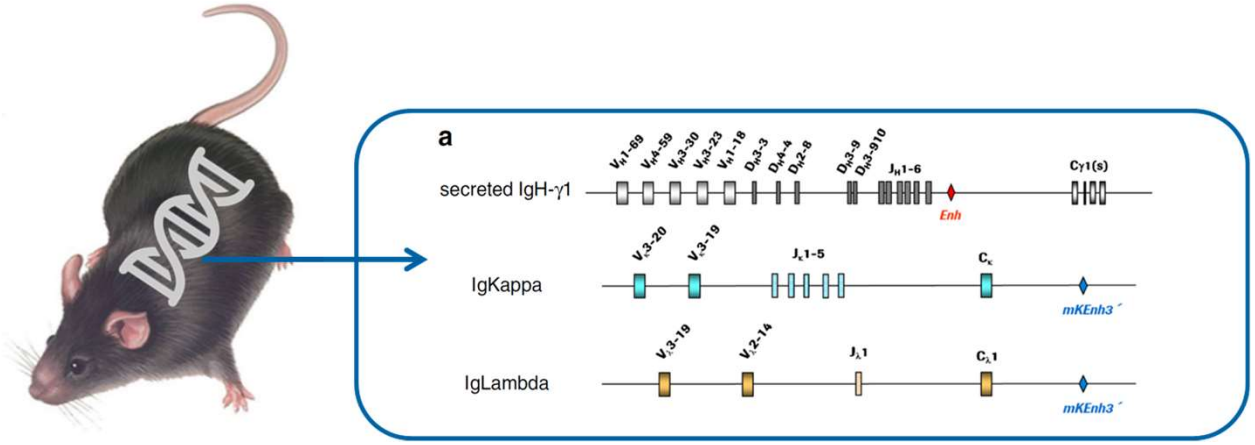
The effect of neo-epitopes on the immunogenicity of antibody aggregates in a human IgG1 Tg mice

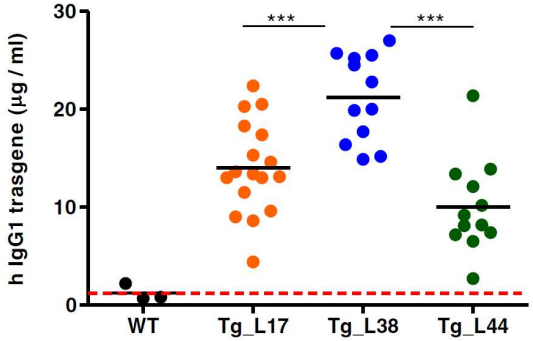
Juliana Bessa
EIP 2019, Lisbon

Pharma Research and Early Development (pRED), Pharmaceutical Sciences, Roche Innovation Center Basel

Roche pRED

Human IgG1 transgenic mouse immunogenicity model

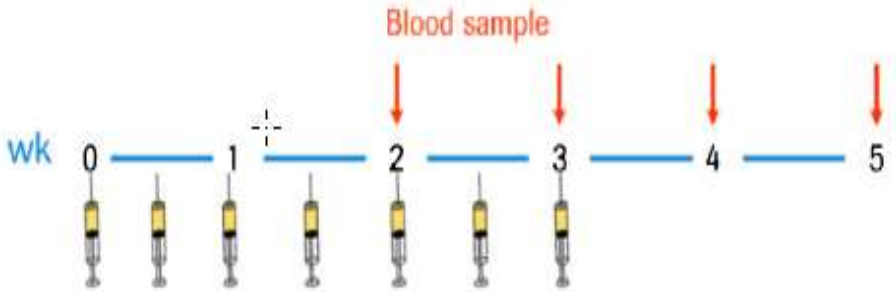


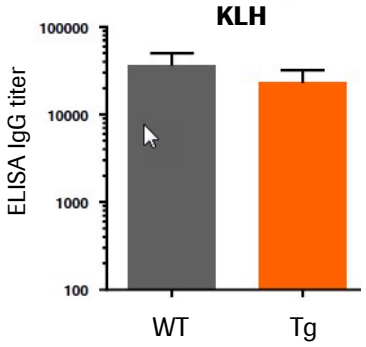


- Mouse model expressing a mini-repertoire of soluble human IgG1 antibodies
- The expressed transgenic repertoire represents most commonly used antibodies (V genes) in humans

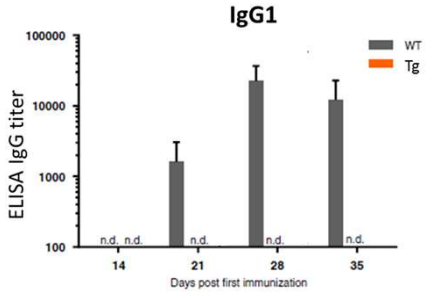
Bessa J et al 2015

Human IgG1 Transgenic mice



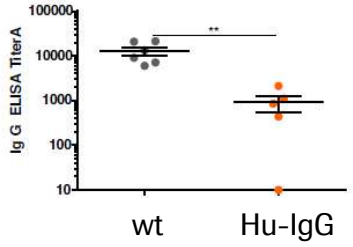


The tg mice can mount an immune response to foreign antigen (KLH) …



… but are tolerant to native monomeric huMAbs.

