



Engineering of a Plant Isoprenyl Diphosphate Synthase for Development of Irregular Coupling Activity

Iryna Gerasymenko,^{*[a, b]} Yuriy V. Sheludko,^[a, b, d] Ismael Navarro Fuertes,^[c] Volker Schmidts,^[d] Lara Steinel,^[a, b] Elisabeth Haumann,^[a, b] and Heribert Warzecha^[a, b]

We performed mutagenesis on a regular isoprenyl diphosphate synthase (IDS), neryl diphosphate synthase from *Solanum lycopersicum* (SINPPS), that has a structurally related analogue performing non-head-to-tail coupling of two dimethylallyl diphosphate (DMAPP) units, lavandulyl diphosphate synthase from *Lavandula x intermedia* (LiLPPS). Wild-type SINPPS catalyses regular coupling of isopentenyl diphosphate (IPP) and DMAPP in *cis*-orientation resulting in the formation of neryl diphosphate. However, if the enzyme is fed with DMAPP only, it is able to catalyse the coupling of two DMAPP units and synthesizes two irregular monoterpene diphosphates; their structures were elucidated by the NMR analysis of their dephosphorylation products. One of the alcohols is lavandulol. The second compound is the *trans*-isomer of planococcol, the first example of an irregular cyclobutane monoterpene with this stereochemical configuration. The irregular activity of SINPPS constitutes 0.4% of its regular activity and is revealed only if the enzyme is supplied with DMAPP in the absence of IPP. The exchange of asparagine 88 for histidine considerably enhanced the non-head-to-tail coupling. While still only observed in the absence of IPP, irregular activity of the mutant reaches 13.1% of its regular activity. The obtained results prove that regular IDS are promising starting points for protein engineering aiming at the development of irregular activities and leading to novel monoterpene structures.

organisms or taxonomic groups and are postulated to take part in the communication with the environment.^[2] Many natural terpenoids and their derivatives are indispensable as medicines.^[3]

Biosynthesis of all terpenes proceeds through the formation of isoprenyl diphosphates containing five carbon atoms (C5 units). Except for hemiterpenes that are produced by elimination of diphosphate from C5 molecules, all other groups of terpenes are formed by the condensation of two or more C5 building blocks. This reaction is catalysed by isoprenyl diphosphate synthases (IDS). In most cases, biosynthesis of a terpene starts with the head-to-tail (1'-4) coupling of isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP). The chain can be elongated by head-to-tail linkage of further IPP units by IDS or *via* head-to-head (1'-1) dimerization of C15 or C20 isoprenyl diphosphates catalysed by squalene or phytoene synthases, respectively. The compounds formed by this mechanism are referred to as regular terpenes. The limited number of basic linear carbon skeletons can be further cyclized and/or modified in multiple ways by terpene synthases and other enzymes (e.g. cytochromes P450) which results in a wide range of regular terpene structures.^[4]

In addition, there exists an alternative way of increasing the structural diversity of terpenes. The so-called irregular monoterpenes are formed by head-to-middle linkage of two C5 units, in this case two DMAPP molecules. This coupling leads directly to the formation of branched or cyclized monoterpenes. While regular terpenes are present in all living organisms, irregular structures (Figure S3.1) are reported from a narrow number of species. However, some of these compounds are of particular interest to humans. Pyrethrins, the derivatives of chrysanthemyl diphosphate with a cyclopropane skeleton that occur in the flowers of *Tanacetum cinerariaefolium*, and chemically produced pyrethroids possess high insecticidal activity but are non-toxic


Terpenes display a vast diversity of structures and functions. This extraordinary group includes primary metabolites that are widely distributed in nature and play essential roles as membrane constituents, hormones, and components of photosynthetic systems.^[1] The majority of terpenes belong to the group of specialized compounds that are specific for certain


[a] I. Gerasymenko, Y. V. Sheludko, L. Steinel, E. Haumann, H. Warzecha
 Technische Universität Darmstadt
 Plant Biotechnology and Metabolic Engineering
 Schnittspahnstraße 4, 64287 Darmstadt (Germany)
 E-mail: gerasymenko@bio.tu-darmstadt.de


