Phenotypic strategies for kinase inhibitor discovery

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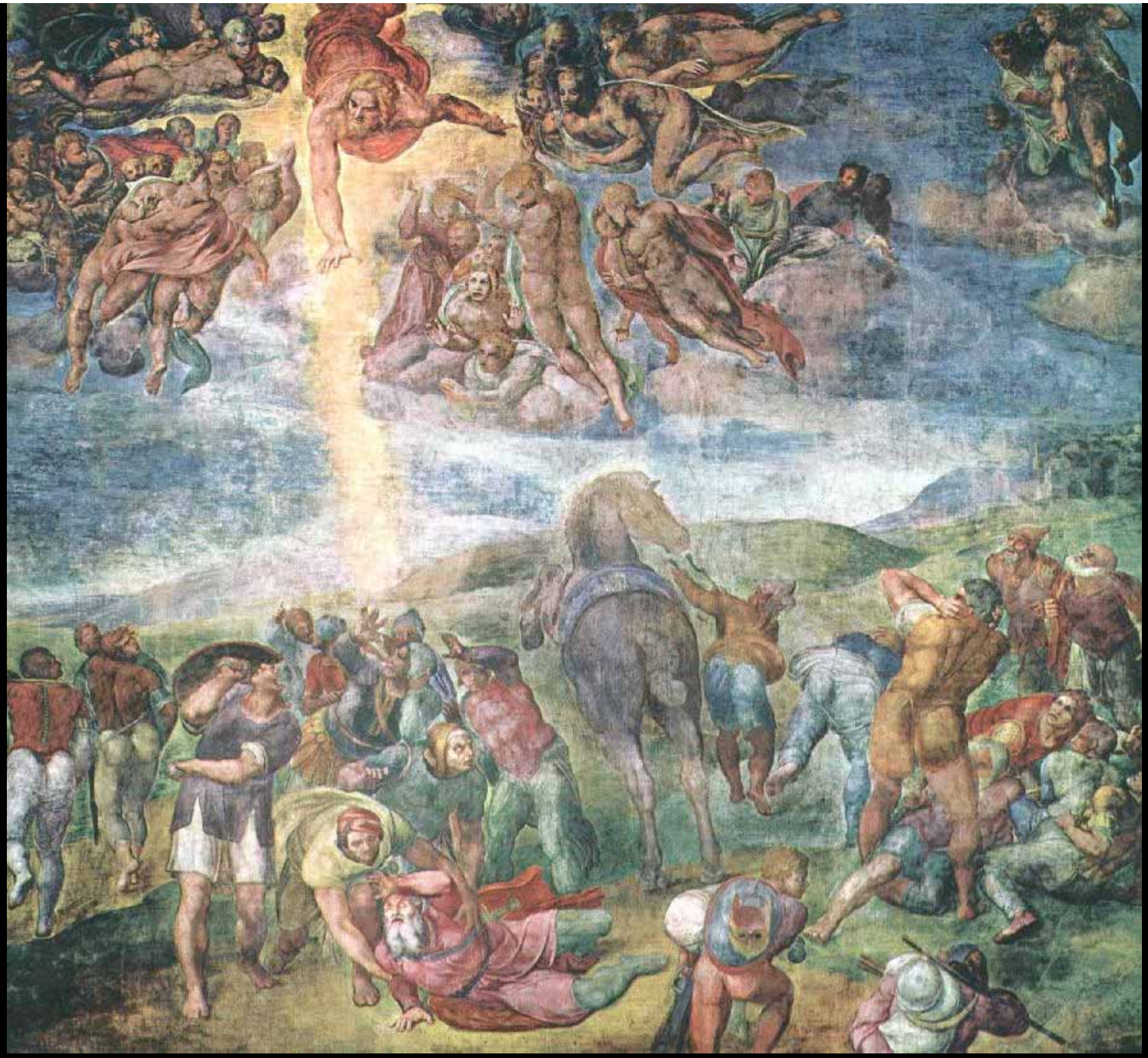
Disease Biology/In vitro methods in Industry

1. ~~Basic cell biology~~
2. ~~Mechanistic biomedical research~~
3. Target research
4. Alternative methods to animal experiments



The conversion of Saul to St Paul (Michelangelo, 1542)

Animal ethics

Here is a brief introduction to our animal ethics philosophy.

Lundbeck uses laboratory animals in order to identify and predict clinical and adverse events in humans. We do this because we want to ensure the highest possible standards of medicinal product safety and efficacy prior to administration to humans.

There is a real need for new medicines to patients with severe psychiatric and neurological diseases. Here at Lundbeck, we are committed to improve the quality of human life by enabling people to do more, feel better, and live longer. We strive to use animal testing only when necessary and when it promises to benefit individual patients in need of new medicinal care and society as a whole.

Animal testing at Lundbeck is performed with great care. The procedures used are in compliance with national and global guidelines on animal welfare. We use the highest industrial standards and all experiments are performed under strict surveillance of a dedicated veterinarian and reviewed by an ethical board. Lundbeck acknowledges the general principles of the 3R rule: Reduction, Refinement, and Replacement of animal studies. Whenever new scientific knowledge arises, we carefully consider these new alternatives as options in our research. As a result, during the latest five-year period, we have attained a 30% reduction in the number of animals used relative to the number of scientists employed at Lundbeck.

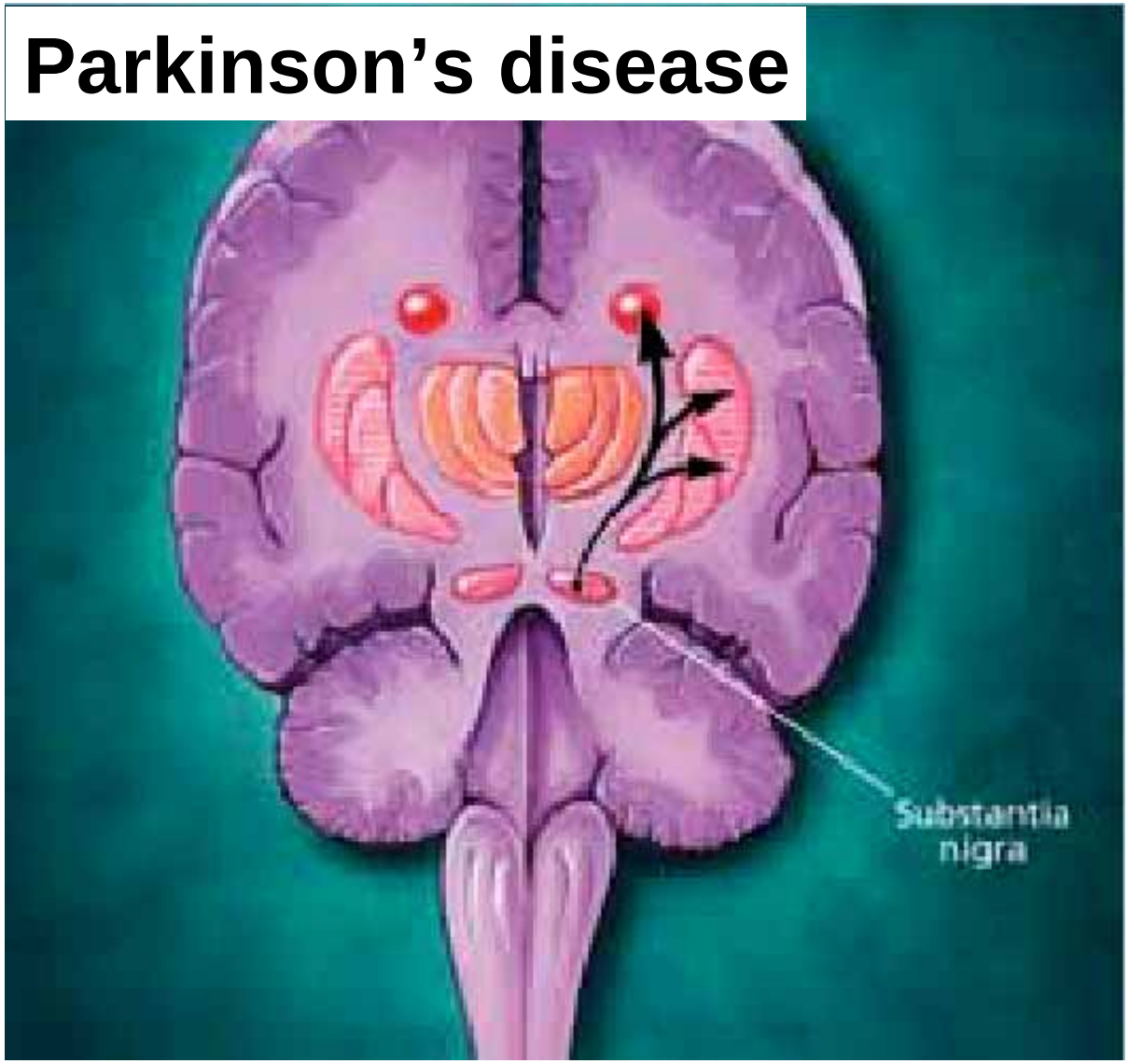
Read also

> Our focus

> Non-clinical safety research

TO A FRIEND

Parkinson’s disease

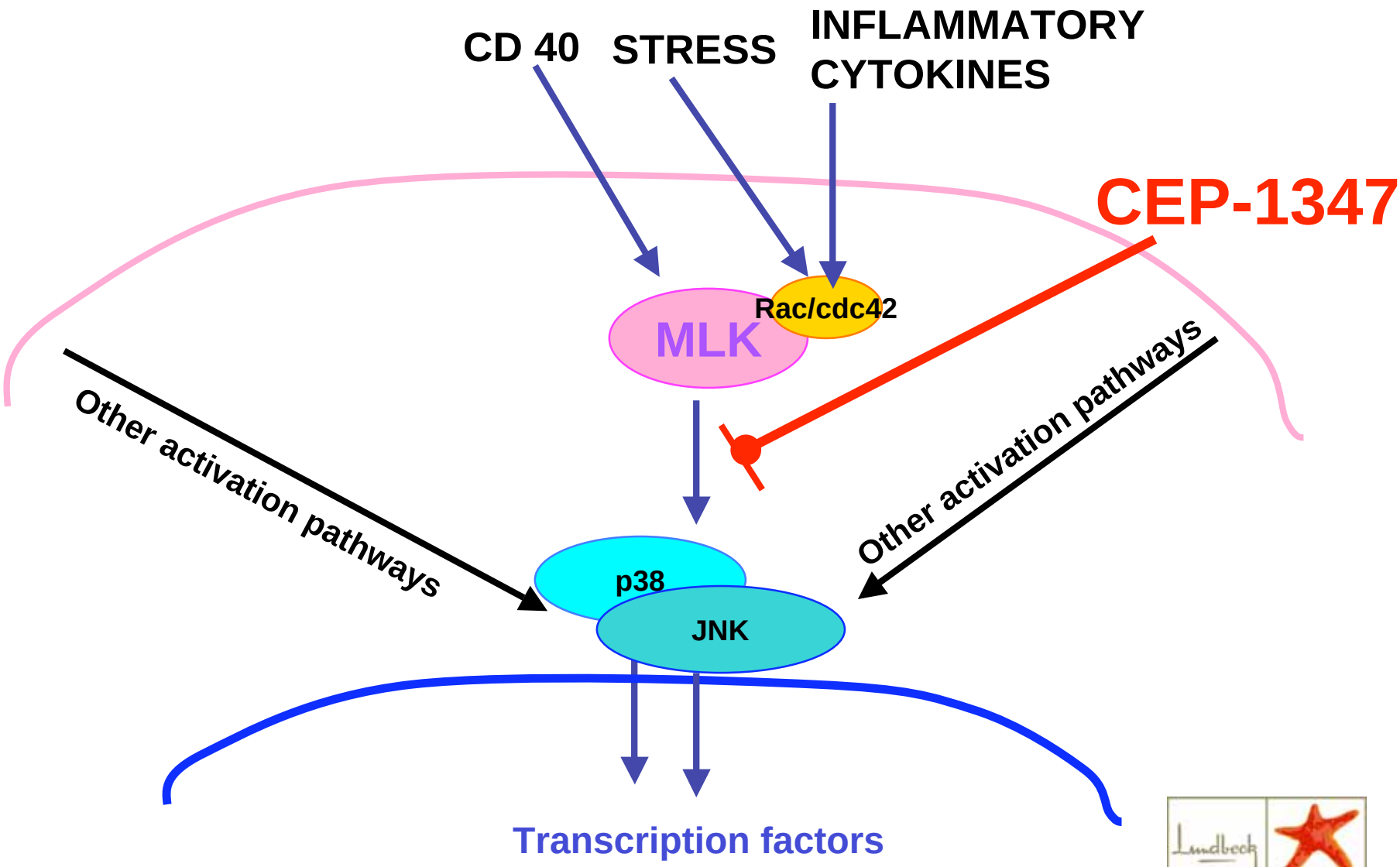


US figures:
50,000 new cases/year
> 800,000 total cases
$ 5,400,000,000 cost

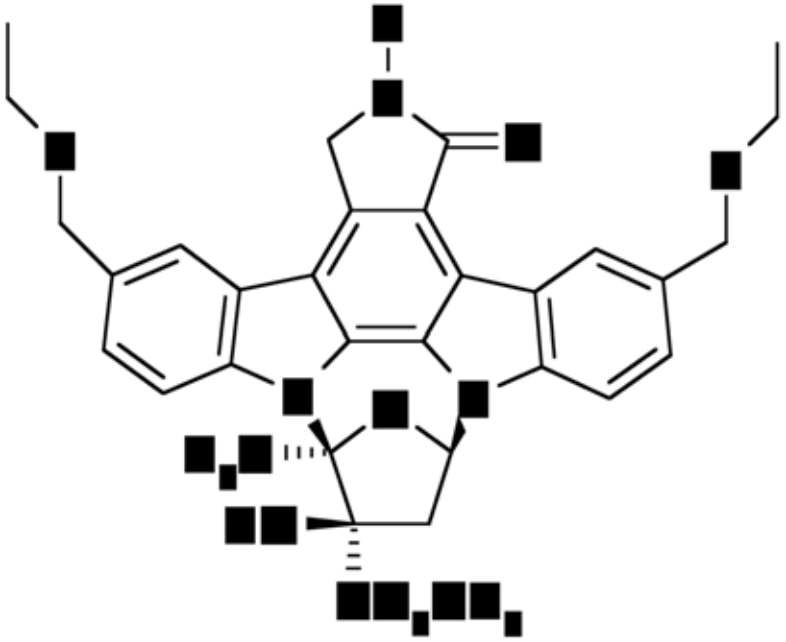
About 1-2 % of elderly population

PD: death of dopaminergic neurons projecting from substantia nigra to striatal nuclei

