

**Communication****STRUCTURAL INSIGHT INTO THE FORMIN BINDING PROPERTY OF HUMAN FORMIN BINDING PROTEIN 4****Amit Das, Simanti Bhattacharya, Angshuman Bagchi\* and Rakhi Dasgupta\****Department of Biochemistry and Biophysics, University of Kalyani, Nadia 741235, West Bengal, India*

**Abstract:** Formin mediated dynamic regulation of actin cytoskeleton is involved in numerous cellular processes, organism growth and development and different diseases. The cellular formin-actin network is further fine tuned by different formin binding proteins (FNBP). Four such FNBP which were initially discovered as formin poly proline rich FH1 domain binding; were found to contain tandem WW domains as their formin interacting module. Despite the fact that WW domains are the smallest known protein module that is involved in multi-protein interactions, the interaction of formins and WW domain containing FNBP were rarely explored and their interactions under physiological contexts became doubtful. However different studies continuously provided indications of greater cellular impacts of these interactions. We have used computational techniques (homology modeling and molecular docking) to investigate the ligand binding, especially formin binding properties of human FNBP4 WW domains. We have found that the triple  $\beta$  sheet structure of the 1<sup>st</sup> WW domain of FNBP4 can bind to the proline rich FH1 region of formin mDia1. However the FNBP4 second WW domain was found to be unable to bind to mDia1 FH1 region. Detailed investigation showed the importance of sequence variations of FNBP4 WW domains in specifying their interactions with formins.

**Keywords:** Formin; Formin binding protein; Homology modeling; Docking; WW domain.

**Introduction**

Formin mediated dynamic regulation of actin cytoskeleton is involved in cellular processes like filopodia formation, cellular transport, signal transduction and many more (Goode and Eck, 2007; Chesarone *et al.*, 2010; Paul and Pollard, 2009; Mellor, 2010; Young and Copeland, 2010). The physiological impacts of this formin-actin interplay have well established effects on organ development, organism growth and survival and different diseases (Liu *et al.*, 2010; DeWard *et al.*, 2010). This formin-actin network, which spans across all groups of eukaryotes i.e. plants, fungi, invertebrates and vertebrates (Chalkia *et al.*, 2008;

Blanchoin and Staiger, 2010), is further functionally regulated by a group of proteins known as formin binding proteins (FNBP) (Aspenström, 2010). Unlike actin binding proteins (ABP) which directly regulate actin (Pollard and Cooper, 1986), FNBP primarily affect the formin mediated actin assembly by regulating formins (Aspenström, 2010). While affecting the cellular actin cytoskeleton is one of the many implications of FNBP – formin interaction, other important functions of FNBP are also known to exist (Aspenström, 2010). However the FNBP classification is based on the outcome of their interactions with formins. Till date the FNBP are primarily categorized into four groups – 1. Profilin like FNBP that directly affect the rate of actin polymerization by formins, 2. FNBP regulating the state of activation of formins (example Small GTPases like RhoA), 3. FBP17 or BAR domain containing FNBP that change the localization of

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formins and 4. FNBP<sub>s</sub> that physically link formins to other nucleators (example. IQGAP<sub>s</sub>) (Aspenström, 2010). As different FNBP<sub>s</sub> regulate formins in different ways, their corresponding formin interacting regions are also different. RhoA interacts with the N terminal regions of formins (Higgs, 2005) while Dishevelled interacts with the C terminal portions (Liu *et al.*, 2008). On the other hand FBP17, profilin is known to interact with the proline rich formin homology 1 (FH1) region. This proline rich FH1 region, which is located immediately at the N terminal to the formins characteristic formin homology 2 (FH2) domain, played the most important role in the discovery of FNBP<sub>s</sub> in the initial stages of formin science.

