Roles of glycosaminoglycans as regulators of ligand/receptor complexes

Robert G. Smock and Rob Meijers

European Molecular Biology Laboratory (EMBL), Notkestrasse 85, 22607 Hamburg, Germany

Glycosaminoglycans (GAGs) play a widespread role in embryonic development, as deletion of enzymes that contribute to GAG synthesis lead to deficiencies in cell migration and tissue modelling. Despite the biochemical and structural characterization of individual protein/GAG interactions, there is no concept available that links the molecular mechanisms of GAG/protein engagements to tissue development. Here, we focus on the role of GAG polymers in mediating interactions between cell surface receptors and their ligands. We categorize several switches that lead to ligand activation, inhibition, selection and addition, based on recent structural studies of select receptor/ligand complexes. Based on these principles, we propose that individual GAG polymers may affect several receptor pathways in parallel, orchestrating a cellular response to an environmental cue. We believe that it is worthwhile to study the role of GAGs as molecular switches, as this may lead to novel drug candidates to target processes such as angiogenesis, neuroregeneration and tumour metastasis.

1. The concept

Glycosaminoglycans (GAGs) are a class of long unbranched polymers of amino and uronic sugars. Many extracellular matrix proteins and receptors bind GAGs, and they are also involved in forming protein–protein complexes [1,2]. GAG molecules are very acidic, and they bind proteins on positive patches often delineated by clusters of arginines and lysines that surround the sugar-associated sulfate groups. When a GAG polymer binds to the positive patch, it neutralizes the protein surface and may facilitate or enhance binding of a protein partner. On the cell surface, the GAG polymer can act as an activator that helps to assemble a receptor/ligand complex (figure 1). There are also receptor/ligand complexes that bind through surfaces that have complementary charges. In this case, the GAG polymer binds to the partner that has a positively charged patch, and it may prevent binding of the partner with a negatively charged patch. The GAG polymer acts as a repressor, inhibiting formation of this specific complex. In addition, combining the roles described above for repressor and activator, binding of the GAG polymer might favour formation of one complex over another, in which case it acts as a selector. There is yet another scenario, where long-chain GAG polymers can string together multiple proteins with positively charged patches. The GAG polymer acts as a concatenator, bringing multiple receptor/ligand complexes together on the cell surface (figure 1).

The mediation of interactions between receptor and ligand through GAG polymers is clearly manifold, and can be categorized as individual molecular switches. The sulfation pattern on GAGs can vary extensively, creating an enormous variety that could be exploited by specific ligand/receptor pairs. Binding studies using chips coated with different GAG families have indicated that individual ligand/receptor pairs may interact stronger with certain GAG families [3], but there is also a lot of cross-reactivity. It can, therefore, be assumed that a singular GAG polymer may interact with different ligand/receptor pairs, leading to a coordinated response between different signalling systems. To our
knowledge, these connections are underexplored but they may be crucial in understanding how different ligand/receptor systems are linked.

GAG polymers can be released into the extracellular matrix (ECM), creating a local chemical environment that will affect neighbouring cells. In addition, cells can present proteoglycans on the cell surface such as glypicans and syndecans, which carry large GAG polymers attached to the ectodomain. As a migrating cell passes through the ECM created by surrounding tissues, it may encounter a shift in the GAG composition that will affect the receptors present on the migrating cell. Some receptors may become blocked and inhibited, other will be activated, and the cell may also accumulate GAG components and carry them along as they migrate further. A shifting response to the GAG composition in the ECM thus provides a rapid mechanism to influence processes within the migrating cell, a mechanism that is much faster than the endocytosis and exocytosis of receptors. A receptor may be present on the surface of a cell, but it is not responsive until it encounters the right GAG composition. It is striking that many systems involved in tissue remodelling and cell migration are regulated by GAG polymers. Based on recent structural characterizations of these systems, we will describe the different GAG switches that we have so far encountered.

2. GAG activators mediate formation of signalling complexes

Fibroblast growth factors (FGFs) are morphogens that activate signalling involved in angiogenesis and tissue remodelling through binding to FGF receptors (FGFRs) [4]. Heparan sulfates (HSs) play an essential role in these processes [5,6]. Moreover, the heparan sulfate proteoglycan (HSPG) syndecan participates in the internalization and endosomal sorting of FGFR in an FGF-dependent manner [7]. As a biophysical basis for these activities, HS was found to mediate oligomerization of FGF1 [8]. A larger assembly of FGF2, FGFR1 and HS was also observed in a symmetric complex with stoichiometry of 2:2:2 [9]. HS is required as a scaffold for an even larger (FGF:FGFR$_2$)$_2$ assembly, such as that of (FGF23:FGFR1:α-Klotho)$_2$ [10]. Taken together, the (FGF:FGFR$_2$)$_2$ assembly forms a large and contiguous positively charged groove bridged by HS (figure 2a). Accordingly, the most highly sulfated chains of HS have the greatest impact on eliciting FGF2/FGFR-mediated ERK1/2 signalling [12], which may relate to tissue specificity of sulfation patterning in the response to morphogens for both FGF2 [12] and FGF1 [13]. HS is an activator of the molecular assembly of FGF with FGFR and consequent activation of downstream intracellular signalling.

