

MUC1 glycopeptide based anti-cancer vaccines

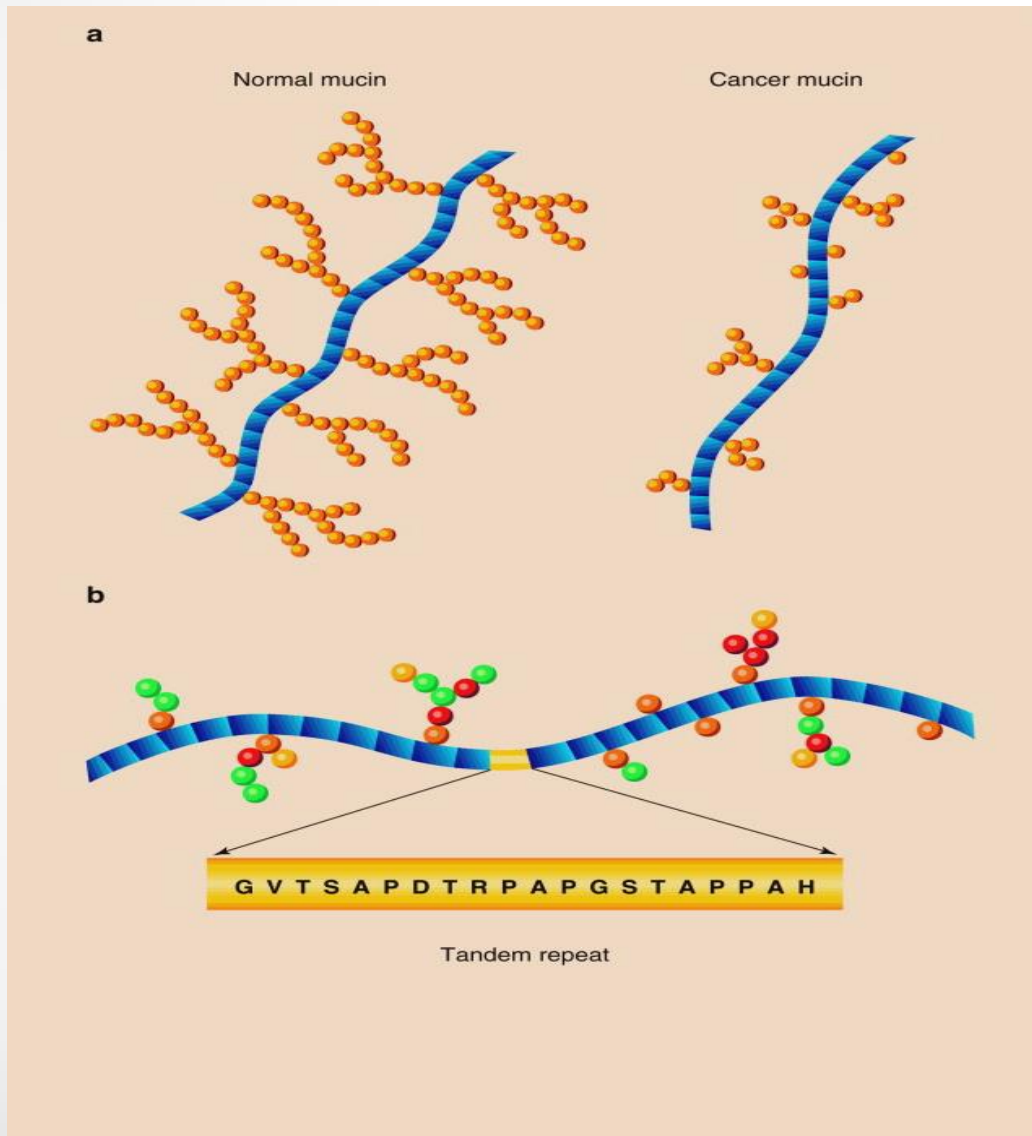
Sourav Sarkar

Glycobiology World Congress

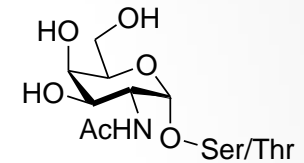
Philadelphia, USA

August 10, 2015

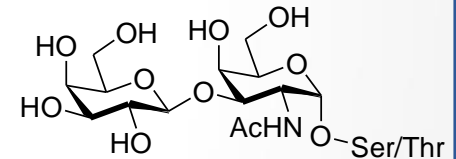
MUC1 on Tumor Cells



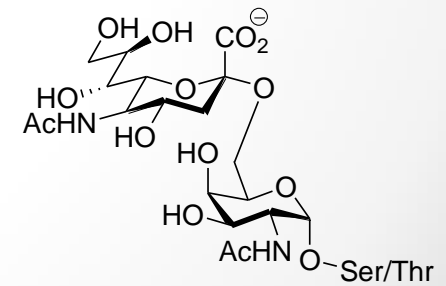
Tumor Associated Carbohydrate Antigens (TACAs)



Tn-Antigen



TF(T)-Antigen

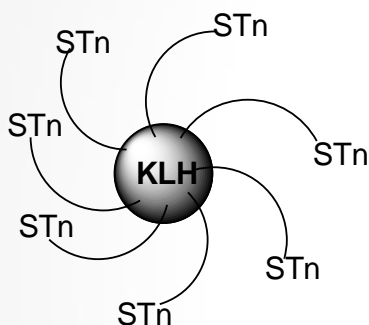


STn-Antigen

The Helper T-epitope

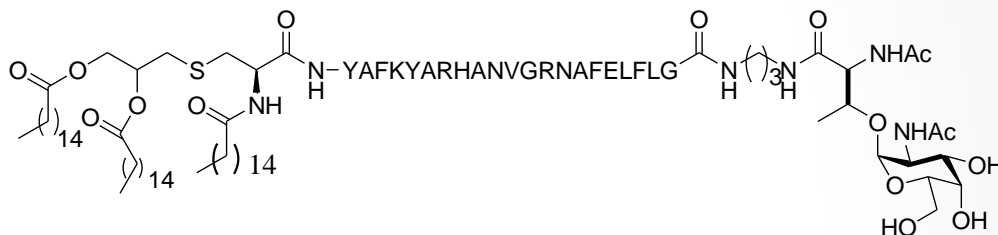
T-cell help is required to obtain high antibody titers against TACAs.

Early Vaccines



Immunoresponse specific to the protein which suppresses the response for the cancer antigen

