Susana Rocha

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Born in Torres Novas, Portugal 26-01-1983



Education

KU Leuven, (Leuven, Belgium)

PhD in Chemistry, *May 2014 Dissertation Topic:* "Single enzyme kinetics"

Instituto Superior Técnico, (Lisbon, Portugal)

Licenciatura (Bachelor and Master) in Chemistry, September 2006 (classification 17/20)

Scientific Career

2014.10-present: Postdoctoral Fellow (FWO)

Molecular Imaging and Photonics, Department of Chemistry, KU Leuven, Belgium

2017.03-2017-06: Visiting Researcher (FWO travel grant)

Molecular Materials, Faculty of Science, Radboud University, Nijmegen, The Netherlands

2014.05-2014.09: Postdoctoral Researcher

Molecular Imaging and Photonics, Department of Chemistry, KU Leuven, Belgium

Research Interests & Expertise

- Cell-matrix interactions and the influence of mechanical stimuli in cellular behavior (expertise
 in molecular cloning, fluorescent labelling, cell culture, transient expression of specific proteins
 and gene editing).
- Structural and mechanical characterization of polymers and hydrogels (using advanced fluorescence microscopy and micro-rheology).
- Interaction between proteins (enzymes, receptors, structural proteins) and the cellular membrane.
- Membrane organization (micro-domains, rafts) and cell signaling.
- Fluorescence microscopy and spectroscopy, more concretely single molecule fluorescence microscopy (single particle tracking, FRET, super-resolution microscopy).
- Software development (using Matlab®)

Invited talks and seminars

- Invited seminar at TU Eindhoven, October 2018, *Imaging the forces driving cellular behaviour*, Eindhoven (The Netherlands)
- Invited talk at the workshop 'Imaging interactions with fluorescence: from the Nano-to-Macro scale', Single-molecule Fluorescence Microscopy: a powerful tool to study proteinprotein interactions September 2018, Hasselt (Belgium)
- Invited seminar at Institute of Molecular Materials, Single-molecule Fluorescence Microscopy: a powerful tool to study molecular dynamics and organization, March 2017, Nijmegen (The Netherlands)

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- Invited seminars at the course of 'Nanobiology' (KU Leuven), Labels and Labelling techniques, March 2018 and February 2017, Leuven (Belgium)
- Invited talk at the Annual meeting of the Belgium Society for Microscopy, *Single Molecule Enzymatics*, September 2016 (Best PhD thesis award)
- Invited talk at the workshop 'Single-molecule fluorescence microscopy meets virology', *Tetherin-HIV interaction*, June 2016, Leuven (Belgium)
- Invited seminar at the symposium 'Groningen Meets Leuven: A One-Day Symposium', Phospholipase C activity visualized by single particle tracking, June 2011, Leuven (Belgium)

In addition, I have given a total of **8 oral presentations** at national and international conferences and have received an award for best student talk (full list at the end of the document).

Research Project Financing

- FWO research grant: "Linking properties of biomimetic polymer matrices to stem cell fate: a microscopy study", **36k€** (promotor: myself), 2018-2020
- FWO project: "Polymer dynamics studied by super-resolution microscopy", **499** k€ (promotor: prof. Johan Hofkens, co-promotors: prof. Peter Dedecker and myself), 2017-2021
- Internal KU Leuven funding, C1 project: "Next-generation microscopy methods for unraveling complex signaling pathways", **583 k€** (promotor: prof. Hideaki Mizuno, co-promotors: Dr. Jelle Hendrix and myself), 2016-2020

In Abril 2018 I have applied for an additional FWO research grant as promotor ("Imaging the forces driving cancer progression", **39 k€**) and a FWO project as co-promotor ("Drug/gene combination delivery system for reversal of multi-drug resistance", **449 k€** promotor: Prof. Hiroshi Uji-i). In addition, 4 out of the 5 PhD students working under my supervision are receiving personal fellowships.