In axon guidance, binding of the guidance cue Slit to the Robo receptor family provides a chemorepulsive signalling mechanism that guides the directional movement of axonal growth cones across the midline. The second leucine-rich repeat domain of the family of Slit proteins contains a relatively flat Arg and Lys-rich C-terminal cap that facilitates HS binding [14–16]. HS forms a ternary complex with Slit-Robo that strengthens the cue–receptor interaction, demonstrating HS activation for chemorepulsive circuit response. Consistent with this scheme, mutations in Slit2 that disrupt HS binding cause loss of the biological activity of chemorepulsive growth cone collapse using axons cultured from Xenopus eye primordia [14]. These positively charged residues are conserved [14] and are also implicated in HS binding to Slit3 [2].

Another HS activator is found in the binding of Sonic hedgehog (Shh), which is also involved in the migration of commissural axons across the midline [17]. The Hedgehog (Hh) guidance cue interacts with several receptors, perhaps most centrally with Patched to relieve inhibition of Smoothed for pathway activation [18,19]. Hh forms a complex with Ihog, an insect co-receptor that potentiates pathway activation and allows stronger binding of Hh to Ihog plus Patched than to either Ihog or Patched alone [20]. HS enabled co-crystallization of Hh–Ihog [21]. While HS is not well ordered in this structure, superposition of the Shh–HS crystal complex reveals that the positively charged HS-binding groove in Hh is

---

**Figure 1.** GAGs as extracellular switches in ligand/receptor complex formation. Schematic of GAG polysaccharides (as strings attached to proteoglycans embedded in the membrane, in red) regulating the formation of ligand/receptor complexes. Ligand-induced receptor pairing leads to a signal across the cell membrane that can trigger cytoskeleton reorganization and a transcriptional response. GAG chains act as an activator when the formation of a ligand/receptor complex is facilitated. GAG chains that block the ligand-binding site on a receptor act as a repressor. Combining the function of activator and repressor, GAG chains can favour a certain ligand/receptor complex, acting as a selector. When a GAG chain is of sufficient length, it can act as a concatenator that strings receptors or ligands together at the cell surface.
contiguously extended across the binding interface to Ihog (figure 2b) [21,22].

3. GAG repressors disrupt signalling complexes

Receptor protein tyrosine phosphatases (RPTPs) act in the repulsion of axonal growth cones from the midline in embryonic development by interacting with the Slit (guidance cue)/Robo (receptor) signalling system [23], for instance, by binding of Drosophila Robo3 and RPTP69d [24]. Moreover, RPTPs acts in the formation of excitatory synapses [25,26]. One such complex is mediated by receptors RPTPα and TrkC, which interact across the synaptic cleft between neurons [27]. The structure of the RPTPα–TrkC binding interaction reveals overlap and binding competition with the RPTPα–HS interaction [1]. In the RPTPα–TrkC assembly, HS demonstrates a biophysical basis of a repressor through competitive binding at the RPTPα molecular surface (figure 3a).

Hedgehog interactions also comprise HS repressors. The coreceptor Hip binds Shh at its HS binding site, indicating a major steric clash when both HS and Hip structures are superimposed that is incompatible with both binding simultaneously (figure 3b) [22,29]. A similar scenario is observed for the Shh–Cdo complex, whose formation was not observed in the presence of HS (figure 3c) [20,22].

4. GAG polymers can act as concatenators in signalling, connecting different signalling complexes within the pathway

Remarkably, HS and chondroitin sulfate (CS) are both ligands for RPTPα and have opposing effects on RPTPα behaviour [28,30,31]. HS oligomerizes RPTPα in solution and HSPGs colocalize with RPTPα on sensory neurons and promote their extension. Whereas HS acts as a concatenator, CS that has a comparable chain length inhibits RPTPα oligomerization, and inhibits neuronal extension (figure 4a) [28].

GAG interaction within the Shh pathway also illustrates how GAG can regulate Hh function as a concatenator.
A recent crystal structure of Shh reveals a characteristic Arg- and Lys-rich surface patch that binds HS and CS, and, given sufficient chain length such as found in the HSPG glypican-3, enables multimeric assembly of Shh along the HS polymer (figure 4b) [22]. As in most Hh structures, calcium and zinc cations shield potentially repulsive interactions between acidic residues and HS and invert the surface electrostatic potential at these sites.

5. A candidate GAG switch for netrin receptor selection in axon guidance

The guidance cue netrin acts as an attractant [32] or repellent for axonal growth cones [33], and its depletion has been linked to apoptosis [34]. How the same guidance molecule can trigger such diverse cellular responses has been under intense investigation. Netrin can bind to a diverse set of cell surface receptors, including DCC [35], UNC5 [33], DSCAM [36], amyloid precursor protein [37] and Cd146, which is involved in angiogenesis [38]. Netrin triggers different signals depending on the receptors present on the cell surface. This has been observed in the turning responses of axons that either contain DCC alone (causing chemoattraction) [35], or both DCC and UNC5 (causing chemorepulsion) [33] (figure 5).

Netrin function has also been associated with HS. In particular, HS affects DCC function as an attractant [42] and forms a complex with netrin and DCC [43]. Recently, it has been shown that a glypican HSPG (Lon2) functions in axon guidance through netrin signalling in *Caenorhabditis elegans* [44].
A microarray containing different forms of HS further indicates that netrin favours the binding of some HS varieties over others [45], suggesting that there may be specific interactions between netrin and HS that affect netrin signalling.

