Title: Extraction and purification of malaria vaccine candidate CLCT produced by transient expression in *Nicotiana benthamiana* plants

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Abbreviations: column volume (CV); downstream processing (DSP); fast flow (FF); flow through (FT); host cell protein (HCP); high-throughput screening (HTS); hydrophobic interaction chromatography (HIC); molecular mass cut off (MMCO); total soluble protein (TSP); ultrafiltration/diafiltration (UF/DF)

Additional file

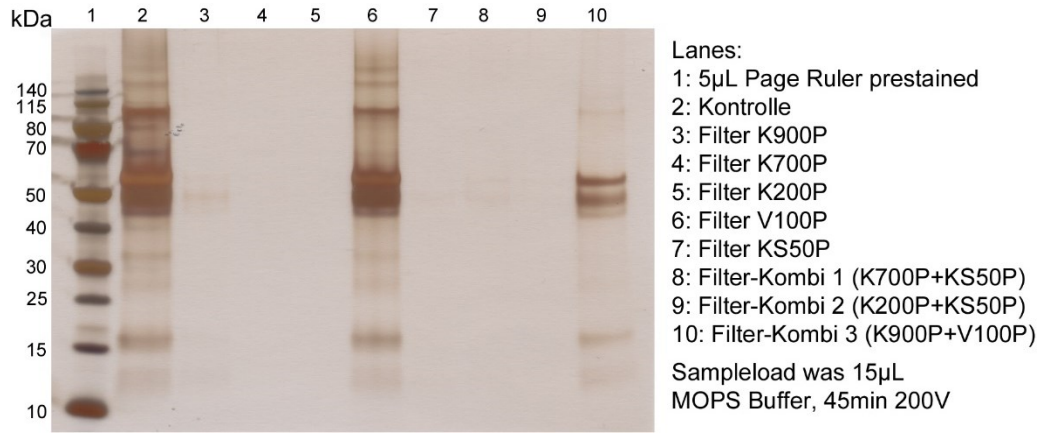

Figure S1: Evaluation of depth filters for the clarification of CLCT from extracts of blanched plant biomass using different types of depth filter sheets (K900P, K700P, K200P, V100P, KS50P; Pall, Dreieich, Germany) or combinations thereof. The filtrate samples were analyzed by LDS-PAGE followed by silver staining as previously described [27].

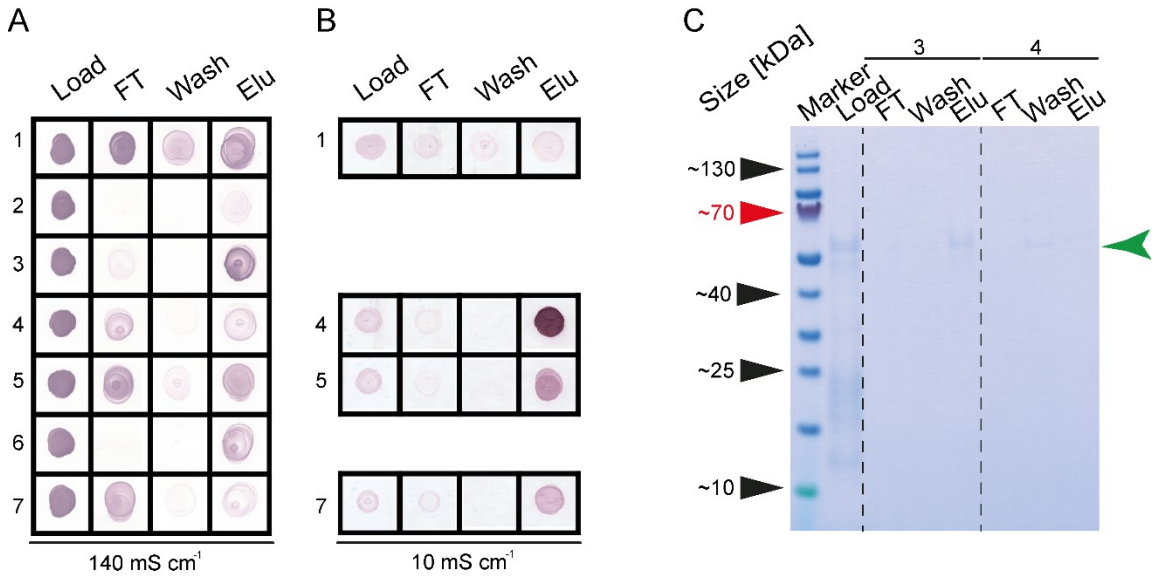

Figure S2: Unmodified version of Figure 5 without contrast adjustments in panel C. High-throughput HIC resin scouting for the purification of CLCT from plant extracts. (A,B) Dot blots of HTS samples representing various HIC resins (1–7) using a high salt load at 140 mS cm^{-1} and (B) a low salt load at ~10 mS cm^{-1} for four selected resins (1, 4, 5 and 7). 1 – Phenyl Sepharose 6FF (HS), 2 – Phenyl Sepharose HP, 3 – Phenyl Sepharose 6 FF (LS), 4 – Octyl Sepharose 4 FF, 5 – Butyl Sepharose 4 FF, 6 – Butyl Sepharose HP, 7 – Butyl-S Sepharose, FT – flow through fraction, Elu – elution fraction. The blots were probed using a mouse primary monoclonal antibody against *Pf*CSP-TSR and an AP-conjugated goat anti-mouse (GAM^{AP}) secondary antibody (Table 1). (C) Samples of a 1.0-mL CV small-scale HIC purification of CLCT (green arrow) analyzed by LDS-PAGE and staining with Coomassie Brilliant Blue. Resins are identified using the same numbers as in panels A and B. Marker – PageRuler pre-stained protein ladder (10–177 kDa), Load – concentrated CLCT at a conductivity of ~140 mS cm^{-1} (in A and C) or at a conductivity of ~10 mS cm^{-1} (in B), Elu – elution, FT – flow through.