[b] I. Gerasymenko, Y. V. Sheludko, L. Steinel, E. Haumann, H. Warzecha
 Centre for Synthetic Biology
 Technical University of Darmstadt, 64287 Darmstadt (Germany)

[c] I. Navarro Fuertes
 Ecología y Protección Agrícola SL
 Pol. Ind. Ciutat de Carlet, 46240 Carlet, Valencia (Spain)

[d] Y. V. Sheludko, V. Schmidts
 Clemens-Schöpf-Institut für Organische Chemie und Biochemie
 Technische Universität Darmstadt
 Alarich-Weiss-Str. 4, 64287 Darmstadt (Germany)

 Supporting information for this article is available on the WWW under <https://doi.org/10.1002/cbic.202100465>

 This article is part of a Special Collection dedicated to the NextGenBiochem 2021 virtual symposium. To view the complete collection, visit our homepage.

 © 2021 The Authors. ChemBioChem published by Wiley-VCH GmbH. This is an open access article under the terms of the Creative Commons Attribution Non-Commercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

for mammals and avians, and rapidly degrade in the fields.^[5] Various irregular monoterpene compounds displaying branched, cyclopropane and cyclobutane skeletons are found among insect pheromones.^[6] The pheromones can be applied for targeted insect pest management directed against the defined harmful species. Lipid-derived pheromones that can be obtained by economically effective methods have been implemented in different controlling strategies, e.g. for mass trapping and mating disruption.^[7] But for irregular terpenes, the chemical synthesis is inefficient and often involves dangerous reagents.^[8,9] An attractive alternative would be the application of enzymes for the formation of irregular monoterpene structures that can render the production of target compounds effective and sustainable.^[10]

IDS enzymes performing the coupling of C5 units fall into two groups according to the stereochemical configuration of their products. Formation of all-*E* chains, e.g. C10 (*E*)-geranyl diphosphate (GPP), C15 (*E,E*)-farnesyl diphosphate (FPP), and C20 (*E,E,E*)-geranylgeranyl diphosphate (GGPP), is catalysed by *trans*-IDS. The products of *cis*-IDS, e.g. C10 neryl diphosphate or C55 undecaprenyl diphosphate, contain one or more double bonds with *Z* configuration. The structures of *trans*- and *cis*-IDS differ considerably in spite of the identical substrates and reaction mechanism.^[11] The coupling reaction starts with the abstraction of diphosphate moiety from DMAPP leading to the formation of an allylic carbocation at C1. The carbocation undergoes nucleophilic attack from the double bond of the second substrate molecule. In the case of regular coupling, the bond between C1 of DMAPP and C4' of IPP is formed. This is followed by deprotonation at C2' that defines *trans*- or *cis*-configuration of the product.^[12] The irregular structures are formed if the carbocation is attacked by the double bond of the second DMAPP unit. The head-to-middle condensation can be performed by IDS of both *trans* and *cis* type.^[13,14]

Examples of enzymes known to be capable of irregular coupling are scant, but they come from all domains of life. Among Eukarya, IDS of plant origin are known to produce irregular monoterpene structures. Enzymes of *trans*-IDS-type from *Tanacetum (Chrysanthemum) cinerariaefolium*^[15] and *Artemisia tridentata*^[16] form chrysanthemyl diphosphate with the cyclopropane ring by 1'-2-3 coupling as the main product along with minor amounts of lavandulyl diphosphate. Branched lavandulyl diphosphate is generated by 1'-2 linkage performed by a *cis*-IDS from *Lavandula x intermedia*.^[17] From Bacteria and Archaea, *cis*-type IDS catalysing 1'-2 linkage, in some cases followed by 4-3'-cyclization, were reported.^[18-21] Enzymes responsible for the formation of irregular monoterpenes with cyclobutane ring have not been identified yet. An inefficient promiscuous chrysanthemyl diphosphate synthase from *Artemisia tridentata* was reported to synthesize trace amounts of macconellyl diphosphate (1 ± 1%, if supplied with 3 mM DMAPP only^[13]). Enzymatic synthesis of macconellyl and planococcyll diphosphates was observed in studies on chimeric proteins composed of parts of regular and irregular *trans*-IDS, but, especially for planococcyll diphosphate, on a very low level.^[13,22] Here we describe the irregular coupling activity of a *cis*-IDS from *Solanum lycopersicum*. This is the first example of a

natural enzyme efficiently producing a cyclobutane ring structure. The rational design of the protein allowed for a considerable increase of irregular activity.

