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Dustin Patterson
University of Texas at Tyler, dpatterson@uttyler.edu

Benjamin Schwarz

Brian Western

Matthew Terra

Paul Krugler

See next page for additional authors

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Authors
Dustin Patterson, Benjamin Schwarz, Brian Western, Matthew Terra, Paul Krugler, Matthew Hicks, Agnieszka Rynda-Apple, Allen G. Harmsen, and Trevor Douglas

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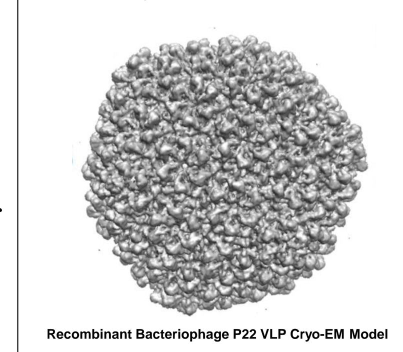
Engineering Virus Like Particles Towards Directing Immunologic Responses

Dustin P. Patterson[1], **Benjamin Schwarz**[2], **Brian Western**[1], **Matthew Terra**[1], **Paul Krugler**[1], **Matthew Hicks**[1], **Agnieszka Rynda-Apple**[3], **Allen G. Harmsen**[3], **& Trevor Douglas**[2].

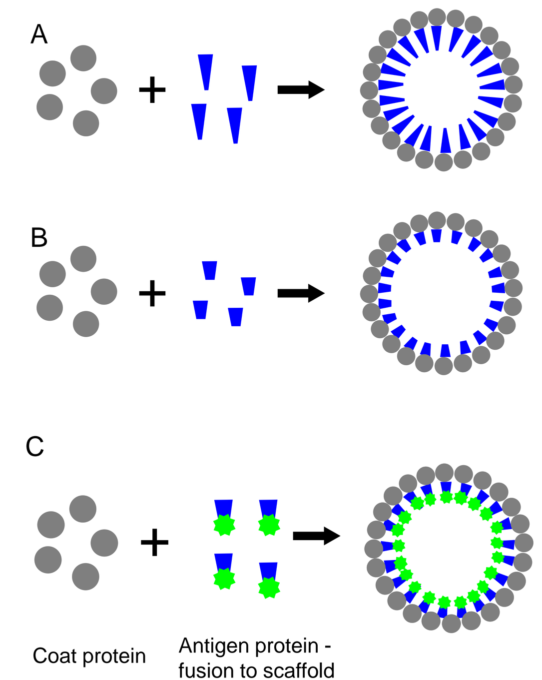
[1]Department of Chemistry & Biochemistry, The University of Texas at Tyler, Tyler, TX 75799, USA
[2]Department of Chemistry, Indiana University, Bloomington, IN 47405, USA
[3]Department of Immunology and Infectious Diseases, Montana State University, Bozeman, MT 59717, USA

Introduction

Virus like particles (VLPs) present a rich biomaterial architecture and platform for displaying antigens in a spatially controlled manner, either on the exterior and/or on the interior, which could be exploited to direct specific immune response pathways. The VLP derived from *Salmonella typhimurium* bacteriophage P22 has been shown to be a versatile platform for the incorporation of proteins on the VLP interior. Heterologous co-expression of a protein or peptide cargo of choice fused to the P22 scaffolding protein (SP) with P22 coat protein leads to assembly of the P22 VLP with the Cargo Protein-SP fusion protein incorporated on the interior. Utilizing this approach P22 VLPs containing the conserved nucleoprotein (NP) from influenza on the interior, in a biomimetic fashion, were shown to elicit a broadly protective CD8+ T cell response to influenza that provided multi-strain protection against 100 times lethal doses of influenza. These results provide strong evidence for utilizing our biomimetic approach, which is amendable to the quick production of vaccines to rapidly emerging pathogens. Investigations into a modular approach for attaching antigenic proteins to exterior of the P22 VLP, allowing display of antigens on the exterior or P22, is described. For proof of concept, green fluorescent protein (GFP) was utilized as a model protein for examining attachment of proteins to the exterior of the P22 VLP via sortase-mediated ligation. Results show that GFP can be effectively attached to the surface of P22, paving the way for attachment of other proteins, including antigenic proteins from pathogens. The ability to display antigens on the outside has the potential to activate alternate immunologic pathways, such as production of neutralizing antibodies that prevent pathogen infections. Tailoring the display of antigens has the potential to allow control over directing the specific immune pathway activated and responses generated.


