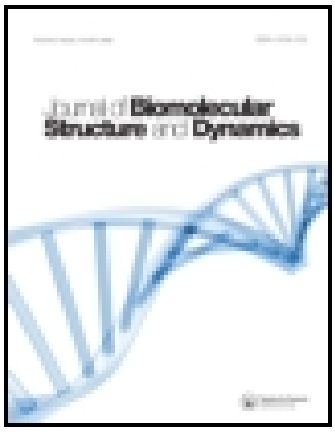

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# Comment on "Complex disruption effect of natural polyphenols on Bcl-2-Bax: Molecular dynamics simulation and essential dynamics study" by S. Verma, A. Singh and A. Mishra

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*Commentary*

# Comment on "Complex disruption effect of natural polyphenols on Bcl-2-Bax: Molecular dynamics simulation and essential dynamics study" by S. Verma, A. Singh and A. Mishra

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Bcl-2-Bax complex, Taxifolin, Quercetin, Obatoclax, Molecular dynamics simulation, post processing, trajectory imaging

## Introduction

In their recent contribution for this journal Verma et al. (Verma, Singh, & Mishra, 2014) investigated the influence of small molecules on the protein-protein complex of Bcl-2 and Bax, which is involved in apoptosis: While Bax induces this process, Bcl-2 blocks it(Adams & Cory, 1998; Reed, 2002). The authors used a computational approach, docking and molecular dynamics (MD), to study the phytochemicals taxifolin and quercetin, which possess anticancerous activity (Lee, Cha, Selenge, Solongo, & Nho, 2007; Seufi, Ibrahim, Elmaghraby, & Hafez, 2009), as well as the broad-spectrum inhibitor obatoclax, which has been reported not to disrupt the Bcl-2-Bax complex(Samuel et al., 2010). In their 12 ns MD simulations of the Bcl-2-Bax complex with a ligand bound they described dissociation events of the protein-protein complex and thus concluded that taxifolin and quercetin induce dissociation of the heterodimer, while obatoclax does not. However, an inspection of the material presented in their contribution casts doubts on the proper analysis of the raw MD data and challenges the authors' conclusions. We have severe concern that technical post-processing issues are the reason of the observed complex dissociation, and not ligand binding.

In order to support our view, we performed several MD simulations of the Bcl-2-Bax system without any ligand bound. This heterodimer is known to be a stable complex, which is also found by Verma et al. (2014) in a control simulation. We intend to show that pseudo-dissociation events occur in the raw MD data, but after proper post-processing all simulations exhibit the expected complex stability.

## Methods

A system setup very similar to that of Verma et al. (2014) was chosen. From the protein

data base(Berman et al., 2002) the initial structure of the Bcl-2-Bax complex were obtained (entry 2XA0, X-ray structure with 0.27 nm resolution(Ku, Liang, Jung, & Oh, 2011)). The system was electrically neutralized by the addition of Na+ ions and then solvated in a TIP3P(Jorgensen, Chandrasekhar, Madura, Impey, & Klein, 1983) water box with at least 1 nm to the border. Two types of boxes common in MD simulations were used here, a rectangular box and a truncated octahedral box. Figure 1 shows the fully solvated system. Like Verma et al. (2014) we performed an unrestrained energy minimization in order to relax the systems: 10,000 steps steepest decent, followed by 10,000 steps of conjugate gradient. Then, the systems were heated up to 300 K in a 300 ps simulation with positional restraints, followed by 40 ns of unrestrained simulation as NVT ensemble using a time step of 2 fs. Long-range interactions were computed via the particle-mesh Ewald method using program default settings. Coordinate snapshots were collected every 10 ps for subsequent analysis. For each of the two differently boxed systems two independent simulations were performed to achieve statistical significance.

All simulations used the parm99SB force field(Cieplak, Cornell, Bayly, & Kollman, 1995; Cornell et al., 1995; Hornak et al., 2006) from the Amber12(Case et al., 2012) suite, and the programs ptraj and cpptraj from there were utilized for post-processing the raw MD data. VMD(Humphrey, Dalke, & Schulten, 1996) was applied for visualization purposes.





