Structural analysis of phosphorylation-associated interactions of human MCC with Scribble PDZ domains

Sofia Caria1,2†, Bryce Z. Stewart1, Ruitao Jin3, Brian J. Smith3, Patrick O. Humbert1,4,5,6 and Marc Kvansakul1,4

1 Department of Biochemistry & Genetics, La Trobe Institute for Molecular Science, La Trobe University, Melbourne, Victoria, Australia
2 SAXS/WAXS, Australian Synchrotron, Clayton, Victoria, Australia
3 Department of Chemistry and Physics, La Trobe Institute for Molecular Sciences, La Trobe University, Melbourne, Victoria, Australia
4 Research Centre for Molecular Cancer Prevention, La Trobe University, Melbourne, Victoria, Australia
5 Department of Biochemistry & Molecular Biology, University of Melbourne, Melbourne, Victoria, Australia
6 Department of Clinical Pathology, University of Melbourne, Melbourne, Victoria, Australia

Keywords
cell polarity; MCC; PDZ domain; phosphorylation; scribble; X-ray crystallography

Correspondence
M. Kvansakul and P. O. Humbert, Department of Biochemistry & Genetics, La Trobe University, Melbourne, Vic. 3086, Australia
Tel: +61 3 9479 2263 (MK); Tel: +61 3 9479 5155 (POH)
E-mails: m.kvansakul@latrobe.edu.au (MK); p.humbert@latrobe.edu.au (POH)

†Present address
Evotec, 114 Innovation Drive, Milton Park, Milton, Abingdon, OX14 4RZ, UK

Sofia Caria and Bryce Z. Stewart contributed equally

(Received 2 January 2019, revised 30 May 2019, accepted 16 July 2019)
doi:10.1111/febs.15002

Introduction

Cell polarity, which manifests itself as the asymmetric distribution of cellular constituents as well as proteins, lipids and carbohydrates into distinct cellular domains [1], is a critical property of eukaryotic cells and pivotal for correct tissue architecture and tissue development. The particular distribution of cellular constituents leads to the establishment of apical-basal cell polarity in epithelial cells, and impacts numerous critical processes ranging from cell migration to immunity and organization of tissue architecture. Scribble harbours a leucine-rich repeat domain and four PDZ domains that mediate most of Scribble’s interactions with other proteins. It has become increasingly clear that post-translational modifications substantially impact Scribble – ligand interactions, with phosphorylation being a major modulator of binding to Scribble. To better understand how Scribble PDZ domains direct cell polarity signalling and how phosphorylation impacts this process, we investigated human Scribble interactions with MCC (Mutated in Colorectal Cancer). We systematically evaluated the ability of all four individual Scribble PDZ domains to bind the PDZ-binding motif (PBM) of MCC as well as MCC phosphorylated at the position. We show that Scribble PDZ1 and PDZ3 are the major interactors with MCC, and that modifications to Ser at the position in the MCC PBM only has a minor effect on binding to Scribble PDZ domains. We then examined the structural basis for these observations by determining the crystal structures of Scribble PDZ1 domain bound to both the unphosphorylated MCC PBM as well as phosphorylated MCC. Our structures indicated that phospho-Ser at the position in MCC is not involved in major contacts with Scribble PDZ1, and in conjunction with our affinity measurements suggest that the impact of phosphorylation at the position of MCC must extend beyond a simple modulation of the affinity for Scribble PDZ domains.

Abbreviations
Dlg, Disks large; ITC, isothermal titration calorimetry; LGL, lethal Giant Larvae; LRR, leucine-rich repeat; MCC, mutated in Colorectal Cancer; PBM, PDZ-binding motif.
cellular signalling pathways including those involved in apoptosis, vesicle trafficking, cell proliferation and migration [2]. Furthermore, loss of cell polarity is recognized as an important hallmark of cancer development [3], underscoring the importance of correct cell polarity for healthy tissues. Apico-basal polarity is controlled by the interplay of three multi-protein complexes, Par, Crumbs and Scribble, with epithelial cell polarity being orchestrated by the antagonistic interaction between Par and Crumbs with the Scribble complex. In mammals, the Scribble complex comprises Scribble (SCRIB), one of four Dlg (Disks Large) homologues (DLG1–4) and two Lgl (Lethal Giant Larvae) homologues (LLGL1, LLGL2) that are highly conserved from the vinegar fly to humans [4]. Scribble was identified in Drosophila melanogaster as a tumour suppressor where loss of Scribble resulted in disrupted epithelial tissue organization accompanied by aberrant growth in the imaginal disks of the larvae [5]. The ability to suppress tumours was shown to be conserved across species, with Scribble knockout promoting tumour initiation, and when coupled with oncogenic drivers including RAS, tumour progression in diverse epithelial tissues including mammary, prostate, skin and the lung [6–10].

Scribble is a large multidomain scaffold protein comprising 16 leucine-rich repeats and 4 PSD-95/Disc-large/ZO-1 (PDZ) domains, and belongs to the LAP family of proteins. These domains enable Scribble to interact with a diverse set of interactors that play a role in a range of discrete signalling pathways [4]. While the majority of interactions are modulated by the four PDZ domains, the Scribble LRR domain also engages a specific subset of interactors, such as Lgl2, during the regulation of cell polarity [11].

Binding to Scribble via its PDZ domains is typically mediated via C-terminally located PDZ-binding motifs (PBMs) on specific interactors. Although these sequences are specific, several studies have shown that while PBMs on Scribble interactors selectively engage Scribble, the Scribble PDZ domains appear to harbour overlapping preferences for certain ligands, with each PDZ domain capable of engaging multiple binding partners (reviewed in [4]). Scribble PDZ domains are classified as Class I PDZ domains, which recognizes a consensus X-T/S-X-ØCOOH motif (where X can be any amino acid residue, and Ø is a hydrophobic residue) in the PDZ of interactors (PDZ residues are denoted from 0 to −8, where Ø represents the C-terminal residue carrying the carboxylate group). Other consensus PBM motifs include class II Ø-X-ØCOOH and class III D/E-X-ØCOOH. In addition to amino acid variations, post-translational modification via phosphorylation has also been shown to impact ligand binding to PDZ domains. Binding of Kir2.3 [12] and stargazin [13] to DLG4 (PSD-95) is regulated by phosphorylation, with phosphorylation of a Thr at the −2 position in stargazin abrogating binding to DLG4. Similarly, phosphorylation of a Ser at −2 prevents Kir2.3 binding to DLG4. More recently phosphorylation of PBMs was examined using proteomics approaches, which suggested that phosphorylation of PBMs occurs frequently and can have diverse impacts on PDZ domain binding [14]. A comprehensive analysis of the effect of phosphorylation in the PBM of β2AR in positions −2, −3, −5 and −6 on the binding to the SNX27 PDZ domain [15] revealed that phosphorylation of the −2 position blocks the interaction, whereas phosphorylation at the −3 and −5 positions significantly increased affinity.

