Ligand Binding at the IP3-Binding Core of the IP3 Receptor

Lessons for small molecule design

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Introduction

When Otto Loewi remarked in 1959, “Ja Kalzium, das ist alles!,” the field of chemical biology was only in its infancy and only some of Ca^{2+}’s many roles as a second messenger in intra- and inter-cellular signalling had been elucidated. Today, Loewi has been perhaps more vindicated than even he could have imagined. Atomic calcium binds to a variety of proteins to deliver a variety of different signals in different cellular contexts - for example, in neurons, it can cause the release of synaptic vesicles, while in muscle cells it leads to contraction. At high levels, calcium can cause apoptotic signalling[1].

Calcium is also regulated by a range of cellular factors. The phospholipase C pathway is perhaps the most common. To initiate this signalling pathway, an extracellular ligand binds to a transmembrane G protein-coupled receptor or a receptor tyrosine kinase, which activates the phospholipase C enzyme (PLC). PLC then catalyzes the hydrolysis of phosphatidylinositol diphosphate (PIP2), a membrane-bound phospholipid, into inositol triphosphate (IP3) and diacylglycerol (DAG), both of which are second messengers in their own right. DAG remains bound to the cell membrane and goes on to recruit protein kinase C. Meanwhile, IP3 diffuses away from the membrane to the endoplasmic reticulum, where it binds to an IP3 receptor glycoprotein complex. This receptor, when activated, functions as a Ca^{2+} channel, and releases calcium from the endoplasmic reticulum into the cytoplasm[2].

The basic structure of the phospholipase C pathway has long been known. However, even now researchers are working to develop ways to understand and manipulate the system at an ever-finer level of control. The system can be chemically interfered with at several levels, but the most promising and direct may be the action of small molecules at the IP3 receptor, causing the release of calcium from the ER directly. This avenue of research presents a problem of target-oriented design: how can a molecule be prepared to specifically have agonist or antagonist action at the IP3 receptor? In order to approach this problem, two steps are necessary. First, the character of the receptor itself, its structure and its nature, must be examined. Second, the existing and known ligands of the receptor, and derivatives of them, must be analyzed to determine the chemical and structural components that determine their activity. Once those two steps are complete, it may be possible to begin the process of rationally designing new small molecule ligands for the IP3 receptor.

Character of the IP3 Receptor: Subtypes

[1] Joseph and Hajnoczky, 2007
[2] Rohacs, 2013

The IP3 receptor is actually, in mammals, expressed in three different subtypes[3]. These share about 60-80% of their primary structure and almost all of their function. They are named IP3 receptor types 1-3, or IP_3R1, IP_3R2, and IP_3R3. Among these subtypes, critical regions, such as the ligand binding domain, are almost perfectly conserved. It is unlikely, but possible, that variation between subtypes will affect the ability of small molecular tools to interface with calcium signalling at the IP3 receptor.

Character of the IP3 Receptor: Tetramericity

It has long been known that individual IP3 receptors are each tetrameric, composed of four subunits, in various cases homomeric or heteromeric. However, it was only determined this year that all four subunits must be bound by IP3 for substantial Ca^{2+} release[4]. Heteromeric receptors containing three functional subunits and one ligand binding-deficient subunit failed to release Ca^{2+} from the endoplasmic reticulum. These data were collected under conditions lacking native-type IP3 receptors and using careful recordings of the calcium response, demonstrating that binding of four separate IP3 molecules is indeed required for activation of the calcium response. Presumably, this finding is also applicable to the binding of artificial small molecule ligands, such as adenophostin A, and such stoichiometry must be remembered in the design of experiments to test potential ligands.

