J Mol Model (2009) 15:91–96
DOI 10.1007/s00894-008-0377-x

ORIGINAL PAPER

# Simulations of a specific inhibitor of the dishevelled PDZ domain

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Received: 9 July 2008 / Accepted: 17 September 2008 / Published online: 25 October 2008


**Abstract** The dishevelled (Dvl) PDZ domain is believed to play an essential role in the canonical and noncanonical Wnt signaling pathways, which are involved in embryo development as well as in tumorigenesis. Also, it binds directly to frizzled (Fz) receptors. An organic molecule (NSC668036) from the National Cancer Institute small-molecule library has been identified to be able to bind to the Dvl PDZ domain. Molecular dynamics simulation was used to provide detailed analyses of the binding between them.

**Keywords** Dishevelled PDZ domain · Molecular dynamics · Protein docking

## Introduction

The PDZ domain is a common structural domain containing about 80 to 90 amino acids which is found in the signaling proteins of bacteria, yeast, plants and animals [1]. PDZ is an acronym combining the first letters of the three proteins which were first discovered to share the domain: post synaptic density protein (PSD95), Drosophila disc large tumor suppressor (DlgA), and zonula occuldens-1 protein (zo-1). PDZ domains are also referred to as DHR (Dlg homologous region) or GLGF (glycine-leucine-glycine-phenylalanine) domains.

Generally, signal transduction in biology means any process by which a cell converts one kind of signal or stimulus into another. Signaling transduction pathways provide critical cell-cell communications which are required to coordinate the activities of vast numbers of cells in every animal's life. Regulations of signaling is crucial, inappropriate activity from a given signal transduction pathway can cause devastating results. Many disease processes such as diabetes, heart disease, autoimmunity, and cancer arise from defects in signal transduction pathways, which further indicate the critical importance of signal transduction pathways.

The Wnt signaling pathway is a major route by which the cell conveys information from its exterior to the nucleus [2]. It describes a complex network of proteins most well known for their roles in embryogenesis and cancer. The name Wnt came from a combination of Wg (wingless) and Int [3]. The wingless gene had originally been identified as a segment polarity gene in Drosophila melanogaster [4]. The INT genes were originally identified as vertebrate genes near several integration sites of mouse mammary tumor virus [5]. The Wnt signaling pathway is believed to be involved in embryonic and postembryonic development as well as in tumorigenesis [6–8].

The canonical Wnt pathway describes a series of events that occur when Wnt proteins bind to cell-surface receptor of the Frizzled family, causing the receptor to activate the dishevelled (Dvl) family protein and ultimately resulting in a change in the amount of beta-catenin (a subunit of the cadherin protein complex) that reaches the nucleus. It is associated with cancers, body axis specification and morphogenic signaling, etc. Non-canonical Wnt signaling is associated with other activities, such as planar cell polarity, axon guidance, stem cells, etc. Dishevelled is a

**Electronic supplementary material** The online version of this article (doi:10.1007/s00894-008-0377-x) contains supplementary material, which is available to authorized users.

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key component of a membrane-associated Wnt receptor complex. It relays the Wnt signals from a membrane-bound receptor to downstream components and thereby plays an essential role in this signaling. The Dvl proteins are composed of an N-terminal DIX domain, a central PDZ motif, and a C-terminal DEP domain [9]. Of the three, the PDZ domain is believed to play an essential role in both the canonical and the noncanonical Wnt signaling pathways.

The PDZ domain we investigated is of mouse Dvl-1 (mDvl1) [10] residues 247-341 [11] as shown in Fig. 1. Because the structure of the Dvl PDZ domain is well studied, it is possible to use structure-based virtual ligand screening to access potential ligands. Shan et al. [11] have found an organic compound, NSC668036, which binds to the Dvl PDZ domain. They used molecular dynamics (MD) simulation to study the binding between them in detail, but their simulations were limited to 5 ns. We extend the simulation to 20 ns which allows us to reveal more interesting phenomena after the initial 5 ns period.

