Biophysical characterization of excipient combinations for mAb formulation development

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Content

- Need for and problems with Subcutaneous biologic administration
- Comera SQore™ platform technology
- Identifying and characterizing excipients
  - Computational screening
- New stabilizing excipients
  - Experimental validation
- Case studies on formulation development of mAb A
    - Formulation Buffer screening
    - Viscosity and stability optimization
    - DOE for formulation optimization
- Conclusions

Conversion of IV to SQ Administration

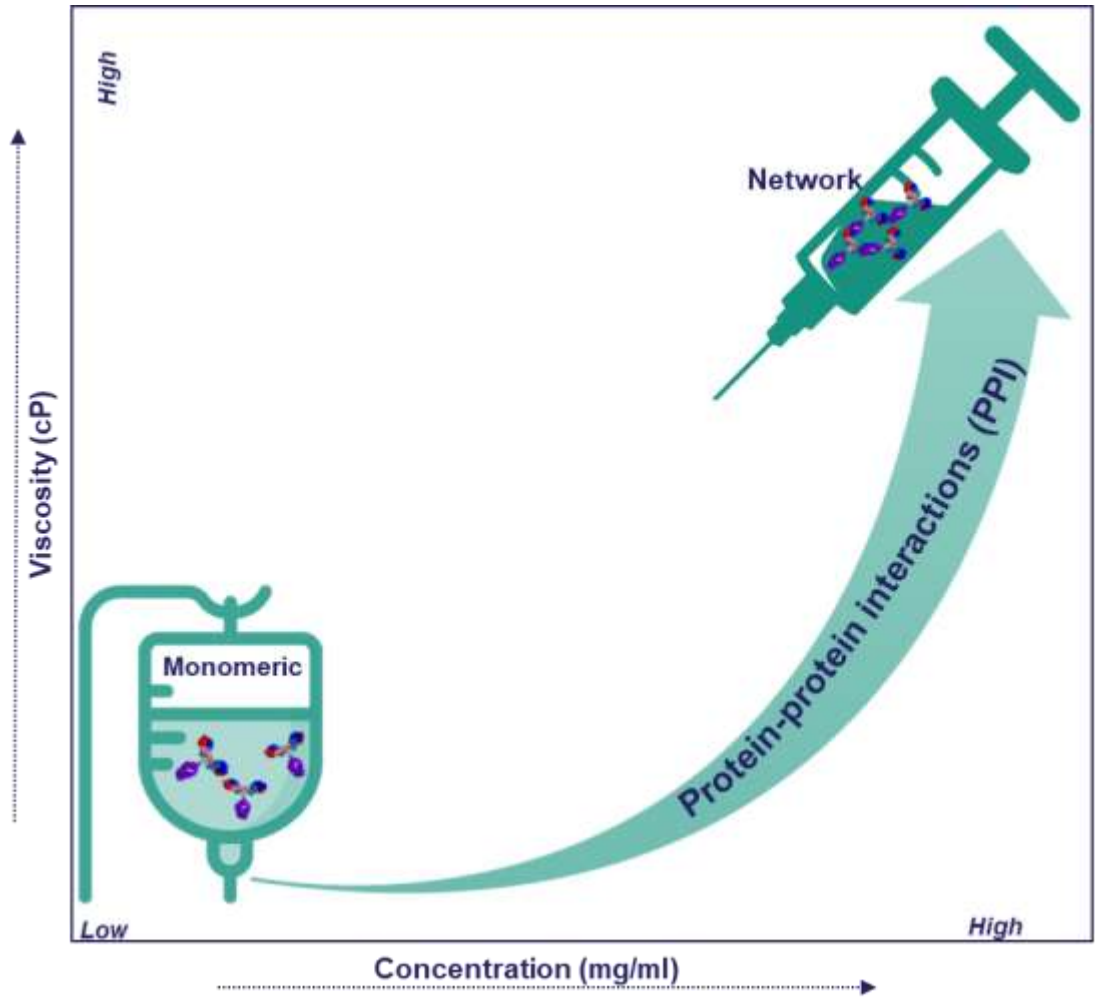
IV

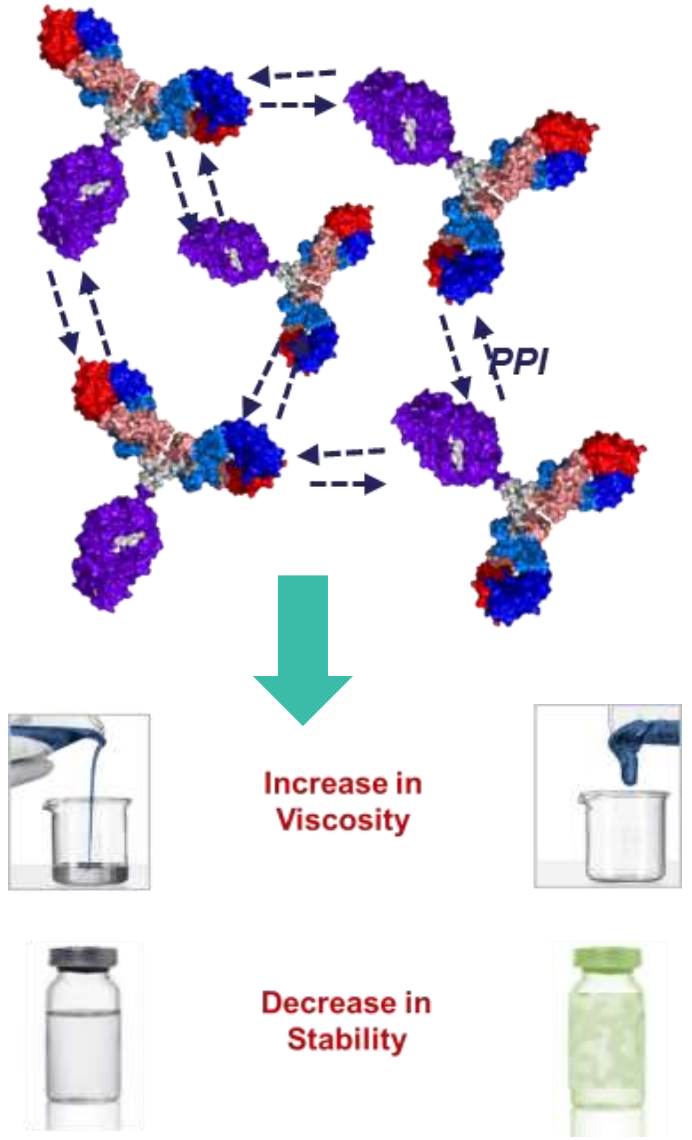
- Pain and discomfort
- Time commitment
- Risk of infection
- Reduced compliance
- Space and nursing time requirements
- Increased costs

SQ

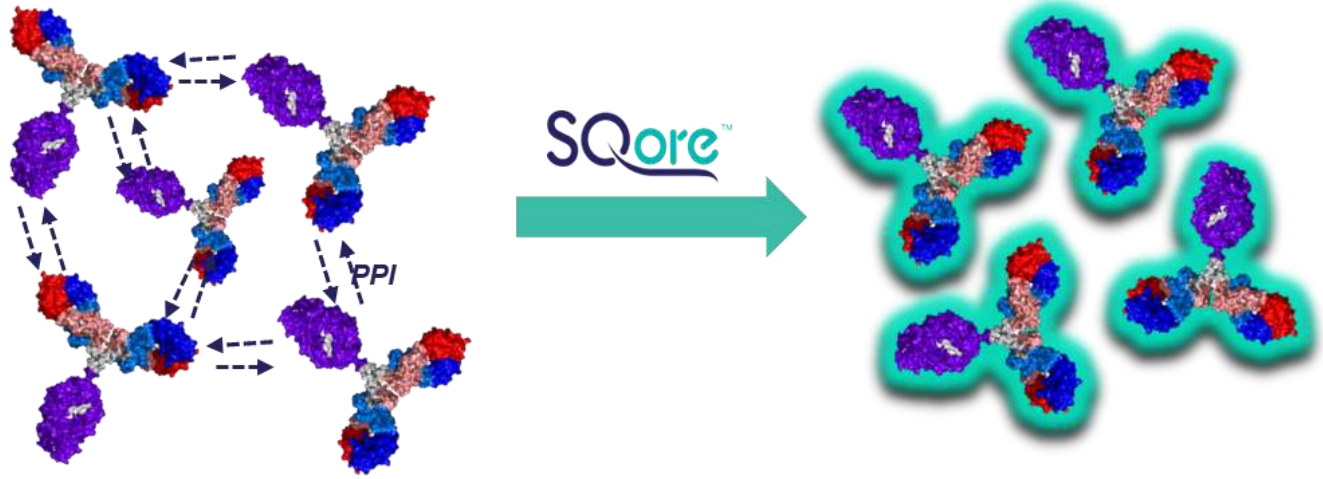
- Less pain, less time
- Reduced risk of infection
- Potential self-administration
- Higher patient satisfaction
- Improved quality of life
- Reduced cost

