The Platform Strategy of Antibody Disulfide Bond Reduction to Reduce the Risk of Antibody Process Development

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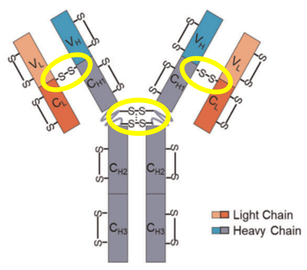
ABSTRACT

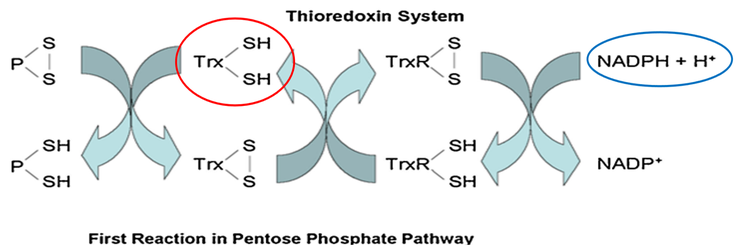
The reduction of disulfide bonds poses a highly challenging issue in antibody production, as it can lead to a decrease in product purity and can impact the safety and efficacy of drugs. Therefore, it is necessary to develop and optimize process strategies to minimize disulfide bond reduction and ensure the quality, efficacy, and safety of the product. In recent years, with the emergence of more complex molecules such as bispecific and trispecific antibodies, the problem of molecular heterogeneity caused by incomplete or mispaired interchain disulfide bonds has become even more daunting.

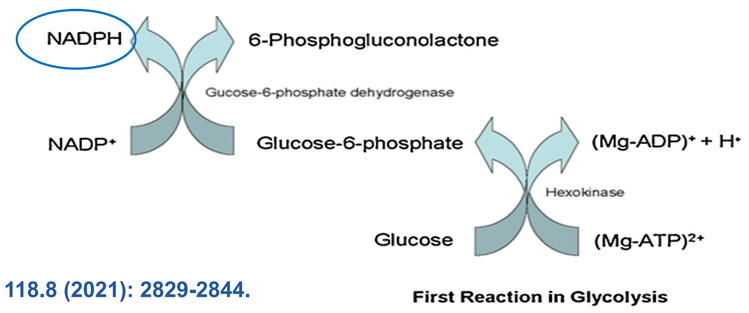
Recently, ProBio has developed the ProBox™ platform, which offers efficient solutions for antibody reduction. This strategy involves initially assessing the risk of molecular reduction and using the resulting score to guide the implementation of additional mitigation strategies.

In summary, we present a risk assessment strategy for antibody disulfide bonds reduction, and the strategy to mitigate the impact of antibody reduction on subsequent purification processes.

ANTIBODY DISULFIDE BOND REDUCTION PRINCIPLE, RISK ASSESSMENT AND SOLUTION STRATEGY



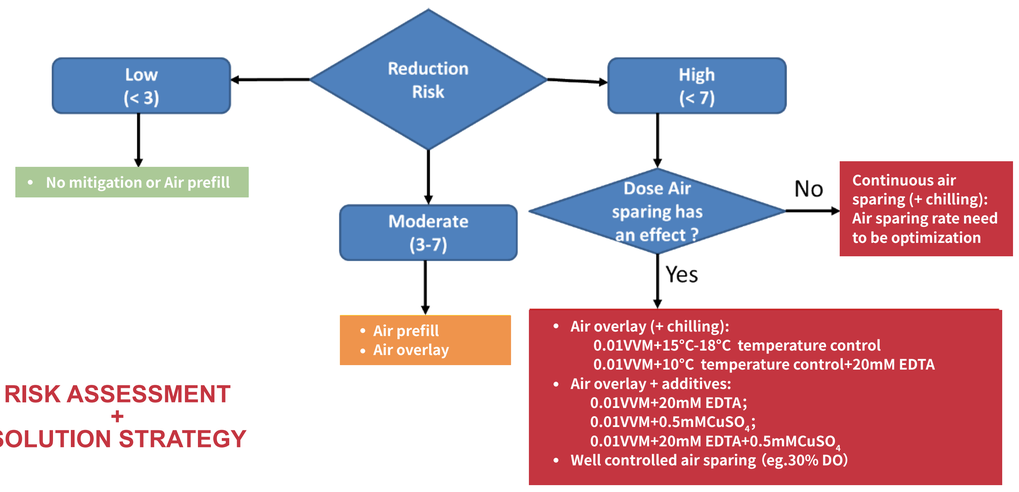




Ren, Tingwei, et al. Biotechnology and Bioengineering 118.8 (2021): 2829-2844.