Neryl diphosphate synthase from *Solanum lycopersicum* (*S/NPPS*) was reported to catalyse the condensation of IPP and DMAPP into a *cis*-configured C10 neryl diphosphate (NPP).^[23] No other products were detected when the enzyme was supplied with both C5 substrates. If fed with DMAPP only, *S/NPPS* formed two C10 products in proportion 2:1, as calculated based on LC-MS peak areas (Figure S3.2). Their retention times on LC-MS chromatogram differed distinctly from those of the regular C10 prenyl diphosphates, *E*-GPP and *Z*-NPP. The retention time of the main product corresponded to that of lavandulyl diphosphate, the branched monoterpene formed by 1'-2 linkage (Figure S3.3).

For structure elucidation, the prenyl diphosphates were treated with alkaline phosphatase and the resulting alcohols were purified by preparative TLC and subjected to NMR analysis. After TLC separation, one of the alcohols showed the R_f value identical to that of the reference sample of lavandulol. The NMR spectra of the compound (Table S1) indicate that it has the branched structure, and ¹H and ¹³C NMR signals were in agreement with the literature data of lavandulol.^[24] The assignments of the signals were confirmed by 2D NMR experiments (Figure S3.4–S3.8).

The TLC mobility of the second compound matched the behaviour of cyclobutane monoterpenes, planococcol and maconelliol, and its structure was elucidated by NMR (Table 1, Figure S3.9–S3.14). In contrast to lavandulol, ¹H NMR spectrum of the second product displayed only two alkene signals. Two protons at δ 4.84 ppm and 4.63 ppm were assigned to the vinyl carbon C7 at δ 109.44. The signal at δ 145.87 ppm belongs to a quaternary carbon C6, suggesting a terminal vinyl group. Two protons with chemical shifts δ 3.86 (dd, *J* = 10.9, 7.6 Hz) and 3.71 (dd, *J* = 10.9, 7.6 Hz), characteristic for an alcohol functional group, were assigned to the carbon C5 at δ 64.45. CLIP-COSY^[25] experiment (Figure S3.13) revealed the interaction between C5 alcohol protons and a proton at δ 1.85 (m, 1H), on the one hand, and long-range coupling between C7 vinyl protons and a signal at δ 2.57 ppm (t, *J* = 9.6 Hz) on the other. In turn, both protons (at δ 1.85 and δ 2.57) exhibited coupling with two protons at δ 1.61 and δ 2.12, attached to a secondary carbon at

Table 1. ¹H and ¹³C NMR data of planococcol in CDCl₃. Values are in ppm. The multiplicities and coupling constants (*J* in Hz) are in parentheses.

Position	¹ H NMR	¹³ C NMR
1	1.85 (m, 1H)	43.22
2	–	39.87
3	2.57 (t, 1H, <i>J</i> = 9.6 Hz)	48.38
4	1.62 ^[a] (m, 1H); 2.12 ^[a] (m, 1H)	22.28
5	3.86 (dd, 1H, <i>J</i> = 10.9, 7.6 Hz); 3.71 (dd, 1H, <i>J</i> = 10.9, 7.6 Hz)	64.45
6	–	145.87
7	4.84 (s, 1H); 4.63 (s, 1H)	109.44
8	1.66 (s, 3H)	23.33
9	1.13 (s, 3H)	24.39
10	0.97 (s, 3H)	24.98

[a] Signal values were retrieved from HSQC spectra.

δ 22.28. The obtained results suggest a six-carbon chain bearing terminal vinyl and alcohol functions. Additional long-range coupling of the vinyl protons with a three proton singlet at δ 1.66 indicates a methyl group in a vicinal position. Two additional methyl groups were identified as singlets at δ 0.97 and δ 1.13. They both did not couple to other hydrogens, but HMBC experiment showed their correlations with C1 and C3 (δ 43.22 and δ 48.38, respectively), a quaternary carbon C2 (δ 39.87) and between themselves (Figure S3.12). Additional HMBC signals were registered between the quaternary carbon C2 (δ 39.87) and hydrogens at C3, C4, and C5. The obtained data suggest the structure of cyclobutane monoterpene planococcol (Figure 1).

