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Molecular modeling of structure-dynamics-function relationships in proteins

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Promotor: dr hab. Jan Brezovsky, prof. UAM

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Contents

List of publications

Publications included in the thesis

1. Research article

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3. Research article

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4. Preprint

   Grulich, M.[‡]; **Surpeta, B.**[‡]; Palyzová, A.; Marešová, H.; Zahradník, J.; Brezovsky, J. Rational engineering of binding pocket's structure and dynamics in penicillin G acylase for selective degradation of bacterial signaling molecules. bioRxiv 2023.05.09.538545; doi: https://doi.org/10.1101/2023.05.09.538545

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1. Review article

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2. Research article

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Abstract

Ongoing advances in science and computational methods and technology make it possible to study proteins better and better. Over the decades, it has become clear that proteins are dynamic entities, and to accurately study their function, it is not enough to look at the structure only, but also at the inherent dynamics component. As a result, the original structure-function paradigm is gradually being replaced by a focus on structure-dynamics-function relationships.

In my doctoral research, I contributed to addressing the global challenge of widespread bacterial resistance to antibiotics and focused on alternative solutions that will undoubtedly be needed in the future. Bacteria exhibit social behavior and use signaling molecules to communicate with each other and respond to environmental changes in a cell-density-dependent manner, a process called quorum sensing. Disruption of this communication is called quorum quenching and is considered a promising alternative to deal with bacterial colonies in various fields of life. It can be achieved enzymatically, by cleavage of signaling compounds, and thus such quorum quenching enzymes were the main interest of my thesis. In addition, these enzymes are useful models for studying the relationships between structure, dynamics and function in proteins.

The dissertation consists of four articles. In the first, I investigated details of the dynamic components that determine and limit the quorum quenching activity in N-terminal serine hydrolases. The second is a literature review that summarizes approaches in protein engineering that consider dynamics as crucial during the design process. The third part presents the TransportTools library, software developed in our laboratory to address the challenge of consistent analysis of interior spaces in protein ensemble data resulting from massive molecular dynamics simulations. Finally, the last part of the thesis combines the insights gained from the study of the wild-type quorum quenching enzymes, approaches for efficient design of protein dynamics, and advantages of the developed software to rationally design variants of *Escherichia coli* penicillin G acylase with improved activities and modulated specificities towards signaling molecules of different pathogenic bacterial species.

Keywords

protein dynamics, structure-dynamics-function relationships, molecular dynamics, protein engineering, quorum quenching

Streszczenie

Stały postęp nauki oraz metod i technologii obliczeniowych pozwala na coraz lepsze badanie białek. W ciągu kilkudziesięciu lat stało się jasne, że białka są dynamicznymi jednostkami i aby dokładnie zbadać ich funkcję, nie wystarczy spojrzeć tylko na strukturę, ale także na nieodłączny element dynamiki. W rezultacie, pierwotny paradygmat struktura-funkcja jest stopniowo zastępowany przez ukierunkowanie na relacje struktura-dynamika-funkcja.

W moich badaniach doktoranckich przyczyniłem się do rozwiązywania globalnego problemu powszechnej oporności bakterii na antybiotyki i skupiłem się na alternatywnych rozwiązaniach, które niewątpliwie będą potrzebne w przyszłości. Bakterie wykazują zachowania społeczne i używają cząsteczek sygnalizacyjnych do komunikowania się ze sobą i reagowania na zmiany środowiskowe w sposób zależny od gęstości komórek, proces ten nazywany jest wykrywaniem kworum (*ang. quorum sensing*). Zakłócenie tej komunikacji nazywane jest wygaszaniem kworum (*ang. quorum quenching*) i jest uważane za obiecującą alternatywę radzenia sobie z koloniami bakterii w różnych dziedzinach życia. Można to osiągnąć enzymatycznie, poprzez inaktywację związków sygnałowych i dlatego takie enzymy wygaszające kworum były głównym przedmiotem zainteresowania mojej pracy doktorskiej. Ponadto enzymy te są użytecznymi modelami do badania zależności pomiędzy strukturą, dynamiką i funkcją w białkach.

Rozprawa składa się z czterech artykułów. W pierwszym z nich szczegółowo badam dynamiczne komponenty, które determinują i ograniczają aktywność wygaszania kworum w N-terminalnych hydrolazach serynowych. Drugi to przegląd literatury podsumowujący podejścia w inżynierii białek, które uwzględniają dynamikę jako kluczową podczas procesu projektowania. Trzecia część przedstawia bibliotekę TransportTools, oprogramowanie opracowane w naszym laboratorium w celu sprostania wyzwaniu spójnej analizy danych zespołów białkowych będących wynikiem masowych symulacji dynamiki molekularnej. W końcu, ostatnia część rozprawy łączy spostrzeżenia uzyskane z badań dzikich enzymów wygaszających kworum, podejścia do efektywnego projektowania dynamiki białek oraz zalety opracowanego oprogramowania w celu racjonalnego zaprojektowania wariantów acylazy penicyliny G *Escherichia coli* o poprawionej aktywności i zmodulowanej specyficzności wobec cząsteczek sygnałowych różnych patogennych gatunków bakterii.

Słowa kluczowe

dynamika białek, zależności struktura-dynamika-funkcja, dynamika molekularna, inżynieria białek, wygaszanie kworum

Abbreviations

NMR	nuclear magnetic resonance
cryo-EM	cryo-electron microscopy
PDB	Protein Data Bank
SFX	serial femtosecond crystallography
XFELs	X-ray free-electron lasers
HDX-MD	hydrogen-deuterium exchange mass spectrometry
FRET	single-molecule fluorescence resonance energy transfer
MD	molecular dynamics
BPTI	bovine pancreatic trypsin inhibitor
GPUs	graphics processing units
QM/MM	quantum mechanics/molecular mechanics
sMD	steered molecular dynamics
US	umbrella sampling
HTMD	high-throughput molecular dynamics
CG	coarse-grained
COVID-19	coronavirus disease 2019
SARS-COV-2	severe acute respiratory syndrome coronavirus 2
QS	quorum sensing
AHL	N-acyl homoserine lactone
HSL	homoserine lactone
C06-HSL	N-hexanoyl-L-homoserine lactone
C08-HSL	N-octanoyl-L-homoserine lactone
C12-3O-HSL	N-3-oxo-dodecanoyl-L-homoserine lactone
QQ	quorum quenching
QSI	quorum sensing inhibition
Ntn	N-terminal
paPvdQ	*Pseudomonas aeruginosa* acyl-homoserine lactone acylase
PGAs	penicillin G acylases
kcPGA	*Klyuvera citrophila* penicillin G acylase
ecPGA	*Escherichia coli* penicillin G acylase
aPGA	*Achromobacter* species penicillin G acylase
PCA	principal component analysis
NMA	normal mode analysis
SPM	shortest path map
VAF	Phe138αVal-Met142αAla-Ile177βPhe ecPGA variant
YAF	Phe138αTyr-Met142αAla-Ile177βPhe ecPGA variant
MSF	Phe138αMet-Met142αSer-Ile177βPhe ecPGA variant

Introduction to structure-dynamics-function relationships in proteins

Living cells are composed primarily of four major types of macromolecules: nucleic acids, carbohydrates, lipids, and proteins. Among these, proteins were found to be the most abundant, accounting for nearly half of the total dry mass of the cell. Although each of these macromolecules plays a key, intrinsic role, proteins are known (at least with current knowledge) to be the most versatile in terms of their types and different functions. These include: signaling (hormones), structural (actin, tubulin, keratin), defense (immunoglobins), transport (hemoglobin, myoglobin), storage (albumin), access control (transporter proteins), and catalysis (enzymes) [1]. The latter, with their ability to accelerate chemical processes and synchronize them with the multitude of other reactions taking place in the cell, are the main focus of my doctoral research. They act as biocatalysts, lowering the barrier to reaction activation and speeding up processes that are often either prohibitively slow or require additional energy, e.g. in the form of heat, which on the other hand would be harmful to the cell. Enzymes also have the ability to create a specific environment for the reaction at hand, protecting the cell from potentially toxic and highly unstable intermediates. Enzymes are not only important macromolecules for living cells, but also show substantial importance in various fields of life, including biotechnology, variable industries, medicine, and others. All these features make these macromolecules immensely exciting, but also very difficult to study. Importantly, with the deepening level of knowledge, the development of experimental techniques, molecular modeling and computational capabilities, it is becoming clear that the function of proteins, including enzymes, is not only defined by the sequence-dependent three-dimensional structure, but also (as much!) by their dynamics, which is the main focus of this dissertation.

Since the first protein structures of hemoglobin and myoglobin were solved by Perutz and Kendrew in the late 1950s [2], and the first enzyme structure – lysozyme – in 1965 [3], the mechanism of their action has been proposed based on the sequence-structure-function paradigm. X-ray crystallography, together with nuclear magnetic resonance (NMR) [4] and cryo-electron microscopy (cryo-EM) [5] resulted in over 200 thousand static structures currently available in the PDB database, which revolutionized the understanding of the molecular biology [6]. Furthermore, the recent breakthrough with the discovery of AlphaFold 2 for protein structure prediction [7], and the subsequent development of the AlphaFold Protein Structure Database with more than 200 million protein structures [8] has been another milestone in the field, expanding the current state of the knowledge and further advancing science. Nevertheless, proteins have been shown not to be static entities, and the dynamic factor has to be considered as crucial, on the same level of importance, moving further towards structure-dynamics-function approaches [9].

The phenomenon of protein dynamics is complex [10]. Several different modes of motion, occurring on versatile time scales, contribute to the overall protein motions, depending on the process, system and environment. The fastest motions are related to the vibrations of covalent bonds, which occur within femtoseconds to picoseconds and are mostly in the amplitude of one angstrom. Side-chain motions and rotations of amino acids, some of which are critical for protein function, can occur rapidly, within hundreds of picoseconds to nanoseconds, depending on the depth of the protein core and the local environment. The coordinated motions of several residues, which usually constitute a process called gating, in which the moving fragment acts as a lid, covering the ligand bound to the active site or influencing its release by exposing the bulk solvent, are in the range of hundreds of nanoseconds to milliseconds. Another two groups of principal motions are related either to ligand transport and binding, where the molecule often has to overcome tens of angstroms to reach the binding site, requiring many structural adjustments at the protein level, or to the collective motions of entire macromolecules or their parts in multi-subunit complexes, which can reach amplitudes of nanometers. The former can occur over hundreds of nanoseconds and last as long as milliseconds, while the latter are typically a matter of at least microseconds and usually considerably longer. Finally, processes related to folding and unfolding of the protein structure are the type of motions that require the most significant changes and rearrangements in the structure, so the duration may even be hours [1,10,11].

Due to the described principal motions, proteins, in this case specifically enzymes, coexist in an ensemble of multiple states that interconvert in dynamic processes [6]. Progress in the field has broadened the understanding of the dynamics-function relationship of enzymes, so that the original *"lock and key"* model, which assumed the enzyme rigidity and complementarity between the substrate and enzyme structure [12], has gradually been replaced by dynamic models such as induced fit or conformational selection [13,14]. In fact, from a recent structural point of view, the function of an enzyme is defined by its conformational landscape. Highly robust enzymes have their conformational landscape evolutionarily optimized for a particular reaction pathway, positioning alternative options as significantly less favorable. On the other hand, more promiscuous enzymes exhibit more sophisticated landscapes with multiple minima separated by moderate energy barriers that are accessible under standard conditions [15]. The existence of such multiple accessible states is also important for the overall process of catalysis, which includes not only the chemical reaction itself but also closely related steps [16]. Considering an enzyme with a buried active site, which will be the focus of the following part of the thesis as a relatively common example [17], additional stages controlled by an enzyme besides the chemical reaction itself are critical as presented in **Figure 1** [18]. This model perfectly illustrates the close relationship between structure-dynamics and function in proteins, and emphasizes the importance of considering the dynamic component on an equal footing with the others.

