

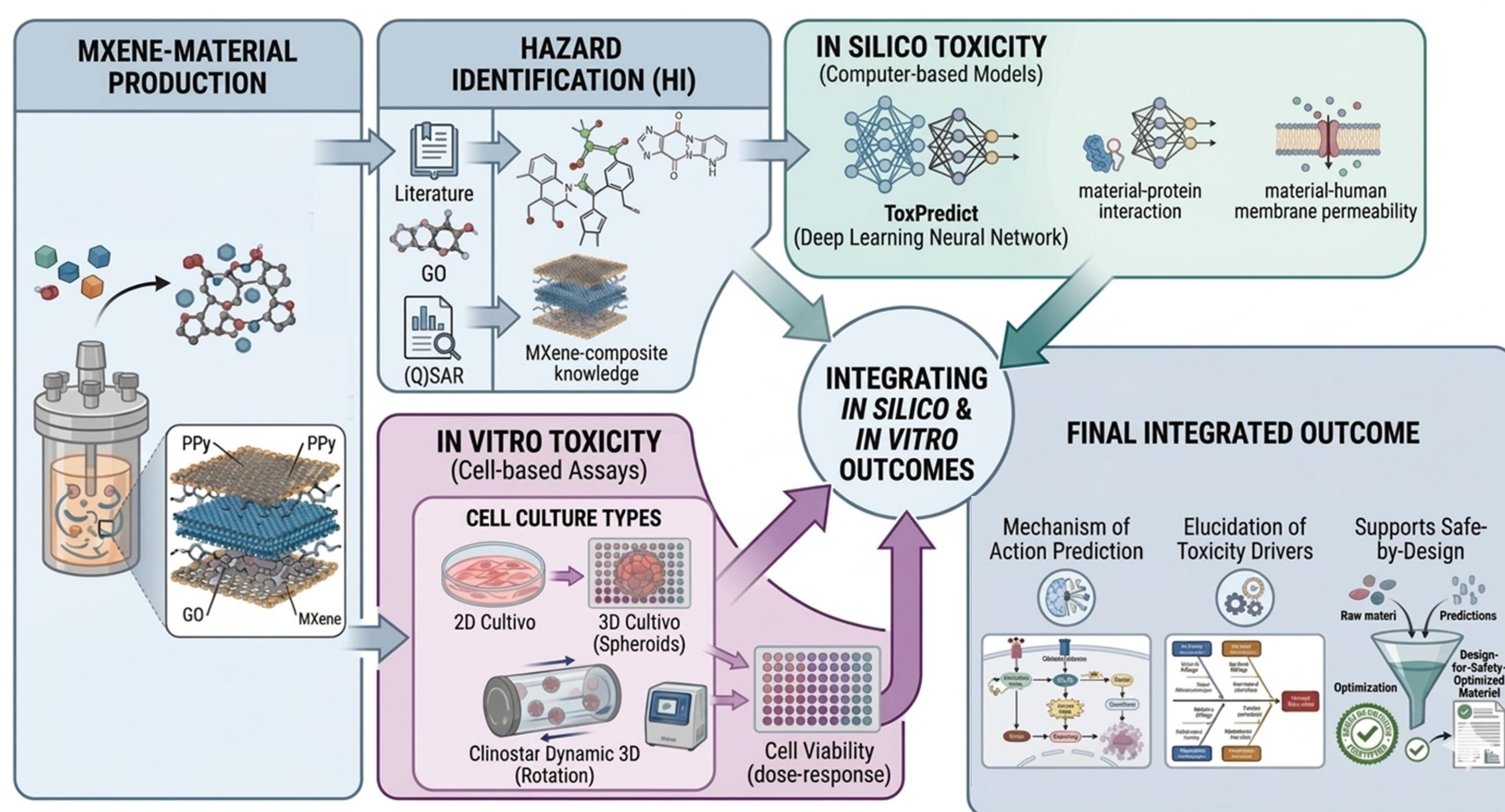
**Background** MXenes and Graphene possess outstanding physicochemical properties (thermal stability, electrical conductivity). They have strong application potential in biosensors, conductive ink, and EMI shielding.

**Challenge** Current (nano)particle regulations present critical gaps, particularly in human health risk assessment. There is a growing need for Safe by Design (SbD) aligned evaluation strategies.

**Aim** Implementing NAMs (*in vitro* and *in silico*) to replace animal testing and effectively bridge the *in vitro-in vivo* gap in safety assessments.

**Approach** Step-1 SbD was implemented via chemical hazard identification. *In vitro* NAMs (static and dynamic 3D spheroids) were compared with 2D cultures in a multi-tiered approach integrating toxicological profiles with predictive *in silico* models for mechanistic cellular analysis.

**Impact** Enables SbD-driven, animal-free predictive toxicology by integrating advanced 3D NAMs with *in silico* mechanistic modeling.



Integrating the SSbD framework with advanced NAMs, including physiologically relevant 3D cell models and *in silico* approaches, improves the predictive assessment of nano-bio interactions and hazards, supporting the development of safer and more sustainable nanomaterials.

