LMU LUDWIG-MAXIMILIANS-UNIVERSITÄT MÜNCHEN
PHARMACEUTICAL TECHNOLOGY AND BIOPHARMACEUTICS
THE FRIESS GROUP

10 SEP 2024 WOLFGANG FRIESS

Lyso-phosphatidylcholine As Surfactant Alternative For Biologics Formulation

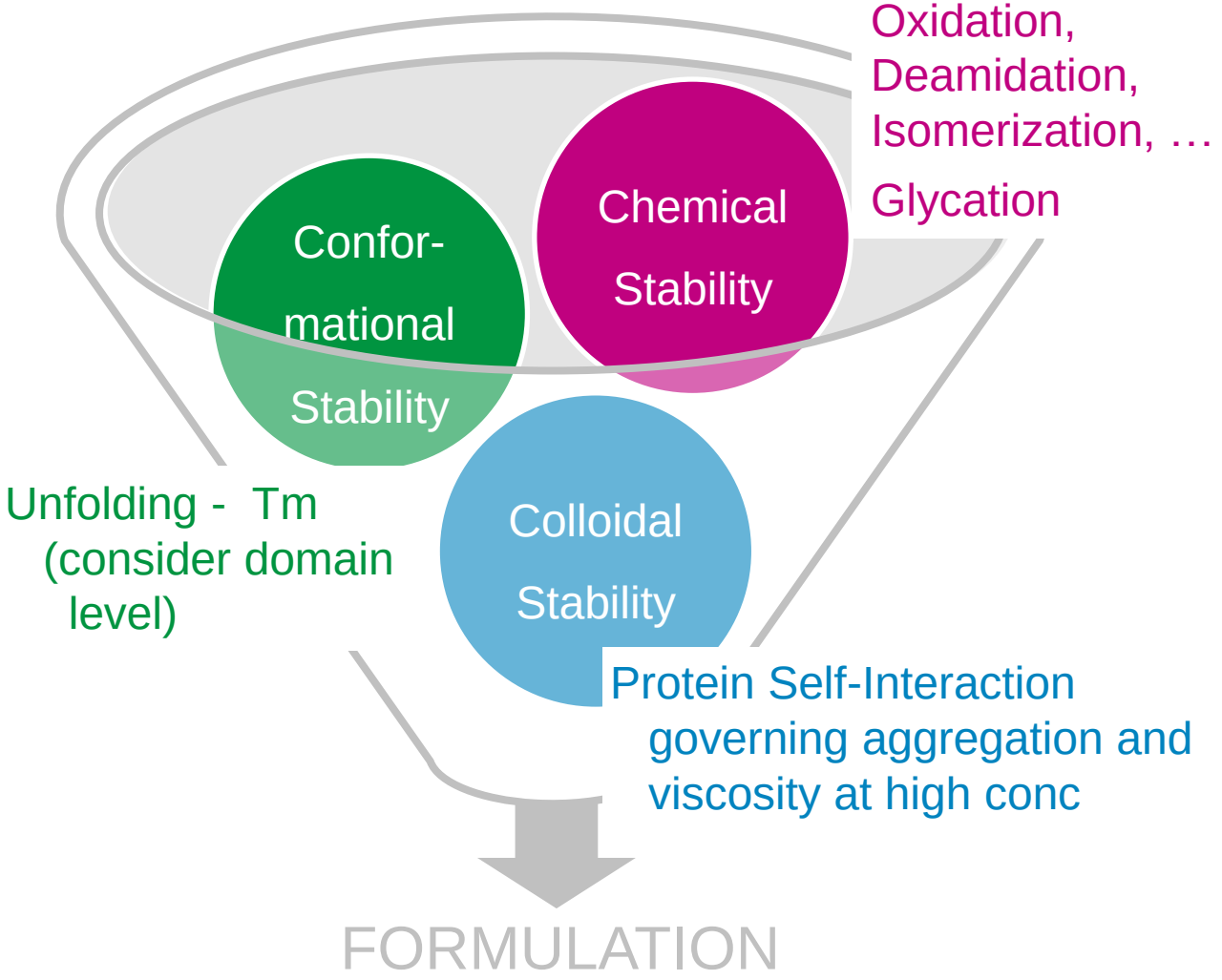
8th International Symposium on Phospholipids in Pharmaceutical Research

Heidelberg

Phospholipid Research Center

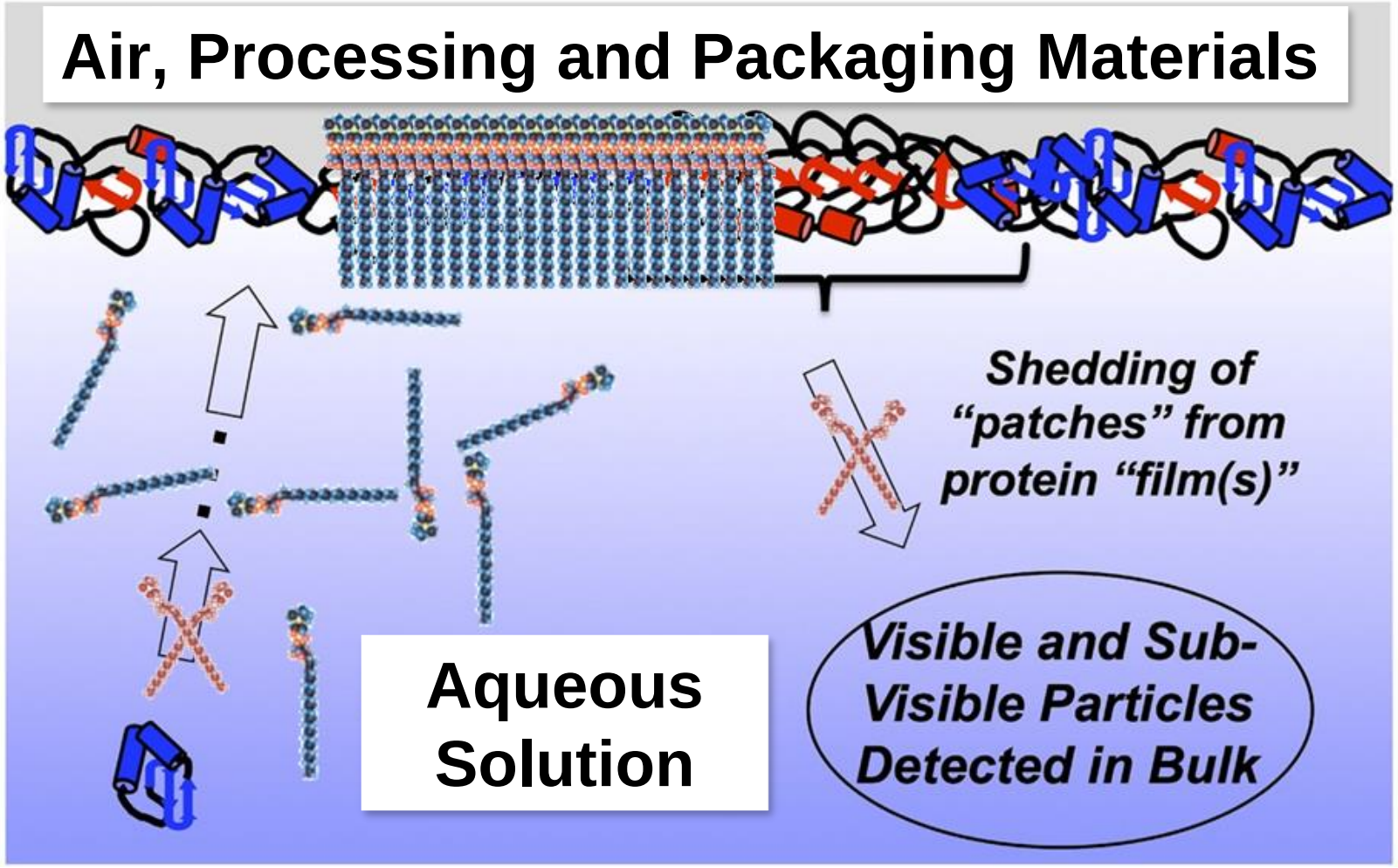
The Importance Of Protein Drugs – Top Sellers 2022

#1 Comirnaty®	SARS-CoV-2 vaccine (29.0 B$)
#2 Humira®	Anti-TNF mAb (21.2 B$) LIQ
#3 Spikevax®	SARS-CoV-2 vaccine (19.5 B$)
#3 Keytruda®	Anti-PD1 mAb (19.5 B$) LYO
#5 Eliquis®	Factor Xa inhibitor (11.9 B$)
#6 Revlimid®	Immunmodulator (11.3 B$)
#7 Stelara®	Anti-Il-12/IL-23 mAb (10.1 B$) LIQ
#8 Biktarvy®	Antiretroviral (9.7 B$)
#9 Opdivo®	Anti-PD1 mAb (8.9 B$) LIQ
#10 Dupixent®	Anti-IL-4/IL-13 mAb (7.4 B$) LIQ



