Immunogenicity of therapeutic antibodies: role of aggregation in T lymphocyte response

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INSERM UMR 996, Inflammation, Microbiome, Immunosurveillance

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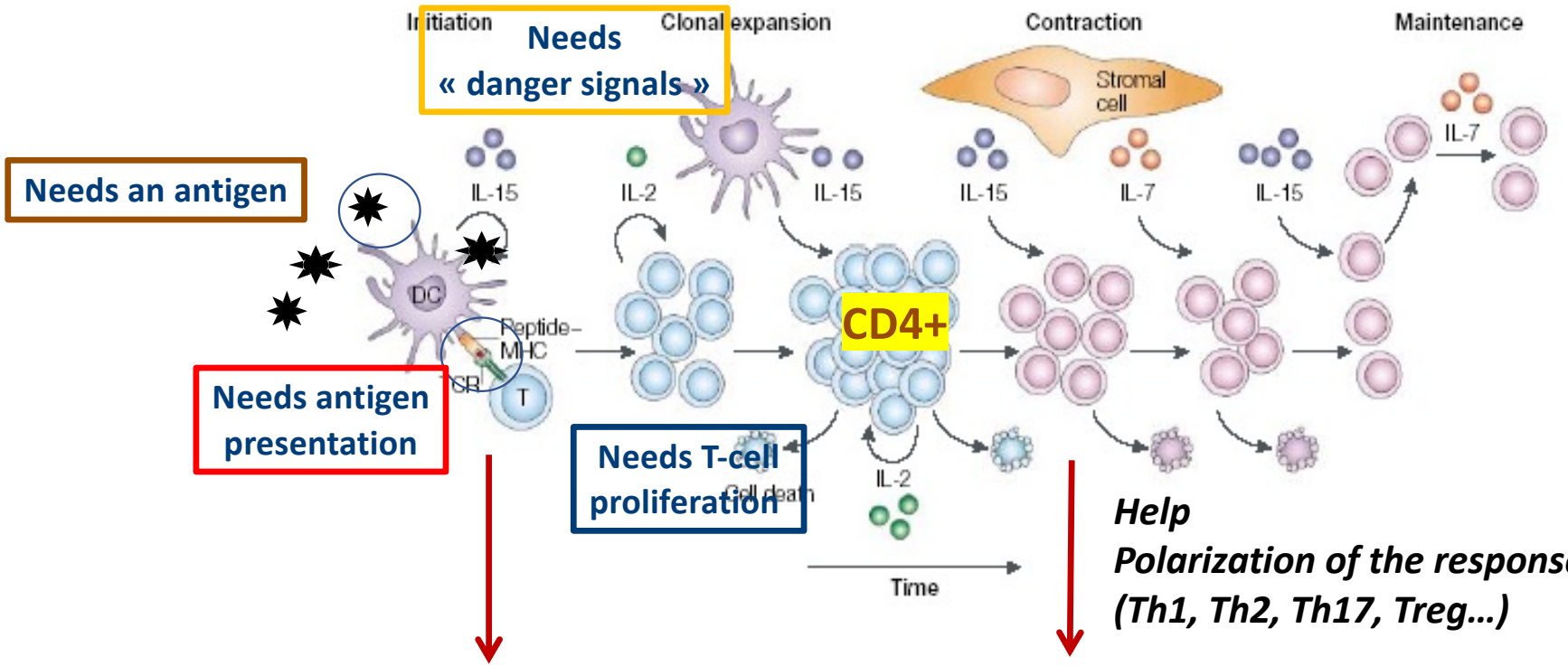
ACCREDIA, PERP, ANR, France (Bernard Maillère, coordinator)

Immunogenicity = recognition by the immune system of defined structures
= needs mobilization of the adaptive immune response = T-cells

Adaptive Immune Response



Cross-presentation/MHC I → CD8+ T lymphocytes: antigen-driven cytotoxicity

Whole antigen → B lymphocytes, plasmocytes → antibodies

Schluns and Lefrançois (2003)

Influencing Factors

Patient features

- Immune status
- Pathology
- Genetic background

Treatment-related

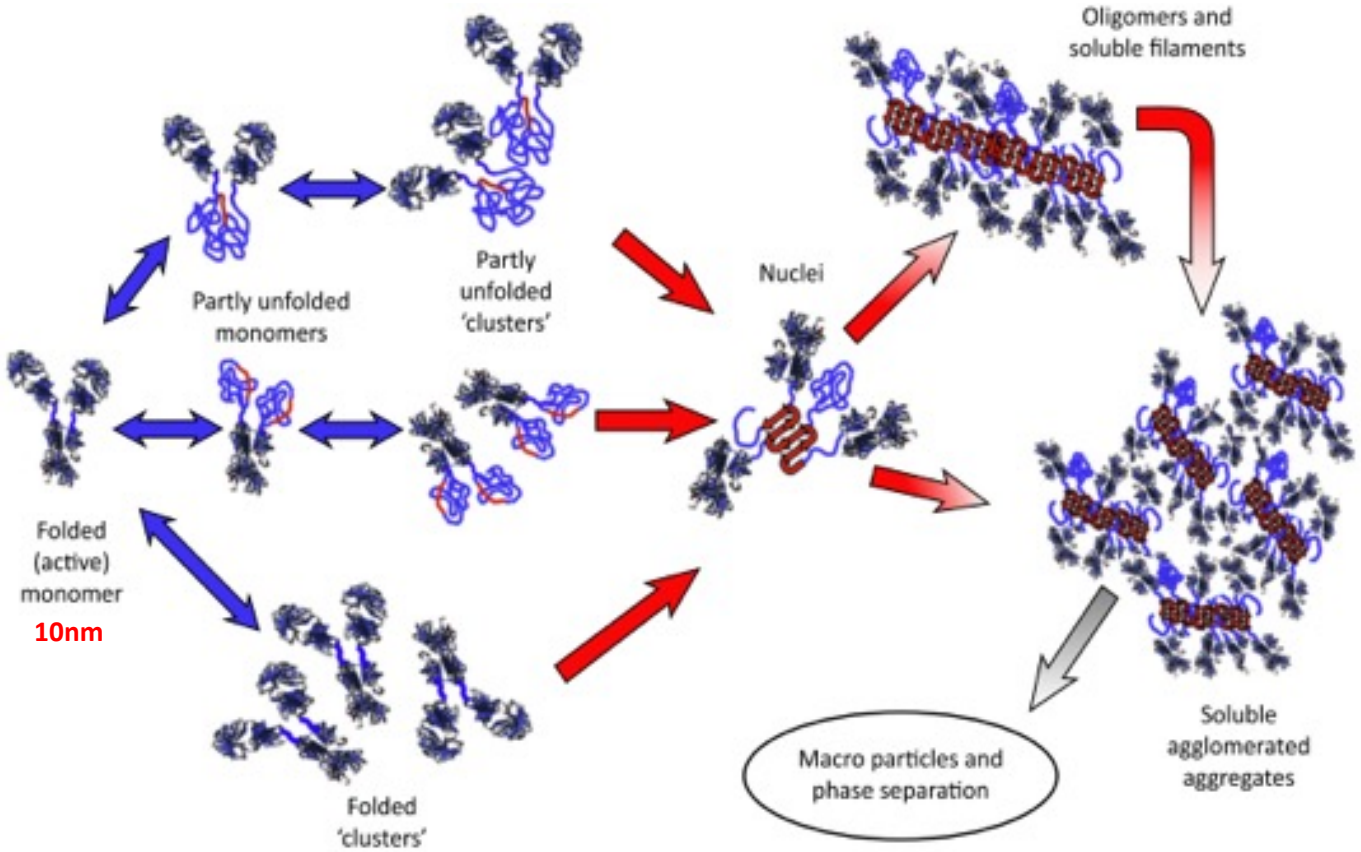
- Length, Frequency
- Dose
- Route of administration
- Immunosuppressive medication

