



*Batsheva de Rothschild seminar on:*

*“Current Challenges in Amyloid Diseases: From molecular mechanisms to the cell and clinics”*

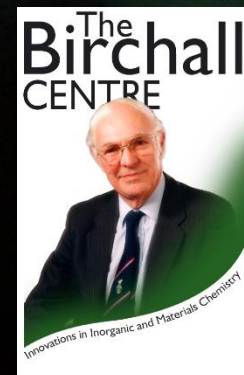
# Intracellular tracing of amyloid vaccines through direct fluorescent labelling

**Dr Matthew J. Mold (MRSB)**

[m.j.mold@keele.ac.uk](mailto:m.j.mold@keele.ac.uk)



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# Amyloid in immunotherapy?

- Interestingly, immunotherapy using A $\beta$  has shown therapeutic promise in developing *passive* treatments for AD, as has been shown for aducanumab (**Sevigny *et al.*, 2016**).
- Phagocytic migratory blood-borne monocytes may predispose them to internalise misfolded amyloid peptides and seed amyloid formation in the brain (**Eisele *et al.*, 2014**).
- Understanding the cellular fate of amyloid may prove key in the development of future vaccination regimes.

# Aluminium based adjuvants (ABA)



*Brenntag Biosector, Denmark*

- **Alhydrogel®**: Aluminium oxyhydroxide,  $Al(O)OH$ 
  - Crystalline rod / needle-like structure
  - Net positive charge

- **Adju-Phos®**: Aluminium hydroxyphosphate
  - Amorphous structure
  - Net negative charge



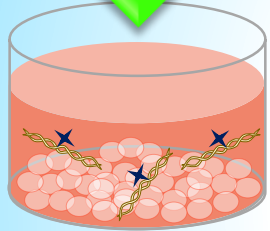
*Imject™ Alum, Thermo scientific*

- **Imject™ Alum**: Magnesium hydroxide aluminium hydroxycarbonate
  - Amorphous structure
  - An experimental adjuvant formulation

# Antigen and adjuvant co-culture

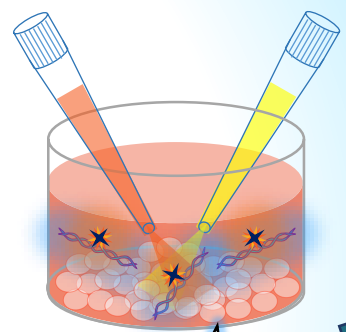
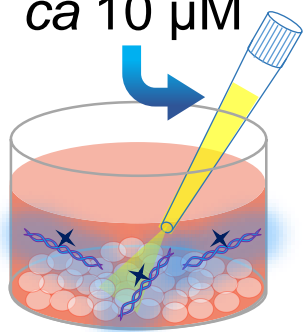
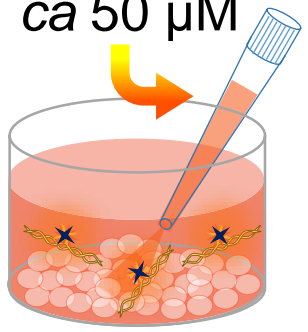
## Sequential pre-labelling of ABA / amyloid

**1 x 10<sup>5</sup> THP-1 cells added 1:1**



**T = 0 h**  
37°C, 5% CO<sub>2</sub>

Lumogallion ca 50 μM      Thioflavin T (ThT) ca 10 μM



Fluorophores added at **21 h**

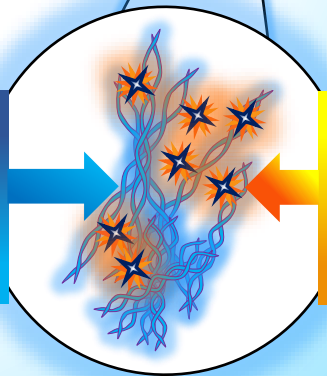
**T = 24 h**  
37°C,  
5% CO<sub>2</sub>

**1** > *Fluorescence microscopy*

**Complementary analyses**

**2** > *Transmission electron microscopy*

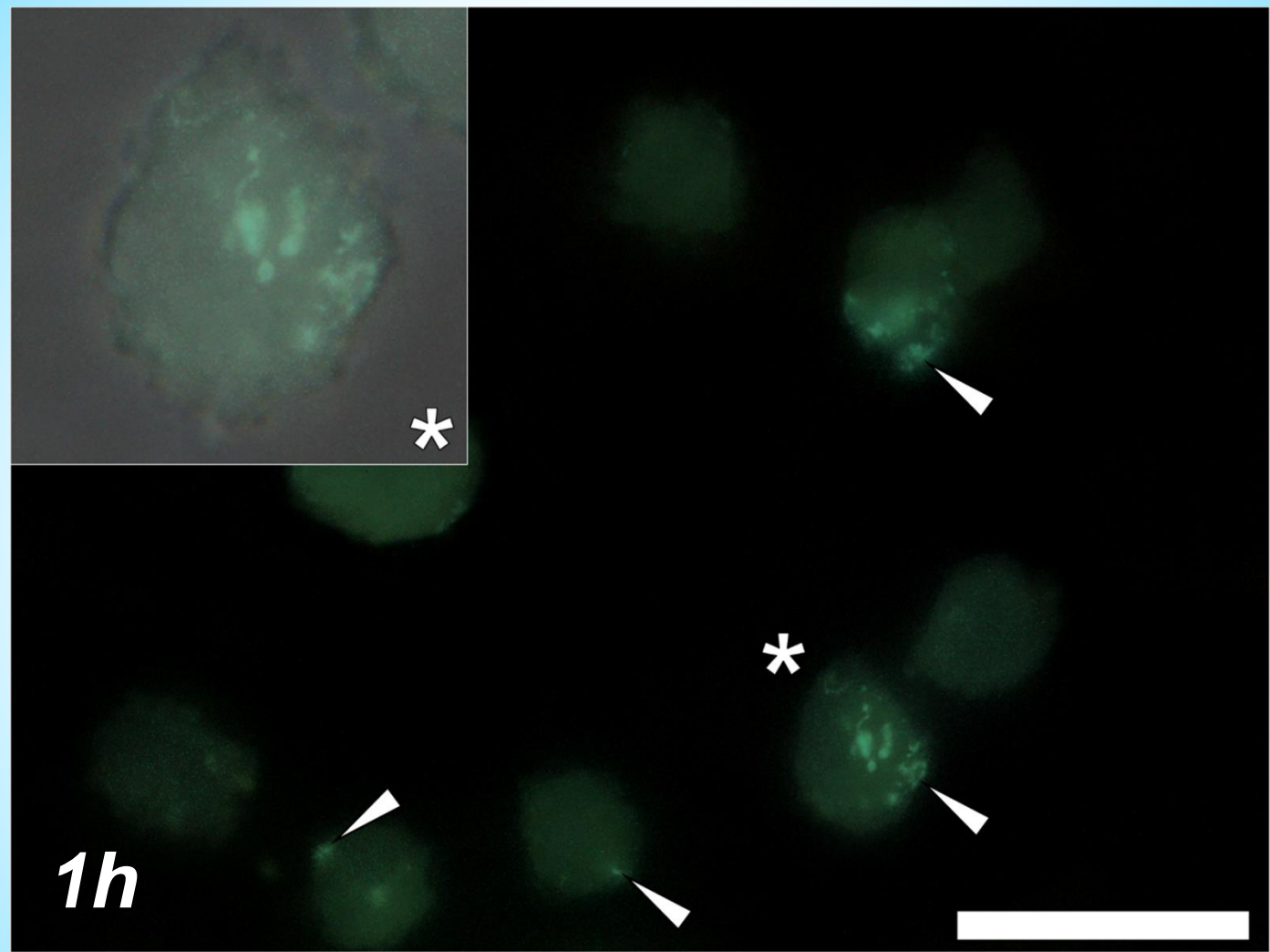
**ThT : Amyloid**  
λ<sub>Em</sub>: 482nm



