Mapping multivalency in the CLIP-170–EB1 microtubule plus-end complex

Received for publication, October 3, 2018, and in revised form, November 9, 2018 Published, Papers in Press, November 19, 2018, DOI 10.1074/jbc.RA118.006125

Yaodong Chen‡, Ping Wang§, and Kevin C. Slep¶

From the ‡Key Laboratory of Resources Biology and Biotechnology in Western China, Ministry of Education, College of Life Sciences, Northwest University, Xi’an, Shaanxi 710069, China, the §Department of Biology, University of North Carolina, Chapel Hill, North Carolina 27599–3280, and the ¶Department of Neurology, University of North Carolina, Chapel Hill, North Carolina 27599

Edited by Peter Cresswell

Cytoplasmic linker protein 170 (CLIP-170) is a microtubule plus-end factor that links vesicles to microtubules and recruits the dynein–dynactin complex to microtubule plus ends. CLIP-170 plus-end localization is end binding 1 (EB1)–dependent. CLIP-170 contains two N-terminal cytoskeleton-associated protein glycine-rich (CAP-Gly) domains flanked by serine-rich regions. The CAP-Gly domains are known EB1-binding domains, and the serine-rich regions have also been implicated in CLIP-170’s microtubule plus-end localization mechanism. However, the determinants in these serine-rich regions have not been identified. Here we elucidated multiple EB1-binding modules in the CLIP-170 N-terminal region. Using isothermal titration calorimetry and size-exclusion chromatography, we mapped and biophysically characterized these EB1-binding modules, including the two CAP-Gly domains, a bridging SXIP motif, and a unique array of divergent SXIP-like motifs located N-terminally to the first CAP-Gly domain. We found that, unlike the EB1-binding mode of the CAP-Gly domain in the dynactin-associated protein p150Glued, which dually engages the EB1 C-terminal EEY motif as well as the EB homology domain and sterically occludes SXIP motif binding, the CLIP-170 CAP-Gly domains engage only the EEY motif, enabling the flanking SXIP and SXIP-like motifs to bind the EB homology domain. These multivalent EB1-binding modules provided avidity to the CLIP-170–EB1 interaction, likely clarifying why CLIP-170 preferentially binds EB1 rather than the α-tubulin C-terminal EEY motif. Our finding that CLIP-170 has multiple non-CAP-Gly EB1-binding modules may explain why autoinhibition of CLIP-170 GAP-Gly domains does not fully abrogate its microtubule plus-end localization. This work expands our understanding of EB1-binding motifs and their multivalent networks.

Microtubules are polarized cellular polymers composed of the building block αβ-tubulin and are central to dynamic cellular processes, including cell migration, ciliogenesis, and mitosis. The microtubule structure is dynamic and undergoes phases of polymerization, pause, depolymerization, and rescue (1). Although microtubules are inherently dynamic, the dynamics are spatially and temporally regulated by a host of microtubule-associated proteins (MAPs). A subset of MAPs preferentially localize to polymerizing microtubule plus ends and are termed plus-end tracking proteins (+TIPs) (2, 3). +TIPs include a diverse set of proteins that have assorted structural domains and functional properties. Many +TIPs regulate microtubule dynamics or serve as adaptors that link microtubule plus ends to other factors, including cell polarity factors and kinetochores (4, 5).

The microtubule EB family members, including human EB1, EB2, and EB3, are central +TIPs, as they directly bind the microtubule plus end and recruit a host of other +TIPs, establishing a +TIP network (3, 6, 7). The EB proteins have a common domain architecture that consists of an N-terminal calponin homology (CH) domain, a central linker, and a C-terminal dimerization domain (EBHD) that confers homodimerization as well as heterodimerization for the EB1–EB3 pair (Fig. 1A) (4, 8–11). The dimerization domain is followed by a conserved C-terminal motif consisting of the residues EEY that is similar to the C-terminal segment of α-tubulin (12). The CH domain binds the microtubule lattice between four tubulin heterodimers, where it specifically engages tubulin in a transition state/post-GTP-hydrolysis state just distal of the microtubule’s polymerizing GTP cap (7). Both the EB1 dimerization domain and the EEY motif serve to bind and recruit factors to the microtubule plus end (12–15).

The EBHD forms a coiled-coil structure that transitions to a four-helix bundle at its C-terminal region (Fig. 1B) (4, 11). The dimerization domain forms two homologous sites at the coiled coil–four-helix bundle interface that are used to bind SXIP or LXXPTPh motif–containing proteins (Fig. 1B) (13, 16, 17). Over 50 proteins have been identified that contain SXIP motifs and show EB1-dependent microtubule plus end–tracking activity (3). The EB1 C-terminal EEY motif is capable of binding CAP-Gly domains such as those found in p150Glued of the dynactin complex as well as CLIP-115 and CLIP-170 (Fig. 1C) (14, 15, 18). The p150Glued CAP-Gly domain dually engages the EB1 EEY motif as well as the EBHD, where its binding site overlaps

2 The abbreviations used are: MAP, microtubule-associated protein; +TIP, plus end tracking protein; CH, calponin homology; EBHD, EB homology domain; ITC, isothermal titration calorimetry; SEC, size-exclusion chromatography; SEC-MALS, SEC with multi-angle static light scattering; Ct, C-terminal; Nt, N-terminal.
with the binding site for SXlP and LXXPTPh motifs, suggesting mutually exclusive binding per site on the EB1 dimer (13–17). Whether CLIP-170 CAP-Gly domains engage the EBHD in a similar manner remains to be determined.

CLIP-170 was the first identified +TIP protein and is involved in linking endocytic vesicles to microtubules and recruiting the dynein–dynactin complex to microtubule plus ends by binding the dynactin component p150Glued (14, 15, 19–23). The domain architecture of CLIP-170 includes three prime parts: an N-terminal region of ~350 residues that encompasses two CAP-Gly domains flanked and separated by serine-rich regions, a central coiled-coil region that mediates homodimerization and is involved in binding the microtubule pause factor CLIP-associated protein (CLASP), and a C-terminal segment that contains two zinc knuckles and an ETF motif that can bind and autoinhibit the CAP-Gly domains (19, 21, 22, 24–27). When not autoinhibited, the CLIP-170 CAP-Gly domains can each bind C-terminal EEY motifs, as found in both the α-tubulin acidic tail and EB1 (26–28). In cells and in vitro, the CLIP-170 N-terminal CAP-Gly domain – containing region (residues 1–350) is sufficient to track polymerizing microtubule plus ends in an EB1-dependent manner (20, 28, 29). In vitro, in the absence of EB1, CLIP-170 mostly binds along the lengths of microtubules (28–30). Why CLIP-170 preferentially engages EB1 versus the tubulin C-terminal tail when EB1 is present remains unclear. One hypothesis is that CLIP-170 has a higher affinity/greater avidity for EB1 than for tubulin.