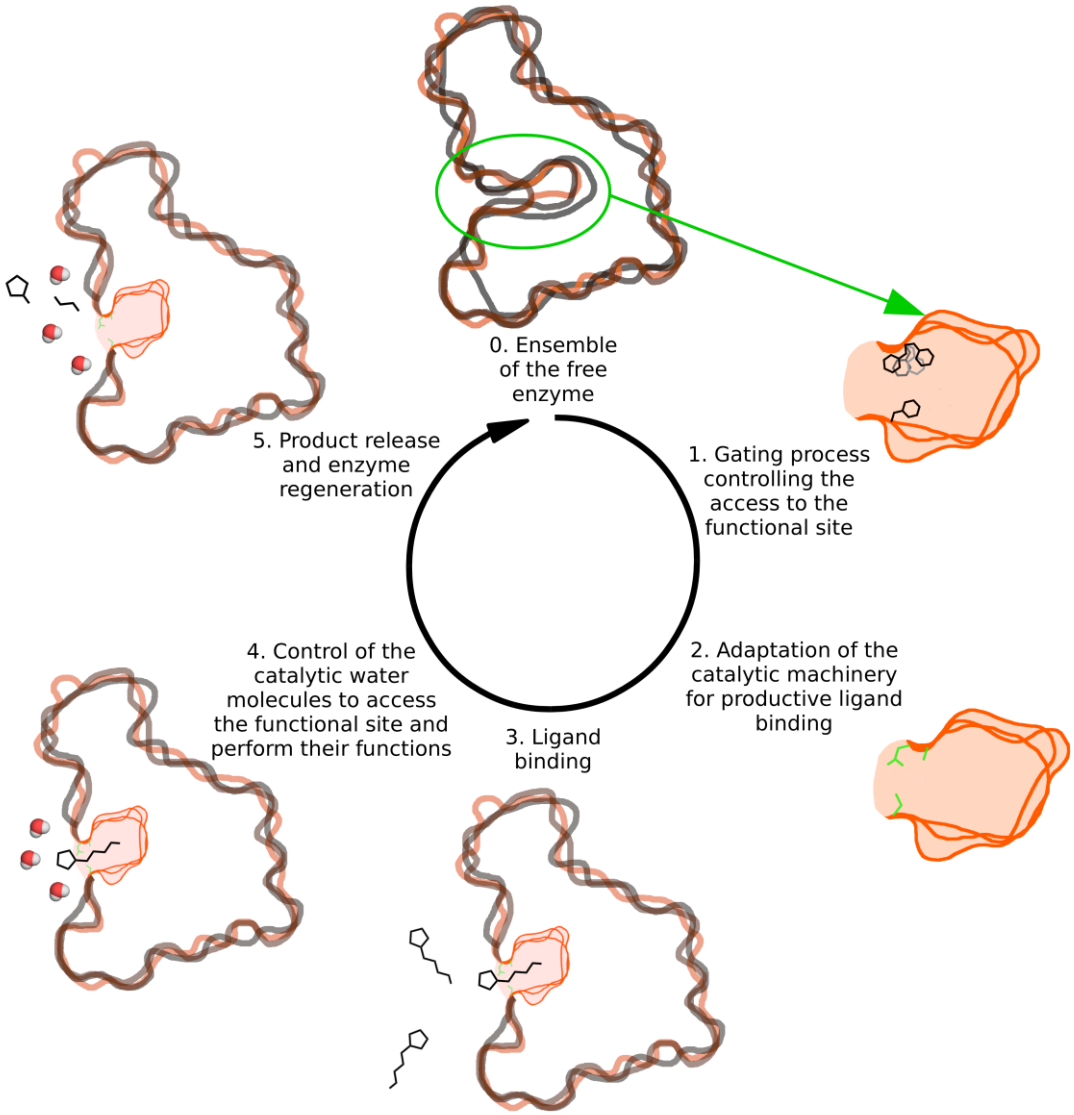

Figure 1. Structure-dynamics-function relationships for the enzyme perfoming catalytic function. *Starting from the ensemble of the enzyme in the free form (**0**), additional stages are critical besides catalytic reaction itself. This includes: gating processes controlling access to various functional sites (**1**), adaptation of the catalytic machinery for productive ligand binding (**2**), an actual binding of the ligand (**3**), and later, during the reaction progress, control of the water molecules to reach the functional site and perform their functions (**4**), and finally product release and enzyme regeneration (**5**) leading to the enzyme in free form, ready for the next cycle (**0**).*

Undoubtedly, the dynamics of proteins in the context of their function play a key role, but *how can the phenomena of structure-dynamics-function interplay be explored in the era of modern science?* The aforementioned standard methods of protein structure determination, such as X-ray crystallography and NMR, although limited, can be combined to provide an atomic resolution view of the transitions between different protein conformations in solution. Ongoing developments in the field have led to variants of these techniques that provide even broader access to functional dynamics. These include time-resolved and real-time variants of X-ray crystallography [19,20], or serial femtosecond

crystallography (SFX) taking an advantage of X-ray free-electron lasers (XFELs) [21], a wide range of NMR variations such as hydrogen/deuterium exchange, spin-relaxation, residual dipolar couplings, and others [4,22,23]. Insights into the functional dynamics of proteins have been further complemented by the development of techniques such as hydrogen-deuterium exchange mass spectrometry (HDX-MS) [24], sub-second time-resolved mass spectroscopy [25], single-molecule fluorescence resonance energy transfer (FRET), or the aforementioned cryo-EM, but enhanced by incorporation of artificial intelligence algorithms [26].

Importantly, in parallel with experimental techniques, molecular modeling methods, especially molecular dynamics simulations (MD), have matured enough to become a distinct influential field for predicting and studying protein structure and dynamics. In fact, considering the complexity, cost and human effort of most of the listed experimental methods, MD often represents a convenient alternative to replace or at least efficiently support experimental efforts. Moreover, some processes in biological systems, such as the study of transport events through biomolecules or the progress of chemical reactions, are difficult or even impossible to directly study experimentally at the atomistic level, and computational methods, such as MD, are the primary alternative [27]. In its early stages, MD was very limited in terms of speed and efficiency. The first MD simulations of a protein - bovine pancreatic trypsin inhibitor (BPTI) - performed by McCannon et al. in the late 1970s, although a milestone in the field, covered a relatively small system and a short time scale of 9.2 ps [28]. Fortunately, the continuous development of efficient algorithms, versatile MD sampling approaches, and increasing computational power, including the use of graphics processing units (GPUs) for MD simulations, has resulted in state-of-the-art methods capable of sampling biologically relevant processes of ever-increasing size [29]. Depending on the process under investigation, researchers have at their disposal a wide range of models and methods of varying complexity, accuracy and speed. Chemical reactions can be studied at the atomistic level using hybrid quantum mechanics/molecular mechanics (QM/MM) methods, which allow the catalytically relevant region to be described by quantum chemical models, while the environment composed of the protein and solvent is described by the less computationally demanding molecular mechanics force fields [30], using improved sampling methods such as steered MD (sMD) or umbrella sampling (US) simulations, which make these processes feasible on the time scale of all-atom simulations [31,32]. Long time scale processes such as ligand (un)binding or allosteric communication, which are challenging due to their infrequent nature, can be approached using enhanced sampling methods such as various biased-potential methods including accelerated MD, metadynamics, adaptive sampling methods based on Markov state model such as high-throughput MD (HTMD), or variants of path sampling methods such as weighted ensemble methods or milestoning, all of which have been extensively described in the recent review by Ahmad *et al.* [33]. Finally, highly complex multi-subunit

systems or transmembrane proteins simulated together with lipid membranes and solvent can be efficiently studied by simulations using computationally less demanding coarse-grained (CG) models [34]. Although computational models and methods are far from perfect and many challenges remain to be solved [27], they represent a powerful toolbox for studying structure-dynamics-function relationships in proteins, and as such inspired their use during my doctoral research for this purpose.

Accordingly, the next chapter contains a summary and highlights of my findings during the dissertation preparation, followed by conclusion of my findings, an outlook and further perspectives related to the field of my investigation. At the end of the thesis sections Publications 1-4 contain full text of the publications.

Summary of the doctoral research

Molecular modeling of quorum quenching enzymes

Antimicrobial resistance is a rapidly growing global health problem that has been prioritized by the World Health Organization [35]. Although dismissively referred to as a silent pandemic, it must be seriously considered and addressed on a global scale and referred to as a true pandemic to avoid being ignored and to spread awareness of the overwhelming danger in society [36]. The last thorough report on mortality in 2019 showed nearly 5 million deaths associated with, and more than 1 million deaths directly caused by, bacterial antibiotic resistance [37]. Strikingly, it is expected to be even more accelerated since 2019 due to the COVID-19 pandemic, as it led to increased antibiotic treatment of SARS-COV-2 patients [38,39]. Widespread antibiotic resistance is due to a variety of factors, including misuse/overuse in the treatment of medical conditions such as viral infections, exposure from the use of antibiotics outside of medical settings in agriculture, aquaculture, and other human uses [37,40]. On the other hand, because antibiotics are mostly lethal to bacteria, they exert a strong selective pressure that promotes the development of highly efficient resistance mechanisms [41,42]. Clearly, global efforts and new alternatives are needed to effectively contain this emerging threat.

One of the recent alternatives to conventional antibiotics, mainly considered as their complement or substitute in non-medical applications [43], is to target a bacterial communication process called quorum sensing (QS). In Gram-negative bacteria, QS is mediated by organic molecules - N-acyl-homoserine lactones (AHLs) of different length (depending on the species) - and enables bacteria social behaviors in cell-density depended manner [44] – Figure 2. Importantly, quorum quenching (QQ), a process in which bacterial communication is interrupted, is of particular interest because it can prevent the expression of genes responsible for bacterial virulence factors, highly problematic biofilm

formation, swarming and resistance – **Figure 2** [45]. QQ can be achieved either by inhibition of signal biosynthesis, signal detection (QSI) or by direct, chemical or enzymatic inactivation of the signaling molecule. Since QSI is more susceptible to drug-like resistance mechanisms due to its mode of action, inactivation is considered to be a more promising strategy, as it acts at the level of the cell environment and is believed to escape common resistance mechanisms, but as a result of limited selective pressure, scientists hope not to rapidly observe the development of new defense mechanisms. Furthermore, enzymatic inactivation has an advantage over the chemical solution in that it is potentially less harmful to the environment, i.e. it is a green and sustainable solution, and, most importantly, it has a much more modifiable specificity to particular bacterial strains by targeting a defined type/size of molecule. Thus, enzymes with QQ activity (hereafter referred to as QQ enzymes) are of particular interest for future antibacterial applications. AHLs can be enzymatically inactivated by three main classes of enzymes that catalyze different modifications (**Figure 2**). Lactonases cleave the homoserine lactone ring, oxidoreductases reduce 3-oxo substituted AHLs, and finally acylases/amidases hydrolyze the amide bond between the homoserine lactone and the acyl chain. Among these, the action of acylases is considered to be the only irreversible modification that cannot be easily reversed by subtle changes in conditions (such as pH), and also represents the group of QQ enzymes with the highest specificity - they are sensitive to the length of the acyl chain [46]. QQ acylases belonging to the subfamily of N-terminal (Ntn) serine hydrolases represent one of the most intensively studied groups of enzymes for antibacterial applications, some of which exhibit truly promising biotechnological properties and thus represent excellent templates for further development and engineering. Therefore, they represent a high-impact research object and, as such, model systems for the computational exploration of structure-dynamics-function relationships in proteins, the main objective of this dissertation.

