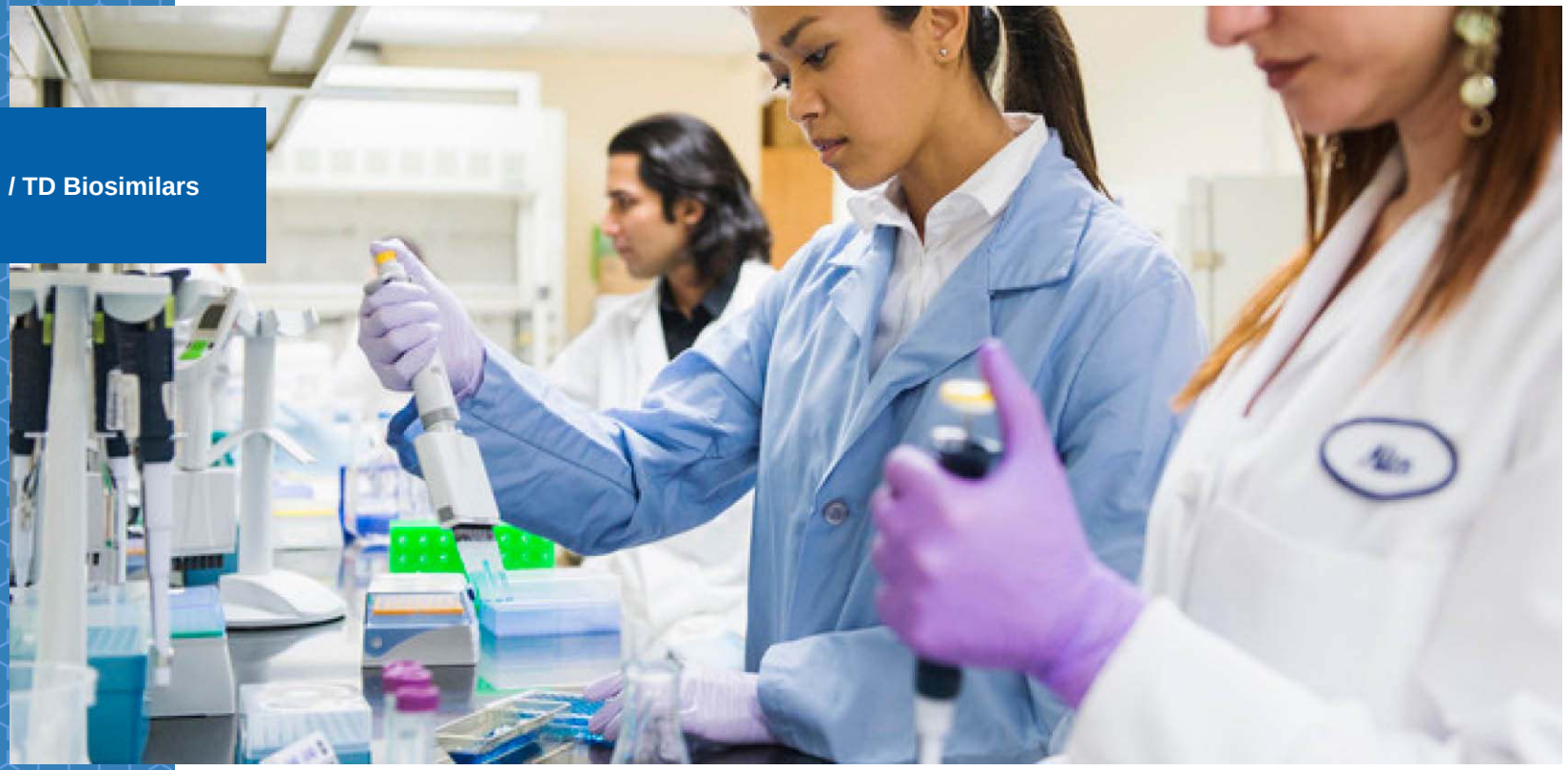
BTDM / TD Biosimilars



Structure-function of GP2015/Erelzi

Robert Mayer, Senior Scientist PCC Kundl

AT Europe 2017

March 17th, 2017

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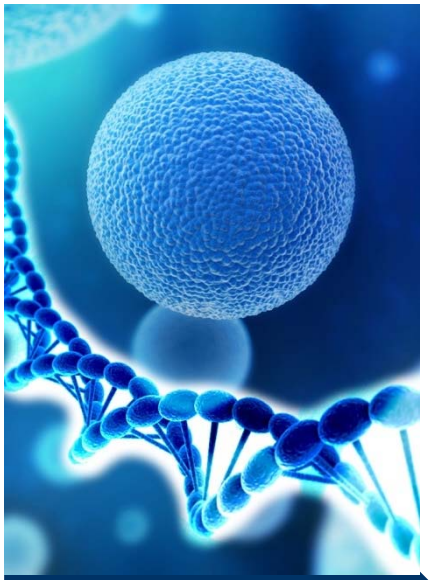
Biopharmaceutical manufacturing



Modify host cells

(e.g. bacteria, yeast, mammalian) to produce recombinant proteins

Grow cells

under controlled conditions (fermentation, upstream process)

Extract, refold, purify

to generate drug substance (downstream process)

Formulate to stable finished drug product

vial, syringe, cartridge

Adapted from EGA Handbook on biosimilar medicines; available from http://www.egagenerics.com/index.php/publications/

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Variability is in the nature of glycoproteins

Batch-to-batch

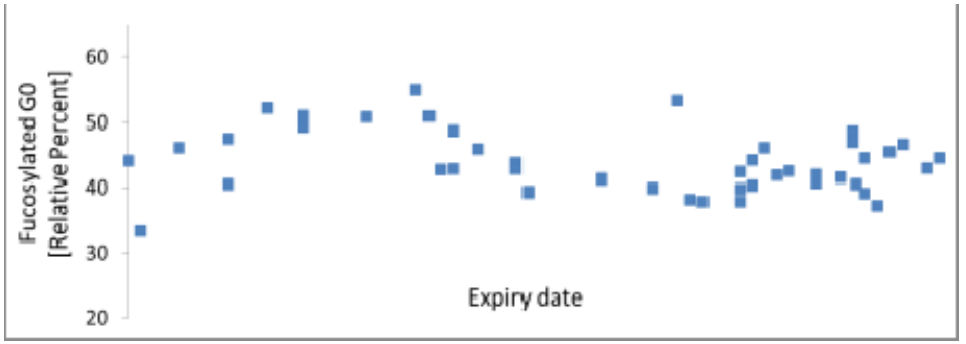
- Non-identicality is a normal principle in glycosylated proteins
- No batch of any biologic is “identical” to the other batches
- Variability is natural even in the human body and usually not problematic

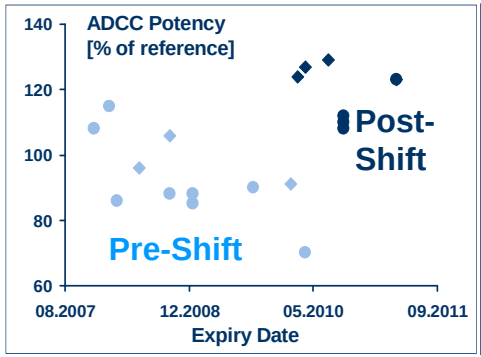
Manufacturing changes

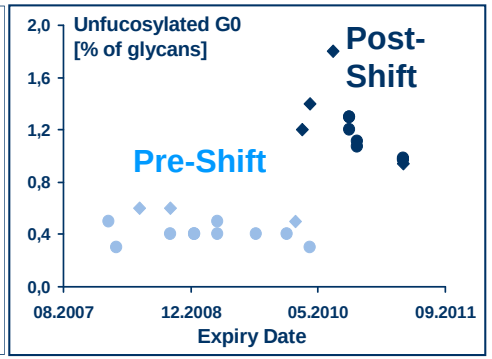
- Manufacturing changes are made frequently
- Differences in attributes often larger than batch-to-batch variability
- Such changes are stringently controlled by regulators and approved only if they do NOT lead to clinically meaningful differences

