The **Glycotechnology Group** at CIC biomaGUNE is engaged in both basic and applied research in **Glycoscience**.

Glycoscience is still a relatively understudied area but has a strong potential to provide groundbreaking solutions to major societal challenges in **personalized medicine, pharmaceuticals, food and biomaterials**.

In our research we focus specifically on **Biomedical Glycoscience**, investigating and exploiting structure and function of carbohydrates in the context of human biology and disease.

We do this by developing specific tools for glycan analysis and glycan-based intervention and by closely collaborating with a growing number of internationally recognized researchers.

The following pages illustrate the active major research lines in **2016** and our efforts in translating our results from basic science into commercially viable products.

Our main areas of research are:

- Carbohydrate Synthesis
- Glycan Microarrays
- Lectin Analysis
- Glycan Analysis
- Chemical Immunology
Glycans play a key role as recognition elements in the communication of cells and other organisms. Thus, the analysis of carbohydrate–protein interactions has gained significant importance. In particular, nuclear magnetic resonance (NMR) techniques are considered powerful tools to detect relevant features in the interaction between sugars and their natural receptors. Here, we present the results obtained in the study on the molecular recognition of different mannose-containing glycans by *Pisum sativum* agglutinin. NMR experiments supported by Corema-ST analysis, isothermal titration calorimetry (ITC) experiments, and molecular dynamics (MD) protocols have been successfully applied to unmask important binding features and especially to determine how a remote branching substituent significantly alters the binding mode of the sugar entity. These results highlight the key influence of common structural modifications in natural glycans on molecular recognition processes and underscore their importance for the development of biomedical applications.

Fluoroacetamide Moieties as NMR Spectroscopy Probes for the Molecular Recognition of GlcNAc-Containing Sugars: Modulation of the CH–π Stacking Interactions by Different Fluorination Patterns

Luca Unione, Marí Alcalá, Begoña Echeverria, Sonia Serna, Ana Ardá, Antonio Franconetti, F. Javier Cañada, Tammo Diercks, Niels Reichardt, Jesús Jiménez-Barbero


We herein propose the use of fluoroacetamide and difluoroacetamide moieties as sensitive tags for the detection of sugar–protein interactions by simple 1H and/or 19F NMR spectroscopy methods. In this process, we have chosen the binding of N,N′-diacetyl chitobiose, a ubiquitous disaccharide fragment in glycoproteins, by wheat-germ agglutinin (WGA), a model lectin. By using saturation-transfer difference (STD)-NMR spectroscopy, we experimentally demonstrate that, under solution conditions, the molecule that contained the CHF2CONH- moiety is the stronger aromatic binder, followed by the analogue with the CH2FCONH- group and the natural molecule (with the CH3CONH- fragment). In contrast, the molecule with the CF3CONH- isoster displayed the weakest intermolecular interaction (one order of magnitude weaker). Because sugar–aromatic CH–π interactions are at the origin of these observations, these results further contribute to the characterization and exploration of these forces and offer an opportunity to use them to unravel complex recognition processes.
**Specific anti-glycan antibodies are sustained during and after parasite clearance in Schistosoma japonicum-infected rhesus macaques**

Y. Y. Michelle, Yang Xiao, Hong Li, Katarzyna Brzezicka, Niels-Christian Reichardt, R. Alan Wilson, Angela van Diepen, Cornelis H. Hokke

PLOS Neglected Tropical Diseases 2017, 11 (2) | e0005339

Human immunity to *Schistosoma* infection requires many years of exposure, and multiple infections and treatments to develop. Unlike humans, rhesus macaques clear an established schistosome infection naturally at the same time acquiring immunity towards re-infection. In macaques, schistosome egg production decreases after 8 weeks post-infection and by week 22, physiological impairment of the worm caused by unclarified antibody-mediated processes is observed. Since strong antibody responses have been observed against schistosome glycan antigens in human and animal infections, we here investigate if anti-glycan antibodies are associated with immunity against schistosome infections in macaques.

**Identification of dominant anti-glycan IgE responses in school-children by glycan microarray**


**Lectin-Array Blotting**
R. Pazos, J. Echevarria, A. Hernández, N.C. Reichardt

Aberrant protein glycosylation is a hallmark of cancer, infectious diseases, and autoimmune or neurodegenerative disorders. Unlocking the potential of glycans as disease markers will require rapid and unbiased glycoproteomics methods for glycan biomarker discovery. The present method is a facile and rapid protocol for qualitative analysis of protein glycosylation in complex biological mixtures. While traditional lectin arrays only provide an average signal for the glycans in the mixture, which is usually dominated by the most abundant proteins, our method provides individual lectin binding profiles for all proteins separated in the gel electrophoresis step. Proteins do not have to be excised from the gel for subsequent analysis via the lectin array but are transferred by contact diffusion from the gel to a glass slide presenting multiple copies of printed lectin arrays. Fluorescently marked glycoproteins are trapped by the printed lectins via specific carbohydrate-lectin interactions and after a washing step their binding profile with up to 20 lectin probes is analyzed with a fluorescent scanner. The method produces the equivalent of 20 lectin blots in a single experiment, giving detailed insight into the binding epitopes present in the fractionated proteins.

