Inside job: ligand-receptor pharmacology beneath the plasma membrane

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Most drugs acting on the cell surface receptors are membrane permeable and thus able to engage their target proteins in different subcellular compartments. However, these drugs’ effects on cell surface receptors have historically been studied on the plasma membrane alone. Increasing evidence suggests that small molecules may also modulate their targeted receptors through membrane trafficking or organelle-localized signaling inside the cell. These additional modes of interaction have been reported for functionally diverse ligands of GPCRs, ion channels, and transporters. Such intracellular drug-target engagements affect cell surface expression. Concurrent intracellular and cell surface signaling may also increase the complexity and therapeutic opportunities of small molecule modulation. Here we discuss examples of ligand-receptor interactions that are present in both intra- and extracellular sites, and the potential therapeutic opportunities presented by this phenomenon.

Keywords: ligand-receptor interaction; pharmacological chaperone; endoplasmic reticulum; GPCR; ion channel; transporter

Introduction

Generally, the activities of most endogenous receptor ligands or exogenous small molecule modulators have been studied at the plasma membrane, where they initiate signaling cascades along intracellular pathways. However, increasing evidence suggests that such interactions may also occur while the target receptor is inside the cell, either in nascent form in the endoplasmic reticulum (ER) or as a mature component residing on intracellular organelle membranes (Table 1).

A number of studies show that compounds may modulate the processing and trafficking efficiency of transmembrane proteins to the cell surface, acting as chemical ‘chaperones’ or ‘pharmacoperones’[1–5]. This terminology is used to describe two classes of modulators.

The first are relatively nonspecific compounds such as dimethyl sulfoxide (DMSO), 4-phenylbutyrate (4-PBA), thapsigargin, and glycerol, which modulate the trafficking of diverse targets. Such nonspecific compounds may act through a number of mechanisms. Modulators of endoplasmic reticulum calcium, such as thapsigargin, may enhance activity of endogenous chaperones and allow exit of misfolded proteins from the ER[6]. Compounds such as 4-PBA may increase expression of heat shock proteins to aid export from the ER[7]. Finally, osmolytes such as glycerol may aid protein folding by solvating hydrophobic regions and preventing aggregation of partially-folded intermediates[8].

The second class of molecules termed ‘pharmacological chaperones’ are selective ligands, which promote the folding and expression of specific targeted proteins. While the first class resemble interactions of native chaperone molecules such as the heat shock proteins, the second presumably engage specific binding site(s) on their target(s). In this perspective we have chosen to distinguish these two classes of small molecule chaperones as ‘general’ and ‘specific’. Independently from these trafficking effects, receptors including dopaminergic D2/D3 or serotonin 5-HT2A receptors may be endogenously targeted to organelles in addition to the cell surface, where they may have functional sub-populations and unique signaling roles on intracellular membranes[9,10].

We speculate that these additional sites of action may help explain the complex therapeutic mechanisms of known drugs[11], or offer potential treatments for pathological defects in trafficking or processing such as long QT type 2 (LQT2) and cystic fibrosis (CF)[11–17]. In this perspective we discuss examples that highlight the intracellular activities of ligands for three major drug target classes on the plasma membrane: G-protein coupled receptors (GPCRs), ion channels, and transporters (Figure 1). Illustrative examples for each group

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including disease associations and relevant ligands are summarized in Table 1.

**G-protein coupled receptors**

A classic example of a pharmacological chaperone capable of restoring surface expression of misfolded receptors is the vasopressin 2 receptor (V2R) antagonist SR121463A[18]. This compound was found to rescue surface expression of trafficking-deficient V2R mutants, while a membrane-impermeable antagonist lacked this effect[18]. This observation was accompanied by a decrease in ER-retained V2R and increased production of full-length glycosylated receptors that are presumably expressed on the cell surface[18]. Besides SR12463A and antagonist SR121463B, less specific compounds such as glycerol, DMSO, thapsigargin, curcumin and ionomycin can also rescue some V2R mutants[19]. However, the spectrum and magnitude of activity appears to be broader for specific compounds[19]. As ~90% of documented V2R mutations linked to diseases such as

![Figure 1. Intracellular drug mechanisms. Hypothetical modes of action for a GPCR ligand at the cell surface, in the trafficking pathway, and at intracellular organelle membranes.](image-url)

| Table 1. Examples of intracellular ligand-receptor targets, molecules, and mechanisms. |

| Class | Target | Ligands | Disease links | Reference(s) |
|-------|--------|---------|---------------|--------------|
| GPCRs | DRD2-4 | Quinpirole, haloperidol, butaclamol, clozapine, domperidone | Attention-deficit hyperactivity disorder | [25] |
| 5-HT₃ | SR46349B | | Schizophrenia | [31] |
| A₁ | DCPX, IBMX | NA | | | [30] |
| GnRHR | Indoles, quinolones, erythromycin derivatives | Congenital hypogonadotropic hypogonadism | | [35, 130, 131] |
| Rhodopsin | β-Ionone | Retinosa pigmentosa | | [39] |
| δ-opioid | Naltrexone | Analgesia/pain | | [37] |
| μ-opioid | Naloxone, etorphine | Analgesia/pain | | [36] |
| hMCHR1 | NBI-A | Anxiety, feeding | | [34] |
| MC4R | ML00253764 | Obesity | | [29] |
| VR | SR49059, SSR149415, SR121463A, B, thapsigargin, glycerol, DMSO, curcumin, ionomycin | Nephrogenic diabetes insipidus | | [18, 19, 132–4] |
| S1P1 | Fingolimod | Multiple sclerosis | | [49] |
| Ion channels | nAChR | Nicotine, cytosine, dihydro-β-erythroidine | Neuroprotection in Parkinson’s | | [1, 5] |
| hERG | Celasrol, E4031, thapsigargin, fluorozalone, fluoxetine, ketoconazole, pentamidine, probucol, cardiac glycosides, astemizole, cisapride | Long QT Syndrome 2 | | [11, 13, 54–6, 58–61, 70] |
| KCNQ2 | Retigabine | Benign neonatal familial convulsions | | [69] |
| CFTR | Quinazolines, thapsigargin | Cystic fibrosis | | [12, 63, 76] |
| Kᵥₑₑₑₑₑₑₑₑₑₑₑₑₑₑ | Tolbutamide, glibenclamide, repaglinide, | Congential hyperinsulinism | | [84] |
| Transporters | ABCA1,3 | 4-PBA, thapsigargin | Cardiovascular disease, respiratory distress syndrome, tangier disease | | [64, 95, 101] |
| ABCD1,2 | 4-PBA | X-linked adrenoleukodystrophy | | | [103] |
| ABCB1,4 | Glycerol, cyclosporin A | Progressive familial intrahepatic cholestasis type 3 | | | [105] |
| ABCG6 | 4-PBA | | | | |
| ABCG2,5,8 | Mitoxantrone, tauroursodeoxycholate | Gout | | [108–10] |
| SERT | Ibotagine | NA | | [111, 112] |
| ATP7B | 4-PBA, curcumin | Wilson’s disease | | [115] |
| MNK | Copper, glycerol | Menke’s disease | | [114] |
| hABST | Cyclosporin A | Cholesterol transport | | [113] |
as nephrogenic diabetes insipidus (NDI) cause ER membrane retention amenable to this manner of correction\[^{20,21}\], this specific pharmacological chaperone was proposed as a novel prospective therapeutic\[^{20}\].

