MAR15-2014-020239

Abstract for an Invited Paper
for the MAR15 Meeting of
the American Physical Society

Dissecting EB1-microtubule interactions from every direction: using single-molecule visualization and static and dynamic binding measurements

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EB1 is an important microtubule associating protein (MAP) that acts as a master coordinator of protein activity at the growing plus-end of the microtubule. We can recapitulate the plus-end binding behavior of EB1 along the entire length of a static microtubule using microtubules polymerized in the presence of the nonhydrolyzable GTP analogs GMPCPP and GTPγS instead of GTP. Through the use of single-molecule TIRF imaging we find that EB1 is highly dynamic (with a sub-second characteristic binding lifetime) and continuously diffusive while bound to the microtubule. We measure the diffusion coefficient, D, through linear fitting to mean-squared displacement of individually labeled proteins, and the binding lifetime, τ, by fitting a single exponential decay to the probability distribution of trajectory lifetimes. In agreement with measurements of other diffusive MAPs, we find that D increases and τ decreases with increasing ionic strength. We also find that D is sensitive to the choice of GTP analog: EB1 proteins bound to GTPγS polymerized microtubules have a D half of that found with GMPCPP polymerized microtubules. To compare these single-molecule measurements to the bulk binding behavior of EB1, we use TIRF imaging to measure the intensity of microtubules coated with EB1-GFP as a function of EB1 concentration. We find that EB1 binding is cooperative and both the quantity of EB1 bound and the dissociation constant are sensitive to GTP analog and ionic concentration. The correlation between binding affinity and D and the cooperative nature of EB1-microtubule binding leads to a decrease in D with increasing EB1 concentration. Interestingly, we also find an increase in τ at high EB1 concentrations, consistent with attractive EB1-microtubule interactions driving the cooperativity. To further understand the nature of the cooperativity we estimate the interaction energy by measuring the association and dissociation rates (k_{on} and k_{off} respectively) at different concentrations of EB1.