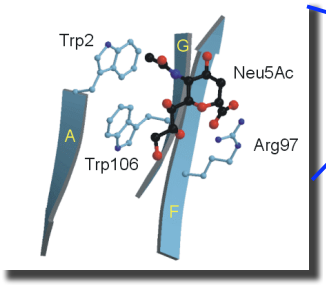
Sialoglyco 2008
July 22, 2008

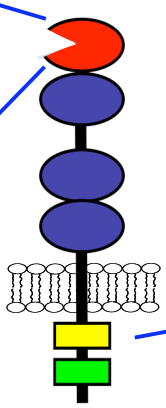
Multivalent ligands of CD22 for targeting of B cells

James C. Paulson
Departments of Chemical Physiology and Molecular Biology
The Scripps Research Institute, La Jolla, CA

Sialic-acid binding Ig-like lectin (Siglec) family:
Functional domains adapt function to diverse biological roles



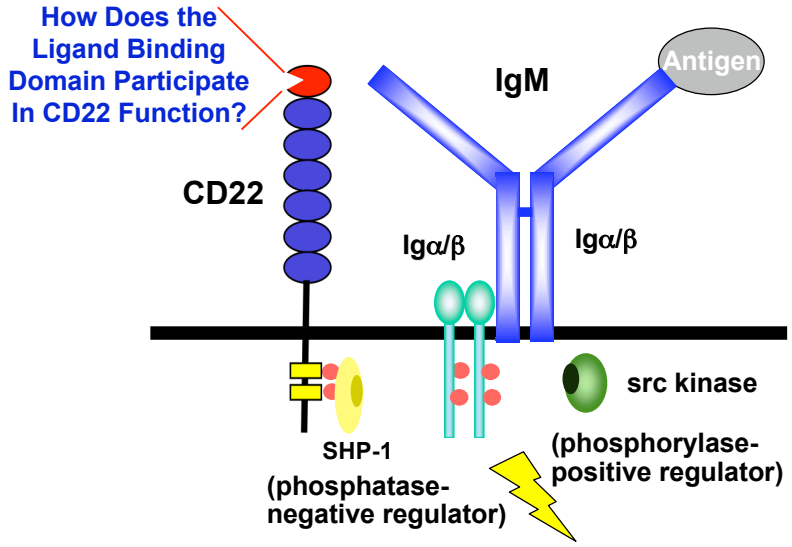
Varied sialoside binding specificity



Siglec family
- Thirteen human and 9 murine members
- Differentially expressed on various white blood cells
- Functional domains adapt function to diverse biological roles in innate and adaptive immunity

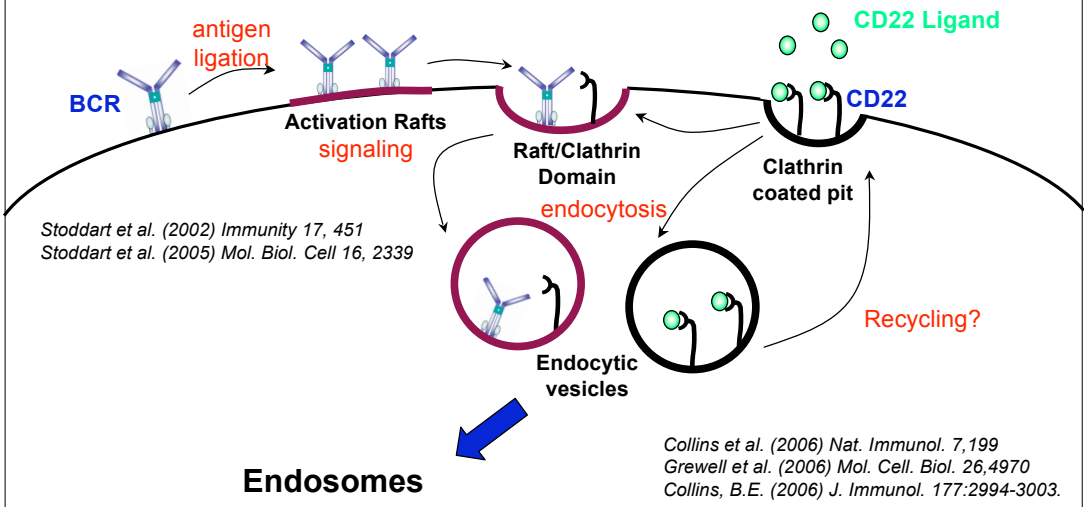
Cytoplasmic domain
- ITIM motifs regulate cell signaling
- Regulation of microdomain localization and endocytic mechanism

CD22 is a regulator of B cell receptor signaling



Sialoglyco 2008

Roles of CD22 Rich Clathrin Domains in Regulation of BCR Signaling and Ligand Endocytosis

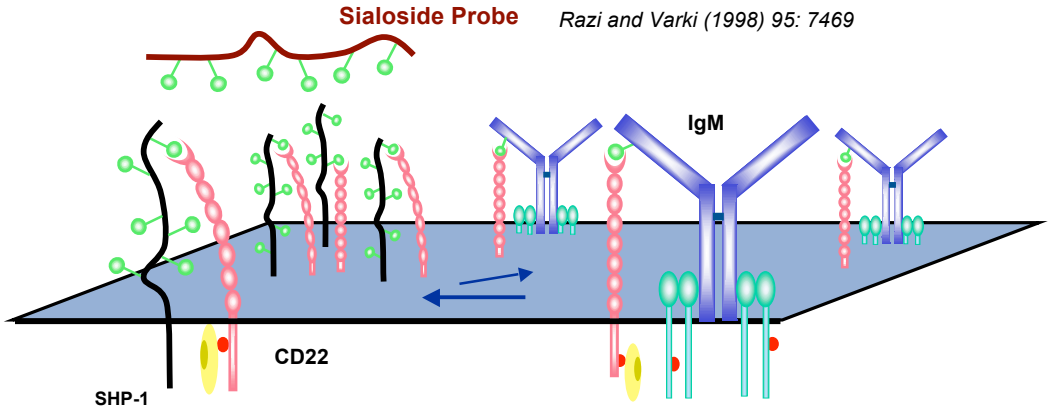


Stoddart et al. (2002) Immunity 17, 451
Stoddart et al. (2005) Mol. Biol. Cell 16, 2339

Collins et al. (2006) Nat. Immunol. 7,199
Grewell et al. (2006) Mol. Cell. Biol. 26,4970
Collins, B.E. (2006) J. Immunol. 177:2994-3003.

