Biomolecular Chemistry
(department visit Molecular Sciences 2022)

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Radboud University

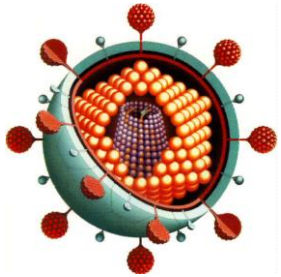
Biomolecular Chemistry

Prof.Dr. Ger Pruijn	Molecular mechanisms in autoimmunity: autoantibodies and autoantigens
Dr. Christian Büll	Protein glycosylation in health and disease

www.biomolecularchemistry.nl

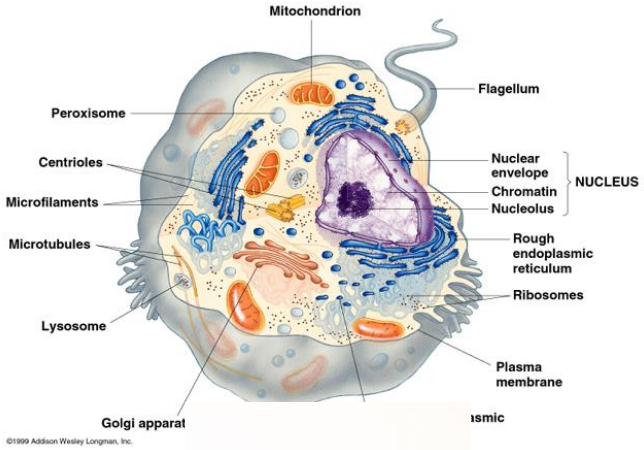
protimo.science.ru.nl

Radiation



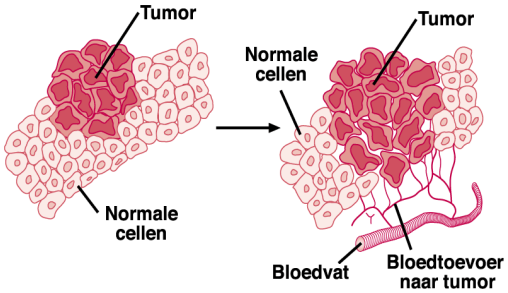
Viruses

Autoimmune diseases



Cellular stress

Autoimmunity



Cancer

Elevated temperature

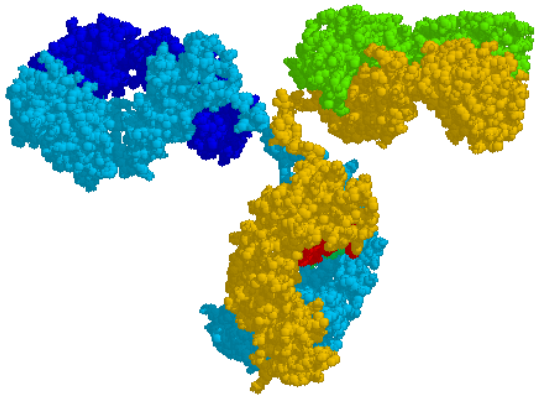
Molecular aspects

Many environmental factors can stress cells and the effects can contribute to disease development

Autoimmunity

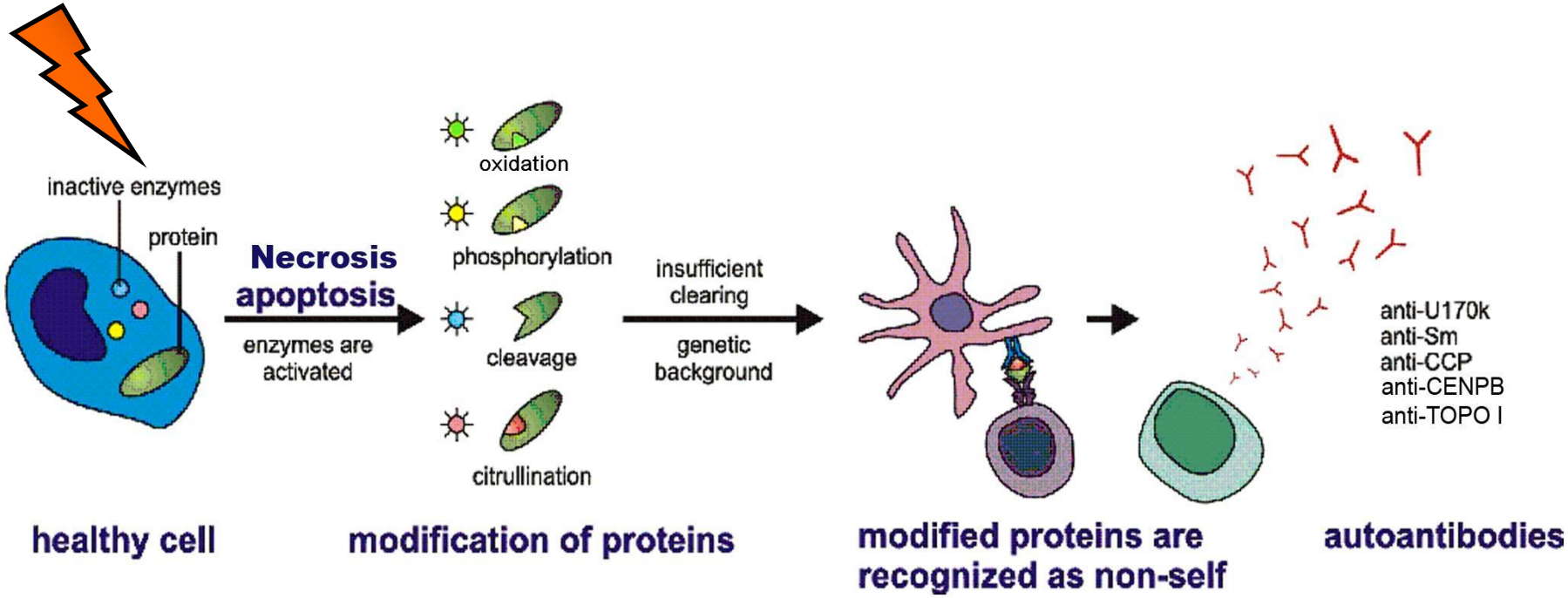
Immunity:
The immune system (immune cells and antibodies) is directed against non-self proteins and compounds (antigens).

