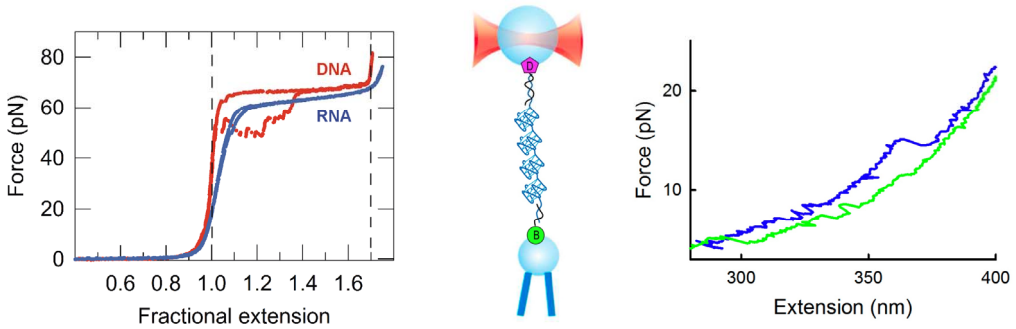
# Single-molecule manipulation of RNA structures: double-stranded helices and G-quadruplexes

J. Ricardo Arias-Gonzalez
IMDEA Nanociencia
Calle Faraday 9, Cantoblanco, 28049 Madrid, Spain
ricardo.arias@imdea.org
Phone: +34 912998860

RNA are ubiquituous macromolecular platforms that develop multiple biological roles in the cell. Here, we present single-molecule investigations on double-stranded (ds) RNA and on RNA G-quadruplex forming sequences.

DsRNA is the genetic material of a variety of viruses and has been recently recognized as a relevant molecule in cells for its regulatory role. Despite that the elastic response of dsDNA has been thoroughly characterized in recent years in single-molecule stretching experiments, an equivalent study with dsRNA was still lacking. Here, we have engineered long dsRNA molecules for their individual characterization contrasting information with dsDNA molecules of the same sequence.[1] It is known that dsRNA is an A-form molecule unlike dsDNA, which exhibits B-form in physiological conditions.[2-4] These structural types are distinguished at the single-molecule level with atomic force microscopy and are the basis to understand their different elastic response. Force-extension curves of dsRNA with optical and magnetic tweezers manifest two main regimes of elasticity, an entropic regime whose end is marked by the A-form contour-length and an intrinsic regime that ends in a low-cooperative overstretching transition in which the molecule extends to 1.7 times its A-form contour-length. DsRNA does not switch between the A and B conformations in the presence of force. Finally, dsRNA presents both a lower stretch modulus and overstretching transition force than dsDNA, whereas the electrostatic and intrinsic contributions to the persistence length are larger.




*Left*, force-extension curves of dsRNA and dsDNA, normalized in the extension axis to the A and B forms, respectively. *Right*, Force-induced unfolding of RNA constructions of GGGUUA repeats by optical tweezers.

G-quadruplexes are nucleic acid sequences that are rich in guanine and are capable of forming a four-stranded conformation. These structures are rare from a biological point of view but it is believed that they play a role in gene expression and telomere regulation. Here, we have studied long human telomeric RNA (TERRA). These non-coding RNA molecules contain subtelomere-derived sequences and an average of 34 GGGUUA repeats at their 3' end.[5] TERRA acts as a scaffold for the assembly of telomeric proteins involved in telomere maintenance and telomeric heterochromatin formation.[6,7] By using optical-tweezers and other biophysical techniques, we have found that long RNA constructions of up to 25 GGGUUA repeats form higher order structures comprised of single parallel G-quadruplex blocks, which unfold at lower forces than their DNA counterparts. [8]

**References**


1. Herrero-Galán, E.; Fuentes-Pérez, M.E.; Carrasco, C.; Valpuesta, J.M.; Carrascosa, J.L.; Moreno-Herrero, F.; Arias-González, J.R. *J. Am. Chem. Soc.* **2013**, 135, 122-131.
2. Hormeno, S.; Moreno-Herrero, F.; Ibarra, B.; Carrascosa, J.L.; Valpuesta, J.M.; Arias-Gonzalez, J. R. *Biophys. J.* **2011**, 100, 2006−2015.
3. Hormeno, S.; Ibarra, B.; Valpuesta, J.M.; Carrascosa, J. L.; Arias-Gonzalez, J.R. *Biopolymers* **2012**, 97, 199−208.
4. Hormeno, S.; Ibarra, B.; Carrascosa, J.L.; Valpuesta, J.M.; Moreno-Herrero, F.; Arias-Gonzalez, J.R. *Biophys. J.* **2011**, 100, 1996−2005.
5. Porro, A.; Feuerhahn, S.; Reichenbach, P.; Lingner, *J. Mol. Cell Biol.* 2010, 30, 4808.
6. Deng, Z.; Norseen, J.; Wiedmer, A.; Riethman, H.; Lieberman, P. M. *Mol Cell* **2009**, 35, 403.
7. Lopez de Silanes, I.; Stagno d'Alcontres, M.; Blasco, M. A. *Nat. Commun.* **2010**, 1, 33.
8. Garavís, M.; Bocanegra, R.; Herrero-Galán, E.; González, C.; Villasante, A.; Arias-Gonzalez, J.R. *Chem Comm.* DOI: 10.1039/c3cc42981d