



# Developing a MAPS vaccine against Salmonella Typhi and Paratyphi



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and Other Invasive Salmonellosis  
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I disclose the following financial relationships with commercial entities that produce health care-related products or services relevant to the content I am planning, developing, or presenting:

<u>Company</u>	<u>Relationship</u>	<u>Content Area</u>
Merck	Scientific Advisor	Vaccines

I also disclose membership on the Scientific Advisory Board of Arsanis Biosciences, and Advanced Inhalation Therapies  
I am a Scientific Founder, consultant and member of the Board of Directors of Affinivax, Inc.

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# Outline of presentation

1. Overview of MAPS technology
2. Studies of monovalent Vi MAPS
3. Studies of monovalent OSP MAPS
4. Immunogenicity testing of combination vaccine in rabbit model





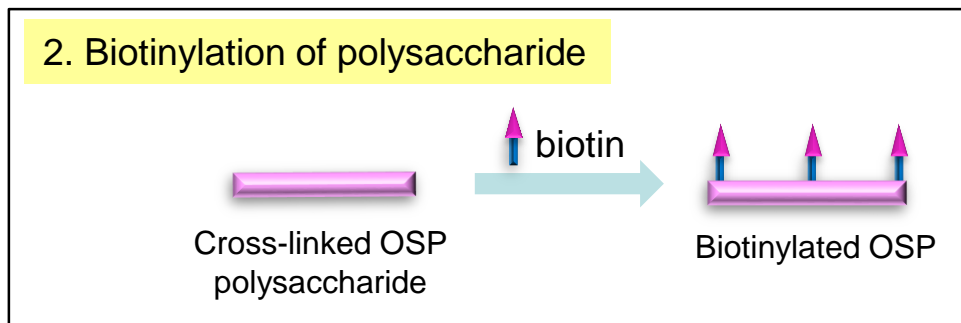
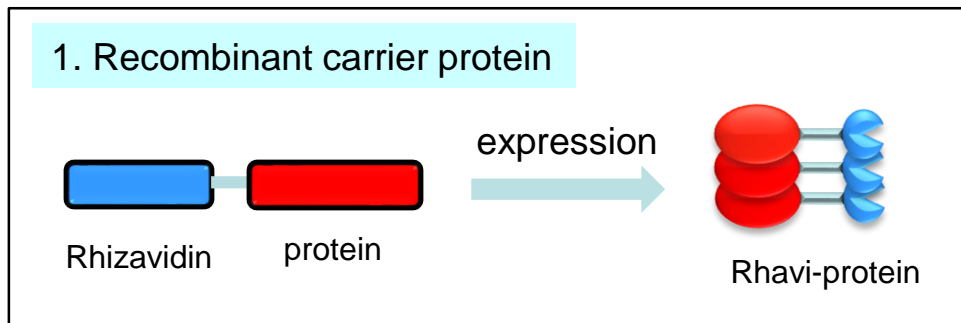
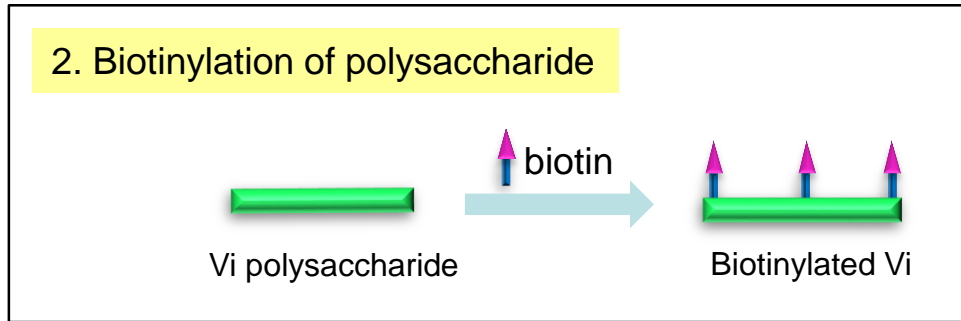
# Some issues with traditional conjugation chemistry

- For multivalent vaccines, containing several PS, each conjugate is essentially a separate vaccine
- Chemically coupling proteins to PS can be inefficient, complex, and subject to variability
- Can result in high cost of goods (COGs), difficulties in technology transfer
- Protein carriers are modified in act of conjugation, resulting in loss of potential protective epitopes. For this reason, most carrier proteins are not selected on basis of potential protection (CRM197, TT)

