Biochimica et Biophysica Acta 1848 (2015) 83–94

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamem

Review

Bacterial and algal orthologs of prostaglandin H_2 synthase: novel insights into the evolution of an integral membrane protein

Kushol Gupta [a], Barry S. Selinsky [b,*]

[a] Department of Biochemistry & Biophysics, Perelman School of Medicine, University of Pennsylvania, 242 Anatomy-Chemistry Building, Philadelphia, PA 19104, USA
[b] Chemistry Department, Villanova University, Villanova, PA 19085, USA

ARTICLE INFO

Article history:
Received 20 May 2014
Received in revised form 9 September 2014
Accepted 23 September 2014
Available online 2 October 2014

Keywords:
Prostaglandin synthase
Membrane binding
Heme
Cyclooxygenase
Peroxidase
Myeloperoxidase
α-Dioxygenase

ABSTRACT

Prostaglandin H_2 synthase (PGHS; EC 1.14.99.1), a bi-functional heme enzyme that contains cyclooxygenase and peroxidase activities, plays a central role in the inflammatory response, pain, and blood clotting in higher eukaryotes. In this review, we discuss the progenitors of the mammalian enzyme by using modern bioinformatics and homology modeling to draw comparisons between this well-studied system and its orthologs from algae and bacterial sources. A clade of bacterial and algal orthologs is described that have salient structural features distinct from eukaryotic counterparts, including the lack of a dimerization and EGF-like domains, the absence of gene duplicates, and minimal membrane-binding domains. The functional implications of shared and variant features are discussed.

Contents

Abbreviations: PGHS, prostaglandin H_2 synthase; ORF, open reading frame; NSAID, non-steroidal anti-inflammatory drug; PDB, protein data bank; EGF, epidermal growth factor; 11*R*-HETE, 11*R*-hydroxy-5*Z*,8*Z*,12*E*,14*Z*-eicosatetraenoic acid; 15*S*-HETE, 15*S*-hydroxy-5*Z*,8*Z*,11*Z*,13*E*-eicosatetraenoic acid; 15*R*-HETE, 15*R*-hydroxy-5*Z*,8*Z*,11*Z*,13*E*-eicosatetraenoic acid

* Corresponding author at: Chemistry Department, Villanova University, 800 E. Lancaster Ave., Villanova, PA 19085, USA. Tel.: +1 610 519 3959; fax: +1 610 519 7249.
E-mail address: barry.selinsky@villanova.edu (B.S. Selinsky).

http://dx.doi.org/10.1016/j.bbamem.2014.09.011
0005-2736/

1. Introduction

Prostaglandin H_2 synthase (PGHS; EC 1.14.99.1) is a monotopic integral membrane protein that resides within the outer leaflet of the endoplasmic reticulum. In mammals, it catalyzes the first step in the formation of prostanoids. This class of potent fatty acid autocoids regulates a wide array of physiological processes, including the pathophysiology of cardiovascular disease [1,2], inflammation [3–5], tumorigenesis [6–10], and Alzheimer's disease [11]. PGHS acts as a bi-functional homodimer; each monomer contributes distinct cyclooxygenase and peroxidase active sites that employ a heme cofactor. Within the dimer, these active sites work in concert via an allosteric "half-of-sites" mechanism [12–16] to transform arachidonic acid into PGH_2 by sequential oxidation and reduction steps [17]. The study of this enzyme is very mature, well-informed by decades of detailed biochemistry, x-ray crystallography, and the extensive development of isoform-specific inhibitors for clinical use (reviewed in [17–19] and many other articles). This wealth of information provides a valuable point of departure from which detailed comparisons with primitive homologs can be made to gain evolutionary insight into structure and function.

In contrast, relatively little is known about the orthologs of PGHS from more primitive organisms, such as bacteria, algae, and fungi. Assignment of these many orthologs to the peroxidase-cyclooxygenase superfamily is based largely on sequence relationships [20]; only most recently have select orthologs been characterized *in vitro* to directly assess this relationship [21,22]. While the endoplasmic reticulum is common to all eukaryotic organisms, the presence of PGHS orthologs in lower organisms invokes a number of intuitive questions regarding evolution, structure, and function. Are all bacterial and algal orthologs of PGHS also integral membrane proteins? Did mammalian PGHS originate from a soluble ancestor? What biological roles do their respective lipid product profiles play in these more primitive organisms? Among prokaryotes, the selection of unique chemical reactions that yield bioactive metabolites is presumably driven in part by persistent immersion within the lipid bilayer, providing access to a concentrated source of hydrophobic substrates. The lipid composition of various prokaryotes varies significantly from species-to-species, even within the same genus [23]. Hence, selective metabolic pressure could drive diversity among prokaryotic PGHS orthologs, both kinetically and structurally, in a way not seen in higher organisms.

The presence of two spatially distinct active sites within the mammalian PGHS open reading frame (ORF) product raises additional questions about the evolutionary path by which these two active sites were married. Given the abundance of peroxidases across genomes, an ancient peroxidase probably incorporated a second active site and a membrane-binding motif through evolution, whereas an alternative hypothesis advocates the advent of a peroxidase active site within a cyclooxygenase precursor [24]. The oligomeric properties of mammalian PGHS underlie its concerted "half-of-sites" mechanism of catalysis and the interplay between both active sites. However, the oligomeric and kinetic properties of the primitive orthologs identified are not as well understood. If the more primitive orthologs of PGHS have different oligomeric properties, how does the mechanism of catalysis change, if at all? In the only algal ortholog characterized *in vitro*, the recombinant protein ably converts arachidonic acid to PGG_2 and PGH_2, but behaves as a tetramer by size-exclusion chromatography [22]. In the only bacterial ortholog characterized to date, the cyanobacterial 'cyclooxygenase' (actually characterized as a dioxygenase) identified from *Nostoc punctiforme* is proposed to work in tandem with a neighboring upstream fatty acid heme hydroperoxide lyase gene rather than provide two distinct activities, and shows strong preference for oleic and linoleic acid over arachidonic acid [21]. While the recombinant algal protein required detergent for solubility, the bacterial protein did not. These initial observations together indicate that these orthologs are diverse and functionally distinct from their eukaryotic counterparts.

In this review, we consider these functional implications from a structural point-of-view, comparing the well-characterized eukaryotic protein with its homologs within the peroxidase-cyclooxygenase superfamily from more primitive species. The structural study of eukaryotic PGHS in itself is a very well-developed area of study, with greater than fifty crystallographic structures available in the Protein Data Bank (PDB); the properties of its structural domains are well-defined and have been recently reviewed [25]. These structural domains guide discussion of more primitive orthologs, with a particular emphasis on a clade of bacterial enzymes already identified by bioinformatics for which no representative experimental crystal structures are currently available. These orthologs present an exciting opportunity to further our knowledge of many aspects of PGHS structure and function, including structure-function relationships, tyrosyl radical formation and substrate reactivity, substrate and inhibitor selectivity, kinetics, and structural allostery.

2. Canonical properties of mammalian prostaglandin H_2 synthases

In mammals, there are two primary isoforms which differ not only in their expression patterns and gene regulation but also in the organization of the cyclooxygenase site. Both mammalian PGHS-1 and PGHS-2 are highly conserved, with 85-90% identity within both isoforms [26]. The first isoform, PGHS-1, is generally described as a constitutively-expressed isoform involved in "housekeeping" functions, while PGHS-2 expression is induced in response to inflammatory stimuli. Both isoforms of this enzyme are the site of action of nonsteroidal anti-inflammatory drugs (NSAIDs, *e.g.* aspirin, ibuprofen, indomethacin), clinically important molecules that, via the inhibition of the cyclooxygenase active site of PGHS, target the regulation of pain, the inflammatory response, and blood clotting (reviewed in [18]). In the same organism, the primary sequences of PGHS-1 and PGHS-2 are approximately 60-65% homologous. Despite this high sequence similarity, a number of small molecules have been successfully engineered that preferentially block the cyclooxygenase active site of one isoform over the other [27–29].

The three-dimensional structure of ovine PGHS-1 was first reported in 1994 at 3.5 Å resolution [24], among the first integral membrane protein structures to be determined by x-ray crystallography (Fig. 1A). Crystal structures of human and mouse PGHS-2 at low and medium resolution followed in 1996 [30,31]. X-ray crystallographic studies have demonstrated that the structures of the eukaryotic PGHS isoforms are nearly superimposable, providing a complete structural view of the domains of PGHS, within the context of a crystallographic dimer.

All mammalian PGHSs display high similarity in sequence and domain structure (Fig. 1B). An N-terminal signal peptide precedes an epidermal growth factor-like (EGF) domain. This domain is followed by a membrane-binding domain, a short dimerization domain [32,33], a globular catalytic peroxidase domain distinguished by a peroxidase fold similar to that found in cytochrome c peroxidase [34] and other classical peroxidases, and a C-terminal segment that is implicated in retention of the protein by the endoplasmic reticulum [25]. We will now

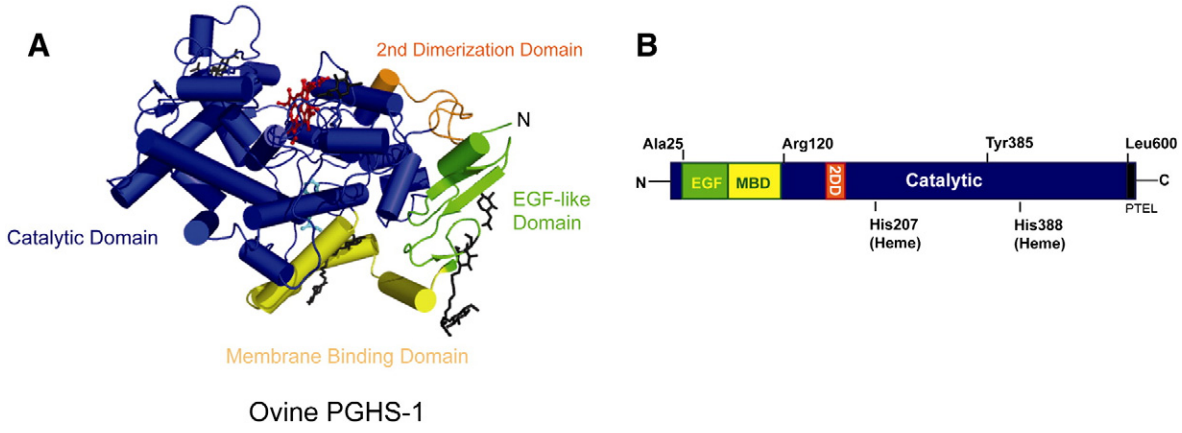

Fig. 1. A. Crystal structure of ovine PGHS-1 (PDB ID: 1Q4G). The structural domains are colored as such: the EGF-like domain, green; the membrane-binding domain, yellow; the second dimerization domain, orange; the catalytic domain, blue. The structural elements (in black) attached to the soluble region of the structure represent glycosylation sites; the unattached structures near the membrane binding domain are molecules of the detergent β-octylglucoside identified during structural refinement. This figure was prepared using PYMOL [105]. B. Schematic diagram of ovine PGHS-1 sequence with structural features denoted. The protein features an N-terminal EGF-like domain (EGF, green), followed by a membrane-binding domain (MBD, yellow), a second dimerization domain (2DD, orange) and a catalytic domain (blue). PTEL refers to the final four residues in the protein sequence associated with an ER retention signal [106].

highlight the most salient features of each domain, using ovine PGHS-1 as the point of reference for discussion of sequence boundaries and structure.

