Synergy assessments of plant extracts used in the treatment of stress and ageing related disorders

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In collaboration with

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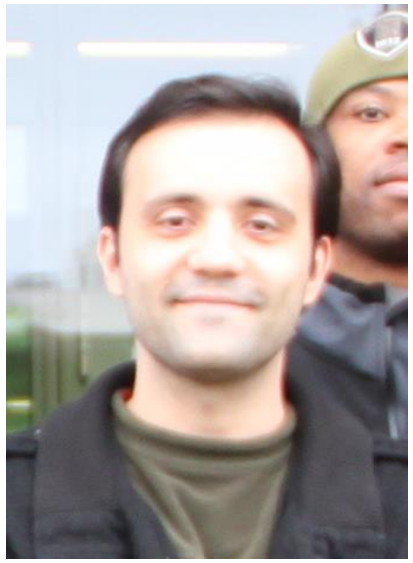
Rebecca Hamm



Onat Kadioglu

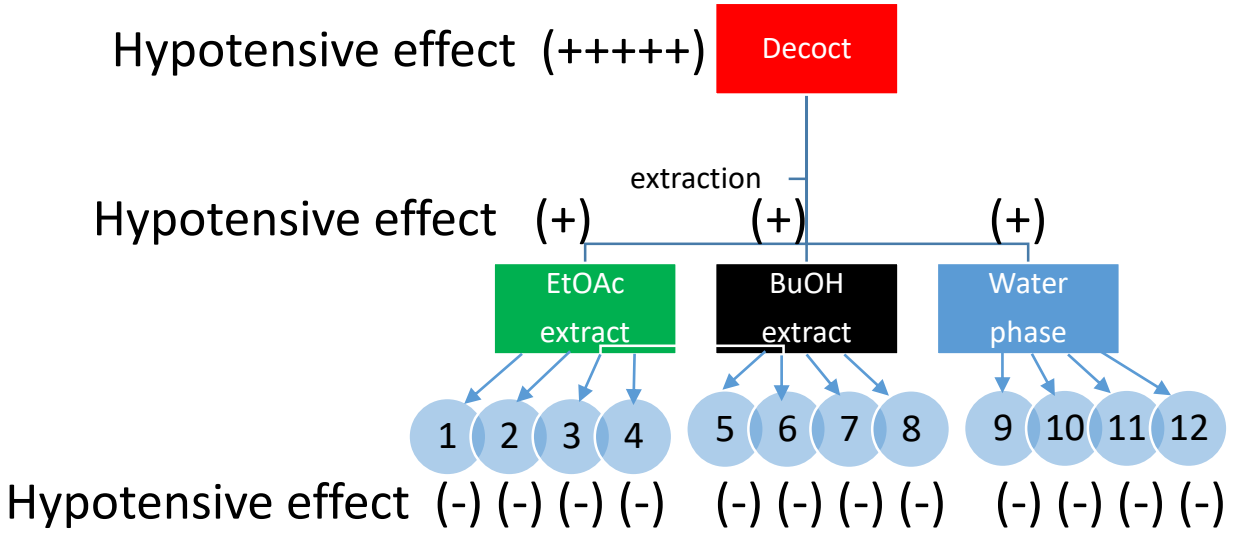
Ean-Jeong Seo

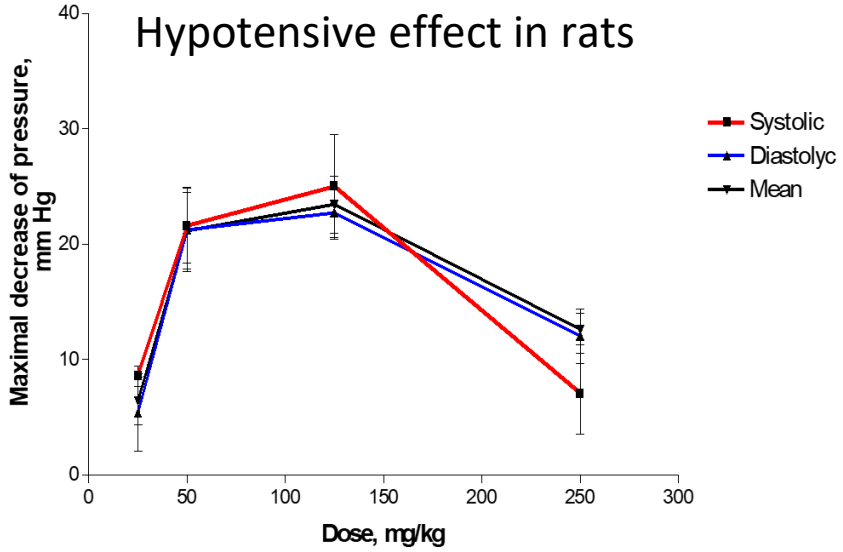
Thomas Efferth

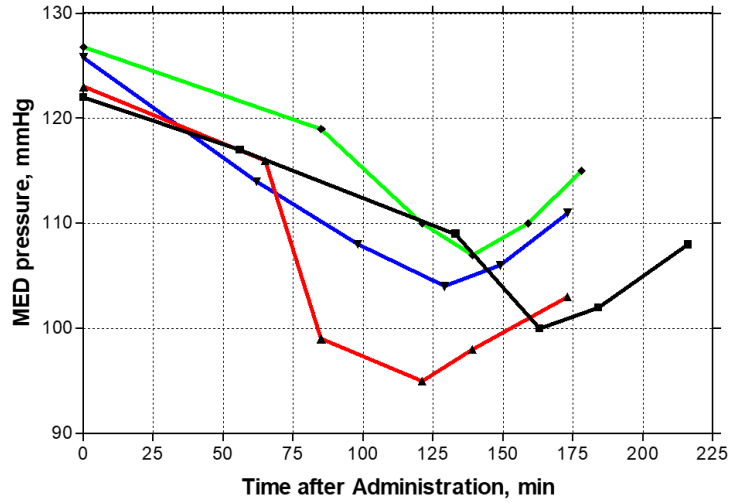
Synergistic interaction > hypotensive effect

Bioassay guided fractionation

Cychorium intybus L. herb decoct

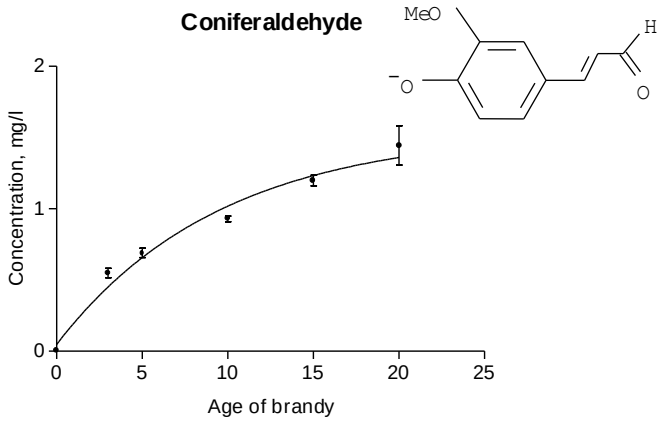


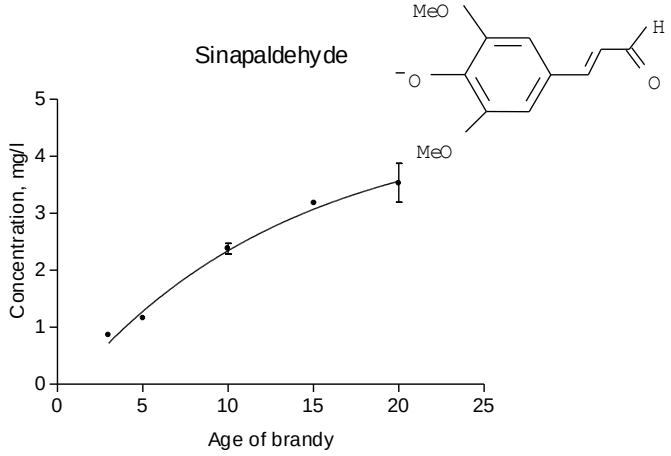


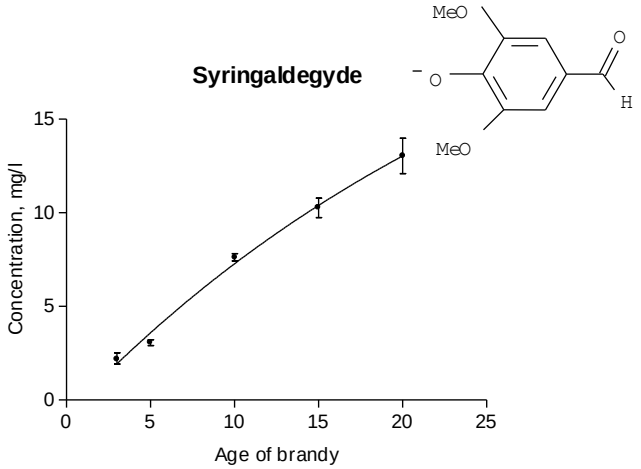


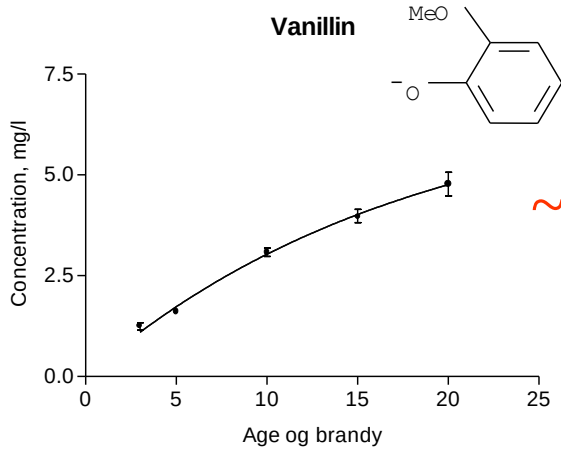
Unpublished data, 1984

Antagonistic effect – vanillin aroma









~ 1 mg

1. Panossian, G. Mamikonyan, M. Torosyan, E. Gabrielyan, S. Mkhitaryan. Analysis of Aromatic Aldehydes in Brandy and Wine by High Performance Capillary Electrophoresis. *Anal Chem*. 2001. Vol. 73, No 17. Pp.4379-4383.
2. Panossian A.G., Mamikonyan G.V., Torosyan M.A., Abrahamyan A.G., Hovanissyan A.S., Gabrielyan E.S., Grigoryants, Mkhitaryan S., Lapin B.V. Determination of phenolic aldehydes in vine and brandy by capillary electrophoresis: new markers of quality of brandy. *J. Analytical Chemistry*. 2001, v. 56, No12, pp. 11-23
3. Panossian A., Gabrielyan E., Gevorgyan Kh. Method of determination of age and quality of alcohol drinks stored in wood barrels. Patent of Armenia No 1137 A2, 2001.

Synergy and antagonism of plant extracts

What is common in the mechanisms of action of various stress response modifiers - adaptogens?

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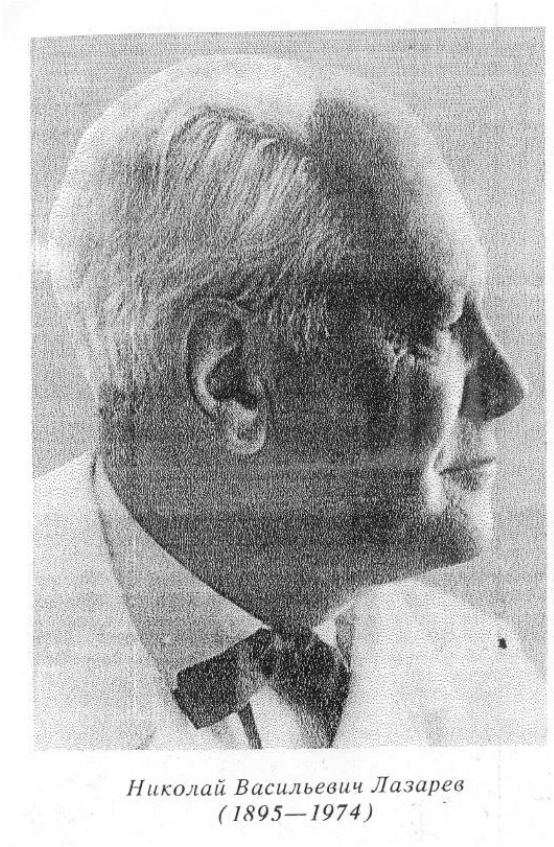
Panossian A, Hamm R, Kadioglu O, Wikman G and Efferth T . 2013. Synergy and antagonism of active constituents of ADAPT-232 on transcriptional level of metabolic regulation of isolated neuroglial cells. ***Front. Neurosci***. **7**:16. doi:10.3389/fnins.**2013.**00016

Introduction of adaptogens: historical background



Nicolay Lazarev

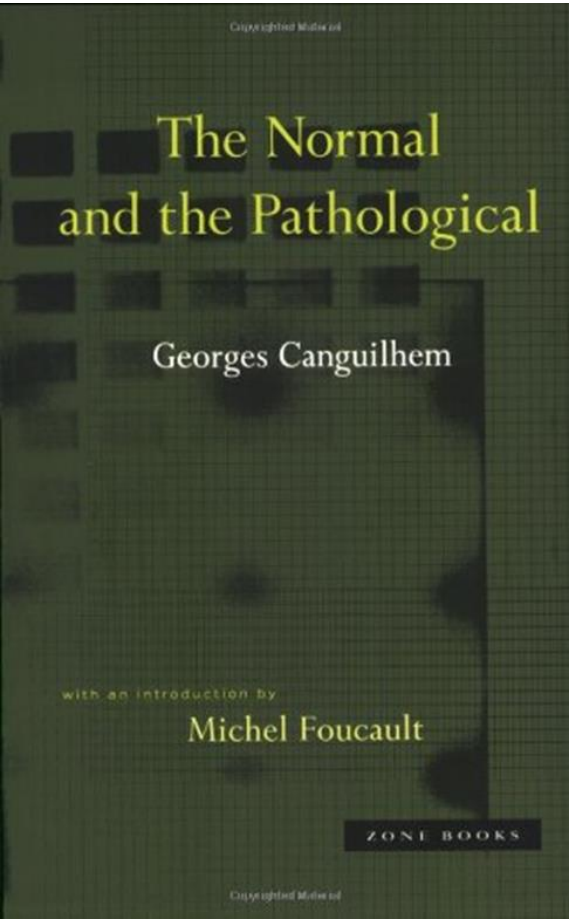
First definition of adaptogens

"Adaptogens" are compounds which increase "the state of non-specific resistance" in stress

Lazarev NV. 1958. General and specific in action of pharmacological agents. Farmacol.Toxicol, 21(3): 81-86.
Lazarev NV. Ljublina EI, Rozin MA. 1959. State of nonspecific resistance. Patol.Fiziol.Experim.Terapia, 3(4): 16-21.

Health is the ability to adapt to one's environment

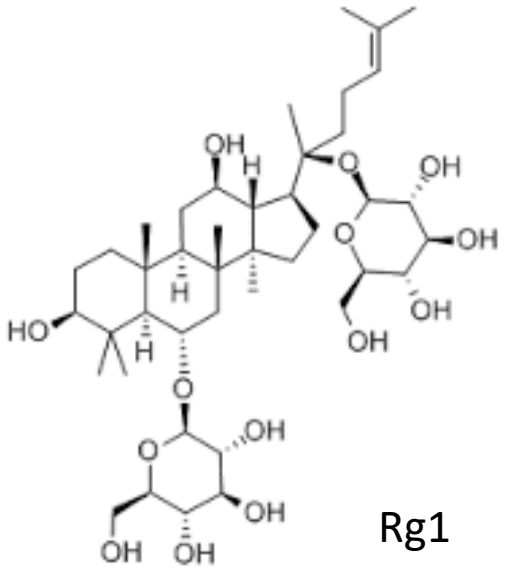
George Canguilhem
1943



Adaptability – test of health
(The Lancet, 2009: 373, 781)

Attenuation of adaptability to stress is crucial for health and survival

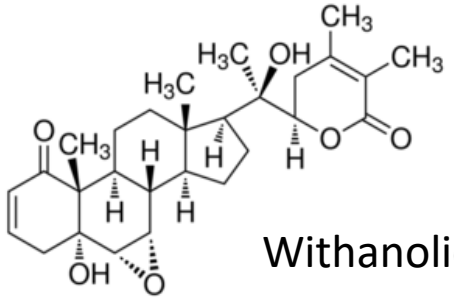
TCM and Ayurveda



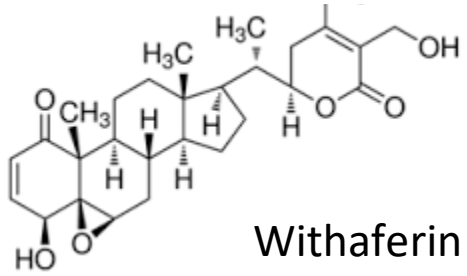
Rg1

Panax ginseng –"superior“, tonic herb in TCM.
used in convalescence and fatigue - Qi deficiency

Qi (Chi) - determines the state of health and lifespan



Withanolide A



Withaferin A

Withania somnifera, Ashwaganda

Prana - determines the life vital energy, activating body and mind, governing emotions, memory, and other functions

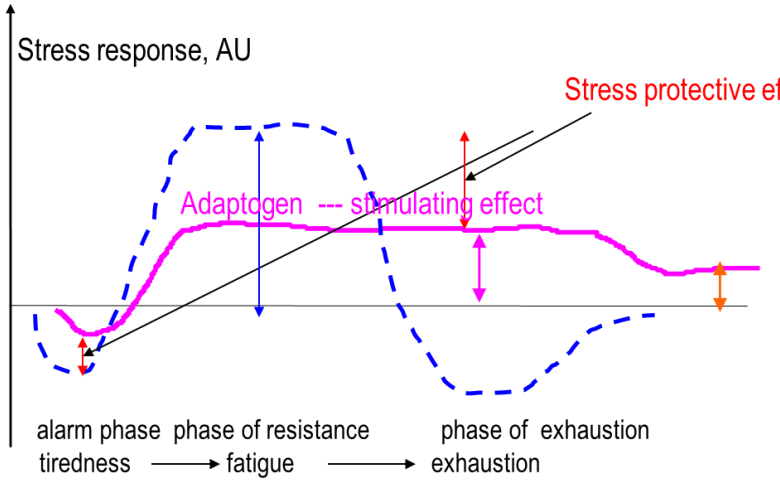
Adaptive stress response

Stress is a state of threatened homeostasis.

