Science For A Better Life

Tailor-made proteins:

A formulation development perspective

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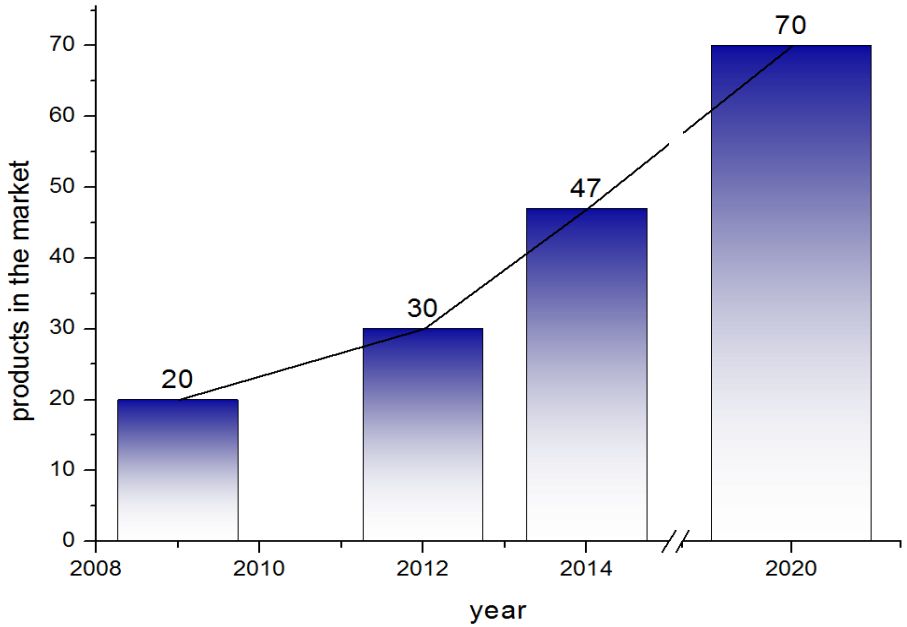
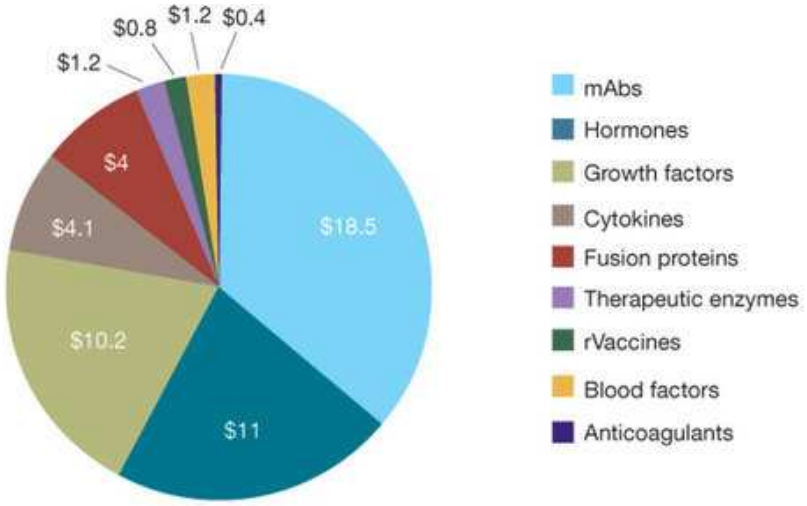
March 11th 2015, Braunschweig

Overview of marketed therapeutic proteins

In 2020 combined world-wide sales are estimated to be nearly $125 billion



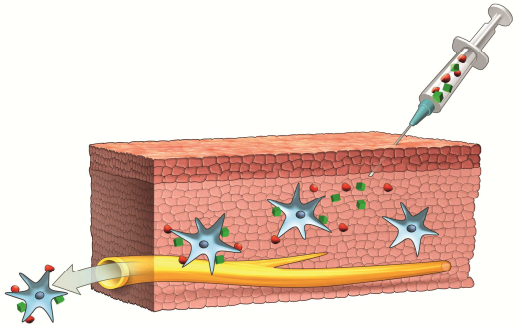
DM Ecker, SD Jones, HL Levine, mAbs 2015, 7:1, 9-14



S. Aggarwal, Nature Biotechnology, 2011, 29 (12), 1083-1089

Early formulation development:
What is expected from us?

Trend: New routes of application are becoming more important!
E.g. subcutaneous injection of proteins



Benefits

→ increased therapeutical compliance

→ reduced healthcare costs

Formulation development challenges

→ highly concentrated protein formulations are needed due to a limited application volume

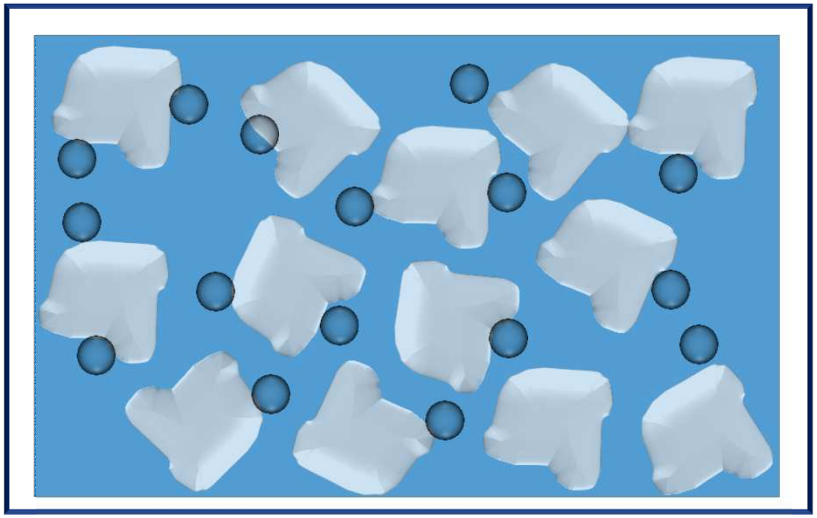
→ consequences:

- Higher viscosity
- Decrease in stability (more hydrophobic interactions, aggregation, subvisible and visible particles)