Immunogenicity

Product-related

- Degree of non-self, Presence of T or B-cell epitopes
- Post-translational modifications (glycosylation…)
- Formulation, production, purification, impurities
- Structural alterations: Oxidation, Deamidation and degradation, Conformational changes
- **Aggregation**

AGGREGATES FORMATION



Insoluble aggregates	
1 - 100 µm	Subvisible micron aggregates
> 100 µm	Visible aggregates

Soluble aggregates	
< 100 nm	Oligomers
100 - 1000 nm	Submicron aggregates

Roberts et al (2014)

AGGREGATES IN BIOLOGICAL PRODUCTS

Production process: *bioreactor, purification, formulation*
⇨ Aggregates elimination well controlled

Injected volume	≤ 100 mL (particles/container)		> 100 mL (particles/mL)	
Particle size	**≥ 10 µm**	**≥ 25 µm**	**≥ 10 µm**	**≥ 25 µm**
Light obscuration	6000	600	25	3
Microscopy	3000	300	12	2

European (Ph. Eur. 2.9.19) & US (USP <788>) Pharmacopea

Handling & administration: T°C variations, shaking, light stress…
⇨ ONLY visual control & filtration

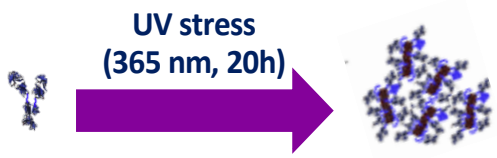
"Rapid aggregation after mixing Avastin® (bevacizumab) or Herceptin® (trastuzumab) with 5% dextrose and human plasma under *in vitro* conditions that simulate the interface of IV infusion" **Arvinte et al. (2013)**

"Nanometer, submicron, and micron protein particles have been evidenced in intravenous saline bags that could inadvertently be delivered to patients" **Pardeshi et al. (2020); Kannan et al. (2020)**

INFLIXIMAB (IFX)

- Chimeric anti-TNFα monoclonal antibody (IgG1).
- High potential for aggregation
- Highly immunogenic: 17 to 58 % of patients developing ADA (IgG1, IgG4, IgE)
- CD4 T-cell epitopes of IFX identified among healthy donors.

Aggregates production



Native IFX **IFX aggregates**

ATEZOLIZUMAB:

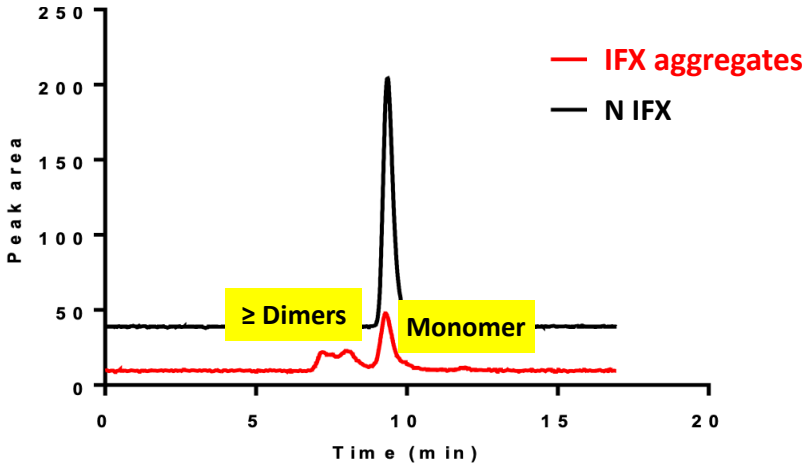
- Humanized anti-PDL1 (IgG1)
- Lacks the N-glycosylation site → favors aggregation
- Highly immunogenic: 13%- 36% of patients developing ADA

BOCOCIZUMAB:

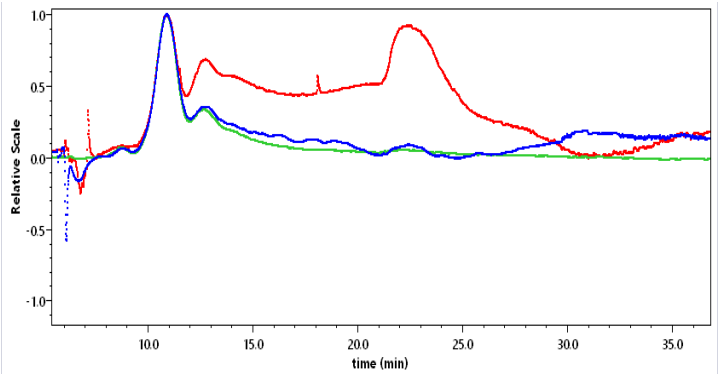
- Humanized PCSK9 inhibitory antibody that reduces LDL cholesterol levels
- High polyreactivity and self-aggregation propensities
- ADAs were detected in 44.0% (155/352) of bococizumab-treated subjects

NANOSIZED AGGREGATES CHARACTERIZATION

Size exclusion chromatography (SEC)*



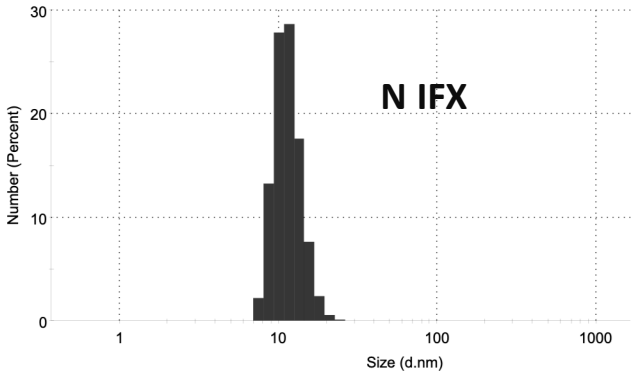
Field-flow fractionation - multi angle light scattering (MALS)

