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Structure-Function Analysis Glyceraldehyde-3-Phosphate Dehydrogenase Homologue GapB in Staphylococcus aureus

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Abstract

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is the most studied reference protein that catalysis the inter-conversion reaction of glyceraldehyde-3-phosphate into 1,3-diphosphoglycerate using NAD+ as coenzyme. GAPDH is also recognized as an important player in DNA repair mechanisms, autophagic and apoptotic cell deaths and posttranslational modifications. Staphylococcus aureus is Gram positive commensal pathogenic bacteria. In the genome of S. aureus, GapA was assumed to be a glycolytic GAPDH and GapB was assumed to be a gluconeogenic GAPDH. The crystal structure of GapA has already been in preceding studies. However, to my knowledge, no structural studies on the gapB homologue is available in the literature. The main aims of this study were to analyze physicochemical properties and generate a homology model structure of GAPDH homologue GapB in S. aureus. This was carried out by Protparam tool, Phyre2 homology modeling server and PSIPRED secondary structure analysis tool. ProtParam predicted that GapB is a stable and liposoluble protein. Homology modeling studies revealed that each subunit of GapB was made up by two domains: the NAD coenzyme binding-domain and the catalytic domain. The NAD binding domain was shown to contain a Rossman fold. On the other hand, the catalytic domain was made up by a mixture of eight strands of beta sheet and seven alfa helices. PSIPRED analysis revealed that the secondary structure of the GapB contains α-helices (29.91%), extended strands (24.63%) and random coil (45.45%).

Key words: Glyceraldehyde-3-phosphate dehydrogenase, Staphylococcus aureus, GapB, Phyre2, PSIPRED

1. Introduction

Staphylococcus aureus is among the most studied Gram-positive, nonmotile, pathogenic bacterial species. It is documented to be one of the most clinical bacteria that has been identified in the human commensal microbiota (Becker et al., 2016; Lee et al., 2018). Several types of staphylococcal bacterias such as *S. aureus*,

Staphylococcus epidermidis, *Staphylococcus xylosus*, *Staphylococcus warneri*, *Staphylococcus hemolyticus*, and *Staphylococcus saprophyticus* are commonly found in microbiata of humans and animals, however among them only *S. aureus* and *S. epidermidis* have potential to result in infection and disease (Plata et al., 2009). The common use of antibiotics has resulted antibiotic resistant strains of *S. aureus* to emerge. Among them the most important being Methicillin Resistant *S. aureus* (MRSA). Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) is one of the most important metabolic enzymes that plays a vital function for metabolism of carbon in living organisms. It catalyses inter-conversion of glyceraldehyde-3-phosphate (G3P) to 1,3-diphosphoglycerate (1,3-dPG) in glycolytic/gluconeogenic pathways using NAD^+ for activity (Oesper, 1954; Ayna, 2016). Nonetheless, the studies in recent decades have demonstrated that homologues proteins GAPDH from eukaryotes and prokaryotes are actually multi-functional as they were shown to involve in DNA repair mechanisms, autophagic and apoptotic cell deaths and posttranslational modifications Tristan et al. (2011) and (Nicholls et al. (2012). GAPDH was acknowledged to be crucial for the catabolism of glucose, and shown to be conserved in bacterial species, yeast and higher eukaryotic organisms. Although several prokaryotic and eukaryotic organisms have only one copy of GAPDH gene that encodes a protein (enzyme) that funtions in glycolysis and gluconeogenesis (Pancholi & Fischetti, 1992), the genome of a lot of organisms contain more than one GAPDH homologues. The genome of some of the organisms including *Saccharomyces cerevisiae*, have more than one homologue all of which have GAPDH activity (Delgado et al., 2001). Three GAPDH homologues have been identified in *E. coli*, but only one of these genes, *GapA*, has been demonstrated to encode the GAPDH enzyme. In addition to these, the additional homologues, known as *GapB* and *GapC*, has been shown to encode a truncated GAPDH with unknown functionality and erythrose-4-phosphate dehydrogenase (Zhao et al., 1995; Hidalgo et al., 1996; Seta et al., 1997). In these organisms GapA has been shown to be involved in both glycolytic and gluconeogenic GAPDH acitivity. Additionally, two gap homologues have been identified in the genome of Gram positive bacteria *Bacillus subtilis*, but in contrast, both of them has been demonstrated to encode GAPDH proteins, each of them with opposite functions in metabolism of glucose rather than the widespread phenomena that one GAPDH functions in both roles (Fillinger et al., 2000). The *B. subtilis* gapA GAPDH has been reported to be only functional in glycolytic pathway in which it converts G3P to 1,3-dPG in the presence of NAD^+. The gapB GAPDH is reported to involve in gluconeogenic pathway, and requires $NADP^+$ as cofactor (Fillinger et al., 2000). The aims of this study were to analyze physicochemical properties and generate a homology model structure of GAPDH homologue *GapB* in *S. aureus.*

