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# Food Web–Specific Biomagnification of Persistent Organic Pollutants

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Substances that accumulate to hazardous levels in living organisms pose environmental and human-health risks, which governments seek to reduce or eliminate. Regulatory authorities identify bioaccumulative substances as hydrophobic, fat-soluble chemicals having high octanol-water partition coefficients ( $K_{OW}$ ) ( $\geq 100,000$ ). Here we show that poorly metabolizable, moderately hydrophobic substances with a  $K_{OW}$  between 100 and 100,000, which do not biomagnify (that is, increase in chemical concentration in organisms with increasing trophic level) in aquatic food webs, can biomagnify to a high degree in food webs containing air-breathing animals (including humans) because of their high octanol-air partition coefficient ( $K_{OA}$ ) and corresponding low rate of respiratory elimination to air. These low  $K_{OW}$ –high  $K_{OA}$  chemicals, representing a third of organic chemicals in commercial use, constitute an unidentified class of potentially bioaccumulative substances that require regulatory assessment to prevent possible ecosystem and human-health consequences.

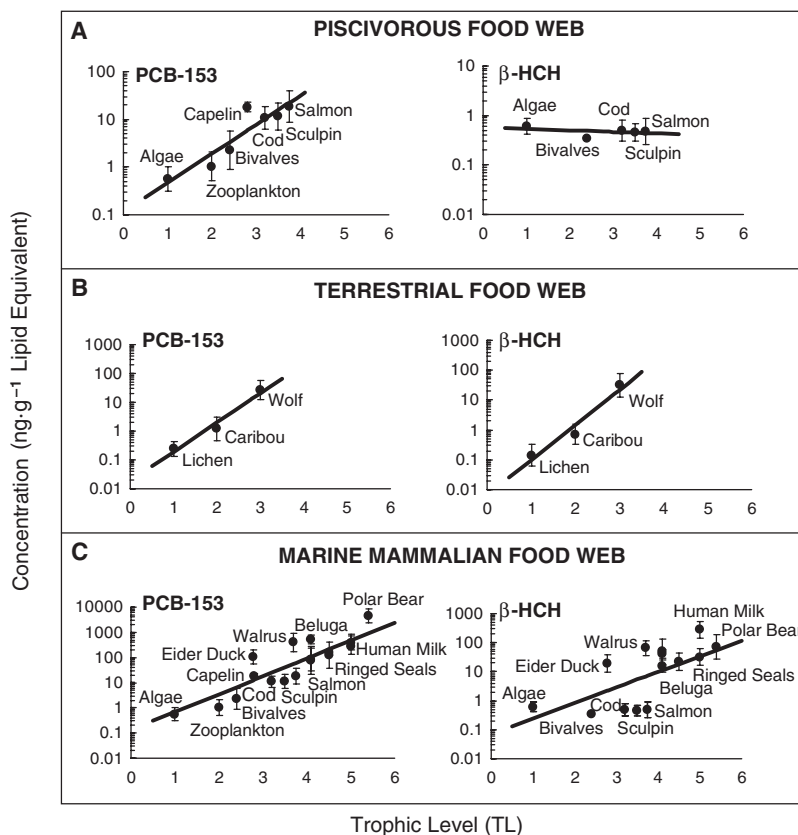
The Stockholm Convention on Persistent Organic Pollutants was endorsed by 131 nations in 2004 to eliminate the world's most persistent bioaccumulative and toxic substances (1). Such substances include polychlorinated biphenyls (PCBs) and dichlorodiphenyltrichloroethanes (DDTs), which are highly accumulative and can cause adverse health effects in fish, wildlife, and humans (2–6). Brominated flame retardants and certain perfluorinated chemicals have recently emerged as new contaminants of concern (7, 8). Governments worldwide are currently evaluating all commercial chemicals, with the goal to identify substances that can biomagnify in food chains and achieve harmful concentrations in high-trophic level organisms including human beings.