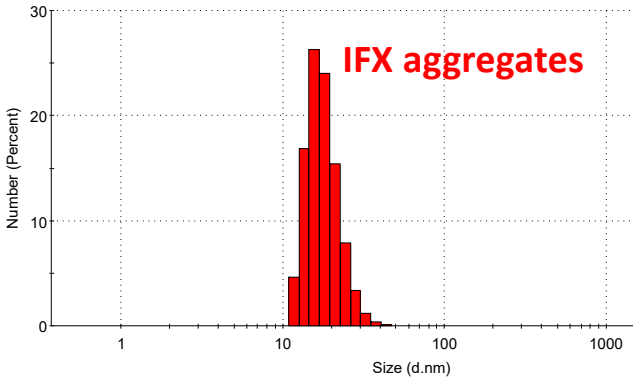


Fragment	2.1%
Monomer	52.3%
Dimers	19.3%
Oligomers	26.3%

- **Mixture monomer/aggregates**
- **Soluble dimers and oligomers <100 nm**

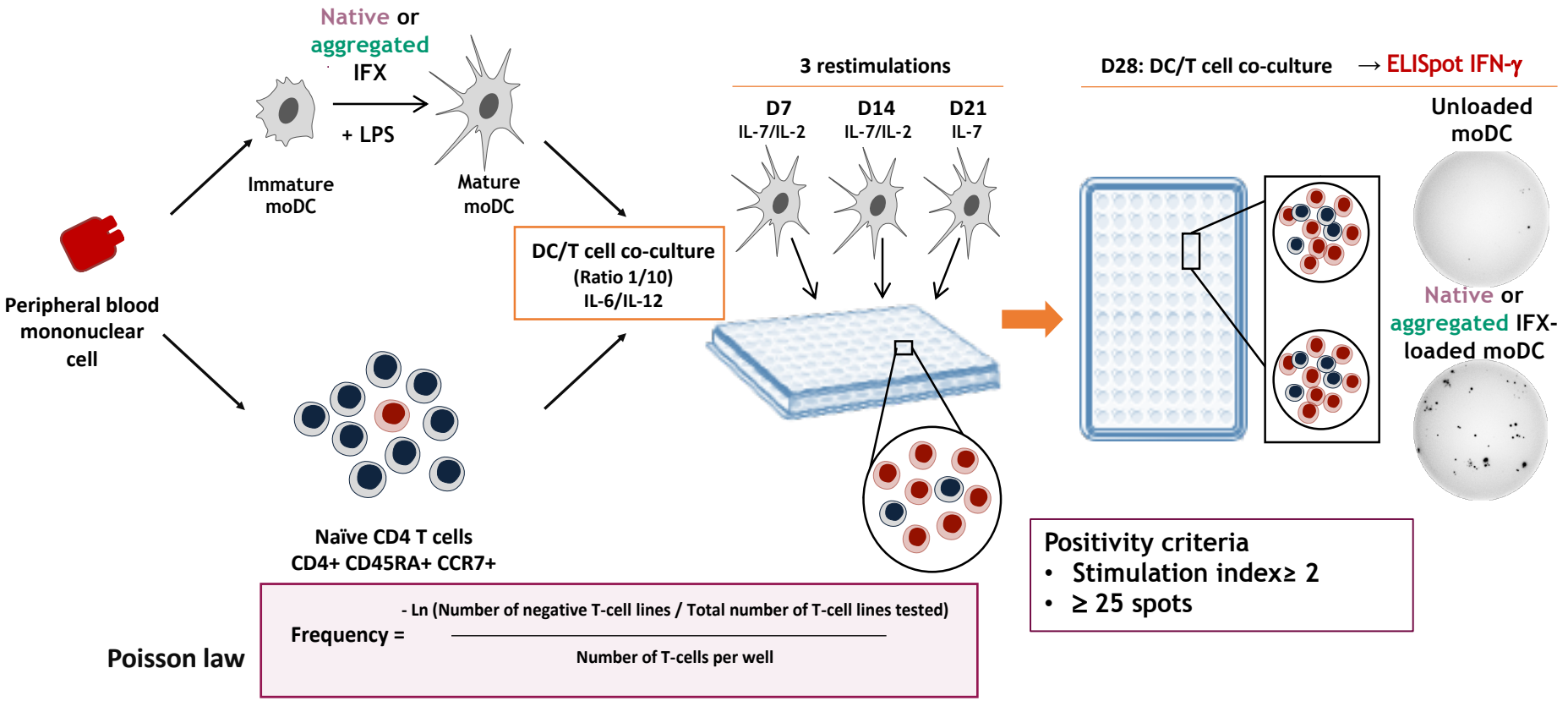
Dynamic light scattering (DLS)

