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REVIEW ON THERAPEUTIC PROTEIN: APPLICATION AND CHALLENGES

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ABSTRACT

Protein-based drugs are now one of the fastest-expanding areas of pharmaceutical development, spanning enzymes, hormones, monoclonal antibodies, clotting factors, cytokines, recombinant vaccines, and engineered fusion proteins [1]. Compared with conventional small-molecule drugs, they tend to act with far greater target specificity and cause less off-target toxicity, which has made them central to treating metabolic disease, cancer, autoimmune disorders, and infection. At the same time, several biological and manufacturing hurdles keep proteins from reaching patients as easily as a typical tablet or capsule would: they can provoke unwanted immune responses [3], they lose structural integrity fairly easily [2], they clear from the bloodstream quickly [5], they are poorly absorbed when swallowed [4], and they are expensive and technically demanding to manufacture [6]. This review walks through the major classes and uses of therapeutic proteins, examines the challenges that continue to limit their development and delivery, and summarizes the strategies currently being used to work around them.

KEYWORDS: Theraoutic Proteins; Monoclonal Antibodies; Enzyme Replacement Therapy; Immunogenicity; Proties Formulation; Biosimilars; Protein Engineering.

1. INTRODUCTION

The approval of recombinant human insulin in 1982 is generally regarded as the starting point of the modern therapeutic protein era, and the field has grown substantially since then [1]. What sets proteins apart from small-molecule drugs is their ability to achieve highly specific target binding, engage in multivalent interactions, and carry out biological functions that are, in practice, very difficult to reproduce with a synthetic small molecule [1]. None of this would have been commercially feasible without parallel advances in recombinant DNA technology, cell-culture engineering, and downstream purification, all of which now allow protein therapeutics to be produced at a scale sufficient to supply

global markets. As a result, biologics now account for a substantial and growing share of newly approved drugs each year [6]. Proteins occupy a unique position in the pharmacopeia. Because they are the direct products of the genes that go wrong in disease, they can replace a missing hormone or enzyme, neutralize a specific pathogenic target, or redirect the immune system with a level of selectively that small chemical molecules rarely achieve.[11,12] Cumuli was the first medicine of any kind derived from recombinant DNA method,and it established that genetically engineered human proteins could be manufactured at scale and used safely in patients.That single approval opened a path that has since produced well over one hundred twenty distinct recombinant protein drug, spanning hormones, clotting factors, enzymes, interferons, and from the late 1990s onward, an expanding family of monoclonal antibodies.

The scale of this shift is reflected in current market figures. The global protein therapeutics market was estimated at roughly 375 billion US dollars in 2024 and is projected to approach 750 billion dollars by the early 2030s , with monoclonal antibodies representing the largest single product category[13,14]

2. Classification and Clinical Applications

2.1 Enzyme Replacement Therapies

These proteins are used to correct inherited metabolic and lysosomal storage disorders in which a particular enzyme is missing or present at insufficient levels. Imiglucerase, used for Gaucher disease, and alglucosidase alfa, used for Pompe disease, are two well-established examples [1]. In both cases, the therapy does not cure the underlying genetic defect but supplies the enzyme activity the body cannot produce on its own.

The earliest and conceptually simplest therapeutic proteins are those that replace an endogenous hormone the body no longer produces in sufficient quantity. Recombinant human insulin remains the prototype , and it is joined on the market by human growth hormone, erythropoiethin for anemia associated with chronic kidney disease and chemotherapy, and various clotting factor concentrates for hemophilia [15] Enzyme replacement therapy (ERT) supplies a functional version of an enzyme that is missing or defective,most notably in the lysosomal storage disorders- a group of more than fitty rare inherited metabolic diseases, including Gaucher, Fabry, Pompe, and the mucopolysaccharidose, in which the absence of a single lysosomal enzyme causes toxic accumulation of undegraded substrates in cells [17,18] ERT for Gaucher disease,approved in 1991,was the first success in this area and remains the most effective example; therapies for other lysosomal disorders have since followed but generally produce more modest clinical benefit, in large part because intravenously asministered enzymes cannot cross the blood –brain barrier and therefore cannot address the neurological manifestations that dominate many of these conditions[19,20]This limitation has motivated substantial research into enzyme engineering ,alternative delivery routes, and combination with gene therapy or hematopoietic steam cell transplantation [18,21]

