# INTESTINAL *IN VITRO* CULTURES AS A VALUABLE TOOL FOR HAZARD ASSESSMENT OF NANOMATERIALS

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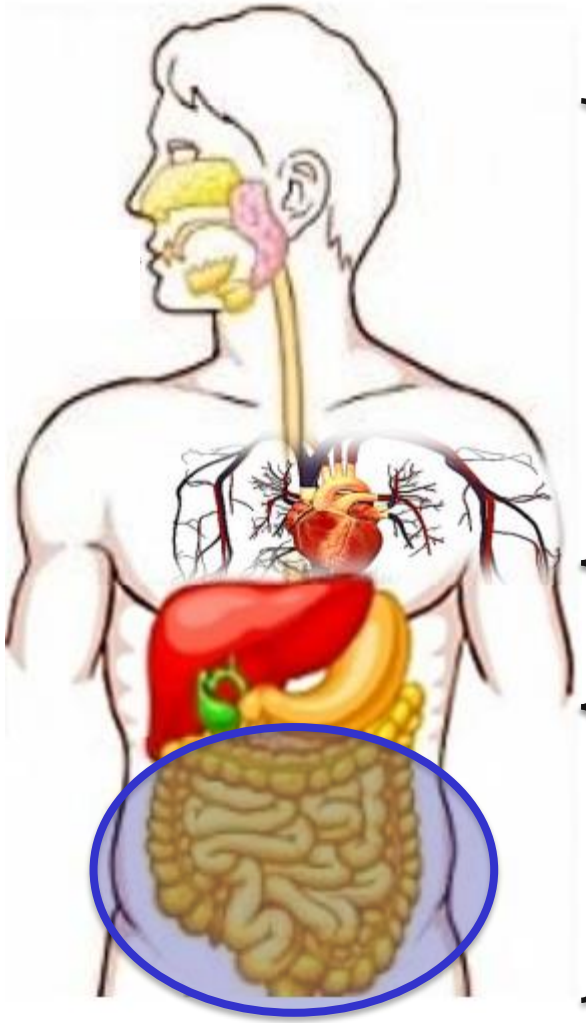
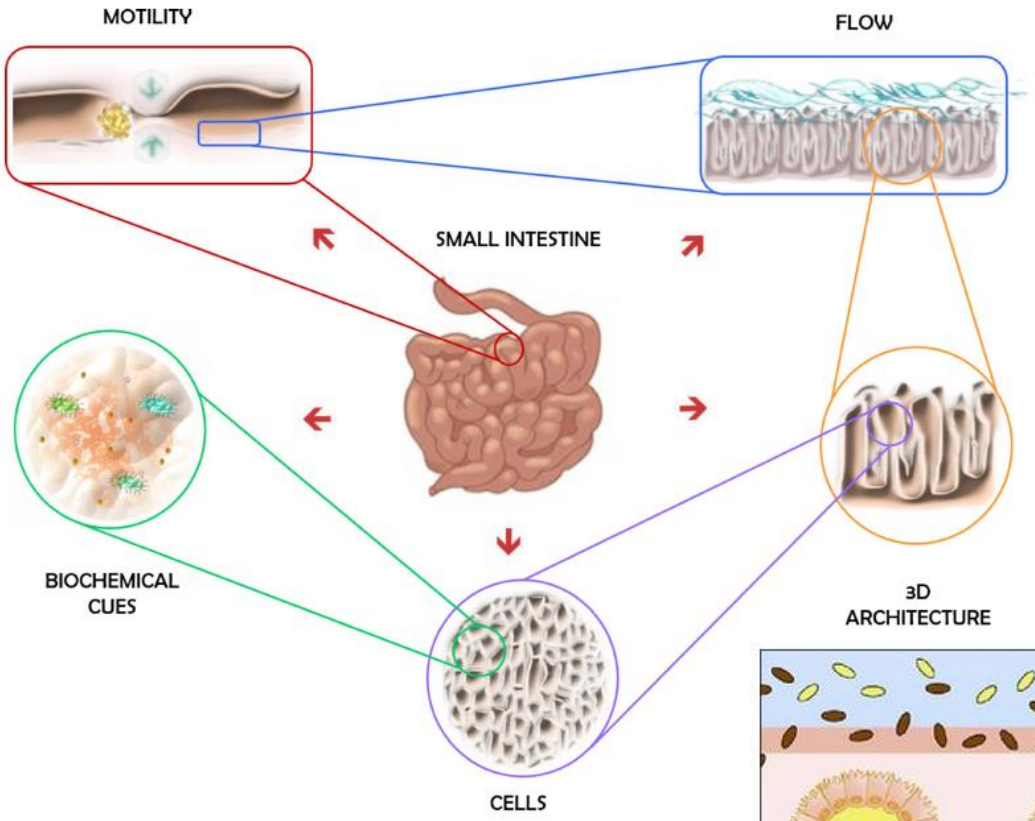
LUXEMBOURG INSTITUTE OF SCIENCE AND TECHNOLOGY | LIST

