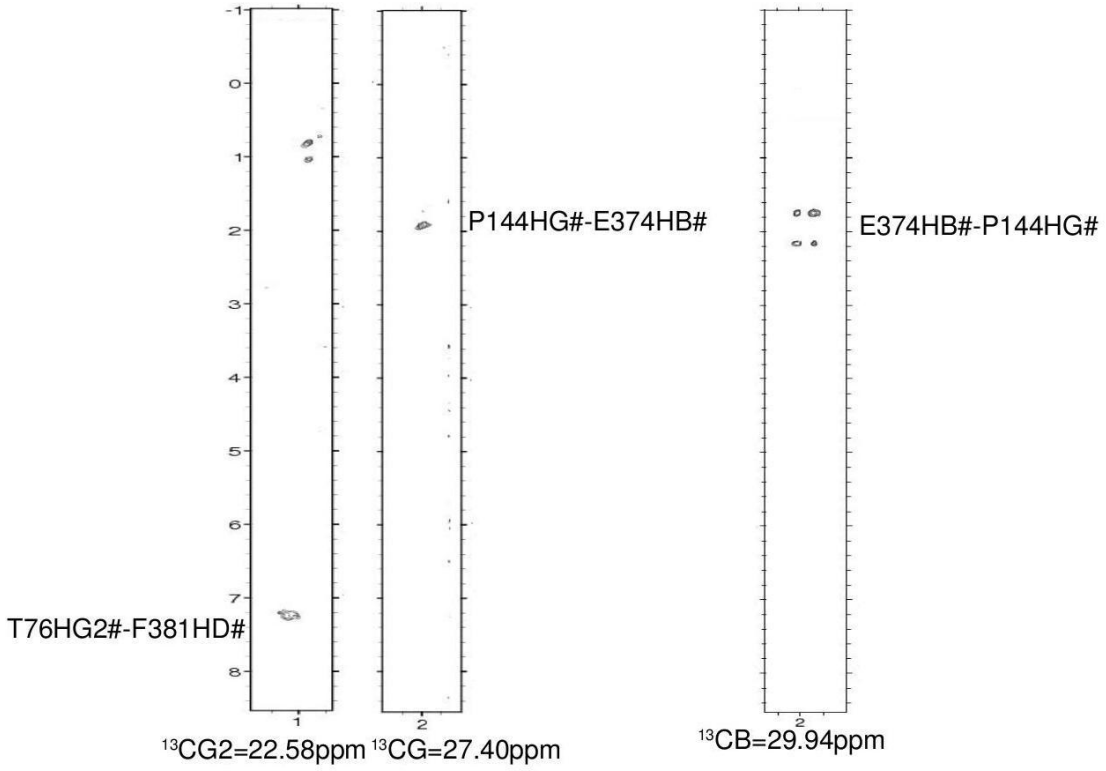
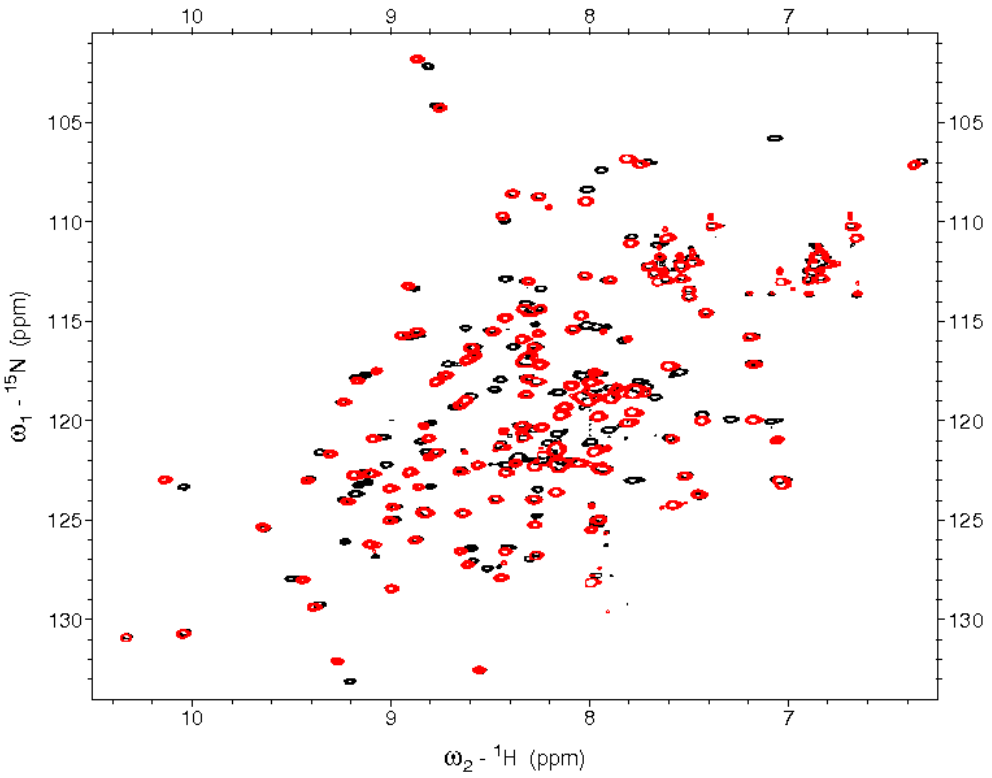
Solution Structure of the PTB Domain of Human Tensin2 in Complex with DLC1 Peptide Reveals a Novel Peptide Binding Mode