Developing SubQ mAb formulations is challenging





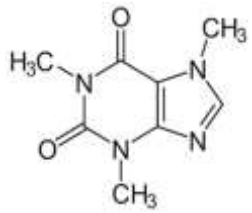
SQore platform helps in IV to SQ Conversion



High Concentration and high PPI
(High viscosity)

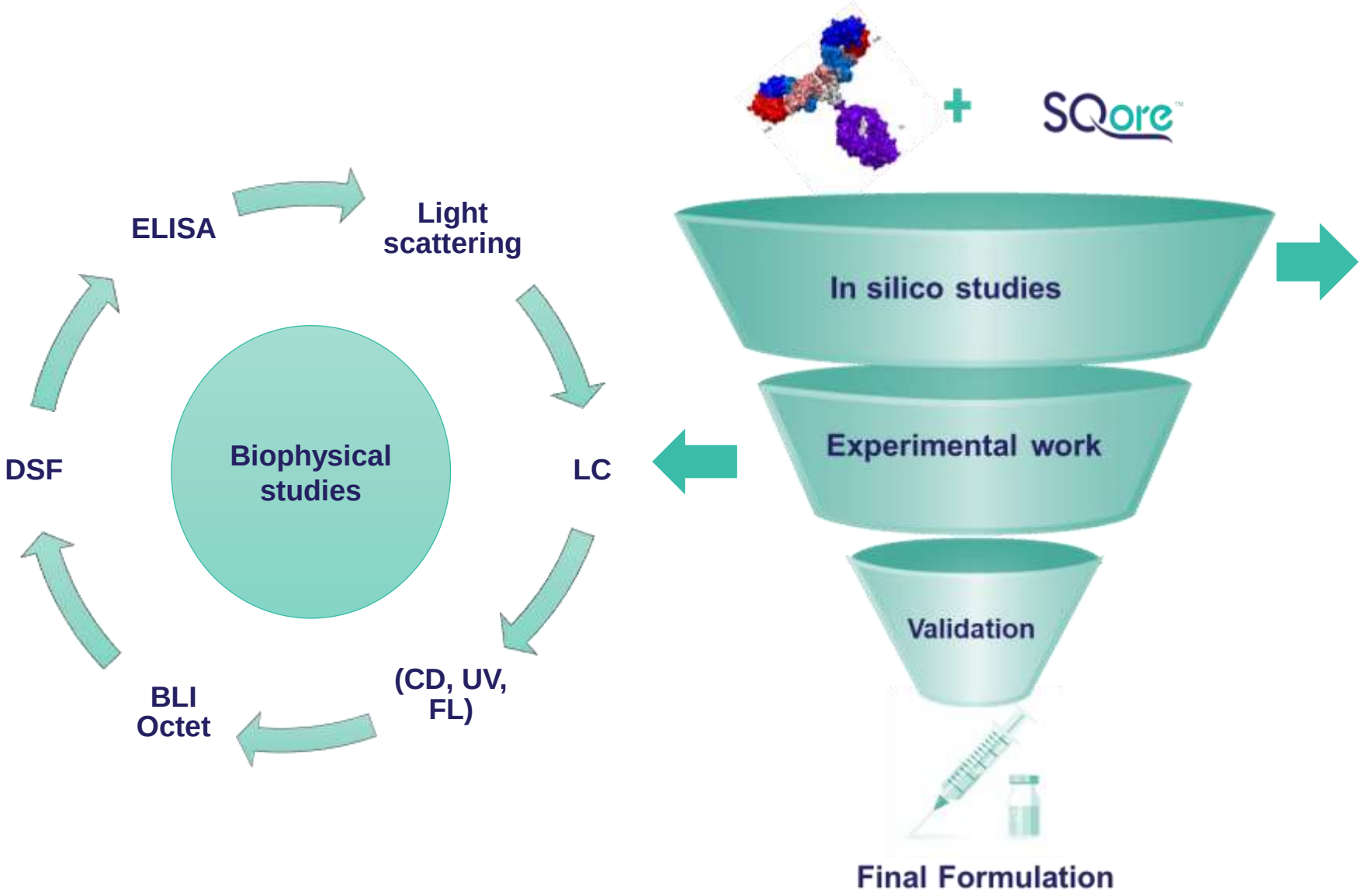
High Concentration and Low PPI
(Low viscosity)

- SQore excipients
  - Viscosity reducers
  - Stabilizers
- They are known chemical structures
- They have well established toxicology profiles



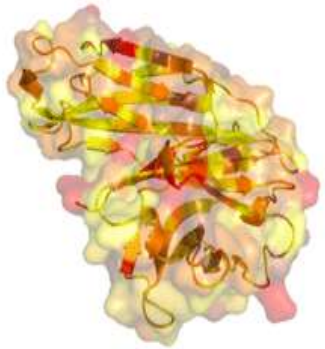
Caffeine (1,3,7-trimethylxanthine)
CAS # [58-08-2]
MW 194.19 g/mol

Developing subQ mAb formulations using SQore platform



Computational studies to identify viscosity hotspots.

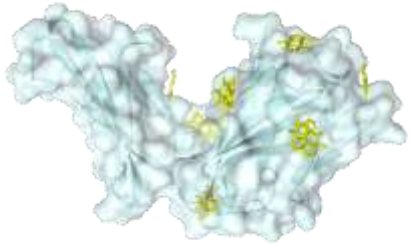
- Protein-protein and protein-excipient blind docking helps identify interaction hotspots and the residues involved.
- Results overlaid with the protein ligand docking to identify excipient binding sites, interfering with mAb self association

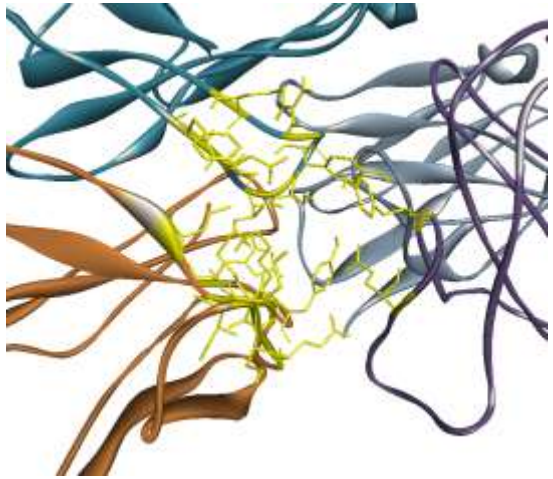


Infliximab fab fragment (pdb ID:5VH3)

***In silico* protein protein interaction**

***In silico* protein ligand interaction**



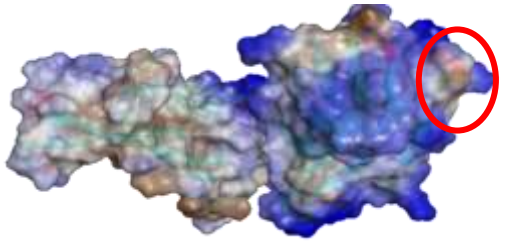


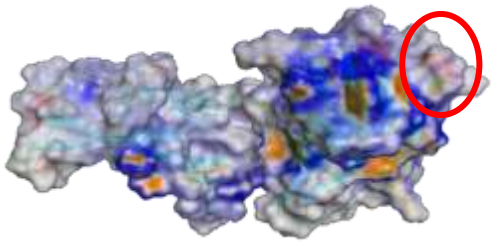
Excipient interfering with residues involved in mAb self association

Identifying screening sites and desired pharmacophores

- **Computational studies allows**
  1. **Identifying self interaction hotspots in mAbs**
  2. **Identifying excipients that decrease self association**
  3. **Identify excipients that can stabilize the mAb**

