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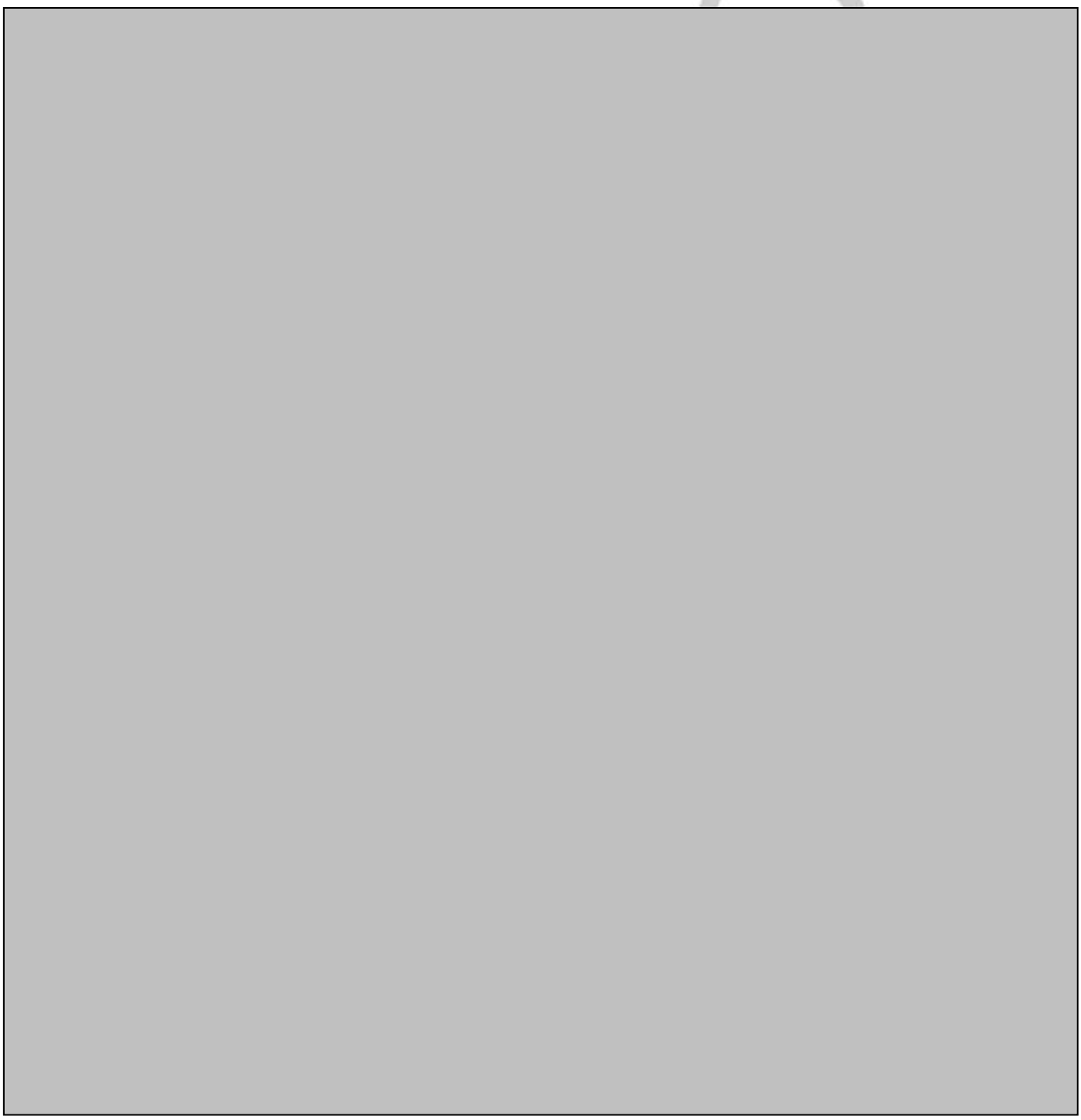
REVIEW

SHIP2: Structure, Function and Inhibition

Mark P. Thomas, [a] Christophe Erneux, [b] and Barry V. L. Potter*[c]



Abstract: SHIP2 is a phosphatase that acts at the 5-position of phosphatidylinositol 3,4,5-trisphosphate. It is one of several enzymes that catalyse dephosphorylation at the 5-position of phosphoinositides or inositol phosphates. SHIP2 has a confirmed role in opsismodysplasia, a disease of bone development, but also interacts with proteins involved in insulin signalling, cytoskeletal function (thus having an impact on endocytosis, adhesion, proliferation and apoptosis) and immune system function. The structure of three domains (constituting about 38% of the protein) is known. Inhibitors of SHIP2 activity have been designed to interact with the catalytic domain with sub-micromolar IC_{50} values: these come from a range of structural classes and have been shown to have *in vivo* effects consistent with SHIP2 inhibition. Much remains unknown about the roles of SHIP2 and possible future directions for research are indicated.

1. Introduction

SHIP2 is an enzyme that catalyses dephosphorylation at the 5-position of mainly phosphatidylinositol 3,4,5-trisphosphate (PtdIns(3,4,5)P3) to generate phosphatidylinositol 3,4-bisphosphate (PtdIns(3,4)P2; Figure 1). In catalysing this reaction, SHIP2 is one of several human enzymes that can dephosphorylate at the 5-position of phosphoinositides or inositol phosphates (Table 1). The inositol polyphosphate 5-phosphatases have been the subject of several general reviews [1].

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Supporting information for this article is given via a link at the end of the document.

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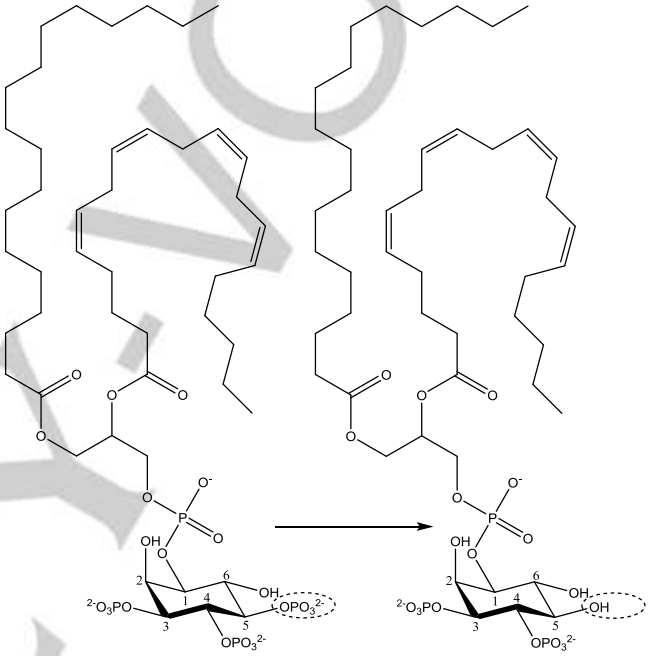
Professor Barry Potter is Professor of Biological and Medicinal Chemistry at Oxford University, a Fellow of University College and a Wellcome Trust Senior Investigator, with interests in cellular signalling and anticancer drug discovery. He studied Chemistry at Oxford, and after postdoctoral research there and in Göttingen, returned to Oxford in 2015 following academic positions at Leicester and Bath universities. He was elected to the UK Academy of Medical Sciences and Academia Europaea.

Table 1. Human 5-phosphatases of phosphatidylinositols or inositol phosphates.

Protein	UniProtKB ID	Gene Name	Number of Residues
Phosphatidylinositol 3,4,5-trisphosphate 5-phosphatase 2 (SHIP2)	O15357	*INPPL1*	1258
Phosphatidylinositol 3,4,5-trisphosphate 5-phosphatase 1 (SHIP1)	Q92835	*INPP5D*	1189
Phosphatidylinositol 4,5-bisphosphate 5-phosphatase A	Q15735	*INPP5J*	1006
Inositol polyphosphate 5-phosphatase OCRL-1	Q01968	*OCRL*	901
Type II inositol 1,4,5-trisphosphate 5-phosphatase	P32019	*INPP5B*	993
72 kDa inositol polyphosphate 5-phosphatase	Q9NRR6	*INPP5E*	644
Inositol polyphosphate 5-phosphatase K (SKIP)	Q9BT40	*INPP5K*	448
Synaptojanin-1	O43426	*SYNJ1*	1573
Synaptojanin-2	O15056	*SYNJ2*	1496
Type I inositol 1,4,5-trisphosphate 5-phosphatase	Q14642	*INPP5A*	412

Phosphoinositides generally [1a], and PtdIns(3,4,5)P_3 more specifically [3], have been the subject of recent reviews: these papers should be referred to for details of the roles of PtdIns(3,4,5)P_3 in the brief summary that follows. PtdIns(3,4,5)P_3 is found embedded in membranes with the phosphorylated inositol headgroup exposed to the cytoplasm. It is predominantly synthesized from phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P_2) in a reaction catalysed by phosphatidylinositol 3-kinase (PI3K) in response to extracellular stimuli. PtdIns(3,4,5)P_3 functions as a second messenger, amplifying an external stimulus to generate (or not) a response. Effector proteins are recruited from the cytoplasm and bind to the inositol trisphosphate headgroup moiety through their PH domain, resulting in activation of the protein and/or the membrane translocation of the protein. Among the processes controlled or influenced by PtdIns(3,4,5)P_3 are cellular growth, proliferation, apoptosis, cytoskeletal rearrangement, chemotaxis and neuronal development and function. Perturbations in PtdIns(3,4,5)P_3 signalling have been linked to roles in cancer, inflammation, cardiovascular disease and diabetes. By dephosphorylating PtdIns(3,4,5)P_3 at the 5-position SHIP2 potentially has an influence on all these processes. It should be noted that dephosphorylation of PtdIns(3,4,5)P_3 at the 3-position by the tumour suppressor PTEN (UniProtKB ID P60484) to generate phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P_2) is also important [4]. The contrasting roles of SHIP2 and PTEN have been studied [5].