**Analysis of Defective Protein Ubiquitylation Associated to Adriamycin Resistant Cells**
V. Lang, F. Aillet, W. Xolalpa, S. Serna, L. Ceccato, R.G. López-Reyes, M.P. López-Mato, R. Januchowski, N.C. Reichardt, M. S. Rodríguez

DNA damage activated by Adriamycin (ADR) promotes ubiquitin-proteasome system-mediated proteolysis by stimulating both the activity of ubiquitylating enzymes and the proteasome. In ADR-resistant breast cancer MCF7 (MCF7ADR) cells, protein ubiquitylation is significantly reduced compared to the parental MCF7 cells. Here, we used tandem ubiquitin-binding entities (TUBEs) to analyze the ubiquitylation pattern observed in MCF7 or MCF7ADR cells. While in MCF7, the level of total ubiquitylation increased up to six-fold in response to ADR, in MCF7ADR cells only a two-fold response was found. To further explore these differences, we looked for cellular factors presenting ubiquitylation defects in MCF7ADR cells. Among them, we found the tumor suppressor p53 and its ubiquitin ligase, Mdm2. We also observed a drastic decrease of proteins known to integrate the TUBE-associated ubiquitin proteome after ADR treatment of MCF7 cells, like histone H2AX, HMGB1 or β-tubulin. Only the proteasome inhibitor MG132, but not the autophagy inhibitor chloroquine partially recovers the levels of total protein ubiquitylation in MCF7ADR cells. p53 ubiquitylation is markedly increased in MCF7ADR cells after proteasome inhibition or a short treatment with the isopeptidase inhibitor PR619, suggesting an active role of these enzymes in the regulation of this tumor suppressor. Notably, MG132 alone increases apoptosis of MCF7ADR and multidrug resistant ovarian cancer A2780DR1 and A2780DR2 cells. Altogether, our results highlight the use of ubiquitylation defects to predict resistance to ADR and underline the potential of proteasome inhibitors to treat these chemoresistant cells.
Presentations at Conferences

*Presentation of the Glycotechnology Laboratory (Invited)*  
N.C. Reichardt  
1st Euskadi Workshop on Exosomes, CIC bioGUNE Bilbao (Spain)  
23 March 2017

*10 years of Glycotechnology at CIC biomaGUNE (Invited)*  
N.C. Reichardt  
1st Glycobasque Meeting, CIC bioGUNE Bilbao (Spain)  
15 May 2017

*Neoglycoproteins role in protein corona formation and immunomodulation*  
B. Kuhn  
1st Glycobasque Meeting, CIC bioGUNE Bilbao (Spain)  
15 May 2017

*Glycan microarrays for studying protein carbohydrate interactions*  
S. Serna  
1st Glycobasque Meeting, CIC bioGUNE Bilbao (Spain)  
15 May 2017

*Synthesis of N- and O-glycan mimetics for CLR targeting*  
A. Cioce & J. Pham  
1st Glycobasque Meeting, CIC bioGUNE Bilbao (Spain)  
15 May 2017

*Parasite Glycans as Lead Structures for Dendritic Cell Targeting (Invited)*  
A. Cioce, J. Pham, A. Bernardi, B. Lepenies, K. Brzezicka, S. Serna, B. Echevarría, N.C. Reichardt  
Molecular aspects of host/microbe dialogue, ITN TOLLerant, Naples (Italy)  
5-7 June 2017

*Synthesis and Biomedical Applications of Parasite glycans (Invited)*  
K. Brzezicka, S. Serna, A. Diepen, C. Hokke, B. Lepenies, N.C. Reichardt  
XXXVI Reunión Bienal de la RSEQ, Sitges (Spain)  
25-28 June 2017

*Novel strategies for the synthesis of glycomimetic libraries, rapid MALDI based method for testing antibiotic resistance, core xylose and fucose*  
N.C. Reichardt  
19th European Carbohydrate Symposium – Eurocarb 19, Barcelona (Spain)  
2-6 July 2017

*On-chip development of N-glycan mimetics for improving CLR targeting*  
A. Cioce, A. Hernández, S. Serna, G. Goti, A. Bernardi, N. C. Reichardt  
19th European Carbohydrate Symposium – Eurocarb 19, Barcelona (Spain)  
2-6 July 2017
**Glycotechnology | Annual Report**