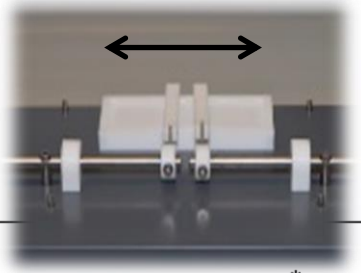
www.senieer.com; Chiu et al 2019

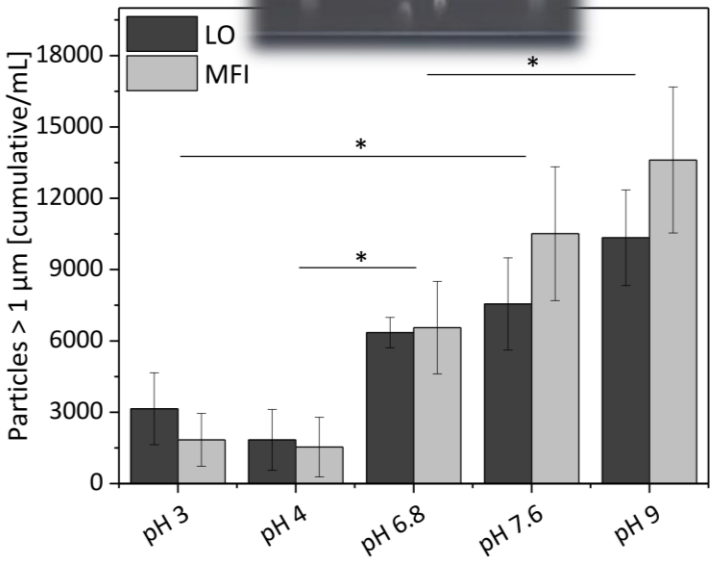
Protein Aggregation At Interfaces

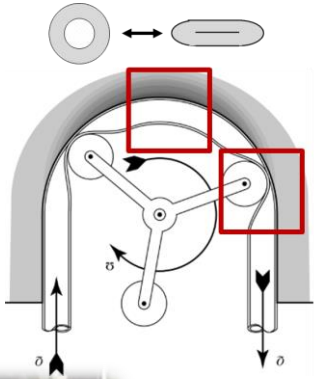


Protein Aggregation At Interfaces

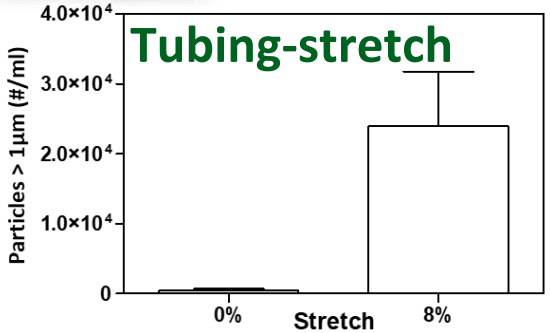
Mini-Trough

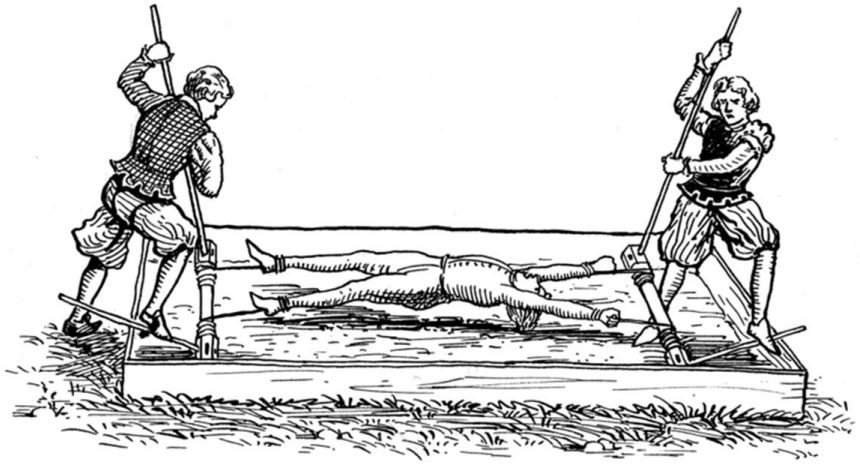






Tubing-stretch

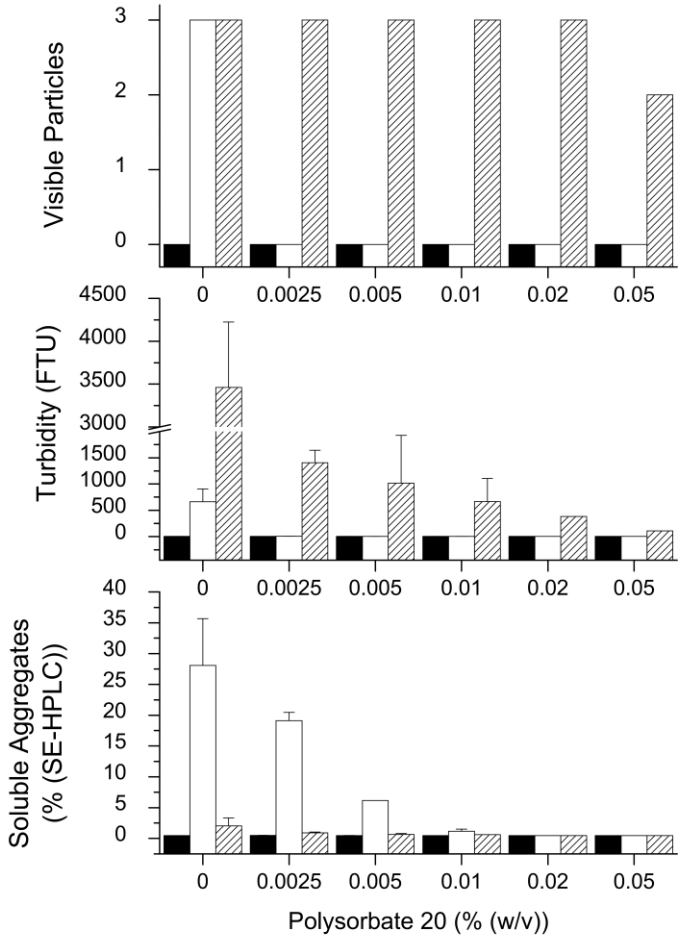




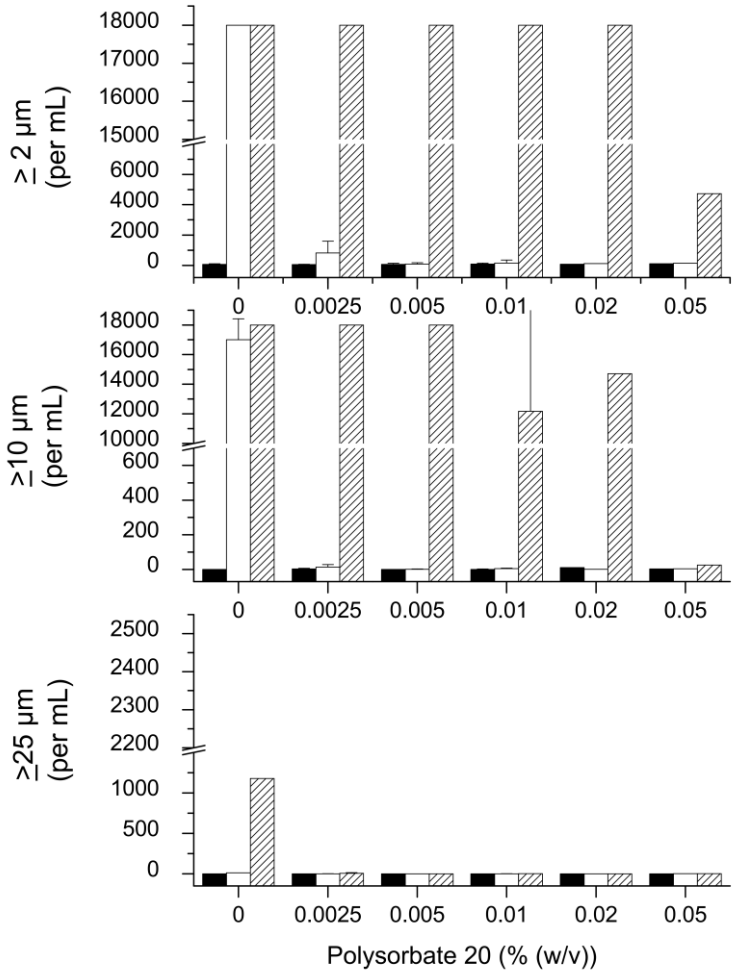
Pearson Scott Foresman

Koepf et al 2018; Deiringer & Friess 2022

Shaken, Not Stirred – Protection By Polysorbate

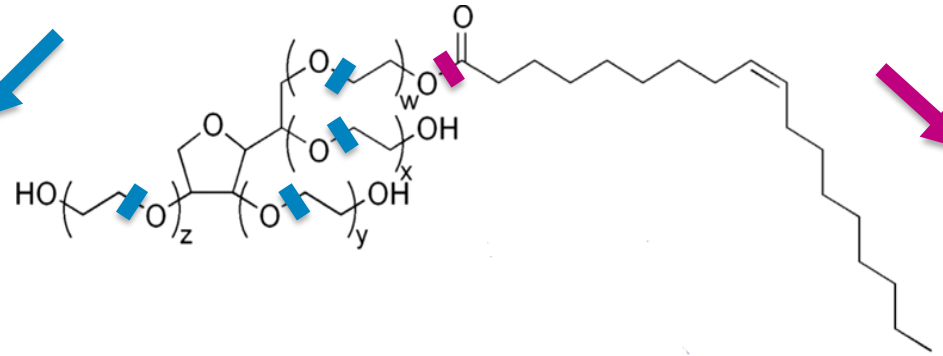


Kiese et al J Pharm Sci 2008



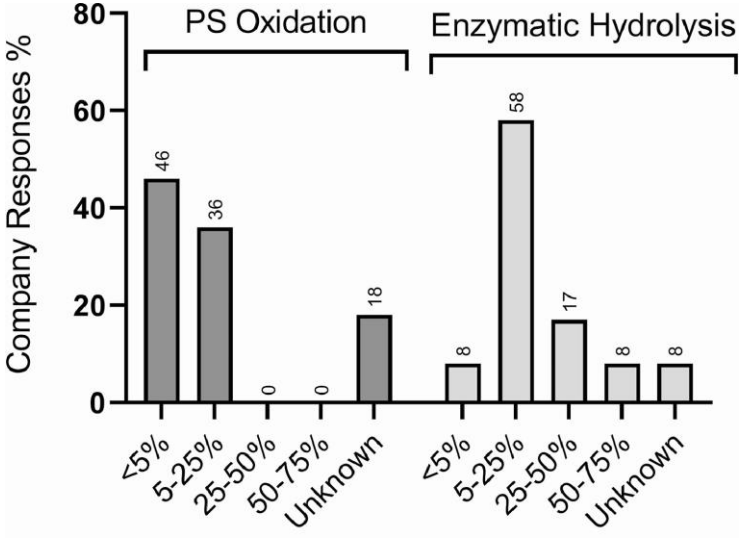
Polysorbate Challenges

Auto-Oxidation
fatty acid esters; aldehydes; acids;
Ketones; n-alkanes; peroxides



