



**הקרן הלאומית למדע**

المؤسسة الإسرائيلية للعلوم

Israel Science Foundation

כ"א בתמוז, תשפ"ה  
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לכבוד  
פרופ' דן תומס מאיור  
כימיה  
אוניברסיטת בר-אילן

לפרופ' מאיור שלום רב,

הנדון: בקשתך למענק מחקר בנושא:  
**חקר של אננטיומרים בביו-סינתזה של חומרי טבע באמצעות מידול מולקולרי**  
**ואינטליגנציה מלאכותית**

הצעת המחקר אשר הגשת לקרן הלאומית למדע לא נכללה, לצערנו, בין ההצעות אשר זכו במענקי מחקר השנה.

מצ"ב עיקרי חוות הדעת.

לתשומת לבך, החלטות הנהלת הקרן מתקבלות על סמך סיקור עמיתים ודיונים בוועדות מקצועיות. ראוי להדגיש כי הוועדה המקצועית אשר בחנה את הצעת המחקר התבססה על מכלול ההערות והציונים שהתקבלו מסוקרים חיצוניים ומחברי הוועדה שהם מומחים בתחום, ונתנה משקל, בדיוניה, רק לאותן ביקורות והערות שהיו מקובלות עליה.

מאחר שכספי ההקצבה השנתית מחולקים עד תום, החלטות הנהלת הקרן הן סופיות ואינן ניתנות לשינוי.

אנו מאחלים לך הצלחה בהמשך דרכך המדעית.

בכבוד רב,

תמר

ד"ר תמר יפה-מיטווד  
מנכ"ל

העתק: רשות המחקר, אוניברסיטת בר-אילן

מוזמנים לבקר באתר קול המדע <https://kolhamada.isf.org.il> - מיזם של הקרן הלאומית למדע, המגיש מידע מדעי מחזית המדע, לציבור ולקהילה המדעית בישראל.

# **Reviewer No. 1**

## **Originality & innovation**

- Role of enantiomers demands more intensive investigation, from this perspective the proposal is rather original and innovative
- The proposed combination of protein structure modelling, reaction modelling, and machine learning approaches is innovative as well, although related approaches have recently been starting to be developed by the applicant as well as others

## **Project importance and contribution to scientific knowledge**

- Given the importance of terpenes, insights into determinants of specificity of their production will be highly valuable
- The methodology to be developed in the proposed project will be applicable in biosynthesis at a much wider scale than only terpenes, adding to the project importance

## **Adequacy of methods**

- A number of methods is proposed, some of which are already in development, others of which have still to be demonstrated. In any case however, the set of methods seems very adequate
- The project seems rather ambitious, possibly slightly overambitious. However the background of the applicant matches very well with the required method development, making it likely that the ambitious goals of the project will be achieved. Even if this would only partially be the case, the project would already strongly contribute to scientific knowledge.

## **Suitability of investigators' scientific background to the project**

- The investigator is very well known for his background in both the terpene synthase enzymes as well as the required computational methodology. He has demonstrated convincingly that his group is the right place for this type of research.

## **Summary (strengths / weaknesses of the proposal)**

### **Strengths:**

- Timely and important project
- Strong emphasis on needed development of computational methodology
- Applicant is world-class leading scientist in this topic

### **Weakness:**

- Experimental validation of predictions seems not to be part of the proposal. However, given the level of connections the applicant has with groups performing experiments on terpene synthases, this will not be a problem; I expect many of the predictions to be very inspiring for groups working on terpene synthases worldwide.
- Possibly the project is a bit overambitious but I don't consider this a strong issue.

## **Reviewer No. 2**

This is a fantastic proposal in all regards. The problem to be tackled – determining the determinants of enantioselectivity for complex reactions in biosynthesis – is challenging and important. The proposed workflow is well-conceived. The proposed database will undoubtedly be useful to many natural products chemists and the proposed ML analysis is worthwhile. The computational methods to be used for mechanistic studies are appropriate and several of them developed by the PI. The PI is a world leader in the area of the proposed research.

## **Reviewer No. 3**

In this proposal Prof Mayor aims to do a machine learning study on the biosynthesis of terpenes. Terpene biosynthesis is very fashionable and I have seen many papers in that area in recent years. They particularly are interested in how the P450s can perform a regioselective or stereospecific reaction mechanism as it may help with the biosynthesis of compounds in biotechnology. Overall that is an ambitious and important goal and will make the proposal and its outcomes relevant.

