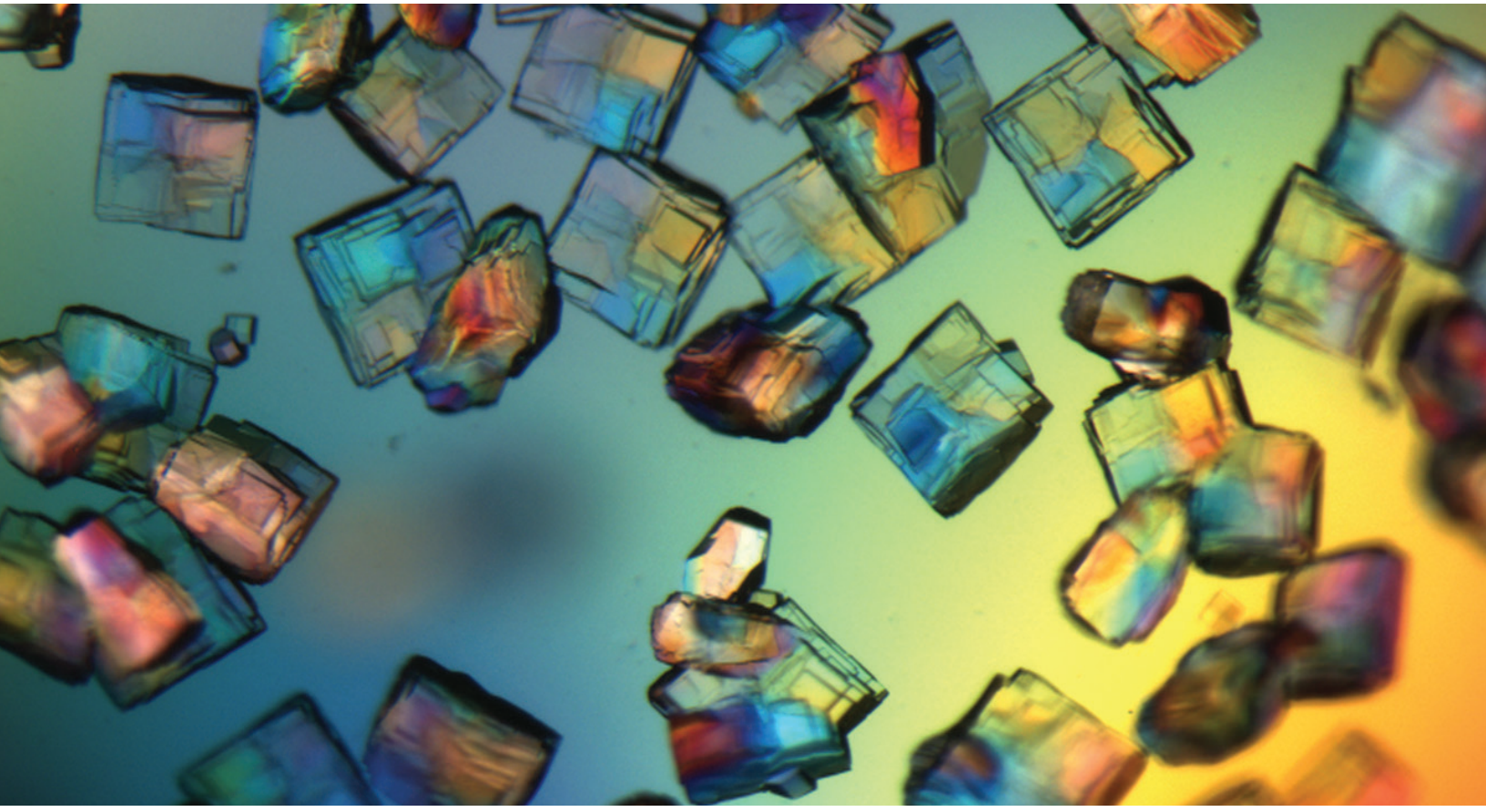
ALTHEA®

THE POWER TO MAKE

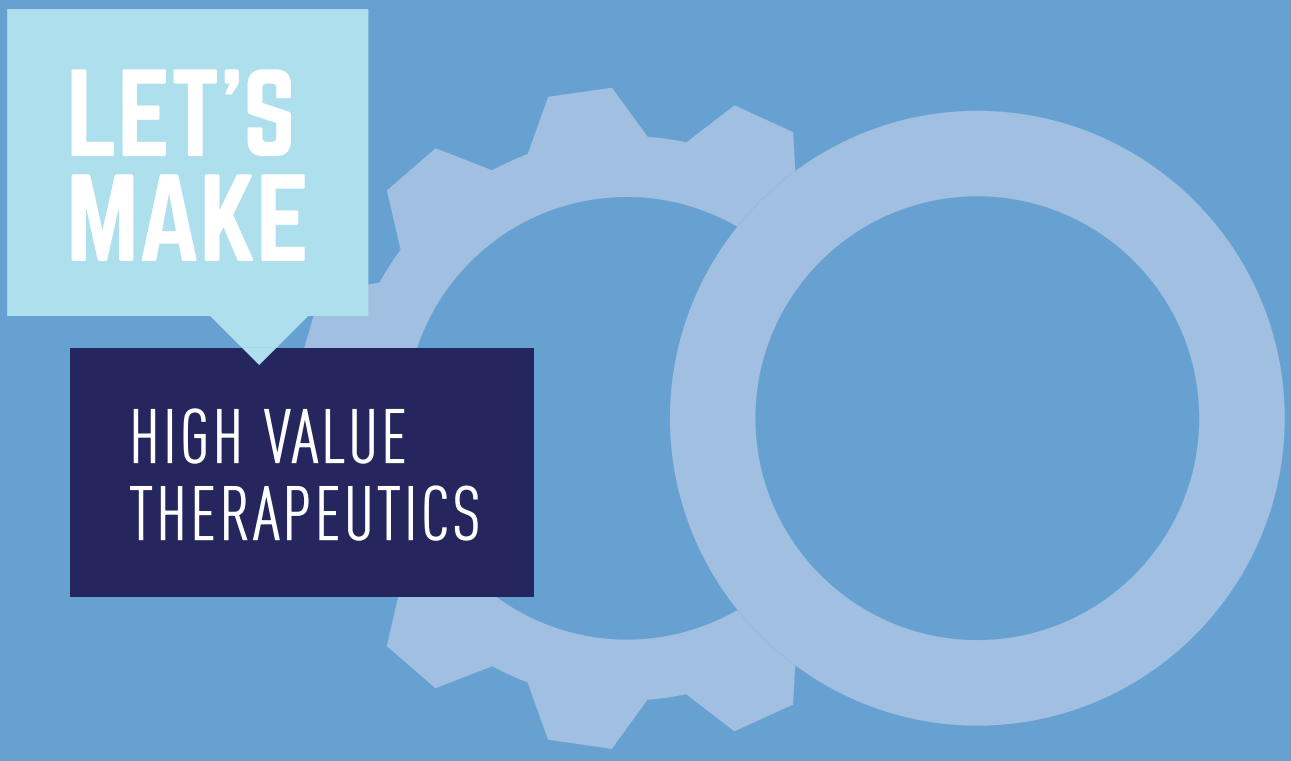
CRYSTALOMICS® FORMULATION TECHNOLOGY



INTRODUCTION

Over the past decade, protein-based therapeutics have emerged as a key driver of growth in the pharmaceutical industry. R&D pipelines have filled with biologics and monoclonal antibodies have become the best-selling drugs around the world. Despite the success of this segment, the size and complexity of protein molecules create specific challenges when developing these types of therapeutics.

To help clients overcome formulation challenges, Althea® offers access to our proprietary Crystalomics® Formulation Technology. The technology produces highly concentrated formulations with low viscosity, enabling low volume administration and increased stability. Crystalline suspensions offer alternative routes of delivery and the opportunity to extend the patent life of high value biologics.