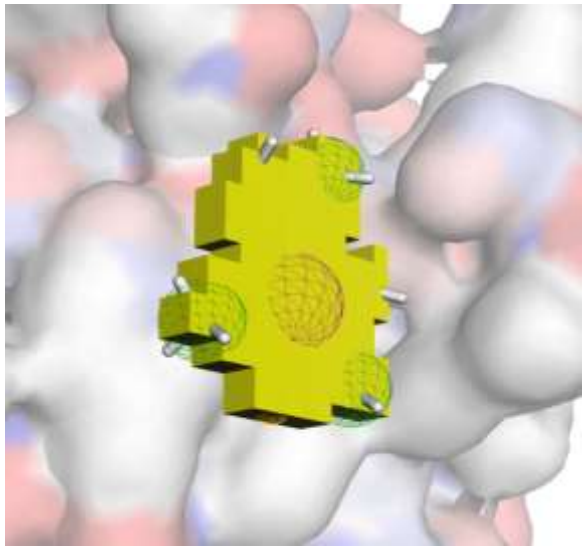
Surface Hydrophobicity





Surface charge

Identifying sites involved with Self interaction

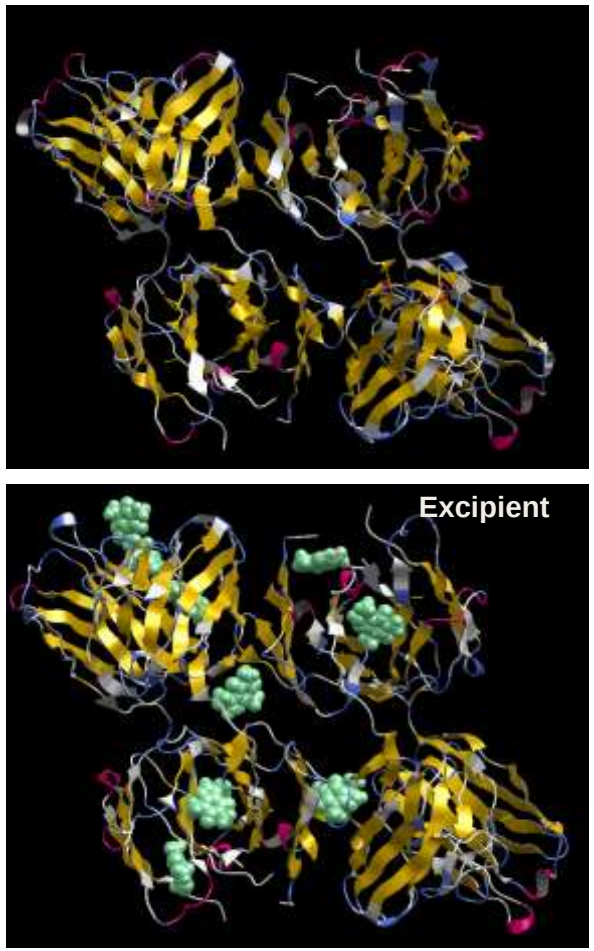


Identify single interaction site and Screen SQore library within the pocket

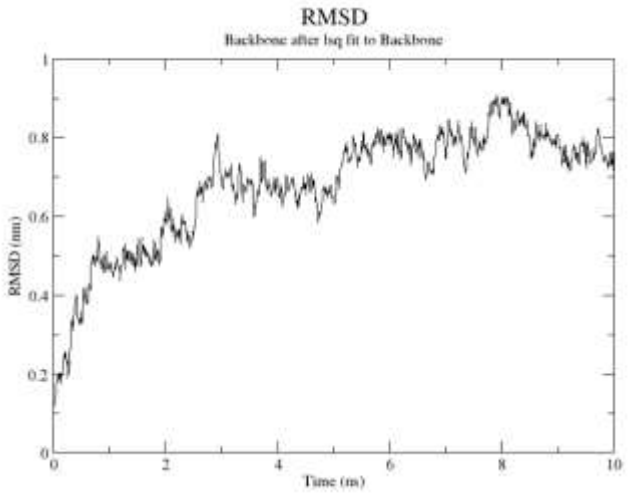
Ranked screening results

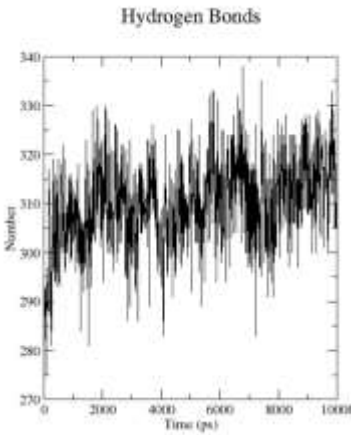
MD simulation data of protein excipient interaction

10 ns time scale

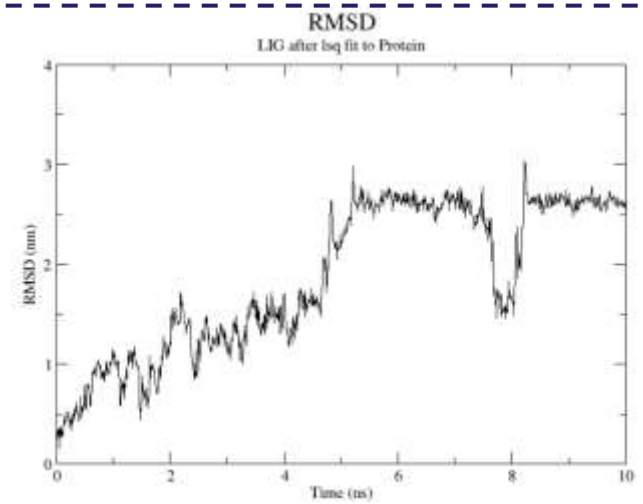


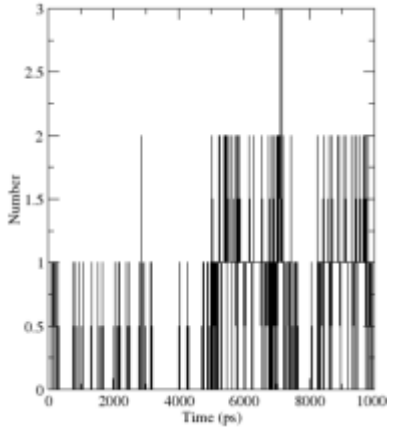
Protein





Ligand



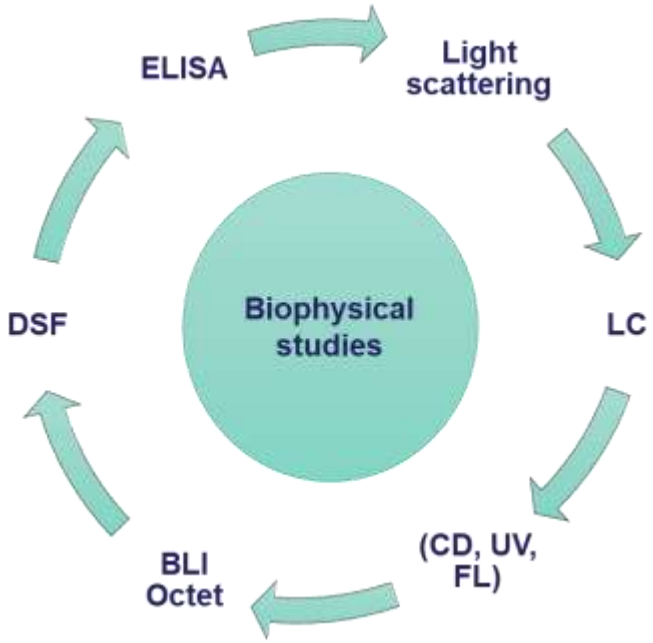


Simulation data allows to identify regions

1. **Where the excipient binds**
2. **How the excipient binds**
3. **Strength of binding**
4. **Interaction time**
5. **Residues it can interact with**

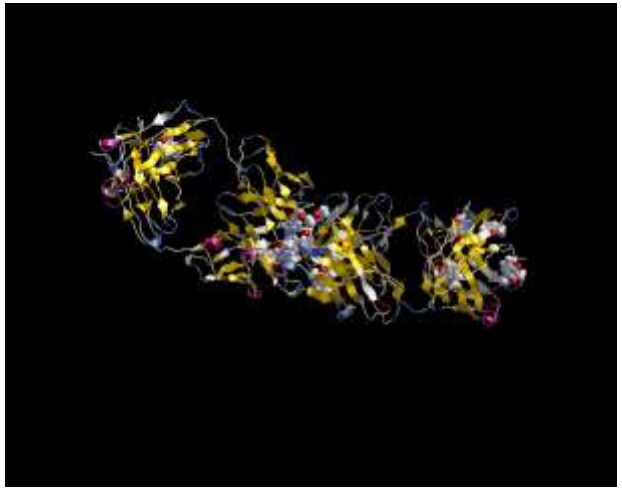
MD Simulation gives a better predictive power and more reliable analysis of protein-ligand dynamics and improve screening results

