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Targeting endosomal receptors, a new direction for polymers in nanomedicine

Paulina D. Ramirez-Garcia,^{*ac} Nicholas A. Veldhuis,^{ib} Nigel W. Bunnett^{cd} and Thomas P. Davis^{ib} ^{*e}

In this perspective, we outline a new opportunity for exploiting nanoparticle delivery of antagonists to target G-protein coupled receptors localized in intracellular compartments. We discuss the specific example of antagonizing endosomal receptors involved in pain to develop long-lasting analgesics but also outline the broader application potential of this delivery approach. We discuss the materials used to target endosomal receptors and indicate the design requirements for future successful applications.

10th Anniversary Statement

Congratulations on a decade of excellence. This current contribution is a forward-looking new opportunity for materials scientists in assisting pharmacological solutions to health challenges and builds on the contribution of TPD to the journal going back to the distant days before the journal split into three a decade ago. TPD serving on the Editorial Board of *J. Mater. Chem.* and then *J. Mater. Chem. B* for many years and having published on numerous aspects of materials chemistry and nanomedicine over three decades as an academic. The strength of the journal is its focus on excellence in materials coupled with bioscience, bringing the best of interdisciplinary science to the forefront supported by an outstanding editorial and referee team committed to high quality.

1. Conventional targets explored in nanomedicine

Since the discovery of living radical polymerization, there has been a drive to exploit the control achieved over macromolecular architectures to design and synthesize innovative materials at the nanoscale. In many cases, the application targeted by researchers is the drug delivery field, which has heavily relied on the development of new structures to exploit the enhanced permeability and retention effect (EPR). Under this principle, nanoparticles are postulated to 'escape' the vasculature and accumulate at tumor sites, altering biodistribution and optimizing the toxic effects of cancer medicines to target tumor cells.¹ However, the heterogeneity of tumors and their multiple biological and pathophysiological barriers

indicate that not all tumors benefit from EPR-based nanomedicines. A solution could rely on combination treatments to enhance the EPR effect.²

The EPR effect has inspired many polymer chemists and dominated the field for more than a decade, resulting in a major focus within polymer nanoscience to provide potential treatments for solid tumors, largely to the neglect of other therapeutic opportunities. This approach has been used to justify many polymer-driven studies, where biological testing has focused on *in vitro* studies such as cell association, particle accumulation and nanotoxicity. These isolated *in vitro* studies can be misleading when attempting to translate findings to the clinic or predict their behavior in an *in vivo* setting. Moreover, when we look at the numerous *in vivo* studies conducted, there are still challenges regarding their accuracy in recapitulating true clinical situations.^{3,4} Despite a rapidly growing number of publications demonstrating clever nanocarrier designs with high potential for drug delivery, more or adequate testing continues to hamper the translation of polymeric nanomedicines.

In this short perspective, we aim to extend the vision of polymer scientists interested in therapeutic delivery to new targets beyond solid tumors and to exploit the need for nanoparticle delivery in unique pathophysiological environments to target a whole new range of medical conditions. Specifically, we

^a Dentistry Translational Research Center, New York University College of Dentistry, New York, 10010, USA. E-mail: pr2336@nyu.edu

^b Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, VIC, 3052, Australia

^c Department of Molecular Pathobiology, New York University College of Dentistry, New York, NY 10010, USA

^d Department of Neuroscience and Physiology, Neuroscience Institute, New York University Langone School of Medicine, New York, NY 10010, USA

^e Australian Institute for Bioengineering and Nanotechnology, The University of Queensland, Brisbane, QLD, 4072, Australia. E-mail: t.davis@uq.edu.au

describe the opportunity for intracellular targeting to deliver antagonists to G-protein coupled receptors (GPCRs) that drive pathologies from intracellular compartments – ideal targets for nanoparticle and bioconjugate delivery strategies in cells that constitutively internalize nanoparticulate materials into the endo-lysosomal network. We will introduce GPCRs and their role in pathophysiological processes, explain the role of receptor internalization, and give examples of the many GPCRs available as novel targets for a new approach to intracellular-targeted delivery. We will particularly focus on endosomal GPCRs involved in pain and give published examples that have shown the validity of the approach.

