蛋白质三维构像矩阵检测技术的开发和应用.

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Array Bridge Inc.

报告内容

1. 为什么需要新的三维构像测定技术?

2. 新型三维构像测定技术的开发.

3. 蛋白质三维构像抗体矩阵技术的应用.

4. 第一个应用范围：InnoBridge ELISA 在新型单克隆抗体和单抗偶联药物开发中的应用.
5. 第二个应用范围: 生物仿制药的开发.
6. 第三个应用范围: p53 and SHP2 的基础研究和小分子抗癌药物的筛选.
7. 第四个应用范围: MouseBridge and RabBridge ELISA 在关键抗体试剂使用中的质量控制.
8. 第五个应用范围: 客户定制任何感兴趣蛋白质的三维构像抗体矩阵ELISA.

9. 总结.

1. 为什么需要新的三维构像测定技术?

新型单抗的开发规模：本技术的市场需求*.

First approved therapeutic monoclonal antibody product in 1986 (Orthoclone, Kidney Disease)
By November 10, 2014, 47 monoclonal antibody with EU/USA Clearance.
At current approval rates by 2020 70 expected with a cap of $125bn

* Ecker et al, The Theraputic Monoclonal Antibody Market. mAbs 7:1, 9--14; January/February 2015;

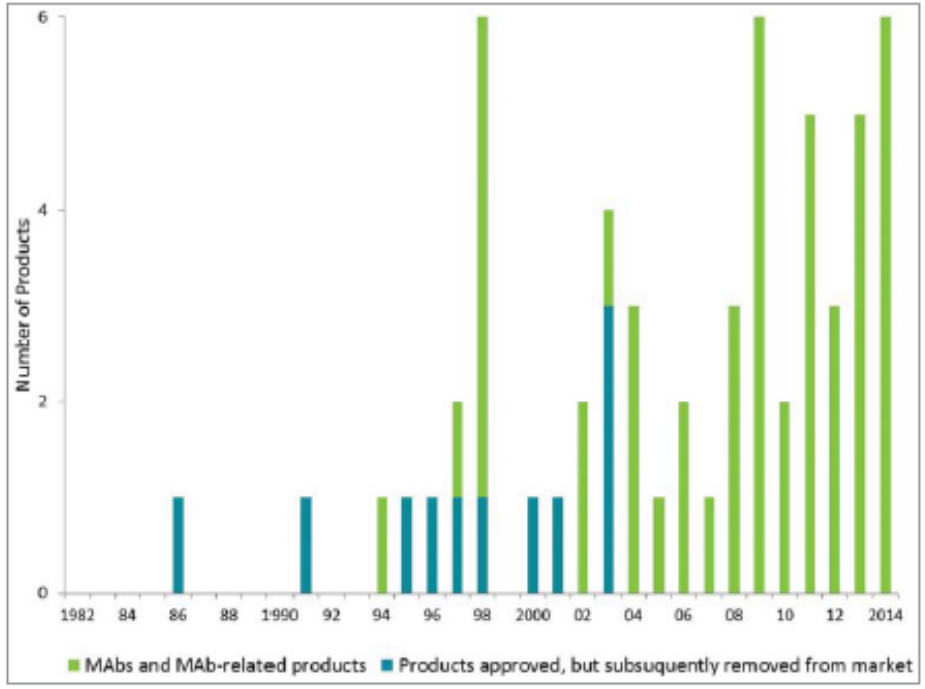


Figure 1. Annual approvals of monoclonal antibody products.[3,4] The number of monoclonal antibody products first approved for commercial sale in the US or Europe each year since 1982 is shown. The totals include all monoclonal antibody and antibody-related products. Products approved but subsequently removed from the market are denoted in blue; products currently marketed are denoted in green. 2014 total is as of November 10, 2014.

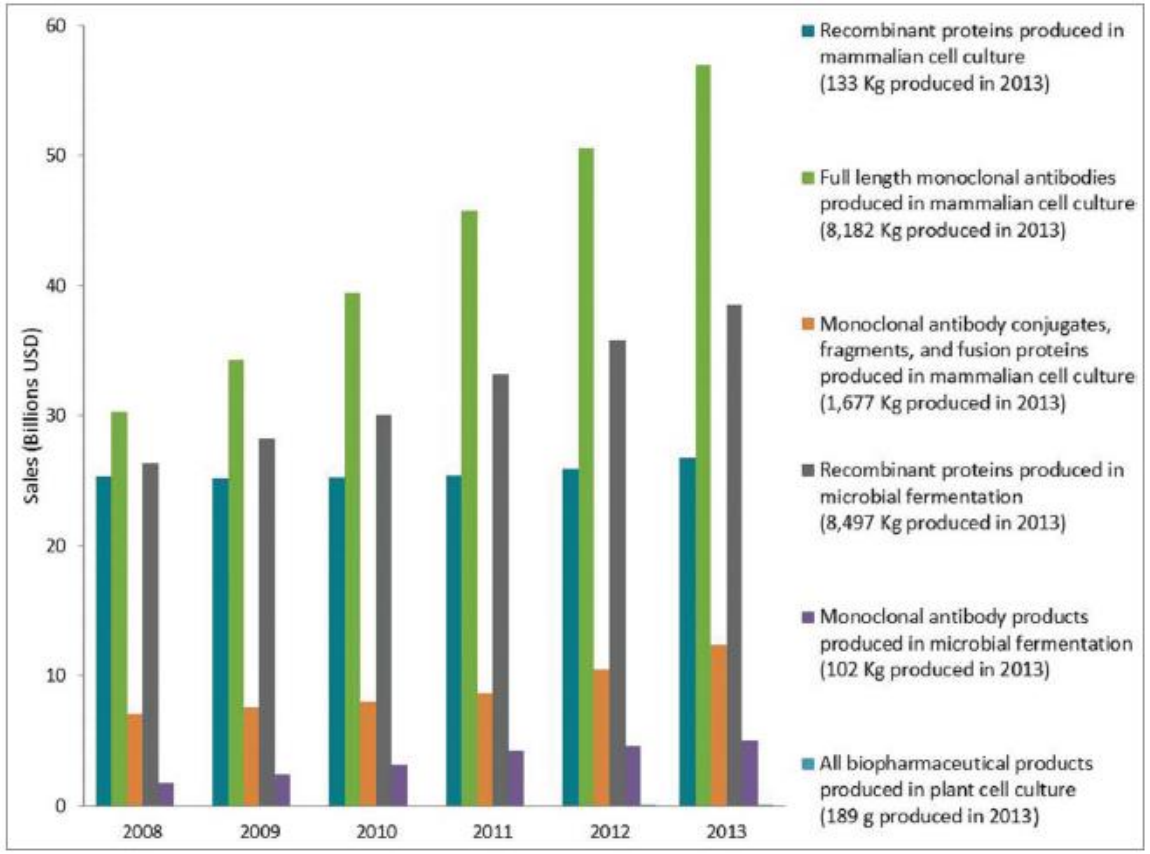
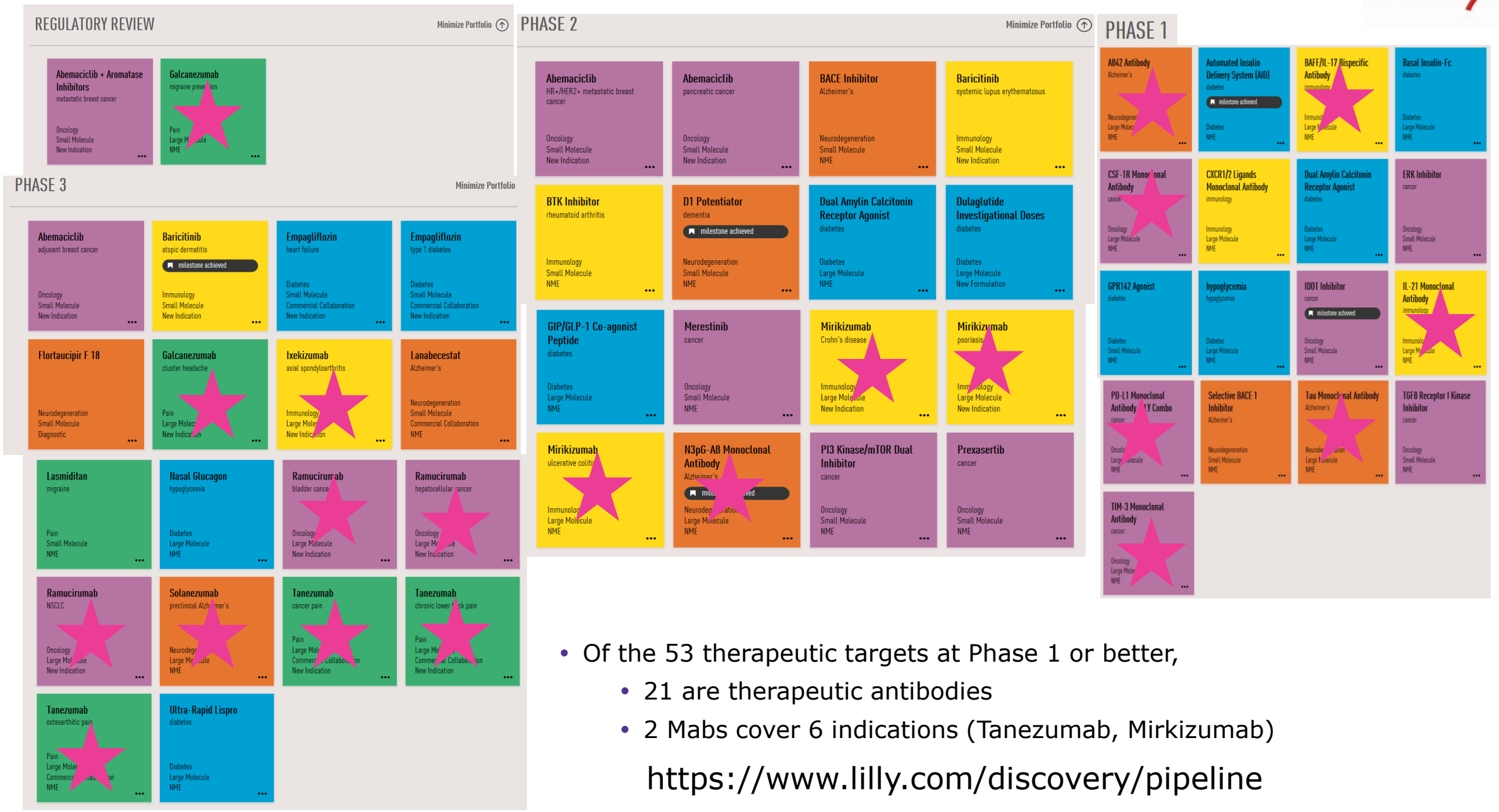


Figure 2. Sales of biopharmaceutical products by product type. Total annual sales of biopharmaceutical products are shown as a function of product type. Note that recombinant proteins produced by microbial fermentation include recombinant human insulin products which represent nearly 50% of the sales and >90% of the material produced in this category.

大型制药公司举例：：美国礼来的管线近一半是抗体药物，其它公司也类似



- Of the 53 therapeutic targets at Phase 1 or better,
  - 21 are therapeutic antibodies
  - 2 Mabs cover 6 indications (Tanezumab, Mirkizumab)

https://www.lilly.com/discovery/pipeline

Table 1. Marketed therapeutic monoclonal antibody products

Brand name (INN)	Original BLA/MAA Applicant	Company Reporting EU Sales	Year of First Approval	2013 Global Sales ($M)[a]
Abthrax (raxibacumab)	Human Genome Sciences	N/A[b]	2012	23
Actemra (tocilizumab)	Roche	Roche	2009	1,119
Adcetris[c] (brentuximab vedotin)	Seattle Genetics	Takeda Pharmaceutical Co.	2011	253
Alprolix[d] (Factor IX Fc fusion protein)	Biogen Idec	N/A	2014	NoM[e]
Arcalyst[f] (rilonacept)	Regeneron Pharmaceuticals	N/A	2008	17
Arzerra (ofatumumab)	GlaxoSmithKline	GlaxoSmithKline	2009	117
Avastin (bevacizumab)	Genentech	Roche	2004	6,748
Benlysta (belimumab)	Human Genome Sciences	GlaxoSmithKline	2011	228
Cimzia[g] (certolizumab pegol)	UCB	UCB	2008	789
Cyramza (ramucirumab)	Eli Lilly and Co.	N/A	2014	NoM[e]
Eloctate[h] (Factor VIII Fc fusion protein)	Biogen Idec	N/A	2014	NoM[e]
Enbrel[i] (etanercept)	Immunex	Pfizer	1998	8,325
Entyvio (vedolizumab)	Takeda Pharmaceuticals U.S.A., Inc	Takeda Pharmaceutical Co.	2014	NoM[e]
Erbitux (cetuximab)	ImClone Systems	Merck KGaA	2004	1,926
Eylea[j] (aflibercept)	Regeneron Pharmaceuticals	Bayer Healthcare Pharmaceuticals	2011	1,851
Gazyva (obinutuzumab)	Genentech	Roche	2013	3
Herceptin (trastuzumab)	Genentech	Roche	1998	6,559
Humira (adalimumab)	Abbott Laboratories	AbbVie	2002	10,659
Ilaris (canakinumab)	Novartis Pharmaceuticals	Novartis Pharmaceuticals	2009	119
Inflectra[k,l] (infliximab [biosimilar])	Hospira	Hospira	2013	<1[m]
Kadcyla[n] (ado-trastuzumab emtansine)	Genentech	Roche	2013	252
Keytruda (pembrolizumab)	Merck & Co.	N/A	2014	NoM[e]
Lemtrada (alemtuzumab)	Genzyme Therapeutics	Sanofi	2013	3
Lucentis[o] (ranibizumab)	Genentech	Novartis Pharmaceuticals	2006	4,205
Nplate[p] (romiplostim)	Amgen	Amgen	2008	427
Nulojix[q] (belatacept)	Bristol-Myers Squibb	Bristol-Myers Squibb	2011	26
Orencia[r] (abatacept)	Bristol-Myers Squibb	Bristol-Myers Squibb	2005	1,444
Perjeta (pertuzumab)	Genentech	Roche	2012	352
Prolia[s] (denosumab)	Amgen	GlaxoSmithKline	2011	824
Remicade (infliximab)	Centocor	Merck & Co.	1998	8,944
Removab[t] (catumaxomab)	Fresenius Biotech	NeoPharm Group	2009	5
Remsima[k,l] (infliximab [biosimilar])	Celltrion	Celltrion	2013	<1[m]
ReoPro[u] (abciximab)	Centocor	N/A	1994	127
Rituxan (rituximab)	Genentech	Roche	1997	7,500
Simponi/ Simponi Aria (golimumab)	Centocor Ortho Biotech	Merck & Co.	2009	1,432
Simulect (basiliximab)	Novartis Pharmaceuticals	Novartis Pharmaceuticals	1998	30[v]
Soliris (eculizumab)	Alexion Pharmaceuticals	Alexion Pharmaceuticals	2007	1,551
Stelara (ustekinumab)	Janssen-Cilag International	Johnson & Johnson	2009	1,504
Sylvant (siltuximab)	Janssen Biotech	Johnson & Johnson	2014	NoM[e]
Synagis (palivizumab)	Abbott Laboratories	Abbvie	1998	1,887
Tysabri (natalizumab)	Biogen Idec	Biogen Idec	2004	1,527
Vectibix (panitumumab)	Amgen	Amgen	2006	389
Xgeva[s] (denosumab)	Amgen	Amgen	2010	1,030

截至2015年被批准的单抗药物名单

- Most of these are novel Mabs.
- However biosimilars are starting to be approved.
- FDA approved 1st full length mAb biosimilar in 2016.
- EMA approval was in 2013.