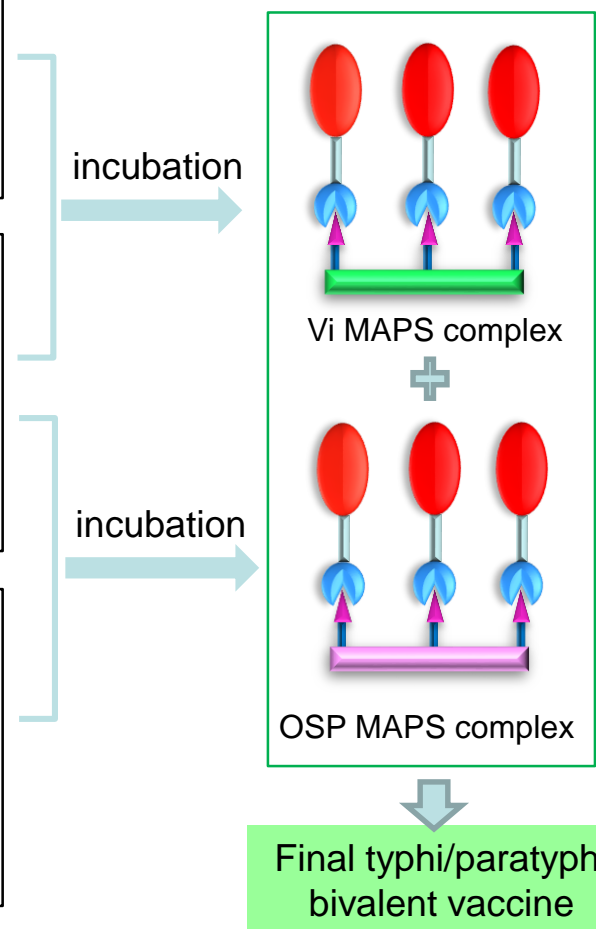




# Multiple Antigen Presenting System (MAPS) for Vi and paratyphi OSP



**3. Complex assembly**





# Comparison with and advantages over conventional conjugation

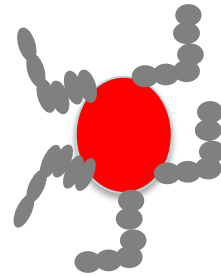
1. Immunological responses to PS indistinguishable from those obtained with traditional conjugates (e.g. pneumococcus)
2. Enhanced efficiency of manufacture of MAPS complex (>85% at laboratory scale and now scale-up, compared to 20-40% for conventional conjugates)
3. Little to no PS or protein epitope modification or damage due to controlled biotinylation of the PS and attachment via affinity-interactions with protein rather than cross-linking (Typhoid toxin for example)
4. Modular process (“plug and play”), allowing for rapid optimization



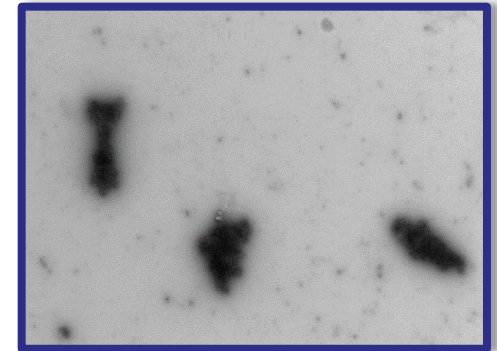
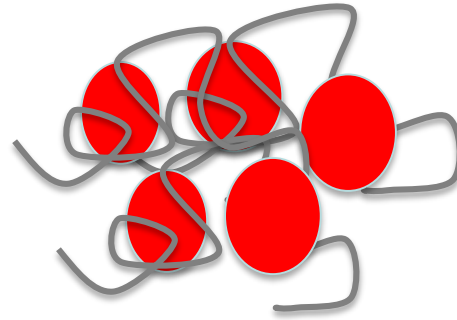


# Structures of various conjugates

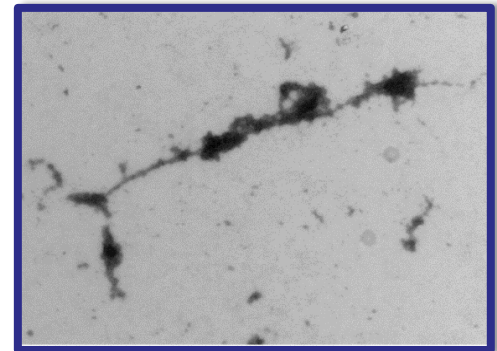
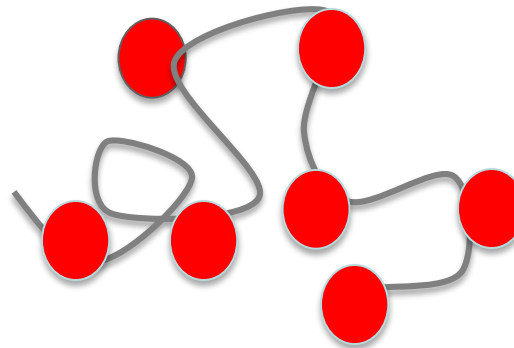
1. **Hairy Cue Ball**  
(neoglycoprotein) e.g. Wyeth's Hib (HbOC), some meningococcal conjugates



2. **Spaghetti and Meat Balls (lattice)** e.g. most conventional conjugates such as PCVs (Prennar, Synflorix)

