Review

Introduction to current and future protein therapeutics: A protein engineering perspective

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Abstract

Protein therapeutics and its enabling sister discipline, protein engineering, have emerged since the early 1980s. The first protein therapeutics were recombinant versions of natural proteins. Proteins purposefully modified to increase their clinical potential soon followed with enhancements derived from protein or glycoengineering, Fc fusion or conjugation to polyethylene glycol. Antibody-based drugs subsequently arose as the largest and fastest growing class of protein therapeutics. The rationale for developing better protein therapeutics with enhanced efficacy, greater safety, reduced immunogenicity or improved delivery comes from the convergence of clinical, scientific, technological and commercial drivers that have identified unmet needs and provided strategies to address them. Future protein drugs seem likely to be more extensively engineered to improve their performance, e.g., antibodies and Fc fusion proteins with enhanced effector functions or extended half-life. Two old concepts for improving antibodies, namely antibody-drug conjugates and bispecific antibodies, have advanced to the cusp of clinical success. As for newer protein therapeutic platform technologies, several engineered protein scaffolds are in early clinical development and offer differences and some potential advantages over antibodies.

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Abbreviations: FDA, Food and Drug Administration; PEG, polyethylene glycol; TPO, thrombopoietin
Introduction

Since the early 1980s proteins have emerged as a major new class of pharmaceuticals with ~200 marketed products that are mainly therapeutics with a small number of diagnostics and vaccines [1]. Protein therapeutics can be classified based upon their pharmacologic activity as drugs that: i) replace a protein that is deficient or abnormal, ii) augment an existing pathway, iii) provide a novel function or activity, iv) interfere with a molecule or organism, or iv) deliver a payload such as a radionuclide, cytotoxic drug, or protein effector [2]. Alternatively, protein therapeutics can be grouped into molecular types that include: antibody-based drugs, anticoagulants, blood factors, bone morphogenic proteins, engineered protein scaffolds, enzymes, Fc fusion proteins, growth factors, hormones, interferons, interleukins, and thrombolytics (Fig. 1) [1,3]. Antibody-based drugs are the largest and fastest growing class of protein therapeutics with 24 marketed antibody drugs in the USA [4] and over 240 more in clinical development [5].

This introductory article provides a brief history of protein therapeutics, offers a rationale for developing better protein drugs, and identifies some major future opportunities as well as emerging technologies that may meet them. The 30-year history of protein drugs is illustrated here with examples that pioneered their class or have high clinical or commercial significance. The rationale for creating better protein drugs, including antibodies [6], comes from the convergence of clinical, scientific, technological and commercial drivers that collectively have identified major unmet needs and provide strategies to address them. Major future opportunities for protein therapeutics, are improved efficacy, greater safety, reduced immunogenicity or improved delivery. A plethora of emerging technologies for the generation and optimization of protein therapeutics are anticipated to help address these opportunities. Space constraints limit examples here to a sampling of technologies of broad applicability — so called platform technologies. The interested reader is referred to many excellent articles on protein therapeutics including those in this special issue and others cited herein.

Brief history of protein therapeutics

Protein therapeutics enabled by protein engineering

Protein therapeutics and its enabling sister discipline, protein engineering, have emerged since the early 1980s. Protein engineering by rational design or molecular evolution allows the systemic dissection of protein structure–function relationships and the generation of novel proteins with modified activities or entirely new properties. Indeed, protein engineering has revolutionized protein therapeutics by providing the tools to customize existing proteins or to create novel proteins for specific clinical applications.

A brief history of protein therapeutics is provided here from a protein engineering perspective. The focus is on technologies with the greatest impact on current protein therapeutics — engineered versions of natural proteins, Fc fusion proteins, conjugation to the polymer, polyethylene glycol (PEG), and antibody therapeutics as the quintessential protein therapeutic platform.

