contaminants and capillary electrophoresis analysis of heparin samples. The authors would like to thank A. d'Avignon of Washington University for the analysis of samples by NMR. The authors also thank J. Esko, E. Conrad, S. Kornfeld, J. Baenziger and D. Tollefsen for their suggestions, comments and critiques of the manuscript. L.Z. thanks E. Unanue, S. Santoro, J. Ladenson and N. Brown for their support in establishing a GAG structure/function laboratory at Washington University. L.Z. thanks J. Metz for critical reading of the original manuscript. This work is supported in part by US National Institutes of Health grant R01GM069968 to L.Z. and a St. Louis Children's Discovery Institute Research Fund to L.Z.

COMPETING INTERESTS STATEMENT
The authors declare no competing financial interests.

Jing Pan[1,2], Yi Qian[1,2], Xiaodong Zhou[1,2], Andrew Pazandak[1], Sarah B Frazier[1], Peter Weiser[1], Hong Lu[1] & Lijuan Zhang[1]

[1]The Department of Pathology and Immunology, Washington University School of Medicine, St. Louis, Missouri, USA. [2]These authors contributed equally to this work.
e-mail: ljzhang@wustl.edu

1. Guerrini, M. *et al. Nat. Biotechnol.* **26**, 669–675 (2008).
2. Guerrini, M. *et al. Proc. Natl. Acad. Sci. USA* **106**, 16956–16961 (2009).
3. Kishimoto, T.K. *et al. N. Engl. J. Med.* **358**, 2457–2467 (2008).
4. Hovingh, P., Piepkorn, M. & Linker, A. *Biochem. J.* **237**, 573–581 (1986).
5. Acostamadiedo, J.M., Iyer, U.G. & Owen, J. *Expert Opin. Pharmacother.* **1**, 803–814 (2000).
6. Anonymous. Danaparoid. <http://en.wikipedia.org/wiki/Orgaran> (accessed 17 February 2010).
7. Frazier, S.B., Roodhouse, K., Hourcade, D.E. & Zhang, L. *Open Glycosci.* **1**, 31–39 (2008).
8. Studelska, D.R., Giljum, K., McDowell, L.M. & Zhang, L. *Glycobiology* **16**, 65–72 (2006).
9. Volpi, N., Maccari, F. & Linhardt, R.J. *Anal. Biochem.* **388**, 140–145 (2009).
10. Zhang, Z. *et al. J. Pharm. Sci.* **98**, 4017–4026 (2009).
11. Liu, H., Zhang, Z. & Linhardt, R.J. *Nat. Prod. Rep.* **26**, 313–321 (2009).
12. Li, F. *et al. Glycoconj. J.* **25**, 603–610 (2008).
13. Fareed, J. *et al. Int. Angiol.* **27**, 457–461 (2008).
14. Volpi, N. *J. Chromatogr. B Biomed. Appl.* **685**, 27–34 (1996).
15. Warda, M., Gouda, E.M., Toida, T., Chi, L. & Linhardt, R.J. *Comp. Biochem. Physiol. C Toxicol. Pharmacol.* **136**, 357–365 (2003).
16. Pan, J. *et al. Glyobiol. Insights* **2**, 1–12 (2010).
17. Maruyama, T., Toida, T., Imanari, T., Yu, G. & Linhardt, R.J. *Carbohydr. Res.* **306**, 35–43 (1998).
18. Toida, T. *et al. Int. J. Biol. Macromol.* **26**, 233–241 (1999).
19. Blossom, D.B. *et al. N. Engl. J. Med.* **359**, 2674–2684 (2008).
20. Ahmad, S. *Front. Biosci.* **12**, 3312–3320 (2007).