Similar pharmacological rescue of trafficking deficiency has also been documented in other GPCRs, suggesting this activity may represent a potential general strategy for diverse pathologies. Indeed, an estimated 65% of the more than 600 documented GPCR mutations are missense mutations\[^{22}\], of which >80% are estimated to affect receptor folding in the ER\[^{23,24}\]. Assuming equal propensity for disease mutations among GPCRs, approximately half of all GPCR mutations may cause such localization defects. As previously indicated, the activity and specificity profiles of compounds rescuing such mutants may also be diverse. This is the case for trafficking-deficient D4-class dopamine receptors, which are rescued by specific chaperones including antagonists (such as domperidone), agonists (eg, quinpirole), or even endogenous ligands such as dopamine itself, independent of effects on translation or transcription\[^{25}\]. Unlike similar effects observed for general chaperone compounds such as glycerol and DMSO\[^{26,27}\], which rescue both D4 and CFTR trafficking mutants, these examples appear to be specific for the dopamine receptor\[^{25}\]. Further, the observation that brefeldin A blockade of Golgi-ER vesicle traffic may be reversed by these ligands suggests that these compounds may play a role in facilitating proper folding of the immature receptor, rather than later steps, in which they might instead promote trafficking of the fully glycosylated, mature protein through the Golgi machinery\[^{25}\]. Pharmacological chaperone activity also appears to rescue D4 receptors from proteasomal as opposed to lysosomal degradation, as suggested by the differential effects of lactacystin and chloroquine treatment on receptor levels in the ER membrane\[^{25}\], results correlated with similar studies of the V2R receptor\[^{28}\].

However, it is not clear whether this is a universal pattern for proteins rescued by such ligands. As variants in D4 have been linked to conditions such as attention deficit hyperactivity disorder (ADHD), modulation of receptor surface expression may offer a therapeutic pathway\[^{25}\]. Intriguingly, the trafficking of the wild type dopamine receptor to the cell surface is also amplified by both specific and general pharmacological chaperones, an observation that may correlate with the relatively inefficient endogenous processing of this receptor leaving a large, latent pool of functional D4 primed in the ER membrane for export\[^{25}\]. Further evidence is offered by the pro-trafficking effects of antagonist ML00253764 on both wild type and mutant melanocortin 4 receptors, which are also inefficiently processed endogenously\[^{29}\]. However, in cases such as the adenosine 1 (A1) receptor, the wild-type protein is unaffected by the specific pharmacological chaperones 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) and isobutylmethylxanthine (IBMX), suggesting this effect may vary with the natural efficiency of the protein’s transport to the cell surface\[^{30}\] and site(s) of drug-target interaction(s).

Another common antipsychotic drug target, the 5-HT serotonin receptor, has also been shown to be up-regulated in response to treatment with antagonist SR46349B\[^{31}\], though it is unclear whether this effect is due to modulation of trafficking or transcription/translation. Similarly, multiple point mutations in the melanin-concentrating hormone receptor 1 (hMCHR1) (whose signaling is associated with stress response\[^{32}\] and metabolism\[^{33}\]) leading to impaired trafficking may be rescued by the antagonist NBI-A\[^{34}\]. The gonadotropin-releasing hormone receptor (GnRHR), for which ~90% of inactivating mutations causing hypogonadotropic hypogonadism are due to impaired trafficking, may also be functionally rescued with diverse compound classes such as indoles, quinolones, thienopyrimidinediones, and erythromycin-derived macrolides\[^{35}\].

Experiments with the rat μ-opioid receptor have demonstrated pharmacological rescue of deletion mutants of transmembrane and carboxyl-terminal motifs using the specific agonist etorphine and antagonist naloxone\[^{36}\]. Likewise, pain and analgesia associated δ-opioid receptors have demonstrated similar enhanced trafficking through interaction with naltrexone\[^{37}\]. Because these receptors form heterodimers, mutants can function as ‘dominant negatives’ to retain wild type receptors in the ER through protein-protein interactions\[^{38}\], allowing small molecule chaperones to rescue both wild type and mutant trafficking. In related studies, higher order-oligomerization of δ\[^{15}\]-adrenoceptors appears to be conserved in some trafficking-deficient mutants of transmembrane segment I, suggesting that such mutations can alter surface expression without effecting intermolecular interactions between receptors retained in the ER/Golgi\[^{38}\]. The potential for systematic discovery of specific small molecule chaperones has been demonstrated for rhodopsin, for which high-throughput in silico docking was combined with in vitro competitive binding studies to discover potential therapeutic compounds for retinosa pigementosa (RP) such as β-ionone\[^{39}\].

Intriguingly, dopamine, serotonin, adrenergic, and thrombin receptors have also been identified in the ER membrane\[^{40-45}\] with D2 receptors appearing to be capable of activating G-protein signaling while retained in the ER and this expression correlating with increased vacuolization\[^{46}\]. In related studies, dopamine receptors have been identified in endosomes through immunohistochemical analysis of the rat cerebral cortex and hippocampus\[^{47}\]. As organelle membranes such as endosomes have been proposed as alternative signaling ‘platforms’ distinct from the plasma membrane\[^{48}\], these observations are suggestive of additional opportunities for tuning the activity of these receptors beyond biogenesis. For instance, the immunomodulator drug fingolimod (FTY720), a therapeutic for multiple sclerosis, promotes internalization of the sphingosine-1-phosphate receptor 1 (S1P1) as well as persistent signaling via extracellular-signal-regulated kinase (ERK) and adenylyl cyclase from endosomes\[^{49}\]. Similarly, experiments with chemical blockers of endocytosis have revealed that thyroid stimulating hormone (TSH) receptor signaling appears to require internalization, suggesting that non-endogenous ligands of the TSH receptor might also promote intracellular activity\[^{50}\].
Ion channels