Multiple binding sites between netrin and DCC demonstrate mechanisms of netrin-mediated DCC homodimerization [39,40]. Based on site-specific mutants of netrin and their influence on axonal turning in the presence of DCC alone or in combination with UNC5, it was proposed that DCC and UNC5 share a common binding site on netrin-1 that lies between domains EGF-1 and EGF-2 [40]. UNC5 and DCC would compete for binding at this site, allowing a DCC/DCC homodimer or a UNC5/DCC heterodimer to form (figure 5). The UNC5 binding site on netrin-1 was confirmed by complementary biophysical studies using domain deletions and mutants [41]. The most important residues on netrin that determine both DCC and UNC5 binding include a cluster of five conserved arginines, and mutation of two of them (Arg349 and Arg351) abolishes binding of both DCC and UNC5 [40,41]. Interestingly, these arginines bind to four sulfate ions in the crystal structure of the netrin–DCCFN5FN6 complex [40]. The cluster of sulfate is at such a close range that it resembles a unit of HS. Indeed, HS can be fitted using the sulfate ion cluster as a guide. It therefore seems likely that HS acts as a switch that alters the binding competition of DCC and UNC5 for netrin and modifies the circuit of axonal attraction and repulsion. However, a precise mechanistic role has not yet been depicted of HS as a switch for netrin receptor selection, and is a promising area of future investigation.

6. GAG circuit complexity, crosstalk and regulation in a broader physiological context

Data mining has uncovered at least 435 human proteins that interact with HS alone. The most prominent enrichment of gene ontology terms are found in cell–cell signalling, development, cell proliferation and immunoresponse [46], which are dependent on complex signal processing behaviour from a mixture of external cues. HSPGs in the membrane-bound extracellular domain of cells are increasingly appreciated for their role in signalling mechanisms and axon guidance of diverse organisms [47]. Modulation of cue–receptor engagements by HS, and restructuring of the sulfation pattern on the extracellular surface [48], suggest clues for how cells respond to the appropriate signals in a complex milieu of extracellular binding.

Figure 5. GAG selector. HS may modulate the selection of netrin receptors in axon guidance signalling. Human netrin-1 (PDB: 4URT) is shown with electrostatic surface potential and three labelled binding sites for the receptors DCC and Unc5 [39 – 41]. Positive surface charge clusters (blue patches) and series of crystallographic sulfate ions (orange spheres) suggest putative HS binding at sites 1 and 2 (marked with *). Moreover, DCC and Unc5 appear to be in binding competition at an Arg-rich, sulfate-binding region of netrin site 2, suggesting a model in which HS acts in netrin receptor selection and axon guidance fates.
partners. Different GAGs may favour different cue/receptor engagements, and the presence of a particular GAG in a confined area may amplify the effect of specific cues and thus regulate certain migration patterns. The so-called sugar code for axon guidance may thus be linked to the regulation of cue/receptor complexes [49–51].

In Shh signalling, the Patched receptor also binds to Shh overlapping the same molecular interface as HS, Hip and Cdo [20,52,53], which may, in part, explain the competition among these proteins for Shh and signalling attenuation by mammalian HSPG Gpc-3 [54]. The HS-binding Arg/Lys surface residues in Shh serve as a crucial site with elaborate HS switching circuitry. Mutation of these residues leads to phenotypes associated with defective binding to HS, reduced Shh multimers, defective binding to Patched, weak signalling and downstream gene transcription activity, and developmental disorder [55–59]. Breaking the myriad interactions by mutation of the HS-binding hotspot on Hedgehog seems difficult to associate with any single causal factor, underscoring the perspective of a GAG-controlled integrated circuit.

Furthermore, the same biophysical mechanisms of GAG repression and activation in signalling are also observed in a variety of other extracellular interactions: for example, trans-cellular receptor binding in synapse formation and in interactions among extracellular matrix proteins. HS, but not CS, concatenates RPTPα and RPTPβ in this interaction. HS seems to provide a contextual switch between different biological activities in neuronal extension (RPTPα–TrkC; HS repressor) (figure 6a), while HS represses RPTPα and TrkC interaction. HS seems to provide a contextual switch between different biological activities in neuronal extension (RPTPα clustering; HS concatenator) and stable synaptic formation (RPTPα–TrkC; HS repressor) (figure 6a).

Larger, more complex circuits and elaborate signal processing behaviour may also emerge from a more complete understanding of extracellular protein interactomes and their modulation by GAGs. Thrombospondin-1 (Tsp-1) is an extracellular protein that binds a wide array of matrix proteins, guidance cues, receptors and proteases. Of more than 80 interaction partners, at least 10 are influenced by HS [60]. Accordingly, Tsp-1 binds the HSPG co-receptors syndecan and glypicican [61] in addition to extracellular matrix CSPGs aggrecan and versican [62]. Many of the Tsp-1 interactions are inhibited by HS, suggesting that HS provides control over the linkages among membrane and extracellular components [60]. For instance, binding of HS blocks the interaction of Tsp-1 with bFGF (FGF2) [63]. Combined with the scheme of HS-mediated assembly of FGF2 with FGFR-1 (HS activator in figure 2a), and the variety of interactions mediated with Tsp-1 that are also influenced by HS, Tsp-1 appears to provide a mechanistic platform for context-dependent crosstalk among signalling systems (figure 6b). The biophysical basis of interaction on Tsp-1 is through HS binding to the Tsp-1 N-terminal laminin G-like domain in an arginine-rich surface patch [64]. Furthermore, HS homodimerizes Tsp-1 G-like domains in alternate configurations, suggesting orientational plasticity among HS-mediated Tsp-1 interactions [65].

