UNIVERSITY of MARYLAND
SCHOOL OF MEDICINE

# *S*. Typhimurium Core-OPS (COPS) glycoconjugate with the homologous serovar phase 1 flagellin as a vaccine to prevent invasive *S*. Typhimurium infections in sub-Saharan Africa

**10th International Conference on Typhoid and Other Invasive Salmonelloses, Kampala, Uganda**

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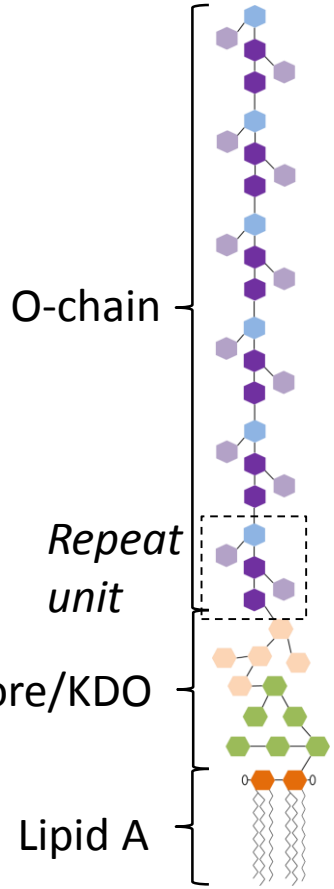
# Glycoconjugate vaccine approach for iNTS

- Polysaccharide:Protein Conjugate Vaccines
  - Elicit immunologic memory (T dependent)
  - Immunogenic & protective in young infants (e.g. Hib, pneumo, MCV)

**Components:**

- **Core + O polysaccharide** (COPS) of lipopolysaccharide
  - Surface polysaccharide of un-encapsulated *Salmonella* serovars
  - Conserved core, OPS repeat structure defines serogroup
- **Flagellin** subunits serve as the carrier protein
  - Target for immune responses
  - Protection by active immunization (Simon et al. 2011. I&I), passive transfer of anti-flagellin antibodies (Ramanchandran et al. 2016. PLOS One)
  - T helper epitopes and immunologic memory for relevant protection against target pathogen indication
- Previously found *S.* Enteritidis COPS:FliC conjugates immunogenic and protective in mouse model

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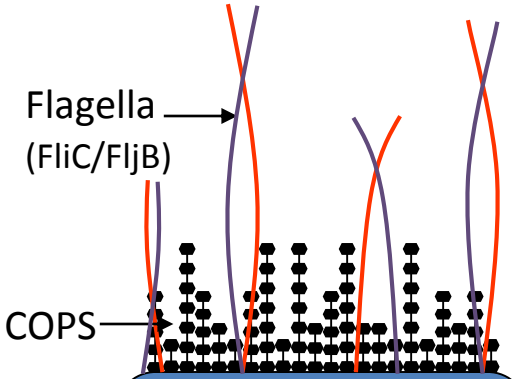
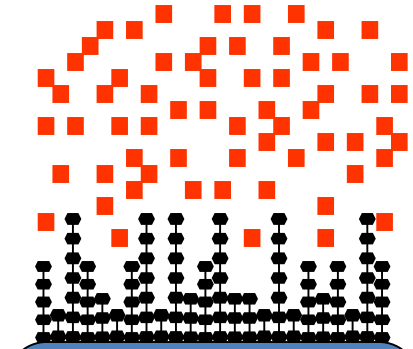
# Phenotypes of CVD NTS strains used as live vaccines and reagent strains to produce conjugate vaccine components




**Wild-type**

***S.* Typhimurium I77**

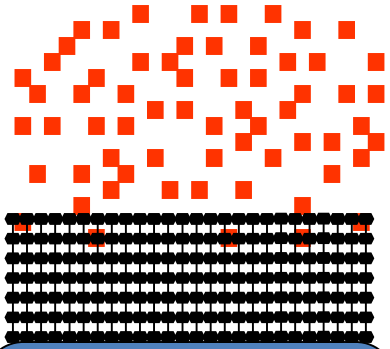
**(Malian Blood isolate)**



**CVD 1925**

I77 Δ*guaBA* Δ*clpPX* Δ*fliD* Δ*fljB*

**Flagellin production strain**



**1925wzzB**

I77 Δ*guaBA* Δ*clpPX* Δ*fliD* Δ*fljB* pSEC10-*wzzB*

**COPS production strain**

<u>Phenotypes of genetically engineered *Salmonella* strains</u>

1. Δ*guaBA* = >5-log attenuation
2. Δ*clpPX* = 2nd attenuating mutation / High-flagella
3. Δ*fliD* = flagellin monomer export
4. Δ*fljB* = phase 1 flagellin only
5. pSEC10-wzzB = uniform long-chain LPS

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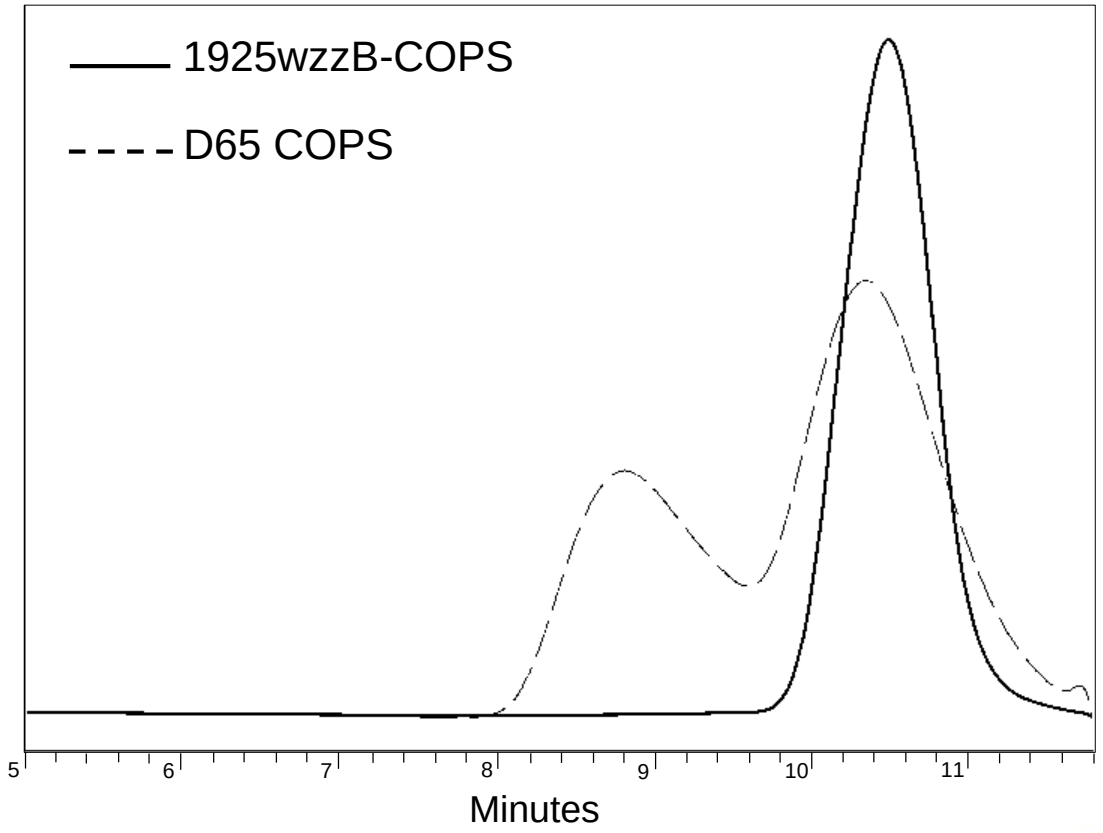
# Molecular size analysis of COPS from *S.* Typhimurium CVD 1925, CVD 1925 (pSEC10-wzzB) and Malian blood isolate D65