K525a derived MLK inhibitors

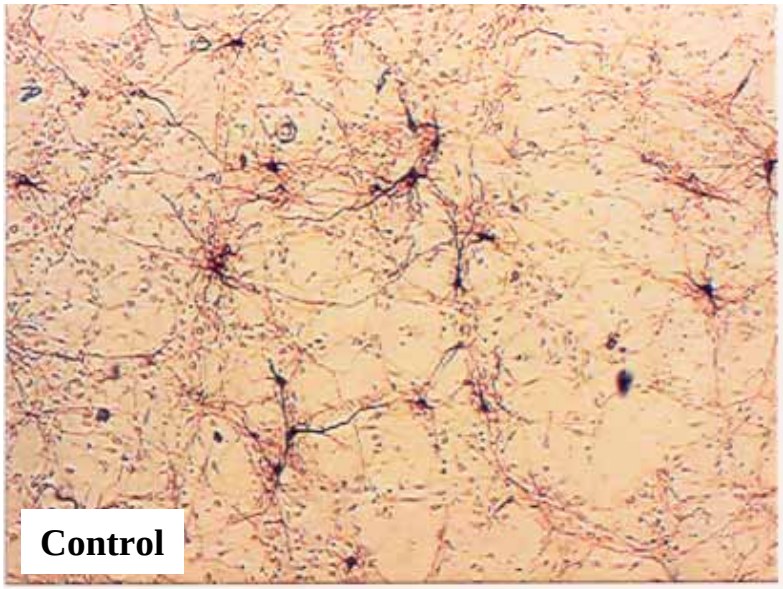


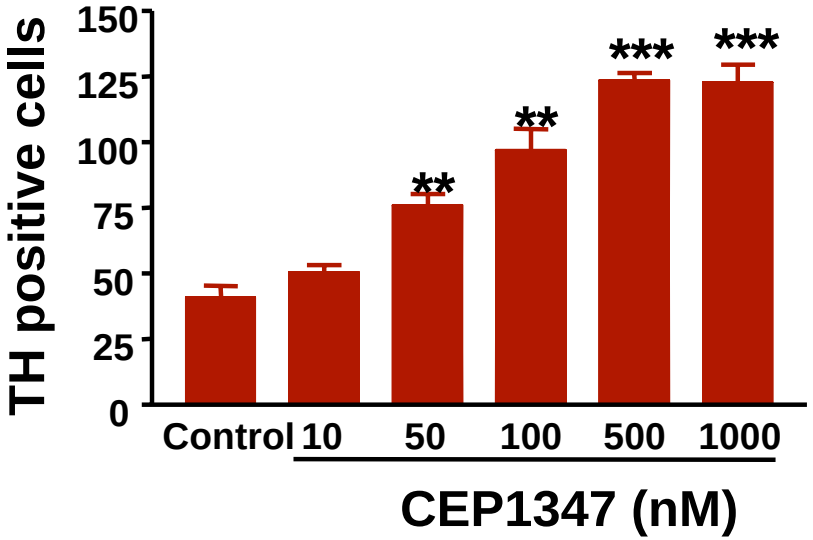
Selectivity of CEP-1347/Lu-02648 for MLK 1-3

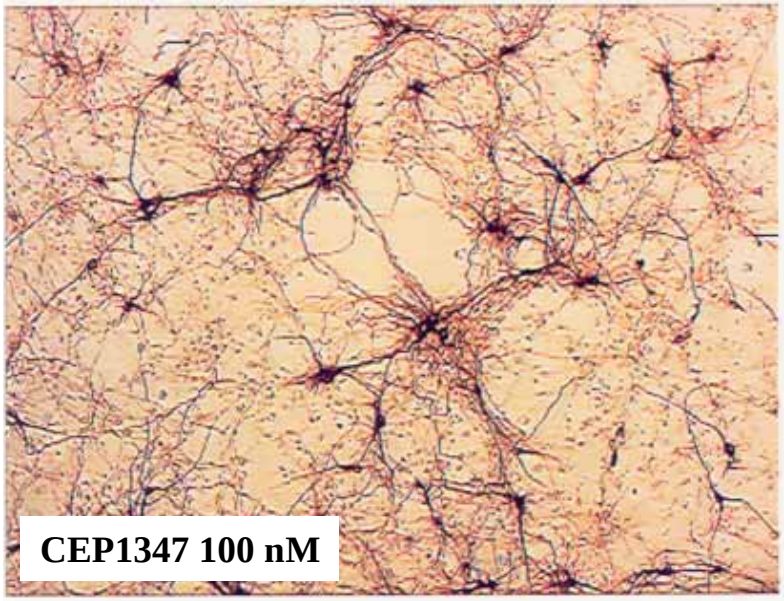


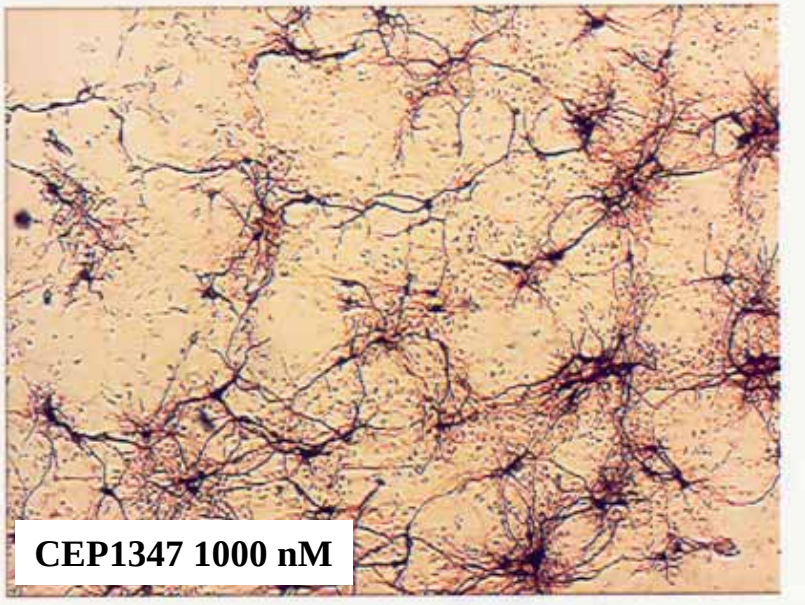
Kinase	IC_{50} nM
MLK1	50
MLK2	64
MLK3	23
PKC	>10000
PKA	>10000
TRKA	>10000
EGFR	>10000
βIRK	>10000
p38	>10000
PDGFRβ	>10000
FGFR1	315

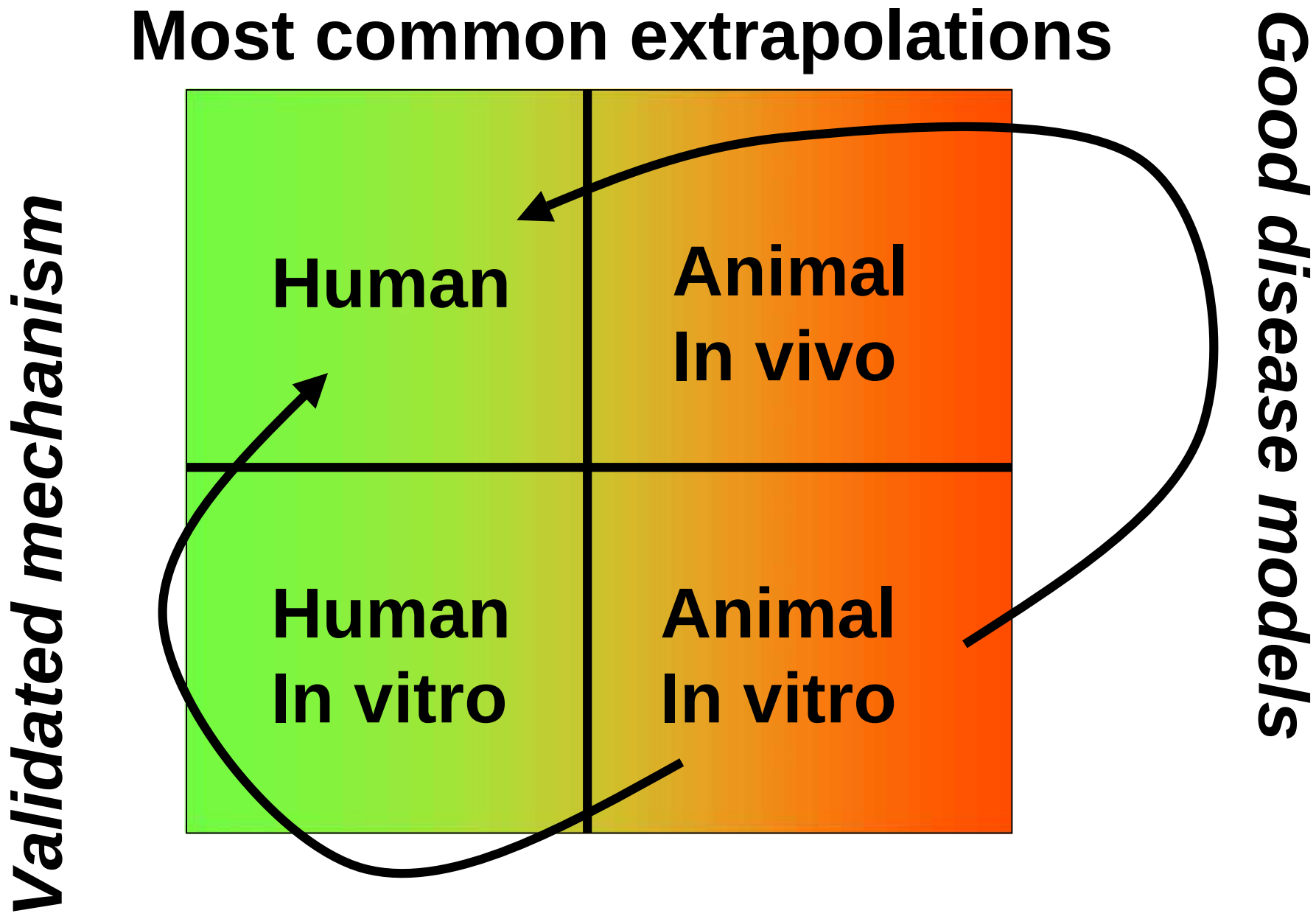
Effect of CEP1347 on survival of dopaminergic cells







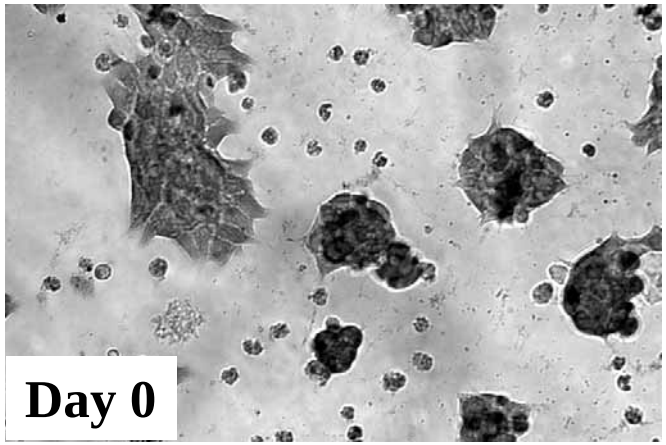



Most common extrapolations
Validated mechanism
Good disease models
Human
Animal
In vivo
Human
In vitro
Animal
In vitro

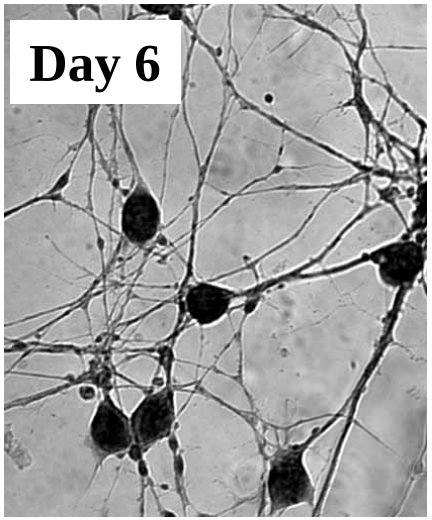
Generation of LUHMES cells

(Lund human mesencephalic neurons)

