Robust Control for Single Molecule Force Spectroscopy

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Single molecular studies often enable estimations of parameters unobservable in bulk studies. In particular, single molecule force spectroscopy is well recognized for its potential in understanding biomolecules since many biological functions are known to be a result of the physical interactions of molecules. The atomic force microscope (AFM) is a powerful tool for force spectroscopy because it can be used to apply and measure forces ranging from pico to nano newtons, under diverse conditions such as under liquids, at physiologically relevant temperatures, and with little or no modifications to the samples being studied.

Force spectroscopy using AFMs involves using a feedback control system to apply a controlled tensile force on a single biomolecule attached between the tip of an AFM cantilever and a substrate. The applied force may be held at a constant value or increased linearly in a ramp. The data from the experiments are then analyzed assuming that the force on the molecule was indeed a true constant or a perfect ramp. Thus, accuracy and reliability of the parameters estimated are dependent on the feedback controller's ability to maintain the applied force close to the desired setpoint.

The design of a force controller is made complicated by many factors. Besides the noise added via the AFM's electronics, the measurements for feedback are subject to the thermal oscillations of the AFM cantilevers and biomolecules. In addition, the stiffness of the biomolecules changes with extension (and thus the applied force). This can be observed from the frequently used worm like chain (WLC) model used to describe the force-extension characteristics of biomolecules. This change in stiffness implies a change in the open loop gain of the system to be controlled. Further, biomolecules may undergo sudden and drastic structural changes under tension. For example, many protein molecules consist of folded domains that unfold under tension, leading to a sudden increase in their contour lengths, and hence a decrease in the stiffness.

In order to address these challenges, we propose a robust force control design for the application of AFM based single molecule force spectroscopy. This would ensure consistent performance under the changing conditions of the force spectroscopy experiments. Moreover, such a controller would be reusable across a wider range of biomolecules without the need for retuning the controller, enabling a greater level of automation of single molecule experiments. This robust control system would be validated on the reference protein titin and applied to study utrophin and dystrophin, whose characterization is important for understanding muscular dystrophy.

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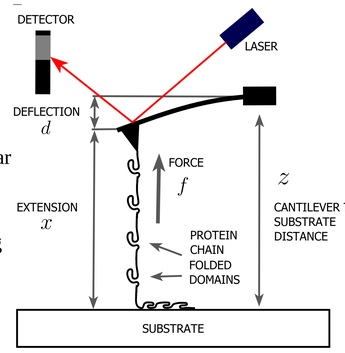
Summary

- Single molecular studies enable estimations of parameters unobservable in bulk studies. Single molecule force spectroscopy is frequently used for studying biomolecules (such as proteins) since many biological functions are known to be a result of the physical interactions of molecules.
- The Atomic Force Microscope (AFM) is a powerful tool for force spectroscopy. It can apply & measure forces from pico to nano newtons. Experiments can be done under liquids, at physiological temperatures, and without modifying the samples being studied. Feedback control is used for applying controlled tensile forces on a molecule attached between the tip of an AFM cantilever and a substrate.
- Folded domains stochastically unfold, measurements are analyzed to build models for the biomolecules, assuming perfect control. Thus, the accuracy of model parameters estimated are dependent on the feedback controller's performance.
- Control design is complicated by non-linearities of protein molecules, abrupt changes during unfolding events, thermal & measurement noise. We explore the use of robust force control design to address these challenges, and further to help in experiment automation.
- This approach enables controlled system to achieve desired nominal performance, further ensures consistency in controller performance across the experimental conditions. This is verified via non-linear simulations (titin model).
- Simulation and control design tools are modular with the intention of release for other users.
- This work is for studying utrophin and dystrophin, whose characterization is important for understanding muscular dystrophy.