## Results and Discussion

### *Bcl-2-Bax complex shows pseudo-dissociation events*

We investigated the complex between Bcl-2 and Bax, which is known from experiment to be stable(Ku et al., 2011). When visualizing the raw trajectory data from the four 40

ns MD simulations, we observed pseudo-dissociation events like those described by Verma et al. (cf Figure 11B and 12B from Verma et al. (2014)). The root mean square deviation (RMSD) of the backbone atoms in the Bcl-2-Bax complex from the initial structure over the course of the simulation is depicted in Figure 2A and shows corresponding immediate peaks in all of our simulations: the RMSD jumps from ca. 0.3 nm to ca. 2.0 nm within 10 ps. However, such instantaneous structural changes, where the RMSD jumps to values > 2.0 nm between two snapshots, are indicative for a technical artifact, because real dissociation events progress in a continuous way and would show a slower ascending of the RMSD curve.

***Periodic boundary conditions require proper post-processing***

What is the reason for the observed RMSD jumps? MD simulations use periodic boundary conditions, to mimick an infinite solution(Allen & Tildesley, 2003): a central water-filled box with the protein system is surrounded by identical boxes at all sides. In that way, solvent molecules near the box border interact with solvent molecules from the neighbouring box; otherwise, boundary effects would cause instabilities in the simulation. The periodic boundary condition thus establishes a super-system of many identical simulation boxes. When a solvent molecule leaves its original solvent box and diffuses into the neighbouring box, an image solvent molecule enters the original box from the opposite site (cf. Figure 3). This happens frequently during MD simulations and is part of all commonly used MD codes, like Amber(Case et al., 2012) or Gromacs(Van der Spoel et al., 2005), but does not affect the energetics of the entire system. As most often the water molecules are removed prior to any further analysis, these effects often occur unnoticed. However, in protein system consisting of more than one amino-acid chain this effect also happens, when a chain crosses the box border during simulation. An image chain then enters the simulation box from the opposite

side. If the MD program saves coordinate snapshots directly of the simulation box and not of the original protein chains, pseudo-dissociation events are observed, which result in instantaneous RMSD jumps, like in Figure 2A.

Common MD programs, of course, provide tools to restore the original complexes. Proper post-processing of the raw MD data from our Bcl-2-Bax simulations resulted in a smooth trajectory and a RMSD curve without discontinuities, as depicted in Figure 2B. Figure 4 shows two consecutive coordinate snapshots from a Bcl-2-Bax simulation, to clarify the structural effect of pseudo-dissociation and re-imaging.

***RMSD jumps indicate a technical issue***

The comparison between our unprocessed RMSD curves (Figure 2A) and those given by Verma et al. (cf Figure 11A, 12A, 13A of Verma et al. (2014)) for their 12 ns simulations of the Bcl-2-Bax complex bound to taxifolin, quercetin, and obatoclax, respectively, show striking similarities: the RMSD plots of all three simulations exhibit immediate jumps, which are less often in the case of obatoclax. However, with the technical MD background in mind, we have belief that the dissociation events described by Verma et al. (2014) are not caused by interaction of the small molecules, but are mere artifacts from trajectory post-processing. The fact that they did not observe such dissociation events for the Bcl-2-Bax complex without any ligand is also in accord with our conclusion, because proteins move differently in independent MD simulations. Obviously, the Bcl-2-Bax protein complex did not leave the original simulation box during their simulation of 12 ns length. In our four simulations we observed such an event at very different times: Once during the first few nanoseconds, twice around 10 ns, and once after ca. 12 ns.

In summary, in our opinion the dissociation events described by Verma et al. (2014) were of mere artificial nature and were caused by post-processing issues. Their conclusions, therefore, about the disrupting effect of the compounds are in our view not supported by their data. Although we cannot exclude any potential anti-cancerous effect of these compounds, we suggest a careful re-analysis of the raw MD data as earlier(A.H.C. Horn, 2014; A. H. C. Horn & Kahler, 2013). This might reveal some more subtle effects of taxiferol, quercetin, and obatoclax upon Bcl-2-Bax complex stability on that time scale.