---

**Posters at Conferences**

*Functionalized CVD Graphene as an efficient MALDI-MS MATRIX for detection of carbohydrates*
Juan Pedro Merino, Sonia Serna, Alejandro Criado, Alba Centeno, Amaia Zurutuza, Niels-Christian Reichardt, Maurizio Prato
XXXVI Reunión Bienal de la RSEQ, Sitges (Spain)
25-28 June 2017

*Preparation of glycan multivalent systems by corona formation for immunomodulation and as candidate vaccines*
Bárbara Kuhn, Begoña Echeverría, Álvaro Hernández, Niels-Christian Reichardt
19th European Carbohydrate Symposium - Eurocarb, Barcelona (Spain)
2-6 July 2017

*Synthesis of S. mansoni inspired O-glycans and mimetics for improved CLR targeting*
Julie Pham, Álvaro Hernández, Niels-Christian Reichardt
19th European Carbohydrate Symposium - Eurocarb, Barcelona (Spain)
2-6 July 2017

*Neoglycoprotein protected gold nanoclusters as fluorescent sensing probes*
Katarzyna Brzezicka, Sonia Serna, Niels-Christian Reichardt
19th European Carbohydrate Symposium - Eurocarb, Barcelona (Spain)
2-6 July 2017

*Synthesis of a Glycomimetic Library for microarray based Screening with C-type Lectin Receptors*
Laura Medve, Sonia Serna, Niels Reichardt, Silvia Achilli, Franck Fieschi, Anna Bernardi
19th European Carbohydrate Symposium - Eurocarb, Barcelona (Spain)
2-6 July 2017

*Artificial Tetrameric Lectins, TetraLEC, as a tool for multivalency enhancement*
Silvia Achilli, Corinne Vives, Michel Thépaut, Laura Medve, Sonia Serna, Niels Reichardt, Anna Bernardi, Franck Fieschi
19th European Carbohydrate Symposium - Eurocarb, Barcelona (Spain)
2-6 July 2017

*Fluoroacetamide Moieties as NMR Spectroscopy Probes for the Molecular Recognition of GlcNAc-Containing Sugars: Modulation of the CH–π Stacking Interactions by Different Fluorination Patterns*
Luca Unione, María Alcalá, Begoña Echeverría, Sonia Serna, Ana Ardá, Antonio Franconetti, F. Javier Cañada, Tammo Diercks, Niels Reichardt, Jesús Jiménez-Barbero
19th European Carbohydrate Symposium - Eurocarb, Barcelona (Spain)
2-6 July 2017
1st Euskadi Workshop on Exosomes
CIC bioGUNE Bilbao (Spain)
23 March 2017
Niels Reichardt & Raquel Pazos participated in the workshop together with Juan Manuel Falcón & Charles Williams

1st Glycobasque Meeting
CIC bioGUNE Bilbao (Spain)
15 May 2017
Inspiring and productive meeting with a day-long program of research talks and a joint lunch of Jimenez-Barbero, Reichardt, Guerin, Fernández-Tejada and Anguita/Prados groups with special guest Manuel Martín-Lomas
Nerea Guedes Carrera

*Solid-Phase Synthesis of Glycosaminoglycans*

Director: Niels-Christian Reichardt
University of Basque Country (UPV), San Sebastian / CIC biomaGUNE
15 September 2017

---

Ioanna Kalograiaki

*Glycosylation Patterns and Recognition by Lectins*

Director: Dolores Solis
UCM (Universidad Complutense de Madrid)
5 June 2017

---

N.C. Reichardt:
- Agencia Andaluza del Conocimiento: *NanoMedPhD Programme 2017*
- *ANEP - Agencia Nacional de Evaluación y Prospectiva*
- *FRNS - National Fund for Scientific Research, Belgium*
- *Horizon 2020*

---

N.C. Reichardt:
- Since 2017: Member of editorial board *Carbohydrate Research (Elsevier)*
- Niels Reichardt was one of the Most Outstanding Referees for *Angewandte Chemie* in 2017
Anna Cioce

*Cell uptake assays using dendritic cells and T cell activation assays with neoglycoproteins*

Host Institution: Immunology Unit, Research Center for Emerging Infections and Zoonoses (RIZ), University of Veterinary Medicine Hannover (Germany)

January 2017

Julie Pham

*Collaboration with the groups of Marcelo Guerin and Ramon Hurtado for the study of the crystallography structure of several enzymes*

Host Institution: CIC bioGUNE

February / March 2017

Antonio Di Maio

*Develop of a new coupling strategy on microarrays using Strain-Promoted Azide-Alkyne Cycloaddition (SPAAC) reaction in order to generate tools to investigate Lectin-Carbohydrate interactions*