SDS-PAGE/Pro-Q




1925wzzB-COPS = ~19.8 kDa (SEC-MALS)

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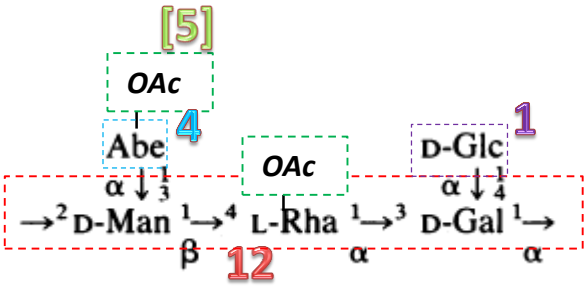
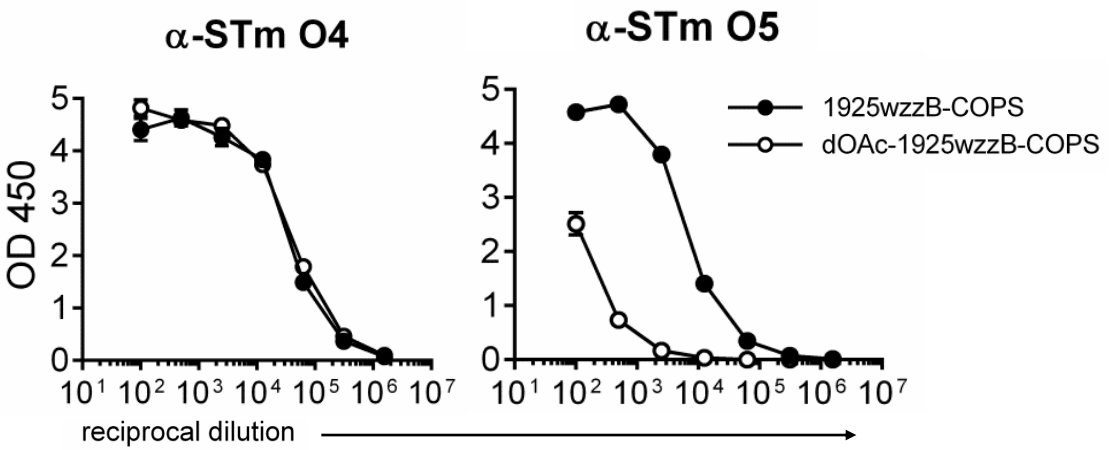
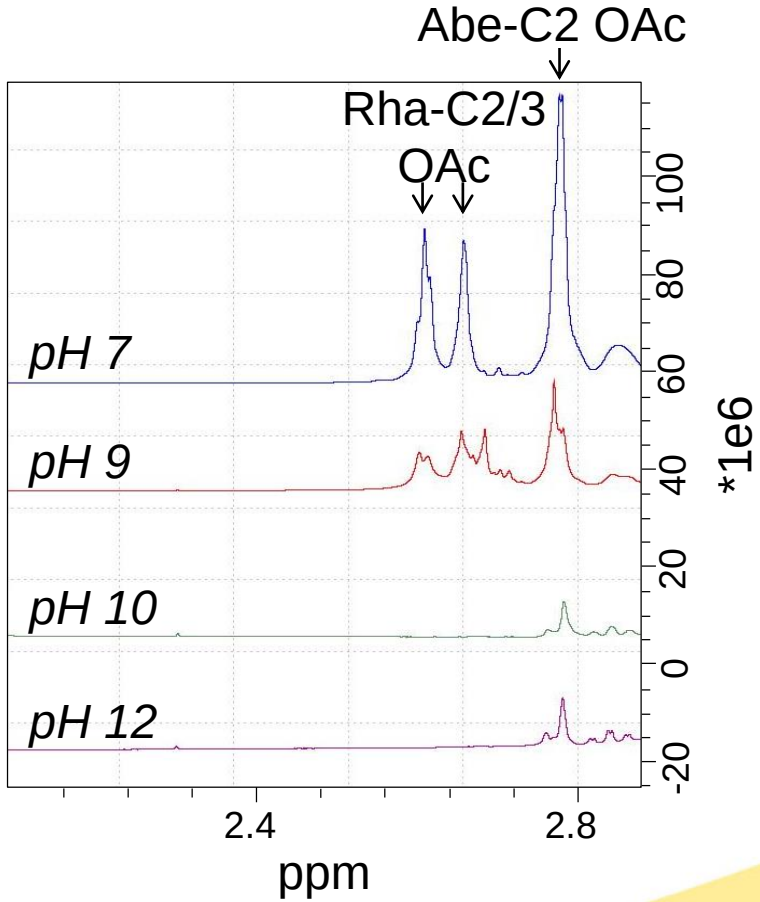
# Biochemical O-acetylation (1H NMR) and antigenicity (ELISA) analyses of purified *S.* Typhimurium 1925wzzB COPS




*S.* Typhimurium (B) – O:1,4,[5],12




- De-O-acetylation at pH10




- Variable O-acetylation at RhaC2/3, Abe C2

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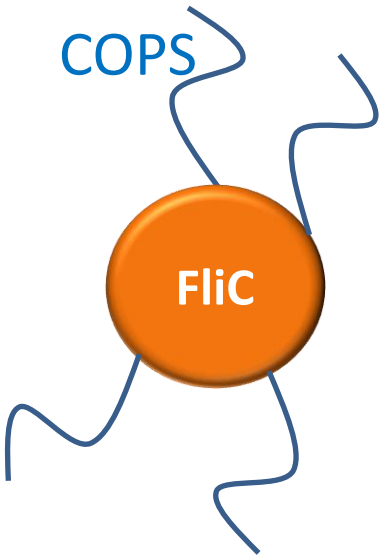
# Summary of conjugates synthesized for this study

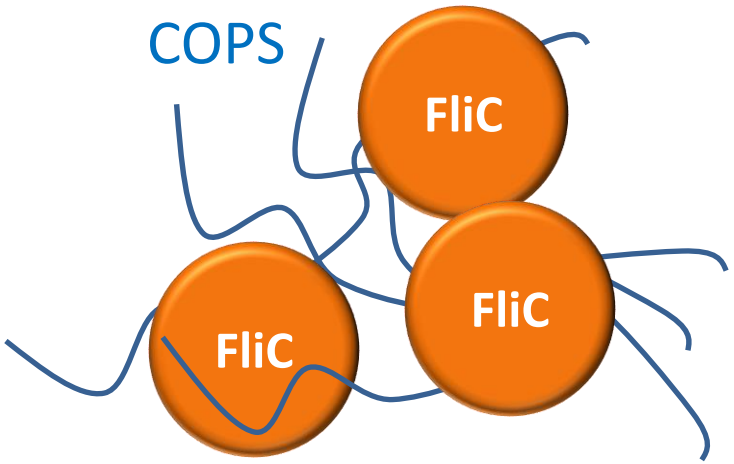
## Sun-type (end-link)

**Chemistry**: thioether
**Linkers**: GMBS (FliC lysines), aminooxy-thiol (COPS-KDO)
**Linkage**: COPS-KDO -> protein amines
**Conjugation pH**: 5-7




## Lattice (multi-point linkage)

**Chemistry**: CDAP cyanylation
**Linkers:** Adipic acid dihydrazide (FliC carboxyls)
**Linkage**: COPS hydroxyls -> protein amines and carboxyls
**Conjugation pH**: 9-10