Screening and MD simulation with Mab A



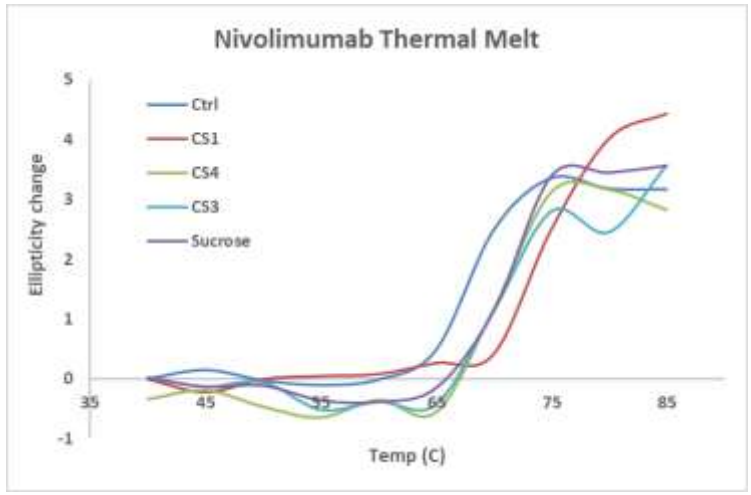
SQore

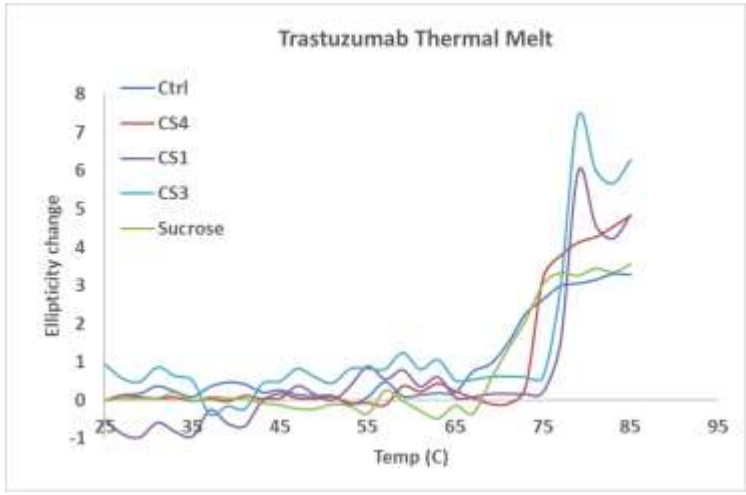
Computational

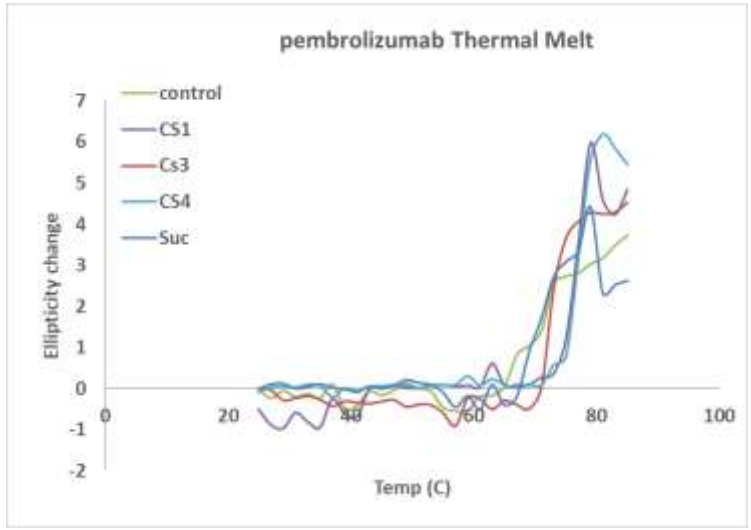


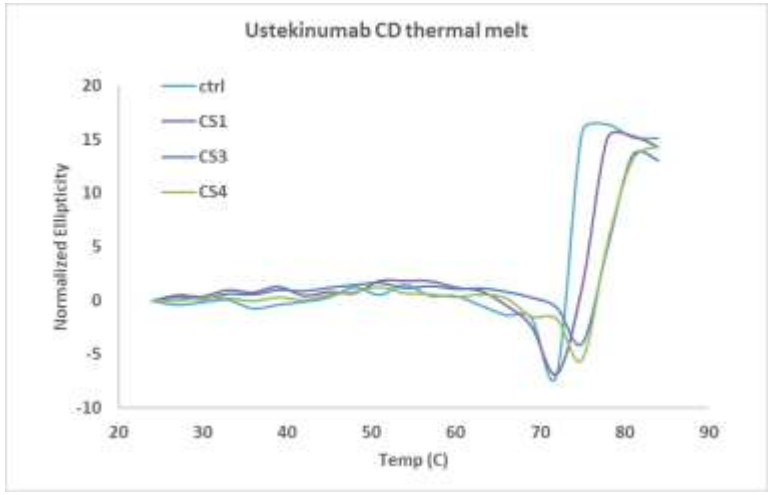
Excipients	FDA Inactive Ingredient	USP / GMP	Prior injectable use	GRAS
CS1	Yes	Yes	Yes	Yes
CS2	No	Yes	No	Yes
CS3	No	Yes	No	Yes
CS4	Yes	Yes	No	Yes
CS5	Yes	Yes	Yes	Yes

Effect of stabilizers on mAb thermal stability





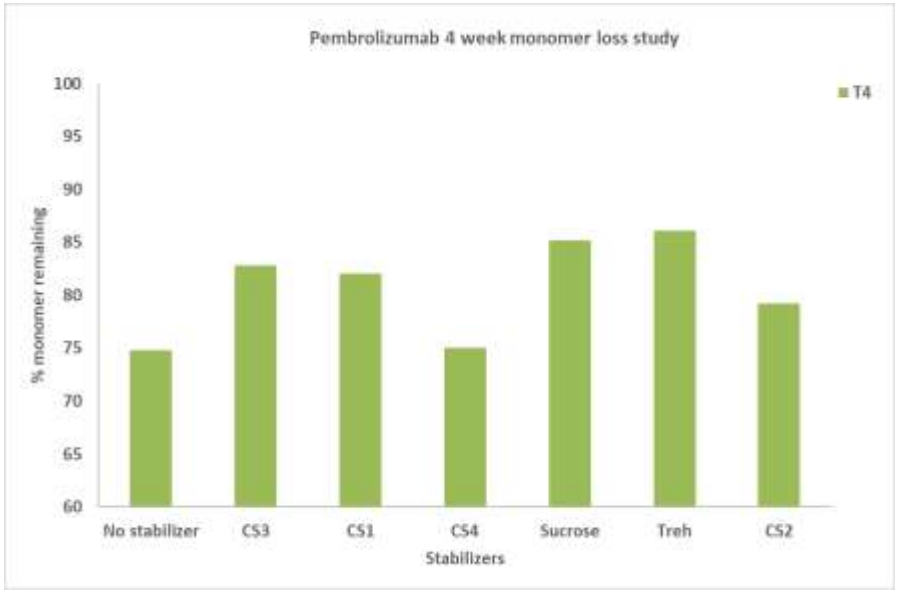


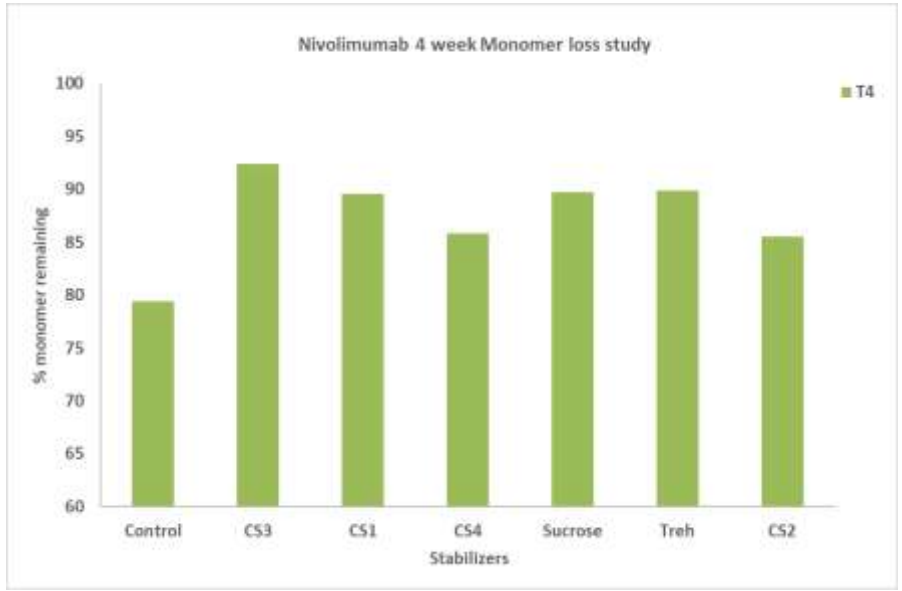


- **Stabilizers validated with CD thermal melt**
- **mAb conc: 0.2 mg/ml mAb**
- **Stabilizer conc: 20 mM**
- **Temp ramp 1C/ min**

- **Novel stabilizers improve the thermal stability by shifting melting temp**
- **Thus they appear to be similar or better than sucrose in stabilizing mAbs against thermal stress**

