Call for 14 Early Stage Researcher PhD fellowships in

"A multidisciplinary training network for the bioinspired development of glycomimetics tuning the Siglec-Sialoglycan axis"

Introduction

GLYTUNES is an Innovative Training Network (ITN) funded by the European Union Horizon 2020 Programme. GLYTUNES represents a novel, pioneering platform for understanding and exploiting the Siglecs-glycans cross-talk by harnessing the synergy of combining chemical to biological, biophysical, immunological research strategies and finally translating this knowledge into novel diagnostics and therapeutics. The GLYTUNES training network involves leading scientists from academia and industry and offers a total of **14 doctoral research projects (PhD projects)** for early stage researchers (ESRs). The research topics range from fundamental to translational science and aim at i) unravelling the mechanisms governing sialo-glycans recognition by the immune surface receptors Siglecs, and ii) enabling the rational design and development of novel therapeutic and diagnostic strategies against immune and infectious diseases. Experts in chemical and structural biology, drug design and computational chemistry, synthetic and medicinal chemistry, biophysics, biochemistry, immunology and molecular and medical microbiology will provide the ESRs with a multidisciplinary and interdisciplinary training. Successful applicants will receive training in **carbohydrate chemistry, glycoimmunology, tumor immunology, microbiology, drug design and development, project management, scientific communication, funding programmes and grant writings, entrepreneurship, ethics and intellectual property.** GLYTUNES will expose ESRs to a unique combination of "hands-on" research training, non-academic placements, courses, and workshops on scientific and complementary skills necessary for their future thriving careers in academia, clinical research or industry and to become the new leaders in the expanding research area of **glycosciences.**

Research projects

The research activities implemented in GLYTUNES have the following objectives:

- **To gain structural, functional, immunological and mechanistic insights into the mechanisms governing sialoglycan recognition by Siglecs.**
- **Host glycocodes: to understand the molecular, immunological and dynamic features of Siglecs-glycans crosstalk.**
- **Sialylated Microbial Glycans: to understand the structure, function and conformation of sialylated envelope, and their interaction with cognate Siglecs.**
- **To perform the rational design, development and optimization of mono- and multivalent glycomimetics active against the Siglecs-sialic acid axis.**

The 14 Early Stage Researchers' (ESRs) projects are listed in the following table.

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ESR	TITLE OF THE PROJECT	HOST INSTITUTION	SHORT NAME	SUPERVISOR	EXPECTED START DATE
1	Full characterization of bacterial sialylated envelope components	University of Naples Federico II (Naples, Italy)	UNINA	Alba Silipo	1st October, 2021
2	The role of Siglecs in the association between Fusobacterium nucleatum strains and colon cancer	Quadram Institute Bioscience (Norwich, UK)	QIB	Nathalie Juge	1st October, 2021
3	Deciphering the structural details of the recognition mode of cancer-associated glycoproteins by Siglec receptors implicated in immune suppression in cancer	Center for Cooperative Research in Biosciences (Derio, Spain)	CIC-bioGUNE	June Ereño-Orbea	1st September, 2021
4	Studying the immunological pathways of the Sialic acid-Siglec axis including the Siglec/TLR cross-talk	VU University Medical Center (Amsterdam, The Netherlands)	VUMC	Yvette van Kooyk Fabrizio Chiodo	1st September, 2021
5	Biophysical characterization of Siglecs and Siglec-sialoglycans complexes	University of Florence Magnetic Resonance Center (Florence, Italy)	UNIFI-CERM	Marco Fragai	1st November, 2021
6	High resolution structural analysis of Siglec oligomers by cryo-EM	Centre National De La Recherche Scientifique (Grenoble, France)	CNRS	Irina Gutsche	1st September, 2021
7	Development and optimization of novel active glycomimetics	Radboud University (Amsterdam, The Netherlands)	RAD	Thomas Boltje	1st September, 2021
8	NMR methods for the characterisation of protein-ligand interactions using 19F-compounds	ATLAS Molecular Pharma (Bilbao, Spain)	ATLAS	Oscar Millet	1st September, 2021
9	Chemoenzymatic synthesis of novel ligands for the detection or inhibition of Siglecs	ICENI Diagnostics (Norwich, UK)	ICENI	Rob Field	1st July, 2021
10	Synthesis of Advanced sialylated glycans libraries and their variant	GlycoUniverse (Potsdam, Germany)	GCU	Kim Le Mai Hoang	1st September, 2021
11	Expression and biophysical characterization of Siglec proteins	Giotto Biotech (Sesto Fiorentino, Italy)	GIOTTO	Tommaso Martelli	1st September, 2021
12	Physico-chemical analysis of Siglecs-Glycan complexes	University of Naples Federico II (Naples, Italy)	UNINA	Alba Silipo	1st October, 2021
13	Looking at different sialylation in cancer tissues using Siglecs (and tissues sialic acids) as biomarkers	VU University Medical Center (Amsterdam, The Netherlands)	VUMC	Yvette van Kooyk Fabrizio Chiodo	1st September, 2021
14	Sialic acid biosynthesis inhibitors to target the sialic acid - Siglec axis	Radboud University (Amsterdam, The Netherlands)	RAD	Thomas Boltje	1st September, 2021

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Training Programme

All the selected students will be involved in a highly stimulating training programme, both at the **local and at the network-wide level.**

The training programme comprises:

1) **The implementation of the individual research project at the host institution. The research project will involve collaborations with other GLYTUNES institutions, to be implemented through secondments.**
2) **Each researcher will be involved in local training sessions.**
3) **Joint scientific courses and meetings will be organised by the GLYTUNES consortium, together with short courses for transferable skills training.**
5) **Enrolment in PhD programmes of the following universities**:

ESR	HOST INSTITUTION	UNIVERSITY RELEASING THE PhD
1	UNINA	University of Naples Federico II (Naples, Italy)
2	QIB	University of East Anglia (Norwich, UK)
3	CIC-bioGUNE	Universidad del País Vasco (Leioa, Spain)
4	VUMC	VU University Medical Center (Amsterdam, The Netherlands)
5	UNIFI-CERM	University of Florence (Florence, Italy)
6	CNRS	University Grenoble-Alps (Grenoble, France)
7	RAD	Radboud University (Nijmegen, The Netherlands)
8	ATLAS	Universidad del País Vasco (Leioa, Spain)
9	ICENI	University of Manchester (Manchester, UK)
10	GCU	Freie Universität Berlin (Berlin, Germany)
11	GIOTTO	University of Florence (Florence, Italy)
12	UNINA	University of Naples Federico II (Naples, Italy)
13	VUMC	VU University Medical Center (Amsterdam, The Netherlands)
14	RAD	Radboud University (Nijmegen, The Netherlands)

Recruitment

The ESRs will be contractually employed for **36 months** by the recruiting organisation and will be covered under the related national social security scheme. ESRs will receive a Monthly Living Allowance plus a Mobility Allowance (where applicable) compliant with the applicable EC Marie Skłodowska - Curie Actions - ITN (https://ec.europa.eu/research/participants/data/ref/h2020/wp/2018-2020/main/h2020-wp1820-mscaen.pdf page 89 and 92).

