Supporting Information

Dong et al. 10.1073/pnas.1104834108

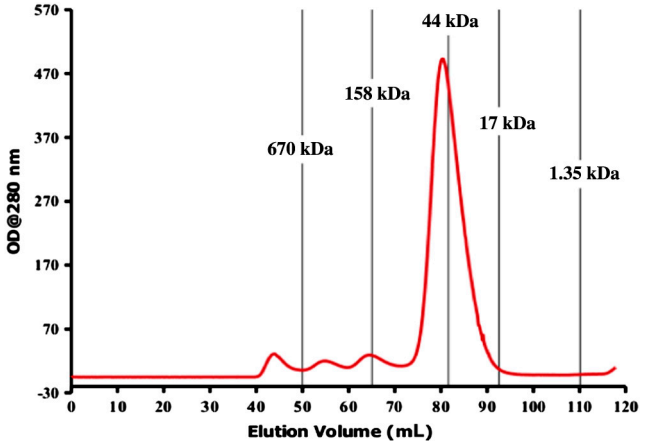

Fig. S1. Astrovirus $P2^{415–646}$ forms dimer in solution. $P2^{415–646}$ was applied to size-exclusion chromatography using a Superdex 200 column (GE Healthcare). $P2^{415–646}$ was eluted at approximately 80.3 mL with an apparent molecular mass of approximately 60 kDa. The droplines show the peak positions of protein standards.

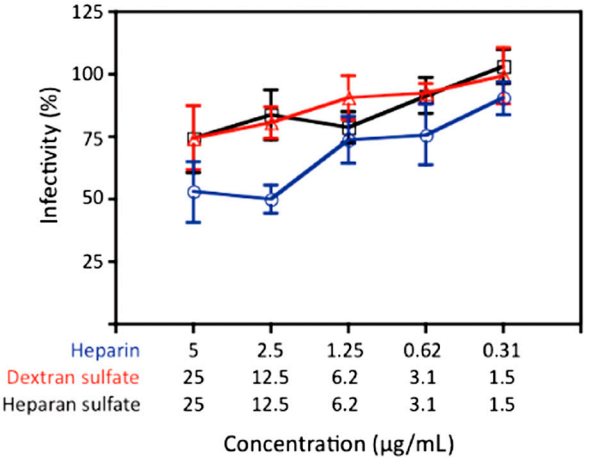

Fig. S2. Partial inhibition of astrovirus infectivity by heparin. Caco-2 cells were cultured in 96-well plates for 3–4 d in media with FBS until cells were 100% confluent. Human astrovirus (serotype 8) was pretreated with 200 μg/mL trypin for 1 h at 37 °C to activate infectivity; after that, soybean trypsin inhibitor was added at the same concentration to avoid cell detachment during virus adsorption. Approximately 4,000 focus forming units of activated virus, *ca.* 50 μL in volume, were mixed with each of the polysaccharides at the indicated concentration and incubated for 30 min at 37 °C. This virus mixture was then added to each well of the Caco-2 cell plate. After incubation at 37 °C for 1 h, the inoculum was removed and cells were washed twice with minimal essential medium without serum. Afterward, the plate was incubated with fresh media for another 16 h. At the end of the infection, cells were fixed and immunostained with anti-HAstV-8 serum to quantify infected cells. The effect of the reagents was reported as the percentage of the virus infectivity observed in the absence of any treatment. All measurements were performed in duplicate. Because the infectivity did not drop to zero with heparin at 5 μg/mL, it appears that this interaction is somehow weak, or that additional cell molecules might also participate in the initial attachment step.

Table S1. Crystallographic data statistics

Selenomethionine-P2$^{415–646}$ *diffraction data collection*

Wavelength, Å	0.97973 (inflection)	0.97911 (peak)	0.96411 (remote)
Space group	$P3_221$		
Unit cell dimensions	$a = b = 48.3$ Å, $c = 167.3$ Å, $\alpha = \beta = 90°$, $\gamma = 120°$		
Resolution, Å	44.00 – 2.02	44.00 – 2.02	44.00 – 2.02
High-resolution shell, Å	2.07 – 2.02	2.07 – 2.02	2.07 – 2.02
R_{sym},* %	6.7 (33.0)	7.2 (42.5)	6.4 (35.5)
Mean $I/\sigma(I)$	25.2 (4.2)	22.5 (2.5)	25.1 (4.1)
Total reflection no.	15,743 (1,266)	15,735 (1,274)	15, 757 (1,280)
Completeness, %	100	100	100
Selenium site no.	6		
Overall figure of merit†	0.55		

P2$^{415–646}$ *native data collection*

	Crystal form 1	Crystal form 2
Unit cell dimensions	$a = b = 48.4$ Å, $c = 167.1$ Å; $\alpha = \beta = 90°$, $\gamma = 120°$	$a = b = c = 166.5$ Å; $\alpha = \beta = \gamma = 90°$
Spacegroup	$P3_221$	$I2_13$
Resolution, Å	25.00 – 1.80	50.00 – 2.80
High-resolution shell, Å	1.83 – 1.80	2.86 – 2.80
R_{sym},* %	8.5 (48.1)	7.6 (58.0)
Mean $I/\sigma(I)$	49.4 (2.2)	59.3 (6.6)
Total reflection no.	21,767 (1,067)	17,920 (1,236)
Refinement statistics		
Completeness, %	98.8	94.7
R_{work}/R_{free},‡ %	17.97/21.54	24.75/28.12
Average B factor, Å^2	25.4	62.5
Total atom no.	1,966	1,735
Water molecule no.	137	18
Bond angles, °	1.2658	1.5184
Bond length, Å	0.0096	0.0080

*$R_{sym} = \sum\sum |I_{hkl} - I_{hkl(j)}| / \sum I_{hkl}$, where $I_{hkl(j)}$ is the observed intensity and I_{hkl} is the final average intensity.
†Figure of merit $= \langle |\sum P(\alpha) e^{i\alpha} / \sum P(\alpha)| \rangle$, where α is the phase and $P(\alpha)$ is the phase probability distribution.
‡$R_{work} = \sum ||F_{obs}| - |F_{calc}|| / \sum |F_{obs}|$ and $R_{free} = \sum ||F_{obs}| - |F_{calc}|| / \sum |F_{obs}|$, where R_{free} and R_{work} are calculated using a randomly selected test set of 5% of the data and all reflections excluding the 5% test data, respectively. Numbers in parentheses are for the high-resolution shell.