Intracellular tracing of amyloid vaccines through direct fluorescent labelling

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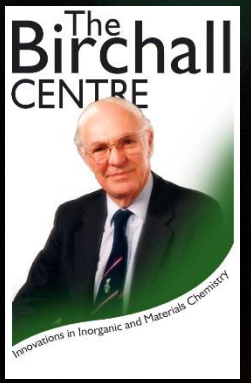


Amyloid in immunotherapy

- The accumulation of the Aβ peptides *in vivo*, is thought to contribute to the pathogenesis of both familial and sporadic forms of Alzheimer's disease (AD).

- Interestingly, immunotherapy using Aβ has shown therapeutic promise in developing *active* and *passive* treatments for AD **(Sevigny *et al*., 2016)**.

- The phagocytic nature of migratory blood-borne monocytes may predispose them to internalise misfolded amyloid peptides and seed amyloid formation in the brain **(Eisele *et al*., 2014)**.

- Therefore, uncovering mechanisms governing the cellular fate of amyloid may prove key in the development of future vaccination regimes.

Vaccine formulations

- **Antigen**:

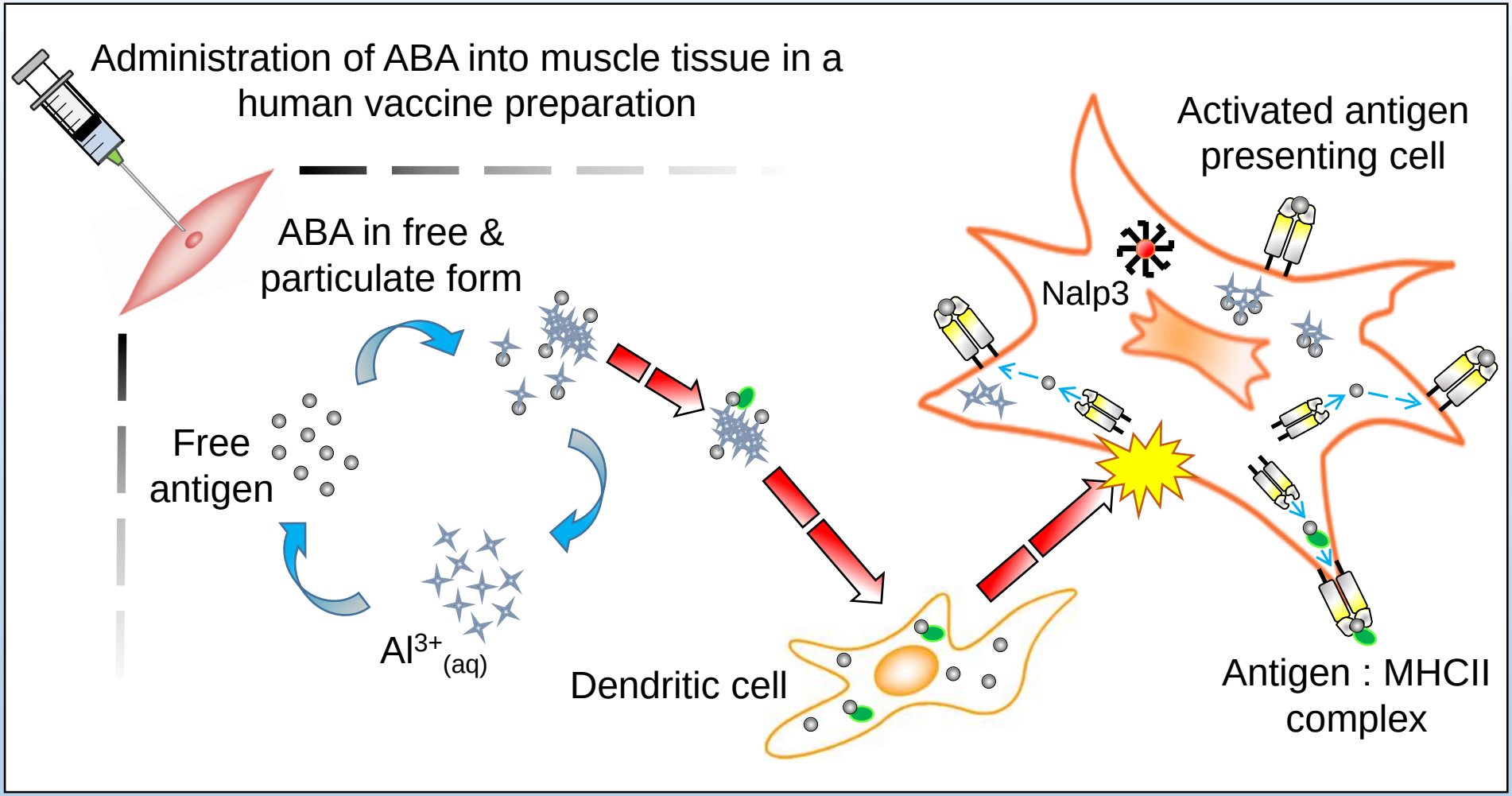
A substance recognised as foreign to the body capable of stimulating an immune response (**Ghimire *et al*., 2015**).

- **Adjuvant**:

Components capable of enhancing and/or shaping antigen specific immune responses (**Reed *et al*., 2013**).

- Vaccine formulations typically contain an antigen and an adjuvant, diluted into autoclaved and sterile 0.9% sodium chloride (GlaxoSmithKline, GSK).

Mechanism of aluminium adjuvanticity



"Current opinion for a mechanism of aluminium adjuvanticity via cognate B-cell activation and T-cell differentiation". *10th Keele Meeting on aluminium, Winchester, 2013*.

Aims and objectives

- Establish direct fluorescent labelling methodologies to identify $A\beta_{42}$ and aluminium based adjuvants (ABA) in a T helper 1 (THP-1) cell line.

- Optimise treatment conditions to allow for the propagation of amyloid fibrils of $A\beta_{42}$ in THP-1 cell culture media.

- Utilise the complementary technique of transmission electron microscopy (TEM) to identify potential intracellular antigen and adjuvant materials.

- Elucidate upon the mechanisms of cellular uptake of amyloid vaccines, underpinning the unusual persistence of intracellular misfolded protein aggregates observed *in vivo*.

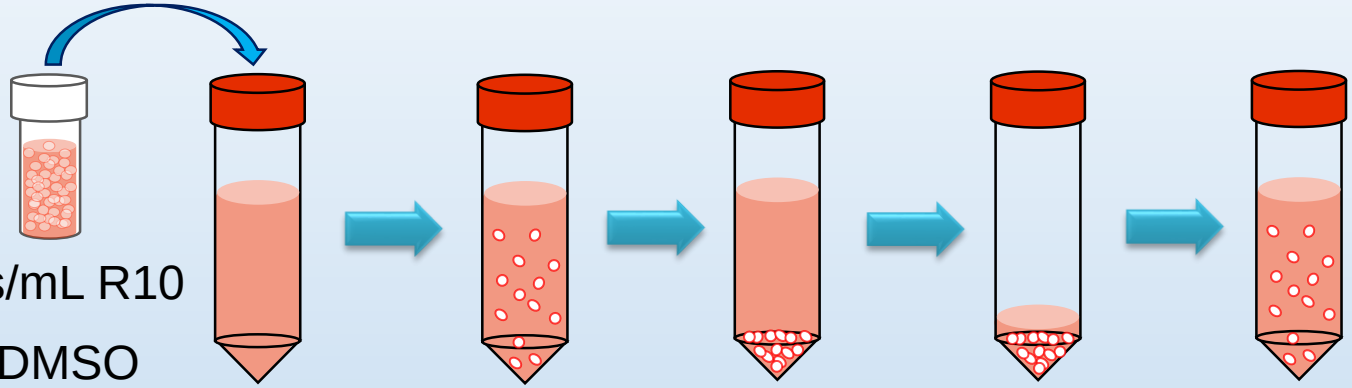
Aluminium based adjuvants (ABA)

