CASSS Consultants' Network 24th March 2025

Black Box, Fantasy, or Gödel's Knot?

Why mass spectrometry in Biopharma is none of these

Agenda

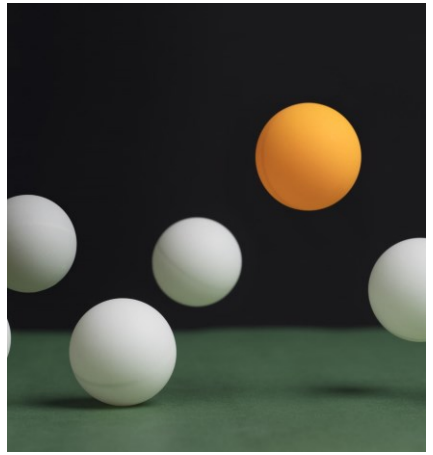
1. Mass Spec Basics and Timeline
2. Case Study 1: It’s not what you think it is
3. Case Study 2: we told you so!
4. Future Planning: there’s a way out …
5. Questions

(Modern) Mass Spec Basics

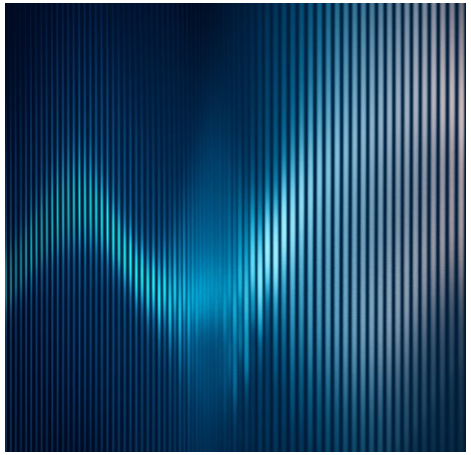
Separation Device

Ionization system

Ion Separation



Ion detection

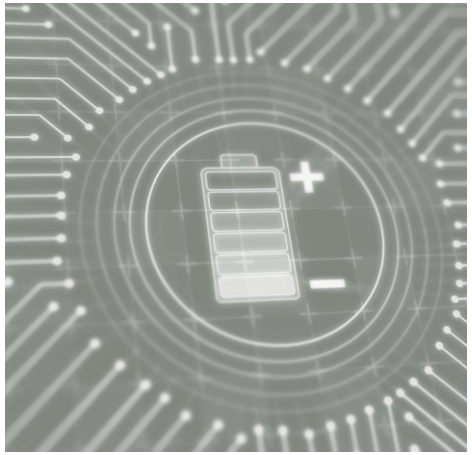


(Spectral) Output

(Modern) Mass Spec Basics - Caveats

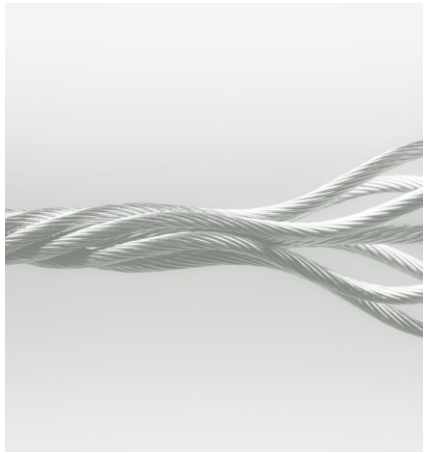
Separation Device

- Molecule specific
- Needs method development
- Interfacing to MS: MATRIX dependency



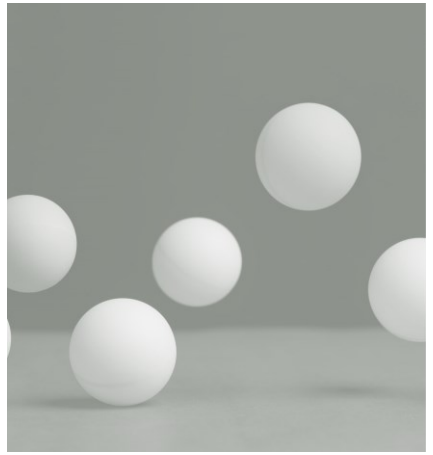
Ionization system

- Technique specific – not universal
- Charge reliance therefore matrix dependent



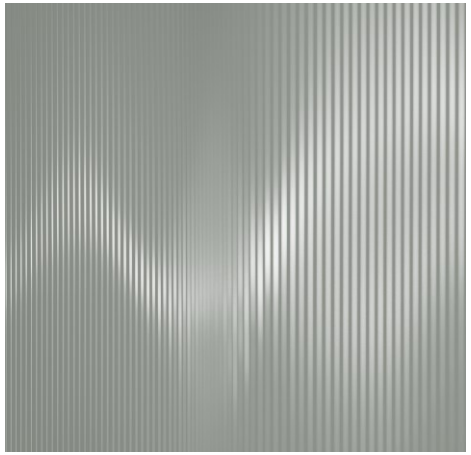
Ion Separation

- SIZE dependency
- Molecule dependency (MS types)
- Mass to charge mechanisms (NOT Mass!)



Ion detection

- CONCENTRATION DEPENDENT - Limited dynamic range
- Subject to INTERFERENCES



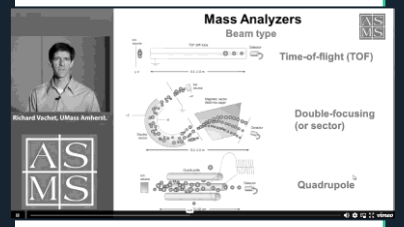
(Spectral) Output

- HUGE data load
- Needs INTERPRETATION (and so now SOFTWARE)

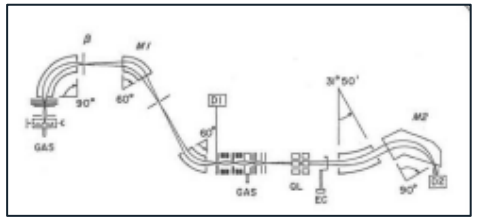
Mass Spectrometry Development Pseudo-Timeline

Wide variety of 'general' tools

Magnetic sectors, Quadrupoles, GC.

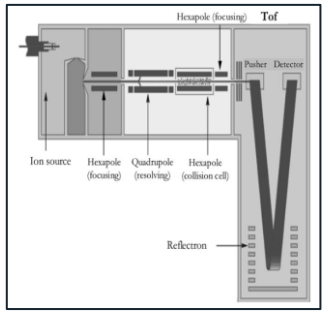


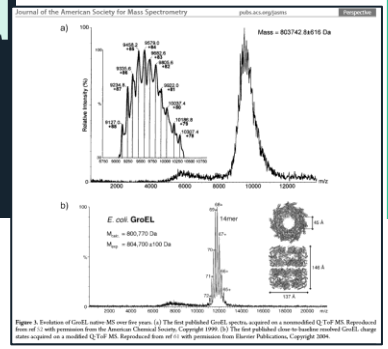
https://player.vimeo.com/video/68728880



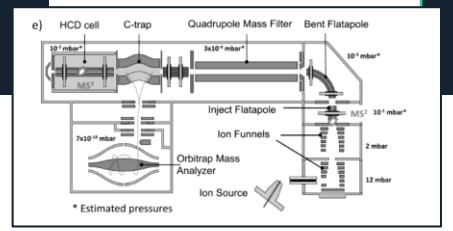
Tandem Mass spec (MSMS) and high Resolution

New Ionization techniques (at atmosphere)



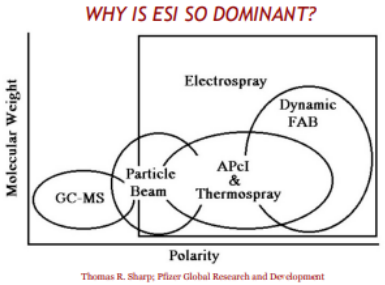


Operations on a separations scale (GC and LC)



https://pubs.acs.org/action/showCitFormats?doi=10.1021/jasms.4c00348&ref=pdf

Very 'High Mass' instruments



https://www.asms.org/docs/default-source/history-posters/tech_asms-lcms-poster_2018_final-(1).pdf?sfvrsn=ef3476c3_0

Dominance of Electrospray (ESI).

- Moves to 'structural biology'

Scientists can Confuse Accuracy and Precision….

Almost always one or the other, not both

- To be ‘both’ they need:
  - Calibration
  - Standards
  - SOPs/ Reproducibility
  - Expertise?
  - RIGOR!

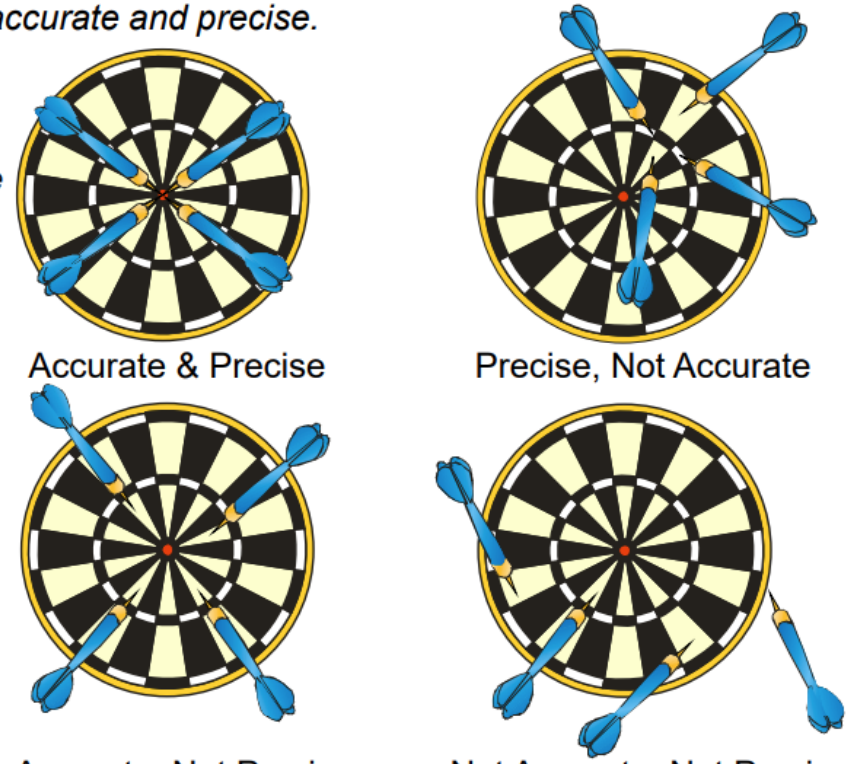
Concept 8: Accuracy and Precision

Mass spectrometrists always say:

Mass spectrometry is very accurate and precise.

Reality:

Mass spectrometrists confuse accuracy and precision.



NC STATE UNIVERSITY

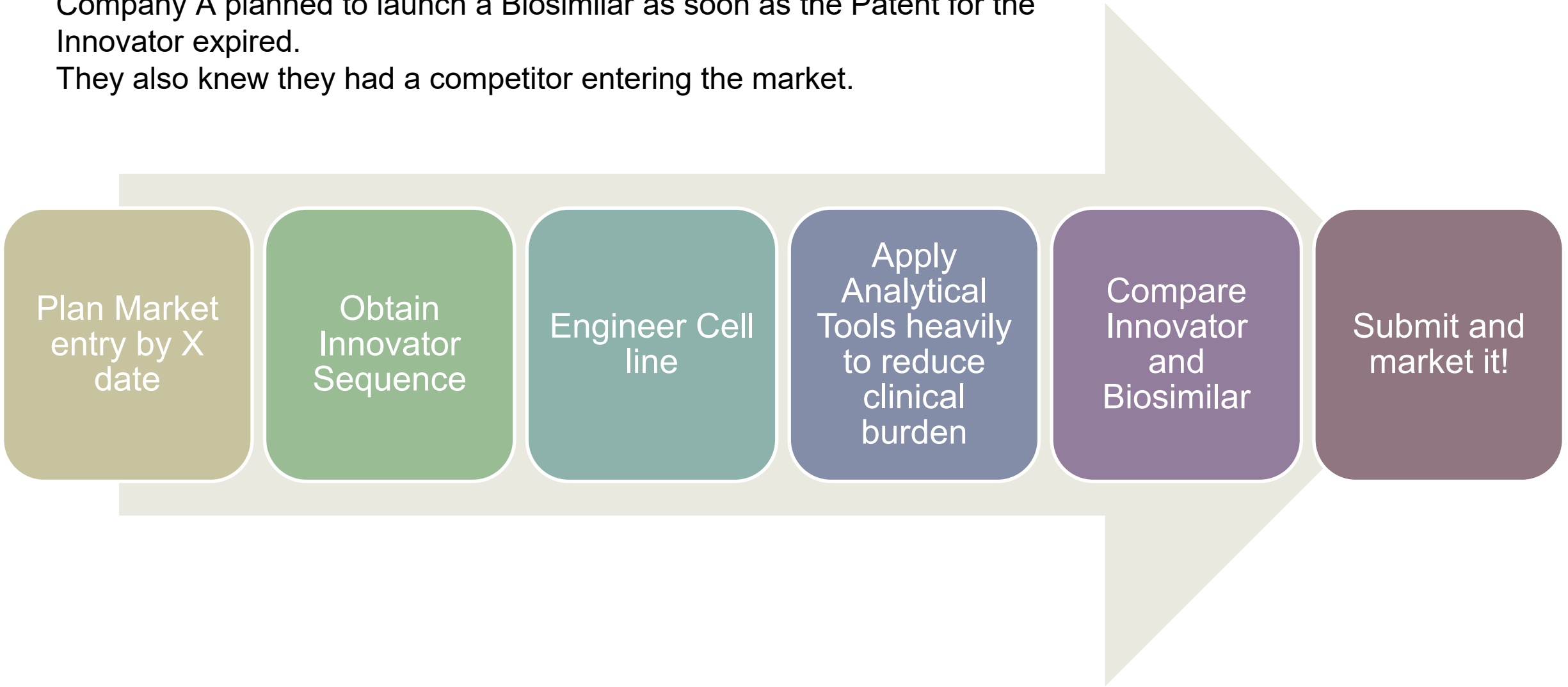
With thanks to David Muddiman, and Diana Ayerhart: https://www.asms.org/docs/default-source/what-is-ms-booklet/whatisms-poster-side-only_landscape.pdf?sfvrsn=117b70c3_0