Autoimmunity:
The immune system is directed against self proteins or other constituents of self tissues.
Patients produce a.o. (pathological) autoantibodies against self proteins, so-called autoantigens.



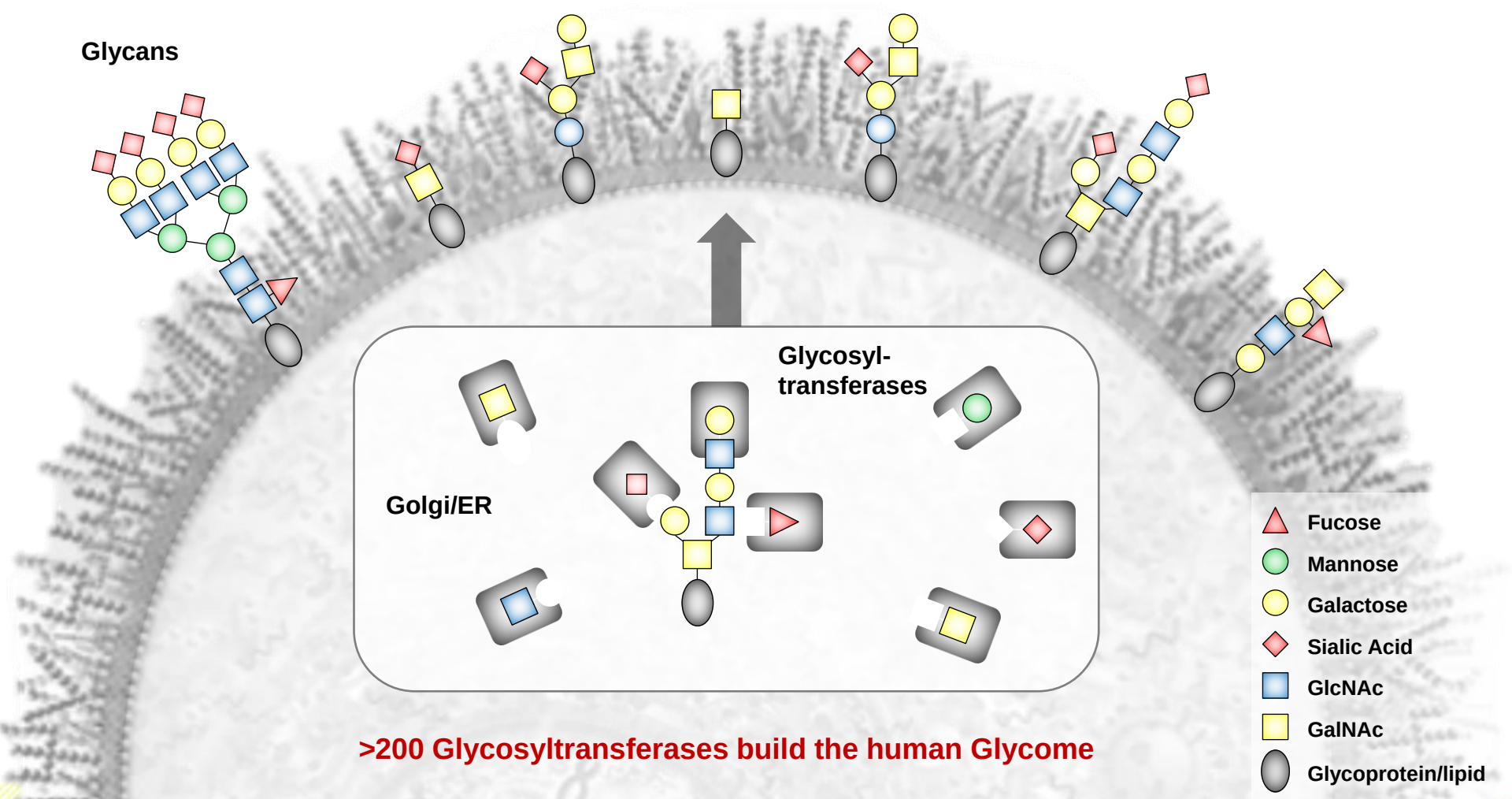
Examples:
- Myositis (muscles)
- Type 1 diabetes (pancreas)
- Multiple sclerosis (central nervous system)
- Rheumatoid arthritis (joints)

Can too much stress induce autoimmunity?