Scheme 1. The reaction catalysed by SHIP2. The site of reaction is identified by the dotted ellipse on the lower right. The exact nature of the fatty acids in the structure is variable [2].

Aside from cleaving the 5-phosphate from PtdIns(3,4,5)P_3 SHIP2, or truncated versions of it, have also been reported to catalyse the *in vitro* and *in vivo* removal of the 5-phosphate from phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P_2) [6], and the *in vitro* removal of the 5-phosphate from inositol 1,3,4,5-tetrakisphosphate (Ins(1,3,4,5)P_4) [7], and Ins(1,4,5,6)P_4, Ins(2,4,5,6)P_4, Ins(1,2,3,4,5)P_5 and diC_4PtdIns(3,5)P_2 [8]. Whether these reactions occur *in vivo* (or even whether some of these substrates clearly exist *in vivo*) is unknown. Similarities and differences between the reaction specificity of SHIP1 and SHIP2 are discussed elsewhere [1a].

However, the role of SHIP2 is not limited to its catalytic activity. As discussed below, SHIP2 is able to interact with many proteins through four different interaction motifs and these interactions influence a number of processes including insulin signalling (and, thus, diabetes and metabolic syndrome), cytoskeletal organisation and function (thus influencing endocytosis, adhesion

and proliferation), and immune system function. Despite indirect evidence for SHIP2 involvement in these processes based on essentially *in vitro* models, genetic evidence has provided a role for SHIP2 only in human bone maturation: indeed, rare mutations in *INPPL1* cause opsismodysplasia, a disease of bone maturation [9].

This paper starts by discussing the sequence and structure of SHIP2. It then goes on to review the role of SHIP2 in bone maturation, insulin signalling, cytoskeletal organisation and function, and immune system function at least partly through a discussion of the proteins with which it interacts. It concludes by reviewing the development of modulators of SHIP2 activity, discussing both their *in vitro* and *in vivo* effects.