Homeostasis is a complex dynamic equilibrium

Cannon,1935: Selye, 1950,

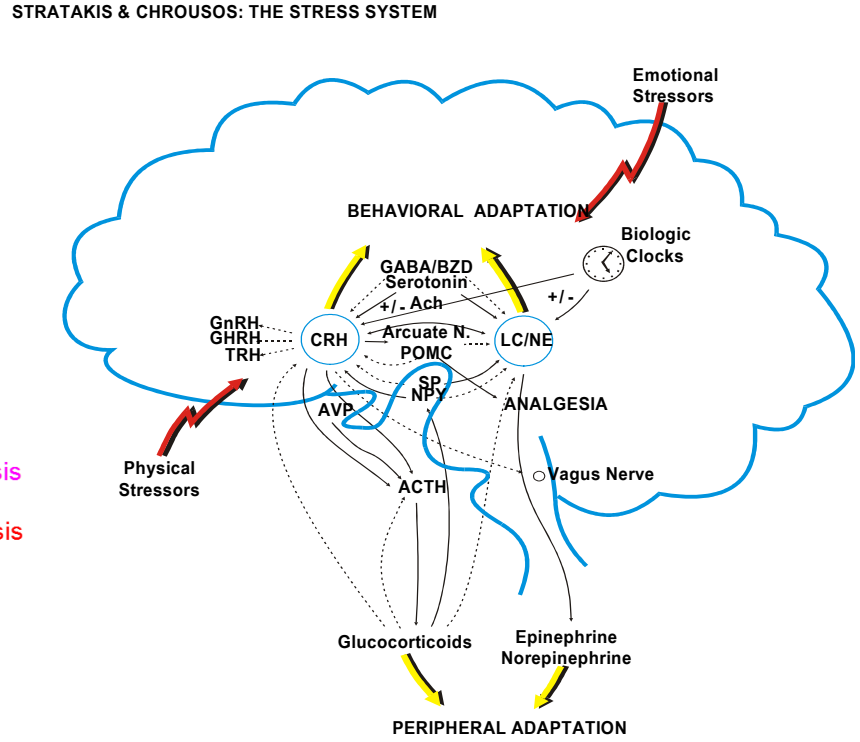
Effect of adaptogens – stress response modifiers increasing the resistance to stress



Heterostasis
adaptation
homeostasis

The Stress system

Neuroendorine – immune complex



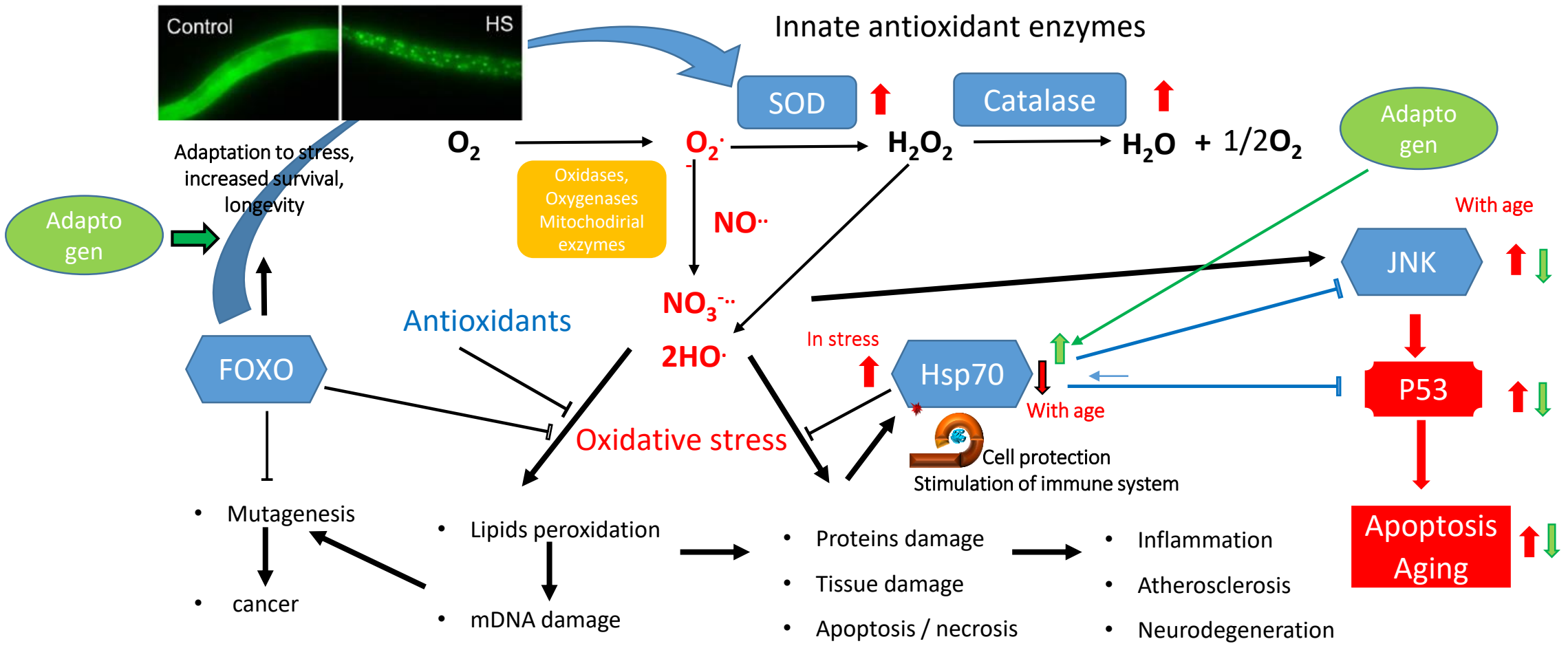
Age related disorders - complications arising from senescence - decreased ability to cope stress and to maintain homeostasis

- atherosclerosis - thrombosis, infarction, stroke
- cardiovascular disease and hypertension
- cancer
- degenerative joint disease (osteoarthritis)
- type 2 diabetes, obesity
- muscle degeneration (sarcopenia)
- senile dementia
- Alzheimer's disease
- Visual loss because of clouding of the lens (cataracts)

Stress

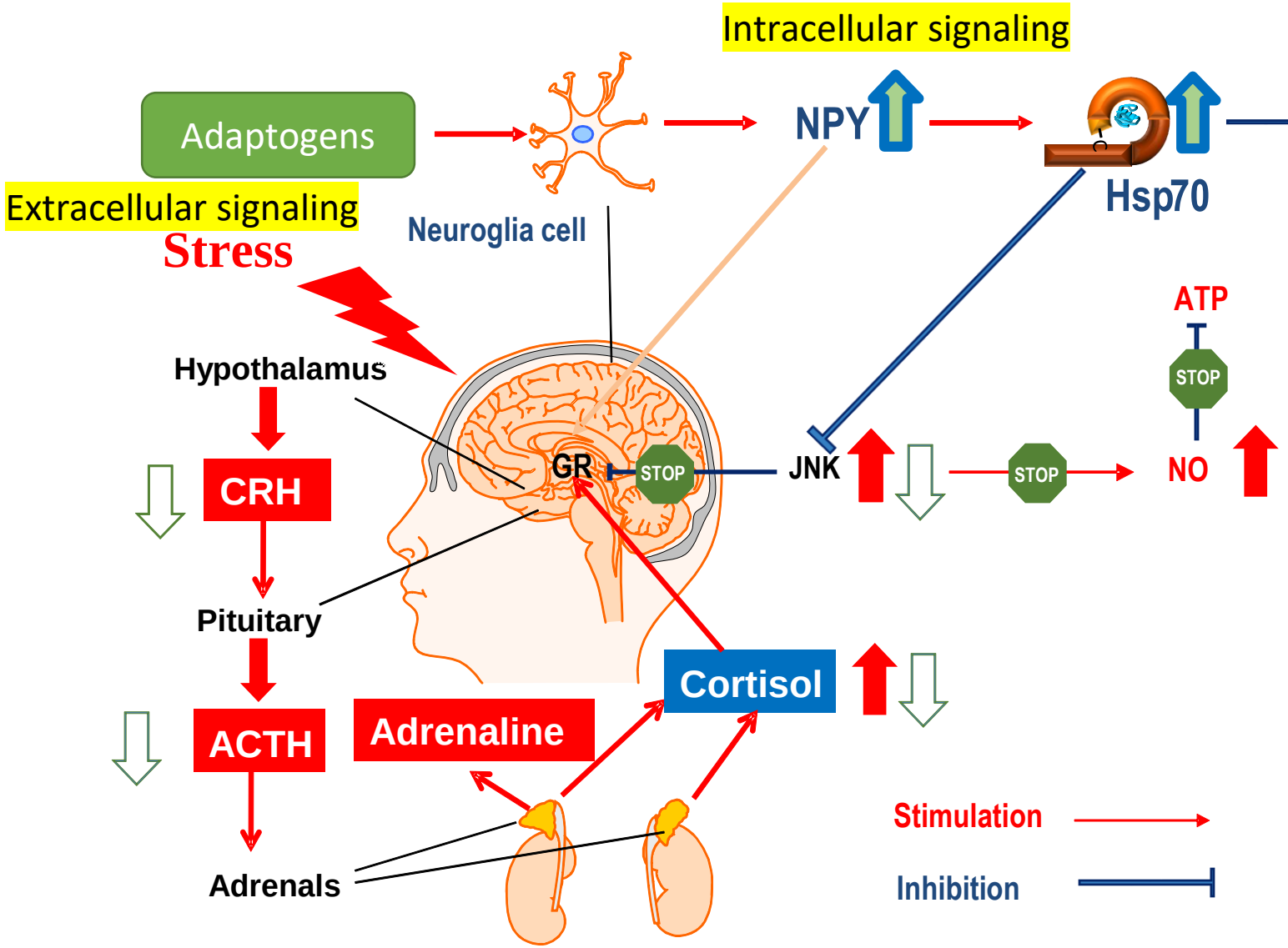
- Chronic inflammation
- Cells degeneration
- **Inflammaging**

The role of Hsp70 and FOXO in current theory of oxidative stress induced inflammaging and the effects of adaptogens



Panossian A.G. 2017. Understanding adaptogenic activity: specificity of the pharmacological action of adaptogens and other phytochemicals. Ann. N.Y. Acad. Sci. 1401(1):49-64.

Effects of adaptogens on adaptive stress response in HPA axis: FOXO, NPY and Hsp70 signaling



Activation of innate immunity, adaptation to stress, survival, longevity, cognitive function

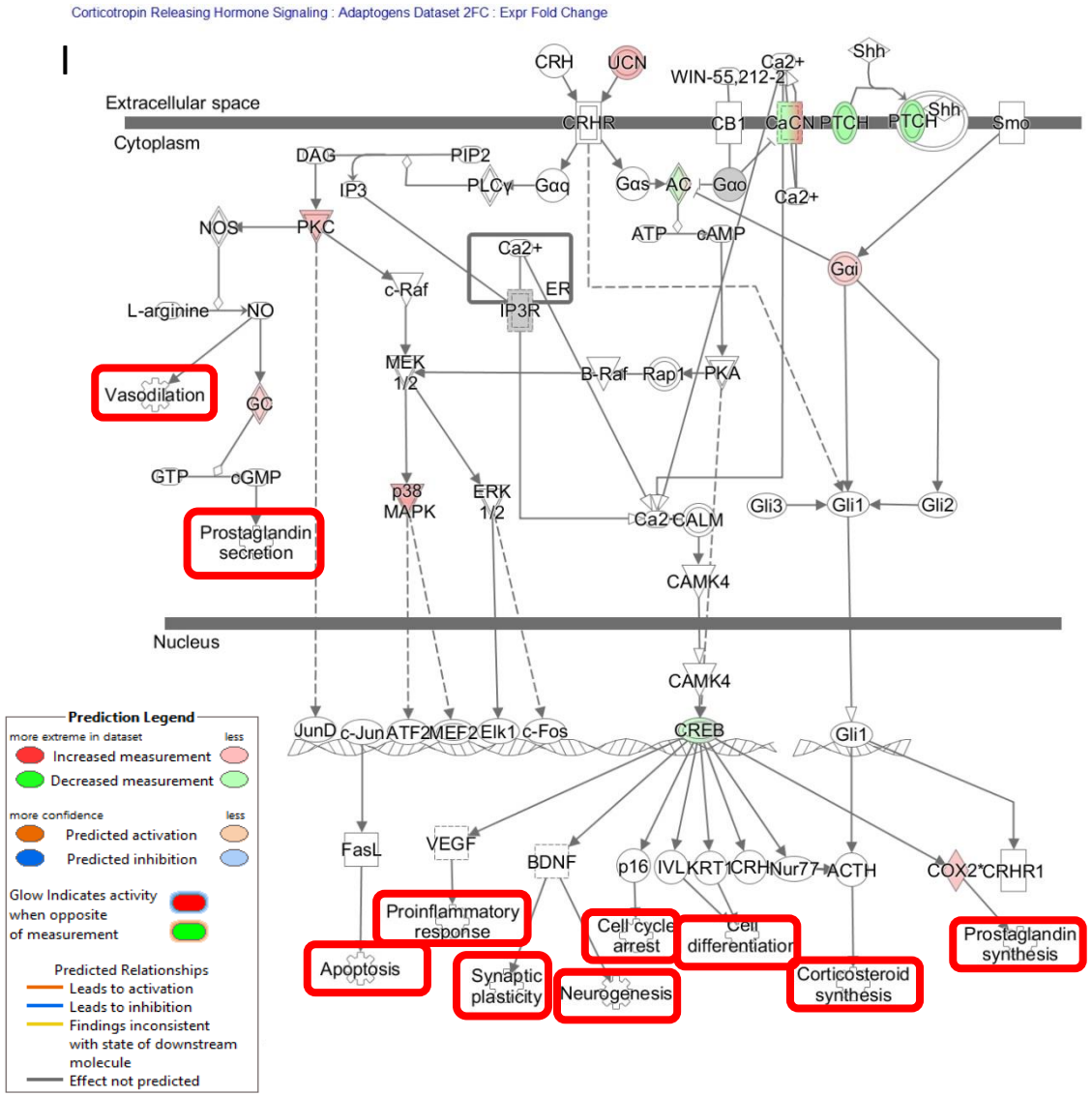
Immune stimulation
cytoprotection

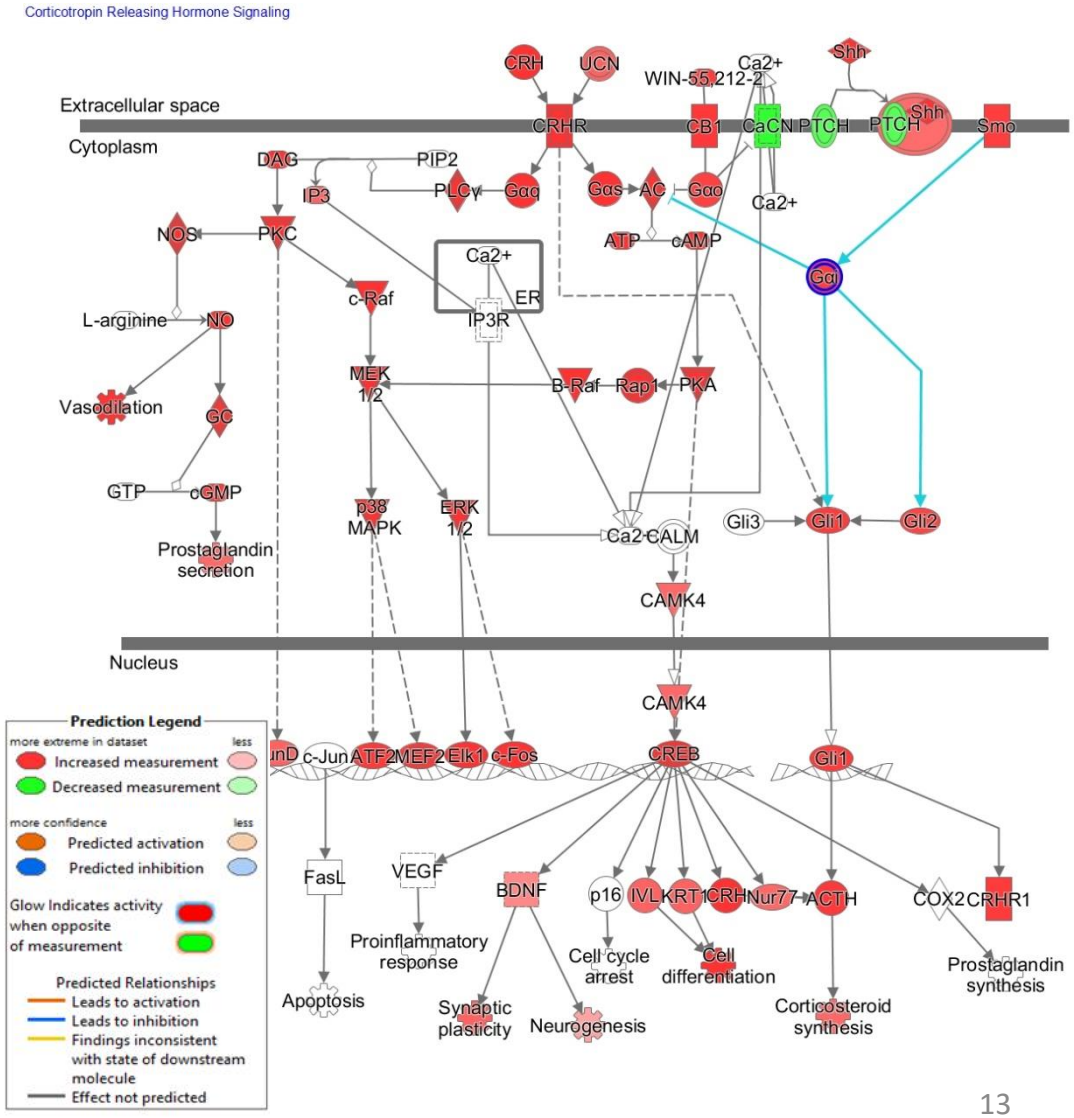
Hsp72 and NPY are directly involved in adaptation to stress, resulting in increased survival, longevity and cognitive function.