Character of the IP3 Receptor: Calcium as a Ligand

Until this point, description of the IP3 receptor has focused, naturally, IP3 itself and its analogues. However, activation of the receptor actually requires a second substance: the calcium ion itself. This has been known since the early days of the receptor's study[5]. Ca^{2+} in the cytoplasm and at the N-terminus of the IP3 receptor modulates the activity of the IP3 receptor biphasically: at low concentrations, it enhances the IP3-response; at high concentrations, it inhibits that response. That is to say, the probability of a particular IP3 receptor's calcium channel opening displays a bell-shaped curve as a function of $[Ca^{2+}]$. This behavior is seen across all three subtypes of the IP3 receptor. Researchers do not seem to be unanimous in their interpretations of this data or in their structural models explaining it, but there is one especially compelling model. This model[6] proposes two separate calcium binding sites: one stimulatory and the other inhibitory. It further suggests that the exclusive role of IP3 or other ligands is not to directly open the ion channel but rather to strongly promote calcium binding to the stimulatory rather than the inhibitory site by causing the

[3] Taylor et al., 1999
[4] Alzayady et al., 2016
[5] Suematsu et al., 1984
[6] Taylor and Tovey, 2010

stimulatory site to become accessible and the inhibitory site to be concealed within the protein. Under this model, IP3, priming the inhibitory Ca^{2+} site, prepares calcium to instantaneously control the opening of the channel.

It is not entirely clear, though, exactly how calcium manifests its stimulatory and inhibitory actions. It may be through direct binding or through an accessory Ca^{2+} binding protein or molecule. There are two potential Ca^{2+} binding sites in the IP3-binding core; however, point mutations of their acidic amino acid residues failed to have any effect on Ca^{2+} regulation of the IP3 receptor calcium channel[7]. A third potential site, in the cytoplasmic region but outside of the binding core, is in the area of the Glu2100 residue. When that residue is altered, sensitivity of the IP3 receptor to calcium decreases[8]. This region may be a critical target for future studies of IP3 receptor ligands: if a small molecule can be synthesized that interacts with this target so as to block or amplify the effect of calcium there, such a molecule could have major utility in experiments to modulate or interfere with calcium signalling at the IP3 receptor.

Structure of the IP3 Receptor

Two recent studies, both published in *Nature*, have identified critical elements of the IP3 receptor's structure. The first, from the Zu laboratory at the University of Pennsylvania, reports the crystal structure of the receptor's ligand-binding domain both in apo and in IP3-bound configurations[9]. The second, from the Taylor laboratory at the University of Cambridge in the United Kingdom, verifies and builds upon the findings of the first.

The ligand-binding domain (LBD) of the IP3 receptor is approximately 600 amino acid residues long and located at the N-terminus of the protein. It is coupled to the transmembrane pore domain and, at the level of secondary structure, is composed of two beta-trefoil folds (B-TF1 and B-TF2) and armadillo repeat fold (ARF). The latter two of those three regions constitute the IP3-binding core which directly interacts with IP3. [see appendix 1, fig. 1] It, alone, binds IP3 more tightly than does the entire LBD or the entire protein. The Ip3-binding core was, almost two decades ago, shown to include residues 226-578, and to rely in its function upon the critical residues Arg265, Lys508, and Arg511[10] as well as seven other important but nonessential side chains. This was elucidated through mutational analysis, a "reverse genetic" approach. It was also noted that binding affinity increased over the pH range 5-9, a range over which protons dissociate from IP3's phosphate groups; this suggests that binding is due to interaction

[7] Joseph et al., 2005
[8] Tu et al., 2003
[9] Lin et al., 2011
[10] Yoshikawa et al., 1996

between the negatively-charged phosphates of IP3 and the positively-charged basic amino acid residues of the receptor[11].

Although this, among other earlier studies, illuminates to some extent the mechanism by which IP3 binds its receptor, it does not fully explain the nature and implications of that binding. To address these questions, the Zu lab produced high-resolution structures of the IP3 receptor under IP3-bound and -unbound conditions [see appendix 1, fig. 2].

The binding of IP3 to the LBD results in a slight change in the relative orientation of the three lobes. B-TF2, a component of the binding core, moves slightly closer to the ARF, binding IP3 between the two regions in an example of induced fit. The interface between ARF and B-TF2 is the most dynamic region of the LBD, showing the greatest movement and change in response to IP3 binding. The flexibility of this region allows the binding of IP3 to impose conformational changes on the LBD and, in fact, on the rest of the protein, causing the LBD to come to a "bound state that favors opening of the ion pore"[12]. As B-TF2 and ARF come together around a ligand molecule, B-TF1 couples that conformational change in the LBD to an opening of the Ca^{2+} gate.

