Abstract

Recent Progress in Molecular Recognition Imaging of Protein Systems at the Nanoscale Level †

Carlos Marcuello [1,2,*] and Anabel Lostao [1,2,3,*]

1 Instituto de Nanociencia y Materiales de Aragón (INMA), CSIC-Universidad de Zaragoza, 50009 Zaragoza, Spain
2 Laboratorio de Microscopias Avanzadas (LMA), Universidad de Zaragoza, 50018 Zaragoza, Spain
3 Fundación ARAID, 50018 Zaragoza, Spain
* Correspondence: cmarcuel@unizar.es (C.M.); aglostao@unizar.es (A.L.)

† Presented at the 2nd International Electronic Conference on Biomolecules: Biomacromolecules and the Modern World Challenges, 1–15 November 2022; Available online: https://iecbm2022.sciforum.net/.

Keywords: molecular recognition imaging; atomic force microscopy; functionalized tips; adhesion maps; protein–protein interactions; single molecule level; jumping mode

Citation: Marcuello, C.; Lostao, A. Recent Progress in Molecular Recognition Imaging of Protein Systems at the Nanoscale Level. *Biol. Life Sci. Forum* **2022**, *20*, 22. https://doi.org/10.3390/IECBM2022-13722

Academic Editor: Cristina Martínez-Villaluenga

Published: 21 November 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

Identification of proteins has received considerable attention in recent years due to the increasing interest in resolving individual biomolecules under physiologically relevant conditions. In this framework, atomic force microscopy (AFM) has shown great potential to acquire a variety of biomolecular physico-chemical properties at the single molecule level [1]. Particularly, force spectroscopy based on AFM (AFM-FS) allows the intermolecular interactions between two biomolecules to be determined, requiring one to be covalently immobilized on a flat surface and the other linked to the AFM tip. Previous work was developed in this field by simultaneous topography and recognition imaging (TREC) [2] and tuning-fork-based transverse dynamic force microscopy (TDFM) [3], although both methods lack quantitive information. To overcome the aforementioned limitations, force–volume (F-V) [4] emerged as promising alternative, but the extremely large data acquisition times can lead to drifting effects during the image recording. Here, we present the intermittent jumping force mode (JM) as suitable approach to gathering quantitative high-resolution force maps at local areas of the scanned sample with fast-acquisition times. Using this mode, by applying very low forces under respulsive regime conditions, simultaneous maps of topography and specific rupture forces corresponding to the unbinding of the protein:ligand complexes are obtained. Two different protein systems are employed to illustrate the capabilities of the built-up methodological improvements. First, the flavoenzyme system formed between flavodoxin NADP$^+$ reductase (FNR) and its redox partners, ferredoxin and flavodoxin [5,6], and second, the strongest non-covalent complexes observed in nature between avidin and streptavidin and biotin [7]. In the first case, the results were optimized when an oriented immobilization procedure was designed. In the second work, discrimination between avidin and streptavidin molecules in a hybrid sample was achieved with a unique sensor ligand. The most relevant scientific outcomes can serve as a proof-of-principle stage to design diagnostic devices with an ultra-sensitivity detection signal for drug screening applications.

Supplementary Materials: The presentation material of this work is available online at https://www.mdpi.com/article/10.3390/IECBM2022-13722/s1.

Author Contributions: Conceptualization, A.L.; methodology, C.M. and A.L.; validation, C.M. and A.L.; formal analysis, C.M. and A.L.; investigation, C.M. and A.L.; resources, A.L.; data curation, C.M.; writing-original draft preparation, C.M. and A.L.; writing-review and editing, C.M. and A.L.; supervision, A.L.; project administration, A.L.; funding acquisition, A.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by grant PID2019-103901GB-I00 funded by MCIN/AEI/10.13039/501100011033, grant QTP2103003 funded by CSIC, and grants E35_20R and LMP58_18 funded by the Government of Aragón-FEDER.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Additional data is available upon reasonable request.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

1. Müller, D.J.; Dumitru, A.C.; Lo Giudice, C.; Gaub, H.E.; Hinterdorfer, P.; Hummer, G.; De Yoreo, J.J.; Alsteens, D. Atomic Force Microscopy-Based Force Spectroscopy and Multiparametric Imaging of Biomolecular and Cellular Systems. *Chem. Rev.* **2020**, *121*, 11701–11725. [CrossRef] [PubMed]
2. Stroh, C.M.; Ebner, A.; Geretschlager, M.; Freudenthale, G.; Kienberger, F.; Kamruzzahan, A.S.M.; Smith-Gill, S.J.; Gruber, H.J.; Hinterdorfer, P. Simultaneous topography and recognition imaging using force microscopy. *Biophys. J.* **2004**, *87*, 1981–1990. [CrossRef] [PubMed]
3. Hofer, M.; Adamsmaier, S.; van Zanten, T.S.; Chtcheglova, L.A.; Manzo, C.; Duman, M.; Mayer, B.; Ebner, A.; Moertelmaier, M.; Kada, G.; et al. Molecular recognition imaging using tuning-fork based transverse dynamic force microscopy. *Ultramicroscopy* **2010**, *110*, 605–611. [CrossRef] [PubMed]
4. Ludwig, M.; Dettmann, W.; Gaub, H.E. Atomic force microscope imaging contrast based on molecular recognition. *Biophys. J.* **1997**, *72*, 445–448. [CrossRef] [PubMed]
5. Marcuello, C.; De Miguel, R.; Gómez-Moreno, C.; Martínez-Júlvez, M.; Lostao, A. An efficient method for enzyme immobilization evidenced by atomic force microscopy. *Protein Eng. Des. Sel.* **2012**, *25*, 715–723. [CrossRef] [PubMed]
6. Marcuello, C.; de Miguel, R.; Martínez-Júlvez, M.; Gómez-Moreno, C.; Lostao, A. Mechanostability of the Single-Electron-Transfer Complexes of Anabaena Ferredoxin-NADP$^+$ Reductase. *ChemPhysChem* **2015**, *16*, 3161–3169. [CrossRef] [PubMed]
7. Marcuello, C.; De Miguel, R.; Lostao, A. Molecular Recognition of Proteins through Quantitative Force Maps at Single Molecule Level. *Biomolecules* **2022**, *12*, 594. [CrossRef] [PubMed]