Safety/efficacy demonstrated

Variability of major glycan variant in commercial mAB







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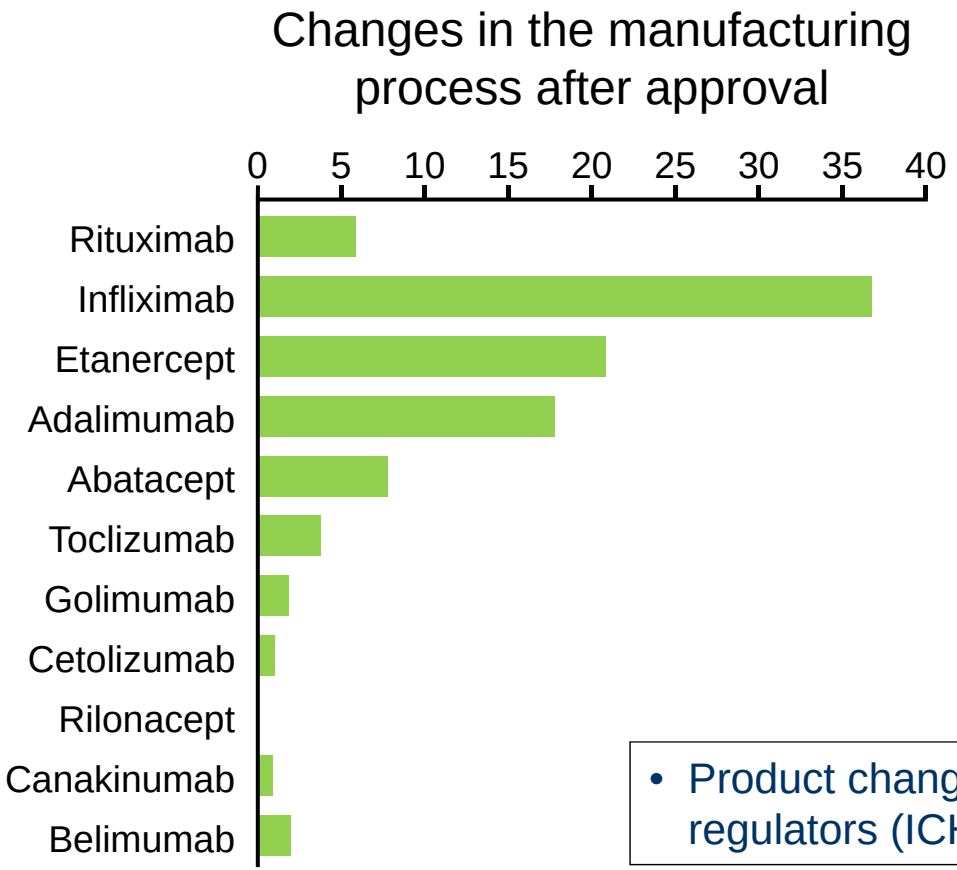
Schiestl M, et al. Nat Biotechnol 2011;29(4):310–2; Sandoz data

Manufacturing changes are made frequently in a biologic's life time



Changes include

- Change in the supplier of a cell culture media
- New purification methods
- New manufacturing sites

- Product changes are closely monitored by the regulators (ICH Q5E)

Schneider C. Biosimilars in rheumatology: the wind of change. Ann Rheum Dis 2013;72:315-318.

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What is a biosimilar?

Biosimilarity means

- that the biological product is **highly similar** to the reference product notwithstanding minor differences in clinically inactive components; and that
- there are **no clinically meaningful differences** between the biological product and the reference product in terms of safety, purity, and potency of the product.[1]

An approved biosimilar medicine and its reference medicine contain **essentially the same** active ingredient and are expected to have the **same** safety and efficacy profile[2]

[1] Section 7002(b)(3) of the Affordable Care Act, adding section 351(i)(2) of the PHS Act
[2] European Comission Consensus Information Document “What you need to know about Biosimilar Medicinal Products“ http://ec.europa.eu/DocsRoom/documents/8242/attachments/1/translations/en/renditions/native

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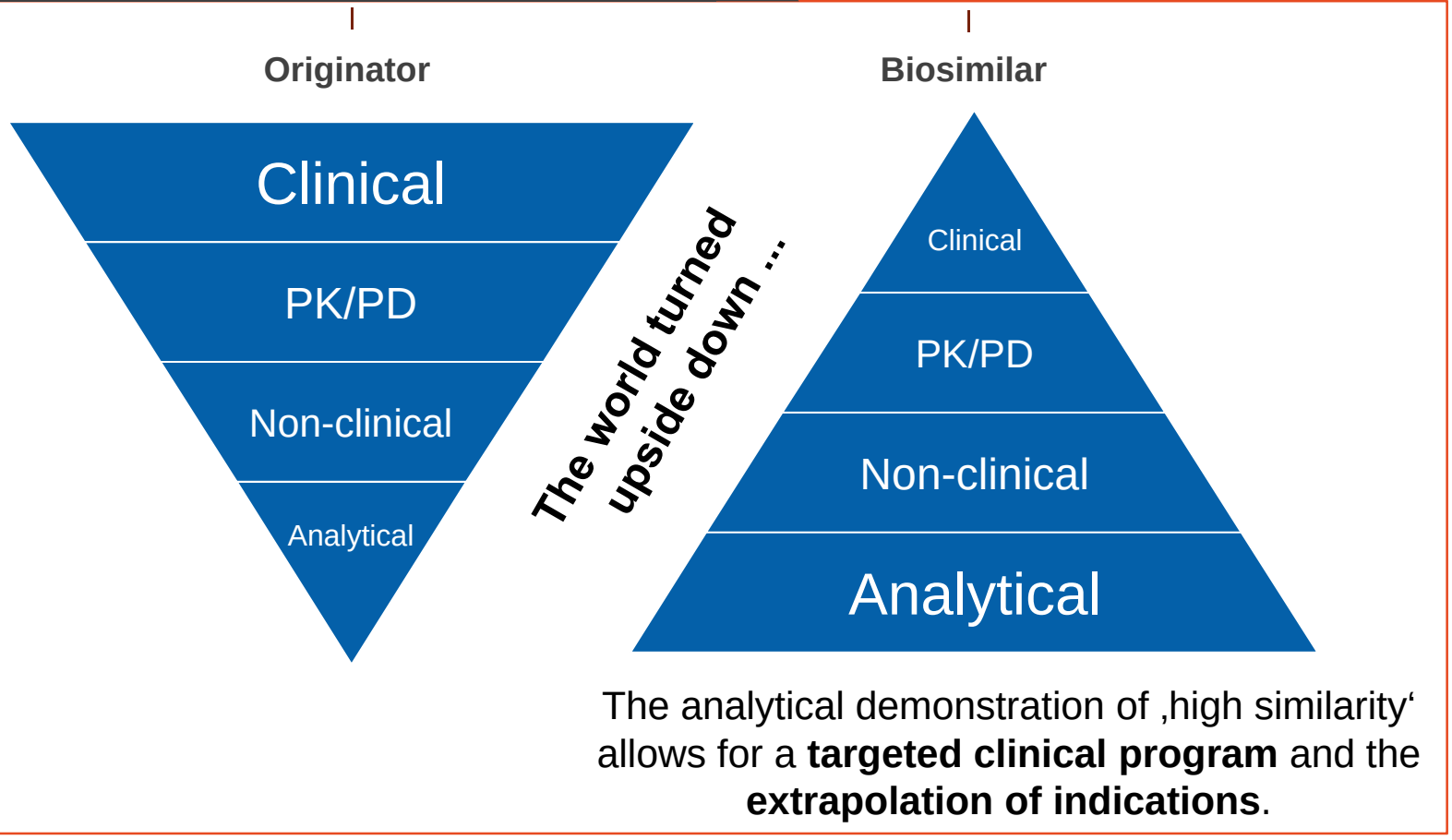
Different focus between originator and biosimilar development

Comparison of the development approach



The analytical demonstration of ‚high similarity' allows for a **targeted clinical program** and the **extrapolation of indications**.

