**COVER LETTER**

**Title**: COZOID: COntact ZOne IDentifier for Visual Analysis of Protein-Protein Interactions

**Date**: February 2nd, 2018

We would like to thank the anonymous reviewers for their helpful comments. We addressed all remarks in the revised version of the paper. In this letter, we give detailed responses to all reviewer comments and describe the corresponding changes in our manuscript.

**Technical comments**

- *Provide email addresses of all authors*

*-Missing List of Abbreviations*

*-Missing Declarations heading*

*-Missing Ethics approval and consent to participate, Consent for publication*

The email addresses of all authors were added to the Author’s contributions section. The List of Abbreviations was added as well. We added the Declarations heading as well as the requested missing parts.

**Editor Comments:**

*As mentioned by the reviewers, availability of the tool is required so that the readership can take advantage of the interesting work reported in this manuscript.*

Upon the request of the reviewers, we made the tool available and it can be downloaded here: http://decibel.fi.muni.cz/cozoid/

To ease the testing of the tool, we made available also a testing dataset, obtained by the web version of the HADDOCK tool.

**Reviewer 1**

1. *In the Conclusion, the authors state that their system allows the proteomics experts to obtain information that was very hard or even impossible to obtain with previous methods. This statement is a bit vague and should be supported by giving examples what this additional information actually is.*

We extended this statement in the Conclusion section in the following way:

“Our aim was to overcome the drawbacks of the existing methods for visual analysis and comparison of configurations, which provide the users only with traditional 3D view and exploration of individual configurations one-by-one. Additionally, specialized techniques enabling to explore the content of the contact zone are completely missing. Therefore, our proposed solution provides the proteomic experts with information that was very hard or even impossible to get using these previously available methods.”

1. *Furthermore, also in the Conclusions, the authors state that with their system conclusion about the proteomical relevance of individual configurations can be obtained \*much faster\*. However, throughout the whole manuscript the authors do not give any timings. So my questions are: (1) How did the authors evaluate the timings? (2) How long does it actually take to come to a satisfying conclusion and how long did it take before without their system? Do such measurements exists? If  
   the authors cannot specify this, the statement should be removed, but it would be good to know for an expert reading the paper, what he or she can expect by applying the tool.*

We completely agree with the reviewer that this statement was very vague. Although the time for the analysis of the input set of configurations was shortened significantly, it would be very hard to come with some satisfying measurement method. Therefore, we decided to remove the sentence from the manuscript.

1. *Apart from this, I would suggest changing the notation CONF\_i(C(P\_1,P\_2)) to C(P\_1,P\_2)\_i.*

After thorough discussion we decided to keep the original notation, as we believe that the order of the configuration (the i parameter) is better readable at the beginning rather than at the end of the expression.

1. *Minor issues – typos and grammar corrections.*

All mentioned errors were corrected.

**Reviewer 2**

1. *The tool should be publicly and freely available on a web site.*

We made the tool available here: http://decibel.fi.muni.cz/cozoid/. We also added the testing dataset obtained by using the HADDOCK tool.

1. *There are some grammatical errors.*

All mentioned errors were corrected.

**Reviewer 3**

1. *The authors propose "a novel tool to proteomic (? replace, use protein structure) experts" to select the most relevant configurations and explore their contact zones on different levels of detail.*

The term “proteomic expert” is commonly used in the domain. Therefore, we kept this notation.

1. *The software or tool should be made available, for download, best plus a simple tutorial on its usage.*

The tool is available here: http://decibel.fi.muni.cz/cozoid/

We added also a simple tutorial how to load the testing dataset and use the interface to test the proposed views.

1. *The authors should think of some use cases (e.g. identify the best predicted interaction faces /contact zones in their chosen example complex) and then explain, why and how their tool is superior to standard techniques such as direct protein visualization tools (e.g. rasmol, pymol, ….) or more complex protein structure investigation tools (e.g. docking software, protein structure investigation software etc.)*

We were trying to demonstrate the usage of the tool in the Results and Discussion section where we showcase examples of three most typical protein-protein interaction modes.

We believe that the benefit of our tool when comparing it with the tools as Rasmol or PyMol is clear – these tools provide the users with standard 3D visualization and alignment of molecules, which leads to problems illustrated in Figure 1. None of the existing tools enables to explore and filter the configurations using additional specialized views like Residue Matrix and Contact Zone Graph. Our tool is based on the core of the CAVER Analyst application [1] which was introduced to the community in 2014 and is freely available for downloading at [www.caver.cz](http://www.caver.cz). It served us as the environment for creating the prototype of the COZOID tool and we are releasing the version containing COZOID with the reviewed version of this manuscript. It is available here: http://decibel.fi.muni.cz/cozoid/

However, as the CAVER Analyst tool was primarily designed for visual analysis of tunnels in proteins, we do not mention this tool in the manuscript, not to confuse the readers.

[1] Kozlíková, B., Šebestová, E., Šustr, V., Brezovský, J., Strnad, O., Daniel, L., Bednář, D., Pavelka, A., Maňák, M., Bezděka, M., Beneš, P., Kotry, M., Gora, A. W., Damborský, J., Sochor, J. CAVER Analyst 1.0: Graphic tool for interactive visualization and analysis of tunnels and channels in protein structures. Bioinformatics, Oxford University Press, 2014, 30(18), 2684-2685. ISSN 1367-4803. doi:10.1093/bioinformatics/btu364.

1. *Currently the reader has the impression that there is a tool written by the authors which helps them to their satisfaction, but for own work one should better use a freely available and easily installable protein structure viewer as the tool is not available and the advantages to such a basic viewer are not made clear.*

As stated in our answer to comment no. 9, the COZOID tool was integrated to the CAVER Analyst tool which contains all standard methods for visualization and exploration of molecules. Upon the request of the reviewers, we released the COZOID tool. Its advantage is that the installation of the tool is not required – the user launches the binary execution file. The release contains also a testing dataset and simple user guide explaining the basic functions of the tool.

1. *Another point is that the tool seems only to help in making predictions for protein complexes. It is maybe better to discuss also an example case where  
   the correct protein complex and interaction face is known (there are many in pdb available) and then one can very well compare the advantages and limitations  
   of the suggested new tool with standard tools for such a task.*

We believe that this is exactly described and demonstrated on the case with the 3NW0 structure (Surface-Surface Interaction section in Results and Discussion).

1. *Important are corrections by a native speaker, this will further improve the English (though it is understandable).*

The manuscript was now checked and corrected by the professionals from the American Journal Experts.