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Dear *Nature Methods* Editors,

We are submitting the following manuscript for consideration for publication as an **Article** in *Nature Methods*:

Bayesian hidden Markov model analysis of single-molecule force spectroscopy: Characterizing kinetics under measurement uncertainty, by John D. Chodera, Phillip Elms, Frank Noé, Bettina Keller, Christian M. Kaiser, Aaron Ewall-Wice, Susan Marqusee, Carlos Bustamante, and Nina Singhal Hinrichs.

In this manuscript, we describe a new technique for the analysis of single-molecule force spectroscopy experiments. Our approach utilizes a *Bayesian* extension of hidden Markov models that allows information about kinetic and equilibrium properties to be extracted simultaneously from force or extension data collected in single-molecule optical or atomic force microscopy experiments. In addition to providing a way to reconstruct hidden state sequences (like standard hidden Markov models), our approach provides much more accurate estimates of characteristics of conformational states and transition rates than previous methods based on simple segmentation of the observed force range using force thresholds. Our approach also provides an excellent assessment of the *confidence intervals* with which various kinetic and mechanical properties are known can be computed, allowing the experimenter to easily judge whether a particular mechanistic hypothesis is borne out by the data. We validate the method on synthetic data, and illustrate the approach applied to real experimental data by characterizing the folding kinetics under force of an RNA hairpin with three distinct conformational states using an optical trap. All of the Matlab code and data used here will be made freely available online as a companion to this work.

We believe this work will be of high interest to the *Nature Methods* readership. A number of articles on single-molecule force spectroscopy have appeared in previous issues—a testament to the interest in these tools:

- Evanko D. *Optimizing your optical tweezers.* Nature Methods 3:584, 2006.
- Dufrêne YF. *Atomic force microscopy of membrane proteins separating two aqueous compartments.* Nature Methods 3:973, 2006.
- Walter NG, Huang C-Y, Manzo AJ, and Sobhy MA. *Do-it-yourself guide: how to use the modern single-molecule toolkit.* Nature Methods 5:475, 2008.
- Neuman KC and Nagy A. *Single-molecule force spectroscopy: optical tweezers, magnetic tweezers and atomic force microscopy.* Nature Methods 5:491, 2008.
- Min TL, Mears PJ, Chubiz LM, Rao CV, Golding I, and Chemla YR. *High-resolution, long-term characterization of bacterial motility using optical tweezers.* Nature Methods 6:831, 2009.
- Lipfert J, Kerssemakers JWJ, Jager T, and Deller NH. *Magnetic torque tweezers: measuring torsional stiffness in DNA and RecA-DNA filaments.* Nature Methods: 7:977, 2010.
- Dufrêne YF, Evans E, Engel A, Helenius J, Gaub HE, and Müller DJ. *Five challenges to bringing single-molecule force spectroscopy into living cells.* Nature Methods 8:123, 2011.

Appropriate reviewers of this work include the following scientists working in the field of single-molecule force spectroscopy:

- Yann R. Chemla (UIUC) - ychemla@illinois.edu
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Kind regards,

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