ACEnano knowledge infrastructure to support data collection, methods optimisation and knowledge sharing in the area of physicochemical characterisation of nanomaterials

Lucian Farcal, Edelweiss Connect (Switzerland)

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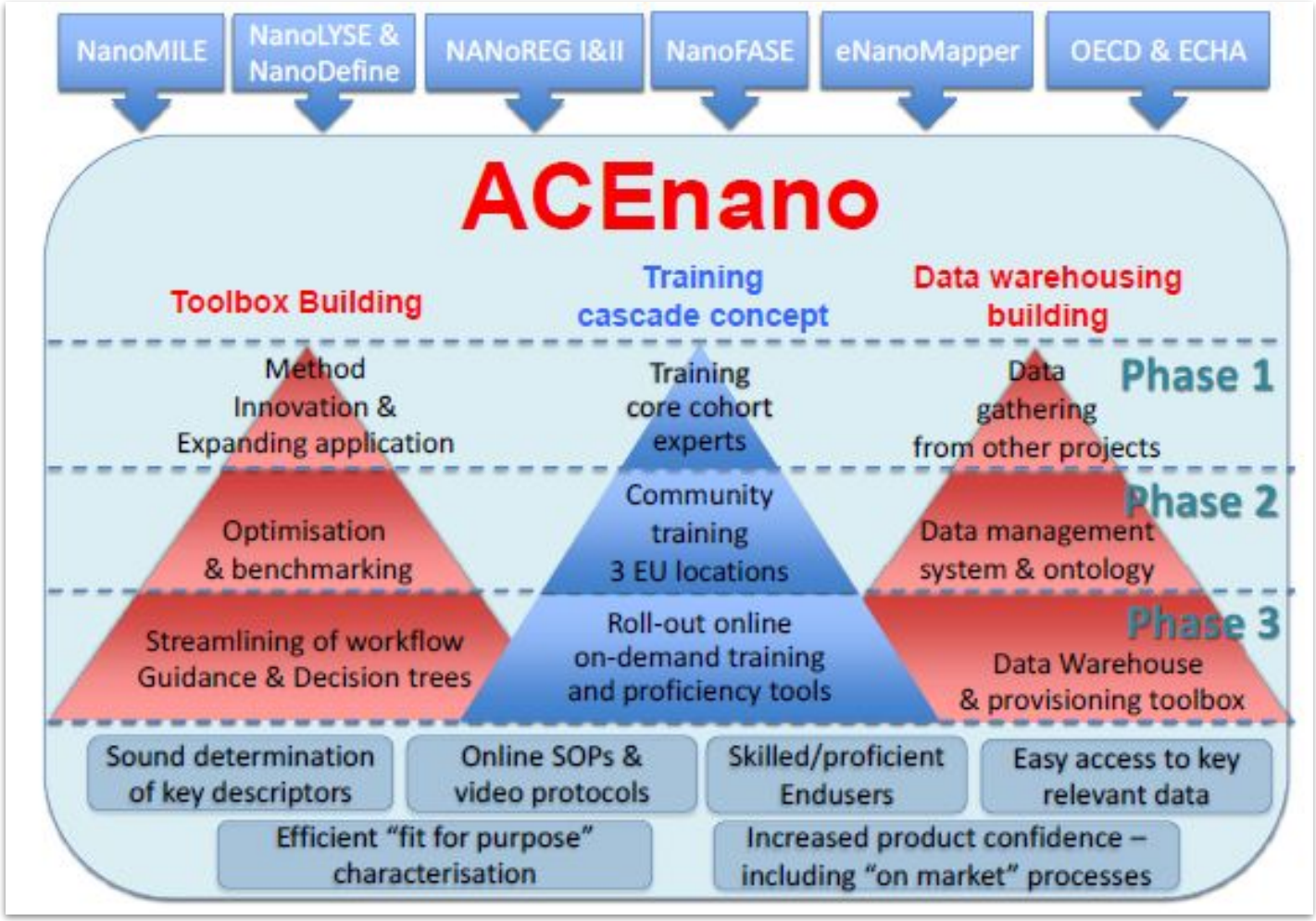
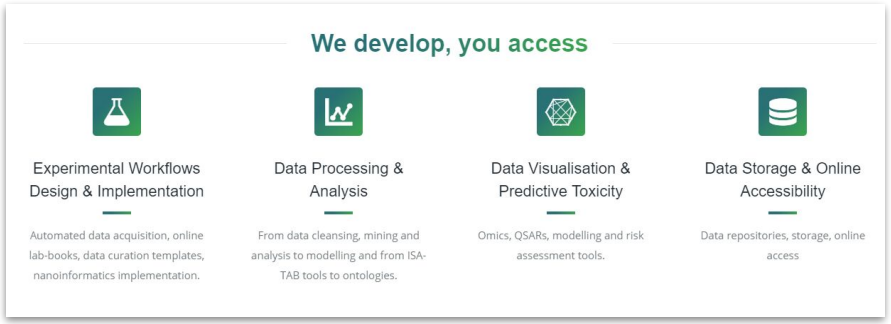
Introduction

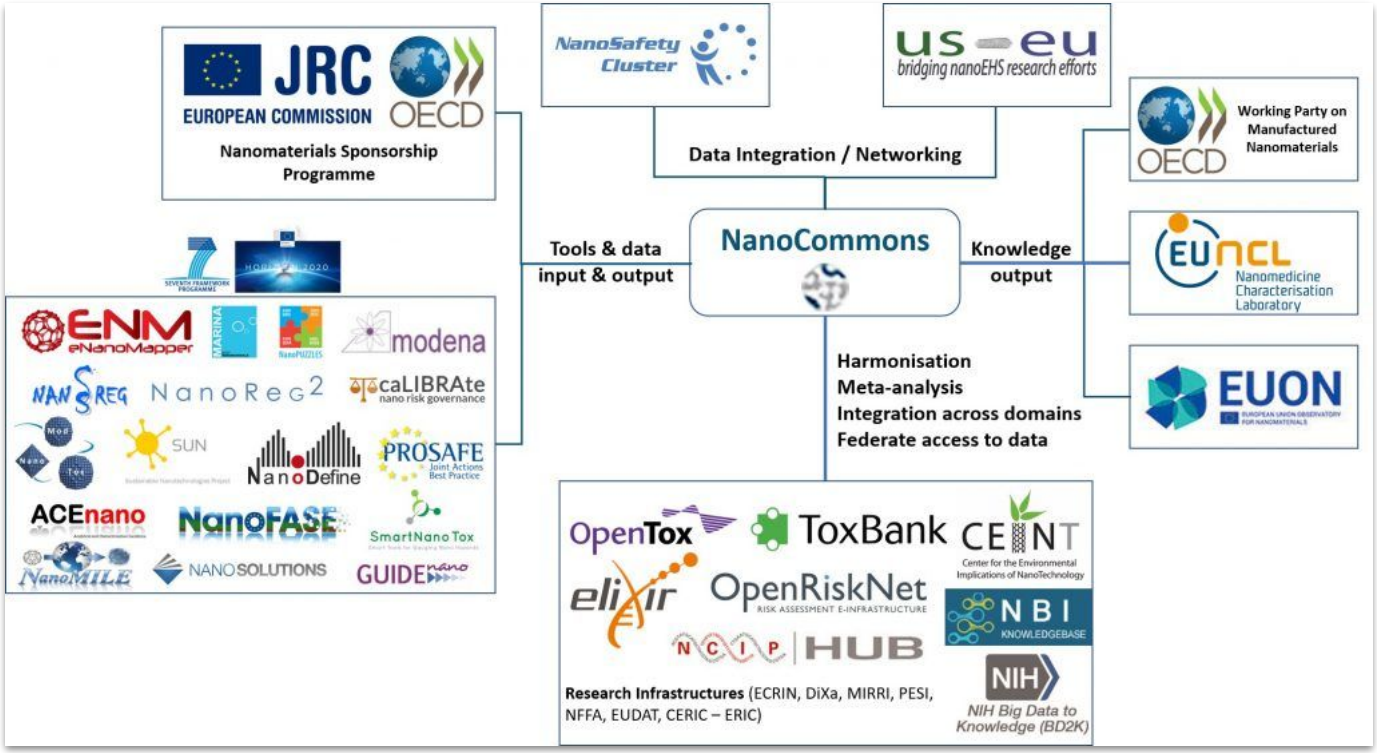
- **ACEnano** (Horizon 2020; Project number 720952) aims to introduce confidence, adaptability and clarity into nanomaterial risk assessment by developing a widely implementable and robust tiered approach to **nanomaterials physicochemical characterisation**.



