Title	Supporting Safe and Sustainable-by-Design of Nanomaterials Using Machine Learning Models and Analytical Methods

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Place, country	1- Netherlands 2- The UK
# months (min.3)	1-3 months 2-3 months

Project description (2 page max):

The state of the art: The Safe and Sustainable by Design (SSbD) strategy is an emerging framework aimed at ensuring that materials (e.g., nanomaterials (NMs)) are developed with a proactive focus on safety, sustainability, and circularity throughout their entire life cycle[1]. This approach integrates hazard and exposure assessments early in the innovation process, encouraging the selection of inherently safer substances, processes, and designs that minimize risks to human health and the environment. For NMs, SSbD is particularly crucial due to their unique properties and potential for unintended biological interactions. To design NMs following the SSbD framework, it is essential to understand their fate within the body of organisms, including humans.

The fate of NMs in the human body is largely unknown, complicating efforts to predict and control their biological interactions. It has been reported that NMs can interact with proteins in biological fluids, forming a dynamic protein corona (Fig 1) that influences their behavior and biodistribution [2]. Yet, the interactions depend heavily on both the physicochemical properties of the NMs and the specific proteins involved, creating an endless number of possible combinations and outcomes. *This complexity presents a major obstacle to reliably assessing the safety and sustainability of NMs* and highlights the urgent need for mechanistic understanding.

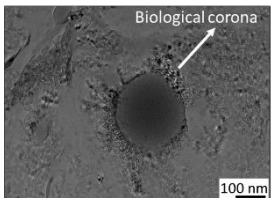

Fig 1. Formayion of biological corona on PS NMs.

Machine learning (ML) models, including tools like Toxicokinetic-Toxicodynamic (TKTD) models and the SimpleBox4Nano (SB4N) framework, have opened new horizons for predicting the fate of NMs in organisms' bodies and their potential interactions with biological molecules such as proteins [3]. These ML enable the integration of complex datasets to simulate absorption, distribution, metabolism, and excretion processes, offering insights into how NMs behave under different biological conditions. By incorporating nanoparticle-specific properties, e.g,. size, shape, and coating—as well as environmental and physiological parameters, these ML help forecast how NMs might behave in organisms' bodies. ML-based approaches present a powerful tool to overcome experimental challenges and support the SSbD development of NMs by guiding safer material design and reducing the need for extensive *in vivo* testing.

Despite their potential, ML models like TKTD and SB4N face key challenges in predicting the fate of NMs [3]. A major issue is the lack of high-quality, standardized datasets needed to train reliable models, largely due to the absence of suitable analytical methods to accurately track and characterize NMs in physiological media. Additionally, the dynamic and complex nature of the protein corona—shaped by both nanoparticle properties and the biological environment—is difficult to model. In the current TKTD models for NMs, the formation of the protein corona is typically disregarded, despite its critical role in influencing NMs uptake, biodistribution, and biological effects [3]. Current models also struggle to integrate multi-scale data and often lack validation across diverse scenarios, limiting their practical and regulatory application.
Recently, a novel analytical method has been developed and validated by the Analytical Chemistry group at the University of Padua, in collaboration with partners from the Netherlands, Germany, the United States, and Finland [4]. This method enables accurate tracking and characterization of NMs in complex physiological media, addressing a key limitation in current modeling approaches. Successfully applied in joint studies, it provides high-quality, standardized data essential for improving predictive tools like SB4N. This advancement marks a significant step toward more reliable modeling of NMs fate and behavior. In this PhD project, all these efforts will be consolidated to validate models specifically tailored to support the SSbD framework for NMs. The resulting tools and datasets will enhance the reliability of both the SB4N and TKTD models, paving the way for safer NMs design and more robust risk assessment strategies.

Objectives (O): The project consists of three main objectives:

1- ***Optimizing the SimpleBox4Nano model by generating experimental data on the interactions of three selected NMs—polystyrene (PS) nanoplastics, titanium dioxide (TiO_2), and silver (Ag) nanoparticles—in human plasma, with a focus on their behavior and protein corona formation.*** *Measurability: number of NMs studied (3 types: PS, TiO_2, Ag), physicochemical characterization parameters measured (e.g., size, surface charge, corona composition), number of experimental datasets generated/collected for model input. Verifiability: publications describing the optimized parameters integrated into SimpleBox4Nano, comparison between experimental results and model predictions*

2- ***Validating the TKTD model by incorporating protein corona formation as a critical factor influencing NMs uptake, distribution, and biological effects, thereby enhancing the model's realism and predictive power.*** *Measurability: number of TKTD simulations with and without corona factor, Quantitative changes in model output after including corona data, Verifiability: documentation of model modifications and input datasets, publications demonstrating enhanced predictive accuracy*