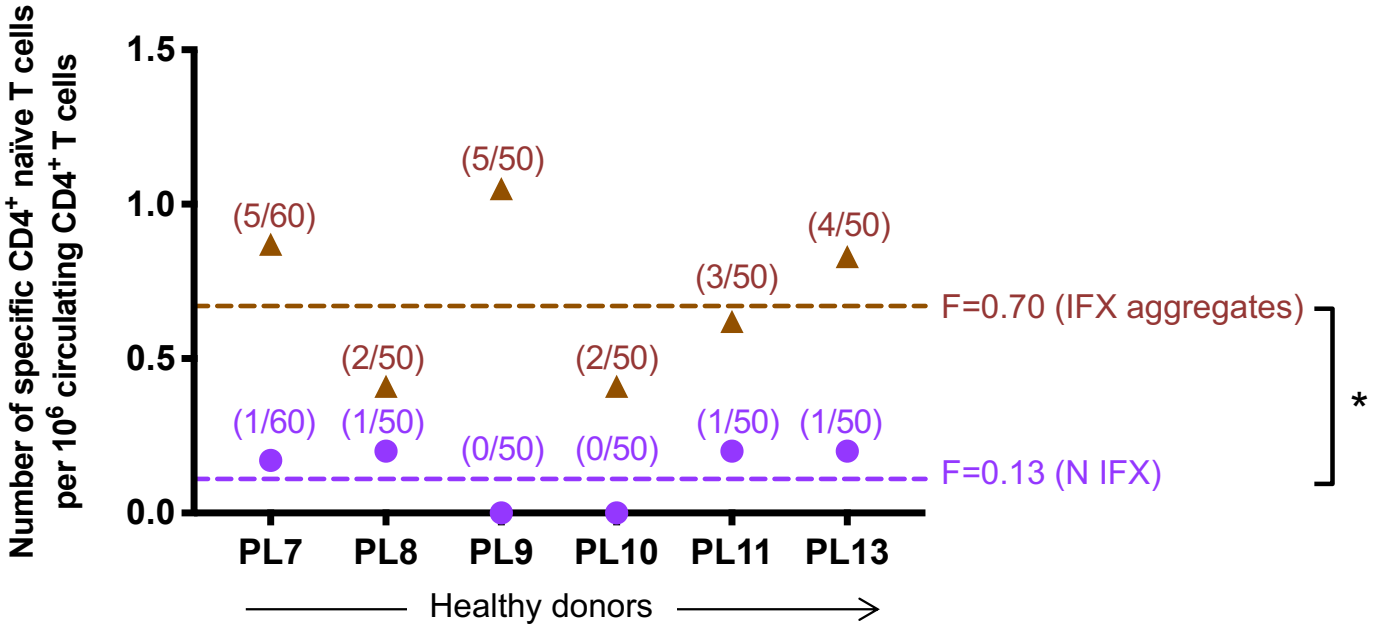




Absence of aggregates>100nm

AUTOLOGOUS CO-CULTURE MODEL TO IDENTIFY NAIVE T CELLS RECOGNIZING N OR AGGREGATED IFX



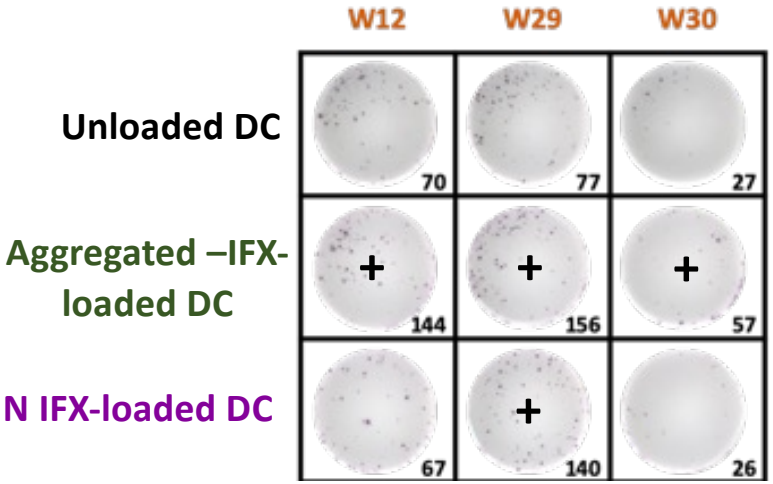


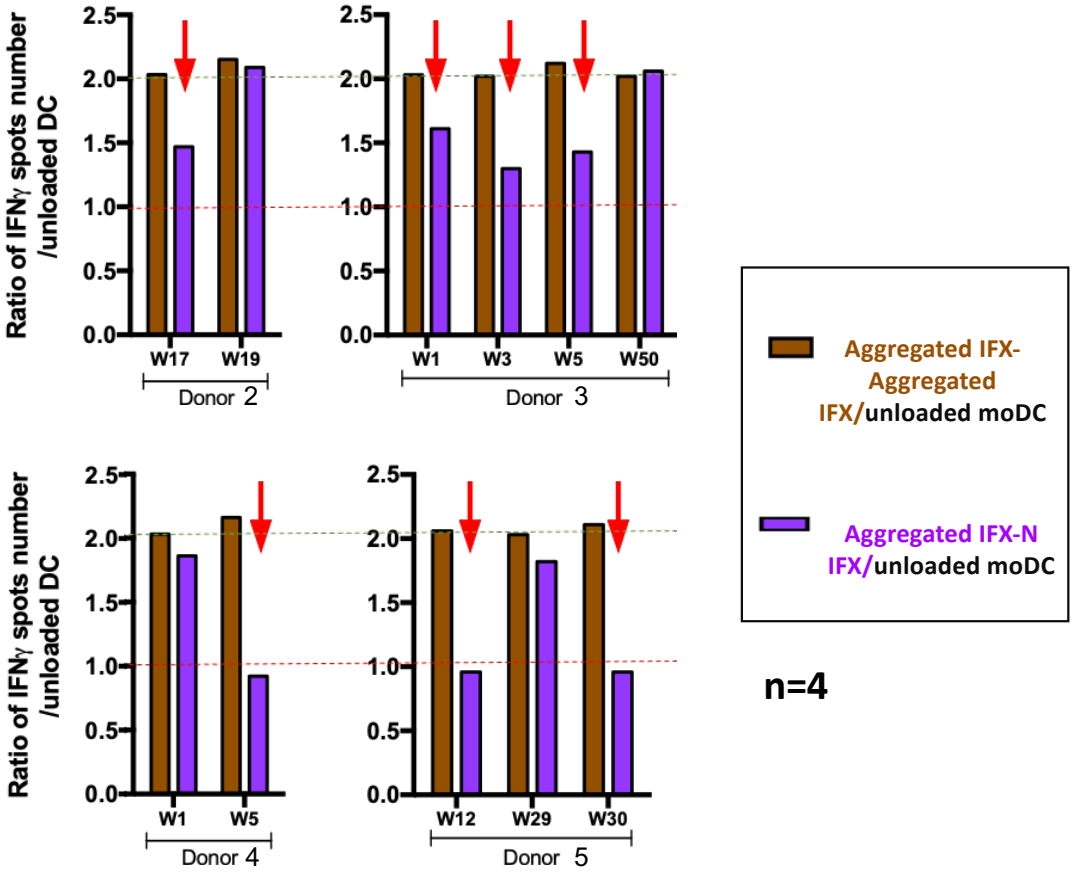
⇨ **Identification of a higher number of specific T cells in response to IFX aggregates for each donor**

CROSS-REACTIVITY OF UV-AGGREGATED IFX-RECOGNIZING T CELLS WITH NATIVE IFX

Donor 5

ELISpot 2: UV IFX-primed naive CD4 T cells (3/50)



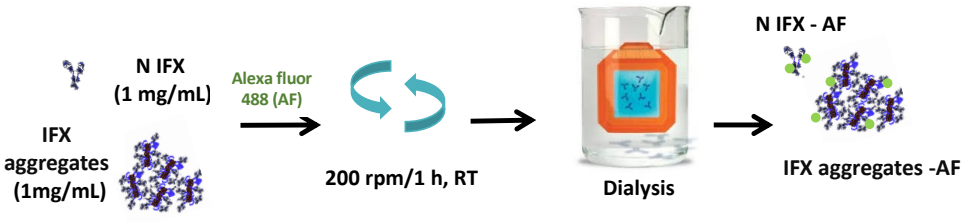


➪Some aggregated IFX-specific T cells can recognize ONLY aggregated-derived peptides

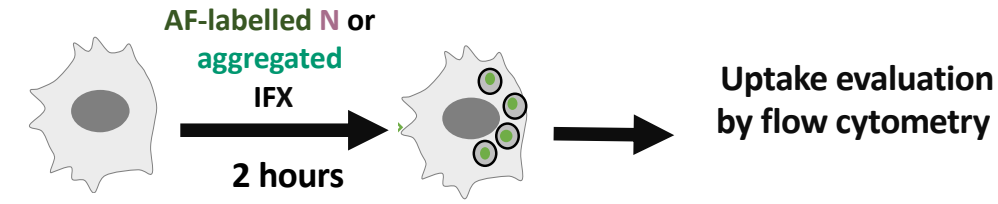
Hypothesis

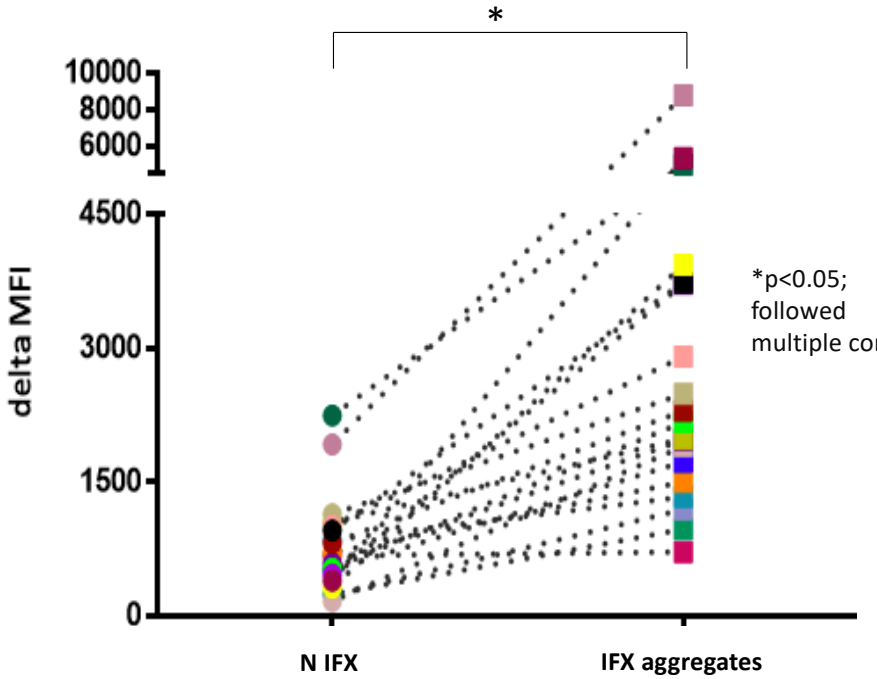
The quantity of IFX entering DC plays a role

