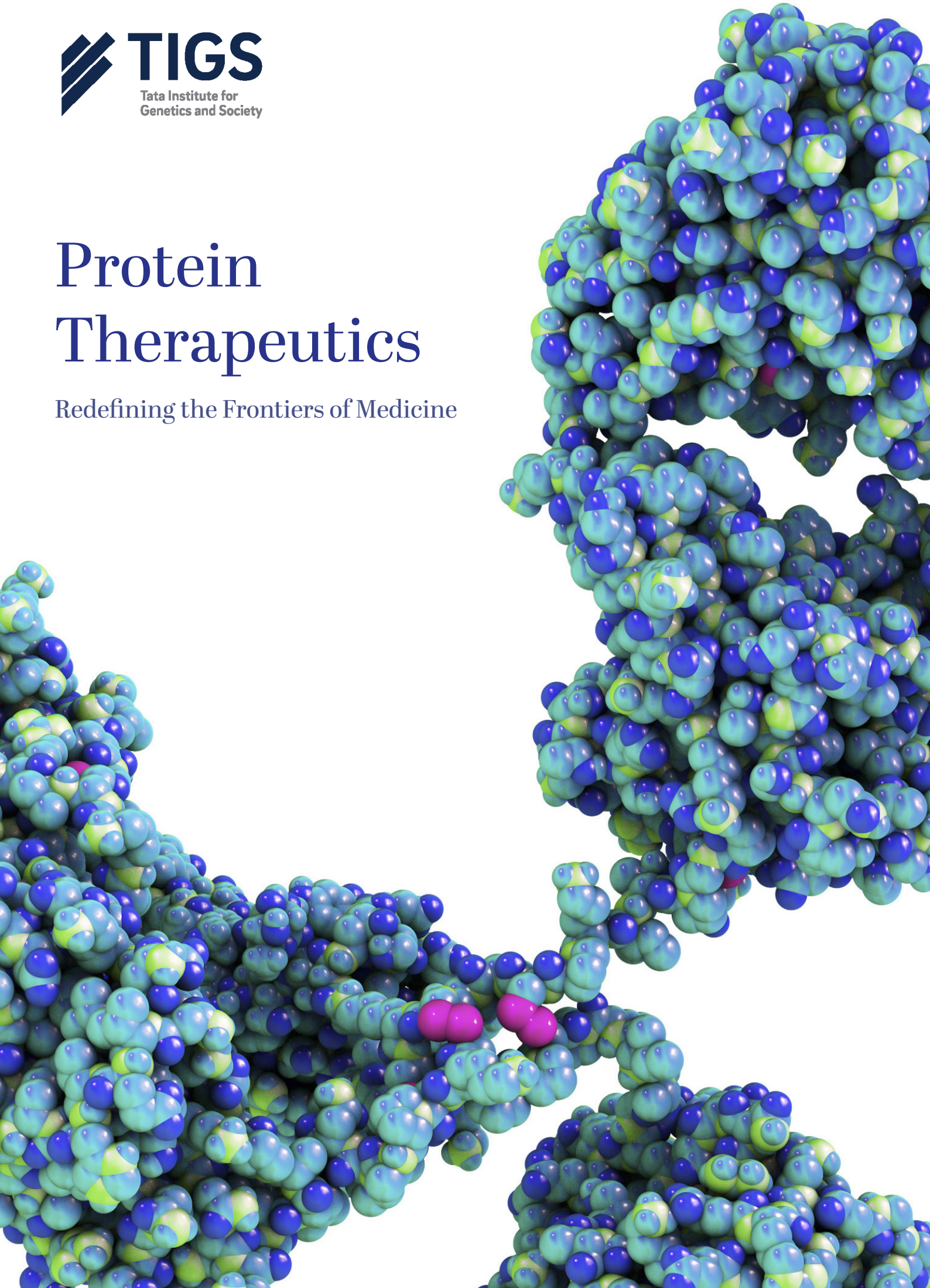
TIGS
Tata Institute for Genetics and Society

Protein Therapeutics

Redefining the Frontiers of Medicine



Protein therapeutics

Proteins comprise a significant percentage of the human body. These long chains of amino acids conformed in three-dimensional structures carry out essential functions and form structural elements of our body.