Philip Leder and his group used the standard pull down assay with an affinity tagged mouse formin poly proline region to pull down proteins interacting with formins, especially to the proline rich FH1 region (Chan *et al.*, 1996). Their study led to the discovery of a series of FH1 interacting proteins (generally termed as formin binding proteins) which were later found to have distinct cellular functions (Bedford *et al.*, 1998). However one common feature among all these FH1 interacting FNBP<sub>s</sub> is that all of them were found to contain either WW domain or Src homology 3 (SH3) domain as their formin interacting module (Chan *et al.*, 1996). Both WW and SH3 domain are known to bind to proline rich ligands (Salah *et al.*, 2012; Morin *et al.*, 2003). Further characterization led to the findings that identified BAR (Bin1, Amphiphysin and Rvs167) domain containing FNBP<sub>s</sub> (like FBP17) possess SH3 domains and their functions were further characterized in details (Morin *et al.*, 2003; Takano *et al.*, 2008). However the formin interacting properties of WW domain containing FNBP<sub>s</sub> (WW-FNBP<sub>s</sub>) have not been documented in details. The four proteins, that are members of this WW-FNBP category are WW domain containing protein 4 (WBP4, also known as FBP21), Pre-mRNA processing factor 40 (Prp40, also known as FBP11), Transcription elongation regulator 1 (TCERG1, also known as Ca150 / FBP28) and Formin binding protein 4 (FNBP4, also known as FBP30). Among these 4 proteins, Prp40 and WBP4 have been further characterized to be a member of eukaryotic splicing machinery (Morris and

Greenleaf, 2000; Klippe *et al.*, 2011) while Ca150 is reported to be associated with eukaryotic transcription regulation (Smith *et al.*, 2004). All these three WW-FNBP<sub>s</sub>, all of which contain two WW domains (as tandem WW domains) are known to mediate multi-protein interactions via their tandem WW domains during transcription and transcript processing.

The fourth WW-FNBP protein i.e. FNBP4 remains to be the only WW-FNBP which is hardly characterized. Depraetere and Goldstein (1999) reported that this protein is transcriptionally up-regulated in a p53 dependent manner during  $\gamma$ -irradiation induced cellular stress. Several studies that investigated the changes and modifications of global cellular proteome during important cellular events like mitosis, T-Cell receptor (TCR) signaling, cellular differentiation have found this protein to be post-translationally modified (phosphorylated) (Olsen *et al.*, 2006; Dephoure *et al.*, 2008; Mayya *et al.*, 2009; Rigbolt *et al.*, 2011). Due to lack of further evidences, FNBP4 is yet to be structurally, biochemically and functionally characterized. In this context, we have found that FNBP4 class of proteins is eukaryotic in origin with a clear trend of evolution separating the plants from animals (unpublished data). We have also found that presence of tandem WW domain is the most common domain architecture of FNBP4 across different taxonomic groups. Due to the lack of scientific evidences, the formin binding ability of WW-FNBP<sub>s</sub> (under physiological context) became questionable in recent years. In this context, to enlighten the scientific basis of the FNBP4 – formin interaction, we have used molecular docking techniques to investigate the formin binding property of human FNBP4 WW domains. Our findings report the differential abilities of the human FNBP4 WW domains to interact with the poly proline ligands of formin mDia1 FH1 region (human genome encodes 15 different formins (Schönichen and Geyer, 2010), mDia1 is the best characterized among all of them).

## Materials and Methods

The models of the human FNBP4 1<sup>st</sup> and 2<sup>nd</sup> WW domains (1<sup>st</sup> WW: 219-246 amino acids; 2<sup>nd</sup> WW: 598-627 amino acids) were previously modeled