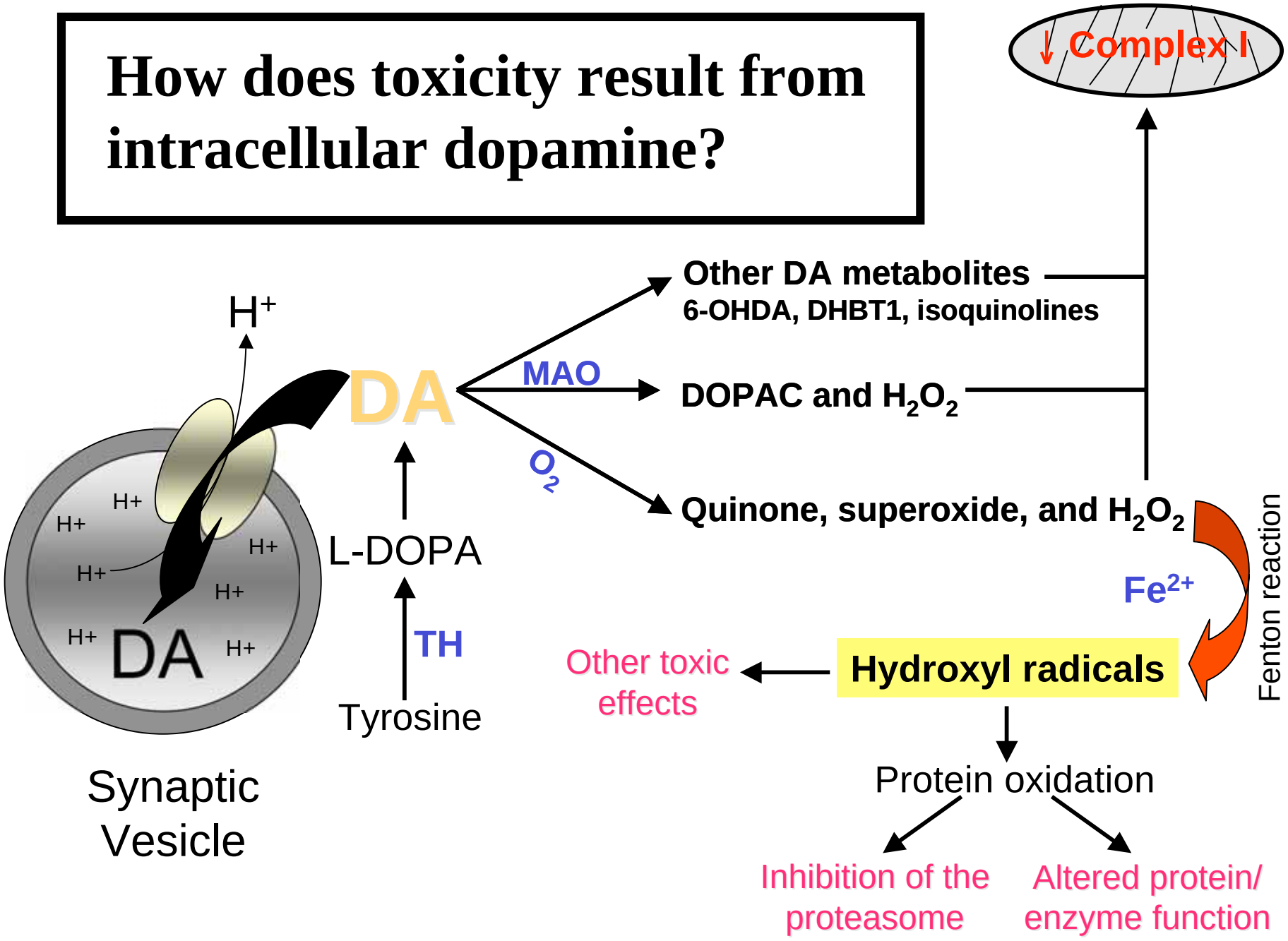
Differentiation to dopaminergic cells Expressing DAT (dopamine transporter), TH (tyrosine hydroxylase) and containing dopamine



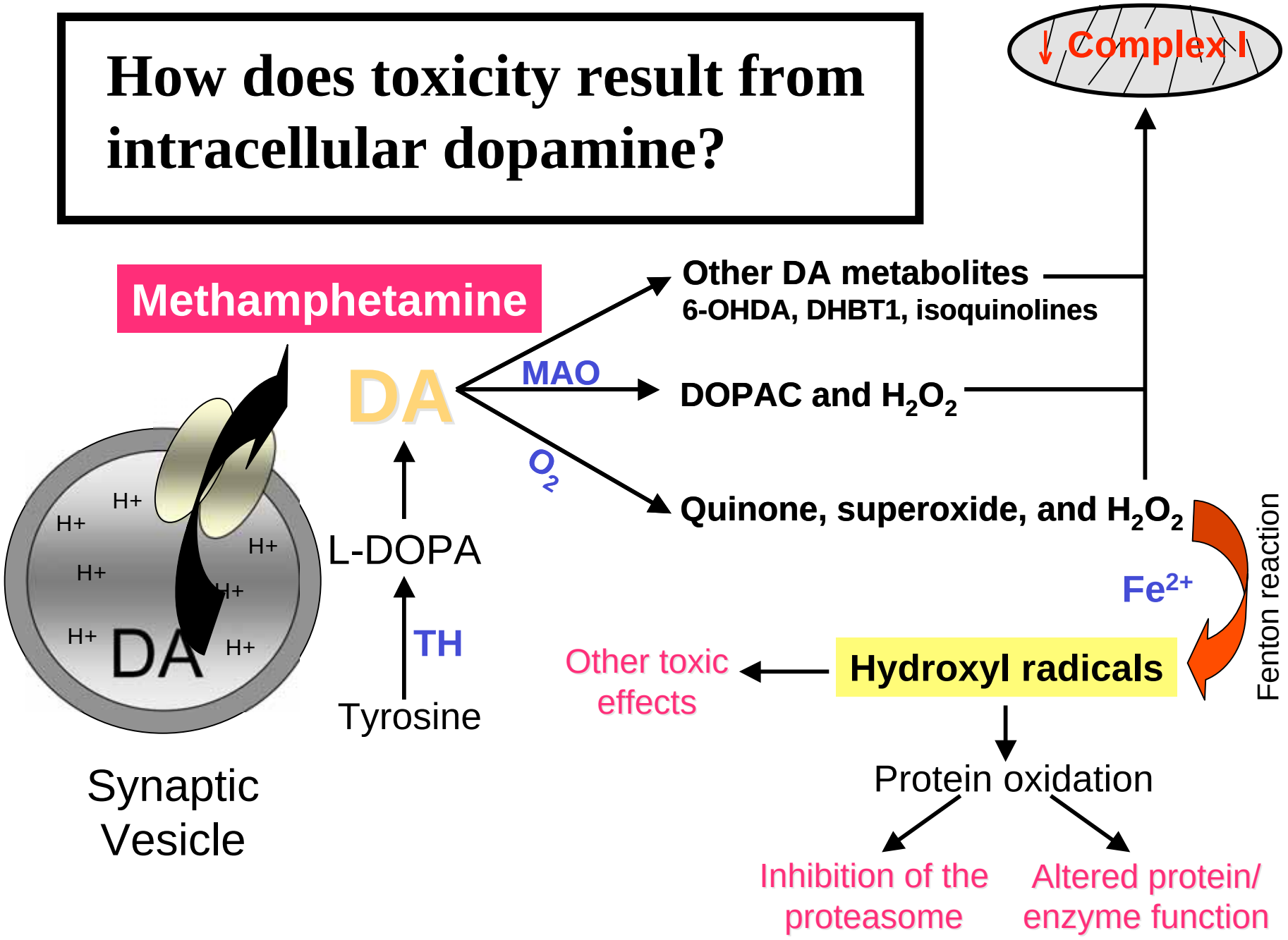
Cytokine mix



How does toxicity result from intracellular dopamine?

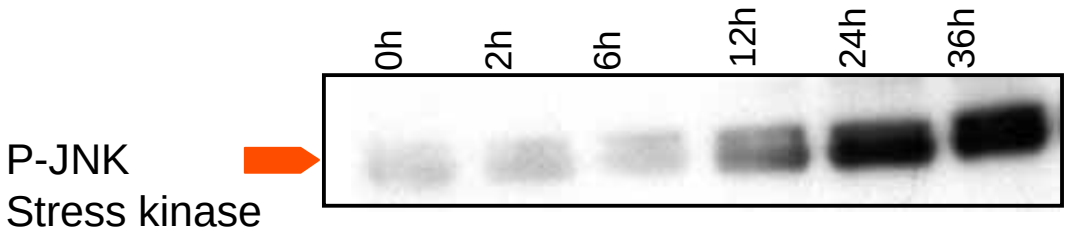


How does toxicity result from intracellular dopamine?

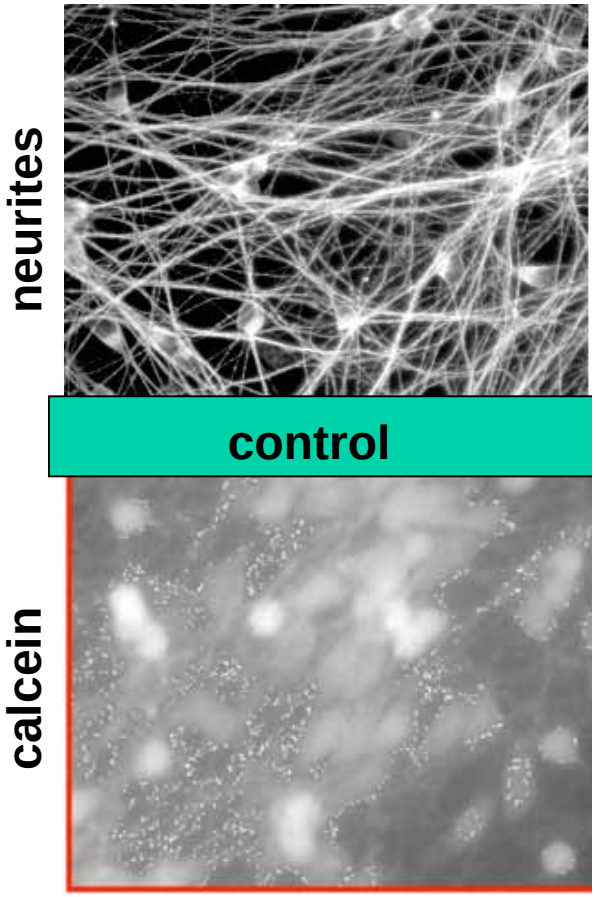


PD-related stress model

Iron plus methamphetamine (Fe/meth) triggers stress via endogenous dopamine in LUHMES



Human mesencephalic stem cell-derived neurons with dopaminergic phenotype: Direct test of neuroprotection in a human model