My research on QQ enzymes was primarily inspired by two members of the Ntn serine hydrolase subfamily that have been described to have activity against AHLs. First, the *Pseudomonas aeruginosa* acyl-homoserine lactone acylase (paPvdQ), which is known to have native activity against long acyl chain AHLs [47], enzyme, which is well studied from an experimental and biochemical point of view, although it lacks biotechnological potential because it is not used in large-scale processes. The second, a representative of penicillin G acylases, enzymes widely used in the pharmaceutical industry because of their application in the production of semi-synthetic beta-lactam antibiotics, *Klyuvera citrophila* penicillin G acylase (kcPGA), recently identified as having QQ activity towards short AHLs [48]. Interestingly, QQ activity was not detected for other penicillin G acylases, including one from *Escherichia coli* (ecPGA), for which activity has even been disputed [49], despite high sequence and structural similarity and the presence of all functional residues in the catalytic site. This prompted me to contrast the prototypical paPvdQ with native QQ activity with the ecPGA with alleged inactivity, used

as a negative control, and another more distant representative from *Achromobacter* species (aPGA). The basic question was - *why, if so similar, are some active and others not?*

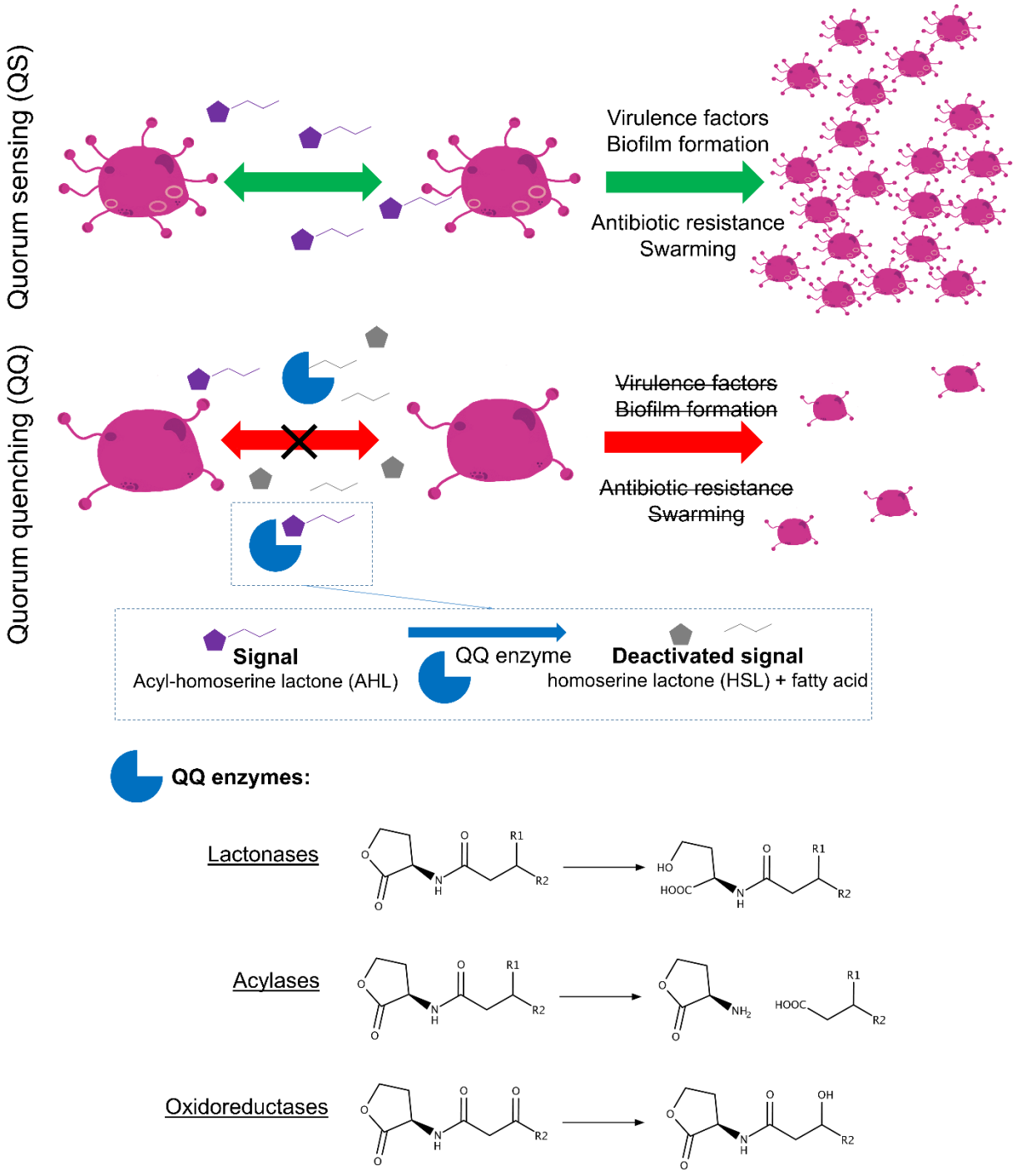

Figure 2. Schematic representation of bacterial communication and its enzymatic interference. *Quorum sensing (QS) and quorum quenching (QQ) processes (**top**). Three classes of enzymes capable to inactivate acyl-homoserine lactones (AHLs) acting as bacterial signaling molecules (**bottom**).*

To answer this question, I have performed multistep molecular modeling of paPvdQ, ecPGA, and aPGA enzymes, gradually increasing the complexity of the methods used. The initial analysis consisted

of a detailed analysis and comparison of static structures, focusing on the acyl-binding pocket, the catalytic machinery, and the gating residues that control access to the active and binding sites. This explains why PGAs are not active towards long substrates, as their binding cavity turned out to be much shallower compared to paPvdQ, suitable for this type of molecules, but did not justify the inactivity for molecules of the size that would geometrically fit the PGAs pocket (short and medium length AHLs with 6 and 8 carbons in the acyl chain). Therefore, the next step was to study the equilibrium dynamics in free form to inspect possible phenomena of permanent closure of the gates or stabilization of the catalytic machinery in a non-productive state from the perspective of AHLs hydrolysis. Since both were eliminated and I observed transient but sufficiently frequent opening of the pocket along with the active site favorably pre-organized for the nucleophile attack reaction, I extracted representatives for molecular docking experiments of C06-HSL and C08-HSLs to verify potential binding difficulties. Protein-ligand complexes were successfully obtained for both substrates with all three enzymes, as determined by the docking binding score as well as crucial stabilization by the catalytic residues, excluding the next potential reason for QQ inactivity. The complexes were subjected to tens of repetitive standard MD simulations with cumulative sampling of 4.5 μs, with the main goal of evaluating the enzymes' ability to maintain the productive stabilization of the AHLs and to estimate the binding energy. The stabilization, although possible in PGAs, was noticeably better in paPvdQ, especially for the longer substrate, which was determined by the relatively wider openings in the cavity entrance in PGAs and caused the ligand to fluctuate more in contrast to paPvdQ. Nevertheless, the binding energies were not significantly different among the studied complexes, so I proceeded with the modeling of the catalytic reaction to explore the structural or energetic obstacles preventing the hydrolysis of AHLs by the studied PGAs. Hundreds of sMD QM/MM simulations of the first two rate-limiting steps of the reaction have shown that paPvdQ, ecPGA and aPGA are capable of hydrolyzing short to medium AHLs according to similar reaction mechanisms, but with the system-dependent energetic differences. Interestingly, the differences observed were mostly related to the different topology of the binding sites, the different dynamics of the gating residues for each of the enzymes studied, and finally the different positioning of the Arg263β(PGAs)/Arg297β(paPvdQ) residue, which plays a crucial role in the second step of the reaction. In addition, the catalytic activity of both PGAs toward modeled substrates, although basal, was experimentally confirmed by our collaborator, Michal Grulich, Ph.D., from the Academy of Sciences of the Czech Republic, adding these two biotechnologically relevant enzymes to the set of known QQ enzymes. Full details of this work can be found in the publication summarizing these results in Publication 1.

Importantly, this research constitutes an example of the structure-dynamics-function relationships that, if not thoroughly investigated, can lead to incomplete and biased conclusions. Furthermore,

considering the potential of PGAs for further exploration towards practical applications as antibacterial agents, and their relatively low native QQ activity, this made me realize that engineering attempts will inevitably be needed to improve their catalytic activity and fine-tune specificity fully described in Publication 4, which will not be effective without taking the dynamic aspect seriously, motivating the literature review, summarized in the next section.

Dynamics as critical aspect for modern rational protein engineering

Before tackling the challenge of modulating PGAs for improved QQ activity, my colleagues and I reviewed the current (2017-2020) *in silico* approaches to protein design that consider the component of dynamics as an important factor in the engineering process. From the collected literature, we noticed mainly a trend towards post-processing analysis of protein structural ensembles, coming mainly from MD simulations, focusing on interactions, allosteric effects as well as ligand transport. On the other hand, we have summarized efforts to incorporate backbone flexibility into the design process and its implementation in various software packages. For the first group of ensemble analysis methods, the common approach included the CG representation of the protein as Cα or Cβ atoms to reduce the complexity of the analysis, and further either the extraction of interaction networks or dimensionality reduction by principal component analysis (PCA), both of which can either provide hotspot residues critical for functional dynamics or, when performed for wild type and considered mutants, indicate introduced changes. Furthermore, the analysis of transient pathways used by water molecules or ligands can indicate the regions of the protein for the usually rare transport events inherent in catalysis. On the other hand, such pathways can first be extracted from the structural ensembles and further explored explicitly [50], albeit by repetitive molecular docking experiments (although known to have limitations) without the need to sample the transport process during the simulation, which is often a non-trivial task and requires efficient sampling. Residues that either form the bottleneck (e.g. gating residues) in the transport pathways or are related to the high energy barriers during transport events are of primary interest for engineering as they contribute to the rate-limiting steps. Second, methods dedicated to incorporate protein dynamics during the design process have been shown to benefit either from the information obtained from pre-generated ensembles (MD or Monte Carlo derived) or knowledge-based prediction of motions learned from the experimental or modeled structures, which will further grow considering the current progress in the development of various improved sampling methods, hardware and breakthrough in protein structure prediction.

In addition, we highlighted the main limitations and challenges associated with approaches that aim to incorporate dynamics in protein design. We pointed out that the mutations introduced by these

methods are mainly located in the vicinity of the catalytic machinery or the functional region, which allows to limit the computational burden and makes them efficient in terms of likely impact on function, but at the same time constitutes their limitation. We highlighted the need to invest effort in developing approaches that take into account distal regions important for allosteric control and/or ligand transport. These can be based either on approximate methods such as normal mode analysis (NMA), which may not be sensitive enough to capture subtle changes, or on highly efficient enhanced sampling MD methods. The latter pose additional challenges related to sufficient sampling of rare events (such as allosteric motions or ligand transport) and the resource demands of such computations, which, even if properly captured, provide immense data sets for analysis and post-processing that need to be addressed in parallel.