Table S1: Malaria vaccine candidates against *P. falciparum* used in active clinical trials. The table lists different malaria vaccine candidates and their corresponding development stage, information about the antigen(s) and platform type.

Product name	Trial phase	Developers	Life cycle stage	Antigen	Platform	Registry ID
BK-SE36/CpG	Phase I	Osaka University	Blood stage	PfSERA5	RS	JPRN-JMA-IIA00109
ChAd63-MVA Rh5	Phase Ia	University of Oxford	Blood stage	PfRh5	RVV	NCT02181088
ChAd63-MVA Rh5	Phase Ib	University of Oxford	Blood stage	PfRh5	RVV	NCT03435874
Chemically attenuated Pf7G8	Phase I	Griffith University	Blood stage	Whole parasite	Whole parasite	ACTRN12618001314213
FMP013/ALFQ	Phase I	WRAIR	Pre-erythrocytic stage	PfCSP	RS	NCT04268420
FMP014/ALFQ	Phase I	USAMRDC	Pre-erythrocytic stage	PfCSP	SaN	NCT04296279
PfGAP3-KO	Phase I	NIAID	Pre-erythrocytic stage	Whole sporozoite	Whole parasite	NCT03168854
Pfs25-IMX313/Matrix M1	Phase Ib	University of Oxford	Sexual stage	Pfs25	RS	NCT04271306
PfSPZ Vaccine	Phase II	NIAID	Pre-erythrocytic stage	Whole sporozoite	Whole parasite	NCT03989102
PfSPZ-CVac	Phase I	University of Tubingen	Pre-erythrocytic stage	Whole sporozoite	Whole parasite	DRKS00017462
PfSPZ-GA1	Phase I	Sanaria	Pre-erythrocytic stage	Whole sporozoite	Whole parasite	NCT03163121
PRIMVAC/Alhydrogel;PRIMVAC/GLA-SE	Phase I	INSRM	Blood stage; Blood stage	VAR2CSA;VAR2CSA	RS	NCT02658253
R21/AS01B	Phase I	University of Oxford	Pre-erythrocytic stage	PfCSP	RS,VLP	NCT02600975
R21/Matrix M1	Phase III	University of Oxford	Pre-erythrocytic stage	PfCSP	RS	NCT04704830
R21/Matrix M1	Phase II	University of Oxford	Pre-erythrocytic stage	PfCSP	RS,VLP	NCT05252845
R21/Matrix M1;ChAd63-MVA ME-TRAP	Phase IIb	University of Oxford	Pre-erythrocytic stage	PfCSP;ME-TRAP	RS,VLP; Viral vector	NCT03947190
rCSP/AP10-602	Phase I	NIAID	Pre-erythrocytic stage	PfCSP	RS	NCT03589794
Rh5.1/Matrix M1	Phase Ib	University of Oxford	Blood stage	PfRh5	RS	NCT04318002
Rh5/AS01B	Phase IIa	University of Oxford	Blood stage	PfRh5	RS	NCT02927145
RTS,S/AS01E	Phase IV	GlaxoSmithKline (GSK)	Pre-erythrocytic stage	PfCSP	RS,VLP	NCT02251704

WRAIR – Walter Reed Army Institute of Research; NIAID – U.S. National Institute of Allergy and Infectious Diseases; INSRM – Institut National de la Sainté Et de la Recherche Médicale, France; USAMRDC – U.S. Army Medical Research and Development Command; RS –

Recombinant subunit; RVV – Recombinant viral vector; VLP – Viral-like particle; SaN – Self-assembling nanoparticles. Data retrieved and modified from (https://www.who.int/observatories/global-observatory-on-health-research-and-development/monitoring/who-review-of-malaria-vaccine-clinical-development#data-sources).

Table S2: Correlation coefficients for modeled response surfaces of CLCT purity and recovery in the HIC elution fraction. Purity was calculated following LDS-PAGE and gel staining. Immunoblot data were used to calculate recoveries.

Modeled response	CLCT purity [% TSP]	CLCT recovery [% load]
R^2	0.8998	0.9184
Adjusted R^2	0.8776	0.9002
Predicted R^2	0.7486	0.8083

HIC – hydrophobic interaction chromatography; LDS – lithium dodecyl sulfate; PAGE – polyacrylamide gel electrophoresis; TSP – total soluble protein.

Table S3: Numerical optimization for the chromatographic purification of CLCT based on the descriptive models. The desirability function of Design Expert was used to optimize responses in the zero (undesired) to one (desired, target) range [64].

Optimized	pH[b] [-]	Conductivity load [mS cm^{-1}]	Conductivity elution [mS cm^{-1}]	Desirability [-]
Purity [%]	7.7	140.0	1.2	0.882
Recovery [%]	8.0	140.0	0.0	0.949
Purity [%] and recovery [%][a]	8.0	140.0	0.0	0.909

[a] Rated equally, [b] No effect on optimization results.