2.1. The EGF-like domain

The EGF-like domain (residues 31–69) contains seven conserved cysteines that are known to participate in three intramolecular disulfide linkages, along with a fourth disulfide bridging the EGF domain to the catalytic domain. The domain is highly conserved across all eukaryotic homologs of PGHS, with >42% identity across all available eukaryotic PGHS sequences and >63% identity between isoforms within a species. A DALI database [35] search of the domain against the holdings of the Protein Data Bank reveals over sixty structures with >30% sequence identity and structural homology, which is not surprising given the ubiquitous distribution of this modular domain across eukaryotic genomes (>1100 cataloged in UniProt). EGF-like domains have only been detected in eukaryotic genomes [36] and in the animal viruses [37]. Glycosylation of EGF-like modules is common in eukaryotes. In PGHS, modified sites at Asn68, Asn144, and Asn410 in PGHS-1 [38,39] and Asn53, Asn130, Asn396, and Asn580 in PGHS-2 have been confirmed [40].

2.2. The membrane-binding domain (MBD)

All of the mammalian PGHS proteins include a membrane binding domain comprised of four amphipathic α-helices (amino acids 69–116 in ovine PGHS-1, denoted helices A–D) that anchor the enzyme to the endoplasmic reticulum. In additional to solubilizing detergents observed within the crystallographic lattice [41], a wealth of biochemical analyses, including detergent binding studies [42], site-directed mutagenesis [43], and molecular modeling [44] strongly support a structural model in which the four amphipathic helices lie adjacent to the membrane acyl chains at specific sites and anchor PGHS to the outer leaflet of the membrane bilayer, parallel to its plane. Hydrophobic residues in the helices are predicted to interact with fatty acyl chains in the membrane, while hydrophilic groups are exposed to the membrane surface and interact with polar phospholipid head groups, and with water. These modeling studies additionally predicted that the fatty acid substrate enclosed within the center of a motif formed by the four helices form an electrostatic interaction with Arg120 in the PGHS active site [44], a critical interaction for both drug-binding and catalysis, which was confirmed by x-ray crystallography.

Of the domains present in PGHS, the membrane binding helices displays the greatest sequence variability between mammalian species, with ~38% sequence homology versus greater than 70% homology for the rest of the protein [43,45]. Sequence variations are predicted to modify the binding energy between the amphipathic helices and a membrane bilayer. Calculations have predicted that each subunit of the ovine PGHS-1 dimer is anchored into the endoplasmic reticulum with approximately 37 kcal/mol of energy per subunit, while in murine PGHS-2 this interaction energy is much lower (approximately 7 kcal/mol) [33]. In agreement, binding experiments demonstrate that ovine PGHS-1 is stably attached to the membrane surface, with significant expansion in the membrane surface to allow for its insertion, while murine PGHS-2 shows less significant perturbation of the lipid bilayer [46].

2.3. The second oligomerization domain of PGHS and dimerization

Residues 116–140 in PGHS, along with the EGF-like domain, comprise the dimeric interface observed by X-ray crystallography. This interface buries 2893 $Å^2$, corresponding to 13% of the total solvent accessible surface [41]. In eukaryotic proteins, the dimeric structure of PGHS has been shown to be required for enzymatic function. Reversible disruption of the PGHS dimer by mild denaturation yields a monomeric-but-folded enzyme that lacks cyclooxygenase activity; activity is regained following removal of denaturant [47]. More recent studies with mammalian PGHS homodimers indicate that occupation of the active site of one monomer in the dimer prevents binding of substrate or inhibitor to the second monomer [12–16].

2.4. The catalytic domain

PGHS belongs to a superfamily that includes both peroxidase and cyclooxygenase enzymes. The catalytic domain of PGHS comprises the bulk of the monomer chain and contains both the cyclooxygenase and peroxidase active sites. These active sites are spatially distinct, located on opposite sides of the catalytic domain, with no evidence for any direct internal connections between the two sites [13,19,48,49].

Peroxidases are ubiquitous and found in all organisms, but a subset of theses peroxidases have developed a second enzymatic activity. Mammalian PGHS displays similarity in sequence and overall fold with thyroid peroxidase and the catalytic domain of canine myeloperoxidase [24,41,50,51]. In the latter case, this structural homology extends across the ovine PGHS catalytic domain, with the main helices involved in heme binding superimposing with a ~1.4 Å RMSD. Plant peroxidases which also display these similarities in fold include

lignin peroxidase and horseradish peroxidase [24,34]. The most significant structural differences between PGHS and myeloperoxidase are the lack of a membrane binding domain in myeloperoxidase, variations in the dimerization domains, lack of a cyclooxygenase active site, and modifications in the catalytic region that allow greater solvent accessibility to the heme. Like PGHS, myeloperoxidase is glycosylated.

2.4.1. The cyclooxygenase active site

A large number of crystallographic structures of both isoforms of eukaryotic PGHS inform our understanding of fatty acid substrate binding and catalysis [52–56] (Fig. 2). To successfully capture the substrate in its Michaelis complex within the cyclooxygenase active sites, alternative heme cofactors (*e.g.*, Co(III) protoporphyrin IX) or apo enzyme was utilized to remove any trace amounts of catalytic activity (Fig. 2).

Site-directed mutagenesis and x-ray crystallography have revealed a number of salient features of the cyclooxygenase active site within the eukaryotic enzyme. The active site resides at the apex of a long, narrow, hydrophobic channel (~8 × 25 Å), which leads from the membrane-binding domain deep into the catalytic domain [24]. The channel narrows at the meeting of the side-chains from residues Arg120, Tyr355, and Glu524; this triad comprises a constriction through which substrate must pass to enter the active site. It is clear from crystallographic analysis that some rearrangement of this constriction must occur to allow for the passage of substrate into and out of the active site. Two residues (Arg120 and Tyr355) have been shown to interact with the substrate carboxylate group [24,57] as well as similar chemical moieties in NSAIDs. The presence of Arg120 is critical for substrate binding to PGHS-1; an Arg120Glu mutation increases the K_M for arachidonic acid by ~100 fold and Arg120Gln by ~825 fold [58,59]. By crystallography, arachidonic acid is found oriented in an extended "L-shape" conformation within the active. Ser530, the residue covalently modified by the inhibitor aspirin [60,61], is buttressed against carbons 12-14 of the substrate [52] (see Fig. 2); similar configurations are observed in PGHS-2 [56] and with alternative fatty acid substrates [54,55]. Carbon 13 of arachidonic acid is positioned 2.8 Å away from the phenolic oxygen of Tyr385, which is converted to the radical species which initiates the cyclooxygenase activity by extraction of the *pro*S hydrogen of carbon 13. Carbons 14–20 (the ω-end) of this substrate is nested within in a hydrophobic cleft adjacent to Ser530. The vast majority of the interactions that the substrate makes with the enzyme are hydrophobic in nature [52].

The active site in eukaryotic PGHS is promiscuous in nature, encompassing a wide variety of alternative fatty acid substrates and small molecules. Substrates that include linoleic acid, di-homo-γ-linoleic acid, and eicosapentaenoic acid have been shown to bind in very similar orientations, corresponding to different trends in catalytic efficiency [54,55]. These catalytic differences are presumably due to misalignment of the substrate relative to Tyr385, which undermines the precise active site arrangement required for *pro*S hydrogen extraction. Furthermore, the fidelity of the reaction is not

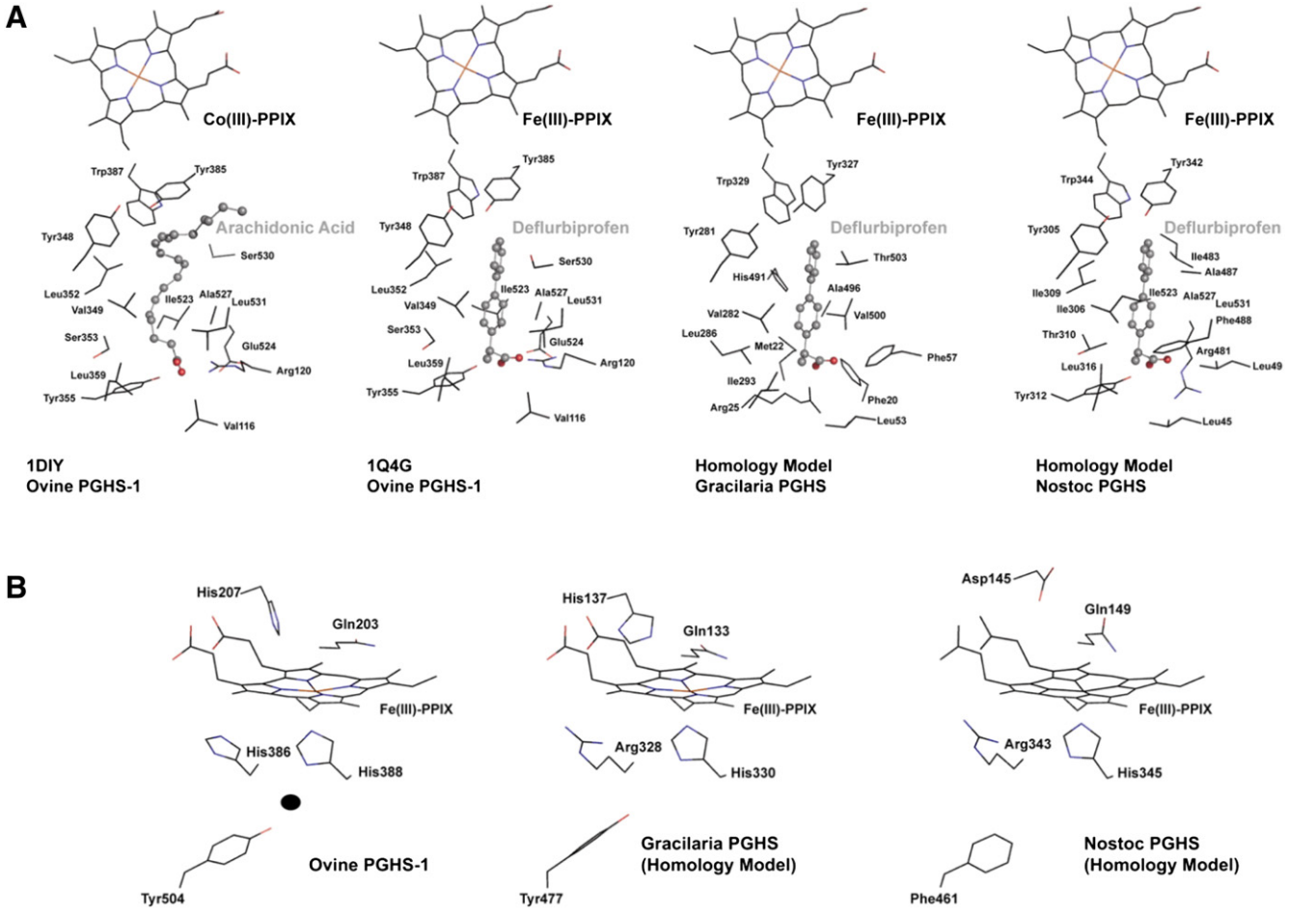

Fig. 2. The prokaryotic homologs of PGHS were threaded onto the ovine PGHS-1 template structure (PDB ID: 1Q4G) using the PHYRE v2.0 server [107]. The resulting models were gradually relaxed by energy minimization in a solvated environment using the programs VMD [108] and NAMD [109], using CHARMM42 force fields. The overall quality of this minimized model was evaluated using the MOLPROBITY server [110]. >90% of the residues resided in the allowable regions of a Ramachandran plot and showed generally good stereochemical properties. The model was rendered using the program PYMOL [105]. A. A comparison of the cyclooxygenase active sites of PGHS from *Ovis aries* in the arachidonic acid (1DIY, [52]) and NSAID-bound (1Q4G, [41]) forms, alongside the homology models for algal (*Gracilaria*) and bacterial (*Nostoc*) PGHS in NSAID-bound states. B. A comparison of the ovine PGHS-1 peroxidase active with those predicted by homology modeling for algal (*Gracilaria*) and bacterial (*Nostoc*) homologs.

absolute: while the predominant cyclooxygenase product of arachidonic acid is prostaglandin G_2, the minor products 11*R*-hydroxy-5*Z*,8*Z*,12*E*,14*Z*-eicosatetraenoic acid (11*R*-HETE), 15*S*-hydroxy-5*Z*,8*Z*,11*Z*,13*E*-eicosatetraenoic acid (15*S*-HETE), and 15*R*-HETE are also produced [62,63]. The relative amounts of these minor products differ between isoforms and species, and can be readily modified by site-directed mutagenesis.

