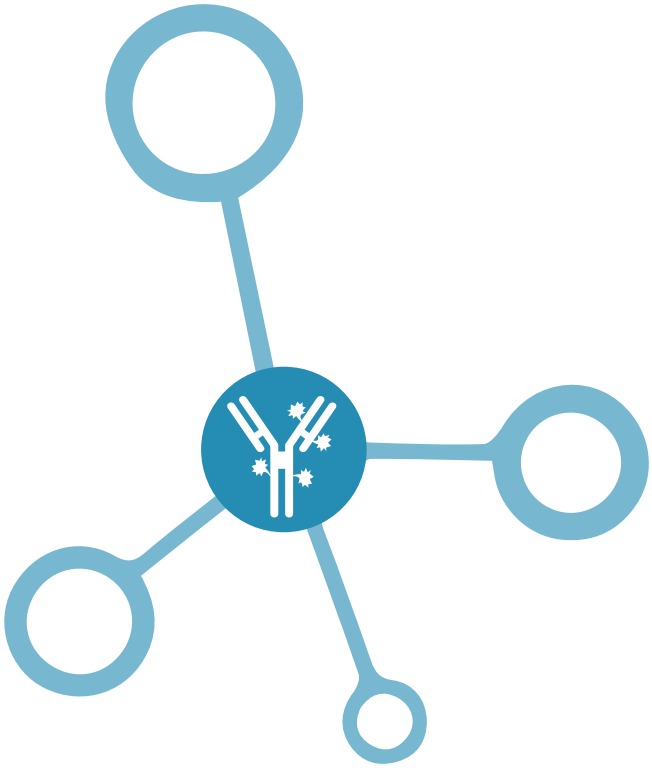
SIMICA

THERAPEUTIC SITE-SELECTIVE PROTEIN-MODIFICATION CHEMISTRIES

OVERVIEW OF THE PROJECT

The SIMICA Project intends to place the Instituto de Medicina Molecular João Lobo Antunes within the core of a European network of laboratories that seeks to produce cutting-edge research in the field of site-selective protein modification.



Did you know that:

Antibody-drug Conjugates or ADCs are highly targeted biopharmaceutical drugs that combine monoclonal antibodies that selectively bind to surface antigens present on specific tumor cells with highly potent anti-cancer agents linked via a chemical linker (**Figure 1**). Because ADCs are capable of delivering highly cytotoxic payloads directly to tumor cells they can be used to achieve high lethality toward the targeted cancer cells while reducing systemic toxicity. The concept of antibody-drug conjugates is relatively easy to understand, however, the design and synthesis of a fully functional and effective antibody-drug conjugate is remarkably challenging.

