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Review

Secreted Phospholipases A_2 – not just Enzymes: Revisited

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Abstract

Secreted phospholipases A_2 (sPLA$_2$s) participate in a very broad spectrum of biological processes through their enzymatic activity and as ligands for membrane and soluble receptors. The physiological roles of sPLA$_2$s as enzymes have been very well described, while their functions as ligands are still poorly known. Since the last overview of sPLA$_2$-binding proteins (sPLA$_2$-BPs) 10 years ago, several important discoveries have occurred in this area. New and more sensitive analytical tools have enabled the discovery of additional sPLA$_2$-BPs, which are presented and critically discussed here. The structural diversity of sPLA$_2$-BPs reveals sPLA$_2$s as very promiscuous proteins, and we offer some structural explanations for this nature that makes these proteins evolutionarily highly advantageous. Three areas of physiological engagement of sPLA$_2$-BPs have appeared most clearly: cellular transport and signalling, and regulation of the enzymatic activity of sPLA$_2$s. Due to the multifunctionality of sPLA$_2$s, they appear to be exceptional pharmacological targets. We reveal the potential to exploit interactions of sPLA$_2$s with other proteins in medical terms, for the development of original diagnostic and therapeutic procedures. We conclude this survey by suggesting the priority questions that need to be answered.

Key words: Secreted phospholipase A_2; binding protein; promiscuity; cell transport; signalling; phospholipase activity regulation

Introduction

Secreted phospholipases A_2 (sPLA$_2$s) (EC 3.1.1.4) are a structurally related group of low-molecular-mass enzymes (14–18 kDa) that catalyse the hydrolysis of glycerophospholipids (phospholipids hereafter) at their *sn*-2 position, to produce lysophospholipids and free fatty acids. sPLA$_2$s contain 6 to 8 disulphide bonds, a highly conserved His/Asp catalytic dyad, and a Ca^{2+}-binding loop [1]. Several sPLA$_2$ isoforms have been described in mammals. Depending on their structural characteristics, the mammalian sPLA$_2$s are divided into 11 groups (G): IB, IIA, IIC, IID, IIE, IIF, III, V, X, XIIA and XIIB [2]. Snake venom sPLA$_2$s are orthologous to mammalian GIIA, GIIB or GIIE sPLA$_2$s, or they belong to the unique GIA sPLA$_2$s [3]. sPLA$_2$s from bee and lizard venoms are homologous to mammalian GIII sPLA$_2$s. Finally, the further GIX sPLA$_2$s are found in venom of marine snails, and GXIA and GXIB sPLA$_2$s are plant proteins.

The importance of sPLA$_2$s as enzymes

The enzymatic activity of sPLA$_2$s define their participation in a very broad spectrum of biological processes. Generally, sPLA$_2$s are secreted from cells and require micromolar to millimolar Ca^{2+} to be catalytically competent. They predominantly target phospholipids in the extracellular space, although they also act intracellularly.

sPLA$_2$s show distinct substrate specificities in terms of the phospholipid polar headgroups and the fatty acids esterified at the *sn*-2 position of the glycerol backbone. For instance, GIII, GV and GX sPLA$_2$s efficiently hydrolyse phosphatidylcholine, while GIIA sPLA$_2$ has higher activity against negatively charged phospholipid substrates, and in particular, phosphatidylserine, phosphatidylglycerol and phosphatidylethanolamine. For their *sn*-2 fatty acid tail specificities, GIB, GIIA and GIIE sPLA$_2$s are promiscuous. GV sPLA$_2$ preferentially targets *sn*-2

fatty acyls with low number of double bonds, such as oleyl, while GIID, GIIF, GIII and GX $sPLA_2$s target polyunsaturated fatty acyls, such as arachidonyl.

$sPLA_2$ isoforms have unique tissue and cellular distributions, and therefore it is evident that individual $sPLA_2$s have distinct enzyme-activity-related biological functions. These include the generation of a variety of lipid mediators, along with membrane remodelling, modification of extracellular non-cellular phospholipid components of pulmonary surfactant, microparticles and lipoproteins, and degradation of microbial membranes and dietary phospholipids. The pathophysiological aspects of $sPLA_2$s as enzymes have been comprehensively reviewed recently (e.g., [2,4]), and therefore we will not focus on these here. Instead, our attention here is focussed on $sPLA_2$s acting as ligands, where fewer of the details have been described.

The existence of $sPLA_2$s without enzymatic activity but with pharmacological activity was an early indication that $sPLA_2$s can participate in physiological settings not just as enzymes, but also as ligands for membrane and soluble receptors. Indeed, in 1982, the first specific $sPLA_2$-binding protein ($sPLA_2$-BP) was discovered [5]. Since then, the number of newly characterized $sPLA_2$-BPs has expanded considerably (Table 1), and due to the development of more sensitive analytical methodologies, this process continues. Several reviews on $sPLA_2$-BPs have been published over the years [6-9], although the most recent is 10 years old [9]. Therefore, now is an appropriate time to survey the advances in this important area of research, and to critically discuss new insights and suggest research directions.

$sPLA_2$s bind very structurally diverse proteins

There are two types of $sPLA_2$-BPs: integral membrane proteins and soluble proteins [9]. The former belong to one of the following protein families: (1) muscle-type $sPLA_2$ receptors (M-type $sPLA_2$Rs); (2) heparan sulphate proteoglycans (HSPGs); (3) integrins; (4) vascular endothelial growth factor receptors (VEGFRs); and (5) ion channels. Two new integral membrane $sPLA_2$-BPs have been described recently, a G-protein-coupled receptor (GPCR), and cytochrome c oxidase (CCOX) [35, 37]. CCOX is the only known intracellular membrane $sPLA_2$-BP, as all of the other integral membrane $sPLA_2$-BPs are located in the plasma membrane. On the other hand, soluble $sPLA_2$-BPs are more frequently found inside cells, in the endoplasmic reticulum (ER), cytosol or nucleus, as well as in the extracellular space. The ER-resident $sPLA_2$-BPs, disulphide isomerase (PDI), taipoxin-associated 49-kDa Ca^{2+}-binding protein (TCBP-49) and crocalbin, and the cytosolic calmodulin (CaM) and 14-3-3 proteins, have been known for some time. Two new soluble $sPLA_2$-BPs were only described more recently: vimentin [26] and nucleolin [27]. Vimentin is an intermediate filament protein, as a constituent of the cytoskeleton, while nucleolin is a nucleolar protein; however, both of these proteins are also found on the cell surface, which is where they are most likely to interact with $sPLA_2$s. Extracellular $sPLA_2$-BPs include pentraxins, α-, β- and γ-type $sPLA_2$ inhibitors (PLIs), blood coagulation factors, pulmonary surfactant protein A (SP-A), metalloproteinase inhibitor DM64, and a serine protease inhibitor (C1 inhibitor protein). The main characteristics of the $sPLA_2$-BPs are shown in Table 1.

Structural aspects of $sPLA_2$ protein-binding-promiscuity

$sPLA_2$-BPs are structurally so diverse that attempts to define their common $sPLA_2$-binding attributes have remained unsuccessful. To solve this problem, it would be most helpful to determine the three-dimensional structures of complexes between $sPLA_2$s and the $sPLA_2$-BPs, and thus to analyse their interaction areas. However, contemporary methodologies are still not optimal for this kind of approach.

The focus has thus mainly been on well-defined structural elements that are present in more than one type of $sPLA_2$-BP, such as the C-type carbohydrate recognition domain (CRD) and CRD-like folds (in M-type $sPLA_2$Rs, SP-A, α-PLIs), the EF-hand Ca^{2+}-binding motif (in CaM, crocalbin, TCBP-49), and the immunoglobulin-like (Ig-like) domain (in VEGFRs, DM64) (Figure 1).