The presentation of HSPGs in extracellular communication networks involves regulated expression of HSPGs themselves and extracellular enzymes that modify GAGs, either by cleaving a GAG from its core protein or altering its pattern of sulfation. Knockout studies on biosynthetic enzymes involved in GAG production in C. elegans have shown a strong effect on the migration behaviours of neurons [66] and their axons [67]. A marked difference in the natural expression of an HS-degrading enzyme, HPSE, was observed in differentiating versus proliferating human olfactory epithelium cells [68], and HPSE has been found to alter Shh and Wnt signalling in human medulloblastoma cells [69]. RNAi-mediated knockdown of extracellular enzymes that modulate HS sulfation patterning, Sulf1 (removes sulfate from HS) and Hs6st (transfers sulfate to HS), have opposing effects on neurotransmission. They result in misregulation of HSPGs such as glypicican (Dlp) and syndecan, high abundance of the guidance cues Wnt and Bmp, and impaired endosomal cycling with the Wnt receptor Frizzled [70,71] in a process that is essential for Wnt signalling [72,73]. HSPGs assemble lipidated, multimeric Shh on the surface of Hh-producing cells [74] and recruit Scube2 for Shh processing and shedding in a manner that is both HS sulfation-dependent and cell-dependent [75]. Understanding the biological reality of systems-level GAG circuits is further challenged by the finding that not all HSPGs behave equally. Hedgehog and Wnt signalling regimes may be either stimulatory or inhibitory in relation to co-expression of a variety of different HSPGs, indicating HSPG specialization that also involves contribution of the protein core [76,77].

7. Evolutionary selection of GAG switch variants

The evolution of the role of GAG switch variants within a ligand/receptor protein family further emphasizes how they can adapt the receptor signalling response to the environment. In the Hh signalling pathway, HS acts as an activator for interaction of insect Ihog and Hh (figure 2b). By contrast, HS serves as a repressor for mammalian Cdo with Shh (figure 3c). Remarkably, the relevant binding regions of these receptors (Ihog Fn1–Fn2 and Cdo Fn2–Fn3) share common ancestry
but diverge in HS circuit modification [20]. Cdo is an HS-binding protein and retains the positively charged HS binding site of Ihog (figure 7a) [78]. However, while Cdo retains the HS binding site in Fn2, its role in mediating interaction is not yet clear, and Cdo has separated its HS and Hh binding sites to different domains. In a mode of Hh binding that is distinct from Ihog, the adjacent Fn3 domain of Cdo binds Shh via the Shh–HS binding site. Assembly of Shh–Cdo is stable in the absence of HS and is structurally incompatible with HS [20]. Therefore, the role of HS in Hh–Ihog/Cdo interaction has undergone evolutionary divergence as an activator in insects and a repressor in mammals.

Evolutionary selection among other GAG switch variants has also been observed. Chemoattraction in the migration of leucocytes is mediated by the binding of CXCL chemokine isoforms to the GPCR receptor CXCR4. The CXCLγ isoform contains a C-terminal motif rich in Lys residues that binds sulfated tyrosine residues of CXCR4 and acts as an inhibitory element that does not result in chemotactic signalling [79]. In the presence of HS, CXCLγ remains bound to CXCR4 and activates signalling, apparently in a conformation that relieves C-terminal inhibition (figure 7b). Alternatively, the CXCL12α isoform has a C-terminal truncation of the Lys-rich region and activates the CXCR4 receptor without requirement for HS to relieve inhibition [79]. The evolutionary selection of isoforms with simple genetic adjustment provide for both HS-dependent (activator) and HS-independent (non-switching) circuits. A related scenario is seen in the HS-dependent trans-synaptic clustering of the HSPG Gpc4 with LRRTM4, but not with its isoform LRRTM2 [80].

8. The potential of GAGs as drug targets

The capacity of sulfated moieties such as HS to function as switches of biological interaction raises their potential for therapeutical intervention. An important question that has to be answered in the future is whether the GAG interactions are specific for particular receptor/ligand complexes. If these interactions are generic, many processes will be linked by a common GAG pool and it will be difficult to identify a particular GAG as a drug target. However, there are several indications that this is not the case, and that GAG specificity is important. The knock-out of certain GAG-modifying enzymes has an effect on specific signalling elements [69], indicating that particular modifications of GAGs will affect only certain signalling pathways. There is also a GAG derivative drug on the market that acts as an anticoagulant with relatively small side effects. Fondaparinux is a pentasaccharide that selectively inhibits a serine endopeptidase Xa [81]. Although it is similar in structure to HS, fondaparinux does not seem to affect all GAG-related pathways. Identification of specific GAGs targeting a particular signalling pathway could, therefore, lead to the development of small molecule drugs that affect protein–protein interactions. Since most of these GAG molecules are biocompatible, toxicity will not be an issue even at high doses. Heparin has been used to treat pre-eclampsia for decades. A cocktail of low-molecular-weight
GAGs may not have been very effective [82], but it showed few side effects. Detailed investigations into the relation between GAG structure and its interactions with specific ligand/receptor complexes will benefit drug discovery and may also reveal which components of signalling pathways are linked by the use of specific GAGs.