2. Material and Method

2.1. Analysis of physicochemical properties of GapB

ProtParam tool, that is used for the in silico analysis of several biophysical and bio-chemical properties of a protein whose sequence is deposited in Swiss-Prot or TrEMBL, was used for biophysiochemical analysis of gapB protein (Gasteiger et al., 2005). Some of the examined parameters are the molecular weight (MW), theoretically calculated isoelectric point (pI), the overall compositions of amino acids, atom contents, extinction coefficient, half-life, instability index, aliphatic index and grand average of hydropathicity.

2.2. Analysis of the secondary structure of the GapB

Analysis of the secondary structure of the GapB was performed by PSIPRED (Jones, 1999) which helps in prediction the secondray structure of protein. The PSIPRED protein structure prediction server helps researchers to submit a protein sequence, perform a prediction of their choice and receive the results of the prediction both textually via e-mail and graphically via the web.

2.3. Structure preparation and homology modelling

In order to generate homology model structure of Glyceraldehyde-3-Phosphate Dehydrogenase Homologue GapB in *S. aureus,* the required sequences were taken from Uniprot (www.uniprot.org/uniprot/Q4L728) as FASTA format. The sequences were then submitted to Phyre2 (Protein Homology/AnalogY Recognition Engine) server to build 3-D computational models in order for protein fold recognision depending on evolutionarily related proteins and homology modelling (Kelley et al., 2015). The Crystal Structure of NADP-Dependent Glyceraldehyde-3-Phosphate Dehydrogenase from *Synechococcus Sp*. complexed with $NADP^+$ (PDB: 2D2I) was used as starting structure for molecular modelling (Kitatani et al., 2006). PyMol was used for molecular visualization.

3. Results

3.1. Analysis of physicochemical properties of GapB

ProtParam results revealed that the MW of the GapB was 36979 Da, the theoretical isoelectric point was 5.95, and the instability index was 25.64. Therefore, it was inferred that GapB was a stable protein. In addition, the value of grand average of hydropathicity was −0.174 and the aliphatic index was 99.77 suggesting that GapB is liposoluble.

3.2. Structural analysis of the GapB

To best of my knowledge, no crystal structure of GapB has been deposited in the PDB. Consequently, the 3-Dimensional structure of the corresponding protein has been produced by the Phyre2, protein sequence based homology modeling server that utilises the Hidden Markov Method to create sequence alignments of a GapB sequence against sequence of the template. The corresponding alignment is given in Figure 1.