To identify bioaccumulative substances, regulatory authorities rely on the chemical's  $K_{OW}$  or, when available, on organism/water chemical concentration ratios measured in laboratory tests [bioconcentration factors (BCFs)] or in the field (bioaccumulation factors) (9). These criteria were derived from laboratory tests in fish, which revealed correlations between the  $K_{OW}$  and the BCF of organic chemicals and showed that bioconcentration could be explained and predicted by lipid-water partitioning (10). Studies in real food webs demonstrated that bioaccumulation in food webs is not solely a lipid-water partitioning process. Dietary accumulation or biomagnification can cause additional bioaccumulation, resulting in an increase in chemical concentration with increasing trophic level in food webs (11). This apparent chemical transport against

the thermodynamic gradient is due to food digestion and absorption, which concentrate ingested chemicals in the gastrointestinal tract (12, 13). Poorly metabolizable, hydrophobic substances with  $K_{OW} \geq 10^5$  have proven particularly susceptible to biomagnification in fish (14), whereas chemicals with lower  $K_{OW}$  do not generally biomagnify in fish.

However, less hydrophobic chemicals (e.g., chlorobenzenes and lindane), with  $K_{OW} < 10^5$  and with BCFs in fish-based experiments below the regulatory criterion of 5000, were found to biomagnify in lichen-caribou-wolf food chains in northern Canada (15, 16). Also, perfluorinated sulfonic acids such as perfluorooctane sulfonate, which (with a calculated  $K_{OW} < 10^5$ ) do not biomagnify in laboratory tests with fish (17), show a high degree of biomagnification in birds and mammals (8, 18, 19). These findings indicate that very hydrophobic chemicals with a  $K_{OW} \geq 10^5$  are not the only chemicals with biomagnification potential and that lipid-water partitioning cannot serve as a universal model for identifying bioaccumulative substances in wild-life and humans.

To test the applicability of current regulatory criteria for identifying bioaccumulative substances, we measured and compiled concentrations of organic contaminants of varying hydrophobicity and  $K_{OW}$  in a piscivorous food web (water-respiring organisms only), a terrestrial food web (air-breathing organisms only), and a combined marine mammalian food web (including water-respiring and air-breathing organisms) from northern Canada (20) (fig. S2). We also used mechanistic food web bioaccumulation models to determine the influence of



**Fig. 1.** Relationship between observed tissue residue concentrations (ng·g<sup>-1</sup> lipid equivalent) and trophic level for PCB 153 (a high  $K_{OW}$ –high  $K_{OA}$  compound) and β-HCH (a low  $K_{OW}$ –high  $K_{OA}$  compound) in Arctic organisms of the piscivorous (A), terrestrial (B), and marine mammalian (C) food webs. Data represent geometric means  $\pm$  1 SD.

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$K_{OW}$  on chemical bioaccumulation in these food chains (20, 21).

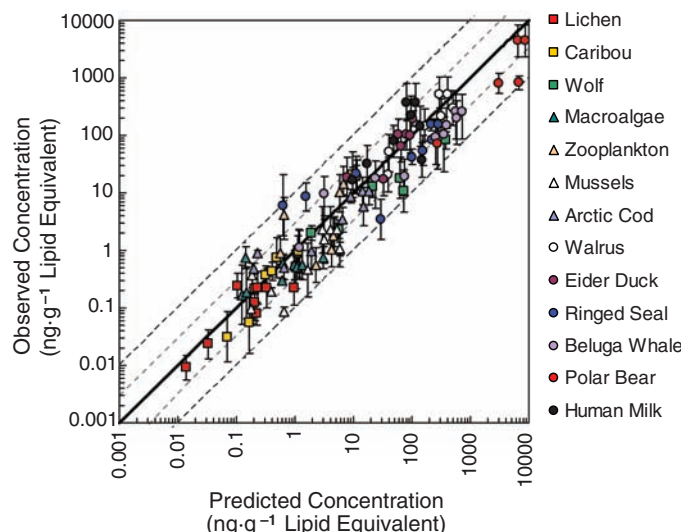
In field studies, we observed strong positive relationships ( $r^2 > 0.8$ ,  $P < 0.001$ ) between trophic level and concentrations of recalcitrant PCB congeners 138, 180, and 153; mirex; hexachlorobenzene (HCBz); dieldrin; and DDTs in