单克隆抗体药物的生产流程

Protein Journey

Originator Mab
Discovery-Non Stable Cell Line

Biosimilar
Reference Standard

MFG Cell Line*
Cell Line Development
Media Optimization
R=Protein Concentration

"Totality of Evidence"

Structural Analytics
- CD Spectrum
- Size Exclusion Chromatography (SEC)
- Analytical Ultracentrifugation (AUC)
- Non-denaturing Electrophoresis
- Hydrogen/deuterium exchange (HDX)
- NMR

R=Similarity to Reference

Sample Analysis
Nonclinical
PK
PD
Clinical Safety
Immunogenicity
R= Safety/Efficacy

*** Cell Line.**
- Work with a well established cell line (avoid Allotypic variation)
- Optimize media to avoid changes in Glycosylation.

Manufacturing Upscale (Downstream)

R=Similarity to Reference

Formulation
Upscale
Bulk Processing

Finished Good
Packaging
Distribution

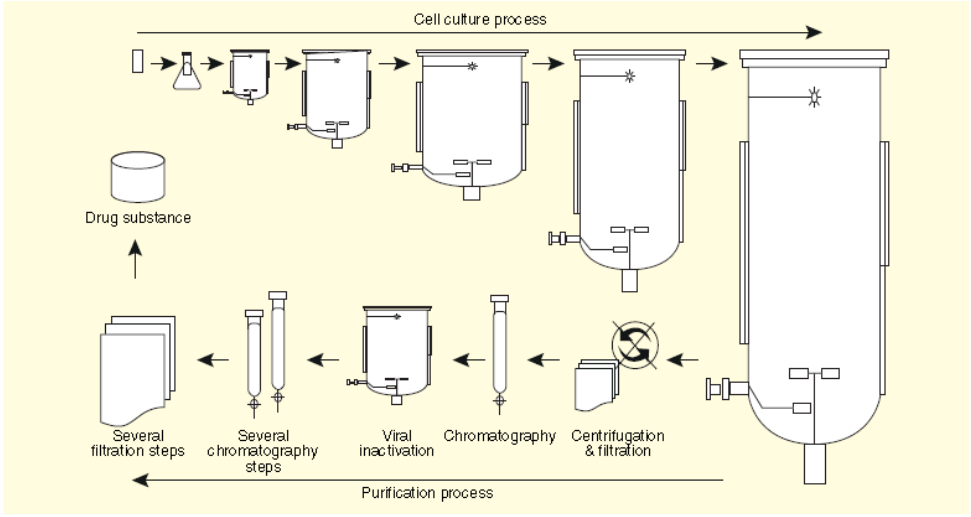


Figure 1. Overview of the manufacturing process of a biologic in a mammalian cell culture system. The manufacturing process consists of a cell culture process and a purification process. The cell culture process is initiated by expansion of a single vial of cell stock to culture flask, after which it is sequentially subcultured to larger bioreactors. In this process, optimized cell culture conditions such as temperature, agitation rate, osmolality, pH, concentration of CO_2 and glucose concentration are tightly maintained, as these conditions are critical to the quality of biologics. The supernatants are harvested and further purified through several steps of chromatography, filtration and viral inactivation in the purification process, which also have potential to influence the quality of biologics.

This may be applied to ANY therapeutic protein

蛋白质三维构像分析方面目前的技术限制：

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

"The three dimensional conformation of a protein is an important factor in its biological function. Protein generally exhibit complex three-dimensional conformations (tertiary structure and, in some cases, quaternary structure) due to their large size and the rotational characteristics of protein alpha carbons. The resulting flexibility enables dynamic, but subtle, changes in protein conformation over time, some of which may be absolutely required for functional activity." "…… at the same time, a protein's three-dimensional conformation can often be difficult to define precisely using current physiochemical analytical technology."

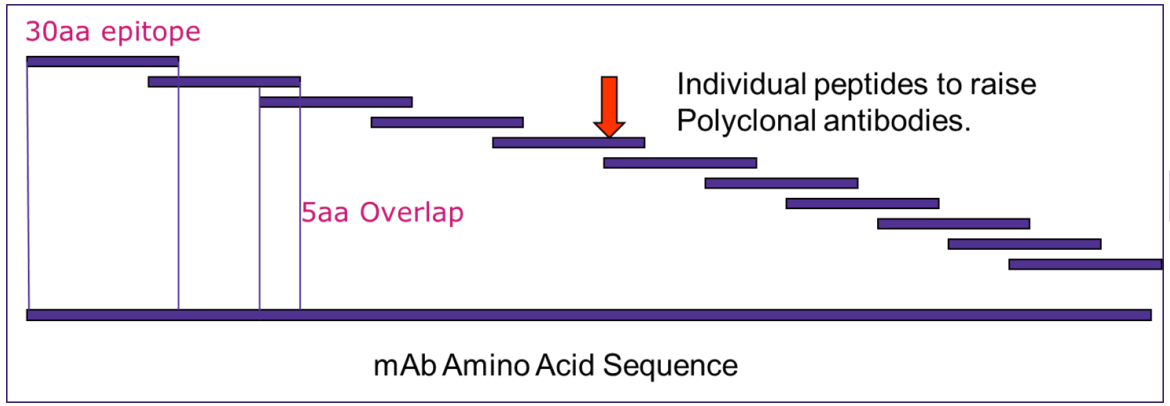
目前应用的蛋白质三维构像分析技术限制：

三维构像分析技术	原理	优点	缺点
CD	肽键与芳香族氨基酸环境	易于使用，成本低	低灵敏度，整个测量样品的平均值
FTIR	肽键	易于使用，成本低	低灵敏度，整个测量样品的平均值
PCA	通过抗体识别表位	易于使用，cGMP友好，系统化，高灵敏度和高通量	市场新技术，价格处于中间水平
HDX-MS	蛋白质表面酰胺基团中的氢-氘交换	高分辨率、完善的应用	成本高，需要专用仪器和培训，通量低
HRF-MS	蛋白质表面羟基的自由基标记	高分辨率	成本高，需要专用仪器和培训，通量低
Bioassay	目标蛋白质识别	技术完善， cGMP友好	低分辨率
X-Ray	原子衍射	高分辨率	成本高，通量低，不适合常规测试
DLS	聚集体和多聚体光散射	成本低，方法成熟	低灵敏度，整个测量样品的平均值
NMR	原子核自旋和电荷	高分辨率，方法成熟	需要特殊的仪器和培训。 成本高，对于小蛋白质分析比较好
Fluorescence	芳香族氨基酸环境	成本低，方法成熟	低灵敏度，整个测量样品的平均值

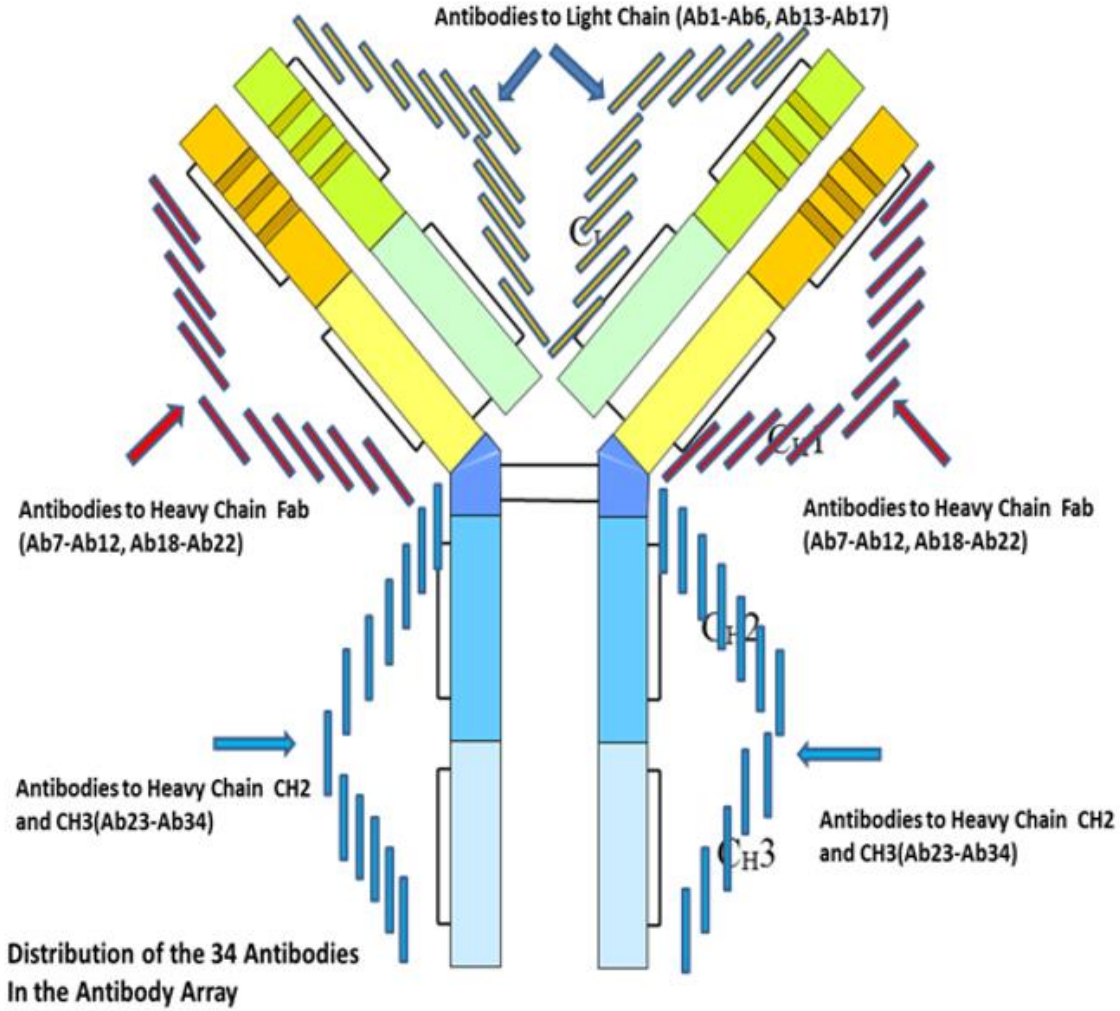
2. 三维构像矩阵技术的开发

PCA 技术涵盖整个单克隆抗体

- **Polyclonal Antibodies (Pab) are raised against 30 amino acid peptides from the amino acid sequence of target therapeutic Mab.**



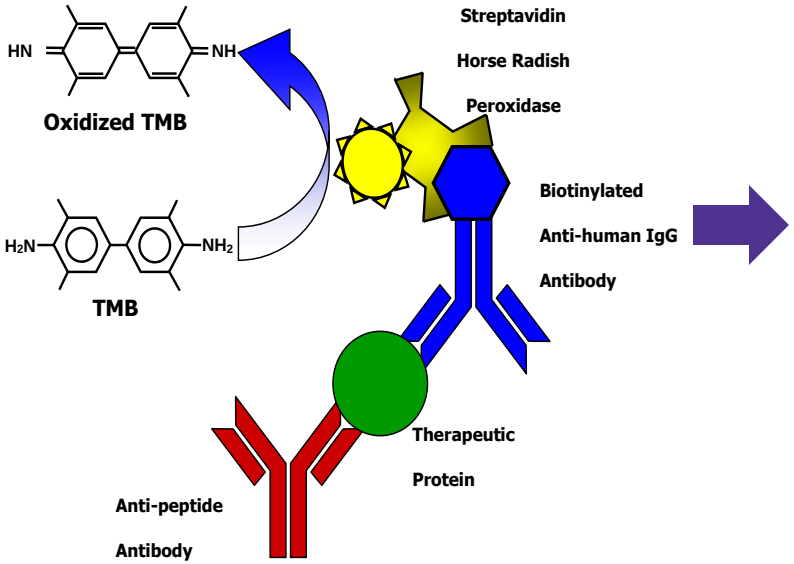
- **Selected Pab's are used to create an array against the structure of the Mab. With a reference molecule providing a figerprint of the properly folded Mab.**