To determine the relative stereochemistry of cyclobutane ring, the data of the NOESY experiment were analysed (Figure S3.14 and S3.15). For H1, NOE signals were observed with H5 and 3H10 while H3 interacted with 3H9 and 3H8. Interpretation of these data using 3D models of *cis*- and *trans*-planococcol led to the assignment of a *trans* configuration of the molecule (Figure S3.16). In *cis* configuration, the calculated distance between H1 and H3 was 2.4 Å which should give a clear NOE signal. However, no corresponding cross peak was detected (Figure S3.15). Moreover, in *cis* configuration, both H1 and H3 must interact with the protons of only one of two (C9 and C10) methyl groups, while in *trans* configuration, these protons interact with the methyl groups at C2 separately (Figure S3.16).

To assign the absolute stereochemistry of the identified irregular terpenes, we considered the biosynthesis of irregular terpenes by *LiLPPS*,^[17] which is structurally homologous to *SiNPPS*. During irregular coupling, dimethylallyl cation in the donor region (S1) undergoes nucleophilic attack by the double bond of the DMAPP located in the acceptor region (S2) of the active site.^[13,14] This leads to the formation of a 1-2' bond and a chiral centre at C2' carbon. The formed tertiary carbocation intermediate undergoes deprotonation, yielding branched lavandulyl diphosphate, or cyclization followed by deprotonation giving *trans*-planococcol. The relative positions of the electrophilic carbocation and DMAPP in the donor and acceptor sites of the active centre determine the chirality at C2' of the formed

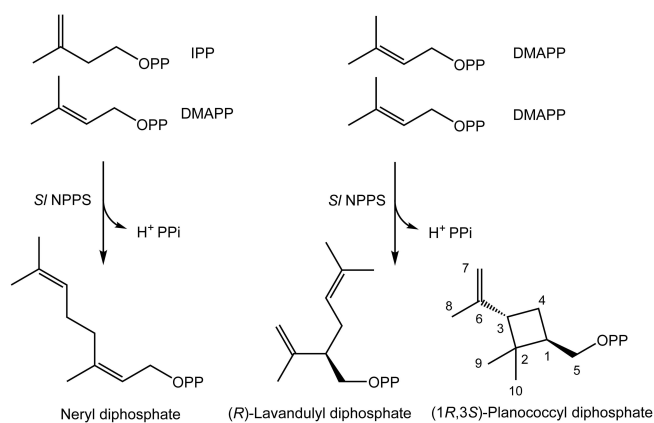


Figure 1. Products of alternative reactions catalysed by *SiNPPS*.

products. Similar positions of key amino acid residues in *LiLPPS* and *SiNPPS* (Figure 2) suggest similar relative positions of the substrates for both enzymes and, consequently, absolute configuration of stereocentre of lavandulyl diphosphate as *R* and *trans*-planococcol diphosphate as *1R* and *3S*.

SiNPPS demonstrated normal Michaelis-Menten saturation curves in assays with two C5 substrates (one kept at a constant concentration of 1000 μM , and the second varied from 50 μM to 1000 μM) and with DMAPP only (in the concentration range from 50 μM to 1000 μM). The K_M values were comparable for both assays. In the reaction with two substrates, the K_M values were calculated as $273 \pm 38 \mu\text{M}$ for IPP and $243 \pm 212 \mu\text{M}$ for DMAPP. For coupling of two DMAPP molecules, the K_M value was determined to be $275 \pm 147 \mu\text{M}$. However, the turnover number (k_{cat}) was significantly lower for the irregular reaction than for the coupling of IPP and DMAPP ($0.42 \pm 0.08 \times 10^{-3} \text{ s}^{-1}$ and $111 \pm 23 \times 10^{-3} \text{ s}^{-1}$, respectively). Specific activity measured at the saturating substrate concentrations (1000 μM of each substrate or 1000 μM of DMAPP) was $1228 \pm 85 \text{ nkat/g}$ for NPP production and $4.52 \pm 0.28 \text{ nkat/g}$ for the irregular coupling. Thus, in spite of the similar substrate binding constants, the irregular activity of *SiNPPS* constitutes *ca.* 0.4% of its regular activity and is revealed only if the enzyme is supplied with DMAPP in the absence of IPP.