Looking back at the observations from our review in the period since its publication, there have been several excellent review articles that are in line with our perspective. Lemay-St-Denis *et al.* raise the importance of integrating dynamics into enzyme engineering and pinpoint experimental and computational methods suitable for targeting it [11]. A particularly intriguing example pointed out by the authors was a successful synergy between experimental exploration by HDX-MS and computational inference by MD simulations that led to a 7000-fold improvement in the catalytic activity of the ancestral luciferase AncLuc [51]. Chen *et al.* and Gu *et al.* referred to the approaches for designing allosteric communication in proteins and described the role of distal sites in enzyme engineering [52,53]. Considerable effort has also been devoted to predicting mutations away from the active sites and engineering the protein conformational landscape to target novel functions [54,55]. Here interesting results were achieved by Maria-Solano *et al.* by *in silico* rationally identifying several point mutations distant from the active site using their shortest path map (SPM) approach [16], which, when modified, led to the transformation of an inefficient conformational ensemble into a productive one for tryptophan synthase [56]. Furthermore, Wu *et al.* provided a comprehensive review of molecular modeling tools and software for computational engineering of enzymatic selectivity [57]. The challenge of intrinsically disordered proteins, which we have addressed in our review, their modeling and related theoretical description have been thoroughly addressed in [58]. Finally, the field of artificial intelligence is still evolving in terms of structure prediction, through the aforementioned AlphaFold and its database of predicted structures, but also in terms of assisting in the protein design process [59–61].

Both the methods described in our review article (full content of the article can be found in Publication 2) and those described in recent methodological and computational reviews often rely heavily on extensive sampling to gain sufficient insight into long-lived processes such as allosteric control or ligand transport. To address this, we developed a Python library for efficient analysis of

massive ensemble-based data of protein transport pathways, summarized in the next section, which I further applied to support the engineering of PGA – **Publication 4**.

Computational burden of ensemble-based protein dynamics data analysis

Because our lab's research focuses specifically on biomolecular transport and interactions, we have experienced the challenges of obtaining sufficient samples to analyze such processes, how to discover transport pathways, how ligands use these pathways, and finally how to consistently analyze or represent these from a set of massive molecular dynamics simulations, often for multiple variants of a given protein.

The first question to ask is: how can we computationally identify and study transport pathways in proteins? Based on their primary approach, these methods can be divided into two main categories: (i) geometry-based tunnel detection by analyzing empty spaces within the protein structure, e.g. by Voronoi diagrams [62], or (ii) ligand-tracking methods that indicate the existence of a pathway based on an observed transport event. Considering the limitations of these methods, the first, although it allows efficient identification of pathways as free spaces between protein atoms, is mainly limited by a spherical representation during the analysis and lacks the ability to determine the function of a given pathway, e.g. in transporting water or ligands. It allows only a geometric description of the pathway, taking into account parameters such as length, average and maximum opening radius, or information about the narrowest point, the so-called bottleneck. In contrast, methods based on tracking ligand molecules provide direct insight into the transport process, but have the disadvantage that not all functional pathways can be detected, e.g. due to insufficient sampling or the fact that unobserved pathways can be used by molecules with different physicochemical properties. This severely limits the detection of relatively closed pathways, which are primarily not used in the native protein/enzyme, but can be engineered as shown by Brezovsky *et al.* [63]. However, no matter which of these two approaches is chosen, efficient sampling is key, and today this can be achieved either by enhanced sampling methods, e.g. HTMD simulations, which have proven to be good for efficient sampling of ligand or solvent dynamics [64,65], or by sufficiently long standard MD simulations. Both lead to a huge amount of data to be analyzed, often derived from multiple simulations or, as already mentioned, for different variants of the system, making it very difficult to analyze in a unified way.

To address both of these issues, either the limitations of each approach or the burden of a unified representation and analysis of these large data sets, we developed the TransportTools library. We decided that since the geometry-based approach and the ligand-tracking approach have different advantages and can satisfactorily complement each other - *why not combine the two* (**Figure 3**)*?*

Therefore, TransportTools takes the set of tunnel networks predicted by the CAVER software [66,67], which can be derived from multiple independent simulations, and combines them into a single, unified tunnel network convenient for investigation. It generates the ensemble-based average properties of all detected paths, their visualization and, most importantly, raw output data that allows tracing the source simulations that sampled a particular path, thus providing the full access to the original data. In addition, our TransportTools library can optionally process the transport events of specific molecule types as detected by the AQUA-DUCT software [68,69] from the same set of simulations, its part, or alternatively from different sets of simulations for the same protein. These transport events are further assigned to corresponding pathways from the unified tunnel network resulting from TransportTools processing of CAVER input data, and provide information on the transport functionality of the pathways by the ligands for which the transport data were provided. Importantly, this software allows comparative analysis by separately generating geometric properties, transport event statistics, and visualization for user-defined groups of simulations or ensembles derived from sources other than MD. This is particularly useful for comparing systems that differ in some way, either in the conditions used to run the simulations (e.g. pH, solvent, temperature, presence of ligands) or in the protein variant itself. The latter is the case of interest for my system, PGA, and the goal of designing different mutants for the protein, which is known to possess the transiently opening pocket gated by residues at its entrance. This requires both efficient sampling and sensitive detection of introduced changes, both of which are perfect challenges for TransportTools and fall within the scope for which the library was designed. The full description of the TransportTools library can be found in Publication 3, while its assistance in the PGA engineering experiment is summarized in the next section.

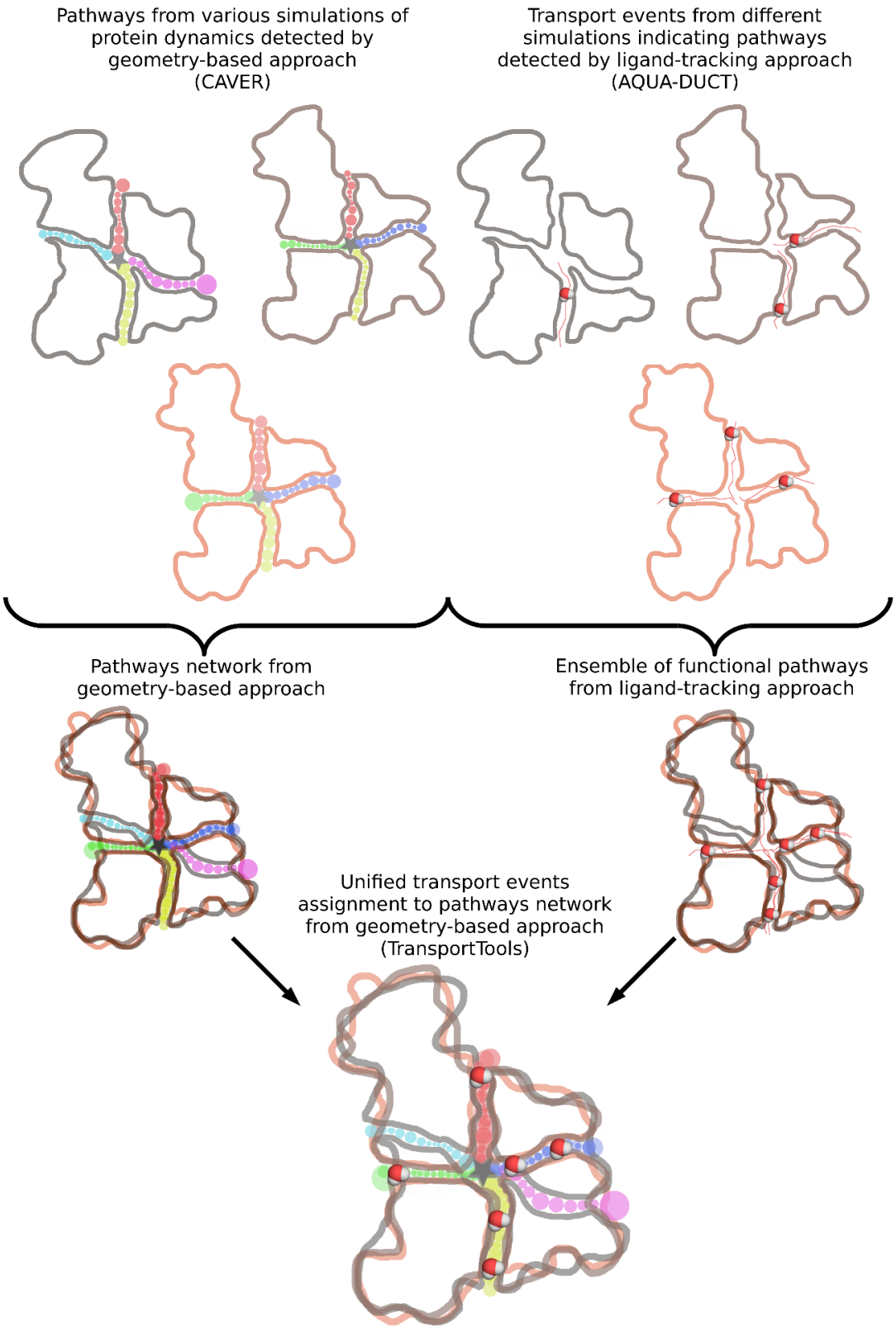

Figure 3. TransportTools library principal concept. *It processes massive datasets of pathways detected by geometry-based approach such as CAVER, and optionally information about functional pathways explored by ligand-tracking method, AQUA-DUCT, and combine these information in unified way into a single dataset.*

Rational engineering of quorum quenching enzyme's dynamic pocket

Before moving on to the engineering itself, it is worth discussing a bit more about the overall potential of quorum quenching enzymes, the proof-of-concept studies that confirm their usefulness, and why I am focusing specifically on PGAs rather than other QQ enzymes. First of all, I have already mentioned the acylases and especially the Ntn serine hydrolases as an interesting group, but I have not mentioned specific applications and prototypical studies that have been successful.

The primary areas of application for QQ enzymes include, but are not limited to, medicine, aquaculture, and membrane-based fields such as industry, biotechnology, and wastewater treatment. To name just a few of the most interesting possibilities: they can be used as wound healing patches, biofilm-preventing agents in catheters, coatings on the surfaces of water-contacting instruments that are subject to biofilm formation and further biofouling, their immobilization can extend the permeability and life of membranes by reducing the effects of bacterial biofilms growing on their surfaces, and many others [43,45,46]. paPvdQ has been extensively studied, particularly with regard to its use in combating bacterial infections. First, it was shown to reduce *Pseudomonas aeruginosa* infection in *Caenorhabditis elegance*, followed by its formulation as a dry powder that, when inhaled in the infected mouse model, led to improved survival rates [70,71]. Interestingly, it has even been successfully engineered to shorter substrates (C08-HSLs), signaling molecules of pathogenic *Burkholderia cenocepacia* and resulted in increased viability on the infected larval model [72]. Another interesting example is the acylase PF2571, which has high QQ activity and can prevent early spoilage of food. Again, a variant of this enzyme was designed to change its specificity towards shorter AHLs [73]. Unfortunately, in the case of both acylases, either paPvdQ, which is useful against infections, or PF2571, which is promising for the prevention of food spoilage, the biotechnological history and their optimization in terms of efficient mass production, storage, immobilization, etc. are lacking. This gives the PGA family of enzymes even greater potential. They have been used in large-scale processes for decades, have the characteristics of excellent biotechnologically optimized enzymes, are good templates for engineering, and finally, their modulation to change specificity towards other non-native substrates has already been successfully demonstrated [74–78].

