Earlier and Novel Findings from Inhalation Studies of Ultrafine Particles: Predictors for Effects and Biokinetics of Inhaled Micro- and Nano-Plastics?

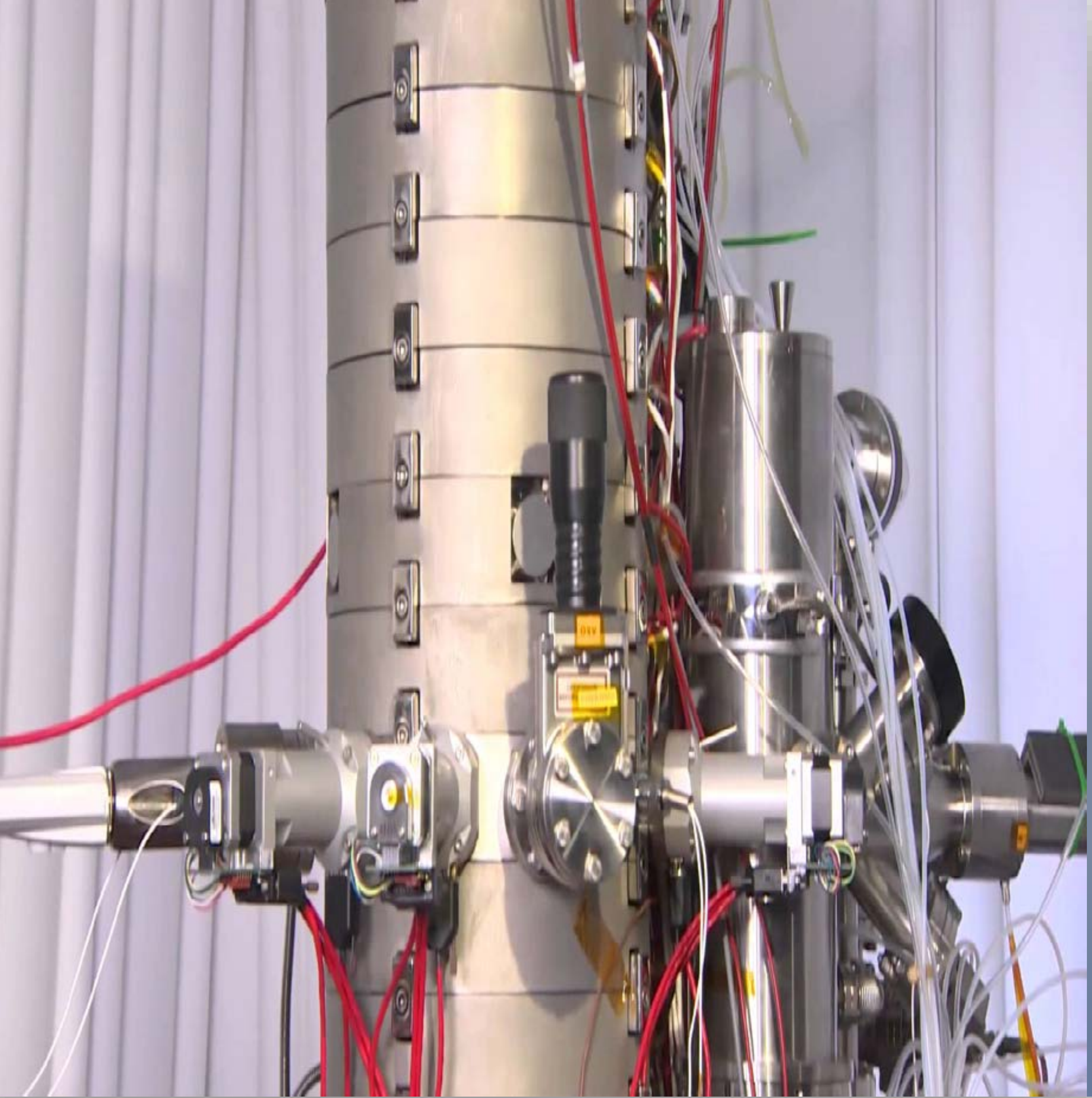
Some thoughts compiled by

Günter Oberdörster and Uschi Graham
University of Rochester, NY *Faraday Energy, KY*

June 13, 2023

UNIVERSITY of ROCHESTER

FARADAY ENERGY



Uschi M Graham, PhD

PhD from Penn State University (Chemistry)

PostDoc at Tohoku University, Japan (Material Sciences)

- Research Director of Faraday Energy, Nanotechnology
- Founder and President of Topasol, Nanotechnology
- Adjunct Professor, University of Kentucky
- Principal Investigator at BioInnovations Institute

Bioprocessing and cell/tissue interaction of nano-particles

- *Sub-nanometer Resolution*
- *In situ Analysis*
- *Oxidation States*
- *3D- Reconstruction*
- *Solubility*
- *Near Atomic Resolution*

High Resolution Analytical Imaging

HRSTEM, EDS, EELS

Environmental Samples, Human and Animal Tissues

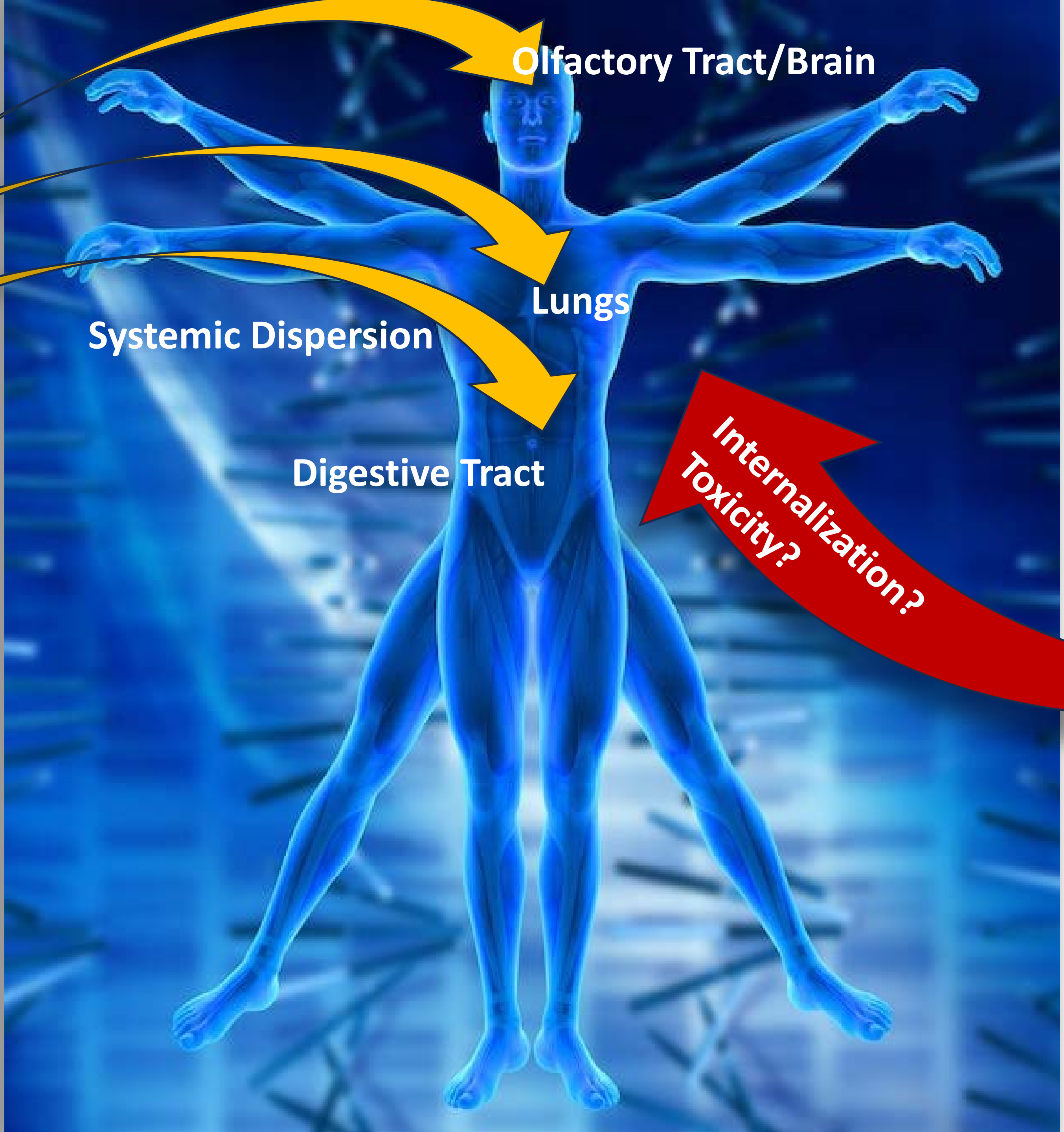
What is known so far:

Nanoparticles

(metals/oxides/carbons)

TOXICITY after:

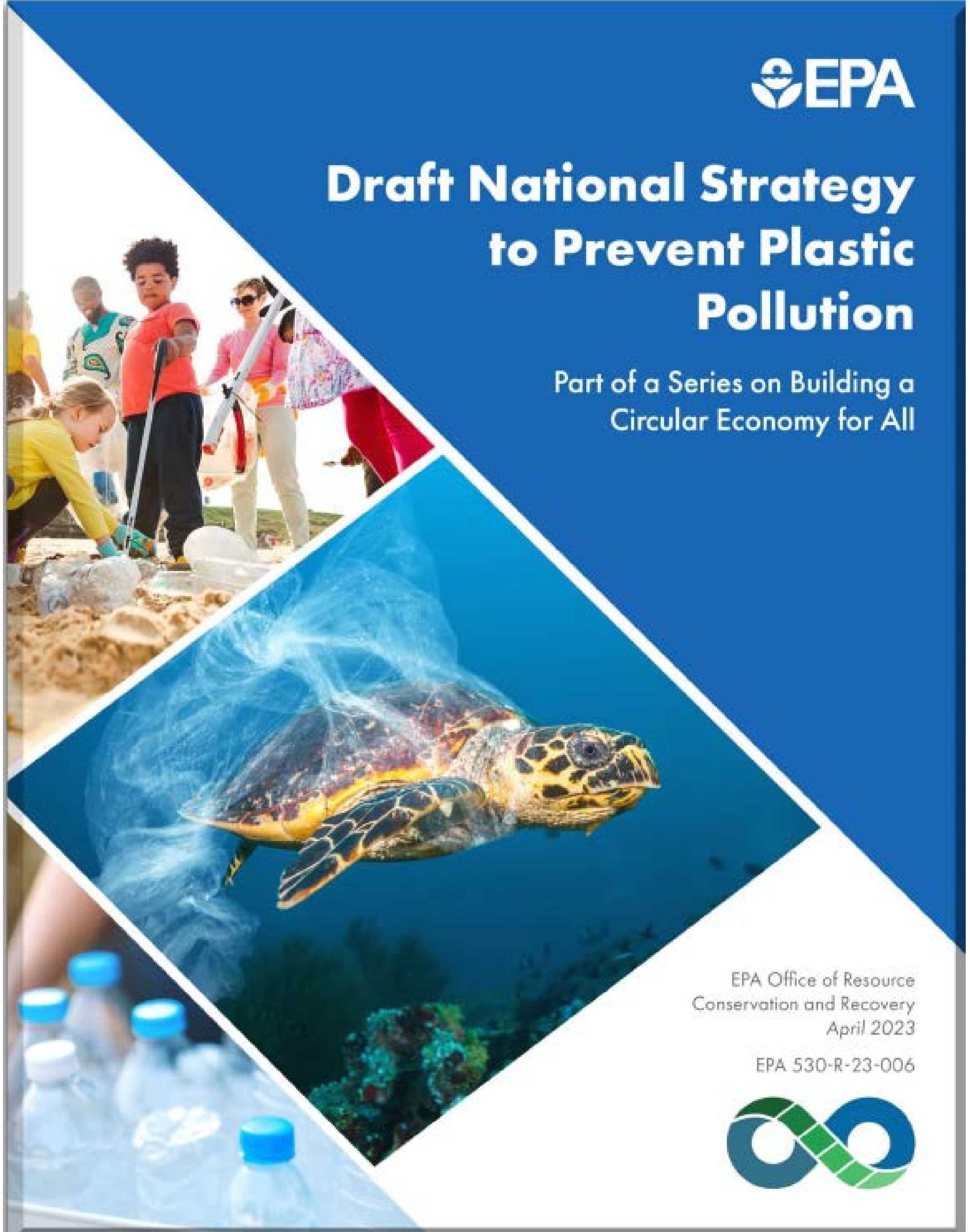
UPTAKE Routes
TRANSLOCATION,
TISSUE INTERACTIONS



What is unknown:

Nano-Plastics

UPTAKE Routes:
TRANSLOCATION?
TISSUE INTERACTIONS?