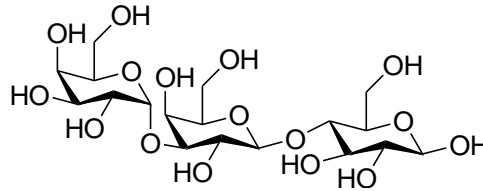
Modern Vaccines



20 Aminoacid sequence derived from the outer membrane protein of *Neisseria meningitides* activate helper T-cells.

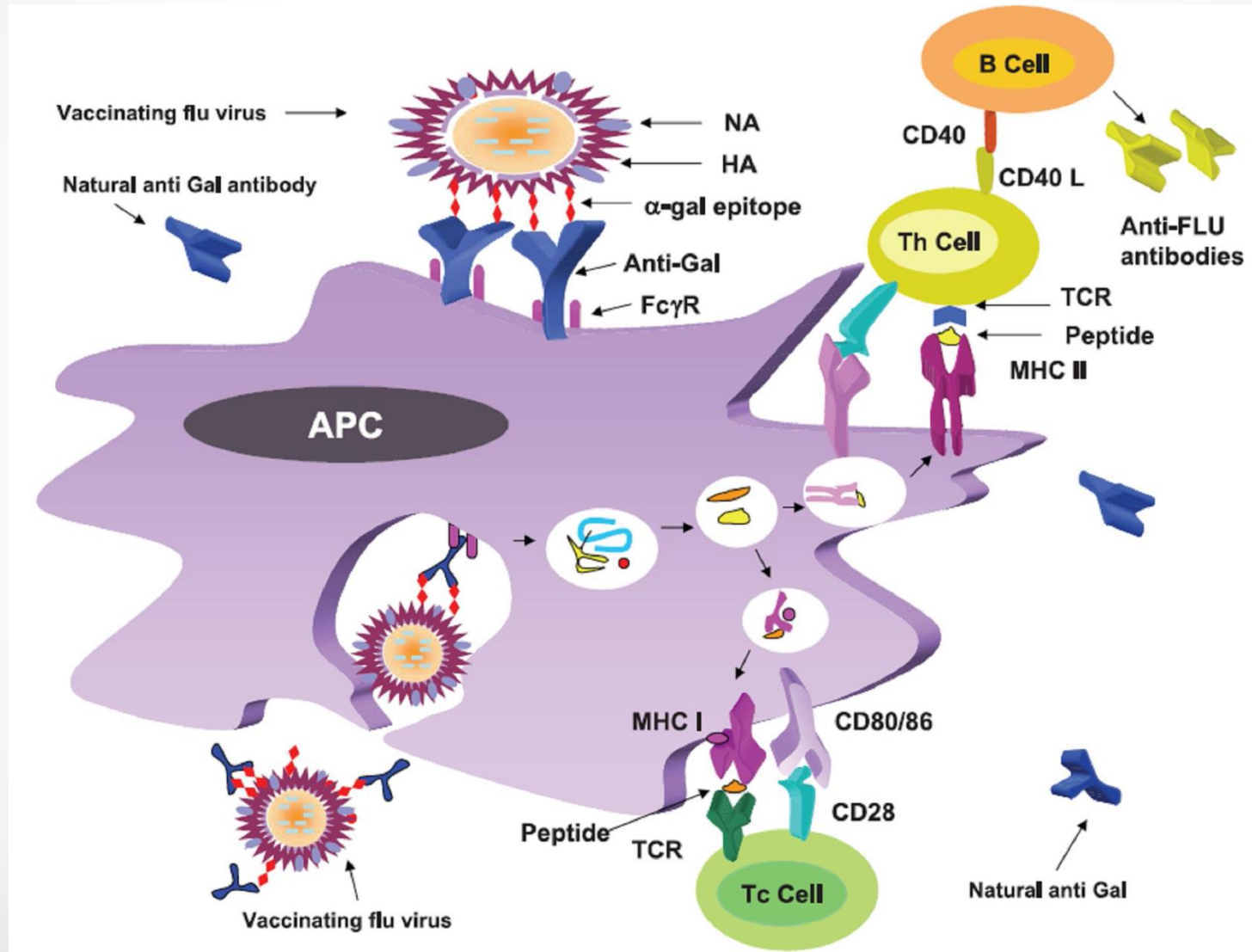
Peptide required to induce T cell dependant immunoresponse with the production of IgG against Tn.

α -Gal Conjugate Vaccines

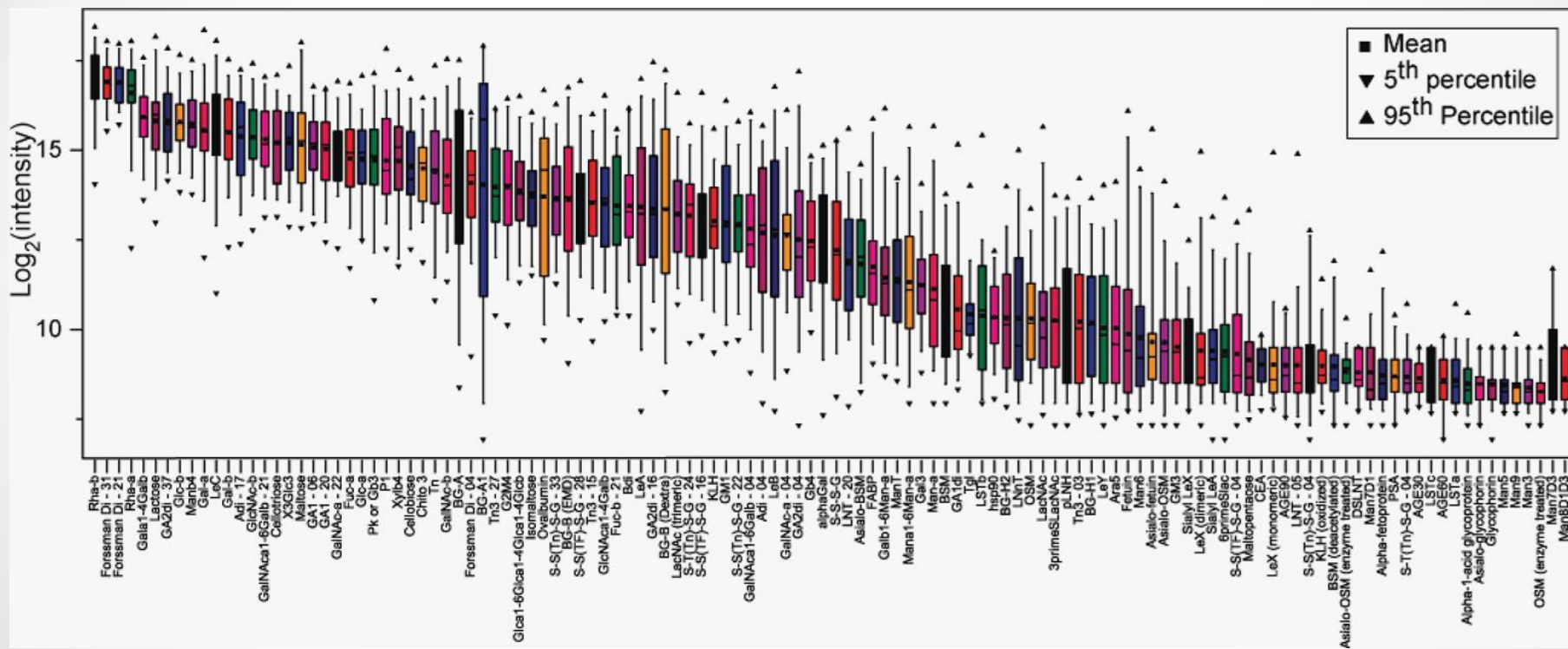


- Immunogenicity against HIV gp120 was increased >100 fold by conjugation with α -gal
 - Galili *et al.* *J Virol* 2006, 6943.
- Vaccination with MUC1 expressing α -gal epitopes elicits effective antibody production and induces tumor-specific T-cell responses.
 - Deguchi, *et al.* *Cancer Res.* 2010, 70, 5259.
- But this only works in a subset of humans and still needs improvement

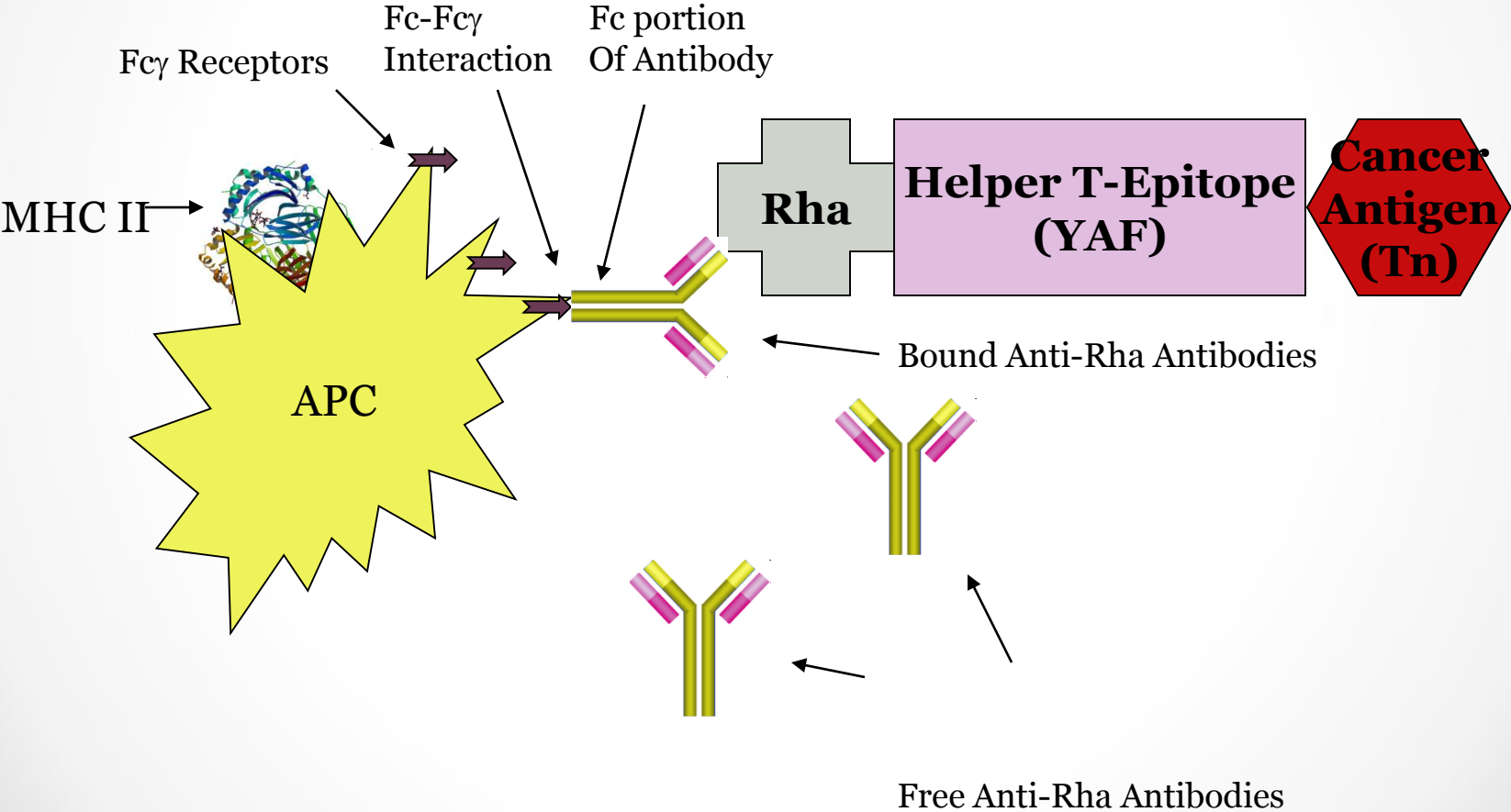
Anti- α -Gal Mediated Targeting of Vaccines to APCs