The CLIP-170 central coiled coil has a predicted hinge region that enables the C-terminal zinc knuckles and ETF motif to engage and autoinhibit the CAP-Gly domains from binding EB1 (21, 27). CLIP-170 autoinhibition is regulated by phosphorylation of Ser-311 (31, 32). Phosphorylated CLIP-170 preferen-

---

**Figure 1. Domain architecture of CLIP-170 and EB1.** A, domain architecture of EB1 and CLIP-170. EB1 contains an N-terminal CH domain that binds the microtubule lattice and a C-terminal dimerization domain termed the EBHD. EB1 also contains a C-terminal EEY motif that binds CAP-Gly domains. CLIP-170 contains two N-terminal CAP-Gly domains (denoted A and B) and a central coiled coil involved in dimerization. Two C-terminal zinc knuckle domains are involved in binding and inhibiting CAP-Gly binding to EEY motifs and a C-terminal ETF motif that also binds CAP-Gly domains in a mode similar to EEY motifs. B, structure of EBHD–MACF2 SXlP complex (PDB code 3GJO) (13). The EBHD is shown in cartoon format in green and yellow, and the MACF2 SXlP motif is shown in stick format in orange. C, composite structural model of the EB1-p150Glued CAP-Gly domain complex (PDB codes 2HKQ and 2HL3 superimposed, dually aligned over their respective CAP-Gly domains) (15). The EBHD is presented as in B, with the EEY motif depicted in stick format. The two p150Glued CAP-Gly domains (shown in cyan with a transparent surface depicted on the molecule on the left) engage the EB1 EEY motif as well as the coiled-coil portion of the EBHD. The CAP-Gly residues that engage the EBHD are colored red. The p150Glued CAP-Gly domain engages the same region on the EBHD as the SXlP peptide (shown in B), suggesting that p150Glued CAP-Gly domain and SXlP motif binding to the EBHD are mutually exclusive. D, sequence comparison of the EBHD binding region of the p150Glued CAP-Gly domain versus CLIP-170 CAP-Gly A and B. p150Glued EBHD-binding residues are shown in red. The position-equivalent residues in CLIP-170 CAP-Gly domains that are not conserved are highlighted in yellow.
Diverse CLIP-170–EB1 interaction modes

...adoption of a folded/autointhibited conformation. Interestingly, when phosphorylated at Ser-311, CLIP-170 still accumulates at the microtubule plus end (albeit with diminished comet length relative to nonphosphorylated CLIP-170), suggesting that it is still bound to EB1 (31, 32). How CLIP-170 accumulates at the microtubule plus end when its CAP-Gly domains are autoinhibited is unclear but suggests that CLIP-170 may contain additional EB1-binding determinants.

In this study, we performed structure/function analysis of the N-terminal CLIP-170 region that displays EB1-dependent microtubule plus-end tracking activity. We determined the affinity of each CAP-Gly domain for EB1 and identified two distinct regions outside of the CAP-Gly domains that bind EB1. One binding element is an SXIP motif located between the two CAP-Gly domains that has a weak binding affinity for the EBHD. Another EB1 binding site includes multiple SXIP-like motifs, is located N-terminally to the first CAP-Gly domain, and includes ~20 conserved residues that bind the EBHD with high affinity. These interactions, when analyzed in the presence of the CAP-Gly domains, strongly enhanced EB1 binding in vitro. Thus, CLIP-170 uses a diverse, multivalent set of elements to engage EB1 and form a stable complex. The multivalent nature of the CLIP-170–EB1 interaction likely clarifies the ability of CLIP-170 to bind EB1 and localize to microtubule plus ends even when its CAP-Gly domains are autoinhibited. The high avidity of the complex is anticipated to preferentially select EB1 binding over α-tubulin binding.

Results

The CLIP-170 CAP-Gly domains engage the EB1 C-terminal tail and do not appreciably affect SXIP motif binding to the EBHD

Previous work suggests that CLIP-170 may be able to interact with EB1 using determinants outside of its CAP-Gly domains. This is based on two sets of data. First, CLIP-170 phosphorylated at residue Ser-311 (a readout for the autoinhibited state in which the CAP-Gly domains are competitively bound) still tracks microtubule plus ends (31, 32). Second, although the p150Glued CAP-Gly domain has a higher EB1 binding affinity than a CLIP-170 construct containing both of its two CAP-Gly domains and the bridging linker, a larger CLIP-170 construct interacts with EB1 more robustly than does p150Glued (26, 33). This caused us to ask the following question: are there determinants outside of the CLIP-170 CAP-Gly domains that engage EB1? Potential non-CAP-Gly EB1 binding modes include SXIP or LXXPTPH motif binding to the EBHD (13, 16, 17). However, the structure of the p150Glued CAP-Gly domain bound to EB1 indicated that this CAP-Gly domain engaged both the EB1 Eey motif as well as the EBHD, which sterically occluded SXIP and LXXPTPH motif binding (Fig. 1, B and C) (14, 15). We questioned whether CLIP-170 CAP-Gly domains could also engage the EBHD or whether they only engaged the Eey motif, leaving the EBHD free to bind SXIP or LXXPTPH motifs that might be found flanking the CLIP-170 CAP-Gly domains. Comparative analysis of the nine p150Glued CAP-Gly residues that contact the EBHD (Fig. 1C, residues colored in red) revealed that only four are conserved in CLIP-170 CAP-Gly A and B, with two of these being conserved glycine residues involved in the domain’s fold (Fig. 1D). This suggested that, unlike p150Glued, CLIP-170 CAP-Gly domains might bind EB1 in a mode that left the EBHD free to bind SXIP or LXXPTPH motifs.

We next used isothermal titration calorimetry (ITC) to determine whether CLIP-170 CAP-Gly domains, like those of p150Glued, bind EB1 through dual engagement of the C-terminal Eey motif and the EBHD. We generated two EB1 constructs. The first included both the EBHD domain and Eey C-terminal tail (EB1268, residues 191–268), and the second contained only the EBHD (EB1260, residues 191–260), and we measured the binding affinity between these constructs and CLIP-170 CAP-Gly A (residues 57–130) and B (residues 211–279) (Fig. 2, A–D). CAP-Gly A and B each showed an exothemeric binding isotherm with EB1268. The binding curves were best fit to a one-site model (two CAP-Gly domains binding independently to one EB1 dimer; potential binding models were evaluated based on characterization analyses) yielding KD values equal to 1.5 μM and 4.0 μM for CAP-Gly A and B, respectively (Fig. 2, A and C). In contrast, neither CAP-Gly domain revealed detectable binding to EB1260 (Fig. 2, B and D), suggesting that CLIP-170 CAP-Gly domains do not robustly engage the EBHD.

We next tested whether CLIP-170 CAP-Gly domain binding to the EBHD affected the binding of an SXIP motif to bind the EBHD (Fig. 3, A–F). We synthesized two peptides corresponding to the SXIP motifs found in SLAIN2 (QSAIIPSPGK-FRSPAY) and CLASP2 (KRKIPRSQGSSRET) (13, 34). We measured the binding affinity of these peptides for EB1268 alone and when EB1268 was prebound to CLIP-170 CAP-Gly A or CAP-Gly B. The SLAIN2 peptide and the CLASP2 peptide bound free EB1268 with a KD equal to 17.9 μM and 5.3 μM, respectively (both fit to a one-site model; two peptides binding independently to one EB1 dimer) (Fig. 3, A and D). Prebinding CAP-Gly A to EB1268 did not appreciably affect the binding affinity of the SLAIN2 peptide (KD = 18.9 μM); however, it did have a moderate effect on the CLASP2 peptide’s affinity, decreasing the apparent affinity to a KD equal to 14.4 μM (Fig. 3, B and E). Similar results were obtained when EB1268 was prebound to CAP-Gly-B; the SLAIN2 peptide bound with a KD of 22.5 μM and the CLASP2 peptide bound with a KD of 14.7 μM (Fig. 3, C and F). This indicates that CLIP-170 CAP-Gly domain binding to EB1 does not occlude the SXIP motif binding site. It is also of note that we did not observe CAP-Gly–dependent cooperative effects on trans SXIP motif binding to the EBHD; i.e., CAP-Gly domain binding to the EB1 Eey motif did not potentiate the binding of an SXIP motif–containing peptide to the EBHD (Fig. 3, A–F).