April 2023

The “Draft National Strategy to Prevent Plastic Pollution,” builds upon EPA’s National Recycling Strategy and focuses on actions to reduce, reuse, collect, and capture plastic waste. New and innovative approaches are necessary to reduce and recover plastic materials and improve economic, social, and environmental impacts. With input from organizations, EPA identified three key objectives for the strategy:

- **Objective A**: Reduce pollution during plastic production.
- **Objective B**: Improve post-use materials management.
- **Objective C**: Prevent trash and micro/nanoplastics from entering waterways and remove escaped trash from the environment.

Deadline for comments is June 16, 2023

Brahney et al, 2020

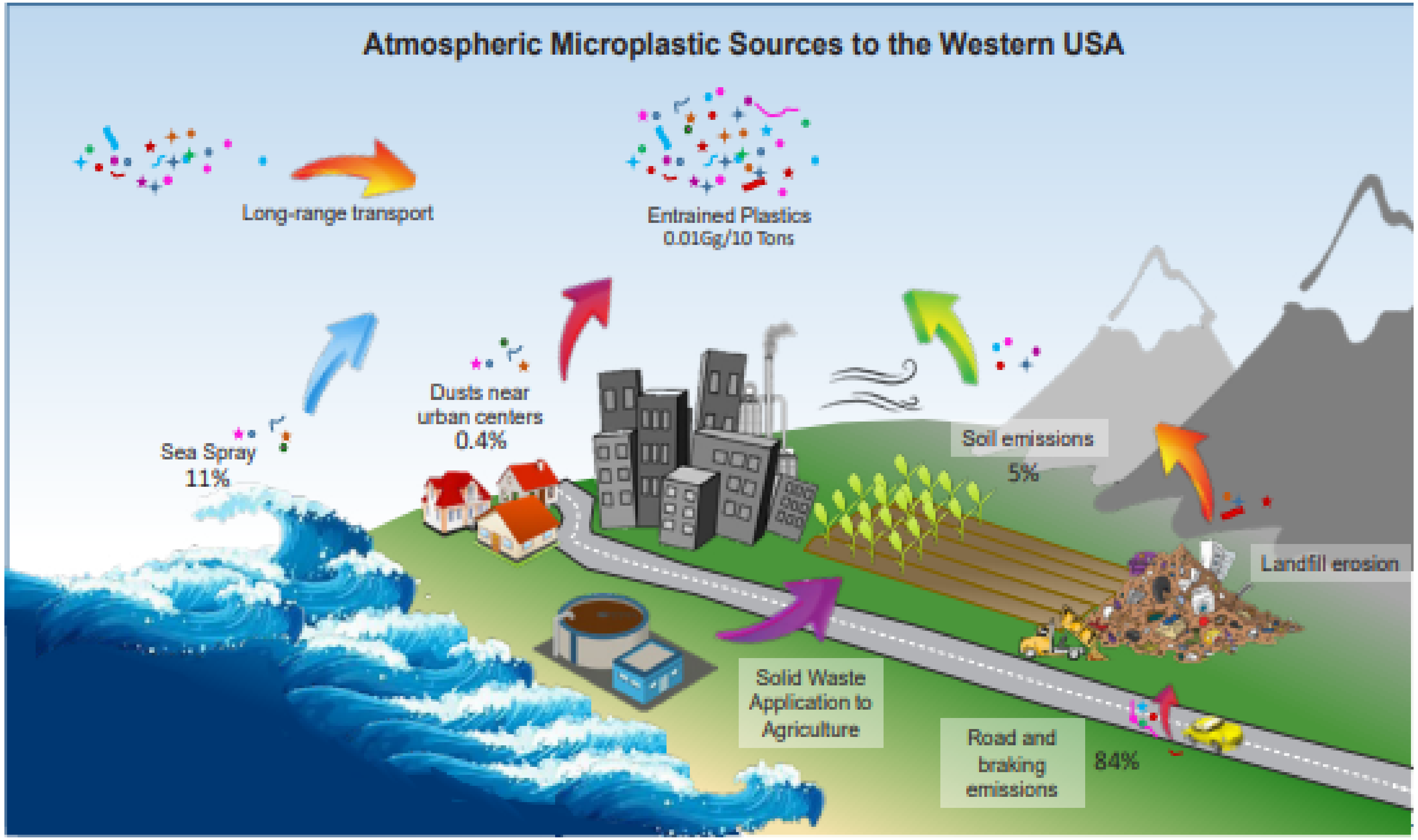
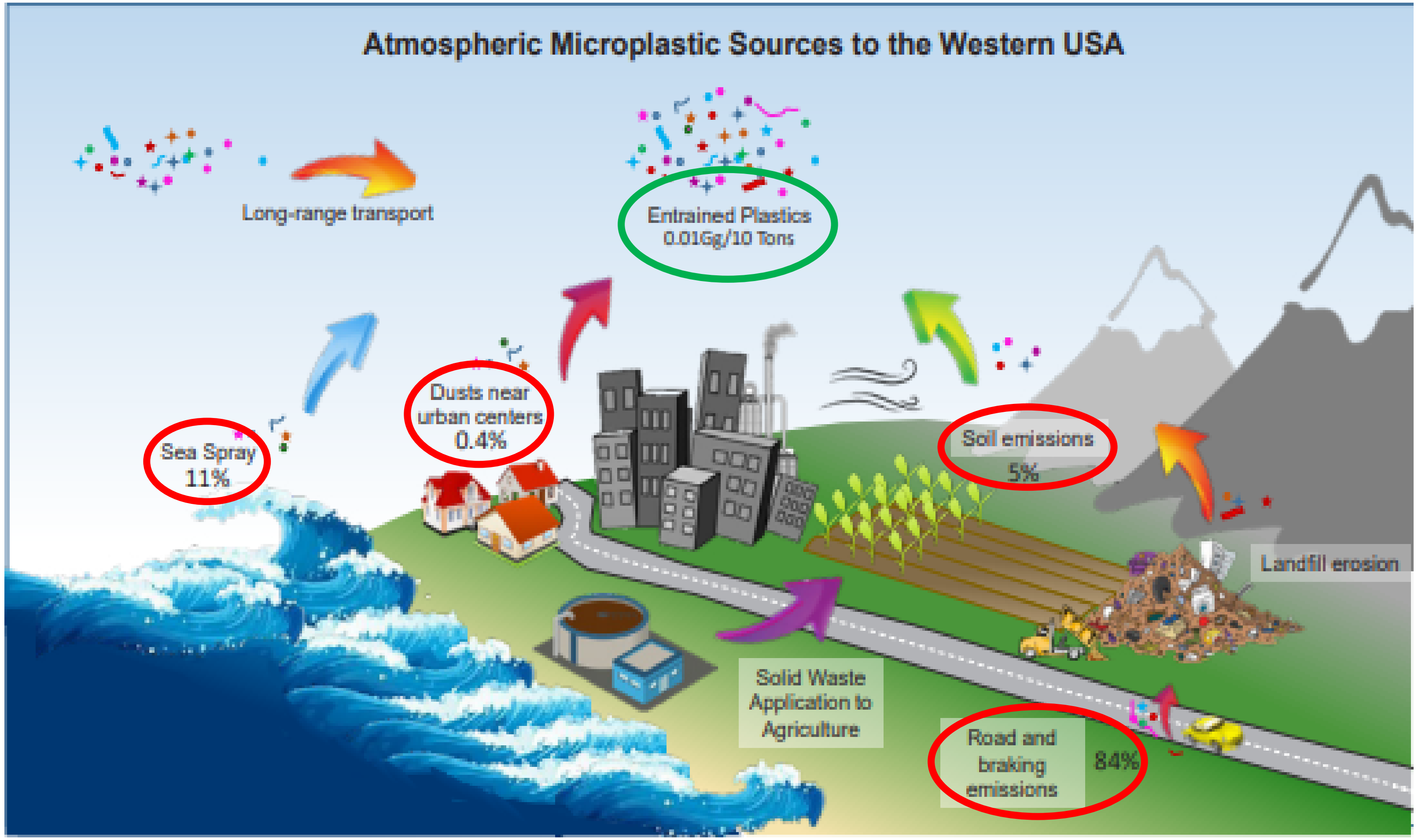


Fig. 1. Representation of the major sources of microplastics to the atmosphere and their relative contributions to deposition to the terrestrial environment over the western United States (30 to 50°N, 120 to 100°W). Over this region, the deposition of microplastics is 84% from roads, 11% from sea spray, 5% from agricultural dust, and 0.4% from dust near population centers. The atmospheric burden above this region is 0.01 Gg.