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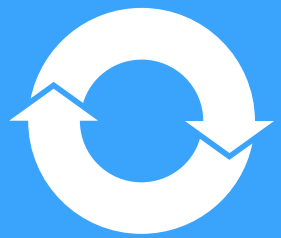
Targeted development of a biosimilar

Target definition - Analyzing numerous batches of the reference product

Iterative optimization of all process steps to match the reference product

1. Cell line development
2. Bioprocess development
3. Protein purification
4. Drug product development



Knowledge of relevance of quality attributes for efficacy and safety

Demonstration of similarity

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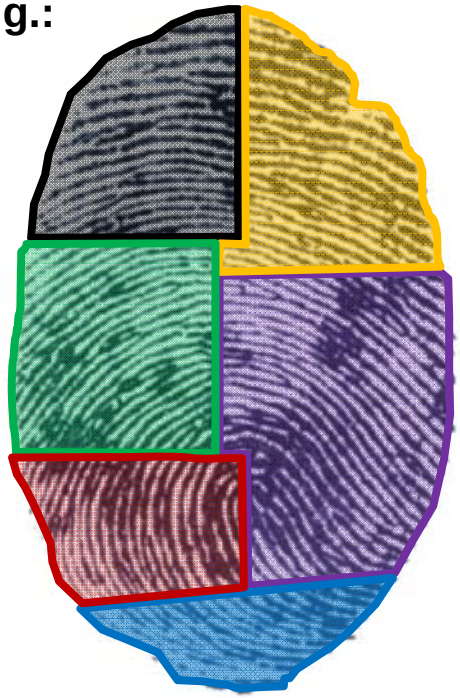
The biosimilar must match the reference product in all relevant structural and functional attributes

Primary structure (identical) e.g.:
- LC-MS intact mass
- LC-MS subunits
- Peptide mapping

Impurities and related substances e.g.:
- CEX, cIEF acidic/basic variants
- LC glycation
- Peptide mapping deamidation,
- oxidation, mutation, glycation
- SEC/FFF/AUC aggregation

Biological activity e.g.:
- Binding assay
- ADCC assay
- CDC assay



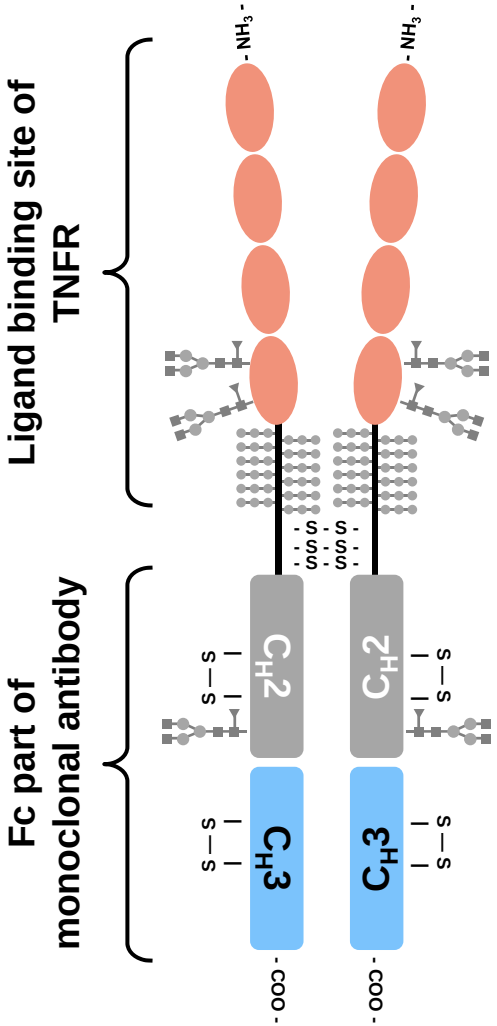
Higher order structure e.g.:
- NMR
- CD spectroscopy
- FT-IR

Glycosylation:
- NP-HPLC-(MS) N-glycans
- AEX N-glycans
- MALDI-TOF N-glycans
- HPAEC-PAD N-glycans
- MALDI-TOF O-glycans
- HPAEC-PAD sialic acids
- RP-HPLC sialic acids

Combination of attributes:
- Evaluated using MVDA, mathematical algorithms
- Checks redundant data for consistency
- Takes additive or subtractive effects of combinations of attributes into account

- Combined data from **~60 different attributes**
- Attributes are ideally measured by more than one method (redundancy)

Etanercept—A well-characterized molecule



- Manufactured by a bioprocess using a well-established recombinant CHO cell line
- Etanercept is a dimeric, secreted, soluble protein
- It has multiple glycosylation sites and disulfide bonds

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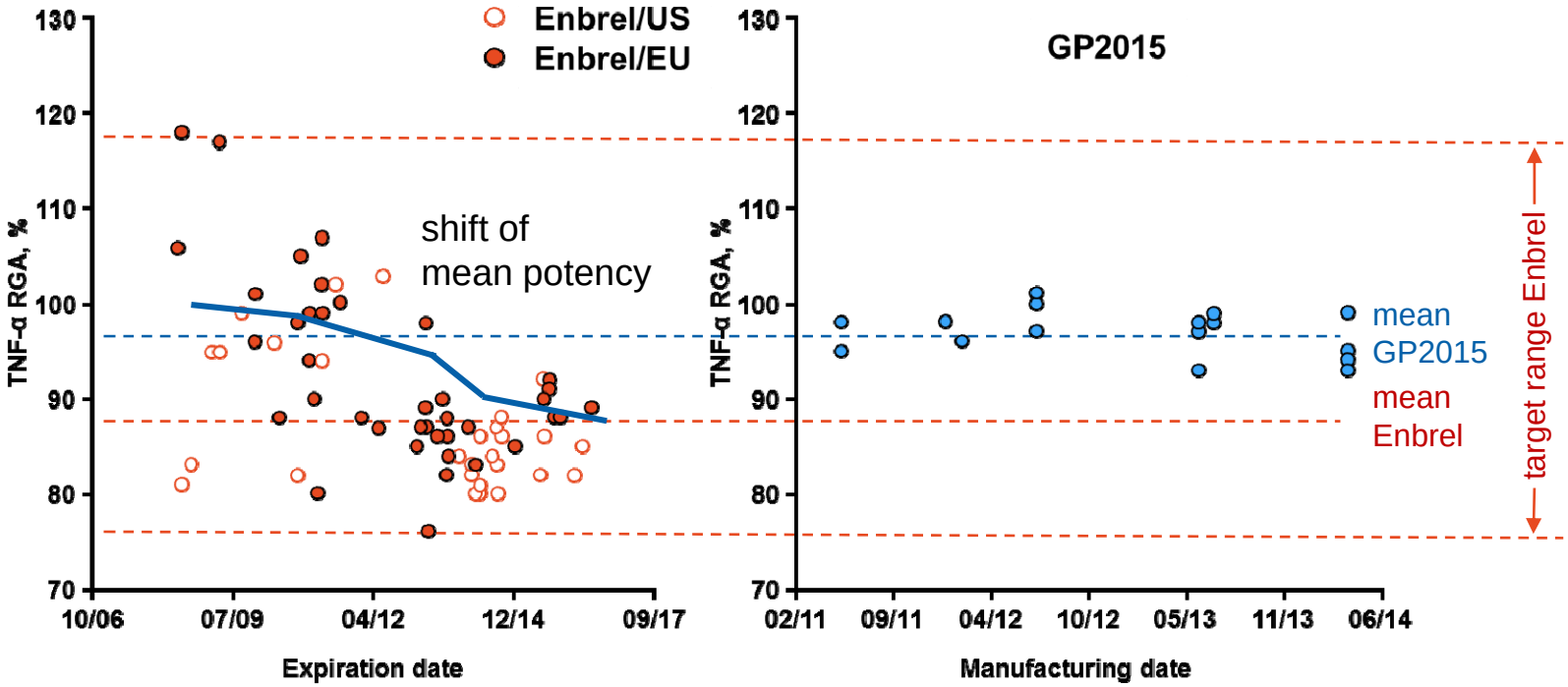
Overlapping potency for GP2015 and Enbrel® in TNF-α Neutralization