2.4.2. The peroxidase active site

The peroxidase cycle of ovine PGHS is as robust as other well-characterized heme peroxidases [33,64], but is distinguished by the residues that comprise the heme binding site. The peroxidase activity of eukaryotic PGHS is primarily dependent upon the presence of Gln203 and His207 at the heme's distal face and His388, the proximal heme ligand (Fig. 2). Mutations of these residues lead to a marked reduction or elimination of peroxidase activity [65,66]. By x-ray crystallography, His388 is seen to participate in a hydrogen bonded network with Tyr504 and His386 via a well-ordered bridging water molecule [41]. In contrast, in related peroxidases such as horseradish peroxidase, the proximal histidine instead forms a strong salt bridge with an adjacent aspartate [67]; in myeloperoxidase, the adjacent residue is an asparagine [68].

The distal face of the heme cofactor in ovine PGHS-1 is largely defined by two residues: the distal heme ligand His207 and Gln203 (Fig. 2). In ovine PGHS-1, His207 is closest to the heme and is believed to assist in the deprotonation of the peroxidase substrate. His207 is conserved throughout all eukaryotic PGHS examples, and also in the related α-dioxygenase (*vida infra*). Heme iron ligands such as carbon monoxide or cyanide bind to the distal side of the iron [69], and glycerol has also been seen to bind to this site [41]. The adjacent glutamine residue (Gln203) also appears to participate in peroxidase substrate binding. PGHS is believed to utilize a "push-and-pull" mechanism for catalysis [70]. In ovine PGHS, Gln203 and His207 have been proposed to work in tandem in this regard to provide the "pull" for this mechanism.

3. The progenitors of mammalian prostaglandin H_2 synthases

3.1. Prostaglandin H_2 synthase homologs from crustaceans, coral, and alga

PeroxiBase [71] curates a collection of peroxidase-related genes from all kingdoms of life, including 95 entries for open-reading frames (ORFs) which are predicted to possess structural homology to ovine PGHS based on primary sequence. In addition to mammalian ORFs, additional PGHS genes have been reported from a number of additional more primitive vertebrate [72] invertebrate, and plant species, including crustacean, coral, and algae [22,68,73–75]. Table 1 provides a synopsis of the level of similarity observed between orthologs of PGHS-1, using ovine PGHS-1 as a point of reference. Coral PGHS sequences are 50% homologous to ovine PGHS-1, and recombinant coral PGHS exhibits both cyclooxygenase and peroxidase enzyme activity [73,74]. Similarly, the single characterized algal PGHS sequence is 25% identical to ovine PGHS-1 and *in vitro* also displays cyclooxygenase and peroxidase activities. Neither coral nor algal enzymes demonstrate significant inhibition by NSAIDs [22,74,76,77].

Among eukaryotic PGHS proteins, crystallographic structures are available only from mammalian examples. Hence, evolutionary insights into structure and function can only be inferred from sequence analysis and enzymology. All eukaryotic orthologs contain sequences consistent with the domains common to mammalian PGHS, and all require detergent to isolate in an active form. Of the known orthologs, all prefer arachidonic acid as substrate, and all but the enzyme from the coral *Plexaura homomalla* generate PGG_2 as their major cyclooxygenase product. Subspecies of *P. homomalla* have been identified that encode for orthologs that prefer either the corresponding 15R or 15S-hydroperoxide [77,78]. A single point mutation (Val349Ile) effectively was found to modulate this stereochemical preference. This observation is somewhat reminiscent of the inability of aspirin-acetylated mammalian PGHS-1 (at residue 530) to produce the minor product 15R-HETE [79] and the associated role of Val349 in the control of stereochemistry at the position 15 of substrate [80]. Together, these results illustrate how subtle changes within the cyclooxygenase active site can affect the dioxygen attack of the lipid substrate and the resulting lipid profile.

3.2. The peroxidase fold is the unifying feature of all prostaglandin H_2 synthase homologs

Phylogenetic analyses of these entries using both the *Phylogeny.fr* resource [81] and prototype animal heme peroxidases as a reference group provide insight into the evolutionary relationships among the structural homologs of mammalian PGHS. Fig. 3 provides a cladogram summarizing the relationships between four distinct clusters in our analysis: (I) prototype animal heme peroxidases, (II) PGHS orthologs from gamma- and delta-proteobacteria, sac fungi, and plants, (III) orthologs from alpha- and betaproteobacteria and algae, and (IV) eukaryotic PGHSs. The full phylogenetic analysis is provided in Supplemental Fig. 1. Where available, structural prototypes for which crystal structures are available are also shown, revealing both conserved and absent features between the clades. We will next discuss the basic features of these prototype peroxidases before drawing contrast with evolutionarily related PGHS enzymes from lower organisms.

3.2.1. Phylogeny of peroxidases

A phylogenetic relationship between the different mammalian peroxidases previously has been proposed [20]. The peroxidase domains of 134 heme-containing enzymes were analyzed using the maximum likelihood method, and divided into seven distinct subfamilies:

Table 1
Sequence comparisons between PGHS and related genes from various organisms compared to ovine PGHS-1. Sequence alignments were performed in BLAST [104]. Identities indicate sites where the same amino acid is conserved, while positives represent identities or residues with similar size or charge properties. Ovine PGHS-1 contains 600 amino acids.

	Identities	Positives	Gaps
Eukaryotes			
Homo sapiens (human)	534/576 (93%)	554/576 (96%)	0/576
Mus musculus (mouse)	504/576 (88%)	537/576 (93%)	0/576
Xenopus laevis (frog)	412/552 (75%)	479/552 (86%)	0/552
Danio rerio (zebrafish)	364/556 (65%)	451/556 (81%)	2/556 (<1%)
Plexaura homomalla (coral)	280/555 (50%)	378/555 (68%)	7/555 (1%)
Gammarus sp. (crustacean)	253/555 (46%)	373/555 (67%)	8/555 (1%)
Gersemia fruticosa (coral)	277/555 (50%)	375/555 (67%)	7/555 (1%)
Gracilaria vermiculophylla (red algae)	132/529 (25%)	240/529 (45%)	83/529 (15%)
Prokaryotes			
Nostoc punctiforme (cyanobacterium)	149/523 (28%)	241/523 (46%)	55/523 (10%)
Rhodobacter sphaeroides (alphaproteobacterium)	141/497 (28%)	221/497 (44%)	57/497 (11%)
Nitrosospira_multiformis (betaproteobacterium)	186/492 (34%)	251/492 (51%)	54/492 (10%)

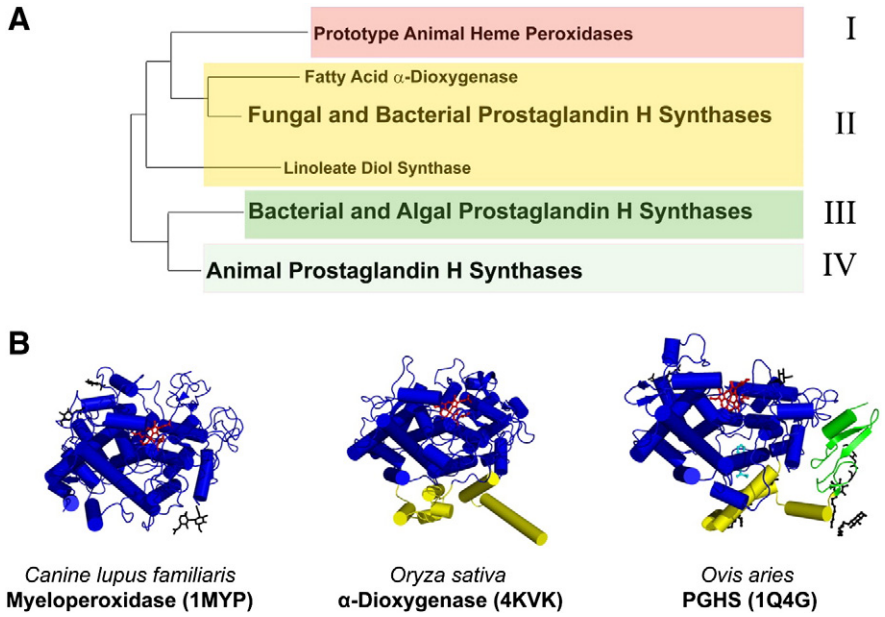

Fig. 3. Evolutionary relationship of PGHS homologs. A. Shown is an unrooted phylogenetic tree that summarizes the sequence relationship between PGHS homology cataloged in PeroxiBase. ORF sequences from 96 cataloged sequences were aligned and a maximum-likelihood tree calculated using PhyML [111]. Alignments were prepared with ClustalW [112] and trees were visualized using TREEDYN [113]. These software packages are available at http://www.phylogeny.fr/ [81]. A detailed phylogenetic tree resulting from this analysis is provided as Supplemental Fig. 1. B. Shown are three available crystal structures representing different evolutionary clades in this group of homologs: *Canine lupus familiaris* myeloperoxidase (1MYP, a prototype animal heme peroxidase), *Oryza sativa* α-dioxygenase (4KVK, an α-dioxygenase-like ortholog), and *Ovis aries* prostaglanding H_2 synthase (1Q4G, the prototype animal PGHS). No experimental structure of a representative from the bacterial and algal PGHS clade (III) is currently available.

peroxidases, peroxidasins, peroxinectins, cyclooxygenases, peroxicins, peroxidockerins, and dual oxidases. In that analysis, the prostaglandin synthases, also referred to as cyclooxygenases, were further subdivided into two clades: the mammalian PGHS proteins and their bacterial homologs represent one clade, while the plant and fungal PGHS proteins comprise the second clade [20]. These results are similar in nature to the relationships forwarded by our phylogenetic analysis (Fig. 3). The unifying feature of the peroxidase superfamily is the classic peroxidase fold with bound heme cofactor. However, most peroxidases lack features that distinguish eukaryotic PGHS, including a dimeric interface, a second distinct active site, and a membrane-binding domain. Within the prostaglandin synthases, sequence similarity has been identified with a number of gene products from a diverse number of species, including linoleate diol synthase, rice α-oxygenase, and the aforementioned cyclooxygenases identified in sea coral [77,82,83].

3.3. Group II: fungal and plant orthologs of PGHS

Putative and known fungal sequences within this clade of PGHS orthologs are remarkably similar to one another (greater than 42% sequence identity and 53% positive correlation in the most disparate cases), highly suggestive of a common origin, structure, and function. Many of the organisms within this clade prosper in fecund environments and may exist in close contact (e.g. mold and bacteria in the soil, alongside plants), raising the distinct possibility of lateral DNA transfer.

