Supporting Information

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SI Text

***E. coli* Recombinant Protein Expression and Purification.** The gene encoding FCR3-DBL3X (accession AY372123), cloned into a modified pET15b vector, was a kind gift from Dr. Matthew K. Higgins; expression was carried out as previously described (1). The gene encoding FCR3-DBL6ε (accession AY372123; residues 2326-2633) was amplified by PCR and cloned into pET15b vector between the NdeI and XhoI restriction sites, in frame with the hexa-His tag. Both proteins were expressed in the Rosetta-Gami or Origami B strains of *E. coli* (Novagen) as soluble proteins at 20 °C for 20 h after IPTG induction. Postinduction cells were centrifuged, resuspended in 20 mM Tris-HCl, 150 mM NaCl, pH 7.5 and lysed with an Emulsiflex homogeniser (Avestin). The proteins were purified using a metal affinity column (TALON, Clontech), followed by gel filtration (S75 16/60, GE Healthcare). The yield for both proteins was ~10 mg per liter of culture.

Circular Dichroism. All measurements were performed at A_{280} 0.2–0.3. Far-UV CD spectra were recorded in a cell with a 0.1 cm path-length from 185 to 260 nm (0.5 nm step) with an averaging time of 3 seconds per step at 25 °C. The spectra, corrected using buffer baselines measured under the same conditions, were normalized to molar peptide bond concentration and path-length as mean molar differential coefficient per residue. Secondary structure estimates were derived from the normalized spectra using the CDSSTR method included in the CDPro software by comparison with a reference set of spectra from 43 different proteins (2). Thermal denaturation was recorded at 222 nm from 10 °C to 95 °C using a temperature increase of 1 °C for each step.

Analytical Ultracentrifugation. The recombinant proteins FCR3-DBL3X, FCR3-DBL6ε, 3D7-DBL4ε-6ε, and 3D7-DBL1X-6ε were used at 0.68 mg/mL, 0.5 mg/mL 0.3 mg/mL and 0.58 mg/mL, respectively. Experiments were carried out in an An-Ti 50 rotor at 20 °C using a 1.2-mm thick two-channel Epon centerpiece at a rotor speed of 42,000, 42,000, 25,000, and 20,000 rpm for 8 hours, for FCR3-DBL3X, FCR3-DBL6ε, 3D7-DBL4ε-6ε, and 3D7-DBL1X-6ε, respectively. C(s) distributions were calculated with a fitted frictional ratio f/f0 and a maximum entropy regularization procedure with a confidence level of 0.95. Theoretical sedimentation values of each protein were calculated with the HYDROPRO 7c program (3) using the published coordinates for FCR3-DBL3X and FCR3-DBL6ε, the extended model or the ab initio SAXS model coordinates for 3D7-DBL1X-6ε.

Small-Angle X-ray Scattering. 30 μL of protein solution, loaded into a 2 mm quartz capillary mounted in vacuum, were measured using an automated robotic system that minimized the effect of radiation damage by passing the sample through the beam during x-ray exposure at a constant speed. Two-dimensional scattering images were collected using a Vantec200 gas-filled detector (Bruker) at a distance of 1.745 m from the sample. Standard data collection time of 5 minutes, split into ten 30 second exposure frames, was used for all samples. Individual time-frames were processed automatically and independently by the data collection software BsxCUBE, developed at the European Synchrotron Radiation Facility (ESRF), yielding individual radially averaged curves of normalized intensity versus scattering angle ($s = 4\pi \sin\theta/\lambda$). These were combined to give the average scattering curve for each measurement. The scattering from the buffer alone was measured before and after each sample measurement and the average was used for background subtraction using the program PRIMUS (4) from the ATSAS software package. Assessment of scattering data collected in ten 30-second frames did not show any changes in the scattering due to radiation induced aggregation. A range of DBL1X-6ε concentrations obtained by dilution (1.1–3.3 mg/mL) was measured in order to assess and remove any concentration dependent interparticle effects. The normalized curve free from radiation or interparticle effects (with the best signal to noise ratio) obtained was then used to determine the model-independent scattering parameters using GNOM from the PRIMUS software package.