Recombinant Bacteriophage P22 VLP Cryo-EM Model

P22 VLP as Platform for Internal Display of Antigens



-Antigen incorporated by *in vivo* co-expression of antigen-scaffolding protein fusion protein (SP, truncated) with P22 Coat Protein (CP).

-*In vivo* expression produces high yields (up to 200 mg/Liter) of homogenous P22 VLPs.

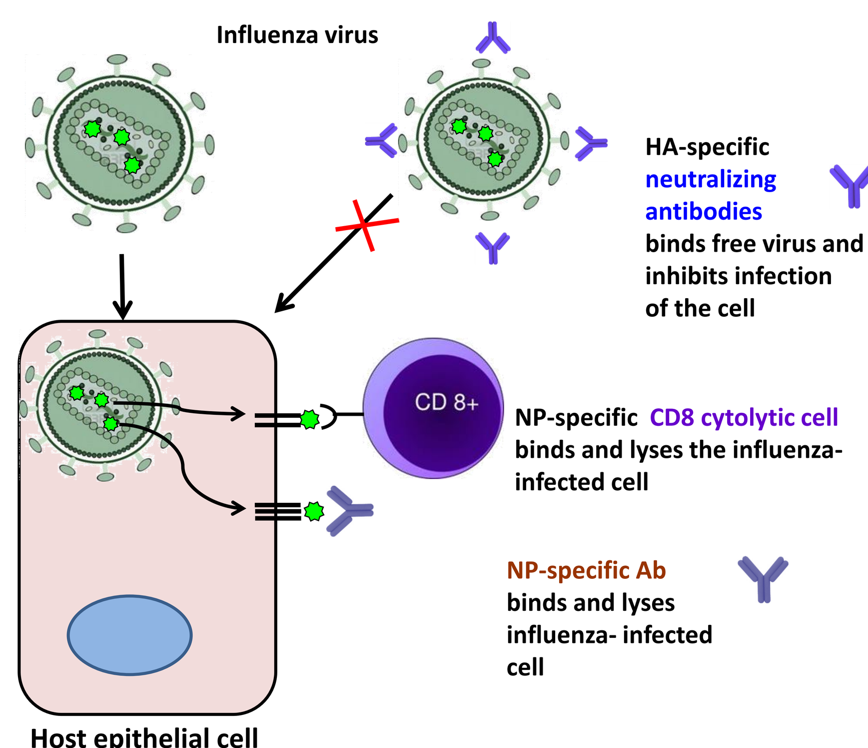
-Rapid generation of new immunogenic nanoparticles using recombinant DNA technology to fight newly emerging pathogens.

Targeting Influenza Immune Responses

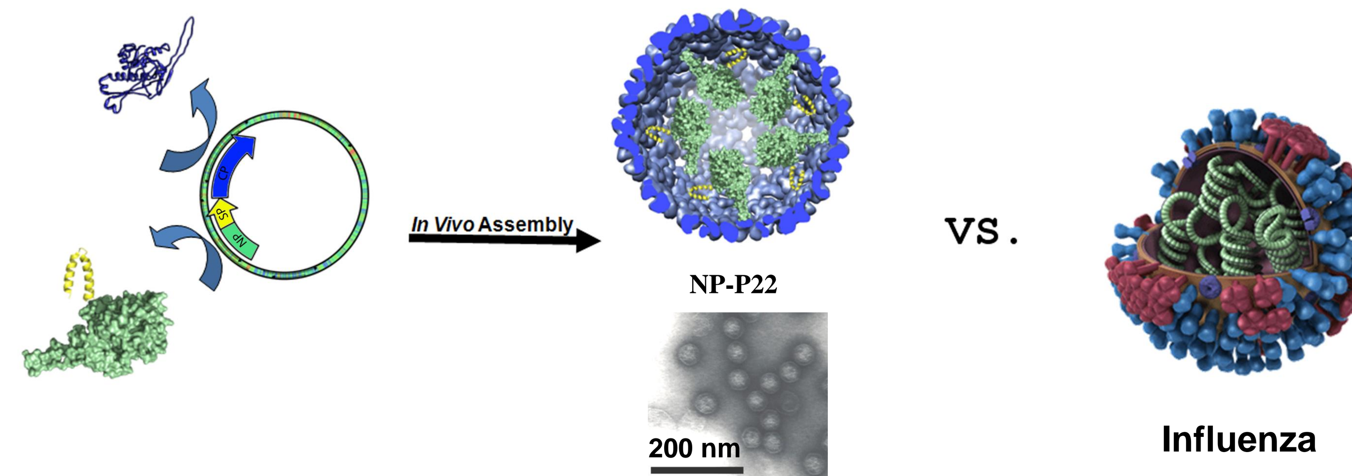
-Current vaccine strategies against influenza largely target non-conserved Hemagglutinin (HA) to generate neutralizing antibody response.