Hydrolysis
free fatty acids; POE sorbitan

Industry Survey: % of Products Affected by Degradation Pathway



Visual Particles in mAb Formulation with PS20 stored at 4°C

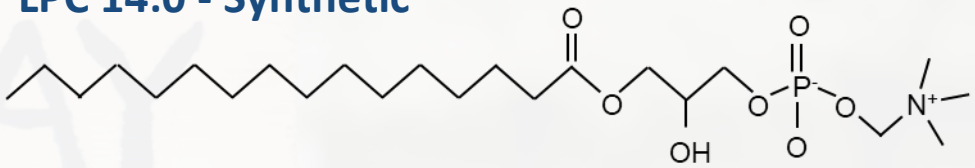
PS20	T0	4w	12w	26w	50w
0%	–	–	–	–	–
0.004%	–	–	–	+	++
0.020%	–	–	++	++	++
0.100%	–	–	–	–	–

Siska et al J Pharm Sci 2015; Wuchner et al J Pharm Sci 2022

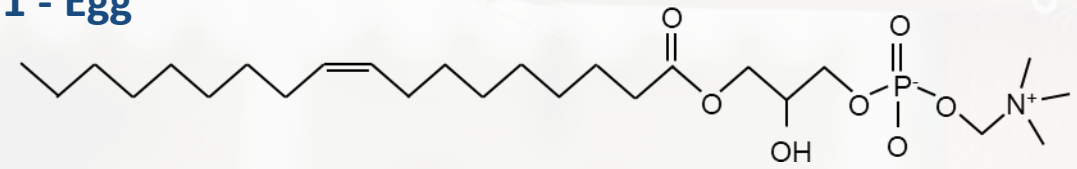
Are LPCs Suitable For Protein Formulation?

S LPC 80: PC 18:2 - Soy

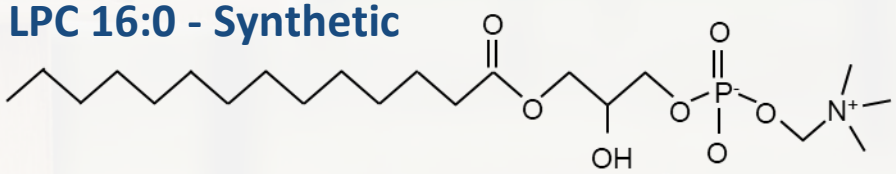
LPC 14:0 - Synthetic



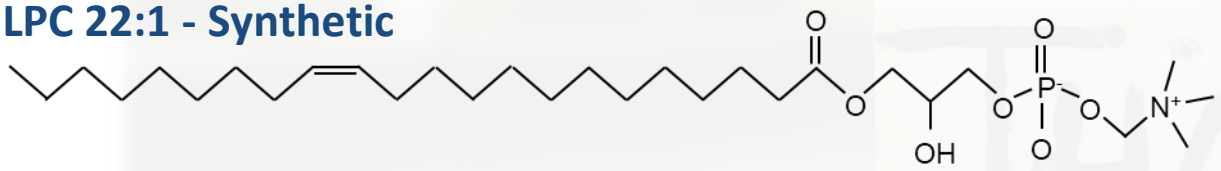
LPC 18:1 - Egg



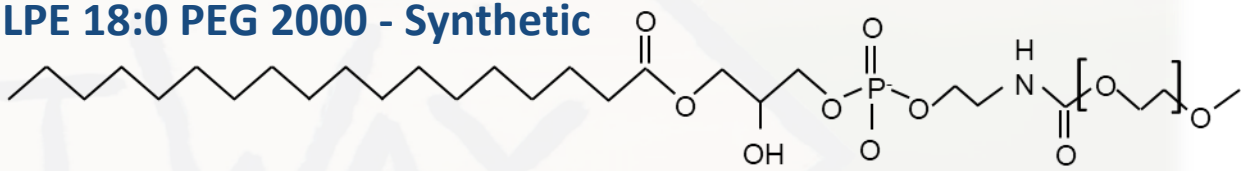
LPC 16:0 - Synthetic



LPC 22:1 - Synthetic

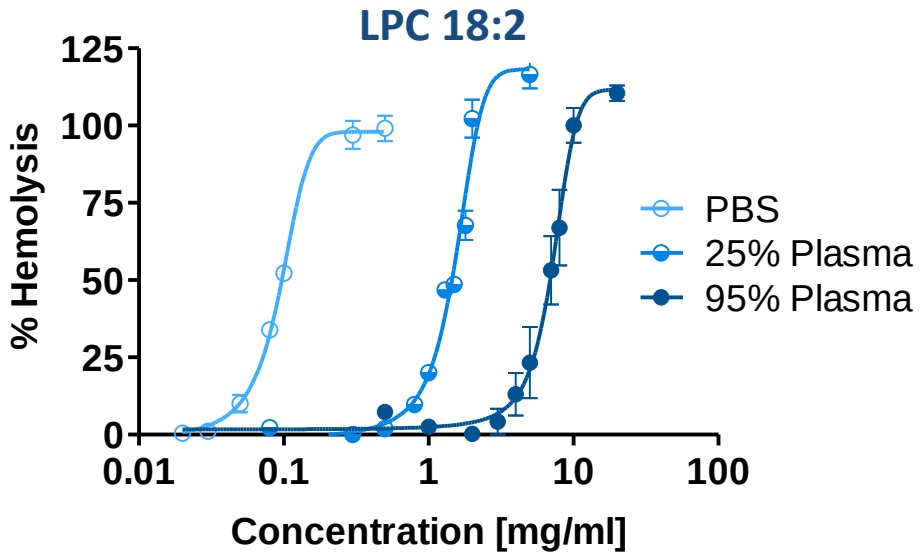


LPE 18:0 PEG 2000 - Synthetic



Is The Hemolytic Activity Of LPCs Critical?

- **Hemolysis in PBS**
  - HC50: 0.00345-0.525 mg/ml
- **Hemolysis in Plasma**



*in 95% plasma

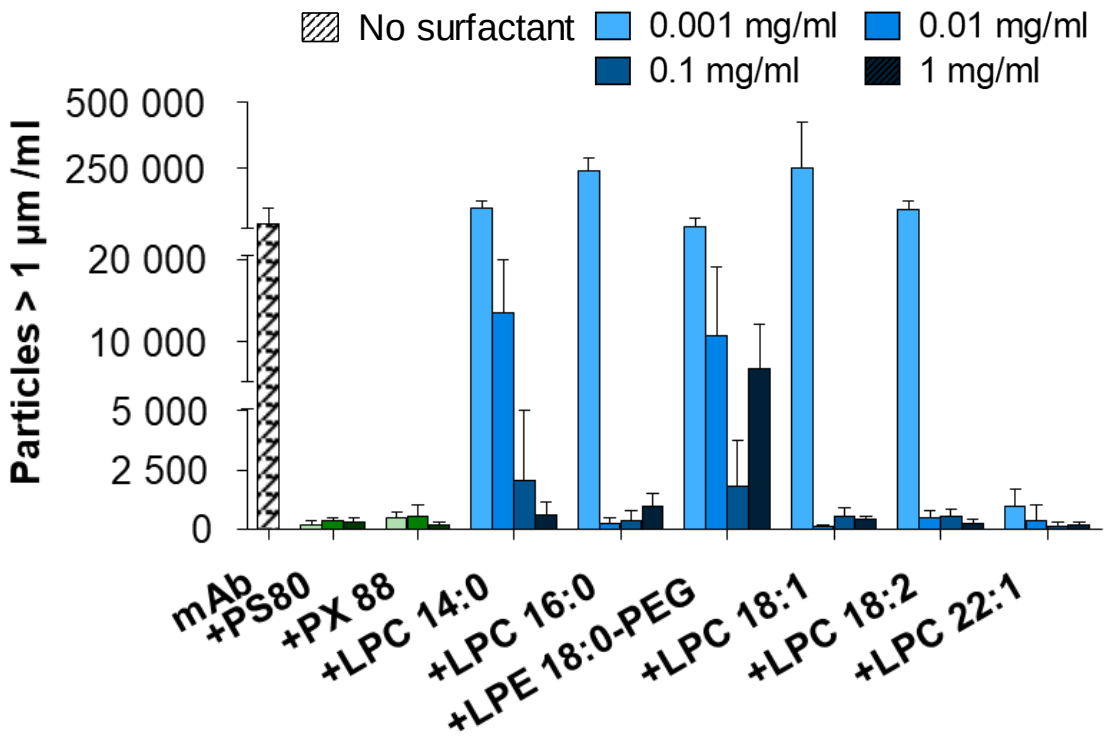
Surfactant	HC5* [mg/ml]	HC50* [mg/ml]	CMC [mg/ml]
LPC 14:0	1.3	2.0	0.006
LPC 16:0	1.9	2.9	0.001
LPE 18:0 PEG	> 20	> 20	0.0003
LPC 18:1	1.4	1.9	0.007
LPC 18:2	2.5	6.9	0.003
LPC 22:1	3.3	4.0	0.0003
PS80	> 20	> 20	0.007
PX188	> 20	> 20	