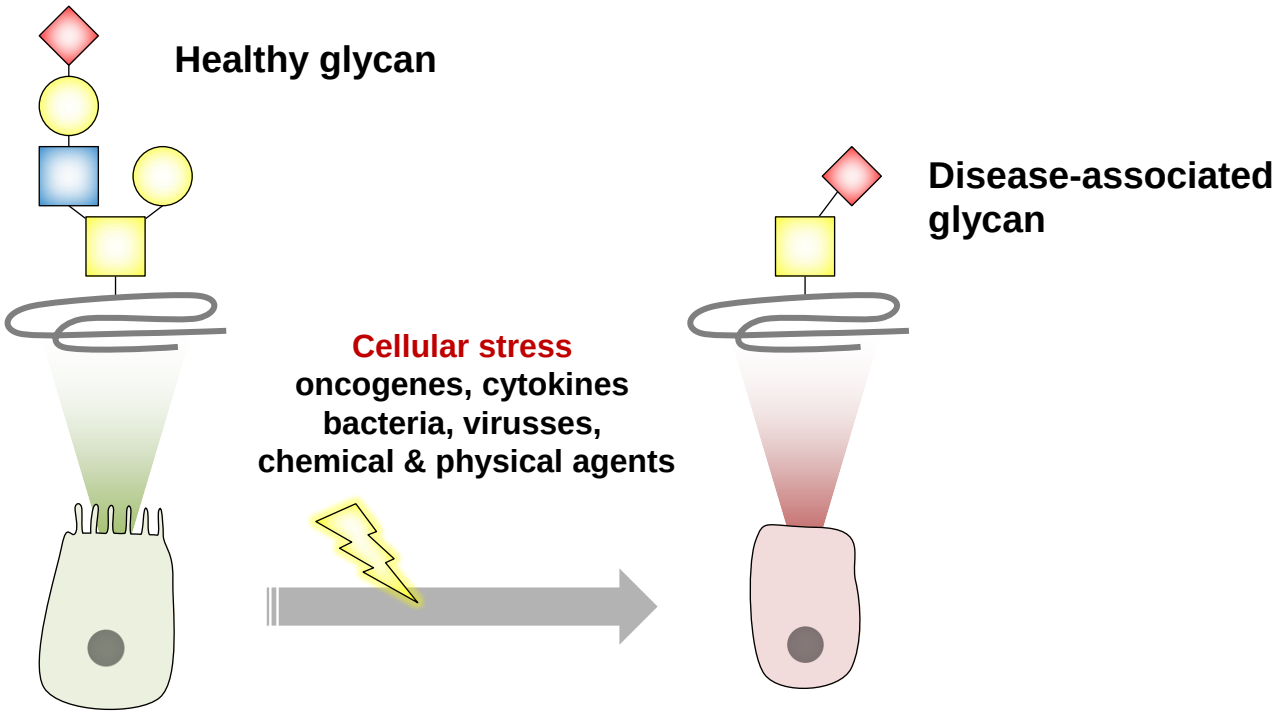
The main factors involved in the development of autoimmune diseases are genetic and environmental factors. It has been hypothesized that in genetically susceptible individuals environmental factors may induce cell death, leading to the exposure of self-components to the immune system and its activation.

Cellular Glycosylation



>200 Glycosyltransferases build the human Glycome

How is glycosylation changed in disease?



Altered glycosylation changes the specific functions of a protein and thus can have an impact on the cell, tissue, and organism level.
The (de)regulation of glycosylation is poorly understood.

Research questions addressed at the BMC dept

Which autoantibodies are specifically associated with a disease?

What is the structure and function of autoantigens?

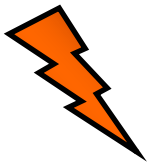
Autoantibodies

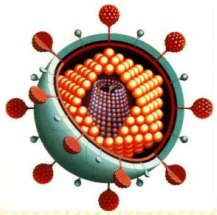
Autoantigens

Cell death

Protein modification

Why is the immune system of autoimmune patients directed to self molecules?





Research questions addressed at the BMC dept

What are the biosynthetic rules underlying protein glycosylation?

Glycosyltransferases

How do ‘healthy’ and ‘diseased’ glycans regulate protein function?

Glycans

Autoimmunity, inflammation & cancer

How does glycosylation contribute to disease and can they be explored as therapeutic targets?

Ongoing research projects at the BMC dept

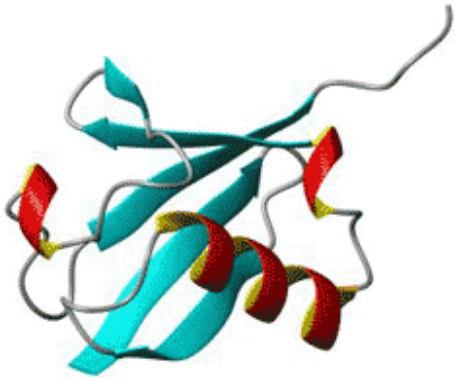
On the next slides you will find examples of related research projects, which may provide opportunities for internships.

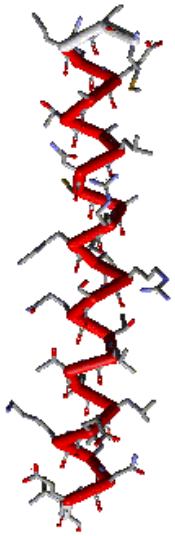
In addition, the separate movie does not only give an idea how our laboratory looks like, but also contains explanations of some of these projects by the respective researchers.