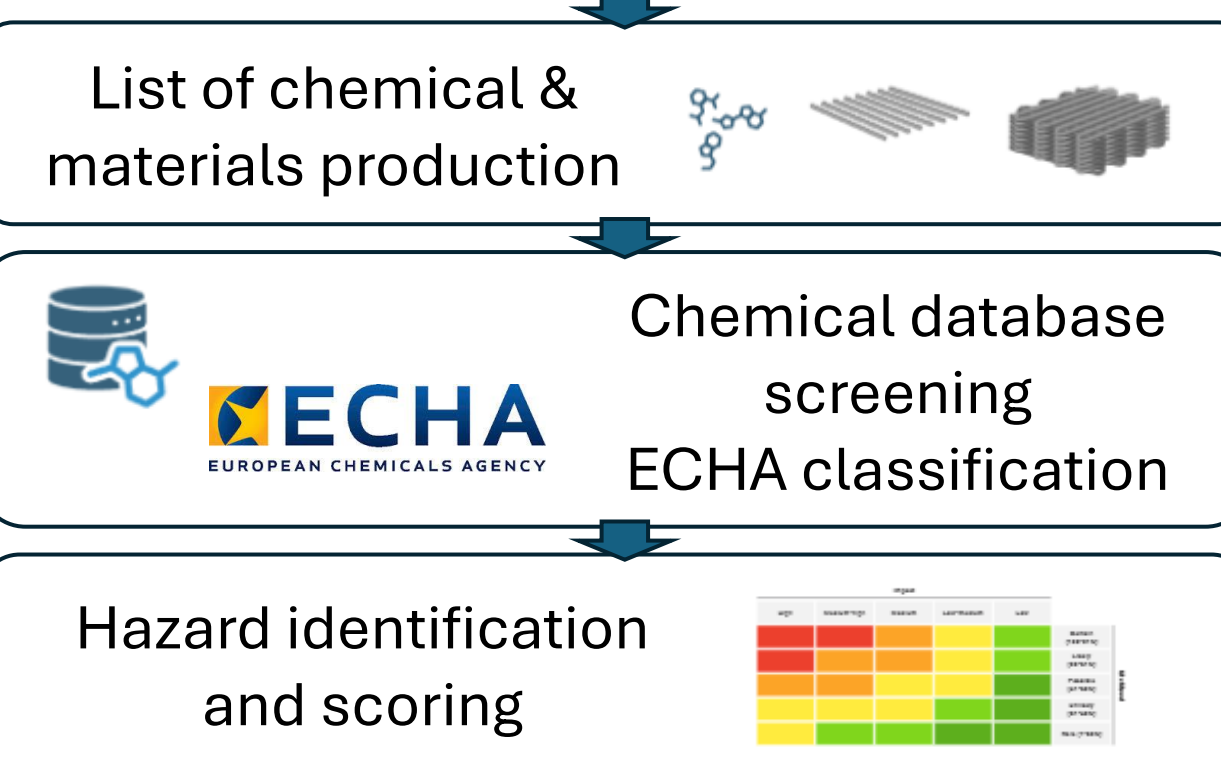
## What was our approach?

## What did we find?

## Key takeaways

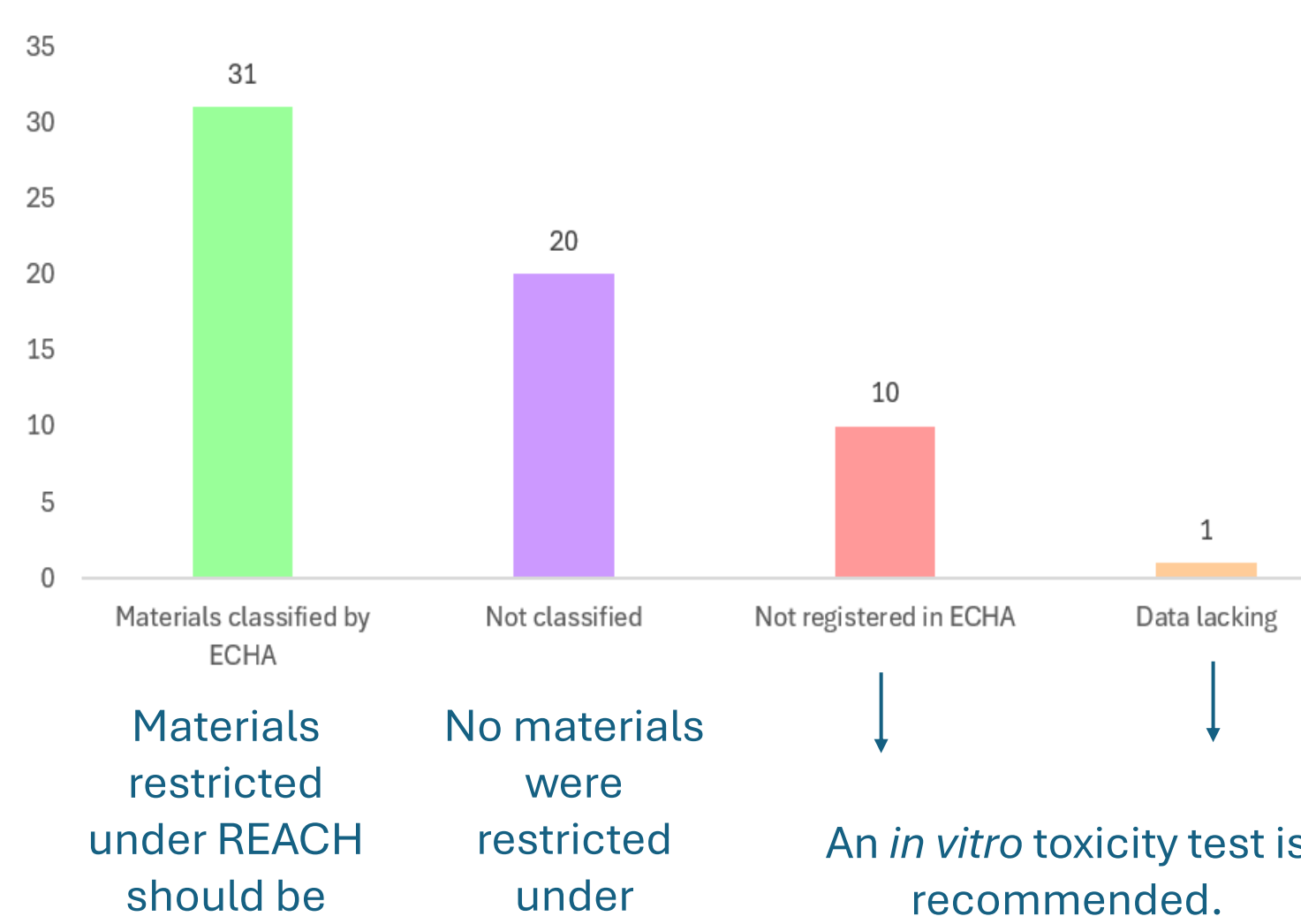
### 1. Hazard Identification of Chemicals and Materials Used in MXene Production