Insulin – the first recombinant human protein therapeutic

The first human protein therapeutic derived from recombinant DNA technology was human insulin (Humulin®) created at Genentech [7], developed by Eli Lilly, and approved by the US Food and Drug Administration (FDA) in 1982. Recombinant insulin...
was used to replace its natural counterpart that can be deficient in diabetes mellitus. Other recombinant human proteins were soon developed as therapeutics to replace the natural proteins deficient in some patients (e.g., growth hormone) or augment existing pathways (e.g., interferon-α, tissue plasminogen activator, and erythropoietin) [2].

**Beginning of engineered proteins as therapeutics**

Beyond recombinant versions of natural proteins, the next major step in the evolution of protein therapeutics was the redesign of proteins to increase their clinical potential. Injected insulin fails to adequately mimic endogenous insulin in two major ways: rapid onset of action after meals and prolonged maintenance of low level insulin between meals [8]. Insulin has been engineered, commonly by one to three amino acid replacements, to create analogs that are either rapid-acting or long-acting and better mimic different properties of endogenous insulin [8]. Multiple insulin analogs have been approved for the treatment of diabetes mellitus including the rapid-acting analogs, insulin lispro (Humalog®), insulin aspart (NovoRapid® and Novolog®) and insulin glulisine (Apidra®) and the long-acting analogs, insulin glargine (Optisulin® and Lantus®) and insulin detemir (Levemir®) [1,8]. Insulin analogs offer multiple benefits over first generation recombinant insulin drugs that include improved physiologic profile, greater convenience for patients, reduced risk of hypoglycemia, and less weight gain in some cases [9]. The commercial importance of engineered insulins is illustrated by Lantus®, which was one of the top ten selling biopharmaceuticals in 2009 [1].

Many protein therapeutics are produced in mammalian cells and have one or more N- and/or O-linked glycans [10]. Engineering a protein with additional glycosylation sites can increase the in vivo serum half-life and thereby target exposure [11]. An early example of such “glycoengineering” of proteins for half-life extension is provided by darbepoetin-α (Aranesp®), administered to anemic patients to stimulate the production of red blood cells in the bone marrow. The first generation drug, epoetin-α, has 3 N-linked glycosylation sites and was engineered with 2 additional glycosylation sites to create darbepoetin-α, a commercially successful longer-acting second generation drug [12]. Protein glycosylation can sometimes reduce bioactivity, albeit offset by longer half-life. Darbepoetin-α has higher specific activity in patients than epoetin-α, reflecting that the 3-fold increase in serum half-life that increases drug exposure more than offsets a 5-fold reduction in receptor binding affinity [12].

**Fc fusion proteins**

Another significant advance in protein therapeutics came with the advent of fusion proteins by joining the genes encoding 2 or more different proteins. Fusion proteins combine properties of their component parts. The most clinically and commercially successful fusion protein therapeutics to date contain the Fc region of immunoglobulins [1]. Such Fc fusions can endow peptides or proteins with the IgG-like property of long serum half-life (days to weeks), by virtue of binding to the salvage receptor, FcRn [13,14]. By contrast, proteins of ~70 kDa and smaller are typically eliminated rapidly from circulation by renal filtration and have half-lives of a few minutes to a few hours, that can in many cases render them marginal or unsuitable for therapeutic applications [15].

Beyond half-life extension, Fc fusion can provide several additional benefits such as facilitating expression and secretion of recombinant protein, enabling facile purification by protein A chromatography, improving solubility and stability, and enhancing potency by increasing valency. Optional additional properties conferred by Fc fusion include binding to Fcγ receptors and/or complement to support secondary immune functions [13,14]. The first demonstration of the Fc fusion protein concept was CD4-Fc [16]. Five Fc fusion protein therapeutics were approved by 2010, the prototypical and most successful drug of this class being etanercept (Enbrel®), a TNF receptor 2-Fc fusion protein [1]. Etanercept was the top-selling protein therapeutic in 2009 with $6.6 billion in world-wide sales in rheumatoid arthritis and other autoimmune diseases [1]. Romiplostim is a thrombopoietin (TPO) receptor agonist approved as a treatment for thrombocytopenia [17].

**Protein conjugation to polyethylene glycol**

Protein conjugation to chemicals such as radionuclide chelators, cytotoxic drugs and PEG can endow proteins with brand new capabilities to increase their clinical potential.