2.2 Hormones

Hormone replacement was among the earliest applications of recombinant protein technology [1]. Recombinant insulin remains the standard treatment for diabetes mellitus, recombinant human growth hormone is used for growth disorders in children and adults, and erythropoietin is prescribed for anemia associated with chronic kidney disease or chemotherapy.These molecules illustrate a general principle of protein replacement therapy: because the drug is structurally identical or nearly identical to the natural human protein , immune tolerance is generally favorable , although rare but serious exception exist, such as antibody – mediated red-cell aplasia that has occasionally followed erythropoietin therapy[16]

2.3 Monoclonal Antibodies (mAbs)

Monoclonal antibodies now represent the largest and fastest-growing category of protein therapeutics [8]. In oncology, agents such as trastuzumab and rituximab target specific tumours antigens; in autoimmune and inflammatory disease, adalimumab and infliximab are widely used; and antibodies are increasingly deployed for infectious disease prophylaxis as well. Because antibody structure is well understood, mAbs can be engineered to enhance effector function or to reduce their potential to trigger an immune response [8].Monoclonal antibodies (mAbs) are now the dominant class of therapeutic protein by both number of approvals and market value.since the 1986 approval of the first therapeutic mAb, muromonab-CD3,for transplant rejection,the filed has progressed from murine antibodies to humanized and fully human,formsts, substantially reducing immunogenicity while broadening clinical use into onacology, autoimmune and inflammatory disease, hematology , and infectious and neurological disease [22,23,24] An analysis of antibodys approvals through 2024 identified 159 distinct antibody-based biologics, incuding conventional IgG antibodies, antibody fragments, Fc-fusion proteins, thirteen antibody-drug conjugates (ADCs), and fourteen bispecific antibodies; by the 2025 the number of approved bispecifics had risen to fifteen and ADCs to twenty worldwise[23] . In oncology,mAbs act through several mechanisms, including blockade of immune checkpoints, direct targeting of tumor-associated antigen, modulation of the tumor microenvironment, and antibody-dependent cellular cytotoxicity; bispecific T-cell engagers such as blinatumomab extend this by simultaneously binding a tumor antigen and a T-cell receptor component to physically bring immune effector cells into contact with malignant cells[25] ADCs combine an approach that has expanded rapidly in the past five years[23,26] Outside oncology, antibodies against inflammatory cytokines such as TNF-alpha and IL-4/IL-13 (for example, adalimumb and dupilumab) are among the best-selling drug in the world, reflecting the broad uitility of antibody-based blockade in chronic autoimmune and allergic disease[27,28].

2.4 Blood Factors

Recombinant clotting factors, particularly Factor VIII and Factor IX, are now the standard of care for hemophilia A and B, respectively. Their main advantage over plasma-derived alternatives is that they eliminate the risk of transmitting blood-borne pathogens [1].

2.5 Cytokines and Interferons

Cytokines and interferons are used to modulate the immune system rather than to replace a missing protein. Interferon-alpha, for example, is used in chronic hepatitis, while interleukins such as IL-2 are used in cancer immunotherapy to stimulate an anti-tumor immune response [1]. Cytokines and interferones, administered either as native recombinant molecules or in engineered long-acting forms,are used to modulate immune and antiviral responses in condition ranging from hepatitis to multiple sclerosis and certain cancers[29]. Collectively, engineered formats such as fusion proteins, bispecifics, and antibody fragmentes have grown to represent an increasing share of newly approved protein therapeutics, reflecting a broader shift in the field from naturally occurring proteins towords deliberately engineered, multi-functional molecules[29,26].