Early material hazard identification applying SSbD principles.



Not ECHA registration, and insufficient data are available for the materials (2, 3).

The intrinsic human health, environmental, and physical hazards of the materials were assessed using the Step 1 of the SSbD framework to support safer material substitution.



| Case Study of Production Materials |             | Score Human Hazard |                     | Score Environmental Hazard |                                    | Score Physical Hazard | Score STEP 1 | Score without PBT, PMT and ED categories |
|------------------------------------|-------------|--------------------|---------------------|----------------------------|------------------------------------|-----------------------|--------------|------------------------------------------|
|                                    |             | All categories     | Without ED category | All categories             | Without PBT, PMT and ED categories |                       |              |                                          |
| Pyrrole                            | 109-97-7    | 1                  | 1                   | 0                          | 3                                  | 2                     | 0            | 1                                        |
| Aniline                            | 62-53-3     | 0                  | 0                   | 0                          | 1                                  | 3                     | 0            | 0                                        |
| Polyaniline                        | 25233-30-1  | 1                  | 1                   | 0                          | 3                                  | 3                     | 0            | 1                                        |
| PEEDOT:PSS                         | 155090-83-3 | 1                  | 1                   | 0                          | 3                                  | 3                     | 0            | 1                                        |
| EDOT                               | 126213-50-1 | 1                  | 1                   | 0                          | 3                                  | 3                     | 0            | 1                                        |
| 1,4-Phenylenedithiocyanate         | 104-49-4    | 0                  | 0                   | 0                          | 1                                  | 3                     | 0            | 0                                        |
| 3-(aminopropyl)triethoxysilane     | 919-30-2    | 2                  | 2                   | 0                          | 3                                  | 3                     | 0            | 2                                        |
| NISO                               | 10101-97-0  | 0                  | 0                   | 1                          | 1                                  | 3                     | 0            | 0                                        |
| NaNO <sub>2</sub>                  | 7632-00-0   | 2                  | 2                   | 2                          | 2                                  | 2                     | 2            | 2                                        |
| Ammonium peroxydisulfate           | 7727-54-0   | 0                  | 0                   | 0                          | 3                                  | 2                     | 0            | 0                                        |
| (mercaptopropyl)triethoxysilane    | 4420-74-0   | 0                  | 0                   | 0                          | 1                                  | 3                     | 0            | 0                                        |
| Potassium iodide                   | 7681-11-0   | 3                  | 3                   | 3                          | 3                                  | 3                     | 3            | 3                                        |
| Phosphoric acid                    | 13598-36-2  | 2                  | 2                   | 3                          | 3                                  | 3                     | 2            | 2                                        |
| Iron(III) chloride                 | 7705-08-0   | 1                  | 1                   | 0                          | 3                                  | 2                     | 0            | 1                                        |

0 Substances should be replaced. If there is not an alternative available, they can be used with special safety and recycling measures.  
1 Substances can be used with special safety measures.  
2 Substances safer than ones with level 1, but also need special safety measures.  
3 Substances safe to use.

