



Screening of anti-inflammatory compounds from soil algae extracts

Outline

- **Terrestrial microalgae**
- **Project outline and approach**
- **Assay for anti-inflammatory compounds**
- **Screening and validation of extracts**
- **Investigation of mode of action**

Terrestrial Microalgae

Algae collection

ASIB, Institute of Botany, University Innsbruck
Prof. Georg Gärtner

- Estimated >500 Culture strains, Slant agar cultures
 - Soil-, Air- and Lichen-algae
 - Origin: alpine area of central europe
- MCI ASIB 505 collection maintains 58 terrestrial microalgae and cyanobacteria strains which are currently under active research



ASIB Algensammlung Schrägagarkulturen

Features of Soil-Algae

- Primary producers
- Grout of the soil
- Symbiosis
- Survival under extreme conditions

Diverse ecological niches:

- Areas with high altitude
- On the surface or beneath soil
- Surface of stones, tree trunks, ...



Ever-changing,
extreme environmental conditions:

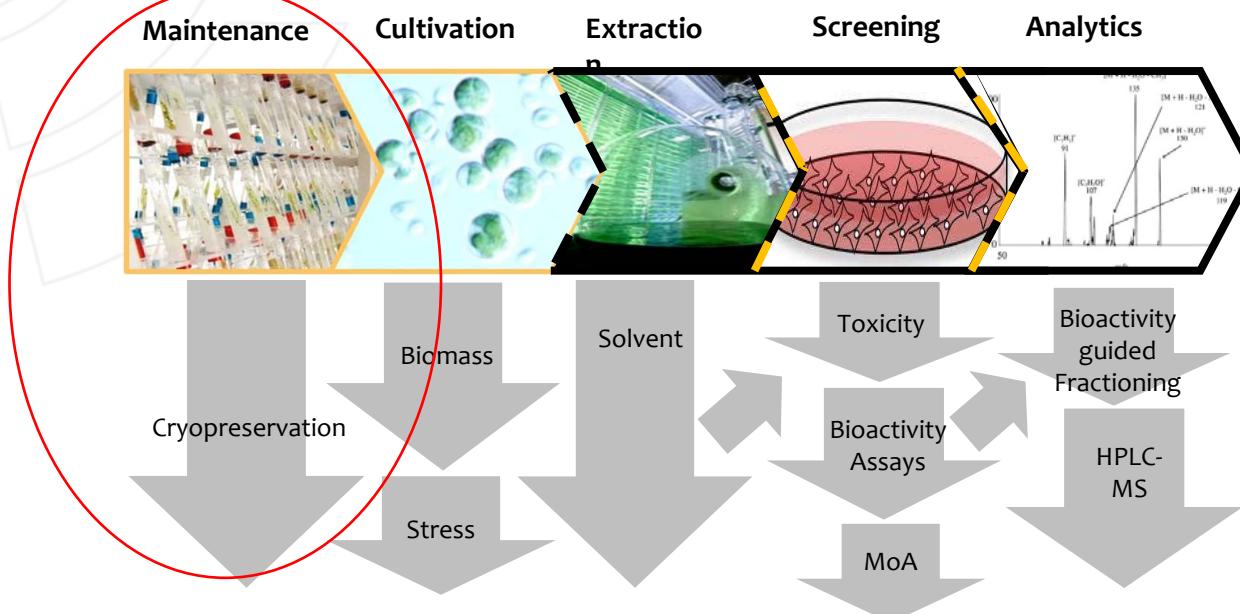
- Temperature
- Nutrient deficiency
- Desiccation
- UV radiation



Source: Getty Images/Lonely Planet Images

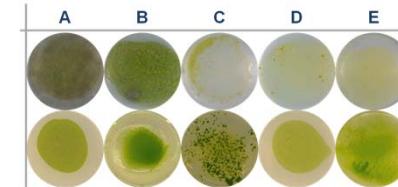


Bioactive metabolites



Generation of unicellular cultures:

- = get rid of contaminations
- = have only 1 algal strain in 1 sample
 - Physical Separation
 - Antibiotics
- take care of possible symbiosis
 - Not necessarily axenic culture!

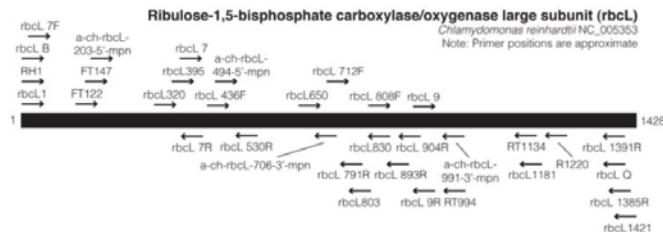


Purified cultures: *Chlorella vulgaris* (**A**), *Chlorococcum hypnosphorum* (**B**), *Geminella* sp. (**C**), IB273 (**D**), and *Nostoc punctiforme* (**E**)

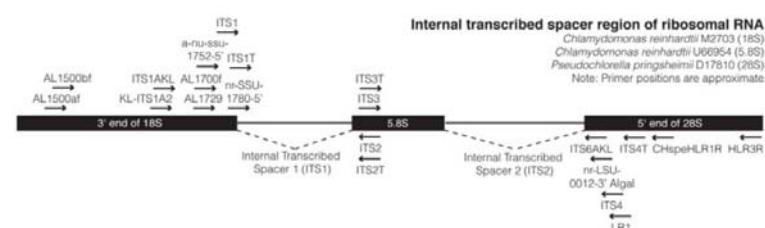
Genotyping

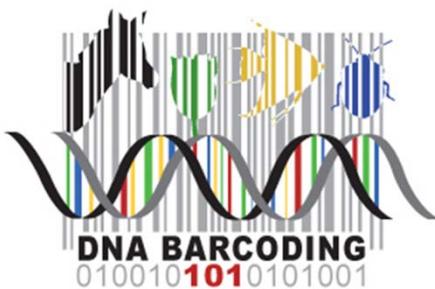
- Ribosomal RNA – Marker Gene for Molecular Phylogeny

Algal Ribulose 1,5-Bisphosphate Carboxylase Large Subunit (rbcL):

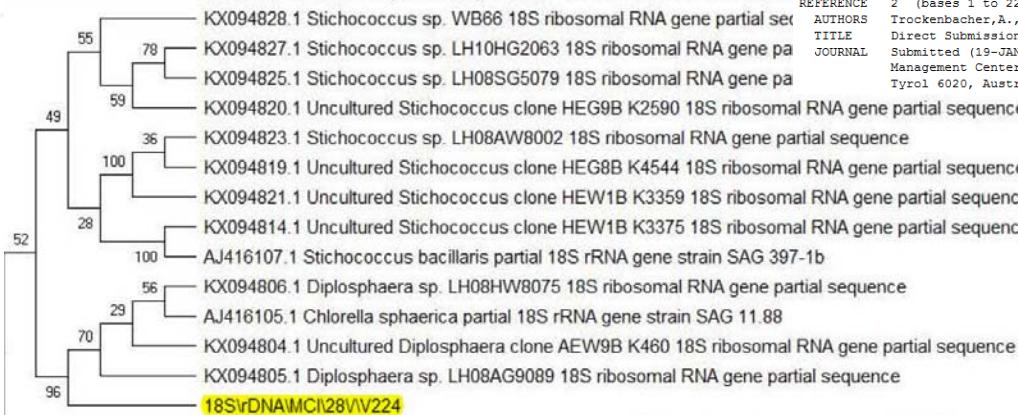


Algal Internal Transcribed Spacer (ITS):





- Molecular Phylogenetic analysis by Maximum Likelihood method



Diplosphaera sp. MCI 28 small subunit ribosomal RNA gene, partial sequence

GenBank: MK425685.1

[FASTA](#) [Graphics](#)

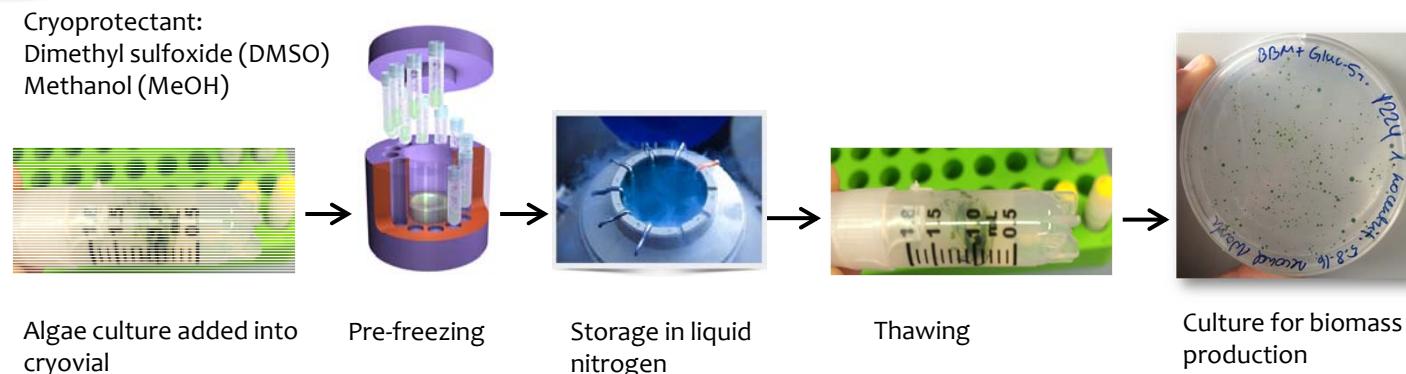
[Go to:](#)