SiNPPS has a structurally related analogue performing non-head-to-tail coupling of two dimethylallyl diphosphate (DMAPP) units, lavandulyl diphosphate synthase from *Lavandula x intermedia* (*LiLPPS*).^[17] The analysis of crystal structure of *LiLPPS* suggested that histidine residue in position 78 plays a crucial role in catalysis by facilitating the release of diphosphate moiety from DMAPP. The H78N mutant of *LiLPPS* lost the irregular activity completely.^[26] Now we demonstrate that the reverse

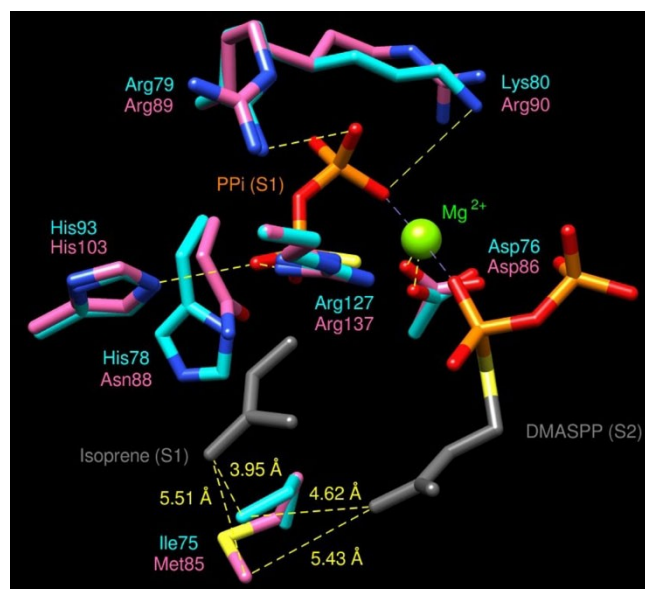


Figure 2. Comparison of active centres of *LiLPPS* and *SiNPPS*. The crystal structure of *LiLPPS*/DMASPP/isoprene/PPI/ Mg^{2+} complex^[24] was superimposed with the model of *SiNPPS*. The amino acid residues of *LiLPPS* and *SiNPPS* are shown in cyan and pink, respectively.

exchange in a regular *cis*-IDS, *SINPPS*, leads to a significant increase of the irregular coupling. *SINPPS* has asparagine in the corresponding position (N88, Figure 2), as is often the case for regular *cis*-IDS enzymes. In the reaction with two substrates, the N88H mutant demonstrated elevated K_M values of $457 \pm 91 \mu\text{M}$ for IPP and $391 \pm 96 \mu\text{M}$ for DMAPP. The specific activity at the saturating concentrations of substrates (1000 μM each) dropped to $914 \pm 120 \text{ nkat/g}$, and the turnover number decreased to $87 \pm 15 \times 10^{-3} \text{ s}^{-1}$. In contrast to this, a considerable enhancement of the irregular activity was observed. To obtain a normal Michaelis-Menten saturation curve, the substrate concentration range was extended to 4000 μM DMAPP. Although this reflected a significantly higher K_M value ($1394 \pm 307 \mu\text{M}$), the turnover number increased about 20-fold, to $8.27 \pm 1.53 \times 10^{-3} \text{ s}^{-1}$. The specific irregular activity of the N88H mutant (measured at 4000 μM DMAPP) reached $120 \pm 28 \text{ nkat/g}$, and constitutes 13.1% of its regular activity (Figure 3), although the coupling of two DMAPP units is still possible only in the absence of IPP. The mutant produces LPP and PPP in the same 2:1 ratio as the wild-type enzyme. Enzyme variants possessing other amino acids in position 88 (glutamine, aspartate, lysine, arginine, tyrosine, phenylalanine, tryptophan, valine, leucine, isoleucine, cysteine, proline) did not display irregular activity. Regular activity of these mutants was not investigated.