Brahney et al, 2020



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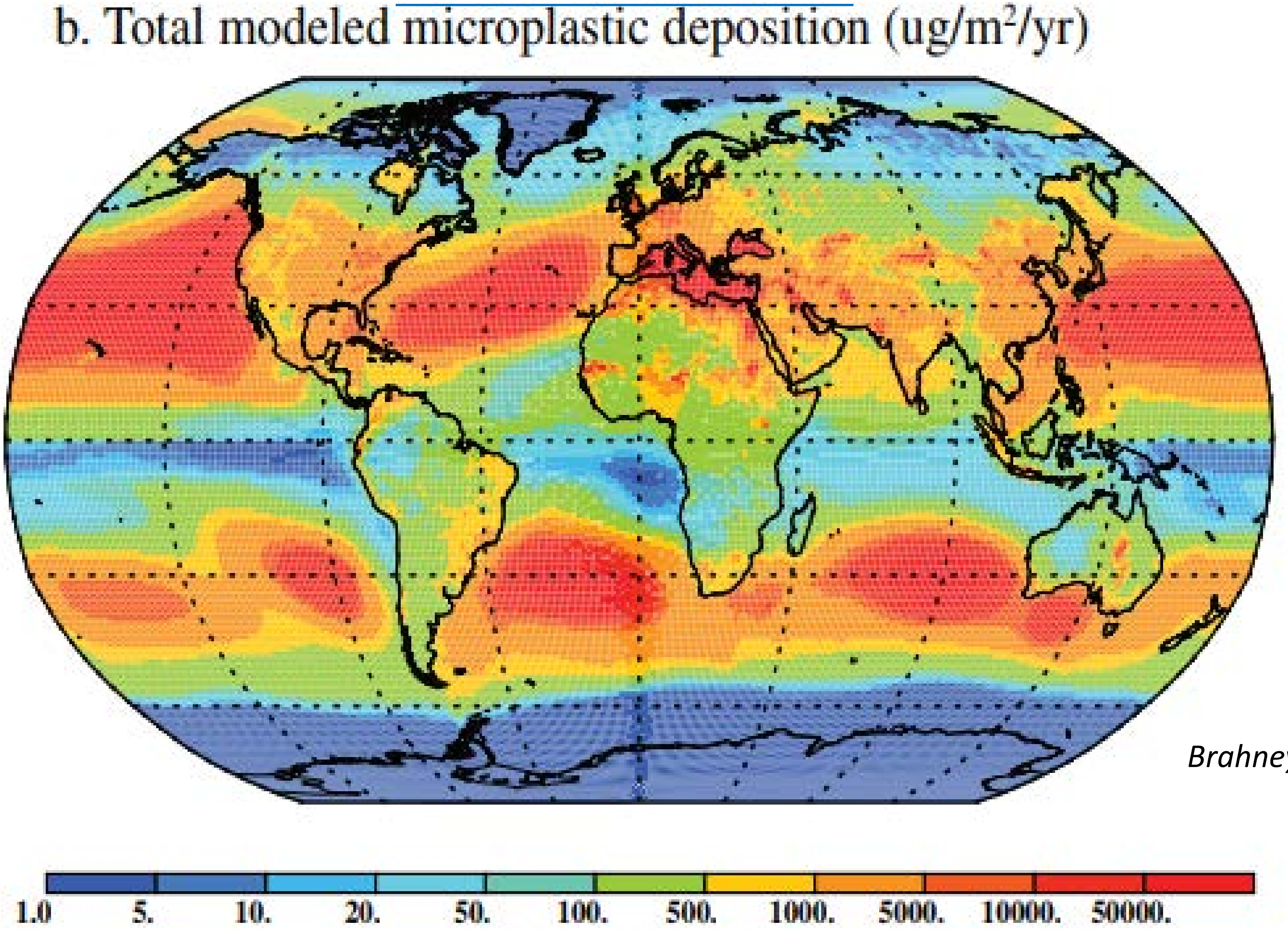
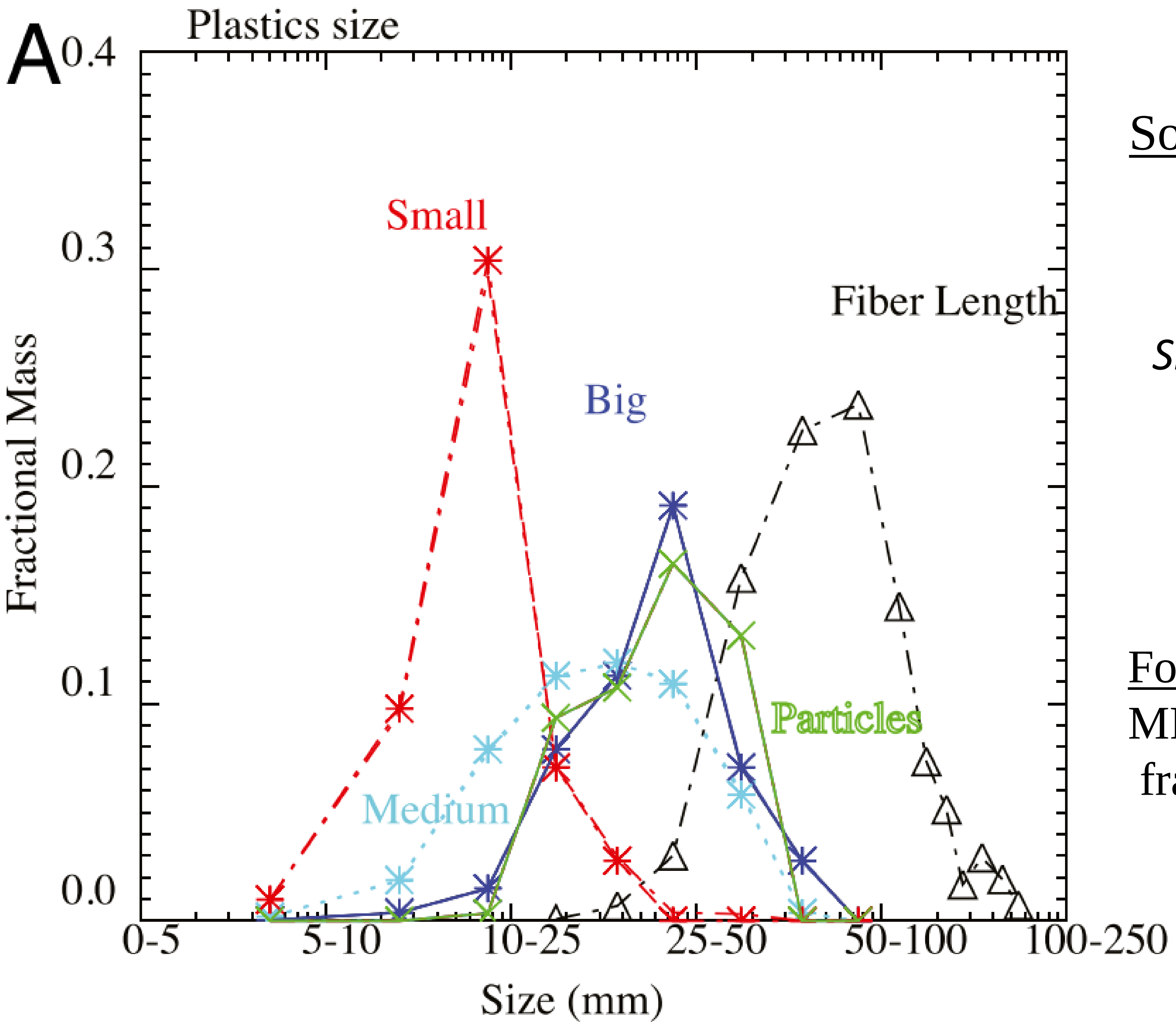
Global Plastics Pollution



Brahney et al, 2021



Brahney et al, 2021:

Sources of Microplastics **(> 5mm)**

modeled into 3 size modes:

Small, Medium, Big

*Size distribution for **Small** mode, estimated from figure:*

MMD = 26 mm; GSD = 2.4

Formation of Nanoplastics **(< 1 μm):**

MPs in environment ➡ continued fragmentation, degradation*

(**photochemical, biotic, abiotic*)

But, there is increasing awareness of direct release of envNPs *(Morales et al 2022)*

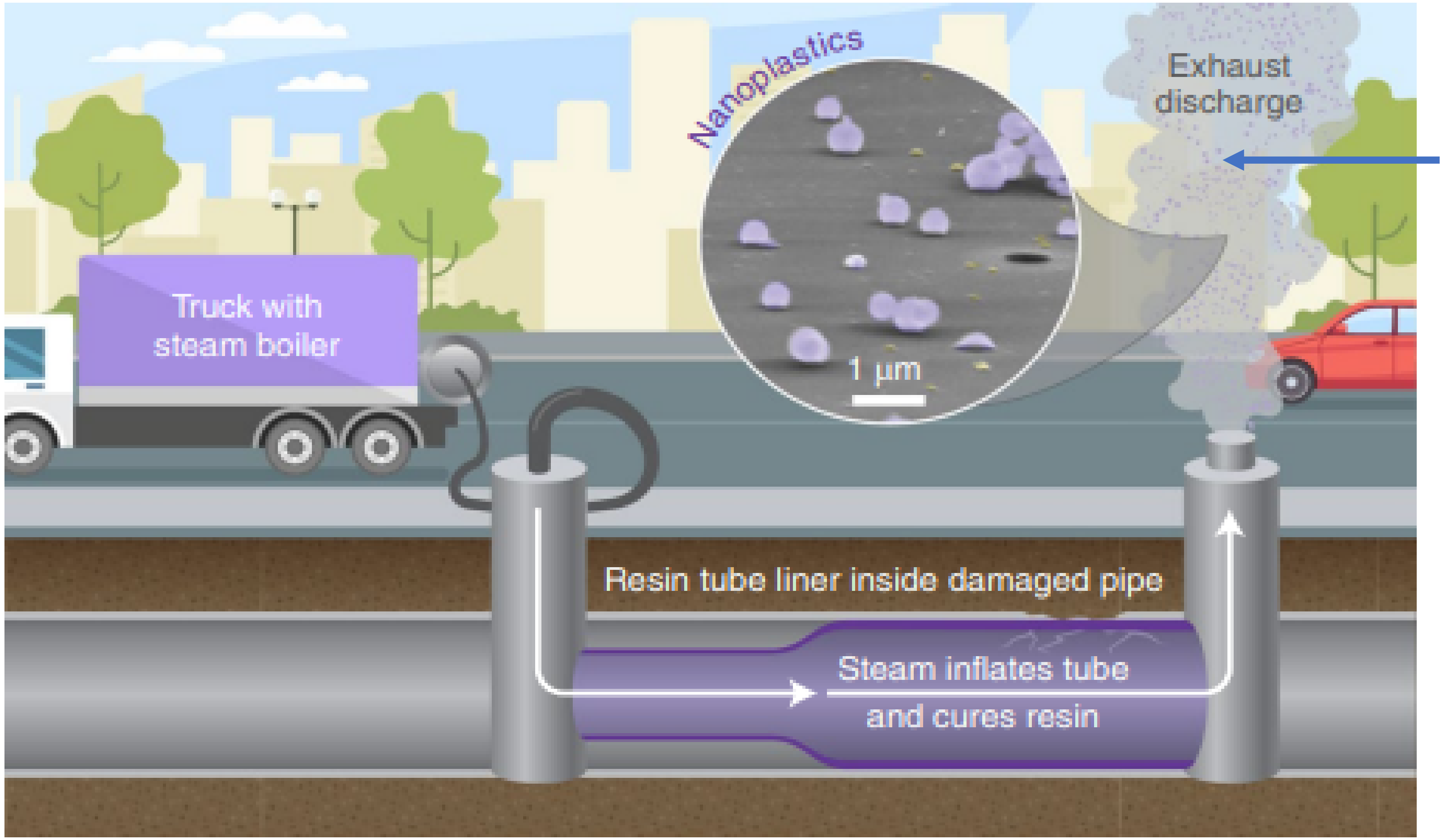


Fig. 1 | A schematic illustration of CIPP installation. A flexible resin-impregnated tube is first inserted into the damaged pipe. This tube is inflated against the damaged pipe wall by pumping ambient air, water and/or steam through the tube. Next, the tube is cured in place using either thermal (hot steam injected into the tube) or ultraviolet curing methods, and the waste is discharged into the environment. After curing, the newly installed plastic pipe is cooled by blowing forced ambient air through the tube, also resulting in the atmospheric discharge of waste laden with EnvNP particles.

