

# K-kit Applications for Biotechnology & Nanopharmaceuticals



The Best R&D Partner

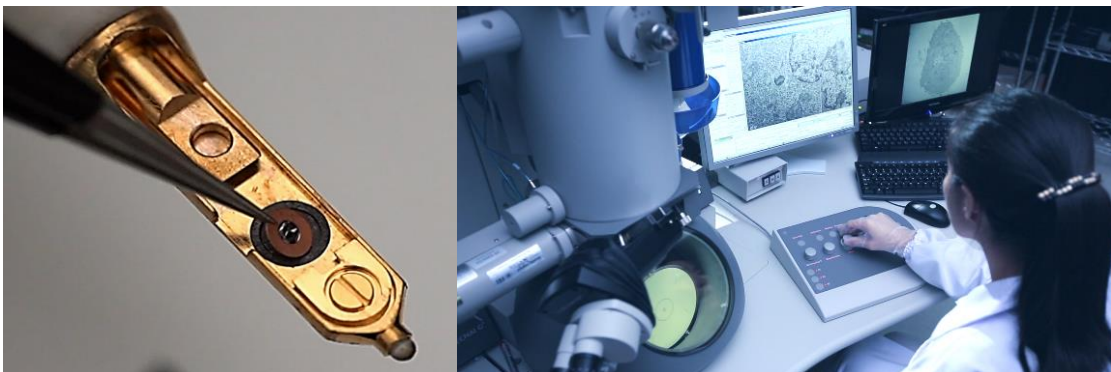
[www.bioma-tek.com](http://www.bioma-tek.com)

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## OUTLINE

- ❑ EM-based imaging for pharma & CRO
- ❑ K-kit for biotechnology & pharmaceuticals
- ❑ What is K-kit
- ❑ Conclusion

# EM-based imaging for pharma & CRO



## Drug discovery and development



- Candidate drug screening
- Structures and sizes
- Stability test
- Pre-clinical study

## Drug Manufacture (CMC)

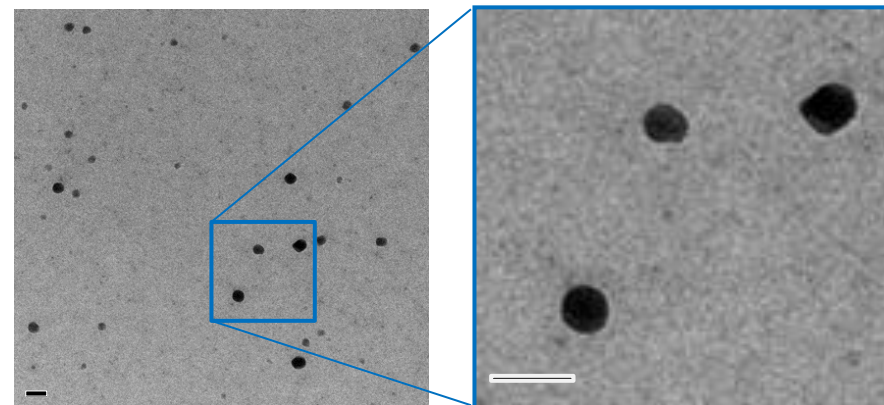
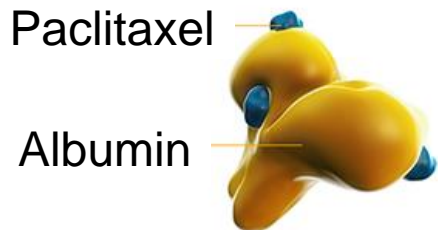
- Raw material IQC
- Drug homogeneity
- Uniformity between batches
- Process monitoring

## Pharmacokinetic studies (PK)

- In vitro stability
- Bioavailability
- Sizes and concentrations
- Drug Interaction
- Excipient effect

## Early-phase clinical studies

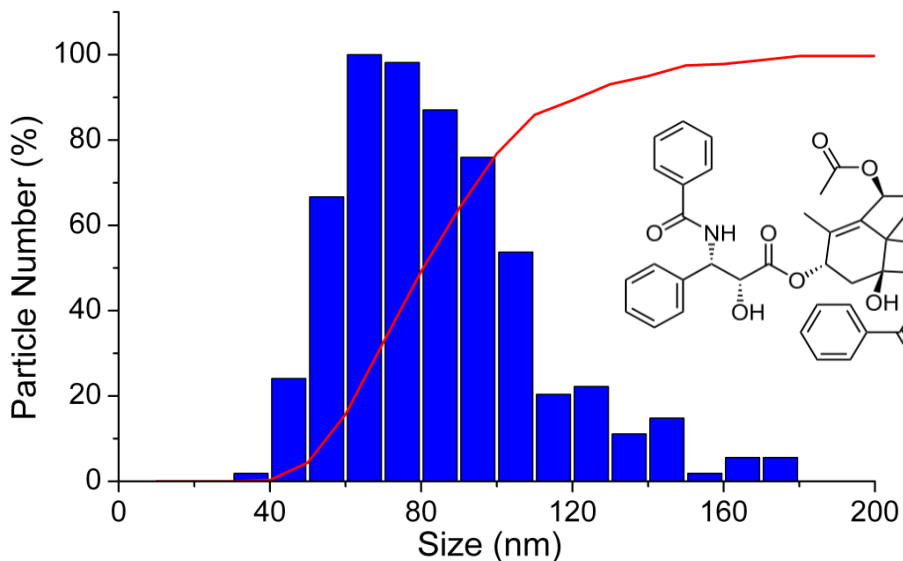
# Protein particles in Abraxane®



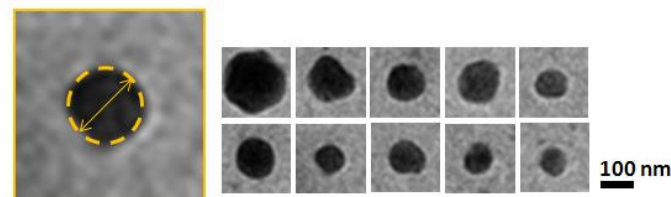
(TEM images by K-kit)

\* Scale bar: 200 nm

- Total calculated particle #: 319
- Average size: 85.1 nm
- Standard deviation: 27.0 nm



Size/size distribution (D10 , D50 , D90 )

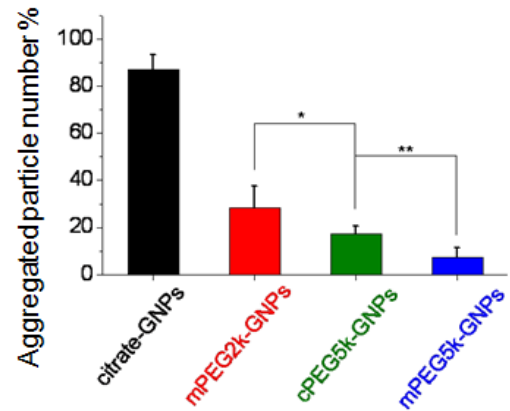
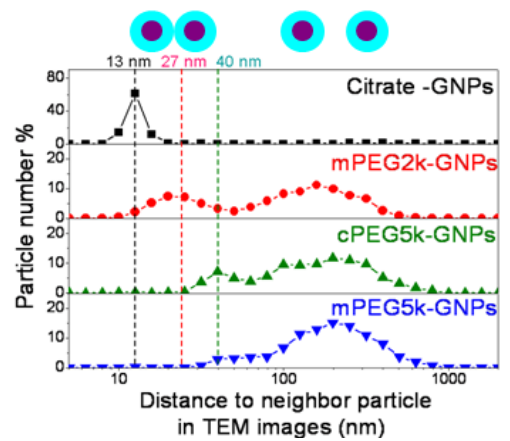
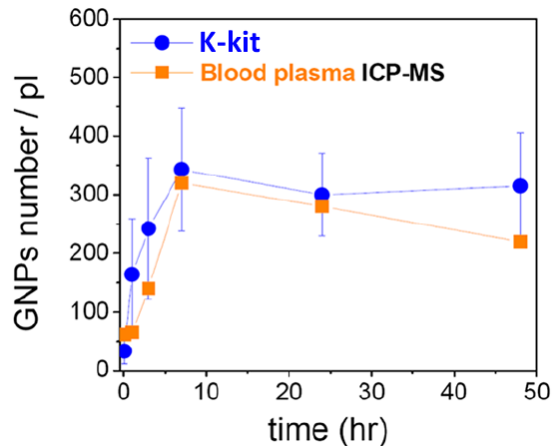
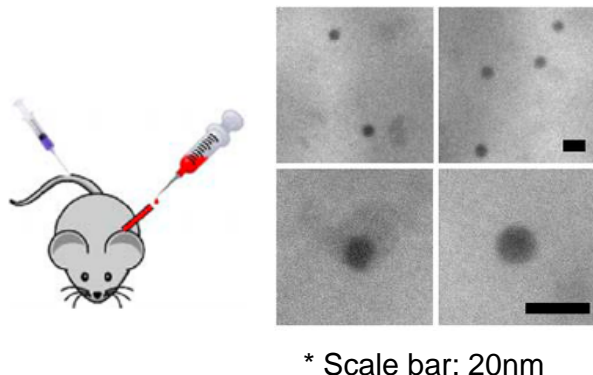
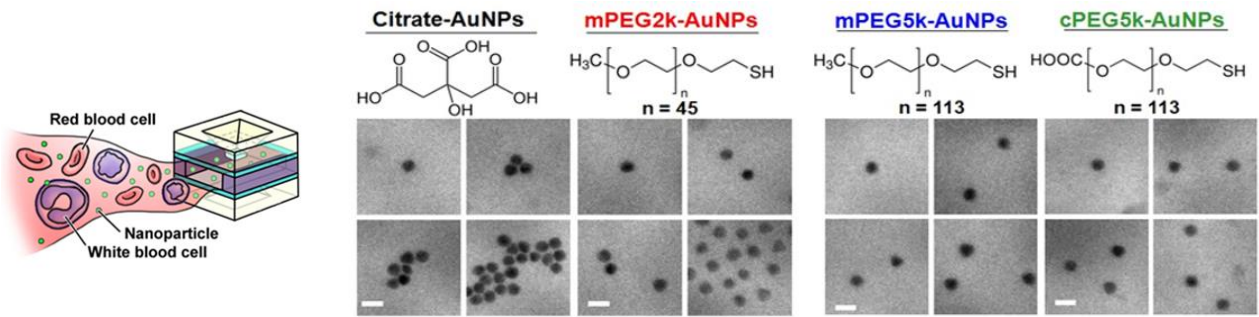


Parameter	Size (nm)
D 10	55.6
D 50	80.1
D 90	122.2
Span: $(D_{90} - D_{10}) / D_{50}$	0.831

# NOAAs of Au Nanoparticles (NPs) in Blood

Image-based statistic analysis of particle concentration (K-kit vs. ICP-MS)

( Tai etc. Anal. Chem. 2012, 84: 6312-6316 )



Statistic analysis of Aggregation and agglomeration of Au NPs in blood

K-kit can be used to analyze physicochemical characteristics of NPs in blood by TEM.