Brenntag Biosector, Denmark

Imject™ Alum, Thermo scientific

- **Alhydrogel®**: Aluminium oxyhydroxide, Al(O)OH
  - Crystalline rod / needle-like structure
  - Net positive charge
- **Adju-Phos®**: Aluminium hydroxyphosphate
  - Amorphous structure
  - Net negative charge
- **Imject™ Alum**: Magnesium hydroxide aluminium hydroxycarbonate
  - Amorphous structure
  - An experimental adjuvant formulation

T helper 1 (THP-1) cell culture



1 x 10^6 cells/mL R10

+ 5% *v/v* DMSO

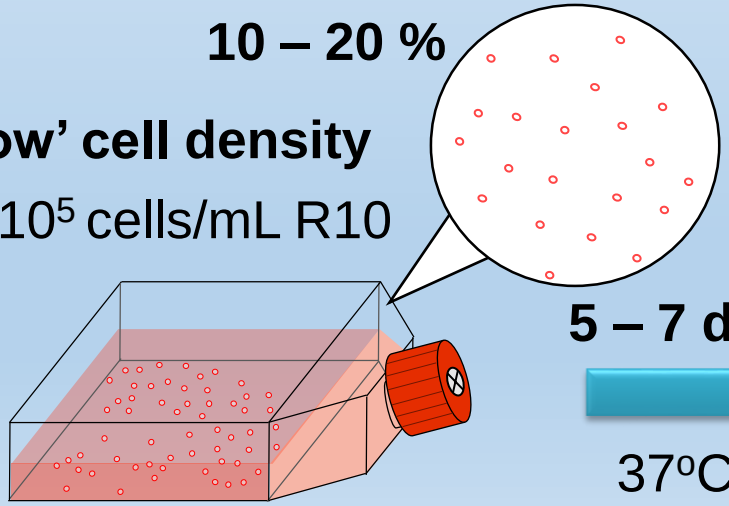
Cells thawed & transferred into warm R10 medium

Cells pelleted and re-suspended in R10

10 – 20 %

'Low' cell density

1 x 10^5 cells/mL R10



5 – 7 days

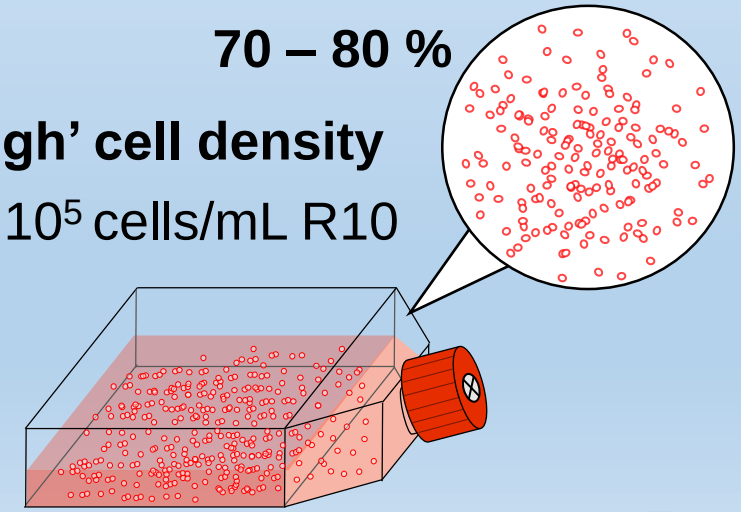
37°C,

5% CO_2

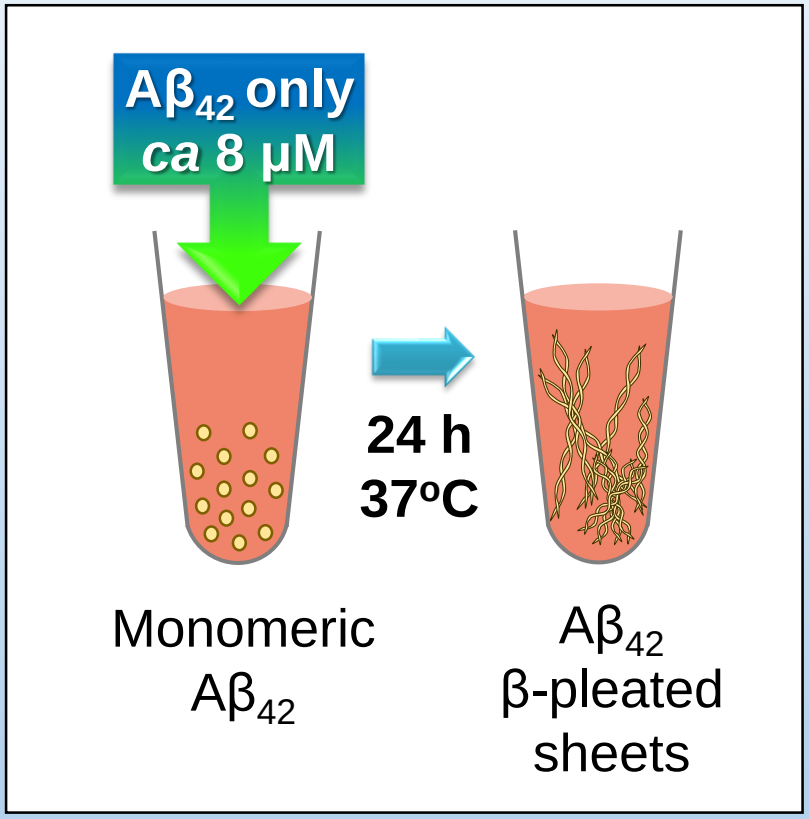
70 – 80 %

'High' cell density

5 x 10^5 cells/mL R10



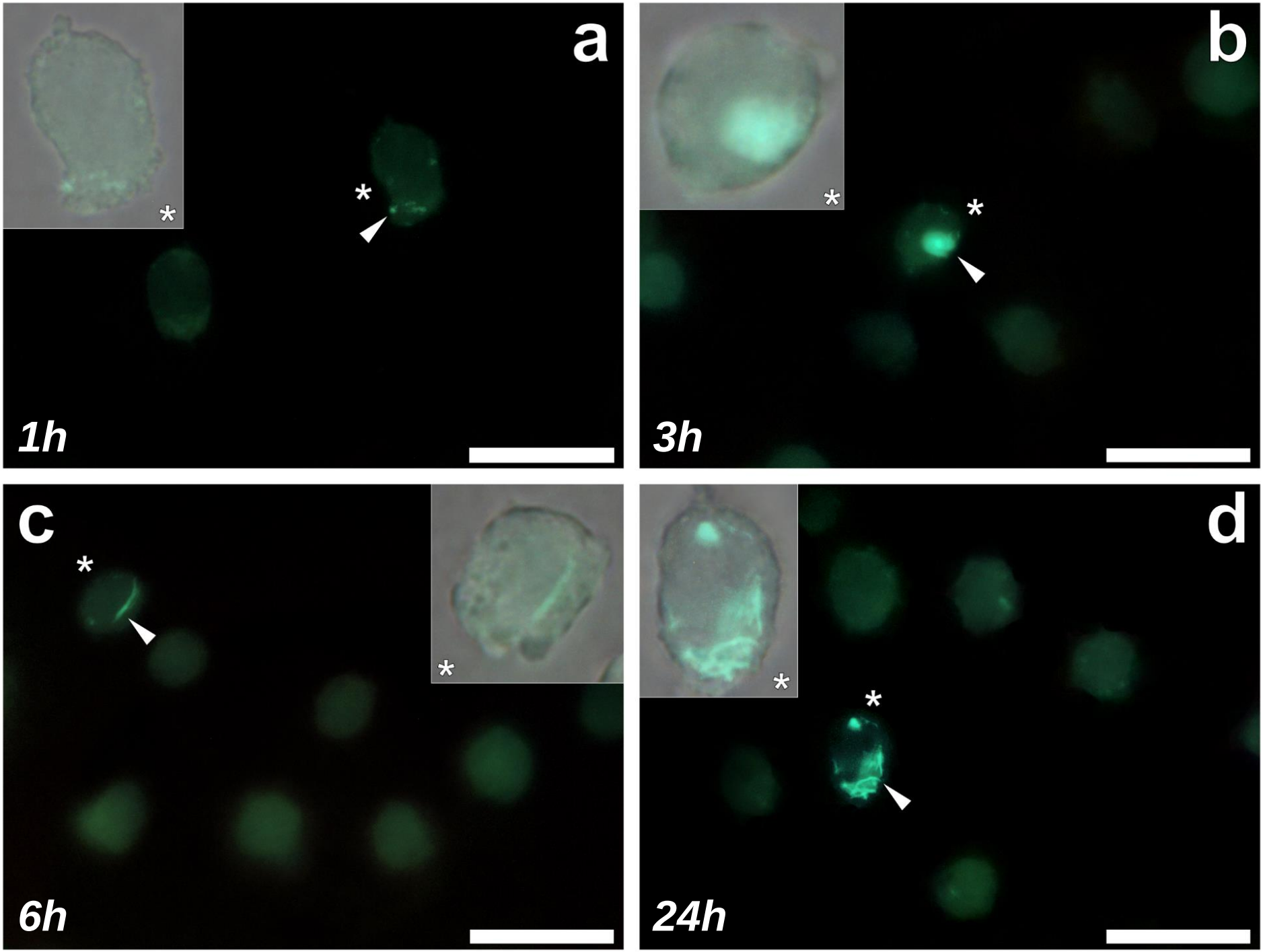
Amyloid fibril formation of $A\beta_{42}$