We generated a simple extended model of the extracellular domain of var2CSA consisting of ten structural modules: six DBL domains and four interdomain regions. To date, the crystal structures of only two DBL domains of PfEMP1-var2CSA are known (DBL3X and DBL6ε lacking the N terminus). There are no known structures containing interdomain regions of PfEMP1. We used the coordinates of DBL homologues of the appropriate length for the DBL domains for our model: DBL1α for DBL1X and DBL2X, DBL3X (3BQK) for DBL3X and DBL4ε. The two-domain structure of EBA175 (1ZRL) was used to model DBL5ε-Int4-DBL6ε. The large interdomain region (Int2) between DBL2X and DBL3X was modeled using the CIDR structure (3C64), as this region is predicted to be highly helical and contains about the same number of residues. We used the interdomain linker from EBA175 to model Int1 and Int3. All modules were then positioned "head-to-tail" (N-terminal to C-terminal between successive modules) by computer graphics.

Surface Plasmon Resonance. CM5 sensor chips, the amine coupling kit, ethanolamine, and surfactant P20 were from GE Healthcare. Placental CSPG (MR4) was covalently immobilized *via* primary amino groups on the sensor chip surface as described elsewhere (5). The amount of immobilized CSPG corresponded to 240 Response Unit. A separate flow channel on the same sensor chip, reserved for control runs, was prepared in the same way but without CSPG. For all SPR measurements, the recombinant domains were dialyzed against PBS buffer (Gibco), 0.005% P20, and centrifuged immediately before the runs to minimize possible effects from nonspecific aggregation. The association was monitored by injecting different concentrations of the DBL analytes at 25 °C at flow rate of 40 μL/minute for 300 seconds to achieve steady-state binding. Between each injection, surfaces were regenerated by four washes with 40 μL of 2 M NaCl. All curves were corrected for nonspecific binding by subtraction of control curves obtained from injection of the corresponding protein through the blank flow channel.

The kinetic association and dissociation constants (k_{on} and k_{off}) could not be estimated to determine K_D because the data did not fit a simple 1:1 kinetic model. CSPG is a heterogeneous ligand, which was furthermore immobilized in a random orientation on the Biacore sensorchip. Quantitative analysis of the kinetic curves was thus limited to the concentration dependence of the steady-state SPR response, which gives a global average estimation of the K_D. The affinity constants were calculated from the plot of the steady-state binding as a function of protein concentration, using the Biacore BIAEVALUATION 3.1 software (Biacore AB).

1. Higgins MK (2008) The structure of a chondroitin sulfate-binding domain important in placental malaria. *J Biol Chem* 283(32):21842–21846.
2. Sreerama N, Woody RW (2000) Estimation of protein secondary structure from circular dichroism spectra: comparison of CONTIN, SELCON, and CDSSTR methods with an expanded reference set. *Anal Biochem* 287(2):252–260.
3. Garcia De La Torre J, Huertas ML, Carrasco B (2000) Calculation of hydrodynamic properties of globular proteins from their atomic-level structure. *Biophys J* 78(2):719–730.
4. Konarev PV, Volkov VV, Sokolova AV, Koch MHJ, Svergun DI (2003) PRIMUS: a Windows PC-based system for small-angle scattering data analysis. *J Appl Crystallogr* 36 1277–1282
5. Badaut C, et al. (2007) Receptor-binding studies of the DBL gamma domain of *Plasmodium falciparum* erythrocyte membrane protein 1 from a placental isolate. *Mol Biochem Parasitol* 151(1):89–99.

