Live and Let Die: insights into pseudoenzyme mechanisms from structure

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Abstract

Pseudoenzymes were first described more than 50 years ago, when it was recognised that a subset of proteins that are structurally homologous to active enzymes lack amino acids necessary for catalytic activity. Recently, interest in pseudoenzymes has surged as it has become apparent that they constitute ~10% of proteomes and perform essential metabolic and signalling functions that can be experimentally distinguished from catalytic outputs of enzymes. Here, we highlight recent structural studies of pseudoenzymes, which have revealed the molecular basis for roles as allosteric regulators of conventional enzymes, as molecular switches and integrators, as hubs for assembling protein complexes, and as competitors of substrate availability and holoenzyme assembly. As structural studies continue to illuminate pseudoenzyme molecular mechanisms, we anticipate that our knowledge of the breadth of their biological functions will expand in parallel.

Introduction

Genomic sequencing of many organisms confirms that nearly all enzyme families include pseudoenzyme homologues, which are predicted to be enzymatically inactive due to the loss of key catalytic amino acid residues that perform a role in co-factor or substrate binding. Pseudoenzymes are already known to be conserved in ~20 different protein families [1], including well-studied examples of pseudokinases, pseudophosphatases and pseudoproteases [2-7]. In these families, incremental changes in catalytic and substrate-binding sites created new evolutionary trajectories that led to the evolution of pseudoenzymes from enzyme templates sharing a similar fold [8,9]. Although an absence of conserved catalytic residues is not proof of catalytic deficiency, very high sequence and/or structural conservation suggests that pseudoenzymes have been functionally selected across all branches of life, and preserved to regulate cell biology in a catalytically-independent manner. Although pseudoenzymes comprise a significant percentage of proteomes, we understand little about individual classes relative to their enzyme counterparts. However, since much of what we know about pseudoenzymes arose from structural studies, and the fold of proteins provides clues to functions, this review will focus on key examples that help illustrate the general pseudoenzyme principles underlying specialised non-catalytic functions. Indeed, as the field grows and matures, it will be interesting to monitor whether different types of pseudoenzymes have diverged structurally from sequence-related enzyme homologues in order to fulfil specific biological niches. Such information will help reveal the direction of evolutionary information flow between enzymes and pseudoenzymes, and have utility for evaluating pseudoenzymes as new targets or anti-targets for pharmaceutical intervention [10].

Four classes of biological pseudoenzyme mechanism

In terms of predicted and experimentally-defined mechanisms, pseudoenzymes fall into four major classes (Figure 1), which include many members of the protein kinase superfamily, whose ubiquity in model organisms, and relative ease of assay, has made them popular amongst experimentalists. Several specific examples of pseudoenzyme classes, including the specific ‘subtype’ to which they belong, are listed in Table 1, alongside a description of their (known) biological function. The first of these, for which multiple examples are established in the protein kinase, phosphatase and ubiquitination fields, still retain a recognisable enzyme-like

architecture, but have evolved an ability to regulate a catalytically active partner that generates a biological output in signalling or metabolism (Figure 1a). The second class acts as “switches” that integrate signals in the form of post-translational modifications or binding to metabolic ligands, which trigger interconversion between inactive and active conformations (Figure 1b). The third category appears to have gained new functions as protein interaction modules through structural specialisation, where they can act as cellular scaffolds to nucleate the assembly of protein complexes or regulate the localization or trafficking of a binding partner (Figure 1c). The fourth category has repurposed canonical features of a protein fold that is shared with active enzyme relatives so that they can act as competitors for either substrate binding (as catalytic ‘traps’) or higher order complex assembly (Figure 1d).

How has structure illuminated the molecular mechanisms of pseudoenzymes?

Building upon a flood of genomic data, structural studies have provided key insights into the mechanisms by which pseudoenzymes operate as protein (and ligand) interaction domains to elicit biological responses. Additionally, pseudoenzyme structures have been instrumental in clarifying the molecular basis for deficient catalytic activity, whether it be: loss of canonical catalytic residues; loss of cofactor binding; loss of allosteric regulatory potential; occlusion of the active site by sequences divergent from those in an active enzyme counterpart; or active site blockade by non-canonical appendages. Importantly, while conventional enzymes are best understood for their catalytic functions, structural studies of pseudoenzymes are also providing an avenue to help understand non-catalytic functions of catalytically active enzymes, and in so-doing, are uncovering new strategies for therapeutic intervention.

1. Allosteric Activators

A conceptually simple mechanism that helps explain the prevalence of pseudoenzymes in biology is the finding that, upon binding, a pseudoenzyme can often impact upon the catalytic activity of a conventional, often related, enzyme (or non-enzyme) protein. The best-characterised examples of such allostery involve pseudoenzymes regulating a structurally-related enzyme counterpart. Good examples include the pseudokinase/kinase pairings KSR/RAF and HER3/EGFR (Figure 2a) [11-15], and the secretory pathway pseudokinase FAM20A, which has

the interesting ability to bind ATP in an 'inverted' conformation in the absence of cations [16]. Underscoring the biological importance of this pseudoenzyme, FAM20A stimulates the catalytic activity of FAM20C, the physiological casein kinase, and mutations in both FAM20A and FAM20C are linked to defective biomineralisation and disease in eukaryotes [17]. As pointed out by others [18], ancestors of both FAM20A and FAM20C are abundant in prokaryotes and slime moulds, suggesting a very ancient origin for this mode of allosteric enzyme activation. The regulation of the apoptotic protease Caspase-8 by the pseudoprotease FLIP (Figure 2b) [19] provides another example of allosteric modulation by an evolutionary-directed pseudoenzyme/enzyme couple. Interestingly, multiple cases of domain duplication have led to tandem pseudoenzyme-enzyme domain architectures within the same polypeptide—for example, kinases like JAK1-3 and TYK2 [12], the ATPase EccC (addressed in scaffolds section below) and the GTPase p190RhoGAP—in which two pseudoenzyme folds with very low sequence identity to active GTPases are sandwiched between neighbouring catalytic GTPase and GAP domains [20].

Further examples of allosteric regulation between inactive and active enzyme homologues have been uncovered in the ubiquitin system, particularly among Really Interesting New Gene (RING) protein homologues that comprise the largest class of ubiquitin ligases. There are numerous examples of RING heterodimers where one component has a functional binding site for a ubiquitin-conjugating (E2) enzyme, and one inactive RING cannot bind E2. Perhaps the best known is MDM2-MDMX, where the active RING MDM2 can form a functional complex with MDMX to promote ubiquitination of p53. MDMX plays this role because it retains a conserved C-terminal hydrophobic residue present in active RINGs, which is essential for stabilisation of the catalytic complex [21]. Additional examples include the BRCA1–BARD1 RING complex [22]; and Polycomb Repressive Complexes (Figure 2c), in which an active RING1a/b component is able to partner with one of 6 different PCGF inactive RINGs (PCGF1–6). In addition to stabilising the catalytic complex, varying pseudoenzyme PCGF RINGs participate in substrate recognition and can modulate the intrinsic catalytic rate of their respective complexes [23,24]. In contrast to ubiquitin conjugation by RINGs, ubiquitin removal by the BRCC36 deubiquitinase is controlled by pseudoDUBs of the KIAA0157 family. KIAA0157 is essential for assembly of a heterotetrameric BRCC36–KIAA0157 complex, in which BRC366 becomes

catalytically active and the catalytic sites of BRCC36 are ideally arranged to act upon their preferred Lys_{63}-linked ubiquitin substrate (Figure 2d; [25]).

The frequency of pseudoenzymes regulating structurally-similar enzymes has been proposed to arise from gene duplications that liberate the second gene from selective pressures that normally ensure it catalyzes a chemical reaction, therefore encouraging regulatory specialisation [8,9]. Interestingly, many enzymes are now appreciated to possess 'pseudoenzyme' modes of allostery, where the catalytic potential of the enzyme is overridden in favour of a pseudoenzyme-like conformational output that supports a specific biological function. Recent examples include a non-enzyme scaffolding function of Caspase-8 in the immune system [26], and a non-catalytic function for the conformationally-flexible canonical kinase Aurora A through N-Myc regulation in neuroblastoma [27].