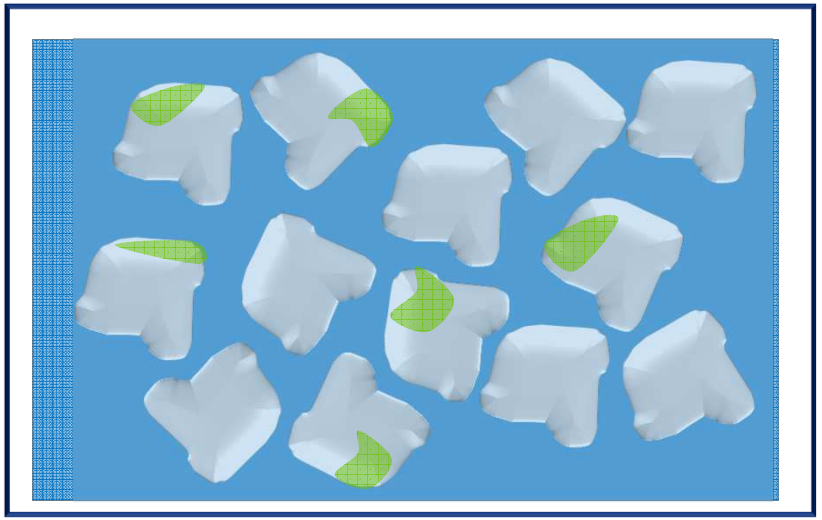
Challenges: High viscosity and low stability

How to address these issues?

Change **extrinsic** factors

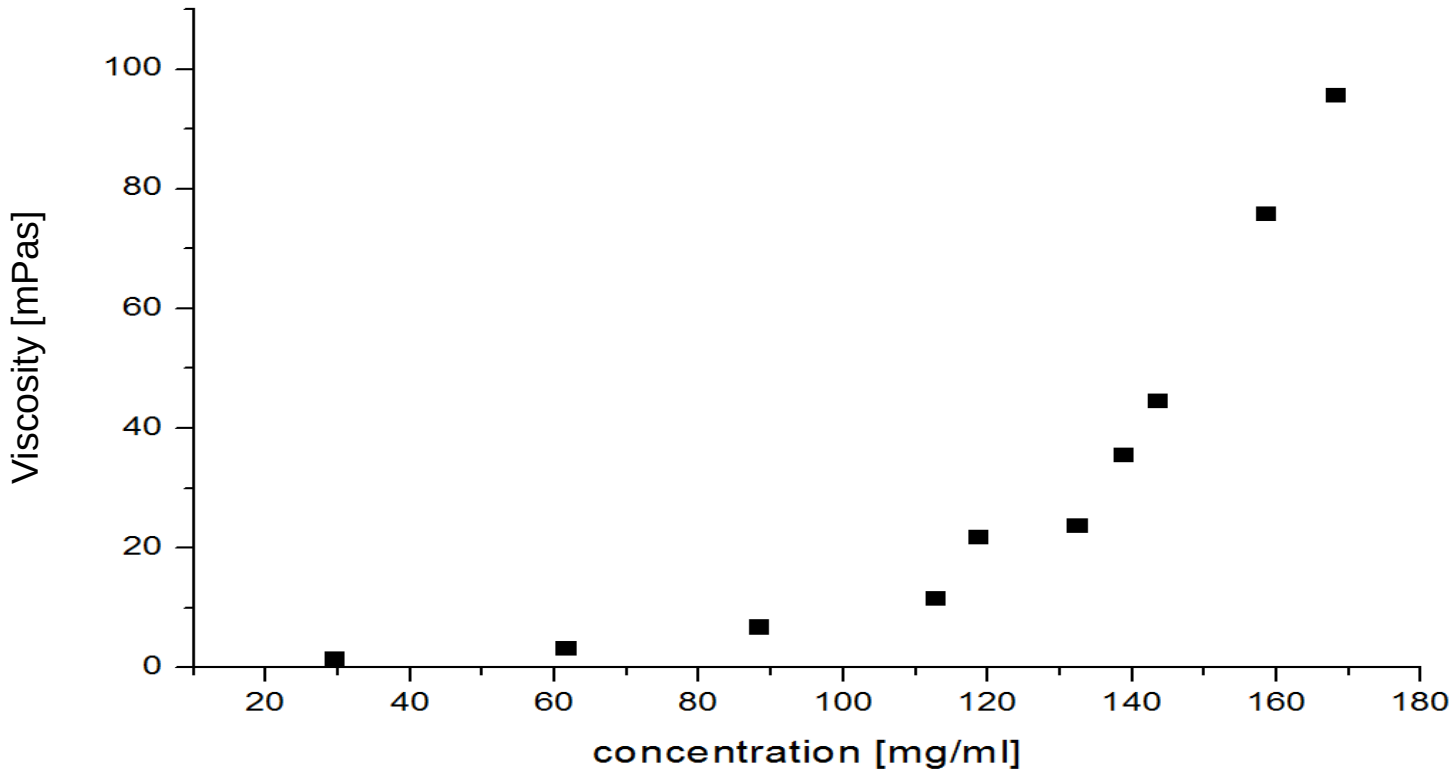


Change **intrinsic** factors



Challenges for formulation development:

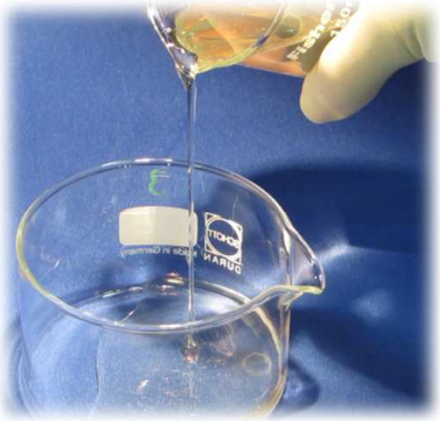
Increasing viscosity with increasing protein concentration

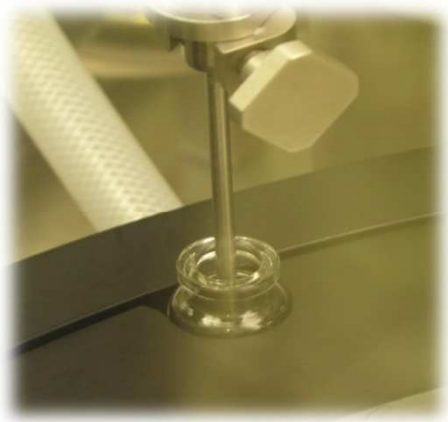


Example of increasing viscosity in dependence of concentration of an IgG1 antibody (Bayer internal data)

