SOFTWARE TOOL ARTICLE

FlyOde - a platform for community curation and interactive visualization of dynamic gene regulatory networks in *Drosophila* eye development [version 1; peer review: 3 approved]

Stefan A. Koestler^{1*}, Begum Alaybeyoglu^{2*}, Christian X. Weichenberger⁴, Arzu Celik^{1,3}

¹Department of Molecular Biology and Genetics, Bogazici University, Istanbul, 34342, Turkey

²Department of Chemical Engineering, Bogazici University, Istanbul, 34342, Turkey

³Life Sciences Center, Bogazici University, Istanbul, 34342, Turkey

⁴Center for Biomedicine, European Academy of Bozen/Bolzano (EURAC), (Affiliated to the University of Lubeck, Lubeck, Germany), Bozen/Bolzano, 39100, Italy

* Equal contributors

V1 First published: 21 Dec 2015, 4:1484
<https://doi.org/10.12688/f1000research.7556.1>

Latest published: 21 Dec 2015, 4:1484
<https://doi.org/10.12688/f1000research.7556.1>

Abstract

Motivation: Understanding the regulatory mechanisms governing eye development of the model organism *Drosophila melanogaster* (*D. m.*) requires structured knowledge of the involved genes and proteins, their interactions, and dynamic expression patterns. Especially the latter information is however to a large extent scattered throughout the literature.

Results: FlyOde is an online platform for the systematic assembly of data on *D. m.* eye development. It consists of data on eye development obtained from the literature, and a web interface for users to interactively display these data as a gene regulatory network. Our manual curation process provides high standard structured data, following a specifically designed ontology. Visualization of gene interactions provides an overview of network topology, and filtering according to user-defined expression patterns makes it a versatile tool for daily tasks, as demonstrated by usage examples. Users are encouraged to submit additional data via a simple online form.

Keywords

Drosophila development, gene regulatory network, protein interaction network, visualization, web-tool, database, community annotation, ontology

Open Peer Review

Approval Status

	1	2	3
version 1			
21 Dec 2015	view	view	view

1. **Pelin Cayirlioglu Volkan**, Duke University, Durham, USA
2. **Bassem A Hassan**, VIB and University of Leuven School of Medicine, Leuven, Belgium
IHU Pitié Salpêtrière, Paris, France
3. **Fernando Casares**, CSIC and Pablo de Olavide University, Seville, Spain

Any reports and responses or comments on the article can be found at the end of the article.

This article is included in the **Eye Health** gateway.

Corresponding author: Stefan A. Koestler (stefan@koestler.at)

Competing interests: No competing interests were disclosed.

Grant information: S.A.K. was supported by TÜBITAK 2236 Co-Funded Brain Circulation Scheme.

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Copyright: © 2015 Koestler SA *et al.* This is an open access article distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. Data associated with the article are available under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original data is properly cited.

How to cite this article: Koestler SA, Alaybeyoglu B, Weichenberger CX and Celik A. **FlyOde - a platform for community curation and interactive visualization of dynamic gene regulatory networks in *Drosophila* eye development [version 1; peer review: 3 approved]** F1000Research 2015, 4:1484 <https://doi.org/10.12688/f1000research.7556.1>

First published: 21 Dec 2015, 4:1484 <https://doi.org/10.12688/f1000research.7556.1>

Introduction

Developmental biology is the study of processes that generate an entire organism from a single cell. A central question in this field is how differentiation produces specific cell types from pluripotent precursors. *Drosophila melanogaster* (*D. m.*) serves as a suitable and well-established model organism to address this question for numerous reasons including a short generation time, the multitude of available genetic methods, and its orthology shared with vertebrates (Reiter *et al.*, 2001). The *D. m.* eye allows the study of morphological rearrangements as well as differentiation of non-neuronal and neuronal cell types like photoreceptors (PRs) on the single cell level (Thomas & Wassarman, 1999).