Only the CRD fold (Figure 1A) has been confirmed as an $sPLA_2$-binding structure [10]. Using radio-iodinated snake venom GIA $sPLA_2$ (^{125}I-OS_1), it was established that one of the eight CRDs in M-type $sPLA_2$R (i.e., CRD5) includes the $sPLA_2$ binding site. This also revealed that not all of the CRDs can bind $sPLA_2$s. M-type $sPLA_2$R bound $sPLA_2$s only under neutral and basic conditions, which implies that positively charged amino acids have important roles in this interaction. At pH 6 or lower, M-type $sPLA_2$R also underwent conformational changes that prevented the binding of $sPLA_2$s [41]. For the other two CRD-fold-containing $sPLA_2$-BPs, as lung SP-A [13] and soluble α-PLIs from sera [12], the $sPLA_2$-binding characteristics are not very clear yet. As M-type $sPLA_2$R and α-PLIs do not require Ca^{2+} to bind $sPLA_2$s [12, 42], while SP-A does require Ca^{2+}, this indicates that CRDs bind $sPLA_2$s in different ways. To date, Ca^{2+}-dependent binding of $sPLA_2$s has been reported only in a case of a single

CRD-containing $sPLA_2$-BP, SP-A, while Ca^{2+}-independent binding of $sPLA_2$s has been shown for $sPLA_2$-BPs with multiple CRDs, where more than one CRD constitutes the $sPLA_2$-binding site. For M-type $sPLA_2R$, three consecutive CRD repeats (repeats 4, 5, 6) were shown to influence $sPLA_2$ binding [10], and consistently, in α-PLIs, the trimeric CRD proteins, the central part, where three CRDs are associated together, has been suggested to provide the $sPLA_2$-binding site [43].

Table 1. Some characteristics of $sPLA_2$-BPs.

$sPLA_2$-BP	Location	Defined structural element	Interacting part of $sPLA_2$	Binding affinity	Effect of interaction
M-type $sPLA_2Rs$ [10]	Plasma membrane, extracellular	CRD-like fold	Ca^{2+}-binding loop	38 pM (OS_1) [11]	Inhibition, clearance, translocation of $sPLA_2$
α-PLIs [12]	Blood		n.d.	n.d.	Inhibition of $sPLA_2$
SP-A [13]	Extracellular		n.d.	n.d.	Inhibition of $sPLA_2$
β-PLIs [14]	Blood	Leucine-rich repeat	N-terminal region, β-wing	n.d.	Inhibition of $sPLA_2$
γ-PLIs [15]	Blood	Three-finger motif	N-terminal region, β-wing	n.d.	Inhibition of $sPLA_2$
CaM [16]	Cytosol	EF-hand Ca^{2+}-binding motif	C-terminal region, α-helices C, E	3.3 nM (Atx) [17]	Stabilization of $sPLA_2$, augmentation of $sPLA_2$ enzymatic activity
TCBP-49 [18]	Endoplasmic reticulum		n.d.	n.d.	Translocation of $sPLA_2$ (proposed)
Crocalbin [19]	Endoplasmic reticulum		n.d.	n.d.	Translocation of $sPLA_2$ (proposed)
PDI [20]	Endoplasmic reticulum	Thioredoxin-like fold	IBS	1.27 μM (Atx) [21]	Translocation of $sPLA_2$ (proposed)
VEGFR-1/Flt-1 [22]	Plasma membrane	Ig-like domain	C-terminal region	74 nM (Lys49 GIIA) [23]	Competitive inhibition of VEGFR
VEGFR-2/KDR [22]	Plasma membrane			10 nM (Lys49 GIIA) [23]	
DM64 [24]	Blood		n.d.	n.d.	Neutralization of $sPLA_2$
HSPGs [25]	Plasma membrane	Negatively charged carbohydrate moiety	Clusters of basic amino acids at C- and N-terminal regions	n.d.	Clearance and translocation of $sPLA_2$
Vimentin [26]	Cytosol, plasma membrane	Rod domain	IBS	n.d.	Internalization and translocation of $sPLA_2$
Nucleolin [27]	Nucleolus, cytoplasm, plasma membrane	RNA recognition motif	n.d.	n.d.	Internalization and translocation of $sPLA_2$
NP1, NP2, NPR [28]	Extracellular	Pentraxin domain	n.d.	n.d.	Translocation of $sPLA_2$ (proposed)
14-3-3γ/ε [29]	Cytosol	14-3-3 domain	C-terminal region	1 μM (Atx) [29]	Positioning of $sPLA_2$ on plasma membrane
L-type voltage-dependent Ca^{2+} channel [30]	Plasma membrane	α domain	n.d.	n.d.	Activation of L-type voltage-dependent Ca^{2+} channel (proposed)
nAChR [31]	Plasma membrane	-	n.d.	120 nM (crotoxin) [31]	Negative allosteric modulation of nAChR
GLIC [32]	Plasma membrane	ECD	IBS	125 nM (CB_c) [32]	Negative allosteric modulation of GLIC
CFTR/ ΔF508CFTR [33]	Plasma membrane	NBD1	IBS, Ca^{2+}-binding loop, C-terminal region	4 nM (CB_c) [33]	Potentiation and correction of ΔF508CFTR
EGFR [34]	Plasma membrane	L domain	n.d.	n.d.	Activation of EGFR
PAR-1 [35]	Plasma membrane	-	n.d.	n.d.	Activation of PAR-1
Integrins [36]	Plasma membrane	-	C-terminal region, α-helices D, E	200 nM (hGIIA) [36]	Induction of integrin-mediated signalling
CCOX-II [37]	Inner mitochondrial membrane	-	C-terminal region	15 nM (Atx) [38]	Inhibition of CCOX (proposed)
C1 inhibitor protein [39]	Extracellular	-	n.d.	n.d.	Impairment of C1 inhibitor protein activity (proposed)
FX, FXa, FIIa (thrombin) [40]	Blood	EGF-like domain of light chain, interface regions I-V, exosite of heavy chain	Basic amino acids in C-terminal region, IBS, loop preceding β-wing	0.6 nM (CB_c) [40]	Noncompetitive inhibition of FX, FXa, FIIa (thrombin)

$sPLA_2$-BP, $sPLA_2$-binding protein; M-type, muscle-type; CRD, carbohydrate recognition domain; OS_1, $sPLA_2$ from *Oxyuranus s. scutellatus* venom; PLIs, $sPLA_2$ inhibitors; n.d., not defined; SP-A, pulmonary surfactant protein A; CaM, calmodulin; Atx, $sPLA_2$ from *Vipera a. ammodytes* venom; TCBP-49, taipoxin-associated 49-kDa Ca^{2+}-binding protein; PDI, protein disulphide isomerase; VEGFR, vascular endothelial growth factor receptor; DM64, metalloproteinase inhibitor from *Didelphis marsupialis* blood; HSPGs, heparan sulphate proteoglycans; IBS, interfacial binding surface; NP, neuronal pentraxin; nAChR, nicotinic acetylcholine receptor; GLIC, proton-gated ion channel from *Gloeobacter violaceus*; ECD, extracellular domain; CB, basic $sPLA_2$ subunit of crotoxin; CFTR, cystic fibrosis transmembrane conductance regulator; NBD; nucleotide-binding domain; EGF(R), epidermal growth factor (receptor); PAR, protease-activated receptor; hGIIA; human group IIA $sPLA_2$; CCOX, cytochrome c oxidase; FX/FXa/FIIa, blood coagulation factors.