-Nucleoprotein (NP) is conserved across serotypes of influenza and generates CD8+ T cell response that aids in clearance of infected cells.

-Conserved components of external proteins could be utilized to construct broadly protective vaccines.



Biomimetic Display of Influenza Nucleoprotein

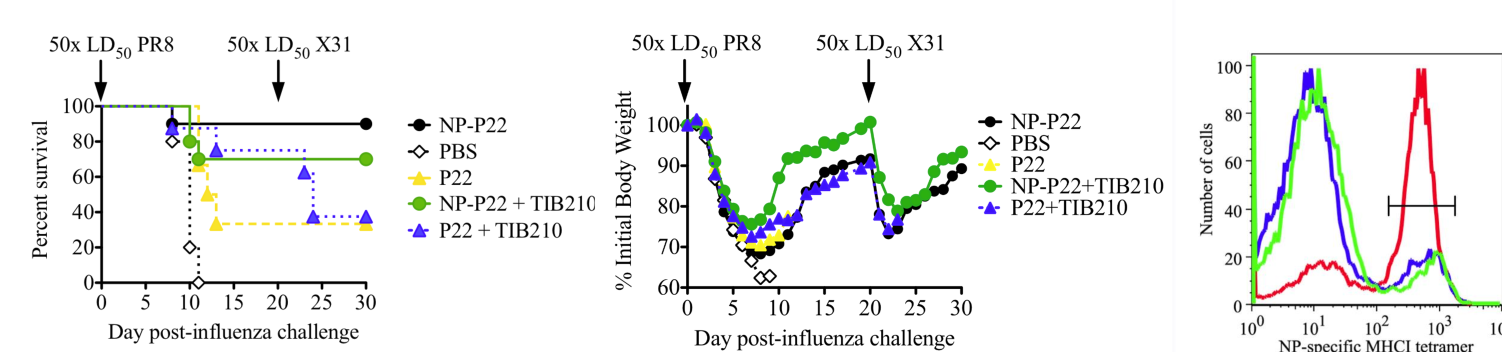


-Biomimetic display of nucleoprotein (NP, green) within P22 is expected to generate a broadly protective CD8+ T cell response.

-Constructs containing full length NP were constructed by *in vivo* expression in *E. coli* (TEM image).

-SDS-PAGE, TEM, and size exclusion chromatography verified NP incorporation and homogeneous particle formation with high internal packaging of 145 copies of NP per capsid.