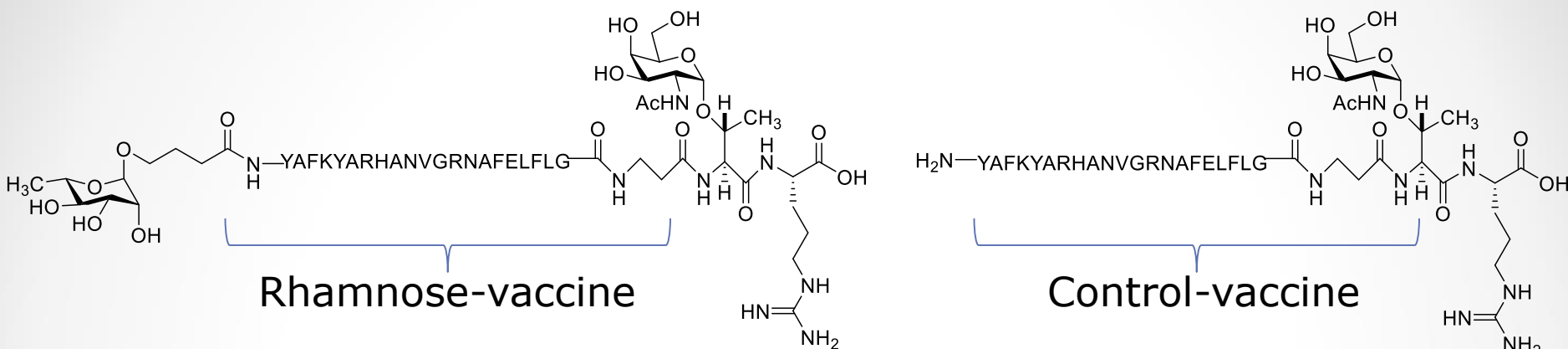
L-Rhamnose: A Promising Xenoantigen



The Three component Rha-Vaccine



The Three component Rha-Vaccine



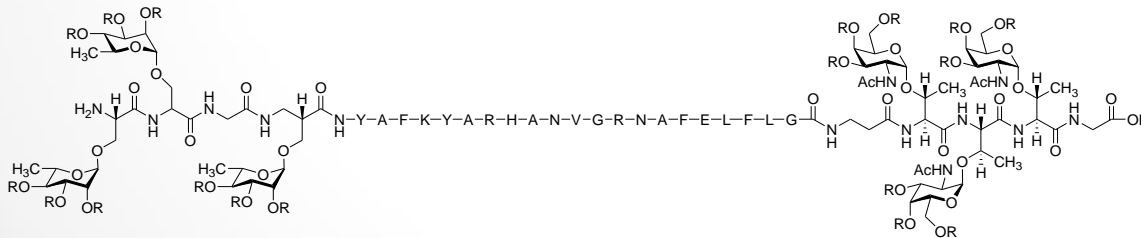
Outcomes:

- Anti-Tn antibody titers were significantly higher in groups of mice previously immunized with Rha-OVA and later challenged with Rha-YAF-Tn.
- Antibodies specific to Tn and evidence of IgG production was provided by the competitive binding experiments.
- T-cell proliferation assay showed that the peptide YAF was better displayed by the MHC in the presence of anti-Rha antibodies.

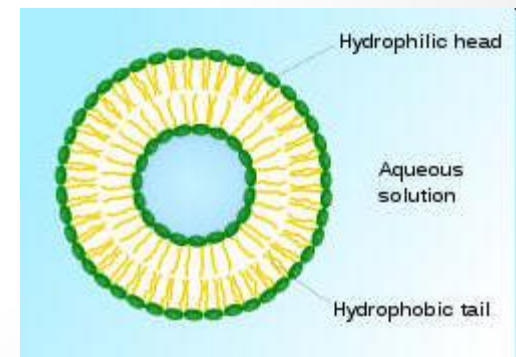
Incorporating Multivalency into Vaccine Design

- Multiple epitope vaccine facilitates better vaccine internalization.
- High antibody titers have been reported against antigen clustered vaccines and multiple antigenic peptides (MAP).

Multivalent Vaccine Strategies



Epitope Clustered Vaccine



Liposome

MUC1 VNTR as Helper T Epitope

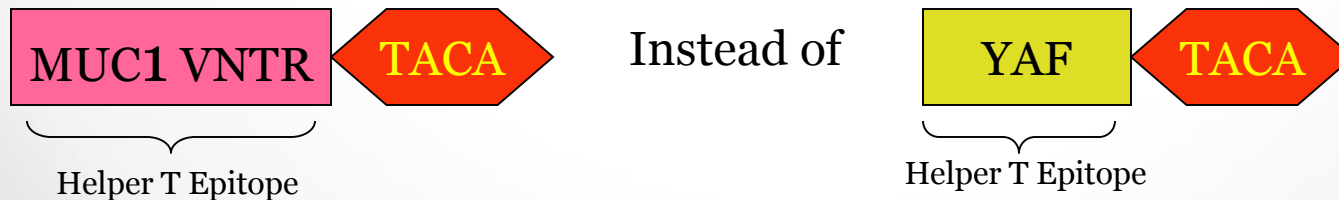
MUC1 : Large polymorphic transmembrane glycoprotein containing numerous 20-amino acid long variable number tandem repeats (VNTR)