The most clinically and commercially successful protein conjugates thus far have been with PEG. Protein conjugation to PEG – “PEGylation” – increases their hydrodynamic volume, prevents rapid renal clearance, and thereby increases the serum half-life, as extensively reviewed [15,18,19]. At least 8 PEGylated proteins are approved as human therapeutics [18]. Indeed, long-acting second generation interferon-α molecules that are PEGylated have largely superseded their first generation non-PEGylated counterparts [20]. Benefits of PEGylation are that it is possible to tune the serum-half of proteins by varying the number of attached PEG molecules, their size and extent of branching [21]. PEGylation can also reduce the immunogenicity of proteins [18,19], although PEGylated proteins can still sometimes be immunogenic [22]. A draw back to PEGylation is that it can impair protein function, a limitation that is offset by the greatly increased half-life, and potentially ameliorated by site-specific PEGylation and/or tailoring the PEG [18,19]. PEG is a non-natural and apparently non-biodegradable polymer, a molecular attribute that has unclear clinical significance. PEGylated proteins can induce renal tubular vacuolation in animals, albeit with unaltered clinical pathology [23] and unknown impact on long term safety.

**The rise of antibody therapeutics**

Antibody-based drugs are the largest and fastest growing class of protein therapeutics with 24 marketed antibody therapeutics in the USA (28 FDA-approved and 4 later withdrawn from the market) [4] and at least 240 more in clinical development [5]. Antibody-based drugs contributed $38 billion of $99 billion in world-wide sales for protein biopharmaceuticals in 2009 [1]. Moreover, 5 of the 10 top-selling protein therapeutics in 2009 were antibodies, namely, infliximab (Remicade®), bevacizumab (Avastin®), rituximab (Rituxan® and MabThera®), adalimumab (Humira®) and trastuzumab (Herceptin®) [1].

The rapid growth of antibody therapeutics since the mid-1990s was made possible by the advent of antibody chimerization and humanization [24] technologies that largely overcome the
limitations of rodent monoclonal antibodies as drugs, as well as the emergence of facile routes to human antibodies using transgenic mice [25,26] or phage display [25,27] technologies. Other advances that have contributed to the success of antibodies as therapeutics include the industrialization of antibody production technologies [28] that can provide sufficient quantities of antibodies to allow higher doses to be used in patients. The development of methods to express and secrete functional antibody fragments from E. coli [29,30] has significantly stimulated the field of antibody engineering and enabled subsequent technologies such as phage display. The next major advance in the expression of antibody fragments in E. coli was high titer (gram per liter) production in a fermentor [31], that facilitated the development of antibody fragment drugs such as the anti-VEGF Fab, ranibizumab (Lucentis®). The emergence of antibody as therapeutics likely also reflects improved choices of antibody and target antigen for intervention in the pathobiology of disease.

The majority of approved antibody drugs are unmodified (“naked”) IgG molecules. However, other antibody formats have been approved for therapy including IgG “armed” with radio-nuclides or cytotoxic drugs (antibody-drug conjugates) for cancer treatment, as well as Fab fragments and a PEGylated Fab fragment [1].

Rationale for next generation protein therapeutics

A major quest with protein therapeutics, especially antibodies [6,32], is the development of even better next generation drugs with enhanced efficacy, greater safety or improved delivery. Current antibody therapeutics reveal major strengths that provide a foundation upon which to build, as well as significant limitations to overcome that offers new opportunities [6,33]. Some antibody strengths and many of their limitations apply more broadly to protein therapeutics and are discussed below as a compass to guide the development of next generation protein therapeutics. The impetus to develop better protein therapeutics is also discussed here as a convergence of clinical, scientific, and commercial forces that identify unmet needs (this section), in conjunction with technological tools that many help address them (next section).

