Versatile allosteric properties in Pex5-like tetratricopeptide repeat proteins to induce diverse downstream function

Jérôme Bürgi1 | Lakhan Ekal1 | Matthias Wilmanns1,2

1European Molecular Biology Laboratory, Hamburg Unit, Hamburg, Germany
2University Hamburg Clinical Center Hamburg-Eppendorf, Hamburg, Germany

Correspondence
Matthias Wilmanns, European Molecular Biology Laboratory, Hamburg Unit, Notkestrasse 85, 22607 Hamburg, Germany. Email: matthias.wilmanns@embl-hamburg.de

Funding information
Seventh Framework Programme, Grant/Award Numbers: 664726, MSCA-COFUND-2014-FP; European Commission; Deutsche Forschungsgemeinschaft, Grant/Award Number: WI 1058/9-1 & WI 1058/9-2

Abstract
Proteins composed of tetratricopeptide repeat (TPR) arrays belong to the α-solenoid tandem-repeat family that have unique properties in terms of their overall conformational flexibility and ability to bind to multiple protein ligands. The peroxisomal matrix protein import receptor Pex5 comprises two TPR triplets that recognize protein cargos with a specific C-terminal Peroxisomal Targeting Signal (PTS) 1 motif. Import of PTS1-containing protein cargos into peroxisomes through a transient pore is mainly driven by allosteric binding, coupling and release mechanisms, without a need for external energy. A very similar TPR architecture is found in the functionally unrelated TRIP8b, a regulator of the hyperpolarization-activated cyclic nucleotide-gated (HCN) ion channel. TRIP8b binds to the HCN ion channel via a C-terminal sequence motif that is nearly identical to the PTS1 motif of Pex5 receptor cargos. Pex5, Pex5-related Pex9, and TRIP8b also share a less conserved N-terminal domain. This domain provides a second protein cargo-binding site and plays a distinct role in allosteric coupling of initial cargo loading by PTS1 motif-mediated interactions and different downstream functional readouts. The data reviewed here highlight the overarching role of molecular allosterity in driving the diverse functions of TPR array proteins, which could form a model for other α-solenoid tandem-repeat proteins involved in translocation processes across membranes.

KEYWORDS
allosteric binding and coupling of function, cargo binding, translocation and release, molecular evolution and functional diversity, peroxisomal targeting signal, tetratricopeptide repeat domain, α-solenoid tandem-repeat protein

1 | INTRODUCTION

Peroxisomes are single-membrane organelles present in virtually all eukaryotic cells that live under aerobic conditions.1 Their central role in cell metabolism is tightly coupled to the functions of mitochondria and the endoplasmic reticulum.2-6 Many severe disorders such as the Zellweger syndrome originate from peroxisome biogenesis defects,7,8 indicating their importance in cell homeostasis and proteostasis. The proteome composition of peroxisomes varies among species; most of them share enzymes involved in the β-oxidation of fatty acids, the generation of hydrogen peroxide and hydrogen peroxide degradation to prevent oxidative damage.9 As these processes involve the use of reactive oxygen species (ROS), which would be toxic to other parts of the cell, they generally require compartmentalization and so are thought to be the driving force for peroxisome evolution.7,10
Peroxisomes are membrane-bound organelles devoid of their own DNA, therefore their function requires the import of proteins synthesized in the cytosol. To ensure that peroxisomes are properly functional, the peroxisome proteome needs to be well-balanced in composition, concentration, posttranslational modification, and activity status under defined physiological conditions.11,12 To achieve this, peroxisomal matrix proteins with two main types of sequence signals, called Peroxisomal Targeting Sequence 1 (PTS1) or 2 (PTS2), are recognized by specific peroxisomal receptors Pex5 and Pex7, respectively, for subsequent import into peroxisomes.2,5,13-18 PTS1 motifs are localized at the C-terminus of target proteins, where the last three highly conserved residues are generally essential for protein import. The most common PTS1 sequence required for high-affinity binding of cargos is serine-lysine-leucine, but numerous variations of this motif have been identified that are associated in part with diminished binding affinity.13,19 A recent study in vivo has shown that differences in affinity for different PTS1 cargos play a critical role in cargo sorting and targeting priority to the peroxisomal matrix.20 The PTS2 motif is more degenerate and is usually located close to the N-terminus, but other localizations have also been observed.21,22 Several cases have been reported where hidden PTS1 and PTS2 motifs may become exposed by splicing or translational read-through, which provides crucial additional mechanisms of regulated cargo transport and sorting and leads to distributed targeting to peroxisomes and mitochondria or, alternatively, the protein remaining in the cytosol.23-26

Although the cargo-binding domains in the two receptors Pex5 and Pex7 are unrelated, they share the same protein components for their integration into the peroxisome docking/translocation machinery (DTM) to form a transient translocon during cargo translocation across the peroxisomal membrane and subsequent cargo release17 (Figure 1). Whereas the core receptors (Pex5, Pex7) and docking components are present in all known peroxisomal translocation machineries across different species, there is a high level of divergence in the composition of most DTM proteins and the presence of additional associated factors, some of them limited to defined groups of species, reflecting the substantial functional divergence of peroxisomes.1,17 Remarkably, the cargo translocation process itself does not require energy consumption by nucleotide turnover.18 By contrast, the subsequent recycling of these import receptors is initiated by mono-ubiquitination and requires ATP turnover. Cargo translocation, triggered by cargo receptor loading, and receptor recycling are well-separated steps in the overall cyclic process. This review aims to integrate the available data that provide mechanistic insight into the first part of the overall cyclic process—recognition, docking, translocation—and to draw generally applicable conclusions where possible. For further details on the subsequent recycling steps, we refer to recent reviews.2,13,15,18,21,27

In this article, we propose and highlight allostery—activity regulation by binding or release of an effector to a site distinct from the site of activity—as a key guiding principle to permit and regulate the level of import of a large number of peroxisomal cargo proteins. This generates a complex and highly species-specific peroxisomal proteome without the requirement of external energy. We postulate that all three major steps during the translocation process of PTS1/PTS2 matrix proteins across the peroxisomal membrane—cargo recognition, translocation and

![FIGURE 1](https://example.com/figure1.png)