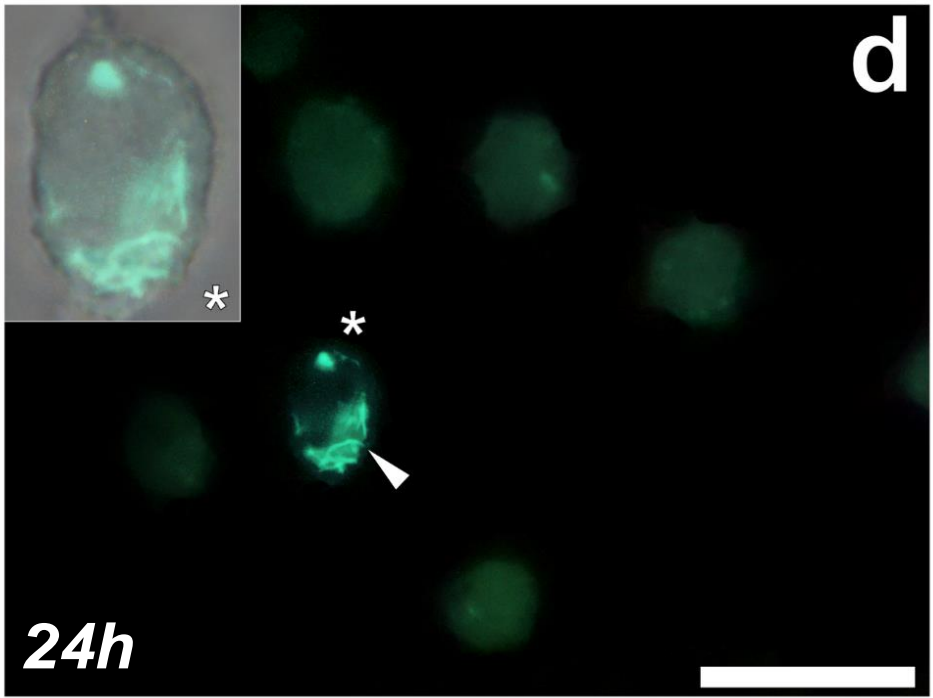
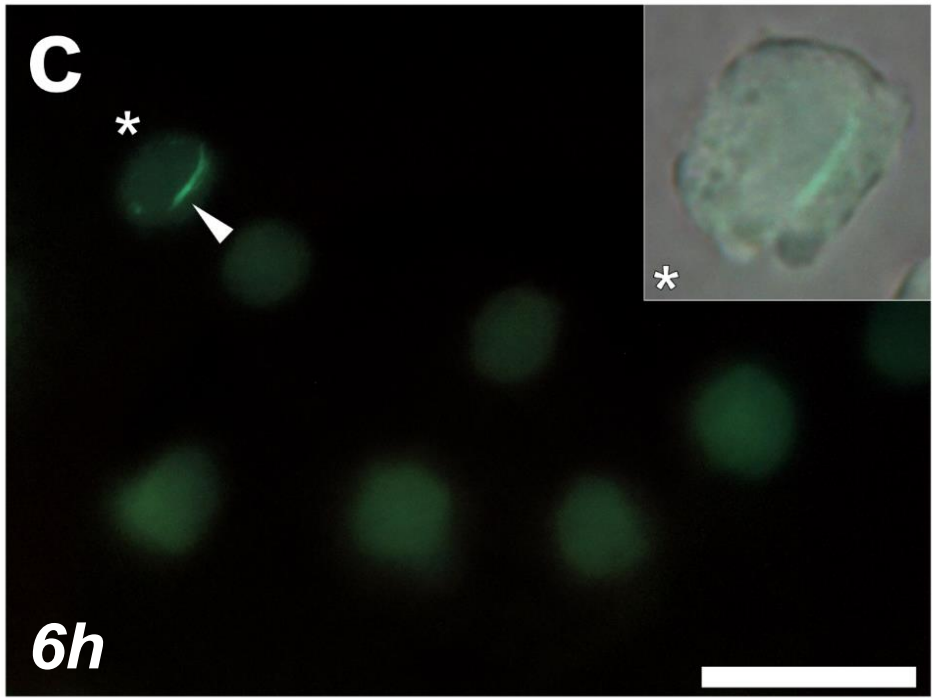
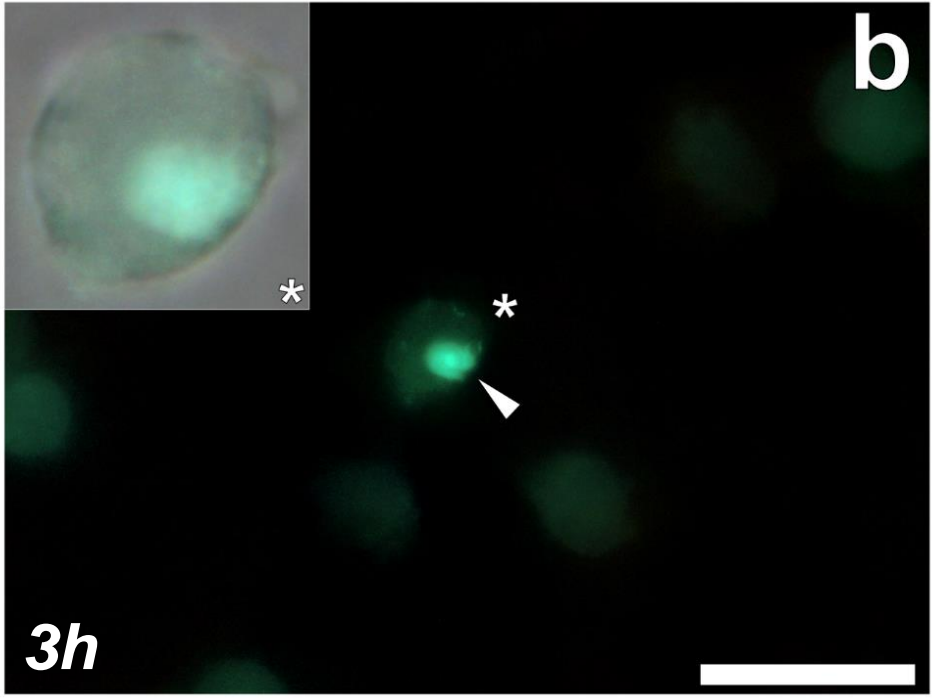
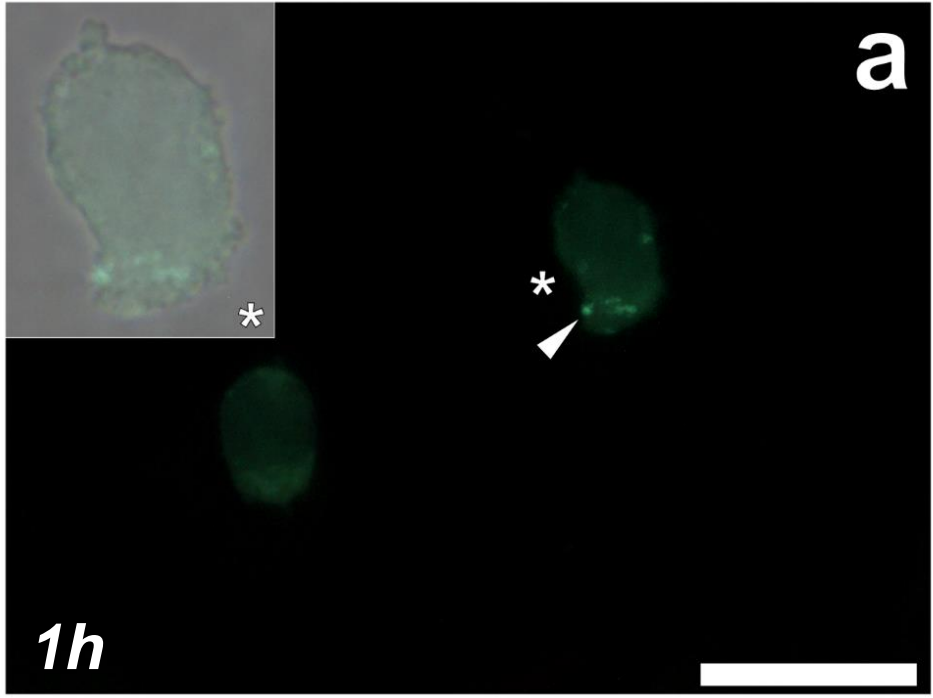
**Lumo : ABA**  
λ<sub>Em</sub>: 590nm



