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**Olamide Jeje; Oluwatobi Otun; Chinyere Aloke; Ikechukwu Achilonu**

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# Exploring NAD$^+$ metabolism and NNAT: Insights from structure, function, and computational modeling

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ABSTRACT

Nicotinamide Adenine Dinucleotide (NAD+), a coenzyme, is ubiquitously distributed and serves crucial functions in diverse biological processes, encompassing redox reactions, energy metabolism, and cellular signalling. This review article explores the intricate realm of NAD + metabolism, with a particular emphasis on the complex relationship between its structure, function, and the pivotal enzyme, Nicotinate Nucleotide Adenylyltransferase (NNAT), also known as nicotinate mononucleotide adenylyltransferase (NaMNAT), in the process of its biosynthesis. Our findings indicate that NAD + biosynthesis in humans and bacteria occurs via the same de novo synthesis route and the pyridine ring salvage pathway. Maintaining NAD homeostasis in bacteria is imperative, as most bacterial species cannot get NAD+ from their surroundings. However, due to lower sequence identity and structurally distant relationship of bacteria, including *E. faecium* and *K. pneumonia*, to its human counterpart, inhibiting NNAT, an indispensable enzyme implicated in NAD + biosynthesis, is a viable alternative in curtailing infections orchestrated by *E. faecium* and *K. pneumonia*. By merging empirical and computational discoveries and connecting the intricate NAD + metabolism network with NNAT's crucial role, it becomes clear that the synergistic effect of these insights may lead to a more profound understanding of the coenzyme's function and its potential applications in the fields of therapeutics and biotechnology.

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## 1. Introduction

The group of pathogens known as "ESKAPE" has been associated with the primary aetiology of nosocomial infections [1]. This group of pathogens encompasses a variety of species, including both Gram-positive and Gram-negative bacteria, including *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp. [2]. However, these pathogens pose a significant challenge, responsible for approximately 40 % of infections in the intensive care unit [3]. These organisms are distinguished by their capacity to evade antibacterial medications' biocidal effects effectively; hence, they pose a significant challenge in eradication [4]. This is quite apparent in the resistance of extended-spectrum cephalosporin and carbapenem by *K. pneumoniae* [5], *E. faecium* to vancomycin (VRE) [6], *P. aeruginosa* to fluoroquinolone and carbapenem [7], *S. aureus* to methicillin (MRSA) [8], *Enterobacteriaceae* to fluoroquinolone and carbapenem [9], and *A. baumannii* to carbapenem [10].

Despite the development of various innovative therapies to address the issue of multi-drug resistance, such as combination therapy, enhancements in β-lactamase inhibitors, the introduction of new antibiotics, utilisation of nanoparticles, phage therapy, and antimicrobial peptides, the prevalence of *K. pneumoniae* and *E. faecium* infections continues to persist [3,11]. This highlights the critical need to promptly discover new alternative targets and efficacious bactericidal agents. In addition, given that the majority of current antibiotics target the synthesis of DNA and cellular functions, such as proteins implicated in cell wall biosynthesis, it is crucial to identify additional alternative targets. Considering this, studying how to disrupt nicotinamide adenine dinucleotide ($NAD^+$) biosynthesis seems an excellent way to find a new therapeutic regimen to treat infections caused by *K. pneumoniae* and *E. faecium*. This approach is justified by the growing recognition of the crucial involvement of $NAD^+$ and its derivatives in a wide range of cellular and biological activities. This entity's functionality encompasses multiple activities, including DNA repair, energy metabolism, redox reactions, protein modification and gene regulation [12]. Hence, it is imperative to maintain $NAD^+$ homeostasis, as most bacterial species cannot acquire $NAD^+$ from their surroundings. Any disruption in the production of $NAD^+$ or breakdown modulation would likely compromise the cellular metabolic state, leading to fatal consequences [13]. The enzymes implicated in the subsequent biosynthesis of $NAD^+$ present promising avenues for exploring novel therapeutic agents. This study explicitly examines the enzyme nicotinate nucleotide adenylyltransferase (NNAT), alternatively known as nicotinate mononucleotide adenylyltransferase (NaMNAT), as the reaction facilitated by NNAT is a critical process in synthesising pyridine dinucleotides. Given the absence of any alternative pathway for the formation of pyridine dinucleotides, it is evident that the activity of NNAT plays a crucial and irreplaceable function in $NAD^+$'s production. In addition, when comparing the enzymes accountable for the subsequent production of $NAD^+$, it is evident that the bacterial NNAT enzyme has the lowest degree of similarity in sequence and structure compared to its human counterpart. This characteristic makes it a more viable target for developing antibacterial agents with specific inhibitory properties. Furthermore, previous research has investigated the development of inhibitors based on protein knockdown techniques to induce a deficiency of NNAT, which has been shown to exhibit antibacterial properties [13–15]. The results of this study support the notion that NNAT is a promising target. While the activity of NNAT is highly conserved across various species, the enzymes possess distinct structural and catalytic features indicating their species-specific nature [16]. The findings from a preliminary investigation into the phylogeny of NNAT, as depicted in Fig. 1, show an evolutionary divergence of NNAT across various bacterial species despite their shared prokaryotic nature [17].

Similarly, the sequence alignment comparison between the various bacteria species (Fig. 2) reveals a discernible divergence in their sequence similarity. The observed variations in enzyme sequences may play a role in determining their unique biophysical characteristics, structural attributes, and potential functional roles.

All NNAT enzymes that have been characterised to date are classified as metal-activated enzymes, which utilise divalent cations to bind co-substrates and facilitate catalytic reactions. Numerous studies have demonstrated that the NNAT isozyme exhibits a discernible inclination towards diverse divalent metal ions [12,18]. The observed variation in metal ion specificity has the potential to influence structural variation, thereby playing a role in the enzyme's distinct function and its choice of substrate.

Hence, it is essential to comprehensively comprehend the biophysical architecture of the specific enzymes, their functionalities, and their catalytic mechanism and dynamics. This understanding should encompass both scenarios, namely the presence and absence of cations. Such knowledge acquisition aims to effectively devise molecular compounds that can specifically impede the NNAT enzyme's functioning in *K. pneumoniae* and *E. faecium*.

## 2. Nicotinamide adeninedinucleotide ($NAD^+$) metabolism

The co-enzyme nicotinamide adenine dinucleotide ($NAD^+$), a metabolite found in prokaryotic and eukaryotic cells, substantially impacts various metabolic pathways owing to its pervasive influence [19]. The essentiality of NAD stems from its role as both an oxidising agent ($NAD^+$) and a reducing agent (NADH), facilitating electron transfer and catalysing several physiological events and metabolic pathways [12]. In addition to its role as a coenzyme in redox reactions, $NAD^+$ is employed as a co-substrate by non-redox $NAD^+$-hydrolyzing enzymes. These enzymes, including bacterial DNA ligases, utilise $NAD^+$ for DNA repair [20]. Furthermore, $NAD^+$ is

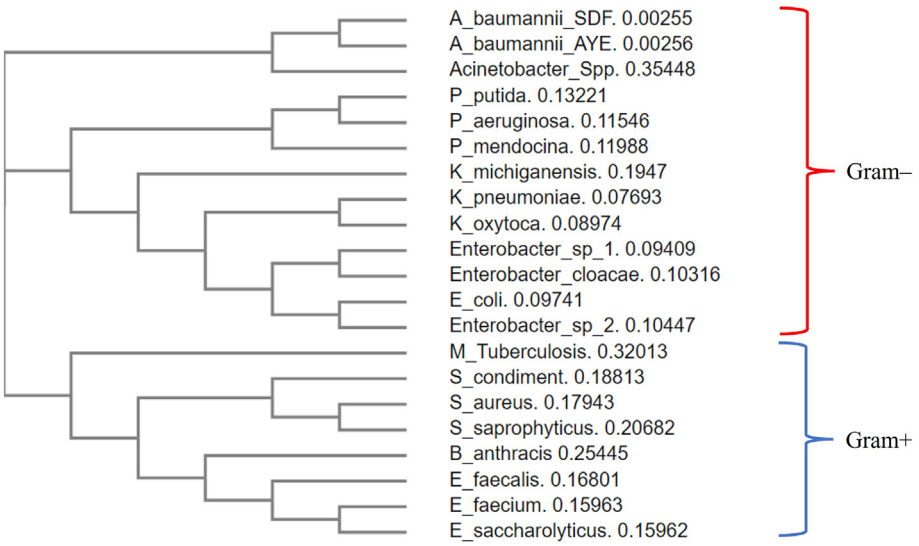



**Fig. 1. A phylogram depicting the evolutionary divergence of NNAT across various bacterial species, highlighting the differentiation between Gram-positive and Gram-negative bacteria.** The numerical values shown in the figure correspond to the distances between sequences. The similarity between sequences increases as the numerical values approach each other. The Clustal Omega multiple sequence alignment tool was utilised to conduct the analysis [17].

used by protein lysine and histone deacetylases belonging to the CobB and Sir2 family (sirtuins) to remove acetyl groups from lysine residues on proteins [21]. Poly ADP-ribose polymerases (PARPs) and ADP ribosyltransferases (ARTs) are enzymes that use $NAD^+$ as a source of ADP-ribose moieties to modify proteins and generate lengthy poly (ADP-ribose) (PAR) chains for gene regulation (Fig. 3). Besides, $NAD^+$ functions as a precursor for cyclic ADP-ribose, which plays a role in maintaining calcium homeostasis [22]. $NAD^+$ also acts as a substrate in cobalamin synthesis [23] and activates diphtheria and cholera toxins [13].

### 2.1. Redox metabolic pathways involving $NAD^+$ in bacteria

$NAD^+$ functions as a cofactor in redox metabolism by efficiently interconverting between its reduced form (NADH) and oxidised form ($NAD^+$) without any overall net expenditure [25]. This interconversion allows $NAD^+$ to facilitate the transport of hydride ions throughout the cell, serving as both an acceptor and donor of hydride ions in energy metabolism. The conversion of $NADP^+$ to NADPH enables its involvement in reductive biosynthesis and the regulation of redox activity ($NADP^+$/NADPH). Numerous dehydrogenases use $NAD^+$ and its derivatives as primary electron acceptors or donors, and almost all metabolic pathways need the presence of these molecules [26]. Fig. 4 illustrates many essential metabolic pathways that rely significantly on utilising NAD(H).

$NAD^+$ is pivotal in facilitating energy production along the glycolytic pathway and Kreb cycle by enabling the transfer of reducing equivalents in NADH. The β-oxidation pathway and glyoxylate cycle rely on $NAD^+$ as a hydride acceptor to carry out their oxidative processes. The process of converting pyruvate to lactate via fermentation involves the use of NADH as a source of hydride ions. The Electron Transport Chain employs NADH created via a sequence of oxidation-reduction electron exchanges to liberate free energy to produce ATP (Fig. 4). Moreover, the conversion of malate to pyruvate and the use of the pentose phosphate pathway includes the utilisation of $NADP^+$ to transfer reducing equivalents in the form of NADPH [29]. This NADPH may then serve as a reducing agent in producing cholesterol, nucleic acids, fatty acids and nucleotides. Preserving the NAD(P) redox pair is crucial to prevent the complete breakdown of the central metabolic system since creating NAD(P) sets limitations on synthesis efficiency and energy production. This is particularly significant due to the pathways' interconnectedness [30].

### 2.2. $NAD^+$ synthesis in bacteria

Bacterial organisms use two distinct metabolic processes, namely de novo biosynthesis and the pyridine ring salvage pathway, to facilitate the manufacture of $NAD^+$ [31]. This process is shown in Fig. 5. Amino acids serve as precursors in the de novo production of $NAD^+$. Bacterial organisms have been observed to engage in the anaerobic synthesis of $NAD^+$ by utilising aspartate and dihydroxyacetone phosphate as precursor molecules [32]. This process involves a two-step enzymatic reaction that ultimately leads to the production of quinolinic acid.

Conversely, a minority of bacterial species have been found to synthesise $NAD^+$ aerobically, employing a five-step enzymatic reaction that utilises tryptophan as a starting material. This aerobic pathway also culminates in quinolinic acid production. Nicotinate mononucleotide (NaMN) is produced by the phosphoribosylation and decarboxylation of quinolinic acid, which may be derived from either tryptophan or aspartate. This enzymatic process is facilitated by the enzyme quinolinic acid phosphoribosyltransferase, encoded as NadC. The formation of NaMN is then catalysed by the enzyme NNAT (NadD) by using ATP as a cofactor, resulting in the production of nicotinate adenine dinucleotide (NaAD). The NaAD produced is then subjected to amidation in the presence of ammonia and ATP, catalysed by the enzyme $NAD^+$ synthetase (NadE) [33,34].