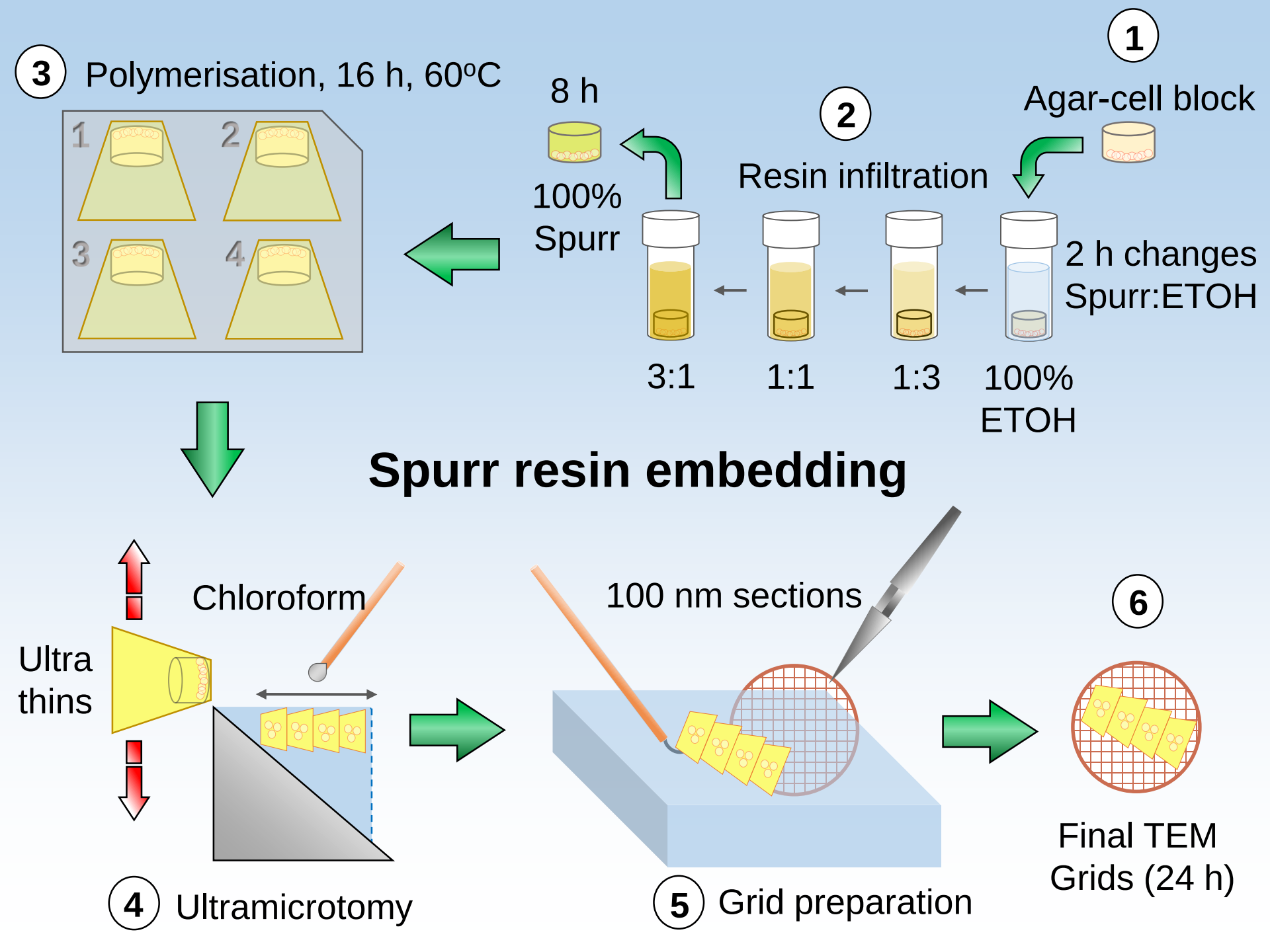


β-pleated sheet propagation in R10 cell culture medium

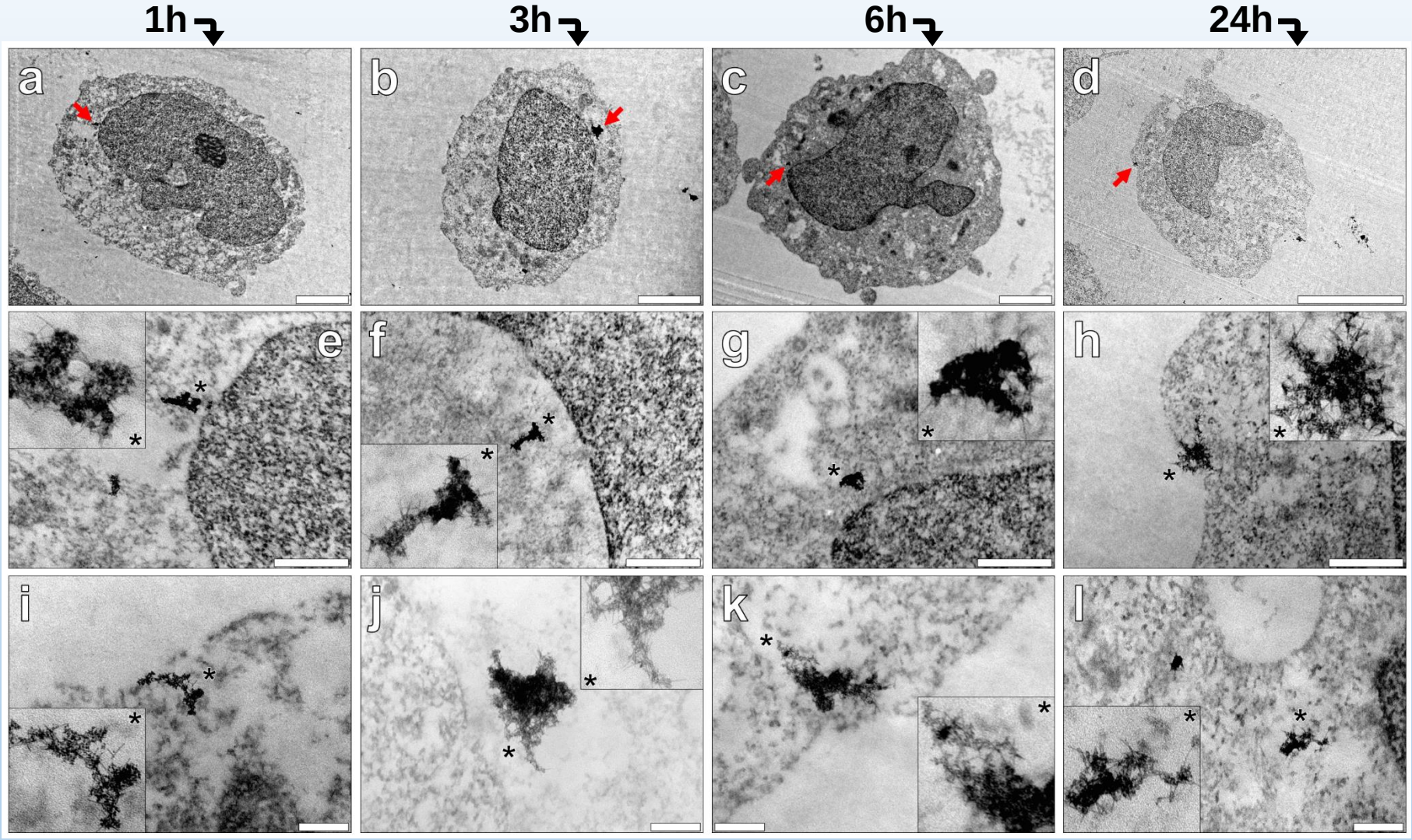
- $A\beta_{42}$ was reconstituted in 0.01M NaOH and diluted into complete R10 medium at *ca* 8µM.
- Peptide only treatments were incubated for 24h at 37ºC to initiate amyloid fibril formation.
- A thioflavin T (ThT) assay indicated a 64.8% increase in fluorescence intensity at 482nm (13.07 ± 0.34, mean ± SD, *n* = 3) above background (4.60 ± 0.85, *n* = 3).