www.acenano-project.eu

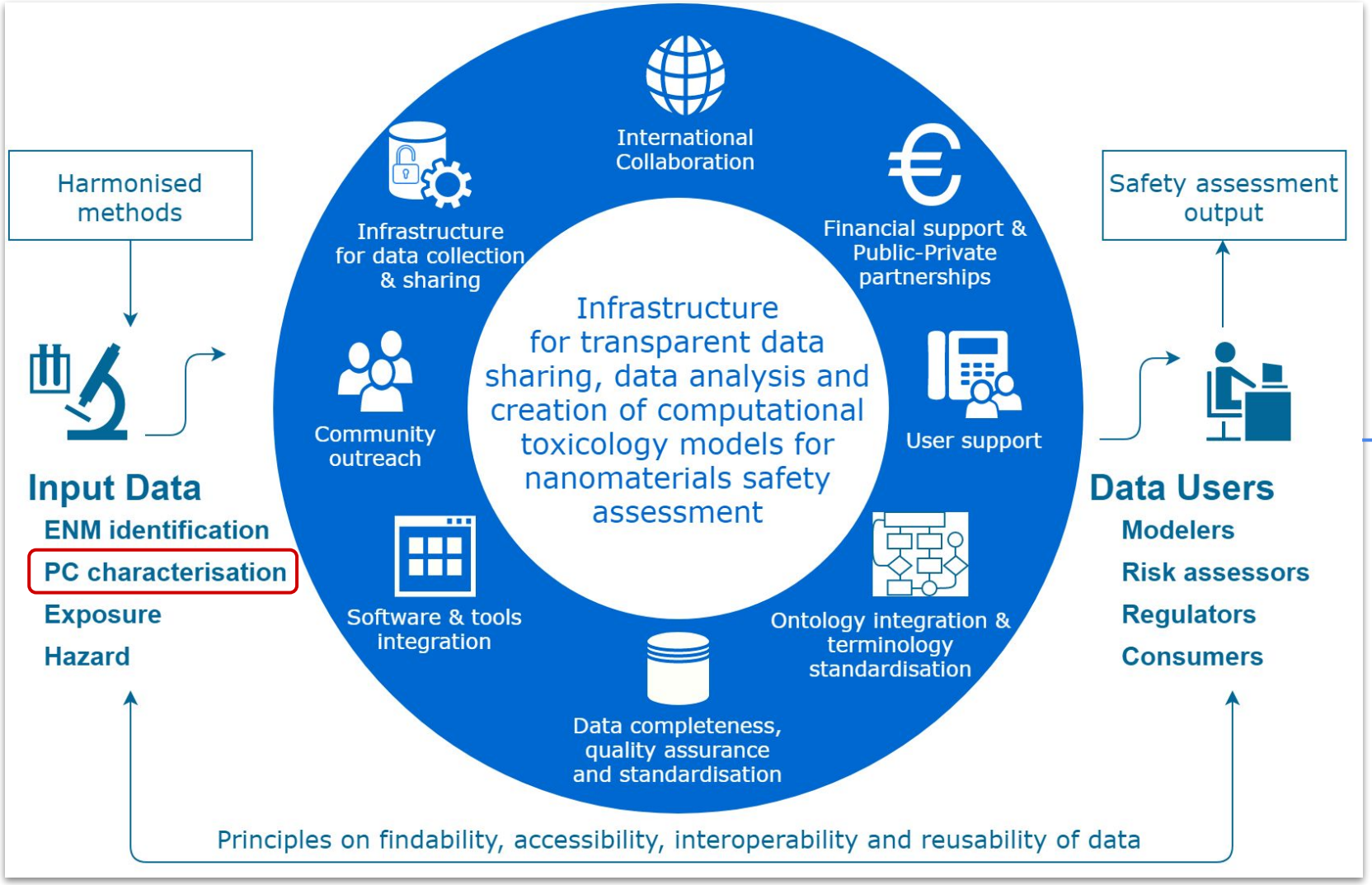
- **NanoCommons** (Horizon 2020; Project number 731032) will deliver a sustainable and openly accessible nanoinformatics framework (knowledgebase and integrated computational tools, supported by expert advice, data interpretation and training), for assessment of the risks of NMs, their products and their formulations.

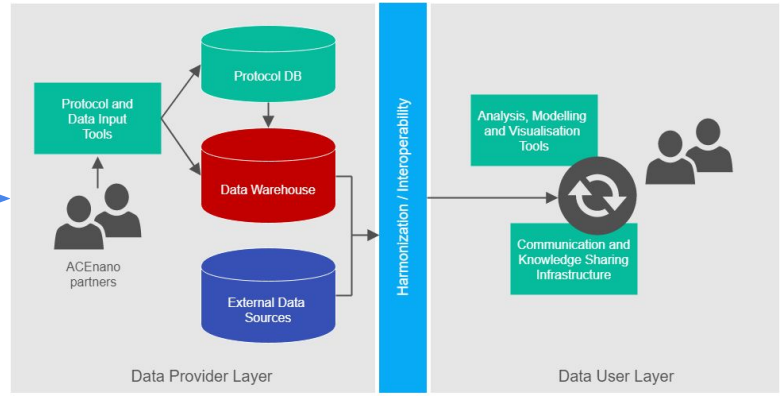




www.nanocommons.eu

Concept





ACEnano Knowledge Infrastructure

Fadeel B, Farcal L, Hardy B, Vázquez-Campos S, Hristozov D, Marcomini A, Lynch I, Valsami-Jones E, Alenius H, Savolainen K, Advanced tools for the safety assessment of nanomaterials, *Nature Nanotechnology 13, 537–543 (2018)*, DOI: 10.1038/s41565-018-0185-0

