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Ranibizumab

Technology from the group of **Rahul Bhambure**
at **CSIR-National Chemical Laboratory, Pune, India**

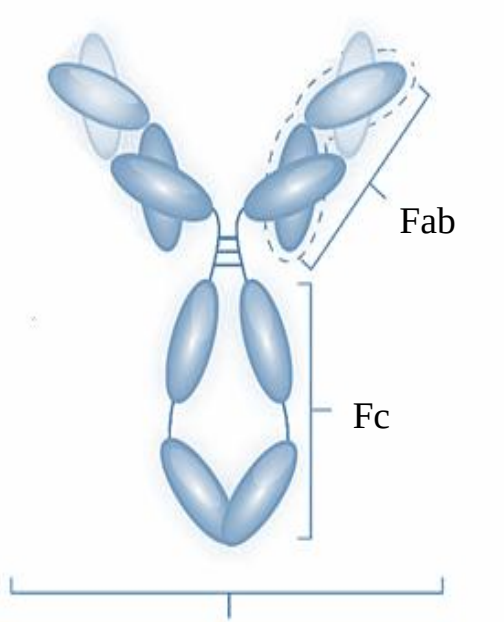
Match Maker/ Biosimilars / 31 Aug 2021/DrBhambure CSIR-NCL

Council of Scientific and Industrial Research
National Chemical Laboratory

TechEx.in Case Manager:

Devanshi Patel,
devanshi@venturecenter.co.in

Primer: Antibody fragments



Immunoglobulin G

- Fab is the multi-domain protein containing:
  - -- **heavy chain** composed of a variable (VH) and the first constant (CH1) domains
  - -- **light chain** composed of the light variable domain (VL) and the constant domain (CL)

- Eight Fab molecules approved by the US Food and Drug Administration
  - -- six of which are produced using **E. coli host cell**, which include rHu Ranibizumab, rHu Certolizumab pegol, Blinatumomab, Moxetumomab pasudotox, rHu Caplacizumab, and rHu Brolucizumab
  - -- Two other antibody fragment rHu Abciximab and rHu Idarucizumab are produced using **mammalian host cell**

Primer: Why antibody fragments?

Advantages

- Easy penetration in tissues
- Elimination of the immunogenicity due to lack of Fc region
- ***Bacterial expression of antibody fragments offers time and cost-effective high throughput manufacturing processes as compared to monoclonal antibody production using mammalian cell systems***

Disadvantages

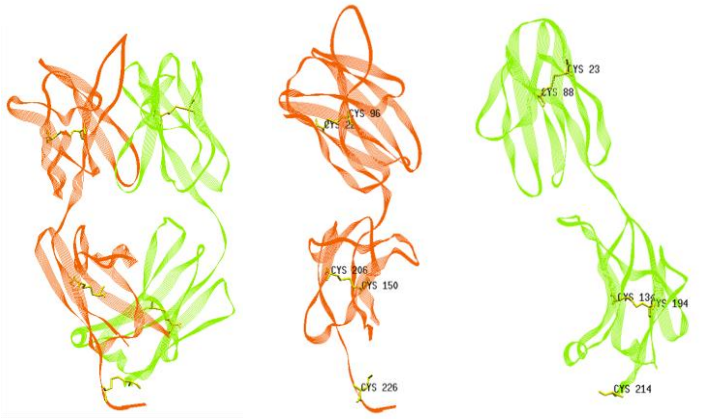
- Reduced stability of the fragments compared to full-length antibodies
- Short circulation half-life
- Requirement of an efficient in-vitro refolding process

About Ranibizumab

Ranibizumab is a **recombinant humanized IgG1** monoclonal antibody fragment and **VEGF-A antagonist**

- **Originator / reference product:** Lucentis, was marketed by Genentech (Roche)/Novartis, approved by the USFDA in June 2006 and by EMA in Jan 2007. The patents on Lucentis **expired** in the **US in June 2020** and will expire in **Europe in 2022.** (Source: GaBI Online)

- **Indications:** Used in treatment of neovascular (wet) **age-related macular degeneration (wAMD),** Neovascular AMD (most severe vision loss), Macular edema following retinal vein occlusion (RVO), Diabetic macular edema (DME), Diabetic retinopathy (DR) and Myopic choroidal neovascularization (mCNV)