using homology modeling using the following structures as templates – **1O6W** and **2DMV** (corresponding PDB IDs) respectively. The models of the proteins were energy minimized in two steps. In first step the steepest decent technique was used and in the next step conjugate gradient technique was used to minimize the overall structure of the three models using the Discovery studio software until the structures reached the final RMS gradient of 0.0001kcal / mole. All energy minimization were done using CHARMM force field and fixing backbone of proteins. The stereo-chemically fit structures checked using ProCheck (for Ramachandran plot), Errart score and Verify\_3D through SAVES server (Lüthy *et al.*, 1992; Bowie *et al.*, 1991; Colovos and Yeates, 1993) were used for docking studies. The only available ligand structure of any formin is of formin mDia1 (PDB ID: **2V8F**). The ligand poly proline region was extracted from the reported structure using Molegro molecule viewer (CLC Bio, Qiagen). Finally ligand interactions with human FNBP4 WW domain models were studied with this extracted ligand structure. First a rigid body docking was performed by docking the ligand to the modeled WW domains of human FNBP4 via Z-DOCK server (Pierce *et al.*, 2011). Since the ligand contains structural constrains from its template structure (**2V8F**, a X ray diffraction mediated solved structure), there could be possible structural limitations that interferes with the best possible interaction between the receptor WW domain and the formin poly proline ligand. Thus the suitable Z-DOCK outputs were further fine tuned via the Flexpepdock server (London *et al.*, 2011) to maximize the docking parameters by removing the structural constrains of the ligand. Flexpepdock outputs were analyzed by Discovery studio client 3.5 (Accelrys) and Protein interaction calculator server (Tina *et al.*, 2007).

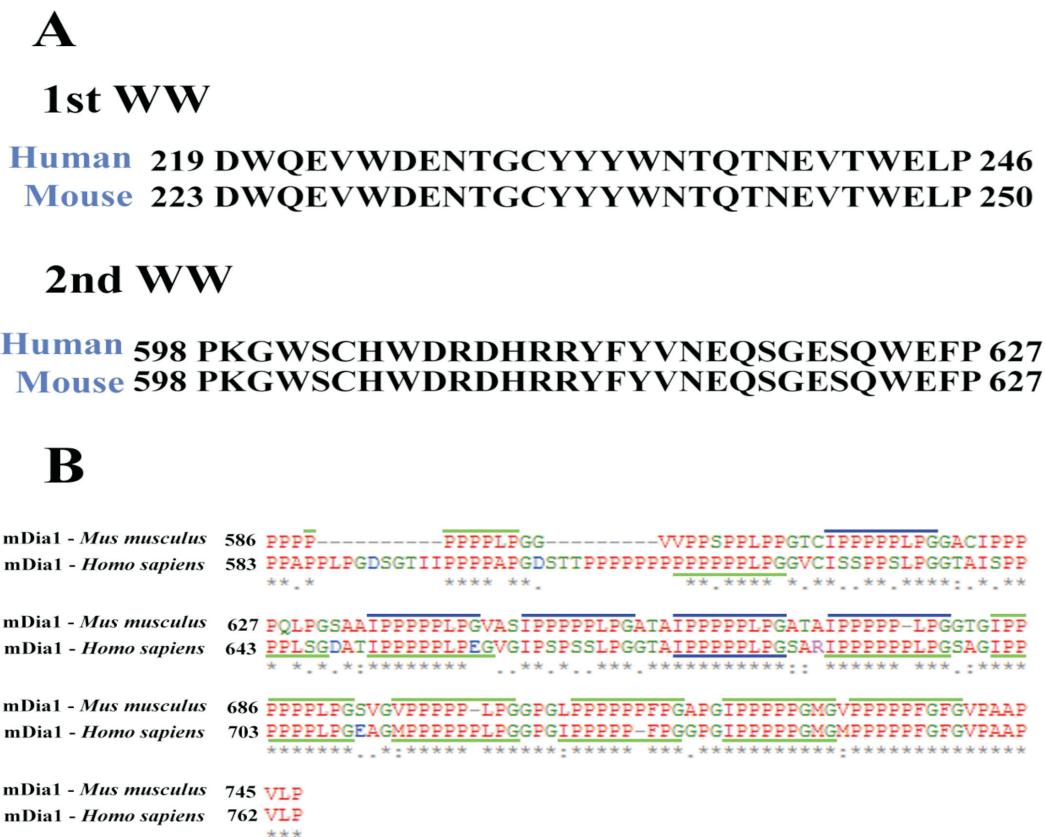
## Results and Discussions

To understand the formin binding properties of human FNBP4 we have used our previously modeled structures of the two WW domains of this protein (unpublished data). As a ligand, we were restricted to the use of mouse mDia1 FH1 region sequence IPPPPPLPG (647 to 655 amino acids) as till date this is the only formin FH1

