nature reviews chemistry

https://doi.org/10.1038/s41570-026-00864-1

Perspective

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Subcellular photochemistry for precision spatial protein targeting

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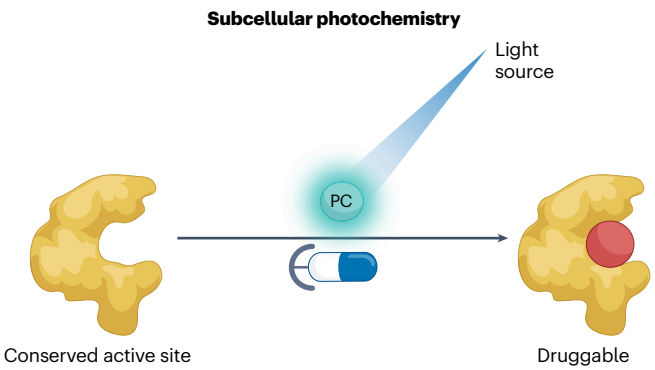
Abstract

Proteins with conserved active sites but distinct subcellular localizations, such as mislocalized variants, splicing isoforms and isozymes, are prevalent in disease. However, conventional drug design lacks the spatial control needed for their selective targeting. Subcellular photochemistry offers a solution. Light-activated catalysts with spatiotemporal control convert inert prodrugs into active drugs at the desired subcellular location, without affecting conserved proteins at native sites. In this Perspective, we discuss challenges of targeting mislocalized proteins, principles of subcellular photochemistry for site-specific intervention, strategies for anchoring photocatalysts and key examples of subcellular photocatalytic prodrug activation for precision targeting. We also examine limitations of this approach and propose future solutions. This strategy is expected to expand the druggable target repertoire and advance precision medicine.



Sections

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Introduction

Identifying therapeutic targets that can be modulated through drug binding is essential for the development of effective disease treatments. As summarized in The Human Protein Atlas[1–4], 4,906 genes encode proteins that are associated with human diseases. However, only 854 of these proteins currently have US Food and Drug Administration-approved drugs, leaving the remaining categorized as 'hard-to-drug' or 'undruggable' (Fig. 1a). Besides the 854 approved protein targets, an additional 1,757 gene-encoded proteins can be considered potential drug targets. This is due to their similarity to approved protein targets belonging to classes of enzymes, transporters, receptors and ion channels, which typically possess bindable pockets[5]. However, many protein target candidates lack distinct expression patterns or the structural features required for selective pharmacological intervention. Even when gene mutations drive pathology[6], they do not always create characteristic binding pockets. Instead, the conserved catalytic or interaction domains they carry are resistant to selective pharmacological targeting[7,8]. Furthermore, some dysregulated enzymes maintain identical amino acid sequences in both healthy and diseased states, even if their function changes. These challenges are further complicated when targeting alternatively spliced isoforms or isozymes, due to their high structural and functional similarities[9].

Although many disease-associated proteins do not exhibit distinct expression patterns or structural features, they may accumulate in subcellular locations where they are not typically observed in healthy cells[10–13]. For example, a 2024 study characterized the localization of 3,400 missense variants of over 1,000 genes. The study found that mislocalization of the encoded proteins to the wrong organelle is a surprisingly common molecular phenotype associated with coding variants. One-sixth of pathogenic mutations showed relocalization[10] (Fig. 1b,c). This finding exceeds previous estimates, which suggested that less than 2% of disease-causing variants become mislocalized within cells[14]. This insight has been reinforced by a recently developed protein language model, ProtGPS[15], that predicts compartment localization of human proteins. In addition, aberrant protein compartmentalization originates not only from primary sequence mutations but also through secondary disruptions. These include dysregulated chaperons, impaired transport receptors or defective trafficking machinery, leading to loss of function, misregulation or toxic gain of function[10,16]. Consequently, protein mislocalization has emerged as a unifying mechanistic thread connecting metabolic disorder[17], cardiovascular dysfunction[18], neurodegeneration[19] and oncogenic transformation[20,21].

Although protein subcellular mislocalization is increasingly recognized as a key factor in disease progression, developing strategies to selectively target proteins that are mislocalized in the cell remains a challenge. Efforts to return a mislocalized protein to its correct subcellular compartment have been explored[13,22]. These include modulating cellular processes that link protein folding, signalling and trafficking. Yet, this approach lacks generality and has achieved only limited success. A promising strategy is to selectively inhibit the proteins mislocalized in the wrong place but without affecting those residing at the native compartment. For decades, drug discovery has focused on identifying protein targets that can be selectively bound. Typically, these targets are characterized by unique expression profiles or mutations that create distinct binding pockets[23]. This approach, exemplified by small-molecule kinase inhibitors, has yielded a remarkable success. However, discriminating between conserved proteins that differ only in subcellular location demands an additional layer of spatial selectivity. Drug molecules are typically constrained by low molecular weight (<500 Da) and moderate lipophilicity (log$P$ < 5) to ensure effective absorption and distribution in vivo[24]. As a result, they are difficult to modify with additional compartment-targeting moieties for the selective inhibition of mislocalized proteins without affecting the normally localized ones in their proper subcellular locations.

Researchers have turned their attention to light-activated prodrug strategies to confine drug activity to a defined disease tissue. In such a system, an active drug is covalently masked by a protecting group, and a photocatalyst (PC) is appended with a tissue-targeting ligand. The prodrug remains inactive until light of a specific wavelength cleaves the mask exclusively within the diseased tissue[25–27]. Although this tissue-specific activation does not control the subcellular fate of the released drug, it offers a promising approach to mitigate 'on-target, off-site' toxicity (Table 1). Based on those works, we argue that a transformative step is to merge spatiotemporal precision of photocatalysis with the concept of targeting subcellularly mislocalized proteins. This enables the modulation of functionally dysregulated yet structurally conserved proteins, without affecting proteins residing at their native subcellular localizations.

This Perspective highlights disease-associated protein relocalization as an underexplored therapeutic vulnerability. We examine the challenges inherent in targeting mislocalized proteins and introduce the principles of subcellular photochemistry as a means of achieving site-specific intervention. Building on this foundation, we discuss strategies for anchoring photocatalysts and showcase key examples of subcellular photocatalytic prodrug activation for precision therapy. Finally, we outline the challenges and future directions of this emerging field, which has the potential to redefine precision medicine through spatial control in therapeutic design.

Challenges in spatial protein targeting

The pursuit of precision medicine in disease treatment has largely focused on molecular specificity, in that drugs are designed to selectively engage a specific protein target over others[23,28]. Although this approach has achieved impressive successes, a critical dimension of precision, that is, the spatial specificity at the subcellular level, remains a formidable challenge for molecular design. The lack of organelle specific drug action represents a substantial source of off-target effects and narrows therapeutic windows[29]. This limitation stems from the following interrelated factors.

Insufficient spatial specificity in drug design

The philosophy of small-molecule drug design centres on affinity and selectivity for a protein's active site or binding pocket. This approach can indeed yield highly effective molecules selectively engaging the target protein and modulating its downstream signalling. However, conventional drug evaluation systems ignore the spatial distribution of compounds within the complex intracellular environment, which may be a critical implication for efficacy and toxicity. Once administered systemically, these molecules passively diffuse across membranes and distribute throughout the cell on the basis of their physicochemical properties (for example, lipophilicity and charge)[30]. Consequently, the drug reaches not only its intended pathogenic target but also other cellular compartments where structurally similar or functionally related isoforms may reside (Fig. 2a). The subcellular address of a protein is not a parameter that can be simply encoded into the structure of a small molecule, leading to potential 'on-target, off-organelle' toxicity[31,32]. This underscores that molecular specificity