The pyridine bases nicotinate (Na) and nicotinamide (Nm), resulting from the breakdown products of $NAD^+$ turnover, are recycled via the pyridine ring salvage route to re-synthesise $NAD^+$. The PncA-PncB salvage route is the predominant salvage pathway observed in bacteria, and it entails two enzymatic steps: firstly, the conversion of nicotinamide to nicotinate through the action of the enzyme nicotinamidase (PncA), followed by the attachment of nicotinate to 5-phosphoribosyl-1-pyrophosphate (PRPP) by the enzyme nicotinate phosphoribosyltransferase (PncB), to form NaMN. The NaMN molecule convergence at the de novo route is subjected to adenylation and amidation processes by NNAT and NAD synthase enzymes. These enzymatic reactions result in the

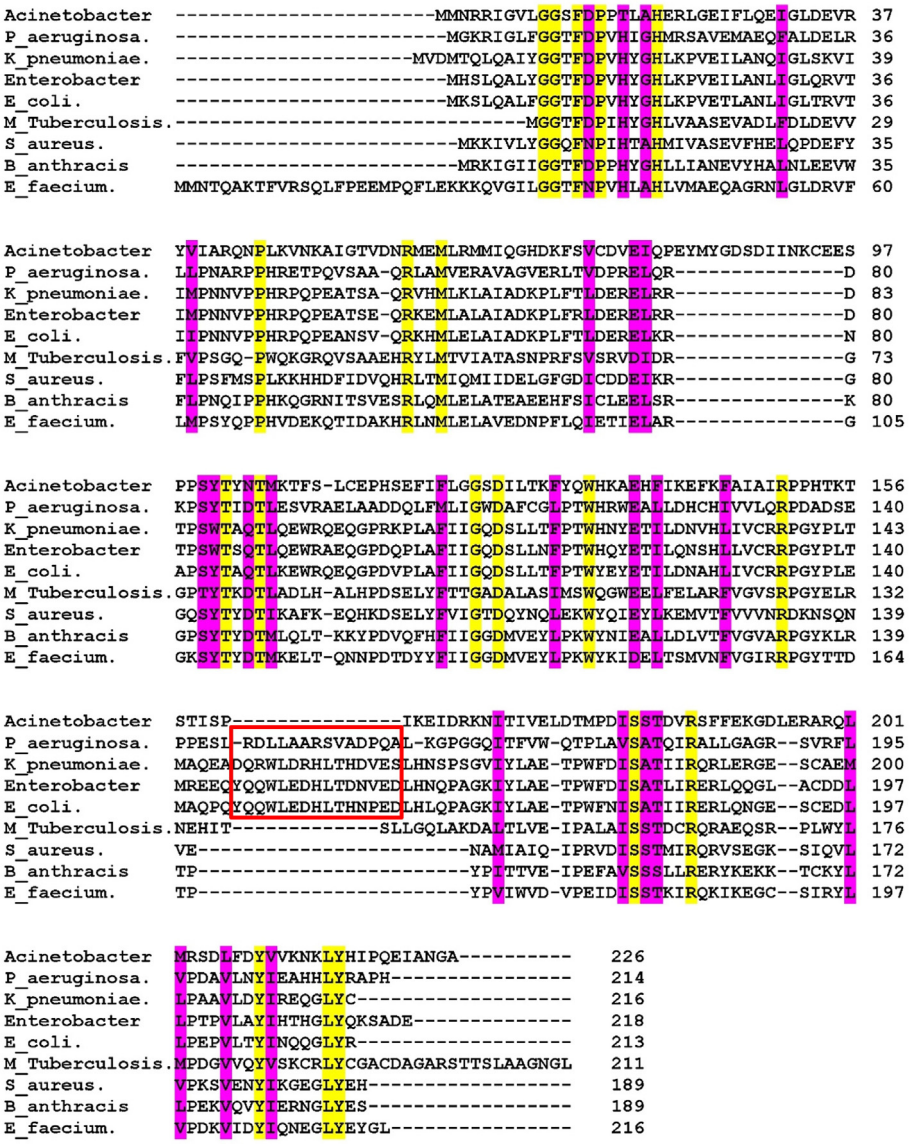



**Fig. 2. The sequence alignment of NNAT from various bacteria species showing variation in the amino acid sequences.** The yellow highlight indicates positions containing conserved residues, while the partially conserved or mutated residues are indicated in purple. The absence of residues in the designated space indicates non-conservation. The region in the red box indicates the additional αβα unit insertion common to the Gram-negative bacteria NNAT structure. The sequence alignment presented in this context was produced utilising the CLUSTAL Omega.

formation of NaAD and $NAD^+$, respectively.

In an alternative pathway, nicotinamide is recycled via nicotinamide mononucleotide (NMN) to produce nicotinamide adenine dinucleotide ($NAD^+$) through the enzymatic actions of nicotinamide phosphoribosyltransferase and NNAT, as seen in Fig. 5. Certain bacteria, such as Haemophilus influenzae, depend on $NAD^+$ and use distinct salvage mechanisms that utilise nicotinamide intermediates, ribosyl nicotinamide, to manufacture $NAD^+$. The process described above is accomplished using the PnuC-NadR nicotinamide riboside salvage route, which involves a two-step transformation of ribosyl nicotinamide into $NAD^+$. This conversion is facilitated by the enzymatic actions of nicotinamide riboside kinase (NmR kinase) and NNAT (NadR), which phosphorylate and adenylate ribosyl nicotinamide, respectively [34].

Thus, NaMN produced via the de novo route, together with NaMN and NMN products formed through the salvage pathway, come together in a process facilitated by the enzyme nicotinate nucleotide adenylyltransferase (NNAT) to produce their respective dinucleotides, namely NaAD and $NAD^+$ [35]. This implies that the enzyme NaMNAT can catalyse the conversion of both NaMN and NMN.

Bacteria employ L-aspartate as the primary amino acid precursor for anaerobic synthesis of $NAD^+$, whereas a subset of bacteria and humans use tryptophan for aerobic synthesis. Nicotinate nucleotide adenylyltransferase (NNAT) utilises NaMN, which undergoes amidation to yield $NAD^+$. The compounds nicotinic acid and nicotinamide and their ribosides, derived from the breakdown of $NAD^+$ and obtained from external sources, are recycled to regenerate $NAD^+$ through NaMN and NMN. This process involves the catalytic reaction facilitated by NMNAT, resulting in the formation of NaAD and $NAD^+$ [36]. QAPRTase (quinolinic acid phosphoribosyltransferase), NamPRTase (nicotinamide

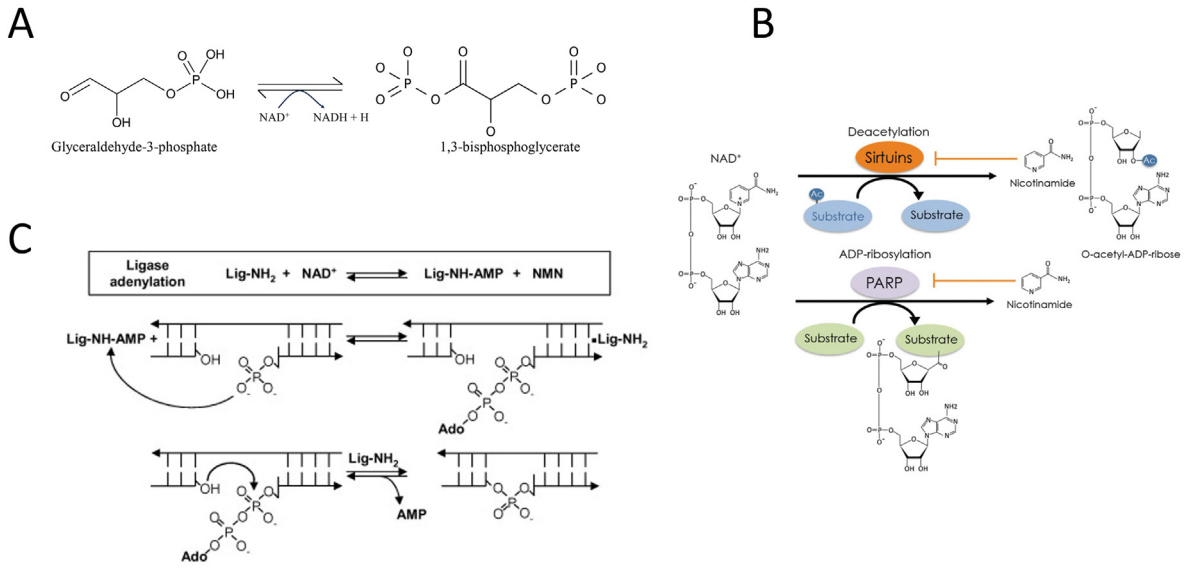



**Fig. 3.** Biochemical reactions demonstrating the biological roles of $NAD^+$. A. NAD $^+$ serves as a coenzyme during the conversion of glyceraldehyde 3-phosphate to 1,3-bisphosphate by the enzyme glyceraldehyde phosphate dehydrogenase, reducing $NAD^+$ to NADH. B. The depiction illustrates $NAD^+$ functioning as a substrate for the enzymes Sirtuins (Sir2) and ADP-ribose transferases, wherein it donates ADP-ribose. C. Bacterial DNA ligases employ $NAD^+$ as a cofactor for the adenylation of the ligase active site, resulting in the formation of an adenylated nicked substrate [24].

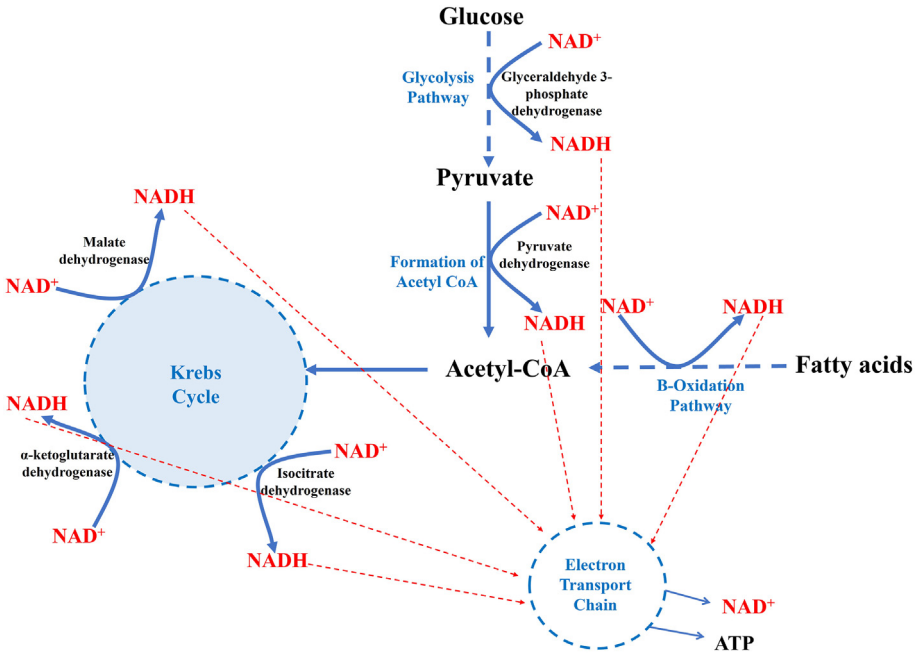



**Fig. 4. A schematic illustration of interconnected metabolic pathways involving the redox activity of $NAD^+$/NADH for energy generation** [27,28].

phosphoribosyltransferase), and NNAT (nicotinate nucleotide adenylyltransferase).

