Gold Functionalized Nano-needles for Angular Protein Movement Visualization

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I. Introduction - State of the art

Motor proteins are a class of proteins that has caught the interest of biologists and engineers alike. These bio-molecules can convert chemical energy into motion and are commonly found in the living world. Two forms of movement can be distinguished: rotation and translation. The size range of these tiny motors is around a few nanometers, which is far below the resolution of an optical microscope. While many techniques exist to characterize proteins despite their small size (scanning cryo-electron microscopy, AFM, atomic modeling with X-ray diffraction, NMR crystallography) for observation of the proteins in action there are few alternatives to the optical microscope. A standard technique to observe the movement of motor-proteins is by means of fluorescence or label beads. Fluorescence as well as beads can give clear information on linear movement. In case of spinning rotational motors like F1 motors, rotation can be clearly observed [1]. However, in case the motors exhibit a slight angular movement, like linear KIF1A motors ones [2] but which are suspected to move with a slight angular movement, these spherical objects cannot give clear information on the movement. If asymmetric structures are used, like long and thin ones, slight angular movement is then amplified.
Various strategies have been applied for the task of making long-thin structures for angular movement observation amplification. However none of these proposals give results corresponding to all of the following requirements: 1) easy fabrication in amounts of millions range; 2) well controllable diameter of 100-200 nm and length of 1-2µm (trade-off between visibility under the microscope and not too high drag load onto the protein moving it); 3) highly localized functionalization at one end to avoid random attachment of the proteins onto the needles. Such structures have been successfully realized, according to the requirements. They have been successfully tested onto F1 ATPase spinning motor-proteins, which exhibit a clearly visible angular movement. Attachment onto KIF1A proteins is now in progress.

II. Needles-like Structures Fabrication and Functionalization

The needles-like structures have been fabricated according to the process described in Fig. 1 [3-5]. This fabrication process is highly reproducible, quick and allows obtaining structures which follow all the requirements. The gold head of the needles allows functionalizing specifically the structures at a highly localized place: the proteins will attach specifically on that gold head more than on other areas. Pictures of the structures are in Fig. 2.

III. Application to F1 ATPase Rotation Motor-proteins

After functionalization of the needles, they are recovered by sonification in phosphate buffer saline PBS . Then, they are attached onto F1 ATPase spinning motor-proteins, which exhibit a clearly visible angular movement. Fig. 3 shows a scheme of an attached needle onto one F1 ATPase protein. Fig. 4 shows pictures extracted from a film, where needles are successfully rotating onto different F1 ATPase proteins.
By using F1 ATPase spinning motors it is possible to demonstrate that those needles can inform clearly on angular movement of the proteins. These needles will next be used onto KIF1A motors in order to amplify suspected slight angular movement during their linear displacement.

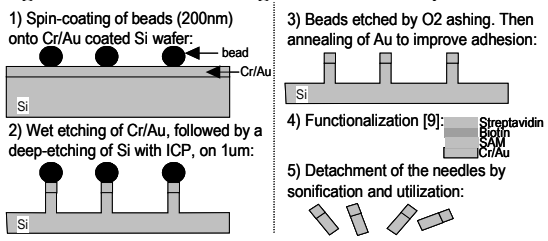
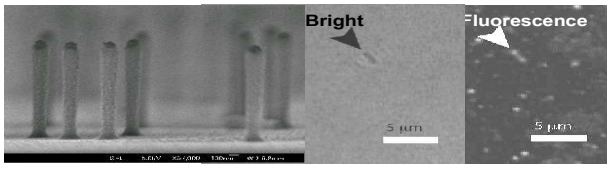


Figure 1: Fabrication process of the needles as well as functionalization.



a) b)

Figure 2: a) SEM picture of needles before sonification. b) One needle observed under bright field then fluorescence image.

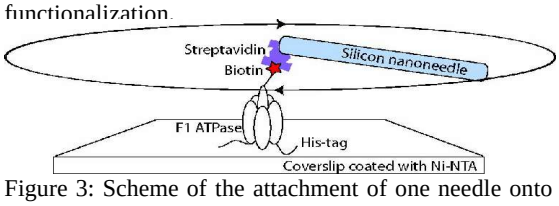


Figure 3: Scheme of the attachment of one needle onto an F1 protein, and rotation.

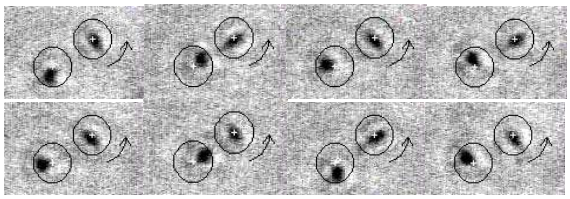


Figure 4: Pictures extracted from a film. Two needles can be observed rotating at the same time.

Acknowledgments

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References

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