(House *et al*., 2004)


a
1h
b
3h
c
6h
d
24h

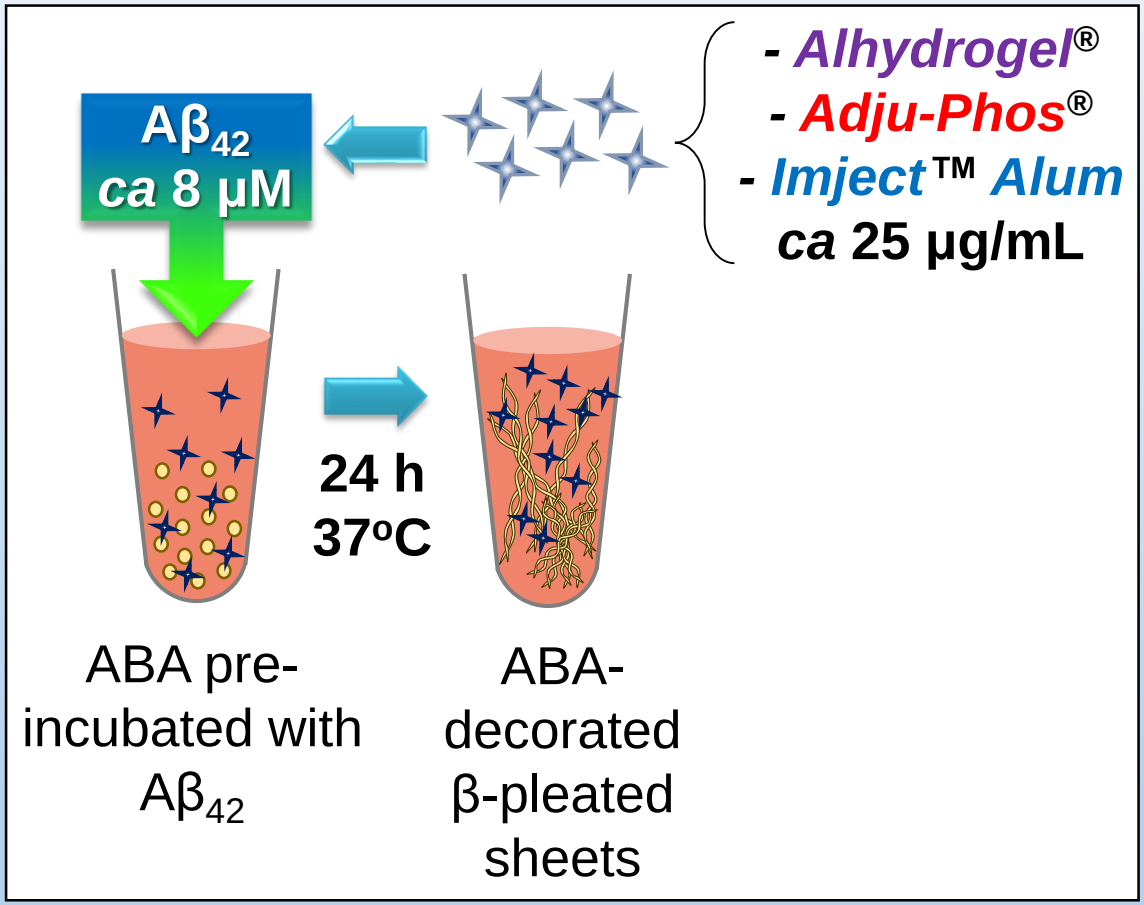

1
Agar-cell block
2
Resin infiltration
2 h changes
Spurr:ETOH
100%
ETOH
1:3
1:1
3:1
100%
Spurr
8 h
3
Polymerisation, 16 h, 60ºC
1
2
3
4
Spurr resin embedding
Ultra
thins
Chloroform
4
Ultramicrotomy
100 nm sections
5
Grid preparation
6
Final TEM
Grids (24 h)

TEM of intracellular $A\beta_{42}$



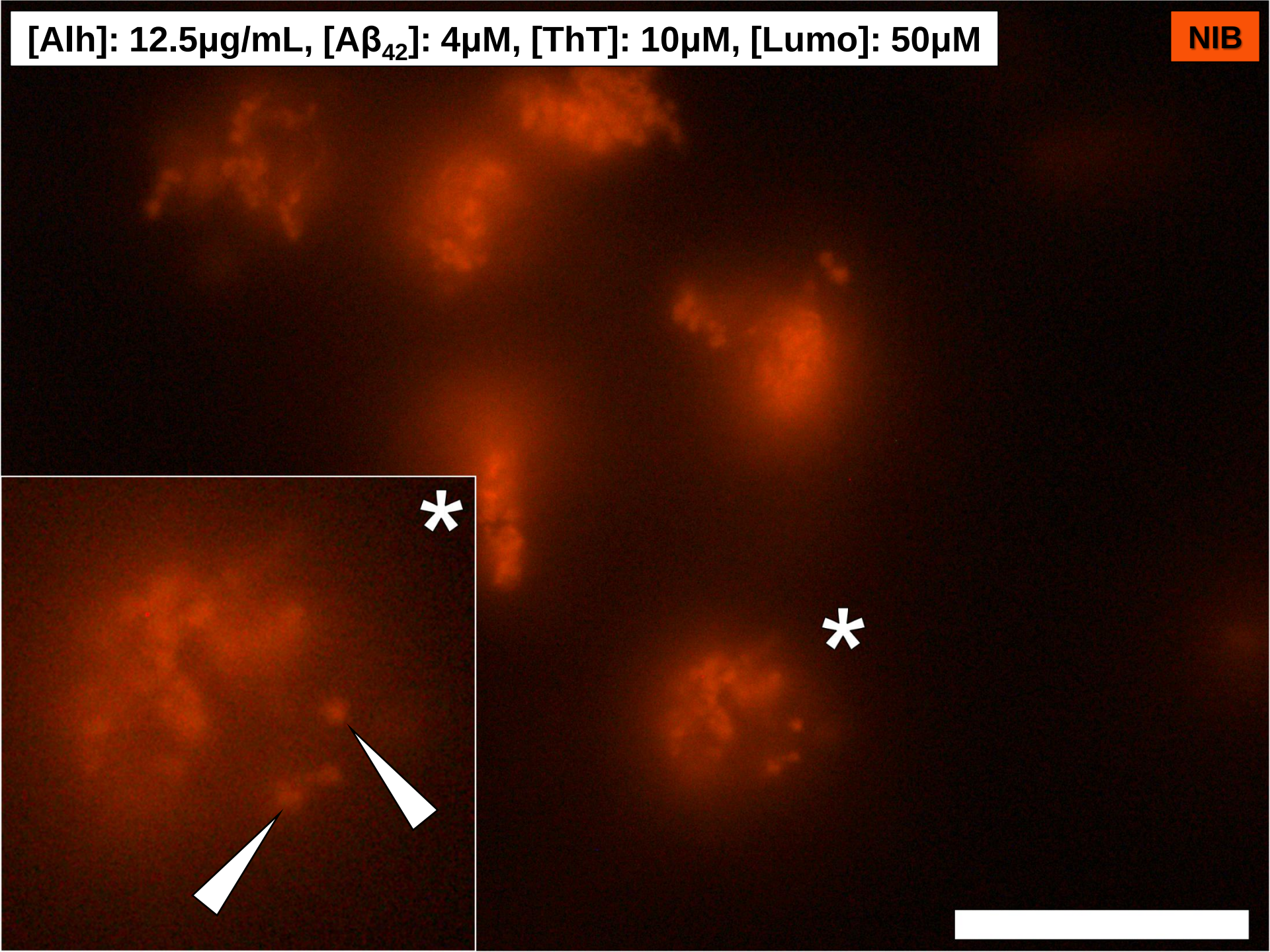
THP-1 cells co-cultured with *ca* 4μM $A\beta_{42}$ over 24h. Mag. & scale bars: **a & c.** X 10K, 2μm, **b.** X 12K, 2μm, **d.** X 8K, 5μm, **e – h.** X 60K, 0.5μm & **i – l.** X 100K, 0.2μm, respectively.