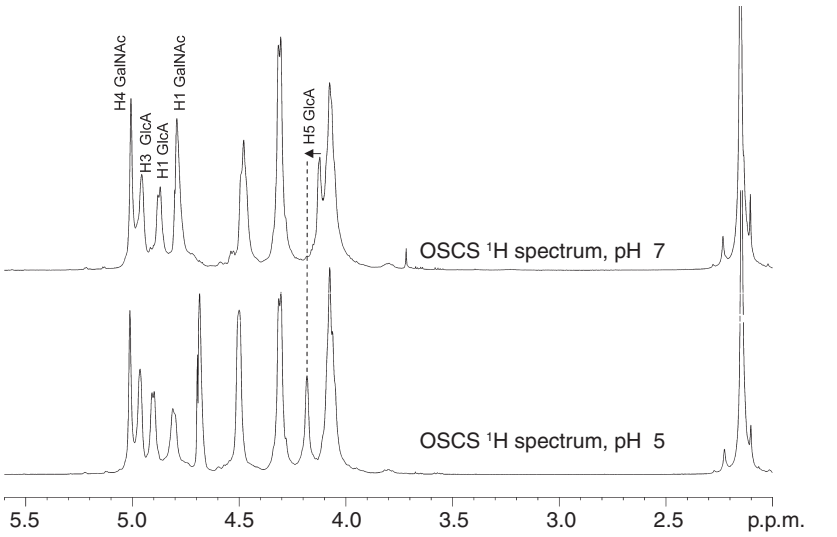

Figure 1 Comparison of the proton NMR spectra of synthesized OSCS at pH 7 and pH 5. The chemical shift of the H5 proton shifts as a function of pH. GalNAc, *N*-acetylgalactosamine; GlcA, glucuronic acid.

Marco Guerrini, Zachary Shriver, Annamaria Naggi, Benito Casu, Robert J Linhardt, Giangiacomo Torri & Ram Sasisekharan reply:

Our paper in *Nature Biotechnology* reported the structural identification of a major contaminant in suspect heparin lots[1]. Notably, the structural assignment of oversulfated chondroitin sulfate (OSCS) was made by four independent laboratories (and has since been confirmed by others[2,3]). In their conclusion and in agreement with our original findings, Pan *et al.* conclude that OSCS is an important contaminant in heparin. The identification of OSCS as a major contaminant within heparin has had a number of ramifications, including providing an underlying logic for the original screening methods developed by the FDA and since implemented into pharmacopeias. Widespread adoption of such methods as screening tools has helped secure the heparin supply and, most importantly, decreased to baseline adverse reactions associated with administration of heparin.

To extend this analysis, we have systematically investigated factors that may influence the signatures associated with OSCS in the proton NMR spectrum. As demonstrated previously, the identity of the counter-ion[4] influences the chemical shifts associated with OSCS. We also find that minor differences between the chemical shifts of the authentic standard and the isolated contaminant displayed in ref. 1, especially the C5 and the H5 of the uronic acid, are likely due to pH differences between the samples (**Fig. 1**). Such pH shifts are not unusual given that samples are typically exchanged and analyzed in unbuffered D_2O. Regardless of minor chemical shift differences, inspection of Table 2 in ref. 1 indicates that the complete chemical shift assignment for OSCS made by two of the laboratories agrees very well with one another and with the original assignment made by Maruyama *et al.*[5]. As such, the structure of OSCS was correctly assigned in Guerrini *et al.*[1]

Additionally, we have completed studies to robustly define how changing the nature of the contaminant, either the monosaccharide identity, linkage or overall sulfation pattern, affects the ability of a variety of analytical techniques to detect a given contaminant. As one part of this investigation, we examined the effect of differential sulfation on the ability of various NMR techniques to detect oversulfated chondroitins. To this end, chondroitins with various degrees of sulfation (ds), ranging from 2.4 to 4.0, were prepared and analyzed by proton NMR. Whereas OSCS (ds = 4.0) is readily detected in the proton NMR in the 2.0–2.2 region, materials with lower ds values are not distinguishable within the same region (**Fig. 2**). Depending on the level of contamination, these materials would not be detected by proton NMR analysis, even upon inspection of the entire spectrum. However, multidimensional NMR is able to distinguish differentially sulfated chondroitin from heparin, owing to several structural signatures, including those of 2, 3-*O*-sulfoglucuronic acid as well as disulfated *N*-acetylgalactosamine (**Fig. 3**). Furthermore, multidimensional NMR can readily detect the presence of oversulfated heparin/heparan sulfate due to several characteristic signals, including 2, 3-*O*-sulphouronic acid (**Fig. 4** and **Supplementary Table 1**). In conclusion, as the acetyl signal of partially sulfated heparin/heparin sulfate or chondroitin show similar proton chemical shifts to that of heparin, a proton NMR method might fail to detect such a contaminant if present in a heparin preparation. Indeed, we have scanned a wide variety of potential sulfated polysaccharide contaminants to