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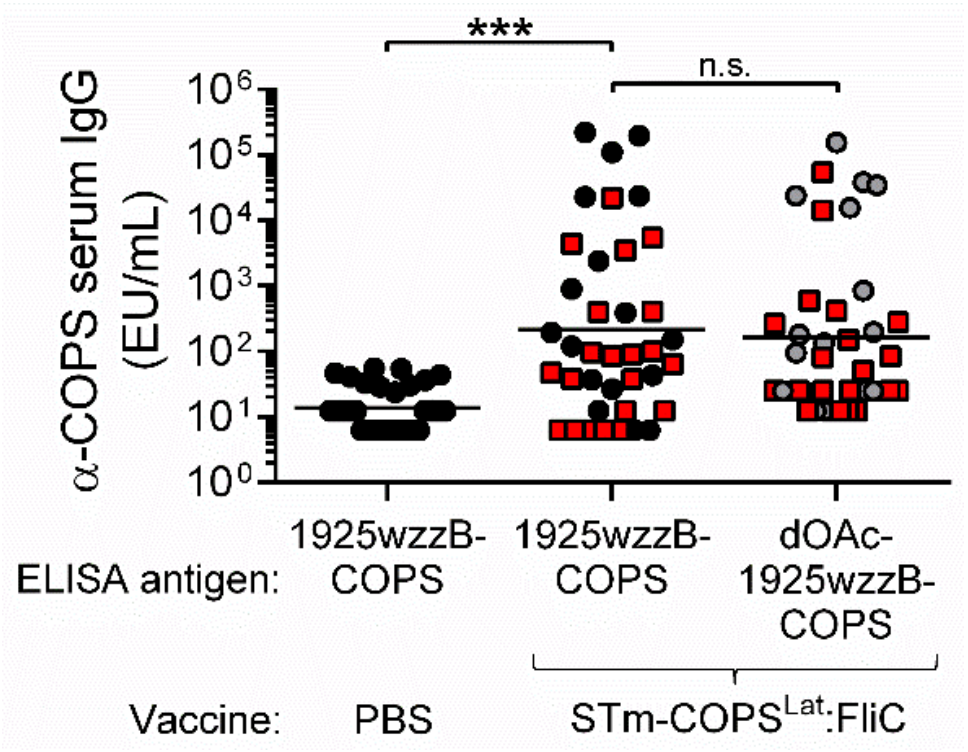
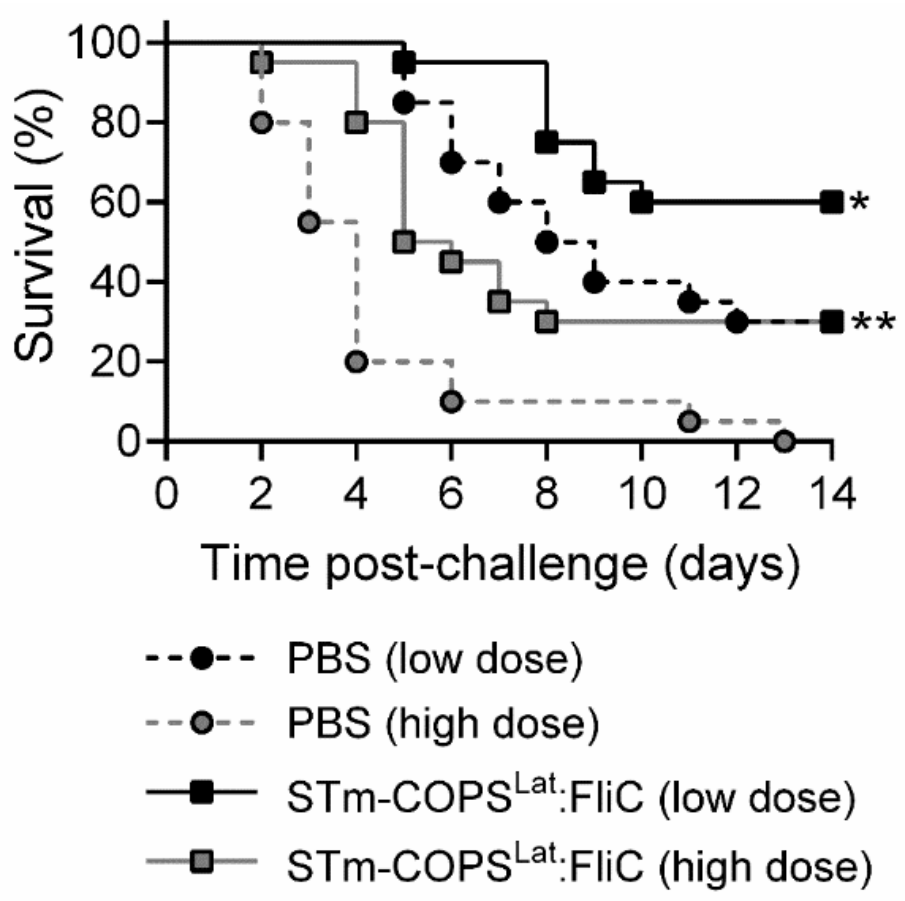
# Immunogenicity and protection against fatal infection with Malian *S.* Typhimurium blood isolate D65 in mice immunized with *S.* Typhimurium COPS$^{Lat}$:FliC