3- ***Enhancing TKTD model integration and applicability by incorporating multi-scale data and validating model performance across diverse biological scenarios.*** *Measurability: Types and levels of biological data integrated (e.g., molecular, cellular, organismal), Improvement in model prediction accuracy across test cases. Verifiability: Documentation of integrated datasets and model adjustments, regulatory feedback supporting broader applicability*

Methodology: The three NM selected for this project represent a diverse range of particle types commonly encountered in consumer products, food, cosmetics, and medical applications to ensure that findings from this project are applicable to a broad range of NMs types relevant to SSbD principles. PS nanoplastics also serve as a model for micro- and nanoplastic pollution, with relevance to environmental and human exposure. The project will be carried out in five tasks.
Task 1 **(O1-2, months 1-6):** Optimization of analytical methods for NM characterization in a physiological medium: This task will be closely linked with the newly funded NanoFage project at DISC, leveraging shared expertise and infrastructure. The focus will be on establishing and refining analytical techniques to track, quantify, and characterize the selected NMs (PS, TiO_2, Ag) in human plasma using single-particle ICP-MS, DLS, SEM, TEM, and protein profiling will be optimized to assess critical parameters including size, surface charge, etc. Task 2 (O1-2, **months 1-12**): Experimental investigation of NM–Protein interactions in plasma: The selected NMs will be incubated in human plasma under controlled conditions to study protein corona formation following the method developed by the PI [5]. The influence of physicochemical properties on corona composition, stability, and biological identity will be assessed to generate high-quality data for model calibration. Task 3 (O1, **months 6-24**): Optimization and parameterization of the SB4N model: Using the data from Tasks 1 and 2, the model will be updated to better simulate NMs fate in physiological environments. The student will conduct a 3-month research stay at RIVM (Netherlands) to collaborate with modeling expert Dr. Joris Quik on parameter integration and validation. Task 4 (O2, **months 18-30**): Adaptation and validation of the TKTD

model with Protein Corona Consideration: This task involves modifying an existing TKTD model to account for protein corona formation as a dynamic factor affecting NM biodistribution. The student will spend 3 months at the University of Birmingham (UK, Prof. Iseult Lynch) to work on model adaptation and validation. Task 5 (O3, **months 25-36**): Integration of multi-scale data and final model validation: The final task integrates molecular, cellular, and systemic-level data into a refined modeling framework. Both SB4N and TKTD models will be validated across diverse scenarios using experimental data. This task ensures the models are fit for supporting SSbD strategies and regulatory applications.

Training Plan and Transferable Skills for the PhD: This PhD will provide the student with a strong foundation to pursue a research career focused on NMs safety and sustainability. A tailored training and Career Development Plan will be established to strengthen his skills in analytical chemistry, environmental modeling, and science communication. He will gain hands-on experience with advanced techniques (e.g., spICP-MS, DLS, TEM) and modeling tools (SimpleBox4Nano, TKTD) through international research visits to RIVM and the University of Birmingham. He will also develop transferable skills in project management and outreach by engaging in training courses and presenting at international conferences. Collaborations with leading institutions across Europe and beyond will help him to build a strong multidisciplinary network and prepare him for a future role in industry, academia or policy-driven research.

Impact: The European Green Deal aims to create a climate-neutral, sustainable, and circular economy, with initiatives like the EU Chemicals Strategy for Sustainability and the New Industrial Strategy driving responsible material design. These strategies emphasize SSbD, focusing on developing inherently safe and sustainable materials throughout their life cycle. Smart NMs (Smart NMs), which are responsive to stimuli, hold promise in sectors like agriculture and cosmetics but pose safety and regulatory challenges. Recent EU efforts, including projects like NanoReg2, underscore the need for proactive regulation and interdisciplinary research. The transition to SSbD requires integrating sustainability, circular economy principles, and the UN's Sustainable Development Goals. Strong regulatory frameworks, like REACH, and new tools are essential for addressing the complexity of Smart NMs. This project supports the SSbD strategy by combining advanced analytical methods and predictive modeling (SimpleBox4Nano, TKTD) to assess the fate and safety of NMs.

References:

1. Mech et al., Regulatory Toxicology and Pharmacology (2022). 128, 105093. **2**. Zhang et al. Nature protocols (2024). 19, pages 3000–3047. **3**. A. Monikh et al., Environmental Science: Nano (2022). 9, 1566-1577. **4**. A. Monikh et al., Environmental Science and Technology (2025). 59, 4674–4683. **5.** A. Monikh et al.., Nano Today (2024). 59, 102466.