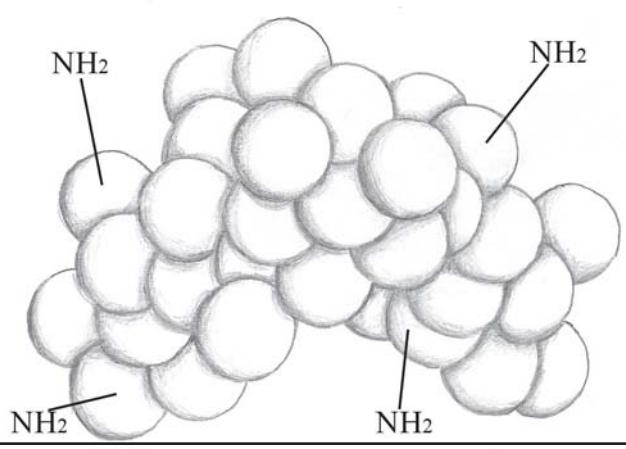


"New information about PDZ interactions emerges at an astounding rate, reinforcing the idea that these specialized protein regions may offer the prospect of novel treatments against diseases ranging from cancer to schizophrenia."

A New Magic Bullet

Targeting PDZ Interactions in Disease Pathways

By Ching Zhu

William James in 1880 made fun of the theory of evolution by suggesting that it "is a change from a no-howish untalkaboutable all-alikeness by continuous sticktogetheration and somethingelsification" (1). While evolution remains controversial to many, there is little doubt that living processes may indeed be defined as a "continuous sticktogetheration." Put another way, dynamic interaction between proteins is the basis of the tiny biochemical engines that constitute the machinery of life. This biological machinery can malfunction when protein interactions go awry, leading to the onset of physiological disease. One increasingly important type of sequence-specific protein interaction involves binding sites known as PSD95/DlgA/ZO-1 protein domains, or PDZ domains. Discovered in the early 1990s by three separate research groups, the PDZ domain has continued to attract attention as a possible target for modulating cellular pathways. New information about PDZ interactions emerges at an astounding rate, reinforcing the idea that these specialized protein regions may offer the prospect of novel treatments against diseases ranging from cancer to schizophrenia (2).

Credit: Chelsea Gordon, HSR

What is the PDZ Domain?

PDZ domains were originally identified as brief, homologous sequences of approximately 90 amino acids within the postsynaptic density-95 protein (PSD95) (3), the *Drosophila* discs large tumor suppressor (DlgA) (4), and the tight-junction-associated protein zonula occludens-1 (ZO-1) (5). The domain thus derives its name from these three proteins. PDZ interactions occur when a PDZ domain binds to a compatible region generally located at the extreme carboxyl terminus of a target protein. The wide variety of proteins that contain PDZ domains and/or compatible PDZ binding regions makes it possible for PDZ interactions to form highly complex protein networks (6).