N AND AGGREGATED IFX LABELLING BY ALEXA FLUOR



N AND AGGREGATED IFX UPTAKE BY MoDC



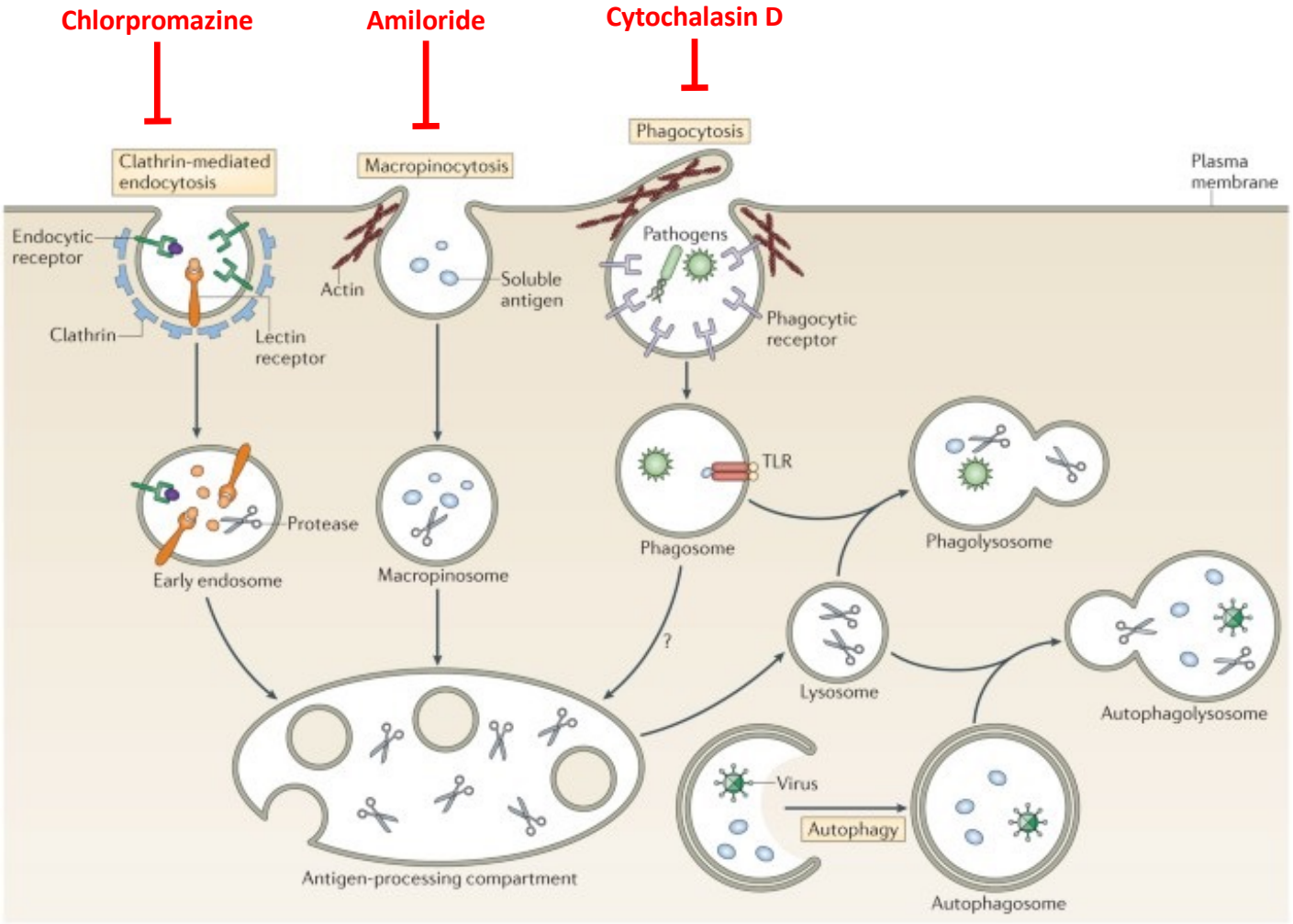


*p<0.05; Friedman test followed by Dunn's multiple comparison

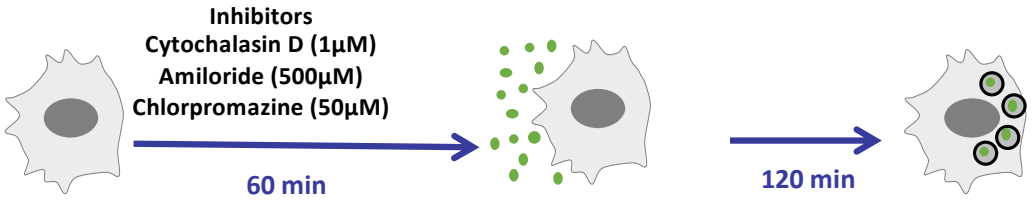
Uptake calculation: difference between mean fluorescence intensity of surface-localized (4°C) and internalized (37°C) N or aggregated IFX.

IFX aggregates tend to be more internalized in comparison to native IFX

ENDOCYTIC PATHWAYS IMPLICATED IN N AND UV IFX INTERNALIZATION



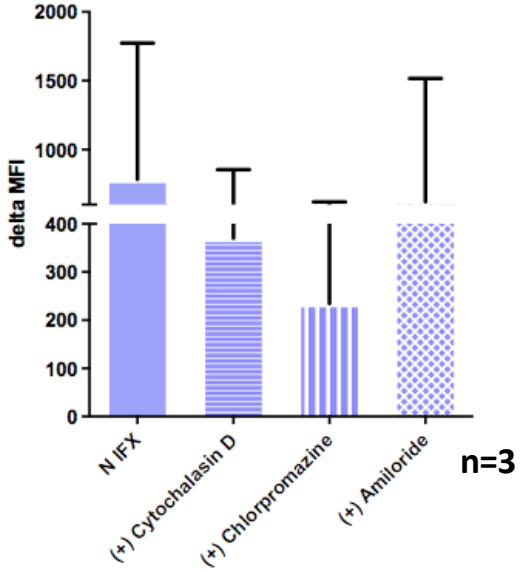
Endocytosis routes can determine cellular trafficking and antigen presentation



