NPL
National Physical Laboratory

Towards de novo peptide-based virus-like particles as biological standards

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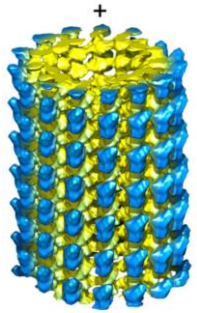
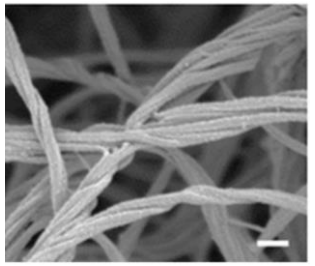
National Physical Laboratory

PAWG 9th April 2019, BIPM

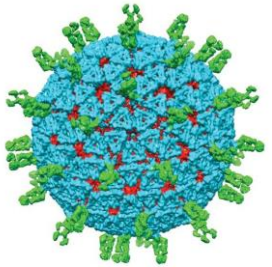
Inspiration from Nature



Microtubules

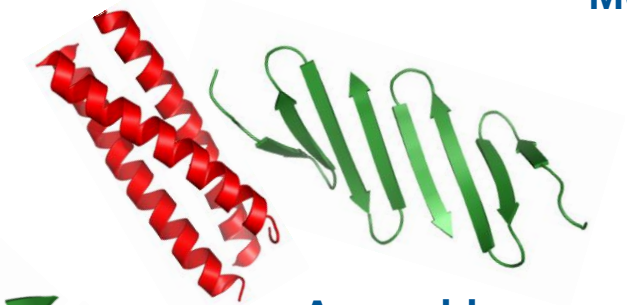


Collagen

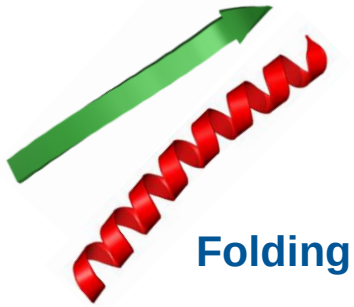


Rhesus rotavirus

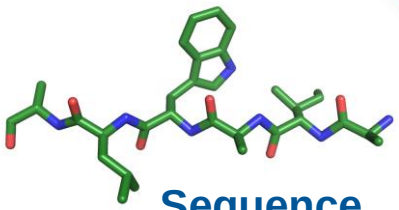
Morphology/Form



Assembly



Folding



Sequence

Functional complexity

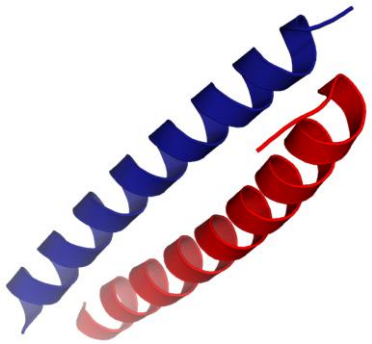
Structural complexity

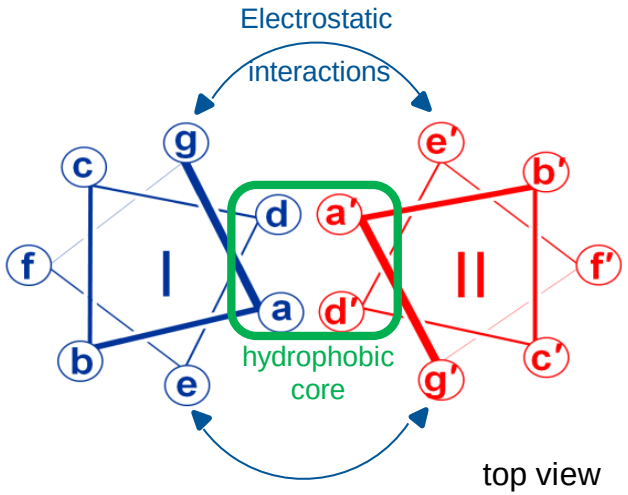
Cell motility and intracellular transport
Mechanical strength
Cell adhesion, migration and proliferation
Angiogenesis
Tissue morphogenesis and repair
Cell transfection

Mimicking Nature: bottom-up design

α-helical coiled-coil

gabcdef
PHPPHPP

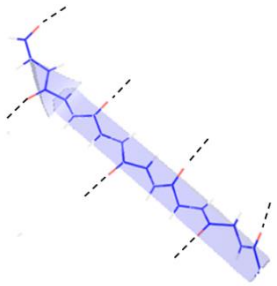


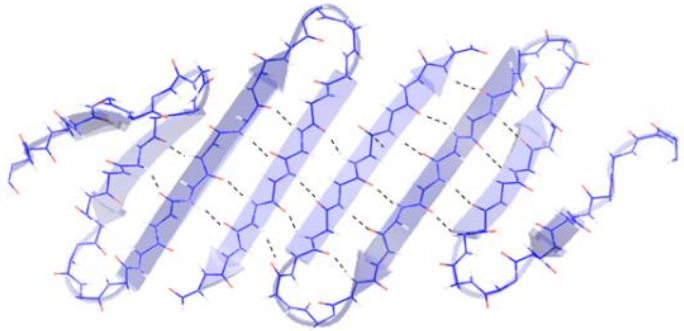


top view

β-sheet

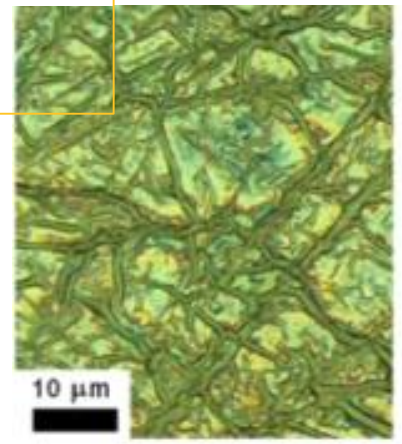
$(HP)_n$





Virus-like particles

Bottom-up design



Matrices

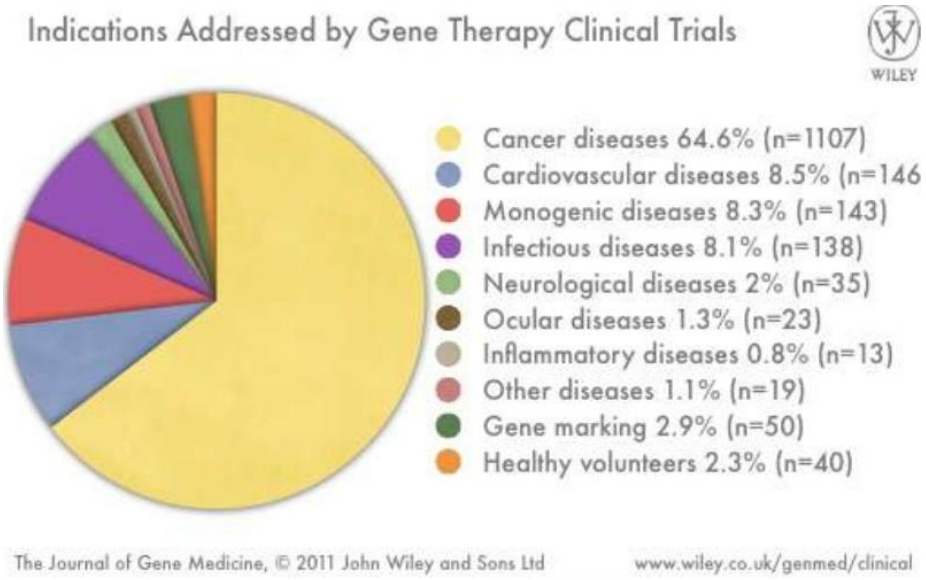
Modularity in peptide design allows for a controlled and predictable assembly.