On the final slides you will find some information on the techniques that are frequently applied in our lab and a list of internship-related issues

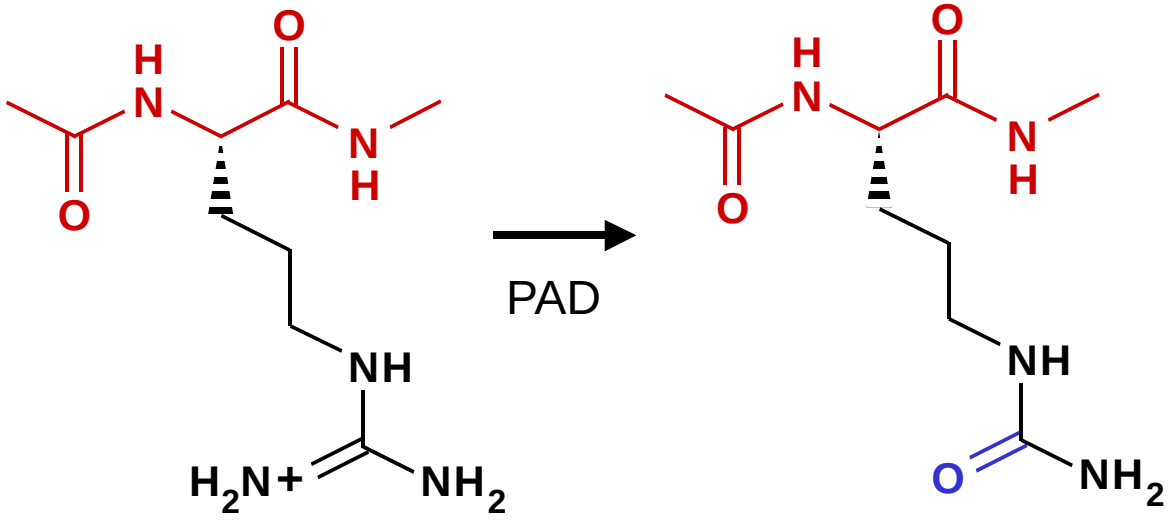
In the Zoom meeting you can ask Ger Pruijn and Christian Büll for more information.

Disease-specific autoantigen modification





Citrullination

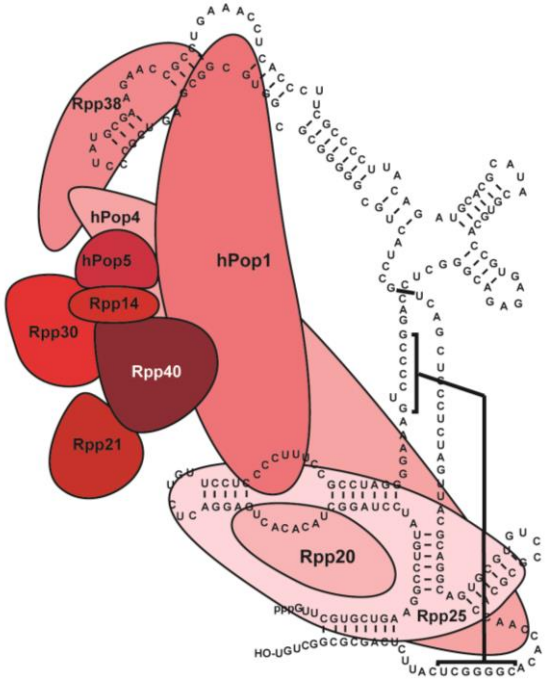


peptidyl-arginine ***peptidyl-citrulline***

Rheumatoid arthritis patients produce antibodies to citrullinated proteins

Functional characterization of RNase MRP

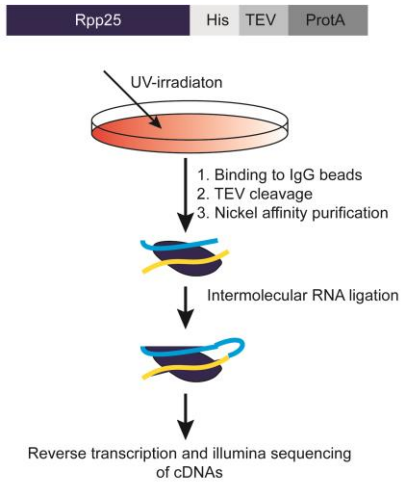
RNase MRP is an endoribonuclease – an enzyme that cleaves specific RNA substrates – which is frequently targeted by the immune system in the autoimmune disease systemic sclerosis.