2. G protein-coupled receptors

G protein-coupled receptors (GPCRs) are the largest family of cell-surface receptors that mediate the communication between cells and the external environment. GPCRs respond to a wide variety of stimuli such as hormones, neurotransmitters, paracrine agents, light, odorants, and tastants.⁵ With almost 800 GPCR sequences in the human genome, these essential receptors are involved in diverse physiological and pathophysiological processes. GPCRs are also well-established therapeutic targets that account for more than 30% of drugs in the clinic.^{6,7}

Upon encountering a stimulus (ligand), GPCRs are activated to promote a cellular response by coupling to G proteins. G proteins are formed by a complex of three subunits: α , β , and γ . In a resting state, the α -subunit remains bound to guanosine diphosphate (GDP) and upon ligand binding GPCR stimulation and conformational changes promote G protein engagement with GTP, resulting in. This GDP-GTP exchange is an essential process for the heterotrimeric G protein complex to dissociate from GPCRs, separating into α from $\beta\gamma$ subunits to subsequently stimulate other downstream effector proteins. A key example is activation of the $G_{\alpha s}$ subunit, leading to increased adenylyl cyclase activity and production of the second messenger molecule cAMP, to promote protein kinase and transcriptional endpoints within cells (Fig. 1).⁸

3. Endosomal signaling of receptors

Endocytosis was classically viewed as a critical mechanism for the desensitization and resensitization of receptors, leading to the termination of GPCR signals. In this process, activated receptors are internalized and dissociated from their ligands (desensitization), followed by sorting in endosomes to be recycled and returned to the plasma membrane (resensitization). Alternatively, receptors can also continue through the endosomal maturation pathway and undergo sorting into lysosomes, to terminate signalling *via* proteolytic degradation.⁹ However, it is now also more widely accepted that this organized network of dynamic intracellular membranes can also serve as sites for organization of signaling platforms or complexes. An increasing number of receptors have been reported to elicit endosomal

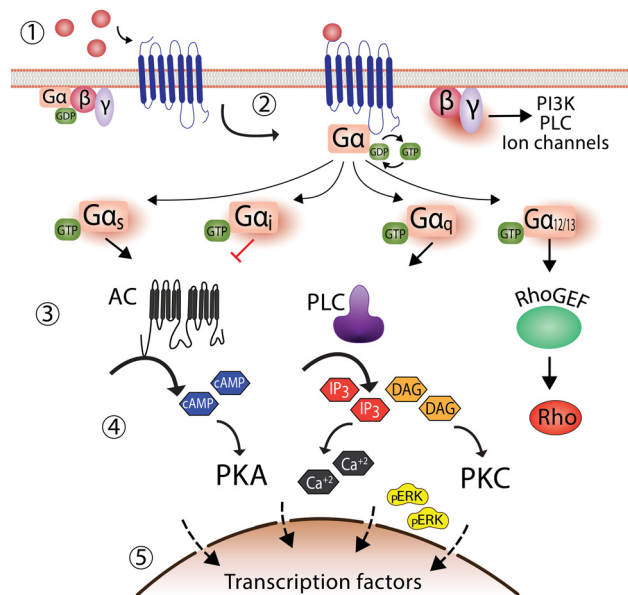


Fig. 1 Classical G-protein dependent signaling of G protein-coupled receptors. (1) In the absence of an agonist, GPCRs remain inactivated, and G-protein is bound to GDP. (2) Following agonist binding, G-protein exchange GDP for GTP, eliciting the dissociation of the G-protein trimeric complex into $G\alpha$ and $G\beta\gamma$, leading to the formation of a complex formed by the agonist bound GPCR and $G\alpha$ subunit. (3) Several effectors are activated depending on the $G\alpha$ subunit recruited. For instance, $G\alpha_s$ activates adenylyl cyclase, while $G\alpha_i$ inhibits it. Similarly, the $G\beta\gamma$ subunit can also activate effectors such as phospholipase C. (4) Activation of effectors increases second messenger molecules, such as cAMP, calcium, and pERK. (5) The outcome is the activation of transcription factors in the nucleus with a concomitant cellular response.

signaling events distinct from those originating at the plasma membrane and regulated by separated mechanisms.^{10–13}

Although this perspective focuses on endosomal GPCRs, it is important to emphasize that endosomal signaling is not an event restricted to GPCRs. In fact, receptor Tyrosine kinases (RTKs) were the first receptor class described to signal from endosomes. First described for the epidermal growth factor receptor (EGFR) and Insulin receptor (IR),^{14,15} it was also later observed that upon nerve growth factor (NGF) stimulation, the tyrosine kinase A receptor (trkA) could also signal from endosomes.¹⁶ Subsequent work reinforced the concept of endosomal signaling by showing that another RTK, the platelet-derived growth factor receptor (PDGFR) could also elicit a biological response from endosomal membranes.¹⁷ Similarly, the Toll-like receptors TLR3, TLR4, and TLR9 are also shown to redistribute into endosomes for optimal innate immune responses.^{18–20}