Consequences of increasing viscosity

DS and DP Manufacturing sites	challenges:	filtration, dosing-accuracy, production speed

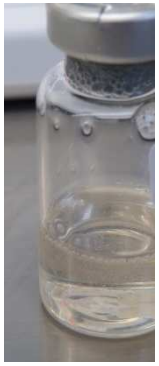




Analytical Departments	challenges:	develop analytical tools to analyze high-conc proteins undiluted

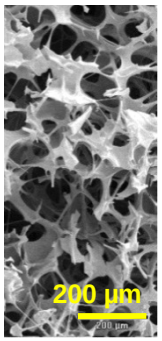
Consequences of increasing viscosity

Clinical departments / end users	challenges:	syringeability, longer reconstitution time

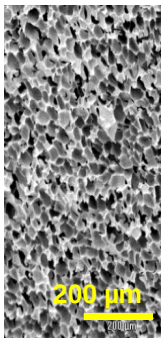


Reconstitution of lyophilisate:
IgG1, 10 mg/ml
reconstitution time:
3-5 min

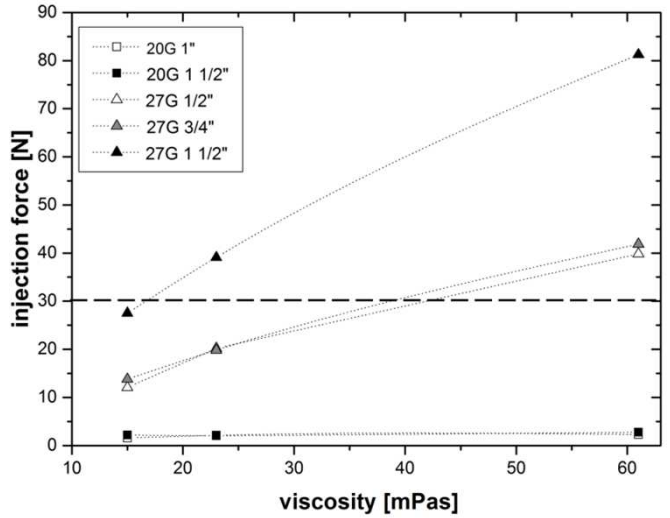
Reconstitution of lyophilisate:
IgG1, 150 mg/ml
reconstitution time:
3h



SEM-picture of a lyophilisated IgG1 (10mg/ml)



SEM-picture of a lyophilisated IgG1 (150mg/ml)



Injection force vs viscosity for an IgG1 solution using different cannulas (Bayer internal data). 30 N is considered as limit for self administration.

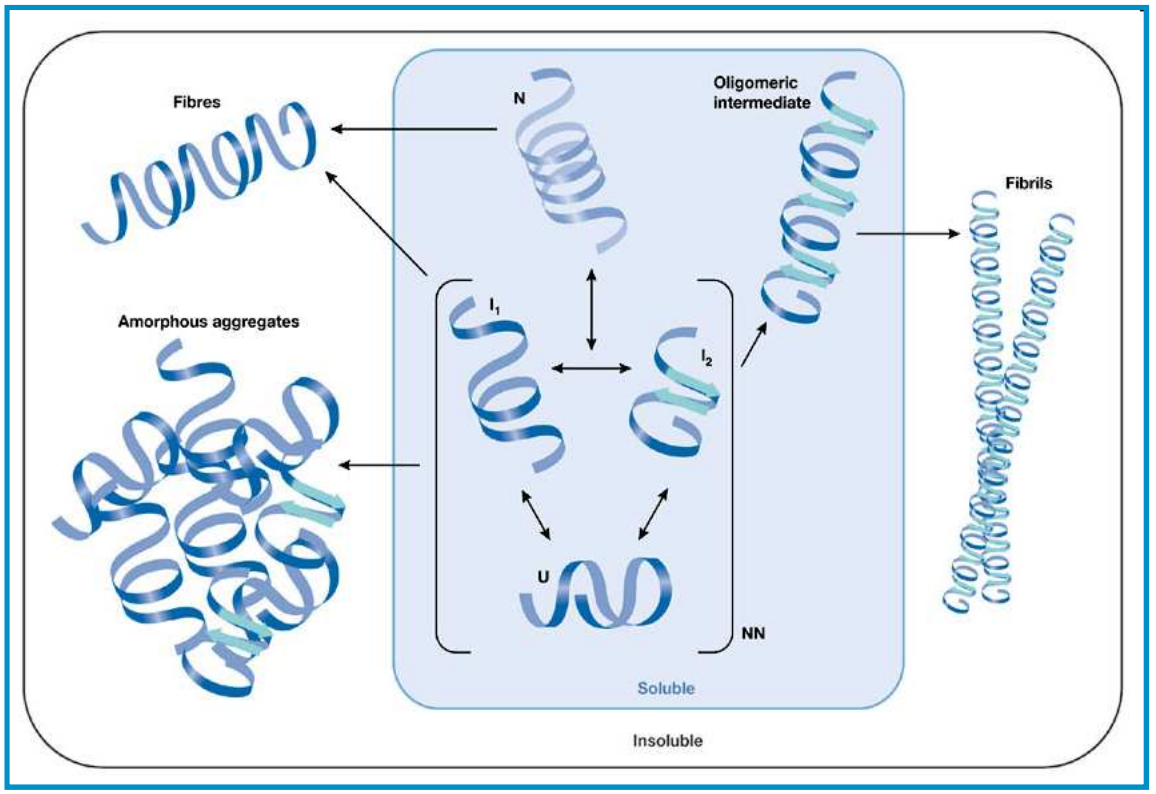
V Burckbuchler et al., EJPB, 2010, 76 (3): 351-356

Challenges for formulation development:
Protein stability at high concentration may decrease

Aggregates are heterogeneous species:

- reversible - irreversible
- native - nonnative,
- dimers - multimers
- few nm - hunderds of µm