Understanding cell differentiation requires knowledge of the involved genes, their temporally varying (dynamic) expression patterns, and interactions. Interaction data from different sources are accessible through e.g. Biogrid, Intact, String, and REDfly (Chatr-Aryamontri *et al.*, 2015; Gallo *et al.*, 2011; Orchard *et al.*, 2014; Szklarczyk *et al.*, 2015) and database collections, e.g. iRefindex or mentha (Calderone *et al.*, 2013) (Razick *et al.*, 2008). Expression data is mostly provided on embryonic development or organ systems, e.g. by FlyBase (dos Santos *et al.*, 2015) and the Berkeley Drosophila Genome Project (BDGP) (Hammonds *et al.*, 2013), but the coverage and precision of expression pattern annotation on the cellular level and the temporal resolution at later stages, e.g. larva and pupa, are limited (see Supplementary Material). On the other hand, a wealth of expression pattern data on these levels is

contained in publications (e.g. (Potier *et al.*, 2014)). Systematic use of these data requires their structured assembly through an extensive curation effort. Since automated curation is prone to errors (Mao *et al.*, 2014), information must be extracted manually from the literature by experts, who can critically interpret the respective types of data like micrographs or expression profiles (Tomancak *et al.*, 2007) and convert these to machine-readable data for further computer-based analyses.

We have thus developed FlyOde, an online hub for the community-driven systematic assembly of data on *D. m.* eye development. FlyOde is a web interface for interactive exploration of gene and protein relationships by combining visualization of the curated gene regulatory network with filters specific to fly development. FlyOde is built on an ontology-driven curation process that stores data in a specifically formatted text file, which can easily be enriched and extended upon arrival of new data.

Implementation

Data

A directed gene interaction network representing eye development of *D. m.* from the third instar larva to the adult with focus on PR differentiation was constructed using Cytoscape (Shannon *et al.*, 2003), based on data extracted manually from 77 publications (Figure 1). Currently, the network contains 146 nodes representing genes/proteins, and 284 edges representing activating or inhibiting genetic, protein-protein, or protein-DNA interactions. The layout

Figure 1. The FlyOde interface displaying the eye developmental network. The red circle indicates the *eye/less* gene (see Example 1). The inset shows selected interactions containing network motifs: negative autoregulation and feedback loop (left) and an incoherent feed forward loop (right).

is generated manually and organized to approximately represent developmental time along the horizontal axis, beginning with early third instar larval stage on the left, and network hierarchy along the vertical axis with master regulators (as defined by (Chan & Kyba, 2013)) placed towards the top. Nodes are associated with their FlyBase symbol, name, alternative names, FlyBase link, dynamic expression pattern, phenotypes, the terms for each of the three Gene Ontologies (GO) (Ashburner *et al.*, 2000), and the literature references. GO annotations were added using Cytoscape. Expression pattern annotation follows a specifically developed ontology which links developmental stage and cell type (Figure 2). We support and encourage annotation from the community to continually extend the dataset.

Web application

The interactive JavaScript based web application renders the manually curated network, which is embedded using Cytoscape Web

(Lopes *et al.*, 2010). Genes are represented as nodes and gene (or protein) interactions are visualized as directed edges. Nodes and edges have been annotated during the curation process (see previous section), and this information is displayed when an item is selected. For the selection of nodes a module for searching gene symbols, names, alternative names, and GO-terms is provided. Additionally, the network can be filtered according to gene expression patterns using dropdown menus whose structure follows the FlyOde ontology (Figure 2). Filters can be combined with the Boolean operators AND, OR, and NOT. Extensions to the network can be submitted to us via a community curation form.