Honors and Awards

2017 Travel grant for a 4-month research stay at Radboud University, Nijmegen (NL), FWO

2016 Best PhD thesis award in the area of Life Sciences, Belgian Society of Microscopy

2007 Best student talk at Single Molecule Spectroscopy Workshop, PicoQuant

2005 Merit Scholarship for excellence in Chemistry studies, Caixa Geral Depósitos¹

2004 Merit Scholarship for excellence in Chemistry studies, Universidade Técnica de Lisboa²

2003 Merit Scholarship for excellence in Chemistry studies, Universidade Técnica de Lisboa²

2002 Merit Scholarship for excellence in Chemistry studies, Universidade Técnica de Lisboa²

Fellowships

2015 Post-doctoral fellowship by FWO (success rate 25%)

2014 Post-doctoral fellowship by KU Leuven (BOF-PDM, success rate 31%)

2006 PhD fellowship from the Portuguese Foundation for Science and Technology (FCT, ranked first for the area of Chemistry, success rate 51%)

2005 Erasmus scholarship for 9-month stay at KU Leuven, Belgium

Teaching Activities

2018

Theoretical lectures on the course "Advanced Light Microscopy" in Hasselt University, on the subjects of super-resolution imaging, single particle tracking and *in vivo* labelling.

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¹ one grant awarded for the second best out of the 200 students of the Chemistry undergraduate program

² one grant awarded for the best out of the 200 students of the Chemistry undergraduate program

2015-present

Theoretical lecture(s) on the course "Advanced Fluorescence Microscopy" in KULeuven (single molecule microscopy, GFP, fluorescent labels, *in vivo* labelling and CRISPR/Cas9 gene editing).

2008-present

Practical class on the course "Advanced Fluorescence Microscopy" in KULeuven on the subjects of super-resolution imaging and single particle tracking

Scientific Output

- 38 articles (5 first author, 4 corresponding author) in peer-reviewed internationally recognized journals including top journals such as Cell, Nature, Nature Communications, Nature Nanotechnology, Langmuir, ACS Nano and PLoS Pathogens. According to Web of Science citation report on September 2018, these contributions have been collectively cited almost 1000 times, with an average of ca. 30 citations per item. This has resulted in a current h-index of 15. I also published 4 book chapters, 2 of which as first author. The complete list of publications can be found at the end of the document.
- Development of freely accessible and user-friendly software for advanced image analysis for different groups within KULeuven and other universities. Some of the most used software includes super-resolution algorithms based on single molecule localization, single particle tracking, FRET using wide-field, AFM/fluorescence correlation among many others.
- Scientific communication: 5 invited talks/seminars, 8 abstract-selected talks, 5 abstract-selected poster presentations (complete list at the end of the document).

Main Publications

• 38 articles (5 first author, 4 corresponding author) in peer-reviewed internationally recognized

International mobility

- Planned 3 month stay at the laboratory of Dr. Rodrigo Barderas (Instituto Salud D. Carlos III, Madrid, Spain), from November 2018 to February 2019 (requested EMBO short-term fellowship and FWO travel grant, awaiting decision)
- On March of 2017 I went for 4 months to the group of Prof. Paul Kouwer (Radboud University Nijmegen, The Netherlands) to learn how to synthesize and manipulate PIC-based hydrogels. From this visit we started a collaboration, involving the exchange of 2 PhD students and several ongoing projects.
- On January of 2009 I went to laboratory of prof. Marcus Sauer (Bielefeld University, Germany),
 where I learned direct stochastic optical reconstruction microscopy (dSTORM). With the
 knowledge acquired there, I returned to the laboratory of prof. Johan Hofkens and
 implemented this new method for super-resolution fluorescence image. dSTORM is currently
 applied in the laboratory as a standard imaging technique.

Supervision

I am currently supervising 5 PhD students and am in the supervisory committee of additional 3 students. In the past I have been co-promotor of 1 PhD student (finished on March 2017) and have been part of the examination committee of 2 other PhD students.

Current PhD students

• Johannes Vandaele, Chemistry Department, KU Leuven

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- "Studying nanoscale heterogeneities and dynamics of new biomimetic polymer gels using advanced microscopy", <u>co-promotor</u> (started November 2017, promotor: prof. Johan Hofkens)
- Indra van Zundert, Chemistry Department, KU Leuven (FWO grant)
 "Next-generation cancer therapy: gene editing meets nanotechnology", co-promotor (started October 2017, promotor: prof. Hiroshi Uji-i)
- Boris Louis, Chemistry Department, KU Leuven (FWO grant)
 "Fluorescence microscopy: a powerfull tool to unravel polymer dynamics", co-promotor (started January 2017, promotor: prof. Johan Hofkens)
- Danai Laskaratou, Chemistry Department, KU Leuven (FLOF grant)
 "Regulation of cell signaling via pseudokinase ErbB3 receptors", co-promotor (started October 2016, promotor: prof. Hideaki Mizuno)
- Monica Ricci, Chemistry Department, KU Leuven (FWO-SB grant)
 "Nanowire based intracellular delivery: a versatile tool for gene therapy", co-promotor (started October 2015, promotor: prof. Hiroshi Uji-i)

