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## GLYCOSAMINOGLYCAN INTERACTION NETWORKS AND DATABASES

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### **ABSTRACT**

Glycosaminoglycans are complex polysaccharides exhibiting a large structural and conformational diversity. These key biological players organize the extracellular matrix, contribute to cell-matrix interactions, and regulate cell signaling. Natural and synthetic libraries of glycosaminoglycans have been spotted on microarrays to find glycosaminoglycan partners, and determine the size and the chemical groups promoting protein binding. Advances in glycosaminoglycan sequencing allow the characterization of glycosaminoglycan sequences interacting with proteins, and glycosaminoglycan-mediated pull-down proteomics can identify glycosaminoglycan binding-proteins at a proteome scale in various biological samples. The analysis of the glycosaminoglycan interaction networks generated using these data gives insights into the molecular and cellular mechanisms underlying glycosaminoglycan functions. These interactomes can also be used to design inhibitors targeting specific GAG interactions for therapeutic purpose.

### **INTRODUCTION**

Glycosaminoglycans (GAGs) are complex linear polysaccharides comprised of disaccharide repeats made of glucose or galactose and a hexuronic acid (glucuronic or iduronic acid). Five out of the six mammalian GAGs, chondroitin sulfate (CS), dermatan sulfate (DS), heparan sulfate (HS), heparin (HP) and keratan sulfate (KS), are covalently attached to core proteins to form proteoglycans (PGs) [1], and undergo several modifications (e.g., sulfation, N-acetylation, epimerization). Hyaluronan (HA) is not sulfated and does not form PGs. GAGs play major biological roles in the extracellular matrix (ECM) and at the cell surface although HS has been found in the nucleus [2]. Throughout their interactions with numerous proteins GAGs contribute to ECM assembly and organization, and to cell-matrix interactions. They are involved in many diseases such as cancer and infections [1,3]. GAG interactions mediate their functions, influence each other at various scales *in vivo*, and form dynamic and context-dependent networks. These networks can be used to study the molecular and cellular mechanisms underlying their functions, and to determine how these functions are regulated. **Figure 1** displays the roadmap to build and analyze GAG interactomes. This review covers the large-scale identification of GAG-binding proteins (GAGBPs), and provides examples of GAG interactomes generated in various molecular and cellular contexts, and of integration of additional data. The perspectives highlight the major challenges to generate 3D specific GAG interactomes.

## IDENTIFICATION OF GAG-BINDING PROTEINS AND GAG-BINDING FEATURES

A number of GAGBPs have been identified by individual binding assays carried out by affinity chromatography, gel mobility shift assays, biophysical techniques [4] such as surface plasmon resonance (SPR) [5–8], bio-layer interferometry (BLI) [5,9], isothermal titration calorimetry, quartz microbalance [10], capillary electrophoresis [11], nuclear magnetic resonance (NMR) [12], and computational approaches (*Samsonov et al., and Desai et al., this volume*). However, the building of comprehensive GAG interactomes requires large-scale, high-throughput, binding assays including GAG microarrays and affinity-proteomics.

Protein arrays can be probed with a single GAG to identify its protein partners as done for KS assayed with 8268 human proteins spotted on a commercially available array [13], or for HP, HS, DS, and CSC with *E. coli* proteome arrays comprised of 4256 proteins [14,15]. Alternatively, GAG arrays can be probed with a single protein to identify its GAG repertoire. Arrays comprised of the six GAGs and selectively desulfated HP have been used to investigate their binding to ECM proteins [5,16], monomer, oligomers, and fibrils of the amyloid A $\beta$ 42 peptide [17] and live pathogens [18]. Natural and synthetic GAG libraries, reflecting the diversity of sizes, sequences and chemical modifications of the glycosaminoglycome (GAGome), are required to generate arrays. This is a challenge given that there are 202 unique GAG disaccharides [19], and that the GAGome composition depends on cell types, tissues, and physio-pathological contexts. GAG libraries are also prepared for combinatorial virtual library screening (CVLS). Two libraries of 400 GAG tetrasaccharides and one library of 1000 GAG hexasaccharides were generated for CVLS against HS 3-O-Sulfotransferase 1 [20], and a library of 1752 CS oligosaccharides (24 disaccharides, 192 tetrasaccharides, and 1536 hexasaccharides) was screened against transforming growth factor  $\beta$ 2 by CVLS [21].