10 mg/m³ - 3.24 g/m³ *solid NPs released into ambient air*

Morales et al, 2022:

Direct source of releasing Nanoplastics into environment:

Cured-In-Place-Pipe (CIPP):

"The cured-in-place-pipe (CIPP) installation of plastic pipes is the most popular, least expensive and most frequently used technology used to cure leaking sanitary and stormwater sewers through the insertion of new plastic pipes inside the existing pipes"

CIPP Installation: Method of determining/confirming release of Nano-Plastics into ambient air

Morales et al, 2022)

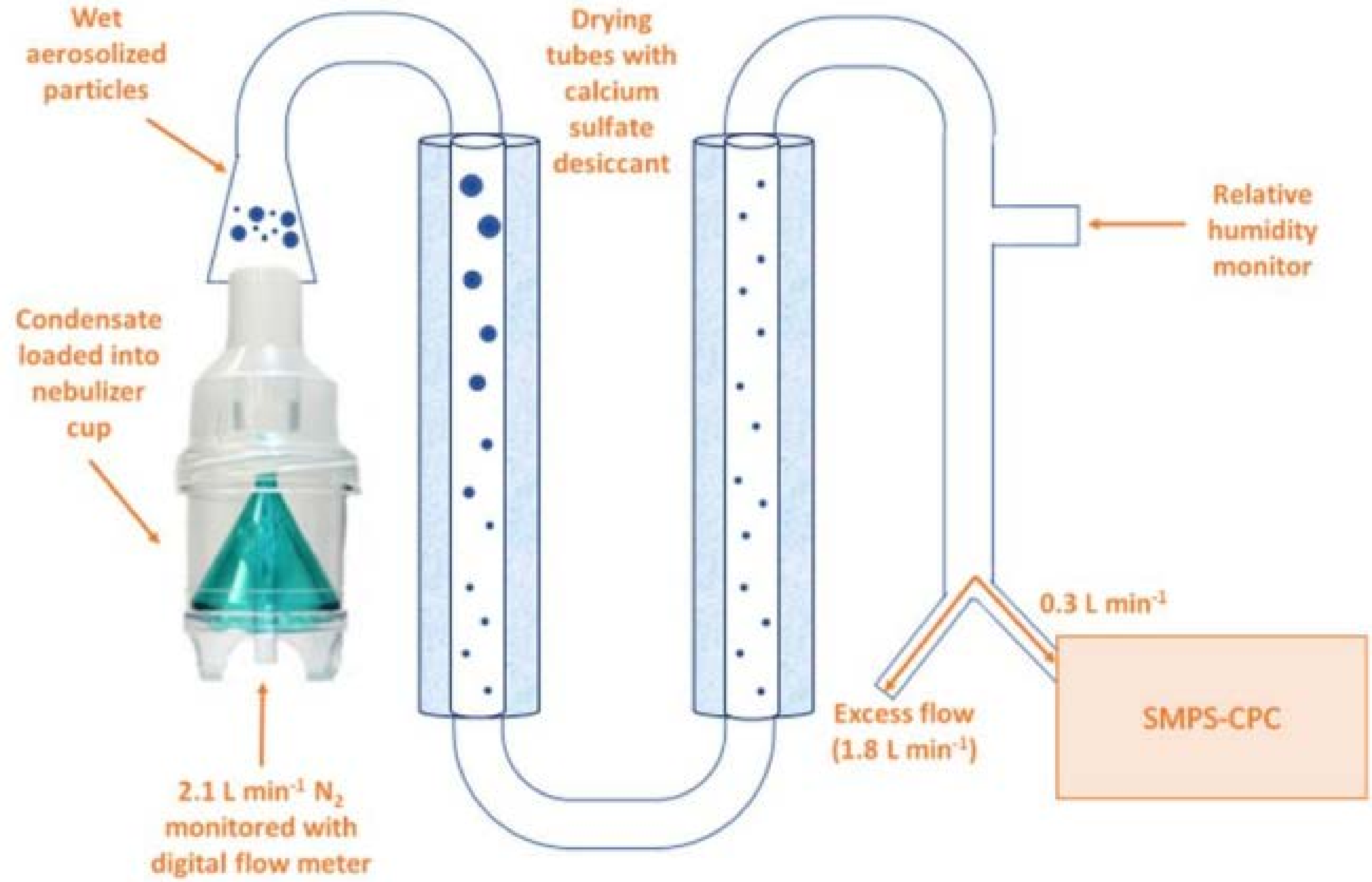


Figure S2. Diagram representing the condensate nebulizing experiment, during flow diagram and detection/counting of dry aerosolized particles.

CIPP waste release of environmental Nano Plastics *(env NPs, airborne)*

Bimodal particle size distributions at 4 locations

Morales et al, 2022

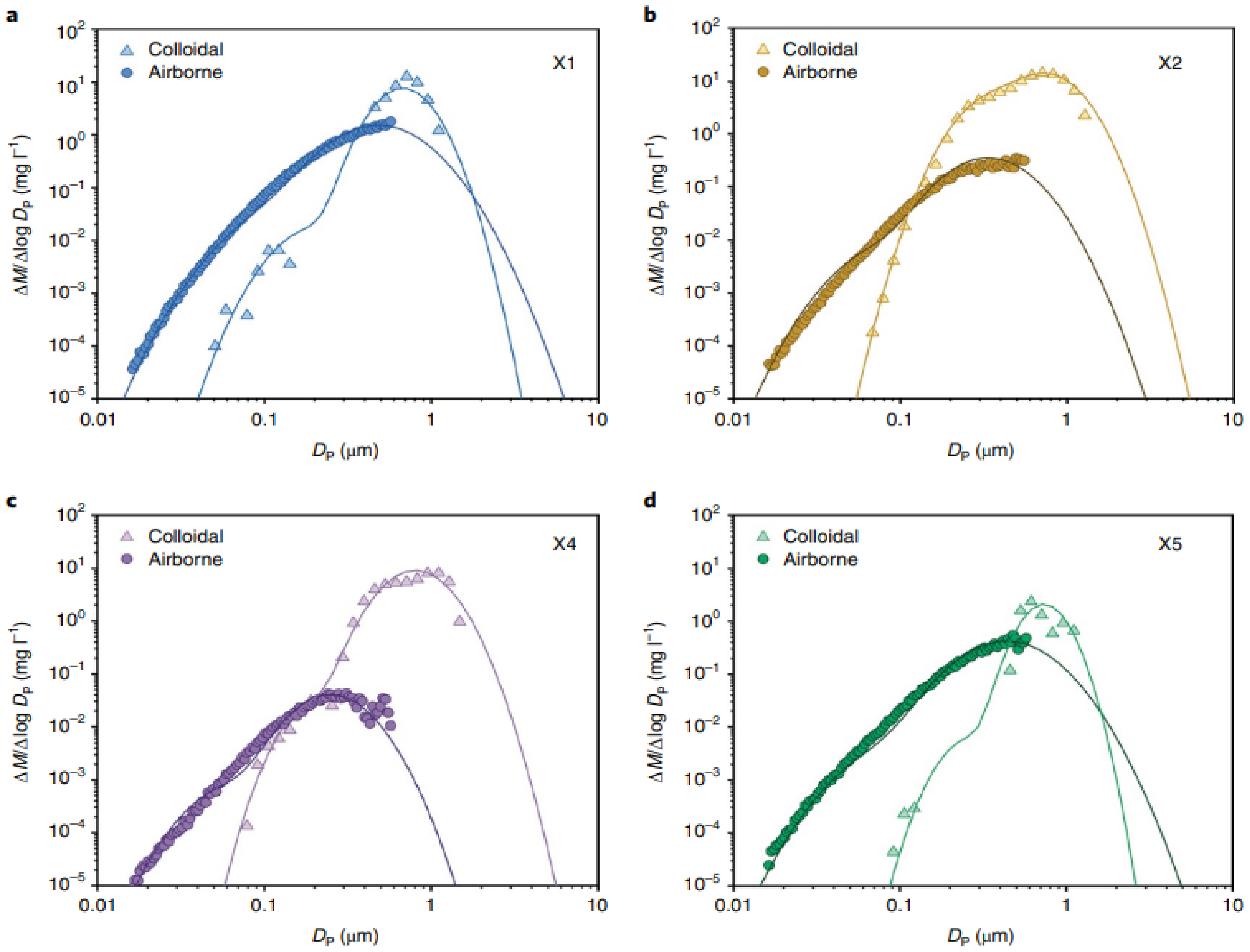


Fig. 2 | Particle mass size distributions of wet colloids and dry particles from CIPP waste. Particle mass size distributions of wet colloids in CIPP waste condensate samples and dry particles aerosolized from the same samples collected at four different operation sites (X1, X2, X4 and X5). The lines show bimodal lognormal data fits; the fitting parameters are tabulated in Supplementary Table 2. D_p, particle diameter; $\Delta M/\Delta \log D_p$, mass concentration. The mass concentration values are reported in units of milligrams of solid material (colloids or particles) per litre of discharged condensate.