bulk IgG1



Bessa J. et al Pharmaceutical Research 32 (2015) 2344-2359

Mab-1 uses the VH gene expressed on Tg mini-repertoire

Transgenic V sequences

	FR1	CDR1	FR2	CDR2	FR3
1-69:	QVQLVQSGAEVKKPGSSVKVSCKASGGTFS	SYAIS	WVRQAPGQGLEWMG	GIIPIFGTANYAQKFQG	RVTITADKSTSTAYMELSSLRSEDTAVYYCAR
1-18:	QVQLVQSGAEVKKPGASVKVSCKASGYTFT	SYGIS	WVRQAPGQGLEWMG	WISAYNGNTNYAQKLQG	RVTMTTDTSTSTAYMELRSLRSDDTAVYYCAR
3-23:	EVQLLESGGGLVQPGGSLRLSCAASGFTFS	SYAMS	WVRQAPGKGLEWVS	AISGSGGSTYYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK
3-30:	QVQLVESGGGVVQPGRSLRLSCAASGFTFS	SYGMH	WVRQAPGKGLEWVA	VISYDGSNKYYADSVKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK
4-59:	QVQLQESGPGLVKPSETLSLTCTVSGGSIS	SYYWS	WIRQPPGKGLEWIG	YIY YSGSTNYNPSLKS	RVTISVDTSKNQFSLKLSSVTAADTAVYYCAR

Conventional Abs

mAb-1:	EVQLVESGGGLVQPGGSLRLSCAASGYTFT	NYGMN	NVRQAPGKGLEWVG	WINTYTGEPTYAADFKR	RFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAK
mAb-2:	EVQLVESGGGLVQPGGSLRLSCAASGYSFT	GHWMN	WVRQAPGKGLEWVG	MIHPSDSETRYNQKFKD	RFTISVDKSKNTLYLQMNSLRAEDTAVYYCAR

→ **Aminoacid mutations not necessarily leads to ADA induction**

Antibody aggregates

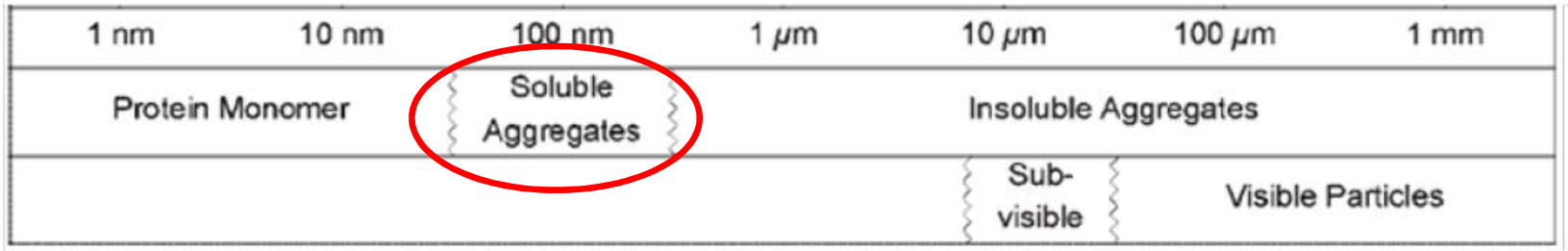
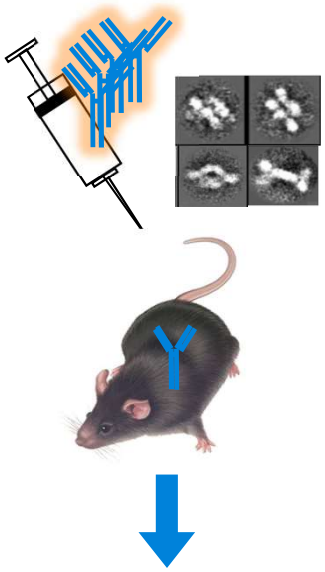


Figure 4: Schematic representation of protein sizes (diameter) classification (Mahler *et al.*, 2009).

Immunogenicity of human IgG1 aggregates

Are **hIgG1** transgenic mice tolerant to Ab aggregates?



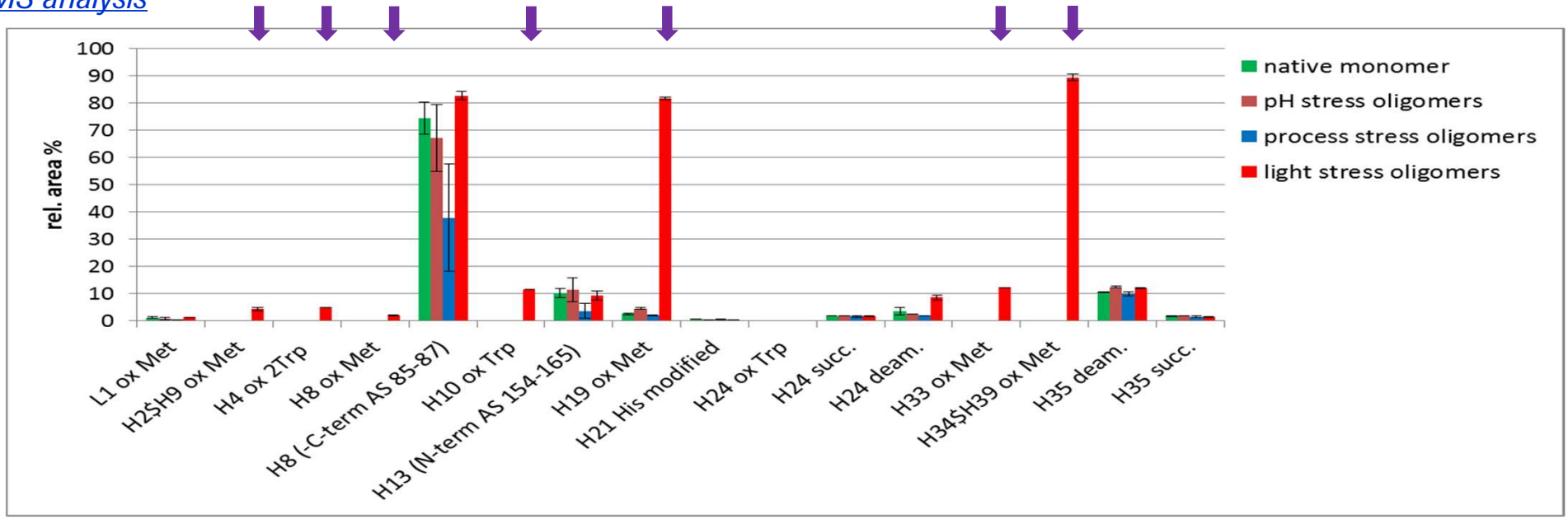
ADA?