2. Signal integrators/molecular switches

Modification of, or ligand binding to, pseudoenzymes can enable them to act as "receivers" of information from upstream regulators to control a downstream output. Structural and biochemical studies of the nucleotide-binding pseudokinase, Mixed lineage kinase domain-like (MLKL; Figure 3a), are illustrative of how post-translational modification, in this case phosphorylation of the pseudokinase domain activation loop by the upstream activator kinase, RIPK3, can toggle a molecular switch to induce a downstream effector function [28,29]. MLKL phosphorylation is proposed to induce a conformational change in the pseudokinase domain that relieves a suppressive interaction between the pseudokinase domain and the N-terminal four-helix bundle domain (Figure 3a). Release of the latter permits MLKL oligomerisation, membrane translocation and death of a cell by the regulated cell death pathway, necroptosis [30].

In addition to covalent modifications, molecular switch functions could analogously be imparted upon ligand binding to pseudoenzymes. For example, structural studies of ADCK3 (COQ8A), considered a pseudokinase because of a contorted active site that confers a preference for ADP over ATP [31], have revealed the importance of nucleotide binding to its function. While precise details are still emerging, nucleotide binding to ADCK3 promotes assembly of a functional Coenyzme Q biosynthetic holoenzyme and, furthermore, induces conformational

changes that expose putative lipid binding pockets adjacent to the substrate-occluding N-terminal helices [32].

Ligand binding has also been shown to exert subtle switching effects on pseudoenzymes that are not always clear from crystal structures. In the case of RNaseL, binding of nucleotide to the pseudokinase domain and/or the cyclic nucleotide second messenger, 2′,5′-oligoadenylate (2-5A), to a channel between the N-terminal ankyrin repeat and pseudokinase domains promotes activity of the C-terminal nuclease domains within the RNaseL homodimer (Figure 3b; [33]). Because *apo* and ligand-bound crystal structures are not obviously different, based on solution scattering data it was proposed that the role of nucleotide binding was to lock the pseudokinase domain into a closed conformation to facilitate nuclease activity [33]. These data suggest that ATP binding by the related Ire1, which contains a conventional protein kinase domain rather than a pseudokinase domain [34], may similarly serve a conformational role to augment nuclease activity. While these examples are illustrative of the propensity of some pseudoenzyme functions to be tuned by modification or ligand binding, not all pseudoenzymes are expected to undergo conformational switching; biochemical studies suggest that fewer than half of all pseudokinases retain binding to conventional nucleotides [35,36].

3. Scaffolds for assembly of protein complexes

Several multimeric complex structures have provided insights into how pseudoenzymes assemble more than one partner simultaneously to create higher order scaffolds or “hubs”. The pseudokinase PAN3 provides an interesting case in point. PAN3 forms an asymmetric dimer [37], which scaffolds the assembly of a higher order complex with the mRNA deadenylation enzyme, PAN2, which itself contains a pseudo-ubiquitin C-terminal hydrolase (UCH) domain (Figure 3c). Beyond simply scaffolding complex formation, PAN3 abuts the PAN2 RNase domain within the complex and promotes RNase activity, whilst the pseudokinase ATP-binding site recruits poly(A) substrates to the holoenzyme [38].

The Tribbles (TRIB) family of proteins (and the homolog SgK495/STK40) also employ an atypical pseudokinase domain to bring a catalytic enzyme into proximity of its substrate. In this case, a ternary complex is formed between TRIB1, COP1 ubiquitin ligase and the C/EBP substrate, which is appropriately positioned for ubiquitination [35,39,40]. The structure of TRIB1 has demonstrated that its C-terminal

tail, which binds COP1, can also self-associate with the pseudokinase domain *in cis* [35], likely through a mutually exclusive mechanism [41]. While the structural mechanism is still not clear, it appears that formation of a Substrate–TRIB1–COP1 holocomplex must involve an evolutionary imprinted conformational change in the TRIB1 pseudokinase, which is likely to have been conserved in other eukaryotic Tribbles proteins. Another pseudokinase, KSR2, serves as a hub to orchestrate RAF communication with the effector kinase, MEK1. KSR2 is able to activate the RAF kinases via a “back-to-back” dimer interface (Figure 2a), but also bind to MEK1 through a “face-to-face” interaction to promote phosphorylation of MEK1 by RAF [42].

In some cases, including the EccC family of hexameric ATPases (see below) and the RBR ubiquitin ligases, pseudoenzyme domains are embedded in the context of tandem arrays with conventional enzyme counterparts, where they have evolved dual functions as both hubs and as allosteric regulators. In RBR ubiquitin ligases, a benign-catalytic region that lacks a catalytic cysteine residue (BRcat; also known as IBR for in-between-RING), lies adjacent to a catalytic RBR RING domain, [43]. Structures of autoinhibited and active RBR ligases (Parkin and HOIP, respectively) have demonstrated important roles that BRcat plays in each state. In inactive Parkin, the BRcat stabilises an autoinhibited conformation that blocks the active site, whereas in active HOIP the BRcat contacts the E2-Ub conjugate and binds an additional ubiquitin that stabilises the catalytically competent complex [44-46].

The EccC protein, a type VII secretion ATPase of bacterial pathogens, is composed of an array of three linked ATPase domains, two of which are pseudoenzymes lacking Walker A Glu residues, permitting them to bind, but not hydrolyse ATP (Figure 3f; [47]). EsxB, a secreted substrate of EccC, is instrumental in the ordered assembly and activation of the active complex. EsxB binding by the terminal pseudoATPase domain simultaneously promotes release of autoinhibition between the ATPase domain and central pseudoATPase, and drives multimerization and activation of the hexameric holoenzyme (Figure 3d; [47]).

Like GTPases, pseudoGTPases are also abundant throughout nature [1,48,49], and structural studies have yielded important insights into their scaffolding and allosteric functions, which have arisen in place of enzymatic functions. For example, crystallographic and cellular data have enhanced our understanding of how pseudoGTPases such as the kinetochore-regulating scaffold CENP-M, which is unable to bind GTP, functions to assemble and regulate a multi-protein complex that

recruits CENP-T/W proteins to the kinetochores of metazoan chromosomes [50]. In addition, the recent structures of two 'cryptic' pseudoGTPase domains (pG1 and pG2) from p190RhoGAP confirms the presence of highly degraded G-motifs, which normally mediate GTP binding and catalysis in GTPases. Interestingly, cellular data supports a regulation role for these pseudoenzyme domains in modulating p190RhoGAP catalytic activity towards its RhoA substrate [20].

4. Competition for substrate or complex assembly

Lastly, examples of pseudoenzymes that act as competitors in various guises have been identified (Figure 4). The structural capacity of some pseudoenzymes to sequester substrates is vividly illustrated by the pseudo-chitinase YKL-39 (Figure 4a; [51,52]). YKL-39 lacks the essential Glu within the DxxDxDxE catalytic motif, meaning the protein can bind chito-oligosaccharides with a nanomolar affinity, but not hydrolyse them, and thus sequester them away from catalytically-active counterparts (or immunological receptors) to prevent processing. Although structures are yet to be reported, another competitive mode of action is illustrated by several pseudophosphatases (Figure 4b), which bind substrates with high affinity to antagonize conventional enzymes. In the case of EGG-4 and EGG-5, interaction with the phosphorylated activation loop of the protein kinase MBK-2 prevents regulatory dephosphorylation [53,54]. A related mode of action is exemplified by the pseudophosphatase STYX, which has been shown to compete with the dual-specificity phosphatase DUSP4 for binding to the canonical protein kinases, ERK1 and ERK2 [55]. As a result, STYX binding precludes DUSP4 engagement and ERK1/2 dephosphorylation and inactivation, which is likely to be important in cells, where compartmentalization of the ERK module regulates transforming potential [56]. Because these substrate "traps" could evolve from simple loss of catalytic functions, we anticipate this group is abundant in nature. This idea is supported by the impressive array of pseudophosphatase domains within both the myotubularin and receptor tyrosine phosphatase families in higher eukaryotes [3,6].