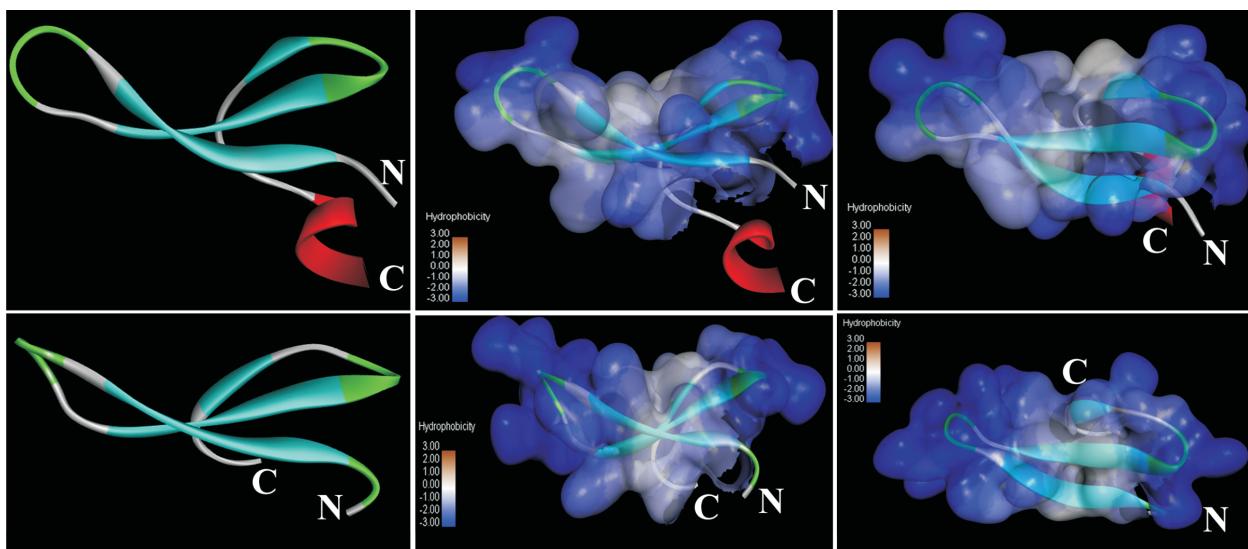
region whose structural co-ordinates are available. However mDia1 is the best characterized formin among all the known formin proteins and this protein have been characterized across many organisms where it was found to have similar conserved functions. Moreover the poly proline ligand i.e. the PPPP or longer proline stretch is a common sequence of almost all the formin FH1 region and this 9 amino acids long stretch is sufficient to cover the small span of WW domains ligand binding grooves. So this mDia1 FH1 region ligand sequence is structurally suitable enough to conduct our intended docking studies. To validate the suitability of cross-species ligand receptor combinations, we have searched the WW domain sequences of human and mouse FNBP4 proteins (Uniprot ID: Q8N3X1 and Q6ZQ03 respectively) and found them to be exactly similar (Figure 1A) and also compared the FH1 regions of human and mouse mDia1 (Uniprot ID: O60610 and O08808 respectively) by sequence alignment (Figure 1B). The FH1 region sequence alignment of these two species shows presence of amino acids stretches with exact ligand sequences (Figure 1B blue highlighted regions) as well as sequences with slight mutations (Figure 1B green highlighted regions). Since both the ligand and receptor sequences are same for both the species, we have continued our docking studies with the human FNBP4 WW domains as receptors and mouse mDia1 FH1 sequence as ligand.