Protection Generated Against Influenza by NP-P22 is NP-Specific

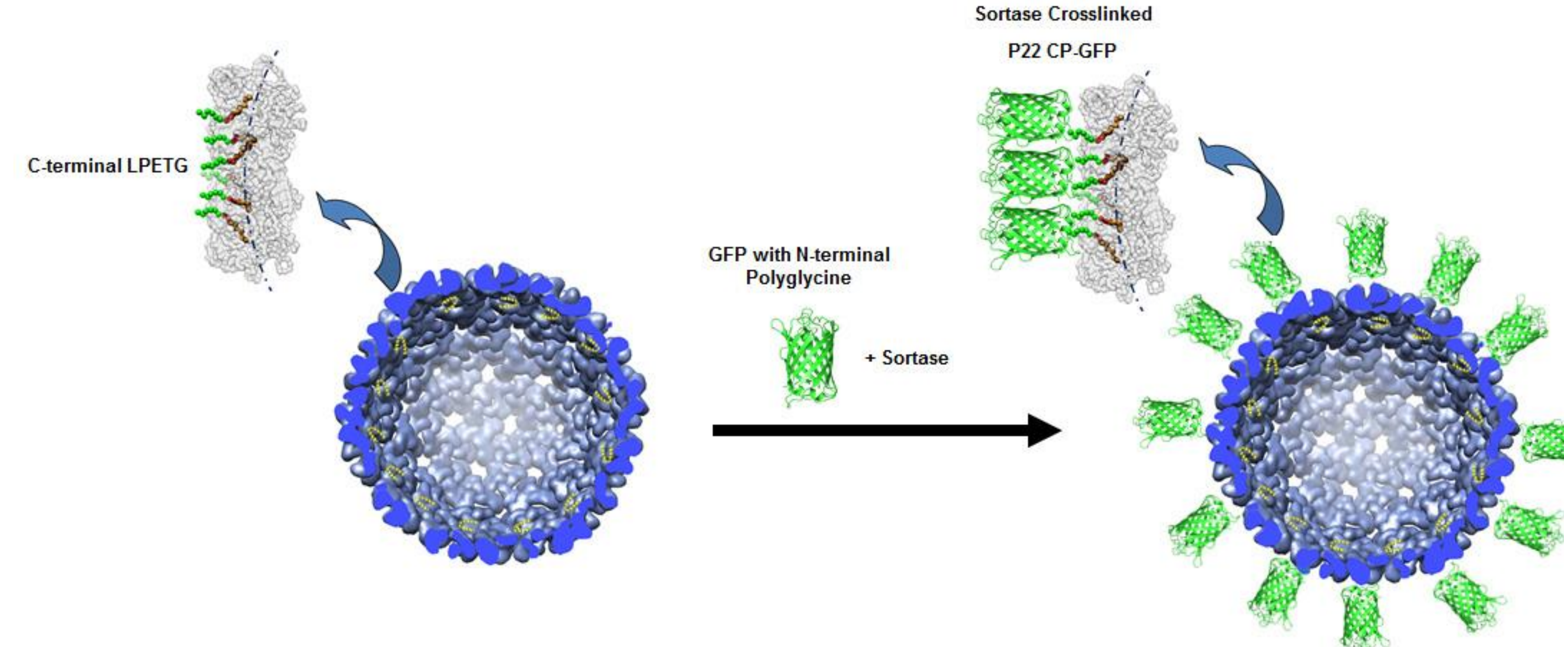


-NP-P22 generates multi-serotype protection in mice treated with high doses (50 times lethal doses) of H1N1 (PR8) and H3N2 (X31).

-Weight data shows recovery by influenza infected mice immunized with NP-P22.

-TIB210 CD8+ T cell depletion combined with MHCI tetramer staining of CD8+ T cells indicate response is CD8+ dependent and NP specific.

Developing Approach for External Display of Antigens on P22



-C-terminus of the P22 CP (green spheres) was experimentally shown to be exposed on the exterior of the P22 capsid (Servid, A. et al. *Biomacromolecules*, 2013, 14 (9), pp 2989–2995).

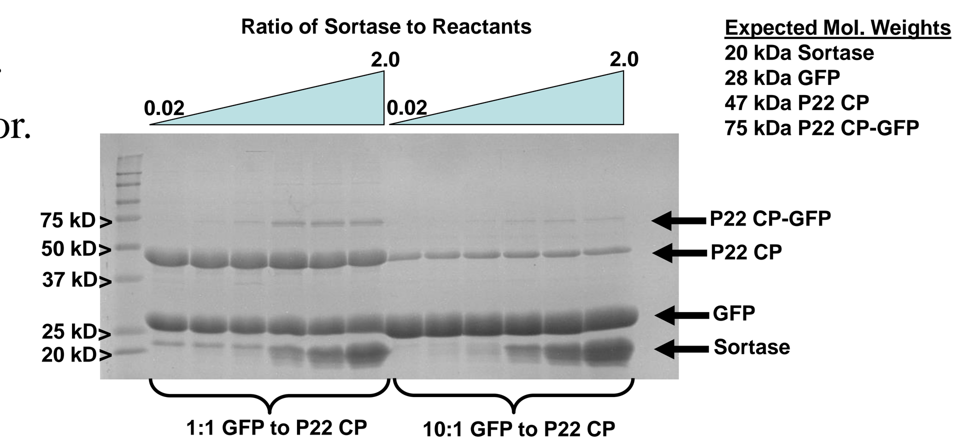
-P22 CP genetically modified to include C-terminal LPETG sequence provides covalent attachment site, for proteins containing N-terminal polyglycine (GFP shown above), to the exterior of P22 via sortase-mediated ligation.