**FIGURE 1** Peroxisomal PTS1 cargo translocation comprises the following main steps: (1) cargo recognition and binding by the peroxisomal receptor Pex5, which is in competition with other cargos indicated by X, Y, Z; (2) cargo-bound receptor recognition and integration into a multi-protein component docking/translocation machinery (DTM) complex within the peroxisomal membrane; (3) cargo translocation across the peroxisomal membrane and subsequent release into the peroxisomal lumen; (4) recycling of the Pex5 receptor in a mono-ubiquitination-dependent and ATP-consuming process, by the ubiquitination (Ub)-mediating Pex2/Pex10/Pex12 RING finger complex and by the Pex1/Pex6/Pex15 ATPase complex, respectively. Participating protein components are labeled by the established numbering scheme for peroxisomal proteins (for details, see text). The principal outline of this scheme also applies to peroxisomal import of PTS2 cargos, which are recognized by the Pex7 receptor.
release—are driven by allostery. As the presently available data on the PTS2 Pex7 receptor largely remain descriptive, we mainly focus on the PTS1 Pex5 receptor. The remarkable conformational flexibility of the C-terminal domain (CTD) of Pex5 points to its unique properties for allosteric cargo recognition and subsequent triggering of cargo translocation and release. We will further relate known findings of the Pex5 receptor and a more recently characterized second PTS1 receptor only found in yeast (Pex9) to a paralogue named TRIP8b that shares the same CTD organization. However, TRIP8b is otherwise functionally unrelated to Pex5 and Pex9 and acts as a hyperpolarization-activated cyclic nucleotide-gated (HCN) ion channel regulator. We refer to Pex5, Pex9 and TRIP8b as Pex5-like proteins. A common hallmark in all three Pex5-like proteins is their bipartite domain structure, where an N-terminal largely disordered domain (NTD) is followed by a highly conserved and identically folded CTD (Figures 2 and 3A). We finally summarize available data on subsequent processes that lead to cargo translocation and cargo release by the Pex5 receptor and regulation of HCN ion channels by TRIP8b (Figure 4). We conclude by comparing emerging general principles of peroxisomal import with those found in other relevant translocation systems, such as nuclear transport factors mediating protein translocation across the nuclear pore. Advancing our knowledge of these processes could considerably improve our understanding on the mechanism and regulation of the translocation of folded proteins across biological membranes in general.

2 | PEX5-LIKE PROTEINS SHARE A COMMON ALLOSTERIC PROTEIN-RECOGNITION MODULE

2.1 | Peroxisomal cargo PTS1 receptor Pex5

The PTS1 receptor Pex5 is one of the few peroxisomal protein components ubiquitously found in all peroxisome-containing eukaryotic

FIGURE 2 Conserved domain structure in Pex5-like proteins. (A) Domain topology of human (Hs) TRIP8b (light orange background), human Pex5 (light green background), and S. cerevisiae (Sc) Pex9 (gray background). Color codes for domains: CORE (only TRIP8b), light red; NTD cargo-binding motif, dark red; WxxxF/Y motif Pex14-binding sites (only Pex5 and Pex9), dark green; first and second TPR triplets 1-3 and 5-7, alternatingly cyan and blue; hinge TPR-4, magenta; C-terminal helical bundle, violet. N-terminal cysteine mono-ubiquitination site, black line. The indicated domain sizes are proportional to their sequence length; residue numbers are indicated on top. (B) Sequence alignment of the conserved NTD cargo-binding site segment (framed in orange) and CTD of human TRIP8b, human Pex5 and yeast Pex9. The alignment of the Pex5 and TRIP8b CTD sequences are based on their high-resolution structures.37,73 Residue positions where the alignment is uncertain are shown by lowercase characters, all others are shown by uppercase characters. Invariant CTD asparagine positions contributing to the composite PTS1 cargo-binding site shared by all three Pex5-like proteins are shown in red; other conserved residues within the PTS1 cargo-binding site are shown in orange; other conserved residues are shown in yellow. Two residues of the TRIP8b NTD binding site for the HCN ion channel that have been used for experimental testing of binding75 are also shown in orange. The positions of TPR helices based on the high-resolution structure of Pex5 are indicated on top of the alignment, using the color codes of panel “A”
organisms, including fungi and plants. In mammals, the PEX5 gene encodes two transcripts known as PEX5S and PEX5L, which differ by a 37-residue insert in the NTD of the corresponding protein. The presence of the Pex5L insert allows this isoform to link to the PTS2 pathway by direct binding to the Pex7 receptor. A similar site impacting PTS2-driven matrix protein import has also been detected and characterized in the plant Pex5 receptor from *Arabidopsis thaliana*. There are conflicting early reports on the Pex5 oligomerization state in the absence and presence of cargos, which appears to also be pH-dependent. Low-resolution electron microscopy structures of human and *Hansenula polymorpha* Pex5, in the absence and presence of the PTS2 co-receptor Pex20, revealed a tetrameric assembly mediated by the NTD. As discussed in more detail below, Pex5 may also form part of other assemblies when the receptor becomes integrated into the DTM for cargo translocation and release.

The folded structure of the Pex5 CTD comprises an array of seven tetratricopeptide repeat (TPR) motifs, followed by a bundle of three helices (Figure 3A). Several high-resolution structures have revealed a snail-like arrangement of two TPR triplets 1-3 and 5-7. By contrast, the middle TPR-4 module is only partly visible in all known structures, suggesting that TPR-4 may function as a flexible hinge. As observed from Pex5 CTD structures in the presence and absence of cargo, this hinge appears to be crucial for adapting the overall TPR array arrangement to allow a snug fit to bound cargos. The structures of the Pex5 CTD in the presence of two different bound cargo proteins—sterol carrier protein-2 (SCP2; Figure 3B) and alanine-glyoxylate aminotransferase (AGT)—illustrate at atomic detail how the C-terminal PTS1 recognition sequences over a range of at least four residues are virtually identically bound into the central and deep cavity that forms the Pex5 binding site. There are further but more diverse interactions between residues preceding the C-terminal PTS1 motif with other residues of the central Pex5 PTS1-binding site. Cargo PTS1-Pex5 interactions are complemented by nonconserved secondary binding sites, involving other receptor CTD parts and cargo. However, these are not sufficient on their own for the binding of cargos whose structures have been determined to date. It is worth noting that the receptor-bound PTS1 motif in SCP2 is autonomous from the remaining SCP2 folded domain, which is in contrast to AGT where most residues next to the PTS1 motif have extensive interactions with the AGT fold as well. Several hyperoxaluria type 1-causing AGT mutations can be explained by coupled defects in mitochondrial and peroxisomal recognition motifs in folded AGT that result in an altered distribution of the protein between the cytosol, mitochondria, and peroxisomes. Pex5 binding to the peroxisomal PTS1 cargo coenzyme A synthetase (Pcs60) appears to be exceptional, as even a PTS1-truncated version of Pcs60 is capable of binding to Pex5 with key contributions from residues next to the PTS1.

Mechanistically, binding of the Pex5 C-terminal TPR array to PTS1 cargos is composite, as key interactions originate from different TPR modules (Figures 2B and 3). Highly conserved interactions observed in all Pex5 cargo peptide and protein complexes are due to a set of invariant asparagine residues situated on TPR-3, TPR-6, and TPR-7. Comparisons of cargo-receptor structures with mutated PTS1 motifs directly explain the modulation of cargo-binding affinities by up to three orders of magnitude as a result of residue-specific interactions. Binding of high-affinity cargo proteins to the Pex5 receptor leads to compaction of the PTS1 cargo-binding site down to one-third of the volume observed in the CTD apo-conformation. Taking these findings together, the type of flexible folding of the Pex5

![Figure 3](image-url)
CTD TPR array and contributions of conserved interactions from different parts of this array provide a plausible model for how effective cargo binding is achieved by allosteric interactions inherent to a specific TPR array arrangement in the Pex5 receptor.