Noble, De Santis et al. JACS, 2015; Faruqui et al. JACS, 2014;

Virus-like particles for gene/drug delivery

Gene delivery depends on adaptable nanoscale carriers to safely handle and deliver therapeutic nucleic acids into human cells.

Viruses are the most efficient gene-transfecting agents in nature and have been an inspiration for the development of novel gene delivery vehicles.



Carrier: viral (e.g. adenovirus) *vs* non-viral (e.g. liposomes, peptides)
Activity: expression (DNA), silencing (siRNA)

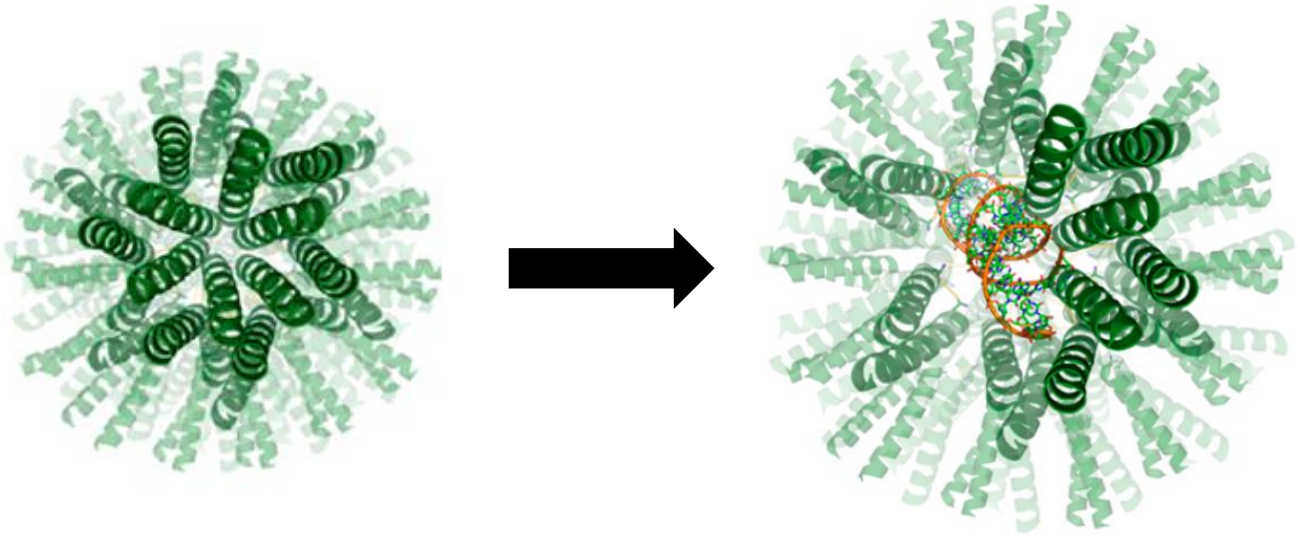
- **Size and mono-dispersity of the carrier (formulation and stability)**
- Amount of cargo (drug/gene) within the carrier (mass fraction)
- Amount of carrier/cargo complex delivered into cells and its fate
- Activity (transfection efficiency, cell viability, activity)

NATURE | VOL 564 | Dec 2018

"This year the FDA expects to receive about 250 applications to start clinical trials for novel cell and gene therapies" (Nature, 2018)

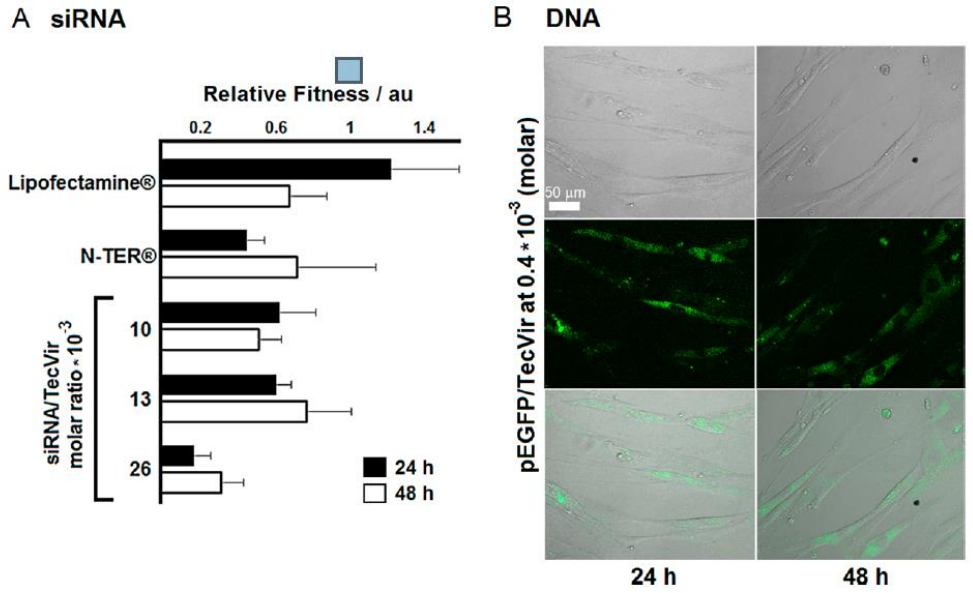
Virus-like particles for gene/drug delivery

We engineer peptide-based viruse-like particles with known, controlled and tuneable properties which are intrinsically biocompatible, biodegradable and underpin nearly all encapsulating systems in biology.



TecVir

TecVir/siRNA



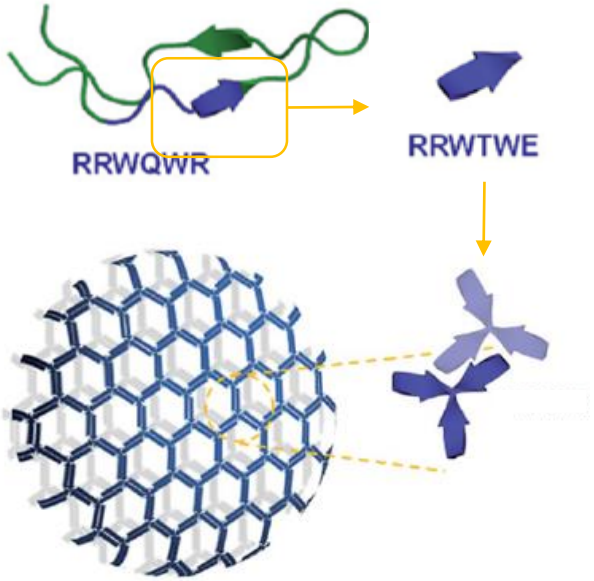
(A) Knockdown fitness of TecVir and commercial Lipofectamine RNAiMAX and N-TER (positive controls) normalized against siRNA alone (negative control) and the total counts of viable cells at different siRNA/TecVir molar ratios at 37 nM siRNA. (B) Widefield (upper), fluorescence (middle), and combined (lower) micrographs of human dermal fibroblasts transfected with plasmid DNA encoding for green fluorescent protein (green). GFP expression measured after 24 and 48 h is shown.

