

Glycotechnology

CICbiomaGUNE

ANNUAL
REPORT



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

2017 GROUP MEMBERS



Dr. Niels-Christian Reichardt
Principal Investigator



Dr. Manuel Martín Lomas
CIC biomaGUNE Founding Director



Dr. Sonia Serna
Postdoctoral Researcher



Dr. Nerea Ruiz
Postdoctoral Researcher



Raquel Pazos
Research Technician



Elisabete Elosegui
Research Technician



María Alcalá
Research Technician
(substitution until Nov. 2017)



Begoña Echeverría
Research Technician



Julie Pham
IMMUNOSHAPE
PhD Student



Anna Cioce
IMMUNOSHAPE
PhD Student



Bárbara Kuhn
PhD Student



Charles Williams
Joint PhD Student
with CIC bioGUNE

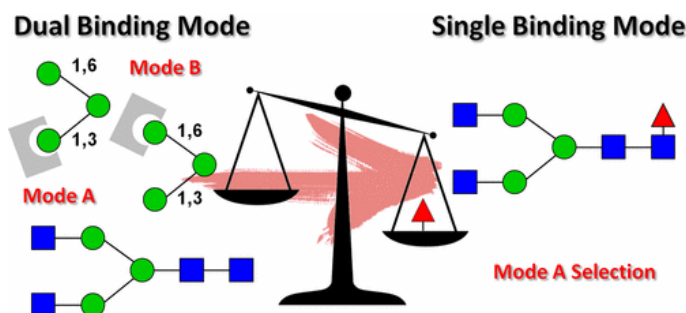


Dr. Cristina Díez
IMMUNOSHAPE
Project Manager

NMR and Molecular Recognition of N-Glycans: Remote Modifications of the Saccharide Chain Modulate Binding Features

Ana Gimeno, Niels-Christian Reichardt, F. Javier Cañada, Lukas Perkams, Carlo Unverzagt, Jesús Jiménez-Barbero, Ana Ardá

ACS Chem. Biol. 2017, 12 (4), pp 1104–1112 | DOI: 10.1021/acscchembio.6b01116



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 Corcema-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, María Alcalá, Begoña Echeverría, Sonia Serna, Ana Ardá, Antonio Franconetti, F. Javier Cañada, Tammo Diercks, Niels Reichardt, Jesús Jiménez-Barbero

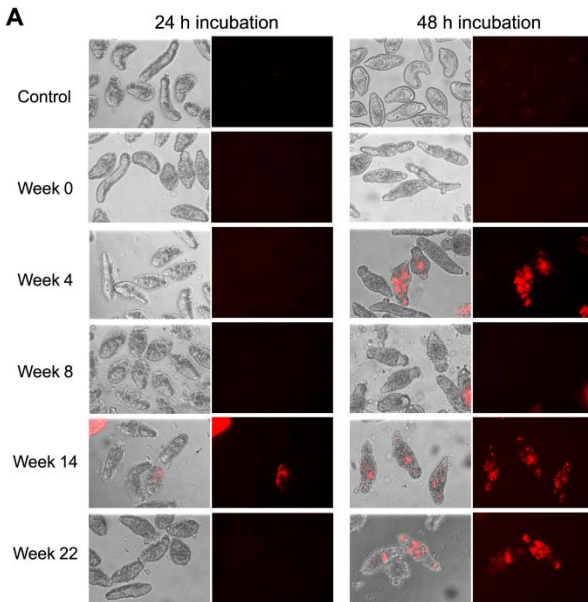
Chem. Eur. J. 2017, 23 (16), 3957–3965 | DOI: 10.1002/chem.201605573

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 ^{19}F 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 $\text{CHF}_2\text{CONH-}$ moiety is the stronger aromatic binder, followed by the analogue with the $\text{CH}_2\text{FCONH-}$ group and the natural molecule (with the $\text{CH}_3\text{CONH-}$ fragment). In contrast, the molecule with the $\text{CF}_3\text{CONH-}$ isoster displayed the weakest intermolecular interaction (one order of magnitude weaker). Because sugar–aromatic $\text{CH-}\pi$ 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.

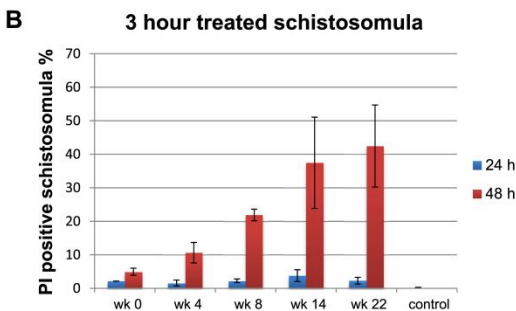
Specific anti-glycan antibodies are sustained during and after parasite clearance in Schistosoma japonicum-infected rhesus macaques

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.

