grown deliberately on the electrodes such that they did not bridge the interelectrode gap. Furthermore, the gating response we observed was reversible without hysteresis, clearly demonstrating the lack of any effect of background currents on our measurements. In contrast, Yates *et al.* observe significant hysteresis and noise in their gating experiments (Fig. 1c and Supplementary Fig. 5C of ref. 1). We performed additional control gating experiments, such as using a potentiostat[7] to evaluate if changes in the reference electrode potential would affect the conductivity response and a four-probe electrode configuration to eliminate contact resistance at the biofilm/electrode interface[7]. These complementary approaches yielded a conductivity versus gating response similar to that using our initial approach. Yates *et al.* did not employ similar controls on biofilms. Particularly, the contact resistance is strongly dependent on the gate potential[8] and it cannot be ruled out from their measurements.

Yates *et al.* claim to have evaluated our experimental approach with polymers. However, the polymers they evaluated were synthesized in a different manner than those reported to yield metallic conductivity[9,10]. Furthermore, Yates *et al.* did not demonstrate that these polymers, which were likely to be quite thin, actually spanned the 50-μm gap between the two electrodes.

Yates *et al.* discuss at length their previous series of studies, which indirectly infer biofilm conductivity over distances of 5 μm. We have previously discussed how these types of measurement do not examine long-range electron transport through biofilms and are unable to distinguish between intra- and extracellular redox-active electron carriers[7,11].

As Yates *et al.* emphasize, the study of long-range electron transport through biological materials is a new field and the concept of metallic-like conductivity in a biological material is unconventional. Elucidation of the mechanisms of long-range biological electron transport is important for optimization of practical applications. To date, the pili-dependent model for long-range electron transport through *G. sulfurreducens* biofilms has led to genetic strategies to increase biofilm conductivity[2,5] and increasing the stacking of aromatic amino acids responsible for metallic-like conductivity dramatically enhances pili conductivity[4,12]. ❐

References
1. Yates, M. D. *et al. Nat. Nanotech.* **11,** 910–913 (2016).
2. Malvankar, N. S. *et al. Nat. Nanotech.* **6,** 573–579 (2011).
3. Reguera, G., Pollina, R. B., Nicoll, J. S. & Lovley, D. R. *J. Bacteriol.* **189,** 2125–2127 (2007).
4. Malvankar, N. S. *et al. mBio* **6,** e00084–e00015 (2015).
5. Leang, C., Malvankar, N. S., Franks, A. E., Nevin, K. P. & Lovley, D. R. *Energy Environ. Sci.* **6,** 1901–1908 (2013).
6. Yi, H. *et al. Biosens. Bioelectron.* **24,** 3498–3503 (2009).
7. Malvankar, N. S., Tuominen, M. T. & Lovley, D. R. *Energy Environ. Sci.* **5,** 8651–8659 (2012).
8. Lange, U. & Mirsky, V. M. *J. Electroanal. Chem.* **622,** 246–251 (2008).
9. Lee, K. *et al. Nature* **441,** 65–68 (2006).
10. Dallas, P. *et al. Polymer* **48,** 3162–3169 (2007).
11. Malvankar, N. S. & Lovley, D. R. *ChemSusChem* **5,** 1039–1046 (2012).
12. Tan, Y. *et al. Small* **12,** 4481–4485 (2016).

Nikhil S. Malvankar[1], Vincent M. Rotello[2], Mark T. Tuominen[3] and Derek R. Lovley[4]*
[1]Department of Molecular Biophysics and Biochemistry, Microbial Sciences Institute, Yale University, Connecticut 06516, USA. [2]Department of Chemistry, University of Massachusetts, Amherst, Massachusetts 01003, USA. [3]Department of Physics, University of Massachusetts, Amherst, Massachusetts 01003, USA. [4]Department of Microbiology, University of Massachusetts, Amherst, Massachusetts 01003, USA.
*e-mail: dlovley@microbio.umass.edu