Proteins can be developed and produced to serve specific therapeutic functions, such as treating metabolic disorders, resisting infections, arresting the spread of cancer, etc. These protein-based drugs can be grouped into the following based on their pharmacological activity:

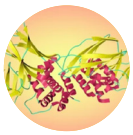
Function type	Pharmacological Activity	Examples
Enzymatic or Regulatory Activity	Replacing a protein that is deficient or abnormal	Insulin for Diabetes mellitus, Growth hormone for growth failure, Coagulation factors for hemophilia
	Augmenting an existing pathway	Erythropoietin to treat anaemic of chronic disease, Sargramostim for leukopenia, Interferons for immunoregulatory and antiviral functions
	Providing a novel function or activity	Botulinum toxin for many types of dystonia and cosmetic uses, L-Asparaginase for acute lymphocytic leukemia, Streptokinase for acute evolving transmural myocardial infarction, pulmonary embolism, deep vein thrombosis, arterial thrombosis or embolism, occlusion of arteriovenous cannula
Special Targeting Activity	Interfering with a molecule or organism	Bevacizumab for colorectal cancer and non-small-cell lung cancer, Trastuzumab for breast cancer, Adalimumab for rheumatoid arthritis, Crohn's disease, etc.
	Delivering other compounds or proteins, stabilizing unstable proteins	Denileukin diftitox for persistent or recurrent cutaneous T-cell lymphoma whose malignant cells express the CD25 component of the IL2 receptor, Ibritumomab tiuxetan for relapsed or refractory low-grade, follicular, or transformed B-cell non-Hodgkin's lymphoma (NHL), including rituximab-refractory follicular NHL
Vaccines	Protecting against a deleterious foreign agent	Hepatitis B surface antigen (HBsAg) for Hepatitis B vaccination
	Treating an autoimmune disease	Anti-Rhesus (Rh) immunoglobulin G for routine antepartum and postpartum prevention of Rh(D) immunization in Rh(D)-negative women; Rh prophylaxis in case of obstetric complications or invasive procedures during pregnancy; suppression of Rh immunization in Rh(D)-negative individuals transfused with Rh(D)-positive red blood cells
	Treating cancer	Sipuleucel-T (Provenge) for treatment of metastatic prostate cancer. Some others are in in clinical trials
Diagnostics	*In vivo* infectious disease diagnostics	Recombinant purified protein derivative (DPPD) for diagnosis of tuberculosis exposure
	Hormones	Glucagon as diagnostic aid to slow gastrointestinal motility in radiographic studies; reversal of hypoglycaemia, Growth hormone releasing hormone (GHRH) for diagnosis of defective growth-hormone secretion
	Imaging agents	Capromab pendetide for prostate cancer detection, HIV antigens for diagnosis of HIV infection

Nature of therapeutic proteins

Therapeutic antibodies are becoming increasingly prominent in the pharmaceutical industry. Several therapeutic antibodies have been approved for the treatment of cancers, autoimmune, metabolic, and infectious diseases. Monoclonal antibodies, antibodies produced by B cells and engineered to target specific proteins that cause disease, are now predominantly used to treat some diseases. Technological advances have also led to the development of bispecific antibodies, antibodies that can treat complex diseases by simultaneously binding to different antigens, and they are being used in the treatment of lymphoblastic leukaemia and haemophilia A.

Enzymes are an important class of protein therapeutics. Deficient or absent enzymes, as seen in the case of lysosomal storage diseases, can be replaced by Enzyme Replacement Therapy (ERT). ERT, manufactured using DNA recombinant technology, is currently available for treating 10 lysosomal storage diseases such as Gaucher disease, Fabry disease, and Pompe disease. However, ERT is not a cure and requires regular and life-long administrations that are very expensive.