NPY plays a major role on the HPA axis and maintaining energy balance, antidepressive anxiolytic and antinarcotic effects. etc.

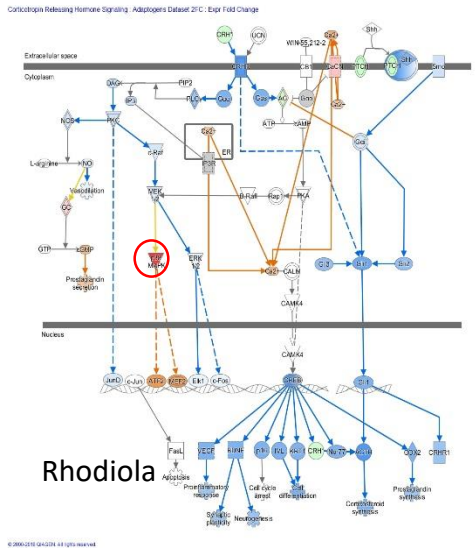
Panossian A. 2013. Adaptogens in Mental and Behavioral Disorders. *Psychiatr. Clin. North Am.*; 36(1):49-64.

Stress induced activation of CRH Canonical Pathway

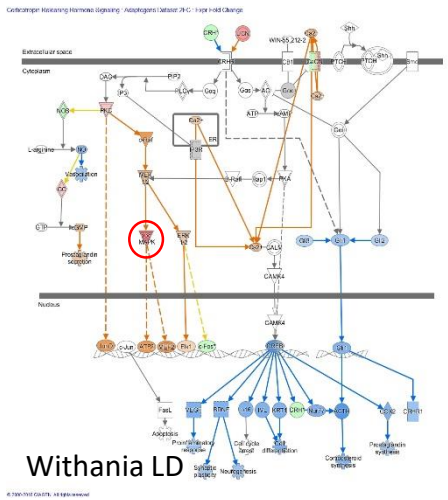




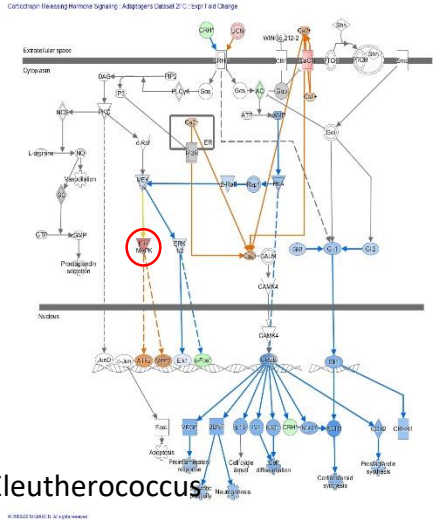
Effects of adaptogenic plants on CRH Signaling Pathway



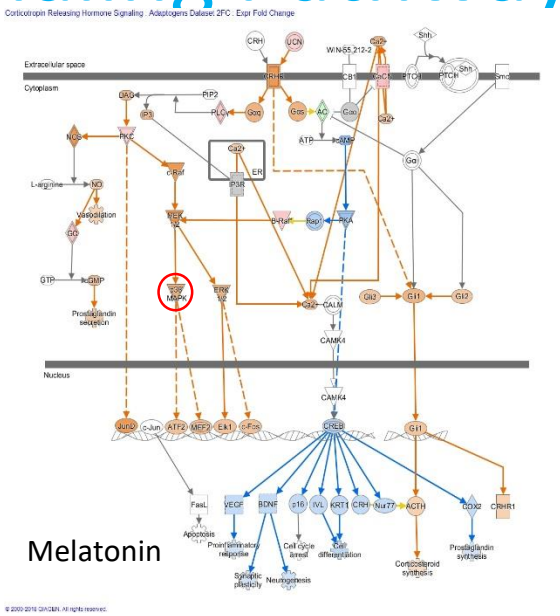
Rhodiola



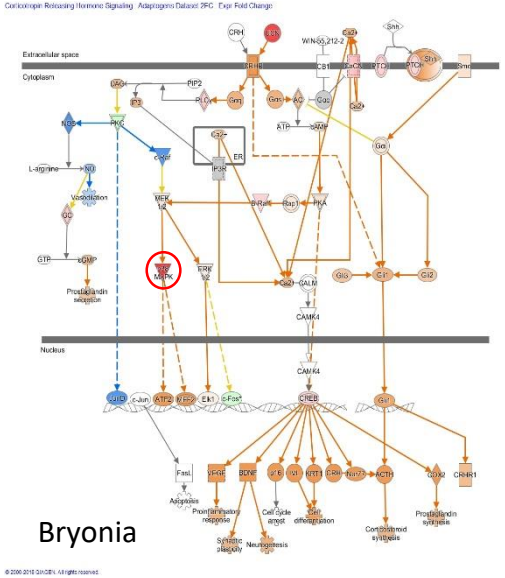
Withania LD



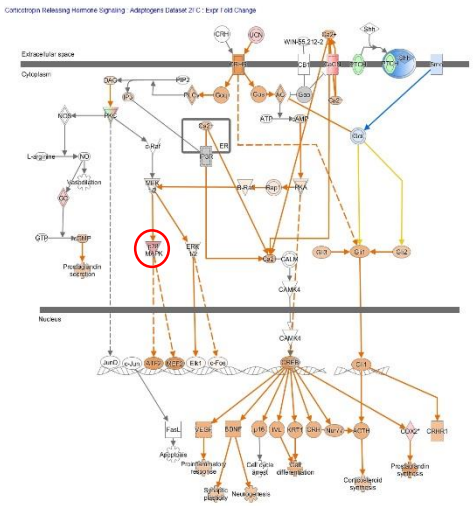
Eleutherococcus



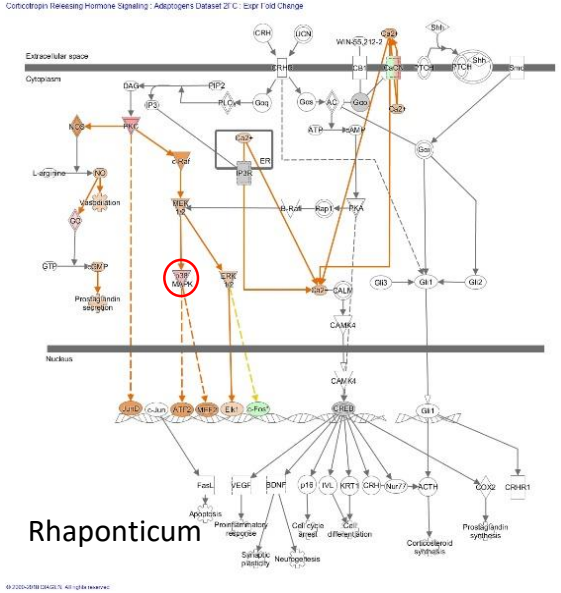
Melatonin



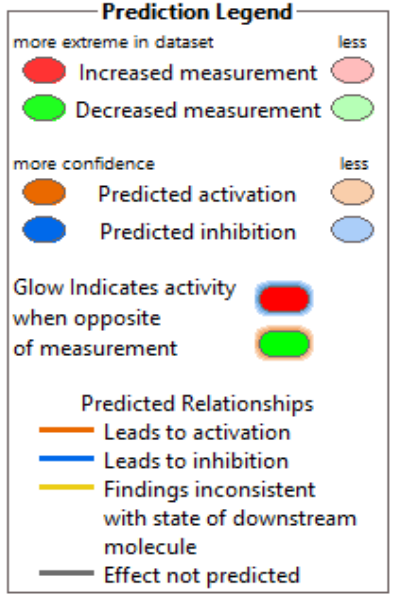
Bryonia



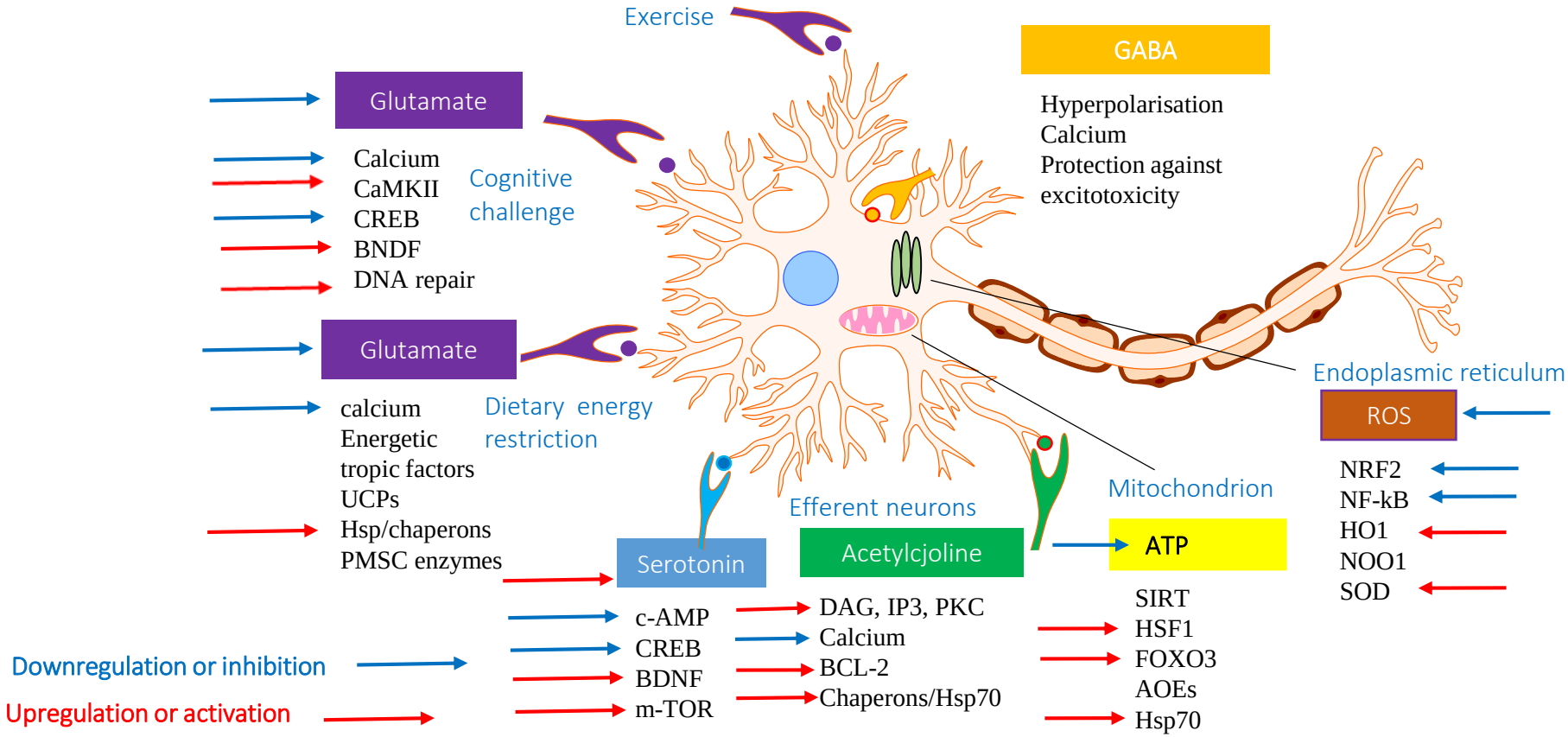
Withania



Rhaponticum



Effects of adaptogens on adaptive stress response signaling pathways that protect neurons against degeneration and promote synaptic plasticity.



A glutamatergic neuron in the hippocampus receiving excitatory inputs from neurons activated in response to exercise, cognitive challenges and dietary energy restriction.

Panossian A.G. 2017. Understanding adaptogenic activity: specificity of the pharmacological action of adaptogens and other phytochemicals. Ann. N.Y. Acad. Sci. 1401(1):49-64.

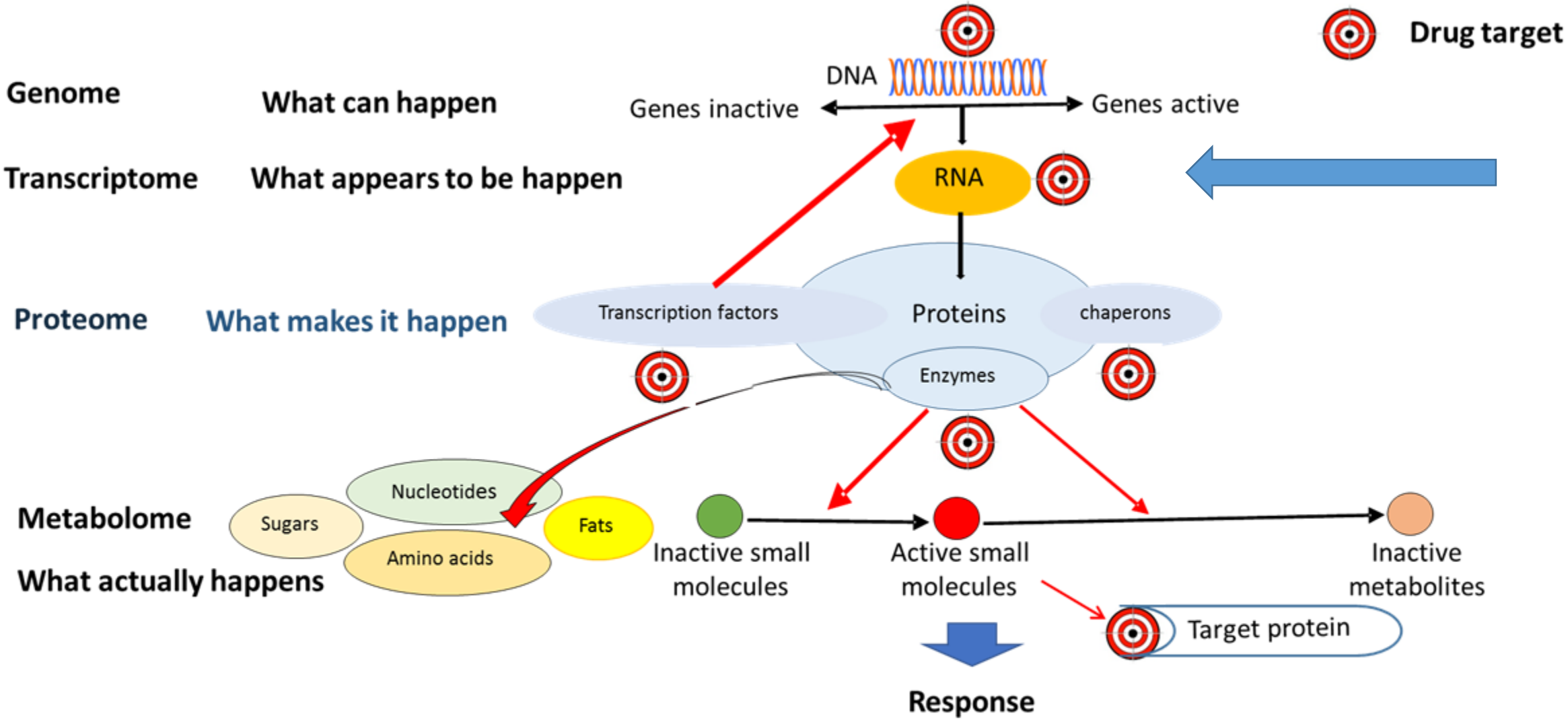
Definitions of adaptive stress response and adaptogens

- Adaptive stress response (hormesis) involves activation of intracellular and extracellular signaling pathways and increased expression of anti-apoptotic proteins, neuropeptides, antioxidant enzymes and defense response of an organism resulting in increased survival .
- Adaptogens are adaptive stress response modifiers of cellular and organismal defense systems, activating intracellular and extracellular signaling pathways, expression of stress-activated proteins, neuropeptides, antioxidant enzymes and anti-apoptotic proteins of an organism resulting in non-specific resistance to various stressors and increased survival .
- Adaptogens, like vitamins and antioxidants constitute a separate category of nutritional and herbal medicinal products.

Panossian A.G. 2017. Understanding adaptogenic activity: specificity of the pharmacological action of adaptogens and other phytochemicals. Ann. N.Y. Acad. Sci. 1401(1):49-64.

- Which of mediators of adaptive stress response are regulated by adaptogens?
- What is common in the mechanisms of action of adaptogens?

Molecular targets of pharmacological intervention



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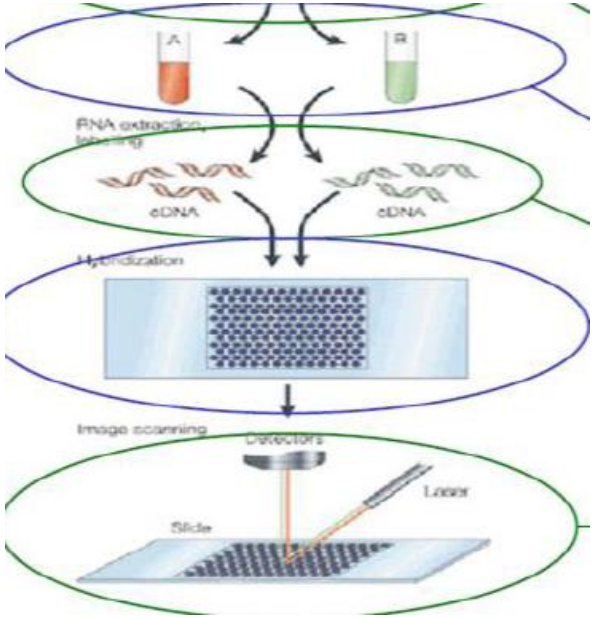
What we observe in our experiments?
Gene expression as drug's efficacy outcome measure

- **Gene expression** is the process by which information from a gene is used in the synthesis of a functional gene product.
- **Regulation of gene expression** includes a wide range of mechanisms that are used by cells to increase or decrease the production of specific gene products - protein or RNA.
- **Up-regulation** is a process that occurs within a cell triggered by an internal or external signal, which results in increased expression of genes and corresponding proteins.
- Up-regulation occurs, when a cell is deficient in some kind of receptor. In this case, more receptor protein is synthesized and transported to the membrane of the cell and, thus, the sensitivity of the cell is increased **back to normal, reestablishing homeostasis**.
- **Down-regulation** is a process resulting in decreased gene and corresponding protein expression.
- Down-regulation occurs, when a cell is overstimulated by a neurotransmitter, hormone, or drug for a prolonged period of time, and the expression of the receptor protein is decreased in order to **protect** the cell and to **reestablish homeostasis**.