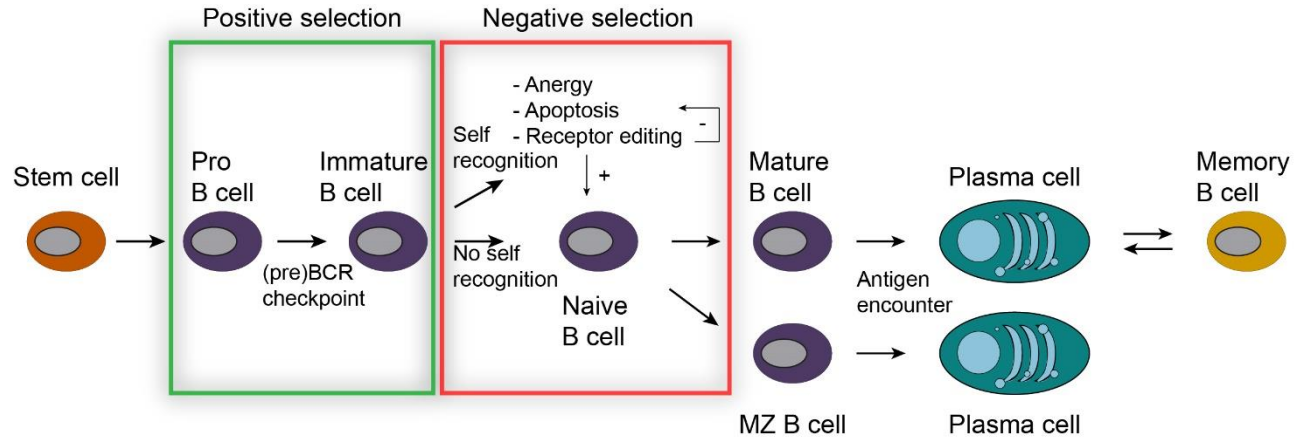
RNase MRP consists of an RNA molecule and 10 protein subunits

RNase MRP cleaves the precursor of ribosomal RNAs.

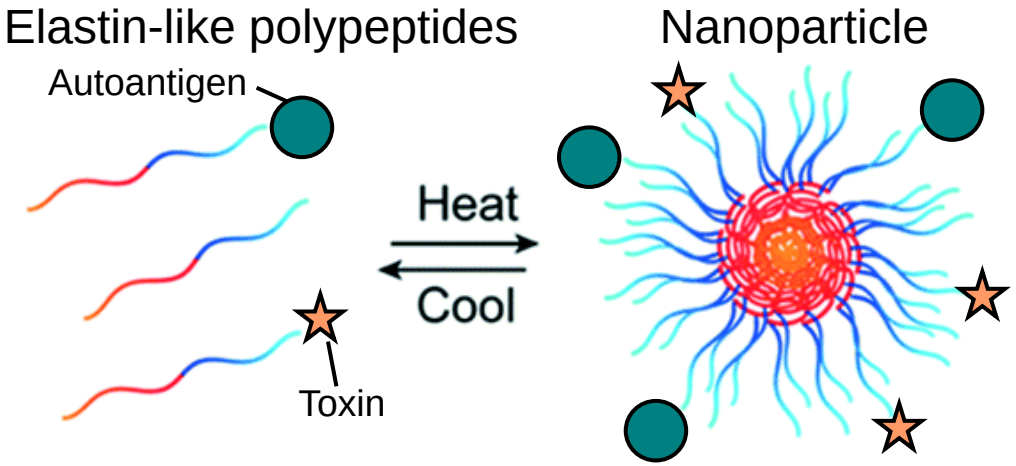
Other substrates are largely unknown. New substrates are identified and characterized in this project.

