



Requiem for Avogadro: recent advances from laboratory research

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Introduction: about the title



**“Requiem aeternam dona eis, Domine,
et lux perpetua luceat eis.”**

(Grant them eternal rest, O Lord,
and may perpetual light shine on them)



St Stephen's cathedral 31 october 2014





Introduction: about the title



**“Requiem aeternam dona eis, Domine,
et lux perpetua luceat eis.”**

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St Stephen's cathedral 31 october 2014

The title I chose is a tribute to Avogadro – an Italian scientist – but especially to this wonderful city, that I consider the cultural capital of Europe.

The last time I came to Vienna was a couple of years ago, following the kind invitation from Michael Frass. After my lecture to medical students I had the opportunity to attend a performance of the Requiem Mass by Mozart in the cathedral.

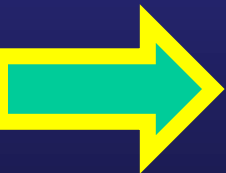
So, this is the right place to connect Mozart requiem and a famous Italian scientist whose name is often linked to homeopathic issues.

My general intention is to demonstrate how and why Avogadro was a great scientist but now he can rest in peace, without his work being used to attack homeopathy





Requiem for Avogadro: recent advances from laboratory research



- 1) Avogadro's *PRINCIPLE*
- 2) Avogadro-Loschmidt's *NUMBER*
- 3) What *EXPERIENCE* tells us



HOMEOPATHIC DRUGS «EXPLAINED» IN TV



Declarations of the Italian most famous and influential pharmacologist at «Ballarò» 26 May 2016

- «Why they don't contain anything?
 - «Because it depends on the preparation procedure:
 - «1 cubic centimeter of that substance...
 - «You shake, for magical reasons...
-
- «You then take another cubic centimeter and put in 99 ccs of water and so on...
 - «At the end, chemistry tells us that according to the principle of Avogadro, there is no longer even a molecule of that product»



Who was Avogadro?

- Amedeo Avogadro graduated in law at the age of 31.
- Soon after, he dedicated himself to physics and mathematics.
- In 1811, he published an article which contains his major contribution to physics.
- He worked as professor at Turin University
- He married Felicita Mazzé and had six children
- His hypothesis was rejected by contemporary scientists and was only recognized after his death.



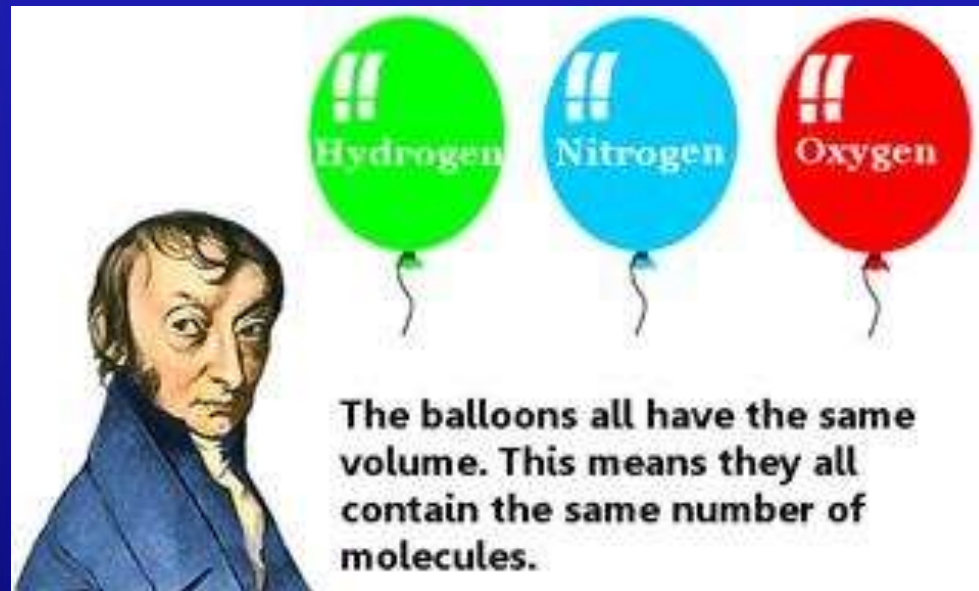
Amedeo Avogadro
1776-1856
(Turin, I)



Avogadro's PRINCIPLE

“Equal volumes of *all* gases, at the same temperature and pressure, have the same number of molecules”.

$$V/n=k$$

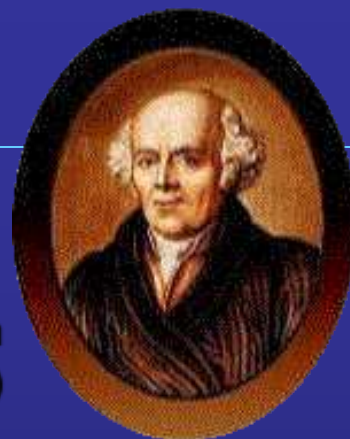


It is important to note that: *THIS PRINCIPLE CONCERNED ONLY GASES.
MOREOVER, THAT HE FORMULATED A PHYSICS HYPOTHESIS BUT DIDN'T
CALCULATE THE NUMBER OF MOLECULES*



A. Avogadro
(1776 – 1856)

Avogadro's principle and Homeopathy



C.F.S. Hahnemann
(1755-1843)

(1)

**AVOGADRO'S «PRINCIPLE»
IS CORRECT
BUT IT HAS NOTHING TO DO WITH
HOMEOPATHIC DRUGS**

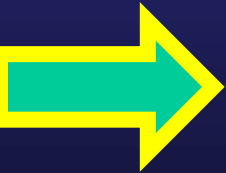
...Requiescat in pace





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Avogadro-Loschmidt's **NUMBER**

The number of molecules in one Mole of substance (es. 12 grams of Carbon) was established in 1865 by Loschmidt and confirmed in 1909 by Perrin

It is approximately 6.022×10^{23}

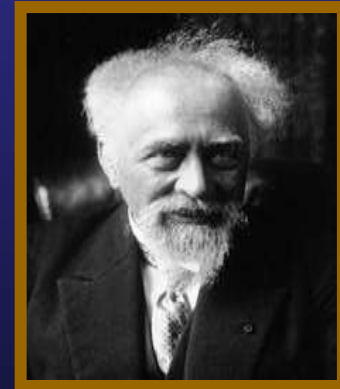
This number – or «constant» - was then named after **Avogadro and Loschmidt** in their honor

Amedeo Avogadro
1776-1856

(Incidentally, Josef Loschmidt was an Austrian high school teacher who later became a professor at the University of Vienna!)



Johann Josef Loschmidt
1821-1895



Jean Baptiste Perrin
1870 –1942

Possibly in Italy only the name of Avogadro is known, but to be honest we must name also Josef Loschmidt!





A. Avogadro
(1776 – 1856)

Avogadro-Loschmidt's number and Homeopathy



C.F.S. Hahnemann
(1755-1843)

If 1 Mole of pure and concentrated substance
= 6.022×10^{23} molecules

Then, a dilution beyond 10^{24} times (24 X or 12CH) = «Zero molecules»

Since many MT are not pure and concentrated substances but often contain low doses (10^{-3} , 10^{-4} Mol) of active principles:

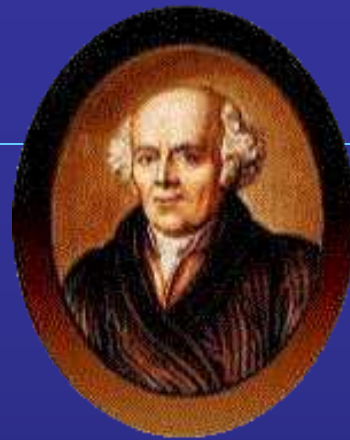
✓ 10^{-20} Mol/L (~potency of 20 X or 10 CH)
corresponds to the molecular limit





A. Avogadro
(1776 – 1856)

Avogadro-Loschmidt's number and Homeopathy



C.F.S. Hahnemann
(1755-1843)

The idea of extreme dilution has become a good opportunity to mock homeopathy. A major example of these vitriolic attacks is Wikipedia, as in this quotation by a so-called “Homeopathy Tutorial” (sic) for university students



«Reference» from Wikipedia chapter on Homeopathy

Faziola L, “Homeopathy Tutorial” (sic)
(Creighton University School of Medicine)

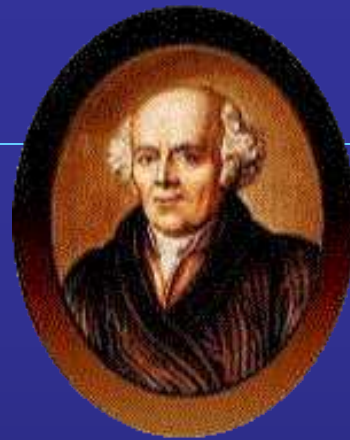
“...For example, a 30C preparation (most preferred by Hahnemann) is a dilution equal to $1:10^{60}$ is equivalent to one molecule diluted in a sphere the size of the orbit of Neptune!”





A. Avogadro
(1776 – 1856)

Avogadro-Loschmidt's number and Homeopathy



C.F.S. Hahnemann
(1755-1843)

Wikipedia is extremely biased against homeopathy, reporting only negative results ignoring positive ones and recent advances in basic research.