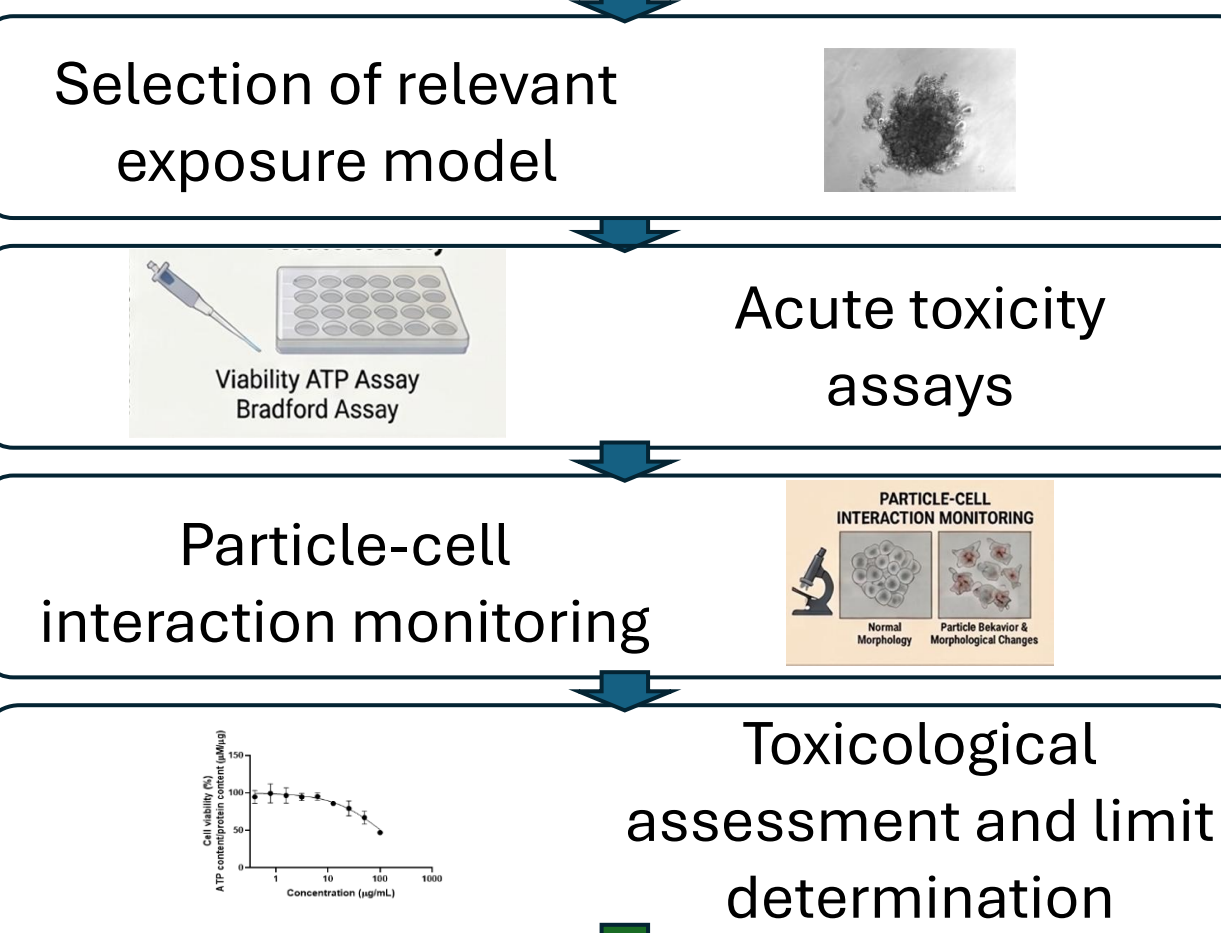
★ Ammonium persulfate was replaced by iron(III) chloride based on product properties and alternative availability.

- The assessment performed in Step 1 of SSbD contributes to reducing hazards, minimizing risks, and lowering environmental impact.
- It enables the identification of substances requiring special attention and the implementation of appropriate safety measures during production.
- It also helps define the *in vitro* endpoints to be evaluated in the final products through the identification of human, environmental, and physicochemical hazards.

MXene derivatives not registered in ECHA and lacking safety data were assessed *in vitro*.

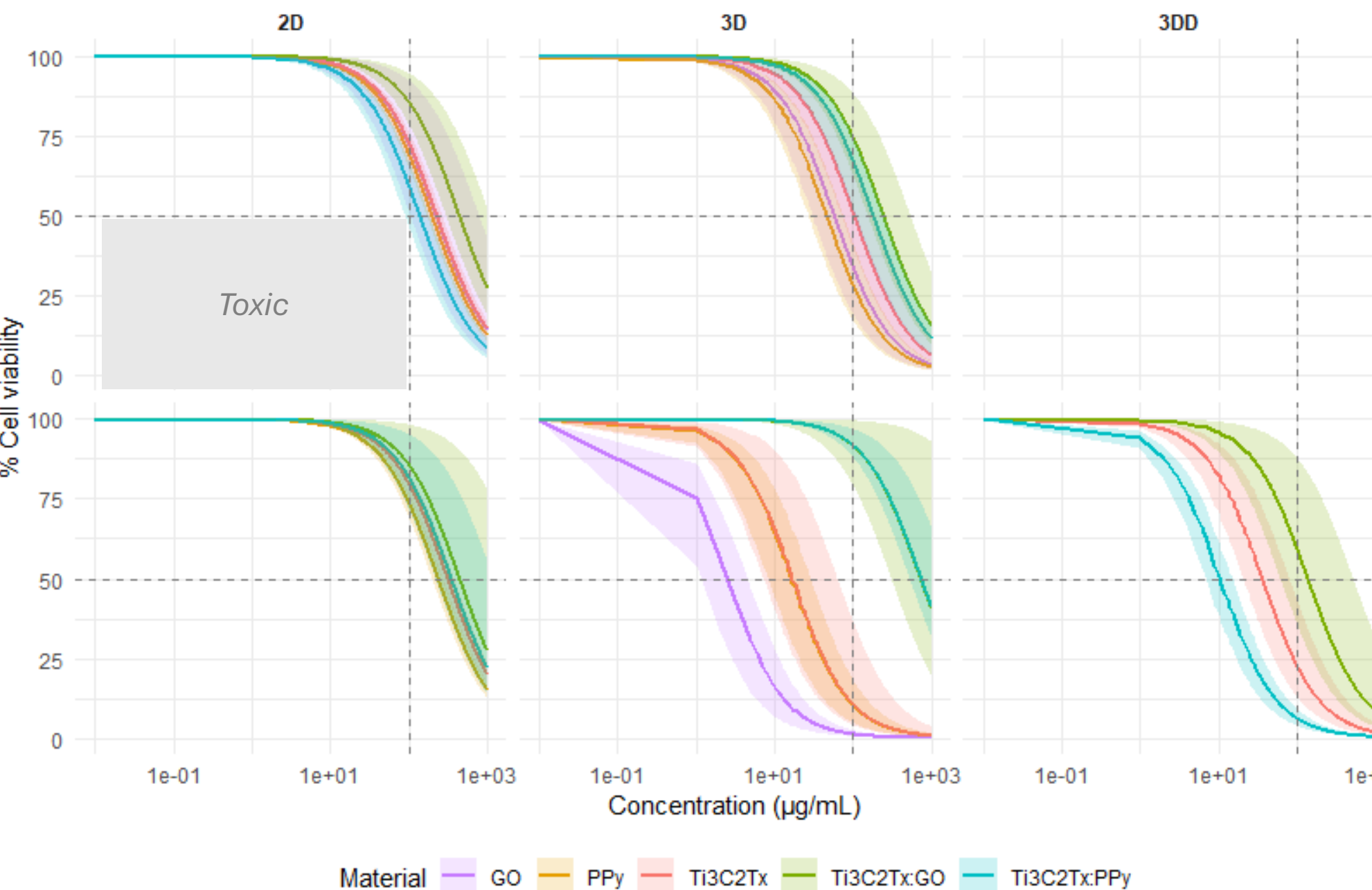
### 2. *In vitro* toxicological profiling of novel 2D materials (MXenes)