Laborwert.info; Weltzien 1973; Reman et al 1969

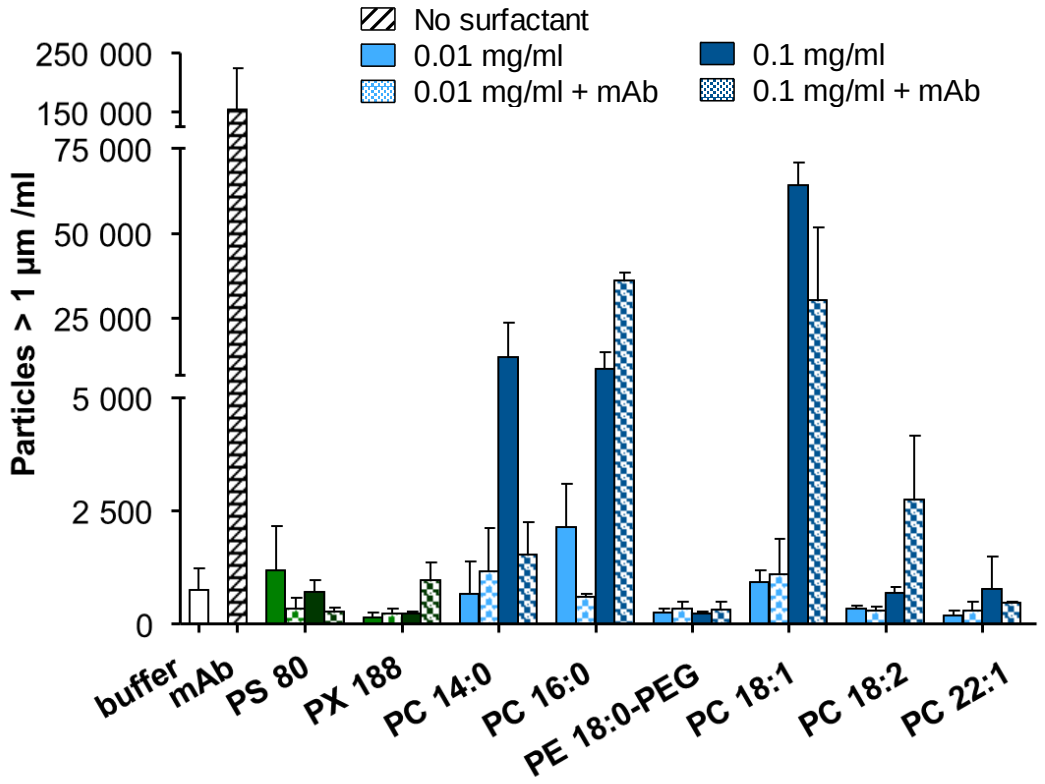
Can LPCs Stabilize Protein Against Interfacial Stress?

Particle Formation Upon Shaking And Freeze/Thaw

Shaking of mAb solution



Freezing and thawing of mAb solution



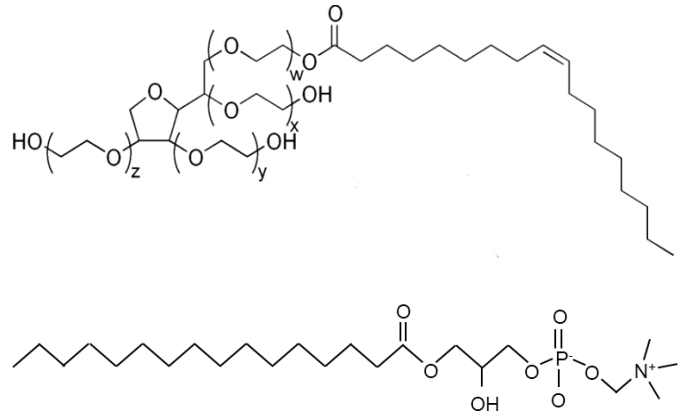
20 mM His pH 5.4; 1 mg/ml mAb

… and no quantifyable interaction with mAb in solution (CG-MALS & ITC)

How Chemically Stable Are LPCs?

Surfactant Remaining After 30 Days Storage

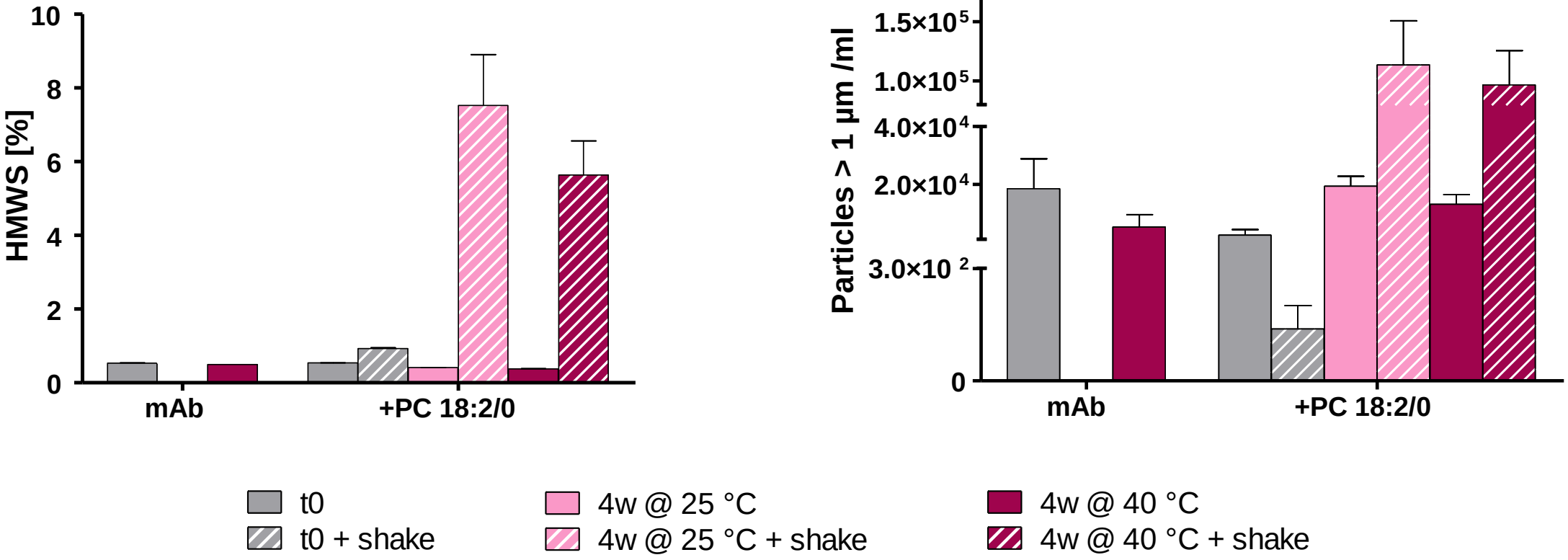
	25 °C				40 °C		
	pH 5.5		pH 7.2		pH 7.2		
	+ E	- E	+ E	- E	-	+ 5mM H_2O_2	+ 25mM Met
PS 80	46	97	47	106			
LPC 14:0	106	103	95	103	97	91	97
LPC 16:0	98	96	99	97	99	90	103
LPE 18:0 PEG	94	99	73	74	15	1	85
LPC 18:1	98	99	108	100	95	77	94
LPC 18:2	71	68	69	66	35	27	79
LPC 22:1	99	98	99	100	101	0	99