Stress type	Size	ADA
None:		
- monomers	150 kD	-
- dimers	300 kD	-
- oligomers	450-900 kD	-
pH 2.5:		
- monomers	150 kD	n. a.
- dimers	300 kD	-
- oligomers	450 kD-3 MD	-
UV 765 W/m^2 :		
- monomers	150 kD	-
- dimers	300 kD	+
- oligomers	450 kD-3 MD	+++

Bessa J. et al Pharmaceutical Research 32 (2015) 2344-2359

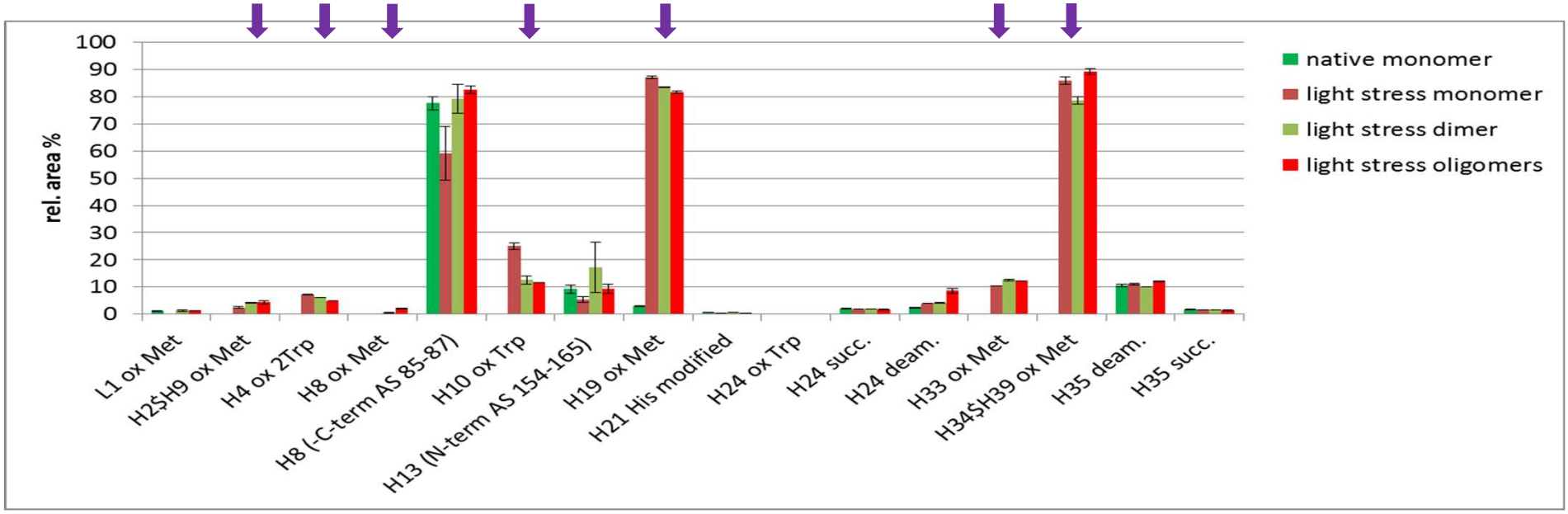
Only UV stressed oligomers carries modifications in the primary structure

LC/MS analysis



→ **Some modifications are only detected in light stress oligomers**

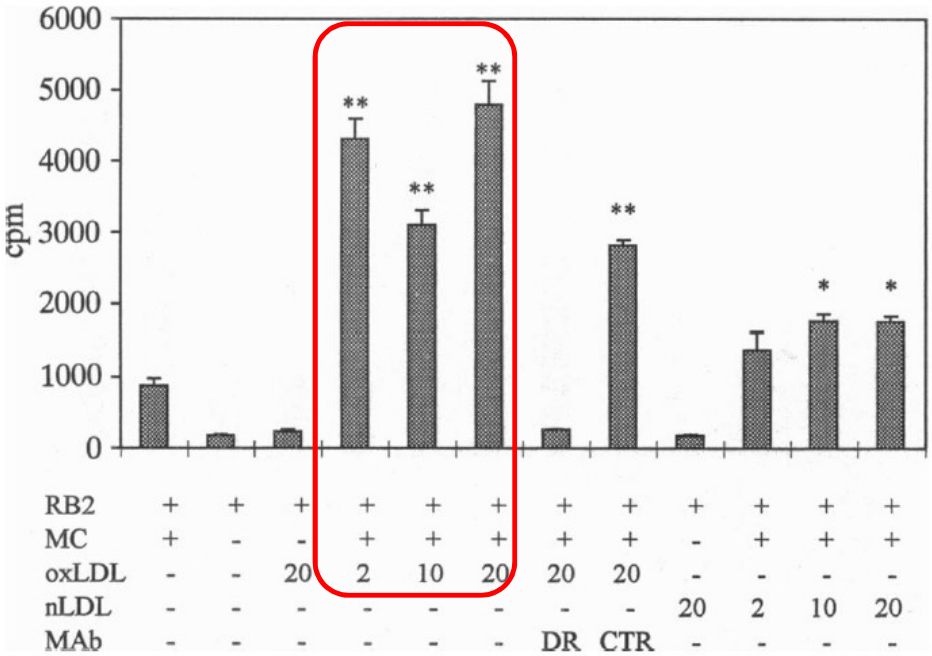
Different fractions of IgG1 UV aggregates have the same modifications