**Vaccine**: COPS$^{Lat}$:FliC (Lattice, ~31% OAc)

**Immunization**: d0, d28, d56 with 2.5 µg polysaccharide or PBS; Sera taken at d77

**Challenge**: IP infection at d84 with 1x$10^5$ or 5x$10^5$ *S.* Typhimurium D65 (IP LD50 = 2 x $10^4$)

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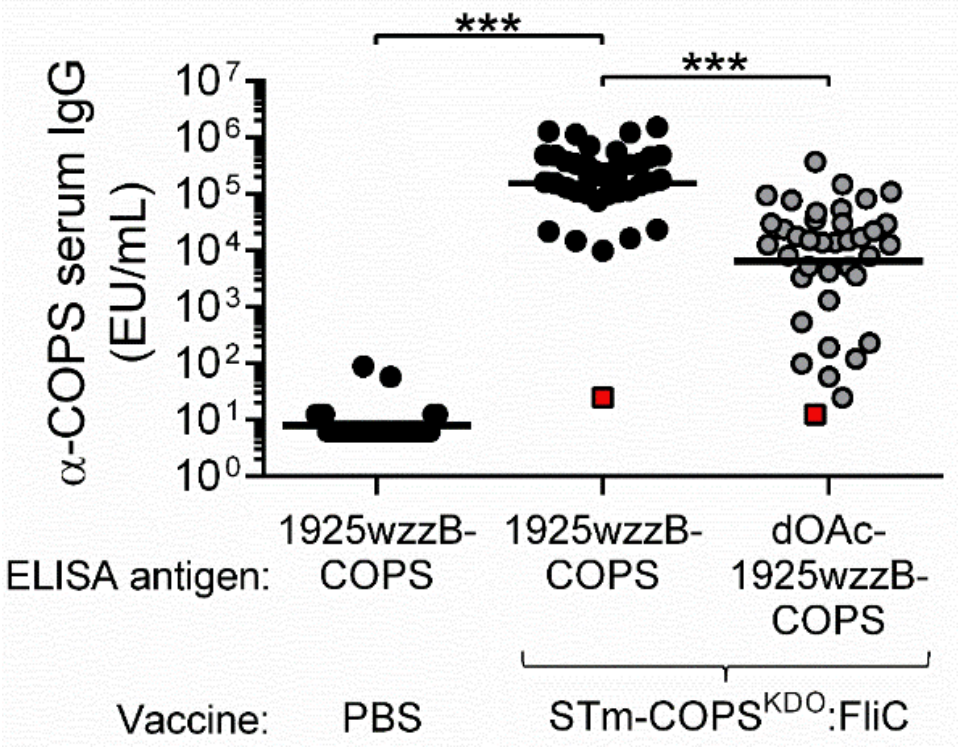
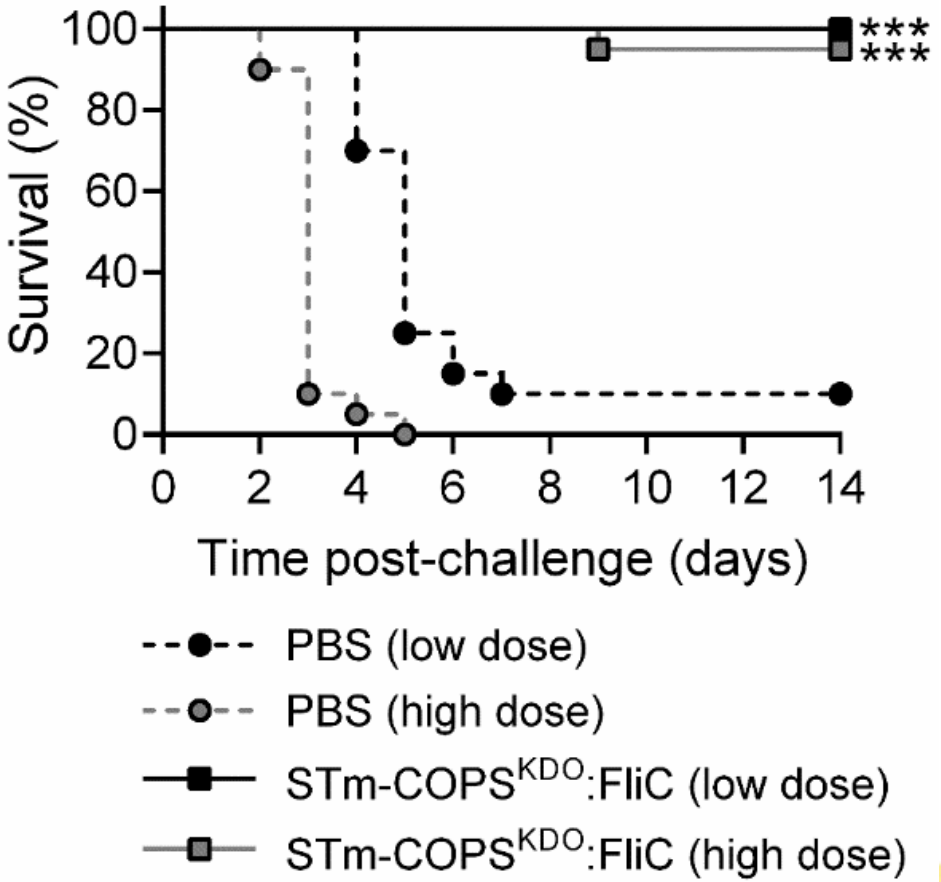
# Immunogenicity and protection against fatal infection with Malian *S.* Typhimurium blood isolate D65 in mice immunized with *S.* Typhimurium COPS$^{KDO}$:FliC




**Vaccine**: COPS$^{KDO}$:FliC (Sun-type, 100% OAc)

**Immunization**: d0, d28, d56 with 2.5 µg polysaccharide or PBS; Sera taken at d77

**Challenge**: IP infection at d84 with 1x$10^5$ or 5x$10^5$ *S.* Typhimurium D65 (IP LD50 = 2 x $10^4$)

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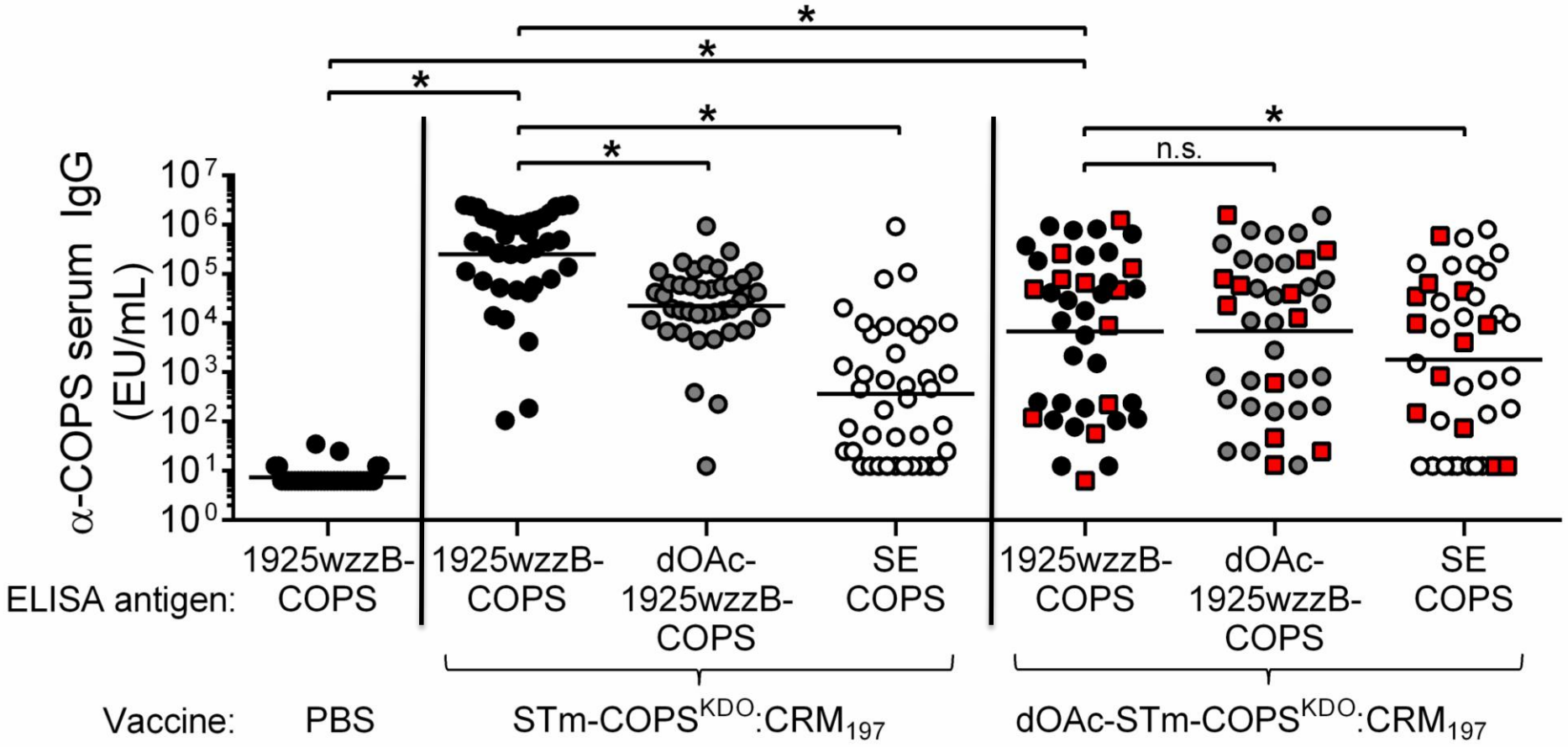
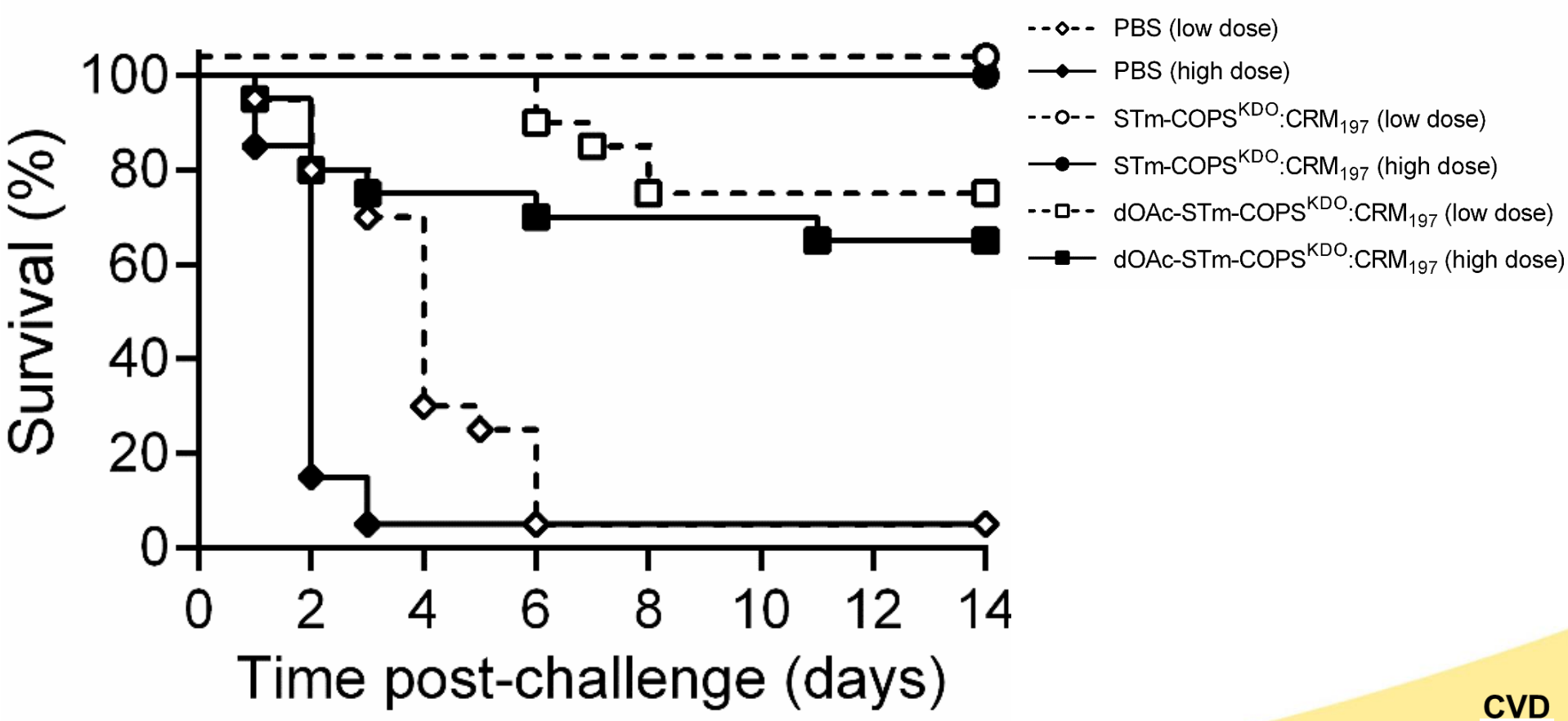
# Immunogenicity and anti-COPS epitope specificity in sera from mice immunized with native or de-O-acetylated 1925wzzB sun-type COPS$^{KDO}$:CRM$_{197}$ conjugates