Other students

- Master thesis (co-)promotor of 3 students (2 more foreseen for the academic year 2018/2019)
- Master internship (co-)promotor of 6 students between 2011 and 2013 (Master Chemistry and Master Nanotechnology)
- Master thesis daily supervisor of 3 master students between 2007 and 2010
- Bachelor thesis promotor of 9 students (KULeuven and Hasselt University)

Collaborations

Current Collaborations

- Prof. Paul Kouwer (Radbound University Nijmegen, The Netherlands): study of biomimetic polymer hydrogels using microscopy
- Prof. Theo Lasser (École Fédérale de Lausanne, Switzerland) and prof. Peter Dedecker (KULeuven): development of a multi-plane wide field microscope
- Prof. Hans van Oosterwyck (KU Leuven) and Prof. Jelle Hendrix (Hasselt University / KULeuven): using FRET sensors to investigate cellular forces during angiogenesis
- Dr. Rodrigo Barderas (Instituto de Salud Carlos III, Madrid, Spain): investigating the role of mechanical forces in the proteomics of cancer
- Prof. Adrian Ranga (KU Leuven): using synthetic materials to direct stem cell differentiation
- Prof. Hideaki Mizuno (KU Leuven) and Prof. Jelle Hendrix (Hasselt University / KU Leuven): development of fluorescence fluctuation based microscopy methods to study protein-protein interactions (granted KU Leuven internal funding)
- Prof. Marcel Ameloot (Hasselt University) and prof. Jochen Meier (TU Braunschweig): analysis of the dynamics and organization of the glycine receptor at the single molecule level; the collaboration is enclosed in the KU Leuven project with prof. Mizuno and Dr. Hendrix.
- Prof. Zeger Debyser (KU Leuven): fluorescence imaging of (single) HIV viral particles

Past collaborations

- European 6th Framework Program "BIOscope: Self reporting biological nanosystems to study and control bio-molecular mechanisms at the single molecule level". Collaboration with Lund University, Novozymes AS, University of Copenhagen, Radboud University Nijmegen and University of Liverpool;
- Prof. Vicent Piguet (now at Cardiff University): super-resolution imaging of HIV-1 virions (resulted in one publication);
- Prof. Karen Vanhoorelbeke (Thrombosis Research, KULeuven): analysis of the mode of action of ADAMTS13 at the single cell/molecule level (resulted in 2 published articles).

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- Prof. Patricia Agostinis (KULeuven): imaging of molecular interactions and protein distribution of the ER-stress sensor PERK (1 publication)
- Prof. Steven De Feyter and Dr. Willem Vanderlinden: correlative AFM/fluorescence imaging of DNA/protein complexes (1 publication and 1 book chapter)

Commissions of trust

I review papers on a regular basis for different peer-reviewed scientific journals, such as Scientific Reports, PlosOne, Angewandte Chemie and IEEE Access.

Organization of conferences

I have helped the practical organization of International Conference of Photochemistry (ICP2013, Leuven, 2013) and of the Methods and Applications in Fluorescence (MAF15, Bruges, 2017).

References

- Prof. Johan Hofkens, Molecular Imaging and Photonics, KU Leuven, Belgium johan.hofkens@kuleuven.be
- Prof. Hiroshi Uji-i, Research Institute for Electronic Science, Hokkaido University, Japan hiroshi.ujii@es.hokudai.ac.jp

Career Breaks

During my scientific career I have had three career breaks, all related to parenthood.

On April of 2013 I gave birth to my first child. Due to some complications during my pregnancy, 3 months before the delivery I worked from home, developing software for image analysis. After the birth, I stayed at home for an additional period of 3 months (Abril 2013 to July 2013).

