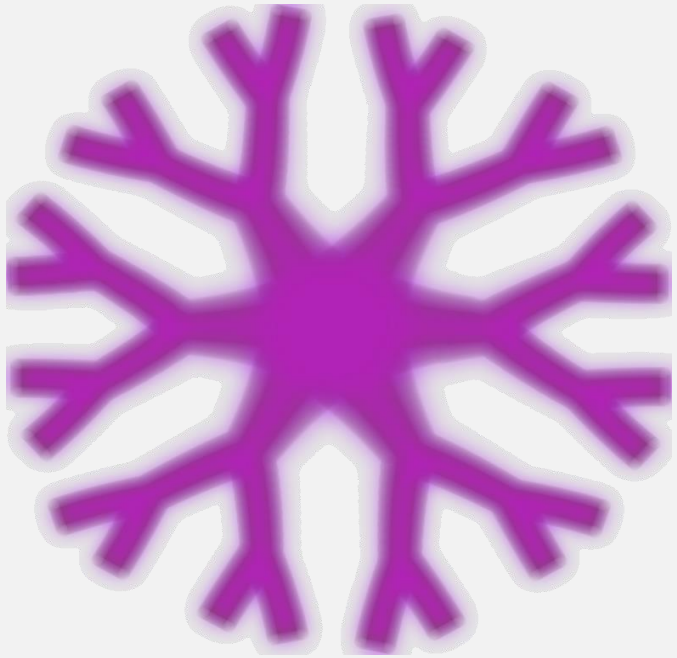
« Targeting monocytes-macrophages for clinical applications »



Prof. Rémy Poupot
Centre de Physiopathologie de Toulouse Purpan
INSERM U1043, Toulouse
"Molecular mechanisms of growth, osteogenesis and osteolysis – Biotherapies"

Disclosures and Conflict of Interest

≈ 20 articles and reviews about the ABP dendrimer

cofounder and shareholder of IMD-Pharma

Chronic Inflammatory Diseases (CID)

❖ **Severe conditions of immunological disorders, most of the time involving auto-immunity**

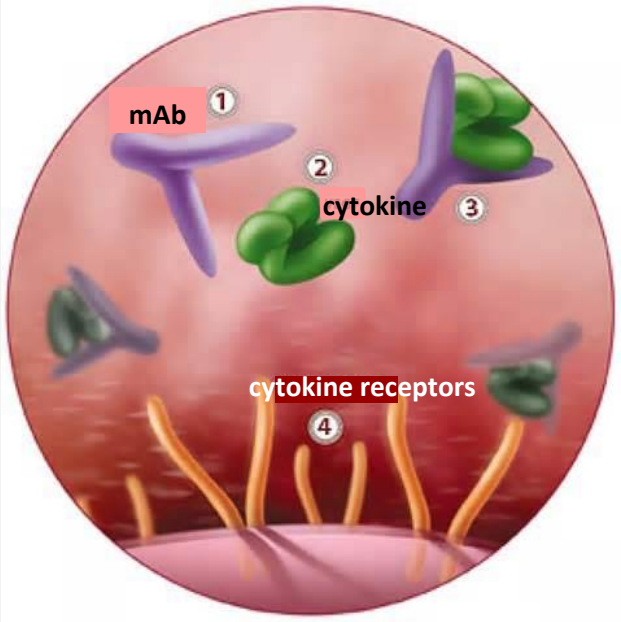
- Multiple Sclerosis (MS)
- Rheumatoid Arthritis (RA)
- Psoriasis
- Inflammatory Bowel Diseases (IBD; Crohn's Disease, Ulcerative Colitis)
- Chronic Obstructive Pulmonary Disease (COPB)

❖ **Current graduate treatments to reduce/resolve inflammation**

- glucocorticoids / Non-Steroidal Anti-Inflammatory Drugs (NSAIDs)
- methotrexate
- bio-engineered proteins (biologics): soluble receptors and monoclonal antibodies (mAbs; especially anti-TNFα)

Chronic Inflammatory Diseases (CID)

❖ **The principle of biologics:** highly specific inhibition of a pro-inflammatory mediator, “ON/OFF” effect



❖ **Opportunities for competitors of biologics:**

- target only ONE cytokine of the inflammatory network
  ⇨ efficacy overcome with time due to redundant functions of cytokines
- immunogen (induce the production of antibodies, although fully humanized)
- immuno-suppressive, potentially enhancing infections and cancers
- contra-indications (eg, heart diseases)
- therapeutic failure for 20% of patients

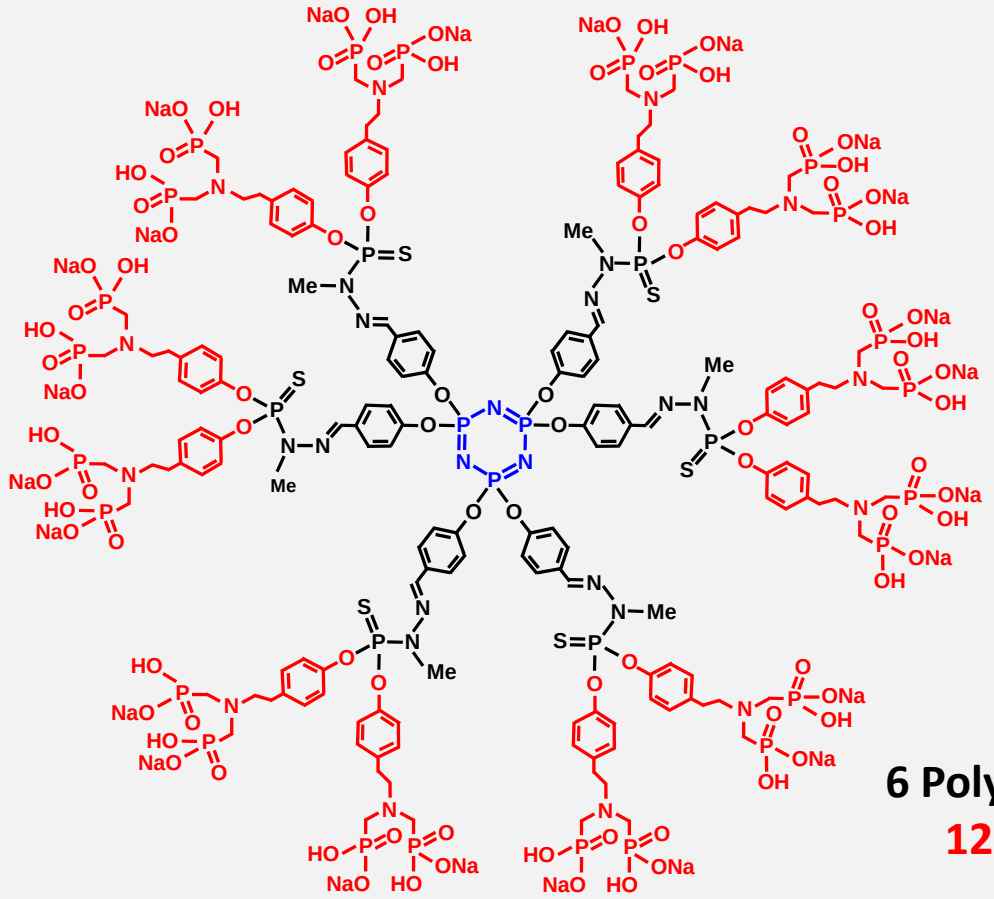
A “lead” dendrimer with anti-inflammatory properties

Dendrimers:

multivalent, non-linear polymers,
perfectly defined size and structure
(iterative stepwise synthesis)

Composed of:

- a core
- 1 or more series of branches
- surface functions



ABP dendrimer:

cyclo-triphosphazene (N_3P_3) core
6 Poly(PhosphorHydrazone) (PPH) branches
12 AzaBisPhosphonate (ABP) end groups

generation 1 dendrimer
(1 series of branches)
MW = 5817 Da, R_h ≈ 2-3 nm

A “lead” dendrimer with anti-inflammatory properties

human PBMC

B lympho

T CD8 lympho

NKT lympho

Tγδ lympho

T CD4 lympho

D. Portevin et al., *J. Transl. Med.* **2009**

DC

Y. Degboé et al., *Arthritis Res. Ther.* **2014**

Griffe L. et al., *Angew. Chem. Int. Ed.* **2007**
Poupot M. et al., *Nanomedicine* **2016**