all three food webs (Fig. 1 and fig. S4, A to C). These and related findings (22–24) support the current regulatory and scientific paradigm that very hydrophobic organic chemicals with a  $K_{OW} \geq 10^5$  can biomagnify in food webs. However, although less hydrophobic compounds ( $K_{OW} < 10^5$ ) such as  $\alpha$ ,  $\beta$ , and  $\gamma$  hexa-

chlorocyclohexanes (HCHs) ( $K_{OW} = 10^{3.8}$ ), tetrachlorobenzenes (TeCBz) ( $K_{OW} = 10^{4.1}$ ), and endosulfans ( $K_{OW} = 10^{3.7}$ ) did not biomagnify in the piscivorous food web, they showed a high degree of biomagnification in the lichen-caribou-wolf food chain and in air-breathing organisms of the marine mammalian food web (Fig. 1 and fig. S4, D to F). The predator-prey biomagnification factors or BMFs (25) of  $\beta$ -HCH in ringed seals of 20 and in beluga whales of 50 exceed the BMFs of PCB 180 in those animals. These findings demonstrate that although substances with a  $K_{OW}$  below  $10^5$  cannot biomagnify in fish, they can biomagnify in birds and mammals.

Bioaccumulation modeling studies (20) showed good agreement between calculated and observed concentrations of recalcitrant substances in all three food webs (Fig. 2). The model shows that air-breathing organisms in this analysis exhibit higher BMFs than those in water-respiring organisms because of their greater ability to absorb and digest their diet (Table 1), which is related to differences in digestive tract physiology and body temperature. The model also shows that the relationship between the BMF and chemical properties is controlled by the rate of elimination. In water-

**Fig. 2.** Model predicted versus observed concentrations ( $\text{ng}\cdot\text{g}^{-1}$  lipid equivalent) in various organisms of the Canadian Arctic terrestrial and marine ecosystems. Observed data represent geometric mean  $\pm$  1 SD. The solid black line represents an ideal model fit (1:1 predicted:observed line). Dashed lines represent a factor of 3 (gray dashes) and 10 (black dashes) above and below the ideal fit.



**Table 1.** Calculated BMFs (25) of selected industrial chemicals in aquatic invertebrates, fish, reptiles, amphibians, birds, nonhuman mammals, and humans for organic chemicals varying in molecular weights ( $M_W$ ,  $\text{g}\cdot\text{mol}^{-1}$ ),  $\log K_{OW}$ , and  $\log K_{OA}$ . No metabolic transformation was assumed. Biomagnification occurs if  $\text{BMF} > 1$ . Low  $K_{OW}$ –low  $K_{OA}$  chemicals show no biomagnification in

water-respiring or air-breathing organisms because of efficient respiratory elimination to air and water. Low  $K_{OW}$  ( $\log K_{OW} \sim 2$  to 5)–high  $K_{OA}$  ( $\log K_{OA} \sim 6$  to 12) chemicals exhibit no biomagnification in water-respiring organisms but biomagnify in air-breathing organisms. High  $K_{OW}$ –high  $K_{OA}$  chemicals biomagnify in water-respiring and air-breathing organisms.