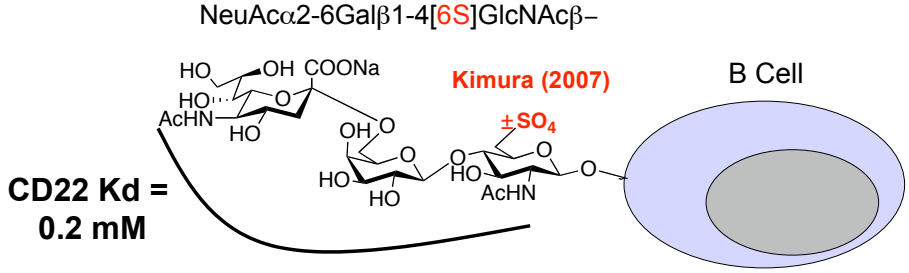
Sialoglyco 2008

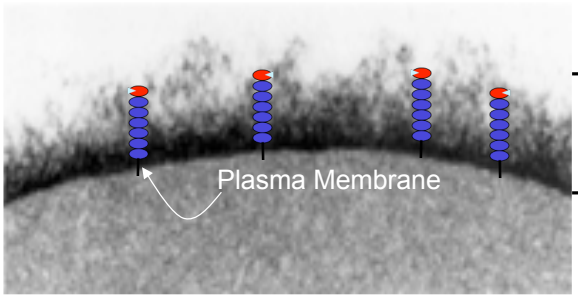
CD22 is masked by *cis* interactions with B cell glycoproteins



Sialoglyco 2008

Cis ligands mask CD22

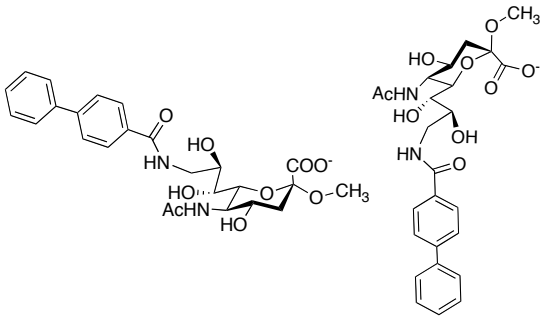




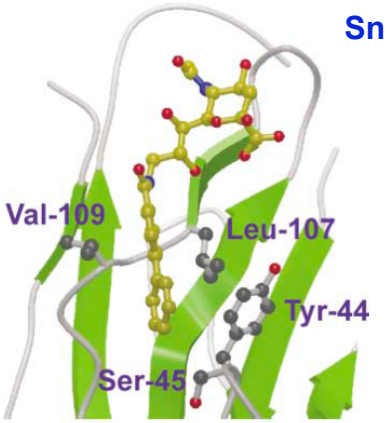
B cell Glycocalyx
[NeuAc] = 100 mM

Sialoglyco 2008

9-biphenyl substituents of sialic acid increase affinity for CD22 and sialoadhesin (Sn)

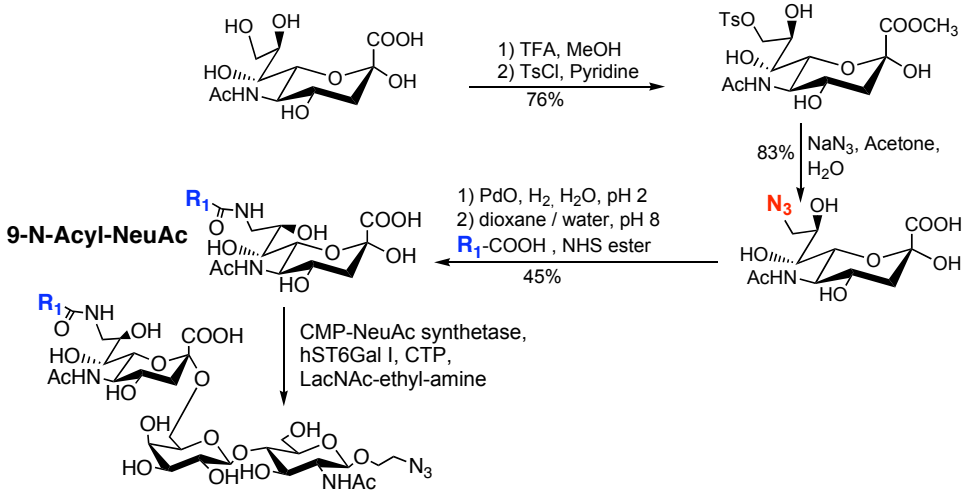


α-methyl-9-N-biphenyl-NeuAc



Zaccai et al. (2003) 11, 557-567

Synthesis of 9-N-Acyl-NeuAc and 9-N-Acyl-Sialoside ligands of CD22

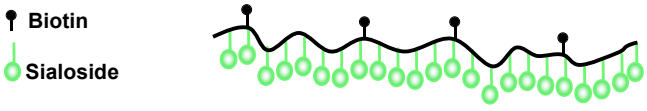


9-N-Acyl-NeuAcα2-6LacNAc-ethylazide

Design of high affinity sialoside-PAA for CD22

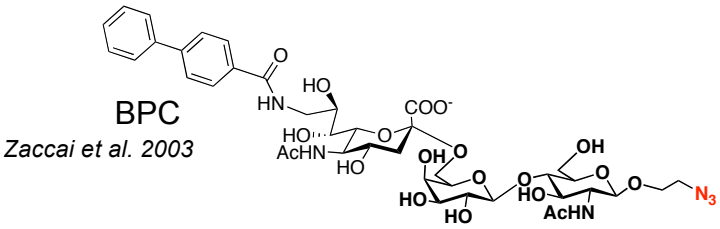
Substituted polyacrylamide polymer:

Shilova et al. 2005



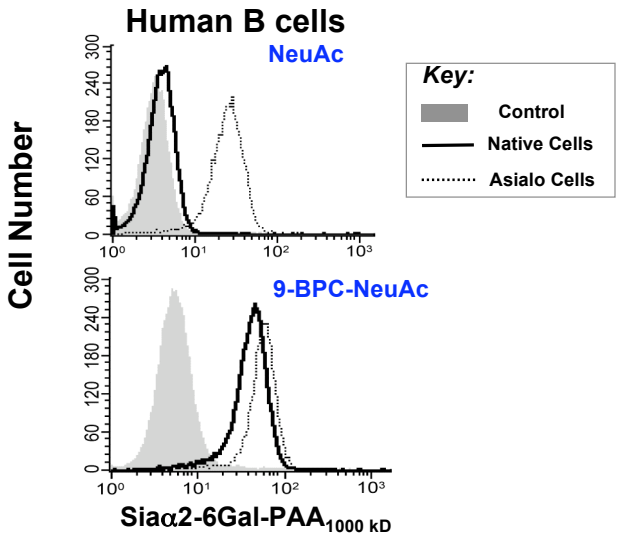
HMW >1000 kDa (n = >500)

Add sialic acid substituents that increase affinity:



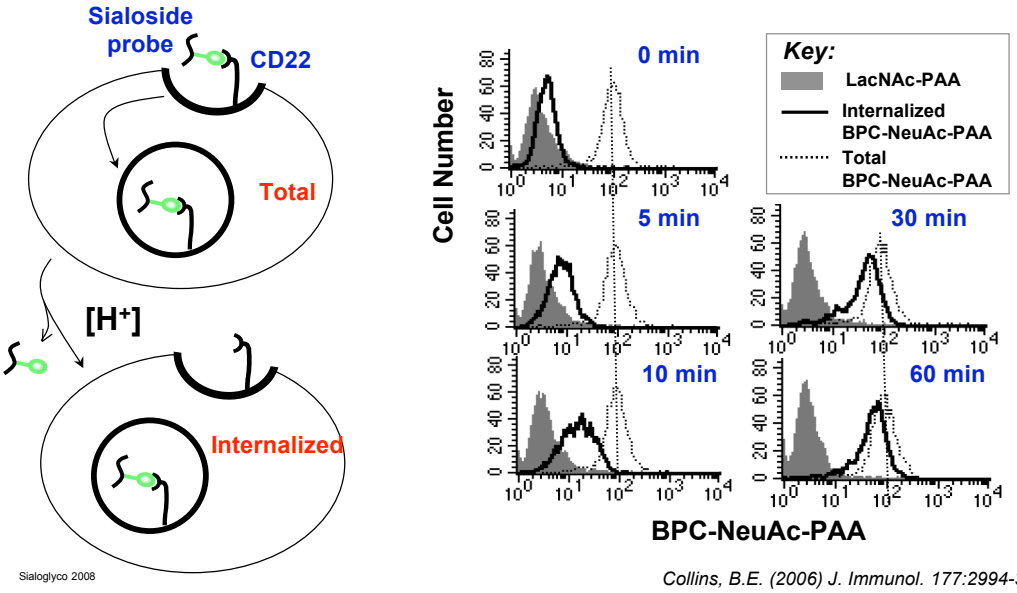
Collins, B.E. (2006) J. Immunol. 177:2994-3003.

B cell *cis* ligands do not 'mask' binding of high affinity sialoside probes to CD22



Collins, B.E. (2006) J. Immunol. 177:2994-3003.

High affinity BPC-NeuAc-PAA is rapidly endocytosed by CD22 on native B cells



Sialoglyco 2008

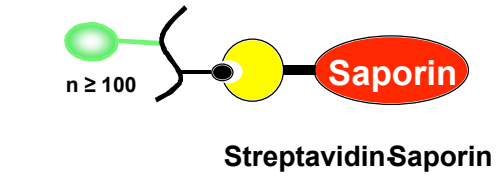
Collins, B.E. (2006) J. Immunol. 177:2994-3003.

Therapeutic implications of targeting siglecs

- Antibodies that block CD22 ligand binding deplete B cells in an *in vivo* murine model (anti-cancer). *Haas et al. (2006) J. Immunol. 177, 3063.*
- Antibodies to Siglec-8 cause apoptosis of eosinophils (anti-allergen). *Nutku et al. (2003) Blood 101, 5014*
- Antibodies to Siglec-9 cause apoptosis of neutrophils (anti-inflammatory). *von Gunten et al. (2006) Blood 108, 4255*