Fab

(48.38 kDa)

Heavy chain

(231-residue heavy chain of 24.95 kDa)

Light chain

(214-residue light chain of 23.43 kDa)

Note: Total five disulfide bond comprises two intra disulfide in each chain with one inter disulfide between light and heavy chain

Market and Industry Overview

Market:

The global age-related macular degeneration (AMD) market stood at $ 1.58 billion in 2020 and is projected to reach **$ 2.64 billion by 2026, growing at CAGR of 8.93%** between 2021 and 2026 (Source: EMR)

Industry players:

- **Global:** Genentech, Novartis
- **India:** Intas

The Opportunity: Why you should be interested?

- **Market interesting**: **AMD Affects nearly 8.7% of the worldwide population,** and the numbers are projected to increase to around 196 million in 2020. Projected number of people with the disease is around **196 million in 2020, increasing to 288 million in 2040**. (Source: All About Vision)
- **Cost still high:** Approximately**, 51% of the patients on VEGF therapy dropout of therapy** after initial injections. The most common reason is non-affordability of the injection followed by no improvement in vision. (Source: The Indian Express).

Price point Global	Price point India
• Razumab: 2.3mg Injection @ ~ $ 270 • Lucentis: 0.5 mg injection @ ~$ 1120	• Razumab: injection $130 • Lucentis (Branded Accentrix): injection $320

- **Industry not yet crowded: 1st ever Biosimilar of Ranibizumab**- 'Razumab' launched by Intas Pharma in 2015. Few players globally.
- **New indications:** A 2021 survey of Indian vitreoretinal specialists showed progressive trend favouring ranibizumab-biosimilar over bevacizumab-biosimilar.
- **Opportunities for process innovations to reduce costs: Novel continuous processing platform r**esults in reduction in Cost of Manufacturing **by 80% for clinical** and **75% for commercial production**.

The Technology Offering

- Clone, upstream and downstream process
- UPSTREAM: Single fermentation batch required: Antibody fragment expression using duet vector system.

  High throughput refolding process : refolding yield of 40-45 %
- DOWNSTREAM: Purification process of recombinant AbF from inclusion bodies
  - Novel multimodal chromatographic purification steps > 2X improvement in productivity
  - Purification platform applicable to: in-vitro refolded and soluble expressed antibody fragments
  - Overcomes requirement for affinity chromatography, a cost center; uses anion and cation exchange, reducing cost by 1/3rd

Related Patents:

A Method For Producing Refolded Recombinant Humanized Ranibizumab
Priority date: 19.05.2017; WO2018211529 - **IN**, **CN**, **KR**, **EP**, **JP**, **BR**, **CA**, **US**, **MX**, **US**

A Process For The Purification Of Recombinant Antibody Fragments
Priority date: 24.03.2017; WO2018173075 - **IN**, **KR**, **CN**, **EP**, **US**, **JP**, **BR**, **CA**, **MX**

***Relevant Publication*:**

K. Gani, R. Bhambure, P. Deulgaonkar, D. Mehta, M. Kamble, Understanding unfolding and refolding of the antibody fragment (Fab). I. In-vitro study, Biochemical Engineering Journal. 164 (2020) 107764.

Selected Data- Clone and upstream details

Upstream

- Codon optimization
- Vector construction; carrying light chain and heavy chain of rHu Ranibizumab
- Transformation of vector into host cell *E.coli* BL21 (DE3)
- High cell density fermentation
- Inclusion body for rHu Ranibizumab
- Solubilization and *in-vitro* Refolding
- Refolded rHu Ranibizumab

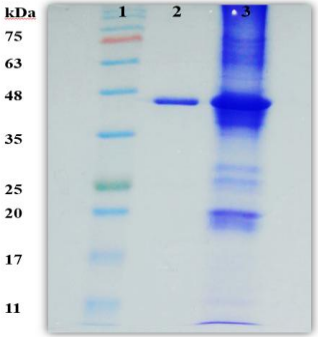
Expression scale: 1 liter bioreactor

Lane 1: NCL-rHu Ranibizumab (Reducing SDS-PAGE showing expressed light and heavy chain)