Characterization

To characterize the content of the current FlyOde network GO enrichment analysis was performed with the ClueGO app in Cytoscape (Bindea *et al.*, 2009). It shows that terms correlated with development and morphogenesis, pattern formation and polarity,

FlyOde Ontology

Annotation syntax:

[organ], [differentiation level], [dev. stage specifier][general dev. stage]_[specialization][subtype][general cell type][specified cell type]

Figure 2. The FlyOde eye-ontology. Hierarchical representation of controlled vocabulary for the gene annotation with dynamic expression patterns is shown. In the annotation syntax terms for cell type and developmental stage are directly linked. Each term-combination represents expression in a specific cell type and developmental stage. As many comma-separated term combinations as needed to describe the expression pattern are given. During retinal development of *Drosophila*, cells at consecutive stages of differentiation coexist at the same developmental stage of the organism. The morphogenetic furrow marks the initiation of differentiation and moves across the eye precursor organ, the eye imaginal disc. Thus, the expression pattern is also defined by the localization relative to the morphogenetic furrow. This and additional terms that are common in the field are assigned to what we call "differentiation level".

apoptosis, mitosis, and regulation of transcription are highly over-represented, as expected (see [Supplementary Materials](#) and [Supplementary Figure 1](#) and [Supplementary Figure 2](#)).

To evaluate the information that can be obtained from FlyOde the FlyOde filter function was compared with the FlyBase QueryBuilder. When queried for general terms like “larva and PR”, FlyBase gave more gene hits than FlyOde, but when increasingly specifying the developmental stage and cell type, like “pupa and dorsal rim area R8 (photoreceptor cell 8)”, more genes were found with FlyOde as compared to FlyBase. This shows that FlyOde already stands out in defining gene expression in a specific cell type and during a specific developmental stage of PR differentiation (see [Supplementary Materials](#) and [Supplementary Figure 3](#)).

To get an idea of the basic mechanisms represented in FlyOde, the occurrence of network motifs was analysed manually in Cytoscape. Motifs commonly found during development of organisms, like autoregulation, feedback and feed forward loops were observed. This indicates that FlyOde at least partially displays the connectivity and level of detail to qualify for representation and detailed analysis of developmental processes (see [Figure 1](#) and [Supplementary Materials](#), [Supplementary Figure 4](#) and [Supplementary Table](#)) (Alon, 2007; Davidson, 2010).

Use cases

Example 1: Obtaining information on a specific gene

In this example we use the search functionality to explore a specific gene in the dataset. In order to do so, *Pax6* is entered into the search box. This gene is known under that name as an important regulator of development in many organisms (van Heyningen, 2002). However, the official name in *D. m.* is *eyeless*. Due to its annotation with alternative names it is found despite the search for the unofficial name *Pax6* and highlighted in the network ([Figure 1](#)). Its position on the left side of the graph indicates that it is mainly expressed early during eye development. In the web interface additional information is displayed in a content related text field below the graph, which we designate “report panel”. A closer look at the expression pattern displayed there shows that it is expressed anterior to the morphogenetic furrow, in all photoreceptors in the early third instar larva, and in outer photoreceptors in the late pupa and adult. The top position in the hierarchy indicates that it is a master regulator. This is supported by its high number of interactions, and by the phenotypes, which are given in the report panel (Chan & Kyba, 2013). We also find that GO annotates *eyeless* as a transcription factor, and that it is involved in developmental processes of other organs. Finally, the literature references and the FlyBase link can be followed for further information.

Example 2: Which genes can be used as markers for PR R8 in the intermediate pupa?

Here we apply filter combinations to display genes that are expressed in R8, but not in any other PR in the intermediate pupa to obtain candidate markers for R8 in immunostaining and cell sorting experiments. In the dropdown menus we choose “pupa”, “intermediate pupa”, “photoreceptor cell”, and “R8”, respectively. We add

another filter line with the Boolean “NOT”, and in the dropdown menus select “intermediate pupa”, “photoreceptor cell”, “R7”, and another filter line with the Boolean “NOT”, “intermediate pupa”, and “at least one outer PR”, which in combination with “NOT” means “no outer PR”. All nodes are removed except for *scabrous* and *senseless*, which are therefore candidates for being R8 markers. The literature references for these two markers provide a starting point for future experimental studies.