Case Study 1

it’s not what you think it is

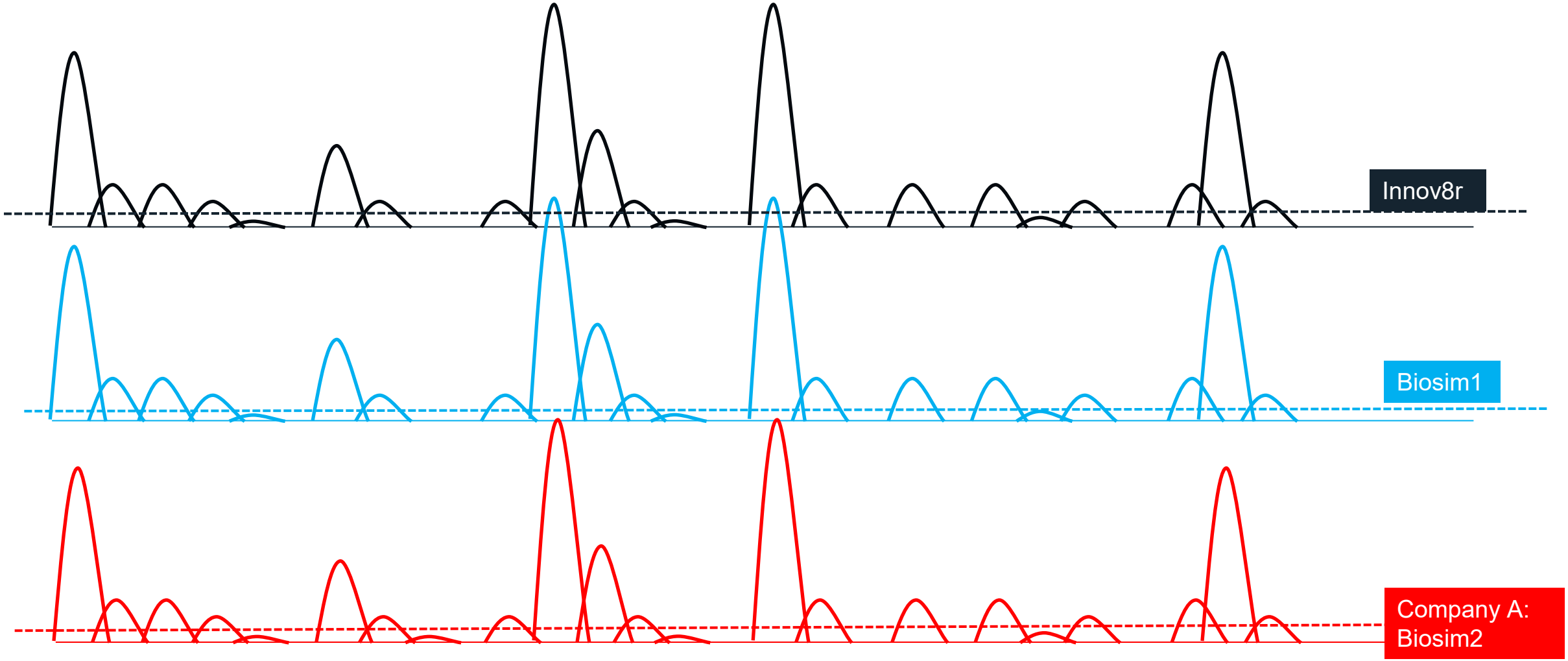
What did the Biosimilar Company do?

Company A planned to launch a Biosimilar as soon as the Patent for the Innovator expired.
They also knew they had a competitor entering the market.



Analytically LC-UV-MS Looks the same…

Peptide Map using LC-UV-MS is no different visually, or even above a given threshold



The Coverage map is just fine

By mass, everything that is expected is indeed matched – well, to 98% anyway …

Protein Coverage View

Protein Coverage

Sample name	Protein name	Protein coverage summary	Protein coverage percent	Protein molecular weight	Protein isoelectric point
Currently checked	gi|001|NISTLC NIST_REFSTD_LC	213 of 213	100.00	23123.59	7.72
Currently checked	gi|002|NISTHC NIST_REFSTD_HC	442 of 449	98.44	49451.66	8.05

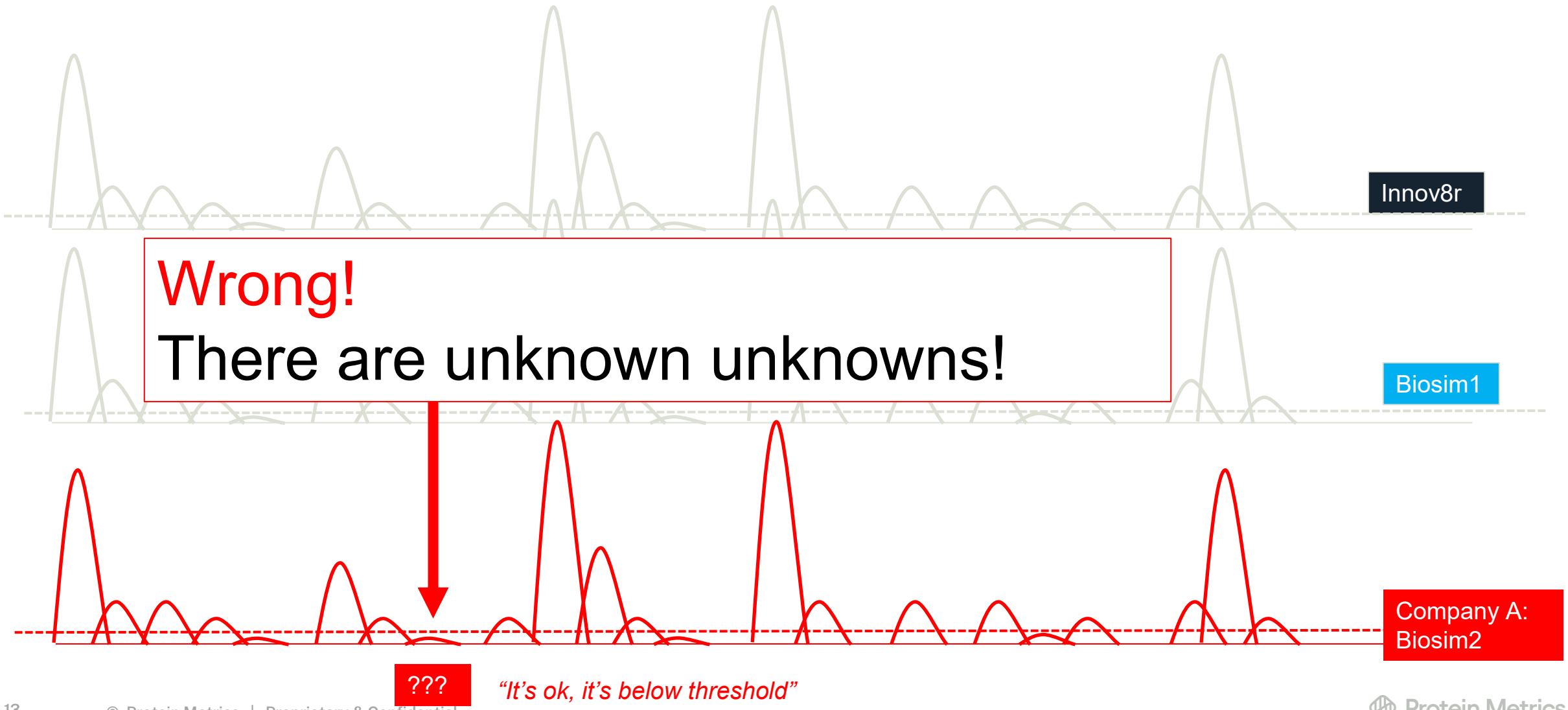
Yes, it's the NIST mAb, but you get the point ;-) …

Key Criteria by mass spec are met:

- ✓ Biosimilar Primary Sequence matches to Innovator by mass
- ✓ Coverage shows all the matches
- ✓ Expected modifications are identified

HOORAY!

So, there’s no problem, right?

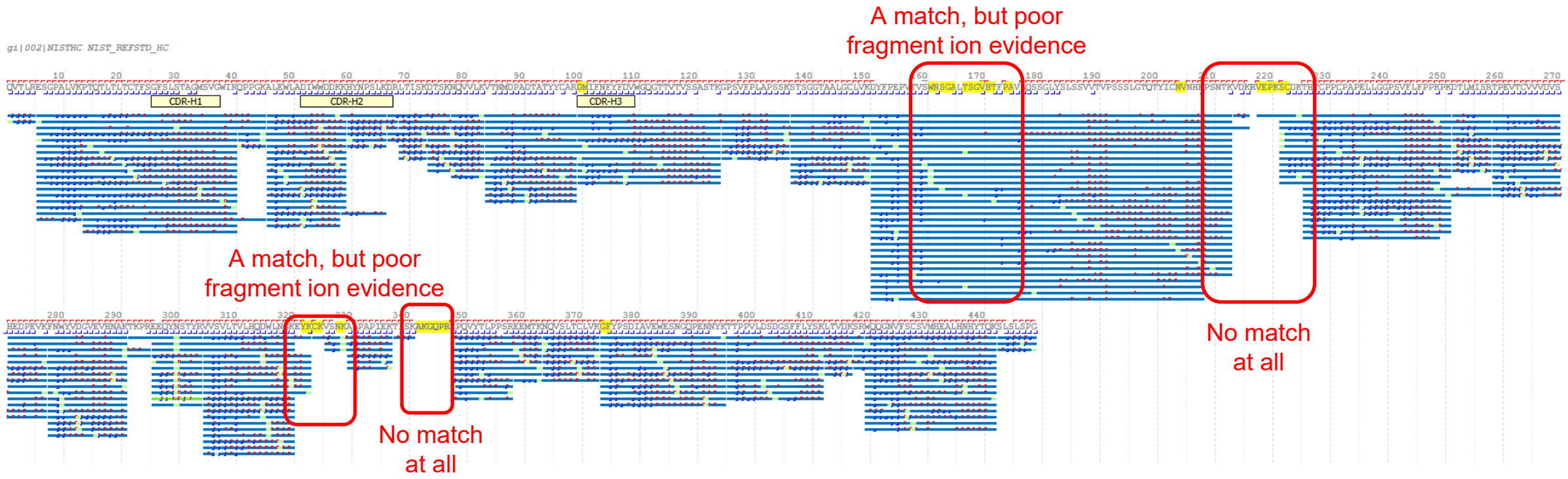


The Coverage map is NOT just fine by MSMS

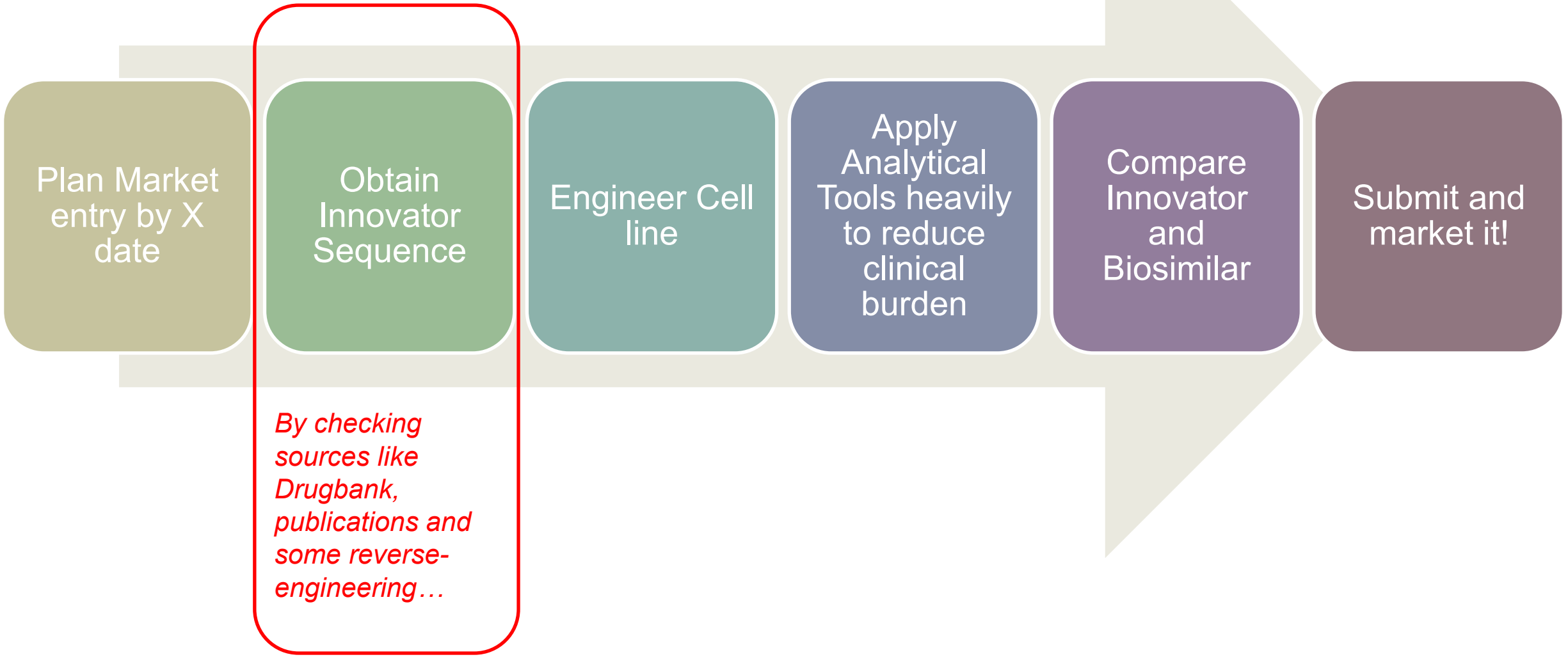
By mass, everything that is expected is indeed matched

But there are sections where there is no fragment information to corroborate the mass by Tandem mass spec (MS/MS)