MUC1 VNTR = PDTRPAPGSTAPPAHGV TSA

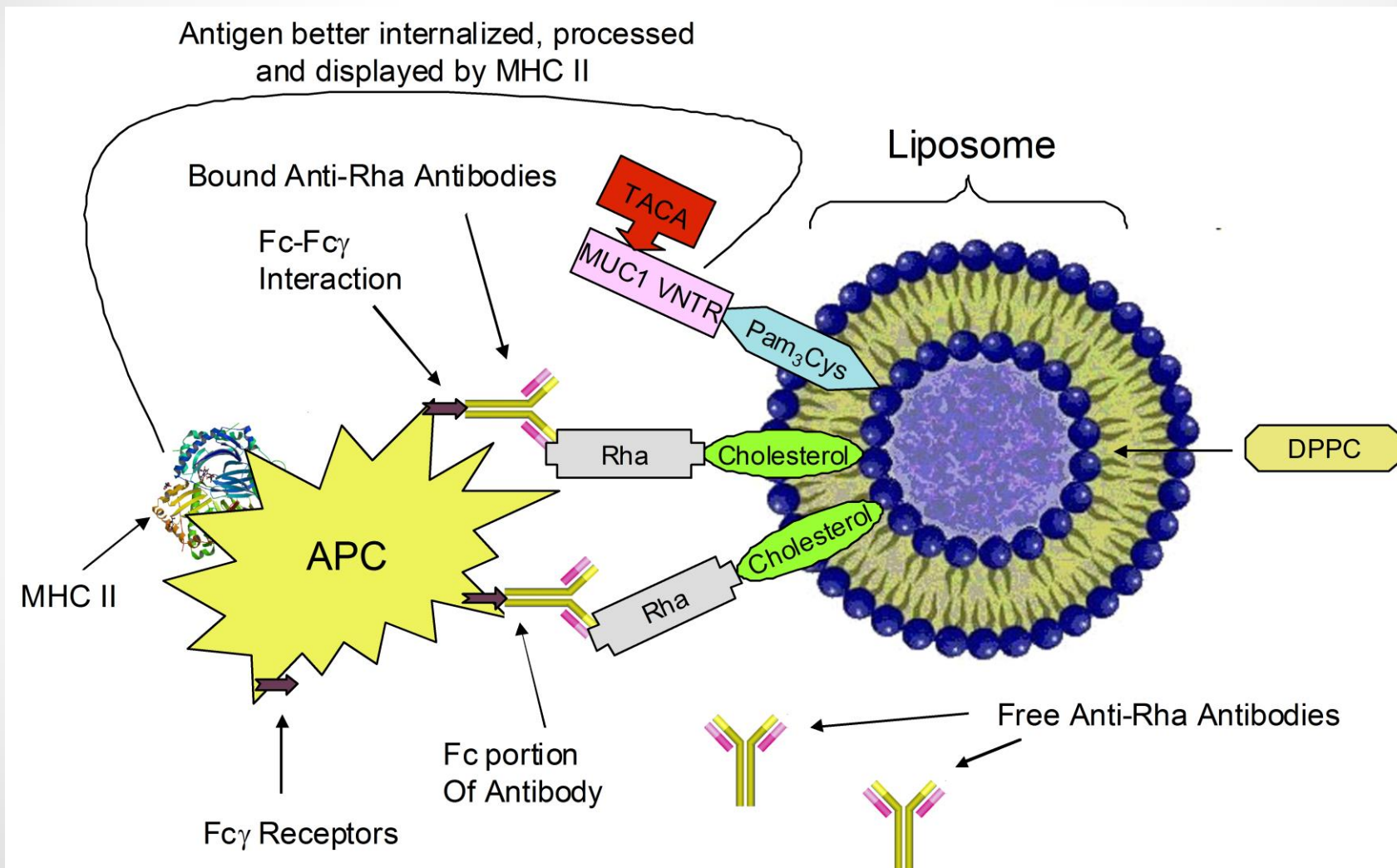
Recent discovery :



Our Hypothesis :



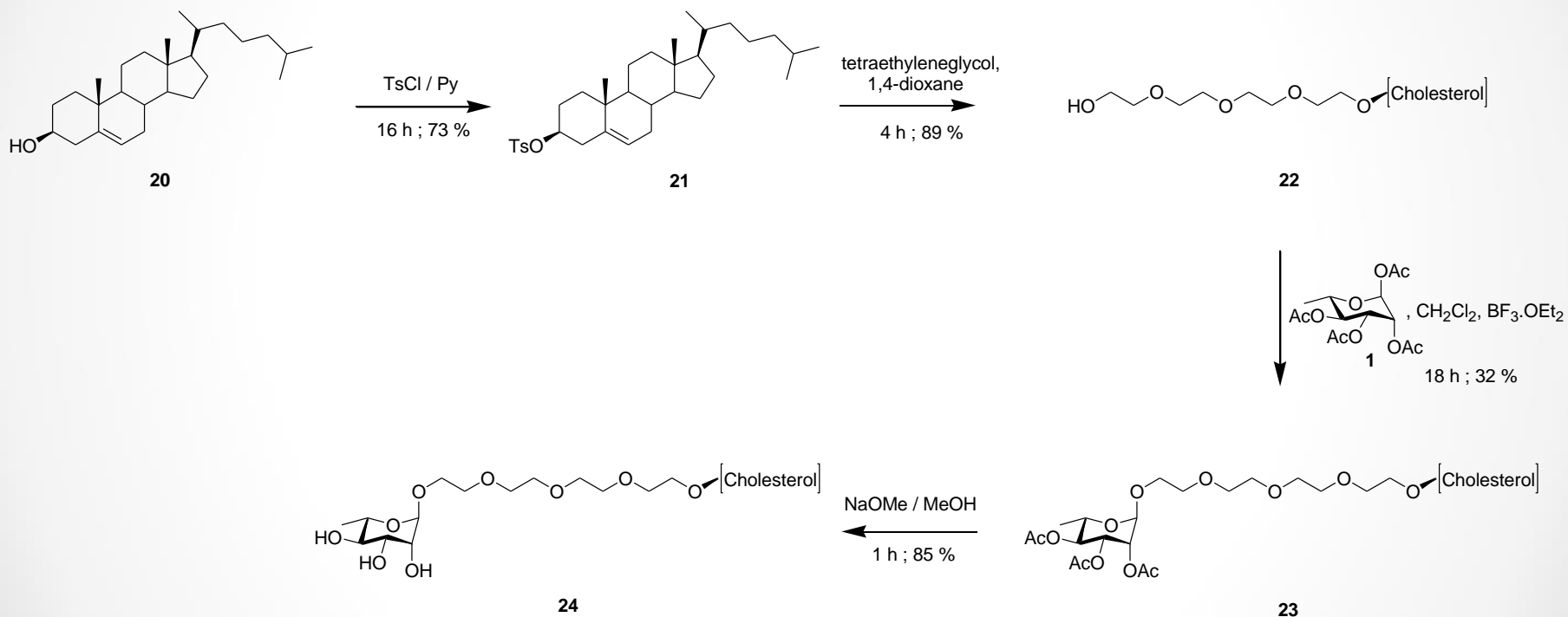
Liposome Design



(1) Sarkar et. al. *Bioconjugate Chem.* **2013**, 24, 363-375.

(2) *PCT Int. Appl.* (2013), WO 2013123282 A1 20130822, **Inventors:** Sucheck, S. J.; Wall, K. A.; Sarkar, S.

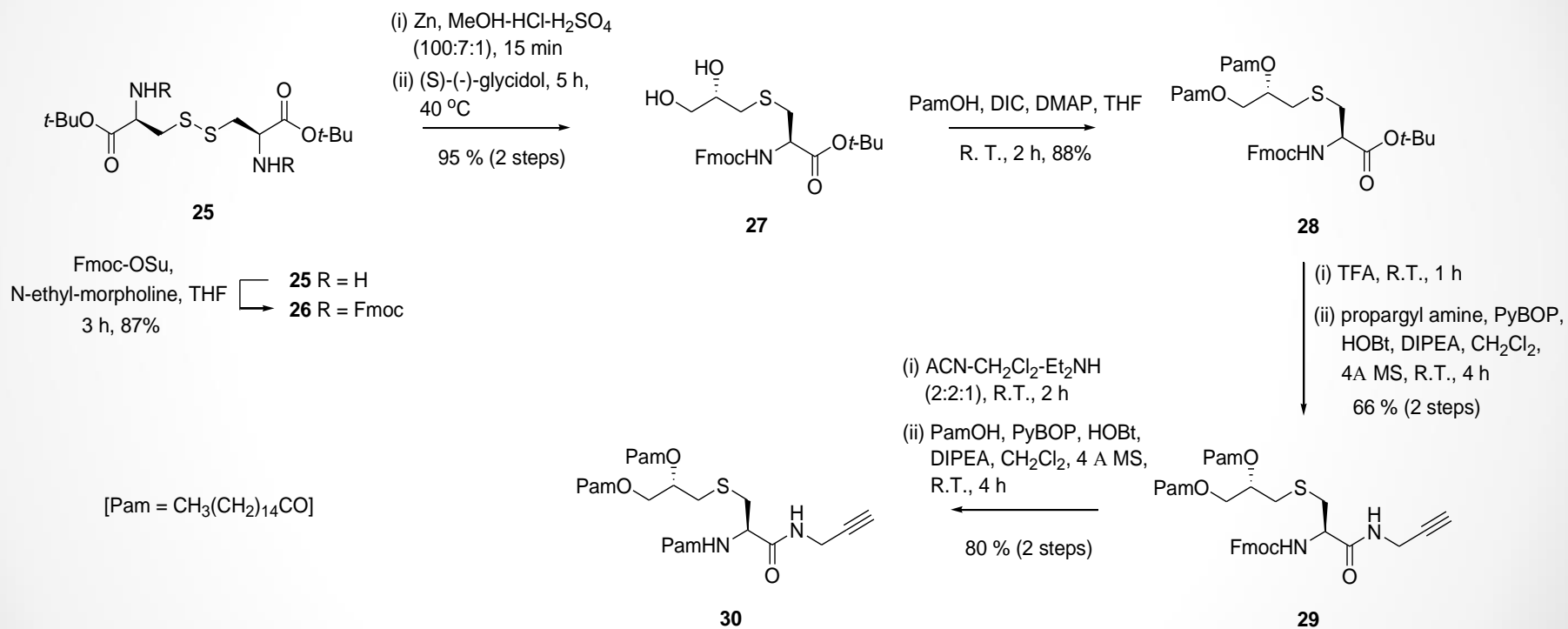
Synthesis of L-Rha Cholesterol Conjugate



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(2) *PCT Int. Appl.* (**2013**), WO 2013123282 A1 20130822, **Inventors:** Sucheck, S. J.; Wall, K. A.; Sarkar, S.