9. Concluding remarks

It has become evident that GAGs play a central role in tissue development, yet it remains a challenge to decipher the GAG code. In vivo investigation of the role of certain GAGs through the knockout of GAG synthesizing enzymes shows many developmental effects, yet lacks mechanistic clarity. Conversely, reductionist experiments of specific GAG complexes provide detailed mechanistic insights but may not be able to recapitulate full biological consequence. Extracellular signalling may be modelled systematically as directional networks and circuits that integrate such codependencies [48,83]. Extending the metaphor of circuitry to a realistic, mechanistic model in cell guidance is appealing because it has the potential to explain complex signal processing behaviour from discrete, modular parts that can be verified by the reductionist experimental investigation of molecular structure and binding modes [84]. Genetic circuit models comprising logic gates, such as those representing transcriptional activators and repressors, recapitulate downstream transcriptional regimes with altered cell behaviour [85,86]. Accordingly, the representation of GAG circuit motifs as extracellular activators and repressors of specific cue–receptor engagements in the ‘front line’ of environmental sensing may expand on an understanding of signal integration. Moreover, the capacity of truncated GAG chains to function as extracellular switches holds great promise for therapeutic intervention in neural repair and tumour metastasis. A better understanding of the signalling modules affected by GAGs may inform a more sophisticated design of GAG-derived drug candidates.

Data accessibility. This article has no additional data.

Competing interests. We declare we have no competing interests.

Funding. R.G.S. was supported by the European Research Council (ERC) under a Horizon 2020 MSCA-IF (702346).

References

1. Coles CH et al. 2014 Structural basis for extracellular cis and trans RPTPα signal competition in synaptogenesis. Nat. Commun. 5, 5209. (doi:10.1038/ncomms6209)

2. Condac E et al. 2012 The C-terminal fragment of axon guidance molecule Slit3 binds heparin and neutralizes heparin’s anticoagulant activity. Glycobiology 22, 1183–1192. (doi:10.1093/glycob/cws087)

3. Griffin ME, Hsieh-Wilson LC. 2013 Synthetic probes of glycosaminoglycan function. Curr. Opin. Chem. Biol. 17, 1014–1022. (doi:10.1016/j.cbpa.2013.03.015)

4. Lecaudy V, Cakan-Akdogan G, Norton WH, Gilmour D. 2008 Dynamic Fgf signaling couples morphogenesis and migration in the zebralight lateral line primordium. Development 135, 2695–2705. (doi:10.1242/dev.025981)

5. Lin X, Buff EM, Perrimon N, Nichelson AM. 1999 Heparan sulfate proteoglycans are essential for FGF receptor signaling during Drosophila embryonic development. Dev. Camb. Engl. 126, 3715–3723.

6. Mahtook K et al. 2006 Heparan sulphate proteoglycans are essential for the myeloma cell growth of EGF-family ligands in multiple myeloma. Oncogene 25, 7180–7191. (doi:10.1038/sj.onc.1209699)

7. Zimmermann P et al. 2005 Syndecan recycling is controlled by syntxin-PIP2 interaction and Arf6. Dev. Cell 9, 377–388. (doi:10.1016/j.devcel.2005.07.011)

8. Brown A, Robinson CJ, Gallagher JT, Blundell TL. 2013 Cooperative heparin-mediated oligomerization of fibroblast growth factor-1 (FGF1) precedes recruitment of FGFFR2 to ternary complexes. Biophys. J. 104, 1720–1730. (doi:10.1016/j.bpj.2013.02.051)

9. Schlessinger J, Plotnikov AN, Ibrahim OA, Eliseenkova AV, Yeh BK, Yazon A, Linhardt RJ, Mohammadi I. 2000 Crystal structure of a ternary FGF-FGFR-heparin complex reveals a dual role for heparin in FGF binding and dimerization. Mol. Cell 6, 743–750. (doi:10.1016/S1097-2765(00)00073-3)

10. Chen G et al. 2018 α-Klotho is a non-enzymatic molecular scaffold for FGF23 hormone signalling. Nature 553, 461–466. (doi:10.1038/s41586-2014-25451)

11. Baker NA, Sept D, Joseph S, Hotl MI, McCammon JA. 2001 Electrostatics of nanosystems: application to microtubules and the ribosome. Proc. Natl Acad. Sci. USA 98, 10 037–10 041. (doi:10.1073/pnas.1010943257)

12. Hussain S-A et al. 2013 Cooperative heparin-mediated oligomerization of fibroblast growth factor-1 (FGF1) precedes recruitment of FGFFR2 to ternary complexes. Biophys. J. 104, 1720–1730. (doi:10.1016/j.bpj.2013.02.051)

13. Ngan TKN, Raman K, Tran VM, Kuberan B. 2011 Investigating the mechanism of the assembly of FGF1-binding heparan sulfate motifs. FEBS Lett. 585, 2698–2702. (doi:10.1016/j.febslet.2011.07.024)

14. Hussain S-A et al. 2006 A molecular mechanism for the heparan sulfate dependent signalization of Slit–Robo signaling. J. Biol. Chem. 281, 39 693–39 698. (doi:10.1074/jbc.M609384200)

15. Morlot C, Thielens NM, Ravelli RBG, Hemrika W, Romijn RA, Goss P, Cussack S, McCarthy AA. 2007 Structural insights into the Slit–Robo complex. Proc. Natl Acad. Sci. USA 104, 14 923–14 928. (doi:10.1073/pnas.0705310104)

16. Howitt JA, Clout NJ, Hohenester E. 2004 Binding site for Robo receptors revealed by dissection of the leucine-rich repeat region of Slit. EMBO J. 23, 4406–4412. (doi:10.1038/sj.emboj.7600446)

