Final Progress Report for Research Projects Funded by Health Research Grants

Instructions: Please complete all of the items as instructed. Do not delete instructions. Do not leave any items blank; responses must be provided for all items. If your response to an item is "None", please specify "None" as your response. "Not applicable" is not an acceptable response for any of the items. There is no limit to the length of your response to any question. Responses should be single-spaced, no smaller than 12-point type. The report **must be completed using MS Word**. Submitted reports must be Word documents; they should not be converted to pdf format. Questions? Contact Health Research Program staff at 717-783-2548.

1. **Grantee Institution:** The Pennsylvania State University

2. **Reporting Period (start and end date of grant award period):** 1/1/2009 – 12/31/2012

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** John Anthony, MPA

4. **Grant Contact Person's Telephone Number:** 814-935-1081

5. **Grant SAP Number:** # 4100047645

6. **Project Number and Title of Research Project:** 34 - Cytoadherence in Maternal Malaria

7. **Start and End Date of Research Project:** 7/20/2010 to 6/30/3012

8. **Name of Principal Investigator for the Research Project:** Channe D. Gowda, PhD

9. **Research Project Expenses**.

   9(A) Please provide the total amount of health research grant funds spent on this project for the entire duration of the grant, including indirect costs and any interest earned that was spent:

   $ 294,436

   9(B) Provide the last names (include first initial if multiple individuals with the same last name are listed) of **all** persons who worked on this research project and were supported with health research funds. Include position titles (Principal Investigator, Graduate Assistant, Post-doctoral Fellow, etc.), percent of effort on project and total health research funds expended for the position. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name	Position	% of Effort on Project	Cost
Goel	Postdoctoral Scholar	35% (duration 7 months)	$ 12,292
Bewley	Associate Professor (Fixed term)	40% (Duration 7 months)	$ 25,276

9(C) Provide the names of **all** persons who worked on this research project, but who *were not* supported with health research funds. Include position titles (Research Assistant, Administrative Assistant, etc.) and percent of effort on project. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name	Position Title	% of Effort on Project
Gowda	Professor	5%
Flanagan	Professor	5%

9(D) Provide a list of **all** scientific equipment purchased as part of this research grant, a short description of the value (benefit) derived by the institution from this equipment, and the cost of the equipment.

Type of Scientific Equipment	Value Derived	Cost
Delta Vision Elite Imaging System model # 52-851244-01 fluorescent microscope (Applied Precision Inc.)	This is a versatile microscope useful for live cell and fixed cell imaging as well as for the tissue histology.	$217,486

10. **Co-funding of Research Project during Health Research Grant Award Period.** Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

Yes____X_____ No_____

If yes, please indicate the source and amount of other funds:

$ 40,389 from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, USA, during the CURE grant period.

11. **Leveraging of Additional Funds**

11(A) As a result of the health research funds provided for this research project, were you able to apply for and/or obtain funding from other sources to continue or expand the research?

Yes____X_____ No_____

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the application was submitted (column C), and the amount of funds requested (column D). If you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert "not funded" in column E.

Do not include funding from your own institution or from CURE (tobacco settlement funds). Do not include grants submitted prior to the start date of the grant as shown in Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement below the table indicating how the data/results from this project were used to secure that grant.

A. Title of research project on grant application	B. Funding agency (check those that apply)	C. Month and Year Submitted	D. Amount of funds requested:	E. Amount of funds to be awarded:
Malaria Parasite Cytoadherence Mechanisms	☒ NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)	March 2011	$ 1,931,250	Not funded
Targeting *Plasmodium falciparum* Cytoadherence for Placental Malaria Treatment	☒NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)	October 2011	$ 1,912,500	Not funded
Therapeutic Targeting of Malarial Placental Treatment	☒NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)	May 2012	$ 1,912,514	To be resubmitted
Assay Development for Identifying Placental Malaria Cytoadherence Inhibitors	☒NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)	June 2012	$ 918,006	To be resubmitted

11(B) Are you planning to apply for additional funding in the future to continue or expand the research?

Yes____X_____ No_____

If yes, please describe your plans:

We are planning to resubmit the two grant applications listed above to NIH.

12. Future of Research Project. What are the future plans for this research project?

We plan to determine the structure of the parasite protein (VAR2CSA) that mediates the adherence of malaria parasite-infected erythrocytes to placental chondroitin 4-sulfate receptors and identify the amino acid residues of VAR2CSA that interact with chondroitin 4-sulfate. We also plan to identify small molecule inhibitors that disrupt the binding of parasite-infected erythrocytes to placental chondroitin 4-sulfate. The results of these studies will be helpful in developing vaccine and therapeutics for pregnancy-associated malaria.

13. New Investigator Training and Development. Did students participate in project supported internships or graduate or post-graduate training for at least one semester or one summer?

Yes_____ No_____X_____

If yes, how many students? Please specify in the tables below:

	Undergraduate	Masters	Pre-doc	Post-doc
Male				
Female				
Unknown				
Total				

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic				
Unknown				
Total				

	Undergraduate	Masters	Pre-doc	Post-doc
White				
Black				
Asian				
Other				
Unknown				
Total				

14. Recruitment of Out-of–State Researchers. Did you bring researchers into Pennsylvania to carry out this research project?

Yes_____ No____X_____

If yes, please list the name and degree of each researcher and his/her previous affiliation:

15. Impact on Research Capacity and Quality. Did the health research project enhance the quality and/or capacity of research at your institution?

Yes____X_____ No_____

If yes, describe how improvements in infrastructure, the addition of new investigators, and other resources have led to more and better research.

Using this funding, we were able to recruit specialists who were not otherwise available to perform the proposed work. As a result of specialist (Drs. Flanagan and Maria Bewley) joining the project, we accomplished the stated goals and increased our research capacity. Also, we were able to submit two new applications to NIH. We are hoping that, upon resubmission, these grants will be funded.

The fluorescent microscope that we participated in purchasing is already extremely beneficial for the institution.

16. Collaboration, business and community involvement.

16(A) Did the health research funds lead to collaboration with research partners outside of your institution (e.g., entire university, entire hospital system)?

Yes___X_____ No_____

If yes, please describe the collaborations:

We are collaborating with Dr. Ali Salanti, University of Copenhagen, Denmark, to obtain important reagents, which have been produced in his laboratory, for the studies proposed in our grant applications to NIH.

16(B) Did the research project result in commercial development of any research products?

Yes_____ No____X_____

If yes, please describe commercial development activities that resulted from the research project:

16(C) Did the research lead to new involvement with the community?

Yes_____ No____X_____

If yes, please describe involvement with community groups that resulted from the research project:

17. Progress in Achieving Research Goals, Objectives and Aims.

List the project goals, objectives and specific aims (as contained in the grant agreement). Summarize the progress made in achieving these goals, objectives and aims for the period that the project was funded (i.e., from project start date through end date). Indicate whether or not each goal/objective/aim was achieved; if something was not achieved, note the reasons why. Describe the methods used. If changes were made to the research goals/objectives/aims, methods, design or timeline since the original grant application was submitted, please describe the changes. Provide detailed results of the project. Include evidence of the data that was generated and analyzed, and provide tables, graphs, and figures of the data. List published abstracts, poster presentations and scientific meeting presentations at the end of the summary of progress; peer-reviewed publications should be listed under item 20.