Therapeutic modulation of intracellular receptor activity via ligands active at the cell surface pertains not just to disease targets, but also to anti-targets linked to drug side effects. An illustrative example is provided by the human ether-a-go-go related (hERG) potassium channel, a frequent target of promiscuous inhibition by small molecules and drug-induced cardiac arrhythmias, for which genetic mutations are linked to long QT syndrome type 2 (LQT2)\(^{[51]}\). Like the GPCRs described above, diverse LQT2 mutations have been documented, which have detrimental effects on surface expression of hERG\(^{[11, 13–16, 52, 53]}\). Intriguingly, these studies also determined that hERG inhibitors such as E4031, astemizole, and cisapride, while potential causes of drug-induced LQT2 through blockade of channel current across the plasma membrane, could also rescue surface expression of LQT2 mutant channels by potentiating biogenesis of the mature, glycosylated protein\(^{[11, 13]}\). In addition to these pharmacological chaperones, many hERG blockers also appear to inhibit trafficking. These effects, like chemical inhibition of hERG channel conductance, appears to be promiscuous, with evidence that acute blockers such as the antifungal fluconazole\(^{[54]}\), the Chinese thunder god vine component celastrol\(^{[55]}\), as well as fluoxetine\(^{[56, 57]}\), and ketoconazole\(^{[58]}\), also inhibit trafficking of the wild-type channel. However other molecules, such as pentamidine\(^{[59]}\), and probucol\(^{[60]}\) and cardiac glycosides including digitoxin\(^{[61]}\) inhibit trafficking without acute effects on current density. Similarly, the sarcoplasmic/endoplasmic reticulum calcium ATPase (SERCA) inhibitor thapsigargin can rescue trafficking without effects on current\(^{[62]}\), though the mechanism may be not be specific to hERG as this compound also rescues the trafficking of CFTR channels\(^{[63]}\) and the transporter ABCA1\(^{[64]}\). Intriguingly, other SERCA inhibitors cannot rescue these hERG mutants, suggesting thapsigargin might have broad activity to modulate trafficking for particular protein classes\(^{[62]}\). While the inhibitory activity of the above ligands on current or trafficking limits their therapeutic application, these observations raise the possibility that activators or ‘silent’, functionally inactive ligands might find value as chemical rescuers of hERG trafficking. Computational studies of estrogen receptor ligands and modulators of translation initiated at the internal ribosome entry site (IRES) of the encephalomyocarditis virus (EMCV) have identified inactive molecular series that are rendered active through small changes in chemical functionalization\(^{[65, 66]}\). While these studies did not biochemically confirm whether the inactive series bind their target, such results provide support for identification of ‘silent’ modulators.

Another voltage-gated potassium channel, KCNQ2, has been linked to conductive disorders such as neonatal seizures, which might be corrected with chemical openers\(^{[67, 68]}\). While it had been assumed to act only by shifting the voltage activation curve of the channel, recent evidence has also suggested the antiepileptic retigabine (RTG) may function as a specific pharmacological chaperone\(^{[69]}\). Incubation with RTG has been shown to correct processing of a folding-defective mutant linked to neonatal seizures, independent of effects on the open-probability of the channel, as demonstrated by increased current density even following RTG washout\(^{[69]}\). While molecular determinants of RTG have been linked to the channel pore\(^{[70–74]}\), direct evidence of compound-ligand interaction is unavailable. Given the availability of many functionally distinct KCNQ2 activators\(^{[75]}\), there exists the possibility that other pharmacological chaperones remain to be characterized for this channel.

A similar example of this phenomenon among channel proteins is the cystic fibrosis transmembrane conductance regulator (CFTR), for which ~90% of North American cases result from an in-frame deletion (AF508) that causes defects in trafficking, membrane half-life, and gating\(^{[12, 76]}\). Thus, correction of CFTR processing for this high-frequency mutation has therapeutic potential for a large patient population. High-throughput screening has been utilized to discover small molecule modulators using the response of membrane potential or halide-sensitive fluorescence dyes as a proxy for activity of the channel\(^{[12, 76]}\). Among other chemical families, these studies discovered a class of quinazolinones that increase the population of core-glycosylated CFTR mutant proteins and surface expression, without modulating either ubiquitin-proteasome activity or transcription levels, suggesting these specific chaperones might increase the efficiency of ER processing\(^{[12, 76]}\). Later studies utilized crosslinking assays and \textit{in vitro} reconstitution of the protein’s ATPase activity to verify that these ‘corrector’ compound activities are indeed due to direct intracellular interactions\(^{[77, 78]}\). Additional evidence suggests these compounds also stabilize CFTR on the cell surface, rather than purely acting as modulators of ER processing\(^{[76, 79]}\). Supportive for the direct chemical-CFTR interaction, the identified quinazoline series promote surface expression and at high concentrations they also appear to inhibit CFTR conductance\(^{[80]}\). Conversely, other series can enhance the chloride current\(^{[76]}\). While the quinazoline series described above and the corrector phenol VRT-532 remain the only chemicals with evidence of direct physical intracellular association with CFTR, other compound classes with similar functional phenotypes await more detailed biochemical characterization\(^{[78, 81]}\). Further, the recent FDA approval of a CFTR potentiator (ivacaftor) as a treatment for conductance mutants comprising 5% of CFTR cases suggests the possibility of combination therapy promoting conductance and proper trafficking of the channel\(^{[82]}\).

Like the V2R receptor, five aquaporin-2 (AQP2) mutants associated with X-linked diabetes insipidus may be rescued by treatment with general chemical chaperones such as glycerol, trimethylamine N-oxide (TMAO), or DMSO when expressed in \textit{Xenopus} oocytes or Chinese Hamster Ovary (CHO) cells\(^{[27]}\). However, the lack of selective aquaporin modulators\(^{[83]}\) places limits on potential therapeutic interventions to date. An example of endogenous intracellular ligand-channel interaction is presented by the nicotinic acetylcholine receptor (nAChR). Experimental evidence suggests that nicotine interactions with the receptor inside the cell promote trafficking to the cell surface\(^{[2–3]}\) and reduce ER stress, as judged by attenuated acti-
vating transcription factor 6 (ATF6) translocation during the unfolded protein response (UPR)\[9\]. This effect may underlie the protective effect of smoking in neurodegenerative conditions such as Parkinson’s, which are characterized by a loss of nAChR activity in the central nervous system\[7\]. Thus, synthetic ligands may also be useful probes of this intracellular nAChR activity. An additional level of complexity is revealed by studies of pharmacological chaperoning of the K\(_{\text{ATP}}\) complex of Kir6.2 and sulfonylurea receptor 1 (SUR1)\[84\], which is expressed as a 4:4 receptor:channel octamer in pancreatic \(\beta\) cells\[85-87\]. Mutations in the transmembrane domains of SUR1 reduces trafficking of the receptor from the ER to the cell surface, but may be rescued by both sulfonylurea and glinide compounds\[84\]. The observation that pharmacological rescue of trafficking-deficient SUR1 or Kir6.2 mutants requires co-expression of both components of the K\(_{\text{ATP}}\) complex suggests that the entire channel complex, rather than either individual subunit, is the target of these small molecules\[84\].

Like GPCRs, channels may also have functional populations at intracellular sites that complicate potential therapeutics. For example, both wild type and AF508-CFTR have been shown to conduct chloride currents across ER membranes as measured by patch clamp of isolated nuclear membranes from CFTR-expressing CHO cells\[88\]. CFTR intracellular localization is also associated with endosome fusion\[89\] and regulation of lysosomal pH\[90\], and thus intracellular modulators may have specialized functional effects within inside the cell. Concurrently, these alternative functional roles may indicate that chemical therapeutics for CFTR, like other receptors with cell surface and intracellular roles, may ameliorate defects across multiple pathways within the cell. Recent evidence has further identified ion channels expressed both on the cell surface and functionally in intracellular compartments such as endosomes. For example, TRPML3 and TRPML2 channels may be expressed at both the plasma membrane as well as membranes of intracellular compartments\[91\]. Recently identified small molecule modulators for these channels may be useful to dissect their functional roles at these sites\[91\]. The identification of increased lysosomal lipid inclusions from knocking down these channels further suggests that chemical modulators may influence not only the receptor itself, but downstream functionality of the organelle in which they are found\[92\]. Similarly, the 2-pore domain channel KCNK9 has been reported in mitochondrial membranes\[93\] as well as the cell surface functions, for which reported small molecule inhibitors\[94\] may be useful probes.