LOCUS MK425685 2214 bp DNA linear PLN 28-JAN-2019
DEFINITION Diplosphaera sp. MCI 28 small subunit ribosomal RNA gene, partial sequence.
ACCESSION MK425685
VERSION MK425685.1
KEYWORDS.
SOURCE Diplosphaera sp. MCI 28
ORGANISM [Diplosphaera sp. MCI 28](#)
Eukaryota; Viridiplantae; Chlorophyta; Chlorophyceae;
Chaetophorales; Chaetophoraceae; Diplosphaera; unclassified
Diplosphaera.
REFERENCE 1 (bases 1 to 2214)
AUTHORS Trockenbacher,A., Rainer,B. and Dapra,L.
TITLE Identification of a unique N-methyl bound mycosporine-like amino acid in terrestrial microalgae Diplosphaera sp. 'Isolate'
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 2214)
AUTHORS Trockenbacher,A., Rainer,B. and Dapra,L.
TITLE Direct Submission
JOURNAL Submitted (19-JAN-2019) Biotechnology & Food Engineering,
Management Center Innsbruck MCI, Maximilianstrasse 2, Innsbruck,
Tyrol 6020, Austria

- NCBI GenBank Entry

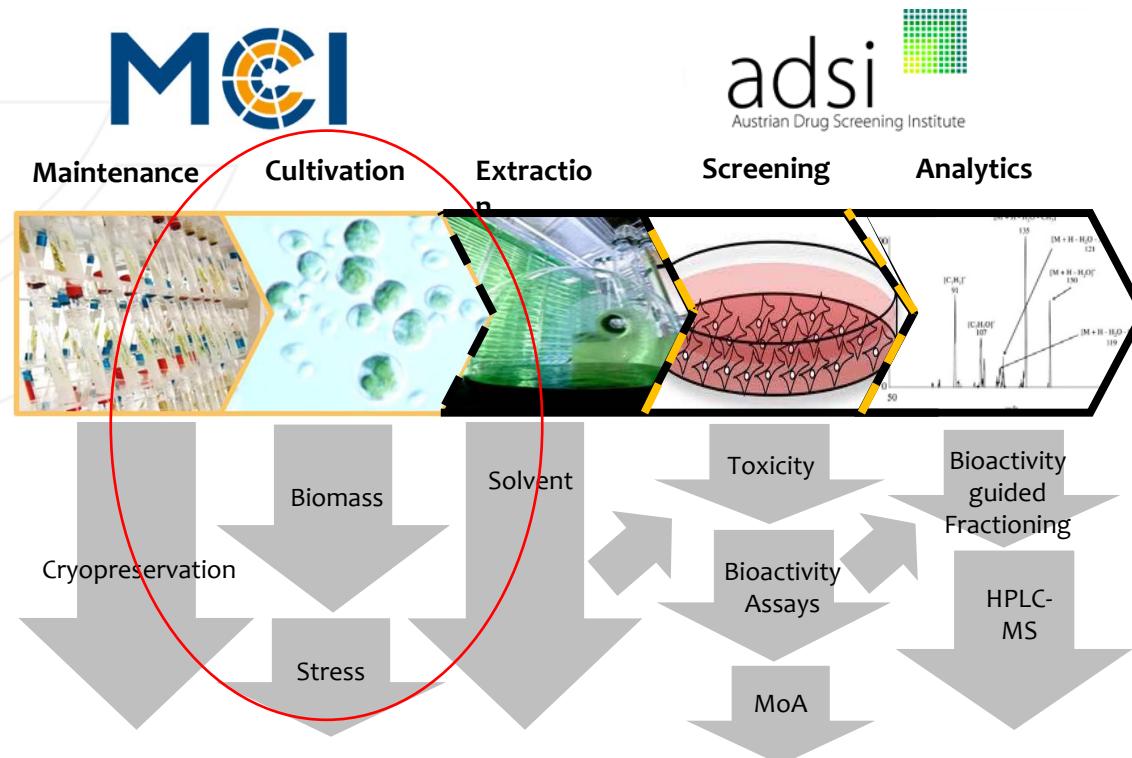
Advantages for long term preservation:

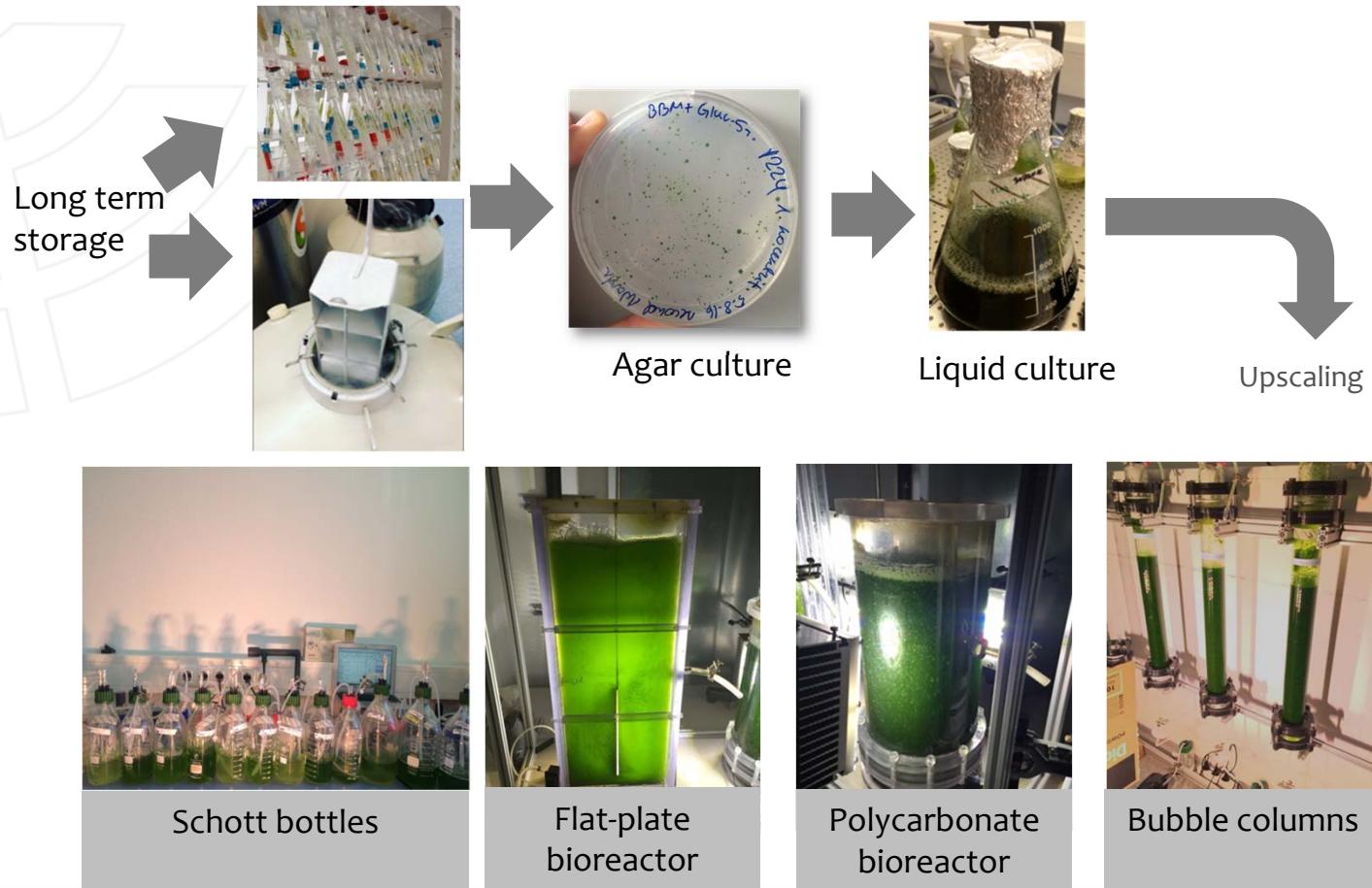
- Reduced maintenance requirements
- Maintain viability and genetic integrity
- Establishment of a biobank with pure algae strains

