

## MIT Open Access Articles

*Heparin sensing: Blue-chip binding*

The MIT Faculty has made this article openly available. **Please share** how this access benefits you. Your story matters.

**Citation:** Shriver, Zachary, and Ram Sasisekharan. "Heparin Sensing: Blue-Chip Binding." Nature Chemistry 5, no. 8 (July 7, 2013): 644–646.

**As Published:** <http://dx.doi.org/10.1038/nchem.1701>

**Publisher:** Nature Publishing Group

**Persistent URL:** <http://hdl.handle.net/1721.1/88988>

**Version:** Author's final manuscript: final author's manuscript post peer review, without publisher's formatting or copy editing

**Terms of use:** Creative Commons Attribution-Noncommercial-Share Alike



Published in final edited form as:

*Nat Chem.* 2013 August ; 5(8): 644–646. doi:10.1038/nchem.1701.

## Blue-chip binding

**Zachary Shriver and Ram Sasisekharan**

Koch Institute for Integrative Cancer Research, Department of Biological Engineering,  
Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA

### Abstract

Heparin is an anionic polysaccharide that has tremendous clinical importance as an anticoagulant. Several dyes have been developed that can detect heparin, and the latest example — named Mallard Blue — has now been shown to have excellent sensing properties under biologically relevant conditions.

---

Heparin and heparan sulfate (HS) are complex linear polysaccharides that modulate a range of normal and disease-related biological functions. The basic disaccharide repeat unit of each consists of a glucosamine linked to a uronic acid<sup>1,2</sup>. Modification of these biosynthesized polymers — primarily by *O*- and *N*-sulfonation — leads to structural heterogeneity within the chains. Heparin can be viewed as a ‘specialized’ version of HS, composed primarily of highly sulfated chains, with ~60–80% of the disaccharide units consisting of 2-*O* sulfo iduronic acid and 6-*O* sulfo, *N*-sulfo glucosamine. In a typical heparin chain, repeats of this trisulfated disaccharide unit are interrupted by other minor sequences — including a 3-*O*-sulfate-containing pentasaccharide sequence that binds to the small protein antithrombin and, as a consequence, is responsible for heparin’s anticoagulant activity<sup>3,4</sup>. Conversely, HS composition can vary more significantly, although there do seem to be six major disaccharide constituents<sup>5</sup> (Fig. 1a).

Substantial effort has been devoted to developing strategies to detect and characterize heparin and HS. Two distinct, but related, approaches have been the focus of these studies. First, a variety of techniques — including antibody-based strategies<sup>6</sup>, dye staining and labelling of the core proteins of HS proteoglycans<sup>7</sup> — have been used to study HS in biological samples, in terms of measuring how much of it is present as well as examining its structure. Second, more application-driven work has focused exclusively on the pharmaceutical applications of heparin, which is used clinically as an agent to prevent or treat thrombosis (blood clotting). In these cases, the goal is to devise techniques that can directly detect heparin chains within a complex biological sample (that is, measure a true concentration) rather than rely on an activity measurement within plasma or serum (units of anticoagulant activity).

Within this framework, the synthesis of a heparin-binding dye — termed Mallard Blue — reported<sup>8</sup> in the *Journal of the American Chemical Society* by David Smith and colleagues provides a potentially important advance. Cationic dyes, such as Alcian Blue and Azure A (Fig. 1b), have historically been used to detect heparin, but it is well known that many other substances interfere with the non-covalent binding that underpins these assays. Although several alternative approaches have been investigated, including substitute dye systems, these have yet to demonstrate superior performance in biological fluids, including urine,

whole blood and blood serum, and sputum. In this current study, Smith and co-workers provide tantalizing evidence that Mallard Blue may overcome many of these challenges.

In their study, Smith and co-workers highlight some important attributes of Mallard Blue that are likely to make it superior to older approaches. Perhaps most importantly, it is shown that despite the fact that electrostatics govern the interaction between Mallard Blue and heparin, the dye ‘recognizes’ heparin even in the presence of significant amounts of electrolyte. This property is critical for a heparin-detection agent to be able to work in many biological samples. Indeed, Mallard Blue is demonstrated to work in serum and, importantly, shows a quantitative linear dose response, in contrast to many other dyes. Finally, given the ease of synthesis, it is likely that Mallard Blue can be a widely used reagent and not confined to synthesis labs.