ALTHEA'S CRYSTALOMICS® FORMULATION TECHNOLOGY: OVERCOMING THE CHALLENGES

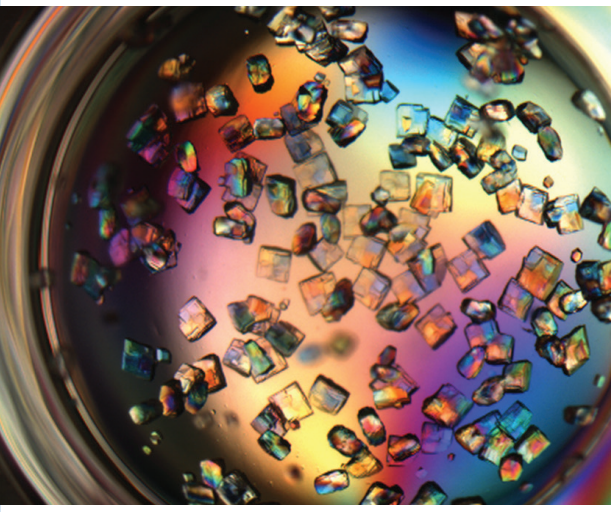
Althea has a unique portfolio of intellectual property encompassing crystallization, cross-linking and complexation of proteins for therapeutic use. It includes patents, proprietary knowledge and expertise to develop ideal crystallization conditions, stable crystalline formulations and scale-up for GMP manufacturing of crystalline suspension drug products. As a result, both small and large pharmaceutical companies have relied on Althea to formulate highly-concentrated, crystallized therapeutic proteins in suspension. Our Crystalomics® Formulation Technology benefits both pharmaceutical developers and, more importantly, patients.

