Introduction: Neurons have branched projections called dendrites that receive signals from other neurons. Defects in dendrite structure negatively impact brain function. Dendrites are stabilized by an internal skeleton made up of microtubules (MTs). Dimers comprise MTs and polymerize into dynamic tube-like structures (**Fig. 1**); these polymers undergo nonequilibrium behavior called dynamic instability, a phenomenon that describes the stochastic interconversion between states of MT growth and shrinkage via cycles of GTP hydrolysis[1]. Though stochastic, MT growth is biased: GTP-tubulin heterodimers are added at the growing plus-ends to form a stabilizing GTP-cap. Local concentrations of microtubule-binding proteins (MBPs) regulate MT dynamics defined by growth and shrinkage parameters. MT growth and shrinkage are tightly regulated by the cell's needs, *e.g.* elongating to serve as highways for the trafficking of building materials in the neuron. MTs are integral to proper brain development and function, but mechanisms of MT development in neurons are not well understood.

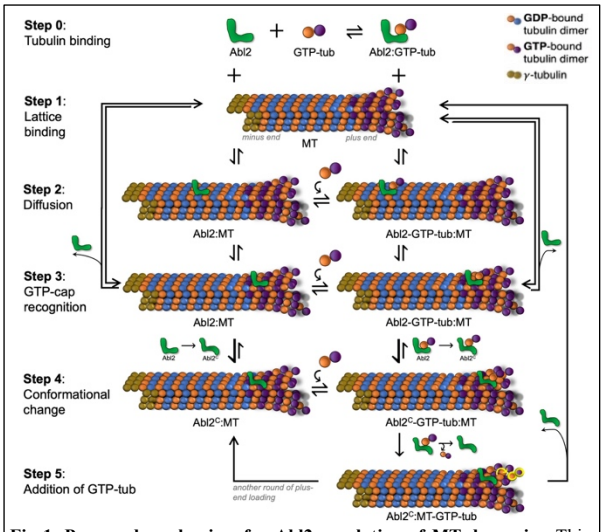

Fig 1. Proposed mechanism for Abl2 regulation of MT dynamics. This model for Abl2 regulation of MT dynamics considers the following possibilities: Abl2 may bind GTP-tubulin dimers in solution before its association with the MT lattice, recruit dimers during its "surf" along the GDP-lattice, or recruit dimers once it reaches the tip. Once Abl2 recognizes the GTP-cap comprised of at least one layer of 13 laterally-associated GTP-tubulin heterodimers, Abl2 may enhance its affinity for the lattice due to a conformational change and load its recruited GTP-tubulin to the growing end.

Abl-family nonreceptor tyrosine kinases play key roles in regulating actin cytoskeleton processes for cell adhesion, motility, and morphogenesis[2]. Abl2 kinase is an essential regulator of dendrite development in neurons. Knockout of Abl2 in mice significantly impairs dendrite development and stability, resulting in learning and memory deficits. Despite it being a kinase, Abl2 has the ability to directly interact with the cytoskeleton independently of its kinase domain[2,3]. The Koleske Group discovered that Abl2 binds to MTs to promote their elongation and suppresses shrinkage events[3]. From unpublished data, Yuhan Hu of the Koleske Group, my thesis lab, showed Abl2 binds tubulin dimers. However, it is still unclear how Abl2 interacts with MTs to regulate their dynamics.

Motivation: Plus-ends undergo frequent addition and subtraction of tubulin, and serve as binding hubs for a subset of MBPs called plus-end tracking proteins (+TIPs)[4]. Coordinated recruitment of +TIPs and other MBPs along the lattices, or bodies excluding the growing ends, of MTs allows cells to couple MT dynamics to specific sites for signaling events. The Koleske Lab provided strong evidence Abl2 is a novel MBP that increases MT growth rate and reduces shrinkage events[3]. However, the mechanism by which Abl2 elongates MTs to regulate cell motility and morphogenesis remains unknown. Hence, I will test if Abl2 recruits tubulin dimers, binds and diffuses along MT lattices, and recognizes the GTP-cap (**Fig. 1, Steps 2-4**).

Hypothesis: I hypothesize that Abl2 binds to the GDP lattice and recruits tubulin dimers using three binding interfaces: MT-binding region (Abl2-MTBR), tubulin-binding region (Abl2-TBR), and E-hook binding region (Abl2-EBR). I propose Abl2 diffuses along the GDP lattice, retains binding to the MT due to a characteristic structural feature of the GTP-cap, and stabilizes the cap by plus-end loading tubulin to increase growth rate and reduce shrinkage events (**Fig. 1**).

Aim 1: Identify Abl2 binding sites for MTs, tubulin dimers, and E-hooks. In order to determine whether binding to MTs, dimers, and E-hooks use different Abl2 interfaces, I will identify which residues mediate binding to all these sites. To address this, I will use molecular cloning to generate Abl2-MTBR/-TBR/-EBR fragments. I will perform co-sedimentation assays to identify the minimal Abl2-MTBR sufficient for MT binding with near-native affinity. To identify the minimal Abl2-TBR and Abl2-EBR with near-native affinities for soluble tubulin and E-hooks, I will use rhodamine-labeled Abl2 fragments and E-hooks in fluorescence anisotropy (FA) assays, respectively. If rhodamine-labelling of fragments yields inconsistent experimental results, I will use another dye-labeling methodology that involves site-specific labeling of a slowly hydrolysable GTP analog, GMPCPP. This label will bind specifically to the nucleotide-binding pocket of tubulin. I hypothesize that these interfaces are not mutually exclusive. *I expect Abl2-EBR and*

Abl2-TBR will not overlap significantly as to permit Abl2 to simultaneously diffuse along the lattice while remain bound to a tubulin dimer for eventual plus-end loading. These results would suggest Abl2 can bind to tubulin dimers while diffusing along the MT filament simultaneously (**Fig. 1, Step 2**).