Types of therapeutic proteins:

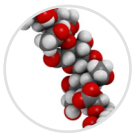


Antibody based drugs

Highly target specific conjugates of antigen specific monoclonal antibodies and a drug, linked though chemical linkers. Mylotarg (Gemtuzumab ozogamicin) is a commercially available antibody drug conjugate used as an antitumor agent.

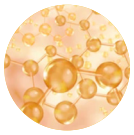
Anti-coagulants

Molecules that prevent the formation of blood clots. They are administered to patients to reduce the chances of developing strokes and heart attacks. Antithrombin (recombinant) is an FDA approved anti-coagulant.



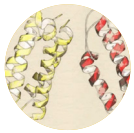
Engineered protein scaffolds

Used to develop antibodies with target specificity, by providing flexible binding sites for the selection of target molecules. Ecallantide (Kalbitor®) is an established plasma kallikrein inhibitor.



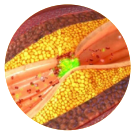
Growth factors

A group of proteins responsible for growth stimulation of cells. Granulocyte-macrophage colony-stimulating factor (GM-CSF) and granulocyte colony-stimulating factor (G-CSF) are widely used among cancer patients to ensure white blood cell recovery after chemotherapy.



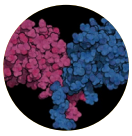
Interferons

Pro and anti-inflammatory cytokines that can actively impart antiviral and anti-tumor effects.



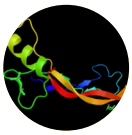
Thrombolytics

Enzymes or drugs used in the treatment of thrombotic diseases. Thrombolytics are cost effective over traditional methods of clot removal. Streptokinase is one of the earliest thrombolytics discovered and is administered to patients with pulmonary embolism.



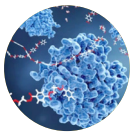
Fc-Fusion protein

Synthetic combination of crystallizable part of a monoclonal antibody and a therapeutically active protein which could deliver enhanced target specificity and deliverance. Romiplostim is an Fc-Fusion peptide (peptibody) used for enhancing platelet production.



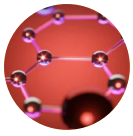
Bone morphogenetic proteins

A family of growth factor proteins, which have been identified to have a potential role in recovery from spinal injury and other reconstructive surgeries. Recombinant BMP-2 is a widely used osteo-inductive growth factor.



Enzymes

Biocatalysts, widely used in industrial and pharmaceutical sectors. Recombinant enzymes can be employed as anticoagulant agents, thrombolytics, antibiotics, anti-inflammatory, and as therapeutics for enzyme deficiency disorders. Marketed recombinant enzymes such as Myozyme (alpha glucosidase) are used in the treatment of lysosomal storage disorders such as Pompe disease.



Hormones

Messenger molecules secreted by multicellular glands. Recombinant hormones are produced and administered to patients with hormonal deficiency disorders such as diabetes.

Interleukins

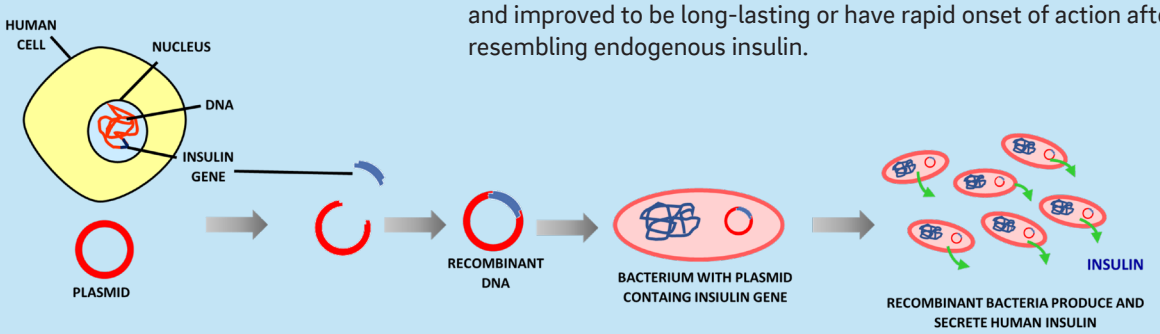
Pro-inflammatory cytokines that can be used to modulate inflammatory responses in allergy and infectious diseases. Aldesleukin/ Proleukin is a recombinant human interleukin-2 administered to cancer patients.