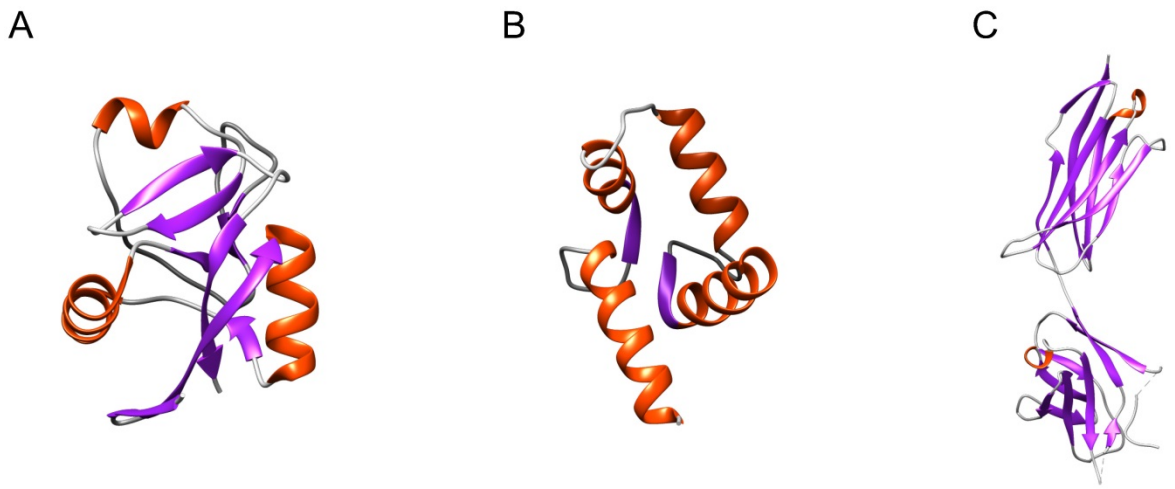

Figure 1. Distinct structural elements present in more than one type of sPLA$_2$-BP. (**A**) CRD (PDB ID: 6JLI) and the CRD-like fold are structural elements found in M-type sPLA$_2$Rs, SP-A and α-PLIs. (**B**) The EF-hand Ca^{2+}-binding motif (PDB ID: 1CLL) is found in CaM, crocalbin and TCBP-49, which all bind sPLA$_2$s. (**C**) The Ig-like domain (PDB ID: 2X1X) is found in VEGFRs and DM64. To date, only the CRD has been experimentally demonstrated to be a sPLA$_2$-binding structure. Red, α-helices; violet, β-sheets; grey, loops. The Figure was prepared using UCSF Chimera v1.15.

There are plenty of other CRD-containing proteins, such as the mannose receptor, Endo180, DEC205 and FcRY. At present, only the human mannose receptor has been demonstrated to interact with bee venom GIII sPLA$_2$ [44], while FcRY did not bind any of the sPLA$_2$s tested [45].

To recognize sPLA$_2$-binding attributes of sPLA$_2$-BPs it is important to also consider the characteristics of the sites on sPLA$_2$s that interact with sPLA$_2$-BPs, as these are complementary to the sPLA$_2$-binding sites on sPLA$_2$-BPs. It has to be borne in mind, however, that a single sPLA$_2$ molecule might harbour multiple protein-binding sites [46], and therefore it is reasonable to consider separately binding sites directed towards particular types of sPLA$_2$-BPs, or those sPLA$_2$s that induce the same interaction-dependent physiological effects.

The interaction of an sPLA$_2$ with a CRD fold is primarily governed by the structure of the interfacial binding surface (IBS) of the sPLA$_2$ [47]. The IBS is defined as the molecular surface with which sPLA$_2$ contacts a phospholipid membrane – its substrate. The IBS is formed by a collar of hydrophobic amino acids around the entrance to the catalytic site of the enzyme, and by mainly positively charged amino acids in more remote positions. The amino acids within or close to the Ca^{2+}-binding loop in sPLA$_2$s were also shown to be involved in the interactions between sPLA$_2$s and CRDs [42]; however, not profoundly, consistent with the evidence that the Ca^{2+}-binding loop is highly conserved among sPLA$_2$s that show considerably different affinities for M-type sPLA$_2$R.

sPLA$_2$-BPs that contain Ig-like domains, such as VEGFRs and DM64, have been shown to associate with catalytically inactive myotoxic sPLA$_2$s. VEGFRs are receptor tyrosine kinases, and they interact with sPLA$_2$s through their extracellular region, which is composed of seven Ig-like domains. Although the interaction of myotoxic sPLA$_2$s and VEGFRs has not been functionally linked with myotoxic effects yet, the C-terminal region of these sPLA$_2$s (known as the 'myotoxic site') was shown to also be responsible for binding to VEGFRs [22, 23]. The myotoxic site of the snake venom myotoxic sPLA$_2$s has been attributed to amino acids 115–129, which form a single α-helical turn at the C-terminus [48-50]. This C-terminal region is firmly attached to the body of the protein by two disulphide bonds, Cys27–Cys126 and Cys50–Cys133, thus constraining its position and orientation. The myotoxicity of sPLA$_2$s has been associated with a cluster of positively charged amino acids in this region (i.e., conserved Lys115 and Arg118, and possibly also Lys122 and Lys127), and with hydrophobic amino acids at positions 121 and 125 [51, 52].

The sPLA$_2$-BP DM64 was originally isolated from marsupial (*Didelphis marsupialis*) blood, and it was shown to inhibit the myotoxic activity of several sPLA$_2$s [24]. DM64 contains five Ig-like domains. Its myotoxicity-inhibitory activity is most likely due to its binding to the myotoxic site of sPLA$_2$s, to thus obstruct their association with the 'myotoxicity receptor', which would be VEGFR. Contrary to what is seen for CRD in M-type sPLA$_2$R, binding of sPLA$_2$s to DM64 does not inhibit their enzymatic activity. This is in agreement with the involvement of the C-terminal of these sPLA$_2$s in the interaction with this Ig-like domain-containing sPLA$_2$-BP, rather than the IBS.

Identification of the PDZ-binding domain at the C-terminus of some myotoxic sPLA$_2$s [53] suggested that there is another group of sPLA$_2$-BPs, as

PDZ-domain-containing proteins. In muscle cells, these proteins include, e.g., LDB3 and α-1-syntrophin [54, 55]; however, their potential $sPLA_2$ binding remains to be tested.

The third type of well-defined structural element present in more than one $sPLA_2$-BP is the EF-hand Ca^{2+}-binding motif. These $sPLA_2$-BPs include CaM, crocalbin and TCBP-49, although the structural aspects of $sPLA_2$ binding have been studied only for CaM. A three-dimensional model of a complex between CaM and the snake venom GIIA $sPLA_2$ ammodytoxin (Atx) [16] revealed that CaM 'clamps' Atx between its N-terminal and C-terminal domains (Figure 2A). Atx contacts CaM most extensively through a distinct patch of hydrophobic and charged amino acids at its C-terminus, which interact with the central part of CaM, and through the α-helices C and E, which contact the C-terminal globular domain of CaM. In the complex, Atx is oriented in such a way that the entrance to its enzymatic pocket remains open wide. In the complex, a new larger membrane-contacting area (IBS) is formed; this comprises parts of both Atx and CaM, and explains the increased enzymatic activity of complexed Atx.

Considerable structural insight has also been gained for the interactions between the anticoagulant GIIA $sPLA_2$s Atx, CB (basic $sPLA_2$ subunit of crotoxin, from venom of the South American rattlesnake [*Crotalus durissus terrificus*]) and daboxin P (from venom of the Indian viper [*Daboia r. russelii*]), and human activated blood coagulation factor X (FXa) [40, 56, 57, 58]. The interaction area between the