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Eligibility Rules

At the time of recruitment applicants must fulfil the following rules:

Experience:

- Applicants must be in possession of the degree (usually the Master Degree) which would formally entitle them to embark on a doctorate, either in the country in which the degree was obtained or in the country in which the researcher will be recruited. In case the degree has not been obtained yet, it is necessary to send a declaration of the university stating that the degree will be obtained before the expected starting date.
- Applicants must be in the first 4 years of their research careers (full-time equivalent research experience) at the signature of the contract (measured from the time the Master's degree has been obtained).
- Eligible applicants must not hold a Doctoral degree already.

Mobility:

- The applicants must not have resided or carried out their main activity (work, studies, etc.) in the country where the research training activities will take place for more than 12 months in the 3 years immediately prior to the recruitment date.
- Exceptions International Organisations: Eligible researchers must not have spent more than 12 months in the 3 years immediately prior to the date of selection in the same appointing international organisation.

How to apply

GLYTUNES will select ESR through a 2-step recruitment process.

Candidates should submit their application for their top two preferred research projects.
Application documents should be sent by email to the relevant project supervisors (see emails indicated in the individual project descriptions below).

Applications (in English) should indicate the preferred research project(s) and should include:

1) **an updated CV; the CV must be without gaps, in order to easily check the mobility and experience requirements. CVs that either do not clearly show the applicant's past experience, or have gaps, will be considered ineligible;**
2) **a letter giving reason for his/her motivation for the position;**
3) **at least 1 reference letter (in English) from one former supervisor and/or lecturer;**
4) **the scan of the degree (usually the Master Degree) which would formally entitle him/her to embark on a doctorate, either in the country in which the degree was obtained or in the country in which the researcher will be recruited. In case the degree has not been obtained yet, it is necessary to send a declaration of the university stating that the degree will be obtained before the expected starting date;**
5) **transcripts of records (document indicating their ranking and marks within their last year at their Master Degree as well as the courses/modules they have followed).**

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Applications must be in English and will be evaluated against the following criteria:
- educational record;
- scientific quality of the applicant's CV;
- expected individual impact and benefit to the fellow and to the project;
- previous experience in the subject of GLYTUNES research programme.

The closing date for applications is 30/04/2021.

Candidates will be evaluated by the recruiting institution on the basis of the received documents.

The best **4 candidates** for each position will be invited for a Skype (or face to face) interview (in the period **15-31 May 2021**). Interviews will be held by the recruiting institution and at least one other member of the respective ESR Board, preferably from the industry.

For each position a short list of top candidates will be prepared and notified to the applicants.
The top candidates will be asked to provide a written acceptance of the studentship; then the following ranked candidates will be requested to confirm. If a successful candidate declines the offer, the studentship will be offered to the next ranked candidate.

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ESR1 - Full characterization of bacterial sialylated envelope components	
Host Institution	**University of Naples Federico II (Naples, Italy) - UNINA**
Primary Supervisor	**Alba Silipo**
Email address	**silipo@unina.it**
Planned duration	**36 months**
Subject Area	**Structural and chemical biology, glycomics and glycosciences**

Introduction: Pathogens exploitation of inhibitory Siglecs. Pathogens can shield their envelope glycans with *self*-ligands, with the aim of dampening host immune responses. Feared human pathogens such as membrane-enveloped viruses and bacteria such as GBS, *C. jejuni, H. influenzae, P. aeruginosa, N. meningitides* and *gonhorreae, F. nucleatus*, are coated with sialic acid containing ligands that can be potentially recognized by inhibitory Siglecs, this allowing to escape host immune responses by averting detection and immune elimination, promoting successful bacterial colonization and tolerance.

Aims: *Science:* The project aims to assess the structure and function of cell envelope components isolated from bacterial sialylated envelope glycans. ii) determine conformation and 3D structure of isolated microbial glycans. *Training:* Expertise in glycomics, microbial glycans structural and chemical biology, organic chemistry, carbohydrate chemistry and biochemistry, analytical chemistry, isolation, purification and characterization of microbial glycans (LPS, EPS, CPS....), NMR spectroscopy and MS spectrometry, computational methods for building 3D complex carbohydrate structures; stage(s) in the industry.

Expected Results: Formation of the ESR with a solid knowledge in glycomics, glycosciences, structural biology of glycoconjugates. Definition of the structure to function relationships of bacterial cell envelope compounds. Establishment of the molecular mimicry of host glycans from different microbial strains. Stage in the industry; PhD thesis.

Planned secondment(s): 3-month secondments at: 1) QIB: approaches to identify Siglecs receptors recognized by different isolated microbial glycans; 2) VUMC: immunological approaches toward the understanding of immunopotential of isolated envelope components 3) ICENI: developments of multivalent glycomimetics based on the structure of isolated microbial glycans.

Enrolment in Doctoral degree(s): ESR1 will be enrolled at University of Naples Federico II.

Project-specific selection criteria: Good skills in glycomics and glycosciences, carbohydrate chemistry and biochemistry, organic chemistry, glycans structure and biology; good knowledge of techniques for glycans structural biology, as isolation, purification methodologies, NMR spectroscopic, biophysical, spectrometric techniques; microbial glycomics.

Recommended reading:

- Chemical synthesis of glycans up to a 128-mer relevant to the O-antigen of Bacteroides vulgatus Zhu Q et al., *Nat Commun.* 2020;11(1):4142. doi: 10.1038/s41467-020-17992-x
- Pairing Bacteroides vulgatus LPS Structure with Its Immunomodulatory Effects on Human Cellular Models. Di Lorenzo F et al., *ACS Cent Sci.* 2020; 6(9):1602-1616. doi: 10.1021/acscentsci.0c00791.
- Solving the structural puzzle of bacterial glycome, Marchetti R et al., *Current Opinion in Structural Biology*, Volume 68, 2021, Pages 74-83. doi: 10.1016/j.sbi.2020.12.003
- The Lipid A from Rhodopseudomonas palustris Strain BisA53 LPS Possesses a Unique Structure and Low Immunostimulant Properties. Di Lorenzo F et al., *Chem. Eur. J.* 2017, Volume 23, Issue 15, 3637–3647. doi: 10.1002/chem.201604379
- Covalently linked hopanoid-lipid A improves outer-membrane resistance of a Bradyrhizobium symbiont of legumes. Silipo A et al., *Nat Commun.* 2014; 5:5106. doi: 10.1038/ncomms6106

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ESR2 - The role of Siglecs in the association between Fusobacterium nucleatum strains and colon cancer	
Host Institution	**Quadram Institute Bioscience (Norwich, UK) - QIB**
Primary Supervisor	**Nathalie Juge**
Email address	**nathalie.juge@quadram.ac.uk**
Planned duration	**36 months**
Subject Area	**Microbiology, glycobiology, cell biology, immunology**

Introduction: Colorectal cancer (CRC) is one of the most frequently diagnosed malignancies worldwide and accounts for approximately 20% of all cancer-related deaths in developed countries. CRC has been associated with an enrichment of microbes in intestinal tissues with the most abundant species being *Fusobacterium nucleatum. F. nucleatum* potentiates intestinal tumorigenesis through immune suppression but the mechanisms underpinning its interaction with immune cells is unknown. We recently showed that *F. nucleatum* interact with Siglecs (sialic acid-binding immunoglobulin-like lectins), opening a new dimension in our understanding of how *F. nucleatum* promotes CRC progression and in the development of therapeutic strategies targeting Siglec axis.