Concept

From ACEnano **Tasks**:

1. Develop new and optimise existing characterisation methods
2. Perform interlaboratory comparison studies
3. Optimise, validate and standardise methodologies

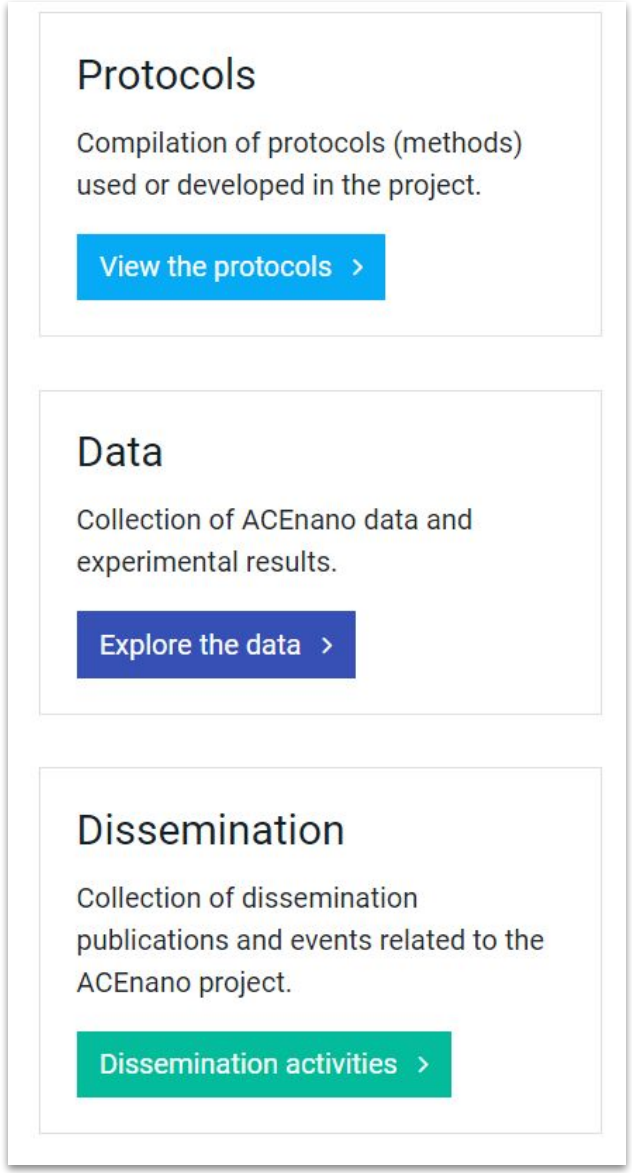
To **Solutions**:

1. Collect (computer-readable) metadata in form of a questionnaire, that are able to show differences between methods applied
2. Link metadata to the measurement results.

Data management approach

ACEnano Knowledge Infrastructure
https://acenano.douglasconnect.com/
A central platform to access harmonised and standardised methods applied for physicochemical characterisation of nanomaterials

- The knowledge infrastructure (KI) supports activities related to **data collection and methodology optimisation, and aims to further disseminate this knowledge** in a re-usable format
- Supports the implementation of Findable, Accessible, Interoperable and Reusable (**FAIR**) data principles, the reproducibility of **results** and **documentation** process
- **Structured protocols** and **metadata** allow for an easier comparison of the experimental setups/protocols used and, in this way, leads to better comparability (support intra- and inter-laboratory reproducibility goal)
- Document all steps performed on a sample from the identification to the final characterisation results, solving issues on **comparability and reproducibility** of results derived from insufficient documentation of the procedures applied
- Support the **optimisation, validation and standardisation** of methods



Targeted users

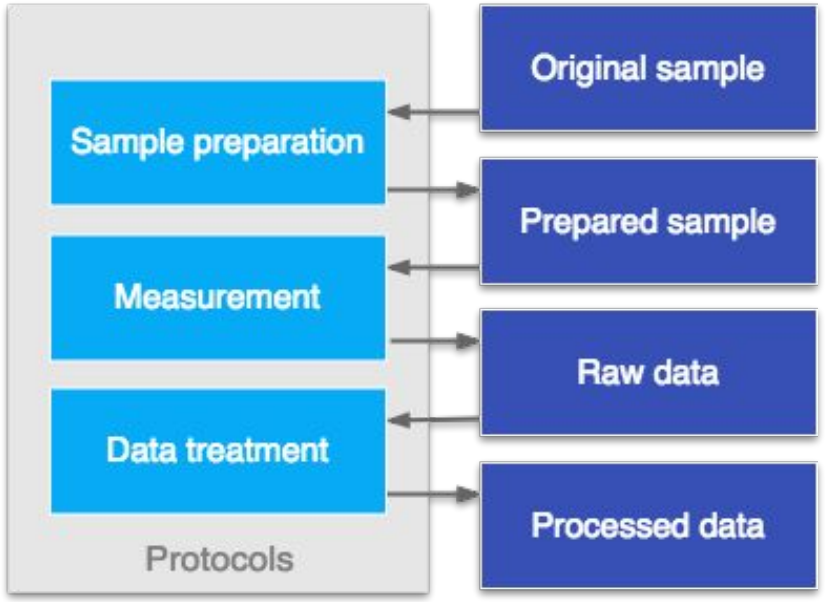
The KI addresses the needs of:

- **Method developers** (e.g., instruments providers, laboratories working on new methods) that aim to store, optimise and validate their protocols;
- **Methods applicants** (e.g., industry or research laboratories) that wish to have access to existing procedures, workflows and datasets in order to apply similar approaches and evaluate them regarding their performance, applicability domain and reproducibility or perform training activities;
- **Laboratories** applying additional methods (e.g., functionalisation of nanomaterials), performing **safety or toxicity assessments** or using **computational modelling** to further analyse the data, that require access to harmonised physicochemical characterisation data;
- **Industry, CROs and consultants** for the preparation of **regulatory dossiers** (e.g., under REACH regulation) by offering access to structured physicochemical characterisation information and datasets on different regulatory-relevant endpoints.

Knowledge warehousing

Protocols

- Access and sharing of methods
- Collection of metadata on the experimental procedure
- Tracking details on the steps performed
- Linked the method with the result
- Comparison of the experimental design
- Searchable and easy to filter database



Data

- Selection and use any of the methods added in the protocols database
- Create and save the full workflow applied
- Support intra- and inter-laboratory reproducibility goal
- Document all steps performed on a sample from the identification to the final characterisation results
- Storage and sharing of data

Protocols

Sample preparation protocol	
Part 1: General information	Protocol name and description
	Contacts
	Technique and Endpoints
Part 2: Steps	Multiple actions and action parameters
→ **Preview protocol, Make more changes & Submit protocol**	

Data treatment protocol	
Part 1: General information	Protocol name and description
	Contacts
	Technique and Endpoints
Part 2: Steps	Steps and algorithm used
→ **Preview protocol, Make more changes & Submit protocol**	

Measurement protocol	
Part 1: General information	Protocol name and description
	Contacts
	Technique and Endpoints
Part 2: Equipment	Instrument settings
	Type of datasets produced
	Measurement quality parameters
Part 3: Steps	Protocol steps
→ **Preview protocol, Make more changes & Submit protocol**	

Protocols: measurement

Protocol original name:

Original/published name or tradename.