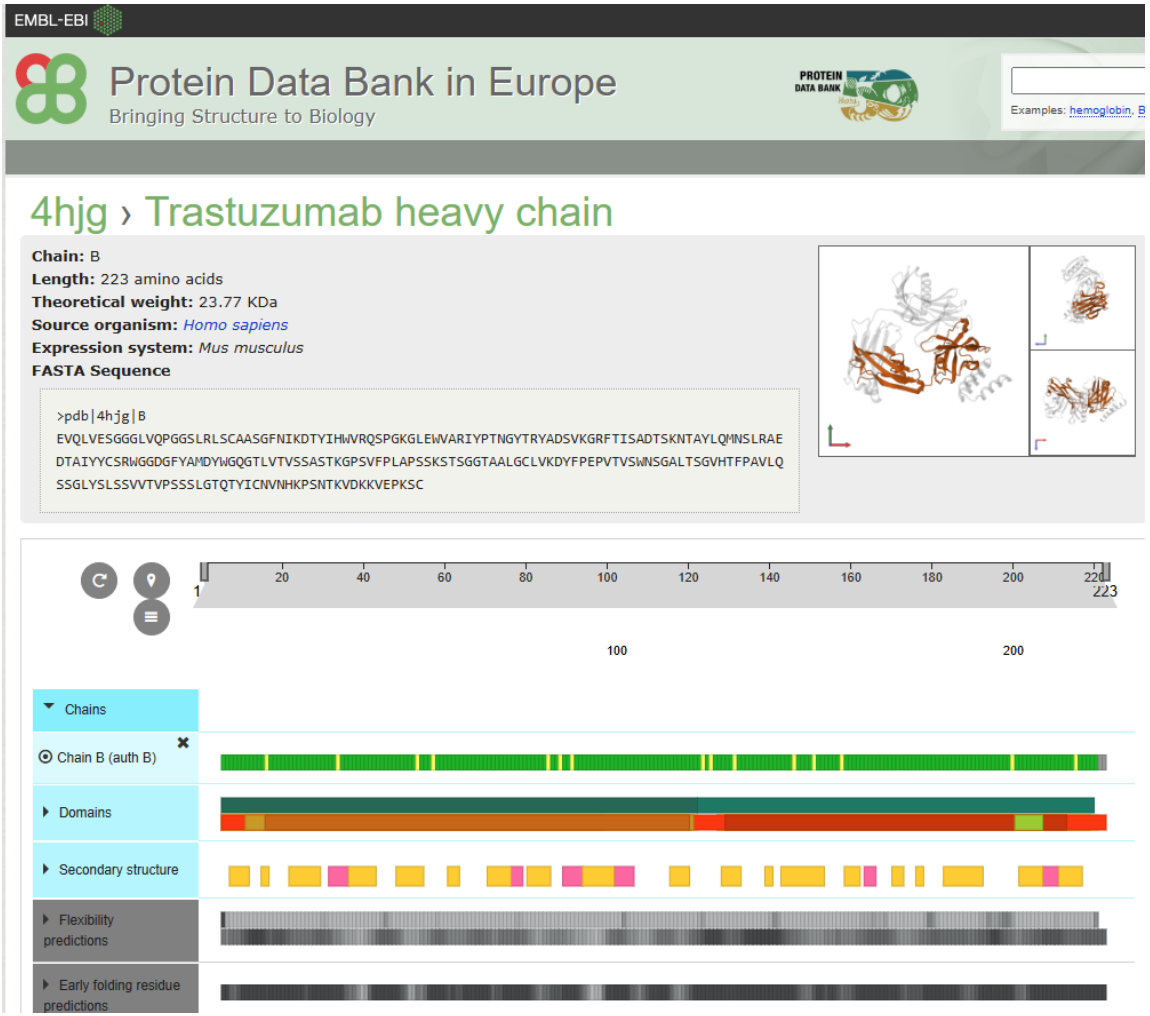
And some small sections in the HC that have no evidence – that may, or may not be, important (unknown)



What did the Biosimilar Company do?



Drugbank, Sequences, and Assumptions



Drugbank sequence for Trastuzumab

- Is this the exact sequence that became a **product**?
- No one promised you that, it's between the innovator and the regulator(s)

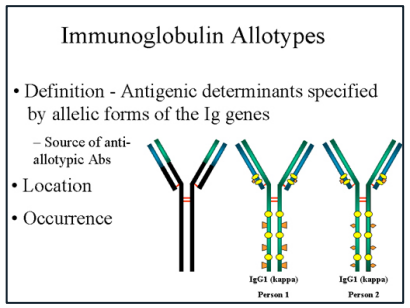
https://www.ebi.ac.uk/pdbe/entry/pdb/4hjg/protein/2

Oh, Allotypes/ Isoforms/ Sequence Variants… who knew…!

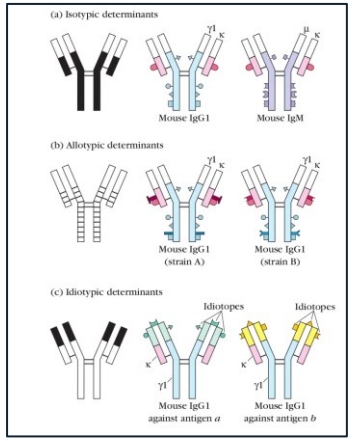
We've known since 1956 !

We also know they bind differently and are hard to identify with reagents.

So Mass spectrometry might help here….

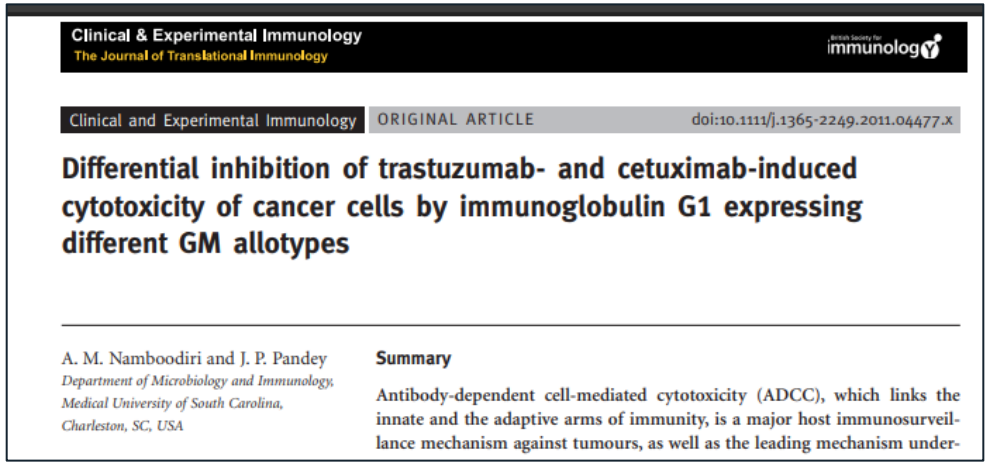


https://www.microbiologybook.org/mayer/IgTypes2000.htm



https://microbeonline.com/isotypes-allotypes-idiotypes/

https://en.wikipedia.org/wiki/Allotype_(immunology)


Clinical & Experimental Immunology
The Journal of Translational Immunology

Clinical and Experimental Immunology ORIGINAL ARTICLE doi:10.1111/j.1365-2249.2011.04477.x

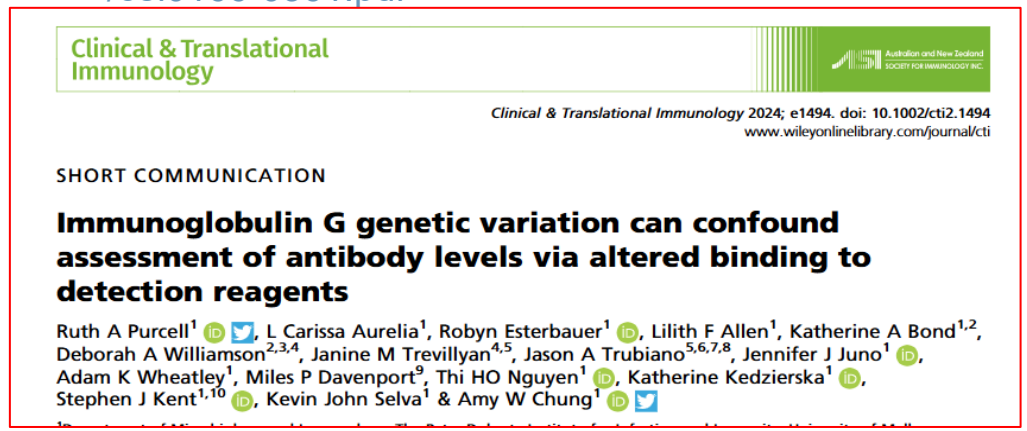
Differential inhibition of trastuzumab- and cetuximab-induced cytotoxicity of cancer cells by immunoglobulin G1 expressing different GM allotypes

A. M. Namboodiri and J. P. Pandey
Department of Microbiology and Immunology, Medical University of South Carolina, Charleston, SC, USA

Summary

Antibody-dependent cell-mediated cytotoxicity (ADCC), which links the innate and the adaptive arms of immunity, is a major host immunosurveillance mechanism against tumours, as well as the leading mechanism under-

https://pmc.ncbi.nlm.nih.gov/articles/PMC3232384/pdf/cei0166-0361.pdf


Clinical & Translational Immunology

Clinical & Translational Immunology 2024; e1494. doi: 10.1002/cti2.1494
www.wileyonlinelibrary.com/journal/cti

SHORT COMMUNICATION

Immunoglobulin G genetic variation can confound assessment of antibody levels via altered binding to detection reagents

Ruth A Purcell[1], L Carissa Aurelia[1], Robyn Esterbauer[1], Lilith F Allen[1], Katherine A Bond[1,2], Deborah A Williamson[2,3,4], Janine M Trevillyan[4,5], Jason A Trubiano[5,6,7,8], Jennifer J Juno[1], Adam K Wheatley[1], Miles P Davenport[9], Thi HO Nguyen[1], Katherine Kedzierska[1], Stephen J Kent[1,10], Kevin John Selva[1] & Amy W Chung[1]

https://onlinelibrary.wiley.com/doi/epdf/10.1002/cti2.1494

Protein Metrics

What did the Biosimilar Company do?

Plan Market entry by X date

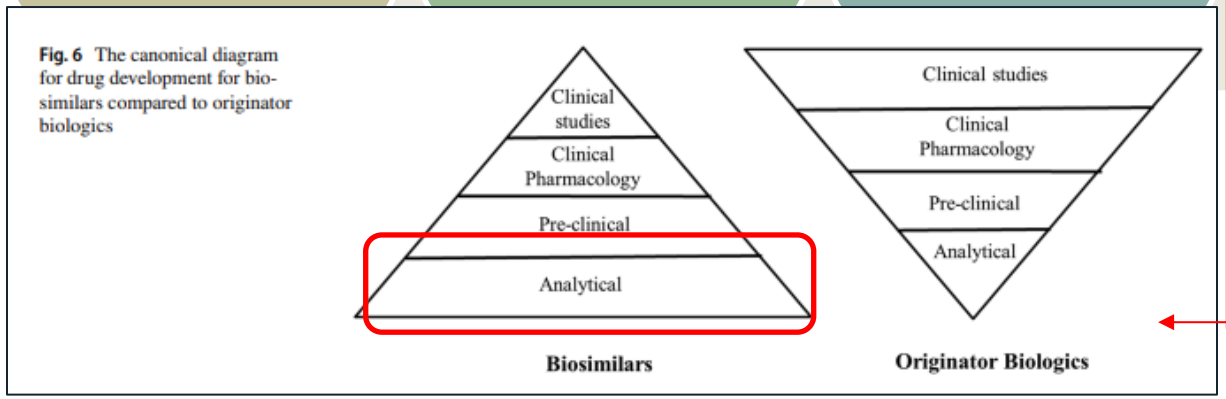
Obtain Innovator Sequence

Engineer Cell line

Apply Analytical Tools heavily to reduce clinical burden

Compare Innovator and Biosimilar

Submit and market it!



But using LC-UV-MS not LC-UV-MS/MS

https://pmc.ncbi.nlm.nih.gov/articles/PMC6791907/pdf/10295_2019_Article_2216.pdf

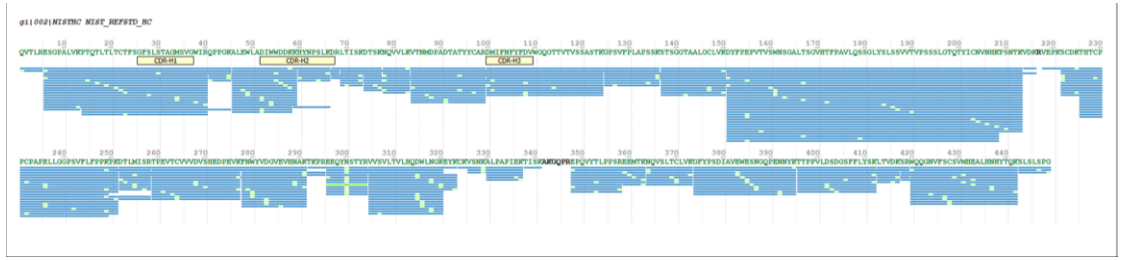
‘Matching’ vs ‘Proving’

MS1 is an excellent tool for **matching**

MSMS is an excellent tool for **proving** sequences of peptides by fragmentation

‘Mass Spec’ is used as a catch-all term that simply captures the general category of instrument – the difference is important.