Aims: *Science:* The project aims to 1) unravel the nature of the *Fusobacterium nucleatum* ligands of Siglecs and 2) their role in modulating host immune response and 3) test compounds derived from WP4 as potential glycomimetics inhibitors of the interaction between *F. nucleatum* and immune cells. *Training* will be provided in molecular microbiology (anaerobic bacterial growth assays), cell biology (mammalian cells and gut-on-chips) and advanced bioimaging techniques (fluorescence and confocal microscopy; imaging flow cytometry), protein purification and protein-carbohydrate interactions (Octet, SPR, ITC). Training will include transferable skills including communication (to scientific and lay audiences), intellectual property impact of the research on human health and outreach activities.

Expected Results: The ESR will gain a solid knowledge in molecular microbiology, cell biology including gut-on-chip technology, biophysical and bioimaging techniques. Scientific outputs include the identification of *F. nucleatum* components interacting with Siglecs and new glycomimetics as inhibitors of *F. nucleatum* interaction with host Siglecs and host immune response; experience in industry; PhD thesis.

Planned secondment(s): 3-month secondments at: 1) GIOTTO: methodologies of Siglecs purification and characterization; 2) RAD: development of glycoinhibitors targeting *Fusobacterium* strains 3) VUMC: use of relevant cancer models on glycomimetics against *Fusobacterium.*

Enrolment in Doctoral degree(s): ESR2 will be enrolled at University of East Anglia.

Project-specific selection criteria: Expertise in microbiology including anaerobic bacteria, knowledge on host immune response, expertise in cell biology and bioimaging, expertise in protein purification and protein-carbohydrate interactions.

Recommended reading:

- Structure of the O-Antigen and the Lipid A from the Lipopolysaccharide of *Fusobacterium nucleatum* ATCC 51191. Garcia-Vello P et al., *Chembiochem.* 2020. doi: 10.1002/cbic.202000751
- *Fusobacterium nucleatum* and colorectal cancer: A review. Shang FM et al., *World J Gastrointest Oncol.* 2018; 10(3):71-81. doi: 10.4251/wjgo.v10.i3.71

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ESR3 - Deciphering the structural details of the recognition mode of cancer-associated glycoproteins by Siglec receptors implicated in immune suppression in cancer	
Host Institution	**Center for Cooperative Research in Biosciences (Derio, Spain) - CICbioGUNE**
Primary Supervisor	**June Ereño-Orbea**
Email address	**jereno@cicbiogune.es**
Planned duration	**36 months**
Subject Area	**Glycobiology, structural biology, biophysics**

Introduction: Siglecs expressed on innate immune cells and tumor-infiltrating T cells, have emerged as alternative immune checkpoint inhibitors. This family of receptors recognize sialic acid (Sia) containing glycans on the surface of cancer cells as self-associated molecular patterns. However, the molecular basis of Siglec binding to its ligands continues to be a puzzle. In this research project we plan to study the glycan binding specificity of Siglec receptors by a range of structural techniques including x-ray crystallography and NMR, and biophysical techniques (e.g. ITC and SPR). Understanding Siglec-sialic acid interactions from an atomic perspective will help us in better understanding their role in cancer development.

Aims: *Science:* 1) high-resolution analysis of the 3D structure of Siglec receptors at atomic resolution using X-ray crystallography. 2) molecular details of the interaction of the Siglecs with host glycans and cancer-associated glycoproteins using structural (X-ray crystallography, NMR, small-angle X-ray scattering (SAXS)) and biophysical (ITC, BLI, SPR) techniques.
Training: Expression and purification of Siglecs and cancer-associated glycoproteins using human and bacterial cell lines. Crystallization of proteins and protein-glycan complexes, X-ray data collection and processing; computational methods; biophysical techniques (ITC, BLI, SPR).

Expected Results: Training of the ESR with a solid knowledge in structural biology and computational methods. Production and 3D structural details of Siglecs; production of cancer-associated glycoproteins; affinity determination and structural details of Siglecs binding to sialylated glycans and cancer-associated glycoproteins.

Planned secondment(s): 3-month secondments at: 1) GCU: synthesis of sialylated glycans for recognition and binding studies; 2) UNINA: structural biology/characterization of glycans; 3) VUMC: explore biochemical approaches (ELISA or WB) to correlate the structural data with preliminary functional assays.

Enrolment in Doctoral degree(s): ESR3 will be enrolled at Universidad del País Vasco.

Project-specific selection criteria: Experience in recombinant protein expression and purification. Good knowledge of techniques in structural biology (i.e. X-ray crystallography, SAXS, NMR) and biophysical techniques (i.e ITC and BLI).

Recommended reading:

- Molecular basis of human CD22 function and therapeutic targeting. Ereño-Orbea J et al., *Nat Commun.* 2017; 8(1):764. doi: 10.1038/s41467-017-00836-6
- Characterization of glycoproteins with the immunoglobulin fold by X-ray crystallography and biophysical techniques. Ereño-Orbea J et al., *J Vis Exp*. 2018; (137):57750. doi: 10.3791/57750
- Structural characterization of N-linked glycans in the receptor binding domain of the SARS-CoV-2 spike protein and their interactions with human lectins. Lenza MP et al., *J. Angew Chem Int Ed Engl.* 2020; 59(52):23763-23771. doi: 10.1002/anie.202011015
- Current status on therapeutic molecules targeting Siglec receptors. Lenza MP et al., *Cells.* 2020; 9(12):E2691. doi: 10.3390/cells9122691

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ESR4 - Studying the immunological pathways of the Sialic acid-Siglec axis including the Siglec/TLR cross-talk	
Host Institution	**VU University Medical Center (Amsterdam, The Netherlands) - VUMC**
Primary Supervisor	**Yvette van Kooyk / Fabrizio Chiodo**
Email address	**Y.vankooyk@amsterdamumc.nl and f.chiodo@amsterdamumc.nl**
Planned duration	**48 months**
Subject Area	**Immunology, chemical biology**

Introduction: Antigen presenting cells such as Dendritic cells are crucial in sensing pathogens resulting in specific T cell responses that correlate with the elimination of that pathogen.This can be achieved by innate receptors like glycan binding receptors CLRs and Siglecs, and TLRs that provide together an integrated signaling pattern that alter surface-markers, cytokine production and T cell responses. Unravelling these interactions is crucial for the proper activation of immunity.