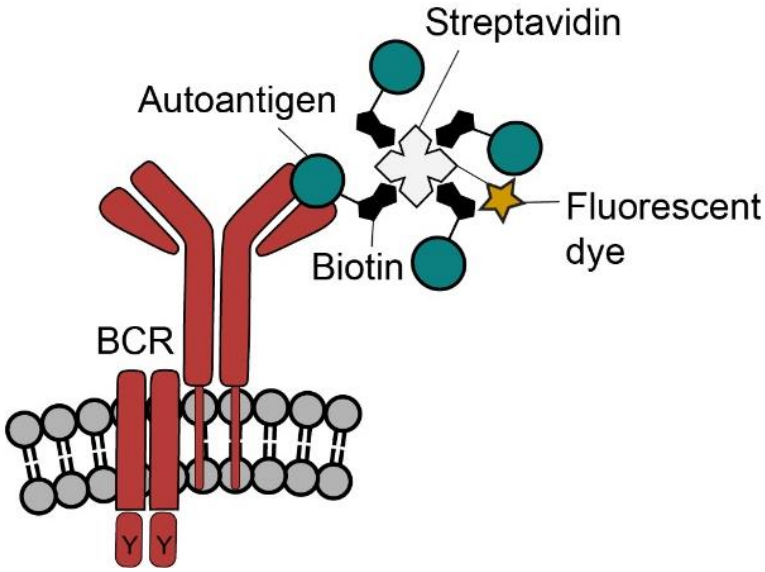


Antigen-specific targeting of autoreactive B-cells

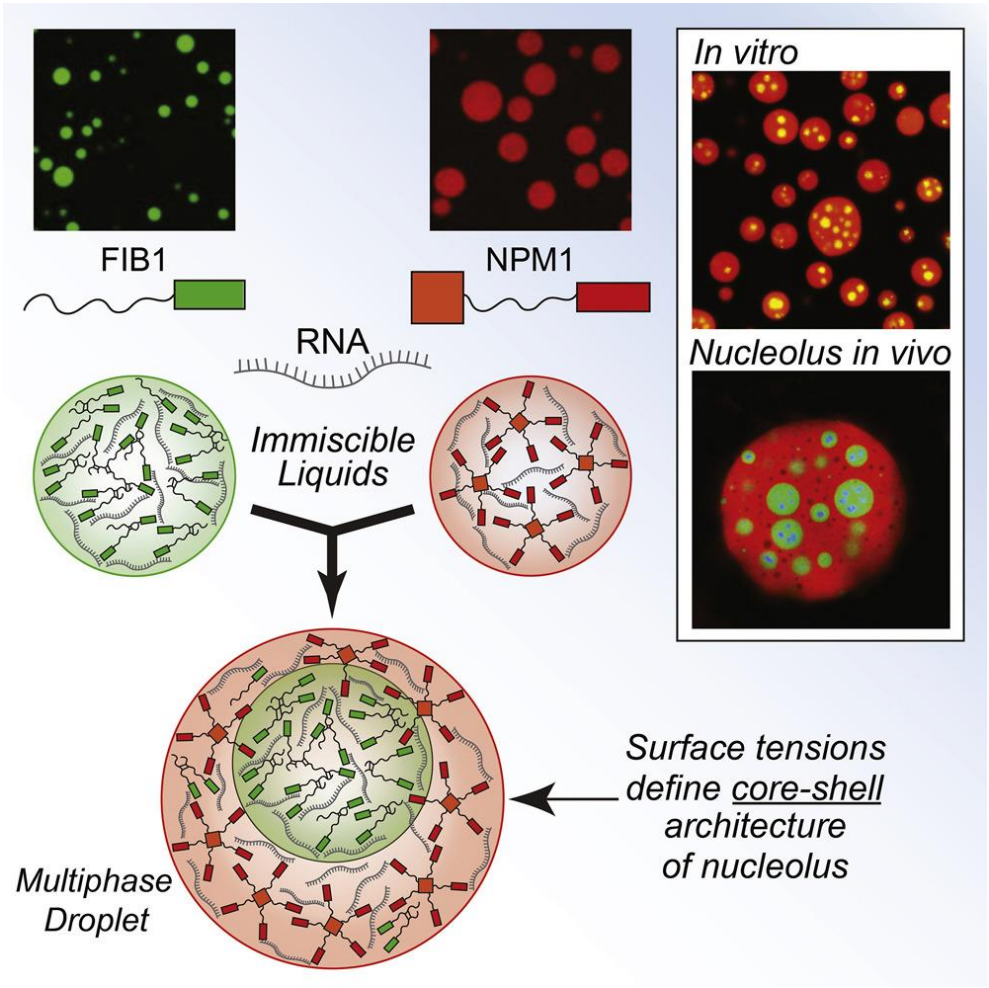


Molecular tools (nanoparticles) are being generated to target autoreactive B-cells in an antigen-specific fashion.

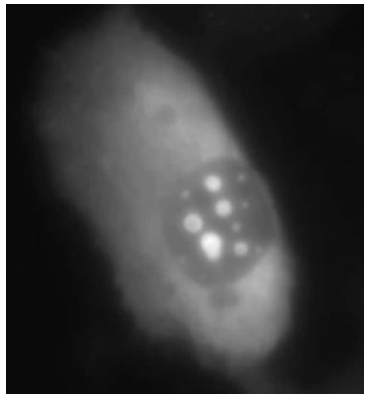




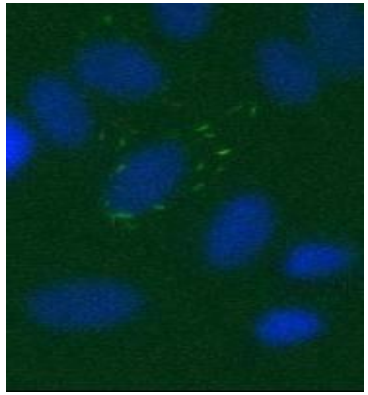
Liquid-liquid phase separation in cells



Special forms of a small heat-shock protein accumulate in liquid droplet-like structures in the nucleus.