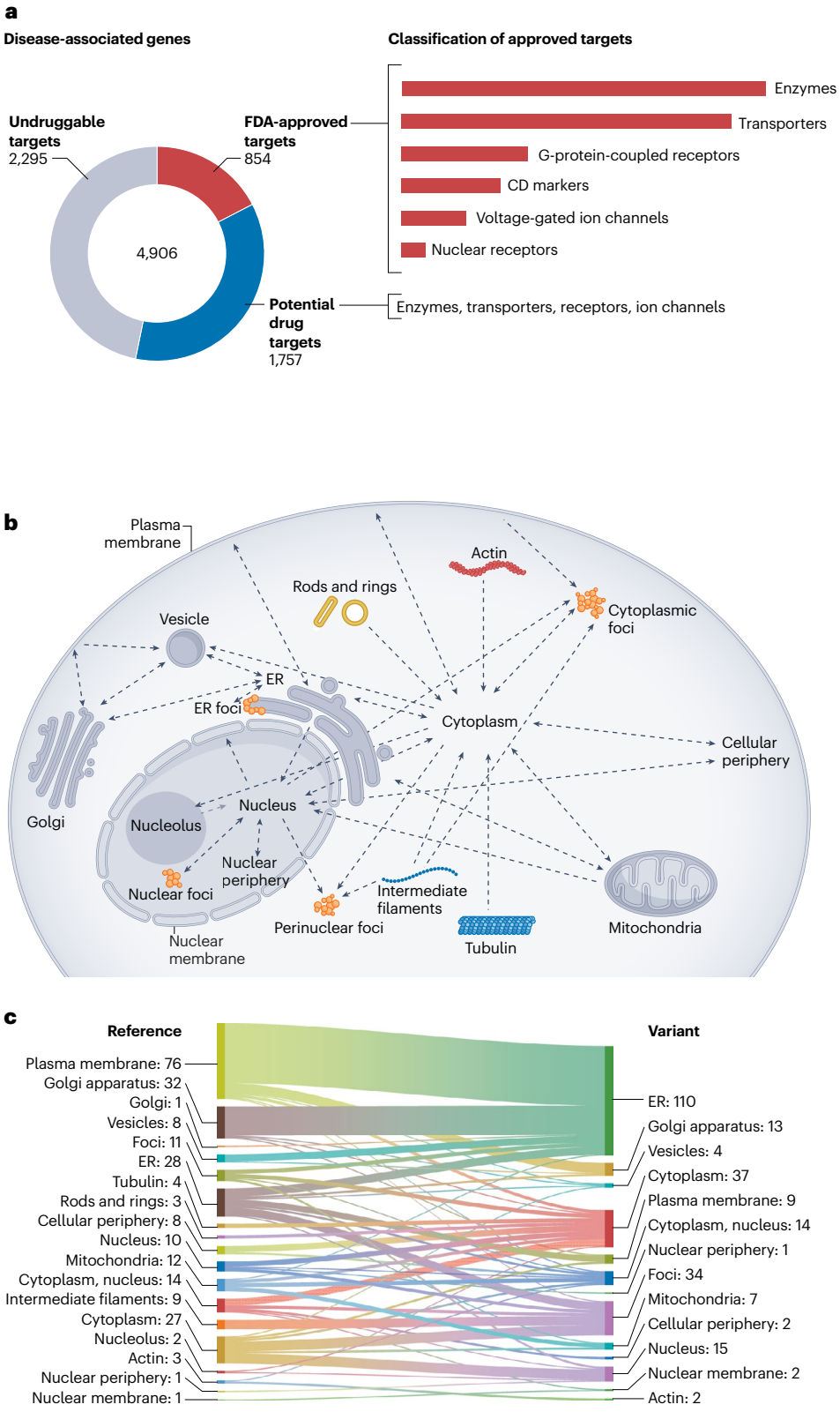


Fig. 1 | Description of the human disease-associated genes and the prevalence of subcellular localization-associated diseases. **a**, There are 4,906 human genes that have experimental evidence to show their associations with diseases. Among them, 854 genes have US Food and Drug Administration (FDA)-approved drugs, which belong to enzymes, transporters, G-protein-coupled receptors, cluster of differentiation (CD) markers, voltage-gated channels and nuclear receptors. Another 1,757 genes are potential drug targets and are classified as enzymes, transporters, receptors and ion channels. The rest of genes are considered undruggable. **b**, The common protein subcellular mislocalization pattern of missense variants for over 3,000 variants across >1,200 genes in diverse human disorders and tumorigenesis. **c**, The Sankey diagram showing the subcellular mislocalization map in 250 missense variants (from reference to variant) based on fluorescence imaging. ER, endoplasmic reticulum. Parts **b** and **c** adapted with permission from ref. 10, Elsevier.

Table 1 | Comparison of tissue-level versus subcellular-level photocatalytic prodrug activation

Features	Tissue-level photoactivation	Subcellular-level photoactivation
Spatial resolution	Low-moderate (mm–cm scale); broad illumination across tissue regions	High (nm–μm scale); specifically targets organelles and suborganelle compartments
Primary objective	Overcome 'on-target, off-tissue' toxicity	Target hard-to-drug or undruggable proteins (by addressing 'on-target, off-organelle' toxicity)
Drug diffusion post-activation	Low to moderate (less diffusion than subcellular activation)	Moderate to high (may be attenuated by introducing covalent drugs, pulsed illumination protocol and so on)
Photocatalyst design	Relatively straightforward; light provides inherent tissue selectivity	Requires careful engineering; subcellular selectivity is derived from the photocatalyst design
Prodrug design	Must consider stability, systemic safety and tissue bioavailability	Similar considerations for stability, systemic safety and bioavailability, but also include intracellular delivery (without inherent subcellular preference)
Clinical practicability	More tractable; can leverage highly specific antibodies for conjugation with photocatalysts	Translation gaps for subcellular anchoring of photocatalysts
Limitations	Primarily effective for superficial or optically accessible tissues	Similarly limited to superficial or optically accessible pathologies; drug diffusion issues

alone is insufficient when the target protein has essential homologues (with identical binding pockets) in other organelles.

Difficulty in compartment-specific delivery

Considerable efforts have been devoted to organelle-targeted delivery systems (including nanoparticles) to improve subcellular specificity. Progress has been made for membrane-enclosed organelles such as mitochondria, lysosomes and endoplasmic reticulum[33,34]. However, efficient delivery to the cytosol remains a challenge[29]. The major bottleneck is endosomal entrapment: systems functionalized with cytosol-directing peptides are largely internalized via endocytosis but exhibit poor endosomal escape, leading to cargo degradation (Fig. 2b). This limitation severely hampers targeting of cytosolic signalling proteins and kinases. Moreover, emerging research reveals that drug targets and their interactors can become enriched within specific biomolecular condensates, forming distinct suborganelle compartments[35]. Yet, strategies to precisely target these condensates remain largely unexplored.

Dynamic nature of protein relocalization

An even more fundamental and often overlooked challenge is the dynamic nature of protein localization. Many proteins change their subcellular position in response to cellular stress, signalling or disease states[16] (Fig. 2c). Proteins at their native sites typically preserve normal function, whereas mislocalized ones can acquire toxic gain of function[10,20]. A therapeutic strategy based on static protein–ligand interactions may become ineffective or even harmful if the protein relocates (Fig. 2c). This dynamic behaviour adds considerable complexity for fixed-dose, systemically administered small molecules, underscoring the urgent need for spatiotemporally controllable therapeutic interventions.

Collectively, the passive distribution of small molecules, inefficient compartment-specific delivery and dynamic protein shuttling constitute a critical barrier in precision medicine. Overcoming these limitations may need a paradigm shift from the simple recognition of molecular targets towards their context-specific intervention within their precise pathological environment.

Principles of subcellular photochemistry

Light offers exceptional spatiotemporal control over chemical reactions[36–57], establishing it as a powerful modality for interrogating and manipulating biological systems with subcellular precision[58–70]. Cells are organized into functionally distinct compartments, which are membrane-bound or based on biomolecular condensate. This feature provides naturally confined environments to conduct chemical reactions with minimal cross-compartment interference. Within this framework, several photochemical strategies have emerged for achieving subcellular modulation. This section introduces the general principles of these photochemical strategies and possible limitations, then focuses on a more versatile and modular subcellular photocatalytic approach.

General principles of subcellular photomodulation

Subcellular photomodulation exploits the ability of light to trigger chemical reactions in defined cellular locations. A straightforward approach is to directly conjugate a photocage with the drug molecule. The spatial confinement is achieved by their intrinsic subcellular localization properties, or by additionally conjugating with directing moieties. Several photochemical approaches have been harnessed for compartment-localized modulation of biological processes (Table 2). Certain photocages[71–92], for instance, can achieve targeted release by leveraging intrinsic organelle-targeting properties or through conjugation with specific targeting groups. A representative example is the mitochondria-targeting H_2S donor, which utilizes the innate localization of cyanine dyes for precision release[93]. Alternative strategies for endogenous protein photomodulation include small molecular ligand-directed caging[94,95] and photo-switchable affinity ligands[96]. Nevertheless, despite their proven potential in achieving spatially precise subcellular delivery of bioactive payloads, their wide applications are hampered. These issues include the need for sophisticated molecular design and synthesis, potential instability in biological environments, off-target binding and a low-efficiency stoichiometric release profile.

Subcellular photocatalysis represents a more powerful strategy for selectively targeting relocalized proteins, pathogenic protein isoforms or isozymes that exhibit distinct subcellular distributions. This approach is realized by combining three modular components. These include a photocatalyst precisely anchored to a specific organelle or compartment, a caged small-molecule prodrug that can freely cross the subcellular barriers, and light (Fig. 3). Upon photoexcitation, the confined photocatalytic process allows localized prodrug activation spatially proximal to the excited photocatalysts. This proximity-driven process ensures that the active drug is released exclusively within the target organelle, enabling compartment-selective inhibition of pathological targets with high spatial precision.