# The digestive track



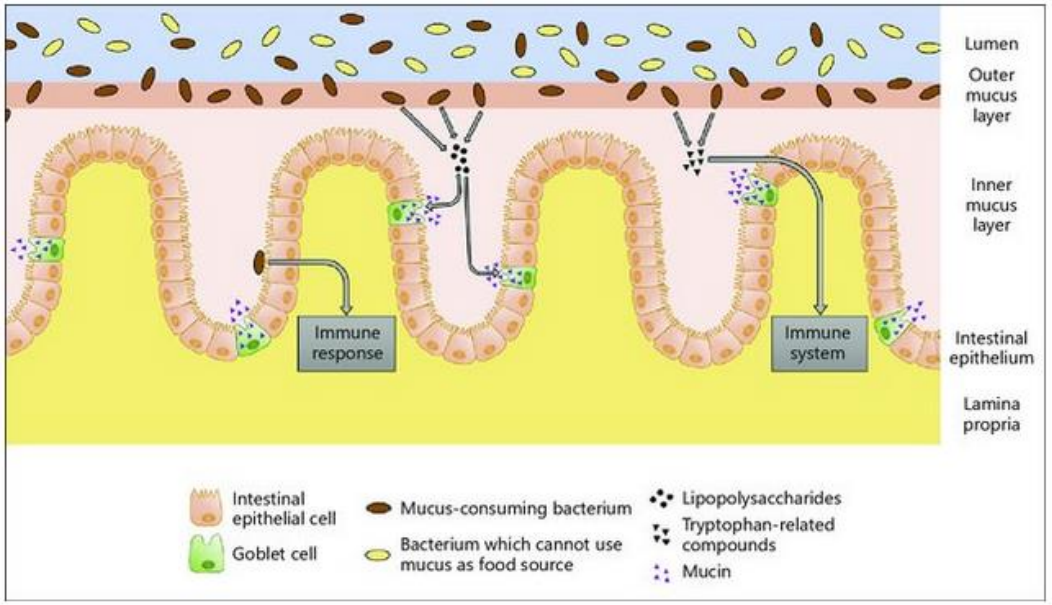
| | | |
|---|---|---|
| Mouth + Oesophagus | 1.5 min | Chewing |
| Stomach | 2-6 hour | Chyme production |
| Intestine | 90 hours | Nutrient absorption |

# Key elements of the Intestinal Environment

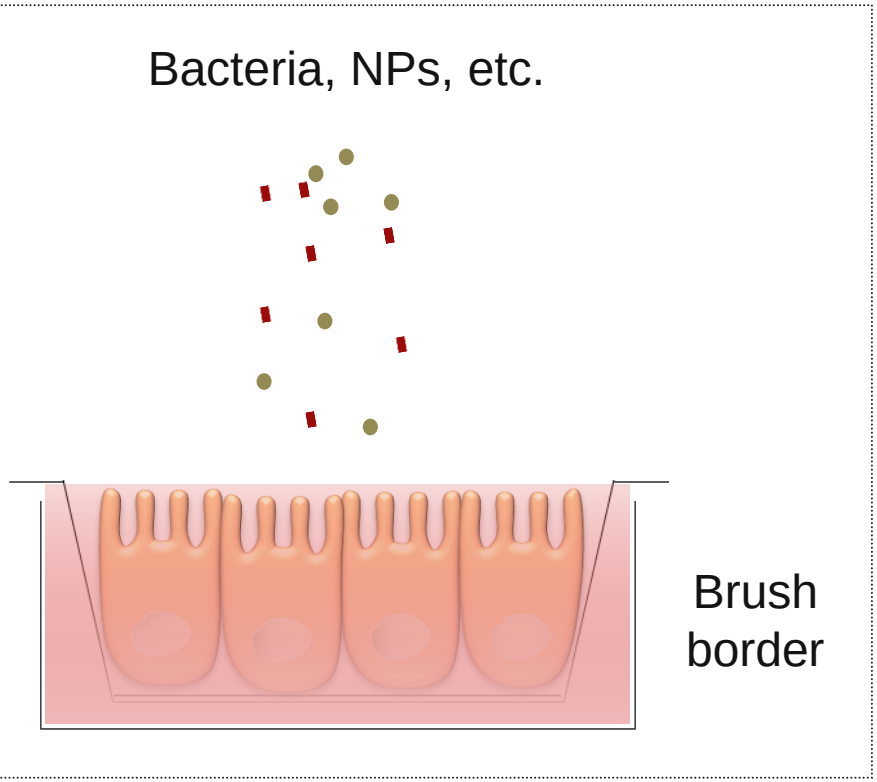



Joana Costa and Arti Ahluwalia, 2019

Esser et al., 2018



# Intestinal *in vitro* model: Monoculture



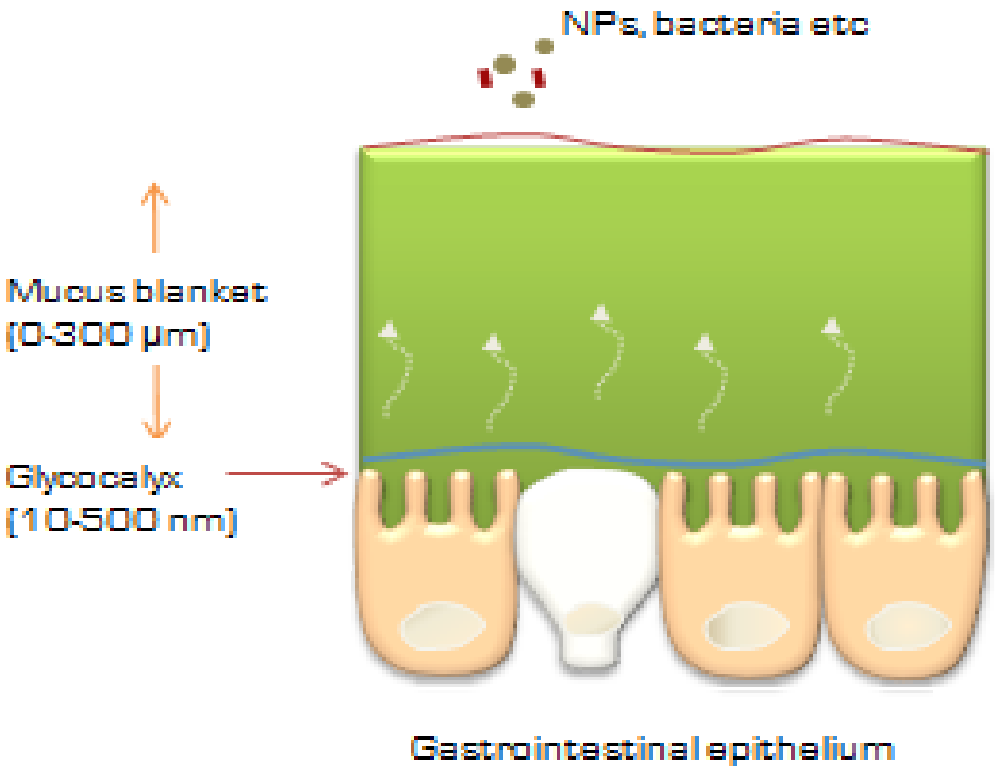

- Standard model for intestinal barrier
- Differentiation into enterocytes

…However...