Lane 2: NCL-rHu Ranibizumab replicate batch

Lane 3: Innovator rHu Ranibizumab (Lucentis)

***In-vitro* dilution based refolding**



***In-vitro* refolding scale: 2 liter reactor**

Lane 1: Molecular weight marker

Lane 2: Innovator rHu Ranibizumab (Lucentis)

Lane 3: NCL refolded rHu Ranibizumab

(Non-reducing SDS-PAGE)

In-vitro refolding: rate limiting step in antibody fragment manufacturing

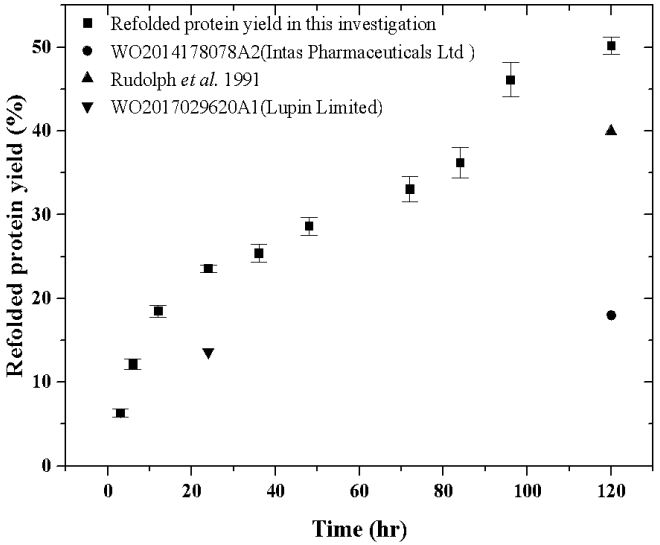
Refolding

- Dilution based refolding is the only scalable alternative for large scale production of antibody fragments
- *In-vitro* refolding process is the key rate limiting step in overall manufacturing of antibody fragments
- Reported *in-vitro* refolding yield for antibody fragments:

  -- Intas: **9.0 refolding yield in 120 hour**

  -- Lupin: **15.0 % refolding yield in 72 hour**

  -- Rudolph *et al.* : **40.0 % refolding yield in 120 hour**

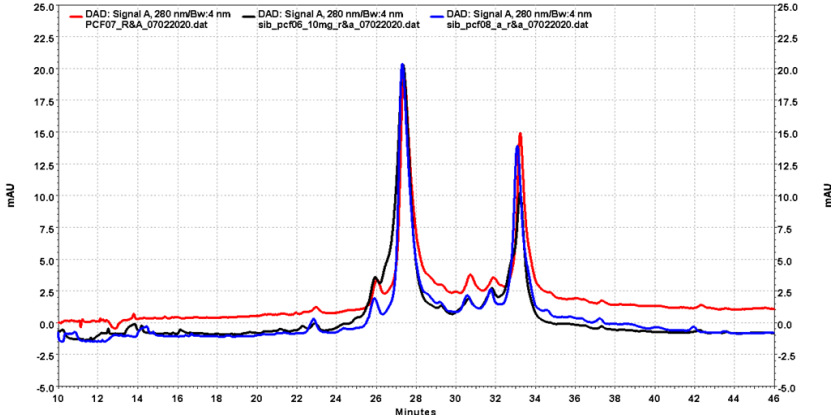


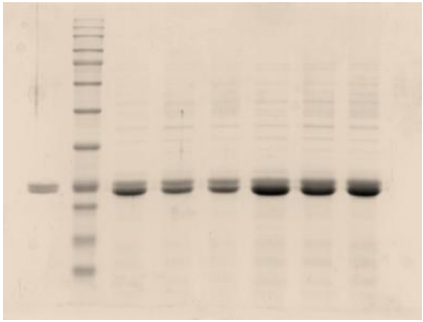
References:

- J. Buchner, R. Rudolph, Renaturation, purification and characterization of recombinant fab-fragments produced in Escherichia coli, Nat. Biotechnol. 9 (1991) 157–162
- H. Shandilya, H. Gadgile, V. Farkade, Cloning, expression & purification method for the preparation of Ranibizumab, US20160289314A1 (2016).
- S. Somani, A. Pandey, A. Nishra, R. Mody, An improved refolding process for antibody's fragments, WO2017029620Al (2017).