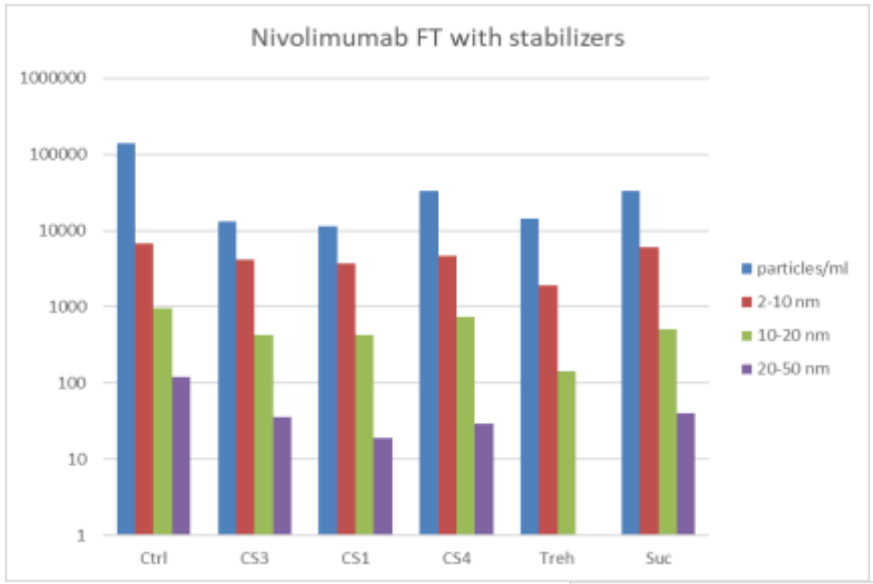
Effect of stabilizers on Isothermal stability of mAb

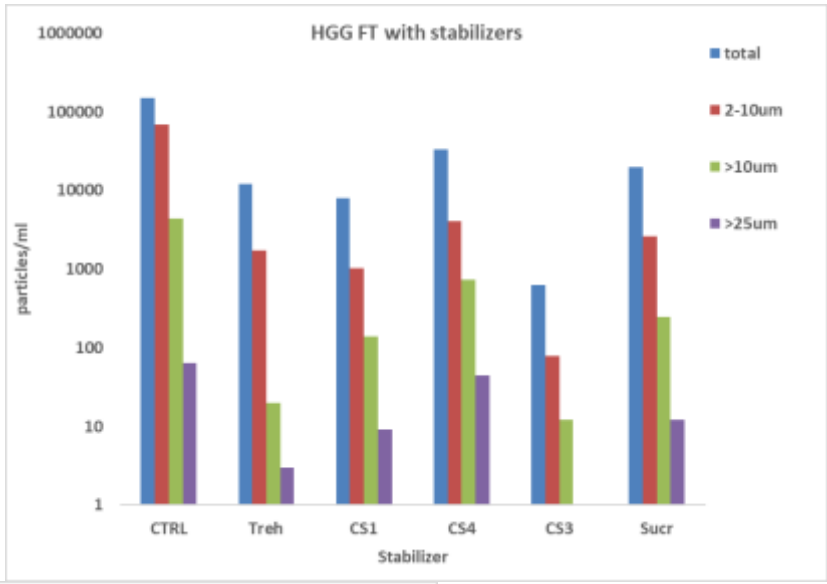


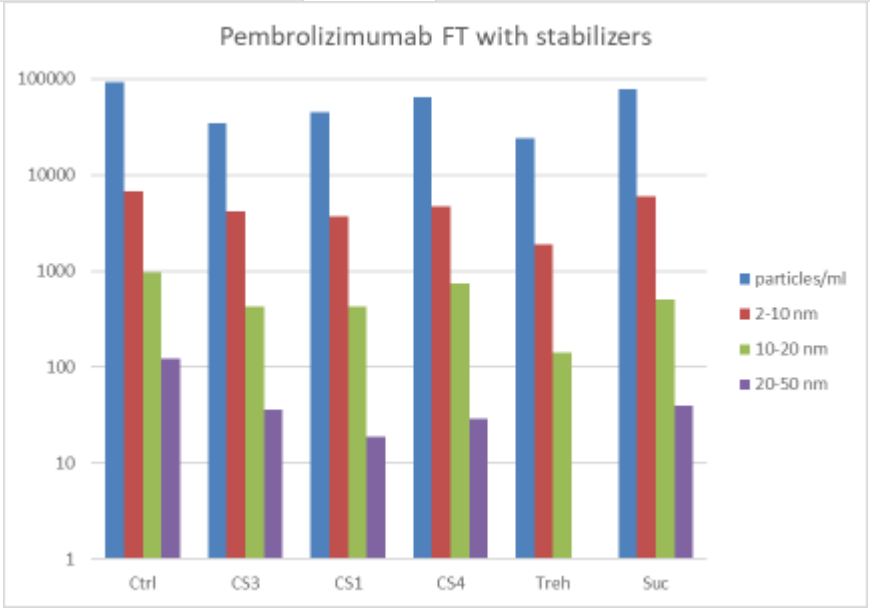


- **mAbs were formulated with the stabilizers and isothermally incubated at 40C for 4 wk**
- **5 mg/ml mAb**
- **saccharide conc: 0.2M**
- **All stabilizers were effective in improving thermal stability as compared to no stabilizer control**

Effect of stabilizers on Freeze thaw stability of mAbs







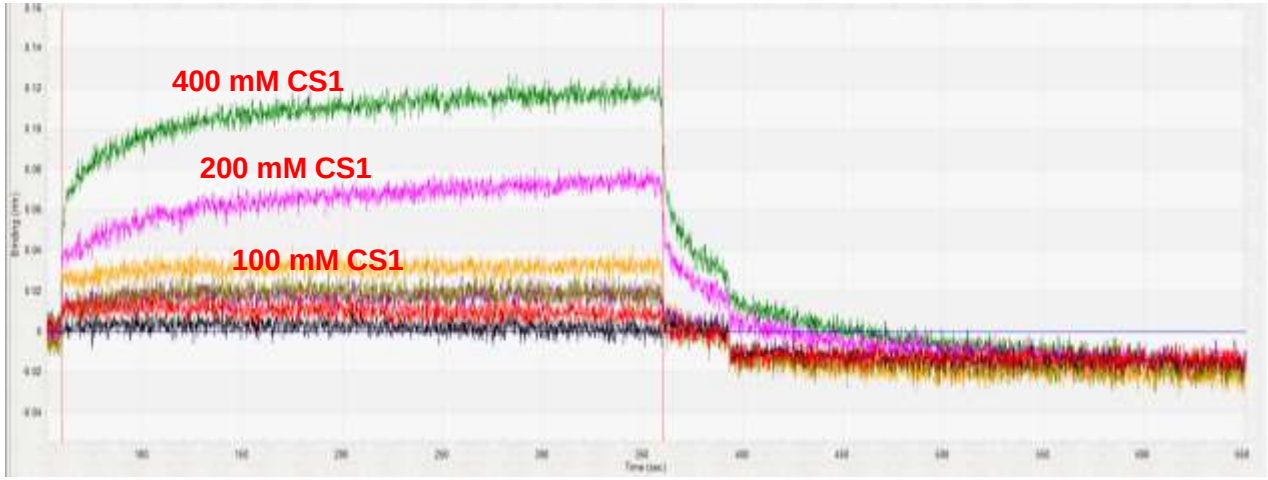
- **mAb conc: 10 mg/ml**
- **saccharide conc: 0.2M**
- **10 FT cycles**
- **Samples analyzed by flowcam**

- **Stabilizers showed effectiveness in preventing particulate formation due to freeze thaw stress**

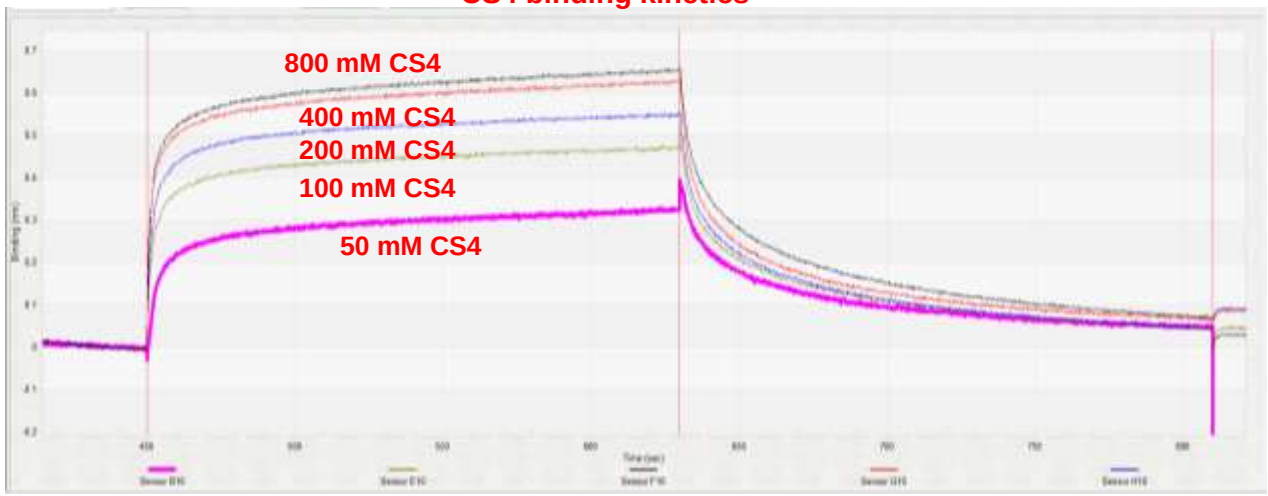
Binding kinetics and mechanism of stabilizers