For GPCRs, initial evidence of endosomal signaling was observed by the activation of the α -factor receptor (Ste2p) in the mating response of *Saccharomyces cerevisiae* yeast.²¹ Later work demonstrated similar endosomal-mediated signaling responses *via* the parathyroid hormone receptor (PTHr) and V2 vasopressin receptor resulted in sustained cAMP production (V2R).^{22,23} The first evidence to suggest that endocytosis can promote acute signaling was demonstrated for the D1

dopamine receptor (DRD1).²⁴ Similarly, studies on the β_2 -adrenergic receptor (β_2 AR) and thyroid-stimulating hormone receptor (TSHR) revealed that unique, sustained signaling profiles were associated with internalized receptors.^{25,26} Regarding pain transmission, the protease-activated receptor 2 (PAR₂) expressed on dorsal root ganglion sensory neurons and the neurokinin 1 receptor (NK₁R) within the spinal cord were the first receptors demonstrated to promote sustained neuroexcitability and nociceptive signals from endosomes.^{27,28}

The discovery of receptors that signal from endosomes has been closely linked to the development of new tools to study these processes. Irannejad and colleagues,³⁴ for example, were the first to use a nanobody that mimics the cognate $G\alpha_s$ protein subunit fused to GFP (Nb80-GFP). This nanobody enabled the direct visualization of activated β_2 AR by live confocal imaging.³⁵ Nb80-GFP showed for the first time that stimulation of the β_2 AR initially promoted $G\alpha_s$ protein recruitment to the plasma membrane, followed by internalization and subsequent recruitment to endosomes.³⁴ Since the development of nanobodies, we now have new biophysical tools that have allowed us to investigate and understand these events in much greater detail,^{29–31,36} helping to establish endosomal signaling as a platform for spatiotemporal regulation of cellular responses.^{32,33}

4. The importance of compartmentalized signaling processes

In last two decades, the biological significance of receptor signaling from distinct locations beyond the plasma membrane has been revealed in much greater detail. This is largely owed to the availability of new, sensitive biophysical tools can simultaneously measure signalling in real time and in different locations, which have demonstrated that shifts in distribution of receptors to discrete subcellular locations (aka receptor compartmentalization) is an essential regulatory process that provides spatial and temporal separation of signals.^{9,37–39} Fig. 2, for example, illustrates how a sustained signalling profile can be promoted exclusively from internalized receptor populations. Indeed, it is hypothesized that cells have evolved this compartmentalization of signals in a spatiotemporal manner, to increase signaling specificity and provide a high order of regulation, to ultimately enable a single receptor to initiate multiple signaling events within a cell.³⁸ This spatial segregation serves to avoid unwanted crosstalk between different signaling cascades, especially since many signaling molecules are shared between different pathways. Beyond the endosomal network, other