Upstream batch consistency for IB production of rHu Ranibizumab

Upstream





Batch fermentation	Protein (mg/L) Batch PC01	Protein (mg/L) Batch PC02	Protein (mg/L) Batch PC03
IBs per litre of media	8806.00	8575.00	8755.00
Light chain	1022.63 ± 71.97	955.08 ± 7.34	948.62 ± 18.38
Heavy chain	402.80 ± 46.97	419.29 ± 3.39	454.08 ± 8.95
Total protein	1425.44	1374.37	1402.70

Lane 1	Standard (4µl)
Lane 2	Molecular weight marker
Lane 3	PC01_IB (4µl)
Lane 4	PC02_IB (4µl)
Lane 5	PC03_IB (4µl)
Lane 6	PC01_IB (7µl)
Lane 7	PC02_IB (7µl)
Lane 8	PC03_IB (7µl)

Downstream platform for rHu Ranibizumab

Downstream

Refolded rHu Ranibizumab

↓

Ultrafiltration and Diafiltration

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Multimodal chromatography I

↓

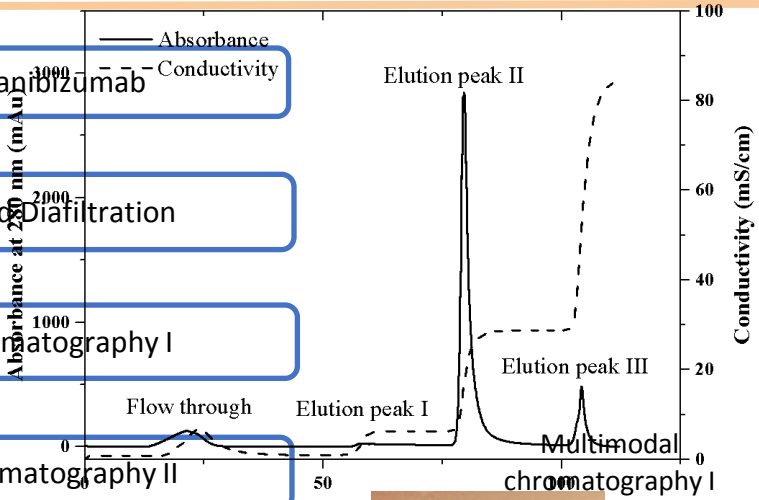
Multimodal chromatography II

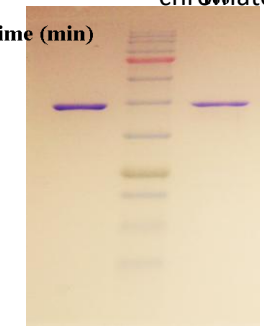
↓

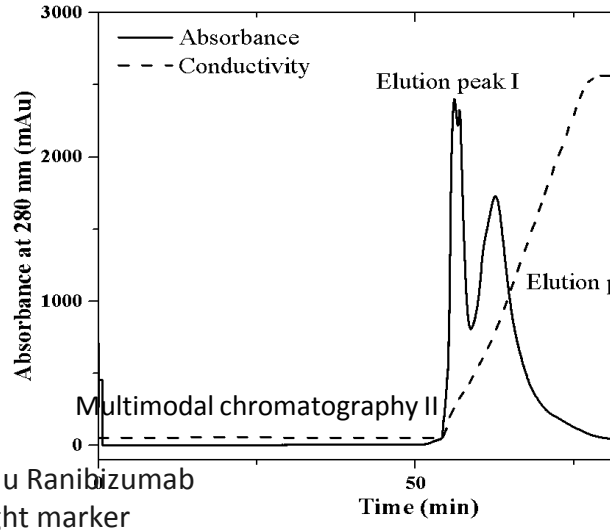