I’ve matched everything I know about



I’ve matched almost everything I know about, and I have proof over a large part of the map

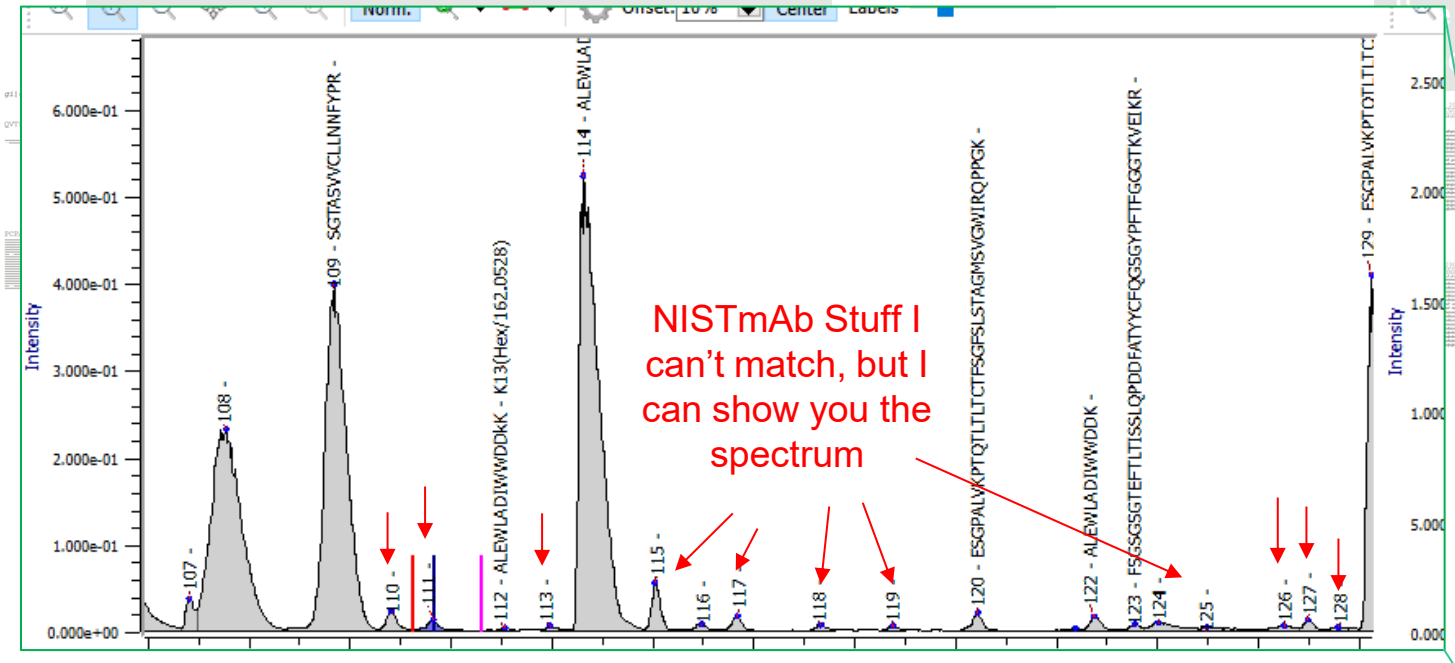
‘Matching’ vs ‘Proving’ vs ‘Finding’

MS1 is an excellent tool for **matching**

MSMS is an excellent tool for **proving** sequences of peptides by fragmentation

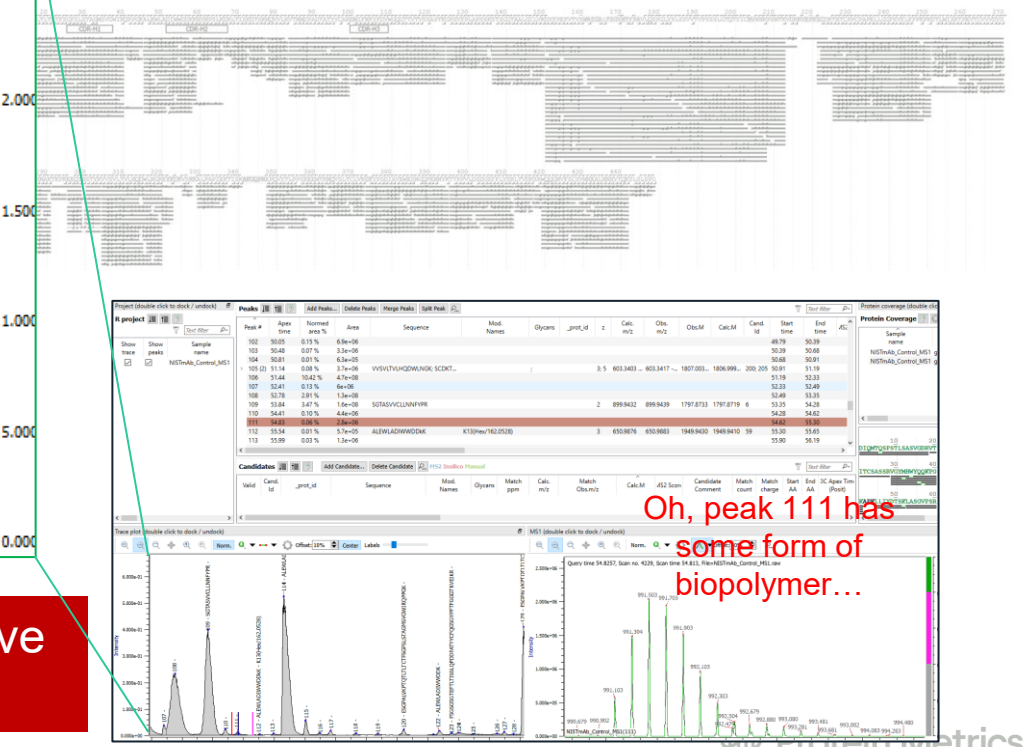
‘MS’ is used as a catch-all term that simply captures the general category of instrument – the difference is important.

To IDENTIFY, you need a lot more information, beyond matching and proving



NISTmAb Stuff I can’t match, but I can show you the spectrum

matched almost everything I know about, and I have
of over a large part of the map

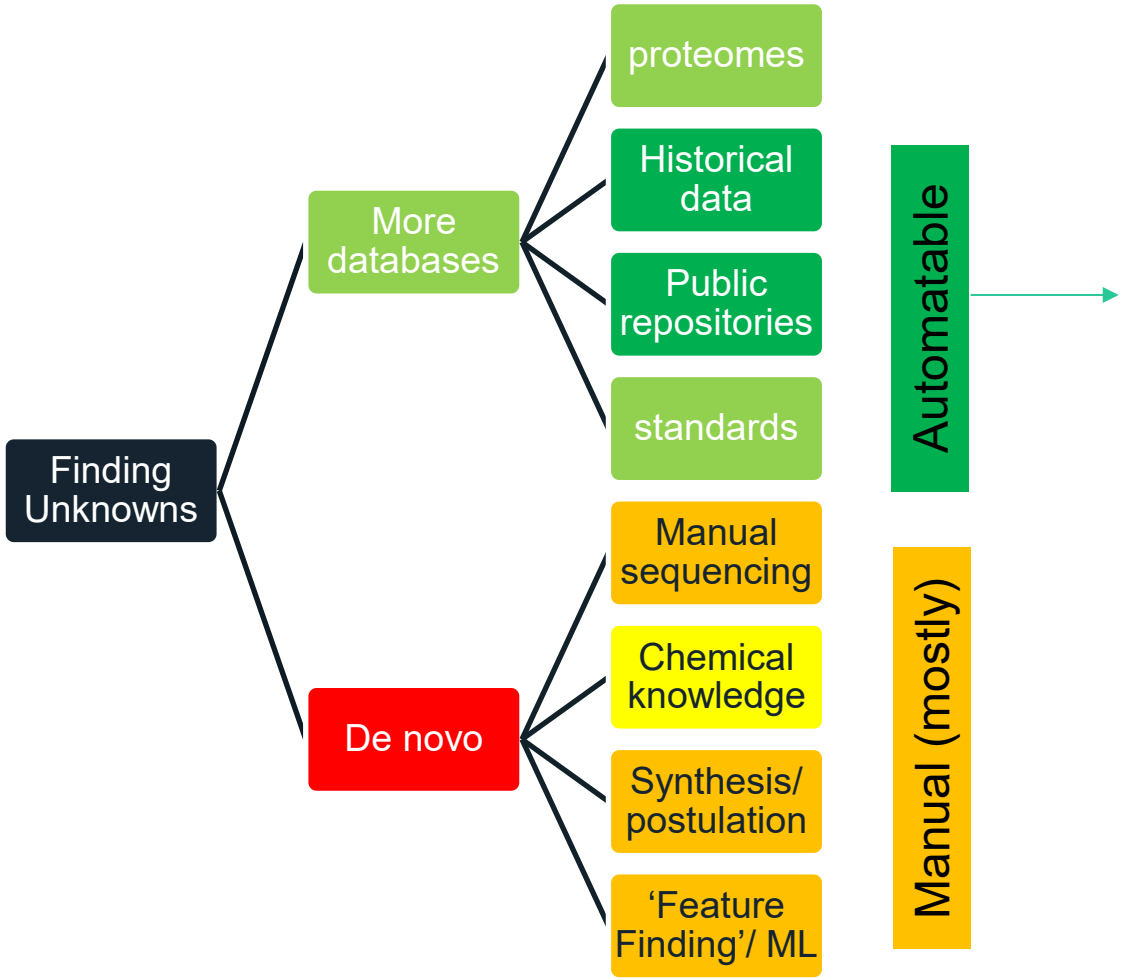


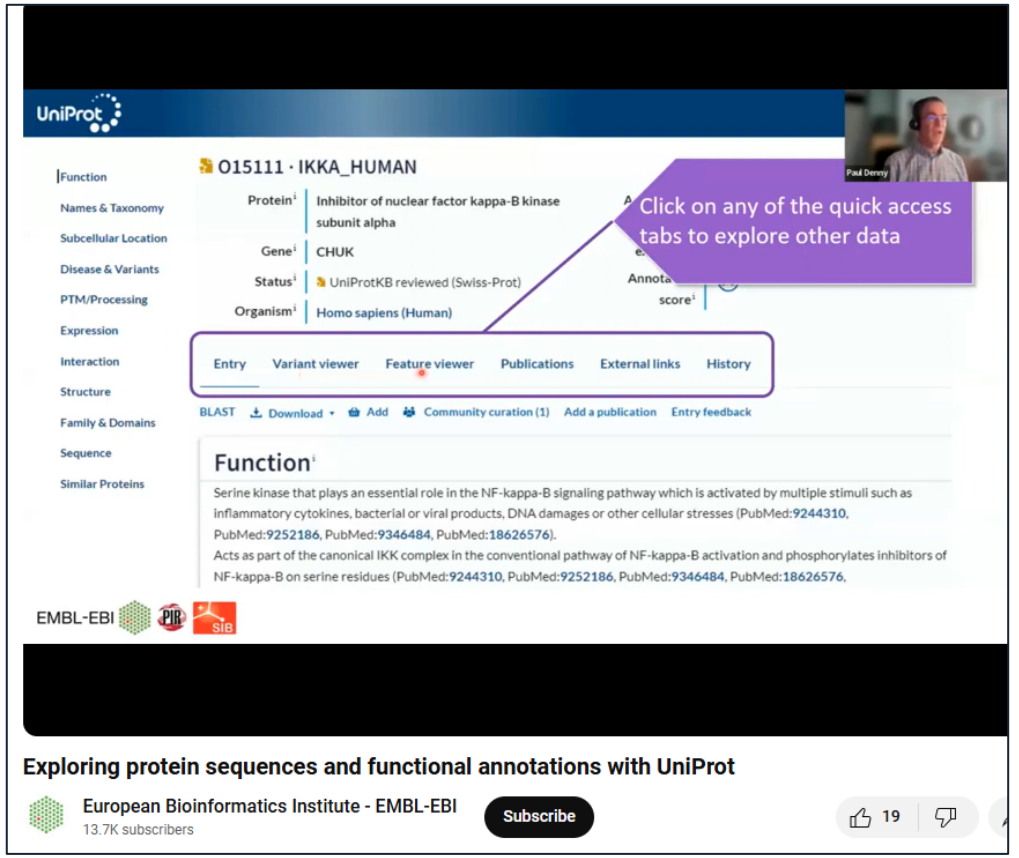
Oh, peak 111 has some form of biopolymer…

I have some idea of what the unknowns are, but I’m going to have to investigate (and there are a lot of them)

Once you know, you know...

We are only as good as the data we have today, if we are doing ‘matching’
If we are ‘searching’, we need to spread the net

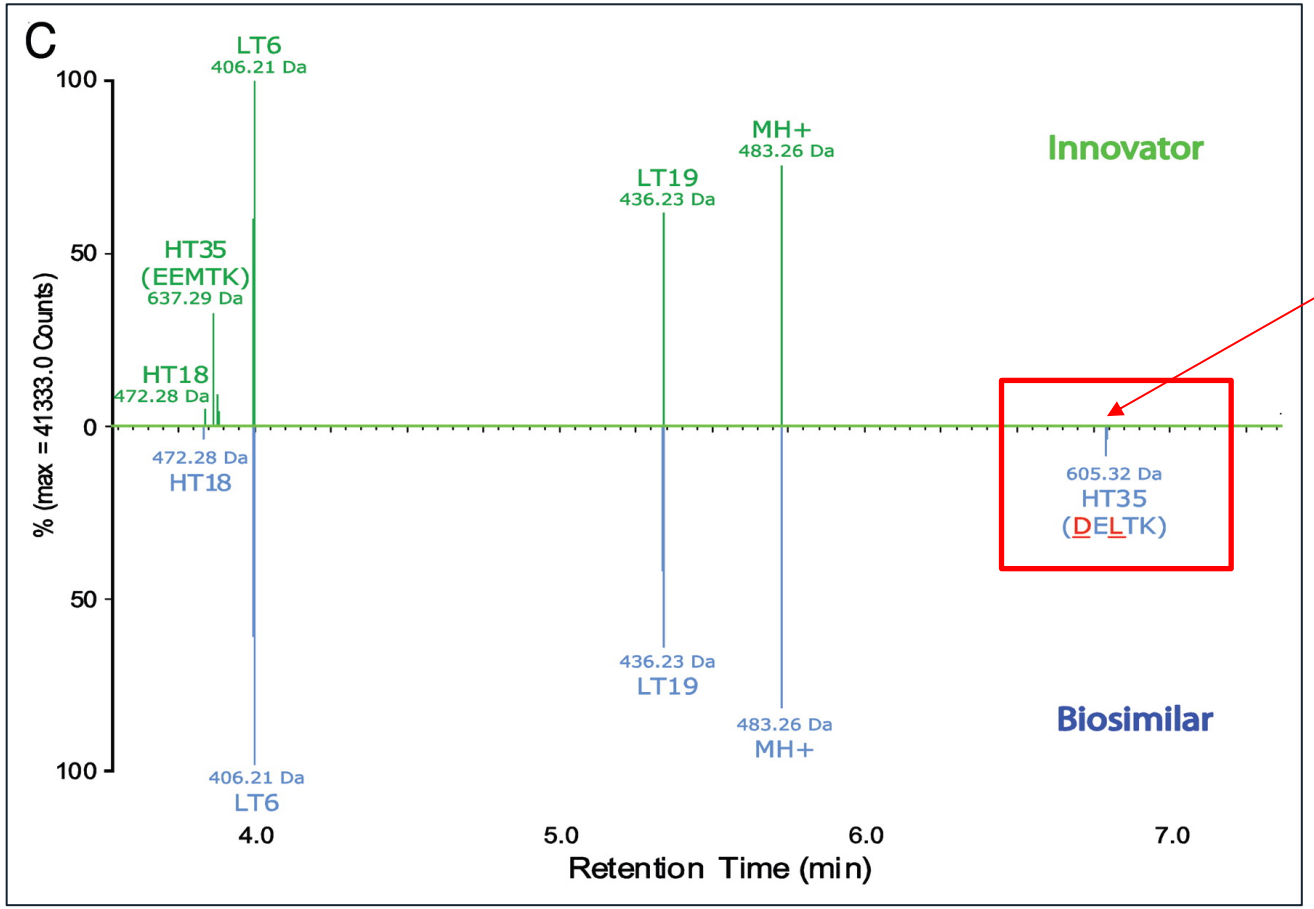




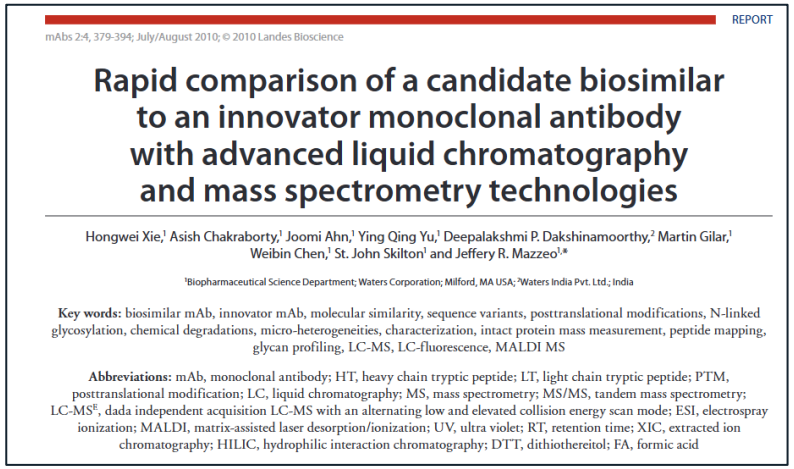
https://www.youtube.com/watch?v=qwW7TuN4-SY

