Pyrococcus furiosus thioredoxin as a platform to express extracellular loops of *Treponema pallidum* outer membrane proteins to assess antigenicity and isolate antigen-specific B-cells: a new structural approach for syphilis vaccine development

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Background

The incidence of syphilis continues to increase in the US and globally. Development of an effective syphilis vaccine is a critical component of strategies to curtail this surging public health threat. Syphilitic infection in rabbits has demonstrated that it is possible to elicit a protective response against *Treponema pallidum*. Immune rabbit serum (IRS) enhances opsonophagocytosis of intact spirochetes by macrophages, indicating the presence of protective antibodies targeting surface epitopes. Our hypothesis is that antibodies directed against extracellular loops (ECLs) of outer membrane proteins (OMPs) are responsible for the opsonic activity observed in IRS. The *Pyrococcus furiosus* thioredoxin (PfTrx) was used as a scaffold to express ECLs (PfTrx^{ECL}), which was then used to assess ECL reactivity with syphilitic sera and isolate ECL-specific B-cells.

PfTrx construct design and cloning

PfTrx was engineered to express BamA-ECL4 (PfTrx$^{BamA/ECL4}$), a highly immunogenic and opsonic loop previously characterized by our group. N-terminal His6- and C-terminal Avi-tags were added to PfTrx$^{BamA/ECL4}$ for Ni-NTA purification and *in vivo* biotinylation, respectively (**Fig. 1**).

PfTrx $^{BamA/ECL4}$ expression and purification

PfTrx$^{BamA/ECL4}$ was then cloned into pET28a expression vector and transformed into *E. coli* BL21 containing the biotin-protein ligase BirA, which allows for *in vivo* biotinylation. Recombinant proteins were purified from the supernatant in three-steps: (i) heat purification, (ii) Ni-NTA affinity column and (iii) size exclusion chromatography (**Fig. 2**).

Screening for ECL antigenicity

The antigenicity of PfTrx$^{BamA/ECL4}$ with IRS (Nichols strain) and human secondary syphilitic sera (HSS) was assessed by immunoblotting. IRS and HSS reacted with PfTrx$^{BamA/ECL4}$; cross-reactivity against PfTrx was not detected. (**Fig 3**).

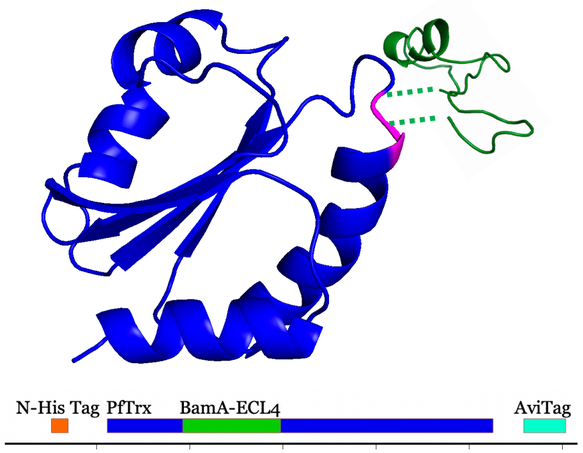

Figure 1. Phyre2 prediction of PfTrx structure showing insertion site for ECLs (in this case, BamA ECL4) and a linear map of the PfTrx$^{BamA/ECL4}$ construct with N-His6- and C-Avi-tag.

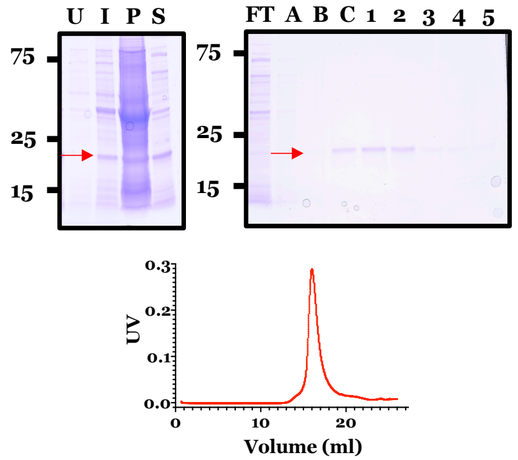

Figure 2. Coomassie Blue-stained SDS-PAGE showing purified PfTrx$^{BamA/ECL4}$ (arrows) (Top left panel). Ni-NTA purification yielded PfTrx$^{BamA/ECL4}$ with high purity (Top right panel). Size exclusion chromatography (SEC) from Ni-NTA elution fractions of PfTrx$^{BamA/ECL4}$ (Bottom panel). (U: uninduced, I: Induced, P: Insoluble pellet, S: Soluble fraction, FT: flow through, A to C: Wash buffers, 1 to 5: Elution fractions).