2.6 Recombinant Vaccines

Subunit vaccines produced through recombinant expression, including the hepatitis B surface antigen vaccine and HPV virus-like particle vaccines, avoid many of the safety concerns associated with live-attenuated or whole inactivated pathogen vaccines, since only a defined antigenic component is administered [1]. Protein – based vaccines, which deliver a purified or recombinant antigen (such as a viral surface protein) rather than a live or inactivated, pathogen,represent a further major application of recombinant protein technology. These subunit vaccines rely on the same expression and purification platforms used for other biologics and share several of their manufacturing and stability challenges,including the need for temperature-controlled distribution[2] Their appeal lies in a generally favorable safety profile, since they contain no genetic material or live organism, at the cost of typically requiring adjuvants or multiple doses to achieve adequate immunogenicity.

2.7 manufacturing of therapeutic proteins

Fusion proteins combine functional domains from two different proteins to create a molecule with novel pharmacological properties. Etanercept, a fusion of the TNF receptor and an antibody Fc domain, is a well-known example used in the treatment of rheumatoid arthritis [5]. Fusion protein combine functional domains from different natural protein-commonly a receptor ectodomain or ligand- binding domain fused to an antibody Fc region- to achieve a desired biological activity together with the extended circulating half-life that Fc fusion confers[15,30].

Unlike small- molecule drugs, which are produced by chemical synthesis, therapeutic proteins must be manufactured inside living cells, a requirement that shapes nearly every downsteam challenge discussed in section 4. Expression platforms have diversified considerably since Escherichia coli was first used for high-thoughput recombinant protein production;because bacterial systems cannot perform the post-translational modifications, particularly glycosylation, required by many therapeutic proteins,production has expanded to yeast,mammalian cell lines (most commonly Chinese hamster ovary, or CHO,cells) insect cells, plant systems, and ,more recently, cell-free synthesis platforms[29]. Each host organism represents a distinct trade-off among yield,folding fidelity, glycosylation pattern,

and cost and the choice of expression system is one of the earliest and most consequential decisions in a protein drug's developments[30]

Upstream processing – the cultivation of the host cells and the induction of protein expression- must contend with the inherent unpredictability of living organisms; modern fed-batch bioreactor processes for monoclonal antibody production can now achieve titers of ten grams per liter or more, aided by advances in cell line engineering , process analaytical technology , and continuous (perfusion-based) culture method[4,31,32]. Downstream processing, which seperates, purifies, and formulates the protein from the harvested cell culture, is equally demanding: it must remove host cell proteins , DNA, and process-related impurities while preserving the proteins native structure, typically through a series of chromatography and filteration stepes[33,34]. Regulatory analyses have found that manufacturing and quality deficiencies were the leading causes of complete response letters issued by the FDA between 2020 and 2024, underscoring how much of the overall risk in protein drug development resides in the manufacturing process itself rather than in the molecule's biological activity[33]

3. Key Challenges in Development and Delivery

3.1 Immunogenicity

Anti-drug antibodies (ADAs) are one of the most consequential risks associated with protein therapeutics [3]. They can neutralize the drug's activity outright, alter its pharmacokinetic profile, or in some cases trigger hypersensitivity reactions in the patient. The likelihood of an immune response depends on several factors, including whether the protein is of foreign or humanized origin, whether it tends to aggregate, its glycosylation pattern, and the presence of process-related impurities left over from manufacturing [3]. Even carefully engineered protein therapeutics can trigger an unwanted immune response in treated patients, most consequentially the generation of anti-drug antibodies (ADAs)[35,36]. ADAs can neutralize the drug and abolish its efficacy, accelerate its clearance from circulation ,or, in rare cases, cross-react with an endogenous protein and causes serious adverse effects[3,36]. The roots of immunogenicity are multifactorial : CD4-positive T-cell responses to peptide epitopes within the protein sequence are considered a central drive of most ADA responses, and these are amplified by memory B cells and long- lived plasma cells[37,38] . Product –releated factors such as aggregated protein is processed by the immune system differently from properly folded, monomeric protein[3] . Mitigation strategies now span the entire development pipeline, including computational and in-vitro predication of T-cell epitopes prior to clinical testing, "deimmunization''Though targeted sequence engineering, and at the formulation level, minimizing aggregation and other quality attributes associated with heightened immune recognition[5,7,8]. Despite this progress, immunogenicity risk assessment remains one of the more difficult aspects of biologics development because the available preclinical models only partially predict what will occur in human patients[35].