Kinesin-1 motors can increase the lifetime of taxol-stabilized microtubules

To the Editor — The application of biological nanosystems has recently been explored for a variety of tasks. However, the success of these systems is crucially influenced by the lifetime of the involved biomolecules[1]. Dumont *et al.*[2] recently studied whether wear occurred and contributed to the failure of a mechanical system at the nanoscale. Their *in vitro* model system consisted of taxol-stabilized microtubules (Tx-MTs) bound to or propelled across a surface coated with high and intermediate densities of kinesin-1 motor proteins. The shrinking rate of the Tx-MTs was then determined and used as a measure of their stability. Stationary Tx-MTs, immobilized to the motor proteins (surface density ≥300 μm^{-2}) with the non-hydrolysable adenosine triphosphate (ATP) analogue adenylyl-imidodiphosphate (AMPPNP), were found to slowly shrink, despite taxol stabilization, at an average rate of 0.068 ± 0.013 nm s^{-1}. This rate was found to be independent of the investigated kinesin-1 surface density. In contrast, for Tx-MTs gliding in the presence of ATP, the shrinking rate was shown to vary depending on the kinesin-1 surface density as well as on the gliding velocity (see Fig. 2 in ref. 2). Most importantly, the average shrinking rate of gliding Tx-MTs of 0.191 ± 0.007 nm s^{-1} was higher than the shrinking rate of stationary Tx-MTs. In addition, it was observed that the gliding Tx-MTs mainly lost material from their leading minus end. The difference in the shrinking rates of stationary and gliding Tx-MTs was attributed to molecular wear caused by stresses associated with individual kinesin-1 motors stepping on the microtubule lattice.

Using the same full-length kinesin-1 construct as Dumont *et al.*, we performed similar experiments and determined the shrinking rates of stationary and gliding Tx-MTs (Fig. 1). Within the range of the published kinesin-1 surface densities our results are in good agreement with those reported in ref. 2. However, when using low motor surface densities (≤20 μm^{-2}), we observed that the shrinking rates of stationary Tx-MTs became dependent on the kinesin-1 surface density; the shrinking rates significantly increased with decreasing motor surface densities (Fig. 1b). Moreover, when we compared the shrinking rates of stationary Tx-MTs in the low-kinesin-density regime to the shrinking rates of Tx-MTs being propelled by an intermediate density of surface-adhered kinesin-1 motors, we found that the stationary Tx-MTs shrank equally fast as the gliding ones (Fig. 1b).

Our results indicate that kinesin-1 motors in the AMPPNP state have a stabilizing effect on Tx-MTs. This finding coincides with formerly observed structural changes on binding of kinesin-1 motor heads to Tx-MTs; increased axial tubulin dimer contacts and a rearrangement of the density within the α-tubulin subunit were suggested to stabilize protofilaments in microtubule/kinesin-1 complexes[3].

Furthermore, recent evidence indicates that the binding of strong-state kinesin-1 motor domains can inhibit the shrinkage of guanosine diphosphate (GDP)-microtubules by up to two orders of magnitude (D. Peet and R. Cross, personal communication). Interestingly, our finding that gliding Tx-MTs (at intermediate kinesin-1 surface density) did not shrink faster than stationary ones (at low kinesin-1 surface density; Fig. 1b), suggests that molecular wear, as proposed by Dumont *et al.*, may not be the only reason for the observed shrinking rates of gliding Tx-MTs. Rather, to explain the difference in the shrinking rates between stationary and gliding Tx-MTs for equal kinesin-1 surface densities (as seen in Fig. 2c in ref. 2, and Fig. 1b), additional possibilities should be taken into account. One main difference in the set-ups used to study the shrinkage of stationary and gliding Tx-MTs is the nucleotide state of the motor heads. As a consequence, the conformational change induced in the microtubule lattice on binding of the motor heads is expected to be different, which is most likely to lead to reduced stabilization of gliding Tx-MTs in the presence of ATP hydrolysis compared with stationary Tx-MTs held by two-head bound kinesin-1 motors in the presence of AMPPNP. Moreover, during gliding, a significant fraction of motor heads is detached from the Tx-MTs, lowering the 'effective' motor density. The faster shrinkage of the minus ends compared with the plus ends observed by Dumont *et al.*[2] for gliding Tx-MTs could alternatively be explained by taxol being generally less effective on microtubule minus ends, in agreement with recent reports[4–6].

To fully understand the shrinkage of stationary and gliding microtubules, further investigations of the underlying molecular mechanisms will be necessary. Desirably, these investigations would differentiate between the impact of external forces (due to the motors being bound to a rigid surface) and internal forces (resulting from the mechanical communication of the motor heads). If successful, the gained insight is expected to deepen our biological understanding of motor–microtubule interactions as well as to allow for devising more efficient protocols to stabilize microtubules *in vitro*. ❐