Mutated in Colorectal Cancer (MCC) has been shown to be a Scribble interactor [16]. The interaction between Scribble and MCC appears to be highly conserved evolutionarily, since Drosophila Scrib and MCC can interact physically [16] and in zebrafish loss of Scrib and MCC interact genetically to regulate convergent extension movements during gastrulation [17]. MCC is a large multi-PDZ domain protein found in epithelial cells that has been shown to participate in several key cellular signalling pathways including canonical and noncanonical Wnt signalling, NFκB signalling as well as cell cycle control [17–20]. More recently, it was shown that MCC harbours a C-terminal PBM that is able to engage human Scribble PDZ1 and 3 domains to impact epithelial cell polarity [16]. Interestingly, the MCC PBM has been shown to be a target for phosphorylation in position −1 which features a Ser residue, with phosphorylation impacting the formation of lamellipodia in colon epithelial cells [21]. Apart from MCC only Syndecan1 has been described to feature phosphorylation a the −1 position of their PBM [22] that promoted binding to the Tiam1 PDZ domain by improving the affinity from 51.8 to 35.1 µM.

Notably, phosphorylation of the −1 Ser in the MCC PBM was proposed to increase binding to Scribble, as evidenced by increased immunoprecipitation of phospho-MCC. The impact on phosphorylation of the MCC PBM on binding to the Scribble PDZ1 domain was recently shown to lead to a nearly twofold increase in affinity from 4.4 µM for MCC to 2.4 µM for phospho-MCC as measured by microscale thermophoresis and ITC [14]; however, NMR chemical shift analysis revealed no significant differences in binding to the Scribble PDZ1 for unphosphorylated and phosphorylated MCC [23].

To understand the impact of phosphorylation on the ability of the MCC PBM to bind to Scribble PDZ...
domains, we systematically examined the affinities of recombinant human Scribble PDZ domains for peptides spanning the MCC PBM with either phosphorylated or unphosphorylated Ser at the −1 position, as well as peptides harbouring a phosphomimetic Ser to Glu mutation. Finally, we determined crystal structures of human Scribble PDZ1 bound to a MCC PBM peptide as well as the MCC PBM with phosphorylated Ser at the −1 position to examine the structural basis for this interaction and the impact of phosphorylation on binding to Scribble PDZ1. Our findings suggest that phosphorylation of the MCC PBM at the −1 position is not a major determinant of interactions with human Scribble PDZ1 and 3 domains.

Results

Isolated human Scribble PDZ domains specifically interact with the β-Pix PBM

Human Scribble has previously been shown to directly interact with the MCC C-terminal PDZ-binding motif (PBM) via its PDZ1 and 3 domains using pull-down and biochemical assays. To understand the impact of phosphorylation on the binding of MCC to Scribble we examined the affinity of recombinant human Scribble PDZ1, 2, 3 and 4 domains for a panel of 8-mer peptides corresponding to the MCC PBM (Fig. 1, Table 1). Our ITC measurements revealed that unphosphorylated MCC bound to Scribble PDZ1 and 3 domains with $K_D$ values of 7.7 and 5.0 µM, respectively, and did not show detectable binding to Scribble PDZ2 and 4 domains. A phosphorylated MCC peptide carrying a phosphorylated Ser at the −1 position bound Scribble PDZ1 with 6.1 µM and PDZ3 with 3.7 µM, whereas two phosphomimetic MCC peptides with a Ser to Glu (MCC_SE) or a Ser to Asp (MCC_SD) mutation bound PDZ1 with 7.2 or 7.0 µM, whereas PDZ3 was bound with 4.5 or 3.4 µM respectively (Fig. 2, Table 1). We also examined binding of MCC and pMCC to a construct encompassing the tandem PDZ3/PDZ4 supramodule [24]. No significant differences were observed for peptide binding, with $K_D$ values of 12.4 µM and 10.3 µM for MCC and pMCC to the PDZ3/4 supramodule respectively.

Examination of the thermodynamic binding parameters of MCC binding to Scribble PDZ3 indicates that the binding of phosphorylated MCC (pMCC) to Scribble PDZ3 compared to the unphosphorylated MCC peptide is largely driven by a more favourable -TAS or entropic term, indicative of more disorder upon pMCC binding (Fig. 3, Table 1) compared to MCC binding.

The crystal structures of SCRIB PDZ1:MCC peptides

To understand the structural implications of Ser phosphorylation at the −1 site of the MCC PBM, we next determined the crystal structure of PDZ1 bound to the

| Table 1. Summary of affinity and thermodynamic binding parameters for Scrib PDZ domain interactions with MCC peptides. NB denotes no binding. Each of the value was calculated from at least three independent experiments |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| PDZ1:McC       | $7715 \pm 499$  | $-4.53 \pm 0.06$| $-2.44 \pm 0.09$| $1.1 \pm 0.05$  |
| PDZ2:McC       | NB              | NB              | NB              | NB              |
| PDZ3:McC       | $4971 \pm 901$  | $-13.38 \pm 0.44$| $6.12 \pm 0.45$ | $1.1 \pm 0.02$  |
| PDZ4:McC       | NB              | NB              | NB              | NB              |
| PDZ3:4:McC     | $12441 \pm 762$ | $-5.21 \pm 0.48$| $-1.48 \pm 0.52$| $1.04 \pm 0.03$ |
| PDZ1:pMCC      | $6083 \pm 555$  | $-3.70 \pm 0.99$| $-3.41 \pm 0.93$| $1.0 \pm 0.032$ |
| PDZ2:pMCC      | NB              | NB              | NB              | NB              |
| PDZ3:pMCC      | $3744 \pm 788$  | $-9.99 \pm 1.00$| $2.58 \pm 1.12$ | $1.0 \pm 0.03$  |
| PDZ4:pMCC      | NB              | NB              | NB              | NB              |
| PDZ3:4:pMCC    | $10295 \pm 1677$| $-2.26 \pm 0.42$| $-4.54 \pm 0.47$| $1.1 \pm 0.08$  |
| PDZ1:McC_SE    | $7185 \pm 884$  | $-3.53 \pm 0.22$| $-3.49 \pm 0.15$| $1.0 \pm 0.05$  |
| PDZ3:McC_SE    | $4476 \pm 510$  | $-8.14 \pm 0.64$| $2.82 \pm 0.84$ | $1.0 \pm 0.05$  |
| PDZ1:McC_SD    | $7033 \pm 754$  | $-3.14 \pm 0.12$| $-3.90 \pm 0.13$| $1.0 \pm 0.02$  |
| PDZ3:McC_SD    | $3441 \pm 632$  | $-8.32 \pm 0.79$| $0.86 \pm 0.69$ | $1.1 \pm 0.01$  |
| PDZ1_R762A:McC | $23399 \pm 1969$| $-5.43 \pm 0.67$| $-0.89 \pm 0.63$| $1.0 \pm 0.06$  |
| PDZ1_R762A:pMCC| $20954 \pm 1732$| $-3.15 \pm 0.11$| $-2.84 \pm 0.56$| $1.1 \pm 0.04$  |
| PDZ1_ph4:2:MCC | $6607 \pm 1435$ | $-2.91 \pm 0.46$| $-3.23 \pm 0.12$| $1.0 \pm 0.00$  |
| PDZ1_ph4:2:pMCC| $4624 \pm 81$   | $-3.57 \pm 0.22$| $-3.72 \pm 0.22$| $1.0 \pm 0.06$  |
| PDZ3_K1040A:MCC| $21869 \pm 1160$| $-7.43 \pm 0.83$| $1.07 \pm 0.85$ | $1.0 \pm 0.06$  |
| PDZ3_K1040A:pMCC| $19809 \pm 1594$| $-3.24 \pm 0.41$| $-3.17 \pm 0.45$| $1.0 \pm 0.05$  |
MCC 8-mer peptide (P^7-H^6-T^5-N^4-E^3-T^2-S^1-L^0; Figs 4 and 5, Table 2). As previously shown [25] PDZ1 adopts a compact globular fold comprising six \(-\)strands and two \(\alpha\)-helices that form a \(\beta\)-sandwich structure. The MCC peptide is bound in the canonical ligand binding groove formed by the \(\beta\) strand and helix \(\alpha\). Compared to the previously determined structure of a Scribble PDZ1:\(\beta\)-PIX complex or ligand-free Scribble PDZ1 (PDB ID 5VWC) [25], binding of MCC does not significantly alter the PDZ1 domain structure, with the root-mean-square deviation (RMSD) between Scribble PDZ1:\(\beta\)-PIX and MCC complexes being 1.3 \(\AA\) over 92 \(\mathrm{Ca}\) atoms, and between PDZ1 alone and PDZ1:MCC being 0.9 \(\AA\) over 99 \(\mathrm{Ca}\) atoms [26].

