DNA mechanical nanoresonator as a tool for structural molecular investigation

PhD candidate:

Francesca Legittimo

Supervisors:

Tutor: Prof. Carlo Ricciardi

Co-tutor: Prof. Enzo di Fabrizio

Summary

This thesis reports a detailed characterization of the structural and mechanical properties of self-assembled DNA bundles, suspended between silicon micropillars of a Super Hydrophobic Substrate (SHS). To the best of our knowledge, these structures are the first nanomechanical resonators entirely composed of biological molecules, paving the way to new fascinating opportunities in the field of DNA analysis and bio-sensing. In fact, the versatile suspension process, described for the first time in 2011 by De Angelis et al. [1], allows for the localization and detection of molecules in extremely diluted solution (up to the attomolar levels). Furthermore, this method provides self-sieved [2] and background-free DNA-fibers, which can provide fundamental information about the DNA molecules composing them: the aim of this PhD project was to establish the vibrometrical analysis of these bio-nanoresonators as an efficient tool for DNA structural and molecular analysis.

In Chapter 1, I reported an introduction on DNA structure and DNA mechanical properties, together with a brief description of the main techniques typically employed to study them, as Optical and Magnetic Tweezers and AFM force spectroscopy. Chapter 2 reviews the DNA bundles suspension process and the main results obtained by means of Raman Spectroscopy and High Resolution Transmission Electron Microscopy in the last 10 years; it is followed by a detailed description of the theory behind their applicability as nanoresonators and by the first results obtained by the NaMes Group in Polito by means of the vibrometrical analysis of these structures. In fact, in 2019 Stassi et al. explored the mechanical response of DNA-nanoresonators composed of different DNA complexes: bare DNA, DNA intercalated with YOYO-1 and GelRed, and DNA interacted with the chemotherapeutic agent CisPt [3]. They successfully determined the Young's moduli of the nanoresonators, unambiguously linking that figures of merit to the rigidity of the molecules composing the suspended bundles: the authors demonstrated that the action of the bis-intercalants stiffens the DNA molecules, while the chemotherapeutic drug significantly lowers the stiffness of the whole structure, by disrupting the base pairs H-bonds.

This thesis project starts from this outstanding result, spanning from the vibrometrical analysis of different DNA-ligand complex, to the measurements of the mechanical response of nanoresonators composed by DNA at a different level of methylation. Chapter 4 in fact reports the extensive statistical study performed

on λDNA molecules 50%, 25% and 0% methylated, while in Chapter 5 the temperature dependence of nanoresonators composed by bare DNA molecules, DNA+YOYO-1 and single stranded DNA is explored.

This part of the thesis is related to the work carried out in the Nanoscience Lab of Politecnico di Torino, while the majority of the experimental work detailed in Chapter 5 and 6 was performed in the Laboratories of the Biological Nanostructure and Imaging and Manipulation of Nanostructures of the Molecular Foundry (Berkeley, California) where I spent my period of research abroad. As reported in Chapter 5, by means of AFM force curve analysis I provided an independent estimation of the Young's modulus of DNA suspended bundles, confirming the values found by Stassi et al. in 2019; furthermore, by means of this technique, I proved the presence of residual tensile stress inside the structures, shading light on some limitations faced by the vibrometrical analysis and detailed in Chapter 3. Always in Berkeley I worked on the production and suspension process of DNA+RecA protein filaments, whose challenges have been described in Chapter 6. As there reported, the vibrometrical response of these new nanoresonators have been explored in Polito, giving interesting results and a further proof that the behavior of the DNA-based nanoresonators is strictly related to the properties of the molecules composing them.

At the end of the dissertation, the Conclusion recalls all the results achieved in these three years, suggesting possible new lines of work for the future of DNA-nanoresonators.

[1] F. De Angelis et al. "Breaking the diffusion limit with super-hydrophobic delivery of molecules to plasmonic nanofocusing SERS structures." In: *Nature Photonics* 5.11 (2011), pp. 682–687. issn: 17494885. doi: 10.1038/nphoton.2011.222
[2] Monica Marini et al. "Self-sieving DNA over superhydrophobic surfaces : A Raman spectroscopy study." In: *Journal of Raman Spectroscopy* March (2022), pp. 1– 9. doi: 10.1002/jrs.6368
[3] Stefano Stassi et al. "Nanomechanical DNA resonators for sensing and structural analysis of DNA-ligand complexes." In: *Nature Communications* 10.1 (2019), pp. 1–10. issn: 20411723. doi: 10.1038/s41467-019-09612-0. url: http://dx.doi.org/10.1038/s41467-019-09612-0