### 2.3. NAD $^+$ synthesis in human

In the human body, the production of $NAD^+$ occurs via both the de novo route and the pyridine salvage pathways. Like the de novo aerobic production of $NAD^+$ in bacteria, as seen in Fig. 5, tryptophan functions as the precursor amino acid. The process by which $NAD^+$ is formed in humans follows a sequential series of identical stages. The $NAD^+$ salvage route is the primary provider of $NAD^+$ in humans. The conversion of nicotinate and nicotinamide, obtained from external sources and via the turnover of $NAD^+$-utilising enzymes, is directly linked to the synthesis of phosphoribosyl pyrophosphate (PRPP) through the actions of NAPRTase and NamPRT. This results in the formation of nicotinate mononucleotide (NaMN) and nicotinamide mononucleotide (NMN). NNAT catalyses the subsequent adenylation of the mononucleotides, culminating in the production of NaAD and $NAD^+$. The formation of NaAD is eventually transformed into $NAD^+$ by the action of $NAD^+$ synthase. Moreover, it is worth noting that exogenous nicotinamide ribosides play a crucial function in synthesising $NAD^+$ via phosphorylation and

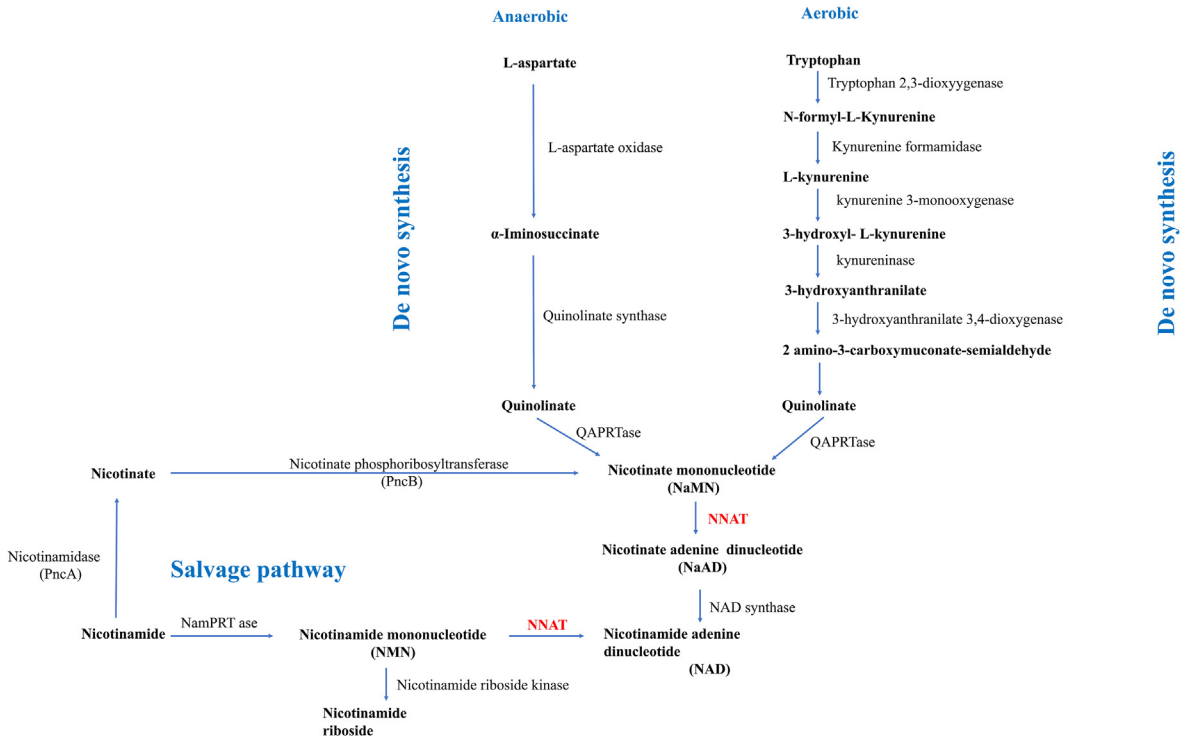



**Fig. 5.** A schematic representation illustrating de novo processes and salvage synthesis of NAD+ in bacteria and humans. Source [36].

adenylation processes, which are catalysed by NRK and NNAT, respectively [37].

Notably, the sequential biosynthetic processes leading to the production of $NAD^+$ from quinolinic acid are well maintained in both bacterial and human organisms. The processes mentioned above are facilitated by comparable enzyme sets but with several noteworthy differences [38]. NNAT is a promising target for antibacterial intervention in the enzymatic pathway responsible for converting quinoline to $NAD^+$. This is due to its much lower sequence identity and structurally distant relationship to its human counterpart [39].

## 3. Ninicotinate nucleotide adenylyltransferase (NNAT): enzyme at the core

The enzyme nicotinate nucleotide adenylyltransferase (NNAT) plays a vital role in the two pathways leading to the production of $NAD^+$, viz: the de novo and salvage pathways [36]. It is essential for catalysing the adenylation step, which leads to the generation of pyridine dinucleotides. The physiological activity of NNAT is thought to be the synthesis of $NAD^+$ due to the convergence of all paths at the reaction catalysed by NNAT, with no alternative routes leading to pyridine dinucleotide production. NNAT belong to the nucleotidyltransferase α/β phosphodiesterase (EC 2.7.7) superfamily, which is distinguished by the presence of a conserved "H/TXGH" signature motif and a Rossmann fold [40]. The Nicotinate nucleotide adenylyltransferases (NNAT) can catalyse the adenylation of both nicotinamide mononucleotide (NMN) and nicotinic acid mononucleotide (NaMN), as seen in Figs. 5 and 6. However, the efficiency level varies with a higher preference for the NaMN substrate over NMN [37], consequently classifying the enzyme into EC 2.7.7.18. The enzyme under consideration facilitates the adenylation process of NMN and NaMN by transferring the adenyl group from ATP to NMN and NaMN, culminating in the production of $NAD^+$ and NaAD, respectively. Simultaneously, this process releases inorganic pyrophosphate. The reaction entails a nucleophilic attack on the α-phosphoryl group of ATP by the mononucleotide substrate, as described by Ghanem et al. [15].

### 3.1. The physicochemical properties of NNAT

This refers to the characteristics and behaviours of the protein to its physical and chemical attributes. The NNAT enzymes demonstrate an optimal pH range of 6.0–9.0. Metal ions activate these enzymes and rely on divalent metal ions to bind to the substrate and facilitate the catalysis of the adenylyl transfer process. Previous studies have shown that NNAT isozymes prefer several divalent metal ions besides magnesium ions [42]. NNATs are a class of globular proteins comprising around 160–400 amino acids. The subunit mass of these proteins typically falls between the range of 20–50 kDa (kDa). Most NNATs are often seen as homo-oligomers with 2–6 identical subunits [16]. The activity of NNAT is highly conserved across several species; nevertheless, there are notable differences in the structural and catalytic features of the enzyme and its isoforms. These differences are attributed to the species, specificity, and additional activities the enzyme possesses [16,43].

### 3.2. A prototypical bacterial nicotinate nucleotide adenylyltransferase (NNAT)

The enzyme NNAT (NadD gene), also known as nicotinate mononucleotide adenylyltransferase (NaMNAT) (EC 2.7.1.18), is widely conserved across bacterial species and has a greater affinity for NaMN than NMN, as Magni et al. [44] reported. The interaction between the carboxylate group of the substrate and an anion-binding pocket determines the substrate selectivity of NadD bacterium NNATs towards NaMN. The main-chain amide groups of threonine and histidine generate this binding pocket. Moreover, the analysis of the quaternary structures reveals significant resemblances and patterns. For example, the Gram-positive bacteria

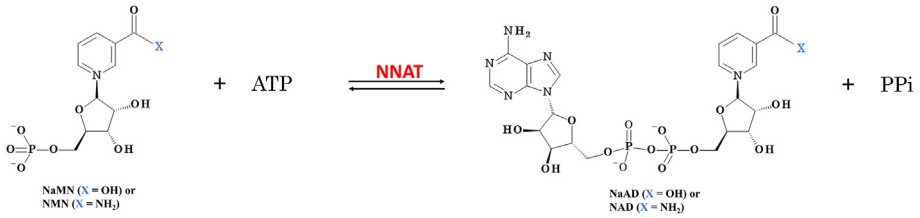



**Fig. 6. The enzymatic reaction facilitated by nicotinate/nicotinamide mononucleotide adenylyltransferase.** The provided diagram illustrates the catalytic reaction of the NNAT enzyme involving two substrates, leading to the synthesis of either $NAD^+$ or NaAD [41].

NNAT, such as saNNAT in *S. aureus,* bsMNAT in *B. subtilis*, and baMNAT in *B. anthracis*, may be differentiated based on their dimerised structure and shared substrate affinity for NaMN. Gram-negative bacteria, such as *Pseudomonas aeruginosa, Escherichia coli* and *Acinetobacter baumannii*, exhibit a characteristic monomeric structure and a preference for the NaMN substrate. This observation has been reported in studies conducted by Yoon et al. [45] for *P. aeruginosa*, Mehl et al. [46], Zhang et al. [47] for *E. coli* and Sorci et al. [48] for *A. baumannii*.

The structure of a typical bacteria NNAT comprises two domains: the N-terminal domain, which is the nuclnucleotide-bindingain characterised by a Rossman fold and the C-terminal domain whi, which contains the ATP binding motif. Considering the first bacterial NNAT discovered, *E. coli* NNAT (Uniprot ID: P0A752), the secondary structure of EcNNAT consists of a central β-sheet composed of seven parallel strands surrounded by multiple helices (Fig. 6). The 25 kDa EcNNAT operates as a monomeric entity, and its crystal structure, as elucidated by Zhang et al., demonstrates that the enzyme has structural adaptability in many locations around the active site. This adaptability facilitates the interaction with the substrate during binding and the subsequent release of the product. The recognition of the NaMN substrate by EcNNAT is facilitated by the interaction between the nicotinate carboxyl group and a positive helix dipole, as well as the primary chain amides of many proproteins [45,49].

### 3.3. Structural overview of KpNNAT and EfNNAT via computational tools

The protein KpNNAT (Uniprot ID: B5XZR5) comprises 216 amino acids and has an estimated molecular weight of around 24.8 kDa (kDa). It is also anticipated to have a theoretical isoelectric point (pI) of 5.82 and an extinction coefficient of 38055 $M^{-1}$ $cm^{-1}$ when considering the formation of cystine from the two cysteine residues. Alternatively, if all cysteine residues remain reduced, the extinction coefficient is reported to be 37930 $M^{-1}$ $cm^{-1}$ (ProtParam ExPASY). In contrast, EfNaMNAT (Uniprot ID: A0A133MWI0) comprises 213 amino acids, with an estimated molecular weight of around 24.6 kDa and a calculated isoelectric point of 5.17. The molar extinction coefficient of the substance, as determined in an aqueous solution at a wavelength of 280 nm, is reported to be 30370 $M^{-1}$ $cm^{-1}$, as estimated via the ProtParam ExPASY database.

According to the homology model built using EcNaMNAT (PDB ID: 1k4k), which exhibits about 7 cent similarity in sequence with KpNNAT, it may be inferred that KpNNAT is a monomeric form. EfNNAT as a homo-dimer may be seen by its homology model, which was built using *Bacillus anthracis* (BaNNAT- PDB ID:2QTN). This model (Fig. 7) indicates that EfNNAT has around 46 % sequence identity with BaNNAT, as reported by Swissmodel.expasy.org. Both proteins have conserved secondary structures, distinguished by a Rossman fold.

The comparison of KpNNAT and EfNNAT via sequence alignment analysis utilising the EMBOSS Stretcher pairwise alignment tool resulted in an observed sequence similarity of around 47 % (Fig. 8). The residues Trp120 and Asp112 in KpNNAT, which are equivalent to Trp138 and Asp130 in EfNNAT, respectively, exhibit conservation and play a role in inducing conformational alterations following the binding of $NAD^+$ [49]. Within the active region of KpNNAT, a key enzyme, resides Cys135, one of the two cysteine residues. This cysteine lived close to Arg136 and Arg137, crucial in substrate binding and catalytic activity. The arginine residues mentioned in the text are identified as Arg154 and Arg155 in the EfNNAT protein. These residues interact with the phosphate group of the nucleotide ligand, as outlined by Olland et al. [50] and Zhang et al. [49]. In addition, the histidine residue at position 48 of KpNNAT, which corresponds to histidine residue 66 in EfNNAT, partially interacts with the mononucleotide substrate. According to Wang et al. [51], the presence of this compound facilitates the stacking of the pyridine ring via π-cation interactions. It generates a negative charge region that enables interactions with exocyclic carboxylate groups.

In addition to the conserved residues, many reported changes or substitutions in the amino acid residues of KpNNAT and EfNNAT have the potential to modify substrate binding or catalytic activity. The motif sequence "H/TXGH" in KpNNAT, crucial for substrate identification and binding [40], is highly conserved. In this enzyme, the motif is designated as "HYGH" and is situated towards the N-terminus, specifically between residues 19 and 22. In the context of EfNNAT, the glycine residue inside the motif is substituted with alanine, resulting in its designation as "HLAH" (Fig. 8). The hallmark motif "HLAH" is positioned within the amino acid residues 37 to 40.