This response should be a DETAILED report of the methods and findings. It is not sufficient to state that the work was completed. Insufficient information may result in an unfavorable performance review, which may jeopardize future funding. If research findings are pending publication you must still include enough detail for the expert peer reviewers to evaluate the progress during the course of the project.

Health research grants funded under the Tobacco Settlement Act will be evaluated via a performance review by an expert panel of researchers and clinicians who will assess project work using this Final Progress Report, all project Annual Reports and the project's strategic plan. After the final performance review of each project is complete, approximately 12-16 months after the end of the grant, this Final Progress Report, as well as the Final Performance Review Report containing the comments of the expert review panel, and the grantee's written response to the Final Performance Review Report, will be posted on the CURE Web site.

There is no limit to the length of your response. Responses must be single-spaced below, no smaller than 12-point type. If you cut and paste text from a publication, be sure symbols print properly, e.g., the Greek symbol for alpha (α) and beta (ß) should not print as boxes (□) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.

Specific Aims, and Goals and Objectives.

The overall goals of the proposed studies in the grant application were to obtain preliminary results for the resubmission of the renewal grant application entitled "Cytoadherence in Maternal Malaria" to NIH. The project was originally initiated in 1997 with a 2-year Burroughs Wellcome Fund seed grant and has been continuously funded by a R01 grant from NIAID, NIH from 1999 to 2009. The study was focused to understand the molecular interactions involved in the adherence of malaria parasite-infected red blood cells (IRBCs) to placental-specific receptor chondroitin-4-sulfate (C4S), the process that leads to pregnancy-associated malaria. Studies have shown that the parasite protein called VAR2CSA expressed on the surface of IRBCs binds to C4S. The structure of VAR2CSA is schematically shown in Figure 1 (see below). The VAR2CSA ectodomain (2634 amino acids) comprises six Duffy binding-like (DBL) domains and two structured linker polypeptides called internal domains, ID1 and ID2. The specific aims

and objectives of the proposed studies are outlined below:

1. Specific Aim 1. To clone and produce proteins corresponding to individual Duffy binding like (DBL) domains of VAR2CSA. The Objectives of this Aim were: (i) To clone and express all six DBL domains of VAR2CSA, (ii) standardize refolding procedures for those DBL domains that expressed as insoluble inclusion bodies, and (iii) produce and purify proteins for preliminary studies on goals stated in Aims 2 and 3 (below).

2. Specific Aim 2. To study DBL domain-domain interactions. The goal of this Aim was to perform preliminary studies to determine which DBL domains interact with one another and perform preliminary analysis of an interaction DBL domain pair (DBL3x and DBL2x) using biophysical techniques such as size-exclusion chromatography, SAXS, fluorescence titration, and plasmon resonance (Biacore) measurements.

Specific Aim 3. Crystallization of DBL domain complexes and NMR analysis of DBL domains. The objectives of this Aim were to: (i) set up crystallization experiments and standardize procedures for crystal formation of an interacting DBL domain pairs in the presence and absence of C4S oligosaccharide receptor, (ii) clone larger fragments of VAR2CSA into mammalian cell expression vectors and standardize procedure for the efficient expression as soluble secretory proteins, and (iii) standardize procedure for the expression of ^{15}N-labeled DBL domains and perform preliminary NMR analysis on a DB2x, DBL3x, and their mixture.

Progress Made

1. Specific Aim 1. To clone and produce proteins corresponding to individual Duffy binding like (DBL) domains of VAR2CSA. The Objectives of this Aim were: (i) To clone express all six DBL domains of VAR2CSA, (ii) standardize refolding procedures for those DBL domains that expressed as insoluble inclusion bodies, and (iii) produce and purify proteins for preliminary studies on goals stated in Aims 2 and 3 (below).

We cloned all six DBL domains of *P. falciparum* (3D7 strain) VAR2CSA into a pET-28aTEV vector at an *N*-terminal His tag and a tobacco etch virus (TEV) protease-cleavable site and expressed them in *E. coli*. The domain boundaries of cloned DBLs were: DBL1x aa 46-430, DBL2x aa 535-961, DBL3x aa 1214-1562, DBL4ε aa 1574-1957, DBL5ε aa 1983-2291, DBL6ε aa 2332-2634, and DBL2x-ID2-DBL3x aa 535-1562). All constructs expressed at high levels; DBL3x and DBL6ε were primarily soluble proteins and were purified by Ni-NTA chromatography and size-exclusion chromatography. DBL1x and DBL2x were expressed as inclusion bodies and were isolated by denaturing Ni-NTA chromatography, refolded to obtain their native structures, and further purified by Ni-NTA affinity and cation-exchange chromatography. On size-exclusion chromatography, DBL1x, DBL2x and DBL3x eluted as single monomeric proteins (Figure 2, and data not shown). MS of DBL2x showed a single molecular ion of predicted mass 44.8 kDa; calculated mass 45.3 kDa (Figure 2). The yields were typically 8-12 mg/L culture.

In the case of DBL2x, we found that the protein containing aa 535-961 found to be unstable and formed aggregates. Therefore, using the published crystal structure of DBL3x as a template and

the modeling programs, I-TASSER (zhanglab.ccmb.med.umich.edu/I-TASSER/) and modeller (www.salilab.org/modeller/) we engineered a longer stable, foldable construct, named DBL2xL (aa 488-961), that covers the exposed hydrophobic patch on the surface of the original DBL2x (Figure 3) by adding 47 amino acids at the N-terminus. On size-exclusion chromatography, the proteins eluted as single monomeric proteins; detected by in-line multi-angle light scattering (Figure 4). As predicted, DBL2xL does not aggregate. CD spectra of DBL1x, DBL2xL, and DBL3x showed high degree of α-helical contents (Figure 5A, and not shown) and were similar to the spectrum of DBL3x used in crystal structure studies and that of DBL6ε, suggesting that they are properly folded. Thermal unfolding of DBL-2x and DBL-3x measured by CD showed a sharp loss of secondary structure at ~70 °C as expected for compact proteins that contain several disulfide bonds (Figure 5B).
Thus, we accomplished all the stated objectives of this Specific Aim.

2. Specific Aim 2. To study DBL domain-domain interactions. The goal of this Aim was to perform preliminary studies to determine which DBL domains interact with one another and perform preliminary analysis of a, interaction DBL domain pair (DBL3x and DBL2x) using biophysical techniques such as size-exclusion chromatography, SAXS, fluorescence titration, and plasmon resonance (Biacore) measurements.