**Transporters**

Like GPCRs and ion channels, transporters regulate important physiological processes at both intracellular sites and the plasma membrane, functions which may be disrupted by mutations causing improper folding or erroneous trafficking. In many cases, however, the primary sites of localization for transporters are on intracellular membranes, with trafficking to organelles being the target of chemical modulation. In the case of the ATP-binding cassette transporter A1 (ABCA1), the transporter is an important regulator of high-density lipoprotein cholesterol (HDLC) formation by exporting lipid to cell-surface apoA-I lipoproteins during the biogenesis of HDL particles\[95\]. Over 150 mutations in ABCA1 have been documented\[96\], many leading to mutants correlated with the cholesteryl transport and cardiovascular disorder Tangier Disease\[97-99\], through reduced protein expression and retention in the ER\[100\]. Experiments employing both heterogeneous expression systems and fibroblasts derived from HDL-deficient patients demonstrate the general chemical chaperone 4-PBA may correct intracellular retention of ABCA1 mutants, but boost expression of the protein in the heterologous system only\[96\]. In the same gene family, the ABCA3 transporter is localized not to the plasma membrane, but to lysosomes and lamellar bodies in lung cells where it mediates secretion of surfactant lipids such as phosphatidylcholine, sphingomyelin, and cholesterol. Mutations are associated with respiratory distress syndrome (RDS), and cellular pathologies include lack of conversion of lysosomes to lamellar-like bodies that store lipids\[101\]. This failure in lamellar body formation may be rescued with 4-PBA in cultured lung and HEK293 cells\[101\]. A similar deficiency in trafficking to predominantly intracellular sites is observed for the lipid transporter ABCD1, for which mutations in the ATPase domain\[102\] block peroxisome proliferation and are associated with X-linked adrenoleukodystrophy (X-ALD)\[103\]. These deficiencies may be rescued by 4-PBA induction of ABCD2 expression in X-ALD patient-derived fibroblasts, though it is unclear whether this results from increased trafficking to the peroxisome or other pleiotropic effects via gene expression\[103\].

Unlike the ABCA transporters, the multidrug resistance (MDR)/ABCB family facilitates the passage of drugs in addition to lipid substrates\[104\]. Despite this functional distinction, trafficking-deficient ABCB1 mutants may also be pharmacologically rescued\[105\]. The efficacy of rescue varies for specific and general small molecule chaperones, with cyclosporin A demonstrating greater effect than glycerol\[105\]. Conversely, neither heat shock protein 70 (Hsp70) overexpression nor thapsigargin treatment affects mutant ABCB1 trafficking\[105\]. As identical mutations in closely related ABCB4 have been identified in progressive familial intrahepatic cholestasis type 3, data from ABCB1 indicates a possible therapeutic strategy for this disorder\[105\]. ABCC6, another liver-localized transporter\[106\], has unknown substrates but its mutation is associated pathologically with deposition and accumulation of minerals within tissues, with several mutants being improperly retained in the ER and restored by 4-PBA treatment\[107\]. Like ABCB transporters, ABCC2 plays an important role in excretion, including regulating intracellular drug concentration\[108\]. The transporter substrate mitoxantrone (MX) has also been shown to serve as a pharmacological chaperone for dimerization-deficient mutants which are normally impaired for trafficking to the cell surface\[108\]. Trafficking-deficient mutants have been further identified in regions of the protein not associated with dimerization, alterations which are genetically linked to gout\[109\]. Two additional transporters in the same gene

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family, ABCG5/8, form heterodimers involved in sterol secretion which have been reported to be downregulated at the cell surface in mouse models of leptin deficiency, a defect rescued by treatment with tauroursodeoxycholate\[109\].

In the case of the serotonin transporter (SERT) [a member of the solute carrier (SLC) family], mutants in the carboxyl-terminal binding motif are impaired for ligand binding as quantified by a radiolabeled-imipramine displacement assay, and are partially rescued by both DMSO and ibogaine, but not other substrates of the protein\[121\]. The binding of ibogaine to the cytoplasmic face of the transporter\[112\], unlike other agonists, suggests that specific binding sites of receptors may be more amenable than others to trafficking modulation and rescue. Additionally, these data highlight the role that C-terminal motifs may play in proper protein folding. Indeed, C-terminal mutations of SERT that disrupt folding also interact with vesicular component Sec24, indicating potential pleiotropic effects of trafficking-deficient mutants\[111\]. Trafficking-deficient mutations in the first transmembrane segment of another SLC family member, the human apical sodium-dependent bile transporter (hABST, SLC10A2), may also be pharmacologically rescued with MG132 or cyclosporin\[119\].

Two diseases of copper transport (Wilson’s and Menkes disease) are associated with trafficking-deficient mutations in copper transporter proteins\[114, 115\]. In the absence of its substrate, the copper-transporting P-type ATPase MNK is localized to the trans-Golgi complex; sharp increases in copper concentration promote its trafficking to the plasma membrane\[114\]. A conditional mutant in the large cytoplasmic loop of MNK is associated with Menkes disease, and causes the transporter to be retained in the ER in the absence of copper\[114\]. However, this mislocalization may be corrected by glycerol or copper itself, suggesting copper supplementation as a possible treatment for Menkes\[114\]. Similarly, mutations in multiple structural domains of the copper transporter, ATP7B, are implicated in deficient copper extrusion by the liver and basal ganglia in Wilson’s disease\[115\]. Many of these mutations impair proper folding and localization of the protein to the trans-Golgi complex, but their improper retention in the ER may be corrected with either 4-PBA or curcumin treatment\[115\].

Besides trafficking, transporters, like GPCRs and channels, may also be dually localized to both the plasma membrane and intracellular compartments where they have distinct signaling roles. For instance, treatment of CHO cells with the vacuolar ATPase inhibitor baflomycin A reduces transferrin receptor trafficking\[116\] by interfering with the function of the vacuolar transporter in the endosomal compartment\[117\]. Similarly, both concanamycin and baflomycin have been found to inhibit influenza virus entry into cells by impairing acidification of vacuoles through transporter inhibition\[118\]. In osteoclasts, however, cell surface activity of the vacuolar ATPase may be implicated in bone reabsorption conditional on N-terminal interactions between subunits, as this reabsorption is blocked by a benzohydrazide derivative which antagonizes this interaction\[119\].

**Therapeutic implications**

Ligand-receptor interactions at both the plasma membrane and intracellular sites suggest a number of intriguing therapeutic possibilities. For example, drug synergies (achieved by combined treatment with trafficking and activity modulators) might offer complementary efficacy or effect targets inaccessible with single compound treatments. In fact this synergy may have already been achieved and unappreciated for current medications, since most therapeutic treatments involve administration of drugs for days, not the seconds or minutes in which most cell-based assays are quantified. The timespans of typical drug administration allow both the acute effects typically measured by functional assays of cell surface targets as well as chronic modulation of intracellularly retained or localized proteins. Additionally, the rescue of trafficking-deficient mutants by ligands which impair the functionality of their target presents a conundrum, as increased surface expression may be offset by functional antagonism. Thus, ‘silent’ modulators that bind their target without functional effect may find utility as modulators of trafficking, and suggest high-throughput screening for such chemicals. Intriguingly, chronic application of such compounds may have medicinal benefits, despite their seeming inertness in acute activity measurements. Alternatively, if the potency for acute and chronic compound effects is sufficiently different, an appropriate dosage window might be defined to avoid functional antagonism while preserving beneficial effects on trafficking. This window may be influenced by the intracellular accumulation of drugs, such as through inhibition of P-glycoprotein transporters\[120-122\], which may lead to chronic doses that are far higher concentrations than acute levels to which the cell is initially exposed.

