KIF1A MOTOR FUNCTION REVISITED: FROM MECHANOCHEMICAL MODELS TO DISEASE MECHANISMS

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My View of the Present State of Research on KIF1A Motor Mechanism and Disease

KIF1A, a microtubule-based kinesin-3 family motor, is vital for the long-range, high-speed transport of synaptic vesicles and other cargo in neurons [1, 2]. Its ability to move with high speed and superprocessivity—unlike other kinesins—has positioned it as a molecular outlier and subject of intense investigation. At the same time, the emergence of KIF1A Associated Neurological Disorder (KAND) [3], caused by mutations in the KIF1A gene, has underscored the clinical urgency of understanding the motor's mechanisms and their perturbation in disease [4].

Historically, KIF1A was thought to function as a monomeric, processive motor [5, 6], an idea later supplanted by the discovery that dimerization *via* cargo binding or artificial leucine zippers is required for long-distance movement [7]. Nevertheless, the field remained intrigued by KIF1A's distinct behavior: a lower force output compared to kinesin-1 [8], rapid velocity, and extraordinary run lengths [9]. These characteristics raised foundational questions: How does KIF1A coordinate its two heads? Does it follow the same hand-over-hand stepping pattern as kinesin-1 [10]? And what features enable or limit its performance *in vivo*?

A widely held model—based on kinetic analyses of truncated dimers—proposed that KIF1A spends most of its stepping cycle in a one-head–bound (1HB) state [11]. This model attributes superprocessivity to the K-loop, a lysine-rich insertion in loop-12 that enhances the microtubule on-rate of the tethered head by interacting electrostatically with the negatively charged tubulin C-terminal tails. However, despite its appeal, this model left several questions unresolved, including how KIF1A maintains coordination between heads, and how disease mutations alter this behavior.

My Recent Research Contributions to KIF1A Motor Mechanism and Disease

In collaboration with Hernando Sosa, we recently addressed these questions using high-resolution cryo-EM and structure-function studies [12]. We resolved KIF1A bound to microtubules in both one- and two-heads–bound configurations, revealing tight inter-head connections and distinct conformations for the leading and trailing heads. Our sequence analysis indeed revealed that KIF1A's neck-linker is shorter than that of kinesin-1 and other kinesins, providing an explanation for increased inter-head tension and tighter coupling in KIF1A. Strikingly, we also found that KIF1A's class-specific K-loop engages the C-terminal tails of both α- and β-tubulin simultaneously, providing structural confirmation of its role in stabilizing the engaged state. The disease-associated P305L mutation, while not disrupting these electrostatic contacts, alters the conformation of loop-12, impairing the motor's ability to enter and maintain the strong-binding state. Structure-function analysis across wild-type and mutant constructs highlights the K-loop and head-head coordination as major determinants of KIF1A's superprocessivity [12]. These results refine our understanding of KIF1A's unique motility and suggest pathways for structure-guided drug development efforts.

Building on these structural insights, we conducted recently an in-depth mechanistic investigation using MINFLUX nanoscopy [13], a single-molecule localization technique that determines fluorophore positions by relating the zero-intensity position of a donut-shaped excitation beam to the fluorophore's unknown location [14, 15]. Contrary to the dominant view, we found that KIF1A predominantly adopts a two-heads–bound (2HB) state throughout its chemo-mechanical cycle. This new paradigm overturns the assumption that processivity depends on rapid reattachment following detachment. Instead, KIF1A's design favors stabilization of the 2HB state through K-loop-mediated interactions and tightly coupled neck-linker dynamics. This configuration supports efficient inter-head coordination and suggests that superprocessivity arises from prolonged tethering of the stepping head in a favorable geometry, rather than from compensation after detachment.

These findings are further supported by our mutational analyses. Pathogenic variants in the conserved 3_{10}-helix (e.g., P305L, identified in KAND patients) result in severe motility defects by altering K-loop conformation and disrupting head-head tension [12]. Single-molecule optical trapping experiments show that such variants reduce force production, impair coordination, or destabilize microtubule binding altogether [16]. Thus, minor structural perturbations at key integrative sites can propagate across the motor domain to disrupt motility, offering a molecular explanation for the broad spectrum of KAND phenotypes.

In complementary work, we investigated how different amino acid substitutions at the same residue—specifically R216H and R216C—give rise to distinct motor phenotypes

and clinical outcomes in KAND patients [4]. By generating and analyzing both homodimeric and heterodimeric KIF1A constructs, we found that the degree of motor impairment depends not only on the specific substitution but also on the dimeric context. Importantly, while KAND is mostly caused by heterozygous mutations, our results show that the functional properties of homodimeric mutant motors are more predictive of clinical severity than those of heterodimers. This is likely because the most severe disease phenotypes are associated with mutant homodimers that exhibit strong loss-of-function or dominant-negative behavior. These findings establish a mechanistic framework for understanding variable disease expressivity and for evaluating the potential efficacy of targeted therapeutic strategies.

An ongoing debate in the field concerns the maximal force output of KIF1A and the role of vertical (z-axis) forces in single-molecule force assays. In our prior work, we demonstrated that both a tail-truncated (390 aa) and full-length (1690 aa) KIF1A construct generate the same stall force of ~3 pN [8], indicating that z-force does not limit force generation in our experimental geometry. Importantly, based on molecular length estimates (~20 nm for the truncated and ~210 nm for the full-length construct, including tags and disordered tail regions), we calculated that the angle between the motor and the microtubule surface decreases from ~68° for the short construct to ~34° for the full-length motor, assuming a 250 nm bead radius. This reduces the associated z-force dramatically—from ~7.4 pN to ~1.9 pN at a constant horizontal force of 3 pN. While the force output remains unchanged, we found that the full-length construct exhibited a significantly longer stall duration compared to the truncated version, consistent with the idea that reduced vertical force decreases the off-rate during stall. This finding suggests that z-force primarily affects stall duration rather than maximal force output.

Nevertheless, Pyrpassopoulos et al. reported that KIF1A stalls at ~5 pN using a three-bead assay with negligible z-force, and Takamatsu et al. recently obtained similar results using a parallel DNA-spring geometry [17,18]. These authors attribute the higher stall forces to the near-zero z-force environment. However, our data challenge this explanation, as full-length KIF1A in our single-bead geometry experiences similarly low z-forces yet does not exceed 3 pN. Thus, the discrepancy remains unresolved. It is possible that other factors—such as compliance in the experimental system, differences in motor density or changing mechanochemical properties of the used microtubules—may underlie the reported differences. Resolving this issue will be essential for establishing a unified understanding of KIF1A's mechanical limits.

Outlook to Future Developments of Research on KIF1A Motor Mechanism and Disease

Looking ahead, several pressing questions remain. How does ATP binding versus hydrolysis regulate transitions between conformational states? How do post-translational modifications or lipid interactions modulate full-length KIF1A's activity in cells? Can structural insights into mutant motors be leveraged to develop small molecules that restore coordination or binding affinity? We believe that ongoing developments in cryo-EM, combined single-molecule fluorescence and optical tweezers setups, and *in vivo* nanoscopy will enable answers to these questions, moving the field closer to mechanism-based treatments for KAND.

In summary, the field of KIF1A motor biology is at a turning point. Recent advances have transformed our understanding of how this unique motor functions at the molecular level and how it fails in disease. The shift from a 1HB to a 2HB-based model of motility redefines the motor's design principles and repositions the K-loop and inter-head tension as central features of KIF1A function. As the field moves toward precision intervention, detailed structural and mechanochemical insight will be the foundation on which new therapies are built.

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