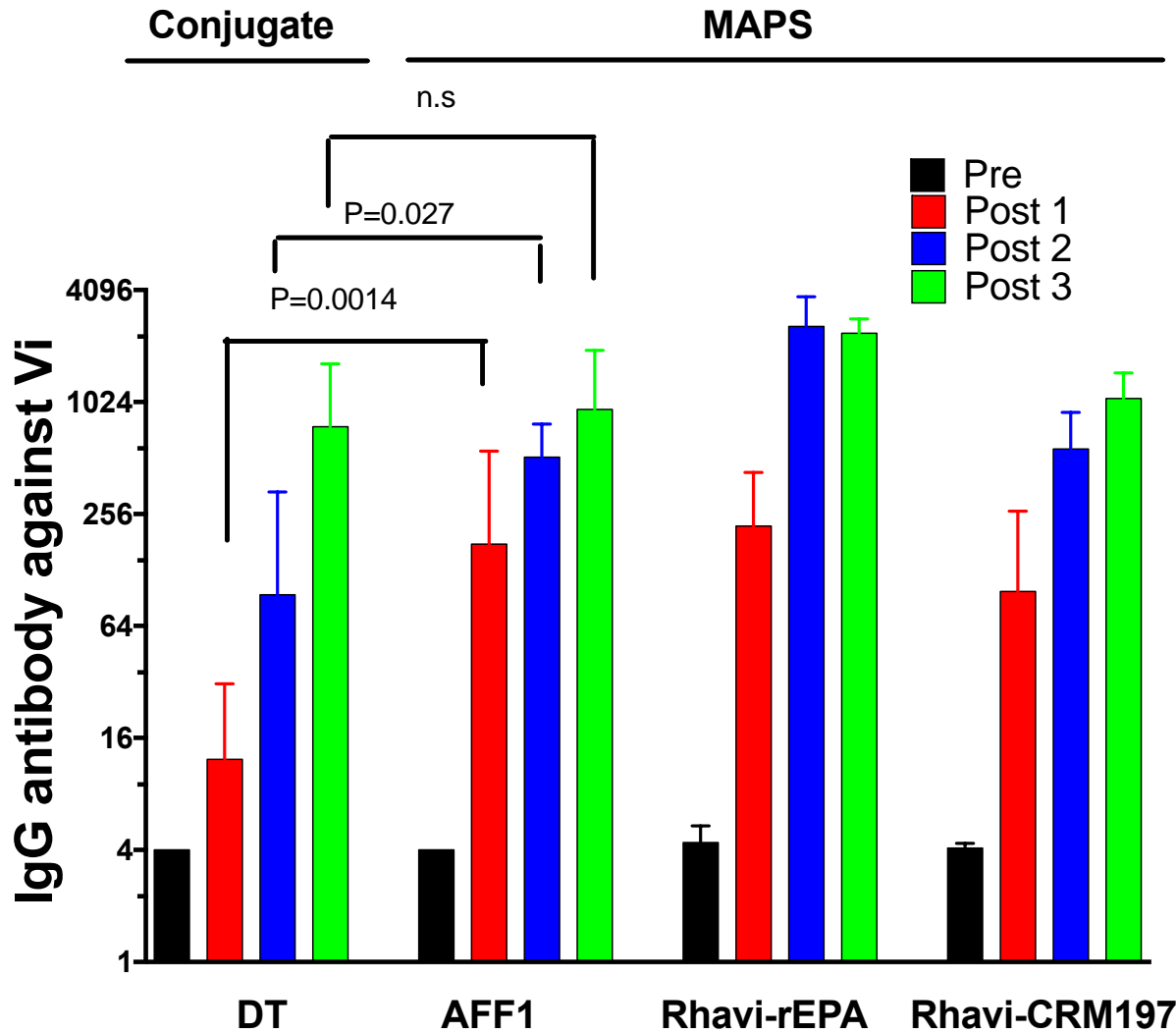


3. **Beads on a string** e.g. MAPS complexes with biotin-avidin





# Vi antibody titers in Guinea pigs after immunizations with Vi MAPS

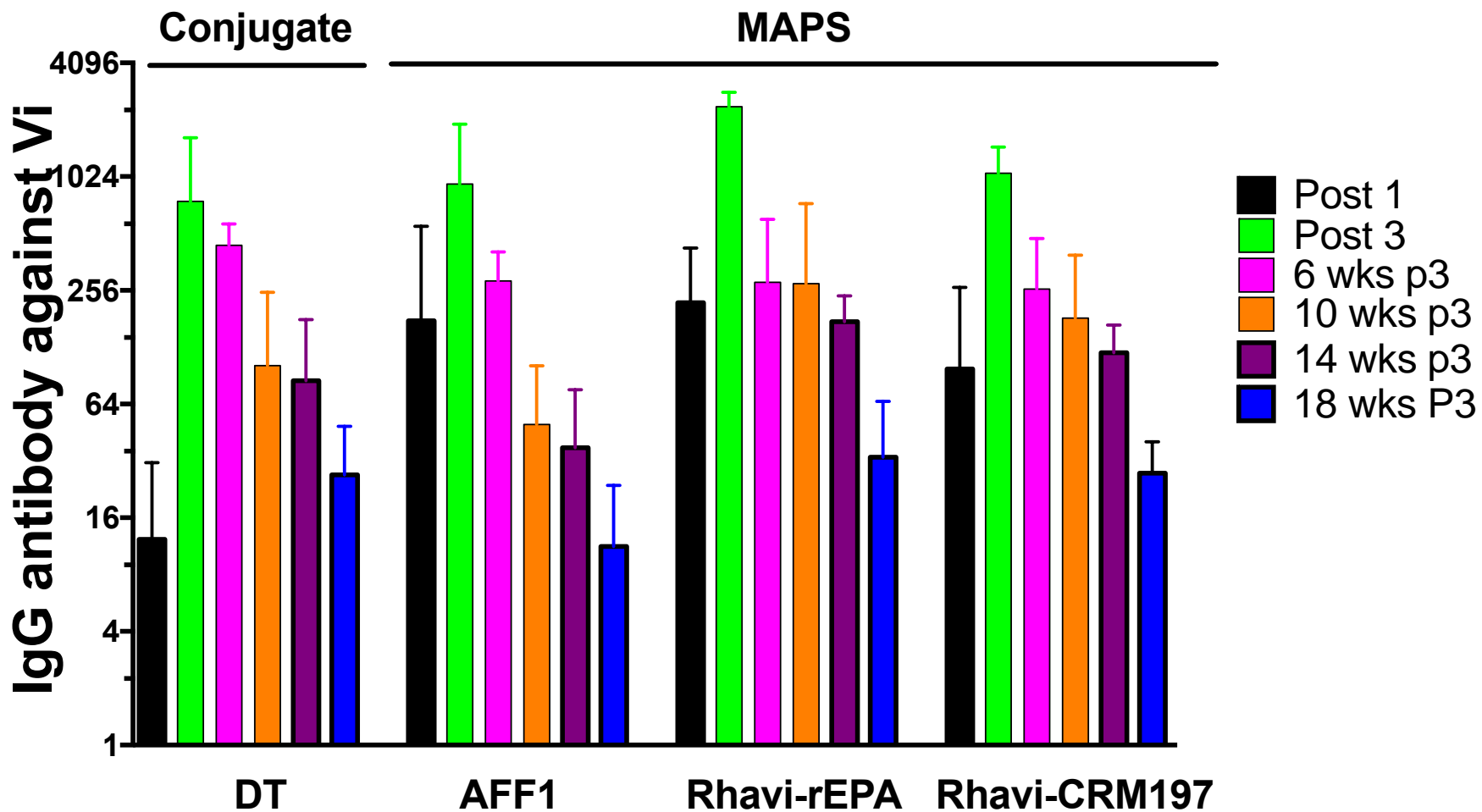


Vi-DT conjugate kindly provided by IVI





# Vi antibody titer duration in Guinea pigs





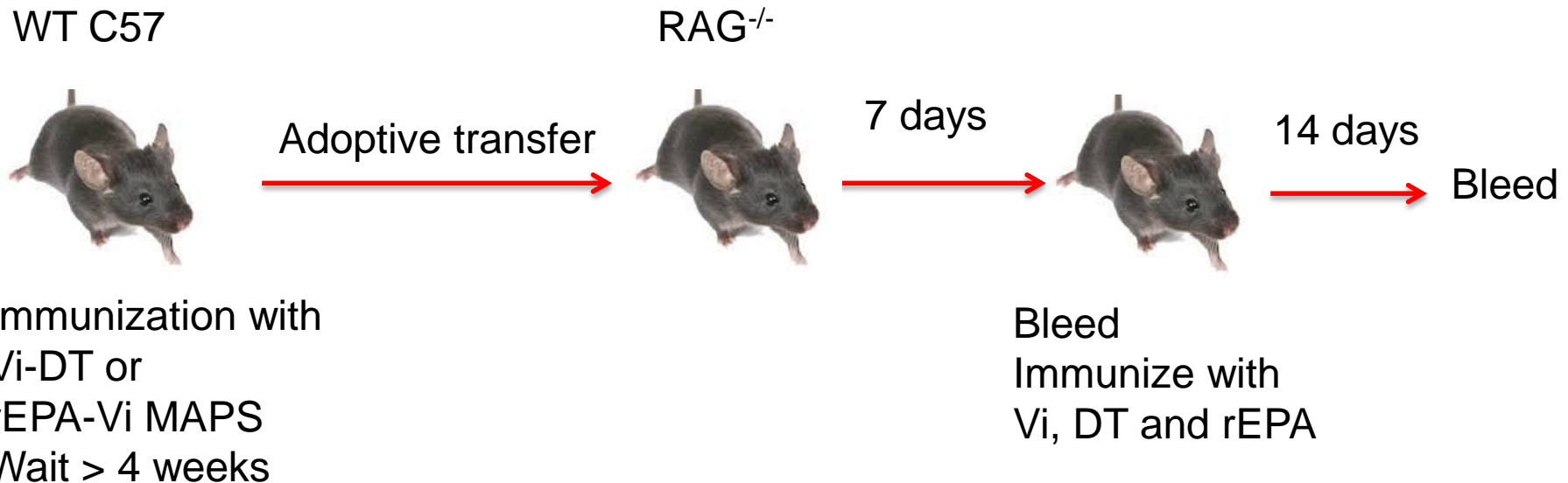