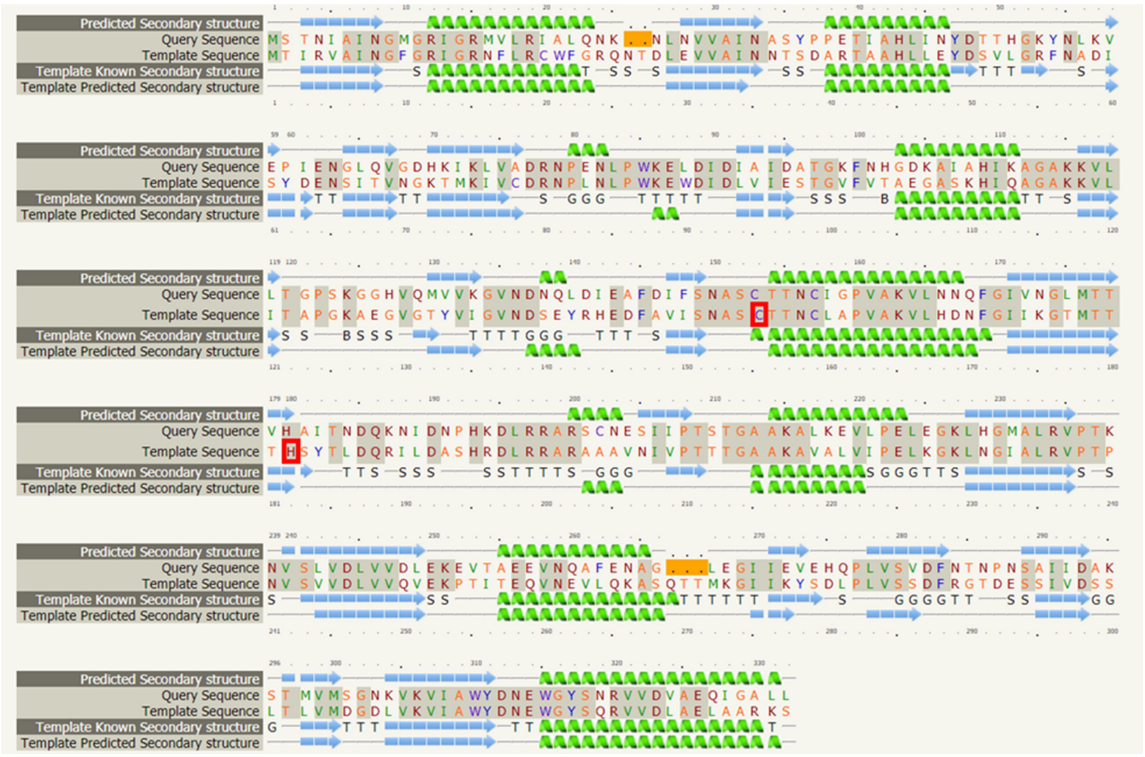

Figure 1. Alignment of GapB sequence against protein with the Glyceraldehyde-3-Phosphate Dehydrogenase from *Synechococcus Sp*. complexed with $NADP^+$ (PDB: 2D2I).

Cartoon illustration of the structure of GapB generated by Phyre2 server is demonstrated in Figure 2. The results demonstrated that every sub-unit of the enzyme is made up by two domians: the NAD^+ coenzyme binding-domain and the catalytic domain. The NAD^+ binding domain (residues 1 to 153) formed of the characteristic Rossman fold- a central parallel β-sheet flanked on both sides by two pairs of α-helices. On the other hand, the catalytic domain is made up by a mixture of beta sheet of 8 strands and 7 α-helices (Figure 2).

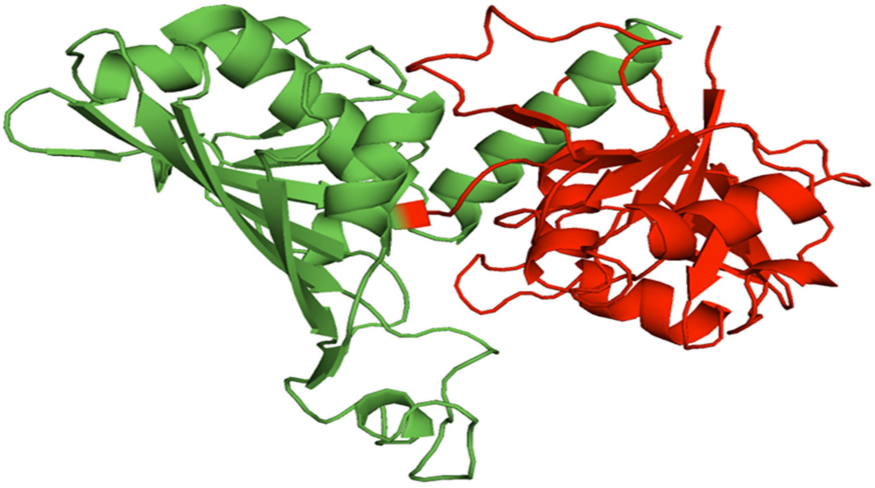

Figure 2. Structure of a monomer of GAPDH. The NAD^+ binding domain, corresponding to residues 1-153 is shown in red, the catalytic domain, corresponding to residues 153-318 is coloured in green.