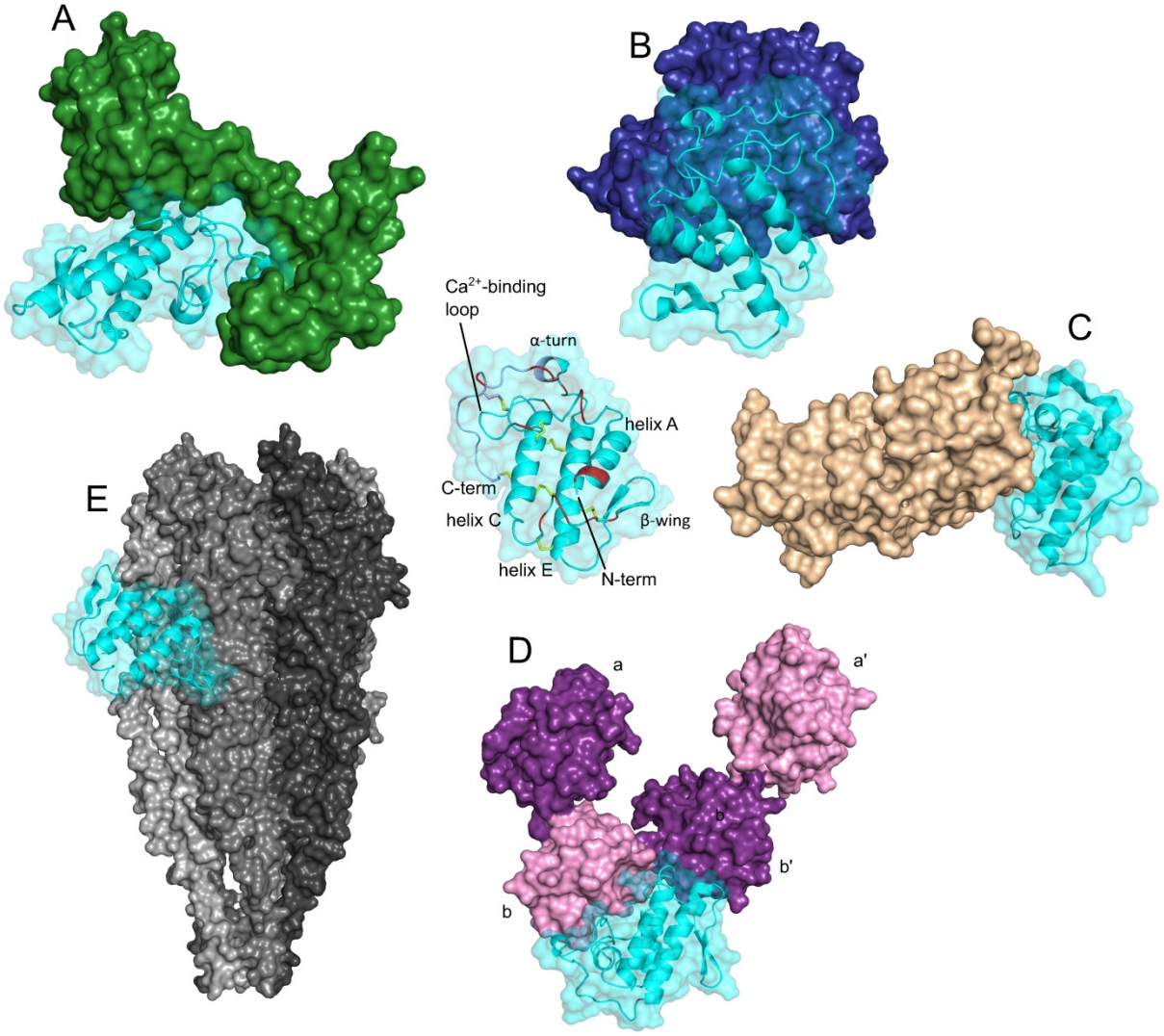

Figure 2. Secreted PLA_2s are ligands of proteins that are structurally very diverse. The three-dimensional models, generated by molecular docking, are showing complexes between the $sPLA_2$ Atx and CaM (green, PDB ID: 1CLL) (**A**), Atx and FXa (blue, PDB ID: 2BOH) (**B**), CB_b and ΔF508NBD1 of CFTR (PDB ID: 1XMJ) (**C**), Atx and PDI (purple/pink, PDB ID: 4EL1) (**D**), and between the $sPLA_2$ vurtoxin and nAChR (grey, PDB ID: 2BG9) (**E**). Centre: The $sPLA_2$ Atx, showing its main structural elements. Red, interfacial binding surface; yellow, disulphide bonds; violet, C-terminal region. Note that the $sPLA_2$s interact with these different $sPLA_2$-BPs in very different ways; i.e., they have multiple protein binding sites, as is characteristic of promiscuous proteins. The Figure was prepared by adaptation of Figures from Kovačič et al. (2010) (**A**), Faure and Saul (2011) (**B**), Faure et al. (2016) (**C**), Oberčkal et al. (2015) (**D**) and Vulfius et al. (2014) (**E**), using PyMOL.

anticoagulant $sPLA_2$s and FXa is extensive, and encompasses the heavy and light chains of FXa. As evident from the model of the complex between Atx and FXa shown in Figure 2B, Atx interacts with the FXa heavy chain through its Ca^{2+}-binding loop, α-helix C, β-wing, and C-terminal region, and with the FXa light chain through its β-wing and α-helices A and B. Electrostatic interactions importantly participate in the efficient binding of $sPLA_2$s to FXa, including in particular some positively charged amino acids of Atx (e.g., Arg72, Lys74, His76, Arg77 in the β-wing; Arg118, Lys128, Lys127, Lys132 in the C-terminal region). However, not all of the basic $sPLA_2$s are strong anticoagulants, which are consistent with the importance also of certain hydrophobic and aromatic $sPLA_2$ amino acids for optimal interactions with FXa (e.g., Atx: Phe124 in the C-terminal region; CB_{a2}: Trp70 in the 65–72 loop).

Molecular docking allowed to propose a model of the complex between ΔF508-NBD1 (nucleotide-binding domain 1), the $sPLA_2$-binding domain of ΔF508CFTR (Phe508 deletion mutant of cystic fibrosis transmembrane conductance regulator), and CB_b (Figure 2C) [33]. From the model, which applies also to the wild type NBD1 (i.e. CFTR), it can be seen that the binding interface of CB with ΔF508NBD1/NBD1 is predominately composed of the hydrophobic residues located in its α-helices A and B, Ca^{2+}-binding loop and the C-terminal region. Hydrophobic interactions are clearly very important for holding both proteins together, nevertheless, also some polar amino acid residues are involved in the interaction. Forming ionic contacts with ΔF508NBD1/NBD1 of ΔF508CFTR/CFTR, the N-terminal His1 and the Asp112 in the C-terminal region of CB are two examples.

A three-dimensional model of the complex between Atx and PDI has also been reported [20]. PDI consists of multiple domains (i.e., a, a', b, b'), and according to the model shown in Figure 2D, the Atx-binding site on PDI is situated between domains b and b'. Atx interacts with PDI across an extensive area that also includes the Atx IBS. The basic amino acids are important for Atx binding to PDI, especially Arg77 and Arg118, but also Lys69, Arg72, Lys74 and Lys86. However, Atx also contacts PDI with some hydrophobic amino acids, Leu3, Leu19 and Phe24, and through two polar amino acids, Asn17 and Asn119.

Molecular docking has also provided some structural insights into the interactions between vurtoxin, another snake venom GIIA $sPLA_2$, and the nicotinic acetylcholine receptor (nAChR) [59]. According to their model, vurtoxin binds at the interface of the α and γ subunits of nAChR, and is oriented with its active site towards the lipid bilayer (Figure 2E). Vurtoxin does not occupy the nAChR binding sites for its classical agonists and competitive antagonists; however, it is located very close to these.

Several structural features allow the $sPLA_2$s to bind to structurally very diverse targets (Table 1; Figure 2), which is characteristic of promiscuous proteins [60, 61]. Despite the generally compact structure of $sPLA_2$s, the flexibility of the exposed side chains of the amino acids at the IBS promotes their optimal binding to phospholipid aggregates, as their substrates, for efficient hydrolysis [62]. As indicated above, the IBS is critically involved also in the interactions of $sPLA_2$s with the CRD-containing $sPLA_2$-BPs and FXa, as also for PDI [20] and vimentin [26]. The association of the $sPLA_2$ IBS with a variety of different protein surfaces is most likely driven by the same principles as the association of the $sPLA_2$ IBS with phospholipid membranes. The flexibility of the $sPLA_2$ β-wing might be another feature that favours their high adaptability for different protein partners. For example, the β-wing of $sPLA_2$s should be involved in the interactions of $sPLA_2$s with β-PLIs and γ-PLIs [63]. $sPLA_2$s are also characterized by patches of basic and hydrophobic amino acids that might also greatly broaden the spectrum of their protein interaction partners. We have already shown that such patches are part of the IBS, but they are also located in other parts of $sPLA_2$s. Indeed, those at the C-terminus [62] are implicated in the binding of $sPLA_2$s to CaM and VEGFR.

However, only the atomic structure of an $sPLA_2$–$sPLA_2$-BP complex will fully disclose the structural requirements for particular association. The present-day rapid advances in techniques for the determination of the three-dimensional structures of proteins might enable this in the near future; e.g., cryo-electron microscopy.