In a structure of only the IP3-binding core of the protein (B-TF2 and ARF) it was found that those two regions approached even closer, when binding IP3, than they do in the full structure; this validates the theory that B-TF1 has an inhibitory effect on the binding of the LBD.

The authors of this structural research in the Zu laboratory conclude that because the linker between B-TF1 and ARF is static while that between ARF and B-TF2 is flexible, the former interface is responsible for coupling to the ion pore such that the LBD allosterically modulates the flow of calcium ions. They also conclude that the former interface, as it is dynamic, spontaneously transitions between states in the absence of IP3 but is locked in a position corresponding to the opening of the channel when IP3 is present.

The other recently-published experiment dealing with the structure of the IP3 receptor LBD upon the binding of ligand, from the Taylor laboratory, came to similar conclusions about the conformational changes induced in the IP3 receptor. Referring to to the B-TF1 region as the "suppressor domain", they identified the same key roles for the inter-region interfaces and for the role of B-TF1 as the linkage coupling the IP3-binding core to the ion channel. Furthermore, they identified a specific region of B-TF1 located opposite the IP3-binding core, its "hotspot loop" as "a critical link between [IP3] binding and gating". Mutation of Tyr167, located on the hotspot loop, attenuated the opening of the calcium channel in response to IP3 binding. This discovery only further validates the structural model of the LBD. Additionally, the presence of Ca^{2+} ion

[11] Yoshikawa et al., 1996
[12] Lin et al., 2011

in the cytoplasm causes this region (specifically, residue Ser171) to become more accessible to the cytoplasm[13].

Analysis of Small Molecule IP3 Receptor Ligands: Adenophostin A

Perhaps the first paper to identify a small molecule ligand for the IP3 receptor, from Japan in 1993, isolated adenophostin A from *Penicillium brevicompactum* and found it to bind to human IP3 receptor an order of magnitude more tightly than IP3 itself. Basing their analysis on the regions of similarity between adenophostin A and IP3, the researchers proposed that the 4- and 5- phosphate groups of IP3 are the regions most responsible for its biochemical activity[14] [see appendix 1, fig. 3]. These results have been corroborated over time. Additionally, several nonspecific inhibitors of the IP3 receptor have long been known, including 2-aminoethoxydiphenyl borate, caffeine, and xestospongins, but none of these are competitive or specific and so are of very limited utility in the laboratory[15].

Analysis of Small Molecule IP3 Receptor Ligands: Inositol Derivatives

More recently, though, in a search for understanding of the mechanism of activation of the IP3 receptor, and target-oriented development and synthesis of a ligand for that receptor, the Taylor laboratory explored a particularly clear avenue: the analysis of analogues of IP3 itself[16]. Researchers in that laboratory synthesized and characterized several small molecules similar to (1,4,5)IP3, the natural ligand: malachite green (1,4,5)IP3, a variant with a chromophore attached at the 1' group; 2-deoxy (1,4,5)IP3; 3-deoxy(1,4,5)IP3; (1,3,4,5)IP4; and (1,4,6)IP3, in which the orientations of the 2- and 3-hydroxy groups had been inverted.

Syntheses for these compounds and the compounds themselves have long been available, and it is not within the scope of this paper - focusing as it must upon recent research - to describe these syntheses. Regardless, the IP3 analogues were evaluated on two criteria: first, their ability to cause the release of Ca^{2+} from the ER; second, their ability to block the binding of ^{3}H labeled IP3 to IP3 receptors.

The various IP3 analogues displayed similar behavior across the various IP3 receptor subtypes. Of the analogues, 2-deoxy (1,4,5)IP3 showed the tightest binding and the highest release of Ca^{2+}. Malachite green (1,4,5)IP3 showed the second-highest affinity (second-lowest EC_{50}) and an only somewhat-lower total release of Ca^{2+}. However, interestingly, (1,4,6)IP3 caused the highest total release of Ca^{2+}, actually

[13] Anyatonwu and Joseph, 2009
[14] Takahashi et al., 1994
[15] Mills et al., 2012
[16] Saleem et al., 2013

greater than that caused by (1,4,5)IP3 itself, but its binding affinity was two orders of magnitude lower than that of the natural ligand.