Caco-2/TC7, epithelial cells

- More cell types present
- Mucus present

# Intestinal *in vitro* model: Co-culture




Caco-2/TC7, epithelial cells

HT29, goblet cells-mucus secreting

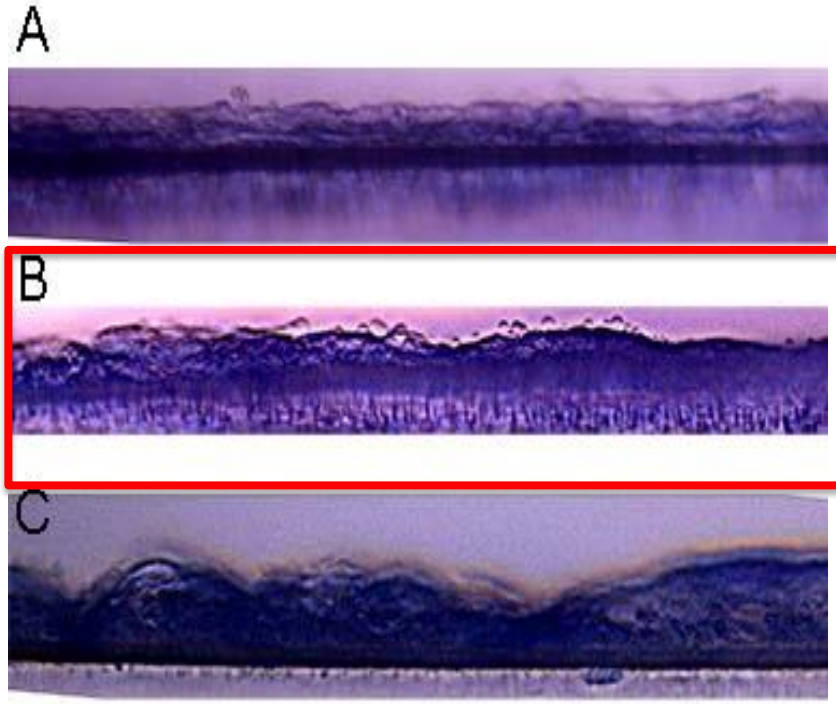
- More cell types present
- Mucus secretion

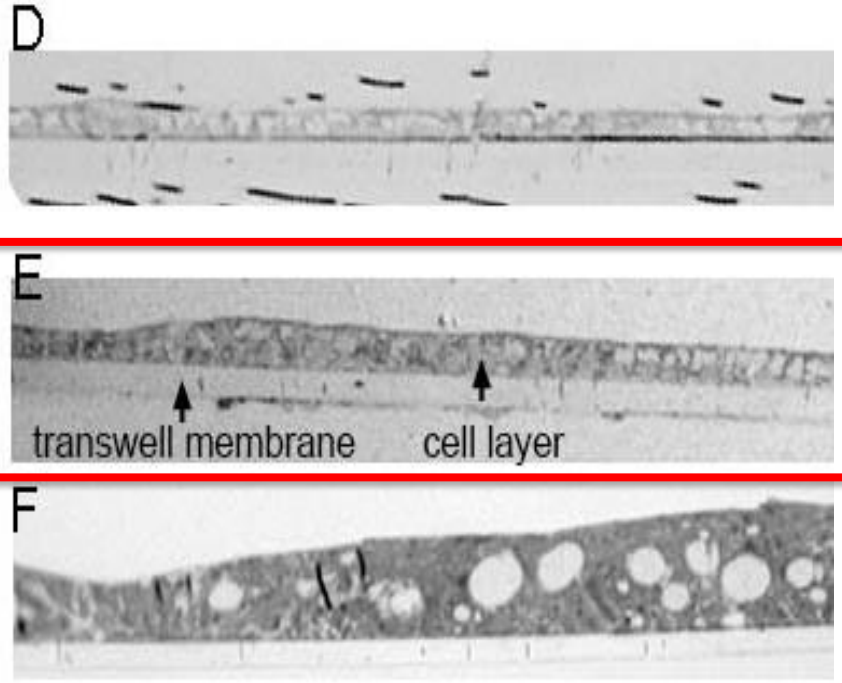
# Intestinal *in vitro* model: Co-culture