An SXIP motif between the CLIP-170 CAP-Gly domains has weak EB1–binding activity

Given that the CLIP-170 CAP-Gly domains do not sterically occlude SXIP motif binding, we inquired how a previously identified SXIP motif located between CAP Gly A and B (residues 165–168) affected CLIP-170 binding to EB1 (33, 35). The motif in human CLIP-170, SNIP, is conserved across many animals from frog to human, with conservation extending to flanking regions (consensus: P(S/N/G)IPXXXS) (Fig. 4A). We first tested the binding of a CLIP-170 construct (CLIP-170 A-SNIP) that embodied both the CAP-Gly domain A and the C-terminally...
flanking SNIP motif (residues 57–210) to EB1260. Although CAP-Gly A did not bind EB1260 (Fig. 2C), CLIP-170 A-SNIP bound EB1260 exothermically and fit best to a one-site model with a $K_D$ equal to 12.7 $\mu$M. When we examined CLIP-170 A-SNIP binding to EB1268, the exothermic binding curve was best fit to a two-site model (two CLIP-170 A-SNIP constructs binding to one EB1 dimer, with each EB1 dimer containing two CAP-Gly domain binding sites (the EEY tail) and two SXIP motif binding sites (on the EBHD)), yielding $K_{D1}$ equal to 19.7 $\mu$M and $K_{D2}$ equal to 1.5 $\mu$M (Fig. 4, C and D). When we mutated the SNIP motif to SNNN (mutations shown previously to ablate EB1 binding (13)), the CLIP-170 A-SNNN construct still bound EB1268 as expected but fit best to a one-site model with a $K_D$ equal to 1.2 $\mu$M. This binding is on par with the value expected for just CAP-Gly A binding to the EB1268 C-terminal EEY motif. ITC analysis of CLIP-170 A-SNIP binding to EB1260 yielded a $K_D$ equal to 15.6 $\mu$M, whereas CLIP-170 A-SNNN did not exhibit binding to EB1260 (Fig. 4E). Similar

![Figure 2. CLIP-170 CAP-Gly domains bind the EB1 EEY motif but do not detectably bind the EBHD.](image)
EB1<sub>268</sub> and EB1<sub>260</sub> binding patterns were observed for a CLIP-170 construct that embodied the SNIP motif and the flanking CAP-Gly B domain (CLIP-170 SNIP-B, residues 156–279) and its mutant SNNN version (CLIP-170 SNNN-B) (Fig. 4F). We next synthesized a peptide corresponding to the human CLIP-170 SNIP-containing sequence (residues 162–176; TPSNIPQKPSQPAAKY) and analyzed the binding of this peptide to EB1<sub>260</sub>. The binding isotherm was best fit to a one-site binding model (two peptides binding independently to one EB1 dimer) with a $K_D$ equal to 19.5 μM (Fig. 4G). This binding affinity is similar to that determined for the SLAIN2 SAIP motif peptide (17.9 μM, Fig. 3A) but slightly weaker than the CLASP2 SKIP motif peptide (5.3 μM, Fig. 3D).

These findings indicate that the EB1 dimer can engage CLIP-170 using multiple binding sites, two C-terminal EEY motifs, used to bind two CAP-Gly domains (either CAP-Gly A or B), as well as the EBHD’s two SXIP motif binding sites, used to bind the CLIP-170 SNIP motif (for which there would be two in the functional CLIP-170 homodimer), and that these binding sites function independently.

### The CLIP-170 CAP-Gly domains and SNIP motif are not the only EB1-binding modules in CLIP-170

We next analyzed whether the CLIP-170 SNIP motif was sufficient to bind and shift the EB1 elution profile using size-
Figure 4. An SXIP motif between the two CAP-Gly domains of CLIP-170 binds the EBHD with moderate affinity. 

A, sequence alignment of CLIP-170 from seven animals focused on the linker region that bridges CAP-Gly domains A and B. The SXIP motif is highlighted in yellow. The image on the right depicts the general CLIP-170 A-SNIP and SNIP-B constructs used in the ITC analyses presented in B-F. 

B, ITC analysis of CLIP-170 A-SNIP titrated into a well containing EB1260. Top panel, thermogram; bottom panel, integrated heats of reaction fit to a one-site binding model yields a $K_D$ of $12.7 \pm 3.9 \mu M$. 

C, CLIP-170 A-SNIP titrated into a well containing EB1268 is best fit to a two-site binding model with $K_{D1}$ of $19.7 \pm 3.5 \mu M$ and $K_{D2}$ of $1.5 \pm 0.3 \mu M$. 

D, comparison of the data in C fit to a one-site versus two-site binding model. 

E, comparative analysis of ITC binding curves for CLIP-170 A-SNIP constructs (WT and a construct in which the SXIP motif was mutated to SNNN) to EB1268 as shown in C (fit to a two-site model) and to EB1260 (fit to a one-site model). CLIP-170 A-SNIP bound to EB1260 with a $K_D$ of $12.7 \pm 3.9 \mu M$. CLIP-170 A-SNNN bound to EB1268 with a $K_D$ of $1.3 \pm 0.2 \mu M$ (one-site binding model) but failed to show detectable binding to EB1260. 

F, comparative analysis of ITC binding curves for CLIP-170 SNIP-B constructs (WT and a construct in which the SXIP motif was mutated to SNNN). CLIP-170 SNIP-B binding to EB1268 fit to a two-site binding model and yielded $K_{D1}$ of $20.0 \pm 6.8 \mu M$ and $K_{D2}$ of $5.1 \pm 0.8 \mu M$. CLIP-170 SNIP-B bound EB1260 with a $K_D$ of $16.4 \pm 8.0 \mu M$ (fit to a one-site model). CLIP-170 SNNN-B bound to EB1268 with a $K_D$ of $4.7 \pm 0.6 \mu M$ (one-site binding model) but failed to show detectable binding to EB1260. 

G, CLIP-170 SNIP peptide titrated into a well containing EB1260 yielded a $K_D$ of $19.5 \pm 4.5 \mu M$ (one-site binding model). For the ITC experiments shown, the injected components are indicated in blue, and components in the well are indicated in green. Errors reported are standard deviation.
exclusion chromatography (SEC). To assay the interaction, we constructed and purified a CLIP-170 fragment containing both CAP-Gly A and B and the intervening SNIP motif–containing linker (CAP-Gly A-B, residues 57–279). Co-injection of an equimolar CAP-Gly A-B:EB1268 mixture yielded a peak that eluted at 28.5 min that was distinct and eluted earlier than either component run individually (Fig. 5A). This peak shift suggested that CAP-Gly A-B and EB1268 formed a robust complex over gel filtration. We next analyzed CAP-Gly A-B binding to EB1260 but did not detect a shift in the elution profile (Fig. 5B). Because the EB1260 construct cannot engage the CAP-Gly domains, this result indicates that the CLIP-170 SNIP motif alone is not sufficient to form a stable complex with the EBHD over gel filtration. This may suggest that the SNIP motif may not be accessible to EB1 when flanked by both CAP-Gly domains. This finding is aligned with previous work demonstrating that the SNIP motif did not contribute significantly to EB1 binding in the context of a CLIP-170 construct containing both CAP-Gly domains (33). Surprisingly, when we analyzed the ability of a larger CLIP-170 construct embodying residues 1–350 to bind EB1260, we found that it robustly interacted with the EBHD, and the proteins co-eluted at 25 min, earlier than either component run individually (Fig. 5C). In agreement with our finding that the CLIP-170 SNIP motif was not sufficient for the interaction with EB1260, when we tested a CLIP-170 1–350 construct in which the central SNIP motif was mutated to SNNN, we still detected a robust interaction with EB1260 over gel filtration (Fig. 5C). This suggested that a region either N-terminal to CAP-Gly A (within residues 1–56) or C-terminal to CAP-Gly B (within residues 280–350) was binding to the EB1260 EBHD.