NK cells

monocytes

anti-inflammatory

Poupot M. et al., *FASEB J.* **2006**
Rolland O. et al., *Chemistry* **2008**
Fruchon S. et al., *J. Leukoc. Biol.* **2009**
Rolland O. et al., *Tetrahedron Lett.* **2009**
Marchand P. et al., *Bioorg. Med. Chem. Lett.* **2009**

A “lead” dendrimer with anti-inflammatory properties

in vivo "Proof of Efficacy": mouse model of **Rheumatoid Arthritis (RA)**

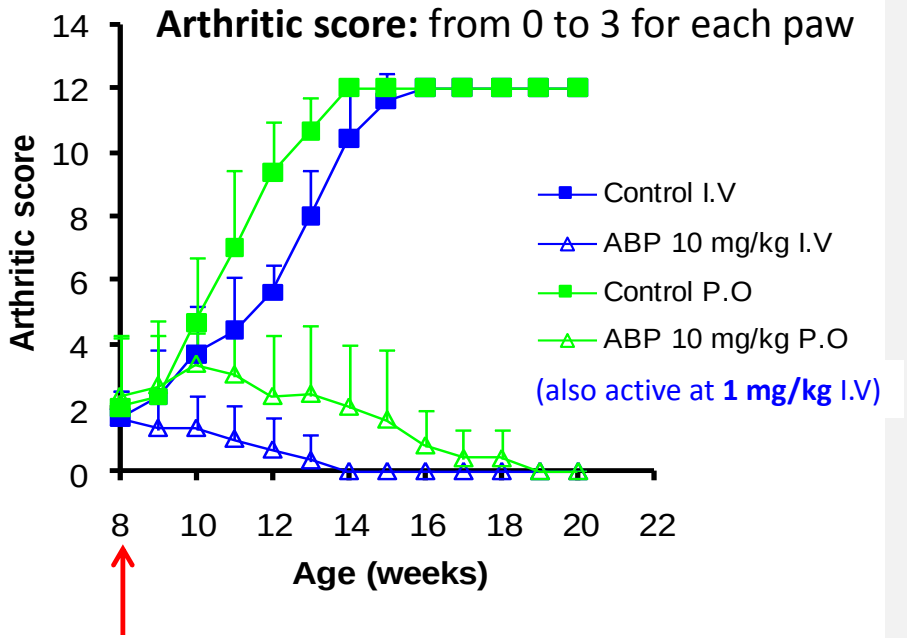
Development of Chronic Inflammatory Arthropathy Resembling Rheumatoid Arthritis in Interleukin 1 Receptor Antagonist–deficient Mice

By Reiko Horai,* Shinobu Saijo,* Hidetoshi Tanioka,‡ Susumu Nakae,* Katsuko Sudo,* Akihiko Okahara,‡ Toshimi Ikuse,‡ Masahide Asano,* and Yoichiro Iwakura*

IL1-ra = IL1-Receptor Antagonist

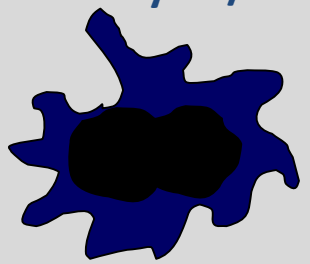
A “lead” dendrimer with anti-inflammatory properties

in vivo "Proof of Efficacy": mouse model of **Rheumatoid Arthritis (RA)**



constitutive inflammatory activation

inflammatory monocytes/macrophages



pro-inflammatory cytokines TNF-α, IL1-β …

MMP secretion (Matrix Metallo-Proteases)

osteoclast differentiation

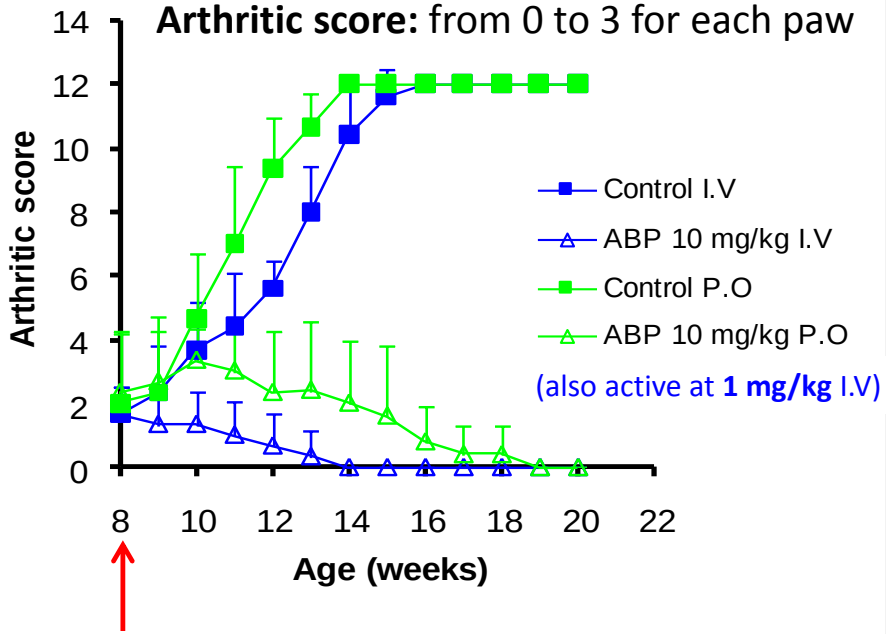
systemic and joint inflammation

cartilage degradation

bone resorption

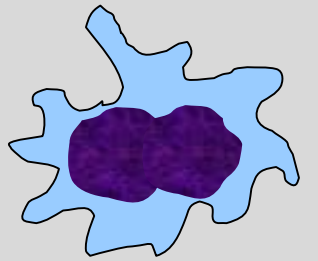
A “lead” dendrimer with anti-inflammatory properties

in vivo "Proof of Efficacy": mouse model of **Rheumatoid Arthritis (RA)**



constitutive inflammatory activation

anti-inflammatory monocytes/macrophages



pro-inflammatory cytokines TNF-α, IL1-β … → **systemic and joint inflammation**

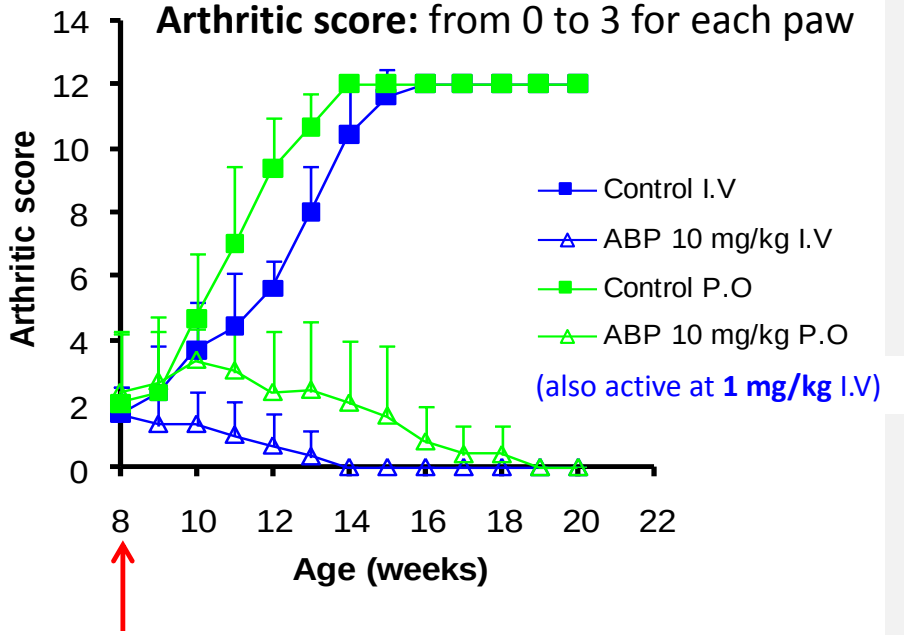
MMP secretion (Matrix Metallo-Proteases) → **cartilage degradation**

