwheel is chosen, and to run GPU simulation, job settings → host: localhost-gpu → run. To run CPU simulation, the following are chosen:

Job settings → host: localhost, Total: enter number on the "processors" box and "run."

To run the system on a computer cluster (high-performance computer, HPC), **steps 1–7** of the above protocol are applied. At the reversed triangle (next to the cogwheel) → write. The "write" command produces a .cfg, a .cms, and an .msj file. These files should be transferred to your HPC account, using scp command of Linux. To continue, Desmond Maestro should be already installed on your HPC cluster account.

1. A pbs script using a preferred editor is created (vi, emacs, nano, etc.) that needs to include the commands below, depending on the case.
2. CPU MD job is launched:

```
$SCHRODINGER/desmond -LOCAL -WAIT -HOST localhost:number-of-
cpu-cores -JOBNAME <jobname> -c <your>.cfg -in <your>.cms
```

(Files had been produced before, by pressing the "Write" button).

3. CPU MD job is restarted as follows:

```
$SCHRODINGER/desmond -LOCAL -WAIT -JOBNAME <job-continue>
-HOST localhost:<number-of-cores> -restore <jobname>.cpt
```

CPU MD job can be extended as follows:

```
$SCHRODINGER/desmond -LOCAL -WAIT -JOBNAME <job-extension>
-HOST localhost:<number-of-cores> -restore .cpt -cfg mdsim.
last_time=<till-xyz-ns>
```

4. GPU MD job is launched:

```
env SCHRODINGER_CUDA_VISIBLE_DEVICES="0" $SCHRODINGER/utili-
ties/multisim -WAIT -LOCAL -JOBNAME $JOBNAME1 -HOST localhost
-maxjob 1 -cpu 1 -m <your-job>.msj -c <your-job>.cfg <your-
job>.cms -mode umbrella -set 'stage[1].set_family.md.
jlaunch_opt=["-gpu"] -o $JOBNAME1-out.cms &
```

and

```
env SCHRODINGER_CUDA_VISIBLE_DEVICES="1" $SCHRODINGER/utili-
ties/multisim -WAIT -LOCAL -JOBNAME $JOBNAME2 -HOST localhost
-maxjob 1 -cpu 1 -m <your-job>.msj -c <your-job>.cfg <your-
job>.cms -mode umbrella -set 'stage[1].set_family.md.
jlaunch_opt=["-gpu"]' -o $JOBNAME2-out.cms.
```

5. GPU MD job is restarted:

```
env SCHRODINGER_CUDA_VISIBLE_DEVICES="0" $SCHRODINGER/desmond
-JOBNAME job_continue -HOST localhost:1 -gpu -restore jobname.
cpt -in job_continue-in.cms &
```

and

```
env SCHRODINGER_CUDA_VISIBLE_DEVICES="1" $SCHRODINGER/desmond
-JOBNAME job_continue -HOST localhost:1 -gpu -restore jobname.
cpt -in job_continue-in.cms.
```

6. GPU MD job is restarted:

```
env SCHRODINGER_CUDA_VISIBLE_DEVICES="0" $SCHRODINGER/desmond
-JOBNAME job_extend -HOST localhost:1 -gpu -restore job_ex-
tend.cpt -in <your_md_job>.cms -cfg mdsim.last_time=<till-
xyz-ns> &
```

and

```
env SCHRODINGER_CUDA_VISIBLE_DEVICES="0" $SCHRODINGER/desmond
-JOBNAME job_extend -HOST localhost:1 -gpu -restore jobname.cpt
-in job_continue-in.cms -cfg mdsim.last_time=<till-xyz-ns>
```

The following protocol using Desmond Maestro (2013-1 or later version) for the non-common solvent hexafluoroisopropanol -HFIP is applied.

1. A cubic array is created for the solvent.
2. The molecule is imported and the title for the entry is given hexafluoroisopropanol.
3. The "project table" is opened and the entry is selected.
4. The entry is duplicated till 16 entries were created by typing Ctrl+A Ctrl+D (i.e., four times).
5. All entries are selected (Ctrl+A) and included in the Workspace (Ctrl+N).
6. "Tile" is typed in the command line box of Desmond Maestro (i.e., the command input area) in the main window. The entries are placed in a grid in the plane of the Workspace, with new coordinates. (Warning: Do not use the Tile toolbar button, as this button does not change the coordinates but only the display.)
7. In the "project table" panel, all entries are selected, and then right click on "merge." A new entry is created with the molecules from the Workspace. This must be the only selected entry.
8. All entries are deleted, apart from the new one created on stage 7.
9. The "new entry" in the Workspace is chosen and then "view" and "align."
10. The "YZ" plane is selected to align three or more atoms in a plane. "Pick for alignment" is then selected to a plane and then the same atom in three of the molecules. Do *not* pick the same atom in three molecules of the same row/column. For instance, pick two from the first row and one from the second one.
11. "Align" and then "Update Coordinates" are selected. The plane of the "square-slices" of molecules will be rotated, and the coordinates will be updated.
12. The entry is duplicated once with Ctrl+A and Ctrl+D.
13. Stages 5–7 are repeated. One entry with two "square-slices" of molecules is formed.
14. The entry is duplicated with Ctrl+A Ctrl+D, and stages 5–7 are repeated again. Now a single entry with four "square-slices" of HFIP solvent molecules that make up a $4 \times 4 \times 4$ box was created. If not, please delete what you have done so far, and restart the procedure from the beginning, i.e., from stage 1.