Homeopathy is defined as a
“pseudoscience”.

Well, if homeopathy is a “pseudoscience”, it means that Wikipedia is a
“pseudoencyclopedia”!



A POPULAR HOMEOPATHIC 6X



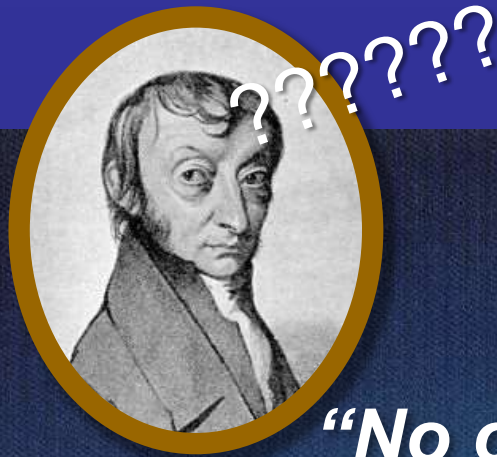
- ✓ Arnica extract (MT) contains 0.36 grams/L of sesquiterpene active principles
→ → → 6x potency = 3.6×10^{-7} grams/L
= 3.6×10^{-10} grams/ml
- ✓ Since sesquiterpenes MW= 340 Da,
= about 10^{24} molecules/340 grams
= about 2.9×10^{21} molecules/gram
- ✓ = 2.9×10^{21} molecules/gram x (3.6×10^{-10} grams/ml) = **10^{12} molecules/ml**
- ✓ Assuming that a minimum of 1 ml 6X is used in 500 tablets, we have a minimum of 2×10^9 (**2 BILLION**) molecules in a tablet



A POPULAR HOMEOPATHIC 6X

- Contains at least 2 BILLIONS active molecules in a tablet
- → THE LIMIT OF AVOGADRO-LOSCHMIDT **HAS NOTHING TO DO** WITH HOMEOPATHIC LOW POTENCIES
- → FURTHERMORE, HOMEODRUGS ARE NOT ONLY DILUTED BUT ALSO **TRITURATED AND SUCCESSED**





HOWEVER: ARE HOMEOPATHIC DRUGS SIMPLY DILUTED MOLECULES?

“No one really understands water.

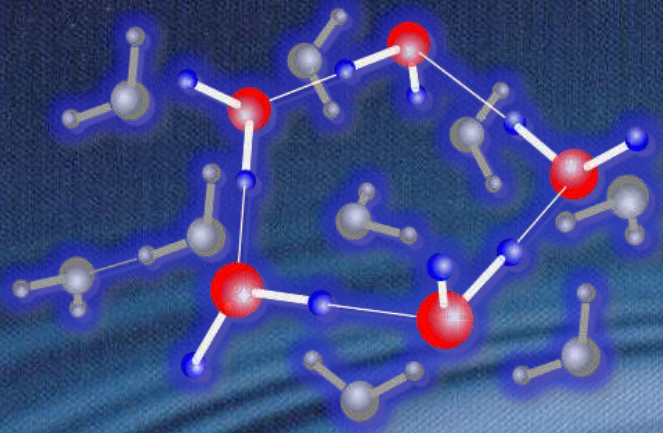
It’s embarrassing to admit it, but the stuff that covers two-thirds of our planet is still a mystery.”

Ball P. “**Water-an enduring mystery**”.
Nature 2008 Mar 20; 452(7185):291-2.

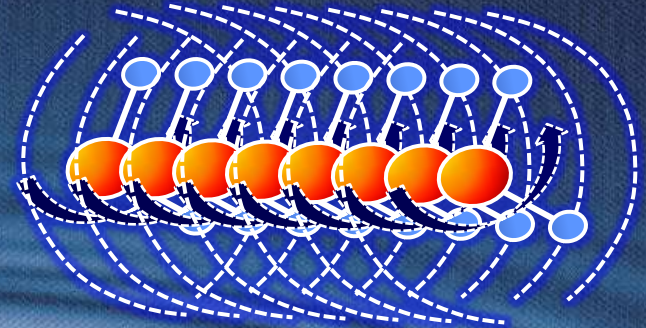
I can’t enter into this problem of water physics, there is no time and it’s too difficult. In connection with our problems, however, it is important to state that pure water does not exist: On the contrary, evidence is emerging that water is heterogeneous liquid at the nanoscale level: This heterogeneity can be represented according three major concepts, or working models as in the next slide.



AND... WATER IS NOT ONLY “FRESH WATER”



Hydrogen-bonded
clusters (clusters)



Coherence domains (CD)
Superradiance



Nanoparticles,
nanobubbles,
aggregates

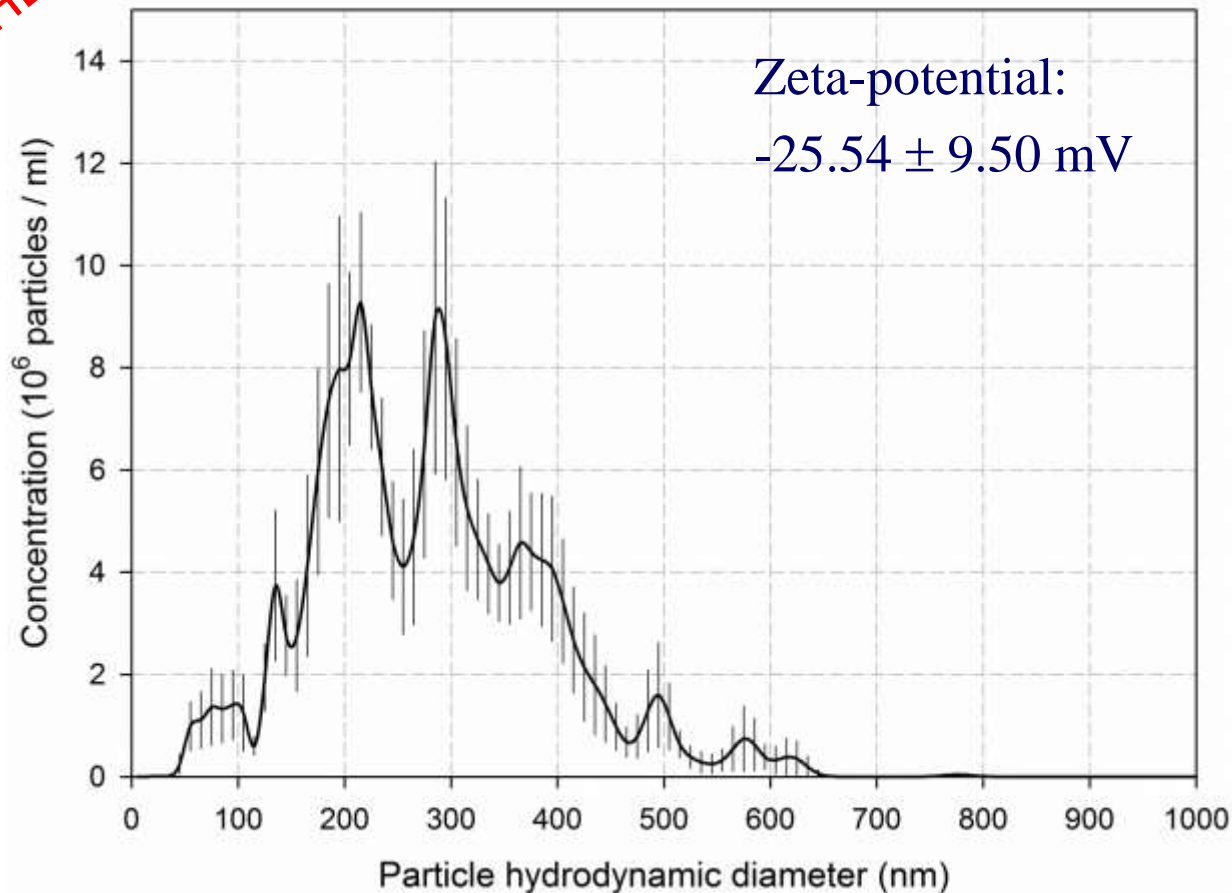
<http://www.ncbi.nlm.nih.gov/pubmed/24439452>

<http://www.ncbi.nlm.nih.gov/pubmed/24439453>



Homeopathic drugs are not «pure» solutions

PlosONE
JUST PUBLISHED!