2. SHIP2 Sequence and Structure

SHIP2 (UniProtKB ID O15357) is the product of the *INPPL1* gene on human chromosome 11 [10]. An earlier paper had partially identified the sequence, but sequencing mistakes resulted in errors in both the *N*- and *C*-termini [11]. Although commonly (and conveniently) called SHIP2 the official name of the enzyme is phosphatidylinositol 3,4,5-trisphosphate 5-phosphatase 2 (EC 3.1.3.86). Two isoforms of the protein, generated by alternative splicing, are known to exist. The longer isoform 1 has 1,258 residues (Figure 2) with the shorter isoform 2 having 1,016 residues, missing residues 1-242 of isoform 1. There are several natural variants that are mutated from the human sequence shown in Figure 2. The R401W, P659S, W688C and F722I variants have all been associated with the genetic disease opsismodysplasia (suggesting a key role in endochondral ossification [9]), and the L632I and N982S variants have been associated with susceptibility to non-insulin dependent diabetes mellitus [12]. The T180A mutation has been found in families suffering from lymphatic dysfunction [13]. Other natural variants are V721M and A1083G [12], and A1114G [10][14].

```
   1 MASACGAPGP GGALGSQAPS WYHRDLSRAA   30
  31 AEELLARAGR DGSFLVRDSE SVAGAFALCV   60
  61 LYQKHVHTYR ILPDGEDFLA VQTSQGVPVR   90
  91 RFQTLGELIG LYAQPNQGLV CALLLPVEGE  120
 121 REPDPPDDRD ASDGEDEKPP LPPRSGSTSI  150
 151 SAPTGPSSPL PAPETPTAPA AESAPNGLST  180
 181 VSHDYLKGSY GLDLEAVRGG ASHLPHLTRT  210
 211 LATSCRRLHS EVDKVLSGLE ILSKVFDQQS  240
 241 SPMVTRLLQQ QNLPQTGEQE LESLVLKLSV  270
 271 LKDFLSGIQK KALKALQDMS STAPPAPQPS  300
 301 TRKAKTIPVQ AFEVKLDVTL GDLTKIGKSQ  330
 331 KFTLSVDVEG GRLVLLRRQR DSQEDWTTFT  360
 361 HDRIRQLIKS QRVQNKLGVV FEKEKDRTQR  390
 391 KDFIFVSARK REAFCQLLQL MKNKHSKQDE  420
 421 PDMISVFIGT WNMGSVPPPK NVTSWFTSKG  450
 451 LGKTLDEVTV TIPHDIYVFG TQENSVGDRE  480
 481 WLDLLRGGLK ELTDLDYRPI AMQSLWNIKV  510
 511 AVLVKPEHEN RISHVSTSSV KTGIANTLGN  540
 541 KGAVGVSFMF NGTSFGFVNC HLTSGNEKTA  570
 571 RRNQNYLDIL RLLSLGDRQL NAFDISLRFT  600
 601 HLFWFGDLNY RLDMDIQEIL NYISRKEFEP  630
 631 LLRVDQLNLE REKHKVFLRF SEEEISFPPT  660
 661 YRYERGSRDT YAWHKQKPTG VRTNVPSWCD  690
 691 RILWKSYPET HIICNSYGCT DDIVTSDHSP  720
 721 VFGTFEVGVT SQFISKKGLS KTSDQAYIEF  750
 751 ESIEAIVKTA SRTKFFIEFY STCLEEYKKS  780
 781 FENDAQSSDN INFLKVQWSS RQLPTLKPIL  810
 811 ADIEYLQDQH LLLTVKSMDG YESYGECVVA  840
 841 LKSMIGSTAQ QFLTFLSHRG EETGNIRGSM  870
 871 KVRVPTERLG TRERLYEWIS IDKDEAGAKS  900
 901 KAPSVSRGSQ EPRSGSRKPA FTEASCPLSR  930
 931 LFEEPEKPPP TGRPPAPPRA APREEPLTPR  960
 961 LKPEGAPEPE GVAAPPPKNS FNNPAYYVLE  990
 991 GVPHQLLPPE PPSPARAPVP SATKNKVAIT 1020
1021 VPAPQLGHHR HPRVGEGSSS DEESGGTLPP 1050
1051 PDFPPPPLPD SAIFLPPSLD PLPGPVVRGR 1080
1081 GGAEARGPPP PKAHPRPPLP PGPSPASTFL 1110
1111 GEVASGDDRS CSVLQMAKTL SEVDYAPAGP 1140
1141 ARSALLPGPL ELQPPRGLPS DYGRPLSFPP 1170
1171 PRIRESIQED LAEEAPCLQG GRASGLGEAG 1200
1201 MSAWLRAIGL ERYEEGLVHN GWDDLEFLSD 1230
1231 ITEEDLEEAG VQDPAHKRLL LDTLQLSK   1258
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Figure 1. The amino acid sequence of human SHIP2. (UniProtKB ID O15357). The first 242 residues (shown in *ITALICS*) are missing from isoform 2. Residues 21-117 (shown in ***BOLD ITALICS***) form an SH2 domain. Residues 139-143 and residues 944-949, constituting SH3-binding motifs, and residues 983-986, forming an NPXY motif, are shown in **UNDERLINED BOLD**. The sterile alpha motif, residues 1196-1258, is shown UNDERLINED.

Residue T165 is reported to be a site of phosphorylation [15], as are residues S132, T1254 and S1258 [16], S241 [15][17], T958 [18], Y986 [19], Y1135 [17][20] and Y1162 [21]. (Further putative phosphorylation sites can be found listed on the PhosphoSitePlus web site: it may be that most of these putative sites have been observed only at very low stoichiometries, and they have often not been confirmed by the use of phospho-specific antibodies.) The reversible phosphorylation of SHIP2 may have an important role to play in the interactions SHIP2 makes with other proteins, in

phosphoinositide signalling, and catalytic activity [22]. Residue K315 is reported to be a site of ubiquitination in EGF-stimulated COS-7 cells [23].

Residues 21-117 form an SH2 domain (missing from isoform 2). Residues 139-142 (sequence PPLP) and 140-143 (sequence PLPP) are both PxxP motifs that can bind SH3 domains. Residues 944-949 (sequence PPAPPR) constitute another SH3-binding motif and residues 983-986 (sequence NPAY) form an NPXY motif: both these motifs lie within a region of compositional bias (residues 935-1105) that has a proline residue at 53 out of 171 positions. Residues 1196-1258 form a SAM (sterile alpha motif) domain. The significance of these domains and motifs is that they can interact with other proteins. SH2 domains bind to phosphotyrosine-containing peptides 3-6 residues *C*-terminal to the phosphorylated tyrosine [24]: a novel hydrophobic *N*-terminal motif for SHIP2 binding through its SH2 domain has been identified [25]. The PPAPPR motif is a ligand for SH3 domains of proteins [26]. The NPXY motif is a sequence highly favoured for forming β-turns [27] and is commonly found as the ligand or substrate for phosphotyrosine binding (PTB) domains, though the tyrosine in the motif does not necessarily need to be phosphorylated to be recognised by the PTB domains [28]: the tyrosine in the SHIP2 NPXY motif, Y986, is a known phosphorylation site [19]. SAM domains are known to interact with a wide range of proteins and nucleic acids [29]. A variety of methods, but principally immunoprecipitation followed by mass spectrometry, have been used to identify proteins that, under the conditions of the experiment, can interact with SHIP2 [6c][19a][30] but whether or not they do so *in vivo* at physiological levels of the proteins has not always been definitively or firmly established. Some of these possible interactions have been discussed previously [1d][22].

Alignment of the human SHIP2 sequence with those from other species (Supplementary Information, Figure S1) shows a high degree of identity with the mouse (*Mus musculus*; 96% sequence identity [31]) and rat (*Rattus norvegicus*; 96% [32]) sequences and a lower degree of identity with two sequences from zebrafish (*Danio rerio*; 69% and 43%). The SHIP2 sequence is also similar to that of SHIP1, having approximately 42% sequence identity (Supplementary Information, Figure S2). In SHIP1 residues 725-863 have been identified as a C2 domain [33], though how the identification was made is not clear: the cited reference [34] describes the sequence of SHIP1 but does not identify a C2 domain. Residues 742-884 of SHIP2 share a similar sequence to the putative C2 domain of SHIP1 so may also be a C2 domain. C2 domains are highly variable in sequence, have an eight-stranded anti-parallel β-sandwich structure (formed in two different ways), and are involved in binding calcium or lipids [35]. The definitive identification of a C2 domain in SHIP2 will have to wait until the structure is solved: a preliminary report has described the crystallization, but not the structure, of this domain [36].

There are two NMR structures of the SAM domain (Figure 3). The 2K4P structure [37] has residues 1194-1258 (65 residues), and the 2KSO structure [38] has residues 1200-1258 (59 residues) in complex with the SAM domain (residues 14-72, 59 residues) of the ephrin-A2 receptor (a tyrosine kinase, UniProtKB ID P29317). The SAM domain is formed of five α-helices forming a 4-5 helical bundle with two orthogonally packed alpha-hairpins. Modelling studies suggest that the SAM domain of ARAP3 (UniProtKB ID Q05CH1) interacts with SHIP2 in the same way as the ephrin A2 receptor [39]. This domain is capable of homo- and hetero-dimerization on two faces, meaning that proteins containing SAM domains can potentially form oligomers [29].

There are also two crystal structures of the ligand binding domain both with residues 419-732 (314 residues). The 3NR8 structure [40] is the apo-enzyme at 2.8Å resolution and the 4A9C structure (Figure 4) [41] is at 2.1Å resolution and contains a synthetic ligand, biphenyl 2,3',4,5',6-pentakisphosphate (Figure 5), in the substrate binding site as a headgroup surrogate. (This ligand and the interactions it makes with the protein are discussed further in the section below on SHIP2 modulators.) The structure of the binding site domain is based on a core of two stacked β-sheets, one with five strands and the other with seven strands. Around this core are a number of α-helices, a couple of short β-sheets and several flexible loops. Despite the absence of the ligand the 3NR8 structure is very similar to the 4A9C structure with just a couple of side chains and a few water molecules in and around the ligand binding site in different positions. Crystals of residues 420-878 have been obtained but the structure has yet to be solved [36].

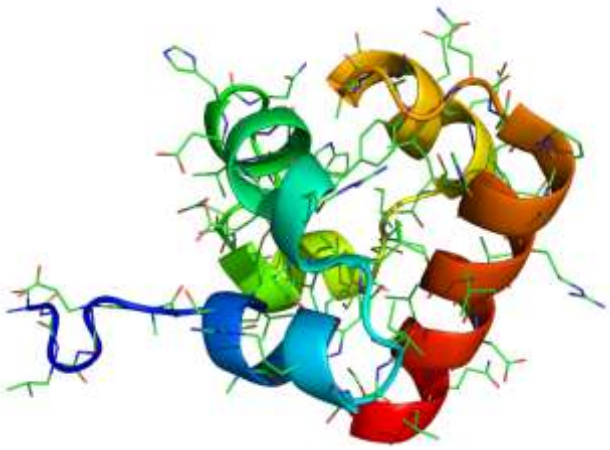


