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PERSPECTIVE

Beyond Binding Affinity: How a Conformation-Specific Salt Bridge Tunes KIF1A Mechanochemistry

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ABSTRACT

Mutations at KIF1A residue R350 are linked to hereditary spastic paraplegia type 30 (SPG30), part of the spectrum of KIF1A-associated neurological disorders (KAND). Recent high-resolution cryo-EM structures of the KIF1A R350G and R350W mutants bound to microtubules in both nucleotide-free (apo) and AMP-PNP–bound states revealed a salt bridge between KIF1A R350 and α-tubulin E415 that forms specifically in the open motor domain conformation and is abolished by both substitutions. Single-molecule motility assays further showed that disruption of this conformation-dependent electrostatic interaction increases motor velocity while reducing processivity and microtubule affinity in the apo state. These findings identify a previously unrecognized mechanism regulating KIF1A motility and highlight how subtle changes in motor mechanochemistry can contribute to KAND pathology.

KIF1A is a neuron-specific motor protein of the Kinesin-3 family, a subgroup of ATP-driven molecular motors that convert the chemical energy of ATP hydrolysis into mechanical work and directional movement along microtubules (Yildiz 2024; Siddiqui and Straube 2020). It is a plus-end-directed motor that plays an essential role in anterograde transport of synaptic vesicle precursors and dense-core vesicles, as well as in interkinetic nuclear migration of radial glial progenitor cells (RGPs) during brain development (Hall and Hedgecock 1991; Okada et al. 1995; Yonekawa et al. 1998; Zahn et al. 2004; Barkus et al. 2008; Lo et al. 2011; Carabalona et al. 2016; Tsai et al. 2010). Pathogenic variants in *KIF1A* cause KIF1A-associated neurological disorder (KAND), a clinical spectrum of conditions ranging from hereditary spastic paraplegia to severe intellectual disability, cerebellar atrophy, and early-onset neurodegeneration (Lin et al. 2025; Kaur et al. 2020). Most KAND-associated mutations are located in the motor domain (Boyle et al. 2021), yet the molecular basis by which many of these mutations affect KIF1A motility remains unclear. Some variants, such as R350G, exhibit gain-of-function behavior, highlighting that KAND mutations do not necessarily abolish KIF1A motility (Chiba et al. 2019).

Using cryo-electron microscopy (cryo-EM) and in vitro single-molecule motility assays, we found that KIF1A R350 mutations disrupt the formation of a salt bridge between KIF1A R350 and α-tubulin E415 (Figure 1) (Shatarupa et al. 2026). Notably, this salt bridge forms only when the nucleotide-binding pocket of the KIF1A motor domain is in its open conformation—one of three discrete states (open, semi-closed, and closed) that the pocket adopts during the mechanochemical cycle (Benoit et al. 2024). The recognition that microtubule-bound KIF1A adopts a distinct open conformation is itself remarkably recent. Although KIF1A has been studied biochemically and structurally for nearly three decades, earlier X-ray crystallography of unbound motor domains and lower-resolution (worse than 6 Å) cryo-EM reconstructions of microtubule-bound complexes could resolve only the closed and semi-closed states; the open state was indistinguishable from semi-closed at these resolutions (Benoit et al. 2024). It was only with the cryo-EM 'resolution revolution' of the past decade (Kühlbrandt 2014; Egelman 2016)—and in particular with sub-4 Å reconstructions of microtubule-bound KIF1A reported in 2024 (Benoit et al. 2024)—that this third conformation could be identified and shown to be induced by microtubule binding itself.