In cases where receptors are functionally present at both the plasma membrane and intracellular sites, optimization of subcellular localization profiles of small molecule modulators, perhaps through conjugation with polymers\[123\], toxins\[124\], or lipids\[124\] directed to particular organelles, may allow fine tuning of their therapeutic phenotype through a balance of extra- and intracellular actions. Finally, the existence of these intracellular chemical activities argues for more systematic exploration of such effects among existing drugs, for possible therapeutic repurposing or to discover mechanistic explanations of chronic physiological side effects.

**Perspective**

While the concept of pharmacological chaperones and intracellular ligand-receptor activities has been well-established for many of the therapeutic targets and target classes described above, in other cases these activities are just beginning to be appreciated. The field will benefit from more systematic evaluation of these activities, using platforms that concurrently evaluate chronic (such as trafficking and intracellular effects) as well as acute actions of molecules in a comprehensive fashion. High-content imaging assays, allowing visualization of both receptor localization and downstream readouts such as
ion flux may offer opportunities for such multiplexed analysis[125]. Another is offered by approaches such as chemobleaching, which functionally inactivates surface receptors to allow chemical effects on trafficking to be quantified independently of acute effects[126]. Further, improved understanding of the biophysical interactions by which ligands stabilize their target receptors during biogenesis may also open new doors for rational drug design in this field. As discussed, it is clear that different drug binding sites could lead to differential rescue efficacy. Some clues have emerged from studies in which specific pharmacological chaperones have been co-crystallized with phenylalanine hydroxylase and β-glucosidase[127, 128]. These data suggest that the ligands may stabilize a particular conformation of the target protein that is optimal for export, and thus improve their processing through the ER. The observation that ligand binding sites are often located at subdomain interfaces may indicate that chemical interactions in these regions are generally conducive to stabilization of macromolecular structure[129].

More broadly, the realization that ligands may interact with receptors not just at the cell surface, but at many points along their maturation process and at distinct intra- and extracellular sites extends the concept of polypharmacology and ‘network medicine’ to not just interactions among diverse proteins, but complex processes involving a single target. Many drugs are being taken by patients for an extended period of time, thus making more likely to induce intracellular pharmacology in addition to the cell surface pharmacology. Further knowledge of all sites of contact between receptors and their corresponding ligands will help to expand both the functional nuance and potential impact of future medicines.

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References
1 Srinivasan R, Richards Cl, Xiao C, Rhee D, Pantoja R, Dougherty DA, et al. Pharmacological chaperoning of nicotinic acetylcholine receptors reduces the endoplasmic reticulum stress response. Mol Pharmacol 2012; 81: 759–69.
2 Kuryatov A, Luo J, Cooper J, Lindstrom J. Nicotine acts as a pharmacological chaperone to up-regulate human alpha 4 beta 2 acetylcholine receptors. Mol Pharmacol 2005; 68: 1839–51.
3 Lester HA, Xiao C, Srinivasan R, Son CD, Miwa J, Pantoja R, et al. Nicotine is a selective pharmacological chaperone of acetylcholine receptor number and stoichiometry. Implications for Drug Discovery. AAPS J 2009; 11: 167–77.
4 Saille J, Pons S, Devillers-Thiery A, Soudant M, de Carvalho LP, Changeux JP, et al. Nicotine upregulates its own receptors through enhanced intracellular maturation. Neuron 2005; 46: 595–607.
5 Lester HA, Miwa JM, Srinivasan R. Psychiatric drugs bind to classical targets within early exocytotic pathways: therapeutic effects. Biol Psychiatry 2012; 72: 907–15.
6 Brostrom MA, Brostrom CO. Calcium dynamics and endoplasmic reticulum function in the regulation of protein synthesis: implications for cell growth and adaptability. Cell Calcium 2003; 34: 345–63.
7 Choo-Kang LR, Zeitlin PL. Induction of HSP70 promotes Delta F508 CFTR trafficking. Am J Physiol Lung Cell Mol Physiol 2001; 281: L58–68.
8 Vagenende V, Yap MGS, Trout BL. Mechanisms of protein stabilization and prevention of protein aggregation by glycerol. Biochemistry 2009; 48: 11084–96.
9 Cho DI, Zheng M, Kim KM. Current perspectives on the selective regulation of dopamine D2 and D3 receptors. Arch Pharm Res 2010; 33: 1521–38.
10 Cornea-Hebert V, Watkins K, Roth B, Kroeze W, Gaudreau P, Leclerc N, et al. Similar ultrastructural distribution of the 5-HT2A serotonin receptor and microtubule-associated protein MAP1A in cortical dendrites of adult rat. Neuroscience 2002; 113: 23–35.
11 Anderson CL, Delisie BP, Anson BD, Kilby JA, Will ML, Tester DJ, et al. Most LQT2 mutations reduce Kv11.1 (hERG) current by a class 2 (trafficking-deficient) mechanism. Circulation 2006; 113: 365–73.
12 Van Goor F, Straley KS, Cao D, Gonzalez J, Hadida S, Hazelwood A, et al. Rescue of DeltaF508-CFTR trafficking and gating in human cystic fibrosis airway primary cultures by small molecules. Am J Physiol Lung Cell Mol Physiol 2006; 290: L1117–30.
13 Zhou Z, Gong Q, January CT. Correction of defective protein trafficking of a mutant HERG potassium channel in human long QT syndrome. Pharmacological and temperature effects. J Biol Chem 1999; 274: 31123–6.
14 Ficker E, Thomas D, Viswanathan PC, Dennis AT, Priori SG, Napolitano C, et al. Novel characteristics of a misprocessed mutant HERG channel linked to hereditary long QT syndrome. Am J Physiol Heart Circ Physiol 2000; 279: H1748–56.
15 Paulusussen A, Raes A, Matthijis G, Snijders DJ, Cohen N, Aersens J. A novel mutation (T65P) in the PAS domain of the human potassium channel HERG results in the long QT syndrome by trafficking deficiency. J Biol Chem 2002; 277: 48610–6.
16 Rossenbacker T, Mubagwa K, Jongbloed RJ, Vereecke J, Devriendt K, Gewillig M, et al. Novel mutation in the Per-Arm-Sim domain of KCNH2 causes a malignant form of long-QT syndrome. Circulation 2005; 111: 961–8.
17 Roque M, Godoy CP, Castellanos M, Pusiel E, Mayorga LS. Population screening of F508del (DeltaF508), the most frequent mutation in the CFTR gene associated with cystic fibrosis in Argentina. Hum Mutat 2001; 18: 167.
18 Morello JP, Salaphour A, Laperriere A, Bernard V, Arthus MF, Lonergan M, et al. Pharmacological chaperones rescue cell-surface expression and function of misfolded V2 vasopressin receptor mutants. J Clin Invest 2000; 105: 887–95.
19 Robben JH, Sze M, Knoers NV, Deen PM. Rescue of vasopressin V2 receptor mutants by chemical chaperones: specificity and mechanism. Mol Biol Cell 2006; 17: 379–86.
20 Wenkert D, Schoneberg T, Merendino JJ Jr, Rodriguez Pena MS, Vinitsky R, Goldsmith PK, et al. Functional characterization of five V2 vasopressin receptor gene mutations. Mol Cell Endocrinol 1996; 124: 43–50.
21 Tsukaguchi H, Matsubara H, Taketani S, Mori Y, Seido T, Adada M. Binding-, intracellular transport-, and biosynthesis-defective mutants of vasopressin type 2 receptor in patients with X-linked nephrogenic diabetes insipidus. J Clin Invest 1995; 96: 2043–50.
22 Schoneberg T, Schulz A, Biehermann H, Hermsdorf T, Rompler H, Sangkhul K. Mutant G-protein-coupled receptors as a cause of human diseases. Pharmacol Ther 2004; 104: 173–206.
23 Leanos-Miranda A, Janovick JA, Conn PM. Receptor-misrouting;
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an unexpectedly prevalent and resuable etiology in gonadotropin-releasing hormone receptor-mediated hypogonadotrophic hypogonadism. J Clin Endocrinol Metab 2002; 87: 4825–8.