The use of structurally defined oligosaccharides to generate GAG microarrays [22,23], and strategies to synthesize anomerically modified HA synthetic oligosaccharides with defined sulfation patterns have been recently reviewed [24]. Consequently, only some recent examples of GAG libraries of various size and structural diversity synthesized by chemical or chemoenzymatic methods, are listed in **Table 1**. CS hexasaccharides synthesized on a solid support with a photolabile linker were used to prepare CS arrays [25]. A combinatorial HS mimetic array can be prepared by the self-assembly of several ratios of two disaccharides with different physicochemical properties (lactose and sulfated lactose) to create a variety of modifications, charges, and topologies accounting for HS structural diversity. This array was able to discriminate the binding of a wild-type protein and its single-point mutants [26]. The molecular diversity of the array could be increased by additional disaccharides with other physicochemical properties. Two HS libraries comprising 27 hexasaccharides with or without 3-O-sulfate groups [27], and 47 tetrasaccharides with 3-O- and N-sulfation [28] have been synthesized to determine the role of these chemical groups in protein binding. The GAG size matters for protein interaction, and a HS library of 93 di-, tetra-, hexa-, and octasaccharides of various sequences and sulfation patterns has been generated to identify the minimal recognition sequence of the spike protein of severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) [29]. GAG may be conjugated to bovine serum albumin (BSA) [30] or to neoglycolipids (NGLs) [31] before spotting on arrays.

Cells can be used to synthesize GAG libraries. Chondroitin, and heparosan (unsulfated HS) were prepared from *E. coli*, which can synthesize <sup>13</sup>C-labeled polysaccharides to be used for NMR experiments when grown in presence of <sup>13</sup>C-labeled glucose [9]. Genes encoding GAG biosynthetic enzymes have been edited, individually or in combination, in Chinese hamster ovary cells to produce a cell-based GAG library (GAGOme) with a large repertoire of modifications [32], and in mouse lung endothelial cells to generate a HS mutant mouse lung endothelial cell library [33]. In addition, two arrays made of 24 HS oligosaccharides and 46 GAG oligosaccharides (HA, CS, CSDS, HP, and HS oligosaccharides ranging from deca- to eicosasaccharides) are commercially available (<https://www.zbiotech.com>).

Fluorescence is the most common detection method on GAG and protein arrays [14,27–31], but GAG arrays have been designed to detect binding events by a label-free method, SPR imaging (SPRi) [5,16,26,34], to calculate kinetics and affinity of the interactions. SPRi arrays can be interfaced with a MALDI mass spectrometer (MS) for on-chip analyses of the oligosaccharides captured by the proteins spotted on the arrays [34]. Proteins captured from biological media on any glycan arrays can be identified by MALDI-time-of-flight MS/MS after *in situ* proteolytic digestion [35]. Time of flight secondary ion mass spectrometry was used to analyze nanogram amounts of the six GAGs (CS, DS, HA, HP, HS and KS) spotted on a microarray [36]. Experiments performed with GAG arrays should be reported following the MIRAGE (Minimum Information Required for A Glycomics Experiment) guidelines [37].

Affinity proteomics-based approaches have been designed to identify GAGBPs in various cells and tissues, and to compare their patterns in health and disease (**Table 2**) [38–40]. An affinity-proteomics approach, named Limited Proteolysis in the absence of

denaturation, Heparin-Affinity chromatography, and high-resolution LC-MS/MS proteomics (LPHAMS), allows the enrichment of low-abundance HSBPs in several cell types, and the mapping of HP-binding protein domains [6]. Another glycan-mediated pull-down proteomics method based on affinity chromatography on immobilized GAGs coupled to nano-LC/MS/MS can evaluate the affinity of GAG-protein interactions by eluting GAGBPs with a salt step-gradient [38]. HSPGs have been immobilized onto nano-affinity columns (internal diameter: 75  $\mu\text{m}$ ) to screen potential partners [41]. A filter-entrapment enrichment pull-down assay with a membrane instead of an affinity column can be used to enrich ultra LMW HP sequences interacting with a protein before analysis by capillary zone electrophoresis MS/MS [8].