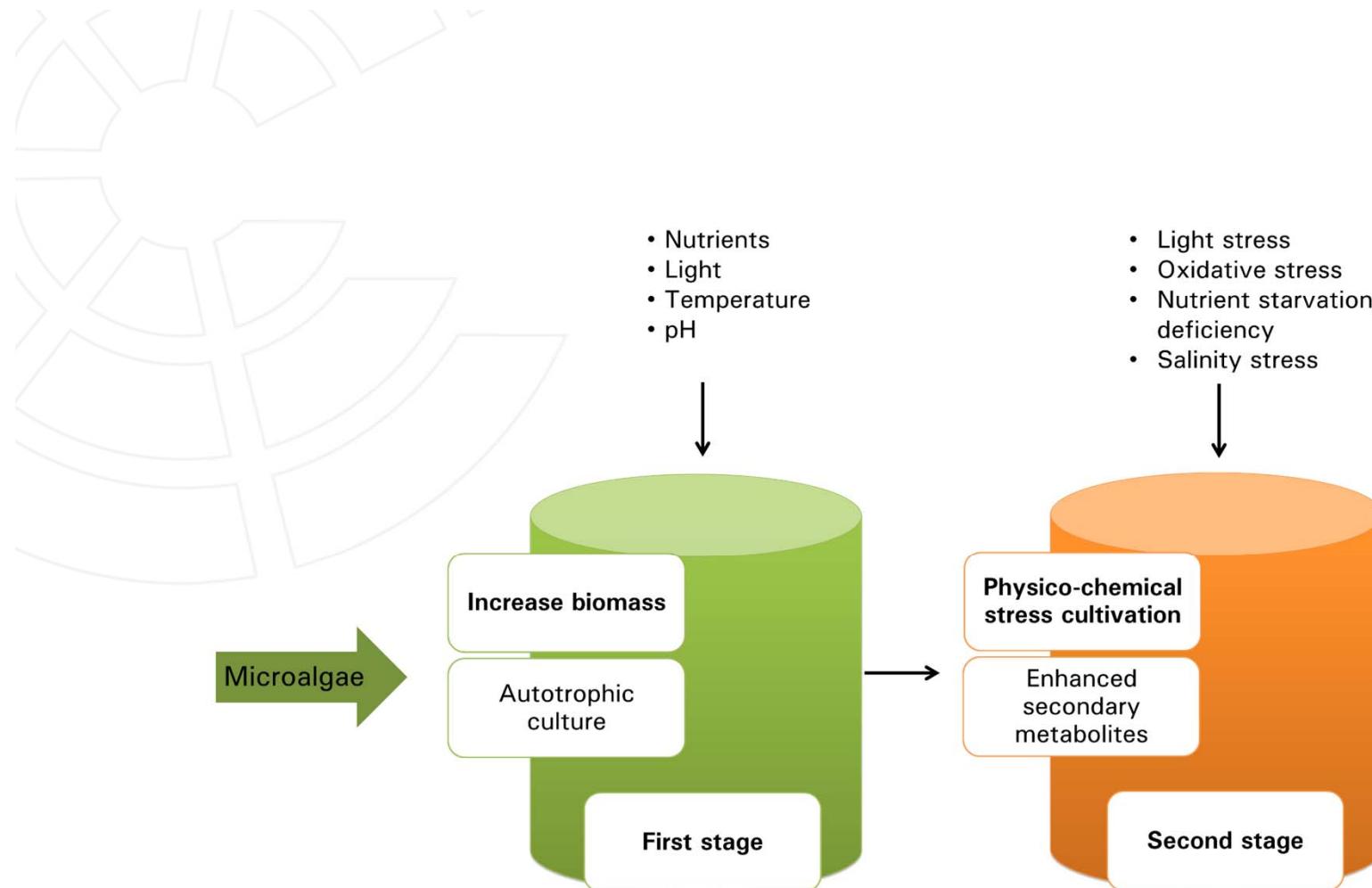


<http://www.biocision.com/products/cool-cell-freezing-containers/>

<http://www.fertilityinstitutesandiego.com/our-services/fertility-treatment/in-vitro-fertilization-ivf/embryo-cryopreservation/>



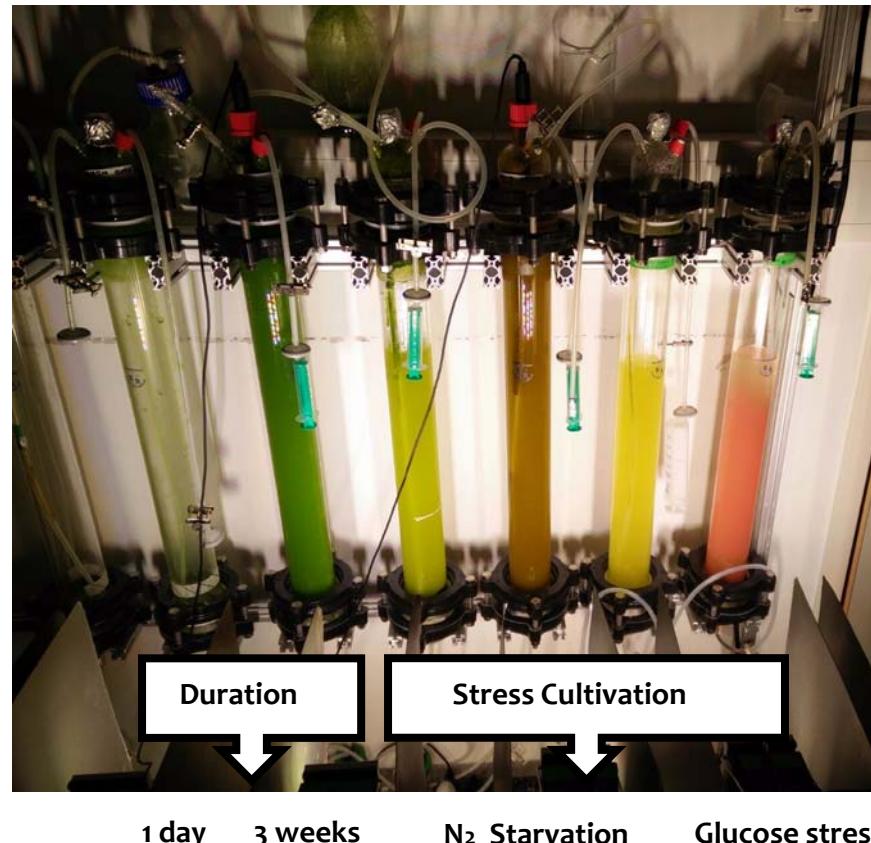


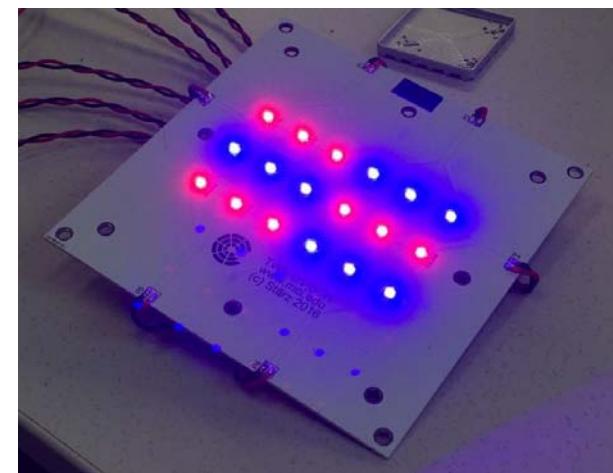
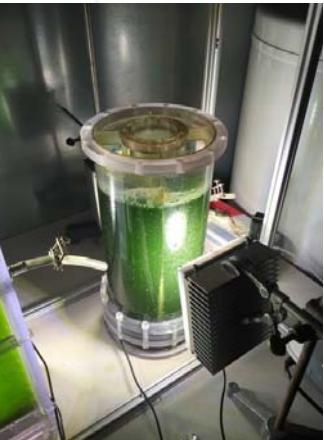


Bubble Columns

- Volume 1 l
- Optimal light conditions
- Gasing in with filtered air and CO₂
- Autoclavable

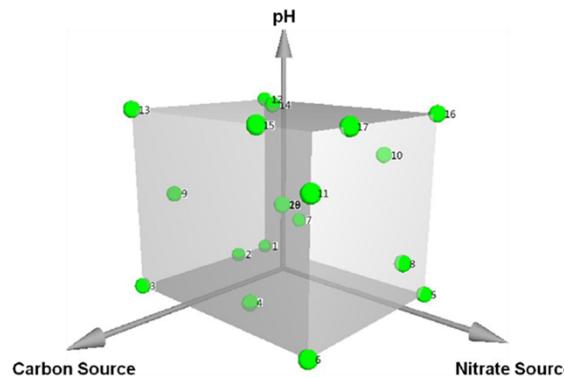
Variation of culture conditions:
Induction and accumulation of bioactive metabolites



