

Dr. Nandita Singh  
Senior Science Editor  
*JoVE*

Dear Dr. Singh,

Thank you for reviewing our invited manuscript entitled “Force spectroscopy of single protein molecules using an atomic force microscope.” We note that the authors are now listed as Zackary N. Scholl, Qing Li, Eric Josephs, Dmitra Apostolidou and Piotr E. Marszalek.

We have closely followed the editor’s comments to adapt our manuscript to the JoVE style - including adding abbreviations, more references, and increased usage of the imperative tense. Also, as suggested, we have clarified the language in the cases where our manuscript had significant overlap with our previous work (these areas are marked in red). Additionally, as suggested by both the editor and reviewers, we have also significantly expanded the Protein purification steps, namely the transformation and purification steps. Finally, as specified by the editor, we have added three new paragraphs to address the critical steps, limitations, applications and significance of this method.

While one reviewer said that our account of AFM was well written, we received several points of consideration to address from all reviewers. Reviewers 1 and 2 suggested making the protocols more general in terms of proteins and methods used. To this end, we have adopted more general terminology where appropriate and we have included references about the construction of our particular AFM setup to make our setup easier to replicate in other laboratories. We have also followed the reviewers concerns to include more information about the DNA sequences used and have introduced a new figure showing a plasmid map for a plasmid we have made available through AddGene that is readily used with our method. Following Reviewer 3’s suggestions we have also included more information on the analysis of AFM data.

We hope that these changes make our manuscript suitable for publication in *JoVE*.

Sincerely,

Zackary Scholl, Ph.D.