‘Similar’ Publication in the Public Domain

In brief, an allotype was indeed created by the biosimilar’s cell line, and not identified by mass (MS1) and UV alone
The new sequence had to be identified manually



Sequence did not exist in the innovator, was in an allotype



REPORT

mAbs 2:4, 379-394; July/August 2010; © 2010 Landes Bioscience

Rapid comparison of a candidate biosimilar to an innovator monoclonal antibody with advanced liquid chromatography and mass spectrometry technologies

Hongwei Xie,[1] Asish Chakraborty,[1] Joomi Ahn,[1] Ying Qing Yu,[1] Deepalakshmi P. Dakshinamoorthy,[2] Martin Gilar,[1] Weibin Chen,[1] St. John Skilton[1] and Jeffery R. Mazzeo[1,*]

[1]Biopharmaceutical Science Department; Waters Corporation; Milford, MA USA; [2]Waters India Pvt. Ltd.; India

Key words: biosimilar mAb, innovator mAb, molecular similarity, sequence variants, posttranslational modifications, N-linked glycosylation, chemical degradations, micro-heterogeneities, characterization, intact protein mass measurement, peptide mapping, glycan profiling, LC-MS, LC-fluorescence, MALDI MS

Abbreviations: mAb, monoclonal antibody; HT, heavy chain tryptic peptide; LT, light chain tryptic peptide; PTM, posttranslational modification; LC, liquid chromatography; MS, mass spectrometry; MS/MS, tandem mass spectrometry; LC-MS^E, dada independent acquisition LC-MS with an alternating low and elevated collision energy scan mode; ESI, electrospray ionization; MALDI, matrix-assisted laser desorption/ionization; UV, ultra violet; RT, retention time; XIC, extracted ion chromatography; HILIC, hydrophilic interaction chromatography; DTT, dithiothereitol; FA, formic acid

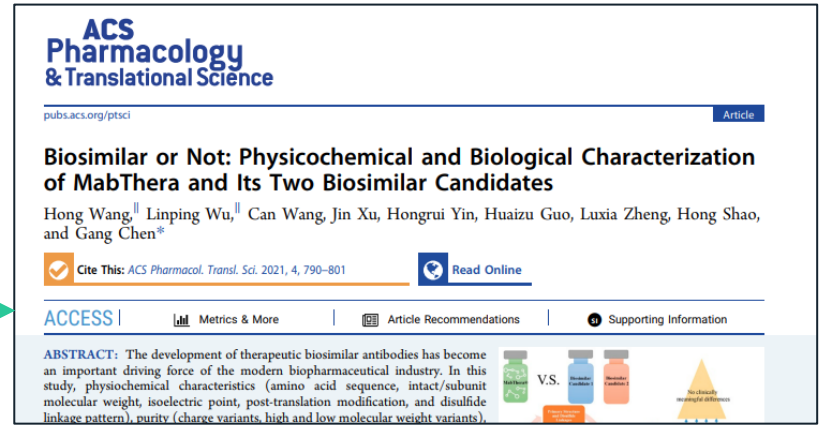
https://doi.org/10.4161/mabs.11986

Is Sequence the only Showstopper?

What about all the other stuff?

"some QAs of BC2 were dissimilar from those of the RP. First, the intact mass and peptide mapping data indicated that alanine at 219 of HC was mutated into valine, which was an allotype of IgG1. Although the allotype is designed to reduce the risk of the potential for immunogenicity, therapeutic IgG1 allotypes located in the constant region influence the pharmacokinetics through FcRn binding.28 Therefore, an in vivo nonclinical study may be required to further evaluate the difference…"

- **Glycosylation**
- **Disulfide bonds**
- **… and many more!**


ACS Pharmacology & Translational Science

pubs.acs.org/ptsci Article

Biosimilar or Not: Physicochemical and Biological Characterization of MabThera and Its Two Biosimilar Candidates

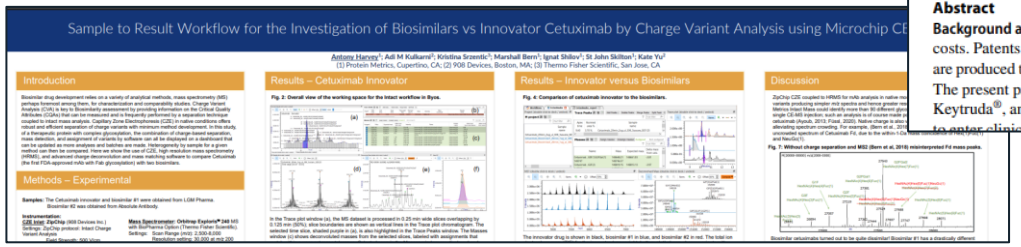
Hong Wang,‖ Linping Wu,‖ Can Wang, Jin Xu, Hongrui Yin, Huaizu Guo, Luxia Zheng, Hong Shao, and Gang Chen*

Cite This: ACS Pharmacol. Transl. Sci. 2021, 4, 790−801 Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: The development of therapeutic biosimilar antibodies has become an important driving force of the modern biopharmaceutical industry. In this study, physiochemical characteristics (amino acid sequence, intact/subunit molecular weight, isoelectric point, post-translation modification, and disulfide linkage pattern), purity (charge variants, high and low molecular weight variants),

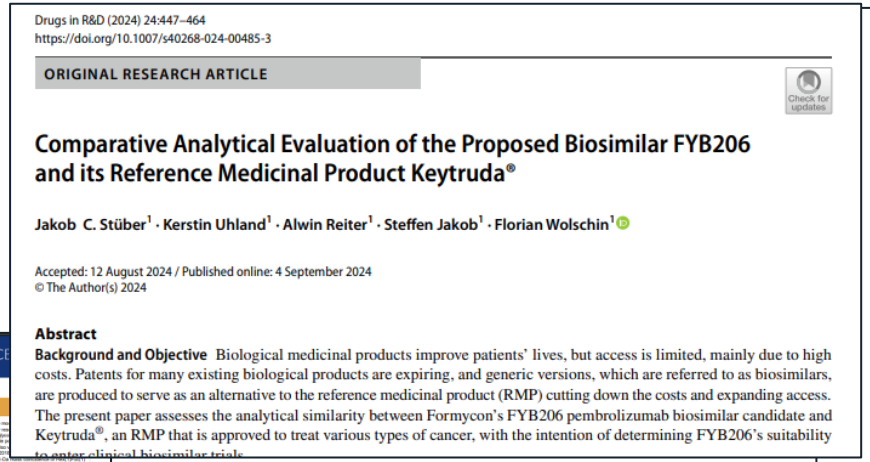
https://pmc.ncbi.nlm.nih.gov/articles/PMC8033751/pdf/pt0c00225.pdf


Sample to Result Workflow for the Investigation of Biosimilars vs Innovator Cetuximab by Charge Variant Analysis using Microchip CE

Introduction | Results – Cetuximab Innovator | Results – Innovator versus Biosimilars | Discussion

Methods – Experimental

https://908devices.com/wp-content/uploads/2023/06/TP-384_CVA_908-PMI_Final.pdf


Drugs in R&D (2024) 24:447–464
https://doi.org/10.1007/s40268-024-00485-3

ORIGINAL RESEARCH ARTICLE

Comparative Analytical Evaluation of the Proposed Biosimilar FYB206 and its Reference Medicinal Product Keytruda®

Jakob C. Stüber[1] · Kerstin Uhland[1] · Alwin Reiter[1] · Steffen Jakob[1] · Florian Wolschin[1]

Accepted: 12 August 2024 / Published online: 4 September 2024
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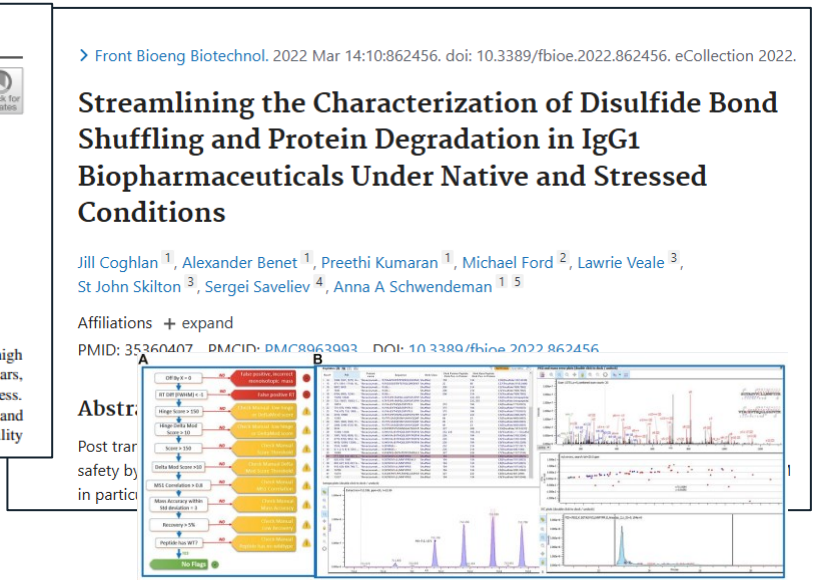
Abstract

Background and Objective Biological medicinal products improve patients' lives, but access is limited, mainly due to high costs. Patents for many existing biological products are expiring, and generic versions, which are referred to as biosimilars, are produced to serve as an alternative to the reference medicinal product (RMP) cutting down the costs and expanding access. The present paper assesses the analytical similarity between Formycon's FYB206 pembrolizumab biosimilar candidate and Keytruda®, an RMP that is approved to treat various types of cancer, with the intention of determining FYB206's suitability

https://pmc.ncbi.nlm.nih.gov/articles/PMC11456019/pdf/40268_2024_Article_485.pdf


Front Bioeng Biotechnol. 2022 Mar 14:10:862456. doi: 10.3389/fbioe.2022.862456. eCollection 2022.

Streamlining the Characterization of Disulfide Bond Shuffling and Protein Degradation in IgG1 Biopharmaceuticals Under Native and Stressed Conditions

Jill Coghlan[1], Alexander Benet[1], Preethi Kumaran[1], Michael Ford[2], Lawrie Veale[3], St John Skilton[3], Sergei Saveliev[4], Anna A Schwendeman[1,5]

Affiliations + expand

PMID: 35360407 PMCID: PMC8963993 DOI: 10.3389/fbioe.2022.862456

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https://pubmed.ncbi.nlm.nih.gov/35360407/

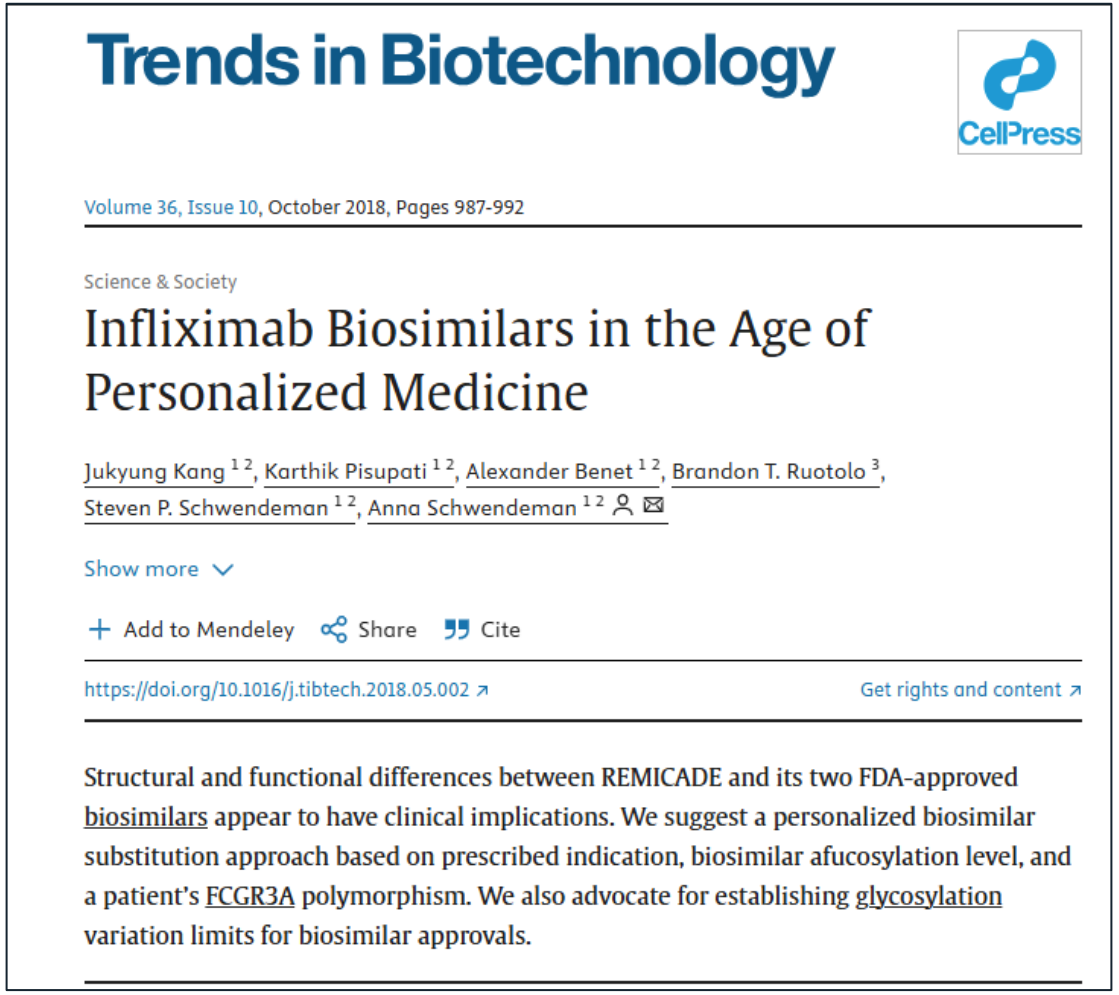
‘Similar’ and ‘Personalized’

Allotypes are not necessarily ‘bad’

Eg Kang et al on Infliximab biosimilars

“Understanding the [...] patient’s genetic makeup could unlock new possibilities ... The human FCGR3A gene, which codes for the FcγR-IIIa receptor, contains an allelic dimorphism to V or F at residue 158. Not only does the FcγR-IIIa-158V isoform possess higher mAb binding affinity relative to FcγR-IIIa-158F [7], but also the immune cells from IBD patients with FCGR3A...”