**Vaccine:** STm-COPS$^{KDO}$:CRM$_{197}$ (100% OAc), dOAc-STm-COPS$^{KDO}$:CRM$_{197}$ (11% OAc)
**Immunization & challenge**: d0, d28, d56 with 2.5 µg polysaccharide or PBS; Sera taken D77; challenge at d84

# Mortality after challenge with high ($5x10^6$) or low ($1x10^6$) *S.* Typhimurium D65 doses in mice immunized with native or de-O-acetylated 1925wzzB COPS:$CRM_{197}$ conjugates

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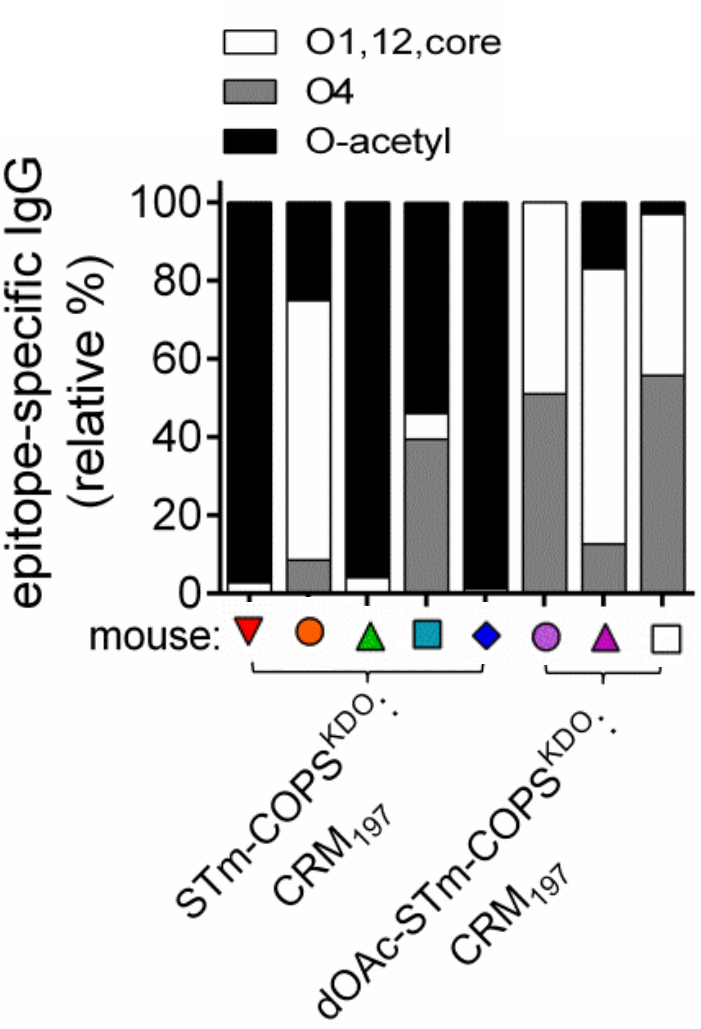
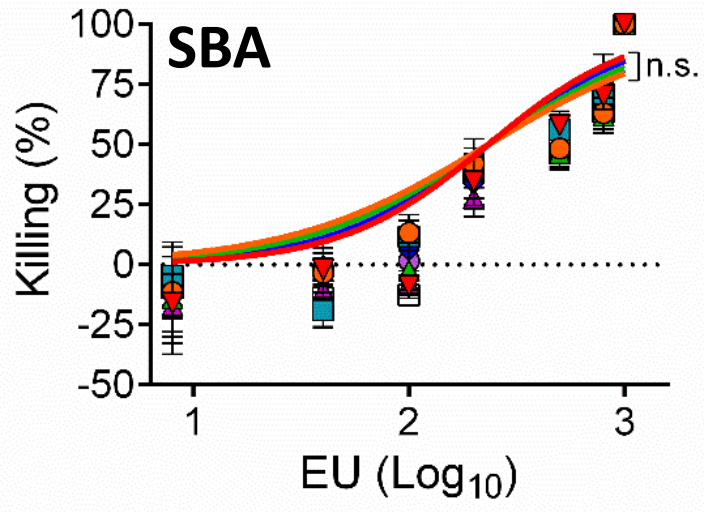
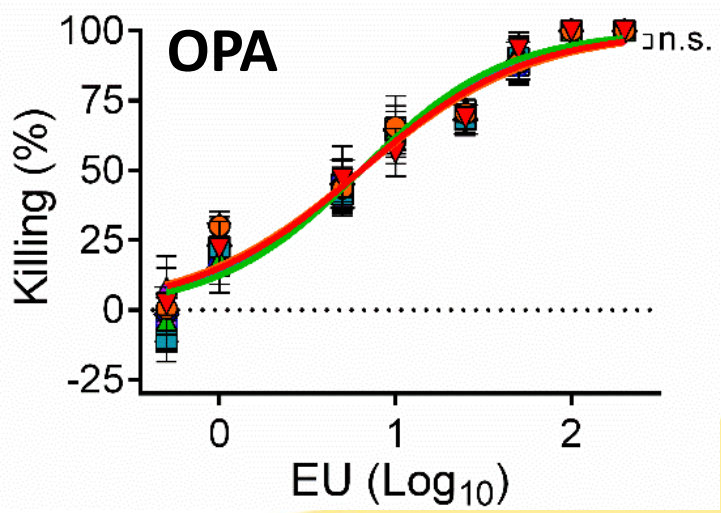
# Complement-mediated (SBA) and opsonophagocytic (OPA) functional bactericidal activity for *S.* Typhimurium with $COPS^{KDO}$:$CRM_{197}$ immune sera manifesting different anti-OPS epitope IgG profiles