## Presence Of The Mucous Layer : HT29-MTX %

### Mucus staining

### Structure observation




0 %

10 %

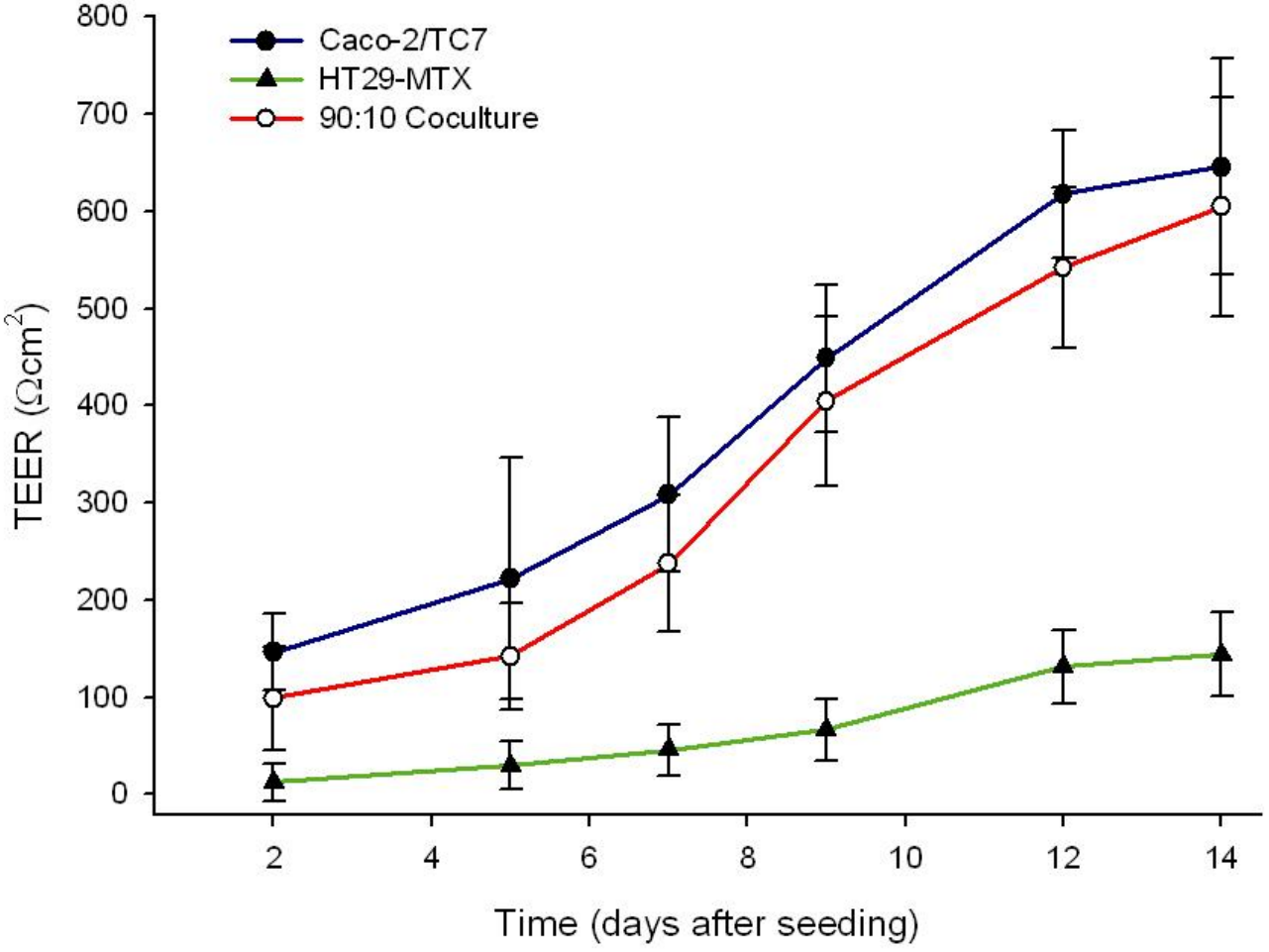
100 %



Georgantzopoulou et al., PFT, 2016

# Intestinal *in vitro* model: Co-culture

## TEER : Trans Epithelial