3.2 Physical and Chemical Instability

Proteins are inherently fragile molecules. During production, storage, and administration they are prone to aggregation, unfolding, oxidation, deamidation, and fragmentation [2]. Any of these degradation pathways can reduce potency and, in the case of aggregation in particular, increase immunogenic risk. Managing this instability requires carefully controlled formulation — appropriate buffers, stabilizing excipients, and often lyophilization — as well as a cold-chain distribution system to maintain product integrity from manufacturing site to patient [2].

3.3 Short Circulatory Half-Life

Many therapeutic proteins are cleared from the bloodstream quickly, whether through renal filtration or proteolytic breakdown, which often forces frequent dosing regimens that reduce patient convenience [5]. To address this, developers rely on techniques such as PEGylation, Fc-fusion, albumin binding, and glycoengineering, all of which are aimed at extending circulating half-life and allowing longer intervals between doses [5].

3.4 protein stability, Aggregation, and Formulation

Because protein are large, conformationally complex molecules held together largely by non-covalent interactions, they are intrinsically less stable than small-molecules drugs. Elevated temperature,mechanical agitation,freeze-thaw cycling, and shifts in PH can all causes a proteins to unfold and aggregate into higher-order species that reduce potency and, as noted above, elevate immunogenic risk. Industy analyses estimate that fail during clinical development. Because most protein formulations remains stable only within a narrow temperature range, the majority require an uninterrupted “cold chain” from the point of manufacture through storage , distribution, and administration; a single temperature excursion can compromise an entire batch, and maintaining this cold chain is both expencive and logistically difficult, particularly in low-resource settings. Formulation scientists mitigate these risk through excipients such as sugars and amino acid stabilizers that limit protein-protein contact, through lyophilisation (freeze-drying) to produce a more thermally stable solid forms, and increasingly though computational modelling used to predict aggregation-prone region of a candidate protein early in development. Newer classes of biologics, including antibody-drug conjugates, mRNA-based product, and fusion protein, introduce additional, format-specific stability concerns beyond those of conventional antibodies[22].

3.5 Delivery and Bioavailability

Oral bioavailability remains one of the toughest problems in the field. Gastrointestinal proteolysis and poor permeability across the intestinal epithelium mean that the overwhelming majority of protein therapeutics must be given parenterally rather than as a pill [4]. Oral, pulmonary, and transdermal delivery systems continue to be studied, but none has yet overcome the formulation hurdles well enough to replace injection for most large proteins [4]. Nearly all protein therapeutics are administered parenterally-by intravenous, subcutaneous, or intramascular injection – because the gastrointestinal tract is specifically designed to break proteins down into their constituent amino acids.oral

administration, which would be far more convenient and would improve patient adherence, is obstructed by four principle barriers –

1. The highly acidic gastric environment
2. Extensiv proteolytic degradation by digestive enzymes
3. The protective mucus layers lining the intestine
4. The tight junctions of the intestinal epithelium that block paracellular transport of large hydrophilic molecules[36,39]

A substantial body of formulation research has pursued strategies to overcome theses barriers, including chemical modification such as PEGylation and lipidation, co-administration of protease inhibitors and permeation enhancers, and encapsulation in nanoparticle, liposomal,or lipid-based carrier systems[22,19,15]. More recent work has explored physical delivery devices such as microneedle-based capsules designeed to inject the drug directly into the intenstinal wall, and "multifunctional'' delivery systems that combine several of these protective strategies simultaneously[40]. Despite extensive preclinical progress, only a small number of oral protein or peptide products have reached clinical use, and the gap between laboratory-scale success and reliable clinical translation remains one of the most persistent open problems in the field[36].

