

**[0018]** One embodiment of the present invention is directed to peptidic nanoparticles comprising self-assembling polypeptides which each comprises a pentameric domain and a trimeric domain; a linker which joins the pentameric domain and the trimeric domain; and an epitope comprising a sequence of a malarial antigen fused to the self-assembling polypeptide. Preferably the epitope is a universal epitope and may be fused to the self-assembling core at an exposed terminus which is an N-terminus or a C-terminus, and the epitope is a T-cell epitope or a B-cell epitope. Preferably, nanoparticles each contain one or more antigens as listed in Table 2. Also preferable, the antigen contains a sequences containing one or more of the SEQ ID NOs listed in table 3. Nanoparticles of the invention may further comprise a second epitope, wherein the second epitope is a T-cell epitope or a B-cell epitope.

**[0019]** Nanoparticles are very thermostable and are candidates for vaccines. Nanoparticles are of roughly homogeneous size, and spherical appearance, with a diameter of about 25 nm. Preferably, the nanoparticle self-assembling polypeptide contains no disulfide cross-linking.

**[0020]** Also preferably, an assembly of nanoparticles remains non-aggregated in solution in the absence of a reducing agent over a period of months. Preferred nanoparticles are useful for the treatment and prevention of malaria and the epitope is derived from the *P. falciparum* sporozoite protein.

**[0021]** Another embodiment of the invention is directed to vaccines for the prevention or treatment of malaria. Vaccines of the invention comprise a self-assembling polypeptide comprising a pentameric domain; a trimeric domain; and a linker that joins the pentameric domain and the trimeric domain; and an epitope of an antigen capable of inducing a protective immune response in a mammal susceptible to infection by a malaria parasite. Preferably the self-assembling polypeptide is a continuous chain comprising peptide oligomerizations of the pentameric domain and the trimeric domain. Vaccines of the invention comprises the antigens and proteins set forth in Table 2 or one or more of the sequences set forth in Table 3. Vaccines preferably contain a pharmaceutically acceptable carrier. Preferred vaccines include a construct containing the circumsporozoite protein antigen of *P. falciparum*.

**[0022]** Another embodiment of the invention is directed to methods for vaccinating against infection from a malaria parasite. These methods comprise administering a functionalized self-assembling polypeptide nanoparticle comprising a self-assembling core; and an epitope fused to the self-assembling core, wherein the self-assembling core comprises a pentameric coiled-coil domain; a trimeric coiled-coil domain; and a linker joining the pentameric coiled-coil domain and the trimeric coiled-coil domain wherein the epitope generates an immunologically protective reaction against infection by a malaria parasite when administered to a mammal. Preferably the nanoparticle is administered without an adjuvant and the epitope is PfCSP. Also preferably, the epitope is a universal epitope comprising the sequence of SEQ ID NO. 8 or SEQ ID NO. 9.

**[0023]** Another embodiment of the invention is directed to an icosahedral particle comprising functionalized self-assembling polypeptide nanoparticles, wherein each self-assembling polypeptide nanoparticle comprises a self-assembling core, and an epitope fused to the self-assembling core, wherein the self-assembling core comprises a pentameric coiled-coil domain, a trimeric coiled-coil domain, and a linker, said linker joining the pentameric coiled-coil domain

and the trimeric coiled-coil domain, and wherein the icosahedral particle is formed by multimerization via the coiled-coil sequences. Particles typically have a diameter of about 25 nm and contain an antigen of a malaria parasite. Preferably the antigen is derived from a protein of *P. falciparum* such as the circumsporozoite protein.

**[0024]** Other embodiments and advantages of the invention are set forth in part in the description, which follows, and in part, may be obvious from this description, or may be learned from the practice of the invention.

#### DESCRIPTIONS OF THE DRAWINGS

**[0025]** FIG. 1. A schematic of a linear self-assembling polypeptide building block of a SAPN vaccine.

**[0026]** FIG. 2 Schematic drawing of "even units" for trimeric and pentameric oligomerization domains [left side, A)] and trimeric and tetrameric oligomerization domains [right side, BA)], respectively. The number of monomers (building blocks) is defined by the least common multiple (LCM) of the oligomerization states of the two oligomerization domains D1 and D2 of the building blocks. In the even units the linker segments of all building blocks will be arranged as closely to each other as possible, i.e. as close to the center of the peptidic nanoparticle as possible and hence the even units will self-assemble to a spherical nanoparticle.

**[0027]** FIG. 3. A model of the SAPN showing: A) a monomer peptide sequence composed of a trimeric coiled-coil, a linker segment, a pentameric coiled-coil and a disulfide bridge; B) the self-assembly of multiple SAPN via trimer and pentamer oligomerization; C) a completely assembled 60 mer icosahedrons SAPN.

**[0028]** FIG. 4. The architecture of a nanoparticles constructed from various elements shown as a figure and as an EM photograph.

**[0029]** FIG. 5. A schematic of the linear, self-assembling polypeptides.

**[0030]** FIG. 6. A bar graph summarizing the survival of mice in two separate experiments totaling 40 mice, 20 C57BL/6 and 20 Balb/c in each group.

**[0031]** FIG. 7. A graph related to parasitemic mice (Balb/c) vaccinated with SAPN with or without adjuvant are provided sterile protection. Groups of 10 mice receiving 3 doses of vaccine were challenged with *P. berghei* sporozoites. Mice receiving PBS, N-Empty or N-Empty/M all developed parasitemia by day 6. Mice receiving N-PbCSP or NPbCSP/M or were vaccinated with irradiated sporozoite vaccination did not demonstrate detectable parasitemia. Similar results were seen in C57BL/6 mice.

**[0032]** FIG. 8. Antibody response to N-PbCSP in Balb/C mice.

**[0033]** FIG. 9. The percent of mice developing parasitemia after *P. berghei* sporozoite challenge. Mice (Balb/c) had received either splenocytes or serum for mice that had been previously immunized with nanoparticles expressing the *P. berghei* B cell epitope (PbCSPr) administered with or without adjuvant. Control mice received no cells or serum.

**[0034]** FIG. 10. A bar graph of IgG Isotype profile of Balb/c and C57BL/6 mice after three immunizations with *P. berghei* CSP B cell repeat containing nanoparticles.

**[0035]** FIG. 11. The potency of N-PbCSP without adjuvant. Mice were given the indicated  $\mu\text{g}$  of protein, in each of 3 doses, 2 wks apart then challenged with sporozoites.

**[0036]** FIG. 12. SAPN made with NCS-PfAMA and NCS-PbCSP in the indicated ratios protected mice from challenge.