Chemical*	$M_W$ (g·mol <sup>-1</sup> )†	Log $K_{OW}$ †	Log $K_{OA}$ †	Water-respiring organisms			Air-breathing organisms						
				Zooplankton	Forage fish	Predatory fish	Reptile	Amphibian	Seabird	Marine mammal	Terrestrial herbivore	Terrestrial carnivore	Human
Low $K_{OW}$ –low $K_{OA}$													
1,4 butadiene	145	1.9	2.9	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
1,3,5 TriCBz	182	3.5	4.7	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
Hexachlorobutadiene	261	4.5	3.9	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
Styrene	150	2.5	4.9	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
Low $K_{OW}$ –high $K_{OA}$													
Dicofol	371	3.5	8.9	<1	<1	<1	6.1	12	16	42	3.9	76	76
β-endosulfan	391	3.7	7.1	<1	<1	<1	4.9	11	10	22	2.5	28	23
β-HCH	291	3.8	8.9	<1	<1	<1	7.5	12	17	45	4.0	85	84
Musk xylene	297	4.1	8.9	<1	<1	<1	8.5	12	18	47	4.1	90	89
Trifluralin	350	4.4	7.2	<1	<1	<1	6.2	11	12	26	2.8	34	28
Tetradifon	356	4.6	11.4	<1	<1	<1	9.4	12	19	49	4.1	97	97
1,2,4,5 TeCBz	247	4.7	6.3	<1	<1	<1	2.4	7.3	3.9	7.3	1.0	7.6	5.9
High $K_{OW}$ –high $K_{OA}$													
PeCBz	247	5	6.9	<1	<1	<1	4.8	10	8.4	17	2.1	21	16
Dieldrin	381	5.4	8.8	1.7	2.4	2.3	9.6	12	19	48	4.1	94	93
HCBz	285	5.5	7.7	1.8	2.7	2.7	8.4	12	16	38	3.6	62	55
PCB 153	361	7.5	9.6	2.5	5.0	7.7	9.6	12	18	49	3.3	97	96
PCB 180	381	7.5	10.9	2.5	5.1	8.0	9.3	12	18	47	2.4	95	95
PBDE 47	308	6.0	9.8	2.2	4.0	5.1	9.7	12	19	49	4.1	98	98
PBDE 99	342	6.8	11.2	2.5	4.9	7.5	9.7	12	19	49	3.6	98	98
Mirex	546	8.1	9.6	1	1	2	8.2	10	14	16	1	82	82
PBDE 209	600	9.9	13.1	1	1	1	1	1	1	3	1	8	8

\*Chemical abbreviations not provided in the text include TriCBz (trichlorobenzene), PeCBz (pentachlorobenzene), PCB 153 (2,2',4,4',5,5' hexachlorobiphenyl), PCB 180 (2,2',3,4,4',5,5' heptachlorobiphenyl), PBDE 47 (2,2',4,4' tetrabromodiphenyl ether), PBDE 99 (2,2',4,4',5 pentabromodiphenyl ether), and PBDE 209 (2,2',3,3',4,4',5,5',6,6' decabromodiphenyl ether). †See supporting online material for physical-chemical properties.

respiring organisms, elimination becomes sufficiently slow to cause biomagnification if the  $K_{OW}$  of the chemical exceeds  $\sim 10^5$ . In the air-breathing organisms of this study, this occurs for chemicals with a high  $K_{OA}$  ( $\geq 10^6$ ), which causes slow respiratory elimination, and a  $K_{OW} > 10^2$ , causing slow elimination in urine or nitrogenous wastes (Table 1). The difference in biomagnification behavior between air-breathing and water-respiring organisms implies that, for substances with a  $K_{OA} \geq 10^6$  and a  $K_{OW} > 10^2$ ,  $K_{OW}$  and the BCF in fish are not good predictors of biomagnification in air-breathing animals.

Application of the bioaccumulation model to identify potentially bioaccumulative substances among commercial chemicals reveals distinct differences in the biomagnification behavior of chemicals in different food webs (Fig. 3). In the piscivorous food web, concentrations of non-metabolizing chemicals with  $K_{OW}$  between  $10^5$  and  $10^8$  biomagnify in top-level predatory fish up to 100-fold (Fig. 3A). No biomagnification occurs for less hydrophobic chemicals with  $K_{OW} < 10^5$ , which are efficiently eliminated by respiration, or for superhydrophobic organic substances with  $K_{OW} > 10^8$ , which are absorbed at very slow rates (26–28). However, in the marine mammalian food web (Fig. 3B), which includes water-respiring invertebrates and fish and air-breathing birds and mammals, poorly metabolizing chemicals with a  $K_{OW} \geq 10^5$  and  $K_{OA} \geq 10^6$  biomagnify, attaining concentrations in top predators (polar bears) up to 10,000 times the concentrations in primary producers. Less hydrophobic chemicals with  $K_{OW} < 10^5$  and  $K_{OA} \geq 10^6$  also biomagnify strongly, with concentrations in polar bears exceeding those in primary producers up to 3000-fold. Chemicals