Ultrafiltration and Diafiltration

↓

Purified rHu Ranibizumab





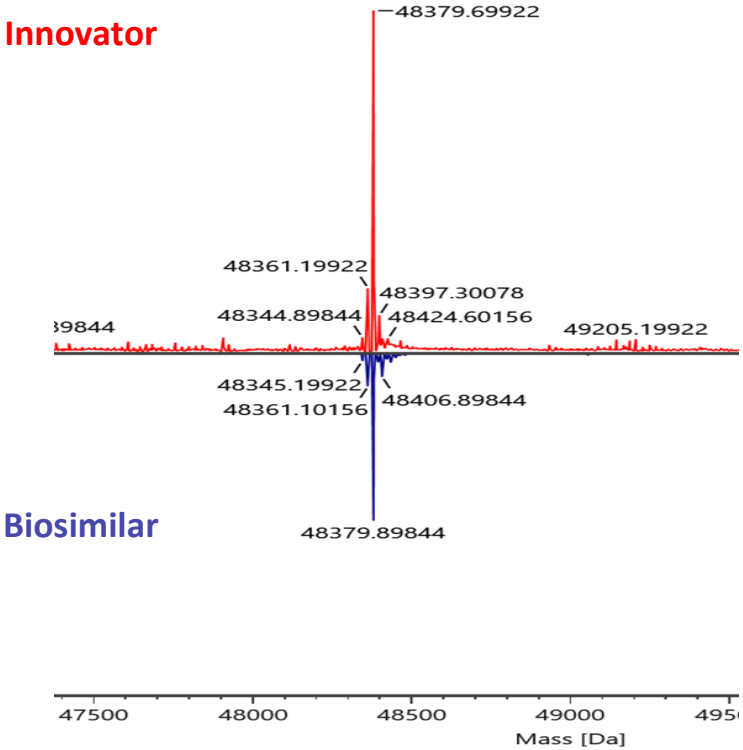


Lane 1: Purified NCL-rHu Ranibizumab
Lane 2: Molecular weight marker
Lane 3: Innovator-Ranibizumab

Conclusion: Designed a novel multimodal chromatography based purification platform for rHu Ranibizumab downstream processing

Biosimilarity data: Intact mass data analysis

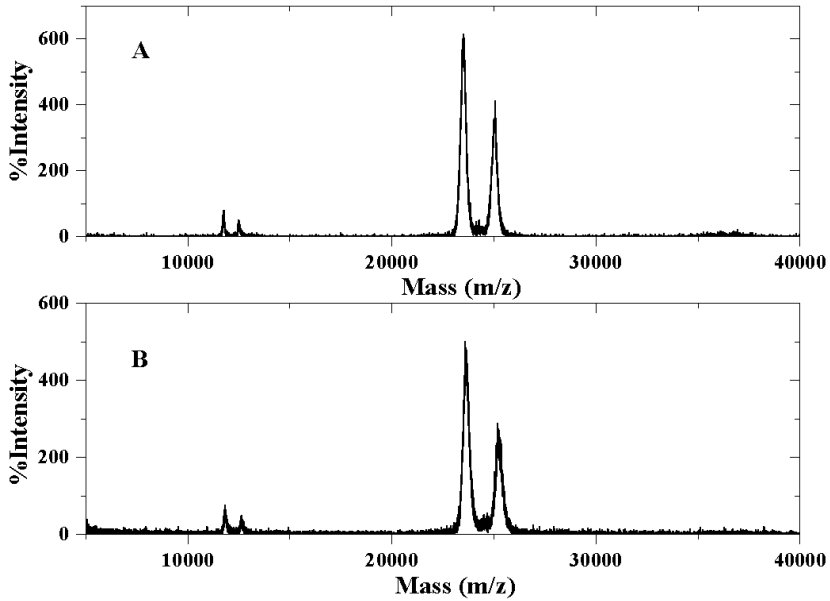
Analytical



Sample	Intact Mass (Da)
Lucentis®	48379.713 ± 0.038
Biosimilar	48379.719 ± 0.023

Biosimilarity data: MALDI-TOF Analysis

Analytical



MALDI-TOF analysis for reduced Ranibizumab molecule

A: Innovator rHu Ranibizumab

B: NCL rHu Ranibizumab

Protein name	Chain Name	Observed mass (Da)
Lucentis®	Light Chain	23428.596±0.002
Lucentis®	Heavy Chain	24952.579±0.013
Biosimilar rHu Ranibizumab	Light Chain	23428.773±0.014
Biosimilar rHu Ranibizumab	Heavy Chain	24952.565±0.010