- Interaction of stabilizers with mAbs was investigated using BLI octet
- Infliximab was biotinylated and loaded onto SSA tips.
- Binding kinetics determined by direct interaction with upto 1M stabilizers
- Kd calculated as 5.7 mM for CS1 and 6.9 for CS4

CS1 binding kinetics



CS4 binding kinetics



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Case Study

Developing an optimal formulation for mAb A using combination of excipients

mAb liquid formulation development: Buffer screening

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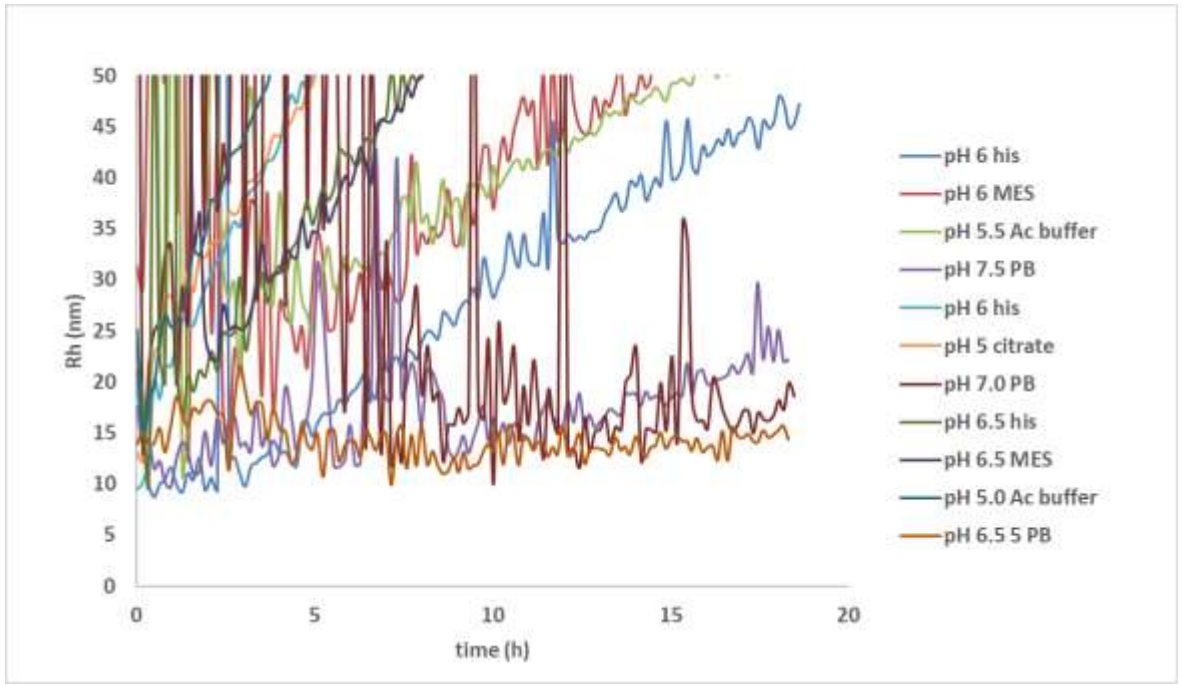
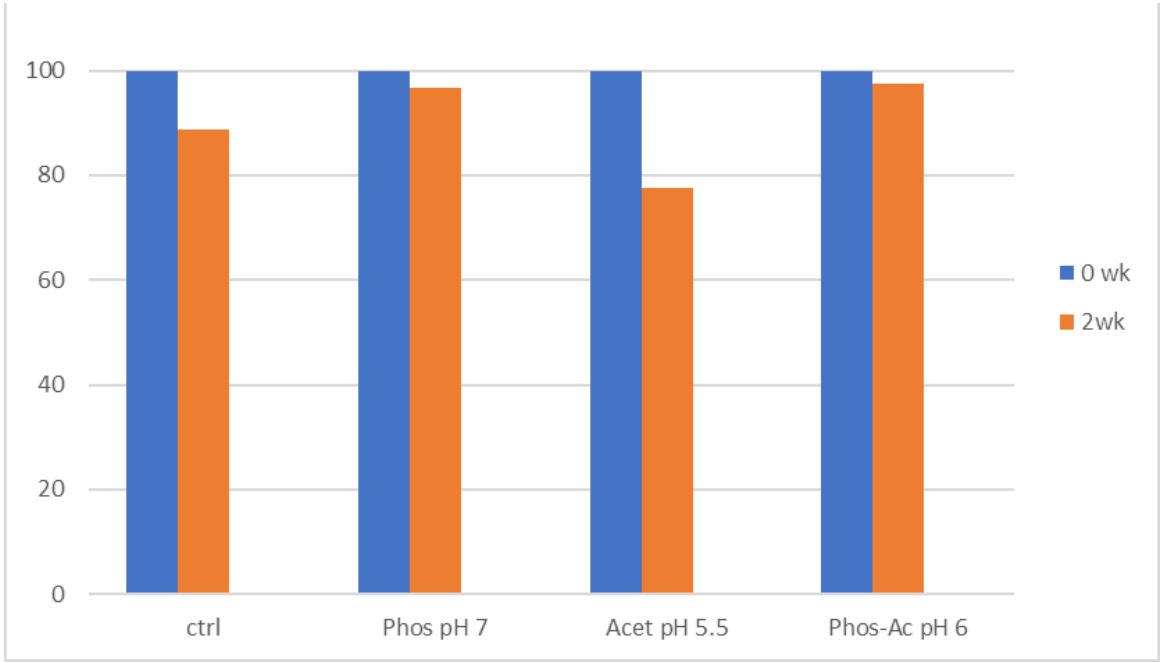
DLS screen