with  $K_{OW} < 10^2$  do not biomagnify in this food web regardless of their high  $K_{OA}$  because air-breathing animals eliminate them through urinary excretion. In terrestrial food webs, chemicals with a  $K_{OW}$  between  $10^2$  and  $10^{10}$  and a  $K_{OA} \geq 10^6$  can biomagnify up to 400-fold if not metabolized (Fig. 3C). Chemicals with a  $K_{OW}$  between  $\sim 10^3$  and  $10^9$  achieve a similar degree of biomagnification, given the same  $K_{OA}$ .

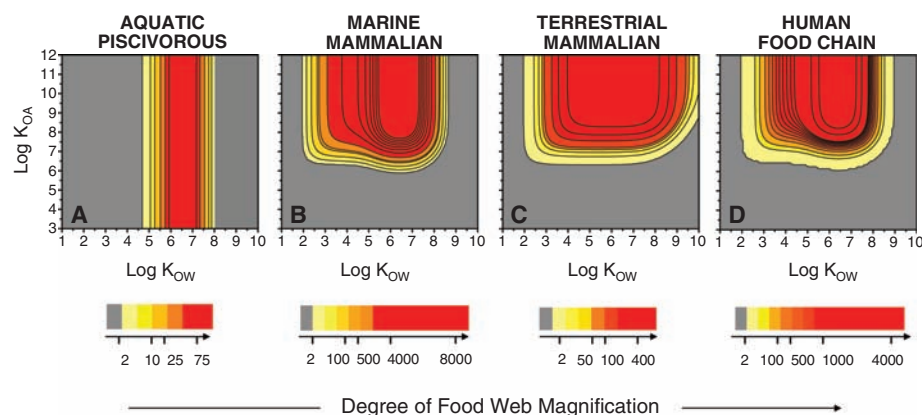
Simulations, representing human dietary exposure of contaminants to the indigenous Inuit population of northern Canada, show that chemical amplifications can reach  $\sim 4000$ -fold for chemicals with a  $\log K_{OW} \geq 5$  and a  $\log K_{OA} \geq 6$  and 2000-fold for chemicals with a  $\log K_{OW}$  between 2 and 5 and a  $\log K_{OA}$  above 6 (Fig. 3D). The modeling results are consistent with empirical observations for PCBs, chlordanes, HCBz, dieldrin, and DDTs, which have both a high  $\log K_{OW} \geq 5$  and a high  $\log K_{OA} \geq 6$  and biomagnify in all three Arctic food webs studied (fig. S4). The results are also consistent with observations of HCHs, TeCBz, and endosulfans, which have a low  $\log K_{OW} < 5$  but a high  $\log K_{OA} \geq 6$  and exhibit no biomagnification in the piscivorous food web but biomagnify in air-breathing organisms.

Organic chemicals with a  $K_{OW} > 10^2$  and a  $K_{OA} \geq 10^6$  (i) have an inherent biomagnification potential in air-breathing organisms of terrestrial, marine mammalian, and human food chains and (ii) include almost two-thirds of all organic chemicals used in commerce (29) (fig. S6). About 40% of chemicals with these properties have a  $K_{OW} > 10^5$  and are currently recognized as potentially bioaccumulative because of their high degree of lipid-water partitioning. The remaining 60% of these chemicals, which include substances with a  $K_{OW}$  between  $10^2$  and  $10^5$  and

a  $K_{OA} \geq 10^6$ , should also be considered for their bioaccumulative potential because of their high degree of lipid-air partitioning. These low  $K_{OW}$ -high  $K_{OA}$  chemicals are susceptible to biomagnification in air-breathing animals, including humans, because of their slow rate of respiratory elimination. Metabolic transformation can reduce or eliminate the anticipated biomagnification potential but only if the metabolic transformation rate is sufficiently high. In those cases, the bioaccumulation behavior of resulting metabolites should also be considered.