Version of this protocol:

Use numbers to identify the versions of the protocol (e.g. 1, 2, ...). A new version is an updated version of the same protocol (e.g. major changes in the protocol steps, change of the instruments used for measurements, etc.).

Variant of this protocol:

Use letters to identify the variants of the protocol (e.g. a, b, ...). A new variant is the same protocol version with different variations in the procedure (e.g. the same protocol with a change in the instrument settings, different volumes used, etc.).

Brief description:

Long description:

References:

Development phase:*

Confidentiality:*

License:

Technique and Endpoints

Technique:*

List of endpoints and techniques covered by the ACEnano project.

Endpoints:*

Average size dimension
Batch dispersion / stability
Corona characterisation
Crystalline phase
Density
Deposition rate
Elemental composition and chemical purity
Functional coating
Homoaggregation rate

Hold down "Control", or "Command" on a Mac, to select more than one.

Phase:*

Please specify for which phase can your instrument measure the endpoint?

Continue to next step

Part 2: Equipment

Equipment

Please describe the equipment used to preform the measurement. Be sure to provide details on any instrument settings that may introduce artefacts in the final result.

Name:*

Model:

Common instrument makes and models.

Instrument type:

Software:

Software version:

Limit of detection upper:

What is the largest value of the endpoint that can be measured? If there are no definite detection limits please mention the particle or medium properties that limits the detectability as a function of size.

Limit of detection lower:

What is the lowest value of the endpoint that can be measured?

Limit of detection unit:

Instrument settings and parameters (optional)

List instrument settings and parameters that might influence the measured value or its accuracy, or are of importance for reproducing the experiment. Where applicable, also give units of these settings.

Setting	Value	Unit	
Setting	Value	Unit	delete
Setting	Value	Unit	delete
Setting	Value	Unit	delete

https://acenano.douglasconnect.com/

Collection of protocols

ACEnano — Analytical and Characterisation Excellence

NanoCommons — Nano-Knowledge Community

1

Sample preparation — NPL spICP-MS round robin dilutions of AuNPs in citrate - v2_a
Dilution of AuNPs in citrate
For technique: Single Particle Inductively Coupled Plasma Mass Spectrometry
For endpoints: Particle Size Distribution, Particle number concentration

Sample preparation — Sample preparation protocol AuNP FFF-ILC - v1.0_b
For technique: Asymmetrical field flow fractionation
For endpoints: Average size dimension, Particle Size Distribution

Sample preparation — Sample preparation protocol AuNP FFF-ILC - v1.0_b
For technique: Asymmetrical field flow fractionation
For endpoints: Average size dimension, Particle Size Distribution

Sample preparation — Sample preparation for Hydrophobic Interaction Chromatography - v1_a
Dilution in 1% PBS
For technique: Hydrophobic interaction chromatography
For endpoint: Hydrophobicity

Sample preparation — Sample preparation protocol AuNP FFF-ILC - v1.0_1.1
For technique: Asymmetrical field flow fractionation
For endpoints: Average size dimension, Particle Size Distribution

Sample preparation — NTA automatic settings - v1.0
Sample preparation for NTA interlaboratory comparisons
For technique: Nanoparticle Tracking Analysis
For endpoints: Average size dimension, Particle Size Distribution, Particle number concentration

Sample preparation — Sample Preparation for BET - v1_a
This protocol describes the sample preparation procedure for powdered TiO2 NMs used to measure the amount of physically adsorbed gas according to the Brunauer, Emmett and Teller (BET) method.
For technique: Brunauer–Emmett–Teller analysis
For endpoint: Volume Specific Surface Area (VSSA) / porosity

Sample preparation — Sample Preparation for UV-Vis - v1_a
This protocol describes the sample preparation procedure for particle size measurements in gold NP suspensions.
For technique: Ultraviolet–visible spectroscopy
For endpoints: Average size dimension, Particle Size Distribution

2

Measurement — Sample Analysis by BET - v1_b
This protocol describes the measuring of the amount of physically adsorbed gas according to the Brunauer, Emmett and Teller (BET) method.
Technique: Brunauer–Emmett–Teller analysis
Endpoint: Volume Specific Surface Area (VSSA) / porosity

Measurement — HIC elution of latex NMs in Phenyl functionalized column - v1_a
Characterization of Latex NMs hydrophobicity
Technique: Hydrophobic interaction chromatography
Endpoint: Hydrophobicity

Measurement — Measurement AuNP FFF-ILC - v1.0_1.1
Size determination of gold nanoparticles by Asymmetrical Flow Field-Flow Fractionation coupled with UV-vis-detection
Technique: Asymmetrical field flow fractionation
Endpoints: Average size dimension, Particle Size Distribution

Measurement — NTA automatic settings - v1.0
NTA Diameter and Number Concentration Measurement Using Automated Capture Settings
Technique: Nanoparticle Tracking Analysis
Endpoints: Average size dimension, Particle Size Distribution, Particle number concentration

Measurement — Sample Analysis by BET - v1_a
This protocol describes the measuring of the amount of physically adsorbed gas according to the Brunauer, Emmett and Teller (BET) method.
Technique: Brunauer–Emmett–Teller analysis
Endpoint: Volume Specific Surface Area (VSSA) / porosity

Measurement — Sample Analysis by UV-Vis - v1_a
This protocol describes quantification of the extinction of light that is measured from the spectral pattern using absorbance.
Technique: Ultraviolet–visible spectroscopy
Endpoint: Average size dimension

3

Data treatment — NTA automatic settings - v1.0
Data analysis for NTA
For endpoints: Average size dimension, Particle Size Distribution, Particle number concentration

Data treatment — Data Processing UV-Vis unknown samples - v1_a
This protocol describes the procedure of reporting results for the unknown samples analysed using Ultraviolet–Visible spectroscopy (UV-Vis) method.
For technique: Ultraviolet–visible spectroscopy
For endpoint: Average size dimension

Data treatment — Data Processing UV-Vis known samples - v1_a
This protocol describes the procedure of reporting results for the known samples analysed using Ultraviolet–Visible spectroscopy (UV-Vis) method.
For technique: Ultraviolet–visible spectroscopy
For endpoint: Average size dimension

Data treatment — Data processing BET
This protocol describes the procedure of reporting results obtained following the measuring of physically adsorbed gas according to the Brunauer, Emmett and Teller (BET) method.
For technique: Brunauer–Emmett–Teller analysis
For endpoint: Volume Specific Surface Area (VSSA) / porosity