Initially, to examine which DBL domains interact with one another, we expressed all DBL domains on the yeast cell surface and tested DBL domains to form complexes. In an ELISA-based reporter assay, DBL1x and DBL2x, isolated from recombinant yeast, bound to *E. c*oli-expressed and purified DBL3x in a dose-dependent manner (Figure 6). The DBL1x expressed in yeast also bound *E. coli*-expressed DBL2x. By contrast, DBL4ε, DBL5ε and DBL6ε did not interact with each other or with either DBL2x or DBL3x with measurable affinity. DBL2x purified from the recombinant yeast efficiently bound immobilized DBL3x (K_d of 10-20 nM) in an ELISA assay. Furthermore, yeast that expressed DBL2x bound to immobilized DBL3x in a dose-dependent manner (Figure 7). The interaction between DBL1x, DBL2xL and DBL3x was also observed by tryptophan fluorescence (Figure 8) and by SPR analyses (Figure 9). Together, these data suggest that DBL1x, DBL2xl, and DBL3x form an interacting unit in VAR2CSA.

We also we measured scattering measurements on DBL2x, DBL3x, and the DBL2x + DBL3x complex at the National Synchrotron Light Source (NSLS, beamline X9), Brookhaven National Laboratory, NY. Small and wide-angle data were collected simultaneously using a MarCCD SAXS detector and a Photonic Science, Pilatus 300K pixel array WAXS detector, respectively. Data were processed using the ATSAS program suite (http://www.embl-hamburg.de/biosaxs/software.html, version 2.4), a web-supported data analysis package. Radius of gyration (R_g) and zero scattering (I(0)) were calculated using both GNOM and Guinier analysis within this suite. Inverse Fourier transform calculations of I(0) to yield P(r) functions, I(0), R_g and the maximum dimension D_{max} were determined from the data between S ($S=4\pi\sin(\theta)/\lambda$) of 0.01 $Å^{-1}$ and 0.3 $Å^{-1}$. A*b initio* shape reconstruction, using the program GASBOR with default parameters, was used to generate 3-D envelopes from the 1-D scattering. In each case, 10 independent models were generated, aligned and averaged using the DAMAVER program. In addition, SASREF was used to compare simulated intensity data derived from the crystal structure of DBL3x with the experimental intensity measured at the beamline.

The scattering curves were flat in the low S range, confirming the absence of aggregates. Profiles obtained at three different protein concentrations have the same shape indicating that there was no significant increase in self-association with increasing concentration. Guinier analysis was used to calculate the R_g (DBL3x; R_g 33 ± 0.8 Å; DBL3x R_g 28 ± 0.7 Å; DBL2xL + DBL3x R_g 44 ± 0.1 Å) and determine the effect of protein concentration upon oligomerization state. The plots are linear over the Guinier region at low S, where SRg <1 and indicated that the proteins were monomeric. The molecular reconstructions showed characteristic compact structures of DBL2xl and DBL3x, and that DBL2x and DBL3x interact side by side to form a more extended molecule (Figure 10).

We modeled DBL1x and DBL2x structures (Figure 11, and data not shown). Superposition of the homology models onto the crystal structure of DBL3x suggested a potential sulfate-binding site (K552, K553 and K798 interacting) on DBL2x. In this model, the 47 aa N-terminal extension (dark blue residues) helps to shape the putative C4S binding site; no such potential sulfate-binding site was evident in DBL1x. These results and those of the DBL3x crystal structure suggest that DBL2x and DBL3x together provide a specific C4S sulfate-binding site. The above data also suggest that *DBL1x, DBL2x, and DBL3x form a C4S-interacting unit in VAR2CSA.* This prediction agrees with the recent reports by other investigators that DBL1x-ID2 and DBL1x-DBL3x bind C4S-containing decorin and that DBL2x is a part of the binding site. Thus, we have accomplished all the stated goals of this Specific Aim.

Specific Aim 3. Crystallization of DBL domain complexes and NMR analysis of DBL domains. *The objectives of this Aim were to: (i) set up crystallization experiments and standardize procedures for crystal formation of interacting DBL domain pairs in the presence and absence of C4S oligosaccharide receptor, (ii) clone larger fragments of VAR2CSA into mammalian cell expression vectors and standardize the procedure for the efficient expression as soluble secretory proteins, and (iii) standardize the procedure for the expression of ^{15}N-labeled DBL domains and perform preliminary NMR analysis on a DB2x, DBL3x, and their mixture*.

(i) Set up crystallization experiments and standardize procedures for crystal formation of an interacting DBL domain pairs. In the NIH grant application, we proposed to study the crystal structures of DBL domains other than DBL3x and DBL5e as well as the crystal structures interacting DBL domain mixtures such as DBL2x + DBL3x, DBL1x + DBL2x, and DBL1x + DBL3x with and without C4S oligosaccharides. We obtained crystals of DBL2xL (Figure 12) using low ionic strength screens. However, the crystals did not diffract sufficiently (at X25 beamline) at National Synchrotron Light Source, Brookhaven National Laboratory, NY. Efforts to crystallize DBL1x and DBL2xL mixture were unsuccessful, and further efforts and additional funds are needed to crystallize DBL domain mixtures with and without C4S oligosaccharides. Nevertheless, we obtained substantial preliminary data on this goal.

(ii) Clone larger fragments of VAR2CSA into mammalian cell expression vectors and standardize the procedure for the efficient expression as soluble secretory proteins. We originally proposed to express proteins in a mammalian system. However, we subsequently realized that baculovirus system would be more economical for producing proteins comprising two or more consecutive DBL domains. Hence, we decided to pursue baculovirus system. We obtained a codon-optimized *VAR2CSA* DNA (GenScript Corp.; aa 1-2649) cloned in frame into

the pACGP67A vector (BD Biosciences) for expression in the baculovirus expression system. We have also cloned DBL1x-DBL2x (aa 1-961), DBL1x-ID2 (aa 1-1213) and DBL1x-DBL3x (1-1595 aa). However, we could not continue the work further to express proteins because of insufficient funds and lack of personnel in the lab.

(iii) Standardize procedure for the expression of ^{15}N-labeled DBL domains and perform preliminary NMR analysis on a DB2x, DBL3x, and their mixture. As an initial step toward studying DBL domain-domain interacting surfaces by NMR spectrometry, we optimized the conditions for the expression of ^{15}N-labeled DBL2xl and DBL3x domains in M9 medium using [^{15}N]NH_4Cl]. We expressed and purified 4-5 mg each of [^{15}N]-DBL2x and [^{15}N]-DBL3x and studied their solution structures by 850-MHz NMR spectrometry. The transverse relaxation optimized spectroscopy (TROSY) spectrum of DBL3x, and TROSY spectrum DBL2xl overlapped with that of DBL2x is shown in Figure 13 and 14, respectively. The spectra indicate that both proteins have well ordered structures. The spectral resolution is consistent with those expected for proteins of 38-kDa (DBL3x) and 52-kDa (DBL2x) sizes. Thus, we were able to obtain significant preliminary data on this goal.