The results of the study shed some light on the importance of various structural aspects of IP3 to its binding with its receptor. For example, removal of the 2- and 3-OH groups from the molecule showed the same effect on binding interactions as their stereochemical inversion caused by the movement of the 5' phosphate group to the 6' location. This equivalence implies that the precise location of those groups is as important to the ligand's function as their presence, though that they are not absolutely essential to binding.

Malachite green (1,4,5)IP3 is an analogue in which 4-carboxy-malachite green (MG) is attached to the 1'-phosphate with an aminopropyl linkage. It functions as a full agonist of the IP3 receptor, though with a seven-fold lower affinity than IP3 itself. This level of efficacy, despite the large moiety attached to the 1'-phosphate, indicates that although that phosphate plays some role in binding to the receptor, it is not critical that it be embedded deeply in the IP3 receptor protein. This interpretation is reinforced by the observation that although (4,5)IP2 binds the receptor at an 83-fold loss in efficiency compared to IP3, it is nonetheless able to compete with ^{3}H-(1,4,5)IP3 for binding. Clearly the 1'-phosphate group is not entirely necessary. This confirms the 1993 conclusion that the 4' and 5' phosphate groups are in fact the functional elements essential to IP3 binding.

A different approach was taken to inositol-based ligands by the Conway group, five years ago[17]. Researchers synthesized four derivatives of IP3, adding four different phosphate bioisosteres at the 5-position [see appendix 1, fig. 4]. The synthesis of the four derivatives was based, first, on the nine-step creation from cyclohexa-hexanol of a inositol-like intermediate, phosphorylated at the 1- and 4-positions, open at the 5-position, and protected with benzyl groups at the remaining positions. To the 5-position was added a variety of functional groups (a methyl phosphate ester, a sulfate group, a methylphosphonate, and a carboxymethyl), and the remaining positions were deprotected to hydroxy groups, resulting in the four derivatives to be tested.

The ability of these small molecules to bind to IP3 receptors was tested through their effect on calcium release. Results of the assays indicated that the methylphosphonate derivative and the carboxymethyl derivative function as inhibitors in the 1-5mM range, blocking IP3-induced calcium release and failing to cause calcium release themselves. However, the methyl phosphate ester and the methylphosphonate functioned as activators of calcium release, the former potently with an EC_{50} of 9.7uM and the latter less potently with an EC_{50} in the 100-300uM range.

[17] Keddie et al., 2011

The comparisons between these four molecules have interesting implications for an understanding of the IP3 receptor binding mechanism. The researchers theorize that, because the two agonists have, in their functional groups, the same number of atoms capable of forming polar contacts as a phosphate group, they interact differently with the IP3 receptor. Specifically, the compounds with fewer sites for polar contacts at the 5-position are capable of occupying the binding site, but not of causing the conformational change in the IP3-binding core that is transmitted by the B-TF1 domain to cause opening of the calcium channel. Specifically, they are not capable of causing the ARF and B-TF2 regions to "clamp" together. This importance attributed to the functionality of the 5-position of the inositol is coherent with the earlier theory that the 4- and 5-phosphates are the groups most critical and necessary for the activation of the IP3 receptor.

Analysis of Small Molecule IP3 Receptor Ligands: Adenophostin A Biphosphates

The evaluation of IP3's mechanism of action and the nature of its chemical binding at the IP3 receptor through comparison with adenophostin A (AdA) was continued twenty years later in a different laboratory halfway around the world. Barry Potter's lab in the United Kingdom sought to understand the IP3 receptor by probing it with synthetic small molecules similar to adenophostin A. Specifically, they synthesized and experimented with all three of its possible biphosphate analogues, aiming to identify which pair of phosphate groups is more critical to the function of the molecule. The three analogues were 3''-dephospho-AdA, 4''-dephospho-AdA, and 2'-dephospho-AdA. Adenophostin A, though, was originally obtained through isolation from bacterial cells, and the synthesis of its analogues presents technical difficulties that the Potter lab was forced to overcome.