Pathophysiological significance of $sPLA_2$ binding to $sPLA_2$-BPs

The structural diversity of $sPLA_2$-BPs is consistent with the wide range of pathophysiological activities that have been associated with $sPLA_2$s. By binding to $sPLA_2$-BPs, $sPLA_2$s have been suggested to be involved in inflammation [64, 65], hormone release [66, 67], neurotoxicity [20, 37, 68, 69] and myotoxicity [22]. It has already been confirmed that $sPLA_2$s are ligands for specific $sPLA_2$-BPs in cytokine production [70], cell proliferation [71-73], cell migration [74], lipid mediator production [75], antibacterial activity [73] and blood coagulation [40]. These (patho)physiological effects of $sPLA_2$s might arise through: (1) specific cellular translocation and associated actions of $sPLA_2$s after their binding to $sPLA_2$-BPs; (2)

triggering of specific signalling following their binding to sPLA$_2$-BPs; and/or (3) regulation of sPLA$_2$ enzymatic activity by sPLA$_2$-BPs (Figure 3). Particular mechanistic possibilities for sPLA$_2$ actions as ligands for sPLA$_2$-BPs are exemplified and discussed below.

Binding of sPLA$_2$s to some sPLA$_2$-BPs does not have any obvious physiological function. Some functional implications of sPLA$_2$s acting as ligands certainly remain to be discovered, while others might be latent. When environmental conditions change radically, these interactions might become functional. Promiscuous proteins, which include sPLA$_2$s, can acquire new functions much more readily than non-promiscuous proteins, which can provide swifter adaptation of the organism to new situations [76, 77].

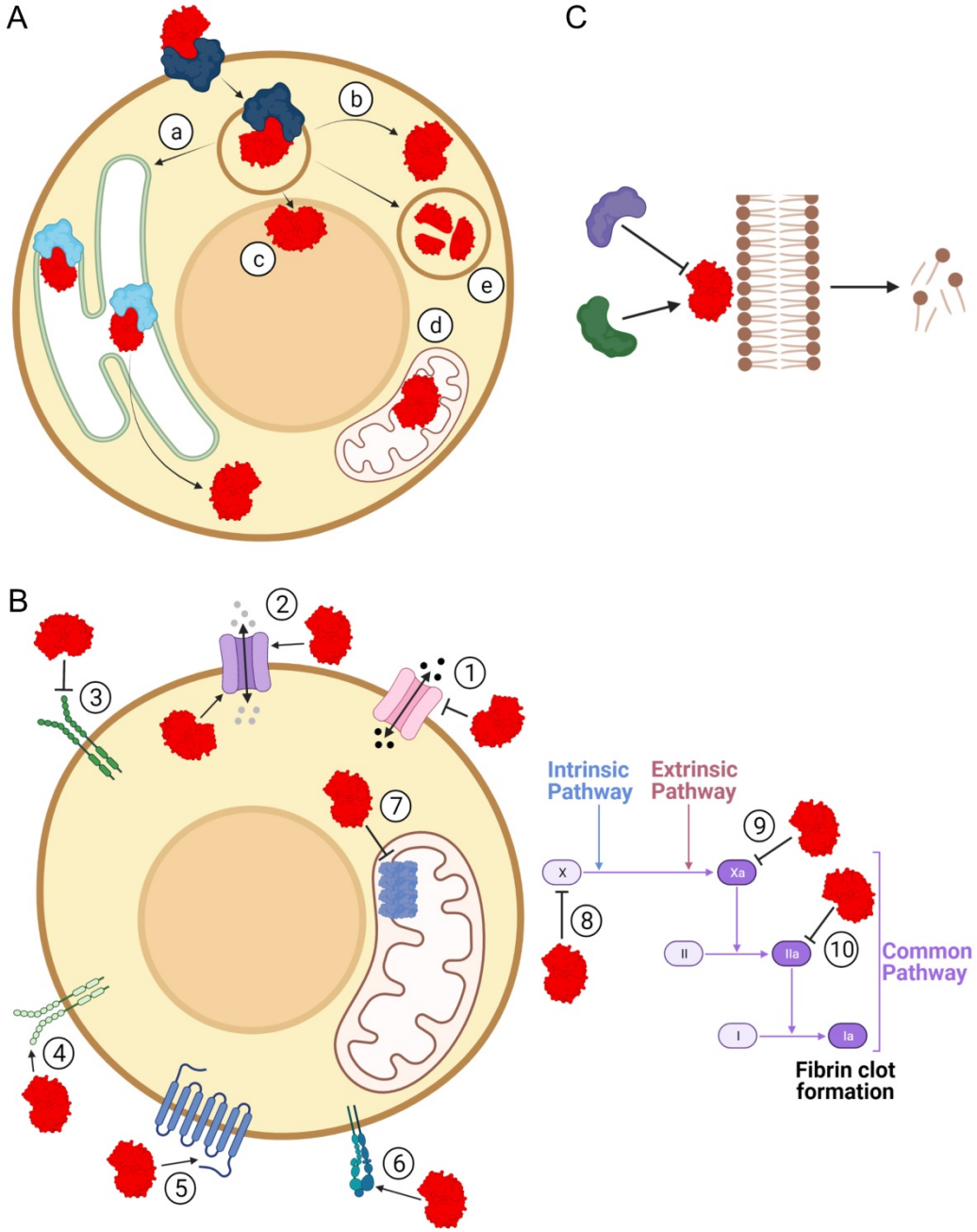

Figure 3. Pathophysiological implications of binding of sPLA$_2$s to sPLA$_2$-BPs. Physiological and/or pathological effects of sPLA$_2$s (red) are also the consequence of their binding to sPLA$_2$-BPs. (**A**) By binding to sPLA$_2$-BPs (blue/cyan), sPLA$_2$s can be translocated to specific intracellular compartments, such as the endoplasmic reticulum (a), cytosol (b), nucleus (c), mitochondria (d) or lysosomes (e). In each of these compartments, they can act as enzymes or ligands for receptors, or they can undergo proteolytic degradation in the lysosome. (**B**) As ligands for receptors, sPLA$_2$s have been specifically implicated in molecular signalling through decreased (1) or increased (2) permeabilities of certain ion channels, inhibition (3) or activation (4) of activities of receptor tyrosine kinases, modulation of activities of GPCR (5), interference in integrin-mediated functions (6), attenuation of ATP production (7) and inhibition of blood coagulation, at different stages (8, 9, 10). (**C**) Binding of sPLA$_2$ to a sPLA$_2$-BP might inhibit or potentiate phospholipolytic activity. The Figure was created with BioRender.com.

Cellular transport of sPLA$_2$s after binding to sPLA$_2$-BPs

Although sPLA$_2$s appear to be secreted into the extracellular space after being synthesized inside the cell, convincing evidence of their intracellular localization and activities has already been provided [69, 71, 78, 79]. sPLA$_2$s can re-enter cells after their synthesis and secretion, to pass into the cytosol and to different organelles, such as the nucleus and mitochondria [80]. Indeed, several sPLA$_2$-BPs have been suggested to assist sPLA$_2$s in their retrograde cellular transport and intracellular translocation (Figure 3A).

The sPLA$_2$-BP in the lumen of the ER, PDI, has been proposed to be involved in such retro-transport of the Atx snake venom GIIA sPLA$_2$, which is a neurotoxin from nose-horned viper venom that acts presynaptically [20]. Molecular docking and heterologous competition assays have suggested that PDI acts in a similar way as on Atx also on some mammalian sPLA$_2$s, such as GIB, GIIA and GV sPLA$_2$s.

Two other candidates for the same function are the ER luminal proteins TCBP-49 and crocalbin, which were discovered through their binding to the hetero-oligomeric snake venom sPLA$_2$s taipoxin and crotoxin [9]. As for PDI, these two EF-hand Ca^{2+}-binding proteins have the characteristic ER-retention motif at their C-termini, which has been suggested to be essential for retention and concentration of sPLA$_2$s in the ER before they are translocated into the cytosol. Some other EF-hand Ca^{2+}-binding proteins might also interact with sPLA$_2$s. For instance, the Miro proteins are mitochondrial adaptor proteins that promote the transportation of mitochondria by connecting them to motor proteins [81]. Binding of sPLA$_2$s to the Miro proteins might concentrate the sPLA$_2$s on the outer mitochondrial membrane, which would explain their colocalization with mitochondria after entering cells [78, 79].