**Response from Verma et al. (2014)**

The Editor-in-Chief Prof. Ramaswamy H. Sarma contacted Verma et al. for their response on this commentary, and they declined to provide this.

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**References:**


Adams, J. M., & Cory, S. (1998). The Bcl-2 protein family: arbiters of cell survival. *Science, 281*(5381), 1322-1326. doi: 10.1126/science.281.5381.1322

Allen, M. P., & Tildesley, D. J. (2003). Computer Simulation of Liquids. Oxford: Clarendon Press.

Berman, H. M., Battistuz, T., Bhat, T. N., Bluhm, W. F., Bourne, P. E., Burkhardt, K., . . . Zardecki, C. (2002). The Protein Data Bank. *Acta Crystallogr D Biol Crystallogr, 58*(Pt 6 No 1), 899-907. doi: 10.1107/S0907444902003451

Case, D. A., Darden, T. A., III, T. E. C., Simmerling, C. L., Wang, J., Duke, R. E., . . . Kollman, P. A. (2012). AMBER12. San Francisco, USA: University of California.

Cieplak, P., Cornell, W. D., Bayly, C., & Kollman, P. A. (1995). Application of the Multimolecule and Multiconformational RESP Methodology to Biopolymers: Charge Derivation for DNA, RNA, and Proteins. *J. Comput. Chem., 16*(11), 1357-1377. doi: 10.1002/jcc.540161106

Cornell, W. D., Cieplak, P., Bayly, C. I., Gould, I. R., Merz, K. M., Ferguson, D. M., . . . Kollman, P. A. (1995). A 2nd Generation Force-Field for the Simulation of Proteins, Nucleic-Acids, and Organic-Molecules. *Journal of the American Chemical Society, 117*(19), 5179-5197. doi: 10.1021/Ja00124a002

Horn, A. H. C. (2014). Letter to the Editor: Quercetin and taxifolin completely break
MDM2-p53 association: molecular dynamics simulation study
by S. Verma, A. Singh, A. Mishra. Med Chem Res 2013, 22(6)
2778–2787. *Medicinal Chemistry Research*. doi: 10.1007/s00044-014-1135-5

Horn, A. H. C., & Kahler, A. (2013). The effect of fulvic acid on pre- and postaggregation state of Abeta17-42: molecular dynamics simulation studies, S. Verma, A. Singh and A. Mishra, Biochim Biophys Acta 1834 (2013) 24-33. *Biochim Biophys Acta, 1834*(12), 2867-2868. doi: 10.1016/j.bbapap.2013.07.012

Hornak, V., Abel, R., Okur, A., Strockbine, B., Roitberg, A., & Simmerling, C. (2006). Comparison of multiple amber force fields and development of improved protein backbone parameters. *Proteins-Structure Function and Bioinformatics, 65*(3), 712-725. doi: 10.1002/Prot.21123

Humphrey, W., Dalke, A., & Schulten, K. (1996). VMD: Visual molecular dynamics. *Journal of Molecular Graphics & Modelling, 14*(1), 33-38. doi: 10.1016/0263-7855(96)00018-5

Jorgensen, W. L., Chandrasekhar, J., Madura, J. D., Impey, R. W., & Klein, M. L. (1983). Comparison of simple potential functions for simulating liquid water. *J. Chem. Phys., 79*, 926-935. doi: 10.1063/1.445869

Ku, B., Liang, C., Jung, J. U., & Oh, B. H. (2011). Evidence that inhibition of BAX activation by BCL-2 involves its tight and preferential interaction with the BH3 domain of BAX. *Cell Res, 21*(4), 627-641. doi: 10.1038/cr.2010.149

Lee, S. B., Cha, K. H., Selenge, D., Solongo, A., & Nho, C. W. (2007). The chemopreventive effect of taxifolin is exerted through ARE-dependent gene regulation. *Biol Pharm Bull, 30*(6), 1074-1079. doi: 10.1248/bpb.30.1074