CHALLENGES IN PROTEIN THERAPEUTICS

- Large doses must be administered due to poor bioavailability
- Frequent injections are often required because of undesirable pharmacokinetics
- Low concentration solutions demand large volume intravenous (IV) infusions
- High viscosity solutions result in poor syringeability and injectability

MANUFACTURING ADVANTAGES OF CRYSTALLINE SUSPENSIONS

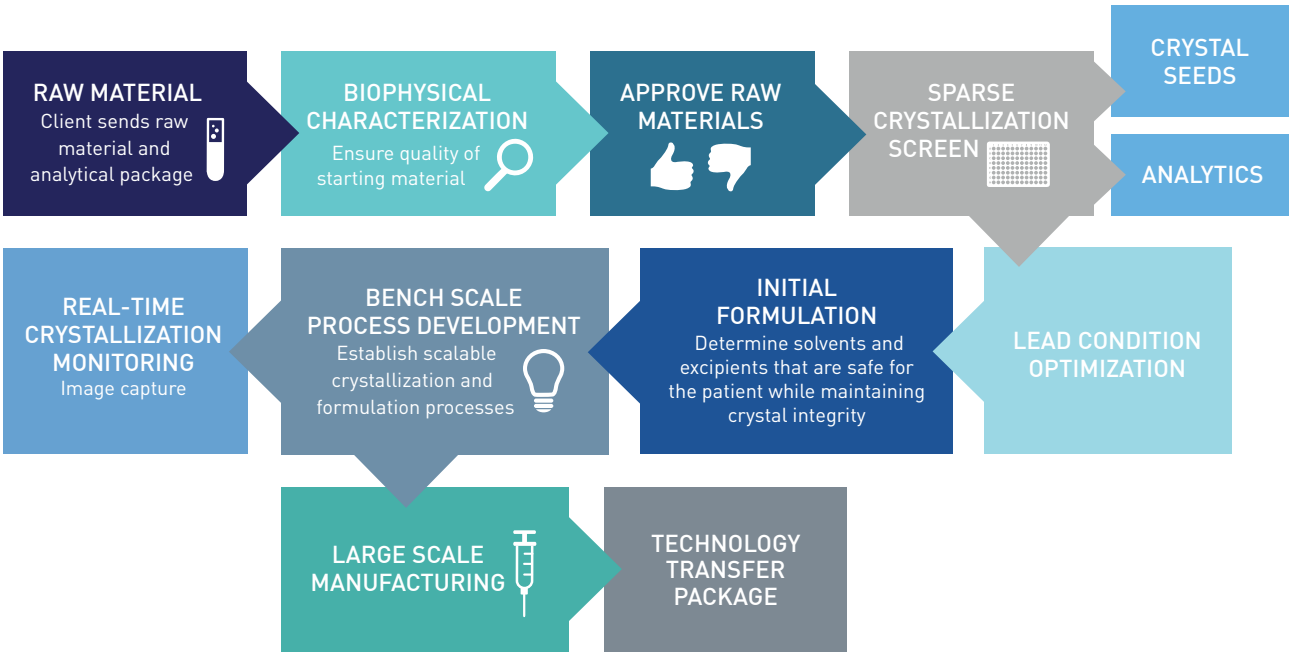
- Ability to formulate highly concentrated proteins in small injection volumes
- Scalable to support both clinical stage and commercial manufacturing
- Maintains biochemical characteristics and bioactivity of the soluble protein
- Improvement in syringibility and injectability
- Flexibility in dosage form–oral, pulmonary, topical and subcutaneous injection possible
- Opportunity to extend patent life of branded protein-based therapeutics



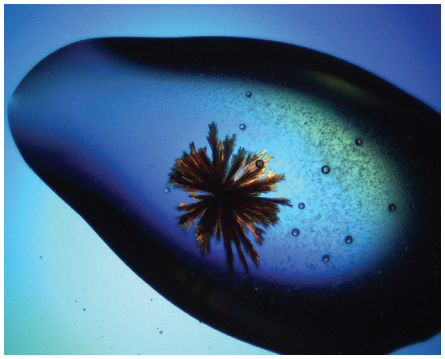
PATIENT BENEFITS OF CRYSTALLINE SUSPENSIONS

- Better compliance without time-consuming IV infusions
- Self-injection that doesn't require trained medical personnel
- Improved patient comfort via use of finer gauge needles
- Non-injection routes of administration possible
- Fewer treatments via controlled and extended release formulations
- Same therapeutic benefits as low concentration formulations

CRYSTALOMICS® WORKFLOW



Althea has successfully crystallized hundreds of proteins of various sizes and types. The following case study outlines the crystallization of Infliximab and provides data demonstrating the crystallization process has no effect on structure or biological activity.