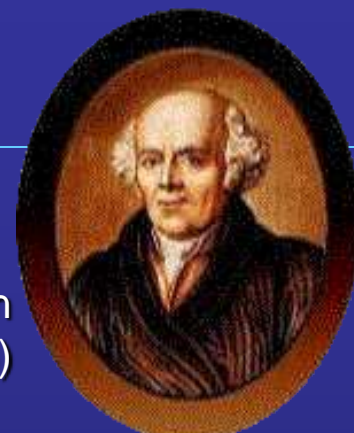


**Nanoparticle spectrum of *Arnica m. 1c* used in experiments with macrophages
(Marzotto et al. PlosONE 2016)**



A. Avogadro
(1776 – 1856)

Avogadro-Loschmidt number and Homeopathy



C.F.S. Hahnemann
(1755-1843)

(2)

**AVOGADRO-LOSCHMIDT'S NUMBER
IS CORRECT
BUT:**

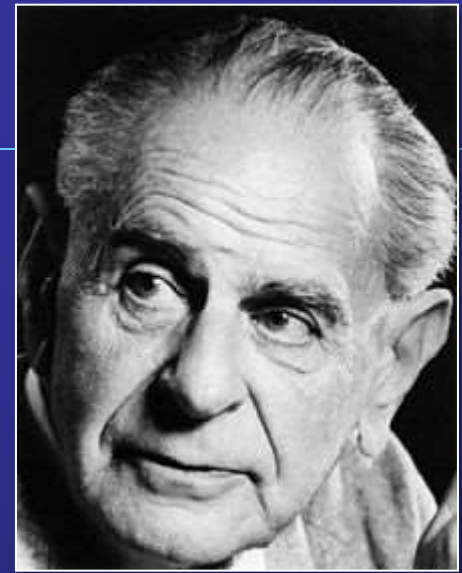
- 1) IT HAS NOTHING TO DO WITH
LOW- POTENCY HOMEODRUGS
- 2) IT ASSUMES HOMOGENEOUS WATER
«SOLUTIONS», BUT THIS IS NOT THE CASE

...Requiescat in pace



Theory versus Experience

*“Whenever a theory appears to you
as the only possible one,
take this as a sign that you have
neither understood the theory
nor the problem which it was
intended to solve.”*



Karl Popper
(1902 –1994)

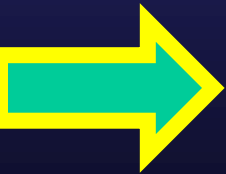
K. Popper, Objective Knowledge: An Evolutionary Approach (1972)



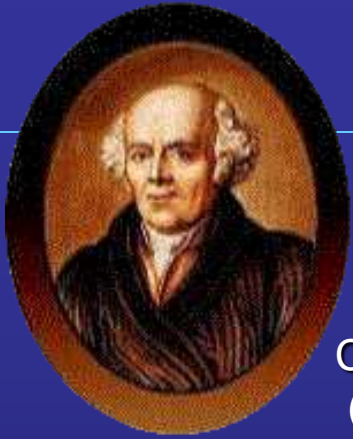


Requiem for Avogadro: recent advances from laboratory research

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HOW SMALL MUST THE DOSE BE?



C.F.S. Hahnemann
(1755-1843)

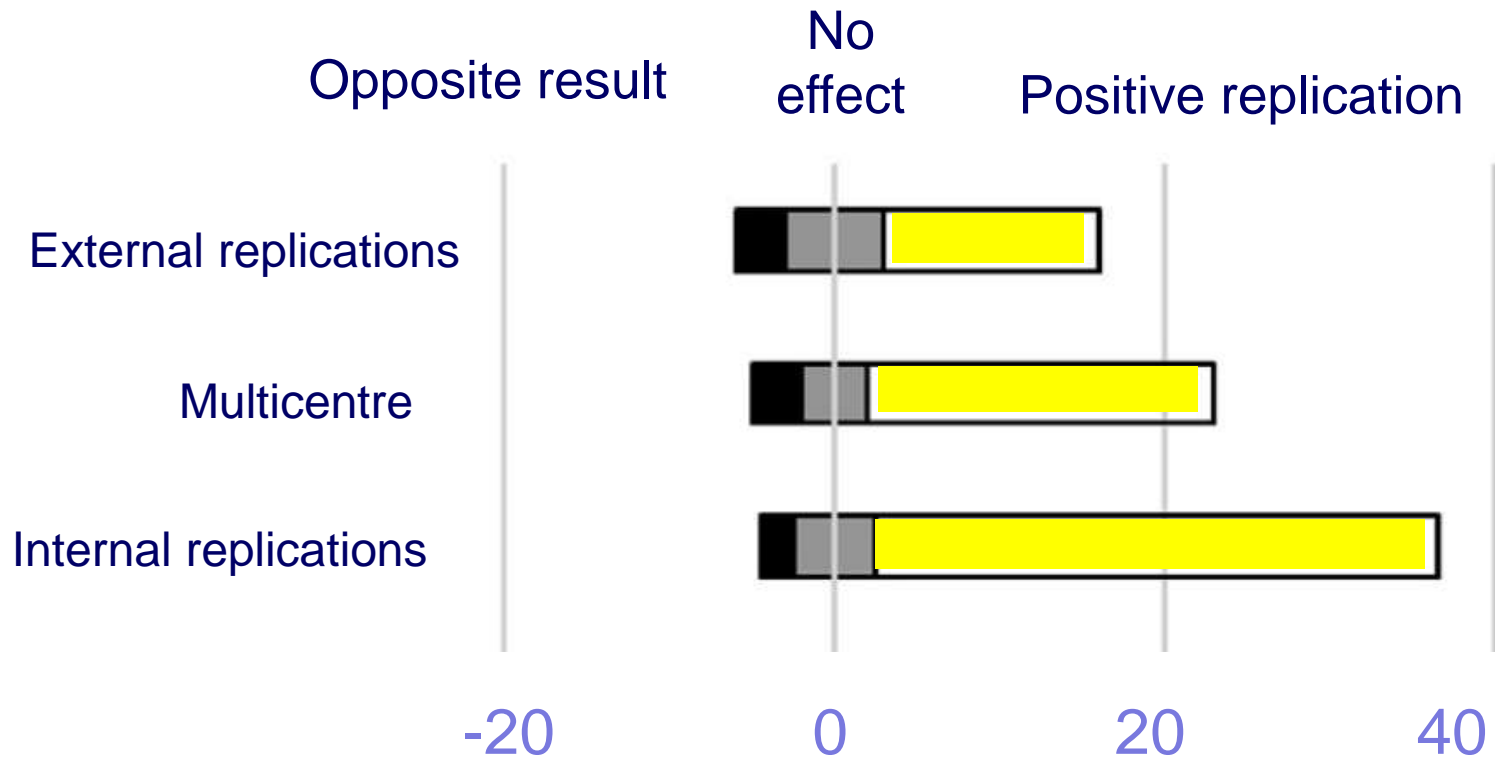
“How small, in other words, must be the dose of each individual medicine, to effect the best cure?”

“This is not the work of theoretical speculation. (...) Pure experiment, careful observation of the sensitiveness of each patient, and accurate experience can alone determine this in each individual case”

Organon, par. 278



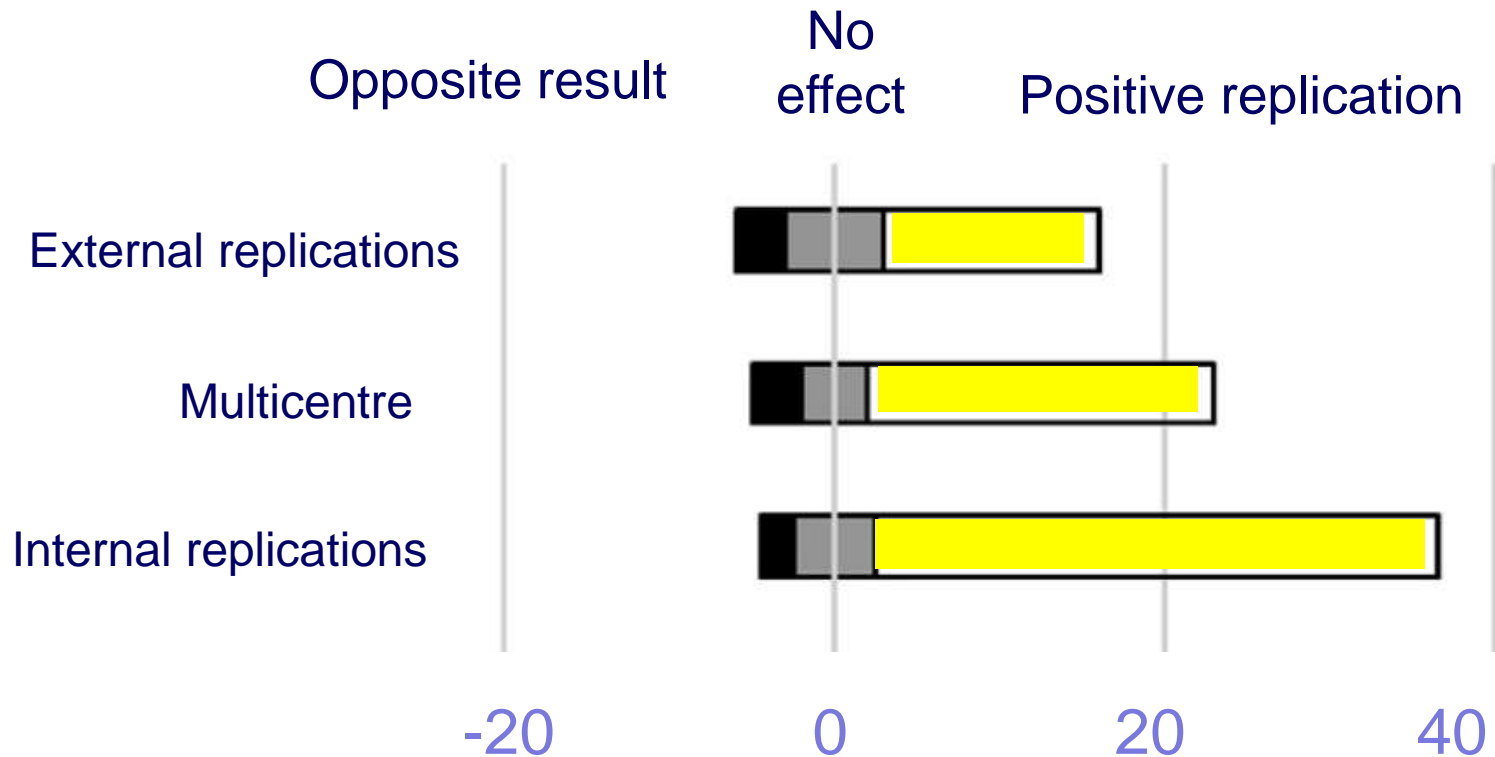
Results of models of basic research submitted to replication (98 studies until 2015)