# Comparison between carriers

Carriers	Advantages	Potential issues	Carrier function
Rhavi-rEPA	Highly immunogenic; rEPA has been tested in multiple human clinical trials, good safety track record	Toxin mutant; not used in licensed vaccines; purification scheme needs to be established	Mice: +++ Guinea pigs: +++ Rabbit: ++
AFF1	Excellent carrier; purification established and yield is high (>1g/L); no toxin concern	Not yet tested in humans (part of pneumo project at Affinivax)	Mice: ++ Guinea pigs: ++ Rabbit: +++
Rhavi-CRM <sub>197</sub>	CRM <sub>197</sub> Used in licensed conjugate vaccines, fewer safety concerns	Yield at lab scale is low. May increase COG	Mice: +++ Guinea pigs: ++ Rabbit: Not tested



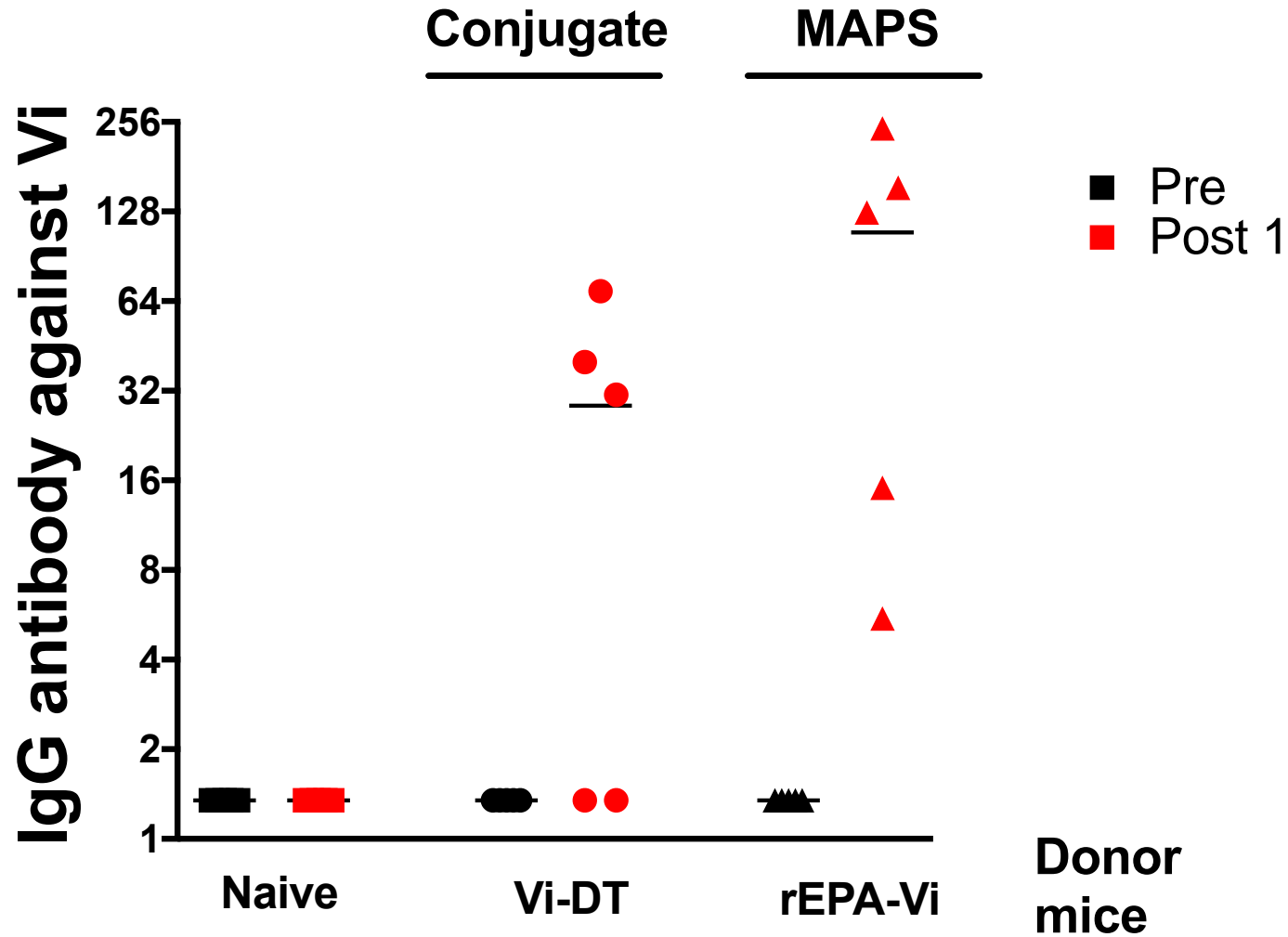
# Testing for immunological memory: Adoptive transfer experiments