- GP2015 perfectly within min-max range of reference product
  → Scientific criteria for biosimilarity is met
- Issue: FDA formally required in addition **equivalence of the means**
- However, the reference product mean may change over time. Biopharmaceuticals including originator products are usually released when they comply with a min/max-specification range. The average of the product is not specified.
- Equivalence testing criteria asks for hunting a moving target and would be an unreasonable requirement

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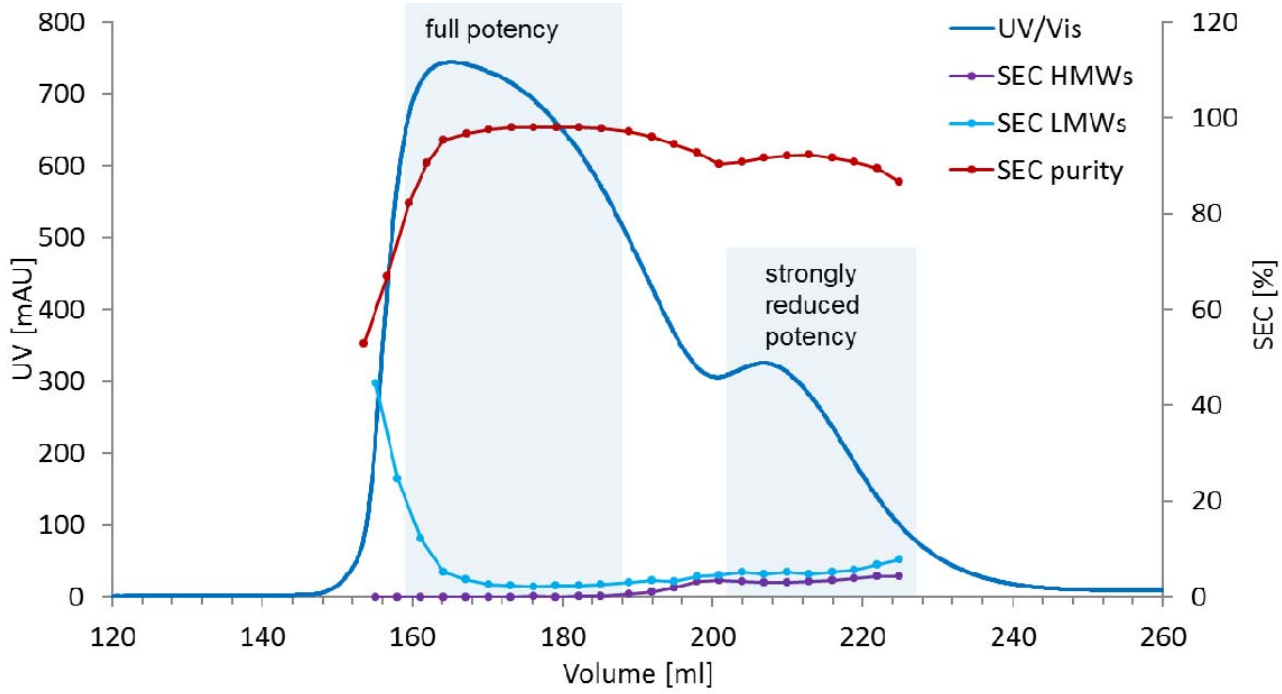
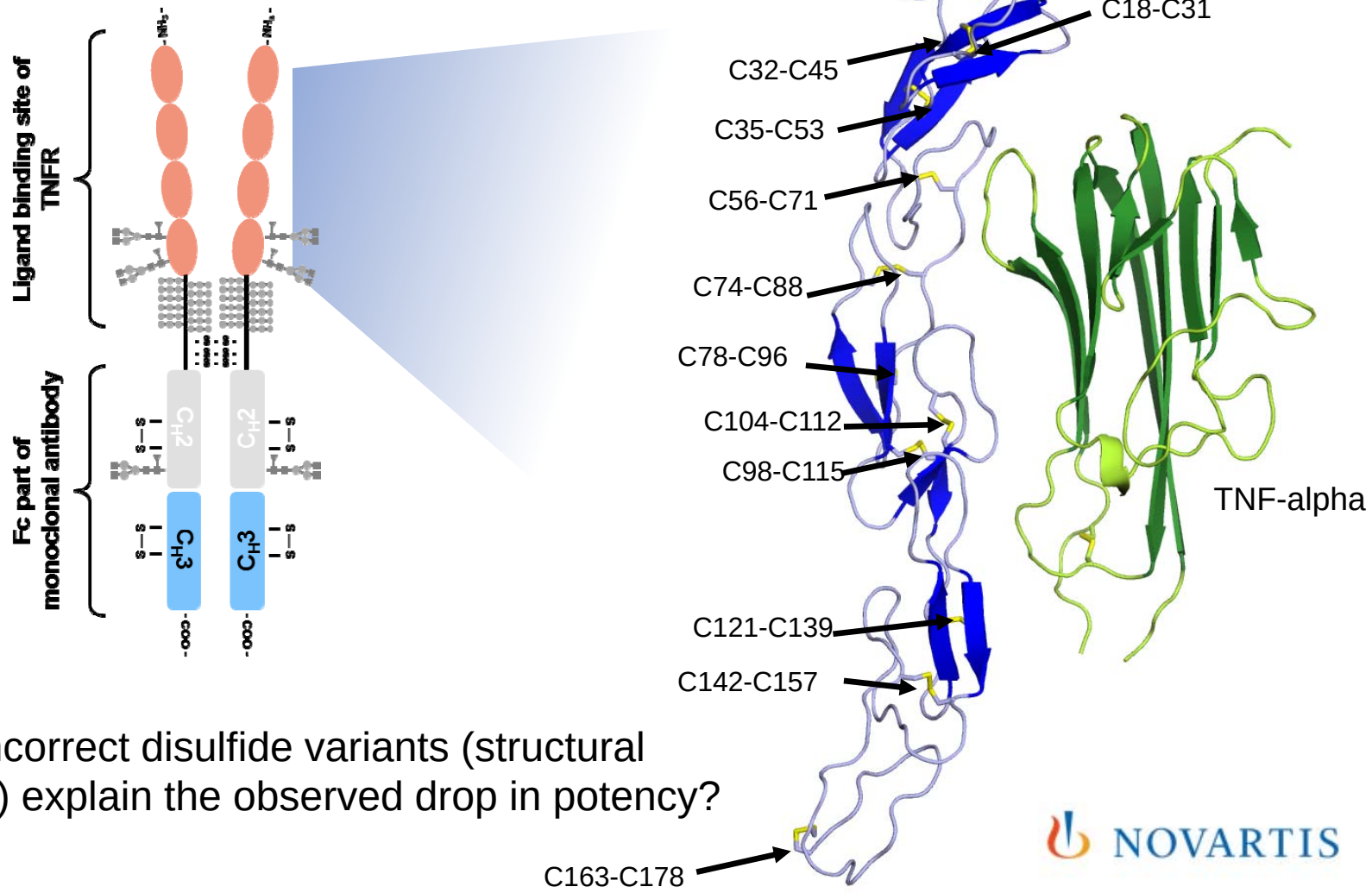
What could explain the shift?
Which quality attributes impact potency?