osteoclast differentiation → **bone resorption**

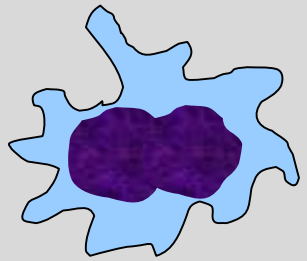
A “lead” dendrimer with anti-inflammatory properties

in vivo "Proof of Efficacy": mouse model of **Rheumatoid Arthritis (RA)**

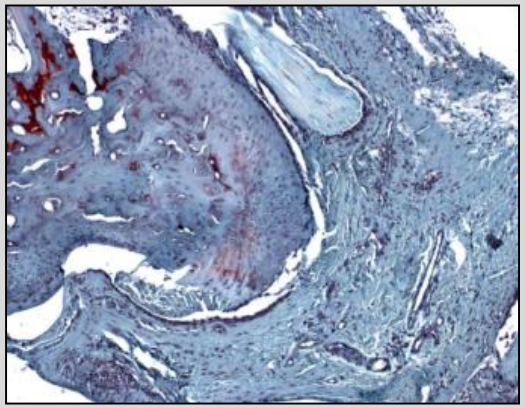
constitutive inflammatory activation



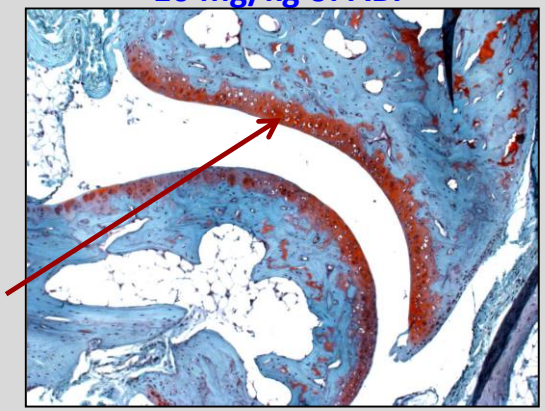
anti-inflammatory monocytes/macrophages



untreated arthritic mouse

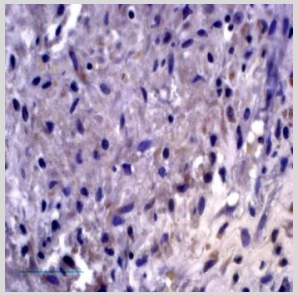
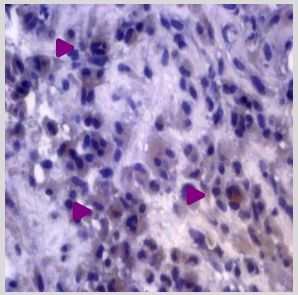
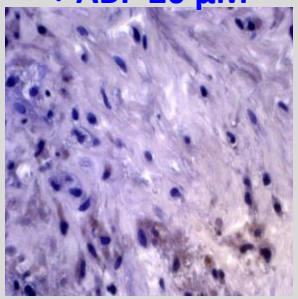


arthritic mouse treated with 10 mg/kg of ABP



A “lead” dendrimer with anti-inflammatory properties

ex vivo "Proof of Efficacy": culture of **human rheumatoid synovial membrane**
(informed consent, Rheumatology Dept, Toulouse Hospital)

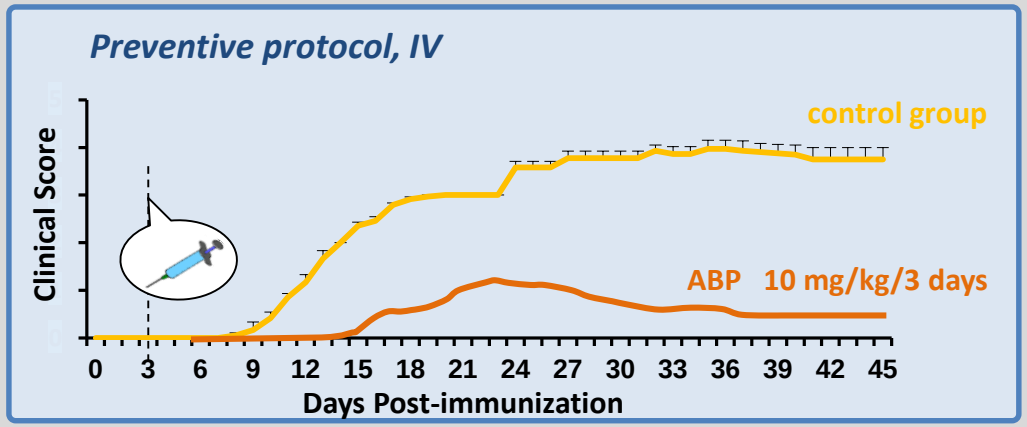
	control medium	differentiation medium	differentiation medium **+ ABP 20 µM**
TRAP labelling			
osteoclast counts:	61 ± 12 / nm²	**202 ± 24 / nm² osteoclast differentiation**	**92 ± 17 / nm² inhibition**

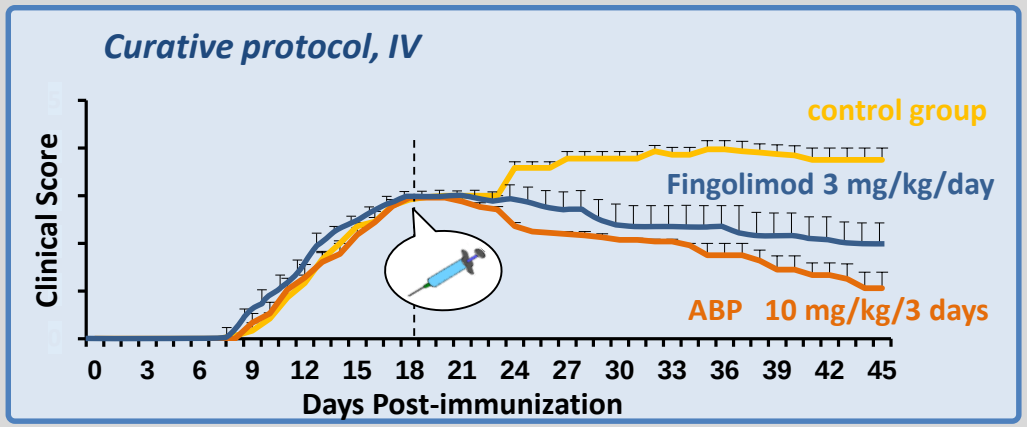
The ABP dendrimer is a drug-candidate for the treatment of Rheumatoid Arthritis

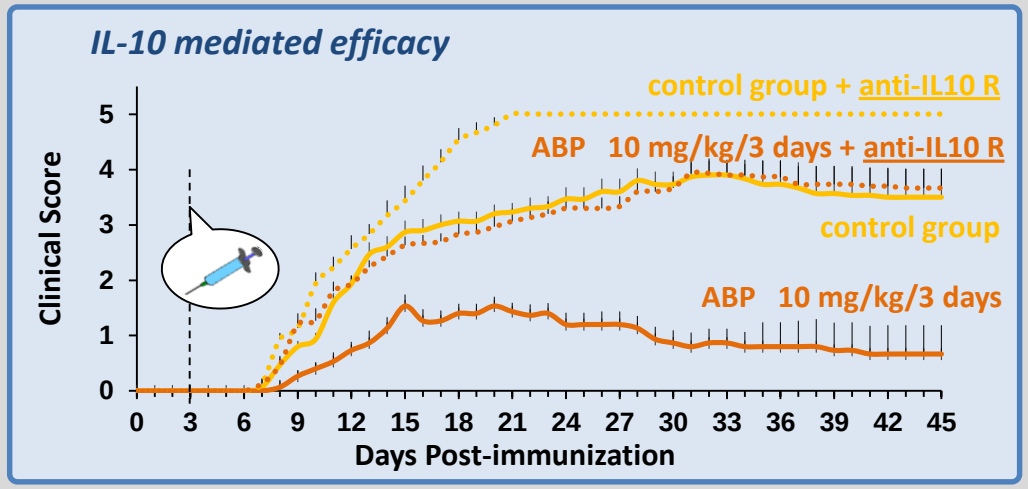
Hayder M. et al., *Sci. Transl. Med.* **2011**

A “lead” dendrimer with anti-inflammatory properties

in vivo "Proof of Efficacy": mouse model of **Multiple Sclerosis** (EAE model)