→ Aggregation may lead to immunogenicity and loss in efficacy



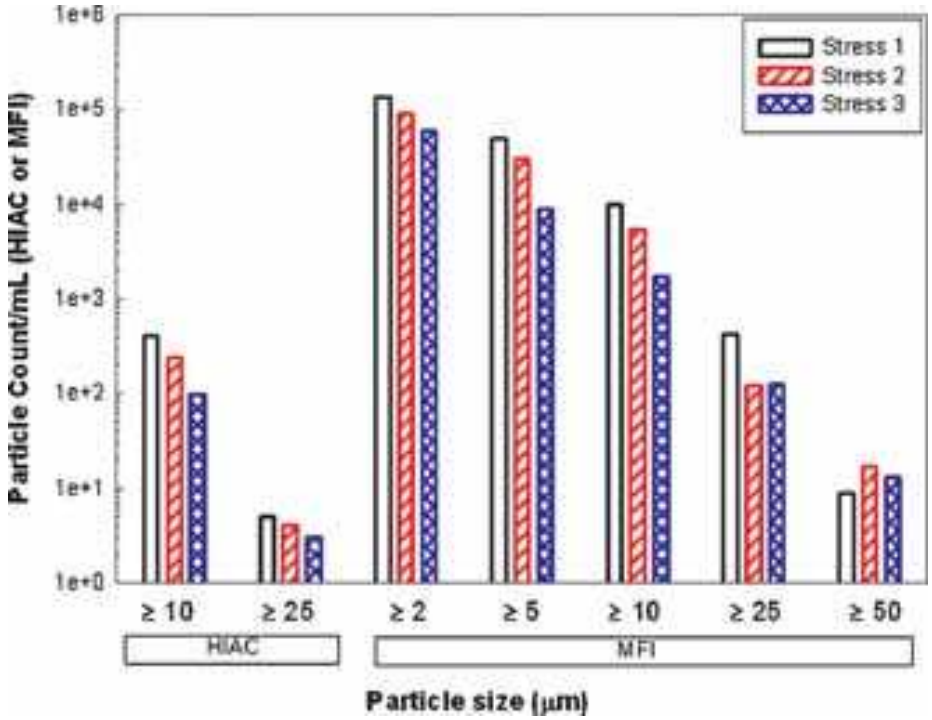
J.J. Yerbury EMBO reports 6, 12; 1131–1136, 2005.

USP requirements light obscuration test <788>

Not more than 6000 particles/container > 10 µm

Not more than 600 particles/container > 25 µm

- Currently, preferred method for specification – light obscuration (HIAC)
- Smaller SVPs (1-10 µm) are not mentioned in the specification
- More sensitive method for particle analysis – micro flow imaging (MFI)



SK Singh et al., Journal of Pharmaceutical Sciences, 2010, 99 (8), 83302–3321

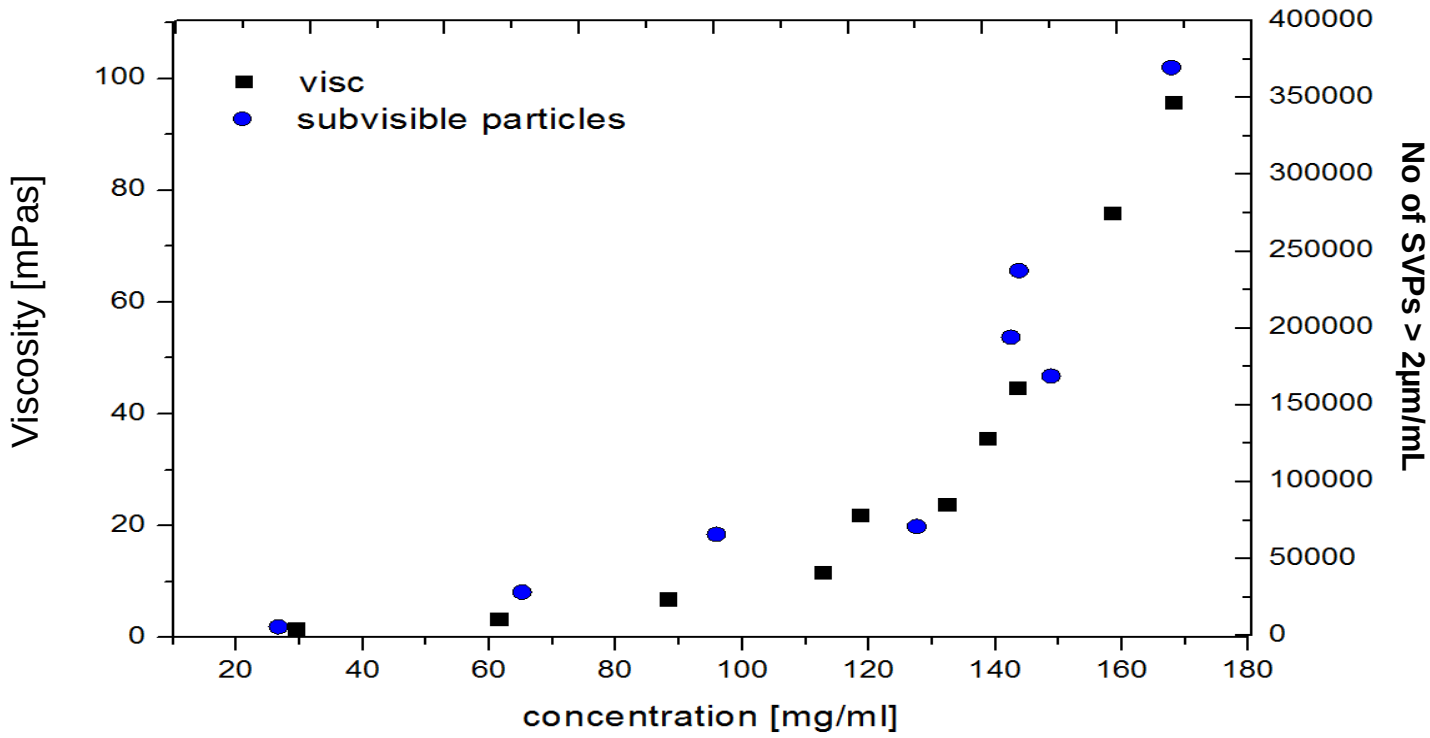
Subvisible particles measured by MFI

IgG1 Ab concentration (mg/mL)	Cumulative number of particles (µm/container)					
	>1	>2	>5	>10	>25	>50
10 mg/mL	10597	2334	247	26	0	0
50 mg/mL	33870	5596	642	56	0	0
100 mg/mL	259936	48624	3496	224	12	0
150 mg/mL	307194	71040	4434	414	60	0