In addition to substrate competition, enzyme sequestration represents another mode of pseudoenzyme action. This is exemplified by a naturally-occurring, catalytically-defective variant of aldehyde dehydrogenase-2 (ALDH2), termed ALDH2*2, which can compete with the catalytically-active counterpart, ALDH2*1, to

‘poison’ complex assembly into an active homo-tetramer, thus inhibiting activity (Figure 4c; [57]).

Conclusions

In this review, we have sought to illustrate the diversity of mechanisms underlying pseudoenzyme functions as revealed from recent structural studies. An underlying theme is that pseudoenzymes behave as protein interaction modules, whether in the guise of allosteric regulators, signal integrators, nucleators of protein complex assembly or as substrate competitors with conventional enzymes. It is also becoming clear that pseudoenzymes can often perform several of these functions simultaneously. We expect that future multidisciplinary studies will reveal new and unexpected modes of action of pseudoenzymes, which will in turn lead to an expansion of knowledge pertaining to the repertoire of non-catalytic functions that are performed by conventional enzymes.

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References

1. Murphy JM, Farhan H, Eyers PA: **Bio-Zombie: the rise of pseudoenzymes in biology**. *Biochem Soc Trans* 2017, **45**:537-544.

•• A description of the ~20 different classes of pseudoenzymes identified to date and their functions in biology.

2. Jacobsen AV, Murphy JM: **The secret life of kinases: insights into non-catalytic signalling functions from pseudokinases**. *Biochem Soc Trans* 2017, **45**:665-681.
3. Chen MJ, Dixon JE, Manning G: **Genomics and evolution of protein phosphatases**. *Sci Signal* 2017, **10**.
4. Adrain C, Freeman M: **New lives for old: evolution of pseudoenzyme function illustrated by iRhoms**. *Nat Rev Mol Cell Biol* 2012, **13**:489-498.
5. Eyers PA, Murphy JM: **The evolving world of pseudoenzymes: proteins, prejudice and zombies**. *BMC Biol* 2016, **14**:98.

• An overview of the rapidly emerging pseudoenzyme field.

6. Reiterer V, Eyers PA, Farhan H: **Day of the dead: pseudokinases and pseudophosphatases in physiology and disease**. *Trends Cell Biol* 2014, **24**:489-505.
7. Eyers PA, Murphy JM: **Dawn of the dead: protein pseudokinases signal new adventures in cell biology**. *Biochemical Society Transactions* 2013, **41**:969-974.
8. Pils B, Schultz J: **Inactive enzyme-homologues find new function in regulatory processes**. *J Mol Biol* 2004, **340**:399-404.
9. Todd AE, Orengo CA, Thornton JM: **Sequence and structural differences between enzyme and nonenzyme homologs**. *Structure* 2002, **10**:1435-1451.
10. Byrne DP, Foulkes DM, Eyers PA: **Pseudokinases: update on their functions and evaluation as new drug targets**. *Future medicinal chemistry* 2017, **9**:245-265.
11. Littlefield P, Liu L, Mysore V, Shan Y, Shaw DE, Jura N: **Structural analysis of the EGFR/HER3 heterodimer reveals the molecular basis for activating HER3 mutations**. *Sci Signal* 2014, **7**:ra114.

• Illustration of pseudoenzyme allostery describing how HER3 activates the EGFR kinase.

12. Lupardus PJ, Ultsch M, Wallweber H, Bir Kohli P, Johnson AR, Eigenbrot C: **Structure of the pseudokinase-kinase domains from protein kinase TYK2 reveals a mechanism for Janus kinase (JAK) autoinhibition**. *Proc Natl Acad Sci U S A* 2014, **111**:8025-8030.

• The first crystal structure of the tandem pseudokinase-kinase domains from a JAK family member.

13. Rajakulendran T, Sahmi M, Lefrancois M, Sicheri F, Therrien M: **A dimerization-dependent mechanism drives RAF catalytic activation**. *Nature* 2009, **461**:542-545.
14. Zeqiraj E, Filippi BM, Goldie S, Navratilova I, Boudeau J, Deak M, Alessi DR, van Aalten DM: **ATP and MO25alpha regulate the conformational state of the STRADalpha pseudokinase and activation of the LKB1 tumour suppressor**. *PLoS Biol* 2009, **7**:e1000126.
15. Zeqiraj E, Filippi BM, Deak M, Alessi DR, van Aalten DM: **Structure of the LKB1-STRAD-MO25 complex reveals an allosteric mechanism of kinase activation**. *Science* 2009, **326**:1707-1711.

16. Cui J, Zhu Q, Zhang H, Cianfrocco MA, Leschziner AE, Dixon JE, Xiao J: **Structure of Fam20A reveals a pseudokinase featuring a unique disulfide pattern and inverted ATP-binding**. *Elife* 2017, **6**:e23990.
• Lack of selective pressure to retain enzymatic active site geometry allows pseudoenzymes to evolve diverse ligand-binding mechanisms.
17. Cui J, Xiao J, Tagliabracci VS, Wen J, Rahdar M, Dixon JE: **A secretory kinase complex regulates extracellular protein phosphorylation**. *Elife* 2015, **4**:e06120.
18. Raju S, Shaw AS: **What is the point of pseudokinases?** *Elife* 2015, **4**:e07771.
19. Yu JW, Jeffrey PD, Shi Y: **Mechanism of procaspase-8 activation by c-FLIPL**. *Proc Natl Acad Sci U S A* 2009, **106**:8169-8174.
20. Stiegler AL, Boggon TJ: **p190RhoGAP proteins contain pseudoGTPase domains**. *Nat Commun* 2017, **In Press**.
21. Linke K, Mace PD, Smith CA, Vaux DL, Silke J, Day CL: **Structure of the MDM2/MDMX RING domain heterodimer reveals dimerization is required for their ubiquitylation in trans**. *Cell Death Differ* 2008, **15**:841-848.
22. Brzovic PS, Rajagopal P, Hoyt DW, King MC, Klevit RE: **Structure of a BRCA1-BARD1 heterodimeric RING-RING complex**. *Nat Struct Biol* 2001, **8**:833-837.
23. McGinty RK, Henrici RC, Tan S: **Crystal structure of the PRC1 ubiquitylation module bound to the nucleosome**. *Nature* 2014, **514**:591-596.
24. Taherbhoy AM, Huang OW, Cochran AG: **BMI1-RING1B is an autoinhibited RING E3 ubiquitin ligase**. *Nat Commun* 2015, **6**:7621.
25. Zeqiraj E, Tian L, Piggott CA, Pillon MC, Duffy NM, Ceccarelli DF, Keszei AF, Lorenzen K, Kurinov I, Orlicky S, et al.: **Higher-Order Assembly of BRCC36-KIAA0157 Is Required for DUB Activity and Biological Function**. *Mol Cell* 2015, **59**:970-983.
•• First structure of a pseudoDUB (KIAA0157), revealing allosteric and hub functions that organize and activate BRCC36 DUB activity in higher order complexes.
26. Henry CM, Martin SJ: **Caspase-8 Acts in a Non-enzymatic Role as a Scaffold for Assembly of a Pro-inflammatory "FADDosome" Complex upon TRAIL Stimulation**. *Mol Cell* 2017, **65**:715-729 e715.
27. Otto T, Horn S, Brockmann M, Eilers U, Schuttrumpf L, Popov N, Kenney AM, Schulte JH, Beijersbergen R, Christiansen H, et al.: **Stabilization of N-Myc is a critical function of Aurora A in human neuroblastoma**. *Cancer Cell* 2009, **15**:67-78.
28. Murphy JM, Czabotar PE, Hildebrand JM, Lucet IS, Zhang JG, Alvarez-Diaz S, Lewis R, Lalaoui N, Metcalf D, Webb AI, et al.: **The pseudokinase MLKL mediates necroptosis via a molecular switch mechanism**. *Immunity* 2013, **39**:443-453.
29. Petrie EJ, Hildebrand JM, Murphy JM: **Insane in the membrane: a structural perspective of MLKL function in necroptosis**. *Immunol Cell Biol* 2017, **95**:152-159.
30. Hildebrand JM, Tanzer MC, Lucet IS, Young SN, Spall SK, Sharma P, Pierotti C, Garnier JM, Dobson RC, Webb AI, et al.: **Activation of the pseudokinase MLKL unleashes the four-helix bundle domain to induce membrane localization and necroptotic cell death**. *Proc Natl Acad Sci U S A* 2014, **111**:15072-15077.