So we really do want to know that they are present!



Trends in Biotechnology

CellPress

Volume 36, Issue 10, October 2018, Pages 987-992

Science & Society

Infliximab Biosimilars in the Age of Personalized Medicine

Jukyung Kang [1,2], Karthik Pisupati [1,2], Alexander Benet [1,2], Brandon T. Ruotolo [3], Steven P. Schwendeman [1,2], Anna Schwendeman [1,2]

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https://doi.org/10.1016/j.tibtech.2018.05.002 Get rights and content

Structural and functional differences between REMICADE and its two FDA-approved biosimilars appear to have clinical implications. We suggest a personalized biosimilar substitution approach based on prescribed indication, biosimilar afucosylation level, and a patient’s FCGR3A polymorphism. We also advocate for establishing glycosylation variation limits for biosimilar approvals.

https://www.sciencedirect.com/science/article/abs/pii/S0167779918301379

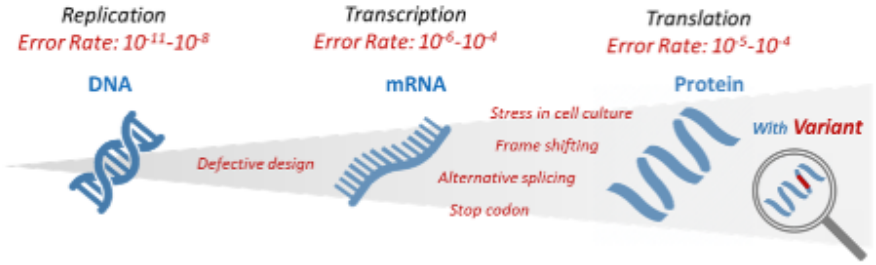
Sequence Variant Analysis ‘Business’

As the market grows, so is the anticipation of double-checking primary sequences

- for example WuXi specifically advertising their capability specifically by mass spec…
- .. With caveats ☺

https://www.wuxibiologics.com/analytical-strategies-for-complex-biologics-webinar/

Figure 1.



Contact Us

Sequence variant analysis (SVA) for risk mitigation

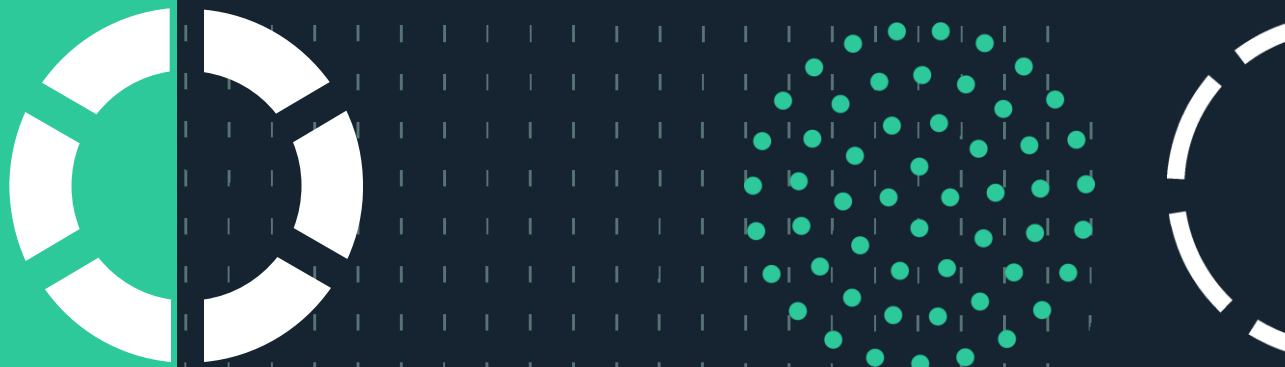
In view of the widespread occurrence of sequence variants (SVs) and the stringent requirements imposed by regulatory authorities, the implementation of the SVA for critical development phases (e.g. *Top clone*, *clone stability*) has become increasingly essential. WuXi Biologics' sequence variant analysis service offers sensitive monitoring of SVs on protein level by LC-MS. This service reliably detects sequence variants with ratios higher than 0.1%. Detailed reference reports are delivered to support further decision-making.

Figure 2.

https://www.wuxibiologics.com/capabilities/analytical-testing/product-characterization-services/

Case Study 2

we told you so!



Titers are HIGH!

Cytiva survey demonstrated the trend to higher titer

Numerous factors increase need for higher titer:

- Delivery mechanisms: IV/ subcutaneous
- Economic models
- Technological improvements
- New formats

With higher titer come new problems…

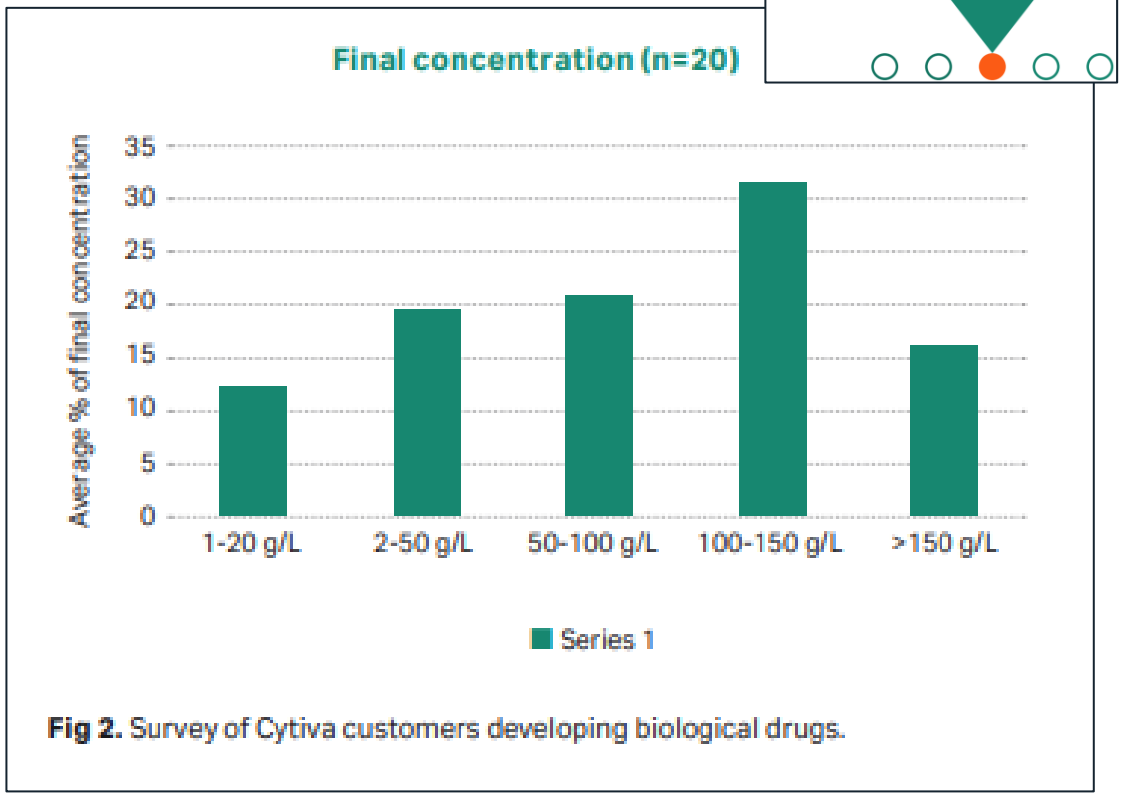
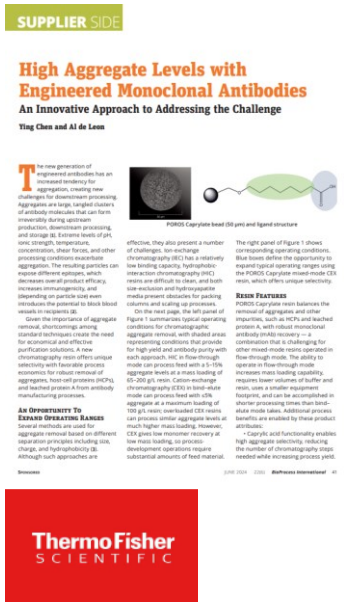


Fig 2. Survey of Cytiva customers developing biological drugs.

https://cdn.cytivalifesciences.com/api/public/content/-17KHaz67EmvKI9FnxYRig-pdf?v=d2325979

It’s hard work, Purification!

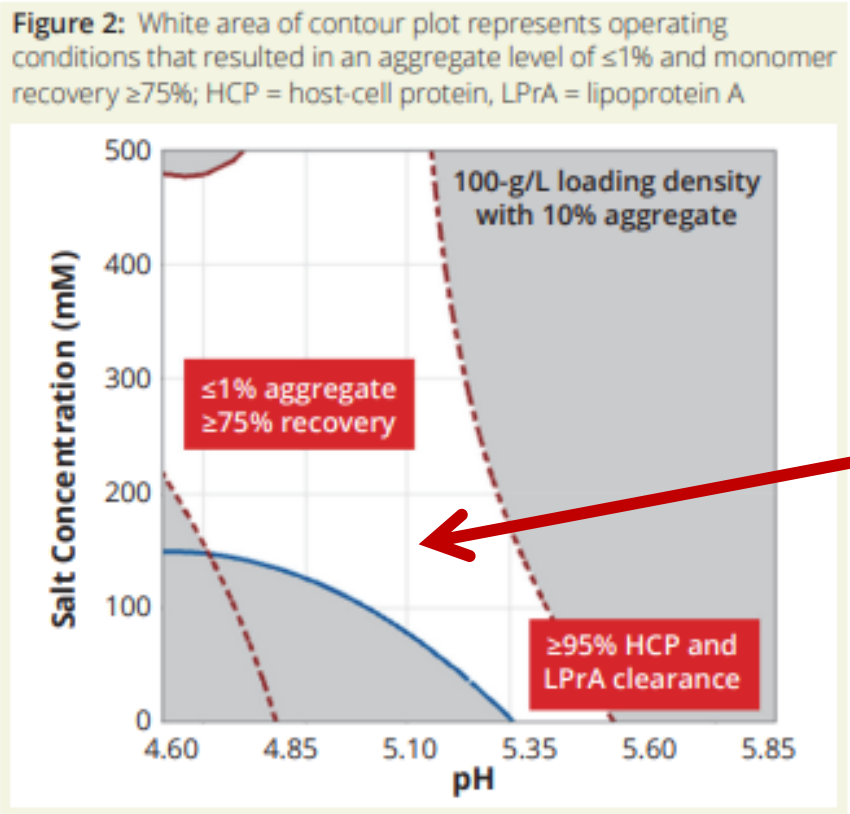

SUPPLIER SIDE

High Aggregate Levels with Engineered Monoclonal Antibodies

An Innovative Approach to Addressing the Challenge

Ying Chen and Al de Leon

ThermoFisher SCIENTIFIC



Manufacturers of Purification resins

know the limits

Evolution by suppliers is constant.

Narrow window for recovery vs clearance

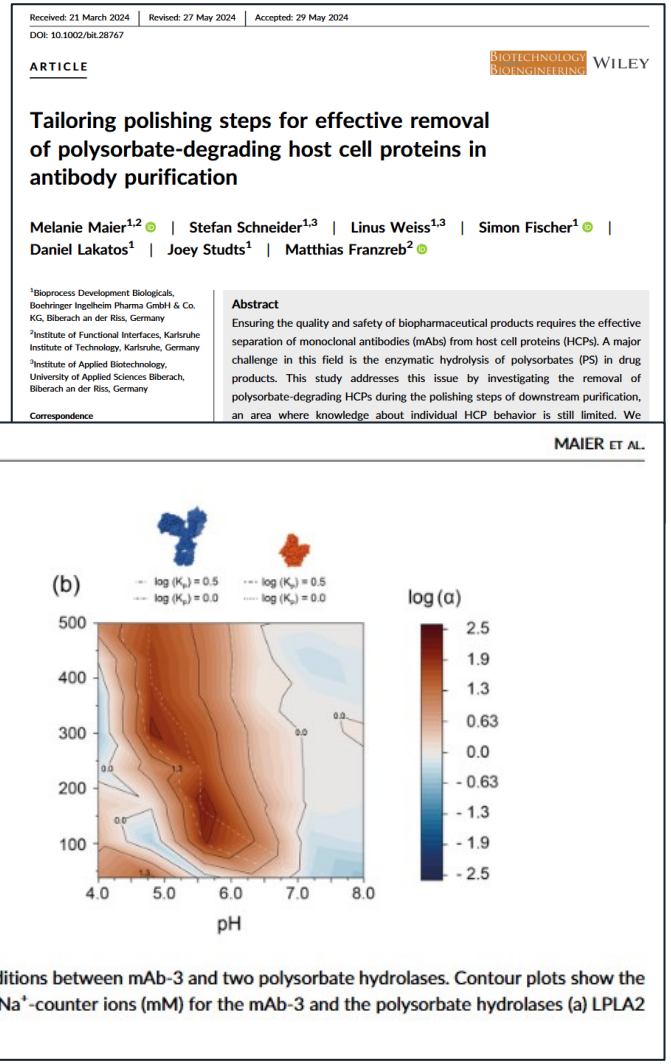
https://547446.fs1.hubspotusercontent-na1.net/hubfs/547446/Technology%20Networks/Landing%20Pages/Thermo/Katie/Article%20High%20Aggregate%20Engineered%20mAbs%20PUR%20May%202024%20(1).pdf

It’s hard work, Optimizing Purification!