SVPs of an IgG1 Mab solution at different concentrations (Bayer internal data)

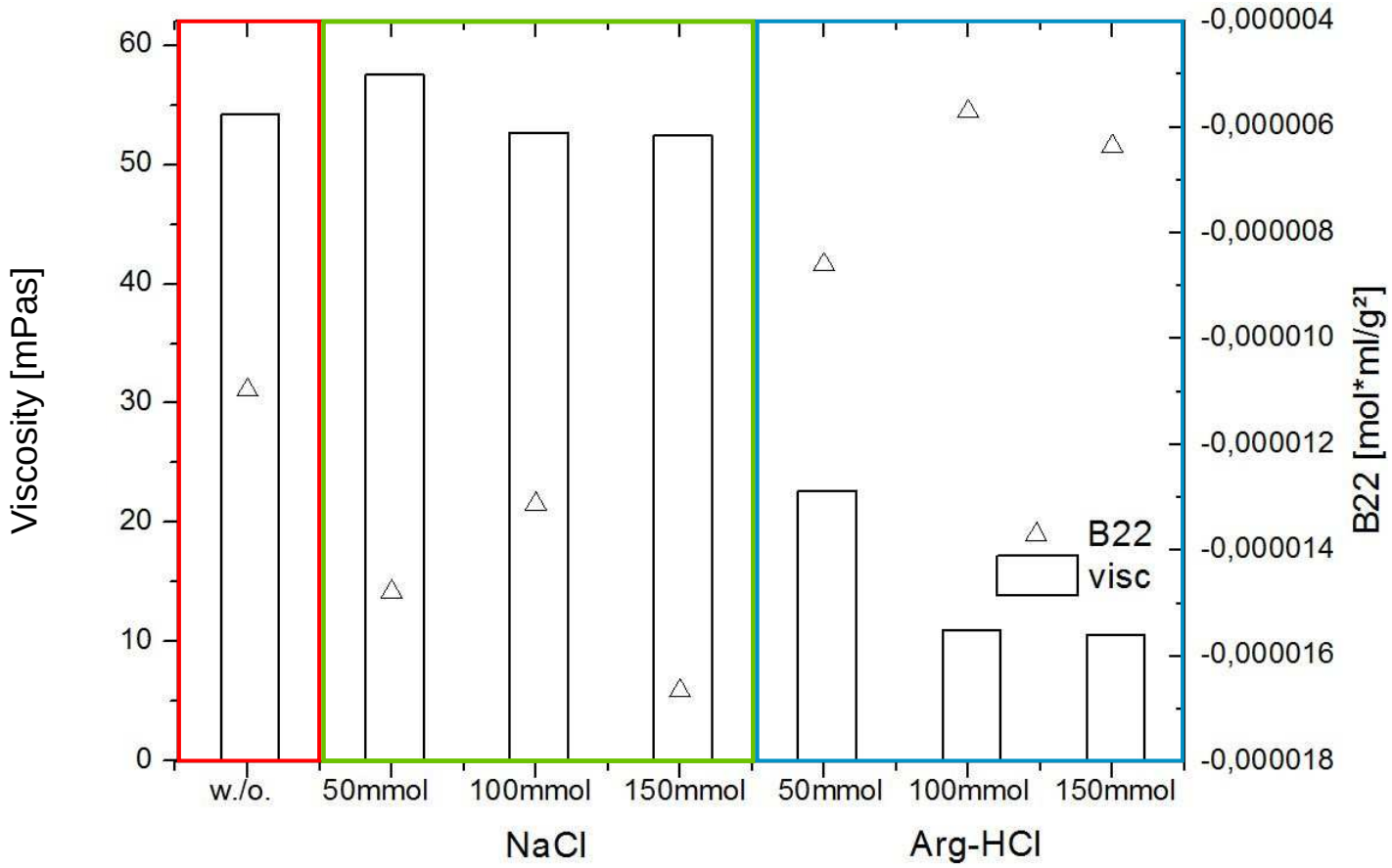
Although the specifications are largely met, large increases in the smaller SVPs are observed with increasing concentration

Increasing amount of subvisible particles (SVPs) with increasing protein concentration



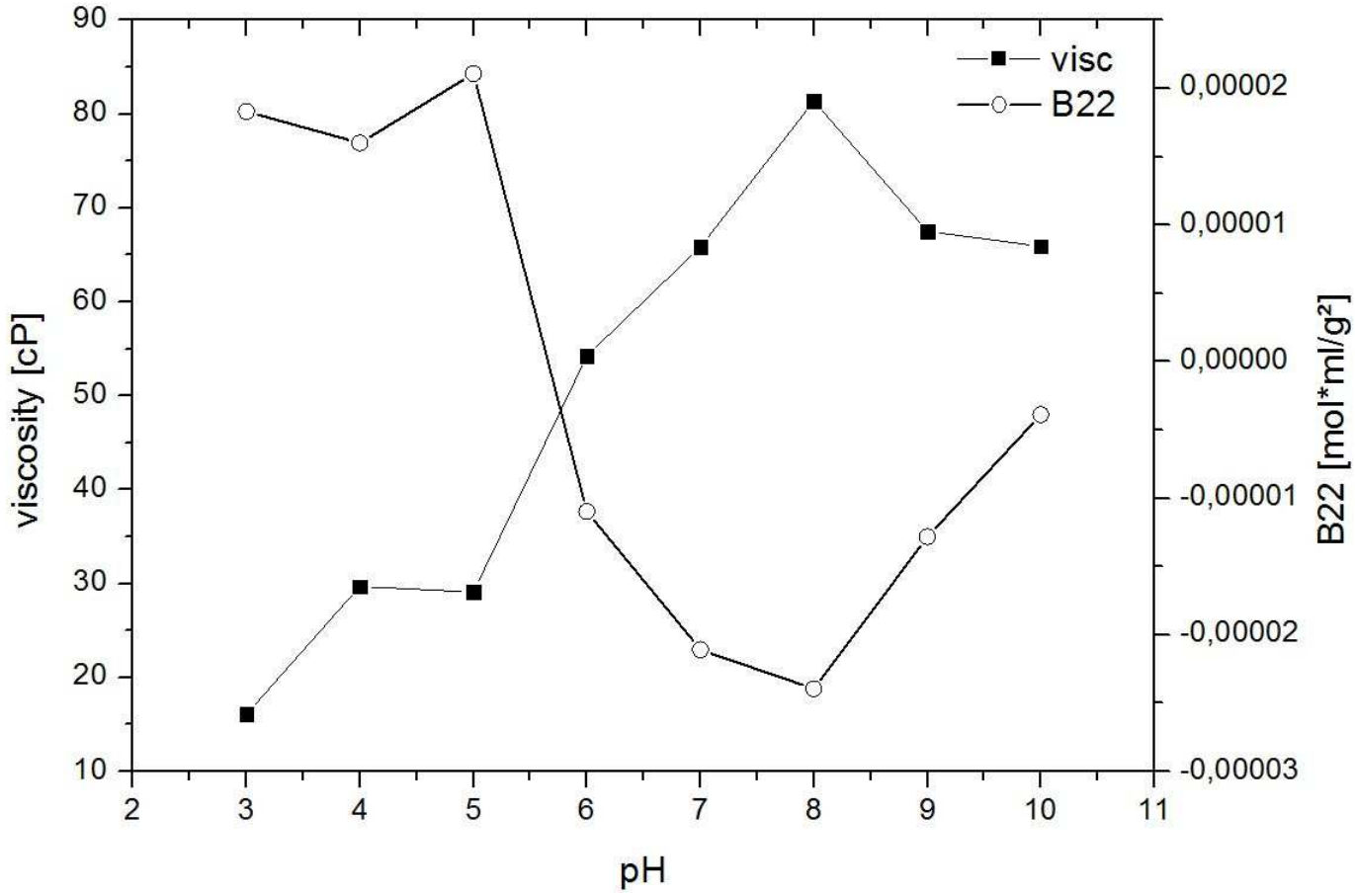
Correlation between rise in viscosity and number of SVPs for an IgG1 Ab with increasing concentration (Bayer internal data)