• With reference 28, proposes a role for the MLKL pseudokinase domain, both as a molecular switch and as an interaction domain that suppresses executioner domain activity.
31. Stefely JA, Reidenbach AG, Ulbrich A, Oruganty K, Floyd BJ, Jochem A, Saunders JM, Johnson IE, Minogue CE, Wrobel RL, et al.: **Mitochondrial ADCK3 employs an atypical protein kinase-like fold to enable coenzyme Q biosynthesis**. *Mol Cell* 2015, **57**:83-94.
32. Stefely JA, Licitra F, Laredj L, Reidenbach AG, Kemmerer ZA, Grangeray A, Jaeg-Ehret T, Minogue CE, Ulbrich A, Hutchins PD, et al.: **Cerebellar Ataxia and Coenzyme Q Deficiency through Loss of Unorthodox Kinase Activity**. *Mol Cell* 2016, **63**:608-620.
33. Huang H, Zeqiraj E, Dong B, Jha BK, Duffy NM, Orlicky S, Thevakumaran N, Talukdar M, Pillon MC, Ceccarelli DF, et al.: **Dimeric structure of pseudokinase RNase L bound to 2-5A reveals a basis for interferon-induced antiviral activity**. *Mol Cell* 2014, **53**:221-234.
•• ATP binding by the RNase L pseudokinase domain is proposed to promote activity of the adjacent RNase domain by locking the structure.
34. Lee KP, Dey M, Neculai D, Cao C, Dever TE, Sicheri F: **Structure of the dual enzyme Ire1 reveals the basis for catalysis and regulation in nonconventional RNA splicing**. *Cell* 2008, **132**:89-100.
35. Murphy JM, Nakatani Y, Jamieson SA, Dai W, Lucet IS, Mace PD: **Molecular Mechanism of CCAAT-Enhancer Binding Protein Recruitment by the TRIB1 Pseudokinase**. *Structure* 2015, **23**:2111-2121.
•• The first structure of a Tribbles family pseudokinase providing important insights into the molecular basis for its scaffolding function.
36. Murphy JM, Zhang Q, Young SN, Reese ML, Bailey FP, Eyers PA, Ungureanu D, Hammaren H, Silvennoinen O, Varghese LN, et al.: **A robust methodology to subclassify pseudokinases based on their nucleotide-binding properties**. *Biochem J* 2014, **457**:323-334.
• Approximately half of 31 pseudokinases surveyed did not bind to nucleotides, indicating they predominantly function as protein interaction modules.
37. Christie M, Boland A, Huntzinger E, Weichenrieder O, Izaurralde E: **Structure of the PAN3 pseudokinase reveals the basis for interactions with the PAN2 deadenylase and the GW182 proteins**. *Mol Cell* 2013, **51**:360-373.
38. Schafer IB, Rode M, Bonneau F, Schussler S, Conti E: **The structure of the Pan2-Pan3 core complex reveals cross-talk between deadenylase and pseudokinase**. *Nat Struct Mol Biol* 2014, **21**:591-598.
•• With reference 37, structural delineation of how an asymmetric dimer of the pseudokinase PAN3 nucleates assembly of the deadenylation complex with the pseudoUCH domain-containing PAN2.
39. Durzynska I, Xu X, Adelmant G, Ficarro SB, Marto JA, Sliz P, Uljon S, Blacklow SC: **STK40 Is a Pseudokinase that Binds the E3 Ubiquitin Ligase COP1**. *Structure* 2017, **25**:287-294.
40. Eyers PA, Keeshan K, Kannan N: **Tribbles in the 21st Century: The Evolving Roles of Tribbles Pseudokinases in Biology and Disease**. *Trends Cell Biol* 2016, 10.1016/j.tcb.2016.11.002.
41. Uljon S, Xu X, Durzynska I, Stein S, Adelmant G, Marto JA, Pear WS, Blacklow SC: **Structural Basis for Substrate Selectivity of the E3 Ligase COP1**. *Structure* 2016, **24**:687-696.

42. Brennan DF, Dar AC, Hertz NT, Chao WC, Burlingame AL, Shokat KM, Barford D: **A Raf-induced allosteric transition of KSR stimulates phosphorylation of MEK**. *Nature* 2011, **472**:366-369.
43. Spratt DE, Walden H, Shaw GS: **RBR E3 ubiquitin ligases: new structures, new insights, new questions**. *Biochem J* 2014, **458**:421-437.
44. Lechtenberg BC, Rajput A, Sanishvili R, Dobaczewska MK, Ware CF, Mace PD, Riedl SJ: **Structure of a HOIP/E2~ubiquitin complex reveals RBR E3 ligase mechanism and regulation**. *Nature* 2016, **529**:546-550.
•• First structure of an active RBR E3 ligase, in which the pseudoenzyme BRcat domain mediates E2~Ub contacts and Ub recruitment to the complex
45. Swatek KN, Komander D: **Ubiquitin modifications**. *Cell Res* 2016, **26**:399-422.
46. Trempe JF, Sauve V, Grenier K, Seirafi M, Tang MY, Menade M, Al-Abdul-Wahid S, Krett J, Wong K, Kozlov G, et al.: **Structure of parkin reveals mechanisms for ubiquitin ligase activation**. *Science* 2013, **340**:1451-1455.
47. Rosenberg OS, Dovala D, Li X, Connolly L, Bendebury A, Finer-Moore J, Holton J, Cheng Y, Stroud RM, Cox JS: **Substrates Control Multimerization and Activation of the Multi-Domain ATPase Motor of Type VII Secretion**. *Cell* 2015, **161**:501-512.
• Structures revealed the molecular basis for allosteric regulation of the ATPase domain by adjacent pseudoATPase domains, and their role in substrate recruitment and protein oligomerization.
48. Chandra M, Mukherjee M, Srivastava VK, Saito-Nakano Y, Nozaki T, Datta S: **Insights into the GTP/GDP cycle of RabX3, a novel GTPase from Entamoeba histolytica with tandem G-domains**. *Biochemistry* 2014, **53**:1191-1205.
49. Artymiuk PJ, Rice DW, Mitchell EM, Willett P: **Structural resemblance between the families of bacterial signal-transduction proteins and of G proteins revealed by graph theoretical techniques**. *Protein engineering* 1990, **4**:39-43.
50. Basilico F, Maffini S, Weir JR, Prumbaum D, Rojas AM, Zimniak T, De Antoni A, Jeganathan S, Voss B, van Gerwen S, et al.: **The pseudo GTPase CENP-M drives human kinetochore assembly**. *Elife* 2014, **3**:e02978.
51. Ranok A, Wongsantichon J, Robinson RC, Suginta W: **Structural and thermodynamic insights into chitooligosaccharide binding to human cartilage chitinase 3-like protein 2 (CHI3L2 or YKL-39)**. *J Biol Chem* 2015, **290**:2617-2629.
• Insights into a pseudochitinase catalytic "trap" from structures bound to chito-oligosaccharide ligands of different lengths.
52. Schimpl M, Rush CL, Betou M, Eggleston IM, Recklies AD, van Aalten DM: **Human YKL-39 is a pseudo-chitinase with retained chitooligosaccharide-binding properties**. *Biochem J* 2012, **446**:149-157.
53. Cheng KC, Klancer R, Singson A, Seydoux G: **Regulation of MBK-2/DYRK by CDK-1 and the pseudophosphatases EGG-4 and EGG-5 during the oocyte-to-embryo transition**. *Cell* 2009, **139**:560-572.
54. Parry JM, Velarde NV, Lefkovith AJ, Zegarek MH, Hang JS, Ohm J, Klancer R, Maruyama R, Druzhinina MK, Grant BD, et al.: **EGG-4 and EGG-5 Link Events of the Oocyte-to-Embryo Transition with Meiotic Progression in C. elegans**. *Curr Biol* 2009, **19**:1752-1757.