Interesting observation during proces development:
Strongly reduced potency observed in waste-fractions of th process purification-step



Degradation products are not causing the drop in potency
What else could cause such a significant reduction in potency?

The TNF-alpha receptor domain is stabilized by 26 disulfide bonding



Could incorrect disulfide variants (structural variants) explain the observed drop in potency?

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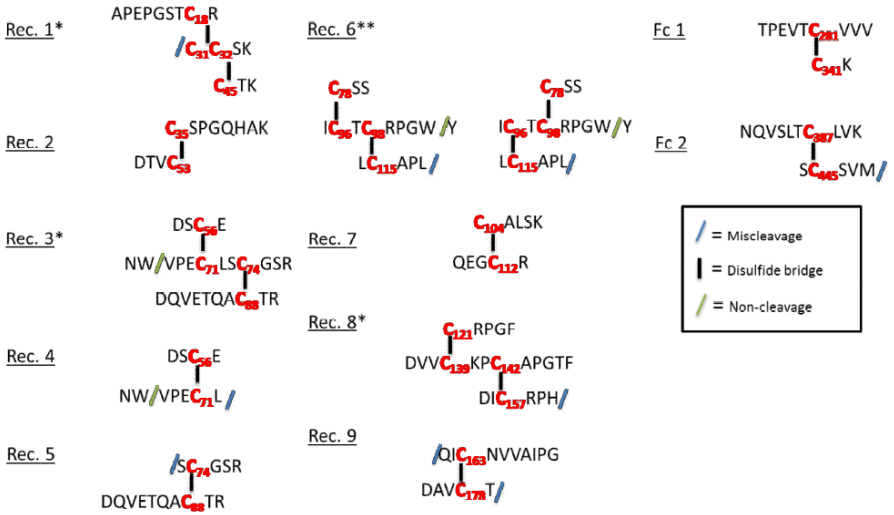
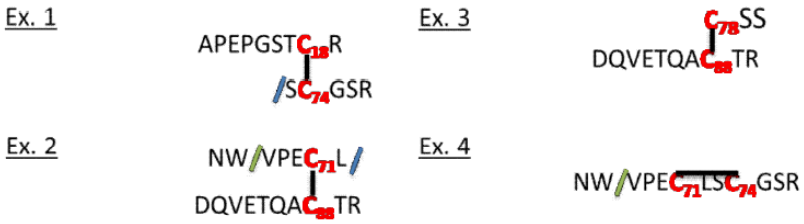
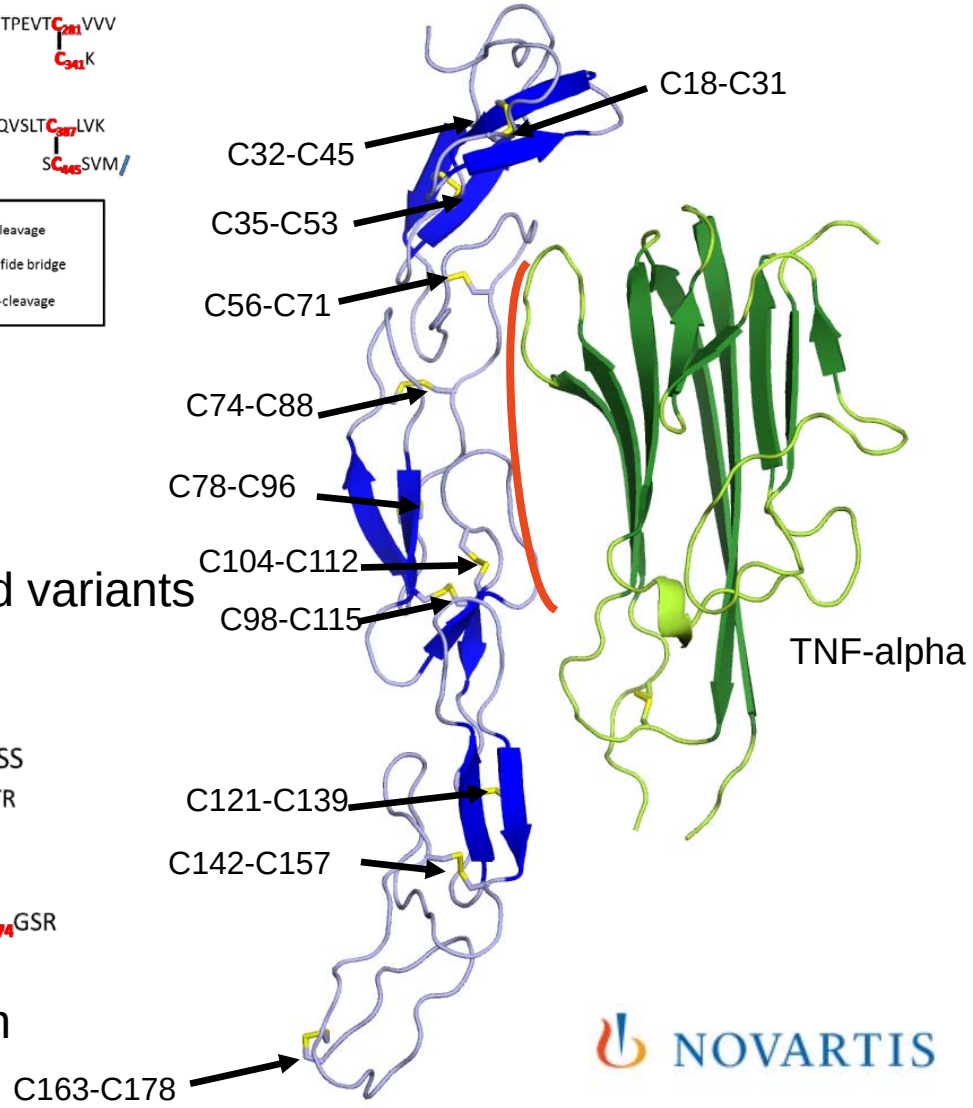
A Mass Spectrometry based assay confirmed the disulfide bonds identified by X-ray



and also identified incorrectly bridged variants in the process purification-step...