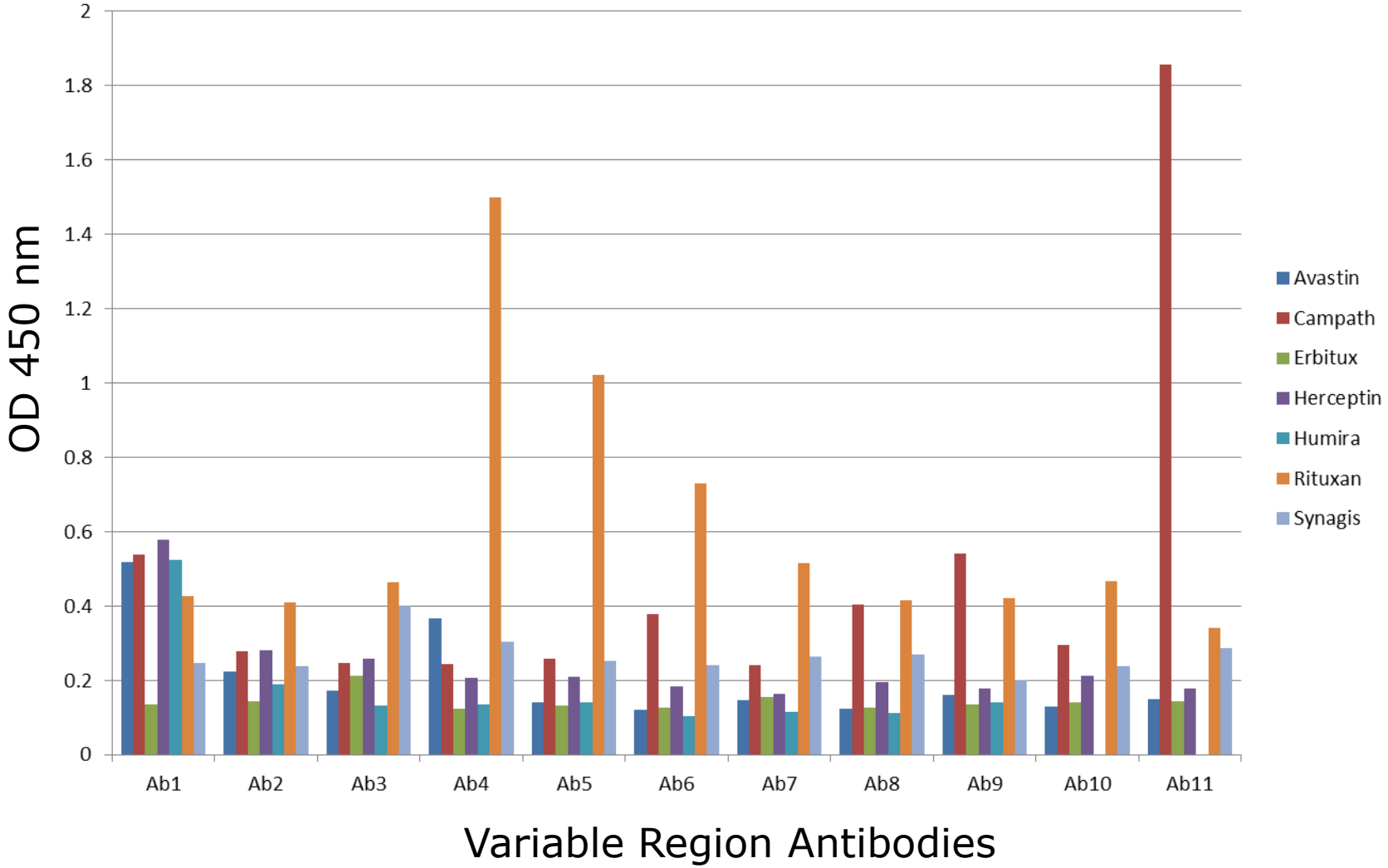


Distribution of the 34 pAb: pAb 1-12 (variable region); pAb 13-34 (constant region)

PCA酶联免疫吸附法

The initial product offering consist of Sandwich ELISA based arrays, allowing the generation of unique therapeutic Mab signatures.



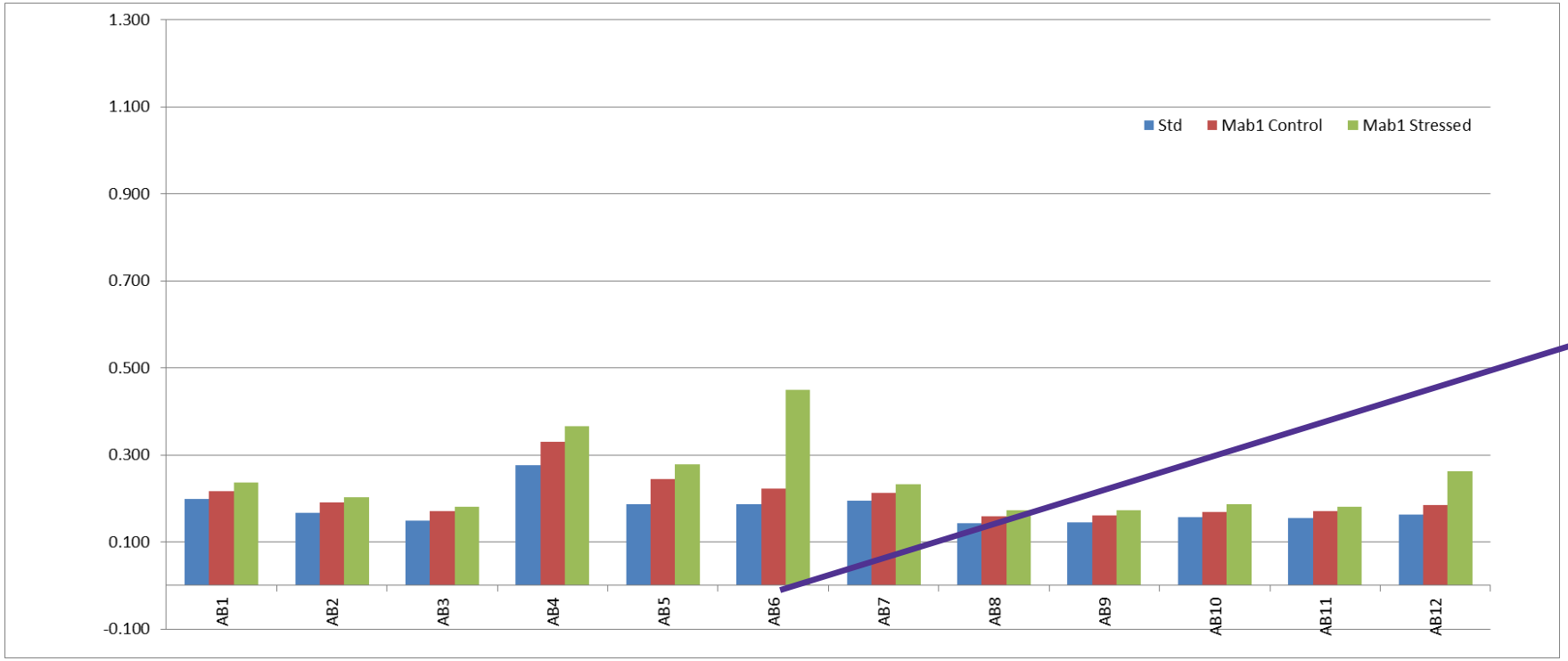


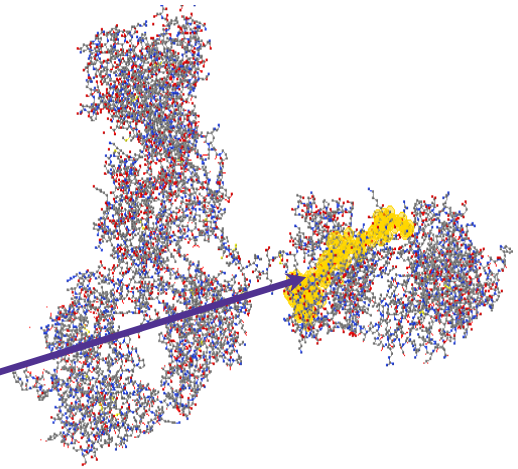
蛋白质三维构像抗体矩阵分析技术（PCA）是一种多面体分析技术 可以全面精确的测定由任何物理化学因素导致的构像变化

Testing Condition	PCA ELISA Detection	Sensitivity	Resolution
Temperature Stress	Yes	0.1% (5 ng impurity in 5 µg testing sample)	Epitope-based, 3-6 Amino Acids
Low pH	Yes	High	Epitopes
High pH	Yes	High	Epitopes
Oxidation	Yes	High	Epitopes
Glycosylation	Yes	High	Epitopes
Aggregation	Yes	High	Epitopes
Bioassay Difference	Yes	High	Epitopes
Light Stress	Yes	High	Epitopes

案例研究：构象与生物活性之间的相关性测试（新型单克隆抗体）

The most significant difference in the variable region was seen at Ab6 suggesting a correlation between this site and the decrease in bioactivity (the more unfolding the higher the signal)