Figures

Figure 1. Schematic representation of VAR2CSA structure

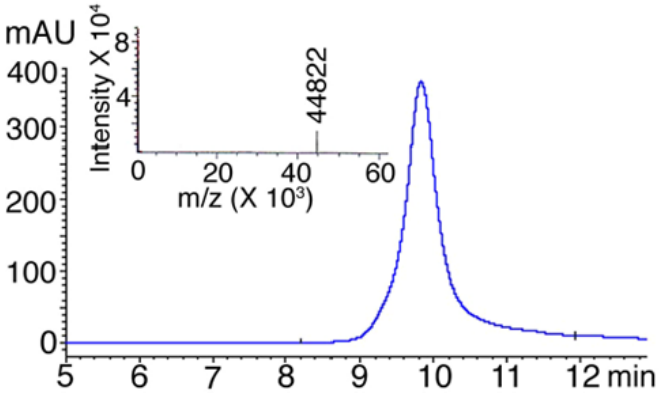

Figure 2. Size-exclusion chromatography of refolded *E. coli* DBL2x; inset, mass analysis by MS. DBL1x and DBL3x also gave symmetrical single peak.

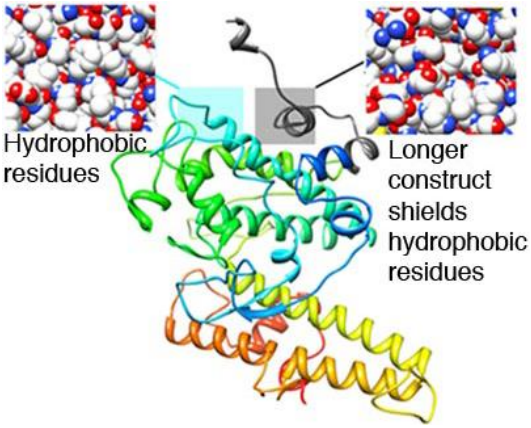

Figure 3. Model of DBL2xL showing that the N-terminus extra sequence (dark gray) masks the exposed hydrophobic surface. The structure is colored rainbow. Gray, carbon; red, oxygen; blue, nitrogen.

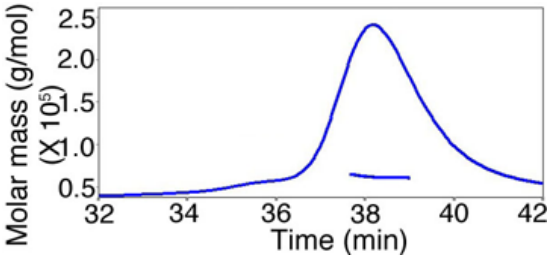

Figure 4. Size-exclusion chromatography-multiangle scattering detection of DBL2xl (52 kDa).

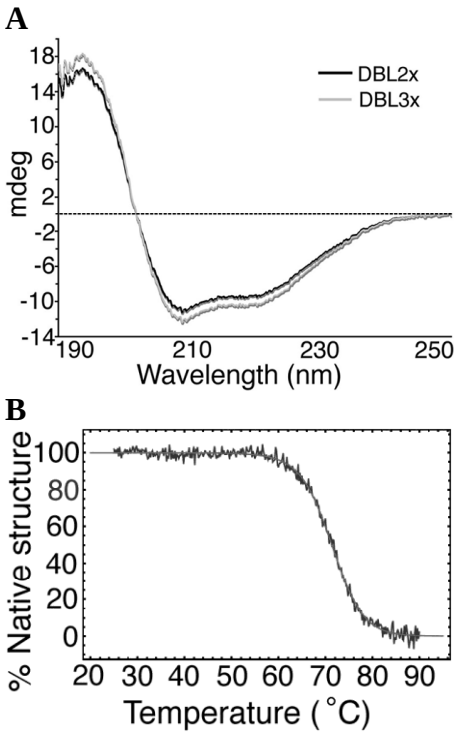

Figure 5. (A), CD spectra of refolded DBL2x and DBL3x. (B), Thermal unfolding was measured at 222 nm. DBL1x was similarly analyzed.

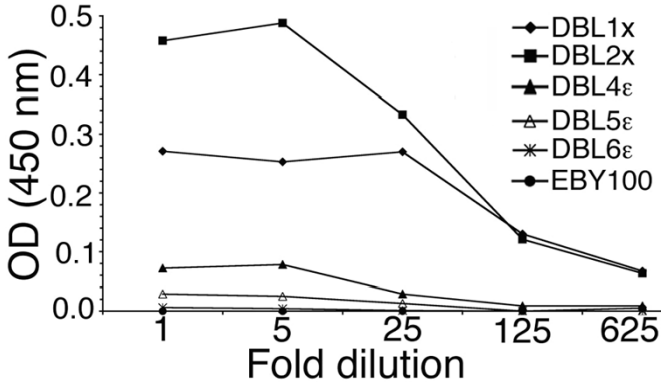

Figure 6. Binding of lysates (at 1:1 to 1:625 dilutions) of yeast expressing various DBL proteins to recombinant DBL3x.

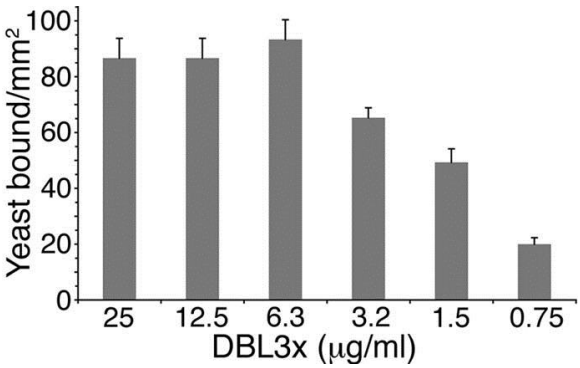

Figure7. Binding of yeast, expressing DBL2x to DBL3x coated to plastic plates.

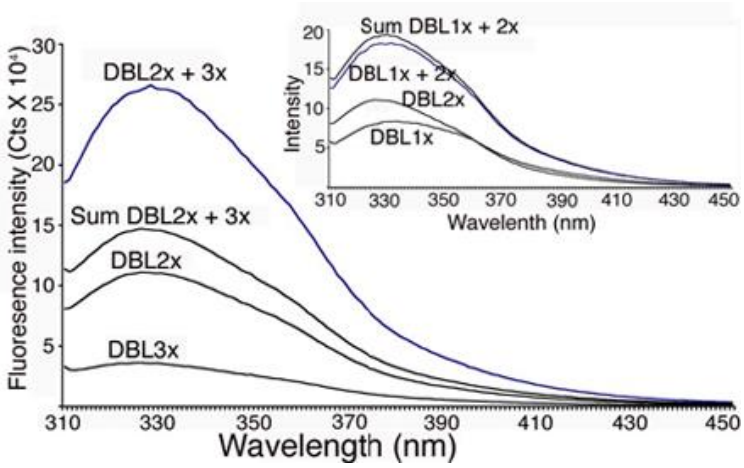

Figure 8. The fluorescence intensity of DBL2x plus DBL3x (1 μM each) is higher than the sum of individual DBLs; the emission λ was also shifted to a higher region: Inset: DBL1x and DBL3x.