In the PDZ1:MCC structure, the pocket accommodating MCC \(L^0\) is formed by PDZ1 L738, I740, I742, V797 and L800. In addition, PDZ1 forms several hydrogen bonds with MCC, including side chain-mediated MCC T^2:H793^PDZ1 and MCC N825^4:S748^PDZ1 (Figs 4 and 5) as well as the main chain contacts from

Fig. 1. Interaction profiles of Scribble PDZ domains with MCC peptide. Binding profiles of isolated Scribble PDZ domain interactions with MCC peptides are displayed. Each profile is represented by a raw thermogram (top panel) and a binding isotherm fitted with a one-site binding model (bottom panels). \(K_D\): dissociation constant; \(\pm\): standard deviation; NB: no binding. Each of the values was calculated from at least three independent experiments.
L738PDZ1, G739PDZ1 and I740PDZ1 with the carboxyl group of MCC L0 and inter main chain contacts between MCC T2:T742 PDZ1. The final hydrogen bond is formed by MCC T3:T749 PDZ1. Examination of the complex of phosphorylated MCC (pMCC) with Scribbel PDZ1 complex reveals two copies of PDZ1: pMCC in the asymmetric unit (denoted as PDZ1-A: pMCC-D and PDZ1-B:pMCC-C) that featured differences in the formation of hydrogen and ionic bonds. A comparison of the PDZ1:MCC complex with PDZ1-A:
pMCC-D reveals only a single major difference in the formation of hydrogen or ionic bonds, where R762PDZ1 forms an ionic bond with the phosphate group on pMCC pS\(^{-1}\) from a neighbouring molecule. Furthermore, the orientation of the N terminus of pMCC-D is different, with two additional residues, H\(^{-6}\) and P\(^{-7}\), being resolved. A comparison of the PDZ1:MCC complex with PDZ1-B:pMCC-C reveals that R762PDZ1 also forms an ionic bond with the phosphate group on pMCC pS\(^{-1}\); however, in this instance the bond is formed with the actual pMCC chain bound in the PDZ1 domain binding groove. In addition, hydrogen bonds between S761PDZ1 and pMCC E\(^{-3}\) as well as S748PDZ1 and pMCC N\(^{-4}\) and G747PDZ1 and pMCC H\(^{-6}\) are found. Interestingly, the MCC T\(^{-5}\):T749PDZ1 hydrogen bond seen in the PDZ1:MCC complex is absent in the PDZ1:pMCC-C, and instead T749PDZ1 engages pMCC P\(^{-7}\). Lastly, the C-terminal pMCC residue L\(^{0}\) only makes two contacts via its carboxyl group with PDZ1 via L738PDZ1 compared to the three contacts in PDZ1: MCC. The overall configuration of pMCC when bound to PDZ1 differs from wild-type MCC bound to PDZ1, with the C terminus from both pMCC

Fig. 5. 2Fo-Fc electron density maps of PDZ1 complexes with wild-type MCC and phosphorylated MCC peptide. (A) Electron density map encompassing the binding groove of Scribble PDZ1 in complex with MCC peptide. PDZ1 is shown as grey sticks, whereas MCC peptide is shown as green sticks. The electron density map is shown as a blue mesh contoured at 1.5 \(\sigma\). (B) Electron density map encompassing the binding groove of Scribble PDZ1 in complex with pMCC. PDZ1 is shown as grey sticks, whereas pMCC is shown as cyan sticks. The electron density map is shown as a blue mesh contoured at 1.5 \(\sigma\). (C) Acidic clamp conformation of the PDZ-binding motif in the SNX27 PDZ:pLRRC3B and SCRIB PDZ1:pMCC complexes. SNX27 PDZ:pLRRC3B (PDZ ID 5EMA) is shown as green cartoon with sticks and magenta sticks, respectively, whereas SCRIB PDZ1:pMCC is shown as yellow cartoon with sticks and cyan sticks respectively. (D) Overlay of ribbon traces of PDZ1 (grey) bound to MCC (green) or pMCC peptides (cyan and coral). (E) Superimposition of Scribble PDZ1 (grey) bound to pMCC (cyan and coral) complexes with \(\beta\)-PIX (yellow, PDB ID 5VWK), p120-like synthetic peptide (green, PDB ID 1N7T) and Gukh (magenta, PDB ID 5WOU) complexes.
completely revealing the position of pMCC H$^{\text{pS}}$ and P$^{|\text{pS}}$ that are disordered in PDZ1:MCC (Fig. 5C).

Binding of a phosphorylated MCC peptide did not induce any significant overall structural changes to Scribble PDZ1 or the ligand binding groove compared to binding of unphosphorylated MCC, with the RMSD of a superimposition of the Scribble PDZ1: MCC complex with both pMCC complexes being 1.0 Å over 96 Ca atoms.

To understand the role of Scribble PDZ1 R762 in binding to MCC in more detail, we performed site-directed mutagenesis to change R762 to alanine and measured the ability of this mutant to bind both MCC and pMCC. Loss of R762 impacts binding to MCC and pMCC essentially equally, with resultant $K_D$ measurements of 23.4 and 21.0 μM respectively. This suggests that R762 does not play a role in discriminating between MCC and pMCC. We next attempted to understand if the equivalent charged Lys residue in Scribble PDZ3 may have a discriminatory effect on MCC over pMCC binding. Superimposition of Scribble PDZ3 on PDZ1 indicated that K1040 is located in the equivalent position in PDZ3 compared to R762 in PDZ1. A Scribble PDZ3 mutant K1040A bound MCC with an affinity of 21.9 μM while binding pMCC with a $K_D$ of 19.8 μM. This suggests that while K1040 contributes significantly to binding of MCC/pMCC by Scribble PDZ3 as indicated by the fourfold reduction in affinity after substitution with alanine, again it does not allow discrimination between MCC and pMCC.