On my second pregnancy there were no complications, and my career break was only the 15 weeks allowed in Belgium (from July 2015 until October 2015).

After a 4-month stay abroad in 2017, I decided to take 3 months of parental leave to spend more time with my family (from July to September of 2017).

In addition to these extended career breaks, due to the limited familiar support arising from living abroad, between 2013 and 2017 I have reduced the travel to conferences and symposium to a minimum. Nowadays I am more engaged in scientific discussions (in 2018 I will attend at least 4 conferences).

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Complete List of Publications and Communications

Papers in international scientific periodicals with referees

- (1) Ross, S.R., Rodríguez-Mora, S., De Wit, F., Bermejo, M., López Huertas, M.R., Mateos, E., Marti, P., Rocha, S., Christ, F., Debeyser, Z., Padilla, J.J.V., Coiras, M., Alcamí, J., The mutation of transportin-3 gene that causes limb girdle muscular dystrophy 1F induces protection against HIV-1 infection, *submitted*
- (2) Ripoll-Lorente, C., Resa, S., Miguel, D., Puente-Muñoz, V., Herrero-Foncubierta, P., Paredes, J.M., Ruedas-Rama, M.J., Martin, M., Roldan, M., Rocha, S., Dekeersmaecker, H., Hofkens, J., Cuerva, J.M., Orte, A., Thiophene ring as a new and noncharged carrier to mitochondria, submitted
- (3) Fortuni, B., Inose, T., Ricci, M., Fujita, Y., Masuhara, A., Fron, E., Mizuno, Hideaki, M., Latterini, L., Rocha, S. □, Uji-i, H., Layer-by-layer surface modification of nanoparticles for highly efficient multifunctional drug delivery systems, *Scientific Reports, under revision*
- (4) De Keersmaecker, H., Camacho, R., Rantasa, D.M., Fron, E., Uji-i, H., Mizuno, H., Rocha, S.

 Mapping transient protein interactions at the nanoscale in living mammalian cells, ACS Nano,
 DOI: 10.1021/acsnano.8b01227
- (5) Eelen, G., Dubois, C., Cantelmo, A.R., Goveia, J., Brüning, U., DeRan, M., Jarugumilli, G., van Rijssel, J., Saladino, G., Comitani, F., Zecchin, A., Rocha, S., Huang, H., Vandekeere, S., Kalucka, J., Lange, C., Morales-Rodriguez, F., Cruys, B., Treps, L., Ramer, L., Vinckier, S., Brepoels, K., Wyns, S., Souffreau, J., Schoonjans, L., Lamers, W., Wu, Y., Haustraete, J., Hofkens, J., Liekens, S., Cubbon, R., Ghesquière, B., Dewerchin, M., Gervasio, F.L., van Buul, J., Wu, X., Carmeliet, P., Unexpected role of glutamine synthetase in vessel sprouting, *Nature*, 561, pp. 63 (2018)
- (6) Parveen, N., Borrenberghs, D., Rocha, S. Hendrix, J., Single Viruses on the Fluorescence Microscope: Imaging Molecular Mobility, Interactions and Structure Sheds New Light on Viral Replication, *Viruses*, 10(5), pp. 250 (2018)
- (7) Dirix, L., Kennes, K., Fron, E., Debeyser, Z., van der Auweraer, M., Hofkens, J., Rocha, S.

 Photoconversion of Far-Red Organic Dyes: Implications for Multicolor Super-Resolution Imaging, ChemPhotoChem, 2, pp. 433 (2018)
- (8) Frederickx, W., Rocha, S., Fujita, Y., Kennes, K., De Keersmaecker, H., De Feyter, S., Uji-i, H., Vanderlinden, W., Orthogonal Probing of Single-Molecule Heterogeneity by Correlative Fluorescence and Force Microscopy, *ACS Nano*, 12(1), pp.168 (2018)
- (9) van Vliet, A.R., Giordano, F., Gerlo, S., Segura, I., Van Eygen, S., Molenberghs, G., Rocha, S., Houcine, A., Derua, R., Verfaillie, T., Vangindertael, J., De Keersmaecker, H., Waelkens, E., Tavernier, J., Hofkens, J., Annaert, W., Carmeliet, P., Samali, A., Mizuno, H., Agostinis, P., The ER Stress Sensor PERK Coordinates ER-Plasma Membrane Contact Site Formation through Interaction with Filamin-A and F-Actin Remodeling, *Molecular Cell*, 65(5), pp. 885-899 (2017)
- (10) De Keersmaecker, H., Fron, E., <u>Rocha, S.,</u> Kogure, T., Miyawaki, A., Hofkens, J. and Mizuno, H., Photoconvertible property of LSSmOrange applicable for single emission band optical highlighting, *Biophysical Journal*, 5(6), pp.1014 (2016)