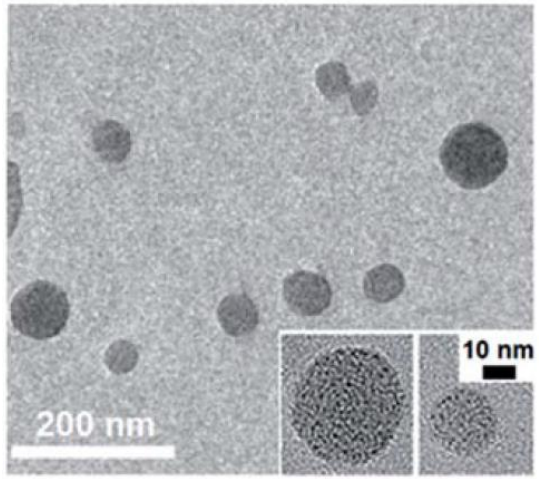
Tools and **methods** for the characterisation of nanocarriers, nanocarriers/cargo complexes and activity.

Noble, De Santis et al. JACS, 2015

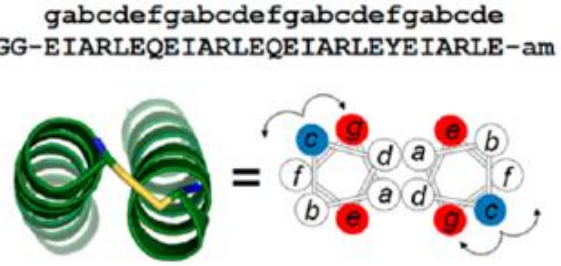
Virus-like particles: designs

Nature-inspired

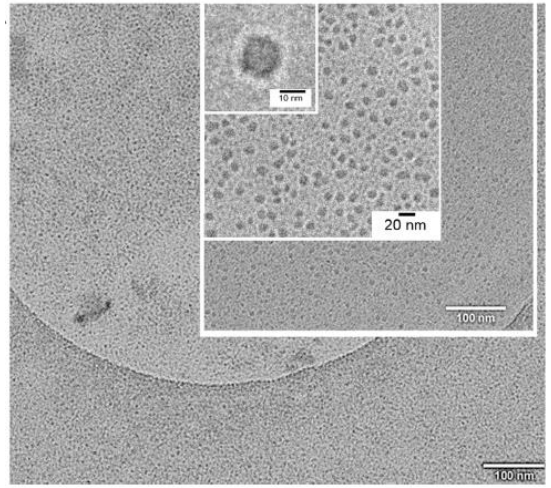


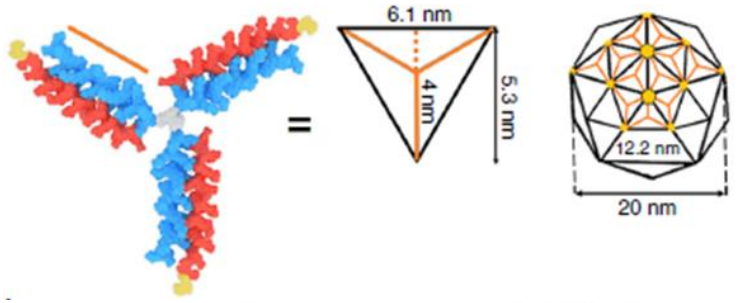


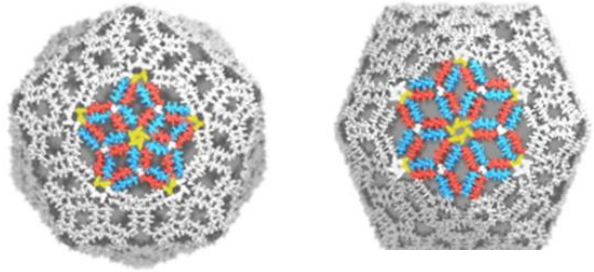
De novo

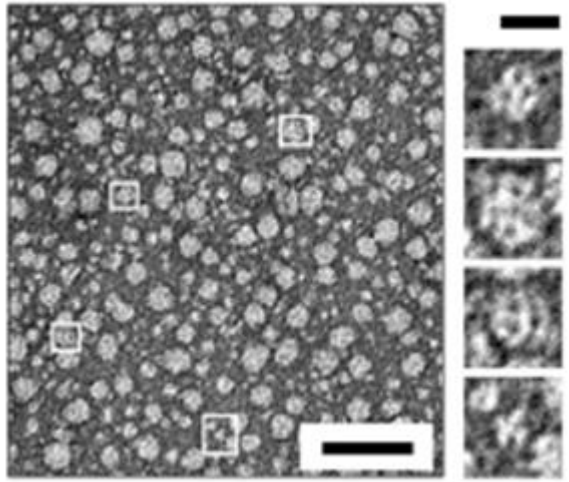


self-assembling subunit



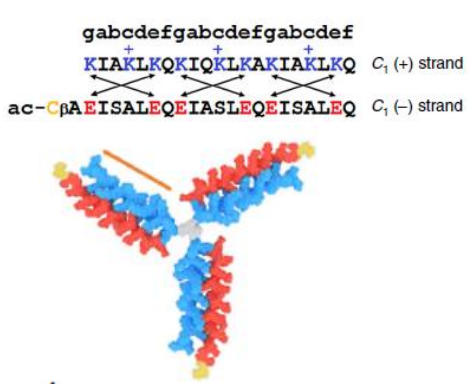


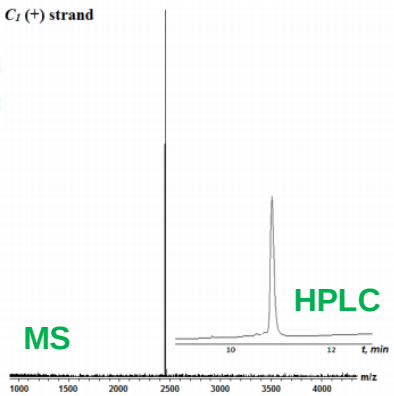




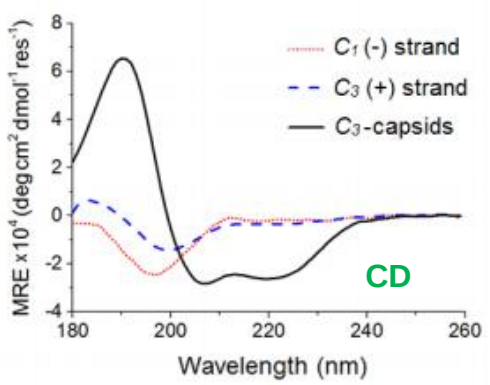
De Santis et al. Nat Comm, 2017; Castelletto et al, Chem Sci, 2015; Noble et al. JACS, 2015,