How to overcome these challenges… Addition of excipients



Viscosity lowering effect of NaCl and Arginine HCl on a
140 mg/mL IgG1 Mab solution in correlation with the B22 value (Bayer internal data)

How to overcome these challenges... Change the pH



Effect of pH on viscosity and B22 value of a 140 mg/ml IgG1 Mab solution (Bayer internal data)

Strategy change:

Extrinsic optimization (pH, excipients) vs intrinsic optimization

Instead of optimizing the environment →

Optimize the protein?

Can computational modeling be used as tool to optimize intrinsic protein properties?

Goals of computational modeling:

Predict which regions of a protein are involved in aggregation

Modify these regions (provided that they are not involved in target binding)

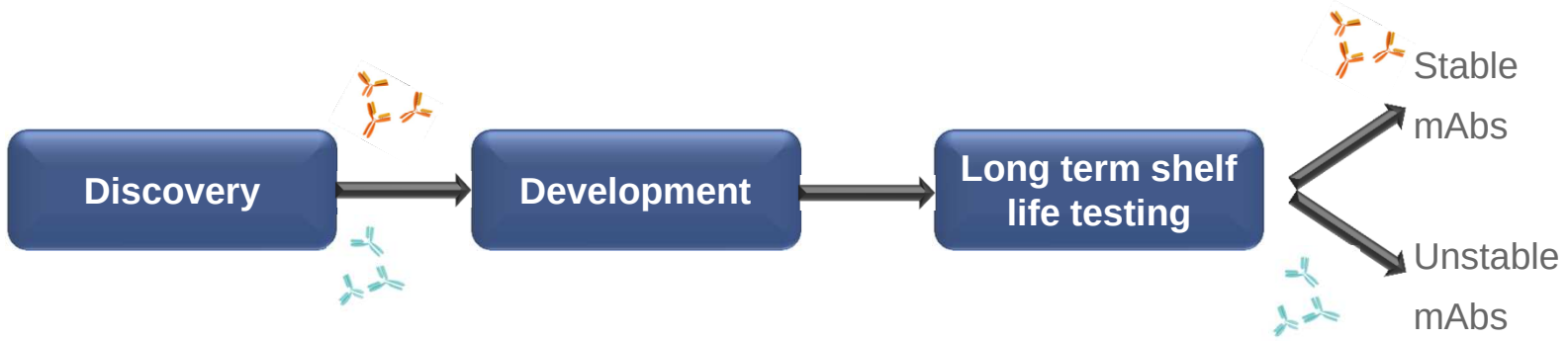
Candidate ranking → low stability towards high stability

If all candidates show equal binding properties to target, select the most stable one

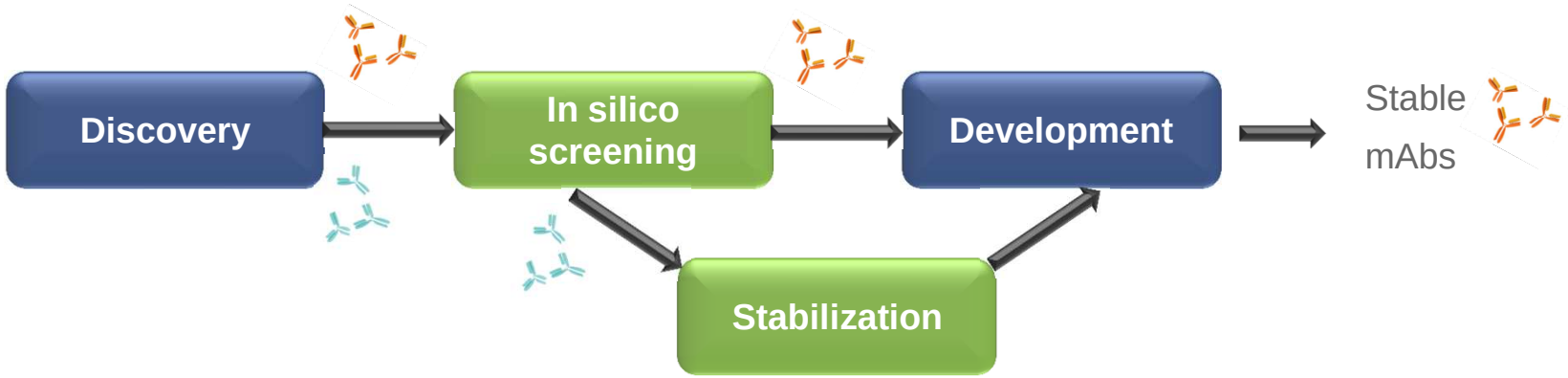
Rational design to reach optimal equilibrium between potency, developability and safety