System Description

- Attempts made to adsorb a single polypeptide molecule to cantilever tip and substrate
- If successful, molecule stretched to apply constant force or force ramp
- Actuation: Piezo extends the molecule along Z-axis to desired length
- Measurements:
  - LVDT for Z-axis displacement
  - Photo detector for lever deflection
  - Force = deflection × spring constant



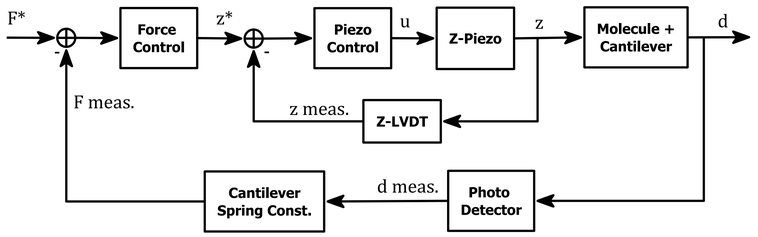
- Polypeptide force extension behavior: Highly non-linear
- Worm-Like Chain : $F = \frac{K_B T}{P}\left[\frac{1}{4}\left(1-\frac{x}{L_c}\right)^{-2} - \frac{1}{4} + \frac{x}{L_c}\right]$
- Folded domains stochastically unfold rapidly, changing contour length (Lc) and persistence length (P)
- **System: Non-linear, Uncertain, and Varying**

Objectives

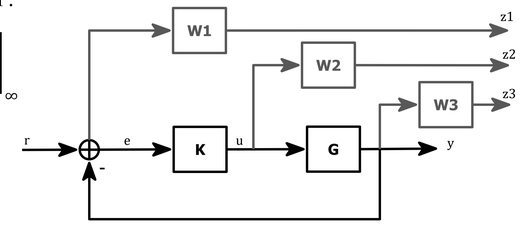
- AFM based Force clamp :
  - No overshoot : Unfolding probability proportional to force
  - Settling Time $\leq$ 10 ms : For high force experiments with rapid unfolding
- Robust to :
  - Wide change in Protein parameters [Lc : 5-6x increase, P: 3-4x increase]
  - Variations in Cantilever Spring Constants [0.002 – 0.02 N/m]
  - Unmodeled system dynamics
  - Thermal & measurement noise
- Automate the single molecule experiments

Design

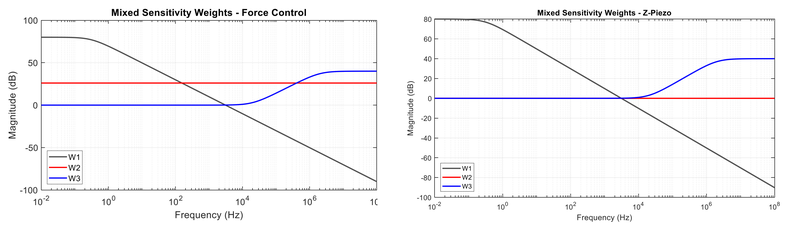
- Cascaded architecture : Inner loop for Z-Piezo position, always ON
- Outer loop for Force, switched ON only upon successful attachment



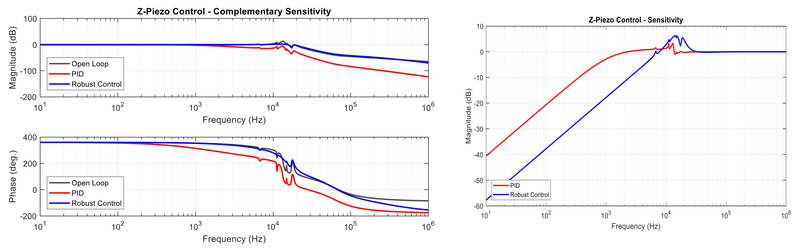
- Mixed-sensitivity H_∞ control :
  - K that minimizes $\left\| \begin{matrix} W_1 S \\ W_2 KS \\ W_3 T \end{matrix} \right\|_\infty$