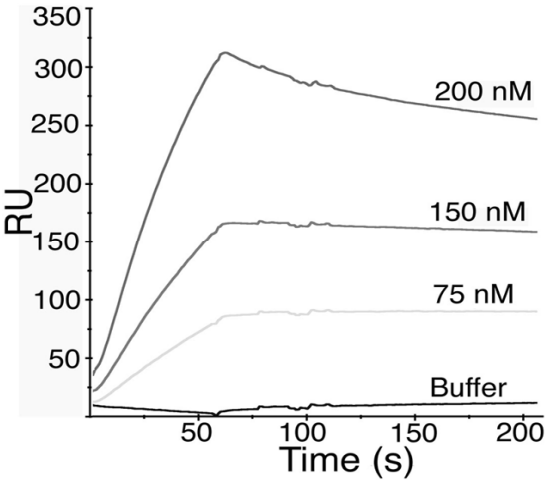

Figure 9. The binding of DBL2x to DBL3x conjugated to a Biacore CM5 sensor chip.

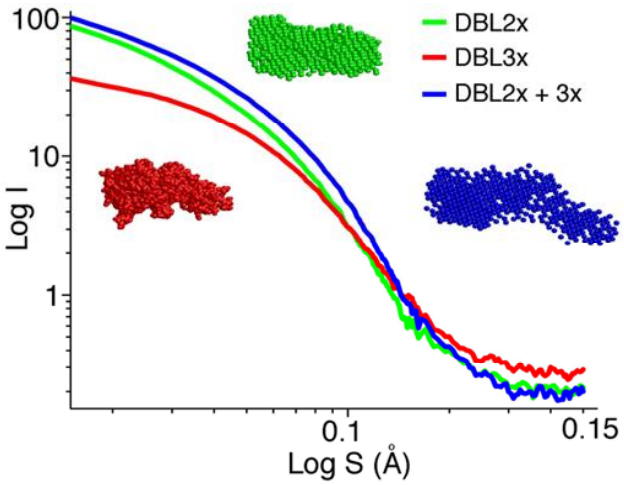

Figure 10. Scattering intensity as a function of the momentum transfer $S = 4\pi\sin(\theta)/\lambda$. SAXS measurements were done in 20 mM Tris, 150 mM NaCl, pH 7.5. The molecular reconstructions are shown.

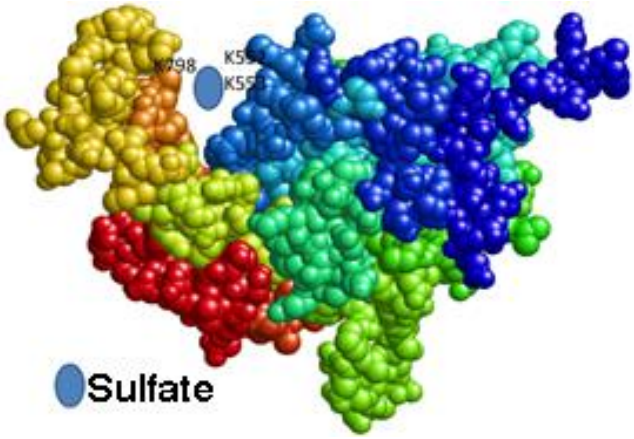

Figure 11. Space filling model of DBL2x, showing the putative sulfate-binding site that comprises K552, K553 and K798. Dark blue, N-terminal 47 aa extension.

Figure 12. Crystals of DBL2xL.

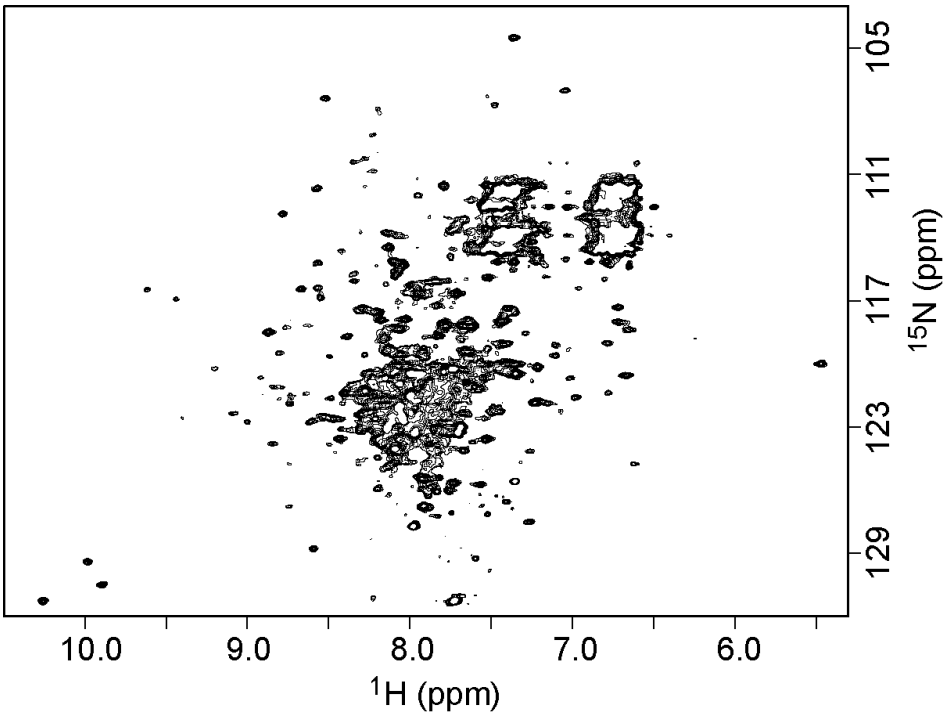

Figure 13. TROSY spectrum of ^{15}N-labeled DBL3x collected at 37 °C using 850-MHz spectrometer.