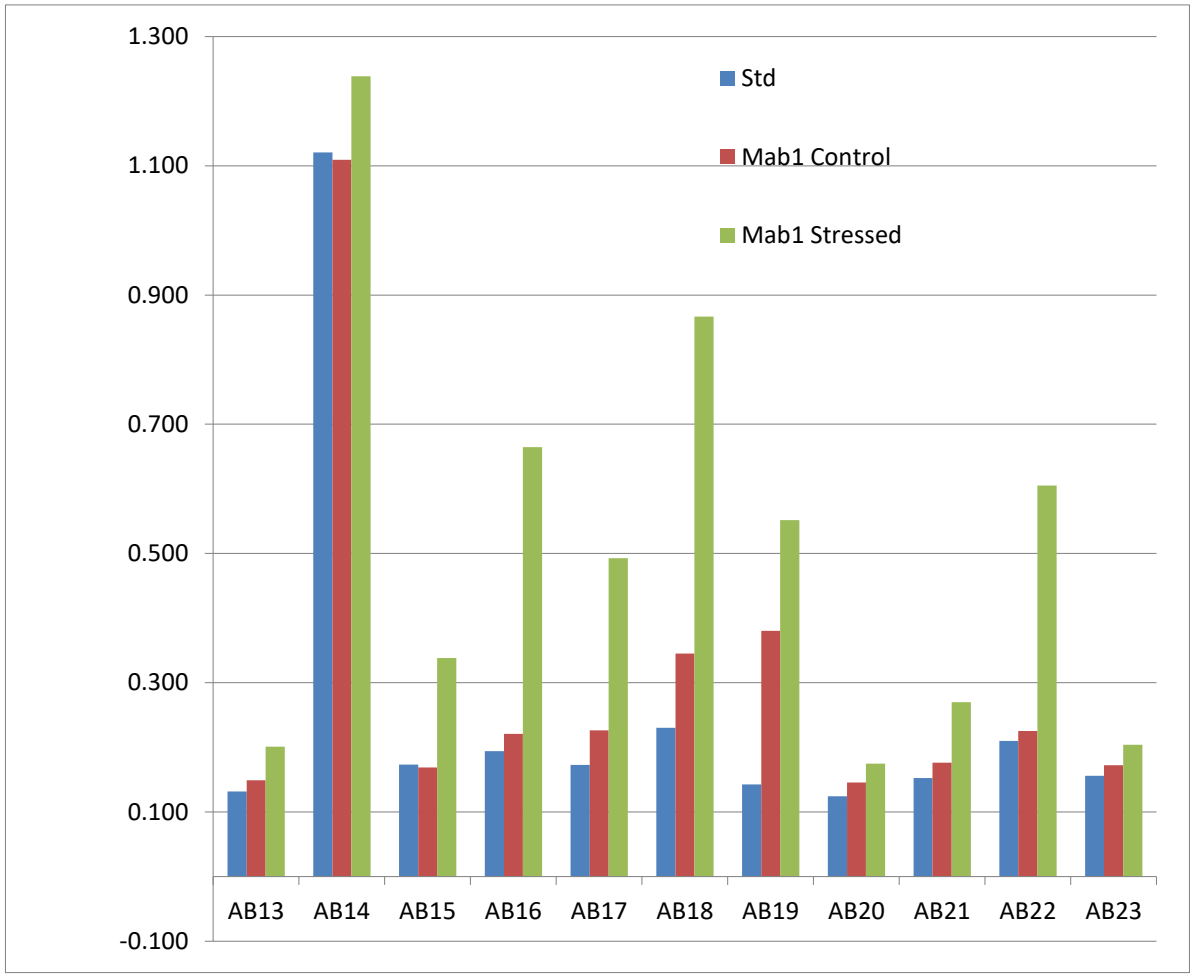


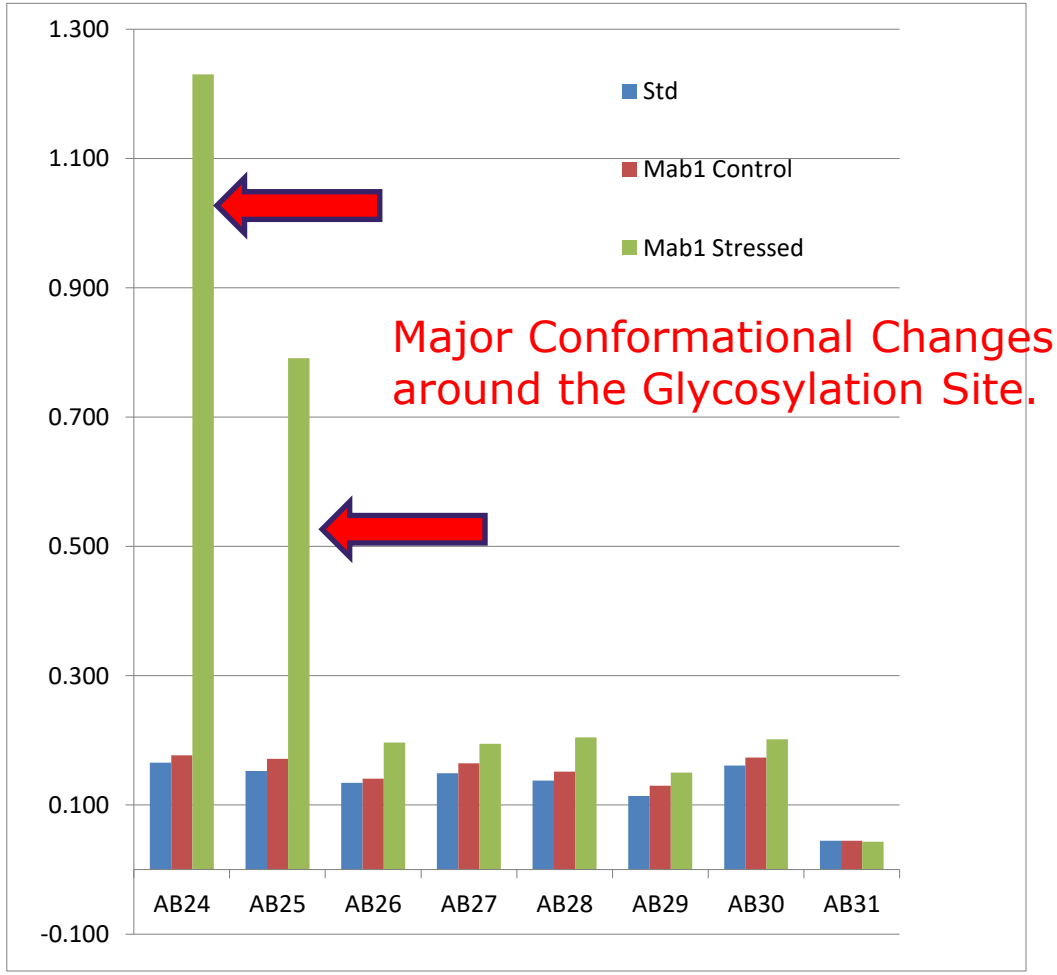


Ab6 is close to light chain CDR3 22% Bioassay Activity Decrease

案例研究：（新型单克隆抗体）中构象与生物活性之间的相关性

FcγRIIIa binding result: 64% Decrease

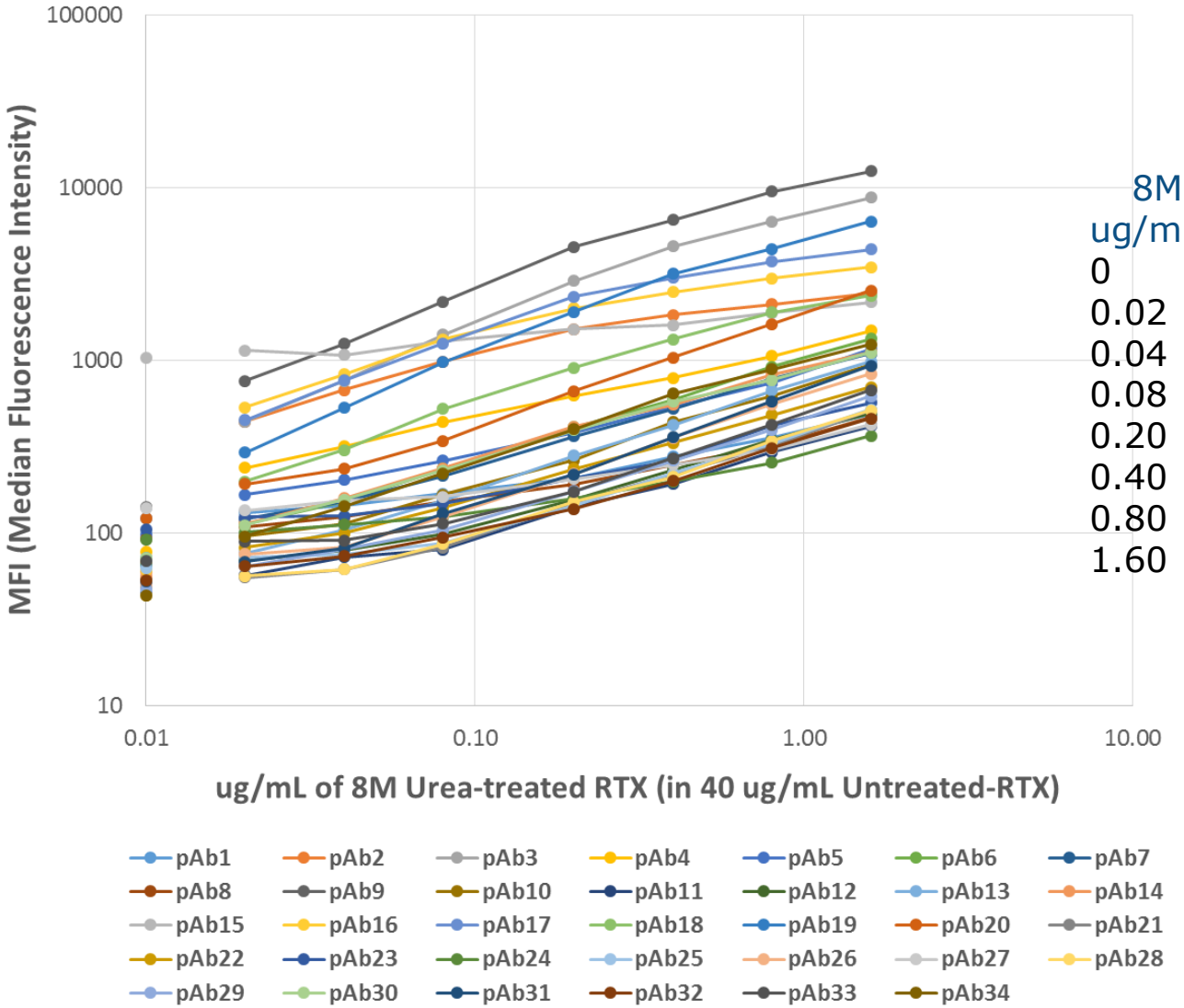




Ab15,16: LC Hinge Region; Ab17,18:HC, Fv-Fc domain
Ab24: HC Hinge Region.; Ab25: HC Glycosylation Site.

灵敏度：使用 8M 尿素处理的单克隆抗体进行灵敏度测定

As low as 0.05% epitope exposure can be detected and quantified



8M Urea-RTX	
ug/mL	% Spiked
0	0
0.02	0.05%
0.04	0.10%
0.08	0.20%
0.20	0.50%
0.40	1%
0.80	2%
1.60	4%

目前可用的生物仿制药构像检测方法，共17个试剂盒。

InnoBridge 试剂盒用于新型单克隆抗体的构像测定。

mAb Name	Trade Name	Composition	IgG Class	Sales ($ billions)
Bevacizumab	Avastin	Humanized mAb	IgG1	7.0
Cetuximab	Erbitux	Humanized mAb	IgG1	2.3
Alemtuzumab	Campath	Humanized mAb	IgG1	0.7
Rituximab	Rituxan	Chimeric mAb	IgG1	8.6
Adalimumab	Humira	Human mAb	IgG1	16.1
Trastuzumab	Herceptin	Humanized mAb	IgG1	6.9
Palivizumab	Synagis	Humanized mAb	IgG1	0.5
Infliximab	Remicade	Chimeric mAb	IgG1	10.2
Etanercept	Enbrel	Fc Fusion Protein	IgG Fusion	9.1
Erythropoietin	EPO	Human protein	Non-mAb	3.4
Pegfilgrastim	Neulasta	Human protein	Non-mAb	4.6
Denosumab	Prolia	Human mAb	IgG2	3.0
Ranibizumab	Lucentis	Humanized mAb	IgG1 Fab	4.3
Golimumab	Simponi	Human mAb	IgG1	2.9
Ustekinumab	Stelara	Human mAb	IgG1	2.5
Aflibercept	Eylea	VEGFR-1-Fc Fusion Protein	IgG Fusion	5.9
Somatropin	Genotropin etc.	Human Growth Hormone	Non-mAb	5.2

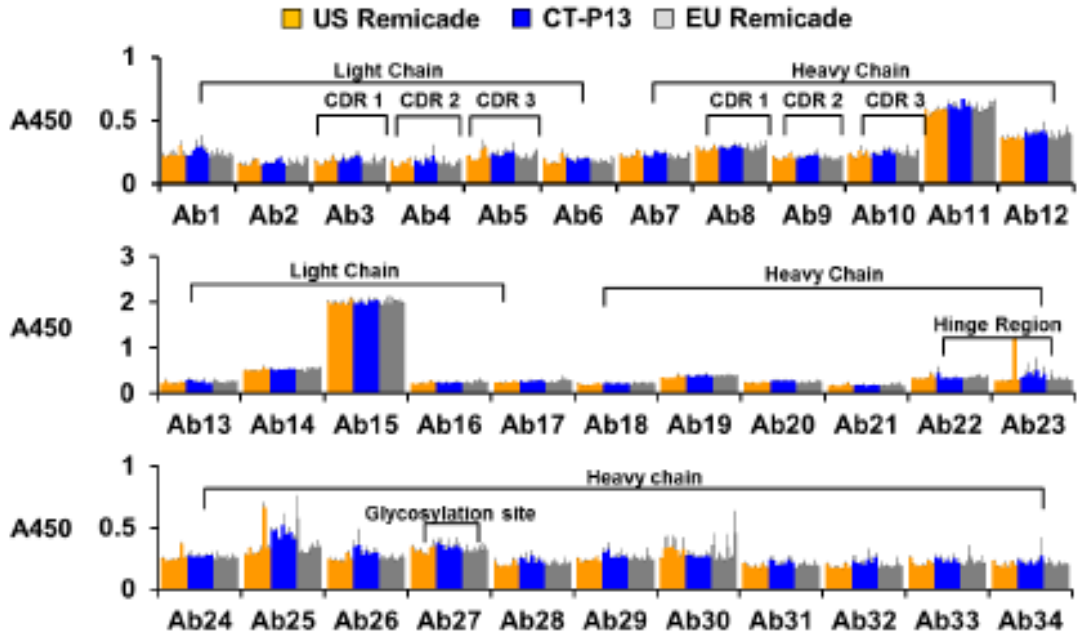
CT-P13 英夫利昔单抗生物仿制药：FDA 提交中使用的 PCA 数据。

8.4 IMMUNOGENICITY RESULTS

8.4.1 Antibody Array Study

Antibody array technology or Protein Conformational Array (PCA) is a technique for comparing structural differences among similar molecules such as monoclonal Antibodies (mAb). The ELISA consists of a pool of 34 pAbs, each raised against a short segment of the linear mAb peptide sequence. Together, this overlapping series of peptides covers the entire peptide sequence of the mAb and were the mAb to exist in a linear or denatured state, each of the 34 pAbs would give a strong signal in the ELISA. With a correctly folded mAb, most of the epitopes are buried and are not strongly recognized by the pAbs. Difference in intensity of the responses for each pAb reflects the exposure of the epitope Ab detects. This technique showed CT-P13, EU Remicade, and US Remicade were consistent with regard to epitope exposure and higher order structure (Figure 54). One batch of US Remicade (CJM76016P1) showed deviations at some epitopes around the hinge region and in the overlapping region of 254-275 aa and 272-293 aa of the HC, suggesting slight unfolding in this region.

ure 54: Antibody Array Data Showing Epitopes Exposed by 7 Lots each of US Remicade, CT-P13, and EU Remicade using 34 Polyclonal Sera



CDR: Complementarity determining region

- Notes differences between US & EU Remicade production and CT-P13.

HOS similarity between CT-P13 (Biosimilar) and its originator manufactured in the EU & USA.