GAGBPs can be retrieved from interaction databases, and more specifically from the extracellular interaction database MatrixDB, which is the only database curating GAG-interactions on a regular basis (<http://matrixdb.univ-lyon1.fr/>) [42]. MatrixDB uses the curation interface of the IntAct database (<https://www.ebi.ac.uk/intact/home>) [43]. Both databases belong to the International Molecular Exchange Consortium (IMEx, <http://www.imexconsortium.org/>) providing an open access unified dataset of molecular interactions [44]. A step-by-step protocol has been set up to build protein-GAG interaction networks using MatrixDB data and its i-Navigator [45]. MatrixDB data have been integrated into several GAG interactomes [1,46,47]. The STRING database (Search Tool for the Retrieval of Interacting Genes, <https://string-db.org/>) [48] was used to build HPBP interactomes in normal and diseased pancreas [39], in cardiovascular diseases [49], in periodontitis [50], and the global HS interactome [51]. HP/HSBP networks can be generated *in silico* as shown for those involved in cardiovascular diseases, using a published dataset [46], and the Open Targets database (<https://www.opentargets.org>) to identify the HP/HSBPs related to four cardiovascular diseases [49], and in periodontal diseases using literature curation and the STRING database [50].

## GAG INTERACTOMES

Interaction networks are analyzed to generate hypotheses, guide the design of new experiments, and define general principles of molecular, supramolecular, cellular, and tissular assembly, organization and functions. Computational tools and resources to analyze ECM networks including GAG interactomes have been recently reviewed [52]. Those used for perform functional enrichment analyses of GAG interactomes are the Database for Annotation, Visualization and Integrated Discovery (DAVID) [38,50,51], the Biological Network Gene Ontology tool (BiNGO) [46], the Functional Enrichment tool FunRich [47], and Metascape (<https://metascape.org>) [49]. Reactome [47] and KEGG [14,46] are specifically used for biological and metabolic pathways. The topology of GAG networks has been determined with STRING [39] or the Cytoscape apps Network analyzer [49,50], whereas the clusters and the most connected proteins have been identified with MCODE and cytoHubba apps respectively [49].

**Table 2** lists the major GAG interactomes available. HP/HSBPs regulating angiogenesis have been identified by literature curation but not analyzed further [53]. The identification of

KSBPs by protein arrays has shown that 75 of them are kinases and 40 nerve-related proteins [13]. A systems biology approach has been used to investigate HP/HS interactomes [46,51] and the global GAG interactome including HA, CS, DS and KS in addition to HP/HS [47]. The HP/HS network is highly interconnected and enriched in biological processes such as cell-cell signaling, regulation of cell proliferation, system development, immune and inflammatory responses, and in pathways controlling biological processes characteristic of multicellular organisms [46]. The link between HS-binding and the evolution toward multicellularity has been confirmed on a larger HP/HS interactome, which was clustered into five classes (*i.e.*, chemokines and complement proteins, growth factors, ion sensing and transport, protease activity and coagulation, lipoprotein metabolism, and ECM assembly) [51]. The HPBPs identified in human microvascular endothelial cells are enriched in functions related to protein degradation and folding, glycan metabolism, inflammatory processes, apoptosis, cytoskeleton regulation, RNA recognition, splicing, and synthesis [38]. The cytosolic HPBPs isolated from human pulmonary fibroblasts are also associated with apoptosis regulation and RNA processing [38]. Signal transduction, cell communication and growth, immune response, ECM organization, hemostasis, development and metabolism of proteins are enriched in the global GAG interactome [47]. In this first draft of the GAG interactome, more than 80% of HP/HSBPs and KSBPs bind only to HP/HS and KS respectively, ~60% of HABPs interact with HA only and ~25% of CSBPs bind to CS only [47]. In contrast, all DSBPs interact with at least another GAG in addition to DS [47]. This low specificity of DS is consistent with the finding that only five proteins out of the 129 GAGBPs identified in human plasma bind only to DS [38]. Affinity purification-proteomics has been applied to several human proteomes to explore GAG-binding specificity and relative affinity [38], and to identify proteins binding to HP and sulfated synthetic HA [40].

