Postdoctoral fellowship:
Coarse-grained models of bispecific antibodies for cancer immunbotherapy: dynamics and binding kinetics at interfaces

Title of the project	Physical and computationally aided design of bispecific antibodies for cancer immunbotherapy (ComPhysAb)
Duration of the project	1st december 2018 – 1st December 2021
Funded by	INSERM
Duration of the fellowship	Two years, 100 %. Starting date: available immediately.
Partners	1. Adhesion and Inflamation lab (LAI) Dr Laurent LIMOZIN (coordinator). https://labadhesioninflammation.org 2. Cancer research center (CRCM) Dr Patrick CHAMES http://crcm.marseille.inserm.fr 3. Centre de Biophysique Moléculaire (CBM) Prof. Francesco PIAZZA http://cbm.cnrs-orleans.fr
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Application	Please send Prof. Piazza file the following documents as a single PDF file 1. Detailed CV with description of expertise in computational biophysics 2. Publication list 3. Master thesis 4. Motivation letter 5. Contact details of three referees

The project

Background
In the recent years many new therapeutic antibodies (Abs) harnessing the immune system have revolutionized cancer treatment. However, their discovery remains empirical, their mechanisms of action are poorly understood, and many patients don't respond to such therapies. In the mean time, single molecules and single cell techniques have started to unveil new physical mechanisms governing biological molecules activity in situ, with profound implications in cell biology and immunology, beyond the reach of classical biochemical approaches. For example, while Abs are selected on the basis of their affinity measured in solution, immune response ultimately rely on multivalent interactions and cell-surface contacts (2D), through the receptors of antibody Fc fragment (CD16) like in Antibody Dependent Cell Cytotoxicity (ADCC) performed by immune cells to kill tumour cells. Therefore, there is a need for improved characterization techniques and rational design of therapeutic antibodies for immunotargeting by taking into account their role as adhesive and multivalent molecules in 2D environments at cell surface.

Project:
Starting from an original library of single domain Abs (nanobodies) targeting HER2 cancerous markers or CD16, we will exploit 2D on/off kinetics under force of these Abs measured with state-of- art biophysical methods based on microfluidics and applicable to panels of molecules. Aided by ultra- coarse grain simulations and single molecule fluorescence to select optimal molecular architectures and nanobodies combinations, we will construct new bispecific HERxCD16 Abs and multivalent derivatives. We will correlate the biophysical molecular parameters with the efficiency of these molecules to mediate ADCC in cellular controlled environment.
Results:
Our principal goal is establish engineering principles for immunotargeting and therapeutics in cancer, and provide as a proof of concept a molecule outperforming trastuzumab in the treatment of breast cancer.

Issue, hypothesis and objective(s) of the investigations

Antibody-based cancer immunotherapy - success and limitations.
Immunotherapy is arguably one of the fastest developing and most promising strategies to fight various pathologies, from infectious diseases to cancer [Batlevi2016]. It encompasses various modalities based on molecular or cellular agents, using tools of synthetic biology, protein engineering and biotechnologies. Among these strategies, those based on antibodies (Abs) offer versatile and powerful tools and a large spectrum of applications. From the clinical point of view, monoclonal Abs (MAbs) have enormously improved the prognostic in several cancers. Therapeutic antibodies can harness the immune system by blocking inhibitory receptors expressed by immune effector cells such as T cells, NK cells, macrophages (checkpoint inhibitors), or by recruiting and reactivating immune cells by targeting activating receptors [Scott 2012; Slikowski 2013; Nimmerjahn 2015]. **While these molecules have a high potential, many patients do not respond to such therapies; there is an urgent need to increase their efficiency** [Chames 2009; Vyas 2014]

A physical approach for antibody design.
Therapeutic antibody discovery and design remains largely the specialty of teams involving immunologists, biochemists, molecular biologists or medical doctors. However,

the isolation and engineering of therapeutic Abs rely on selection with loosely defined criteria; additionally, their mechanisms of action are often poorly understood. Without minimizing the wide range of biological phenomena involved in the efficiency of therapeutic molecules, **some fundamental physical questions regarding Ab structure, binding properties and mechanisms of action are relevant and still open**. For example, the roles of 3D scaffold and intramolecular flexibility are not systematically explored. When molecular recognition occurs between proteins linked to cellular structures, chemical and mechanical phenomena at the molecular scales are intertwined, providing a physical basis for mechanosensing [Bustamante 2011; Bongrand 2012; Zhu 2014]. The elusive governing physical principles provide a new paradigm to study bond formation and rupture in physiological contexts. These dynamical and mechanical considerations may have some profound consequences in cell biology and immunology [Robert 2007; Hoffmann 2011; Malissen 2015]. **How dynamics and forces can affect Abs recognition and action in the previously described context, or whether those factors can be exploited *in vitro* and *in silico* for antibody selection and design, has not been explored yet, to our knowledge.**