Beyond the ability to analyse specifically the behaviour of heparin in biological systems, work such as this offers exciting possibilities in a more general sense, in that we may be moving closer to developing a systematic understanding of the biology of this class of complex polysaccharides in their natural context, namely at the cell/extracellular matrix interface. We have previously described how a convergence of -omics-based analysis of biological samples (a bottom-up approach) with a structure-based analysis of protein–glycan interactions (a top-down approach) is helping to achieve this goal<sup>9</sup>. In the case of complex linear glycans such as heparin and HS, however, it will require determination of the sequence of individual HS chains, and how the known domains within heparin or heparan (that is, highly sulfated versus undersulfated domains) are organized to completely understand the binding preference of dyes such as Mallard Blue. Most recently, techniques such as mass spectrometry have been applied to the sequencing of simpler linear complex polysaccharides, such as the chondroitin sulfate chains from the proteoglycan bikunin<sup>10,11</sup>. Further development of such approaches to more complicated HS structures, in combination with *in vitro* and *in vivo* analysis of HS function, provides a potential path to addressing the diverse biochemical functions of HS.

Some questions remain regarding the work reported by Smith and colleagues, and the answers to these will influence how Mallard Blue will be used. First, in several clinical settings, including those geared towards the prevention of venous thrombosis or pulmonary embolism, low-molecular-weight heparins (with reduced chain lengths) are preferred over full-length, undigested heparin. How does molecular weight affect the performance of Mallard Blue — and what influence does sulfation density have? For example, with the low-molecular-weight heparins, the depolymerization chemistry is known to affect sulfation levels<sup>12</sup>. Also, heparin is known to contain other related polysaccharides, such as dermatan sulfate as an impurity and, in some cases, oversulfated chondroitin sulfate as a contaminant<sup>13,14</sup>. How does the presence of these similar polymers affect the performance of Mallard Blue? Given the molecular dynamics work, it is clear that sulfation density plays a role in the stoichiometry and strength of binding (Fig. 1c) but, for example, how does positioning versus density matter? This question is especially important given that heparin composition is known to be altered after *in vivo* administration because of differential filtration in the kidney<sup>15</sup>. Finally, can the use of Mallard Blue be extended to the analysis of HS from biological samples, including tissue sections? If so, this dye could prove to be an important reagent for probing the biological function of HS.

In summary, given the performance of Mallard Blue — its ability to detect heparin in serum in a linear dose-response manner, its superior performance to Azure A, its specificity, and its relative resistance to higher ionic strength conditions — this work bodes well for its continued use in the detection of heparin. Moreover, this study suggests that new reagents that could be used to probe heparin, HS and related polysaccharides, both in

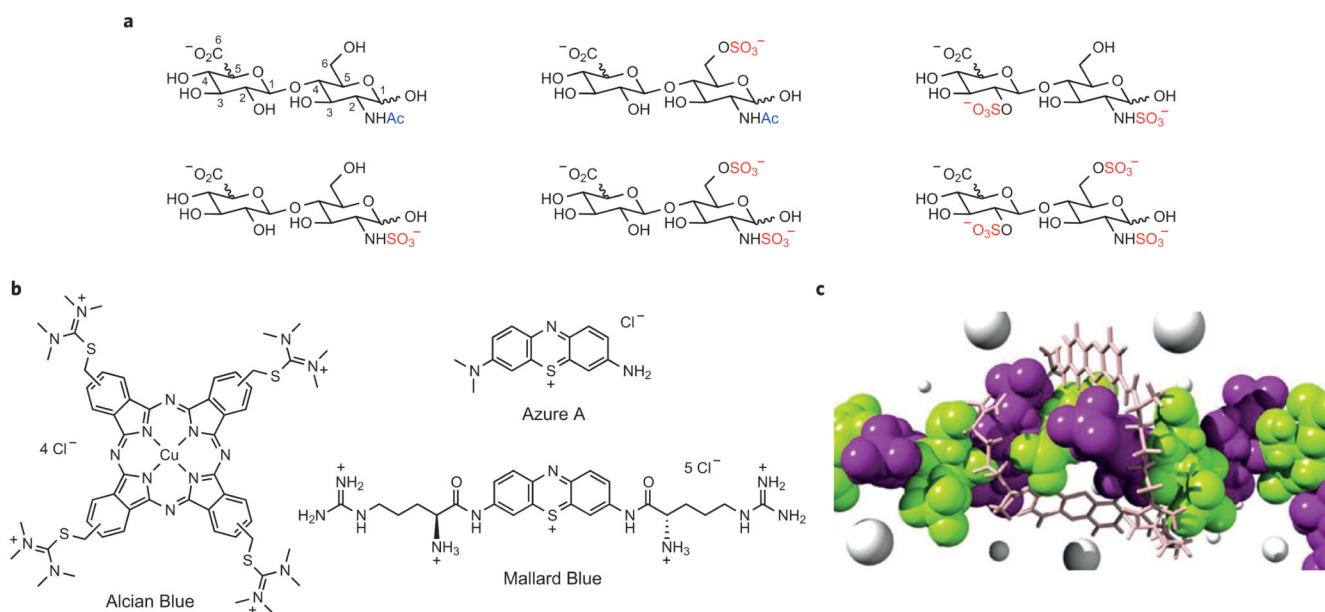
pharmacological and biological contexts, can be created inexpensively. Because heparin is an important medicinal agent, the development of new and effective schemes for its detection are crucial. If such techniques can be further extended for the analysis of heparin in tissue samples, they could prove to be very useful methods for studies aimed at broadening the use of heparin or heparin-related oligosaccharides to other diseases, including inflammatory and immunological conditions.

