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Computational Approaches in Antibody-Drug Conjugate Optimization for Targeted Cancer Therapy

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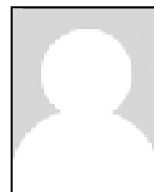

Abstract: Cancer has become one of the main leading causes of morbidity and mortality worldwide. One of the critical drawbacks of current cancer therapeutics has been the lack of the target-selectivity, as these drugs should have an effect exclusively on cancer cells while not perturbing healthy ones. In addition, their mechanism of action should be sufficiently fast to avoid the invasion of neighbouring healthy tissues by cancer cells. The use of conventional chemotherapeutic agents and other traditional therapies, such as surgery and radiotherapy, leads to off-target interactions with serious side effects. In this respect, recently developed target-selective Antibody-Drug Conjugates (ADCs) are more effective than traditional therapies, presumably due to their modular structures that combine many chemical properties simultaneously. In particular, ADCs are made up of three different units: a highly selective Monoclonal antibody (Mab) which is developed against a tumour-associated antigen, the payload (cytotoxic agent), and the linker. The latter should be stable in circulation while allowing the release of the cytotoxic agent in target cells. The modular nature of these drugs provides a platform to manipulate and improve selectivity and the toxicity of these molecules independently from each other. This in turn leads to generation of second- and third-generation ADCs, which have been more effective than the previous ones in terms of either selectivity or toxicity or both. Development of ADCs with improved efficacy requires knowledge at the atomic level regarding the structure and dynamics of the molecule. As such, we reviewed all the most recent computational methods used to attain all-atom description of the structure, energetics and dynamics of these systems. In particular, this includes homology modelling, molecular docking and refinement, atomistic and coarse-grained molecular dynamics simulations, principal component and cross-correlation analysis. The full characterization of the structure-activity relationship devoted to ADCs is critical for antibody-drug conjugate research and development.

Keywords: Antibody-Drug Conjugate; Cancer; Molecular Docking; Homology Modelling; Molecular Dynamics

1. INTRODUCTION

Cancer is the most wide-spread disease across countries, cultures and ethnicities, affecting both developed and developing regions [1]. It is defined as the formation of abnormal cells caused by uncontrolled cell division, and it is the leading cause of morbidity and mortality with an estimated 14,100,000 new cases in 2012 and 8,200,000 deaths worldwide [2]. Cancer has a complex aetiology that often starts with a mutation in cell's DNA in which the cell loses normal functionality and instead gains the ability to indefinitely multiply until impairing normal tissue properties [3]. Many aspects that contribute to its development and progression are still not fully understood. Therefore, there is an emerging need for development of novel approaches that help potentiate innovative therapeutic targets and effective drugs. There are currently ca. 200 different types of cancer identified, including solid tumours such as breast and lung cancer, as well as blood-based malignancies, namely, leukaemia and lymphoma [4]. Breast, prostate and lung cancer are common types whereas 22% of rest of cancers are considered as rare [5] appearing in a small percentage of the population [6]. The "hallmarks of cancer" can be categorized in 6 sections as indicated in Hanahan *et al.* [7]. The first is *Self-Sufficiency in Growth Signals*. Unlike normal cells, cancer cells are able to move to an active proliferative state without extracellular signalling which are received by transmembrane receptors. Second, *insensitivity towards antigrowth signals- meaning that* in cancer cells antigrowth

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XXX-XXX/14 $58.00+.00 © 2014 Bentham Science Publishers

mechanisms do not work properly, but rather they provoke the cells to grow and replicate continuously. The third hallmark is the, *evading apoptosis*, while cell proliferation is a stimulatory factor in the onset of cancer, the cell death avoidance is an alternative way for the cancer development. Fourth, *Limitless replicative potential*: this acquired capability emerges as a combination of the previous three stimulators, that result in lack of communication between the cell and environment, either environmental or self-cell related. This causes tumour cells to escape from a limit that usually applies to normal cells, above which they can no longer replicate. *Sustained angiogenesis* is the fifth hallmark. Angiogenesis, which is known as the promotion of the generation of new blood vessels, allows cancer cells to provide enough supplies. Cancer cells can interfere with angiogenesis by releasing angiogenesis promoting factors, such as Vascular Endothelial Growth Factor (VEGF) or by regulating proteases, that can degrade the existing factors[8]. Lastly, *tissue invasion and the metastasis*: the previous steps result in an overstepping of a set of cells' boundaries into new areas where they should not be in high numbers otherwise. This occurrence requires changes in cells' interactions with surroundings, otherwise they would be recognized by the immune system and would be forced to undergo apoptosis. Cancer cells tend to have proteins responsible for communication with the altered environment, such as Cell-cell Adhesion Molecules (CAM) – members of the immunoglobulin and calcium-dependent cadherin families that mediate cell-cell interactions – and integrins that facilitate cell-extracellular adhesion. Furthermore, extracellular proteases can be activated, thus promoting the elimination of extracellular communication factors.

Despite the genetic factors, the high incidence of cancer is widely related to changes in diet, physical inactivity, smoking and excessive alcohol consumption as well as environmental changes [9]. To combat with this disease, prevention is always a better approach than treatment. This can be done, primarily, by avoiding the external factors mentioned above. Furthermore, early detection of primary tumours should be done to avoid cancer progression and metastases. Medical imaging technologies, which provide a better diagnosis, have grown rapidly over the past few years [10], and can be used in all phases of cancer management including diagnosis and staging, using specific markers, as well as treatment follow-up [11]. Recently, the simultaneous combination of imaging with therapy, named theranostic, has gained importance in both research and in the clinical field [12] as it provides an understanding of underlying molecular mechanisms, better diagnosis strategies and therapeutic efficiency [13]. That leads to a shift from traditional chemotherapy to targeted cancer therapy. This new approach has been enhanced by distinguishing specific features of tumour cells to provide a framework for development of more selective drugs [14].

1.1. The concept of Antibody-drug conjugates (ADCs)

ADCs are humanized or fully human monoclonal antibodies (mAbs) that are covalently bound to highly cytotoxic small molecules (cytotoxic payloads) through chemical linkers. Due to the high binding specificity of mAbs for tumour-specific cell-surface antigens (found uniquely on the surface of tumour cells) and for tumour-associated cell-surface antigens (found overexpressed on the surface of tumour cells, but also present in healthy cells) [15-16], these immunoconjugates may combine the anticancer efficacy of small-molecule chemotherapeutics with high selectivity, stability, and favourable Pharmacokinetics/Pharmacodynamics (PK/PD) profile of mAbs. The ADCs can be recognized as sophisticated delivery systems for drugs with anticancer activity, in which the antibody may effectively guide the cytotoxic drug to target tumour cells, where the drug can be chemically and/or enzymatically released from the immunoconjugate to induce the cytotoxic activity [17]. Structurally, the ADCs can be divided into three main structural units: a humanized or a fully human mAb, a stable linker, and a cytotoxic payload (Figure 1).

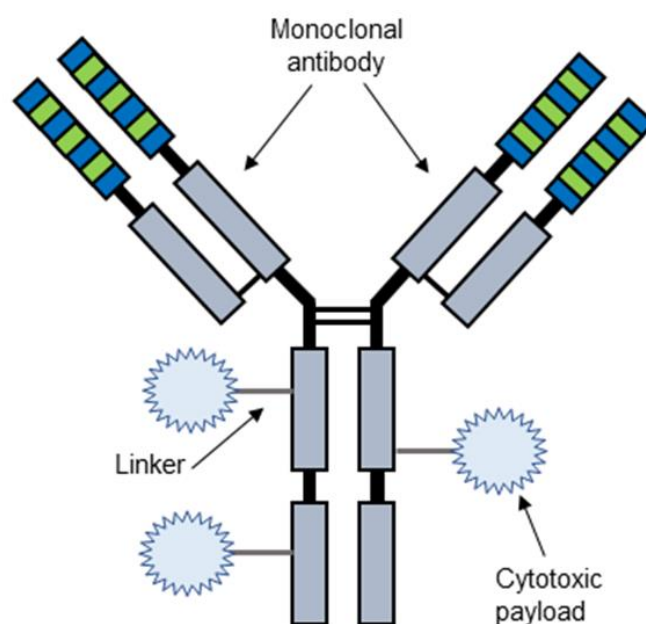

Figure 1. Schematic description of an ADC.