Replications of fundamental research models in ultra high dilutions 1994 and 2015
update on a bibliometric study

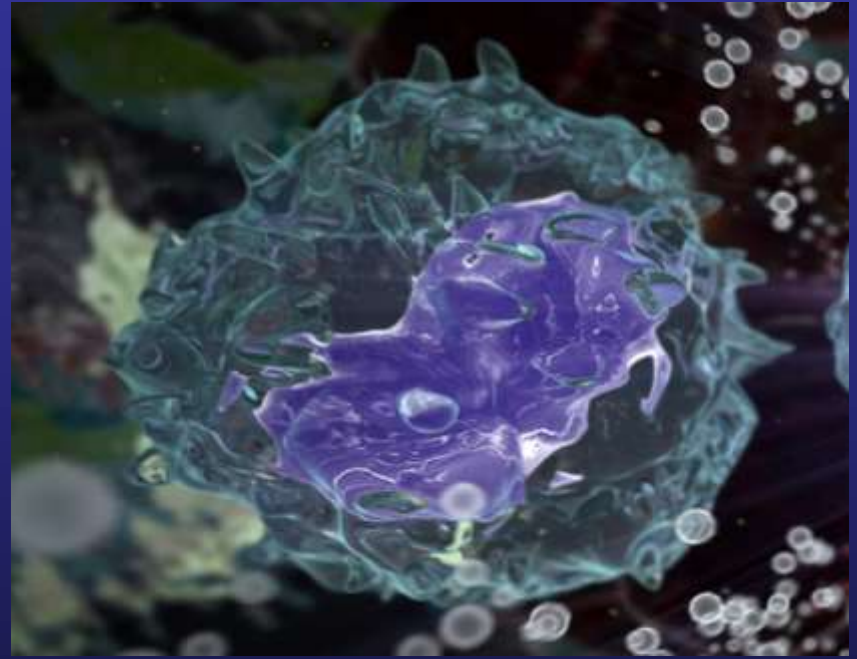
P. C. Endler et al. Homeopathy (2015) 104, 234e245

Results of models of basic research submitted to replication (98 studies until 2015)



“The null-hypothesis (dominance of zero results) should be rejected.”
“We encourage further replications of studies in order to learn more about the model systems used.”

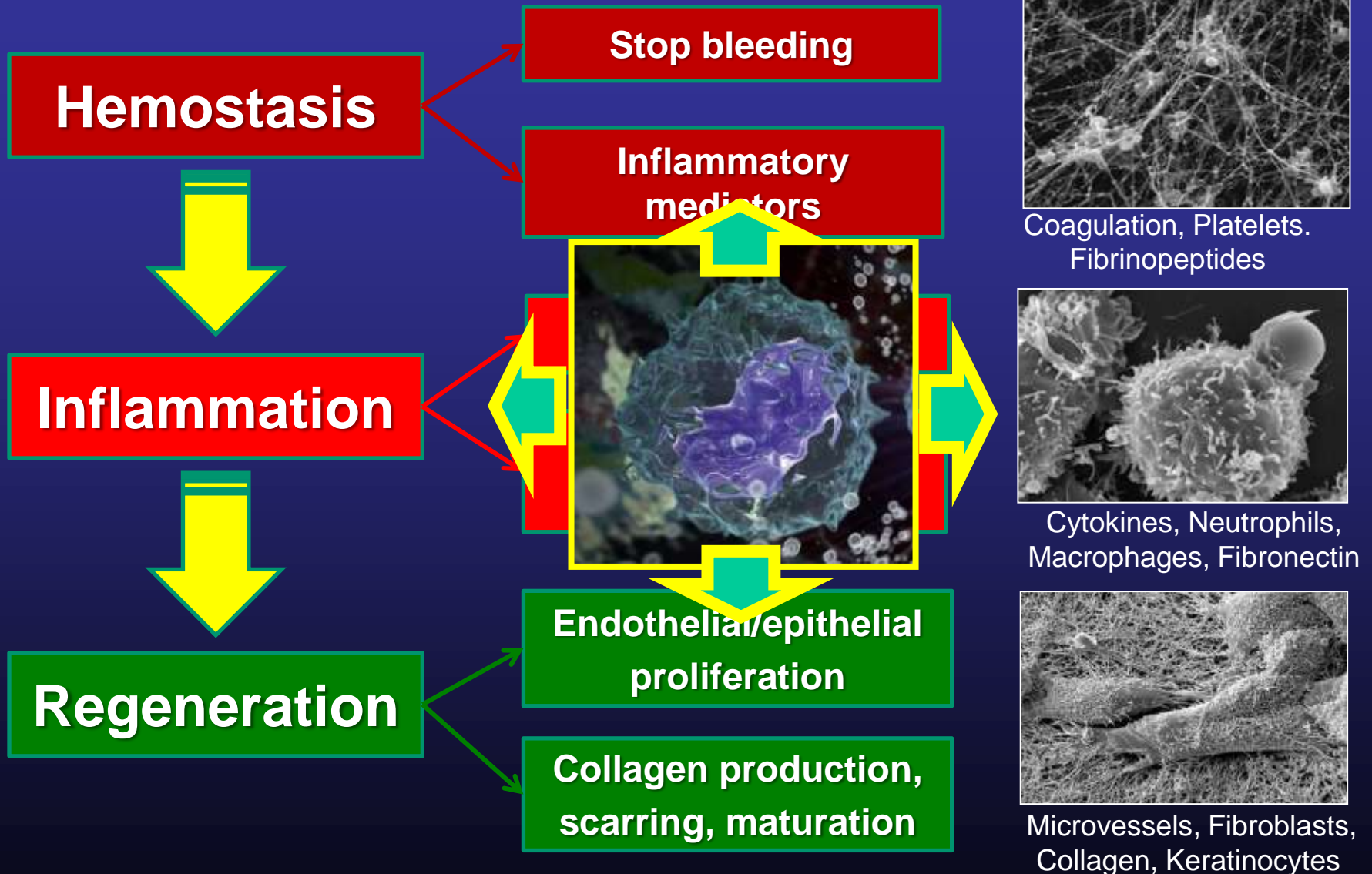
Evaluation of *Arnica montana* dilutions on gene expression in a human macrophage cell line



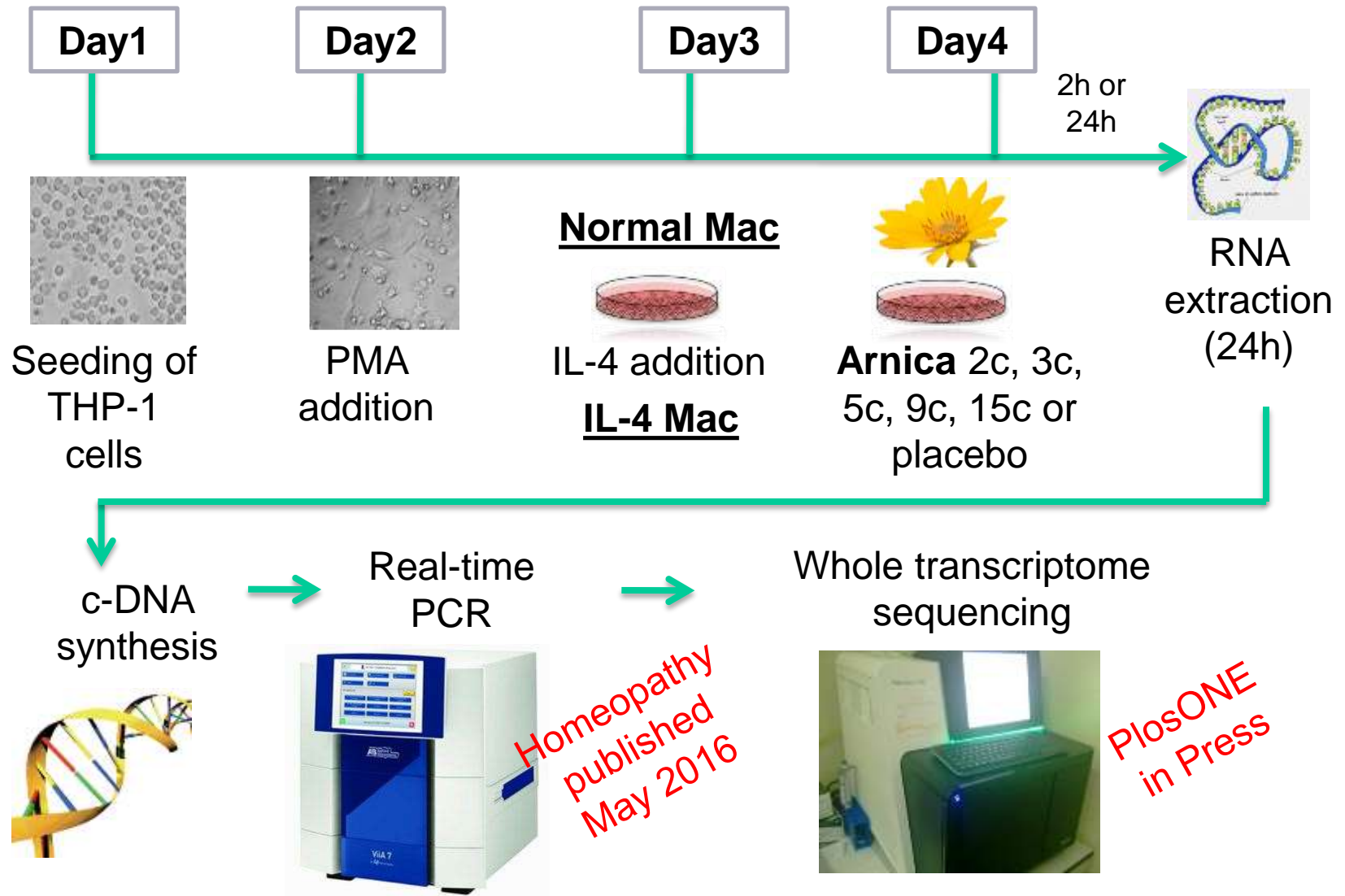
Macrophages have a central role in inflammatory processes, immunity and even in wound healing in all tissues.