The general scheme of the Potter lab synthesis of the adenophostin A biphosphates began with the preparation of a disaccharide base, carefully differentiating the hydroxy groups intended to become phosphorylated from the rest. This differentiation was accomplished by using two different types of protecting groups on the hydroxy groups - one that could be removed easily and one that was more stable. Then a Vorbrüggen condensation was used to attach a purine at the appropriate location. The synthetic pathway was concluded with deprotection of the more-labile hydroxy protecting groups, those groups' conversation into phosphates, and finally deprotection of the remaining hydroxy groups.

The exact syntheses of the three biphosphates were each complex procedures of at least a dozen steps each, but many of those steps were common to all three syntheses. Each one started with a polyacylated glucose. Butane 2,3-diacetals were added to protect certain oxygen atoms of the glucose, including those destined for phosphorylation, while others were left unprotected. At this point the 1-acetoxy group

was also converted to ethyl sulfide. The unprotected oxygen atoms were then benzylated. Benzyl had been chosen as the stronger protecting group, designated to remain present through phosphorylation. The butane diacetals were removed. If necessary, one of the resulting hydroxy groups was also selectively benzylated using tetrabutylammonium hydrogen sulfate under biphasic conditions for 74% selectivity. The remaining hydroxy group(s) were acylated, the acylation serving as a more labile protecting group to be later replaced by phosphorylation. N-Iodosuccinimide was used to glycosylate a prepared ribofuranose with the prepared glycosyl group, relying the presence of the 1'-ethyl sulfide and resulting the presence of the proper alpha-glycoside. After treatment with trifluoroacetic acid, this alpha-glycoside was prepared for the Vorbrüggen condensation and the attachment of adenine. Then the two acetate protecting groups, wherever they were located of the three possible positions, were ammonolytically removed. The resulting hydroxy groups were phosphitylated with a phosphoramidite, and in the following step oxidized to phosphates. Finally, the remaining oxygen atoms were deprotected to hydroxy groups by the removal of the benzylation.

This basic procedure was used in the synthesis of 3''-dephospho-AdA and of 4''-dephospho-AdA, with only minor differences between the two, but a somewhat different approach was needed for 2'-dephospho-Ada due to the necessity of a 2' hydroxy group. Solution of this difficulty required synthesis of a benzylated triphosphate, followed by treatment with a mild base, potassium carbonate, in phenol. Because of the 2' phosphate's greater stereoavailability and accessibility to nucleophilic attack relative to the 3'' and 4'' phosphates, it was disproportionately formed "in excellent yield". In this case, as well as the cases of the other biphosphates, results were confirmed and the product was isolated by ion exchange column chromatography.

Upon synthesis of these compounds, the next step was their testing for efficacy as ligands of the IP3 receptor. All three were found to have some affinity as agonists at the receptor, leading to release of Ca^{2+} into the cytoplasm, but both affinity and potency varied. 2'-dephospho-AdA was the highest in potency, resulting in release of about ¾ of the stored calcium from the ER - the same proportion as AdA or IP3, which are considered full agonists, while 3''-dephospho-AdA released only 53% and 4''-dephospho-AdA only 8%. However, although 2'-dephospho-AdA was resulted in the same summative effect as the known full agonists IP3 and AdA, its binding affinity was lower; its EC_{50} for binding to the IP3 receptor was four-fold lower than IP3 and 40-fold lower than AdA.

The significantly higher efficacy of 2'-dephospho-AdA relative to the other biphosphates indicates, again, the greater importance of the 4- and 5-position phosphates in IP3 relative to the 1-position phosphate. Another important implication of the use of adenophostin A as an agonist at the IP3 receptor, though, has to do with its

potency even greater than that of IP3 itself. Some researchers have accredited this long-known but surprising result to cation-pi interactions between the adenine group (which can, in some cases, be replaced by an aromatic equivalent) and the Arg504 residue in the IP3-binding core of the receptor[18] [see appendix 1, fig 5]. As it seems that the primary importance of the 2'-phosphate in AdA, corresponding to the 1-phosphate of IP3, is more necessary for anchoring the ligand to the receptor than for causing the change in conformation that actually opens the ion channel, it is sensible that the presence of the adenine, fulfilling an equivalent function, would compensate in part for loss of that phosphate group in 2'-dephospho-AdA.