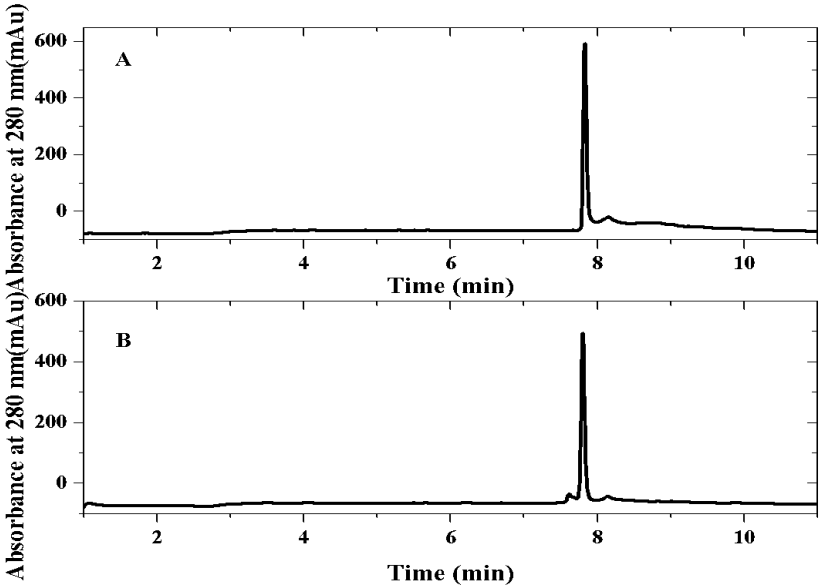
MALDI-TOF analysis for reduced Ranibizumab molecule