More examples are provided in the tutorial at <http://flyode.boun.edu.tr/quickguide.html>.

Discussion

Here we have presented FlyOde, which provides a platform for combining published data on gene regulatory networks (GRNs) of *Drosophila* organ development. As a starting point, we have equipped FlyOde with extensive GRN data for *D. m.* eye development, such that the web interface serves as a versatile tool for everyday tasks a fly researcher encounters.

FlyOde delivers high quality data standards by manual curation. We expect to achieve efficient data collection by distributing the annotation workload among community members with minimal effort for the individual contributors, who only need to submit a simple form to add new nodes, interactions, or annotation. In return, they directly profit from the improved tool by linking their data of interest with the shared knowledge.

We are constantly improving the ontology and web application, in parallel to ongoing data curation and dataset extension. FlyOde will be expanded to include other organs with the ultimate goal to compare their GRNs.

Future work will profit from the obtained data by constraining network inference from gene expression data (Hecker *et al.*, 2009). Another anticipated approach is to assign quantitative expression data to the established network to facilitate mathematical modelling (Graham *et al.*, 2010).

We envision FlyOde as a companion that guides researchers through developmental processes, for example while studying a paper, and believe that the community-curated dataset and its analysis will add significant knowledge to developmental biology.

Data availability

FlyOde, including all network data, annotations and their corresponding references can be freely accessed via the web-application at <http://flyode.boun.edu.tr/>.

Software availability

Software access

The source code for the web application can be downloaded from Github (<https://github.com/begum-alaybeyoglu/FlyOde>).

Source code as at the time of publication

<https://github.com/F1000Research/FlyOde>

Archived source code as at the time of publication

<http://dx.doi.org/10.5281/zenodo.35227>

Software license

The FlyOde web application is released under the MIT License.

Author contributions

S.A.K. established the concept, network, annotation and ontology, performed analysis and wrote the manuscript. B.A. generated the web application. C.X.W. performed analysis and provided input during manuscript preparation. A.C. coordinated the project. All authors contributed to proofreading the manuscript and have agreed to the final content of the manuscript.

Competing interests

No competing interests were disclosed.

Grant information

S.A.K. was supported by TÜBİTAK 2236 Co-Funded Brain Circulation Scheme.

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Acknowledgements

We thank Stefan Fuss and the Bogazici University Computer Centre for help with the server and Elif Özkirimli (all Bogazici University, Istanbul) for proofreading the paper.

Supplementary Material

Supplementary Figure 1.

GO-term enrichment analysis with ClueGO.

[Click here to access the data.](#)

Analysis and characterization of the FlyOde network.

Includes Supplementary Figures 2, 3, 4.

[Click here to access the data.](#)