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Collection of protocols

Sample preparation protocol AuNP FFF-ILC - v1.0_b

Sample preparation protocol

Identical protocol to the initial one, difference: filtered water filtered with membrane with 100 nm pore

For technique: Asymmetrical field flow fractionation
For endpoints: Average size dimension, Particle Size Distribution

1 Vortexing Mixing and dispersing of nanoparticles that are suspended in a liquid phase

Speed:	/
Duration:	2 minute
Phase change:	Liquid → Liquid

2 Dilution Additional dilution of original sample for analysis

Dilution scale factor:	1:4
Medium:	Filtered Ultrapure Water
	Ultrapure water provided by Millipore unit vacuum filtered using membrane with 0.1 um pore size (diameter 47 m PVDF) at 100 mBar
Other particles:	under LOD
Purity (resistivity):	18.2 MΩ.cm MΩ·cm
Medium volume:	0.9 milliliter
Sample concentration within medium:	12.5 milligram per liter
Phase change:	Liquid → Liquid

3 Vortexing Mixing and dispersing of nanoparticles that are suspended in a liquid phase

Speed:	/
Duration:	2 minute
Phase change:	Liquid → Liquid

Measurement AuNP FFF-ILC - v1.0_1.1

Create a copy

Measurement protocol

Size determination of gold nanoparticles by Asymmetrical Flow Field-Flow Fractionation coupled with UV-vis-detection

This protocol describes the sample preparation procedure for gold nanoparticle suspensions and subsequent particle size determination by means of AF4-UV-vis using external size calibration. This method enables the determination of the hydrodynamic diameter and the recovery of the sample. The herein described procedure is applicable to gold nanoparticles in suspension in a size range between 20 and 100 nm using BBI gold size standards for external calibration.

Measurement

Endpoints	Average size dimension Particle Size Distribution
Technique	Asymmetrical field flow fractionation (aF4)
Measurement quality parameters	Average mass recovery (percent) – *common setting:* > 80 Squared correlation coefficient (R^2) of relative size calibration – *common setting:* > 0.99 Repeatability (relative standard devitation of retention time) (percent) – *common setting:* < 2
Phase in which the measurement is performed	Liquid

Equipment

Instrument	**AF4**
Type of instrument	Asymmetrical Flow Field-Flow Fractionation
Instrument model	AF2000 MT Postnova Analytics
Settings and parameters	**Setting** membrane type: regenerated cellulose membrane, off channel temperature spacer thickness injection volume detector flow rate

Steps

1 Eluent preparation

Filtering NovaChem100 through a 0.22 µm syringe filter. Adding 0.50 mL of filtered NovaChem100 to 2 L of filtered and degassed ultrapure water to obtain the eluent (0.025% (v/v) NovaChem100, pH ~ 9.4).

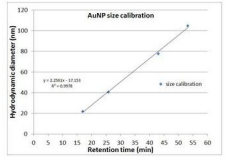
2 System qualification

In order to determine the mass recovery rate of the AF4-UV-vis system, three consecutive direct injections (n = 3, cross flow rate = 0 mL/min, elution time = 10 min, no focussing step) of the 12.5 mg L-1 suspension of one AuNP size standard shall be performed.
Furthermore, the same sample shall be measured (n = 3) using the fractionation conditions. The internal pressure of the system during these measurements must be within 4-12 bars. Based on the obtained sample peak area (without void piak and field-off peak) the mass recovery can be determined.
The system will be considered qualified for subsequent measurements if the obtained parameters are within the following values
- Repeatability: relative standard deviation of retention time (RSD) ≤ 2%
- Mass recovery ≥ 80%

3 Construction of external size calibration

The AF4 size-retention time external calibration shall be performed using BBI AuNP size standards with at least 4 calibrants in the working range of 20 nm to 100 nm. Each point of the calibration curve is defined by different AuNP sizes (20, 40, 80 and 100 nm). Measurements for each size shall be performed in triplicate (n = 3).
Based on the obtained retention times at the UV-vis-peak maximum and the hydrodynamic diameter Dh of the respective AuNP known from previously performed batch DLS measurements , a size-retention time calibration curve can be established.

Note: For proper sizing via the calibration curve equation, all AuNP samples including the unknown sample shall be fractionated using the same AF4 fractionation method!

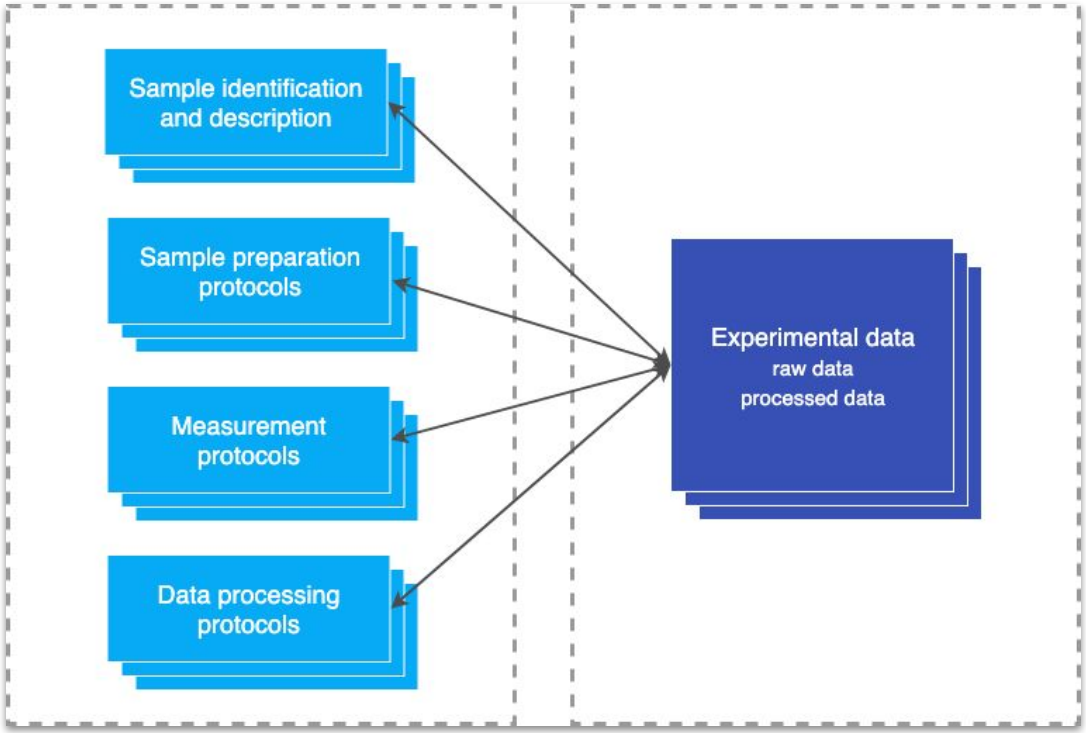


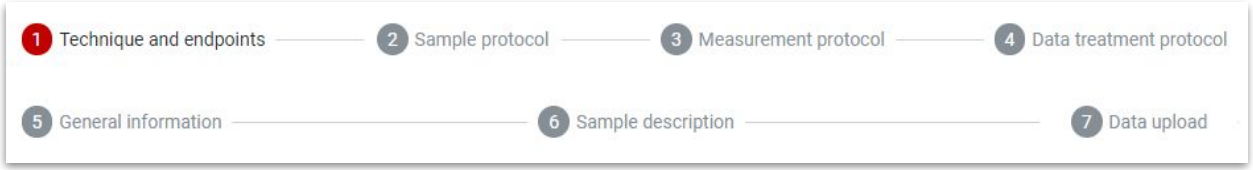
https://acenano.douglasconnect.com/

Data

Data upload process:

1. Select the technique used in the analysis and which endpoints were measured.
2. Select which sample preparation protocol was used.
3. Select the measurement protocol.
4. Select which data treatment protocol was used.
5. Provide details such as analysis name, description, and contact information.
6. Provide description of the sample that was used in the measurement.
7. Upload raw and processed data files.