Figure 2. The SAM domain of SHIP2. The overall structure is shown by the cartoon coloured blue at the N-terminus through green, yellow and orange to red at the C-terminus. Taken from the 2K4P structure [37].

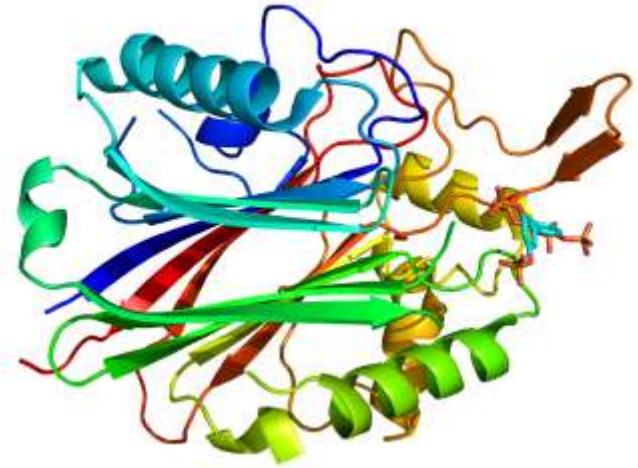


Figure 3. The structure of the ligand binding domain of SHIP2. The structure is shown by the cartoon coloured blue at the *N*-terminus through green, yellow and orange to red at the *C*-terminus. The ligand, biphenyl 2,3',4,5',6-pentakisphosphate with cyan carbon atoms, is visible on the right. Taken from the 4A9C structure [41].

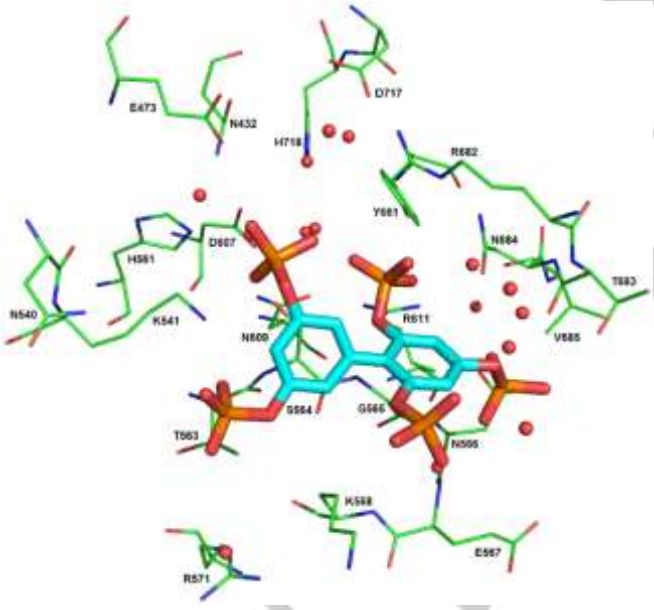


Figure 4. The residues around the ligand in the 4A9C crystal structure of SHIP2. The ligand, biphenyl 2,3',4,5',6-pentakisphosphate, is coloured with cyan carbon atoms. The red spheres are the oxygen atoms of water molecules.

Finally, there is an NMR structure of the SH2 domain, residues 20-117, 2MK2 (Northeast Structural Genomics Consortium, unpublished; Figure 6). This has two helices sandwiching a three-stranded antiparallel *β*-sheet. This is a typical SH2 domain structure with the residues commonly involved in the binding of phosphotyrosine-containing peptides conserved (Arg27 in α-helix A, Arg47 in β-sheet B, Ser49 and Glu50 in the loop between β-sheet B and β-sheet C, Val60 in β-sheet C, and His67 in β-sheet D). In SH2 domains the phosphotyrosine-containing peptide usually (but not always) binds with the residues' *C*-terminal to the phosphotyrosine lying perpendicular to and across the core β-strands [24a][42]. It is not known if the SHIP2 ligands bind in this canonical fashion.

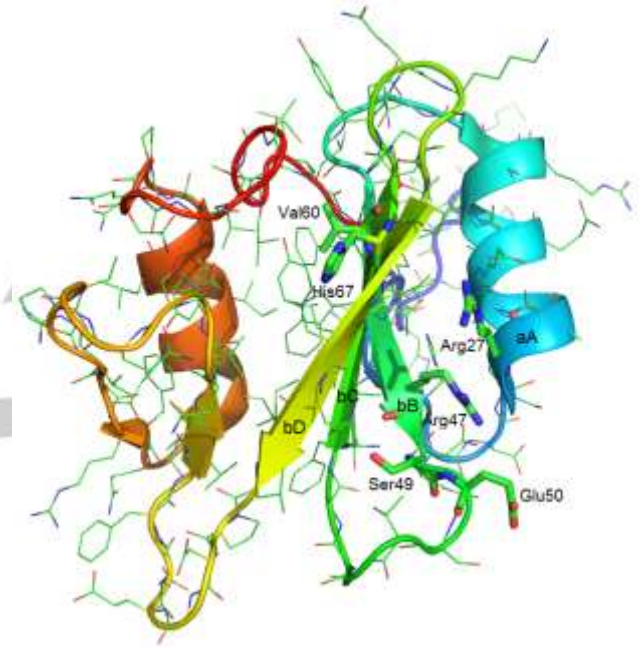


Figure 5. The SH2 domain of SHIP2. The overall structure is shown by the cartoon coloured blue at the *N*-terminus through green, yellow and orange to red at the *C*-terminus. Some of the α-helices (aA) and β-sheets (bB, bC and bD) are identified as are the residues (shown as sticks and labelled) that are involved in binding the phosphotyrosine-containing peptide. Taken from the 2MK2 structure (Northeast Structural Genomics Consortium, unpublished).

3. SHIP2 in Opsismodysplasia

The established role of SHIP2 in human disease or pathology is in opsismodysplasia [43]. Opsismodysplasia is a congenital autosomal recessive disease [44] that is characterized by a delay in ossification, the maturation process whereby cartilage is replaced by bone. Whole genome sequencing of three members of the same family suffering from opsismodysplasia revealed mutations in the *INPPL1* gene, with analysis of a further twelve families suggesting that *INPPL1* mutations account for 60% of cases of opsismodysplasia [45]. Frameshift, deletion and missense mutations in the *INPPL1* gene have been shown to be responsible for some, but not all, cases of opsismodysplasia [9][45][46]. SHIP1 has also been shown to play a role in bone development [47].

4. SHIP2 in Insulin Signalling

Early work on SHIP2 ascribed to it roles in insulin signalling and diabetes [48]. However, insulin signalling is no longer regarded as being the main pathway where SHIP2 is involved [49]. Despite this, research into a possible role of SHIP2 in insulin signalling and diabetes continues because some genetic studies suggest SHIP2 does have a role to play.

An argument that SHIP2 is involved in insulin signalling came from the finding that mutations in the SHIP2 gene, *INPPL1*, contribute to the genetic susceptibility of rats and humans to type 2 diabetes [12][50] and other symptoms of metabolic syndrome [51]. In a dietary rat model of metabolic syndrome antisense oligonucleotides against SHIP2 improve the muscle insulin sensitivity [52]. In men with type 1 diabetes mutation of the *INPPL1* gene may contribute to susceptibility to metabolic syndrome [53]. Small molecule inhibitors of SHIP1 have been shown to reverse diet-associated obesity and metabolic syndrome in mice, though the possible inhibition of SHIP2 cannot be excluded [54]. The absence of SHIP2 in mice also confers resistance to dietary obesity [55].

In the mouse brain insulin has neuroprotective effects that are reduced when SHIP2 is overexpressed [56]. Mice overexpressing SHIP2 exhibited impaired performance in tests of memory, avoidance and recognition. Diabetic mice had higher levels of SHIP2 in the brain than non-diabetic mice, and the impairment of brain function in the diabetic mice was reduced in mice expressing catalytically dead SHIP2. Metabolic syndrome, of which type 2 diabetes is a component, is a risk factor for Alzheimer's disease (AD) and single nucleotide polymorphisms in the *INPPL1* gene have suggested a possible association between SHIP2 activity and AD [57]: the possible role of SHIP2 in AD is discussed further below.

Mice expressing a germline catalytically inactive SHIP2 mutant protein are viable, but have defects in the development of muscle, adipose tissue and the female genital tract, and in somatic growth [49]. Glucose tolerance, insulin sensitivity and insulin-induced Akt/PKB phosphorylation were unaffected, but lipid metabolism and insulin secretion were. Variants of these mice expressing also a catalytically inactive PI3K might be expected to have a phenotype where the two mutations cancel each other out, but this was not the case, possibly suggesting that some of the effects of an inactive SHIP2 may be due to a lack of PtdIns(3,4)P_2 [49].

5. SHIP2 and the Cytoskeleton: Endocytosis, Adhesion, Proliferation and Apoptosis

In some cell types the downregulation of *INPPL1* expression or the inhibition of SHIP2 activity has been shown to induce apoptosis. Virusecurinine, a plant alkaloid, downregulates *INPPL1* expression (thus reducing the amount of SHIP2) in K562 cells and induces apoptosis [58]. SHIP2-expressing multiple myeloma breast cancer cells lacking SHIP1 expression, when treated with pan-SHIP1/2 inhibitors, undergo apoptosis [59]. Palmitate induces apoptosis in HepG2 cells which was increased when wild-type SHIP2 was overexpressed and decreased if SHIP2 was inhibited [60]. However, contrarily, in other cell types the overexpression of *INPPL1* expression has been shown to increase apoptosis. Thus, in gastric cancer where *INPPL1* expression is usually downregulated the overexpression of *INPPL1* induced apoptosis (as well as inhibiting cell proliferation and suppressing cell motility and invasion) [61].

In colorectal cancer samples SHIP2 is overexpressed and has an increased level of activity [62]. Inhibition of SHIP2 reduced cell viability, and both SHIP2 inhibition and knockdown reduced the amount of phosphorylated PKB resulting in sensitivity to chemotherapeutics. SHIP2 knockdown also reduced cell migration and invasive capacity but had no effect on cell adhesion.

SHIP2 plays an important role in cell adhesion and spreading. It could do so at least in part through an interaction with the p130^{Cas} adaptor protein (UniProtKB ID P56945), also known as the breast cancer anti-estrogen resistance protein 1, that is a mediator of actin cytoskeleton organization and associates with phosphorylated SHIP2 through the SHIP2 SH2 domain [30g]. HeLa cells expressing SHIP2 exhibited increased adhesion that was not observed when the R47G SH2 domain SHIP2 mutant was expressed: cells with mutant SHIP2 adhered between a third and a half of the time of those cells expressing the wild-type protein, and the mutant cells spread more slowly than wild-type cells [30g]. Catalytically dead SHIP2 also inhibited cell spreading [30g]. These roles of SHIP2 are dependent on the phosphorylation status: the phosphorylations are catalysed by Src kinases [19b]. If Src is inhibited then phosphorylation of SHIP2 tyrosine residues falls [63]. HeLa cells with SHIP2 expression suppressed by RNA interference (RNAi) had defects in cell spreading associated with disruption of cytoskeletal proteins [64].

Vinexin (UniProtKB ID O60504) is a cytoskeletal protein involved in cell spreading and cytoskeletal organization [65]. The *C*-terminus of SHIP2 interacts with vinexin: the interaction has no effect on SHIP2 catalytic activity [30l]. The interaction between SHIP2 and vinexin promotes localization of SHIP2 at the cell periphery and increases cellular adhesion.

Intersectins are scaffold proteins that have roles to play in signal transduction, cytoskeletal rearrangements and cytosis [66]. In response to EGF, SHIP2 associates with intersectin-1 (UniProtKB ID Q15811) and may recruit it to the plasma membrane of COS-7 cells [30n]: the interaction occurs through the SHIP2 proline-rich region. In COS-7 cells the interaction with intersectin concentrates SHIP2 at endocytic clathrin-coated pits (CCPs) early in pit formation, though dissociation occurs before fission [6b]. CCP lifetime is shortened by reducing the expression of SHIP2 or increasing production of PtdIns(3,4,5)P_3 and/or PtdIns(4,5)P_2, both of which result in an increase in the rate of CCP maturation, though it was unclear which of these scenarios had this effect. More recent work has suggested that in N1 glioblastoma cells a fall in the amount of PtdIns(3,4,5)P_3 controls proliferation and a