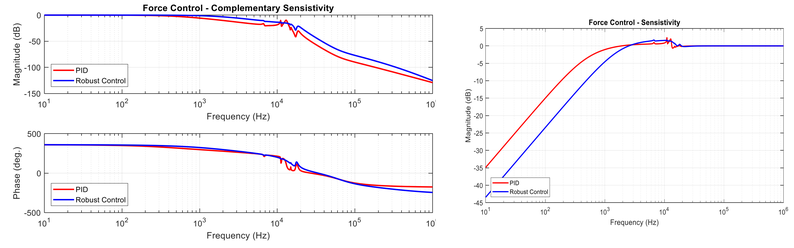
- Weight selection



- Inner loop control : Flattens peak, maintains bandwidth of 2.58 kHz (A PID tuned using MathWorks® pidTuner inset for a benchmark, with bandwidth of 0.19 kHz)

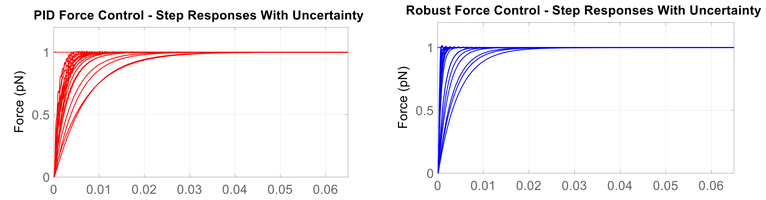


- Outer loop control : Nominal bandwidth - 290 Hz (Bandwidth with PID - 92 Hz)

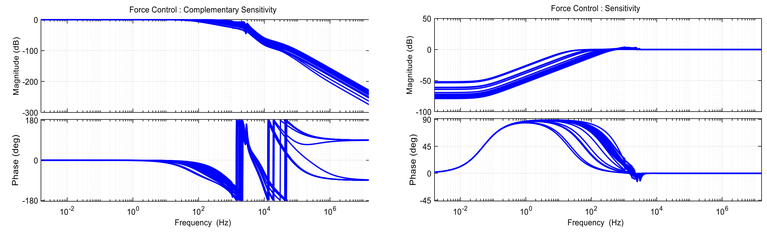


Results & Conclusions

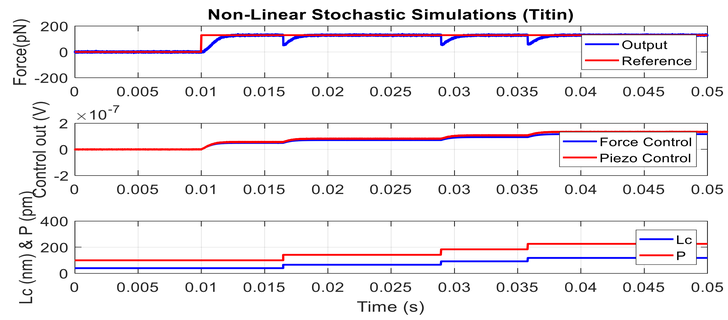
- Step responses with model variations



- Sensitivity and Complementary Sensitivity over model variations (Stability retained)



- Simulation with WLC model and stotchastic unfolding behavior



- **H_∞ Control achieves 3x faster control while maintaining similar margins.**

	Nom. Bandwidth (Hz)	Settling Time (ms)		% Overshoot		GM (dB)	PM (deg.)
		Nom.	WC*	Nom.	WC*		
PID	92	7.1	41.6	0	1.4	22.2	∞
Mixed-Sen. H_∞	294	2.3	35	0	0.9	17.3	∞

* Worst case averaged over 5 random sampling

In Progress

- Detection of unfolding events and refolding
- Implementation using FPGA (Ni CRIO-9039)
- 'Shake' piezo based control
- Validation on Titin, experiments on utrophin & dystrophin

References

- [1] Oberhauser, Andres F., et al. "Stepwise unfolding of titin under force-clamp atomic force microscopy." *Proceedings of the National Academy of Sciences* 98.2 (2001): 468-472.
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