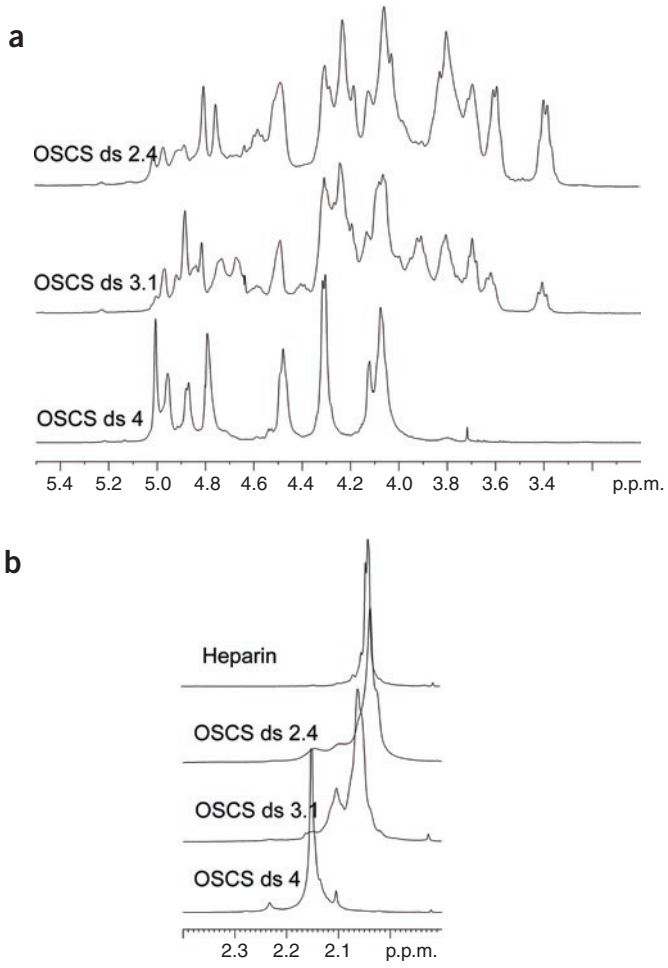

Figure 2 NMR spectra of sulfated chondroitins. (**a**) NMR spectra of sulfated chondroitins with various degrees of sulfation of the disaccharide repeat. (**b**) Zoom of the *N*-acetyl region from 1.9–2.4 p.p.m. and comparison of the various chondroitin products with heparin.

address which are detectable by proton NMR and which are not[6,7]. Taken together, these results reinforce the importance of using orthogonal approaches, including multidimensional NMR (for example, heteronuclear single quantum coherence (HSQC) methods), such as were used in our original paper[1] to ensure accurate identification of contaminants as well as ensure, to as great an extent as is practical, their absence in heparin.

Within this framework, Pan *et al.* argue in their Correspondence that the major contaminant present in heparin could be oversulfated heparin by-products (or components thereof), presumably obtained through chemical sulfonation. At the outset, we find the authors' claim that their data support the presence of other contaminants, beyond OSCS, in suspect heparin lots analyzed in early 2008 unsubstantiated. We would note that Pan *et al.* alternatively use the phrase "NMR" in a way to mean either one-dimensional (1D) proton NMR or the approach used by us[1]. In their letter, they present only 1D proton data, whereas we used both 1D and two-dimensional (2D) NMR experiments to definitively identify major impurities and contaminants in suspect heparin lots. Furthermore, with no experimental substantiation, they claim that, in the context of signals associated with 'native' heparin, contaminants are not always apparent upon inspection of the NMR spectrum and, accordingly, NMR is not useful as a quality-control test. However, in contradiction of this assertion, we and others have shown that multidimensional NMR can detect and quantify contamination that might arise from sulfonation of numerous sidestream components, either alone or mixed with one another[8–10]. Indeed, other persulfonated polysaccharide components could serve a similar role to OSCS, and thus an appropriate testing strategy for heparin should account for not just OSCS but also other potential persulfonated polysaccharide derivatives. This theme is something that has been explored by us (either individually or in collaboration) since the initial structural elucidation of OSCS[6,7,11].