Subcellular photocatalysis: modular by design

A key strength of subcellular photocatalysis lies in its highly modular architecture. This architecture decouples targeting, therapeutic and

control elements into three distinct, changeable components: the photocatalyst, the prodrug and the light source. This modularity provides exceptional versatility and facilitates optimization for diverse applications.

The photocatalyst module is responsible for subcellular targeting and photonic energy conversion. This module offers considerable flexibility. It can be selected from genetically encoded photosensitive proteins[58], which ensure precise targeting and homogeneous cellular expression. Alternatively, synthetic agents such as organic dyes or inorganic nanoparticles can be used[97,98]. Many of these agents possess an intrinsic propensity to localize to specific organelles or can be functionalized with organelle-targeting motifs to direct their localization. Examples of organelle-localizing photocatalysts include cyanines for mitochondria[99], acridine orange for the nucleus[100] and DND-189 for lysosomes[101]. The choice of catalyst determines the activation wavelength, efficiency and biocompatibility, allowing it to be tailored to the specific biological context.

The prodrug module carries the therapeutic payload (Box 1). Its design is independent of the targeting mechanism, following a 'caging' strategy. A potent drug molecule is rendered biologically inert by covalently attaching a photocatalytically labile protecting group (for example, aryl azides[56,102,103], *o*-nitrobenzyl derivatives[104], azo[105], *N*-methylpicolinium[106,107] and boronic acids[108–111]). The linker between the cage and the drug is engineered for specific cleavage via a photosensitized process mediated by the selected photocatalysts. In addition, a suitable lipophilicity (for example, with a low-to-moderate log*P* value) is required to assist the prodrug in freely crossing the cellular compartments without accumulation. This modular architecture is highly versatile, as a single photocatalytic activation mechanism can be applied to diverse drug molecules with distinct pharmacological activities.

The light source module provides external spatiotemporal control by acting as a remote trigger. This confers unparalleled precision, and on-demand control over when and where the drug is activated. Key parameters include wavelength (chosen to match the photocatalyst's absorption and maximize tissue penetration), intensity and duration of irradiation. The ability to precisely deliver light onto a specific tissue region allows unmatched control over the therapeutic intervention[112]. This enables both temporal regulation and excellent spatial specificity.

Subcellular anchoring of photocatalysts

As mentioned above, spatial selectivity is governed by the precise localization of the photocatalyst, not the prodrug. Accordingly, the development of robust and reliable strategies for precisely anchoring photocatalysts to objective subcellular compartments constitutes a cornerstone of this methodology. This section delves into approaches for achieving such spatial control, each offering distinct mechanisms and practical applicability.

Passive physicochemical anchoring

Several commercially available organic dyes and metal-based photocatalysts exhibit organelle-targeting capabilities (Fig. 4a). Capitalizing on this intrinsic targetability offers a relatively straightforward strategy for subcellular anchoring. A prominent example is the work by Zhu, Chen and coworkers, who used organelle-selective organic dyes to achieve confined photocatalysis. This enables the quantitative profiling of lipid transport between mitochondria, endoplasmic reticulum, nucleus and lysosomes[113]. Moreover, most such dyes are amenable to structural modification to enhance or redirect their targeting specificity. For instance, Fan, Chen and coworkers reported a modified eosin derivative with improved lysosome-targeting ability, facilitating in situ lysosomal proteomics[114]. Nevertheless, it should be noted that the targeting property of dyes is often suboptimal. Although chemical modification with targeting motifs can improve their precision, they are generally restricted to common membrane organelles[97]. For more specific subcellular compartments, targeting is difficult due to a lack of functional groups. Furthermore, a more notable limitation of these dyes is their susceptibility to concentration-dependent/photoinduced relocalization and photobleaching[115,116].

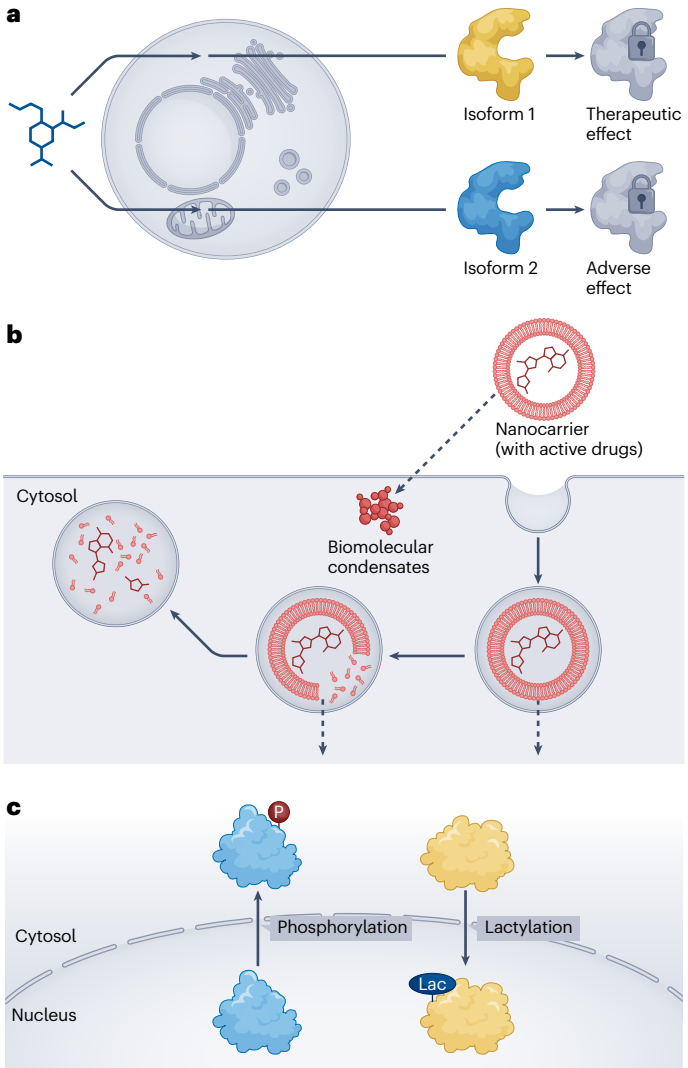


Fig. 2 | Challenges in targeting mislocalized proteins with conventional drug design. a, Conventional drug design prioritizes binding affinity and selectivity for protein targets, although drug molecules are also constrained by parameters such as molecular weight and lipophilicity. Introducing an additional layer of specificity based on subcellular localization is therefore challenging. **b**, Nanoparticle-based delivery systems are often trapped within endosomes, limiting their ability to deliver cargo to other organelles. **c**, Protein localization within cellular compartments is highly dynamic. Consequently, a static protein–ligand interaction may become ineffective or even harmful if the protein relocates.

Table 2 | Comparison of different photochemistry in subcellular biological modulation

Photochemistry	Ease of subcellular targeting	Advantages	Limitations
Photocatalysis	High (via photocatalyst localization)	Proven versatility in organelle-specific prodrug activation; modular design flexibility	Potential ROS-mediated cytotoxicity
Direct photouncaging	Moderate to low (limited by chemical constraints on prodrug modification)	Highly controllable; potentially minimal off-target toxicity (for example, little ROS generation by Ru(II) photocages[a])	Stoichiometric release mechanism (low efficiency); complicated prodrug design
Ligand-directed caging	Medium to high (by ligand–receptor specificity)	Controllable modulation of protein functions	Limited applicability for prodrug activation

[a]Those Ru(II) polypyridyl photocages are typical photoactivated chemotherapy compounds that undergo photo-substitution via an easily accessible, antibonding metal-centred triplet excited state. ROS, reactive oxygen species.

Directing group-mediated subcellular anchoring

Small molecules (for example, ligands), peptides or antibodies can act as directing groups to localize photosensitizers within specific subcellular compartments (Fig. 4b). For instance, the derivatives of O^6-nitrobenzylguanine (O^6-NBG3 as an example) have been engineered to selectively modify O^6-alkylguanine-DNA alkyltransferase with a photocage[94]. Similarly, conjugating a Bcl-2 homology 3 (BH3) peptide of phorbol-12-myristate-13-acetate-induced protein 1 (PMAIP1, or NOXA) with a Ru(II) photocatalyst enables targeted immobilization on BCL-2, leveraging protein–peptide interaction specificity[117]. Another approach uses opto-covalent inhibitors, such as the ibrutinib derivative OCM-1. It forms irreversible bonds with Bruton's tyrosine kinase, allowing photocontrolled modulation of enzymatic activity[95]. Conjugating a photocatalyst to an antibody represents a feasible strategy for its direct immobilization on cell membranes. A representative example is an alkyne-bearing iridium photocatalyst conjugated to a secondary antibody (targeting, for example, PD-L1 and CD45) for spatially targeted photocatalytic proximity labelling on cell surfaces[61].