# K-kit for biotechnology & pharmaceuticals

*Does ANDA ≈ RLD?*

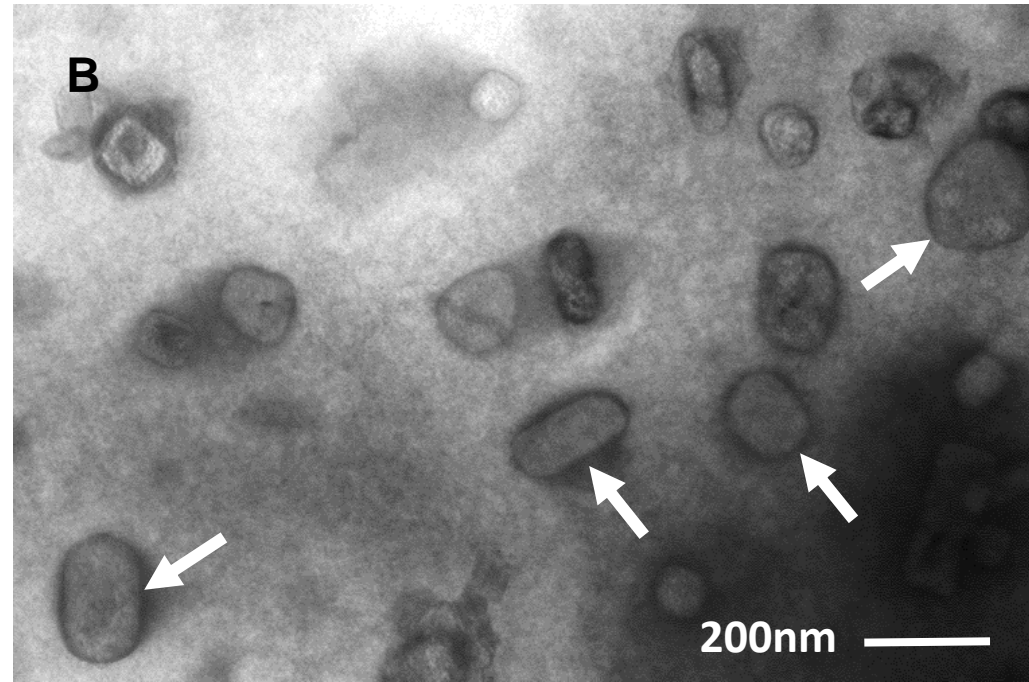
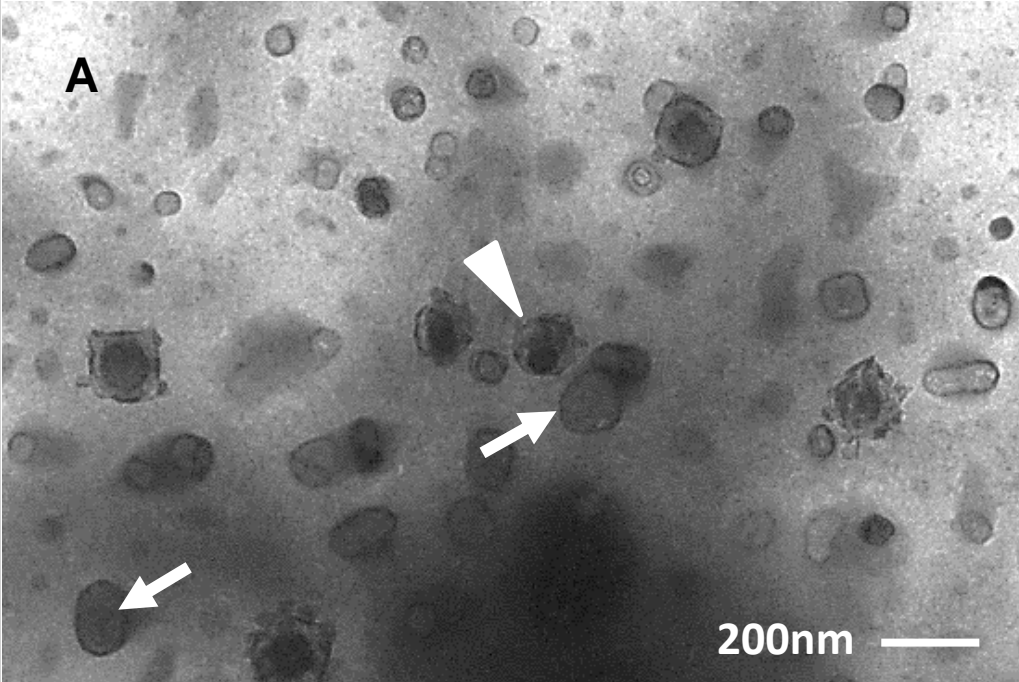
Observing the morphology and size distribution by electron microscopy



## What we can do by using K-kit:

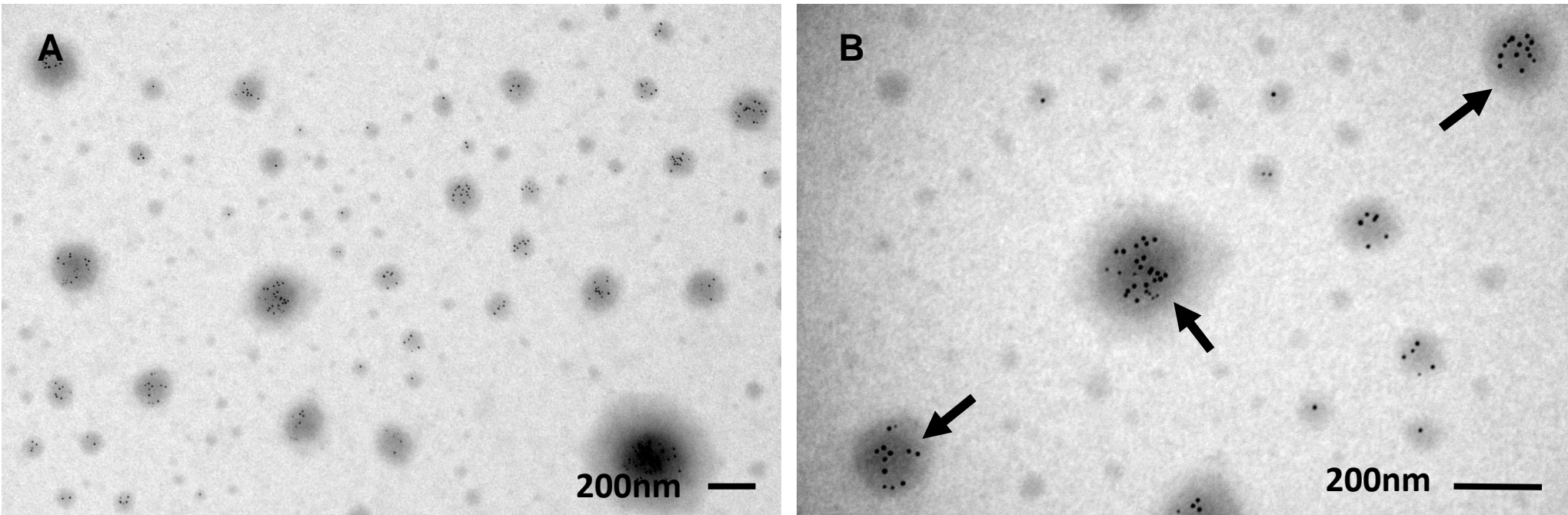
- In-situ EM Imaging of biological specimens and Nanopharmaceuticals in aqueous solutions
- Immunoelectron microscopy to identify nanogranules in liquid
- Image-based statistic analysis allowing nanoobjects, aggregates, and agglomerates (NOAAs) in aqueous condition to be characterized

## Isolated platelet granules in K-kit



(A)  $\alpha$ -granules are large and round or oval in shape (indicated by white arrows). A dense granule with a high-density core was also identified (by a white arrowhead). The small circular vesicles with low electron density are lysosomes or exosomes. Some broken granules could also be observed. (B) The  $\alpha$ -granules (by white arrows) could be identified with a long axis length of about 200 nm.