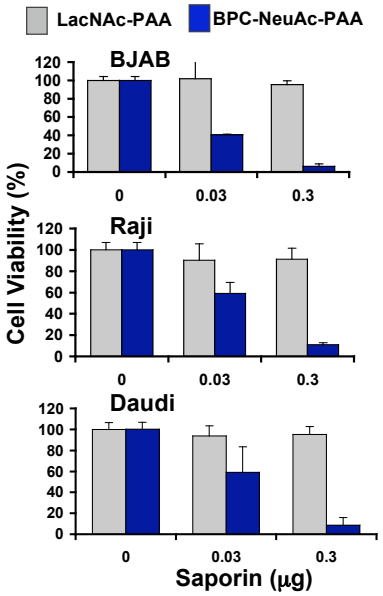
Sialoglyco 2008

A Trojan Horse for B cells: Endocytosis of BPC-NeuAc-PAA/Saporin conjugate by CD22 promotes killing of B cell lymphoma cells



LacNAc
(Galβ1-4GlcNAc)

BPC-NeuAc
(9-BPC-NeuAcα2-6Galβ1-4GlcNAc)

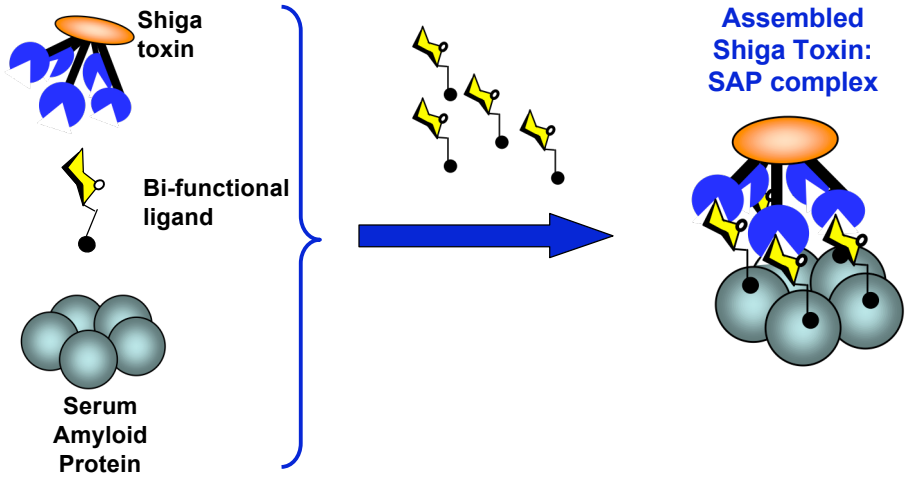


Collins, B.E. (2006) J. Immunol. 177:2994-3003.

Targeting B cells with ligands of CD22

Carrier/Scaffold	Comments
Polyacrylamide	• High valency • Particle size and ligand spacing unknown
Virus capsid (Finn collaboration)	• High valency nanoparticle • Potential to vary surface spacing
Immunoglobulin	• Small molecule hetero-bi-functional ligand induces Ig-CD22 assembly on the cell • Adaptable to immuno-toxin approach
Liposome	• Attach ligand to lipid • Incorporate into liposome • Encapsulate toxin/drug in liposome

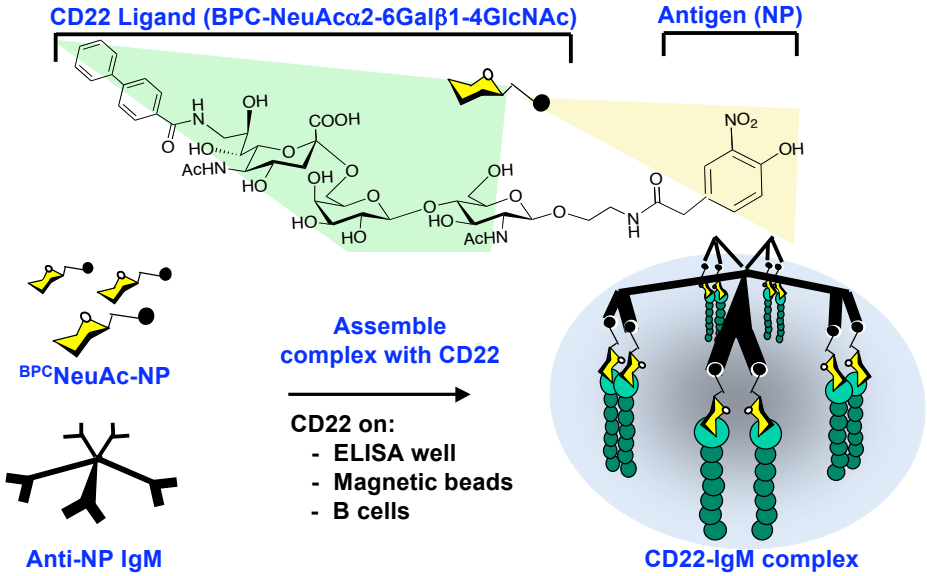
Docking of two pentameric binding proteins with bi-functional ligands



Liu, J. et al. (2005) J. Am. Chem. Soc. ***127****, 2044.*

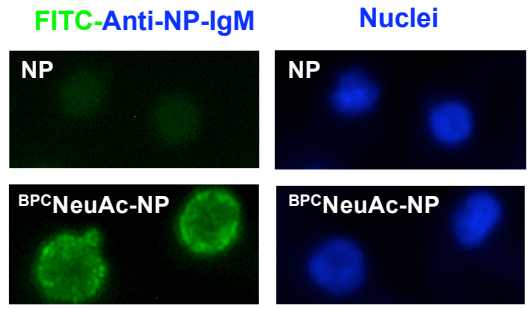
 Solomon, D. et al. (2005) Org. Lett. ***7****, 4369.*

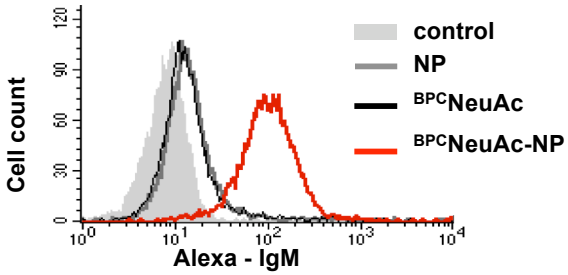
Bi-functional ligand that uses IgM as a decavalent protein scaffold for assembly of CD22-IgM complexes