It is utmost importance to understand the key features of each of the three components, and so the mechanism of action, in order to be able to develop ADCs with clinical efficacy. A general mechanism of action of ADCs is represented in Figure 2. In order to avoid the proteolytic degradation of mAbs by gastric acids and proteolytic enzymes, the ADCs are preferentially administered via the intravenous route and released into the bloodstream (Figure 2, step 1). When circulating into the bloodstream the mAb component of ADCs is recognized by tumour-specific or tumour-associated cell-surface antigens (Figure 2, step 2) and, subsequently, the ADC-target antigen complex is primarily internalized via clathrin-mediated endocytosis (Figure 2, step 3) [18]. After the formation of a clathrin-coated early endosome, the acidic environment of endosomes promoted by an influx of H^{+} ions enables the association of the mAb unit of a fraction of ADCs to human neonatal Fc Receptors (FcRns) present in early endosomes. The ADC-FcRn complexes are released into the

bloodstream, where the physiological pH of 7.4 facilitates the dissociation of the ADC from the FcRn (Figure 2, step 4) [19]. Inside the cancer cell, the remaining unbound ADCs in the endosome form the late endosome. Afterwards, the late endosomes fuse with the cell lysosomes, allowing the lysosomal degradation of the immunoconjugate (Figure 2, step 5) and subsequent release of the cytotoxic drug in its bioactive form (Figure 2, step 6). The released cytotoxic drug interferes with the cellular machinery through various mechanisms of cell death, depending on the class of the cytotoxic drugs [20].

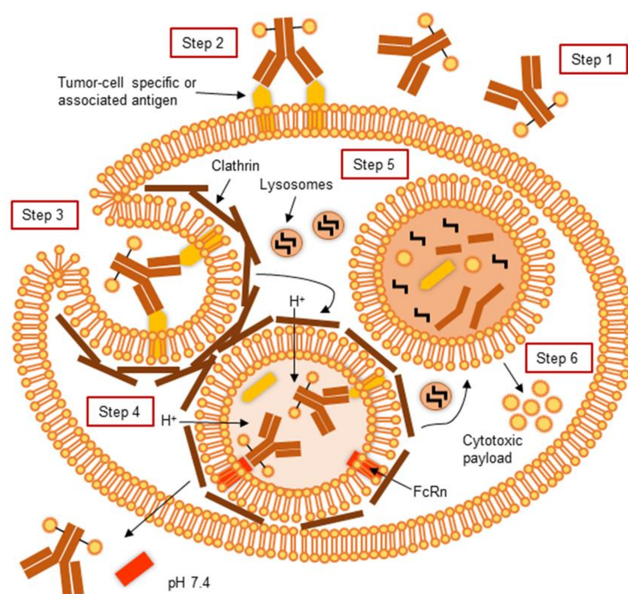

Figure 2. Mechanism of action of ADCs.

From the intravenous administration of ADCs and circulation into the bloodstream to the molecular target located in the tumour tissues, the ADCs are exposed to different conditions on their journey. The understanding of the mechanism of action of ADCs at cellular and molecular level as well as the distinct challenges faced by ADCs in each step may provide new insights for the design of novel immunoconjugates with desirable pharmacokinetic and pharmacodynamics properties through optimization of each of ADC components [21]. The first-generation of ADCs were typically used to selectively deliver clinically approved chemotherapeutic drugs such as methotrexate (a competitive inhibitor of dihydrofolate reductase), doxorubicin (a DNA intercalating agent), and vinblastine (an inhibitor of microtubule formation) with a well-established mechanism of action and a well-known toxicity profile [22-23]. These early experiments which were made to create an effective ADC have revealed to be unsuccessful for various reasons including the low potency of the loaded chemotherapeutic drugs, the restricted number of cytotoxic drug molecules that can be carried on the mAb without impairing the antibody-antigen interaction, the use of unstable linkers, the low antigen selectivity, and the restricted number of antigen molecules on the surface of the tumour cells, all of which limit the therapeutic efficacy of these immunoconjugates [24]. Moreover, the first-generation ADCs, which were achieved by using murine mAbs, have shown to induce a significant immunogenicity, which is a major determinant for half-life of ADCs when circulating in the blood. The unwanted immunogenicity compromises the clinical efficacy of these immunoconjugates [25-26]. To overcome these challenges and failures, several technical approaches have been focused intensively on the optimization of the functionality of each component of the ADCs (the antibody, the cytotoxic payload, and the linker) for the construction of drug delivery systems with improved efficacy and tolerability.

The selection and optimization of antibodies have been extremely relevant in the ADC design. For direct delivery of cytotoxic drugs to tumour cells, the humanized and/or fully human mAbs with high target-affinity and target-specificity to the same antigen determinant are preferentially used to minimize immunogenicity issues [25-26]. The ideal antibody also needs to target well-characterized and tumour-specific or tumour-associated antigens with sufficient binding affinity and specificity. In addition, the antigen-binding characteristics of antibodies are needed to be maintained when connected to the required number of cytotoxic drug molecules via linker [27].

The choice of cytotoxic payloads is of maximum importance for the development of highly effective ADCs [28]. It is imperative that the cytotoxic drug loaded on the ADC possesses a sustained stability for circulation in the blood stream in order to avoid the damage of non-tumour cells and increase the drug bioavailability in tumour cells. Most of these cytotoxic drugs target mainly the DNA (these are cytotoxic for tumour and non-tumour cells) or the microtubules (these are cytotoxic for tumour cells), and should possess a cytotoxic potency in the nano-molar or pico-molar concentrations so that only a small number of cytotoxic drug molecules can be loaded [29]. In addition, the solubility of the cytotoxic drugs is also a critical factor. In fact, the lipophilic drugs can pass the cell membranes and, therefore, after the lysosomal degradation of the ADC complex, the cytotoxic drug has the ability to escape from the lysosome. On the other hand, the cytotoxic payload must be sufficiently hydrophilic to enable conjugation with the antibody in aqueous solutions since the use of high concentrations of organic solvent can promote the denaturation of mAbs. The problem of low water solubility of many drug candidates may be solved by using hydrophilic linkers [30].