The Pex5 NTD, in contrast, is largely unfolded.43 The NTD of the mammalian protein comprises seven diaromatic WxxxF/Y motifs that serve to different degrees as binding sites to the main DTM component Pex14 by helix/helix interactions (Figure 2A).44-47 Degenerate versions of the WxxxF/Y motif, such as an N-terminal LVAEF sequence motif and inverted F/YxxxW motifs have also been shown to bind to Pex14 in yeast.48-50 Higher order Pex14:Pex5 assemblies with up to 8:1 stoichiometry have been observed biochemically and structurally with purified mammalian proteins.48,51 Cooperativity in binding, however, was not detected, suggesting that these short sequence motifs bind independently to Pex14 and there is no evidence that the resulting interactions are protein context-dependent.47 As the number and sequence positions of di-aromatic F/YxxxW motifs are not taxonomically conserved in other Pex5 receptors—the Pex5 NTDs from different yeast species, for instance, have only two of these motifs—it seems unlikely that complexes of related stoichiometry and subsequent mechanism of DTM integration are preserved. As discussed in more detail below, a model for how such assembly could become integrated into the peroxisomal DTM has been proposed but mechanistic details of this process still remain elusive.16

In yeast, the inverted F/YxxxW motif is part of a larger moderately conserved sequence stretch that serves as an alternative binding site referred to as PTS3 for a range of peroxisomal cargos, including acyl-CoA oxidase (AOx), carnitine acetyltransferase (Cat2p), enoyl-CoA hydratase (Fox2p), and peroxisomal catalase A.50,52-54 AOX, for
instance, lacks a PTS1 motif and its peroxisomal import is dependent on binding to this NTD PTS3-binding site. In contrast to canonical PTS1-mediated cargo recognition that is permissive for oligomeric cargo assemblies,\textsuperscript{39,42,54} NTD-mediated cargo recognition requires cargo monomerization and is sensitive to the presence of co-factors, suggesting that recognition depends on specific cargo fold features.\textsuperscript{55-58} Although mutagenesis data indicate different residue-specific preferences for Pex14 and PTS3-mediated cargo binding, there are no conclusive data showing whether there is competition for binding to or next to the inverted F/YxxxF motif.\textsuperscript{52,53} Interestingly, the only evidence that a diaromatic WxxxF/Y motif is directly involved in peroxisomal cargo recognition and binding is from AOx of Yarrowia lipolytica. This cargo binds to a WxxxxF/Y motif of the PTS2 co-receptor Pex20, which together with the PTS2 receptor Pex7, functions similarly to the PTS1 receptor Pex5.\textsuperscript{59} Other Pex5 NTD-mediated cargo recognition effects are likely to be indirect because of either altered cargo-binding properties of the receptor in the presence of Pex14 or cargo piggybacking on other cargos.\textsuperscript{2,5,60,61}

Finally, the Pex5 NTD comprises a highly conserved cysteine residue at the N-terminus that serves as a redox-sensitive mono-ubiquitination site to trigger Pex5 receptor recycling (Figures 1 and 2A).\textsuperscript{62} These data explain diminished peroxisomal cargo import efficiency under aging conditions, which is correlated with an increase in mitochondrial production of reactive oxygen species (ROS).\textsuperscript{63,64} Mutation of the cysteine to lysine converts Pex5 into a target for redox-independent mono-ubiquitination, confirming the role of this site as a crucial redox switch for Pex5-dependent cargo import into peroxisomes.\textsuperscript{62} Similar observations were made in a parallel study, but led to an alternative mechanistic proposal in which reducing conditions within peroxisomes induce a Pex5 conformational state with reduced affinity for cargos and ultimately promote cargo release.\textsuperscript{65}

2.2 Alternative yeast Pex5-like PTS1 cargo receptor Pex9

In yeast, a Pex5 parologue was recently characterized and termed Pex9 because of its Pex5-like receptor properties resulting in peroxisomal import of specific PTS1 cargos.\textsuperscript{66-68} In contrast to Pex5, Pex9 expression is strongly increased under conditions in which oleate is the sole carbon source, suggesting a particular role in adaptation to specific metabolic needs.\textsuperscript{66,67} Under these conditions, Pex9 specifically recognizes two different yeast malate synthases, Mls1p and Mls2p.\textsuperscript{67}

The CTDs of Pex5 and Pex9 share a high level of sequence similarity (Figure 2B). A structural homology model of the Pex9 CTD using the Pex5 CTD as a structural template suggests that both proteins have the same CTD organization: a 7-fold repeated TPR array followed by a C-terminal bundle domain (Figure 3A). In addition to the four invariant asparagine residues from TPR-3, TPR-6, and TPR-7 crucial for PTS1 binding in Pex5, various other residues within the PTS1-binding site are conserved in Pex9 (Figure 2B), suggesting a preserved mechanism of PTS1 cargo binding in Pex5 and Pex9. However, the differential properties that lead to such different cargo substrate specificities remain largely elusive in the absence of high-resolution structural data.

The Pex9 NTD is approximately 100 residues shorter than the Pex5 NTD (Figure 2A). However, key features such as an N-terminal cysteine-mediated mono-ubiquitination site, two WxxxF/Y motifs identical in number to those observed in Pex5 from different yeast species, and a putative di-aromatic PTS3-binding site are all present in Pex9 (Figure 2). Given that these NTD hallmarks are found in both Pex9 and Pex5, in subsequent sections of this review we work on the assumption that further functional features of the Pex5 receptor are likely to also apply to the Pex9 receptor, which is much less characterized in terms of mechanistic detail to date.

2.3 Pex5-like HCN ion channel accessory factor TRIP8b

Tetratricopeptide repeat-containing Rab8b-interacting protein (TRIP8b), initially also identified as a Pex5-related protein,\textsuperscript{69} functions as an accessory subunit of hyperpolarization-activated cyclic nucleotide-gated (HCN) ion channels by altering both HCN cell surface expression and cyclic nucleotide dependence.\textsuperscript{70,71} Its inclusion in the Pex5-like protein family is due to its closely related CTD and a conserved sequence stretch in the NTD that is equivalent to the diaromatic PTS3-binding site in Pex5 (Figure 2).\textsuperscript{72} The structure of the TRIP8b CTD in the presence of a C-terminal peptide from the HCN ion channel cytoplasmic cyclic nucleotide-binding domain (CNBD) revealed an overall organization that is virtually identical to known Pex5-PTS1 complexes\textsuperscript{73} (Figure 3). In contrast to most Pex5-protein cargo complexes where PTS1-mediated receptor recognition is sufficient for binding, HCN ion channel binding by TRIP8b requires a second binding site, which has been characterized as a conserved “core” domain of about 80 residues in length.\textsuperscript{72} Unlike the C-terminal HCN ion channel PTS1-like interaction, the core domain-mediated interaction partially competes with cAMP binding to the HCN ion channel CNBD and thus acts as an agonist for cAMP-dependent regulation of HCN ion channel function.\textsuperscript{74-76} Remarkably, both binding sites together lead to allosteric coupling and hence enhancement of HCN ion channel CNBD binding.\textsuperscript{77}