...which localize to the binding region



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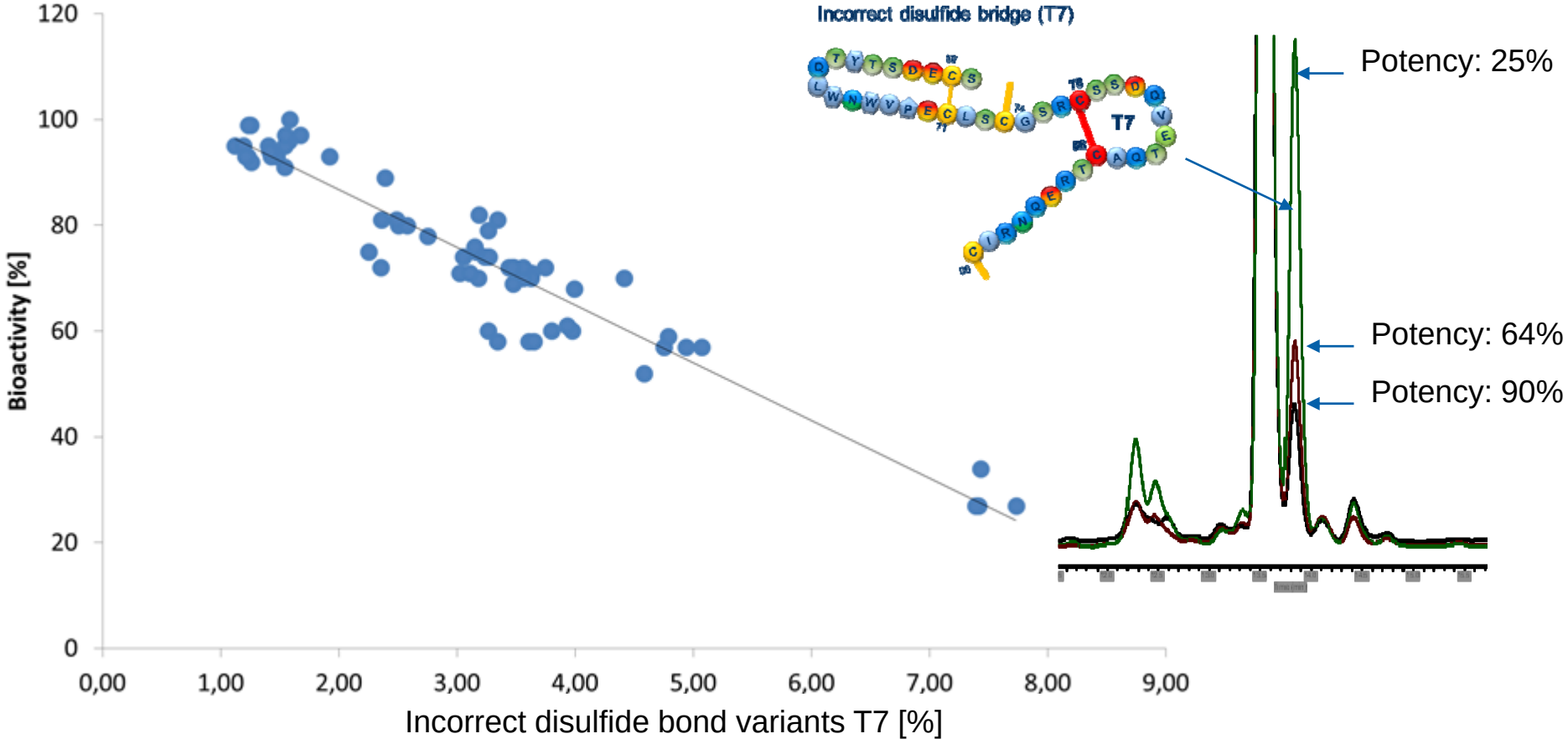
Structure-function relationship between incorrect disulfide bonds and potency



Incorrect disulfide bond variants strongly impact the potency

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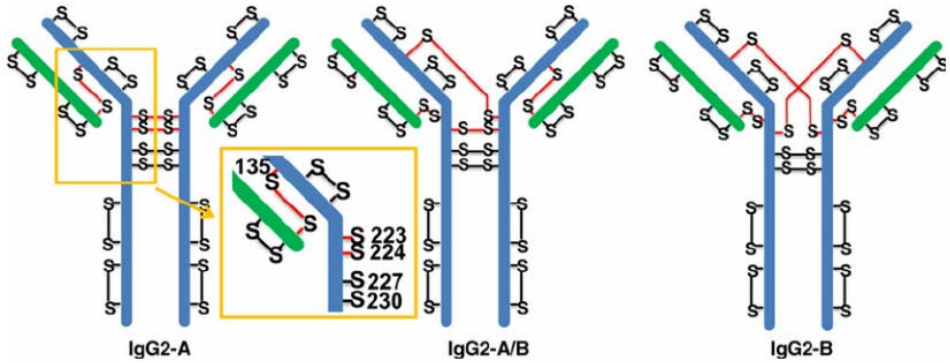
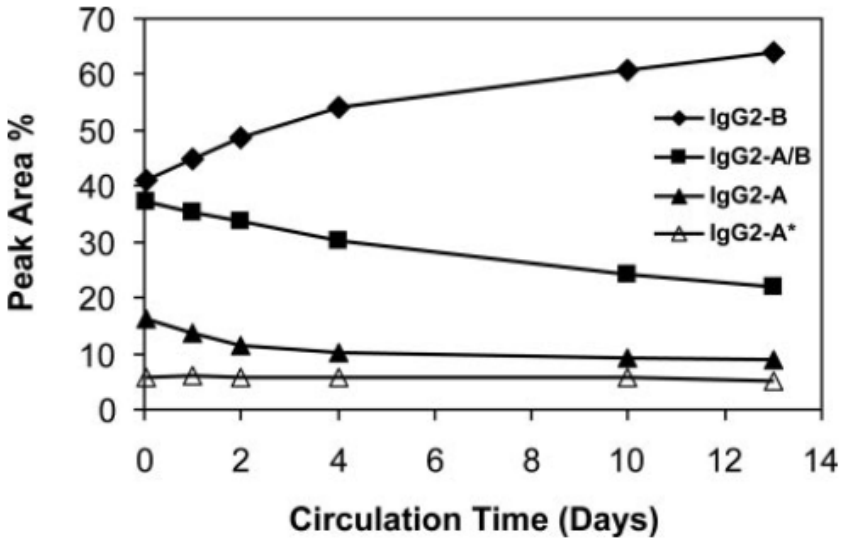
Could the incorrectly bonded molecules revert back and thereby restore potency?