The linker chemistry plays an important role since it may greatly influence the safety, the therapeutic index, the specificity, the pharmacokinetic, and the pharmacodynamics profiles of ADC species [26, 31]. The ideal linkers must fulfil a set of requirements towards the development of ADCs with potential clinical efficacy [32]. Firstly, they need to be stable in the bloodstream to ensure that the ADCs remain intact until being recognized by tumour-specific or tumour-associated antigens of cancer cells and reaching the molecular target. Instability of the linker moiety can induce the premature release of the cytotoxic drug, leading to an undesired damage of healthy cells and to other adverse

effects as well [33-34]. Simultaneously, once the ADC species are internalized into the cancer cells, the linkers need to be labile to allow a rapid cleavage and release of the cytotoxic drug in the active form [33-34]. Based on these parameters, research efforts have been focused in the design of novel ADC linker structures. The latter can be classified into two major classes based on the mechanism of release of the cytotoxic drug from immunoconjugate: cleavable and non-cleavable linkers. Cleavable linkers have the characteristic to be cleaved by proteolytic enzymes in the lysosome or by responding to environmental differences present between conditions of blood stream and the intracellular region within tumour cells. The majority of ADC species possess cleavable linkers. Once the ADC-antigen complex is internalized, the change of environmental conditions promotes the cleavage of the linker and subsequent release of the cytotoxic drug molecules in their active form [35]. Cleavable linkers have the ability to respond to different cancer-specific intracellular conditions and they include the linkers that are sensitive to lysosomal degradation (e.g. dipeptide linkage consisting of valine and citrulline along with a *p*-aminobenzylcarbamate linker [36]), which are sensitive to an acidic pH (e.g. hydrazones) [37], and reduced by glutathione such as disulfide linkers [38]. Contrary to the cleavable linkers, the non-cleavable linkers rely on cytosolic and lysosomal proteases to ensure the complete cleavage of the mAb component of ADC species. After the cleavage, a single amino acid residue (usually a lysine or a cysteine) derived from the degraded mAb is still attached to the linker and the cytotoxic drug molecule [39]. Subsequently, the cytotoxic drug-linker-amino acid residue complex is then liberated into the cytoplasm to become the active drug. Examples of non-cleavable linkers include the thioether linker Succinimidyl-4-(*N*-Maleimidomethyl) Cyclohexane-1-Carboxylate (SMCC) and maleimidocaproic acid linker [40]. In this case, the structure of the cytotoxic drug must be precisely designed so that the cytotoxic drug can induce a similar or an improved anticancer activity in a modified form. Additionally, the toxicity, pharmacodynamic, and pharmacokinetic profiles of all possible products of degradation of ADC species containing non-cleavable linkers need to be carefully examined [39].

The conjugation chemistry has been also a crucial component for the construction and activity of ADCs and novel conjugation techniques are continuously being developed. Chemical and enzymatic conjugation are two techniques that are presently in use for the association of mAb and the cytotoxic payload components. [41]. Traditional strategies for the association of cytotoxic drugs to mAbs have been focused on the presence of reactive side chains of solvent accessible naturally occurring amino acid residues including the epsilon-amino end of the lysine residues and the thiol groups of cysteine residues in the reduced form, as attractive sites for conjugation [42-44]. This linking strategy involving native amino acid residues does not require a prior modification or processing of the antibody. However, the conjugation of the cytotoxic drug is restricted to the amino acid sequence of mAb, limiting the control over the number and the site of the loaded cytotoxic drugs. Consequently, a heterogenous mixture of ADC species with variable Drug-Antibody Ratios (DARs) and distinct tethering positions will be generated. The heterogeneity of DAR among ADC species is a major shortcoming of these types of non-specific conjugation techniques, influencing the stability, the efficacy, and the toxicity of ADC species [45-46]. In fact, a broad distribution of the number of cytotoxic drug molecules tethered per antibody, leads to a small percentage of ADC species being therapeutically active and, therefore, contributes to a reduced therapeutic efficacy. The attachment of too few cytotoxic molecules will reduce the efficacy. On the other hand, loading too many cytotoxic molecules on the antibody may affect the stability of ADCs, may lead to the premature release of the cytotoxic payload into the bloodstream and alter the Pharmacokinetics (PK) properties, including high plasma clearance and reduced half-life [45-46]. Moreover, the inactive ADC species can indirectly reduce the ADC efficacy by interacting with a restricted number of tumour-specific or tumour-associated antigens of tumour cells or by blocking the interaction of active ADC species [45-46]. The development of site-specific drug conjugation strategies has emerged as a promising strategy for the production of homogenous ADC species with the desired DAR, potentially enhancing the therapeutic window, decreasing the off-target toxicity, and improving the PK profile. Various approaches used to increase the site specificity of ADC conjugation involve the incorporation of more discriminate residues, in particular non-natural amino acids (e.g. Selenocysteine (Sec) [47], *p*-AcetylPhenylalanine (pAcPhe) [48], *p*-AzidoMethyl-L-phenylalanine (pAMF) [49], N^6-((2-azidoethoxy)carbonyl)-L-lysine [50]), the use of ligating enzymes to catalyse bond formation between specific amino acid sequences or chemical groups (e.g. Sortase A (Sort A) [51], Bacterial TransGlutaminases (BTGs) [52-53], Formylglycine-Generating Enzyme (FGE) [54]), the incorporation of aldehyde groups on *N*-glycan terminus of Asp297 residue of IgG using β-1,4-GalactosylTransferase (GalT) and α-2,6-SialylTransferase (SialT) [55] [56]. In general, the introduction of selectively reactive molecules at specific positions enables a more control over the number and the position of the loaded cytotoxic drug molecules.

1.2. Applications of ADCs in clinical therapeutics

Advances in the research and development of novel ADCs have allowed the approval of several of this type of targeted drugs by the US Food and Drug Administration (FDA) and European Medicines Agency (EMA) for clinical use [57]. Moreover, a large number of complexes are still under clinical trials. The ADCs are being tested in both haematological malignancies such as leukaemia and in solid tumours [58] in which breast cancer is the most common target [59-60]. Currently approved ADCs and those under advanced clinical development (Phases III and II) are listed in Table 1.

Table 1. Selected ADCs in clinical development.

ADC	Main indication	Target antigen	Antibody type	Cytotoxic drug	Status/Phase	Reference
Lorvotuzumab mertansine	Small-cell lung	CD56	Humanized IgG1	DM1	Phase II	[73-74]
Coltuximab Ravtansine	DLBCL; acute	CD19	Chimeric IgG1	DM4	Phase II	[69-72]
Glembatumumab vedotin	Breast cancer,	Glycoprotein NMB	Human IgG2	MMAE	Phase II	[68]
Lifastuzumab vedotin	Non-small-cell	NaPi2b	Humanized IgG1	MMAE	Phase II	[24]
Polatuzumab Vedotin (DCDS450	DLBCL and	CD79b	Humanized IgG1	MMAE	Phase II	[67]
Pinatuzumab vedotin (RG-7593)	Diffuse large B-	CD22	Humanized IgG1	Monomethyl Auristatin	Phase I/II	[66]
Depatuxizumab Mafodotin	Glioblastoma	EGFR	ABT-806	Monomethyl Auristatin	Phase II/III	[64-65]
Inotuzumab ozogamici	Acute lymphobla	CD22	Humanized IgG4	Calicheamicin	Phase III	[63]
Trastuzumab emtansine	Degenerated or	HER2	Humanized IgG1	Emtansine (DM1)	FDA approved	[62]
Brentuximab vedotin	Relapsed Hodgkin	CD30	Chimeric IgG1	Monomethyl Auristatin	FDA approved	[61]
Gemtuzumab ozogamici	acute myelogeno	CD33	Humanized IgG4	Calicheamicin	FDA approved	[24, 58]
MM-302	Epithelial cancer	HER2	anti-HER2 scFv	Liposomal doxorubicin	Phase II	[82-85]
IMMU-130	Colorectal cancer	CEACAM5	Labetuzumab	SN-38	Phase II	[80-81]
MLN-0264	Gastrointestinal	Guanylyl cyclase C	Human IgG	MMAE	Phase II	[79]
Labetuzumab-SN-38	Colorectal cancer	carcinoembryonic	Humanized IgG1	Irinotecan metabolite (SN-38)	Phase II	[78]
PSMA	Prostate cancer	Prostate-specific	Human IgG1	MMAE	Phase II	[77]
Indatuximab Ravtansine	Multiple myeloma	CD138	Chimeric IgG	DM4	Phase II	[75-76]

The knowledge of the detailed three-dimensional (3D) structure is fundamental for full understanding and development of ADCs with higher efficacy. In this review, we focused on a variety of up-to-date methods that can be used to attain 3D structure, energetics and dynamics of these emerging systems including their interactions with the target protein (antigen).