Comparison of the active sites of *LilPPS* and *SINPPS* shows that, while the residues that coordinate magnesium ion (D76/D86) and diphosphate moiety in S1 site (R79/R89, K80/R90, H93/H103, R127/R137) are identical or similar, the binding of isoprene parts is different (Figure 2). The side chain of isoleucine 75 in *LilPPS* is inserted between the dimethyl groups of substrates, and strongly influences the outcome of the reaction.^[27] In *SINPPS*, this position is occupied by methionine with the side chain predicted to be shifted to longer distances from the isoprene units. When the exchange of asparagine 88 to histidine leads to faster detachment of PPI and isoprene in

S1,^[26] the introduction of isoleucine into position 85 may be necessary to ensure better binding of the isoprene moiety. For irregular *cis*-IDS enzymes, an extensive analysis of structure-function relationship has been carried out, and the residues defining the shape of the active sites were suggested.^[14] Further mutagenesis in the analogous positions of *SINPPS*-N88H may improve the catalytic properties of the enzyme.

The broad distribution of irregular monoterpenes within the higher taxonomic ranks is in the same time restricted to a limited number of genera and species. It leads to the assumption that the enzymes producing irregular monoterpenes have evolved repeatedly from their regular counterparts. Comparison of two *trans*-IDS from *Tanacetum cinerariifolium*, regular farnesyl diphosphate synthase (*TcFPPS*) and irregular chrysanthemyl diphosphate synthase (*TcCPS*), shows that only few amino acid exchanges are sufficient to switch the coupling pattern of the enzyme.^[15] Four mutations accumulated in the *TcCPS* gene were suggested to be responsible for the shift of activity.^[28] It should be noted, however, that the attempts to metamorphose a regular *trans*-IDSs from *Artemisia tridentata* ssp., farnesyl diphosphate synthase (*AtFPPS*), into an irregular enzyme by point mutations in the active site were unsuccessful. None of the *AtFPPS* mutants with single or multiple replacements in the active site designed by comparison with a promiscuous chrysanthemyl diphosphate synthase from the same plant (*AtCPS*) were able to synthesize irregular products. Contrariwise, the introduction of the amino acids of *AtFPPS* into the active site of *AtCPS* enhanced regular activity and substantially diminished irregular coupling.^[13]

We performed mutagenesis on a regular *cis*-IDS, neryl diphosphate synthase from *Solanum lycopersicum* (*SINPPS*), that has a structurally related analogue performing non-head-to-tail coupling of two dimethylallyl diphosphate (DMAPP) units, lavandulyl diphosphate synthase from *Lavandula x intermedia* (*LilPPS*). The discovered irregular side activity of *SINPPS* shows that this enzyme has potential for the development of new functions.^[29] *SINPPS* is the first example of a natural protein capable of efficient 1'-2-3-2' coupling of two DMAPP units resulting in cyclobutane irregular monoterpenes. *SINPPS* produces a *trans*-isomer of planococcyll diphosphate. It is noteworthy, that all compounds with this skeleton obtained previously by enzyme-catalysed reactions or from natural sources had *cis* configuration. These structures have been found only in mealybugs that use derivatives of *cis*-planococcol and *cis*-maconelliol as sex pheromones.^[6] The biological activity of *trans*-isomer of planococcyll acetate was reported to be approximately 10 times lower.^[30] However, enzymes responsible for the biosynthesis of cyclobutane monoterpenes in mealybugs have not been identified yet. Enzymatic synthesis of low amounts of *cis*-maconelliol and *cis*-planococcyll diphosphate was observed in studies on chimeric proteins constructed by combining parts of a regular and an irregular *trans*-IDS, FPPS and CPS from *Artemisia tridentata*.^[13,22] It indicates that IDS of *cis* and *trans* type give rise to products with different stereochemical configurations not only in regular, but also in the irregular coupling reactions.