55. Reiterer V, Fey D, Kolch W, Kholodenko BN, Farhan H: **Pseudophosphatase STYX modulates cell-fate decisions and cell migration by spatiotemporal regulation of ERK1/2**. *Proc Natl Acad Sci U S A* 2013, **110**:E2934-2943.
56. Kidger AM, Rushworth LK, Stellzig J, Davidson J, Bryant CJ, Bayley C, Caddye E, Rogers T, Keyse SM, Caunt CJ: **Dual-specificity phosphatase 5 controls the localized inhibition, propagation, and transforming potential of ERK signaling**. *Proc Natl Acad Sci U S A* 2017, **114**:E317-E326.
57. Larson HN, Weiner H, Hurley TD: **Disruption of the coenzyme binding site and dimer interface revealed in the crystal structure of mitochondrial aldehyde dehydrogenase "Asian" variant**. *J Biol Chem* 2005, **280**:30550-30556.
58. Dhawan NS, Scopton AP, Dar AC: **Small molecule stabilization of the KSR inactive state antagonizes oncogenic Ras signalling**. *Nature* 2016, **537**:112-116.
59. Bollag G, Hirth P, Tsai J, Zhang J, Ibrahim PN, Cho H, Spevak W, Zhang C, Zhang Y, Habets G, et al.: **Clinical efficacy of a RAF inhibitor needs broad target blockade in BRAF-mutant melanoma**. *Nature* 2010, **467**:596-599.
60. Plechanovova A, Jaffray EG, Tatham MH, Naismith JH, Hay RT: **Structure of a RING E3 ligase and ubiquitin-loaded E2 primed for catalysis**. *Nature* 2012, **489**:115-120.
61. Sato Y, Yoshikawa A, Yamagata A, Mimura H, Yamashita M, Ookata K, Nureki O, Iwai K, Komada M, Fukai S: **Structural basis for specific cleavage of Lys 63-linked polyubiquitin chains**. *Nature* 2008, **455**:358-362.
62. Dong B, Silverman RH: **Alternative function of a protein kinase homology domain in 2', 5'-oligoadenylate dependent RNase L**. *Nucleic Acids Res* 1999, **27**:439-445.
63. Jura N, Shan Y, Cao X, Shaw DE, Kuriyan J: **Structural analysis of the catalytically inactive kinase domain of the human EGF receptor 3**. *Proc Natl Acad Sci U S A* 2009, **106**:21608-21613.
64. Shi F, Telesco SE, Liu Y, Radhakrishnan R, Lemmon MA: **ErbB3/HER3 intracellular domain is competent to bind ATP and catalyze autophosphorylation**. *Proc Natl Acad Sci U S A* 2010, **107**:7692-7697.
65. Chang DW, Xing Z, Pan Y, Algeciras-Schimnich A, Barnhart BC, Yaish-Ohad S, Peter ME, Yang X: **c-FLIP(L) is a dual function regulator for caspase-8 activation and CD95-mediated apoptosis**. *EMBO J* 2002, **21**:3704-3714.
66. Chardin P: **Function and regulation of Rnd proteins**. *Nat Rev Mol Cell Biol* 2006, **7**:54-62.
67. Foster R, Hu KQ, Lu Y, Nolan KM, Thissen J, Settleman J: **Identification of a novel human Rho protein with unusual properties: GTPase deficiency and in vivo farnesylation**. *Mol Cell Biol* 1996, **16**:2689-2699.
68. Wennerberg K, Forget MA, Ellerbroek SM, Arthur WT, Burridge K, Settleman J, Der CJ, Hansen SH: **Rnd proteins function as RhoA antagonists by activating p190 RhoGAP**. *Curr Biol* 2003, **13**:1106-1115.
69. Schroeder CM, Ostrem JM, Hertz NT, Vale RD: **A Ras-like domain in the light intermediate chain bridges the dynein motor to a cargo-binding region**. *Elife* 2014, **3**:e03351.
70. Adrain C, Zettl M, Christova Y, Taylor N, Freeman M: **Tumor necrosis factor signaling requires iRhom2 to promote trafficking and activation of TACE**. *Science* 2012, **335**:225-228.

71. Christova Y, Adrain C, Bambrough P, Ibrahim A, Freeman M: **Mammalian iRhoms have distinct physiological functions including an essential role in TACE regulation**. *EMBO Rep* 2013, **14**:884-890.
72. Siggs OM, Grieve A, Xu H, Bambrough P, Christova Y, Freeman M: **Genetic interaction implicates iRhom2 in the regulation of EGF receptor signalling in mice**. *Biol Open* 2014, **3**:1151-1157.
73. Grieve AG, Xu H, Kunzel U, Bambrough P, Sieber B, Freeman M: **Phosphorylation of iRhom2 at the plasma membrane controls mammalian TACE-dependent inflammatory and growth factor signalling**. *Elife* 2017, **6**.
74. Reiterer V, Figueras-Puig C, Le Guerroue F, Confalonieri S, Vecchi M, Jalapothu D, Kanse SM, Deshaies RJ, Di Fiore PP, Behrends C, et al.: **The pseudophosphatase STYX targets the F-box of FBXW7 and inhibits SCFFBXW7 function**. *EMBO J* 2017, **36**:260-273.
75. Reiterer V, Pawlowski K, Farhan H: **STYX: a versatile pseudophosphatase**. *Biochem Soc Trans* 2017, **45**:449-456.

Figure Legends

Figure 1. Modes of pseudoenzyme function

Pseudoenzymes (green) exert their effects on signal transduction or metabolism through interactions with other proteins, including client enzymes (grey), or substrates (yellow).

(a) Allosteric binding of a pseudoenzyme can positively or negatively regulate the catalytic activity of a client protein. This client is classically a related enzyme, however it could be an unrelated, potentially non-enzyme, protein.

(b) Pseudoenzyme domains can act as receivers for post-translational modifications (star), such as (de)ubiquitylation, (de)phosphorylation or proteolytic cleavage, which can promote conformational switching and effector functions.

(c) As protein interaction domains, pseudoenzymes can nucleate the assembly of protein complexes to bring enzyme and substrate pairs into proximity (top), or regulate protein localization, stability or quality control in a particular trafficking pathway or organelle (bottom).

(d) Pseudoenzymes can compete with conventional (active) enzymes to prevent assembly of higher order protein complexes (top), or sequester substrates to protect them from enzymatic processing (bottom).

Figure 2. Allosteric regulation of active enzymes

(a) *Stabilisation of active kinases by pseudokinase partners.* Shown are complexes between pseudokinase KSR–kinase BRAF (based on superposition of KSR2 onto the BRAF homodimer; PDBs 5kkr and 3og7; [58] and [59] respectively) and the crystal structure of HER3 pseudokinase bound to the EGFR kinase domain (PDB 4riw). Bound ATP is shown as spheres.

(b) *Regulation of Caspase-8 activity by a pseudocaspase partner*. Structure of the complex between proCaspase-8 (grey) and pseudoprotease FLIP (green) (PDB 3h11; [19]). The intersubunit linker of Caspase-8, which undergoes proteolysis upon activation, is not visible in the crystal structure but is represented by a dotted line.

(c) *Active–inactive RING heterocomplexes regulate RING activity.* Model of the putative RING1b-Bmi1–E2-Ub substrate complex, based on the structure from PDB 4r8p; [23]. Ubiquitin is modelled by superposition of primed E2-Ub conjugate from

PDB 4ap4; [60]. The non-E2 binding Bmi1 pseudo-RING domain stabilises the E2-Ub conjugate in the closed conformation primed for attack by a substrate lysine from the histone substrate (not shown).