Supplementary Information



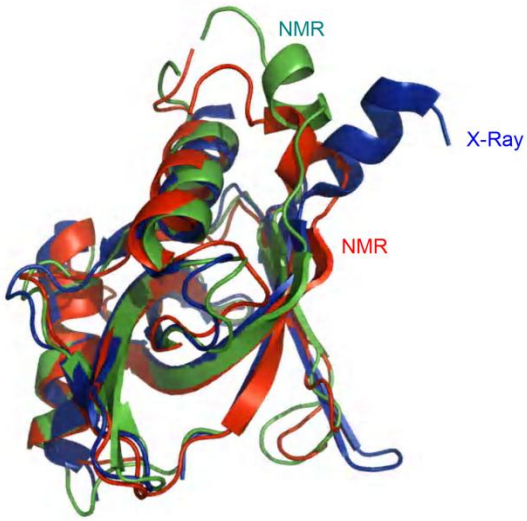
Supplementary Figure 1.

Intermolecular NOEs between PTB and DLC1. The left two strips are of a 3D F_1 $^{13}C,^{15}N$-filtered, F_2 ^{13}C-edited NOESY-HSQC spectrum (150 ms mixing time) of a sample containing $^{13}C,^{15}N$-labeled PTB and unlabeled DLC1 showing NOEs from the PTB (T76, P144) to the DLC1 (E374, F381). The right strip is from the same experiment of a sample containing ^{13}C, ^{15}N-labeled DLC1 and unlabeled PTB showing NOEs from the DLC1 (E374) to the PTB (P144).