→ **Same chemical modifications are detected in light stress mono, dimer and oligomers**

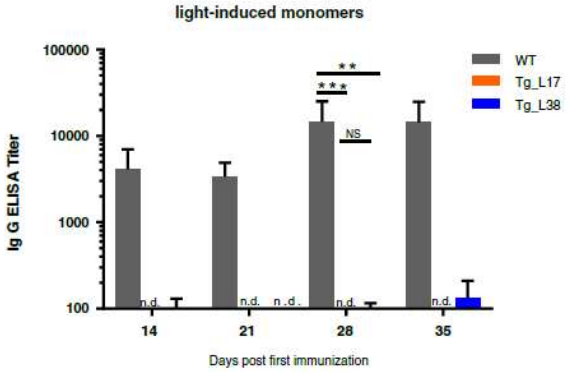
→ **Both, size and neo-epitopes are required for breakage of immune tolerance**

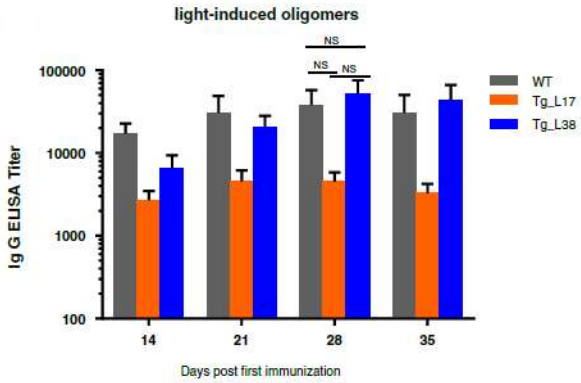
T cells proliferate in response to oxidized LDL

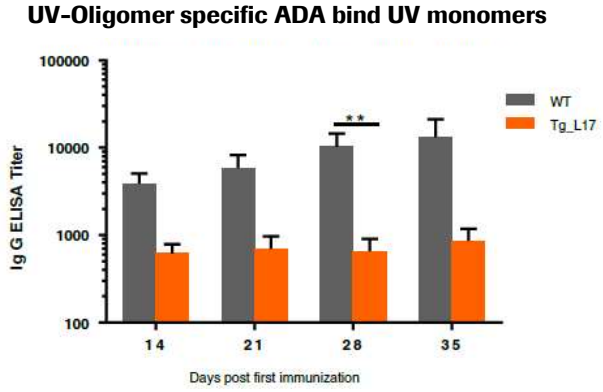


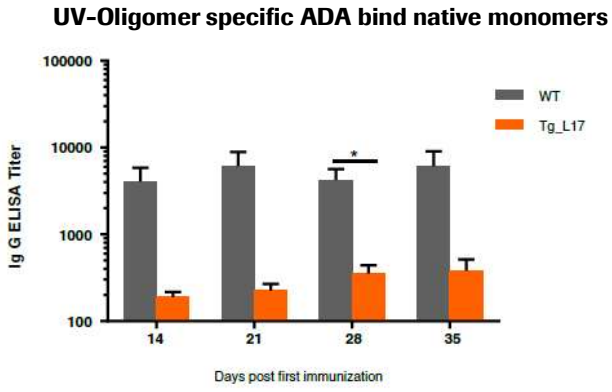
Stemme S et al PNAS 1995

UV aggregates break immune tolerance to native mAb-1





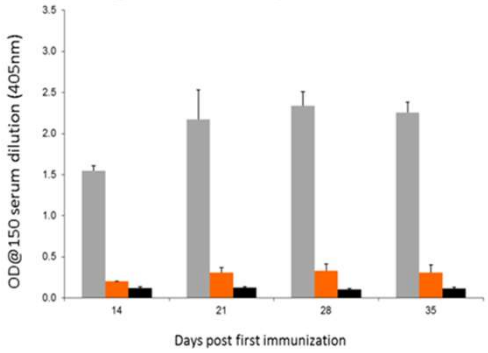




→ **IgG1-specific B cells also consist the repertoire of IgG1 Tg mice**

Immunogenicity of light-stressed IgG1 oligomeric aggregates

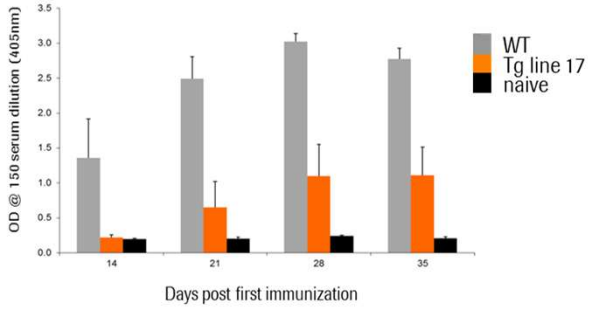
C1 mix: 19% HMW



15.9% monomer
27.2% dimer
37.6% trimer mostly (~ 450kDa)
14.2 % tetramer-hexamer (~ 600 – 900kDa)
4.8% aggregates (up to ~ 3MDa)