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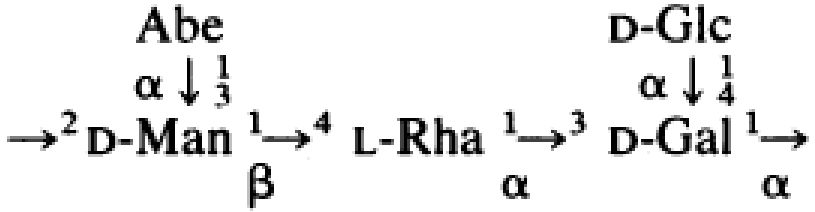
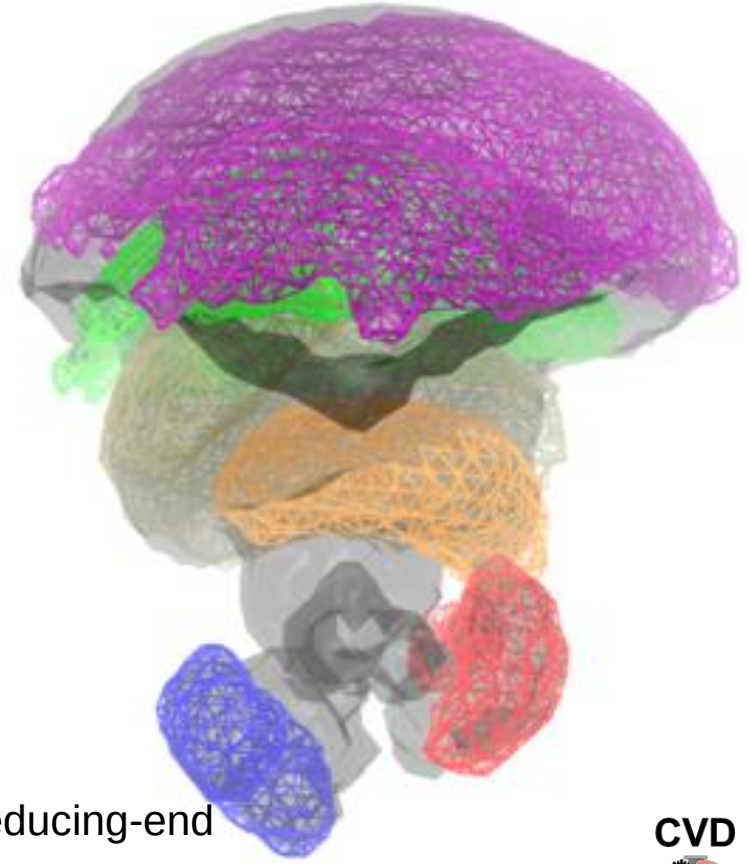
# *In-silico* enhanced molecular dynamics simulations of *S.* Typhimurium 3-repeat OPS to determine effect of O-acetylation on conformation

**Modeled 3-repeat OPS:**

- Variable glucosylation, O-acetylation (Rha+Abe)
- Determined possible conformations and residence time (steric accessibility)
- Dominant conformation shared between all types (>90% of sampled time), similar conformational volumes
- O-acetyls extend outwards, highly solvent accessible




**Total PS volume**: O-acetyls (color) overlaid with PS backbone (grey)



Reducing-end

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# Summary & conclusions for *in-vitro*/*in-vivo* studies

- Immunization with flagellin alone, passive transfer of anti-flagellin antibody is protective (~30% VE)
- 1925wzzB-COPS sun-type conjugates highly immunogenic, provide 100% protection against fatal challenge in mice with a Malian *S*. Typhimurium blood isolate
- End-linked sun-type conjugates of *S*. Typhimurium CVD 1925wzzB-COPS more immunogenic and protective than multipoint lattice type conjugates
- O-acetyls comprise strongly immunogenic COPS epitopes, however sera with different anti-OPS epitope profiles have similar anti-bacterial activity in-vitro, de-O-acetylated 1925wzzB-COPS:$CRM_{197}$ sun-type conjugate protected against D65 challenge (~70% VE)
- Molecular modeling analyses suggest O-acetylation does not affect conformational properties of OPS

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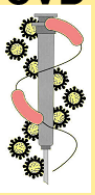

Baliban et al. 2017. PLOS NTD. *In-press*

# Trivalent formulation for invasive *Salmonella* infections in sub-Saharan Africa

UK Wellcome Trust Strategic Translational funding to UMB-CVD with Bharat Biotech (Hyderabad, India) as industrial partner for phase 1 & 2 clinical trials with trivalent S. Enteritidis COPS:FliC/S. Typhimurium COPS:FliC/Tybar-TCV (Vi:TT) formulation

UNIVERSITY of MARYLAND
THE FOUNDING CAMPUS

wellcome trust

Bharat Biotech

# Team & Funding

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Nageswara Rao
Janardhana Chary