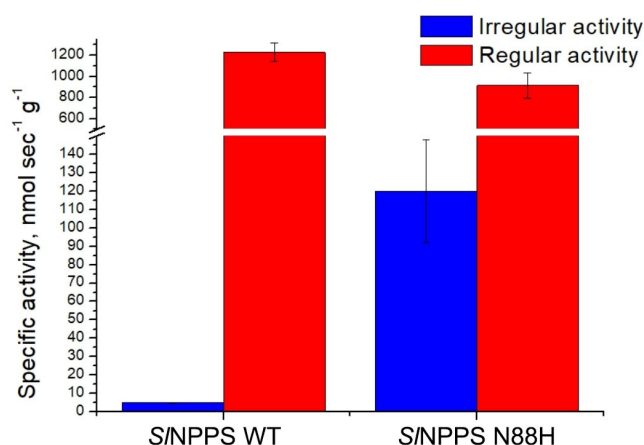


Figure 3. Specific activity of the wild-type *SINPPS* and N88H mutant. Regular activity: quantity of NPP (in nmol) produced in an assay with 1 mM IPP and 1 mM DMAPP as substrates in 1 sec per 1 g of the enzyme; irregular activity: sum of LPP and PPP (in nmol) produced in an assay with only DMAPP as substrate (1 mM for wild type *SINPPS*; 4 mM for N88H mutant) in 1 sec per 1 g of the enzyme. Bars represent the standard deviation for three measurements.

Another example of the development of irregular activity in a regular *cis*-IDS enzyme was described in the study of *Z,Z*-farnesyl diphosphate synthase from tomato. Exchange of histidine 103 for tyrosine resulted in the synthesis of lavandulyl diphosphate and presumably another irregular monoterpene, although its structure was not resolved.^[31] Together with our results, these findings prove that regular IDS are promising starting points for protein engineering aiming at the development of irregular activities and leading to novel monoterpene structures.

Acknowledgements

The research was supported by the European Research Area Cofund Action 'ERACoBioTech' under Horizon 2020, the project SUSPHIRE (Sustainable Production of Pheromones for Insect Pest Control in Agriculture). I.G., E.H., and H.W. acknowledge the support by German Federal Ministry of Education and Research (BMBF), grant number 031B0605. I.N.F. acknowledges the support of the Centre for the Development of Industrial Technology (CDTI) of the Spanish Ministry of Science and Innovation. The authors thank Prof. Christina M. Thiele, Technische Universität Darmstadt, for measurement time on the 700 MHz NMR spectrometer. Open Access funding enabled and organized by Projekt DEAL.

Conflict of Interest

The authors declare no conflict of interest.

Keywords: irregular terpenes · isoprenyl diphosphate synthase · planococcol · protein engineering · terpenoids

- [1] E. Pichersky, R. A. Raguso, *New Phytol.* **2018**, *220*, 692–702.
- [2] J. Gershenzon, N. Dudareva, *Nat. Chem. Biol.* **2007**, *3*, 408–414.
- [3] M. E. Bergman, B. Davis, M. A. Phillips, *Molecules* **2019**, *24*.
- [4] A. M. Boutanaev, T. Moses, J. Zi, D. R. Nelson, S. T. Mugford, R. J. Peters, A. Osbourn, *Proc. Natl. Acad. Sci. USA* **2015**, *112*, E81–88.
- [5] N. Matsuo, *Proc. Jpn. Acad. Ser. B* **2019**, *95*, 378–400.
- [6] Y. Zou, J. G. Millar, *Nat. Prod. Rep.* **2015**, *32*, 1067–1113.
- [7] S. A. H. Rizvi, J. George, G. V. P. Reddy, X. Zeng, A. Guerrero, *Insect Sci.* **2021**, *12*.