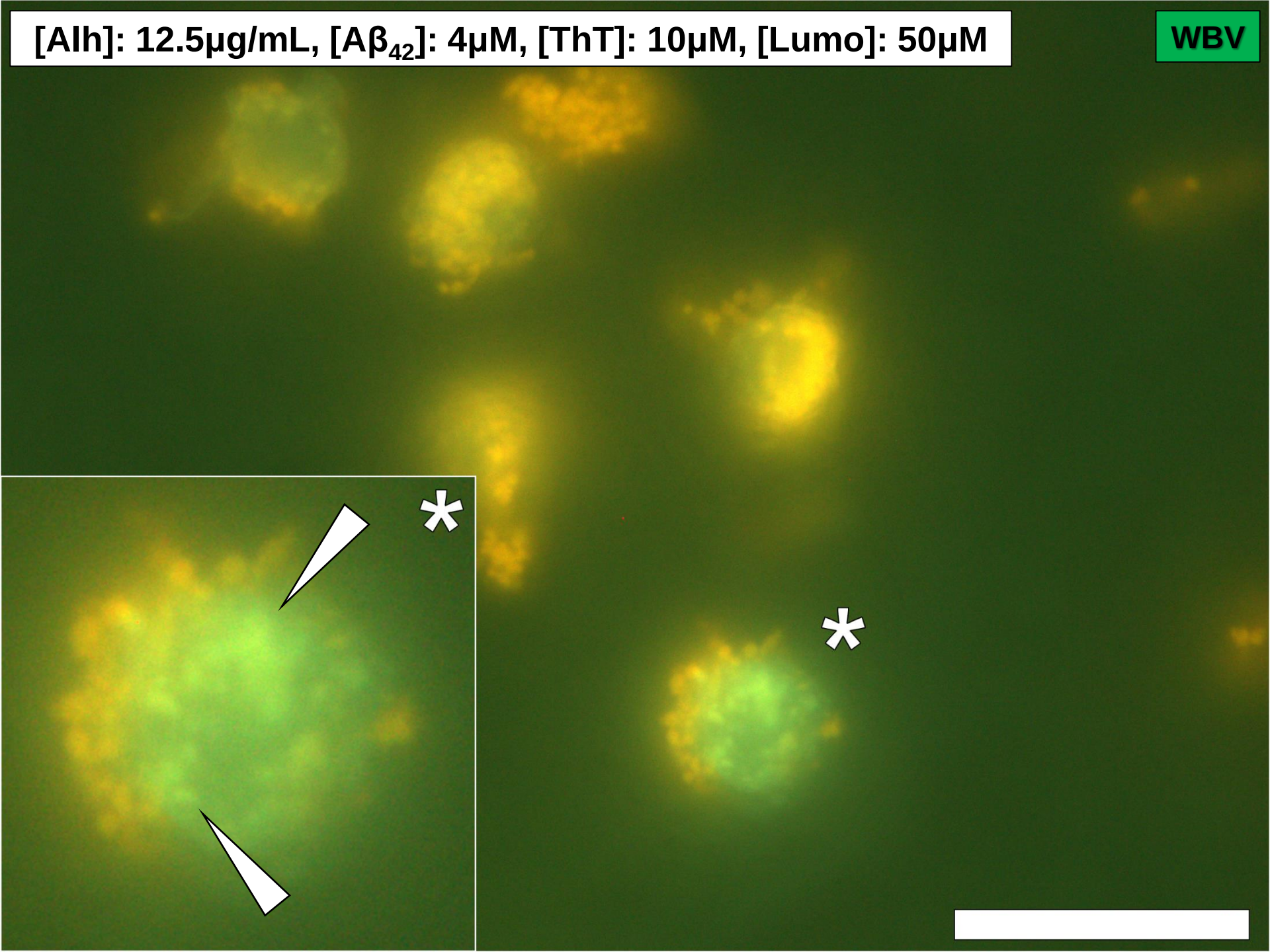
Simulated vaccine formulations



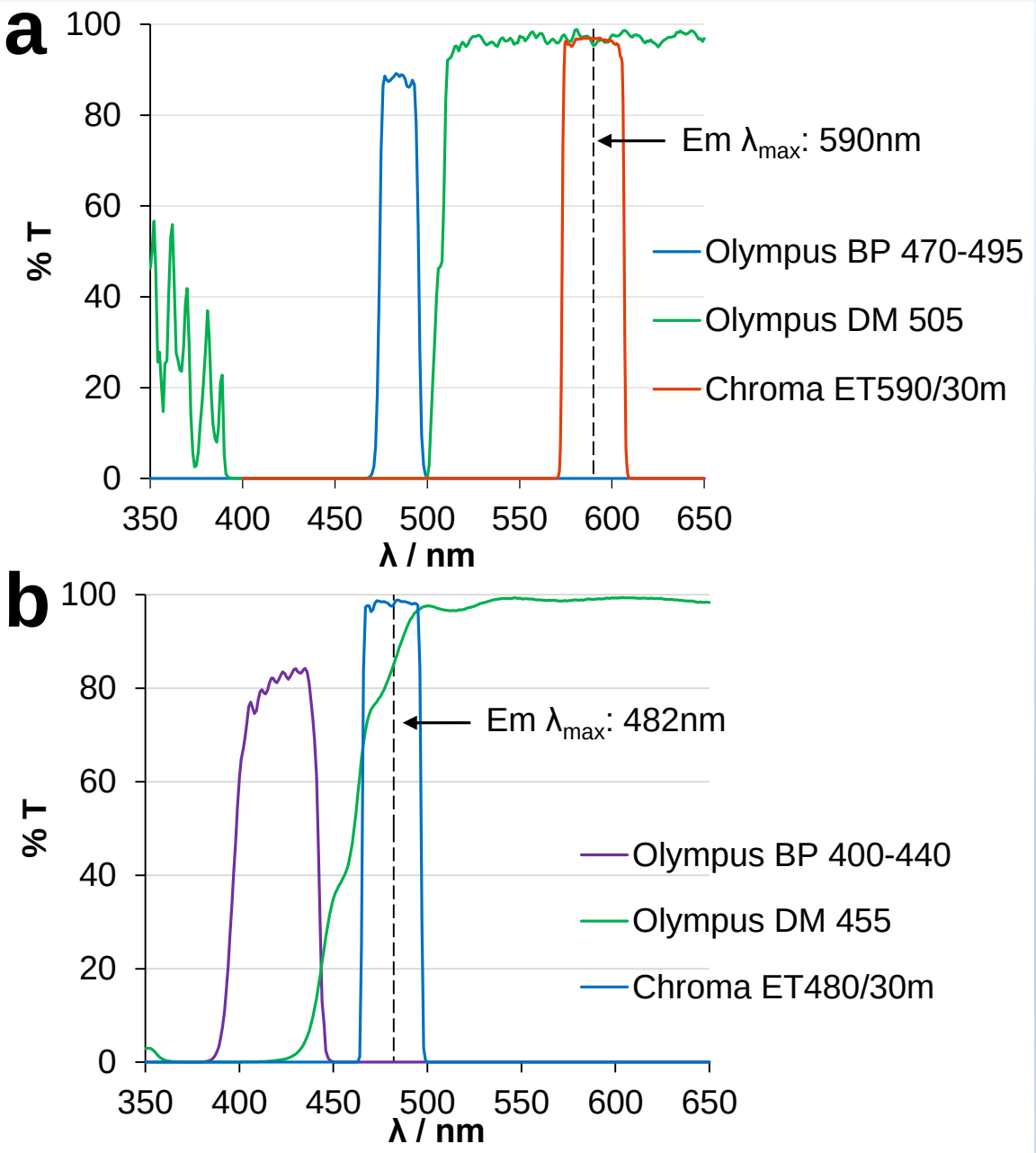
ABA formulated with monomeric $A\beta_{42}$ in R10 cell culture medium.