Although the essential requirement for both HCN ion channel binding sites in TRIP8b explains its role as an HCN ion channel-specific accessory factor, the mechanism underlying the broad versatility of cargo recognition by the peroxisomal Pex5 receptor as opposed to the highly HCN ion channel-specific binding properties of TRIP8b remains an open question. As TRIP8b is capable of binding to other PTS1 peptides in vitro,\textsuperscript{77} it is conceivable that there are additional mechanisms to preclude TRIP8b from unintended binding to other PTS1 cargos in vivo. Distinct and highly specific expression patterns of both proteins partly explain cargo recognition differences but would not safeguard one vs the other function in cases of overlap. Distinct phosphorylation patterns and sites observed in both proteins could also lead to both differential regulation of ligand recognition...
and ligand specificity. The sequence similarity of the PTS3-binding motif of the Pex5 NTD with part of the TRIP8b core domain (Figure 2B) also suggests related modes of ligand binding in both proteins—PTS3 cargos for Pex5 and HCN ion channel CNBD for TRIP8b—and coupled allostery caused by multiple binding sites. However, in the absence of high-resolution structures of the respective interfaces, their structural similarities remain speculative. In contrast to Pex5 and Pex9, the TRIP8b NTD does not comprise any WxxxF/Y motifs, which is a key feature contributing to the role of Pex5 as a peroxisomal translocation receptor by binding and subsequent integration into the peroxisomal DTM. Whether there is a common evolutionary origin of an NTD-driven protein-binding site for TRIP8b and Pex5/Pex9 remains an open question at present.

2.4 | Peroxisomal cargo PTS2 receptor Pex7 with an unrelated cargo-binding domain

In contrast to the PTS1 receptor Pex5 that binds cargo via its CTD and integrates into the DTM through its NTD, the function of the PTS2 receptor Pex7 is restricted to PTS2 cargo recognition and binding alone. Its inability to integrate into the DTM is supplemented by binding to co-receptors, such as the long isofrom of the PTS1 receptor Pex5L in higher vertebrates and plants. In fungi, this function is carried by different co-receptors (Pex18, Pex21, and Pex20). These protein components have in common one or more WxxxF/Y motifs that are required for interaction with Pex14 and subsequent docking and pore formation. A high-resolution structure of Saccharomyces cerevisiae Pex7 in the presence of the Pex7-binding domain of the co-receptor Pex21 and the PTS2 motif of the cargo Fox3 (thiolase) revealed how the Fox3 PTS2 forms an amphipathic helix that is sandwiched between the Pex7 6-fold repeated WD repeat domain and Pex21.12 Similar to the requirement for PTS1 cargo binding to the Pex5 CTD for DTM integration and cargo translocation, Pex7 DTM integration requires both binding to its co-receptor (Pex5L in human, Pex18 or Pex21 in S. cerevisiae) and PTS2 cargo loading. Interestingly in yeast, only the co-receptor Pex21 but not Pex18 provides an additional binding site for the PTS2 cargo glycerol-3-phosphate dehydrogenase (Gpd1p). In addition, Pex20 from both H. polymorpha and Y. lipolytica provides a binding site for AOx and mediates WxxyF/Y-mediated DTM integration by binding to Pex14. Whether there is any analogy to PTS3-mediated cargo binding by the Pex5 NTD has not yet been investigated. A piggyback mechanism has been recently observed for pyrazinamidase/nicotinamidase I (Pnc1), which depends on PTS2-dependent import of Gpd1p.

3 | POSSIBLE ROLES OF ALLOSTERY IN PEX5 RECEPTOR CARGO TRANSLLOCATION

In higher eukaryotes, the DTM for both PTS1 and PTS2 cargos, although recognized by different receptors (Pex5, Pex7), consists of the same major membrane proteins Pex13 and Pex14, as well as the RING domain proteins Pex2, Pex10, and Pex12 (Figures 1 and 4). The latter are also involved in the Pex5 recovery process for subsequent import cycles by mono-ubiquitination. Pex13 and Pex14 play a direct role in docking of cargo-loaded Pex5 into the DTM and subsequent cargo release. In fungi, there are two additional factors Pex8 and Pex17 that contribute to PTS1 cargo translocation and release. A recent structural study showed that the yeast Pex14/17 docking complex forms an elongated stalk directed toward the cytosol, potentially to capture cargo-loaded Pex5. In filamentous fungi, the Pex33 component appears to combine the functions of Pex14 and Pex17, and hence is also referred to as Pex14/17. At present, it remains unknown to what extent these components in other higher eukaryotes are either replaced by unrelated proteins or are undetectable due to evolutionary divergence.

It is well established that peroxisomal matrix cargo loading of the Pex5 receptor is critical for switching the NTD conformation of the receptor allowing its integration into the DTM. Truncated Pex5 lacking the PTS1 cargo-binding CTD, as well as a Pex5 variant mimicking a cargo-bound state, are constitutively integrated into the DTM, suggesting a repressor function of the CTD in the absence of cargo. However, how this repression is lifted—by loss of NTD/CTD interactions upon cargo binding or other conformational changes—remains an open question. Presently, available data allow different interpretations. In summary, both cargo loading and DTM integration trigger a unique metamorphosis of Pex5 from a highly soluble import receptor to an integrated, quasi-membranous component within the multi-subunit peroxisomal DTM (Figure 4). This NTD-mediated DTM integration coupled to allosteric cargo loading of the Pex5 CTD may be analogous to the dual binding of the TRIP8b NTD and CTD sites to the HCN ion channel that leads to allosterically coupled regulation of cAMP-dependent HCN ion channel activity. A crucial difference between the two systems is that, whereas allosteric activation of TRIP8b is caused by interacting with and targets the same protein (HCN ion channels), the Pex5 receptor is activated by proteins (PTS1 cargos) that are distinct from its target for integration, the peroxisomal multi-component DTM.

The ability of the Pex5 receptor to bind to both DTM core components Pex14 and Pex13 seems to be crucial for its integration into the DTM. While the structural basis of WxxyF/Y motif-mediated Pex5/Pex14 interactions has been established, the extent to which these interactions are impacted by cargo loading and other potential conformational transitions during DTM integration, such as transient pore formation and translocation as well as cargo release, remains unknown. Analysis of the structure of the cytosolic C-terminal SH3 domain from Pex13 from yeast revealed binding to both WxxyF motifs of the Pex5 NTD as well as to a PxxP motif in Pex14. The two binding motifs are nonoverlapping and non-competitive based on experiments with purified protein components, but data from Pichia (P. pastoris) have shown that binding of the Pex13 SH3 domain attenuates the interaction of the Pex5 receptor with Pex14. The interaction between Pex13 and Pex14 is further enhanced through a second interaction site involving the N-terminal...
region of Pex13, and blocking of both sites leads to loss of Pex13 from the DTM.\textsuperscript{100} In the human Pex5 receptor, a different WxxxF/Y motif-mediated Pex13-binding site has been found, which binds to the N-terminal region instead of the C-terminal SH3 domain of Pex13.\textsuperscript{46} This binding site appears to be required for the import of oligomeric PTS1 cargos as shown by the loss of import of tetrameric catalase.\textsuperscript{101} Finally, DTM phosphorylation may also have an important role as it has been shown that specific Pex14 phosphorylation sites may affect interactions between DTM components, which could also affect the Pex5 export and subsequent recycling process.\textsuperscript{102-104} The function of these phosphorylation sites remains to be determined, and thus far only defects in the import of certain cargos (catalase, citrate synthase 2) under specific conditions such as mitosis and oxidative stress have been detected, rather than a more general effect on cargo import.\textsuperscript{103,104}