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- (11) Borrenberghs, D.*, Dirix, L.*, De Wit, F., Rocha, S., Blokken, J., De Houwer, S., Gijsbers, R., Christ, F., Hofkens, J., Hendrix, J. and Debyser, Z., Dynamic integrase oligomerization orchestrates HIV nuclear entry, *Scientific Reports*, 6, 36485 (2016)
- (12) Su, L., Yuan, H., Lu, G., Rocha, S., Orrit, M., Hofkens, J. and Uji-I, H. Super-resolution localization and defocused fluorescence microscopy on resonantly coupled single-molecules single-nanorods hybrids, *ACS Nano*, DOI: 10.1021/acsnano.5b07294
- (13) Fron, E., De Keersmaecker, H., Rocha, S., Baeten, Y., Lu, G., Uji-i, H., Van der Auweraer, M., Hofkens, J., Mizuno, H., Mechanism Behind the Apparent Large Stokes Shift in LSSmOrange Investigated by Time-Resolved Spectroscopy, *The Journal of Physical Chemistry B*, 119 (47), pp. 14880-91 (2015)
- (14) Vangindertael, J., Beets, I., <u>Rocha, S.</u>, Dedecker, P., Schoofs, L., Vanhoorelbeeke, K., Hofkens, J., Mizuno, H., Super-resolution mapping of glutamate receptors in C. elegans by confocal correlated PALM, *Scientific Reports*, <u>5</u>, 13532 (2015)
- (15) Rocha, S., De Keersmaecker, H., Uji-i, H., Hofkens, J., Mizuno, H. Photoswitchable fluorescent proteins for superresolution fluorescence microscopy circumventing the diffraction limit of light, *Methods in Molecular Biology*, 1076, pp. 793-812 (2014)
- (16) Su, L., Lu, G., Kenens, B., Rocha, S., Fron, E., Yuan, H., Chen, C., Van Dorpe, P., Roeffaers, M.B.J., Mizuno, H., Hofkens, J., Hutchison, J.A., Uji-i, H., Visualization of molecular fluorescence point spread functions via remote excitation switching fluorescence microscopy, *Nature Communications*, 6, 6287 (2015)
- (17) Lu, G., De Keersmaecker, H., Su, L., Kenens, B., Rocha, S., Fron, E., Chen, C., Van Dorpe, P., Mizuno, M., Hofkens, J., Hutchison, J.A., Uji-i, H., Live-Cell SERS Endoscopy Using Plasmonic Nanowire Waveguides. *Advanced Materials* 26(30), pp. 5124-28 (2014)
- (18) Borrenberghs, D., Thys, W., <u>Rocha, S.</u>, Demeulemeester, J., Dedecker, P., Hofkens, J., Debyser, Z., Hendrix, J. Viruses as nanoscopic test tubes for quantifying protein-protein interactions: Feasibility and application to the HIV-1 integrase enzyme oligomerization. *ACS Nano*, <u>8</u>(4), pp.3531-45 (2014)
- (19) De Houwer, S., Demeulemeester, J., Thys, W., <u>Rocha, S.</u>, Dirix, L., Gijsbers, R., Christ, F., Debyser, Z. The HIV-1 Integrase Mutant R263A/K264A Is 2-fold Defective for TRN-SR2 Binding and Viral Nuclear Import, *Journal of Biological Chemistry*, <u>289</u>(36), pp. 25351-61 (2014)
- (20) Hutchison , J., Uji-I, H., Deres, A., Vosch, T., <u>Rocha, S.</u>, Mueller, S., Bastian, A., Enderlein, J., Nourouzi, H., Chen, L., Herrmann, A., Muellen, K., De Schryver, F., Hofkens, J., A surface-bound molecule undergoing optically-biased Brownian rotation. *Nature Nanotechnology*, <u>9</u>(2), pp. 131-136 (2014)
- (21) Notelaers, K., Rocha, S., Paesen, R., Swinnen, N., Vangindertael, J., Meier, J.C., Rigo, J.M., Ameloot, M., Hofkens, J., Membrane distribution of the glycine receptor alpha3 studied by optical super-resolution microscopy, *Histochemistry and Cell Biology*, 142(1), pp.79-90 (2014)
- (22) Ghosh, A., Passaris, I., Mebrhatu, M. T., <u>Rocha, S.</u>, Vanoirbeek, K., Hofkens, J., Aertsen, A., Cellular localization and dynamics of the Mrr Type IV restriction endonuclease of *Escherichia coli*, *Nucleic Acid Research*, <u>42</u>(6), pp.3908-18 (2014)