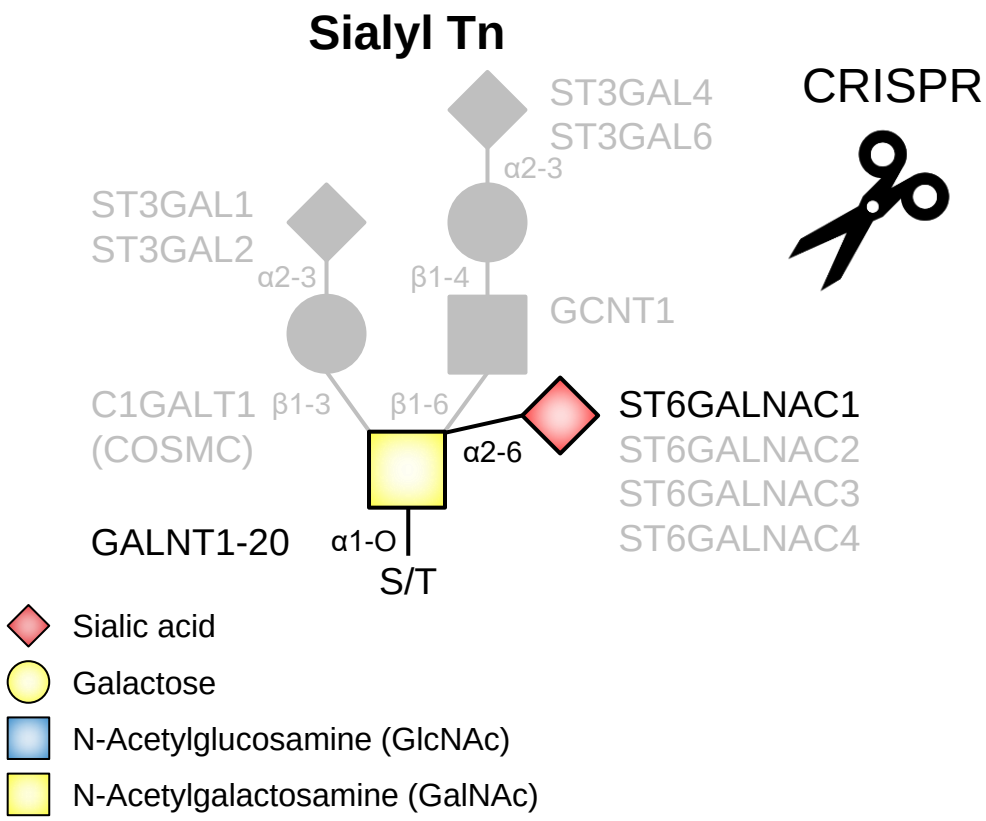


Cytosolic 5’-nucleotidase 1A, a myositis autoantigen, accumulates in filaments (see movie), which may be related to liquid droplets.

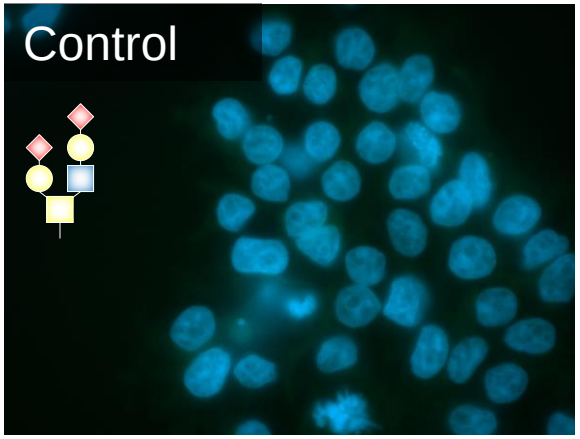


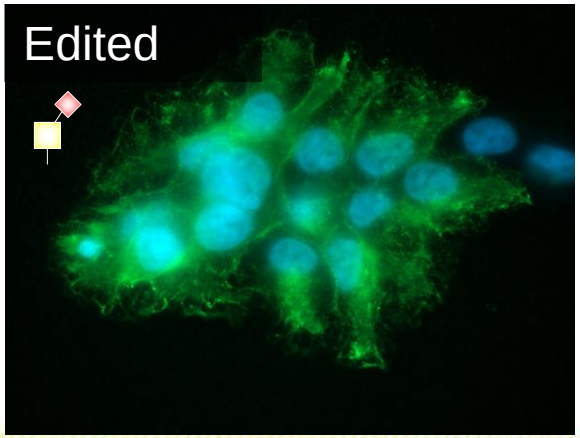
Genetic Engineering of Glycosylation

Gene editing allows to remove/induce disease-associated glycans.



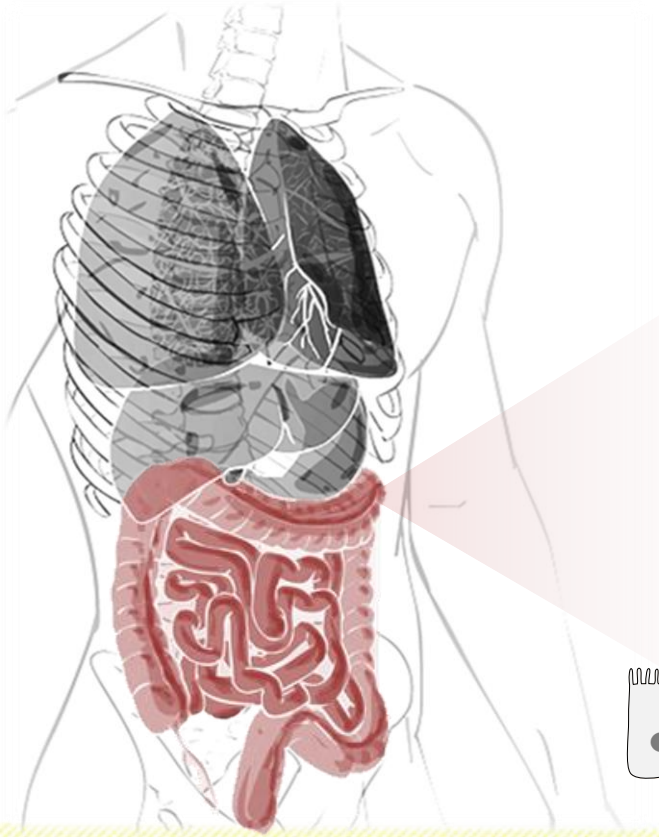
CRISPR



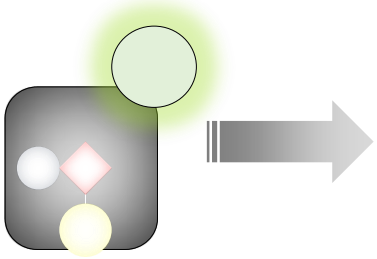


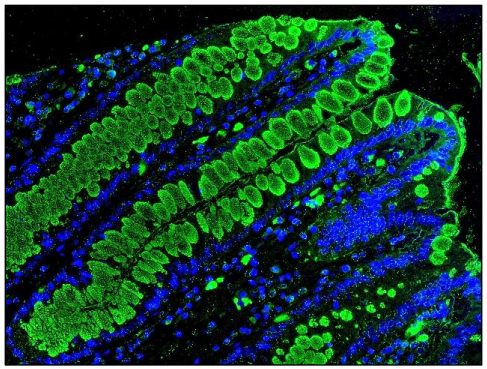
Dissecting colon-specific glycosylation features

Novel probes are developed to detect unique glycan structures in the colon.