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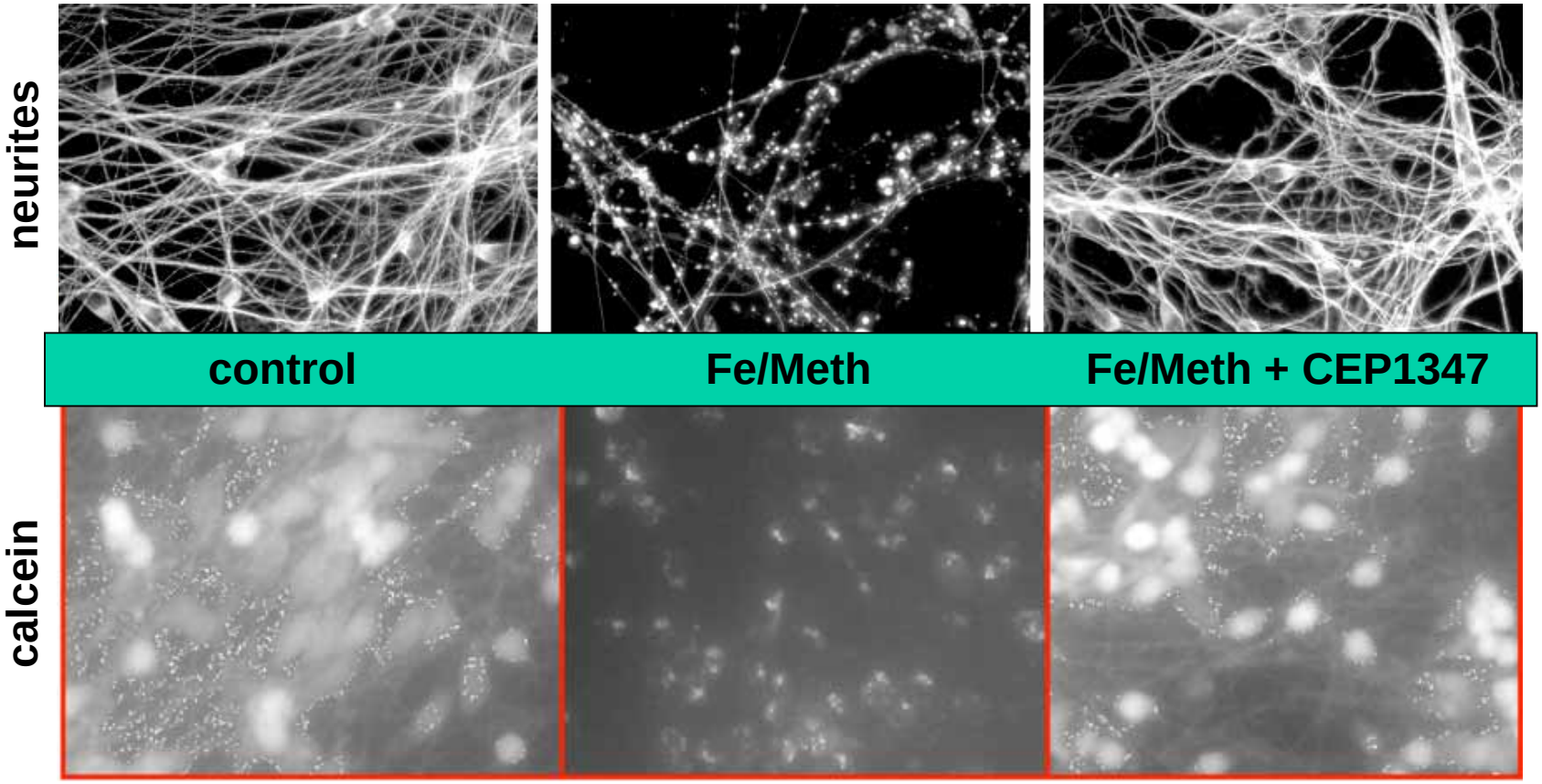
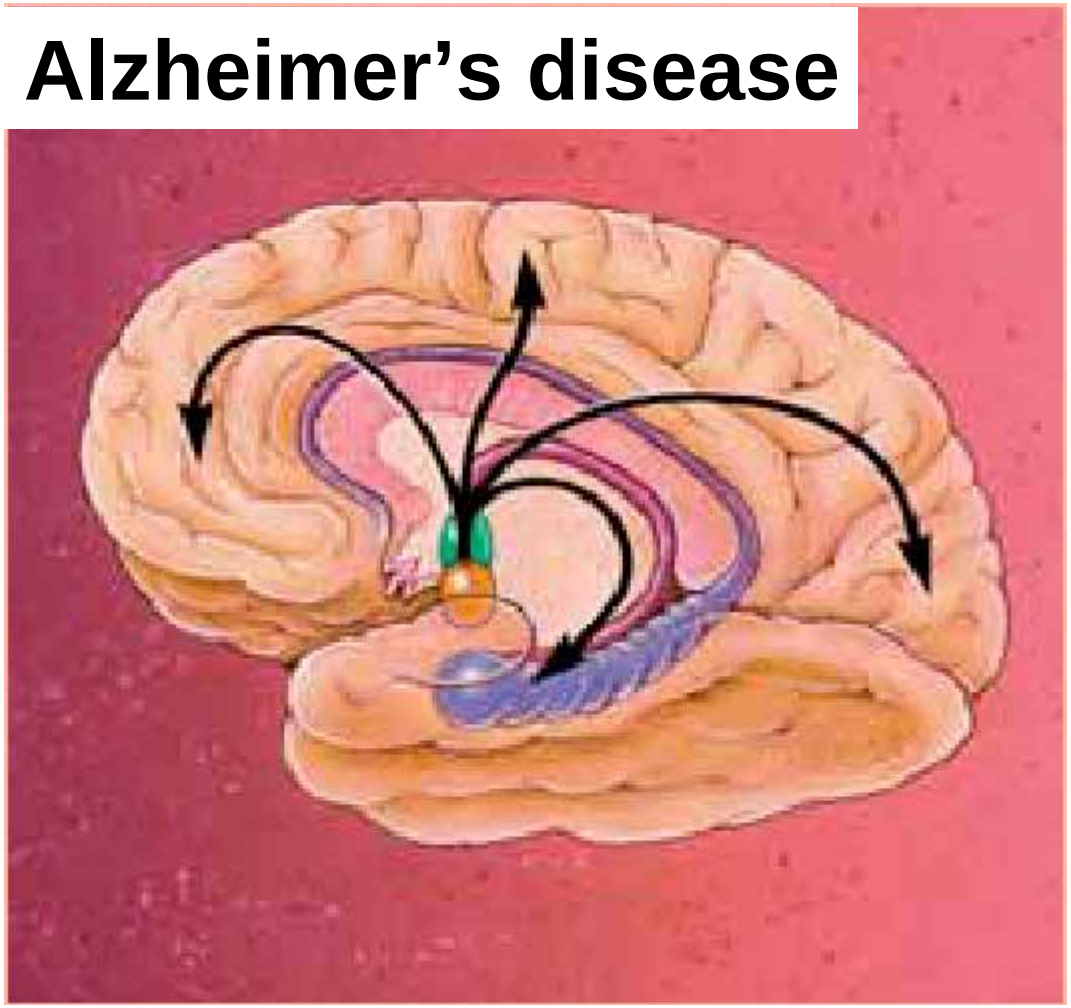


Alzheimer's disease



US numbers:
3-5 million patients
$ 100,000,000,000 cost
Strongly growing
2- 15 % of elderly with AD
< 2 % of AD patients treated

AD: Deficiency of cholinergic input from basal forebrain

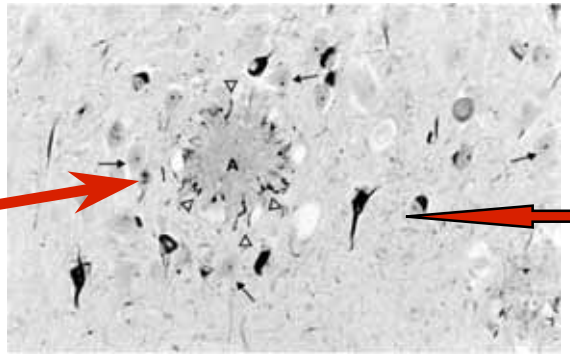
Example: Neuroprotection in AD/PD

3-5 % over 65 get AD (Alzheimer's disease)

2 % of patients are treated
(with poor symptomatic drugs)

Disease-modifying drugs are not available

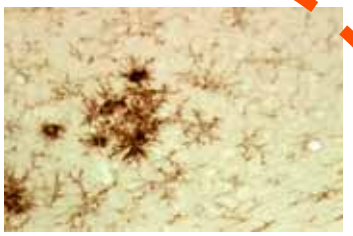
Neuritic plaques



Neurofibrillary tangles

Alzheimer´s disease:

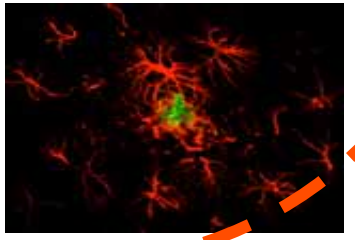
Dissection into individual processes



Neurodegeneration

A-beta toxicity
Neurotrophin loss

Neuroinflammation

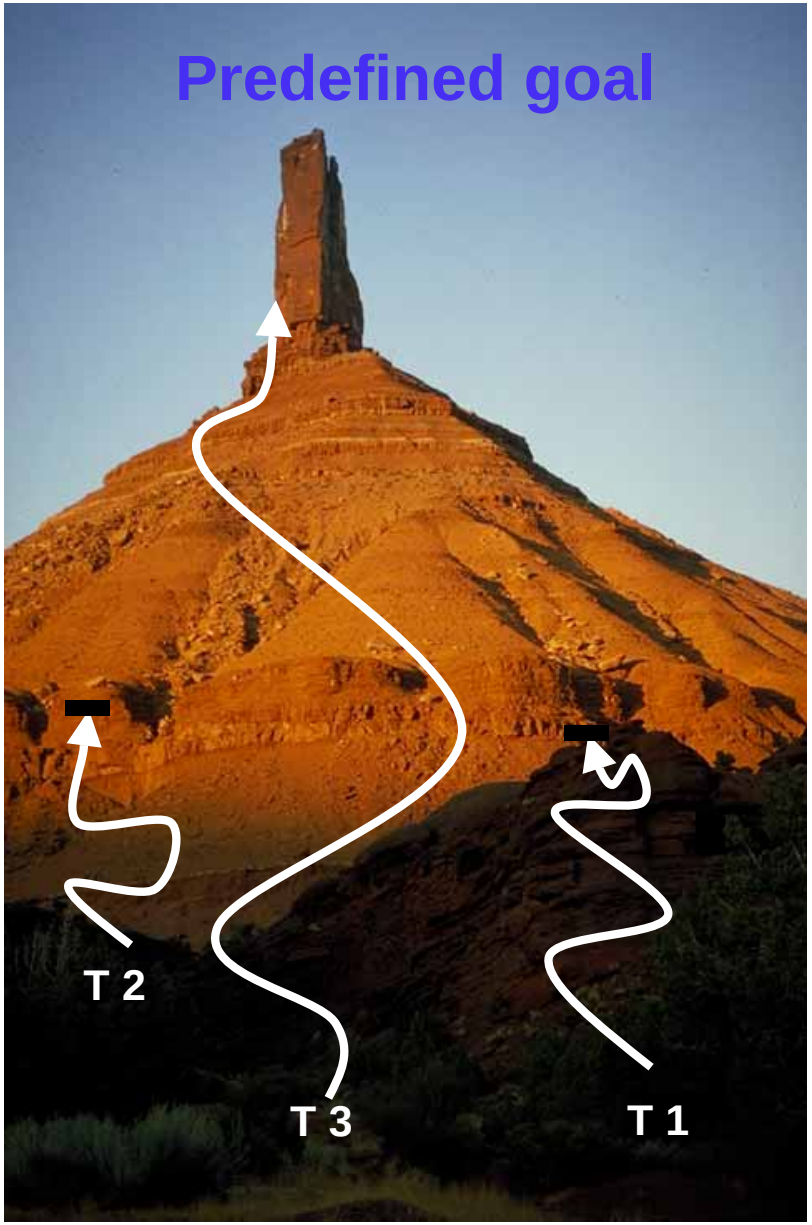


Modelling
In vitro

Mixed kinase inhibitors

A discovery program independent of animal disease models

Drug discovery as filtering process



5000 compounds

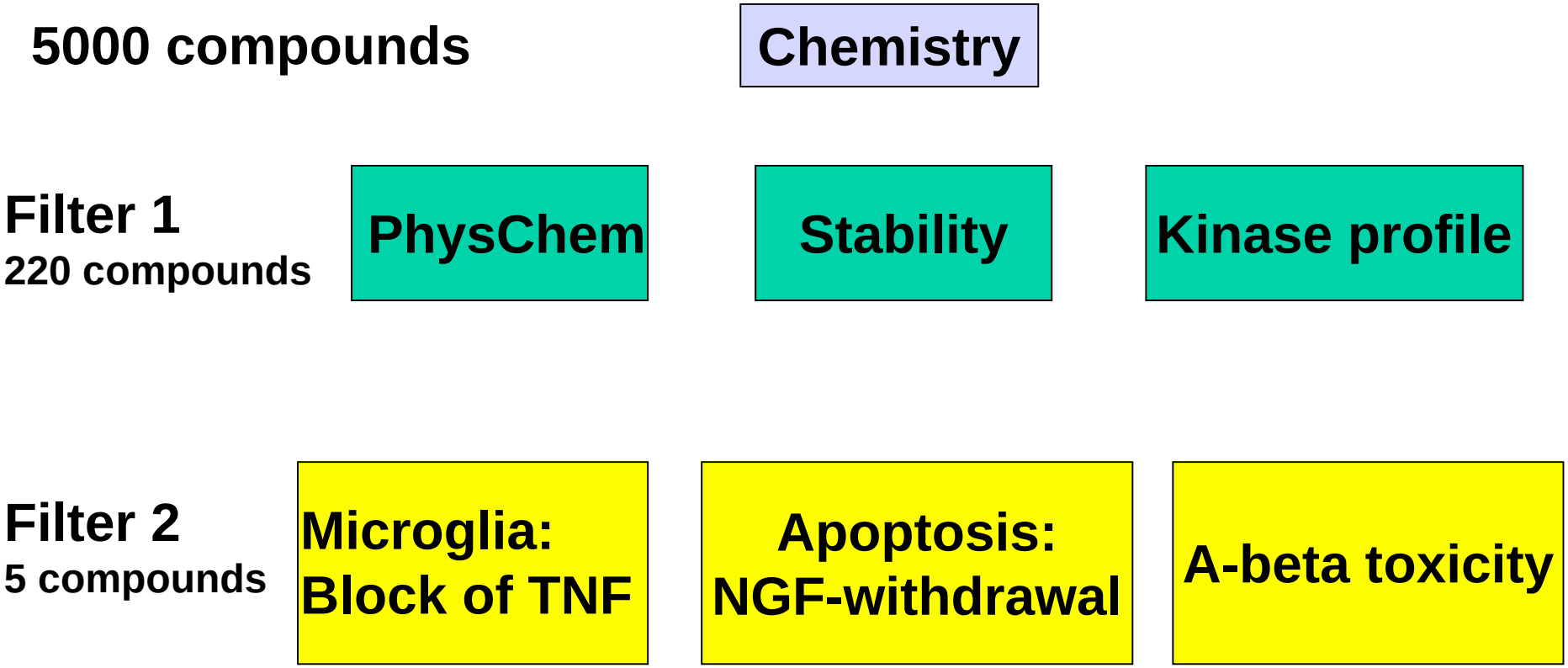
Chemistry

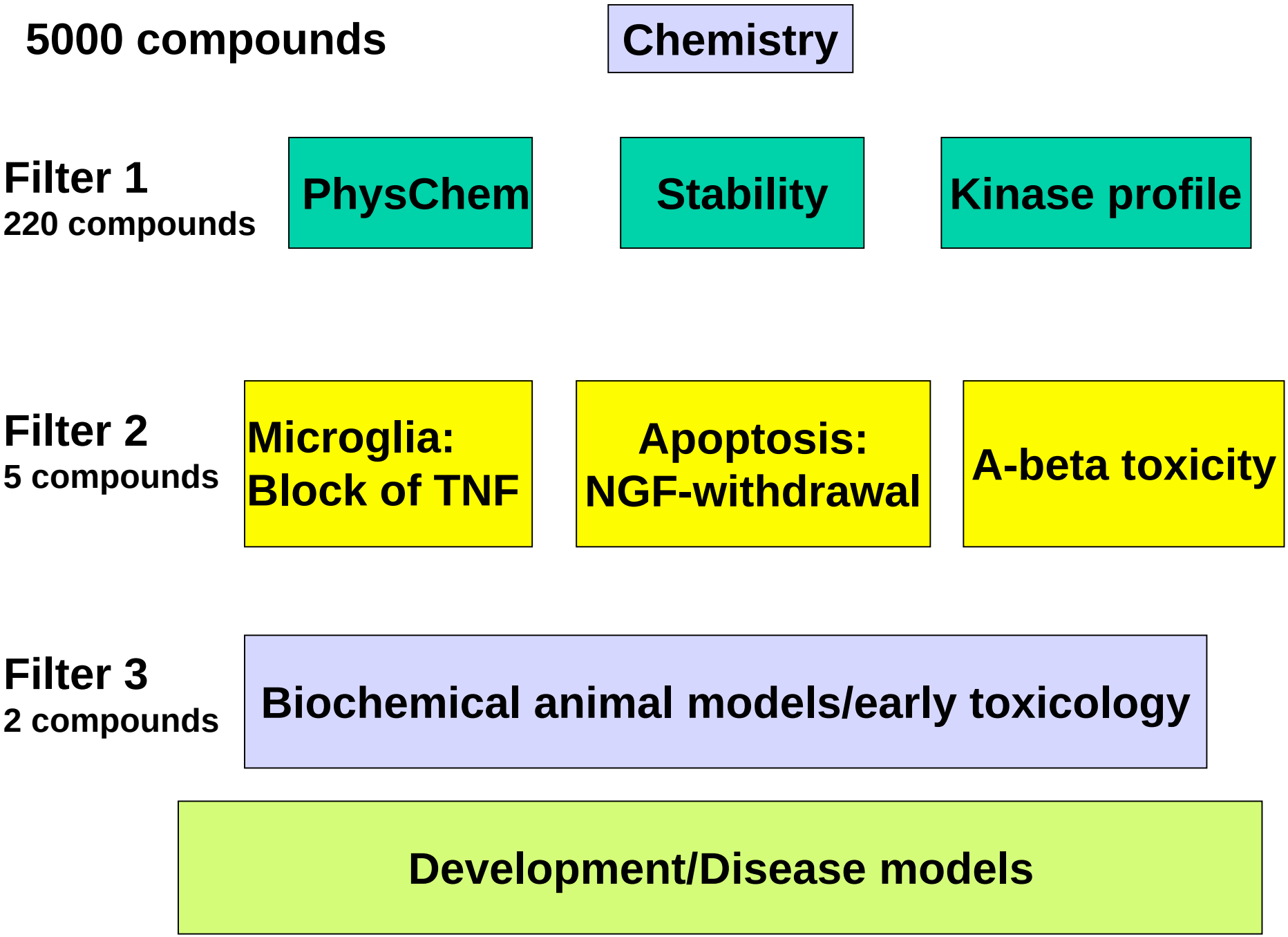
Filter 1
220 compounds

PhysChem

Stability

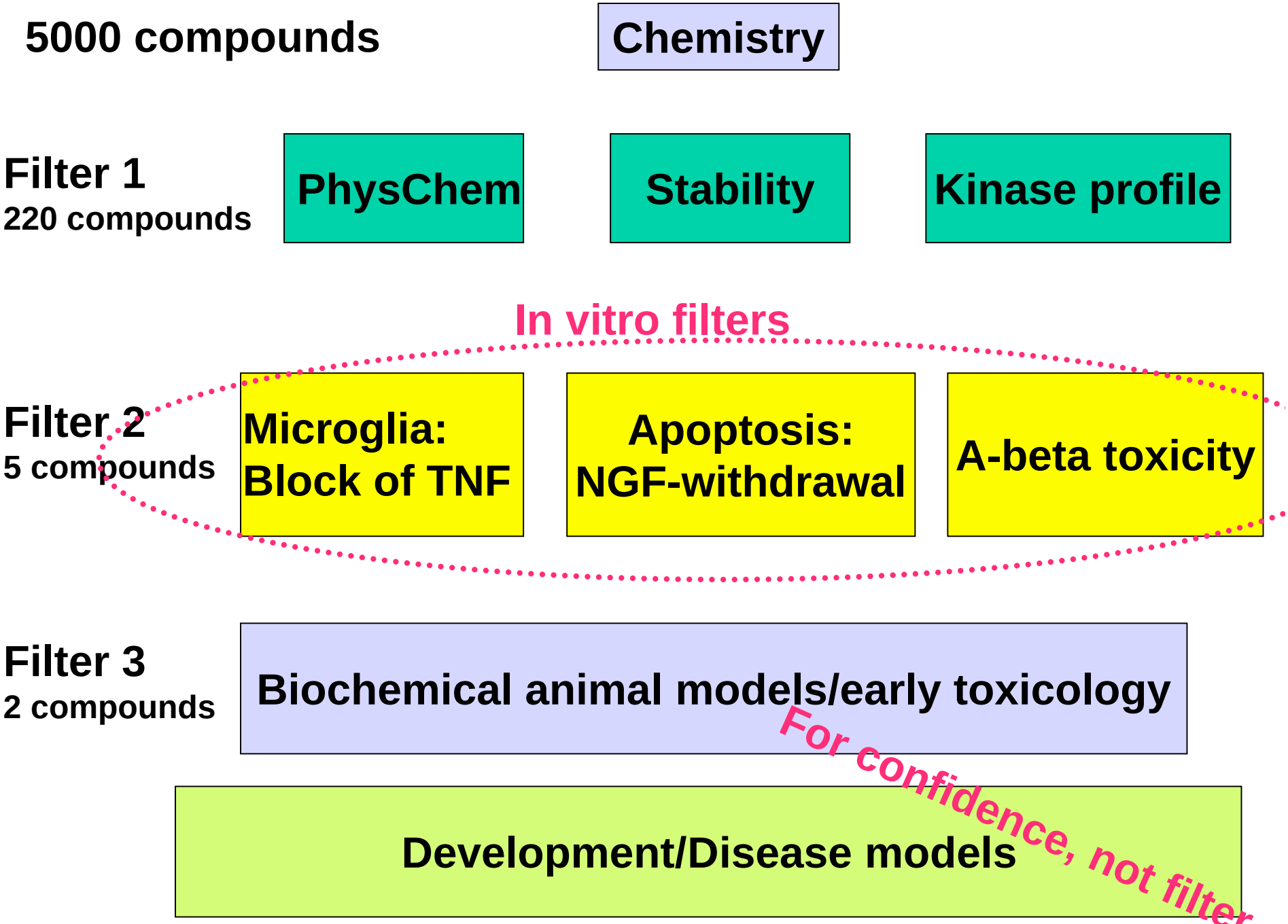
Kinase profile


5000 compounds
Chemistry
Filter 1
220 compounds
PhysChem
Stability
Kinase profile
Filter 2
5 compounds
Microglia:
Block of TNF
Apoptosis:
NGF-withdrawal
A-beta toxicity

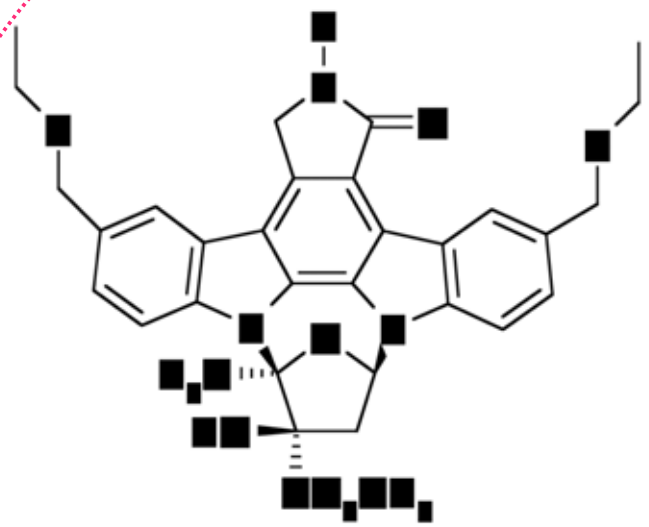

5000 compounds
Chemistry
Filter 1
220 compounds
PhysChem
Stability
Kinase profile
Filter 2
5 compounds
Microglia:
Block of TNF
Apoptosis:
NGF-withdrawal
A-beta toxicity
Filter 3
2 compounds
Biochemical animal models/early toxicology
Development/Disease models

Drug discovery, NEARLY without mice

(Van Beyeren, 1667)


5000 compounds
Chemistry
Filter 1
220 compounds
PhysChem
Stability
Kinase profile
In vitro filters
Filter 2
5 compounds
Microglia:
Block of TNF
Apoptosis:
NGF-withdrawal
A-beta toxicity
Filter 3
2 compounds
Biochemical animal models/early toxicology
Development/Disease models
For confidence, not filter

Further profiling of drugs in *in vitro* assays



CEP1347
(Development for Parkinson's disease (PD))

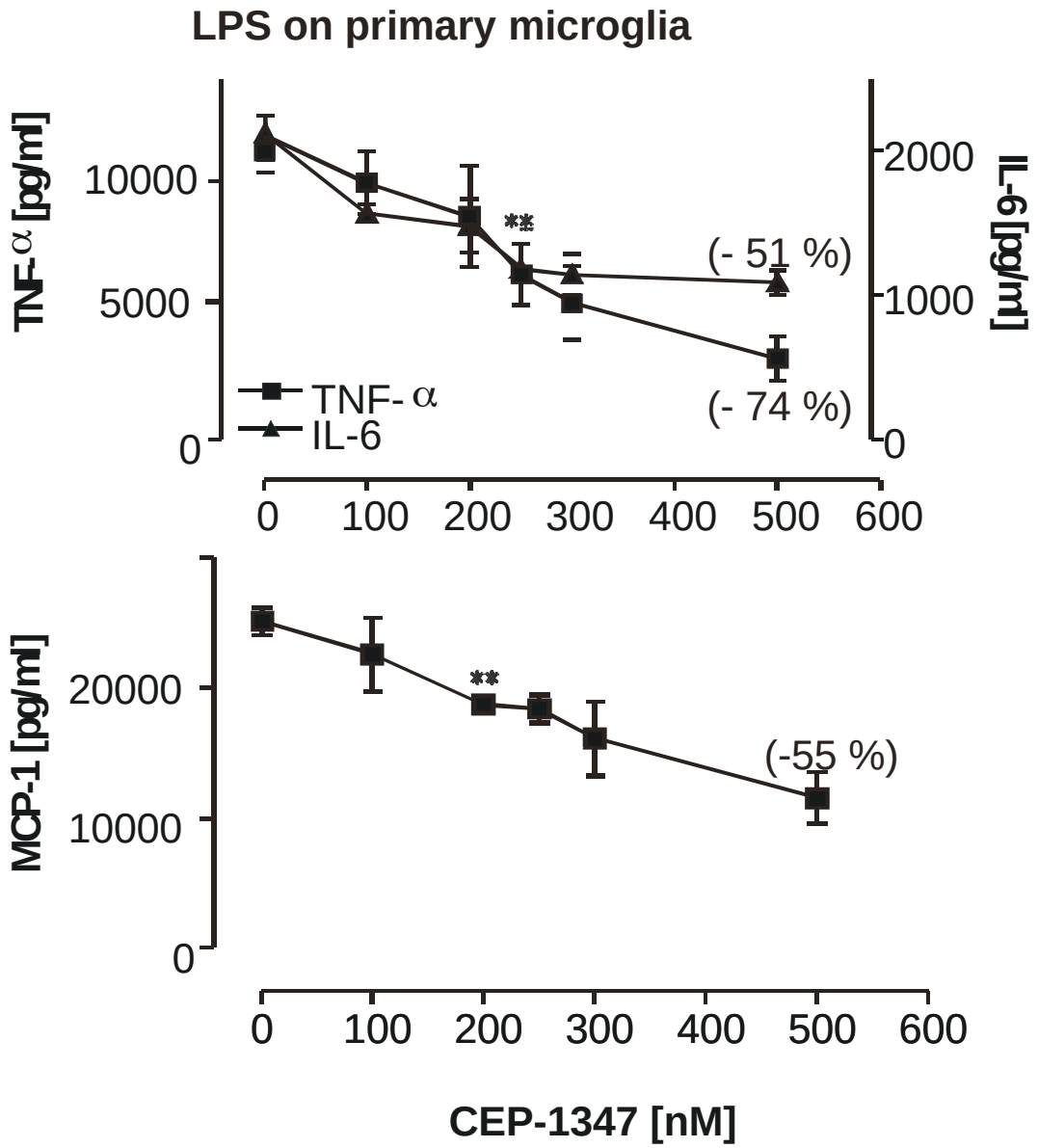
Non-glycoside
(Development for AD)

Due to setup of screening filters:

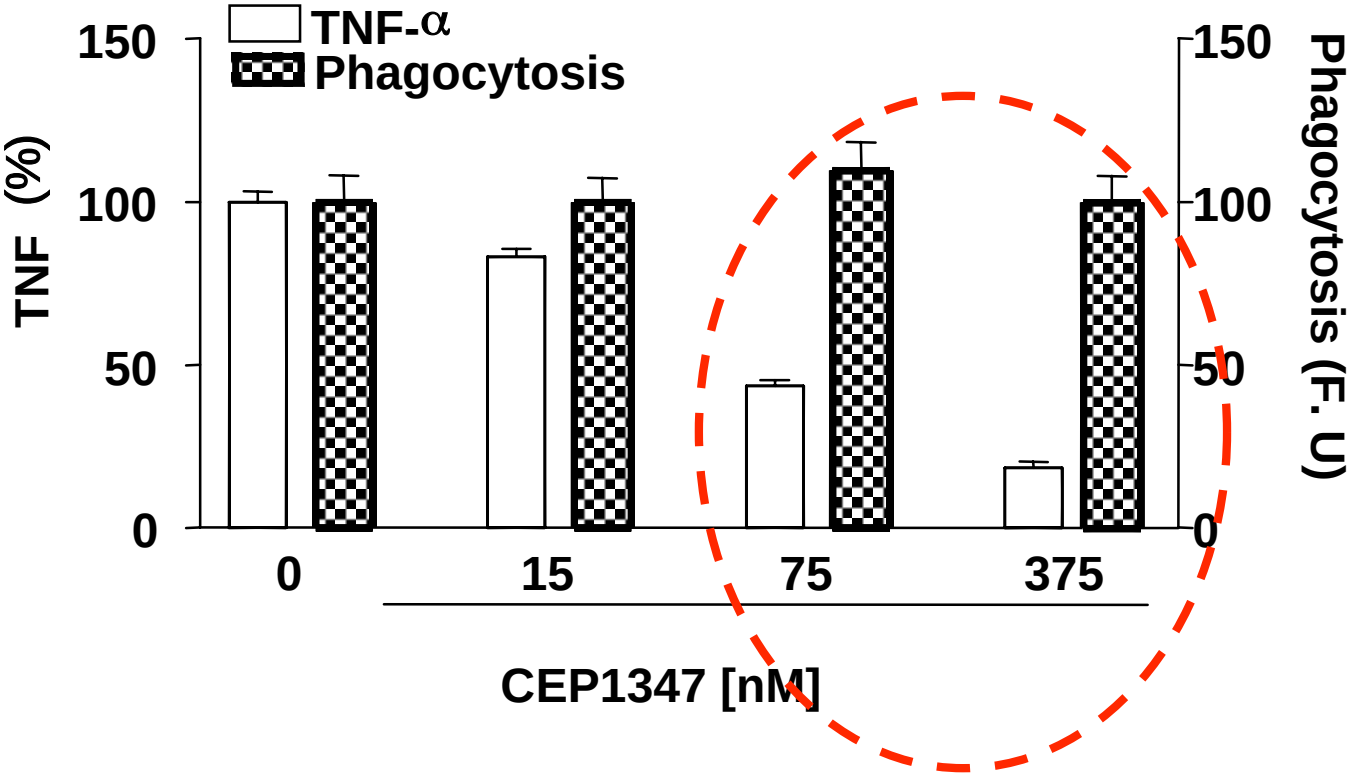
Compound should be neuroprotective AND anti-inflammatory

→ **Further evaluation of profile before any in vivo use**

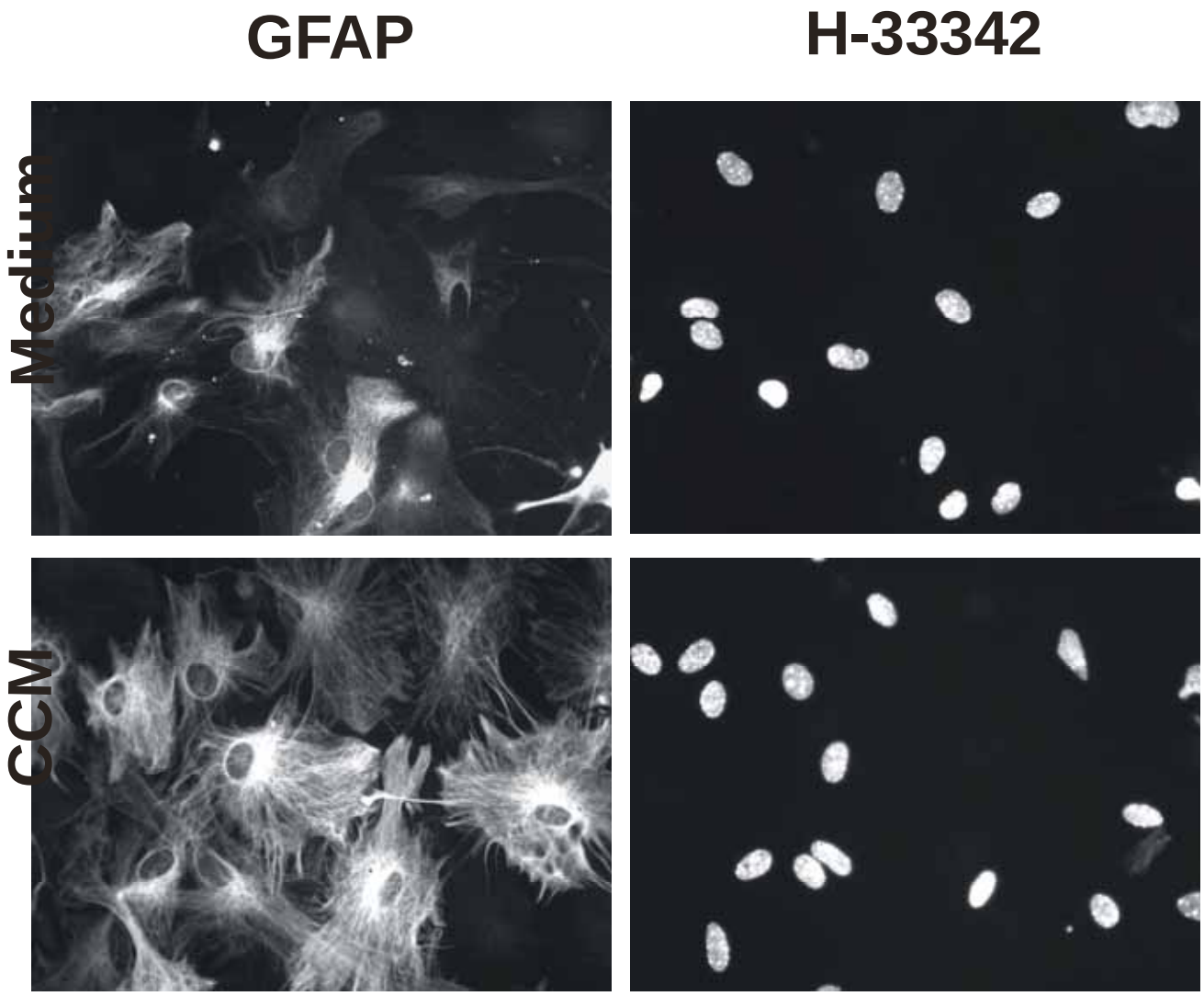
Reduced microglial inflammation



Selective anti-inflammatory properties of CEP1347 in microglia

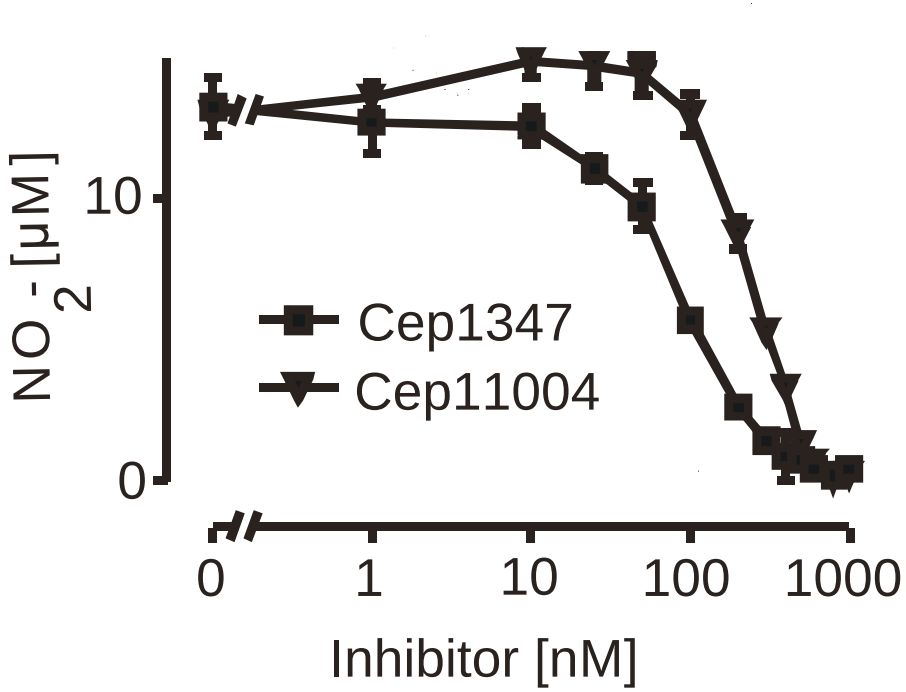


Activation of astrocyte cultures



CCM = TNF + IL-1 + IFN-gamma

Reduction of NO production in astrocytes



Selective transcriptional effects on inflamed astrocytes

Fold induction: > 5 ■: 2-5 ■: 0.51-2 ■: <0.50 ■			
Gene name/product	Regulation by CEP1347:		Genbank
activating transcription factor 4**, ATF-4**	**up**	2.3 ± 0.3	NM_001675
Birc2, baculoviral IAP repeat-containing 2		2.3 ± 0.1	NM_007464
B2m, beta-2 microglobulin		1.4 ± 0.2	NM_009735
Caspase 11		1.4 ± 0.1	NM_007609
FAS, Tnfrsf6, CD95		1.1 ± 0.1	NM_007987
M-CSF, colony stimulating factor 1		1.1 ± 0.0	NM_007778
GSK3, glycogen synthase kinase 3	**No regulation**	1.0 ± 0.3	XM_133269
NF-kappaB1,		1.0 ± 0.3	NM_008689
MnSOD, superoxide dismutase 2		1.0 ± 0.2	NM_013671
MCP-1, CCL2		0.7 ± 0.3	NM_011333
RANTES, CCL5		0.6 ± 0.1	NM_013653
IL6, interleukin 6		0.4 ± 0.1	NM_031168
GM-CSF, colony stimulating factor 2	**down**	0.4 ± 0.0	XM_109951
Nos2, iNOS		0.4 ± 0.0	NM_010927
Cox-2, prostaglandin-endoperoxide synthase 2		0.4 ± 0.1	NM_011198

Falsig et al.: Differential modulation of astrocyte inflammation by CEP1347 (J. Immunol, in press)

Use of cell lines for inflammation studies to further reduce the use of animals

1. Design of an inflammation-specific gene chip

Use of cell lines for inflammation studies to further reduce the use of animals

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2. **Characterisation of the inflammation response of astrocytes, microglia and brain in vivo.**

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3. **Relatively good correlation of upregulated genes**

Use of cell lines for inflammation studies to further reduce the use of animals

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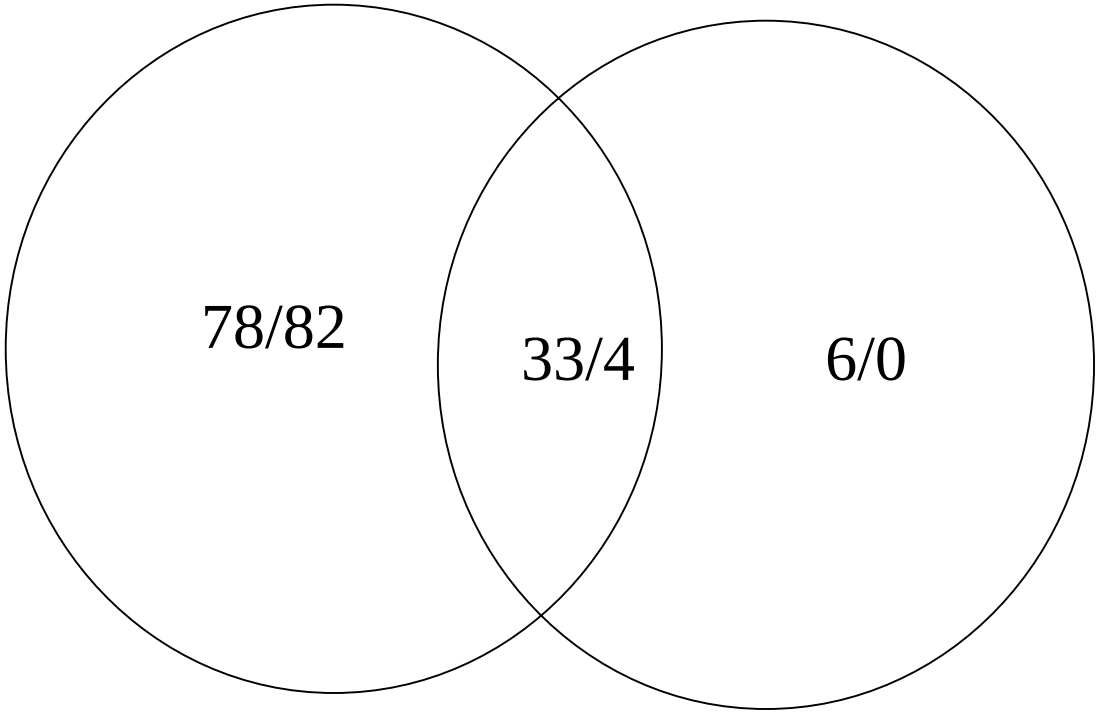
2. **Characterisation of the inflammation response of astrocytes, microglia and brain in vivo.**
3. **Relatively good correlation of upregulated genes**