Gene expression degree: RNA microarray assay

- mRNA extraction from treatment and control samples
- RNA converted to fluorescence-labeled transcripts (for detection)
- Labeled RNA fragments hybridize (bind to their matching DNA sequence) with DNA for GeneChip array. Each labelled gene “matches” with its specific “partner” DNA on the GeneChip array
- Fluorescence intensities captured/scanned on the image of Gene Chip array that reflect gene expression level: higher gene expression => higher fluorescence intensity.
- CHIPSTER Software: transformation of the intensity values to fold changes => efficiently statistical analysis and differential gene expression profiling of a microarray data => identification of deregulated genes => deregulated gene sets.
- Venn diagram to show sets of deregulated genes
- *Ingenuity Knowledge Data Base software =>* Interactive Pathways Analysis (IPA) - downstream analysis for influenced signalling pathways and protein networks
- Validation of microarray-based mRNA expression by quantitative real-time RT-PCR.

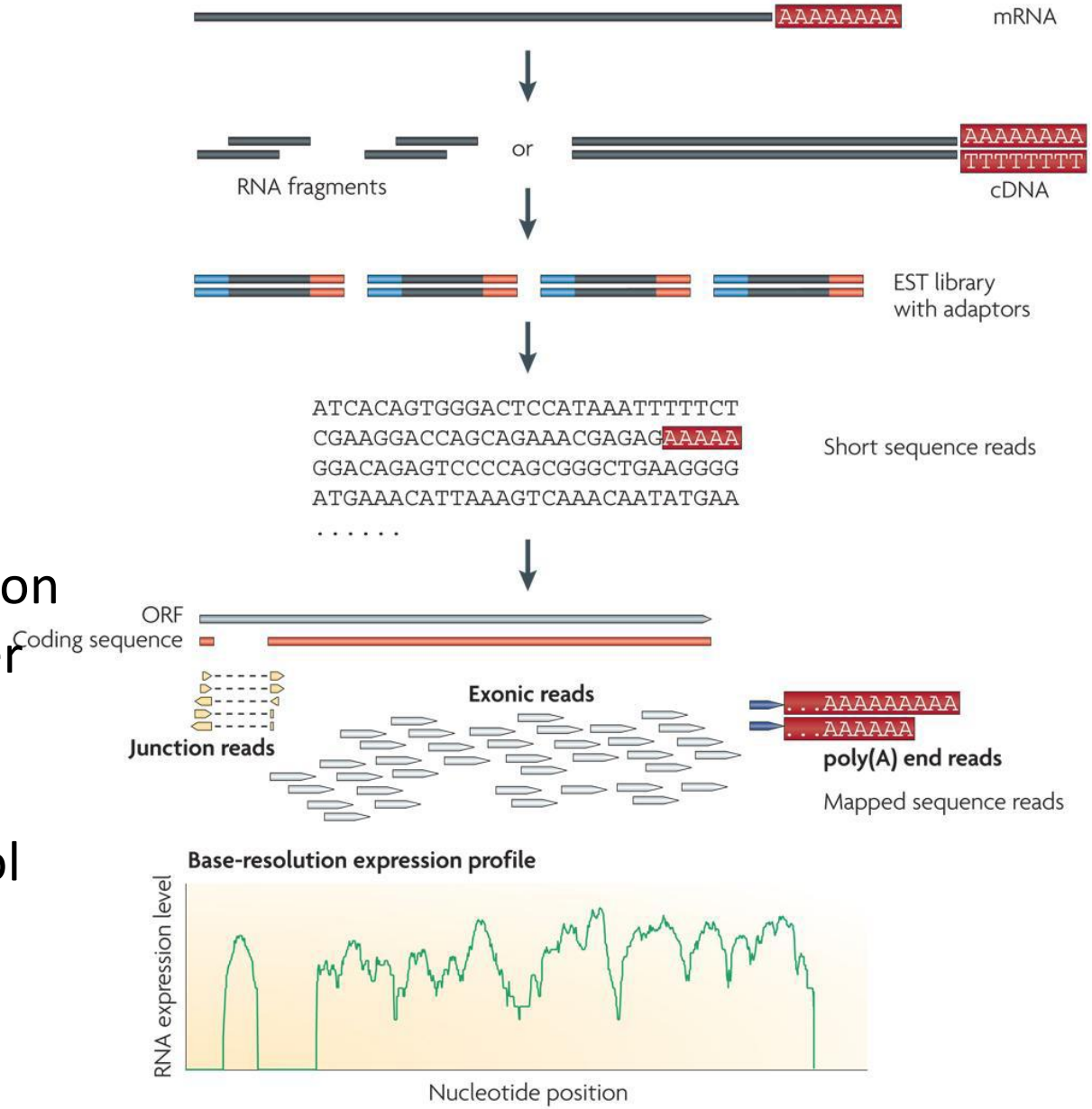


CSC Chipster
Open source platform for data analysis

INGENUITY
PATHWAY ANALYSIS

Gene expression degree: RNA Sequencing

- No hybridization and labeling steps are required,
- After the alignment to reference genome, RNA read numbers determine the expression degree of each gene RPKM value (reads per kilobase per million mapped reads),
- Fold changes are calculated by using the ratio of each gene's RPKM values to control sample.



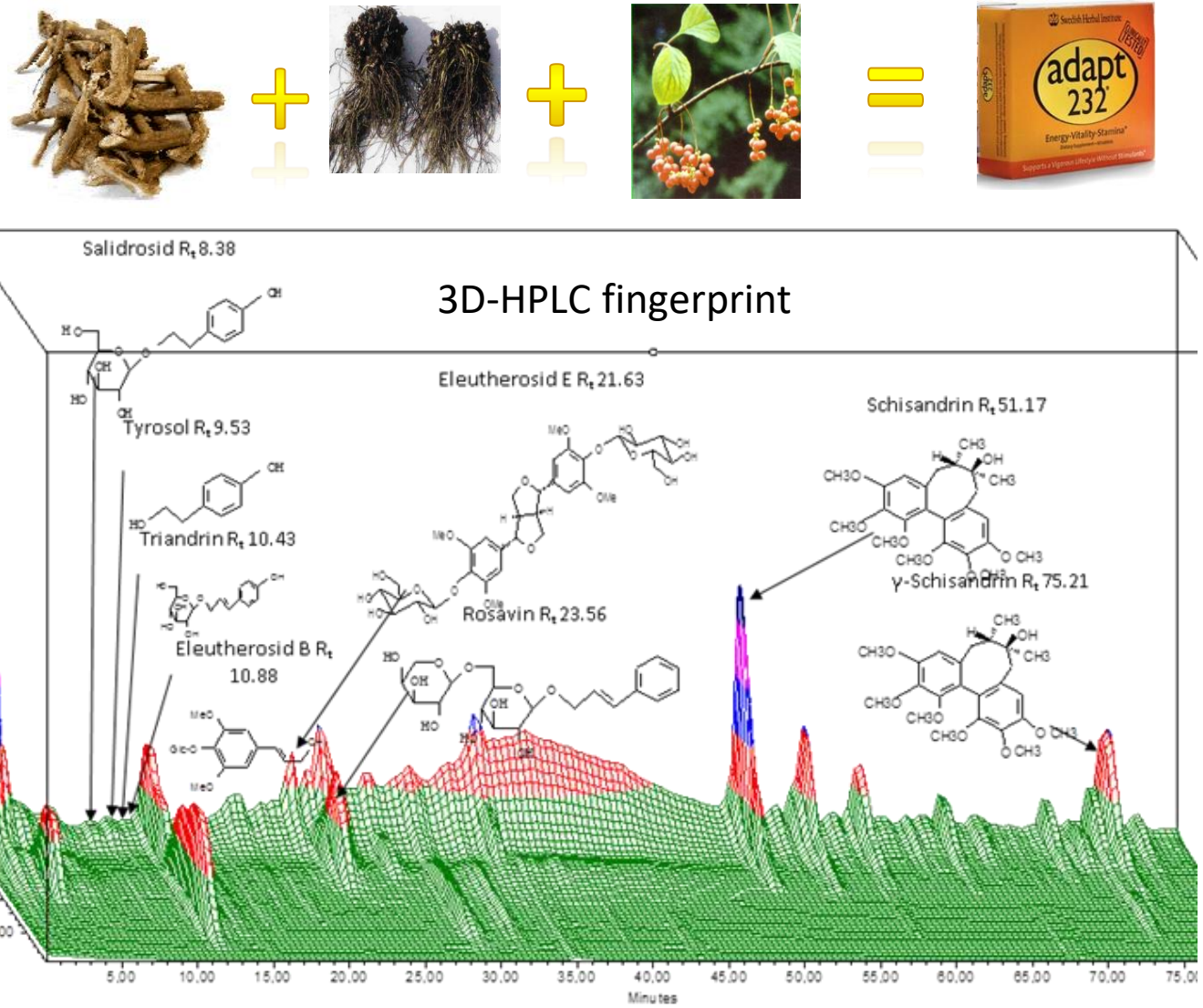
Complete list of plant names declared as adaptogenic in scientific literature

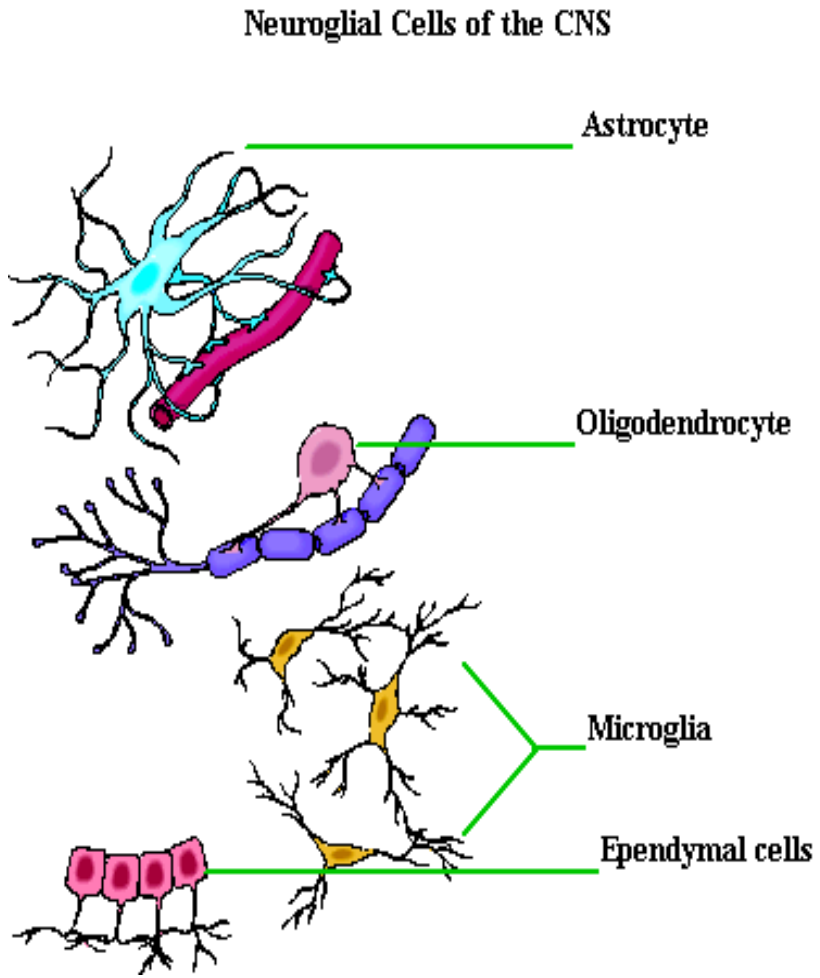
Ajuga turkestanica (Regel) Briq.	*Emblica officinalis* Gaetrn.	*Piper longum* L.
Alstonia scholaris (L.) R. Br.	*Eucommia ulmoides* Oliv.	*Potentilla alba* L.
Anacyclus pyrethrum (L.) Lag.	*Evolvulus alsinoides* (L.) L.	*Ptychopetalum olacoides* Benth.
Andrographis paniculata (Burm.f.) Nees	*Firmiana simplex* (L.) W.Wight	*Rhaponticum carthamoides* (Willd.) Iljin
Aralia mandshurica Rupr. & Maxim	*Gentiana pedicellata* (D.Don) Wall	*Rhodiola heterodonta* (Hook. f. & Thomson) Boriss.
Argyreia nervosa (Burm. f.) Bojer	*Glycyrrhiza glabra* L.	*Rhodiola rosea* L.
Argyreia speciosa (L. f.) Sweet	*Heteropterys aphrodisiaca* Machado	*Rostellularia diffusa* (Willd.) Nees .
Asparagus racemosus Wild	*Hippophae rhamnoides* L.	*Salvia miltiorrhiza* Bunge
Bacopa monnieri (L.) Wettst	*Holoptelea integrifolia* Planch	*Schisandra chinensis* (Turcz.) Baill.
Bergenia crassifolia (L.) Fritsch	*Hoppea dichotoma* Willd.	*Scutellaria baicalensis* Georgi
Bryonia alba L.	*Hypericum perforatum* L.	*Serratula inermis* Poir
Caesalpinia bonduc (L.) Roxb	*Lepidium peruvianum/ Lepidium meyenii* Walp.	*Sida cordifolia* L.
Centella asiatica (L.) Urb.	*Ligusticum striatum* DC.	*Silene italica* (L.) Pers.
Chlorophytum borivilianum Santapau & R.R.Fern.	*Melilotus officinalis* (L.) Pall.	*Sinomenium acutum* (Thunb.) Rehder & E.H.Wilson
Chrysactinia mexicana A. Gray	*Morus alba* L.	*Solanum torvum* SW.
Cicer arietinum L.	*Mucuna pruriens* (L.) DC.	*Sutherlandia frutescens* (L.) R.Br.
Codonopsis pilosula (Franch.) Nannf.	*Nelumbo nucifera* Gaertn.	*Terminalia chebula* Retz.
Convolvulus prostratus Forssk.	*Ocimum sanctum* L.	*Tinospora cordifolia* (Willd.) Miers
Curculigo orchioides Gaertn.	*Oplopanax elatus* (Nakai) Nakai	*Trichilia catigua* A.Juss.
Curcuma longa L., Curcumin	*Panax ginseng* C.A.Mey.	*Trichopus zeylanicus* Gaertn.
Dioscorea deltoidea Wall. ex Griseb.	*Panax pseudoginseng* Wall.	*Turnera diffusa* Willd. ex Schult.
Drypetes roxburghii (Wall.) Hurus.	*Pandanus odoratissimus* L.f.	*Vitis vinifera* L.
Echinopanax elatus Nakai	*Paullinia cupana* Kunth	*Withania somnifera* (L.) Dunal
Eleutherococcus senticosus (Rupr. & Maxim.) Maxim.	*Pfaffia paniculata* (Mart.) Kuntze	

Combination A	Combination B	Combination C	Combination D
Andrographis paniculata (Burm.f.) Nees • Andrographolide	*Rhodiola rosea* L. • Salidroside • Tyrosol	*Rhaponticum carthamoides* (Willd.) Iljin	*Rhodiola rosea* L. • Salidroside • Tyrosol
Eleutherococcus senticosus (Rupr. & Maxim.) Maxim. • Eleutheroside E	*Bryonia alba* L.	*Eleutherococcus senticosus (Rupr. & Maxim.) Maxim.* • Eleutheroside E	*Eleutherococcus senticosus* (Rupr. & Maxim.) Maxim. • Eleutheroside E
		Withania somnifera (L.) Dunal	*Schisandra chinensis* (Turcz.) Baill. • Schizandrin

Incubation of herbal extracts with neuroglial cells

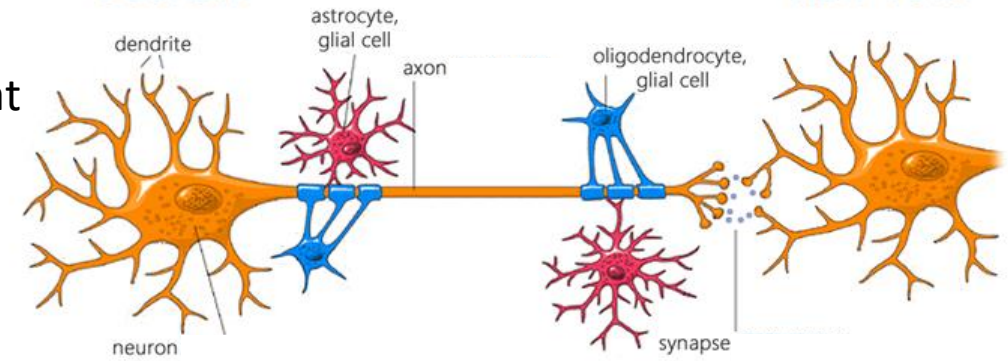
Eleutherococcus + *Rhodiola* + *Schisandra* = **Combination D**





Glial cells do NOT have chemical synapses. But….

Neurons HAVE synapses that use neurotransmitters...



Glia contributes to the defense of the brain through:

- the expression of the innate immune response,
- promoting the clearance of neurotoxic proteins and apoptotic cells from the CNS,
- regulating the entry of inflammatory systemic cells into the brain at the blood brain barrier.

