Supplementary Information

Crystal structure of histidine-rich glycoprotein N2 domain reveals redox activity at an interdomain disulfide bridge: Implications for angiogenic regulation

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Table S1: Collection statistics for the highest resolution shells of crystallographic data.

Figure S1: Free thiol measurements of rabbit serum, plasma and HRG preparations.

Figure S2: Mass spectrometric analysis of rabbit serum- and plasma-derived HRG preparations.

Table S1. Collection statistics for the highest resolution shells of crystallographic data. These data illustrate where the completeness drops below 90%.

Resolution (Å)	R_{merge}	Completeness (%)	Multiplicity	I / σ (I)
2.56 – 2.43	0.113	99.0	4.6	7.5
2.43 – 2.31	0.133	99.4	4.6	6.9
2.31 – 2.20	0.162	99.7	4.4	5.6
2.20 – 2.10	0.192	96.2	3.7	4.2
2.10 – 2.03	0.248	82.9	3.0	2.8
2.03 – 1.93	0.329	69.2	2.7	2.1

Figure S1. Free thiol measurements of rabbit serum, plasma and HRG preparations. The samples were diluted/reconstituted in PBS, pH 7.2 and thiol determination carried out using 5,5'-dithiobis(2-nitrobenzoic acid) following a modified method based upon that of Ellman.[48] **(**A) The free thiol concentrations in rabbit serum and plasma differed and were determined to be 250 µM and 208 µM, respectively. (B) The free thiol contents of HRG purified from both rabbit serum and plasma were very similar ~0.7 mol/mol, with this likely corresponding to the reduced form of Cys407. Error bars indicate standard deviation. Statistical analyses were performed using a T-test (** indicates $p<0.01$; n=3).

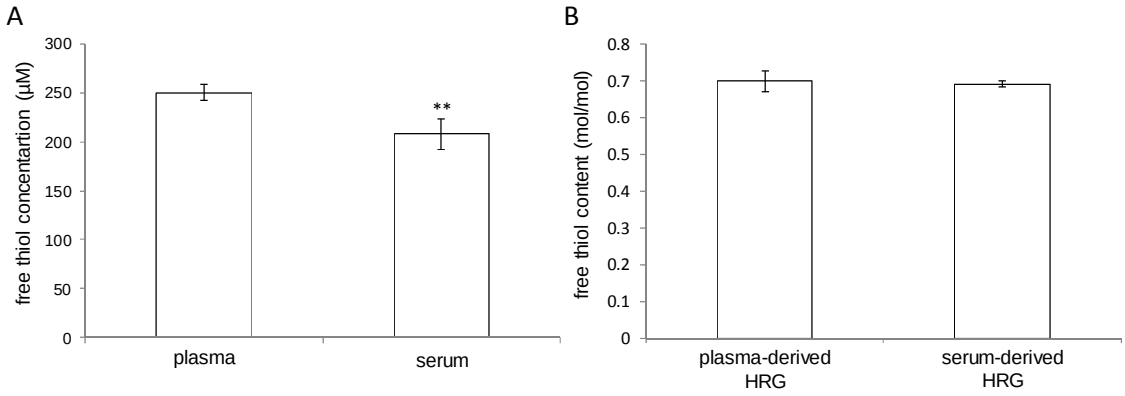

Figure S2. Mass spectrometric analysis of rabbit serum- and plasma-derived HRG preparations. The protein sample (20 µl, 1 mg/mL) was desalted on-line through a MassPrep On-Line Desalting Cartridge 2.1 x 10 mm, eluting at 50 µl/min, with an increasing acetonitrile concentration (2 % acetonitrile, 98 % aqueous 1 % formic acid to 98 % acetonitrile, 2 % aqueous 1 % formic acid) and delivered to a Waters LCT electrospray ionisation mass spectrometer which had previously been calibrated using myoglobin. An envelope of multiply charged signals was obtained and deconvoluted using MaxEnt1 software to give the molecular mass of the protein. The spectra reveal the serum- and plasma-derived HRG preparations to be almost identical in composition. One major form of HRG can be observed, which in each sample has essentially the same molecular mass (*ca.* 72,179 Da). A minor form can also be observed (*ca.* 72,835 Da), which likely corresponds to an alternative glycosylated form of the protein.