References

- Alon U: **Network motifs: theory and experimental approaches.** *Nat Rev Genet.* 2007; **8**(6): 450–461.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Ashburner M, Ball CA, Blake JA, *et al.*: **Gene ontology: tool for the unification of biology.** *The Gene Ontology Consortium. Nat Genet.* 2000; **25**(1): 25–9.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Bindea G, Mlecnik B, Hackl H, *et al.*: **ClueGO: a Cytoscape plug-in to decipher functionally grouped gene ontology and pathway annotation networks.** *Bioinformatics.* 2009; **25**(8): 1091–3.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Calderone A, Castagnoli L, Cesareni G: **mentha: a resource for browsing integrated protein-interaction networks.** *Nat Methods.* 2013; **10**(8): 690–1.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Chan SS, Kyba M: **What is a Master Regulator?** *J Stem Cell Res Ther.* 2013; **3**: pii: 114.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Chatr-Aryamontri A, Breitkreutz BJ, Oughtred R, *et al.*: **The BioGRID interaction database: 2015 update.** *Nucleic Acids Res.* 2015; **43**(Database issue): D470–8.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Davidson EH: **Gene Regulatory Networks.** In *Development And Evolution.* Academic Press, 2010.
- Gallo SM, Gerrard DT, Miner D, *et al.*: **REDfly v3.0: toward a comprehensive database of transcriptional regulatory elements in Drosophila.** *Nucleic Acids Res.* 2011; **39**(Database issue): D118–23.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Graham TG, Tabei SM, Dinner AR, *et al.*: **Modeling bistable cell-fate choices in the Drosophila eye: qualitative and quantitative perspectives.** *Development.* 2010; **137**(14): 2265–78.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Hammonds AS, Bristow CA, Fisher WW, *et al.*: **Spatial expression of transcription factors in Drosophila embryonic organ development.** *Genome Biol.* 2013; **14**(12): R140.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Hecker M, Lambeck S, Toepper S, *et al.*: **Gene regulatory network inference: data integration in dynamic models-a review.** *Biosystems.* 2009; **96**(1): 86–103.
[PubMed Abstract](#) | [Publisher Full Text](#)
- van Heyningen V, Williamson KA: **PAX6 in sensory development.** *Hum Mol Genet.* 2002; **11**(10): 1161–1167.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Lopes CT, Franz M, Kazi F, *et al.*: **Cytoscape Web: an interactive web-based network browser.** *Bioinformatics.* 2010; **26**(18): 2347–8.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Mao Y, Van Auken K, Li D, *et al.*: **Overview of the gene ontology task at BioCreative IV.** *Database (Oxford).* 2014; **2014**: pii: bau086.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Orchard S, Ammari M, Aranda B, *et al.*: **The MintAct project—IntAct as a common curation platform for 11 molecular interaction databases.** *Nucleic Acids Res.* 2014; **42**(Database issue): D358–63.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Pinoli P, Chicco D, Masseroli M: **Computational algorithms to predict Gene Ontology annotations.** *BMC Bioinformatics.* 2015; **16**(Suppl 6): S4.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Potier D, Davie K, Hulselmans G, *et al.*: **Mapping gene regulatory networks in Drosophila eye development by large-scale transcriptome perturbations and**

motif inference. *Cell Rep.* 2014; 9(6): 2290–2303.

[PubMed Abstract](#) | [Publisher Full Text](#)

Razick S, Magklaras G, Donaldson IM: **iRefIndex: a consolidated protein interaction database with provenance.** *BMC Bioinformatics.* 2008; 9: 405.

[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

Reiter LT, Potocki L, Chien S, *et al.*: **A systematic analysis of human disease-associated gene sequences in *Drosophila melanogaster*.** *Genome Res.* 2001; 11(6): 1114–25.

[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

dos Santos G, Schroeder AJ, Goodman JL, *et al.*: **FlyBase: introduction of the *Drosophila melanogaster* Release 6 reference genome assembly and large-scale migration of genome annotations.** *Nucleic Acids Res.* 2015; 43(Database issue): D690–7.

[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

Shannon P, Markiel A, Ozier O, *et al.*: **Cytoscape: a software environment for integrated models of biomolecular interaction networks.** *Genome Res.* 2003; 13(11): 2498–504.

[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

Szklarczyk D, Franceschini A, Wyder S, *et al.*: **STRING v10: protein-protein interaction networks, integrated over the tree of life.** *Nucleic Acids Res.* 2015; 43(Database issue): D447–52.

[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

Thomas BJ, Wassarman DA: **A fly's eye view of biology.** *Trends Genet.* 1999; 15(5): 184–190.

[PubMed Abstract](#) | [Publisher Full Text](#)

Tomancak P, Berman BP, Beaton A, *et al.*: **Global analysis of patterns of gene expression during *Drosophila* embryogenesis.** *Genome Biol.* 2007; 8(7): R145.