24. Schullein R. The early stages of the intracellular transport of membrane proteins: clinical and pharmacological implications. Rev Physiol Biochem Pharmacol 2004; 151: 45–91.

25. Van Craenenbroeck K, Clark SD, Cox MJ, Oak JN, Liu F, Van Tol HH. Folding efficiency is rate-limiting in dopamine D4 receptor biogenesis. J Biol Chem 2005; 280: 19350–7.

26. Sato S, Ward CL, Krouse ME, Kopito RR. Glycerol reverses the misfolding phenotype of the most common cystic fibrosis mutation. J Biol Chem 1996; 271: 635–8.

27. Tamarappoo BK, Verkman AS. Defective aquaporin-2 trafficking in nephrogenic diabetes insipidus and correction by chemical chaperones. J Clin Invest 1998; 101: 2257–67.

28. Schwieger I, Lautz K, Krause E, Rosenthal W, Wiesner B, Hermosilla R. Derlin-1 and p97/valosin-containing protein mediate the endoplasmic reticulum-associated degradation of human V2 vasopressin receptors. Mol Pharmacol 2008; 73: 697–708.

29. Tao YX. The melanocortin-4 receptor: physiology, pharmacology, and pathophysiology. Endocr Rev 2010; 31: 506–43.

30. Málaga-Díéguez L, Yang Q, Bauer J, Pankevych H, Freissmuth M, Tao YX. The melanocortin-4 receptor: physiology, pharmacology, and pathophysiology. Endocr Rev 2010; 31: 506–43.

31. Borowsky B, Durkin MM, Ogozalek K, Marzabadi MR, DeLeon J, Petaja-Repo UE. Cys-27 variant of human delta-opioid receptor modulates maturation and cell surface delivery of Phe-27 variant via endosomal location of dopamine receptors. J Biol Chem 1994; 269: 27719–26.

32. Griffond B, Baker BI. Cell and molecular cell biology of melanin-concentrating hormone-1 receptor antagonist. Nat Med 2002; 8: 825–30.

33. Rajamani S, Eckhardt LL, Valdivia CR, Klemens CA, Gillman BM, Anderson OL, et al. Drug-induced long QT syndrome type 2 ether-a-gogo-related gene (HERG) mutations. J Biol Chem 2000; 275: 2327–37.

34. Han S, Zhang Y, Chen Q, Duan Y, Zheng T, Hu X, et al. Fluconazole inhibits HERG K+ channel by direct block and disruption of protein trafficking. Eur J Pharmacol 2011; 650: 138–44.

35. Sun H, Liu X, Xiong Q, Shikano S, Li M. Chronic inhibition of cardiac Kir2.1 and HERG potassium channels by celestrol with dual effects on both ion conductivity and protein trafficking. J Biol Chem 2006; 281: 5877–84.

36. Rajamani S, Eckhardt LL, Valdivia CR, Klemens CA, Gillman BM, Anderson OL, et al. Drug-induced long QT syndrome: hERG K+ channel block and disruption of protein trafficking by fluoxetine and norfluoxetine. Br J Pharmacol 2006; 149: 481–9.

37. Thomas D, Gut B, Wendt-Nordahl G, Kiehn J. The antidepressant drug fluoxetine is an inhibitor of human ether-a-go-go-related gene (HERG) potassium channels. J Pharmacol Exp Ther 2002; 300: 543–8.
protein trafficking in ketoconazole-induced long QT syndrome. Br J Pharmacol 2008; 153: 439–47.

59 Cordes JS, Sun Z, Lloyd DB, Bradley JA, Opsahl AC, Tengowski MW, et al. Pentamidine reduces hERG expression to prolong the QT interval. Br J Pharmacol 2005; 145: 15–23.

60 Guo J, Massaehi H, Li W, Xu J, Luo T, Shaw J, et al. Identification of $I_{\text{KV7}}$ and its trafficking disruption induced by procollan in cultured neonatal rat cardiomyocytes. J Pharmacol Exp Ther 2007; 321: 911–20.

61 Wang L, Wible BA, Wan X, Ficker E. Cardiac glycosides as novel inhibitors of human ether-a-go-go-related gene channel trafficking. J Pharmacol Exp Ther 2007; 320: 525–34.

62 Delisle BP, Anderson CL, Ballajepalli RC, Anson BD, Kamp TJ, January CT. Thapsigargin selectively rescues the trafficking defective LQT2 channels G601S and F805C. J Biol Chem 2003; 278: 35749–54.

63 Egan ME, Glickner-Pager J, Ambrose C, Cahill PA, Pappoe L, Balamuth N, et al. Calcium-pump inhibitors induce functional surface expression of Delta F508-CFTR protein in cystic fibrosis epithelial cells. Nat Med 2002; 8: 485–92.

64 Tanaka AR, Fano K, Ueda K, Murata M. The ABCA1 Q597R mutant undergoes trafficking from the ER upon ER stress. Biochem Biophys Res Commun 2008; 369: 1174–8.

65 Varin T, Didiot MC, Parker CN, Schuffenecker A. Latent hit series hidden in high-throughput screening data. J Med Chem 2012; 55: 1161–70.

66 Mestres J, Veeneman GH. Identification of “latent hits” in compound screening collections. J Med Chem 2003; 46: 3441–4.

67 Xiong Q, Sun H, Li M. Zinc pyrithione-mediated activation of voltage-gated KCNQ potassium channels rescues epileptogenic mutants. Nat Chem Biol 2007; 3: 287–96.

68 Rundfeldt C, Netzer R. The novel anticonvulsant retigabine activates M-currents in Chinese hamster ovary-cells transfected with human KCNQ2/3 subunits. Neurobiol Lett 2000; 282: 73–6.

69 Maljevic S, Naros G, Yalcin O, Blazevic D, Loeffler H, Caglayan H, et al. Molecular determinants of KCNQ (Kv7) K+ channel sensitivity to the anticonvulsant retigabine. J Neurosci 2005; 25: 12347–51.