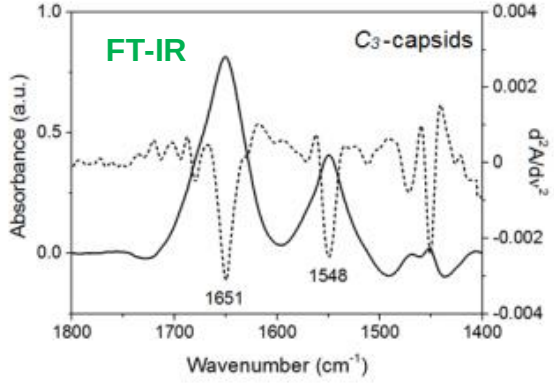
Virus-like particles: measurement continuum





Chemical identity/purity





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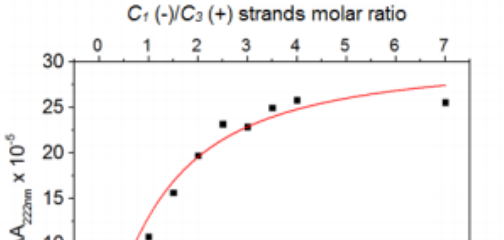
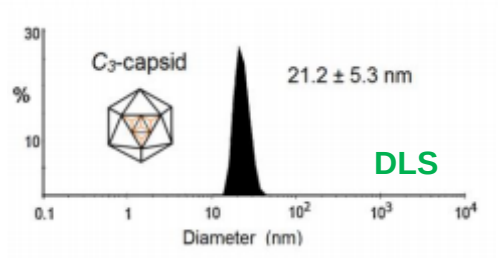
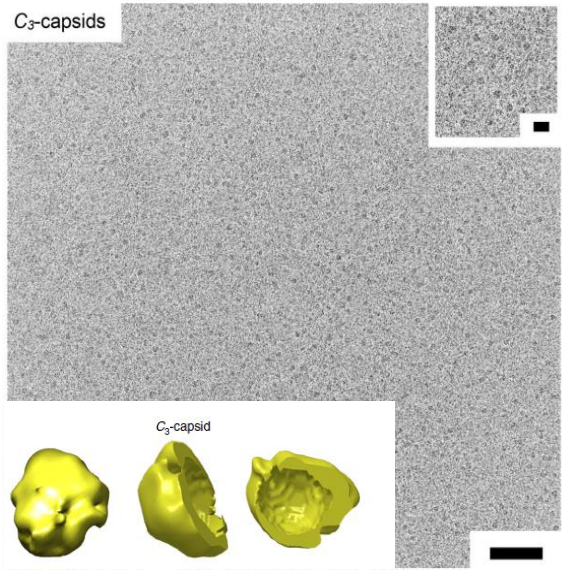
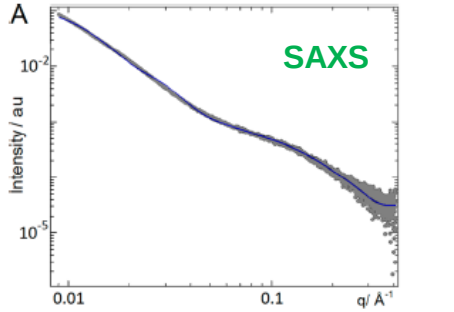
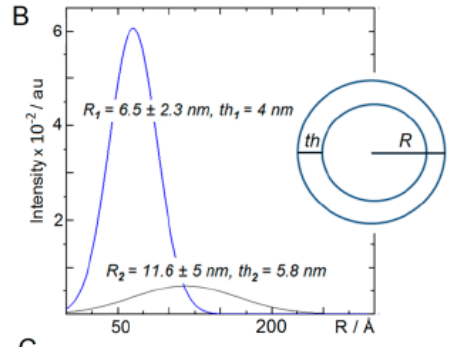




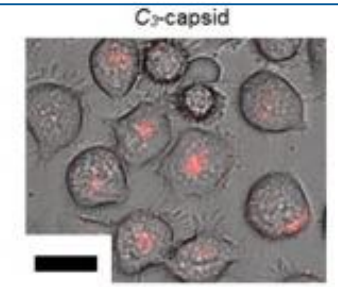


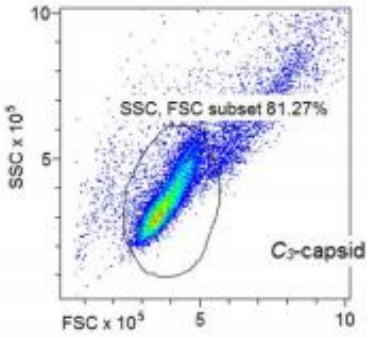
Cryo-EM and tomography





Assembly





Activity

Virus-like particles metrology

VAMAS

Synthetic Biomaterials
Technical Work Area 40

Versailles Project on Advanced Materials and Standards (VAMAS)
Participants accepted

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VAMAS supports world trade in products dependent on advanced materials technologies, through International collaborative projects aimed at providing the TECHNICAL BASIS FOR HARMONIZED MEASUREMENTS, TESTING, SPECIFICATIONS, AND STANDARDS.

Size and mono-dispersity of the carrier (formulation and stability)

International intercomparison studies for the validation of candidate reference materials and protocols