### Description of the ligand binding region of the 1<sup>st</sup> and 2<sup>nd</sup> WW domain of human FNBP4

The ligand binding groove of human WW domains are situated at the top concave surface formed by the triple  $\beta$  sheets of a WW domain (Salah *et al.*, 2012). In case of our receptor domains, both of them appeared to have more or less similar ligand binding groove (Figure 2) despite their large sequence variations (Figure 1A). Slight changes in the lengths of  $\beta$  sheets and their connecting  $\beta$  turns (Figure 2 left column) resulted in minute variations in their respective ligand binding grooves. Also the ligand binding surfaces of the two WW domains did not show major diversions from each other (Figure 2 middle and right column). In case of both 1<sup>st</sup> and 2<sup>nd</sup> WW domains, the connecting  $\beta$  turn comprising



**Figure 1: Sequences of human and mouse FNBP4 WW domains and mDia1 FH1 region.** (A) The WW domain containing regions of human and mouse FNBP4 1<sup>st</sup> and 2<sup>nd</sup> WW domains are exactly same (as identified by pfam (Punta *et al.*, 2012) searches). The amino acids regions comprising the corresponding WW domains are marked on either side of the sequences. (B) The poly proline rich FH1 regions of human and mouse mDia1 are presented after alignment. The conserved ligand motif i.e. IPPPPPLPG sequences are marked in blue while similar regions with some mutations are marked in green.



**Figure 2: Description of the ligand binding surface of human FNBP4 WW domains.** Upper panel: 1<sup>st</sup> WW domain, Lower panel: 2<sup>nd</sup> WW domain of FNBP4. The N and C terminal regions of each WW domain are marked with N and C, respectively. First column shows the side view of the triple  $\beta$  sheet structure of the two WW domains. The upper concave grooves of the two WW domains are the main ligand binding regions. Second and third column shows the side and top view of the ligand binding surfaces (colored according to hydrophobicity) of the WW domains. As evident from the images, the two sides of the ligand binding region which mainly harbors the connecting  $\beta$  strands (joining the  $\beta$  sheets of each WW domain) are more hydrophilic in nature (blue regions) when compared to the central region of the ligand binding surface (grey color).

regions were found to be hydrophilic in nature (blue) while the center region comprising the deepest region of the ligand binding groove appearing neutral (grey) from hydrophobicity point of view.

### **Formin FH1 binding ability of human FNBP4 WW domains**

While both the WW domains of human FNBP4 appeared to bear similar surfaces and ligand binding grooves, their ligand binding abilities were found to differ significantly. The 1<sup>st</sup> WW domain was found to successfully bind to the mouse mDia1 ligand via its ligand binding groove, the 2<sup>nd</sup> WW always failed to dock with the ligand through its ligand binding groove. The 2<sup>nd</sup> WW domain was not even selected by the docking programs as a potential site of interaction even when we used the amino acids residues of the ligand binding region of the receptor (to restrict the docking algorithm from searching elsewhere of the receptor). This finding proved that despite having overall similar structural appearances, WW domains pose significant structural constraints which are essential for binding to their specific ligands. These variations in ligand choices, although all are proline rich, are the basis of WW domains classification (Salah *et al.*, 2012). These finding also proves that the two WW domains of human FNBP4 are different in nature (both sequence wise and functionally), a common feature of proteins with two or more WW domains.