81%
19%

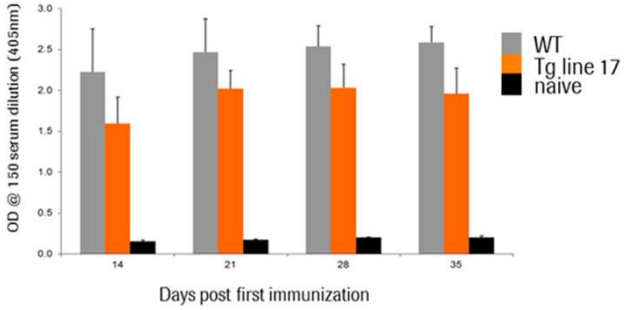
B1 mix: 34% HMW



14% monomer
18.5% dimer
33.1% trimer mostly (~ 450kDa)
24.6 % tetramer-hexamer (~ 600 – 900kDa)
9.4% aggregates (up to ~ 3MDa)

65.6%
34%

B2 mix: 52% HMW

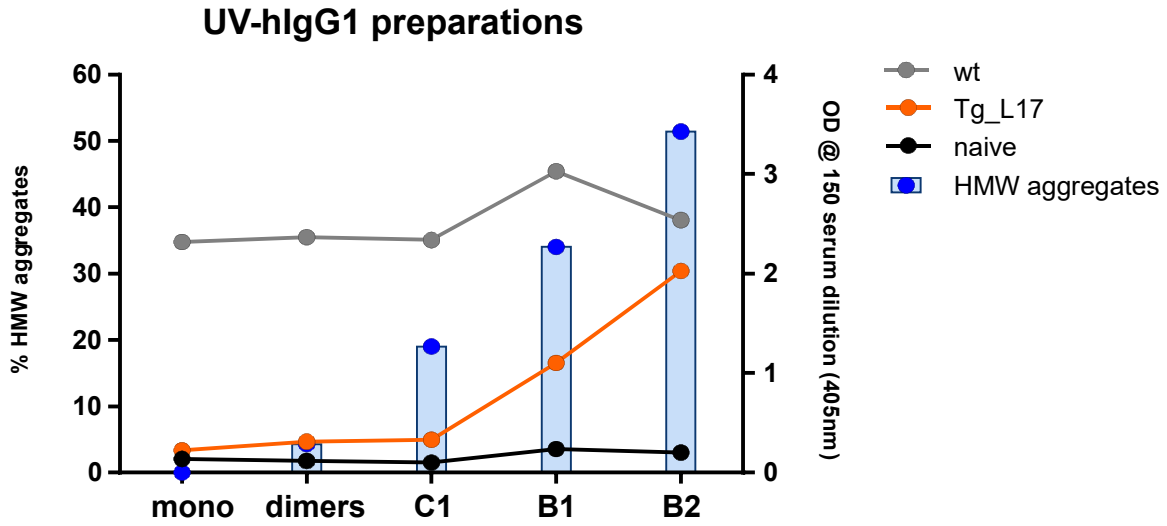


13.3% monomer
15.5% dimer
18.4% trimer mostly (~ 450kDa)
36 % tetramer-hexamer (~ 600 – 900kDa)
16.4% aggregates (up to ~ 3MDa)

51.7%
52.4%

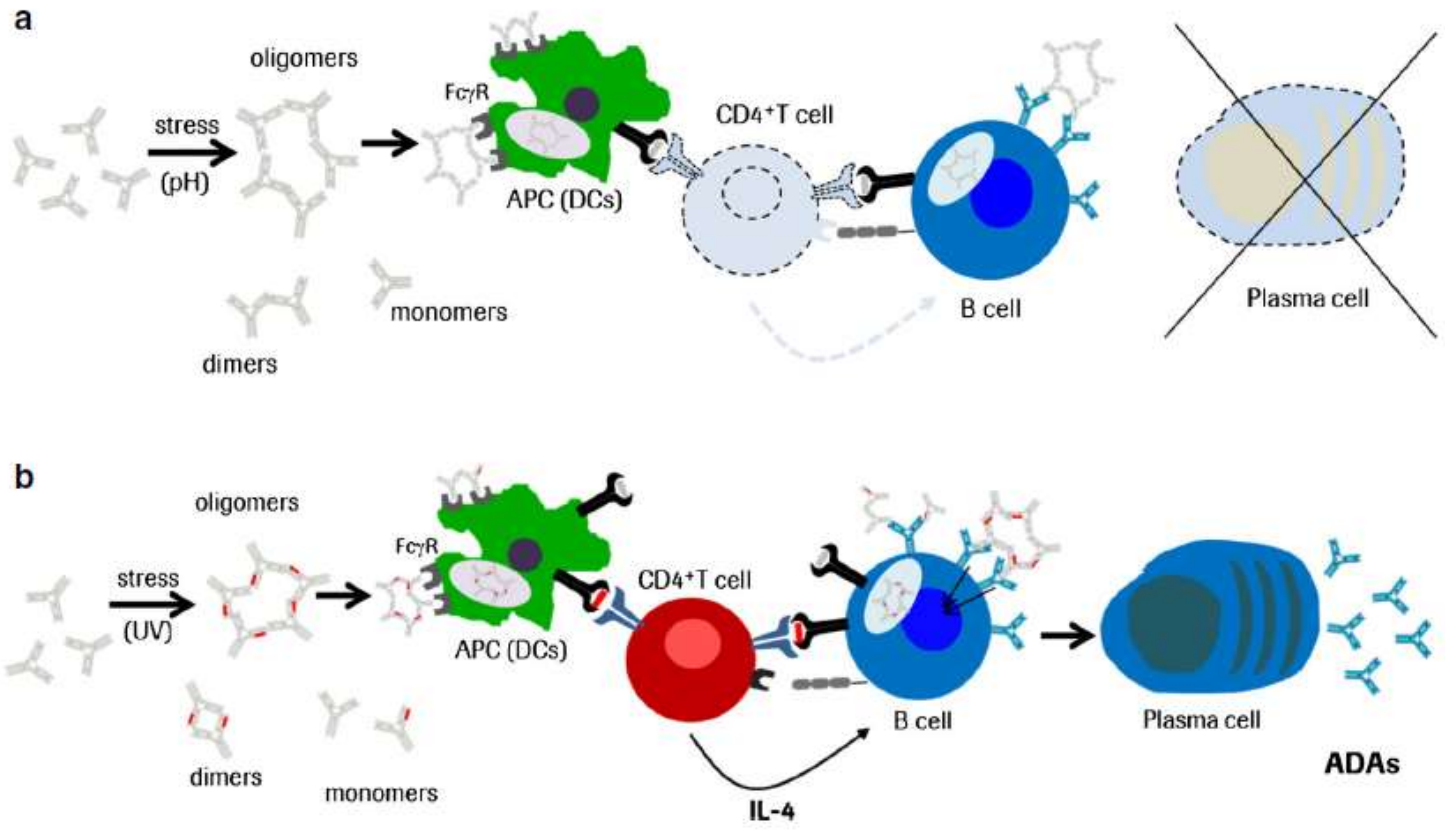
→ ADA increases along with increased content of HMW species