M. O'Reilly, B. Collins, S. Han, M., B. Liang, C. Rillahan, P. Kitov, D. Bundle, J. Paulson, 2008, JACS in press

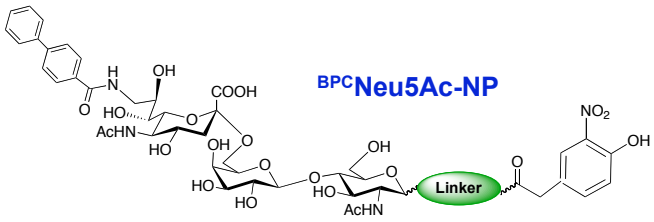
^{BPC}NeuAc-NP drives binding of anti-NP-IgM to CD22 on B cells

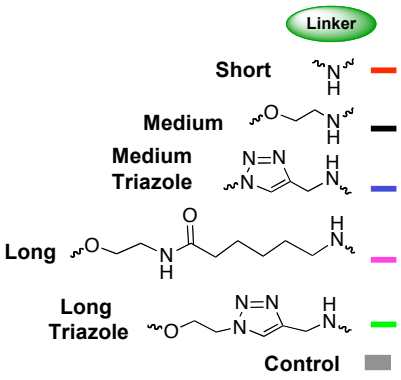


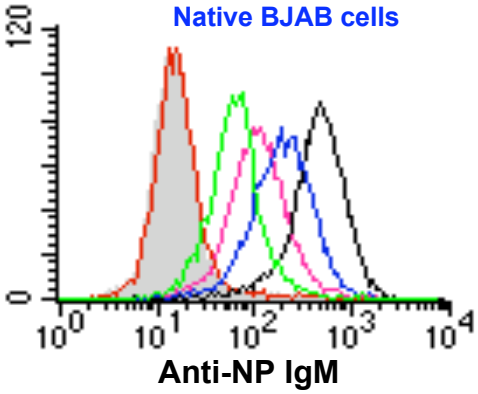


Sialoglyco 2008

Complex formation is sensitive to linker length