Proposed changes in strategy

Current strategy

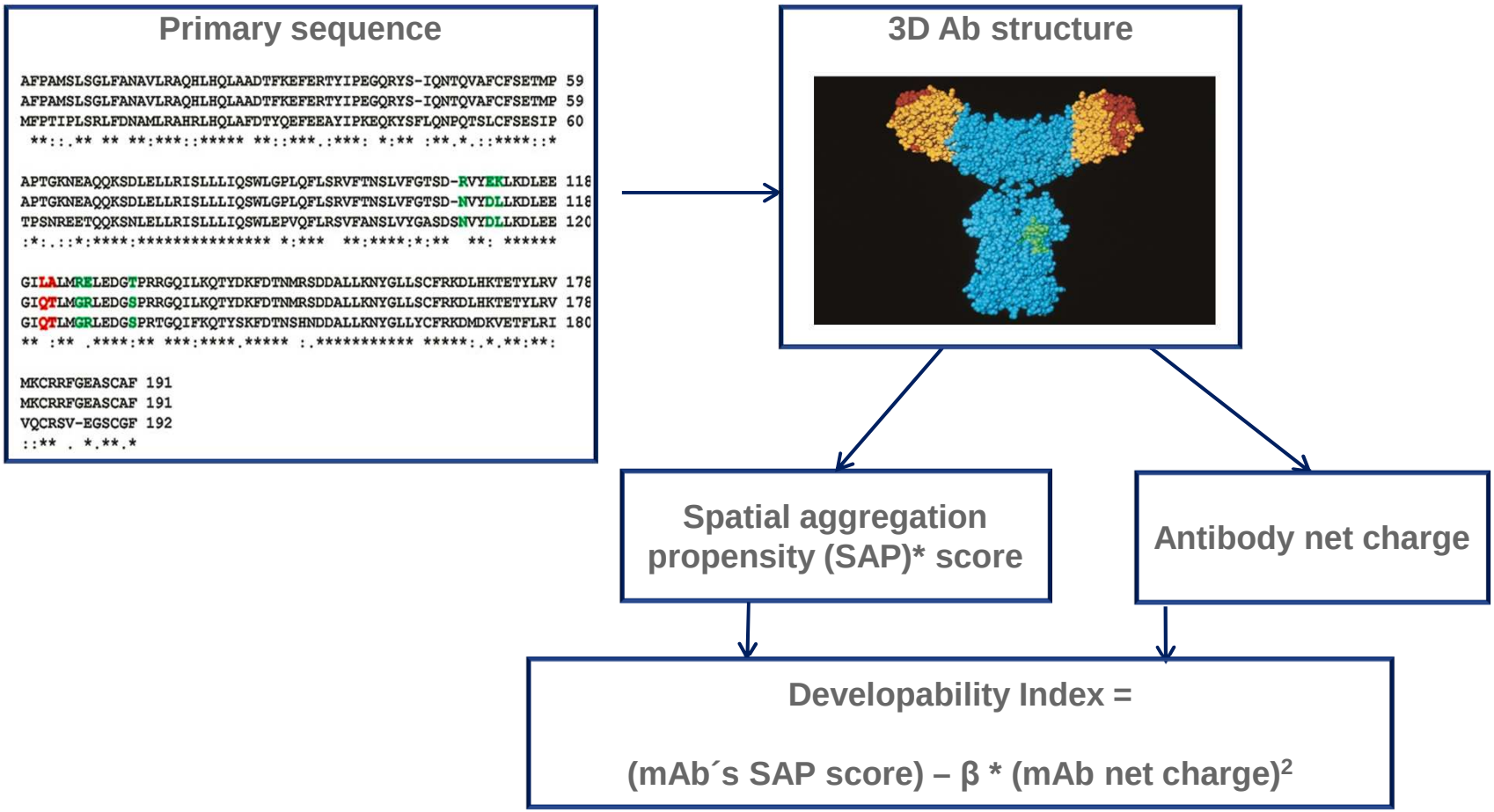


Proposed strategy



After Agrawal NJ et al, J Pharm Sci 100:5081–5095, 2011

Developability Index (DI)

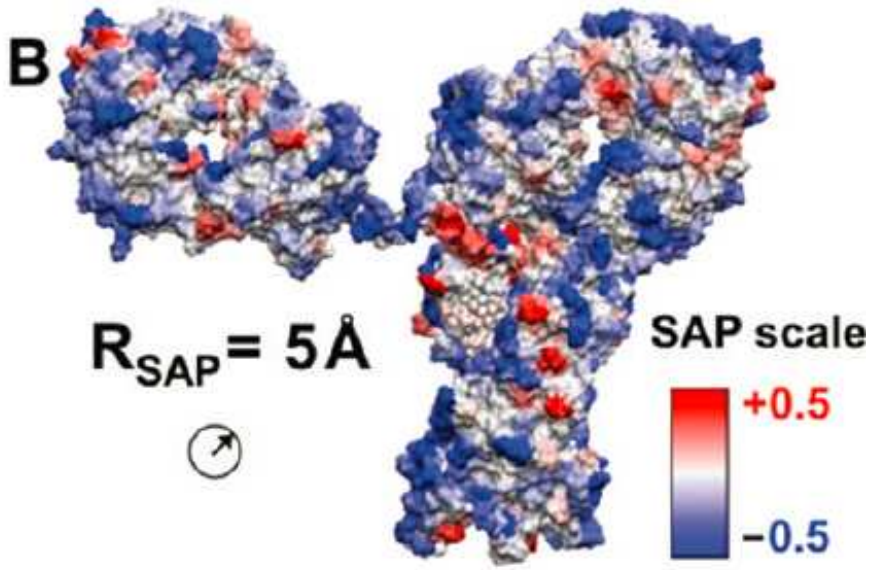


* Identifies regions of hydrophobic patches on the protein surface

Lauer TM et al, J Pharm Sci 101:102-115, 2012
Buck PM et al, Ther Proteins: Methods and Protocols, 2012, vol. 899: 425-451