Fungal ORFs display the least sequence homology to their mammalian counterparts. Notably, this clade does bear a close sequence relationship to α-dioxygenase (α-DOX) from rice, suggesting that the members of this clade are evolutionary precursors closer to α-DOX in structure and function than eukaryotic PGHS.

3.3.1. α-Dioxygenase

α-Dioxygenases (α-DOX) catalyze the oxidation of fatty acids into 2-hydroperoxides. The reaction proceeds via a radical mechanism by removal of the *pro-R* hydrogen from C2 of a fatty acid substrate, followed by addition of molecular oxygen to generate the hydroperoxide. The unstable product can spontaneously lose a carboxylate moiety to form the corresponding aldehyde, or can be reduced by peroxidases to form a 2-hydroxy fatty acid [84]. The structure of α-DOX isolated from *Arabidopsis thaliana* was recently determined to 1.5 Å resolution [85], providing a unique high-resolution insight into the evolution of PGHS. At the N-terminus of α-DOX is a series of eight amphipathic helices, reminiscent of the PGHS membrane binding domain. From its crystal structure, α-DOX is apparently monomeric [85], while mammalian PGHS proteins are dimeric both in solution and the crystal lattice.

With regards to the first active site, α-DOX displays similar architectural features to PGHS, with a catalytic tyrosine (Tyr386) required for hydrogen atom abstraction from the fatty acid substrate. However, the architecture of the fatty acid binding site is different in α-DOX relative to PGHS, resulting in hydrogen abstraction and oxygen addition at C2 of the fatty acid. The structure observed at the two faces of the bound heme of α-DOX is also similar to PGHS, with His389 (analogous to His388 of PGHS) at the proximal side of the porphyrin ring and His163 and Gln159 (analogous to His207 and Gln203 in PGHS) on the distal face. The mutation Gln159Arg in α-dioxygenase completely abolishes oxygenase activity [86], but mutation of the same residue to Asn, Ser, or Val generates enzymes with greater than 75% of native activity [85].

In contrast to PGHS, α-DOX exhibits virtually no peroxidase activity, which is attributed to two peptide loops that limit the access of peroxidase substrates to the distal face of the heme [85]. This result, along with sequence alignments of other members of this clade might suggest that not all of the orthologs of this group may have retained *bona fide* peroxidase activity. For example, a sequence analysis of a putative PGHS identified in the *Neurospora crassa* genome indicates while the cyclooxygenase site appears largely intact, signature sequences associated with heme binding (including the proximal and distal histidine ligands) are not evident. This suggests that the peroxidase activity in this PGHS ortholog might be altered and the heme binding site remains only as a vestigial element.

3.3.2. Linoleate diol synthase

Linoleate diol synthases (LDS) are prevalent in fungi, and catalyze the addition of molecular oxygen to several sites in unsaturated C18

lipids [87]. Oliw's group has noted the mechanistic similarities between LDS and PGHS. Their recent work has concentrated upon two experimental systems: 7,8-LDS from *Gaeumannomyces graminis*, and the 5,8-LDS from *Aspergillus fumigatus*. In the *G. graminis* LDS, the authors performed a sequence alignment with ovine PGHS-1, identifying regions of functional overlap [82]. Later, amino acids in *G. graminis* LDS were mutagenized that correspond to residues known to be critical for PGHS cyclooxygenase activity. Several mutations exhibited reduced dioxygenase activity, as predicted from comparison with PGHS mutations, while others did not behave as expected [88]. Later, similar sequence homology was observed between ovine PGHS-1 and the 5,8-LDS of *Aspergillus fumigatus* [89]. Based upon sequence alignments, a truncated version of 5,8-LDS was prepared; the shorter construct retained 8-LDS dioxygenase activity. Mutagenesis of a critical tyrosine residue necessary for hydrogen atom abstraction completely abolished enzyme activity, as predicted from comparison with PGHS-1 [90]. While there is some limited sequence homology between LDS and PGHS-1, there is no structural data available for this class of enzymes. Structure prediction does not generate the structural elements typically associated with PGHS.

3.4. Group III: bacterial and algal orthologs of PGHS

The PeroxiBase collection contains eleven structural homologs to PGHS that are bacterial in origin. Unfortunately, there are no available high-resolution structures of members within this group that directly reveal their structural properties. As aforementioned, the enzyme from *Nostoc punctiforme* was recently expressed and characterized as a 10S-dioxygenase [21]. The dioxygenase is proposed to work in tandem with an upstream ORF for a catalase that converts the 10S hydroperoxide product of the dioxygenase enzyme to the corresponding alcohol. Notably, the recombinant *Nostoc* enzyme can be expressed and isolated as a soluble enzyme, in contrast to eukaryotic counterparts that require detergent for solubilization [21].

The degree of amino acid sequence homology between bacterial and eukaryotic PGHS orthologs is shown in Table 1, and a more detailed sequence alignment of two bacterial and one algal PGHS ORFs is provided in Fig. 4. In general, the bacterial orthologs display approximately ~20–30% sequence homologies relative to ovine PGHS and one another. For example, a pairwise comparison of the *Nostoc* 10S-dioxygenase with sequences for putative PGHS proteins from *Gracilaria* and *Rhodobacter* yield overall identities of 27% and 36%, respectively. The observed activity in the *Nostoc* enzyme suggests that the other identified ORFs also encode for dioxygenases, but the poor sequence homology decreases confidence in this assumption.

This group of bacterial orthologs can be further segregated by size. Two of the ORFs (from *Streptomyces avermitilis* and *Oceanobacter_sp.*) have greater than ~900 amino acids; these align poorly with the other 11 examples and for that reason have been eliminated from this analysis. The remaining ORFs display reasonable sequence homology and are further distinguished by length: one group ("medium") has four members (*Mycobacterium smegmatis*; *Acaryochloris marina*; *Microcoleus chthonoplastes*; *Solibacter usitatus*) with between 583 to 618 amino acids, while the other group ("small") has seven members (*Mycobacterium vanbaalenii*; *Nostoc punctiforme*; *Roseobacter denitrificans*; *Rhodobacter sphaeroides*; *Nitrosomonas europaea*; *Nitrosospira multiformis* (2 entries)) with between 528 and 550 amino acids. All of the bacterial genes are weakly homologous with mammalian PGHS genes due to the lack of the EGF-like domain and most of the dimerization domain found in eukaryotes. The bacterial genes do contain amino acids weakly analogous to the membrane binding domain, but with significant alteration in sequence and length.

The aforementioned discovery of a cyclooxygenase within the genome of the red algae *Gracilaria vermiculophylla* [22] was the first such non-animal cyclooxygenase to be identified and the only characterized from this class of organisms. The observed homology between the algal and mammalian enzymes is low relative to other pairwise comparisons (Table 1). While the coral PGHS shows ~50% homology to ovine PGHS-1, the algal PGHS only displays 25% homology. The algal protein shows limited sequence homology to the bacterial ORFs, with 20-30% sequence homology. The most striking feature of these comparisons is the size of the gaps in the alignment, ranging from ~1% in the coral enzyme to ~13% in the algal enzyme when compared to ovine PGHS.

The algal PGHS is a membrane-associated protein that requires detergent extraction to complete its purification and detergent in solution to maintain solubility. Accordingly, the authors present homology modeling demonstrating the concordance between the algal sequence and the ovine PGHS structure, except that the EGF-like domain and one of the membrane-binding helices are missing from the algal protein [22]. In further support of their structural model, recombinant preparations of this algal homolog were shown to possess both cyclooxygenase and peroxidase activities, utilizing a variety of C20 unsaturated fatty acids as substrates.

4. Predicted relationship between sequence and structure in the Group III PGHS orthologs

The recent characterization of PGHS structural homologs from algae and cyanobacteria, with different substrate specificities and solubility properties, provides a unique opportunity to speculate on the subtle structural modifications that underlie their functional differences. We next present a sequence and structural comparison of the well-known structures of ovine PGHS with the sequence and predicted structures of algal and bacterial homologs. The numbering convention corresponding to ovine PGHS-1 provides the basis for comparison by amino acid. To visually facilitate this comparison, we employed standard homology modeling methods to create models of the active sites and heme binding regions of the PGHS orthologs from *Gracilaria* (algae) PGHS and *Nostoc* (cyanobacteria) 10S-dioxygenase, using ovine PGHS-1 as the template for threading (see Fig. 2 legend). All-atom superpositions of these homology models with available eukaryotic experimental x-ray structures are provided in Fig. 2 to underscore the predicted differences in overall structure versus the mammalian prototype. These models guide our subsequent discussion of these predicted differences.

4.1. Absence of an EGF-like domain region

A careful comparison and sequence alignment of the EGF-like region in PGHS-1 and -2 from five mammalian species, trout, and zebrafish was previously reported [33]. If these sequences are aligned additionally with the sequences for the coral cyclooxygenases (*Plexaura* and *Gersemia*), strong sequence homology remains evident. Conserved cysteines are also found in the coral and crustacean PGHS enzyme sequences, indicating that the disulfide bonds identified in the mammalian proteins are conserved and suggest that the three dimensional structure of the EGF-like domain is largely retained through all vertebrate and invertebrate PGHS enzymes.

In contrast, when the sequences from the algal and bacterial genes are compared to ovine PGHS-1, no sequence correspondence to the EGF-like domain is apparent. Also, unlike their eukaryotic counterparts, the ORFs of the bacterial PGHS homologs do not include signal peptide sequences [91], suggesting that the bacterial PGHS does not undergo such targeting. In ovine PGHS-1, the EGF-like region ends near amino acid 64. When the primary sequence of the *Nostoc* 10S-dioxygenase ORF is aligned with ovine PGHS-1, we find that the first residue of the *Nostoc* protein coincides with amino acid 59 of ovine PGHS-2, but with very limited sequence homology in the N-terminal region. Alignment of the algal PGHS with ovine PGHS-1 begins with amino acid 56. Hence, the bacterial and algal orthologs lack the EGF and first dimerization domains common to the mammalian PGHS prototypes.