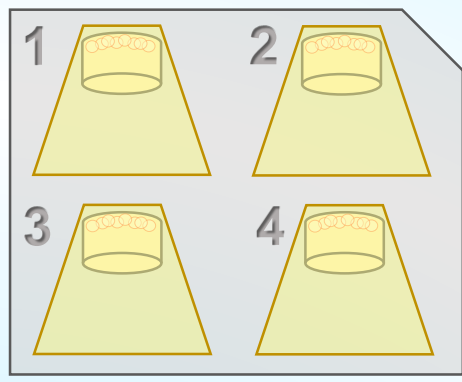
# ThT fluorescence of intracellular A $\beta_{42}$



**THP-1 cells cultured for 1 h in R10 medium containing 10  $\mu$ M thioflavin T (ThT) in the presence of 4  $\mu$ M A $\beta_{42}$ . ThT fluorescence (green) is depicted (Olympus U-WMBV2 filter cube). Mag. X 1000, scale bar: 20  $\mu$ m.**



③ Polymerisation, 16 h, 60°C



8 h  
100% Spurr

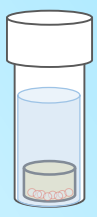
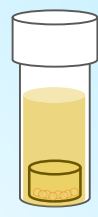
②

Resin infiltration

①

Agar-cell block

2 h changes  
Spurr:ETOH



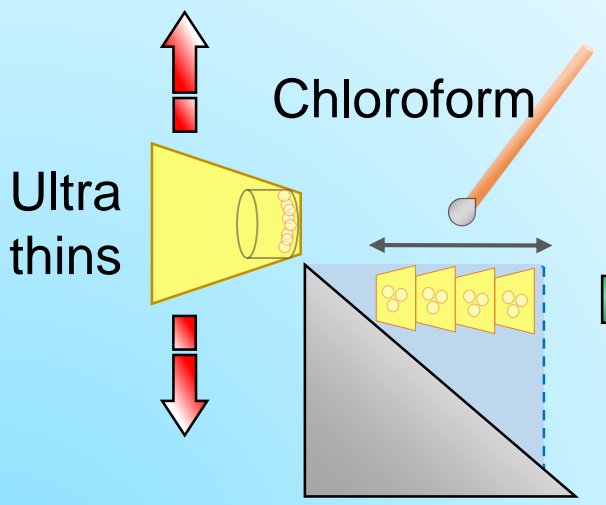
3:1

1:1

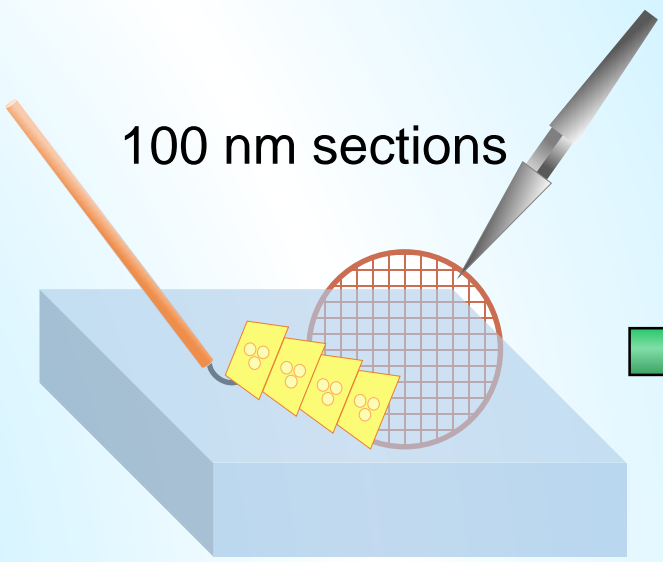
1:3

100%  
ETOH

# Spurr resin embedding



④ Ultramicrotomy



⑤ Grid preparation

⑥

Final TEM  
Grids (24 h)