Removal of lipases and hydrolases is a huge challenge. Boehringer Ingelheim 2024 example is instructive:

- *“[We] underscore the need to tailor the purification process to leverage the slight differences in binding behavior and elution profiles between mAbs and specific HCPs”*

- *“[Our] antibodies with lower pI’s may present challenges during purification from .. hydrolases. [eg] mAb-3, where no separation window was identified in this process step.”*



Received: 21 March 2024 | Revised: 27 May 2024 | Accepted: 29 May 2024
DOI: 10.1002/bit.28767

ARTICLE

BIOTECHNOLOGY BIOENGINEERING WILEY

Tailoring polishing steps for effective removal of polysorbate-degrading host cell proteins in antibody purification

Melanie Maier[1,2] | Stefan Schneider[1,3] | Linus Weiss[1,3] | Simon Fischer[1] | Daniel Lakatos[1] | Joey Studts[1] | Matthias Franzreb[2]

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[2]Institute of Functional Interfaces, Karlsruhe Institute of Technology, Karlsruhe, Germany
[3]Institute of Applied Biotechnology, University of Applied Sciences Biberach, Biberach an der Riss, Germany

Correspondence

Abstract
Ensuring the quality and safety of biopharmaceutical products requires the effective separation of monoclonal antibodies (mAbs) from host cell proteins (HCPs). A major challenge in this field is the enzymatic hydrolysis of polysorbates (PS) in drug products. This study addresses this issue by investigating the removal of polysorbate-degrading HCPs during the polishing steps of downstream purification, an area where knowledge about individual HCP behavior is still limited. We

FIGURE 6 Identification of cation exchange separation conditions between mAb-3 and two polysorbate hydrolases. Contour plots show the logarithmic separation factor α (log (α)) as a function of pH and Na^+-counter ions (mM) for the mAb-3 and the polysorbate hydrolases (a) LPLA2 and (b) CES2C. mAb, monoclonal antibody.

https://analyticalsciencejournals.onlinelibrary.wiley.com/doi/epdf/10.1002/bit.28767

New Formats are even harder

Singapore BTI example of a knob-in-hole bispecific; <90% recovery, high HCP levels, with very hard work:

"…we demonstrate that through the employment of (1) Protein A chromatography step and (2) flow-through polishing steps, a final product containing < 1% HMW species, < 1% LMW species and < 100 ppm HCP can be obtained with an overall process recovery of 56–87%."

Research | Open access | Published: 14 September 2022

Effective flow-through polishing strategies for knob-into-hole bispecific antibodies

Serene W. Chen, Kong Meng Hoi, Farouq Bin Mahfut, Yuansheng Yang & Wei Zhang

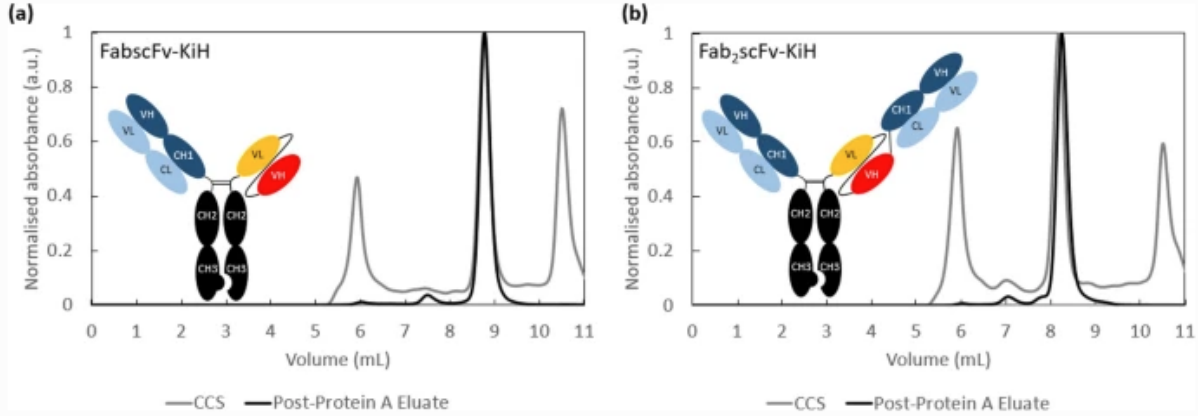
Bioresources and Bioprocessing **9**, Article number: 98 (2022) | Cite this article

5356 Accesses | **1** Altmetric | Metrics

Abstract

Bispecific antibodies (bsAbs), though possessing great therapeutic potential, are extremely challenging to obtain at high purity within a limited number of scalable downstream processing steps. Complementary to Protein A chromatography, polishing strategies play a

Fig. 1

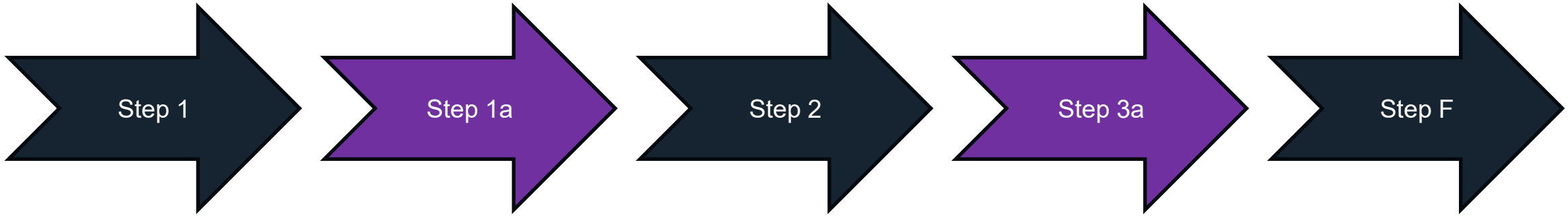


Schematic representation of model bsAbs—FabscFv-KiH (**a**) and Fab_2scFv-KiH (**b**)—used in this study, along with their representative HPLC-SEC purity profiles of cell culture supernatant (CCS) and post-Protein A eluates

https://bioresourcesbioprocessing.springeropen.com/articles/10.1186/s40643-022-00590-8#Abs1

Company B – [downstream] Purification Strategy

Senior VP: ***"This process is way too expensive, get rid of some steps"***



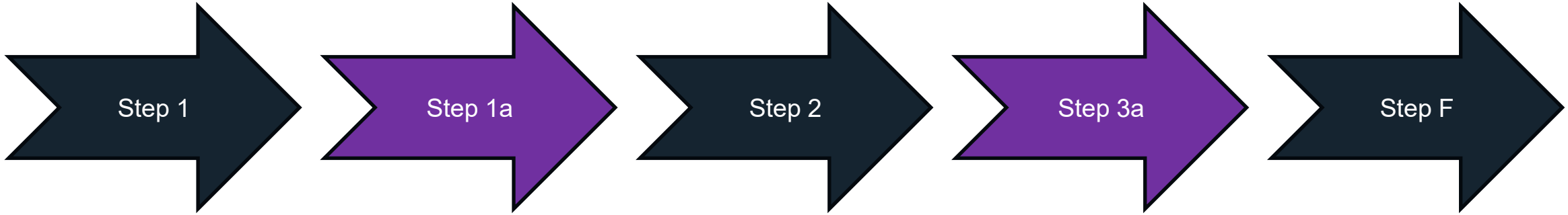
11,500 proteins
- Hydrolases
- Lipases
- Housekeeping proteins
- …

17,200 proteins
- Hydrolases
- Lipases
- Housekeeping proteins
- …

What???

Company B – [downstream] Purification Strategy

Senior VP: ***"This process is way too expensive, get rid of some steps"***



~~11,500 proteins~~
LOTS OF PROTEINS
- Hydrolases
- Lipases
- Housekeeping proteins
- …

~~17,200 proteins~~
STILL LOTS OF PROTEINS
- Hydrolases
- Lipases
- Housekeeping proteins
- …

What???

14,100 proteins
- Some NEW hydrolases!
- Fewer Lipases
- A bunch of NEW unknown proteins
- Product fragments
- …

Approx 600 proteins
- The same NEW hydrolases!
- Product fragments
- (cell death proteins)

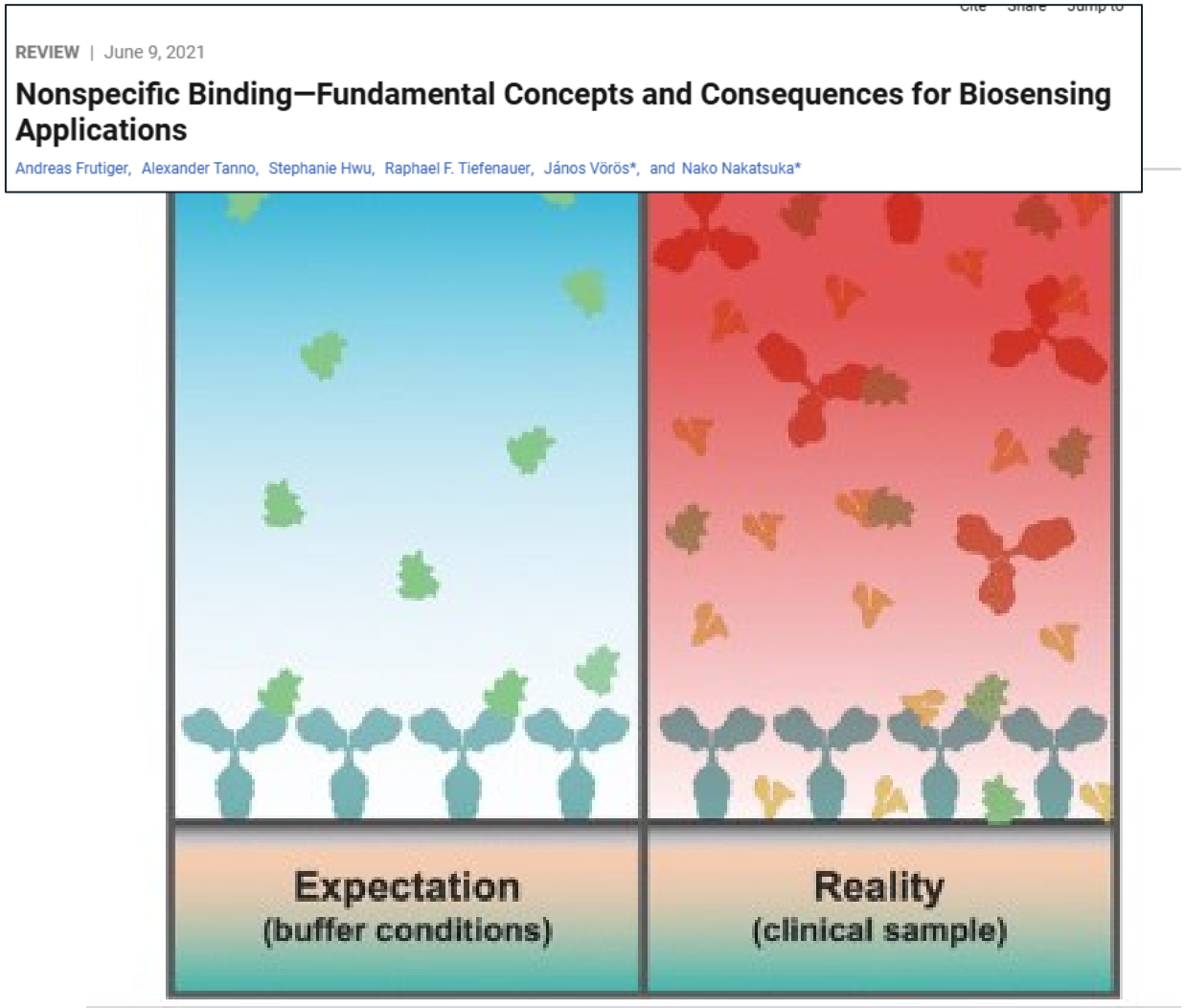
Approx 40 HCPs to be monitored
- A dozen that are painful
- The rest are 'safe'

Senior VP: ***"Mass Spectrometry is useless, we can't increase HCPs during the process! It's not right"***

Company B – [downstream] Purification Strategy

The Mass Spec is telling the ‘truth’ as it sees it

There is so much in the sample, that the method simply ‘masks’ the low abundance species, until the **CONCENTRATION** is lower, because Mass Spec is a concentration-dependent technique in electrospray



https://pubs.acs.org/doi/10.1021/acs.chemrev.1c00044

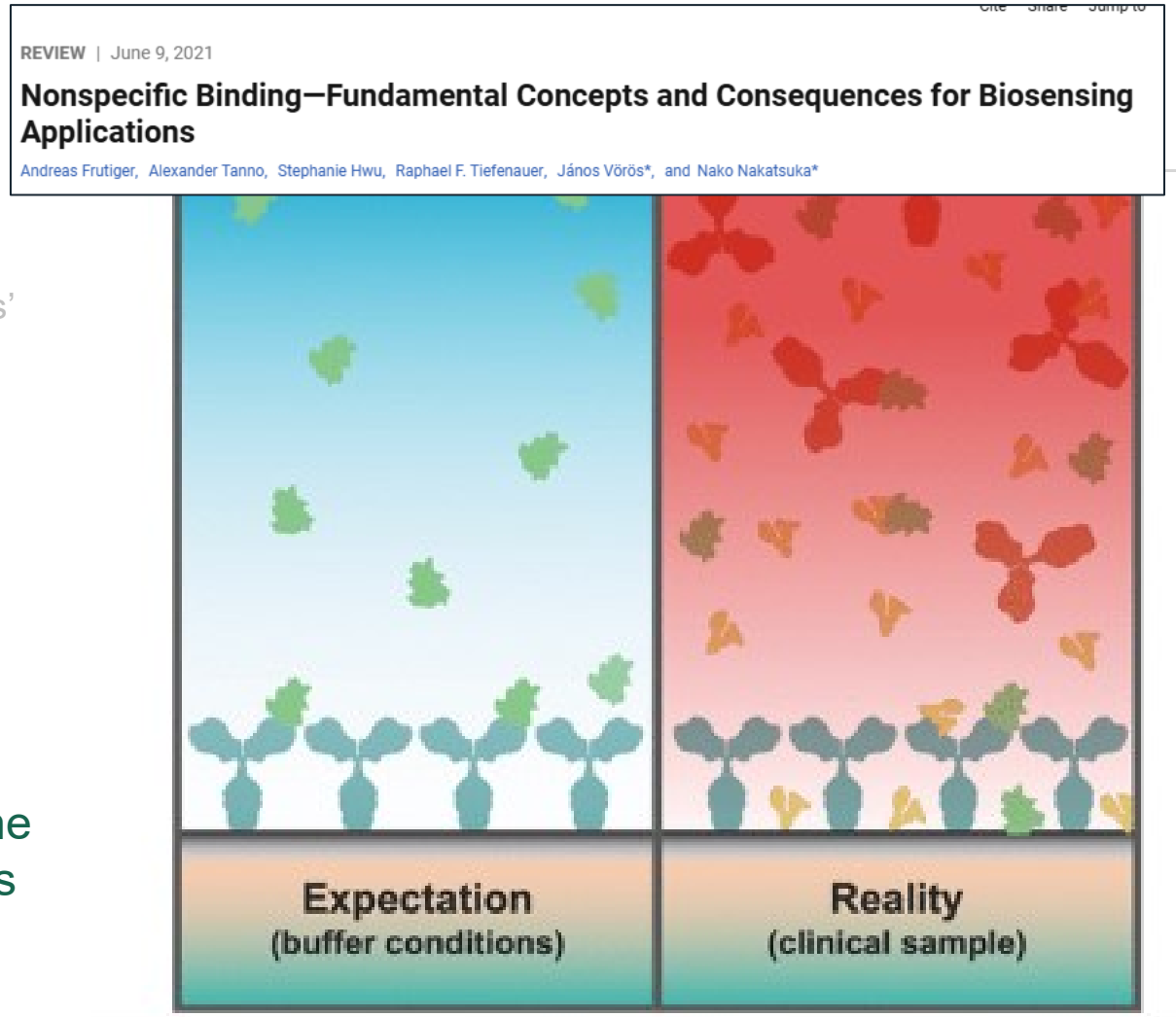
Company B – [downstream] Purification Strategy

The Mass Spec is telling the ‘truth’ as it sees it

There is so much in the sample, that the method simply ‘masks’ the low abundance species, until the **CONCENTRATION** is lower, because Mass Spec is a concentration-dependent technique in electrospray

Senior VP: ***“Oh, so we have non-specific binding in our high titer? … urgh”***

Yes, they figured out how to change the pH in one of the steps to eliminate a step by using pI values of the hydrolases.



https://pubs.acs.org/doi/10.1021/acs.chemrev.1c00044

The Mass Spectrometer was Precise

The mass spec told us there was a protein soup

… but maybe it was not ‘Accurate’? [in telling us how many]

Real world example: Are two distinguishable Actins the same, or are they different?