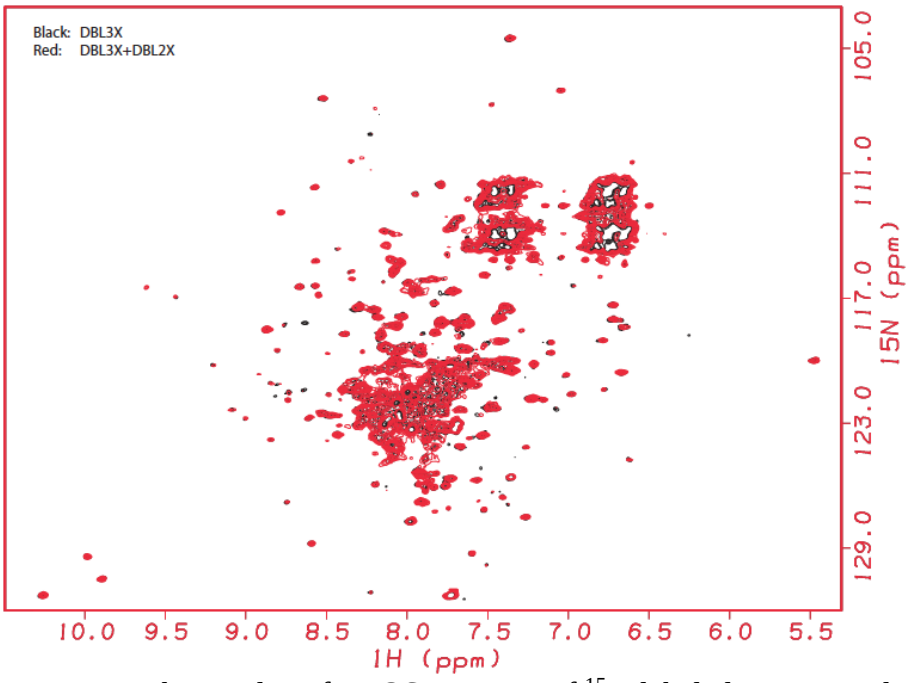

Figure 14. The overlay of TROSY spectra of ^{15}N-labeled DBL2x and DBN2x collected at 37 °C using 850-MHz spectrometer.

Presentation of the results produced in this study at seminars and symposia/workshop

1. Chondroitin 4-Sulfate-mediated Sequestration of Malaria Parasite in The Placenta Sixth Annual Georgia Glycoscience Symposium and CFG Workshop: A Molecular View of Glycan Signaling in Development and Disease at the Complex Carbohydrate Research Center, University of Georgia, Athens, Georgia. March 14-15, 2011.

2. What did we learn from one and a half decade of research on malaria glycobiology and pathogenesis? International Symposium on Synthetic and Structural Chemistry, Mangalore University, Mangalore, India, December 8-10, 2011.

3. Parasite-Host Interactions in Pregnancy-associated Malaria. Malaria Research Training: Indo-US Seminar/ Workshop, Jawaharlal Nehru Center for Advanced Scientific Research, Bangalore, India. January 17, 2012.

18. Extent of Clinical Activities Initiated and Completed. Items 18(A) and 18(B) should be completed for all research projects. If the project was restricted to secondary analysis of clinical data or data analysis of clinical research, then responses to 18(A) and 18(B) should be "No."

18(A) Did you initiate a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
___X__No

18(B) Did you complete a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
___X__No

If "Yes" to either 18(A) or 18(B), items 18(C) – (F) must also be completed. (Do NOT complete 18(C-F) if 18(A) and 18(B) are both "No.")

18(C) How many hospital and health care professionals were involved in the research project?

_____Number of hospital and health care professionals involved in the research project

18(D) How many subjects were included in the study compared to targeted goals?

_____Number of subjects originally targeted to be included in the study
_____Number of subjects enrolled in the study

Note: Studies that fall dramatically short on recruitment are encouraged to provide the details of their recruitment efforts in Item 17, Progress in Achieving

Research Goals, Objectives and Aims. For example, the number of eligible subjects approached, the number that refused to participate and the reasons for refusal. Without this information it is difficult to discern whether eligibility criteria were too restrictive or the study simply did not appeal to subjects.

18(E) How many subjects were enrolled in the study by gender, ethnicity and race?

Gender:
_____Males
_____Females
_____Unknown

Ethnicity:
_____Latinos or Hispanics
_____Not Latinos or Hispanics
_____Unknown

Race:
_____American Indian or Alaska Native
_____Asian
_____Blacks or African American
_____Native Hawaiian or Other Pacific Islander
_____White
_____Other, specify:_____
_____Unknown

18(F) Where was the research study conducted? (List the county where the research study was conducted. If the treatment, prevention and diagnostic tests were offered in more than one county, list all of the counties where the research study was conducted.)

19. Human Embryonic Stem Cell Research. Item 19(A) should be completed for all research projects. If the research project involved human embryonic stem cells, items 19(B) and 19(C) must also be completed.

19(A) Did this project involve, in any capacity, human embryonic stem cells?
_____Yes
__X__ No

19(B) Were these stem cell lines NIH-approved lines that were derived outside of Pennsylvania?
_____Yes
_____ No

19(C) Please describe how this project involved human embryonic stem cells:

20. Articles Submitted to Peer-Reviewed Publications.

20(A) Identify all publications that resulted from the research performed during the funding period and that have been submitted to peer-reviewed publications. Do not list journal abstracts or presentations at professional meetings; abstract and meeting presentations should be listed at the end of item 17. **Include only those publications that acknowledge the Pennsylvania Department of Health as a funding source** (as required in the grant agreement). List the title of the journal article, the authors, the name of the peer-reviewed publication, the month and year when it was submitted, and the status of publication (submitted for publication, accepted for publication or published.). Submit an electronic copy of each publication or paper submitted for publication, listed in the table, in a PDF version 5.0.5 (or greater) format, 1,200 dpi. Filenames for each publication should include the number of the research project, the last name of the PI, the number of the publication and an abbreviated research project title. For example, if you submit two publications for PI Smith for the "Cognition and MRI in Older Adults" research project (Project 1), and two publications for PI Zhang for the "Lung Cancer" research project (Project 3), the filenames should be:

Project 1 – Smith – Publication 1 – Cognition and MRI
Project 1 – Smith – Publication 2 – Cognition and MRI
Project 3 – Zhang – Publication 1 – Lung Cancer
Project 3 – Zhang – Publication 2 – Lung Cancer

If the publication is not available electronically, provide 5 paper copies of the publication.

Note: The grant agreement requires that recipients acknowledge the Pennsylvania Department of Health funding in all publications. Please ensure that all publications listed acknowledge the Department of Health funding. If a publication does not acknowledge the funding from the Commonwealth, do not list the publication.

Title of Journal Article:	Authors:	Name of Peer-reviewed Publication:	Month and Year Submitted:	Publication Status (check appropriate box below):
1. None				☐Submitted ☐Accepted ☐Published

20(B) Based on this project, are you planning to submit articles to peer-reviewed publications in the future?

Yes___X_____ No_____

If yes, please describe your plans:

The results obtained from this grant were preliminary and were mainly intended for supporting the grant applications to NIH. We are planning to continue the project and are seeking funds from NIH. We are reasonably confident that NIH will fund this project. The

results that we have generated from these CURE funds will be published together with those that we will obtain from future funding.

21. Changes in Outcome, Impact and Effectiveness Attributable to the Research Project. Describe the outcome, impact, and effectiveness of the research project by summarizing its impact on the incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of outcome, impact or effectiveness of the research project. If there were no changes, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None

22. Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment. Describe major discoveries, new drugs, and new approaches for prevention, diagnosis and treatment that are attributable to the completed research project. If there were no major discoveries, drugs or approaches, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

Understanding of structural interactions involved in VAR2CSA binding to C4S will be useful in designing novel vaccine candidates and/or therapeutics for pregnancy-associated malaria. The data that we obtained from this study provide foundation for further studies on this project aimed at developing a vaccine and/or therapeutics.