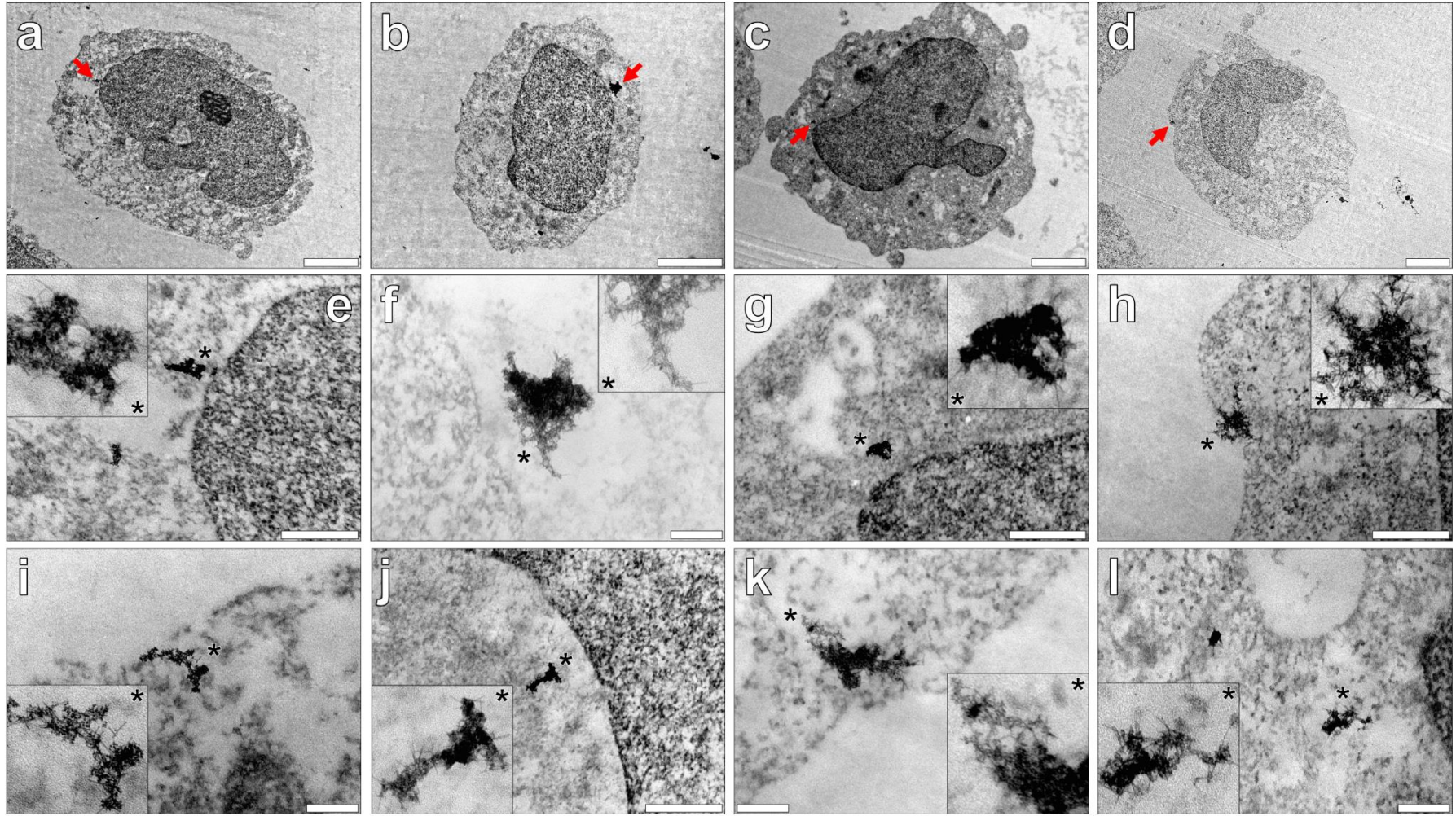
# TEM of intracellular A $\beta_{42}$

1h  $\rightarrow$

3h  $\rightarrow$

6h  $\rightarrow$

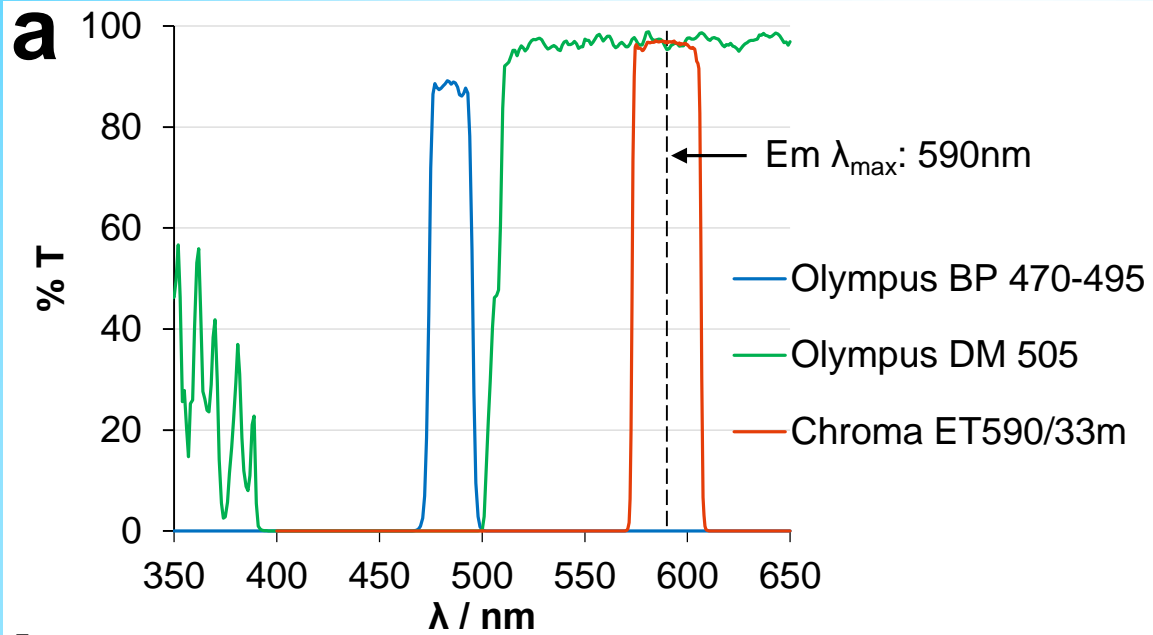
24h  $\rightarrow$



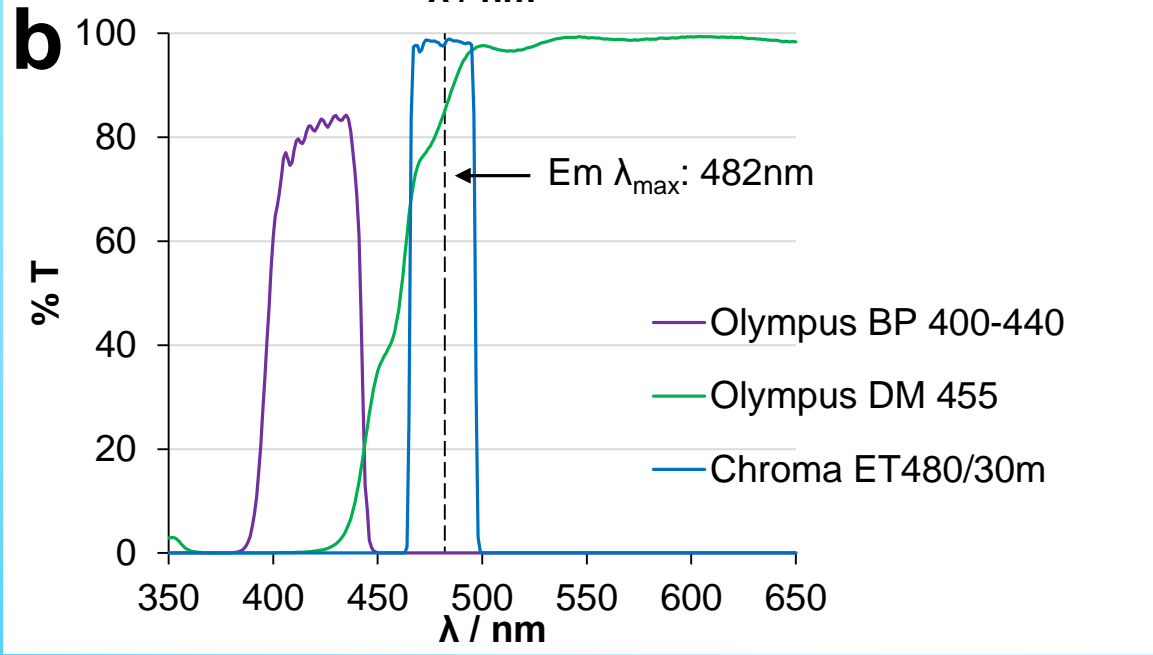
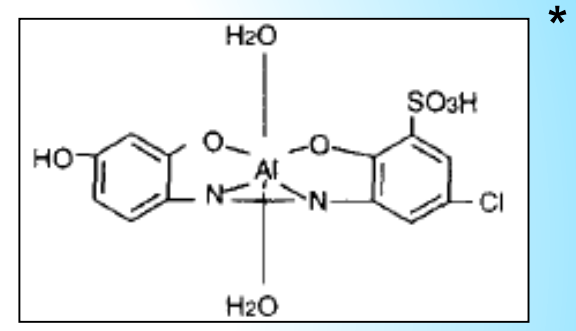
**THP-1 cells co-cultured with ca 4 $\mu$ M A $\beta_{42}$  over 24h. Mag. & scale bars: a & c. X 10 K, 2  $\mu$ m, b. X 12 K, 2  $\mu$ m, d. X 8 K, 2  $\mu$ m, e – h. X 60 K, 0.5  $\mu$ m & i – l. X 100 K, 0.2  $\mu$ m, respectively.**