This stimulates both tissue repair and the rapid restoration of tissue homeostasis.

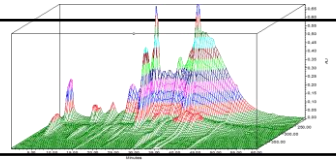
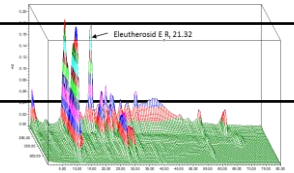
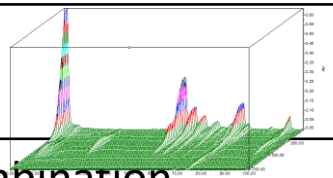
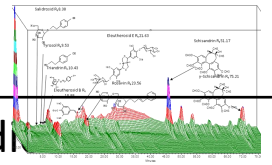
An important physiological function of neuroglial cells is metabolic supply of energy and other substances, maintaining brain homeostasis.

Astrocytes, but not neurons, prevent macrophage and T-Cell inflammation in the CNS, to attenuate axonal loss and gliosis resulting in neuroprotection.

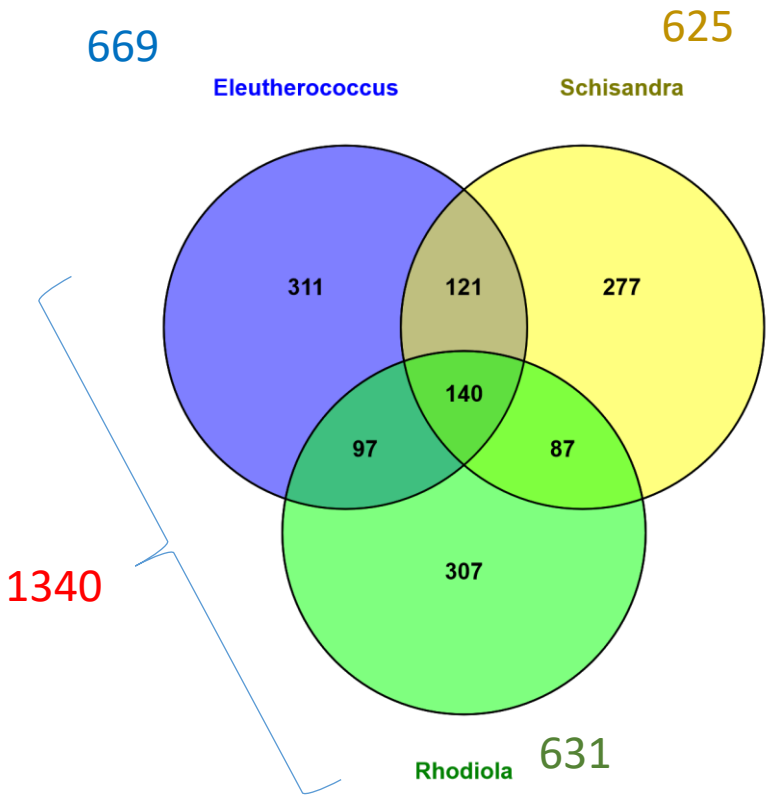
Glia has been shown to have several functions, including:

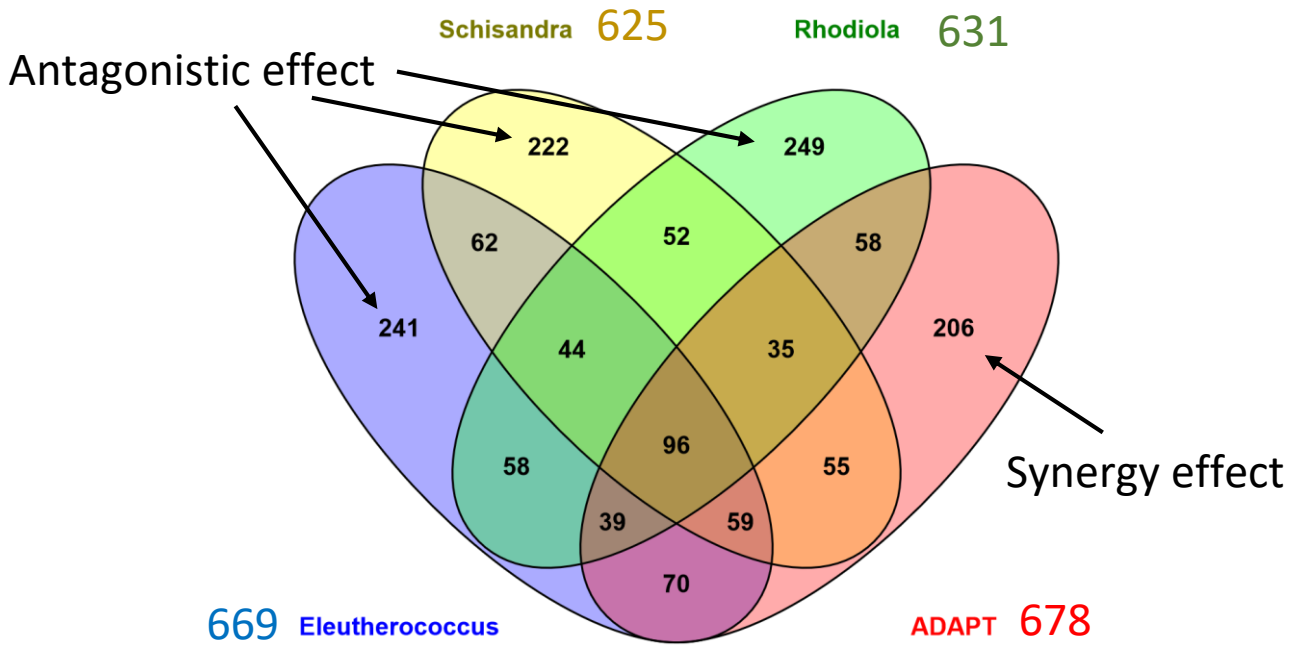
- serving as a transportation link between the bloodstream and neurons, uptake of neurotransmitters,
- synthesis and release of neurotrophic factors,
- immune regulation, and
- modulation of synaptic activity .

How many targets has one purified active compound?

Herbal extracts	Chemical composition "Fingerprint" Number of plant secondary metabolites identified in extracts, n A+B+C=ABC	Pharmacological effect, "Signature", on transcriptome level of regulation in target cells, Number of deregulated genes in host cells, N A+B+C=D
A - Rhodiola 	140	631
B - Eleutherococcus 	35	669
C – Schisandra 	32	625
D - ADAPT combination 	A+B+C=ABC 140+32+35=207	A+B+C=D 678
E – Salidroside isolated from Rhodi	1	640

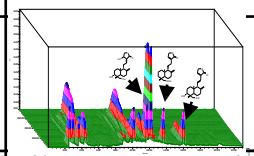
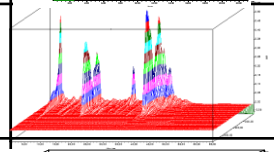
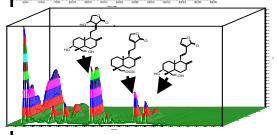
Venn diagram to show sets of deregulated genes





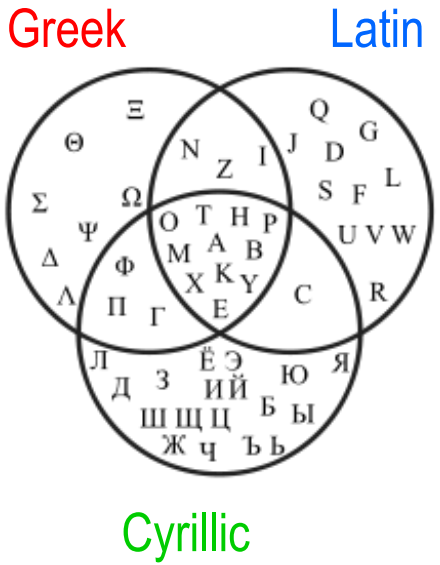
206 synergetic interactions resulted in activation of genes that none of the constituents alone could deregulate

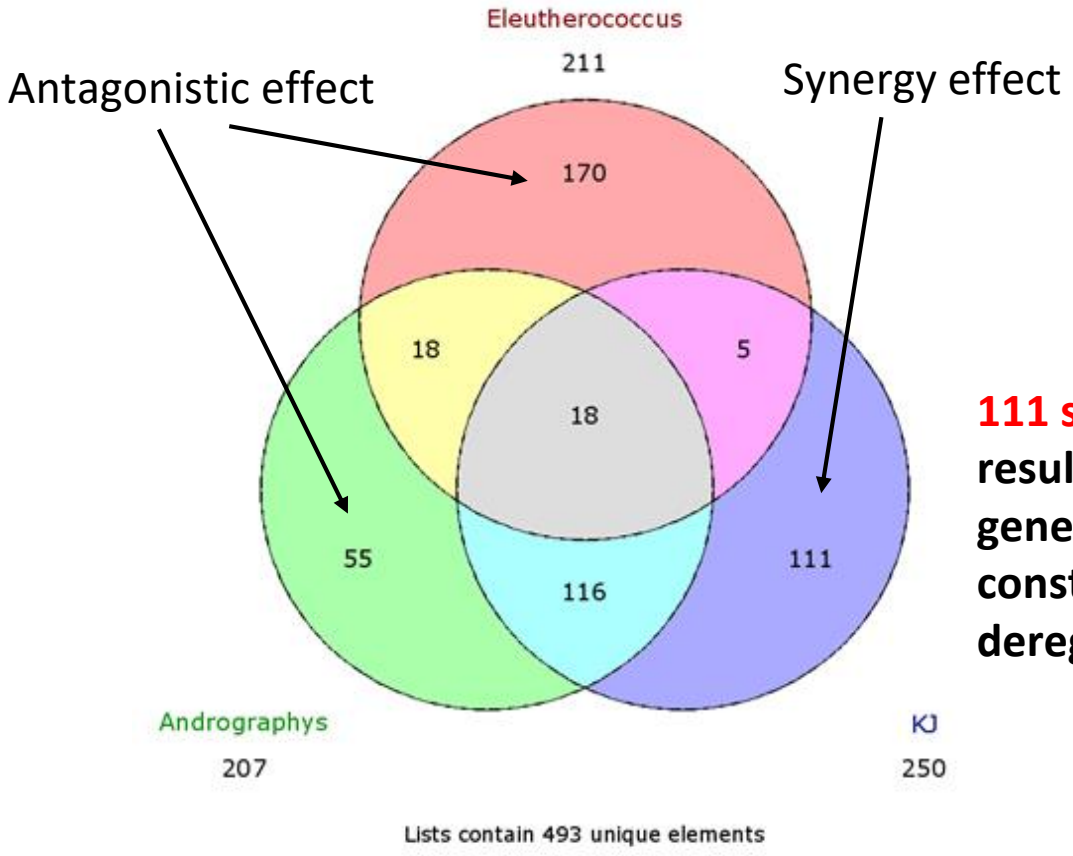
Panossian, A., Hamm, R., Kadioglu, O., Wikman, G., Efferth, T., 2013. Synergy and antagonism of active constituents of ADAPT-232 on transcriptional level of metabolic regulation of isolated neuroglial cells. Front Neurosci. 7:16. http://journal.frontiersin.org/article/10.3389/fnins.2013.00016/abstract

Herbal extracts	Chemical composition “Fingerprint” Number of plant secondary metabolites identified in extracts, n A+B=AB	Pharmacological effect, “Signature”, on transcriptome level of regulation in target cells, Number of deregulated genes in host cells, N A+B=C
A - Andrographis	 39	207
B - Eleutherococcus	 35	211
C – A+B combination	 39+35=74	250
D – Andrographolide from Andrographis	1	626

Synergy and antagonism

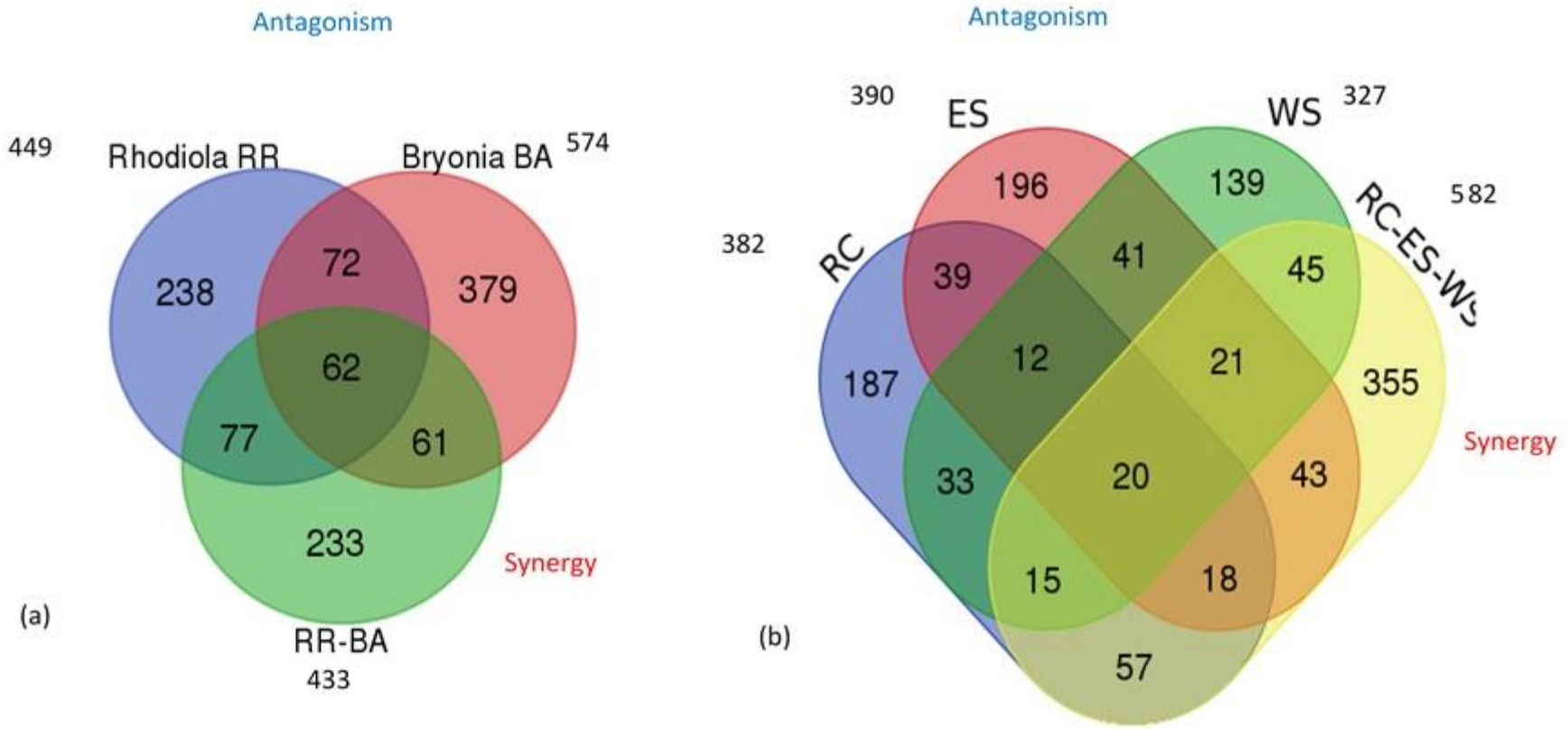
Venn diagram to show sets of deregulated genes





111 synergetic interactions resulted in activation of genes that none of the constituents alone could deregulate

Venn diagram to show sets of deregulated genes

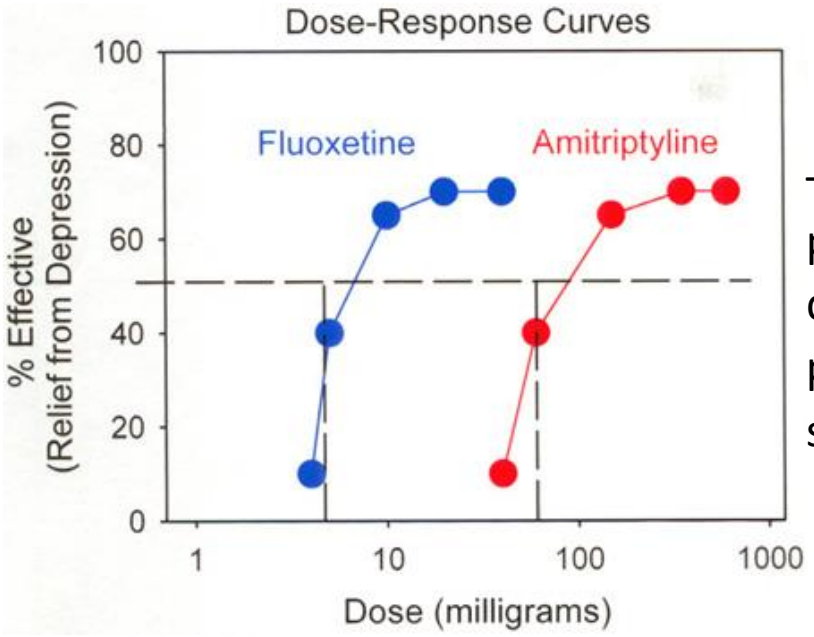


What is synergism? Current Definition # 1

- “Synergy and antagonism imply that the different constituents affect each other’s actions” assuming that “If it is synergistic, the drugs will be more effective in combination than separately” (Goldin and Mantel, 1957).
- “A combination of agents that is more effective than is expected from the effectiveness of its constituents is said to show synergy” (Berenbaum, 1977).
- This definition of synergy is related to effectiveness of the combinations, which is associated with the concentration of an agonist needed to elicit half of the maximum biological response of the agonist. The effectiveness of a drug usually is considered relative to its safety (therapeutic index).
- This definition of synergy is not associated with the efficacy of the combination, where maximum possible effect relative to the ingredients is considered, particularly when the effect is 0 or negative for the ingredients of the combination.