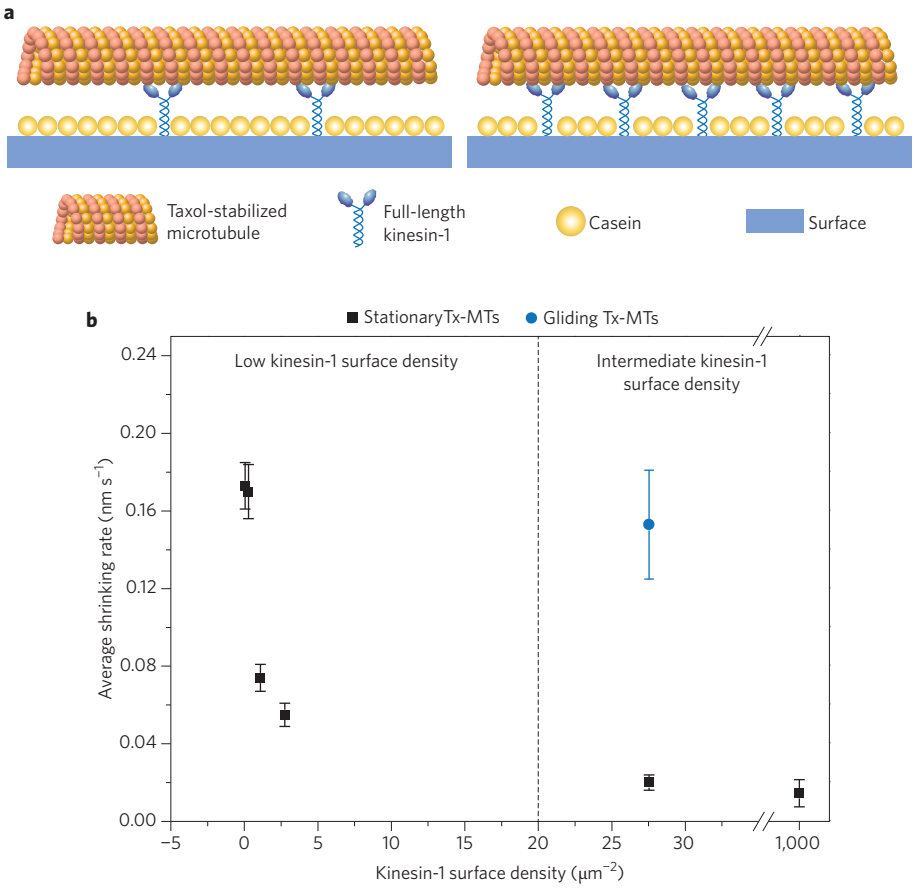

Figure 1 | The shrinkage of taxol-stabilized microtubules (Tx-MTs) depends on the kinesin-1 surface density. **a**, Schematic of the experimental set-up at low (left) and intermediate (right) kinesin-1 surface densities. The surface was passivated with casein before adhering kinesin-1 molecules non-specifically at defined surface densities. Tx-MTs were added in tubulin-free buffer solutions containing AMPPNP (stationary) or ATP (gliding). **b**, Average shrinking rates and standard errors of the mean for stationary as well as for gliding Tx-MTs (tracked by Fluorescence Image Evaluation Software for Tracking and Analysis (FIESTA); ref. 7) versus the kinesin-1 surface density. The surface densities of kinesin-1 were determined by landing-rate measurements of Tx-MTs for different dilutions of the kinesin-1 stock solution. For each data point for the stationary group, between 53 and 122 Tx-MTs were evaluated. The gliding Tx-MTs moved in the presence of 10 μM ATP with a velocity of 123 ± 11 nm s^{-1} (mean ± standard deviation; N = 35 Tx-MTs).

References
1. Brunner, C., Ernst, K.-H., Hess, H. & Vogel, V. *Nanotechnology* **15,** S540–S548 (2004).
2. Dumont, E. L. P., Do, C. & Hess, H. *Nat. Nanotech.* **10,** 166–169 (2015).
3. Krebs, A., Goldie, K. N. & Hoenger, A. *J. Mol. Biol.* **335,** 139–153 (2004).
4. Derry, W. B., Wilson, L. & Jordan, M. A. *Cancer Res.* **58,** 1177–1184 (1998).
5. Nogales, E. *Annu. Rev. Biochem.* **69,** 277–302 (2000).
6. Mitra, A. & Sept, D. *Biophys. J.* **95,** 3252–3258 (2008).
7. Ruhnow, F., Zwicker, D. & Diez, S. *Biophys. J.* **100,** 2820–2828 (2011).

Cordula Reuther[1,2†], Alejandra Laguillo Diego[1,2†] and Stefan Diez[1,2*]
[1]B CUBE — Center for Molecular Bioengineering, Technische Universität Dresden, 01069 Dresden, Germany. [2]Max Planck Institute of Molecular Cell Biology and Genetics, 01307 Dresden, Germany. [†]These authors contributed equally.
*e-mail: stefan.diez@tu-dresden.de