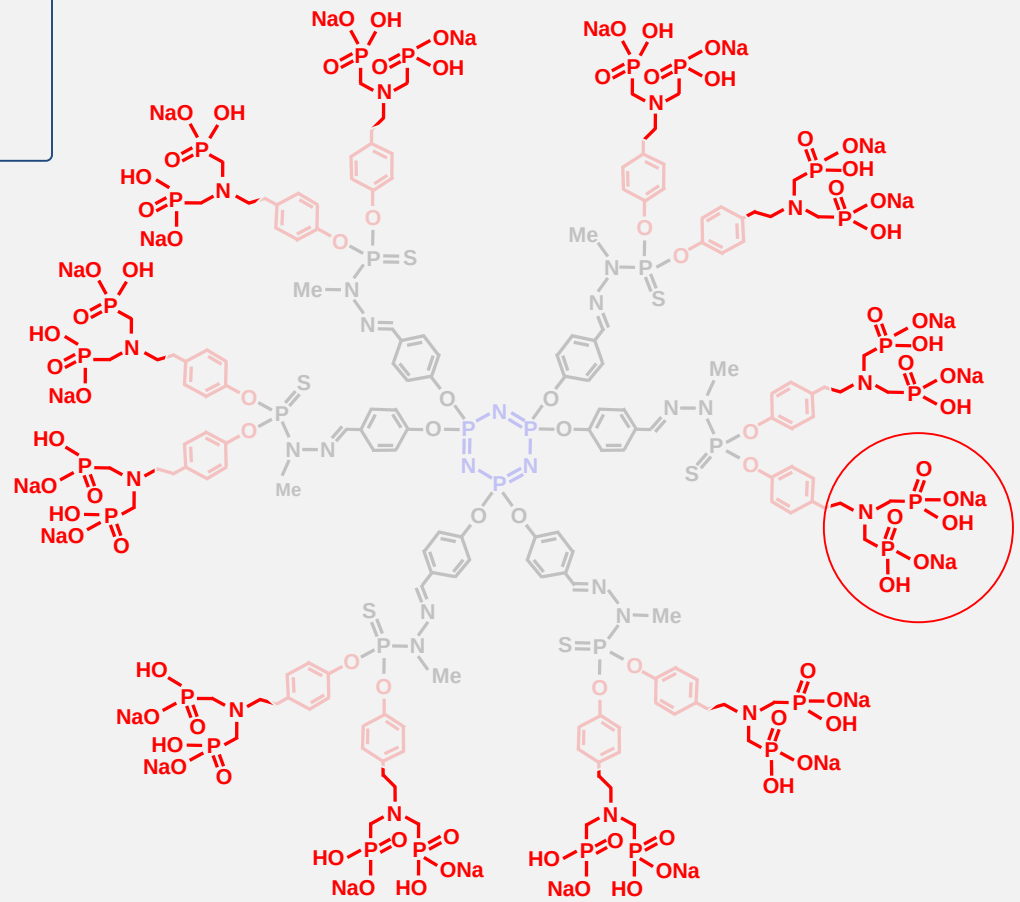


The ABP dendrimer is a drug-candidate for the treatment of Multiple Sclerosis

Hayder M. et al., *Biomacromolecules* **2015**

A “lead” dendrimer with anti-inflammatory properties

surface function = variable
size/generation = constant
outer shell density = constant
internal scaffold = constant



optimized surface function:
AzaBisPhosphonate (ABP)

A “lead” dendrimer with anti-inflammatory properties

surface function = optimized
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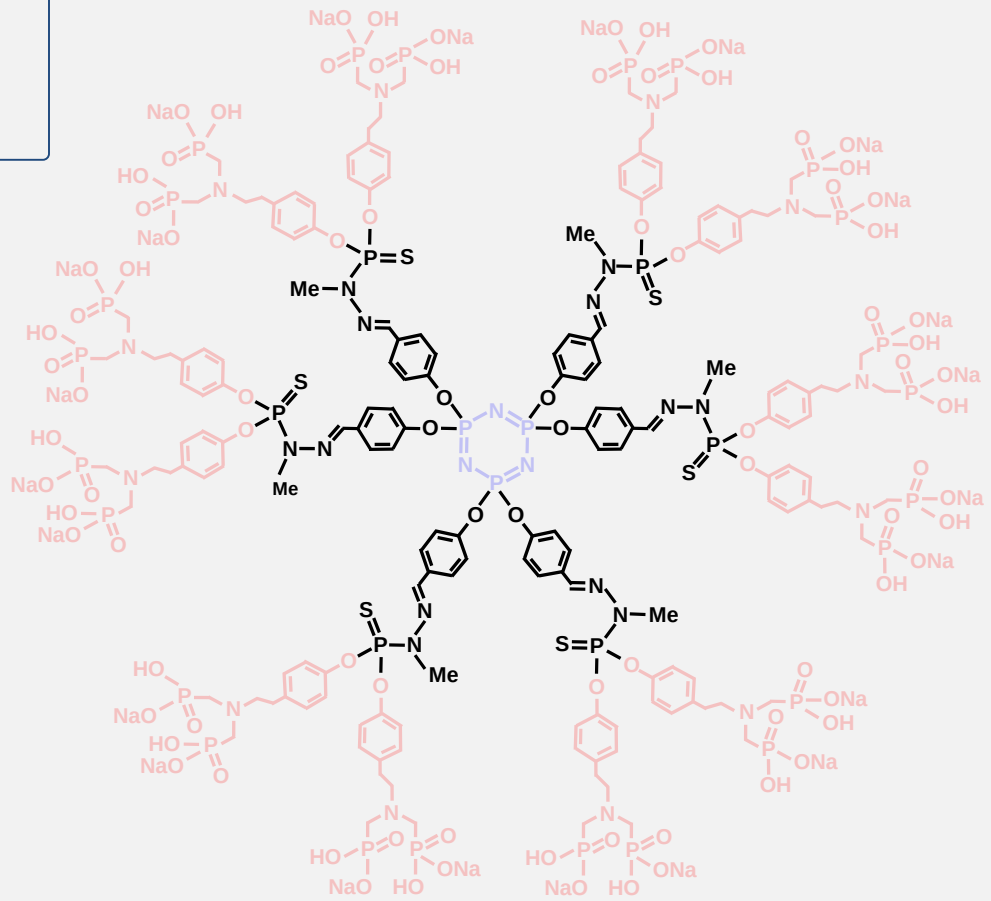

optimized generation:
generation 1

6 PPH branches
(12 ABP surface functions)

A “lead” dendrimer with anti-inflammatory properties

surface function = optimized
size/generation = optimized
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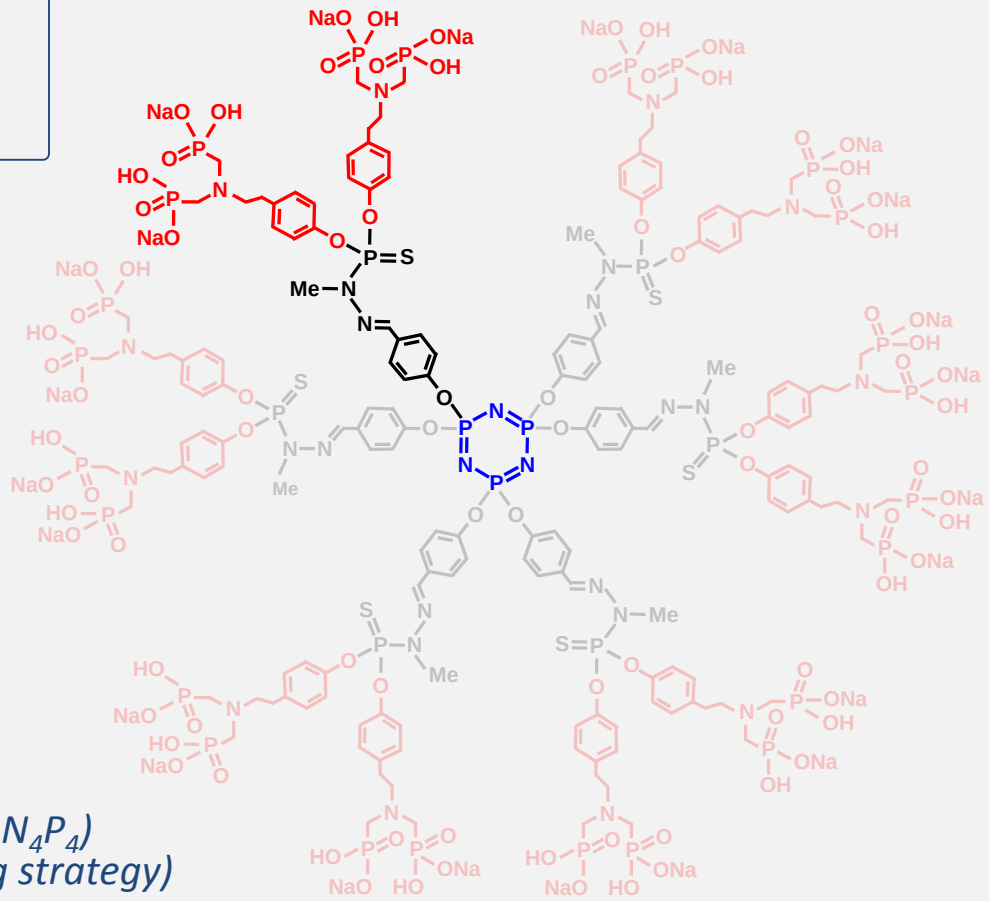
internal scaffold = constant