Genetically encoded photoenzyme

Genetically encoded methods achieve high precision by using genetically fused targeting motifs (for example, protein markers or signal peptides). These motifs direct protein-based photocatalysts to objective organelles via endogenous expression pathways[58] (Fig. 4c). For example, the compact and genetically encodable mini Singlet Oxygen Generator (miniSOG), a miniature photocatalytic protein, has been harnessed for spatial targeting. By directing miniSOG to diverse subcellular locations, such as the cytoplasm, mitochondria, endoplasmic reticulum and stress granules, organelle-specific proteomes have been spatially resolved. Notably, when miniSOG was anchored in the mitochondrial matrix, more than 477 mitochondrial proteins were captured with 94% specificity, highlighting the exceptional compartmental accuracy[118]. Furthermore, the introduction of a HaloTag-based platform enables the construction of a more versatile photocatalytic system with protein-level precision. A representative example is reported recently by MacMillan and colleagues. They profiled the stress granule proteome by engineering a HaloTag–G3BP1 fusion protein to specifically anchor an iridium-based photocatalyst within these granules[119]. In addition, genetic code expansion technology allows the site-specific incorporation of non-canonical amino acids with single-residue precision[120]. These non-canonical amino acids serve as small, bioorthogonal handles for the subsequent targeted installation of organic dyes[121]. Although these approaches afford high specificity and sustained expression, they rely on genetic manipulation. This remains experimental and may cause risks due to uncontrolled gene modification, thus limiting their practical applicability.

To bridge this gap, the adeno-associated virus (AAV) system may be a promising alternative, which has been successfully used in gene therapy with a favourable safety profile[122,123]. AAV is a non-pathogenic vector with low immunogenicity that delivers a single-stranded DNA genome, which typically persists in the nucleus as an independent episome. This enables stable, long-lasting protein production without integration into the host genome. Currently, there are seven US Food and Drug Administration-approved AAV gene therapies and over 300 clinical trials targeting more than 50 diseases.

The mRNA delivery system

The field of mRNA therapeutics has reached maturity, as underscored by the global approval of mRNA vaccines against COVID-19[124]. Unlike vaccines, which rely on transient antigen expression, mRNA for gene delivery faces limitations in sustaining long-term and high-level protein production. Fortunately, advances in mRNA design and formulation technologies, along with improved tissue targeting and chronic dosing strategies, continue to enhance mRNA stability and translational efficiency[125,126]. Building on the progress, several clinical trials have been initiated for genetic diseases. Examples include mRNA-3927 for propionic acidemia (NCT04159103), mRNA-3745 for glycogen storage disease 1a (NCT05095727) and MRT5005 and mRNA-3692/VX-522 for cystic fibrosis (NCT03375047 and NCT05668741). In their trials, therapeutic mRNA expression achieves clinically relevant levels to effectively function. These advances highlight the potential of mRNA delivery systems for safe anchoring of compartment-specific proteins carrying photocatalytic properties (Fig. 4d).

Photochemical internalization

Photochemical internalization has emerged as a promising strategy for subcellular targeting[127]. This strategy leverages the compartment-specific accumulation of certain small-molecule photocatalysts such as $TPPS_4$ and $AlPcS_2a$ in endosomes and lysosomes. Upon low-dose visible-light irradiation, these agents generate reactive oxygen species (ROS), predominantly singlet oxygen ($^1O_2$). Owing to its ultrashort lifetime (~4 μs) and limited diffusion distance (~10 nm)[128,129], $^1O_2$ acts locally, permeabilizing endo-lysosomal membranes without inducing global cellular damage. This controlled disruption facilitates the release of encapsulated cargo into the cytosol[130,131]. Once liberated, the photocatalysts can either remain diffusely distributed in the cytosol or relocate to other organelles such as mitochondria[132] or nucleus[133,134] (Fig. 4e). Such light-triggered translocation offers potential for spatially confined activation of prodrugs within cytosol or common organelles, enabling targeted interventions with minimal off-target effects.

Subcellular photochemistry for therapy

Researchers have developed a variety of complementary strategies that exploit subcellular photochemistry to confine therapeutic activity

to defined organelles. Using organelle-targeted photocatalysts, ranging from small-molecule photosensitizers and genetically encoded photoenzymes to fluorophore–drug conjugates, each approach triggers prodrug activation exclusively at the intended location. The released drug molecules include enzyme inhibitors, receptor agonists and protein modulators. The examples below demonstrate how such spatial control enables (1) selective inhibition of cytosolic thioredoxin reductase 1 (TrxR1) while sparing mitochondrial thioredoxin reductase 2 (TrxR2); (2) tunable mitochondrial uncoupling with minimal off-target effects; and (3) light-controlled modulation of microtubule dynamics.

Tumour-targeted inhibition of TrxR1 over TrxR2

TrxR maintains cellular redox balance by catalysing NADPH-dependent thioredoxin reduction, thereby facilitating protein disulfide bond reduction. Due to its regulatory roles closely linked to cancer development, TrxR has become an attractive target for therapeutic intervention[135]. Notably, mammalian cells express two isoforms of TrxR: cytosolic TrxR1 and mitochondrial TrxR2[136]. In many cancers, TrxR1 is often upregulated and implicated in a tumour promotion, with its elevated levels frequently correlating with poor patient survival[137] (Fig. 5a). By contrast, TrxR2 is crucial for preserving mitochondrial redox homeostasis and has a protective role generally associated with better patient prognoses[137–140] (Fig. 5a). This functional distinction underpins selective TrxR1 inhibition in cancer cells to impede tumour growth while avoiding adverse effects from TrxR2 inhibition. Nevertheless, despite being encoded by different genes, the two isoforms share high sequence homology, including identical catalytic active sites (Gly–Cys–Sec–Gly)[141]. Consequently, achieving isoform selectivity with conventional small-molecule inhibitors poses a fundamental challenge. In 2018, Arnér's group identified Tri-1 as a specific TrxR1 inhibitor via high-throughput screening, whereas it exhibits modest selectivity (approximately five- to tenfold) for TrxR1 over TrxR2[137].

Given that thiol-reactive gold-based therapeutics are potent TrxR inhibitors and the two isoforms TrxR1 and TrxR2 reside in distinct subcellular compartments[135,136,142–145], our group developed a subcellular photocatalytic strategy for spatially selective gold(I) prodrug activation. This strategy enables selective inhibition of tumour-promoting cytosolic TrxR1 while sparing its mitochondrial counterpart, TrxR2[146] (Fig. 5a). In this design, a stable NHC–Au(I)–alkynyl complex (**1a**) was combined with hydrophilic photocatalysts such as rose bengal (RB) or methylene blue. Compound **1a** can freely distribute across cancer cells without organelle specificity. These photocatalysts are internalized into cancer cells via endocytosis and accumulate in endo/lysosomal compartments. Upon irradiation with a non-cytotoxic light dose (530 nm, 100 mW cm^{-2}, 10 min irradiation), they readily escape into the cytosol, where they catalyse the transformation of **1a** into thiol-reactive gold species. Mechanistic studies revealed that the photocatalytic activation of **1a** by RB proceeds via a radical-mediated bimolecular reductive elimination pathway. Notably, the '**1a** + RB' combo enabled precise, light-controlled inhibition of cytosolic TrxR1 over mitochondrial TrxR2 in living cells. This leads to a striking cytotoxicity selectivity advantage over auranofin, an established pan-TrxR inhibitor. These results were confirmed by using selective TrxR-responsive fluorescent probes and colorimetric assays on isolated cytosolic and mitochondrial fractions. Moreover, the '**1a** + RB' treatment regimen exhibited substantial light-induced antitumour activity in zebrafish and mouse xenograft models.