fall in the amount of PtdIns(4,5)P_2 controls migration [6c]. SHIP2 is also involved in a clathrin-independent endocytic pathway that utilises endophilin A (UniProtKB IDs Q99962, Q99961 and Q99963) and is involved in the ligand-stimulated uptake of several G-protein-coupled receptors [67].

There are three types of filamin in human cells, filamins A, B and C (UniProtKB IDs P21333, O75369 and Q14315, respectively). These proteins are involved in the organization of the actin cytoskeleton, contributing to the mechanical stability of the plasma membrane and the cell cortex, and also have roles in cell migration and adhesion [68]. In fulfilling these roles they have been shown to interact with more than ninety other proteins, one of which is SHIP2 [30f][69]. Filamin and SHIP2 co-localize at Z-lines and the sarcolemma in striated muscle and at membrane ruffles in COS-7 cells. (Membrane ruffling is the formation of a motile cell surface that contains a network of freshly polymerised actin filaments.) Levels of PtdIns(3,4,5)P_3 and the presence of sub-membranous actin at membrane ruffles were dependent on SHIP2 catalytic activity [30f]. The regulation of cortical and sub-membranous actin by the localised hydrolysis of PtdIns(3,4,5)P_3 may be orchestrated by a complex between SHIP2, filamin, actin and the platelet receptor for the von Willebrand factor glycoprotein 1b-IX-V (GPIb-IX-V) [69].

The formation of lamellipodia can be dependent on PtdIns(3,4,5)P_3. The sub-cellular location of LL5*β* (PHLDB2; UniProtKB ID Q86SQ0) is dictated by PtdIns(3,4,5)P_3: it interacts with the actin cross-linking filamin A at the PtdIns(3,4,5)P_3-enriched leading edge of lamellipodia. Filamin A co-localizes with SHIP2 which dephosphorylates PtdIns(3,4,5)P_3 at the same location [70]. Lamellipodia formation is associated with a protein complex that includes SHIP2, lamellipodin (UniProtKB ID Q70E73) and filamin: the assembling of this complex is regulated by nephrin (UniProtKB ID O60500) [71]. SHIP2 is also important for PtdIns(3,4)P_2 production which is a specific ligand of lamellipodin [72].

SHIP2 binds directly to the HGF receptor, c-met (UniProtKB ID P08581) [30k]. HGF stimulates cell spreading and scattering, but this is suppressed if a catalytically inactive mutant of SHIP2 is present in the cell.

SHIP2 binds to actin tails that form beneath enveloped poxviruses following their fusion with the plasma membrane [73]. The binding requires phosphotyrosine, neural Wiskott-Aldrich syndrome protein (N-WASP; UniProtKB ID O00401) and tyrosine kinases from the Abl and Src families. Cells lacking SHIP2 have normal actin tails but release more virus particles. N-WASP also plays a role in the formation of invadopodia which are actin-rich protrusions from cell membranes that extend into the extracellular matrix, and which may play a role in some cancers. Invadopodia formation is associated with increased activity of PI3K and SHIP2 and the consequent increased levels of PtdIns(3,4)P_2 [74]. SHIP2 regulates invadopodium maturation, but not initiation, by associating (along with Tks5 (UniProtKB ID Q5TCZ1)) with an invadopodium initiating complex consisting of N-WASP, cortactin (UniProtKB ID Q14247), cofilin (UniProtKB ID P23528, Q9Y281) and actin [75].

In U251 glioma cells a GTP-dependent interaction between RhoA (UniProtKB ID P61586) and SHIP2 is associated with spreading and migration [76]. RhoA exhibits polarization in migrating cells: this polarization and migration are attenuated in cells expressing SHIP2 mutants defective in RhoA binding. SHIP2-depleted cells have improper PtdIns(3,4,5)P_3 localization which is not restored by a SHIP2 mutant defective in RhoA binding. In MDCK cells expressing the hepatitis C virus core protein, apicobasal polarity is disrupted, this being associated with decreased expression of SHIP2 and the polarity protein Dlg1 (UniProtKB ID Q12959) and decreased activity of RhoA [77]. Increasing the expression of SHIP2 restored cell polarity and RhoA activity. ARAP3 (UniProtKB ID Q8WWN8) is a PtdIns(3,4,5)P_3-dependent GTPase-activating protein that regulates Arf and Rho proteins thus modulating actin cytoskeleton remodelling. ARAP3 and SHIP2 bind to each other in a heterodimeric interaction between their SAM domains [78].

The proliferation rate of K562 erythroleukemia cells is reduced by overexpression of SHIP2 with cells accumulating in the G_2/M phase of the cell cycle [79]. The decrease in proliferation rate is accompanied by an increase in the amount of PtdIns(3,4)P_2.

Neuritogenesis following stimulation with nerve growth factor (NGF) is preceded by an accumulation of PtdIns(3,4,5)P_3 and a consequent activation of Rac1/Cdc42. In SHIP2-deficient PC12 cells NGF-induced Rac1/Cdc42 activation and PtdIns(3,4,5)P_3 accumulation are increased with a consequent increase in the number and length of neurites [80].

In 3T3-L1 preadipocytes platelet-derived growth factor (PDGF) stimulates proliferation and SHIP2 tyrosine phosphorylation. One of the pathways that regulates proliferation involves PI3K, PtdIns(3,4,5)P_3 and Akt, and another involves Shc, Ras and Erk 1/2. PDGF stimulates SHIP2 tyrosine phosphorylation and association with Shc [18]. By failing to dephosphorylate PtdIns(3,4,5)P_3 catalytically inactive SHIP2 attenuates PDGF signalling and inhibits proliferation [81]. The presence of inactive SHIP2 resulted in greater ubiquitination, and subsequent lysosomal degradation, of the PDGF receptor.

In multiple clinical samples of laryngeal squamous cell carcinoma (LSCC) elevated levels of SHIP2 have been detected [82]. SHIP2 expression correlated with clinical stage, metastasis, recurrence, and poor survival. Similarly, elevated SHIP2 expression is associated with poor prognosis in non-small cell lung cancer [83] and in hepatocellular carcinoma [84]. These results suggest that SHIP2 plays an important role in cancer development and progression, and may be useful in diagnostics and as a therapeutic target. In colorectal cancer cells the expression of SHIP2 is significantly higher than in equivalent non-cancerous cells and is correlated with metastasis and overall survival [85].

In oestrogen receptor-negative breast cancer stem cells (BCSCs) SHIP2 is expressed at higher levels than in non-BCSCs: the higher expression is associated with increased expression of JNK1 (UniProtKB ID P45983), JNK2 (UniProtKB ID P45984) and vimentin (UniProtKB ID P08670), and increased tumourogenicity [86].

Treatment of HeLa cells and of MDA-MB-231 breast cancer cells (both with RNAi suppression of SHIP2 expression) with EGF led to increased EGF receptor (EGFR) internalization and degradation consequent upon increased association of EGFR with c-Cbl ubiquitin ligase and EGFR ubiquitination [87]. In these cells the EGF-stimulated activation of Akt was reduced, and the cytokine receptor CXCR4 (UniProtKB ID P61073) is down-regulated [88]: CXCR4 is on the EGF-Akt pathway and plays an important role in metastasis. SHIP2 can be ubiquitinated on K315 but the ubiquitination is catalysed by neither c-Cbl nor Nedd4-1: ubiquitination increases within thirty minutes of stimulation with EGF, correlating with a loss of interaction between the SHIP2 SH2 domain and c-Cbl, suggesting that the association of c-Cbl with SHIP2 masks the ubiquitination site [23]. The PR130 regulatory subunit of protein phosphatase 2A (UniProtKB ID Q06190) has been reported to bind to the SHIP2 regulatory domain and prevent EGF-induced EGFR degradation, thus sustaining EGF-mediated signalling [89]. In serum-maintained cells SHIP2 has a perinuclear location: upon stimulation with EGF part of SHIP2 translocates to the plasma membrane of HeLa cells [90].

6. SHIP2 and the Immune System

The FcγR proteins are low affinity receptors for immunoglobulin G (IgG) that function as modulators of immune responses [91]. They are membrane proteins in monocytes and macrophages that either, (a), have a partner adaptor protein to bring about an intracellular effect, or which, (b), contain, in an intracellular cytoplasmic tail, either an immunoreceptor tyrosine-based inhibition motif (ITIM) or the activation motif equivalent (ITAM). SHIP2 has been shown to interact with two types of FcγR, the ITAM-containing FcγRIIa (UniProtKB ID P12318) [30h], and the ITIM-containing FcγRIIb (UniProtKB ID P31994) [30d,e]. After the receptor binds its ligand the FcγRIIa ITAM becomes phosphorylated which allows other proteins to bind resulting in the activation of molecules involved in cell signalling leading to phagocytosis. In contrast to FcγRIIa with its ITAM is FcγRIIb which in place of the ITAM has an ITIM.