Data workflow and data upload

1

Technique and Endpoints

In the lists below mark techniques and endpoints for which this analysis was done.
(List of endpoints and techniques covered by the ACEnano project).

Technique:*

Ultraviolet–visible spectroscopy

Endpoints:*

Homoaggregation rate
Hydrophobicity
Isoelectric Point
NP-cell interaction
Particle Size Distribution
Particle number concentration
Particle shape
ROS generation
Redox speciation

Hold down "Control", or "Command" on a Mac, to select more than one.

2

Protocols

Select a sample preparation protocol if one was used as part of your analysis.

Sample preparation protocol:

Continue to next step

Select a protocol for preview

3

Protocols

Select the measurement protocol used in your analysis.

Measurement protocol:

Continue to next step

Select a protocol for preview

4

Protocols

Select the data treatment protocol if one was used as part of your analysis.

Data treatment protocol:

Continue to next step

Select a protocol for preview

https://acenano.douglasconnect.com/

Data workflow and data upload

5

General information

Organisation submitting the analysis:
EwC - Edelweiss Connect GmbH

Analysis name:*

Brief description:

Long description:

References:

Confidentiality:*

License:

If the information and data provided are Open Access, please select one option from the Creative Commons copyright licenses.

Contacts

Name and email of contact person for the analysis:

First name *

Last name *

Email *

6

Sample description

Sample

Name*:

Code:

Supplier:

Batch number:

Sample phase:

Medium:

+Add a new medium

Sample volume:

Volume units:

Sample weight:

Weight units:

Concentration of material in sample:

Concentration units:

Nanoparticles in sample

Name:

CAS number:

Coating:

Crystalline phase:

Shape:

Size:

Size units:

Surface area:

Surface area units:

Coating thickness:

Units:

Delete this nanoparticle

+ Add nanoparticle

https://acenano.douglasconnect.com/

Data workflow and data upload

7

Data files

Dataset type:* Raw | Dataset name:* | Upload dataset:* Choose File No file chosen

Delete this dataset

Dataset type:* Processed | Dataset name:* | Upload dataset:* Choose File No file chosen

Delete this dataset

+ Add dataset

Submit your data

Technique | Organisation | Filter | Reset

Analysis	Data	Submitted
Characterisation of gold nanoparticles Technique: Ultraviolet–visible spectroscopy Endpoint: Average size dimension	ACEnano_UVVis_Raw data_AuNP 20190605 Raw ACEnano_UVVis_Results_AuNP 20190605 Processed	5 Jun 2019
Particle Size Distribution by UV-Vis 20190603 Technique: Ultraviolet–visible spectroscopy Endpoint: Particle Size Distribution	Particle size distribution test 20190603 Processed Particle size distribution test 20190603 Raw	3 Jun 2019

View complete workflow

Characterisation of gold nanoparticles

This workflow describes the sample preparation, measurement and data treatment procedures for particle size measurements of gold NP suspensions. The procedure involves quantification of the extinction of light that is measured from the spectral pattern using absorbance. UV-Vis refers to the ultraviolet to visible spectral region of light, the absorption of which is size dependent at the nanoscale. UV-Vis is therefore an ideal technique for the size characterisation of NP suspensions through absorbance at an appropriate wavelength. The settings defined below will be refined to optimise results during the subsequent runs.

Technique: Ultraviolet–visible spectroscopy

Endpoints: Average size dimension

Datasets

ACEnano_UVVis_Raw data_AuNP 20190605 - raw

ACEnano_UVVis_Results_AuNP 20190605 - processed

Sample description

Name:	Gold nanoparticles
Code:	AuNP BBI Unknown
Supplier:	BBI Solutions OEM Ltd
Medium:	Unknown
Phase:	Aqueous liquid
Sample volume:	1 mL
Concentration of material in sample:	50 mg/L

Nanoparticle

Core chemistry	Au
CAS number	7440-57-5
Size	Unknown nm

Sample preparation protocol

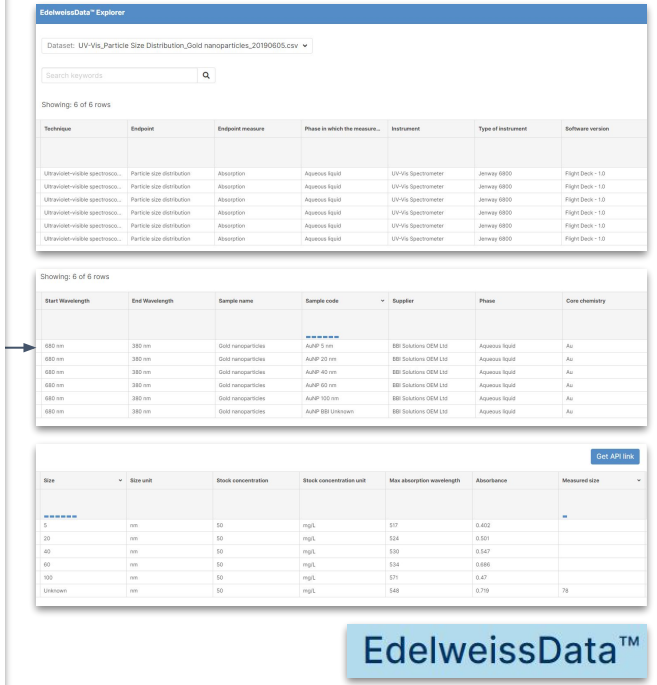
For technique: Ultraviolet–visible spectroscopy
For endpoints: Average size dimension, Particle Size Distribution

1 **Vortexing** Mixing and dispersing of nanoparticles that are suspended in a liquid phase