Tool to rank Ab candidates and/or mutate aggregation prone regions (APRs)

SAP values of an aglycosylated IgG1



Red regions are prone to aggregation

Increasing DI implies increase in aggregation propensity

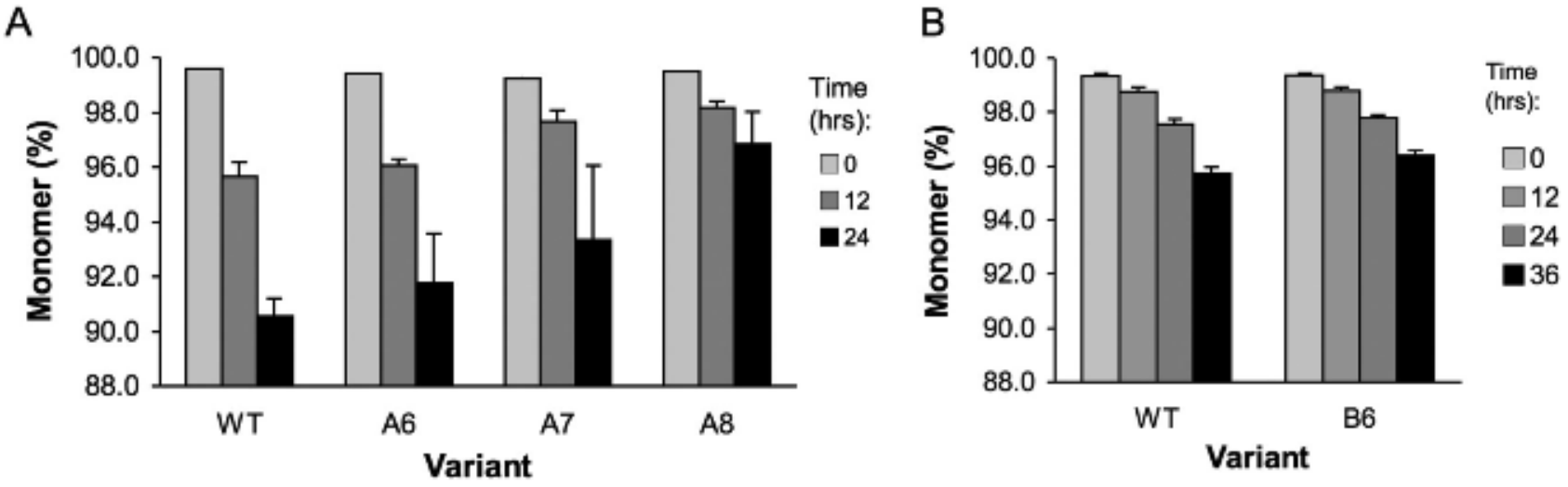
Lauer TM et al, J Pharm Sci, 2012; 101:102-115
Chennamsetty N et al, J Phys Chem B 2010 144(19); 6614-6624

Mutation of APRs: *in vitro* validation

In vitro stability study:

Monomer loss of antibody A and antibody B wild type and variants after storage at 58℃ and 52℃, respectively.

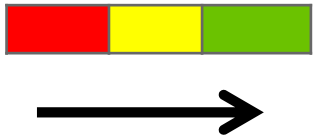
- variations of aggregation prone regions → increased monomer content
- **Remark:** variations are also made in the CDR-region, binding may be influenced



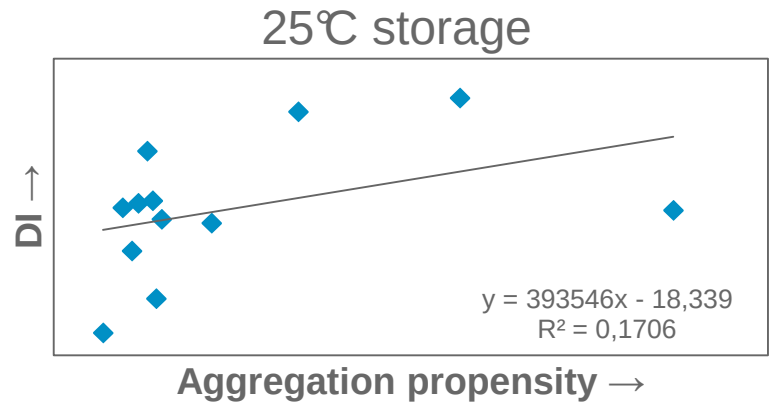
Chennamsetty N et al, J Phys Chem B 2010 144(19); 6614-6624

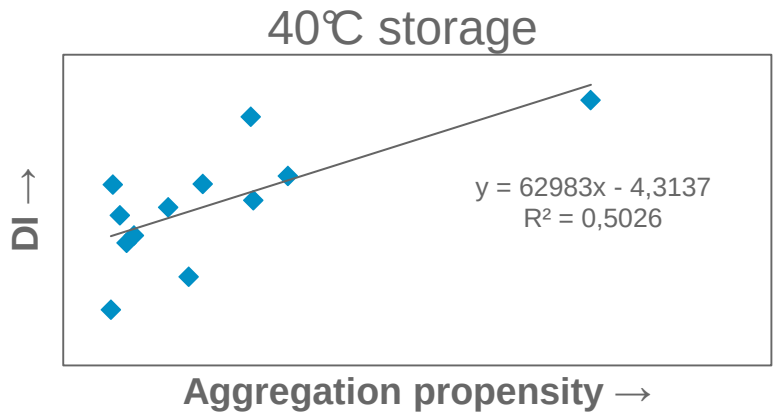
Ranking: *in silico* vs *in vitro*

DI classifications of long-term stability				
mAb	25℃		40℃	
	Expt.	DI	Expt.	DI
mAb1				
mAb2				
mAb3				
mAb4				
mAb5				
mAb6				
mAb7				
mAb8				
mAb9				
mAb10				
mAb11				
mAb12				



Increasing stability





Lauer TM et al, J Pharm Sci, 2012; 101:102-115

Key messages and conclusions

- To develop a stable highly concentrated protein formulation, ideally both extrinsic and intrinsic tools are investigated.

- Using computational modeling to change APRs is becoming more important, however not as stand-alone → *in vitro* validation essential!

- An optimal equilibrium between potency, developability and safety is pivotal for succesful development of high-conc proteins

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Thank you!

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