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Reply to Comment on "Direct and Real-Time Visualization of the Disassembly of a Single RecA-DNA-ATPγS Complex Using AFM Imaging in Fluid"

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We appreciate the comments regarding our paper entitled "Direct and Real-Time Visualization of the Disassembly of a Single RecA-DNA-ATPγS Complex Using AFM Imaging in Fluid".[1] In general, we share the concern of Heijden et al. about atomic force microscopy (AFM) imaging studies of soft matter, which is why we make sure that certain control experiments are performed. We will address these later. But we should note that we reported three different types of experiments. In all cases, the recA-DNA complexes were first formed in the presence of appropriate conditions of buffer, ions, and ATPγS, as described previously. We then examined these complexes using AFM imaging under physiological conditions, as well as upon drying. We then initiated disassembly by the removal of ATPγS from solution; this was done by exchanging the solution either with deionized water or with the original buffer solution but without ATPγS. We perfomed AFM imaging at various times both in fluid (deionized water, buffer) and on dried samples. Our conclusions are based on this series of experiments.

1. Tip−Sample Interaction Effects on RecA Filament Disassembly. We have remarked in our original paper[2] that we had been concerned with possible tip effects that could strongly influence the process of disassembly. To address this, we performed two control experiments, one of which, already described in ref 2, is a comparison of real-time imaging in fluid vs allowing the disassembly to proceed in a vial and taking aliquots with time. A second series of control experiments that we have not described previously concerns repeated scanning (either continuous or intermittent) of the recA/DNA complex in the presence of the solution of ATPγS in deionized water. In this case, no obvious disassembly was observed for a period of 40 min (a time period comparable to that used in Figure 1 of ref 2). An example is shown in Figure 1. Note that if the tip "bumps" the complex strongly, it could trigger disassembly. However, when imaging under controlled conditions, we clearly observed a difference in behavior as a function of the absence or presence of ATPγS. These observations were replicated numerous times by two different scientists. On the basis of the results of these control experiments, we conclude that the initiation of the disassembly is at least primarily due to the removal of ATPγS from solution rather than merely a tip effect.

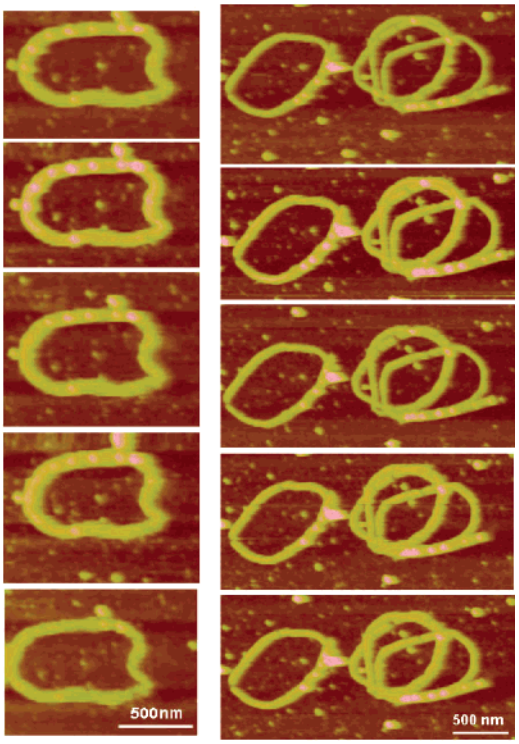

Figure 1. RecA/DNA complexes in the presence of ATPγS in deionized water, observed as a function of time. The time lapse between the first and last images is 42 min.

Common to all our experiments is the removal of ATPγS from solution. That different disassembly rates were observed in buffer and in deionized water also suggest that the

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disassembly is not due to tip effect. Heijden et al.'s experiments were conducted in buffer, which we agree is a more relevant condition. But in all cases, ATPγS was present. Hence, if disassembly were to happen, it can only be triggered by either the tip initiating it or another unpredictable random event. Heijden et al.'s conditions are equivalent to our experimental time zero, before the removal of ATPγS. In these cases, we found our complexes quite stable over at least a time period comparable to our experiment.

In AFM imaging, if a type of feature occurs always at a particular angle, one has to be suspicious of potential artifacts. But contrary to Heijden et al.'s results, we do not see a relationship between the fast scanning direction and the observed gaps. In Figure 1 of our original paper,[2] for example, gaps are seen occurring in various directions; this is typical of our many images. The gaps observed by Heijden et al. may have been due to higher imaging forces.

Notwithstanding the above arguments, we believe there is always a tip effect associated with AFM imaging. The tip has to interact with the sample in order to provide a measurement; hence it can never be fully nonperturbative. In this particular case, it might enhance the rate of disassembly by loosening recA binding from DNA binding, resulting in either knocking some recA off or at least facilitating the escape of ATPγS, which serves as a glue in the complex. Nevertheless, it is the hope of any one using AFM that perturbations such as these can be minimized by working hard to optimize imaging conditions. Furthermore, by designing appropriate experiments and their controls, we expect that significant information can still be obtained about many systems, this one included.

2. Size Determination from AFM Images. The imaging conditions and tip used are given in ref 2 (Figure 1 caption). While we have not performed in situ tip inspection,[3] this type of tip typically has a radius of ~20 nm, which is indeed much larger than the recA molecules. Tip convolution effects in AFM are well-known, just as it is well-known that a larger tip can be used to visualize much smaller objects. The aspect ratio of this probe tip is such that it cannot prove the existence of a hole that is ~2 nm in diameter; it can, however, visualize corrugations at that scale. In this work, we are not attempting to determine size and structure of recA molecules and aggregates de novo. Our interpretation in terms of hexamers is simply based on comparison with existing models, such as shown in Figure 2 of ref 2. By performing section analysis of various objects, we are able to obtain a height above the mica substrate (which is less affected by tip convolution). We can also see small dips, as well as make a relative determination that the objects in Figures 2 and 5 differ in dimensions.

It is possible that the objects in Figure 5 are nonspecific aggregates—we certainly cannot discount that—but their dimensions and features are similar to what is expected of hexamers. Hence, we chose to interpret our images in terms of specific aggregates—hexamers—that are expected for recA.

We should add that determination of size and structures of very small objects in solution using AFM is equivocal not only because of tip convolution effects but also because of factors such as charges and bound water molecules. One has to remember that what is being visualized is a reflection of the interactions between tip and sample, and even a detailed knowledge of the tip geometry is not necessarily useful information. But we strongly disagree with the contention that the lack of absolute determination of sizes means that the data cannot be properly interpreted.

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