Electric Resistance

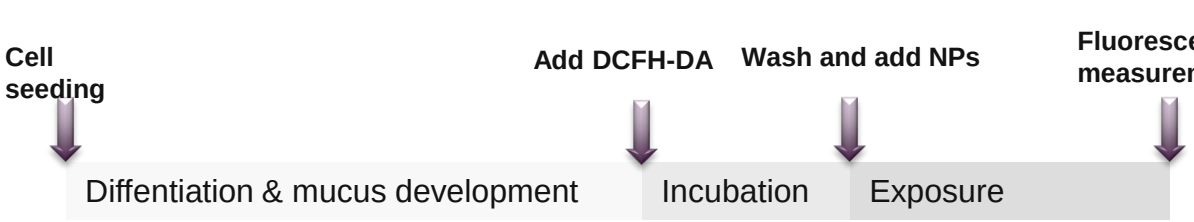


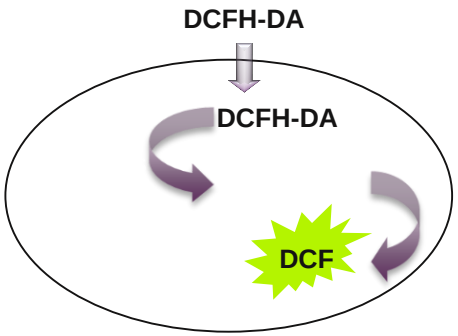

TEER

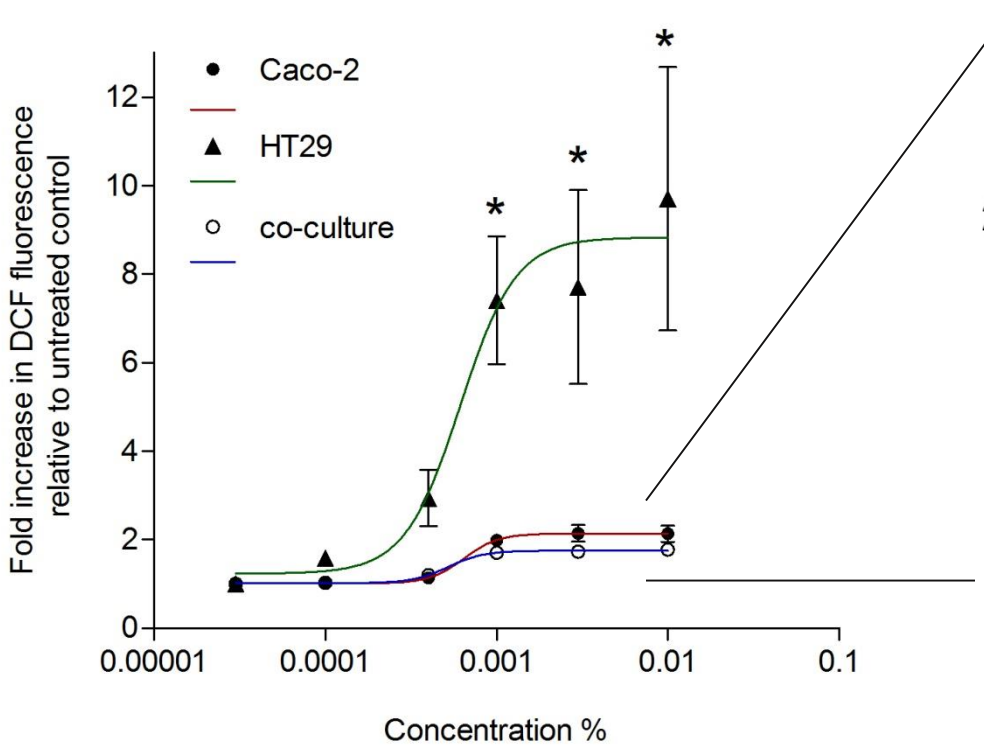
Real epithelial barrier

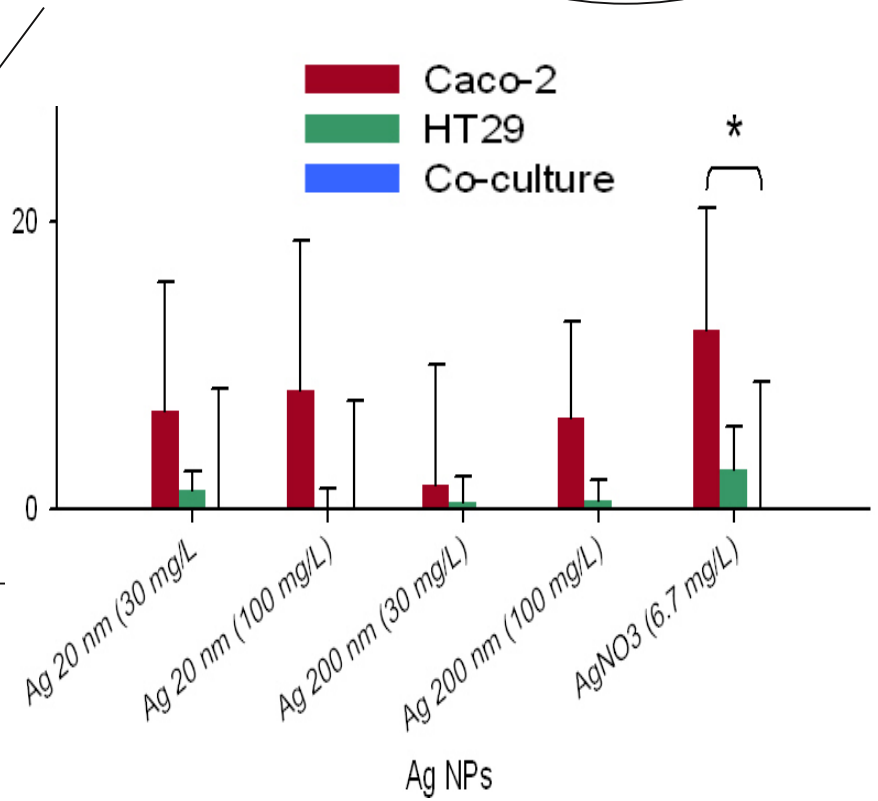
# Intestinal *in vitro* model: Co-culture