Antibody disulfide bond reduction is essentially an oxidation–reduction (redox) reaction that involves redox enzyme. Glutathione (GSH) and thioredoxin (Trx) system (comprising Trx, thioredoxin reductase [TrxR] and nicotinamide adenine dinucleotide phosphate (NADPH) are the known enzymes, and enzyme systems, that contribute to disulfide reduction. NADPH is generated from the pentose phosphate pathway and serves as an electron source in the disulfide bond reduction reaction. Electrons first transfer from NADPH to TrxR and reduce the TrxR disulfide bond, then move to the oxidized Trx to form the reduced Trx, and finally reduce the disulfide bond. GSH catalyzes the disulfide bond reduction in a similar way as Trx system.

Impact factor		Grading standards	score	percentage	weighted score (score*percentage)
Reduction Sensitivity	**Reducing agent test*1 (0.5 and 2mM Cysteine)**	high sensitivity	10	30 %	
		moderately sensitive	5		
		low sensitivity	0		
Scale down study	TrxR activity (U/ml)	< 100	Report value/10	12 %	
		≥ 100	10		
	Reducing agent test*2	high sensitivity	10	12 %	
		moderately sensitive	5		
		low sensitivity	0		
	HCCF Stability under purged with Nitrogen	reduction	10	26 %	
		Non-reduction	0		
Total score	**High Risk (>7)**		**Moderate Risk (3-7)**		**Low Risk (<3)**

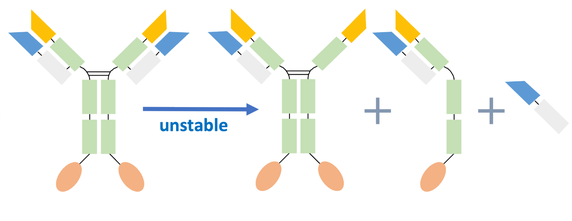


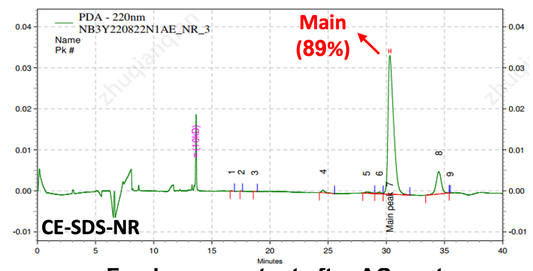
RISK ASSESSMENT + SOLUTION STRATEGY

CASE STUDY: FUSION PROTEIN

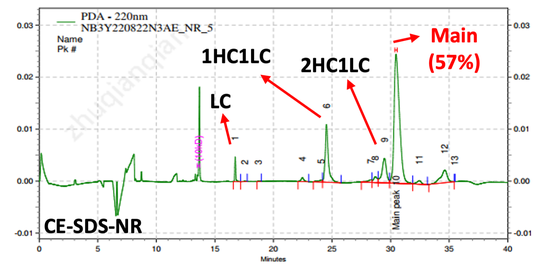
Challenges:

- The molecule has high reduction tendency
- CE main peak drops from 89% to 57% in 7 h when stored at RT





Fresh supernatant after AC capture



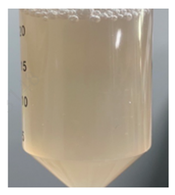
Place at RT for 7 h after AC capture

Solutions:

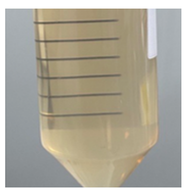
- ✓ Increase dissolved oxygen to consume NADPH
- ✓ Add metal ions to inhibit Trx activity
- ✓ Add oxidant consuming Trx

No.	Sample	CE-SDS-NR (%)		
		pre-peaks	Main Peak	post-peaks
0	Fresh supernatant	2.5	88.8	8.8
1	Place at RT for 7 h	36.0	56.7	7.3
2	Continuous air sparging 20 h	1.4	90.6	8.1
3	Insulation 24 h	40.9	56.4	2.7
4	Add 1 mM $CuSO_4$ 27 h	2.1	89.7	8.2
5	Add 1 mM cystamine 30 h	1.5	91.5	7.0

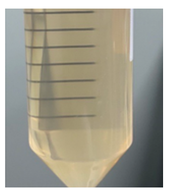
Continuous Air sparging 20 h



Insulate the air 24 h



Add 1 mM $CuSO_4$ 27 h



Add 1 mM cystamine 30 h

CONCLUSION

We have established a comprehensive platform for antibody disulfide bond reduction, which assesses the risk of disulfide bond reduction during the early stages of antibody development. Based on the severity of the risk, we recommend appropriate solution strategies to mitigate potential risks in later stages of development. This platform has been successfully applied to antibody projects, ensuring smooth process development and obtaining highly stable therapeutic products.