INFLIXIMAB CASE STUDY–MAKING SUBCUTANEOUS INJECTIONS POSSIBLE

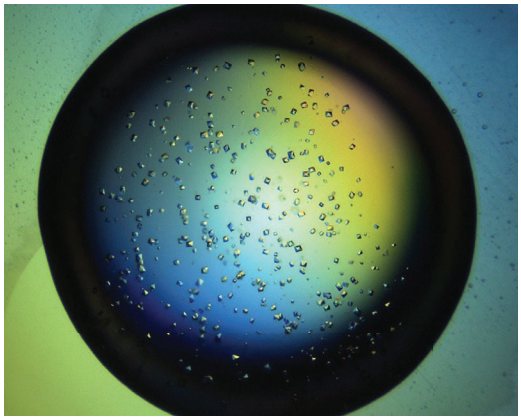
Infliximab is a monoclonal antibody against tumor necrosis factor alpha (TNF-α). It is marketed under the trade names Remicade® (Janssen Biotech), Remsima™ (Celltrion), and Inflectra™ (Hospira) for the treatment of Crohn's disease, psoriasis and other autoimmune diseases. Infliximab is administered at a dose of 3-5 mg/kg via intravenous infusion every six to eight weeks. IV infusion at a hospital or clinic is necessary because the maximum deliverable antibody concentration is limited to 10 mg/mL. Therefore, a large volume is required to deliver the therapeutic dose. Using Crystalomics® Formulation Technology, Althea successfully formulated a 250 mg/mL suspension, thereby enabling self-administered subcutaneous injections that have the potential to significantly reduce dosing frequency and improve patient compliance in the future.

	Concentration (mg/mL)	Administration Volume (mL)
Infliximab		
Commercial	10	35
Crystalline	250	1.5
Change	**+25x**	**-96%**

TABLE A: Crystalomics® Formulation Technology increased protein concentration 240 mg/mL over the soluble dosage form while also reducing the volume approximately 96%.

CRYSTALLIZATION OF INFLIXIMAB

Althea typically screens over a thousand crystallization conditions. Variables include the types and concentrations of salts, precipitants and buffers as well as combinations of pH and temperature. The best crystallization conditions use pharmaceutically acceptable ingredients and are selected to give the highest yield of uniform crystals of a narrow size range in a relatively short amount of time (usually <24 hours).

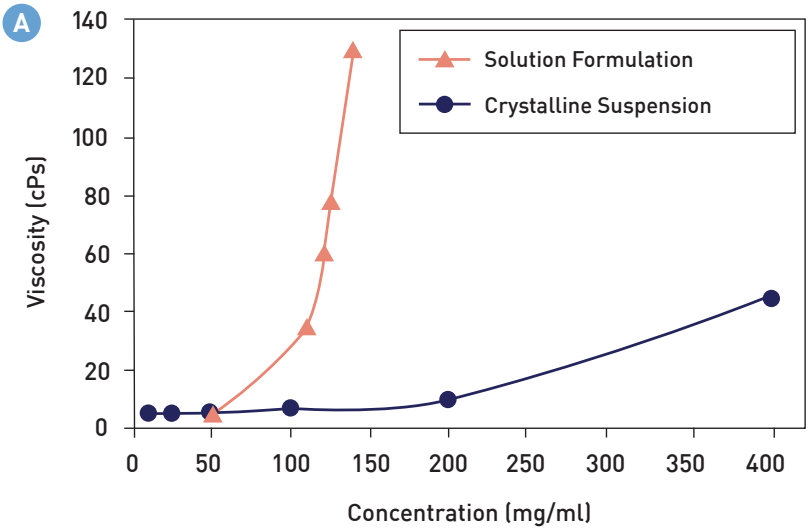


Commercially available Infliximab (5g) was desalted on a column. The crystallization solution was added dropwise to the desalted antibody and stirred at room temperature. Star-shaped crystals began appearing overnight and crystallization was continued for 24 hours. Upon completion, the yield (percent conversion) of crystals was approximately 90%.