## Assessment of Ag NPs effects : Oxidative stress



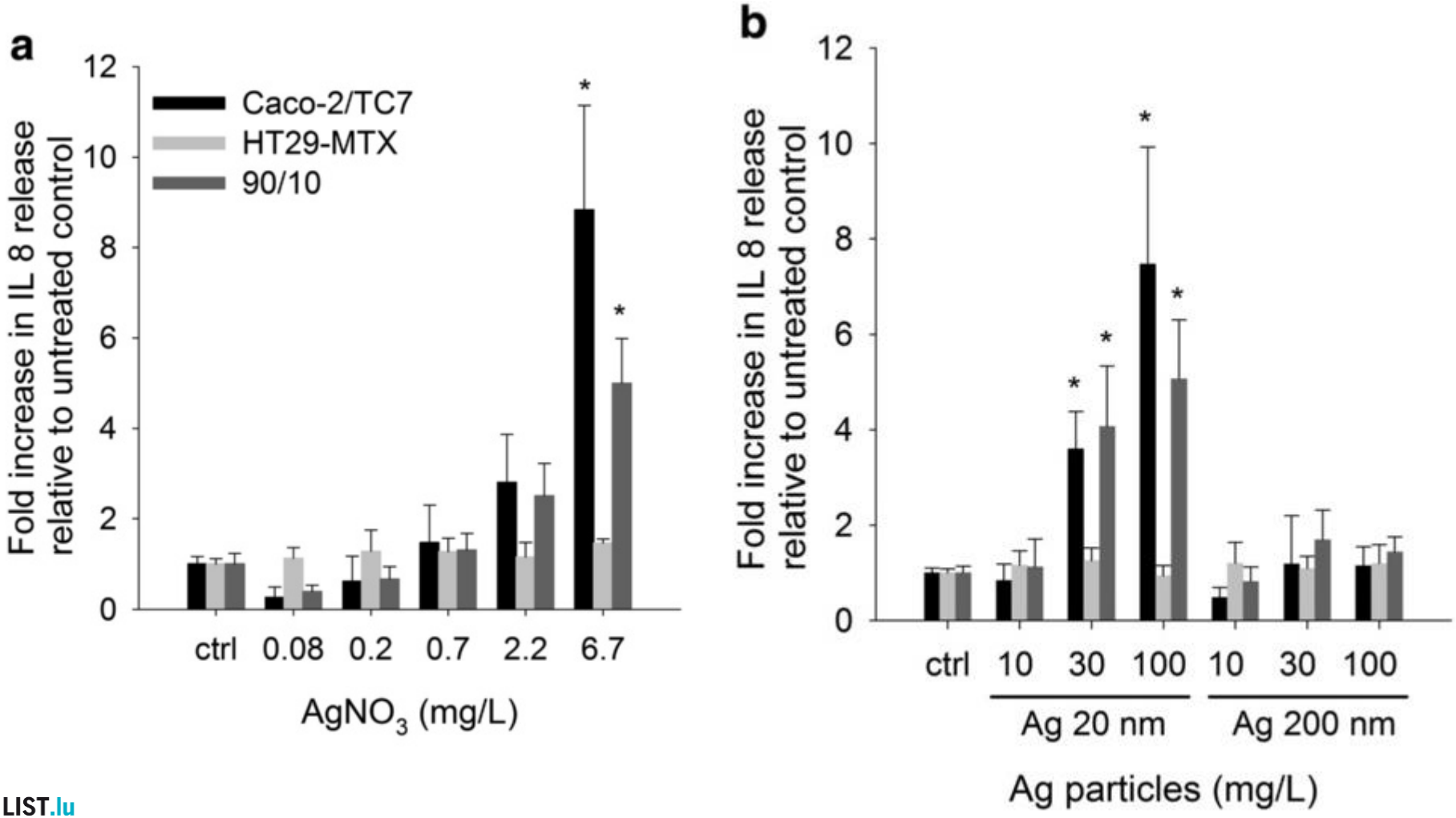




Values are mean ± SD of 3 independent experiments

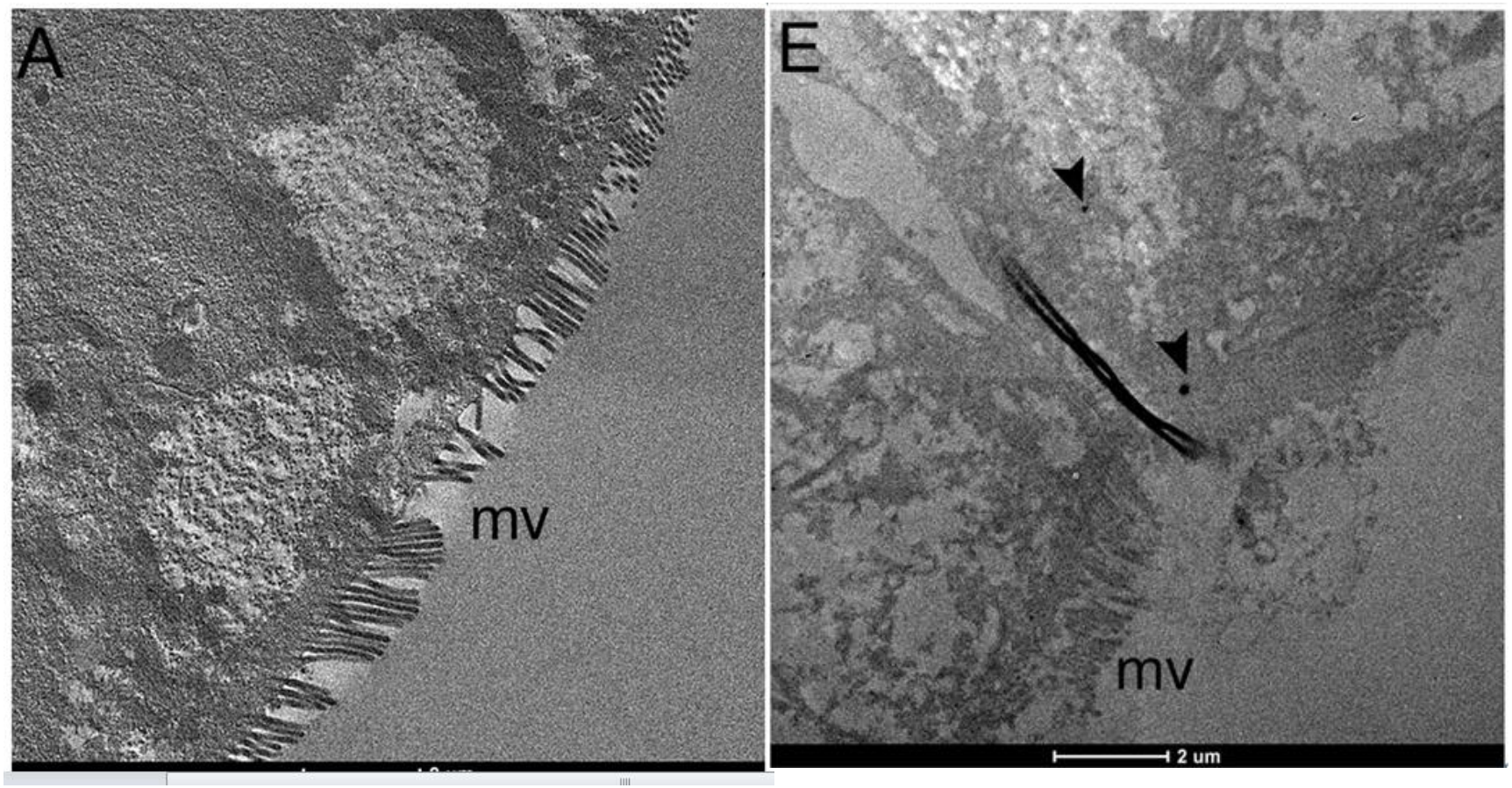
# Intestinal *in vitro* model: Co-culture