**Molecular dynamics simulations**

To gain further insight into the alternative confirmations observed for Scribble PDZ1 R762 in complex with pMCC, and into differences in the mode of binding of PDZ1 to MCC and pMCC, we performed molecular dynamics simulations. Simulations were run on the ligand-free apo PDZ1 domain (a) and the complexes with MCC (b) and pMCC (c,d). X-ray crystal structures determined herein were used as initial structures. For the complexes with pMCC, both the structure of pMCC-D in complex with PDZ1-A where R762$^{\text{PDZ1}}$ interacts with the phosphate of pMCC p$^{|\text{S}}$ from a neighbouring complex in the crystal structure, and pMCC-C in complex with PDZ1-B where this interaction was observed within the same complex, were used as starting structures. Following initial minimisation and equilibration, each system was simulated for 1 μs. In the complex that began with R762$^{\text{PDZ1}}$ and pMCC p$^{|\text{S}}$ separated (PDZ1-A Arg(C$_\text{l}$) - pMCC-D pSer(P) 5.5 Å), the arginine side chain immediately altered conformation and formed a stable salt bridge with the phosphate of pMCC p$^{|\text{S}}$. This salt bridge remained stable throughout the 1-μs-long MD simulation of both systems (Fig. 6B). The RMSD between C$_\alpha$ atoms in the PDZ1 domain of the two structures at the end of the 1-μs MD simulation was 1.1 Å. Only minor differences are observed between ligand-free and ligand-bound structures at the end of the 1-μs simulations; the RMSD for C$_\alpha$ atoms in the PDZ1 domain between apo and MCC or pMCC ligands was ~ 1.9 Å.

The structures of each of the four systems studied (i–iv) at the completion of 1 μs MD are presented in Fig. 6C. Principal component analysis (PCA) was conducted on the 1 μs MD trajectory of each system to explore the effect of peptide binding and of peptide phosphorylation on the dynamics of the complexes. Structural variances along the first two dominant principal components, PC1 and PC2, confirmed that both the loop connecting beta strands β1 and β2, as well as alpha helix α1, were stabilized by the changes. The extent of the motions of these regions throughout the trajectory is illustrated in Fig. 6D. In the ligand-free apo form of the PDZ1, both regions exhibit significant flexibility. Binding of MCC reduces slightly the mobility of the β1–β2 loop but not of helix α1. In both simulations with pMCC bound, the mobility of both regions is significantly damped.

**Discussion**

Post-translational modifications can alter the affinity with which Scribble binds to its partners including phosphorylation [14,21]. However, phosphorylation does not always impact the affinity of Scribble PDZ domain binding. Phosphorylation of APC seems to have little effect on Scribble binding [14,27], whereas phosphorylation of RPS6KA2 leads to a fourfold increase in affinity for Scribble PDZ1. In contrast, phosphorylation of MAPK12 completely abolishes Scribble PDZ1 interaction [14].

Our data suggest that while Scribble PDZ domains 1 and 3 are able to bind the PBM of MCC, the affinities were not significantly changed after phosphorylation of a Ser residue at the –1 position of the MCC PBM. These findings are compatible with the NMR chemical shift report but are unexpected, since previous reports indicated that phosphorylation of Ser at –1 of the MCC PBM is important for the interaction of MCC with Scribble and control of lamellipodia formation, and that loss of phosphorylation reduced the amount of MCC bound to Scribble in immunoprecipitation experiments. Our affinity measurements are broadly corroborated by crystal structures of Scribble PDZ1 domain bound to unphosphorylated and
phosphorylated PBMs of MCC. While interactions between Scribble PDZ1 and MCC including T-2, H793PDZ1, N-4:S748PDZ1, T-2:I742PDZ1, and L738PDZ1, G739PDZ1 and I740PDZ1 with the carboxyl group of L0 are conserved in the pMCC complex, the phospho-Ser at position C0 in the pMCC PBM is involved in one major contact with R762 of Scribble PDZ1, as well as two additional hydrogen bonds between S762PDZ1 and pMCC E-3 and as G747PDZ1 with pMCC H-6 that are not seen in the Scribble PDZ1 complex with unphosphorylated MCC.

These interactions, however, are not uniform across both copies of the PDZ1:pMCC complexes present in the asymmetric unit, initially suggesting that the interplay between PDZ1 and pMCC and the required intermolecular interactions are flexible, thus providing a rationale for the lack of significant change in affinity of phospho-MCC to Scribble PDZ1 compared to unphosphorylated MCC. However, molecular dynamics simulations revealed that SCRIB PDZ1 R762 switches to a single conformer where pMCC pS-1 is engaged, suggesting that the observation of two different R762 conformations is likely a crystallization artefact. The lack of discrimination at the binding affinity and structural level we found is in agreement with findings from Sundell et al. [23], who based on chemical shift data speculated that phosphorylation at the C0 position of the MCC PBM may not be a major functional switch. Examination of the phosphorylated Ser at the C0 position of the MCC PBM bound to Scribble PDZ1 reveals that although pMCC is able to adopt an acidic clamp configuration with C0 (pS828) and C2 (E826), Scribble PDZ1 does not harbour a corresponding charged residue that could be targeted.

Table 2. Data collection and refinement statistics

|                      | PDZ1:MCC | PDZ1:pMCC |
|----------------------|----------|-----------|
| **Data collection**   |          |           |
| Space group          | I 1 2 I | I 4 1     |
| No. of molecules in AU | 2 + 2   | 2 + 2     |
| **Cell dimensions**  |          |           |
| a, b, c (Å)          | 57.40, 55.66, 61.78 | 53.60, 53.60, 214.45 |
| α, β, γ (°)          | 90.00, 116.12, 90.00 | 90.00, 90.00, 90.00 |
| Wavelength (Å)       | 0.9537   | 0.9537    |
| Resolution (Å)       | 39.25–2.60 (2.71–2.60) | 4288–2.14 (2.48–2.14) |
| Rsym or Rmerge<sup>a</sup> | 0.085 (0.936) | 0.082 (0.750) |
| i/σ<sup>i</sup>       | 7.3 (1.8) | 12.6 (1.5) |
| CC(1/2)              | 0.995 (0.573) | 0.999 (0.641) |
| Completeness spherical (%)<sup>a</sup> | 97.6 (93.8) | 53.3 (8.0) |
| Completeness ellipsoidal (%)<sup>a</sup> | – | 91.5 (69.1) |
| Redundancy<sup>a</sup> | 2.8 (2.9) | 5.7 (4.5) |
| Wilson B-factor       | 22.02    | 41.65     |
| **Refinement**       |          |           |
| Resolution (Å)       | 37.79–2.60 | 42.88–2.14 |
| No. of reflections   | 5311     | 8810      |
| Rwork/Rfree          | 0.227/0.265 | 0.197/0.255 |
| **No. of nonhydrogen atoms** |          |           |
| Protein              | 1574     | 1611      |
| Ligand/ion           | 22       | 75        |
| Water                | 4        | 44        |
| **B-factors**        |          |           |
| Protein              | 66.58    | 43.86     |
| Ligand/ion           | 81.52    | 52.00     |
| Water                | 68.81    | 39.42     |
| **R.m.s. deviations**|          |           |
| Bond lengths (Å)     | 0.002    | 0.003     |
| Bond angle (°)       | 0.53     | 0.52      |
| Ramachandran plot (%)|         |           |
| Favoured             | 95.92    | 97.0      |
| Allowed              | 4.08     | 3.0       |
| Disallowed           | 0        | 0         |

<sup>a</sup>Data in parentheses are for highest resolution shell.
by the pMCC acidic clamp in a manner previously observed in the SNX27 PDZ in complex with 5-HT_4 (a)R or in complex with LRRC3B-pSer(-3), which does feature an acidic clamp formed by -4 and -6 PBM [15] (Fig. 5D). Intriguingly however, our molecular dynamics simulation did suggest that binding of pMCC stabilizes the loop connecting beta strands β1 and β2, as well as alpha helix α1, whereas bound MCC only led to a small reduction in mobility of the β1/β2 loop. This differential modulation of dynamics may provide an alternative mode for phosphorylation of MCC to impact Scribble signalling.