## 4. Comparison of NNAT in bacteria and humans

Despite belonging to the same nucleotidyltransferase superfamily, NNAT in bacteria humans are two unique subfamilies with a sequence identity of less than 20 humans; the enzyme is called nicotinamide mononucleotide adenylyltransferase (NMNAT). Multiple biochemical investigations have shown that NMNAT exhibits distinct characteristics compared to the bacteria NNAT. Specifically, bacteria and humans' NNAT subfamilies vary regarding sequence conservation patterns, selectivity towards NMN and NaMN substrates, oligomeric states, and other biochemical and enzymatic features. This information is depicted in Fig. 9.

### 4.1. Mononucleotide substrate specificity

In contrast to the bacterial enzyme NNAT (EC 2.7.1.18), which prefers NaMN over N [24], the human NMNAT (hNMNAT EC 2.7.1.1) demonstrates almost equal recognition of both mononucleotides. As a result, hNMNAT can participate in both pathways involved in $NAD^+$ production with flexibility. The substrate specificities of the enzymes are determined by the carboxyl and carboxamide groups present in the nicotinate and nicotinamide substrates, contributing to their broad range of interactions. Considering the first bacterial

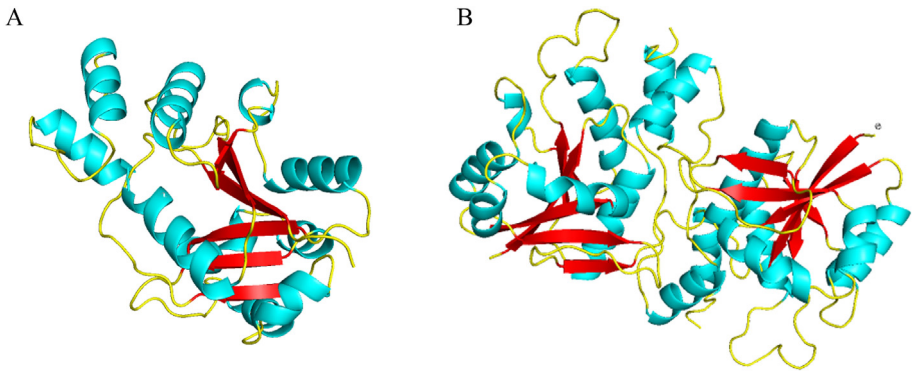



**Fig. 7. Homology Model of (A) KpNNAT and (B) EfNNAT. The KpNNAT model was constructed using the 1k4k.4.A template and EfNNAT using the 2qtn.1.**A model. The α-helices are depicted in cyan, red repr; reds the β-strands, and the ordered coils in yellow. Both homology models were generated via SwissModel.

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K_pneumoniae.      1 MV---------------DMTQLQ------AIYGGTFDPVHYGHLKPVEIL    29
E_faecium.         1 MMNTQAKTFVRSQLFPEEMPQFLEKKKQVGILGGTFNPVHLAHLVMAEQA    50

K_pneumoniae.     30 ANQIGLSKVIIMPNNVPPHRPQPEATSAQ-RVHMLKLAIADKPLFTLDER    78
E_faecium.        51 GRNLGLDRVFLMPSYQPPHVDEKQTIDAKHRLNMLELAVEDNPFLQIETI   100

K_pneumoniae.     79 ELRRDTPSWTAQTLQEWRQEQGPRKPLAFIIGQDSLLTFPTWHNYETILD   128
E_faecium.       101 ELARGGKSYTYDTMKELTQNN-PDTDYYFIIGGDMVEYLPKWYKIDELTS   149

K_pneumoniae.    129 NVHLIVCRRPGYPLTMAQEADQRWLDRHLTHDVESLHNSPSGVIYLAETP   178
E_faecium.       150 MVNFVGIRRPGY----------------TTD------TPYPVIWV-DVP   175