Immunogenicity of light-stressed IgG1 aggregates



→ **ADA formation requires a high content (≥ 20%) of HMW oligomers**

UV IgG1aggregates activate neoepitope-specific T cells



Are Sub-visible Particles (SvP) immunogenic?

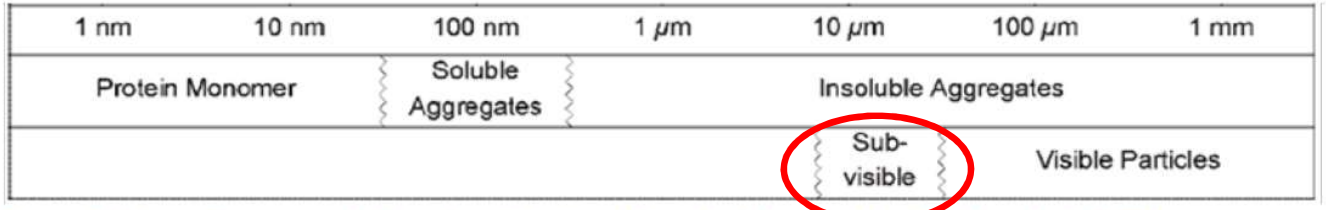
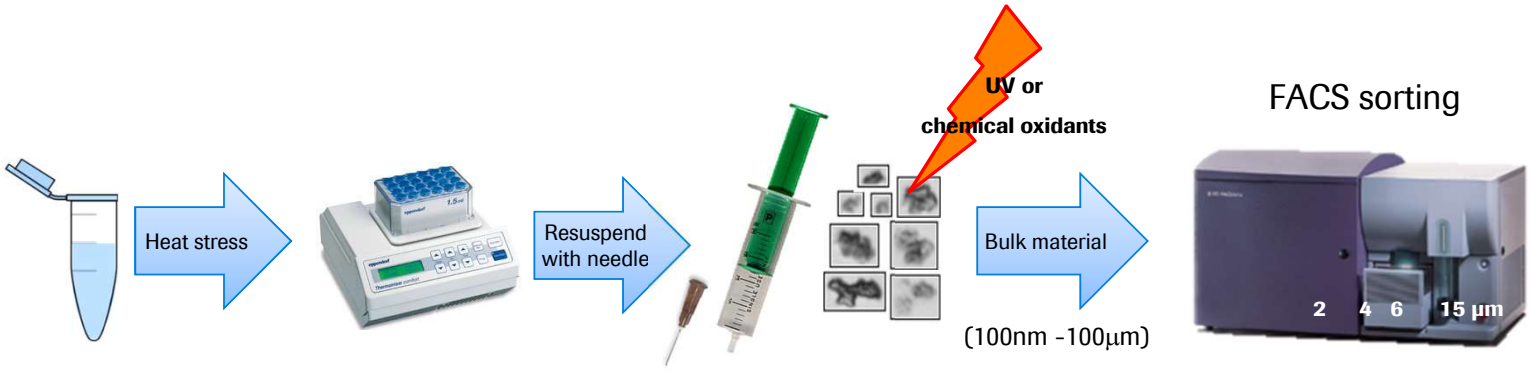


Figure 4: Schematic representation of protein sizes (diameter) classification (Mahler *et al.*, 2009).

