Through this 3 months stay in the Netherlands, I had a great chance to encounter with different people having various cultural and scientific diversities. The primary purpose of my stay in the Netherlands was in 2 categories. Firstly, I wanted to expand my protein nanotube system to be visualized under state-of-art stochastic optical reconstruction microscopy (STORM). From the previous visit supported by MERIT Errantry to TU/e, I already confirmed that it is possible to visualize GroEL nanotubes by STORM under certain condition. By combining the toolbox in Tokyo and STORM in the Netherlands, I wanted to find a story that cannot be proven by the conventional techniques. Another important point of my visit is to explore other possible collaboration of my research topics with the tools my colleagues in TU/e had. I truly enjoyed every single moment of my stay, my exploration and the great scientific discussions I had with the people in TU/e.

Eindhoven University of Technology (TU/e)

TU/e is famous academic institution for their specialties in supramolecular chemistry. Many renowned scientists including professor Meijer had become a great stimulation for me to think in a different way than I used to. The organization I officially belonged to was institute for complex molecular systems (ICMS) and professor Meijer was the scientific director of the entire ICMS. ICMS was a complex mixture of experts from different backgrounds: ranging from supramolecular chemistry to molecular biology. It was highly encouraged to have casual brain storming discussions to those who affiliated to different fields for the aim of multidisciplinary collaborations and I highly enjoyed the atmosphere.

Stochastic Optical Reconstruction Microscopy (STORM)

1) Optimization of Dye-labeling to GroEL

   To visualize objects under STORM, labeling dyes to those objects is the essentially required steps. Some criteria to the sample preparation including labeling process for successful imaging were as follows: [1)] labeling steps and the following purification should not hinder the normal function or structure of the object and [2)] the interface of the object and the surface should be carefully modulated to not disrupt the structure of the object.

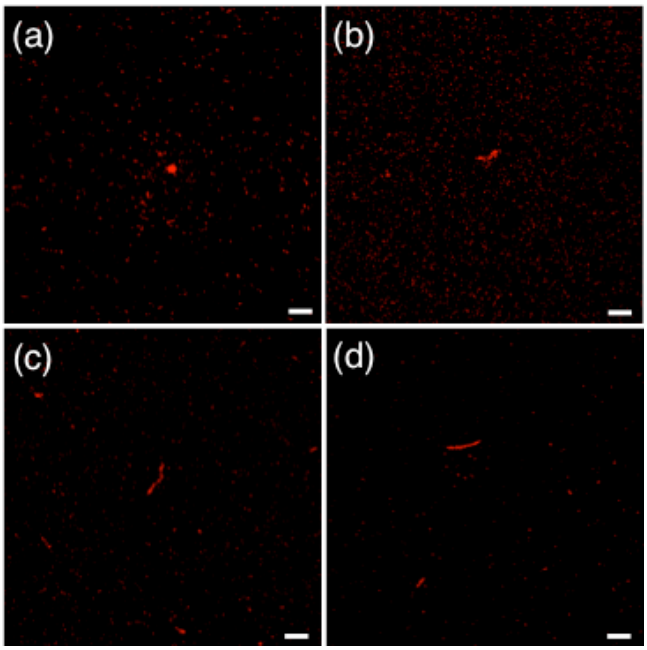

Figure 2 Optimization process of GroEL nanotube imaging under STORM. All scale bars are 1 μm. (a) as-prepared solution, (b) as-prepared nanotube with polylysine surface, (c) mildly crosslinked nanotube with polylysine surface, (d) sample (c) quenched with glycine.

(before and after: figure c and d).

These optimization processes were done for 2 different types of dyes therefore yielding two-color respectively well-constructed images as in figure 2.

Figure 1 was the resulting STORM images made from the different chemical environments. There is an intrinsic electrostatic repulsion between negatively charged protein GroEL and the piranha-etched glass surface. To reduce those repulsing nature and remain the integrity of GroEL nanotubes, 3 additional steps had been made before imaging. First, I needed to dope the glass surface with cationic polymer poly-$_L$-lysine to make the objects down to the surface (before and after: figure a and b). Second, mild-cross linking after labeling the object was necessary to get the better anisotropic objects on the surface (before and after: figure b and c). Lastly, quenching steps were necessary to get the better quality images of those GroEL nanotubes on the surface

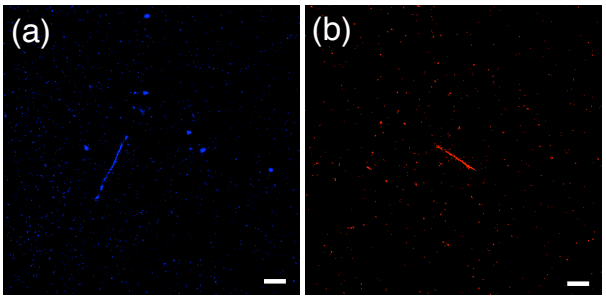

Figure 1 Two color images of GroEL nanotube labeled with (a) Atto 488 dye and (b) Cy5.