Strengths and limitations of antibody and other protein therapeutics

One of the greatest strengths of antibodies as a therapeutic platform is the potential ease of generating high affinity and specificity human antibodies to virtually any target of therapeutic interest, e.g., using transgenic mice [25,26] or phage display [25,27] technologies. Moreover, antibodies are readily tailored for therapeutic applications by modulating (enhancing or attenuating) their existing properties or endowing them with new activities as discussed in a later section and reviewed more extensively elsewhere [33,34]. As for other protein therapeutics, antibodies are commonly well tolerated by patients, but not invariably so. Antibody drug development benefits from recombinant production methods that are well-established, broadly applicable and commonly support expression at high titer (multi-gram per liter) [28]. Widely available knowledge and experience across all facets of antibody drug development also facilitates the generation of future antibody therapeutics. An advantage of antibodies over other drug formats is that the success rate has been somewhat higher — 17% for humanized antibodies from the first clinical trial to regulatory approval [35].

Proteins have several significant limitations as therapeutics. For example, protein therapeutics are very expensive, reflecting high production costs, that may limit patient access and also clinical applications [28]. This high cost issue is exacerbated for protein therapeutics where multi-gram doses are needed for a treatment course, as is the case for some antibodies. Intracellular delivery of proteins is possible in the laboratory setting, e.g., by protein transduction [36]. However, targeted pharmacetic delivery of proteins to reach intracellular targets is in its infancy, so protein therapeutics are currently limited to cell surface or extracellular targets.

Another shortcoming of proteins therapeutics is that they are denatured and/or proteolyzed in the gut and are thus not orally bioavailable. Proteins, including antibodies [37], show very poor ability to penetrate the specialized endothelial structure of the neurovasculature known as the blood-brain barrier. The integrity of the blood-brain barrier can be partially compromised in some pathological settings such as chronic neurodegenerative diseases [38]. Nevertheless, proteins with current technologies are poorly suited to the treatment of chronic neurodegenerative diseases or tumors that originate or have metastasized to the brain.

Antibodies are relatively large (IgG ~150 kDa) compared to other proteins and this large size may limit their tissue penetration and ability to localize to their targets. For example, antibody localization to tumors in patients is typically very inefficient: \(\leq 0.01\%\) of the injected dose per gram of tumor [39]. Comparison of mathematical modeling with experimental data, strongly suggests that molecular size and binding affinity are major determinants of tumor targeting [40]. Many other factors likely contribute to tumor penetration including antigen expression level and turnover rate [41].

Clinical rationale

From a clinical standpoint, first generation protein drugs can provide substantial patient benefit. However, in many cases there is room for improvement in efficacy and/or safety. For example, several anti-cancer antibodies provide an overall survival benefit, including bevacizumab [42] and trastuzumab [43] in combination with chemotherapy in metastatic colorectal and breast cancers, respectively. Nevertheless, there remains plenty of opportunity for enhancing antibodies for cancer treatment, such as elevating the overall survival rate of patients, as well as many strategies being explored to do so [44]. All therapeutic proteins are potentially immunogenic in patients and occasionally an anti-drug antibody response can lead to a major safety issue. For example, a truncated and PEGylated form of TPO (MGDF-PEG) elicited an antibody response in some patients that neutralized endogenous TPO leading to severe and persistent anemia, i.e., the opposite of the desired effect of increasing platelet counts [22]. This issue was overcome with the peptibody, Romiplostim (see Fc fusion proteins section) [17].

Scientific rationale

Scientifically, our expanding knowledge suggests drug mechanisms of action to enhance, and mechanisms of resistance or toxicity to
overcome. For example, anti-CD3 antibodies illustrate the benefits of understanding and overcoming mechanisms of drug toxicity and resistance. In 1986 the anti-CD3 antibody, muromonab-CD3 (OKT3®), became the first antibody approved by the FDA for human therapy — for the treatment of acute allograft rejection in renal transplant patients. In 1993 muromonab-CD3 was approved for the treatment of acute rejection of heart and liver transplants in patients resistant to steroid treatment. Unfortunately, muromonab-CD3 activates T cells resulting in transient cytokine release and acute and severe flu-like symptoms [45]. Another major limitation of muromonab-CD3 is that its therapeutic benefit is reduced by neutralizing antibodies that develop in ~50% of patients [45]. Muromonab-CD3 has since been voluntarily withdrawn from the US market due to decreased sales. These major limitations of muromonab-CD3 have been greatly reduced, if not overcome, in some second generation anti-CD3 antibodies that have advanced to late stage clinical development [6]. For example, the anti-CD3 antibodies, teplizumab and otelixizumab, combine Fc engineering to attenuate Fcγ receptor binding with chimerization or humanization to reduce immunogenicity [34].