E: Esterase

Stability Of mAb Formulations with LPC 18:2

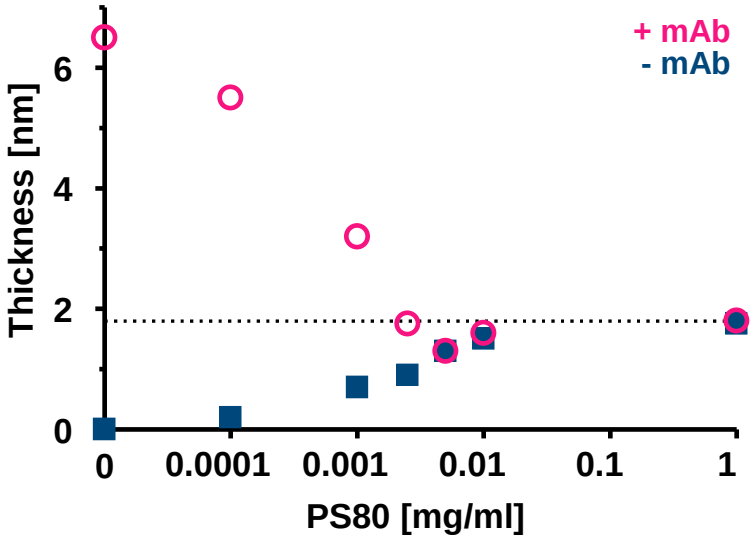
Liquid Up To 4 weeks

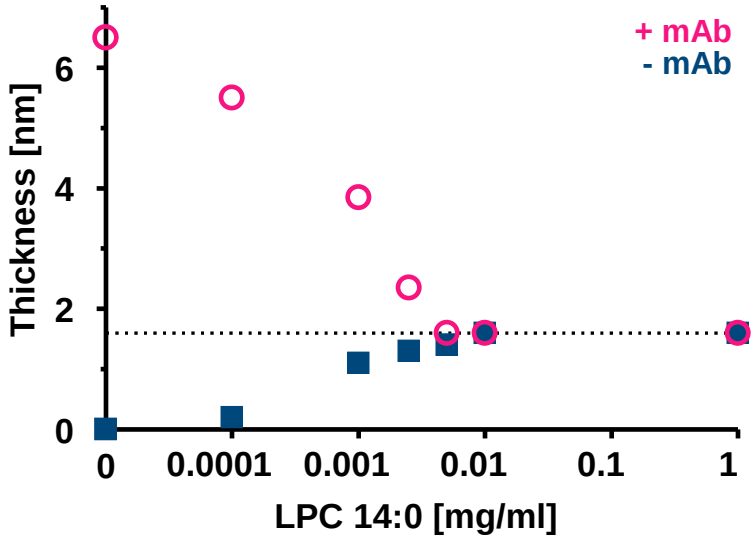


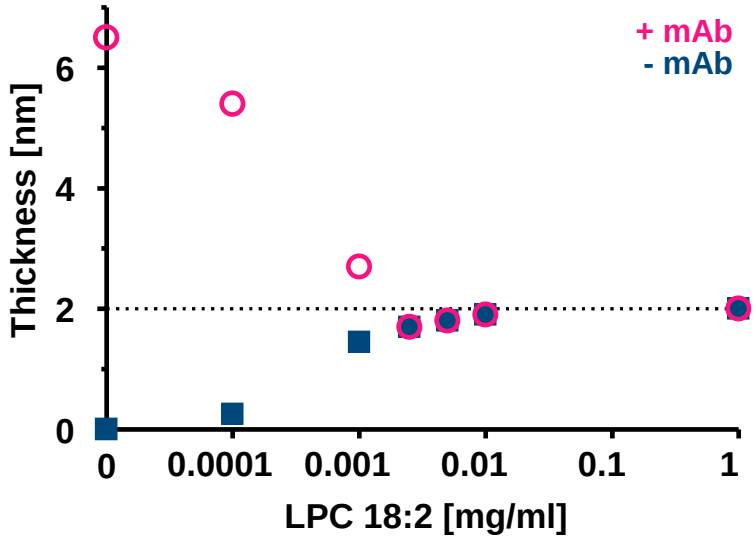
1 mg/ml mAb + 0.01 mg/ml LPC

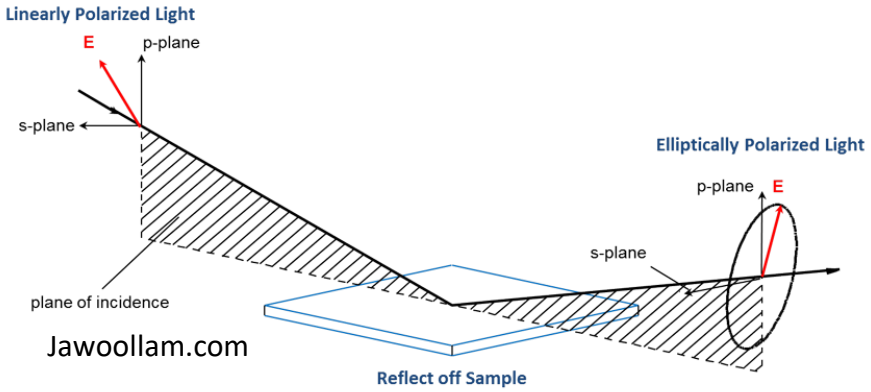
Do LPCs Form A Stabilizing Film?

LPC Film Characterization Using Ellipsometry

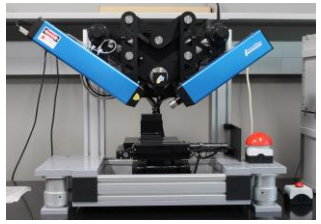








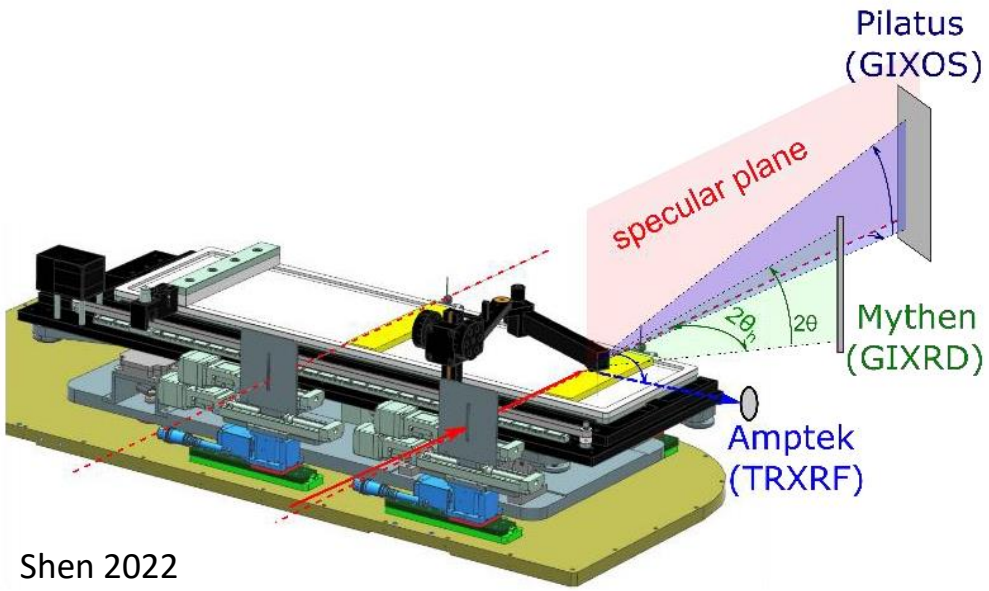
20 mM His pH 5.4; 1 mg/ml mAb



TECHNISCHE UNIVERSITÄT DARMSTADT

Prof. Dr. Emanuel Schneck
Prof. Dr. Gerald Brezesinski
Lukas Bange

LPC Film Characterization Using X-ray Techniques



Shen 2022

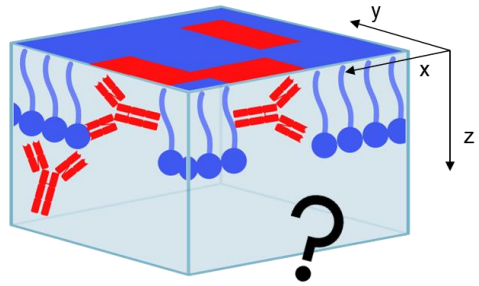
- Total Reflection X-ray Fluorescence spectroscopy (TRXF)

  Elemental composition in X-Y plane

Element	K_α [keV]
Na	1.04
Si	1.74
P (LPC)	**2.01**
S (mAb)	**2.31**
Cl	2.62

- Grazing Incidence X-ray Off-specular Scattering (GIXOS)

  Film thickness and structure in Z direction using electron density profile



Prof. Dr. Emanuel Schneck
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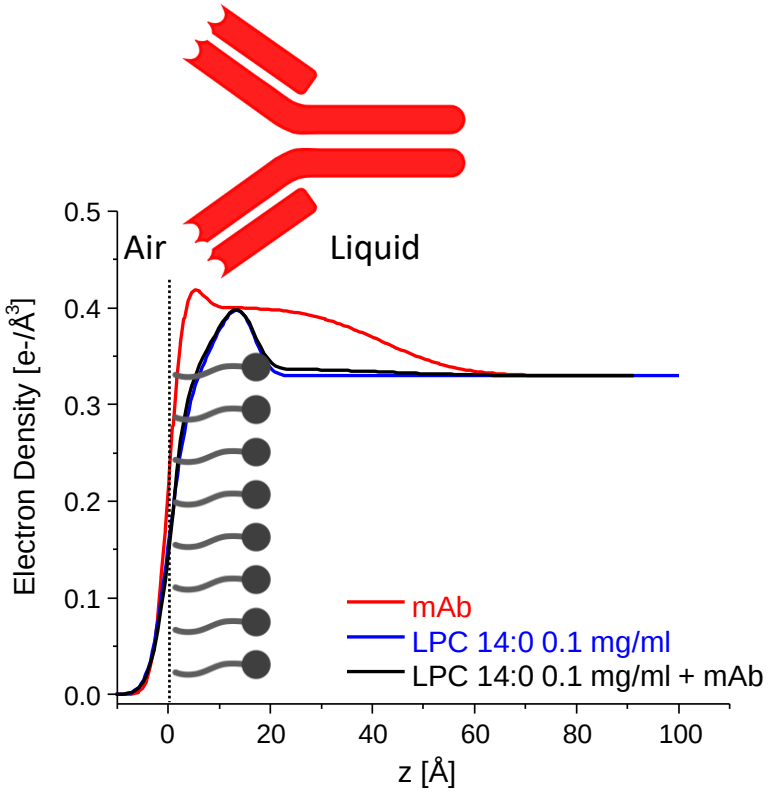
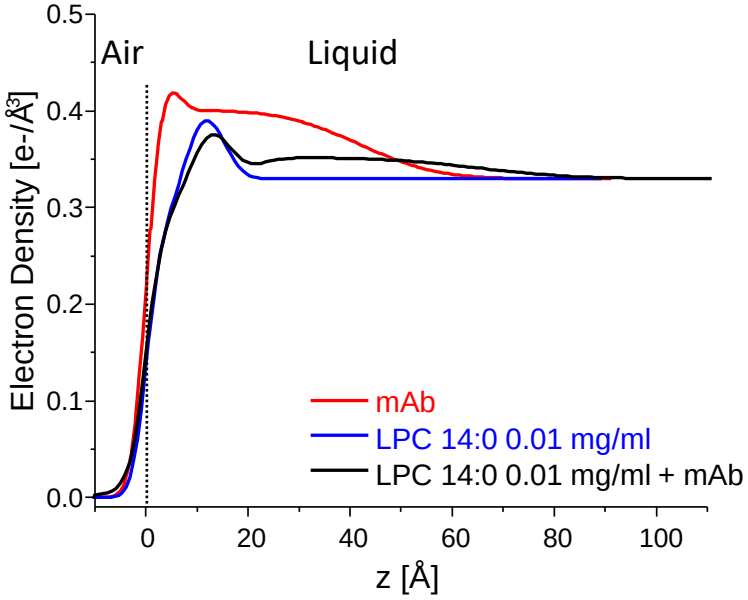
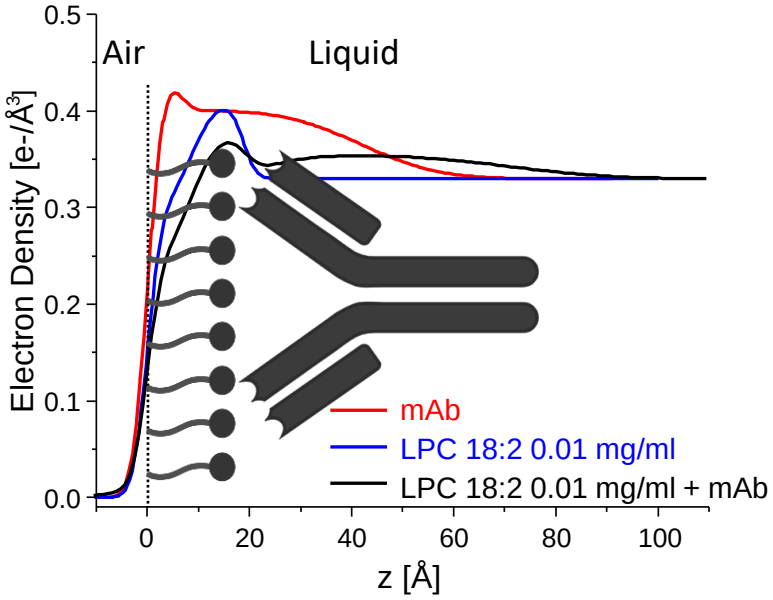
Lukas Bange

Do LPCs Form A Stabilizing Film?