4. **Comparison of microglia with microglia cell lines**

Microglia, LPS/control 4h

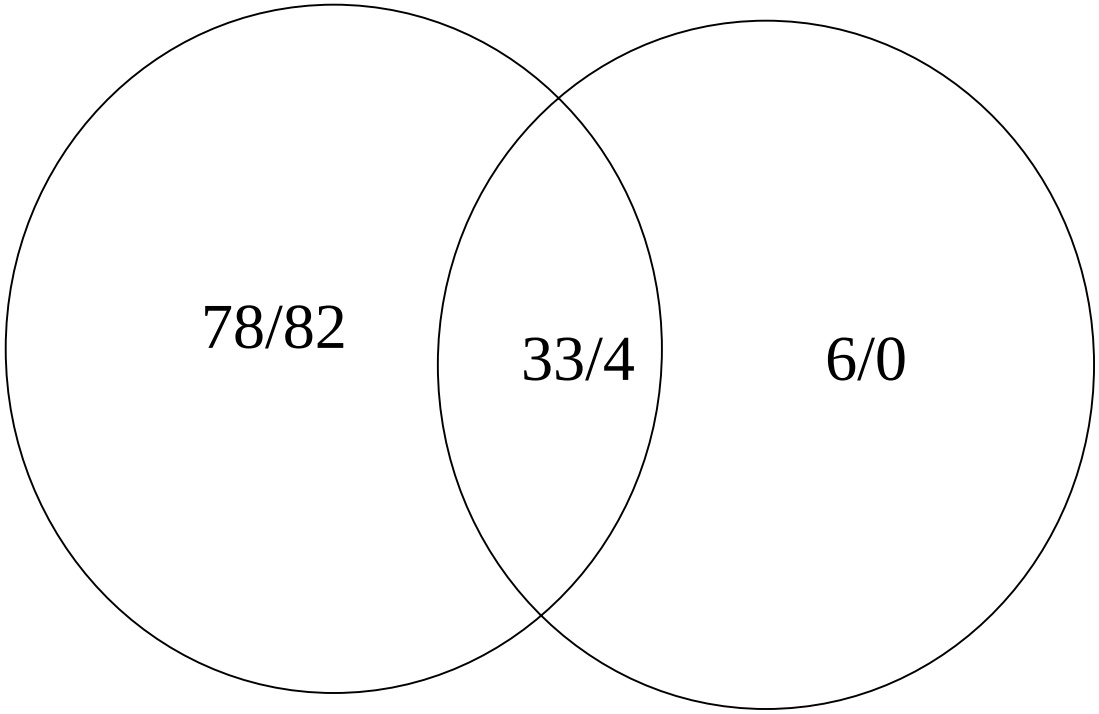
BV-2, LPS/control 4h



X/Y:
X = upregulations
Y= downregulations

Microglia, LPS/control 4h

BV-2, LPS/control 4h



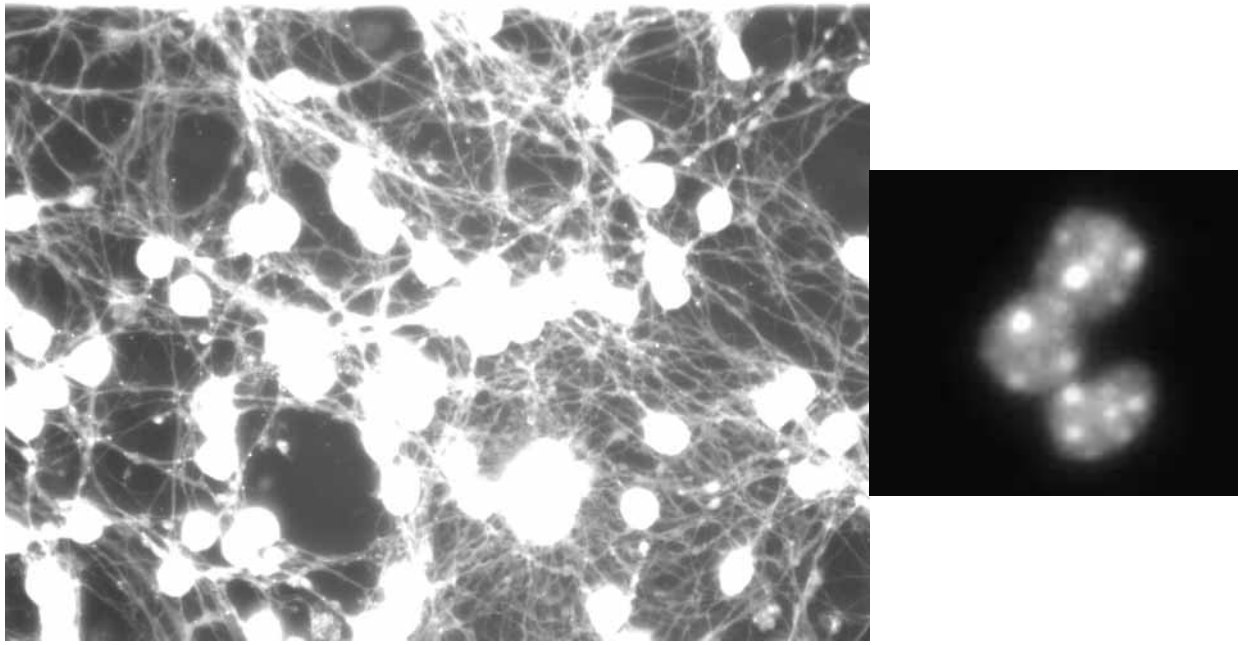
X/Y:
X = upregulations
Y= downregulations

→ **Poor overlap**
Use of primary cells for profiling
Use of cell line if mechanism of interest overlaps

Due to setup of screening filters:

Compound should be neuroprotective AND anti-inflammatory

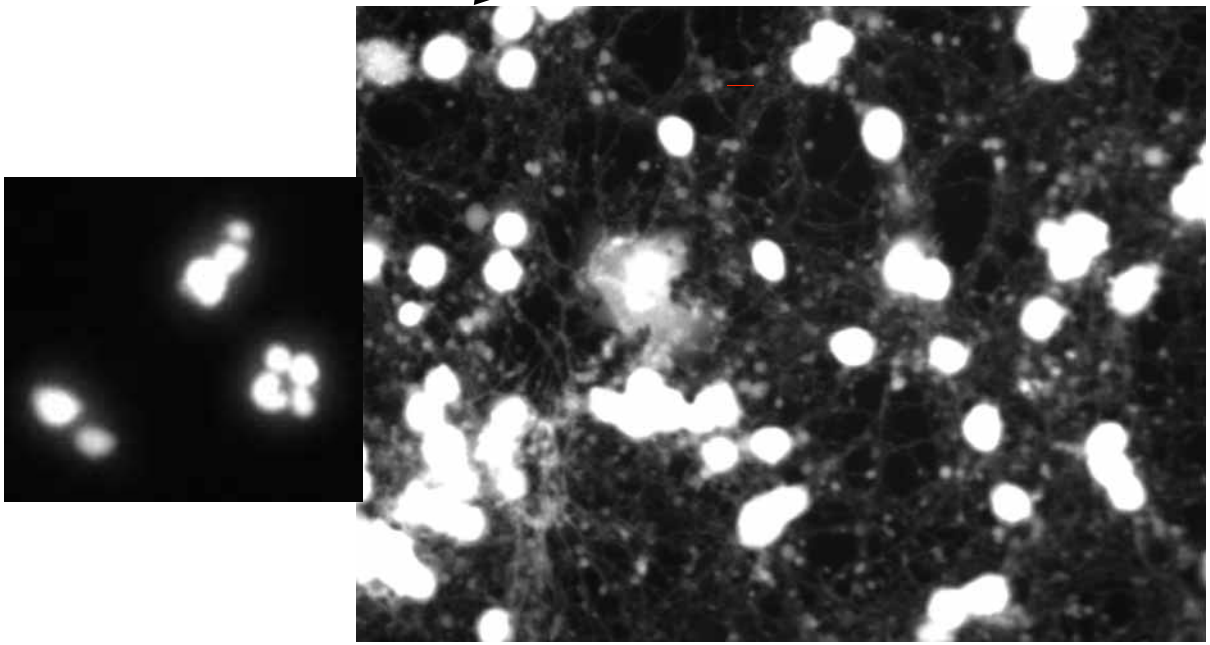
→ **Further evaluation of profile before any in vivo use**

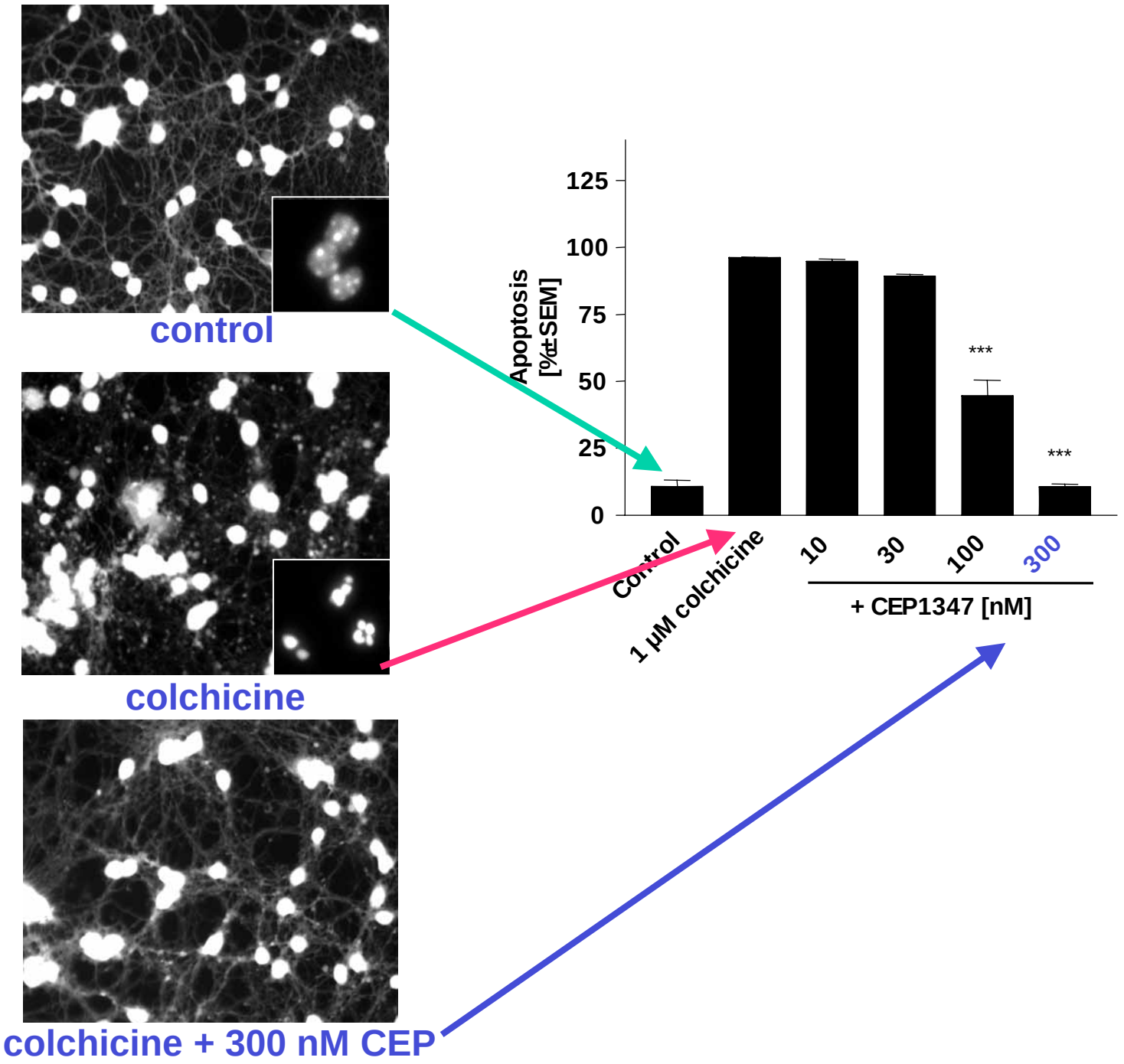


Intact neurites
Intact nuclei

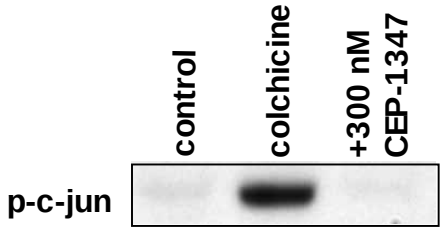
1 µM Colchicine

Lost neurites
Apoptotic nuclei

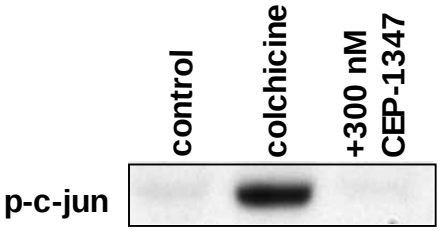



control
colchicine
colchicine + 300 nM CEP
Apoptosis [%±SEM]
125
100
75
50
25
0
***
***
Control
1 µM colchicine
10
30
100
300
+ CEP1347 [nM]

Block of JNK activation:



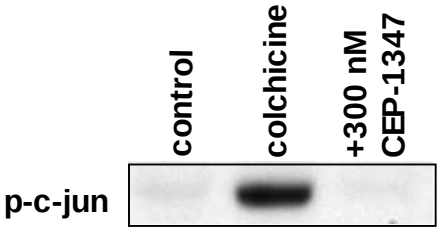
Block of JNK activation:



Algorhythm for automatic detection of individual cells and nuclear compartment

Staining and automatic quantitation of Phosphorylated c-jun

Block of JNK activation:



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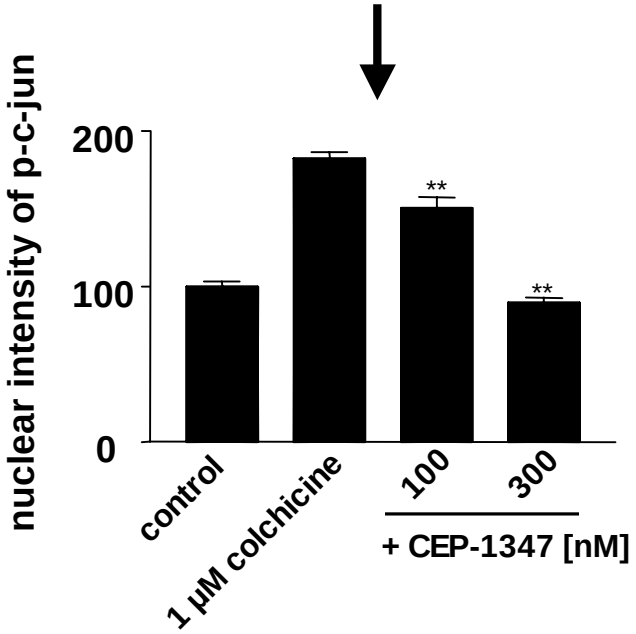
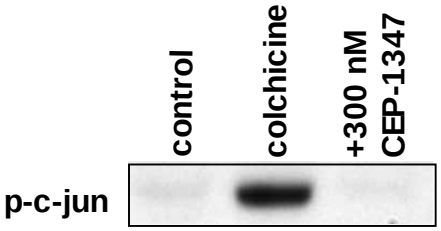


