

*Title:* **Stretching of weak polyelectrolytes at the single-molecule level**

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The goal of this project consists in the study of the mechanical response of weak polyelectrolytes at the single-molecule level in order to evaluate the interplay between Charge Regulation (CR) and mechanical stretching. Specifically, the chosen weak polyelectrolyte has been hyaluronic acid HA, also called hyaluronan. The mechanical response during stretching of weak polyelectrolytes is an area still under study. Weak polyelectrolytes can modulate their charge in response of external stimuli (such as pH changes, ionic strength or by electrostatic interactions with other charged species) and, for this reason, it is expected that their mechanical properties will also change. The present work was carried out by single molecule force spectroscopy (SMFS) using an atomic force microscope (AFM) under liquid environmental control and Monte Carlo based computational simulations in order to investigate the influence of pH changes and salt concentration on the behavior of the elasticity of HA single chains.

For the nanomechanical AFM-SMFS characterization of single HA (physically adsorbed onto glass, mica and silicon substrate), an AFM tip was used to pick up and stretch the single chains. Upon separation of the tip and the substrate, the macromolecule attached onto the substrate is first uncoiled and then stretched or desorbed. At larger extensions, the force increases strongly with the extension in a nonlinear manner until pulling force becomes at one point so large that the molecule detaches from the tip. In this case, when the macromolecule is strongly attached to the AFM tip and substrate, stretching (also called pulling) events were observed which has a characteristic spike in the force curve profile. On the other hand, if the macromolecule is weakly attached to the substrate and the tip is retracted, the macromolecule is being peeled away. In this new situation, desorption events were observed with various force plateaus. These results were obtained in pH 4 and 5.7, and pH 5.7 with 0.1 M NaCl solution, where macromolecules are in different ionized degrees. Force spectroscopy clearly reflects the influence of the different environments. The obtained force extension curves suggest that the measured force needed to

stretch a single chain up to certain extension decrease at pH 5.7 compared to pH 4 and no differences were observed comparing pH 5.7 with and without added salt. In the case of desorption events, the plateau force increased from pH 4 to pH 5.7. This suggests that the affinity between macromolecule and the surface is better in the solvent with the higher pH-value.

Finally, the experimental data recorded were compared using mechano-statistical theories (specifically Worm-Like Chain model, WLC) and simulation Monte Carlo (MC) computational methods. With this aim, we used a minimal model that captures the fundamental aspects present in the stretching of a weak linear polyelectrolyte: bond stretching, bond bending and proton binding. Mechanical stretching was performed using Grand Canonical Monte Carlo (GCMC) simulations at different pH and ionic strength values. The force-extension curves show the effect of pH and ionic strength on polyelectrolyte stretching. Simultaneously, these simulations also provide conformational (persistent length  $l_p$  and chain elongation  $L_z$ ) and protonation properties (degree of protonation  $\theta$  and the effective acidity constant  $pKa$ ). It can be observed that at large force regimes, the force-extension curves were approximately independent of the pH and the ionic strength  $I$ . Finally, the comparison between the experimental and computational force-extensions curves suggest that both follow the same general trend but in different force regimes.

**Keywords:** polyelectrolyte, stretching, desorption, force spectroscopy, atomic force microscope, Monte Carlo simulation.