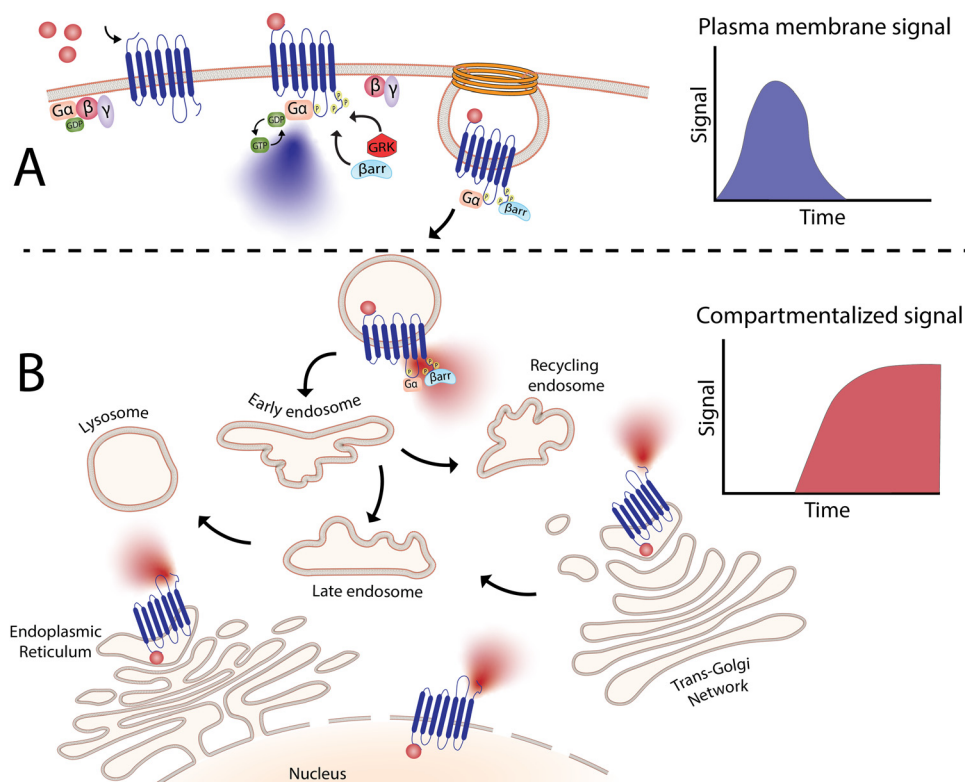


Fig. 2 Compartmentalized signaling of G-protein coupled receptors. (A) Agonist binding results in the formation of a complex between $G\alpha$ and agonist-bound GPCR, giving rise to classical G-protein-dependent signaling. (B) After GPCR activation, receptors are internalized in a process thought to mediate only desensitization and resensitization of receptors. However, signaling molecules can also be recruited into intracellular compartments where receptors can continue to signal. This signaling differs from the signals elicited by plasma membrane receptors in terms of duration and outcome. This spatiotemporal regulation allows receptors to produce highly complex signaling processes where the same receptor can now trigger diverse physiological processes mediated by signals elicited from different locations.

intracellular locations, such as the Golgi apparatus, mitochondria and nuclei are also key membranes for these compartmentalized signaling events. Each site presumably can also promote formation of unique protein complexes to allow receptors to initiate distinct signaling processes to achieve a physiological or disease-relevant outcomes.^{13,40–42} To this end, nanomaterials designed to accumulate in specific organelles have tremendous potential to deliver drugs in intracellular locations of pathophysiological relevance, to “fine-tune” cellular behavior. However, this application of nanomaterials is not only limited to spatial control – as discussed below, temporal regulation through sustained drug release may also be beneficial.

5. Endosomal receptors involved in pain

Cancer has been the focus of nanomedicine for a long time. However, cancer is associated with high levels of pain that are often untreatable, reducing the quality of life of cancer patients tremendously. Each year, 14 million new cancer cases are diagnosed worldwide, with 52–77% of patients reporting pain triggered by the disease or its treatment. These numbers increase to 60–90% for patients with advanced cancer, with as many as 50% of patients manifesting inadequate analgesia.^{43,44} Moreover, chronic pain affects 28.4% of adults in the USA (79.6 million),⁴⁵ with costs fluctuating between \$560 and \$635 billion annually.⁴⁶ In Australia, pain was reported to affect 3.24 million people, with an estimated cost of \$73.2 billion per year.^{47,48} Surveys have identified that 17–30% of adults suffer pain at any given time with increasing prevalence linked to advancing age.^{49–53}

It has been demonstrated that GPCRs can trigger intracellular signals associated with pain transmission, with receptors on its majority described to signal from endosomes.^{37,54} A key exception is the metabotropic glutamate receptor 5 (mGluR5), which show no apparent endosomal signalling and instead signals from the nucleus.^{54,55} A wide variety of receptors, have been reported to signal from other intracellular compartments such as Golgi, mitochondria, and endoplasmic reticulum (Table 1). However, these beyond the scope of this perspective, especially due to the fact that many nonparticulate systems can naturally accumulate within endosomes, even in the absence of ligand-directed nanoparticle uptake.⁵⁶ We therefore focus on GPCRs that are associated with pain transmission and are also known to signal from endosomes – PAR₂, the calcitonin receptor-like receptor (CLR), NK₁R, and the delta (δ) opioid receptor (DOR).⁵⁶ Preclinical work examining these receptors shows promise in the development of improved analgesics.

