Project Proposal
(*Instructions: this proposal will appear on the PhD webpage https://cqb.dieti.unina.it/ and will be forwarded to specialized sites. Please give a synthetic background and two to four research objectives. Include some information about the expected background of the candidate and relevant references. Please fill the part regarding the funding source/grants of the research activity needed for equipment, reagents, travels, conferences, traineeships etc.*)

Decoding the structural details of the recognition mode of sialoglycans by Siglec receptors implicated in infection and cancer

Project Description (*max 500 words*)

Background The research project is located in the field of structural and computational biology.

Siglecs[1] (sialic acid–binding immunoglobulin-like lectins) are proteins on the front line of the immune defense, increasingly recognized as promising therapeutic target and alternative immune checkpoint inhibitors. Most Siglecs display inhibitory signaling properties, acting as negative regulator of the immune system after sensing sialic acid-containing glycans and inducing tolerance to self-antigens.

Aberrant sialic acid–Siglec interactions are associated with an increasing number of pathologies including infection and cancer[2]. Immune cells expressing Siglecs are inhibited upon binding to their ligands on cancer cells. Siglecs recognize highly abundant sialylated glycans on the surface of cancer cells as self-associated molecular patterns, thereby inhibits immunosurveillance and mediate immune evasion of tumor cells. Siglecs are also targeted by human pathogens such as membrane-enveloped viruses and bacteria to escape host immune responses, promoting successful bacterial colonization and tolerance. In this frame, there is a growing interest in the potential role inflammation and bacterial infections have on the initiation and progression of cancer. Colorectal Cancer (CRC), one of the most frequently diagnosed malignancies worldwide, is characterized by gut microbiota dysbiosis, and an increase in cancer-associated bacteria such the most abundant species *Fusobacterium nucleatum*. *F. nucleatum* is a Gram negative obligate oral anaerobe, identified as one of the pathobionts that outgrow during dysbiosis and important actor of CRC etiology[3]. Indeed *F. nucleatum e*volved the ability for displaying Siglecs ligands on the cell surface, apparently to evade immunosurveillance via molecular mimicry, promoting CRC progression through the generation of a pro-inflammatory environment[4].

***Research objectives*:** The molecular basis of Siglec binding to its (endogenous and exogenous) ligands continue to be a puzzle. Understanding Siglec-sialic acid interactions from an atomic perspective will help us in better understanding their role in the etiology of immune-related diseases, as cancer development and infections, and will be of great value toward the development of diagnostic/therapeutic tools.

In this research project the aims are unravelling structural, functional mechanisms governing glycoconjugates and glycoprotein recognition and binding specificity by Siglec receptors. To this aim, an integral and multidisciplinary approach will be used, including solution and solid-state NMR methodologies, emerging computational methods, integrated with biochemical, biophysical techniques. Specific aims: - Expression and purification of Siglecs and glycoproteins using human and bacterial cell lines. -High-resolution analysis of the 3D structure of Siglec receptors at atomic resolution. - Molecular details and affinity determination of Siglecs binding to host glycans, cancer-associated glycoproteins and bacterial glycans using structural (NMR, small-angle X-ray scattering (SAXS)) and biophysical (ITC, BLI, SPR) techniques.

References:

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Expected background of the candidate Good knowledge of techniques in structural biology (NMR spectroscopy), computational biology (MD simulation, docking….), and biophysical techniques (i.e ITC and BLI). Experience in recombinant protein expression and purification. Knowledge in glycomics and glycosciences is highly appreciated.

Supervisor(s), Lab/Group details, other additional info. (*please provide information about the group and available facilities*)

Prof. Alba Silipo, full professor of organic chemistry
Department of Chemical Sciences, University of Naples Federico II.
Expertise: NMR spectroscopy; Structural biology; glycosciences; structure to function relationships; High-resolution analysis of the 3D structure of protein-ligand(glycans) systems by means of NMR techniques, computational studies, docking and biophysical approaches.

Available facilities: fully equipped organic and biological laboratories; Laboratory for protein expression and purification (in prokaryotic and eukaryotic systems); ÄKTA™ protein purification system; NMR instruments: 600 MHz BRUKER AVANCE NEO equipped with cryoprobe; 500 MHz; 400 MHz AvanceIII Bruker BBFO probe; 400 MHz Wide Bore with CP-MAS; AMBER16 and AMBER20 LINUX VIXION CERTIFIED GPU Workstation, SCHRÖDINGER MD workstation; Departmental Computing Center (2 Intel(R) Xeon(R) Gold 5218R CPU @ 2.10GHz (20 core), 196 GB di RAM). Bruker EPR spectrometer Elexys E500; LC-MS ESI-TOF 1260/6230DA Agilent, MALDI TOF-TOF 5800 ABI SCIEX, nano LC-CHIP-MS/MS system with a Q-TOF analyzer, LC-MS/MS system equipped with ORBITRAP analyzer (ThermoFisher), nanoLC-MS/MS 4000 Q-TRAP with a linear ion trap analyzer from ABI SCIEX; Wide range of spectroscopic (UV-Vis, Fluorescence, Circular dichroism, Raman, EPR) and calorimetric Instruments (DSC, ITC, SPR), Access to European facilities for neutron scattering experiments

Funding (*how the reagents, equipments, conference and travelrs will be supported)*

Selected funding sources
H2020-MSCA-ITN-2020 (Coordinator);
PRIN 2017 (2017XZ2ZBK) (Coordinator);
MSCA-ITN-ETN 2018
COST actions (INNOGLY)
Other local, national grants and EU Grants

Selected publications o

✓ Shao X, Zheng C, Xu P, Shiraishi T, Kuzuyama T, Molinaro A, Silipo A*, Biao Y*. Total Synthesis and Stereochemistry Assignment of Nucleoside Antibiotic A-94964. Yu B. *Angew Chem Int Ed Engl.* 2022 doi: 10.1002/anie.202200818. **IF: 15.336**
✓ Vanacore A, Vitiello G, Wanke A, Cavasso D, Clifton LA, Mahdi L, Campanero-Rhodes MA, Solís D, Wuhrer M, Nicolardi S, Molinaro A, Marchetti R, Zuccaro A, Paduano L, Silipo A*. Lipopolysaccharide O-antigen molecular and supramolecular modifications of plant root microbiota are pivotal for host recognition, *Carbohydrate Polymers*, 2022; 277: 118839. **IF: 9.381**
✓ Forgione RE, Nieto FF, Di Carluccio C, Milanesi F, Fruscella M, Papi F, Nativi C, Molinaro A, Palladino P, Scarano S, Minunni M, Montefiori M, Civera M, Sattin S, Francesconi O, Marchetti R, Silipo A.

Conformationally Constrained Sialyl Analogues as New Potential Binders of h-CD22. *Chembiochem*. 2022 **IF: 3.164**

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✓ F. Di Lorenzo, M.D. Pither, M. Martufi, I. Scarinci, J. Guzman Caldentey, E. Łakomiec, W. Jachymek, S.C.M. Bruijns, S. Martín Santamaría, J.S. Frick, Y. van Kooyk, F. Chiodo, A. Silipo, M.L. Bernardini, A. Molinaro, Pairing Bacteroides vulgatus LPS structure with its immunomodulatory effects on human cellular models *ACS Cent Sci.* **2020***;6(9):1602-1616.* **IF: 14.553**

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