CIPP waste release of environmental Nano Plastics *(env NPs, airborne)*

Bimodal particle size distributions at 4 locations

Morales et al, 2022

X1

Mode 1		*Mode 2*	
nm	σ_g	*nm*	σ_g
55	*1.4*	*210*	*1.35*

Mass Percentage of Total

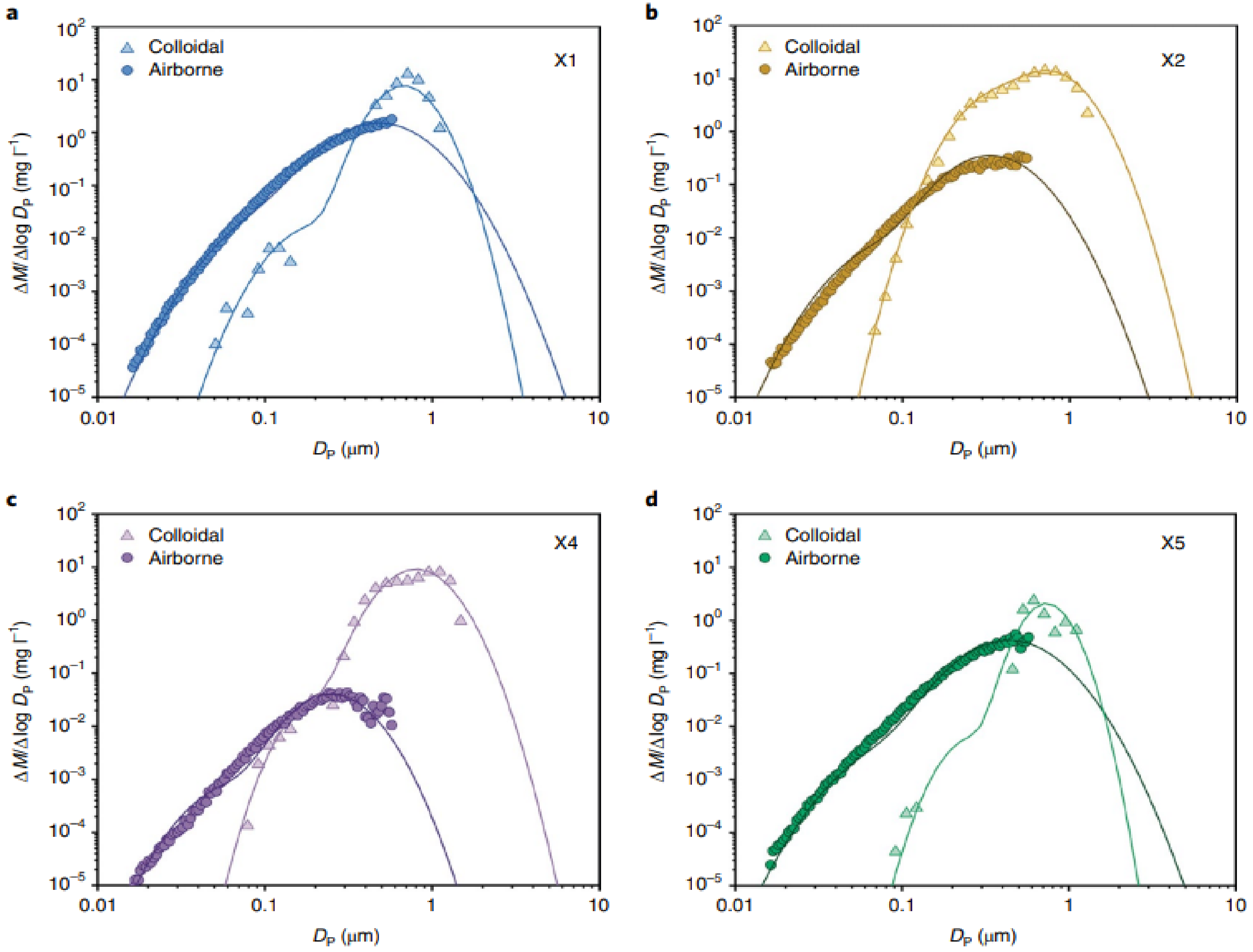
Mode 1	*Mode 2*
2.3%	*97.7%*

X2

Mode 1		*Mode 2*	
nm	σ_g	*nm*	σ_g
40	*1.45*	*170*	*1.4*

Mass Percentage of Total

Mode 1	*Mode 2*
2.3%	*97.7%*



X4

Mode 1		*Mode 2*	
nm	σ_g	*nm*	σ_g
35	*1.4*	*145*	*1.35*

Mass Percentage of Total

Mode 1	*Mode 2*
2.5%	*97.5%*

X5

Mode 1		*Mode 2*	
nm	σ_g	*nm*	σ_g
40	*1.5*	*190*	*1.45*

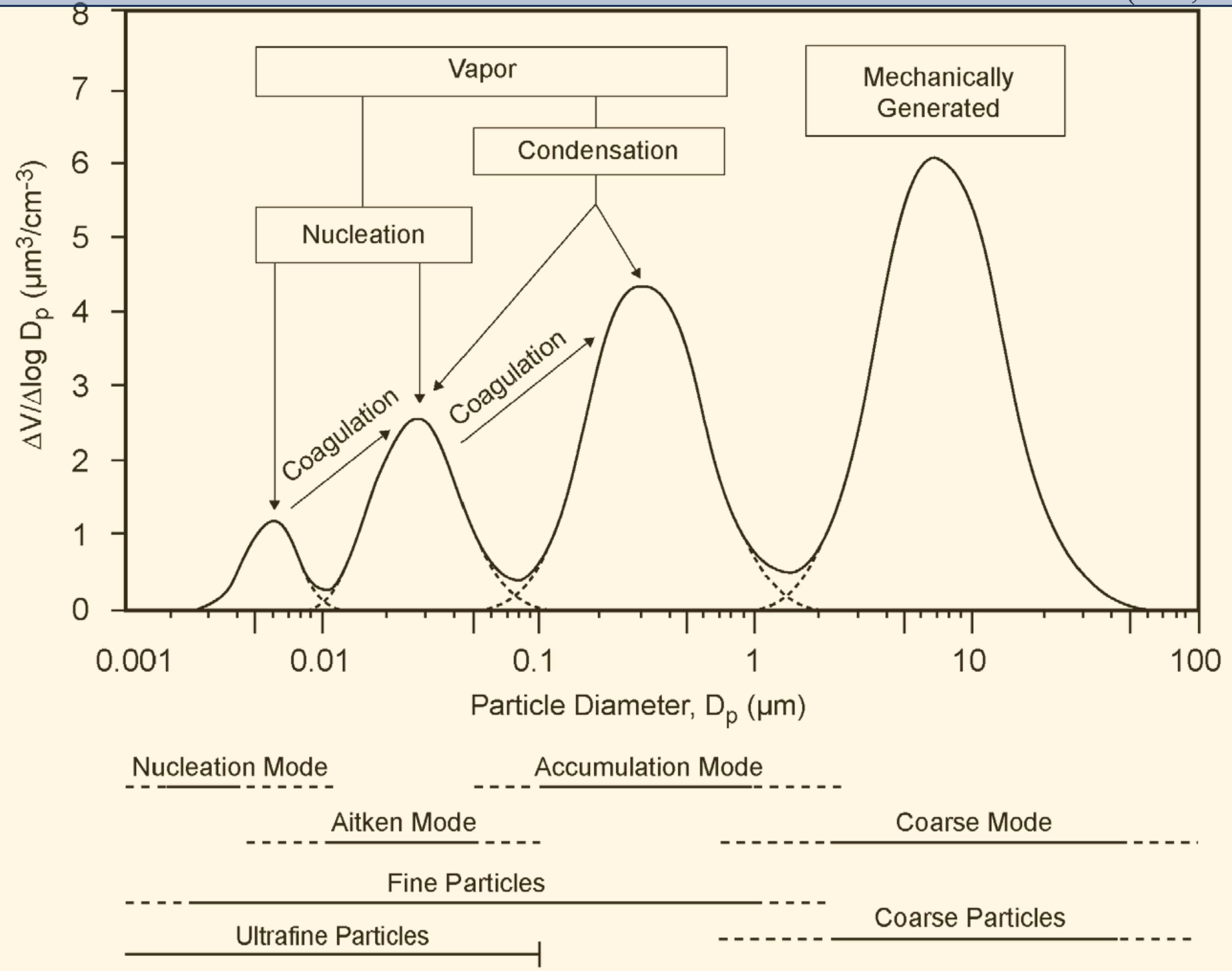
Mass Percentage of Total

Mode 1	*Mode 2*
1.7%	*98.9%*

Fig. 2 | Particle mass size distributions of wet colloids and dry particles from CIPP waste. Particle mass size distributions of wet colloids in CIPP waste condensate samples and dry particles aerosolized from the same samples collected at four different operation sites (X1, X2, X4 and X5). The lines show bimodal lognormal data fits; the fitting parameters are tabulated in Supplementary Table 2. D_p, particle diameter; $\Delta M/\Delta \log D_p$, mass concentration. The mass concentration values are reported in units of milligrams of solid material (colloids or particles) per litre of discharged condensate.

Idealized Size Distribution of Traffic-Related Particulate Matter

(EPA, 2004)



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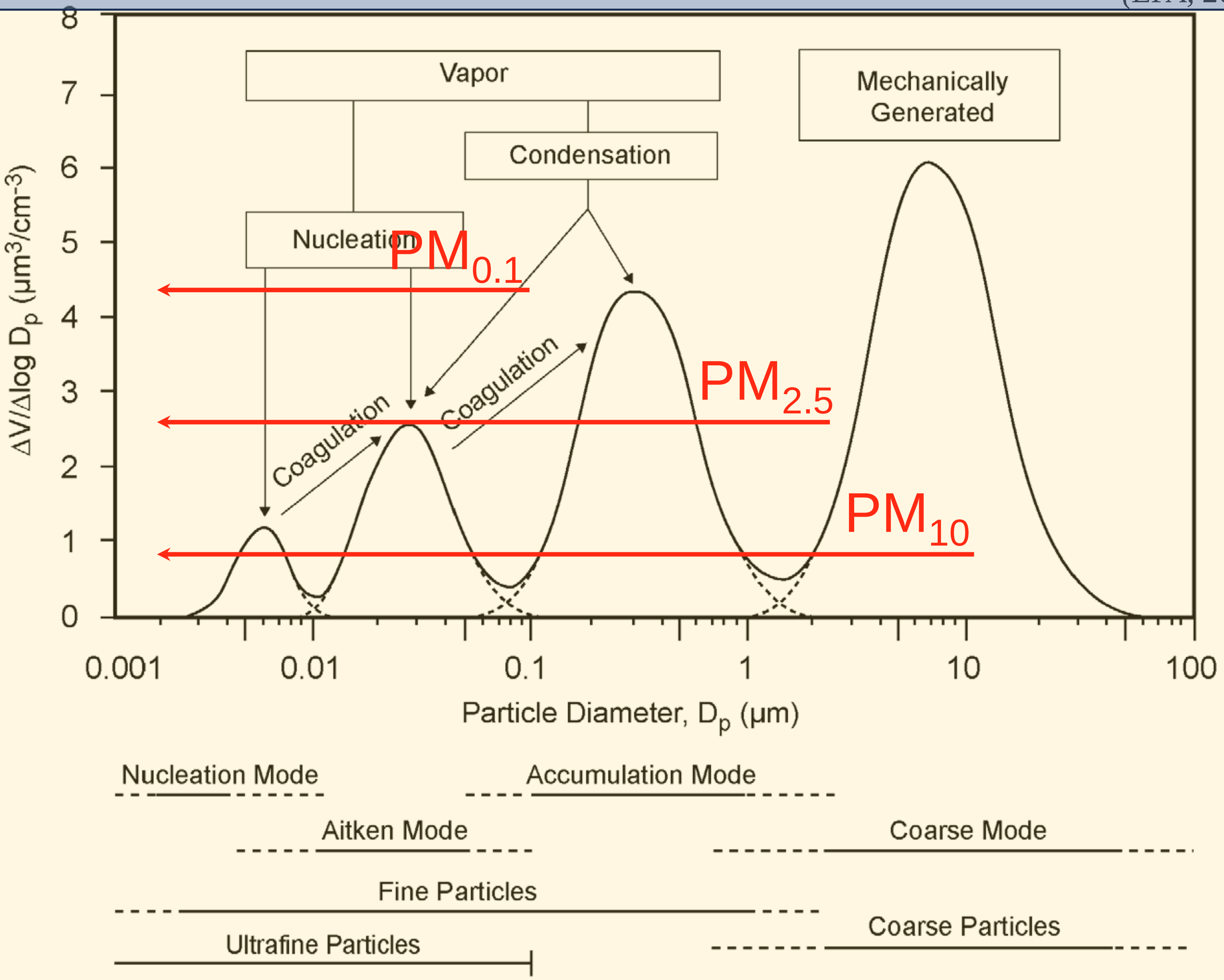

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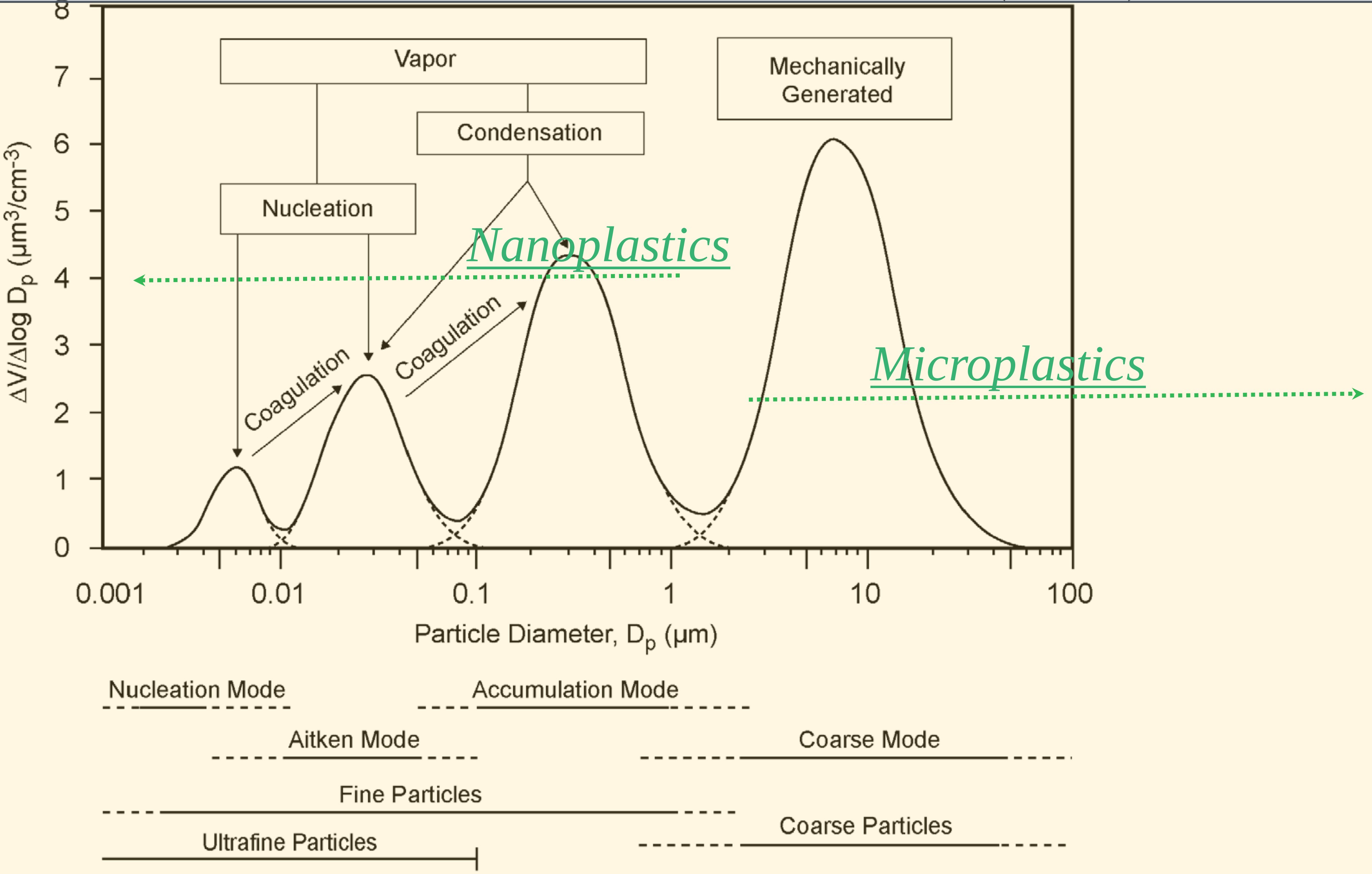