To provide a non-exhaustive overview of these receptors, PAR₂ is present in pain-sensing (nociceptive) neurons and promotes neurogenic inflammation and pain.^{57,58} This receptor is activated by proteases released after injury and inflammation, plays a role in oral cancer pain,⁵⁹ and has been demonstrated to produce signalling from endosomes in sensory neurons.^{27,60}

Table 1 Compartmentalized GPCR signaling^{56,75,76}

Subcellular localization	Receptor
Endosomes	β ₂ -adrenergic receptor (β ₂ AR) Angiotensin receptor 1 (AT ₁ R) Calcitonin receptor-like receptor (CLR) Calcium-sensing receptor (CaSR) Dopamine D1 receptor (D1DR) Delta opioid receptor (DOR) 5-Hydroxytryptamine receptor 2 (5-HTR2) Mu opioid receptor (MOR) Neurokinin 1 receptor (NK ₁ R) Parathyroid hormone receptor (PTHr) Protease-activated receptor-2 (PAR ₂) Vasopressin type 2 receptor (V2R)
Endoplasmic reticulum	G protein-coupled estrogen receptor 1 (GPR30)
Golgi	β ₁ -adrenergic receptor (β ₁ AR) Dopamine D1 receptor (D1DR) Mu opioid receptor (MOR) Sphingosine-1-phosphate 1 receptor (S1P1R) Thyroid-stimulating hormone receptor (TSHR)
Mitochondria	5-Hydroxytryptamine receptor (5-HTR3 & 5-HTR4) Angiotensin II receptor type 1 (AT ₁ R) Angiotensin II receptor type 2 (AT ₂ R) Cannabinoid type 1 receptor 1 (CB ₁ R) Melatonin MT ₁ receptor (MT ₁ R) Purinoreceptor 1 like receptor (P2Y ₁) Purinoreceptor 2 like receptor (P2Y ₂)

A number of studies have demonstrated that this is of particular relevance to the contribution of PAR₂ endosomal signalling to chronic pain associated with irritable bowel syndrome⁶¹ and also colitis-evoked pain.⁶²

CLR forms a complex with the receptor activity modifying protein 1 (RAMP1).⁶³ When the body is exposed to noxious stimuli such as heat of chemical irritants, activation of sensory neurons leads to release of the neuropeptide calcitonin gene-related peptide (CGRP), which activates CLR/RAMP1 to endothelial cells to facilitating edema and inflammation.⁶⁴ CGRP and CLR/RAMP1 receptors are also involved in migraine pain, and it was recently demonstrated that CLR/RAMP1 activity within the endosomes of Schwann cells contributes migraine and may be a valuable, unique therapeutic target.⁶⁵

NK₁R is predominantly distributed in endothelial cells of the airways, myenteric neurons and also in neurons of the central nervous system. This receptor is activated by the neuropeptide substance P (SP) to elicit plasma leakage, inflammation, and pain transmission.^{66–68} SP release and NK₁R stimulation initiate receptor internalization on spinal neurons.^{69,70} From this location, the receptor elicits an endosomal signal that mediates pain transmission in acute, inflammatory, and neuropathic preclinical pain models.⁷¹

Opioid receptors are expressed in the central, peripheral, and enteric nervous systems, and unlike PAR₂, CLR, and NK₁R, their activation leads to pain relief. There are three classes of opioid receptors: mu (μ, MOR), delta (δ, DOR), and kappa (κ, KOR). These are activated by endogenous opioids, such as enkephalins and endorphins, and by synthetic compounds, like

morphine and fentanyl.⁷² The most commonly used opioids act on the MOR, leading to analgesia and also side effects such as respiratory depression, addiction, and tolerance. The analgesic actions of KOR are mediated by agonists that engage G_{xi} signaling, whereas internalization of the receptor favors the side effects of dysphoria and sedation.⁷³ In inflamed colons, activation of endosomal DOR was found to reduce inflammatory pain in mouse models.⁷⁴

6. Utilising nanoparticle-based drug delivery to target endosomal receptors

The targeting of plasma membrane-localized receptors depends on drugs distributing in such a way that sufficient levels of therapeutic agent remain in the cell exterior and engage with a receptor, before diffusing across membranes and throughout cells. However, several biological barriers make delivery more challenging when the targeted receptor has moved away from the cell surface, and instead, is “hidden” inside the cell. In particular, the therapeutic agent must cross the plasma membrane and sufficiently accumulate within the endosomal network to allow efficacious blockade of the endosomal signaling without disrupting endosomes – a feat that is unlikely, especially for lipophilic small molecules that can indiscriminately distribute across all membranes. In contrast, nanoparticle-based drug delivery may offer significant advantages. Engineering nanomaterials with tunable drug release properties, for example, has been a common approach for delivery of