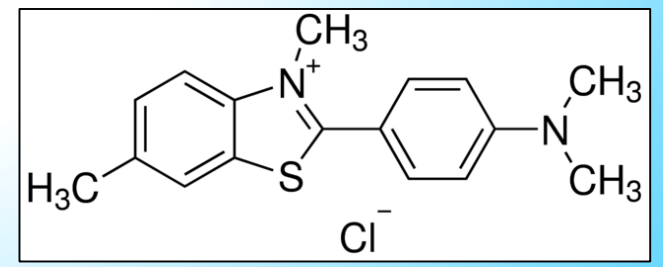
# Fluorescent labelling of amyloid vaccines



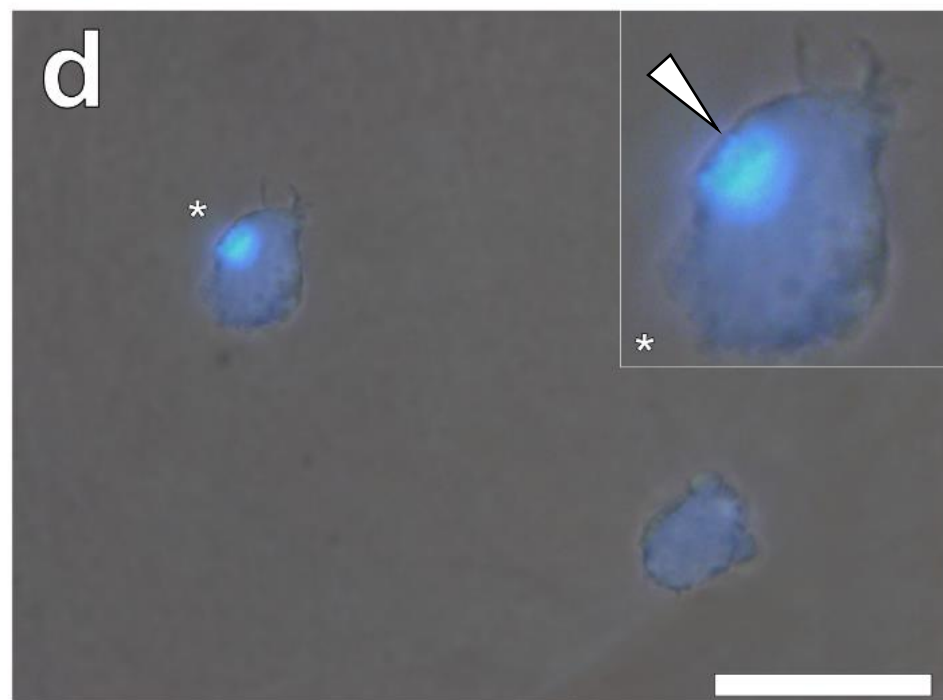
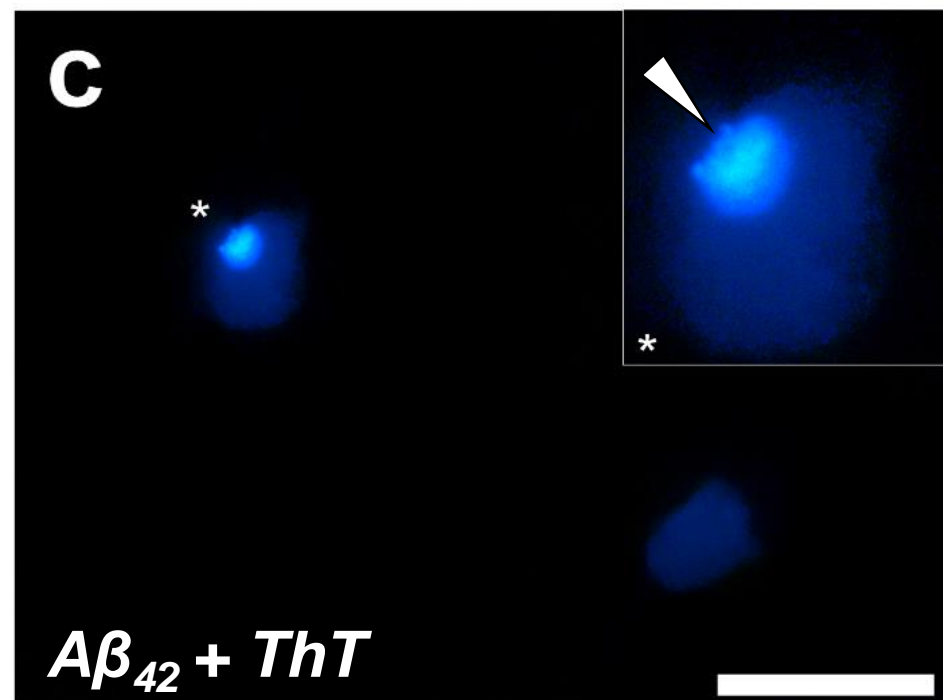
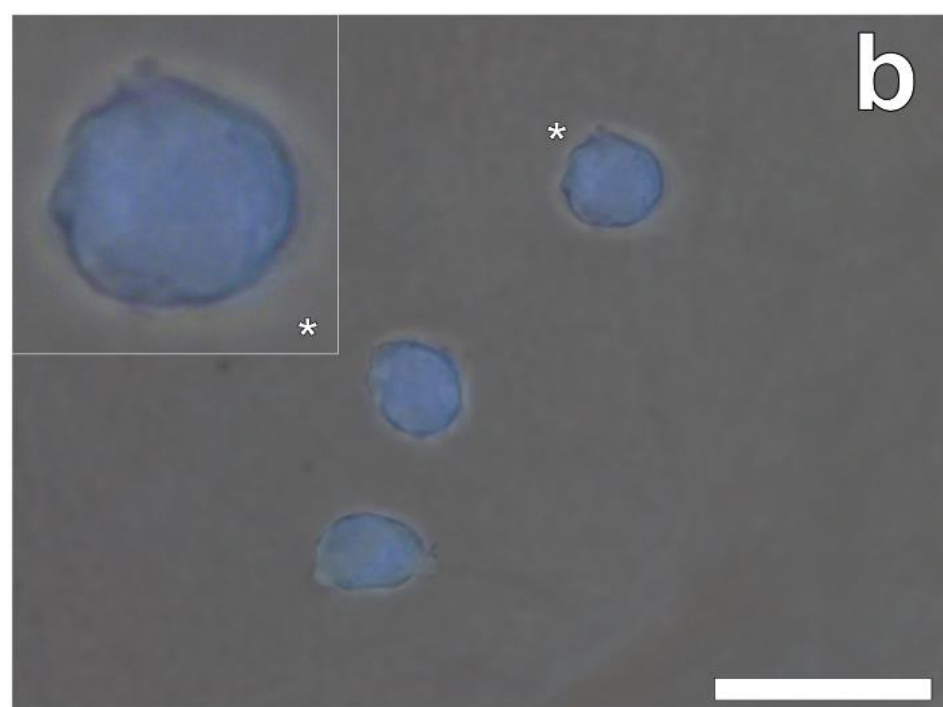
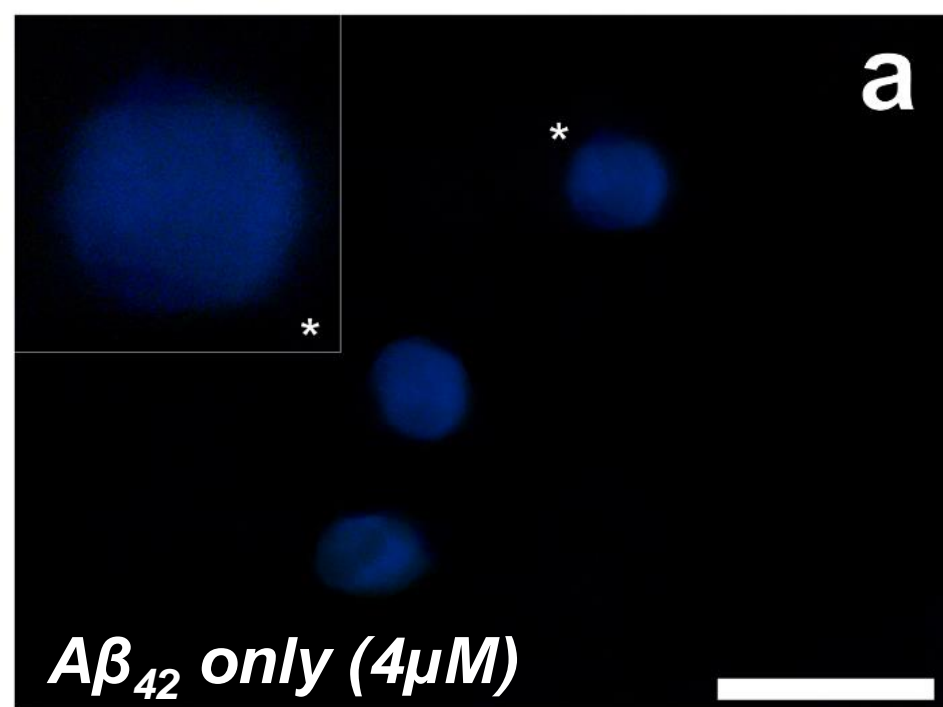
**(a) Lumogallion fluorescence**  
 U-MNIB3 (Olympus) filter cube and a ET590/33m SBP emission filter (Chroma®, Vermont, US).