In our laboratory we cultivate human cell lines that can be considered as good models for this type of research since they are very stable and can be differentiated in various types. For example if we add interleukin4 to these cells, they differentiate into a type that is called macrophage of “wound-healing” type.

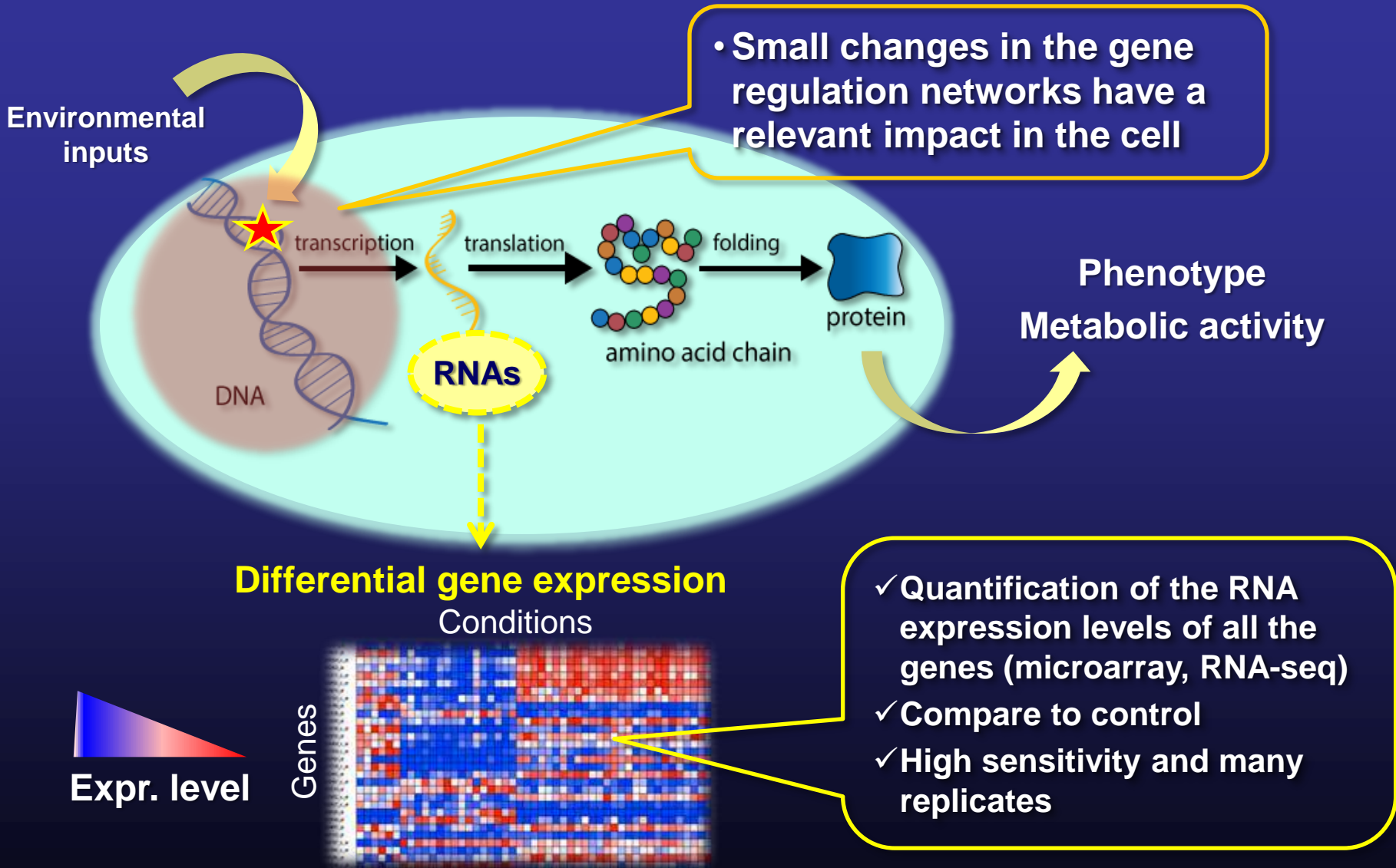
Central role of the Macrophage in wound healing



Macrophages treatment with *Arnica m.* and gene expression (Verona group 2015-2016)

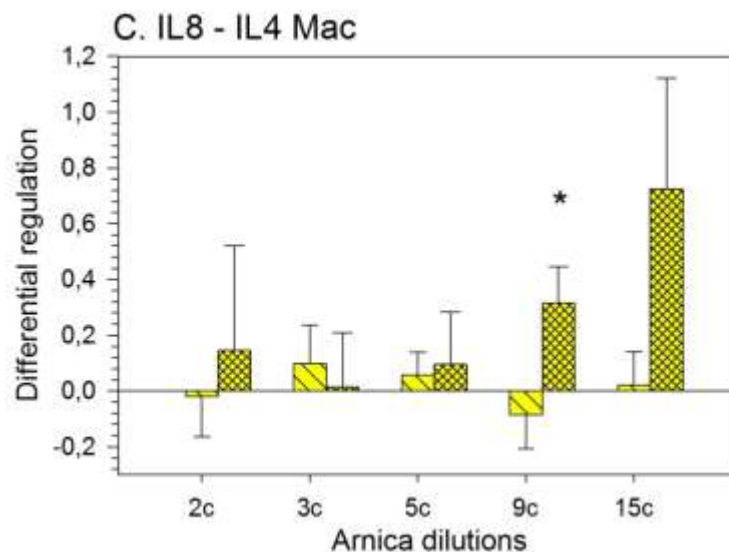
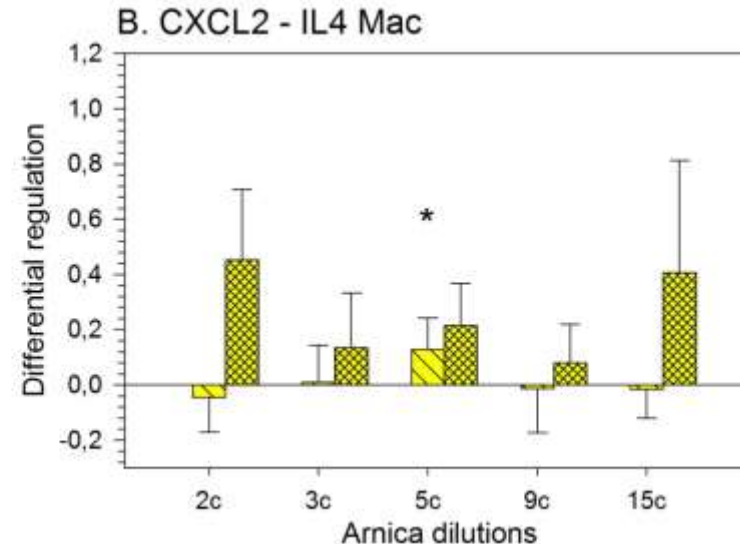
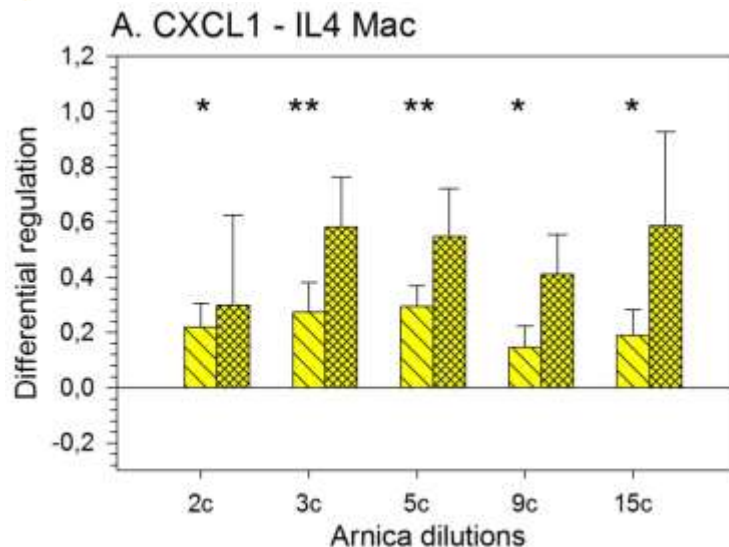