What’s more important, that we know the ‘Actin’ abundance as a group, or that we know that there may be multiple different Actin types?

Condition | Replicate | MS Alias name

[HCP study at Protein Metrics – Credit Antony Harvey]

			Condition	Actin					
			Replicate	55	61	62	[Avg]	[RSD]	[StdDev]
			MS Alias name →	T_055 (%)	T_061 (%)	T_062 (%)	value (%)	value (%)	value (%)
Protein name	Protein Accession ↑	Sequence (unformatted) ↑							
sp|P60709|ACTB_HUMAN Actin, cytoplasmic 1 OS=Homo sapiens OX=9606 GN=ACTB PE=1 SV=1	P60709	DLYANTVLSGGTTMYPGIADR				1.58	1.58	0.00	0.00
		GYSFTTTAER				11.38	11.38	0.00	0.00
		VAPEEHPVLLTEAPLNPK				20.76	20.76	0.00	0.00
sp|P63261|ACTG_HUMAN Actin, cytoplasmic 2 OS=Homo sapiens OX=9606 GN=ACTG1 PE=1 SV=1	P63261	DLYANTVLSGGTTMYPGIADR		0.72	1.40		1.06	45.02	0.48
		GYSFTTTAER		3.75	9.17		6.46	59.28	3.83
		VAPEEHPVLLTEAPLNPK		10.13	18.16		14.15	40.13	5.68

Ok, So the Problems are insurmountable?

No! There is mitigation available

Mass Spec Conditions – and Models!

- Understand the limits of your experiment
- Understand the parameters that ‘bound’ the experiment
- Understand the specific instrument you are using (yes, individual mass specs may be different, even from the same vendor)

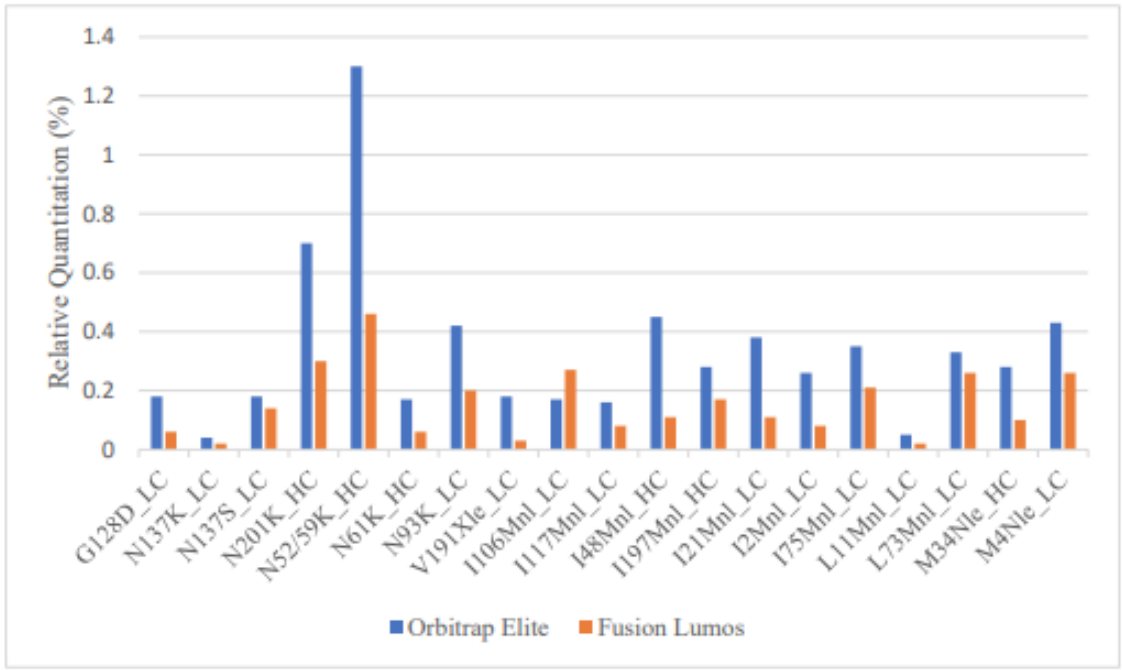
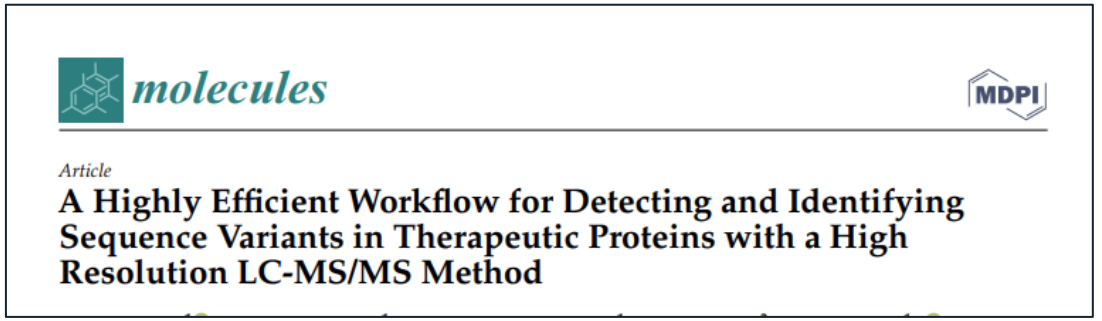


Figure 2. Comparison of the relative quantitation of true positive sequence variants obtained in Orbitrap Elite vs. Fusion Lumos.



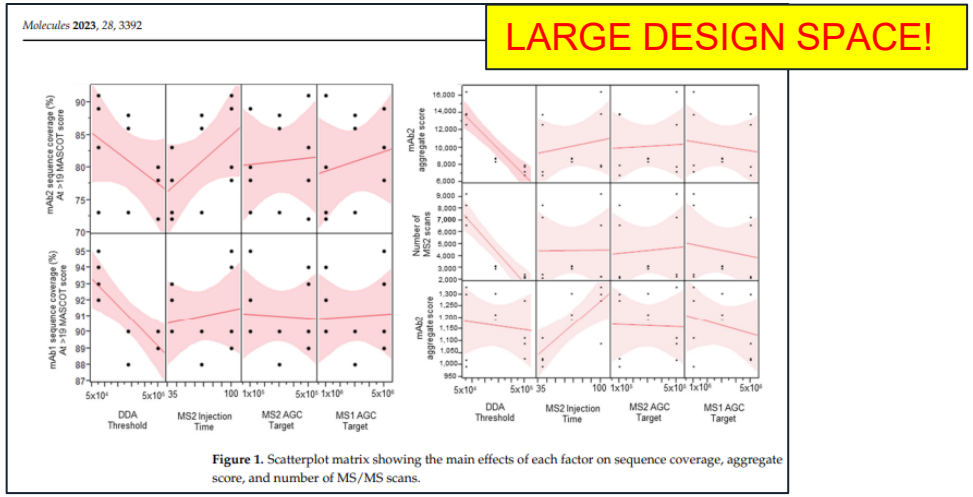


Figure 1. Scatterplot matrix showing the main effects of each factor on sequence coverage, aggregate score, and number of MS/MS scans.

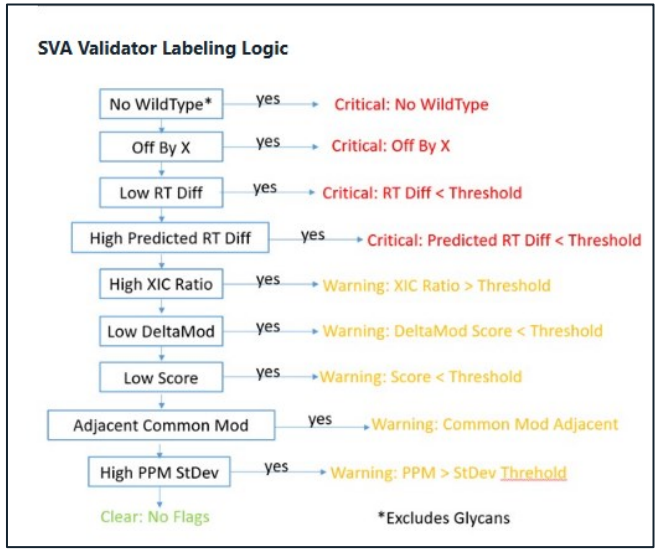
https://pmc.ncbi.nlm.nih.gov/articles/PMC10144261/pdf/molecules-28-03392.pdf

Logic for Humans and within Software

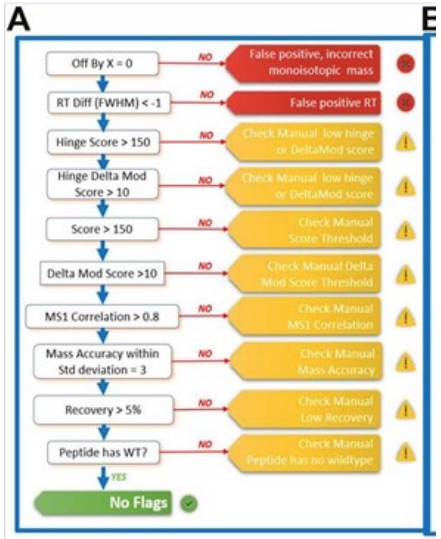
Many logic tools are built into software such as ‘Sequence Variants Analysis’ that use rule-based algorithms, appropriate for the right study

- Ability to ‘triage’ based on ‘likelihood’
- Validator logic provides a rapid review system, using multiple, weighted mass spec parameters.
- Like any logic tree, it is made for a certain process: highly purified biopharmaceutical products
- NB: this is a way of coping with substitutions, it does NOT ‘discover’ anything.

Moral: Understand your experiment!



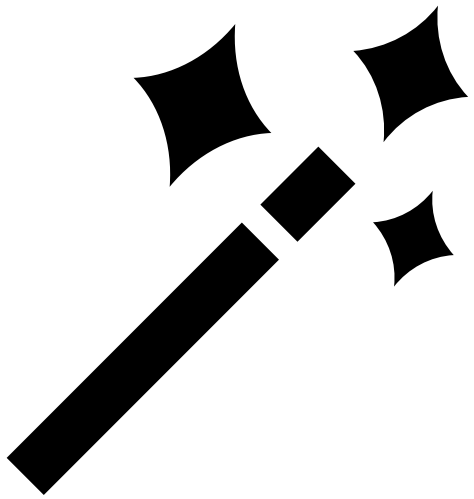
Protein Metrics App note: https://support.proteinmetrics.com/hc/en-us/articles/11608220246932-Accelerating-Sequence-Variant-Analysis-with-Byos-Validators



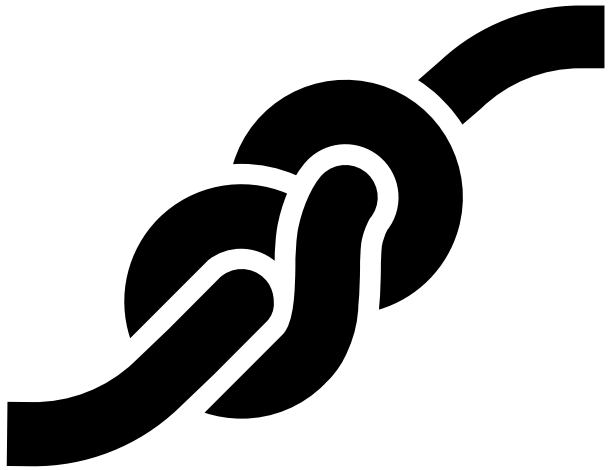
Data ‘triage’ with flags to alert a reviewer

Summary

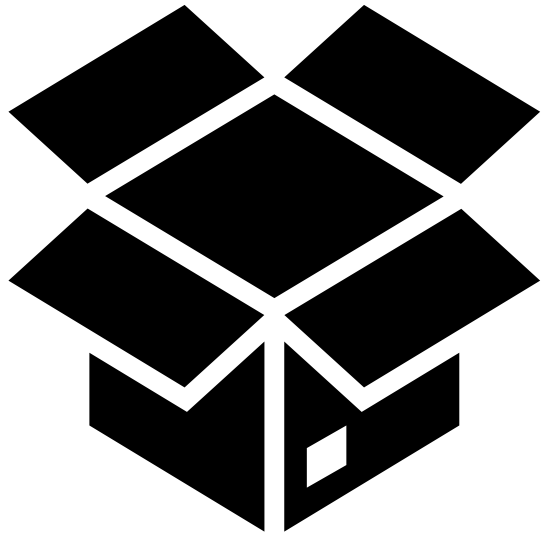
Not ‘Magic’: it’s a device with SPECIALIZATIONS



It’s only knotty because it provides DENSE information



Not a black box, it’s just SCIENCE, not Rocket Science



Questions?

“There is a crack, a crack in everything, that’s how the light gets in”.
Leonard Cohen - Anthem