Björn Boll

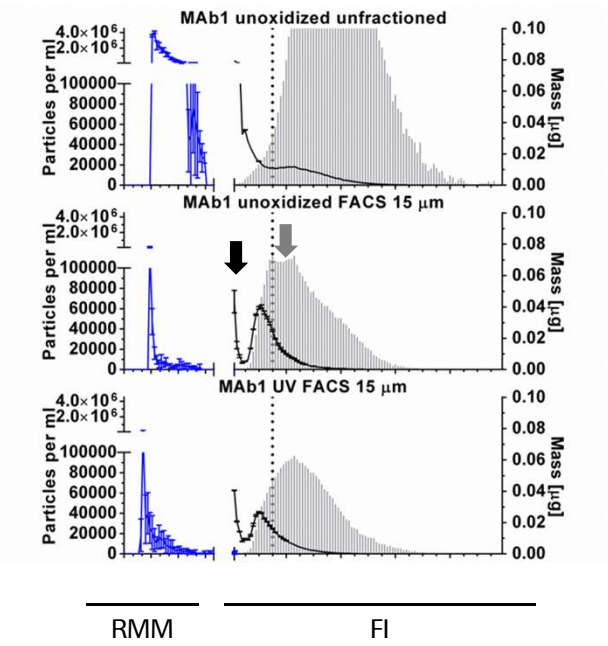
Boll B et al 2017

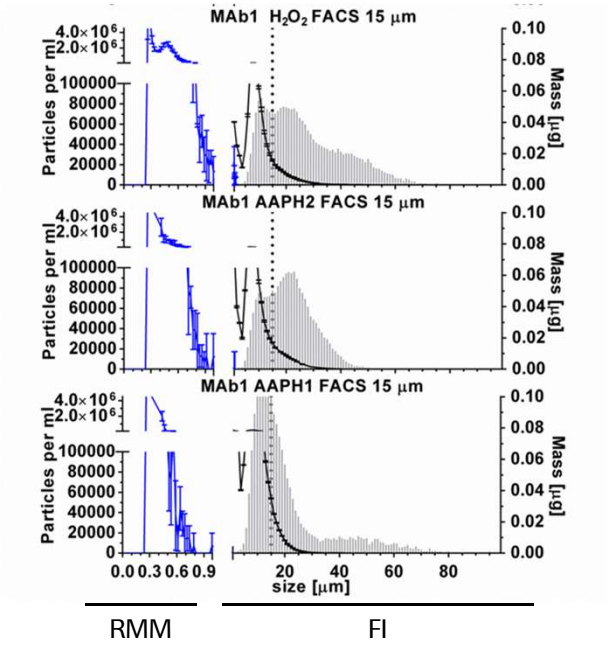
Generation of SvP of human IgG1 Mab-1



	Stress type	Oxidation
UV	**765 W/m², 30 hr**	broad
H2O2	**1 % at 5 °C / 24 h**	Not Trp
AAPH2	**5 %/ 40 °C/ 120 h + free L-Met**	Not Met
AAPH1	**5 %/ 40 °C/ 120 h**	extensive

Biophysical Characterization: Size and mass distribution



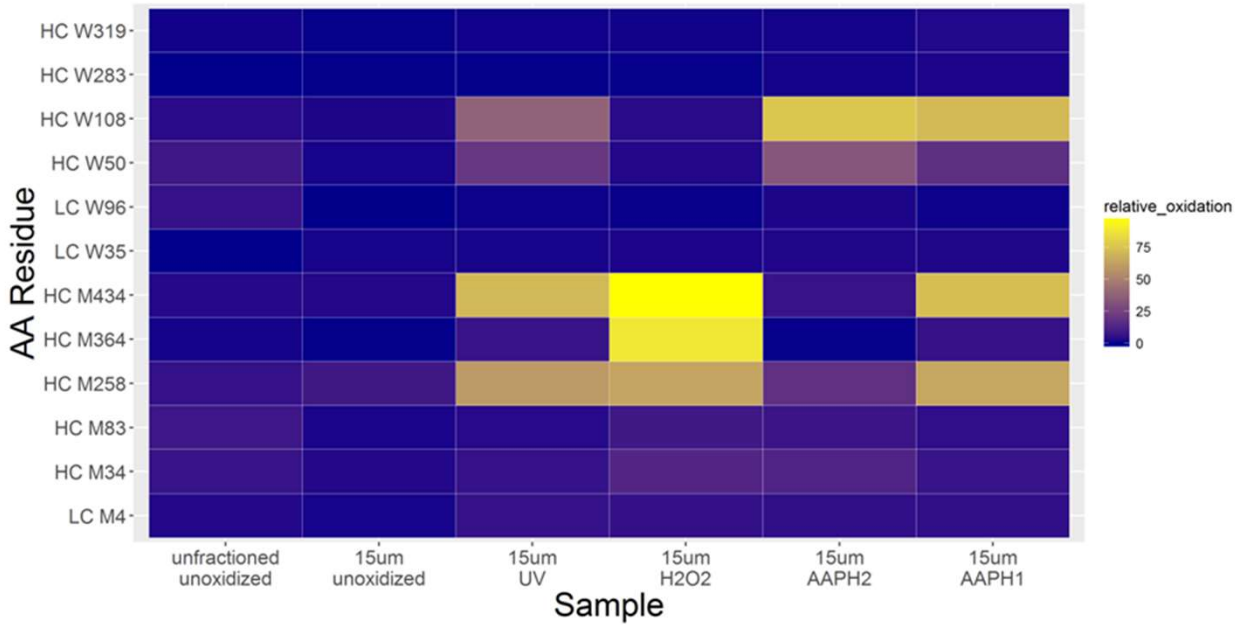


→ Highest particle count around 1 µm size

→ Highest mass distribution around 15 µm size

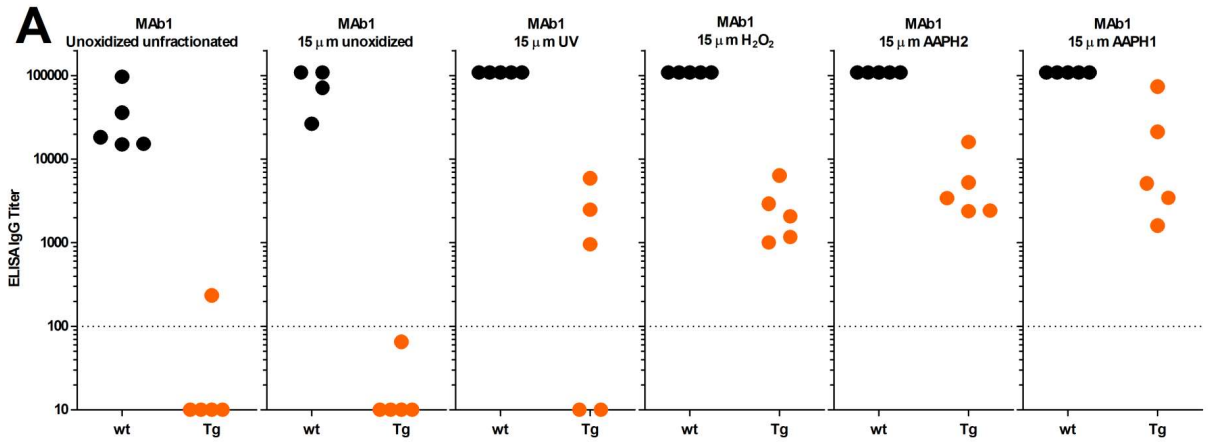
RMM= resonant mass measurements (0.013-5 µm); FI= flow imaging (1-100µm)