17. Belgacem YH, Hamilton AM, Shim S, Spencer KA, Borodinsky LN. 2016 The many hats of sonic hedgehog in nervous system development and disease. J. Dev. Biol. 4, 35. (doi:10.3930/jdb.2040035)

18. Qi X, Schmiege P, Coutavas E, Wang J, Li X. 2018 Structures of human Patched and its complex with native palmitoylated Sonic hedgehog. Nature 560, 128–132. (doi:10.1038/s41586-018-0308-7)

19. Song G, Qian H, Cao P, Zhao X, Zhou Q, Lei J, Yan N. 2018 Structural basis for the recognition of Sonic Hedgehog by human Patched1. Science 361, eaas8935. (doi:10.1126/science.aas8935)

20. McLellan JS, Zheng X, Hauk G, Ghirlanda R, Beachy PA, Leahy DJ. 2008 The mode of Hedgehog binding to Ihog homologues is not conserved across different phyla. Nature 455, 979–983. (doi:10.1038/nature07338)

21. McLellan JS, Yao S, Zheng X, Geisbrecht BV, Ghirlanda R, Beachy PA, Leahy DJ. 2006 Structure of a heparin-dependent complex of Hedgehog and Ihog. Proc. Natl Acad. Sci. USA 103, 17 208–17 213. (doi:10.1073/pnas.0606738103)

22. Whalen DM, Malinauskas T, Gilbert RJ, Siebold C. 2013 Structural insights into proteoglycan-shaped Hedgehog signaling. Proc. Natl Acad. Sci. USA 110, 16 420–16 425. (doi:10.1073/pnas.1310097110)

23. Sun Q, Bahri S, Schmid A, Chia W, Zinn K. 2000 Receptor tyrosine phosphatases regulate axon guidance across the midline of the Drosophila embryo. Dev. Camb. Engl. 127, 801–812.

24. Oliva C et al. 2016 Regulation of Drosophila brain wiring by neuropil interactions via a Slit-Robo-RPTP signalling complex. Dev. Cell 39, 267–278. (doi:10.1016/j.devcel.2016.09.028)

25. Dunah AW, Hueske E, Wyszynski M, Hoogenaar CC, Jaworski J, Pak DT, Simonetta A, Liu G, Sheng M. 2005 LAR receptor protein tyrosine phosphatases in the development and maintenance of excitatory
38. Tu T et al. 2015 CD146 acts as a novel receptor for netrin-1 in promoting angiogenesis and vascular development. Cell Res. 25, 275 – 287. (doi:10.1038/cr.2015.15)

39. Xu K et al. 2014 Neural migration. Structures of netrin-1 bound to two receptors provide insight into its axon guidance mechanism. Science 344, 1275 – 1279. (doi:10.1126/science.1252149)

40. Finzi Li et al. 2014 The crystal structure of netrin-1 in complex with DCC reveals the bifunctionality of netrin-1 as a guidance cue. Neuron 83, 839 – 849. (doi:10.1016/j.neuron.2014.07.010)

41. Grandin M et al. 2016 Structural decoding of the Netrin-1/UNCs interaction and its therapeutic implications in cancers. Cancer Cell 27, 173 – 185. (doi:10.1016/j.ccell.2016.01.001)

42. Bennett KL, Badshah J, Youngman T, Rodgers J, Greenfield B, Arruffo A, Limusis PS. 1997 Deleted in colorectal carcinoma (DCC) binds heparin via its fifth fibronectin type III domain. J. Biol. Chem. 272, 2946 – 2956. (doi:10.1016/j.jbc.2002.01.002)

43. Geisbrecht BV, Dowd KA, Barfield RW, Longo PA, Levy DJ. 2002 Netrin is a ligand-gated association between cytoplasmic domains of UNC5 and DCC and UNC5 and mediates interactions between DCC and heparin. J. Biol. Chem. 278, 32 561 – 32 568. (doi:10.1074/jbc.M203094200)

44. Blanchette CR, Perras PN, Thackeray A, Benard CY. 2015 Glycans of UNC5 and DCC bind netrin-1 via their fundamental interaction. PLoS Biol. 13, e1002183. (doi:10.1371/journal.pbio.1002183)

45. Shipp EL, Hsieh-Wilson LC. 2007 Profiling the sulfation specificities of glycosaminoglycan interactions with growth factors and hematocytic proteins using microarrays. Chem. Biol. 14, 195 – 208. (doi:10.1016/j.chembiol.2006.12.009)

46. Ott A, Wilkinson MC, Fering DG. 2011 A systems biology approach for the investigation of the heparin/heparan sulfate interaction. J. Biol. Chem. 286, 19 892 – 19 904. (doi:10.1074/jbc.M111.228114)

47. Lee J-S, Chien C-B. 2004 When sugars guide axons: insights from heparin sulfate proteoglycan factors for commissural axons in the embryonic spinal cord. Cell 78, 425 – 435. (doi:10.1016/S0092-8674(94)00421-9)

48. Hong K, Hinck L, Nishiyama M, Poo MM, Tessier-Lavigne M. 1994 Netrins are diffusible chemotropic factors for commissural axons in the embryonic spinal cord. Cell 78, 425 – 435. (doi:10.1016/S0092-8674(94)00421-9)