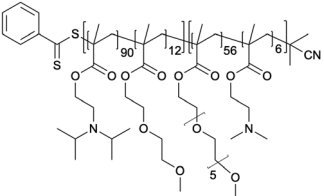
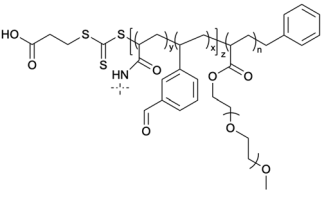
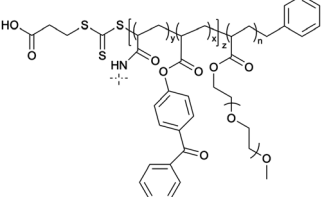
cytotoxic agents to tumors while shielding healthy tissue, as a strategy to improve efficacy and safety profiles of chemotherapeutic agents.² With a focus in this perspective on internalized receptors, these same systems are also advantageous for shielding drug cargo from cell surface receptors and promoting localized delivery and controlled release of cargo, as a strategy to enhance endosomal accumulation of drugs to control endosomal receptors, as is discussed in more detail below (Table 2).

6.1. The use of pH-responsive materials to control internalized receptors

The need to precisely control the spatiotemporal delivery of drugs has led to the development of a wide variety of stimulus-responsive delivery systems that can promote drug release at specific sites when exposed to altered tissue environments specific to disease states.^{79,80} A common feature of pathologies such as cancer or chronic inflammation, for example, include highly localized acidic environments that have been widely exploited *via* application of pH-sensitive drug delivery systems.^{81,82} Interestingly, when considering strategies that may be valuable for selective targeting of endosomal receptors discussed above, the endo-lysosomal network is also a highly acidified micro-environment that can be exploited as a trigger for effective and selective intracellular drug release or accumulation.⁸³

Block copolymer materials synthesized by two-step sequential polymerization by the reversible addition-fragmentation chain (RAFT) method have played an essential role in exploring

Table 2 Drug delivery structures for endosomal delivery of antagonist

Nanoparticle	Polymer/lipid	M_n (g mol ⁻¹)	PDI
DIPMA-AP ⁷⁷ DIPMA-MK3207 ⁶⁵		17 581	1.36
VBA-AP ⁷⁸		115 000	1.22
Benzo-AP ⁷⁸		111 000	1.33
DADLE-LipoMSN-DADLE ⁷⁴	Core: mesoporous silica Coating: liposome assembly 1,2-Dioleoyl-3-trimethylammonium propane:cholesterol:dioleoylphosphatidylethanolamine: 1,2-distearoyl- <i>sn</i> -glycero-3-phosphoethanolamine- <i>N</i> -[amino(polyethylene glycol)-2000]	—	—

the design and development of stimulus-responsive nanoparticulate delivery systems.^{84–86} The chemical diversity of monomers that are utilized as the building blocks of copolymers offers a myriad of possibilities to tune the physicochemical properties of nanoparticles (size, morphology, stability, and surface properties) and subsequently design customized drug delivery systems.⁸⁷ With respect to targeting endosomal receptors, polymeric pH-responsive nanoparticles made with block copolymers that incorporated units of P(PEGMA-*co*-DMAEMA) in the outer hydrophilic shell and pH-sensitive ($\sim pK_a$ 6.1) monomers of 2-[*N,N*-(diisopropylamino)ethyl] methacrylate (DIPMA) within the hydrophobic portion, provides one of the first demonstrations of utilising the acidity of endosomes as a trigger for release of drugs that specifically modulate GPCR activity.⁷⁷ These micellar-based systems were further loaded with the lipophilic NK₁R antagonist, aprepitant, as an approach for targeting the pain-transmitting endosomal pools of the NK₁R (Fig. 3).⁷⁷ These nanoparticles were rapidly internalized by dynamin and clathrin-dependent endocytosis and showed a fast release of aprepitant into endosomes within five minutes of addition *in vitro*. DIPMA nanoparticles abolished the NK₁R endosomal signaling *in vitro*. A single intrathecal dose showed

superior analgesic properties compared to free aprepitant on *in vivo* models of acute, inflammatory, and neuropathic pain.⁷⁷ This work was instrumental in demonstrating the potential of drug delivery systems to target endosomal receptors selectively. These findings were later endorsed using DIPMA nanoparticles to successfully target endosomal CLR/RAMP1 in Schwann cells in preclinical models of migraine pain.⁶⁵

In addition to pH-responsive nanoparticles – a number of other polymeric nanoparticles have been designed⁸⁸ using different mechanisms for pH release, including pH-sensitive crosslinking in nanoparticles and nanostars^{89,90} and hydrolysis of acetylated dextran.^{91,92} Interestingly, nanostars loaded with the NK₁R antagonist, aprepitant, provided sustained release of aprepitant, which produced longer-lasting analgesia than DIPMA nanoparticles,⁷⁸ indicating that the kinetics of release within endosomes plays a vital role in the duration of the analgesic effect.