Tunable biointervention in confined organelles

Mitochondrial uncoupler 2,4-dinitrophenol (DNP) disrupts cellular energy metabolism by binding to uncoupling protein 1 (UCP1), thereby decoupling oxidative phosphorylation[147]. This mechanism underlies the therapeutic potential of DNP for metabolic disorders such as non-alcoholic fatty liver disease and obesity. However, DNP exhibits promiscuous interactions with multiple cellular targets,

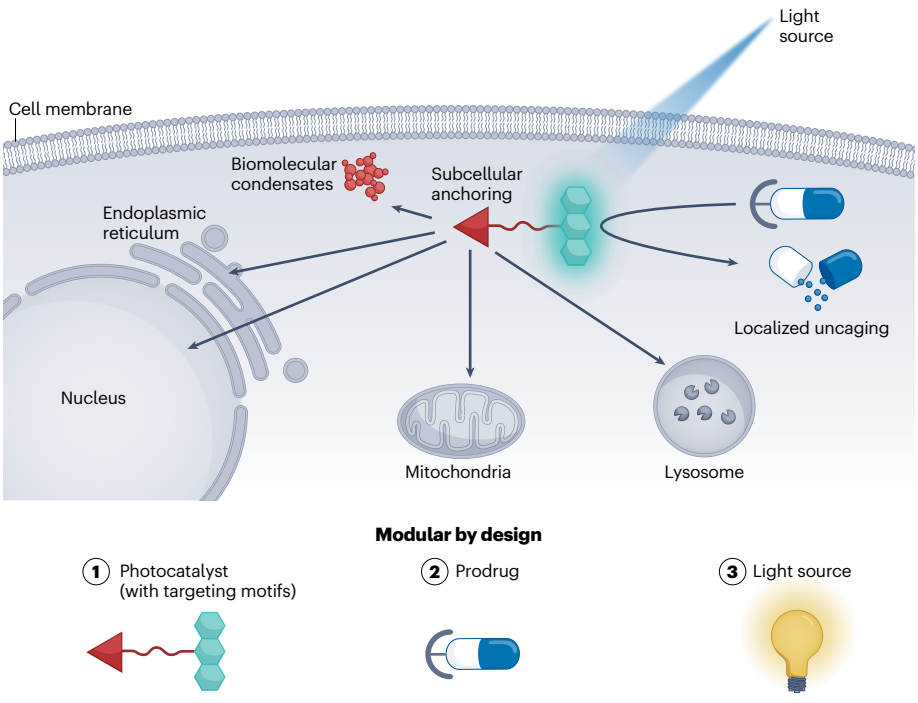
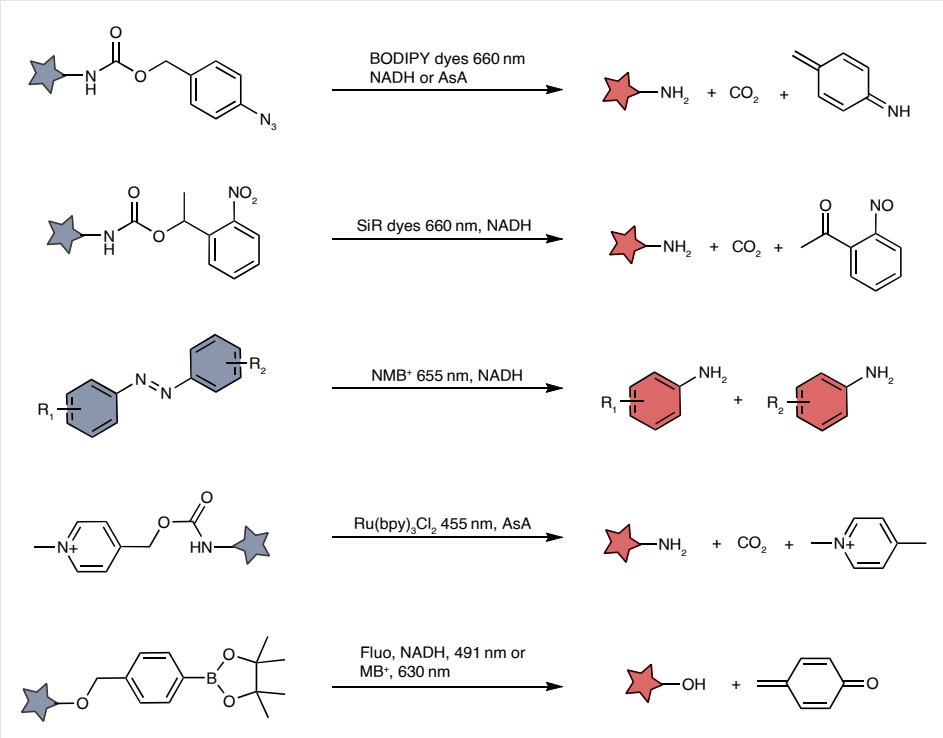


Fig. 3 | Principles of subcellular photocatalytic prodrug activation for precision targeting. The system comprises an organelle-/compartment-targeting photocatalyst, a prodrug and light source. The prodrug freely diffuses across the membrane barriers; upon light irradiation, the photocatalyst anchored at specific cellular compartments activates the prodrug at defined locations. This enables spatiotemporally controlled drug release for targeted intervention of localized protein target. The photocatalyst, prodrug and light source can be modularly designed to provide a general platform for subcellular targeting.

Box 1 | Examples of photocatalytic prodrug activation reactions

Photocatalytic reactions, which typically proceed via radical mechanisms, have demonstrated high efficiency and good biocompatibility in biological environments. Common functional groups in drug molecules, such as amino, hydroxyl and carboxyl moieties, can be masked with photocatalytically labile protecting groups in prodrug design. The following lists the typical caging groups and the photocatalytic reactions for drug activation.



Aryl azide. This functional group was first introduced by Liu and coworkers for a visible-light-induced azide reduction under mild physiological conditions, using $Ru(bpy)_3Cl_2$ as the photocatalyst[102]. This transformation showed broad functional-group tolerance and has been harnessed to achieve spatiotemporally controlled activation of an azide-caged doxorubicin prodrug in living systems[103].

***ortho*-Nitrobenzyl (ONB).** The ONB group is a classic moiety for UV/blue light-triggered molecular release. Recently, Chu and coworkers developed an efficient red-light-mediated decaging strategy by using silicon rhodamine as a photocatalyst and NADH as a sacrificial reductant. This leads to precise ONB cleavage across diverse in vitro and in vivo settings[104].

Azobenzene. Redox-active azo is a stable functional group under physiological conditions. A solution to efficiently break the N=N bond involves a red-light photocatalytic approach using new methylene blue (NMB+) as a photocatalyst and NADH as a reductant. This approach has been demonstrated to facilitate the release of a fluorescent probe and the drug mesalazine[105].

Methylpicolinium. The *N*-methylpicolinium carbamate unit is another photocleavable linker for releasing amine payloads. The system uses $Ru(bpy)_3Cl_2$ as the photocatalyst and ascorbic acid as the stoichiometric reductant via triggering C–O bond cleavage and spontaneous CO_2 release to liberate free amine[106,107]. This mild, aqueous-compatible protocol has been successfully applied to release a range of aliphatic and aromatic primary amines.

Organoboron. The aerobic oxidative hydroxylation of arylboronic acids through a type I photocatalytic mechanism was first established by the Xiao group in 2012[108]. The first demonstration of visible-light-induced deboronative hydroxylation in live cells and neurons was reported by the Chen group in 2019[109] and was subsequently extended to near-infrared light-mediated activation by the Fan groups in 2023[110]. Expanding on this precedent, our group introduced in 2023 a red-light photocatalytic strategy. This enables efficient decaging of organoboron prodrugs under hypoxic conditions via a different phenyl-radical mechanism[111].

leading to off-target effects and systemic toxicity that limits its clinical application[148]. To address this problem, Chen and coworkers constructed a genetically encoded photocatalyst, the mito-SNAP-FL protein[149] (Fig. 5b). Upon light exposure (520 nm, 10 mW cm^{-2}, 10 min irradiation), HeLa cells expressing mitochondria-localized mito-SNAP-FL protein is capable of converting boronate-caged DNP (**1b**) into its active form via deboronative hydroxylation. This conversion was monitored by the membrane potential-sensitive dye tetramethylrhodamine ethyl ester (TMRE). By contrast, cells expressing SNAP-FL without mitochondrial targeting property displayed negligible change in TMRE staining. The efficacy of this strategy was further clarified in mouse cortical neurons, with minimal off-target effects on neighbouring glial cells. Importantly, selective prodrug activation at nucleus, membrane, cytosol and endoplasmic reticulum has also been achieved by transfection with different organelle-localized SNAP genes. Moreover, a membrane-anchored, extracellularly oriented SNAP-tag has been used to achieve photocontrolled activation of a boronate-caged baclofen prodrug, enabling precise $GABA_BR$ activation on $Na_V1.8$-expressing nociceptor terminals[149]. This subcellular photochemistry platform further validated its therapeutic potential in vivo by enabling nociceptor-specific baclofen release in the peripheral nervous system of $Na_V1.8$-Cre mice. Notably, this approach

provided the first direct evidence that $GABA_BR$ activation selectively in $Na_V1.8$-expressing nociceptor terminals mediates antipruritic (anti-itch) effects. In a 2025 study, the same group creatively repurposed miniSOG, a genetically encodable, endogenously expressible flavoprotein, as a self-contained miniature photoenzyme (12 kDa) for broad-scope bioorthogonal catalysis[150]. Upon genetic anchoring, this system robustly mediates deboronative hydroxylation reaction with subcellular precision. These results collectively indicate the essential role of organelle-confined photocatalysis for precision modulation of drug activity.