Insulin: The first commercial recombinant therapeutic protein

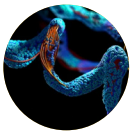
Diabetes mellitus (type I and II), when untreated, leads to wasting and death due to the lack of a protein hormone 'insulin', which signals cells to perform numerous functions related to glucose homeostasis and intermediary metabolism. In 1922, insulin was first purified from bovine and porcine pancreas and used as a life-saving daily injection for patients with type I diabetes. Producing insulin from direct animal sources had several issues:

a) availability of pancreases b) cost of purification
c) immunological reaction in some patients.

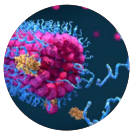
These issues were addressed with the invention of recombinant insulin developed by isolating the human insulin gene and mass producing the hormone through engineered ***Escherichia coli.*** This was the first commercially available recombinant protein therapeutic approved by US FDA in 1982. In later years it was redesigned and improved to be long-lasting or have rapid onset of action after meals, resembling endogenous insulin.



Engineered proteins for enhanced utility

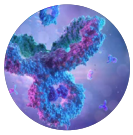


Higher specific activity of the protein and a lower chance of immunological rejection.

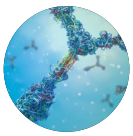


Produced more efficiently and inexpensively and in potentially limitless quantities.

Reduced exposure to animal or human diseases when proteins are recombinantly expressed.



Modification of a protein or the selection of a particular gene variant to improve function or specificity.



Allows the production of proteins that have novel function or activity.

Strategies for protein engineering

Protein engineering is the process of modifying an existing protein or synthesizing a new protein to perform specific functions. Strategies used in protein engineering are broadly classified into: Rational design, Semi-rational design, Directed evolution, and De novo design.

Rational design involves making changes to amino acids in a protein through site-directed mutagenesis to yield a specific protein structure. This method necessitates that the three-dimensional structure and the relationship between the structure and function of the protein are well-known.

In the **semi-rational** strategy, bioinformatics tools are used to provide the direction for mutation. The key to semi-rational evolution is positioning the candidate mutation sites by using computer simulations and then constructing an appropriate mutant library leading to the creation of smaller, smarter libraries and enhancing the efficiency of the process.

In recent years, we've also seen much progress in ***de novo*** protein design or computationally designing proteins with predetermined structures and functions from scratch. Although a more difficult process, ***de novo*** protein design allows us to build proteins that are not found in nature. This was made possible with new advancements in molecular modelling tools that can predict the structure of a protein from its sequence.

Directed evolution employs random mutagenesis and high-throughput screening to induce mutations in a controlled environment and select variants that have desired properties. This method is therefore more commonly used when the structure and mechanisms of the target protein are unknown.