Definitions of effectiveness and efficacy

Effectiveness

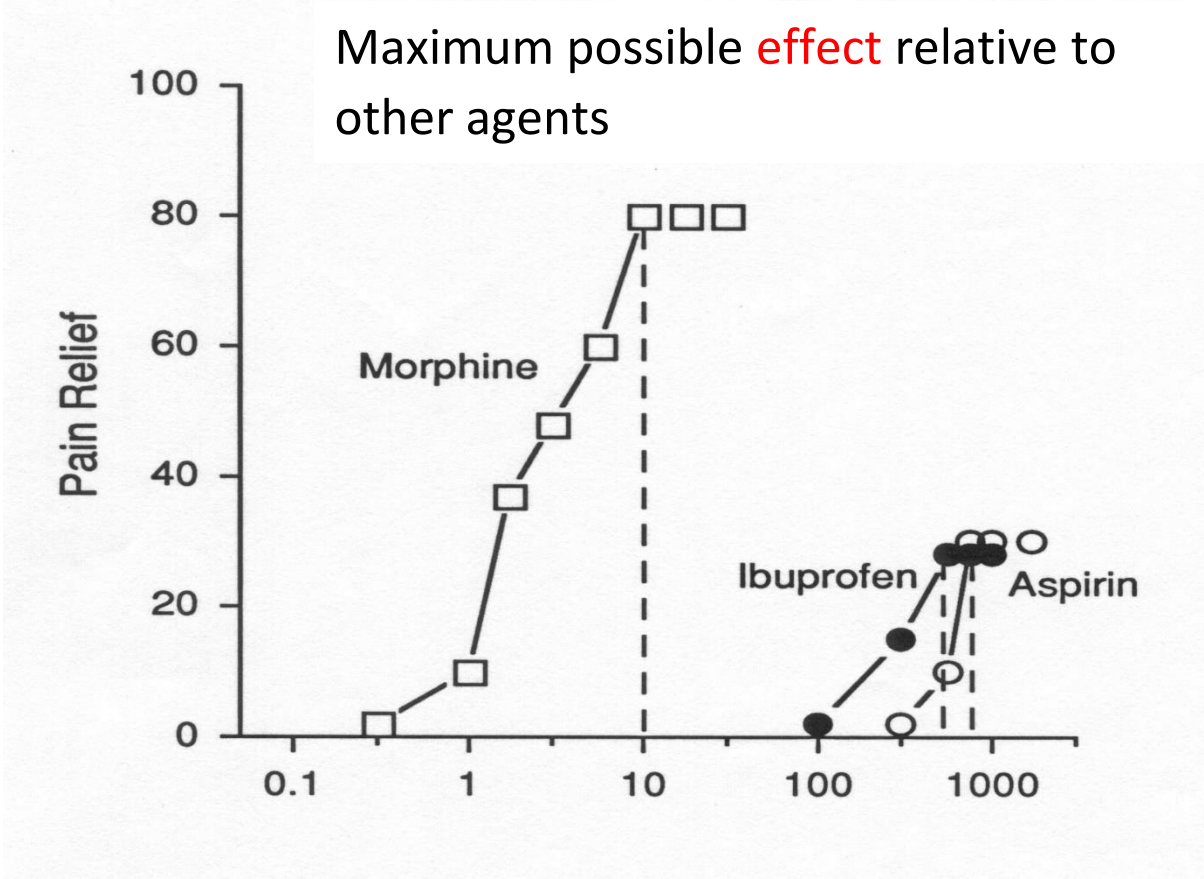


– After this point increasing doses do not produce a stronger effect

- Effective concentration (EC50) is the concentration of an agonist needed to elicit half of the maximum biological response of the agonist
- The effectiveness of a drug is considered relative to its safety (therapeutic index)

Efficacy

Maximum possible effect relative to other agents



Definition # 1 Update

In this context, it would be reasonable to distinguish among definitions of synergy and implement more relevant definitions for assessing efficacy and effectiveness of fixed combinations of active agents, such as:

- Amplification, if 1+1>2, or **synergistic amplification** instead of a synergy
- Attenuation, if 1+1<2
- Addition, if 1+1=2
- Potentiation, if 0+1>1
- **Bionetwork synergy**, If 0+0>0

Limitation of Definition # 1

- The models of assessment of synergy, e.g., the isobole method (Berenbaum, 1977; Greco et al., 1995; Chow, 2010; Roel; et al., 2017), are not suitable for assessing complex interactions of molecular networks involved in drug-induced synergistic or antagonistic response. They cannot be used to predict the effects of multi-target interactions or homeostatic feedback on the pharmacological response.

What is synergism? Current Definition # 2

"Two or more agents working together to produce a result not obtainable by any of the agents independently"

(Skirven et.al., 2011; https://www.theopedia.com/synergism; http://googledictionary.freecollocation.com/meaning?word=synergy).

can be interpreted as

Generation of new pharmacological activity, which is specific only for the combination of two or more agents

Definition # 2 Update

- The term **bionetwork synergy** is more suitable for network interactions of two or more agents resulting in qualitatively new pharmacological effects that cannot be obtained by any single constituent independently, regardless of dose.
- Similarly, antagonism results from bionetwork interactions of several constituents in combination, leading to lack, reduction, or prevention of effects that any individual ingredient in that combination yields.

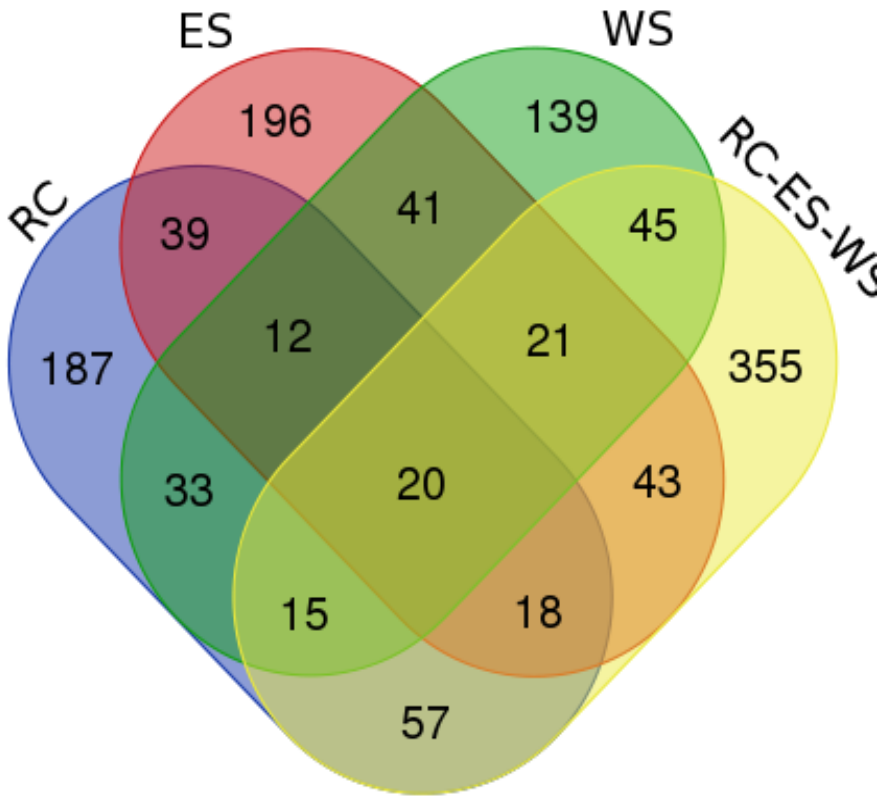
In other words:

- **Bionetwork synergy** results from synergistic interaction of ingredients A and B of the combination AB, inducing new pharmacological activity c: a+b=c;
  - where (a) is the activity of substance A, (b) is the activity of substance B, and (c) is bionetwork synergy–induced activity of the combination AB = the hybrid substance C.
- **Bionetwork antagonism** results from antagonistic interaction of ingredients A and B of the combination AB (the hybrid substance C), resulting in suppression of (a) and (b): – a+b=0

Applications

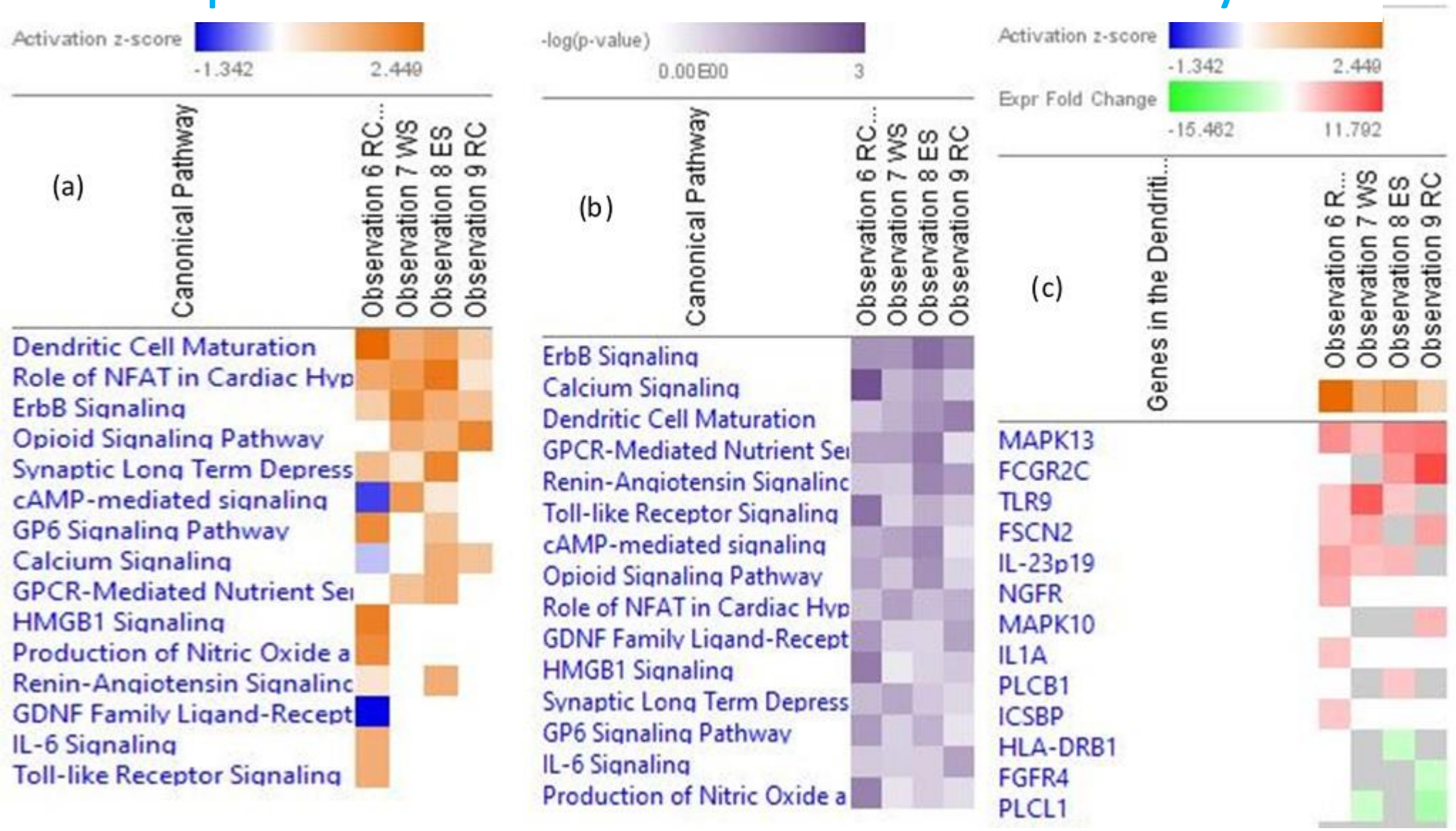
- This definition is suitable and relevant to pharmacological studies of combinations of plant extracts where the focus is the discovery of unexpected potential indications and toxic effects of the combinations from complex intracellular and extracellular interactions of molecular networks with many players at several phases of development of final pharmacological outcomes.

Further evidences from IPA analysis

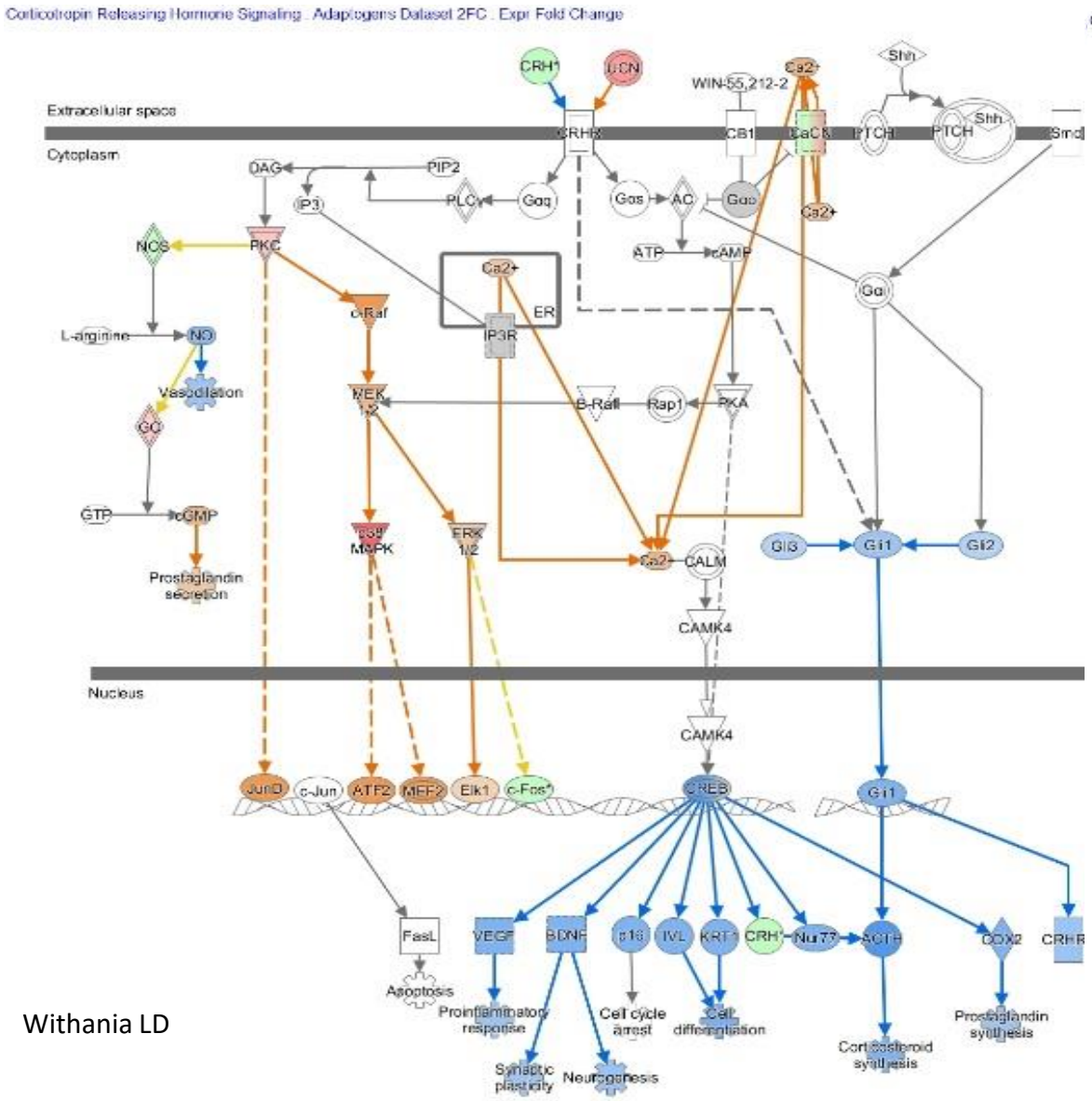


RC - *Rhaponticum carthamoides* (Willd.) Iljin
ES - *Eleutherococcus senticosus* (Rupr. & Maxim.) Maxim.
WS - *Withania somnifera* (L.) Dunal
RC-ES-WS – Their Combination

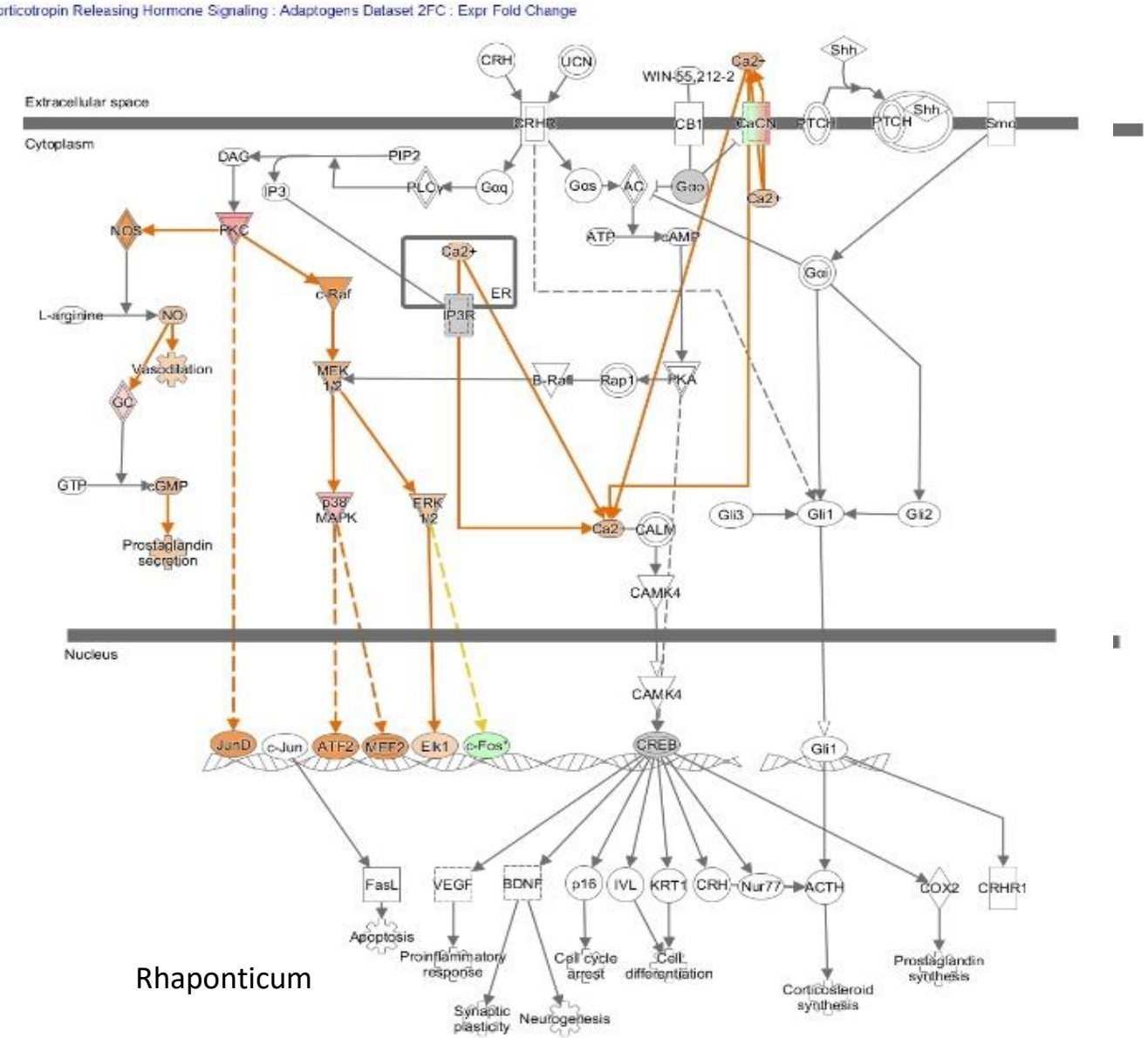
Comparison effects on Canonical Pathways