We study the PDZ domain because we believe an inhibitor of the Dvl PDZ domain is likely to effectively block the Wnt signaling pathway at the Dvl level [12, 13], thus making it an ideal pharmaceutical target [14, 15]. Small organic inhibitors of the PDZ domain in the Dvl might be useful in dissecting molecular mechanisms and formulating agents that target cancers or other disease in which Wnt signaling is involved. We simulate the interactions between it and the PDZ domain with MD.

MD is widely used by physicists, chemists, and biologists in different fields. It is a kind of computational simulation where atoms and molecules of the systems are allowed to interact for a period of time under the laws of physics. Comparing to in vitro experiment, MD can often provide information which is hard if not impossible to observe.

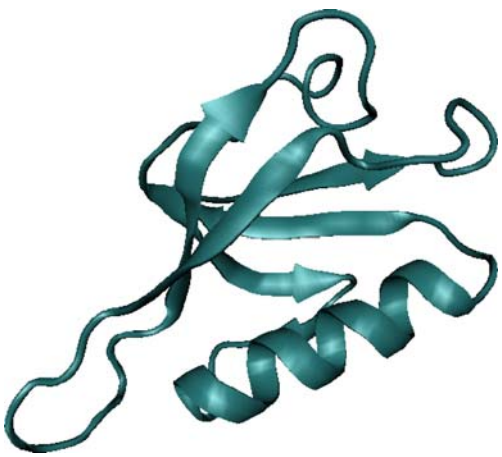


**Fig. 1** The PDZ domain

## Simulations procedures

Charge assignment for the ligand was done using the Gaussian program [16]. The MD simulations and minimizations were done using the sander program in Amber version 8 [17] using the e16 modified parm99 force field. The systems were minimized using an eight step approach: (1) Steepest descent minimization restraining the complex with a weight (force constant) of 5 kcal/mol $Å^{-2}$. (2) NPT (constant number of molecules, pressure and temperature) MD equilibration of 15 ps at 1 bar and 300 K while restraining the complex with a weight of 5 kcal/mol $Å^{-2}$. (3) Steepest descent minimization restraining the complex with a weight of 2 kcal/mol $Å^{-2}$. (4) An MD equilibration of NPT of 1.5 ps at 1 bar and 300 K while restraining the complex with a weight of 5 kcal/mol $Å^{-2}$. (5) NPT MD equilibration of 5 ps at 1 bar and 300 K while restraining the complex with a weight of 1 kcal/mol $Å^{-2}$. (6) NPT MD equilibration of 5 ps at 1 bar and 300 K while restraining the complex with a weight of 0.5 kcal/mol $Å^{-2}$. (7) NPT MD equilibration of 10 ps at 1 bar and 300 K while restraining all the carbon (including backbone alpha carbons) and nitrogen atoms with a weight of 0.5 kcal/mol $Å^{-2}$. (8) NPT MD equilibration of 5 ps at 1 bar and 300 K with no restraints.

All MD simulations were done with a time step of 1 fs and a cutoff of 8 Å for non-bonded force. The force field we used AMBER force field ff99 [18]. Particle mesh Ewald (PME) summation was used to reduce the computing need for the electrostatic interactions in force calculation. After minimization, the actual MD simulations were performed by NAMD with a time step of 2 fs and the nonbonded cutoff set to 9.0 Å at a temperature of 300 K, 1 atm of constant pressure with PME.

Our simulation was performed on the Beowulf cluster NankaiStars at Nankai University in China with 800 Intel Xeon processors running at 3.06 GHz. It was ranked 42nd in the Top500 list as of June 2004 after it was built, which has a real performance of 3.23 Tflops in sustained high performance Linpack (HPL) test with overall peak performance of 4.7 Tflops.