K_pneumoniae.    179 WFDISATIIRQRLERGESCAEMLPAAVLDYIREQGLY---C     216
E_faecium.       176 EIDISSTKIRQKIKEGCSIRYLVPDKVIDYIQNEGLYEYGL     216
```

**Fig. 8. The amino acid sequence alignment of KpNNAT and EfNNAT.** Conserved residues are coloured red, and the semi-conserved residues in blue., A black and red box shows the conserved ATP and the NMN/NaMN binding motifs. The sequence alignment is the EMBOSS Stretcher pairwise alignment tool.

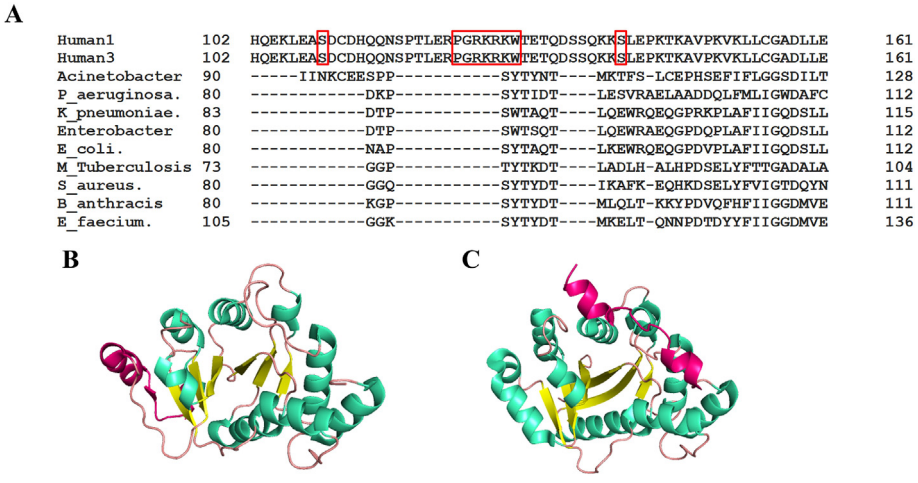



**Fig. 9. Sequence Alignment and structural comparison of bacteria NNAT and human NMNAT.** (A) The nuclear localisation signal (NLS) sequence and the two phosphorylation site serine residues conserved to the hNMNAT are indicated in the red box. The regions with no corresponding residues are designated in space. Ribbon representation of (B) *E. coli* NNAT (1k4k) and (C) human NMNAT (1kq0) protomer structure showing their structural difference. The secondary structures are labelled green for helices, yellow for strands, and salmon for the random coils. The region in hot-pink indicates the portions that are not present in both structures (αβα insertion in *E.coli* NNAT and the C-terminal helical structure extension in the human NMANT). RMSD = 15.755 Å (492–492 atoms) [49].

NNAT discovered, *E. coli* NNAT (Uniprot ID: P0A752), hs an anion-binding pocket formed by the positive dipole of α-helix C and the main chain amide groups of Tyr85, Ala86, and Leu117. This pocket is essential for recognising the negative charge present in the NaMN carboxylate group. The electrophilic character of this cavity is augmented by the adjacent hydrophobic amino acid residues, enhancing its capacity to differentiate between a carboxamide (NMN) and a carboxylate (NaMN) and consequently leading to a

more considerable affinity for NaMN compared to NMN.

Contrarily, the hNMNAT enzyme can efficiently identify NaMN and NMN without requiring significant alterations in spatial arrangement [16]. This capability is attributed to a negatively charged aspartic acid residue (Asp173) and many conserved water molecules inside the pyridine-binding region of the hNMNAT enzyme. This phenomenon enables the enzyme to accommodate substrates with varying electrostatic characteristics by modifying the precise charge distributions inside the binding site [52]. Consequently, hNMNAT exhibits reduced rigidity in facilitating diverse metabolic transformations of NMN/NaMN to manufacture $NAD^+$.

### 4.2. Sequence conservation and secondary structure

The crystallographic analysis conducted on recombinant hNMNAT (Fig. 9) demonstrates that the complex structures exhibit a disordered region that includes a nuclear localisation signal (NLS) sequence PGRKRKW, as well as two serine residues (Ser109 and Ser136) phosphorylation site. These elements engage with proteins responsible for nuclear transport. Nevertheless, the bacterial NNAT lacks the sequence that complements this specific area, as shown by previous works described by Schweiger et al. [53] and Zhang et al. [49]. In addition, the monomer structure of hNMNAT displays a dinucleotide-binding fold characterised by a central parallel β-sheet composed of six strands, surrounded by several helices. This differs from the structure of the bacterial enzyme EcNNAT, which features an additional αβαβ (D″fE'g) segment inserted at the C-terminal end of the β-sheet, resulting in a central β-sheet with seven strands (Fig. 9) [49]. Moreover, it is noteworthy to mention that the hNMNAT C-terminal extension has a brief helical structure (residues 267–274) near the pyridine-binding site. This structural feature potentially influences substrates' recognition, as Zhou et al. [49] suggested. It is essential to highlight that this helical extension is not present in the bacterial NNAT, as reported by Zhang et al. [49].

### 4.3. Oligomeric assemblies

Previous research has shown the existence of three separate isoforms of NMNAT, which may be differentiated based on their cellular activity and distribution among various tissues. These isoforms demonstrate distinct functional components. In contrast to the monomeric and dimeric configurations often seen in bacterial NNAT, hNMNAT-1 displays a homo-hexameric structure, hNMNAT-2 exists as a monomer, and hNMNAT-3 adopts a tetrameric shape. The dissimilarities observed in the active site, subunit interfaces, and limited sequence identity between bacteria and human NNAT indicate the potential for discovering or designing highly selective inhibitors [49].

### 4.4. Metal ion selectivity variability in NMNATs

So far, it has been observed that all defined NMNATs are classified as metal-activated enzymes since they need the presence of divalent cations to facilitate the catalytic process of the adenylyl transfer reaction. The presence of divalent metals is crucial for the interaction and orientation of nucleotides and for streamlining catalytic processes. In addition to the physiologically significant $Mg^{2+}$ ion, many divalent metals like $Ca^{2+}$, $Zn^{2+}$, and $Mn^{2+}$ have been shown to have a role in phosphoryl transfer and substrate interaction. However, it should be noted that these metals may not necessarily exhibit high efficiency in substrate turnover or product release [54]. The primary factor contributing to this phenomenon is forming a stable complex between the enzyme and its product. This complex exhibits a binding solid affinity, impeding the product's release.

Numerous investigations have shown that the NNAT isozyme has a discernible inclination towards diverse divalent metal ions. For instance, the work conducted by Jeje et al. [18] demonstrates that *K. pneumonia* NNAT has a greater affinity for $Mg^{2+}$ and $Ni^{2+}$. Similarly, Di Stefano et al. [55] found that NMNAT1 prefers $Zn^{2+}$ and $Ni^{2+}$ over $Mg^{2+}$. On the other hand, Raffaelli et al. [56] and Sorci et al. [48] discovered that NMNAT2 and NMNAT3 achieve optimum activity when bound to $Mg^{2+}$. Similarly, research performed by Emanuelli et al. in Ref. [57] indicated that yeast NMNAT (yNaMNAT1) had the highest activity level when paired with $Ni^{2+}$. Again, in a subsequent study by Emanuelli et al. [54], it was found that yNMNAT2 showed a preference for $Co^{2+}$.

Metal ions have several roles in proteins, including reinforcing structural stability necessary for their biological activity, alteration of protein structures, and control or catalysis of enzymatic activities. Enzymes impacted by metal ions are categorised into metalloenzymes and metal-enzyme complexes. Metalloenzymes have a strong binding affinity towards metal ions, which remains intact even when separated. On the other hand, metal-enzyme complexes are characterised by their relatively weak binding to metal ions, resulting in lower affinity interactions that may be readily disrupted during separation [58].

Divalent metal ions interact with proteins, including metal-ligand and enzyme-bridge complexes. These ions may fulfil several roles within the protein structure, acting as Lewis acids, regulators of structural integrity, as well as electron donors or acceptors [59]. Equations (1–3)below depict the many modes of interaction between metal ions and enzymes. In metal-activated enzymes, it is customary for metal ions to interact with the substrate, forming a substrate-metal complex that assumes the role of the active substrate. The creation of this complex may occur either before or concomitantly with the formation of the enzyme-substrate complex.

In many cases, as often found in most metalloenzymes, the first interaction involves binding the metal to the protein. This binding event is a crucial point of contact between the metal and the substrate. Under some circumstances, the metal can serve as a binding site and be a component of the enzyme's catalytic mechanism or fulfil both roles simultaneously. Metal ions may also engage with enzymes by exerting their effects at a location separate from the active site, causing a rearrangement in the enzyme's structure. In instances of this kind, the metal may fulfil two potential roles: preserving the structural integrity of the enzyme while concurrently exerting an indirect impact on catalytic activity or modulating activity by maintaining the enzyme's active conformations.

1) $M + S = MS$, $E + MS = EMS$
2) $E + M = EM$, $EM + S = EMS$
3) $E + M = ME$, $ME + S = MES$

The equations above illustrate the interplay among metal ions (M), enzyme (E), and substrate (S). According to Riordan [59], there are three possible mechanisms by which a metal ion can interact with an enzyme: (1) the metal ion can form a substrate-metal complex, which then acts as the actual substrate; (2) the metal ion can bind to the enzyme first, serving as a site of interaction for the substrate; and (3) the metal ion can interact with the enzyme at a site that is distant from the active site.

Metals can engage in redox (30 %) and non-redox (70 %) reactions during enzyme catalysis. In the context of redox catalysis, metal ions play a direct function in the transfer of electrons and undergo a cyclic transformation in their oxidation state. On the other hand, in non-redox catalysis, metals function as Lewis acids, attracting electrons through interactions with electronegative

donors from the ligand. This interaction enhances the polarity of reactive chemical bonds, thereby increasing the electrophilic nature of the metal and facilitating the transfer of atoms or groups [59].

Divalent cations, such as $Mg^{2+}$, play a vital role in the functionality of ATP-binding proteins. These cations are involved in the binding of co-substrates and properly aligning nucleotides while aiding in the catalytic process. Although the metal is not a constituent of the enzyme's active site, its presence is used for catalysis. During the process of enzymatic catalysis, the ATP substrate undergoes binding with $Mg^{2+}$ to generate the $ATPMg^{2+}$ complex. This complex represents the activated form of the substrate that is necessary for the ATP-binding enzymes to function [59]. The ATP-$Mg^{2+}$ complex formation increases the binding energy, improving the specificity of the interactions between enzymes and substrates. The prevention of nonspecific ionic interactions between the enzyme and the phosphoryl group of ATP may be achieved by counteracting certain negative charges on the phosphoryl group by the presence of $Mg^{2+}$. Furthermore, the precise binding of the nucleotide by the enzyme is facilitated by the well-defined conformations maintained by the interactions between $Mg^{2+}$ and the oxygen atoms of the phosphoryl group (Fig. 10) [60].

In addition, the presence of $Mg^{2+}$ ions enhances the affinity between the enzyme and the $ATPMg^{2+}$ complex by promoting the formation of supplementary interaction sites. In some enzymes, such as DNA polymerases, the binding of $Mg^{2+}$ ions occurs 'directly to the side chains of the enzyme, namely to the glutamate and aspartate residues. In addition to the enzymes above, the adenylate kinases engage with $Mg^{2+}$ ions indirectly, mainly by establishing hydrogen bonds with the coordinated water molecules [60]. The interaction with ATP is facilitated by glycine and other positively charged residues, such as lysine, arginine, and histidine [61].

In the NNAT enzyme, it is postulated that Mg2+ serves to polarise the active site, thus aiding in the nucleophilic assault on ATP's α-phosphorus. This, in turn, disrupts the ester linkage between ATP's α- and β-phosphates and results in the transfer of the adenyl group to NMN [18].

### 4.5. The suppression of nicotinamide mononucleotide adenylyltransferase (NaMNAT) and its physiologic importance

The concept posits that the inhibition of NNAT inside the cellular environment will impede the synthesis of $NAD^+$, ultimately leading to cellular demise. Their study Rodionova et al. [62] demonstrated the abovementioned phenomenon in the *Mycobacterium smegmatis* model system. They used a protein knockdown technique to cause the deprivation of the target enzyme NNAT, resulting in a pronounced bactericidal impact. This effect was accompanied by alterations in the levels of NaMN and eventual NAD(H) pool depletion. The depletion effect was particularly notable in crucial metabolic pathways, including the Krebs cycle, cholesterol catabolism, fatty acid synthesis, and production of aromatic chemicals, which heavily rely on the NAD(P) redox reaction. The researchers found that the metabolic processes were inhibited, leading to a subsequent impact on the generation of ATP energy [62]. According to Sorci et al. [48,63], it is anticipated that inhibitors targeting the NNAT enzyme should be able to deplete the $NAD^+$ pool, leading to the complete disruption of the central metabolic system and suppressing bacterial growth.

Bacterial virulence regulation can occur through direct control of their $NAD^+$ levels or indirect modulation of oxidases and associated enzymes. Evidence suggests a consistent positive relationship between $NAD^+$ levels and the virulence of bacteria [64]. During the investigation into the synthesis of virulence factors in multidrug-resistant *P. aeruginosa*, it was observed that citrate and pyruvate could disrupt the central metabolism of bacteria. Leveraging this finding, scientists utilised it to impede the interaction between $NAD^+$ and enzymes, leading to a decrease in total $NAD^+$ production and a subsequent reduction in the virulence of *P. aeruginosa* [65].