We next analyzed the binding affinity between EB1260 and CLIP-170 constructs containing both CAP-Gly domains using ITC. The CLIP-170 CAP-Gly A-B construct (residues 57–279)
bound to EB1_{260} with a $K_D$ equal to 30.4 $\mu M$, presumably mediated exclusively by the central SNIP motif (Fig. 5D). This binding affinity is slightly weaker than that detected for CAP-Gly A-SNIP (12.7 $\mu M$, Fig. 4B) or SNIP-CAP-Gly B (15.6 $\mu M$, Fig. 4F) and may reflect binding hindrance when both CAP-Gly domains are present and flank the SNIP motif. In contrast, the CLIP-170 1–350 construct bound to EB1_{260} robustly with a $K_D$ value equal to 0.5 $\mu M$ (Fig. 5E). In agreement with our size exclusion chromatography data, this suggested that a region outside of the CLIP-170 A-B (57–279) construct but present in the CLIP-170 1–350 construct was binding EB1 with high affinity. We purified a CLIP-170 construct embodying CAP-Gly B and residues C-terminal to it (residues 205–350) but failed to detect binding to EB1_{260} (Fig. 5F). This suggested that determinants N-terminal to CLIP-170 CAP-Gly A were capable of binding the EB1_{260} EBHD.

**The CLIP-170 N-terminal region contains a unique EB1-binding motif**

To map specific EB1 binding determinants in the CLIP-170 N-terminal region, we generated a number of CLIP-170 N-terminal deletion constructs that spanned through the end of CAP-Gly A and tested the ability of these constructs to bind EB1_{260} (Fig. 6, A–E). As EB1_{260} cannot bind CAP-Gly A, we reasoned that any EBHD binding activity would be due to the region N-terminal to CAP-Gly A. Inclusion of CAP-Gly domain A in the constructs facilitated ease of detection during purification (construct size and determination of protein concentration because of residues in the CAP-Gly domain that absorb at 280 nm). A CLIP-170 construct spanning residues 1–130 bound EB1_{260} with a $K_D$ equal to 1.3 $\mu M$ (Fig. 6A). Deleting the first 12 residues of CLIP-170 (construct, residues 13–130) did not affect EB1_{260} binding ($K_D = 1.4 \mu M$, Fig. 6B); however, deleting the first 18 residues ablated detectable binding (Fig. 6E). This suggested that the CLIP-170 13–18 region contained key EBHD binding residues.

To gain insight into whether residues in the CLIP-170 N-terminal region were conserved, we used Clustal 2.1 to align CLIP-170 homologs from a diverse set of animals, including mammals, birds, reptiles, fish, and insects (tarantula and bee) (Fig. 6D) (36). Across these species, the first 20 amino acids of CLIP-170 are well conserved. The alignment reveals a trimeric array of SXIP-like motifs: SMLKP, SGLKAP, and TKILKP. The first motif takes the form (S/T)(X/I/L)XP, whereas the second and third motifs take the form (S/T)(X/I/L)XP, where X denotes any amino acid (excluding those with acidic side chains) with at least one of the Xs in the motif being a basic amino acid (lysine or arginine). To examine the potential role of these motifs in EBHD binding, we introduced mutations into these SXIP-like motifs and tested how these mutations affected binding to EB1_{260}. First, we introduced two point mutations in the CLIP-170 13–130 construct (K17N and P18L) and did not detect binding to EB1_{260} (Fig. 6E). Similarly, introducing a triple point mutation in the CLIP-170 13–130 construct (T13N, K14N, and I15N) resulted in no detectable binding to EB1_{260}. Interestingly, when we engineered the T13N, K14N, and I15N point mutations in a CLIP-170 6–130 construct, we found EB1_{260} binding activity ($K_D = 5.6 \mu M$), indicative that additional determinants in the 6–12 region were contributing to EBHD binding. The introduction of three point mutations into the 6–12 region (S7N, L9N, and K10N) in concert with the T13N, K14N, and I15N mutations ablated the ability of the CLIP-170 6–130 construct to bind EB1_{260}. However, introducing these six point mutations into the larger CLIP-170 1–130 construct yielded EB1_{260} binding with a $K_D$ value equal to 10.8 $\mu M$. Collectively, these data indicate that determinants spanning CLIP-170 residues 1–18 are involved in binding the EBHD. To test whether this region was sufficient for EBHD binding, we synthesized a peptide corresponding to CLIP-170 residues 1–24. This CLIP-170 N-terminal peptide bound to EB1_{260} with a $K_D$ equal to 1.4 $\mu M$, on par with the binding activity of the CLIP-170 1–130 construct (Fig. 6, A, C, and E).

We next confirmed the interaction between the CLIP-170 N-terminal segment and the EB1 EBHD using a SEC multi-angle static light scattering (SEC-MALS) assay. In this assay, components and potential complexes are separated on a SEC column based on molecular size and radius of gyration. The eluate is then run in-line through a MALS system that calculates an average experimental mass based on the component’s or the complex’s multi-angle light scattering profile. The calculated experimental mass provides information regarding oligomeric states, potential binding stoichiometries, and complex dissociation during the SEC run and aids the characterization of elution profiles in which the macromolecule’s shape is elongated. CLIP-170 1–130 was able to robustly bind and co-elute with EB1_{268} after incubation and co-injection of a 1:1 protein ratio (Fig. 7A). The elution profile of this peak was nearly Gaussian in form and was shifted relative to the peaks of the individual components. The experimentally determined mass of this peak was 41.3 kDa, a value that is between that of a 2:2 (47.5 kDa) and a 2:1 (33.2 kDa) EB1_{268}–CLIP-170 1–130 complex, indicative that some dissociation may have occurred during the run. Presumably, formation of this complex relied on interactions between CAP-Gly A and the EB1 EEY motif as well as SXIP-like interactions with the EBHD. We next analyzed whether CLIP-170 1–130 could bind and shift EB1_{260} over gel filtration. The use of EB1_{260} would prevent complex formation via CAP-Gly A binding to the EEY motif. In agreement with our ITC results, CLIP-170 1–130 was able to bind and shift EB1_{260} over gel filtration, indicative that the N-terminal SXIP-like array was sufficient for EBHD binding (Fig. 7B). The elution profile of the peak was not Gaussian but had a trailing tail, indicative of complex dissociation. The experimentally determined average mass across the peak was 27 kDa, which is less than the calculated mass of a 2:1 EB1_{260}–CLIP-170 1–130 complex (31.2 kDa). Although the main peak gave an experimental mass of ~31 kDa, the trailing tail yielded a lower mass, bringing the overall average down. Thus, although the N-terminal SXIP-like motifs of CLIP-170 are sufficient for EB1 binding, binding is enhanced through avidity, mediated by CAP-Gly domain binding to the EB1 EEY motif. We next used SEC to test whether deleting or mutating some of the CLIP-170 N-terminal SXIP-like motifs affected EB1 binding. Using excess of the CLIP-170 construct, we found that both CLIP-170 13–130 (lacking the first two SXIP-like motifs) and CLIP-170 6–130 (T13N, K14N, I15N) (lacking the first SXIP-like motif and containing muta-
Diverse CLIP-170–EB1 interaction modes

**A**

![Graph A](image)

**B**

![Graph B](image)

**C**

![Graph C](image)

**D**

| Species          | Sequence Information                                                                 |
|------------------|--------------------------------------------------------------------------------------|
| *H. sapiens*     | MSLKPSGLKAPTKILPGSTALKTPAA---VAVPVEKTISSEKASSTPSSETQEFVD 56                         |
| *M. musculus*    | MSLKPSGLKAPTKILPGSTALKTPAA---AAPVETIPSEKASGPPSETQEFVD 56                          |
| *G. gallus*      | MSLKPSGLKAPSKTKHGSTLKLKAPAS---VATAAPEKAPSSSEKSSSTTADAHDDFDV 57                    |
| *O. anatinus*    | MSLKPSGLKAPTKISKPGPSASKAASAV---AAPVKEAVSEKTSSTPSSETQDEFVD 56                      |
| *P. bivittatus*  | MSLKPSGLKAPTKISPVSVLKAAAPVAASPEKTPASEKPSAAAPPEAQDEFVD 60                         |
| *C. p. bellii*   | MSLKPSGLKAPTKISPVSVLKAAAPVAASPEKTPASEKPSAAAPPEAQDEFVD 60                         |
| *X. tropicalis*  | MSLKPSGLKAPSKTIAPGTAACKSAV---TAPTVKTTAEKPSAPSETAEFDV 53                           |
| *D. reio*        | MSGKPSGLKPSIGKPIATAPKTSPT--------------------------------------------------------|
| *P. formosa*     | MSLKPSGLKAPSKTRPPPVTGAPKTPNPS---------------------------------------------------|
| *M. rotundata*   | MSLKPSGLKAPSKTAGPCNPRPSNPPAPVP-----------------------------------------------|