**Fina BioSolutions**
Andrew Lees
John Van Druff

**Funding**
NIH/NIAID
UK Wellcome Trust

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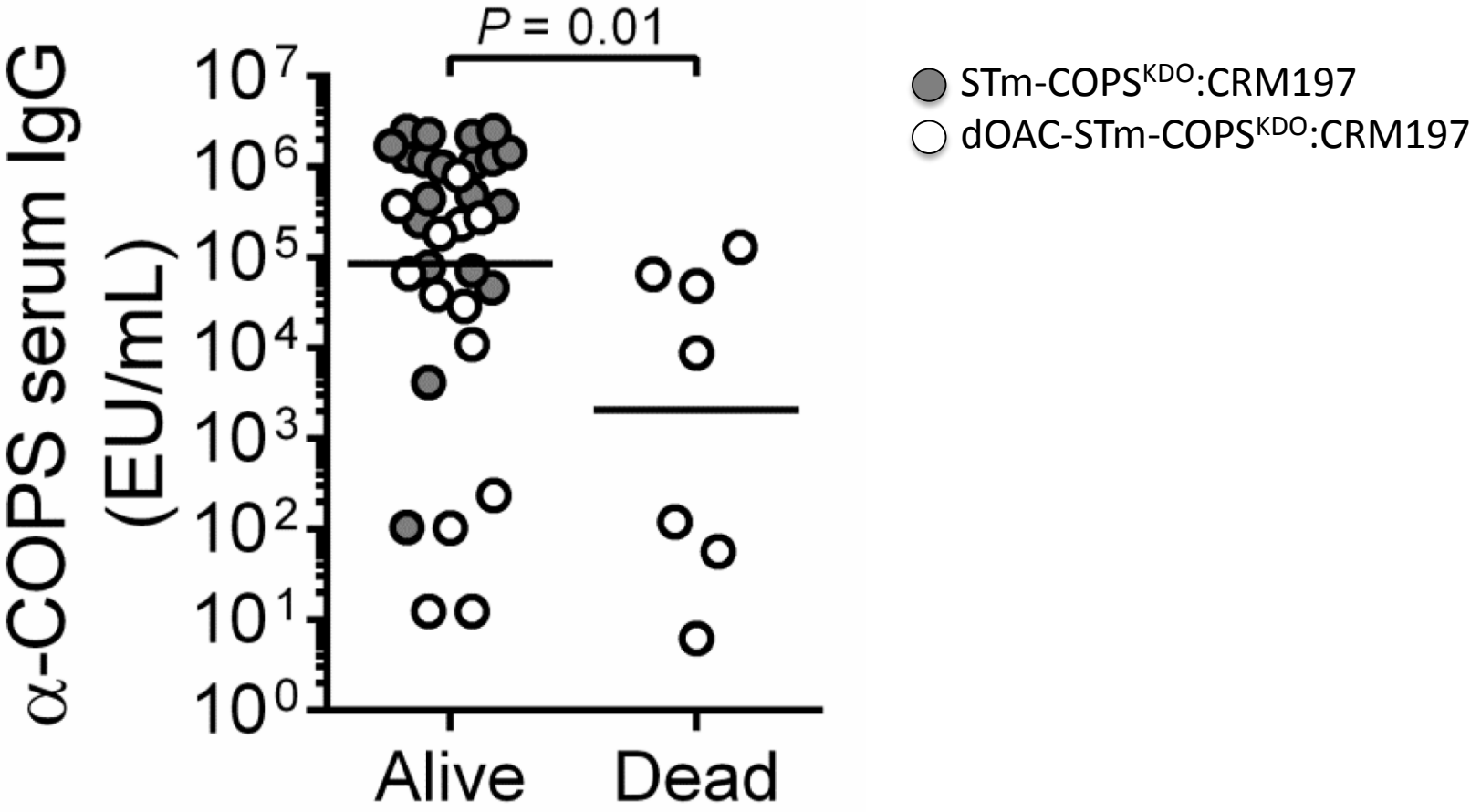
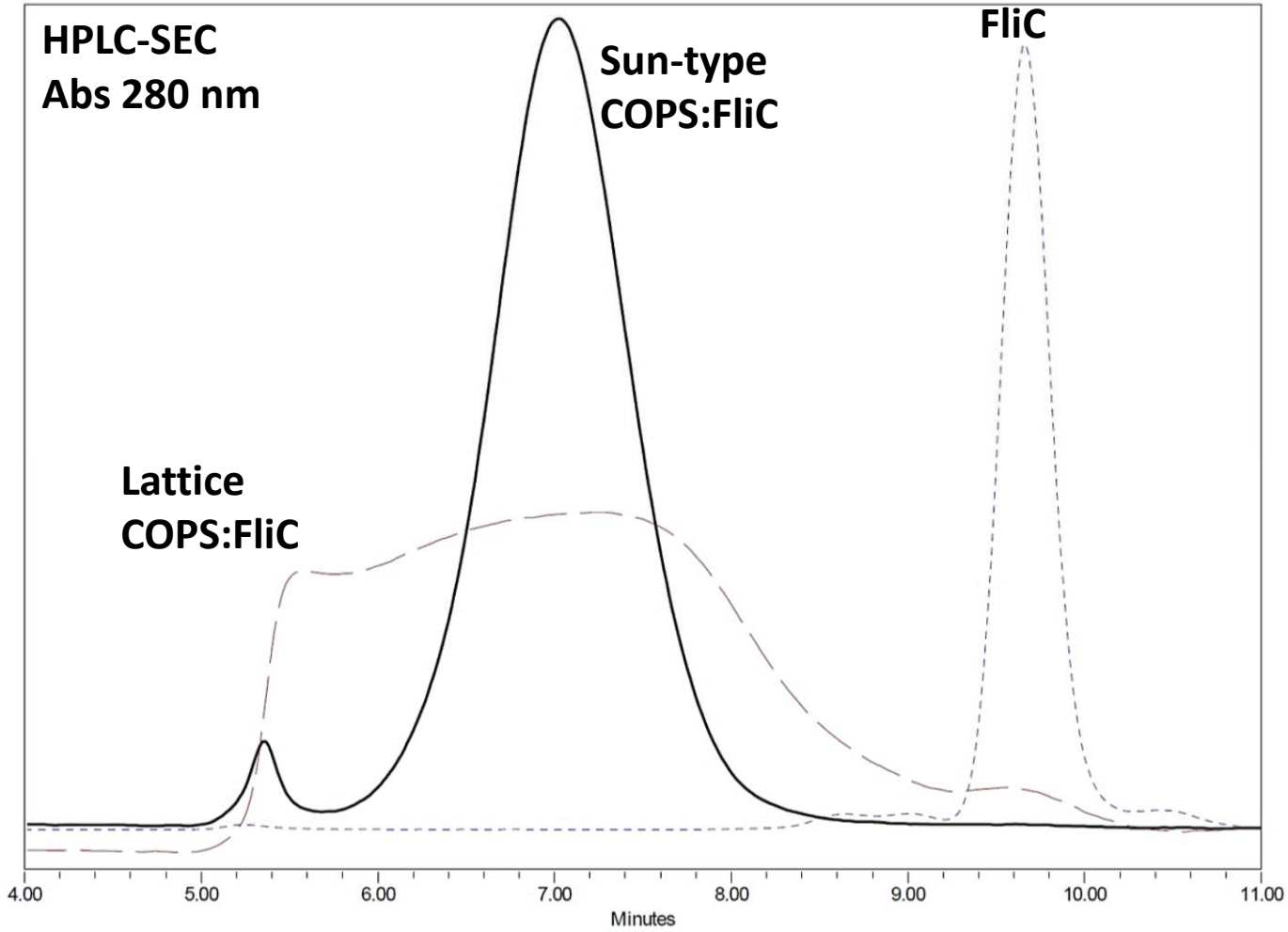
# Relationship between anti-COPS serum IgG titers induced by native and dOAc COPS:CRM197 conjugates and protection after challenge with $5x10^6$ CFU D65



# Biochemical and biophysical analyses of *S.* Typhimurium COPS:FliC conjugates




| Conjugate | PS:protein (wt:wt) | O-acetyl level (retained) |
|---|---|---|
| STm-COPS$^{Lat}$:FliC | 0.75 | 31% |
| STm-COPS$^{KDO}$:FliC | 1.4 | 100% |