1.3. Computational methodologies used for optimization of ADCs

With the aim of developing ADCs with high specificity and efficacy various computational approaches are currently in use. In particular, the computational methods which rely on Machine-Learning (ML) approaches, can be used as effective tools for the selection of the best target for ADCs. Also, homology modelling and molecular docking and refinement can help devise the most probable fit between antibodies and antigens and to understand the linker-drug interaction. This is a crucial step in ADC development, as it determines the specificity of the molecules in the overall

system. Molecular dynamics (MD) is also another tool to clarify aspects that consider antibody and antigen. Particularly, MD can be used to help for the selection of the best drug candidate by exploring possible conformations that the antibody might acquire, rendering important information on drug-antibody complementarity. Other tools have been also discussed in order to assess the best approaches for selecting the best ADC candidates by considering their selectivity and effectivity, which is mostly inherent to the drug.

1.3.1. Computational selection of ADC targets

Within the scope of targeted cancer treatment, we should also take into consideration the properties of the target antigen in addition to those of the ADCs [86]. Target selection is based on the following criteria: i) target expression on tumour cells - the target must be overexpressed exclusively on the respective tumour cell while its population must be as low as possible on other cells. This allows the ADC to be specific; ii) the outcome associated with the target's expression – chemogenomics information is the key aspect regarding this step. Fundamentally, it is an umbrella term that encompasses high-throughput techniques for simultaneous screening of both cell and compound libraries. By doing so, a high dimensional information can be produced regarding genomic information of cells and biological activity of compounds studied [87]; iii) extraction of relevant data regarding the target from the literature – several approaches for Text-Mining (TM) have been developed and are reviewed in Yang *et. al* [88], iv) target's subcellular location. TMs typically focus on determining likely target-disease or network-disease associations. Some tools also provide a mean to analyse data that come from microarray and mass spectrometry and to establish important phenotype-genotype relations.

A recent ML approach, which is based on the existence of some receptors on the cell surface, microarray data and cell characterization methods for epithelial, mesenchymal or mixed, have been developed for the selection of targets of ADC [89]. ML was used mainly to classify the cells' receptor expression using gene expression data and to classify the cells as epithelial, mesenchymal and mixed. Genes were identified as good targets for ADC therapy considering their normal/cancer expression ratio. The method reported Human Epidermal growth factor Receptor 2 (HER2) as the most probable target, for which trastuzumab emtansine – an ADC – interacts as given in Table 1.

Pharmacokinetics-pharmacodynamics models have also been used for development of ADCs. The key aspect of these models is the determination of the stability, permanence, binding kinetics and efficacy of ADC *in vivo*. While these priorities can be assessed via *in vitro* models, *in silico* approaches, which are based on mathematical modelling of ADC pharmacokinetics and pharmacodynamics [90], can also be used. The Conjugation in ADC development refers to the computational process used to investigate the structural basis of antibody-drug assembly. For this process, a linker can also be used. A simple minimization step could be implemented to ensure that the most realistic conformation is achieved [91-92]. There is also an interest in statistical models to correlate the Drug-to-Antibody Ratio (DAR) with other ADC characteristics, such as drug load distribution [93]. This kind of approaches allow researchers to perform a lower number of experiments to investigate the optimal DAR when aiming to reach an optimal value.

The study of ADCs must also focus on drug resistance mechanisms. Proteins associated with drug resistance, such as Multi-drug Resistance Proteins (MRP), Permeability-Glycoprotein (P-gp) and Breast Cancer Resistance Protein (BCRP) [94] are needed to be studied in detail, in order to prevent expelling of drugs/toxins from the inside of the cell to the outside which causes an increase in the dosage used [95]. For instance, there are strategies to blockade these proteins by adjuvant drugs that prove that conjugated cytotoxic drugs are a poor substrate for the drug transporter [29]. To this and, an MD simulation on the protein, which displays drug resistance, together with the bound drug can be performed to determine the binding affinity and the expulsion possibility of the drug from the cell as done for P-gp for optimal drug delivery [96].

The current methods used for production of ADCs can lead to a heterogeneous mix where some of the molecules have poor performance. For such, more complex methods are required to increase the homogeneity of the conjugates [97]. For example, site-specific cysteine conjugation can be used to increase the homogeneity of the produced conjugates. To this end, three coordinate files regarding the drug, the antibody and the linker were selected from the Protein Data Bank (PDB). Subsequently, these could serve as input for THIOMAB^{TM} [98], which refers to antibodies with engineered reactive cysteine residues. According to it, the molecules are chosen and engineered to include cysteine residues that can be used to link the three components. The molecules can then be aligned according to well-defined equations, changing the relative positions of the atoms by Affine transformations. This procedure allows the preservation of the points, lines and planes while rotating and translating the atoms and residues to a proper conformation [99]. A more thorough explanation of the whole procedure can be found at Filntisi *et al.* [100]. Voynov *et al*. has also computationally designed antibody cysteine variants through homology modelling and evaluated them for their Spatial Aggregation Propensity (SAP) [101]. Besides using antibody-cysteine variants, it is also possible to incorporate amino acids into the backbone of the antibody or enzyme, an approach that exploit short peptide sequences involved in posttranslational modifications [102]. Independent of the method, molecular modelling approaches –in general- provide a platform for systematic ADC generation, thus shortening the experimental time required for development of effective ADCs.

1.3.2. Molecular docking of antibody-antigen complexes

The successful application of ADC as anticancer therapeutics is particularly dependent on the cytotoxic potency of the cytotoxic payload and the ability of antibodies or immunoglobulins to selectively recognize unique conformations and spatial hot points located at the surface of antigens with exquisite specificity and a high binding affinity. The association of the antibody molecule to the cognate antigen is driven by a considerable number of non-covalent interactions taking place between the binding site of antigens and antibodies, which are known as respectively the epitope and the paratope. Among them are electrostatic interactions, hydrogen bonds, van der Waals interactions,