Objectives.
Current protocols for the design of therapeutic monoclonal Ab (mAb) relies on selection on the basis of affinity measurements with soluble mAbs (3D). The previous discussion shows that there is a clear need for improved characterization techniques and rational design of therapeutic mAbs for immunotargeting, by taking into account their role as adhesive molecules in 2D environments. **The main goal of this project is to improve the design of bispecific antibodies, and thus their therapeutic efficiency in cancer. For this, we will characterize and decipher *in vitro* and *in silico* the role of their ultrastructure, conformational dynamics and binding properties under mechanical and geometrical constraints.** We are forming a unique consortium to study antibodies, mastering a wide range of complementary expertise:
- advanced single molecule fluorescence and manipulation techniques (Partners 1 and 3) - antibody engineering based on the use of single domain as building blocks and bispecific Abs (P2) - ultra-coarse-grained models and many-body Brownian dynamics for Abs conformations and reactions (P3).

The postdoc will work in the group of Prof. Piazza @ CBM Orléans and will be involved mainly in the following tasks in the project.

Subtask 1.4 : Parameterization and validation of coarse-grained models of modified antibodies.

The aim of this task, together with subtask 2,2 is to provide a flexible in-silico platform to assist the design of optimal molecular constructs by assessing the role on the binding kinetics of structural and dynamical determinants, such as intra-molecular flexibility and size, contour and persistence length of linking units. More precisely, we will use the computational tools such as those developed in Ref. [De Michele, 2016] to the study of the molecular constructs synthesized and analyzed in this project. Coarse-grained models of (i) Tandom, (ii) bsFab and (iii) SdAb-Fc and (iv) tandom+sdAb/bsFab+sdAb antibodies will be developed and parameterized through the results of single-molecule FRET experiments (task 1) and kinetic measurements (preliminary results). Successively, we will integrate these molecular models into many-body simulation schemes to investigate the kinetics in different geometries, concentrating on crucial features such as avidity, intra-molecular flexibility and the role of confinement.
Our coarse-grained models of antibodies and multi-domain constructs will be built by

assembling through flexible linkers coarse-grained domains built following the shape-based coarse-graining algorithm[1] [Arkhipov 2008] (see IFg. 1). Snapshots from preliminary simulations of a diabody are shown in Fig. 2. Poly(Gly4-Ser) Linkers in the case of Tandom constructs will be modeled as chains of spheres [Kremer 1990] plus angle-bending terms, so as to reproduce the correct persistence lengths (taken from the literature or obtained by separate sets of atomistic simulations). For our large-scale simulations we will use the powerful simulation framework LAMMPS [Plimpton 1995] coupled to state-of-the-art multi-scale coarse-graining techniques [Noid 2008]. The computational models of our multi-domain molecules will be parameterized from smFRET data (subtask 1.3).

An important task of the postdoctoral fellow will be to implement methods to investigate the kinetics of binding of the multivalent CG molecules to epitope at interfaces with different geometries [see e.g. De Michele, 2016]. Special attention will be paid to models of the synaptic interface between cancer cells on one side and immune effector cells (such as NK cells) on the other. Our multivalent molecules shall be designed to bind on both sides, thus stabilizing the synapse with the aim of boosting the immune response in the tumor microenvironment. It will be crucial to assess how the dimensions (cell-cell distance can be as short as a few nanometers) and geometry (surface density and mobility of receptors on either side) of the interface modulate the binding kinetics. We are particularly interested in establishing the optimal constructs able to boost the binding cooperativity (avidity).