The outer shell density is:
↗ by modifying the core dimension (N_3P_3 → N_4P_4)
↘ with the number of branches (core locking strategy)

A “lead” dendrimer with anti-inflammatory properties

surface function = optimized
size/generation = optimized
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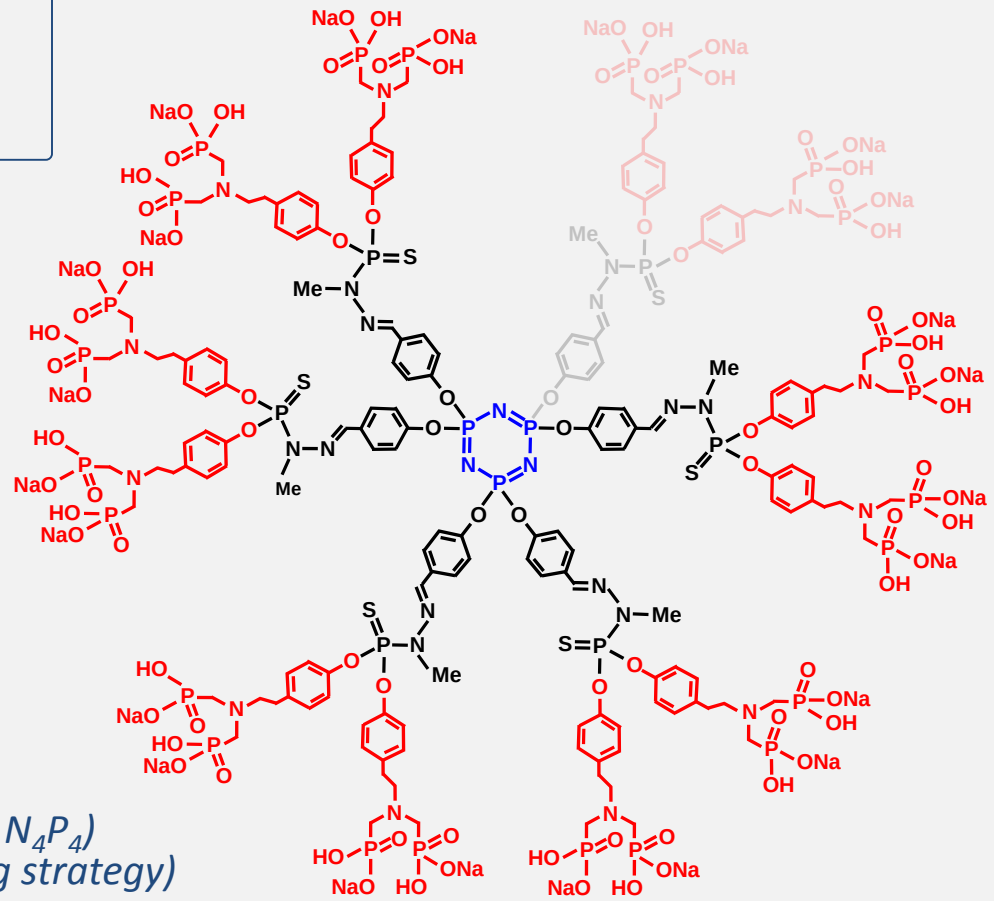


optimized outer shell density:
10 and 12 ABP surface functions

The outer shell density is:
↗ by modifying the core dimension ($N_3P_3 \rightarrow N_4P_4$)
↘ with the number of branches (core locking strategy)

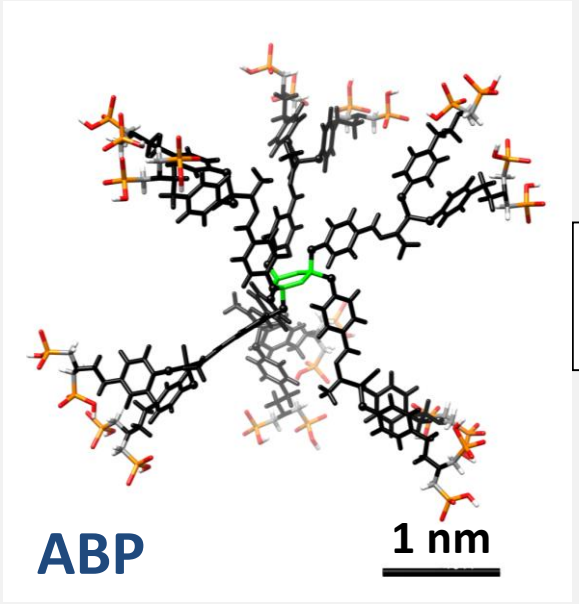
A “lead” dendrimer with anti-inflammatory properties

surface function = optimized
size/generation = optimized
outer shell density = optimized
internal scaffold = variable

assay of 13 different scaffolds having approximately the same size and number of surface functions as the ABP dendrimer

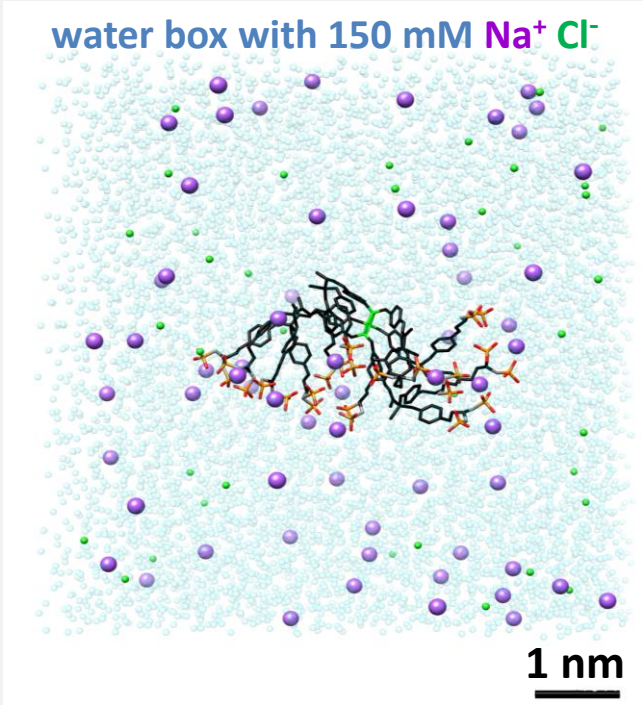
molecular simulations *vs* **anti-inflammatory bioactivity**