**E**

**CLIP-170 Constructs**

- **CLIP-170 1-130**
- **CLIP-170 19-130**
- **CLIP-170 13-130**
- **CLIP-170 13-130 (K17N, P18L)**
- **CLIP-170 13-130 (T13N, K14N, I15N)**
- **CLIP-170 6-130 (T13N, K14N, I15N)**
- **CLIP-170 6-130 (S7N, L9N, K10N, T13N, K14N, I15N)**
- **CLIP-170 1-130 (S7N, L9N, K10N, T13N, K14N, I15N)**
- **CLIP-170 Nterm Peptide (M = N)***

**CAP-Gly A**

- **130**
- **A**

**Binding Affinity with EB1<sub>260</sub>**

- **K<sub>D</sub>(µM)**
- **1.3 ± 0.1**
- **N/A**
- **1.4 ± 0.2**
- **N/A or very weak**
- **5.6 ± 1.4**
- **N/A**
- **10.8 ± 2.8**
- **1.4 ± 0.3**
Diverse CLIP-170–EB1 interaction modes

Figure 7. Multiple SXIP-like motifs in the CLIP-170 N-terminal region can stably bind the EBHD over gel filtration. A, SEC-MALS analysis of the CLIP-170 1–130 fragment binding to EB1$_{260}$. EB1$_{260}$ alone eluted at 29 min with an experimental mass of 18.6 kDa, on par with the calculated mass of a dimer (18.9 kDa). CLIP-170 1–130 eluted at 33.5 min with an experimental mass of 14.8 kDa, on par with the calculated mass of a monomer (14.3 kDa). When co-incubated and injected, the proteins co-eluted at 27.5 min with an experimental mass of 41.3 kDa, a value that is between that of a 2:2 (47.5 kDa) and a 2:1 (33.2 kDa) EB1$_{260}$–CLIP-170 1–130 complex, indicative of some dissociation during the run. B, SEC-MALS analysis of the CLIP-170 1–130 fragment binding to EB1$_{260}$, EB1$_{260}$ and CLIP-170 1–130 behaved similarly as the individual components in A (EB1$_{260}$ calculated dimer mass, 16.9 kDa). When co-incubated and injected, the proteins co-eluted at 28.5 min with a tail to the peak, indicative of dissociation. The peak yielded an experimentally determined mass of 27 kDa (less than the calculated mass (31.2 kDa) of a 2:1 EB1$_{260}$–CLIP-170 1–130 complex), indicating that the interaction with EB1 is not as robust when the CAP-Gly domain A interacts with the EB1 EEF motif is removed. C, gel filtration analysis of CLIP-170 N-terminal fragment constructs binding to EB1$_{260}$. Excess amounts of CLIP-170 13–130 (which only contains one of the three SXIP-like motifs) was co-incubated with EB1$_{260}$ and injected onto a size-exclusion column. All EB1$_{260}$ was shifted to a higher mass species, indicating that the third SXIP-like motif is sufficient for detectable EB1 binding in this assay. A CLIP-170 6–130 construct containing the second SXIP-like motif with mutations in the third SXIP-like motif (T13N, K14N, I15N) also produced a stable shift of EB1$_{260}$ to a higher mass species, indicating that the second motif can also bind the EBHD, although not as robustly as the third repeat (compare relative shifts).

Discussion

We delineated multiple, distinct EB1-binding determinants in the CLIP-170 N-terminal region. These include the two known CAP-Gly domains (A and B) that engage C-terminal EEF motifs as found in EB1 and EBHD-binding determinants that include an SXIP motif in the linker bridging CAP-Gly domains A and B and a unique array of SXIP-like motifs located N-terminally to CAP-Gly A. These N-terminal motifs bear some resemblance to SXIP motifs and LXXPTPh motifs that have been structurally characterized to date (Fig. 8, A and B). Here we describe SXIP and LXXPTPh EBHD binding modes and compare these binding determinants with the CLIP-170 SXIP-like array to gain insight into how this region of CLIP-170 might bind the EBHD. SXIP and LXXPTPh motifs both engage the EBHD at a hydrophobic site at the domain’s coiled coil and four-helix bundle junction (Fig. 8A) (13, 16, 17). Although each motif takes a slightly different trajectory on the domain, three residues structurally overlap at this site and involve the serine-lysine-isoleucine residues (SXI) of the SXIP motif, as observed in the EB1–MACF2 complex, and the threonine-arginine-leucine residues of the LXXPTPh motif involving the first leucine of the motif and the two residues proceeding the leucine (-2 –1L), as observed in the Bim1-Kar9 complex. In the CLIP-170 SXIP-like array, we conjecture that the three corresponding structurally homologous residues in each motif are methionine-serine-methionine (MSM, first motif), serine-glycine-leucine (SGL, second motif), and threonine-lysine-isoleucine (TKI, third motif). We anticipate that these three residues bind the EBHD in a mode similar to the SXI of the SXIP motif and the -2 –1L of the LXXPTPh motif. Across all motifs, there is a conserved proline at a position equivalent to the first proline in the LXXPTPh motif. Structural studies of Dis1 bound to the Mal3 EBHD reveals that Dis1 uses an LXXPTPh-like motif where the second proline is replaced with a glutamine, demonstrating residue variability at this site, but followed by a hydrophobic residue (phenylalanine). In line with Dis1, none of the CLIP-170 motifs have a second proline; however, the first and second motifs in the array do have a final hydrophobic residue (leucine and isoleucine, respectively). The motifs in the CLIP-170 array overlap, suggesting that EBHD binding along the array may occur in different frames, as binding to one site likely precludes the binding of either another EBHD dimer or the second site on the same EBHD to a second motif in the array. Across vertebrates, the SXIP-like motif array is well conserved (Fig. 6D). When compared with more distant animals or with other CLIP family protein structures such as CLIP-115, the residues of the second SXIP-like motif are best conserved (Fig. 8C). We note that fly species (e.g. Drosophila melanogaster and Drosophila busckii) that lack the first and third SXIP-like motifs have...
other SXIP motifs that flank this region, potentially suggesting convergent use of diverse EB1-binding motifs in the sequence located N-terminally to CAP-Gly A.

Delineation of multiple distinct EB1-binding determinants in CLIP-170 suggests that CLIP-170 can bind EB1 in a variety of modes that range from the use of a single determinant (no avidity) up to four determinants (high avidity), at which point it saturates the known, available EB1 dimer’s binding sites. Alternatively (or additionally), CLIP-170’s multiple EB1-binding determinants can be used to engage more than one EB1 dimer. Our data indicate that CLIP-170 CAP-Gly domains only engage the EB1 EEY motif and do not dramatically occlude the EBHD, leaving the domain accessible to SXIP, SXIP-like, or LXXPTPh motif binding. This interaction mode is distinct from p150Glued CAP-Gly domain binding, which dually engages the EEY motif and the EBHD and competes for SXIP binding (14, 15). Our work aligns with a recent analysis of the yeast Bik1-Bim1 (CLIP-170 and EB1 homologs, respectively) interaction, demonstrating that Bik1 CAP-Gly domain binding does not occlude SXIP motif binding to Bim1 (37). It was thought previously that CLIP-170 CAP-Gly domain binding to EB1 occluded SXIP motif binding and was thus mutually exclusive. This is based on the titration of a SXIP peptide that displaced CLIP-170 from growing microtubule plus ends in vitro in the presence of EB1 as well as the reverse: titrated CLIP-170 displaced an SXIP peptide from growing microtubule plus ends (33). Although one interpretation is steric occlusion, another possibility is that the titrated trans SXIP peptide displaced the CLIP-170 SXIP and/or SXIP-like motifs from the EBHD, which lowered the CLIP-170–EB1 binding affinity, leading to dissociation. Likewise, the CLIP-170 SXIP or SXIP-like motifs, when titrated, could displace a trans SXIP peptide.