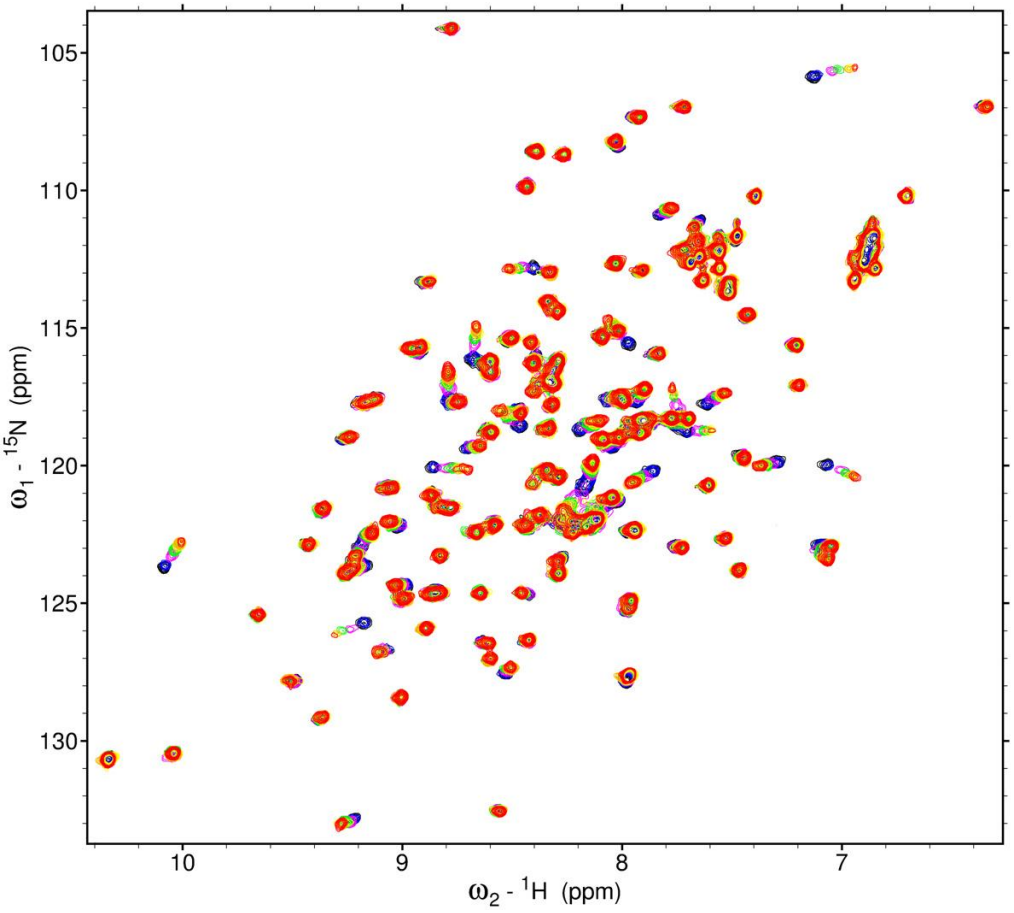
Supplementary Figure 2.

The two-dimensional ^{1}H-^{15}N HSQC spectrum of the wild-type PTB (black) and F151A mutant (red). The similarity of these two spectra indicates that the tertiary structure of the PTB F151A mutant is not affected by the mutation.



Supplementary Figure 3.

Comparison with the PTB structures of the same molecule deposited in the PDB. Blue: X-ray, (3HQC); green: NMR (2DKQ); red: NMR presented in this study. The orientation of the N-terminal -helix displays differences among the x-ray and NMR structures obtained for apo- and complexed forms of the PTB domain of Tensin2.



Supplementary Figure 4.

The overlaid full ^{1}H-^{15}N HSQC spectra of ^{15}N labeled PTB in free form (*black*) and titrated with non-labeled DLC1 peptide at different molar ratios: 1:1 (*blue*), 1:2 (*magenta*), 1:3 (*green*), 1:4 (*yellow*), 1:5 (*orange*) and 1:6 (*red*). Figure 1D is a zoom in section of these spectra.