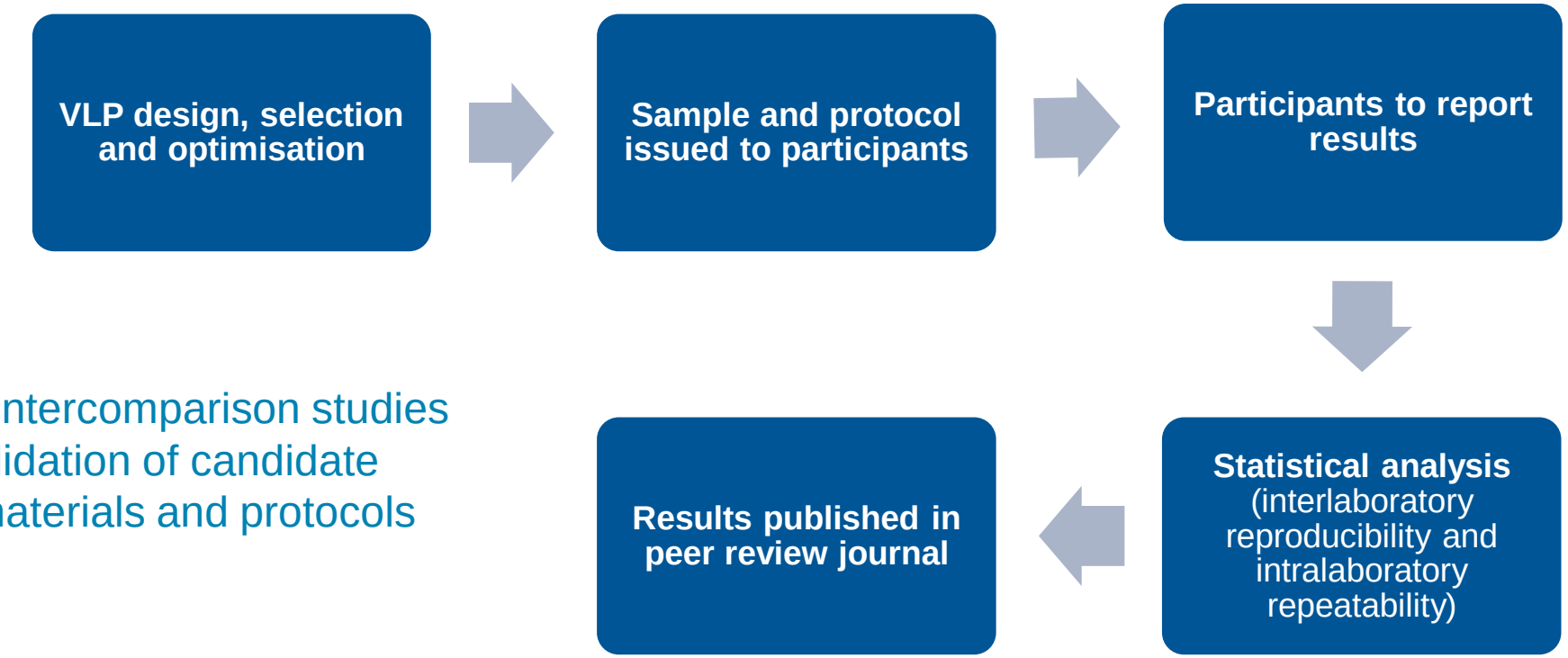

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Table I: Summarising the most common techniques used in nanoparticle sizing applications.

Technique	Size Range in nm	Ensemble or single particle	Sample condition	Additional Comments	Refs	Standards
Transmission electron microscopy	1 – 1000	Single Particle	Deposited onto a film	Provides additional information on the shape, internal structure and chemical composition of individual nanoparticles. Due to this it is often seen as the ultimate technique of nanoparticle measurement. However the high set up cost, the requirements for highly trained personnel and issues such as particle agglomeration means that this technique cannot be used for routine analysis	*[40-45]*	*[46]*
Scanning electron microscopy	10 – 10000	Single Particle	Deposited onto a film	Similar to TEM except with lower overall resolution (but with a higher lower limit) but can successfully resolve size larger particles. In addition there are less sample preparation issues.	*[42][44][47]*	*[46]*
Scanning probe Microscopy	0.5 – 3000	Single Particle	Deposited onto a film	The major advantages over TEM and SEM are the lower setup and running costs and offers a direct route to traceability via the use of a metrological instrument. In addition particle agglomeration is not as a big an issue. Image artefacts mean that little or no shape information can be obtained.	*[42][48][49]*	
Dynamic light scattering	0.5 – 1000	Ensemble	In suspension	Well-established technique, which provides reliable measurements of monodispersed, non-agglomerated particles in suspension. Confusion often arises as it measures the hydrodynamic radius and not the physical size. For poly dispersed samples the analysis is weighted towards larger particles and/or agglomerates leading to misleading results.	*[20][42][45] [47][50-52]*	*[53]*
Nanoparticle tracking analysis	30 – 1000	Single particle	In suspension	Based on the same principle as DLS but tracking the behaviour of individual nanoparticles, which can be related back to the hydrodynamic radius. Overcomes the problems of polydispersity and agglomeration that	*[54-56]*	

…etc

Good Practice Guide No. 119: *Good Practice Guide for the Determination of the Size and Size Distribution of Spherical Nanoparticle samples (NPL)*

Virus-like particles metrology

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Scope

Characterisation of the size and size distribution of chemically synthesised peptide-based virus-like particles in the nanoscale range.

Measurands

- Count: number of particles/μm^2
- Size and size distribution: Feret diameter
- Shape: roundness

Sources of uncertainty (by TEM)

- Image acquisition
  - Instrument calibration
  - Geometric errors, e.g. internal or external misalignment of the microscope
  - Number of particles measured (ISO 13322-1:2004 - size distribution and degree of confidence)
- Image analysis
  - Thresholding (under or oversampling)
  - Pixel size and noise (difficulty in assigning the edges of the particles)
  - Touching particles

Virus-like particles: selection and optimisation

From one sample

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A metrology checklist established by **ISO/TC 229** was used to assess and design the protocol

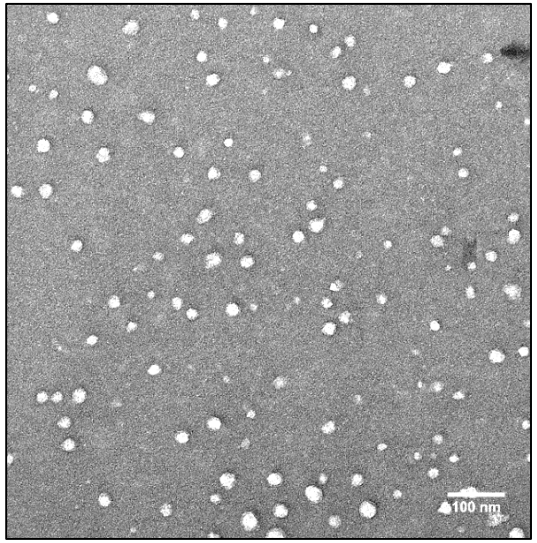
ISO 13322-1:2004: Instrument set up and image acquisition

Sample deposited on 3 mm TEM grids (formvar/carbon), stained using uranyl acetate (UA) and imaged by TEM.

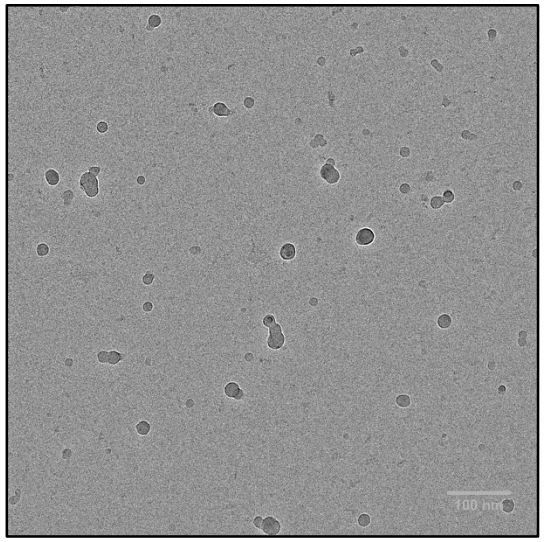
Optimisation:

- Sample concentration (100 µM and 200 µM)
- Incubation time (prior to deposition): 12h, 24h, 48h
- Concentration of uranyl acetate
- Staining protocol

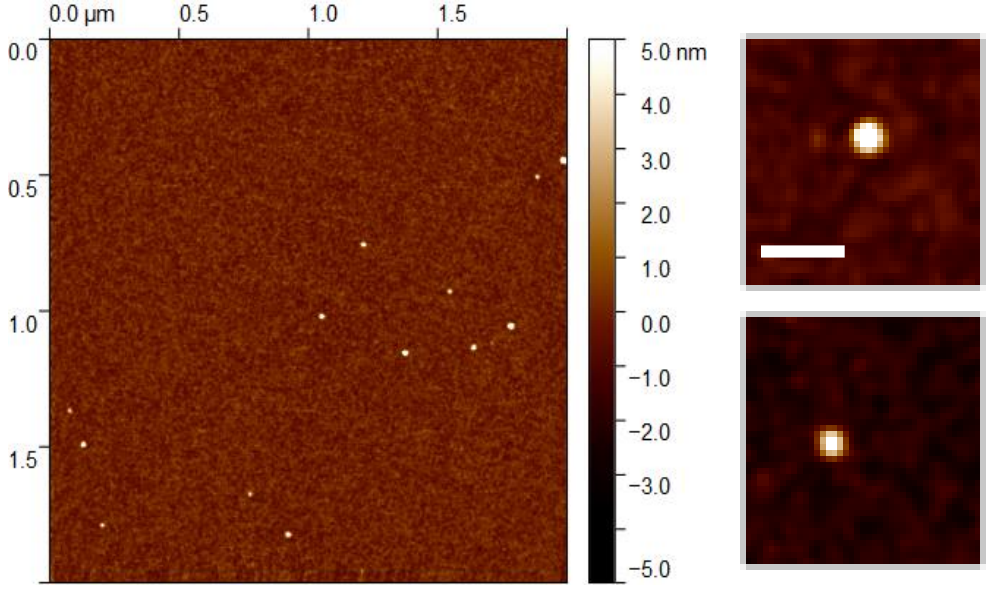
Representative images
100 µM, 48 hrs incubation.