To understand the basis of such discrimination of ligand (mDia1 FH1 ligand) by the two WW domains of human FNBP4 we analyzed the 1<sup>st</sup> WW domain's docked complex in details. To maximize the docking parameters of this complex, we first tried to eliminate out the structural constraints of the ligand molecule (from its previous structure) through Flexpepdock. The best output of the Flexpepdock docking (Figure 3A) was analyzed further. Details of hydrogen bonds (H-bonds) and other hydrophobic interactions between the ligand and receptor WW domain is mentioned in details in Table 1. The receptor (1<sup>st</sup> WW domain) – ligand complex formed 3 hydrogen bonds (H-bonds) (green dashed lines in Figure 3B) which were found to

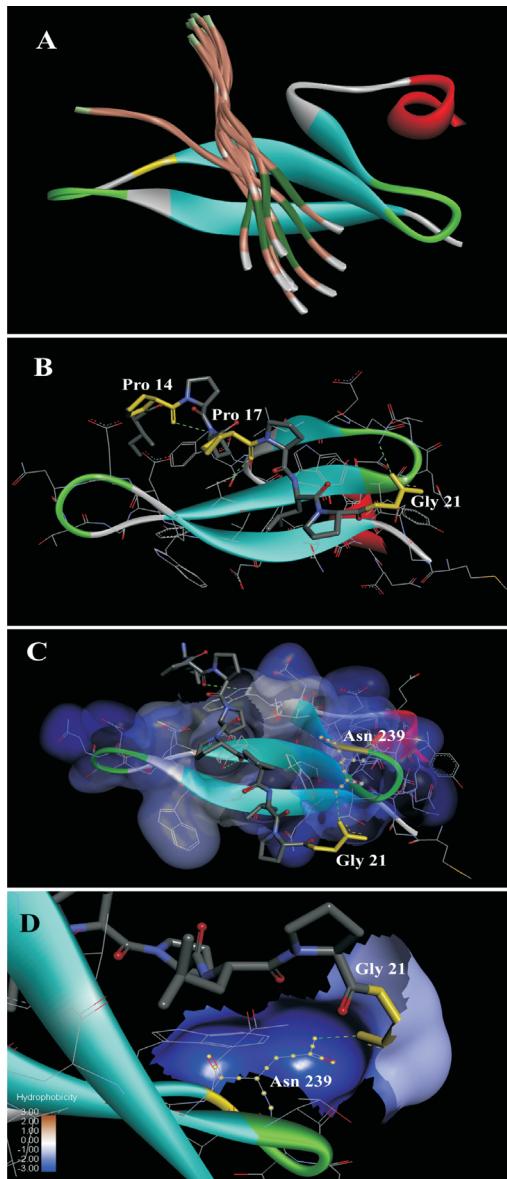
**Table 1**

**Details of interactions between the FNBP4 1<sup>st</sup> WW domain and mDia1 FH1 region proline rich ligand. The H-bonds and other hydrophobic interactions are mentioned in details with the interacting residues of receptor and ligands (R – receptor, L – ligand) and interaction distances. In case of H-bonds, concerned bond energies are also mentioned**

Interacting Atoms	Length (Å)	Energy (kCal)
<b>H bonds:</b>		
R-TYR232: OH – L-PRO17: O	2.48	-1.53
R-ASN239: ND2 – L-GLY21: O	2.67	-2.5
R-TRP243: NE1 – L-PRO14: O	3.5	-0.3
<b>Hydrophobic Interactions:</b>		
R:TYR232:CD2 - L:PRO16:CB	2.15	
R:TYR232:CD2 - L:PRO16:CG	1.9	
R:TYR232:CE2 - L:PRO16:CB	2.13	
R:VAL241:CG2 - L:PRO16:CG	2.28	

be crucial for stabilization of this complex (Figure 3B). Among these three H-bonds, the one formed between 239 Asn (of receptor WW domain) and 655 Gly (of ligand) proved to be the most important H-bond (Figure 3C). It also had the smallest distance and maximum energy among all the three H-bonds. Docking with ligand sequence after removal of 655 Gly severely impacted the stabilization of the docked complex. This particular H-bond formed between the 239 Asn which provides a hydrophilic surface (Blue surface of Figure 3D) at the β turn between 2<sup>nd</sup> and 3<sup>rd</sup> β sheet of the receptor WW domain, showed the importance of specific variation of sequences in receptor WW domains and ligand poly proline regions (in the form of 655 Gly). A closer look at the FH1 poly proline stretches of both human and mouse mDia1 (Figure 1B blue and green highlighted regions) shows the presence of a conserved Gly residue after almost all the proline stretches, separated by 2 or 3 amino acids.