ANALYTICAL CHARACTERIZATION OF CRYSTALLIZED INFLIXIMAB

COMPARISON OF VISCOSITIES – SOLUBLE VS. CRYSTALLINE

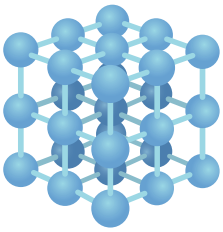
A



CRYSTALS ARE HIGHLY ORGANIZED MOLECULAR STRUCTURES

B

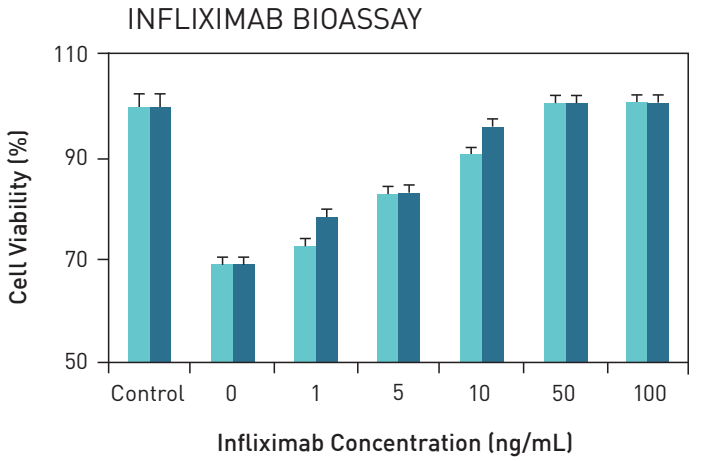
Crystal Size (µm)	Number of Protein Molecules
0.1	10^4
1	10^7
10	10^{10}
100	10^{13}



CRYSTALLINE SUSPENSIONS ALLOW FOR MUCH HIGHER PROTEIN CONCENTRATION WITHOUT INCREASING VISCOSITY

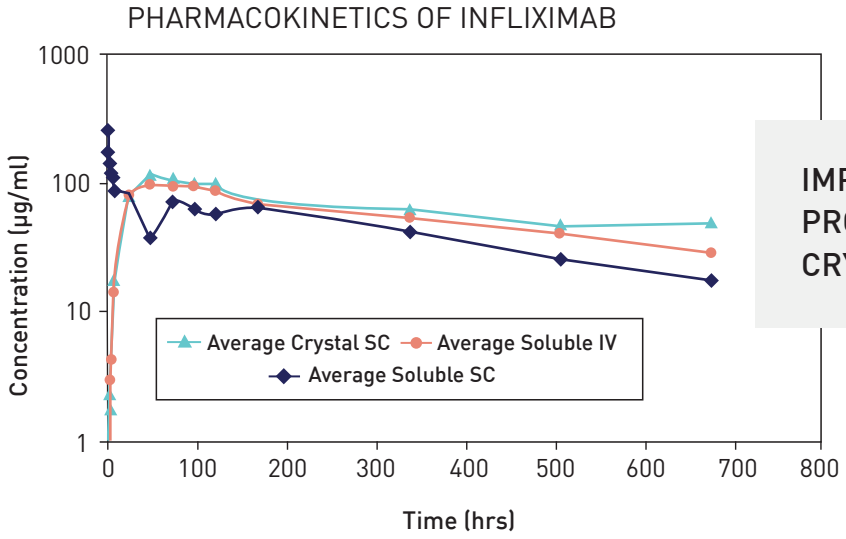
FIGURE 1. A: Viscosity of Infliximab. The viscosity of soluble and crystalline suspensions of Infliximab was measured by using a Cannon-Fenske viscometer according to the manufacturer's instructions. **B:** Crystallization reduces viscosity. Protein crystals are highly organized structures of densely packed protein molecules.

BIOLOGICAL CHARACTERIZATION OF CRYSTALLIZED INFLIXIMAB



BIOLOGICAL ACTIVITY OF CRYSTALLIZED INFLIXIMAB IS INDISTINGUISHABLE FROM THE SOLUBLE FORM

FIGURE 2. *In vitro* bioactivity of Infliximab. Cultured L-929 mouse fibroblast cells were detached, diluted to $2x10^5$ cells per mL and added to 96-well plates (100 µL per well). TNF-α neutralization assays were performed by incubating mouse fibroblast cells overnight in the presence of 100 pg/mL TNF-α and various concentrations of Infliximab or dissolved crystals of Infliximab. The number of viable cells was determined by using a commercially available cell proliferation assay kit.



IMPROVED PHARMACOKINETIC PROFILE WITH SUBCUTANEOUS CRYSTALLINE INFLIXIMAB

FIGURE 3. PK studies of Infliximab. Approximately 100 µL of Infliximab (20 mg/mL) was administered to BBDR_Wor rats having a body weight of 250 g to provide a dose of 8 mg/kg. The mAb was administered in a soluble form either intravenously (IV) or subcutaneously (SC) or as a crystalline suspension SC. The maximum concentration (Cmax) for soluble Infliximab was 257 µg/mL for the IV sample and 95 and 112 µg/mL for the SC soluble and crystalline samples, respectively. The half-life of soluble Infliximab was 270 h for IV sample; the half-life of the SC soluble and crystalline Infliximab was 390 and 779 h, respectively. The total Area Under the Curve (AUC_{0-t}) for soluble Infliximab was 29.3 mg-h-mL^{-1} for the IV sample and 37.5 and 42.8 mg-h-mL^{-1} for the SC soluble and crystalline samples, respectively.