A: Innovator rHu Ranibizumab

B: NCL rHu Ranibizumab

Biosimilarity data: RP-HPLC and SEC-HPLC

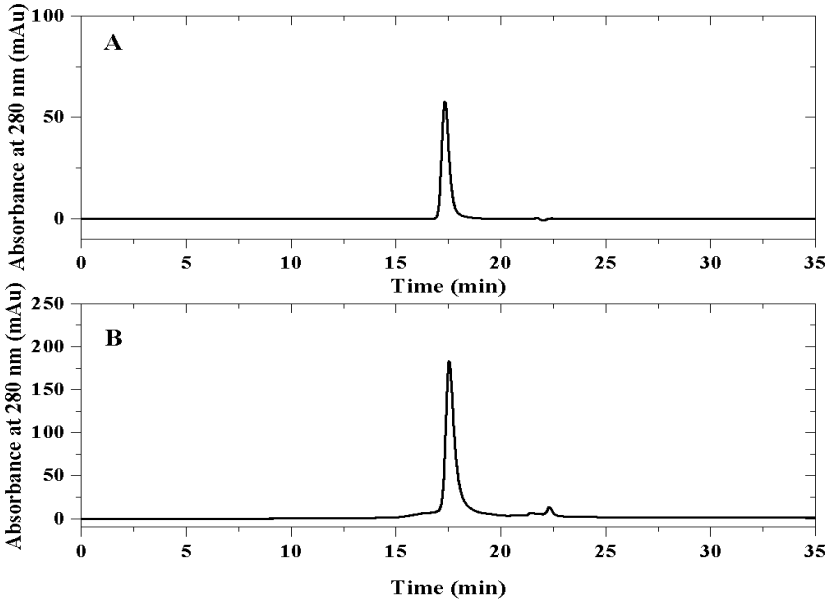
Analytical



Size exclusion chromatogram of purified rHu Ranibizumab

A: Novartis rHu Ranibizumab

B: Refolded rHu Ranibizumab



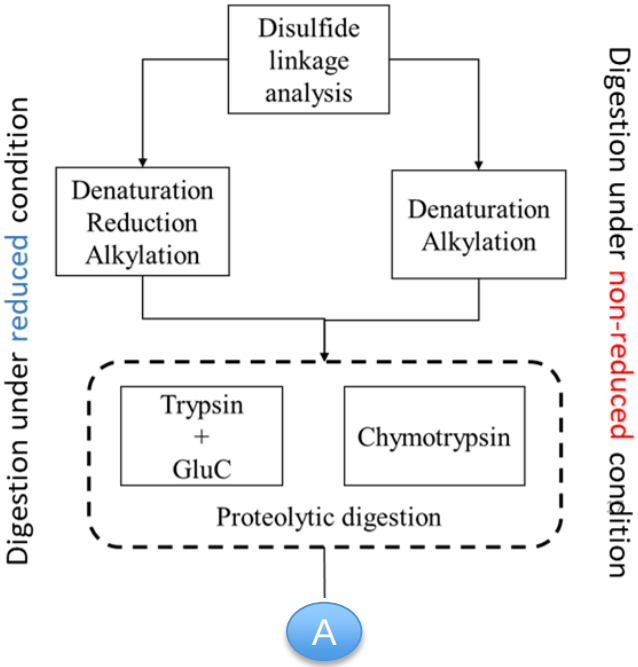
Reversed phase HPLC chromatogram of purified rHu Ranibizumab.

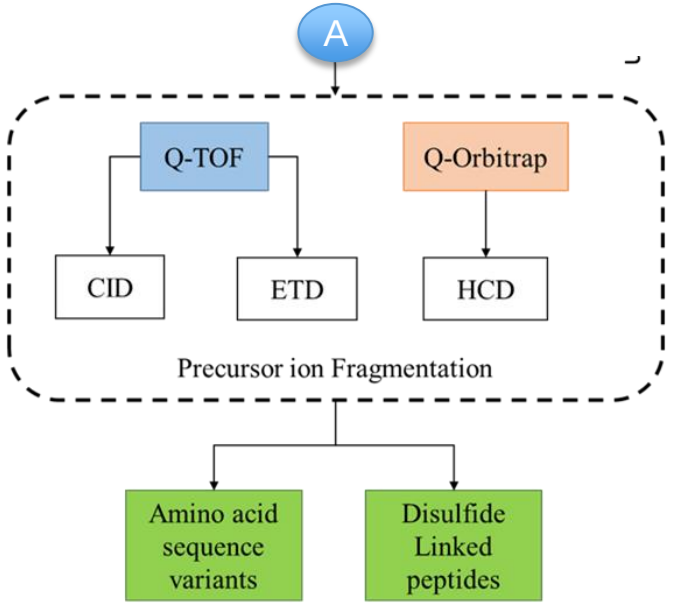
A: Novartis rHu Ranibizumab

B: Refolded rHu Ranibizumab.