Figure 8. The CLIP-170 N-terminal EB1 binding region bears homology to both SXIP motifs and LXXPTPh motifs. A, structural comparison of SXIP and LXXPTPh motif binding to the EBHD. The MACF2 SXIP motif is shown in stick format in cyan (from PDB code 3GJO) (13). The KAR9 LXXPTPh motif is shown in stick format, colored magenta (from PDB code 5N74) (17). Structures were superimposed using the coordinates of the respective EBHD from each structure. Only the EBHD from the KAR9 complex is depicted, shown in surface format, colored light gray. The sequence of the two peptides is shown below each structure. The peptides structurally align over the region boxed in black (residues SKI (SX) from the MACF2 SXIP motif and residues TRL from the KAR9 LXXPTPh motif, which include the initial leucine in the motif and the two residues N-terminal to it). The alignment of the MACF2 SXIP motif as well as the LXXPTPh motifs from structures determined to date: KAR9 and Dis1 (PDB codes 5N74 and 5M9E) (16, 17) compared with the three SXIP-like motifs in the CLIP-170 N-terminal region. B, comparison of the human CLIP-170, CLIP-115, and fly CLIP-190 N-terminal regions. The second SXIP-like motif of CLIP-170 is conserved in CLIP-115 and in the fly species D. melanogaster and D. busckii (highlighted in purple). Although the fly homologs lack the other SXIP-like motifs found in the CLIP-170 N-terminal region, they do contain other SXIP motifs (highlighted in green), suggesting a potential conserved multivalent EB1 interaction mechanism. D, model depicting the interaction modes between CLIP-170 and EB1. CLIP-170 CAP-Gly domains bind the EB1 C-terminal EEY motif. The CLIP-170 N-terminal region and the SXIP motif located between CAP-Gly A and B bind the EBHD. The ability of the CLIP-170 CAP-Gly domains to bind the EB1 EEY motif is auto-inhibited by the C-terminal zinc knuckles. When the CAP-Gly domains are auto-inhibited, the EBHD binding motifs may serve to confer EB1 binding, albeit weaker than when they can engage EB1 in concert with the CAP-Gly domains.
As CLIP-170 CAP-Gly domain binding to the EB1 EEY motif leaves the EBHD free, the EBHD might be used to engage CLIP-170 SXIP or SXIP-like motifs or to form a higher-order complex involving CLIP-170, EB1, and a third factor. Examples of higher-order complexes that may implement this interaction mode include CLASP2 and SLAIN2. CLASP2 has an EB1-binding SXIP motif as well as a C-terminal domain that binds the CLIP-170 coiled coil (13, 24). SLAIN2 has multiple SXIP motifs as well as a C-terminal WRDGCY sequence that can bind the EB1 domain. Thus, multivalency in the EB1–CLIP-170 complex can afford avidity and/or alternative binding modes that accommodate higher-order complex formation. It is likely that a high-avidity, multivalent interaction with the EB1 dimer outcompetes CLIP-170 CAP-Gly binding to the α-tubulin C-terminal EEY motif (Fig. 8D). Preferential binding to EB1 would localize CLIP-170 to the microtubule plus end rather than along the length of the microtubule lattice by α-tubulin binding. This may explain why, in the absence of EB1, CLIP-170 primarily localizes along the lengths of microtubules in vitro but, upon addition of EB1, preferentially localizes to growing microtubule plus ends (28–30).

Interestingly, previous work suggested that CLIP-170 in its autoinhibited state still localized to microtubule plus ends (21, 32). How CLIP-170, with its CAP-Gly domains bound in cis to the zinc knuckle motifs in an autoinhibited state could still engage EB1 to localize to microtubule plus ends was a mystery. In these experiments, the cellular readout for CLIP-170 was its phosphorylation state, as phosphorylation of Ser-311 promoted autoinhibition. It is possible that not all CAP-Gly domains were bound to zinc knuckles, enabling some CAP-Gly domains in the functional dimer to bind EB1 and therefore localize to microtubule plus ends. Alternatively, autoinhibited CLIP-170 may use the SXIP motif and/or the SXIP-like motifs we delineate here to bind the EBHD and localize to microtubule plus ends independent of its inhibited state (Fig. 8D). Future cellular studies in which distinct CLIP-170 EB1-binding modes are systematically ablated will help address this question. The unique EB1-binding SXIP-like motifs we delineate in the CLIP-170 N-terminal region expand our understanding of EB1-interacting determinants and how diverse networks of MAPs in distinct complexes and stoichiometries can be recruited to microtubule plus ends.

**Experimental procedures**

### Cloning and purification of EB1 and CLIP-170 constructs

EB1 C-terminal (Ct) fragments and CLIP-170 N-terminal (Nt) fragments were inserted into plasmid pET28a (Millipore Sigma, Burlington, MA) using PCR and engineered NdeI and BamHI restriction sites. EB1 Ct clones included EB1268 (residues 191–268) and EB1260 (residues 191–260). An array of CLIP-170 Nt constructs was generated, including constructs with point mutations generated using the QuikChange method (Agilent Technologies, Santa Clara, CA). Plasmids containing EB1 Ct and CLIP-170 Nt fragments were transformed into BL21 DE3 PlysS *Escherichia coli* and grown in 6 liters of Luria-Bertani medium at 37 °C under kanamycin selection. At an optical density of 0.8 (600 nm), protein expression was induced using 0.1 mM isopropyl-1-thio-β-β-d-galactopyranoside for overnight induction at 18 °C. Cells were harvested and lysed by sonication at 4 °C. After centrifugation at 23,000 × g for 45 min, supernatant was loaded onto a nickel-nitrilotriacetic acid (Qiagen, Hilden, Germany) chromatography column and eluted with a linear gradient between 10 mM and 300 mM imidazole (25 mM Tris (pH 8.0), 300 mM NaCl, and 0.1% β-mercaptoethanol). EB1 Ct constructs were dialyzed against 25 mM Tris (pH 8.0) and 0.1% β-mercaptoethanol with either α-thrombin (0.1 mg, Hematologic Technologies, Essex Junction, VT) included in the dialysis tube or added after dialysis to cleave off the N-terminal His6 tag. EB1 Ct constructs were then filtered over benzamidine-Sepharose (GE Healthcare, Waukesha, WI). The cleaved EB1 Ct protein was loaded onto a Q-Sepharose Fast Flow column (GE Healthcare) and eluted over a linear 0–1 M NaCl gradient. After dialyzing into 25 mM MES (pH 6.5) and 0.1% β-mercaptoethanol, the CLIP-170 Nt fragments were digested by α-thrombin (0.1 mg, Hematologic Technologies), filtered over benzamidine-Sepharose (GE Healthcare), loaded onto an SP-Sepharose Fast Flow column (GE Healthcare), and eluted over a linear 0–1 M NaCl gradient. Proteins were exchanged into 25 mM Tris pH 8.0, 150 mM NaCl, and 0.1% β-mercaptoethanol, concentrated in a 3000 molecular weight cutoff Millipore concentrator (Millipore Sigma), and flash-frozen in liquid nitrogen for storage.