70 Schenzer A, Friedrich T, Pusch M, Saftig P, Jentsch TJ, Grotzinger J, et al. Temperature and pharmacological rescue of a folding-defective, dominant-negative KV 7.2 mutation associated with neonatal seizures. Hum Mutat 2011; 32: E2283–93.

71 Rostock A, Tober C, Rundfeldt C, Bartsch R, Engell J, Polymeropoulos EE, et al. D-23129: a new anticonvulsant with a broad spectrum activity in animal models of epileptic seizures. Epilepsy Res 1996; 23: 211–23.

72 Tober C, Rostock A, Rundfeldt C, Bartsch R. D-23129: a potent anticonvulsant in the amygdala kindling model of complex partial seizures. Eur J Pharmacol 1996; 303: 163–9.

73 Rundfeldt C. The new anticonvulsant retigabine (D-23129) acts as an opener of K+ channels in neuronal cells. Eur J Pharmacol 1997; 336: 243–9.

74 Tatinil L, Delmas P, Abogadie FC, Brown DA. Activation of expressed KCNQ potassium currents and native neuronal M-type potassium currents by the anti-convulsant drug retigabine. J Neurosci 2001; 21: 5535–45.

75 Xiong Q, Gao Z, Wang W, Li M. Activation of Kv7 (KCNQ) voltage-gated potassium channels by synthetic compounds. Trends Pharmacol Sci 2008; 29: 99–107.

76 Pedemonte N, Lukacs GL, Du K, Caci E, Zegarra-Moran O, Galletta LJ, et al. Small-molecule correctors of defective DeltaF508-CFTR cellular processing identified by high-throughput screening. J Clin Invest 2005; 115: 2564–71.

77 Wang Y, Loo TW, Bartlett MC, Clarke DM. Correctors promote maturation of cystic fibrosis transmembrane conductance regulator (CFTR)-processing mutants by binding to the protein. J Biol Chem 2007; 282: 33247–51.

78 Wellhauser L, Kim Chiaw P, Pasyk S, Li C, Ramjeesingh M, Bear CE. A small-molecule modulator interacts directly with deltaPhe508-CFTR to modify its ATPase activity and conformational stability. Mol Pharmacol 2009; 75: 1430–8.

79 Varga K, Goldstein R, Jurkvenaite A, Chen L, Matalon S, Sorscher E, et al. Enhanced cell-surface stability of rescued DeltaF508 cystic fibrosis transmembrane conductance regulator (CFTR) by pharmacological chaperones. Biochem J 2008; 410: 555–64.

80 Kim Chiaw P, Wellhauser L, Huan LJ, Ramjeesingh M, Bear CE. A chemical corrector modifies the channel function of F508del-CFTR. Mol Pharmacol 2010; 78: 411–8.

81 Molinski S, Eckford PD, Pasyk S, Ahmadi S, Chin S, Bear CE. Functional rescue of F508del-CFTR using small molecule correctors. Front Pharmacol 2012; 3: 160.

82 Ramsey BW, Davies J, McElvaney NG, Tullis E, Bell SC, Drevinek P, et al. A CFTR potentiator in patients with cystic fibrosis and the G551D mutation. N Engl J Med 2011; 365: 1663–72.

83 Verkman AS. Aquaporins in clinical medicine. Annu Rev Med 2012; 63: 303–16.

84 Yan FF, Casey J, Shyng SL. Sulfonylureas correct trafficking defects of disease-causing ATP-sensitive potassium channels by binding to the channel complex. J Biol Chem 2006; 281: 33403–13.

85 Clement JP 4th, Kunjilwar K, Gonzalez G, Schwanstecher M, Panten U, Aguilar-Bryan L, et al. Association and stoichiometry of K(ATP) channel subunits. Neuron 1997; 18: 827–38.

86 Inagaki N, Gonoi T, Seino S. Subunit stoichiometry of the pancreatic beta-cell ATP-sensitive K+ channel. FEBS Lett 1997; 409: 232–6.

87 Shyng S, Nichols CG. Octameric stoichiometry of the K_{ATP} channel complex. J Gen Physiol 1997; 110: 655–64.

88 Pasyk EA, Foskett JK. Mutant (delta F508) cystic fibrosis transmembrane conductance regulator Cl− channel is functional when retained in endoplasmic reticulum of mammalian cells. J Biol Chem 1995; 270: 12347–50.

89 Biewers J, Emanus N, Verkman AS. Cystic fibrosis transmembrane conductance regulator activation stimulates endosome fusion in vivo. Proc Natl Acad Sci U S A 1996; 93: 12484–9.

90 Liu J, Lu W, Gupta S, Baltazar GC, Coffey EE, Laties AM, et al. Calcium-pump inhibitors induce functional correction of DeltaF508-CFTR to modify its ATPase activity and conformational stability. Mol Pharmacol 2007; 329: 9–17.

91 Grimm C, Jors S, Saldanha SA, Obukhov AG, Pan B, Oshima K, et al. Small molecule activators of TRPML3. Chem Biol 2010; 17: 135–48.

92 Szeve DA, Frumkin A, Offen-Glaser V, Kogot-Levin A, Bach G. A potentially dynamic lysosomal role for the endogenous TRPML3 protein. J Cell Sci 2011; 124: 3082–3087.

93 Edelstein DY, Cai M, Wang J, Soll R, Dong J, Babcock JJ. Identification of small-molecule correctors of defective M-currents in Chinese hamster ovary-cells tranfected with human KCNQ9.1 (TASK-3). ChemMedChem 2012; 7: 123–33.
ABC1 gene with high-density lipoprotein cholesterol levels and risk of ischemic heart disease. JAMA 2008; 299: 2524–32.

Brooks-Wilson A, Marcił M, Clee SM, Zhang LH, Roopp K, van Dam M, et al. Mutations in ABC1 in Tangier disease and familial high-density lipoprotein deficiency. Nature Genet 1999; 22: 336–45.

Bodzioch M, Orso E, Klucken T, Langmann T, Bottcher L, Diederich W, et al. The gene encoding ATP-binding cassette transporter 1 is mutated in Tangier disease. Nature Genet 1999; 22: 347–51.

Rust S, Rosier M, Funke H, Real J, Amoura Z, Piette JC, et al. Tangier disease is caused by mutations in the gene encoding ATP-binding cassette transporter 1. Nature Genet 1999; 22: 352–55.

Candini C, Schimmel AW, Bochem AE, Holleboom AG, Vergeer Li Q, Jiang Q, Pfendner E, Varadi A, Uitto J. Pseudoxanthoma elasticum: clinical phenotypes, molecular genetics and putative pathomechanisms. Exp Dermatol 2009; 18: 1–11.

Cheong N, Madesh M, Gonzales LW, Zhao M, Yu K, Ballard PL, et al. Identification and characterization of novel loss of function mutations in ATP-binding cassette transporter A1 in patients with low plasma high-density lipoprotein cholesterol. Atherosclerosis 2010; 213: 492–8.

Cheong N, Madesh M, Gonzales LW, Zhao M, Yu K, Ballard PL, et al. Functional and trafficking defects in ATP binding cassette A3 mutants associated with respiratory distress syndrome. J Biol Chem 2006; 281: 9791–800.