Aims: *Science:* The immunological interactions between the sialylated exogenous and endogenous conjugates prepared by ESR1, ESR10 and ESR7, will be studied on human antigen-presenting cells (or injected in mice) (ESR4). Monocytes-derived dendritic cells, macrophages, and antigenpresenting cells isolated from human skin-biopsies will be stimulated with the sialylated compounds. The analysis of released cytokines, co-stimulatory surface-markers, immunological pathways and the intracellular routing of the sialylated compounds will be studied by flow cytometry, confocal microscopy and ELISA. The outcome of the dendritic cells/T-cells interaction will be also studied characterizing the type of T-cells generated (Th1, Th2 or Th17). In addition, the sialylated conjugates will be incubated with cells also in co-stimulation with Toll-like receptor (TLR) ligands like LPS, Pam3CSK4 etc., to study the cross-talk between Siglecs and TLRs. *Training*: The ESR will be trained on biochemistry, cellular immunology, flow cytometry, animal facilities and confocal microscopy. Also, the ESR will learn how to analyze big data with significant relevance. The ESR will learn how to write scientific publications, reports, posters and she/he will learn to present data at any type of audience (group meetings, department meetings conferences, press releases, etc.).

Expected Results: Formation of the ESR with a solid knowledge in cell biology and immunology; Science related: At the end of this project we will have a full understating on the immune-modulatory properties of sialylated conjugates. Some of them will be used to actively modulate DC, or to change the Th1/Th2 balance, important for different diseases (cancer, autoimmunity etc.). We will be able to have a full set of new immune modulators. A successful PhD thesis is expected from the ESR as well as the dissemination of the scientific works produced during the PhD training.

Planned secondment(s): 1) UNINA (3 months): to isolate and purify microbial sialylated glycans 2) ATLAS (4 months): to explore ATLAS cell screening assay to finally have in hands a selected library of sialylated compounds.

Enrolment in Doctoral degree(s): ESR4 will be enrolled at University Medical Center.

Project-specific selection criteria: Good skills in immunology, culturing, T cell responses, flowcytometry, microscopy, affinity with glycomics and glycosciences, interest in working in the field of cellular biology and chemistry.

Recommended reading:

- Targeting of the C-Type Lectin Receptor Langerin Using Bifunctional Mannosylated Antigens. Li RE et al., *Front Cell Dev Biol.* 2020; 8:556. doi: 10.3389/fcell.2020.00556
- Modulation of Immune Tolerance via Siglec-Sialic Acid Interactions. Lübbers J et al., *Front Immunol.* 2018; 9:2807. doi: 10.3389/fimmu.2018.02807

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ESR5 - Biophysical characterization of Siglecs and Siglec- sialoglycans complexes	
Host Institution	**University of Florence - Magnetic Resonance Center (Florence, Italy) - UNIFI-CERM**
Primary Supervisor	**Marco Fragai**
Email address	**fragai@cerm.unifi.it**
Planned duration	**36 months**
Subject Area	**NMR spectroscopy and structural biology**

Introduction: Solution NMR is a powerful tool for structural studies and an indispensable enabling technology for determining weak and transient macromolecule interactions as well as for characterizing functional processes. Recently, solid-state NMR is emerging as an important technique to obtain information on the structure and dynamics of protein complexes that, due to solubility and size limitations, cannot be achieved by solution NMR or other methods. The integration of NMR spectroscopy with other biophysical methodologies and computational methods is thus clearly the strategy of choice to investigate the structural determinants of Siglec-sialoglycans interaction.

Aims: *Science:* 1) NMR assignment of selected Siglecs in solution and at the solid-state NMR 2) Analysis of the Siglec-sialoglycans interactions to determine the binding specificity and affinity. 3) Determination of the key protein residues and functional surfaces driving the assembling of the Siglecsialoglycans complexes by NMR spectroscopy and using biophysical methods and site-directed mutagenesis data. 4) Characterization of the interaction between Siglecs and microbial envelope components isolated by ultrafast magic angle spinning (MAS) solid-state NMR spectroscopy. 5) Calculation of docking models to predict the structure of Siglec-sialoglycans complexes through the use of experimental NMR contacts and site-directed mutagenesis. *Training*: Expertise in i) preparation of biological sample for solid-state NMR spectroscopy; ii) NMR spectroscopy and methodologies for structural biology; iii) protocols to integrate NMR and site-directed mutagenesis data in docking calculation; iv) thermodynamic characterization of the binding between the selected Siglecs and sialoglycans. Secondments will provide a perspective of possible synergies that can be exploited by the ESR for his/her future career.

Expected Results: Formation of the ESR with a solid knowledge in NMR-based and integrative structural biology of proteins and glycoproteins, molecular modeling and docking calculations. NMR assignment of Siglec proteins. Structural models of Siglec-sialoglycans complexes. Identification of the structural determinants of binding selectivity in Siglec-glycans complexes; Posters and publications on siglecs in relation to the their interaction with sialoglycans; Ph.D. thesis.

Planned secondment(s): 1) IBS, (4 months): Cryo-EM sample preparation and analysis and to relate the NMR analysis to Cryo-EM characterization; 2) ICENI, (4 months): glycans conjugation to nanoparticles/quantum dots in order to develop new strategies for the evaluation of protein-carbohydrates interactions based on solid-state NMR. 3) QIB, (3 months): recombinant expression and purification of Siglecs from CHO cell lines.

Enrolment in Doctoral degree(s): ESR5 will be enrolled at University of Florence.

Project-specific selection criteria: Candidates must have a master degree in any of the relevant disciplines: structural biology, chemistry, medicinal chemistry, physics and bio-physics. Good skill/experience in NMR spectroscopy will be considered an asset.

Recommended reading:

- Characterization of PEGylated Asparaginase: New Opportunities from NMR Analysis of Large PEGylated Therapeutics. Cerofolini L et al., *Chemistry.* 2019; 25(8):1984-1991. doi: 10.1002/chem.201804488
- Characterization of the Conjugation Pattern in Large Polysaccharide-Protein Conjugates by NMR Spectroscopy. Giuntini S et al., *Angew Chem Int Ed Engl.* 2017; 56(47):14997-15001. doi: 10.1002/anie.201709274
- Structural characterization of a protein adsorbed on aluminum hydroxide adjuvant in vaccine formulation. Cerofolini L et al., *J Vaccines.* 2019; 4:20. doi: 10.1038/s41541-019-0115-7
- Fucosylated ubiquitin and orthogonally glycosylated mutant A28C: conceptually new ligands for Burkholderia ambifaria lectin (BambL). Kuhaudomlarp S et al., *Chemical Science*, Pub Date : 2020-10-21. doi: 10.1039/d0sc03741a

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ESR6 - High resolution structural analysis of Siglec oligomers by cryo-EM	
Host Institution	**Centre National De La Recherche Scientifique (Grenoble, France) - CNRS**
Primary Supervisor	**Irina Gutsche**
Email address	**irina.gutsche@ibs.fr**
Planned duration	**36 months**
Subject Area	**Structural biology, high resolution cryo-electron microscopy**

Introduction: Siglecs are transmembrane receptors expressed on surfaces of innate immune cells. They recognise sialic acids used by vertebrates as signatures of "self" but also by some microbial pathogens that thereby modulate host immune responses. Aberrant interactions between Siglecs and their ligands lead to a variety of pathologies including infection, autoimmune diseases and cancer. Understanding the structural basic of Siglec-ligand binding is therefore a crucial step towards the rational design and development of novel therapeutic and diagnostic strategies against numerous diseases.