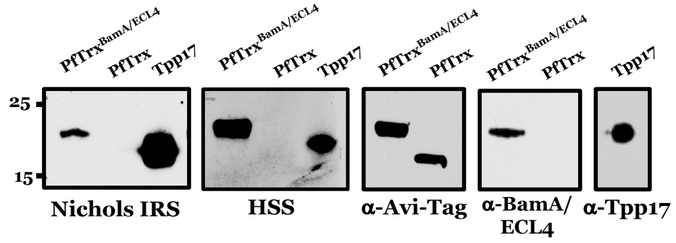

Figure 3. PfTrx$^{BamA/ECL4}$ reactivity with immune rabbit serum and human syphilitic serum by immunoblotting; ross-reactivity against PfTrx was not detected. Mouse α-Avi Tag, rat α-BamA-ECL4 and rat α-Tpp17 were used as controls.

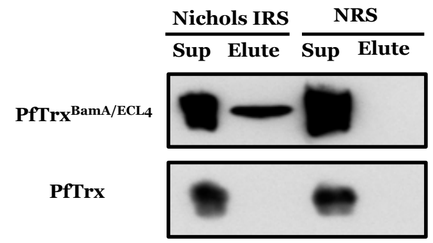

Figure 4. Pull down assay performed to assess the interaction of PfTrx$^{BamA/ECL4}$ with antibodies present in IRS. NRS was used as control. Detection was done with mouse α-Avi Tag mAb and developed with α-mouse IgG-Horse Radish Peroxidase (HRP).

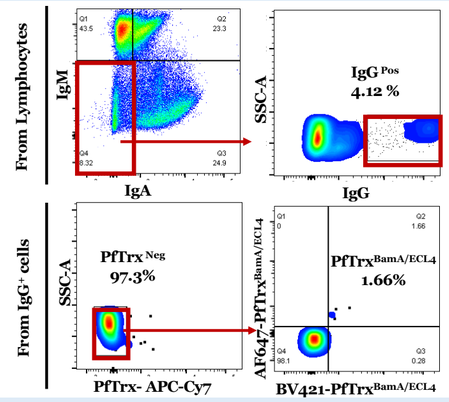

Figure 5. Identification of BamA/ECL4-specific B-cells from immune rabbit's PBMCs by using PfTrx$^{BamA/CL4}$ conjugated to AF-647 and BV-421. PfTrx was conjugated to APC-Cy7 to eliminate cells bound to the thioredoxin scaffold.

ECL accessibility for antibody recognition

A pull-down assay was done evaluate the ability of PfTrx$^{BamA/ECL4}$ to present ECLs in an antibody accessible form, PfTrx$^{BamA/ECL4}$ (or PfTrx alone) was incubated with IRS or NRS and protein G agarose beads. PfTrx$^{BamA/ECL4}$ was eluted from Nichols IRS but not NRS. Importantly, PfTrx alone was not pulled down with either IRS or NRS (**Fig. 4**).

BamA/ECL4-specific B-cells

PfTrx$^{BamA/ECL4}$ then was used to identify Ag-specific B-cells from PBMCs of an immune rabbit by flow cytometry. Rabbit B-cells of the IgG isotype were identified as shown in **Fig. 5.** 1.66% of the PfTrx^{Neg} IgG^{Pos} cells were PfTrx$^{BamA/ECL4}$-specific.

Conclusions

- The PfTrx^{ECL} system described represents a major step forward to assess antigenicity of ECLs and dissect the opsonic activity of IRS.
- PfTrx present ECLs in an accessible form for antibody recognition as confirmed by the pull-down assay.
- PfTrx^{ECL} constructs can be used to identify ECL-specific B-cells in rabbit PBMCs and generate ECL-specific mAbs.
- This system will help to identify highly immunogenic and opsonic ECLs as potential syphilis vaccine candidates.

Funding

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References

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