Concept: Infuse B cells from immunized wild type mice into RAG immunodeficient mice and evaluate response to boost in RAG mice





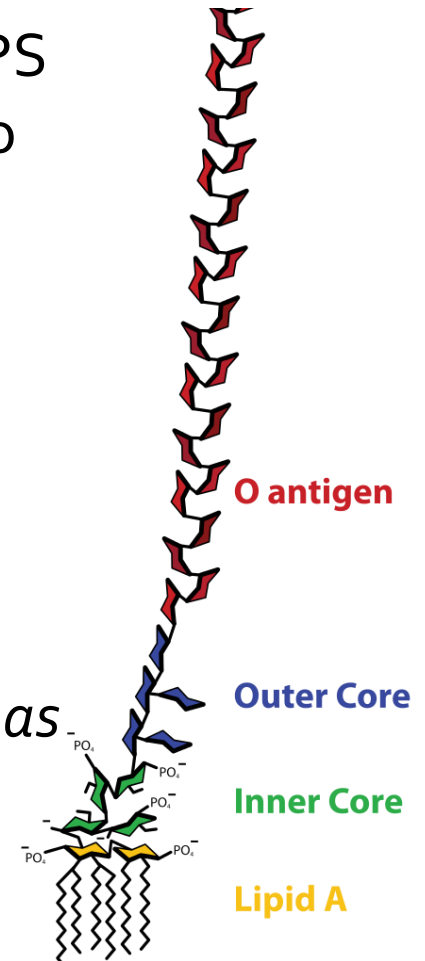
# Both conventional conjugate and MAPS generate Vi-memory B cells





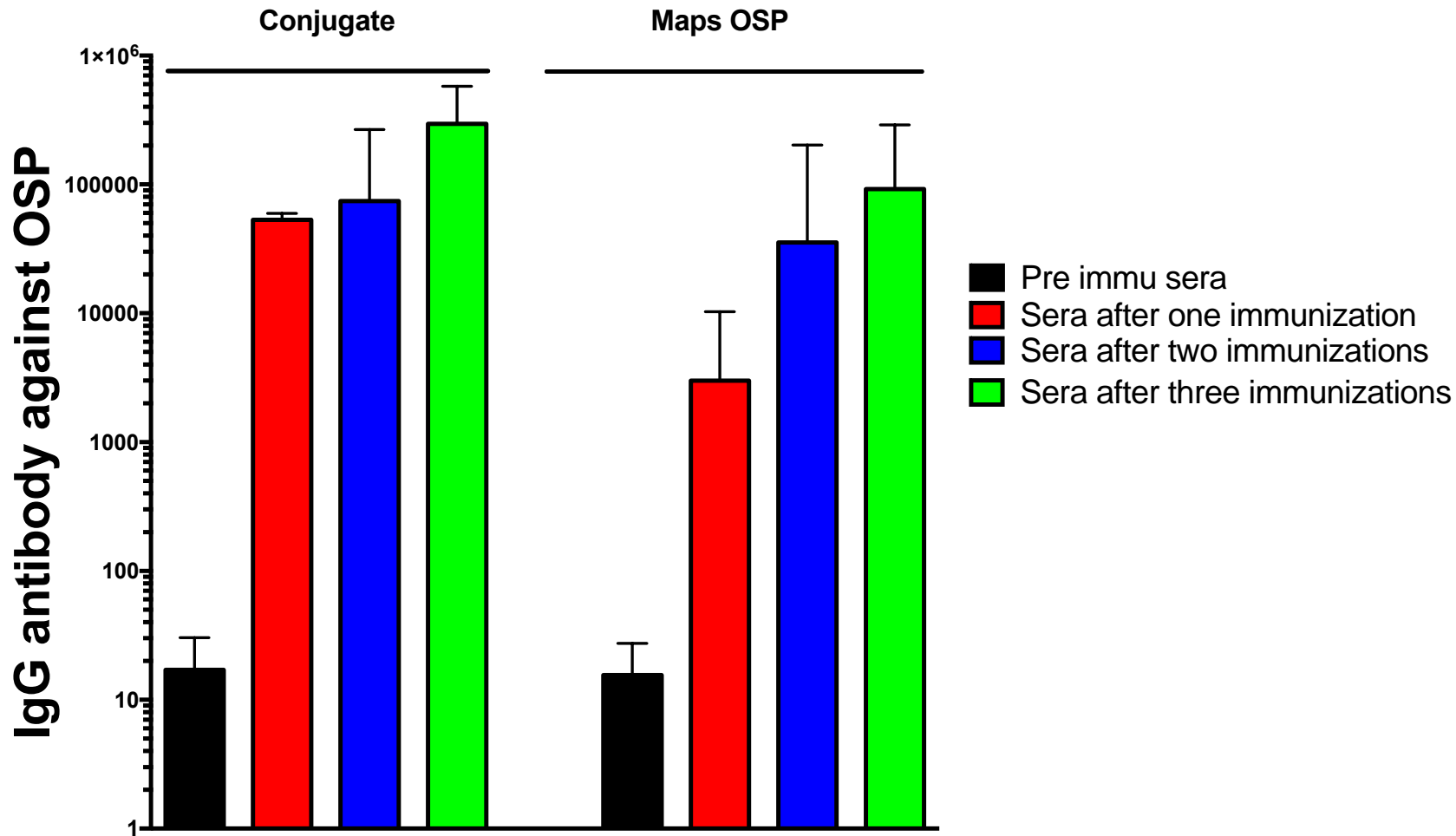
# Design of OSP MAPS for Paratyphi: Challenges