Speed:	/
Duration:	2 min
Phase change:	Aqueous liquid → Aqueous liquid

2 **Dilution** Additional dilution of original sample for analysis

Data - view, filter, analyse, API

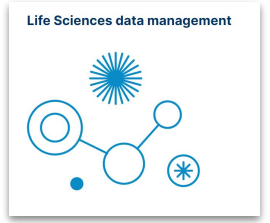


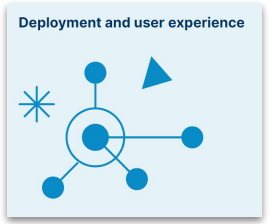
EdelweissData™

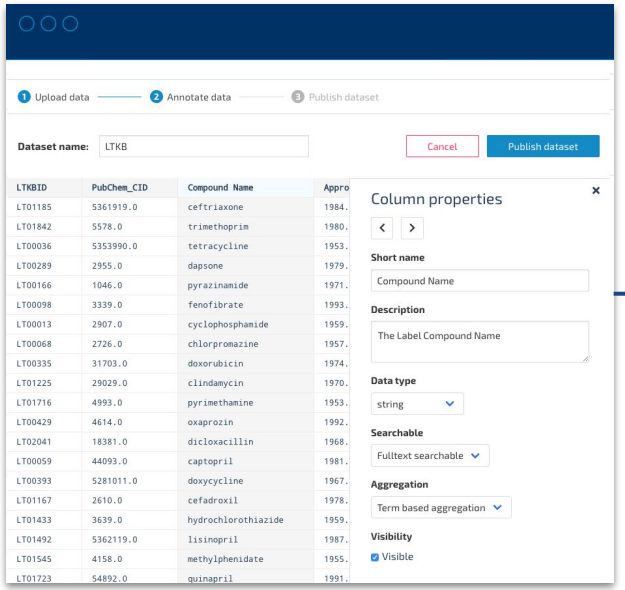
https://acenano.douglasconnect.com/

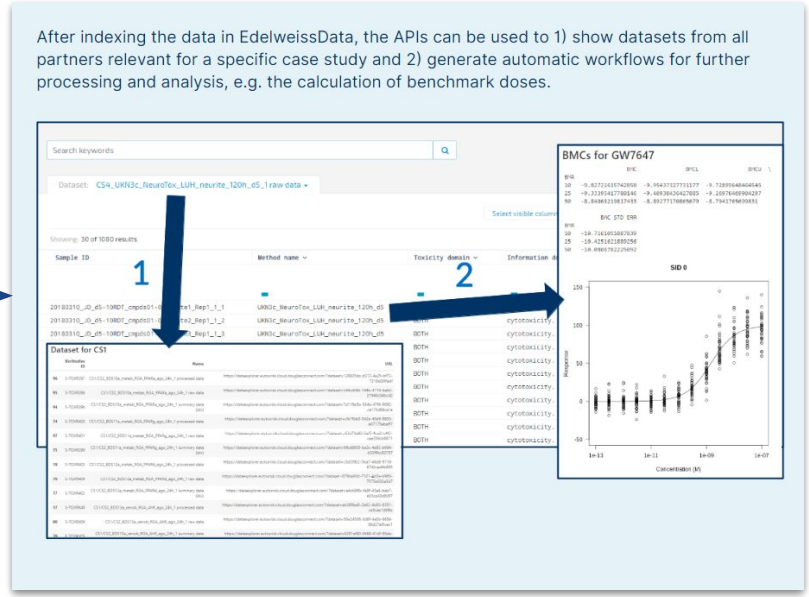
EdelweissData™

- A comprehensive tabular data and metadata environment
- Supports annotation, organisation and storage of primary data and metadata
- Provides domain data types (e.g. understand chemical's SMILES)
- Facilitates the analysis, visualisation and sharing of data
- Provides interactive exploration of the data via web-based tools
- Implements the FAIR data principles of Findability, Accessibility, Interoperability and Reusability.
- Allows the upload of data directly onto a secure, cloud-based platform
- Provides harmonised and interoperable access to different knowledge sources including publicly available databases
- Provides a rich application programming interface (API)
- Helps creating a culture of data sharing by making sharing easy
- Replace manual error-prone, time consuming and costly processes with lean data solutions and processing workflows



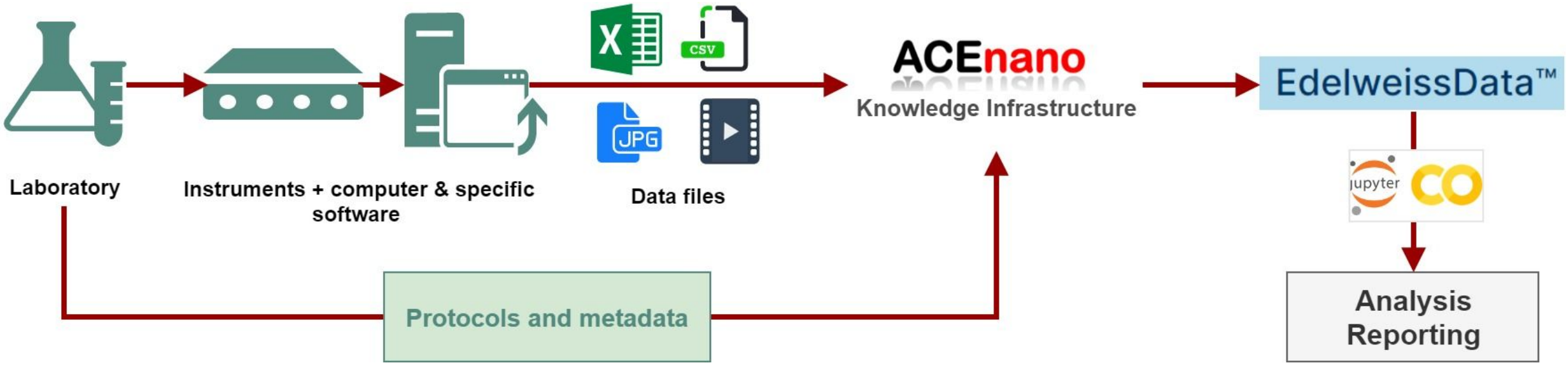






https://saferworldbydesign.com/edelweissdata/

From bench-to-analysis workflow



https://acenano.douglasconnect.com/

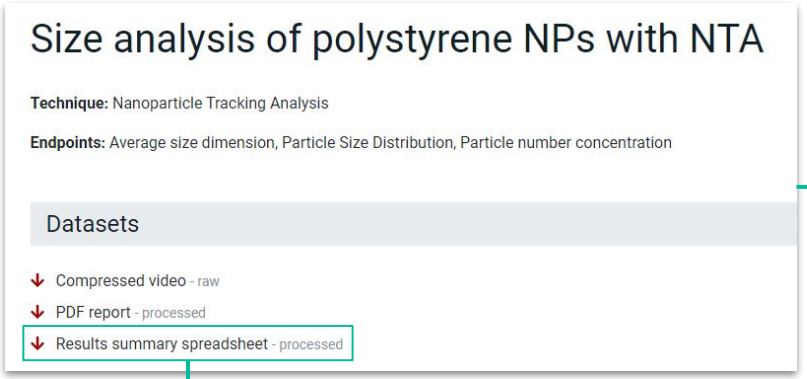
Use case: Nanoparticle Tracking Analysis (NTA)