Drug development, including for protein therapeutics, seems likely to gain from advances in the understanding of targets and their role in the pathobiology of disease. For example, there is a growing appreciation that targets are not isolated gene products but integral components of networks of interconnected signaling pathways [46,47].

Commercial rationale

Clinical and commercial success with protein therapeutics has bred intense competition between different organizations striving to develop protein therapeutics to the same antigen and/or overlapping therapeutic indications. Indeed, this is already reflected in marketed drugs as illustrated by 5 different protein therapeutics that block TNF including 3 different IgG (infliximab, adalimumab and golimumab), a PEGylated Fab′ fragment (certolizumab pegol) and a receptor-Fc fusion (etanercept) [6]. Such competition is increasingly commonplace as patents dominating individual targets expire. The generation of high affinity and potency IgG is often possible, arguably commoditized. This provides a strong commercial incentive to develop superior-performing antibody drugs to differentiate products from those of competitors. The success of innovator protein therapeutics has brought a rise in biosimilar efforts (i.e., products that are deemed highly similar to innovator protein therapeutics according to drug regulatory authorities). Indeed, several biosimilar approval applications have been filed in the European Union with 13 biosimilars being approved by European drug regulators by early 2009 [48]. For creators of innovator drugs, the impetus to develop improved next generation protein therapeutics that out perform the previous generation drugs has increased substantially [48].

Platform technologies for next generation protein therapeutics

Many protein engineering tools have been developed that allow one to optimize favorable properties of proteins, attenuate undesired attributes and create proteins with entirely novel activities. Space constraints limit the discussion here to next generation antibody and Fc fusion protein therapeutics and the emerging technologies of engineered protein scaffolds.

Next generation antibodies and Fc fusion proteins

Many promising strategies are emerging to enhance the existing properties of antibodies or to endow them with new activities, as reviewed in detail elsewhere [6,32,33]. Antibodies (IgG) have multiple binding partners including antigen, Fcγ receptors, complement and FcRn. Each of these interactions can be tailored in ways that may improve the clinical properties of antibodies [32,34,49]. Platform technologies that seem likely to impact the next generation of antibody therapeutics include old concepts that are finally coming to fruition, such as antibody-drug conjugates and bispecific antibodies. Technologies for improving Fc-mediated functions, such as half-life extension and effector function enhancement, seem likely to benefit the next generation of antibody therapeutics. Additionally, these technologies appear directly applicable to the optimization of Fc fusion proteins.

The 50-year old concept of bispecific antibodies [50] came of age in 2009 with the first regulatory approval of a bispecific antibody — catumaxomab (Removab®, anti-EpCAM x anti-CD3) in Europe. Catumaxomab is approved for the treatment of malignant ascites in patients with EpCAM-positive carcinomas [1,51]. Recruitment of T cells via the anti-CD3 arm of catumaxomab and other immune effector cells via its Fc region may contribute to antitumor activity. A tandem bispecific scFv format known as a “BiTE”, that binds CD3 and a tumor-associated antigen, is a particularly effective way to retarget T cells to kill tumor cells. Blinatumomab, a BiTE specific for CD19 and CD3, gave several complete responses in a phase I clinical trial in non-Hodgkin’s lymphoma [52] and is currently in multiple phase II clinical trials. Another major area of current interest with bispecific antibodies is in the simultaneous blockade of 2 disease mediators, which may lead to greater therapeutic benefit than targeting individual disease mediators [53]. Until recently, a major bottleneck in the development of bispecific antibodies as therapeutics was the challenge of producing these complex molecules in sufficient quantity and quality for clinical applications. This issue has been largely overcome by the advent of many alternative formats and production methods for bispecific antibodies [6].