To assess cellular responses and potential toxic effects using *in vitro* cell-based models.



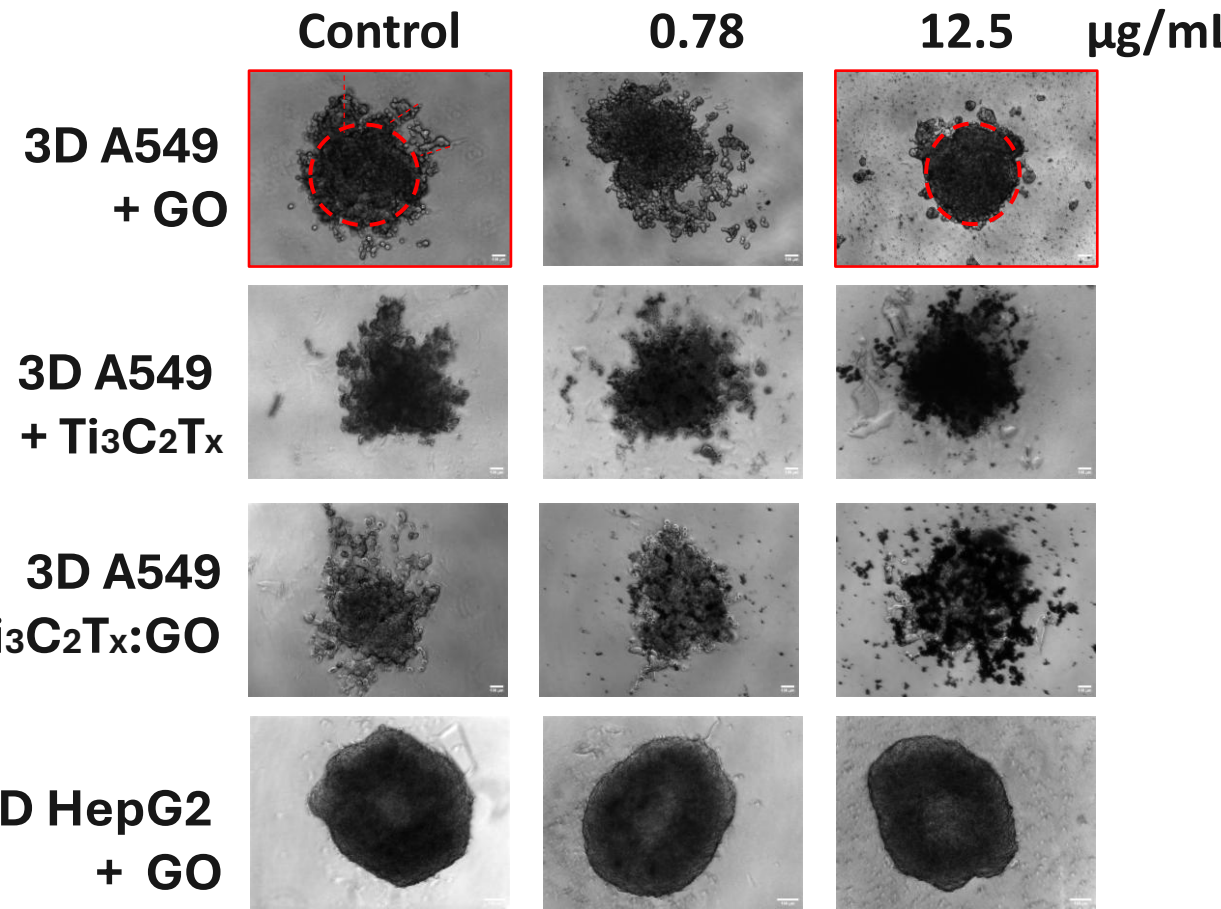
Mechanisms underlying dose-response remain to be elucidated

Cellular dose-response assessment using 2D, static 3D, and dynamic 3D (3DD) models of lung (A549) and liver (HepG2) cells.



Cell viability is expressed as intracellular ATP content normalized to total protein content (µM/µg).

Impact of GO-Based Materials on Spheroid Architecture



GO (> 6.5 µg/mL) increased spheroid compaction in A549 cells without affecting HepG2 morphology, whereas MXene-GO hybrids showed no effect. This morphological change was not observed in the 2D model.