GAG-protein interactions play also a role in diseases [3]. GAG-pathogen interactions have been investigated at a proteome scale in one strain of *E. coli* [14,15], and in 24 strains of intact, live, *Leishmania* parasites at the ECM scale [18]. *E. coli* HPBPs and HSBPs play a role in bacterial amino acid metabolism, and in enhanced lipopolysaccharide biosynthesis, whereas lipopolysaccharide biosynthesis and ribosome are enriched in the entire *E. coli* GAGBP dataset [14]. The interactome of the three IdoA-containing GAGs (HP, HS and DS), which can mediate host-bacteria interactions, has been generated using CSC, a non-IdoA-containing GAG, as a negative control, and shown to be enriched in functions related to protein synthesis and metabolism [15]. The 15 proteins interacting with the IdoA-containing GAGs share a conserved amino acid sequence [15]. Most *Leishmania* strains bind to two IdoA-containing GAGs, high molecular weight (HMW) HP (83%) and HS (62%), whereas less than 10% of them interact with DS, the third IdoA-containing GAG [18]. HP/HSBPs involved in cardiovascular diseases are associated with cell activation, movement, migration, and adhesion, and they upregulate the expression of ECM-affiliated proteins, ECM regulators, and secreted factors [49]. In acute pancreatitis, HPBPs are associated with acute phase response signaling, complement activation and mitochondrial dysfunction in addition to molecular transport and cellular movement found in normal pancreas [39]. The analysis of the interactome of HPBPs differentially expressed in periodontal diseases, including matrix metalloproteases, cytokines

and chemokines, highlights the importance a multi-targeted approach in periodontitis treatment [50].

### INTEGRATION OF ADDITIONAL DATA IN GAG NETWORKS

The biological context promotes or abolishes interactions, which rewires the interactomes. GAG interactomes associated with specific proteomes can be built by experimentally targeting proteomes with protein arrays [13,14] and affinity-based methods [6,38,40]. Alternatively, context-specific networks can be generated by integrating GAGomic [54] and proteoglycanomic [55] data collected in various biological samples into GAG networks combining interaction data from different tissues, species, and methods. The molecular context also matters for interactions established by sulfated GAGs, which are attached to proteins *in vivo* to form PGs. The core protein of PGs may modulate protein affinity for HS as reported for an ECM bioactive fragment, endostatin [56]. The building of PG interactomes would thus provide a molecular context to discriminate GAG interactions mediated by protein cores from those mediated by their GAG chains as done for the interactome of the four membrane PG syndecans [57]. Affinity and kinetics of interactions can also be added to GAG networks. HP/HSBPs have been ranked according to their affinity in the endostatin network regulating angiogenesis at the endothelial cell surface [56]. Lipoproteins and non-ECM secreted proteins have the lowest dissociation rates, whereas matrisome-associated proteins and lipoproteins bind faster to HP than other proteins and have the highest affinity for HP [56].

Another critical area of investigation is the identification of GAG sequences interacting with proteins. There are 202 unique GAG disaccharides, including 48 HS disaccharides, which highlights the structural diversity of GAGs, and makes their sequencing challenging [19]. GAG patterns of chemical modifications form glycodes, which are cell-, tissue- or disease-specific. The identification of GAG sequences interacting with proteins is thus of crucial importance to decipher the glycode specificity. Few binding assays were performed with defined GAG sequences, but the availability of GAG libraries and their use to prepare GAG arrays will help to determine GAG sequences binding to proteins. Structural domains can be mapped in complex GAG mixtures using the GAGDoMa approach based on the enzymatic depolymerization of GAGs and on the analysis of the internal, terminating, and initiating GAG domains generated by LC-MS/MS [58]. The advances in MS and infrared spectroscopy should also ease the analysis of GAG sequences binding to proteins [59–61]. Another limitation to investigate the glycode specificity is that GAG sequences are reported in publications in heterogeneous formats, ranging from free-text and images of chemical formulae to sequences written in IUPAC, GlycoCT, and WURCS formats. This prevents GAG interaction data mining with computational tools. We have thus designed a pipeline consistent with the standards of the IMEx consortium and glycan repositories and databases such as GlyTouCan (<https://glytoucan.org/>) [62] and the Chemical Entities of Biological Interest database (ChEBI, <https://www.ebi.ac.uk/chebi/>) [63] to ease the conversion of the various GAG formats. MatrixDB is interoperable with these databases, and with the Glygen portal providing an interface to explore glycoscience data (<https://data.glygen.org>) [64]. A workflow has been

designed to improve the interoperability of the glycan-containing databases ChEBI, PubChem, and GlyGen [65].