Although MCC is able to bind to both Scribble PDZ1 and 3 domains, the interaction with PDZ3 is modestly tighter. Interestingly, PDZ3 has been shown to be the primary binding site for MCC [16], with phosphorylation of S828 at the -1 position predicted to lead to tighter binding compared to unphosphorylated MCC [21] based on functional data where enzymatically dephosphorylated MCC showed reduced levels of immunoprecipitation with full-length Scribble. Unexpectedly, our data suggest that phosphorylation of MCC S-1 does not significantly alter its affinity for Scribble PDZ3. Pangon et al. [21] previously observed
that there is no significant difference in immunoprecipitation levels between unphosphorylated MCC and phosphorylated MCC with Scribble, whereas an MCC S828A mutant displayed reduced Scribble binding. These findings were interpreted as evidence that MCC is usually phosphorylated. Our data suggest that MCC may not necessarily be phosphorylated to account for the lack of significant differences in immunoprecipitation levels observed, and may be indicative of an effect that is not directly linked to Scribble binding. This is in contrast to MCC binding to Dlg, where phosphorylation of MCC leads to a decrease in affinity from 2.7 to 95 µM for Dlg PDZ2 [14]. A comparison of the DLG PDZ2:APC PBM complex [28,29] with the Scribble PDZ3:β-PIX complex [25] indicates that key residues that form the βB strand that constitutes one side of the canonical ligand binding groove are conserved. Furthermore, the putative pSer binding residue in Scribble PDZ3, K1040, is also conserved (K352 in DLG PDZ2), so in principle a suitable recognition residue is present in DLG PDZ2. A better understanding of the differences in DLG PDZ2 and Scribble PDZ3 discrimination between MCC and pMCC binding is likely to require experimental structure determination of the relevant complexes.

Examination of the thermodynamics of MCC and pMCC binding to PDZ3 reveals that the minor difference in binding of pMCC to PDZ3 is due to more favourable -TΔS or entropy terms rather than enthalpy terms, which remained comparable. We speculate that few or no major additional hydrogen bonds are formed with the phosphate group on Ser –1; however, this would have to be confirmed by direct structure determination of a PDZ3:pMCC complex. When comparing the thermodynamic parameters of PDZ1 and PDZ3 binding to MCC and pMCC, substantial differences in the mode of binding become apparent. Although PDZ1 binding to MCC is driven by both moderately favourable enthalpic and entropic contributions, binding of MCC to PDZ3 is dominated by the enthalpy component that is accompanied by significant unfavourable entropy changes. These findings suggest that the resultant hydrogen bond network in a putative PDZ3:MCC complex is more extensive compared to PDZ1:MCC. Interestingly, our mutagenesis data suggest that K1040 from PDZ3 makes a significant contribution to MCC and pMCC binding, since substitution of K1040 by alanine results in an approximately fourfold loss of affinity. Furthermore, this also suggests that the mode of binding of MCC to PDZ3 may be different from the one observed for a synthetic peptide to the Scribble PDZ3/4 supramodule [24], where K1040 was occupied in the interdomain interface. This notion is further supported by the lack of significant differences in the affinity of PDZ3 alone compared to PDZ3/4 to MCC and pMCC. Further clarification of the role of PDZ4 during MCC binding to PDZ3 is likely to require determination of the relevant complex structure.

Compared to other previously established affinities of Scribble PDZ1 domain for interactors, MCC is not a particularly high-affinity interactor, Gukh engages Dro sophila Scribble PDZ1 with 660 nM affinity [30], and β-PIX binds human Scribble PDZ1 with 2 µM affinity [25,31]. MCC only binds to Scribble PDZ1 with 7.7 µM affinity, and examination of the different molecular contacts between MCC and Scribble PDZ1 indicate that the lower affinity compared to β-PIX may be due to the lack of a hydrophobic residue engaging the PDZ1 β2-3 loop, as is achieved by β-PIX via β-PIX W641 [25] or Trp at position –4 in a high-affinity nanomolar complex of a synthetic peptide with Erbin [32] (see Fig. 5E). An alternative configuration that enables high-affinity interactions is observed in the Scribble PDZ1:Gukh complex, where F1784 in Gukh contacts H796 on the opposite side of the ligand binding groove [30].

In summary, we show that Scribble PDZ3 domain binds MCC with an approximately twofold higher affinity compared to Scribble PDZ1 domain. Furthermore, phosphorylation of MCC at the –1 position of the PBM has no significant impact on binding to Scribble PDZ1 and PDZ3 domains, suggesting that the impact of phosphorylation of the MCC PBM at the –1 position extends beyond a simple change in binding affinity to Scribble PDZ domains and that could be dependent on a Scribble PDZ interdomain interaction to favour or block MCC binding. Another possibility is that –1 phosphorylation of the MCC PBM primarily affects an interactor that indirectly affects Scribble such as Dlg, which has been shown to display substantial modulation of affinity for MCC depending on the phosphorylation status of the MCC PBM. Our findings thus provide a clear structural basis for Scribble PDZ1:MCC interactions, and will enable more detailed structure-guided investigations to understand the precise effect of MCC phosphorylation on the control of cell polarity and directed migration.

Materials and methods

Protein expression and purification

Protein expression constructs encoding the PDZ domains of human Scribble (Uniprot accession number: Q14160) PDZ1 (728–815); PDZ2 (833–965); PDZ3 (1005–1094) and PDZ4 (1099–1203)) were obtained as synthetic cDNA codon
with 200 μg mL⁻¹ ampicillin (AMRESCO, West Chester, PA, USA) using auto-induction media (10 mM Tris-Cl pH 7.6, 100 mM NaCl, 1 mM MgSO₄, 0.2% (w/v) D-lactose, 0.05% (w/v) glucose, 0.5% (v/v) glycerol) [34] at 37 °C until the optical density at 600 nm (OD600) reached 1.0 before transferring cultures to 20°C for 24 hours for protein expression. Bacterial cells were harvested by centrifugation and lysed in the presence of deoxyribonuclease I (Sigma-Aldrich, St Louis, MI, USA) from bovine pancreas using TS Series 0.75 kw model cabinet (Constant Systems Ltd., Davenport, UK) at 25 kPis, Qsonica Q700 sonicator at amplitude 50 for a 4-min process time on ice (5-s pulse-on time and 30-s pulse-off time). Lysates were clarified by centrifugation at 20 000 g for 20 min using an Avanti® J-E (Beckman Coulter, Pasadena, CA, USA). The resulting supernatant was filtered using Millex-GP syringe filter unit 0.22 μm (Merck Millipore, Burlington, MA, USA) prior to loading onto equilibrated columns for affinity purification.

