InnoPlex: High throughput PCA based HOS confirmation for Novel therapeutic Mabs.

InnoPlex Team

Primary Contributors

Wen-Rong Lie (EMD Millipore)
Xing Wang (Array Bridge)
Lawrence Rentoul (EMD Millipore)
Mike Godney (EMD Millipore)
Shane Curran (EMD Millipore)

Introduction

Therapeutic Monoclonal Antibodies (Mab) represent a growth market within pharmaceutical research. By 2020 there are expected to be 70 approved with a market cap of $125bn, with the majority expected to be full length Mabs*1.

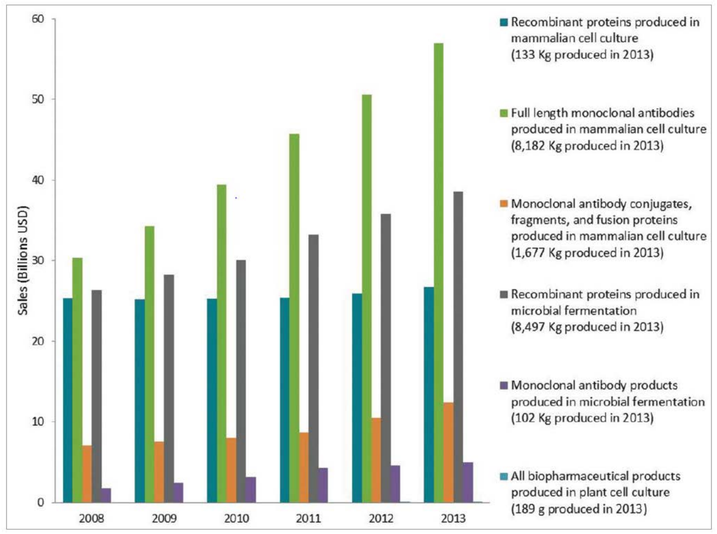
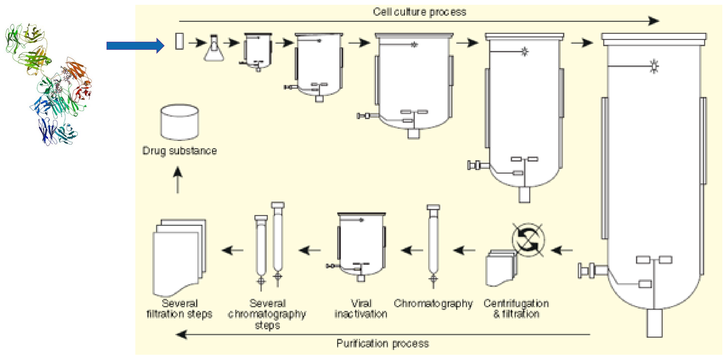


Fig 1*1 Sales of biopharmaceutical products by product type. Total annual sales of biopharmaceutical products are shown as a function of product type. Note that recombinant proteins produced by microbial fermentation include recombinant human insulin products which represent nearly 50% of the sales and >90% of the material produced in this category.

The process to create these molecules differs at the discovery phase however the majority of industrial processes are aiming to create a well folded IgG within a mammalian cell line.



Mammalian cell culture (Fig 2 above*2) from establishment and media optimization through down stream processing and formulation, thousands of samples may be generated. At each stage potential miss folding may occur causing a change in Higher Order Structure (HOS) and potentially increasing immunogenicity. Current technologies used to monitor these changes fall into two categories:

Technique:

- CD Spectrum
- Size Exclusion Chromatography (SEC)
- Analytical Ultracentrifugation (AUC)
- Non-denaturing Electrophoresis

Cheap but NOT Sensitive Moderate throughput.

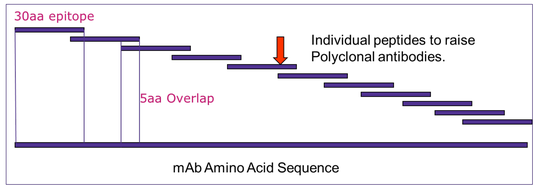
- Hydrogen/deuterium exchange Mass Spec (HDX/MS)
- NMR

Sensitive but NOT Cheap Really low throughput.

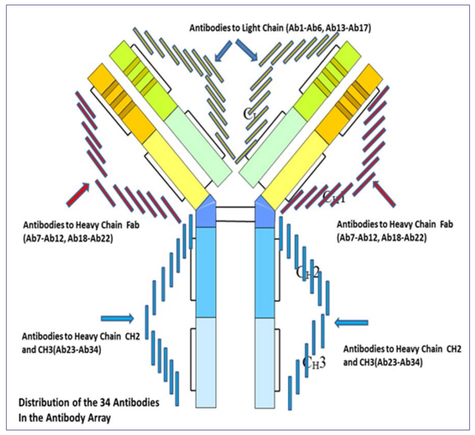
So there is a market place for a technology which is cheaper, sensitive and faster.

Creating InnoPlex

1. Polyclonal Antibodies (Pab) are raised against 30 amino acid epitopes from the amino acid sequence of an IgG1 therapeutic Mab raised in a CHO line. IgGs share a 90% homology allowing for the creation of a universal tool.