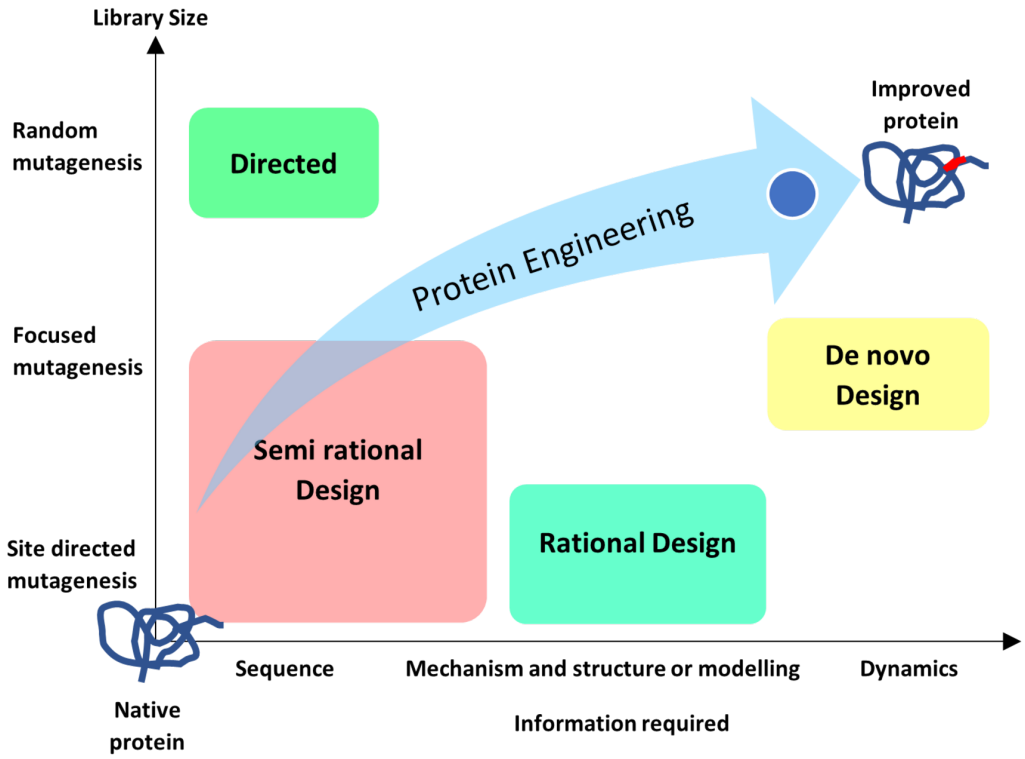


Figure: Comparison of the information and library size required by the four common methods in protein engineering

Challenges in protein engineering

- Protein solubility, route of administration, distribution, and stability
- Immune response against the therapeutic protein
- Appropriate post translational modifications required to be made physiologically active
- Cost of development
- Ethical considerations in allocation of resources and the use of protein therapeutics for cosmetic alterations

Future directions

Protein therapeutics have an increasingly important role today, and several have been approved for treatment globally. For some diseases, recombinant proteins are the only available treatment. They can also be used in combination with small-molecule drugs to provide additive or synergistic benefits. For example, in the treatment of EGFR-positive colon cancer, combination therapy with the small-molecule drug irinotecan and the recombinant monoclonal antibody cetuximab results in increased survival in patients with colorectal cancer by synergistically inhibiting the EGFR signalling pathway at different locations.

In the last few decades, efforts have intensified in making the development of therapeutic proteins more economical and easier to produce and in making alternate routes of administration more viable. Given the specificity and range of functions they can perform therapeutic proteins are poised to play a more significant role in the future of medicine.

Protein Therapeutic Platform at TIGS

Ever since the development of the first recombinant protein therapeutic, human insulin, 38 years ago, many protein molecules have been approved by regulatory agencies for various disease indications. Enzymes, being the most important class of proteins, when defective, lead to a series of abnormalities that can be corrected by introducing the concomitant functional recombinant enzyme in the system. At TIGS, we're working on a protein therapeutics project on enzyme replacement therapy (ERT). Our aim is to reduce the cost of treatment for lysosomal storage diseases (LSDs) by manufacturing biologics at the point of care.

Therapeutic proteins of interest are expressed in periplasm, cytoplasm, or secreted into the supernatant of the media based on the nature of the protein, in bacterial, yeast, insect, and mammalian systems. This is followed by multi-step purification of recombinantly expressed proteins with affinity chromatography, cation/anion exchange chromatography and size exclusion chromatography employing FPLC. The process is optimized for high level expression and purification of homogenous proteins, for end applications. Purified proteins can be characterized with biophysical and biochemical techniques like Circular dichroism (CD), Differential scanning fluorimetry (DSF), differential scanning calorimetry (DSC), and functional assays. The end-to-end capabilities include expression construct generation, expression, purification, and characterization, including optimization of the process of protein production, which can be translated for GMP scale manufacturing. Bioanalytical analysis, efficacy and non-clinical toxicity studies are performed in collaboration with a third party, followed by GMP scale manufacturing.