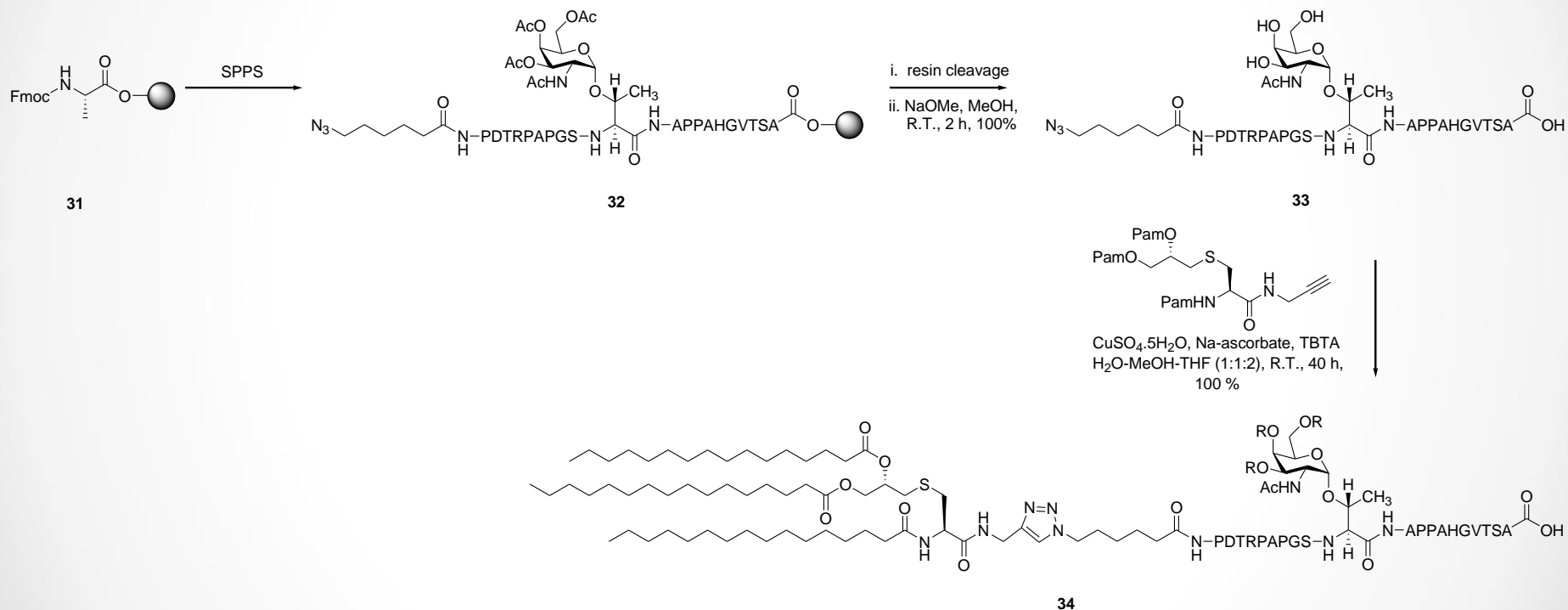
Synthesis of alkyne functionalized Pam₃Cys



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(2) *PCT Int. Appl.* (**2013**), WO 2013123282 A1 20130822, **Inventors**: Sucheck, S. J.; Wall, K. A.; Sarkar, S.

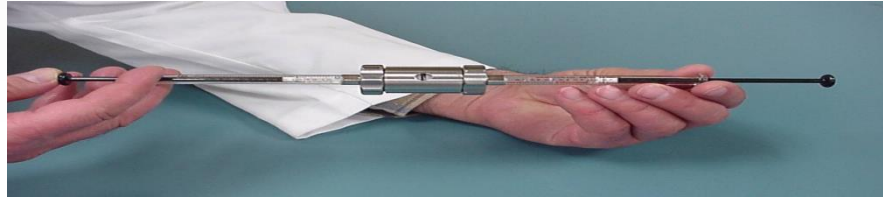
Synthesis of Pam₃Cys-MUC1 VNTR-TACA



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(2) *PCT Int. Appl.* (**2013**), WO 2013123282 A1 20130822, **Inventors:** Sucheck, S. J.; Wall, K. A.; Sarkar, S.

Liposome Preparation Procedure



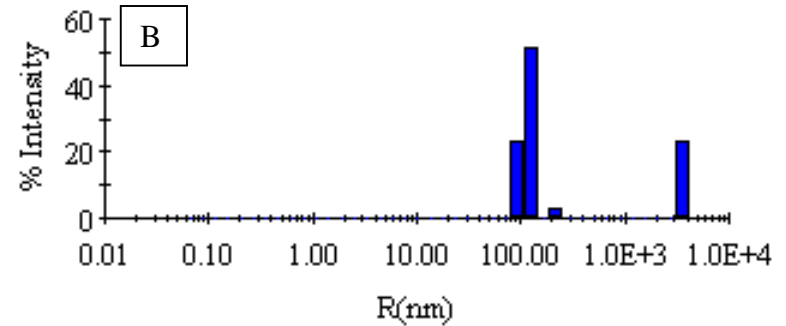
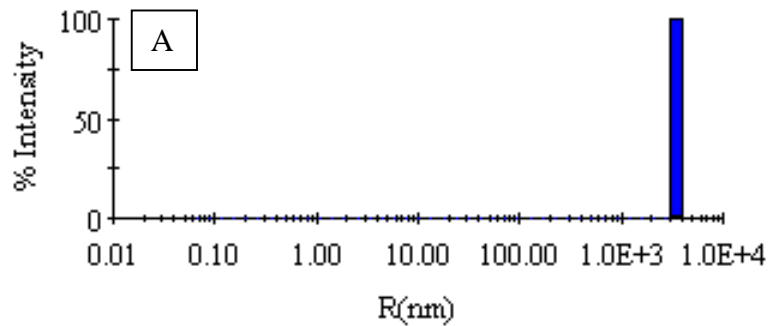
- Lipid stock solutions prepared in chloroform and mixed in glass vials (total lipid conc. 30 mM).
- Chloroform layer evaporated and lipid film dried overnight under vacuum.
- Hydrated with HEPES buffer (pH 7.4).
- Agitated at 43 °C for 40 mins.
- Subjected to 10 freeze thaw cycles.
- Extruded 21 times through lipofast basic fitted with 100 nM polycarbonate membrane.

Characterization of Liposomes

- A. Stability, Homogeneity and Size characterization
 - i. Dynamic Light Scattering (DLS) measurements.
 - ii. Scanning Electron Microscope (SEM) imaging.

- B. Surface Display of MUC1 VNTR and L-Rha epitopes
 - i. Human anti-MUC1 antibody binding experiment.
 - ii. Anti-L-Rha antibody binding experiment.