The analysis of conserved basic amino acid sequences among 437 HP/HSBPs has shown that HP/HS-binding likely results from the 3D arrangement on the protein surface of small, independent basic amino acid sequences [66]. The integration of the 3D structures of GAGs and GAG-protein complexes in GAG networks is thus a prerequisite to build 3D GAG interactomes. GAGs have broad conformational landscapes, and conformational changes occur upon GAG binding to proteins. They contribute to the specificity of the binding but are still poorly investigated. The analysis of 75 protein–GAG complexes has shown that the glycosidic  $\Phi$  and  $\Psi$  torsion angles of HP/HS and HA oligosaccharides complexed with proteins are clustered into two major areas. The sulfate groups have a minor influence on GAG conformational space whereas the conformation of iduronic acid induces a spatial restriction when located at the reducing end of disaccharides [19]. GAG structures are available in generic databases such as the Protein Data Bank (<https://www.rcsb.org/>), and the Cambridge Structural Database (<https://www.ccdc.cam.ac.uk/>). The dedicated database GAG-DB (<https://gagdb.glycopedia.eu/>) [67] covers the three-dimensional landscape of GAGs. It contains 123 entries reporting GAG composition, sequence, and topology, and provides the 3D visualization of contacts between GAGs and their partners using the protein-ligand interaction profiler [68]. GAG-DB also displays the structure adopted by GAGs in solid-state or in solution, alone or complexed with their partners. Experimental 3D structures of GAG-binding sequences are also on MatrixDB web site. The 3D models of these sequences can be generated on MatrixDB web site *via* the polysaccharide builder program POLYS [69] implemented in the database [42].

## CONCLUSIONS AND PERSPECTIVES

The creation of GAG libraries by various approaches paves the way to the identification of additional GAGBPs and GAG oligosaccharides binding to proteins. The synthesis of CS by engineered *E. coli* provides an alternative to the purification of GAGs from animal tissues. In addition this approach can be used to prepare isotope-labeled GAGs for structural studies [70]. The recent advances in GAG sequencing by MS and infrared spectroscopy [59–61] (*Greis et al., this volume*) will ease the determination of GAG glycocodes for a variety of structural and functional protein families. The design of GAG arrays could be improved by mimicking the multivalent display occurring *in vivo*. Affinity-based techniques were successfully used to explore GAGBPs in various proteomes. They will be applied to further healthy and diseased proteomes in order to map GAGs at an organism level, and to determine disease-related changes in GAG interactomes, with a focus on GAG–pathogen interactomes, which are currently underexplored. GAG interaction datasets should be consistent with existing standard glycan formats [19] and experimental guidelines [37], and computational tools should be developed to explore these datasets. Integrating structural data in GAG interactomes is a prerequisite to generate a 3D interactome, and to investigate the relationships between glycocodes, involving both linear sequences and 3D motifs, and GABP

localization and molecular or biological functions. The structure of GAGs of various sizes and sequences and of GAG-protein complexes can be generated experimentally by X-ray and NMR, or by combining small angle X-ray scattering data with molecular modeling approaches [71] (*Samsonov et al., and Desai et al., this volume*). In absence of experimental data, 3D atomic-resolution models of non-sulfated GAGs with lengths up to 200 mers will be built using molecular dynamics [72]. Another topic worth of investigation is the study of GAG-GAG interactions, which should provide new insights into GAG functions. GAG self-interaction has been suggested to occur in tendon where adjacent rings formed by GAGs around collagen fibrils merge to form a planar network [73]. Integrating the above data in GAG interactomes generated from healthy and diseased cells and tissues will be of major importance to design inhibitors targeting specific GAG interactions for therapeutic purpose.