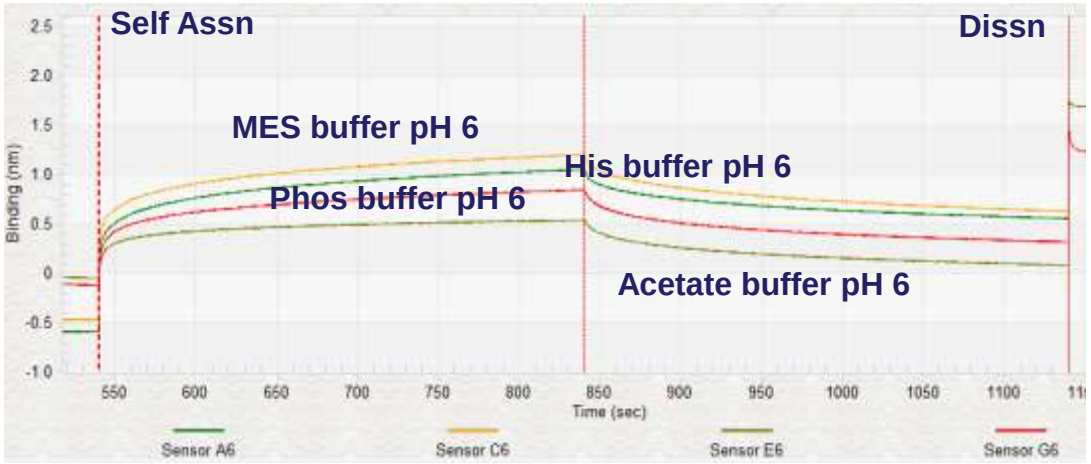
Accelerated stress screen

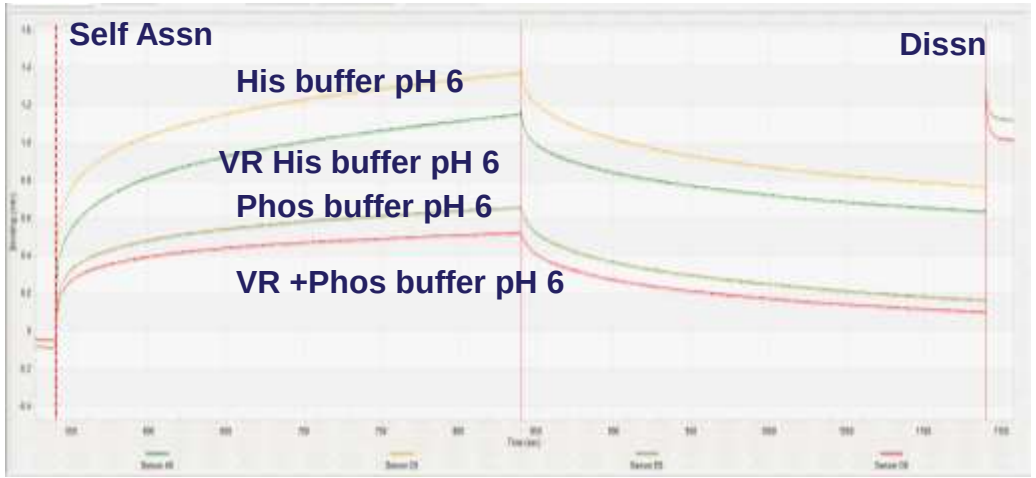


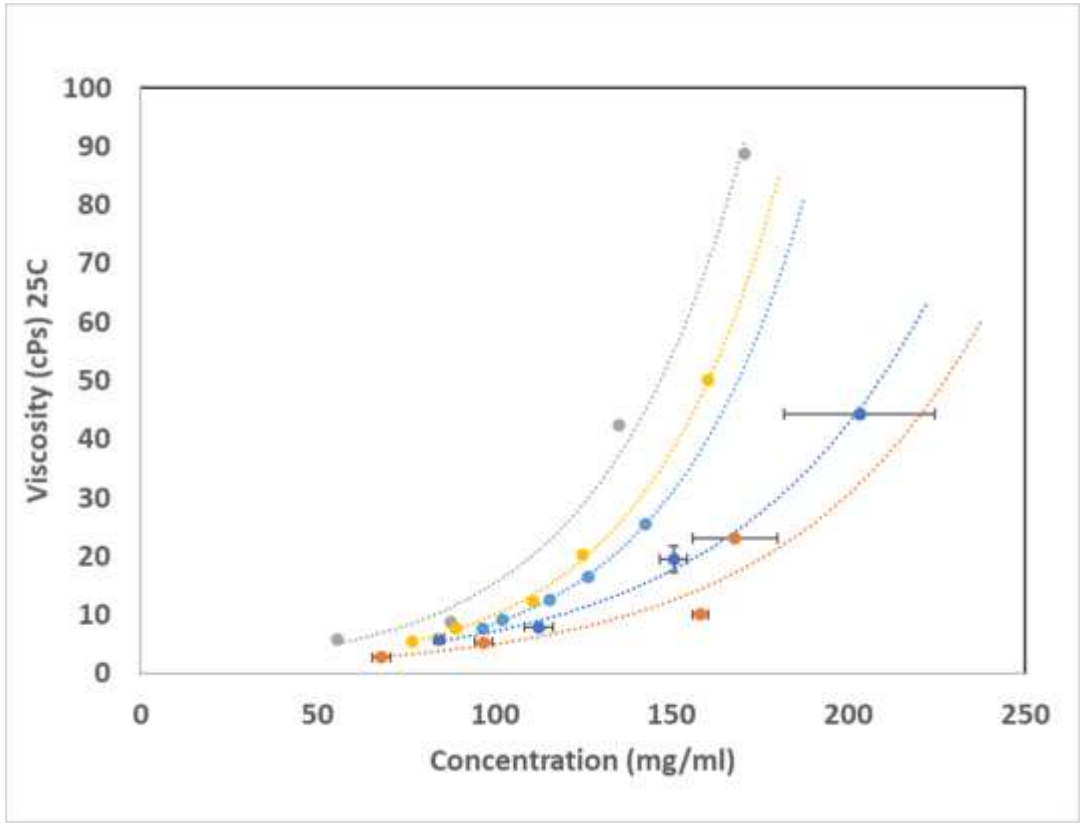
- DLS and BLI octet screening to identify pH and buffer
- Short Accelerated isothermal hold stress screening for 1-2 week
- Phosphate buffer was observed to improve stability

mAb liquid formulation development: viscosity screen

BLI octet screen



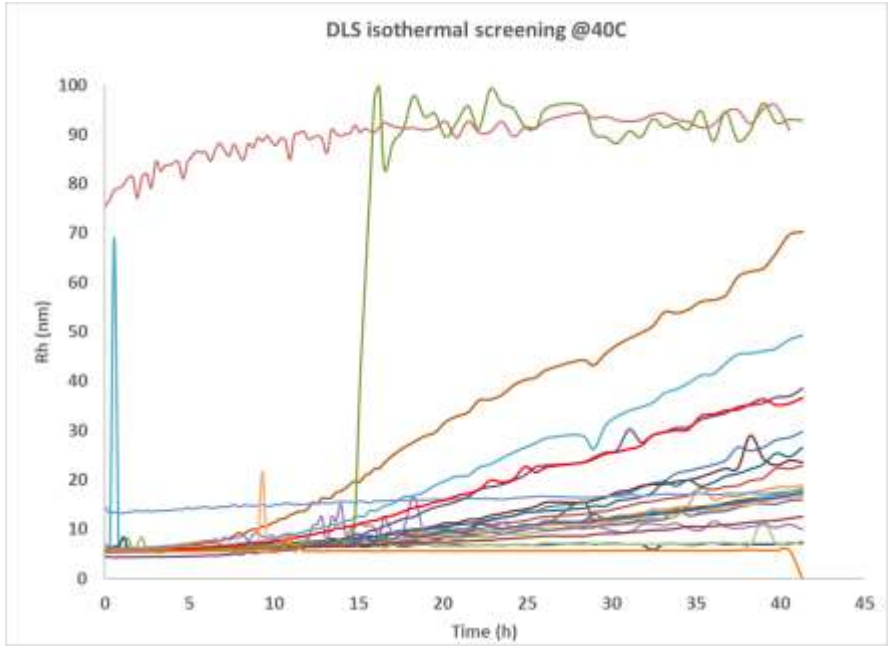




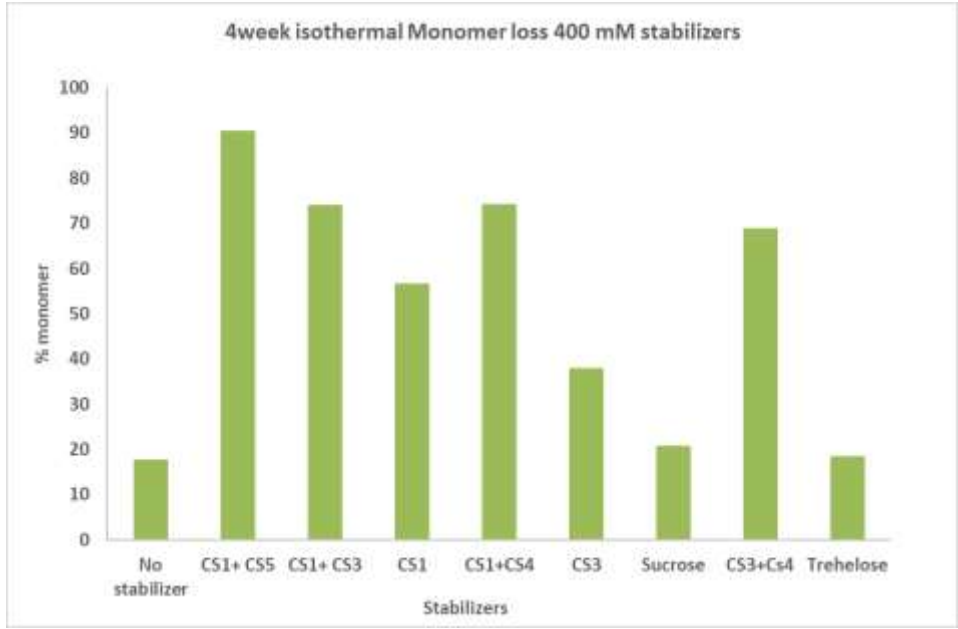
DLS, BLI octet and microvisc help to identify and validate viscosity reducing excipients

mAb liquid formulation development

- **DLS screening to identify stabilizing excipients**
- **Isothermal Accelerated stress screening at 45C**