Purification of heparin is a multistep process[12]. Previous studies indicate that (1) the composition and identity of the waste at each purification step is different; and (2) different manufacturing processes can generate waste streams that are structurally and compositionally distinct from one another[9,12]. Pan *et al.* use these points to claim that heparin by-product may contain several major components, including heparan sulfate(HS) and/or chondroitin sulfate (CS) and/or dermatan sulfate (DS). Unfortunately, they present no quantitative analysis of the percentages of these components, but rather cite data from analysis of heparin purified from camel intestine and bovine lung (refs. 15 and 17 of Pan *et al.*). However, pharmaceutical heparin, used clinically, is derived from porcine intestine (or intestinal mucosa). As such, findings regarding products from species other than pig have no real relevance to the identity of sidestream products produced as part of the purification of pharmaceutical heparin. Furthermore, Pan *et al.* attempt to support their argument by stating that the content and sequence of component glycosaminoglycans present in porcine mucosa can change based on subspecies, age and environment, without providing supporting data. In addition, the references they cite (refs. 11, 12 and 14 within Pan *et al.*) do not support this point. In follow-up studies, completed after initial publication of the structure of OSCS, we demonstrate that for one porcine-based process, dermatan sulfate is a major impurity and sidestream product produced from crude heparin[9]. Conversely, very little CS is found as a waste product in the manufacture of heparin from porcine intestines. Persulfonation of this sidestream material, enriched in DS, would accordingly lead to primarily oversulfated DS, which would be detected in NMR analysis and is readily differentiated from OSCS (Figure S8 and Figure 3 of ref. 9).

Notably, the quality of the NMR spectra and results reported by Pan *et al* is poor (including, but not limited to, a noticeable lack of splitting of the H2 glucosamine signal and low resolution of glucosamine and iduronic acid anomeric signals). We, and others, have found that accurate quantification of proton signals is strongly related to the quality and resolution of the spectrum[9,13]. Proper magnetic field (≥500 MHz), sample concentration and solution pH value, together with the absence of multivalent cations are conditions that must be carefully controlled to ensure appropriate NMR measurements. For example, in Supplementary Figure 5b of Pan *et al.*, there is significant line broadening of the H1 proton of 2-*O*-sulfo iduronic acid (5.20–5.30 p.p.m.), indicative of suboptimal sample preparation and/or analysis conditions. Significant and selective line broadening, such as is observed in Supplementary Figure 5 of Pan *et al.*, is typically observed in samples contaminated by multivalent cations. Furthermore, the authors assert that proton signals of A-2, A-3 and A-4 (ring positions of *N*-sulfoglucosamine) are much higher than those of the rest of the protons. As shown in previous studies using HSQC analysis[13], only the H2 of *N*-sulfoglucosamine is usually sufficiently resolved from the rest of protons to be quantified accurately in the 1D proton spectra. Conversely, the H3 and H4 protons severely overlap with other signals belonging to unsulfated uronic acid (H2 of iduronic acid and H3, H4 and H5 of glucosamine) and thus show an overall higher intensity in the corresponding 1D spectrum[10,14].

In addition, at several points in their Correspondence, Pan *et al.* state that different data sets, derived from Supplementary Table 1 in the present Correspondence, support one another and thus confirm their results, citing specific examples. However, looking at the data as a whole, there are some puzzling findings that raise important questions. For example, analysis of the data for the 32 samples that have both hexosamine and heparinase results, indicates that the heparinase data do not correlate with the galactosamine data (**Supplementary Fig. 1**). The data clearly form two clusters, on the basis of whether there is an early-migrating peak (ostensibly OSCS) in capillary electrophoresis; however, in neither cluster is there a significant correlation between increasing galactosamine content and decreased susceptibility to heparinase digestion (r^2 values of 0.098 and 0.014 for early and late migrating peaks, respectively). On the basis of this analysis, the data sets do not, in fact, support one another, nor do they support the actual presence of another major contaminant in heparin.