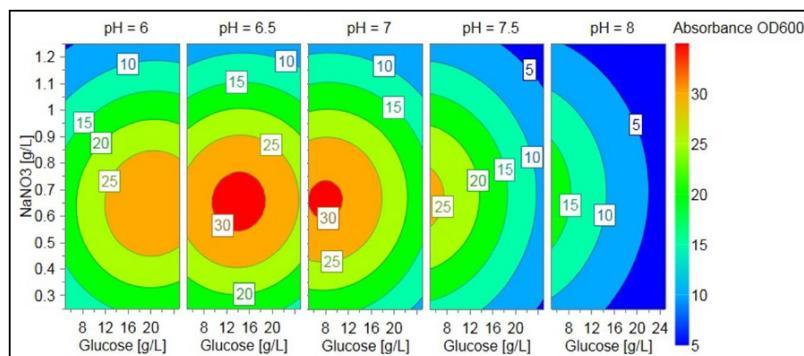
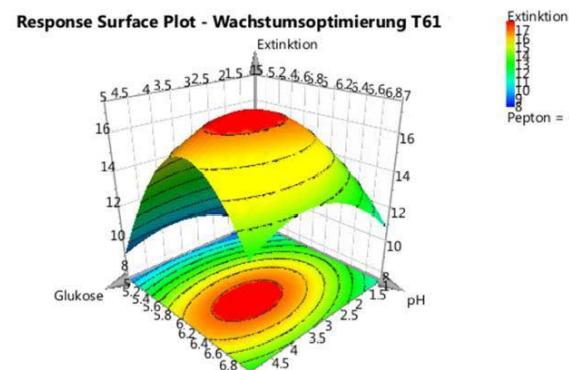


Example of stress cultivation: factors for DoE

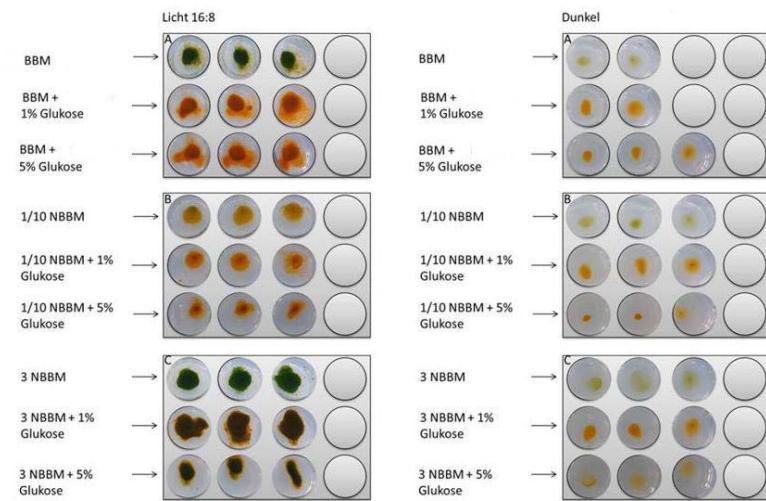
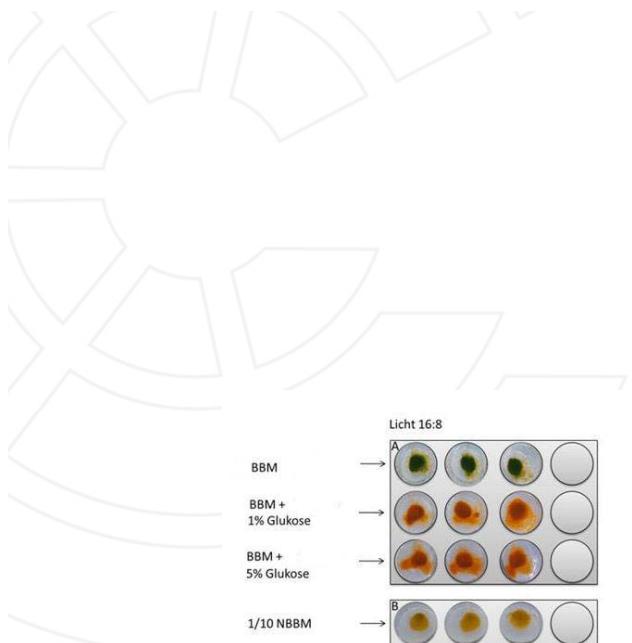
Factors:	Carbon Source	pH	Nitrogen Source			
Assay	Assay A-D	Assay A-D	Assay A	Assay B	Assay C	Assay D
Name	Glucose	pH	NaNO ₃	NH ₄ Cl	Urea	Peptone
Abbr.	Glu	pH	NO ₃	NH ₄	U	P
Unit	g L ⁻¹		g L ⁻¹	g L ⁻¹	g L ⁻¹	g L ⁻¹
Type	Multilevel	Multilevel	Multilevel	Multilevel	Multilevel	Multilevel
Settings	5/ 10/ 15/ 20/ 25	6/ 6.5/ 7/ 7.5/ 8	0.25/ 0.5/ 0.75/1/1.25	0.25/ 0.5/ 0.75/ 1/1.25	0.125/ 0.25/ 0.5/ 0.75/ 1	0.5/ 1.5/ 2.5/ 3.5/ 4.5
Precision	0.5	0.05	0.025	0.025	0.0219	0.1

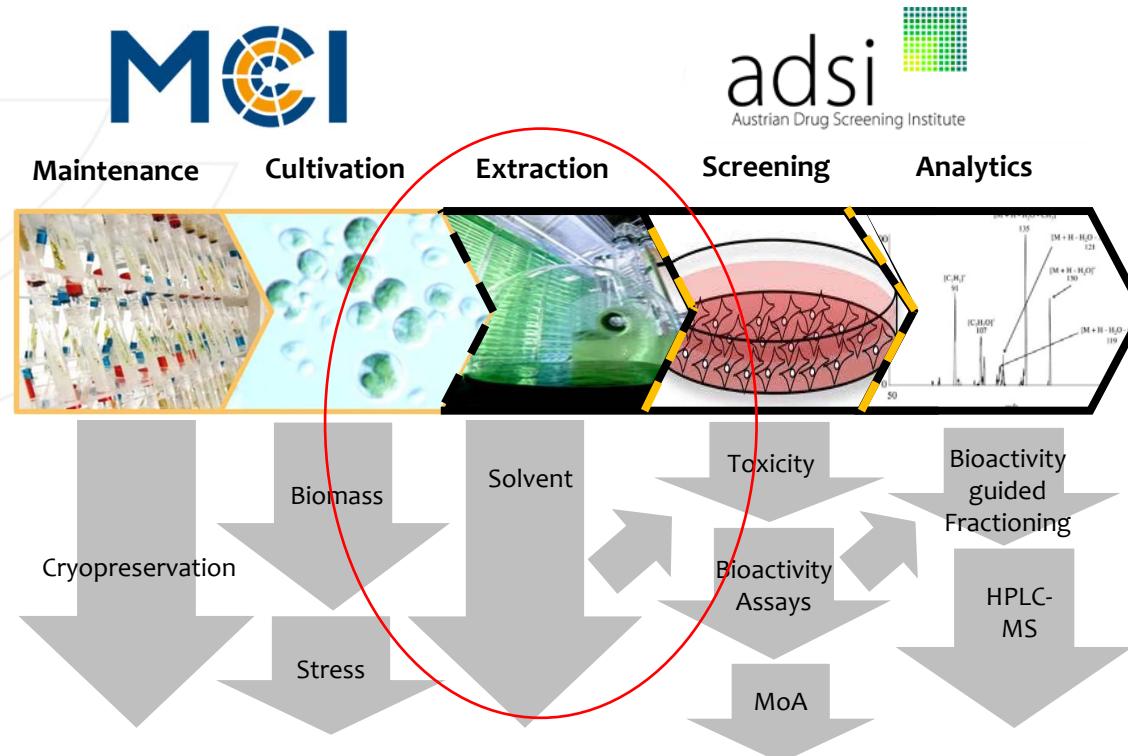


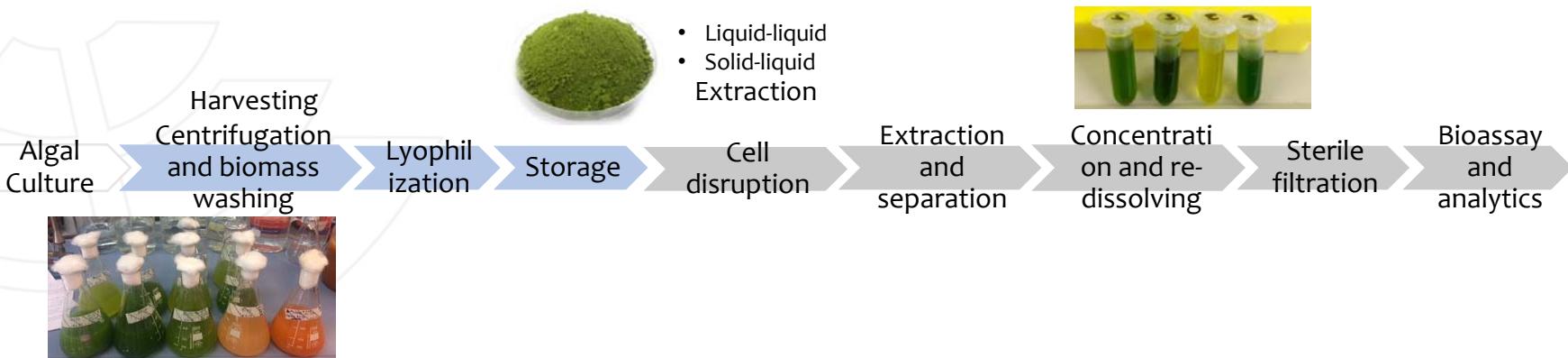
Example of stress cultivation: DoE Contour Plot