# Immunolectron micrographs of isolated platelet granules

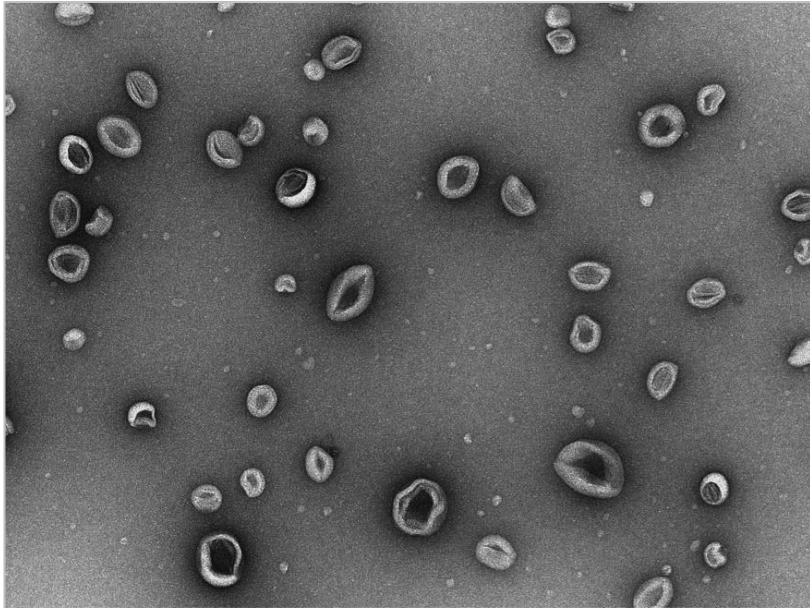


(A) This picture shows that the 6-nm gold particle-labeled granules are  $\alpha$ -granules. The other vesicles not significantly labeled by gold particles might be lysosomes or exosomes. (B) There are abundant gold particles located on the surface of  $\alpha$ -granules (indicated by arrows).

( [Appl. Sci. 2020, 10, 4946](https://doi.org/10.3390/app10144946); doi:10.3390/app10144946 )



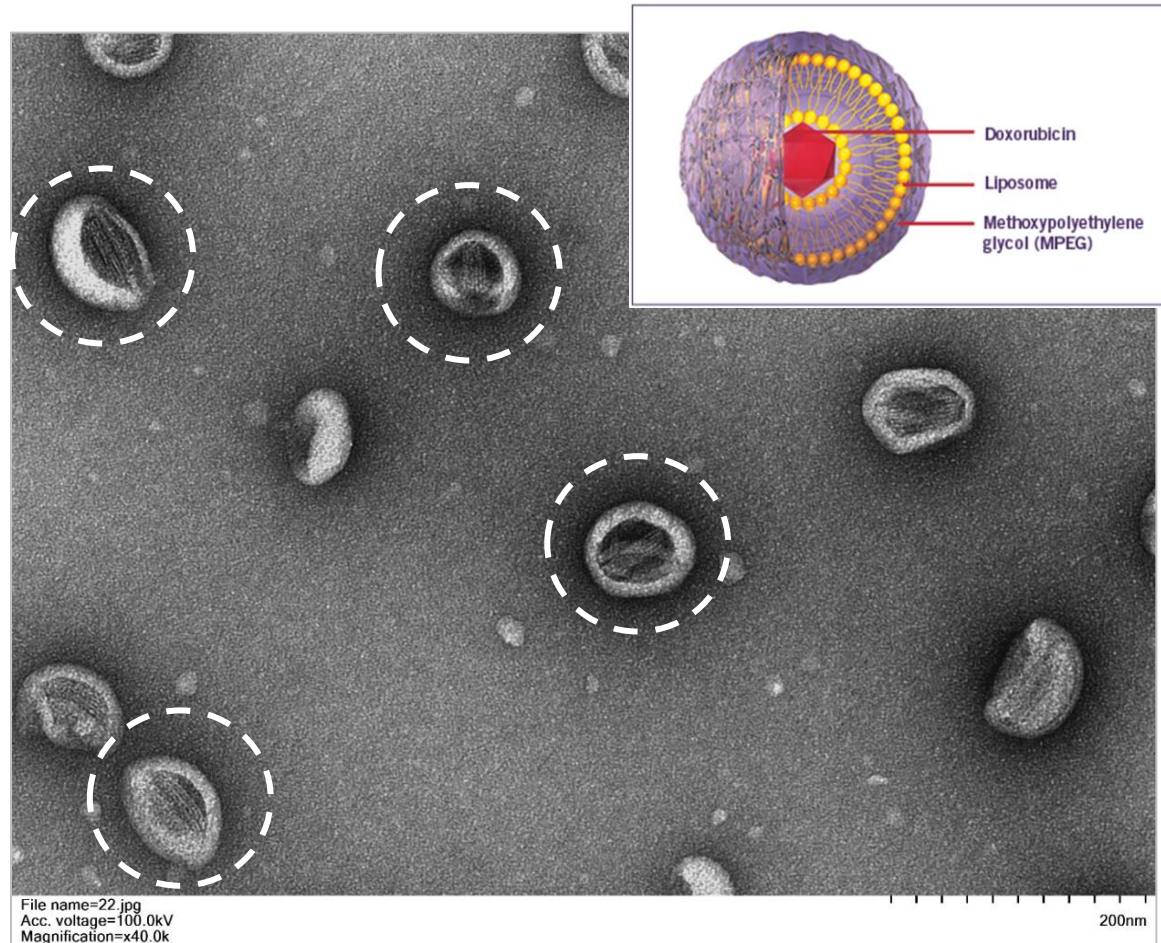
# Drug crystals of Doxorubicin in liposomes



File name=14.jpg  
Acc. voltage=100.0kV  
Magnification=x20.0k

500nm

(with negative staining)

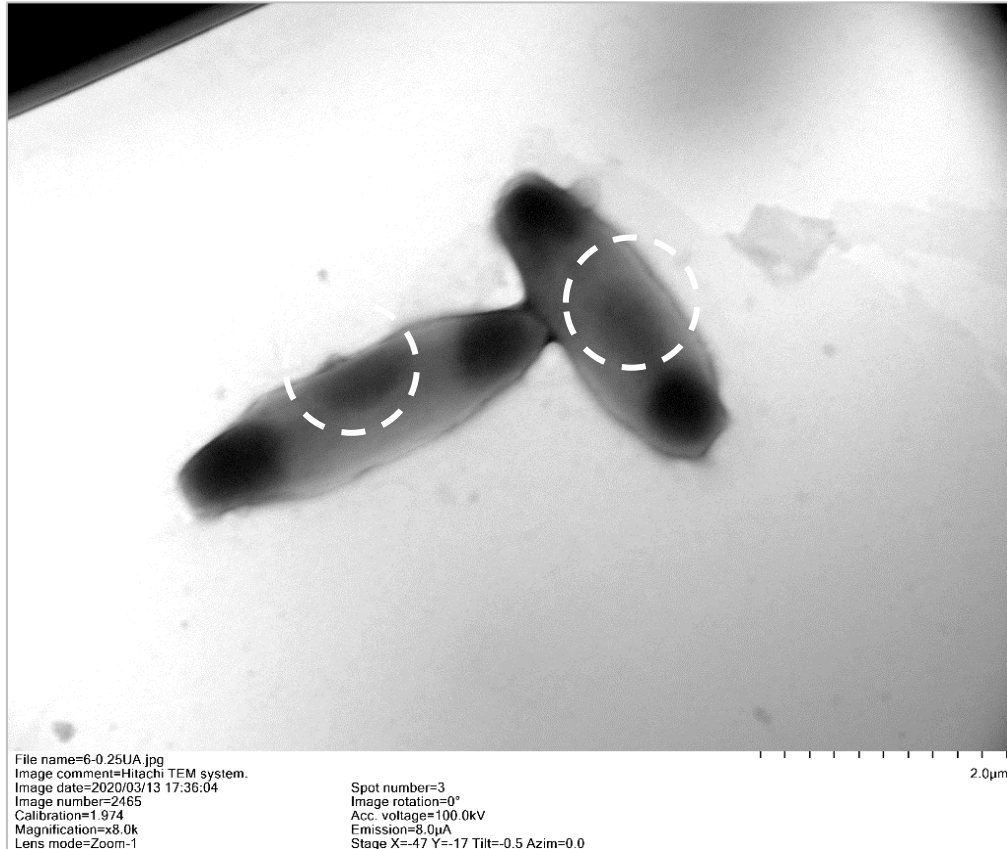


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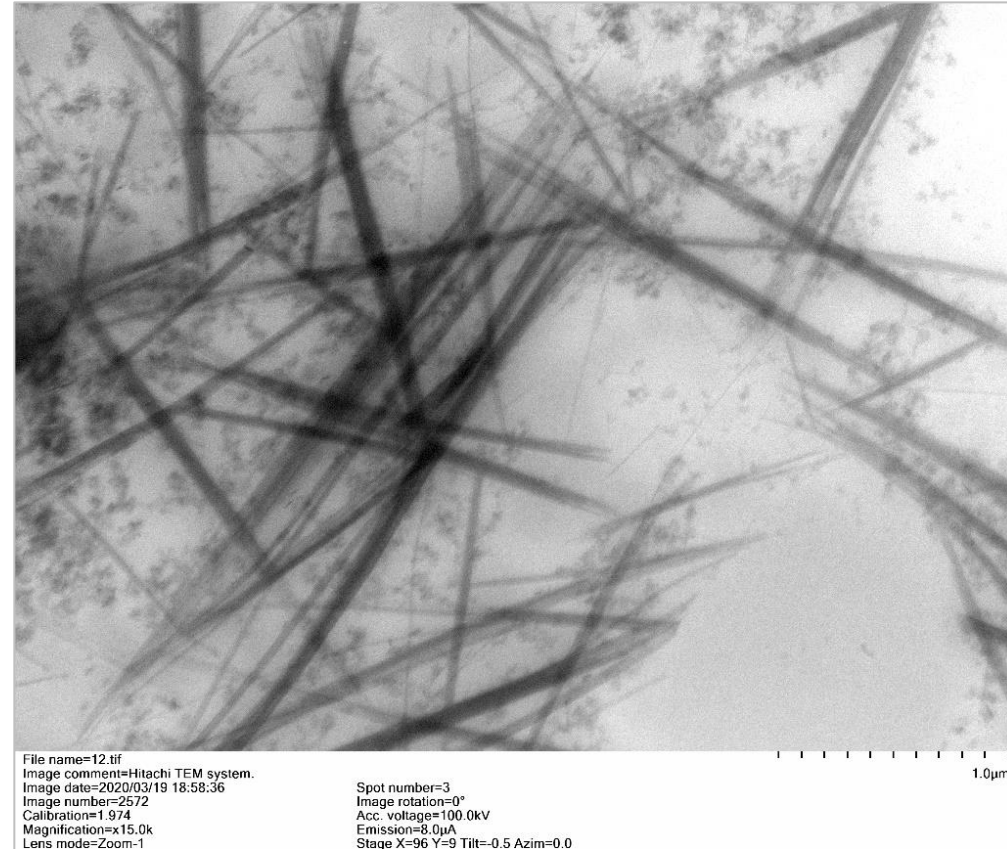
200nm

The enclosed drug crystals in liposomes can be well identified with K-kit.