What is different about airborne ultrafine particles?

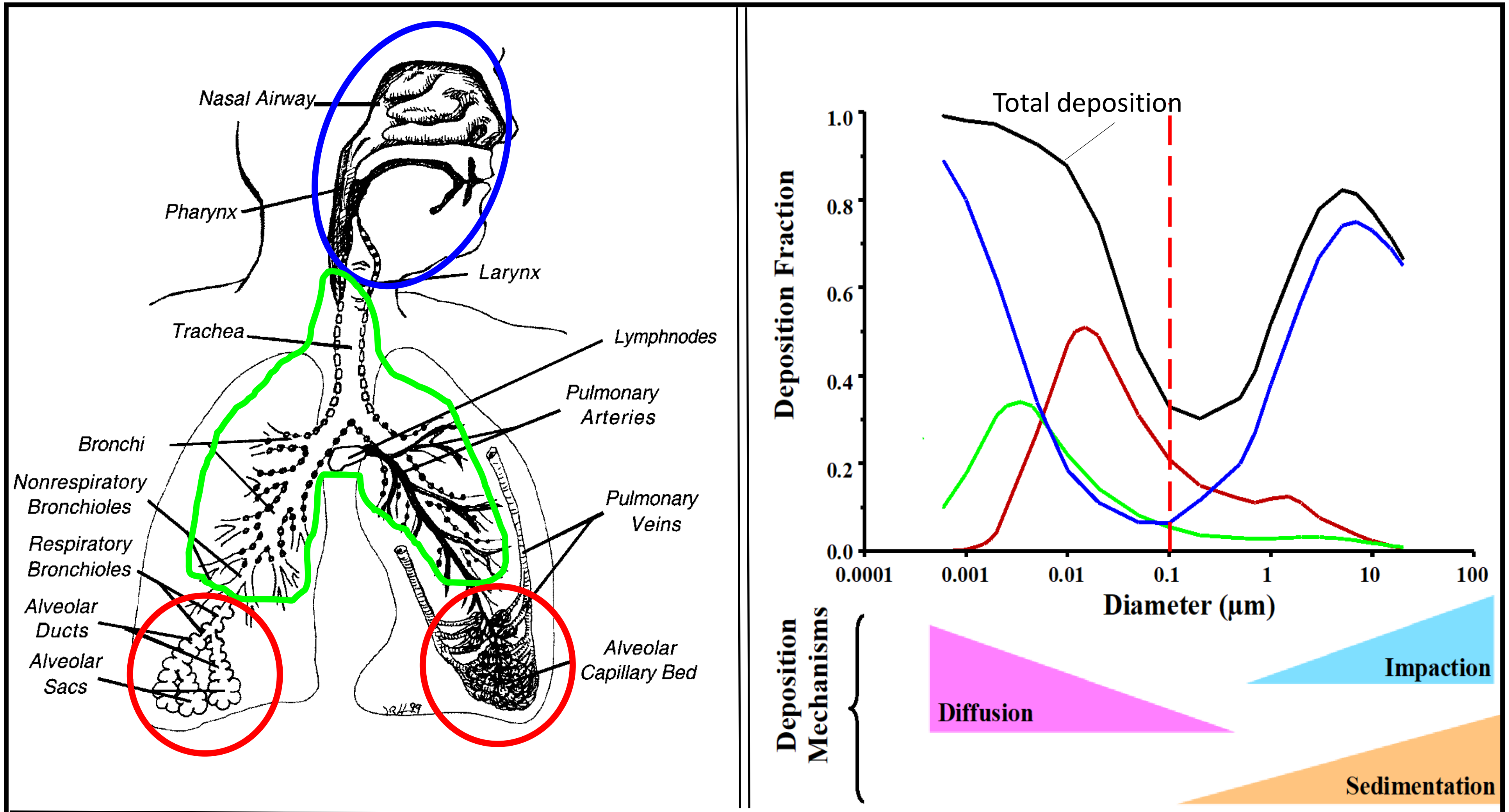
- **Large Number and Surface Area per Volume/Mass**
  – *potential for greater reactivity (ROS; more surface atoms or molecules per mass)*

- **Deposition in Respiratory Tract**
  – *by diffusion*
  – *all regions of the respiratory tract are targeted*

- **Disposition/Biokinetics**
  – *translocation: across cell barriers into cells (subcell. structures)*
  along axons/dendrites

Fractional Deposition of Inhaled Particles in the Human Respiratory Tract

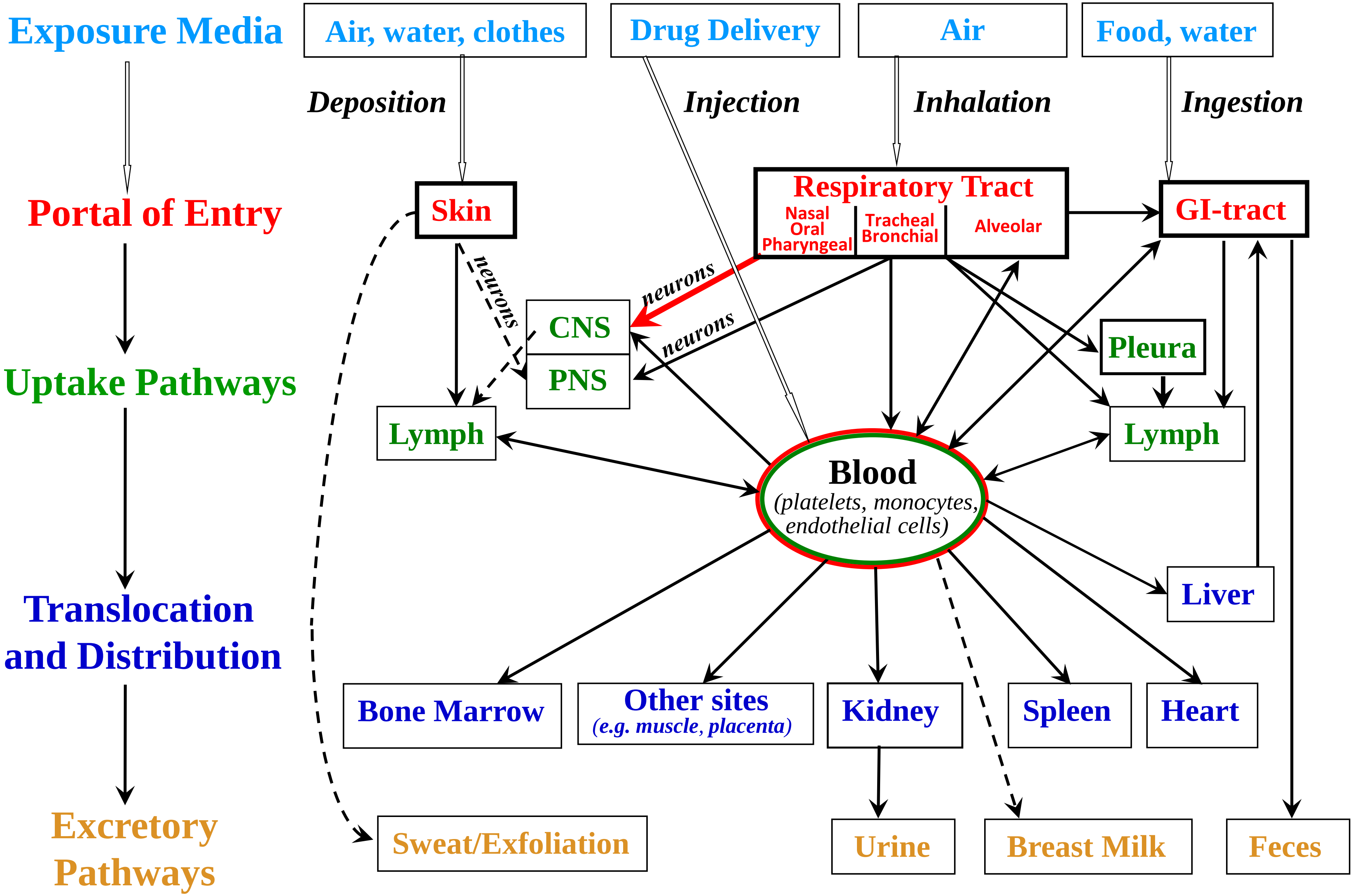
(ICRP Model, 1994; Nose-breathing)



Naso-Oro-Pharyngeal **Tracheo-Broncheal** **Alveolar**

Regions of Respiratory Tract

Exposure and Biokinetics of Nanoparticles



Updated from Oberdörster et al., 2005

Translocation and rates are very low!

→ Confirmed routes; - - → Potential routes

Physico-Chemical and Functional Particle Properties of Relevance for InhalationToxicology

Size *(aerodynamic, hydrodynamic)*

Size distribution

Shape

Agglomeration/aggregation

Density *(material, bulk)*

Surface properties:

- area *(porosity)*
- charge
- chemistry *(coatings, contaminants)*
- defects

Crystallinity

Biol. contaminants (e.g. endotoxin)

Solubility/dissol-rate (*physiol. fluid*, ***in vivo***)

Surface reactivity *(ROS inducing capacity)*

Biotransformation *(intracellular breakdown)*

Properties can change

-with: method of production
preparation process
storage

-when introduced into
physiol. media, organism

-Biopersistence

Key parameter: Dose!