As NaMNAT is a universally preserved enzyme crucial for the survival of all bacteria examined so far, and it displays notable structural and functional distinctions from a closely related human enzyme (NMNAT), it suggests that inhibiting bacterial NaMNAT is unlikely to induce substantial toxic effects on human cells [66]. Hence, it is feasible to create therapeutic targets with small molecules by focusing on essential residues within the NaMNAT binding site, aiming to identify inhibitors of $NAD^+$ synthase. This approach is hypothesised to represent a potential, particular, and efficient therapeutic strategy [67]. Furthermore, the intricate network of bacterial $NAD^+$ metabolism unveils a range of opportunities for essential scientific inquiry and transformative breakthroughs. Exploring these pathways deepens our understanding of bacterial physiology and virulence and reinforces our ability to combat bacterial infections and address the challenge of antibiotic resistance. Effectively managing bacterial diseases requires innovative strategies that tap into the multifaceted landscape of $NAD^+$ metabolism [67].

## 5. Computational modelling

Computational modelling uses computers to simulate and analyse complex systems [68]. Theoretical science is an interdisciplinary field that integrates mathematics, physics, and computer science and has shown to be a very valuable adjunct to experimental inquiries. Over time, researchers have created several advanced applications and approaches to simulate realistic scenarios and investigate the molecular recognition and dynamics of biomolecular interactions between a ligand and a receptor site [69]. These strategies include a variety of physical techniques, such as classical molecular mechanics (MM) and quantum mechanics (QM). Incorporating computational modelling alongside empirical investigations has been extensively embraced to augment

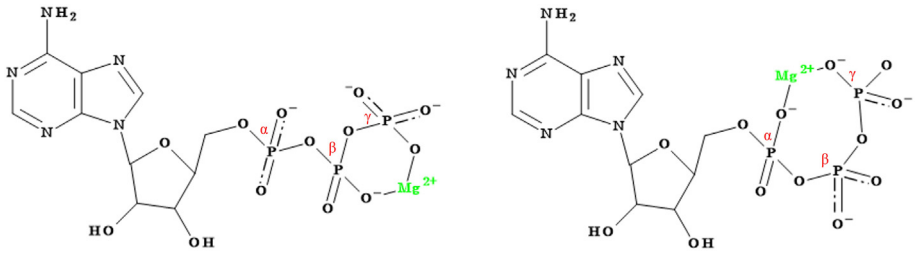



**Fig. 10. The structural representation of the various isomeric configurations of the ATP-$Mg^{2+}$ complex.** According to Jeremy M. Berg [60]), enzyme binding can be facilitated by the direct coordination of $Mg^{2+}$ with either of the two oxygen atoms in the phosphate group, depending on the specific enzyme.

experimental data and attain a more profound comprehension of biological phenomena at the atomic scale [70]. This approach holds significant value owing to the predictive capabilities exhibited by computational biochemistry, which can provide insights and information that may not be readily accessible through experimentation alone. Researchers can extrapolate findings by combining computational and empirical approaches to inform rational drug design [71].

This review suggests that computational methodologies such as homology modelling, molecular docking, and molecular dynamic simulation could be employed to evaluate and examine the molecular interactions and ligand bioactivity associated with other proteins/enzymes, including the NNAT proteins (KpNNAT and EfNNAT) as well as ATP, NMN, and $NAD^+$.

### 5.1. Homology modelling

To do computational analysis or calculation of protein-ligand interactions, it is essential to possess the protein structure under investigation readily available. As a result of the scarcity of actual three-dimensional (3-D) crystal structures of proteins, computational modelling has emerged as an alternative method for predicting or deriving protein structures that are not readily accessible [72]. Constructing a three-dimensional protein structure can be accomplished by employing homology modelling techniques. This method uses the amino acid sequence of the protein of interest and a crystal structure of a closely related homologous protein obtained by experimental means as a model. The level of accuracy with which homology modelling predicts the three-dimensional structures of proteins is comparable to that achieved using practical means. To construct a particular protein structure, a collection of experimentally derived protein structures is examined to identify suitable templates [73]. The selection of this template is contingent upon the structural and sequence alignment similarities with the query sequence. Therefore, the quality of a homology model is contingent upon the quality of both the sequence alignment and the template structure used. According to Zheng et al. [73] a positive correlation exists between sequence identity and model quality. As the sequence identity increases, the quality of the model also increases.

Conversely, as the sequence identity falls, the quality of the model diminishes. Homology modelling is relevant in several applications, such as predicting protein-ligand interactions and facilitating drug discovery when access to atomic-resolution data is necessary [74].

### 5.2. Molecular docking

This computational methodology predicts how the ligand interacts with and forms a bond with its target while also gauging the strength of this interaction [75]. Molecule docking is a scientific approach to investigating the alignment and interlocking of two or more molecule structures. This procedure entails using in-silico techniques to simulate the binding of ligands, including substrates, medicines, or inhibitors, inside the active site of macromolecules, such as proteins or nucleic acids [76].

Analysing the interactions between a specific ligand and its binding sites through molecular docking enables understanding its behaviour within the target protein receptor [77]. This examination also sheds light on the underlying biochemical processes, thereby facilitating the development of new ligands. In contrast to the docking approach based on the lock and fundamental model, the induced-fit mechanism could assess the dynamic nature of the interaction between the nucleotide ligands ATP, NMN, and $NAD^+$ and the KpNNAT protein receptor.

### 5.3. Molecular dynamics (MD) simulation

Molecular dynamics (MD) simulation is a computer method used to replicate intricate molecular systems at the atomic scale and calculate the movements of atoms inside the authentic surroundings of individual molecules [78]. This study examines the kinetic behaviour and temporal variations of atoms and molecules during a specific period, with a particular focus on the dynamic development of the system. Using molecular dynamics (MD), simulation allows for exploring the conformational space of protein molecules and characterising their kinetic and thermodynamic properties. Consequently, this approach establishes a link between proteins' structural and dynamic aspects, facilitating the comprehension of their structural and mechanical attributes [79]. To establish a molecular dynamics (MD) system for simulation, it is necessary to compute the collective forces and energies exerted by all particles inside the system [80]. Molecular dynamics (MD) uses molecular mechanics force fields to approximate the potential energies and forces inside a system of interacting particles. This is achieved by numerically solving the classical Newtonian equations of motion, enabling the determination of the trajectories of atoms and molecules within and around the system [81].

The mathematical representation of the interaction between atoms is represented as:

Bonded

Non-bonded.

$$E_{total} = \boldsymbol{Estretching} + \boldsymbol{Ebending} + \boldsymbol{Etorsional} + \boldsymbol{Ecross{-}terms} + \boldsymbol{Ecoulombic} + \boldsymbol{Evdw}.$$

The force field function consists of both bonded and non-bonded interaction variables. The bonded interactions include several molecular phenomena, such as bond stretching, valence angle bending, torsional dihedral angles (referred to as soft terms), and incorrect dihedrals (referred to as hard terms). On the other hand, it should be noted that electrostatic (Coulomb) and Van der Waals (vdw) interactions contribute to the nonbonded interactions [82].

Various force field models have been developed, each characterised by different properties. The force field selection is contingent upon the specific circumstances and characteristics of the system under investigation. The molecular force fields often used in biomolecular simulations include AMBER [80]. The molecular dynamics simulations of proteins need a series of sequential procedures. The processes above include energy minimisation, solvation, system heating, equilibration, and production phase, as seen in Fig. 11. The simulation of proteins is conducted to replicate experimental settings, necessitating the consideration of several physical factors such as pressure and temperature. Protein simulations often use the isothermal-isobaric (NPT) or the canonical ensemble (NVT). The solutes are solvated using explicit solvent molecules and confined inside a single cell. There are a variety of clear water models, including TIP5P [83], SPC, and SPC/E [84], as well as TIP3P and TIP4P [85]. The water models mentioned above are established through Quantum Mechanics-Driven Molecular Mechanics and are confirmed through experimental approaches. These models are employed to simulate the distinctive characteristics and intricacies of molecule hydration, encompassing the alignment of solvent dipoles, effective electrostatic shielding, nuanced rearrangements in the hydrogen bond network, clathration of hydrophobic surfaces, and the consequential alterations in entropy [86].

The energy minimisation process entails discovering the global minimum energy by adjusting the positions of side chain atoms to achieve optimal geometric arrangements of atoms, where the cumulative attractive force on each atom reaches its peak. Several methods exist for computing the minimum energy, but the steepest

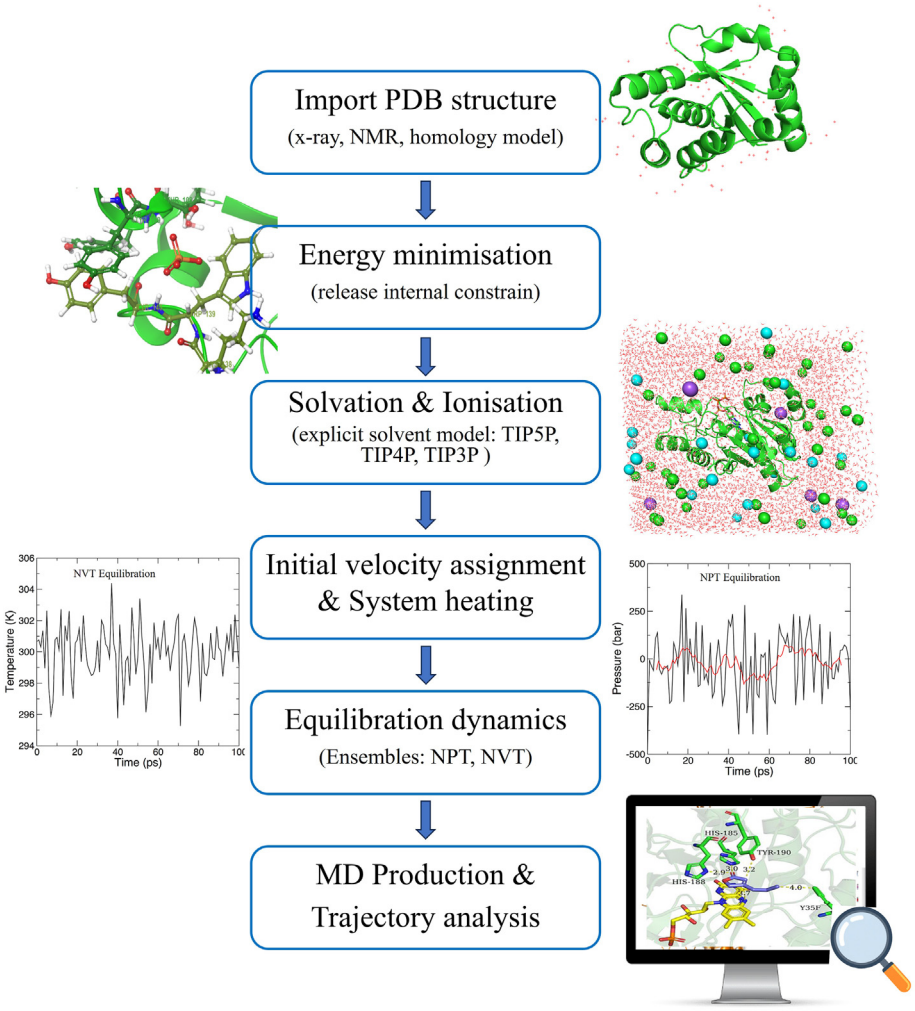



**Fig. 11.** Illustrates a schematic depiction of the sequential steps encompassed in molecular dynamics (MD) simulation of a protein according to Patodia [86]**,** NMR, Nuclear magnetic resonance; MD: Molecular Dynamics; NVT: Number of molecules/atoms in assemby, Volume, Temperature; NPT: Number of molecules/atoms in assembly, Pressure, Temperature; TIP3P/4P/5P: Transferable Intermolecular Potential 3/4/5-site water models.

descent and conjugate gradient techniques are the most prevalent. The most vertical descent method, categorised as a first-order iterative descent approach, uses the gradient derived from the potential energy surface [86]. It is closely tied to the forces described in molecular mechanics for molecular systems, directing the exploration path toward the nearest energy minimum [28]. An important consideration is rectifying the protonation state of titratable residues, and this can be achieved through either free energy of perturbation (FEP) molecular dynamics (MD) simulations or the application of continuum electrostatics models such as finite differences Poisson-Boltzmann (FDPB) or protein dipole-Langevin dipole (PDLD) [86].

During the heating phase, the system minimises energy, with each atom receiving initial velocities at 0 K. The system's time evolution is then numerically integrated using Newton's equations of motion. New velocities corresponding to a slightly higher temperature are assigned at defined intervals, and the simulation continues until the desired temperature is attained. The release of force constraints on various subdomains occurs gradually as structural tensions dissipate during heating. Thermalisation is commonly achieved at constant volume through Langevin dynamics. During the equilibration stage, efforts are made to balance kinetic and potential energies, ensuring the distribution of the kinetic energy introduced into the system during heating across all degrees of freedom. In explicit solvent simulations, protein positions are initially fixed, and waters adjust accordingly. Once the solvent is equilibrated, constraints on the protein can be removed, allowing the entire system (protein + solvent) to evolve [86].

The production phase marks the final step in the simulation methodology, aiming to eliminate constraints on the protein. This phase is executed over a specified time scale to generate a protein molecule trajectory, adhering to specific equilibrium conditions such as NVT, NPT, and NVE. The duration of this phase can vary from several hundred picoseconds to microseconds or more. To mitigate significant trajectory artefacts during extended simulations, recent versions of CHARMM protein parameter files incorporate a 2D grid correction map based on backbone $\varphi$ and $\psi$ parameters derived from alanine, proline, and glycine dipeptide surfaces, known as CMAP correction [86].

After completing a molecular dynamics (MD) simulation, the trajectories obtained from the production phase of the simulation run are subjected to analysis and visualisation. This analysis aims to investigate the atomic-level motions and conformational fluctuations by utilising visualisation software capable of revealing the time-dependent structural parameters. The parameters typically assessed encompass the time-averaged structure, total system

energy, root mean square deviation (RMSD) between two structures, root mean square fluctuation (RMSF), radius of gyration, and interface-related measures like order parameter, electrostatic potential, and density of groups. The present investigation used the OPLS force field and TIP3 explicit solvent model to perform parameterisation and solvation of the KpNNAT-ligand complex systems.