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- (23) Rocha, S., De Keersmaecker, H., Vanhoorelbeke, K., Martens, J.A., Hofkens, J., Uji-i, H., Membrane remodeling processes induced by phospholipase action. *Langmuir*, 30(16), pp.4743-51 (2014)
- (24) Rocha, S.*, De Ceunynck, K.*, De Meyer, S.F., Sadler, E., Uji-i, H., Deckmyn, H., Hofkens, J., Vanhoorelbeke, K., Single particle tracking of ADAMTS13 (a disintegrin and metalloprotease with thrombospondin type-1 repeats) molecules on endothelial von Willebrand factor strings, *Journal of Biological Chemistry*, 289(13), pp.8903-15 (2014)
- (25) Moeyaert, B., Bich, N.N., de Zitter, E., Rocha, S., Clays, K., Mizuno, H., van Meervelt, L., Hofkens, J., Dedecker, P. A green-to-red photoconver-tible Dronpa mutant for multimodal superresolution fluorescence microscopy. *ACS Nano*, 8(2), pp. 1664-73 (2014)
- (26) Fron, E., Sliwa, M., Adam, V., Michiels, J., Rocha, S., Dedecker, P., Hofkens, J., Mizuno, H. Excited state dynamics of photoconvertible fluorescent protein Kaede revealed by ultrafast spectroscopy. *Photochemical & Photobiological Sciences*, 13, pp.867-874 (2014)
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- (34) De Ceunynck, K., Rocha, S., Feys, H.B., De Meyer, S.F., Uji-i, H., Deckmyn, H., Hofkens, J., Vanhoorelbeke, K. Local elongation of endothelial cell anchored von Willebrand factor strings precedes ADAMTS13 mediated proteolysis. *Journal of Biological Chemistry* 286(42) pp.36361-36367 (2011)
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 - * authors with equal contribution; [™] corresponding author

Chapters in books

- (1) De Keersmaecker, H., Frederickx, W. Fujita, Y., De Feyter, S., Uji-i, H., Rocha, S. → Vanderlinden, W., Correlative Atomic Force and Single-Molecule Fluorescence Microscopy of Nucleoprotein Complexes in Nanoscale Imaging in Methods Molecular Biology (Springer), accepted for publication
- (2) Rocha, S., De Keersmaecker, H., Fron, E., Uji-i, H., Hofkens, J., Mizuno, H., Membrane distribution, organization and dynamics of EGF receptor studied by super-resolution imaging in Super-resolution imaging in Medicine and Biology (Taylor & Francis), 2016, chap 17, pages 347-371
- (3) Blank, K., Rocha, S., De Cremer, G., Roeffaers, M.B.J., Uji-i, H., Hofkens, J. Watching individual enzymes at work in Single Molecule Spectroscopy In Chemistry, Physics And Biology (Elsevier), 2010, vol. 96 Pages 495-511

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- (1) Louis, B., Vandaele, J., Liu, K., Kouwer, P., Camacho, R., Rocha, S., *Microscopy toolbox for structural and mechanical characterisation of new biomimetic materials*, Oral presentation at the Annual Dutch meeting on Molecular and Cellular Biophysics, 2-3 October 2018, Veldhoven, (the Nederlands)
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- (6) Rocha, S., Hutchison, J.A., Hofkens, J., Uji-i, H., Oral communication 'watching single enymes at work', on Young scientists towards the challenges of modern technology, Warsaw (Poland), 21-23 September 2009
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