One of the proteins shown to bind to the phosphorylated ITIM of FcγRIIb is SHIP2 [30d,e][92]. This followed earlier work demonstrating the binding of SHIP1 to FcγRIIb [30a]. It was initially thought that the ITIM of FcγRIIb was sufficient for SHIP2 binding [93]. However, later work showed that sixteen *C*-terminal residues (containing a tyrosine-based motif) are also necessary [94] as are the adaptor proteins Grap (UniProtKB ID Q13588) and Grb2 (UniProtKB ID P62993) [95]. (As mentioned above Grb2 also plays a role in cell developmental processes.) SHIP2 inhibits the proliferative response that is the downstream biological consequence of FcγRIIb signalling [96]. SHIP2 binds to the phosphorylated FcγRIIb that is found in the brains of Alzheimer's disease (AD) sufferers. In a mouse model of AD the pharmacological inhibition of SHIP2 has been shown to reduce the memory impairments observed in AD [97]: dysregulation of PtdIns(3,4)P_2 metabolism disrupts the phosphorylation of the tau protein that is associated with memory impairments.

Enteropathogenic *Escherichia coli* bind to cell membranes and insert the bacterial translocated intimin receptor (Tir) into the host plasma membrane. The *C*-terminal region of Tir has a sequence that is homologous to ITIMs. SHIP2 binds to Tir and controls F-actin-pedestal formation by interacting with Shc and generating a PtdIns(3,4)P_2-enriched lipid platform to which the cytoskeletal regulator lamellipodin is recruited [98].

SHIP2 has been shown to interact with the T-cell surface glycoprotein CD4 (UniProtKB ID P01730) [30r]. CD4 is a co-receptor that helps the T-cell receptor communicate with an antigen-presenting cell. The consequences of the interaction of SHIP2 with CD4 are unknown. However, SHIP2 appears to be important in immune cell recognition and interaction as it also binds to vascular cell adhesion protein 1 (VCAM-1; UniProtKB ID P19320) [30s] which is involved in leukocyte-endothelial cell adhesion.

Basophils are granulocytic cells involved in inflammatory reactions and the allergic response. Cellular sensitivity and histamine release have been weakly correlated with SHIP2 expression in basophils [99]. In contrast to basophils, which are the rarest type of granulocyte, the neutrophils are the most common type of granulocyte. Neutrophils have phagocytic activity and are recruited to sites of injury or infection. PtdIns(3,4,5)P_3 is necessary for neutrophil motility: by dephosphorylating PtdIns(3,4,5)P_3 SHIP2 limits neutrophil activity [100].

Another type of phagocyte is the macrophage. When macrophages are stimulated with macrophage colony-stimulating factor (M-CSF; UniProtKB ID P09603) SHIP2 associates with the M-CSF receptor (UniProtKB ID P07333) at the cell membrane, becomes phosphorylated on a tyrosine residue, and associates with the actin-binding protein filamin in an interaction that requires the proline-rich domain but not the SH2 domain [101]. In response to M-CSF stimulation the activation of Akt/PKB by SHIP2 was reduced and NF-κB-mediated gene transcription was inhibited.

SHIP2 is widely expressed, but SHIP1 is predominantly found in cells of haematopoietic origin. In murine platelets SHIP1 plays the major role in regulating the response to thrombin or collagen activation through controlling PtdIns(3,4,5)P_3 levels [102].

Following antigen receptor stimulation phospholipase Cγ (PLCγ; UniProtKB IDs P19174, P16885) is activated in a process that involves the Tec tyrosine kinase (UniProtKB ID P42680). This process requires the activation of PI3K which stimulates the membrane localization of Tec. SHIP1 and SHIP2 interact with,

and negatively regulate, Tec [103]. The interaction occurs through the Tec SH3 domain, removal of which generates a hyperactive form of Tec.

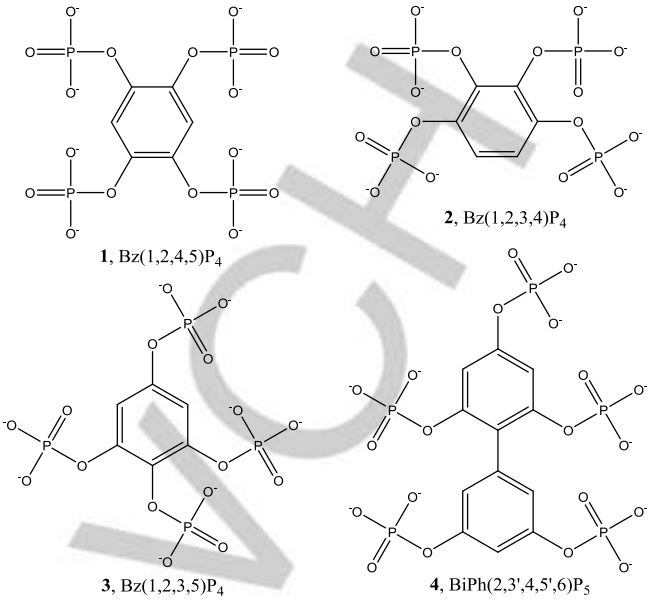
SHIP2 has been shown to interact with discoidin domain receptor 1 (UniProtKB ID Q08345) [30t], a tyrosine kinase that by upregulating matrix metalloproteinases, regulates remodelling of the extracellular matrix and wound healing.

7. SHIP2 Modulators: Studies on the Isolated Enzyme

This section discusses the discovery of SHIP2 modulators, their structures and any *in vitro* data that have been revealed. The consequences of the inhibition for cells and organisms are discussed in the next section. For comparative purposes data for SHIP1 modulators are briefly mentioned. Detailed studies of the SHIP2 reaction mechanism have not been carried out, though results from a study of type II inositol 1,4,5-trisphosphate 5-phosphatase (INPP5B) have been used to identify some of the residues that might be involved [40].

The first mention of SHIP2 inhibitors in the literature came in 2006 when a microfluidic assay for SHIP2 was described [104]. A library of 91,060 compounds was screened in this assay, with 1,343 causing inhibition ≥70% (a 1.5% hit rate). Retesting of 1,116 of these compounds confirmed 721 (64%) as being SHIP2 inhibitors. With IC_{50} = 0.37μM inhibition data for only one of these compounds have been released: similar levels of inhibition by this compound were observed for SHIP1 and PTEN. The structure of none of the inhibitors has been revealed.

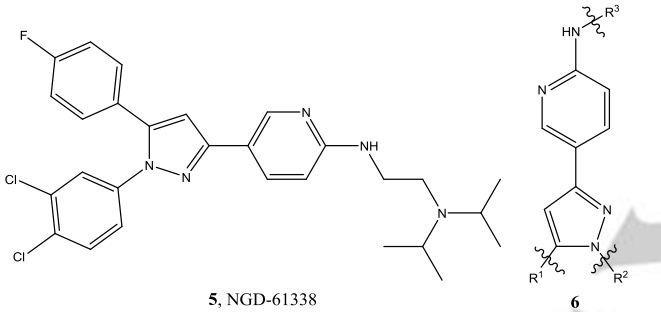
Phosphorylated benzene and biphenyl polyols have been studied as potential inhibitors of inositol phosphatases because they have phosphate groups positioned around a planar six-membered ring with the phosphate regiochemistry similar to, but more rigid than that of the natural inositol phosphates, and it was thought that they would be able to bind to inositol phosphate binding proteins. Using inositol 1,3,4,5-tetrakisphosphate (Ins(1,3,4,5)P_4) as the substrate three phosphorylated benzene derivatives (benzene 1,2,4,5-tetrakisphosphate (Bz(1,2,4,5)P_4, **1**), benzene 1,2,3,4-tetrakisphosphate (Bz(1,2,3,4)P_4, **2**) and benzene 1,2,3,5-tetrakisphosphate (Bz(1,2,3,5)P_4, **3**)) were found to be inhibitors of the SHIP2 catalytic domain with IC_{50} values of 11.2μM, 19.6μM and 40.0μM, respectively [105]. However, in the same study, a phosphorylated biphenyl compound, biphenyl 2,3',4,5',6-pentakisphosphate (BiPh(2,3',4,5',6)P_5, **4**), was found to be a more potent inhibitor of SHIP2 with IC_{50} = 1.8μM, though subsequent work has reported an IC_{50} value of 24.8μM under the same conditions [41]. The inhibitor was not dephosphorylated, i.e. it is not a SHIP2 substrate. It also inhibited type I inositol 1,4,5-trisphosphate 5-phosphatase (UniProtKB ID Q14642), inhibited the binding of Ins(1,4,5)P_3 to the type I inositol 1,4,5-trisphosphate receptor (UniProtKB ID Q14643), but did not inhibit Ins(1,4,5)P_3 3-kinase A (UniProtKB ID P23677) [105].



BiPh(2,3',4,5',6)P_5 (**4**) is the only ligand reported to have been crystallised with the catalytic domain of SHIP2 [41]. The crystal structure contains two SHIP2 catalytic domain monomers but only one molecule of BiPh(2,3',4,5',6)P_5 which is bound in a shallow pocket. The ligand has several direct contacts with the protein and other hydrogen bonds to water molecules that, in turn, are hydrogen bonded to the protein, other water molecules, or another part of the ligand. The 2-phosphate moiety is engaged in three water-mediated hydrogen bonds, one to the 4-phosphate and the others to the surrounding solvent. The 4-phosphate has hydrogen bonds to both the sidechain and backbone of Asn566 as well as to two water molecules. The 6-phosphate has hydrogen bonds to the sidechains of three residues: Arg611, Tyr661 and Arg682. It also forms a water-mediated hydrogen bond to the sidechain of Asn684 and forms another hydrogen bond to a water molecule. The 5'-phosphate has hydrogen bonds to two solvent-exposed water molecules and both it and the 3'-phosphate form hydrogen bonds to both Lys541 and Ser564: the hydrogen bond from the 3'-phosphate is to the backbone of Ser564.

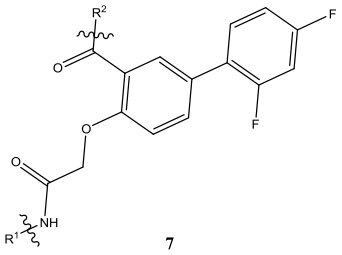
Several of the residues that interact with the ligand are in a loop that, in the crystal structure, contacts a symmetry related protein molecule. Removal of this symmetry related protein molecule followed by a molecular dynamics study of the loop suggest that it has the flexibility to close over the ligand and make additional interactions. Hence, the sidechain of Lys568 can form a π-π stacking interaction with one of the rings, and the sidechains of Lys541 and Lys677 can also make π-π stacking interactions, from opposite sides, with the other ring. Additional hydrogen bonds are formed between the protein and the ligand and some of the water-mediated hydrogen bonds are lost [41]. The flexibility of this loop needs to be taken into consideration when designing ligands to inhibit SHIP2.