Cellular retro-transport of sPLA$_2$s might also be associated with two recently discovered sPLA$_2$-BPs, vimentin and nucleolin. Vimentin belongs to the family of intermediate filaments, although it is also present on the cell surface and in extracellular fluids. Indeed, in recent years, vimentin has been shown to have a much wider role in cell physiology, rather than just being an inert scaffold protein [82]. Vimentin was identified as the receptor for GIIA sPLA$_2$ on the surface of apoptotic human T lymphocytes [65]. Interactions between these two proteins was also shown in rheumatoid fibroblast-like synoviocytes, which associated rapid internalization of sPLA$_2$ with arachidonic acid metabolism in synovial inflammation [83]. Recently, vimentin was reported to bind an acidic sPLA$_2$ (NnPLA$_2$-I) from venom of the Indian cobra (*Naja naja*) [26]. Binding of NnPLA$_2$-I to vimentin resulted in its internalization into partially differentiated myoblasts. The involvement of vimentin in cellular uptake mechanisms has already been shown for C3, a *Clostridium botulinum* toxin [84, 85], and several viruses, such as human immunodeficiency virus type 1, vaccinia virus and the severe acute respiratory syndrome coronavirus [86]. In all of these cases, vimentin acts as a component of the cellular attachment mechanism, either as a receptor or a co-receptor. In a similar way, vimentin might mediate internalization of sPLA$_2$s. This is further supported by the similar location of the binding sites on vimentin for dengue virus DENV-2 envelope protein domain III, *Clostridium botulinum* C3 exoenzyme and NnPLA$_2$-I, all of which have been proposed to bind to the rod domain of vimentin [26, 84, 87].

Nucleolin was described recently as an sPLA$_2$-BP due to its binding to the myotoxin MT-II, a Lys49 sPLA$_2$ from venom of a pit viper, the terciopelo (*Bothrops asper*) [27]. It appears most likely that nucleolin participates in the toxic mechanism of MT-II through promotion of its translocation from the outside into the perinuclear and nuclear areas of myotubes and macrophages. Nucleolin was previously reported to mediate the internalization of several other molecules from the cell surface to the nucleus through an active Ca^{2+}-dependent transport mechanism [88], which might also be the mechanism for sPLA$_2$ internalization. It would be interesting to investigate the potential involvement of nucleolin in GV sPLA$_2$ translocation to the nucleus, where this sPLA$_2$ has been shown to act on the nuclear envelope [89]. Nucleolin has been shown to act as a chaperone for histones and TDP-43 [90, 91], so it might also stabilize sPLA$_2$s in the reducing environment of the cytosol.

Neuronal pentraxins (i.e., NP1, NP2/Narp) are sPLA$_2$-BPs that are homologous to acute phase proteins, and these might also function in retrograde transport of sPLA$_2$s from the cell surface [28, 92]. An sPLA$_2$-shuttling function has also been suggested for the ubiquitously expressed eukaryotic cytosolic 14-3-3 proteins, which were discovered through their interactions with Atx [29]. The neurotoxicity of Atx also involves its binding to the 14-3-3γ and 14-3-3ε isoforms, to correctly position it at the plasma membrane to hinder the function of amphiphysin, and thus of vesicle endocytosis [69].

HSPGs such as the GPI-anchored glypican I, biglycan, syndecan and perlecan, can bind

mammalian $sPLA_2$s, e.g. GIIA, GIID and GV $sPLA_2$s. Using transfected human embryonic kidney 293 cells, it was shown that a GPI-anchored HSPG facilitated the shuttling of GIIA $sPLA_2$ into certain subcellular compartments where the $sPLA_2$ then released arachidonic acid for prostaglandin synthesis [93]. Binding of human GIIA $sPLA_2$ to HSPGs was further demonstrated in human primary T-lymphocytes [25]. Recently, it was suggested that GIIA $sPLA_2$ can increase endothelial cell permeability after binding to HSPGs on the surface of these cells, in a process that is dependent on the $sPLA_2$ phospholipase activity [39]. In a similar way, HSPGs might be receptors for heparin-binding snake venom $sPLA_2$s. By binding to HSPGs, these toxins might enhance inflammation and permeability of the endothelium, to allow the venom to spread more efficiently in the tissue.

M-type $sPLA_2R$ can rapidly internalize $sPLA_2$s and direct them to the lysosomes, where they are then degraded [94, 95]. In this way, M-type $sPLA_2R$ can down-regulate the activity of $sPLA_2$s. M-type $sPLA_2R$ can also mediate $sPLA_2$ signalling by transporting $sPLA_2$s into specific intracellular compartments [96, 97]. Recent structural studies of the human M-type $sPLA_2R$ ectodomain using cryo-electron microscopy showed pH-dependent conformational changes that might have important roles in the control of the functional properties of this receptor, including its $sPLA_2$ binding [41].

Signalling triggered by $sPLA_2$s as ligands

As proteins that are secreted into the extracellular space, $sPLA_2$s encounter various plasma-membrane receptors on target cells. By binding to some of these receptors, $sPLA_2$s can induce intracellular responses that are associated with different physiological processes, such as vascular permeability, cell growth, migration and senescence, hormone release, cytokine and NO production, inflammation, cell adhesion and angiogenicity (Figure 3B).

In this context, $sPLA_2$s have been reported to interact with different ion channels (K^+, Na^+ or Ca^{2+}). β-Butx is a neurotoxic $sPLA_2$ from venom of the many-banded krait (*Bungarus multicinctus*), and it has been shown to interact with voltage-dependent K^+ channels. Further, MitTx from venom of the Texas coral snake (*Micrurus t. tener*) was shown to bind to voltage-insensitive Na^+-conducting acid-sensing ion channels [98]. However, both of these toxins are heterodimers that are composed of the $sPLA_2$ subunit and a subunit homologous to the Kunitz-type serine protease inhibitor and, with the ion channel binding attributed to the latter. Therefore, these ion channels are not $sPLA_2$-BPs. Nonetheless, the neurotoxicity with β-Butx and the pain with MitTx did not occur in the absence of the $sPLA_2$ subunit. Some $sPLA_2$s can influence the conductance of Ca^{2+} channels, the N-methyl-D-aspartate receptor or L-type voltage-dependent Ca^{2+} channels. Direct binding of $sPLA_2$s to these channels has, however, not been demonstrated to date [30, 99].

Recently, pentameric ligand-gated ion channels, nAChRs [31, 59] and their bacterial homologue GLIC from the cyanobacterium *Gloeobacter violaceus* [32], were reported to be $sPLA_2$-BPs. By binding to nAChRs, $sPLA_2$s might affect nAChR-related functions, such as neuromuscular transmission and cell proliferation. Interestingly, nAChRs have also been described for the outer mitochondrial membrane, and were associated with regulation of the formation of mitochondrial permeability transition pores, which release pro-apoptotic substances like cytochrome c and reactive oxygen species [100]. Antagonists of nAChR were shown to attenuate the release of cytochrome c. The antagonistic effect of certain $sPLA_2$s on some nAChRs subtypes might therefore link these $sPLA_2$s to control of cellular viability through these nAChRs [31, 101].

CFTR is a Cl^- channel that was defined as an $sPLA_2$-BP using CB, the basic $sPLA_2$ subunit of crotoxin [33]. CB was shown to bind and allosterically potentiate the activity of CFTR. Of a very high medical significance, CB was found to bind with nanomolar affinity also to ΔF508CFTR mutant, the causative factor of cystic fibrosis, thus augmenting its activity. Importantly, by binding to ΔF508CFTR, CB acts also as a corrector, facilitating trafficking and delivery of the abnormal protein to the plasma membrane.