23. Inventions, Patents and Commercial Development Opportunities.

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance of work under this health research grant? Yes_____ No___X_____

If "Yes" to 23(A), complete items a – g below for each invention. (Do NOT complete items a - g if 23(A) is "No.")

a. Title of Invention:

b. Name of Inventor(s):

c. Technical Description of Invention (describe nature, purpose, operation and physical, chemical, biological or electrical characteristics of the invention):

d. Was a patent filed for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?

Yes_____ No___

If yes, indicate date patent was filed:

e. Was a patent issued for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No___
If yes, indicate number of patent, title and date issued:
Patent number:
Title of patent:
Date issued:

f. Were any licenses granted for the patent obtained as a result of work performed under this health research grant? Yes_____ No___

If yes, how many licenses were granted?_____

g. Were any commercial development activities taken to develop the invention into a commercial product or service for manufacture or sale? Yes__ No___

If yes, describe the commercial development activities:

23(B) Based on the results of this project, are you planning to file for any licenses or patents, or undertake any commercial development opportunities in the future?

Yes_____ No____X_____

If yes, please describe your plans:

24. Key Investigator Qualifications. Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators. In place of narrative you may insert the NIH biosketch form here; however, please limit each biosketch to 1-2 pages. *For Nonformula grants only – include information for only those key investigators whose biosketches were not included in the original grant application.*

BIOGRAPHICAL SKETCH

NAME Gowda, Channe D	POSITION TITLE Professor of Biochemistry and Molecular Biology
eRA COMMONS USER NAME: cgowda	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University of Mysore, Mysore, India	BS	1971	Physics, Chem., Math
University of Mysore, Mysore, India	MS	1973	Organic Chemistry
University of Mysore, Mysore, India	PhD	1978	Organic Chemistry

A. Personal Statement

For the past several years, my research interest has been to study malaria parasite-host interactions in the context of malaria pathophysiology and immunology. Specifically, we have been studying: (i) adherence of parasite-infected red blood cells (IRBCs) in the placenta, and (ii) parasite-host interactions that initiate cell signaling and regulate innate immune responses. In both projects, our work has been very productive and represents important original contributions to the malaria field. The current proposal is based on the knowledge that we gained in previous studies on how the malaria parasite, *Plasmodium falciparum*, sequesters in the placenta contributing to placental malaria (PM). Our previous accomplishments include the identification, localization and structural characterization of placental chondroitin sulfate proteoglycan (CSPG) receptor that interact with VAR2CSA, the *P. falciparum* erythrocyte membrane protein 1 that mediates the adherence of IRBCs to the placenta. We have also elucidated the chondroitin 4-sulfae (C4S) structural motif that interacts with IRBCs and determined various functional groups of this C4S motif that are involved in IRBC binding. Further, we have developed methods for purification of the placental CSPG and C4S oligosaccharides of defined size and sulfate contents. These studies have significantly contributed to the understanding of placental malaria cytoadherence and substantially impacted the field. In this grant application we propose to: (i) define the precise structure of C4S motif that is involved in IRBC binding, (ii) identify small molecule inhibitors that block the interaction of VAR2CSA/IRBC with C4S, and (iii) gain insight into domain organization in VAR2CSA and C4S-binding site by using NMR, X-ray crystallography and other biophysical techniques and use this information to validate inhibition of VAR2CSA-C4S interactions by small molecule inhibitors. To achieve these goals, we collaborate with investigators who have expertise and proven track records of accomplishments. In addition to malaria research, I am a recognized expert in glycobiology and have a proven record of accomplishment in studying the structures and functions of glycoproteins and proteoglycans. These expertise and those of the proposed collaborators put us at the competitive edge to productively accomplish the goals of the proposed studies.

B. Positions and Honors

Positions and Employment

1975-1986 Lecturer, Department of Chemistry, University of Mysore, Mysore, India.

1980-1981	Alexander von Humboldt Foundation Fellow, Institute of Biochemistry, Univ. of Kiel, Germany.
1983-1985	Postdoc. Scholar, Dept. Biological Chemistry, Hershey Med Center, Hershey, PA.
1986-1989	Instructor, Dept. of Pharmacology, New York University Medical Center, NY.
1989-1994	Assistant Professor, Department of Biochemistry and Molecular Biology, Georgetown University Medical Center, Washington, DC.
1994-2000	Associate Professor, Department of Biochemistry and Molecular Biology, Georgetown University Medical Center, Washington, DC.
2001-present	Professor, Department of Biological Chemistry and Molecular Biology, Penn State University College of Medicine, Hershey, PA.

Other Experience and Professional Memberships

Society Membership

1987-present	Society for Glycobiology, USA.
1995-present	American Society for Biochemistry and Molecular Biology.
1995-present	American Chemical Society.
1999-present	American Association for the Advancement of Science.
2000-present	American Society of Tropical Medicine and Hygiene.
2003-present	American Society of Microbiology.
2004-present	American Society for Investigative Pathology.
2011-present	American Association of Immunologists.

Study Sections and Grant Review Panels

1996	Ad-hoc Member, Cell Biology Study Section, US Army Breast Cancer Program.
2000	Ad-hoc Member, Comparative Medicine Study Section, NIH.
2000	Ad-hoc Member, Targeted Drug Discovery for Cancer Study Section, NCI, NIH.
2001	Ad-hoc Member, Biochemistry Study Section, NIGM, NIH.
2002-	Grant Review, The Wellcome Trust, London, UK - three times during this period.
2005	Member, GCRC Site Visit to Univ. of Rochester, Rochester, NY, NCRR, NIH.
2005	Ad-hoc Member, Pathogenic Eukaryotes (PTHE) Study Section, NIAID, NIH.
2005-present	Member, Promotion and Tenure Committee, Department of Biological Chemistry, Penn State University College of Medicine, Hershey, PA, Hershey.
2006	Member, Study Section, Special Panel for Innate Immunity to Parasites, NIH.
2006 and 09	Grant Reviewer, Swiss National Science Foundation, Switzerland.
2007	Member, Special Panel for Infectious, Reproductive, Asthma & Pulmonary Diseases Study Section, NIH.
2008	Member, Study Section on Special Emphasis Panel for Development of Novel Interventions and Tools for the Control of Malaria, Neglected Tropical Diseases and their Vectors.
2009	Grant Reviewer, Maryland Industry and University Joint Research Program, MD.
2010	Grant Reviewer, The Netherlands Org. for Health Research and Development
2011-present	Member, Scientific-Advisory groups, International Center of Excellence in Malaria Research, Thailand.

Journal's Editorial Board member

2003-2008 & 2011-present	*Journal of Biological Chemistry*, American society for Biochemistry and Molecular Biology.
2006-present	*Current Chemical Biology*, Bentham Publishers.