# Biological specimens observed with K-kit



The nucleoid of E.coli



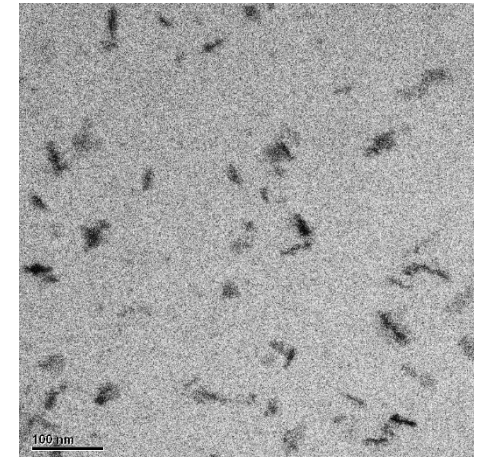
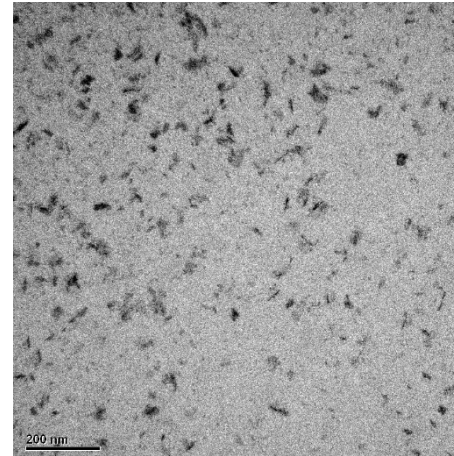
The nanofiber structures of collagen



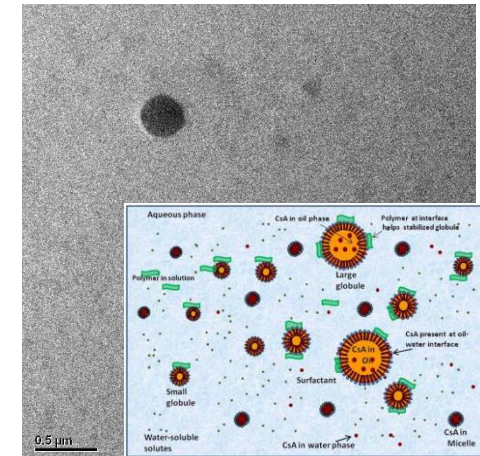
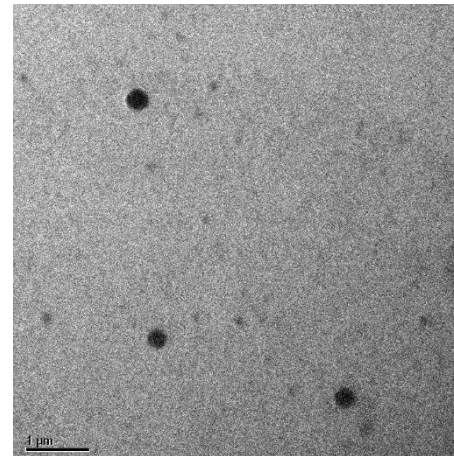
# Nanopharmaceuticals observed with K-kit



Resovist<sup>®</sup>, which is a human used MRI T2 contrast agent with iron oxide nanoparticles in the solution



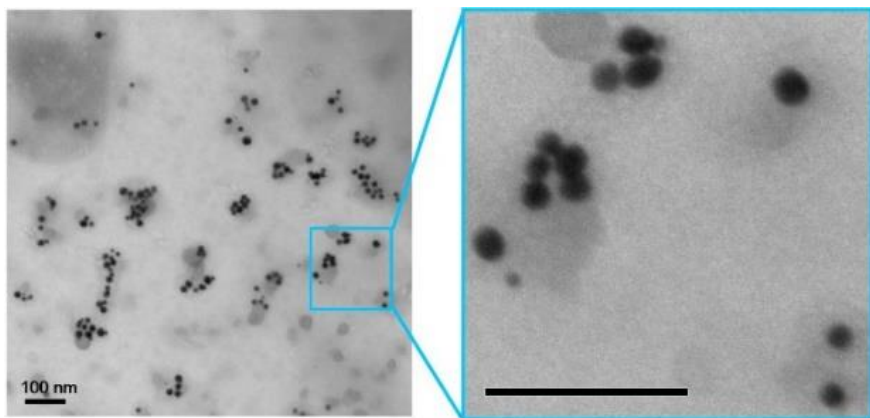
Restasis<sup>®</sup>, cyclosporine ophthalmic emulsion



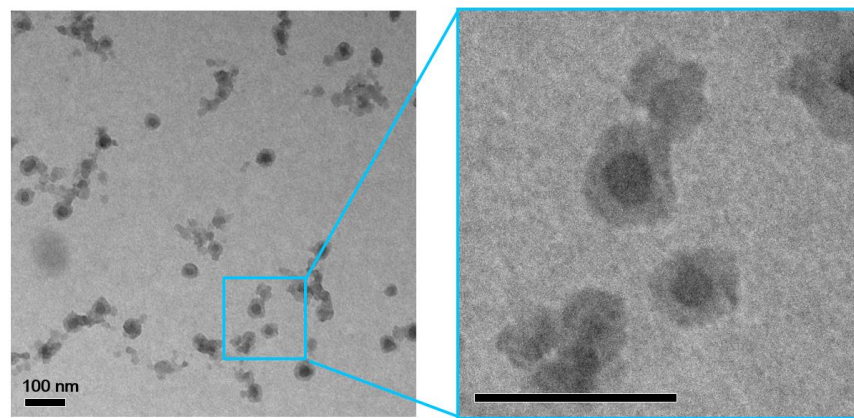


## Suitable for most Nanopharmaceuticals

- Applicable particle concentration for K-kit:  $10^{11} \sim 10^{14}$  particles/ml



AuroVist® solution was directly loaded and sealed in a K-kit in liquid form.



Oil emulsion in water was loaded and sealed in a K-kit in liquid form.

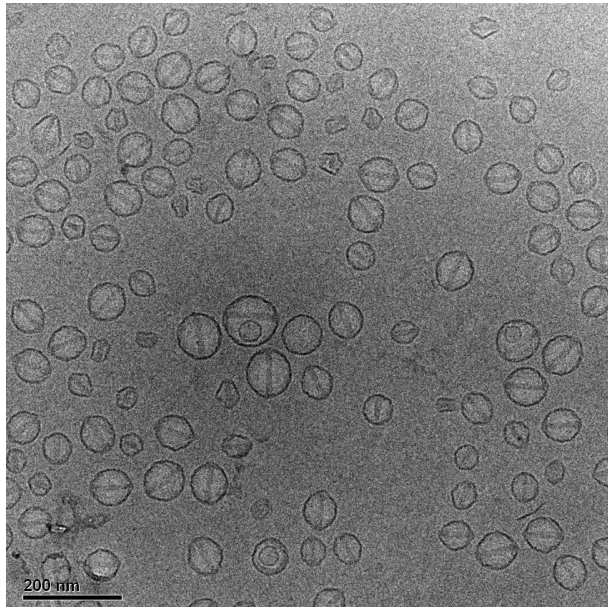
Brand Name of Pharmaceuticals	<b>Doxil®</b> (1995 approved)	<b>Abraxane®</b> (2005 approved)	<b>Aurimune®</b> (Phase II)	<b>Resovist®</b>	<b>Rexin-G®</b> (Phase II)
Particle Size	80-100 nm	~ 130 nm	~ 27 nm (AuNPs core), ~ 30-40 nm as hydrated	~ 45-60 nm (Hydradynamic diameter)	~ 100 nm
Particle Concentrations	$1.0 \times 10^{14}$ liposome /ml	$4.3 \times 10^{13}$ albumin particles /ml	$\leq 1.7 \times 10^{12}$ gold particles /ml	$1 \times 10^{14}$ particles /ml	$1-4 \times 10^{11}$ cfu