Precision modulation of microtubule dynamics

Microtubules serve as essential cytoskeletal components that support numerous critical cellular functions in eukaryotes, including mitosis and migration. Agents that interfere with microtubule dynamics, such as Taxol, are widely used in cell biology research. However,

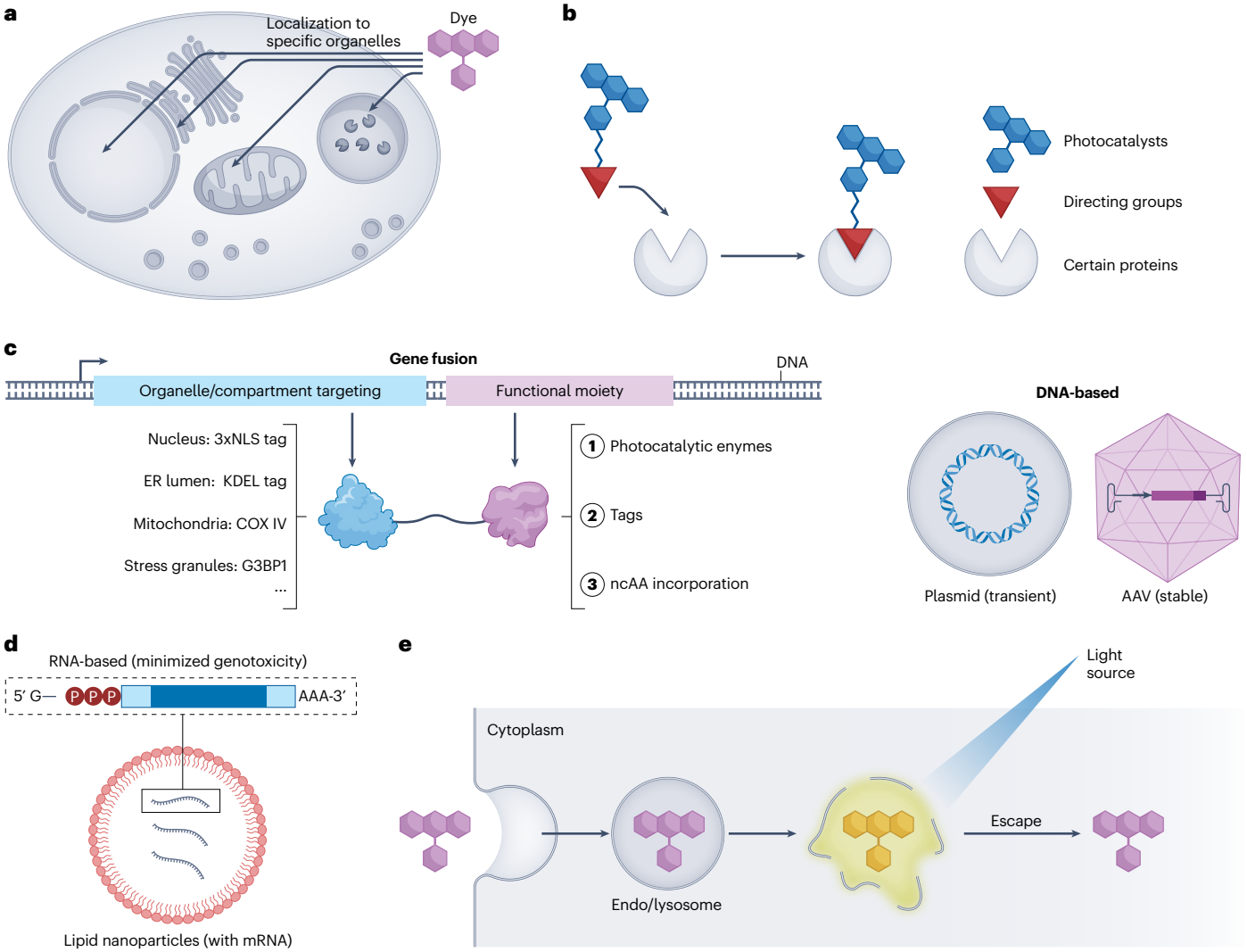
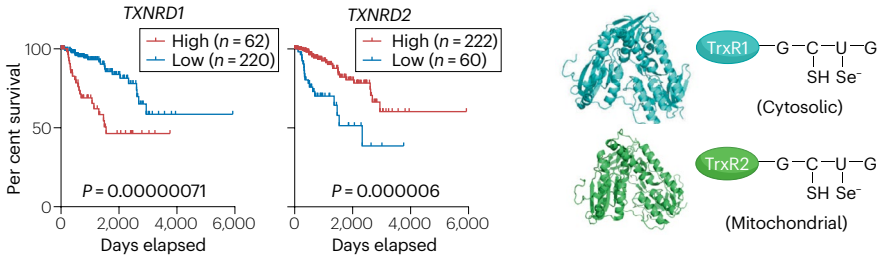
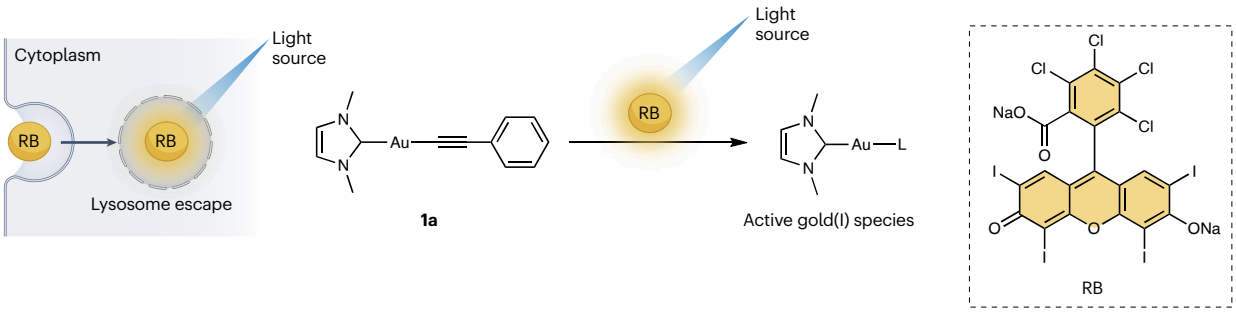


Fig. 4 | Towards compartment-specific anchoring of photocatalyst. a, Passive anchoring of photocatalyst. Certain commercially available dyes naturally localize to specific organelles based their charge, lipophilicity, pK_a and so on. **b**, Anchoring of photocatalysts by conjugating to directing groups such as ligands, peptides and antibodies. **c**, Anchoring by gene-expressing systems. In principle, a fusion protein containing both the organelle-/compartment-targeting gene and the functional moiety is constituted for transfection through either viral vector or plasmid. The targeting protein contain the protein scaffold with known subcellular localization such as nucleus (3× nuclear localization signal/NLS tag), endoplasmic reticulum (ER) lumen (Lys-Asp-Glu-Leu/KDEL tag), mitochondria (cytochrome c oxidase subunit IV/COX IV), stress granules (Ras GTPase-activating protein-binding protein 1/G3BP1) and so on. The functional moiety can be either a direct photoenzyme such as mini Singlet Oxygen Generator (miniSOG), a bindable tag (for example, haloalkane dehalogenase-/HaloTag, mutant of O^6-alkylguanine-DNA alkyltransferase-/SNAP-Tag) or non-canonical amino acids. Please note that the targeting moiety can be fused at either the N terminus or the C terminus of the protein. For brevity, only one representative gene fusion isoform is depicted in this figure. **d**, To avoid the potential genotoxicity of gene transfection, a lipid nanoparticle-encapsulated messenger ribonucleic acid (LNP–mRNA) system can be harnessed for transient expression of photoenzymes at specific locations without causing gene modifications. **e**, Certain dyes prefer to accumulate in endosomes/lysosomes. A low dose of light irradiation can oxidize and increase the permeability of the endo-lysosomal membrane. This causes escape into the cytosol or relocation to other organelles that are difficult to directly target with anchoring strategies. AAV, adeno-associated virus. ncAA, non-canonical amino acids.