and hydrophobic interactions [103-104]. Structural understanding of antibody-antigen interactions has been the focus in the field of immunological research and pharmaceutical applications, including the design and synthesis of novel epitopes that can be used as vaccines, and novel antibodies with optimized properties. For a holistic understanding of the structural basis of antigen-antibody complexes, the knowledge regarding the 3D structure of these complexes is fundamental. The structure determination of protein-protein complexes has been mainly accomplished by two experimental techniques: X-ray crystallography and Nuclear Magnetic Resonance (NMR) Spectroscopy. In fact, inherent fluctuations of atoms that make up protein-protein complexes make the crystallization difficult, while the structures of complexes formed by high-molecular weight proteins are difficult to study with NMR Spectroscopy [105]. Such technical shortcomings associated with these experimental approaches have been reflected by the disparity between the number of experimentally solved protein-protein complexes which are deposited in the PDB and the number of complexes of structures of the individual proteins [106]. Over the past decade, the development of a large number of algorithms for predicting structure of protein-protein complexes by computational docking has contributed to gain additional insights on the structure of biologically/biochemically relevant protein-protein interactions, as in the case of antigen-antibody complexes. In fact, protein-protein docking has emerged as one of the most focal points in computational proteomics and structural biology. It predicts the most likely quaternary structure for protein-protein complexes using the information coming from individual proteins that make up the complex [107-109]. The first key step is the generation of the structures of the individual antigen and the antibody. In a "bound" docking procedure, the protein structures within a co-crystalized complex are dissociated and re-docked using a docking algorithm. No conformational changes are involved in this procedure, so that the interfaces of the protein structures can match ideally. The "bound" docking is unlikely to provide additional structural information if an experimentally determined protein-protein complex is already available. In an "unbound" docking procedure, the separated proteins are originated from experimentally determined structures either in the free form or associated with a different binding partner. The approaches of "unbound" docking have to deal with the drastic conformational changes occurring between the unbound and bound protein structures, as in the case of antibody-antigen complexes [110]. When no 3D structure information of the interacting proteins is available, the accurate prediction of the most likely bound conformation of protein-protein complexes can be a major bottleneck, particularly due to the inclusion of errors associated with "double modeling" (the modeling of the separated proteins and the modeling of the protein-protein complex) [109]. Homology modeling techniques can be employed for the construction of atomic resolution model of the target proteins by using its query amino acid sequence and an experimentally available 3D structure of a related homologous protein, which can be used as a template. Modeller software, which is one of the widely used homology modelling tools [111], provides a simplified approach for modelling by using a single template. There are also more complex endeavors which consider multiple templates, and include various parameters such as secondary structure, salt-bridges, and many other characteristics. In the context of antibody-antigen complexes, a plethora of computational tools has been used for the prediction of the structure of antibodies and for the mapping of epitopic regions. A special focus to the prediction of antibody structure and antigenic epitopes is given below. Having chosen or generated the starting structures of antibody and the antigen, both proteins are then brought together by a specific docking algorithm. The identification of the most likely conformations of both proteins to form a stable complex must involve the exploration of a large conformational space representing various potential binding poses of the binding partners and the prediction of the interaction energy associated to each of the predicted binding poses [107-109]. In order to find the correct orientation, the relative position of the binding partners is constantly changed through a cyclic and iterative process, in which the different binding conformations are evaluated by scoring functions, until converging to a minimum energy conformation. The speed and effectiveness are two critical parameters in a conformational search procedure, in order to cover the relevant conformational space [107-109]. Subsequently, the most likely conformations of the docking partners of a protein-protein complex can be discriminated from the inaccurate ones by using other scoring functions. The correct binding conformation is assumed to be the most energetically favored, and thus the conformation with the lowest energy. In sum, scoring functions are estimated mathematical functions that should include and appropriately weigh all the physicochemical parameters, including intermolecular interactions, desolvation, and entropic effects. In principle, the greater the number of evaluated physicochemical parameters or the better they are evaluated, the greater the accuracy of the scoring function [107-109]. Nevertheless, as the computational cost also increases proportionally with the number of included parameters, an effective scoring function should provide a perfect combination between the accuracy and the speed of the calculation. Several software packages have been used for antibody-antigen docking, including Profacgen [112], SnugDock [113], surFIT [114], PIPER [115] with the antibody-Decoy As the Reference State (antibody-DARS) potential [116], Zhiping DOCKing (ZDOCK) [117] with the antibody i-Patch potential [118].

1.3.3. Prediction of antibody structure

Antibodies or immunoglobulins are large glycoproteins that consist of a tetramer of two identical pairs of polypeptide chains, namely the heavy and the light chains, which are linked by disulphide bonds to form the arms of a Y-shaped structure (Figure 3).

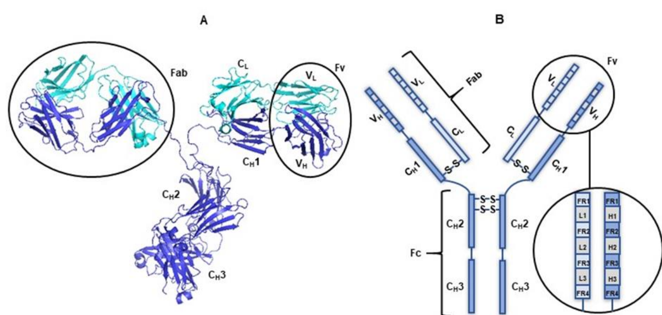

Figure 3. A) The 3D structure of an antibody molecule (PDBid: 1IGT) [119]. **B)** A schematic representation of the antibody scaffold.

Each arm is composed of two variable domains (V_H in the heavy chain and V_L in the light chain) and two constant domains (C_H1 in the heavy chain and C_L in the light chain) [120]. The interaction of C_H1 and V_H domains of heavy chains with the C_L and V_L domains of light chains composes the Fragment, antigen binding (F_{ab}) or the "arms" of the Y. Within the F_{ab}, the V_H and V_L domains dimerize to make up the F_V fragment which is located at the NH_2-terminal domain of each arm and it is responsible for antigen binding [120]. The F_V fragment is a central region for the occurrence of complex processes of V(D)J recombination and somatic hypermutation [121-122]. These events are responsible for the production of a highly diverse repertoire of antibodies, which are able to recognize a variety of antigenic determinants, the so-called epitopes. The V_H and V_L domains of F_V fragment are subdivided into the HyperVariable (HV) and the Framework Regions (FRs). While HV regions display a high amino acid sequence variability among different antibodies, the FR regions are highly conserved both in sequence and in conformation. Within the V_H and V_L domains, three HV regions of each chain (L1-L3 for the light chain and H1-H3 in the heavy chain), often referred as Complementarity Determining Regions (CDR), form the region of the antibody, the so-called paratope, which is in direct contact with the surface of the antigen. The four FR regions of variable domains form *β*-sheets that provide the structural scaffolding to hold the HV loops in contact with the antigen. For each heavy chain, two additional constant domains, C_H2 and C_H3, build up the crystallizable (F_c) region which is able to bind to various cell receptors and determines the mechanism of the immune system depending on the antibody isoform (IgG, IgM, IgA, IgE, and IgD).

The primary differences among distinct antibody molecules reside on the conformation, structural context, and the amino acid sequence of the *β*-sheet part of the variable domains of the F_V fragment that governs the specific binding of the antibody. The prediction methods which are developed for prediction of structures of antibodies have been focused on modeling the F_V region. This prediction is critical for elucidation of the principles that govern antibody-antigen recognition and also for development of novel antibodies with an enhanced affinity and specificity. From an experimental point of view, the task of predicting the structure of an antibody involves mainly two fundamental steps: i) the prediction of the structurally conserved FRs and ii) that of the HV loops. The structural conservation of FRs is remarkably high among the F_V fragments that belonging to distinct antibodies which makes construction of reliable models of FRs possible [123-125]. However, the FRs of light and heavy chains might need to be modelled using different antibody templates, which can be problematic for assembling the heavy and the light chains of the variable domains. A correct packing of the heavy and the light chains is crucial for an accurate orientation of the antigen-binding site of the F_V domain [126].

On the other hand, the structure prediction of CDR loops either by homology modeling techniques or *ab initio* calculations still remains as a challenging task [127-128]. Despite the high amino acid sequence variability, the investigation of crystal structures of antibodies has shown that the conformational diversity in five of the six CDR loops (L1, L2, L3, H1, and H2) is limited. In fact, these five loops can adopt a few number of different conformations, forming a set of discrete conformational classes, which is known as canonical structures [129-130]. Interestingly, the canonical conformations adopted by a specific CDR loop depend exclusively on its length and the identity of specific amino acid residues which are located in key positions both within and outside of the loops. The identification of a specific pattern of amino acid residues at certain positions, which are thought to dictate the structure, can be used to predict the canonical class of a CDR loop with unknown structure with high accuracy solely from its amino acid sequence [131-132]. In contrast to the other five CDR loops, no such canonical structures have ever been established for the H3 loops due to the large variability on the loop length, amino acid sequence, and the structure. Additionally, the H3 loop is located at the interface of V_H and V_L domains and interacts with residues located at both chains. The preservation of V_L–V_H domain orientation is of utmost importance in the process of antibody engineering in order to maintain the original topology of the antigen-binding site. Therefore, the V_L–V_H domain orientation needs to be optimized during the modelling of H3. The accurate modeling of H3 loops and the preservation of V_L–V_H domain orientation remain as the most challenging tasks in the field of antibody structure prediction [133-134]. Several algorithms developed for predicting the protein loop structures can be mainly divided into two categories, depending on how they generate the pool of conformations: i) knowledge-based, where databases of fragments are searched to find possible conformations and ii) *ab initio*, where conformations are generated computationally from scratch. These algorithms include the AntiBody structure GENeration algorithm (ABGEN) [135], Accelrys Tools [136], BioLuminate and Prime [137], CODA [138], FREAD [139], H3Loopred [140], Kotal Antibody Builder [141], Molecular Operating Environment (MOE) [142], Prediction of ImmunoGlobulin Structure (PIGS) [143], RosettaAntibody [144], SmrtAntibody [145], Sphinx [146], Web Antibody Modeling (WAM) [147] (Figure 4).