Combinatorial libraries have been screened by a high throughput affinity selection-mass spectrometry technique [106]. Over three million compounds from 2,000 mixture-based combinatorial libraries were evaluated in the screen and 242 compounds were identified as being inhibitors. The structures of seventeen of these compounds, based on three different core structures, have been revealed with SHIP2 IC_{50} values ranging from 1.1μM to 50μM. Of the compounds revealed compound NGD-61338 (**5**, based on core structure **6**) was one of the most potent with IC_{50} = 1.1μM. Competitive binding data suggest that NGD-61338 binds to the same site in SHIP2 as the substrate, phosphatidylinositol 3,4,5-trisphosphate (PtdIns(3,4,5)P_3). Other core structures revealed were **7** and **8**. One of the compounds based on core structure **7** was shown to compete with NGD-61338 (**5**) for binding to SHIP2 suggesting that compounds with this very different core structure are also able to bind in the same binding site as the substrate.

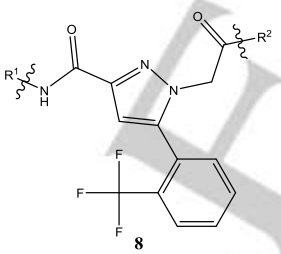


5, NGD-61338

6



7



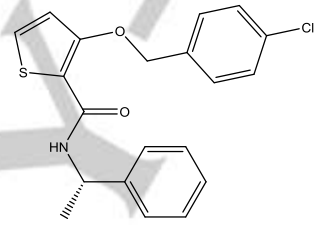
8

High-throughput screening of a compound library resulted in the identification of AS1949490 (**9**) as a competitive inhibitor of SHIP2 with IC_{50} = 0.62μM and K_i = 0.44μM when using Ins(1,3,4,5)P_4 as the substrate [107]. Also identified was AS1938909 (**10**) with IC_{50} = 0.57μM and Ki = 0.44μM [108]. Both were over twenty times more potent against SHIP2 than SHIP1 and failed to inhibit a number of other phosphatases suggesting they are selective for SHIP2.

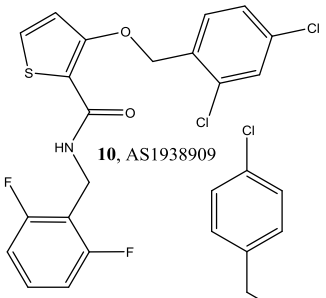
A ligand-based drug discovery programme used NGD-61338 (**5**) and AS1949490 (**9**) as templates for potential new pharmacophores [109]. This yielded four related core structures (**11**-**14**) but no enzyme inhibition data have been released for them. However, some *in vivo* data (discussed below) have been released for one of the compounds, CPDA (**15**). Patent WO 2012/169571 [110] exemplifies thirty compounds with these core structures but also provides no inhibition data. In a more recent patent the same group has exemplified twenty compounds based on a (benzenesulfonylamino) benzamide core structure (**16**) but, again, no inhibition data are provided [111].

A high-throughput screen of a chemical library identified three compounds as being inhibitors of both SHIP1 and SHIP2 [59]. Compounds 1PIE (**17**), 6PTQ (**18**) and 2PIQ (also known as K103, **19**) inhibited SHIP2 with IC_{50} values of 30μM, 63μM and 500μM, respectively, and SHIP1 with IC_{50} values of 30μM, 35μM, 500μM, respectively. Another compound, K149 (**20**), at a concentration of 500μM, reduces SHIP2 activity by about 40% and has been shown to have significant effects in colorectal cancer cells [62].

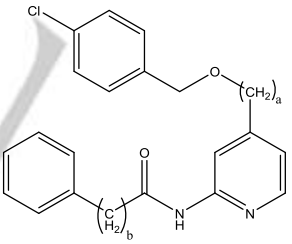
The high-throughput screen that led to the identification of **17**, **18** and **19** was originally designed to find inhibitors of SHIP1 [112]. This had identified 3-α-aminocholestane (3AC, **21**) as a SHIP1 inhibitor with IC_{50} = 10μM; it does not inhibit SHIP2. Some stabilized PtdIns(3,4,5)P_3 analogues (**22**-**26**) have been tested against both SHIP1 and SHIP2 [113]. Compounds **22** and **23** were partially dephosphorylated by SHIP2 but no such hydrolysis of **24**, **25** or **26** was observed. Compounds **22**, **23** and **26** were found to inhibit the SHIP1-catalysed hydrolysis of Ins(1,3,4,5)P_4 but none of them inhibited SHIP2.



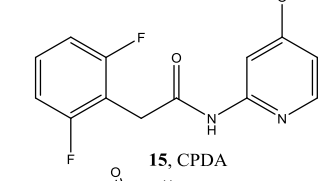
9, AS1949490



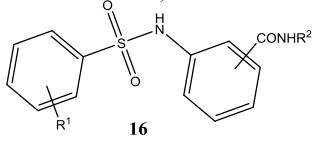
10, AS1938909



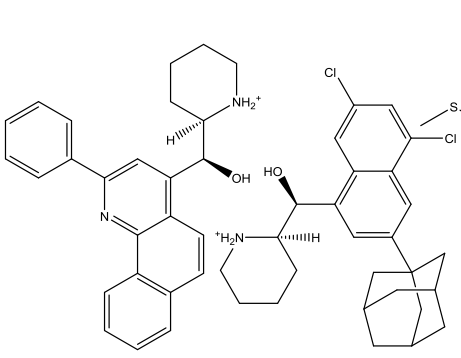
	a	b
11	0	1
12	0	2
13	1	1
14	1	2



15, CPDA



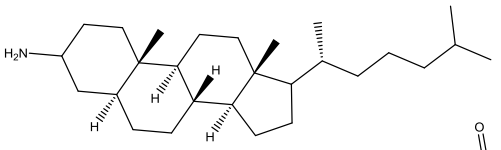
16



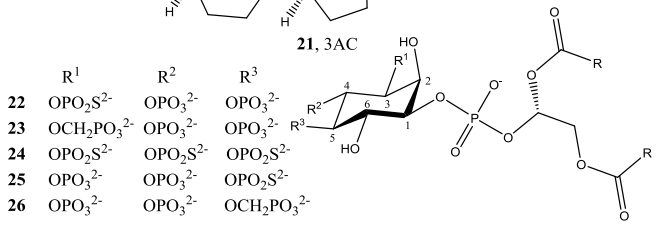
17, 1PIE

18, 6PTQ

19, R = H, 2PIQ, K103
20, R = Cl, K149

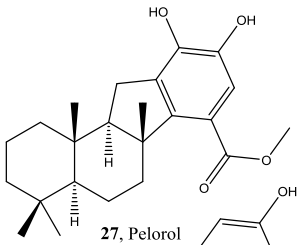


21, 3AC

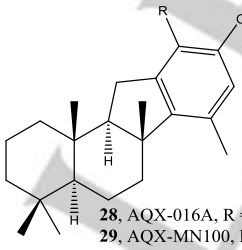


	R^1	R^2	R^3
22	OPO_2S^{2-}	OPO_3^{2-}	OPO_3^{2-}
23	$OCH_2PO_3^{2-}$	OPO_3^{2-}	OPO_3^{2-}
24	OPO_2S^{2-}	OPO_2S^{2-}	OPO_2S^{2-}
25	OPO_3^{2-}	OPO_3^{2-}	OPO_2S^{2-}
26	OPO_3^{2-}	OPO_3^{2-}	$OCH_2PO_3^{2-}$

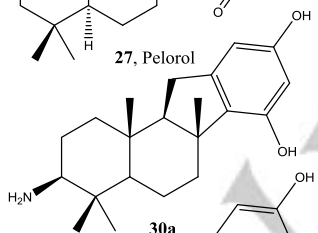
As well as inhibitors of SHIP1 there are also several reports of SHIP1 activators. Pelorol (**27**), a natural product, and a number of synthesized analogues have been found to have *in vivo* effects consistent with activation of SHIP1 [114]. The *in vitro* effects of pelorol were reported alongside those of the more potent SHIP1 activator, and pelorol analogue, AQX-016A (**28**) which also has *in vivo* effects consistent with SHIP1 activation: it is only a poor activator of SHIP2 [33]. The *in vivo* effects of the related compound AQX-MN100 (**29**) have also been reported but without any data pertaining to SHIP2 activity [115]. It has been suggested that these compounds allosterically activate SHIP1 by binding to the putative C2 domain [33]. A racemic pelerol analogue (**30a** and **30b**) is as potent as AQX-MN100 in activating SHIP1 but has better pharmacological properties [116]. Pelorol derivatives with the core structure **31** have been the subject of a patent application [117] but little biological data are given.



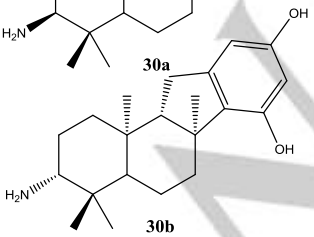
27, Pelorol



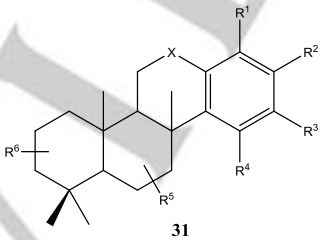
28, AQX-016A, R = OH
29, AQX-MN100, R = H



30a



30b

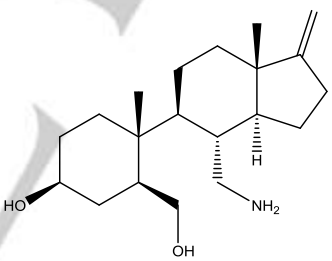


31
X = O, S, SO or SO_2
R^1-R^6 are independently H, OH, halogen, cyano, alkyl, alkoxy, and more complex substituents.

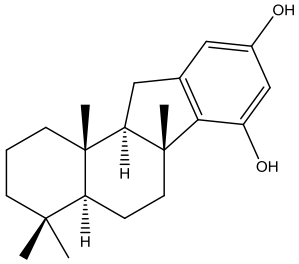
Another SHIP1 activator, AQX-1125 (**32**), is orally active, reduces the response to allergen challenge, and reduces airway inflammation in asthma [118]. Pharmacokinetic studies of AQX-1125 in dogs and rats have been carried out and the tissue distribution in rats has been analysed [119]. At a concentration of 300μM it increases SHIP1 activity by 20% and does so by decreasing K_M and increasing k_{cat}/K_M [119]. Another compound, AQX-MN115 (**33**), under the same conditions increases SHIP1 activity by 77% and does so by having similar effects on the kinetic parameters [119].

A natural product isolated from a *Bacillus* species, turnagainolide B (**34**), was shown to activate SHIP1 to the same extent as AQX-MN100 [120]. A methanolic extract of a soft coral species was active in a SHIP1 assay and purification yielded four diterpenoids, one of which, australin E (**35**), was shown to activate SHIP1 [121].

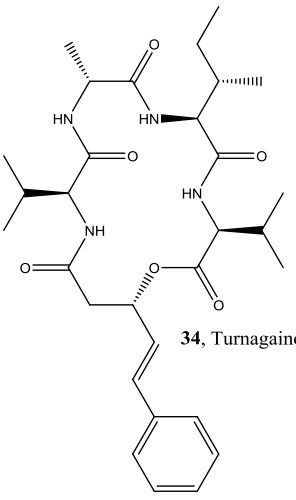
With the exception of the limited data for AQX-016A none of these SHIP1 activators appears to have been evaluated against SHIP2. One SHIP2 activator has been found: anionic lipids in the form of vesicles of phosphatidylserine (**36**) are able to stimulate an increase of up to 9-fold in catalytic activity of both intact SHIP2 and the isolated catalytic domain when using di-C8-PtdIns(3,4,5)P_3 as the substrate but not when using PtdIns(1,3,4,5)P_4 as the substrate [5a].



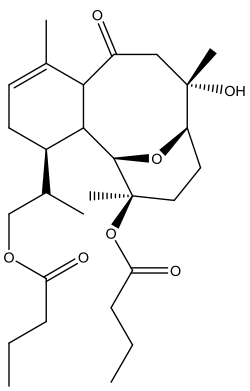
32, AQX-1125



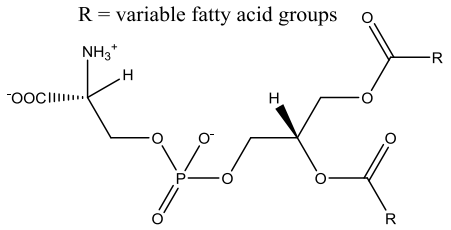
33, AQX-MN115



34, Turnagainolide B



35, Australin E



36

8. SHIP2 Modulators: Effects on Cells and Organisms

The roles of SHIP2 in insulin signalling, apoptosis and immune system function have been described above. The *in vivo* consequences of SHIP2 inhibition have been studied with respect to the effects on either insulin signalling (compounds **9**, **10** and **15**) or apoptosis (compounds **17**, **18** and **19**). No studies have looked at what effects, if any, SHIP2 inhibition may have on immune system function or bone ossification.

The *in vivo* effects of BiPh(2,3',4,5',6)P_5 (**4**) have been studied only in so far as they pertain to the modulation of Ca^{2+} responses in rat hepatocytes [105]. Since this is related to the inhibition of type 1 inositol 1,4,5-trisphosphate 5-phosphatase and the inhibition of binding of Ins(1,4,5)P_3 to the type 1 inositol 1,4,5-trisphosphate receptor rather than to the inhibition of SHIP2 the *in vivo* effects of this compound will not be discussed further.

Compounds AS1949490 (**9**) and AS1938909 (**10**) cause a dose-dependent increase in insulin-induced phosphorylation of Akt/PKB (UniProtKB ID P31749, P31751, Q9Y243) in L6 myotubes [107][108]. This phosphorylation activates Akt/PKB leading to an increase in glucose uptake and metabolism in L6 myotubes, this increased uptake being associated with greater expression of GLUT1 mRNA and more GLUT1 protein (UniProtKB ID P11166). In cultured hepatocyte FAO cells AS1949490 decreased insulin-induced gluconeogenesis. When given to normal mice AS1949490 was found to do this by suppressing the expression of proteins involved in gluconeogenesis: the levels of mRNA coding for PEPCK (UniProtKB ID P35558, Q16822) and G6Pase (UniProtKB ID P35575, Q9NQR9, Q9BUM1) were reduced by approximately 50%. In diabetic *db*/*db* mice the twice daily oral administration of AS1949490 significantly reduced plasma glucose levels with no effect on food intake, body weight or insulin levels, and in an oral glucose tolerance test significantly reduced both fasting blood glucose and the area under the blood glucose concentration time curve. These antidiabetic effects were found to be a consequence of an enhancement of insulin signalling: the phosphorylation and, hence, activity of GSK3β (UniProtKB ID P49841) was increased without changing the amount of GSK3β.

