Hybrid nanoscopy of hybrid nanomaterials

Patricia Bondia,[1] Santiago Casado,[1] Rocío Jurado,[2] José M. Domínguez-Vera,[2] Natividad Gálvez,[2] Cristina Flors[1]

[1]IMDEA Nanoscience, Ciudad Universitaria de Cantoblanco. Faraday 9, 28049, Madrid, Spain

[2]Department of Inorganic Chemistry, University of Granada. Av. Fuentenueva Fuente s/n, 18071, Granada, Spain.

Email: patricia.bondia@imdea.org

Recently, research in the field of new nanomaterials has focused its attention on proteins and peptides as nanoscale building blocks that provide a simple route to fabricate structures from zero to three-dimensions (0–3D) [1]. These structures are rich in functional groups allowing specific coupling with different molecules, which is an attractive approach for templating nanoscale elements [2]. In particular, amyloid-like fibers are emerging as an important class of functional materials since they stand out for their rigidity, mechanical strength and controllable fabrication process [3]. Moreover, they have shown excellent properties as templates for the production of 1D inorganic nanostructures [4]. The combination of fibers with inorganic particles confers these hybrid nanomaterials extraordinary properties such as conductivity, magnetism, and complex optical responses [5].

With the aim of improving the design of these hybrid materials, different complementary techniques are necessary to gain a complete picture of their structure and function. Here we applied atomic force microscopy (AFM), which offers high spatial resolution images and topography of the sample surface, in combination with super-resolution fluorescence microscopy to characterize luminescent amyloid-like fibrils. These fibers were made by heat and pH-denatured β-lactoglobulin (βLG) functionalized with organic fluorophores (Alexa488) or quantum dots (QD) [3]. Both fluorescence molecules possess specific photophysical properties, namely photoswitching or blinking, enabling to perform super-resolution fluorescence microcopy by single molecule localization techniques [6]. We show that hybrid nanoscopy is specially useful to optimize super-resolution image reconstruction since the topography image is used as a “ground truth” [7]. This is particularly important in the case of QDs, which show suboptimal blinking. Importantly, the correlative images provide nanoscale insight into the structure and functionalization of this hybrid material revealing heterogeneous labeling of Alexa488-fibrils which was not detected by standard fluorescence microscopy. Finally, we have achieved two-color super-resolution fluorescence imaging in correlation with topography to characterize fibrils functionalized with QDs of different color. This is the first time that the latter study has been performed and represents an important step forward in the characterization of multi-functionalized hybrid materials, a key challenge in nanoscience.

References:

1. Reches, M. and Gazit, E., *Nature nanotechnology*, **2006**, 1(3): p. 195-200.
2. Lakshmanan, A.; Zhang, S.; Hauser, C. A., *Trends in biotechnology*, **2012**, 30(3): p. 155-165.
3. Jurado, R.; Castello, F.; Bondia, P.; Casado, S.; Flors, C.; Cuesta, R.; Domínguez-Vera, J. M.; Orte, A.; Gálvez, N., *Nanoscale*, **2016**, 8(18): p. 9648-9656.
4. Wang, X.; Li, Y.; Zhong, C., *Journal of Materials Chemistry B*, **2015**, 3(25): p. 4953-4958.
5. Knowles, T. P. and Mezzenga, R., *Advanced Materials*, **2016**.
6. Huang, B.; Bates, M.; Zhuang, X., *Annual review of biochemistry*, **2009**, 78: p. 993.
7. Monserrate, A.; Casado, S.; Flors, C., *ChemPhysChem*, **2014**, 15(4): p. 647-650.

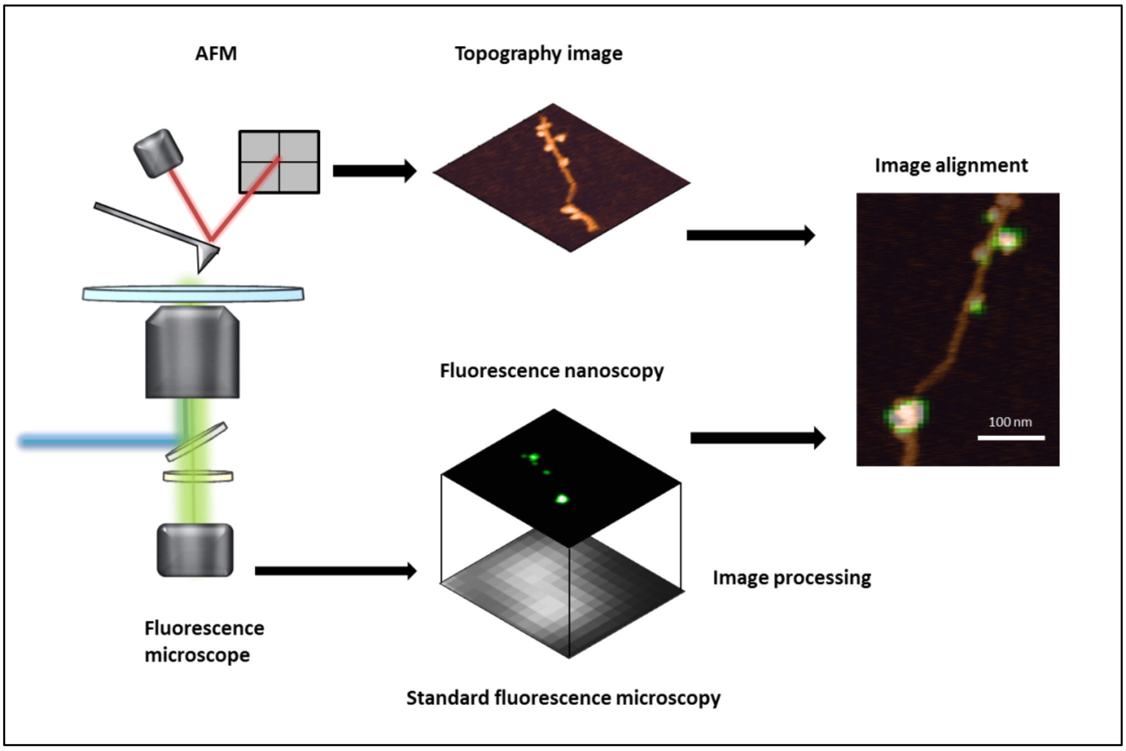

Figure 1. Correlative AFM and fluorescence nanoscopy for the characterization βLG fibers functionalized with QDs. AFM and standard fluorescence images are acquired *in situ*. The AFM image provides additional information to the super-resolution fluorescence image and is useful to optimize image reconstruction of the latter.