1.3.4. Prediction of antigen epitopes

Antigen epitopes, often referred to as B-cell epitopes, are molecular structures contained in the antigen that make specific interactions with the antibody paratopes. On the antigen side of the interaction, an accurate identification and characterization of epitopes on target antigens is of utmost importance for immunological research and other medical applications. Experimental methodologies used for the identification of antigenic epitopes, namely X-ray crystallography, phage display, mass spectrometry, and

mutagenesis analysis have shown to be expensive, labour-intensive, time-consuming, and ineffective for the identification of many epitopes. Therefore, there is an urgent need for development of manageable and reliable computational tools for the prediction of the presence and the location of antigenic epitopes. The problem of predicting potential epitopes for antibody-antigen complexes has been widely explored by several research groups using various *in silico* tools [149-150]. In general, the epitopes are described as linear or continuous, when the antibody interacts with a continuous stretch of amino acid residues located on the surface of an antigen, and as conformational or discontinuous, when the antibody interacts with segments of amino acid residues that are distantly separated in the protein sequence, but are brought into physical proximity within the folded protein 3D structure (Figure 5) [151]. The specificity of Linear Epitopes (LEs) is driven by the sequence and conformation of amino acid residues that make up the protein antigen. On the other hand, the specificity of Conformational Epitopes (CEs) is dependent on the 3D folding and conformation of LEs [152]. In the past, researchers had been focusing on the development of algorithms for the prediction of LEs that rely on properties that can be extracted from the linear sequence of the antigen. Currently, several algorithms are available for the prediction of LEs, including ABCpred [153], BCEPred [154], BCPreds [155], BepiPred [156], BEPITOPE [157], B-cell Epitope prediction using Support vector machine Tool (BEST) [158], COBEpro [159], Linear B-cell epitope (LBtope) [160], Linear Epitope Prediction System (LEPS) [161-162], Predictive Estimation Of Protein Linear Epitopes (PEOPLE) [163]. In general, most of these algorithms evaluate a number of physicochemical properties, namely hydrophilicity, secondary structure, segmental mobility, flexibility, antigenicity, and surface accessibility to predict antigenicity, the amino acid residues of antigen sequence, and then employ several ML algorithms, such as Support Vector Machine (SVM) or Artificial Neural Network (ANN) algorithms, to predict LEs. However, it has been estimated that approximately 90% of all antigenic epitopes are CEs [164], and therefore the focus on the identification of CEs is a more practical and profitable approach. The prediction of CEs has demonstrated to be a tremendous challenge in bioinformatics. The existing *in silico* methodologies for the prediction of CEs require the 3D structures of the antigen and/or antigen-antibody complexes. The use of CEs derived from available X-ray structures can be extremely complex. The reduced available data on CEs in different antigens compared to the data of LEs and the relatively small number of solved structures of antigen-antibody complexes can restrain the development of reliable and accurate methods for the prediction of CEs [165]. For CE prediction, several algorithms have been developed including B-cell Epitope prediction by Evolutionary information and Propensity (BEEPro) [166], BepiPred-2.0 [167], B-Pred [168], Bpredictor [169], Conformational B-cell epitope (CBtope) [170], Conformational Epitope prediction based on Knowledge-based Energy and Geometrical neighbouring residue contents (CE-KEG) [171], Conformational Epitope Prediction (CEP) [172], DiscoTope [165], DiscoTope-2.0 [173], ElliPro [174], Epitope Prediction by ConsEnsus Scoring (EPCES) [175], EPITOPIA [176], Epitope Prediction by Support Vector Regression (EPSVR) [177], PEPITO [178], PEPOP [179], Spatial Epitope Prediction of Protein Antigens (SEPPA) [180], SEPPA-2.0 [181].

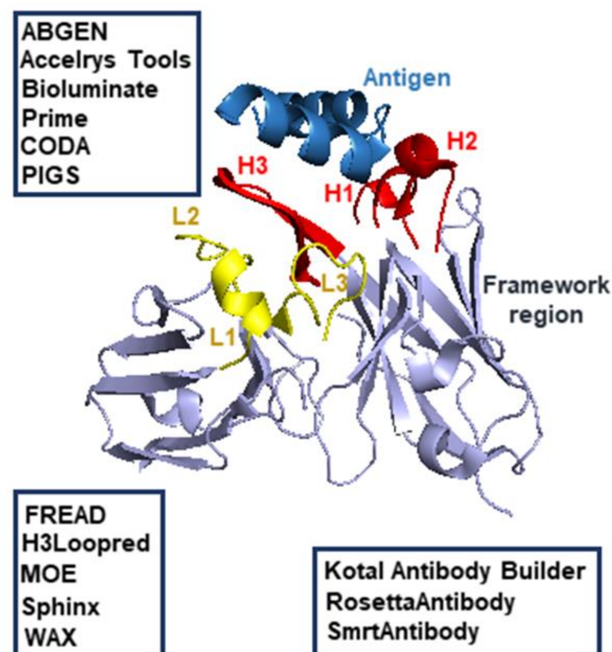

Figure 4. Representation of the variable region of the antibody (PDBid 4G6F) [148]. Algorithms which are used for the structure prediction of CDR loops of antibodies are shown in blue rectangles.

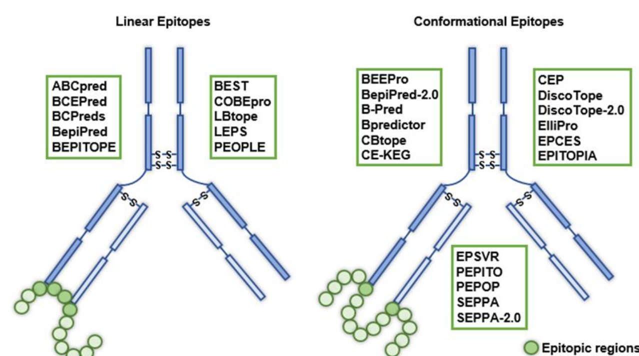

Figure 5. Schematic representation of the antibody-linear epitope and antibody-conformational epitope interactions. The distinct *in silico* tools used for the prediction of linear and conformational epitopes of a protein antigen are described in green rectangles.

1.3.5. Computational methods used to calculate energy of ADCs

In this section, we reviewed computational tools which are used to estimate binding free energy of an ADC to its target antigen which is exclusively expressed on the surface of the tumour cell. In particular, we focus on methods such as umbrella sampling and steered MD simulations, to which Jarzynski's equality is applied, for estimation of the free energy. Moreover, we also focus on computational techniques used to investigate changes in membrane

dynamics upon ADC-antigen complex formation. This kind of knowledge will not only allow getting an insight on the global properties of the system but also help dissect details of the interaction between the ADC and the antigen at the atomic level, thus guiding experimental studies to improve binding affinities and physicochemical properties of this emerging class of therapeutic molecules.