TEM

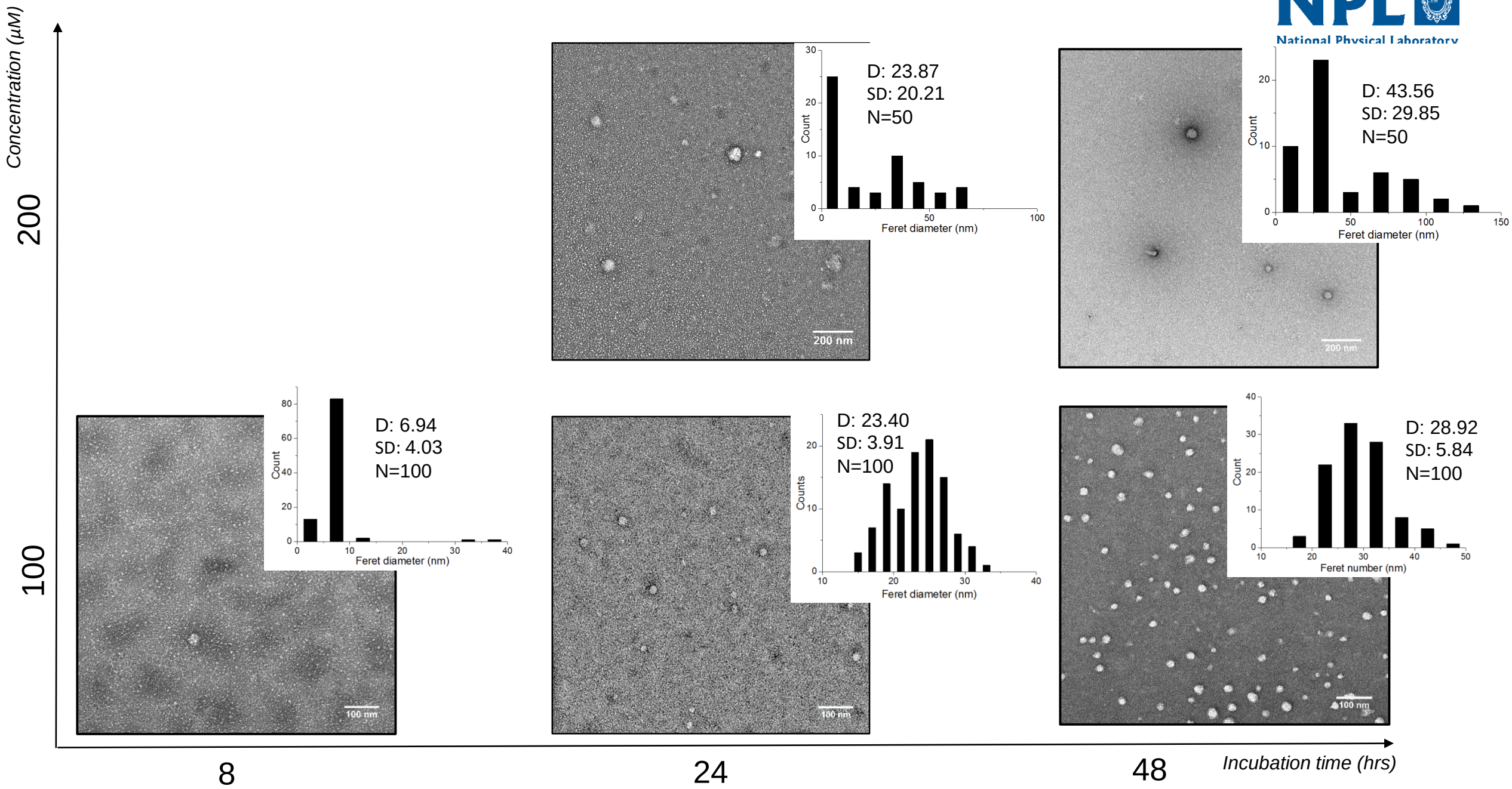


Cryo-EM



Liquid AFM (scale bar is 50 nm)

Virus-like particles: selection and optimisation



Virus-like particles: imaging protocol

ISO 13322-1:2004: Particle size analysis — Image analysis methods

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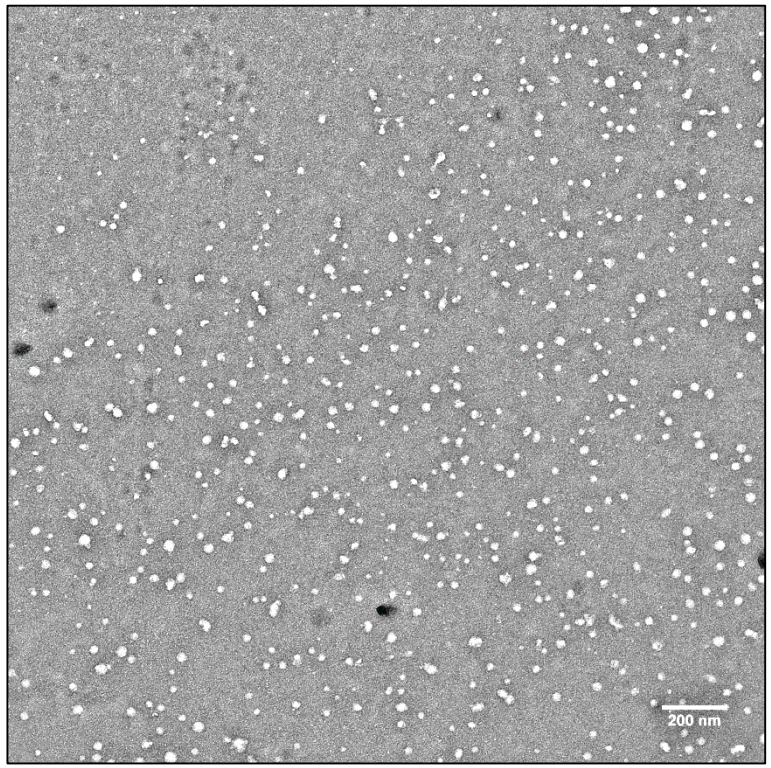
Each participant is sent 3 TEM grids with deposited peptide-based virus-like particles (p-VLPs)