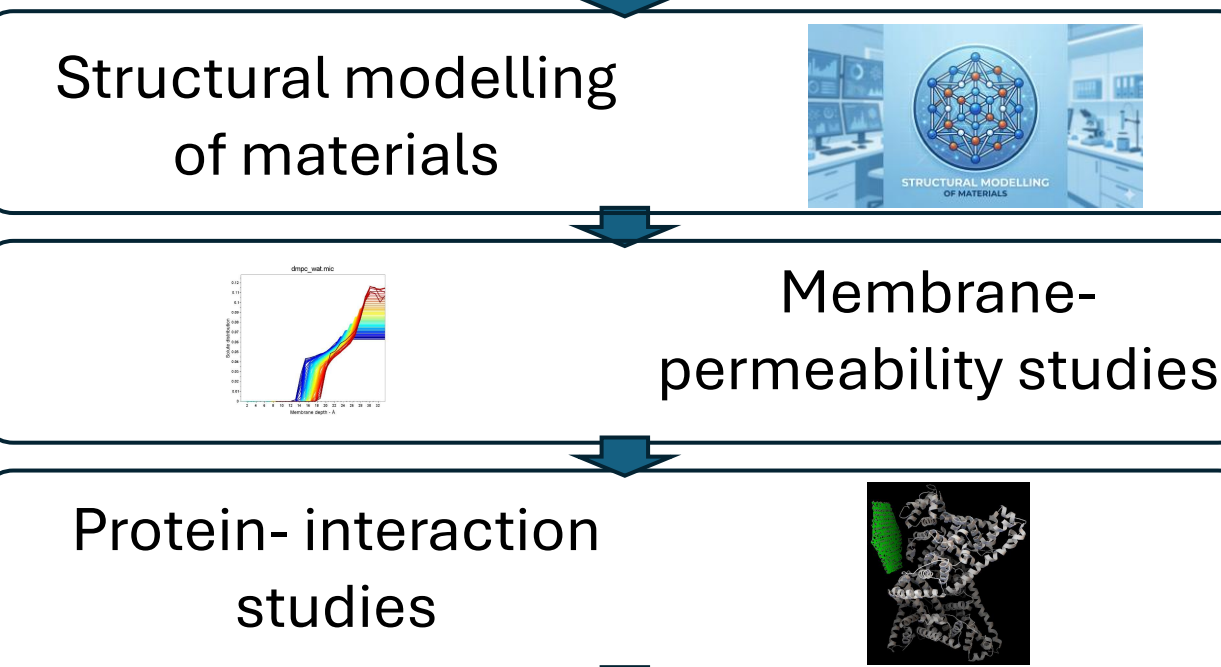
- 2D model: IC<sub>50</sub> > 100 µg/mL → traditionally considered non-toxic for inhalation exposure of nanomaterials [1,2,3].
- 3D static model: Ti<sub>3</sub>C<sub>2</sub>Tx:GO and Ti<sub>3</sub>C<sub>2</sub>Tx:PPY were identified as comparatively safer materials than pristine MXene and their individual components (GO and PPY, IC<sub>50</sub> < 100 µg/mL).
- 3D dynamic model : both Ti<sub>3</sub>C<sub>2</sub>Tx and Ti<sub>3</sub>C<sub>2</sub>Tx:PPY exhibited lower safety profiles than Ti<sub>3</sub>C<sub>2</sub>Tx:GO. This observation warrants further investigation to elucidate the underlying mechanisms.

- Results highlight the limitations of 2D systems and the potential higher physiological relevance and predictive capacity of static and dynamic 3D models.
- Conventional exposure guidelines [1,2,3] may overlook subtle (nano)particle-cell interactions that are captured by New Approach Methodologies (NAMs).

An *in silico* approach was used to further elucidate the mechanisms of MXene toxicity.

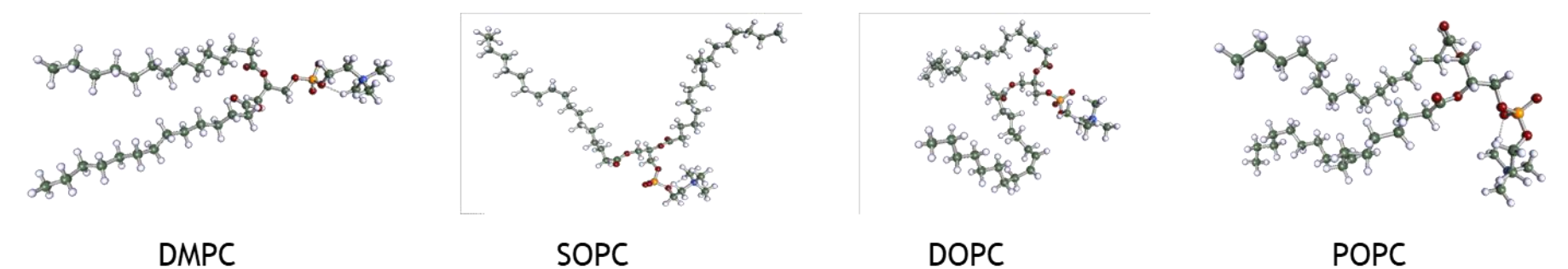
### 3. *In silico* toxicological profiling of novel materials

To use *in silico* toxicological tools to investigate material-cell mechanisms of action.