# Available for low-contrast sample observation

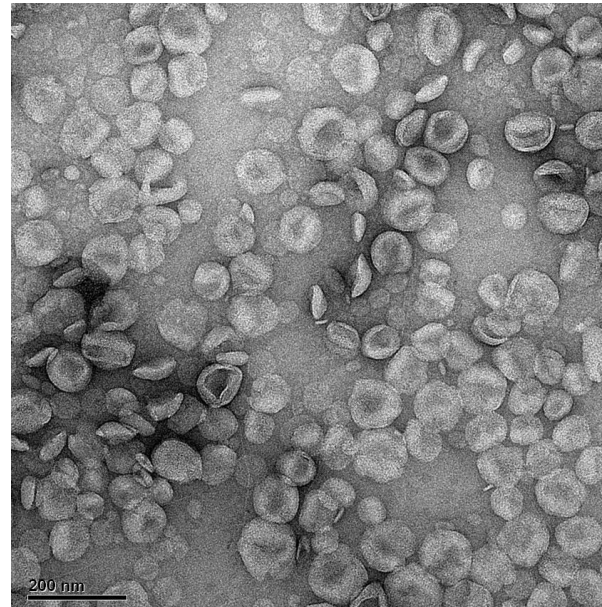


## TEM images of liposomes in Doxil®



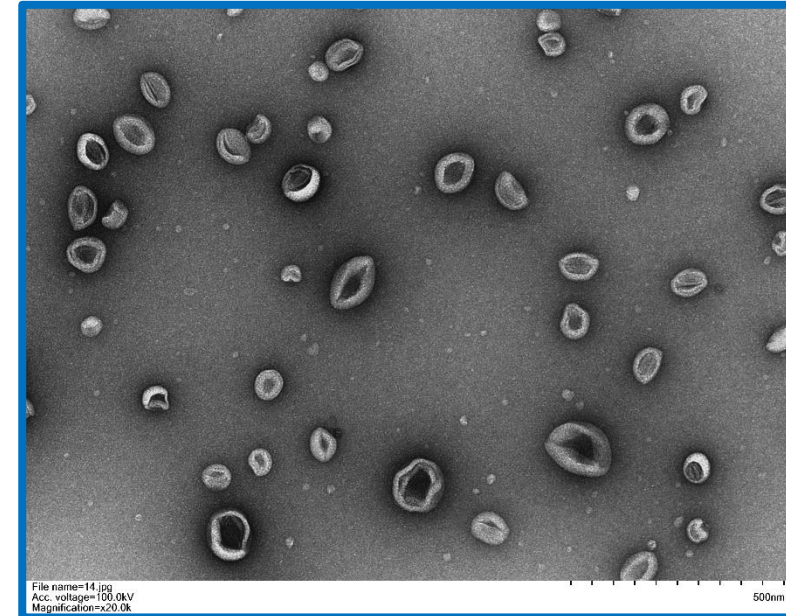
### Cryo-TEM (In formula)

- High cost & difficult
- Cryo-holder required
- Low success rate



### On Cu grid (With N.S.)

- Low cost & easy
- At dry state
- Serious aggregates



### In K-kit (With N.S.)

- Low cost & easy
- In-situ liquid state

( N.S. - Negative Staining )



# The latest news about K-kit

The poster on right side was published in June at RCCM Banyuls 2019 meeting in France; K-kit has been considered as an EM-based imaging solution for new drug development by the famous pharmaceutical company **SANOFI**.

## Contribution of electron microscopy to industrial multimodal characterization of products and raw materials

ML. Sgarra, S. Fayard, L. Petit, C. Girardon, C. Peyrot, F. Greco, A. Deliot, MC. Nicolai, F. Ronzon, S. Marco, H. Ponceblanc

Analytical Sciences, Sanofi Pasteur, Marcy L'Etoile and Neuville sur Saône, France.



### Background

Electron microscopy is used by the pharmaceutical industry for the characterization of products and raw materials at the level of:

- research and development
  - product characterization
  - quality control

This implies overcoming technological barriers for:

- the technical adjustment or developments of tailored tools
- the use of multimodal approaches
- the automatization of image acquisition, processing and analysis

### Tailored tools

- Hydrated samples observation in TEM



Main challenge: to filling in the system with high reticulated filaments

Chosen solution: <http://www.biocma-tek.com/biocma-tek/en/goods.php?act=view&no=22> distributed by <http://www.itg-distribution.fr/>

- TEM-MEB correlation

Single negatively stained grid of viral particles can be observed by both TEM and SEM

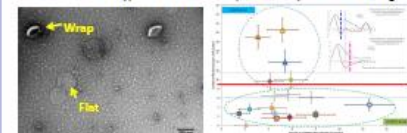


Support to transfer TEM grids to SEM for correlative microscopy

Chosen solution: <https://www.microtonano.com/EM-TEM-grid-holders-and-STEM-imaging-holders.php>

- Software development

Identification of atypical and standard split viruses processed in ImageJ



In some cases requires standalone Z1 CFR part 11 compliant software

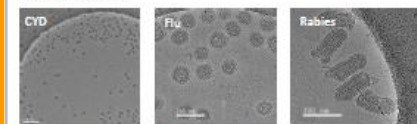
Chosen solution: VAS from VIRIONOVA <https://www.vironova.com/our-offering/vas/>

### Examples of applications

- Protein complexes



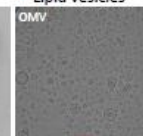
- Viral particles



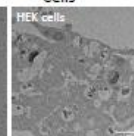
- Bacteria



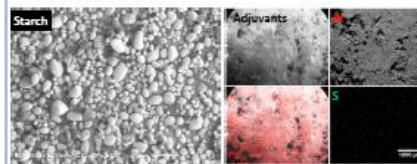
- Lipid vesicles



- Cells



- Raw materials



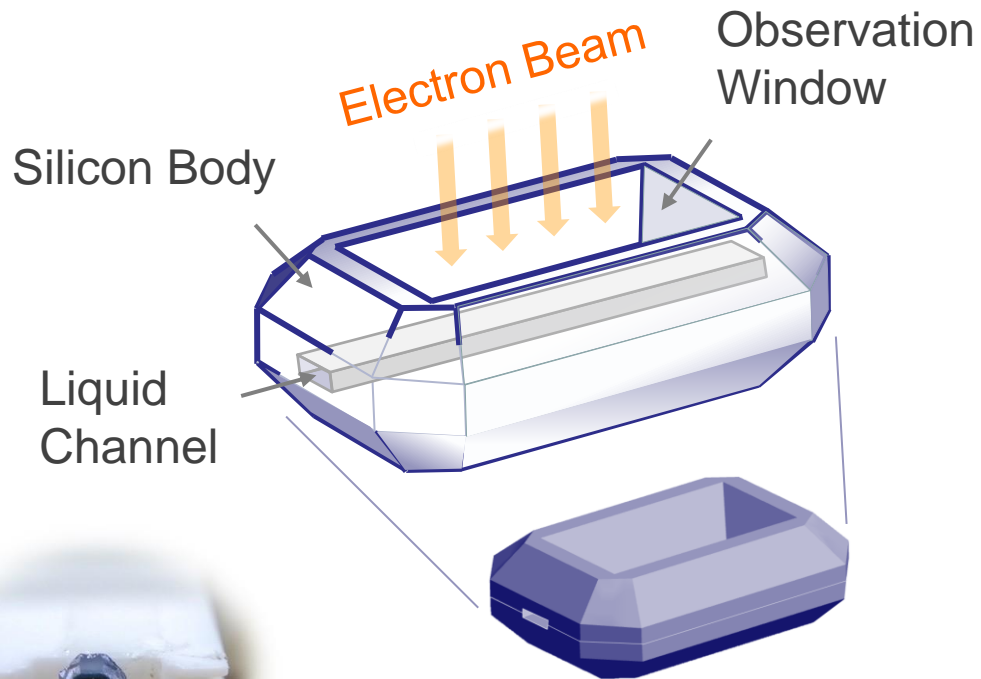
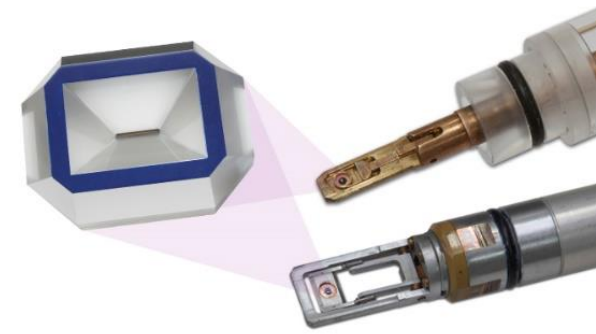
### Challenges

- From subjective image interpretation to quantitative analysis
- From manual acquisition to automatized image recording
- Towards a GMP compatible approach

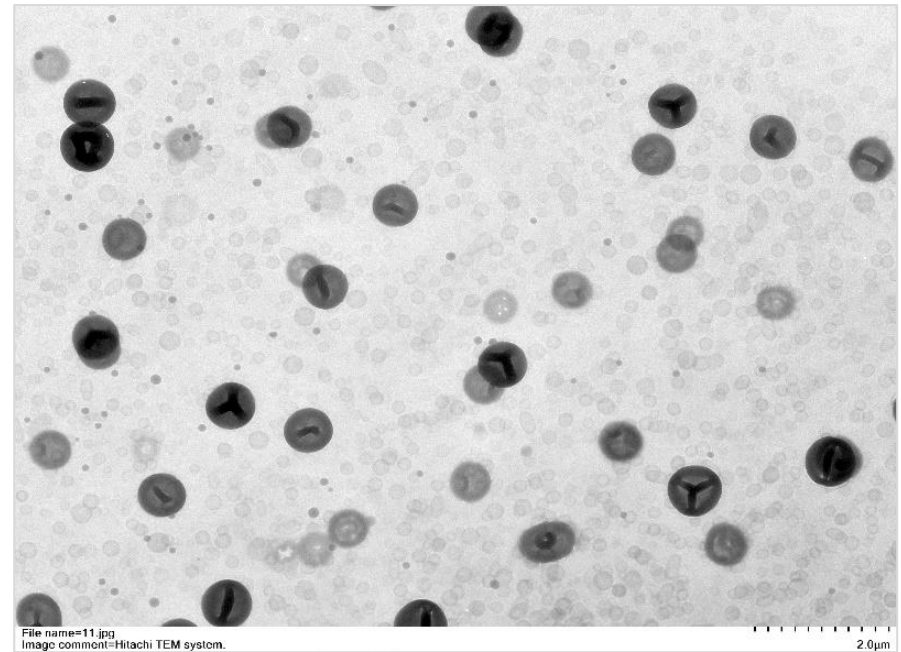
SANOFI PASTEUR

# What is K-kit ?

A disposable device for liquid sample analysis in TEM, SEM and FIB, free of cross contamination issue.