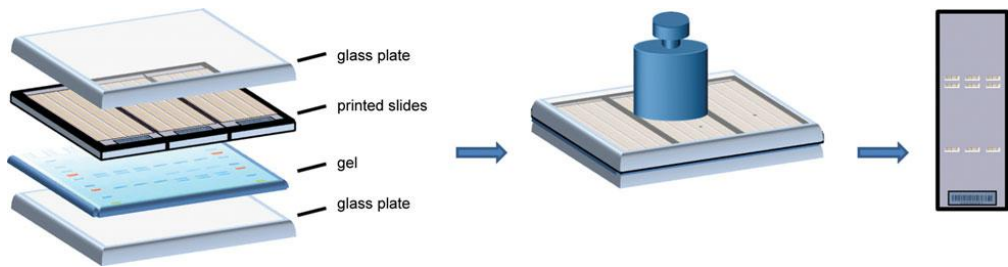


Identification of dominant anti-glycan IgE responses in school-children by glycan microarray

Abena S. Amoah, Elias K. Asuming-Brempong, Benedicta B. Obeng, Serge A. Versteeg, Irene A. Larbi, Yvonne Aryeetey, Thomas A.E. Platts-Mills, Adriano Mari, Katarzyna Brzezicka, Ben A. Gyan, Mohamed Mutocheluh, Daniel A. Boakye, Niels-Christian Reichardt, Ronald van Ree, Cornelis H. Hokke, Angela van Diepen, Maria Yazdanbakhsh
J. Allergy Clin. Immunol. 2017 | In press, DOI: 10.1016/j.jaci.2017.09.040

Lectin-Array Blotting

R. Pazos, J. Echevarria, A. Hernández, N.C. Reichardt

Current Protocols in Cell Biology 2017, 76, 6.12.1–6.12.12. | DOI: 10.1002/cpcb.20

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

Cell Cycle 2017 Nov 3:1-8 | DOI: 10.1080/15384101.2017.1387694

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 Bial 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 CLRs 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

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

Workshops



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



PhD Theses

Nerea Guedes Carrera

Solid-Phase Synthesis of Glycosaminoglycans

Director: Niels-Christian Reichardt

University of Basque Country (UPV), San Sebastian / CIC biomaGUNE

15 September 2017



Thesis Jury

Ioanna Kalograiaki

Glycosylation Patterns and Recognition by Lectins

Director: Dolores Solis

UCM (Universidad Complutense de Madrid)

5 June 2017

Evaluator

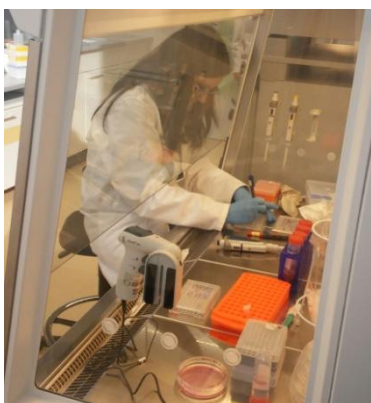
N.C. Reichardt:

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

Editorial Activity / Reviewer

N.C. Reichardt:

- Since 2017: Member of editorial board *Carbohydrate Research (Elsevier)*
- Peer reviewer for *Nature, Angewandte Chemie, JACS, Anal Chem, Carb Res., Chemistry, Accounts of Chemical Research.*
- 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

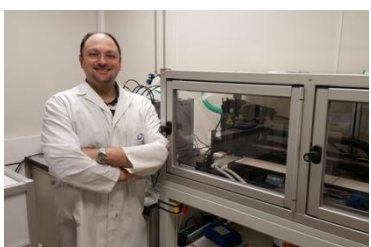


Julie Pham

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

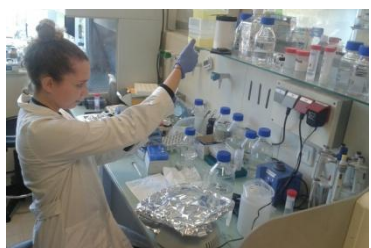


Antonio Di Maio

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*Luminescent 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

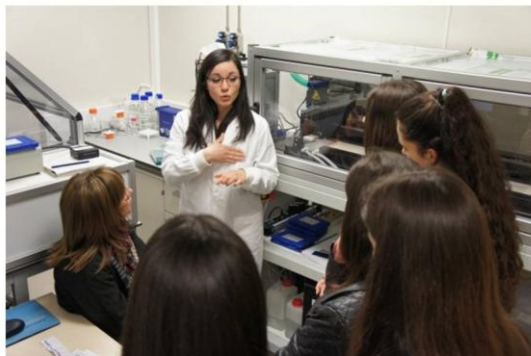
Alberto Fernández-Tejada*Chemical synthesis and immunological studies for the identification of improved saponin vaccine adjuvants*

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](#), Departament 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](#), Departament 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 biomaGUNE and Università degli Studi di Trieste, Italy

National



Spanish MINECO

GLYCOIMMUNOTECH

Grant No. CTQ2014-58779-R

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

2015-2018



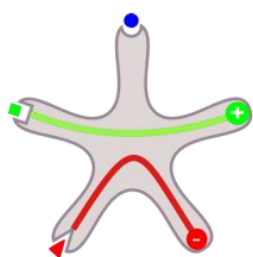
CIBER-BBN

Glyco Thyroid Cancer

Intramural project

2015-2017

European



IMMUNOSHAPe

European Commission

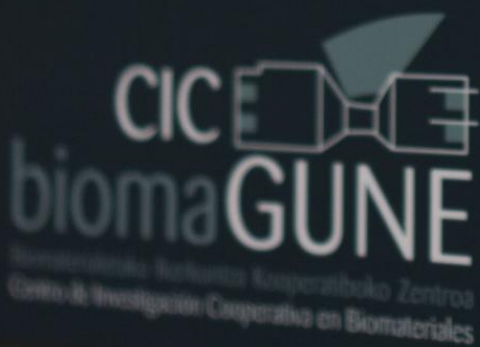
IMMUNOSHAPe (Coordination)

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



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