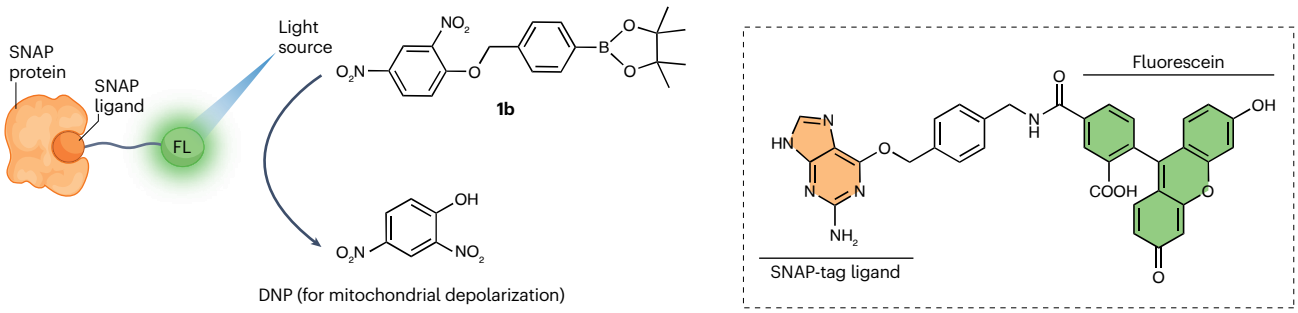
a **Cytosolic photocatalysis for selective inhibition of cytosolic TrxR1**



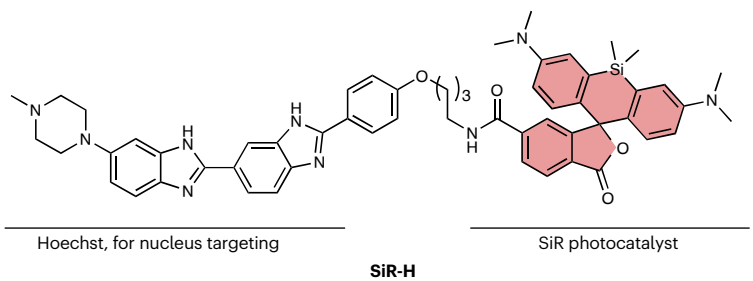
- Opposite roles in most cancers
- The same active sites
- Different subcellular localization



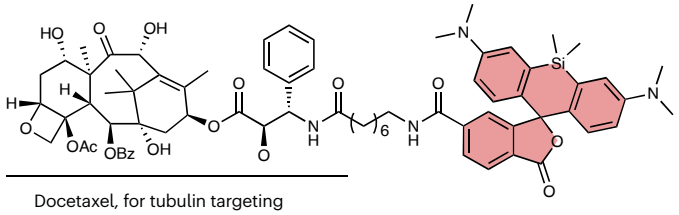
b **Organelle-targeted photocatalysis for localized prodrug activation**



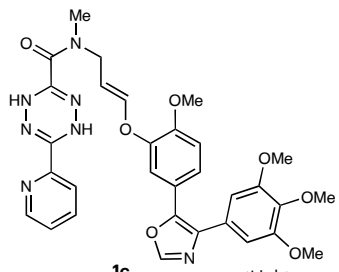
c **Nuclear/tubulin-localized photocatalysis for targeted tubulin depolymerization**



SiR-H



SiR-T



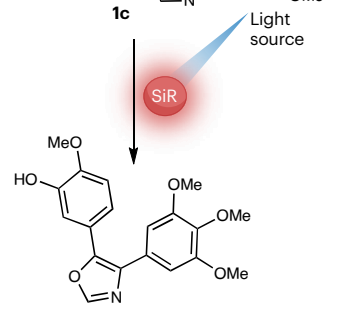


Fig. 5 | Examples of subcellular photocatalytic prodrug activation for targeted inhibition. a, The expressions of *TXNRD1* and *TXNRD2* in patients with kidney renal papillary cell carcinoma (KIRP) and their correlation with the overall survival. The two genes encode proteins with the identical active site sequences. Selective inhibition of thioredoxin reductase 1 (TrxR1) by a hydrophilic photocatalyst rose Bengal (RB), which is trapped by endosome; upon light irradiation, it can escape from endosome to cytosol, enabling selective photocatalytic conversion of the NHC–Au(I)–alkynyl complex (**1a**) into active gold(I) species for targeted TrxR1 inhibition without affecting mitochondrial TrxR2 (bottom panel). **b**, Functionalizing fluorescein (FL) with a SNAP-tag ligand enables its organelle-specific anchoring, where it acts as a photocatalyst to convert organoboron prodrug **1b** into the mitochondrial uncoupler 2,4-dinitrophenol (DNP). This genetically encoded system allows for subcellularly targeted photoactivation, enabling selective modulation of neuron function. **c**, Functionalization of a silicon rhodamine (**SiR**) photocatalyst with a nucleus-targeting (**SiR-H**) or tubulin-targeting (**SiR-T**) moiety achieves the spatially controlled photocatalytic activation of a dihydrotetrazine-cagerd prodrug **1c**, leading to controlled release of microtubule-destabilizing agent in different subcellular compartments. Part **a** reprinted with permission from ref. 146, ACS.

strategies for achieving subcellular-resolution control over microtubule behaviour remain scarce. In 2023, Fox and colleagues introduced a novel photoactivatable bioorthogonal uncaging platform for the spatially controlled activation of prodrugs at the subcellular level[151] (Fig. 5c). The biocompatible silarhodamine (SiR) fluorophore was conjugated to either the Hoechst dye (SiR-H) for nuclear localization or to docetaxel (SiR-T) for microtubule targeting. SiR was chosen for its low $^1O_2$ generation. Upon irradiation with 660-nm red light (400 mW cm^{-2}, 20 min irradiation), the SiR-H/T conjugates act as localized photocatalysts to initiate a bioorthogonal uncaging reaction. Specifically, they catalyse the oxidation of a dihydrotetrazine moiety within prodrug **1c**, thereby triggering an intramolecular Diels–Alder/aromatization cascade process. This approach releases the potent microtubule-depolymerizing payload from **1c**, enabling the precise, spatiotemporally controlled modulation of microtubule organization in cells.

Conclusions and outlook

The past years have greatly advanced our understanding of protein mislocalization as the driver of human diseases[10]. These mislocalized proteins typically show unaltered expression levels and retain the conserved binding pockets of their normally localized counterparts. Although this renders a key challenge for conventional structure-based drug design, it opens a new avenue to target those proteins at the subcellular resolution level[13,22].

In this Perspective, we highlight subcellular photochemistry as a particularly promising route for achieving spatial precision in this therapeutic challenge. This approach enables a layer of spatial control over drug action, stemming from the confinement of prodrug activation to specific cellular compartments. By leveraging targeted photocatalysts, this strategy overcomes a fundamental constraint of traditional molecular design, which is based on systemic drug distribution. Such designed compounds cannot distinguish between protein isoforms localized in different organelles. We have highlighted three examples: (1) how subcellular photocatalysis facilitates the selective inhibition of tumour-associated TrxR1 while preserving the normal function of TrxR2[146]; (2) how a genetically encoded system can enable the targeted anchoring of photocatalysts to specific organelles for drug release[149]; and (3) how ligand-directed subcellular photocatalysis enables precision modulation of microtubule dynamics[151]. The spatial precision achieved through this paradigm minimizes on-target, off-organelle toxicity, thereby offering a powerful means to modulate targets of interest.

Nevertheless, when designing subcellular photocatalytic systems for prodrug activation, several challenges remain. First, the current arsenal of photocatalysts presents a trade-off between precision and broad applicability. Genetically encoded photosensitive proteins enable unparalleled subcellular targeting but rely on gene operation. The repertoire of photosensitive proteins amenable to precise genetic manipulation remains limited; most established systems are activated by blue or green light, which is associated with poor tissue penetration and phototoxicity. Organic small-molecule dyes are generally more amenable to screening and application. However, their targeting specificity is often suboptimal. For example, lysosome-targeting dyes may induce lysosomal membrane damage via ROS production, leading to dye leakage from the lysosomal lumen. Although such leakage may be tolerable for applications like proteomics, it can cause unintended prodrug activation at non-targeted sites, which severely undermines spatial precision. In practice, achieving biologically meaningful specificity with these dyes is generally more feasible for common membrane-bound organelles such as mitochondria and the endoplasmic reticulum. In addition, toxicity and pharmacokinetic profiles of these dyes also warrant thorough investigation. It should be noted that the clinical advancement of AAV-based and mRNA-based therapies for genetic diseases suggests a promising strategy. This strategy involves utilizing these delivery systems to express photosensitive proteins. This enables controllable protein production[131] along with highly precise anchoring at subcellular or even suborganelle compartments[15].

