

erably Montanide ISA 720 adjuvant (Seppic Inc, France). In further aspects, vaccination with the soluble protein of the present technology and at least one adjuvant confers partial or full protection in a vaccinated subject against a malaria challenge, and in some aspects provides sterile protection against malaria challenge.

**[0018]** In another aspect, anti-rCSP antibodies produced after immunization with the soluble protein of the present technology can also recognize native CSP on sporozoites. The present technology includes those anti-rCSP antibodies.

**[0019]** In some aspects, the final recombinant CSP protein of the present technology is of high purity and suitable for human vaccination against malaria. Further, the protein of the present technology can be produced under current good manufacturing practices to produce a vaccine grade protein composition made in animal-free media, a media free of animal-derived components. A human-grade vaccine suitable for administration to human subjects can be produced.

**[0020]** The purified rCSP product of the present technology meets the purity criteria for an injectable for human administration (>95% purity by gel densitometry, low or undetectable levels of host cell proteins, as detected by western blot, and less than 5 endotoxin units/microgram total protein. Further, the rCSP protein product is structurally homogeneous as observed on reduced and non-reduced SDS-page and is stable at 4° C. for at least a week.

**[0021]** In some aspects, the novel rCSP of the present technology is strongly immunogenic and provides protection against challenge of sporozoites.

**[0022]** In some aspects, the present technology provides a method of producing a near full-length soluble CSP (such as the peptide sequence of SEQ ID NO:2) that is stable at high concentrations in aqueous buffer and suitable nucleotide sequences for producing the soluble protein in *E. coli*.

**[0023]** In a further aspect, the present technology provides a PfCSP gene nucleotide sequence that in combination with an *E. coli* host strain produce correctly folded and soluble CSP without requiring a denaturing and refolding step in the production process of the proteins.

**[0024]** In yet a further aspect, the present technology provides a fermentation process that promotes the growth of a CSP-producing bacterial clone in non-animal derived media.

**[0025]** In still another aspect, the present technology provides a two-step purification process which results in a greater than 95% pure product of soluble recombinant CSP with low endotoxin levels (for example, less than 5 endotoxin units per microgram) and low or undetectable levels of host proteins.

**[0026]** In yet another aspect, the present technology provides an *E. coli* host cell that produces a soluble recombinant CSP that can be used for vaccination against malaria. The vaccine containing the CSP can offer improved immunogenicity over yeast derived CSP vaccines which may be post-translationally modified, truncated or glycosylated. Thus, in some embodiments, the present vaccines comprise rCSP that is not glycosylated or post-translationally modified or truncated.

**[0027]** In another aspect, the present technology provides a vaccine that can be economically produced by expression in *E. coli* and a two-step purification system that results in a substantially pure soluble protein.

**[0028]** Other aspects and advantages of the present technology are set forth in part in the description, as follows, and in part, may be obvious from the description, or may be learned from practice of the present technology.

#### BRIEF DESCRIPTION OF THE FIGURES

**[0029]** FIG. 1 is a cartoon representation of the protein expression constructs CS/A, CS/B, CS/C, CS/D, and CS/E shown in relation to native *P. falciparum* CSP gene 3D7 strain (top). The number of NANP and NVDP repeats and relative position of the cysteine residues (C) are also shown (figure not drawn to scale).

**[0030]** FIGS. 2A, 2B and 2C depict the thermal stability of CS/A and CS/B soluble protein. FIG. 2A shows purified CS/A (lane 1) and CSB (lane 2) protein as analyzed by SDS-PAGE via coomassie blue staining and western blot analysis using an anti-6xHIS monoclonal antibody after one freeze-thaw cycle. FIG. 2B shows an image of precipitated CS/A protein having a fibrillar structure upon negative staining and electron microscopy imaging. FIG. 2C demonstrates thermal stability of CS/B protein following storage for one week at 37° C. (lane 1), 4° C. (lane 2) and -80° C. (lane 3) as demonstrated by SDS-PAGE and coomassie blue staining.

**[0031]** FIG. 3 illustrates the comparative expression levels of CS/C, CS/D, and CS/E in *E. coli*. Uninduced (U) and IPTG induced (In) whole *E. coli* cell pellets were analyzed by SDS-PAGE and stained by coomassie blue. CS/C (5 NANP repeat containing), CS/D (19 NANP repeat containing) and CS/E (38 NANP repeat containing) proteins are shown by the arrows.

**[0032]** FIGS. 4A, 4B and 4C illustrate the immunogenicity of rCSP proteins based on the number of NANP repeats. Groups of mice (9 per group) were immunized with either CS/C (a 5 NANP repeat rCS protein) or CS/D (a 19 NANP repeat CS protein). End-point ELISA titers were determined for the mice two weeks after immunization with the second dose of either the CS/C or CS/D protein. Antibody titers were measured using ELISA plates coated with CS/C (FIG. 4A), CS/D (FIG. 4B), or a 6xNANP repeat peptide (FIG. 4C).

**[0033]** FIG. 5 depicts the nucleotide sequence of the recombinant CSP gene of the present technology with and without two expression 6xHIS tags.

**[0034]** FIG. 6 depicts the translated protein sequence of the soluble CSP and the N and C-terminal tags.

**[0035]** FIGS. 7A, 7B, 7C, and 7D depict the results of purity and identity studies on a laboratory grade CS/D protein. FIG. 7A is a SDS-PAGE analysis of the Ni-NTA Ni affinity elution and Q-sepharose elution stained by coomassie blue. FIG. 7B is the SDS-PAGE analysis of the elution from a Superdex-75 gel filtration column stained with coomassie blue. FIG. 7C is SDS-PAGE analysis of the CS/D purified protein with 2 µg, 1 µg, 0.5 µg, 0.25 µg and 0.1 µg protein loaded per lane (lanes 1-5 respectively) analyzed under non-reducing and reducing conditions and visualized by silver staining (Snap Silver Kit™, Pierce). FIG. 7D is an anti-CSP mAb Western Blot showing 1 µg CS/D protein positively reacting with the anti-*P. falciparum* CSP specific mAb 49-1B2 (left panel, lane 1). Western blot was also performed to determine purity of the protein using an anti-*E. coli* polyclonal antibody (Dako Corp). Lane 1 and 2 contain 1 µg and 2 µg purified CS/D protein respectively and lane 3 contains the lysate of bacteria (right panel).

**[0036]** FIGS. 8A and 8B depict the immunogenicity and protection efficacy of lab-grade CS/D shown in FIG. 7. FIG. 8A shows specific end-point ELISA titers against CS/D protein coated plates. Titers of individual CSP vaccinated mice were plotted against time (end point defined as serum dilution that results in an OD=0.5). Days of vaccination are shown by arrows. FIG. 8B shows the survival curve of the CSP vacci-