### Peptide synthesis

Three different SXIP peptides and a CLIP-170 Nt peptide were synthesized at the University of North Carolina Microprotein Sequencing and Peptide Synthesis Facility. The SXIP peptides included SAIP (QASAIPSPGKFRSAP, human SLAIN2, residues 450–464), SKIP (KKSIPRSQGSRETY, human CLASP2, residues 726–739, in which the native cysteine at position 736 was replaced with a serine), and SNIP (TPSNIPQKPSQPAAKY, human CLIP-170, residues 162–176). A CLIP-170 Nt peptide was synthesized (Nle-S-Nle-LKPSGLAPTKLPGSTALKY, human CLIP-170, residues 1–24) in which norleucine (Nle) was substituted for the two native methionines at positions 1 and 3. A tyrosine residue was added at the end of each peptide to determine the concentration using absorption at 280 nm. In the case of the human CLASP2 SKIP peptide, a penultimate threonine was added before the tyrosine.

### ITC

ITC experiments were carried out at 16 °C in 25 mM HEPES (pH 6.8), 50 mM NaCl, and 0.1% β-mercaptoethanol on a MicroCal AutoITC200 (GE Healthcare). Peptides were exchanged into ITC buffer using G-25 Sephadex quick spin columns (Roche Applied Science, Penzberg, Germany). 2-μl volumes of 0.5 to 1 μm protein or peptide were automatically injected into a well containing 360 μl of a 30–50 μm EB1 Ct construct. The resulting binding isotherms were fit to a one-site

---

**Diverse CLIP-170–EB1 interaction modes**

---

**J. Biol. Chem. (2019) 294(3) 918–931**
or two-site binding model using the Origin 7.0 software package, and the respective fits were evaluated using $\chi^2$ analysis in Origin 7.0 (OriginLab, Northampton, MA). Reported $K_D$ values are the average of the $K_D$ values determined from two or three independent experiments. The standard deviation reported was calculated using the STDEV function in Microsoft Excel (Microsoft Corp., Redmond, WA).

**SEC and SEC-MALS measurements**

Before injection onto the SEC column, proteins were incubated at room temperature for 20 min. 100 $\mu$l of the respective protein solution (each protein component at 30–100 $\mu$M) was injected onto a Superdex 2000 column (GE Healthcare) in 50 mM NaCl, 25 mM HEPES (pH 6.8), 0.1% β-mercaptoethanol, and 0.2 g/liter sodium azide with a flow rate of 0.5 ml/min. The sample was then passed through a Wyatt Optilab rES refractometer (Wyatt Technology Corp., Santa Barbara, CA). When conducted in-line with MALS, the sample was also passed through a DAWN HELES II light-scattering instrument (Wyatt Technology Corp., Santa Barbara, CA, USA). The light-scattering and refractive index data were used to calculate the weight-averaged molar mass using the Astra V software program (Wyatt Technology Corp.). SEC and SEC-MALS data shown are representative of independent experiments conducted in duplicate.

**Author contributions**—Y. C. and K. C. S. conceptualization; Y. C. data curation; Y. C., P. W., and K. C. S. formal analysis; Y. C. and K. C. S. validation; Y. C. investigation; Y. C. and K. C. S. visualization; Y. C., P. W., and K. C. S. methodology; Y. C. and K. C. S. writing—original draft; Y. C., P. W., and K. C. S. writing—review and editing; K. C. S. resources; K. C. S. supervision; K. C. S. funding acquisition; K. C. S. project administration.

**Acknowledgments**—We thank Dr. Ashutosh Tripathy for assistance with the ITC and SEC-MALS experiments and Dr. Rebecca Adikes for discussions.