It also seems to be the case that 3''-dephospho-AdA, corresponding to (1,5)IP2, is a somewhat stronger agonist of the IP3 receptor than is 4''-dephospho-AdA. Although neither small molecule is useful as an agonist, this difference points to the critical importance of the 5-phosphate group in IP3. This is the same group that was so thoroughly examined by the Conway group and found to cause differentiation between stimulatory and inhibitory activities at the IP3 receptor. In this case, the Potter laboratory confirms the importance of specific functionality at that location for not only binding between the ARF and the B-TF2 domains, but also for causing the conformational change that opens the ion channel.

Analysis of Small Molecule IP3 Receptor Ligands: Aromatic Polyphosphates

Non-inositol based aromatic polyphosphates are the third category of substance that show promise as ligands of the IP3 receptor. Their synthesis is often simpler than that of IP3 or AdA molecules, avoiding the issue of differentiation between hydroxy groups only some of which are to be phosphorylated.

The first such molecule synthesized and tested was biphenyl 2,3',4,5',6-pentakisphosphate, by the Erneux laboratory in Belgium in 2007[19] [see appendix 1, fig. 6]. This molecule was synthesized in two steps - a much more straightforward process than the creation of the adenophostin A analogues. In the first step, biphenyl-2,4,6,3',5'-pentanol was treated with diethyl chlorophosphite under conditions of CH_2Cl_2 and N,N-diisopropylethylamine. In the second step, 3-chloroperoxybenzoic acid was added, and the product was purified with flash chromatography at 76% yield. The researchers also experimented with benzyl (1,2,4,5)-tetrakisphosphate.

Binding to the IP3 receptor was measured with Ca^{2+} release and with an ^{3}H-IP3 competition assay and it was found that the pentakisphosphate significantly bound the IP3 receptor, with 50% displacement observed at a concentration of only 1.4uM. Bz(1,2,4,5)-tetrakisphosphate was also found to result in 43% inhibition at a

[18] Sureshan et al., 2012
[19] Vandeput et al., 2007

concentration of 10uM. These compounds, though, unlike AdA, did not serve to activate the IP3 receptor and lead to calcium release from the ER. Rather, they functioned as competitive inhibitors of the IP3-motivated opening of the IP3 receptor calcium channels, blocking the release of ionic Ca^{2+}.

This approach was continued by the Potter laboratory in 2012[20]. Researchers in that laboratory analyzed benzyl (1,2,4)-triphosphate and 3-hydroxybenzyl (1,2,4)-triphosphate, along with related compounds, including several linked polyphosphorylated biphenyls. They first found that addition of another phosphate group to Bz(1,2,4)3P, forming Bz(1,2,4,5)4P (as discussed above), significantly increased its binding affinity and inhibitory effect at the IP3 receptor. This led those researchers to hypothesize that "increasing the number of phosphate groups around a core could enhance antagonism" and to test that hypothesis with linked polyphosphorylated biphenyls - such as the previously-discussed biphenyl (2,3',4,5',6)-pentakisphosphate, as well as biphenyl (2,2',4,4',5,5')-hexakisphosphate and others.

BiPh(2,2',4,4',5,5')P6 was synthesized from two molecules of 1,3,4-trimethoxybenzene, joined together by an aryl-aryl bond with a molybdenum(V) chloride reagent. This dibenzene was then stripped of its six methoxy groups, which were ultimately replaced with phosphates to form the final active product.

That product, BiPh(2,2',4,4',5,5')P6, was found to be the most potent inhibitor of the IP3 receptor, with an IC_{50} half that of Bz(2,3',4,5',6)5P and of the tested Bz(1,2,4)P3 dimer. In order to chemically explain this inhibition, it is helpful to compare this compound to the known stimulatory ligands of the IP3 receptor: IP3 itself and the adenophostins. BiPh(2,2',4,4',5,5')P6, and the other aromatic polyphosphorylated inhibitors, are like these compounds in the presence of phosphate groups around their central rings, but perhaps the key difference is the flexibility of those central rings. Both IP3 and AdA, though they tend to remain in one particular conformation, are flexible; the polyphosphorylated aromatics are rigid. Phosphate groups attached to a benzyl core have fewer degrees of freedom. It is also perhaps noteworthy that, of the several biphenyls tested by the Potter group, the substances with less flexibility between their aromatic cores are the more potent inhibitors.