3.6 Manufacturing Complexity and Cost

Protein therapeutics are produced using living expression systems — commonly CHO cells, E. coli, or yeast — and this biological basis introduces a level of batch-to-batch variability that has no real equivalent in small-molecule chemical synthesis [6]. Extensive quality control is required to confirm purity, potency, and the correctness of post-translational modifications for every batch, and this complexity is a major driver of the high production costs associated with biologics relative to conventional drugs [6]. The biological complexity described in section 3 translates directly into cost: producing a therapeutic protein requires living cell cultivation, multi-step purification, extensive analaytical characterization, and continuous quality control , all conducted under current Good manufacturing Practice standards. These requrirement make biologics manufacturing substantially more capital-and labor-intensive than small-molecule drug synthesis and are a major contributor to the high list prices of protein therapeutics[33,39] Because the fermentation or cell-culture process used to produce a given protein is itself part of the product's glycosylation pattern, aggregation propensity, or other quality attributes- a phenomenon captured in the biopharmaceutical Industy's maxim that "the process is the product."

This reality is central to the difficulty of manufacturing biosimilars, discussed below, and also explains why supply disruptions, such as those experienced for certain raw materials and chromatography resins during the COVID-19 pandemic, can have an outsized effect on the availability of biologic medicines[34,16]

3.7 Biosimilar Development

Because a protein's structure and biological activity are so closely tied to the specifics of how it is manufactured, a biosimilar can never be shown to be truly identical to its reference product in the way a small-molecule generic can be [7]. Regulators instead require developers to demonstrate bio-similarity through extensive analytical and clinical comparison, a process that is scientifically and regulatorily far more involved than generic drug approval [10].

The high cost of biologics has motivated the development of biosimilars – protein drugs shown to be highly similar to an already – approved reference biologic – as a mechanism to introduce price competition once the reference product's patents and exclusivity periods expire. In the united, states, biosimilars are authorized under the 2009 biologics price competition and Innovation Act, and more than seventy-five had been approved by mid-2025. Unlike small- molecule generic drugs, which can be shown to be chemically identical to the branded product, biosimilars require extensive analytical, and in some cases clinical, comparability studies because the manufacturing process itself can subtly influence the final protein's structure and function; this raises development costs and timelines relative to generics. A further access barrier is " interchangeability" status, which in the united states permits pharmacy-level subsitituton of a biosimilar for its reference product without a new prescription ; as of mid-2025 only a minority of approved biosimilares had received this designation , and biosimilar uptake has varied considerably across payers,with adoption highest in Medicaid and lowest in medicare in analysis of infliximab biosimilar use . Analysts have projected that a large share of biologics losing patent protection over the coming decade may nonetheless lack any biosimilar competitor at all – a gap sometimes termed the "biosimilar void" - reflecting how manufacturing complexity, patent thickets around reference products, and inconsistent regulatory requrirements across countries continue to slow the translation of biosimilar approval into real-world affordability and access[19].

3.8 Targeting and Tissue Penetration

The same large molecular size that gives proteins their specificity can also work against them. Antibodies and other large proteins often struggle to penetrate deep into solid tumors or to cross the blood-brain barrier, which can limit their effectiveness in certain disease settings even when target specificity is excellent [9].

3.9 Cost and Global Access

The cumulative effect of expensive development and complex manufacturing is that protein therapeutics tend to carry high price tags, which creates real disparities in access — particularly in low- and middle-income countries where healthcare budgets are limited[6].

4. Emerging Strategies to Address Key Challenges

The table below summarizes some of the main strategies currently being used or investigated to address the challenges describe above.