## Assessment of Ag NPs effects : inflammatory response



# Intestinal *in vitro* model: Co-culture

## Detection of Ag NPs : TEM



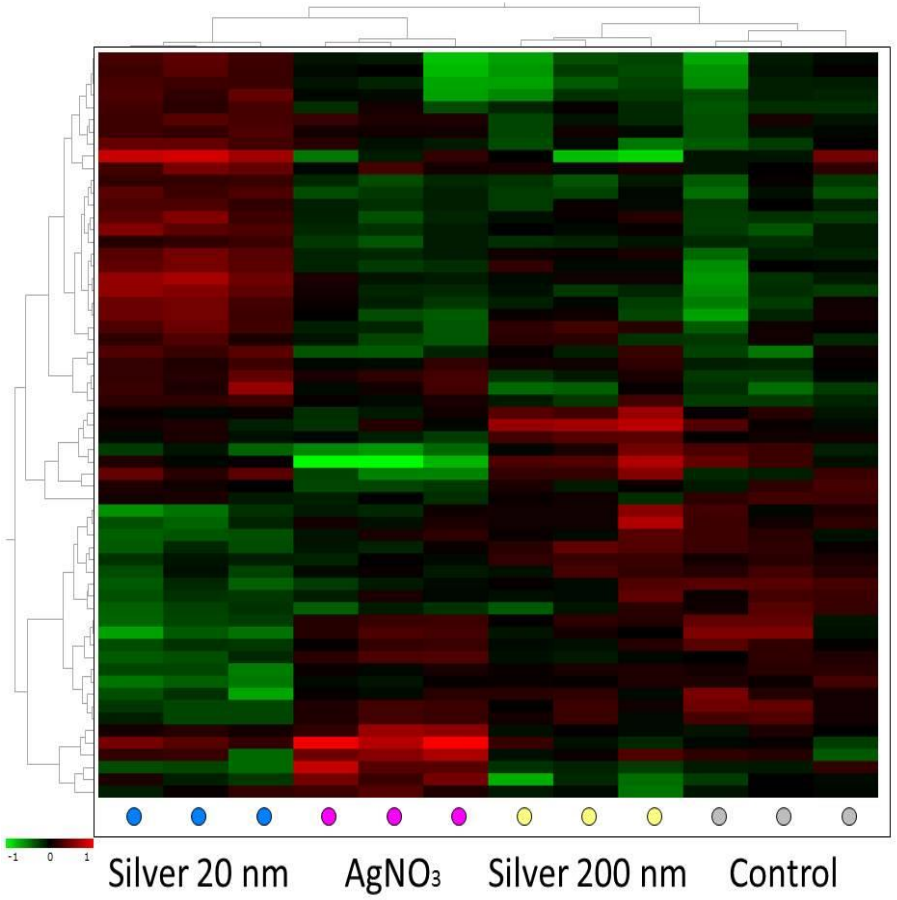



Georgantzopoulou et al., PFT, 2016

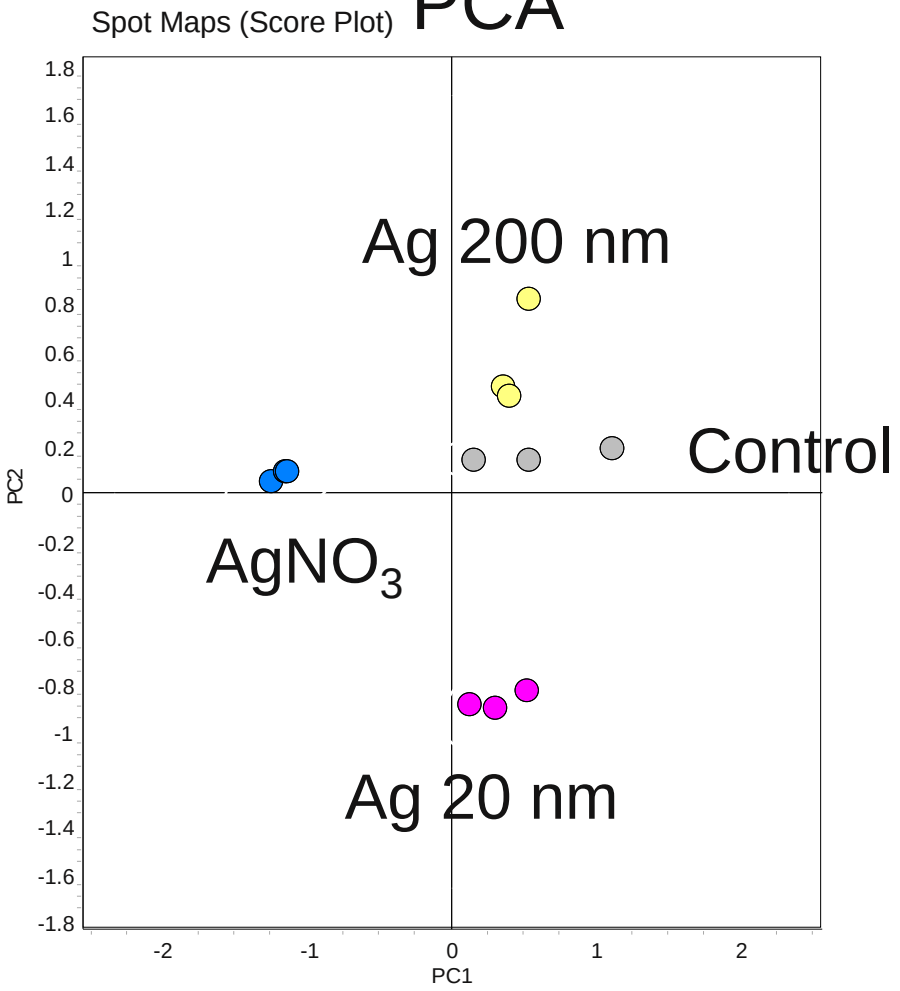
# Intestinal *in vitro* model: Co-culture

## Effects Ag NPs : Proteomics

### Hierarchical clustering




### PCA

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Georgantzopoulou et al., 2016

# Intestinal *in vitro* model: Co-culture

## CONCLUSIONS

*In vitro* assays should be physiologically relevant

- ❖ Coculture to be closer to real condition
- ❖ Mucus reduces the effects of exposure

Ag NPs effects are not only caused by Ag-ions

# How do we usually test?

**OECD Guidelines for the Testing of Chemicals**

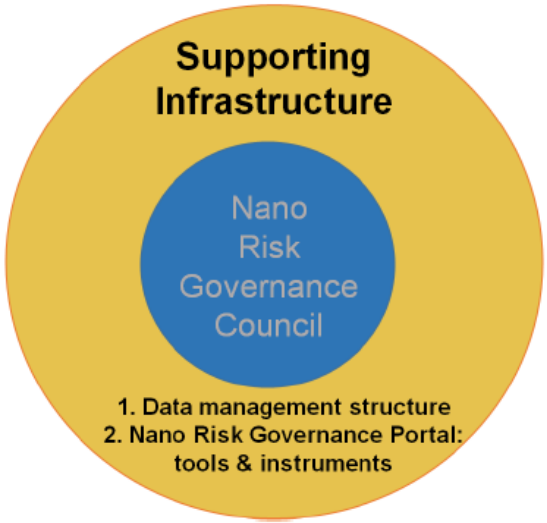
OECD

- **No OECD guideline for *in vitro* intestinal testing**
- **No *in vitro* Digestion OECD guidelines**
- **No OECD guidelines for Nanotoxicology**