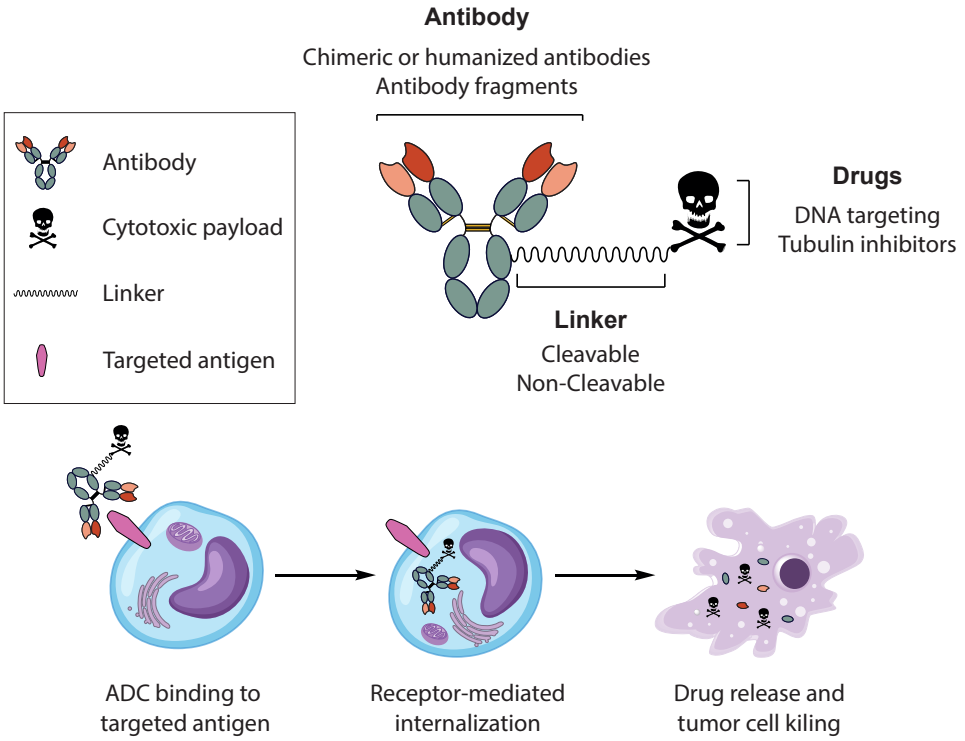


Figure 1: Antibody-drug conjugates deliver cytotoxins to specific cancer cells. Once in the tumor cells the ADC is internalized and degraded by lysosomal digestion leading to release of the cytotoxic agent, resulting in rapid cell death.

Meet the SIMICA

Dr Luis Cruz leads an experienced team of scientists at LUMC, including technicians, PhD students, fellows and postdoctoral scientists, who specialises in synthesis of nanoparticles and their subsequent functionalisation. This includes conjugating targeting moieties to the SLNP based upon computer assisted-drug designs from iMM, where we anticipate a high level of synergy and complementarity between LUMC and iMM with respect to targeting different diseases.

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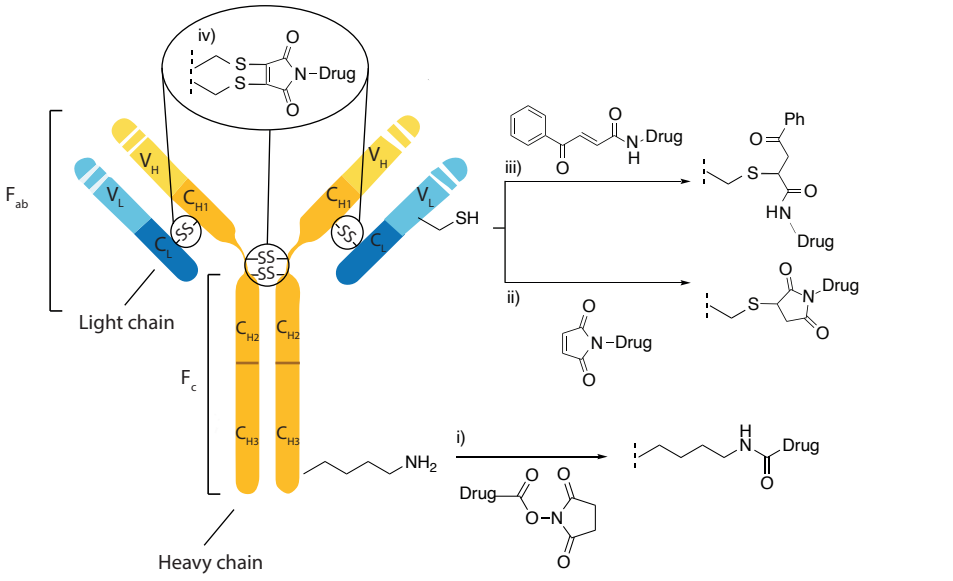
The Make-up of an ADC

Antibodies in ADCs

High specificity of targeting and minimal immunogenicity are the main characteristics for the antibody component in ADCs. Rapid internalization is also important for both ADC efficacy and safety, since it reduces the opportunity of the ADC for off-target release. Additionally, therapeutic activity of ADCs is also mediated via immune effector functions such as Antibody-Dependent Cellular Cytotoxicity (ADCC), Antibody-Dependent Cellular Phagocytosis (ADCP), Complement Dependent Cytotoxicity (CDC), and cytokine signaling modulation (Lancet 2019, 394, 793).