***CT-P13 (infliximab biosimilar) BRIEFING DOCUMENT FOR THE ARTHRITIS ADVISORY COMMITTEE, MEETING DATE: February 9, 2016.**

FDA Advisory Committee Briefing Document, February 9, 2016, Page **1** of **349 (pg179)**

施贵宝公司用PCA 试剂盒发表的文章

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

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REPORT

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

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Bristol-Myers Squibb

ABSTRACT

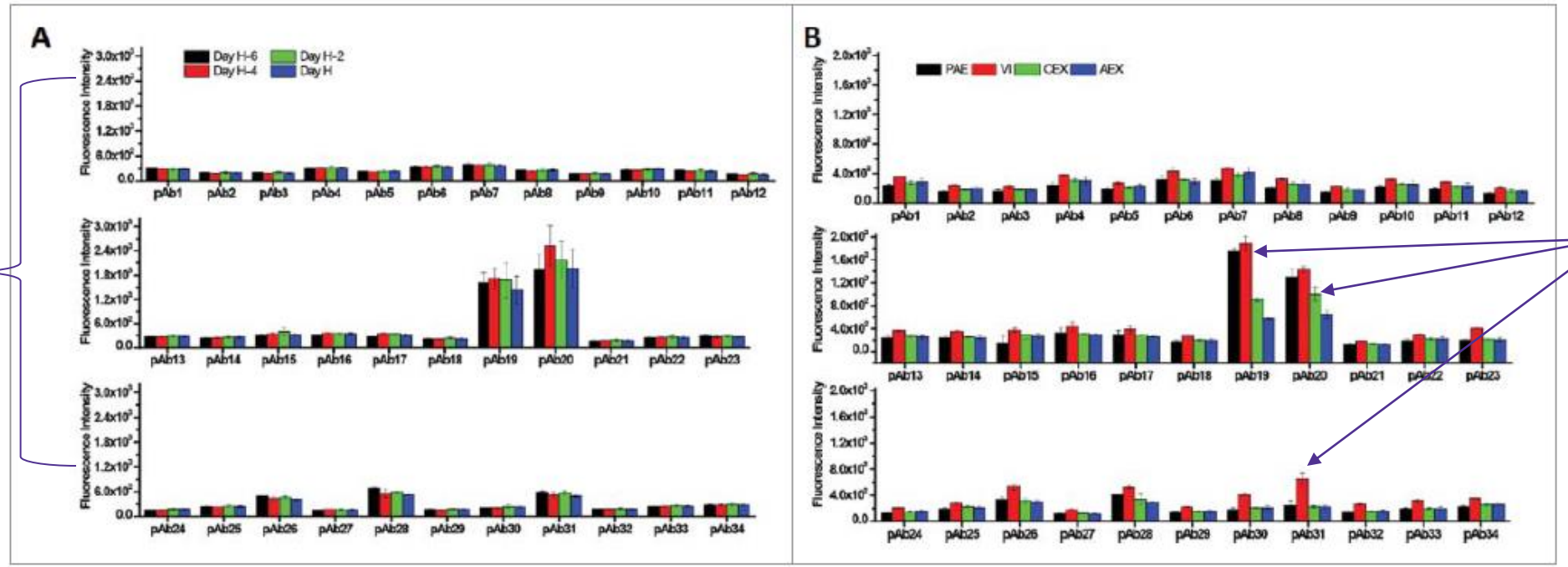
The elucidation of antibody higher order structure (HOS) is critical in therapeutic antibody development. Since HOS determines the protein bioactivity and chemo-physical properties, this knowledge can help to ensure that the safety and efficacy attributes are not compromised. Protein conformational array (PCA) is a novel method for determining the HOS of monoclonal antibodies. Previously, we successfully utilized an enzymelinked immunosorbent assay (ELISA)-based PCA along with other bioanalytical tools to elucidate the structures of antibody aggregates. In this study, applying **a new multiplex-based PCA with 48-fold higher throughput** than the ELISA-based one **we revealed structural differences between different antibody molecules and antibody structure changes affected by various processing conditions**. The PCA analysis of antibody molecules clearly demonstrated significant differences between IgG1 and IgG4 subclasses in epitope exposure and folding status. Furthermore**, we applied small angle X-ray scattering to decipher mechanistic insights of PCA technology and validate structural information obtained using PCA**. These findings enhance our fundamental understanding of mAbs' HOS in general. **The PCA analysis of antibody samples from various processing conditions also revealed that antibody aggregation caused significantly higher exposure of antibody epitopes, which potentially led to a "foreign" molecule that could cause immunogenicity**. **The PCA data correlated well with protein stability results from traditional methods such as size-exclusion chromatography and protein thermal shift assay**. Our study demonstrated that high throughput PCA is a suitable method for HOS analysis in the discovery and development of therapeutic antibodies.

*Monoclonal antibody higher order structure analysis by high throughput protein conformational array. Y.Song *et al*, MABS 2018 Jan 9, 1-9.

MABS 杂志中突出显示PCA 在生物药工艺开发中的应用

Cell culture

Seems stable



Process driven changes

Figure 14: (Fig 5)(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"

3. 结构与功能（免疫原性）研究

证明3-D结构对免疫原性和蛋白稳定性重要性的研究

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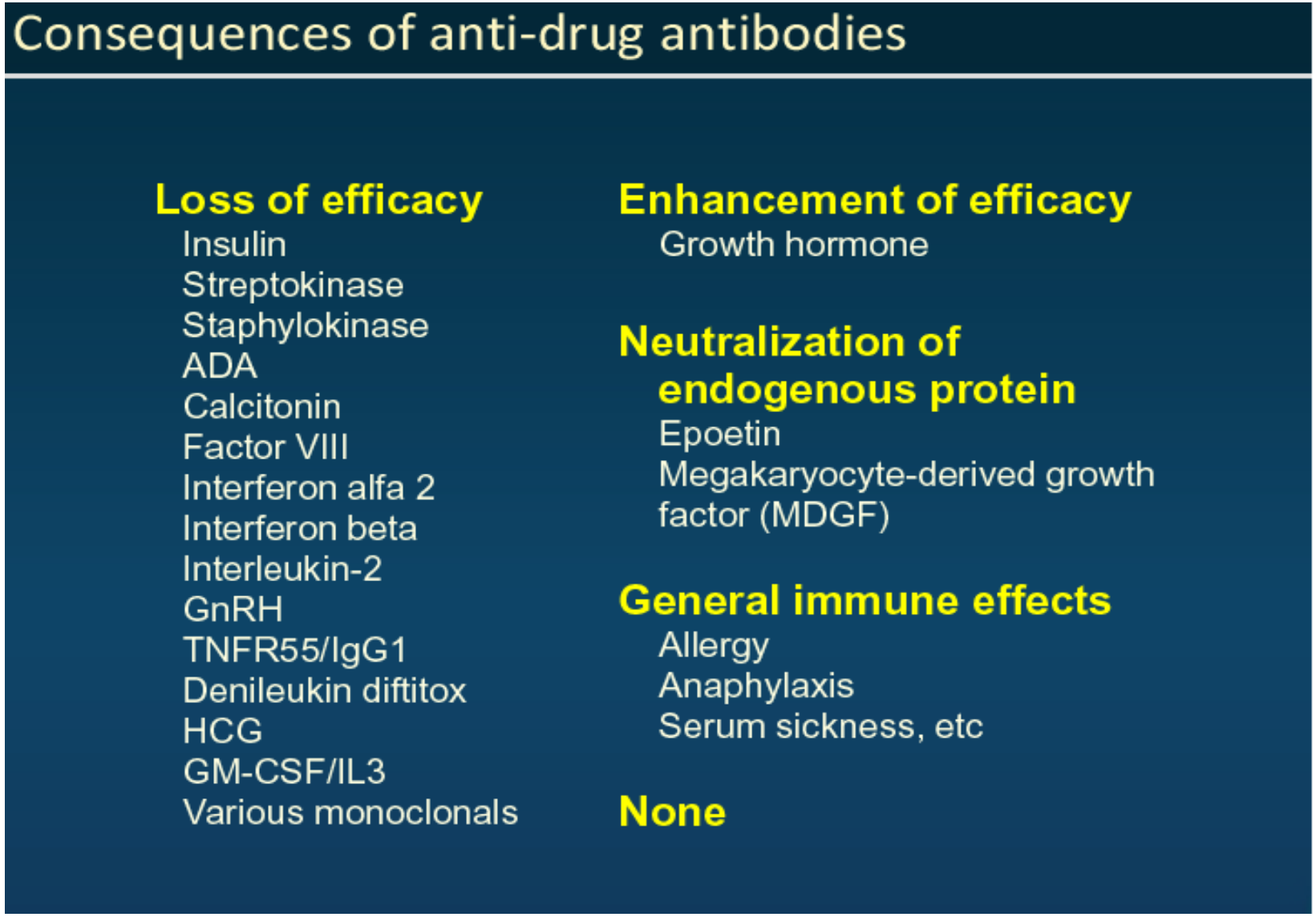
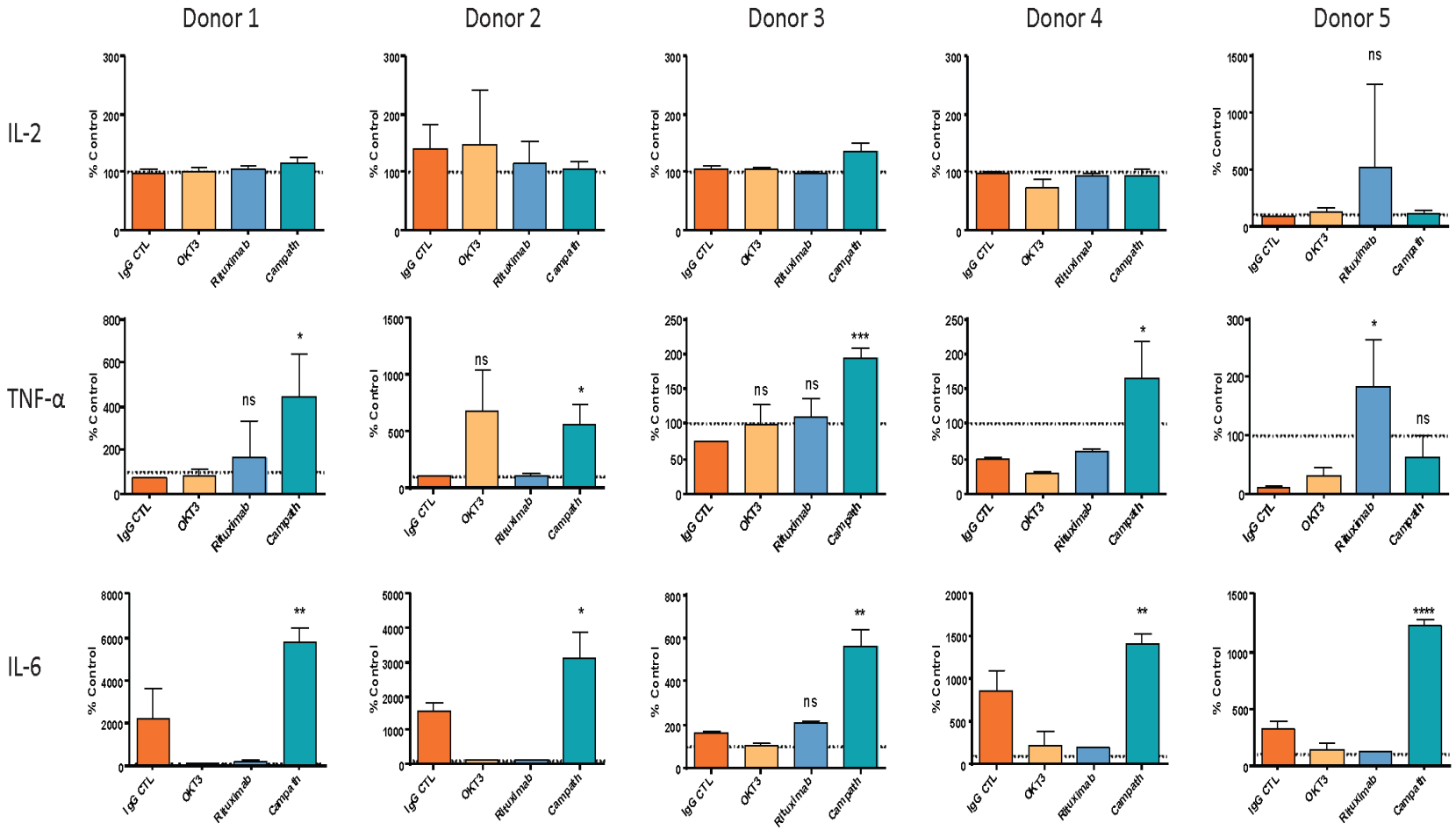
因为免疫原性产生的抗生物药抗体带来的后果



From Wim Jiskoot, NBC, Seattle, 2009

Okt3、利妥昔单抗和坎帕斯的细胞因子释放测试
使用人全血测定（来自Singulex）



CamCampath诱导TNF-α（4/5）和IL-6（5/5）释放;
利妥昔单抗诱导TNF-α（1/5）释放

细胞因子释放实验设计：

1. 3 antibodies used: IgGs from human plasma; Herceptin, Rituxan.

2. 6 different conditions tested: no treatment; 8 M urea-induced unfolding; Heat treatment; Oxidation; Higher pH (pH 9.5); Lower pH (pH3).

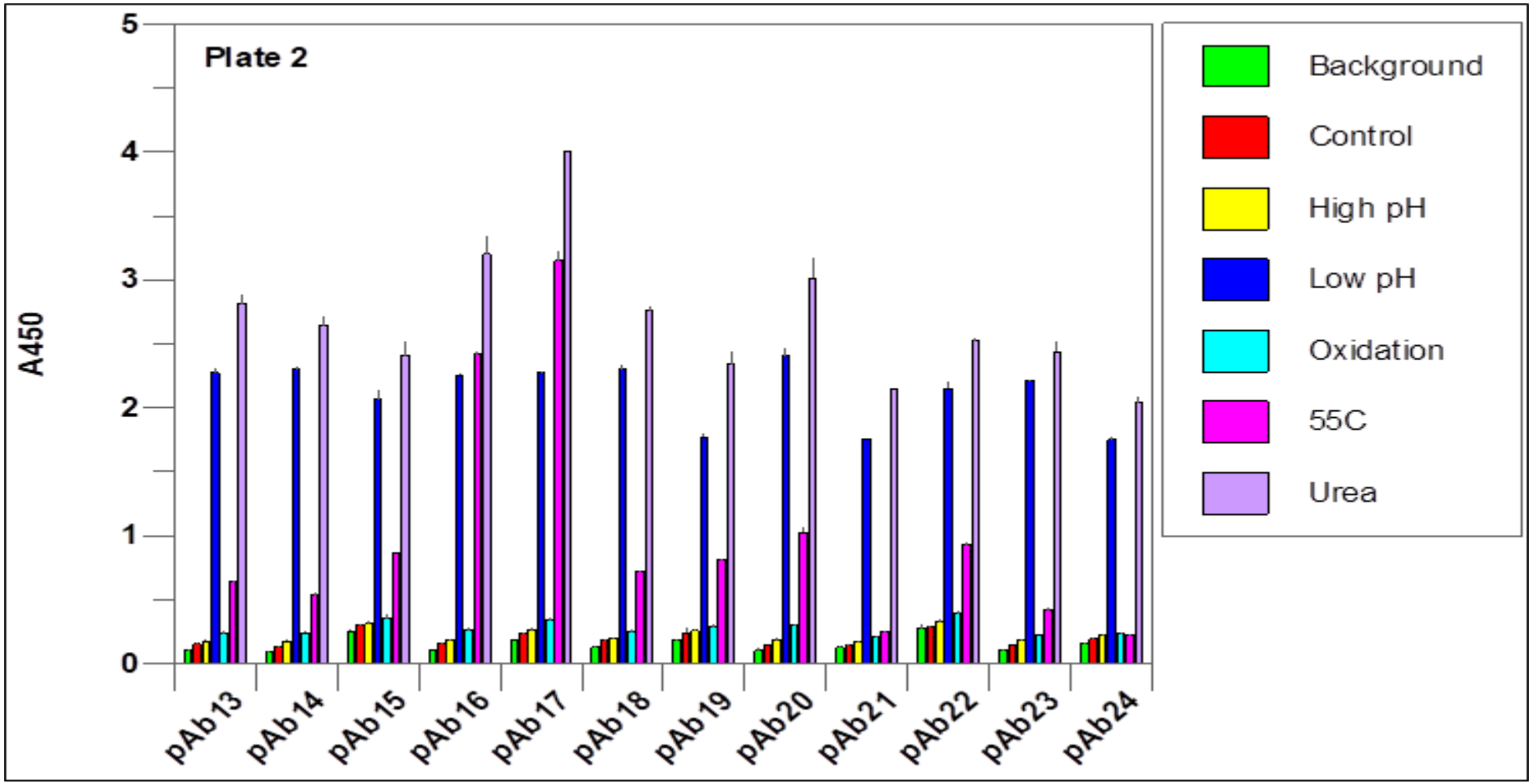
3. Three antibody levels tested: 100 μg/mL; 10 μg/mL; 1 μg/mL (mimic the levels in the human blood during actual treatment).