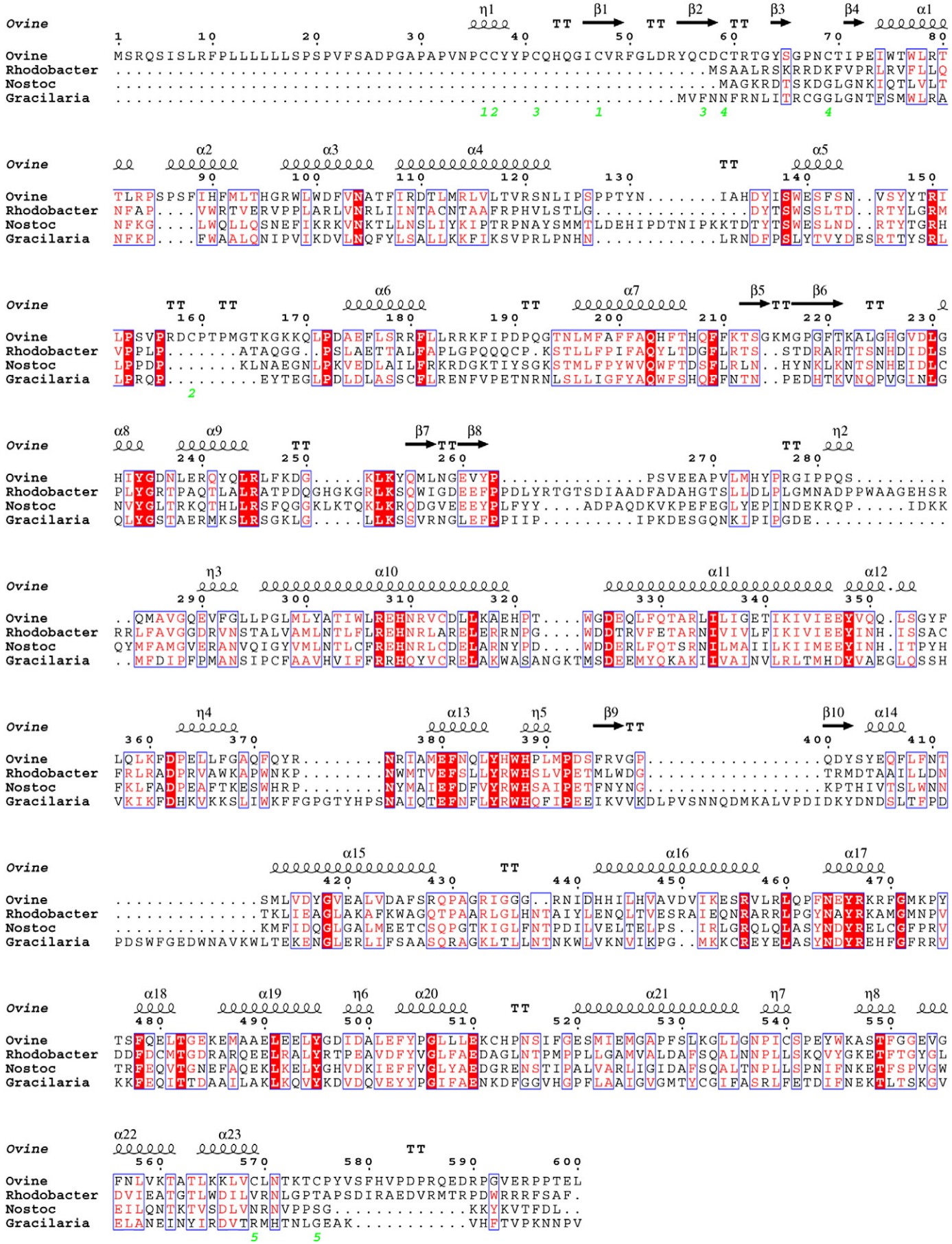

Fig. 4. An alignment of the PGHS open reading frames from *Rhodobacter sphaeroides*; *Nostoc punctiforme*; and *Gracilaria vermiculophylla* relative to ovine PGHS-1. Alignments were performed using ClustalX [112] and the figure generated using ESPript [114].

Accordingly, the homology models generated from the sequences lack this structural feature.

4.2. Is there a membrane binding domain in bacterial and algal orthologs?

As noted in Section 2.2, the membrane binding helices of PGHS display extensive sequence variability between mammalian species, with ~38% sequence homology versus greater than 70% homology for the rest of the protein [43,45]. Amphipathic helices are predicted to occur within the same regions of eukaryotic orthologs from both vertebrates and invertebrates. Hydrophobic residues are found within this region in all of the mammalian and fish PGHS proteins in their expected positions within the predicted helices, as well as within the coral orthologs. These analyses strongly suggest that all eukaryotic PGHS examples exist as monotopic membrane proteins.

In contrast, Group III bacterial and algal PGHS proteins display only modest homology across regions corresponding with the membrane binding domains of its eukaryotic counterparts. Fig. 4 compares the sequence of the membrane binding domain in ovine PGHS-1 with the aligned amino acids from *Nostoc*, *Rhodobacter*, and the algal species *Gracilaria*. While limited in sequence homology versus eukaryotic counterparts, the aligned sequences in bacteria and algae are still predicted to form α-helical structures. Secondary structure algorithms predict multiple amphipathic helices in the N-terminal region of algal PGHS, and homology modeling generates helices that correspond to the MBD of ovine PGHS-1. The requirement of sodium deoxycholate to extract recombinant algal PGHS from the membranes of bacterial cells supports this prediction [22]. In the absence of an experimental structure, available evidence strongly suggests that the algal PGHS contains a MBD in its N-terminus similar to its mammalian counterpart.

In contrast, the recombinant preparations of *Nostoc* 10S-dioxygenase are soluble in the absence of detergents [21]. Residues 15 to 60 of the *Nostoc* 10S-dioxygenase strongly correlate with the first four helices of the membrane binding domain of ovine PGHS-1. While different in composition, the first three predicted *Nostoc* helices, analogous to amphipathic helices A-C in ovine PGHS-1, appear to be amphipathic helices by secondary structure prediction. The structural prediction program PredictProtein [92] also predicts partial membrane burial of many of the residues in the helices, with less than 25% solvent accessibility, consistent with the helices interacting on the membrane surface. In mammalian PGHS proteins, a fourth amphipathic helix is oriented towards the active site relative to the plane of the membrane with only the distal half of the helix interacting with the membrane, and is believed to be less important in membrane binding than the first three helices [34]. Similarly, bioinformatics analysis of the *Rhodobacter* ortholog also predicts four amphipathic helices in this region with similar solvent accessibility.

Overall, these proteins contain structural elements that tentatively assigned as membranes binding domains and more broadly, three groups of structurally related proteins (plant α-dioxygenase, bacterial linoleate dioxygenase, and algal PGHS) have been highlighted which contain similar N-terminal amphipathic helices. However, the apparent soluble property of the *Nostoc* enzyme suggests that membrane binding is not obligatory in this case. As noted previously, it is expected that both composition in lipid and the protein component is expected to have measureable impact on the protein association with membrane bilayer. By virtue of comparison with membrane-bound relatives, this model system may provide a unique opportunity to discern the minimal structural requirements for the transition from a soluble to

4.3. Bacterial and algal orthologs are predicted to be monomeric

Limited sequence homology is predicted between the bacterial and mammalian proteins in part of the second dimerization domain (amino acids 156-177 in ovine PGHS-1), whereas in contrast, ovine PGHS-1 and PGHS-2 show very strong identity in this region. This region is largely devoid of secondary structure in available eukaryotic structures, and is partially absent among this group of bacterial and algal homologs.

When the bacterial and algal sequences in this region are examined by sequence alignment (Fig. 4), little resemblance to either eukaryotic isoform is apparent between amino acids 157-170, including sizeable gaps. The absence of a second dimerization domain, coupled with the lack of an EGF-like domain believed to contribute to dimerization, strongly predicts that PGHS found in lower organisms exist as monomers, rather than as the dimer observed in its mammalian counterparts. While more quantitative analysis is needed, gel filtration in the presence of solubilizing detergents suggests that algal PGHS is tetrameric in solution [22]. Together, these observations might imply that the oligomers formed by homologs from more primitive species rely on interfaces unique to those observed in eukaryotic counterparts.

In eukaryotic proteins, the dimeric structure of PGHS has been shown to be required for enzymatic function. Reversible disruption of the PGHS dimer by mild denaturation yields a monomeric-but-folded enzyme that lacks cyclooxygenase activity; activity is regained following removal of denaturant [47]. More recent studies with mammalian PGHS homodimers indicate that occupation of the active site of one monomer in the dimer prevents binding of substrate or inhibitor to the second monomer [12–16]. While the oligomeric properties of a bacterial PGHS remain to be determined, detailed side-by-side biochemical comparison of bacterial and eukaryotic PGHS would likely provide meaningful insight into the relationship between oligomeric state and catalysis, and the including the advantage of a “half of sites” reaction mechanism observed in the oligomer state.

4.4. The catalytic domain

Gracilaria PGHS, *Nostoc* 10S-dioxygenase, and the *Rhodobacter* ortholog align reasonably well over all residues to ovine PGHS-1, with approximately 25-28% sequence identity and 44–46% positively correlated amino acids (Table 1). Sequence alignments indicate that many of the amino acid residues critical for catalytic activity in mammalian PGHS are conserved in the bacterial and algal proteins. The quality of the theoretical alignment and resulting structural models allows for speculation on the significance of conservation in the lipid binding channel and peroxidase active site between the bacterial and algal proteins and their mammalian counterpart.

4.4.1. The cyclooxygenase active site

Next, we compare the substrate binding sites of one algal and two bacterial proteins to the same site in mammalian PGHS. The algal PGHS prefers arachidonic acid as substrate, but can also oxidize other C20 substrates with varying efficiencies. The substrate with the lowest K_M is 2-arachidonylglycerol, but this substrate is turned over at a rate less than 20% of that seen with arachidonic acid [22]. In contrast, the *Nostoc* 10S-dioxygenase binds most tightly to linolenic acid, and has virtually no activity toward C20 substrates [21]. The selective pressure that promotes diverse substrate specificity may be the availability of lipid substrates. The algal PGHS prefers arachidonic acid as a substrate, and arachidonic acid comprises more than 40% of the total fatty acid content in some species of *Gracilaria* [93]. Lipid analyses of the cyanobacteria *Nostoc punctiforme* indicate that arachidonic acid is not synthesized in this organism [94], and that linoleic acid is the major fatty acid seen in *Nostoc* cells in stationary phase [95]. Hence, despite sequence similarities noted, it stands to reason that the native substrate for *Nostoc* PGHS is different from its other counterparts. A comparison of the substrate binding sites between these two enzymes should reveal amino acid residues critical for adjusting the size of the lipid binding cavity and the orientation of catalytic residues relative to the bound lipid.

In mammalian PGHS proteins, Arg120 plays a key role in substrate and inhibitor binding, more so in PGHS-1 than PGHS-2. Mutational studies demonstrate that the Arg120Gln mutant of PGHS-1 has similar

kinetic properties to wild type PGHS-2, but the Arg120Leu mutant has approximately 20% of the cyclooxygenase activity of the wild type [96] . The region containing Arg120 in ovine PGHS-1 is poorly conserved through the bacterial and algal homologs (Fig. 4). In the mammalian structures, Arg120 resides in the C-terminal half of α-helix D of the membrane binding domain. In *Rhodobacter,* position 120 is occupied by a histidine, while in *Nostoc*, Asn occupies this position in the alignment. While it could be envisioned that the hydrogen bonding capacity could be retained by these residues, these substitutions do not arrive in the same spatial position ideal for interaction with substrate in our homology modeling (Fig. 2). Curiously, the *Gracilaria* ortholog has a Pro in this position by sequence alignment but yet is most active towards the substrate arachidonic acid; dihomo-γ-linolenic acid and arachidonoylglycerol also show significant activity [22]. Hence, the sequence requirements for substrate binding in these more primitive orthologs may be less reliant on the hydrogen bonding network provided in part by the triad of residues that includes Arg120 in eukaryotes.

Another determinant of substrate specificity in ovine PGHS-1 is Ile 523. In PGHS-2, this residue exists as Val and underlies the basis for isoform specificity of NSAIDs and different substrates. For example, arachidonoylglycerol is an excellent substrate for human and murine PGHS-2, but a very poor substrate for PGHS-1 [97], with the difference in activity attributed to the identity at this position. Sequence alignments of the bacterial and algal proteins indicate a lack of consensus at that position, favoring smaller hydrophobic residues (Fig. 4). This might indicate variability in substrate specificity between species.