Roeri P, Mayerhofer P, Holzinger A, Gartner J. Characterization and functional analysis of the nucleotide binding fold in human peroxisomal ATP binding cassette transporters. FEBS Lett 2001; 492: 66–72.

Gondcaled C, Depreter M, Fourcade S, Lecca MR, Leclercq S, Martin PG, et al. Phenybutyrate up-regulates the adrenoleukodystrophy-related gene as a nonclassical peroxisome proliferator. J Cell Biol 2005; 169: 93–104.

Schinkel AH, Jonker JW. Mammalian drug efflux transporters of the ATP binding cassette (ABC) family: an overview. Adv Drug Deliv Rev 2003; 55: 3–29.

Gautherot J, Durand-Schneider AM, Delautier D, Delaunay JL, Rada A, Gabillot J, et al. Effects of cellular, chemical, and pharmacological chaperones on the rescue of a trafficking-defective mutant of the ATP-binding cassette transporter proteins ABCB1/ABCB4. J Biol Chem 2012; 287: 5070–8.

Li Q, Jiang Q, Pfendner E, Varadi A, Uitto J. Pseudoxanthoma elasticum: clinical phenotypes, molecular genetics and putative pathomechanisms. Exp Dermatol 2009; 18: 1–11.

Le Saux O, Fulop K, Yamaguchi Y, Illas A, Szabo Z, Brampton CN, et al. Expression and in vivo rescue of human ABCG6 disease-causing mutants in mouse liver. Plos One 2011; 6: e24738.

Polgar O, Ierano C, Tamaki A, Stanley B, Ward Y, Xia D, et al. Muta
tional analysis of threonine 402 adjacent to the GXXG dimerization motif in transmembrane segment 1 of ABCG2. Biochemistry 2010; 49: 2235–45.

Baselleve A, Bates SE. Gout, genetics and ABC transporters. F1000 Biol Rep 2011; 3: 23.

Sabeva NS, Rouse EJ, Graf GA. Defects in the levin axis reduce abundance of the ABCG5-ABCG8 sterol transporter in liver. J Biol Chem 2007; 282: 22397–405.

El-Kasaby A, Just H, Malle E, Stölt-Bergner PC, Sitte HH, Freimuth M, et al. Mutations in the carboxyl-terminal SEC24 binding motif of the serotinin transporter impair folding of the transporter. J Biol Chem 2010; 285: 39201–10.

Jacobs MT, Zhang YW, Campbell SD, Rudnick G. Ibogaine, a noncompetitive inhibitor of serotonin transport, acts by stabilizing the cytoplasm-facing state of the transporter. J Biol Chem 2007; 282: 29441–7.

da Silva TC, Hussainzada N, Khawtal CM, Polli JE, Swaan PW. Transmembrane helix 1 contributes to substrate translocation and protein stability of bile acid transporter SLC10A2. J Biol Chem 2011; 286: 27322–32.

Kim BE, Smith K, Meagher CK, Petris MJ. A conditional mutation affecting localization of the Menkes disease copper ATPase. Suppression by copper supplementation. J Biol Chem 2002; 277: 44079–84.

van den Berghe PVE, Stapelbroek JM, Krieger E, de Bie P, van de Graaf SFJ, de Groot REA, et al. Reduced expression of ABTB7 affected by Wilson disease-causing mutations is rescued by pharmacological folding chaperones 4-phenylbutyrate and curcumin. Hepatology 2009; 50: 1783–95.

Presley MJ, Mayor S, McGraw TE, Dunn KW, Maxfield FR. Bafloimycin A1 treatment retards transferrin receptor recycling more than bulk membrane recycling. J Biol Chem 1997; 272: 13929–36.

Yoshimori T, Yamamoto A, Moriyama Y, Futai M, Tashiro Y. Bafloimycin A1, a specific inhibitor of vacuolar-type H(+) ATPase, inhibits acidifica
tion and protein degradation in lysosomes of cultured cells. J Biol Chem 1991; 266: 17707–12.

Guinea R, Carrasco L. Requirement for vacular proton-ATPase activity during entry of influenza-virus into cells. J Virol 1995; 69: 2306–12.

Kartner N, Yao Y, Li K, Crasto GJ, Datti A, Manolson MF. Inhibition of osteoclast bone resorption by disrupting vacuolar H(+) ATPase a3-B2 subunit interaction. J Biol Chem 2010; 285: 37476–90.

Keizer HG, Schuurhuis GJ, Broxterman HJ, Lankelma J, Schoonen WG, van Rijn J, et al. Correlation of multidrug resistance with decreased drug accumulation, altered subcellular drug distribution, and increased P-glycoprotein expression in cultured SW-1573 human lung tumor cells. Cancer Res 1989; 49: 2988–93.

Smit JW, Huisman MT, van Tellingen O, Wiltshire HR, Schinkel AH. Absence or pharmacological blocking of placental P-glycoprotein profoundly increases fetal drug exposure. J Clin Invest 1999; 104: 1441–7.

Thomas H, Coley HM. Overcoming multidrug resistance in cancer: an update on the clinical strategy of inhibiting p-glycoprotein. Cancer Control 2003; 10: 159–65.

Wang B, Yuan RX, Zhu CL, Yang Q, Lv FT, Liu LB, et al. Polymer
drug conjugates for intracellular molecule-targeted photoinduced inactivation of protein and growth inhibition of cancer cells. Sci Reports 2012; 2.

Rajendran L, Knolker HJ, Simons K. Subcellular targeting strategies for drug design and delivery. Nat Rev Drug Discov 2010; 9: 29–42.

Korn K, Krausz E. Cell-based high-content screening of small
component libraries. Curr Opin Chem Biol 2007; 11: 503–10.

Lieberman RL, Wustman BA, Huertas P, Powe AC Jr, Pine CW, Khanna R, et al. Structure of acid beta-glucosidase with pharmacological chaperone provides insight into Gaucher disease. Nat Chem Biol 2007; 3: 101–7.

Petsko GA, Ringe D. Protein structure and function. London: Sunderland, MA; Oxford New Science Press; 2004.

Janovick JA, Goulet M, Bush E, Greer J, Wettlaufer DG, Conn PM. Structure-activity relations of successful pharmacologic chaperones for rescue of naturally occurring and manufactured mutants of the gonadotropin-releasing hormone receptor. J Pharmacol Exp Ther
131 Ulloa-Aguirre A, Janovick JA, Brothers SP, Conn PM. Pharmacologic rescue of conformationally-defective proteins: implications for the treatment of human disease. Traffic 2004; 5: 821–37.

132 Hawtin SR. Pharmacological chaperone activity of SR49059 to functionally recover misfolded mutations of the vasopressin V1a receptor. J Biol Chem 2006; 281: 14604–14.

133 Robert J, Auzan C, Ventura MA, Clauser E. Mechanisms of cell-surface rerouting of an endoplasmic reticulum-retained mutant of the vasopressin V1b/V3 receptor by a pharmacological chaperone. J Biol Chem 2005; 280: 42198–206.

134 Wuller S, Wiesner B, Loffler A, Furkert J, Krause G, Hermosilla R, et al. Pharmacochaperones post-translationally enhance cell surface expression by increasing conformational stability of wild-type and mutant vasopressin V2 receptors. J Biol Chem 2004; 279: 47254–63.