Effects on CRH Canonical Pathway



Withania LD

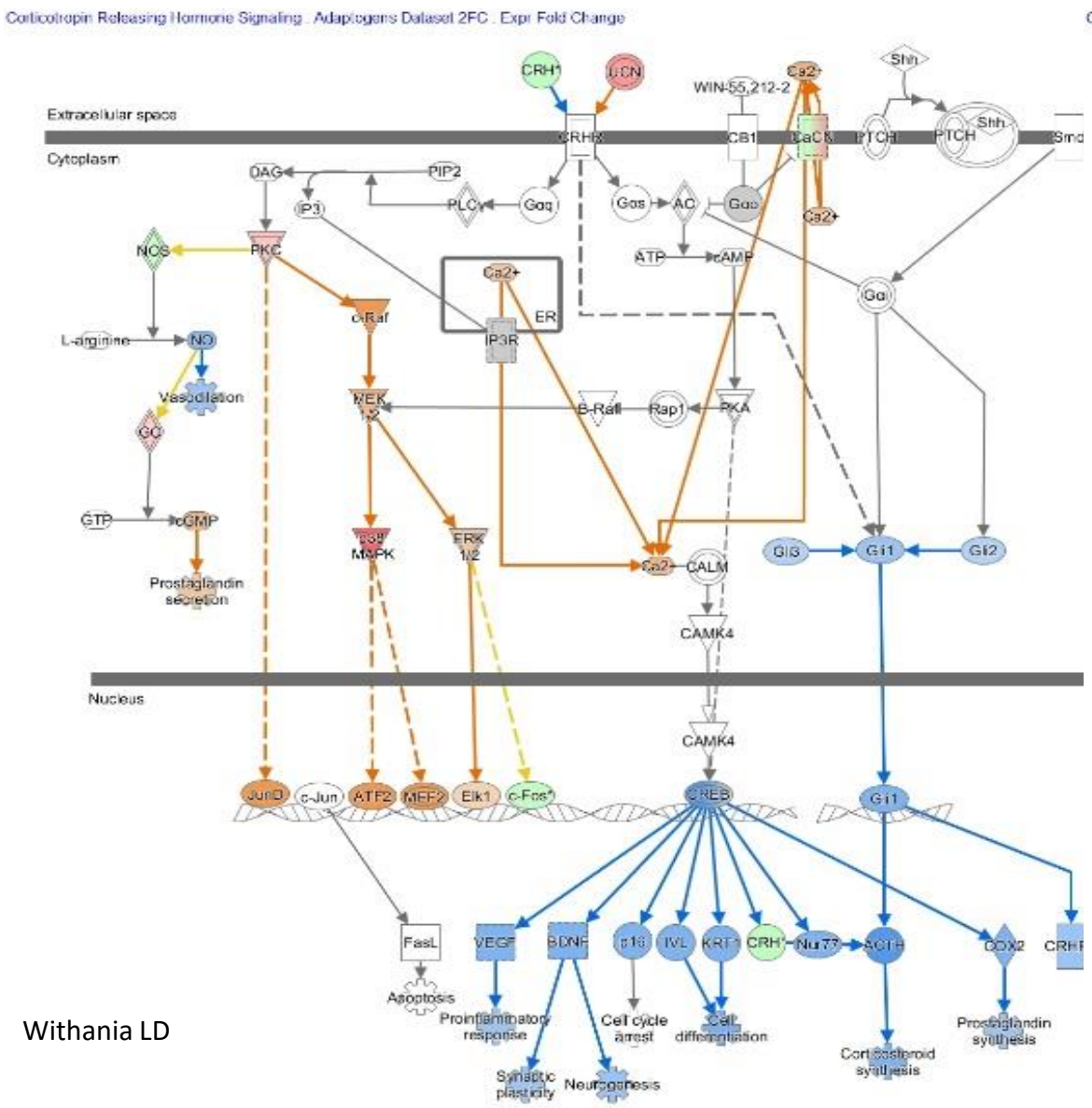


Rhaponticum

Effects on CRH Canonical Pathway

antagonism

Synergy

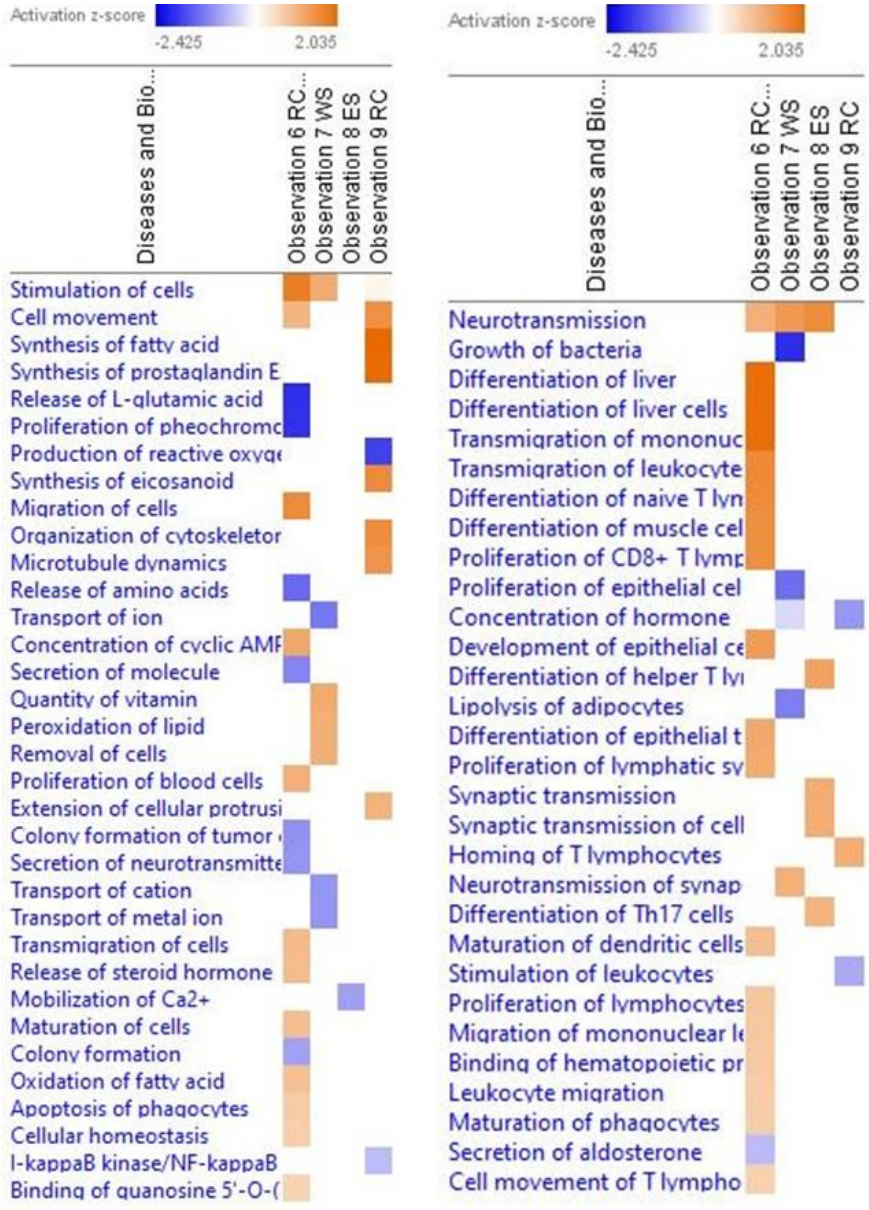


Withania LD

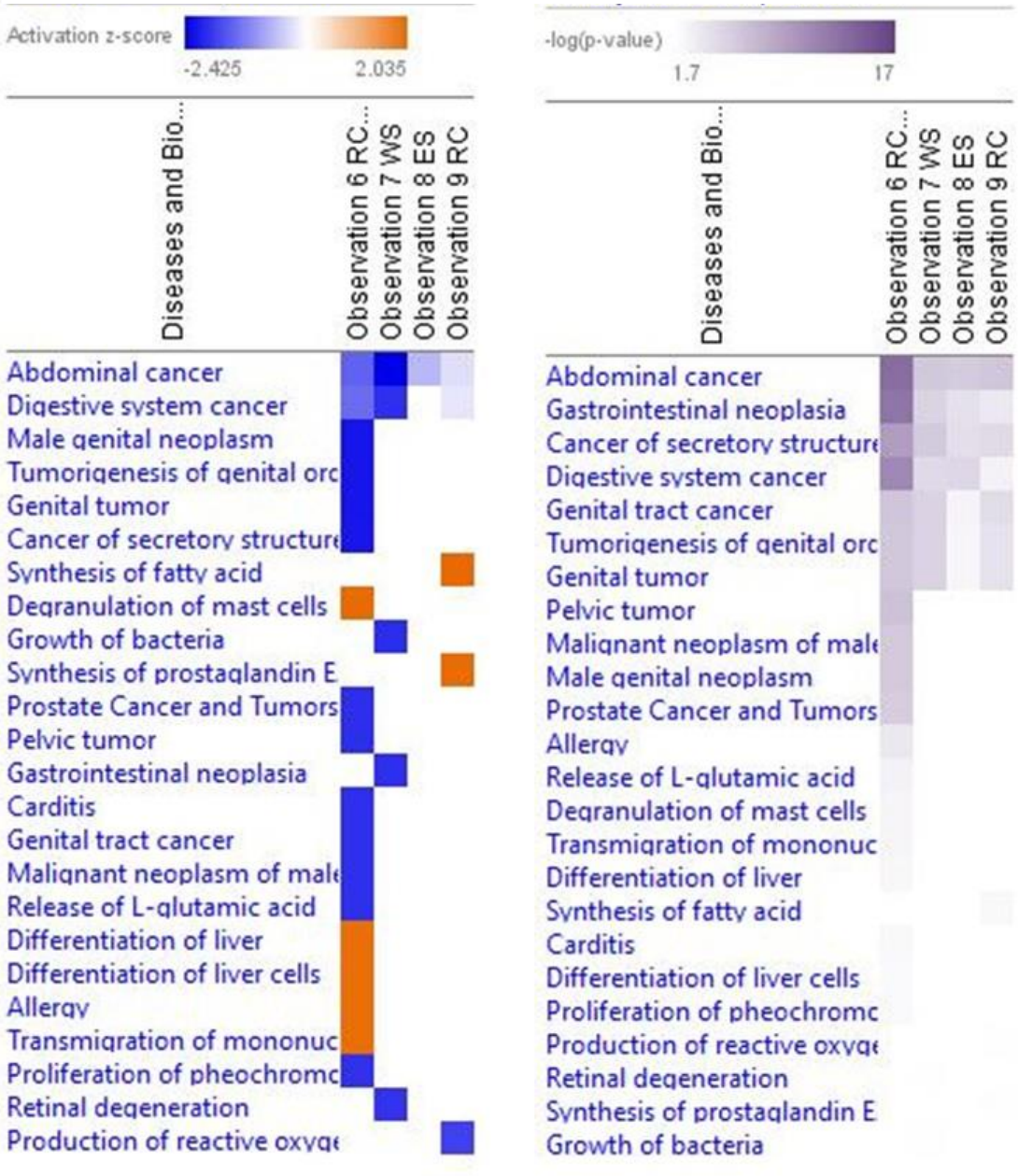
Combination

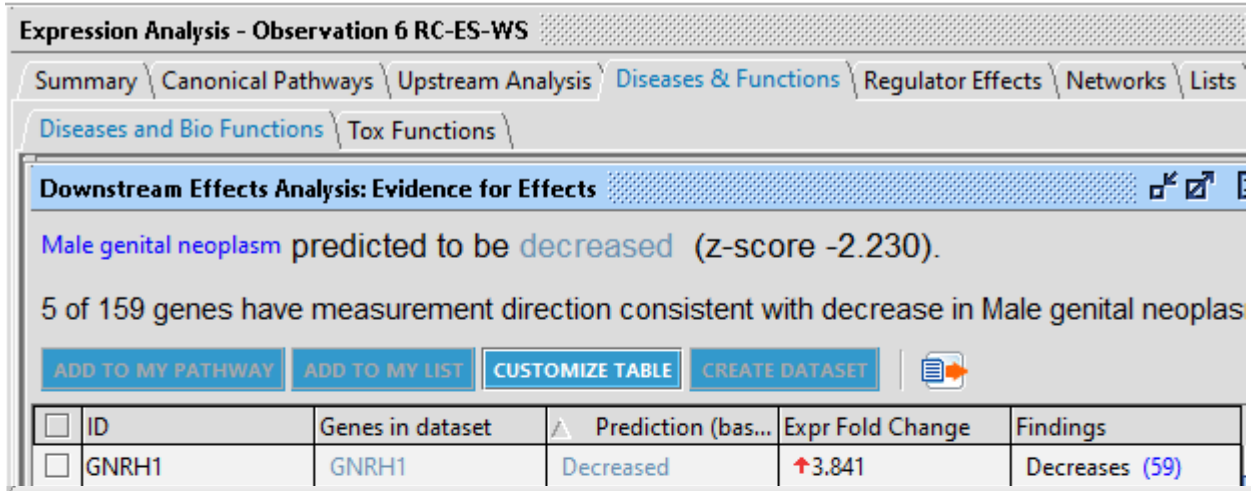
- Withania
- Eleutherococcus
- Rhaponticum

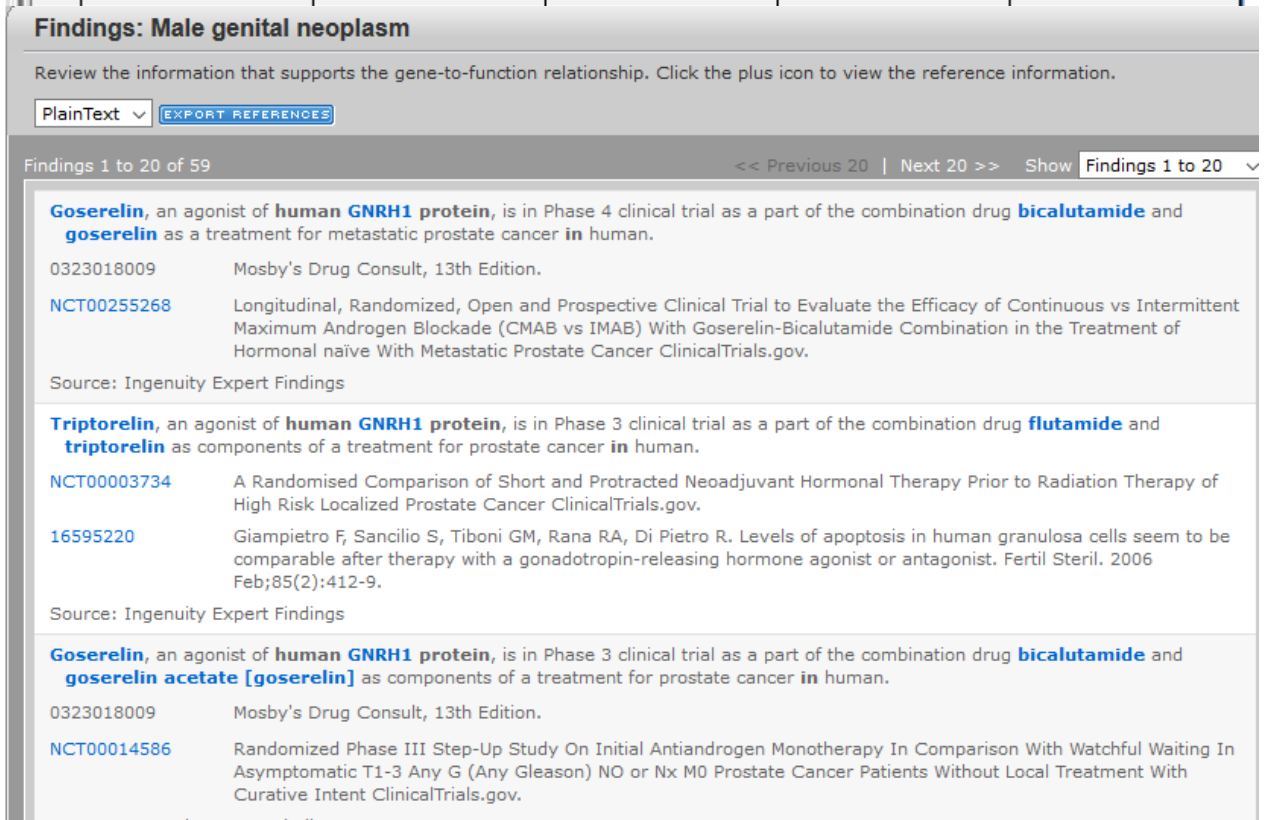
Comparison of effects on biological and cellular functions