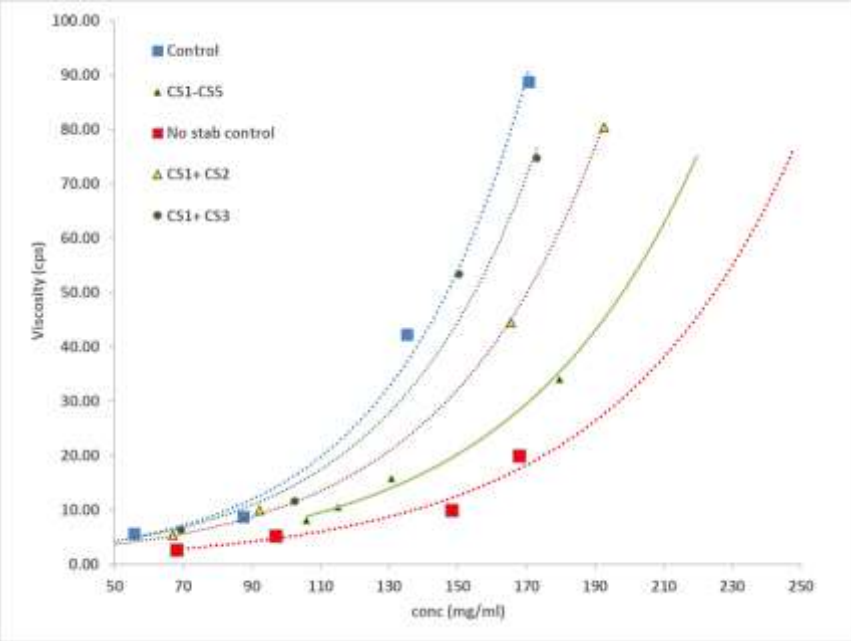


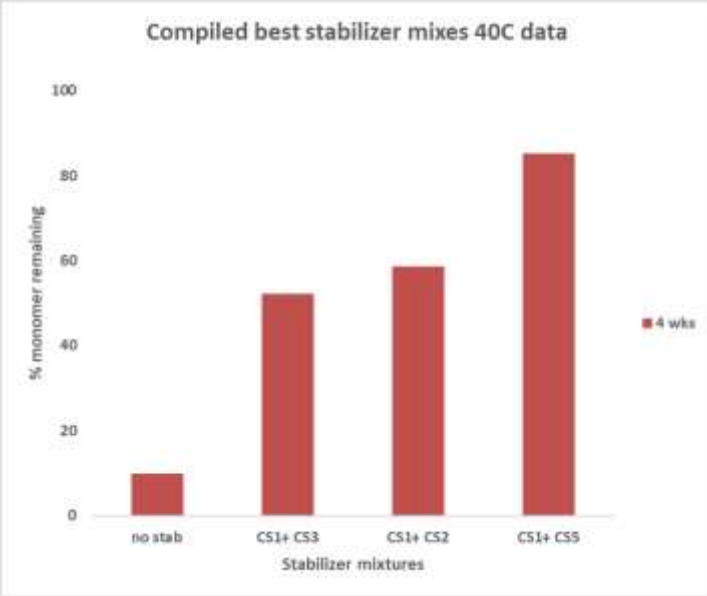
Selected excipients



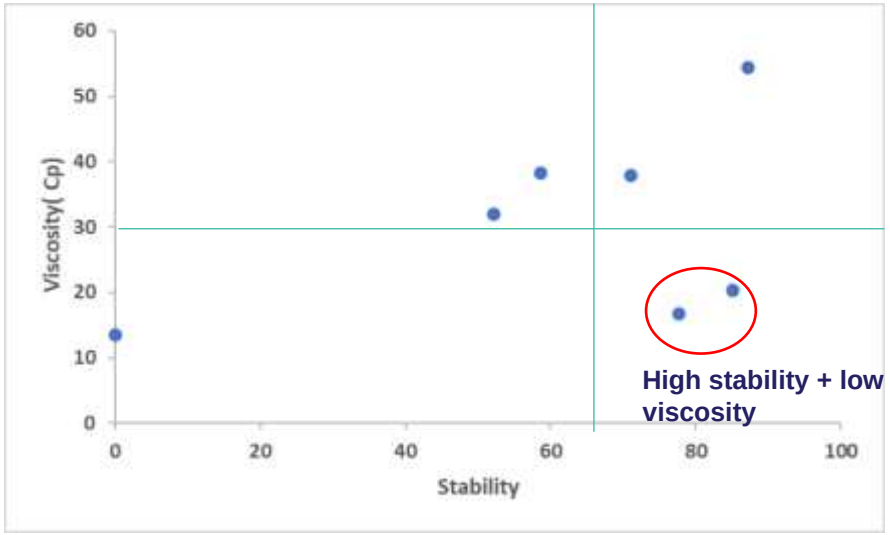
Mixtures were observed to improve stability better than single excipients

mAb liquid formulation development using SQore excipients





Excipient combination	Monomer stability 40C	Viscosity at 150 mg/ml
CS1+ CS2	52.19	32.05
CS1+ CS3	58.63	38.21
CS1+CS5	85.21	20.25
No stabilizer	20	13.5
No viscosity reducer	87.3	54.38

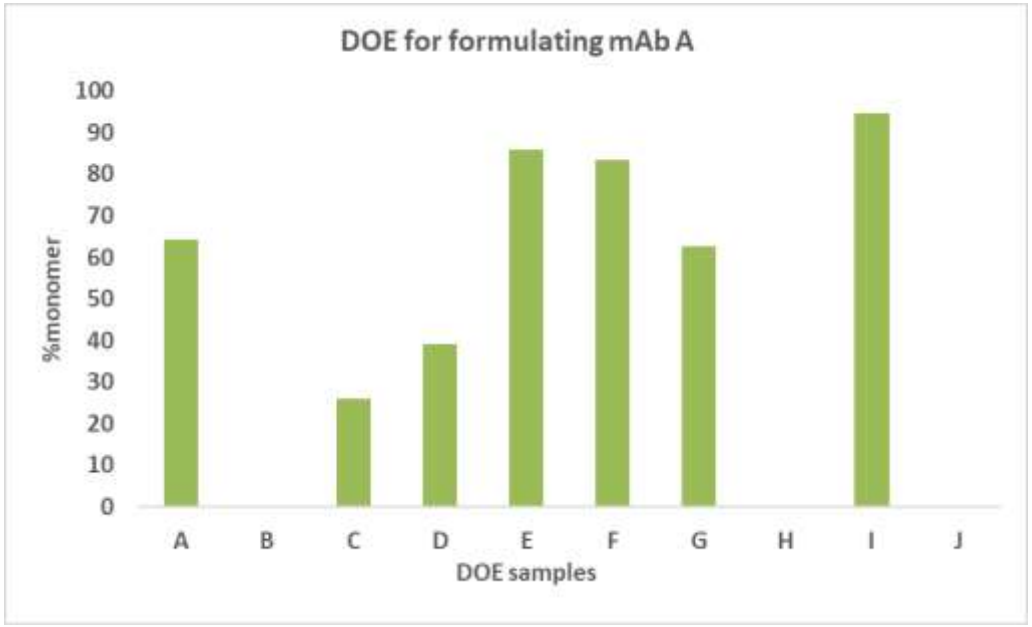


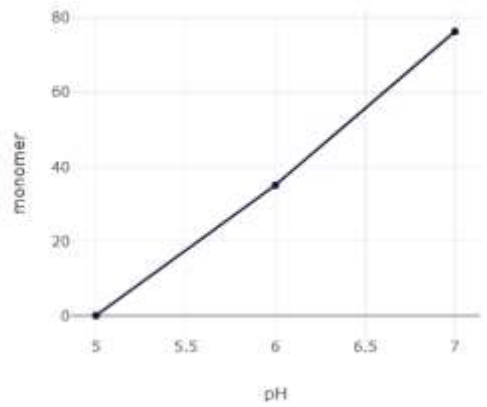
mAb A liquid formulation optimization by DOE

- Isothermal stability studies for optimizing mAb A formulation
- Set up isothermal hold studies at 40C for 4 weeks
  - mAb A: 150 mg/ml
  - Sugars total conc: 200-500 mM

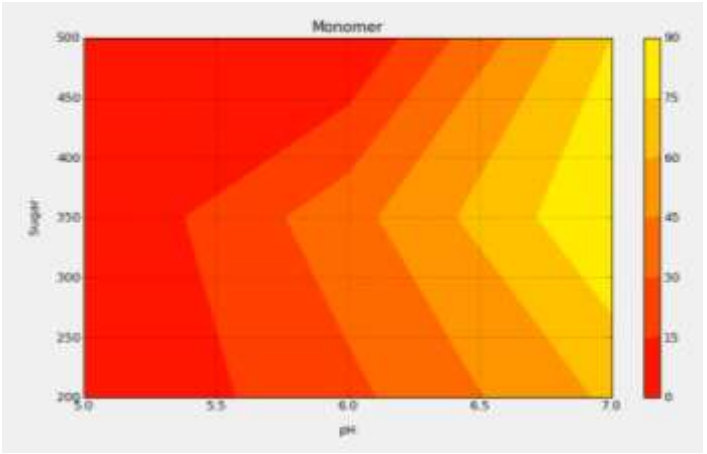
Formulation	pH	Sugars (mM)
A	7	500
B	5	500
C	6	200
D	6	350
E	6.5	500
F	7	350
G	7	200
H	5	350
I	6.5	350
J	5	500

- Recommended Formulation:
  - mAb A conc (150 mg/ml)
  - 5 mM phosphate buffer, pH 6.5
  - 350 mM sugar mix





Lower pH detrimental for stability



> 350 mM sugar mix helps stability

Summary and Conclusions

- Comera SQore™ platform provides excipient technology to address viscosity as well as stability issues for highly concentrated protein formulations enabling SQ administration of biopharmaceuticals.
- Comera can utilize computational as well as traditional screening to identify excipients
- Novel stabilizers show similar/improved profile as compared to sucrose or trehalose
- A mixture of stabilizers showed better stability profile as compared to single stabilizer at equivalent conc
- mAb can be easily optimized by DOE to obtain relatively stable low viscosity subQ formulation

Thank you!

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