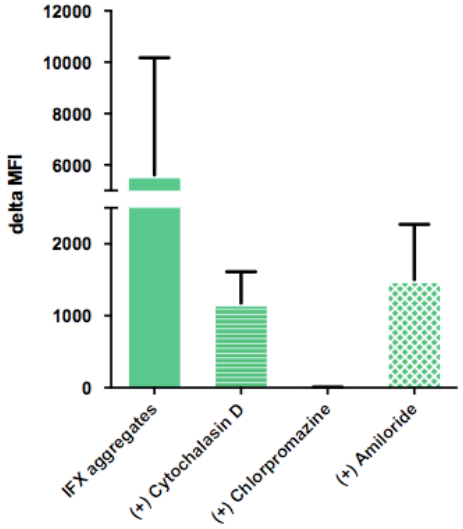
Immature moDCs

N IFX – AF (200 µg/mL)
UV IFX- AF (200 µg/mL)

Cytochalasin D → Inhibits phagocytosis ;
Chlorpromazine→ Inhibits endocytosis ;
Amiloride→ inhibits micropinocytosis ;



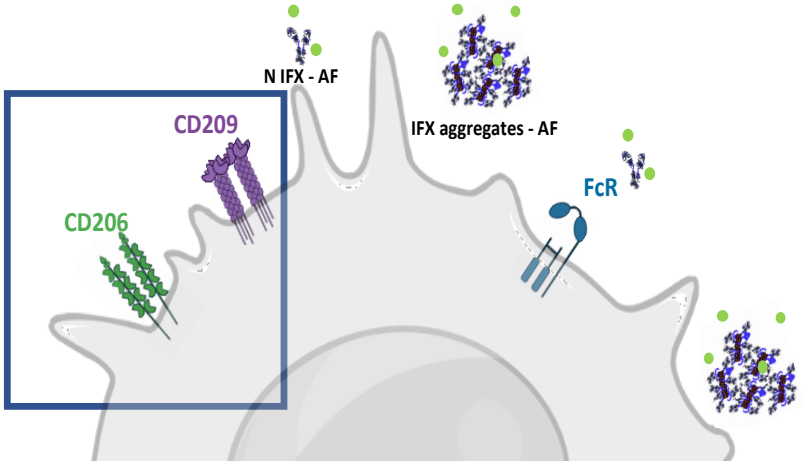
n=3



n=3

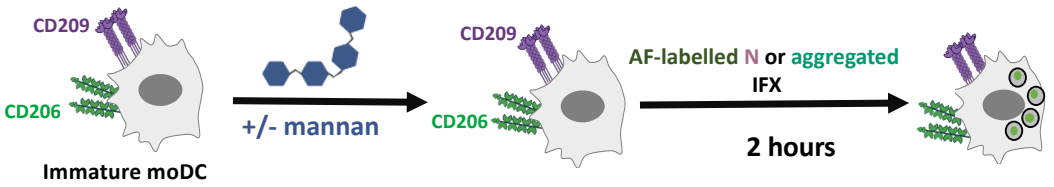
Major inhibition with chlorpromazine: major role for endocytosis ?

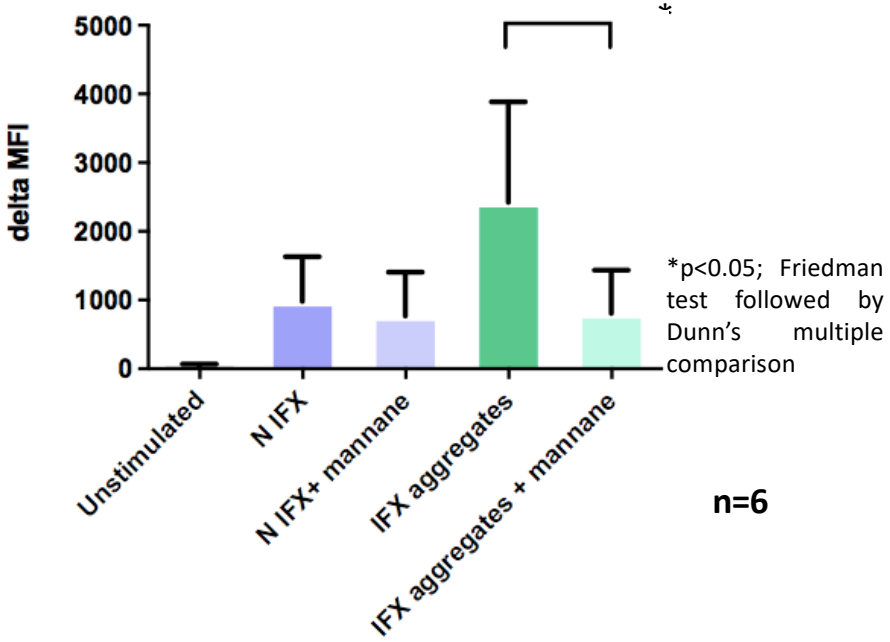
A role for mannose receptors ?



≈5.5% high mannose (M5-M6) in IFX (Fc)

Mannosylated antigens present an **enhanced endocytosis** by DCs and subsequently **an enhanced presentation** to **antigen specific T lymphocyte.**





Internalization of aggregated IFX occurs mainly via mannose-dependent endocytosis ?

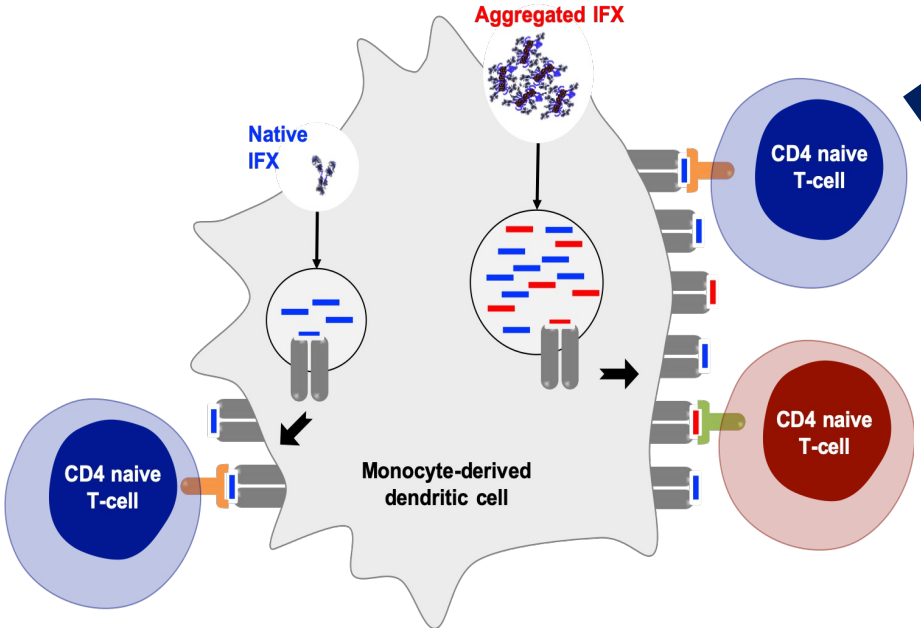
In resume for these small aggregates

Dendritic cell

- No modification moDC phenotype
- Higher internalization of IFX aggregates by moDC
- Internalization of aggregates via a mannose-dependent endocytosis →impact on peptide presentation and T cell response ?