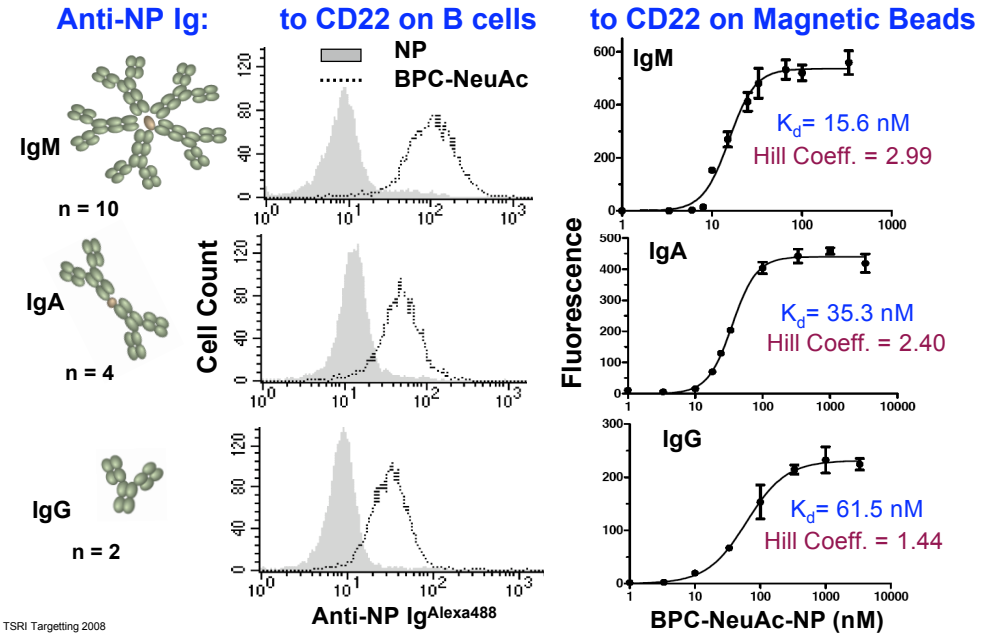






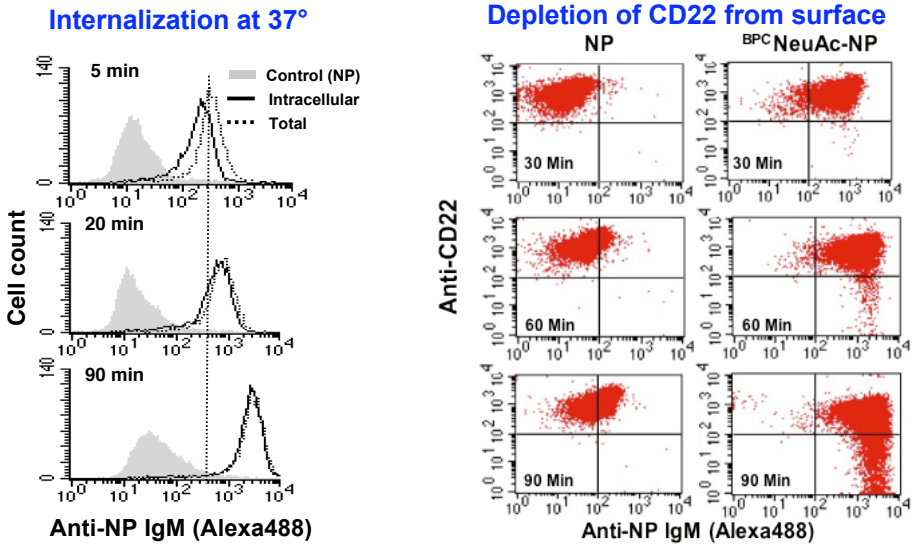
Sialoglyco 2008

Bi-functional ligand driven docking of anti-NP Ig to CD22

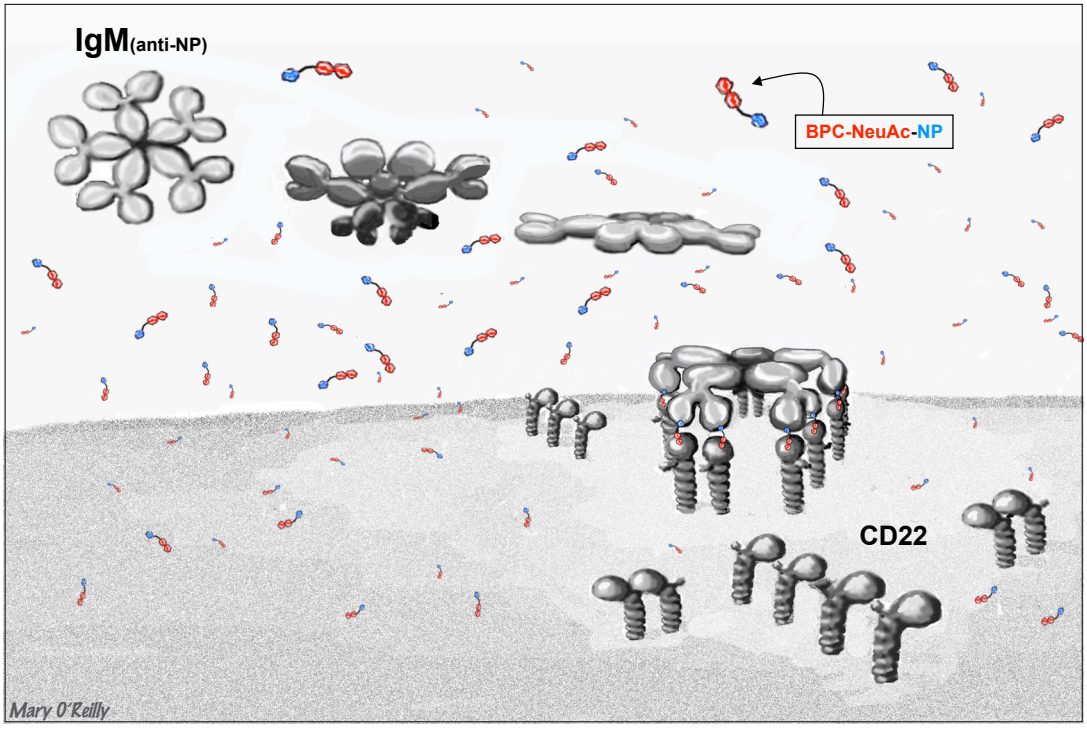


TSRI Targetting 2008

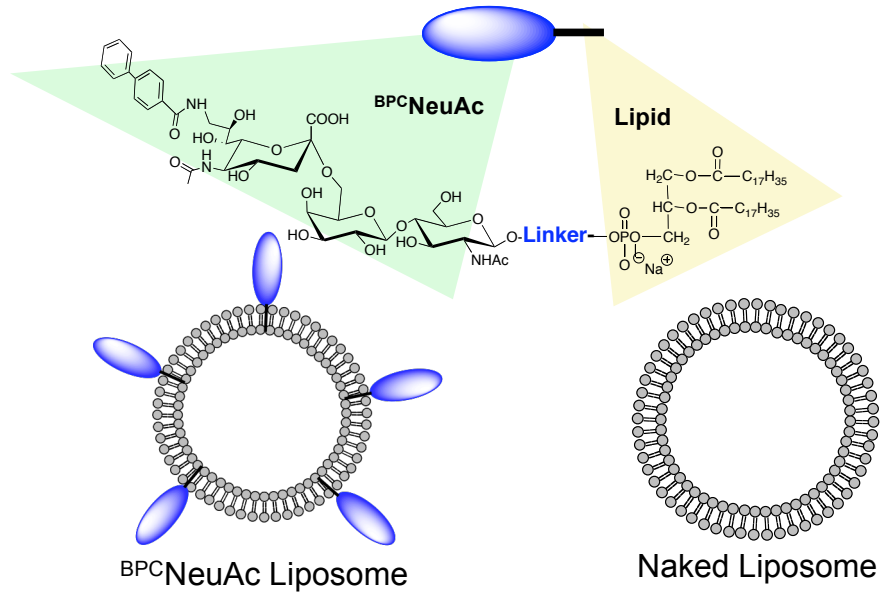
Depletion of cell surface CD22 from BJAB cells with anti-NP IgM and ^{BPC}NeuAc-NP