Aims: *Science:* The project aims to: i) Optimize biochemical conditions for obtaining dimers of the extracellular domain (ECD) of selected Siglecs, homogeneous both in terms of glycosylation and oligomeric state (secondment CIC-bioGUNE). ii) Solve the structures of the best ECD dimers by single particle cryo-EM. iii) Attempt to produce transmembrane or full-length constructs in lipid nanodiscs or liposomes (secondment GIOTTO), with the goal to solve structures of native membrane-bound states by cryo-EM or cryo-ET. iv) Analyse interaction of different soluble and membrane constructs with ligands (secondment UNIFI), define the most suitable targets for high resolution structural studies and solve the resulting structures by cryo-EM. *Training*: Purification and biochemical characterization of ECDs and transmembrane Siglecs; experimental cryo-EM and, if appropriate, cryo-ET; advanced cryo-EM 3D image processing, atomic model building and structure analysis.

Expected Results: Atomic structures of selected ECD dimers alone and in complex with a ligand. If feasible, first insights into the assembly of transmembrane Siglec constructs into model membranes and the effect of ligand binding. Structural analysis and definition of the molecular basis for the Siglec dimerisation, ligand binding and membrane insertion for therapeutic targeting. Secondment in industry.

Planned secondment(s): 3-month secondments at: 1) CIC-bioGUNE: optimisation of biochemical preparation of ECD constructs homogeneous in terms of glycosylation and oligomeric state. 2) GIOTTO: optimization of the expression and purification of transmembrane Siglecs. 3) UNIFI: spectroscopic and biophysical analysis of the interactions of the corresponding Siglec constructs with ligands.

Enrolment in Doctoral degree(s): ESR6 will be enrolled at University Grenoble-Alps.

Project-specific selection criteria: Experience in recombinant protein expression, purification and biochemical characterization techniques. Previous experience in electron microscopy would be an asset. No computational experience is required but interest in computing and image analysis is essential.

Recommended reading:

- Cryo-EM as a powerful tool for drug discovery. Van Drie J et al., *Bioorg Med Chem Lett.* 2020; 30(22):127524. doi: 10.1016/j.bmcl.2020.127524
- How cryo-EM is revolutionizing structural biology. Bai XC et al., *Trends Biochem Sci.* 2015; 40(1):49-57. doi: 10.1016/j.tibs.2014.10.005
- A primer to single-particle cryo-electron microscopy. Cheng Y et al., *Cell.* 2015;161(3):438-449. doi: 10.1016/j.cell.2015.03.050
- Cryo-electron microscopy and the amazing race to atomic resolution. Binshtein E et al., *Biochemistry.* 2015;54(20):3133-41. doi: 10.1021/acs.biochem.5b00114

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ESR7 - Development and optimization of novel active glycomimetics	
Host Institution	**Radboud University (Amsterdam, The Netherlands) - RAD**
Primary Supervisor	**Thomas Boltje**
Email address	**t.boltje@ru.nl**
Planned duration	**48 months**
Subject Area	**Organic (carbohydrate) chemistry, medicinal chemistry, chemical biology**

Introduction: Siglec receptors bind sialoglycans, glycans that contain sialic acid residues. The recognition of sialic acid residues by Siglecs depends on their structure, linkage type and the underlying glycan scaffold. Typically, Siglecs binding leads to immune suppression and represents an important mechanism to modulate immune system activity. However, various pathologies are associated with an imbalance in Siglec immune suppression and hence Siglec receptors are also promising therapeutic targets.

Aims: *Science:* The aim of this project is to develop new glycomimetics based on sialic acid to inhibit Siglec binding. To this end, new sialic acid glycomimetics will be designed, synthesized and tested in cellular assays. Finally, the developed inhibitors will be used in collaboration with project partners to evaluate the functional consequences of Siglec receptor inhibition. *Training*: Expertise in the synthesis, purification and characterization of sialic acid mimetics by means of organic chemistry, MS spectrometry and NMR spectroscopy. Basic cell biology skills such as cell culture and cellular assays. Secondments will provide a perspective of possible synergies that can be exploited by the ESR for his/her future career.

Expected Results: Formation of the ESR with a solid knowledge in chemical synthesis and chemical biology of sialic acid mimetics. Definition of the structure to function relationships of newly synthesized sialic acid mimetics for Siglec receptors. In collaboration with the ITN partners, functional use of the developed sialic acid mimetics in an immunological setting.

Planned secondment(s): 3-month secondments at: 1) QIB: immunological evaluation of novel glycomimetics; 2) UNINA: evaluation of glycomimetics binding via NMR 3) CICbioGUNE: X-ray crystallography, small-angle X-ray scattering (SAXS) of protein-glycomimetic complexes.

Enrolment in Doctoral degree(s): ESR7 will be enrolled at Radboud University.

Project-specific selection criteria: Good knowledge/skills/experience in: organic (carbohydrate) chemistry, medicinal chemistry, chemical biology, basic cell biology, *in silico* molecular docking.

Recommended reading:

- Sialic Acid Mimetics to Target the Sialic Acid-Siglec Axis. Büll C et al., *Trends Biochem Sci.* 2016; 41(6):519-531. doi: 10.1016/j.tibs.2016.03.007
- Steering Siglec-Sialic Acid Interactions on Living Cells using Bioorthogonal Chemistry. Büll C et al., *Angew Chem Int Ed Engl.* 2017; 56(12):3309-3313. doi: 10.1002/anie.201612193
- Sialic Acid Glycoengineering Using an Unnatural Sialic Acid for the Detection of Sialoglycan Biosynthesis Defects and On-Cell Synthesis of Siglec Ligands. Büll C et al., *ACS Chem Biol.* 2015; 10(10):2353-63. doi: 10.1021/acschembio.5b00501
- Siglecs as Immune Cell Checkpoints in Disease. Duan S et al., *Annu Rev Immunol.* 2020; 38:365-395. doi: 10.1146/annurev-immunol-102419-035900

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ESR8 - NMR methods for the characterisation of protein-ligand interactions using 19F-compounds	
Host Institution	**ATLAS Molecular Pharma (Bilbao, Spain) - ATLAS**
Primary Supervisor	**Oscar Millet**
Email address	**omillet@cicbiogune.es**
Planned duration	**36 months**
Subject Area	**High resolution NMR spectroscopy; drug discovery; protein-ligand interaction; molecular dynamics simulations; biophysics; pharmacology, pharmacological chaperones, protein isotope labeling.**

Introduction: In the fields of medicine, biotechnology and pharmacology, drug discovery is the process by which new candidate medications are discovered. We employ NMR spectroscopy, a very versatile and powerful technique to cover most of the stages in the process of drug discovery. We develop methodology and apply it to relevant biomedical problems, namely congenital metabolic disorders.