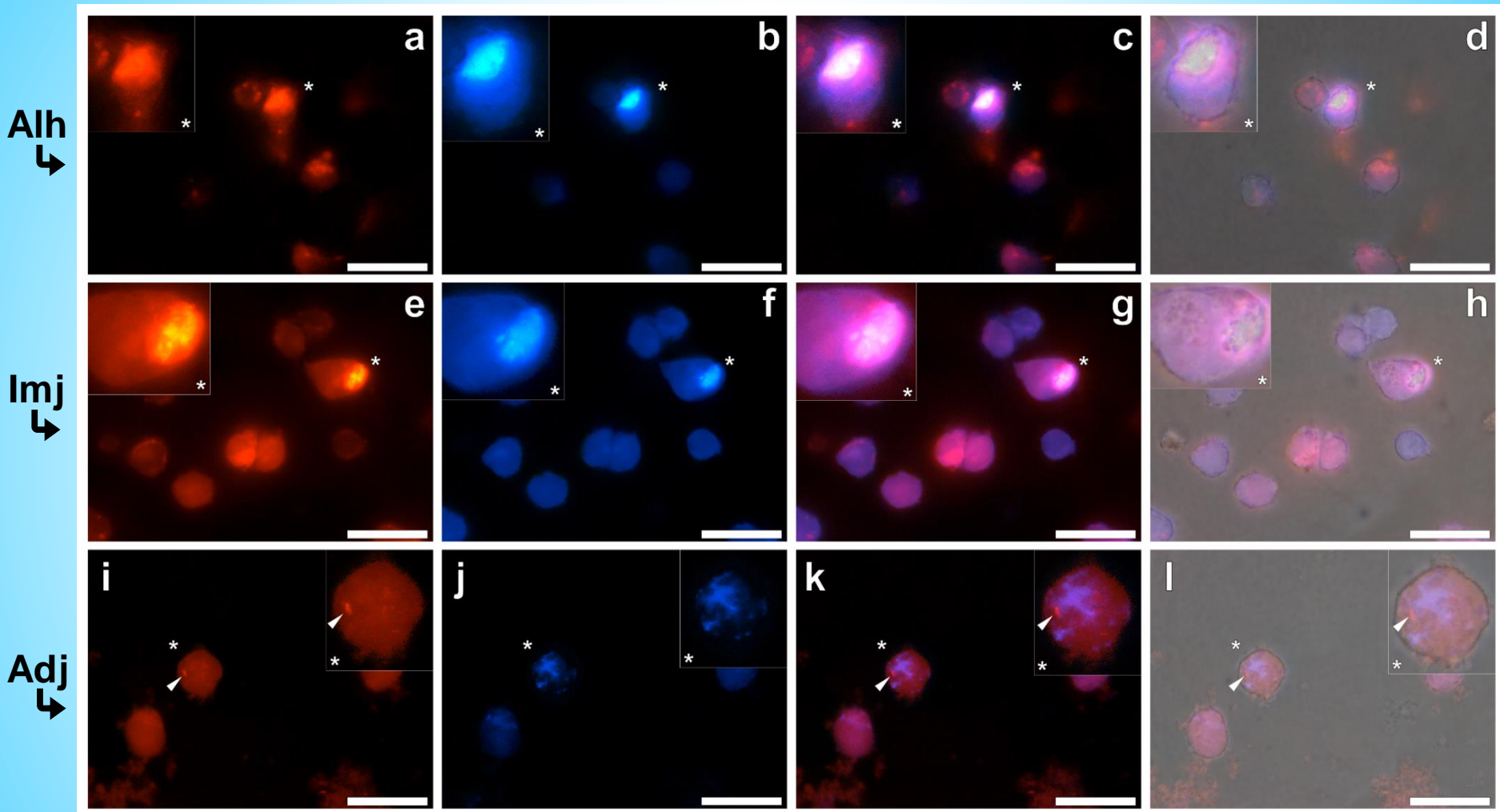
**(b) ThT fluorescence**  
 U-MWBV2 cube equipped with a Chroma® ET480/30m SBP emission filter.