Sialoglyco 2008

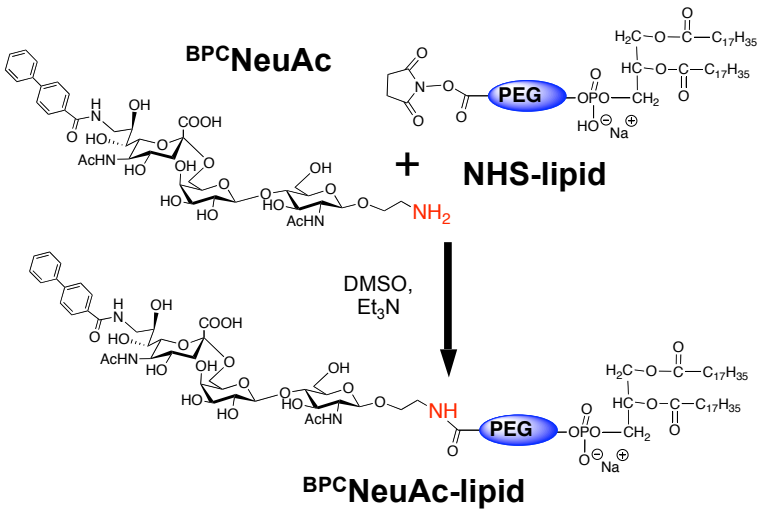


Liposomes with ^{BPC}NeuAc-lipid for targeting B cells



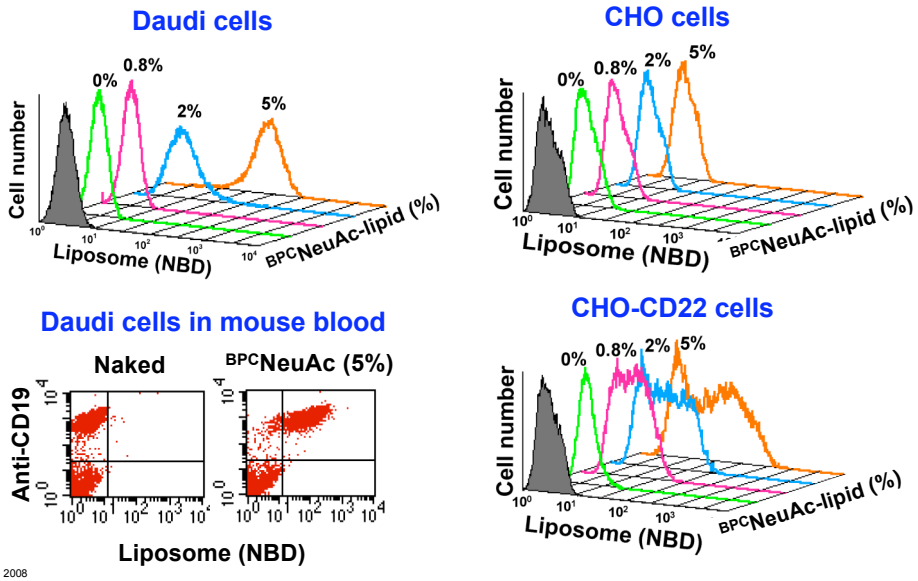
Sialoglyco 2008

Synthesis of ^{BPC}NeuAc-lipid



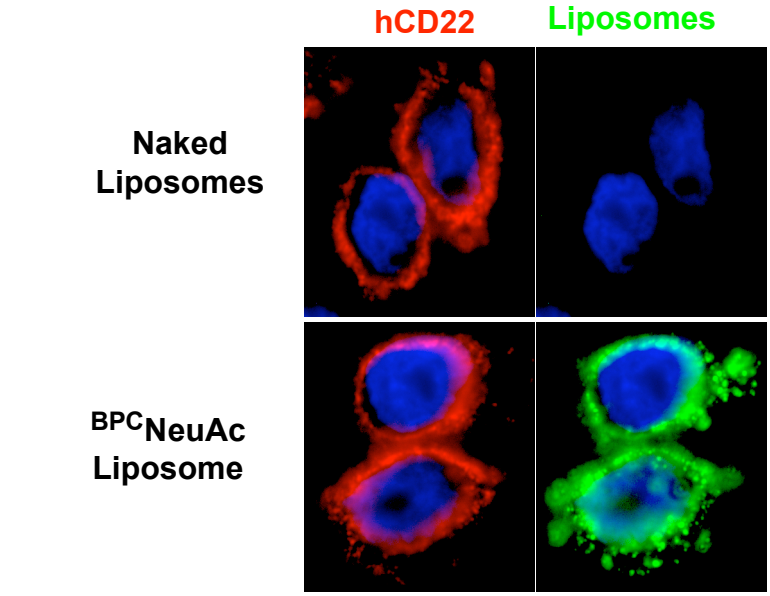
Sialoglyco 2008

Binding of ^{BPC}NeuAc-liposomes to CD22 on cells



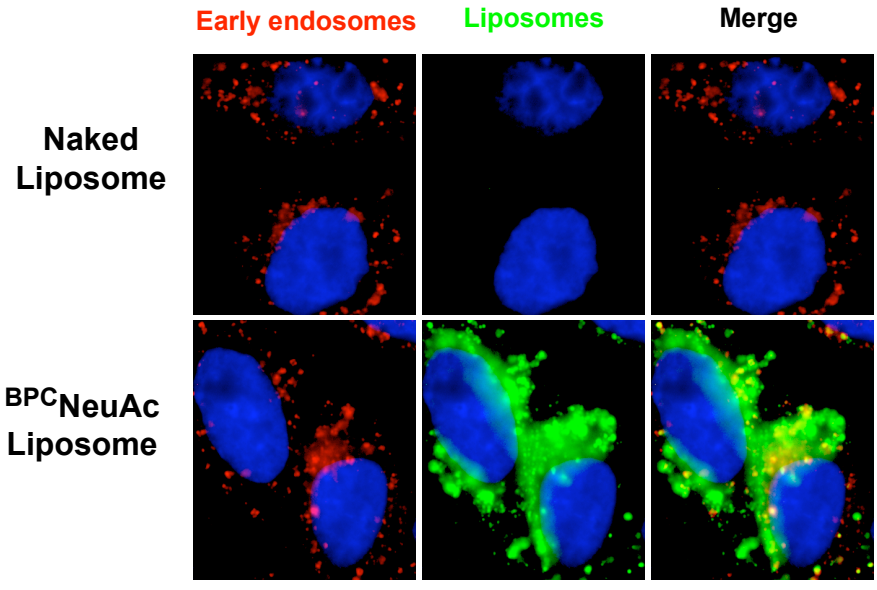
Sialoglyco 2008

Targeting of ^{BPC}NeuAc Liposomes to CD22 CHO cells



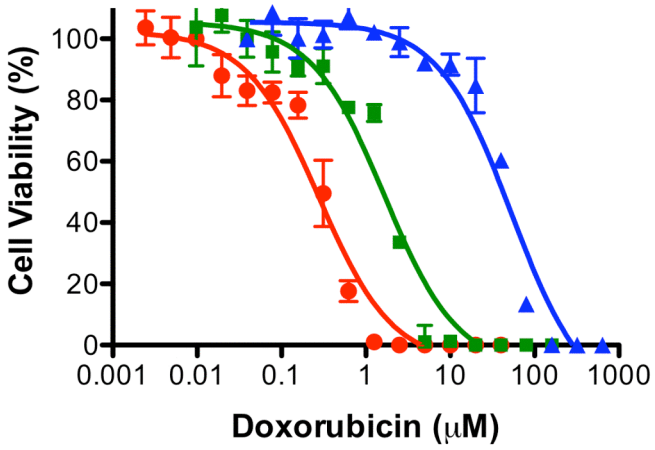
Sialoglyco 2008

Targeted ^{BPC}NeuAc liposomes co-localize with early endosomes in CD22-CHO cells



Sialoglyco 2008

In vitro cytotoxicity of doxorubicin loaded ^{BPC}NeuAc-liposomes to Daudi cells

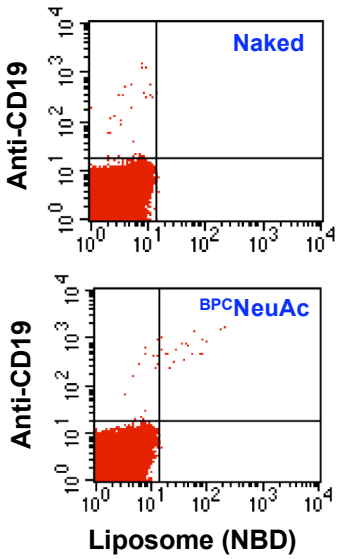


Key	Doxorubicin	IC_{50} (μM)
●	Free Dox	0.3
▲	Naked liposomes	53.0
■	^{BPC}NeuAc liposomes	1.6

Sialoglyco 2008

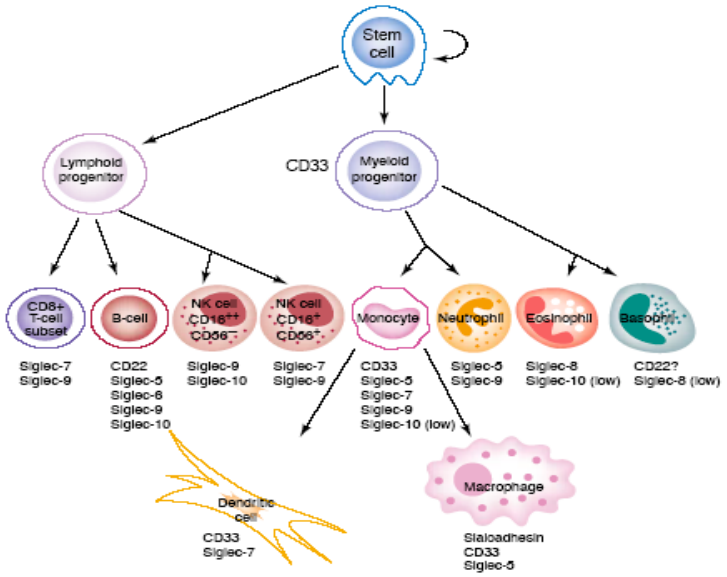
^{BPC}NeuAc-liposomes find Daudi cells *in vivo*

- Inject 5 x 10^6 Daudi cells in mouse tail vein
- After 30 min, inject NBD-labeled liposomes
- At 2.5 hr, draw blood
- Detect Daudi cells with anti-hCD19