Aims: *Science:* The project aims to develop biophysical methods for the characterization of chemical libraries and their interactions with the target protein: i) To obtain an NMR based method to screen libraries of compounds that selectively associate to a predefined binding site. This will be applied to fluorinated glycomimetics that may compete with the natural ligands. ii) To provide innovative tools for the characterization of chemical libraries using NMR spectroscopy. *Training*: To train the ESRs in novel 19F-NMR methods for the characterization of protein-ligand interactions.

Expected Results: NMR methodology to the characterization of receptor-drug interactions at the thermodynamic and structural level, using NMR spectroscopy and other biophysical techniques. Application to several relevant systems associated to metabolic diseases. Specifically, New pulse sequences and/or methods for NMR-based drug discovery exploiting the potential and orthogonality of the 19F isotope. An integral training in NMR spectroscopy and the associated techniques, related to drug discovery.

Planned secondment(s): 1) GCU (3 months): to train on the synthesis and purification of complex glycan libraries; VUMC (4 months): to explore basic cellular experiments based on flow-cytometry to find a functional readout that will correlate with the structural data.

Enrolment in Doctoral degree(s): ESR8 will be enrolled at Universidad del País Vasco.

Project-specific selection criteria: Good skills/experience in NMR spectroscopy of structural biology in general; biophysics and enzymology; protein expression and purification; and/or computational skills applied to bioinformatics.

Recommended reading:

- Repurposing ciclopirox as a pharmacological chaperone in a model of congenital erythropoietic porphyria. Urquiza P et al., *Sci Transl Med.* 2018; 10(459):eaat7467. doi: 10.1126/scitranslmed.aat7467
- Metabolic landscape of the mouse liver by quantitative 31 P-NMR analysis of the phosphorome. Bernardo-Seisdedos G et al., *Hepatology.* 2020. doi: 10.1002/hep.31676

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ESR9 - Chemoenzymatic synthesis of novel ligands for the detection or inhibition of Siglecs	
Host Institution	**ICENI Diagnostics (Norwich, UK) - ICENI**
Primary Supervisor	**Rob Field**
Email address	**info@icenidiagnostics.com**
Planned duration	**36 months**
Subject Area	**Organic chemistry, carbohydrate chemistry, protein chemistry, molecular diagnostics, assay development, lateral flow assay**

Introduction: Sialic acid-Siglec interactions are keys for a wide variety of biological processes, from infection to immune response, with sialic acid being a crucial carbohydrate component of the extracellular matrix and responsible of critical contact point with the Siglec counterpart. The nature of the interaction is ruled by a high selectivity and dictated by the type of sialic acid, in terms of its linkage or modification of the core structure. Exploring new routes to access Sialic acid derivatives, both natural and un-natural, can open new avenues in the development of diagnostic tools and therapeutics.

Aims: *Science:* The project aims to develop a library of carbohydrate derivatives, based on known Siglec ligands but with enhanced affinity and improved discrimination between Siglec isoforms. This will be achieved through a combination of (i) chemical and enzymatic synthesis, with molecules set up for (ii) conjugation to nanoparticles/quantum dots or proteins to (iii) support biological evaluation through microscopic imaging, ELISA or lateral flow assays. The above will be integrated with (iv) experimental and computational evaluation of ligand structure and dynamics, in solution and complex with Siglec targets. *Training*: Expertise in carbohydrate-based click chemistry, enzymatic synthesis, purification and characterization of carbohydrate derivatives, including by mass spectrometry and NMR spectroscopy. Secondments will provide academic and biological context for chemistry-based industry, to ensure full integration with the GLYTUNES program and to support broader chemical biology education in line with career development.

Expected Results: Definition of new Siglec ligands, with enhanced affinity and selectivity, for use in fundamental cell biology, as well as diagnostic or therapeutic applications. A well-trained ESR, equipped with skills and knowledge in carbohydrate and glycoconjugation chemistry, allied to the detection of carbohydrate-binding proteins.

Planned secondment(s): 1) VUMC (4 months): immunological techniques and approaches to evaluate the novel compounds 2) UNIFI (4 months): spectroscopic and biophysical approached to study the novel ligands interacting with Siglecs.

Enrolment in Doctoral degree(s): ESR9 will be enrolled at University of Manchester.

Project-specific selection criteria: Experience in organic chemistry, ability to design and perform multistep synthesis process, hands-on experience of common purification and analytical techniques (flash chromatography, NMR, ESI-MS), experience in carbohydrate chemistry, enzymatic process or assay development would be a distinct advantage.

Recommended reading:

- Chemoenzymatic Synthesis of Fluorinated Cellodextrins Identifies a New Allomorph for Cellulose-Like Materials. de Andrade P et al., *Chemistry.* 2020. doi: 10.1002/chem.202003604
- Preparative and Kinetic Analysis of ß-1,4- and ß-1,3-Glucan Phosphorylases Informs Access to Human Milk Oligosaccharide Fragments and Analogues Thereof. Singh RP et al., *Chembiochem*. 2020;21(7):1043-1049. doi: 10.1002/cbic.201900440

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ESR10 - Synthesis of Advanced sialylated glycans libraries and their variant	
Host Institution	**GlycoUniverse (Potsdam, Germany) - GCU**
Primary Supervisor	**Kim Le Mai Hoang**
Email address	**kim.lemaihoang@glycouniverse.de**
Planned duration	**36 months**
Subject Area	**Glycomics and glycosciences, synthetic and analytical carbohydrate chemistry, enzymatic synthesis, automated solid phase synthesis**

Introduction: An understanding the molecular basis of carbohydrate-protein interactions can be gained by identifying the molecular features essential for molecular recognition. To this end, molecular editing through synthesizing a library of carbohydrates that present a variety of molecular features can help to understand exactly how that recognition works. In addition, synthetic chemistry can be used to introduce molecular labels that can act as a beacon to indicate when and where recognition takes place and to study the kinetics and thermodynamics of the interaction.

Aims: *Science:* The project aims to synthesize well-defined complex glycans to be used to assess their interactions with Siglecs proteins. Suitable monomeric building blocks will be assembled, making use of in-house expertise on identifying optimal protective group schemes and anomeric leaving groups. The glycans will be assembled by means of automated oligosaccharide synthesis using the automated synthesis platforms as well as chemoenzymatic methodologies to assemble sialic acids and analogues. Glycans of interest having various types of labels for biophysical studies (NMR, X-ray crystallography, Cryo-EM) will be prepared. Compounds representing specific portions of selected LPS/microbial glycans will be synthetized, to fully appreciate the molecular features essential for recognition and binding. *Training*: Synthetic organic chemistry; enzymatic chemistry; purification methodologies; automated glycan synthesis; carbohydrate synthesis.

Expected Results: To synthesize focused glycan libraries, advanced sialylated glycans libraries, labelled or unlabeled, will be developed by a combination of organic and chemo-enzymatic approaches, the design of which will be guided in part by studies/epitopes identified by WP2 members; a well-trained ESR, equipped with skills and knowledge in PhD thesis.