More broadly, of the 19 residues that line the cyclooxygenase active site [57], eighteen are identical between human PGHS-1 and PGHS-2; only position 523 differs (Ile523 in PGHS-1 and Val523 in PGHS-2) [98]. Sequence alignments between the algal, bacterial, and ovine PGHS-1 show similar homology, with the exception of Arg120, as aforementioned. The region of amino acids corresponding to amino acids 348-355 and 521-525 also contribute significant surface near and within the entrance of the hydrophobic substrate channel. The annulus of large planar hydrophobic residues flanking Tyr385 at the apex of the cyclooxygenase channel is also predicted to be preserved by sequence alignment and homology modeling (Fig. 4). Also, Ser530, conserved in the eukaryotic enzymes, is universally changed to Ala in the bacterial enzymes and Thr in the algal enzyme. In eukaryotic PGHS-2, a Ser530Ala mutation causes little change in substrate oxidation [60], while Ser530Thr changes product production from PGG_2 to 15*R*-HETE. In PGHS-1, a Ser530Thr mutation inactivates the cyclooxygenase activity [99].

Thus, a simple comparison of active site amino acid residues is insufficient to rationalize the observed differences in substrate specificity between the *Gracilaria* and *Nostoc* enzymes. The factors dictating binding site architecture are certainly complex. For example, mutagenesis of non-active site amino acid residues in *Plexaura* PGHS invert the stereochemistry of dioxygen addition at C15 [100]. Experimental structures of the various enzymes discussed will be required to completely understand the structural basis for these substrate preferences.

4.4.2. The peroxidase active site

Bioinformatics and modeling also suggest unique features within the heme binding site of the prokaryotic and algal PGHS homologs relative to the ovine PGHS prototype. As mentioned earlier, His388 participates in a hydrogen bonded network with Tyr504 and His386 via a well-ordered bridging water molecule [41]. While *Rhodobacter* and *Gracilaria* enzymes preserve Tyr at position 504, the *Nostoc* enzyme features Phe at that position. Other bacterial orthologs similarly feature Phe or the bulkier Trp at the same position. The mechanistic implications of this substitution are two-fold. Firstly, the large hydrophobic substitution of Phe or Trp at this position would be predicted to undermine the reduction potential conferred by the Tyr 504-mediated hydrogen bonding network. Mutagenesis of this residue in ovine PGHS reduces but does not abolish peroxidase activity. His386 is conserved throughout eukaryotic species, but this position is substituted with Arg in the bacterial and algal enzymes, further arguing against the existence of the hydrogen bonding network as seen in available eukaryotic crystal structures. These compositional differences might explain why the *Nostoc* 10S-dioxygenase lacks the peroxidase activity observed in algal and eukaryotic PGHS. The *Rhodobacter* ortholog has the same network as the algal PGHS, and knowing if this enzyme possesses peroxidase activity would be of interest. The algal PGHS suggests that the His386Arg substitution is not critical for peroxidase activity. The cyclooxygenase and peroxidase activities in mammalian PGHS are coupled [101]; this peroxidase cycle is prerequisite for the formation of the tyrosyl radical needed to catalyzes cyclooxygenase activity and subsequent self-inactivation. Second, a second tyrosine radical species has been characterized that originates at Tyr504 and is functionally important for both isoforms of mammalian PGHS [102,103]. Hence, the *Nostoc* enzyme might have a simpler radical structure than that observed in the eukaryotic homologs, which could help to explain its dioxygenase activity relative to the cyclooxygenase activity observed in the algal enzyme.

The distal face of the heme cofactor in ovine PGHS-1 is largely defined by two residues: the distal heme ligand His207 and Gln203 (Fig. 2). The distal histidine is conserved in the algal protein, but in the bacterial homologs, the distal histidine is replaced by an aspartic acid residue. In all cases, Gln203 is absolutely conserved. The His207Asp substitution seen in the bacterial proteins may also help to explain the lack of peroxidase activity.

5. Summary and future directions

Bacterial and algal proteins have been highlighted that possess the potential to perform both cyclooxygenase and peroxidase activities distinct from that observed in eukaryotic counterparts. Already, one such bacterial protein has been demonstrated to act as a 10S-dioxygenase but is surprisingly soluble (rather than membrane-associated). However, while bioinformatics strongly indicates a common structural fold that relates these many orthologs, only experimental structures of can truly establish these structure-function relationships. Careful enzymology, site-directed mutagenesis, biophysics, and x-ray crystallography can help to further our understanding of the critical elements associated with cyclooxygenase activity, peroxidase activity, oligomerization, and membrane binding.

Not only do these orthologs provide a promising experimental system to approach specific mechanistic and structural questions, but also the opportunity to discern the biological roles that they play in these lower organism. Further motivation in this area of study may come from commercial providers of bioactive lipids. Access to recombinant preparations of these diverse enzymes may facilitate large-scale production of an array of bioactive lipids that are otherwise difficult and expensive to obtain from mammalian sources, or remain inaccessible to total chemical synthetic approaches.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.bbamem.2014.09.011.

Acknowledgements

The authors would like to thank Dr. Jennifer Palenchar for her critical reading of an earlier version of this review.

References

[1] P. Patrignani, M.R. Panara, A. Greco, O. Fusco, C. Natoli, S. Iacobelli, F. Cipollone, A. Ganci, C. Creminon, J. Maclouf, et al., Biochemical and pharmacological characterization of the cyclooxygenase activity of human blood prostaglandin endoperoxide synthases, J. Pharmacol. Exp. Ther. 271 (1994) 1705–1712.
[2] C. Patrono, Aspirin as an antiplatelet drug, N. Engl. J. Med. 330 (1994) 1287–1294.
[3] D. Riendeau, M.D. Percival, S. Boyce, C. Brideau, S. Charleson, W. Cromlish, D. Ethier, J. Evans, J.P. Falgueyret, A.W. Ford-Hutchinson, R. Gordon, G. Greig, M. Gresser, J. Guay, S. Kargman, S. Leger, J.A. Mancini, G. O'Neill, M. Ouellet, I.W. Rodger, M. Therien, Z. Wang, J.K. Webb, E. Wong, C.C. Chan, et al., Biochemical and