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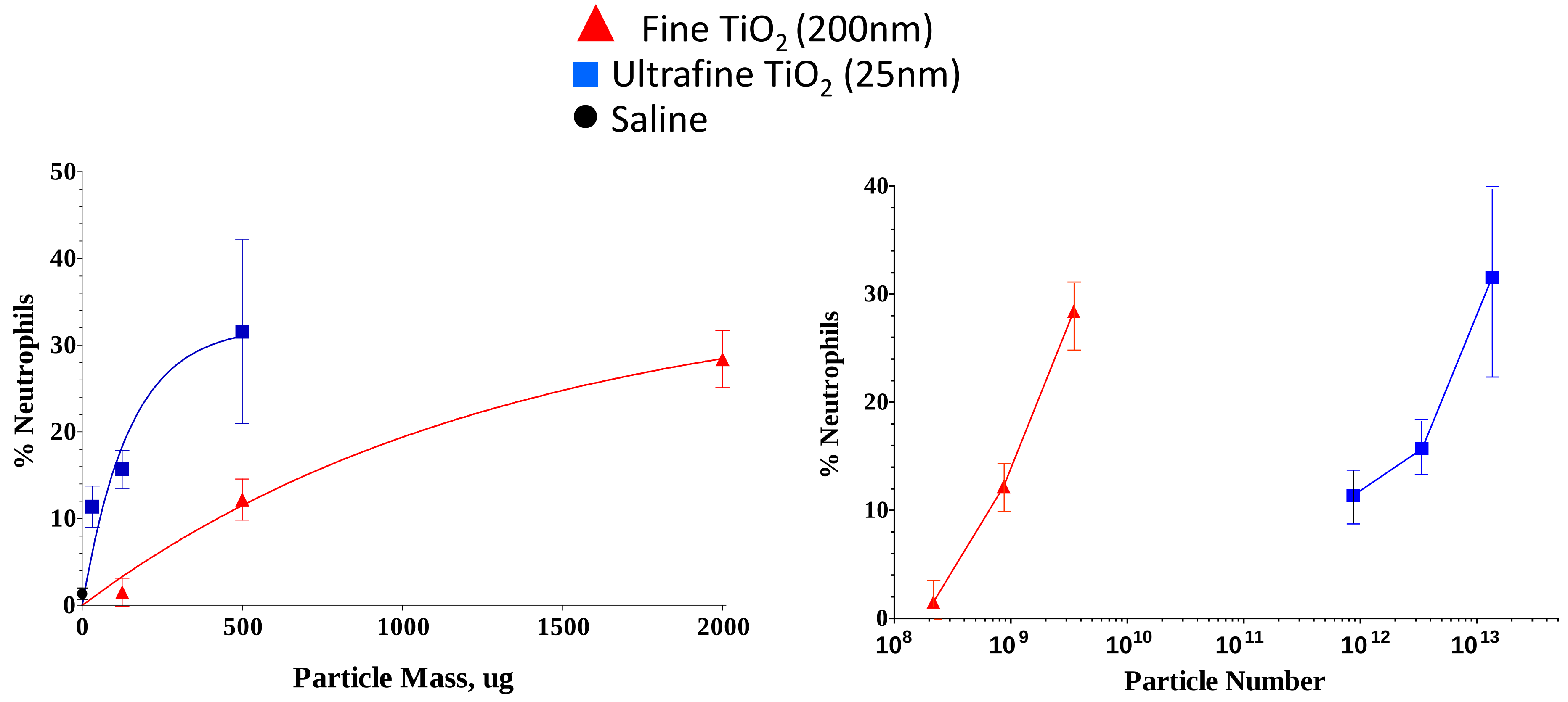
-Biopersistence ←

Key parameter: Dose!

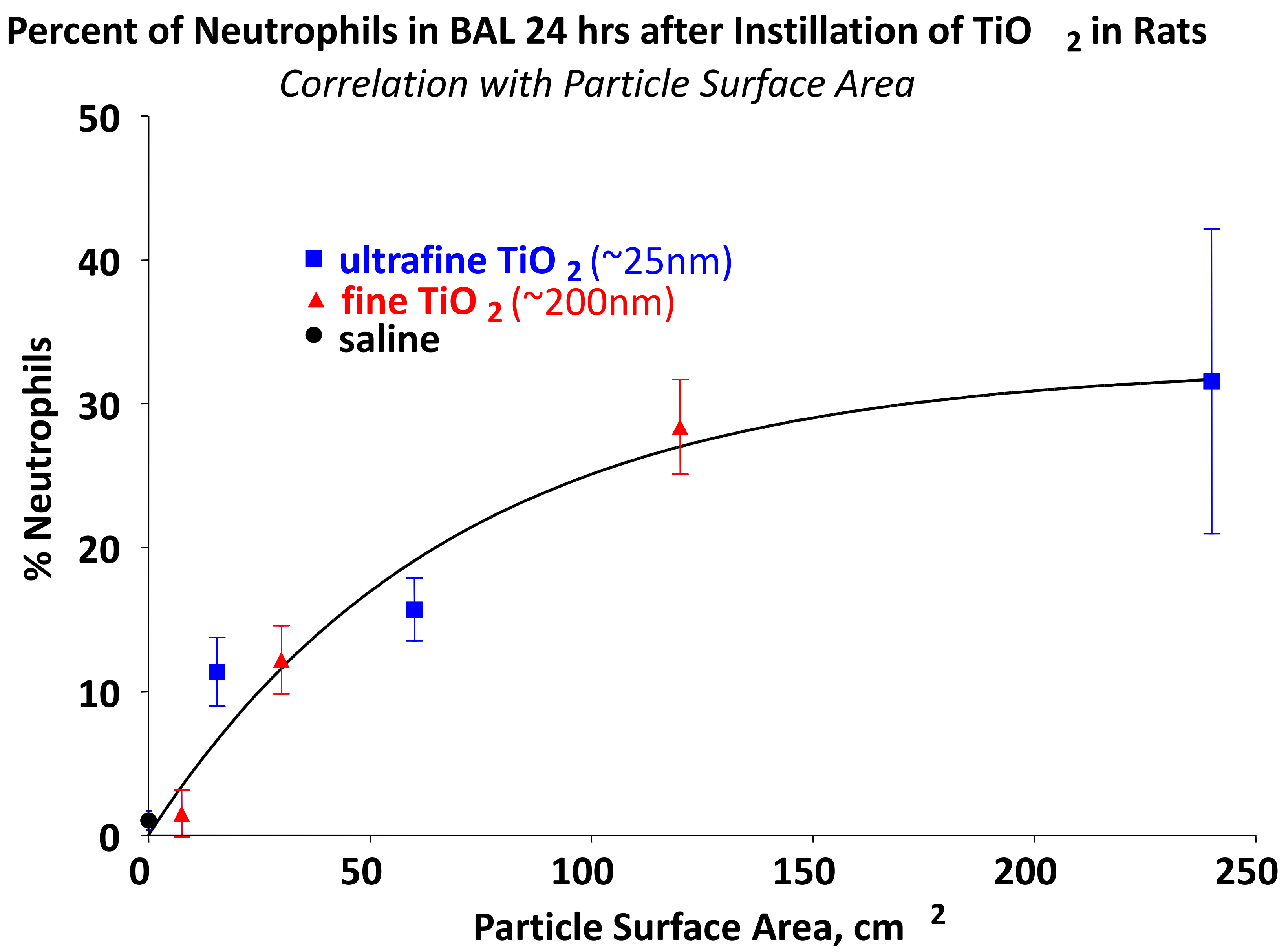
Which Dose-Metric?

Percent of Neutrophils in Lung Lavage 24 hrs after Intratrachael Dosing of Ultrafine and Fine TiO_2 in Rats

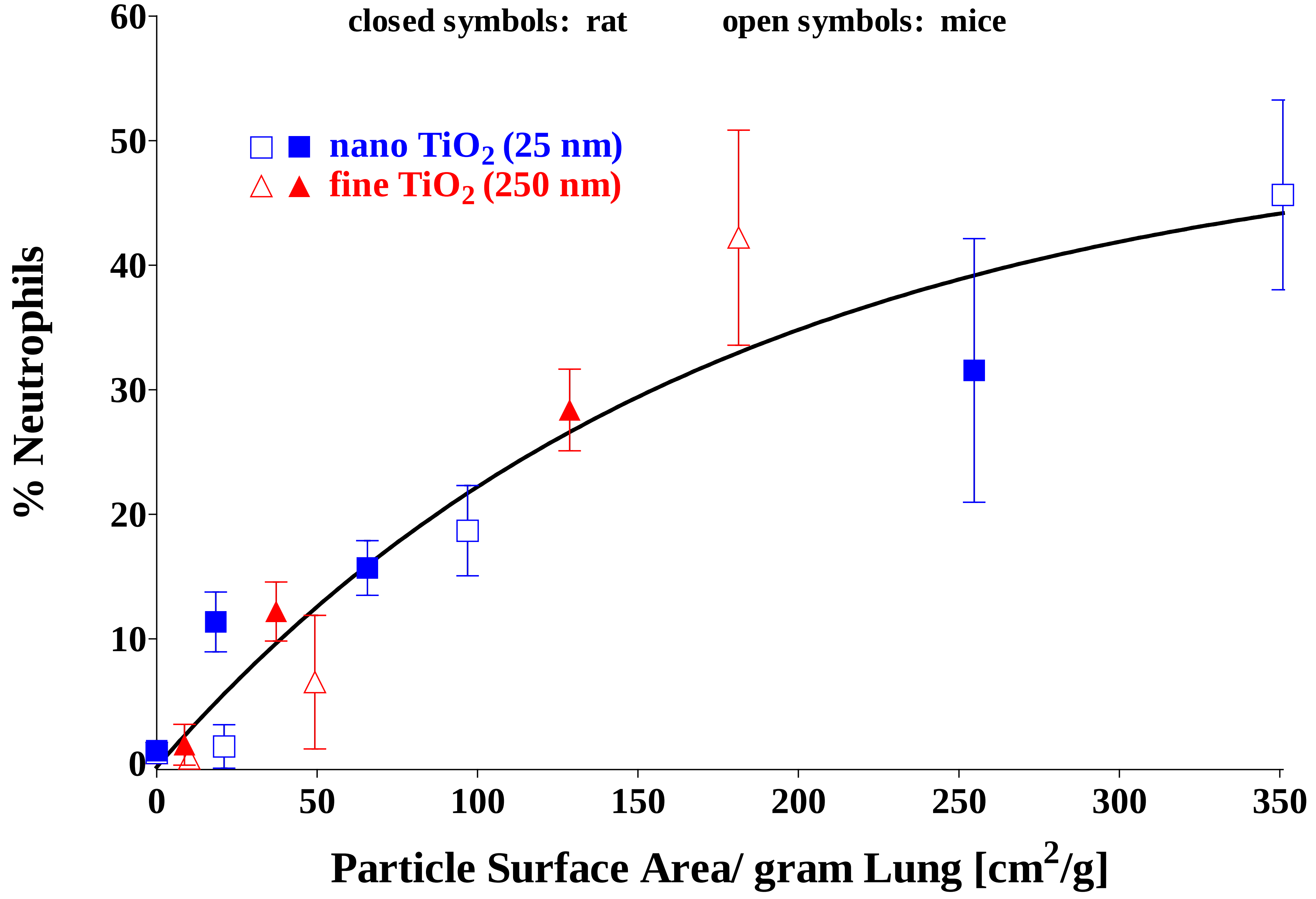


Particle Mass ***vs*** ***Particle Number***

Which Dose-Metric?