Of the two wild-type PGAs with newly confirmed QQ activity, I chose ecPGA because of the availability of several high-resolution X-ray structures, which is a desirable starting point for modeling, as opposed to aPGA, for which a homology model was used in the initial investigation. Using the previously discovered dynamic determinants that condition the limited activity of ecPGA against bacterial signaling molecules, I directed my engineering to reduce or even preferentially eliminate them. Simplified scheme of the engineering procedure in shown in **Figure 4**. I aimed to deepen relatively shallow pocket natively suitable for aromatic groups, inspired by the deep pocket of paPvdQ, which is

favorable for long AHLs. In addition, I targeted the entrance to the acyl-binding cavity, which is located near the gating residues that I have shown to be suboptimal in ecPGA, allowing the substrate to fluctuate more, thus compromising the stabilization in the organization favorable for catalytic action. To achieve this, I first introduced two substitutions at positions Met142α and Ile177β to deepen and narrow the pocket, respectively, followed by a third substitution at position Phe138α to further expand the binding site. This was not a trivial task, as simple combinatorial substitutions considering only 20 naturally occurring amino acids yield nearly 7000 possible variants. Since this was already a substantial set for in-depth investigation, even computationally, I applied a series of rationally driven filtering steps to generate a smart library of the most promising variants for extensive molecular modeling, followed by selection of best hits for experimental validation. These included the following types of filtering: (i) restriction at the mutation introduction stage, as in the case of Ile177β, where to introduce bulkier residues and avoid unpredictable polar groups, I was restricted to phenylalanine only, (ii) destabilization energy-based filters to avoid highly unstable constructs, and (iii) ensemble-based geometry evaluations to promote only mutants with desired modulation of the designed region. The latter was performed using the TransportTools library and its comparative analysis functionality, where hundreds of pathways resulting from repeated CAVER calculations on ensembles of designed mutants were unified into a single pathway network. This allowed consistent identification of the pathway leading to the binding pocket in all designed variants, their comparison with the wild-type structure as well as among each other, and finally a methodologically transparent selection of the best variants with the desired properties, resulting in the selection of six candidates for further detailed study.

Shortlisted variants were used as receptors for molecular docking experiments with a series of six signaling molecules of varying acyl chain length (from C06- to C12-3O-HSLs) to probe designed variants for compounds mediating communication in different bacterial species. In addition, starting from docked poses properly organized for hydrolysis, I performed repetitive standard MD simulations for each complex, resulting in approximately 1000 trajectories cumulatively totaling 5 μs of sampling. At this point, I evaluated the ability of each mutant to productively stabilize the set of AHLs studied, taking into account both the fraction of simulations in which such stabilization occurred and the average number of frames. This, when compared to the capabilities of the wild-type simulated in an analogous manner as a control system, allowed the selection of the three most interesting variants for further experimental validation. These included the following substitution combinations: Phe138αVal-Met142αAla-Ile177βPhe (VAF), with an appealing specificity for medium-length AHLs, Phe138αTyr-Met142αAla-Ile177βPhe (YAF), with a shifted specificity from shorter to longer ligands, and finally the variant Phe138αMet-Met142αSer-Ile177βPhe (MSF), which showed the most significant improvement for the longest substrate studied - C12-3O-HSL. The designed enzymes were successfully expressed,

and biochemical assays of catalytic activity against the same set of AHLs indicated modulated activities compared to wild-type ecPGA. Indeed, we obtained a satisfactory agreement between the computational activity estimation and the experimental parameter K_M, reaching a Spearman's correlation coefficient of -0.69, confirming the predictive power of the metric used in the modeling. Interestingly, VAF showed a 2- to 5-fold improvement in activity towards medium sized AHLs, YAF almost a 3-fold improvement for longer substrates, similar to MSF with C12-3O-HSLs.

This synergistic agreement between modeling and experiments motivated me to analyze in depth the dynamic determinants that contributed to the change in activity and specificity of the designed variants. I performed an additional round of free enzyme standard MD simulations for all three variants and thoroughly explored the dynamics and behavior of the binding pocket, its entrance, including gating residues, catalytic machinery, and paying special attention to the substituted residues. Going straight to the most relevant observations, I found that the pocket volumes of all proposed variants were significantly increased compared to wild-type ecPGA, reaching or even exceeding that observed for paPvdQ. Interestingly, although VAF sampled the deepest openings, it represented the variant with the highest fluctuations of the bottom part of the pocket related to the modulated dynamics of modified residues, explaining its inability to effectively process long substrates. This analysis also shows that I failed to narrow the entrance to the cavity, while at the same time introducing a constriction in the initial part of the pocket, which most likely limited the significant improvement in catalytic rates. Furthermore, by introducing aromatic substitution at position 177β, the dynamics of two gating phenylalanines were modulated, making them mostly more stable. The full description of this work can be found in **Publication 4.**

The study on the engineering of ecPGA pocket dynamics has shown that a relatively simple rational approach has already led to three variants with improved activities and modulated specificities, highlighting its further potential and designability towards future antibacterial agents. We also highlighted the importance of the dynamics aspect in the design process, which cannot be overlooked. Furthermore, we pointed out the remaining limitations to be addressed in the next studies, mainly related to the constriction in the initial part of the pocket. In addition, we hypothesize that the introduced mutations may have shifted the rate-limiting steps of the reaction of AHLs hydrolysis to later stages, therefore I am currently performing extensive sMD QM/MM simulations of the complete catalytic cycle of wild-type ecPGA, paPvdQ and VAF with medium length AHLs, which will clarify the uncertainty of the stage that is directly rate-limiting.

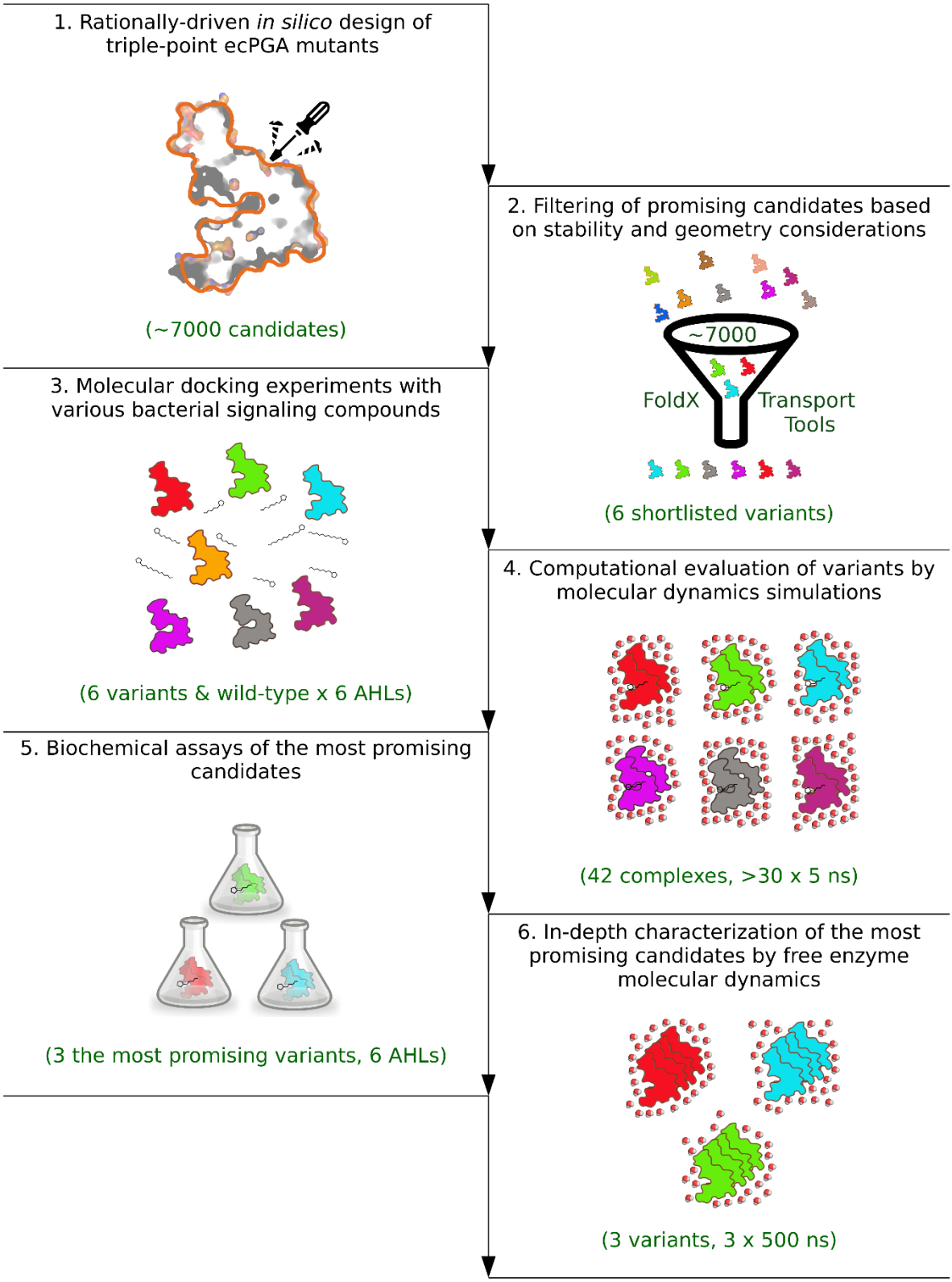

Figure 4. Engineering of quorum quenching enzyme ecPGA for improved activity and modulated specificity towards various bacterial signaling molecules (AHLs).

Conclusions and future perspectives

Based on the QQ enzymes, members of the Ntn serine hydrolases family used as a model system, I have illustrated the importance of the dynamic component for the function - catalytic activity. The first step of the study focused on the static structures and molecular docking experiments, which have not provided the details on the observed experimental low activities. Relevant differences were noted only from repetitive MD simulations and sMD QM/MM MD simulations of protein-ligand complexes and their reaction, respectively, which revealed dynamic determinants responsible for QQ activity. These required extensive sampling, consistent analysis and comparison to track the elucidated system-dependent differences.

Based on the literature review on the dynamic component of protein design, all approaches agree that efficient sampling of the conformational ensemble of functionally relevant states is critical for successful design and evaluation of engineered protein variants. This is becoming more affordable with the current development of experimental techniques, computational methods, technology/hardware and artificial intelligence algorithms, but raises the next challenge of efficient and consistent analysis of massive data sets.

To address these challenges, in the context of transport pathways in proteins and their detection, analysis and associated transport events, we have developed a TransportTools Python library for consistent and unified analysis of these from large ensemble-based protein dynamics data resulting from massive MD simulations. During development, we considered the dynamics component, which requires sufficient sampling and thus large datasets. In addition, we combined the advantages of geometry-based and ligand-tracking-based approaches to benefit from their complementarity, which had not been shown before.

Finally, I utilized the insights gained from the dynamic study of the wild-type with respect to determinants conditioning the native QQ activity of the paPvdQ enzyme, and following recent trends in protein engineering that integrate dynamics into this process, I transplanted these and designed a series of ecPGA variants with modulated specificity and activity towards different bacterial signaling molecules. This was aided and simplified by the TransportTools library, which was able to unify the ensemble-based data. Extensive MD simulations provided details of the dynamic changes contributing to the altered function that were not accessible from the static structures of the designed mutants nor their complexes with signaling molecules obtained by molecular docking, further emphasizing the importance of the dynamic component. Furthermore, our study elucidated the remaining limitations associated with the designed mutants, which motivates and guides their further investigation and

improvement towards efficient antibacterial agents – an alternative to increasingly ineffective antibiotics.

