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AFM Tip-Induced Dissociation of RecA-dsDNA Filaments

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In the paper entitled "Direct and real-time visualization of the disassembly of a single RecA-DNA-ATPγS complex using AFM imaging in fluid" Li et al. concluded that RecA-DNA filaments disassembled in a biologically relevant manner with greatly varying rates and that the dissociation units were hexameric.[1] In our comment on this work, we outlined the influence of tip−sample interaction on the analysis of RecA-DNA filament dissociation.[2] The interaction between tip and sample influences the stability of the RecA-DNA filament, likely causing the great variety observed in dissociation rates. In the response by Goh and Li, they share this concern but show new data supplementing their initial conclusion that disassembly is caused by ATPγS hydrolysis.

In order to firmly draw this conclusion from the new control experiment, however, one should compare the images with and without ATPγS for the same sample and imaging conditions. Information about image conditions and scan parameters, e.g., tapping forces, are crucial. As also noted previously, the use of deionized water is highly problematic from a biochemical perspective. Proteins often lose their relevant biochemical activity in unbuffered deionized conditions. In the vast literature on RecA biochemistry, there is, to our knowledge, no indication that activity is normal in these conditions. It is therefore required to demonstrate by standard biochemical assays that these conditions would be relevant for biochemical function. Similarly, the hydrolysis and release of ATPγS in these conditions are assumed but not supported by reference to previous biochemical data or biochemical assays preformed by these authors. As information on details of the current imaging experiments and confirmation of biochemical relevance is lacking, in both the original communication and the response, the interpretation of the observed behavior is disputable.

Furthermore, we criticized the determination of sizes in AFM images by Li et al. They chose to interpret the RecA dissociation complex to be a hexamer, based upon a published model but without any quantitative analysis of their AFM data. Such analysis methods are well documented,[3] and should be used to circumvent erroneous deductions due to different image conditions and AFM parameters, in particular tip size, to extract the size of the protein complex.

Summing up, AFM imaging is a powerful tool for visualizing DNA−protein interaction, but only when one carefully accounts for the influence of tip−sample interactions and tip convolution. Moreover, when imaging in buffer one should try to resemble, as closely as possible, bulk conditions where proteins and other biological systems are well documented. Only then can biologically relevant information be drawn from AFM studies.

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