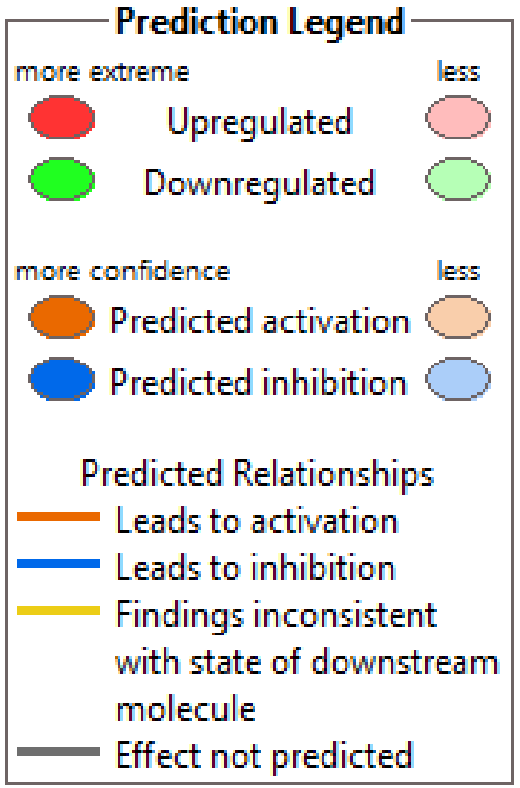
Comparison of effects on diseases

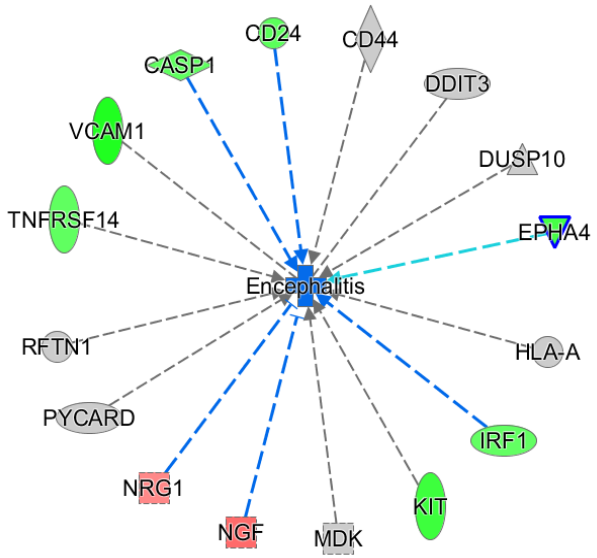






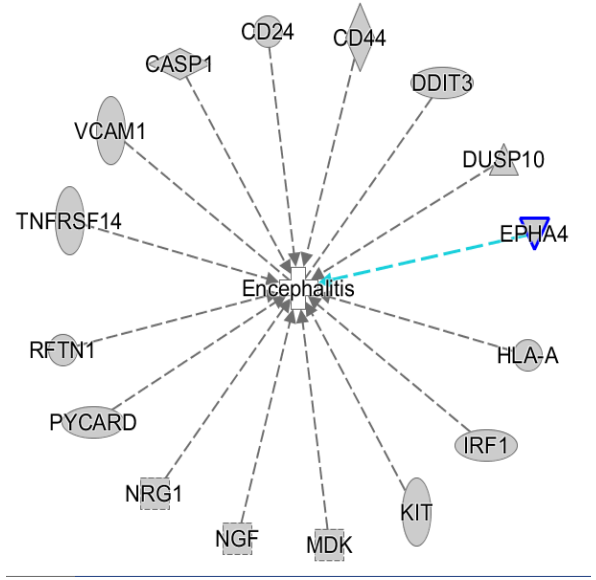
Product specific predictable effects on molecular network associated with encephalitis





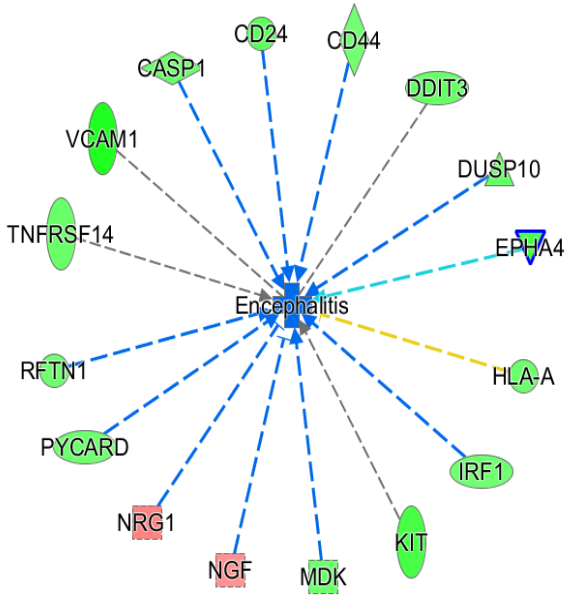
Andrographis

6 of 9 deregulated genes lead to predicted inhibition of encephalitis



Eleutherococcus

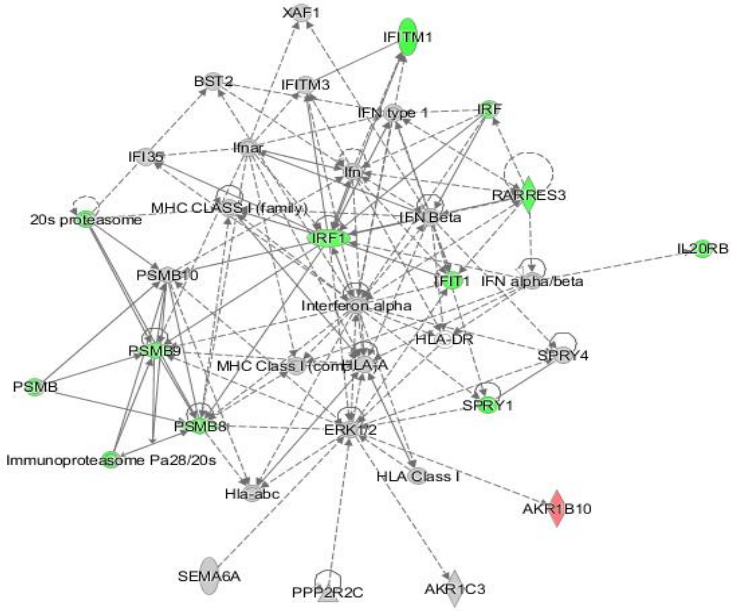
Only 1 gene is deregulated presumably with predicted inhibition of encephalitis



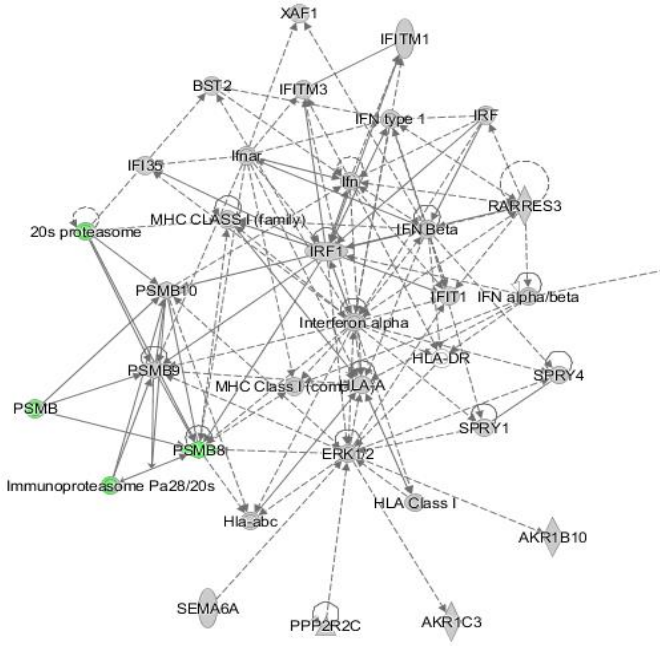
Kan Jang

11 of 16 deregulated genes lead to predicted inhibition of encephalitis

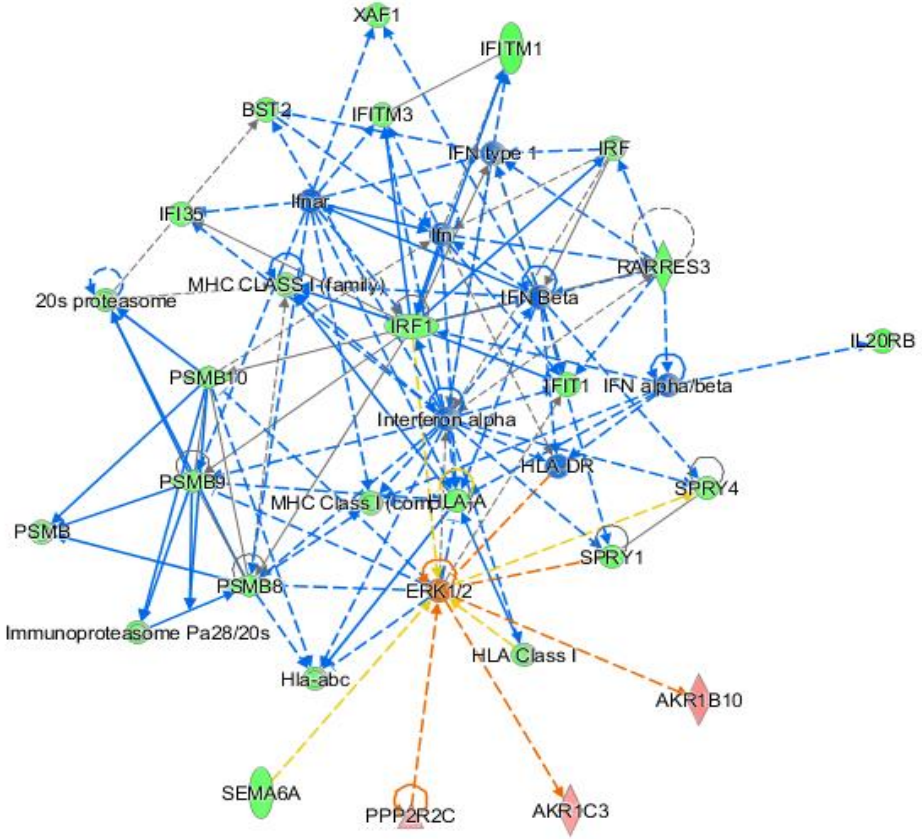
Inhibitory effects of Andrographis, Eleutherococcus extracts and their combination (AE) on molecular network associated with anti-inflammatory response



Andrographis



Eleutherococcus



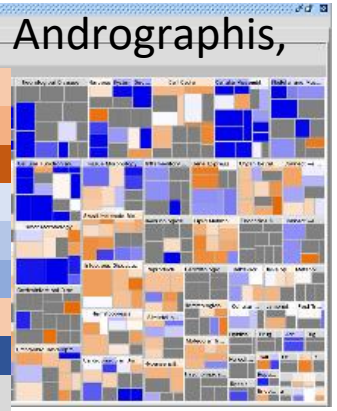
Combination Andrographis &Eleutherococcuc

Conditional “biological signatures” of Andrographis, Eleutherococcus and their combination (AE)

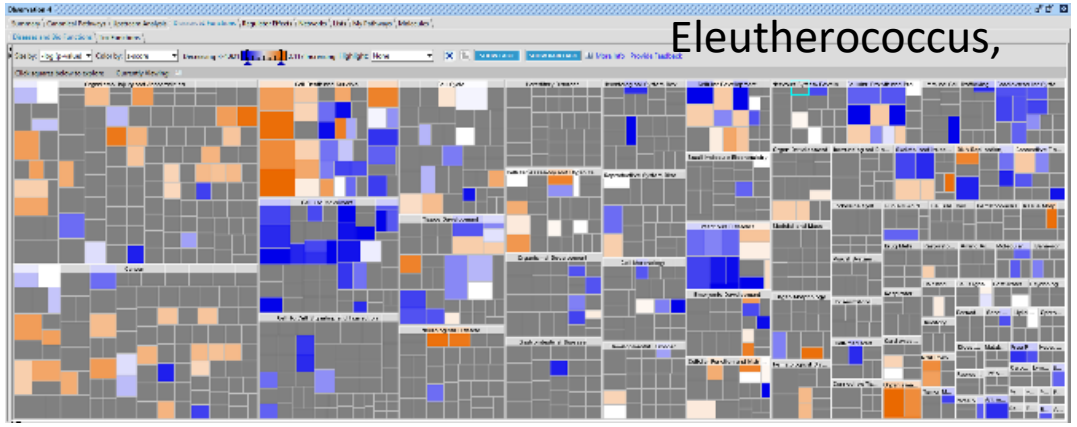
the color-coded heat-maps identify **functions and diseases** that are expected to increase or decrease.

Diseases or Functions Annotation

- antigen presentation
- clearance of virus
- antiviral response of myeloid cells
- emotional behavior
- locomotion
- active avoidance response
- sexual receptivity
- aggressive behavior
- appetite, etc.

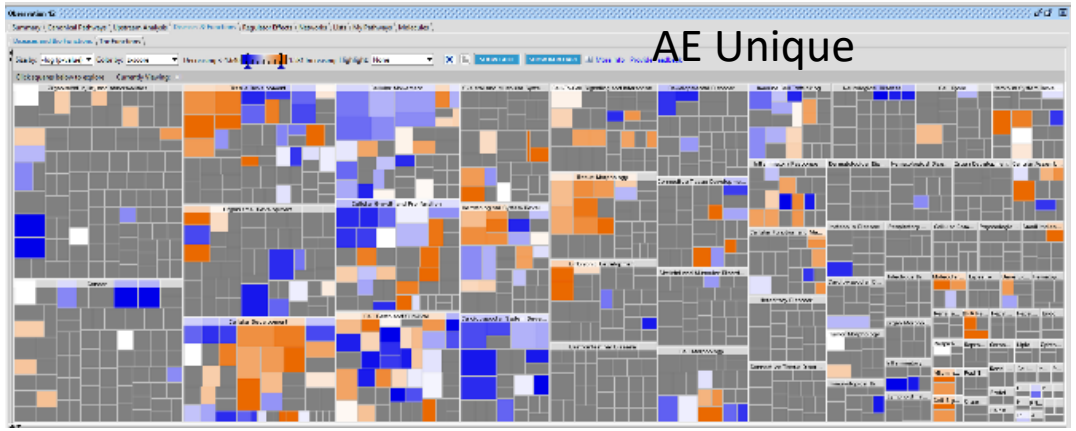


Andrographis,



Eleutherococcus,

AE



AE Unique

Unique signature

- Unlike to chemical reactions, when the chemical properties of purified compounds remain the same as in their mixture with other chemicals, biological activity/signature of their combination differs from signatures of ingredients.
- Combination of two or more plant extracts in one provides a qualitatively new substance, quite similar but different from its constituents.
- In fact, it is a “offspring” unique biologically active substance, a hybrid of “parent” ingredients.
- The expectations to have a sum of biological activities from two ingredients are illusive. The biological activity of their combination could be qualitatively different.

Conclusions related to observed synergistic and antagonistic interactions

- The specificity of a complex biological activity does not necessarily arise from the specificity of individual molecules, as these may act in different processes.
- Biological specificity results from the way in which these components assemble and function collectively.
- Component interactions, as well as the environment, gives rise to new features, such as network behavior, which are absent when considering the components.

Potential effects of adaptogens in ageing and stress induced disorders

❖ Inflammation – atherosclerosis
- Down regulation of CETP,
- Deregulation of GPCR,

❖ Neurodegeneration – impaired cognitive functions (learning, memory, abstract thinking, planning, organizing)
- Down regulation of cAMP
- Down regulation of ESR1
- Up-regulation of PKC
- Up-regulation of serpine
- Deregulation of GPCR,

❖ Metabolic disorders and energy metabolism–
- Down regulation of cAMP
- Inhibition of ATP metabolism

❖ Impaired apoptosis – Cancer
- Down regulation of ESR1, OLFM
- Up regulation of MAPK, IP3, PLC, DAG, PI3K
- Deregulation of GPCR

❖ Stress-induced mental and behavioral disorders
- Down-regulation of serotonin 5-HT3 GPCR
- Up regulation of IP3
- Down regulation of ESR1

New method of assessment of synergy of combinations of herbal extracts by transcriptome-wide microarray profiling

- Analysis of RNA microarray data from isolated cells and the comparison of the number of genes regulated by plant extracts and their fixed herbal formulation might be a useful tool/method for assessment of synergistic and antagonistic interactions of herbal extracts in human organism.

- Panossian A, Seo E-J, Wikman G. Efferth T. 2015. Synergy assessment of fixed combinations of Herba Andrographidis and Radix Eleutherococci extracts by transcriptome-wide microarray profiling. Phytomedicine, 22: 981-992
- Panossian, A., Hamm, R., Kadioglu, O., Wikman, G., Efferth, T., 2013. Synergy and antagonism of active constituents of ADAPT-232 on transcriptional level of metabolic regulation of isolated neuroglial cells. Front Neurosci. 7:16. http://journal.frontiersin.org/article/10.3389/fnins.2013.00016/abstract

Conclusions

- ❖Analysis of effects of herbal extracts on RNA microarray profiles of isolated cell lines is a valuable tool of drug discovery, understanding the mechanisms of action of herbal drugs and their possible clinical benefits.
- ❖Core Comparison Analysis, using:
  - the RNA sequencing or microarray data obtained in vitro experiments on isolated cells incubated with various herbal extracts, their combinations and purified compounds,
  - Integrative OMICS profiling database related to physiological functions and diseases, allows to predict their pharmacological activity and potential indications in medicine.