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Homogeneously modified immunoglobulin domains for therapeutic application

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The field of therapeutic antibodies has been revolutionized over the past decade, led by the development of novel antibody-modification technologies. Besides the huge success achieved by therapeutic monoclonal antibodies, a diversity of antibody derivatives have emerged with hope to outperform their parental antibodies. Here we review the recent development of methodologies to modify immunoglobulin domains and their therapeutic applications. The innovative genetic and chemical approaches enable novel and controllable modifications on immunoglobulin domains, producing homogeneous therapeutics with new functionalities or enhanced therapeutic profiles. Such therapeutics, including antibody–drug conjugates, bispecific antibodies, and antibody/Fc fusion proteins, have demonstrated great prospects in the treatment of cancer, auto-immune diseases, infectious diseases, and many other disorders.

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Introduction

The last two decades have witnessed the rise of therapeutic monoclonal antibodies [1]. To date, over 30 monoclonal antibodies have been approved for the treatment of a variety of disorders, including cancer [2,3], autoimmune diseases [4], infectious diseases [5], among others. Meanwhile, a powerful antibody engineering toolset, with chemical and genetic modification strategies, has been developed for new therapeutic applications. These strategies have successfully yielded diverse antibody-based therapeutic agents. With more and more approved antibody derivatives entering the pharmaceutical market, academic and industrial researchers become increasingly enthusiastic in developing both new strategies to modify immunoglobulin domains and novel applications for the constructs. Here, we will review recent progress in strategies and therapeutic applications for immunoglobulin modification.

Strategies to homogeneously modify immunoglobulin domains

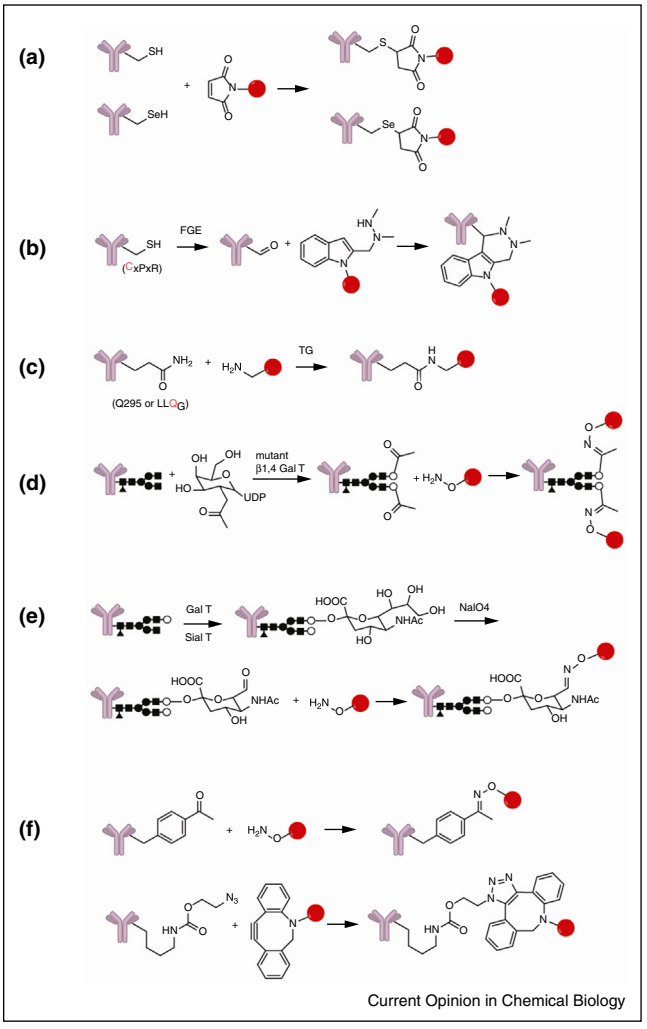
Site-specific chemical conjugation to immunoglobulin domains

Antibodies are excellent vehicles for targeted delivery of functional molecules, including drugs [6], nucleic acids [7,8] and polymers [9]. Recently, it has been demonstrated that the conjugation site has a significant impact on stability and pharmacokinetics of the resulting conjugate [10••]. Thus, current efforts on the development of immunoconjugates toward therapeutic applications have mainly focused on site-specific modifications on immunoglobulin domains to achieve homogeneity, and will be reviewed here.