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



# Fluorescence microscopy of amyloid vaccines



**THP-1 cells cultured for 24h in vaccine formulations containing 4 $\mu$ M A $\beta$ <sub>42</sub> & 12.5 $\mu$ g/mL ABA. Lumogallion (orange,  $\lambda_{em}$ : 590nm) and ThT (blue,  $\lambda_{em}$ : 482nm) fluorescence is depicted. Mag. X 1000, scale bars: 20 $\mu$ m.**

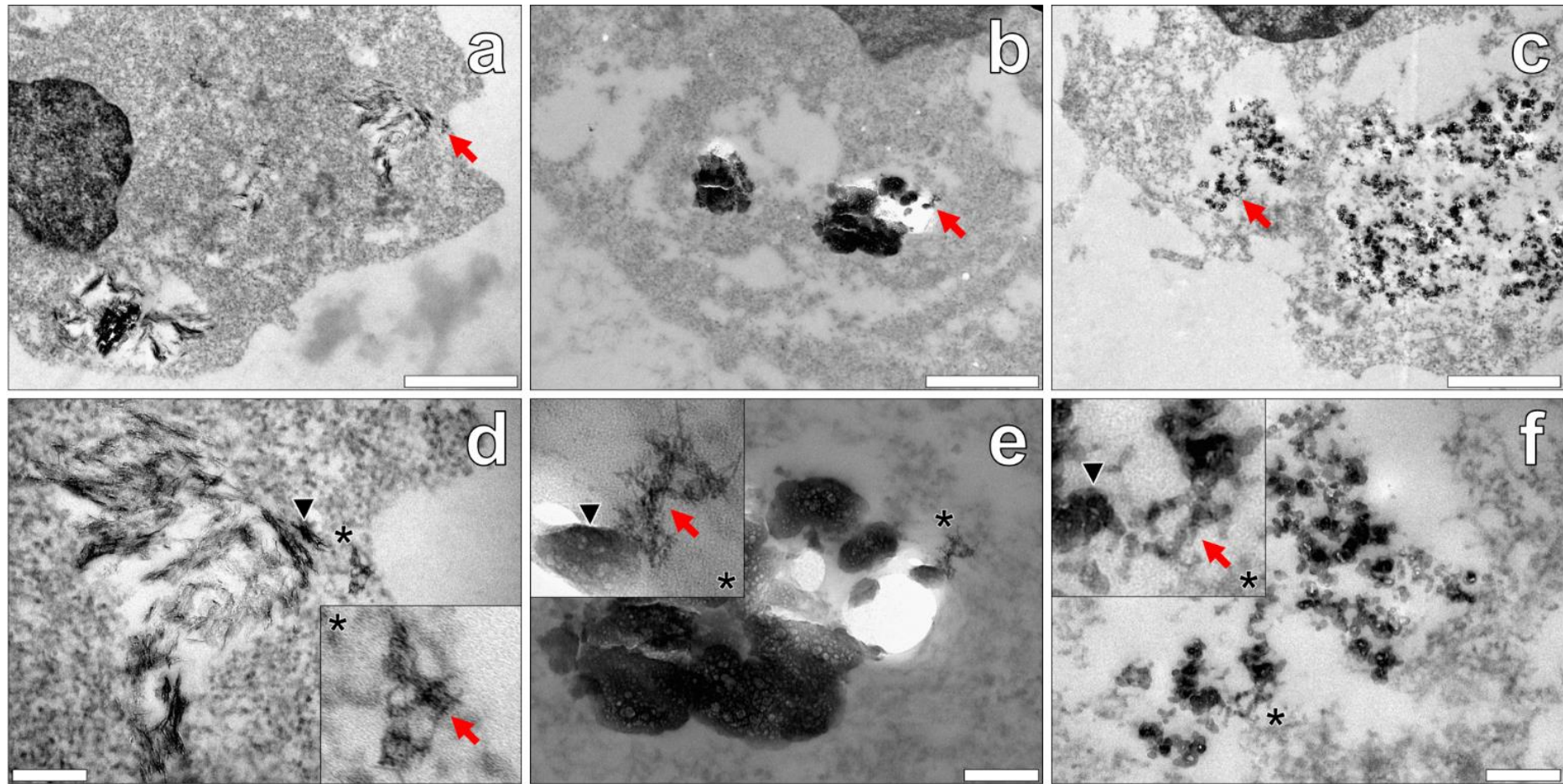


# TEM of amyloid vaccines

Alh ↘

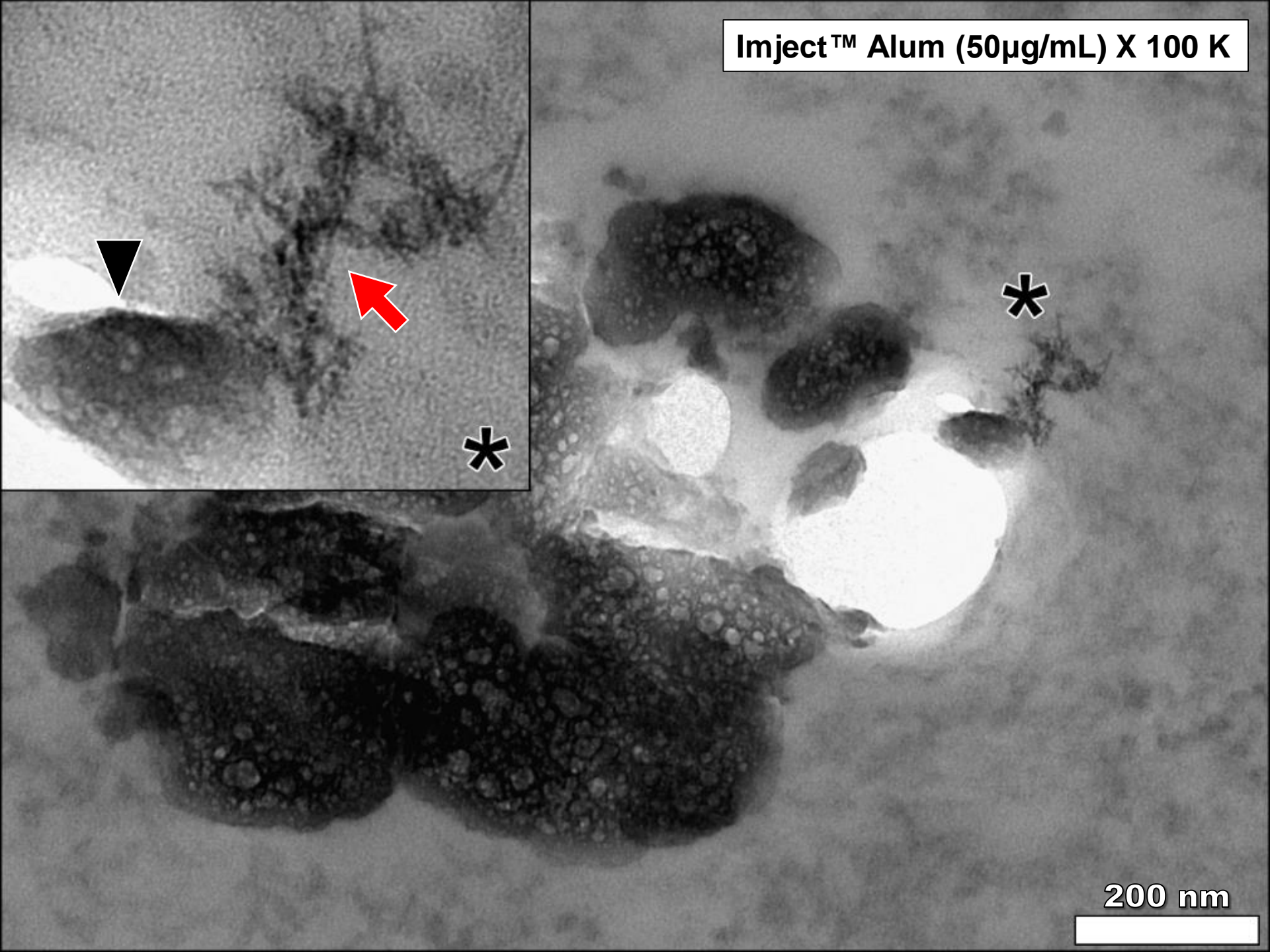
Imj ↘

Adj ↘



**THP-1 cells, co-cultured for 24h with simulated vaccines containing ca 4 $\mu$ M A $\beta$ <sub>42</sub> and 50 $\mu$ g/mL ABA. Mag. & scale bars: a – c. X 30K, 1 $\mu$ m, d – f. X 100K, 0.2 $\mu$ m, respectively.**

Imject™ Alum (50µg/mL) X 100 K



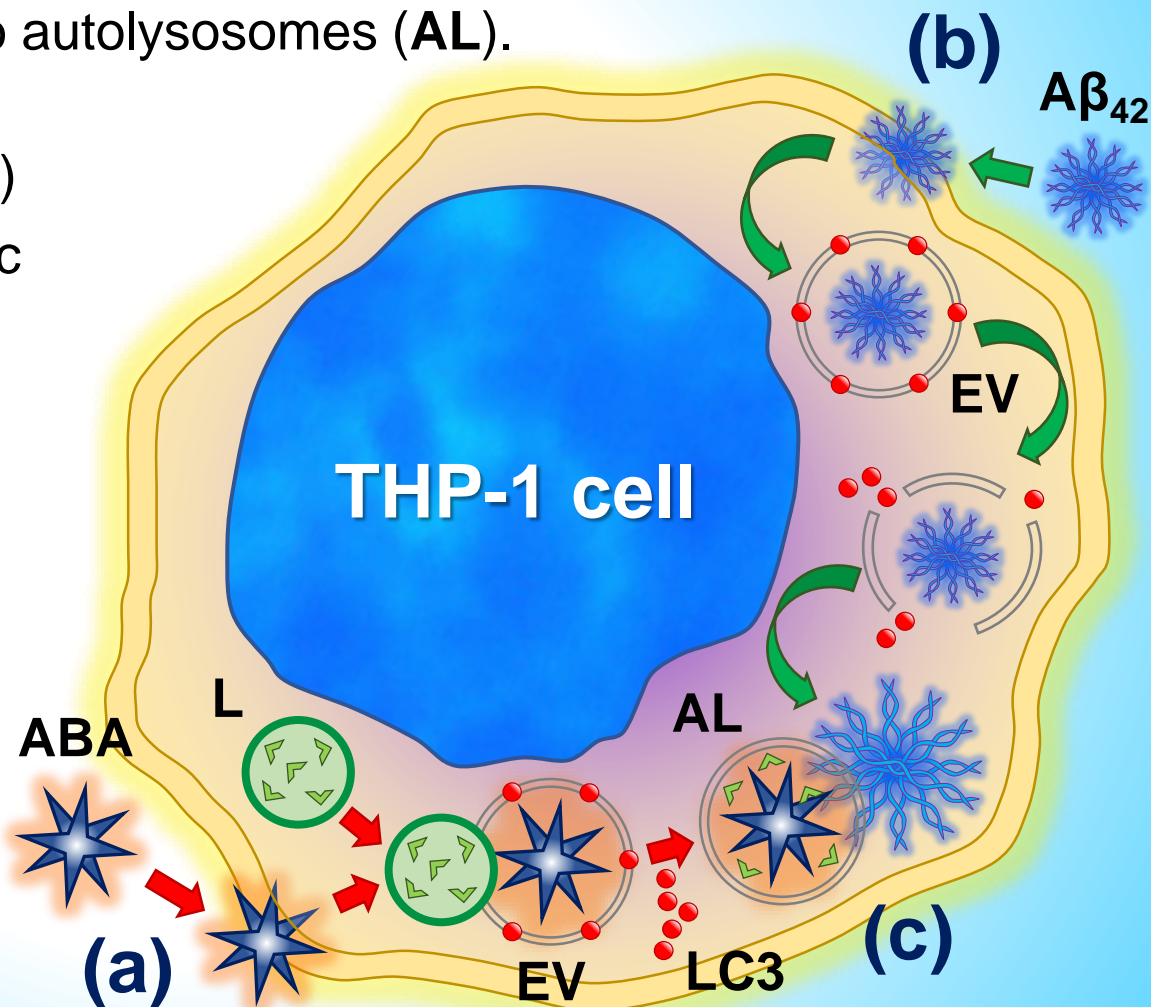


# Conclusions

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

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

**(c)**  $A\beta_{42}$  as a model 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)**



# References

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- Flavin, W. P. *et al.* Endocytic vesicle rupture is a conserved mechanism of cellular invasion by amyloid proteins. *Acta Neuropathol.* **134**, 629-653 (2017).
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- Sevigny, J. *et al.* The antibody aducanumab reduces A $\beta$  plaques in Alzheimer's disease. *Nature* **537**, 50-56 (2016).
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# Acknowledgements

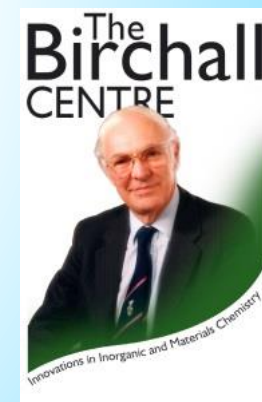
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