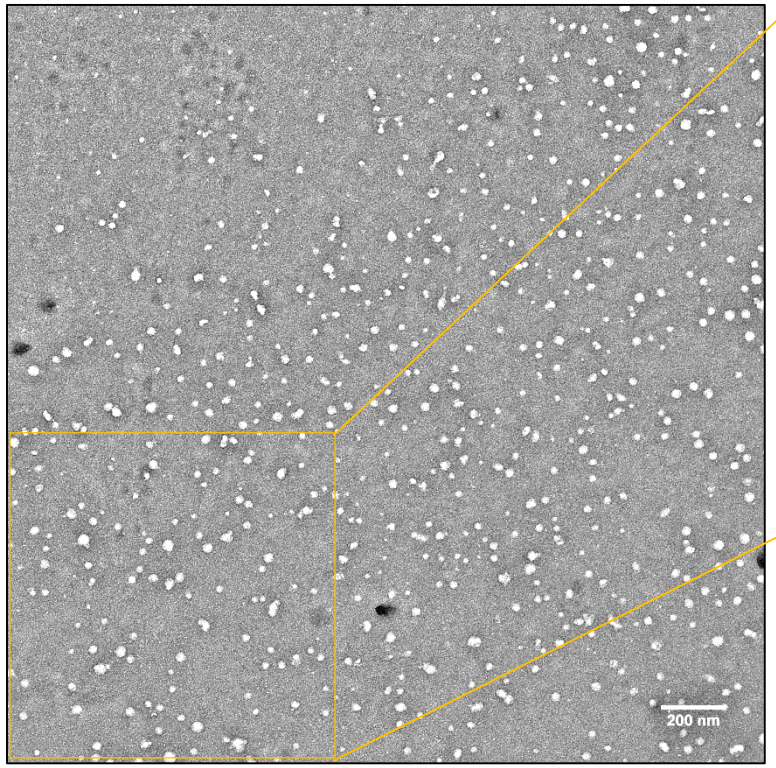


Representative TEM image

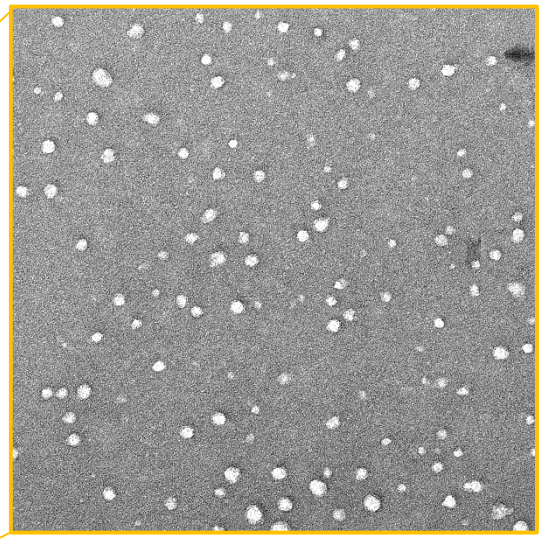
- Select a magnification/image resolution combination that will provide a minimum of **2 pixels/nm** or <0.5 nm/pixel.
- Do not report data for any touching particles or cut by the frame.
- Image different frames well spaced across the sample
- Count and report at least **500 particles** in frames that are well spaced across the sample.
- For all selected particles in each frame, report the particle number, the frame number and all measurand data.

Virus-like particles: data analysis protocol

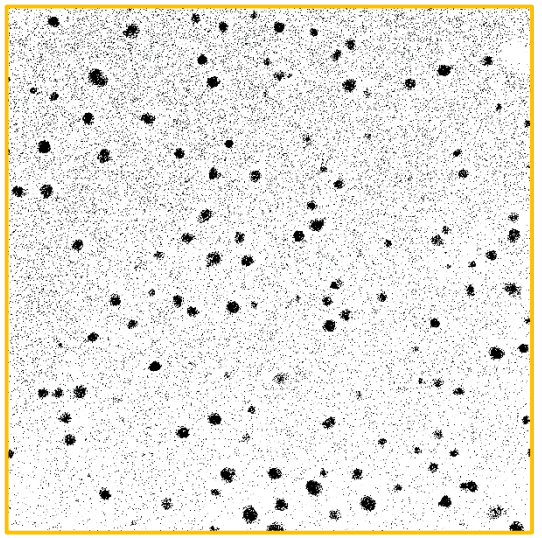
ISO 9267-6: representation of results of particle size analysis



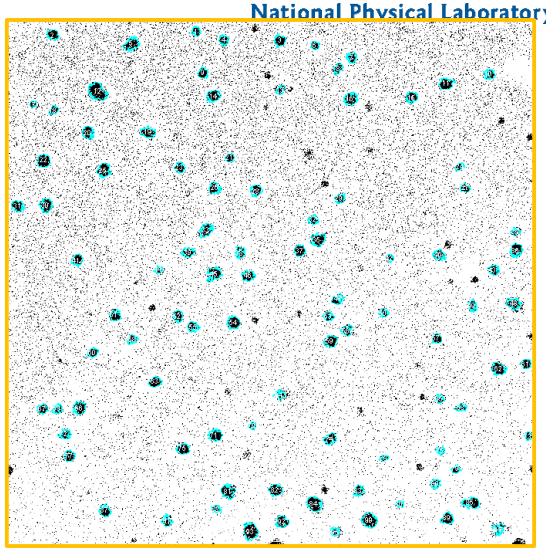
Data acquisition
(from different frames)



Select a 1µm^2 region



Convert the image to binary or threshold



Use automatic particle analysis tools from ImageJ

- Crop the image to remove scale bars and other image artefacts that might affect contrast or particle analysis.
- Convert the image to binary file or threshold manually.
- Select the measurands
- Analyse the particles
- Save each image file that shows particle outlines and their number sequence and the spreadsheet, which reports all measurand values, the particle number and the frame number associated with each particle.

Results from participants will be analysed to establish reproducibility and repeatability

Summary

- Need for tools and materials for the characterisation of carriers for gene/drug delivery
- Selected a suitable candidate for the development of tools and methods for the characterisation of the size and size distribution for biological nanocarriers.
- Described an imaging and data processing protocol for the analysis by TEM.
- Preparing the materials to be sent to participants (currently being recruited).

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Department for Business, Energy & Industrial Strategy

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Worked example – repeatability (One way ANOVA)