In comparison to the earlier evaluation of the nature of IP3 receptor inhibitors formed by substitution of functional groups at the 5-position of inositol sheds further light on the features that distinguish IP3 receptor stimulators from inhibitors by illustrating that flexibility of the ligand is necessary for stimulatory function.

[20] Mills et al., 2012

Conclusion

By examining the lessons learned from the three various types of ligand tested for the IP3 receptor, it is possible to understand some of the chemical characteristics needed for stimulatory activity at that receptor. Although the precise details of the interaction between the ligand and the receptor remain unknown, it is possible to venture certain conclusions based on the primary literature.

It may be the case that for a small molecule to show agonist activity at that receptor, it must have three characteristics. First, it must fit within the binding site of the IP3 receptor. This implies the presence of negatively-charged groups at the equivalents of the 1,4, and 5-positions of IP3, though the 1-position is of somewhat lesser importance. This fit is encouraged by the presence of adenine or a similar aromatic ring, as in adenophostin A. Second, it must have the correct functionality, with two sites for polar binding activity, at the equivalent of the 5-position of IP3. This group is responsible for fully forming the bridge between the ARF and B-TF2 regions of the IP3-binding core of the receptor and for causing the conformational change that pulls together those two regions and opens the ion channel to release Ca^{2+}. Third, and finally, may be necessary for the ligand to be flexible enough to change, though slightly, in conformation along with the receptor. If that flexibility is not present, the receptor will perhaps not be able to “clamp down” on the ligand and change its conformation.

Appendix 1: Figures. All figures are taken from the paper being discussed in the corresponding section of the essay.

Figure 1: Conformational change in the LBD of the IP3 receptor in response to IP3 binding (left: unbound, right: bound)

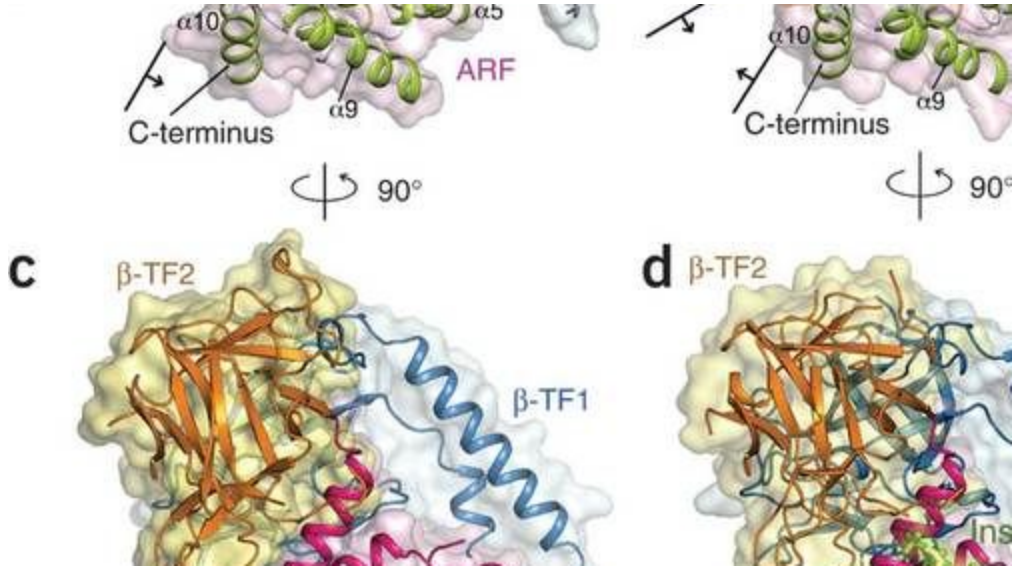


Figure 2: Residues to which IP3 binds in the LBD of the IP3 receptor (left: bound, right: unbound)