Supplementary Materials and Methods

NMR spectroscopy

All of the NMR experiments were performed on protein samples in buffer (50 mM sodium phosphate, 100 mM NaCl, 1 mM EDTA, pH 7.0) with 10% D_2O for lock purpose. The backbone and side chain ^{1}H, ^{13}C and ^{15}N resonances were assigned first on free PTB domain and DLC1 based on triple-resonance experiment spectra of HNCACB, CBCA(CO)NH, HNCO, HBHA(CO)NH, H(CCO)NH, C(CCO)NH, HCCH-COSY and HCCH-TOCSY (1,2,3). The initial backbone assignment was generated by MARS (4) and manually checked in combination with ^{15}N-edited 3D NOESY. Distance restraints were obtained from ^{15}N- or ^{13}C-edited 3D NOESY (1,2,3) spectra and two-dimensional NOESY (mixing time 150ms). The same experimental procedure was performed on either uniformly ^{15}N, ^{13}C-labeled PTB domain with/without unlabeled DLC1 or uniformly ^{15}N, ^{13}C-labeled DLC1 with/without unlabeled PTB domain at a ratio 1:2 between ^{15}N, ^{13}C-labeled and unlabeled components. To identify intermolecular contacts between the PTB domain and DLC1 peptide, intermolecular NOE distance restraints for the complex calculation were obtained from $^{15}N/^{13}C$-edited ^{15}N, ^{13}C-filtered 3D NOESY (5) acquired under identical conditions on a Varian Unity Inova 500 MHz spectrometer(mixing time 150ms). All other spectra were collected on a Varian Unity Inova 750-MHz spectrometer. All spectra were processed using NMRPipe (6,7) and analyzed using SPARKY 3 (Goddard and Kneller, University of California, San Francisco). For titration experiments, the ^{15}N-labeled PTB domain was prepared in 0.2 mM, and DLC1 was prepared in 3 mM, under identical buffer conditions. The PTB domain was titrated by stepwise addition of 1:0.5–1:6 molar equivalents of DLC1. Chemical shift perturbations were monitored from a series of 2D ^{1}H-^{15}N Heteronuclear single quantum correlation (HSQC) spectra using a Varian Unity Inova 500 spectrometer equipped with a triple-resonance Z-gradient probe at 37^{o}C.

Structure calculation methodology

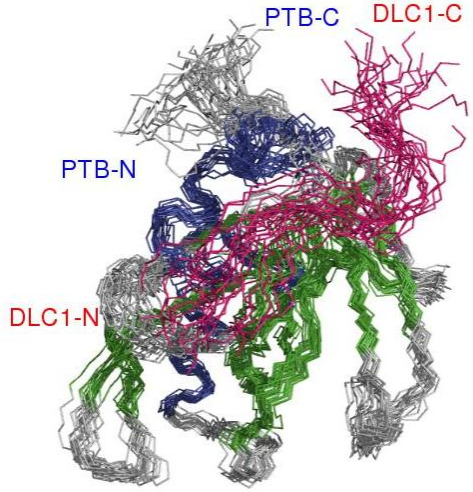
We first calculated structures of PTB domain alone in its free and DLC1-bound form using CYANA2.1 (8) and vice versa for DLC1 peptide. Next, CNS1.1 (9) was used to generate initial structures of the complex with manually assigned unambiguous intermolecular distance restraints. Then, HADDOCK-type (10) protocol without rigid body docking was used in order to do refinement. It is starting from our initial structures of the complex and refined incorporating both unambiguous (NOE, dihedral angle) and ambiguous (chemical shift perturbation) restraints, followed by high-temperature simulated annealing and molecular dynamics using explicit water solvation and full electrostatics. HADDOCK which stands for high ambiguity driven protein-protein docking is a suite of programs dedicated to docking, one of major advantages of this approach explicitly includes backbone flexibility and the movement the interface side chains in the refinement process and biochemical data can be used directly (11). It has been proven reliable and widely used to study protein-protein interactions. To compensate for the relatively low number of unambiguous intermolecular distance restraints, ambiguous

interaction restraints (AIR) (10) based on chemical shift perturbation studies were also added to improve the quality of the structures which were established based on well-known NOEs in HADDOCK1.3 program. Finally, to confirm our structure, we used site-directed spin labeling to confirm the orientation of the DLC1 peptide in the PTB domain bound complex. The introduction of paramagnetic electrons was expected to result in severe line-broadening of resonances close (<14 Å) to the spin label (12). A spin label was introduced to the DLC1 peptide by reacting MTSL ((1-oxyl-2,2,5,5-tetramethyl-Δ3-pyrroline-3-methyl) methanethiosulphonate) with a modified DLC1 peptide a free cysteine at the N- and C- terminus respectively. These sites, were chosen to provide information on the orientation of the DLC1 peptide relative to its binding partner.