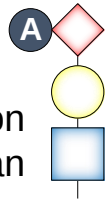


Specific probe

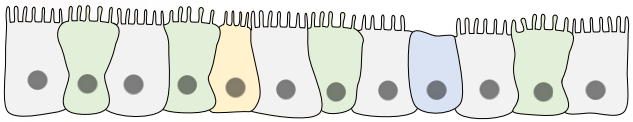




Colon epithlium stained for glycans



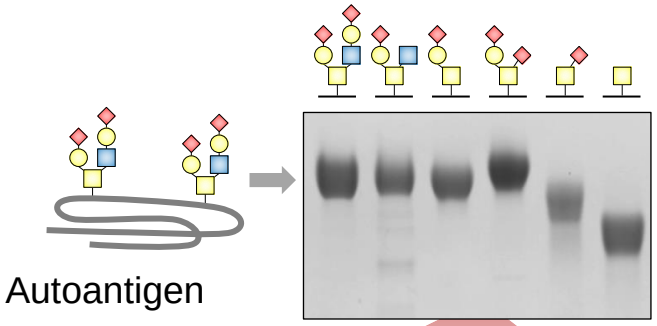
Colon glycan

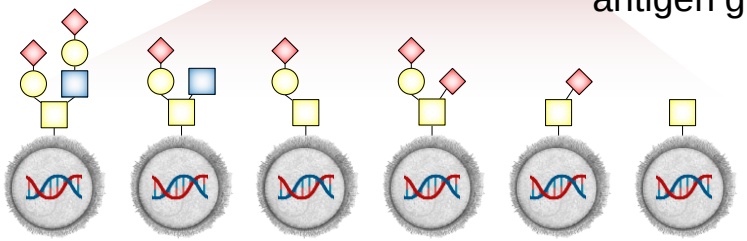


Colon epithelium cultures

Role of glycosylation on autoantibody binding

Production of glycosylated autoantigens

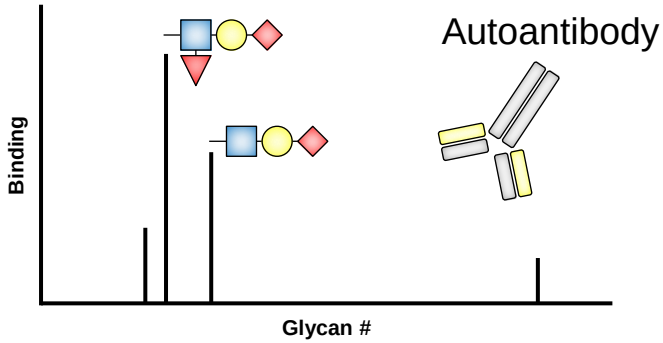




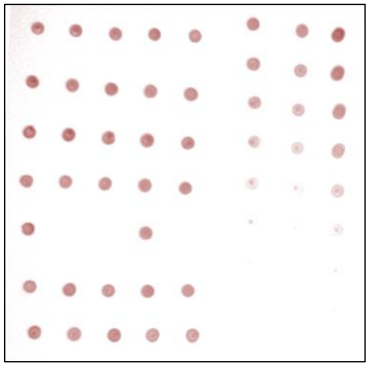
Production of antigen glycoforms

CRISPR gene editing changes glycosylation of cells

Autoantibody binding to antigen glycoforms



Effect of glycosylation on antigen recognition



Methodology

Molecular biological methods
- Recombinant DNA
- RNA-Seq (single cell)
- CRISPR-Cas9

Bioinformatics
- Big data (transcriptomics)

Cell biological methods
- Cell culture
- Transfection
- Cell activation / stress induction

Microscopical methods
- Fluorescence
- Real-time imaging

Immunological methods
- Immunoblotting
- Immunofluorescence
- ELISA
- (Glycan) Epitope mapping

Biochemical analyses
- (Glyco)Proteomics
- Interactomics
- RNA-protein interactions
- Enzyme activity determination
- RNA interference
- Protein purification
- Chemical biological techniques

Patient material (blood; biopsies)

Internship possibilities

Research projects

- Characterization and function of autoantigens
- Antigen-specific B-cell targeting
- Intracellular accumulation of autoantigens
- Protein glycosylation

Application: staff / secretariat (see www.biomolecularchemistry.nl)

- Preferentially at least 3 months before the intended start

Intern can indicate preferred project

- Biochemical / molecular biological
- Cell biological
- Immunological

Supervision of internship: promovendus or post-doc

- In principle not more than 1 intern per supervisor

Prerequisites

- Advanced Molecular Biology