Specific T cell activation

- Autologous moDC/T-cell co-culture model
- Detection of naïve T-cell recognizing aggregates but not the native protein



Increased number of presented peptides derived from the native antibody?
Increase of the number of T-cells recruited

And/Or

Neo peptides generated by the aggregation process?
New T-cells recruited

ACKNOWLEDGEMENTS

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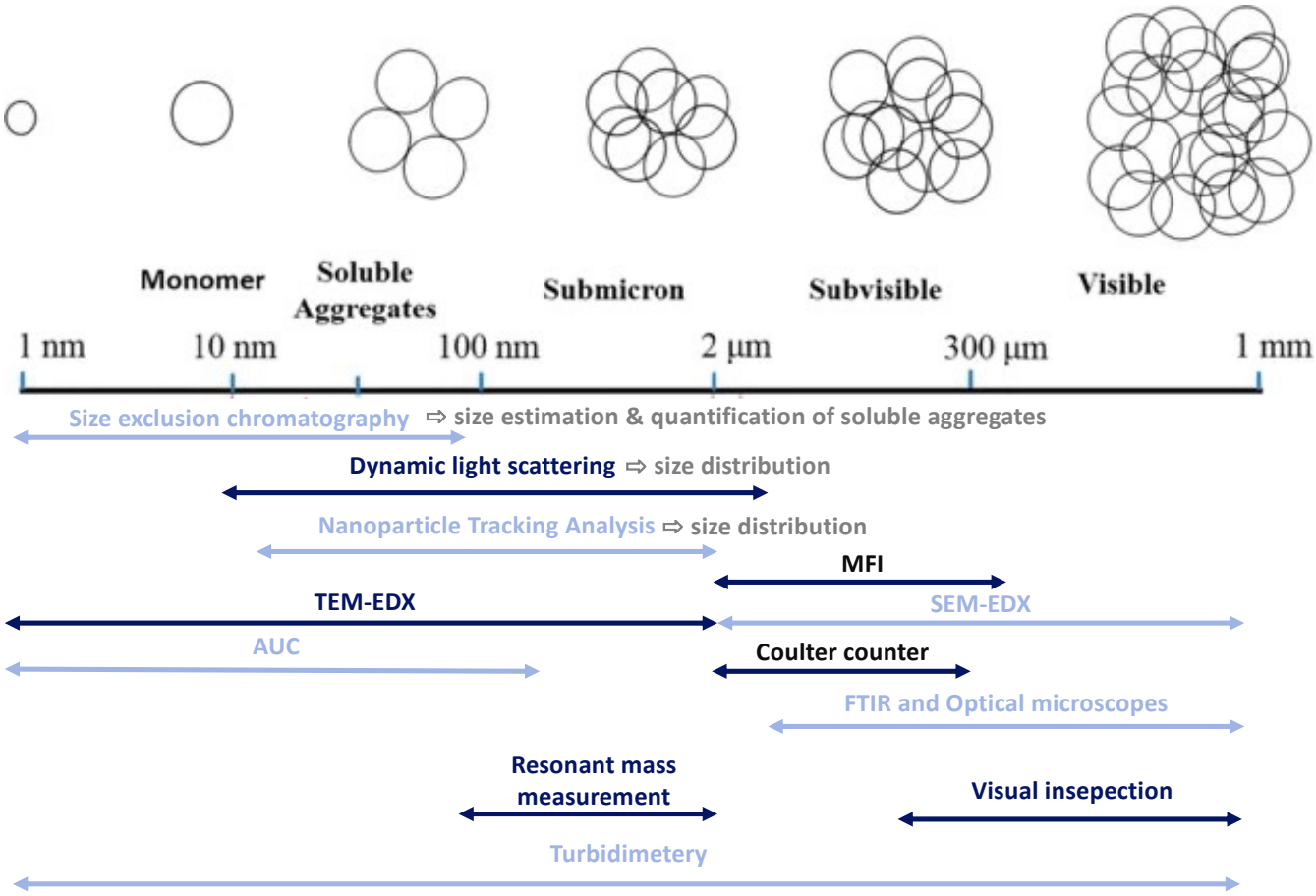
université PARIS-SACLAY | FACULTÉ DE PHARMACIE

Back-Up slides

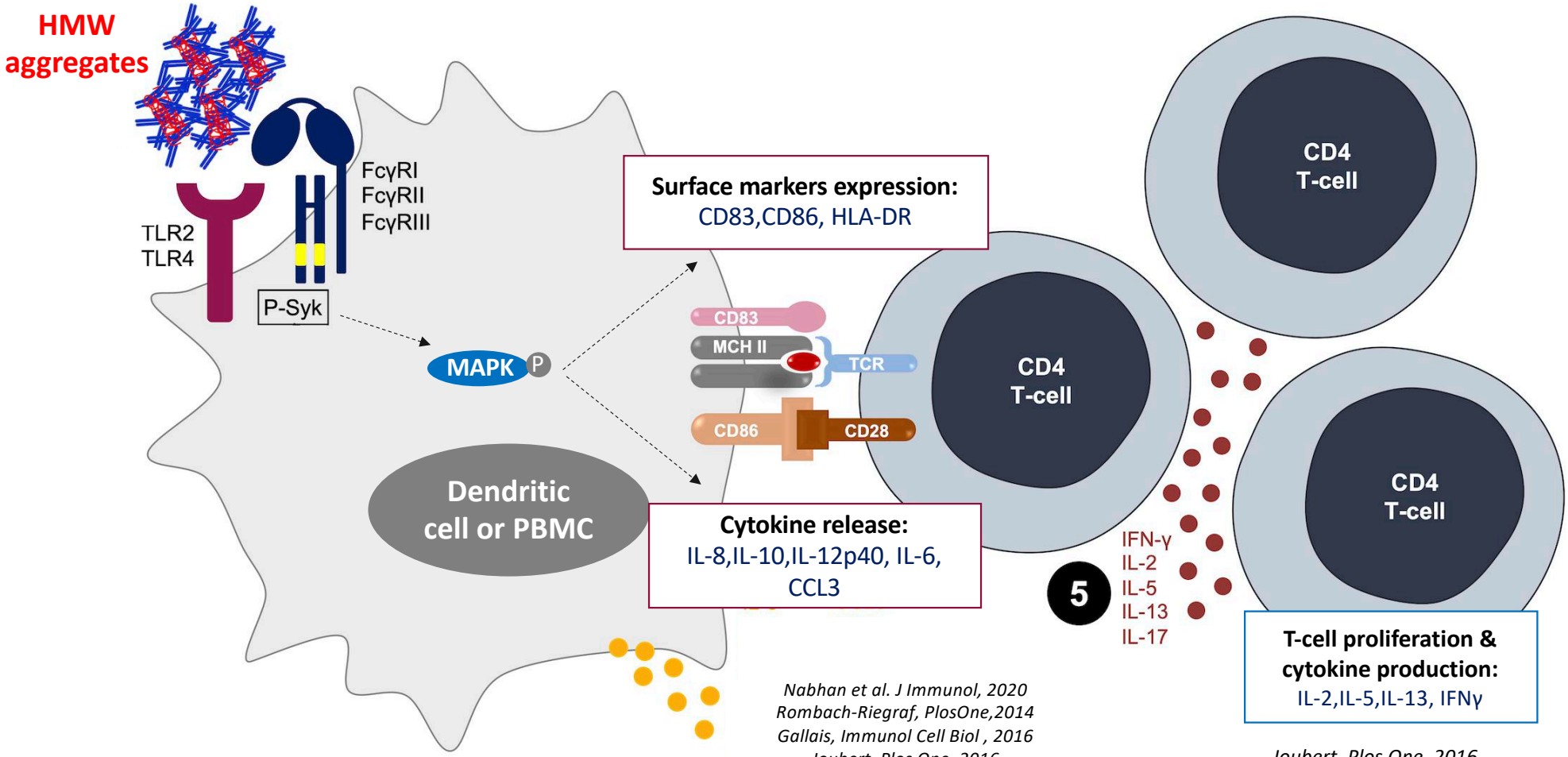
AGGREGATES CHARACTERIZATION



Adapted from Bansal et al, Pharmaceutical Research, 2019

AUC: Analytical Ultra Centrifugation ; FTIR: Fourier Transform Infra-Red Spectroscopy ; MFI: Micro Flow Imaging; SEM-EDX:Scanning Electron Microscopy – Energy Dispersive X-ray Spectroscopy