Antibody-drug conjugates, a concept that dates back to the 1970s, use antibodies to deliver a cytotoxic payload to kill a target such as tumor cells [54,55]. One antibody-drug conjugate, gemtuzumab ozogamicin (Mylotarg®), was approved by the FDA for the treatment of acute myeloid leukemia. Gemtuzumab ozogamicin was withdrawn from the market in 2010 due to safety concerns and insufficient patient benefit in post approval clinical trials. Much progress has been made over the decades in many different facets of antibody-drug conjugates, including better choices of target antigen and antibody, and the development of more potent drugs and linkers of greater stability [54,55]. Pivotal clinical trials are in progress for brentuximab vedotin (SGN-35, [56]) in Hodgkin’s disease and trastuzumab emtansine (T-DM1, [57]) in metastatic breast cancer over-expressing HER2/neu following demonstration of robust antitumor activity and acceptable safety profiles in earlier clinical trials.

Engineering proteins to increase their serum half-life has the potential benefits of increased exposure leading to greater target localization and greater efficacy, lower or less frequent dosing and
lower cost. In the case of antibodies, the serum half-life in mice can be extended by at least a few fold by engineering the Fc region for higher affinity binding to the salvage receptor (FcRn), whilst preserving the pH dependence of binding [58]. Half-life extension of Fc-engineered antibodies in non-human primates was subsequently demonstrated by several different groups [59]. Antibody half-life extension can result in increased target exposure and improved preclinical in vivo efficacy as recently shown for anti-EGFR and anti-VEGF antibodies [60]. Engineering antibodies for pH-dependent interaction with their target antigen has also been shown to extend antibody half-life in vivo [61]. The first half-life extended antibody to enter clinical trials was the humanized anti-RSV antibody, MEDI-557, although pharmacokinetic data have yet to be reported.

Another major area of interest for improving antibody performance is effector function enhancement, as reviewed extensively elsewhere [34,62] and discussed briefly below. Antibody effector functions include ADCC (antibody-dependent cellular cytotoxicity), ADCP (antibody-dependent cellular phagocytosis) and complement-dependent cytotoxicity (CDC). Effector functions may be advantageous where destruction of the target cells is desired, as is often the case for oncology targets.

Indeed many antibodies approved for oncological indications (e.g., rituximab, trastuzumab, alemtuzumab, cetuximab and ofatumumab) support one or many effector functions that may contribute to their clinical antitumor activity, with the strongest evidence being for rituximab [63].

For antibody and Fc fusion protein therapeutics potential benefits from enhancing effector functions include greater efficacy, lower or less frequent dosing and decreased cost-of-goods. As for risks, these include more frequent or severe adverse events. ADCC, ADCP and CDC have been augmented by Fc protein engineering as extensively reviewed elsewhere [62]. ADCC has also been augmented to a similar level by modifying the glycan attached to the Fc, e.g., afofcosylation achieved through cell line engineering [62]. At least 8 effector function enhanced antibodies have advanced into early clinical development [34].

**Engineered protein scaffolds**

The success of antibodies as protein therapeutics has inspired the development of so-called “engineered protein scaffolds” as alternative platform technologies for the development of novel next generation protein therapeutics [64–66]. Engineered protein scaffolds provide binding sites that can be tailored to specifically recognize desired target molecules, in an analogous way that antibodies can be developed to recognize their target antigens. Here the basic features of engineered protein scaffolds are described, together with their strengths, limitations and some possible future directions.

Protein scaffolds chosen for engineering have known 3-dimensional structures that together represent diverse protein folds [64–66]. Protein scaffolds are commonly selected with the following attributes: small, monomeric, high solubility and stability, and that lack disulfide bonds and glycosylation sites. Protein scaffolds are typically readily expressed in microbial hosts. Sequence diversity is introduced into the protein scaffold, e.g., by randomizing surface accessible residues, to create a so-called library. Binders to a target of therapeutic interest are then identified by selection using technologies such as phage, yeast or ribosome display. Increased binding affinity – affinity maturation – is often needed and readily accomplished by further library building and selection.