pharmacological profile of a tetrasubstituted furanone as a highly selective COX-2 inhibitor, Brit. J. Pharmacol. 121 (1997) 105–117.
[4] C.J. Smith, Y. Zhang, C.M. Koboldt, J. Muhammad, B.S. Zweifel, A. Shaffer, J.J. Talley, J.L. Masferrer, K. Seibert, P.C. Isakson, Pharmacological analysis of cyclooxygenase-1 in inflammation, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 13313–13318.
[5] Y. Zhang, A. Shaffer, J. Portanova, K. Seibert, P.C. Isakson, Inhibition of cyclooxygenase-2 rapidly reverses inflammatory hyperalgesia and prostaglandin E2 production, J. Pharmacol. Exp. Ther. 283 (1997) 1069–1075.
[6] H. Sheng, J. Shao, S.C. Kirkland, P. Isakson, R.J. Coffey, J. Morrow, R.D. Beauchamp, R.N. DuBois, Inhibition of human colon cancer cell growth by selective inhibition of cyclooxygenase-2, J. Clin. Invest. 99 (1997) 2254–2259.
[7] G.N. Levy, Prostaglandin H synthases, nonsteroidal anti-inflammatory drugs, and colon cancer, FASEB J. 11 (1997) 234–247.
[8] S.L. Kargman, G.P. O'Neill, P.J. Vickers, J.F. Evans, J.A. Mancini, S. Jothy, Expression of prostaglandin G/H synthase-1 and -2 protein in human colon cancer, Cancer Res. 55 (1995) 2556–2559.
[9] W. Kutchera, D.A. Jones, N. Matsunami, J. Groden, T.M. McIntyre, G.A. Zimmerman, R.L. White, S.M. Prescott, Prostaglandin H synthase 2 is expressed abnormally in human colon cancer: evidence for a transcriptional effect, Proc. Natl. Acad. Sci. U. S. A. 93 (1996) 4816–4820.
[10] D. Hwang, D. Scollard, J. Byrne, E. Levine, Expression of cyclooxygenase-1 and cyclooxygenase-2 in human breast cancer, J. Natl. Cancer Inst. 90 (1998) 455–460.
[11] P.L. McGeer, E.G. McGeer, Inflammation of the brain in Alzheimer's disease: implications for therapy, J. Leukoc. Biol. 65 (1999) 409–415.
[12] L. Dong, N.P. Sharma, B.J. Jurban, W.L. Smith, Pre-existent asymmetry in the human cyclooxygenase-2 sequence homodimer, J. Biol. Chem. 288 (2013) 28641–28655.
[13] L. Dong, A.J. Vecchio, N.P. Sharma, B.J. Jurban, M.G. Malkowski, W.L. Smith, Human cyclooxygenase-2 is a sequence homodimer that functions as a conformational heterodimer, J. Biol. Chem. 286 (2011) 19035–19046.
[14] C. Yuan, C.J. Rieke, G. Rimon, B.A. Wingerd, W.L. Smith, Partnering between monomers of cyclooxygenase-2 homodimers, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 6142–6147.
[15] C. Yuan, R.S. Sidhu, D.V. Kuklev, Y. Kado, M. Wada, I. Song, W.L. Smith, Cyclooxygenase allosterism, fatty acid-mediated cross-talk between monomers of cyclooxygenase homodimers, J. Biol. Chem. 284 (2009) 10046–10055.
[16] H. Zou, C. Yuan, L. Dong, R.S. Sidhu, Y.H. Hong, D.V. Kuklev, W.L. Smith, Human cyclooxygenase-1 activity and its responses to COX inhibitors are allosterically regulated by nonsubstrate fatty acids, J. Lipid Res. 53 (2012) 1336–1347.
[17] A.-L. Tsai, R.J. Kulmacz, Prostaglandin H synthase: resolved and unresolved mechanistic issues, Arch. Biochem. Biophys. 493 (2010) 103–124.
[18] A.L. Blobaum, L.J. Marnett, Structural and functional basis of cyclooxygenase inhibition, J. Med. Chem. 50 (2007) 1425–1441.
[19] C.A. Rouzer, L.J. Marnett, Cyclooxygenases: structural and functional insights, J. Lipid Res. 50 (2009) S29–S34.
[20] M. Zamocky, C. Jakopitsch, P.G. Furtmuller, C. Dunand, C. Obinger, The peroxidase-cyclooxygenase superfamily: Reconstructed evolution of critical enzymes of the innate immune system, Proteins 72 (2008) 589–605.
[21] A.R. Brash, N.P. Niraula, W.E. Boeglin, Z. Mashhadi, An ancient relative of cyclooxygenase in cyanobacteria Is a linoleate 10S-dioxygenase that works in tandem with a catalase-related protein with specific 10S-hydroperoxide lyase activity, J. Biol. Chem. 289 (2014) 13101–13111.
[22] K. Varvas, S. Kasvandik, K. Hansen, I. Järving, I. Morell, N. Samel, Structural and catalytic insights into the algal prostaglandin H synthase reveal atypical features of the first non-animal cyclooxygenase, Biochim. Biophys. Acta 1831 (2013) 863–871.
[23] H. Goldfine, Lipids of prokaryotes – structure and distribution, in: F. Bronner, A. Kleinteller (Eds.), Current Topics in Membranes and Transport, vol. 17, Academic Press, New York, 1982, pp. 1–43.
[24] D. Picot, P.J. Loll, R.M. Garavito, The X-ray crystal structure of the membrane protein prostaglandin H2 synthase-1, Nature 367 (1994) 243–249.
[25] W.L. Smith, Y. Urade, P.-J. Jakobsson, Enzymes of the cyclooxygenase pathways of prostanoid biosynthesis, Chem. Rev. 111 (2011) 5821–5865.
[26] D.L. Simmons, R.M. Botting, T. Hla, Cyclooxygenase isozymes: The biology of prostaglandin synthesis and inhibition, Pharmacol. Rev. 56 (2004) 387–437.
[27] R.M. Garavito, D.L. DeWitt, The cyclooxygenase isoforms: Structural insights into the conversion of arachidonic acid to prostaglandins, Biochim. Biophys. Acta 1441 (1999) 278–287.
[28] C. Luong, A. Miller, J. Barnett, J. Chow, C. Ramesha, M.F. Browner, Flexibility of the NSAID binding site in the structure of human cyclooxygenase-2, Nat. Struct. Biol. 3 (1996) 927–933.
[29] R.G. Kurumbail, A.M. Stevens, J.K. Gierse, J.J. McDonald, R.A. Stegeman, J.Y. Pak, D. Gildehaus, J.M. Miyashiro, T.D. Penning, K. Seibert, P.C. Isakson, W.C. Stallings, Structural basis for selective inhibition of cyclooxygenase-2 by anti-inflammatory agents, Nature 384 (1996) 644–648.
[30] M. Zamocky, P.G. Furtmuller, C. Obinger, Evolution of structure and function of Class I peroxidases, Arch. Biochem. Biophys. 500 (2010) 45–57.
[31] M. Hofrichter, R. Ullrich, M.J. Pecyna, C. Liers, T. Lundell, New and classic families of secreted fungal heme peroxidases, Appl. Microbiol. Biotechnol. 87 (2010) 871–897.
[32] N.V. Chandrasekharan, D.L. Simmons, The cyclooxygenases, Genome Biol. 5 (2004) 241.
[33] R.J. Kulmacz, W.A. van der Donk, A.-L. Tsai, Comparison of the properties of prostaglandin H synthase-1 and -2, Prog. Lipid Res. 42 (2003) 377–404.
[34] R.M. Garavito, D. Picot, P.J. Loll, Prostaglandin H synthase, Curr. Opin. Struct. Biol. 4 (1994) 529–535.
[35] L. Holm, P. Rosenstrom, Dali server: conservation mapping in 3D, Nucleic Acids Res. 38 (2010) W545–W549.
[36] C.P. Ponting, L. Aravind, J. Schultz, P. Bork, E.V. Koonin, Eukaryotic signalling domain homologues in archaea and bacteria. Ancient ancestry and horizontal gene transfer, J. Mol. Biol. 289 (1999) 729–745.
[37] I.K. Damon, Genus *Orthopoxvirus*: Variola virus, Birkhauser Verlag, Boston, 2007.
[38] J.C. Otto, D.L. DeWitt, W.L. Smith, N-glycosylation of prostaglandin endoperoxide synthases-1 and -2 and their orientations in the endoplasmic reticulum, J. Biol. Chem. 268 (1993) 18234–18242.
[39] G.M. Greig, D.A. Francis, J.P. Falgueyret, M. Ouellet, M.D. Percival, P. Roy, C. Bayly, J.A. Mancini, G.P. O'Neill, The interaction of arginine 106 of human prostaglandin G/H synthase-2 with inhibitors is not a universal component of inhibition mediated by nonsteroidal anti-inflammatory drugs, Mol. Pharmacol. 52 (1997) 829–838.
[40] J.F. Nemeth, G.P. Hochgesang Jr., L.J. Marnett, R.M. Caprioli, Characterization of the glycosylation sites in cyclooxygenase-2 using mass spectrometry, Biochemistry 40 (2001) 3109–3116.
[41] K. Gupta, B.S. Selinsky, C.J. Kaub, A.K. Katz, P.J. Loll, The 2.0 A resolution crystal structure of prostaglandin H2 synthase-1: structural insights into an unusual peroxidase, J. Mol. Biol. 335 (2004) 503–518.
[42] D. Picot, R.M. Garavito, Prostaglandin H synthase: Implications for membrane structure, FEBS Lett. 346 (1994) 21–25.
[43] A.G. Spencer, E. Thuresson, J.C. Otto, I. Song, T. Smith, D.L. DeWitt, R.M. Garavito, W.L. Smith, The membrane binding domains of prostaglandin endoperoxide H synthases 1 and 2, J. Biol. Chem. 274 (1999) 32936–32942.
[44] M. Nina, S. Berneche, B. Roux, Anchoring of a monotopic membrane protein: the binding of prostaglandin H2 synthase-1 to the surface of a phospholipid bilayer, Eur. Biophys. J. 29 (2000) 439–454.
[45] J.C. Otto, W.L. Smith, Photolabeling of prostaglandin endoperoxide H synthase-1 with 3-trifluoro-3-(m-iodophenyl)diazirine as a probe of membrane association and the cyclooxygenase active site, J. Biol. Chem. 271 (1996) 9906–9910.
[46] S. Wan, P.V. Coveney, A comparative study of the COX-1 and COX-2 isozymes bound to lipid membranes, J. Comput. Chem. 30 (2009) 1038–1050.
[47] G. Xiao, W. Chen, R.J. Kulmacz, Comparison of structural stabilities of prostaglandin H synthase-1 and -2, J. Biol. Chem. 273 (1998) 6801–6811.
[48] R.M. Garavito, A.M. Mulichak, The structure of mammalian cyclooxygenases, Annu. Rev. Biophys. Biomol. Struct. 32 (2003) 183–206.
[49] R.S. Sidhu, J.Y. Lee, C. Yuan, W.L. Smith, Comparison of cyclooxygenase-1 crystal structures: Cross-talk between monomers comprising cyclooxygenase-1 homodimers, Biochemistry 49 (2010) 7069–7079.
[50] H. Daiyasu, H. Toh, Molecular evolution of the myeloperoxidase family, J. Mol. Evol. 51 (2000) 433–445.
[51] J. Zeng, R.E. Fenna, X-ray crystal structure of canine myeloperoxidase at 3 Å resolution, J. Mol. Biol. 226 (1992) 185–207.
[52] M.G. Malkowski, S.L. Ginell, W.L. Smith, R.M. Garavito, The productive conformation of arachidonic acid bound to prostaglandin synthase, Science 289 (2000) 1933–1937.
[53] M.G. Malkowski, M.J. Theisen, A. Scharmen, R.M. Garavito, The formation of stable fatty acid substrate complexes in prostaglandin H(2) synthase-1, Arch. Biochem. Biophys. 380 (2000) 39–45.
[54] M.G. Malkowski, E.D. Thuresson, K.M. Lakkides, C.J. Rieke, R. Micielli, W.L. Smith, R.M. Garavito, Structure of eicosapentaenoic and linoleic acids in the cyclooxygenase site of prostaglandin endoperoxide H synthase-1, J. Biol. Chem. 276 (2001) 37547–37555.
[55] E.D. Thuresson, M.G. Malkowski, K.M. Lakkides, C.J. Rieke, A.M. Mulichak, S.L. Ginell, R.M. Garavito, W.L. Smith, Mutational and X-ray crystallographic analysis of the interaction of dihomo-γ-linolenic acid with prostaglandin endoperoxide H synthases, J. Biol. Chem. 276 (2001) 10358–10365.
[56] A.J. Vecchio, D.M. Simmons, M.G. Malkowski, Structural basis of fatty acid substrate binding to cyclooxygenase-2, J. Biol. Chem. 285 (2010) 22152–22163.
[57] E.D. Thuresson, K.M. Lakkides, C.J. Rieke, Y. Sun, B.A. Wingerd, R. Micielli, A.M. Mulichak, M.G. Malkowski, R.M. Garavito, W.L. Smith, Prostaglandin endoperoxide H synthase-1, J. Biol. Chem. 276 (2001) 10347–10357.
[58] D.K. Bhattacharyya, M. Lecomte, C.J. Rieke, M. Garavito, W.L. Smith, Involvement of arginine 120, glutamate 524, and tyrosine 355 in the binding of arachidonate and 2-phenylpropionic acid inhibitors to the cyclooxygenase active site of ovine prostaglandin endoperoxide H synthase-1, J. Biol. Chem. 271 (1996) 2179–2184.
[59] J.A. Mancini, D. Riendeau, J.-P. Falgueyret, P.J. Vickers, G.P. O'Neill, Arginine 120 of prostaglandin G/H synthase-1 Is required for the inhibition by nonsteroidal anti-inflammatory drugs containing a carboxylic acid moiety, J. Biol. Chem. 270 (1995) 29372–29377.
[60] D.L. DeWitt, E.A. el-Harith, S.A. Kraemer, M.J. Andrews, E.F. Yao, R.L. Armstrong, W.L. Smith, The aspirin and heme-binding sites of ovine and murine prostaglandin endoperoxide synthases, J. Biol. Chem. 265 (1990) 5192–5198.
[61] P.J. Loll, D. Picot, R.M. Garavito, The structural basis of aspirin activity inferred from the crystal structure of inactivated prostaglandin H2 synthase, Nat. Struct. Biol. 2 (1995) 637–643.
[62] E.A. Meade, W.L. Smith, D.L. DeWitt, Differential inhibition of prostaglandin endoperoxide synthase (cyclooxygenase) isozymes by aspirin and other non-steroidal anti-inflammatory drugs, J. Biol. Chem. 268 (1993) 6610–6614.
[63] E.D. Thuresson, K.M. Lakkides, W.L. Smith, Different catalytically competent arrangements of arachidonic acid within the cyclooxygenase active site of prostaglandin endoperoxide H synthase-1 lead to the formation of different oxygenated products, J. Biol. Chem. 275 (2000) 8501–8507.
[64] W.A. van der Donk, A.-L. Tsai, R.J. Kulmacz, The cyclooxygenase reaction mechanism, Biochemistry 41 (2002) 15451–15458.