- ABA were prepared at *ca* 1mg/mL in 0.9% *w/v* NaCl and diluted to *ca* 25µg/mL in R10 medium.
- $A\beta_{42}$ was added at *ca* 8µM in the presence or absence of a given ABA.
- Treatments were incubated for 24h at 37°C and added 1:1 to THP-1 cells.


[Alh]: 12.5µg/mL, [Aβ42]: 4µM, [ThT]: 10µM, [Lumo]: 50µM
NIB

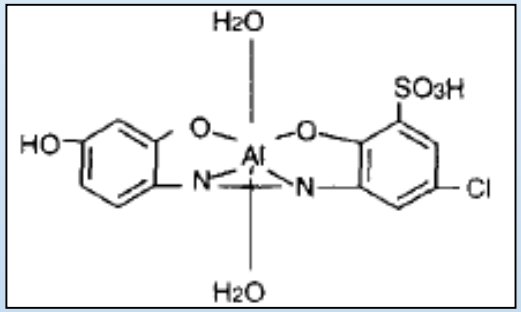

[Alh]: 12.5µg/mL, [Aβ42]: 4µM, [ThT]: 10µM, [Lumo]: 50µM
WBV

Spectral profiles of single bandpass filters



(a) Lumogallion fluorescence

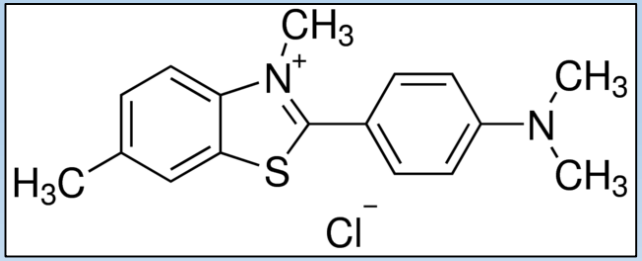
U-MNIB3 (Olympus) filter cube and a ET590/30m SBP emission filter (Chroma®, Vermont, US).