## 6. Synthesis of insights: integrating structure, function, and modelling

Previous studies indicate that integrating the empirical and computational findings, weaving together the intricate web of NAD + metabolism and NNAT, plays a pivotal role. The synergistic nature of these insights fosters a deeper comprehension of the coenzyme's function and potential applications in therapeutics and biotechnology. Homology modelling, molecular docking, and molecular dynamics simulations have facilitated examining the dynamic aspects of $NAD^+$ metabolism and NNAT behaviour [87]. Digital technologies have allowed us to highlight and analyse tiny features, thoroughly analysing binding affinities, conformational changes, and interactions with substrates. Integrating empirical and computational research results establishes a cohesive framework, shedding light on the complex network of $NAD^+$ metabolism. Computational simulations serve as a valuable addition to this approach by providing dynamic insights into the behaviour of enzymes [88].

### 6.1. Implications and applications

The discoveries obtained possess significant ramifications. The thorough comprehension of $NAD^+$ metabolism and the involvement of NNAT lays the foundation for novel treatment approaches. Promising applications arise from creating specific inhibitors and engineering microbial systems for the synthesis of $NAD^+$ [89]. These pragmatic improvements bridge the divide between the fundamental study of enzymology and its practical use in real-world scenarios. Many challenges and potential prospects lie ahead concerning integrating multi-omics data and using modern computational approaches. The ethical issues surrounding using our recently acquired knowledge emphasise the need to do responsible research. Within this ever-evolving domain, the amalgamation of empirical and computational methodologies serves as a driving force behind our progress towards groundbreaking innovations. This statement serves as a reminder that, in the endeavour to acquire scientific knowledge, the effective use of interdisciplinary cooperation and the incorporation of many techniques continue to be very effective instruments.

### 6.2. Therapeutic potential of NNAT-targeting strategies

Research focused on the structure of NNAT presents encouraging opportunities for advancing treatments. Through the elucidation of NNAT's three-dimensional structure, medication design may be meticulously tailored. Understanding the binding locations on NNAT enables the development of medicines customised to interact with areas, changing their action selectively [89]. Suppose the structure of NNAT is altered due to mutations in neurodevelopmental diseases. In that case, creating medications that rectify these alterations is possible, reducing the condition's effect. In the context of disorders such as cancer, if NNAT is involved, medications that specifically target its structure may hinder its activity, providing a possible opportunity for therapeutic intervention [67]. The field of personalised medicine may use insights into individual differences in NNAT to develop drugs tailored explicitly to distinct structural profiles. In addition, the combination of medications targeting NNAT with others that impact adjacent pathways may result in synergistic effects, improving treatment results [66]. Although these examples are hypothetical, the actual influence of NNAT structural research on therapeutics is yet to be determined by definitive results from ongoing studies. This highlights the importance of further investigation in this rapidly evolving field.

## 7. Challenges, future directions, and conclusion

The exploration of $NAD^+$ metabolism and the elucidation of NNAT function have encountered several obstacles. In anticipation of the future, it is essential to recognise and acknowledge these obstacles. Integrating multi-omics data, including genomes, transcriptomics, proteomics, and metabolomics, continues to be challenging [90]. The intricate interaction among different strata within the framework of $NAD^+$ metabolism poses a perplexing problem that necessitates novel methodologies for thorough comprehension.

**Advanced Computational Methods:** The domain of computational modelling presents both opportunities and obstacles. The pursuit of enhancing accuracy and efficiency persists in the field despite the valuable insights provided by methods such as molecular dynamics simulations. Using sophisticated force fields, improved sampling techniques, and algorithms that include quantum mechanical intricacies will undeniably augment our capacity to replicate the behaviour of enzymes precisely.

**Validation of In Vivo and Functional Studies:** Translating in vitro observations into life systems is a stimulating but complex route. Thorough testing is necessary to investigate the physiological implications of NNAT and interactions within the cellular environment [90]. The validation of hypothesised pathways and establishing an enzyme's significance in vivo need genetic modifications, functional tests, and research conducted in live bacterial cells.

## 8. Conclusion: An insightful outlook

The field of $NAD^+$ research has immense potential for future advancements. The amalgamation of empirical and computational methodologies, as shown in this comprehensive analysis, provides a fundamental basis for groundbreaking revelations. The integration of form and function offers valuable perspectives that have the potential to stimulate advancements in drug design, biotechnology, and other related fields. Therefore, integrating empirical and computational methodologies has provided valuable insights into the complex network of $NAD^+$ metabolism and the specific function of NNAT. This complete comprehension provides scientific enrichment as well as practical applications.

In concluding this evaluation, we engage in a retrospective analysis of the attempted trip. The exploration of $NAD^+$ metabolism and the significant contribution of NNAT exemplify the collaborative nature of scientific inquiry, where experimental findings and computer analyses intersect to provide a more profound comprehension of biological mechanisms. Through successfully resolving various challenges and acquiring valuable insights, we gradually approach a deeper understanding of the intricate mechanisms underlying enzyme activity. This ongoing pursuit of knowledge catalyses the exploration of other domains and the practical use of this information.

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## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## References


[1] S. Santajit, N. Indrawattana, Mechanisms of antimicrobial resistance in ESKAPE pathogens, BioMed Res. Int. (2016) 1–8, https://doi.org/10.1155/2016/2475067, 2016.

[2] P. Snetkov, E. Rogacheva, A. Kremleva, S. Morozkina, M. Uspenskaya, L. Kraeva, In-vitro antibacterial activity of curcumin-loaded nanofibers based on hyaluronic acid against multidrug-resistant ESKAPE pathogens, Pharmaceutics 14 (2022) 1186, https://doi.org/10.3390/pharmaceutics14061186.

[3] C. Aloke, I. Achilonu, Coping with the ESKAPE pathogens: evolving strategies, challenges and future prospects, Microb. Pathog. 175 (2023) 105963, https://doi.org/10.1016/j.micpath.2022.105963.

[4] K. Kaur, P. Barathe, S. Reddy, V. Shriram, A. Dey, S. Gosavi, V. Kumar, Nanoformulations against Multidrug-Resistant Members of ESKAPE Pathogens, 2022, pp. 385–411, https://doi.org/10.1007/978-3-031-10220-2_12.

[5] N. Wang, M. Zhan, T. Wang, J. Liu, C. Li, B. Li, X. Han, H. Li, S. Liu, J. Cao, X. Zhong, C. Lei, W. Zhang, Z. Zhang, Long term characteristics of clinical distribution and resistance trends of carbapenem-resistant and extended-spectrum β-lactamase Klebsiella pneumoniae infections: 2014–2022, Infect. Drug Resist. 16 (2023) 1279–1295, https://doi.org/10.2147/IDR.S401807.

[6] C. Gorrie, C. Higgs, G. Carter, T.P. Stinear, B. Howden, Genomics of vancomycin-resistant Enterococcus faecium, Microb. Genom. 5 (2019), https://doi.org/10.1099/mgen.0.000283.

[7] S. Boni, G.H. Marin, L. Campaña, L. Marin, S. Risso-Patrón, G. Marin, F. Gabriel, A. Corso, V. Garay, M. Limeres, Association between consumption of fluoroquinolones and carbapenems and their resistance rates in Pseudomonas aeruginosa in Argentina, Interdiscip Perspect Infect Dis (2022) 1–7, https://doi.org/10.1155/2022/3924212, 2022.

[8] P. Nandhini, P. Kumar, S. Mickymaray, A.S. Alothaim, J. Somasundaram, M. Rajan, Recent developments in methicillin-resistant Staphylococcus aureus (MRSA) treatment: a review, Antibiotics 11 (2022) 606, https://doi.org/10.3390/antibiotics11050606.

[9] Z.I. Kimera, F.X. Mgaya, S.E. Mshana, E.D. Karimuribo, M.I.N. Matee, Occurrence of extended spectrum beta lactamase (ESBL) producers, quinolone and carbapenem resistant Enterobacteriaceae isolated from environmental samples along msimbazi river basin ecosystem in Tanzania, Int. J. Environ. Res. Publ. Health 18 (2021) 8264, https://doi.org/10.3390/ijerph18168264.

[10] M. Nguyen, S.G. Joshi, Carbapenem resistance in *Acinetobacter baumannii* , and their importance in hospital-acquired infections: a scientific review, J. Appl. Microbiol. 131 (2021) 2715–2738, https://doi.org/10.1111/jam.15130.

[11] H. Alaoui Mdarhri, R. Benmessaoud, H. Yacoubi, L. Seffar, H. Guennouni Assimi, M. Hamam, R. Boussettine, N. Filali-Ansari, F.A. Lahlou, I. Diawara, M.M. Ennaji, M. Kettani-Halabi, Alternatives therapeutic approaches to conventional antibiotics: advantages, limitations and potential application in medicine, Antibiotics 11 (2022) 1826, https://doi.org/10.3390/antibiotics11121826.

[12] N. Xie, L. Zhang, W. Gao, C. Huang, P.E. Huber, X. Zhou, C. Li, G. Shen, B. Zou, NAD+ metabolism: pathophysiologic mechanisms and therapeutic potential, Signal Transduct. Targeted Ther. 5 (2020) 227, https://doi.org/10.1038/s41392-020-00311-7.

[13] M. Roussin, S.P. Salcedo, NAD+-targeting by bacteria: an emerging weapon in pathogenesis, FEMS Microbiol. Rev. 45 (2021), https://doi.org/10.1093/femsre/fuab037.

[14] A.J. Covarrubias, R. Perrone, A. Grozio, E. Verdin, NAD+ metabolism and its roles in cellular processes during ageing, Nat. Rev. Mol. Cell Biol. 22 (2021) 119–141, https://doi.org/10.1038/s41580-020-00313-x.

[15] M.S. Ghanem, F. Monacelli, A. Nencioni, Advances in NAD-lowering agents for cancer treatment, Nutrients 13 (2021) 1665, https://doi.org/10.3390/nu13051665.

[16] C. Lau, M. Niere, M. Ziegler, The NMN/NaMN Adenylyltransferase (NMNAT) Protein Family, 2009. http://www.ebi.ac.uk/clustalw/.

[17] H.K. Evans, Comparative phylogenetic analysis of blcap/nnat reveals eutherian-specific imprinted gene, Mol. Biol. Evol. 22 (2005) 1740–1748, https://doi.org/10.1093/molbev/msi165.

[18] O. Jeje, R. Maake, R. van Deventer, V. Esau, E.A. Iwuchukwu, V. Meyer, T. Khoza, I. Achilonu, Effect of divalent metal ion on the structure, stability and function of Klebsiella pneumoniae nicotinate-nucleotide adenylyltransferase: empirical and computational studies, Int. J. Mol. Sci. 23 (2021) 116, https://doi.org/10.3390/ijms23010116.

[19] R. Petrelli, K. Felczak, L. Cappellacci, NMN/NaMN adenylyltransferase (NMNAT) and NAD kinase (NADK) inhibitors: chemistry and potential therapeutic applications, Curr. Med. Chem. 18 (2011) 1973–1992, https://doi.org/10.2174/092986711795590048.

[20] A.E. Tomkinson, S. Vijayakumar, J.M. Pascal, T. Ellenberger, DNA ligases: structure, reaction mechanism, and function, Chem. Rev. 106 (2006) 687–699, https://doi.org/10.1021/cr040498d.

[21] S. Imai, C.M. Armstrong, M. Kaeberlein, L. Guarente, Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase, Nature 403 (2000) 795–800, https://doi.org/10.1038/35001622.

[22] M. Ziegler, New functions of a long-known molecule, Eur. J. Biochem. 267 (2000) 1550–1564, https://doi.org/10.1046/j.1432-1327.2000.01187.x.

[23] S. Wagner, R. Manickam, M. Brotto, S.M. Tipparaju, NAD+ centric mechanisms and molecular determinants of skeletal muscle disease and aging, Mol. Cell. Biochem. 477 (2022) 1829–1848, https://doi.org/10.1007/s11010-022-04408-1.

[24] F. Gazzaniga, R. Stebbins, S.Z. Chang, M.A. McPeek, C. Brenner, Microbial NAD metabolism: lessons from comparative genomics, Microbiol. Mol. Biol. Rev. 73 (2009) 529–541, https://doi.org/10.1128/MMBR.00042-08.

[25] J.M. Berthiaume, J.G. Kurdys, D.M. Muntean, M.G. Rosca, Mitochondrial NAD $^{+}$/NADH redox state and diabetic cardiomyopathy, Antioxidants Redox Signal. 30 (2019) 375–398, https://doi.org/10.1089/ars.2017.7415.

[26] L. Sellés Vidal, C.L. Kelly, P.M. Mordaka, J.T. Heap, Review of NAD(P)H-dependent oxidoreductases: properties, engineering and application, Biochim. Biophys. Acta Protein Proteonomics (2018) 327–347, https://doi.org/10.1016/j.bbapap.2017.11.005, 1866.

[27] D. Brekasis, M.S.B. Paget, A novel sensor of NADH/NAD+ redox poise in Streptomyces coelicolor A3 (2), EMBO J. 22 (2003) 4856–4865.

[28] A.A. Sauve, NAD+ and vitamin B3: from metabolism to therapies, J. Pharmacol. Exp. Therapeut. 324 (2008) 883–893.

[29] T.S. Blacker, M.R. Duchen, Investigating mitochondrial redox state using NADH and NADPH autofluorescence, Free Radic. Biol. Med. 100 (2016) 53–65, https://doi.org/10.1016/j.freeradbiomed.2016.08.010.

[30] R.H. Houtkooper, C. Cantó, R.J. Wanders, J. Auwerx, The secret life of NAD+: an old metabolite controlling new metabolic signaling pathways, Endocr. Rev. 31 (2010) 194–223, https://doi.org/10.1210/er.2009-0026.

[31] Y. Yang, A.A. Sauve, NAD + metabolism: bioenergetics, signaling and manipulation for therapy, Biochim. Biophys. Acta Protein Proteonomics 1864 (2016) 1787–1800, https://doi.org/10.1016/j.bbapap.2016.06.014.