1.3.5.1. In silico estimation of binding free energy of ADC-Antigen Complex

In silico estimation of free energy of binding of an ADC to its antigen requires Cartesian coordinates of the complex, which as already mentioned can be provided by either X-crystallography or NMR data. In the absence of any experimental structure, homology modeling can be done as long as an appropriate template is available as discussed in detail in Section 1.3.1**.** If only the coordinates of individual components of the system are available, but not the complex as a whole, then molecular docking can be used to get possible optimum conformation of the ADC. Before any calculation is made the system should be first minimized to eliminate bad atomic contacts. Subsequently, it should also be relaxed in the presence of both water and membrane to let atoms reorganize themselves in a physiologically similar environment.

1.3.5.2. Umbrella Sampling

Upon obtaining the equilibrated ADC/antigen complex, the free energy of binding can be estimated via umbrella sampling [182-183]. In this technique, the reaction coordinate, ξ , which best describes the process studied, can be restrained, but not constrained, via biased potentials to drive ADC/antigen complex from one thermodynamic state to another (bound and unbound). It should be chosen properly otherwise artificially lower/higher energy values may be obtained [184]. In general, ξ , is defined as distance, torsion, or the Root-Mean-Square Deviation (RMSD) between given two reference points. The probability distribution of the system along ξ can be calculated by integrating out all degrees of freedom but ξ:

$$Q(\xi) = \frac{\int \delta[\xi(r) - \xi]\exp[(-\beta E)d^N r]}{\int \exp[(-\beta E)d^N r]} \quad (1)$$

where $\beta = 1/kT$, and k and T correspond, respectively, to Boltzmann constant and temperature. $Q(\xi)d\xi$ can be given as the probability of finding the system around ξ within a cut-off of $d\xi$. From this, the free energy along the reaction coordinate can be expressed as follows:

$$A(\xi) = -1/\beta \ln Q(\xi) \quad (2)$$

The phase-space integrals, which are given in Equation 1, are impossible to calculate in computer simulations. On the other hand, if the system is ergodic, that is to say, if ensemble average is equal to the time-average, then the ensemble average, $Q(\xi)$, becomes equal to the time-average, $P(\xi)$, which is given as the following:

$$P(\xi) = \lim_{t\to\infty} \frac{1}{t} \int_0^t \int \rho[\xi(t')]dt' \quad (3)$$

The intermediate states in the given process, in particular unbinding of ADC from its antigen, can be covered by certain numbers of windows, each of which is subjected to a separate MD run. This is done to ensure efficient sampling in all regions of ξ . In each window, the system is kept close to the reference point ξ_i^{ref} of the respective window i by means of, usually, a simple harmonic biased potential with strength of K:

$$w_i(\xi) = K/2(\xi - \xi_i^{ref})^2 \quad (4)$$

The strength of the bias potential, K, has to be determined *a priori.* In general, it should be large enough to drive the system from its starting state towards the target state. On the other hand, too large K may cause very narrow distributions leading to generation of non-overlapping windows. This, in turn, necessitates addition of extra windows to fill the gap present in distributions of the neighbouring windows, which is costly in terms of Central Processing Unit (CPU) time. In general, it is suggested to have many windows rather than having fewer windows which are subjected to longer simulation times. This leads to better overlap between windows and thus having smaller statistical errors [185]. If available, experimental data can also be used to determine the most appropriate bias parameters [186]. After simulations are done, as long as overlapping distributions are achieved, the free energy curves obtained in each window can be combined together by using either Weighted Histogram Analysis Method (WHAM) or umbrella integration. The main difference between WHAM and umbrella integration is that the unbiased distributions of the conformations obtained in each window are averaged out in the former, whereas the mean force is averaged out in the latter. This, in turn, allows estimation of the statistical error associated with the free energy in umbrella integration [187]. Subsequently, this can be used for determination of appropriate strength of the bias, K, as well as the optimum number of windows. As a side note, due to the modular nature of ADCs and the assumption that binding of ADC to the target antigen is not affected by the linker and the payload the abovementioned calculations can be done by considering only the antibody part of the ADC.

1.3.5.3. Steered MD simulations with application of Jarzynski's equality

In contrast to umbrella sampling, steered MD simulations rely on non-equilibrium dynamics of the system, in which the motion is driven continuously along the reaction coordinate, ξ , by an external potential function, u. This is done to drive the system from state A to B (in the case of ADC-antigen complex, bound-unbound). The original system is called the intrinsic system and described by the Hamiltonian, $H_0(q,p)$. On the other hand, the perturbed system is called as the extended system and described as the following:

$$H(r,p,\xi_0(t)) = H_0(r,p) + u(r,\xi_0(t)) \quad (5)$$

where $\xi_0(t)$ is used to restrain the system in state A at time 0, and in state B at time τ. If the reaction coordinate $\xi(r)$ is a function of atom positions, $u(\xi(r),t)$ is known as a steering function, which is, in general, centered on a given reference reaction coordinate, $\xi_0(t)$ and chosen harmonic:

$$u(r,t) = k/2\,(\xi(r) - \xi_0(t))^2 \tag{6}$$

where k is the harmonic constant. In this technique, pulling of molecules is usually done by applying a force on one single atom [188-190] in an effort to mimic an Atomic Force Microscopy (AFM) experiment, where ξ is given as the distance between the pulled and a fixed atom. Alternatively, ξ can also be given as the distance between the center of Mass (CM) of protein (e.g. target antigen) and the CM of the ligand (e.g. antibody part of the ADC). In the latter, this corresponds to uniformly applying a force to each atom in the given molecule, which is proportional to its mass. On the other hand, these approaches are not appropriate for big protein complexes bound by a strong interaction like ADC/antigen complex since these two methods can induce either distortions of the tertiary structure or partial unfolding before unbinding. In addition, if the interaction between the ADC and the antigen is spread over a large surface perpendicular to the pulling direction this leads to rotation of the proteins, instead of separating them from each other. To avoid possible distortions and rolling artefacts an alternative scheme can be used [191]. According to it, the reference position of an atom is determined with respect to CM of the respective unit to which it belongs (e.g., ADC if one considers the antibody part). A harmonic potential energy, which is centered on the reference point, is applied only to the z coordinate of the atom, while the movements on the other directions remain free. The positions of the restrained atoms in the two proteins (the ADC and the antigen) are shifted uniformly along the z coordinate in opposite directions. By doing so, in each unit, the reference regions do not change while the CM distance is increased. The free energy differences from steered MD simulations can be recovered using the Jarzynski's identity [192]. In a non-equilibrium process, the external work done on the system from time 0 to τ can be given as follows:

$$W(\tau) = \int_0^{\tau} dt \frac{\partial H}{\partial t}(r, p, \xi_0(t)) \tag{7}$$

where $w(\tau)$ corresponds to the work done by the extended system. The work, $w(\tau)$, in a non-equilibrium process depends on the path taken between the initial and the target state, and hence on the starting condition at time 0. According to the second law of thermodynamics the average work cannot be smaller than the free energy difference between the initial and the final state, $\Delta G \leq \langle W \rangle$. Equality holds only if the process is reversible that is to say, if the work is independent of the path. On the other hand, Jarzynski demonstrated that this equality holds regardless of the speed of the process:

$$e^{-\beta \Delta G} = \left\langle e^{-\beta W} \right\rangle_0 \tag{8}$$

where $\beta = 1/k_B T$. Here, the average is taken over different trajectories each of which starts with different initial velocity, thus having independent canonical distributions. In order to have an accurate estimation of the exponential average it is required to have a large number of trajectories [193-196]. The initial conformations which will be used in individual Steered Molecular Dynamics (SMD) runs can be obtained in two ways. Either independent configurations can be selected from a long reference simulation at equilibrium or, alternatively, different replicas can be run in parallel each of which is started with a different initial random velocity. With the latter approach one can provide a better convergence because: 1) the structures obtained at the end of each short run do not deviate much from the reference one as opposed to those obtained from a long trajectory due to inherent MD inaccuracies, and 2) more diversity is obtained at the end of independent equilibrations than that can be obtained from consecutive frames of a long run. Finally, the bias and errors can be calculated using the scheme developed in [197] and used in [198] for systems having small number of pulling experiments as long as the collection of individual runs displays Gaussian-like distributions.