Second, unlike tissue-specific prodrug activation, which operates at millimetre to centimetre scales, subcellular-level activation functions at the micrometre scale. It is therefore governed by fundamentally different physical constraints. The spatial fidelity of this approach is challenged by the potential diffusion of the activated bioactive molecules away from the targeted organelle. This can lead to toxicity and undermines therapeutic efficacy. To maintain high 'spatial fidelity', a multifaceted optimization strategy is imperative. For instance, covalent inhibitors, which constitute approximately 30% of approved drugs[152], can be harnessed in prodrug design. Following photocatalytic activation, the released covalent inhibitors can be trapped through covalent bonding[68,153]. For non-covalent inhibitors, unintended diffusion poses greater challenges. Nonetheless, the spatiotemporal precision of light activation offers a solution. We propose that optimizing the illumination protocol, for instance, by using pulsed or intermittent lighting, could sustain therapeutic concentrations locally. This creates time-restricted concentration windows, while simultaneously suppressing off-site accumulation. Such a strategy balances efficacy at targets against off-site exposure risks via free-diffusion pathways. Moreover, the diffusion kinetics are governed by both drug-specific physicochemical properties (for example, log*P*, pK_a, charge state and molecular weight) and the local microenvironment surrounding the target (for example, pH gradients, viscosity and macromolecular crowding). Therefore, optimization must be tailored to each specific case (Box 2).

Box 2 | Minimizing drug diffusion after subcellular photoactivation

According to Fick's law of diffusion[185],

$$J = -D\frac{d\varphi}{dx}$$

molecular movement occurs along a concentration gradient $d\varphi/dx$. Although drug diffusion is inevitable, it is possible to achieve meaningful local retention if release kinetics and molecular properties are carefully orchestrated. The diffusion coefficient D depends heavily on the drug's physicochemical properties (for example, lipophilicity, pK_a and charge state) and the subcellular environment (including membrane barriers, viscosity and macromolecular crowding). This gives rise to various trapping mechanisms that promote retention.

Ionic trapping. Over half of small-molecule inhibitors contain ionizable amine groups[186]. Whereas acidic or neutral molecules diffuse rapidly, basic molecules show negligible diffusion[187]. Importantly, acetylation of amine groups, which eliminates protonatable sites, dramatically accelerates cytoplasmic diffusion. This observation carries direct relevance for prodrug design: masking an amine group (for example, as a carbamate ester) temporarily abolishes basicity, enabling efficient membrane permeation. Upon activation, the free amine is regenerated, restoring its protonatable character and thereby triggering ionic trapping. This 'activation–retention' sequence offers a rational strategy to couple prodrug activation with subcellular anchoring.

Covalent binding. Conventional non-covalent inhibitors exist in equilibrium between bound and free forms, leaving a substantial free fraction that can diffuse. However, when the inhibitor is designed to form a covalent bond with its target (equilibrium shown below), particularly with fast association kinetics (k_{on}) and rapid bond formation (k_1), the concentration of free inhibitor can be dramatically reduced. This lowers the concentration gradient driving diffusion, effectively retaining the drug at the site of activation[188].

$$E + I \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} E:I \underset{k_2}{\overset{k_1}{\longrightarrow}} EI,$$

where E is enzyme and I is inhibitor.

High-affinity non-covalent binding. When irreversible covalent binding is not feasible, engineering drugs with exceptionally slow dissociation rates (k_{off}) represents a powerful alternative. High-affinity interactions prolong the residence time of the drug on its target, ensuring that the free drug concentration remains low and minimizing the driving force for diffusion.

Physical microenvironment barriers. The intracellular environment is heterogeneous and crowded. Lipid bilayers create partitioning effects: highly lipophilic drugs partition extensively into membranes, generating steep concentration gradients that drive rapid transmembrane movement, whereas less lipophilic drugs show weaker partitioning, resulting in smaller gradients and slower diffusion[185]. This principle enables the design of hydrophobic prodrugs that yield more hydrophilic active species upon activation, enhancing retention within membrane-bound compartments. In addition, the crowded intracellular environment, comprising cytoskeletal networks, organelles and biomolecular condensates, heavily retards molecular movement. Many drugs and probes exhibit restricted diffusion in specific subcellular compartments[35].

Pulsed versus continuous illumination. Pulsed illumination introduces a temporal dimension that can mitigate drug diffusion issues. This could be realized by keeping the prodrug release rate ($k_{release}$) slower than or comparable to the rate of local binding or trapping. Then, the dark intervals between pulses are created. This allows released drug to bind its target, undergo ionic trapping or diffuse only minimally before the next pulse. Because each pulse releases a limited amount, the concentration gradient remains shallow, thereby promoting cumulative retention.

Third, looking beyond canonical organelles, the next frontier involves pushing the limits of precision to target suborganelle compartments. Specifically, biomolecular condensates — protein-rich, membraneless assemblies formed by liquid–liquid phase separation — are emerging to be disease-associated[154]. These membraneless compartments are increasingly implicated in cancer, yet are notoriously difficult to target[35,155,156]. Achieving photocatalytic activity within a specific condensate represents a monumental challenge. It demands the rational design of catalysts that not only localize selectively to the condensate but also function compatibly within its distinct physicochemical environment. Encouragingly, through in-depth computational prediction and rational structural modification, certain organic dyes have demonstrated the potential to partition selectively into specific biomolecular condensates[35]. Success in this endeavour could enable precise intervention into pathogenic protein activity at a previously challenging spatial scale.

Another concern relates to photoinduced ROS, specifically $^1O_2$ and superoxide ($O_2^{\bullet-}$), which may cause unwanted toxicity during prodrug activation. Previous studies have shown that $^1O_2$ is a major contributor for light-induced cell death in photodynamic therapy. However, superoxide serves as an endogenous signalling molecule that is generally more biocompatible and less toxic[109,157]. In this regard, several organic dyes have demonstrated low cellular phototoxicity while maintaining excellent photocatalytic performance. For instance, fluorescein and rhodamine B, which barely generate $^1O_2$ (ref. 158), exhibit strong photocatalytic activity in mediating uncaging reactions such as deboronative hydroxylation in living systems[109]. Similarly, silicon rhodamine, a common fluorophore for live-cell super-resolution imaging[159], displays low phototoxicity and holds considerable promise for driving prodrug activation[104,151]. To fully circumvent light-induced ROS, certain metal-based photocages can be harnessed in a strategy known as photoactivated chemotherapy. In this system, the lifetime of excited states of photoactivated chemotherapy compounds is too short lived to sensitize oxygen, and thus ROS generated from those photocages is negligible[81]. In addition, there are hypoxic pathologies such as tumours[160], inflammatory sites[161] and ischaemic regions[162] that naturally attenuate oxygen sensitization. Therefore, although ROS-mediated toxicity warrants consideration,

it cannot preclude implementation of photoactivatable prodrug strategy.

Finally, similar to other phototherapies, the penetration of light in biological tissues presents a fundamental constraint for broader applications. Therefore, the proposed subcellular targeting approach using photoinduced prodrug activation is best suited for superficial or optically accessible pathologies. To address this limitation and broaden clinical applicability, we propose three strategies: replacing exogenous photonic triggers with endogenous bioluminescent systems compatible with genetic encoding[163]; extending activation mechanisms towards deeper-penetrating modalities such as ultrasound[164–166] or X-ray irradiation[167–169]; or leveraging alternative stimulus-responsive chemistries such as bioorthogonal or catalytic uncaging reactions[170–184]. Although these approaches mitigate light-dependent limitations, practical implementation faces the challenges. These challenges include stoichiometric limitations for bioorthogonal uncaging and low activation efficiency for sonochemistry and radiochemistry. Nevertheless, the toolkit for chemical manipulation within living systems is expanding. We anticipate that subcellular (photo)chemistry combined with a prodrug approach will expand the repertoire of druggable targets by exploiting organelle-/compartment-specific localization patterns unique to pathogenic states.

Published online: 20 August 2026

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Acknowledgements

This work was finically supported by the National Natural Science Foundation of China (grant nos. 22377154, 22122706 and 22501297), Noncommunicable Chronic Diseases-National Science and Technology Major Project (grant no. 2026ZD0552800), Major Program of Guangzhou National Laboratory (grant no. GZNL2023A02012), Guangdong Science and Technology Department (no. 2019QN01C125), Guangdong Basic and Applied Basic Research Foundation (grant nos. 2024A1515010721 and 2024B1515040028), Guangzhou Science and Technology Projects (grant no. 2024A04J6478) and Guangdong Provincial Key Laboratory of Construction Foundation (grant no. 2023B1212060022).

Author contributions

M.L. and X.X. researched data for the article and contributed to discussion of content and writing. A.Z and T.Z. conceived the idea of subcellular targeting and contributed to researching data, discussion, writing and reviewing/editing the manuscript before submission.

Competing interests

The authors declare no competing interests.

Additional information

Peer review information *Nature Reviews Chemistry* thanks Marc Vendrell and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.