Compound CPDA (**15**) has also been investigated for its effects on insulin signalling [122]. In 3T3-L1 adipocytes CPDA antagonizes the attenuation of the insulin-induced phosphorylation of Akt2 (UniProtKB ID P31751) caused by tumour necrosis factor α (TNFα, UniProtKB ID P01375), and does so more potently than AS1949490. This greater potency of CPDA in stimulating the phosphorylation of Akt was observed, also, in primary cultured neuronal cells from the rat cerebral cortex. In C57BL/6J mice the twice daily oral administration of 300mg/kg had no effect on fasting blood glucose levels, and normal glucose tolerance was observed in a glucose tolerance test. However, in *db*/*db* mice fasting blood glucose levels fell significantly and, during the glucose tolerance test, CPDA significantly reduced the rise in blood glucose levels compared to controls. As with AS1949490 the amounts of PEPCK and G6Pase were significantly reduced in CPDA-treated mice.

Compounds 1PIE (**17**), 6PTQ (**18**) and 2PIQ (**19**) inhibit both SHIP1 and SHIP2 [59]. In three multiple myeloma cell lines (RPMI8226, OPM2 and U266) all three inhibitors at a concentration of 7.5μM caused statistically significant levels of cell death with some variation between inhibitor and cell line. The more selective SHIP1 inhibitor 3AC (**21**) was less effective in killing the cells. Treatment of the multiple myeloma cells with 10μM 1PIE (**16**) found that the cells went into cell cycle arrest at the G_2/M phase with an increase in the number of cells in the sub-G_0/G_1 phase and activation of the intrinsic apoptotic pathways. The viability of two breast cancer cell lines (MDA-MB-231 and MCF-7) was severely reduced by treating them with 1PIE (**17**), 6PTQ (**18**) or 2PIQ (**19**). This is significant because these cell lines do not express SHIP1, so the cell-killing effects of these compounds are probably due to SHIP2 inhibition. This is supported by the finding that the addition of PtdIns(3,4)P_2 (the product of the SHIP2-catalysed reaction) to MCF-7 cells treated with these inhibitors rescued the cells while having little effect on cells that had not been treated with the inhibitor.

The finding that SHIP2 activity is stimulated in the presence of phosphatidylserine vesicles [5a] suggests that interaction with membranes may stimulate SHIP2 activity. However, since the SHIP2 substrate is found embedded in membranes it is possible that this enhanced activity is actually the default level of activity *in vivo* because the enzyme will necessarily be interacting with, or at least be in very close proximity to, the membrane. Phosphatidylserine is normally held facing the cytosolic side of the cell membrane. During apoptosis it flips to face the cell exterior where it acts as a signal for macrophages to engulf cells [123]. It is possible that this is of significance because, as discussed above, SHIP2 is known to play a role in apoptosis both through its interactions with a number of proteins involved in the apoptotic process, and because inhibition of SHIP2 by **17**, **18** and **19** causes apoptosis [59]. Furthermore, if SHIP2 does interact with phosphatidylserine *in vivo* then it has to dissociate before the phosphatidylserine can flip to face the cell exterior.

9. Summary and Outlook

In the sections above what is known about the structure of SHIP2 has been discussed, as have the interactions a number of proteins can make with the various SHIP2 domains and motifs. It should be stressed that several of these interactions have been observed only under the conditions of *in vitro* experiments and there might be, as yet, little or no evidence that these interactions occur *in vivo*.

The interactions have been discussed on the basis of the role of the proteins, i.e. some are involved in cytoskeletal function and apoptosis, and others in immune system function: others, not discussed, might be involved in insulin signalling. It should be noted that some of the proteins with which SHIP2 has been reported to interact have a role in more than one of these areas. All the studies of SHIP2 interactions with other proteins have been performed with isoform 1 of the protein, i.e. the full-length protein. There is little information about the interactions made by isoform 2 which lacks the *N*-terminal 242 residues of isoform 1 and, hence, the SH2 domain. It is not known if the expression of isoform 2 is temporally or spatially regulated, or what proportion of the SHIP2 in a cell is isoform 2.

The *in vitro* and *in vivo* modulation of SHIP2 activity by inhibitors and activators are then discussed. The known inhibitors of SHIP2 have been assayed either for their ability to interfere with insulin signalling or to induce apoptosis, but none of them for both. (The role of SHIP2 inhibitors in the modulation of immune system function or bone ossification has not been studied.) The specificity and selectivity of most of the inhibitors have not been determined to any real extent, and most have IC_{50} values in the low-to-mid micromolar range so, for at least some of them, may not be potent enough for medicinal use: any progress towards medicinal use has not been revealed.

Several activators of SHIP1 are known and it is disappointing that there is SHIP2 activation data for only one of these compounds (AQX-16A, **28**) [33]. The increase in SHIP2 activity in the presence of phosphatidylserine vesicles raises the possibility that this greater level of activity could be the basal level of activity in cells. It is not known how phosphatidylserine interacts with SHIP2, but it is reasonable to suppose that it is not in the substrate binding site. PTEN is stimulated by phosphatidylserine which induces a conformational change in the protein structure [124] suggesting that allosteric activation of SHIP2 is a possibility. It is not known whether, or to what extent, SHIP2 inhibitors will inhibit phosphatidylserine-activated SHIP2. If SHIP2 is allosterically activated by phosphatidylserine and the activation changes the size or shape of the substrate binding site then compounds that inhibit the non-activated form of the enzyme may not inhibit the activated form (and *vice versa*) or have a different level of inhibitory activity.

The ligand-binding domain has a flexible loop that can close over the substrate binding site [41], though the crystal structure of the apo-enzyme has the same open structure as the structure with a ligand in the substrate binding site. When the loop is closed it makes more interactions with the ligand and expels some waters from the binding site. This greatly complicates the use of the protein structure in structure-based drug discovery programs: to what extent should the loop be closed over the binding site when embarking on a virtual screening program? Depending on the size of the ligand the loop will be able to close over it to different extents.

Many small molecule, peptidic and peptidomimetic inhibitors of SH2 domains have been described [42][125] though none appears to have been tested for its effects on SHIP2. The effects of a SHIP2 SH2 inhibitor are hard to predict because of the current lack of knowledge of the function of isoform 2 of SHIP2.

It is over twenty years since SHIP2 was discovered. In that time much has been learnt about the role of SHIP2, but there are still many gaps in our knowledge. Do all the proteins shown by *in vitro* experiments to interact with SHIP2 do so *in vivo* and, if so, with what effect on cellular function? What are the (presumably differing) roles of the two SHIP2 isoforms? Will roles for SHIP2 be confirmed in areas other than bone ossification and opsismodysplasia? What role does SHIP2 over- or under-expression play in disease development? What role, if any, will SHIP2 inhibitors play in the treatment of disease? Can more potent and/or selective SHIP2 inhibitors be developed? These, and other questions, will, hopefully, be answered in the next few years, leading to a greater understanding of the role of SHIP2.

Link to Supporting Information.

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Keywords: enzyme • inhibitor • structure • phospholipid • phosphatase

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Layout 1:

REVIEW

The SHIP2 phosphatase catalyses dephosphorylation at the 5-position of phosphoinositides, mediating diverse biological effects, but much still remains unknown. Inhibitors come from a range of structural classes, but still merit further development. This review brings together interdisciplinary facets of SHIP2 progress and discusses possible future directions.

*Mark P. Thomas, Christophe Erneux & Barry V. L. Potter**

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SHIP2: Structure, Function and Inhibition

Layout 2:

REVIEW

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