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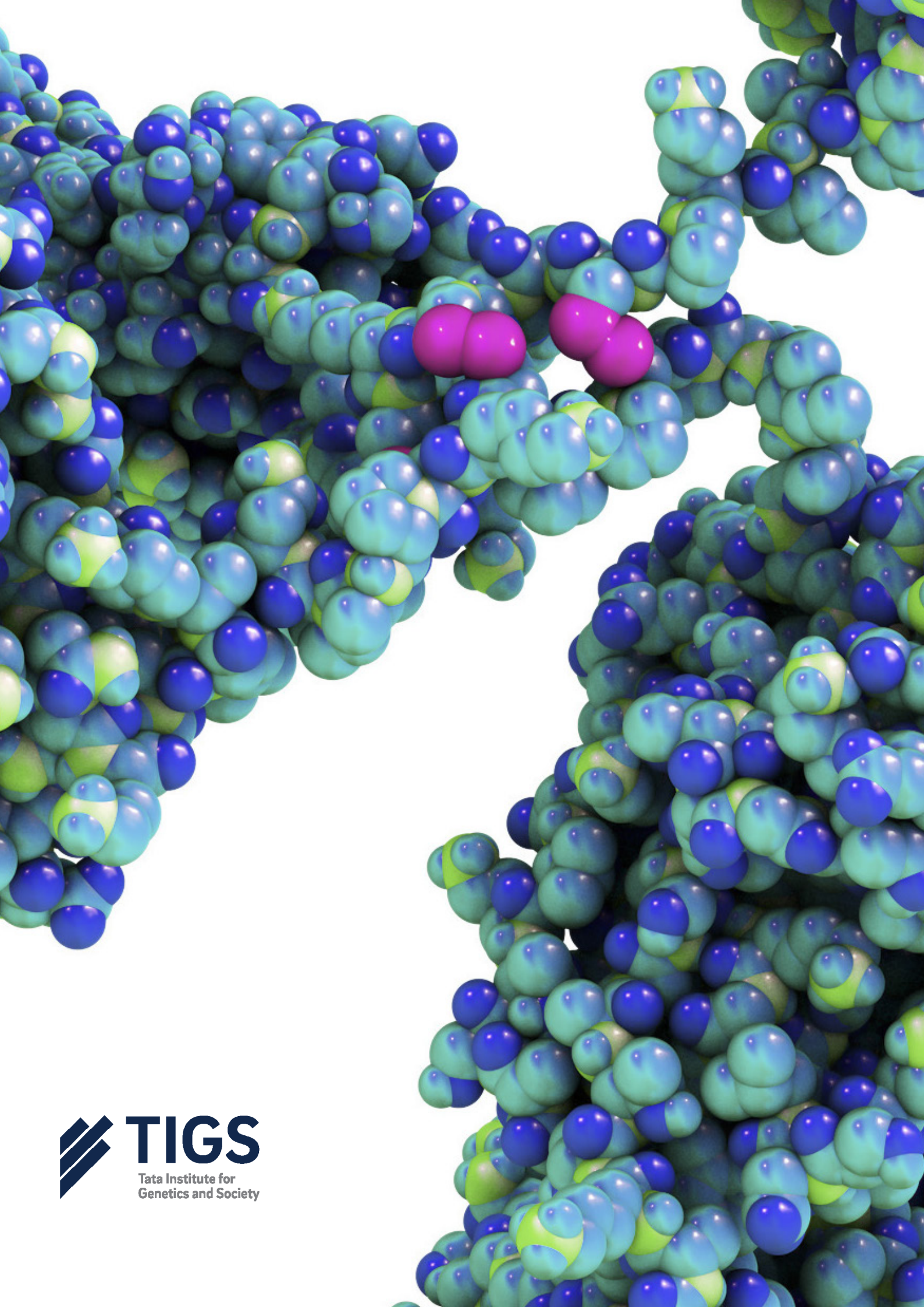

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