- Rationale: OSP purified from *paratyphi* is small (around 40-50 Kd) which is not optimal for MAPS construct (no crosslinking occurs, as opposed to traditional conjugates)
- Three strategies for making larger OSP
  - Clone the whole *S. paratyphi* operon (24kb) and integrate into *E. coli*
  - Delete short chain OSP enzyme WZZ and overexpress long chain OSP enzyme Fepe from *S. paratyphi* or WZZ2 from *Pseudomonas aeruginosa*
  - Chemical crosslinking of OSP





# Anti-OSP antibody titer after one to three immunizations in rabbits





# Evaluation of combination of Vi- and OSP-MAPS in rabbits

Vaccines:

Conjugate: Vi-DT (from IVI) and Rhavi-rEPA-OSP (BCH).

MAPS: AFF<sub>1</sub>-Vi, AFF<sub>1</sub>-OSP dimer

Immunization schedule:

Day 0, pre bleed and immunization;

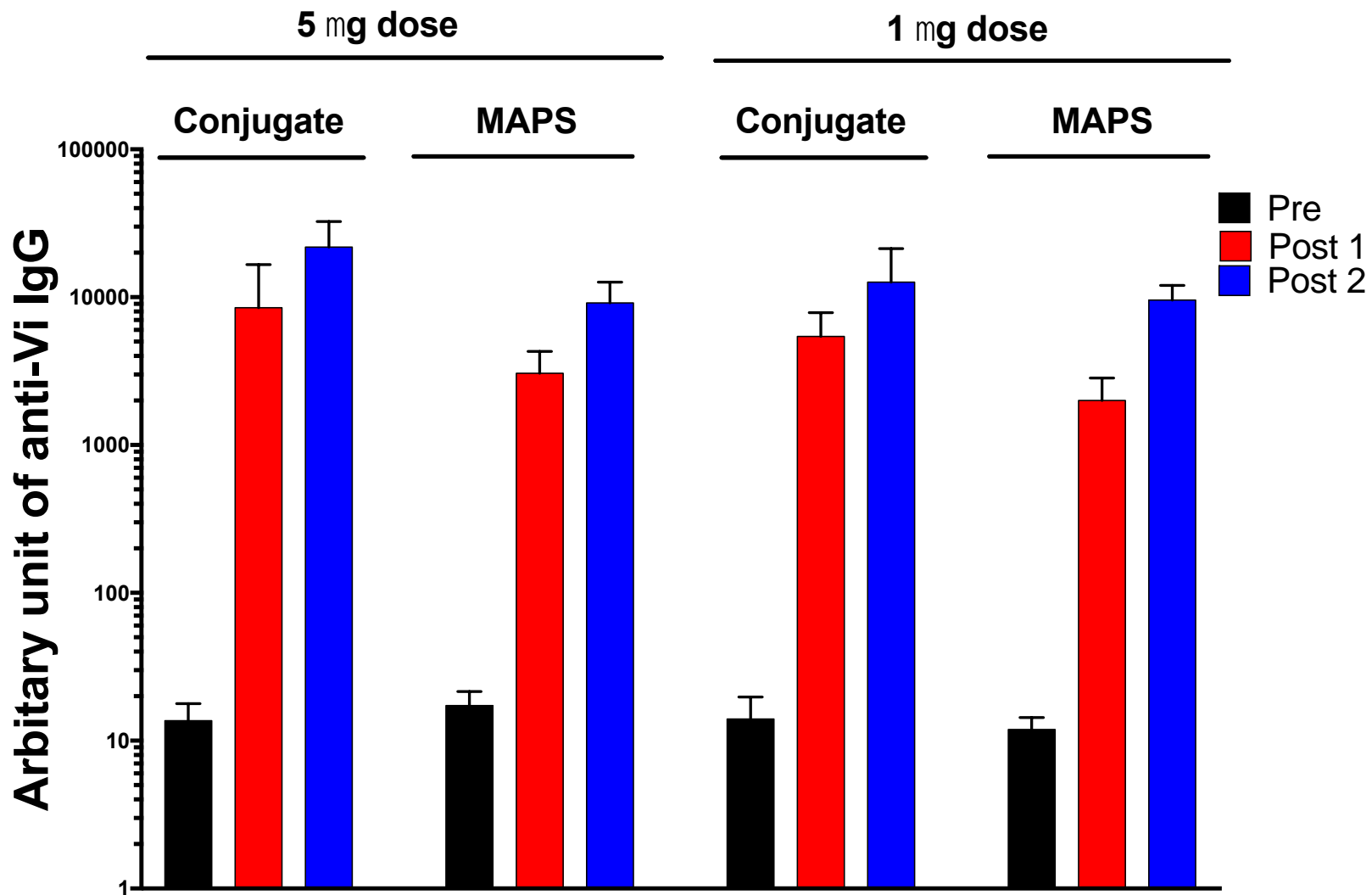
Day 14, p1 bleed and immunization;

Day 28, p2 bleed.