Mapping intra and inter-chain disulfide bonds

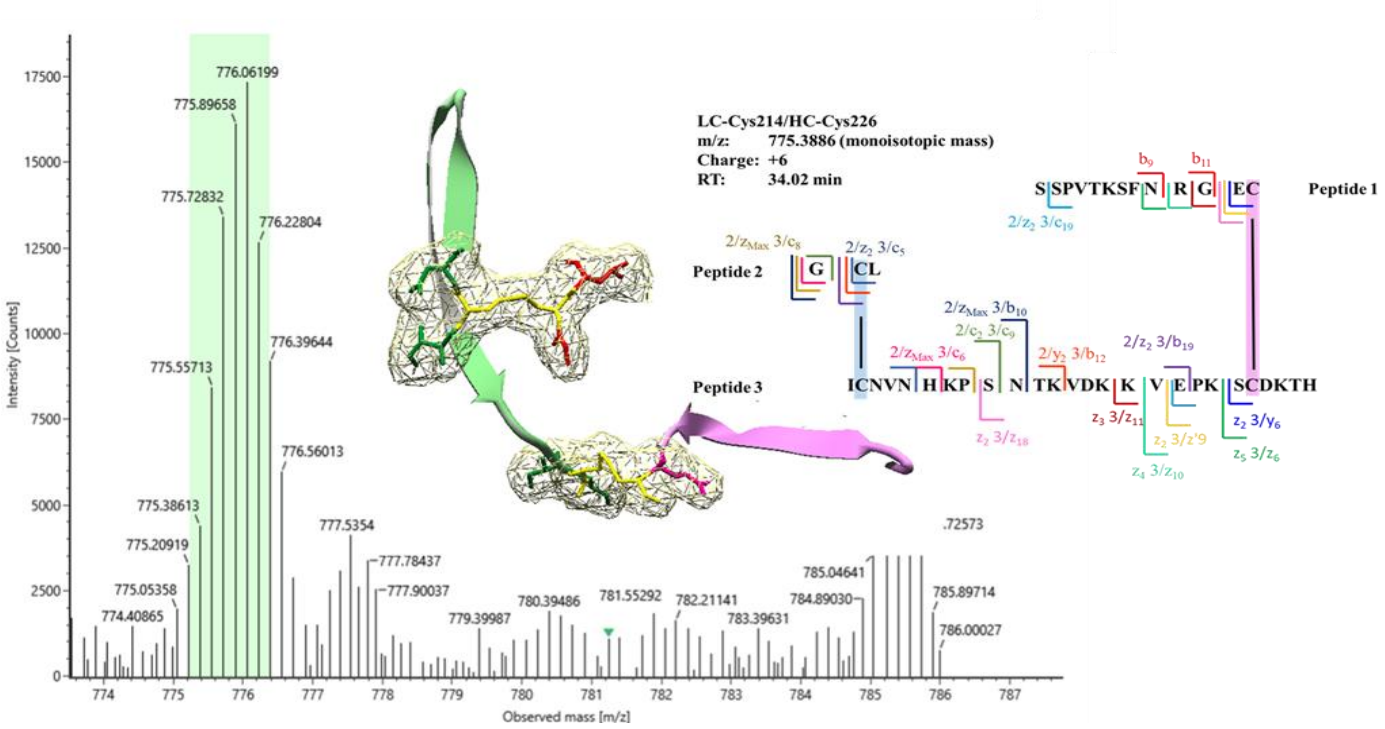
Analytical





Inter-chain disulfide bond: LC-Cys214-HC-Cys226

Analytical



Summary of Biosimilarity Analysis

Test	Test performed at CSIR-NCL
Molecular weight	SDS- PAGE, MALDI-TOF, SEC, ESI-MS/MS
Secondary structure	CD Spectroscopy & Fluorescence Spectroscopy
Carbohydrate content and details of component	Not applicable for this molecule
Aggregate quantification	MALDI-TOF and SEC analysis
HCP quantification	ELISA based assay < 100 ppm in DS
Residual DNA	Picogreen assay < 10 ng/dose in DS
Amino acid sequence	LC-MS/MS
Disulfide bond mapping	LC-MS/MS
Pyrogenic testing	Not applicable for work at CSIR-NCL

- Completed all the biosimilarity analysis required for RCGM submission
- Good agreement between an innovator and developed biosimilar protein

Current Status of Technology

Stage of Development

- Protein expressed at 10 L scale reactor
- Completed five consistency batches at 10 liter scale

Key process parameters

- Achieved yield of **2.81 ± 0.10 g/L**

Next steps

Bioprocess Engg Group at CSIR-NCL is keen to forge industry partnerships for

- Advancing the biosimilar technologies presented today through *in vivo* and clinical studies.

Seeking Industrial partners interested in:

- Licensing technology knowhow with patents
- Joint development, technology advancement and scale-up projects
- Sponsored projects for process development for other biopharmaceuticals
- Industry projects utilizing expertise, capabilities and facilities with the group
- Consulting projects

Bioprocess Engineering Group

Dr Rahul Bhambure
Senior Scientist
Chemical Engineering and Process Development Division,
CSIR-NCL, Pune, India

Recognitions:
DST Early Career Research Award

Past affiliations:
University of Delaware, IIT Delhi, ICT Mumbai

Expertise:
Biochemical engineering; Bioprocess development;
Biopharmaceutical manufacturing (upstream and downstream);
Applied protein biophysics

Fact file of Dr Bhambure's Lab:

- More that 10 years of experience in the field of biosimilars
- Current team strength: 6
- Well equipped labs and analytical facilities including continuous processing platform for monoclonal antibody therapeutics, high resolution and high definition mass spectrometer

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National Chemical Laboratory

For more information contact:

Case Manager:
Devanshi Patel
devanshi@venturecenter.co.in
+91-74100-45655

Lead Scientist:
Dr. Rahul Bhambure
rs.bhambure@ncl.res.in
+91-20-2590 2318

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