**References**

1. Desai, A., and Mitchison, T. J. (1997) Microtubule polymerization dynamics. *Annu. Rev. Cell Dev. Biol.* 13, 83–117 [CrossRef](Medline)
2. Akhmanova, A., and Steinmetz, M. O. (2008) Tracking the ends: a dynamic protein network controls the fate of microtubule tips. *Nat. Rev. Mol. Cell Biol.* 9, 309–322 [CrossRef](Medline)
3. Jiang, K., Toedt, G., Montenegro Gouveia, S., Davey, N. E., Hua, S., van der Vaart, B., Grigoriev, I., Larsen, J., Pedersen, L. B., Beztarostori, K., Linke-Faria, M., Demmers, J., Steinmetz, M. O., Gibson, T. J., and Akhmanova, A. (2012) A proteome-wide screen for mammalian SxIP motif-containing microtubule plus-end tracking proteins. *Curr. Biol. CB.* 22, 1800–1807 [CrossRef](Medline)
4. Slepet, K. C., Rogers, S. L., Elliott, S. L., Ohkura, H., Koloedziej, P. A., and Vale, R. D. (2005) Structural determinants for the microtubule EB1-mediated recruitment of APC and spectraplakins to the microtubule plus end. *J. Cell Biol.* 168, 587–598 [CrossRef](Medline)
5. Maiato, H., Fairley, E. A., Rieder, C. L., Swedlow, J. R., Sunkel, C. E., and Earnshaw, W. C. (2003) Human CLASP1 is an outer kinetochore component that regulates spindle microtubule dynamics. *Cell* 113, 891–904 [CrossRef](Medline)
6. Biedling, P., Laan, L., Schek, H., Munteanu, E. L., Sandblad, L., Dogterom, M., Brunner, D., and Surrey, T. (2007) Reconstitution of a microtubule plus-end tracking system in vitro. *Nature* 450, 1100–1105 [CrossRef](Medline)
7. Maurer, S. P., Fourniol, F. J., Bohner, G., Moores, C. A., and Surrey, T. (2012) EBs recognize a nucleotide-dependent structural cap at growing microtubule ends. *Cell* 149, 371–382 [CrossRef](Medline)
8. Hayashi, I., and Ikura, M. (2003) Crystal structure of the amino-terminal microtubule-binding domain of end-binding protein 1 (EB1). *J. Biol. Chem.* 278, 36430–36434 [CrossRef](Medline)
9. de Groot, C. O., Jelezarov, I., Damberger, F. F., Bjelić, S., Schärer, M. A., Bhaves, N. S., Grigoriev, I., Buey, R. M., Wüthrich, K., Capitani, G., Akhmanova, A., and Steinmetz, M. O. (2010) Molecular insights into mammalian end binding protein heterodimerization. *J. Biol. Chem.* 285, 5802–5814 [CrossRef](Medline)
10. Slepet, K. C., and Vale, R. D. (2007) Structural basis of microtubule plus end tracking by XMAP215, CLIP-170, and EB1. *Mol. Cell* 27, 976–991 [CrossRef](Medline)
11. Honnappa, S., John, C. M., Kostrewa, D., Winkler, F. K., and Steinmetz, M. O. (2005) Structural insights into the EB1–APC interaction. *EMBO J.* 24, 261–269 [CrossRef](Medline)
12. Bu, W., and Su, L.-K. (2003) Characterization of functional domains of human EB1 family proteins. *J. Biol. Chem.* 278, 49721–49731 [CrossRef](Medline)
13. Honnappa, S., Gouveia, S. M., Weibrich, A., Damberger, F. F., Bhaves, N. S., Jawhari, H., Grigoriev, I., van Rijssel, F. J., Buey, R. M., Lawera, A., Jelezarov, I., Winkler, F. K., Wüthrich, K., Akhmanova, A., and Steinmetz, M. O. (2009) An EB1-binding motif acts as a microtubule tip localization signal. *Cell* 138, 366–376 [CrossRef](Medline)
14. Hayashi, I., Wilde, A., Mal, T. K., and Ikura, M. (2005) Structural basis for the activation of microtubule assembly by the EB1 and p150Glued complex. *Mol. Cell* 19, 449–460 [CrossRef](Medline)
15. Honnappa, S., Okhrimenko, O., Jaussi, R., Jawhari, H., Jelezarov, I., Winkler, F. K., and Steinmetz, M. O. (2006) Key interaction modes of dynamic +TIP networks. *Mol. Cell* 23, 663–671 [CrossRef](Medline)
16. Matsuo, Y., Maurer, S. P., Yukawa, M., Zakian, S., Singleton, M. R., Surrey, T., and Toda, T. (2016) An unconventional interaction between Dsi1/T0G and Mab/EB1 in fission yeast promotes the fidelity of chromosome segregation. *J. Cell Sci.* 129, 4592–4606 [CrossRef](Medline)
17. Kumar, A., Manatschal, C., Rai, A., Grigoriev, I., Degen, M. S., Jaussi, R., Kretzschmar, L., Prota, A. E., Volkmer, R., Kammerer, R. A., Akhmanova, A., and Steinmetz, M. O. (2017) Short linear sequence motif LaxPTh targets diverse proteins to growing microtubule ends. *Structure* 25, 924–932.e4 [CrossRef](Medline)
18. Steinmetz, M. O., and Akhmanova, A. (2008) Capturing protein tails by CAP-Gly domains. *Trends Biochem. Sci.* 33, 535–545 [CrossRef](Medline)
19. Pierre, P., Scheel, J., Rickard, J. E., and Kreis, T. E. (1999) CLIP-170 links microtubules and dynactin localization. *Science* 285, 156–173 [CrossRef](Medline)
20. Perez, F., Diamantopoulos, G. S., Stalder, R., and Kreis, T. E. (1992) CLIP-170 links the APC-binding protein EB1 by different mechanisms. *J. Cell Biol.* 119, 249–260 [CrossRef](Medline)
21. Lansbergen, G., Komarova, Y., Modesti, M., Wyman, C., Hoogenraad, C. C., Goodson, H. V., Lemaître, R. P., Drehsel, D. N., van Munster, E., Gadella, T. W., Jr., Grosfeld, F., Galjart, N., Borisuy, G. G., and Akhmanova, A. (2004) Conformational changes in CLIP-170 highlights growing microtubule ends in vivo. *Cell* 96, 517–527 [CrossRef](Medline)
22. Steinmetz, M. O., and Akhmanova, A. (2005) EBs recognize a nucleotide-dependent structural cap at growing microtubule ends. *Cell* 70, 887–900 [CrossRef](Medline)
23. Perez, F., Diamantopoulos, G. S., Stalder, R., and Kreis, T. E. (1999) CLIP-170 links endocytic vesicles to microtubules. *Cell* 70, 887–900 [CrossRef](Medline)
24. Watson, P., and Stephans, D. J. (2006) Microtubule plus-end loading of p150(Glued) is mediated by EB1 and CLIP-170 but is not required for intracellular membrane traffic in mammalian cells. *J. Cell Sci.* 119, 2758–2767 [CrossRef](Medline)
25. Akhmanova, A., Hoogenraad, C. C., Drabek, K., Stepanova, T., Dortland, B., Verkerk, T., Vermeulen, W., Burgering, B. M., De Zeeuw, C. I., Grosfeld, F., and Galjart, N. (2001) CLASPs are CLIP-115 and -170 associating proteins involved in the regional regulation of microtubule dynamics in motile fibroblasts. *Cell* 104, 923–935 [CrossRef](Medline)
25. Hayashi, I., Plevin, M. J., and Ikura, M. (2007) CLIP170 autoinhibition mimics intermolecular interactions with p150Glued or EB1. Nat. Struct. Mol. Biol. 14, 980–981 CrossRef Medline
26. Weisbrich, A., Honnappa, S., Jaussi, R., Okhrimenko, O., Frey, D., Ilepsarov, I., Akhmanova, A., and Steinmetz, M. O. (2007) Structure-function relationship of CAP-Gly domains. Nat. Struct. Mol. Biol. 14, 959–967 CrossRef Medline
27. Mishima, M., Maesaki, R., Kasa, M., Watanabe, T., Fukata, M., Kaibuchi, K., and Hakoshima, T. (2007) Structural basis for tubulin recognition by cytoplasmic linker protein 170 and its autoinhibition. Proc. Natl. Acad. Sci. U.S.A. 104, 10346–10351 CrossRef Medline
28. Bieling, P., Kandels-Lewis, S., Telley, I. A., van Dijk, J., Janke, C., and Surrey, T. (2008) CLIP-170 tracks growing microtubule ends by dynamically recognizing composite EB1/tubulin-binding sites. J. Cell Biol. 183, 1223–1233 CrossRef Medline
29. Dixit, R., Barnett, B., Lazarus, J. E., Tokito, M., Goldman, Y. E., and Holzbaur, E. L. (2009) Microtubule plus-end tracking by CLIP-170 requires EB1. Proc. Natl. Acad. Sci. U.S.A. 106, 492–497 CrossRef Medline
30. Folker, E. S., Baker, B. M., and Goodson, H. V. (2005) Interactions between CLIP-170, tubulin, and microtubules: implications for the mechanism of Clip-170 plus-end tracking behavior. Mol. Biol. Cell 16, 5373–5384 CrossRef Medline
31. Lee, H.-S., Komarova, Y. A., Nadezhdina, E. S., Anjum, R., Peloquin, J. G., Schober, J. M., Danici, O., van Haren, J., Galjart, N., Cygi, S. P., Akhmanova, A., and Boris, G. G. (2010) Phosphorylation controls autoinhibition of cytoplasmic linker protein-170. Mol. Biol. Cell 21, 2661–2673 CrossRef Medline
32. Nakano, A., Kato, H., Watanabe, T., Min, K.-D., Yamazaki, S., Asano, Y., Seguchi, O., Higo, S., Shintani, Y., Asanuma, H., Asakura, M., Minamino, T., Kaibuchi, K., Mochizuki, N., Kitakaze, M., and Takashima, S. (2010) AMPK controls the speed of microtubule polymerization and directional cell migration through CLIP-170 phosphorylation. Nat. Cell Biol. 12, 583–590 CrossRef Medline
33. Duellberg, C., Trokter, M., Jha, R., Sen, I., Steinmetz, M. O., and Surrey, T. (2014) Reconstitution of a hierarchical + TIP interaction network controlling microtubule end tracking of dynein. Nat. Cell Biol. 16, 804–811 CrossRef Medline
34. van der Vaart, B., Manatschal, C., Grigoriev, I., Olieric, V., Gouveia, S. M., Bjelic, S., Demmers, J., Vorobjiev, I., Hoogenraad, C. C., Steinmetz, M. O., and Akhmanova, A. (2011) SLAIN2 links microtubule plus end-tracking proteins and controls microtubule growth in interphase. J. Cell Biol. 193, 1083–1099 CrossRef Medline
35. Buey, R. M., Sen, I., Kortt, O., Mohan, R., Gfeller, D., Veprintsev, D., Kretzschmar, I., Scheuermann, J., Neri, D., Zoete, V., Michelini, O., de Pereda, J. M., Akhmanova, A., Volkmer, R., and Steinmetz, M. O. (2012) Sequence determinants of a microtubule tip localization signal (MtLS). J. Biol. Chem. 287, 28227–28242 CrossRef Medline
36. Larkin, M. A., Blackshields, G., Brown, N. P., Chenna, R., McGettigan, P. A., McWilliam, H., Valentin, F., Wallace, I. M., Wilm, A., Lopez, R., Thompson, J. D., Gibson, T. I., and Higgins, D. G. (2007) Clustal W and Clustal X version 2.0. Bioinformatics 23, 2947–2948 CrossRef Medline
37. Stangier, M. M., Kumar, A., Chen, X., Farcas, A.-M., Barral, Y., and Steinmetz, M. O. (2018) Structure-function relationship of the BIK1-BIM1 complex. Structure 26, 607–616.e4 CrossRef Medline