DTM integration of Pex5 leads to the formation of a distinct transient pore to promote PTS1 cargo translocation (Figure 4).\textsuperscript{105} Molecular weight estimates suggest that under pore-forming conditions, Pex5 assembles into a higher-order oligomeric state. It has yet to be determined if this assembly impacts Pex5 oligomeric arrangements observed for the soluble receptor.\textsuperscript{35} A similar, but less dynamic, pore can be formed upon DTM integration of the PTS2 Pex7 receptor.\textsuperscript{106} Both pores have been identified and characterized by electrophysiological measurements, but no structural data are available at this time.\textsuperscript{17}

In the absence of structural data, it is unknown whether the observed 1:1 Pex5 receptor/cargo stoichiometry prior to receptor docking and cargo translocation remains preserved during the transient pore-based translocation process, which could allow simultaneous translocation of multiple cargos. Another open question surrounds the orientation of the cargo-loaded Pex5 receptor during translocation. Assuming that the snail-like TPR array structurally aligns with the transient pore, two different models are conceivable: one in which the cargo precedes the receptor during translocation, or an alternative model where the cargo follows the receptor during translocation, or a mixture of both. The orientation could also be different depending on the type of receptor recognition signal—PTS1 or PTS3—or combinations of both. Finally, PTS3-mediated cargo loading on the NTD of the Pex5 receptor appears to have more restricted requirements for the cargo association state during the translocation process than PTS1-mediated CTD cargo loading. These questions are relevant as they have a direct impact on the mechanism of cargo release. While cargo-in-front-of-the-receptor translocation is suggestive of a release mechanism directly targeting the cargo/receptor binding site from the luminal side (Figure 4), the cargo-following-the-receptor is compatible with a model of allosteric release targeting one or more receptor sites distinct from the cargo-binding site.

4 | CARGO RELEASE FROM THE PEX5 RECEPTOR: A MECHANISTIC CONUNDRUM

In addition to its central function as the DTM hub for both PTS receptors Pex5 and Pex7, Pex14 has also been reported to be involved in cargo release. In Leishmania, binding of an extended 26-residue PTS1 peptide to Pex5 is considerably reduced in the presence of Pex14.\textsuperscript{107} Similarly, binding of human catalase to Pex5 is impaired in the presence of Pex14.\textsuperscript{57} As only monomeric catalase interacts with the Pex5 NTD PTS3-binding site, there could be direct competition between the cargo and Pex14 for the same binding site on Pex5. In contrast to Pex14, there are no reports to date that suggest a direct involvement of Pex13 in cargo release.

Perhaps the strongest evidence for an active role in PTS cargo release can be ascribed to Pex8, a peroxisomal protein component only found in yeast species that display a high level of sequence diversity.\textsuperscript{108,109} Most Pex8 proteins characterized to date contain both a PTS1 and PTS2 motif, and redundant PTS1/Pex5 and PTS2/Pex7-dependent import pathways have been confirmed.\textsuperscript{110} Notably, all organisms containing Pex8 also contain the Pex7 co-receptor Pex20, suggesting functional linkage between both proteins.\textsuperscript{110} The PTS2-mediated import of Pex8 requires binding to both the Pex7 WD repeat domain and the Pex20 C-terminal domain.\textsuperscript{110} However, in Y. lipolytica, Pex8 interacts with the Pex20 N-terminal domain independently of Pex7, and a role for this interaction in cargo release has been suggested.\textsuperscript{111} A proposed function as a peroxisomal import organizer in S. cerevisiae, connecting the RING finger protein components and the core DTM docking components Pex13, Pex14 and Pex17,\textsuperscript{86} could not be confirmed in experiments in P. pastoris, indicating that the presence of Pex5 and Pex14 alone is sufficient for Pex8 import.\textsuperscript{110,112} Surprisingly in S. cerevisiae, a direct interaction can be detected between Pex8 and the C-terminal SH3 domain of Pex13, but not between Pex8 and Pex14. This result suggests that the established role of Pex14 within the DTM for Pex8 import could be mediated through Pex13.\textsuperscript{113} The Pex8-binding site on the Pex13 SH3 domain does not overlap with the canonical PexP\textsuperscript{14} motif-mediated Pex14 interaction site, and a potential overlap with the second non-canonical Pex5-binding site\textsuperscript{98,99,113} has not yet been tested. Taking the data together, a picture of an intricate interaction network between Pex5, Pex14, Pex13, and Pex8 is emerging. However, deconvoluting these interactions in terms of their precise function during translocation and cargo release remains a task for the future.

The first evidence for direct Pex8 involvement in the cargo release process came from fluorescence correlation spectroscopy (FCS) studies where the presence of Pex8 decreased the amount of PTS1 peptide bound to Pex5.\textsuperscript{34} Noticeably, this effect is independent of the presence of the Pex8 PTS1 motif. This observation was further corroborated by cargo release data where Pex8 facilitates the release of GFP-SKL from Pex5 under reducing conditions.\textsuperscript{65} In the same study, it was also shown that the C-terminal region of Pex8 is involved in cargo release. Furthermore, Pex8 interacts with the Pex5 NTD independently from the TPR domain.\textsuperscript{65,109,110} The interaction seems to be promoted by reducing conditions as this avoids disulfide bridge-mediated blockage of an invariant cysteine at the N-terminus of the NTD, which also serves as a redox-sensitive mono-ubiquitination site to induce Pex5 receptor recycling.\textsuperscript{65} This is in line with earlier observations that the role of Pex8 in peroxisomal import is downstream of DTM integration since it is dispensable for PTS receptor targeting to...
the docking complexes.\textsuperscript{109} To what extent there is cross-talk between redox sensitivity, cargo release and initiation of Pex5 receptor recycling by mono-ubiquitination remains a fascinating question for future studies.\textsuperscript{114}

The N-terminal Pex5 WxxxF/Y motif-binding site on Pex14 also serves as a binding site for the peroxisomal membrane protein (PMP) chaperone/receptor component Pex19.\textsuperscript{44} Whether Pex19 functions primarily to target Pex14 to the peroxisomal membrane, or to regulate Pex5 translocation and cargo release remains an important topic for future research.\textsuperscript{115-119}

5 | CONCLUSIONS AND FUTURE PERSPECTIVES

About 15 years ago, the Erdmann group made a first systematic attempt to integrate the available data supporting a transient pore model to describe the import of peroxisomal cargo proteins across the peroxisomal membrane\textsuperscript{120} over alternative models such as transport through endocytic peroxisomal membrane vesicles.\textsuperscript{121,122} The transient pore model requires one or more receptors that have the ability to: (1) specifically recognize protein cargos destined for import; (2) temporarily integrate within the peroxisomal membrane; (3) form a pore large enough for translocation of folded cargos; and (4) promote cargo translocation and release with the support of additional regulatory factors. Various pieces of evidence since then have illustrated that both PTS receptors Pex5 and Pex7 (with the requirement of additional factors that substitute for the Pex5 NTD domain missing in Pex7) satisfy most of these conditions. Although direct structural data are still missing, the most convincing evidence for the existence of two related peroxisomal transient pores for both receptors sharing the docking components Pex13 and Pex14 originates from electrophysiological measurements, demonstrating that indeed large and dynamic pores can be formed.\textsuperscript{17} However, mechanistic insight on how permanent opening of the pore is avoided remains incomplete at present and poses central questions about their overall regulation.