Maximum biomass production:
Initial pH of 6.6, 12.4 g L⁻¹ glucose,
and 0.66 g L⁻¹ NaNO₃







Main parameters driving the selection of an extraction method:

- Extracted molecules
- Velocity
- Amount of solvent use /toxicity of solvent
- Reproducibility
- Extraction yield
- Extraction sensitivity (pigments)
- Selectivity
- Protection of extraction molecules against chemical transformation
- Dimension
- Cost
- Easiness
- Solvent safe equipments

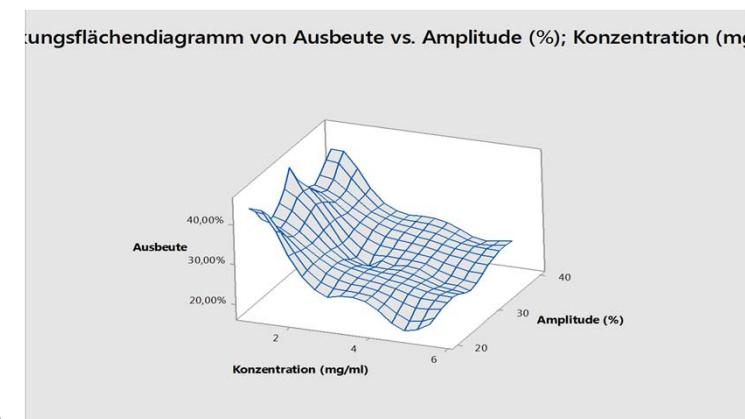
Mechanical cell disruption:

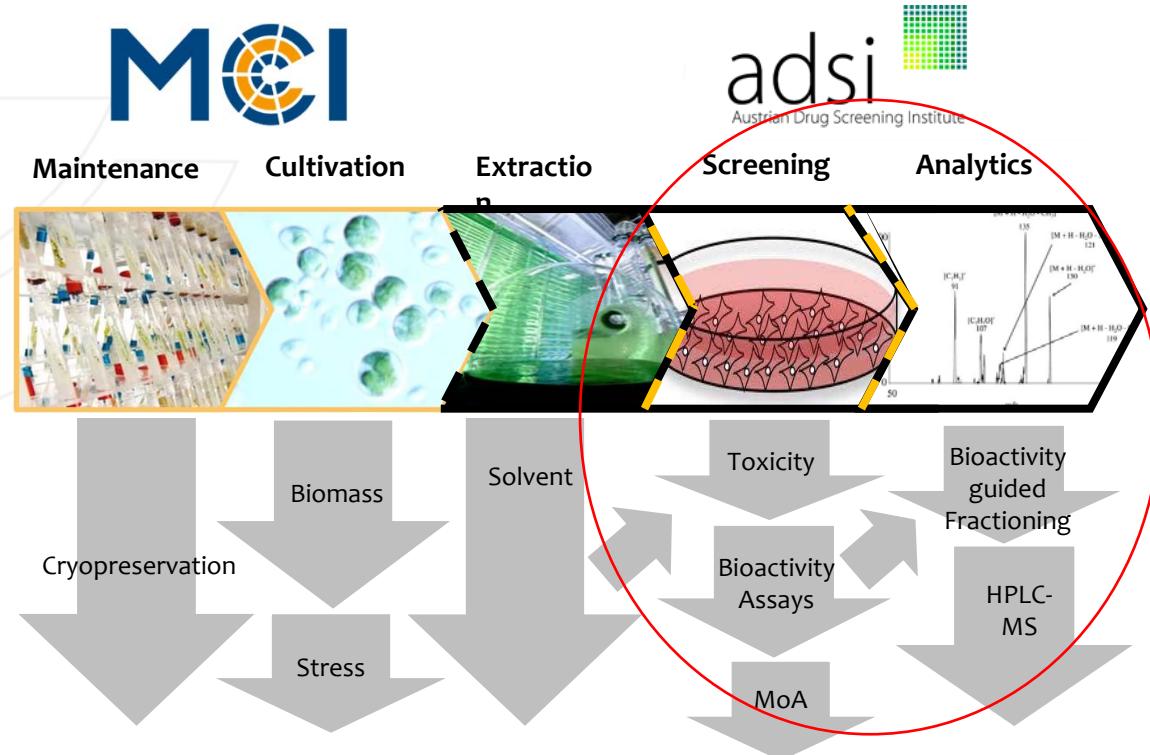
- Bead mill
- Homogenization
- Ultrasound
- Sonication
- Glass beads
- Grinding (mortar and pestle)
- French pressure

Disruption solvents:

- Methanol
- Ethanol
- Hexane
- Dimethylformamid (DMF)
- Dichloromethane (DCM)
- Acetone
- Dimethyl sulfoxide (DMSO)

- **Assessment of cell disruption using Bradford assay (protein concentration)**
- **Comparison of extraction methods**
 - Bead mill
 - Ultraturrax
 - Ultrasound bath
 - Ultra sound probe
- **Design of Experiments – Optimization of parameters**
 - time
 - amplitude
 - concentration dry mass/solvent





Screening methods



Bioactivity

Disk Diffusion Method for Antibacterial/antibiotics and Antifungal Susceptibility Testing

Cell-based Screening Assays:
Toxicity, Inflammation, Oxidative Stress

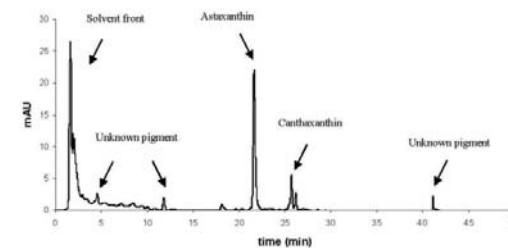


<http://www.asmscience.org/content/education/imagegallery/image.2716>



Analytics

- uHPLC-qTOF-MS/MS
- GC-MS
- PUFAs (DHA, EPA)
- Mycosporine-like amino acids (MAAs)
- Pigments (Astaxanthin, Canthaxanthin)