Taken together, the evolution of science, experimental and computational methods, and artificial intelligence has brought us into an era in which structural studies of enzymes and proteins in general are not limited to single-state insights from static frames, but also allow the integration of protein dynamics as a major component. This allows an incredible synergy between experiments and theoretical predictions that is leading to significant discoveries. The further development of molecular modeling methods, while posing new challenges, especially those related to the systematic analysis and representation of large data sets, will allow the analysis of ever larger systems over much longer time scales, which are increasingly approaching the time scale of real processes in the cell.

References

[1] A. Kessel, N. Ben-Tal, Introduction to Proteins: Structure, Function, and Motion, Second Edition, 2nd ed., Chapman and Hall/CRC, New York, 2018. https://doi.org/10.1201/9781315113876.

[2] J.C. Brooks-Bartlett, E.F. Garman, The Nobel Science: One Hundred Years of Crystallography, Interdisciplinary Science Reviews. 40 (2015) 244–264. https://doi.org/10.1179/0308018815Z.000000000116.

[3] C.C.F. Blake, D.F. Koenig, G.A. Mair, A.C.T. North, D.C. Phillips, V.R. Sarma, Structure of Hen Egg-White Lysozyme: A Three-dimensional Fourier Synthesis at 2 Å Resolution, Nature. 206 (1965) 757–761. https://doi.org/10.1038/206757a0.

[4] G.C.K. Roberts, Protein NMR – Introduction, in: G.C.K. Roberts (Ed.), Encyclopedia of Biophysics, Springer, Berlin, Heidelberg, 2013: pp. 2027–2033. https://doi.org/10.1007/978-3-642-16712-6_303.

[5] W. Kühlbrandt, Forty years in cryoEM of membrane proteins, Microscopy. 71 (2022) i30–i50. https://doi.org/10.1093/jmicro/dfab041.

[6] M.D. Miller, G.N. Phillips, Moving beyond static snapshots: Protein dynamics and the Protein Data Bank, Journal of Biological Chemistry. 296 (2021) 100749. https://doi.org/10.1016/j.jbc.2021.100749.

[7] J. Jumper, R. Evans, A. Pritzel, T. Green, M. Figurnov, O. Ronneberger, K. Tunyasuvunakool, R. Bates, A. Žídek, A. Potapenko, A. Bridgland, C. Meyer, S.A.A. Kohl, A.J. Ballard, A. Cowie, B. Romera-Paredes, S. Nikolov, R. Jain, J. Adler, T. Back, S. Petersen, D. Reiman, E. Clancy, M. Zielinski, M. Steinegger, M. Pacholska, T. Berghammer, S. Bodenstein, D. Silver, O. Vinyals, A.W. Senior, K. Kavukcuoglu, P. Kohli, D. Hassabis, Highly accurate protein structure prediction with AlphaFold, Nature. 596 (2021) 583–589. https://doi.org/10.1038/s41586-021-03819-2.

[8] M. Varadi, S. Anyango, M. Deshpande, S. Nair, C. Natassia, G. Yordanova, D. Yuan, O. Stroe, G. Wood, A. Laydon, A. Žídek, T. Green, K. Tunyasuvunakool, S. Petersen, J. Jumper, E. Clancy, R. Green, A. Vora, M. Lutfi, M. Figurnov, A. Cowie, N. Hobbs, P. Kohli, G. Kleywegt, E. Birney, D. Hassabis, S. Velankar, AlphaFold Protein Structure Database: massively expanding the structural coverage of protein-sequence space with high-accuracy models, Nucleic Acids Research. 50 (2022) D439–D444. https://doi.org/10.1093/nar/gkab1061.

[9] E. Medina, D. R. Latham, H. Sanabria, Unraveling protein's structural dynamics: from configurational dynamics to ensemble switching guides functional mesoscale assemblies, Current Opinion in Structural Biology. 66 (2021) 129–138. https://doi.org/10.1016/j.sbi.2020.10.016.

[10] B. Surpeta, C.E. Sequeiros-Borja, J. Brezovsky, Dynamics, a Powerful Component of Current and Future in Silico Approaches for Protein Design and Engineering, International Journal of Molecular Sciences. 21 (2020) 2713. https://doi.org/10.3390/ijms21082713.
[11] C. Lemay-St-Denis, N. Doucet, J.N. Pelletier, Integrating dynamics into enzyme engineering, Protein Engineering, Design and Selection. 35 (2022) gzac015. https://doi.org/10.1093/protein/gzac015.
[12] E. Fischer, Einfluss der Configuration auf die Wirkung der Enzyme, Berichte Der Deutschen Chemischen Gesellschaft. 27 (1894) 2985–2993. https://doi.org/10.1002/cber.18940270364.
[13] D.E. Koshland, Application of a Theory of Enzyme Specificity to Protein Synthesis*, Proc Natl Acad Sci U S A. 44 (1958) 98–104.
[14] T.R. Weikl, F. Paul, Conformational selection in protein binding and function, Protein Sci. 23 (2014) 1508–1518. https://doi.org/10.1002/pro.2539.
[15] M.A. Maria-Solano, E. Serrano-Hervás, A. Romero-Rivera, J. Iglesias-Fernández, S. Osuna, Role of conformational dynamics in the evolution of novel enzyme function, Chem. Commun. 54 (2018) 6622–6634. https://doi.org/10.1039/C8CC02426J.
[16] A. Romero-Rivera, M. Garcia-Borràs, S. Osuna, Role of Conformational Dynamics in the Evolution of Retro-Aldolase Activity, ACS Catal. 7 (2017) 8524–8532. https://doi.org/10.1021/acscatal.7b02954.
[17] A. Gora, J. Brezovsky, J. Damborsky, Gates of Enzymes, Chem. Rev. 113 (2013) 5871–5923. https://doi.org/10.1021/cr300384w.
[18] B. Surpeta, M. Grulich, A. Palyzová, H. Marešová, J. Brezovsky, Common Dynamic Determinants Govern Quorum Quenching Activity in N-Terminal Serine Hydrolases, ACS Catal. 12 (2022) 6359–6374. https://doi.org/10.1021/acscatal.2c00569.
[19] Y. Lee, H. Lee, H. Ihee, Structural dynamics of proteins explored via time-resolved x-ray liquidography, Chem. Phys. Rev. 3 (2022) 041304. https://doi.org/10.1063/5.0101155.
[20] J.M. Martin-Garcia, Protein Dynamics and Time Resolved Protein Crystallography at Synchrotron Radiation Sources: Past, Present and Future, Crystals. 11 (2021) 521. https://doi.org/10.3390/cryst11050521.
[21] T.R.M. Barends, B. Stauch, V. Cherezov, I. Schlichting, Serial femtosecond crystallography, Nat Rev Methods Primers. 2 (2022) 1–24. https://doi.org/10.1038/s43586-022-00141-7.
[22] M. Kovermann, P. Rogne, M. Wolf-Watz, Protein dynamics and function from solution state NMR spectroscopy, Quarterly Reviews of Biophysics. 49 (2016) e6. https://doi.org/10.1017/S0033583516000019.
[23] A.A. Kawale, B.M. Burmann, Chapter 5 - Advanced NMR spectroscopy methods to study protein structure and dynamics, in: P. Saudagar, T. Tripathi (Eds.), Advanced Spectroscopic Methods to Study Biomolecular Structure and Dynamics, Academic Press, 2023: pp. 125–152. https://doi.org/10.1016/B978-0-323-99127-8.00010-6.
[24] E.A. Hodge, M.A. Benhaim, K.K. Lee, Bridging protein structure, dynamics, and function using hydrogen/deuterium-exchange mass spectrometry, Protein Science. 29 (2020) 843–855. https://doi.org/10.1002/pro.3790.
[25] C. Lento, D.J. Wilson, Subsecond Time-Resolved Mass Spectrometry in Dynamic Structural Biology, Chem. Rev. 122 (2022) 7624–7646. https://doi.org/10.1021/acs.chemrev.1c00222.
[26] S. Matsumoto, S. Ishida, M. Araki, T. Kato, K. Terayama, Y. Okuno, Extraction of protein dynamics information from cryo-EM maps using deep learning, Nat Mach Intell. 3 (2021) 153–160. https://doi.org/10.1038/s42256-020-00290-y.
[27] S.A. Hollingsworth, R.O. Dror, Molecular Dynamics Simulation for All, Neuron. 99 (2018) 1129–1143. https://doi.org/10.1016/j.neuron.2018.08.011.
[28] J.A. McCammon, B.R. Gelin, M. Karplus, Dynamics of folded proteins, Nature. 267 (1977) 585–590. https://doi.org/10.1038/267585a0.

[29] A.R. Atilgan, C. Atilgan, Computational strategies for protein conformational ensemble detection, Current Opinion in Structural Biology. 72 (2022) 79–87. https://doi.org/10.1016/j.sbi.2021.08.007.
[30] H.M. Senn, W. Thiel, QM/MM Methods for Biomolecular Systems, Angew. Chem. Int. Ed. 48 (2009) 1198–1229. https://doi.org/10.1002/anie.200802019.
[31] P.-C. Do, E.H. Lee, L. Le, Steered Molecular Dynamics Simulation in Rational Drug Design, J. Chem. Inf. Model. 58 (2018) 1473–1482. https://doi.org/10.1021/acs.jcim.8b00261.
[32] Y. Yang, L. Pan, F.C. Lightstone, K.M. Merz, Chapter One - The Role of Molecular Dynamics Potential of Mean Force Calculations in the Investigation of Enzyme Catalysis, in: G.A. Voth (Ed.), Methods in Enzymology, Academic Press, 2016: pp. 1–29. https://doi.org/10.1016/bs.mie.2016.05.040.
[33] K. Ahmad, A. Rizzi, R. Capelli, D. Mandelli, W. Lyu, P. Carloni, Enhanced-Sampling Simulations for the Estimation of Ligand Binding Kinetics: Current Status and Perspective, Frontiers in Molecular Biosciences. 9 (2022). https://www.frontiersin.org/articles/10.3389/fmolb.2022.899805 (accessed February 13, 2023).
[34] A.J. Pak, G.A. Voth, Advances in coarse-grained modeling of macromolecular complexes, Current Opinion in Structural Biology. 52 (2018) 119–126. https://doi.org/10.1016/j.sbi.2018.11.005.
[35] World Health Organization, Antimicrobial resistance: global report on surveillance, World Health Organization, Geneva, Switzerland, 2014.
[36] M. Mendelson, M. Sharland, M. Mpundu, Antibiotic resistance: calling time on the 'silent pandemic,' JAC-Antimicrobial Resistance. 4 (2022) dlac016. https://doi.org/10.1093/jacamr/dlac016.
[37] C.J. Murray, K.S. Ikuta, F. Sharara, L. Swetschinski, G.R. Aguilar, A. Gray, C. Han, C. Bisignano, P. Rao, E. Wool, S.C. Johnson, A.J. Browne, M.G. Chipeta, F. Fell, S. Hackett, G. Haines-Woodhouse, B.H.K. Hamadani, E.A.P. Kumaran, B. McManigal, R. Agarwal, S. Akech, S. Albertson, J. Amuasi, J. Andrews, A. Aravkin, E. Ashley, F. Bailey, S. Baker, B. Basnyat, A. Bekker, R. Bender, A. Bethou, J. Bielicki, S. Boonkasidecha, J. Bukosia, C. Carvalheiro, C. Castañeda-Orjuela, V. Chansamouth, S. Chaurasia, S. Chiurchiù, F. Chowdhury, A.J. Cook, B. Cooper, T.R. Cressey, E. Criollo-Mora, M. Cunningham, S. Darboe, N.P.J. Day, M.D. Luca, K. Dokova, A. Dramowski, S.J. Dunachie, T. Eckmanns, D. Eibach, A. Emami, N. Feasey, N. Fisher-Pearson, K. Forrest, D. Garrett, P. Gastmeier, A.Z. Giref, R.C. Greer, V. Gupta, S. Haller, A. Haselbeck, S.I. Hay, M. Holm, S. Hopkins, K.C. Iregbu, J. Jacobs, D. Jarovsky, F. Javanmardi, M. Khorana, N. Kissoon, E. Kobeissi, T. Kostyanev, F. Krapp, R. Krumkamp, A. Kumar, H.H. Kyu, C. Lim, D. Limmathurotsakul, M.J. Loftus, M. Lunn, J. Ma, N. Mturi, T. Munera-Huertas, P. Musicha, M.M. Mussi-Pinhata, T. Nakamura, R. Nanavati, S. Nangia, P. Newton, C. Ngoun, A. Novotney, D. Nwakanma, C.W. Obiero, A. Olivas-Martinez, P. Olliaro, E. Ooko, E. Ortiz-Brizuela, A.Y. Peleg, C. Perrone, N. Plakkal, A. Ponce-de-Leon, M. Raad, T. Ramdin, A. Riddell, T. Roberts, J.V. Robotham, A. Roca, K.E. Rudd, N. Russell, J. Schnall, J.A.G. Scott, M. Shivamallappa, J. Sifuentes-Osornio, N. Steenkeste, A.J. Stewardson, T. Stoeva, N. Tasak, A. Thaiprakong, G. Thwaites, C. Turner, P. Turner, H.R. van Doorn, S. Velaphi, A. Vongpradith, H. Vu, T. Walsh, S. Waner, T. Wangrangsimakul, T. Wozniak, P. Zheng, B. Sartorius, A.D. Lopez, A. Stergachis, C. Moore, C. Dolecek, M. Naghavi, Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis, The Lancet. 399 (2022) 629–655. https://doi.org/10.1016/S0140-6736(21)02724-0.
[38] R.W. Eisinger, M.P. Williams, S.H. Choe, E. Krofah, A call to action—stopping antimicrobial resistance, JAC-Antimicrobial Resistance. 5 (2023) dlac142. https://doi.org/10.1093/jacamr/dlac142.
[39] R. Laxminarayan, The overlooked pandemic of antimicrobial resistance, The Lancet. 399 (2022) 606–607. https://doi.org/10.1016/S0140-6736(22)00087-3.
[40] D.G.J. Larsson, C.-F. Flach, Antibiotic resistance in the environment, Nat Rev Microbiol. 20 (2022) 257–269. https://doi.org/10.1038/s41579-021-00649-x.