To date, our understanding about the first steps of the cargo translocation process—cargo recognition and cargo-loaded receptor docking—has most advanced for the Pex5 receptor and is supported by several high-resolution structural studies.\textsuperscript{16} In essence, available findings demonstrate that this is driven by allosteric interactions for cargo recognition and allosteric coupling as a key principle, leading to integration of the receptor into the DTM. Conversely, the remaining steps—cargo translocation and release—still remain largely enigmatic from a mechanistic perspective. Structural insight into these processes is not yet available. Unraveling common principles of the underlying mechanisms becomes even more challenging due to the lack of a known universal cargo release candidate protein, as Pex8—as being a strong candidate for cargo release—is found in yeast species but not in higher vertebrates.

Despite a lack of mechanistic data on Pex5-like translocation receptors, it is attractive to extrapolate from known mechanistic principles of other relevant translocation receptors. Known systems that allow translocation (secretion) of folded proteins are cargo import/export across the nuclear pore, as well as highly specialized twin-arginine translocation and type VII secretion systems, although the latter is found only in prokaryotic organisms.\textsuperscript{123-125} However, all bacterial secretion systems that allow the translocation of folded proteins have unrelated and in general more rigid architectures. By contrast, one of the key principles outlined for the three Pex5-like receptors in this contribution—allosteric regulation and coupling by \(\alpha\)-helical tandem repeat structures—is also a key mechanism in protein transport via the nuclear pore. In this analogy, the equivalent of the Pex5 receptor in PTS1-mediated import would be nuclear transport factors, which are most broadly represented by a large family of \(\beta\)-karyopherins.\textsuperscript{125,126} Similar to the Pex5 CTD, they have an extended helical tandem repeat structure in common that is composed of HEAT rather than TPR repeats, both of which belong to \(\alpha\)-solenoid tandem-repeat proteins that represent one of the most frequently occurring fold categories in the biological universe.\textsuperscript{127-129} In \(\beta\)-karyopherins, the key regulator for both cargo recognition and release is Ran bound either to GDP or GTP, leading to substantially different conformations of Ran. The gradient of both forms has been shown to serve as a crucial element for the directionality of nucleo-cytoplasmic transport, import vs export. In addition, various structures of \(\beta\)-karyopherins in the presence of the RanGTP/RanGDP regulator, cargos and adaptors as well as in the absence of Nup components for docking into the nuclear pore, impressively illustrate the enormous versatility of solenoid-type tandem repeats for both binding and release. Most of these interactions are observed at the concave surface of the overall HEAT repeat array architecture.\textsuperscript{125} The main mechanism behind this versatility is in the overall flexibility inherent to \(\beta\)-karyopherins leading to multiple levels of allostery, ranging from local to global, and being both competitive and non-competitive.\textsuperscript{130,131} Using these \(\beta\)-karyopherin data as a template, we speculate that the TPR array of the Pex5 CTD may ultimately have more direct but not yet characterized protein binding partners in addition to well-established PTS1 cargos, with the ability to regulate cargo binding and ultimately triggering cargo release. It is worth mentioning that the Pex5-like protein TRIP8b binds to a cAMP-dependent binding domain of the HCN ion channel and thus controls its gating function, which creates a potential analogy to nucleotide binding domain-mediated regulation of \(\beta\)-karyopherin function via RanGTP/GDP. Similar to observations on Pex5 and \(\beta\)-karyopherin-mediated cargo translocation processes, the key underlying mechanism by TRIP8b-mediated activity regulation occurs by both direct and indirect allostery.\textsuperscript{76}

In summary, the translocation of peroxisomal matrix proteins supported by two different types of receptors, Pex5/Pex9 (in yeast only) and Pex7 together with its co-receptors Pex18/Pex21, appears to be as unique as the translocation process itself. Based on presently available knowledge, it does not require external energy and appears to be mainly driven by allosteric binding, coupling and release mechanisms. This is surprising, as peroxisomal cargo import is unidirectional and most likely against a cargo concentration gradient, assuming lower cargo abundance in the cytosol where they are synthesized by
the ribosomal machinery. Whether the energy-dependent receptor recycling process is a driving force during cargo translocation remains an open and fascinating question. Direct association of the ribosomal machinery for cargo biosynthesis with the peroxisomal membrane and DTM could also help to overcome the conundrum of energy-independent cargo import against an apparent concentration gradient. Advancing our understanding of translocation processes in terms of general principles such as allostery, composite vs linear binding, and requirements for energy consumption will be greatly beneficial to unravel functional synergy and diversity in systems with related modules, exemplified here for members of Pex5-like proteins with a common TPR array domain structure.

ACKNOWLEDGMENTS
This study has been supported by the Deutsche Forschungsgemeinschaft to Matthias Wilmanns (WI 1058/9-1 and WI 1058/9-2) via the joint research group PERTRANS. Jérôme Bürgi has been supported by an EIAPOD postdoctoral fellowship from the European Commission (664726, MSCA-COFUND-2014-FP). Open access funding enabled and organized by ProjektDEAL.

PEER REVIEW
The peer review history for this article is available at https://publons.com/publon/10.1111/tra.12785.

ORCID
Jérôme Bürgi https://orcid.org/0000-0003-1171-3455
Lakhan Ekal https://orcid.org/0000-0001-8916-4201
Matthias Wilmanns https://orcid.org/0000-0002-4643-5435