Introducing an unpaired cysteine residue into the immunoglobulin scaffold is a conceptually simple but technically challenging strategy for site-specific antibody modification at industrial scale [11,12]. Using a phage display-based strategy, scientists at Genentech screened and identified suitable positions to introduce unpaired cysteines on the Fab surface [13]. The resulting antibody (THIOMAB) can be subsequently modified with thio-reactive reagents [14]. Alternatively, selenocysteine (Sec), which has higher nucleophilic reactivity and acidity than cysteine, is more selective toward maleimide and iodoacetamide under bio-orthogonal conditions [15,16]. Engineering of a Sec insertion sequence at the 3' end of the antibody-encoding genes allows genetic incorporation of one or even two [17] Sec at the C-terminus of antibody in response to opal codon [18] that can then be utilized for site-specific conjugation [15–17] (Figure 1a).

To site-specifically modify an antibody, one can also introduce a sequence-specific tag, which can be recognized and subsequently modified by a corresponding enzyme [19–21]. One such example is 'aldehyde tag' (Redwood Bioscience), which utilizes a formylglycine generating enzyme (FGE) to specifically recognize a

Figure 1



Representative examples of site-specific chemical modification to immunoglobulin domains. **(a)** Engineering of cysteine and selenocysteine; chemo-enzymatic approaches using **(b)** formylglycine generating enzyme (FGE), **(c)** bacterial transglutaminase (TG) and **(d, e)** glycoenzyme β1,4-galactosyltransferase (Gal T) and α2,6-sialyltransferase (Sial T); **(f)** genetic incorporation of unnatural amino acids.

sequence tag (CxPxR) and to catalyze the oxidation of the cysteine residue to an aldehyde-containing formylglycine (fGly) residue. This technique allows site-specific insertion of an aldehyde group into the immunoglobulin domain, available for subsequent chemical conjugation [22,23], to yield hydrolytically stable conjugates [24] (Figure 1b).

Another example is the use of bacterial transglutaminase (bTG) to catalyze the formation of an isopeptide bond between a glutamine side chain and the ε-amino group of a lysine residue [25]. Scientists at ETH Zurich found that bTG specifically recognizes Gln295 within the heavy chain of deglycosylated IgGs and conjugates primary amine functionalized small molecules [26,27]. On the other hand, although bTG does not modify glycosylated antibodies [26], an engineered glutamine, within a sequence tag LLQG (glutamine tag), can be site-specifically modified by bTG. Scientists at Rinat-Pfizer scanned 90 modification sites on antibodies, and found 12 sites with good biophysical properties and high conjugation yields [10••,28] (Figure 1c).

Glycoengineering represents another elegant chemo-enzymatic approach to site-specifically modify immunoglobulin domains without introducing mutations in the sequence. Researchers at NCI-Frederick have developed a mutant glycotransferase [29,30], which galactosylates monoclonal antibodies at Asn297 with modified galactoses containing ketone or azide groups [31], suitable for the subsequent bio-orthogonal chemical conjugation [32] (Figure 1d). Alternatively, scientists at Sanofi-Genzyme reported that a mixture of β1,4-galactosyltransferase and α2,6-sialyltransferase grafted terminal sialic acids onto the native glycans of IgG1, which can be oxidized to introduce an aldehyde group [33] (Figure 1e).

Genetic incorporation of unnatural amino acids (UAAs) represents a novel and unique strategy to site-specifically modify immunoglobulin. Our lab and others have reprogrammed the cell's translational machinery by evolving orthogonal tRNA/aminoacyl tRNA synthetase (aaRS) to site-specifically incorporate more than 100 structurally and functionally diverse UAAs into recombinant proteins in response to non-sense codons [34,35]. The modification can be introduced into any position of choice on the immunoglobulin scaffold assuming the folding is not disrupted. In addition, the chemistry is not limited to one type of reaction. In fact, we have demonstrated in several examples that UAAs with chemically reactive groups can be site-specially incorporated into immunoglobulin domains, including (but not limited to) ketones (for oxime reaction) [36,37•], azides (for click chemistry) [38,39•], and acrylamides and vinyl sulfonamides (for Michael reaction) [40]. Moreover, we have shown that two different functionalities can be incorporated simultaneously into one antibody using two orthogonal tRNA/aaRS pairs, which allows the site-specific conjugation of two chemically orthogonal small molecules onto the same antibody [39•] (Figure 1f). Ambrx Inc. has optimized this technique for industrial-scale synthesis of various ADCs [37•]. Alternatively, Sutro Biopharma is developing a cell-free expression system to site-specifically incorporate UAA into antibodies [41].

Genetic modification of immunoglobulin domains

Next-generation antibody-based therapeutics also include genetically modified antibodies or antibody fragments, the