Structure calculation details

NOE assignment and preliminary structure calculations were performed using the CANDID **(**13**)** module of the program CYANA2.1 (8) based on the distance restraints of either PTB domain alone in its free and DLC1-bound form or DLC1 peptide alone in free and PTB-bound form. The standard CYANA protocol of 7 iterative cycles of calculations was performed starting from a set of manually assigned NOEs. In each iterations, 100 structures started from random torsion angle values were calculated with 10,000 steps of torsion angle dynamics-driven simulated annealing. Torsion angle restraints, φ and ψ, were generated from an analysis of C′, Cα, Cβ, Hα and amide nitrogen chemical shifts by using the TALOS program (14). 378 dihedral angle restraints were used from this analysis. The final set of NOE restraints together with dihedral restraints from TALOS (14) were recalculated in CNS1.1 (9).

Initial 200 structures of the PTB domain/DLC1 peptide complex were calculated by using 1802 intraprotein, 18 intrapeptide, 8 protein–peptide NOE constraints by using a simulated annealing protocol with the program CNS1.1 (9), as shown in the following figure. The 120 top scoring complex structures from CNS were further refined with additional chemical shift perturbation data using program HADDOCK 1.3 (10). Two stages of calculations (semi-flexible simulated annealing, and a final refinement in water) were executed sequentially. The calculations were performed with ambiguous interaction restraints (AIR) derived from the chemical shift perturbation, unambiguous intra- and intermolecular NOE restraints and dihedral angle restraints.



An ensemble of 20 lowest energy structures calculated by using 1802 intraprotein, 18 intrapeptide, 8 protein–peptide NOE constraints by using a simulated annealing protocol with the program CNS1.1.

For AIR calculations, only residues that exhibited significant chemical shift changes upon titration (>0.04 ppm) and solvent accessibility over 50% determined by the program NACCESS (15) were defined as active residues. Passive residues were selected from neighboring active residues that have solvent accessibility of over 40%. Active residues for PTB domain included A19, D20, L22, R23, Q24, A27, C28, S29, K69, S71, A72, Q73, T76, H89, L139, D140, D142, Q143, A147, V155, L157, G158 and passive residues included M15, S16, T17, P63, V65, H67, R88, P91, D104, R106, W107, G112, T113, T114, S115, Q159, R160. Active residues for DLC1 peptide included the E374, D375, H376, K377, P378, G379, T380, F381, P382, K383, A384, L385 and passive residues T386, N387 were defined from the biological mutational data (16) and residues which give the intermolecular NOEs following the description in HADDOCK1.3. The HADDOCK 'interface' (residues allowed to move during the semi-flexible simulated annealing) was defined as all active and passive residues plus-minus 2 sequential residues.

DLC1 were allowed to be fully flexible during the calculations. Residues on HADDOCK 'interface' were allowed to be semi-flexible. Complex structures were sorted according to the intermolecular interaction energy (the sum of intermolecular van der Waals and electrostatic energies and restraint energies). A family of 10 lowest-energy PTB domain/DLC1 peptide structures in which none showed dihedral-angle violations >5 ° and NOE violations >0.5 Å was used for statistical analysis to represent the structure of the complex. Structures were analyzed for chirality and stereochemistry using the programs Procheck-NMR (17), MOLMOL (18). All of the figures representing the structures were generated by Pymol (http://www.pymol.org).