Home Institution: IIQ-CSIC Seville (Spain)

March / May 2017

Silvia Achilli

*Mannose-based glycan glycomimetic array with non commercial human TetraLEC. Dendrimer array with canonical CLRs*

Home Institution: Institut de Biologie Structurale, IBS – Université Grenoble Alpes (France)

March 2017
Activities

Andreas Seifert
*Photonic methods in medical diagnostics*
Professor of Nanoengineering – CIC nanoGUNE, San Sebastian (Spain)
16 February 2017

Luis D. Carlos
*Luminiscent nanothermometers: What’s next?*
Departamento de Física and CICECO – Aveiro Institute of Materials, Universidade de Aveiro (Portugal)
24 February 2017

Pedro Merino
*Chemical Challenges in Glycosyltransferase Modulation*
Instituto de Biocomputación y Física de Sistemas Complejos – Universidad de Zaragoza (Spain)
9 June 2017

Marcelo Guerín
*Membrane enzymes: Working at the water-lipid interface*
Ikerbasque – CIC bioGUNE, Bilbao (Spain)
30 June 2017

Antony Fairbanks
*ENGase catalysed production of bioactive glycopeptides and glycoproteins*
Department of Chemistry, University of Canterbury, Christchurch (New Zealand)
18 July 2017

Arkaitz Carracedo
*Fuel and oil for the engine of prostate cancer: Metabolic basis for tumor progression*
CIC bioGUNE, Bilbao (Spain)
27 July 2017

Alberto Fernández-Tejada
*Chemical synthesis and immunological studies for the identification of improved saponin vaccine adjuvants*
CIC bioGUNE, Bilbao (Spain)
25 October 2017
Anna Cioce and Julie Pham (IMMUNOSHAPE fellows) showed to Portuguese high school students from the Agrupamento de Escolas de Penacova, Coimbra, the microarray technology and the use of MALDI for the chemoenzymatic synthesis of sugars.
3 March 2017
Our multidisciplinary research is only possible in collaboration with a growing number of international recognized researchers. Many current collaborations are managed within the IMMUNOSHAPE project that runs until 2018.

- **Prof. Bernd Lepenies**, Infection Immunology. University of Veterinary Medicine Hannover. Research Center for Emerging Infections and Zoonoses (RIZ), Germany
- **Anna Bernardi**, Chemistry Department. Università degli Studi di Milano, Italy
- **Sabine Flitsch**, Manchester Institute of Biotechnology (MIB). The University of Manchester, UK

Other current collaborations include:

- **Dr. Ron Hokke**, Parasite Glycobiology Group. Department of Parasitology. University Medical Center Leiden, Netherlands
- **Prof. Jesús Jiménez-Barbero**, Chemical Glycobiology Lab. CIC bioGUNE, Bilbao, Spain
- **Dr. Jesús Angulo**, School of Pharmacy. University of East Anglia, Norwich, UK
- **Prof. Juan Falcón**, Exosomes Lab. CIC bioGUNE, Bilbao, Spain
- **Dr. Alberto de Leiva**, Department of Medicine and Director of Department of Endocrinology and Nutrition. Hospital San Pau, Barcelona, Spain
- **Dr. Emilio Pérez-Trallero**, University Hospital Donostia, Spain
- **Dr. Dolores Gonzales**, Department of Biochemistry and Molecular Pharmacology. Instituto de Parasitología y Biomedicina-Lopez Neyra, Granada, Spain
- **Dr. Héctor Peinado**, Microenvironment and Metastasis Group. CNIO, Madrid, Spain
- **Dr. Katie Doores**, Infectious Diseases. Faculty of Life Sciences and Medicine. Kings College London, UK
- **Prof. Manfred Wuhrer**, Center for Proteomics and Metabolomics. Leiden University Medical Center, Netherlands
- **Prof. Maurizio Prato**, Carbon Bionanotechnology Group at CIC bioGUNE and Università degli Studi di Trieste, Italy
Spanish MINECO

**GLYCOIMMUNOTECH**
Grant No. CTQ2014-58779-R

*Tools to study and exploit the role of glycans in adaptive and innate immunity*

2015-2018

---

**CIBER-BBN**

**Glyco Thyroid Cancer**

*Intramural project*

2015-2017

---

**European Commission**

**IMMUNOSHAPE** *(Coordination)*

H2020-MSCA-ITN-2014-ETN-642870

*Development of Selective Carbohydrate Immunomodulators Targeting C-type Lectin Receptors on Antigen Presenting Cells*

2015-2018

[www.immunoshape.eu](http://www.immunoshape.eu)
Glycotechnology Lab
CIC biomaGUNE
Paseo de Miramón 182
20014 San Sebastian (Spain)
Tel. +34 943 00 53 09
www.glycotechnology.net