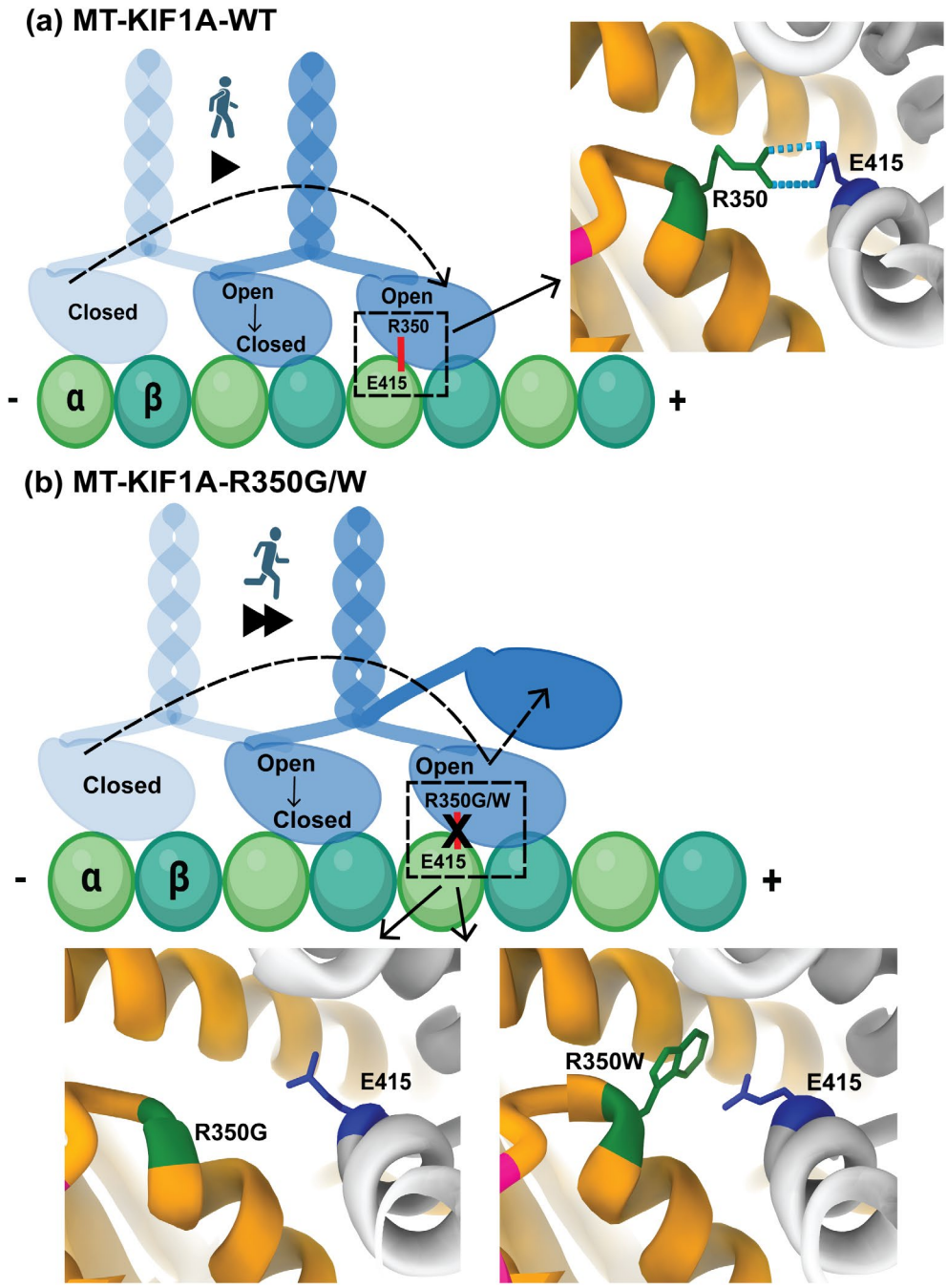

FIGURE 1 | Open-conformation-specific KIF1A–microtubule salt bridge disrupted by pathogenic R350 mutations. (a) Schematic representation of KIF1A stepping along the microtubule, illustrating transitions between closed and open catalytic motor-head conformations during processive movement. α-Tubulin and β-tubulin are shown in light and dark green, respectively, and KIF1A motor domains are colored in different shades of blue to distinguish their positions over time. In the wild-type motor, residue R350 forms a salt bridge with α-tubulin E415 (red line) when the motor domain is in the open conformation. The inset shows a magnified atomic ribbon representation of the interaction, with α-tubulin in light gray, the KIF1A motor domain in orange, R350 in green, and E415 in blue; blue dashed lines indicate the salt bridge. (b) Corresponding models for the R350G and R350W mutants are shown in a similar format. In both cases, the R350–E415 salt bridge is disrupted, as illustrated in the enlarged mutant interfaces below (left, R350G; right, R350W). Loss of this interaction is associated with increased motor velocity and reduced microtubule dwell time, illustrated schematically by a fast-forward symbol and an increased likelihood of microtubule detachment.

In dimeric KIF1A, the open conformation occurs in one-head-bound complexes in the apo or ADP states, and in the leading head of two-heads-bound complexes in the presence of the non-hydrolyzable ATP analog AMP-PNP. Consistent with disruption of the salt bridge in the open conformation, we found that the R350G and R350W mutations reduce microtubule affinity in the apo (open-conformation) state. Both mutations also exhibit increased stepping velocity relative to wild-type KIF1A, consistent with previous gain-of-function reports (Chiba et al. 2019), but at the expense of reduced run lengths (processivity). Strikingly, the choice of the substituting residue matters considerably: R350W increases the apparent dissociation constant for microtubule

binding ~24-fold relative to wild-type, compared with only ~4-fold for R350G—a roughly 6-fold difference in affinity arising from the identity of the substitution alone (Shatarupa et al. 2026). This likely reflects the additional steric disruption imposed by the bulky tryptophan side chain beyond the simple loss of the salt bridge and provides a structural rationale for the growing recognition that different amino acid substitutions at the same KIF1A residue can produce distinct clinical phenotypes (Rao et al. 2025).