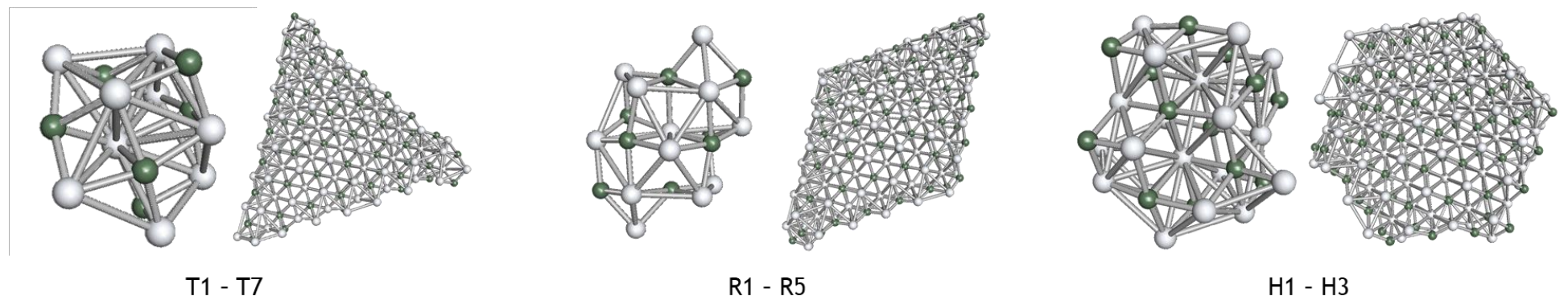


Mechanistic interpretation of MXene toxicity

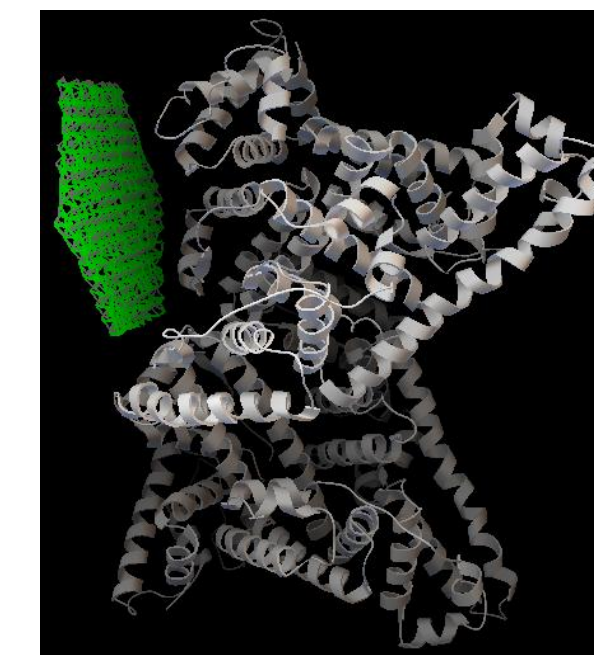
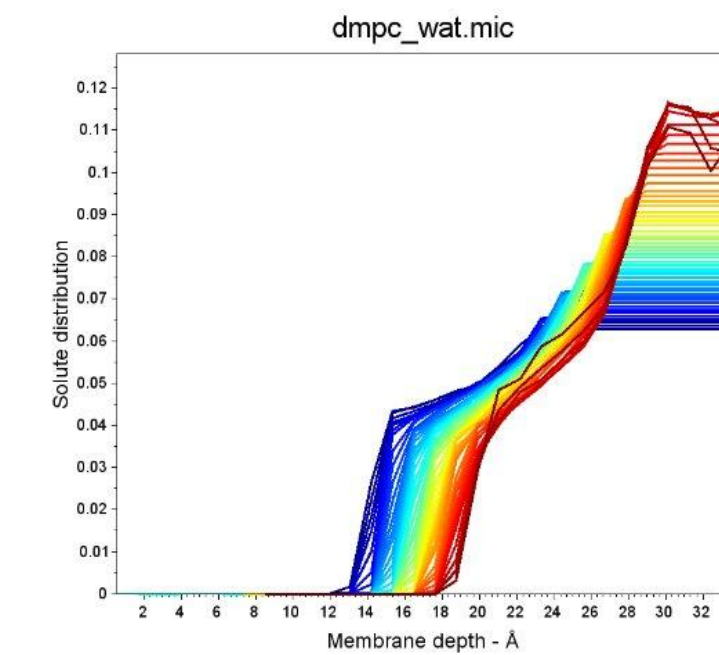
Modelling of archetypal phospholipid bilayer membranes in water.



Modelling of Ti<sub>3</sub>C<sub>2</sub>Tx, Ti<sub>3</sub>C<sub>2</sub>Tx:GO, and Ti<sub>3</sub>C<sub>2</sub>Tx:PPY nanoflakes of various shapes and sizes.