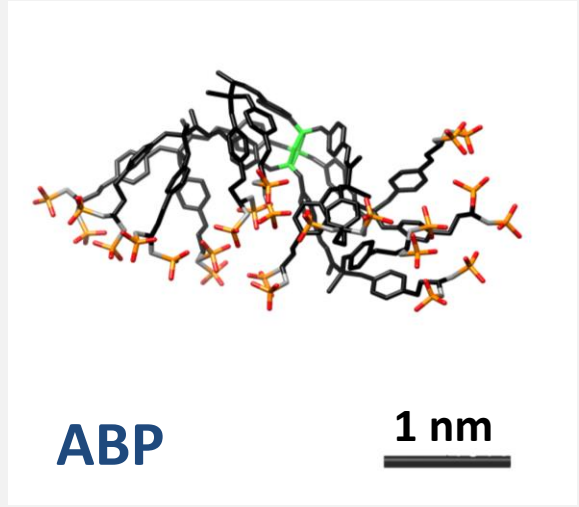
initial conformation



Molecular Dynamics

equilibrated conformation in the experimental conditions





A “lead” dendrimer with anti-inflammatory properties

surface function = optimized
size/generation = optimized
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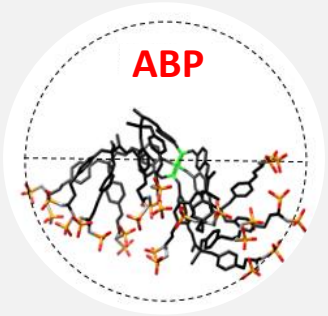
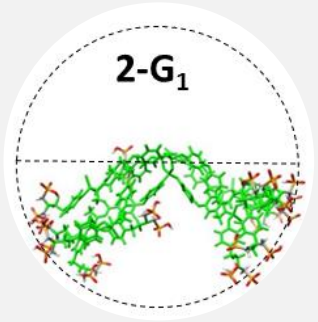
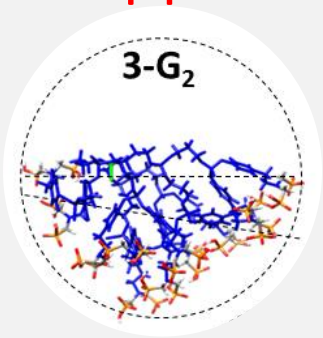
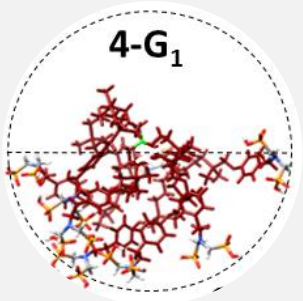
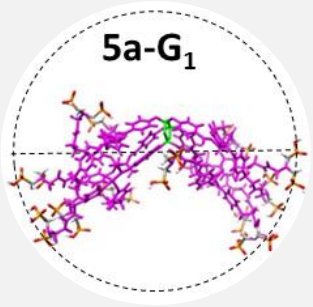
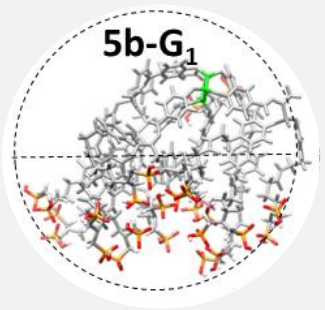
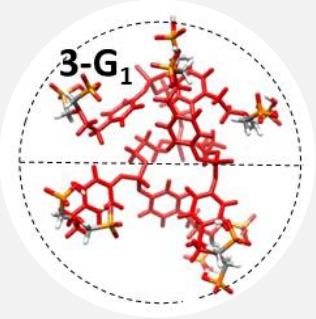
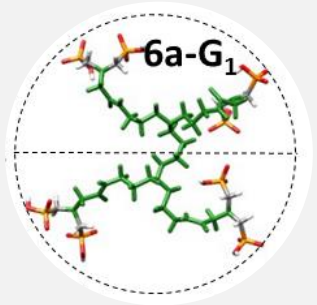
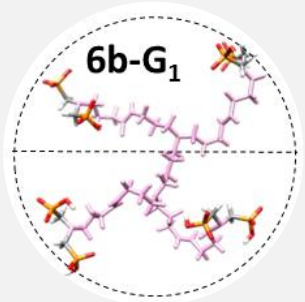
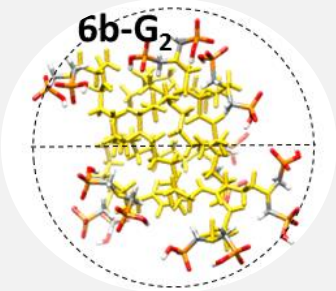
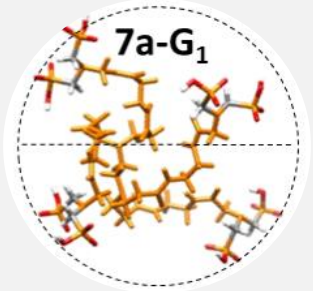
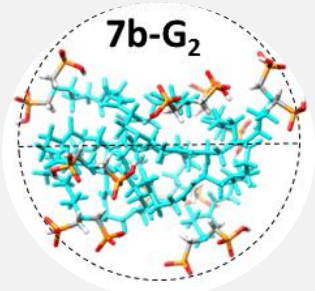
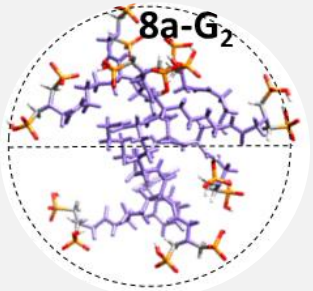
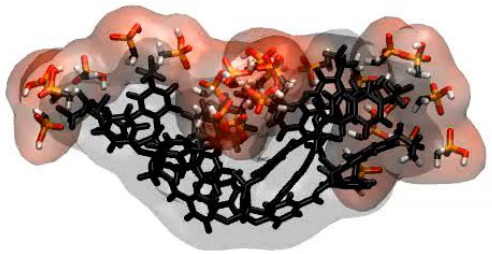
molecular simulations *vs* **anti-inflammatory bioactivity**

Bioactivity towards human monocytes

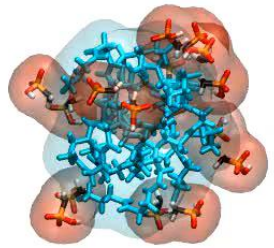
+ + +	+ +	+ +	+ +	+ +	+ +	+
ABP	2-G_1	3-G_2	4-G_1	5a-G_1	5b-G_1	3-G_1
						
0	**0**	**0**	**0**	**0**	**0**	
6a-G_1	6b-G_1	6b-G_2	7a-G_1	7b-G_2	8a-G_2	
						

A “lead” dendrimer with anti-inflammatory properties

ABP dendrimer (active)



6b-G_2 dendrimer (inactive)