Step 1. Addition of protocols

Step 2. Creation of data workflow

Step 3. Transfer of data to EdelweissData

- Selection of the dataset(s)
- Preparation of data file compatible with EdelweissData technology (*reading the original csv file, extracting relevant information, collecting metadata, creating the final csv summary data*)
- Automatic transfer (upload) of data
- Data visualisation



EdelweissData™

Replicate	Distribution	Weighting	Mean	Mode
1	Size	Number	97.1	97.1
2	Size	Number	95.8	95.3
3	Size	Number	96.9	97.8
4	Size	Number	96.6	96.6
5	Size	Number	95.6	95.9
1	Size	Surface Area	97.8	97.7
2	Size	Surface Area	96.4	95.8
3	Size	Surface Area	97.7	98.4
4	Size	Surface Area	97.4	97.2
5	Size	Surface Area	96.3	96.3
1	Size	Volume	98.1	97.9
2	Size	Volume	96.7	96
3	Size	Volume	98.1	98.7
4	Size	Volume	97.8	97.6
5	Size	Volume	96.5	96.5
1	Diffusion	Number	484.3	480.1
2	Diffusion	Number	492.2	489.5
3	Diffusion	Number	487.6	478.1
4	Diffusion	Number	489.9	484.7
5	Diffusion	Number	496	490.5
1	Diffusion	Surface Area	487.8	482.8
2	Diffusion	Surface Area	497.4	494
3	Diffusion	Surface Area	492.4	480.7
4	Diffusion	Surface Area	494.2	487.8
5	Diffusion	Surface Area	501.1	492.6
1	Diffusion	Volume	489.6	484.2
2	Diffusion	Volume	500.1	496.2
3	Diffusion	Volume	495.1	482.1
4	Diffusion	Volume	496.4	489.5
5	Diffusion	Volume	504.6	493.6

Step 4. Data analysis

File type: csv
Instrument: NanoSight NS300
Software: NTA 3.4 Build 3.4.003

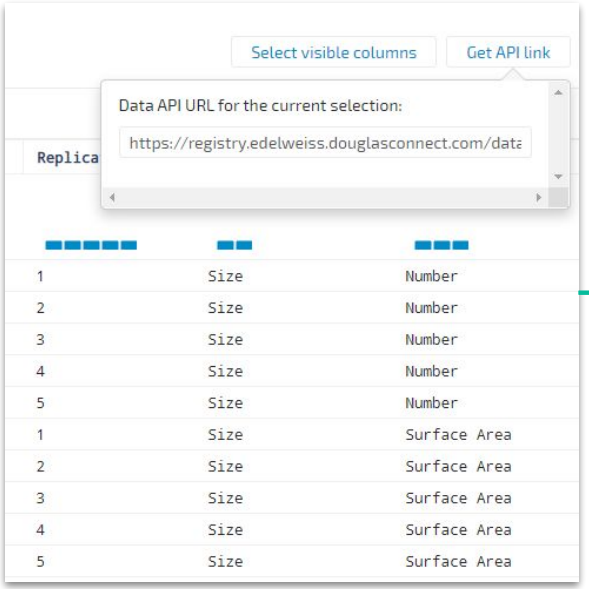
Example of summary file:
https://dataexplorer.edelweiss.douglasconnect.com/?dataset=1180f560-1eef-48d7-8fd5-f9f8bfec4446

Use case: Nanoparticle Tracking Analysis (NTA)

Step 4. Data analysis

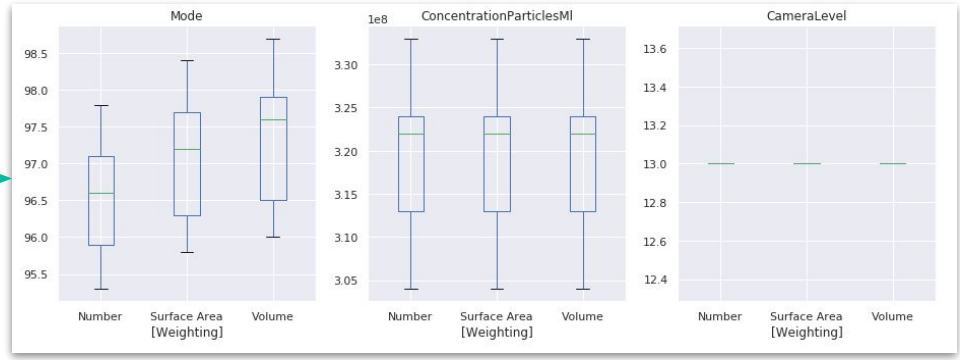
- Selection of dataset(s) to be analysed: data API
- Use the data API url (e.g. in JupyterLab or Google Colaboratory tools): extraction of relevant data, analysis, plotting, etc.
- Generation of a study report

EdelweissData™

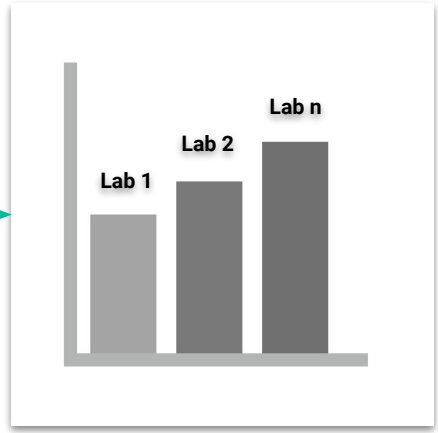


jupyter CO

Single dataset analysis



Analysis and comparison of multiple datasets (intra- or inter-laboratory comparison studies)



Study report

https://acenano.douglasconnect.com/

Why should I use it?

Main features offered by the Knowledge Infrastructure:

- Simple and data protected log-in system;
- Addition, storage and sharing of protocols and procedures;
- Creation of complete physicochemical characterisation workflows (from sample preparation, measurement and data treatment);
- Upload and download raw, processed and summary datasets;
- Harmonisation of the methodology within organisations or projects;
- Supports intra- and interlaboratory comparison of protocols and results towards achieving reproducibility and validation goals;
- Automatically use of data for analysis and computational modelling via the application programming interface (API);
- Direct access to EdelweissData™ technology (metadata integration, data searching, browsing and selection, data APIs selection, etc.);
- Combination of physicochemical data with other hazard and exposure data via linked data approaches based on common terminology and ontologies;
- Multiple endpoints and techniques covered.

Documentation and training materials

- User manual: https://github.com/NanoCommons/tutorials/tree/master/ACEnano manuals
- Presentations and posters: https://acenano.douglasconnect.com/dissemination/

Contact

- For user support, business enquiries or feedback, please contact us at: acenano@edelweissconnect.com

Thank you for your attention!

ACEnano partners (EU Horizon 2020 NMBP project no. 720952)
NanoCommons (Horizon 2020 INFRAIA project no. 731032)

Edelweiss Connect team

- Maja Brajnik, Ian Steeter, Oana Florean, Joh Dokler and Thomas Exner

Partners

- Geert Cornelis and Jani Tuoriniemi (Swedish University of Agricultural Sciences, Uppsala, Sweden)
- Anastasios Papadiamantis and Iseult Lynch (University of Birmingham, Birmingham, United Kingdom)