4. Positive and negative controls: PBS will be used as the negative control to determine assay background and base-line cytokine levels, LPS stimulation will be used as a positive control for measuring cytokine release. Urea (screen at 3 different concentrations) will be tested as the vehicle control for urea-induced unfolding.

5. Plasma samples from the whole blood incubation are analyzed with HSTCMAG384-PX21 High Sensitivity Human Cytokine Assay kit from MilliporeSigma (measuring 21 cytokines simultaneously) on a Luminex FlexMap3D Analyzer.

6. Human Whole Blood Cytokine Release Test (n=5) (Preliminary analysis of 11 selective cytokines from the 21 cytokines measured)

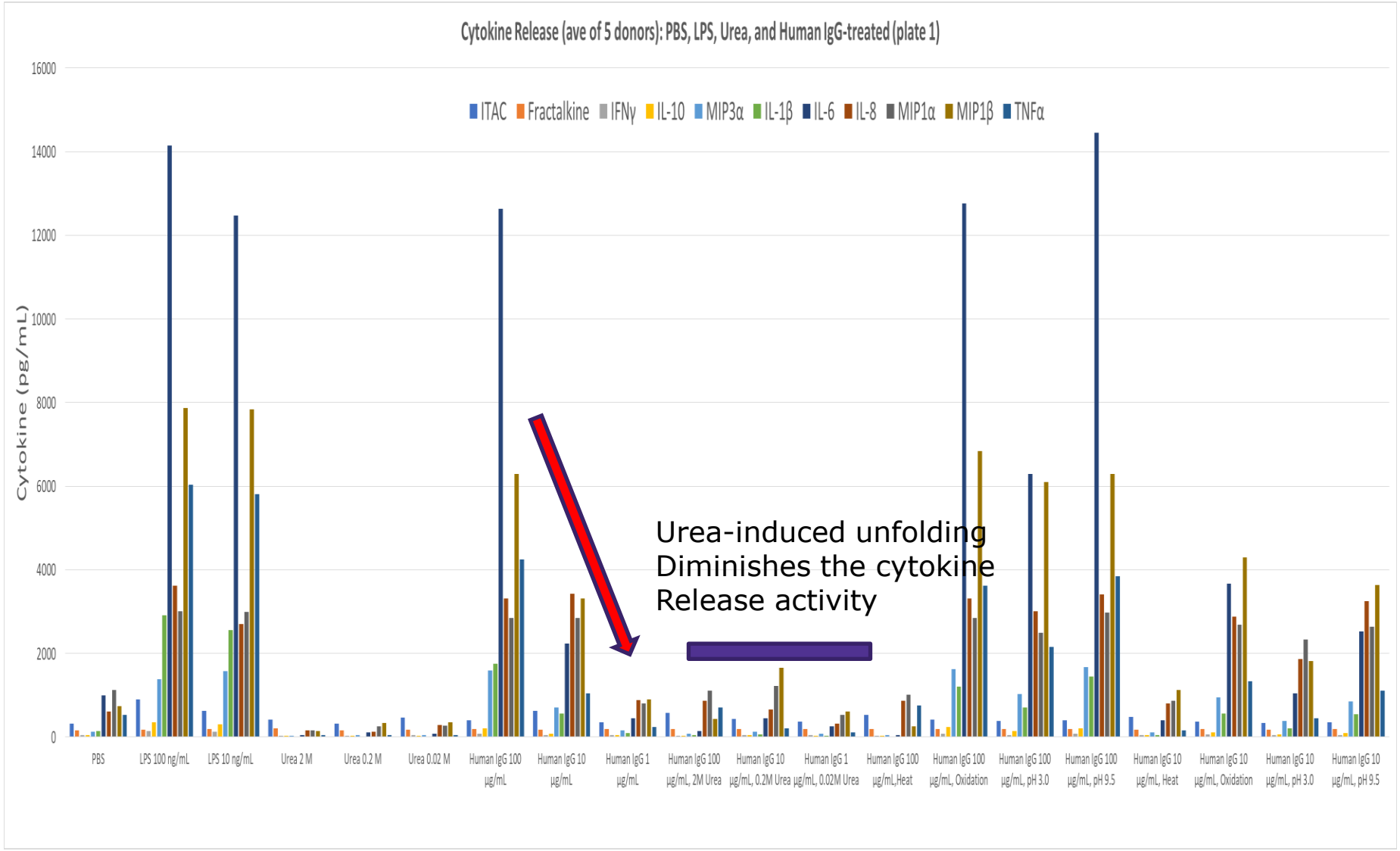
血清来源的人IgG的三维构像(HOS)在不同的物理化学条件下的变化



The HOS of Human serum-derived IgGs are stable in high pH (pH9.5) and oxidation, Changes at different region with low pH (pH 3.0) and heat treatment (55°C, O/N).

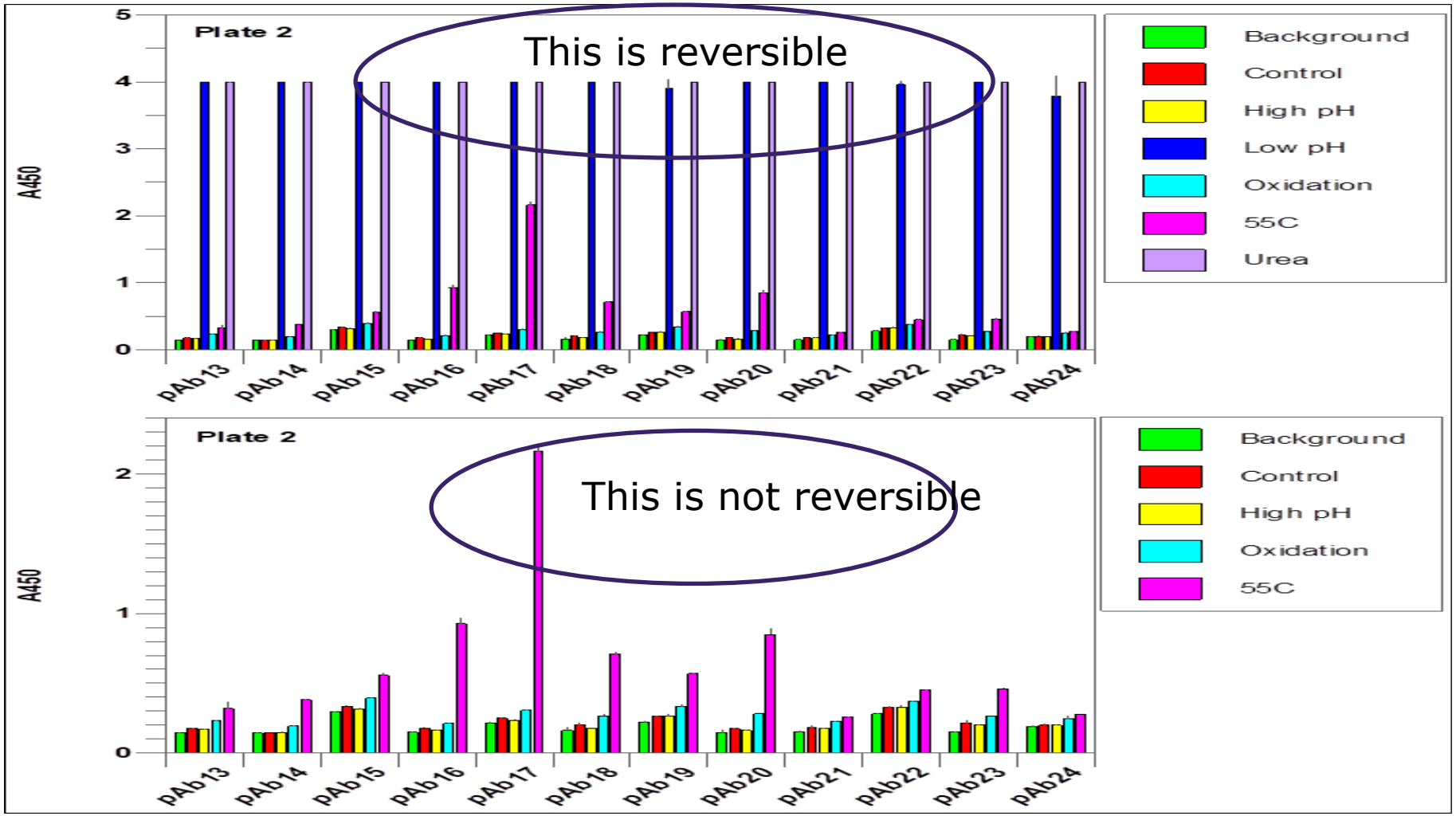
There is general unfolding in 8 M urea but refolding is very fast.

人血清中纯化的IgG诱导释放人全血中的细胞因子



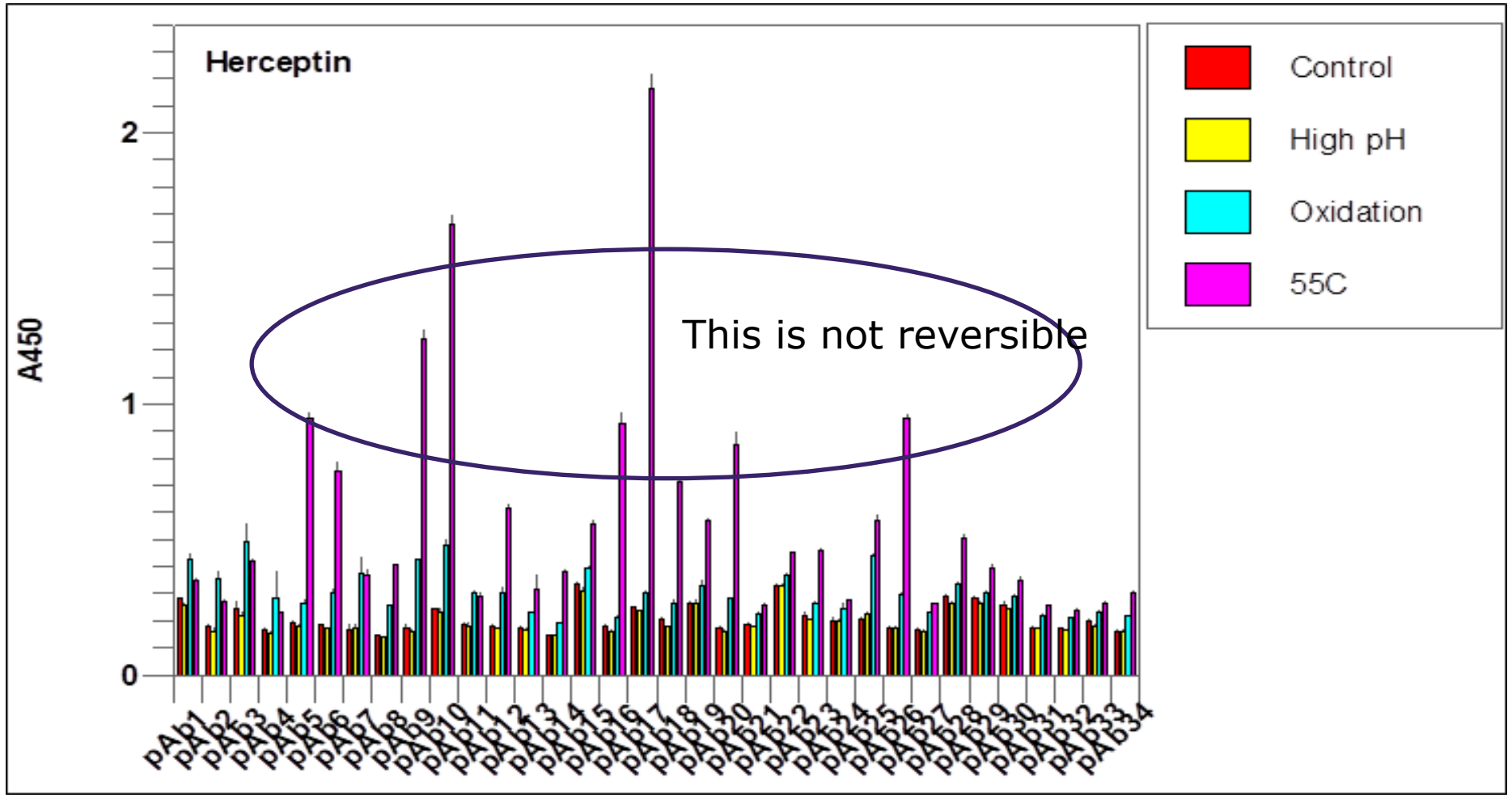
Only 11 of the 21 cytokines measured was shown here.