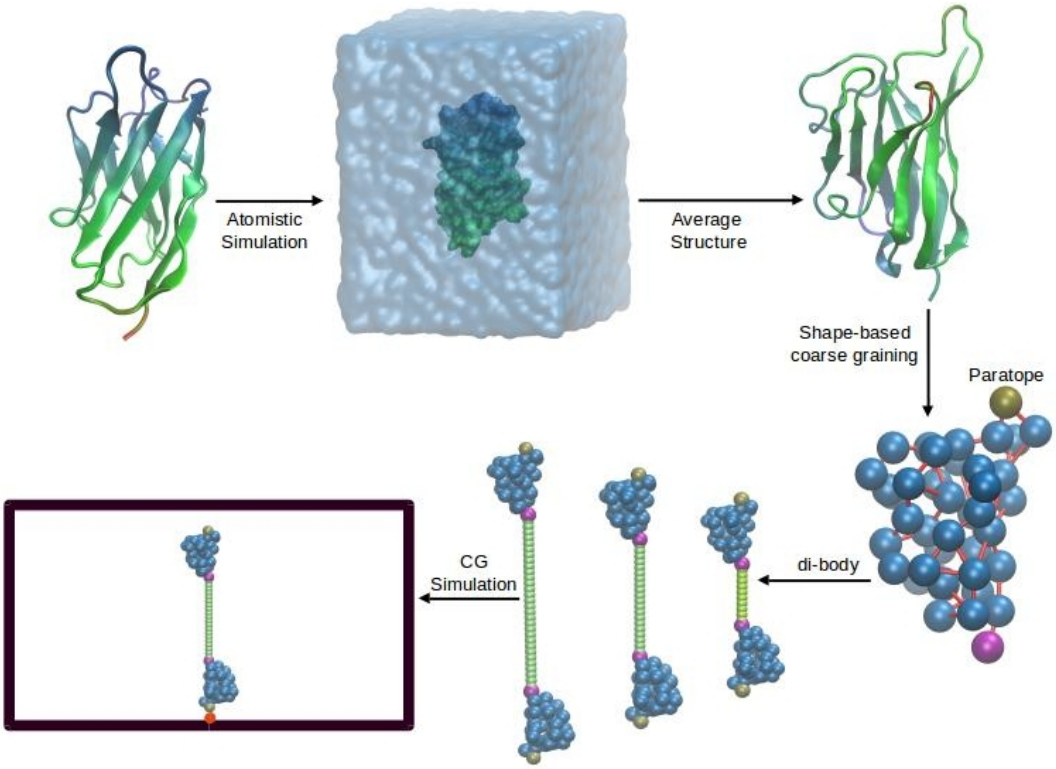

Fig. 1. Illustration of the shape-based coarse-graining technique to model a divalent molecule obtained by joining two single-domain antibodies via a flexible (PEG) linker.

[1] See also the excellent tutorial at
http://www.ks.uiuc.edu/Training/Tutorials/science/coarse-graining/sbcg-tutorial.pdf

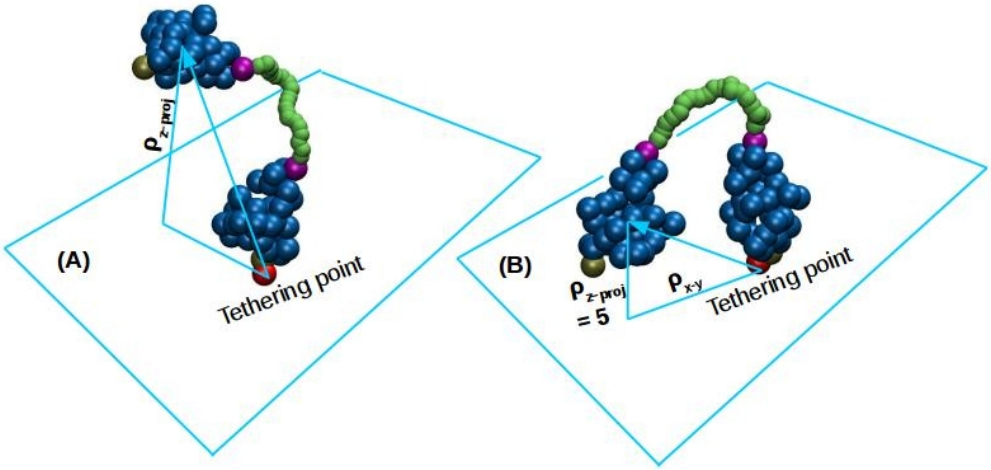

Fig. 2. Coarse-grained model of a divalent molecule obtained by joining two single-domain antibodies via a flexible (PEG) linker. The molecule is tethered on a a surface through one of its paratopes so as to characterize the binding kinetics of the second paratope.

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