The functional and structural results could be accounted for by a motility model in which disruption of the open-conformation-specific salt bridge between KIF1A and the microtubule lowers the energetic barrier for transition to the closed conformation following nucleotide binding, thereby accelerating progression through the stepping cycle and increasing stepping velocity. At the same time, loss of the salt bridge increases the probability of premature detachment from the microtubule in the open conformation (Figure 1b), thereby reducing run length, as observed.

KIF1A residue R350 is located in helix α6, which is adjacent to the neck linker, a structural element whose docking onto the motor domain drives the force-generating power stroke (Rice et al. 1999). Importantly, we found that the mutations do not have a major impact on neck linker conformations or other regions near the mutation site, arguing against altered neck linker dynamics as an explanation for the pathogenic effects of these mutants (Klebe et al. 2012; Lee et al. 2015). Because this study was not performed with full-length KIF1A, potential effects of the R350 mutations on the folded autoinhibited state (Chiba et al. 2019) cannot be completely ruled out. Structural models of full-length KIF1A based on related kinesin structures (Wang et al. 2022) and AlphaFold predictions place R350 far from the intramolecular interfaces that stabilize the autoinhibited state, arguing against an autoinhibition-disruption mechanism, though future studies with full-length constructs will be needed to confirm this.

Notably, this is the second recent example of a KAND mutation affecting the motor–microtubule interface. KIF1A contains a lysine-rich insertion within loop-12, the K-loop (KNKKKKK), that electrostatically interacts with the C-terminal tails of tubulin and is important for KIF1A superprocessive motility (Benoit et al. 2024). The pathogenic P305L mutation alters the conformation of this loop and reduces strong microtubule binding (Shatarupa et al. 2026). Similarly, the R350 mutations described by Shatarupa et al. disrupt a conformation-specific salt bridge between KIF1A and tubulin, resulting in accelerated stepping and reduced processivity. Together, these studies highlight how subtle perturbations at the motor–microtubule interface can strongly alter KIF1A motility.

Beyond KIF1A itself, the positively charged character of this position is striking in its conservation: an arginine or lysine is present at the equivalent position in essentially all kinesin families, from transport kinesins such as Kinesin-1 (KIF5B) and Kinesin-3 (KIF1A) to mitotic kinesins such as Kinesin-5 (Eg5) and Kinesin-13 (MCAK). Consistent with this idea, our finding of a conformation-specific salt bridge between α-helix 6 in the KIF1A motor domain and E415 in α-tubulin fits well with and helps explain previous observations. Computational studies have implicated the equivalent residue in Kinesin-1 (KIF5B) in microtubule binding (Li and Zheng 2011). On the tubulin side of this interface, mutation of α-tubulin E415 has been shown to alter Kinesin-1 microtubule-dependent ATPase activation (Uchimura et al. 2010), and a clinical variant in *TUBA4A* carrying the substitution E415K has been linked to spastic ataxia (Torella et al. 2023). Together, these observations support a coherent structural model in which a conserved electrostatic interaction between a kinesin α-helix 6 arginine/lysine residue and α-tubulin E415 is an indispensable functional element at the motor–microtubule interface, with perturbations on either side capable of producing disease phenotypes. From a therapeutic standpoint, conformation-specific interface contacts such as the R350–E415 salt bridge and the K-loop–tubulin tail interaction are appealing targets. Because these interactions are sampled only transiently during the mechanochemical cycle, a small molecule that selectively stabilizes a particular conformational state could, in principle, rebalance the kinetics of mutant KIF1A without globally inhibiting kinesin function—an approach that high-resolution structures of the kind discussed here now make tractable.

In summary, the work of Shatarupa et al. (Shatarupa et al. 2026) establishes the R350–E415 salt bridge as a conformationally regulated element of the kinesin–microtubule interface that contributes to the mechanochemical tuning of KIF1A. By demonstrating that its disruption by R350G and R350W accelerates motor velocity while compromising processivity and microtubule-binding affinity in KIF1A's open conformation—with quantitative differences between the two substitutions that mirror the allele-specific clinical heterogeneity seen across KAND—the study reveals a previously underappreciated functional dimension of kinesin–tubulin interactions: specific contacts at the interface do not merely govern binding affinity but actively shape the kinetics of the conformational transitions that drive motility. As the structural resolution of kinesin–microtubule complexes continues to improve and the catalog of KAND-associated mutations expands, this work provides a conceptual framework for understanding how point mutations at the motor–microtubule interface can affect motor function in ways that, while subtle at the molecular level, are sufficient to cause neurological diseases.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

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