Aim 2: Determine how Abl2 impacts MT dynamics *in vitro.* The nucleotide state of MT plus-ends determines the polymer's fate for continuous growth (GTP, GDP·P_i) or rapid shrinkage (GDP). It is unclear whether and which of Abl2 binding interfaces for MT, soluble tubulin, and E-hooks are necessary and sufficient for MT elongation. Hence, it is important to investigate if Abl2 binding to impact MT elongation is MT nucleotide-state dependent. To test whether Abl2 stabilizes MT assembly, I will measure turbidity, or cloudiness of the solution quantified by optical density (OD), using Abl2 and Abl2-MTBR/-EBR/-TBR respectively. Total internal reflection fluorescence (TIRF) provides the resolution for elucidating where Abl2 binding occurs on single MT filaments. Using TIRF, I will determine if Abl2 recruits tubulin dimers at the lattice and/or tips while bound onto MTs in a specific nucleotide-state, using immobilized GMPCPP-/GDP-AlF_4^-/GDP-lattices in the presence of soluble fluorescently labeled-tubulin. Nonhydrolyzable GMPCPP and GDP-AlF_4^- analogs will be used to mimic the GTP and GDP·P_i states of tubulin, respectively. These analogs reflect the stabilizing GTP-cap that prevents shrinkage events (**Fig. 1, Step 4**). I will elucidate how Abl2 and Abl2-MTBR/-TBR/-EBR modulate MT dynamics by quantifying growth and shrinkage parameters, and growth lifetimes from kymograph analyses. Under my hypothesis that Abl2-MTBR contains overlapping residues with Abl2-TBR and Abl2-EBR, length variability of MTs grown in presence of Abl2-MTBR will be lower than that of MTs grown in presence of Abl2-TBR and Abl2-EBR. These results would suggest Abl2-MTBR prevents transient episodes of shortening by stabilizing MT plus-ends. *I also predict tubulin would diffuse along MT filaments, suggesting Abl2-TBR and Abl2-EBR mediate tubulin-binding and diffusion respectively.* Turbidity results will support my hypothesis if plateaued OD values in presence of Abl2-MTBR are higher than those observed in presence of tubulin alone or tubulin with Abl2-TBR/-EBR, suggesting greater polymer mass hence Abl2-MTBR stabilizes assembly.

Intellectual Merit: Our current understanding of Abl2 surrounds its regulation of the actin cytoskeleton to stabilize filaments as a scaffolding protein for cell morphogenesis and migration. The Koleske Lab was the first to demonstrate Abl2 impacts not only actin, but MTs. My proposed work aims to elucidate the key mechanism that controls MT dynamics to ensure proper dendrite development. I have extensive experience with protein engineering and purification, and reconstitution of the actin cytoskeleton *in vitro*, similar to the reconstitution of the MT cytoskeleton, making proposed single-molecule TIRF experiments feasible. My findings will open doors for testing new hypotheses surrounding how Abl2:MT interactions affect cell morphogenesis and motility; and make possible future projects studying the crosstalk between actin and MTs under Abl2 regulation for stabilizing dendrite development in neurons.

Broader Impacts: Identifying the mechanism by which Abl2 regulates MT dynamics will enhance the repertoire of MBPs and advance the MT dynamics field at large. There is currently not an MBP identified that both increases MT growth rate and suppresses shrinkage events aside from the classical microtubule-associated protein tau and tubulin itself[5]. Additionally, Abl2 is essential for neuronal stability but how Abl2 stabilizes dendrites also remains unresolved. My complete examination of the impact Abl2 has on MT growth will push forward how researchers understand molecular mechanisms underlying dendrite stability and development. Throughout the course of my project, I will share my findings at national conferences such as the Biophysical Society Annual Meeting, Yale departmental seminars, and retreats. As a student in Yale's Integrated Graduate Program in Physical and Engineering Biology (PEB), I will present research-in-progress (RIP) talks with the PEB community. I will establish a student-led Yale Cytoskeleton Club and organize biweekly RIP talks to provide senior undergraduate and graduate students opportunities for sharing their work related to cytoskeletal dynamics and gaining oral and poster presentation experience. As Director of Education and Outreach of STEM organization, Científico Latino, I will share with the Científico Latino community my passion for studying MT dynamics through webinars. As an NSF Fellow, I am excited to pursue this cutting-edge project while continuing to engage in science education and outreach.

References: [1]Desai A and T J Mitchison. Annu Rev Cell Dev Bi (1997).[2]Lapetina S*, et al.* J Cell Biol (2009).[3]Hu Y*, et al.* J Cell Biol (2019).[4]Akhmanova A and M O Steinmetz. Nat Rev Mol Cell Bio (2008).[5]Bowne-Anderson, *et al.* Trends in Cell Biol (2015).