Planned secondment(s): 1) CICbioGUNE (4 months): interaction studies with complex glycans libraries; 2) VUMC (4 months): studies of effects of glycans libraries on the host.

Enrolment in Doctoral degree(s): ESR10 will be enrolled at Freie Universität Berlin.

Project-specific selection criteria: Good skills/experience in synthetic organic and preferably carbohydrate chemistry, purification and analysis techniques; some experience with enzymatic organic chemistry as well as solid-phase synthesis and automation is preferable.

Recommended reading:

- Automated Glycan Assembly: A Perspective. Guberman M et al., *J Am Chem Soc.* 2019; 141(14):5581-5592. doi: 10.1021/jacs.9b00638
- Siglecs: A journey through the evolution of sialic acid-binding immunoglobulin-type lectins. Bornhöfft KF et al., *Dev Comp Immunol.* 2018; 86:219-231. doi: 10.1016/j.dci.2018.05.008

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ESR11 - Expression and biophysical characterization of Siglec proteins	
Host Institution	**Giotto Biotech (Sesto Fiorentino, Italy) - GIOTTO**
Primary Supervisor	**Tommaso Martelli**
Email address	**martelli@giottobiotech.com**
Planned duration	**36 months**
Subject Area	**Molecular Biology, biochemistry, biology, chemistry, strucutral biology**

Introduction: The definition of a good protocol for the expression and sample preparation for the selected Siglecs is mandatory for their study and analysis with in-vitro assays and also for their structural and interaction studies. Deep studies will be also focused on the definition of protocols for the expression of isotopically enriched samples (with 2H/13C/15N) for solution and solid-state NMR.

Aims: *Science:* Optimization of the expression and purification of the selected Siglecs for biophysical studies and for x-ray and cryo-EM structural characterization. Expression and purification of isotopically enriched samples (2H/13C/15N) of Siglec proteins for NMR studies. Biophysical characterization of Siglecs. *Training*: expertise in molecular biology and in the expression and purification of isotopically enriched proteins in *E.coli*; in biophysical techniques (FPLC, HPLC, CD, MALS-QELS).

Expected Results: Formation of the ESR with a solid knowledge in molecular biology and in the expression, purification and biophysical characterization of recombinant proteins in *E.coli* expression system hhigh yield production of isotopically enriched Siglecs-2, 9, -10 and -15 and of mutants for NMR and Cryo-EM studies; Posters and publications on siglecs in relation to the their interaction with sialoglycans; Ph.D. thesis; stage in industry.

Planned secondment(s): 1) CICbioGUNE (6 months): to learn about expression and purification of proteins and glycoproteins using human cell lines; 2) QIB (4 months): to learn about methodologies (surface plasmon resonance and mass spectrometry) to assess protein-glycans interaction and heterologous expression approaches.

Enrolment in Doctoral degree(s): ESR11 will be enrolled at University of Florence.

Project-specific selection criteria: Candidates must have a master degree in any of the relevant disciplines: molecular biology, biology, biochemistry, structural biology, chemistry and medicinal chemistry. Good skill/experience in protein expression from prokaryotic and eukaryotic systems and protein purification and characterization will be considered an asset.

Recommended reading:

- Algal autolysate medium to label proteins for NMR in mammalian cells. Fuccio C et al., *J Biomol NMR.* 2016; 64(4):275-80. doi: 10.1007/s10858-016-0026-0
- (1)H-detected solid-state NMR of proteins entrapped in bioinspired silica: a new tool for biomaterials characterization. Ravera E et al., *Sci Rep.* 2016; 6:27851. doi: 10.1038/srep27851
- In-cell NMR reveals potential precursor of toxic species from SOD1 fALS mutants. Luchinat E et al., *Nat Commun.* 2014; 5:5502. doi: 10.1038/ncomms6502

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ESR12 - Physico-chemical analysis of Siglecs-Glycan complexes	
Host Institution	**University of Naples Federico II (Naples, Italy) - UNINA**
Primary Supervisor	**Alba Silipo**
Email address	**silipo@unina.it**
Planned duration	**36 months**
Subject Area	**Structural and chemical biology, glycomics and glycosciences**

Introduction: Sialoglycans cross-talk/interaction with host immune Siglecs, description of 3D complexes. Molecular basis of recognition and binding of Siglecs to natural endogenous and exogenous substrates through ortogonal approaches (NMR spectroscopy, computational and biophysical techniques), with the aims to: get a detailed molecular picture of the Siglecs-glycans complexes, describe both protein and glycans key portions critical in the recognition and binding events; analyse the energetics and thermodynamics of the molecular interactions; fast screen possible binders with respect to a specific target receptor.

Aims: *Science:* Study of molecular recognition events in the Siglecs-sialoglycan interaction, comprehension of the binding properties and specificity of Siglecs receptors upon sialoglycans binding; analysis of sialoglycans and proteins region involved in the recognition and binding evente description of the and analysis of the binding regions by NMR spectroscopy, computational approaches and biophysical approaches. Analysis of 3D structures of Siglecs in complex with microbial sialoglycans; the newly synthesized ligands will be analyzed in their interaction with target Siglecs and the ligands with improved binding affinity will be further developed. *Training*: in structural biology, NMR spectroscopy to define protein-ligand complexes, biophysical and computational techniques and their applications, with extensive impact in both pharmaceutical industry and academia environments applied to the structural characterization of the complexes of antimicrobials with bacterial envelope targets.

Expected Results: Learning how the conformation and presentation of epitopes is achieved by using NMR and other biophysical techniques. Learning how this presentation regulates the molecular recognition issues. The integrated approach based on NMR, docking and molecular modeling will profile the ligands' epitope in their bound conformations and to provide consistent 3D-models of the interaction. With microbial ligands identification of key molecular actors of interactions between microbial ligands and Siglecs.

Planned secondment(s): 3-month secondments at: 1) UNIFI: ultrafast HR-MAS NMR applied to complex systems. 2) RAD: to learn how to further develop ligands with improved binding affinity 2) ICENI: without perturbing their binding properties, selected glycomimetics by adding a lipid chain suitable for the multivalent presentation.

Enrolment in Doctoral degree(s): ESR12 will be enrolled at University of Naples Federico II.

Project-specific selection criteria: Good skills/experience in glycomics and glycosciences, glycans structure and conformation; structural biology (NMR spectroscopy); spectroscopic, biophysical, computational, spectrometric techniques for the characterization of 3D complexes, carbohydrate biochemistry, study of protein-ligand interaction via NMR spectroscopy, molecular modelling and biophysical techniques.