Linkage strategy in ADCs

- Conjugation site
- Homogeneity
- Chemical stability
- Cleavable/ non-cleavable linker
- Smart cleavage mechanism
- Site-specific conjugation

F_{ab} - Fragment, antigen-binding
F_c - Fragment, crystalizable
C_L - Constant domain, light chain
C_H - Constant domain, heavy chain
V_L - Variable domain, light chain
V_H - Variable domain, heavy chain

Hypervariable region
Variable region
Constant region

Figure 2: Chemical methods to produce Antibody-Drug Conjugates

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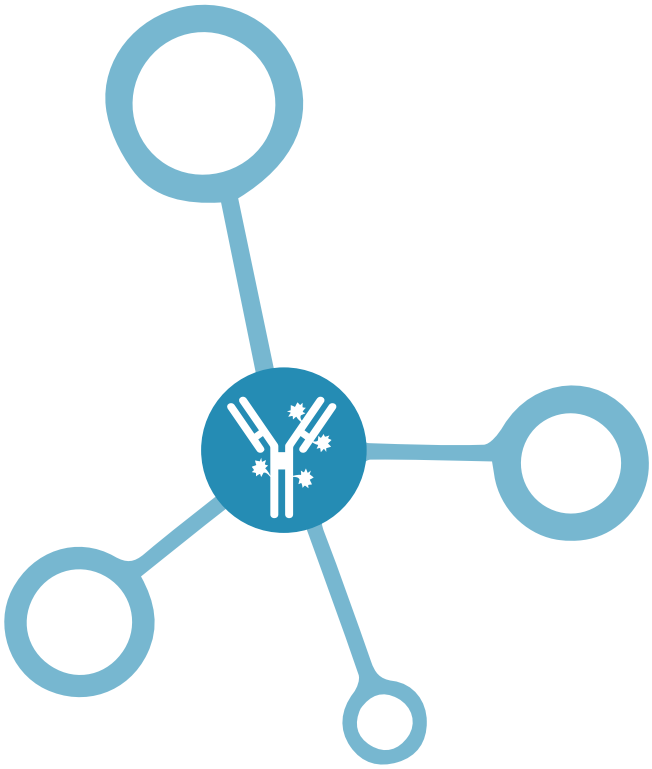

Cytotoxic payload

ADC payloads on FDA approved drugs target mostly DNA or tubulin. The drugs that damage DNA are natural products (e.g., duocarmycins, calicheamicins) that induce double-strand breaks or cause DNA alkylation. The tubulin inhibitors (e.g. monomethyl auristatin E and monomethyl auristatin F) block microtubule polymerisation, causing G2/M phase cell-cycle arrest (Lancet 2019, 394, 793).

Linkers

The chemical linker connects the cytotoxic payload to the antibody. Properties of the linker should include (1) sufficient stability to enable ADC molecules to circulate in the bloodstream and localise to the target site without premature cleavage; and (2) the ability to be rapidly cleaved on internalisation to release the payload. Currently available linkers are categorised as either cleavable or non-cleavable. Cleavable linkers depend on the physiological environment, such as low pH (acid-labile linkers), proteolysis (protease-cleavable linkers e.g. hydrolysis of valine-citrulline), or high intracellular glutathione concentrations (disulphide linkers), to release the payload from its ADC carrier. Non-cleavable linkers form non-reducible bonds with the amino acid residues of the antibody and depend on lysosomal degradation for payload release. These ADCs require an efficient internalisation process and optimal trafficking to lysosomes.

New chemical methods for drug conjugation to antibodies

Linker chemistries have a crucial role in ADC performance in terms of stability, pharmacokinetics and efficacy. One of the dynamic research fields in ADC design is the development of new methods for drug conjugation to antibodies. The key concerns in linkage chemistry are presented in Figure 2. Conjugation site on the antibody, control of the Drug to Antibody Ratio (DAR), stability of the linkage and preparation of homogeneous entities are important parameters that need to be considered (Lancet 2019, 394, 793). Initially, unspecific site-selective conjugation strategies were used to attach drugs to antibodies, which yielded heterogeneous species with different drug loading at different sites. The chemistry behind these methods was based on the use of NHS activated esters for conjugation to lysines on the antibodies (Figure 2 i). An alternative approach being explored are reagents that target cysteine residues on native proteins and antibodies for bioconjugation of different biophysical tags. Typically, maleimides have been extensively used for cysteine modification as they react rapidly and selectively with thiols (Figure 2 ii). However, it also undergoes deconjugation through a retro-Michael pathway, leading to loss of cargo and reduction in efficacy (Chemistry 2019, 25, 43). Alternatively, we have employed distinct thiol reactive molecules, such as carbonyl acrylates that after rapid bioconjugation form an opened succinamic moiety that is resistant to deconjugation (Figure 2 iii)(Nat Commun 2016, 7, 13128).