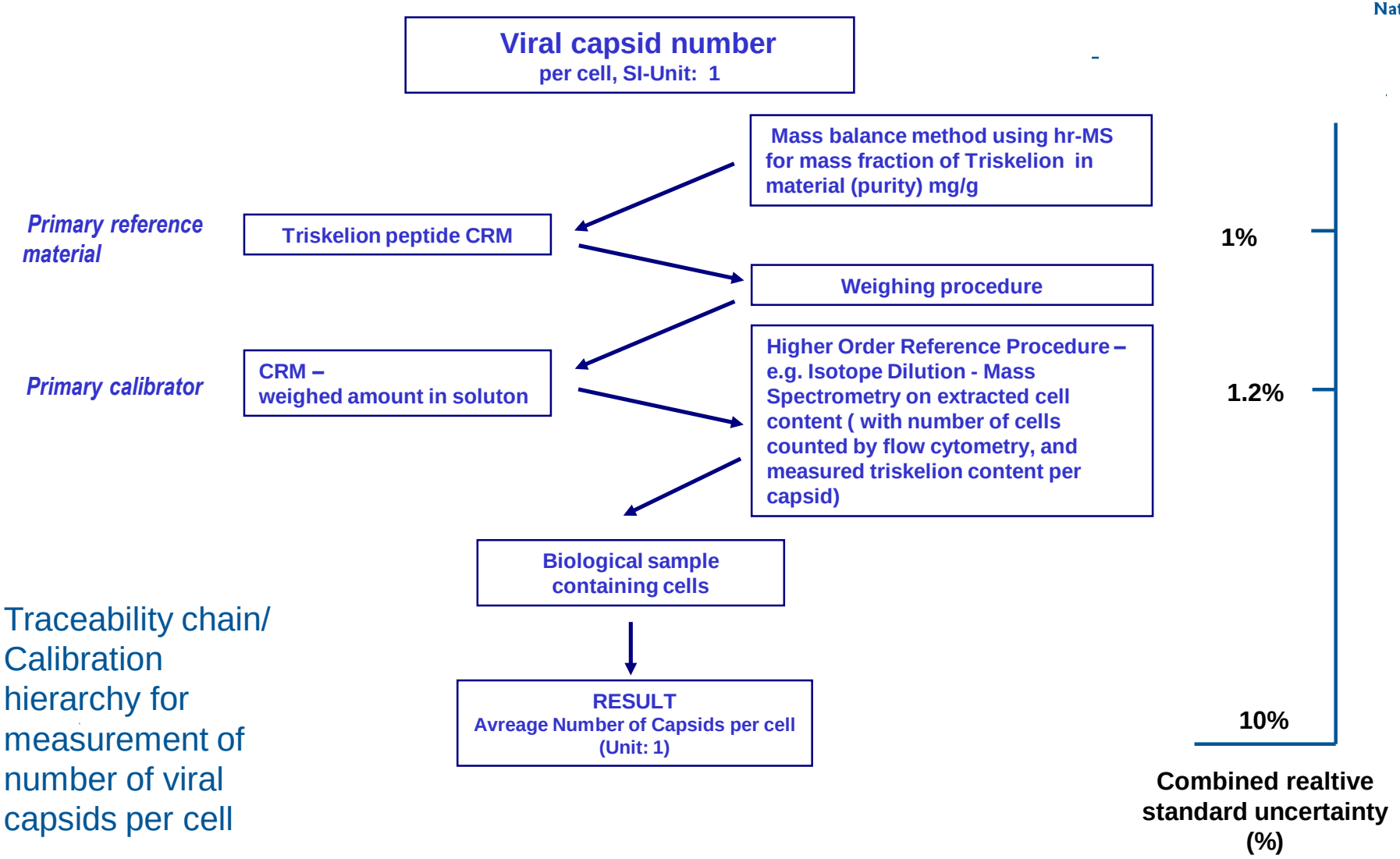
Objective: assess if all frames within one lab are best represented by the mean

Null hypothesis: for each lab, all frames have the same mean

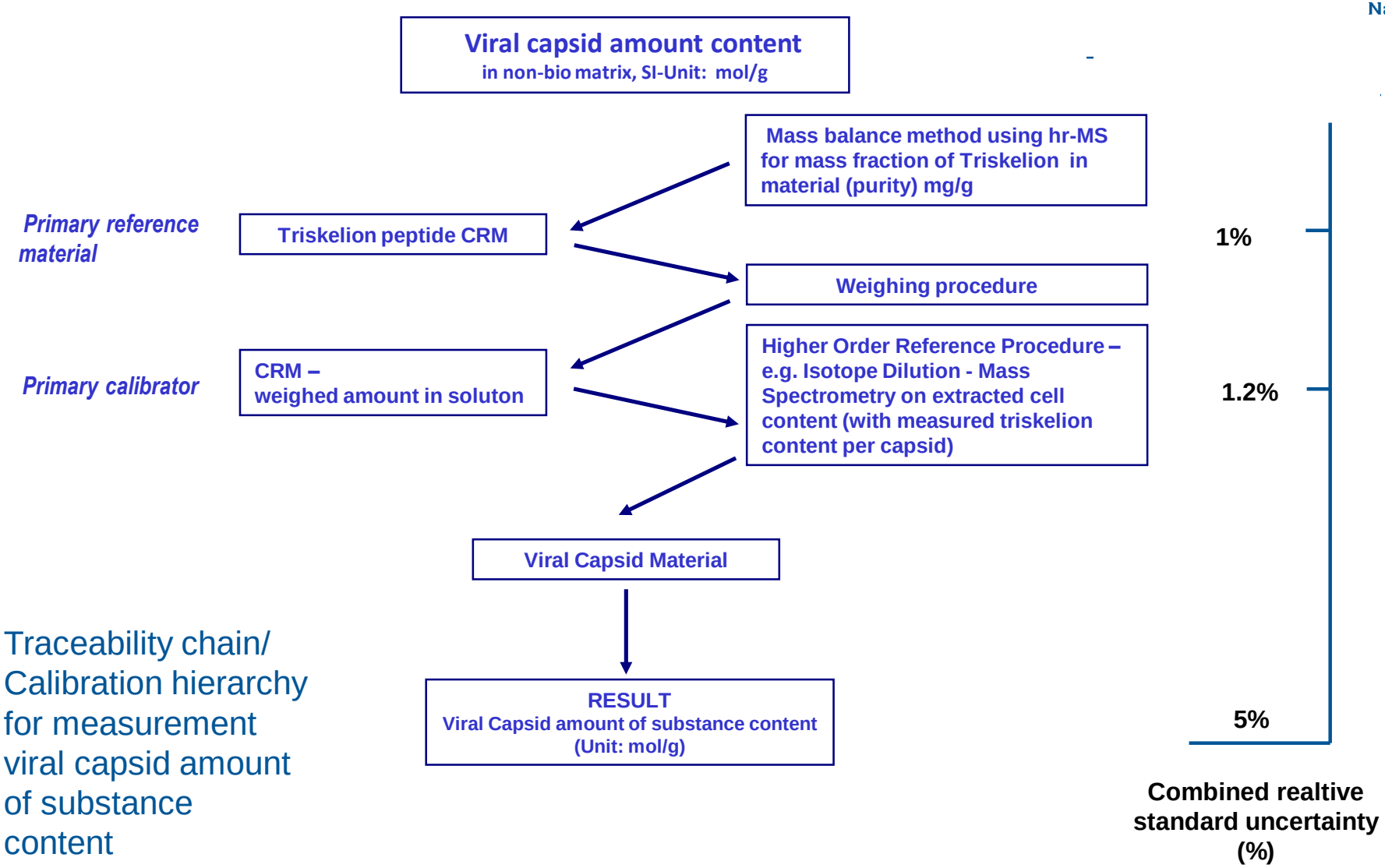
Alternative hypothesis: for each lab, not all frames have the same mean

Metric: if the p-value<0.05, the null hypotheis is rejected.

Lab	Frames	Tot particles (per frame)	Feret diameter	p-value	Cv	SE
A	3	576 (186, 187, 201)	25.53	0.11	0.28	0.41



Traceability chain/ Calibration hierarchy for measurement of number of viral capsids per cell



Traceability chain/ Calibration hierarchy for measurement viral capsid amount of substance content