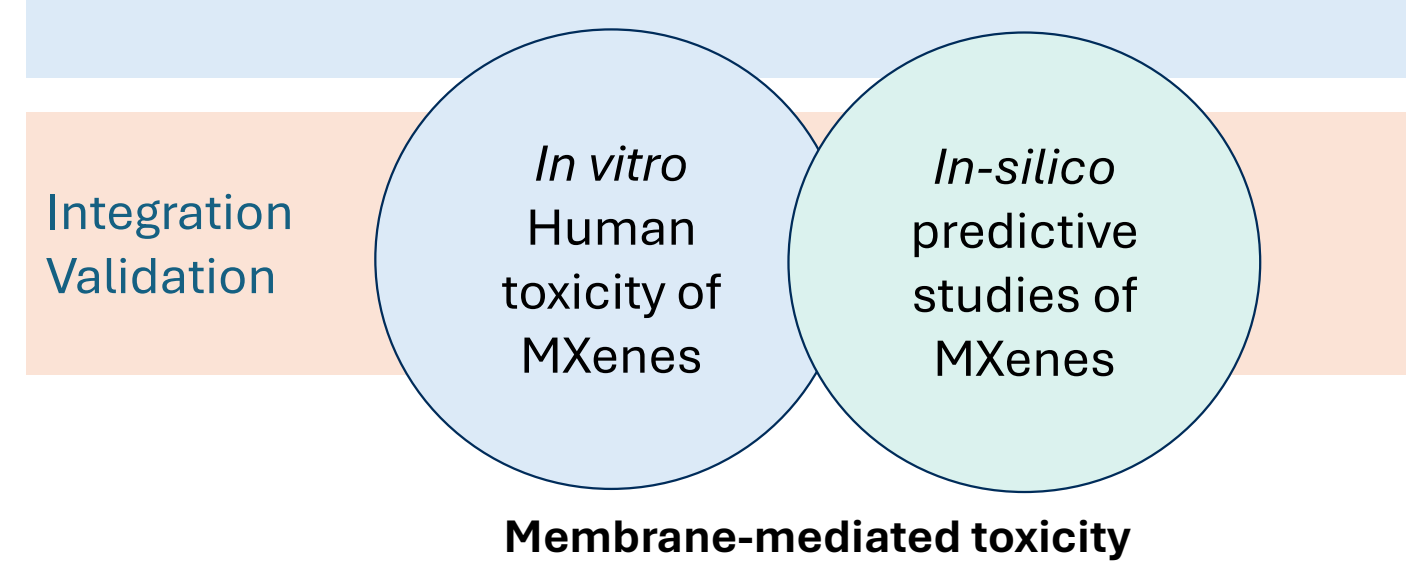


- Membrane permeability studies as a function of temperature, membrane phospholipid composition, and particle size.
- Studies of protein interactions.



- For Ti<sub>3</sub>C<sub>2</sub>Tx, no full membrane translocation is required, with predominant membrane interaction and surface accumulation.

- Ti<sub>3</sub>C<sub>2</sub>Tx:GO and Ti<sub>3</sub>C<sub>2</sub>Tx:PPY were retained in the lipid bilayer, resulting in the retention within the ECM and outer cell layers.

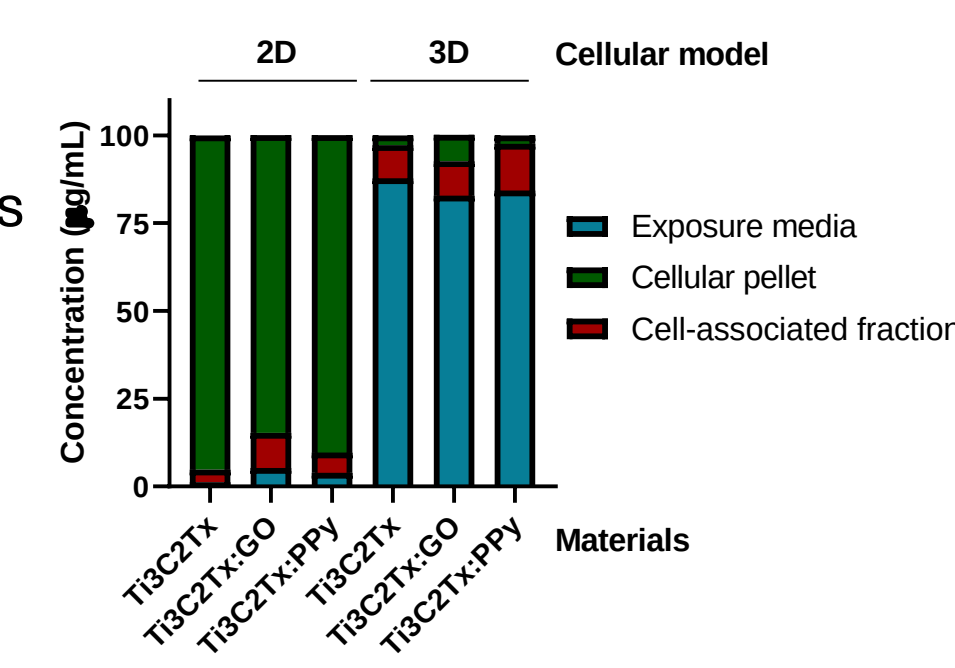
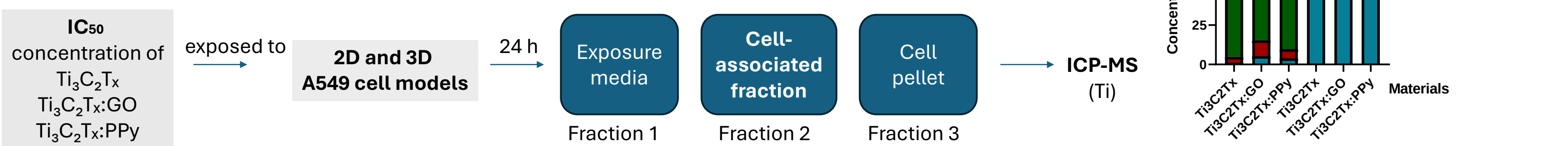


### 4. Mechanistic insights into the toxicity of MXenes

To validate the predicted mechanisms of MXene toxicity in relevant *in vitro* models



Validation of *in silico* predictions through ICP-MS-based discrimination of internalized versus membrane-bound MXenes in cells.



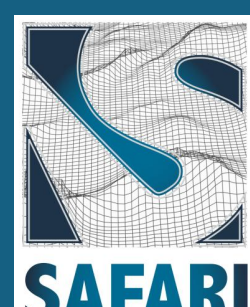
- 2D model: higher MXene bioaccumulation.
- 3D static model: majority of MXene observed in the exposure media.

## References

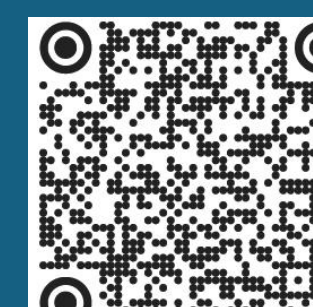
- doi.org/10.2760/487955
  - 10.1371/journal.pone.0127174
  - 10.1021/acsnano.6b03013
- Introductory image generated with Gemini AI

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