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**Fig. 3.** Contour plots illustrating the relationship between chemical  $K_{OW}$  (x axis),  $K_{OA}$  (y axis), and food web magnification (z dimension represented as contours) in the aquatic piscivorous food web (A), marine mammalian food web (B), terrestrial mammalian food chain (C), and Arctic indigenous human food chain (D). The data represent the combined magnification of the chemical concentrations ( $\text{ng}\cdot\text{g}^{-1}$  lipid equivalent) in the top predator over the concentrations at the base of the food web [e.g., primary producers at trophic level (TL) = 1 to polar bear at TL = 5.4]. A matrix table was generated with  $\sim 30,000$   $K_{OW}$ - $K_{OA}$  combinations over a  $\log K_{OW}$  range of 1 to 10 and a  $\log K_{OA}$  range of 3 to 12. These data demonstrate the combined effect of  $K_{OW}$  and  $K_{OA}$  on chemical bioaccumulation.



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#### Supporting Online Material

www.sciencemag.org/cgi/content/full/317/5835/236/DC1  
Materials and Methods

Figs. S1 to S6  
Tables S1 to S11  
References

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# Mechanism of Two Classes of Cancer Mutations in the Phosphoinositide 3-Kinase Catalytic Subunit

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Many human cancers involve up-regulation of the phosphoinositide 3-kinase PI3K $\alpha$ , with oncogenic mutations identified in both the p110 $\alpha$  catalytic and the p85 $\alpha$  regulatory subunits. We used crystallographic and biochemical approaches to gain insight into activating mutations in two noncatalytic p110 $\alpha$  domains—the adaptor-binding and the helical domains. A structure of the adaptor-binding domain of p110 $\alpha$  in a complex with the p85 $\alpha$  inter-Src homology 2 (inter-SH2) domain shows that oncogenic mutations in the adaptor-binding domain are not at the inter-SH2 interface but in a polar surface patch that is a plausible docking site for other domains in the holo p110/p85 complex. We also examined helical domain mutations and found that the Glu<sup>545</sup> to Lys<sup>545</sup> (E545K) oncogenic mutant disrupts an inhibitory charge-charge interaction with the p85 N-terminal SH2 domain. These studies extend our understanding of the architecture of PI3Ks and provide insight into how two classes of mutations that cause a gain in function can lead to cancer.

Phosphoinositide 3-kinases and their lipid product, phosphatidylinositol-(3,4,5)-trisphosphate [PtdIns(3,4,5)P<sub>3</sub>], play key roles in a variety of cellular processes (1–3). Aberrations in PtdIns(3,4,5)P<sub>3</sub> levels, either through activation of PI3Ks or through inactivation of lipid phosphatase PTEN, occur frequently in numerous forms of cancers. For example, recent data suggest that at least 50% of human breast cancers involve mutations in either PI3K $\alpha$  or PTEN (4, 5). Broad-spectrum PI3K inhibitors such as LY294002 or wortmannin result in increased apoptosis, decreased proliferation, and reduced metastasis in vitro and in tumor models [reviewed in (6–8)]. Understanding the structural mechanisms of PI3K regulation may facilitate development of isozyme-specific therapeutics.