Challenge	Representative strategies
Short Circulatory half-life	PEGylation,Fc-fusion,albumin fusion/binding, glycoengineering[5].
Immunogenicity	Humanization, deimmunization,epitope mapping,glycol-optimization[3]
Instability	Lyophilization, excipient and buffer optimization, novel stabilizers[2]
Oral delivery	Permeation enhancers, nanoparticle encapsulation, protease inhibitors[4]
Manufacturing cost	Continuous bioprocessing, single-use system, cell-line engineering[6]
Tissue penetration	Antibody fragments (Fab,scfv),nanobodies, bispecific formats[9]

5. Emerging solution and future Directions

5.1 Artificial –intelligence–Guided Protein Design

The award of the 2024 nobel prize in chemistry to Demis Hassabis and John jumper for Alphafold, and to David Baker for RF diffusion , marked formal recognition of how deep learining has transformed protein science . Alphafold and its successor Alphafold3 predict a protein's three-dimensional structure directly froms its amino acid acid sequence Alphafold database now contain prediction for more than two hundred million proteins, providing a resource used throughout structural biology and drug discovery. RF diffusion and related generative models take the opposite approach , designing entirely novel protein sequences and structures to meet a specified funcation, such as binding a particular disease target with high affinity; complementary tools such as proteinMPNN generate amino acid sequences predicted to fold into a desired shape . applied to therapeutics, theses tools are being used to degien smaller, more stable antibody fragments and binders, to engineer proteins with reduced immunogenicity or extended half-life , and to accelerate enzyme engineering for the both manufacturing and enzyme replacement applications. While still an emerging areas, AI-guided design in increasingly viewed as a route to shortening development timelines and expanding the range of achievable protein architectures beyond what natural evoluation has produced[37,39]

5.2 Next-Generation Delivery and Formulation Technologies

Beyond the oral- delivery research summarized in section 4.3, formulation scientists are pursuing delivery approaches intended to reduce dependence on cold-chain infrastructures and injection- based administration more broadly. Theses include engineered excipient systems and non-aqueous liguid formulations designed to maintain protein stability at room temperature, continuous (rather than batch) freeze-drying processes intended to improve manufacturing efficiency, and noncarrier and hydrogel-based systems intended to enable less frequent, longer-acting dosing[4]. Should these approaches mature, they could meaningfully reduce both the logistical burden and the cost of distributing protein therapeutics, particularly in regions where reliable cold-chain infrastructure is not readily available.

5.3 Toward Broader Access

Addressing the cost and access challenges outlined in section 4.5 will likely require parallel progress on several fronts: continued regulatory harmonization to reduce duplicative biosimilar development requriements across jurisdiction, policy reforms addressing patent thikets and reimbursement incentives that currently slow biosimilar uptake, and manufacturing innovations- such as continuous bioprocessing and alternative expression hosts- that lower the underlying cost of production[20] "Biobetter" molecules , which re-engineer an existing biologic for improved stability,potency, or half-life, represent a related strategy for extracting greater clinical value from established therapeutic targets even as reference products face biosimilar competition.

6.CONCLUSION

Therapeutic proteins have fundamentally broadened what modern medicine can achieve, providing targeted treatment options across metabolic, oncologic, autoimmune, and infectious diseases that would be difficult to manage with small-molecule drugs alone [1]. The obstacles that remain — immunogenicity, structural instability, short half-life, limited delivery options, and high manufacturing cost — are being addressed steadily through progress in protein engineering, formulation science, and bioprocessing technology [2], [5]. Looking ahead, the field is likely to keep moving toward next-generation delivery platforms, wider biosimilar accessibility, and engineering strategies that improve tissue-specific targeting while keeping immune recognition to a minimum [9], [10]. In little more than four decades, therapeutic proteins have progressed from a single recombinant hormones to a diverse and rapidly expanding pharmacopeia that include engineered antibodies, enzyme replacement therapies, fusion proteins, and protein-based vaccines, collectively representing hundreds of billons of dollars in annual global sales[11,30] . This growth has been made possible by parallel advances in molecular biology, bioprocess engineering , and , most recently, artificial intelligence-guided protein design. Yet the fundamental biology of proteins- their size , conformational complexity, and susceptibility to immune recognition and degradation- continues to impose real constraints on how these medicines are manufactured , formulated, delivered, and priced. Continued progress in reducing immunogenicity, improving stability and non-parenteral delivery, streamlining manufacturing , and rationalizing the regulatory and economic pathway for biosimilars will togheter determine how fully the promise of protein-based medicine is translated into accessible clinical benefit over the coming decade..

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