Sialoglyco 2008

Distribution of Siglecs in the immune system



Sialoglyco 2008

Crocker and Varki (2001) Trends Immunol.22,337

Chemical Glycobiology
Roles of Siglecs in immune function

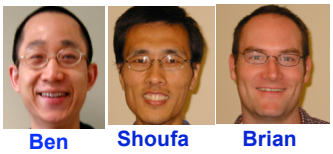
- **Synthesis of sialoside probes of Siglec function.**

Corwin Nycholat, Ying Zeng, Gladys Completo, Cory Rillahan

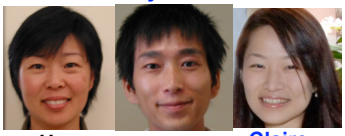
- **Siglec-ligand interactions mediating cell signaling and microdomain localization.**

Mary O'Reilly, Ramya TNC

Past members:



Ben Liao, Shoufa Han, Brian Collins



Hua Tian, Norihito Kawasaki, Claire Chen

TSRI Targetting 2008


The Scripps Research Institute
TSRI
Ben Cravatt - E. Weerapana
Phil Dawson - Anouk Dirkson
MG Finn - Eiton Kaltgrad
David Nemazee - Bao Duong
Barry Sharpless, Larry Folkin - Jason Hein, Larissa Krasnova
Ian Wilson - Daniel Calarese, James Stevens
Chi-Huey Wong - Fabio Fazio, Marian Bryan
Shemyakin Institute
Nicolai Bovin
U. C. San Diego
Jamey Marth
University of Dundee
Paul Crocker
Univ. of Alberta
David Bundle
Pavel Kitov
Funding
NIGMS NIAID