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## AUTHOR CONTRIBUTIONS

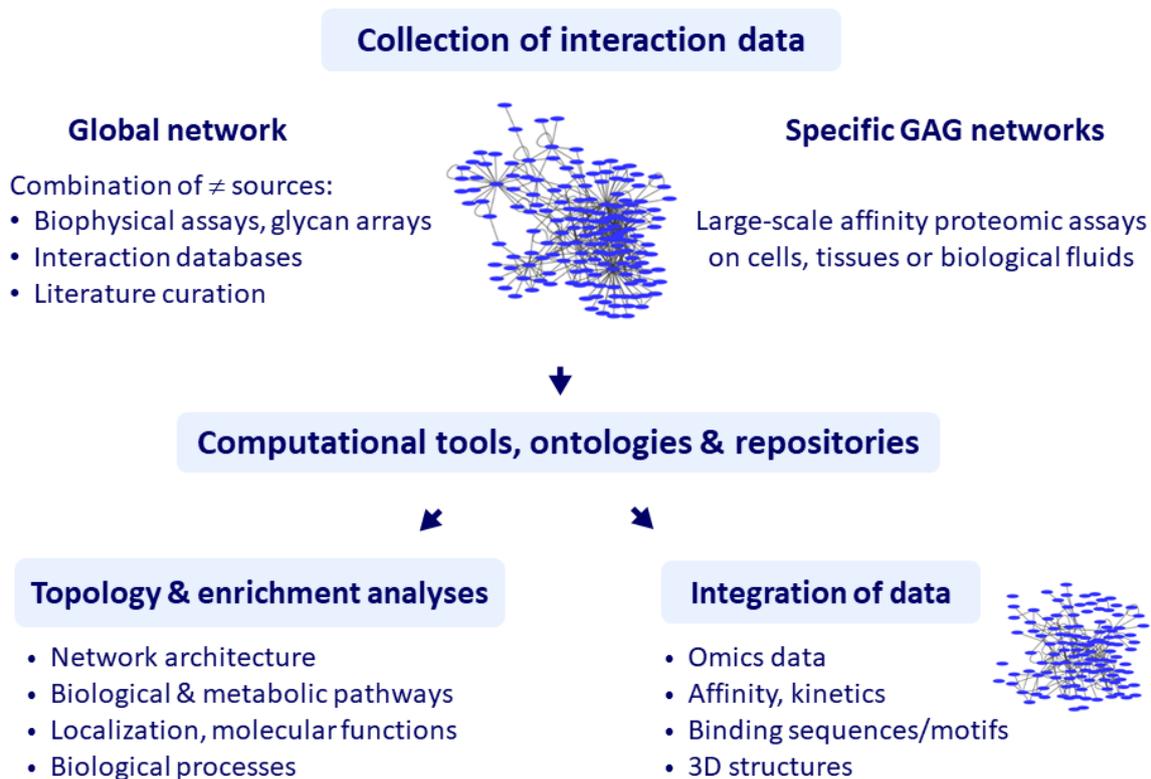
SRB wrote the original draft of the manuscript, reviewed and edited it. SP reviewed and edited the manuscript.

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## FIGURE LEGENDS

**Figure 1:** The roadmap designed to generate and analyze glycosaminoglycan interactomes



**Table 1: Synthetic and natural GAG libraries recently developed** (dp = degree of polymerization, *i.e.*, the number of monosaccharides, CHO: Chinese Hamster Ovary).

GAGs	GAG size and modifications	Applications	Proteins	References
47 dp4	Different backbones and chemical modifications (e.g., O- and N-sulfation)	Arrays	FGF2, CCL and CXCL chemokines	[28]
27 dp6	With and without O-sulfate groups	Arrays	Heparin lyases	[27]
HS 93 oligosaccharides (dp2, dp4, dp6, dp8)	Various sequences and sulfation patterns	Arrays	SARS-CoV-2 spike protein	[29]
CS dp6		Arrays		[25]
20 natural GAGs, including CSA, CSC, CSD, DS, HA, CSE, HP, HS, heparosan	Various Mw, and HP sulfation pattern conjugated with BSA	Arrays	SARS-CoV-2 spike protein	[30]
Chondroitin, and heparosan ( <i>E. coli</i> K12 and K5 strains)	<sup>13</sup> C-labeled polysaccharides	NMR spectroscopy experiments		[9]
CHO cell-based GAG libraries	Broad repertoire of GAG modifications (KO and KI of the 28 genes involved in GAG biosynthesis)	Cell-based assays Arrays Expression of recombinant PGs	VAR2CSA protein ( <i>P. falciparum</i> ) FGF2 CS-56 monoclonal antibody	[32]
A HS mutant mouse lung endothelial cell library	18 cell lines (systematic deletion of HS related genes)	Analysis of HS structure-function relationships	FGF2-FGFR1 signaling Anti-HS phage display antibody epitopes	[33]
63 lipid-GAGs (CSA, CSC, DS, HA, HP, HS)	Various GAG sizes	Array based on the NGL technology	Complement Factor H - Related Protein 5	[31]