The author has set up computational approaches previously and aims to apply these to the P450s. That may not work as Prof Shaik has shown that these systems are challenging and the catalysis is affected by small perturbations within the protein or beyond. Strangely, Prof Mayor does not seem to be aware of the bulk of work of Prof Shaik and I recommend him to study the P450 literature that shows how the catalysis is influenced by the second-coordination sphere including charges and electric field effects. Also, some P450s have tight substrate binding pockets whereas other P450s have a large and open substrate binding pocket, which influences substrate positioning and catalysis.

How are the authors going to validate their results? Little is mentioned on how accurate the approaches are and what may be expected.

Finally, k-clustering is not a machine learning method but a statistical method.

## **Reviewer No. 4**

This proposal to investigate the fascinating reactions catalyzed by terpene synthases from Prof. Dan Major, who is a well-established expert in the application of computational approaches to the structure-function relationships underlying the complex chemistry catalyzed by these enzymes, seems somewhat misguided. While the PI is known for his sophisticated application of quantum mechanics (QM) and molecular mechanics (MM) to terpene synthases, as evidenced by the publication of several very insightful papers over his previous funding period, the focus here on enantiomeric pairs is not well justified. In particular, it is evident these simply arise from alternative (enantiomeric) pre-catalytic configuration of the relevant substrate, which would require substantial differences between the relevant active sites, even in those few cases where there is a relatively close phylogenetic relationship between the two enzymes. Thus, it seems almost certain that there is no broadly applicable rule enabling distinction of such enantiomeric biosynthesis to be discovered. It is also disappointing that the distinct mechanistic differences between terpene synthases, which catalyze carbocation cascades, relative to cytochrome P450 mono-oxygenases, work on which is additionally proposed here, but catalyze radical formation instead, is not acknowledged. Instead, the proposal exclusively refers to carbocations. Similarly, while consideration of homology (subfamilies) for the terpene synthases is presented, it is not for the cytochromes P450, where this is almost certainly even more important given the wider phylogenetic differences in this superfamily. The proposed database assembly and associated mechanistic work (at least for the terpene synthases), would seem to provide some broader utility to the proposed work. However, this wider appeal would require ready accessibility. While Prof. Major touts previous development of the EnzDock approach to investigating terpene synthases, no evidence is presented here that this has been more widely adopted. Unfortunately, this leaves such broader applicability for the work proposed here in question. Although the proposed database would almost certainly provide some insight into terpene synthases (if not also cytochromes P450), even this aspect requires further clarification – e.g., there are almost certainly too many terpene synthases and cytochromes P450 to reasonably investigate via the proposed approach. Thus, while this proposal generates some enthusiasm, it is strongly muted by these myriad issues.

## **Reviewer No. 5**

This project aims at building an extensive database of isoprenoid occurrence and enzymatic derivation throughout living organisms. Several databases on natural isoprenoids exist, but none of them is fully comprehensive, and the first part of the project aims at combining them into a consistent single inventory. Given the expertise of the team, this will be a relatively trivial task. Much more complex will be the following one, namely the one focused on the various enzymes involved in isoprenoid synthesis, with a special focus on the origin of enantiomeric compounds in distinct phyla. This is not a unique feature of isoprenoids, and a remarkable example in the field of sugar is fucose, which occurs in different enantiomeric forms in plants and in animals. Nevertheless, this chirality fluidity is by far more represented in isoprenoids than in any other class of secondary metabolites. In addition, a single organism can produce an uneven ratio of specific isoprenoids, resulting in a scalemic state. For the second part of the project, AI and molecular modelling will be critical, since most enzymes involved in isoprenoid biosynthesis are unknown or poorly characterized, and the elaboration of the current enzymatic databases into a consistent landscape involve a multifactorial analysis well suited to AI-guided modelling. The results of this analysis will also be important to validate AI as a guide for molecular modelling in the pursuit of the identification of classes of enzymatic mechanisms, something that, at cursory examination, seems overambitious.

The end-result of this project will be generation of publicly available databases on isoprenoids and isoprenoid-generating enzymes. The whole isoprenoid community will greatly benefit for this work, with critical impact in the burgeoning field of synthetic biology, a major user of these data. Another issue of relevance will be the validation of AI-guided molecular modelling at enzyme level to capture general mechanistic features for such a diverse class of enzymes like those P-450 based.

The methods on which the project is based are state-of-the art, and the expertise of the investigator excellent. The potential of AI to address the compounds and enzymes complexity involved in the project is unknown, or at least, not yet validated. While this makes the end-results somewhat iffy, at the same time it will be a litmus-test for the methodology, and I do not know if to consider this as a strength or a weakness of the proposal, which is original, innovative and poised to serve at the basis for the synthetic-biology based production of isoprenoids of commercial relevance.