[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

Open Peer Review

Current Peer Review Status:

Version 1

Reviewer Report 15 February 2016

<https://doi.org/10.5256/f1000research.8136.r11677>

© 2016 Casares F. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Fernando Casares

CABD (Andalusian Centre for Developmental Biology), CSIC and Pablo de Olavide University, Seville, Spain

The *Drosophila* eye gene regulatory network ranks among the best textbook examples of organogenetic GRNs. FlyODE, by Koestler and colleagues is a welcomed attempt at integrating the many bits of expression, regulation, molecular interactions and functional analyses obtained to date, by providing a cytoscape-like tool in the form of a curated, directed graph network. FlyODE's also allows the expansion of this network through community-based contributions –which is a great philosophy. Therefore FlyODE is a valuable tool for those working on gene networks in general and on fly eyes in particular, and will help in expanding the network (and our knowledge of the system) further. FlyODE includes references to spatial gene expression patterns. It would be good if the authors tried to unify the somehow ambiguous terms used by different labs, such as “preproneural/precursors/anterior to the MF” or “proneural/immediately anterior to the MF”, or perhaps provide some “equivalence” terms. When sorting genes according to their expression along third instar, I found that the network includes more genes at early L3 than at mid-L3, which shouldn't be the case –as the process unfolds more genes are called into action.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 15 Feb 2016

Stefan A. Koestler, Bogazici University, Istanbul, Turkey

Thank you very much for your feedback!

We are working on improving the ontology, e.g. by adjusting it to the Gene Ontology, and will incorporate your suggestions.

The layout is currently done manually and is certainly to some extent biased depending on

the references used for annotation. This can be expected to improve with an increasing number of annotations/references. In addition, we are planning an automatic layout to calculate the position from the annotations. In any case one has to keep in mind that it will only indicate a single (mean) value, while a gene might display a more complex expression pattern. Thus, currently one has to look at the report panel below the network for more detailed information. We are thinking about adding a graph displaying the expression profile when hovering over a node to make this information more intuitively accessible.

Competing Interests: No competing interests were disclosed.

Reviewer Report 12 February 2016

<https://doi.org/10.5256/f1000research.8136.r12188>

© 2016 Hassan B. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Bassem A Hassan

¹ Center for the Biology of Disease, VIB and University of Leuven School of Medicine, Leuven, Belgium

² Institut du Cerveau et de la Moelle epiniere (ICM), IHU Pitié Salpêtrière, Paris, France

This is a very nice and useful tool to quickly and efficiently access information about fly retina development. The fact that it can be extended to any tissue and is an open platform for community-based annotation makes it all the more useful. I applaud the authors for their efforts and thank them for making such a cool and intuitive tool available. The video tutorial is very nice and helpful as well.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 11 February 2016

<https://doi.org/10.5256/f1000research.8136.r12186>

© 2016 Volkan P. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Pelin Cayirlioglu Volkan

Department of Biology, Duke University, Durham, NC, USA

FlyOde is a very nice resource for labs, which are interested in visualizing developmental dynamics of gene regulatory networks required for the development for many neuronal types in the *Drosophila* eye, in addition to finding lineage specific markers. It will be valuable in the future to expand this to other sensory systems such as the olfactory system. The title and the abstract are appropriate. The video presentation is very useful.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Comments on this article

Version 1

Author Response 19 Feb 2016

Stefan A. Koestler, Bogazici University, Istanbul, Turkey

We thank all referees for their feedback!

Competing Interests: No competing interests were disclosed.

The benefits of publishing with F1000Research:

- Your article is published within days, with no editorial bias
- You can publish traditional articles, null/negative results, case reports, data notes and more
- The peer review process is transparent and collaborative
- Your article is indexed in PubMed after passing peer review
- Dedicated customer support at every stage

For pre-submission enquiries, contact research@f1000.com

F1000Research