VEGFRs are receptor tyrosine kinases that can interact with some myotoxic snake venom $sPLA_2$s. Although some of these toxins are potent antagonists of VEGFRs, it is still not clear whether their binding to VEGFRs is directly involved in their $sPLA_2$ myotoxicity [22, 23]. Nevertheless, the interactions between snake venom $sPLA_2$s and VEGFRs might be important to enhance vascular permeability, to facilitate penetration of the snake venoms into tissues.

Epidermal growth factor receptor (EGFR) is another receptor tyrosine kinase that interacts with $sPLA_2$s. It was reported to interact with human GIIA $sPLA_2$ [34]. However, in this case, the $sPLA_2$ acted as an agonist, as it up-regulated HER (human EGFR)/HER2-elicited signalling in lung cancer, thus stimulating cancer cell growth.

M-type $sPLA_2R$ has already been mentioned as an $sPLA_2$-translocator (see section 4), and it can also act as a signalling receptor to transduce $sPLA_2$-dependent signals independent of $sPLA_2$

catalytic activity. Through M-type $sPLA_2R$, $sPLA_2s$ have been implicated in cell proliferation, migration and senescence, and in hormone release and cytokine and NO production. It was also suggested that human GIB $sPLA_2$ can induce kidney glomerular podocyte apoptosis via M-type $sPLA_2R$ [102].

PA2-Vb is an acidic $sPLA_2$ from venom of the Chinese green tree viper (*Trimeresurus stejnegeri*), and it was shown to induce mouse aorta contraction independent of its enzymatic activity, by acting on a protease-activated receptor (PAR-1), which is a GPCR [35]. GPCRs are the largest and most diverse class of membrane receptors in eukaryotes, and their primary function is to transduce extracellular stimuli into intracellular signals, which lead to different cell responses.

Integrins are extracellular plasma membrane (transmembrane) proteins that are responsible for cell adhesion to the extracellular matrix. They are dimers, as combinations of one of 18 α-subunits and one of 8 β-subunits. Integrins $\alpha_v\beta_3$ and $\alpha_4\beta_1$ have been shown to bind mammalian GIIA $sPLA_2$, while an acidic GIIA $sPLA_2$ from venom of the viper *Macrovipera lebetina transmediterranea* was shown to interact with integrins $\alpha_5\beta_1$, $\alpha_v\beta_3$ and $\alpha_v\beta_6$, to induce inflammation and inhibition of cell adhesion and migration and angiogenicity [36, 103].

CCOX is an essential constituent of the respiratory chain complex, and was characterized as an $sPLA_2$-BP due to its binding to Atx [37]. Through binding to subunit II, Atx was shown to inhibit the oxidase activity of CCOX, independent of its phospholipase activity. This might explain the inhibition of ATP production in nerve endings poisoned by the snake venom neurotoxic $sPLA_2s$ [104]. These findings also provide novel indications for the potential functions and malfunctions of the orthologous mammalian GIIA $sPLA_2$ in mitochondria.

The blood coagulation system consists of a strictly regulated proteolytic cascade of blood coagulation factors that convey signals downstream to fibrinogen, which is then transformed into the insoluble fibrin network, and to activate platelets. Ultimately, a blood clot is formed to prevent blood loss from the injured blood vessel [105]. Several $sPLA_2s$ can affect this process by binding to one of these coagulation factors, to thus inhibit their functions (Figure 3B). Some snake venom $sPLA_2s$ bind to FX, or to its activated form FXa, or to thrombin (FIIa); e.g., crotoxin and Atx can induce anticoagulant effects through binding to FXa, to thus prevent formation of the prothrombinase complex [40, 106]. Human GIIA $sPLA_2$ acts on blood coagulation in exactly the same way [107]. Daboxin is an anticoagulant $sPLA_2$ that can bind to both FXa and FX [57]. On the other hand, Nk-$PLA_2\alpha$ and Nk-$PLA_2\beta$ from venom of the monocled cobra (*Naja kaouthia*) act as anticoagulants through their binding to thrombin and their consequent inhibition of its proteolytic activity [108].

Regulation of $sPLA_2$ enzymatic activity by $sPLA_2$-BPs

$sPLA_2s$ participate in many important physiological processes through their enzymatic activity [2]. These range from those dependent on the composition and flexibility of biological membranes, to those regulated by the products of the $sPLA_2$ phospholipolytic activity, which include lysophospholipids, free fatty acids and their metabolites – the whole range of signalling hormones [109,110]. Regulation of the enzymatic activity of $sPLA_2s$ is thus extremely important for the correct functioning of an organism, and this regulation is also mediated through the binding of $sPLA_2s$ to particular $sPLA_2$-BPs (Figure 3C).

M-type $sPLA_2R$ has been shown to regulate the activity of $sPLA_2s$ in two ways: through acting as an $sPLA_2$ inhibitor; and through mediating $sPLA_2$ endocytosis, which leads to $sPLA_2$ degradation in lysosomes [111]. Soluble form of M-type $sPLA_2R$ that is detected in the blood can function only in the first way [8]. Pulmonary SP-A is structurally and functionally similar to the soluble form of M-type $sPLA_2R$. SP-A belongs to the CRD-containing family of proteins, and it inhibits the enzymatic activity of GIIA and GX $sPLA_2s$ in pulmonary surfactant [9].

Soluble proteins that inhibit $sPLA_2s$ have been isolated from the blood of snakes and some other animals. These are ecologically connected with venomous snakes, and are known as the α-PLIs, β-PLIs and γ-PLIs [9].

$sPLA_2$ inhibitors have also been found in plants. *Withania somnifera* glycoprotein (WSG) is an acidic glycoprotein that is similar to the α-chain of the γ-PLIs, and it was isolated from the medicinal plant known variously as Ashwagandha, Indian ginseng and winter cherry. WSG inhibits the enzymatic activity and toxicity of NN-XIa-PLA_2, an $sPLA_2$ myotoxin from venom of the Indian cobra [112-114].

Interestingly, $sPLA_2$-BPs have also been shown to increase the enzymatic activity of $sPLA_2s$. One such $sPLA_2$-BP is the EF-hand Ca^{2+}-binding intracellular protein CaM. When $sPLA_2s$ are in a complex with CaM, they also become more resistant to chemical denaturation [16, 17]. Two other $sPLA_2$-BPs that can potentiate the catalytic activity of $sPLA_2s$ are vimentin [65] and GLIC [32]. The potentiating effect appears to occur because the interactions with these three $sPLA_2$-BPs position the complexed $sPLA_2$ on the

membrane in a way that provides more efficient catalytic function.

Medical potential of sPLA$_2$s as ligands for receptors

sPLA$_2$-BPs have diverse physiological functions, and therefore their interactions with sPLA$_2$s can be insightful and potentially helpful for medical applications. Important roles of M-type sPLA$_2$R in cancers have been outlined recently, with a tumour suppressive role demonstrated [74, 115]. As ligands of M-type sPLA$_2$R, sPLA$_2$s can be used to study or to regulate processes in which M-type sPLA$_2$R is involved. By binding to M-type sPLA$_2$R, sPLA$_2$s have already been indicated to have roles in cell proliferation, migration and senescence, hormone release, and cytokine and NO production. sPLA$_2$s were also demonstrated to induce kidney glomerular podocyte apoptosis via M-type sPLA$_2$R [102]. A recently generated conditional transgenic mouse that expresses human M-type sPLA$_2$R1 will certainly facilitate future investigations into this sPLA$_2$-BP under different pathophysiological conditions [116].

VEGFRs are sPLA$_2$-BPs that have also been shown to be involved in cancer development. By binding to VEGFRs, sPLA$_2$s might inhibit angiogenesis, which is an essential process for cancer metastasis formation [117]. Therefore, the use of sPLA$_2$s in chemotherapy has been proposed. In addition, the angiogenic pathways of VEGFRs and endothelial nAChRs have been shown to have cross-talk, which suggests that as antagonists of nAChRs, sPLA$_2$s might further inhibit angiogenesis in this way [31, 118]. In contrast to the antagonistic effects on VEGFRs and nAChRs, GIIA sPLA$_2$ can activate EGFRs in lung cancer cells [34]. This leads to elevated HER /HER2-elicited signalling, which contributes to overexpression of GIIA sPLA$_2$. Plasma concentration of GIIA sPLA$_2$ might therefore serve as a biomarker for lung cancer, and GIIA sPLA$_2$ might represent a therapeutic target to treat patients with lung cancer.