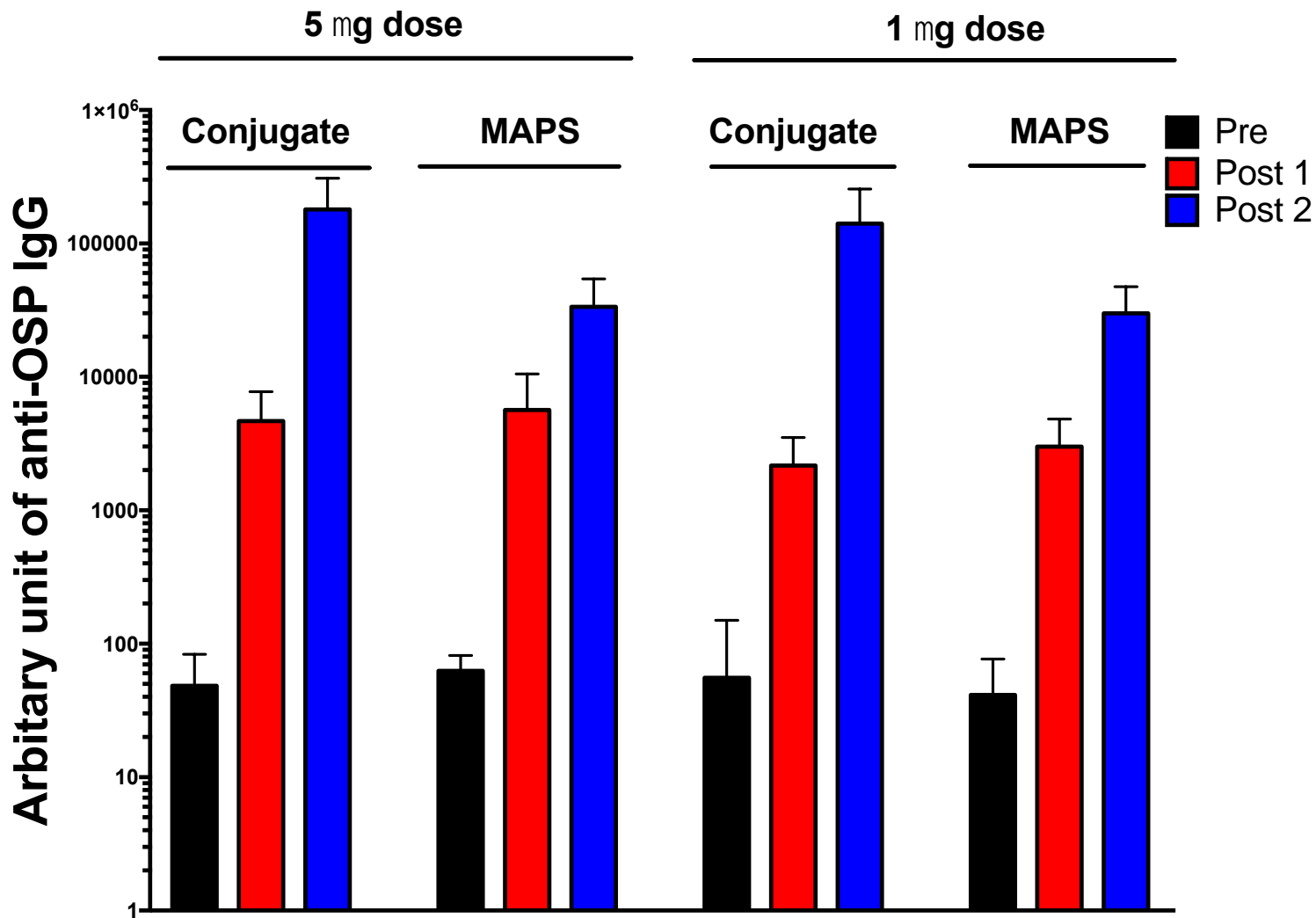
# Immunogenicity of Vi in combination vaccines







# Immunogenicity of OSP in combination vaccines





# Summary

- Vi-MAPS generated Vi-specific memory response
- Both Vi and OSP MAPS are highly immunogenic in animal models.
  - Mice, IgG titer rise: 100-400 fold rise after two immunizations
  - Guinea pigs: 50 fold rise after one and 100 fold rise after two
  - Rabbits: 100-200 fold rise after one and 400-800 fold rise after two
- Functional antibody generated (correlates with titer)
- Scale-up processes in progress



# Acknowledgements

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# Questions/Comments?



"Hmmm, slightly sludgy, with a delicate touch of typhoid."



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