## Results and analysis

The simulation results were analyzed by calculating the root-mean-square deviations (RMSDs) which we calculate with only the backbone of PDZ domain and the NSC668036. It characterizes the amount by which a given selection of the molecule deviates from a defined position. The back bone of NSC668036 was defined as the 13 atoms in the main chain between and including the carbonyl carbon of the carboxylate group and the carbonyl carbon at

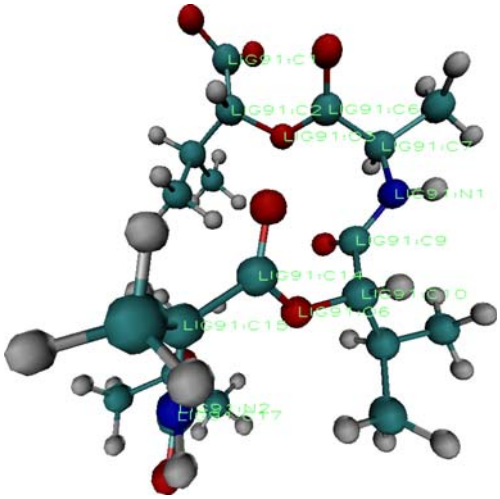


**Fig. 2** Backbone of NSC668036

the other end of NSC668036, as shown in Fig. 2. We measured the difference of the back bone of NSC668036 between the initial structure and the structure after certain period time of simulation. We also measured the difference of the back bone between different structures at the same time after a certain period time of simulation to study the convergence of all the structures.

We simulated all 30 conformational structures of NSC668036 provided by Shan et al. after we docked them to the PDZ domain. Figure 3 shows the structure of the PDZ domain with one NSC668036 molecule. The RMSD of the PDZ domain and NSC668036 for the 20 ns simulations are shown in the figures.

From these graphs, we can figure out that some of the structures are stable after the docking process, while others are not. For example, if we take a closer look at structure #10 shown in Fig. 4, we find that the ligand remains rather stable after the docking, which is evident by its low and stable RMSD.

To study the convergence of final states of the PDZ domain after the 20 ns simulation, we calculate the RMSD between each structure to find the divergence of all these structures as time evolves. Data matrix was made according to the results in Fig. 5. In which, the X and Y axes stand for the 30 different structures, and the color represents the values of the RMSD between the two structures labeled in X and Y axes.

Since the structures have zero RMSD when comparing with themselves, the diagonal elements of the matrix are zero. An island with small numbers and thus blue color in the matrix means that the relevant structures have similar conformation, and tend to converge to a similar structure, like the structures #8, 22, 27. On the contrary, an island with large numbers and thus red color in the matrix indicates that this structure does not converge well to other structures, like structures #16, 19, 28. A more accurate numerical analysis shows that the following group of structures tend to converge after the simulation: group of structures #3, 4, 5, 7; group of structures #2, 9, 10, 12; group of structures #5, 14, 17, 18, 21, group of structure #7, 8, 30.

To understand the time evolvement of the convergence, we also made the matrix at time 10 ns. A series of the matrices clearly showed the formation of a small number of islands in the matrix. From Fig. 6, the matrix at 10 ns, we can see that the number of "red-island" is significantly less than that at 20 ns, which indicates that these 30 structures tends to evolve into several groups, the differences between the groups become large as time progresses. On the other hand, the yellow areas in Fig. 6 are larger than Fig. 5, which suggests that those similar members within the groups evolve into more similar structures as time progresses.

This result can also be confirmed if we take a look for the 30 structure RMSD graphs in the supplemental materials. For example, the group of structures #3, 4, 5, 7 which we found to be converged also shows similar behavior in the RMSD plot. Their total RMSD near 20 ns all converge to a value near 2 Å.

Similar results can also be seen for other group consisting of similar structures.