- IgG2 and IgG4 antibodies have disulfide bonds that can shuffle *in-vivo*
- **Shuffeling occuring *in-vivo* can be mimicked using a redox system *in-vitro***



Jones et al., 2013



Liu et al., 2008

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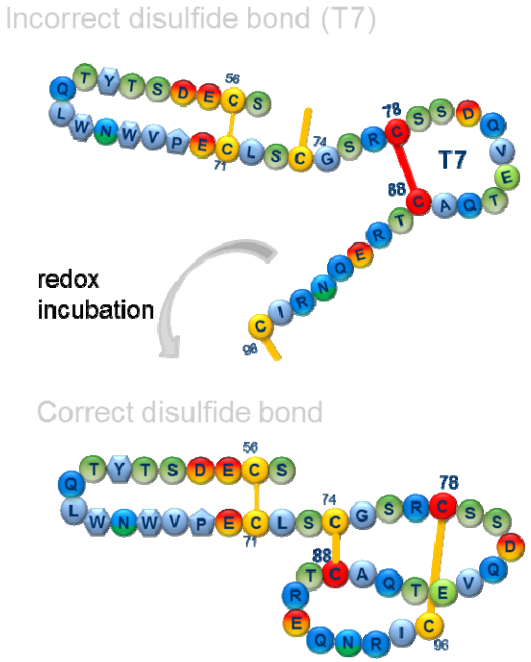
Restoration of *in-vitro* potency after incubation in a redox system mimicking *in-vivo* conditions

Sample	Control		Redox Incubation	
	% T7	% Potency	% T7	% Potency
GP2015 waste fraction	**3.4**	**76**	**1.6**	**98**
GP2015 waste fraction	**5.5**	**58**	**2.0**	**93**



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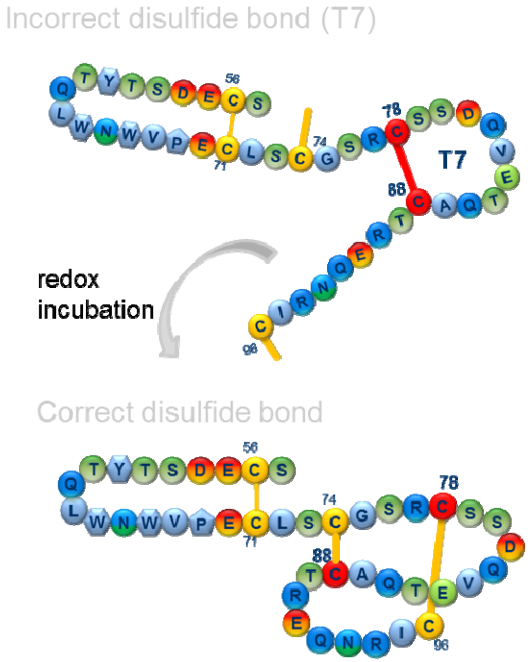
Sample	Control		Redox Incubation	
	% T7	% Potency	% T7	% Potency
GP2015 waste fraction	3.4	76	1.6	98
GP2015 waste fraction	5.5	58	2.0	93
Enbrel US 1	**2.6**	**89**	**1.7**	**107**
Enbrel US 2	**2.5**	**85**	**1.8**	**98**
Enbrel US 3	**2.8**	**81**	**1.8**	**96**
Enbrel US 4	**2.5**	**85**	**1.8**	**95**
Enbrel EU 1	**2.3**	**92**	**1.6**	**100**



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How good is the prediction of the *in-vivo* potency?

	Experimentally determined potency prior treatment	Experimentally determined potency after redox treatment	Computed potency (100% refold)
GP2015 DS	97	102	96
GP2015 CAP.E	66	97	91
GP2015 HIC.E	52	84	88
GP2015 DS	104	103	101
GP2015 CAP.E	70	92	93
GP2015 HIC.E	65	90	93
GP2015 DS	99	103	93
GP2015 CAP.E	76	98	95
GP2015 HIC.E	58	93	99
GP2015 DP Batch 1	98	103	94
GP2015 DP Batch 2	97	101	99
GP2015 DP Batch 3	100	98	96
Enbrel/US #1040542	89	107	99
Enbrel/US #1062728	85	98	94
Enbrel/US #1034018	81	96	93
Enbrel/US #1034842	85	95	94
Enbrel/EU #J13793	92	100	99
AVERAGE		***98 ±6***	***95 ±3***

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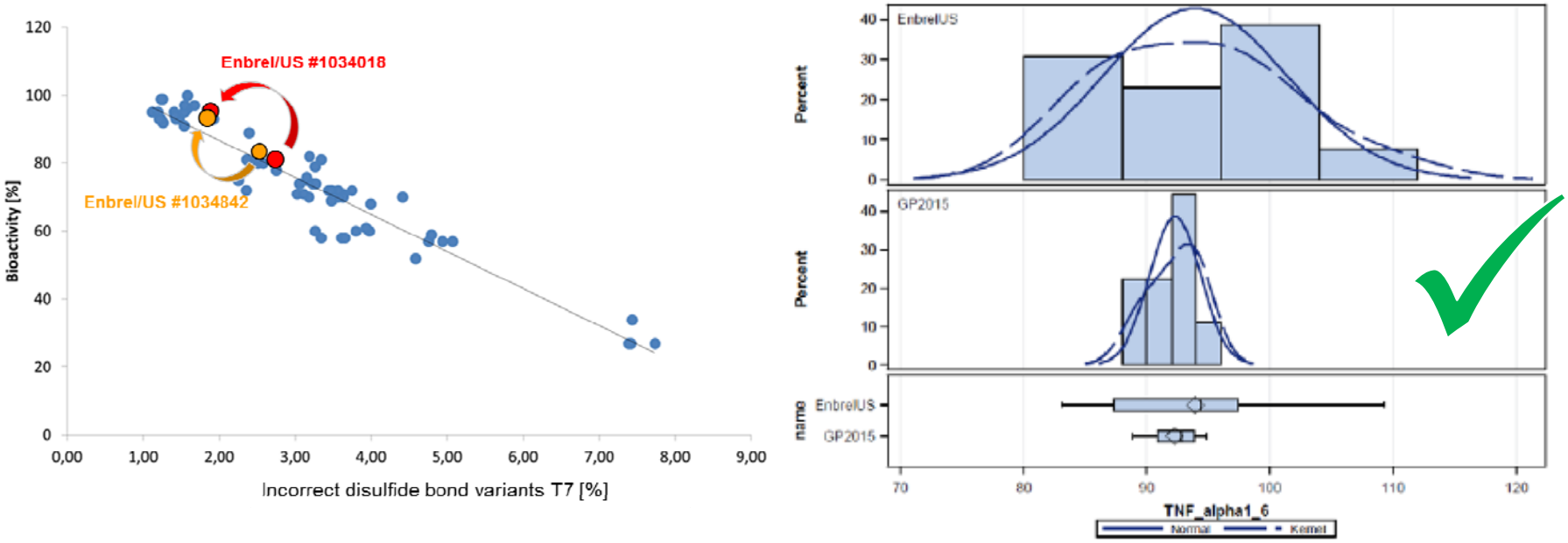
Applying the structure-function knowledge to the biosimilarity assessment



The potency of Enbrel corrected for the incorrectly bridged disulfide variants is equivalent to the potency of GP2015
→ FDA requirement of ‚high similarity' fulfilled