Dynamic Light Scattering Measurements

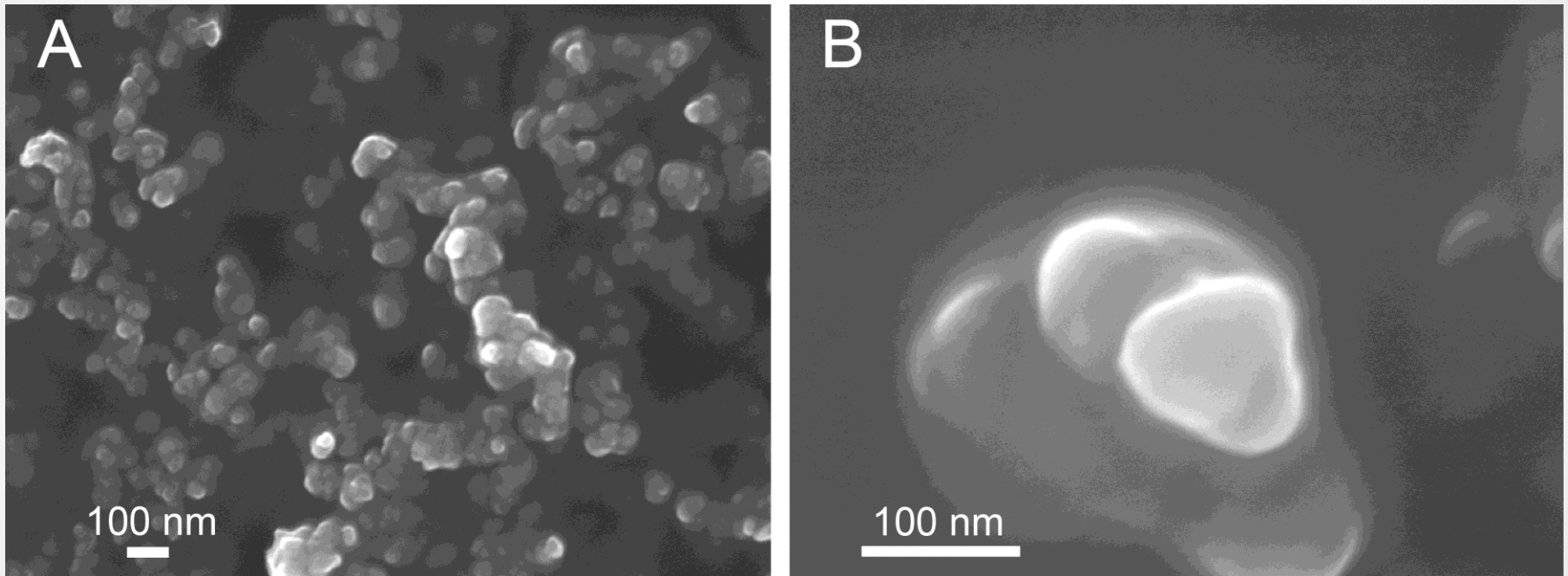


DLS Measurements at 1/1000 Dilution (A) Buffer (HEPES pH = 7.4) (B) liposomes

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SEM Images of L-Rha Displaying Liposomes

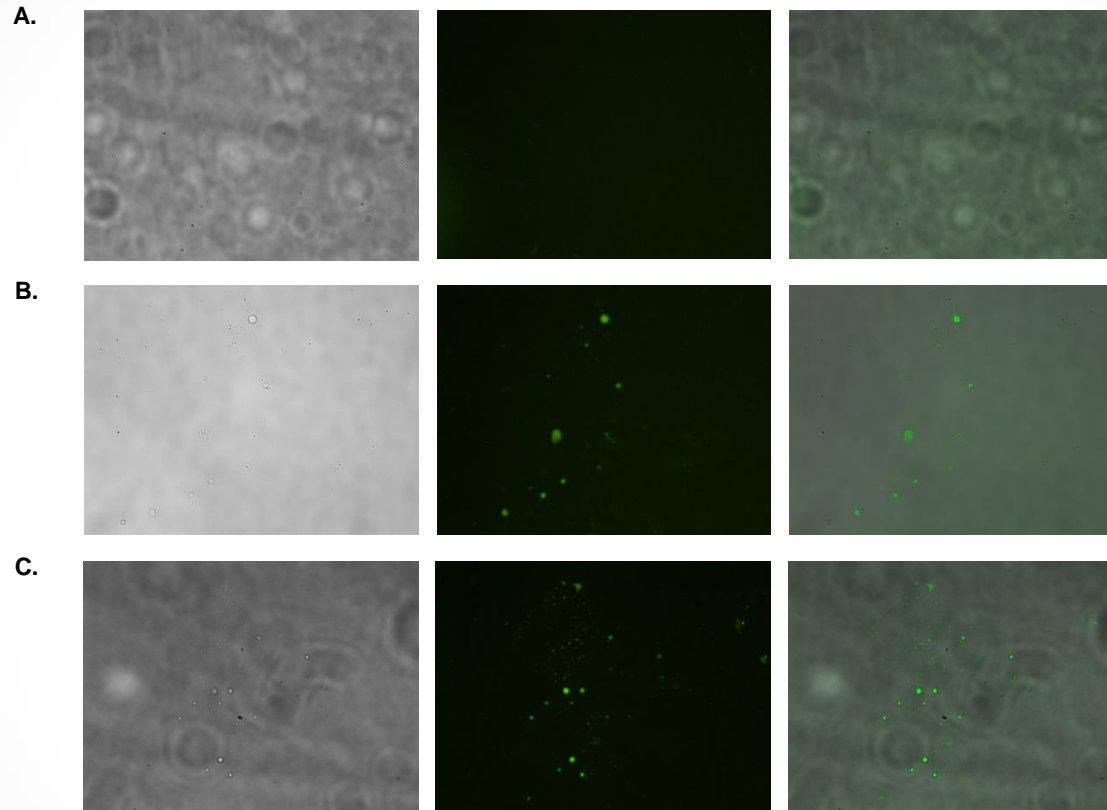


SEM images at 5 kV acceleration voltage (A) liposomes under 50000 X magnification, (B) liposomes under 250000 X magnification.

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(2) *PCT Int. Appl.* (**2013**), WO 2013123282 A1 20130822, **Inventors:** Sucheck, S. J.; Wall, K. A.; Sarkar, S.

Anti-L-Rha and Anti-Human MUC1 antibody binding with Liposomes



Fluorescence microscope images under 60 X magnification (A) Images with control antibodies (antibodies isolated from pre-immunization serum) **(B)** Images with anti-rhamnose antibodies **(C)** Images with anti-MUC1 antibodies [1st , 2nd and 3rd images: brightfield, FITC and overlay]

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(2) *PCT Int. Appl.* (**2013**), WO 2013123282 A1 20130822, **Inventors:** Sucheck, S. J.; Wall, K. A.; Sarkar, S.

Immunization Plan

Group
A1

Group
A2

Group
B1

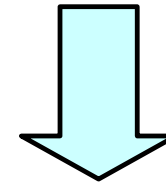
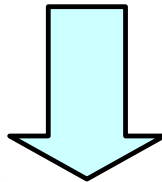
Group
B2