Except for structure analysis, energy analysis during simulation should also be monitored. We monitored the change of Van der Waals (more accurately, the Lennard-Jones) energy and the electrostatic energy as a function of time as shown in Fig. 7 and Fig. 8. From these 2 graphs we can see that the energies are rather constant during the docking simulation process.

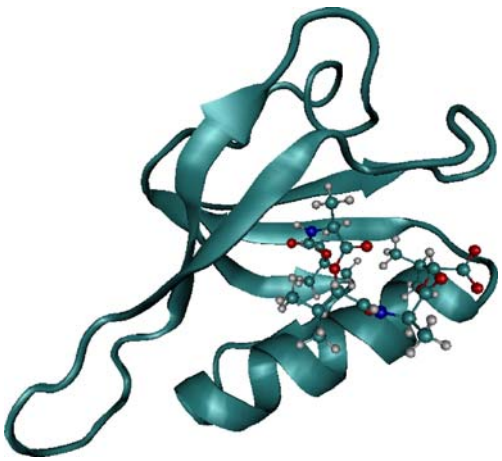


**Fig. 3** The PDZ domain with NSC668036

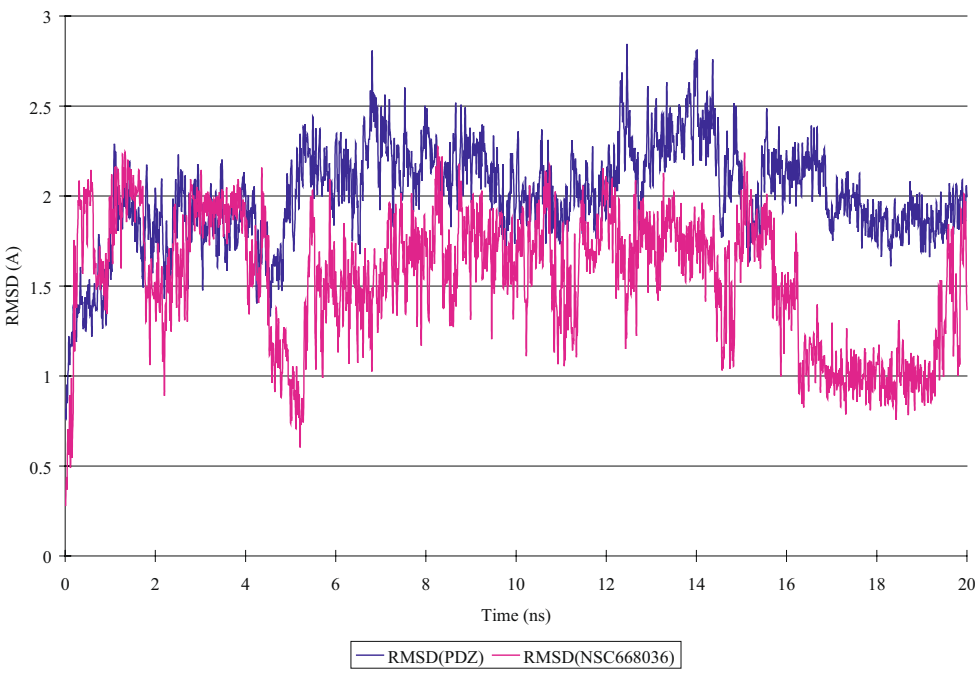



**Fig. 4** RMSD of PDZ domain and ligand #10

## Summary

We extended the simulation by Shan et al. [11] from 5 ns to 20 ns, and found many interesting properties of docking process between the PDZ domain and the NSC668036 molecule. Some RMSD graphs of the PDZ domain and NSC668036 for the 20 ns simulations, like structures #15, 25 and 30, show that there may be dramatic changes for the RMSD value after the 5 ns period. It indicates that the docking process may need a longer time to achieve the equilibrium state, thus, in the study of docking problem using molecular dynamics, a longer simulation time may be necessary.