Glutathione-S-transferase (GST)-tagged recombinant PDZ1 protein was captured using glutathione sepharose 4B (GE Healthcare) in buffer A (50 mM Tris-Cl pH 8.0, 150 mM NaCl and 1 mM EDTA) and was cleaved on-column with HRV 3C protease to remove the GST tag. Cleaved protein was retrieved with buffer A and GST-tagged protein was eluted with buffer A supplemented with 20 mM reduced l-glutathione.

Hexahistidine maltose-binding protein (His-MBP)-tagged recombinant proteins (PDZ2, PDZ3 and PDZ4) were purified using 5 mL HisTrap HP columns (GE Healthcare) in buffer B (50 mM Tris-Cl pH 8.0, 300 mM NaCl) and washed with buffer B supplemented with 20 mM Imidazole before eluting in buffer B supplemented with 300 mM Imidazole. His-MBP-tagged recombinant proteins were cleaved with TEV protease in buffer B supplemented with 0.5 mM EDTA and 1 mM DTT before being subjected to a second round of affinity chromatography to remove cleaved HisMBP tag and uncleaved fusion protein. All cleaved target proteins were subjected to size exclusion chromatography using the HiLoad 16/600 Superdex 75 (GE Healthcare) equilibrated in 25 mM Tris-Cl pH 8.0, 150 mM NaCl and eluted as a single peak.

**Isothermal titration calorimetry**

Purified human Scribble PDZ domains were used in titration experiments against 8-mer peptides spanning the C terminus of human MCC isoform-2 (Uniprot accession number: P23508; PHTNETSL), a modified MCC peptide (pMCC) bearing a phosphorylated Ser residue at position –1 (PHTNET(phoS)L), as well as MCC_SD (PHTNETSDL) bearing Ser to Asp substitution on position –1 and MCC_SE (PHTNETTEL) bearing a Ser to Glu substitution at position –1 to determine the effect of phosphorylation on MCC affinity for Scribble PDZ domains. PDZ1 concentration was quantitated at 280 nm absorbance (A280 nm) using a NanoDrop 2000/2000c UV-Vis Spectrophotometer (Thermo Scientific, Waltham, MA, USA). Since the concentration of PDZ2, PDZ3 and PDZ4 could not be determined by measurements of Abs280 nm due to lack of useful aromatic amino acids, protein concentrations were calculated using the Scope method [35] by measuring absorbance at 205 and 280 nm using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

Titrations were performed at 25°C with a stirring speed of 750 rpm using the MicroCalTM iTC200 System (GE Healthcare). A total of 20 injections with 2 μL each and a spacing of 180 s were titrated into the 200 μL protein sample (25 mM Tris pH 8.0, 150 mM NaCl), except for the first injection which was only 0.4 μL. A protein concentration of 75 μM against a peptide concentration of 0.9 mM was used. Peptides were purchased from Mimotopes (Mulgrave, Australia). Raw thermograms were processed with MicroCal Origin® version 7.0 software (OriginLabTM Corporation) to obtain the binding parameters of each interaction. A synthetic pan-PDZ binding peptide referred to as superpeptide (RSWFETWV) was used as a positive control [30].

**Protein crystallization, data collection and refinement**

Complexes of PDZ1 with MCC and pMCC peptides were reconstituted by mixing protein and peptide at a 1:2 molar ratio. The dilute protein complexes were then concentrated to 15 and 23 mg·mL⁻¹, respectively, using a 3-kDa molecular mass cut-off centrifugal concentrator (Millipore), flash-cooled and stored under liquid nitrogen. Crystallization trials were carried out using 96-well sitting-drop trays (Swissi, Neuheim, Switzerland) and vapour diffusion at 20°C either in-house or at the CSIRO C3 Collaborative Crystallization Centre, Melbourne, Australia. 0.15 μL of protein–peptide complexes were mixed with 0.15 μL of various crystallization conditions using a Phoenix nanodispenser robot (Art Robbins, Sunnyvale, CA, USA). Commercially available screening kits (PACT Suite and JCSG-plus Screen) were used for the initial crystallization screening, with hit optimization performed using a 96-well plate at the CSIRO C3 Centre. Crystals of PDZ1 in complex with MCC peptide were obtained at 15 mg·mL⁻¹ in 25% (w/v) polyethylene glycol 1000, 0.15 mM lithium sulphate monohydrate and 0.1 mM phosphate/citrate pH 4.5. The PDZ1-MCC crystals were cryo-protected using 20% (w/v) glucose and flash cooled at 100 K using liquid nitrogen. Plate shaped crystals were obtained belonging to space group I121. Crystals of PDZ1 in complex with pMCC peptide were obtained at 23 mg·mL⁻¹ in 0.1 mM Phosphate/citrate 4.2 and 40% (v/v) poly(ethylene glycol) 300. The
PDZ1-pMCC crystals were cryoprotected using 30% (w/v) ethylene glycol and flash-cooled at 100 K using liquid nitrogen. Plate-like crystals were obtained belonging to space group $I4_1$ with twin law -h,k,-l (structure refinement was not possible at $I4_22$ as suggested by Pointless).

All diffraction data were collected on the MX2 beamline at the Australian Synchrotron using an Eiger 16M detector with an oscillation range of 0.1° per frame using a wavelength of 0.9537 Å. PDZ1:MCC diffraction data were integrated with Xia2 [36] and scaled using AIMLESS [37] while PDZ1-pMCC data were integrated with XDSme [38] to detector resolution. PDZ1-pMCC diffraction was anisotropic with resolution limits of 2.8 Å along the a* and b* orientation and 2.14 Å on the c* orientation. Therefore, the data were elliptically truncated and corrected using the Staraniso server (http://staraniso.globalphasing.org).

Both structures were solved by molecular replacement using Phaser [39] with the structure of human Scribble PDZ1 (PDB code 5VWC [25] or 2W4F) as a search model. The final TFZ and LLG values were 16.7 and 253 for PDZ1-MCC and 25.6 and 723 for PDZ1-pMCC respectively. The solutions produced by Phaser were manually rebuilt over multiple cycles using Coot [40] and refined using PHENIX [41]. Data collection and refinement statistics details are summarized in Table 2. MolProbity scores were obtained from the MolProbity web server [42]. Coordinate files have been deposited in the Protein Data Bank under the accession codes 6MTU and 6MTV. All images were generated using the PyMOL Molecular Graphics System, Version 1.8 Schrödinger, LLC. All software was accessed using the SBGrid suite [43]. All raw diffraction images were deposited on the SBGrid Data Bank [44] using their PDB accession code 6MTU and 6MTV.

**Molecular modelling**

The X-ray crystallographic structures determined herein were prepared for MD simulation using the MODELLER software (version 9.14) [45] to build missing residues of the PDZ1 domain. All MD simulations were performed using Gromacs software package (version 2018.3) [46] with the CHARMM36 m force field [47]. Molecular systems were prepared using the CHARMM-GUI server [48]. Steepest descent energy minimization was performed before short 100 ps NVT and NPT equilibration molecular dynamics simulations; production MD was performed from the previous NPT ensemble. The TIP3P water model was used to solvate systems, and all ionizable residues were assumed to be the standard state at pH 7; sodium and chloride ions were introduced by replacing water molecules to ensure the systems were electrically neutral and at a 0.1 M ionic strength. A cut-off of 12 Å in real space was applied to account for nonbonded interactions, and the particle-mesh Ewald method [49] was applied for long-range electrostatics. The temperature was maintained at 303K using the velocity-rescale thermostat coupling method; protein and nonprotein groups were treated independently; the time constant for coupling was set as 1.0 ps. The pressure of system was maintained at 1 bar with the Parrinello–Rahman barostat. The LINCS algorithm [50] was used to constrain all bond lengths, permitting a 2 fs time step for MD integration. The Verlet grid cut-off scheme was applied for neighbour searching with an update frequency of 20 steps. Periodic boundaries were applied in all directions. Principal component analysis was performed using the ProDy package [51]. Figures were created using the R package and Chimera software [52].