Block of JNK activation:



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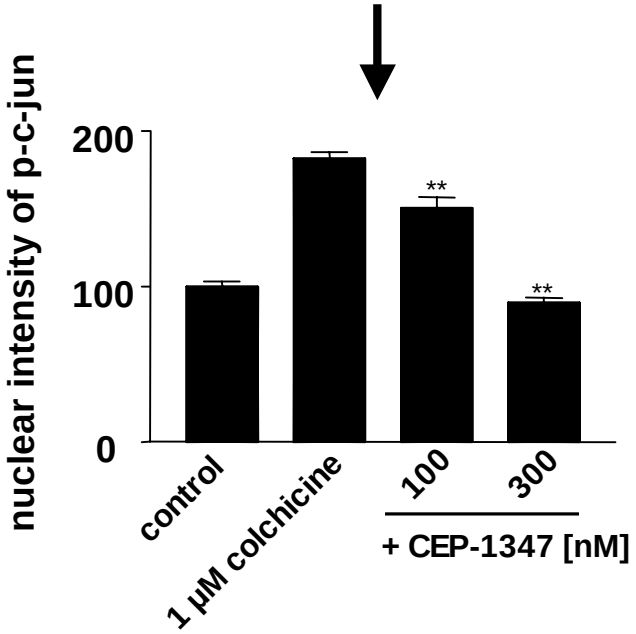


Suitable as mechanistic screen; Correlation with neuroprotection

Summary

Combination of mechanistic filters for drug screen
Replacement of animal testing by in vitro test battery

Development of a human cell model of PD
Reduction of need for animal models

Chip profiling of reaction pattern
Rational choice on use of animals

Acknowledgement

J Falsig
P Pörzgen
S Lund
J Lotharius

M Geist
C Volbracht
J Boll

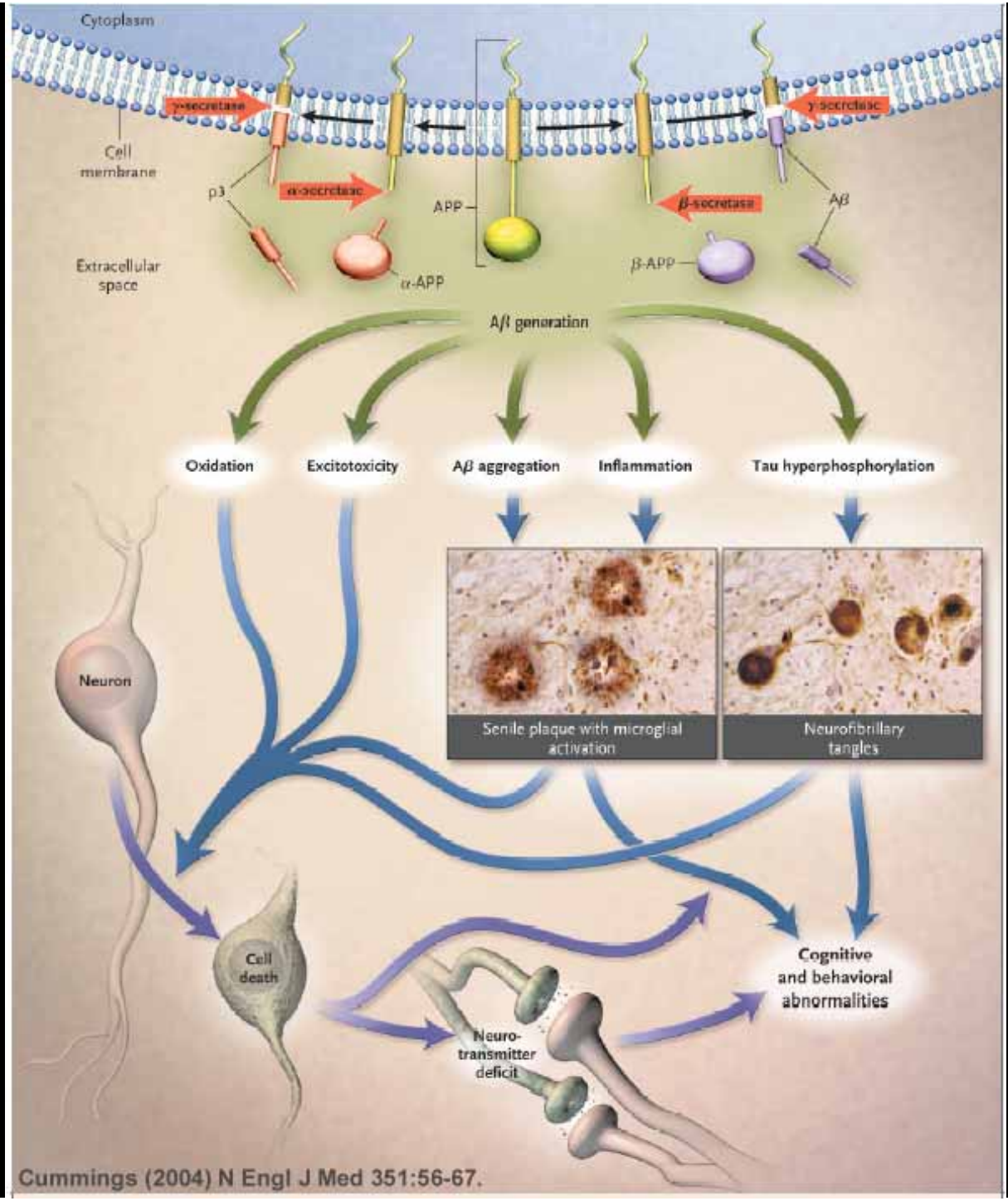
…

Cephalon

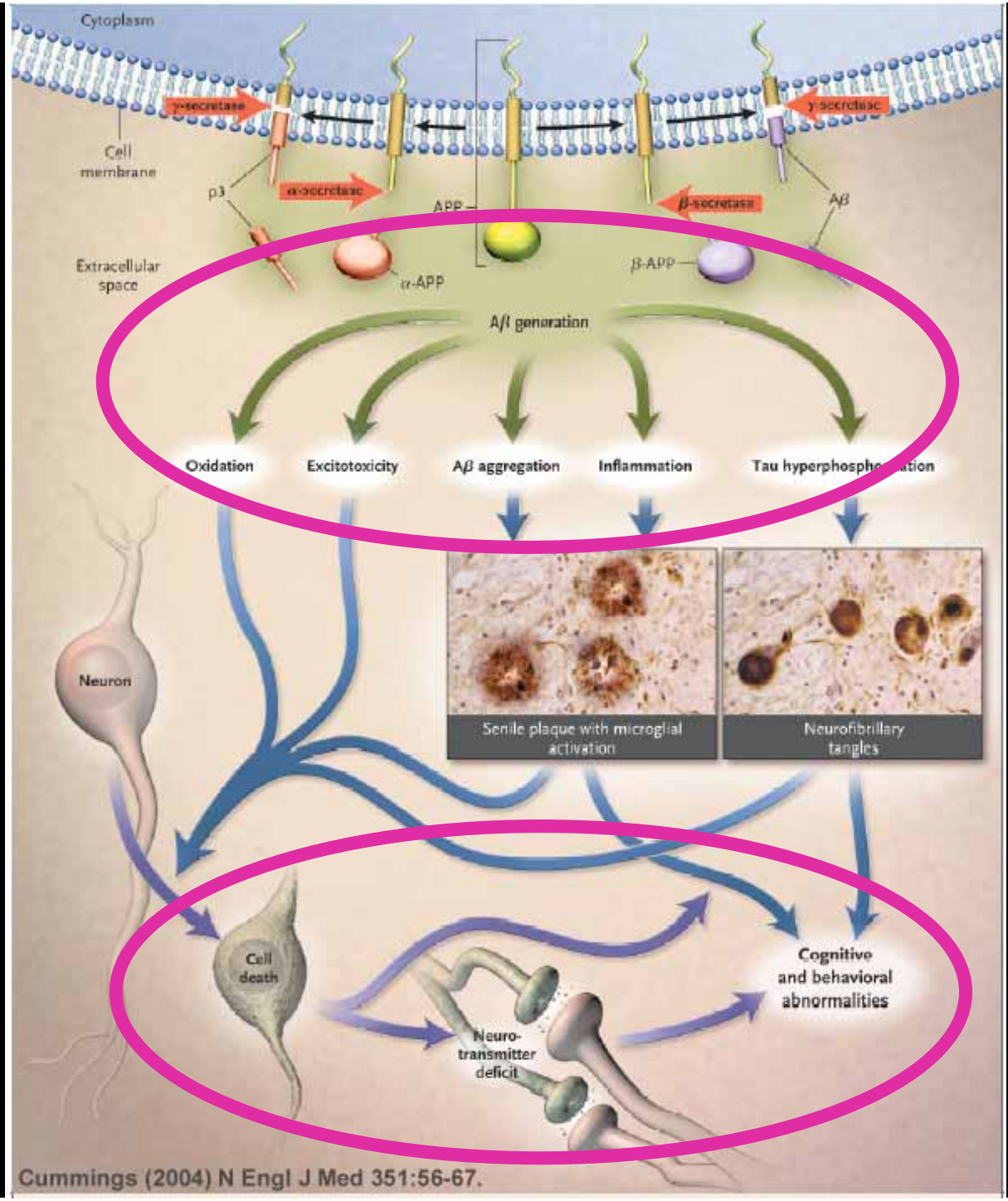
D Bozyczko-Coyne

…

End

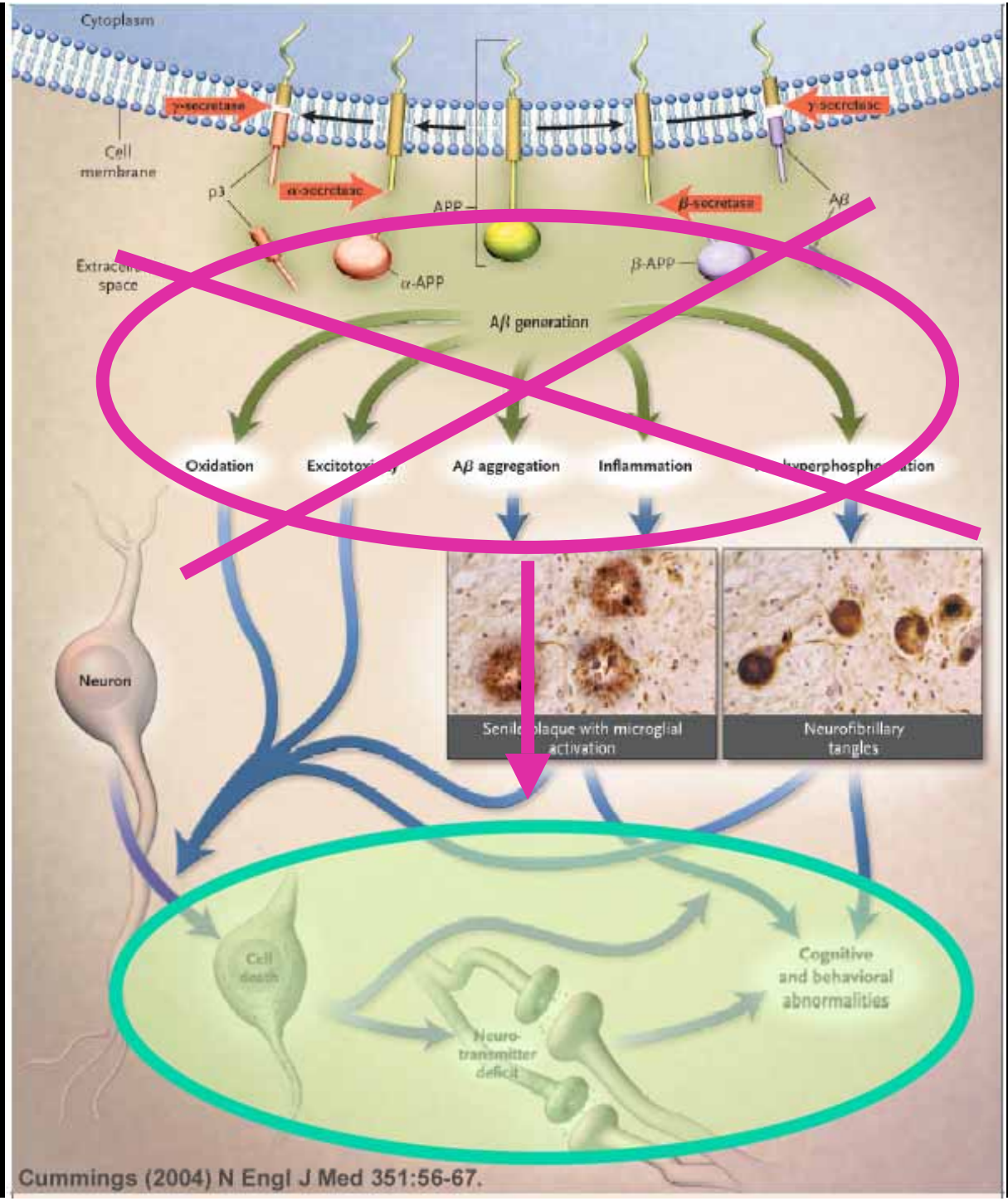


Cummings (2004) N Engl J Med 351:56-67.



Disease cause

Disease symptoms



Cummings (2004) N Engl J Med 351:56-67.

Disease cause

Disease blocked