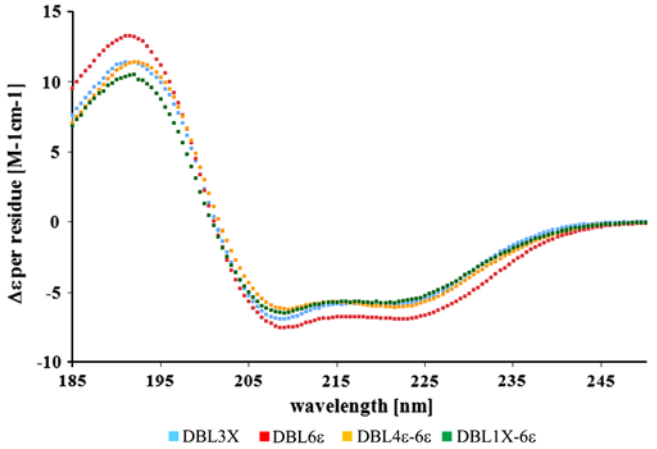

Fig. S1. Secondary structure analysis by CD. Deconvolution of the far-UV spectra shows that var2CSA derived proteins are highly α-helical with a secondary structure content similar to that observed in other DBL proteins.

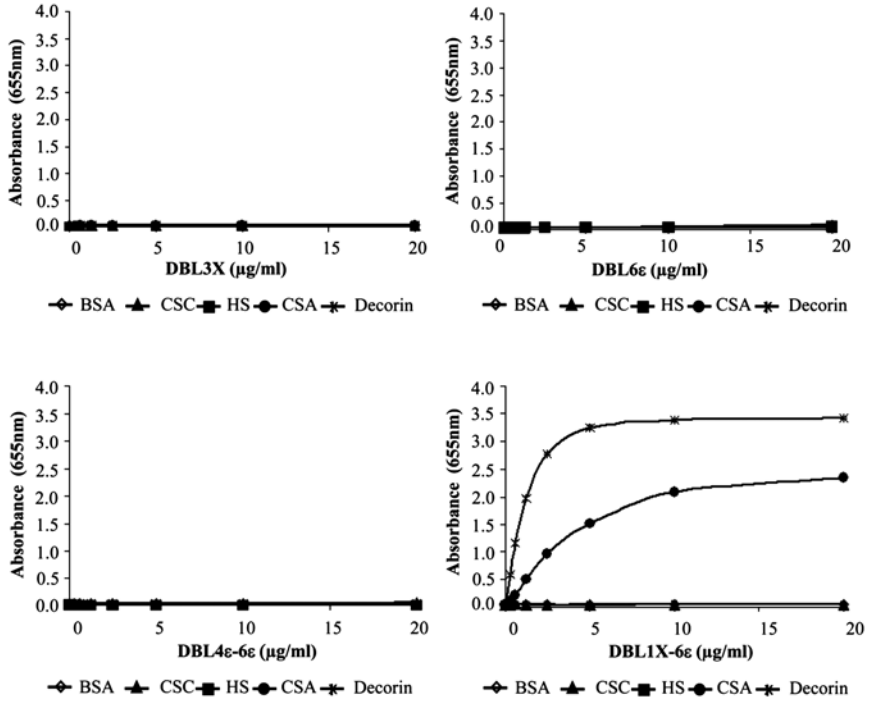

Fig. S2. ELISA-based direct binding assay: binding of the recombinant DBL domains to different glycosaminoglycans. Increasing concentrations of each recombinant protein at serial dilutions of 0.31–20 μg/mL were added to wells previously coated with different glycosaminoglycans: decorin, CSA, CSC, heparan sulfate (HS). (*A*) DBL3X, (*B*) DBL6ε, (*C*) DBL4ε-6ε, (*D*) DBL1X-6ε.

Table S1. N-Glycosylation sites mutated in HEK293 expressed recombinant proteins

Domain	Boundaries	Total sites	N-Glycosylation sites mutated
DBL1X-6ε	59–2630	20	S148A
			T357A
			S381A
			S407A
			T447A
			S506A
			S510A
			S517A
			T692A
			S1013A
			S1576L
DBL4ε-6ε	1578–2630		T1661A
			T1735A
			S1740A
			T1841A
			T1912A
			T2130A
			T2206A
			S2490A
			S2559A

Serine or threonine residues were substituted by alanine or leucine

Table S2. Calculated values obtained from the SAXS data and the theoretical extended model

	Radius (nm)	Maximum length (nm)	Volume (nm^3)
SAXS	6.24	18.5	335
Theoretical extended model	12.7	43.8	333