49. Keino-Masuk M, Musa M, Hinck L, Leonard ED, Chan SS, Culloty JG, Tessier-Lavigne M. 1996 Deleted in colorectal cancer (DCC) encodes a netrin receptor. Cell 87, 927 – 941. (doi:10.1016/S0092-8674(00)00804-1)

50. Liu G, Li W, Wang L, Kar A, Guan K-L, Rao Y, Wu YJ. 2009 DSCAM functions as a netrin receptor in commissural axon pathfinding. Proc. Natl Acad. Sci. USA 106, 2951 – 2956. (doi:10.1073/pnas.0810831106)

51. Lourenço FC, Galvan V, Fombonne J, Cosej V, Llambr E, Reden DE, Meijer P. 2009 Netrin-1 interacts with amyloid precursor protein and regulates amyloid-beta production. Cell Death Differ. 16, 655 – 663. (doi:10.1038/cdd.2008.191)

52. Tu T et al. 2015 CD146 acts as a novel receptor for netrin-1 in promoting angiogenesis and vascular development. Cell Res. 25, 275 – 287. (doi:10.1038/cr.2015.15)

53. Maun HR, Wen X, Lingel A, de Sauvage FJ, Lazarus RA, Scales SJ, Hymowitz SG. 2010 Hedgehog pathway antagonist SEI1 binds hedgehog at the pseudo-active site. J. Biol. Chem. 285, 26 570 – 26 580. (doi:10.1074/jbc.M110.112284)

54. Capurro MI, Xu P, Shi W, Li F, Jia A, Filmus J. 2008 Glypican-3 inhibits Hedgehog signaling during development by competing with Patched for Hedgehog binding. Dev. Cell 14, 700 – 711. (doi:10.1016/j.devcel.2008.03.006)

55. Bymes MA et al. 2009 Brachyury C mutation restricted to the central region of the N-terminal active fragment of Indian Hedgehog. Eur. J. Hum. Genet. 17, 1112 – 1120. (doi:10.1038/ejhg.2009.18)

56. Chang S-C, Mulloly B, Magee AJ, Coumach JR. 2011 Two distinct sites in Sonic Hedgehog combine for heparan sulfate interactions and cell signaling functions. J. Biol. Chem. 286, 44 391 – 44 402. (doi:10.1074/jbc.M111.285361)

57. Nisse M, Tatula S, Page A, Hall TM, Leahy DJ, Brady PA. 1999 Sonic hedgehog protein signals not as a hydrolytic enzyme but as an apparent ligand for Patched. Proc. Natl Acad. Sci. USA 96, 10 992 – 10 999. (doi:10.1073/pnas.96.20.10992)

58. Ohlig S et al. 2011 Sonic hedgehog shedding results in functional activation of the solubilized protein. Dev. Cell 20, 764 – 774. (doi:10.1016/j.devcel.2011.05.010)

59. Statin E-L, Lindqv L, Börnerholm T, Schuster J, Dahl N. 2009 Brachyury type A1 associated with unusual radiological findings and a novel Arg158Ser mutation in the Indian hedgehog (IHH) gene. Eur. J. Med. Genet. 52, 297 – 302. (doi:10.1016/j.ejmg.2009.05.008)

60. Resov A, Pinesi D, Chronios G, Tarabottii G. 2014 Current understanding of the thrombospondin-1 interactor. Matrix Biol. 37, 83 – 91. (doi:10.1016/j.matbio.2014.01.012)

61. Hamdon ME, Stipp CS, Lander AD. 1999 Interactions of neural glycosaminoglycans and proteoglycans with protein ligands: assessment of selectivity, heterogeneity and the participation of core proteins in binding. Glycobiology 9, 143 – 155. (doi:10.1093/glycob/9.2.143)

62. Kuznetsova SA et al. 2006 Versican-thrombospondin-1 binding in vitro and co-localization in microfils induced by inflammation on vascular smooth muscle cells. J. Cell Sci. 119, 4499 – 4509. (doi:10.1242/jcs.017117)

63. Tarabottii G, Beletti D, Borsotti F, Vergani G, Ruscetti M, Presta M, Giavazzi R. 1997 The 140-kilodalton antiangiogenic fragment of thrombospondin-1 binds to basic fibroblast growth factor. Cell Growth Differ. 8, 471 – 479.

64. Tan K, Duquette M, Liu J-H, Zhang R, Joachimiak A, Wang J, Lawler J. 2006 The structures of the thrombospondin-1 N-terminal domain and its complex with a synthetic pentameric heparin. Struct. Land. 14, 170 – 170. (doi:10.1016/j.str.2006.12.005)

65. Tan K, Duquette M, Liu J-H, Shamugasundaram K, Joachimiak A, Gallagher JT, Rigby AC, Wang J, Joachimiak A, Gallagher JT, Rigby AC, Wang J.
Lawler J. 2008 Heparin-induced cis- and trans-dimerization modes of the thrombospondin-1 N-terminal domain. J. Biol. Chem. 283, 3932 – 3941. (doi:10.1074/jbc.M705203200)

66. Kinnunen TK. 2014 Combinatorial roles of heparan sulfate proteoglycans and heparan sulfates in Caenorhabditis elegans neural development. PLoS ONE 9, e102919. (doi:10.1371/journal.pone.0102919)

Bułow HE, Berry KL, Topper LH, Peles E, Hobert O. 2008 Heparan sulfate proteoglycan-dependent induction of axon branching and axon misrouting by the Kallmann syndrome gene kal-1. Proc. Natl Acad. Sci. USA 99, 6346 – 6351. (doi:10.1073/pnas.092128099)