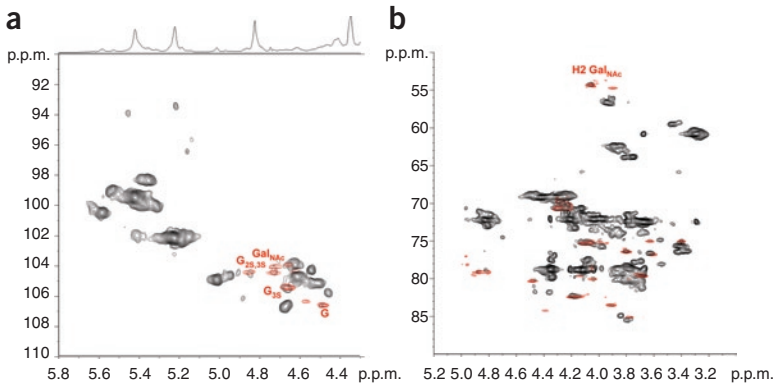

Figure 3 Two-dimensional HSQC analysis of a 10% wt/wt mixture of sulfated chondroitin (ds = 3.1) in heparin compared to a reference standard of sulfated chondroitin. (**a**) The anomeric region. (**b**) Signals from outside the anomeric region. Clearly identifiable signals associated with 2,3-di-*O*-sulfoglucuronic acid ($G_{2S,3S}$) and sulfated *N*-acetylgalactosamine (Gal_{NAc}) are distinct from those of heparin. G_{2S}, 2-*O*-sulfoglucuronic acid; G, glucuronic acid; black, heparin; red, reference standard of sulfated chondroitin.

The enzymatic studies presented by Pan *et al.* are confounded, as evidenced by the fact that in their Supplementary Figure 1 (and Supplementary Table 1 of Pan *et al.*), the authors use inhibition of heparinases as a readout of contamination/impurity levels. Nevertheless, in Table 1 of their Correspondence, they use enzyme susceptibility to determine the percentage of heparin-like components and chondroitin/dermatan sulfate–like components in partially purified impurities/contaminants (for example, so-called low-sulfated contaminants). How could they use such an enzymatic assay to determine the percentage of individual components if the enzymes used to benchmark such determinations are inhibited by the presence of other components? Indeed, enzymatic digestion is a valuable tool for both characterization and quality control of heparin material; however, the data presented by Pan *et al.* are not supportive of their conclusions.

Finally, interwoven in their argument around heparin by-product, they also attempt to advance an argument that oversulfated heparan sulfate could be present in heparin. First, as mentioned above, persulfonated heparan sulfate, if present, would have characteristic structural signatures that would be readily detected, especially in a multidimensional NMR experiment. Indeed, a recent study by us demonstrated that, in contradiction to the assertion of Pan *et al.*, a well-controlled proton NMR spectrum, or, preferably, a multidimensional NMR experiment, can readily distinguish oversulfated heparin/heparan sulfate in the context of a mixture (respectively, Supplementary Fig. S10 and Fig. 5 of ref. 9). Second, there is also ambiguity in the definition of "heparan sulfate" as used by Pan *et al.* In the context of the biology, Pan *et al.* seem to refer to heparan sulfate as porcine intestinal heparan sulfate that has a degree of sulfation of ~0.3 sulfates per disaccharide[15]. However, in the context of the precipitation experiments, "heparan sulfate" likely refers to a different set of compounds; namely, undersulfated chains of heparin. Because heparin is a mixture of polysaccharide chains, which differ in terms of overall sulfation and sulfation pattern, isolated components of the mixture (for example, by molecular mass, sulfation density) have been shown to have different properties from the whole mixture[12]. Purification of OSCS from heparin is a rather difficult process, given the overall polydispersity of heparin. As such, one plausible explanation for the glucosamine/galactosamine content of the 'purified' contaminant presented by Pan *et al.* is that the contaminant is only partially purified and thus still contains chains of heparin with less sulfate density. This interpretation would also explain the data presented in Supplementary Figure 5 of their Correspondence. Here, the major

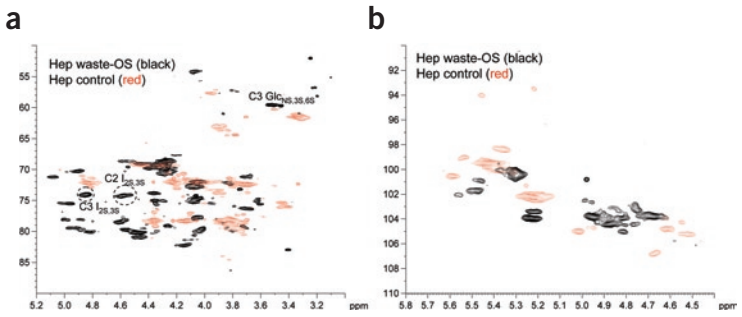

Figure 4 Two-dimensional HSQC analysis of oversulfated heparin waste compared to authentic heparin. (**a**) The HSQC spectrum excluding the anomeric region. Clearly observed signals from oversulfated heparin/heparan sulfate include disulfated uronic acid. (**b**) HSQC spectrum of the anomeric region.

resonances due to polysaccharides in spectrum B labeled "Purified Oversulfated Heparin Contaminant from Lot 2007-23" arise from just two sets of signals, one of which can clearly be assigned to heparin and the other of which can be assigned to OSCS.