Differential gene expression analysis with high throughput technologies





Arnica m. effects on Macrophages (Oliosio et al., RT-PCR study)



 2h treatment
  24h treatment

Arnica m. effects on IL-4 Macrophages (RT-PCR study)

This is the main result of the study with RT-PCR that was published in Homeopathy

One of the most striking and unexpected effects of *Arnica m.* on our macrophages was the increase of chemokines of the CXC family (CXCL1, CXCL2, IL8). The most consistent findings concerned the CXCL1 gene whose expression was increased in all conditions. This finding suggests a new property of this plant in wound healing processes. Cytokines and chemokines promote inflammation, angiogenesis, facilitate the passage of leukocytes from circulation into the tissue, and contribute to regulation of the events involved in the healing process.





Effect of *Arnica m. 2c* on gene expression by human macrophages (Marzotto et al., RNA-seq study)

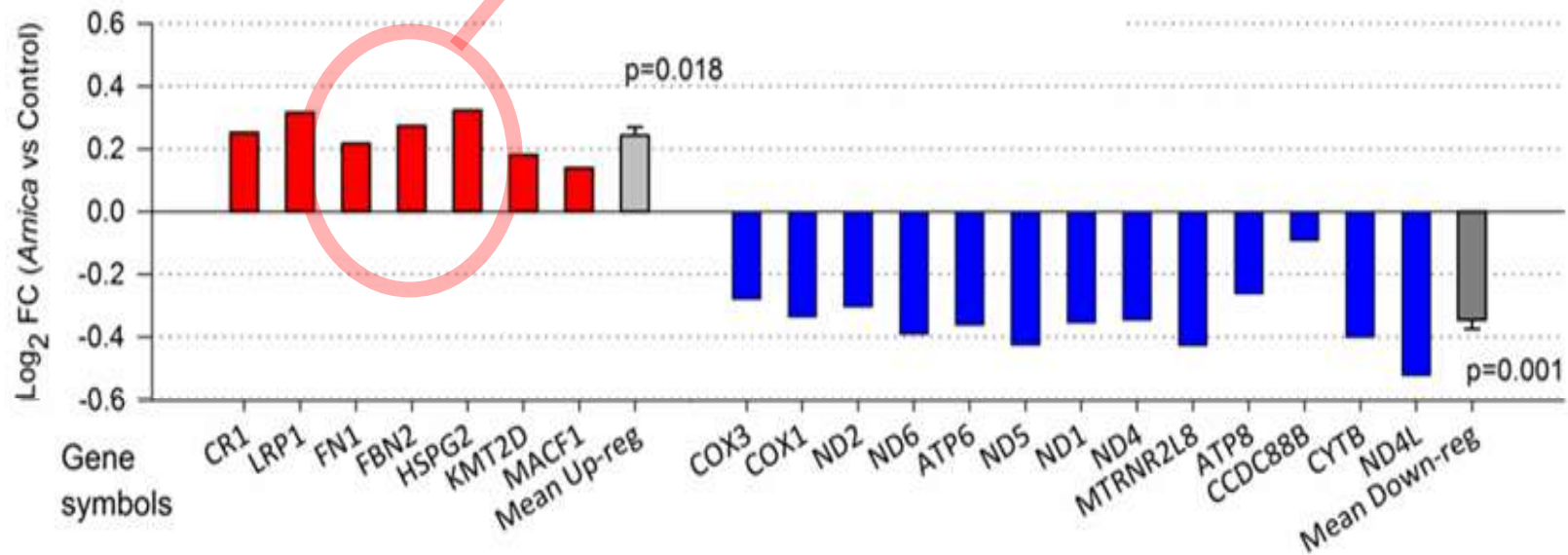


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fibronectin ($p < 0.001$)

fibrillin ($p < 0.05$)

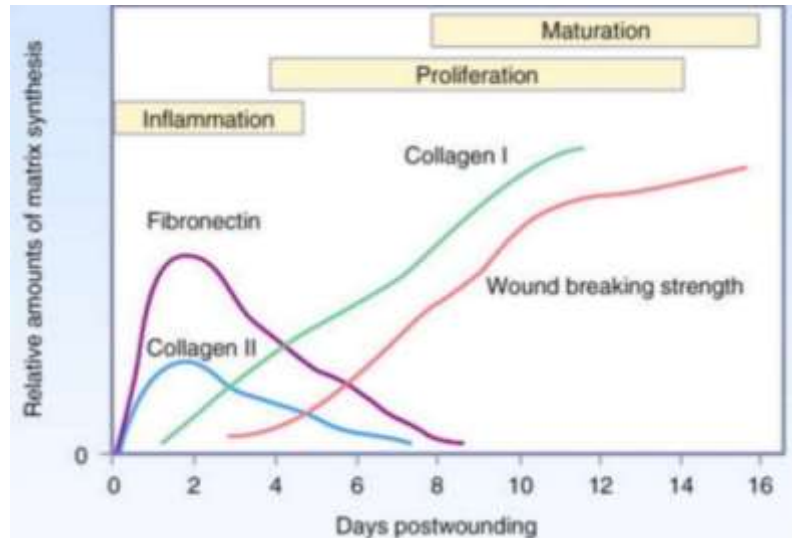
heparan sulfate proteoglycan ($p < 0.05$)



<http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0166340>



Fibronectin is the major protein that organizes the connective tissue during the first phase of wound healing and inflammation



Kinetics of protein production in connective tissue during wound healing

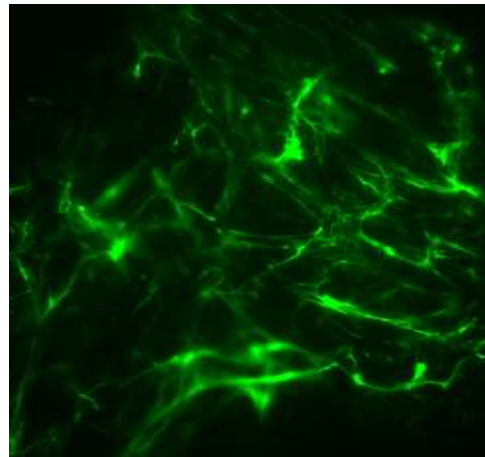
<http://www.slideshare.net/fullscreen/smileycty/wound-healing/1>

- Fibronectin has the ability to **specifically bind a large number of molecules**
- Cellular interactions with fibronectin lead to **bidirectional crosstalk** that directs **cell functions** such as:

- Proliferation
- cell migration
- survival and growth

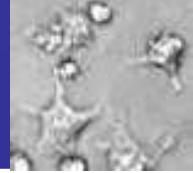
Immunofluorescence image of cells in culture