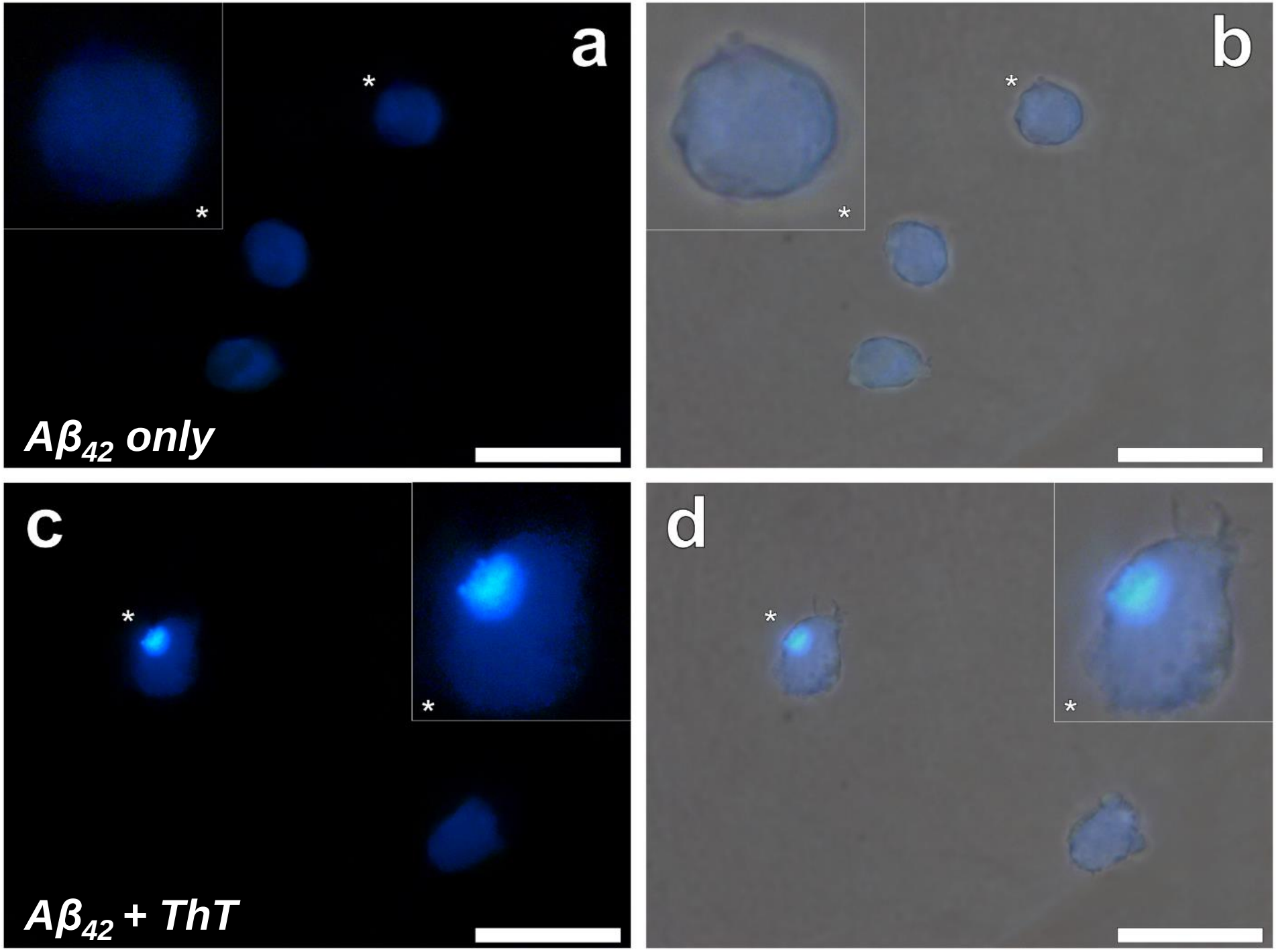
*

(b) ThT fluorescence

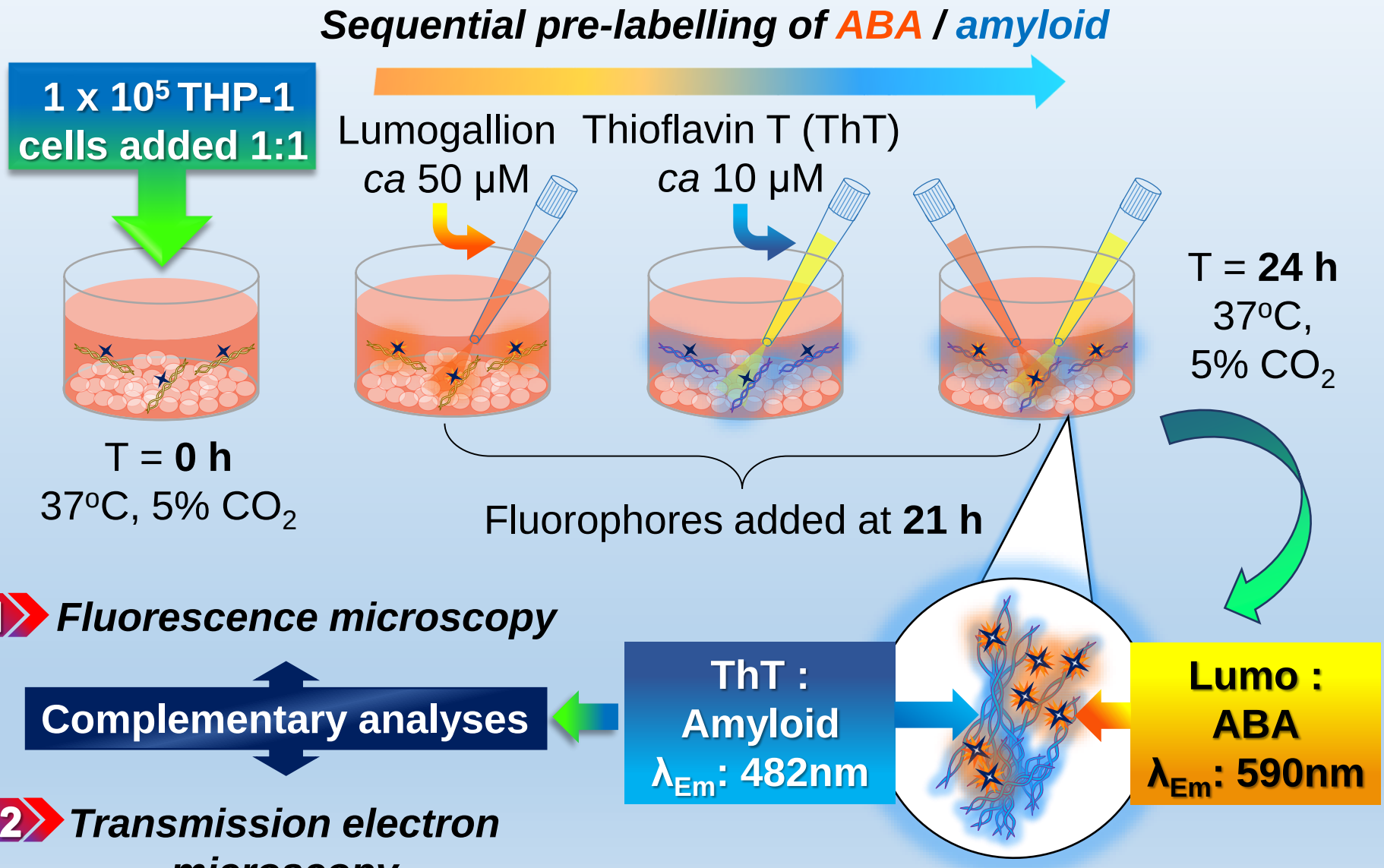
U-MWBV2 cube equipped with a Chroma® ET480/30m SBP emission filter.