Screening Sortase-Mediated Ligation of GFP to P22

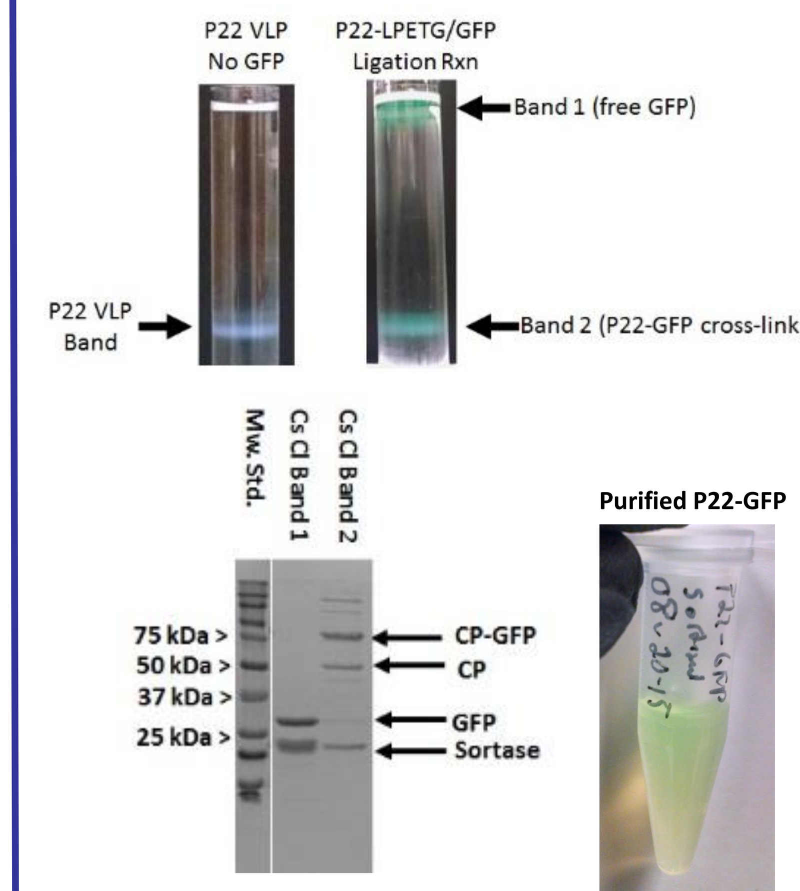
-Polygylcine-GFP was used as a model protein/antigen to screen conditions for sortase-mediated ligation to P22 exterior.

-Ratios of reactants GFP and P22 (total conc. 60 μM) and sortase examined to find best conditions.

-Effective crosslinking observed, especially at higher concentrations of sortase.



Scaling Up Sortase-Mediated Ligation of GFP to P22



-Based on reaction screens, reaction size was scaled up (~4 mL) and performed for optimal time (4 hours) at 1:10 P22 to GFP ratios at higher protein concentrations (120 μM).

-Ultracentrifugation of P22/GFP reactions through a cesium chloride (CsCl) gradient (0.2 mg/mL-0.4 mg/mL) resulted in green colored bands where white P22 VLP bands are normally observed.

-Fractionation and analysis of CsCl bands confirmed that GFP was covalently cross-linked to P22 via sortase.

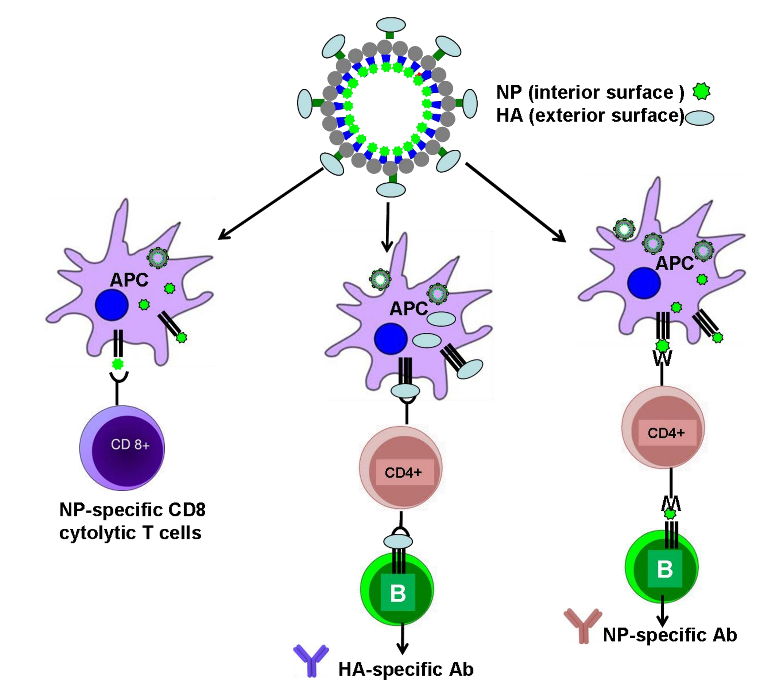
-Results validate sortase-mediated approach for attachment of proteins to the exterior of the P22 VLP platform.

Future Directions

- Further evaluate P22-GFP VLP materials constructed.

-Use sortase method to incorporate influenza antigen on exterior to provide broadly protective neutralizing antibody response.

-Explore how antigen display on interior vs. exterior modulates the response generated to antigens.



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-Immunology results published in: Patterson, D. P., et al. Biomimetic Antigenic Nanoparticles Elicit Controlled Protective Immune Response to Influenza. *ACS Nano*, **2013**, 7 (4), 3036-3044.