[41] A.H. Holmes, L.S.P. Moore, A. Sundsfjord, M. Steinbakk, S. Regmi, A. Karkey, P.J. Guerin, L.J.V. Piddock, Understanding the mechanisms and drivers of antimicrobial resistance, The Lancet. 387 (2016) 176–187. https://doi.org/10.1016/S0140-6736(15)00473-0.
[42] J.M.A. Blair, M.A. Webber, A.J. Baylay, D.O. Ogbolu, L.J.V. Piddock, Molecular mechanisms of antibiotic resistance, Nature Reviews Microbiology. 13 (2015) 42–51. https://doi.org/10.1038/nrmicro3380.
[43] J. Bzdrenga, D. Daudé, B. Rémy, P. Jacquet, L. Plener, M. Elias, E. Chabrière, Biotechnological applications of quorum quenching enzymes, Chemico-Biological Interactions. 267 (2017) 104–115. https://doi.org/10.1016/j.cbi.2016.05.028.
[44] P. Williams, K. Winzer, W.C. Chan, M. Cámara, Look who's talking: communication and quorum sensing in the bacterial world, Philosophical Transactions of the Royal Society B: Biological Sciences. 362 (2007) 1119–1134. https://doi.org/10.1098/rstb.2007.2039.
[45] C. Grandclément, M. Tannières, S. Moréra, Y. Dessaux, D. Faure, Quorum quenching: role in nature and applied developments, FEMS Microbiol Rev. 40 (2016) 86–116. https://doi.org/10.1093/femsre/fuv038.
[46] S. Fetzner, Quorum quenching enzymes, Journal of Biotechnology. 201 (2015) 2–14. https://doi.org/10.1016/j.jbiotec.2014.09.001.
[47] M. Bokhove, P.N. Jimenez, W.J. Quax, B.W. Dijkstra, The quorum-quenching N-acyl homoserine lactone acylase PvdQ is an Ntn-hydrolase with an unusual substrate-binding pocket, Proceedings of the National Academy of Sciences. 107 (2010) 686–691. https://doi.org/10.1073/pnas.0911839107.
[48] R. Mukherji, N.K. Varshney, P. Panigrahi, C.G. Suresh, A. Prabhune, A new role for penicillin acylases: Degradation of acyl homoserine lactone quorum sensing signals by Kluyvera citrophila penicillin G acylase, Enzyme and Microbial Technology. 56 (2014) 1–7. https://doi.org/10.1016/j.enzmictec.2013.12.010.
[49] Y.-H. Lin, J.-L. Xu, J. Hu, L.-H. Wang, S.L. Ong, J.R. Leadbetter, L.-H. Zhang, Acyl-homoserine lactone acylase from Ralstonia strain XJ12B represents a novel and potent class of quorum-quenching enzymes, Molecular Microbiology. 47 (2003) 849–860. https://doi.org/10.1046/j.1365-2958.2003.03351.x.
[50] J. Filipovič, O. Vávra, J. Plhák, D. Bednář, S.M. Marques, J. Brezovský, L. Matyska, J. Damborský, CaverDock: A Novel Method for the Fast Analysis of Ligand Transport, ArXiv:1809.03453 [Physics]. (2018). http://arxiv.org/abs/1809.03453 (accessed May 22, 2019).
[51] A. Schenkmayerova, G.P. Pinto, M. Toul, M. Marek, L. Hernychova, J. Planas-Iglesias, V. Daniel Liskova, D. Pluskal, M. Vasina, S. Emond, M. Dörr, R. Chaloupkova, D. Bednar, Z. Prokop, F. Hollfelder, U.T. Bornscheuer, J. Damborsky, Engineering the protein dynamics of an ancestral luciferase, Nat Commun. 12 (2021) 3616. https://doi.org/10.1038/s41467-021-23450-z.
[52] J. Chen, Y.L. Vishweshwaraiah, N.V. Dokholyan, Design and engineering of allosteric communications in proteins, Current Opinion in Structural Biology. 73 (2022) 102334. https://doi.org/10.1016/j.sbi.2022.102334.
[53] J. Gu, Y. Xu, Y. Nie, Role of distal sites in enzyme engineering, Biotechnology Advances. 63 (2023) 108094. https://doi.org/10.1016/j.biotechadv.2023.108094.
[54] S. Osuna, The challenge of predicting distal active site mutations in computational enzyme design, WIREs Computational Molecular Science. 11 (2021) e1502. https://doi.org/10.1002/wcms.1502.
[55] A.M. Damry, C.J. Jackson, The evolution and engineering of enzyme activity through tuning conformational landscapes, Protein Engineering, Design and Selection. 34 (2021) gzab009. https://doi.org/10.1093/protein/gzab009.
[56] M.A. Maria-Solano, T. Kinateder, J. Iglesias-Fernández, R. Sterner, S. Osuna, In Silico Identification and Experimental Validation of Distal Activity-Enhancing Mutations in Tryptophan Synthase, ACS Catal. 11 (2021) 13733–13743. https://doi.org/10.1021/acscatal.1c03950.

[57] L. Wu, L. Qin, Y. Nie, Y. Xu, Y.-L. Zhao, Computer-aided understanding and engineering of enzymatic selectivity, Biotechnology Advances. 54 (2022) 107793. https://doi.org/10.1016/j.biotechadv.2021.107793.
[58] P. Kulkarni, S. Bhattacharya, S. Achuthan, A. Behal, M.K. Jolly, S. Kotnala, A. Mohanty, G. Rangarajan, R. Salgia, V. Uversky, Intrinsically Disordered Proteins: Critical Components of the Wetware, Chem. Rev. 122 (2022) 6614–6633. https://doi.org/10.1021/acs.chemrev.1c00848.
[59] W. Ding, K. Nakai, H. Gong, Protein design via deep learning, Briefings in Bioinformatics. 23 (2022) bbac102. https://doi.org/10.1093/bib/bbac102.
[60] J. Horne, D. Shukla, Recent Advances in Machine Learning Variant Effect Prediction Tools for Protein Engineering, Ind. Eng. Chem. Res. 61 (2022) 6235–6245. https://doi.org/10.1021/acs.iecr.1c04943.
[61] S. Mazurenko, Z. Prokop, J. Damborsky, Machine Learning in Enzyme Engineering, ACS Catal. 10 (2020) 1210–1223. https://doi.org/10.1021/acscatal.9b04321.
[62] A. Okabe, Barry Boots, Kokichi Sugihara, Sung Nok Chiu, D. G. Kendall, Spatial Tessellations: Concepts and Applications of Voronoi Diagrams, 2nd ed., Wiley, 2000.
[63] J. Brezovsky, P. Babkova, O. Degtjarik, A. Fortova, A. Gora, I. Iermak, P. Rezacova, P. Dvorak, I.K. Smatanova, Z. Prokop, R. Chaloupkova, J. Damborsky, Engineering a de Novo Transport Tunnel, ACS Catal. 6 (2016) 7597–7610. https://doi.org/10.1021/acscatal.6b02081.
[64] S. Doerr, M.J. Harvey, F. Noé, G. De Fabritiis, HTMD: High-Throughput Molecular Dynamics for Molecular Discovery, J. Chem. Theory Comput. 12 (2016) 1845–1852. https://doi.org/10.1021/acs.jctc.6b00049.
[65] S. Decherchi, A. Cavalli, Thermodynamics and Kinetics of Drug-Target Binding by Molecular Simulation, Chem. Rev. 120 (2020) 12788–12833. https://doi.org/10.1021/acs.chemrev.0c00534.
[66] A. Pavelka, E. Sebestova, B. Kozlikova, J. Brezovsky, J. Sochor, J. Damborsky, CAVER: Algorithms for Analyzing Dynamics of Tunnels in Macromolecules, IEEE/ACM Transactions on Computational Biology and Bioinformatics. 13 (2016) 505–517. https://doi.org/10.1109/TCBB.2015.2459680.
[67] E. Chovancova, A. Pavelka, P. Benes, O. Strnad, J. Brezovsky, B. Kozlikova, A. Gora, V. Sustr, M. Klvana, P. Medek, L. Biedermannova, J. Sochor, J. Damborsky, CAVER 3.0: A Tool for the Analysis of Transport Pathways in Dynamic Protein Structures, PLoS Computational Biology. 8 (2012) e1002708. https://doi.org/10.1371/journal.pcbi.1002708.
[68] T. Magdziarz, K. Mitusińska, S. Gołdowska, A. Płuciennik, M. Stolarczyk, M. Ługowska, A. Góra, AQUA-DUCT: a ligands tracking tool, Bioinformatics. 33 (2017) 2045–2046. https://doi.org/10.1093/bioinformatics/btx125.
[69] T. Magdziarz, K. Mitusińska, M. Bzówka, A. Raczyńska, A. Stańczak, M. Banas, W. Bagrowska, A. Góra, AQUA-DUCT 1.0: structural and functional analysis of macromolecules from an intramolecular voids perspective, Bioinformatics. 36 (2020) 2599–2601. https://doi.org/10.1093/bioinformatics/btz946.
[70] E. Papaioannou, M. Wahjudi, P. Nadal-Jimenez, G. Koch, R. Setroikromo, W.J. Quax, Quorum-Quenching Acylase Reduces the Virulence of Pseudomonas aeruginosa in a Caenorhabditis elegans Infection Model, Antimicrobial Agents and Chemotherapy. 53 (2009) 4891–4897. https://doi.org/10.1128/AAC.00380-09.
[71] M. Wahjudi, S. Murugappan, R. van Merkerk, A.C. Eissens, M.R. Visser, W.L.J. Hinrichs, W.J. Quax, Development of a dry, stable and inhalable acyl–homoserine–lactone–acylase powder formulation for the treatment of pulmonary Pseudomonas aeruginosa infections, European Journal of Pharmaceutical Sciences. 48 (2013) 637–643. https://doi.org/10.1016/j.ejps.2012.12.015.
[72] G. Koch, P. Nadal-Jimenez, C.R. Reis, R. Muntendam, M. Bokhove, E. Melillo, B.W. Dijkstra, R.H. Cool, W.J. Quax, Reducing virulence of the human pathogen Burkholderia by altering the substrate specificity of the quorum-quenching acylase PvdQ, Proceedings of the National Academy of Sciences. 111 (2014) 1568–1573. https://doi.org/10.1073/pnas.1311263111.