To indicate the residues involved in the interaction between the coenzyme , NAD^+, and the GAPDHs, multiple sequence alignment methodology was utiised using Omega Clustal . At the beginning, the conservation of the GAPDH sequences were analysed in order to compare residues involved in coenzyme binding in GapA and its equivalent in GapB (Figure 3). These two homologs share 46 % identity as calculated by protein blast.

```
gapB      MSTNIAINGMGRIGRMVLRIALQNKNLNVVAINASYPPETIAHLINYDTTHGKYNLKVEP
gapA      MAVKVAINGFGRIGRLAFRRIQEVEGLEVVAVNDLTDDDMLAHLLKYDTMQGRFTGEVEV
          *:.::****:*****:.:*    : :.*:***:*      : :***::*** :*::. :**

gapB      IENGLQVGDHKIKLVADRNPENLPWKELDIDIAIDATGKFNHGDKAIAHIKAGAKKVLLT
gapA      VDGGFRVNGKEVKSFSEPDASKLPWKDLNIDVVLECTGFYTDKDKAQAHIEAGAKKVLIS
          ::.*::*..:::* .:: : .:****:*:**:.::.** :.. *** ***:*******::

gapB      GPSKGGHVQMVVKGVNDNQLDIEAFDIFSNASCTTNCIGPVAKVLNNQFGIVNGLMTTVH
gapA      APATGDLKTIVFNT-NHQELD-GSETVVSGASCTTNSLAPVAKVLNDDFGLVEGLMTTIH
          .*:.*.   :*.:  *.::**  :  :.*.******.:.*******::**:*:*****:*

gapB      AITNDQKNIDNPHK--DLRRARSCNESIIPTSTGAAKALKEVLPELEGKLHGMALRVPTK
gapA      AYTGDQNTQDAPHRKGDKRRARAAAENIIPNSTGAAKAIGKVIPEIDGKLDGGAQRVPVA
          * *.**:. * **:  * ****:. *.***.*******: :*:**::***.* * ***.

gapB      NVSLVDLVVDLEKE-VTAEEVNQAFENAGLEGIIEVEHQPLVSVDFNTNPNSAIIDAKST
gapA      TGSLTELTVVLEKQDVTVEQVNEAMKNASNESFGYT-EDEIVSSDVVGMTYGSLFDATQT
          . **.:*.* ***: **.*:**:*::**. *.:  . .: :** *.     .:::**..*

gapB      MVM---SGNKVKVIAWYDNEWGYSNRVVDVAEQIGALLTSKETVSAS
gapA      RVMSVGDRQLVKVAAWYDNEMSYTAQLVRTLAYLAELSK--------
           **   . : *** ****** .*: ::* .   :. * .
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Figure 3. Multiple sequence alignment of *Staphylococcus aureus* GapA and GapB.The sequences were obtained from NCBI website and alignment was carried out using omega clustal.

The superimposition of the structures of both GapA and GapB revealed that generally overall fold of GapA and GapB are the same (Figure 4).

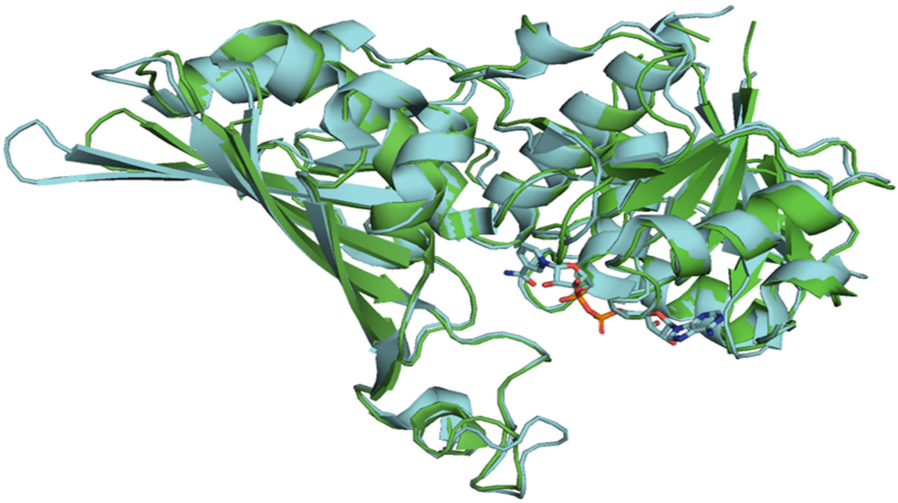

Figure 4. Superimposition of the overall fold of GapA (green) and GapB (cyan).