Non-
Immunized

Rha-Ficoll
Immunized

Non-
Immunized

Rha-Ficoll
Immunized



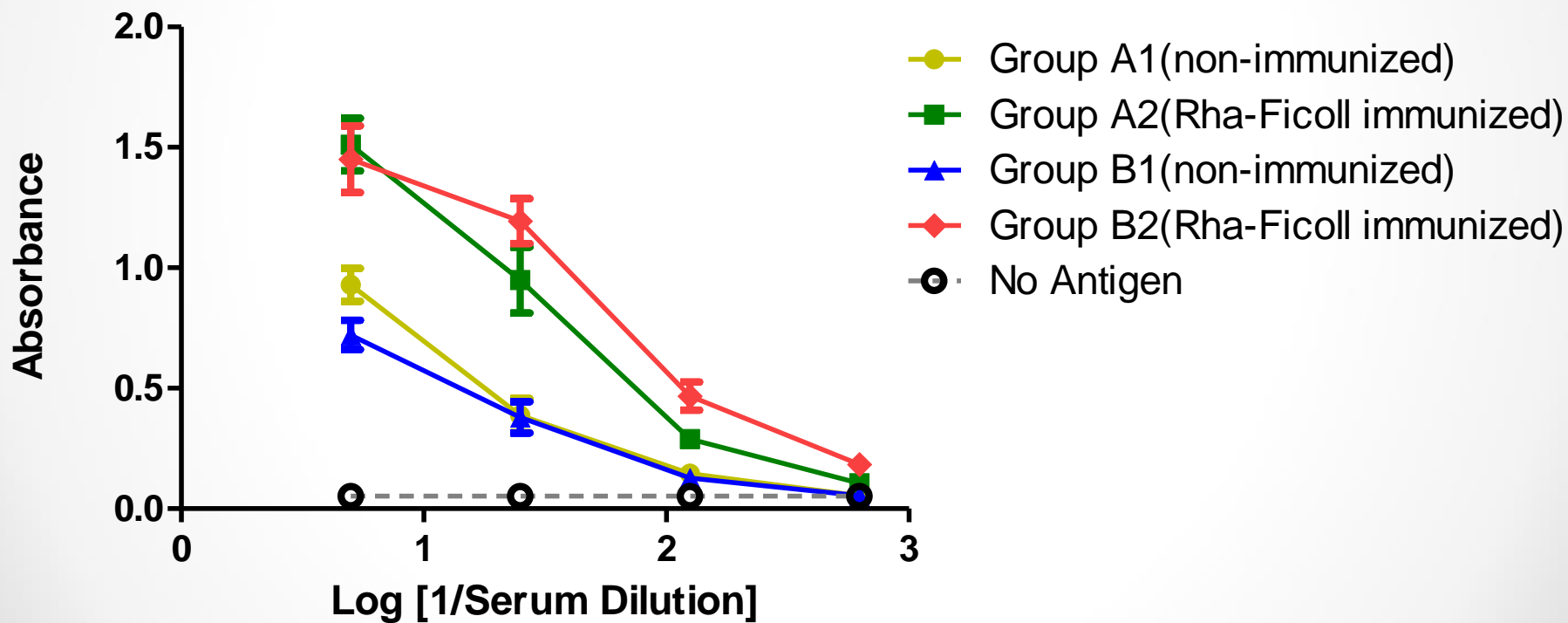
Pam₃Cys-MUC1-Tn
liposomes

Pam₃Cys-MUC1-Tn
+ Rha liposomes

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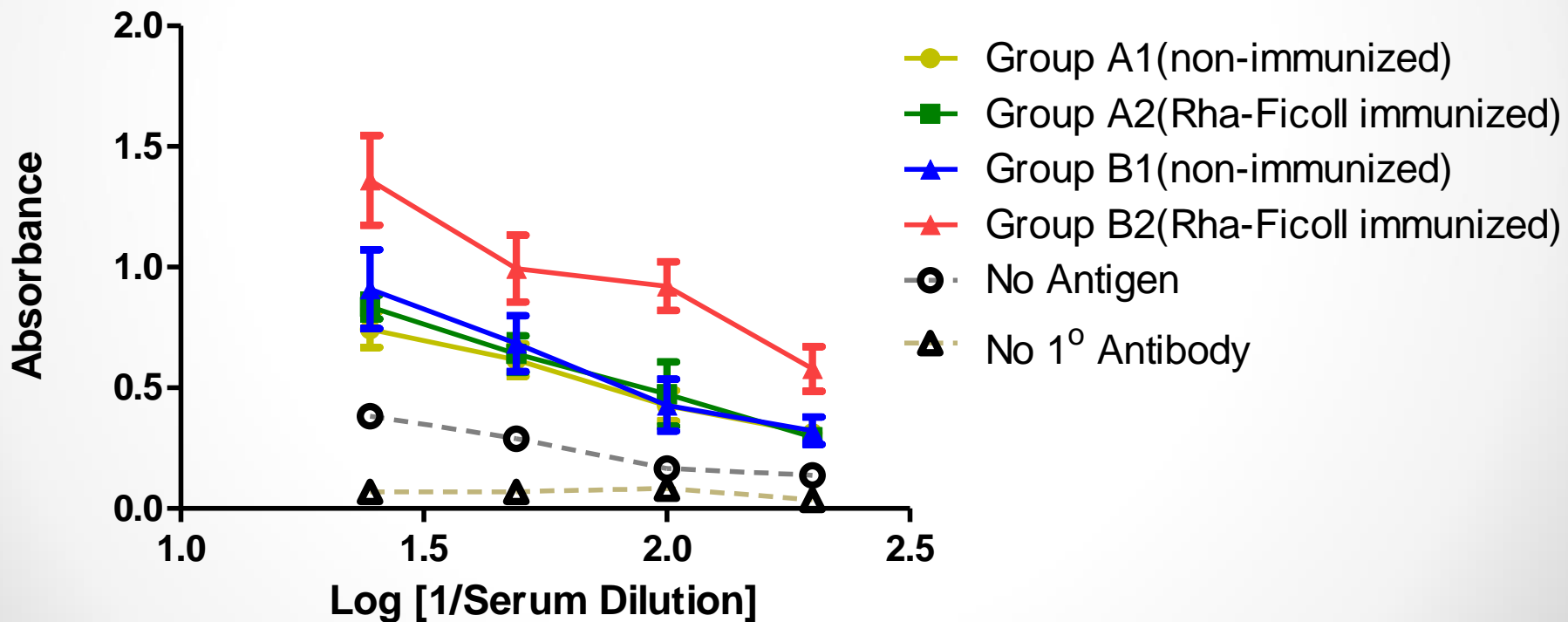
Anti-Rha Ab titers after 4th boost with Rha-Ficoll



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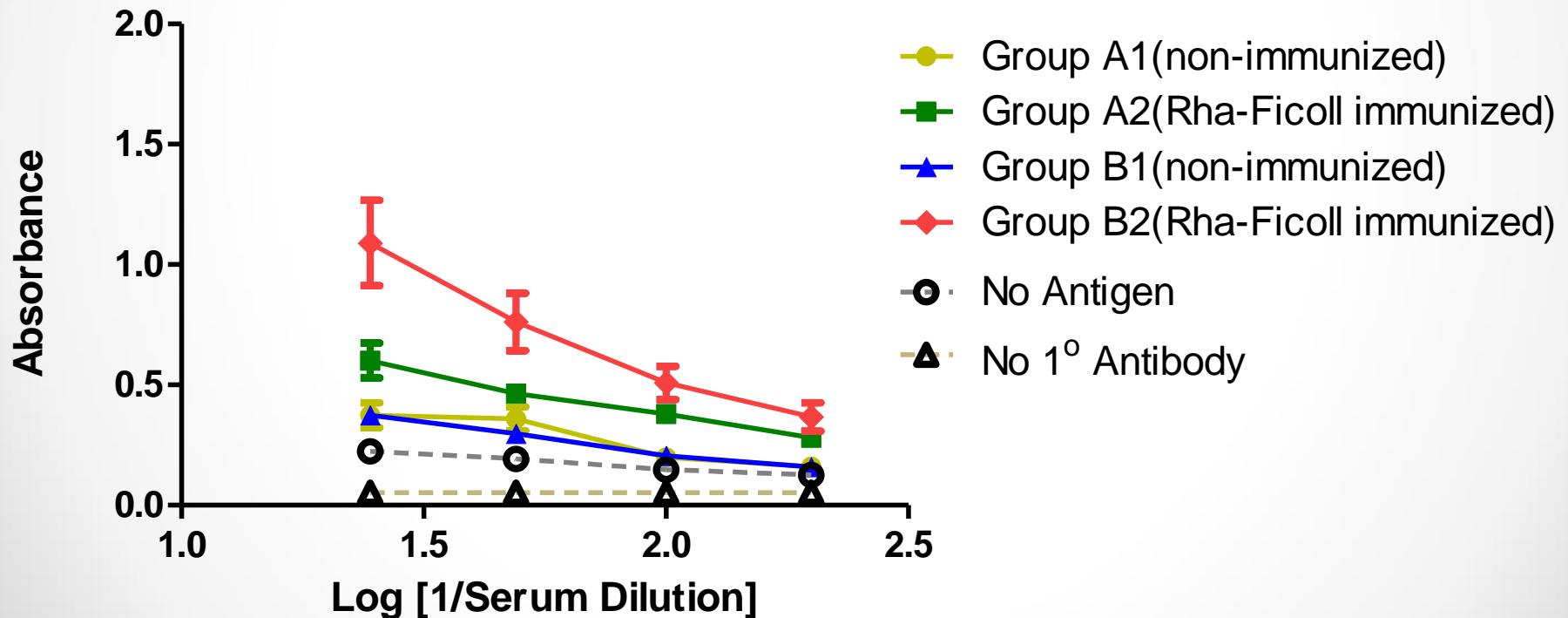
Anti-MUC1-Tn Antibody Titers after 1st boost with Pam₃Cys-MUC1-Tn or Pam₃Cys-MUC1-Tn + Rha Liposomes