**Acknowledgements**

We thank staff at the MX beamlines, in particular Tom Caradoc-Davis, at the Australian Synchrotron for help with X-ray data collection, and the CSIRO C3 Collaborative Crystallization Centre for assistance with crystallization and the Comprehensive Proteomics Platform at La Trobe University for core instrument support. We thank the ACRF for their support of the Eiger MX detector at the Australian Synchrotron MX2 beamline. This work was supported in whole or part by the National Health and Medical Research Council Australia (Project Grant APP1103871 to MK, POH; Senior Research Fellowship APP1079133 to POH), Australian Research Council (Fellowship FT130101349 to MK and La Trobe University (Research focus area “Understanding Disease” project grant and Scholarship to BZS). RJ acknowledges receipt of Australian Research Training Scholarships. Part of this work was undertaken using resources from the National Computational Infrastructure, which is supported by the Australian Government and provided through Intersect Australia Ltd, and through the HPC-GPGPU Facility, which was established with the assistance of LIEF Grant LE170100200.

**Conflict of interests**

The authors declare no conflict of interest.

**Author contributions**

SC and BJS were involved in acquisition of data, analysis and interpretation of data, drafting and revising the article. BZS and RJ were involved in acquisition of data, analysis and interpretation of data. POH and MK were involved in conception and design,
acquisition of data, analysis and interpretation of data; drafting and revising the article.

References

1. Nelson WJ (2003) Adaptation of core mechanisms to generate cell polarity. Nature 422, 766–774.
2. McCaffrey LM & Macara IG (2012) Signaling pathways in cell polarity. Cold Spring Harb Perspect Biol 4, 1–16.
3. Halaoiu R & McCaffrey L (2015) Rewiring cell polarity signaling in cancer. Oncogene 34, 939–950.
4. Stephens R, Lim KYB, Portela M, Kvansakul M, Humbert PO & Richardson HE (2018) The scribble cell polarity module in the regulation of cell signalling in tissue development and tumourigenesis. J Mol Biol 430, 3585–3612.
5. Bilder D, Li M & Perrimon N (2000) Cooperative regulation of cell polarity and growth by Drosophila tumor suppressors. Science 289, 113–116.
6. Zhan L, Rosenberg A, Bergami KC, Yu M, Xuan Z, Jaffe AB, Allred C & Muthuswamy SK (2008) Deregulation of scribble promotes mammary tumourigenesis and reveals a role for cell polarity in carcinoma. Cell 135, 865–878.
7. Pearson HB, McGinn E, Phesse TJ, Schluter H, Srikumar A, Godde NJ, Woelwer CB, Ryan A, Phillips WA, Ernst M et al. (2015) The polarity protein Scrib mediates epidermal development and exerts a tumor suppressive function during skin carcinogenesis. Mol Cancer 14, 169.
8. Pearson HB, Perez-Manera PA, Dow LE, Ryan A, Tennstedt P, Bogani D, Elsum I, Greenfield A, Tuveson DA, Simon R et al. (2011) SCRIB expression is deregulated in human prostate cancer, and its deficiency in mice promotes prostate neoplasia. J Clin Invest 121, 4257–4267.
9. Godde NJ, Sheridan JM, Smith LK, Pearson HB, Britt KL, Galea RC, Yates LL, Visvader JE & Humbert PO (2014) Scribble modulates the MAPK/Fra1 pathway to disrupt luminal and ductal integrity and suppress tumour formation in the mammary gland. PLoS Genet 10, e1004323.
10. Elsum IA, Yates LL, Pearson HB, Phesse TJ, Long F, O'Donoghue R, Ernst M, Cullinan C & Humbert PO (2014) Scribble heterozygosity predisposes to lung cancer and cooperates with KRas hyperactivation to accelerate lung cancer progression in vivo. Oncogene 33, 5523–5533.
11. Kallay LM, McNickle A, Brennwald PJ, Hubbard AL & Braiterman LT (2006) Scribble associates with two polarity proteins, Lgl2 and Vangl2, via distinct molecular domains. J Cell Biochem 99, 647–664.
12. Cohen NA, Brennan JE, Snyder SH & Bredt DS (1996) Binding of the inward rectifier K+ channel Kir 2.3 to PSD-95 is regulated by protein kinase A phosphorylation. Neuron 17, 759–767.
13. Chetkovich DM, Chen L, Stocker TJ, Nicoll RA & Bredt DS (2002) Phosphorylation of the postsynaptic density-95 (PSD-95)/discs large/zona occludens-1 binding site of stargazin regulates binding to PSD-95 and synaptic targeting of AMPA receptors. J Neurosci 22, 5791–5796.
14. Sundell GN, Arnold R, Ali M, Naksukpaiboon P, Orts J, Guntert P, Chi CN & Ivarsson Y (2018) Proteome-wide analysis of phospho-regulated PDZ domain interactions. Mol Syst Biol 14, e8129.
15. Clairfouille T, Mas C, Chan AS, Yang Z, Tello-Lafoz M, Chandra M, Widagdo J, Kerr MC, Paul B, Merida I et al. (2016) A molecular code for endosomal recycling of phosphorylated cargos by the SNX27- retromer complex. Nat Struct Mol Biol 23, 921–932.
16. Arnaud C, Sebbagh M, Nola S, Audebert S, Bidaut G, Hermant A, Gayet O, Dusetti NJ, Ollendorff V, Santoni MJ et al. (2009) MCC, a new interacting protein for Scrib, is required for cell migration in epithelial cells. FEBS Lett 583, 2326–2332.
17. Young T, Poobalan Y, Tan EK, Tao S, Ong S, Wehner P, Schwen-Lara J, Lim CY, Sadasivam A, Lovatt M et al. (2014) The PDZ domain protein Mec is a novel effector of non-canonical Wnt signaling during convergence and extension in zebrafish. Development 141, 3505–3516.
18. Fukuyama R, Niculaita R, Ng KP, Obusez E, Sanchez J, Kalady M, Aung PP, Casey G & Szemere N (2008) Mutated in colorectal cancer, a putative tumor suppressor for serrated colorectal cancer, selectively represses beta-catenin-dependent transcription. Oncogene 27, 6044–6055.
19. Bouwmeester T, Bauch A, Ruffner H, Angrand PO, Bergamini G, Croughton K, Cruciat C, Eberhard D, Gagneur J, Ghidelli S et al. (2004) A physical and functional map of the human TNF-alpha/NF-kappa B signal transduction pathway. Nat Cell Biol 6, 97–105.
20. Matsumine A, Senda T, Baeg GH, Roy BC, Nakamura Y, Noda M, Toyoshima K & Akiyama T (1996) MCC, a cytoplasmic protein that blocks cell cycle progression from the G0/G1 to S phase. J Biol Chem 271, 10341–10346.
21. Pangan L, Van Kralingen C, Abas M, Daly RJ, Musgrove EA & Kohonen-Corish MR (2012) The PDZ-binding motif of MCC is phosphorylated at position -1 and controls lamellipodia formation in colon epithelial cells. Biochim Biophys Acta 1823, 1058–1067.
22. Liu X, Shepherd TR, Murray AM, Xu Z & Fuentes EJ (2013) The structure of the Tian1 PDZ domain/ phospho-syndecan1 complex reveals a ligand conformation that modulates protein dynamics. Structure 21, 342–354.
Crystal structure of Scribble PDZ1 with phosphoMCC peptide

S. Caria et al.

23 Sundell GN, Vogeli B, Ivarsson Y & Chi CN (2018) The sign of nuclear magnetic resonance chemical shift difference as a determinant of the origin of binding selectivity: elucidation of the position dependence of phosphorylation in ligands binding to scribble PDZ1. Biochemistry 57, 66–71.