不同应激条件下赫赛汀的HOS变化



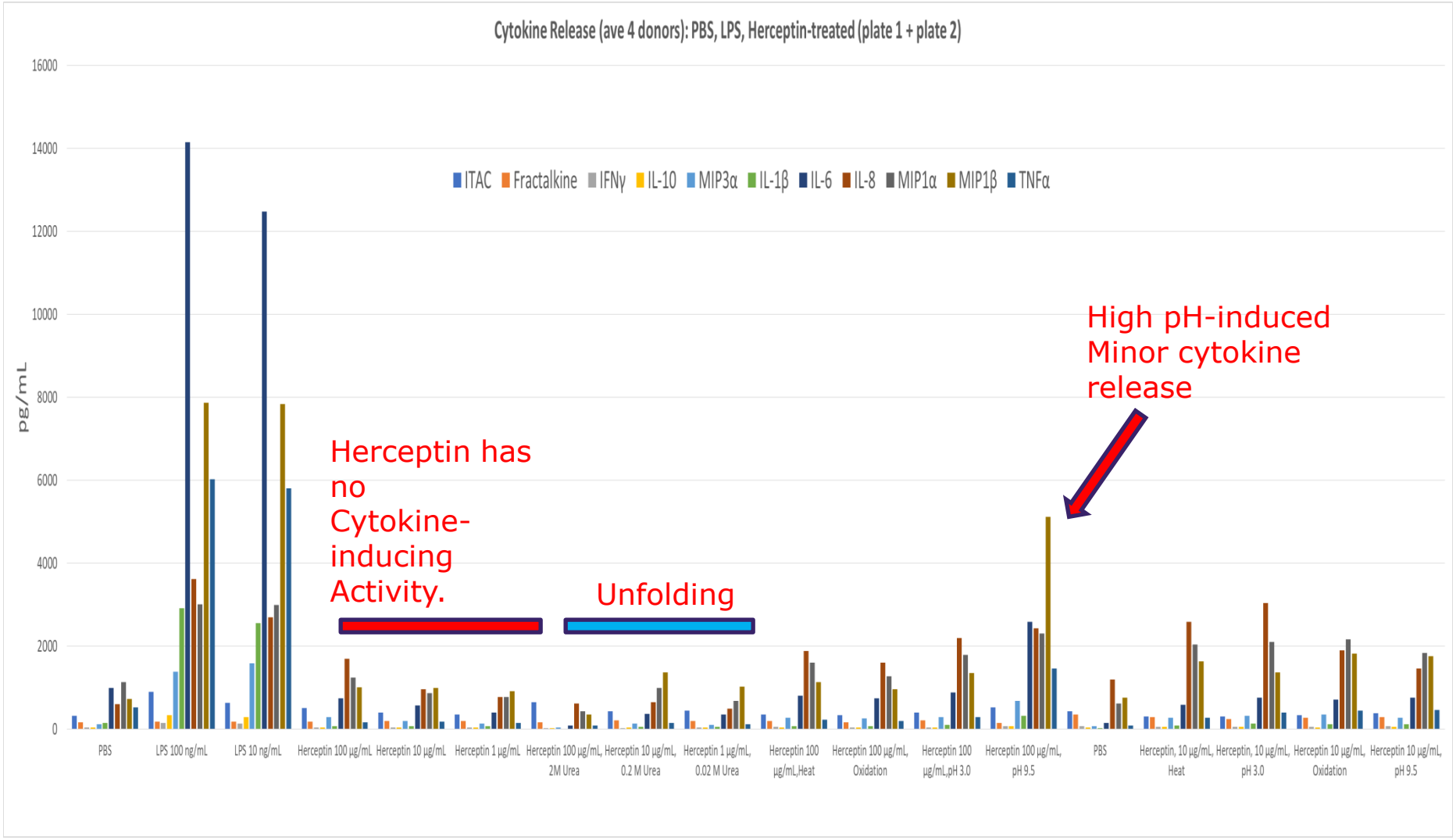
The HOS of Herceptin is stable in high pH (pH9.5). Changes at different region with low pH (pH 3.0), oxidation and heat treatment (55°C, O/N). There is general unfolding in 8 M urea but refolding is fast. Low pH and urea-treated sample has saturated signal but this HOS change is reversible, epitope exposure resumes normal when pH is back to 7.4 .

不同应激条件下赫赛汀的HOS变化



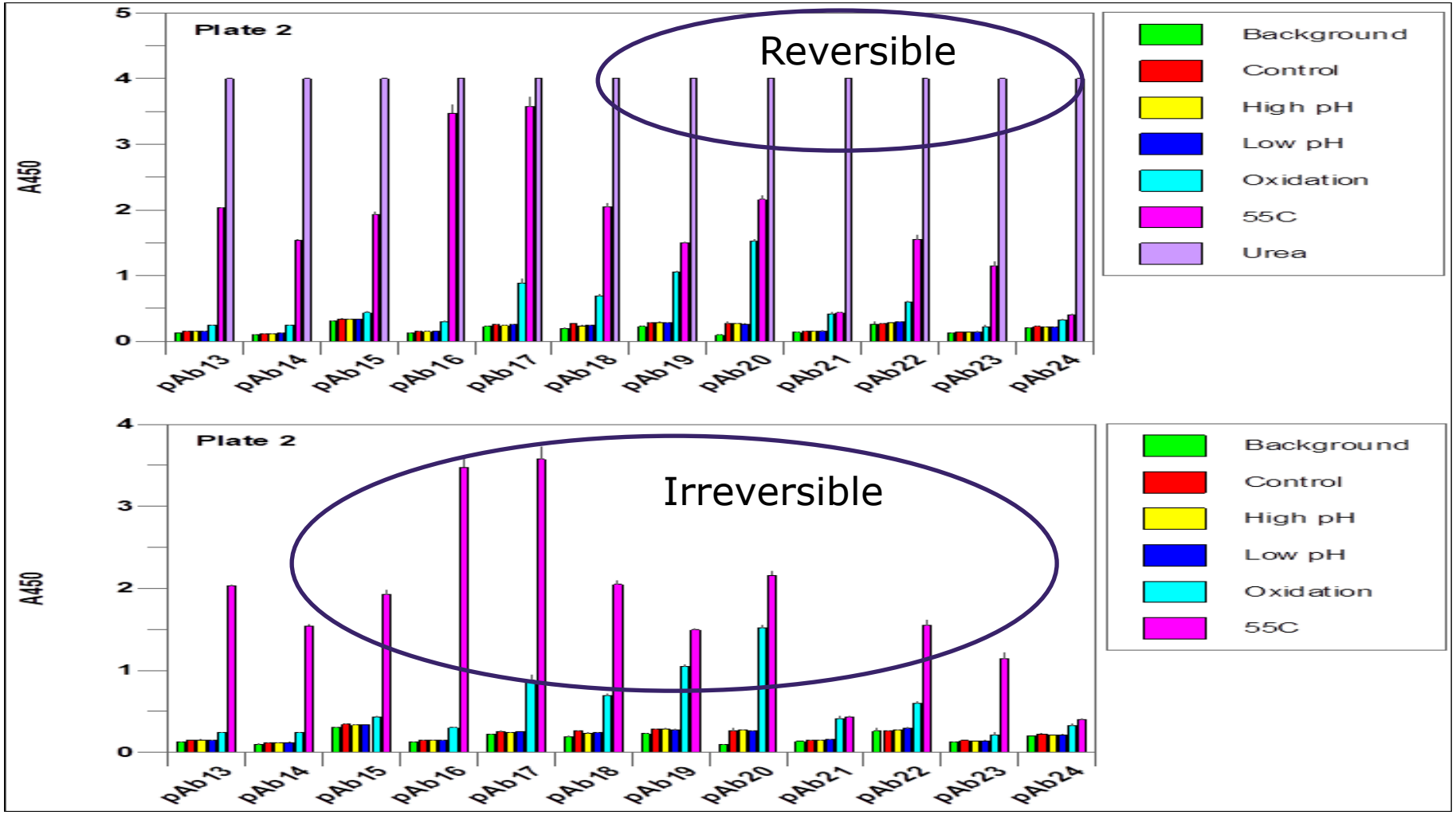
The HOS of Herceptin is stable in high pH (pH9.5). Changes at different region with low pH (pH 3.0), oxidation and heat treatment (55°C, O/N). There is general unfolding in 8 M urea but refolding is fast. Low pH and urea-treated sample has saturated signal.

赫赛汀在不同的压力条件下诱导释放人全血的细胞因子



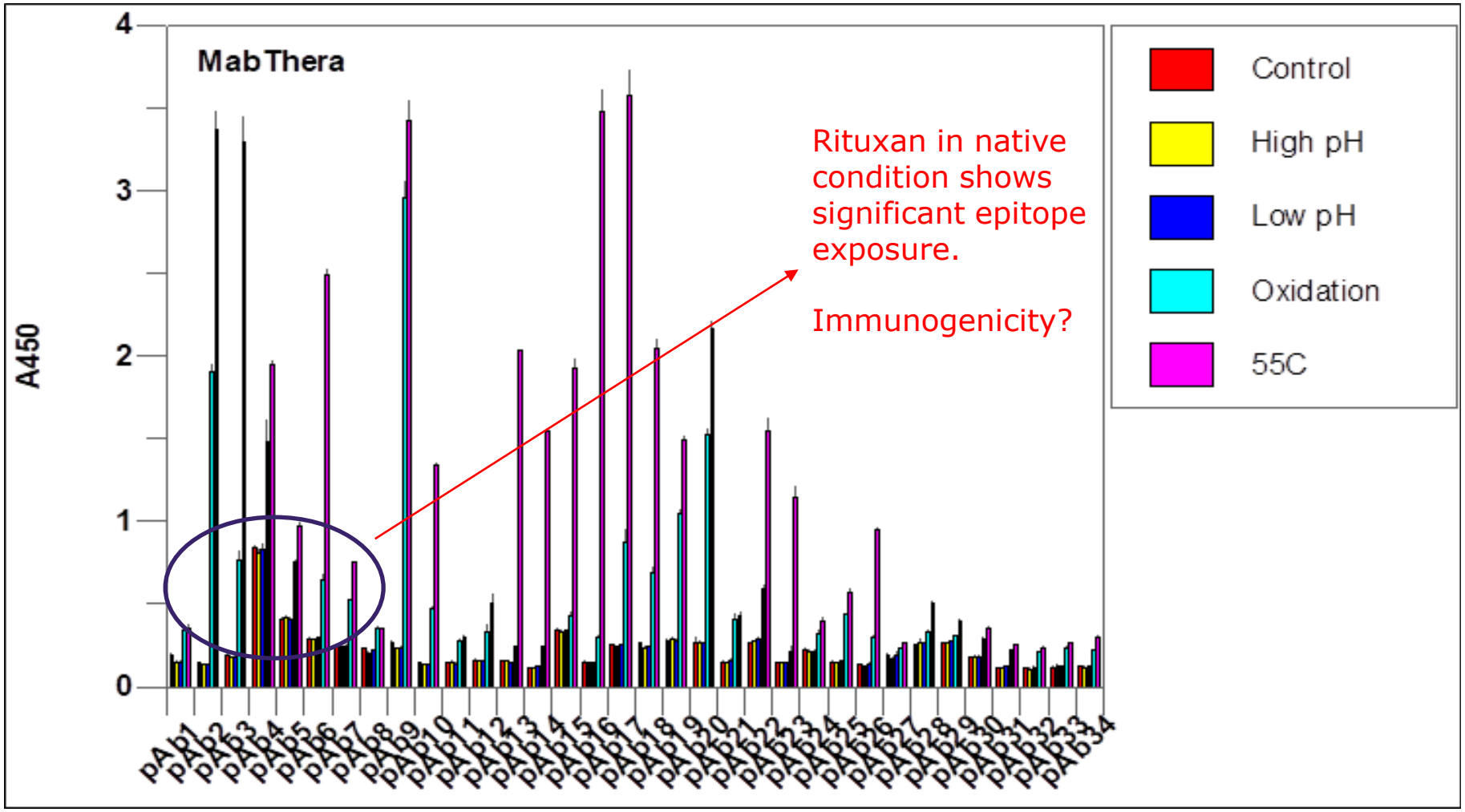
Only 11 of the 23 cytokines measured was shown here.

不同压力条件下利妥昔的HOS变化



The HOS Changes at different region with high and low pH (pH 9.5 and 3.0 respectively), oxidation and heat treatment (55°C, O/N). There is general unfolding in 8 M urea and refolding is slow.

不同压力条件下利妥昔的HOS变化



The HOS Changes at different region with high and low pH (pH 9.5 and 3.0 respectively), oxidation and heat treatment (55°C, O/N). There is general unfolding in 8 M urea and refolding is slow.