[65] L.M. Landino, B.C. Crews, J.K. Gierse, S.D. Hauser, L.J. Marnett, Mutational analysis of the role of the distal histidine and glutamine residues of prostaglandin-endoperoxide synthase-2 in peroxidase catalysis, hydroperoxide reduction, and cyclooxygenase activation, J. Biol. Chem. 272 (1997) 21565–21574.
[66] T. Shimokawa, W.L. Smith, Essential histidines of prostaglandin endoperoxide synthase. His-309 is involved in heme binding, J. Biol. Chem. 266 (1991) 6168–6173.
[67] M. Gajhede, D.J. Schuller, A. Henriksen, A.T. Smith, T.L. Poulos, Crystal structure of horseradish peroxidase C at 2.15 A resolution, Nature Struct Mol. Biol. 4 (1997) 1032–1038.
[68] C.A. Davey, R.E. Fenna, 2.3 A resolution x-ray crystal structure of the bisubstrate analogue inhibitor salicylhydroxamic acid bound to human myeloperoxidase: a model for a prereaction complex with hydrogen peroxide, Biochemistry 35 (1996) 10967–10973.
[69] S.A. Seibold, J.F. Cerda, A.M. Mulichak, I. Song, R.M. Garavito, T. Arakawa, W.L. Smith, G.T. Babcock, Peroxidase activity in prostaglandin endoperoxide H synthase-1 occurs with a neutral histidine proximal heme ligand, Biochemistry 39 (2000) 6616–6624.
[70] T. Poulos, Heme enzyme crystal structures, Adv. Inorg. Biochem. 7 (1988) 1–36.
[71] N. Fawal, Q. Li, B. Savelli, M. Brette, G. Passaia, M. Fabre, C. Mathe, C. Dunand, PeroxiBase: a database for large-scale evolutionary analysis of peroxidases, Nucleic Acids Res. 41 (2013) D441–D444.
[72] J.C. Havird, M.M. Miyamoto, K.P. Choe, D.H. Evans, Gene duplications and losses within the cyclooxygenase family of teleosts and other chordates, Mol. Biol. Evol. 25 (2008) 2349–2359.
[73] R. Järving, I. Järving, R. Kurg, A.R. Brash, N. Samel, On the evolutionary origin of cyclooxygenase (COX) isozymes, J. Biol. Chem. 279 (2004) 13624–13633.
[74] R. Koljak, I. Järving, R. Kurg, W.E. Boeglin, K. Varvas, K. Valmsen, M. Ustav, A.R. Brash, N. Samel, The basis of prostaglandin synthesis in coral: Molecular cloning and expression of a cyclooxygenase from the arctic soft coral Gersemia fruticosa, J. Biol. Chem. 276 (2001) 7033–7040.
[75] K. Varvas, R. Kurg, K. Hansen, R. Jarving, I. Jarving, K. Valmsen, H. Lohelaid, N. Samel, Direct evidence of the cyclooxygenase pathway of prostaglandin synthesis in arthropods: Genetic and biochemical characterization of two crustacean cyclooxygenases, Insect Biochem. Mol. Biol. 39 (2009) 851–860.
[76] H. Kanamoto, M. Takemura, K. Ohyama, Identification of a cyclooxygenase gene from the red alga Gracilaria vermiculophylla and bioconversion of arachidonic acid to PGF2α in engineered Escherichia coli, Appl. Microbiol. Biotechnol. 91 (2011) 1121–1129.
[77] K. Valmsen, I. Järving, W.E. Boeglin, K. Varvas, R. Koljak, T. Pehk, A.R. Brash, N. Samel, The origin of 15R-prostaglandins in the Caribbean coral *Plexaura homomalla*: Molecular cloning and expression of a novel cyclooxygenase, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 7700–7705.
[78] K. Valmsen, W.E. Boeglin, I. Järving, C. Schneider, K. Varvas, A.R. Brash, N. Samel, Structural and functional comparison of 15S- and 15R-specific cyclooxygenases from the coral *Plexaura homomalla*, Eur. J. Biochem. 271 (2004) 3533–3538.
[79] S.W. Rowlinson, B.C. Crews, D.C. Goodwin, C. Schneider, J.K. Gierse, L.J. Marnett, Spatial requirements for 15-(R)-hydroxy-5Z,8Z,11Z, 13E-eicosatetraenoic acid synthesis within the cyclooxygenase active site of murine COX-2. Why acetylated COX-1 does not synthesize 15-(R)-hete, J. Biol. Chem. 275 (2000) 6586–6591.
[80] C. Schneider, W.E. Boeglin, A.R. Brash, Analysis of cyclooxygenase-substrate interactions using stereospecifically-labeled arachidonic acids, Adv. Exp. Med. Biol. 507 (2002) 49–53.
[81] A. Dereeper, V. Guignon, G. Blanc, S. Audic, S. Buffet, F. Chevenet, J.F. Dufayard, S. Guindon, V. Lefort, M. Lescot, J.M. Claverie, O. Gascuel, Phylogeny.fr: robust phylogenetic analysis for the non-specialist, Nucleic Acids Res. 36 (2008) W465–W469.
[82] L. Hörnsten, C. Su, A.E. Osbourn, P. Garosi, U. Hellman, C. Wernstedt, E.H. Oliw, Cloning of linoleate diol synthase reveals homology with prostaglandin H synthases, J. Biol. Chem. 274 (1999) 28219–28224.
[83] T. Koeduka, K. Matsui, Y. Akakabe, T. Kajiwara, Catalytic properties of rice α-oxygenase, J. Biol. Chem. 277 (2002) 22648–22655.
[84] M. Hamberg, I. Ponce de Leon, M.J. Rodriguez, C. Castresana, α-Dioxygenases, Biochem. Biophys. Res. Commun. 338 (2005) 169–174.
[85] C.C. Goulah, G. Zhu, M. Koszelak-Rosenblum, M.G. Malkowski, The crystal structure of α-dioxygenase provides insight into diversity in the cyclooxygenase-peroxidase superfamily, Biochemistry 52 (2013) 1364–1372.
[86] M. Hamberg, A. Sanz, C. Castresana, α-Dioxygenase, a new enzyme in fatty acid metabolism, International Congress Series, 1233, 2002, pp. 307–317.
[87] A. Andreou, F. Brodhun, I. Feussner, Biosynthesis of oxylipins in non-mammals, Prog. Lipid Res. 48 (2009) 148–170.
[88] U. Garscha, E.H. Oliw, Critical amino acids for the 8(R)-dioxygenase activity of linoleate diol synthase. A comparison with cyclooxygenases, FEBS Lett. 582 (2008) 3547–3551.
[89] F. Jernerén, U. Garscha, I. Hoffmann, M. Hamberg, E.H. Oliw, Reaction mechanism of 5,8-linoleate diol synthase, 10R-dioxygenase, and 8,11-hydroperoxide isomerase of *Aspergillus clavatus*, Biochim. Biophys. Acta 1801 (2010) 503–507.
[90] I. Hoffmann, F. Jernerén, U. Garscha, E.H. Oliw, Expression of 5,8-LDS of Aspergillus fumigatus and its dioxygenase domain. A comparison with 7,8-LDS, 10-dioxygenase, and cyclooxygenase, Arch. Biochem. Biophys. 506 (2011) 216–222.
[91] T.N. Petersen, S. Brunak, G. von Heijne, H. Nielsen, SignalP 4.0: discriminating signal peptides from transmembrane regions, Nat. Methods 8 (2011) 785–786.
[92] G. Yachdav, E. Kloppmann, L. Kajan, M. Hecht, T. Goldberg, T. Hamp, P. Hönigschmid, A. Schafferhans, M. Roos, M. Bernhofer, L. Richter, H. Ashkenazy, M. Punta, A. Schlessinger, Y. Bromberg, R. Schneider, G. Vriend, C. Sander, N. Ben-Tal, B. Rost, PredictProtein – an open resource for online prediction of protein structural and functional features, Nucleic Acids Res. 42 (2014) W337–W343.
[93] A.B. Imbs, N.A. Latyshev, V.I. Svetashev, A.V. Skriptsova, T. Le, M. Pham, V. Nguyen, L. Pham, Distribution of polyunsaturated fatty acids in red algae of the genus *Gracilaria*, a promising source of prostaglandins, Russ. J. Mar. Biol. 38 (2012) 339–345.
[94] M.A. Vargas, H. Rodríguez, J. Moreno, H. Olivares, J.A.D. Campo, J. Rivas, M.G. Guerrero, Biochemical composition and fatty acid contant of filamentous nitrogen-fixing cyanobacteria, J. Phycol. 34 (1998) 812–817.
[95] A. Peramuna, M. Summers, Composition and occurrence of lipid droplets in the cyanobacterium Nostoc punctiforme, Arch. Microbiol. 1–10 (2014).
[96] C.J. Rieke, A.M. Mulichak, R.M. Garavito, W.L. Smith, The role of arginine 120 of human prostaglandin endoperoxide H synthase-2 in the interaction with fatty acid substrates and inhibitors, J. Biol. Chem. 274 (1999) 17109–17114.
[97] M. Yu, D. Ives, C.S. Ramesha, Synthesis of prostaglandin E2 ethanolamide from anandamide by cyclooxygenase-2, J. Biol. Chem. 272 (1997) 21181–21186.
[98] W.L. Smith, D.L. DeWitt, R.M. Garavito, Cyclooxygenases: Structural, cellular, and molecular biology, Ann. Rev. Biochem. 69 (2000) 145–182.
[99] C. Schneider, W.E. Boeglin, J.J. Prusakiewicz, S.W. Rowlinson, L.J. Marnett, N. Samel, A.R. Brash, Control of prostaglandin stereochemistry at the 15-carbon by cyclooxygenases-1 and -2, J. Biol. Chem. 277 (2002) 478–485.
[100] K. Valmsen, W.E. Boeglin, R. Järving, I. Järving, K. Varvas, A.R. Brash, N. Samel, A critical role of non-active site residues on cyclooxygenase helices 5 and 6 in the control of prostaglandin stereochemistry at carbon 15, J. Biol. Chem. 282 (2007) 28157–28163.
[101] M. Bakovic, H.B. Dunford, Intimate relation between cyclooxygenase and peroxidase activities of prostaglandin H synthase. Peroxidase reaction of ferulic acid and Its influence on the reaction of arachidonic acid, Biochemistry 33 (1994) 6475–6482.
[102] C.E. Rogge, W. Liu, R.J. Kulmacz, A.-L. Tsai, Peroxide-induced radical formation at TYR385 and TYR504 in human PGHS-1, J. Inorg. Biochem. 103 (2009) 912–922.
[103] C.E. Rogge, W. Liu, G. Wu, L.-H. Wang, R.J. Kulmacz, A.-L. Tsai, Identification of Tyr504 as an alternative tyrosyl radical site in human prostaglandin H synthase-2, Biochemistry 43 (2004) 1560–1568.
[104] C. Camacho, G. Couloris, V. Avagyan, N. Ma, J. Papadopoulos, K. Bealer, T.L. Madden, BLAST+; architecture and applications, BMC Bioinformatics 10 (2009) 421.
[105] Schrodinger, LLC, The PyMOL Molecular Graphics System, Version 1.3r1, 2010.
[106] I. Song, W.L. Smith, C-terminal Ser/Pro-Thr-Glu-Leu tetrapeptides of prostaglandin endoperoxide H synthases-1 and -2 target the enzymes to the endoplasmic reticulum, Arch. Biochem. Biophys. 334 (1996) 67–72.
[107] L.A. Kelley, M.J. Sternberg, Protein structure prediction on the Web: a case study using the Phyre server, Nat. Protoc. 4 (2009) 363–371.
[108] W. Humphrey, A. Dalke, K. Schulten, VMD – Visual molecular dynamics, J. Mol. Graph. 14 (1996) 33–38.
[109] J.C. Phillips, B. Rosemary, W. Wang, J. Gumbart, E. Tajkhorshid, E. Villa, C. Chipot, R.D. Skeel, L. Kale, K. Schulten, Scalable molecular dynamics with NAMD, J. Comput. Chem. 26 (2005) 1781–1802.
[110] H. Chen, A. Engelman, The barrier-to-autointegration protein is a host factor for HIV type 1 integration, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 15270–15274.
[111] S. Guindon, J.F. Dufayard, V. Lefort, M. Anisimova, W. Hordijk, O. Gascuel, New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0, Syst. Biol. 59 (2010) 307–321.
[112] M.A. Larkin, G. Blackshields, N.P. Brown, R. Chenna, P.A. McGettigan, H. McWilliam, F. Valentin, I.M. Wallace, A. Wilm, R. Lopez, J.D. Thompson, T.J. Gibson, D.G. Higgins, Clustal W and Clustal X version 2.0, Bioinformatics 23 (2007) 2947–2948.
[113] F. Chevenet, C. Brun, A.L. Banuls, B. Jacq, R. Christen, TreeDyn: towards dynamic graphics and annotations for analyses of trees, BMC Bioinformatics 7 (2006) 439.
[114] P. Gouet, X. Robert, E. Courcelle, ESPript/ENDscript: Extracting and rendering sequence and 3D information from atomic structures of proteins, Nucleic Acids Res. 31 (2003) 3320–3323.