# NANOHARMONY

## Scientific support for harmonised testguidelines

- **Collaboration with Malta Initiative to join (EU) forces**
- **Nano-specific adaptations of OECD TG/GD**
- **Dedicated regulatory risk research & experimental work**
- **Specific endpoints to be addressed: surface chemistry, solubility, reactivity and dustiness of nanomaterials**

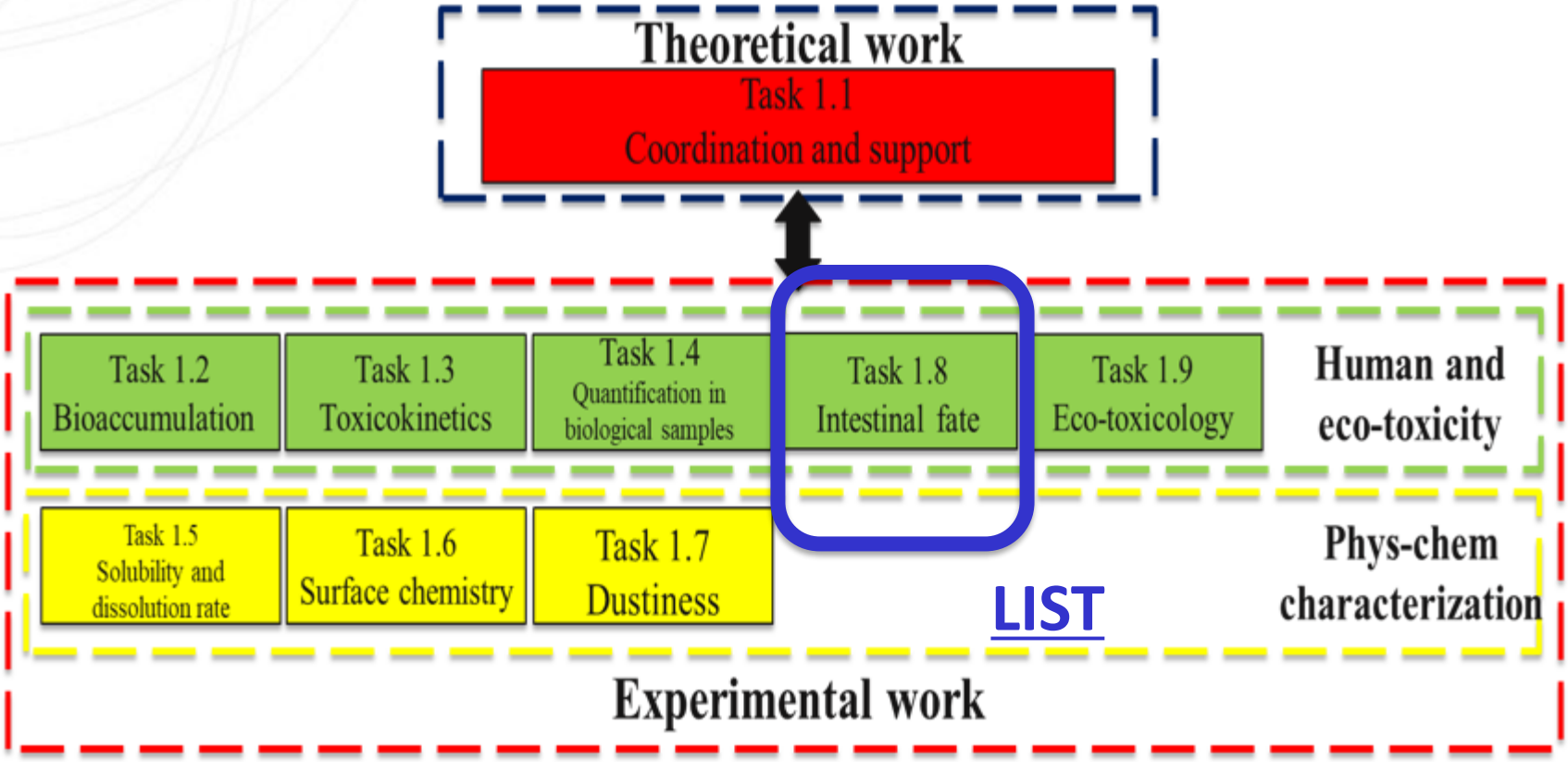



TG for *in vitro* toxicity

European Union's Horizon 2020 research and innovation programme grant

LIST will have the main role

# NanoHarmony structure



# NanoHarmony

## In Vitro Approach for Intestinal Fate of Orally Ingested NMs

- *What is the status of the task description?*

It is ongoing

- *What are the (associated) partners/subcontractors for the task?*

To achieve the task objectives ISS will rely on the following *associated* partners

| | | | |
|---|---|---|---|
| Federico BENETTI | EcamRicert | IT | f.benetti@ecamricert.com |
| Stefania SABELLA | IIT | IT | stefania. sabella@iit.it |
| Juan RIEGO SINTES, Jessica PONTI | JRC | EU | juan.riego-sintes@ec.europa.eu<br>Jessica.PONTI@ec.europa.eu |
| Alba HERNANDEZ | Univ. Barcelona | ES | alba.hernandez@uab.cat |
| Arno GUTLEB (Consortium Partner) | List | LU | arno.gutleb@list.lu |

} coordinators

- *What are your plans for workshops/interaction with the other WPs/tasks?*

We have planned a possible international workshop on our task topics and we believe to have fruitful interactions with tasks 2.1, 2.2, 2.5, 2.6, 2.7 and with WP3

- *How is your contact with the national representatives (WPMN head of delegation and WNT national coordinator)?*

Very close, Isabella De Angelis is the Head of Italian delegation at WPMN while the WNT National Coordinator, Gabriele Aquilina, is member of ISS permanent staff

# CONCLUSIONS

*In vitro* assays should be physiologically relevant

- ❖Coculture to be closer to real condition
- ❖Mucus reduces the effects of exposure
- ❖Harmonized by intra/inter laboratory round robin

**TG will become available for *in vitro* intestinal effects**

# Merci villmols