6.2. Other types of nanoparticles

Mesoporous silica nanoparticles have also been used to deliver DADLE, a DOR agonist. DADLE was incorporated in the core, for endosomal delivery, and on the surface, to provide active

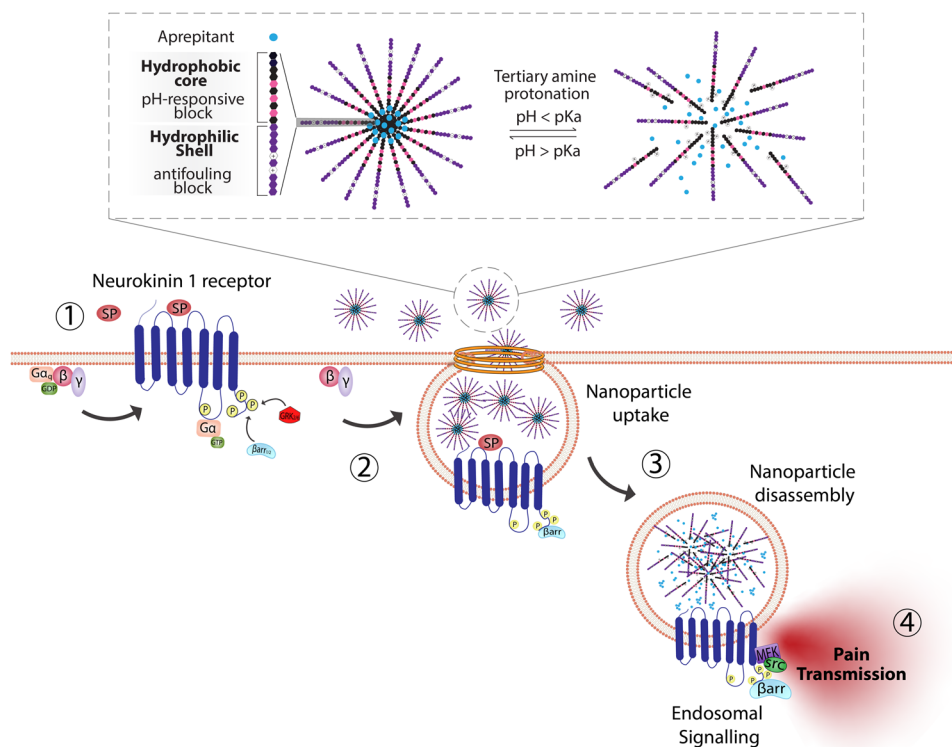


Fig. 3 pH-responsive nanoparticles for endosomal targeting of the NK₁R. pH-responsive nanoparticles were designed using amphiphilic diblock copolymers formed by four monomers. The hydrophobic portion of the diblock copolymer is formed by di(ethylene glycol) methyl ether methacrylate (DEGMA) and the pH-responsive monomer 2-(diethylamino)ethyl methacrylate (DIPMA). The hydrophilic portion is formed by poly(ethylene glycol) monomethyl ether methacrylate (PEGMA) and a positively charged monomer, 2-(dimethylamino)ethyl methacrylate (DEAEMA). The antagonist aprepitant was physically entrapped in the core of the nanoparticles. (1) The NK₁R is activated by substance p (SP) and (2) couples to G-protein, followed by (3) rapid endocytosis. (4) From this location, NK₁R transmits pain. Nanoparticles are passively endocytosed, and the acidity of endosomes triggers the disassembly of nanoparticles and release of aprepitant from the core. Released aprepitant can now antagonize the endosomal signal produced by the NK₁R to decrease pain transmission.

Table 3 Nanoparticles for endosomal targeting of GPCRs involved in pain

Nanoparticle	Diameter	ζ Potential (mV)	Drug	GPCR target
DIPMA-AP ⁷⁷	40.4 $\pm$ 5.1	-0.2 $\pm$ 1.6	Aprepitant	NK ₁ R
DIPMA-MK3207 ⁶⁵	30.7 $\pm$ 1.3	-1.3 $\pm$ 1.6	MK3207	CLR/RAMP1
VBA-AP ⁷⁸	14.5 $\pm$ 1.9	-5.8 $\pm$ 1.0	Aprepitant -NH ₂	NK ₁ R
Benzo-AP ⁷⁸	21.1 $\pm$ 2.6	-2.1 $\pm$ 0.3	Aprepitant- NH ₂	NK ₁ R
DADLE-LipoMSN-DADLE ⁷⁴	206.5 $\pm$ 3.3	29.9 $\pm$ 3.07	DADLE	DOR