While the ligand was same for both WW domains of human FNBP4, it's the specific amino acids that form the crucial bond interactions that stabilize the ligand-receptor complex. It is the 1<sup>st</sup> WW domain of human FNBP4 that harbors these specific residues and thus binds of mDia1 FH1 ligand while absence of such residues limits the



**Figure 3: Docking of formin mDia1 poly-proline rich FH1 region with the 1<sup>st</sup> WW domain of human FNBP4.** The 1<sup>st</sup> WW domain of human FNBP4 successfully docked with the formin mDia1 poly proline rich region. (A) The Flexpepdock output shows the flexible docking of the ligand (to the FNBP4 1<sup>st</sup> WW domain) to maximize the docking qualities. Top 10 poses of the ligand are shown. Analyses of the docking parameters were done with the lowest energy docked structure. (B) The three hydrogen bonds (green dashed lines) that were formed between the ligands and the receptor WW domain are shown here. The corresponding H-bond forming amino acids of the ligand are highlighted in yellow (see text for details). (C) The participating amino acids (Gly for ligand and Asn for receptor) that formed the strongest H bond which played the most important role in stabilizing the interaction between the ligand and the receptor WW domain are highlighted in yellow. (D) Importance of sequence composition of WW domains in ligand interactions are shown for the above mentioned amino acids. The Asn residue which creates a hydrophilic surface (blue) at the β turn between the 2<sup>nd</sup> and 3<sup>rd</sup> β sheet forms a H bond with the neutral Gly residue of the ligand (grey surface)

human FNBP4 second WW domain from binding to mDia1 FH1 region. While this study highlights the ability of human FNBP4 1<sup>st</sup> WW domain to bind to the FH1 region of mDia1, it also opens up areas to investigate about the ligand choices of the 2<sup>nd</sup> WW domain of human FNBP4. Moreover this study also confirms the previously known fact that tandem WW domain containing proteins often contain WW domain with different ligand preferences which in turn widens the interactome of such proteins. Overall this study, for the first time, investigates the formin binding property of FNBP4 from structural point of view as well as highlights the difference of human FNBP4 WW domains from one functional point of view.

## Conclusions

WW-FNBPs were the first ever discovered FNBPs. However studies focusing their interaction with formin were never well reported. In this first ever report addressing this fundamental issue, we have proposed that the human FNBP4 1<sup>st</sup> WW domain can bind to the poly proline rich FH1 region of mDia1. The binding motif IPPPLPG is the most common amino acids stretch for both mouse and human mDia1 (Uniprot ID: O08808 and O60610 respectively) FH1 region. While this region is well conserved in mouse and occurs multiple times in the mouse mDia1 FH1 region, in case of human it is a bit mutated but keeps the main IPPPPP and last Gly residue intact (with mutations or insertions at the intermediate region). Mouse and human FNBP4 WW domains which are composed of exactly similar amino acid residues (Figure 1A) remove the species specific barrier to undertake this study. While structurally and hydrophobic surface wise both the 1<sup>st</sup> and 2<sup>nd</sup> WW domain of human FNBP4 appeared similar, sequence variation led to differences in their interactions with this mDia1 poly proline ligand. As a result the 1<sup>st</sup> WW domain was able to successfully dock with the mouse mDia1 FH1 region while the 2<sup>nd</sup> WW failed to do so. These results led to the conclusion that the human FNBP4 which was discovered as a formin poly proline rich FH1 region interacting protein, actually mediates its interaction with formins (at least for formin mDia1) via its 1<sup>st</sup> WW domain.

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### Abbreviations

FNBP/FBP, Fomin binding protein; WW-CP, WW domain containing protein; WW-FNBP, WW domain containing formin binding protein; ABP, Actin binding protein.

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