CRYSTALLIZATION DOES NOT DECREASE THE EFFICACY OF INFLIXIMAB

IN VIVO EFFICACY OF INFLIXIMAB IN TNF-α MODEL

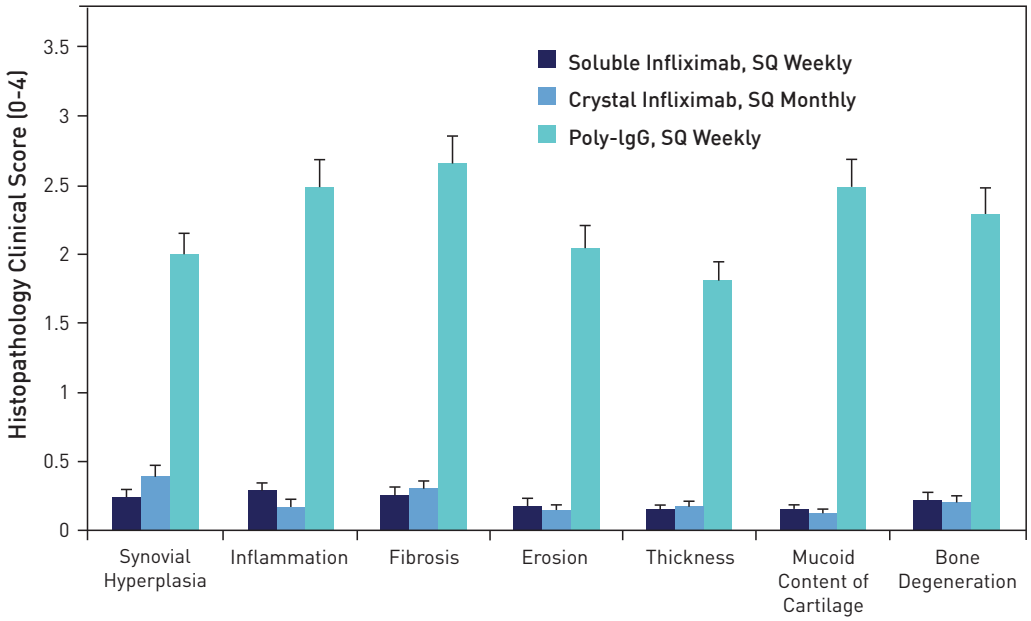
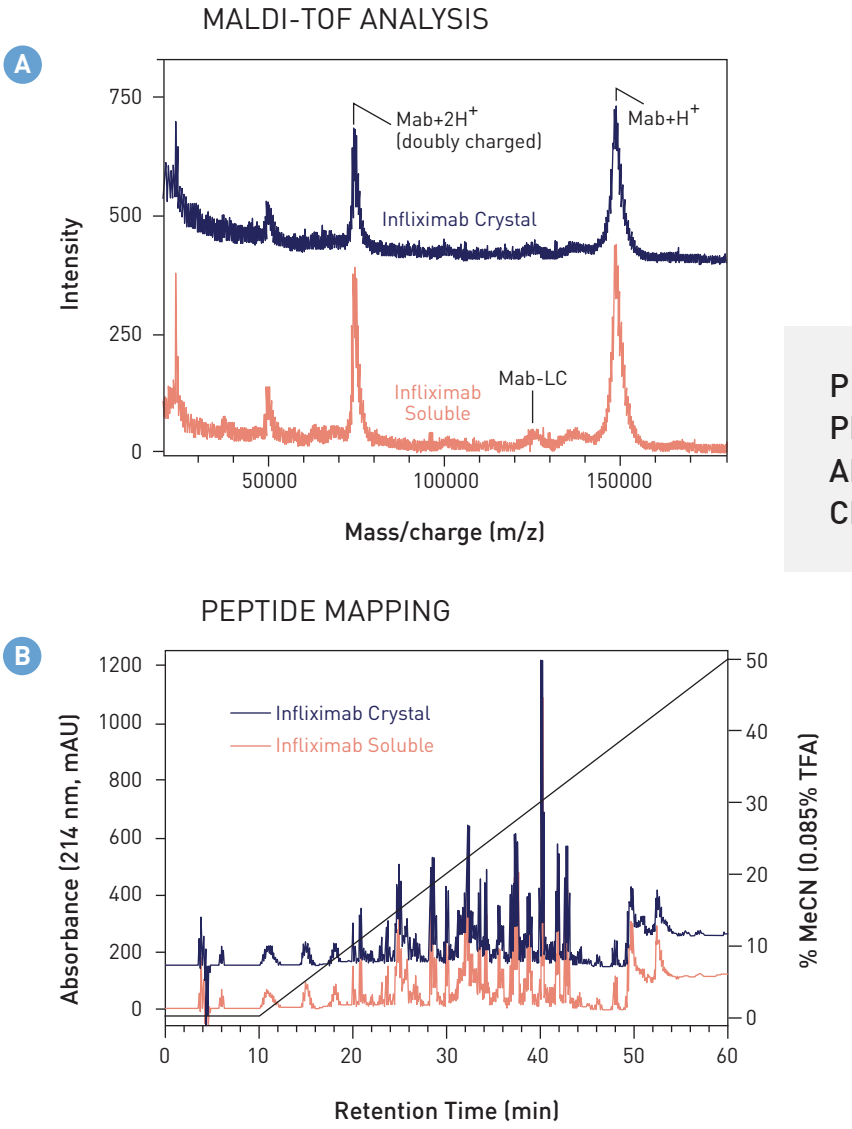