Our simulation was performed at constant temperature and pressure with non-periodic boundary conditions. Further study could be done in periodic boundary condition with explicit water molecules. Also, AMBER force field ff99 was applied to both the ligand and the PDZ domain, for more accuracy, further simulation could be done with QM force field applied to the ligand, which should provide more interesting insights to the problem.

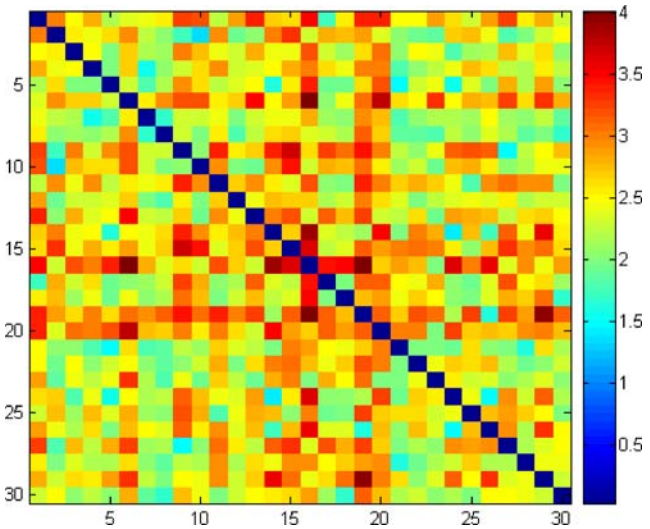


**Fig. 5** Final RMSD(Å) of PDZ domain after 20 ns simulation

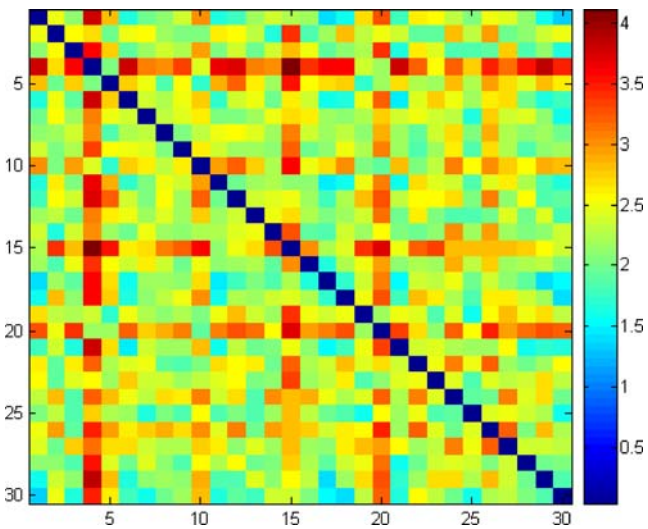


**Fig. 6** Final RMSD(Å) of PDZ domain after 10 ns simulation

**Fig. 7** Van der Waals energy

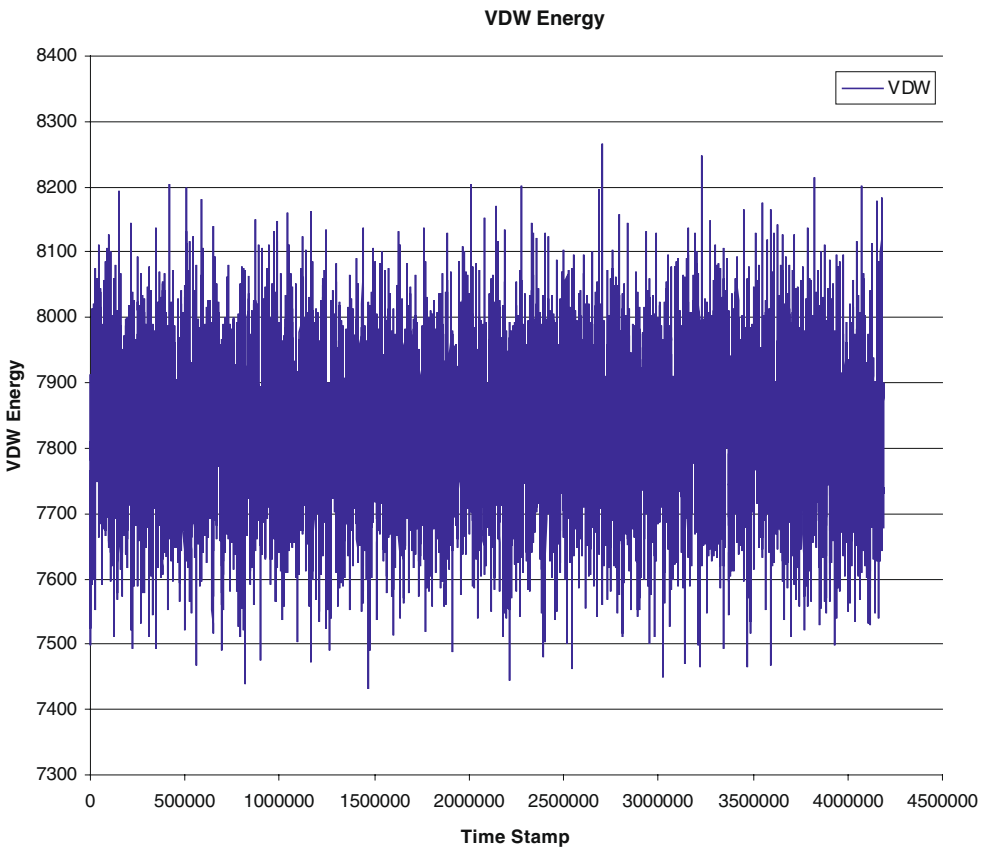
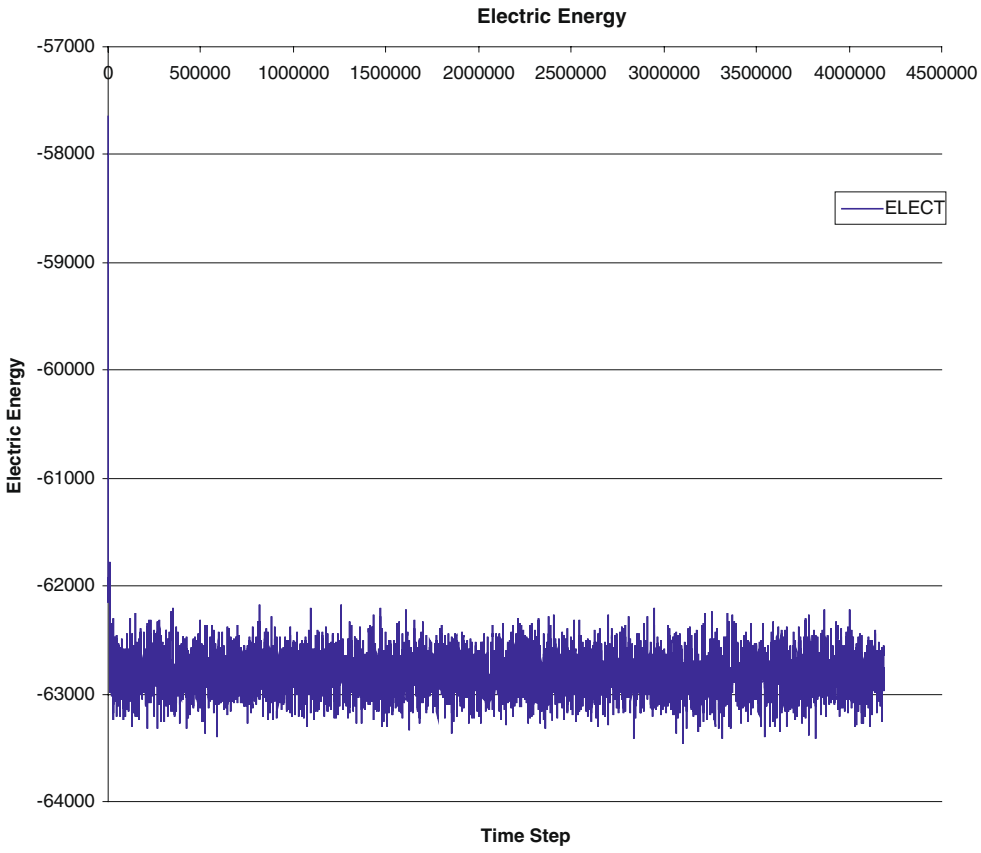