Reed, J. C. (2002). Apoptosis-based therapies. *Nat Rev Drug Discov, 1*(2), 111-121. doi: 10.1038/nrd726

Samuel, S., Tumilasci, V. F., Oliere, S., Nguyen, T. L., Shamy, A., Bell, J., & Hiscott, J. (2010). VSV oncolysis in combination with the BCL-2 inhibitor obatoclax overcomes apoptosis resistance in chronic lymphocytic leukemia. *Mol Ther, 18*(12), 2094-2103. doi: 10.1038/mt.2010.188

Seufi, A. M., Ibrahim, S. S., Elmaghraby, T. K., & Hafez, E. E. (2009). Preventive effect of the flavonoid, quercetin, on hepatic cancer in rats via oxidant/antioxidant activity: molecular and histological evidences. *J Exp Clin Cancer Res, 28*, 80. doi: 10.1186/1756-9966-28-80

Van der Spoel, D., Lindahl, E., Hess, B., Groenhof, G., Mark, A. E., & Berendsen, H. J. C. (2005). GROMACS: Fast, flexible, and free. *Journal of Computational Chemistry, 26*(16), 1701-1718. doi: 10.1002/Jcc.20291

Verma, S., Singh, A., & Mishra, A. (2014). Complex disruption effect of natural polyphenols on Bcl-2-Bax: molecular dynamics simulation and essential dynamics study. *Journal of Biomolecular Structure & Dynamics*, 1-13. doi: 10.1080/07391102.2014.931823

**Captions**

Figure 1. Bcl-2-Bax system from PDB entry 2XA0 in a rectangular solvent box. The protein chains of Bcl-2 and Bax are depicted as black and gray ribbons, respectively, while the water molecules are represented as gray dots.

Figure 2. Root-mean-square deviation (RMSD) in nm of the Bcl-2-Bax system (backbone atoms) over the course of the simulation. A) Raw data from MD simulation. B) post-processed data. Simulations in a capped-octahedral or a rectangular box are shown in the top or bottoms graphs, respectively. The two independent simulations runs are colored black and gray.

Figure 3. Periodic boundary conditions in two dimensions. A) Small isolated molecules (e.g. water). B) Protein-protein complex. The central cell contains the coordinates to be saved for further analysis.

Figure 4. Pseudo-dissociation of the stable Bcl-2-Bax complex. The intact complex (left) seems to dissociate during only 10 ps of simulation time (center); this structure is, however, cured after proper post-processing (right) to form an intact complex again.