FIGURE 4. *In vivo* efficacy of Infliximab. Approximately 100 µL of Infliximab (20 mg/mL) was subcutaneously administered to C57BL/6NTac-TgN(TNF-α) mice at a dose of 8 mg/kg in both soluble (weekly) and crystallized (monthly) forms. Non-specific IgG was used a control.

CRYSTALLINE INFLIXIMAB ADMINISTERED MONTHLY HAS THE SAME EFFECT AS THE SOLUBLE FORM ADMINISTERED WEEKLY

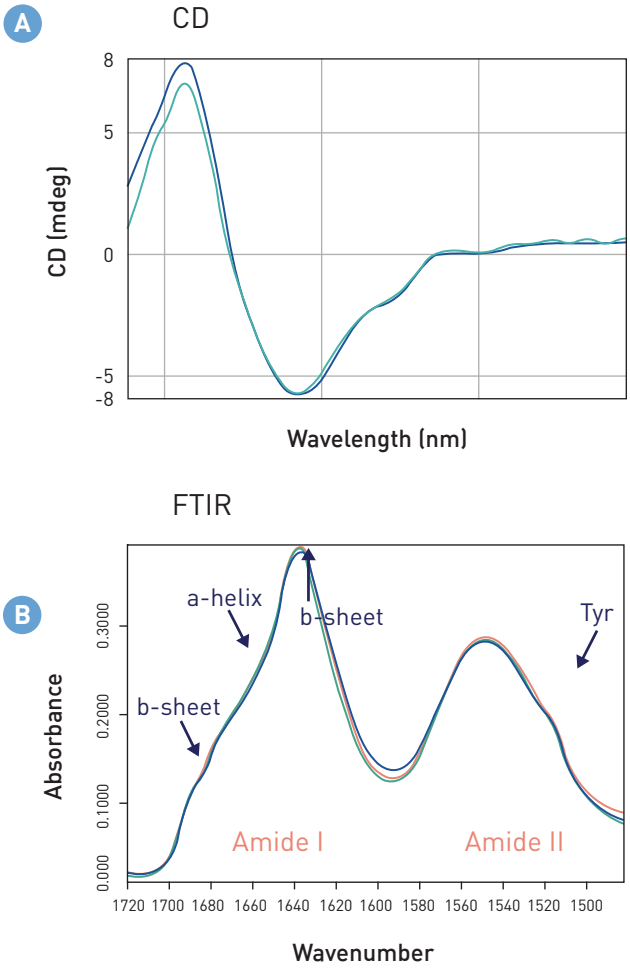
BIOPHYSICAL CHARACTERIZATION OF INFLIXIMAB



PHYSICOCHEMICAL PROPERTIES OF INFLIXIMAB ARE NOT CHANGED BY THE CRYSTALLIZATION PROCESS

FIGURE 5. A: MALDI-TOF analysis of Infliximab. ABI Voyager DE Pro and UV laser. High laser power was used to acquire spectra in one 100-scan experiment and caused the fragmentation into light chain (LC, 25 kDa) and heavy chain (HC, 50 kDa). **B:** Peptide map of soluble and crystalline Infliximab. Upper and lower chromatograms show crystalline and soluble Infliximab, respectively. The soluble and crystalline Infliximab were digested with trypsin and the resulting peptides were separated on a C8 reversed-phase column. Tryptic digest; 74 peaks, Complete match; Vydac C8, 208TP104, 250 X 4.6 mm I.D. Buffer A: 0.1% TFA/H20; Buffer B: 0.085% TFA/MeCN 0.9 mL/min, 1% buffer B/min gradient (after 10 min at 0% B).

STRUCTURAL ANALYSIS OF INFLIXIMAB



Blue, Soluble; **Green**, reconstituted crystals of Infliximab.

Red, Soluble; **Blue**, reconstituted crystals and **Green**, crystals of Infliximab.