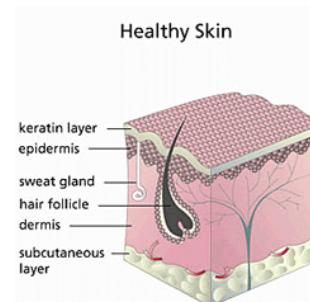


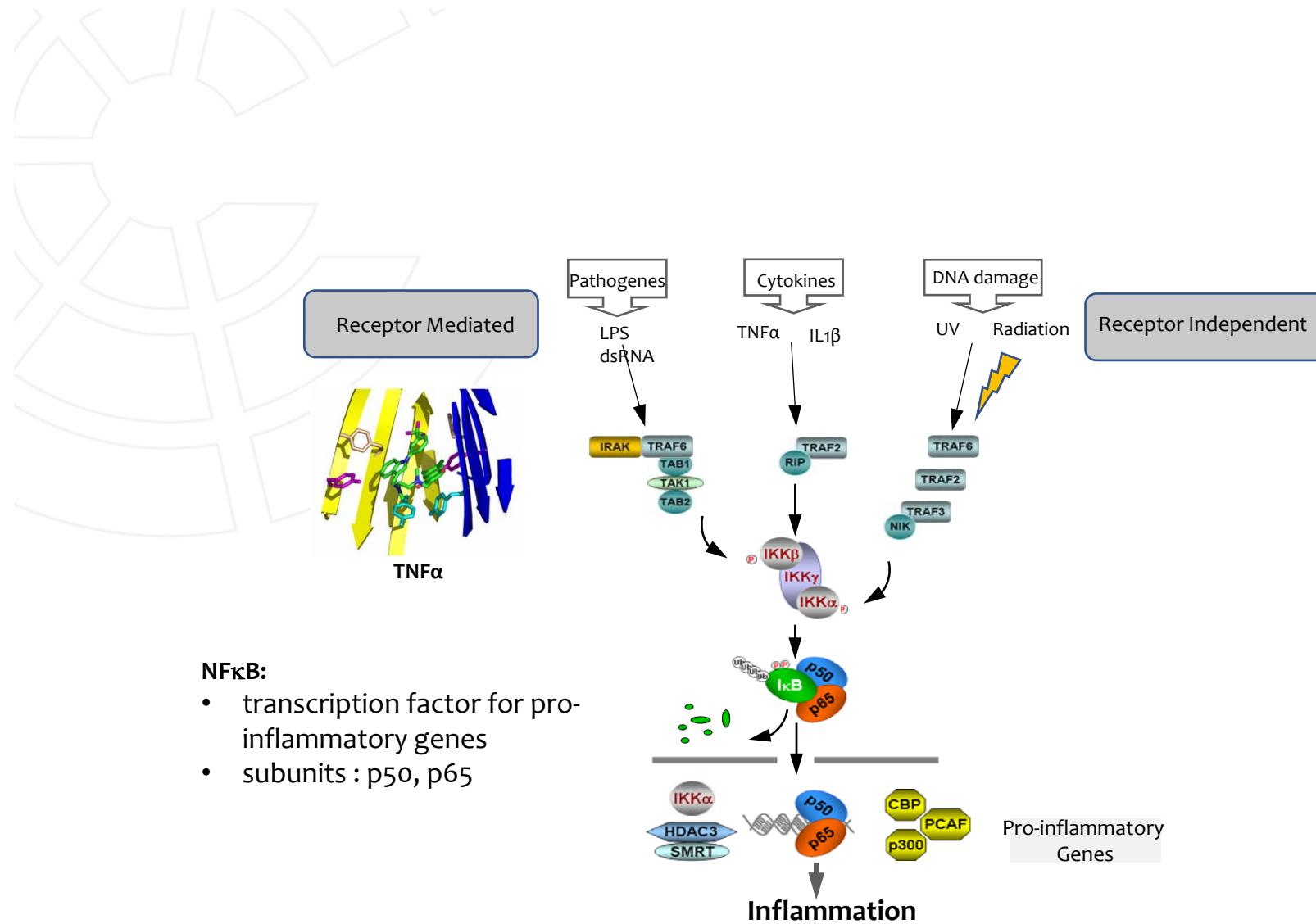
<http://www.mdpi.com/1660-3397/10/6/1400/htm>

Inflammation and skin

Inflammation is a crucial biological process for maintaining the body's homeostasis → necessary for fighting pathogens and for the repair of damaged tissue

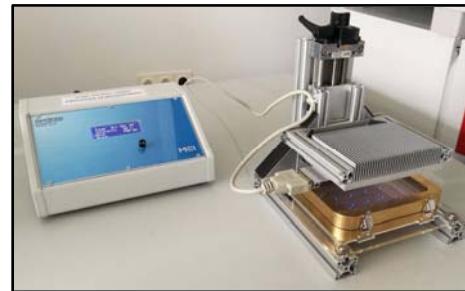
- Approved anti-inflammatory agents → therapy is often not effective enough or is hampered by intolerable side effects
- NF-κB has a central role in the regulation of cellular responses to inflammatory stimuli





MCI UVB LED Box prototype

- Designed and build at the MCI
- UVB LED's (peak wavelength 310 nm)
- Adjustable intensity and irradiation time



VILBER BIO-SUN

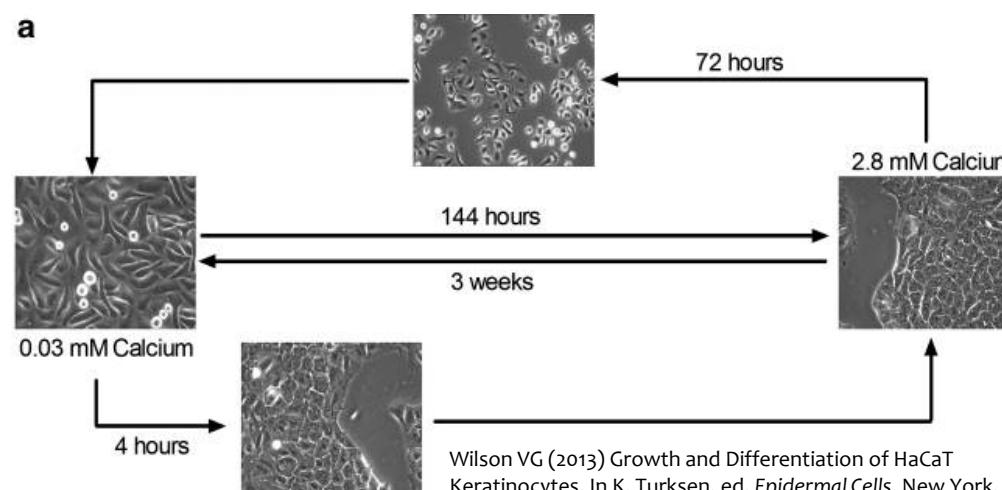
- UVA (peak wavelength 365 nm)
- UVB (peak wavelength 312 nm)
- UVC (for sterilization)
- Sensor-triggered radiation
- => UV dose in J/cm²



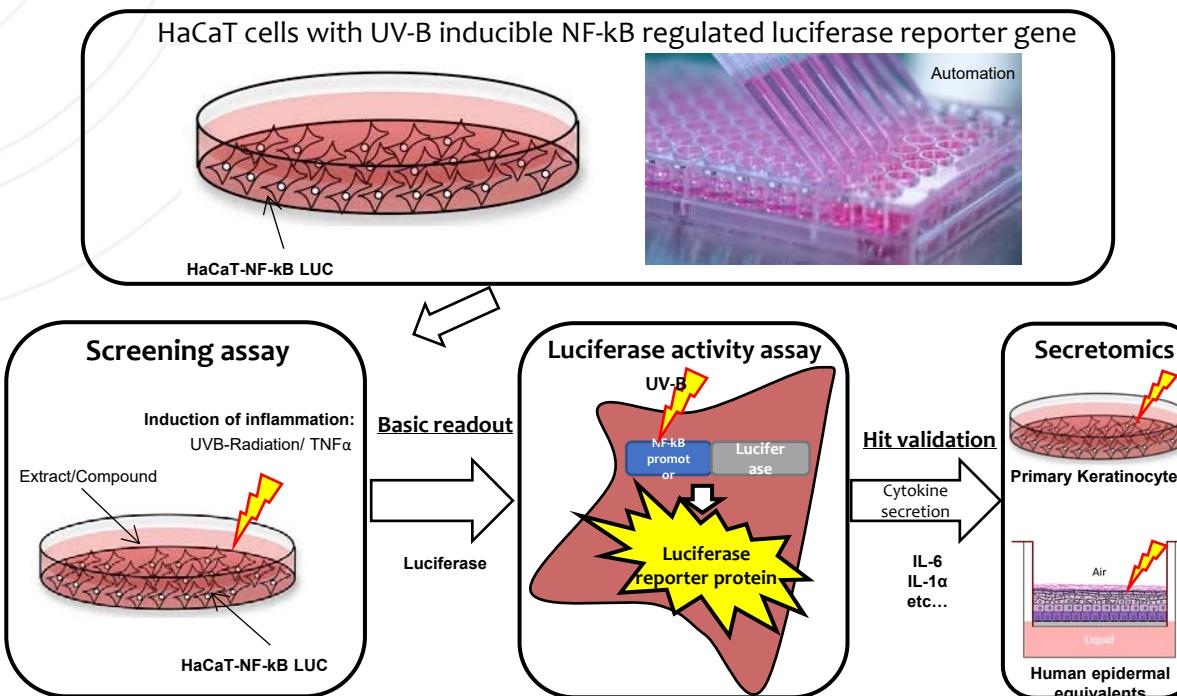
HaCaT cells are a **spontaneously immortalized, human keratinocyte** cell line that has been widely used for studies of skin biology and differentiation.