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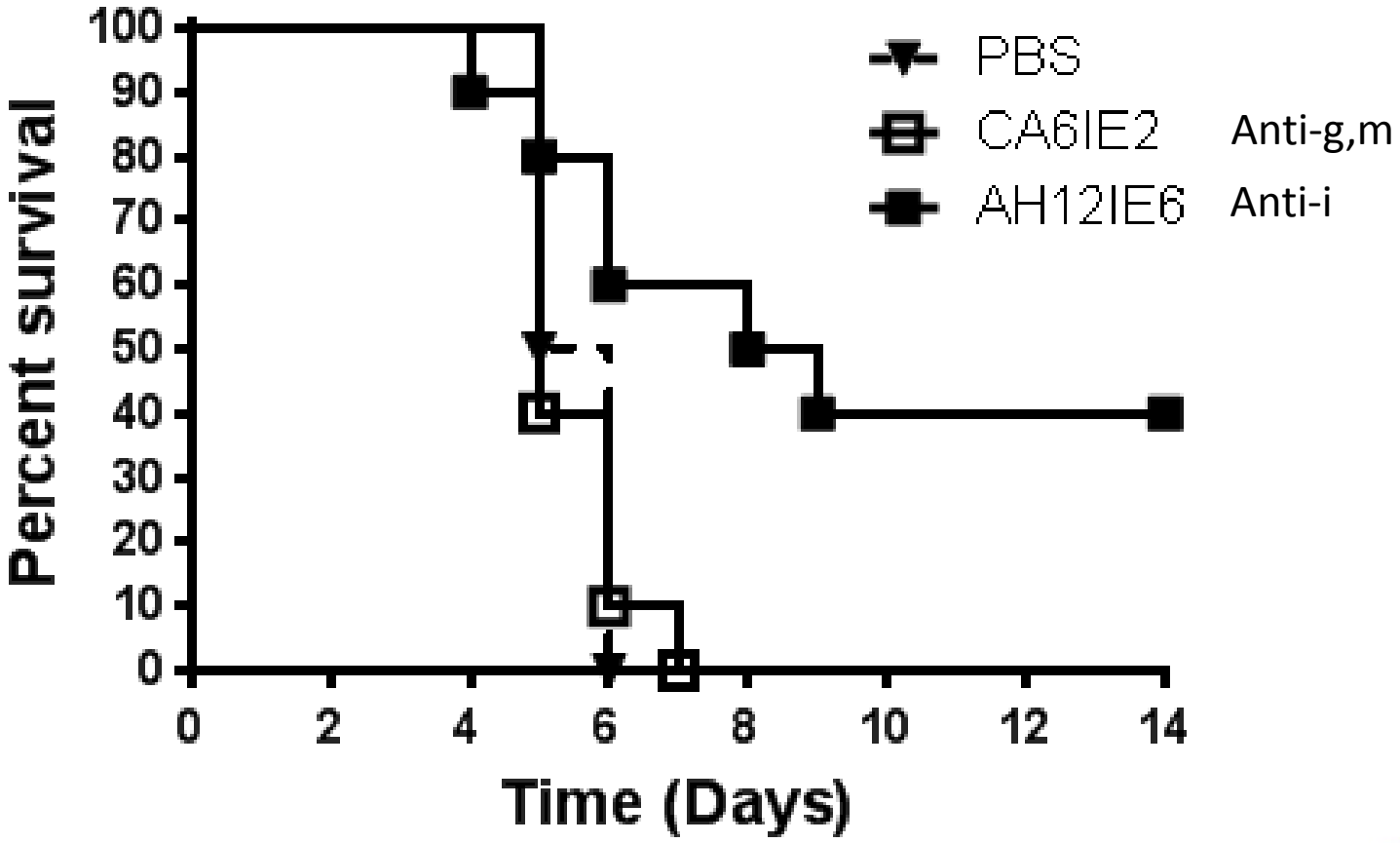
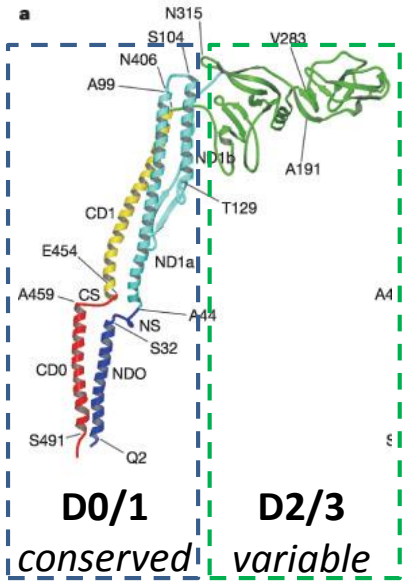
Baliban et al. 2017. PLOS NTD. *In-press*

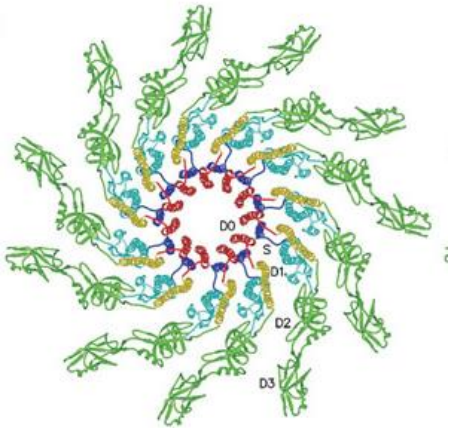
# Protection against *S.* Typhimurium D65 infection in mice passively transferred monoclonal anti-i IgG AH12IE6

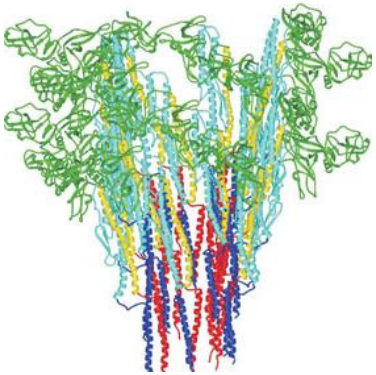


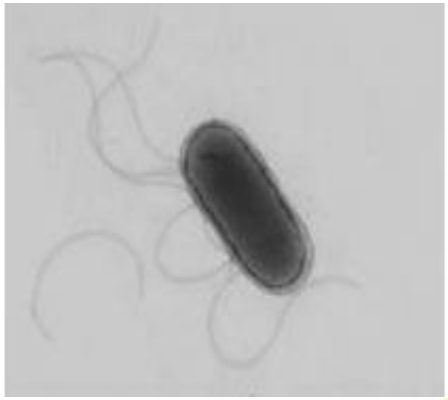

Ramachandran et al. 2016. PLoS One.

# Flagellin as carrier protein and vaccine antigen



*S*. Enteritidis FliC = “g,m” epitopes

*S*. Typhimurium FliC = “i” epitope

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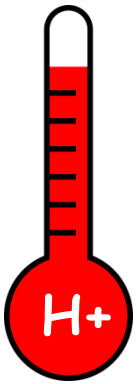
Namba, 2003

# Overview of Core-O polysaccharide (COPS) production process

**Stage I**
bacterial growth

**Stage II**
bacterial growth

**Fermentation**
18 hrs

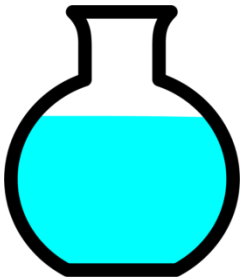



**Extraction**
100°C/pH 3-4

**Clarification**
Centrifugation/filtration

**Tangential flow filtration**
Sizing/buffer exchange

**Anion-exchange chromatography**
NA/protein/endotoxin removal



**Ammonium Sulfate**
Protein precipitation

**Tangential flow filtration**
Concentration/buffer exchange

# Overview of flagellin production process

**Stage I**
bacterial growth

**Stage II**
bacterial growth

**Fermentation**
8 hrs

**Clarification**
Centrifugation/filtration

*pH/conductivity adjustment*

**Cation Ion-exchange chromatography**
Capture/Wash/Elute

**Anion-exchange chromatography**
NA/Endotoxin polishing


**Tangential flow filtration**
Concentration/buffer exchange

*pH 2 monomerization*


**Sterile filtration**