**Fig. 8** Electrostatic energy



## References


1. Ponting C (1997) Evidence for PDZ domains in bacteria, yeast, and plants. Protein Sci 6(2):464–468
2. Speesea SD, Budnik V (2007) Wnts: up-and-coming at the synapse. Trends Neurosci 30(6):268–275. doi:10.1016/j.tins.2007.04.003
3. Rijsewijk F et al. (1987) The Drosophila homolog of the mouse mammary oncogene int-1 is identical to the segment polarity gene wingless. Cell 50(4):649–657. doi:10.1016/0092-8674(87)90038-9
4. Nüsslein-Volhard C, Wieschaus E (1980) Mutations affecting segment number and polarity in Drosophila. Nature 287 (5785):795–801. doi:10.1038/287795a0
5. Nusse R et al. (1984) Mode of proviral activation of a putative mammary oncogene (int-1) on mouse chromosome 15. Nature 307(5947):131–136. doi:10.1038/307131a0
6. Polakis P (2000) Wnt signaling and cancer. Genes Dev 14:1837–1851
7. Moon RT et al. (2002) The promise and perils of Wnt signaling through β-catenin. Science 296:1644–1646. doi:10.1126/science.1071549
8. Wodarz A, Nusse R (1998) Mechanisms of Wnt signaling in development. Annu Rev Cell Dev Biol 14:59–88. doi:10.1146/annurev.cellbio.14.1.59
9. Wong HC et al. (2000) Stuctural basis of the recognition of the dishevelled DEP domain in the Wnt signaling pathway. Nat Struct Biol 7:1178–1184. doi:10.1038/82047
10. Cheyette BN et al. (2002) Dapper, a Dishevelled-associated antagonist of â-catenin and JNK signaling, is required for notochord formation. Dev Cell 2:449–461. doi:10.1016/S1534-5807(02)00140-5
11. Shan J et al. (2005) Identification of a Specific Inhibitor of the Dishevelled PDZ Domain. Biochemistry 44(47):15495–15503. doi:10.1021/bi0512602
12. Brown JD, Moon RT (1998) Wnt signaling: Why is everything so negative? Curr Opin Cell Biol 10:182–187. doi:10.1016/S0955-0674(98)80140-3
13. Potter JD (1999) Colorectal cancer: Molecules and populations. J Natl Cancer Inst 91:916–932. doi:10.1093/jnci/91.11.916
14. Giles RH, van Es JH, Clevers H (2003) Caught up in a Wnt storm: Wnt signaling in cancer. Biochim Biophys Acta 1653:1–24
15. Kreidberg JA (1996) Gene targeting in kidney development. Med Pediatr Oncol 27:445–452. doi:10.1002/(SICI)1096-911X(199611)27:5<445::AID-MPO10>3.0.CO;2-9
16. Frisch MJ et al. (2004) Gaussian 03. Gaussian, Inc., Wallingford CT
17. Pearlman DA et al. (1995) AMBER, a computer program for applying molecular mechanics, normal mode analysis, molecular dynamics and free energy calculations to elucidate the structures and energies of molecules. Comput Phys Commun 91:1–41. doi:10.1016/0010-4655(95)00041-D
18. Case DA et al. (2004) Amber 8 Users' Manual