1.3.5.4. In Silico investigation of changes in membrane dynamics upon formation of ADC/Antigen complex: Possible hint on the capability of the ADC to undergo receptor-mediated endocytosis

In order for ADCs to act properly on the target tissue they must be efficiently taken inside of the cell. Since the formation of the ADC/antigen complex initiates receptor-mediated endocytosis, it is crucial to consider interactions between the complex and the membrane at the atomic level for efficient design of this class of emerging molecules. On the other hand, considering both the time- (on the minute time-scale) and length-scales at which endocytosis occur in mammalian cell lines it is apparent that the process is far beyond the reach of atomistic MD simulations. Therefore, instead of representing whole process, representative part of it, namely remarkable increase in the membrane curvature, can be used as a hint to get an insight on the intrinsic capability of the ADC for undergoing receptor-mediated endocytosis. For instance, the more the membrane curved it is more likely for ADC/receptor complex to undergo endocytosis.

Related to this, a multiscale computational approach has been developed to quantify remodelling of membrane bilayers by multi-helical membrane proteins [199]. In particular, G-Protein-Coupled Receptors (GPCRs) have been shown to display ligand-dependent membrane deformations. Here, the details of the method will not be given and can be found in Shan *et al.* [200]. A similar approach can be applied for investigating changes in the membrane curvature by using a collection of ADCs bound to the target antigen. To this end, as a first step, the coarse-grained representation of the system is constructed, which can be done by using Martini force field [201], and the system is simulated until equilibrium is reached in terms of membrane curvature. Subsequently, the system can be back-transformed to

atomistic representation to get insight into the global properties at the atomistic level. Similar methodology regarding back transforming has been shown to capture successfully the membrane insertion preferences of GPCR transmembrane helices [202-203].

CONCLUSION

Cancer is one of the lethal diseases worldwide, for which the development of novel therapeutics with clinical efficacy is challenging. Currently, targeted immunotherapy acts as an emergent approach as the immune system plays a crucial role in cancer progression. In fact, the use of antibodies provides a selective recognition of specific structures in the body which makes them an attractive tool for selective drug delivery. This constitutes the general basis of ADCs as antibodies are used to transport a cytotoxic drug directly to the specific cancer cell releasing the drug inside of them. Due to its specificity, ADC development is a complex, time-consuming, and expensive process. On the other hand, computational methods can be used to optimize each of these steps effectively. The knowledge of the 3D structure of these complexes as well as their conformational dynamics are fundamental for developing selective antibodies with high affinity. They also allow for a deeper understanding of the interaction between antibodies and their antigens, and the ways to manipulate it. In particular, ML algorithms, molecular docking and MD simulations can be used to predict the best antigen target *in silico*. To elucidate the molecular mechanism in which antibody, cytotoxic drug and target antigen interact with each other, more robust quantum mechanics approaches should be considered. Furthermore, in order to estimate binding free energy of an ADC to its target antigen umbrella sampling and steered MD simulations can be used. Changes in membrane dynamics upon ADC-antigen complex formation can be predicted using these methodologies which may give a hint on the tendency of the complex to undergo endocytosis. To conclude, developing a functional ADC is remarkably challenging but it can still be achieved as long as the experimental methods are complemented with appropriate computational ones.

LIST OF ABBREVIATIONS

ABGEN – the AntiBody structure GENeration algorithm

ADC – Antibody-Drug Conjugate

AFM – Atomic Force Microscopy

ANN – Artificial Neural Networks

Bcl-2 – B-cell lymphoma-2

BCRP – Breast Cancer Resistance Protein

BEEPro - B-cell Epitope prediction by Evolutionary information and Propensity

BEST – B-cell Epitope prediction using Support vector machine Tool

BTG - Bacterial TransGlutaminase

CAM – Cell-cell Adhesion Molecules

CBtope - Conformational B-cell epitope

CDR – Complementarity Determining Region

CE – Conformational Epitopes

CE-KEG – Conformational Epitope prediction based on Knowledge-based Energy and Geometrical neighboring residue contents

CEP – Conformational Epitope Prediction

CM – Center of Mass

CPU – Central Processing Unit

DAR – Drug-Antibody Ratio

DARS – Decoy As the Reference State

DLBCL – Diffuse Large B-Cell Lymphoma

EMA – European Medicines Agency

EPCES – Epitope Prediction by ConsEnsus Scoring

EPSVR – Epitope Prediction by Support Vector Regression

FcRn – Neonatal Fc Receptor

FDA – Food and Drug Administration

FGE - Formylglycine-Generating Enzyme

FR – Framework Region

GalT - β-1,4-GalactosylTransferase

GPCR – G-Protein-Coupled Receptor

HER 2 – Human Epidermal growth factor Receptor 2

HV - HyperVariable

LBtope - Linear B-cell epitope

LEPS - Linear Epitope Prediction System

MAb – Monoclonal Antibody

MD – Molecular Dynamics

ML – Machine Learning

MOE – Molecular Operating Environment

MRP – Multidrug Resistance Protein

P-gp – Permeability-glycoprotein

pAcPhe - *p*-AcetylPhenylalanine

pAMF - *p*-AzidoMethyl-L-phenylalanine

PD - PharmacoDynamics

PDB – Protein Data Bank

PDGF – Platelet-Derived Growth Factor

PEOPLE - Predictive Estimation Of Protein Linear Epitopes

PK - PharmacoKinetics

PIGS – Prediction of ImmunoGlobulin Structure

pRb – Retinoblastoma protein

SAP - Spatial Aggregation Propensity

SE – Sequential Epitopes

Sec - Selenocysteine

SEPPA - Spatial Epitope Prediction of Protein Antigens

SialT - α-2,6-SialylTransferase

SMCC - Succinimidyl-4-(*N*-Maleimidomethyl) Cyclohexane-1-Carboxylate

SMD – Steered Molecular Dynamics

SortA – Sortase A

SVM – Support Vector Machine

TGFα – Tumour Growth Factor α

TM – Text Mining

VEGF – Vascular Endothelial Growth Factor

WAM - Web Antibody Modeling

WHAM - Weighted Histogram Analysis Method

ZDOCK – Zhiping DOCKing

CONFLICT OF INTEREST

The authors declare no conflict of interests.

ACKNOWLEDGEMENTS

Irina S. Moreira acknowledges support by the Fundação para a Ciência e a Tecnologia (FCT) Investigator programme - IF/00578/2014 (co-financed by European Social Fund and Programa Operacional Potencial Humano), and a Marie Skłodowska-Curie Individual Fellowship MSCA-IF-2015 [MEMBRANEPROT 659826]. This work was also financed by the European Regional Development Fund (ERDF), through the Centro 2020 Regional Operational Programme under project CENTRO-01-0145-FEDER-000008: BrainHealth 2020, and through the COMPETE 2020 - Operational Programme for Competitiveness and Internationalisation and Portuguese national funds via FCT, under project POCI-01-0145-FEDER-007440. Rita Melo acknowledges support from the FCT (FCT—SFRH/BPD/97650/2013). This work has been partially supported by the Fundação para a Ciência e Tecnologia (FCT), Portugal, through the UID/Multi/04349/2013 project in Centre for Nuclear Sciences and Technologies (C^2TN).

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Received: March 20, 2014 Revised: April 16, 2014 Accepted: April 20, 2014

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