PSIPRED was used to analyze the secondary structure of the GapB (Figure 5). Results showed that structure of GapB was madeup by α-helices (29.91%), extended strands (24.63%) and random coil (45.45%).

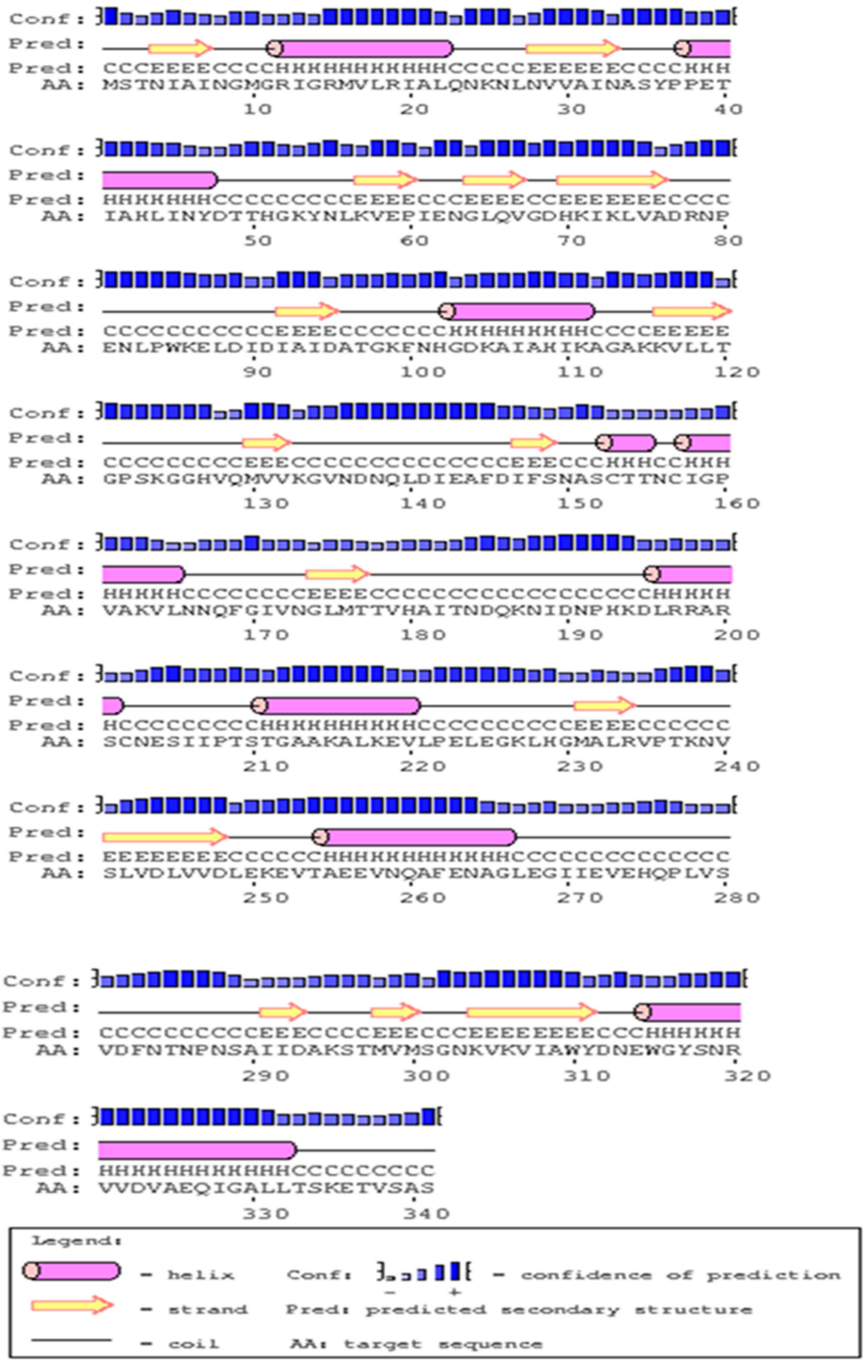

Figure 5. PSIPRED were used to analyze the secondary structure of the GapB.

4. Discussion

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is a well-known house-keeping protein, which has been made up by NAD^+ binding domain (N-terminal) and a catalytic domain (C-terminal) (Nagarajan et al., 2019). The catalytic domain also has an “S- loop” that makes interaction to the NAD^+ coenzyme of adacent sub-unit and is considered to work cooperatively (Ayna, 2016). GAPDH converts glyceraldehyde-3-phosphate into

glycerate-1,3-bisphosphate in the presence NAD^+ as a coenzyme (Park et al., 2019). In addition to its crucial function in glycolytic/gluconeogenic pathways, this enzyme is also involved in DNA repair process, autophagic and apoptotic cellular death (Tristan et al., 2011). Similar to β-Actin, it is commonly used in western blotting and quantiative real time PCR experiments as house keeping (reference) gene or protein (Aykutoglu et al., 2020; Kucukler et al., 2020; Özbolat and Ayna, 2020; Ayna et al., 2020).

S. aureus is documented as a Gram-positive commensal pathogen organism. This bacteria mostly colonise on the mucous membranes and skin. About 30% of the healthy population are reported to be colonized by *S. aureus*. In spite of primarily being a commensal microorganism, *S. aureus* has the potential to cause a wide range of diseases that can significantly differ in terms of severity (Jenul and Horswill, 2019). In *S. aureus*, the researchers have assumed GapA as a glycolytic GAPDH and that GapB as a gluconeogenic GAPDH (Gimpel and Brantl, 2016). The crystal structure of GapA was solved previously (Mukherjee et al., 2010). However, to my literature knowledge, no structural studies on the GapB homologue is available. The aim of this study was to generate a homology model structure of GAPDH homologue gapB in *S. aureus* in order to understand its structure and possible function and analyze its physicochemical properties.

