

# Molecular Motors: Kinesin's Interesting Limp

## Dispatch

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**An ingenious new experiment has used a form of kinesin with one slow head and one fast head to demonstrate definitively that this motor protein moves along a microtubule using alternating left and right steps.**

The motor protein kinesin is a nanometer-scale walking machine. So much is clear, but the question is, what kind of walking machine? The problem is fascinating and continues to generate ingenious, incisive experiments in single-molecule biophysics. The latest of these, from Kaseda *et al.* [1], has used an engineered kinesin heterodimer with fast and slow heads. The heterodimer moves along a microtubule with a marked limp, with alternating fast and slow molecular steps. These new results definitively demonstrate that kinesin has an underlying left–right walking action.

Early observations of microtubules sliding over cover slips that were coated sparsely with brain kinesin molecules indicated that these polymers can move for distances of up to several microns without detaching, over what were probably single kinesin molecules [2]. It was known that kinesin has two identical microtubule-binding heads, so the observation of long runs of continuous (mechanically processive) movement immediately suggested some sort of walking mechanism, in which the heads interact alternately with the microtubule. Subsequent work using optical trapping confirmed that single kinesin molecules can indeed move long distances along microtubules without detaching; that they move in steps of about 8 nm [3] at a time, equal to the axial distance between tubulin subunits in the microtubule; and that stepping slows down under load, stalling at about 6–7 pN [4,5].

That kinesin moves in repetitive steps of about 8 nm is by now axiomatic, but controversy, speculation and a degree of flummery still surround the detailed mechanics of stepping. What is quite clear is that kinesin tracks microtubule protofilaments [6]. Arguably the simplest way it could do this is the ‘protofilament tightrope’ model (Figure 1), in which the motor moves along a single protofilament by touching down with alternate microtubule-binding heads. But this apparently straightforward notion belies unexpected complications. Suppose for a moment that the two heads moved alternately but in exactly the same manner – for example, suppose at every step the trailing head releases and moves clockwise around

the bound head. In this case, the kinesin molecule would gradually wind itself up, twisting the two strands of its coiled coil tail around one another. The resulting torsion would cause microtubules moving over single immobilised kinesin molecules to rotate unidirectionally about their attachment point.

Although microtubules sliding over single kinesin molecules typically undergo large thermally driven rotations about their attachment [7], sustained unidirectional rotation has never been observed. We can therefore infer, either that torsion never occurs, or that if it does occur, it dissipates immediately. To be consistent with this observation, models need either to have exactly equivalent steps that do not generate torsion, or have pairs of non-equivalent steps that generate opposite torsions that cancel each other out.

The new work of Kaseda *et al.* [1] distinguishes between these possibilities. Their results establish directly that kinesin moves in pairs of non-equivalent steps. The innovation was to use kinesin molecules engineered to have two different types of head, a fast one and a slow one. In single molecule tracking in the optical trap, remarkable behaviour emerged: rather than the standard ~8 nm steps, the mutant molecule appeared to take 16 nm steps. But closer inspection (Figure 1 inset) revealed that each 16 nm displacement actually consists of two 8 nm steps with a brief pause between them. What is happening is that the molecule is taking alternate slow and fast steps, as its slow and fast heads alternately bind to the microtubule. This is a powerful result, because it allows us to exclude all models with equivalent steps, such as the inchworm model proposed last year by Hua *et al.* [8].

This inchworm model sprang out of experiments that used a truncated kinesin dimer with two equivalent heads attached via an avidin–biotin link to the surface. Microtubules sliding over such molecules not only failed to rotate unidirectionally about their attachment point, but showed appreciable torsional stiffness. Hua *et al.* [8] argued that this could only be explained by a model with symmetrical steps, and proposed that the leading kinesin head moved in 8 nm steps along the protofilament, towing the other head passively along behind it. The results of Kaseda *et al.* [1] definitively exclude all schemes with symmetrical steps, of which Hua *et al.*'s [8] inchworm model is one. But the observations reported by Hua *et al.* [8] remain to be explained. It will be as well to bear this in mind when considering what kinds of model remain plausible.