REFERENCES
1. Gabaldon T. Peroxisome diversity and evolution. Philos Trans R Soc Lond B Biol Sci. 2010;365(1541):765-773.
2. Kim PK, Hetta EH. Multiple pathways for protein transport to peroxisomes. J Mol Biol. 2015;427(6 Pt A):1176-1190.
3. Costello J, Schrader M. Unloosing the Gordian knot of peroxisome formation. Curr Opin Cell Biol. 2018;50:50-56.
4. Kao YT, Gonzalez KL, Bartel B. Peroxisome function, biogenesis, and dynamics in plants. Plant Physiol. 2018;176(1):162-177.
5. Islinger M, Voelkl A, Fahimi HD, Schrader M. The peroxisome: an update on mysteries 2.0. Histochem Cell Biol. 2018;150(5):443-471.
6. Sugiuara A, Mattie S, Prudent J, McBride HM. Newly born peroxisomes are a hybrid of mitochondrial and ER-derived pre-peroxisomes. Nature. 2017;542(7640):251-254.
7. Wanders RJA. Peroxosomal disorders: improved laboratory diagnosis, new defects and the complicated route to treatment. Mol Cell Probes. 2018;40:60-69.
8. Argyriou C, D’Agostino MD, Braverman N. Peroxisome biogenesis disorders. Transl Sci Rare Dis. 2016;1(2):111-144.
9. Walker CL, Pomatto LCD, Davies KJA. Redox regulation of homeostasis and proteostasis in peroxisomes. Physiol Rev. 2018;98(1):89-115.
10. Lismont C, Nordgren M, van Veldhoven PP, Fransen M. Redox interplay between mitochondria and peroxisomes. Front Cell Dev Biol. 2015;3:35.
11. Sandallo LM, Gotor C, Romero LC, Romero-Puertas MC. Multilevel regulation of peroxisomal proteome by post-translational modifications. Int J Mol Sci. 2019;20(19):4881.
12. Pan D, Nakatsu T, Kato H. Crystal structure of peroxisomal targeting signal-2 bound to its receptor complex Pex7p-Pex21p. Nat Struct Mol Biol. 2013;20(8):987-993.
13. Baker A, Lanyon-Hogg T, Warriner SL. Peroxisome protein import: a complex journey. Biochem Soc Trans. 2016;44(3):783-789.
14. Hasan S, Platta HW, Erdmann R. Import of proteins into the peroxisomal matrix. Front Physiol. 2013;4:261.
15. Francisco T, Rodrigues TA, Dias AF, Barros-Barbosa A, Bicho D, Azevedo JE. Protein transport into peroxisomes: knowns and unknowns. Bioessays. 2017;39(10):1700047.
16. Emmanouilidis L, Gopalswamy M, Passon DM, Wilmanns M, Sattler M. Structural biology of the import pathways of peroxisome matrix proteins. Biochim Biophys Acta. 2016;1863(5):804-813.
17. Meinecke M, Bartsch P, Wagner R. Peroxisomal protein import pores. Biochim Biophys Acta. 2016;1863(5):821-827.
18. Walter T, Erdmann R. Current advances in protein import into peroxisomes. Protein J. 2019;38(3):351-362.
19. Ghosh D, Berg JM. A proteome-wide perspective on peroxisome targeting signal 1(PT1S)-Pex5p affinities. J Am Chem Soc. 2010;132(11):3973-3979.
20. Rosenthal M, Metzl-Raz E, Bürgi J, et al. Uncovering targeting priority to yeast peroxisomes using an in-cell competition assay. Proc Natl Acad Sci U S A. 2020;117(35):21432-21440.
21. Kunze M. The type-2 peroxisomal targeting signal. Biochim Biophys Acta Mol Cell Res. 2020;1867(2):118609.
22. Rodrigues TA, Grou CP, Azevedo JE. Revisiting the intraperoxisomal pathway of mammalian PEX7. Sci Rep. 2015;5:11806.
23. Freitag J, Ast J, Bolker M. Cryptic peroxisomal targeting via alternative splicing and stop codon read-through in fungi. Nature. 2012;485(7399):522-525.
24. Schüeren F, Thoms S. Functional translational readthrough: a systems biology perspective. PLoS Genet. 2016;12(8):e1006196.
25. Ast J, Stiebler AC, Freitag J, Bolker M. Dual targeting of peroxisomal proteins. Front Physiol. 2013;4:297.
26. Kunze M, Berger J. The similarity between N-terminal targeting signals for protein import into different organelles and its evolutionary relevance. Front Physiol. 2015;6:259.
27. Farre JC, Mahalingam SS, Projeto M, Subramani S. Peroxisome biogenesis, membrane contact sites, and quality control. EMBO Rep. 2019;20(1):e4684.
28. Braverman N, Dodt G, Gould SJ, Valle D. An isoform of pex5p, the human PTS1 receptor, is required for the import of PTS2 proteins into peroxisomes. Hum Mol Genet. 1998;7(8):1195-1205.
29. Otera H, Harano T, Honsho M, et al. The mammalian peroxin Pex5pL, the longer isoform of the mobile peroxisome targeting signal (PTS) type 1 transporter, translocates the Pex7p:PT52 protein complex into peroxisomes via its initial docking site, Pex14p. J Biol Chem. 2000;275(28):21703-21714.
30. Woodward AW, Bartel B. The Arabidopsis peroxisomal targeting signal type 2 receptor PEX7 is necessary for peroxisome function and dependent on PEX5. Mol Biol Cell. 2005;16(2):573-583.
31. Hayashi M, Yagi M, Nito K, Kamada T, Nishimura M. Differential contribution of two peroxisomal protein receptors to the maintenance of peroxisomal functions in Arabidopsis. J Biol Chem. 2005;280(15):14829-14835.
32. Schliebs W, Sawdowsky J, Agianian B, Dodt G, Herberg FW, Kunau WH. Recombinant human peroxisomal targeting signal receptor PEX5. Structural basis for interaction of PEX5 with PEX14. J Biol Chem. 1999;274(9):5666-5673.
33. Boteva R, Koek A, Visser NV, et al. Fluorescence analysis of the Hansenula polymorpha peroxisomal targeting signal-1 receptor, Pex5p. Eur J Biochem. 2003;270(21):4332-4338.
34. Wang D, Visser NV, Veenhuis M, van der Klei U. Physical interactions of the peroxisomal targeting signal 1 receptor pex5p, studied by fluorescence correlation spectroscopy. J Biol Chem. 2003;278(44):43340-43345.
Moslicka KB, Klompmaker SH, Wang D, van der Klei IJ, Boekema EJ. The Hansenula polymorpha peroxisomal targeting signal 1 receptor, Pex5p, functions as a tetramer. FEBS Lett. 2007;581(9):1758-1762.

Gatto GJ Jr, Geisbrecht BV, Gould SJ, Berg JM. Peroxisomal targeting signal-1 recognition by the TPR domains of human PEX5. Nat Struct Biol. 2000;7(12):1091-1095.

Stanley WA, Filipp FV, Kursula P, et al. Recognition of a functional peroxisome type 1 target by the dynamic import receptor pex5p. Mol Cell. 2006;24(5):653-663.

Stanley WA, Pursiainen NV, Garman EF, Juffer AH, Wilmanns M, Klein AT, van den Berg M, Bottger G, Tabak HF, Distel B. Saccharomyces cerevisiae acyl-CoA oxidase follows a novel, non-PTS1, import pathway into peroxisomes that is dependent on Pex5p. J Biol Chem. 2002;277(28):25011-25019.

Rymer L, Kempsinski B, Chelstowska A, Skoneczny M. The budding yeast Pex5p receptor directs Fox2p and Cta1p into peroxisomes via its N-terminal region near the FxxxFW domain. J Cell Sci. 2018;131(17):jcs216986.

Kempsinski B, Chelstowska A, Poznanski J, et al. The peroxisomal targeting signal 3 (PTS3) of the budding yeast acyl-CoA oxidase is a signal patch. Front Cell Dev Biol. 2020;8:198.

Gunkel K, van Dijk R, Veenhuis M, van der Klei IJ. Routing of Hansenula polymorpha alcohol oxidase: an alternative peroxisomal protein-sorting machinery. Mol Biol Cell. 2004;15(3):1347-1355.

Freitas MO, Francisco T, Rodrigues TA, et al. The peroxisomal protein import machinery displays a preference for monomeric substrates. Open Biol. 2015;5(4):140236.