Recommended reading:

- Behaviour of glycolylated sialoglycans in the binding pockets of murine and human CD22. Di Carluccio C et al., *iScience* 2021, Volume 24, Issue 1, 101998 https://doi.org/10.1016/j.isci.2020.101998
- Unveiling molecular recognition of sialoglycans by human Siglec-10. Forgione RE et al., *iScience*, 2020, 23 (6), 101231. oi: 10.1016/j.isci.2020.101231
- The Core Fucose on an IgG Antibody is an Endogenous Ligand of Dectin-1. Manabe Y et al., *Angew Chem Int Ed Engl.* 2019; 58(51):18697-18702. doi: 10.1002/anie.201911875
- Characterization of the dynamic interactions between complex N-glycans and human CD22. Di Carluccio C et al., *Chembiochem* 2020, 21, 129 – 140. doi: 10.1002/cbic.201900295
- Burkholderia pseudomallei Capsular Polysaccharide Recognition by a Monoclonal Antibody Reveals Key Details toward a Biodefense Vaccine and Diagnostics against Melioidosis. Marchetti R et al., *ACS Chem Biol.* 2015; 10(10):2295-302. doi: 10.1021/acschembio.5b00502

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ESR13 - Looking at different sialylation in cancer tissues using Siglecs (and tissues sialic acids) as biomarkers	
Host Institution	**University Medical Center (Amsterdam, The Netherlands) - VUMC**
Primary Supervisor	**Yvette van Kooyk / Fabrizio Chiodo**
Email address	**Y.vankooyk@amsterdamumc.nl and f.chiodo@amsterdamumc.nl**
Planned duration	**48 months**
Subject Area	**Cancer immunology, glycosciences**

Introduction: Tumor sialylation has great impact on tumor growth and immune defence. By altering sialylation tumors silence many different immune cells which as a consequence show deficient attack of the tumor. The molecular interplay between various Siglec receptors expressed on immune cells that sense sialic acids expression in the tumor environments needs to be unraveled. Moreover therapeutic strategies aimed to alter sialylation in the tumor microenvironment need to be tested, to restore immune activation and killing of the tumor.

Aims: *Science:* This ESR will be involved with the functional aspects developed by the colleagues in ESR 1-3-9-10 and 11. Aberrant (or modified) sialylation has been described in the cancer microenvironment as well as the expression (up or down-regulation) of different Siglecs. The ESR will use the Siglecs studied and expressed in WP2 to stain different cancer tissues trying to develop a glyco-cancer code (based on sialic acids) able to differentiate tumors at a different stage (or resistant to therapy). In addition, to study and visualize the sialic acids in the tumor microenvironment, the ESR will also track and study the location and expression of different Siglecs (cancer tissues) using the chemical tools developed in ESR 9-10. *Training*: The ESR will learn to explore different techniques to visualize glycans and glycans receptors. Confocal microscopy, mass cytometry, ELISA and tissues staining will give the ESR the basics to develop glyco-diagnostic tools and to study glyco-biomarkers.

Expected Results: PhD thesis the ESR will develop unique and unreported tools for diagnostics in cancer using glycans and glycans-receptors

Planned secondment(s): 3-month secondments at: 1) CICbioGUNE: to study the expression of different Siglecs and glycoproteins. 2) GCU: to design and pre-screen glycomimetics targeting the Siglec-axis.

Enrolment in Doctoral degree(s): ESR13 will be enrolled at VU University Medical Center.

Project-specific selection criteria: Cancer biology and immunology, mouse model systems, immunotherapy, immunohistochemistry, flowcytometry, cell culturing, molecular biology.

Recommended reading:

- Modulation of Immune Tolerance via Siglec-Sialic Acid Interactions. Lübbers J et al., *Front Immunol.* 2018; 9:2807. doi: 10.3389/fimmu.2018.02807
- Sialic acid-modified antigens impose tolerance via inhibition of T-cell proliferation and de novo induction of regulatory T cells. Perdicchio M et al., *Proc Natl Acad Sci U S A.* 2016; 113(12):3329-34. doi: 10.1073/pnas.1507706113
- Tumor sialylation impedes T cell mediated anti-tumor responses while promoting tumor associated-regulatory T cells. Perdicchio M et al., *Oncotarget.* 2016;7(8):8771-82. doi: 10.18632/oncotarget.6822

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ESR14 - Sialic acid biosynthesis inhibitors to target the sialic acid - Siglec axis	
Host Institution	**Radboud University (Amsterdam, The Netherlands) - RAD**
Primary Supervisor	**Thomas Boltje**
Email address	**t.boltje@ru.nl**
Planned duration	**48 months**
Subject Area	**Organic (carbohydrate) chemistry, medicinal chemistry, chemical biology**

Introduction: Siglec receptors bind sialoglycans, glycans that contain sialic acid residues. The recognition of sialic acid residues by Siglecs depends on their structure, linkage type and the underlying glycan scaffold. Typically, Siglecs binding leads to immune suppression and represents an important mechanism to modulate immune system activity. However, various pathologies are associated with an imbalance in Siglec immune suppression and hence Siglec receptors are also promising therapeutic targets.

Aims: *Science:* The project aims to: i) Identify new sialic acid biosynthesis inhibitors to abrogate Siglec binding and signaling. To this end, new sialic acid glycomimetics will be designed, synthesized and tested for activity in cellular assays. Active inhibitors will be used in functional assays in collaboration with various project partners. *Training*: Expertise in the synthesis, purification and characterization of sialic acid mimetics by means of organic chemistry, MS spectrometry and NMR spectroscopy. Basic cell biology skills such as cell culture and cellular assays. Secondments will provide a perspective of possible synergies that can be exploited by the ESR for his/her future career.

Expected Results: Formation of the ESR with a solid knowledge in chemical synthesis and chemical biology of sialic acid mimetics. Definition of the structure to function relationships of newly synthesized sialic acid mimetics for Siglec receptors. In collaboration with the ITN partners, functional use of the developed sialic acid mimetics in an immunological setting.

Planned secondment(s): 3-month secondments at: 1) QIB: evaluation of the activity of newly synthetized inhibitors on microbial cells; 3) VUMC: as synthetic tools to interfere with the immunomodulatory events triggered in the TME as new therapeutic agents 2) GIOTTO: NMR analysis of novel biosynthesis inhibitors.

Enrolment in Doctoral degree(s): ESR14 will be enrolled at Radboud University.

Project-specific selection criteria: Good knowledge/skills/experience in: organic (carbohydrate) chemistry, medicinal chemistry, chemical biology, basic cell biology, in silico molecular docking.

Recommended reading:

- Sialic Acid Mimetics to Target the Sialic Acid-Siglec Axis. Büll C et al., *Trends Biochem Sci.* 2016; 41(6):519-531. doi: 10.1016/j.tibs.2016.03.007
- Siglecs as Immune Cell Checkpoints in Disease. Duan S et al., *Annu Rev Immunol.* 2020; 38:365-395. doi: 10.1146/annurev-immunol-102419-035900
- Selective Inhibition of Sialic Acid-Based Molecular Mimicry in Haemophilus influenzae Abrogates Serum Resistance. Heise T et al., J.Cell Chem Biol. 2018; 25(10):1279-1285.e8. doi: 10.1016/j.chembiol.2018.05.018.
- Potent Metabolic Sialylation Inhibitors Based on C-5-Modified Fluorinated Sialic Acids. Heise T et al., *J Med Chem.* 2019; 62(2):1014-1021. doi: 10.1021/acs.jmedchem.8b01757

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