Pan *et al.* conclude their Correspondence stating, "This heterogeneity complicates the analysis and evaluation of…each component of oversulfated heparin by-product. Clearly, further analysis would benefit from the use of 2D NMR methods to resolve these findings." Such an approach is precisely the type of approach that was completed, first in 2008 (ref. 1) and later in 2009 (ref. 9). In these papers, multidimensional NMR, run in multiple laboratories, allowed us to identify or confirm signatures that arise from oversulfated glycosaminoglycans (GAGs), either in isolation or in the presence of heparin. To extend our results, we also reanalyzed our HSQC data presented in ref. 1 to determine the monosaccharide composition of samples C1–C2 and S1–S6 based upon a published methodology (**Table 1**)[13,16]. Consistent with our original findings, quantification of the monosaccharide composition of the heparin-like component (which would include heparan sulfate or oversulfated heparin/heparan sulfate, if present) indicates no appreciable differences between samples C1–C2 and S1–S6. If, for example, heparan sulfate was a significant impurity, we would expect to see a higher level of *N*-acetylglucosamine in one or more of samples S1–S6 and a correspondingly lower level of overall sulfation, including 6-*O*-sulfoglucosamine and 2-*O*-sulfoiduronic acid. These signatures are not observed. Finally, in none of the samples are the signatures observed for 2,3-di-*O*-sulfouronic acid, a key signature for oversulfated heparin-like components.

In addition, we also would like to comment on and clarify a set of statements made by the authors with regards to how

Table 1 Quantitative HSQC analysis of samples C1, C2 and S1–S6

Constituent (mole%)	Sample							
Glucosamines[a]	**C1**	**C2**	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**
N-sulfoglucosamine–2-*O*-sulfoiduronic acid disaccharide	58.0	62.7	61.9	61.5	66.2	59.3	59.4	66.4
N-sulfoglucosamine–iduronic acid disaccharide	9.1	9.3	8.7	12.9	8.3	10.8	9.7	8.4
N-sulfoglucosamine–glucuronic acid disaccharide	10.7	6.9	8.9	9.7	6.3	10.0	8.4	8.4
N-acetylglucosamine	14.6	13.6	14.6	11.8	13.1	12.3	14.2	10.6
3-*O*-sulfo, *N*-sulfoglucosamine	6.7	6.6	5.9	4.1	6.1	7.6	8.2	5.6
N-sulfoglucosamine at the reducing end of the GAG chain	1.0	0.8	0.0	0.0	0.0	0.0	0.0	0.6
6-*O*-sulfoglucosamine[a]	77.0	76.0	84.8	87.2	82.1	83.0	84.0	81.4
Uronates[b]								
2-*O*-sulfoiduronic acid	73.6	66.1	73.2	71.1	70.7	67.0	68.8	71.7
Iduronic acid–6-*O*-sulfo, *N*-acetyl/sulfoglucosamine disaccharide	7.6	8.1	6.8	5.6	8.1	6.1	6.1	6.3
Iduronic acid–*N*-acetyl/sulfoglucosamine disaccharide[c]	2.1	1.4	4.2	3.7	5.2	4.9	2.3	2.7
Glucuronic acid–*N*-sulfoglucosamine disaccharide	8.5	11.2	8.6	7.7	7.6	8.3	8.2	8.3
Glucuronic acid–3-*O*-sulfo, *N*-sulfoglucosamine	2.7	4.5	2.7	3.2	1.6	3.4	3.6	2.1
Glucuronic acid–*N*-acetyl glucosamine disaccharide	5.4	5.9	4.4	5.2	6.6	7.4	6.3	7.2
Galacturonic acid	0.0	2.8	0.0	1.8	0.0	1.4	2.6	1.2
Epoxide	0.0	0.0	0.0	1.7	0.0	1.5	2.0	0.5
Linkage region	5.3	2.6	4.4	3.2	4.3	3.6	3.5	3.4