68. Moretti M, Sinnamon-Kang ND, Toller M, Curcio F, Marchetti D. 2006 HPSE-1 expression and functionality in differentiating neural cells. J. Neurosci. Res. 83, 694 – 701. (doi:10.1002/jnr.20753)

69. Ridgway LD, Wetzel MD, Marchetti D. 2011 Heparanase modulates Shh and Wnt3a signaling in human medulloblastoma cells. Exp. Ther. Med. 2, 229 – 238. (doi:10.3892/etm.2010.189)

70. Dani N, Nahm M, Lee S, Brodie K. 2012 A targeted glycan-related gene screen reveals heparan sulfate proteoglycan sulfation regulates WNT and BMP trans-synaptic signaling. PLoS Genet. 8, e1003031. (doi:10.1371/journal.pgen.1003031)

71. Gallet A, Staccioli-Lavenant L, Théondon P. 2008 Cellular trafficking of the glypicanc Dali-like is required for full-strength Hedgehog signaling and wingless transcytosis. Dev. Cell 14, 712 – 725. (doi:10.1016/j.devcel.2008.03.001)

72. Blitzer JT, Nusse R. 2006 A critical role for endocytosis in Wnt signaling. BMC Cell Biol. 7, 28. (doi:10.1186/1471-2121-7-7-28)

73. Seto ES, Bellen HJ. 2006 Internalization is required for proper Wingless signaling in Drosophila melanogaster. J. Cell Biol. 173, 95 – 106. (doi:10.1083/jcb.200510123)

74. Vyas N, Goswami D, Manonmani A, Sharma P, Ranganath HA, VijayRaghavan K, Shashidhara LS, Sowdhamini R, Mayor S. 2008 Nanoscale organization of hedgehog is essential for long-range signaling. Cell 133, 1214 – 1227. (doi:10.1016/j.cell.2008.05.026)

75. Jakobs P, Schulz P, Ottmann C, Schürmann S, Exner E. 2008.05.026)

76. Saire-Santiago K, Townley RA, Attonito JD, Cunha S, Díaz-Balzac CA, Tecle E, Bułow HE. 2017 Coordination of heparan sulfate proteoglycans with Wnt signaling to control cellular migrations and positioning in Caenorhabditis elegans. Genetics 206, 1951 – 1967. (doi:10.1534/genetics.116.198739)

77. Williams EH, Pappano WN, Saunders AM, Kim M-S, Leahy DJ, Beachy PA. 2010 Dally-like core protein and its mammalian homologues mediate stimulatory and inhibitory effects on Hedgehog signal response. Proc. Natl Acad. Sci. USA 107, 5869 – 5874. (doi:10.1073/pnas.1001777107)

78. Zhang F, McLellan JS, Ayala AM, Leahy DJ, Linhardt RJ. 2007 Kinetic and structural studies on interactions between heparin or heparan sulfate and proteins of the Hedgehog signaling pathway. Biochemistry 46, 3933 – 3941. (doi:10.1021/bi6025424)

79. Connell BJ, Sadir R, Baleux F, Laguri C, Kleman J-P, Loo L, Azenzana-Seisedos F, Lortat-Jacob H. 2016 Heparan sulfate differentially controls CXCL12α- and CXCL12γ-mediated cell migration through differential presentation to their receptor CXCR4. Sci. Signal. 9, ra107. (doi:10.1126/scisignal.aaf1839)

80. de Witt J, O’Sullivan ML, Savas JN, Cendronetti G, Caccese MC, Vennekens KM, Yates JR, Ghosh A. 2013 Unbiased discovery of gypican as a receptor for LRTM4 in regulating excitatory synapse development. Neuron 79, 696 – 711. (doi:10.1016/j.neuron.2013.06.049)

81. Bauer KA, Hawkins DW, Peters PC, Petitou M, Herbert J-M, van Boeckel CA, Meuleman DG. 2002 Fondaparinux, a synthetic pentasaccharide: the first in a new class of antithrombotic agents—the selective factor Xa inhibitors. Cardiovasc. Drug Rev. 20, 37 – 52. (doi:10.1111/j.1527-3466.2002.tb00081.x)

82. Rodger MA et al. 2016 Low-molecular-weight heparin and recurrent placenta-mediated pregnancy complications: a meta-analysis of individual patient data from randomised controlled trials. The Lancet 388, 2629 – 2641. (doi:10.1016/S0140-6736(16)31394-9)

83. Chitrououchadze Z, Ye Z, Sheng Z, Laflue S, Fry RC, Lauffenburger DA, Janes KA. 2016 TNF-insulin crosstalk at the transcription factor GATA6 is revealed by a model that links signaling and transcriptomic data tensors. Sci. Signal. 9, ra59. (doi:10.1126/scisignal.aad3373)

84. Pomin VH, Mulloy B. 2015 Current structural biology of the heparin interactome. Curr. Opin. Struct. Biol. 34, 17 – 25. (doi:10.1016/j.sbi.2015.05.007)

85. Brophy JAN, Voigt CA. 2014 Principles of genetic circuit design. Nat. Methods 11, 508 – 520. (doi:10.1038/nmeth.2926)

86. Wang B, Buck M. 2012 Customizing cell signaling using engineered genetic logic circuits. Trends Microbiol. 20, 376 – 384. (doi:10.1016/j.tmicr.2012.05.001)