**Table 2 : Major GAG interaction datasets collected by different approaches.** (CHO: Chinese Hamster Ovary, GO: Gene Ontology, LMW: low molecular weight)

<b>GAGs</b>	<b>Biological sources</b>	<b>Techniques</b>	<b>Number of GAGBPs identified</b>	<b>References</b>
CSA	Human proteins	Protein arrays	24	[13]
CSA	Nerve-related proteins (human, mouse, rat, chicken)	Protein arrays	9	[13]
HA	Human proteins	Protein arrays	6	[13]
Low sulfated polymeric HA (sHA1)	Human bone marrow stromal cells	AP-MS	125	[40]
High-sulfated polymeric hyaluronan (sHA3)	Human bone marrow stromal cells	AP-MS	126	[40]
Trideca-sulfo- HA dp6 (13sHA6-TAMRA)	Human bone marrow stromal cells	AP-MS	393	[40]
KS	Human proteins	Protein arrays	217	[13]
KS	Nerve-related proteins (human, mouse, rat, chicken)	Protein arrays	40	[13]
HP/HS	Plasma membrane enriched fractions from rat liver	Literature curation Databases GO terms UniProtKB keywords Affinity proteomics	435	[46]
HS	Human vein endothelial cells (HUVEC)	LPHAMS	32	[6]
HS	Murine brain microvascular endothelial cells	LPHAMS	12	[6]
HS	Murine lung microvascular endothelial cells	LPHAMS	9	[6]

LMW HP	Human microvascular endothelial cells	GAG-mediated pull-down proteomics	296 (269 novel)	[38]
LMW HP	Human pulmonary fibroblast	GAG-mediated pull-down proteomics	87 (75 novel)	[38]
HP	Human macrophage cell line U937	LPHAMS	20	[6]
HP	Murine pancreas	HP affinity proteomics	320 (extracellular)	[39]
HP	Murine pancreas (model of acute pancreatitis)	HP affinity proteomics	345 (extracellular)	[39]
HP	Murine plasma	HP affinity proteomics	161	[39]
HP	Murine pancreas (model of acute pancreatitis)	HP affinity proteomics	151	[39]
DS, LMW HP, HS	Human plasma	GAG-mediated pull-down proteomics	129 (71 novel)	[38]
HP	Human bone marrow stromal cells	AP-MS	43	[40]
HP (10-15 kDa)	<i>E. coli</i> proteins	Protein arrays	185	[14]
HS (10-15 kDa)	<i>E. coli</i> proteins	Protein arrays	62	[14]
DS (CSB) (10-15 kDa)	<i>E. coli</i> proteins	Protein arrays	98	[14]
CSC (10-15 kDa)	<i>E. coli</i> proteins	Protein arrays	101	[14]
IdoA-containing GAGs (DS, HP, HS)	<i>E. coli</i> proteins	Protein arrays	157	[15]
HP/HS	Angiogenesis	Literature curation	60	[53]
HP/HS	Combination of various sources and species	Literature curation	530	[51]
HP	Differentially expressed HBPs in periodontal disease	Literature curation + database	48	[50]

HP/HS	Heart diseases	[46] dataset + Open Targets Database	66 (atherosclerosis) 21 (myocarditis) 71 (myocardial infection) 34 (myocardial ischemia)	[49]
CS, DS, HA, HP/HS, KS	Combination of various sources and species	Literature curation, GO terms, UniProtKB keywords, databases, SPR, SPRi and BLI assays	827	[47]