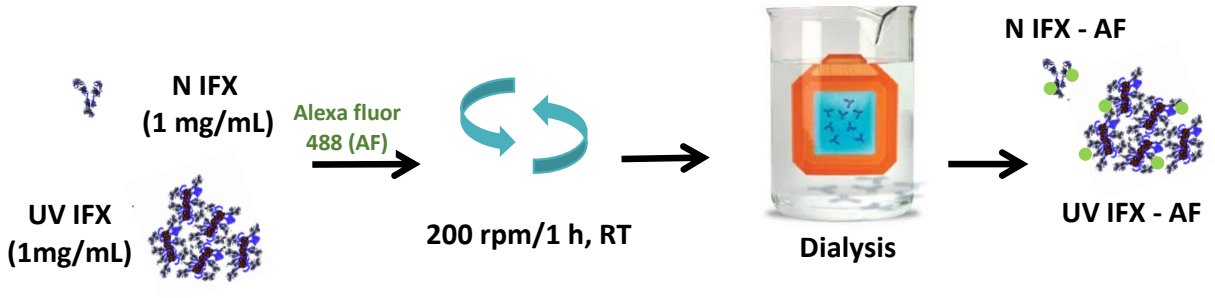
PREVIOUS *IN VITRO* STUDIES: HIGH MW AGGREGATES ACT AS DANGER SIGNAL: phenotype modifications with consequences on T cell proliferation



Nabhan et al. J Immunol, 2020
Rombach-Riegraf, PlosOne,2014
Gallais, Immunol Cell Biol , 2016
Joubert, Plos One, 2016

Joubert, Plos One, 2016
Gallais, Immunol Cell Biol , 2016

ANTIBODY LABELLING



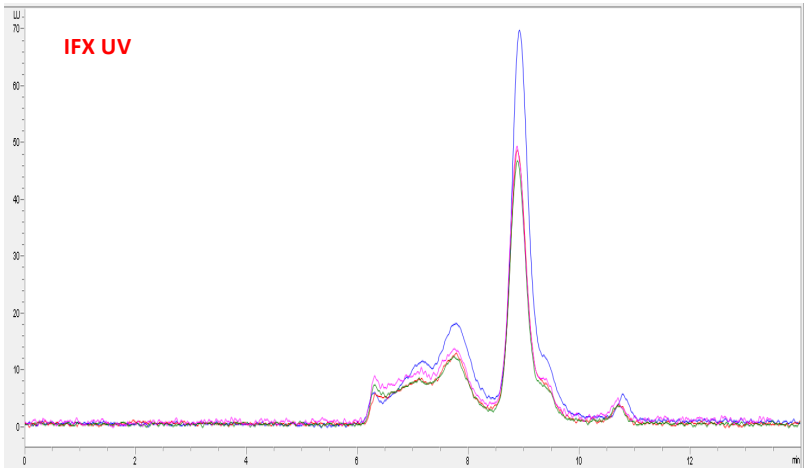
$$\text{Protein concentration (M)} = \frac{[A_{280} - (A_{494} \times 0.11)] \times \text{dilution factor}}{203{,}000}$$

$$\text{Moles dye per mole protein} = \frac{A_{494} \times \text{dilution factor}}{71{,}000 \times \text{protein concentration (M)}}$$

SAMPLE	A 280 nm	A 494 nm	Moles dye per mole protein
IFX N - AF	1.273	1.307	3.31
IFX UV - AF	0.773	0.978	4.20

1.2 times more labeled

n=4



Size exclusion chromatography

Blue: IFX UV
Red: IFX UV-AF before dialysis
Green: IFX UV-AF after dialysis

Labelling do not induce the formation of further aggregates; however, it results in some protein loss.

MHC ASSOCIATED PEPTIDE PROTEOMICS (MAPPS)

Identification of peptides (determination of the aa sequence) via a MHC ASSOCIATED PEPTIDE PROTEOMICS (MAPPS) assay and comparison to database

1. Peptide isolation

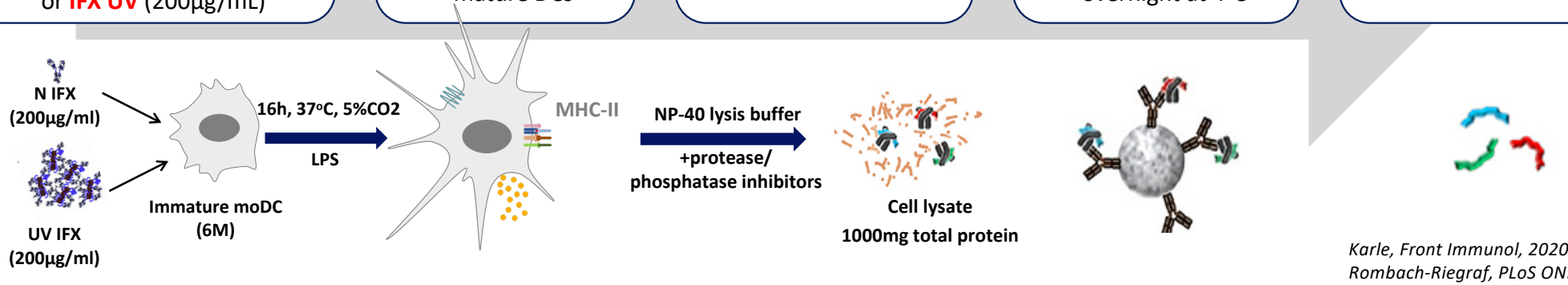
Immature DCs
Immature DCs (6M) loaded with **LPS** (1µg/mL) and **IFX N** (200µg/mL) or **IFX UV** (200µg/mL)

Mature DCs
Presentation of peptides via HLA class II molecules by mature DCs

Cell lysis
in hypotonic buffer with a soft detergent

Immunoprecipitation
Incubation of cell lysate with anti-HLA-DR conjugated beads overnight at 4°C

Peptide isolation
- Peptide elution via acidification (acetic acid)
- Peptide lyophilization



Karle, Front Immunol, 2020
Rombach-Riegraf, PLoS ONE 9, 2014

2. Mass spectrometry
in collaboration with Servier Laboratories

- Determine HLA-DR presented peptide amino acid sequences