Figure 1

Figure 2

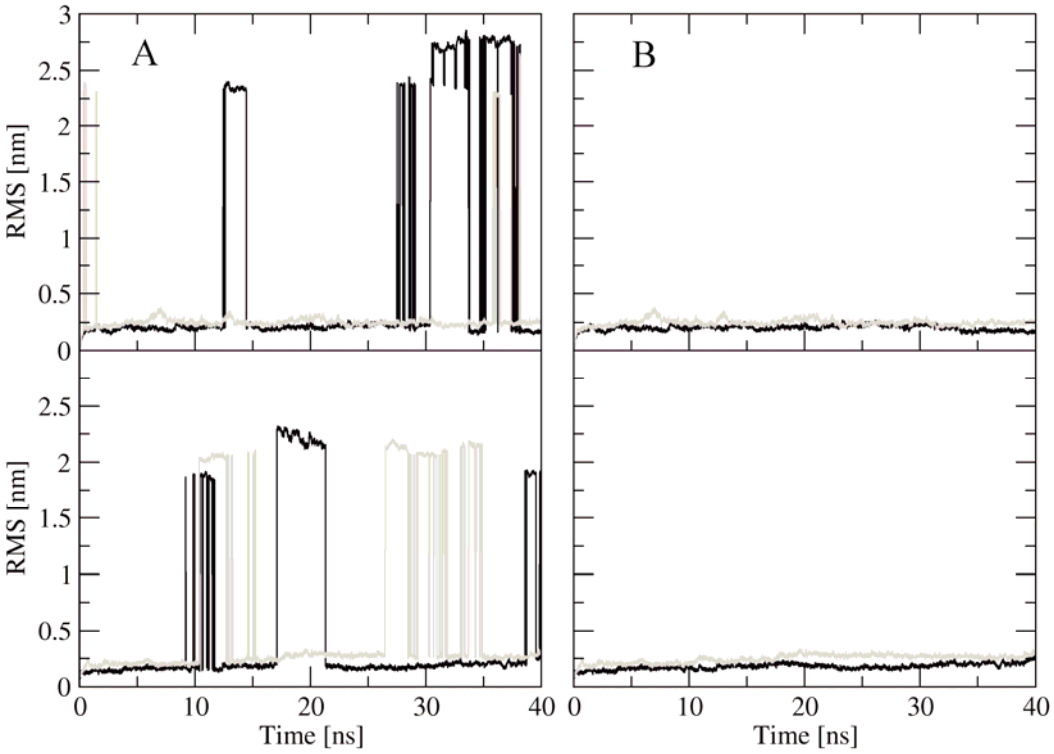

Figure 3

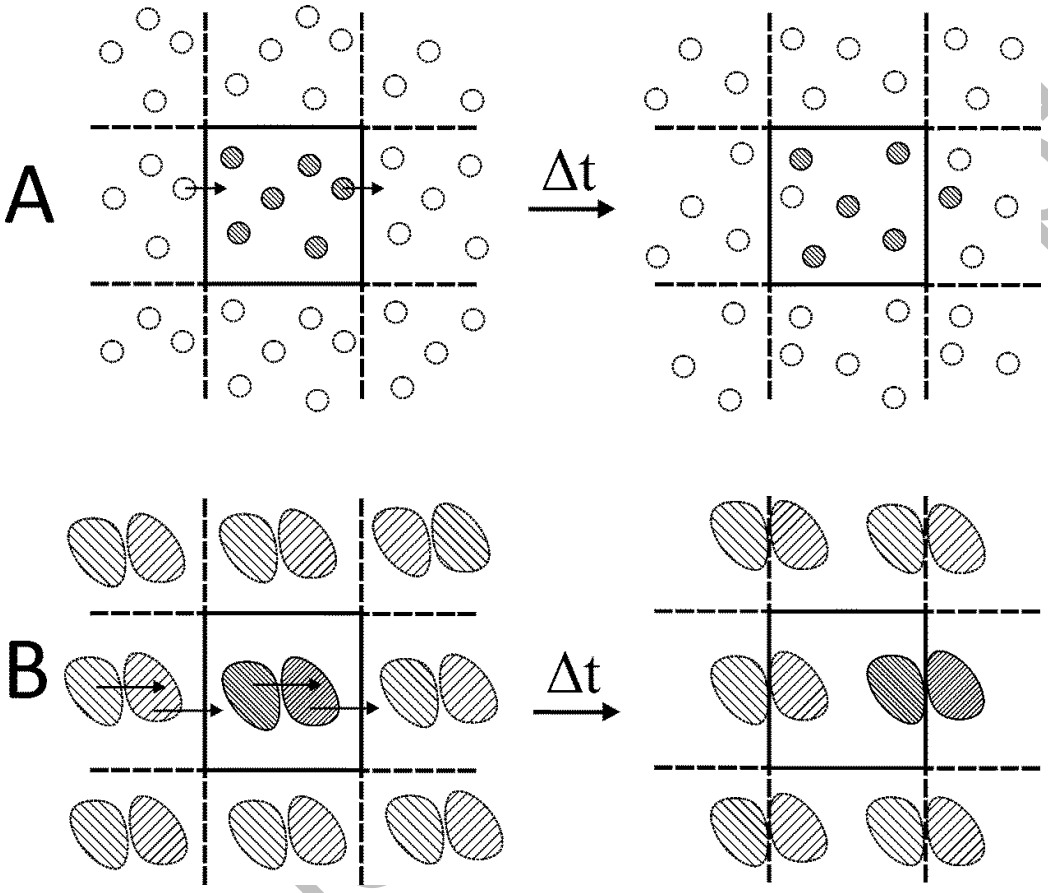

Figure 4