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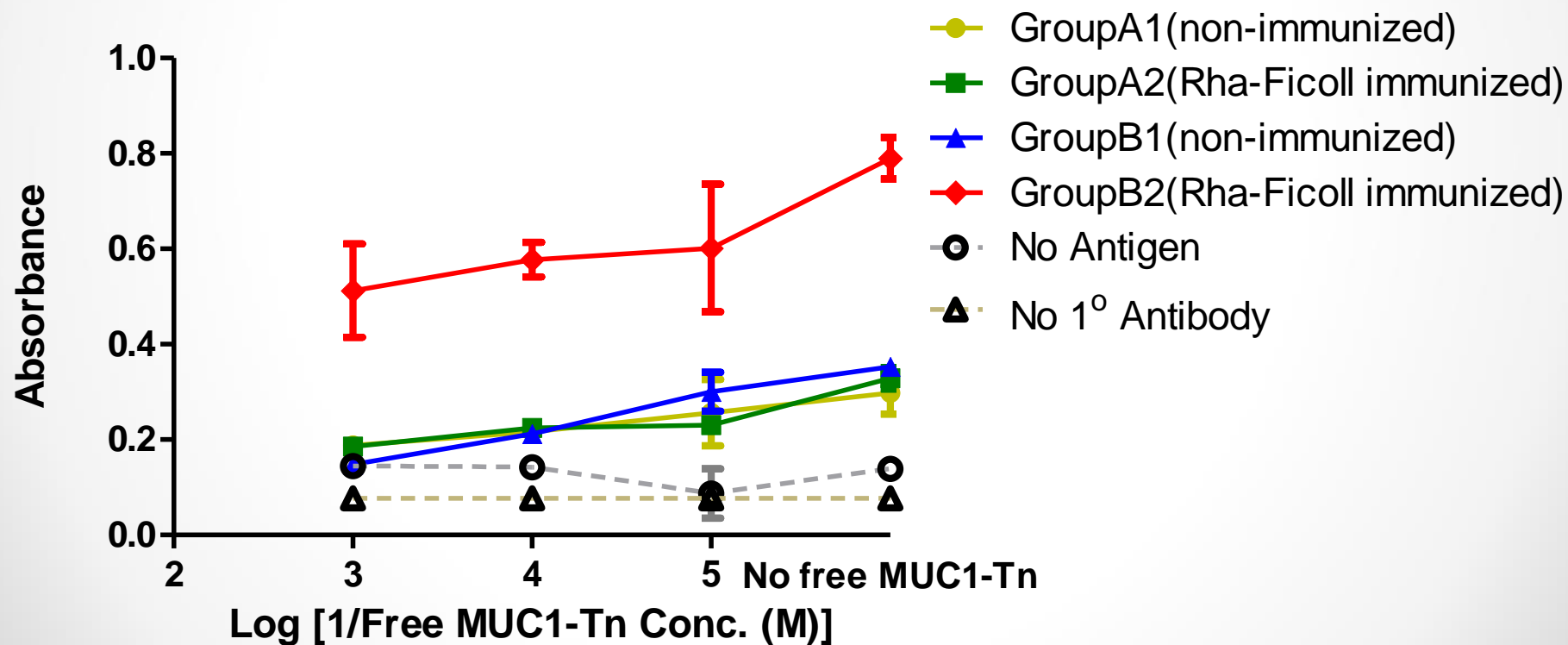
Anti-Tn Antibody Titer after 1st boost with Pam₃Cys-MUC1-Tn or Pam₃Cys-MUC1-Tn + Rha Liposomes



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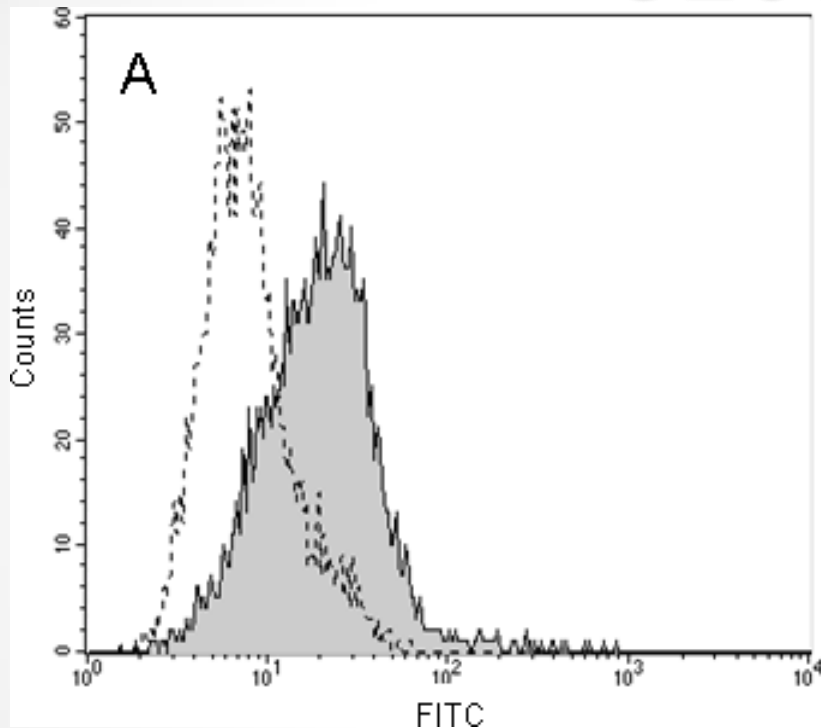
Competitive Binding of Anti-MUC1-Tn Antibodies with bound MUC1-Tn in presence of free MUC1-Tn



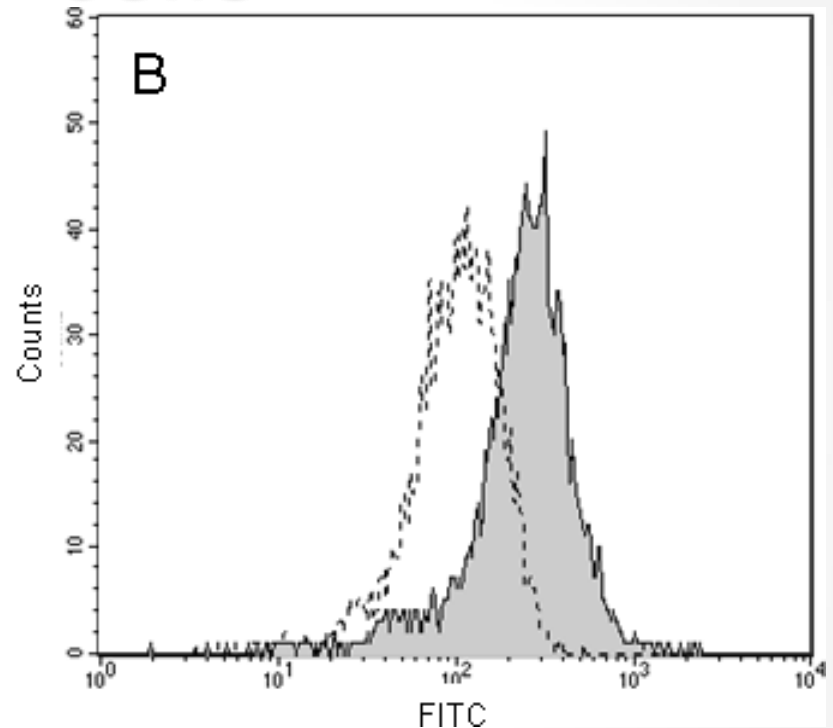
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Binding of Anti-MUC1-Tn Antibodies to Human Leukemia U266 cells



(A) 2nd Antibody alone,.....; with mouse Anti-Human MUC1 Antibodies,_____



(B) with 1/5 dilution of non-immunized mouse serum,.....; with 1/5 dilution of group B2 mice serum,_____

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(2) *PCT Int. Appl.* (2013), WO 2013123282 A1 20130822, **Inventors:** Sucheck, S. J.; Wall, K. A.; Sarkar, S.

Conclusions

- A fully synthetic two component vaccine construct containing the lipopeptide adjuvant Pam₃Cys, 20-amino acid MUC1 VNTR conjugated cancer antigen GalNAc-O-Thr (Tn) was synthesized.
- The synthesized two component lipo-glycopeptide was successfully incorporated into L-Rha displaying liposomes.
- The formulated liposomes were homogenous in size and were stable at 4 °C for two days.
- Binding studies with both anti-rhamnose and mouse anti-human MUC1 antibodies revealed that the rhamnose and the MUC1 VNTR glycopeptide epitopes were surface displayed on the liposomes.
- Generation of glycosylated anti-MUC1 and anti-Tn antibodies and the anti sera bound human leukemia U266 cells.

Acknowledgements

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Parijat Srivastava, Vishwanath Gaitonde, Partha Karmakar, Kevin
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