Grazing Incidence X-ray Off-specular Scattering Of Adsorbed LPC Film

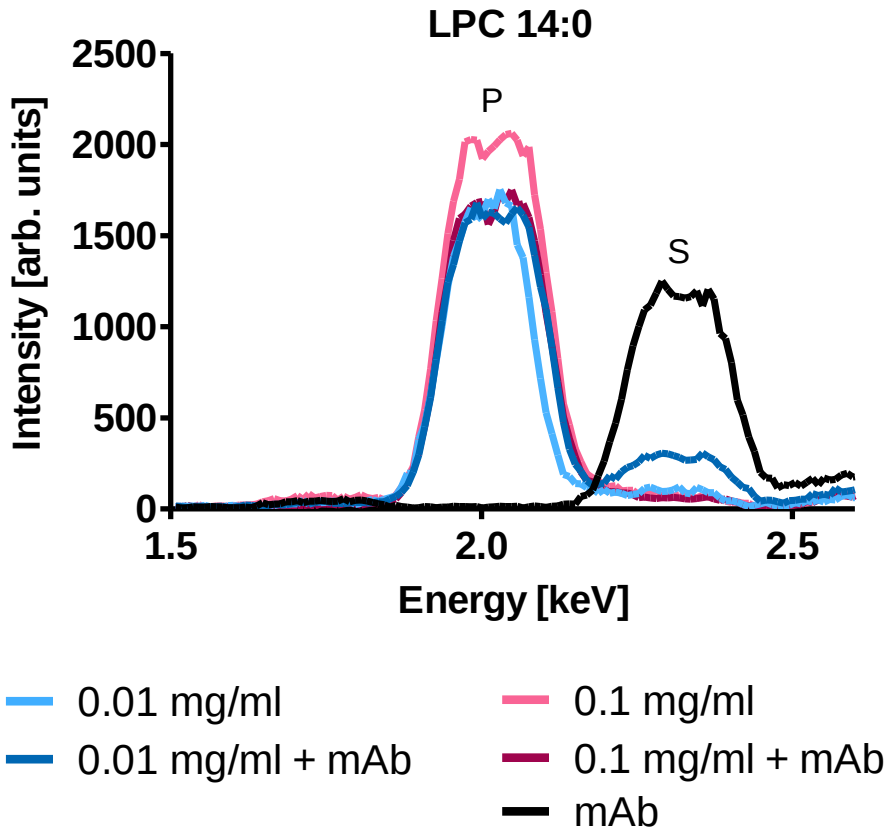






Do LPCs Form A Stabilizing Film?

Total Reflection X-ray Fluorescence Spectroscopy Of Adsorbed LPC Film

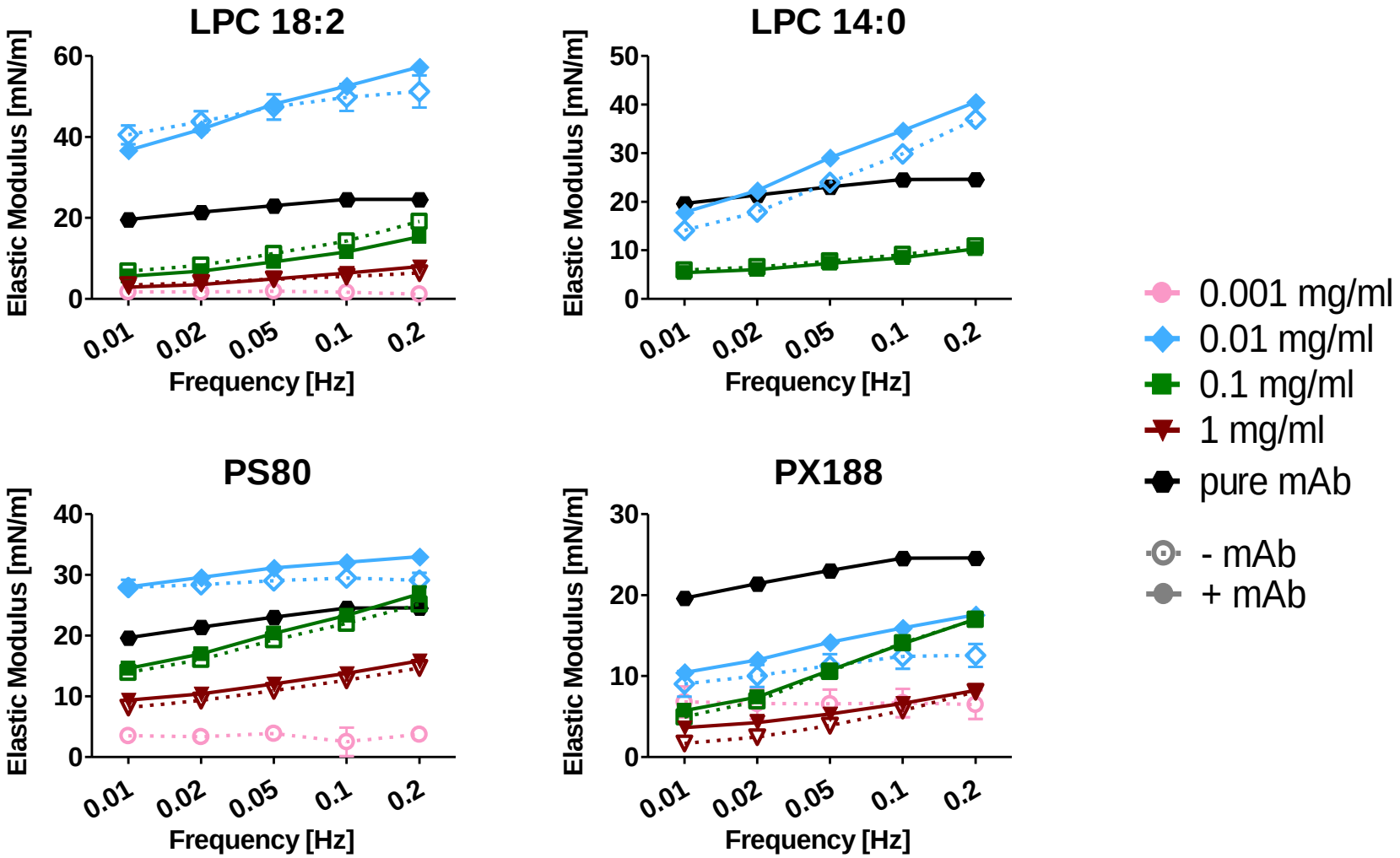


mAb molecules per 1000 nm²	
mAb	69
PS80 0.01 mg/ml	17
PS80 0.1 mg/ml	<LOD
LPC 14:0 0.01 mg/ml	14
LPC 14:0 0.1 mg/ml	<LOD
LPC 18:2 0.01 mg/ml	13
LPC 18:2 0.1 mg/ml	<LOD

1 mg/ml mAb

Do LPCs Form A Stabilizing Film?

Ipat Analysis +- 1g/L mAb

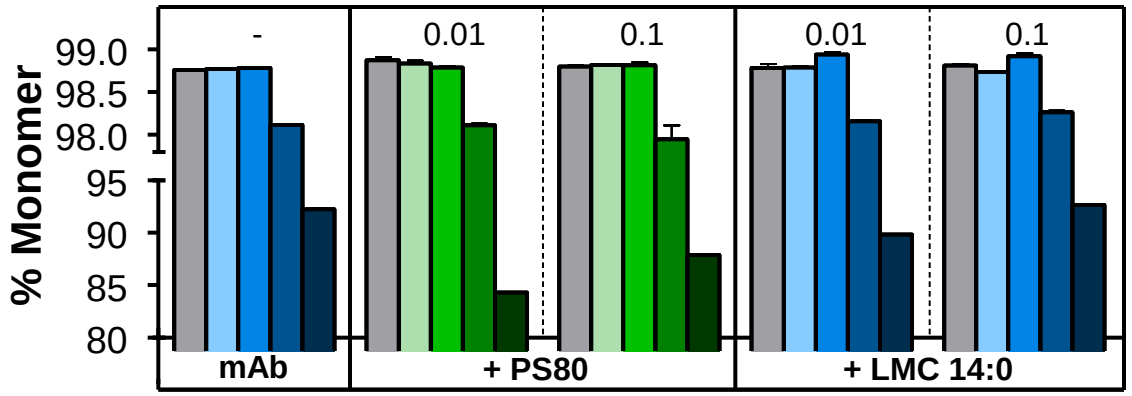


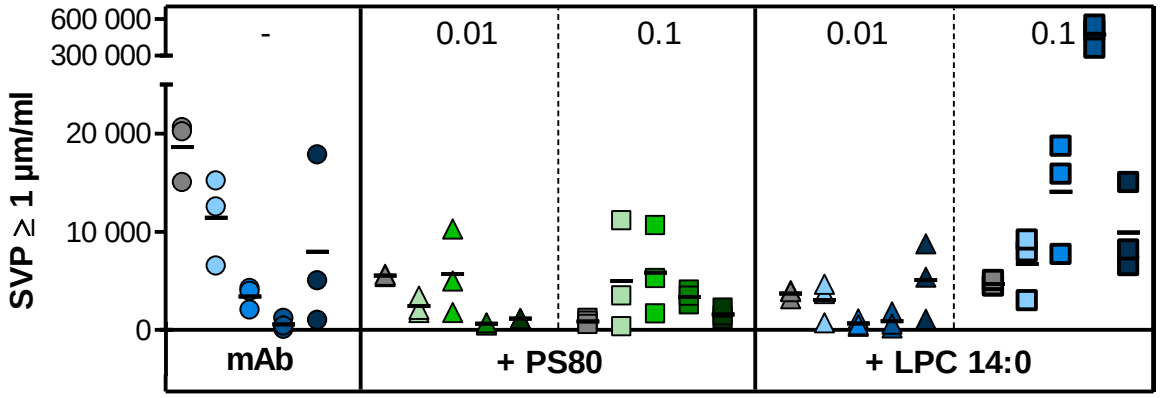
NOTE:

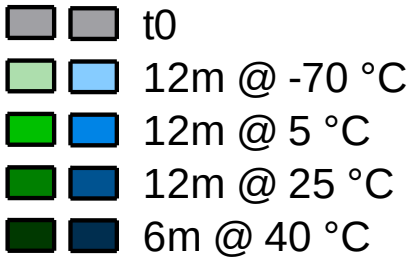
No Interaction between LPCs and mAb in solution (GC-MALS and ITC)

Long Term Stability Of mAb Formulations with LPC 14:0

Liquid Up To 12 months





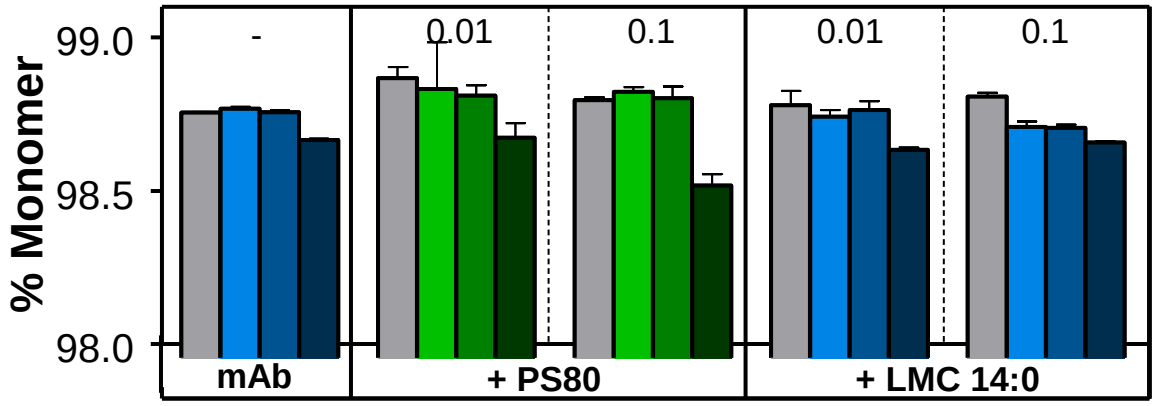


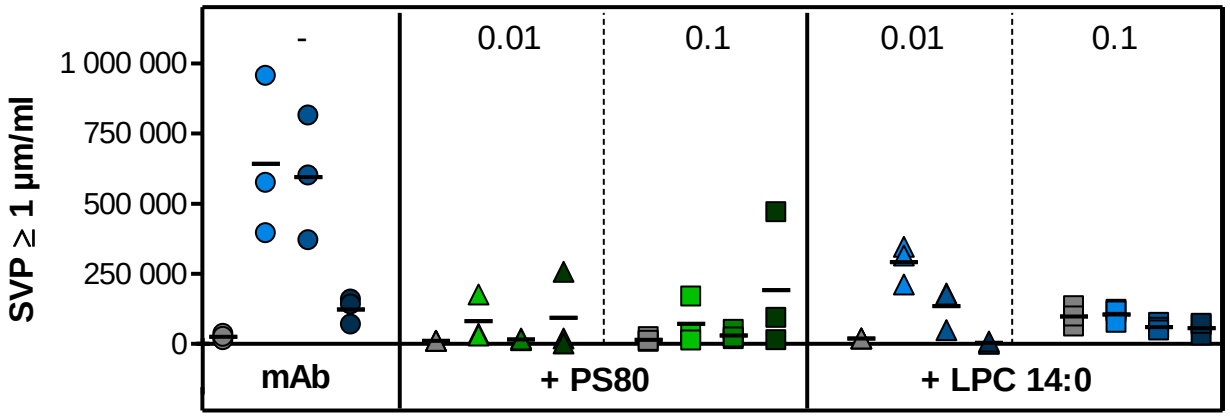
	-70 °C 12 mo	5 °C 12 mo	25 °C 12 mo	40 °C 6 mo
PS80 0.01 mg/ml	27	10	19	17
PS80 0.1 mg/ml	95	74	49	2
LPC 14:0 0.01 mg/ml	93	107	90	48
LPC 14:0 0.1 mg/ml	111	103	35	44

2 mg/ml mAb, 10% sucrose, 20 mM His pH 5.4

Long Term Stability Of mAb Formulations with LPC 14:0

Lyophilisate Up To 12 months



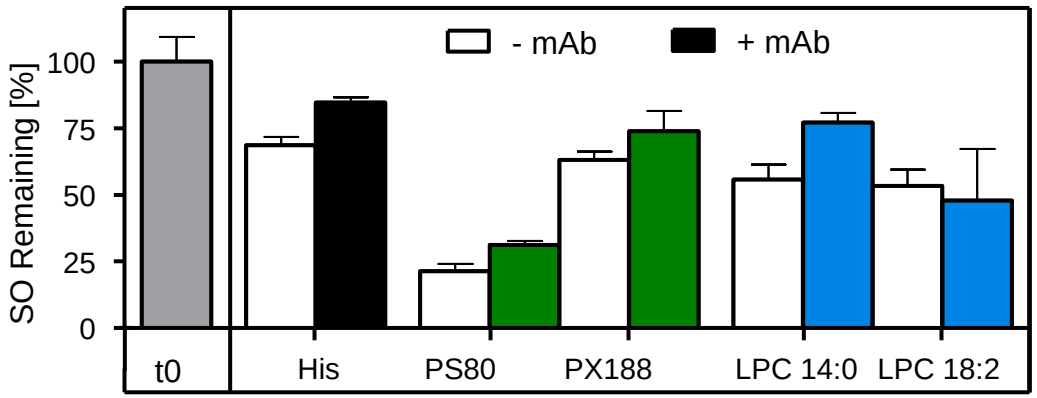


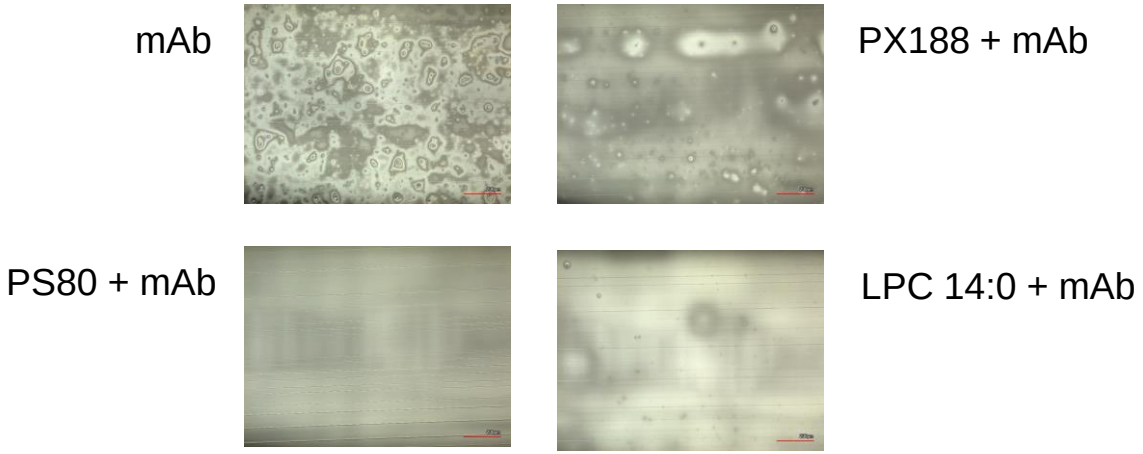
	5 °C 12 mo	25 °C 12 mo	40 °C 6 mo
PS80 0.01 mg/ml	43	50	38
PS80 0.1 mg/ml	86	91	48
LPC 14:0 0.01 mg/ml	95	96	90
LPC 14:0 0.1 mg/ml	100	101	100

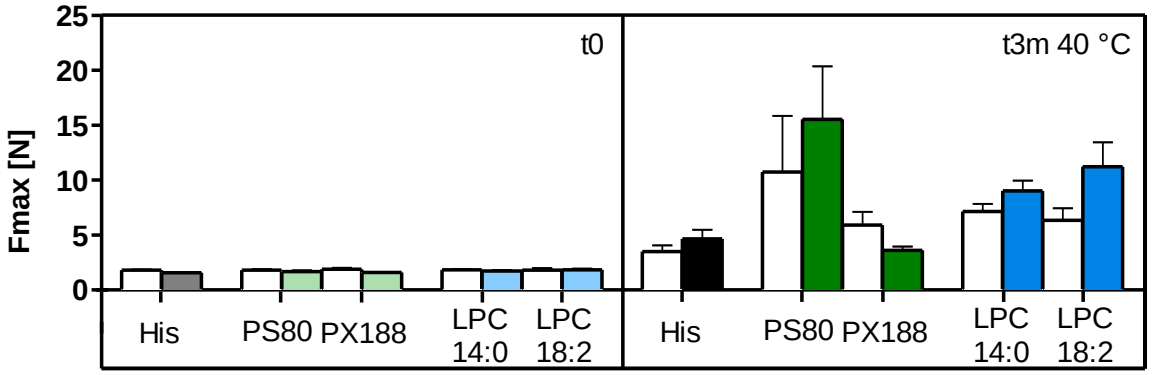
2 mg/ml mAb, 10% sucrose, 20 mM His pH 5.4