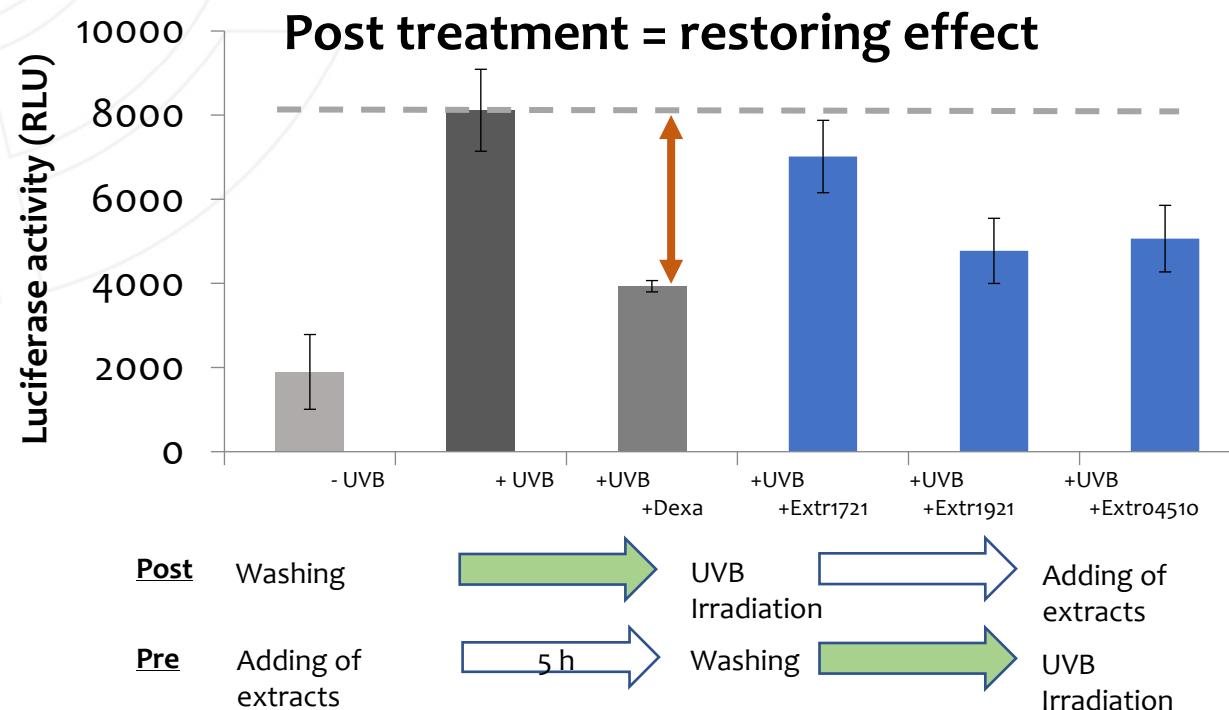
Retain all the **functional differentiation properties** of normal keratinocytes

HaCaT cells in culture can **revert back and forth** between a differentiated and a basal state upon changes in calcium concentration in the medium.



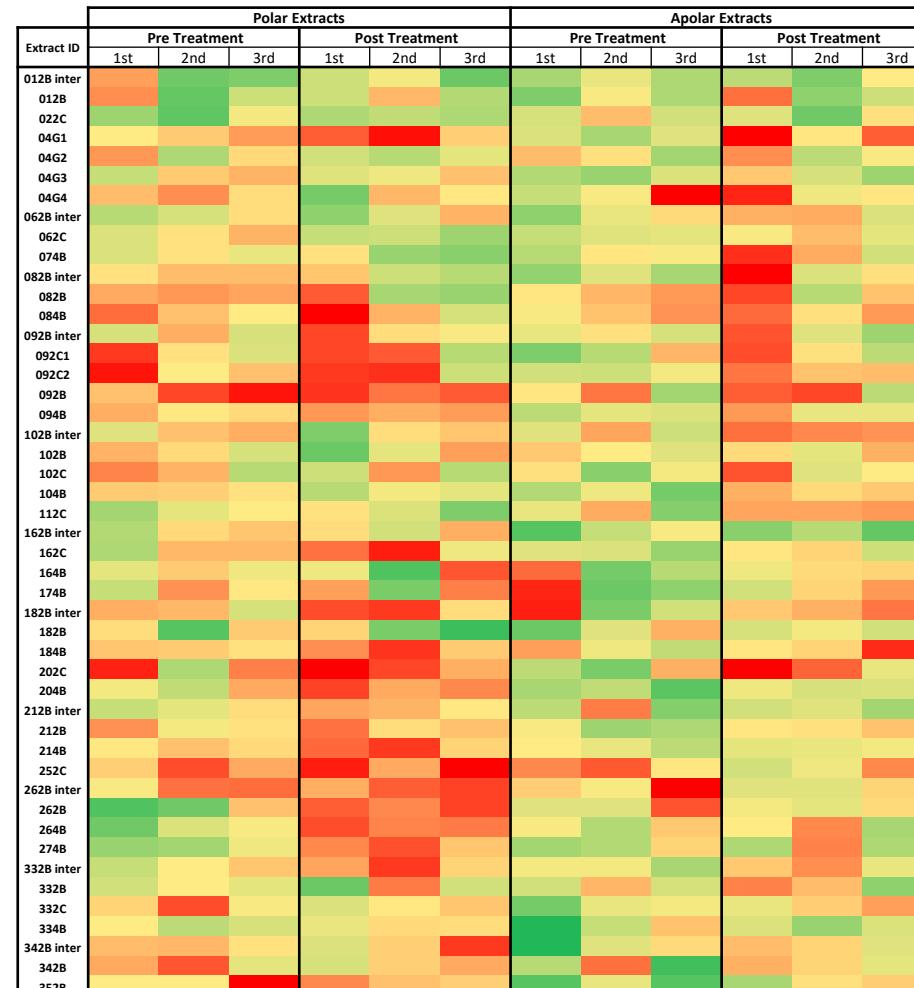
Wilson VG (2013) Growth and Differentiation of HaCaT Keratinocytes. In K. Turksen, ed. *Epidermal Cells*. New York, NY: Springer New York, pp.33–41.





Hits:

- Apolar:
 - Pre-Treatment: 19
 - Post-Treatment: 8
- Polar:
 - Pre-Treatment: 9
 - Post-Treatment: 12
- Overlap
 - Polar: 6
 - Apolar: 6



Extract ID	Polar Extracts						Apolar Extracts					
	Pre Treatment			Post Treatment			Pre Treatment			Post Treatment		
	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
012B inter												
012B	orange	green	yellow	green	orange	green	green	yellow	green	green	yellow	yellow
022C	green	green	yellow	green	green	green	green	green	green	green	green	yellow
04G1												
04G3										orange	green	green
062B inter	green	yellow	green	green	yellow	orange	green	green	green	green	yellow	yellow
062C				green	green	green	green	green	green			
074B				yellow	green	green	green	green	green			
082B inter							green	green	green			
092C2							green	green	yellow			
094B							green	green	yellow			
102B	green	yellow	green	green	yellow	orange	green	green	green			
104B				green	yellow	green	green	yellow	green			
112C	green	yellow	green	green	yellow	green	green	yellow	green			
162B inter							green	green	yellow	green	green	green
162C							green	green	yellow	green	green	green
182B				yellow	green	green	green	green	green	green	yellow	yellow
202C							green	green	green	green	green	green
204B							yellow	green	green	green	green	green
212B							green	green	green	green	green	green
212B inter							green	green	yellow			
262B	green	green	orange									
264B	green	yellow	green									
274B	yellow	green	green									
332B				green	yellow	green	green	yellow	green			
334B	yellow	green	green									
352B							green	yellow	green			



Validation of hits → Dose dependency

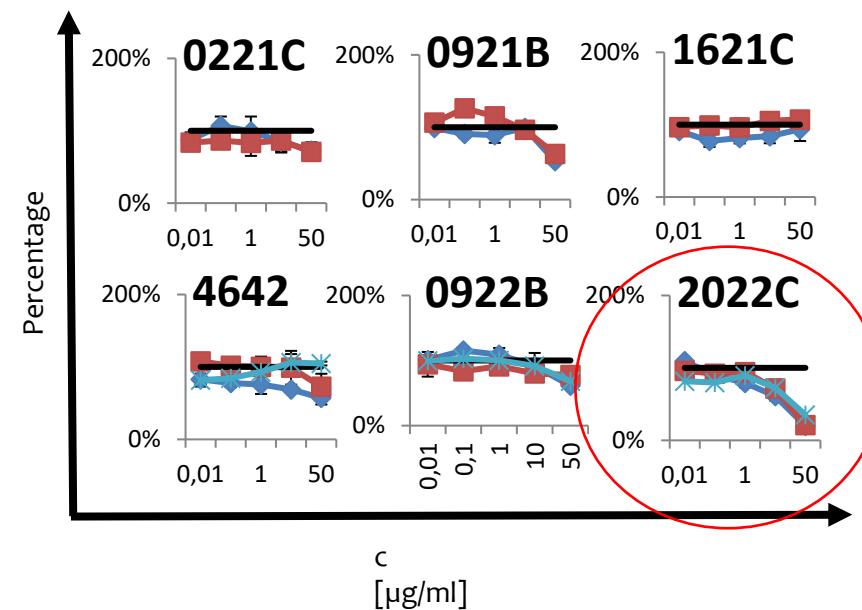
- Investigation of dose dependency of each hit
- Effect of inflammatory response with 5 concentrations:

50, 10, 1, 0.1, 0.01 µg/ml

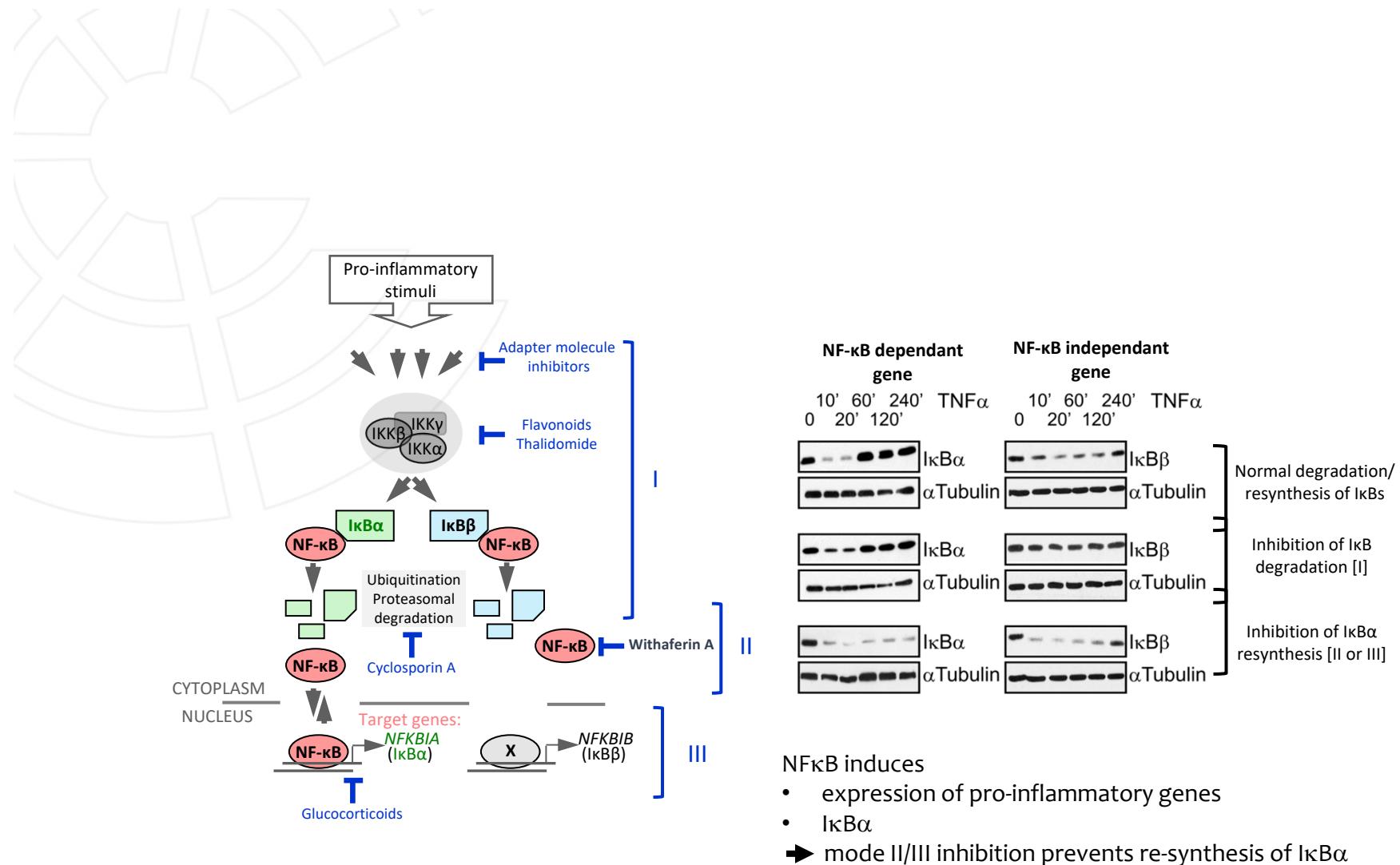
Investigation of dose dependence

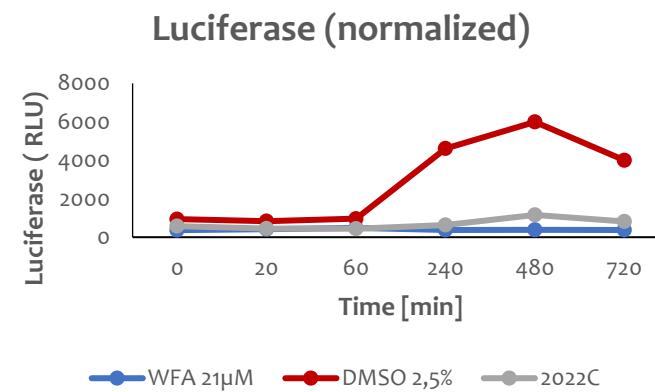
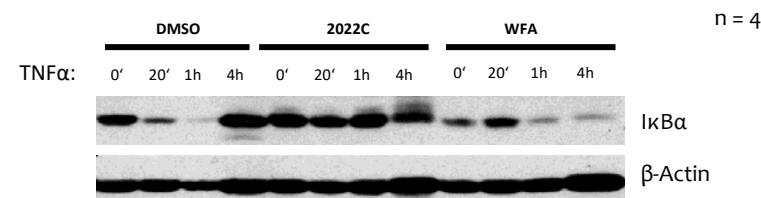
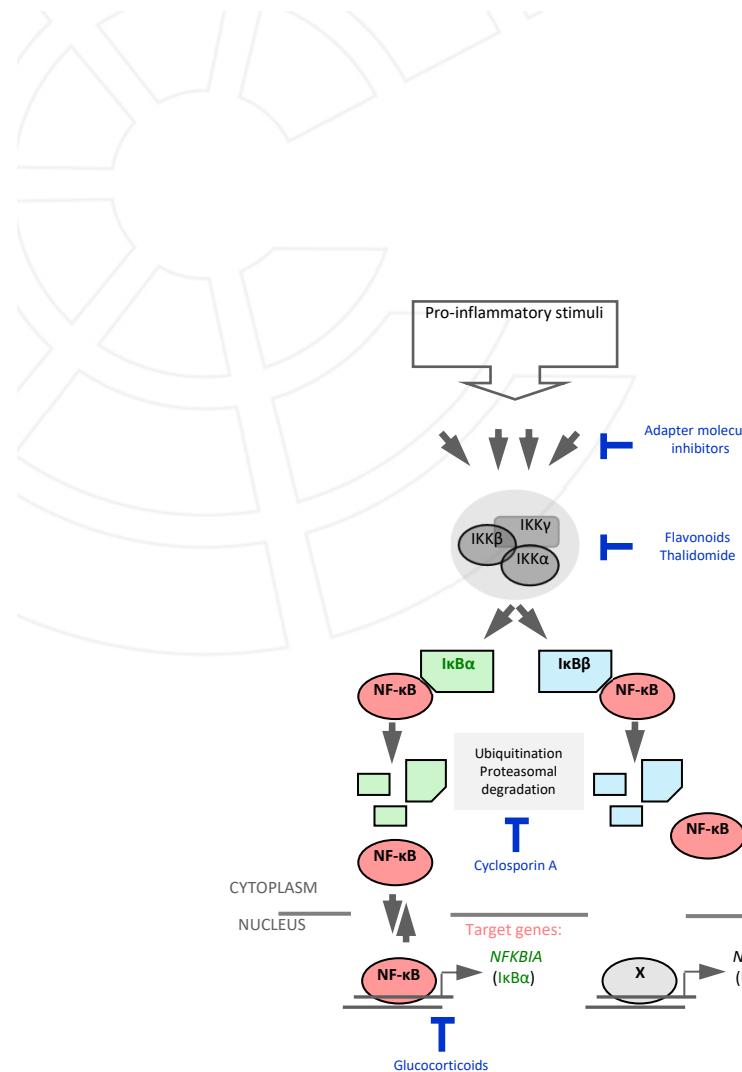
Effect of inflammatory response with
5 concentrations:
50, 10, 1, 0.1, 0.01 µg/ml

Normalized to Control



Extract 2022C as promising inhibitor of inflammation





Extract 2022C inhibits in mode I:
at IKK complex

- ✓ focus on terrestrial microalgae (mainly from collection ASIB 505)
- ✓ purification and characterization of strains
- ✓ cultivation under optimal conditions for biomass generation
- ✓ application of stress conditions (DoE) and extraction
- ✓ establishment of inflammation model in skin cells
- ✓ screening of extracts for anti-inflammatory effects
- ✓ hit: extract 2022C
- ✓ elucidation of mode of action
- ✓ extract 2022C: patent applied for



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Thank you for your attention!

mentoring the motivated.