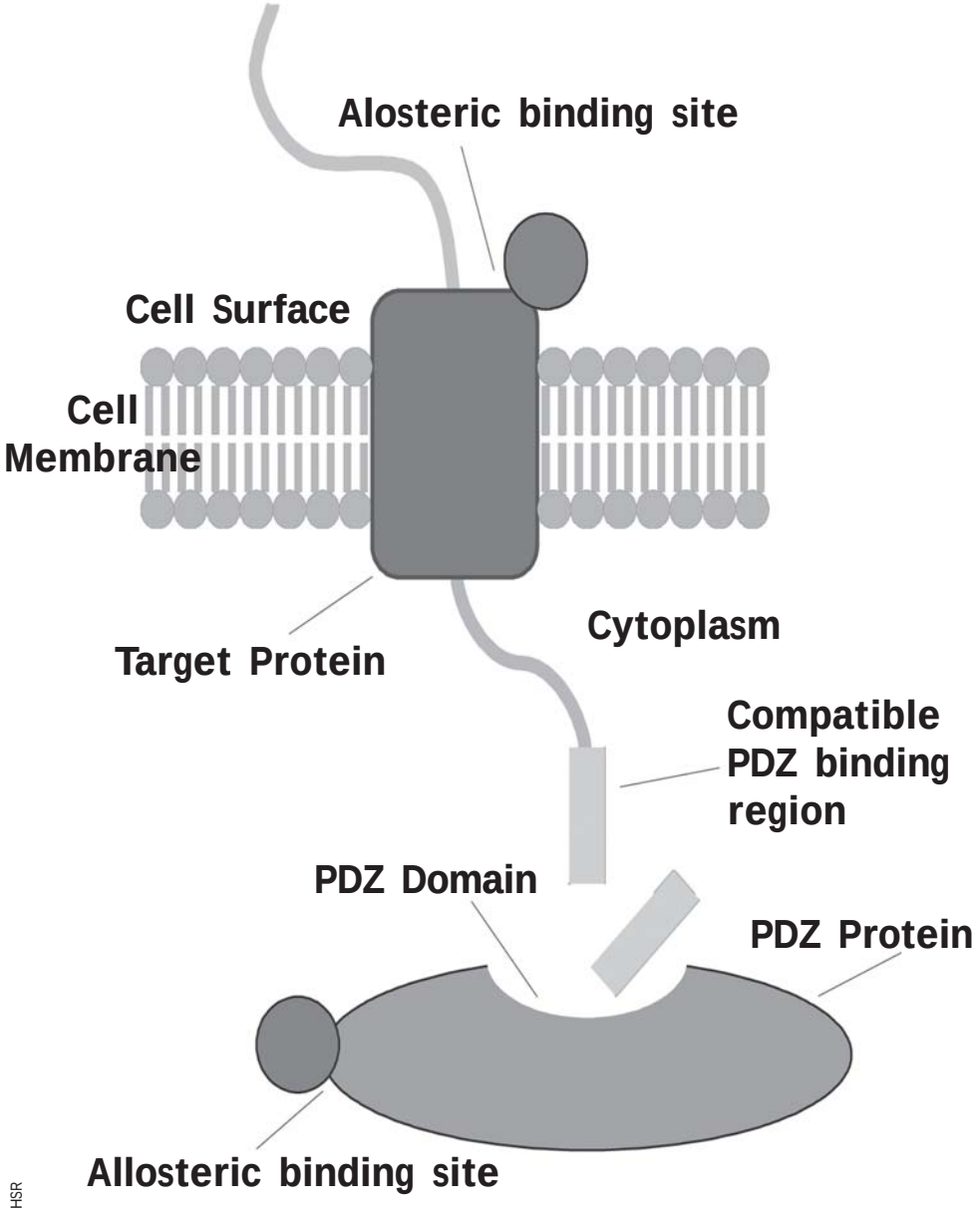
Well over a hundred functionally unrelated proteins contain one or more PDZ regions. These proteins are almost always associated with cell membranes and appear in organisms as diverse as metazoans, bacteria, and plants. Such specific localization within the cell and widespread species distribution suggest that PDZ mechanisms evolved early as important elements in plasma membrane processes governing cell morphology, cell migration, and intercellular contact (6).

Physiological Roles of PDZ Interactions

Countless connections between PDZ activity and physiological disorders have been suggested over the years. For example, the PDZ interaction between the proteins connexin43 and ZO-1 may be a crucial element in cardiac pathologies such as arrhythmia. Proper contraction of the heart depends on the circuitry of intercellular gap junction proteins called connexins, which allow the flow of electrical current throughout cardiac muscle (7). In 1998, it was discovered that the second PDZ domain of ZO-1 binds to the last five amino acids of connexin43 (8), the most abundant gap junction protein in the human heart (9). Because ZO-1 is also connected to the actin cytoskeleton, a transport system for cellular proteins, it may act as a mediating protein, anchoring connexin43 to the cytoskeleton. Thus, the dynamic networking of cardiac gap junctions, vital to proper contraction of heart muscle, may depend upon the PDZ interaction between ZO-1 and connexin43 (10).

Perhaps one of the best examples of the extensive pathological importance of a single PDZ domain is the protein PICK1 (Protein Interacting with C-al-

pha Kinase). The PDZ domain of PICK1 binds with proteins involved in numerous psychiatric, neurological, and neurodegenerative disorders (Figure 2). For example, PICK1 interacts with monoamine transporters, which are vital to neurotransmission. Irregularities in monoamine neurotransmission have been linked to depression, attention-deficit disorder, schizophrenia, and Parkinson's disease (11). Because PICK1 has been shown to increase ion channel activity in neurons, it may also play a role in pain sensitization by boosting the excitability of sensory neurons (12). Moreover, PICK1 has been shown to interact with three types of proteins involved in cancer (2). That a single PDZ protein may play a role in so many diseases is a testament to the far-reaching consequences of PDZ malfunction.



