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## Exploring proteins unbinding by acoustic force spectroscopy

Single-molecule data are of great significance in biology, chemistry, and medicine. However, new experimental tools to characterize, in a multiplexed manner, protein bond rupture under force are still needed.

Acoustic Force Spectroscopy (AFS) is a novel single molecule force spectroscopy technique that uses standing acoustic waves to robustly apply piconewton forces in parallel to several tens of receptor-ligand complexes for several hours and measure their unbinding forces.

We have used it to apply repetitive constant force steps on DNA scaffolds to probe the unbinding kinetics of receptor-ligand complexes. We also use it to study streptavidin-biotin unbinding under ultra-low loading rates.

Through this work, we have implemented protocols for high-throughput studies for the AFS which can be adapted for receptor-ligand complexes. We have established novel force calibration strategies that allow to determine the unbinding forces in-situ. It allows the comparison of individual bonds of same nature and the monitoring of the behaviour of individual bonds under repeated actuation. In addition, it enables to explore the near to equilibrium conditions, poorly accessible by most of the experimental approaches which have low throughput and consequently only give limited statistics on an inherently stochastic process. Therefore, AFS has the potential to both answer fundamental questions and open the way to systematic chemomechanical characterization of biomedically relevant interactions.

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