## Acknowledgments

R.S. would like to acknowledge support from National Institutes of Health (R37 GM057073-13).

## References

1. Carlsson P, et al. *J. Biol. Chem.* 2008; 283:20008–20014. [PubMed: 18487608]
2. Sasisekharan R, Venkataraman G. *Curr. Opin. Chem. Biol.* 2000; 4:626–631. [PubMed: 11102866]
3. Atha DH, et al. *Biochemistry.* 1987; 26:6454–6461. [PubMed: 3427019]
4. Lindahl U, et al. *Proc. Natl Acad. Sci. USA.* 1980; 77:6551–6555. [PubMed: 6935668]
5. Esko JD, Lindahl U. *J. Clin. Invest.* 2001; 108:169–173. [PubMed: 11457867]
6. Thompson SM, et al. *J. Biol. Chem.* 2009; 284:35621–35631. [PubMed: 19837661]
7. Nackaerts K, et al. *Int. J. Cancer.* 1997; 74:335–345. [PubMed: 9221815]
8. Bromfield SM, et al. *J. Am. Chem. Soc.* 2013; 135:2911–2914. [PubMed: 23406254]
9. Robinson LN, et al. *Electrophoresis.* 2012; 33:797–814. [PubMed: 22522536]
10. Ly M, et al. *Nature Chem. Biol.* 2011; 7:827–833. [PubMed: 21983600]
11. Chi L, et al. *J. Am. Chem. Soc.* 2008; 130:2617–2625. [PubMed: 18247611]
12. Linhardt RJ, Gunay NS. *Semin. Thromb. Hemost.* 1999; 25(suppl. 3):5–16. [PubMed: 10549711]
13. Guerrini M, et al. *Nature Biotechnol.* 2008; 26:669–675. [PubMed: 18437154]
14. Kishimoto TK, et al. *New Engl. J. Med.* 2008; 358:2457–2467. [PubMed: 18434646]
15. Kandrotas RJ. *Clin. Pharmacokinet.* 1992; 22:359–374. [PubMed: 1505142]

**Figure 1.**

Molecular make-up of the complex polysaccharides heparin and heparan sulfate, and the structures of heparin-binding dyes. **a**, Heparin and heparan sulfate are primarily composed of six different types of disaccharide units in different ratios to one another. Numbering of the carbon atoms is shown for the top leftmost disaccharide; sulfation is shown in red; acetylation of the N-2 position is shown in blue. **b**, Structures of heparin-binding dyes: Alcian Blue, Azure A and Mallard Blue. **c**, Molecular dynamics simulations show that Mallard Blue interacts in a nearly stoichiometric ratio with the disaccharide unit within heparin primarily through ionic interactions between the sulfates in the polysaccharide backbone and the guanidinium groups of the dye. Part **c** reproduced with permission from ref. 8, © 2013 ACS.