[a]Normalized mole percentage of various glucosamine moieties. Measurement of total 6-*O* sulfation is completed separately and itself is normalized to 100%. Also, quantification of 6-*O* sulfoglucosamine in heavily contaminated samples is increased owing to the signal being proximate to an additional signal at 4.3/69.0 p.p.m. [b]Normalized mole percentage of various uronic acid moieties. Measurement of the linkage region is completed separately and is not included in the overall normalization. [c]Quantification of iduronic acid linked to *N*-sulfoglucosamine or *N*-acetylglucosamine in heavily contaminated samples is increased owing to the signal being close to another signal at 4.9/104.2 p.p.m.

they frame and/or interpret the data and conclusions from previous studies[1,17]. First, they repeatedly attribute to us a claim as to the source of OSCS, namely porcine cartilage. OSCS may very well originate from persulfonation of porcine cartilage; however, we have not asserted such, nor have we presented data that can be used to definitively assign source. In our set of analyses, persulfonation removes any information, such as composition, that could be obtained regarding the source of the chondroitin sulfate.

Furthermore, they claim that in the study of Kishimoto *et al.*[17], quantification of the amount of dermatan sulfate and OSCS requires two assumptions: first, each of the 15 protons in each of the disaccharide units of the polysaccharides produces signals of the same intensity; and second, dermatan sulfate consists of 100% iduronic acid and *N*-acetylgalactosamine residues. The calculation does not require making either of these assumptions. The first assumption, namely, equivalence in signal intensity, is most assuredly not the case, owing to signal overlap and sequence heterogeneity. Second, several studies have identified that dermatan sulfate has a major *N*-acetyl signal at ~2.08 p.p.m., arising from the *N*-acetyl galactosamine attached to iduronic acid[18]. Previously, it has been shown that porcine intestinal mucosa dermatan sulfate has ≥90% iduronic acid[19], a value that agrees well with the 87% calculated by Pan *et al.* Furthermore, we conducted proton NMR analysis of dermatan sulfate to confirm this fact (**Supplementary Fig. 2**). Finally, the calculation presented in ref. 17 is well-founded and is similar to one previously used by Perlin and colleagues[20] to calculate the amount of dermatan sulfate in a heparin preparation. We have confirmed the quantitative analysis of samples through spike and recovery experiments (Supplementary Fig. 3). In addition, it is important to note two points on this analysis. First, the small separation in proton NMR of the $-CH_3$ signal of the *N*-acetyl peak of heparin and dermatan sulfate requires a high-field instrument and careful experimental control to ensure accurate results. Second, although Pan *et al.* equate not determined (ND) to '0.0' in the "published results"[17] column of Table 1 in their Correspondence, this need not be the case, owing to the limit of quantification for both the proton NMR method and the galactosamine method of Pan *et al.* As such, direct comparison of these results to one another is not warranted.

Therefore, based on the data presented in our original paper[1], the scientific literature on the topic and data presented herein, we conclude the following:

• OSCS is a major contaminant in suspect heparin lots collected in February/March of 2008.

• Analysis of heparin samples using orthogonal analytical techniques, including high-resolution capillary electrophoresis and multidimensional NMR, indicates that oversulfated GAG mixtures are not a major contaminant in tested samples[1,9,17]. Nevertheless, analytical control tests for heparin should be assessed for their ability to detect oversulfated GAG mixtures, including individual components, most pertinently oversulfated dermatan sulfate. We have shown that a combination of 1D and 2D NMR, in conjunction with other analytical procedures, can readily detect such compounds, if they are present.

• Oversulfation of heparin/heparin sulfate has been shown to lead to signature monosaccharides, including 3-*O* sulfoiduronic acid, 2,3-di-*O*-sulfouronic acid, and potentially N-desulfonation[10,21] (**Fig. 4** and **Supplementary Table 1**). Multidimensional NMR can readily detect signatures for oversulfated heparin (including the aforementioned 2,3-di-*O*-sulfouronic acid), if they are present in a heparin preparation[9]. We observed none of these signals in any of the tested samples, indicating that oversulfated heparin/heparan sulfate is not a major contaminant.