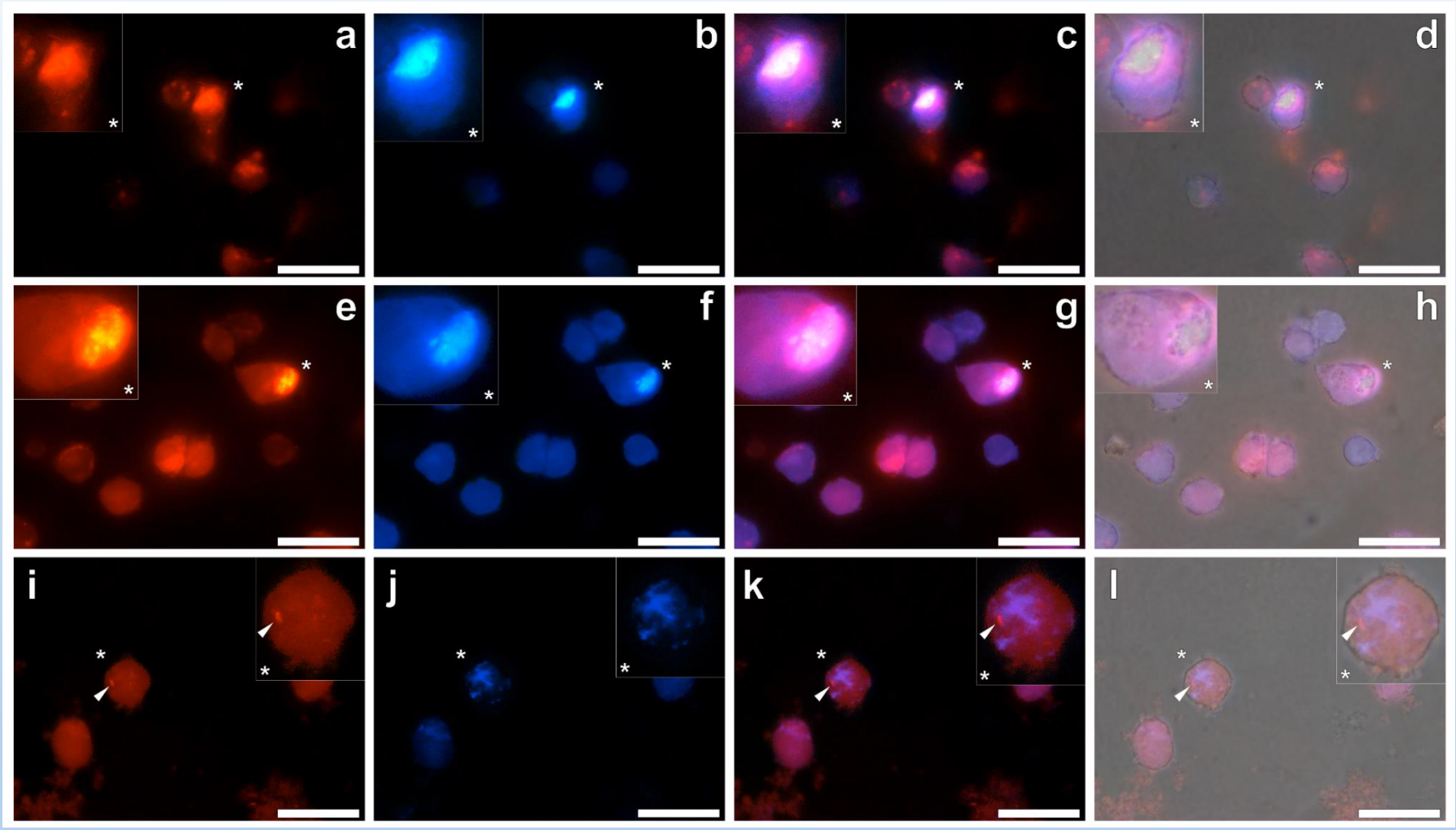
***(Wu *et al.*, 1995)**


a
b
c
d
Aβ42 only
Aβ42 + ThT

Antigen and adjuvant co-culture

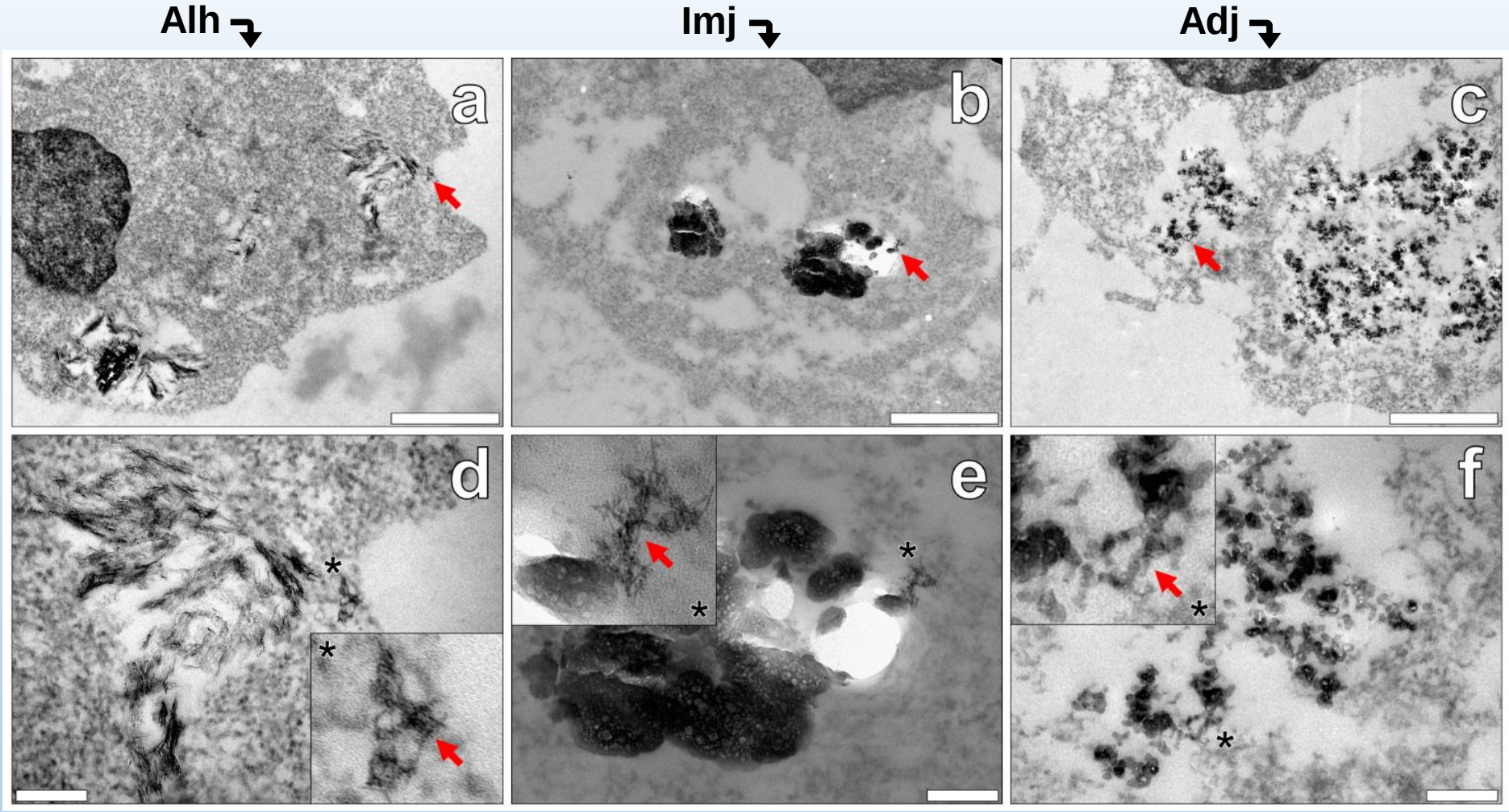


Fluorescence microscopy of amyloid vaccines



THP-1 cells cultured for 24h in simulated vaccine formulations containing *ca* 4μM $A\beta_{42}$. Lumogallion (orange, λ_{em}: 590nm) and ThT (blue, λ_{em}: 482nm) fluorescence is depicted. Mag. X 1000, scale bars: 20μm.

TEM of amyloid vaccines



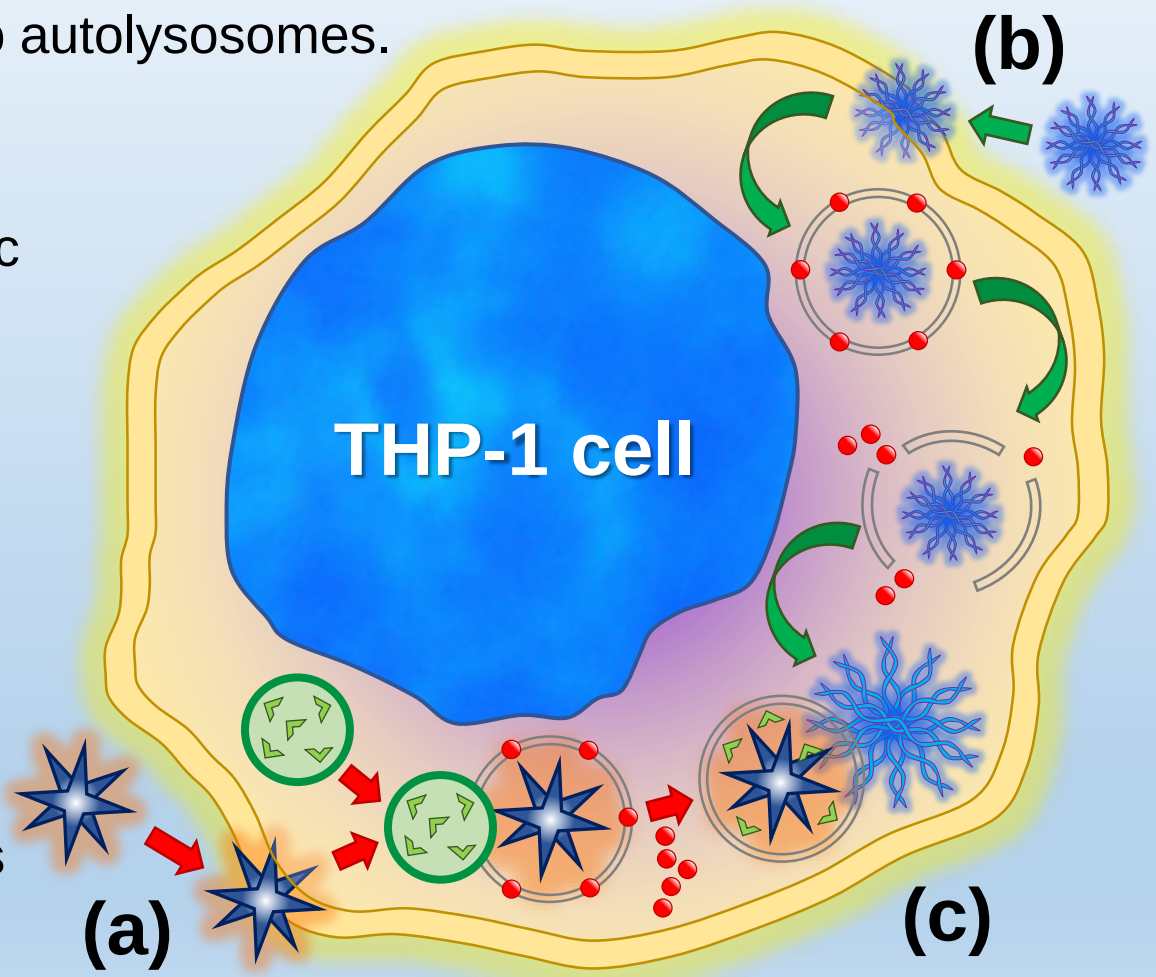
THP-1 cells, co-cultured for 24h with simulated vaccines containing *ca* 4μM $A\beta_{42}$ and 50μg/mL ABA. Mag. & scale bars: **a – c.** X 30K, 1μm, **d – f.** X 100K, 0.2μm, respectively.

Conclusions

(a) Aluminium based adjuvants are internalised via autophagy and processed into autolysosomes.

(b) $A\beta_{42}$ evades lysosomal capture via rupturing endocytic vesicles, releasing cytosolic light chain 3 (LC3).

(c) $A\beta_{42}$ as a model peptide antigen and ABA are suggested to be internalised via the differing endocytic pathways of macropinocytosis and autophagy, respectively.



(Han *et al*., 2017 & Flavin *et al*., 2017)

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