Credit: Dana Lazarus, HSR

Figure 1. The many methods of targeting PDZ interactions include (a) using competitive peptides that mimic the compatible PDZ binding region of a target protein, (b) altering the conformation of the compatible PDZ binding region by allosteric inhibition of the target protein, or (c) altering the conformation of the PDZ domain by allosteric inhibition of the PDZ protein (2).

Targeting the PDZ Domain to Regulate Disease Pathways

The numerous links between PDZ mechanisms and physiological disorders provide significant impetus for developing drugs to specifically target the PDZ domain in disease pathways. Many approaches could be taken to fine-tune faulty PDZ machinery (See Figure 1). The high specificity of PDZ binding makes the domain a good target for inhibition by peptides, or active "mini-proteins." Such peptides would be designed to mimic PDZ binding regions and would thus "stick" to PDZ domains, preventing them from interacting with their target proteins (2).

Because PDZ interactions occur in the cytoplasm (13), disrupting these interactions may require drugs that reach deep inside the cell. This presents a complication to the peptide inhibition approach: the peptides must be able to pass through the cell membrane (a formidable barrier against large molecules) and reach proteins within the cell. Possible methods of transporting peptides across the membrane do exist and include direct injection into the cell (14) and delivery by carrier molecules such as Tat protein from human immunodeficiency virus (15). But these procedures also face technical challenges.

An alternative approach which sidesteps the problem of membrane permeability is the use of small molecules to occupy allosteric sites on a target protein, since many of these allosteric sites are located outside the cell membrane. Allosteric binding alters the configuration of the compatible region on the target protein and can thus disrupt the PDZ interaction without actually coming into contact with the PDZ domain. Additionally, inhibitory molecules such as antisense and RNA interference (RNAi) may also be used to regulate PDZ protein activity. However, the use of such molecules will require additional gene delivery and expression (2).

Target protein (to which PDZ domain of PICK1 binds)	Physiological or pathological significance of PICK1 interaction	Reference
DAT	**Depression, schizophrenia, Parkinson's disease**	11
ASIC2a	**Pain**	12
ErbB2	**Cancer**	2
Glu_2	**Sound stimulation, pain sensitization**	2
Glu_5-2b	**Epilepsy**	19
PrRP	**Food intake**	20

Credit: Ching Zhu, HSR

Figure 2. The single PDZ domain of PICK1 has extensive physiological importance.

Implications for Drug Discovery

Complex diseases such as cancer often disrupt more than one physiological pathway (16), posing a serious challenge to drug developers. However, drugs targeting PDZ interactions might be especially effective against diseases affecting multiple signaling pathways because the PDZ domain is so ubiquitous. That is, a single pathway may involve numerous PDZ-containing proteins (6). Thus, the regulation of a specific protein *interaction* rather than a specific protein makes it possible to inhibit more than one protein and influence more than one signaling cascade involved in a disease (2).

Because side effects present a major threat to the success of any drug, specificity is a key factor in the development of new drugs (17). The regulation of PDZ activity has the advantages of many dimensions of specificity. Drugs made to specifically target the binding partner of a PDZ protein would avoid disrupting other vital pathways involving the same PDZ protein. Furthermore, factors such as domain structure, electrostatic interactions, and hydrogen bonding may also play a role in determining PDZ binding properties (2). Currently, how these factors work in concert remains an open question (18). A clearer picture of PDZ binding dynamics would allow even higher degrees of specificity in developing treatment strategies (2).

The Potential of PDZ Domains

Since its discovery over ten years ago, a wealth of scientific data has called attention to the significance of the PDZ domain in driving life processes. The possibility of influencing PDZ interactions at the molecular level makes this domain a promising target for drug development. However, there is still much to learn about PDZ machinery. Though the protein sequences involved may be short, PDZ interactions are anything but simple, and much remains to be explored in pushing this important protein domain to the forefront of drug discovery. H

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