2008-present	*Open Glycosciences*, Bentham Publishers.
2009-present	*Pathology and Laboratory Medicine International*, Dove Med. Press.
2009-present	*Hematology Research and Reviews*, Dove Medical Press.
2009-present	Molecular Biology International, SAGE-Hindawi Publishers.
2010-present	*Infection and Immunity*, American Society for Microbiology.

Awards

1974	Gold medalist and cash award by the University of Mysore, India, for First Rank in MS.
1980-1981	The Alexander von Humboldt Foundation Fellowship, Germany
1993	Shannon Grant Award from NIH.
1998-2000	Burroughs Wellcome Fund award for New Initiatives in Malaria Research.
2006-08	Outstanding Mentor Award for serving in American Society Microbiology. Mentoring Program.
2007 and 08	Star Award Recognition for Teaching by PSU Hershey Medical Students.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2. Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME Flanagan, John M.	POSITION TITLE Professor of Biochemistry and Molecular Biology
eRA COMMONS USER NAME jflanagan	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Earlham College, Richmond, IN	B.A.	1981	Chemistry and Physics
University of Tennessee, Oak Ridge, TN	Ph.D.	1986	Molecular Biophysics
Yale University, New Haven, CT	Postdoctoral Associate	1986–1987	Molecular Biophysics and Biochemistry
Yale University, New Haven, CT	Postdoctoral Fellow	1987–1989	Molecular Biophysics and Biochemistry

Personal Statement

The purpose of this application is to understand how the malaria proteins, VAR2CSA expressed on the surface of malaria parasite-infected erythrocytes binds the carbohydrate C4S. I am a broadly trained structural biologist (NMR, X-ray crystallography, scanning transmission electron microscopy, small angle X-ray scattering, SAXS, as well as a range of spectroscopic techniques) and have applied these techniques to study a number of complex systems including chaperone dependent protein folding, the bacterial ATP dependent ClpAP protease, the structural basis for polyamine regulation in mammals, the interaction of adenovirus with its cellular receptor CAR and identifying early intermediates in RSV retroviral assembly. I am currently a member of the design team for the SAXS beamlines at the National Synchrotron Light Source-II (Brookhaven Natl. Laboratory), and on the review panel for the SAXS lines at the Advanced Photon Source (Argonne Natl. Laboratory) and thus have extensive knowledge of this methodology. I was also the principal investigator on the NIH proposal that funded the purchase of the 500 and 600 MHz NMR spectrometers in the Penn State University College of Medicine. This broad background, experience and training have allowed me help design, produce and purify the E. coli expressed DBL constructs that are soluble, stable, and suitable for biochemical and structural studies. The efforts to determine the structures of the VAR2CSA single and multidomain constructs are proceeding in my laboratory, as are the development of a rapid and sensitive fluorescence-based carbohydrate-binding assay for these constructs. Our recent work has shown that this assay can be adapted to a high throughput screen (HTS), which is suitable for the identification of small molecule inhibitors of the VAR2CSA carbohydrate interaction. I have been involved in the design of several other HTS screens including one for HIV reverse transcriptase activity and another for the binding of the dopamine D2 receptor to one of its regulators, the neuronal calcium sensor-1 (NCS-1) protein. This latter screen has led to the identification of several new inhibitors of this interaction, as well as shown that several inhibitors that target D2 receptor function actually block this interaction (manuscript in preparation). The

collaboration with Dr. Gowda provides an ideal environment for multidisciplinary studies to develop specific inhibitors that block IRBC attachment to placenta and may have therapeutic benefit in malaria endemic areas. The reagents and assays that we have already developed open up new avenues for this type of research. We feel that these studies will establish a novel model for identifying key protein-carbohydrate interactions required for VAR2CSA function and ultimately guide the development of therapeutics that specifically target these interactions.

Positions and Honors

1989–1993	Associate Research Scientist, Molecular Biophysics and Biochemistry, Yale University
1991–1993	Lecturer, Molecular Biophysics and Biochemistry, Yale University
1993–2003	Biology Department, Brookhaven National Laboratory, Upton, NY
1993–1995	Assistant Scientist, Biology Department, Brookhaven National Laboratory, Upton, NY
1995–1997	Associate Scientist, Biology Department, Brookhaven National Laboratory, Upton, NY
1997–2000	Biophysicist, Biology Department, Brookhaven National Laboratory, Upton, NY
2000–2003	Biophysicist (with tenure), Biology Department, Brookhaven National Laboratory, Upton, NY
1996–2003	Adjunct Asst. Professor, Dept. of Physiology & Biophysics, State Univ. of NY at Stony Brook
2003–present	Professor, Department of Biochemistry and Molecular Biology, Penn State University College of Medicine, Hershey, PA
2011-present	Director of the Biochemistry and Molecular Genetics option of the Biomedical Sciences Graduate program.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors in the order listed on Form Page 2. Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME Maria C. Bewley	POSITION TITLE Associate Professor
eRA COMMONS USER NAME (credential, e.g., agency login) MBEWLEY	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
University of Leeds, United Kingdom	B.Sc.	1990	Molecular Biophysics
University of Leeds, United Kingdom	Ph.D.	1994	Protein Crystallography
Massey University, Palmerston N., New Zealand	Post-doc	1994-1997	Protein crystallography
Brookhaven National Laboratory, Upton, NY	Post-doc	1997-1999	Molecular and Structural Biology

Personal Statement

The goal of the proposed research is to obtain a structural understanding of var2CSA. I have a documented expertise in determining structures by x-ray crystallography. I was a scientist at Brookhaven National Laboratory for 6 years, where I spent most of my time collecting data at the National Synchrotron Light Source, gaining expertise in growing crystals and collecting useable data on small fragile samples. I continue to collaborate with the beamline scientists who are developing techniques for collecting data on microcrystalline arrays using micro supports. I have successfully used modeling algorithms to predict domain boundaries that result in more stable proteins. Recently, I have gained expertise in Small Angle X-ray Scattering (SAXS) that allow the quaternary structures to be determined in solution. I was recently appointed to the proposal review panel for SAXS (beginning June 2011), indicative of my recognized competence in this field. All of my approved proposals have generated manuscripts that are either published or in the later stages of preparation and describe hybrid methods (modeling, NMR, SAXS and X-ray crystallography) that exploit all of the structural information.

Positions and Honors

1999-2001 Assistant Biochemist, Biology Department, Brookhaven National Laboratory, Upton, NY

2001–2003 Associate Biochemist, Biology Department, Brookhaven National Laboratory, Upton, NY

2003–2007 Assistant Professor, Department of Biochemistry and Molecular Biology, PSU College of Medicine, Hershey. PA

2007- present Associate Professor, Department of Biochemistry and Molecular Biology, PSU College of Medicine, Hershey, PA

2011-present Member of proposal review panel for Structural Biology in Solution, NSLS, Brookhaven National Lab, Upton, NY