(d) *DUB regulation within a DUB-pseudoDUB complex*. Structure of the complex between BRCC36 (grey surface) and the KIAA0157 pseudoDUB (green cartoon) (PDB 5cw3; [25]). A putative Lys_{63}-linked Ub substrate is positioned by superposition into the active sites of BRC366 (based on PDB 2znv; [61]), which demonstrates the compatibility of the tetrameric BRCC36–KIAA0157 complex with its preferred substrate. Pseudoenzymes are shown as green ribbons diagrams, and active enzyme partners in grey thoughout.

Figure 3. Assembly of signalling complexes

(a) *The MLKL pseudokinase*. MLKL is thought to exist in a basal state (left) where the N-terminal four-helix bundle domain is sequestered by the C-terminal pseudokinase domain (green). Upon phosphorylation of the pseudokinase domain activation loop, MLKL is proposed to undergo a conformational change (middle; PDB 4btf; [28]), leading to exposure of the four-helix bundle, oligomerisation, membrane translocation, permeabilisation of plasma membranes and cell death (right).

(b) *RNase L pseudokinase.* RNase L is a homodimeric assembly containing ankyrin repeat domains linked to dual pseudokinase (PsK)-ribonuclease domains. The pseudoenzyme domain (green) contains non-canonical adaptions in both N and C-lobes, which prevent catalytic activity [62]. RNAse L drives the IFN-induced antiviral response in humans, and is activated by the 2,'5'-oligoadenylate (2-5A) second messenger, which binds in a cleft between the ankyrin repeats and pseudokinase domain. Crystal structures of RNase L (PDBs 4o1o and 4o1p) demonstrate how 2-5A and the pseudokinase region to drive the RNase domain (yellow) into an enzyme conformation compatible with catalysis [33].

(c) *PseudoDUB and pseudokinase modules orient activity of the PAN2/3 complex*. The PAN2/3 complex is shown with the dimer of PAN3 in cartoon representation, and PAN2 as a surface (PDB 4q8j; [38]). The pseudokinase domains of PAN3 are shown in green, with the nucleotide-binding site (proposed to bind to polyA tails) indicated with spheres. The PseudoDUB domain of PAN2 (green) makes extensive contacts

with the RNase domain that contains the deadenylase active site (indicated with a black sphere).

(d) *Oligomerisation of an active hexameric ATPase via pseudo-ATPase modules*. EccC is comprised of an N-terminal active ATPase (grey) with two N-terminal pseudo-ATPase domains (green). The C-terminal pseudo-ATPase domain of EccC binds to peptides, which themselves emanate from dimeric substrates, and hence promote association of the active EccC ATPase hexamer [47].

Figure 4. Competition for substrate or enzyme binding

(a) *Sequestration of chito-oligosaccharides by the pseudochitinase, YKL-39.* The crystal structure of YKL-39 has been solved bound to chito-oligosaccharides of varying residue length [51,52], the longest being polymeric $GlcNAC_6$ (yellow) (PDB 4p8x).

(b) *Pseudophosphatases that bind to phosphorylated canonical kinases occlude conventional phosphatases.* Left, binding of the pseudophosphatase, EGG-4 or EGG-5, shields the activation loop of the kinase MBK-2 from dephosphorylation [53,54]. Right, pseudophosphatase STYX regulates ERK activation by competing with the conventional phosphatase, DUSP4, for substrate binding [55].

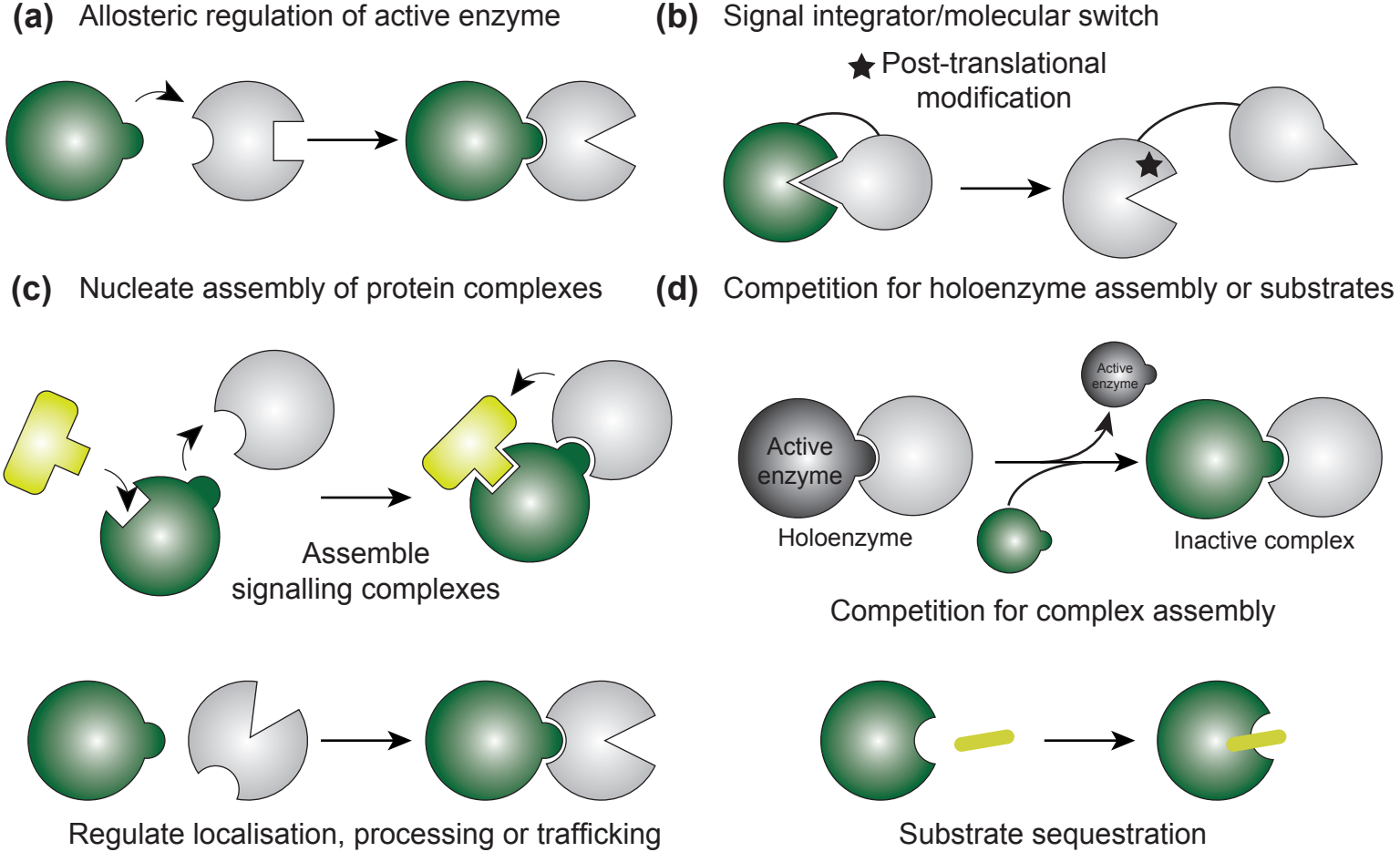
(c) *Pseudodehydrogenase can block assembly higher order enzyme or signalling complexes.* Left, schematic of how the catalytically-defective variant, ALDH2*2, might compete with the active paralog, ALDH2*1, to prevent assembly of, and allosteric activation within, an ALDH2*1 homo-tetramer [57]. Right, homo-tetrameric structures of ALDH2*2 (green; PDB 1zum) and ALDH2*1 (grey; PDB 1nzz).