2) STORM and Nanotubular topology: proof of highly confined nature of GroEL nanotube

Principle mechanism of getting reconstruction in STORM mainly relies upon consecutive switching on and off the dyes by thiol-GluOx pair. To get the good reconstruction beyond the conventional optical huddles, it is mandatory to make sure that only the small subset of the dyes are ON in the same time frame. That means on the other hand, if the system has highly confined structure that even the small thiol from the outside could not physically get in, the dyes should be always ON state, giving almost no reconstruction.

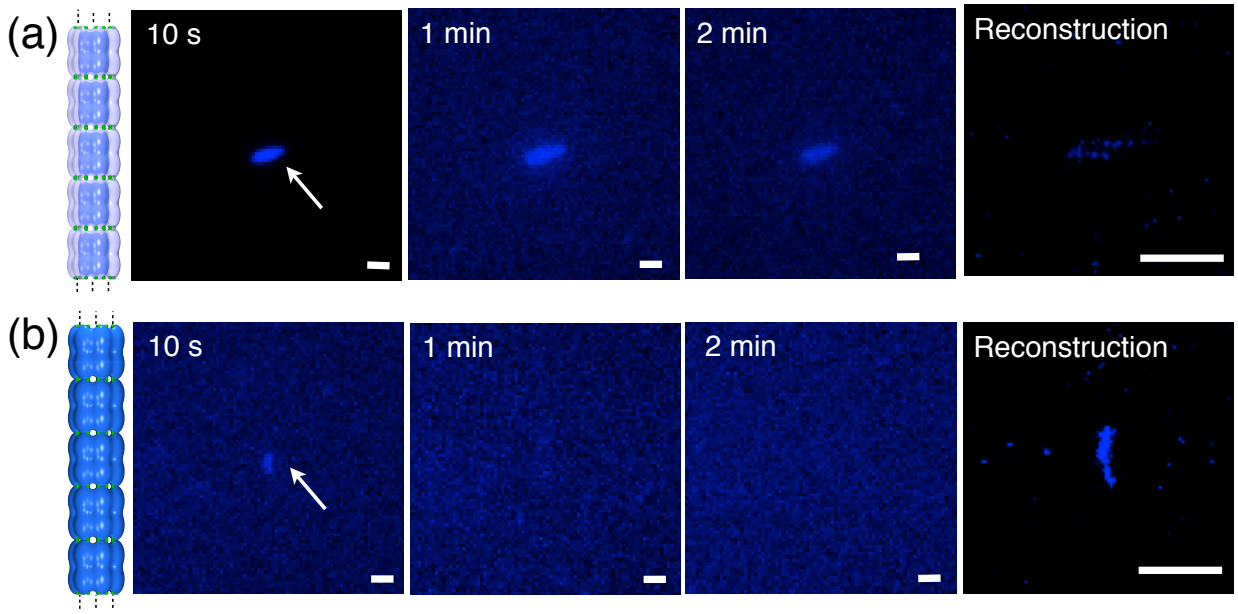

Figure 3 Different dye bleaching behavior of the protein nanotubes labeled (a) internally and (b) externally with atto 488. White arrow indicates the focus of reconstruction process.

By harnessing hydrophobic dye, atto 488, I was able to selectively label the internal site of GroEL cylinder. I could label the exterior of the nanotubes as well by changing the order of sample preparation, making the nanotubes first to make sure that all interior amine site were blocked and adding the dyes afterwards. STORM results of them were clearly juxtaposed in figure 3. When the dyes were inside the nanotubes, they were not bleached at all by the laser stimulation due to the lack of physical contact to the thiol (Figure 3a). The dyes were bleached overtime in the case of external labeling and yielded nicely reconstructed images (Figure 3b). This is a first and clear demonstration that the nanotubular topology affects the reconstruction behavior of the dyes. From this experiments, my colleagues and I concluded that the GroEL nanotube has a very confined structure so that it prohibits small chemicals to diffuse in from the outer medium.

This program was a great inspiration for me to learn more about other fields of chemistry and the power of interdisciplinary approach. I would like to thank professor Meijer who welcomed me with warm hospitality and the MERIT office that kindly arranged everything.

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