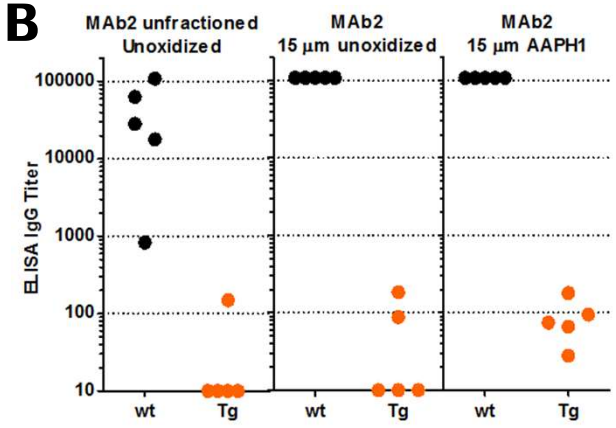
Characterization chemical modifications of Mab-1 SvP by LC-MS



→ Increasing harshness leads to increase oxidation (AAPH1 leads to most broadly modified sample)

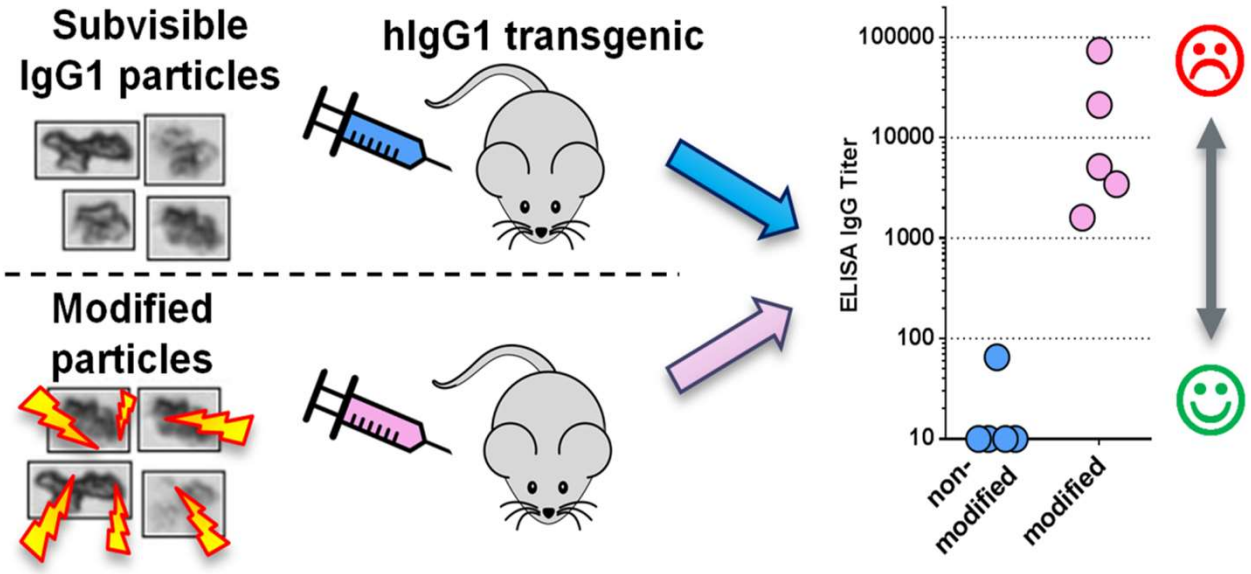
Immunogenicity of mAb-1 & mAb-2 SvP





→ **ADA titer increases along increased oxidation level**

Only modified SvP leads to ADA induction in hIgG Tg mice



Boll B. et al Mol Pharm (2017) ; 1292-99

Taken all together…

- human IgG1 Tg mice are tolerant to a broad range of human antibodies (commonly used V genes)
- Unresponsiveness to IgG1 is preserved mainly by T cell tolerance
- Only IgG1 aggregates bearing extensive chemical modifications (neoepitopes) are expected to cause ADA responses in this system
- Subvisible particles (SvP) of human IgG1 can be processed and recognized by the immune system
- Only SvP bearing extensive chemical modifications (neoepitopes) were immunogenic and induce ADA responses in the IgG1 transgenic mice

Acknowledgements

Roche pRED
Antonio Iglesias
Laetitia Petersen
Guido Steiner
Celine-Marban Doran
Jessica Langer
Ursula Nelböck
Anna Kiialainen
Martin Ebeling
Thomas Weiser
Thomas Singer

Roche PTDE
Björn Boll
Atanas Koulov
Anacelia Ríos
Sabine Boeckle
Emilien Folzer
Roland Schmidt
Patrick Bulau
Christof Finkler
Hermann Beck
Thomas Buckel
Hanns-Christian Mahler

University of Basel
Ton Rolink

Doing now what patients need next