Liquid loading by capillary action

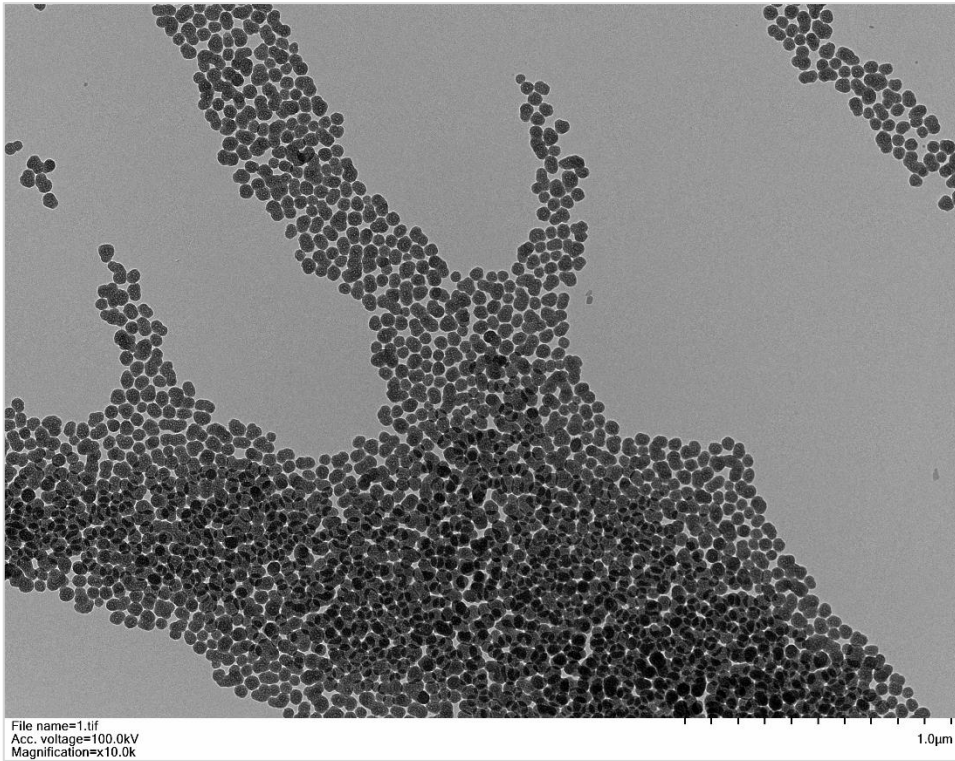


Extracellular vesicles of platelets  
(With negative staining in K-kit)

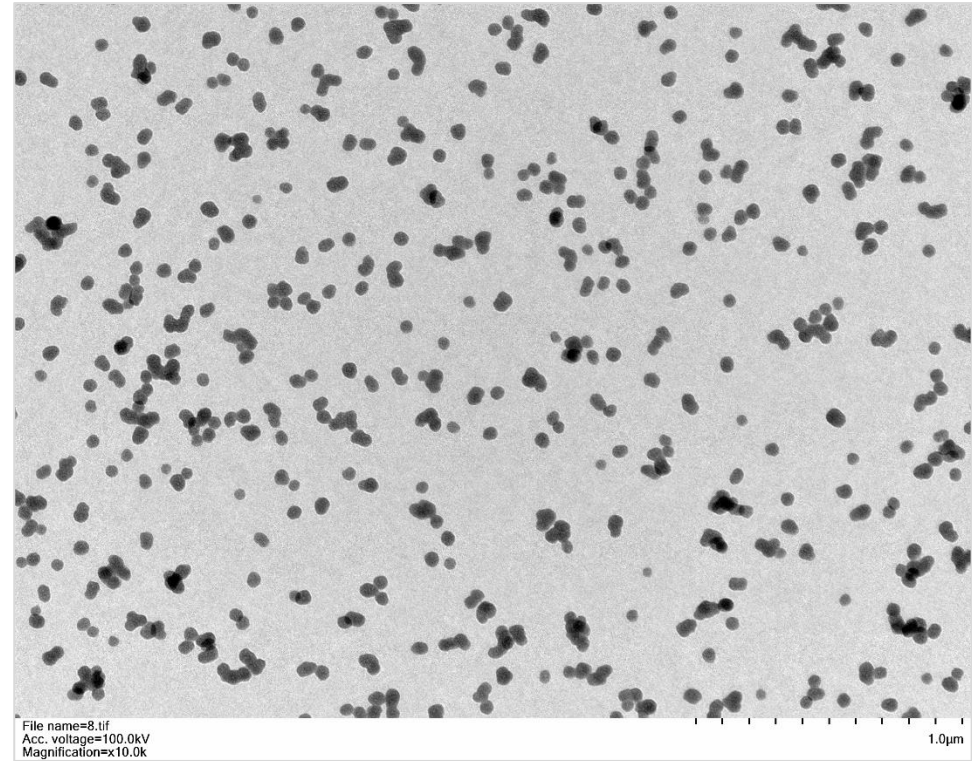


# Imaging at original liquid state

✘  Aggregated as drying on Cu grid



✔  Nanoparticles in liquid by K-kit



By using K-kit, one can observe the particles that distributed uniformly in liquid, preventing the nano particles from dry-induced aggregation and agglomeration.

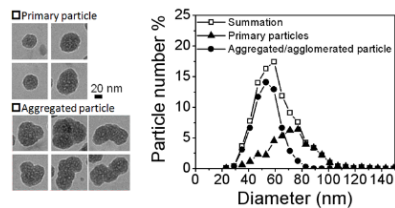
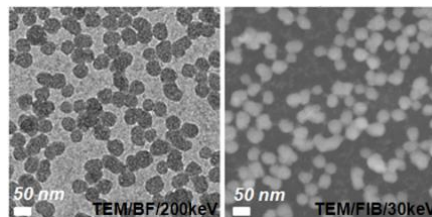


# K-kit Application



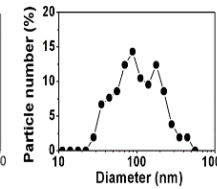
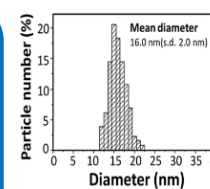
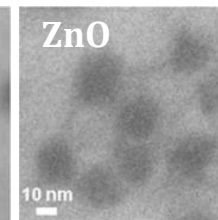
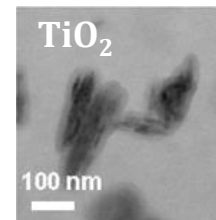
## Slurry

- SiO<sub>2</sub> Nanoparticles in CMP Slurry



## Lotion

- TiO<sub>2</sub> and ZnO Nanoparticles in Sunscreen



## Electronics



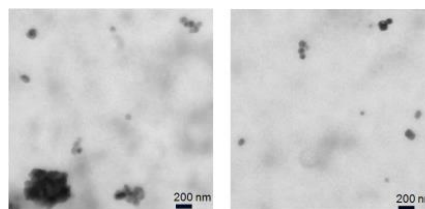
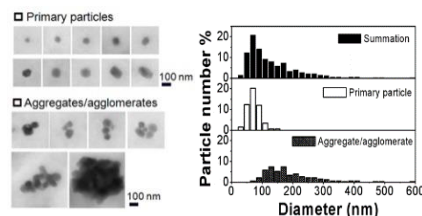
## Cosmetics



## Food



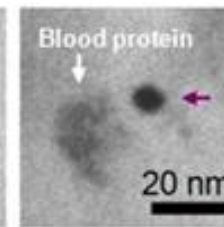
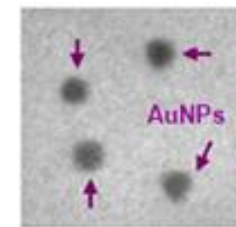
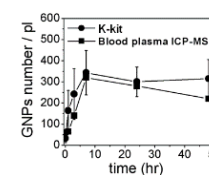
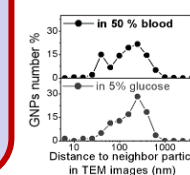
## Bio-Med