<http://www.bme.rochester.edu/research/fibronectin-matrix-remodeling.html>

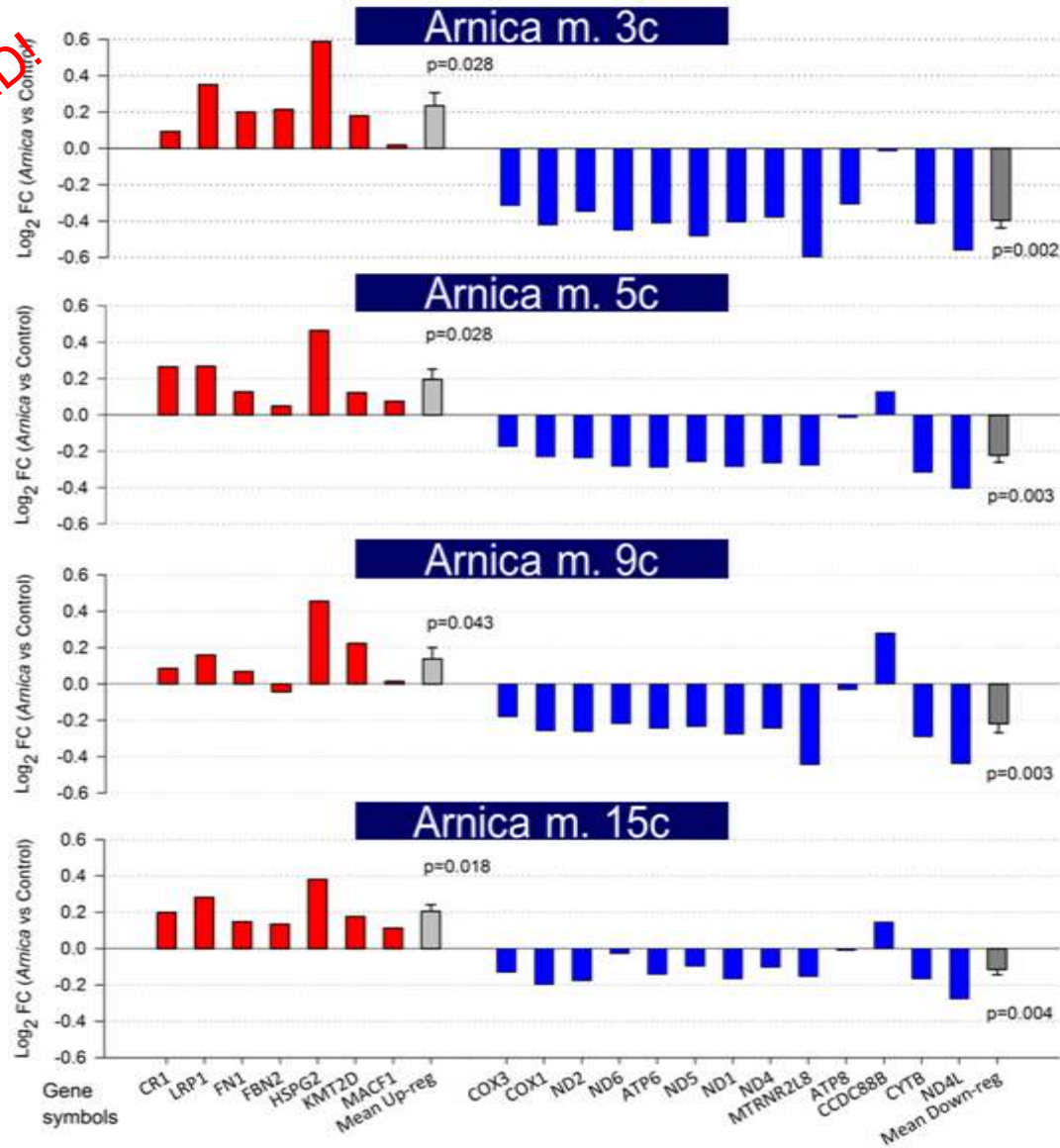
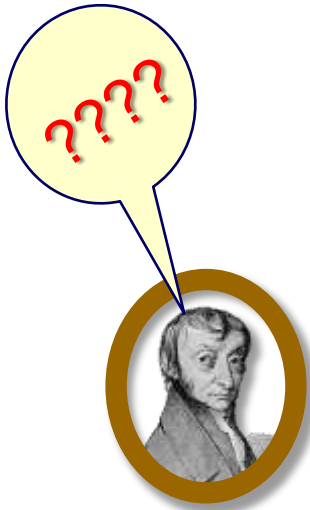




Effect of *Arnica m.* increasing dilutions on IL-4-polarized human macrophages (pool of 5 experiments)



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3c

10⁻¹⁰ Mol/L
sesquiterpene

5c

10⁻¹⁴ Mol/L
sesquiterpene

9c

10⁻²² Mol/L
sesquiterpene

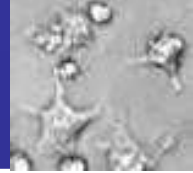
15c

10⁻³⁴ Mol/L
sesquiterpene

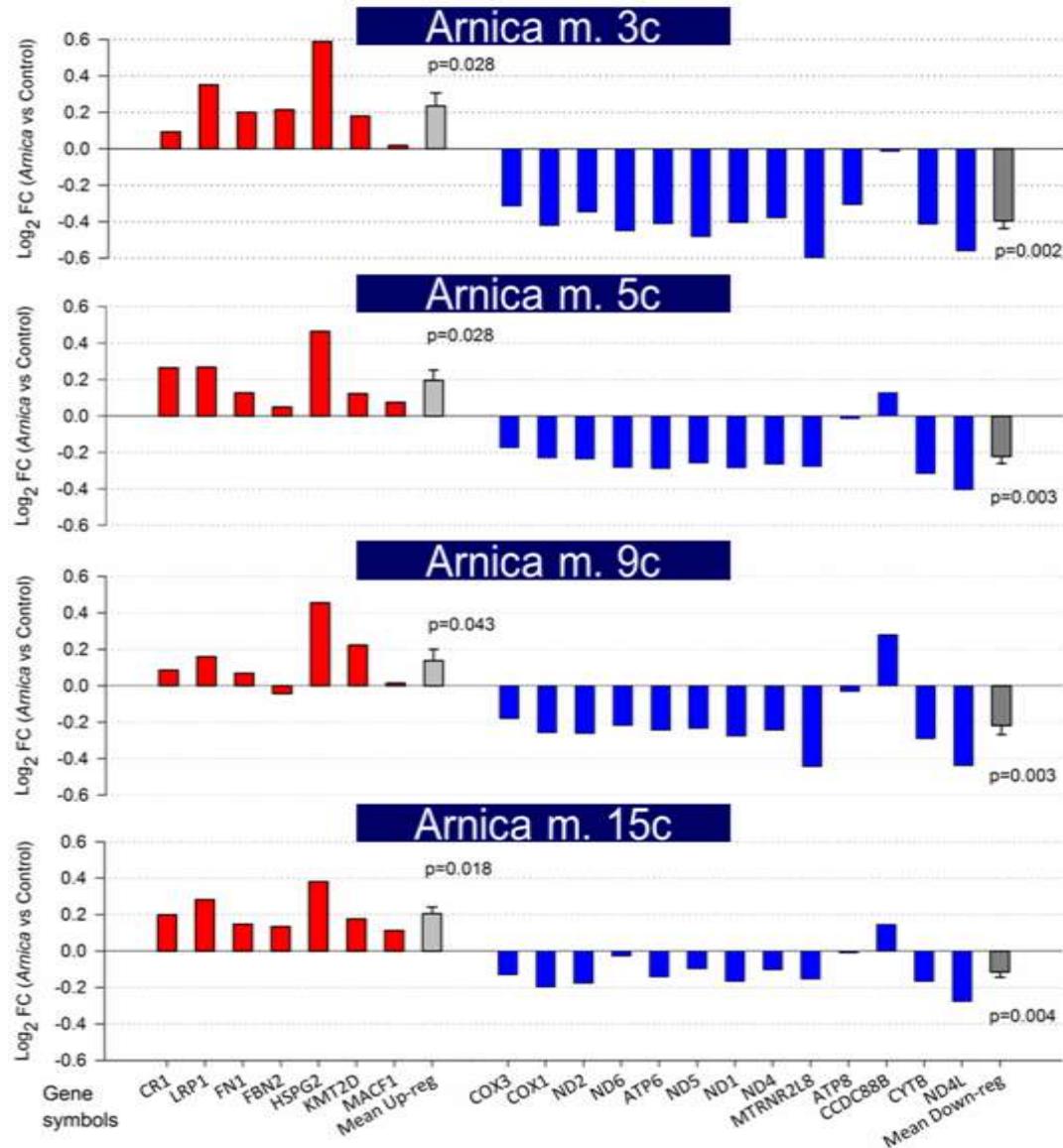




Effect of *Arnica m.* increasing dilutions on IL-4-polarized human macrophages (pool of 5 experiments)



The responses of cells to Arnica at high dilutions were in the same direction of those observed with 2c. Upregulated genes remained upregulated and downregulated remained downregulated, with few exceptions. This trend of the group of genes was always statistically significant and can't be due to chance. If the observed changes were due to chance or to noise in the cell treatments, the genes would have responded in a random way, that is some in up and other in down direction, without any order and any distinction between the two groups. I am proud to say that this paper has just been published the great Scientific Journal PlosONE, that is not a homeopathic journal.