CRYSTALLIZATION PRESERVES A PROTEIN'S SECONDARY STRUCTURE

FIGURE 6. Analysis of secondary structure of Infliximab by CD and FTIR spectroscopy confirms secondary structure is unchanged after crystallization. **A:** CD Plot. Soluble Infliximab (Blue) and reconstituted Infliximab crystals (Green) spectra were collected using JASCO J-810 CD spectropolarimeter. **B:** FTIR Plot. Soluble Infliximab (Red) and reconstituted crystals (Blue; crystals were dissolved before the spectra were taken) and crystal suspension (Green) of Infliximab. The spectra were collected by using a Bruker FTIR instrument (Helios microanalysis) equipped with an ATR accessory. The content of α-helix (≈7%), β-structure (≈47%) and random coil (≈46%) remained unchanged before and after crystallization, as well as in the crystalline state.

SUCCESSFUL CRYSTALLIZATION OF RITUXIMAB AND TRASTUZUMAB

	Concentration (mg/mL)	Administration Volume (mL)
Rituximab		
Commercial	10	75
Crystalline	320	2.3
Change	**+32x**	**-97%**
Trastuzumab		
Commercial	21	13
Crystalline	350	0.8
Change	**+17x**	**-94%**

TABLE B: Crystalomics® Formulation Technology achieved similar increases in protein concentration and reductions in dosage volume for both Rituximab and Trastuzumab.

CRYSTALOMICS® FORMULATION TECHNOLOGY: A TRACK RECORD OF SUCCESS

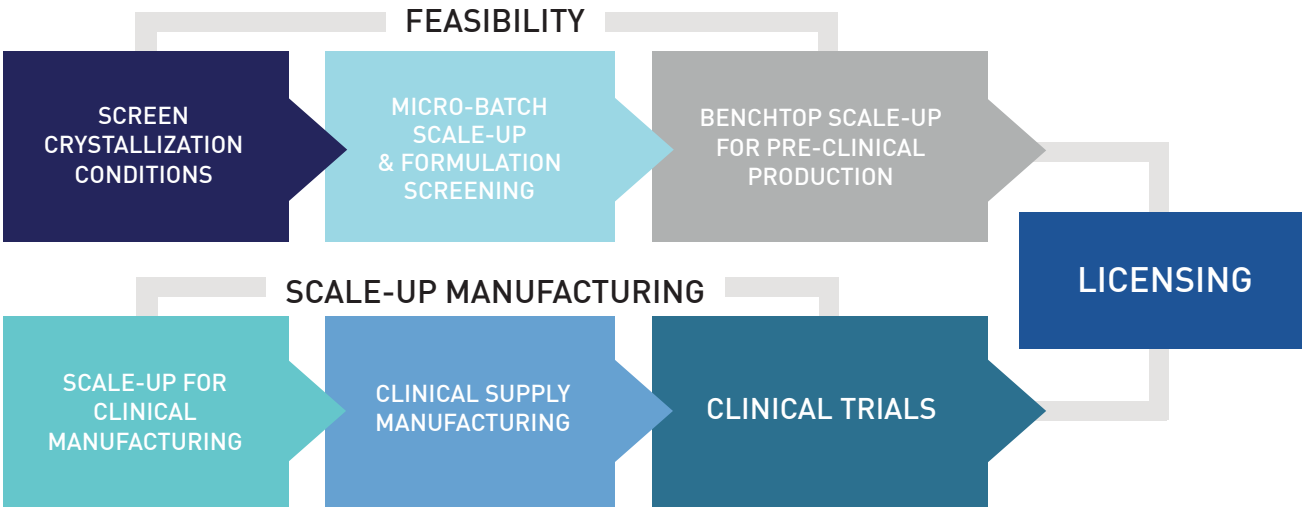
- Over 100 proteins have been crystallized including antibodies, hormones, enzymes and peptides
- Protein Sources: human, microbial, animal, recombinant
- >20-fold increase in concentration: mAbs 200-400 mg/mL
- Characteristics of successfully crystallized proteins:
  - Molecular weights: 1.5-500 kDa
  - No. of subunits: 1-6
  - Glycoproteins: 5-18% carbohydrate
  - Crystal size: 1-500 µm
- Althea has worked with some of the largest pharmaceutical and biotech companies in the world

WHY WORK WITH ALTHEA?

Althea is a leading expert in aseptic filling of drug product in vials and syringes, and our focused expertise and capabilities make us one of the industry's top leaders for cGMP microbial-based manufacturing of recombinant proteins and plasmid DNA. In conjunction with these manufacturing operations, Althea offers comprehensive services including: upstream and downstream process development, complex formulations, lyophilization cycle, analytical development, product release and ICH-compliant stability testing.

Althea has an excellent track record with global regulatory agencies, including the FDA, EMA, PMDA (Japan) and RP (Germany). We have successfully completed seven drug approval inspections covering applications in the US, EU, Japan and Canada demonstrating our drug product manufacturing processes and quality control testing, and our overall quality systems are in compliance with European cGMP standards and FDA guidelines. We take pride in keeping our facility and processes operating in a state of control in order to assure your product will meet the highest possible quality standards.

HOW TO GET STARTED



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