For smaller molecules, you should probably double the size of the box, to produce a box with dimensions approximately 30 Å. To achieve this, follow the procedure above from stage 4, using the

current entry as the original "molecule." In **step 4**, duplicate the molecule with Ctrl+A Ctrl+D a couple of times in order to create 4 entries only (rather than 16), and stop after stage 13—stage 14 can be omitted (17).

3.2.3 Molecular Dynamics of the HFIP System

Now that the box is created, Desmond can be used to create the solvent model box, as follows:

1. A model system without solvent, ions, or membrane in the system builder panel is created. The "cubic," buffer method for the box dimensions, and a distance of 1.0 (or more if you wish) are used. If the disordered system builder is used, this step is skipped, as the model system is already created.
2. The .cms file is edited and solute is changed to solvent.
3. The "molecular dynamics" option from "tasks" menu is opened.
4. In the "model system," the "import from file" from the option menu was chosen, and the .cms file is imported.
5. Ensemble class "NPT" is used.
6. Relax model system before simulation is selected (click on the "default relaxation" box. If not, the MD will probably fail during stage 4 of relaxation).
7. Simulation time used: 1 ns (but you can use the defaults if you want).
8. The simulation was started by clicking on "Run" button.

When the simulation results are incorporated, a property is added as follows:

1. "Property" and then "Add" are chosen.
2. It was named in the "num_component" and "integer" is typed.
3. "1" within the "Initial value" was added and the "add" was clicked. The internal name of the property must be edited to change the "family."

From Suite 2013-1 on the internal name in Maestro, it can be edited as follows (though editing the file is probably quicker):

1. "Property," "Columns," "Edit Name/Type," and then the property "Num_component" are selected.
2. "Num_component" in the visible name text box is entered, and "edit internal data" is selected.
3. In the "Family option" menu, "other" is selected. Enter "ffio" within the box. Finally, click on "Save Changes."
4. The HFIP solvent entry is exported to a .mae file "... and this .mae file will be used as a custom solvent file."

3.3 Results Summary

As the NMR spectroscopy did not provide any constraints, the molecule is built and minimized using the available minimization algorithms of Schrodinger Maestro until the energy convergence threshold of 0.05 Kcal mol^{-1} was achieved. The minimized structure was subjected to MD simulations using dielectric constant $\varepsilon = 46$ that simulates DMSO environment. The conformers obtained with equal propensity for both the "opened" and the "closed" structure (*see* Fig. 2).

Molecular dynamics experiments in explicit solvent DMSO showed the open structure of the molecule in accordance with NMR data. In 80% of the trajectory, isoleucine and leucine were far away (Fig. 3a) and only 20% were in proximity (Fig. 3b). Phenylalanine was far away from all amino acids and did not have any spatial correlations with isoleucine and leucine in accordance with NMR results.

Details of the clusters generated in the explicit HFIP solvent (not shown for simplicity) are depicted in Fig. 4b. Similar trends are found as with DMSO, and again all these clusters are in accordance with NMR results. The above clusters in the two solvents and NMR results clearly show the following: (a) in a polar environment Leu, Ile, and Phe are not in spatial proximity; (b) the peptide is most of the time in a linear form and when it adopts turns the key three amino acids Leu, Ile, and Phe not in a spatial proximity; and (c) Leu and Ile have a freedom to be far away or in spatial proximity.

MD simulations were applied in a more biologically relevant environment of POPC bilayers. In Fig. 5 are shown four clusters of the peptide (POPC bilayer is not shown for simplicity), and in Fig. 6 a snapshot of the whole system is observed.

NFGAILSS is clearly shown that in vacuum or in a membrane simulating environment can adopt hydrophobic interactions between Phe and Ile or Leu that enhance its ability to aggregate. These interactions are not observed when a polar environment is used as it is shown both experimentally by NMR spectroscopy using the two solvents DMSO or HFIP or MD simulations using the same explicit solvents. This information is valuable for drug design and discovery as it points out that (a) the environment may be decisive to aggregation for the peptide NFGAILSS, (b) the hydrophobic interactions between the key amino acids Phe and Ile or Leu may play a pivotal role in this aggregation, and (c) it provides means of using structural modifications to avoid the undesired aggregation.

4 Notes

1. The chirality of the structure was checked, and it was confirmed that all amino acids bear S configuration.