## Beverage

- CaCO<sub>3</sub> Nanoparticles in Milk

Aggregation/agglomeration Particle concentration



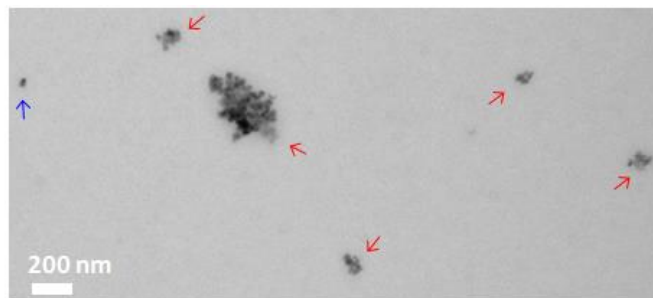
## Bio Sample

- Au Nanoparticles in Blood

# (Example) NOAAs of ZnO NPs in sunscreen

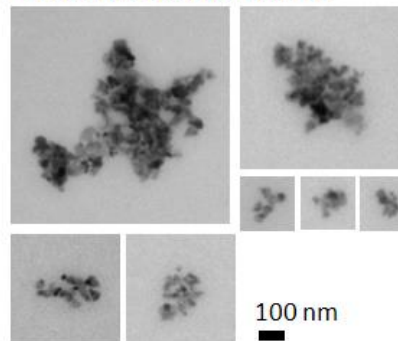
## Lotion

ZnO NOAAs in sunscreen



□ Nano-objects

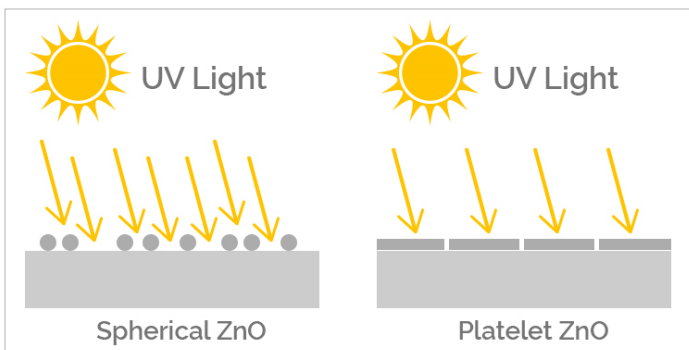
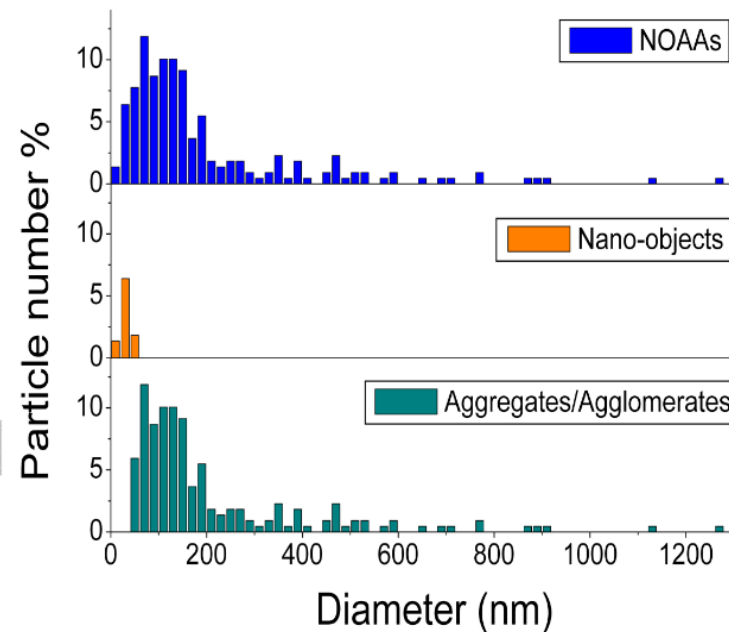
□ Aggregates/Agglomerates



Lotion

Cream

Powder

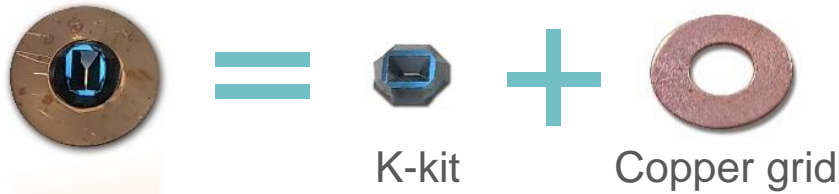


TEM images

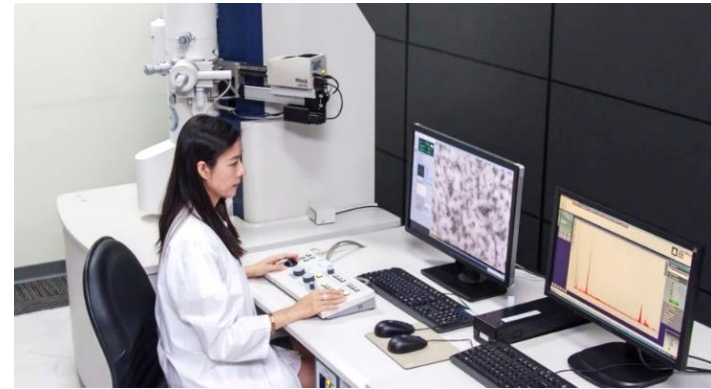
Size and size distribution

K-kit can be used for characterizing NOAAs of cosmetics in final product forms, to assess the safety risks of nanomaterials in cosmetic ingredients.

# Quick, easy, and budget friendly



(Compatible with all kinds of TEM holders)



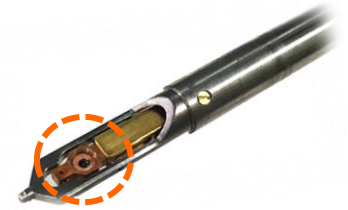
Hitachi  
H-7501 SS holder



JEOL  
EM31640 STHB holder



FEI  
TECNAI F20 holder



Gatan  
CT3500HT holder

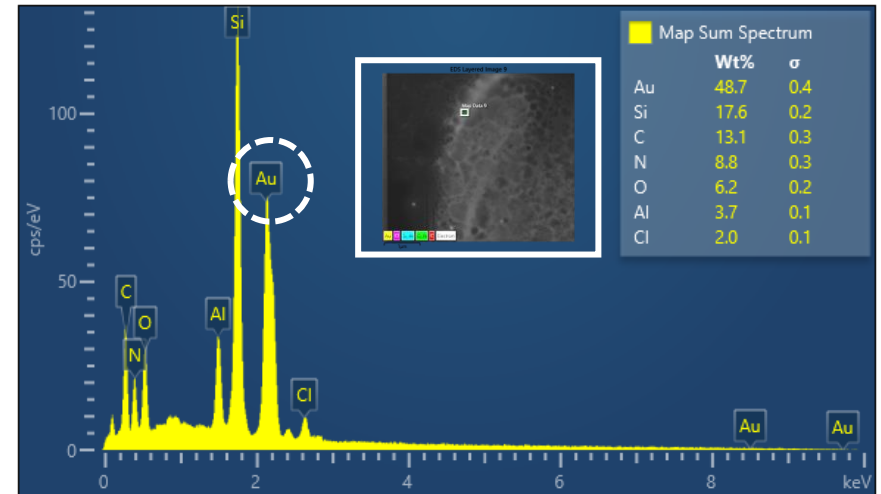
K-kit is disposable and with a vacuum-encapsulated channel structure so that no any cross-contamination risk and no need to do further cleaning before the use.



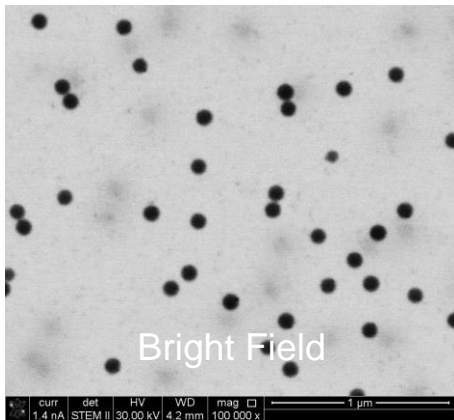
# Available for SEM and EDX analysis



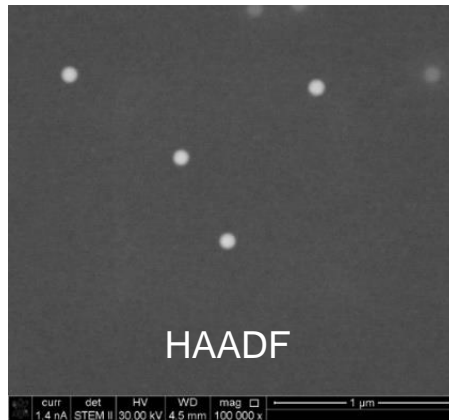
**SEM/ STEM by FEI Helios 400**



100nm Polystyrene beads

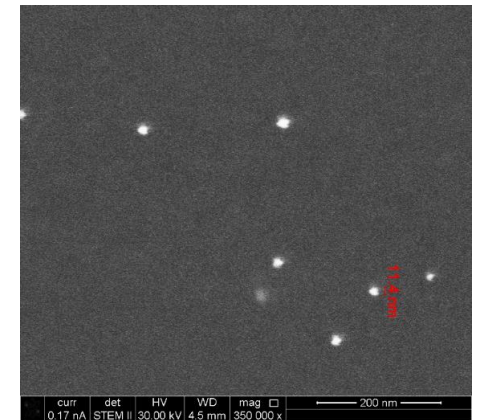
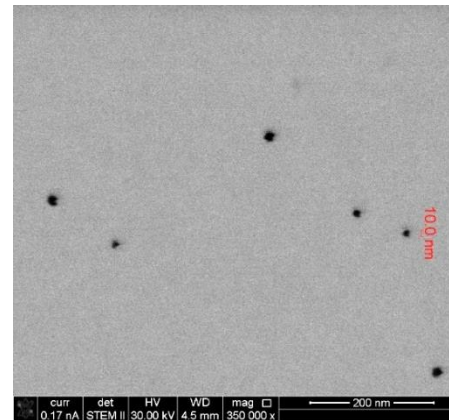


Bright Field



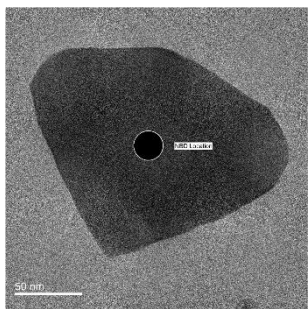
HAADF

10nm Au nanoparticles

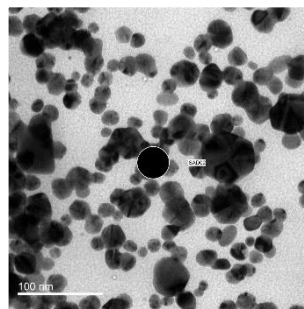




# Available for TEM diffraction analysis



NBD (Nano-beam diffraction)

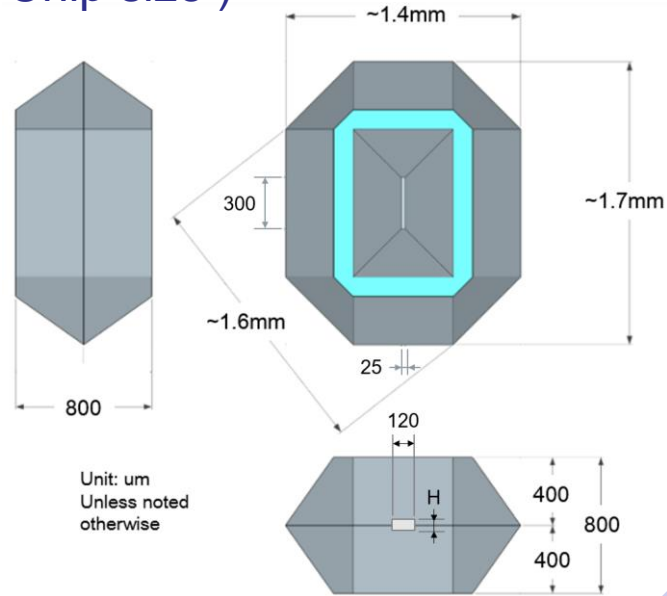


SAD (Selective area diffraction)

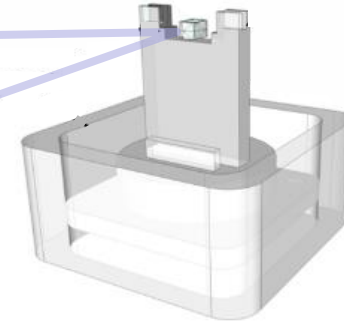
As shown the examples here, Au nanoparticles in K-kit were formed by the reduction process from  $\text{AuCl}_4$  solution in TEM.