Over 50 different engineered protein scaffolds have been described with a few advancing into clinical development [64–66] including: Adnectins® from type III fibronectin domains, Affibodies® from the synthetic Z domain of staphylococcal protein A, Anticalins® from lipocalins, Avimers® from LDLR-A modules, DARPin®s from ankyrin repeat proteins, and engineered Kunitz domains. Engineered protein scaffolds also include single-domain antibodies from human (dAbs®) and camelids (Nanobodies®). One engineered protein scaffold protein has been approved for therapy, namely, ecallantide (Kalbitor®), a potent and selective inhibitor of plasma kallikrein based upon a Kunitz domain [67].

Many potential advantages of engineered protein scaffolds over antibodies have been proposed [64–66], albeit with limited supporting evidence to date in the context of developing protein therapeutics. For example, the smaller size and higher stability of some engineered protein scaffolds may allow for alternative routes of administration such as pulmonary delivery. The smaller size of engineered protein scaffolds over antibodies favors more efficient tissue penetration, but this can be more than offset by much faster clearance. Engineered protein scaffolds may be more readily expressed in microbial hosts reflecting their high stability and solubility, small size, simple structure (single polypeptide), and that they commonly lack disulfide bonds and/or glycosylation sites. The small and modular architecture of engineered protein scaffolds seems particularly well suited to the construction of multivalent and/or multispecific protein therapeutics (Fig. 2). Engineered proteins are commonly associated with independent intellectual property that is outside the scope of antibody patents. Several of these proposed advantages of engineered protein scaffolds have been achieved with antibodies by engineering for improved stability or expression, or by exploiting the modular architecture of antibodies to create different formats including ones that are smaller and simpler than IgG [6,32,33].

Engineered protein scaffolds lack Fc-mediated properties of antibodies such as long serum half-life and effector functions. This does not appear to be a major limitation, as many different strategies have been developed to extend the half-life of proteins as comprehensively reviewed [15]. For example, single-domain antibodies binding to the long-circulating protein, serum albumin, have been identified and fused to small proteins, including other single-domain antibodies to extend their half-life [68,69]. As for effector functions, these are desired in some clinical settings but not others. Fc fusion offers a simple way to endow engineered protein scaffolds with effector functions if needed. Engineered protein scaffolds, like antibodies, offer significant opportunity for sequence diversity but individual protein scaffolds appear to provide less opportunity for structural diversity than do antibodies. The functional impact of lower structural diversity on the ease of being able to generate binding proteins to specific sites on targets of interests remains to be determined.

All protein therapeutics, including engineered protein scaffolds, are potentially immunogenic in patients and may elicit an anti-drug antibody response that may neutralize or otherwise interfere with the drug’s activity. This issue can be more significant for chronic therapy involving repeated drug administration. Clinical trials are ultimately needed to understand the extent to which individual engineered protein scaffold drugs are immunogenic in...
Engineered scaffold proteins are often selected for engineered protein scaffolds, which may help reduce the risk of immunogenicity in patients for therapeutic applications.

**Conclusions**

Proteins are now well established as a clinically and commercially important class of therapeutics. Experience with protein therapeutics has provided an understanding of their strengths and limitations and ways in which they might be improved. Clinical and commercial success with protein therapeutics provides strong motivation to develop better protein drugs, whereas new tools, especially platform technologies provide the likely means to do so. The next generation of protein therapeutics will likely include some of the many enhanced antibodies in early clinical development, optimized Fc fusion proteins, as well as new formats such as engineered protein scaffolds.

**References**


**Fig. 2** – Modular assembly of engineered protein scaffolds into multivalent and multispecific fusion proteins. Engineered scaffold proteins binding to different targets or different epitopes on the same target are identified by selection from libraries. The modular nature of engineered protein scaffolds allows them to be readily assembled into multimers with specific valency for each target [64–66]. However, steric considerations may prevent all modules from being able to bind their respective targets simultaneously. Serum half-life extension is commonly required for engineered scaffold proteins to obtain sufficient exposure to support therapeutic applications. Many different half-life extension strategies for small proteins have been developed [15], including for engineered protein scaffolds (shown), e.g., binding to serum albumin. Linkers between modules may be included but are omitted for simplicity in this figure.