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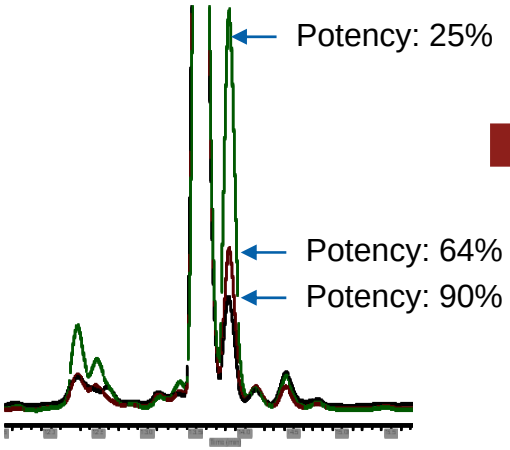
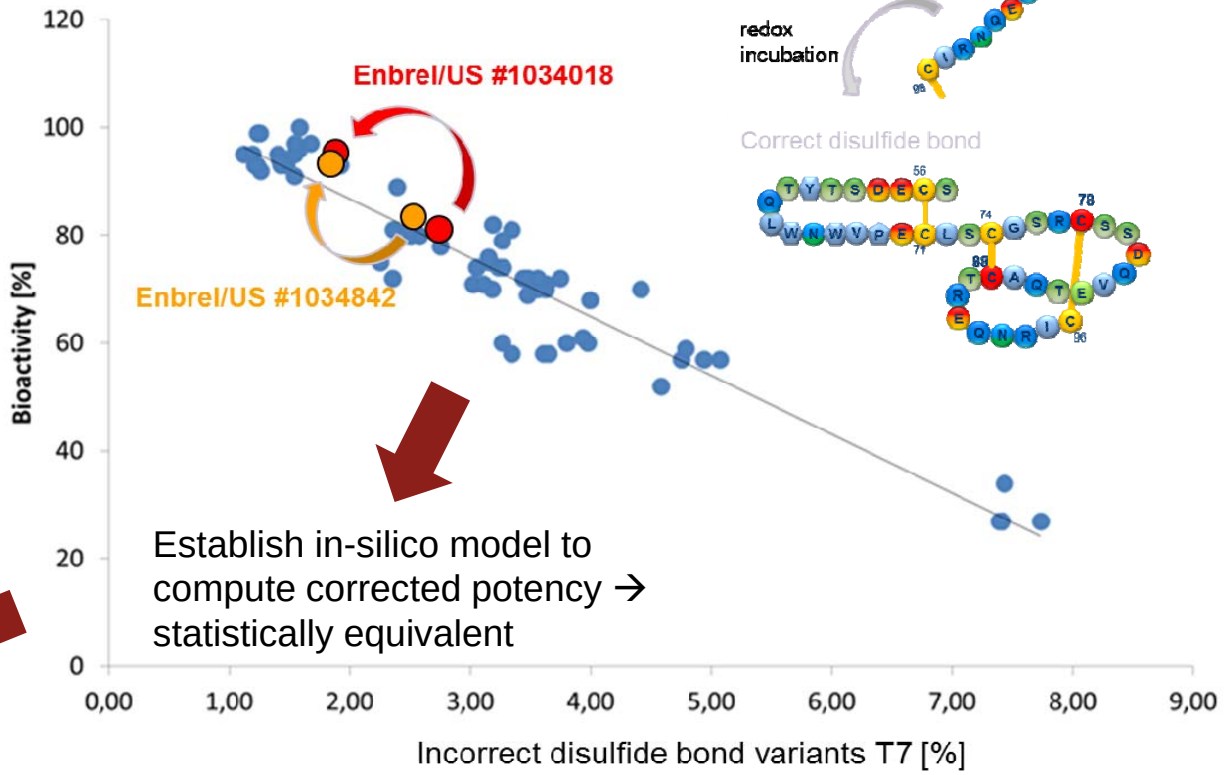
Summary

Identification of incorrect disulfide variants (**3 years**)



Establish linear structure-function relationship (**2 years**)
Establish and perform *in-vitro* reshuffling experiments (**6 months**)



Establish in-silico model to compute corrected potency → statistically equivalent

Successful FDA AdCom (20:0) Approval of GP2015

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and lessons learned

- GP2015 perfectly within min-max range of the reference product Enbrel for potency
- Equivalence testing criteria would require hunting a moving target – meaningless as quality attributes are specified by upper and lower limits but not by means
- Structure function relationship between potency and incorrect disulfide bond variants was well understood
- Disulfide variants refold under physiological conditions
- Equivalence could be demonstrated although it is an unreasonable requirement

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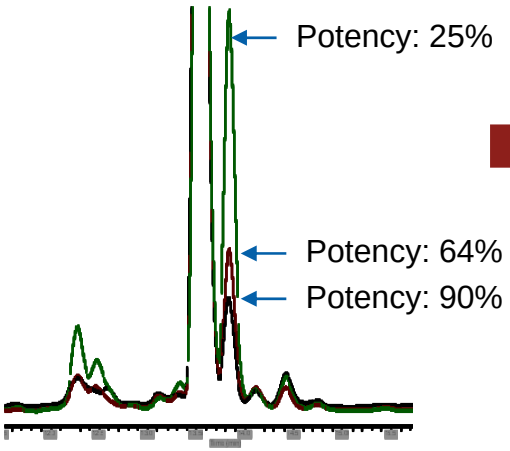
Acknowledgements – TEAMWORK !

Alfred Rupprechter
Hansjörg Toll



Michael Fuchs
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Fabian Higel
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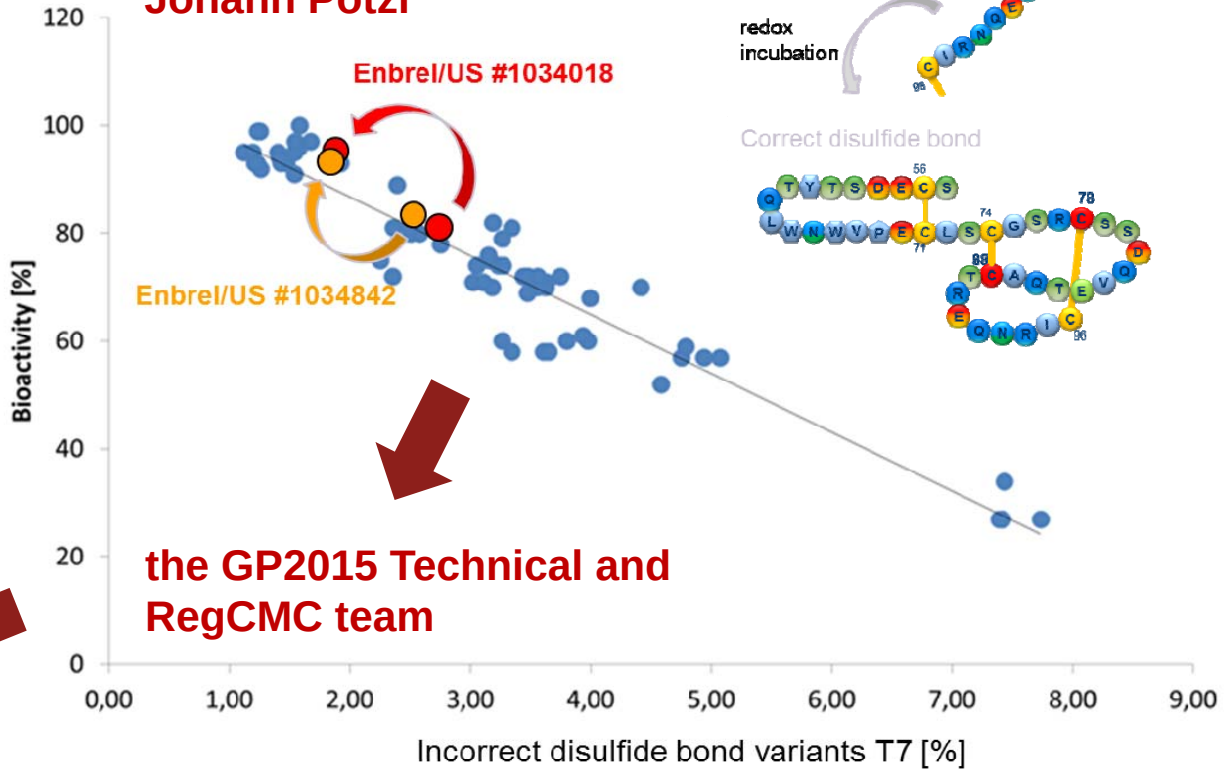

the GP2015 Technical and RegCMC team

Successful FDA AdCom (20:0) Approval of GP2015

Johann Holzmann, Andreas Seidl, Uros Urleb, Martin Schiestl and many many others

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