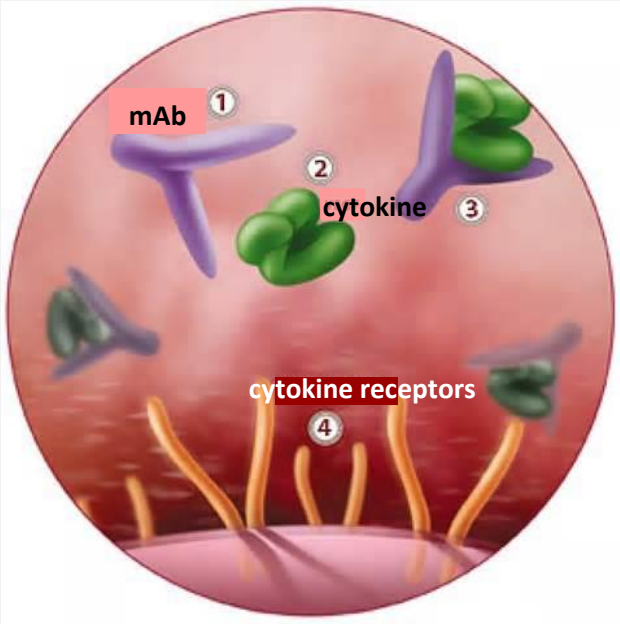


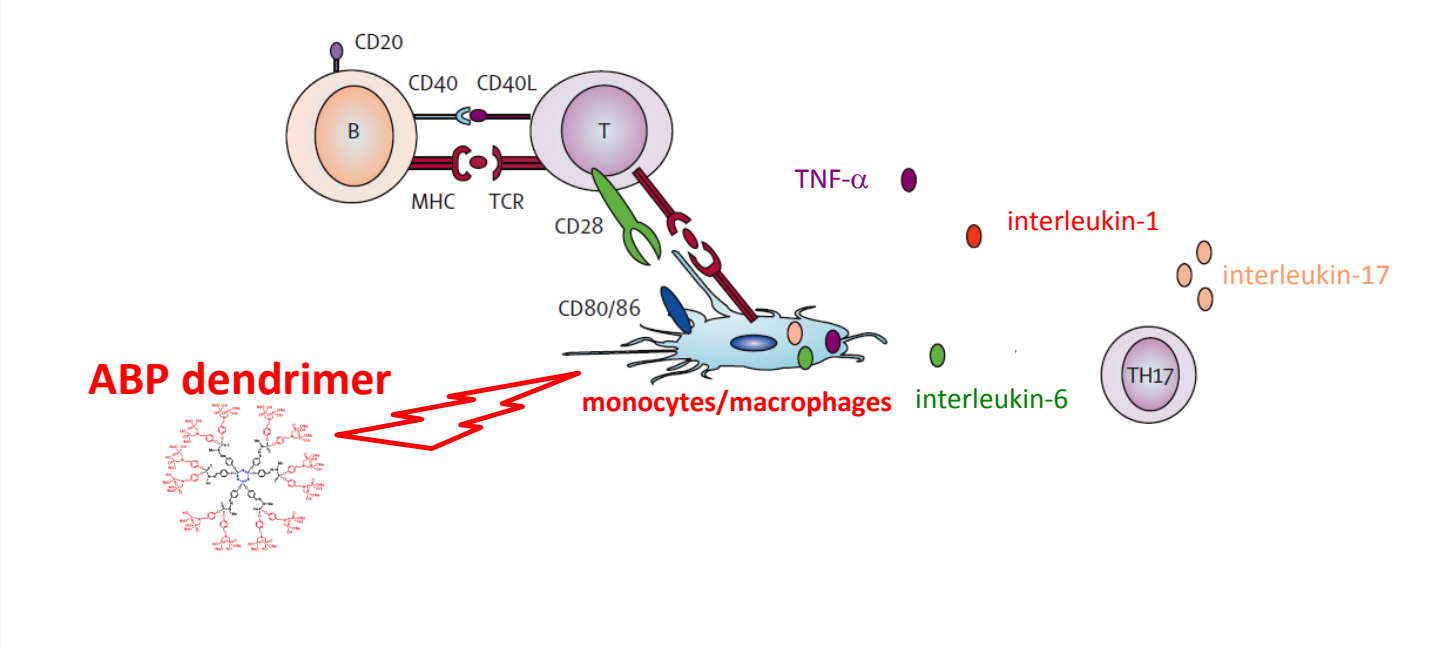
3D-directionality is key to have anti-inflammatory ABP-capped dendrimers

Caminade A.M. et al., *Nat. Commun.* **2015**

Opportunities …

- **The principle of biologics:** highly specific inhibition of a pro-inflammatory mediator, "ON/OFF" effect
- **New concept: rehabilitation of inflammatory monocytes/macrophages with the ABP dendrimer**





… and challenges

The “nanoworld” *versus* regulatory agencies

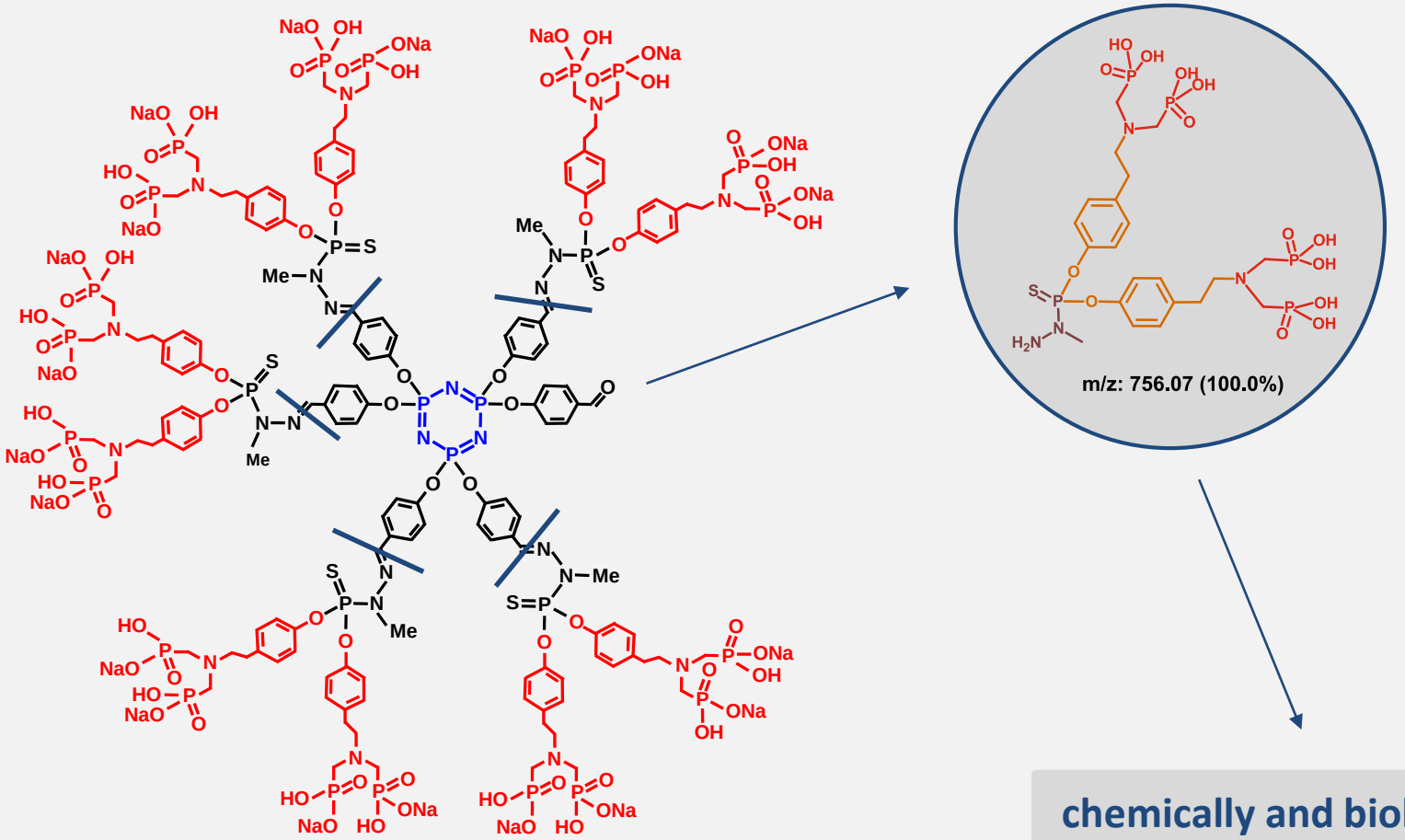
Non-biodegradable nano-objects

- long term effects related to bio-accumulation?
- genotoxicity, immune response (sustained micro-inflammation)?

Biodegradable nano-objects

- they are eliminated/secreted
- genotoxicity, immuno-safety?

… and challenges



chemically and biologically characterized

… and challenges

The “nanoworld” *versus* regulatory agencies

Non-biodegradable nano-objects

- long term effects related to bio-accumulation?
- genotoxicity, immune response (sustained micro-inflammation)?

Biodegradable nano-objects

- they are eliminated/secreted
- genotoxicity, immuno-safety?