[73] D. Wang, F. Cui, L. Ren, X. Tan, Q. Li, J. Li, T. Li, Enhancing the Inhibition Potential of AHL Acylase PF2571 against Food Spoilage by Remodeling Its Substrate Scope via a Computationally Driven Protein Design, J. Agric. Food Chem. 70 (2022) 14510–14521. https://doi.org/10.1021/acs.jafc.2c05753.
[74] M. Grulich, V. Štěpánek, P. Kyslík, Perspectives and industrial potential of PGA selectivity and promiscuity, Biotechnology Advances. 31 (2013) 1458–1472. https://doi.org/10.1016/j.biotechadv.2013.07.005.
[75] M. Arroyo, I. de la Mata, C. Acebal, M. Pilar Castillón, Biotechnological applications of penicillin acylases: state-of-the-art, Appl Microbiol Biotechnol. 60 (2003) 507–514. https://doi.org/10.1007/s00253-002-1113-6.
[76] A. Bruggink, E.C. Roos, E. de Vroom, Penicillin Acylase in the Industrial Production of β-Lactam Antibiotics, Org. Process Res. Dev. 2 (1998) 128–133. https://doi.org/10.1021/op9700643.
[77] M. Grulich, J. Brezovský, V. Štěpánek, A. Palyzová, H. Marešová, J. Zahradník, E. Kyslíková, P. Kyslík, In-silico driven engineering of enantioselectivity of a penicillin G acylase towards active pharmaceutical ingredients, Journal of Molecular Catalysis B: Enzymatic. 133 (2016) S53–S59. https://doi.org/10.1016/j.molcatb.2016.11.014.
[78] M. Grulich, J. Brezovský, V. ŠtL·pánek, A. Palyzová, E. Kyslíková, P. Kyslík, Resolution of α/β-amino acids by enantioselective penicillin G acylase from Achromobacter sp ., Journal of Molecular Catalysis B: Enzymatic. 122 (2015) 240–247. https://doi.org/10.1016/j.molcatb.2015.09.008.

PUBLICATIONS

Publication 1

Common Dynamic Determinants Govern Quorum Quenching Activity in N-Terminal Serine Hydrolases

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Publication 2

Dynamics, a Powerful Component of Current and Future in Silico Approaches for Protein Design and Engineering

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Source code

https://github.com/labbit-eu/transport_tools

Repository

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Common Dynamic Determinants Govern Quorum Quenching Activity in N-Terminal Serine Hydrolases

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I declare the following contribution to this publication:

performed all computational experiments, i.e., preparation of structures for modeling, molecular docking, molecular dynamics simulations of enzymes in the free state and their complexes with bacterial signaling molecules, and hybrid quantum mechanics and molecular mechanics simulations of the chemical reaction of the studied complexes; analyzed, interpreted and visualized the data; and co-wrote the manuscript.

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I declare the following contribution to this publication:

Conceived and coordinated the project; designed computational analyses, co-analyzed and co-interpreted data; co-wrote the manuscript & revised its final version.

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Dynamics, a Powerful Component of Current and Future in Silico Approaches for Protein Design and Engineering

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TransportTools: A Library for High-Throughput Analyses of Internal Voids in Biomolecules and Ligand Transport through Them

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Poznan, March 14, 2023

CO-AUTHOR CONTRIBUTION STATEMENT

Hereby, I certify that I am the co-author of the following publication:

Brezovsky J, Thirunavukarasu AS, Surpeta B, Sequeiros-Borja CE, Mandal N, Sarkar DK, Dongmo Foumthuim CJ, Agrawal N, 2021: TransportTools: A Library for High-Throughput Analyses of Internal Voids in Biomolecules and Ligand Transport Through Them. *Bioinformatics 38:* 1752-1753, DOI: 10.1093/bioinformatics/btab872.

I declare the following contribution to this publication:

contributed to user-testing of the developed tools; wrote the draft of state of the art overview in Supplementary File 1; conceived and written the tutorial included in the user guide.

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Promotor
dr hab. Jan Brezovsky, prof. UAM

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Brezovsky J, Thirunavukarasu AS, Surpeta B, Sequeiros-Borja CE, Mandal N, Sarkar DK, Dongmo Foumthuim CJ, Agrawal N, 2021: TransportTools: A Library for High-Throughput Analyses of Internal Voids in Biomolecules and Ligand Transport Through Them. *Bioinformatics 38:* 1752-1753, DOI: 10.1093/bioinformatics/btab872.

I declare the following contribution to this publication:

conceived the research project and the new computational tool; designed and implemented a large majority of algorithms and tests, and prepared the user and technical documentation for the tool; coordinated the work of the project team; analyzed and interpreted the data; and wrote the manuscript.

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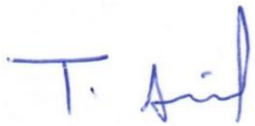
Brezovsky J, Thirunavukarasu AS, Surpeta B, Sequeiros-Borja CE, Mandal N, Sarkar DK, Dongmo Foumthuim CJ, Agrawal N, 2021: TransportTools: A Library for High-Throughput Analyses of Internal Voids in Biomolecules and Ligand Transport Through Them. *Bioinformatics 38:* 1752-1753, DOI: 10.1093/bioinformatics/btab872.

I declare the following contribution to this publication:

contributed to user-testing of the developed tools; generated data for performance evaluation of the TransportTools and Use-case 1 summarized in Supplementary File 5 and 6 and wrote drafts of these two documents.



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Brezovsky J, Thirunavukarasu AS, Surpeta B, Sequeiros-Borja CE, Mandal N, Sarkar DK, Dongmo Foumthuim CJ, Agrawal N, 2021: TransportTools: A Library for High-Throughput Analyses of Internal Voids in Biomolecules and Ligand Transport Through Them. *Bioinformatics 38:* 1752-1753, DOI: 10.1093/bioinformatics/btab872.

I declare the following contribution to this publication:

contributed to user-testing of the developed tools; implemented algorithms for structural alignment and surface visualization of tunnel clusters; edited the manuscript.

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Brezovsky J, Thirunavukarasu AS, Surpeta B, Sequeiros-Borja CE, Mandal N, Sarkar DK, Dongmo Foumthuim CJ, Agrawal N, 2021: TransportTools: A Library for High-Throughput Analyses of Internal Voids in Biomolecules and Ligand Transport Through Them. *Bioinformatics 38:* 1752-1753, DOI: 10.1093/bioinformatics/btab872.

I declare the following contribution to this publication:

contributed to user-testing of the developed tools; generated data for Use-case 2 in Supplementary File 7 and wrote the draft of this document.

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I declare the following contribution to this publication:

contributed to user-testing of the developed tools; generated data for Use-case 3 in Supplementary File 8 and wrote the draft of this document.

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Rational engineering of binding pocket's structure and dynamics in penicillin G acylase for selective degradation of bacterial signaling molecules

1. Surpeta, B.;
2. Grulich, M.;
3. Palyzová, A.;
4. Marešová, H.;
5. Zahradník, J.;
6. Brezovsky, J.

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Hereby, I certify that I am the co-author of the following publication:

Grulich, M.; Surpeta, B.; Palyzová, A.; Marešová, H.; Zahradník, J.; Brezovsky, J. Rational engineering of binding pocket's structure and dynamics in penicillin G acylase for selective degradation of bacterial signaling molecules. bioRxiv 2023.05.09.538545; doi: https://doi.org/10.1101/2023.05.09.538545

I declare the following contribution to this publication:

performed all computational experiments, i.e., preparation of structures for modeling, in silico design of mutants and their filtering, molecular docking, molecular dynamics simulations of enzymes in the free state and their complexes with bacterial signaling molecules; analyzed, interpreted and visualized the data; and co-wrote the manuscript.

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Grulich, M.; Surpeta, B.; Palyzová, A.; Marešová, H.; Zahradník, J.; Brezovsky, J. Rational engineering of binding pocket's structure and dynamics in penicillin G acylase for selective degradation of bacterial signaling molecules. bioRxiv 2023.05.09.538545; doi: https://doi.org/10.1101/2023.05.09.538545

I declare the following contribution to this publication:

designed and coordinated the experimental work, performed detailed characterization of all the ecPGA designed mutants (YAF, VAF and MSF), and analyzed and interpreted data; and co-wrote the manuscript.

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Hereby, I certify that I am the co-author of the following publication:

Grulich, M.; Surpeta, B.; Palyzová, A.; Marešová, H.; Zahradník, J.; Brezovsky, J. Rational engineering of binding pocket's structure and dynamics in penicillin G acylase for selective degradation of bacterial signaling molecules. bioRxiv 2023.05.09.538545; doi: https://doi.org/10.1101/2023.05.09.538545

I declare the following contribution to this publication:

executed all of the bacterial flasks and fed-batch cultivation in a bioreactor to produce enzymes.

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Grulich, M.; Surpeta, B.; Palyzová, A.; Marešová, H.; Zahradník, J.; Brezovsky, J. Rational engineering of binding pocket's structure and dynamics in penicillin G acylase for selective degradation of bacterial signaling molecules. bioRxiv 2023.05.09.538545; doi: https://doi.org/10.1101/2023.05.09.538545

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Hereby, I certify that I am the co-author of the following publication:

Grulich, M.; Surpeta, B.; Palyzová, A.; Marešová, H.; Zahradník, J.; Brezovsky, J. Rational engineering of binding pocket's structure and dynamics in penicillin G acylase for selective degradation of bacterial signaling molecules. bioRxiv 2023.05.09.538545; doi: https://doi.org/10.1101/2023.05.09.538545

I declare the following contribution to this publication:

designed the set of used primers for the ecPGA mutant variants.

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RNDr. Jiří Zahradník, Ph.D.
Principal Investigator

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Hereby, I certify that I am the co-author of the following publication:

Grulich, M.; Surpeta, B.; Palyzová, A.; Marešová, H.; Zahradník, J.; Brezovsky, J. Rational engineering of binding pocket's structure and dynamics in penicillin G acylase for selective degradation of bacterial signaling molecules. bioRxiv 2023.05.09.538545; doi: https://doi.org/10.1101/2023.05.09.538545

I declare the following contribution to this publication:

conceived and coordinated the project, designed computational analyses, and analyzed and interpreted data; co-wrote the manuscript.

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