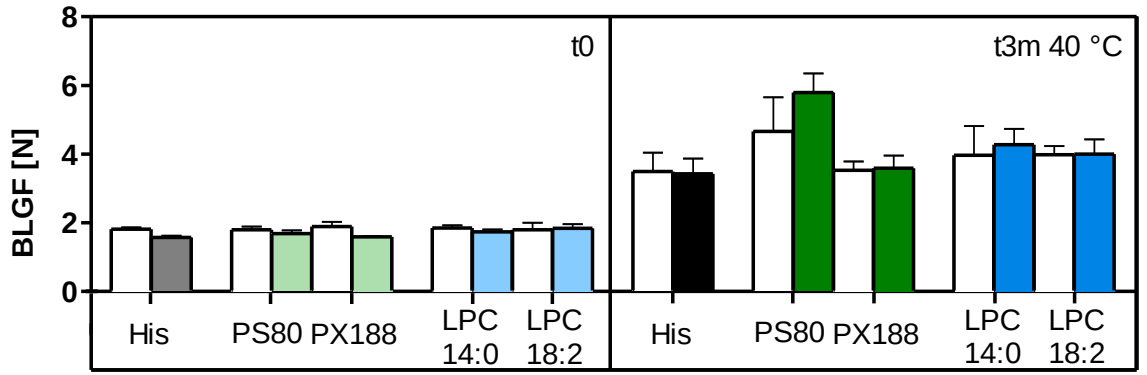
Stability Of The Siliconization Of PFSs Against Attack

3 Months Storage at 40 °C



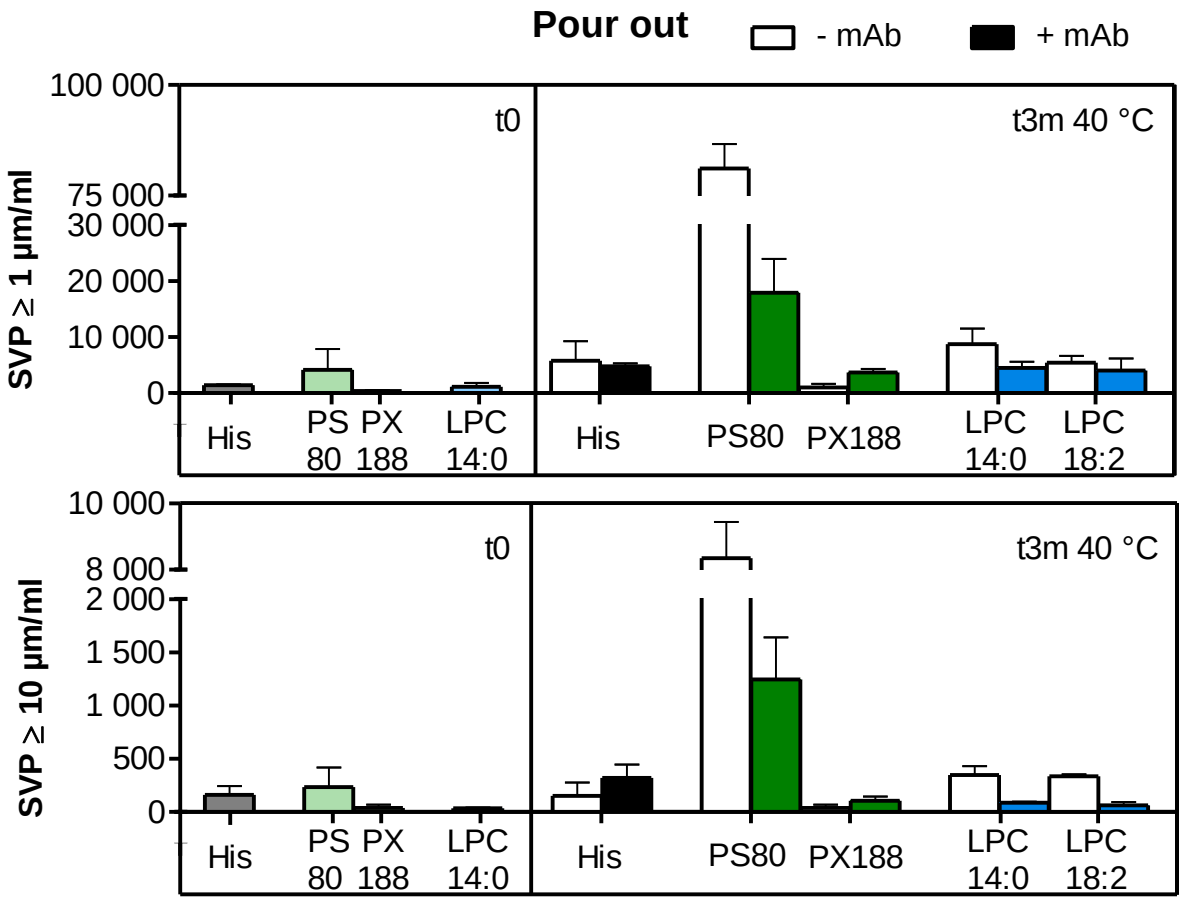




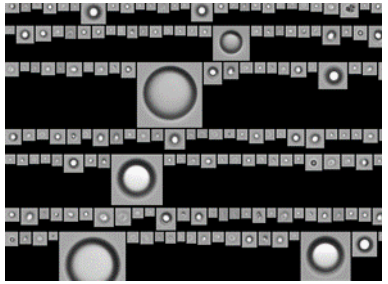


5 mg/ml mAb, 0.6 mg/ml surfactant, 20 mM His pH 5.4

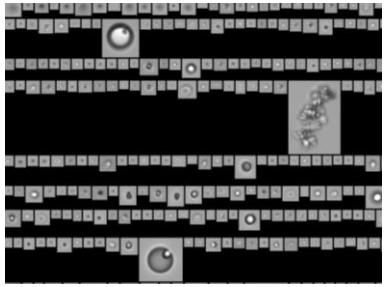
Stability Of The Siliconization Of PFSs Against Attack



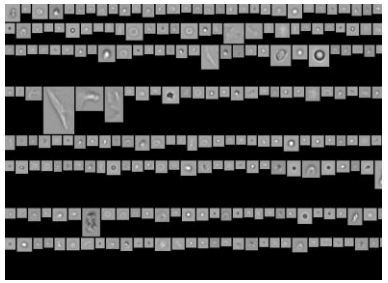
PS80 + mAb



LPC 14:0



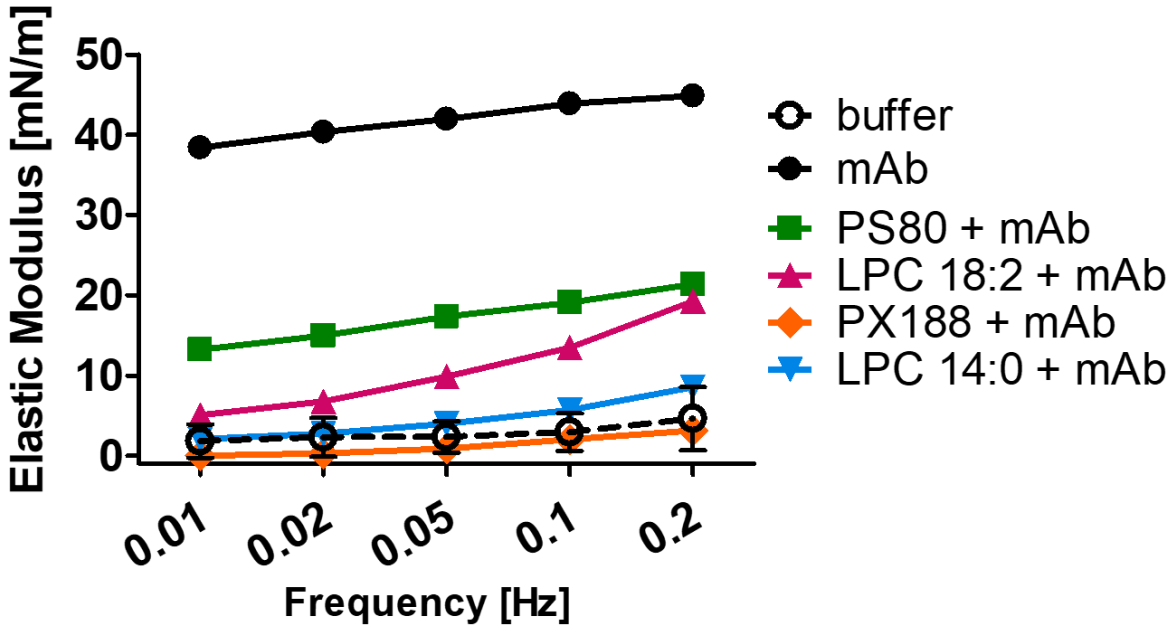
LPC 14:0 + mAb



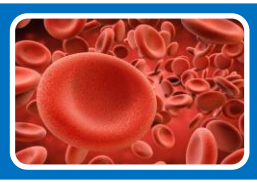
How Do The LPCs Behave At The Silicone Oil Surface?

0.1 mg/ml surfactant ± 1 mg/ml mab

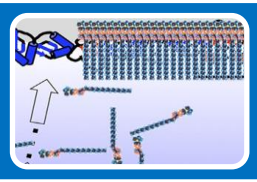
	IFT [mN/m]	IFT + mab [mN/m]
-	34.4	26.7
PS80	12.8	12.3
PX188	24.7	22.6
LPC 14:0	10.2	10.1
LPC 18:2	8.8	10.3



Summary – LPCs Are Suitable For Protein Formulation!



Hemolytic Activity of LPCs Uncritical



LPCs Stabilize Mabs Against Interfacial Stress

LPCs Do Not Interact With Mabs in Solution But Form A Protecting Elastic Interfacial Film

Sensitivity of LPCs To Oxidation And Solubility Limits The Variants Of Choice (LPC 14:0; LPC 18:1?, PC 22:1?)

Stability of mAb formulations LPC 14:0 comparable to PS80; superior in PFS

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Thank you!

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