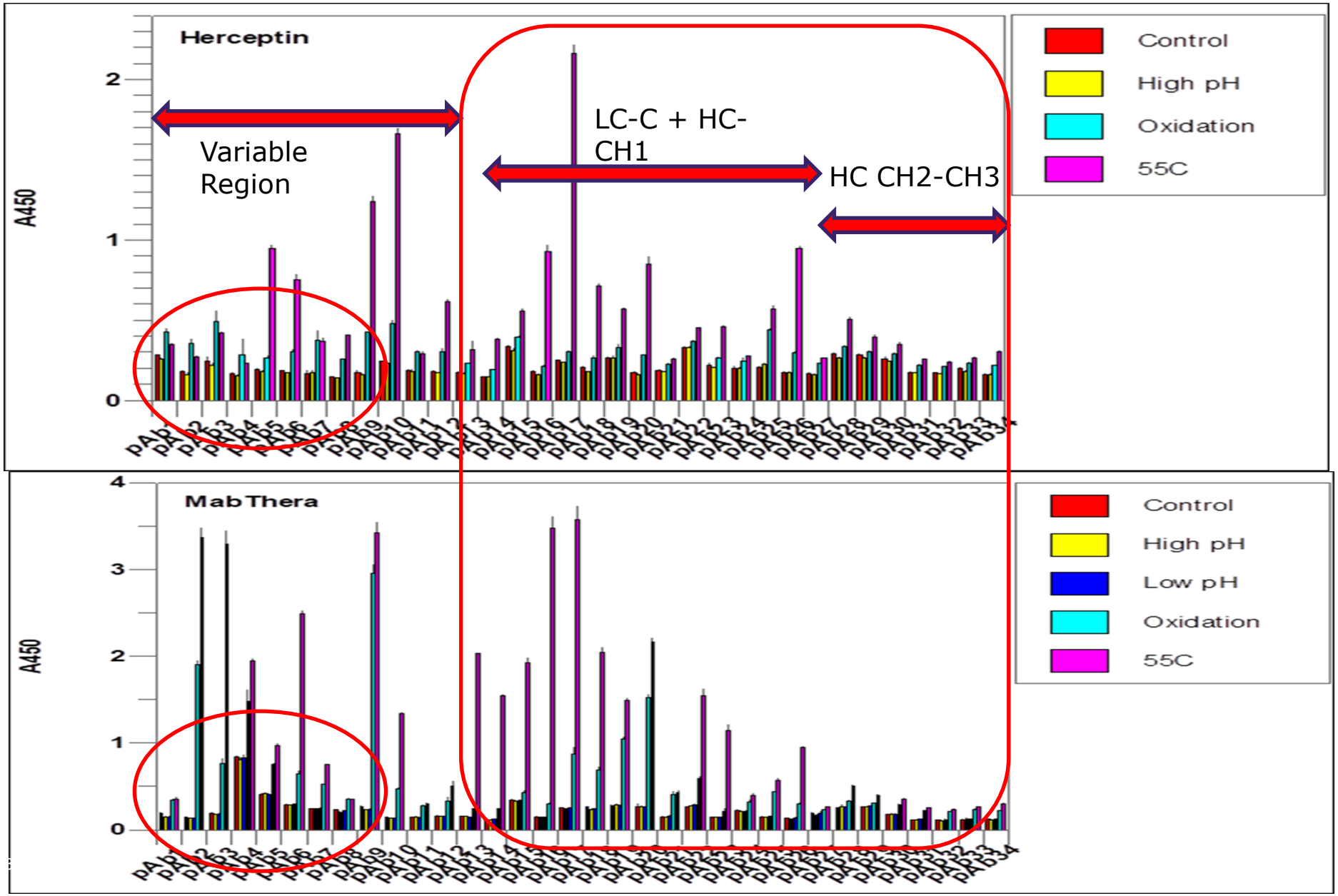
赫赛汀和利妥昔轻链比较，恒定区域是完全相同的。

```
Herceptin_zu DIQMTQSPSSLSASVGDRVTITCRASQDVNT-----AVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQHYTTPPTFGQGTKVEIKRTVAAPSV
Rituxan___xi QIVLSQSPAILSASPGEKVTMTCRASSSVSY------MHWYQQKPGSSPKPWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWSFNPPTFGAGTKLELKRTVAAPSV
                          20*                 40*                 60*                 80*                 100*                120*

Herceptin_zu FIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC 214
Rituxan___xi FIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR--- 210
                          140*                160*                180*                200*
```

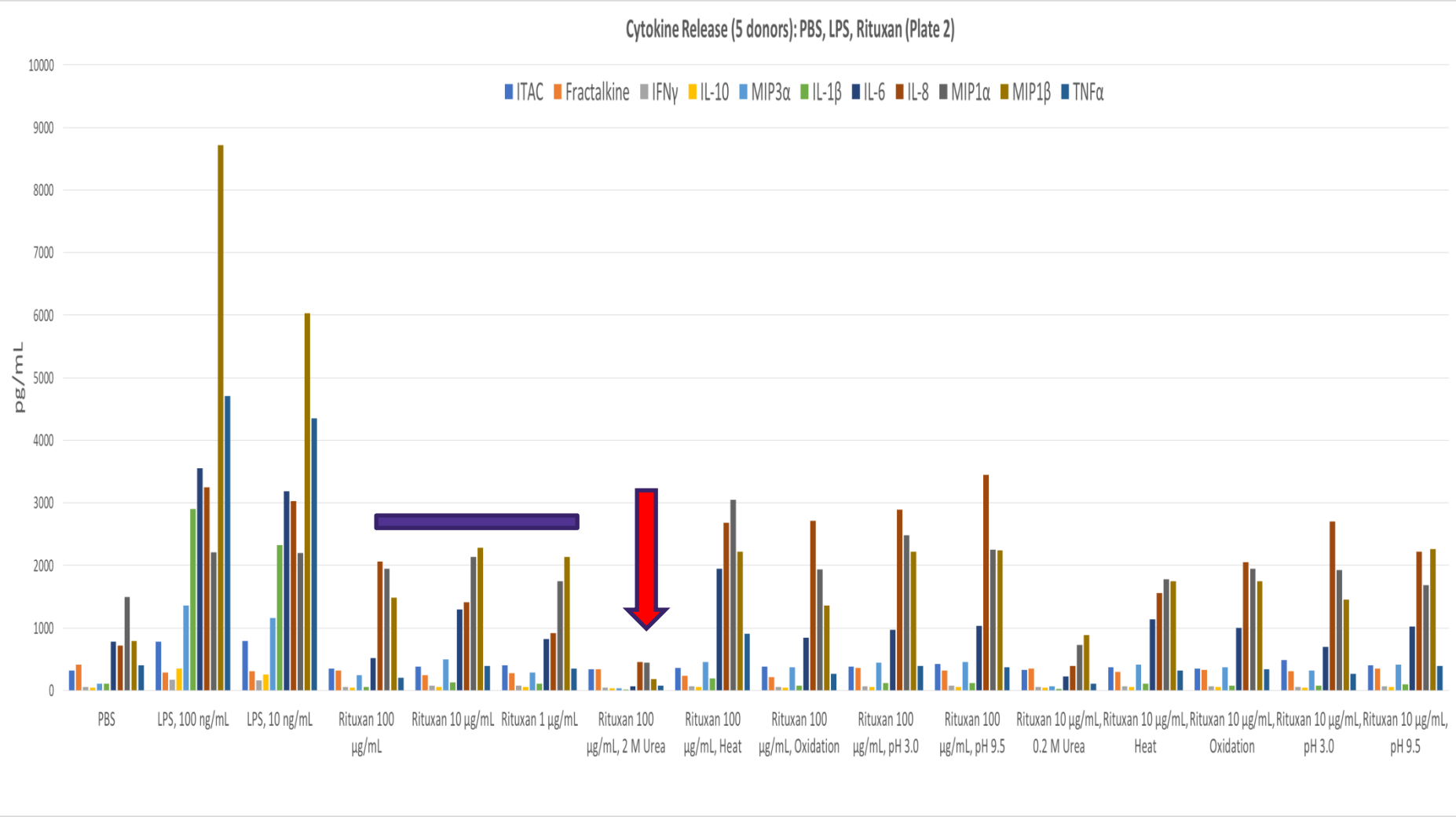
赫赛汀和利妥昔重链比较，恒定区域是完全相同的。

```
Herceptin_G1_zu  EVQLVESGGGLVQPGGSLRLSCAASGFNIKD--TYIHWVRQAPGKGLEWVARIYPTNGY--TRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWG-GDGFYA--MDYWGQG
Rituxan___G1_xi  QAYLQQSGAELVRPGASVKMSCKASGYTFTS--YNMHWVKQTPRQGLEWIGAIYPGNGD--TSYNQKFKGKATLTVDKSSSTAYMQLSSLTSEDSAVYFCAR-VVYYSNSYWYFDVWGTG
                                 20*                 40*                 60*                 80*                 100*                120*

Herceptin_G1_zu  TLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPP--KSC--DKTHT
Rituxan___G1_xi  TTVTVS-----GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKAEP---KSC--DKTHT
                          140*                160*                180*                200*                220*                240*

Herceptin_G1_zu  CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE
Rituxan___G1_xi  CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE
                          260*                280*                300*                320*                340*                360*

Herceptin_G1_zu  PQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK 451
Rituxan___G1_xi  PQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK 447
```

赫赛汀和利妥森HOS稳定性的比较：赫赛汀在压力条件下似乎比利妥昔更稳定

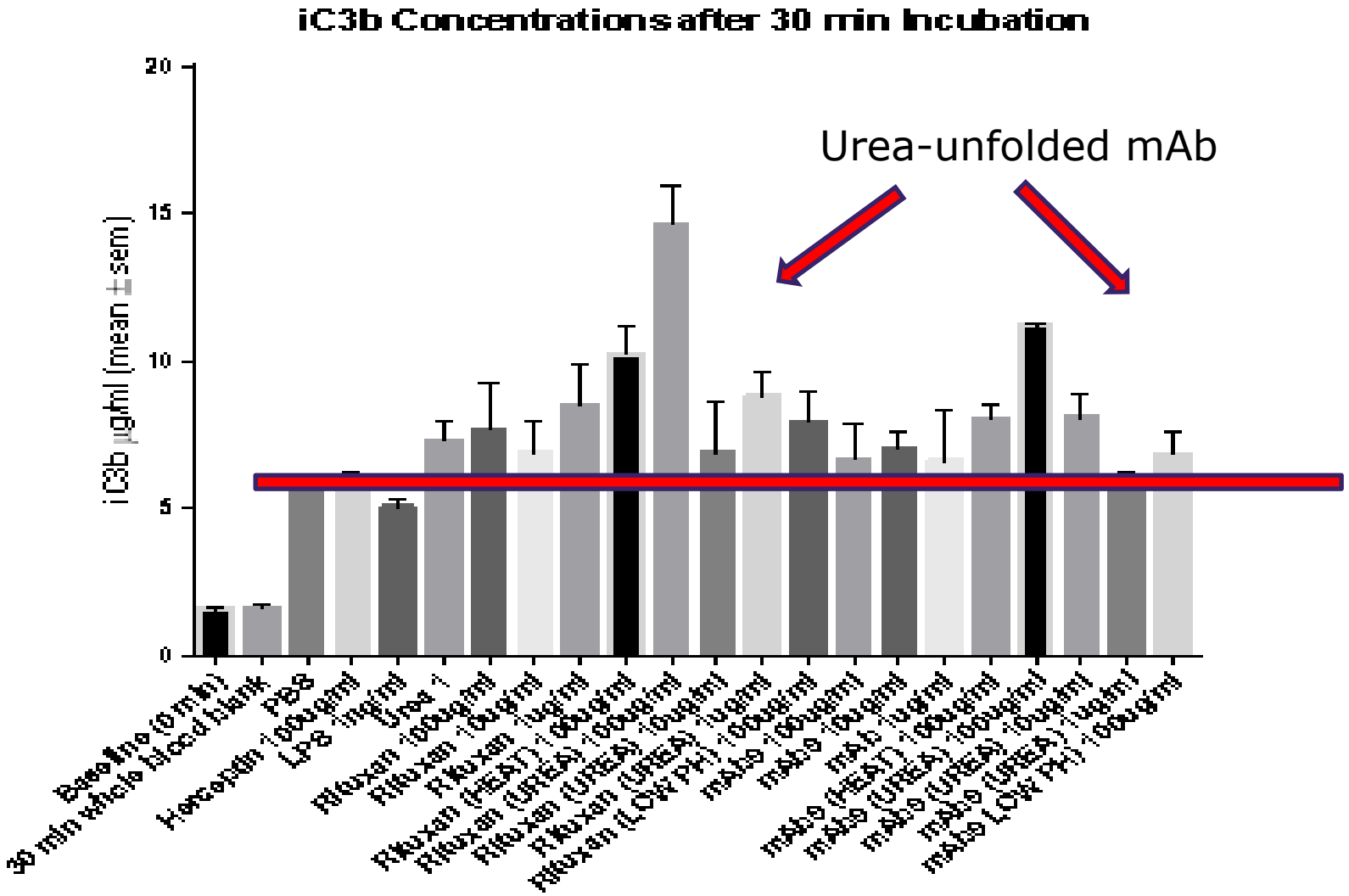


利妥昔在不同的压力条件下对人全血中细胞因子的诱导释放



Only 11 of the 23 cytokines measured was shown here.

补体活性测定--- iC3b （N=3）



免疫原性检测摘要

1. 赫赛汀和PBS在细胞因子释放试验中作为阴性对照效果良好。
2. 观察到细胞因子反应多态性（来自6个供体的两个不同反应组）。
3. 利妥昔单抗即使在1μg/ mL下也能引发多种细胞因子释放，目前尚不清楚是什么原因导致这种强活性。
4. 8 M尿素处理导致单抗降低细胞因子释放活性，但增加iC3b补体活化（在利妥昔单抗和mAb9中均观察到）。
5. 人血清来源的IgG显示出剂量依赖性细胞因子释放活性。

结论

1. 抗体矩阵ELISA现有产品包括针对17种生物仿制药和一种用于新型单克隆抗体的开发。
2. 每个抗体矩阵ELISA都为单克隆抗体提供独特的 HOS 特征，反映其表面免疫原点的位置和数量。
3. 抗体矩阵ELISA灵敏度高、系统化且通量相对较高。
4. 它与稳定性和生物测定数据具有良好的相关性。
5. 它可以检测生物测定可能无法检测到的构像变化。
6. 它可以应用于生物药开发的许多阶段，从细胞系选择到产品放行。