targeting toward DOR-positive neurons.⁷⁴ These nanoparticles provided long-lasting inhibition of pain receptors on human colon biopsies and a superior analgesic effect compared to free DADLE in mice models of inflammatory pain.⁷⁴ The properties of the nanoparticles discussed in this perspective are found in Table 3. In addition, the feasibility of dual-release mechanisms for multiple release profiles with pH control has been published,⁹³ exploiting not just nanoparticle degradation but the use of pH-sensitive linkers to release drugs from the nano-scaffolds. Theranostic nanoparticles have also been described to induce changes in MRI signals on pH-induced drug release,⁶² all of which could be utilized for applications involving the release of antagonists to target endosomal signaling.

7. The future of endosomal signaling

The high level of regulation provided by compartmentalized signaling of receptors is an ideal opportunity for nanomaterials to demonstrate their potential to achieve selective drug delivery in pathologies beyond cancer. Here we discussed the potential applicability of nanomaterials to target locations within the cell, with particular focus on endosomes as an important target site within cells. Numerous GPCRs are known to be activated and rapidly internalize into this membrane network and are likely to continue signaling from this location, thus suggesting that endosomal receptors is a unique target location that could significantly benefit from the pH-responsive delivery offered by nanomaterials. Furthermore, it is also important to note that intracellular signaling events differ from those originating at the plasma membrane, which may explain the clinical failure of many drugs that may have limited access to intracellular locations. At present, most therapeutic drugs are designed to target receptors at the cell surface and do not consider sub-cellular locations that are harder to target when testing drugs that allowed to freely distribute within cells or tissues. Hence, altering the intracellular distribution of drugs could be viewed as a distinct and potentially valuable opportunity for nanomaterials, and may also offer a strategy for repurposing approved drugs that previously failed for specific conditions such as pain, where it is now appreciated the neuroexcitation and pain transmission is likely to be driven by receptors that have internalised. A key example is the NK₁R antagonist aprepitant (Emend[®]), which is currently used for treating emesis and nausea but has failed in clinical trials for pain.^{94,95} While there are likely to be many factors for prior failures in pain-specific trials, it is also tempting to speculate that the limited capacity for aprepitant to accumulate in endomes and directly control

endosomal pools of the NK₁R may have also contributed to the lack of success. Together, nanomaterials may have valuable utility beyond their use in exploiting EPR effect, and instead offer potential benefits for targeting trafficking receptors, to “fine-tune” specific cellular processes. It is also noted that endosomes are but one of many distinct membranes within the cell where disease-relevant signaling complexes can form and be targeted. Indeed, a wide variety of receptors, have been reported to signal from compartments such as the Golgi network, mitochondria, and nuclei.^{41,42,54,96} Moreover, since nanomaterials are often endocytosed and retained within the endo-lysosomal network, lysosomes are also potential targets due to their involvement in several diseases classified as lysosomal storage diseases (LSDs). LSDs comprise more than 50 genetic disorders, mostly involving dysfunction of lysosomal hydrolases.^{97,98} Accumulation of macromolecules in endosomes and lysosomes in various LSDs is of potential interest for the delivery of enzyme replacement therapies,^{98,99} where nanomaterials could be of great advantage.

Although we do not fully understand all the nanoparticle-specific characteristics that govern intracellular delivery- *e.g.*, the optimum nanoparticle size and the optimal residence time in the endosomes have yet to be determined. Still, we know that high cellular uptake and localized accumulation in the desired site of action are essential to target unique intracellular signals and achieve efficient drug delivery. We also know that sustained drug release favors prolonged interaction between the drug and the target. This localized and sustained release of drugs is one of the main contributors to the superior biological actions of drug-loaded nanoparticles.

Lastly, we have exemplified the potential of nanoparticles for intracellular targeting using pain as the disease of interest. For this disease setting, pH was exploited as the environmental stimulus of choice to trigger drug release in endosomes where nociceptive receptors reside. Pain served as an example of the benefits of guiding the design of nanoparticles by a specific disease mechanism. Still, the same principle can be applied to many other diseases driven by intracellular receptors. If we first identify the target and understand the disease environment. Then, we can fully exploit the potential of nanomaterials and design nanoparticles with the specific properties required to deliver drugs to our target of interest. This disease mechanism-based strategy may help us achieve the selectivity necessary to improve drug efficacy to either design new therapies or improve the existing ones.

Conflicts of interest

There are no conflicts to declare.

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