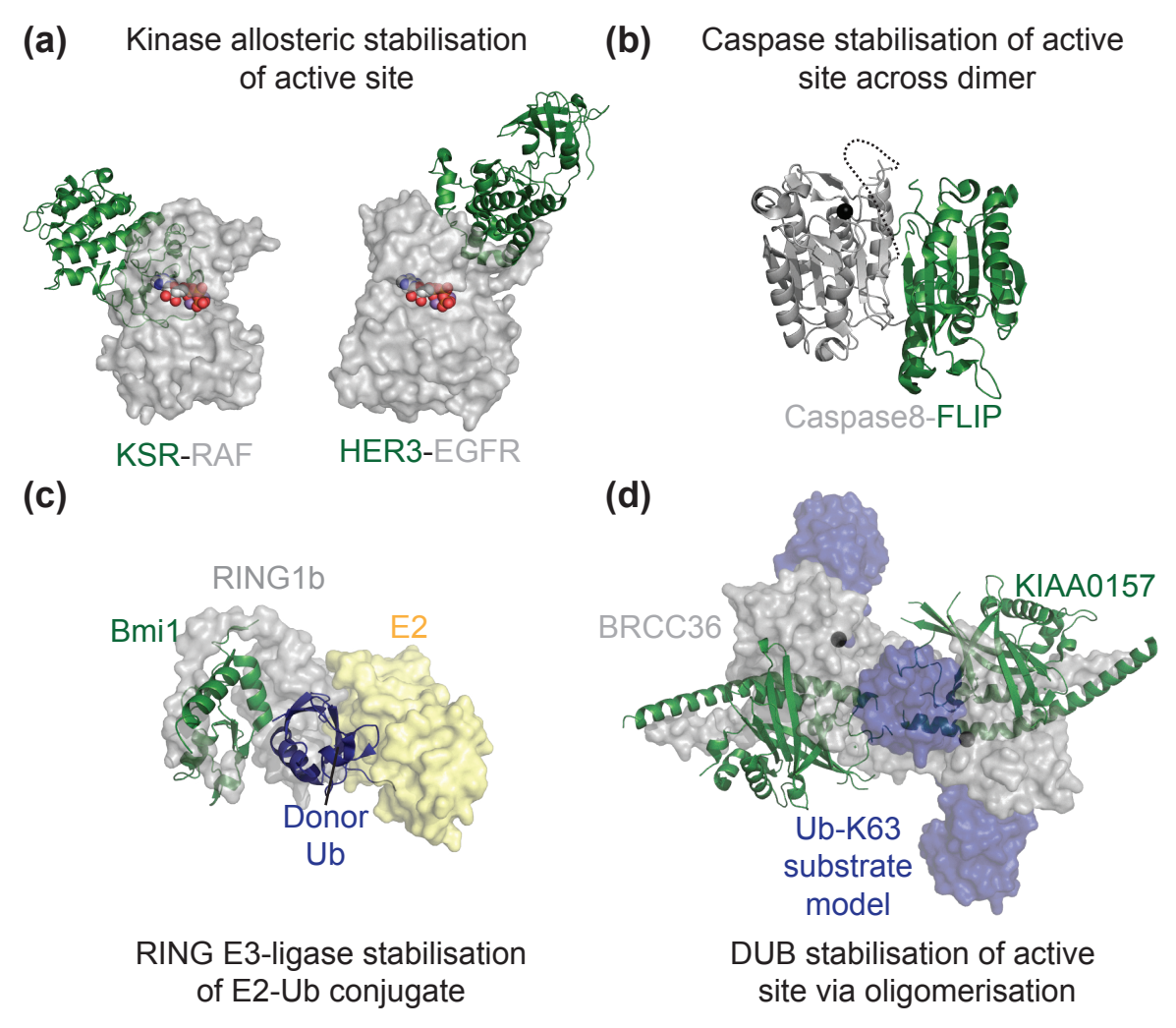
Table 1: **Diversity of biological functions mediated by pseudoenzyme families**

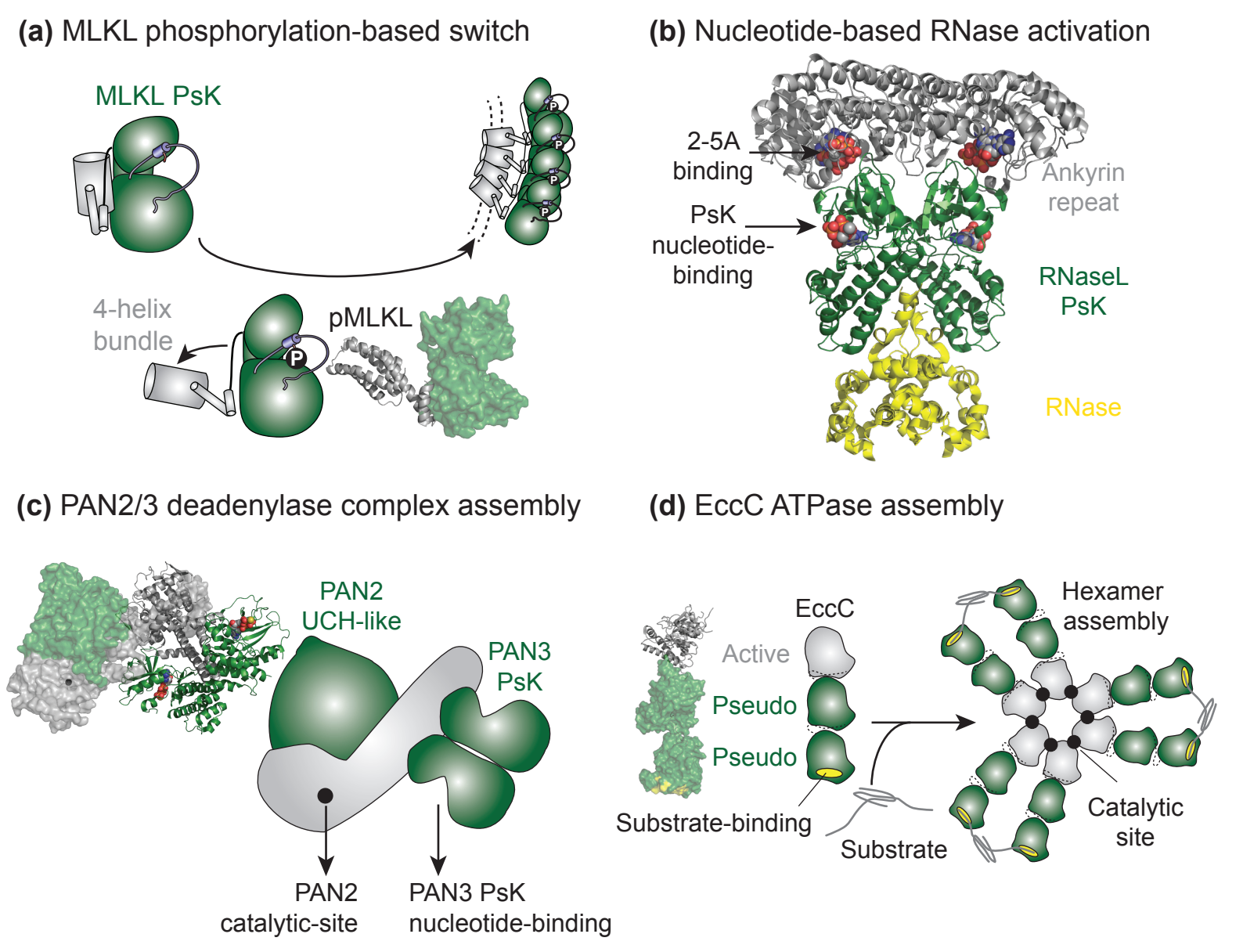
Function	Pseudoenzyme type	Examples	References
Allosteric regulation of an active enzyme	Pseudokinase	The pseudokinases, KSR1/2, HER3, STRADα and FAM20A, promote activity of their conventional active kinase interaction partners. In contrast, the pseudokinase domains of the JAK family, such as JAK2 and TYK2, negatively regulate the catalytic activity of the adjacent conventional active tyrosine kinase domain.	[2,12,14-16,42,63,64]
	Pseudoprotease	Pseudoprotease, cFLIP, binds and inhibits the conventional cysteine protease, Caspase-8	[65]
	Pseudo-deubiquitinase (PseudoDUB)	PseudoDUB, KIAA0157, facilitates assembly of heterotetramer with conventional DUB, BRCC36, and DUB activity	[25]
	PseudoGTPase	PseudoGTPases, Rnd1 or Rnd3/RhoE, bind p190RhoGAP to regulate the catalytic activity of the conventional GTPase, RhoA	[66-68]
		PseudoGTPase domains in p190RhoGAP potentially regulate its RhoGAP activity towards the conventional GTPase, RhoA	[20]
	Pseudo-E3 Ub ligase	Pseudo-E3s, RING proteins MDMX, BARD1 and PCGF1-6, can partner with active RING proteins to regulate substrate recognition and enhance catalysis	[21–24]
Signal integrator/molecular switch	Pseudokinase	Phosphorylation of the MLKL pseudokinase domain by	[28,30]