ABP slowly degrades *in vitro* in physiological solutions

ansm Agence nationale de sécurité du médicament et des produits de santé

EUROPEAN MEDICINES AGENCY SCIENCE MEDICINES HEALTH

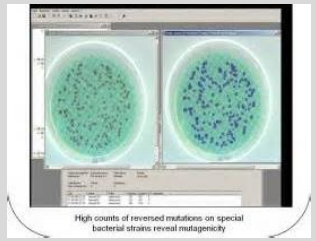
FDA U.S. Food and Drug Administration Protecting and Promoting *Your* Health

… and challenges

❖ Genotoxicity: BN Ames' test

• *Salmonella typhimurium,* 3 mutants, with or without metabolic activation

no significant increase of revertants ⇨ **no genotoxicity**



❖ Early toxicity: Maximal Tolerated Dose (MTD)

• single IV administration: **MTD = 100 mg/kg** (next dose: 150 mg/kg)


Pasteur Institute, 2004

Therapeutic Index > 25

• single IV administration: **MTD = 100 mg/kg** (next dose: 200 mg/kg)

• repeated IV administrations: **MTD = 60 mg/kg/day** (daily, 7 consecutive days) (next dose: 120 mg/kg/day)


Ricerca Biosciences, 2011

… and challenges

❖ Early toxicity and immuno-safety in Non-Human Primates

- 4 monkeys, 4 IV injections each, ABP at 10 mg/kg with 1 week intervals (sub-chronic toxicity)
- a 56 day follow-up:

clinical observations: body weight & temperature, behaviour, food uptake …
local reactions at the site of injection
clinical pathology: 10 biochemical & 16 hematologic/clotting parameters
immunological studies: 23 serum immune mediators, *ex vivo* assessment
histo-pathological study of main thorax and abdomen organs

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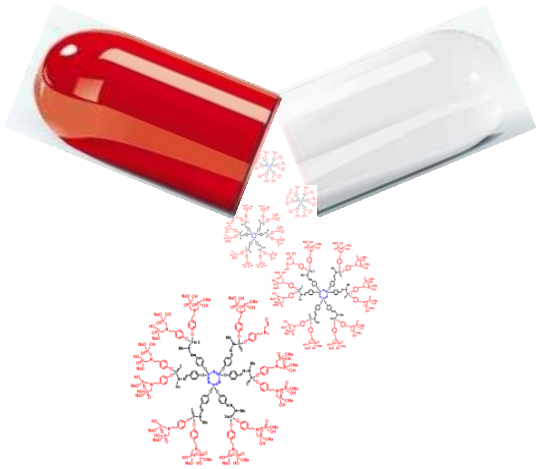
- **values in the physiological range, some subacute variations**
- **back to normal level within 2/3 days**
- **no cumulative effect during the time-course of the injections**

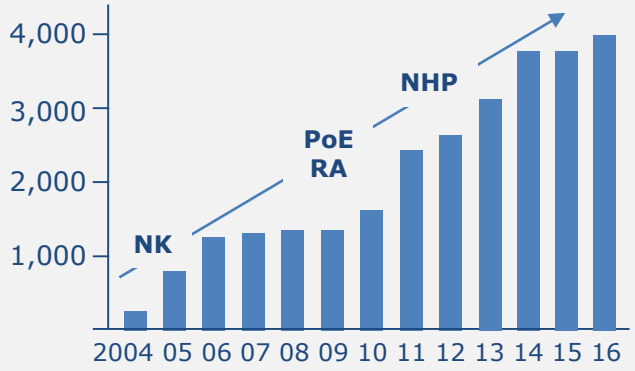
no immuno-suppression, no adverse effect

Fruchon S. et al., *Nanotoxicology* **2015**

What is next?



cumulative granted funding (k€) … only institutional so far …



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Immuno-modulating dendrimers

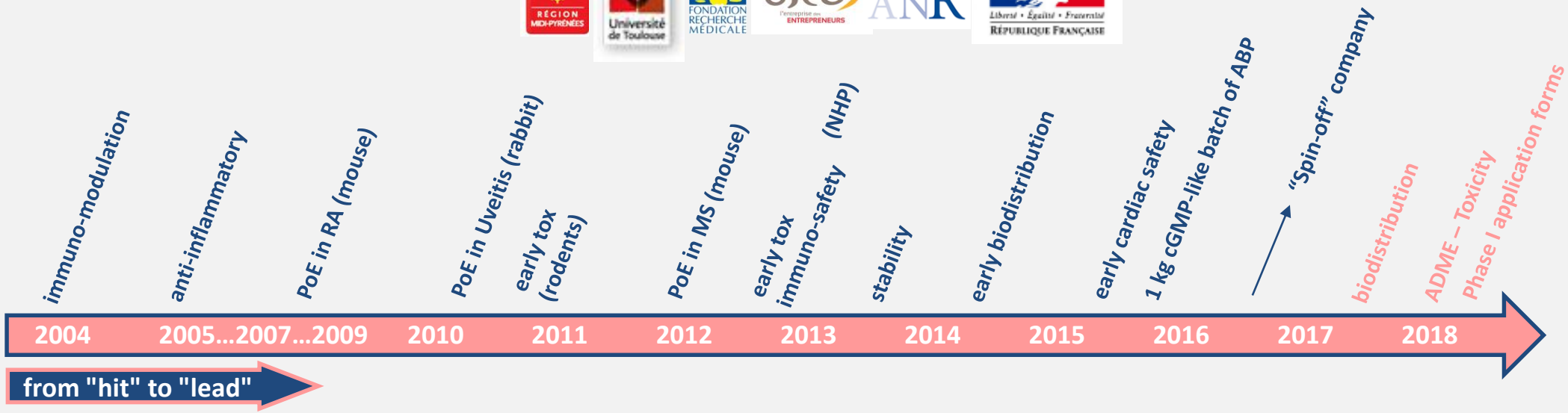
http://www.imd-pharma.com

Founders:

Serge Calet (CEO)
+33 642 828 003
serge.calet@imd-pharma.com

Rémy Poupot
remy.poupot@imd-pharma.com
+33 661 251 899

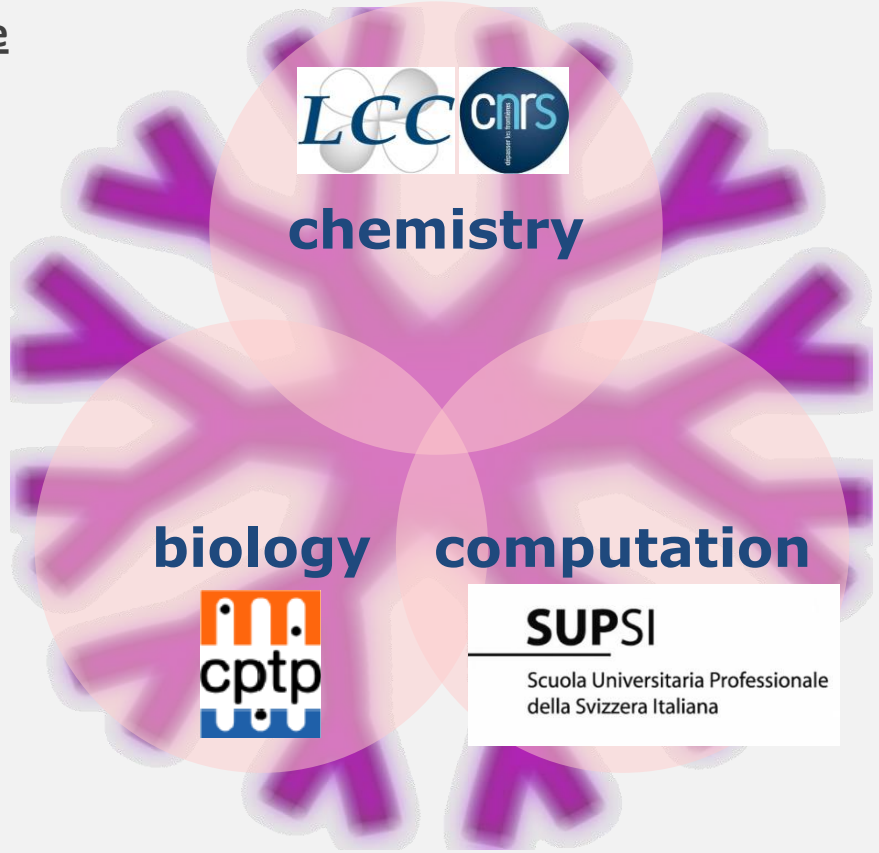
Cédric-Olivier Turrin
cedric-olivier.turrin@imd-pharma.com
+33 648 168 539



Working in synergy on frontiers

Cédric-Olivier Turrin / Anne-Marie Caminade
L. Griffe, O. Rolland, A. Ouali, A. Maraval, P. Marchand

Laboratoire de Chimie de Coordination
CNRS UPR8241, Toulouse



Rémy Poupot
Séverine Fruchon, Myriam Hayder

M. Poupot, D. Portevin, Y. Degboé,
J.-L. Davignon, J. Ledall, Annie Behar,
C. Goursat, R. Jebbawi, N. Beton

Roland Liblau / Abdel Saoudi

Centre de Physiopathologie de Toulouse Purpan
INSERM U1043, Toulouse

Giovanni M. Pavan
M. Garzoni, D. Bochicchio

Department of Innovative Technologies
SUPSI, Lugano