The structure of *S. aureus* was analysed by Phyre2 server, a suite of tools designed for the prediction and analysis of protein structures and functions. The aim of designing Phyre2 was to provide structural biochemists with an easy interface to in silico protein bioinformatics tools. Phyre2 utilizes advansed homology identification methodologies to generate 3-D models, predict inhibitor/activator ligand binding sites) for a suplied protein sequence (Kelley et al., 2015). Until recently structure of thousands of proteins and enzymes were solved by Phyre2. Some of these include structure of glucose-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase (Bayindir et al., 2018; Bayindir et al., 2018; Temel et al., 2020). The structures and functions of GAPDHs have been well documented and the crystal and/or NMR structures were deposited in PDB for a variety of sources some of which include human, archaea, *Bacillus stearothermophilus,* rabbit, *E. coli,* plant and protozoa (Skarzyński et al., 1987; van der Oost et al., 1998; Pavão et al., 2002; ; Cowan-Jacob et al., 2003; Falini et al., 2003; Jenkins and Tanner, 2006 Cook et al., 2009). Generally, the 3-D structures of the GAPDHs for which the structures have been solved revealed to be similar to each other. In each crystal structure solved, crystal structure consists of one tetramer of GAPDH with sub-units related by a 222 non-crystallographic symmetry (Skarzyński et al., 1987). The overall structure of gapB is similar to these as each monomer is composed of two domains; one NAD^+ binding domain and one catalytic domain (Figure 2).

5. Conclusions

In this study physicochemical properties and homology model structure of GAPDH homologue GapB in *S. aureus* were analysed via Protparam tool, Phyre2 homology modeling server and PSIPRED secondary structure analysis tool. ProtParam predicted that GapB was a stable and liposoluble protein. Homology modeling studies revealed that each subunit of GapB is composed of two domians: the NADP coenzyme binding-domain (residues 1 to 153) and the catalytic domain. PSIPRED analysis revealed that the secondary structure of the GapB was madeup by α-helixes (29.91%), extended strands (24.63%) and random coil (45.45%).

Conflict of interest

The author declares that there is no conflict of interests

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