The class IA PI3Ks are obligate heterodimers (9), consisting of a p110 catalytic subunit and a regulatory subunit. Any of the three class IA catalytic subunits (p110 $\alpha$ , p110 $\beta$ , and p110 $\delta$ )

can bind any of the p85-related regulatory subunits. Regulatory subunits have multiple roles in the function of PI3K: down-regulation of the basal activity, stabilization of the catalytic subunit, activation downstream of receptor tyrosine kinases, and sequential activation by tyrosine kinases and Ras (10–13). Common to all regulatory subunits are two SH2 domains (nSH2 and cSH2) that flank an intervening domain (iSH2), and common to all catalytic subunits are the N-terminal adaptor-binding domain (ABD), the Ras-binding domain (RBD), the putative membrane-binding domain (C2), and the helical and catalytic domains (Fig. 1A). The iSH2 domain is responsible for tight binding to the ABD (14). The nSH2 and cSH2 domains bind phosphorylated tyrosines in Tyr-X-X-Met motifs found in activated receptors and adaptor proteins, and this interaction activates the heterodimeric PI3K. The nSH2-iSH2 unit constitutes the minimal fragment capable of regulating the PI3K activity (15): It both inhibits the basal activity and facilitates activation by binding phosphotyrosine peptides. In contrast, the isolated iSH2 only minimally affects the PI3K activity, although it tightly binds the p110 subunit.

A number of studies have identified a high frequency of somatic point mutations in the gene encoding the p110 $\alpha$  catalytic subunit in different human cancers (16, 17). An increased lipid kinase activity in vitro and the ability to induce oncogenic transformation in vivo were shown both for the most frequently mutated, “hotspot” residues (16, 18–20) and for 14 rare cancer-specific mutations in p110 $\alpha$  (21). The

cancer-specific mutations can be grouped into four classes defined by the four domains of the catalytic subunit in which they occur—the ABD, C2, helical, and catalytic domains—and it has been proposed that these classes may increase PI3K activity by different mechanisms (21, 22). Hotspot mutations in the catalytic domain cluster around the “activation loop” involved in substrate recognition (23) and are likely to share a common mechanism (21). The ABD binds tightly to the regulatory subunit, the C2 domain is thought to interact with the plasma membrane, and the helical domain appears to act as a rigid scaffold around which the RBD, C2, and catalytic domains are mounted (24). The catalytic and C2 domain mutations may up-regulate PI3K by increasing the affinity for substrate-containing membranes. However, it is not immediately clear what might be the mechanism of helical domain mutations. We used structural and biochemical approaches to understand the basis for gain-of-function mutations in the ABD and helical domains of p110 $\alpha$ . Because the ABD is the only p110 domain for which there is no known structure, we crystallized it in a complex with the iSH2 domain from p85. This structure suggested a rough preliminary model for the p110/p85 heterodimer, which led us to hypothesize that the nSH2 domain might contact the helical domain. To understand how helical domain oncogenic mutations function, we created a series of site-specific mutations in the nSH2 domain, resulting in an adaptor subunit that specifically counteracts the p110 helical domain hotspot E545K mutant.

We determined the crystal structure of a complex between the bovine p110 $\alpha$  ABD (residues 1 to 108) and the human p85 $\alpha$  iSH2 domain (residues 431 to 600) at 2.4 Å resolution (25), revealing the interaction of the small, globular ABD (35 by 25 by 15 Å) with one end of the long, rodlike iSH2 (115 Å long) (Fig. 1B). The ABD is similar to many ubiquitin-like domains: It superimposes on ubiquitin with a root mean square deviation of 1.4 Å for 60 out of 76 residues (Fig. 1C). The  $\beta$ -grasp fold of both the ABD and ubiquitin is a common fold, and neither sequence nor function suggests a common origin for the ABD and ubiquitin. The ABD ranks as one of the least conserved domains among class I catalytic subunits. Conversely, the iSH2 domain is highly conserved in vertebrates, sharing >90% sequence similarity from human to zebrafish (fig. S1). Despite a lack of sequence similarity, secondary and tertiary structure predictions for the class IB p110 $\gamma$  ABD are consistent with a ubiquitin-like fold, similar to the class IA p110 ABD. However, the greater sequence divergence of the p110 $\gamma$  ABD makes it

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