Perhaps the most salient question is whether the asymmetrical left–right walking action of kinesin takes place along a single protofilament, or whether the kinesin molecule straddles one or more protofilaments. It is clear that kinesin tracks the protofilament axis, but not that it moves along just one protofilament; the track might consist of two protofilaments, or even three. It is even possible that a kind of drunken

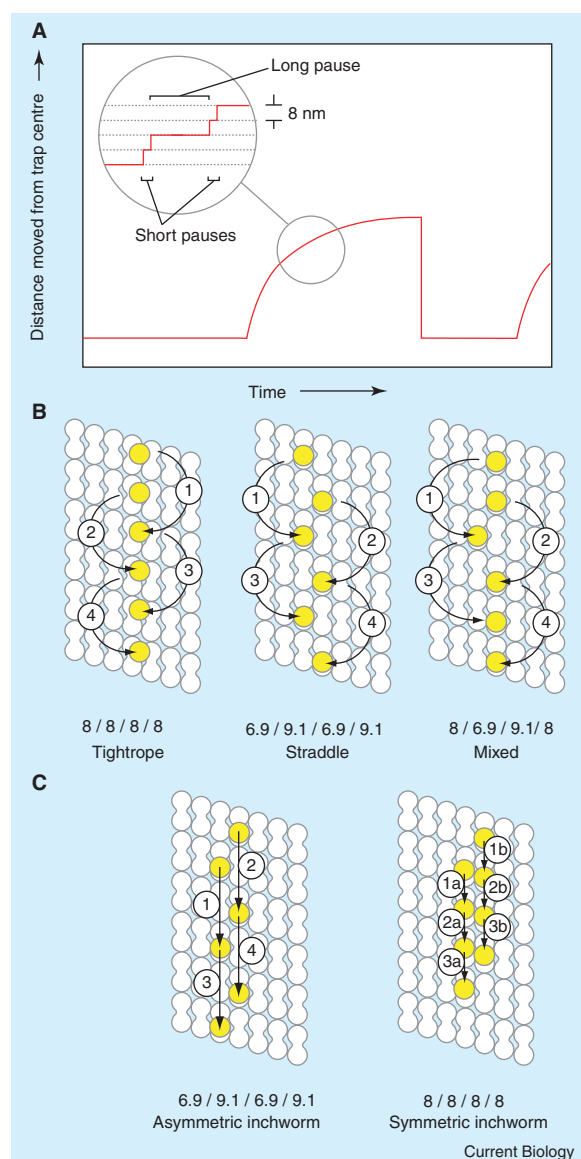


Figure 1. The mechanism by which kinesin walks along microtubules.

(A) An idealised single molecule optical trapping record. Events are initiated as the motor binds to the microtubule. The motor then walks out from the centre of the optical trap, progressively slowing down as the opposing force becomes progressively greater. Eventually it stalls, and then falls off the microtubule and back into the trap centre. In the experiments of Kaseda *et al.* using a kinesin heterodimer with one 'fast' and one 'slow' head, there was an initial impression that the molecule takes alternately fast and slow 8 nm steps (inset). (B) Asymmetrical hand-over-hand models. The yellow circles indicate binding sites, and the arrows the sequence of stepping between these sites. (C) Asymmetric (left) and symmetric (right) inchworm models. The new work of Kaseda *et al.* [1] rules out symmetrical inchworm models.

walk occurs, in which the molecule steps randomly between protofilaments but on average follows the protofilament axis (Figure 1B).

The main reason to think that the protofilament-tightrope model is correct is that it predicts 8 nm steps. Straddling between protofilaments and stepping with alternate heads predicts alternating 7 nm

and 9 nm steps [9]. These have not been seen, but it is not clear that any existing study has the resolution to exclude alternating steps of 7 nm and 9 nm. Another important possibility is an asymmetric inchworm model. A symmetric inchworm model, in which each step is identical and the heads never pass each other (Figure 1C, right), is excluded by the results of Kaseda *et al.* [1]. But an asymmetric inchworm model (Figure 1C, left), in which the heads move along different protofilaments with one head always in the lead, remains a possibility.

Numerous questions thus remain. Are alternate steps of equal size, or is there a 7 nm–9 nm pattern? How do backsteps occur? Are there rapid substeps within the 8 nm steps? Any substeps must certainly be fast, otherwise we would have seen them already. An early report of substeps in the millisecond regime [10] has not been confirmed in other labs, but Nishiyama *et al.* [11] recently observed 2 x 4 nm substeps on the microsecond time scale. Now we know that alternate kinesin steps are different, we can ask whether alternate steps are differently affected by applied force. Indeed, Block *et al.* [12] have recently reported that pulling to the left is more effective at slowing kinesin down than is pulling to the right.

We now know for certain that kinesin heterodimers limp. What about homodimers? The two heads of wild-type kinesin have identical sequences but are structurally non-equivalent. Furthermore, 'left' and 'right' trajectories over the microtubule surface are non-equivalent. Perhaps homodimers too might limp, albeit less detectably? Asbury *et al.* [13] have very recently reported observations of limping by some, though not all, homodimeric kinesin constructs. Perhaps the major challenge for the field now is to understand how this asymmetrical stepping behaviour arises at the molecular level. Unpacking the structure of individual steps will require both further improvements in instrumentation, and the kind of bold protein engineering epitomised by the work of Kaseda *et al.* [1].

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