References for Supplementary Materials and Methods

1. Bax, A., and Grzesiek, S. (1993) Methodological Advances in Protein Nmr. *Acc. Chem. Res.* **26**, 131-138
2. Clore, G.M., and Gronenborn, A.M. (1998) Determining the structures of large proteins and protein complexes by NMR. *Trends Biotechnol.* **16**, 22-34
3. Sattler, M., Schleucher, J., and Griesinger, C. (1999) Heteronuclear multidimensional NMR experiments for the structure determination of proteins in solution employing pulsed field gradients. *Prog. Nucl. Magn. Reson. Spectrosc.* **34**, 93-158
4. Jung, Y.S., and Zweckstetter, M. (2004) Mars - robust automatic backbone assignment of proteins. *J. Biomol. NMR* **30**, 11-23
5. Zwahlen, C., Legault, P., Vincent, S.J.F., Greenblatt, J., Konrat, R., and Kay, L.E. (1997) Methods for measurement of intermolecular NOEs by multinuclear NMR spectroscopy: Application to a bacteriophage lambda N-peptide/boxB RNA complex. *J. Am. Chem. Soc.* **119**, 6711-6721
6. Delaglio, F., Grzesiek, S., Vuister, G.W., Zhu, G., Pfeifer, J., and Bax, A. (1995) Nmrpipe - a Multidimensional Spectral Processing System Based on Unix Pipes. *J. Biomol. NMR* **6**, 277-293

7. Zhu, G., and Bax, A. (1992) Improved Linear Prediction of Damped Nmr Signals Using Modified Forward Backward Linear Prediction. *J. Magn. Reson.* **100**, 202-207
8. Guntert, P., Mumenthaler, C., and Wuthrich, K. (1997) Torsion angle dynamics for NMR structure calculation with the new program DYANA. *J. Mol. Biol.* **273**, 283-298
9. Brunger, A.T., Adams, P.D., Clore, G.M., DeLano, W.L., Gros, P., Grosse-Kunstleve, R.W., Jiang, J.S., Kuszewski, J., Nilges, M., Pannu, N.S., Read, R.J., Rice, L.M., Simonson, T., and Warren, G.L. (1998) Crystallography & NMR system: A new software suite for macromolecular structure determination. *Acta Crystallogr., Sect. D: Biol. Crystallogr.* **54**, 905-921
10. Bonvin, A.M.J.J., Dominguez, C., and Boelens, R. (2003) HADDOCK: A protein-protein docking approach based on biochemical or biophysical information. *J. Am. Chem. Soc.***125**, 1731-1737
11. van, D.M., van Dijk, A.D., Hsu, V., Boelens, R., and Bonvin, A.M.J.J. (2006) Information-driven Protein-DNA Docking using HADDOCK: it is a matter of flexibility. *Nucl Acids Res.* **34**, 3317-3325
12. Battiste, J.L., Wagner, G. (2000) Utilization of site-directed spin labeling and high-resolution heteronuclear nuclear magnetic resonance for global fold determination of large proteins with limited nuclear overhauser effect data. *Biochemistry* **39,** 5355–5365
13. Herrmann, T., Guntert, P., and Wuthrich, K. (2002) Protein NMR structure determination with automated NOE assignment using the new software CANDID and the torsion angle dynamics algorithm DYANA. *J. Mol. Biol.* **319**, 209-227
14. Cornilescu, G., Delaglio, F., and Bax, A. (1999) Protein backbone angle restraints from searching a database for chemical shift and sequence homology. *J. Biomol. NMR* **13**, 289-302
15. Hubbard, S.J., and Thornton, J.M. (1993) 'NACCESS', Computer Program, Department of Biochemistry and Molecular Biology, University College London
16. Kawai, K., Kitamura, S., Maehira, K., Seike, J., and Yagisawa, H. (2010) START-GAP1/DLC1 is localized in focal adhesions through interaction with the PTB domain of tensin2. *Adv. Enzyme Regul.* **50**, 202-215
17. Laskowski, R.A., Rullmann, J.A.C., MacArthur, M.W., Kaptein, R., and Thornton, J.M. (1996) AQUA and PROCHECK-NMR: Programs for checking the quality of protein structures solved by NMR. *J. Biomol. NMR* **8**, 477-486
18. Koradi, R., Billeter, M., and Wuthrich, K. (1996) MOLMOL: A program for display and analysis of macromolecular structures. *J. Mol. Graphics Modell.* **14**, 51-55