24 Ren J, Feng L, Bai Y, Pei H, Yuan Z & Feng W (2015) Interdomain interface-mediated target recognition by the Scribble PDZ34 supermodule. Biochem J 468, 133–144.

25 Lim KYB, Godde NJ, Humbert PO & Kvansakul M (2017) Structural basis for the differential interaction of Scribble PDZ domains with the guanine nucleotide exchange factor beta-PIX. J Biol Chem 292, 20425–20436.

26 Holm L & Laakso LM (2016) Dali server update.

27 How JY, Caria S, Humbert PO & Kvansakul M (2019) Crystal structure of the human Scribble PDZ1 domain bound to the PDZ-binding motif of APC. FEBS Lett 593, 533–542.

28 Slep KC (2012) Structure of the human discs large 1 PDZ2- adenomatous polyposis coli cytoskeletal polarity complex: insight into peptide engagement and PDZ clustering. PLoS ONE 7, e50097.

29 Zhang Z, Li H, Chen L, Lu X, Zhang J, Xu P, Lin K & Wu G (2011) Molecular basis for the recognition of adenomatous polyposis coli by the Discs Large 1 protein. PLoS ONE 6, e23507.

30 Caria S, Magtoto CM, Samiee T, Portela M, Lim KYB, How JY, Stewart BZ, Humbert PO, Richardson HE & Kvansakul M (2018) Drosophila melanogaster Guk-holder interacts with the Scribbled PDZ1 domain and regulates epithelial development with Scribbled and Discs Large. J Biol Chem 293, 4519–4531.

31 Ivarsson Y, Arnold R, McLaughlin M, Nim S, Joshi R, Ray D, Liu B, Teyra J, Pawson T, Moffat J et al. (2014) Large-scale interaction profiling of PDZ domains through proteomic peptide-phage display using human and viral phage peptideodom. Proc Natl Acad Sci USA 111, 2542–2547.

32 Appleton BA, Zhang Y, Wu P, Yin JP, Hunziker W, Skelton NJ, Sidhu SS & Wiesmann C (2006) Comparative structural analysis of the Erbin PDZ domain and the first PDZ domain of ZO-1. Insights into determinants of PDZ domain specificity. J Biol Chem 281, 22312–22320.

33 Rautureau GJ, Yabal M, Yang H, Huang DC, Kvansakul M & Hinds MG (2012) The restricted binding repertoire of Bel-B leaves Bim as the universal BH3-only prosurvival Bel-2 protein antagonist. Cell Death Dis 3, e443.

34 Studier FW (2005) Protein production by autoinduction in high density shaking cultures. Protein Expr Purif 41, 207–234.

35 Scopes RK (1974) Measurement of protein by spectrophotometry at 205 nm. Anal Biochem 59, 277–282.

36 Winter G (2010) xia2: an expert system for macromolecular crystallography data reduction. J Appl Crystallogr 43, 186–190.

37 Winn MD, Ballard CC, Cowtan KD, Dodson EJ, Emsley P, Evans PR, Keegan RM, Krissinel EB, Leslie AG, McCoy A et al. (2011) Overview of the CCP4 suite and current developments. Acta Crystallogr D Biol Crystallogr 67, 235–242.

38 Kabsch W (2010) Xds. Acta Crystallogr D Biol Crystallogr 66, 125–132.

39 Storoni LC, McCoy AJ & Read RJ (2004) Likelihood-enhanced fast rotation functions. Acta Crystallogr D Biol Crystallogr 60, 432–438.

40 Emsley P & Cowtan K (2004) Coot: model-building tools for molecular graphics. Acta Crystallogr D Biol Crystallogr 60, 2126–2132.

41 Adams PD, Afonine PV, Bunkoczi G, Chen VB, Davis IW, Echols N, Headd JJ, Hung LW, Kapral GJ, Grosse-Kunstleve RW et al. (2010) PHENIX: a comprehensive Python-based system for macromolecular structure solution. Acta Crystallogr D Biol Crystallogr 66, 213–221.

42 Chen VB, Arendall WB 3rd, Headd JJ, Keedy DA, Immormino RM, Kapral GJ, Murray LW, Richardson JS & Richardson DC (2010) MolProbity: all-atom structure validation for macromolecular crystallography. Acta Crystallogr D Biol Crystallogr 66, 12–21.

43 Morin A, Eisenbraun B, Key J, Sanschagrin PC, Timony MA, Ottaviano M & Sliz P (2013) Collaboration gets the most out of software. Elife 2, e01456.

44 Meyer PA, Socias S, Key J, Ransey E, Tjon EC, Buschiazzo A, Lei M, Botka C, Withrow J, Neau D et al. (2016) Data publication with the structural biology data grid supports live analysis. Nat Commun 7, 10882.

45 Sali A & Blundell TL (1993) Comparative protein modelling by satisfaction of spatial restraints. J Mol Biol 234, 779–815.

46 Van Der Spoel D, Lindahl E, Hess B, Groenhof G, Mark AE & Berendsen HJ (2005) GROMACS: fast, flexible, and free. J Comput Chem 26, 1701–1718.

47 Huang J, Rauscher S, Nawrocki G, Ran T, Feig M, de Groot BL, Grubmüller H & MacKerell AD Jr (2017) CHARMM36 m: an improved force field for folded and intrinsically disordered proteins. Nat Methods 14, 71–73.

48 Lee J, Cheng X, Swails JM, Yeom MS, Eastman PK, Lemkul JA, Wei S, Buckner J, Jeong JC, Qi Y et al. (2016) CHARMM-GUI input generator for NAMD, GROMACS, AMBER, OpenMM, and CHARMM/
OpenMM simulations using the CHARMM36 additive force field. *J Chem Theory Comput* **12**, 405–413.

49. Essmann U, Perera L & Berkowitz ML (1995) A smooth particle mesh Ewald method. *J Chem Phys* **103**, 8577.

50. Hess B, Bekker H, Berendsen HJC & Fraaije JGE (1997) LINCS: a linear constraint solver for molecular simulations. *J Comput Chem* **18**, 1463–1472.

51. Bakan A, Meireles LM & Bahar I (2011) ProDy: protein dynamics inferred from theory and experiments. *Bioinformatics* **27**, 1575–1577.

52. Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC & Ferrin TE (2004) UCSF Chimera – a visualization system for exploratory research and analysis. *J Comput Chem* **25**, 1605–1612.