• In 1D NMR experiments, certain signals may be masked if heparin contains a heparan sulfate impurity or is contaminated with oversulfated heparan sulfate. This is not a result of an interaction between polysaccharide species, it is simply because these species share close chemical identity. This is an important argument as to why orthogonal analytical approaches, including multidimensional NMR measurements[1], should be used for the identification of contaminants and/or impurities in heparin.

In conclusion, the most important result of the efforts in late 2007 and early 2008 of many individuals and organizations, including scientists from government, industry and academia, is an improvement in clinical outcomes for heparin treatment: since capillary electrophoresis and NMR tests to screen for OSCS were introduced by the Food and Drug Administration, the number of adverse events associated with heparin administration has been reduced to baseline once again. As such, starting with the introduction of screening methods, through the structural and biological work to identify and understand OSCS, to the monograph revisions instituted by the various pharmacopeias, the heparin supply chain has been secured, though constant vigilance is warranted.

Note: Supplementary information is available on the Nature Biotechnology website.

Note added in proof: since the submission of this letter, another study has confirmed the findings in Guerrini et al.[1] and Kishimoto et al.[17] that OSCS is a major contaminant in heparin (McKee et al. Structure Elucidation and Biological Activity of the Oversulfated Chondroitin Sulfate Contaminant in Baxter Heparin, J. Clin. Pharm. (epub ahead of print, 10 February 2010, doi:10.1177/0091270009355158)

ACKNOWLEDGEMENTS

The authors thank G. Cassinelli, M. Nasr and L. Buhse for discussions and the US National Institutes of Health for grant HL101721 to R.S. and R.J.L. to support this work.

COMPETING INTERESTS STATEMENT

The authors declare competing financial interests: details accompany the full-text HTML version of the paper at http://www.nature.com/naturebiotechnology/.

1. Guerrini, M. *et al. Nat. Biotechnol.* **26**, 669–675 (2008).
2. Trehy, M.L., Reepmeyer, J.C., Kolinski, R.E., Westenberger, B.J. & Buhse, L.F. *J. Pharm. Biomed. Anal.* **49**, 670–673 (2009).
3. Viskov, C. *et al. Clin. Appl. Thromb. Hemost.* **15**, 395–401 (2009).
4. McEwen, I. *et al. J. Pharm. Biomed. Anal.* **49**, 816–819 (2009).
5. Maruyama, T., Toida, T., Imanari, T., Yu, G. & Linhardt, R.J. *Carbohydr. Res.* **306**, 35–43 (1998).
6. Zhang, Z. *et al. J. Pharm. Sci.* (in the press).
7. Guerrini, M. *et al. Thromb. Haemost.* **102**, 907–911 (2009).
8. Toida, T. *et al. Int. J. Biol. Macromol.* **26**, 233–241 (1999).
9. Guerrini, M. *et al. Proc. Natl. Acad. Sci. USA* **106**, 16956–16961 (2009).
10. Casu, B. *et al. Carbohydr. Res.* **263**, 271–284 (1994).
11. Li, B. *et al. Biochem. Pharmacol.* **78**, 292–300 (2009).
12. Griffin, C.C. *et al. Carbohydr. Res.* **276**, 183–197 (1995).
13. Guerrini, M., Naggi, A., Guglieri, S., Santarsiero, R. & Torri, G. *Anal. Biochem.* **337**, 35–47 (2005).
14. Yates, E.A. *et al. Carbohydr. Res.* **294**, 15–27 (1996).
15. Toida, T. *et al. Biochem. J.* **322 (Pt 2)**, 499–506 (1997).
16. Bisio, A. *et al. Thromb. Haemost.* **102**, 865–873 (2009).
17. Kishimoto, T.K. *et al. N. Engl. J. Med.* **358**, 2457–2467 (2008).
18. Linhardt, R.J. *et al. Biochem. Pharmacol.* **42**, 1609–1619 (1991).
19. Sudo, M. *et al. Anal. Biochem.* **297**, 42–51 (2001).
20. Neville, G.A., Mori, F., Holme, K.R. & Perlin, A.S. *J. Pharm. Sci.* **78**, 101–104 (1989).
21. Yates, E.A. *et al. Carbohydr. Res.* **329**, 239–247 (2000).