The integrin binding of snake venom sPLA$_2$s has also been exploited for development of new anti-cancer agents that target cell proliferation and migration [36, 103]. MT-II binding to nucleolin appears to explain the higher toxicity of MT-II against cancer cells, as nucleolin is more abundant on the surface of cancer cells than of normal cells [90]. Nucleolin also participates in internalization of many viruses, which suggested that the anti-viral activity of some sPLA$_2$s might be due to their nucleolin binding [27].

The involvement of GIIA sPLA$_2$ in synovial inflammation through liberation of arachidonic acid for production of inflammatory eicosanoids has long been known. Efforts have been made to develop inhibitors of GIIA sPLA$_2$ to attenuate rheumatoid arthritis and sepsis, but without substantial success so far [119, 120]. Colocalization of GIIA sPLA$_2$ and vimentin is, however, associated with phospholipase-activity-independent mechanisms of signalling through arachidonic acid metabolism [83], which provides the way for targeted studies of GIIA sPLA$_2$ signalling, and for the development of new therapeutic strategies based on inhibition of GIIA sPLA$_2$. Lee et al. (2013) [83] also identified vimentin as an interesting player in some other diseases where the involvement of GIIA sPLA$_2$ has been indicated, and particularly in cancers. GIIA sPLA$_2$ is overexpressed in many types of cancers, while vimentin is one of the signature biomarkers of tumour dedifferentiation through epithelial-mesenchymal transition [121, 122].

Abundant expression of vimentin has also been observed in adult neurons as a response to injury, such as in Alzheimer's disease [123]. As vimentin might assist the internalization of GIIA sPLA$_2$ into neurons, this suggests why GIIA sPLA$_2$ is involved in the aetiology of Alzheimer's disease. GIIA sPLA$_2$ might directly damage neurons or boost inflammation by releasing excessive arachidonic acid. Targeting of vimentin is, therefore, potentially interesting as a new therapeutic approach to treat patients with Alzheimer's disease.

A phospholipase-activity-independent mode of action is also characteristic for snake venom catalytically inactive sPLA$_2$s. These can induce inflammation [124]; e.g., MT-II can stimulate the production and release of inflammatory mediators, such as interleukin-6 [125], interleukin-1, tumour necrosis factor α, leukotriene B4, thromboxane A2 and prostaglandins E2 and D2 [126-129]. Indeed, this activity of MT-II was recently used to establish a new experimental model of acute arthritis [130].

Mammalian GIB, GIIA, GV and GX sPLA$_2$s induce the production of pro-inflammatory cytokines and chemokines, whereby these sPLA$_2$s increase inflammation after binding to HSPGs or to M-type sPLA$_2$R on macrophages, neutrophils, eosinophils, monocytes and endothelial cells [131]. This binding to HSPGs also participates in clearance of these sPLA$_2$s and in reduction of their enzymatic activity towards low-density lipoprotein, which is an important factor in atherosclerosis [3, 132].

sPLA$_2$s also mediate pro-inflammatory actions via integrins, as binding of GIIA sPLA$_2$ to human integrins $\alpha_v\beta_3$, $\alpha_4\beta_1$ and $\alpha_5\beta_1$ trigger signalling that leads to inflammation [36, 133]. The effects of sPLA$_2$s on haemostasis have great potential for medical applications as well. The induction of vasoconstriction

by PA2-Vb binding to PAR-1 makes PA2-Vb interesting for the development of new therapeutic approaches against hypertension, atherosclerosis and diabetes-related vascular problems [35]. Moreover, snake venom $sPLA_2$s can induce strong anticoagulant effects through competitive binding to constituents of the prothrombinase complex, which suggests their great potential for the development of therapeutic procedures to attenuate or prevent blood-clot formation [108].

As already mentioned, GIIA $sPLA_2$ has been associated with the aetiology of some neurodegenerative diseases, such as Alzheimer's disease [134-137]. A hallmark of the induction of Alzheimer's disease is the elevated expression of GIIA $sPLA_2$ in the affected tissue, with concomitant dysfunction of the neuronal mitochondria. As the pathological effects of presynaptically neurotoxic $sPLA_2$s from snake venoms and GIIA $sPLA_2$ on mitochondria are similar, a description of the mode by which neurotoxic $sPLA_2$s encounter and affect neuronal mitochondria at the molecular level is expected to advance the study of the role of endogenous GIIA $sPLA_2$ in this and other related destructive diseases.

These findings indicate a way to the development of original diagnostic and therapeutic solutions. An important breakthrough in this direction was the recent identification of CCOX as the mitochondrial receptor for Atx [37]. Application-wise, a very promising discovery was also the binding of crotoxin to ΔF508CFTR, responsible for cystic fibrosis. The $sPLA_2$ subunit of crotoxin has been used as a template to develop a new line of anti-cystic fibrosis agents [33].

Conclusions and outlook

As well as participating in a very broad spectrum of biological processes through their enzymatic activity, $sPLA_2$s participate in many physiological settings as ligands for membrane or soluble receptors. As new and more sensitive analytical tools are developed, the number of newly discovered $sPLA_2$-BPs is growing. Further technical advances will promote the discovery of even more $sPLA_2$-BPs.

$sPLA_2$-BPs are structurally diverse integral membrane and soluble proteins. Despite many attempts, their common $sPLA_2$-binding attributes remain largely obscure. The solution to this puzzle will enable targeted searches of additional $sPLA_2$-BPs. A straightforward approach to solve this problem would be the determination of the three-dimensional structures of complexes between $sPLA_2$s and their $sPLA_2$-BPs. With the development of powerful new technologies, such as cryo-electron microscopy, this is becoming more and more realistic.

$sPLA_2$s are very useful molecules from the evolutionary point of view. Particular structural features have made the $sPLA_2$s promiscuous, and promiscuous proteins can acquire new functions more readily than other proteins. In this way, these can enable organisms to adapt more successfully to environmental changes.

Finally, due to their enzymatic activity and extensive interactomes, $sPLA_2$s are exceptional pharmacological targets. Detailed descriptions of their actions at the molecular level will initiate the development of a plethora of original diagnostic and therapeutic approaches.

Abbreviations

Atx: $sPLA_2$ from *Vipera a. ammodytes* venom; CaM: calmodulin; CB: basic $sPLA_2$ subunit of crotoxin; CCOX: cytochrome c oxidase; CFTR: cystic fibrosis transmembrane conductance regulator; CRD: carbohydrate recognition domain; DM64: metallo-proteinase inhibitor from *Didelphis marsupialis* blood; ECD: extracellular domain; EGF(R): epidermal growth factor (receptor); ER: endoplasmic reticulum; FX/FXa/FIIa: blood coagulation factors; GLIC: proton-gated ion channel from *Gloeobacter violaceus*; GPCR: G-protein-coupled receptor; HER: human EGFR; hGIIA: human group IIA $sPLA_2$; HSPGs: heparan sulphate proteoglycans; IBS: interfacial binding surface; Ig-like: immunoglobulin-like; M-type $sPLA_2$Rs: muscle-type $sPLA_2$ receptors; nAChR: nicotinic acetylcholine receptor; NBD: nucleotide-binding domain; NP: neuronal pentraxin; OS_1: $sPLA_2$ from *Oxyuranus s. scutellatus* venom; PAR: protease-activated receptor; PDI: protein disulphide isomerase; PLIs: $sPLA_2$ inhibitors; SP-A: pulmonary surfactant protein A; $sPLA_2$: secreted phospholipase A_2; $sPLA_2$-BP: $sPLA_2$-binding protein; TCBP-49: taipoxin-associated 49-kDa Ca^{2+}-binding protein; VEGFR: vascular endothelial growth factor receptor; WSG: *Withania somnifera* glycoprotein.

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Competing Interests

The authors have declared that no competing interest exists.

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