[32] V.J. Klein, M. Irla, M. Gil López, T. Brautaset, L. Fernandes Brito, Unravelling formaldehyde metabolism in bacteria: road towards synthetic methylotrophy, Microorganisms 10 (2022) 220, https://doi.org/10.3390/microorganisms10020220.

[33] T.P. Begley, C. Kinsland, R.A. Mehl, A. Osterman, P. Dorrestein, The Biosynthesis of Nicotinamide Adenine Dinucleotides in Bacteria, 2001, pp. 103–119, https://doi.org/10.1016/S0083-6729(01)61003-3.

[34] O. Kurnasov, V. Goral, K. Colabroy, S. Gerdes, S. Anantha, A. Osterman, T.P. Begley, NAD biosynthesis, Chem. Biol. 10 (2003) 1195–1204, https://doi.org/10.1016/j.chembiol.2003.11.011.

[35] D. Purich, Advances in Enzymology and Related Areas of Molecular Biology, Wiley, 1999, https://doi.org/10.1002/9780470123195.

[36] H.N. Jayaram, P. Kusumanchi, J.A. Yalowitz, NMNAT expression and its relation to NAD metabolism, Curr. Med. Chem. 18 (2011) 1962–1972, https://doi.org/10.2174/092986711795590138.

[37] P. Bieganowski, C. Brenner, Discoveries of nicotinamide riboside as a nutrient and conserved NRK genes establish a preiss-handler independent route to NAD+ in fungi and humans, Cell 117 (2004) 495–502, https://doi.org/10.1016/S0092-8674(04)00416-7.

[38] A. Katoh, Molecular biology of pyridine nucleotide and nicotine biosynthesis, Front. Biosci. 9 (2004) 1577, https://doi.org/10.2741/1350.

[39] E. Katsyuba, J. Auwerx, Modulating <scp>NAD</scp> $^{+}$ metabolism, from bench to bedside, EMBO J. 36 (2017) 2670–2683, https://doi.org/10.15252/embj.201797135.

[40] T. Izard, The crystal structure of a novel bacterial adenylyltransferase reveals half of sites reactivity, EMBO J. 18 (1999) 2021–2030, https://doi.org/10.1093/emboj/18.8.2021.

[41] H. Zhang, T. Zhou, O. Kurnasov, S. Cheek, N. V Grishin, A. Osterman, Crystal structures of E. coli nicotinate mononucleotide adenylyltransferase and its complex with deamido-NAD, Structure 10 (2002) 69–79.

[42] C. Fortunato, F. Mazzola, N. Raffaelli, The key role of the NAD biosynthetic enzyme nicotinamide mononucleotide adenylyltransferase in regulating cell functions, IUBMB Life 74 (2022) 562–572, https://doi.org/10.1002/iub.2584.

[43] O. Jeje, R. Pandian, Y. Sayed, I. Achilonu, Obtaining high yield recombinant Enterococcus faecium nicotinate nucleotide adenylyltransferase for X-ray crystallography and biophysical studies, Int. J. Biol. Macromol. 250 (2023) 126066, https://doi.org/10.1016/j.ijbiomac.2023.126066.

[44] N. Raffaelli, T. Lorenzi, P.L. Mariani, M. Emanuelli, A. Amici, S. Ruggieri, G. Magni, The *Escherichia coli* NadR regulator is endowed with nicotinamide mononucleotide adenylyltransferase activity, J. Bacteriol. 181 (1999) 5509–5511, https://doi.org/10.1128/JB.181.17.5509-5511.1999.

[45] M.Y. Yoon, K.-M. Lee, S.H. Jeong, J. Kim, S.S. Yoon, Heterogeneous virulence potential and high antibiotic resistance of Pseudomonas aeruginosa strains isolated from Korean pneumonia patients, J. Microbiol. 48 (2010) 518–525, https://doi.org/10.1007/s12275-010-9388-2.

[46] A. Mehl, P. Ghorbani, D. Douda, H. Huang, N. Palaniyar, F. Ratjen, H. Grasemann, Effect of arginase inhibition on pulmonary L-arginine metabolism in murine Pseudomonas pneumonia, PLoS One 9 (2014) e90232, https://doi.org/10.1371/journal.pone.0090232.

[47] W. Zhang, M. Zhao, L. Ruesch, A. Omot, D. Francis, Prevalence of virulence genes in Escherichia coli strains recently isolated from young pigs with

diarrhea in the US, Vet. Microbiol. 123 (2007) 145–152, https://doi.org/10.1016/j.vetmic.2007.02.018.
[48] L. Sorci, F. Cimadamore, S. Scotti, R. Petrelli, L. Cappellacci, P. Franchetti, G. Orsomando, G. Magni, Initial-rate kinetics of human NMN-adenylyltransferases: substrate and metal ion specificity, inhibition by Products and multisubstrate analogues, and isozyme contributions to NAD $^{+}$ biosynthesis, Biochemistry 46 (2007) 4912–4922, https://doi.org/10.1021/bi6023379.
[49] H. Zhang, T. Zhou, O. Kurnasov, S. Cheek, N. V Grishin, A. Osterman, Crystal structures of E. coli nicotinate mononucleotide adenylyltransferase and its complex with deamido-NAD, Structure 10 (2002) 69–79.
[50] A.M. Olland, K.W. Underwood, R.M. Czerwinski, M.-C. Lo, A. Aulabaugh, J. Bard, M.L. Stahl, W.S. Somers, F.X. Sullivan, R. Chopra, Identification, characterization, and crystal structure ofBacillus subtilis nicotinic acid mononucleotide adenylyltransferase, J. Biol. Chem. 277 (2002) 3698–3707, https://doi.org/10.1074/jbc.M109670200.
[51] J. bo Wang, G. Li, M.T. Reetz, Enzymatic site-selectivity enabled by structure-guided directed evolution, Chem. Commun. 53 (2017) 3916–3928, https://doi.org/10.1039/C7CC00368D.
[52] T. Mittag, L.E. Kay, J.D. Forman-Kay, Protein dynamics and conformational disorder in molecular recognition, J. Mol. Recogn. 23 (2010) 105–116, https://doi.org/10.1002/jmr.961.
[53] M. Schweiger, K. Hennig, F. Lerner, M. Niere, M. Hirsch-Kauffmann, T. Specht, C. Weise, S.L. Oei, M. Ziegler, Characterization of recombinant human nicotinamide mononucleotide adenylyl transferase (NMNAT), a nuclear enzyme essential for NAD synthesis, FEBS Lett. 492 (2001) 95–100, https://doi.org/10.1016/S0014-5793(01)02180-9.
[54] M.J. Knape, L.G. Ahuja, D. Bertinetti, N.C.G. Burghardt, B. Zimmermann, S.S. Taylor, F.W. Herberg, Divalent metal ions Mg $^{2+}$ and Ca $^{2+}$ have distinct effects on protein kinase A activity and regulation, ACS Chem. Biol. 10 (2015) 2303–2315, https://doi.org/10.1021/acschembio.5b00271.
[55] M. Di Stefano, L. Galassi, G. Magni, Unique expression pattern of human nicotinamide mononucleotide adenylyltransferase isozymes in red blood cells, Blood Cells Mol. Dis. 45 (2010) 33–39, https://doi.org/10.1016/j.bcmd.2010.04.003.
[56] N. Raffaelli, L. Sorci, A. Amici, M. Emanuelli, F. Mazzola, G. Magni, Identification of a novel human nicotinamide mononucleotide adenylyltransferase, Biochem. Biophys. Res. Commun. 297 (2002) 835–840, https://doi.org/10.1016/S0006-291X(02)02285-4.
[57] M. Emanuelli, A. Amici, F. Carnevali, F. Pierella, N. Raffaelli, G. Magni, Identification and characterization of a second NMN adenylyltransferase gene in Saccharomyces cerevisiae, Protein Expr. Purif. 27 (2003) 357–364, https://doi.org/10.1016/S1046-5928(02)00645-9.
[58] B.L. Vallee, Zinc and Metalloenzymes, 1955, pp. 317–384, https://doi.org/10.1016/S0065-3233(08)60108-4.
[59] J.F. Riordan, K.D. McElvany, C.L. Borders Jr., Arginyl residues: anion recognition sites in enzymes, Science 195 (1977) (1979) 884–886.
[60] D.K. Berg, W.G. Conroy, Nicotinic α7 receptors: synaptic options and downstream signaling in neurons, J. Neurobiol. 53 (2002) 512–523, https://doi.org/10.1002/neu.10116.
[61] J.S. Chauhan, N.K. Mishra, G.P. Raghava, Identification of ATP binding residues of a protein from its primary sequence, BMC Bioinf. 10 (2009) 434, https://doi.org/10.1186/1471-2105-10-434.
[62] I.A. Rodionova, B.M. Schuster, K.M. Guinn, L. Sorci, D.A. Scott, X. Li, I. Kheterpal, C. Shoen, M. Cynamon, C. Locher, E.J. Rubin, A.L. Osterman, Metabolic and bactericidal effects of targeted suppression of NadD and NadE enzymes in mycobacteria, mBio 5 (2014), https://doi.org/10.1128/mBio.00747-13.
[63] L. Sorci, I. Blaby, J. De Ingeniis, S. Gerdes, N. Raffaelli, V. de Crécy Lagard, A. Osterman, Genomics-driven reconstruction of acinetobacter NAD metabolism, J. Biol. Chem. 285 (2010) 39490–39499, https://doi.org/10.1074/jbc.M110.185629.
[64] T. Franza, A. Rogstam, S. Thiyagarajan, M.J. Sullivan, A. Derré-Bobillot, M.C. Bauer, K.G.K. Goh, V. Da Cunha, P. Glaser, D.T. Logan, G.C. Ulett, C. von Wachenfeldt, P. Gaudu, NAD+ pool depletion as a signal for the Rex regulon involved in Streptococcus agalactiae virulence, PLoS Pathog. 17 (2021) e1009791, https://doi.org/10.1371/journal.ppat.1009791.
[65] K. Perinbam, J.V. Chacko, A. Kannan, M.A. Digman, A. Siryaporn, A shift in central metabolism accompanies virulence activation in Pseudomonas aeruginosa, mBio 11 (2020), https://doi.org/10.1128/mBio.02730-18.
[66] J. Yoshino, J.A. Baur, S. Imai, NAD+ intermediates: the biology and therapeutic potential of NMN and NR, Cell Metabol. 27 (2018) 513–528, https://doi.org/10.1016/j.cmet.2017.11.002.
[67] Y. Chen, Y. Ying, J. Lalsiamthara, Y. Zhao, S. Imani, X. Li, S. Liu, Q. Wang, From bacteria to biomedicine: developing therapies exploiting NAD+ metabolism, Bioorg. Chem. 142 (2024) 106974, https://doi.org/10.1016/j.bioorg.2023.106974.
[68] A. Vidal-Limon, J.E. Aguilar-Toalá, A.M. Liceaga, Integration of molecular docking analysis and molecular dynamics simulations for studying food proteins and bioactive peptides, J. Agric. Food Chem. 70 (2022) 934–943, https://doi.org/10.1021/acs.jafc.1c06110.
[69] K.C. Sivakumar, J. Haixiao, C.B. Naman, T.P. Sajeevan, Prospects of <scp>multitarget</scp> drug designing strategies by linking molecular docking and molecular dynamics to explore the <scp>protein–ligand</scp> recognition process, Drug Dev. Res. 81 (2020) 685–699, https://doi.org/10.1002/ddr.21673.
[70] H. Askr, E. Elgeldawi, H. Aboul Ella, Y.A.M.M. Elshaier, M.M. Gomaa, A.E. Hassanien, Deep learning in drug discovery: an integrative review and future challenges, Artif. Intell. Rev. 56 (2023) 5975–6037, https://doi.org/10.1007/s10462-022-10306-1.
[71] A.U. Rehman, B. Khurshid, Y. Ali, S. Rasheed, A. Wadood, H.-L. Ng, H.-F. Chen, Z. Wei, R. Luo, J. Zhang, Computational approaches for the design of modulators targeting protein-protein interactions, Expet Opin. Drug Discov. 18 (2023) 315–333, https://doi.org/10.1080/17460441.2023.2171396.
[72] B. Robson, De novo protein folding on computers. Benefits and challenges, Comput. Biol. Med. 143 (2022) 105292, https://doi.org/10.1016/j.compbiomed.2022.105292.
[73] W. Zheng, Y. Li, C. Zhang, X. Zhou, R. Pearce, E.W. Bell, X. Huang, Y. Zhang, Protein structure prediction using deep learning distance and hydrogen-bonding restraints in <scp>CASP14</scp&gt, Proteins: Struct., Funct., Bioinf. 89 (2021) 1734–1751, https://doi.org/10.1002/prot.26193.
[74] F. Ballante, A.J. Kooistra, S. Kampen, C. de Graaf, J. Carlsson, Structure-based virtual screening for ligands of G protein–coupled receptors: what can molecular docking do for you? Pharmacol. Rev. 73 (2021) 1698–1736, https://doi.org/10.1124/pharmrev.120.000246.
[75] E.D. Boittier, Y.Y. Tang, M.E. Buckley, Z.P. Schuurs, D.J. Richard, N.S. Gandhi, Assessing molecular docking tools to guide targeted drug discovery of CD38 inhibitors, Int. J. Mol. Sci. 21 (2020) 5183, https://doi.org/10.3390/ijms21155183.
[76] S. Singh, Q. Bani Baker, D.B. Singh, Molecular docking and molecular dynamics simulation, in: Bioinformatics, Elsevier, 2022, pp. 291–304, https://doi.org/10.1016/B978-0-323-89775-4.00014-6.
[77] T.M. Menchaca, C. Juárez-Portilla, R.C. Zepeda, Past, present, and future of molecular docking, in: Drug Discovery and Development-New Advances, IntechOpen, 2020.
[78] Q. Mao, M. Feng, X.Z. Jiang, Y. Ren, K.H. Luo, A.C.T. van Duin, Classical and reactive molecular dynamics: principles and applications in combustion and energy systems, Prog. Energy Combust. Sci. 97 (2023) 101084, https://doi.org/10.1016/j.pecs.2023.101084.
[79] M. Karplus, J.A. McCammon, Molecular dynamics simulations of biomolecules, Nat. Struct. Biol. 9 (2002) 646–652, https://doi.org/10.1038/nsb0902-646.
[80] J.C. Phillips, R. Braun, W. Wang, J. Gumbart, E. Tajkhorshid, E. Villa, C. Chipot, R.D. Skeel, L. Kalé, K. Schulten, Scalable molecular dynamics with NAMD, J. Comput. Chem. 26 (2005) 1781–1802, https://doi.org/10.1002/jcc.20289.
[81] L.E. Scriven, Dynamics of a fluid interface Equation of motion for Newtonian surface fluids, Chem. Eng. Sci. 12 (1960) 98–108, https://doi.org/10.1016/0009-2509(60)87003-0.
[82] O. Guvench, A.D. MacKerell, Comparison of Protein Force Fields for Molecular Dynamics Simulations, 2008, pp. 63–88, https://doi.org/10.1007/978-1-59745-177-2_4.
[83] M.W. Mahoney, W.L. Jorgensen, Diffusion constant of the TIP5P model of liquid water, J. Chem. Phys. 114 (2001) 363–366, https://doi.org/10.1063/1.1329346.
[84] H.J.C. Berendsen, J.R. Grigera, T.P. Straatsma, The missing term in effective pair potentials, J. Phys. Chem. 91 (1987) 6269–6271.
[85] W.L. Jorgensen, J.F. Blake, J.K. Buckner, Free energy of TIP4P water and the free energies of hydration of CH4 and Cl- from statistical perturbation theory, Chem. Phys. 129 (1989) 193–200, https://doi.org/10.1016/0301-0104(89)80004-7.
[86] S. Patodia, Molecular dynamics simulation of proteins: a brief overview, J. Phys. Chem. Biophys. 4 (2014), https://doi.org/10.4172/2161-0398.1000166.
[87] G. Eren, Homology modeling of human nicotinamide/nicotinic acid mononucleotide adenylyltransferase 2: insights into isoenzyme-specific differences using molecular docking simulatons, Lett. Drug Des. Discov. 14 (2017), https://doi.org/10.2174/1570180814666161110112859.
[88] X. Zhao, X. Meng, A.J. Ragauskas, C. Lai, Z. Ling, C. Huang, Q. Yong, Unlocking the secret of lignin-enzyme interactions: recent advances in developing state-of-the-art analytical techniques, Biotechnol. Adv. 54 (2022) 107830, https://doi.org/10.1016/j.biotechadv.2021.107830.
[89] H. Yang, X. Jia, Y. Han, Microbial redox coenzyme engineering and applications in biosynthesis, Trends Microbiol. 30 (2022) 318–321, https://doi.org/10.1016/j.tim.2022.01.012.
[90] F.R. Pinu, D.J. Beale, A.M. Paten, K. Kouremenos, S. Swarup, H.J. Schirra, D. Wishart, Systems biology and multi-omics integration: viewpoints from the metabolomics research community, Metabolites 9 (2019) 76, https://doi.org/10.3390/metabo9040076.