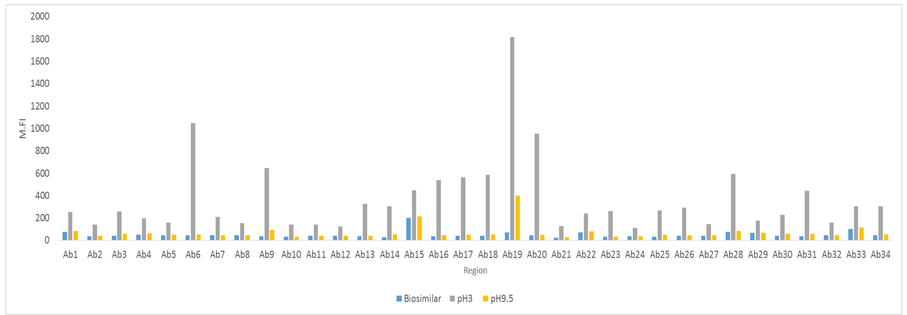


2. Select those that cover the structure of the Mab, usually around 34 targets, from CH-1 to CH-3 regions. This is now a Protein Conformational Array (PCA).



3. InnoPlex: Take the Pabs mount them on xMAP beads and achieve 40+ Samples per plate in a MagPix (Luminex® Corporation).

4. A signal is generated when the structure changes and this epitope of the PCA becomes exposed or hidden. This further provides information as to the location of the deviation as each bead locates to unique sequence of the Mab. E.g. Fig 6 Below represents the change in a candidate biosimilar under pH stress.



InnoPlex & Process Evaluation

Our α-kit was sent to Bristol-Meyers Squibb, for use in their Mab process development group. They published it in January 2018*3 .

MABS
2018, VOL. 0, NO. 0, 1–9
https://doi.org/10.1080/19420862.2017.1421880

Bristol-Myers Squibb

Taylor & Francis
Taylor & Francis Group

REPORT

Check for updates

Monoclonal antibody higher order structure analysis by high throughput protein conformational array

Yuanli Song, Deqiang Yu, Mukesh Mayani†, Nesredin Mussa, and Zheng Jian Li

Biologics Process Development, Bristol-Myers Squibb, 38 Jackson Road, Devens, MA, USA

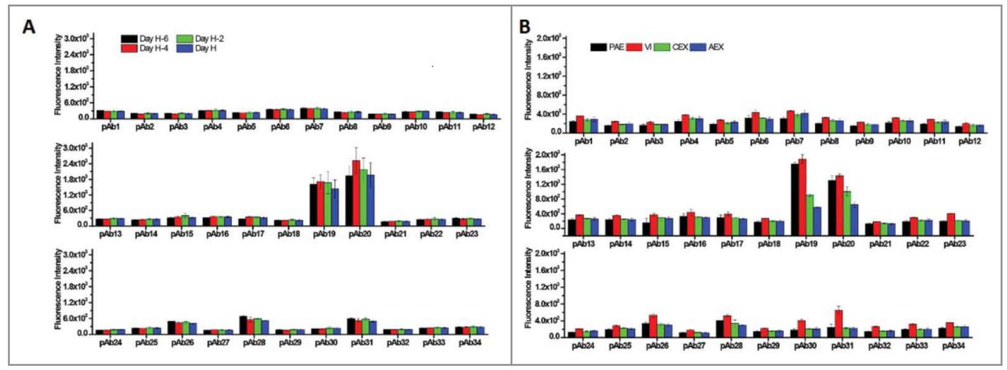


Figure 7. (A) PCA data of mAb5 samples from upstream process development. Samples were collected during the cell culture on Day H-6, H-4, H-2, and H as labeled (Day H is the harvest day). The error bar is the standard deviation from two repeats. (B) PCA data of mAb5 samples from downstream process. Samples collected include ProteinA Elution (PAE), Virus Inactivation (VI), Cation Exchange Elution (CEX), and Anion Exchange Flow-through (AEX). The error bar is the standard deviation from two repeats.

"we revealed structural differences between different antibody molecules and antibody structure changes affected by various processing conditions"

Changing signals with changing conditions = changing HOS

FDA

The future

.."a protein's three-dimensional conformation can often be difficult to define precisely using current physiochemical analytical technology."

Quality Considerations in Demonstrating Biosimilarity of a Therapeutic Protein Product to a Reference Product. Guidance for Industry. FDA, April 2015.

We have a technology which can be customized to fit this need for any Mab, as evidenced by our existing PCA ELISA portfolio

Potentially a customer can start with InnoPlex and if required, customize a Mab specific tool.

Conclusion

- **InnoPlex can determine between induced Mab changes under different processing conditions.**
- **It fits a suggested gap in FDA Guidance.**
- **Custom R&D has optimized it to a high throughput method based on Luminex xMAP technology.**
- **Potential down stream applications may be customized for a particular customers target molecule.**

*1 Ecker et al ,The therapeutic monoclonal antibody market. *mAbs* 7:1 pp9-14, 2015.
*2 Müller-Ladner et al, The scientific rational behind the development and approval of biosimilar infliximab (CT-P13) in Europe. *Expert Rev. Clin. Immunol*. 11 (51) ppS5-S14. 2015
*3 Song et al, Monoclonal antibody higher order structure analysis by high throughput protein conformational array. *mAbs* 10 (2) pp 397-405. 2018.