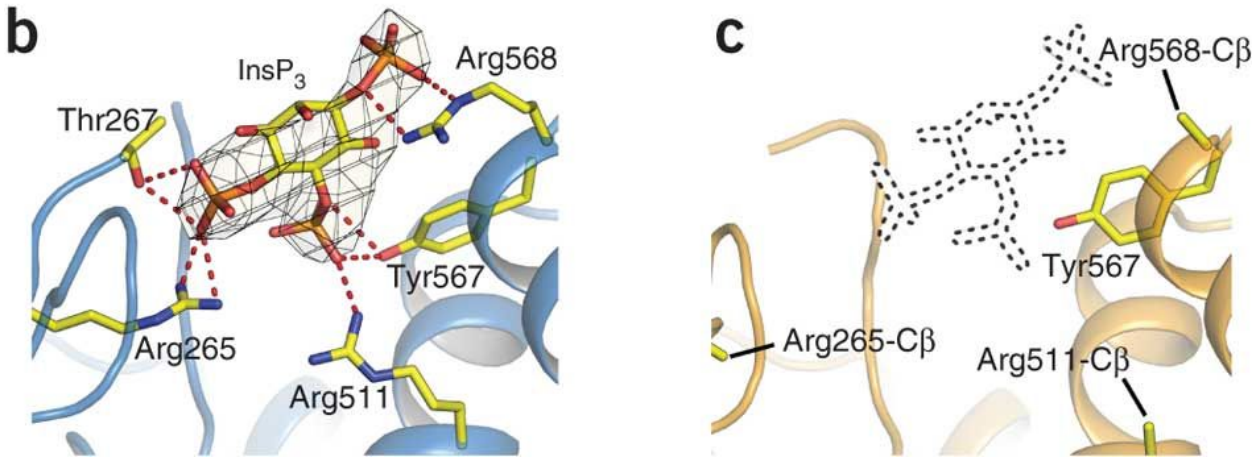


Figure 3: IP3 (left), adenophostin A (right)

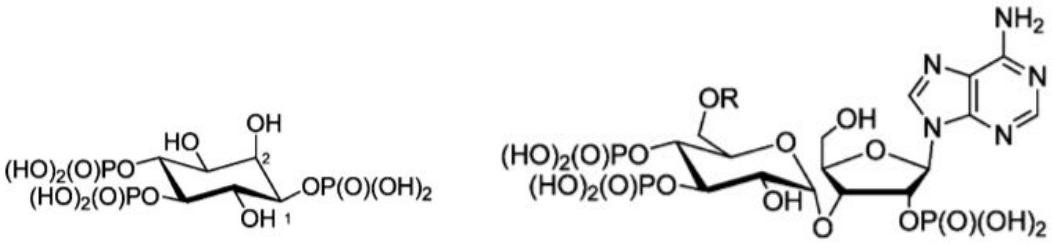


Figure 4: Derivatives of IP3

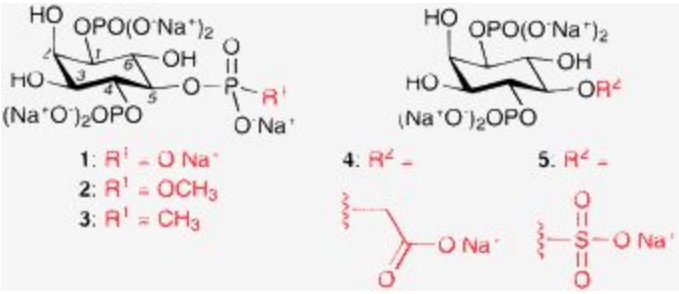
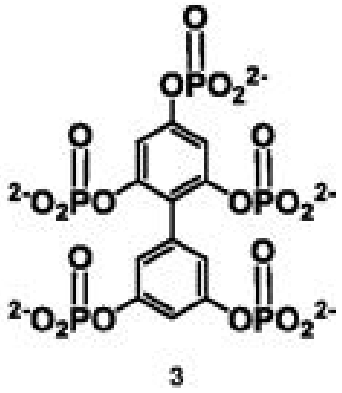


Figure 5: Interaction between R504 and the adenine group of AdA

Figure 6: Biphenyl 2,3',4,5',6-pentakisphosphate



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Appendix 2: Sources

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