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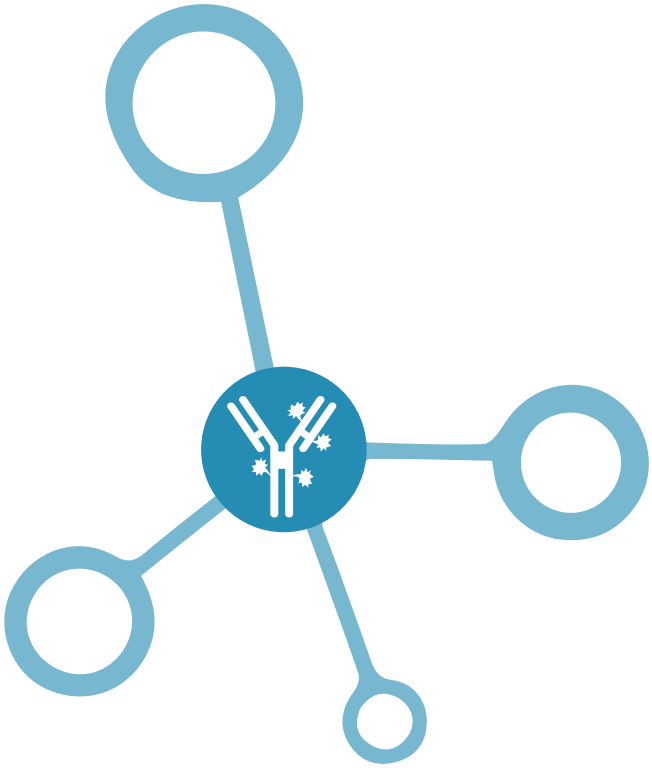

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Disulfide rebridging agents are also a class of linkers used for protein modification. Disulfide rebridging involves the reduction of the interchain disulfide bonds in the antibody followed by reaction with a cysteine-selective cross-linking reagent which can bear different payloads including drugs (Figure 2 iv)(Chem Soc Rev, 2021, 50, 1305).

SIMICA news

- We are happy to announce that our recent publication (Bioconjugate Chemistry 2021 32, 121) has been selected to appear in a virtual issue on “Bioorthogonal Chemistry and Bioconjugation” at Bioconjugate Chemistry.
- We are proud to know that @Allcyte was acquired by @exscientiaAI to join forces on the discovery of better drugs using patient-first AI. It is a privilege to have Allcyte/Exscientia on board.
- Another successful mentorship programme established. On the 3rd call Luis Monteiro, a MD doing his PhD in the group of João Barata at IMM chose Bruno Oliveira as his mentor.
- Our coordinator Bruno Oliveira just got his first grant as PI from the Portuguese Foundation for Science and Technology (FCT) focused on the combination of click chemistry and sequencing approaches to study potential roles of glycoRNA in cancer biology.
- Silvia Sobol from PERC organized a 1 day course in September about bioluminescent imaging at iMM. This course offered an integrated overview of the mechanisms and applications of bioluminescence for in vivo imaging, by covering all the key issues from the chemistry of bioluminescence to biological applications (e.g. visualization of tumor progression and metastasis).
- Dr. Daniel Zaidman, a Blavatnik Postdoctoral Fellow from Cambridge, visited iMM to explain how bioinformatics methods can be explored to design Antibody-Drug Conjugates with increased target specificity and affinity.
- Claúdia Afonso, a PhD student at iMM, just started her exchange visit in the Chemistry Department of the University of Cambridge to work with Gonçalo Bernardes and Michele Vendruscolo.

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