3c

10^{-10} Mol/L
sesquiterpene

5c

10^{-14} Mol/L
sesquiterpene

9c

10^{-22} Mol/L
sesquiterpene

15c

10^{-34} Mol/L
sesquiterpene



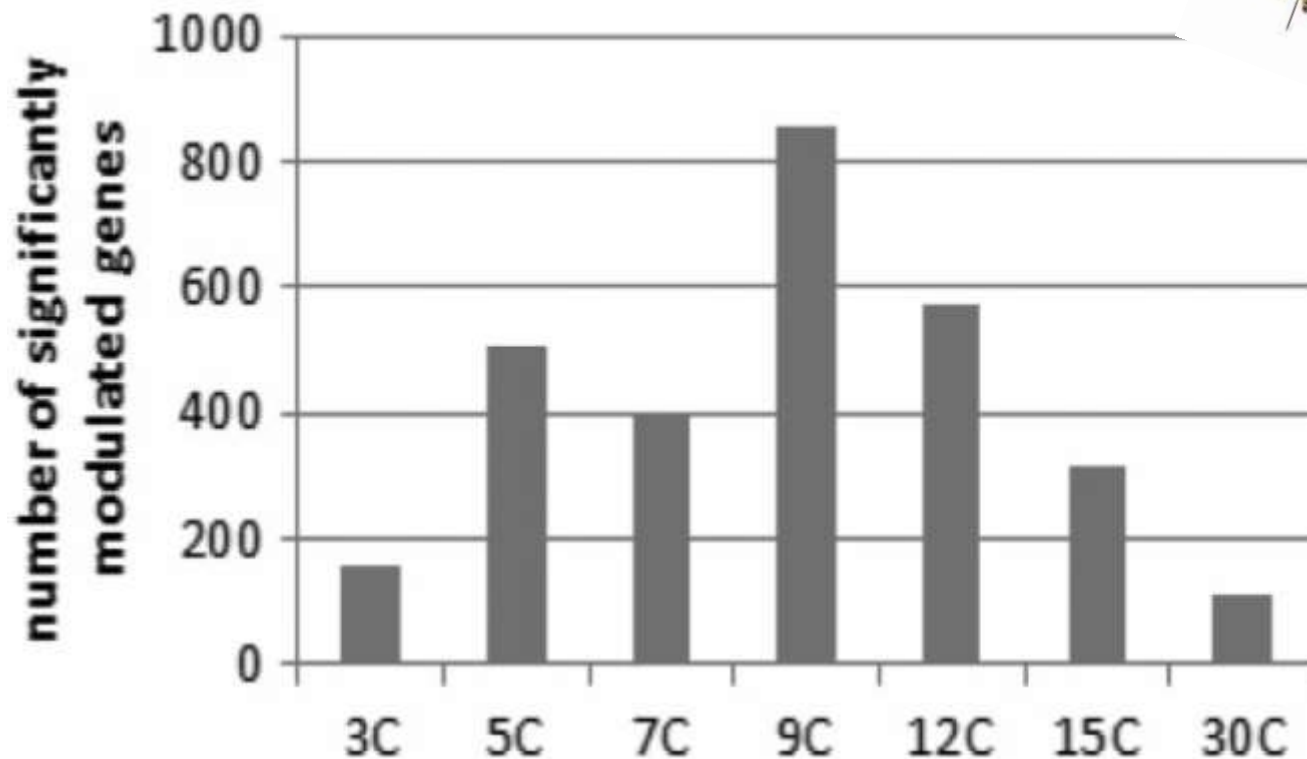


Effects of Extreme Dilutions of *Apis mellifica* Preparations on Gene Expression Profiles of Human Cells

Elisabetta Bigagli¹, Cristina Luceri¹, Andrea Dei², Simonetta Bernardini³, and Piero Dolara¹

E. Bigagli

Apis vs ethanol:water



Dose-Response:
An International Journal
January-March 2016;1-7

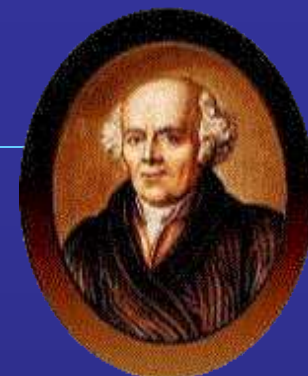
Number of genes significantly up- or downregulated comparing cells exposed to *Apis mellifica* preparations versus cells exposed to the reference ethanol:water solutions (Apis vs ethanol-water).

Reproduced with permission





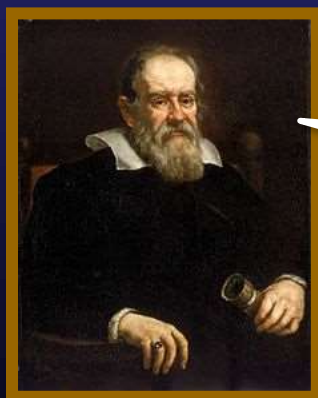
Avogadro-Loschmidt's number and Homeopathy



(3)

AVOGADRO-LOSCHMIDT'S NUMBER
IS THEORETICALLY CORRECT
BUT CAN'T BE USED TO EXPLAIN HOMEOPATHIC
PHENOMENA.

IN FACT, EXPERIMENTAL EVIDENCE SHOWS THAT
THIS LIMIT IS OVERCOME BY HOMEOPATHIC DRUGS!



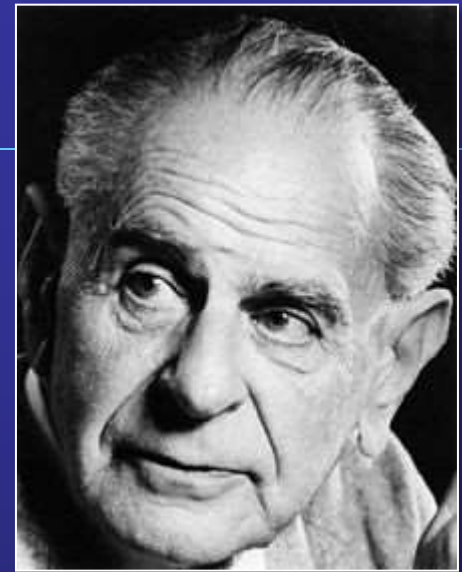
Galileo
Galilei
(1564–1642)

«Eppur si muove!»
(and yet it moves!)



The game of science

***“The game of science
is, in principle,
without end.”***

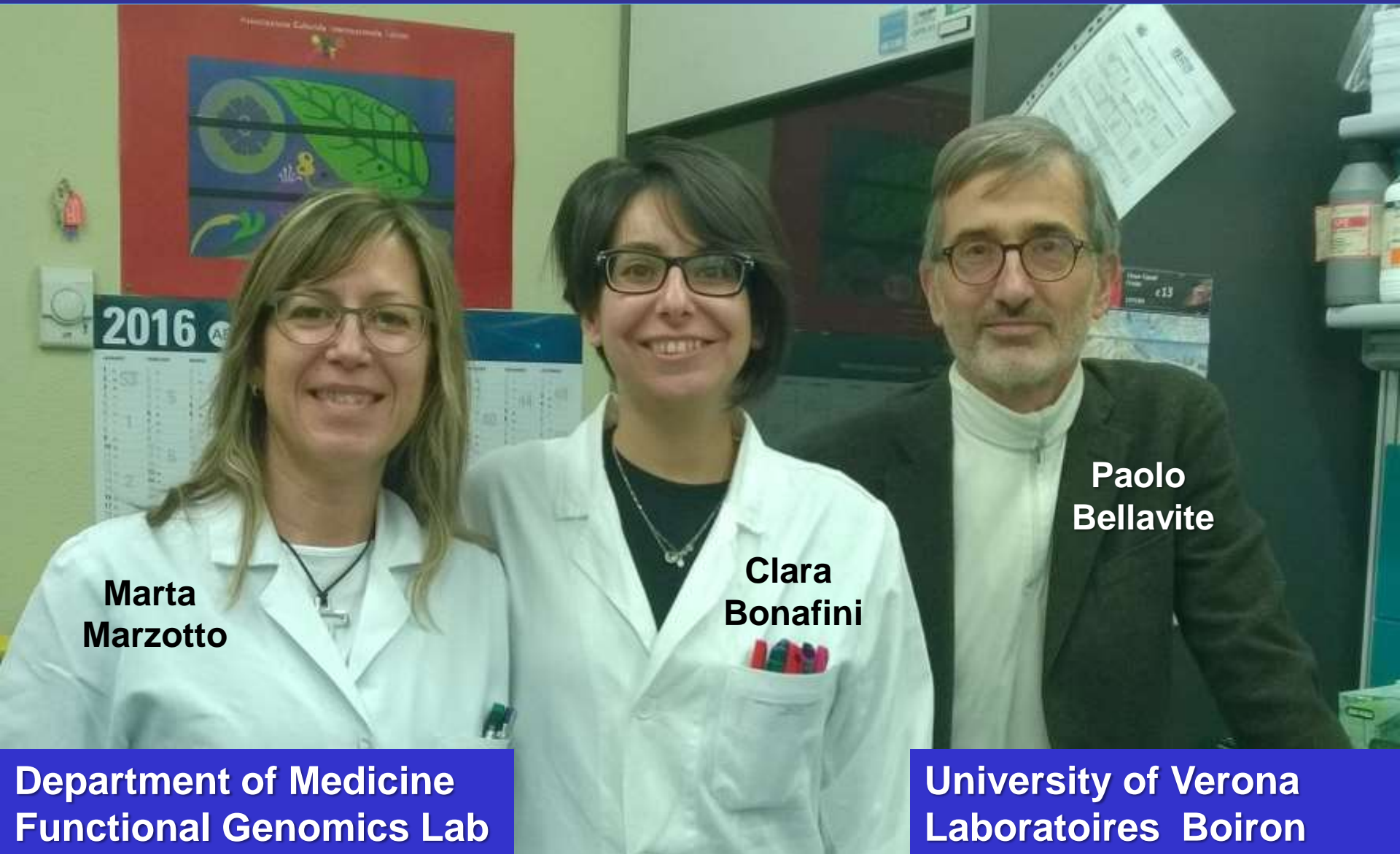


Karl Popper
(1902 –1994)

*K. Popper, Ch. 2 "On the Problem of a Theory of Scientific Method",
Section XI: Methodological Rules as Conventions*



Auf Wiedersehen!



**Marta
Marzotto**

**Clara
Bonafini**

**Paolo
Bellavite**

**Department of Medicine
Functional Genomics Lab**

**University of Verona
Laboratoires Boiron**

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