- [8] L. C. Passaro, F. X. Webster, *J. Agric. Food Chem.* **2004**, *52*, 2896–2899.
- [9] A. Zada, E. Dunkelblum, M. Harel, F. Assael, S. Gross, Z. Mendel, *J. Econ. Entomol.* **2004**, *97*, 361–368.
- [10] R. A. Sheldon, J. M. Woodley, *Chem. Rev.* **2018**, *118*, 801–838.
- [11] Y. Gao, R. B. Honzatko, R. J. Peters, *Nat. Prod. Rep.* **2012**, *29*, 1153–1175.
- [12] Y. Kharel, T. Koyama, *Nat. Prod. Rep.* **2003**, *20*, 111–118.
- [13] J. S. Lee, J. J. Pan, G. Ramamoorthy, C. D. Poulter, *J. Am. Chem. Soc.* **2017**, *139*, 14556–14567.
- [14] M. Kobayashi, T. Kuzuyama, *ChemBioChem* **2019**, *20*, 29–33.
- [15] S. B. Rivera, B. D. Swedlund, G. J. King, R. N. Bell, C. E. Hussey, Jr., D. M. Shattuck-Eidens, W. M. Wrobel, G. D. Peiser, C. D. Poulter, *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 4373–4378.
- [16] A. Hemmerlin, S. B. Rivera, H. K. Erickson, C. D. Poulter, *J. Biol. Chem.* **2003**, *278*, 32132–32140.
- [17] Z. A. Demissie, L. A. Erland, M. R. Rheault, S. S. Mahmoud, *J. Biol. Chem.* **2013**, *288*, 6333–6341.
- [18] T. Ozaki, P. Zhao, T. Shinada, M. Nishiyama, T. Kuzuyama, *J. Am. Chem. Soc.* **2014**, *136*, 4837–4840.
- [19] R. Teufel, L. Kaysser, M. T. Villaume, S. Diethelm, M. K. Carbullido, P. S. Baran, B. S. Moore, *Angew. Chem. Int. Ed.* **2014**, *53*, 11019–11022; *Angew. Chem.* **2014**, *126*, 11199–11202.
- [20] T. Ogawa, K. Emi, K. Koga, T. Yoshimura, H. Hemmi, *FEBS J.* **2016**, *283*, 2369–2383.
- [21] K. I. Emi, K. Sompiyachoke, M. Okada, H. Hemmi, *Biochem. Biophys. Res. Commun.* **2019**, *520*, 291–296.
- [22] H. V. Thulasiram, H. K. Erickson, C. D. Poulter, *Science* **2007**, *316*, 73–76.
- [23] A. L. Schillmiller, I. Schauvinhold, M. Larson, R. Xu, A. L. Charbonneau, A. Schmidt, C. Wilkerson, R. L. Last, E. Pichersky, *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 10865–10870.
- [24] H. J. Kim, L. Su, H. Jung, S. Koo, *Org. Lett.* **2011**, *13*, 2682–2685.
- [25] M. R. M. Koos, G. Kummerlowe, L. Kaltschnee, C. M. Thiele, B. Luy, *Angew. Chem. Int. Ed.* **2016**, *55*, 7655–7659; *Angew. Chem.* **2016**, *128*, 7785–7789.
- [26] M. Liu, C. C. Chen, L. Chen, X. Xiao, Y. Zheng, J. W. Huang, W. Liu, T. P. Ko, Y. S. Cheng, X. Feng, E. Oldfield, R. T. Guo, Y. Ma, *Angew. Chem. Int. Ed.* **2016**, *55*, 4721–4724; *Angew. Chem.* **2016**, *128*, 4799–4802.
- [27] T. Tomita, M. Kobayashi, Y. Karita, Y. Yasuno, T. Shinada, M. Nishiyama, T. Kuzuyama, *Angew. Chem. Int. Ed.* **2017**, *56*, 14913–14917; *Angew. Chem.* **2017**, *129*, 15109–15113.
- [28] P. L. Liu, J. N. Wan, Y. P. Guo, S. Ge, G. Y. Rao, *BMC Evol. Biol.* **2012**, *12*, 214.
- [29] F. H. Arnold, *Q. Rev. Biophys.* **2015**, *48*, 404–410.
- [30] B. A. Bierl-Leonhardt, D. S. Moreno, M. Schwarz, J. Fargerlund, J. R. Plimmer, *Tetrahedron Lett.* **1981**, *22*, 389–392.
- [31] Y. T. Chan, T. P. Ko, S. H. Yao, Y. W. Chen, C. C. Lee, A. H. Wang, *ACS Omega* **2017**, *2*, 930–936.

Manuscript received: August 31, 2021
Revised manuscript received: October 20, 2021
Accepted manuscript online: October 21, 2021
Version of record online: November 5, 2021