# Product features

( Chip size )



- Window length 300  $\mu\text{m}$ , Width 25  $\mu\text{m}$
- Channel height (H):  
**0.2 and 2.0 standard**; 0.1, 0.5, 1.0 and 5.0 available

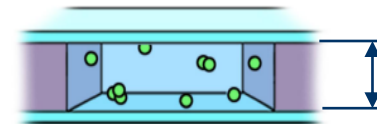


K-kit



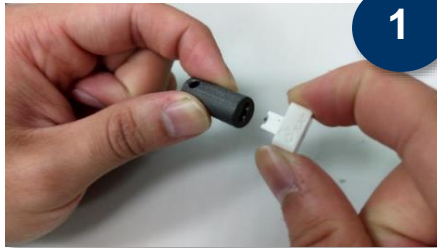
( Shipping packages )

( K-kit carrier )



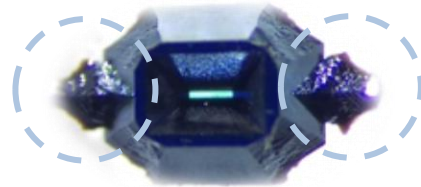
H = 0.1, 0.2, 0.5, 1, 2, 5 ( $\mu\text{m}$ )

# Quick and easy process in sample preparation

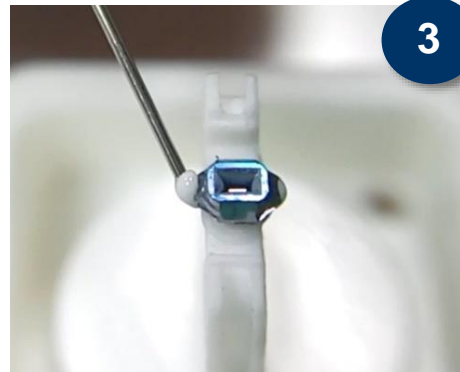


Channel tips, to ensure the cleanness of the channel before use.

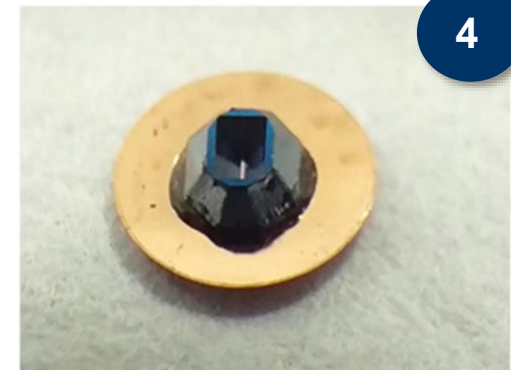
Remove the channel tips



Liquid loading



Gluing



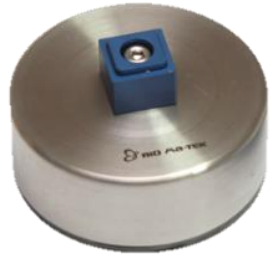
Copper grid



# K-kit Tool Box for Sample Preparation



K-kit by tools



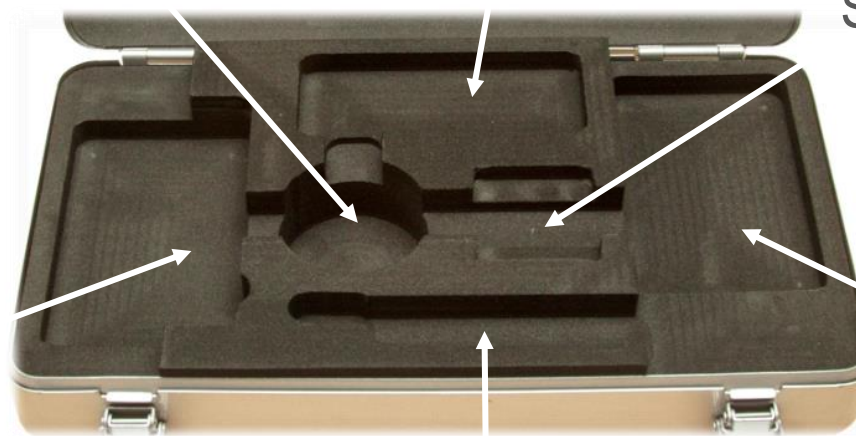
Gluing stand



Glass-slide pack



Sample-loading stage



K-kit holder & needle pen



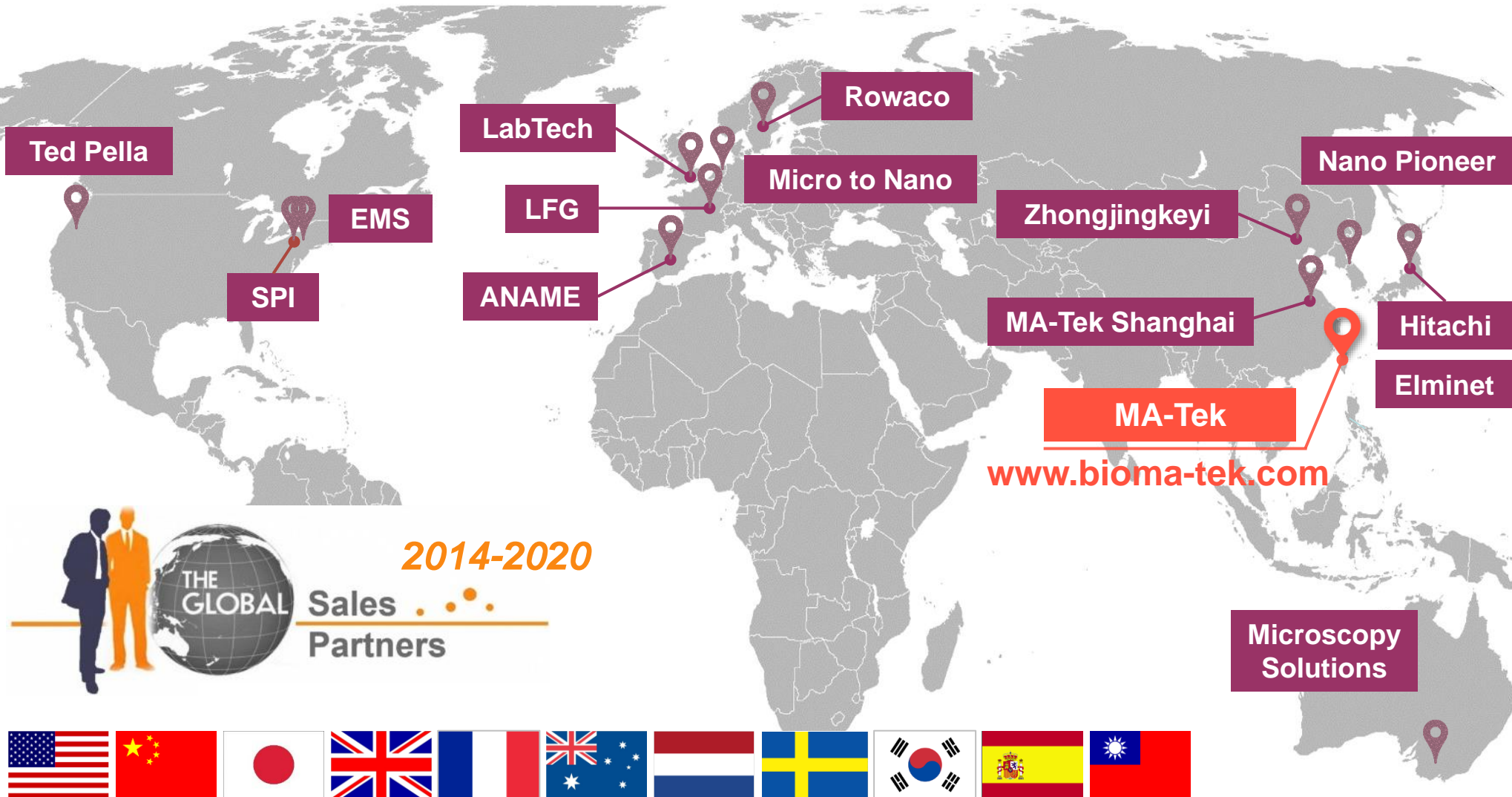
Accessory box



Shipping package



# K-kit distributors worldwide



[www.bioma-tek.com](http://www.bioma-tek.com)

# Conclusion

1. K-kit is a liquid cell designed to facilitate convenient TEM observation of liquid samples, allowing nanoobjects, aggregates, and agglomerates (NOAAs) in liquid samples to be characterized.
2. K-kit is a Silicon chip; it fits on a 3 mm diameter of copper grid and, hence, is compatible with most existing TEM holders such like FEI, JEOL, Hitachi and Gatan etc.; it's an affordable and the best option, bringing Liquid TEM accessible to general researchers and industries.
3. The possible applications of K-kit for pharma & CRO are basically on both fields in-situ EM imaging and immunoelectron microscopy, which the former allowing Nanopharmaceuticals to be observed the morphology and size distribution in aqueous condition and the latter providing unique insights into biological systems (e.g., to label and identify the proteins in human cells) for the development and future applications in biomedicine.

**Thank you!**