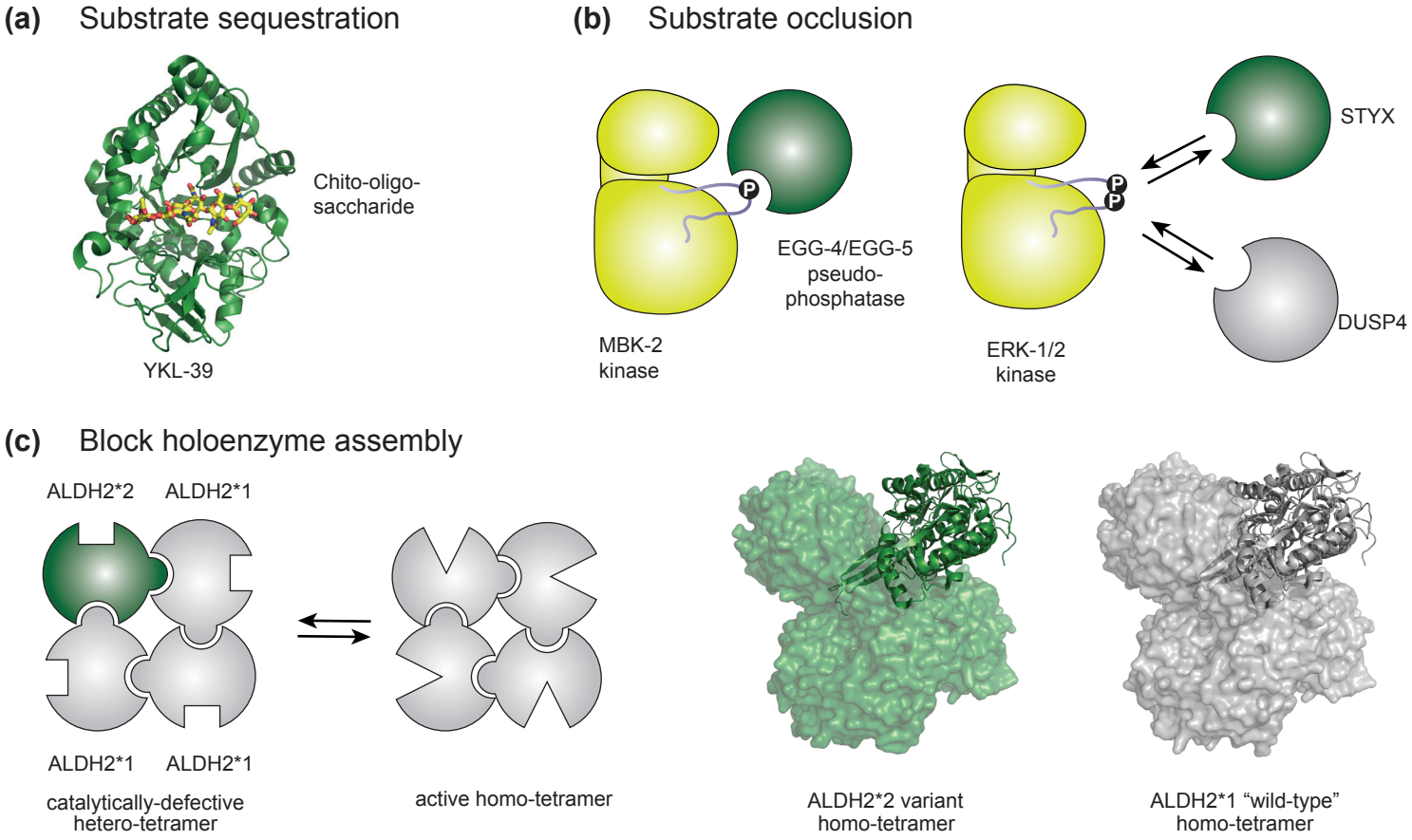
		the conventional protein kinase, RIPK3, leads to exposure of, and cell death by, its N-terminal executioner domain	
		Nucleotide binding by the pseudokinase domain induces activation of the adjacent nuclease domain within the RNaseL homodimer	[33]
Protein Interaction modules: nucleate assembly of protein complexes	Pseudokinase	Pseudokinases, Trib1, Trib2, Trib3 and SgK40, each mediate assembly of a complex comprising a substrate (C/EBPα) and the E3 Ubiquitin ligase, COP1	[35,39]
	Pseudokinase/ pseudoDUB	The pseudokinase PAN3 recruits polyA substrates via its ATP binding site; PAN3 promotes RNase activity of PAN2, which itself contains a pseudoDUB domain	[37,38]
	PseudoATPase	Substrate binding via the tandem pseudoATPase domains in EccC promotes assembly of an active homohexamer	[47]
Regulation of localization, processing and trafficking	PseudoGTPase	PseudoGTPase, light intermediate domain (LIC), binds the dynein motor to cargo.	[69]
	Pseudoprotease	Pseudoproteases, the iRhom proteins, regulate trafficking and stability of single pass transmembrane proteins at the plasma membrane	[4,70-73]
	Pseudophosphatase	Pseudophosphatase STYX anchors ERK1/2 kinase in the nucleus	[74,75]
Competition for holoenzyme assembly	Pseudochitinase	Pseudochitinase YKL-39 binds chitooligosaccharides, but does not catalyse their conversion	[51,52]

Substrate sequestration	Pseudophosphatase	Pseudophosphatase EGG-4/EGG-5 binds to the phosphorylated activation loop of the kinase, MBK-2	[53,54]
		Pseudophosphatase STYX competes with conventional phosphatase DUSP4 for binding to ERK1/2 kinase	[55]
	Pseudo-oxidoreductase	Pseudoenzyme ALDH2*2 “poisons” assembly and allosteric activation within a homotetramer of the catalytically-active counterpart, ALDH2*1	[57]


(a) Allosteric regulation of active enzyme
(b) Signal integrator/molecular switch
Post-translational modification
(c) Nucleate assembly of protein complexes
Assemble signalling complexes
(d) Competition for holoenzyme assembly or substrates
Active enzyme
Active enzyme
Holoenzyme
Inactive complex
Competition for complex assembly
Regulate localisation, processing or trafficking
Substrate sequestration


(a)
Kinase allosteric stabilisation of active site
KSR-RAF
HER3-EGFR
(b)
Caspase stabilisation of active site across dimer
Caspase8-FLIP
(c)
RING1b
Bmi1
E2
Donor Ub
RING E3-ligase stabilisation of E2-Ub conjugate
(d)
BRCC36
KIAA0157
Ub-K63 substrate model
DUB stabilisation of active site via oligomerisation


(a) MLKL phosphorylation-based switch
MLKL PsK
P
4-helix bundle
pMLKL
(b) Nucleotide-based RNase activation
2-5A binding
PsK nucleotide-binding
Ankyrin repeat
RNaseL PsK
RNase
(c) PAN2/3 deadenylase complex assembly
PAN2 UCH-like
PAN3 PsK
PAN2 catalytic-site
PAN3 PsK nucleotide-binding
(d) EccC ATPase assembly
EccC
Active
Pseudo
Pseudo
Substrate-binding
Substrate
Hexamer assembly
Catalytic site


(a) Substrate sequestration
Chito-oligo-saccharide
YKL-39
(b) Substrate occlusion
P
EGG-4/EGG-5 pseudo-phosphatase
MBK-2 kinase
P
P
STYX
DUSP4
ERK-1/2 kinase
(c) Block holoenzyme assembly
ALDH2*2
ALDH2*1
ALDH2*1
ALDH2*1
catalytically-defective hetero-tetramer
active homo-tetramer
ALDH2*2 variant homo-tetramer
ALDH2*1 “wild-type” homo-tetramer