Freitas MO, Francisco T, Rodrigues TA, et al. PEX5 protein binds monomeric catalase blocking its tetramerization and releases it upon binding the N-terminal domain of PEX14. J Biol Chem. 2011;286(47):40509-40519.

Dias AF, Francisco T, Rodrigues TA, Grou CP, Azevedo JE. The first minutes in the life of a peroxisomal matrix protein. Biochim Biophys Acta. 2016;1863(5):814-820.

Chang J, Rachubinski RA. Pex20p functions as the receptor for non-PTS1/non-PTS2 acyl-CoA oxidase import into peroxisomes of the yeast Yarrowia lipolytica. Traffic. 2019;20(7):504-515.

Oshima Y, Kamigaki A, Nakamori C, et al. Plant catalase is imported into peroxisomes by Pex5p but is distinct from typical PTS1 import. Plant Cell Physiol. 2008;49(4):671-677.

Yang J, Pieuchot L, Jedd G. Artificial import substrates reveal an omnivorous peroxisomal importor. Traffic. 2018;19(10):786-797.

Apanasets O, Grou CP, van Veldhoven PP, et al. PEX5, the shuttling receptor-binding cavity impacts protein import efficiency into peroxisomes. Traffic. 2015;16(1):85-98.

Oppici E, Fodor K, Paiardini A, et al. Crystal structure of the S187F variant of human liver alanine:glyoxylate aminotransferase as an essential determinant in primary hyperoxaluria type 1. J Mol Biol. 2015;290(44):26610-26626.

Carvalho AF, Costa-Rodrigues J, Correia I, et al. The N-terminal half of the peroxisomal cycling receptor Pex5p is a natively unfolded domain. J Mol Biol. 2006;356(4):864-875.

Neufeld C, Filipp FV, Simon B, et al. Structural basis for competitive interactions of Pex14 with the import receptors Pex5 and Pex19. EMBO J. 2009;28(6):745-754.

Su JR, Takeda K, Tamura S, Fujiki Y, Miki K. Crystal structure of the conserved N-terminal domain of the peroxisomal matrix protein import receptor, Pex14p. Proc Natl Acad Sci U S A. 2009;106(2):417-421.

Otera H, Setoguchi K, Hamasaki M, Kumashiro T, Shimizu N, Fujiki Y. Peroxisomal targeting signal receptor Pex5p interacts with cargoes and import machinery components in a spatiotemporally differentiated manner: conserved Pex5p WXXXF/Y motifs are critical for matrix protein import. Mol Cell Biol. 2002;22(6):1639-1655.

Kempinski B, Chelstowska A, Poznanski J, et al. The peroxisomal targeting receptor. J Biol Chem. 2013;288(38):27220-27231.

Ivashchenko O, van Veldhoven PP, Brees C, Ho YS, Terlecky SR, Zagotta WN. Structure and stoichiometry of an accessory subunit of TRIP8b interaction with hyperpolarization-activated cyclic nucleotide-gated channels. Channels (Austin). 2020;14(1):110-122.

Santoro B, Hu L, Liu H, et al. TRIP8b regulates HCN1 channel trafficking and gating through two distinct C-terminal interaction sites. J Neurosci. 2011;31(11):4074-4086.

Bankston JR, Camp SS, DilMaio F, Lewis AS, Chetkovich DM, Zagota WN. Structure and stoichiometry of an accessory subunit TRIP8b interaction with hyperpolarization-activated cyclic nucleotide-gated channels. Proc Natl Acad Sci U S A. 2012;109(20):7899-7904.
114. Wang W, Subramani S. Role of PEX5 ubiquitination in maintaining peroxisome dynamics and homeostasis. *Cell Cycle*. 2017;16(21):2037-2045.

115. Fransen M, Vastiau I, Brees C, Brys V, Mannaerts GP, Van Veldhoven PP. Potential role for Pex19p in assembly of PTS-receptor docking complexes. *J Biol Chem*. 2004;279(13):12615-12624.

116. Itoh R, Fujiki Y. Functional domains and dynamic assembly of the peroxin Pex14p, the entry site of matrix proteins. *J Biol Chem*. 2006;281(15):10196-10205.

117. Veenhuis M, van der Klei IJ. A critical reflection on the principles of peroxisome formation in yeast. *Front Physiol*. 2014;5:110.

118. Knoops K, Manivannan S, Cepinska MN, et al. Preperoxisomal vesicles can form in the absence of Pex3. *J Cell Biol*. 2014;204(5):659-668.

119. Giannopoulou EA, Emmanouilidis I, Sattler M, Dodt G, Wilmanns M. Towards the molecular mechanism of the integration of peroxisomal membrane proteins. *Biochim Biophys Acta*. 2016;1863(5):863-869.

120. Erdmann R, Schliebs W. Peroxisomal matrix protein import: the transient pore model. *Nat Rev Mol Cell Biol*. 2005;6(9):738-742.

121. Purdue PE, Lazarow PB. Peroxisome biogenesis. *Annu Rev Cell Dev Biol*. 2001;17:701-752.

122. McNew JA, Goodman JM. The targeting and assembly of peroxisomal proteins: some old rules do not apply. *Trends Biochem Sci*. 1996;21(2):54-58.

123. Palmer T, Stansfeld PJ. Targeting of proteins to the twin-arginine translocation pathway. *Mol Microbiol*. 2020;113(5):861-871.

124. Groschel MI, Sayes F, Simeone R, Majlessi L, Brosch R. ESX secretion systems: mycobacterial evolution to counter host immunity. *Nat Rev Microbiol*. 2016;14(11):677-691.

125. Christie M, Chang CW, Rona G, et al. Structural biology and regulation of protein import into the nucleus. *J Mol Biol*. 2016;428(10 Pt A):2060-2090.

126. Cook AG, Conti E. Nuclear export complexes in the frame. *Curr Opin Struct Biol*. 2010;20(2):247-252.

127. Marcotte EM, Pellegrini M, Yeates TO, Eisenberg D. A census of protein repeats. *J Mol Biol*. 1999;293(1):151-160.

128. Kajava AV. Tandem repeats in proteins: from sequence to structure. *J Struct Biol*. 2012;179(3):279-288.

129. Andrade MA, Perez-Iratxeta C, Ponting CP. Protein repeats: structures, functions, and evolution. *J Struct Biol*. 2001;134(2–3):117-131.

130. Perez-Riba A, Synakewicz M, Itzhaki LS. Folding cooperativity and allosteric function in the tandem-repeat protein class. *Philos Trans R Soc Lond B Biol Sci*. 2018;373(1749):20170188.

131. Forwood JK, Lange A, Zacharäe U, et al. Quantitative structural analysis of importin-beta flexibility: paradigm for solenoid protein structures. *Structure*. 2010;18(9):1171-1183.

**How to cite this article:** Bürgi J, Ekal L, Wilmanns M. Versatile allosteric properties in Pex5-like tetratricopeptide repeat proteins to induce diverse downstream function. *Traffic*. 2021;22:140-152. [https://doi.org/10.1111/tra.12785](https://doi.org/10.1111/tra.12785)