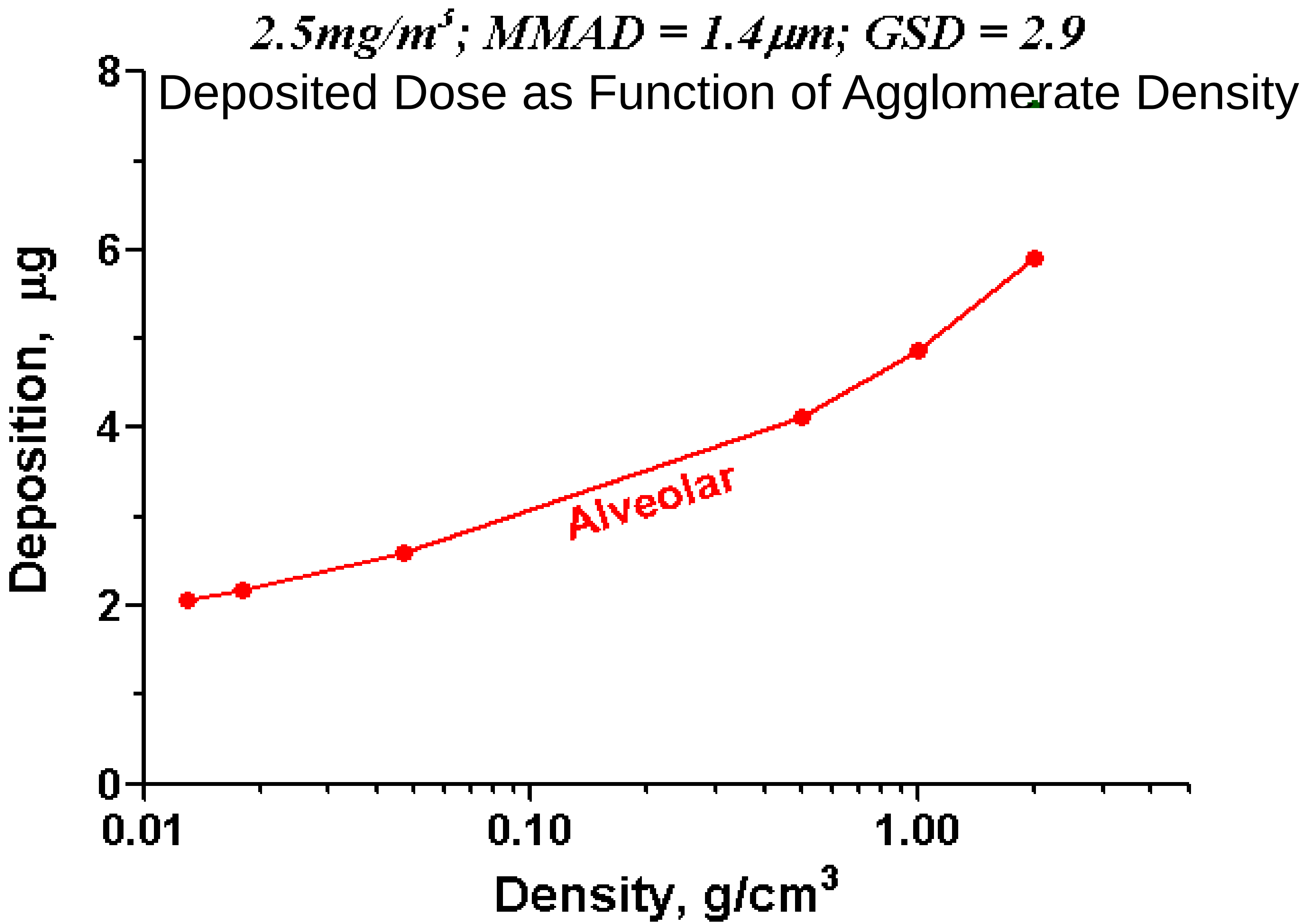


Percent of Neutrophils in BAL 24 hrs after Instillation



Impact of Aerosol Density on Lung Deposition of Inhaled Agglomerated Particles:

MPPD Prediction, Rat, 4 hour Inhalation of carbon aerosol



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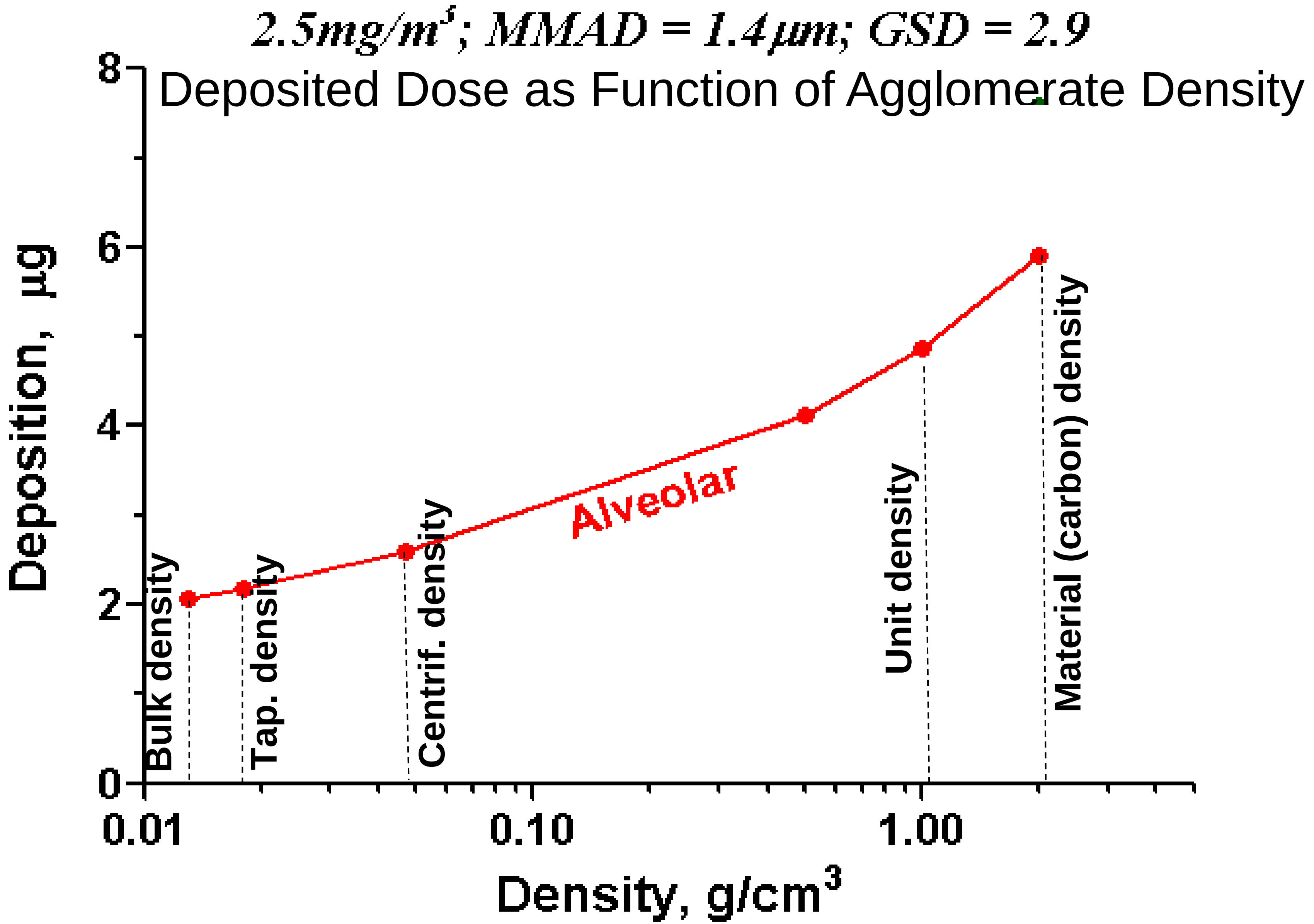


Determinants of Pulmonary Biopersistence of Inhaled Particles

Physiological Clearance Processes → **Particle Biopersistence** ← **Physicochemical Processes**

AM mediated; Translocation

↓

Larynx
Interstitium
Pleura

↓

Species Differences

Particle Biopersistence

↓

Retention $T_{1/2}$
Effects

Biodurability: dissolution; leaching; bioprocessing
(intra-, extra-cellular)

↓

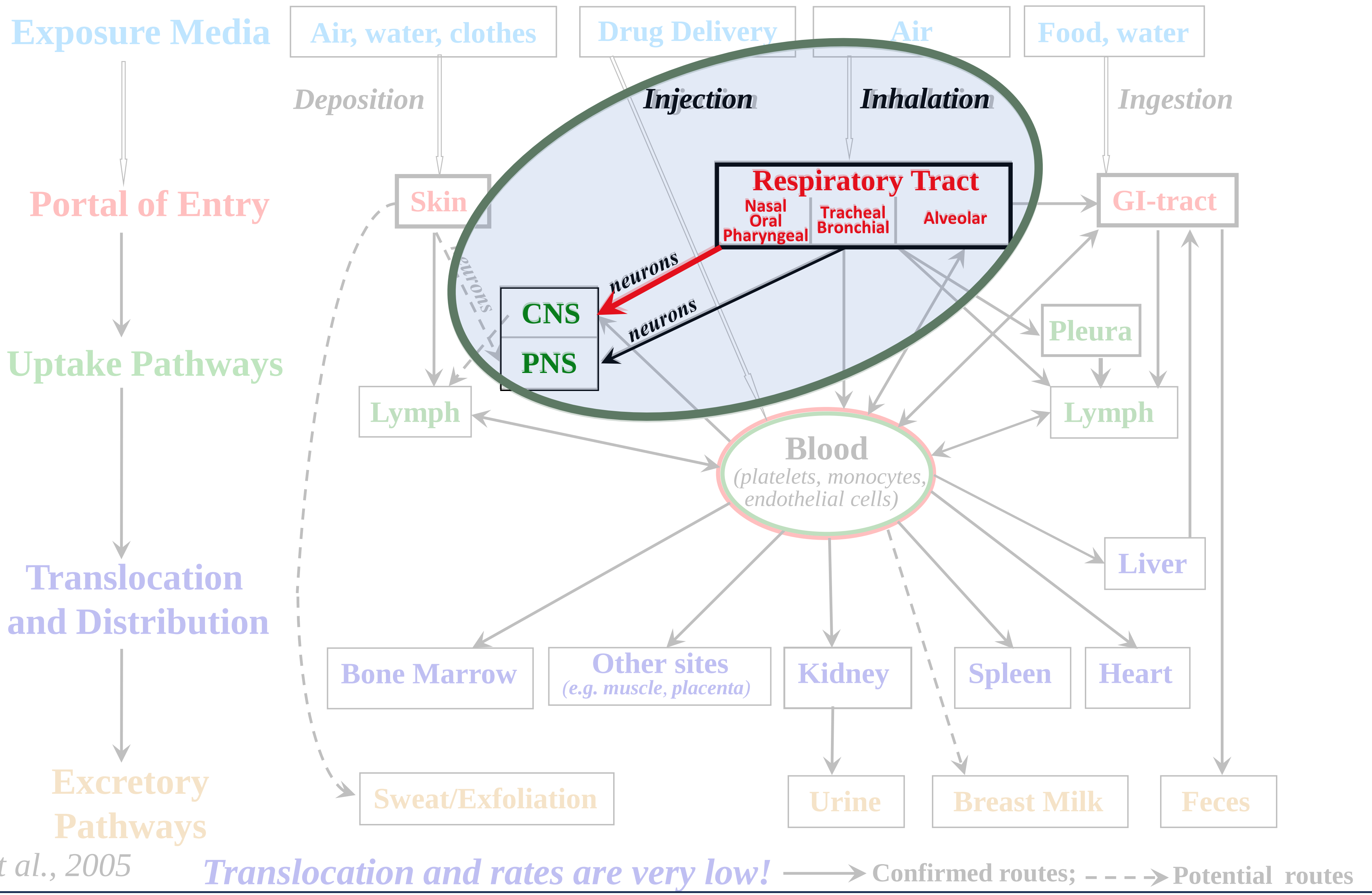
No Species Differences

Biopersistence = f (Physiological Clearance; Biodurability)

Overall clearance rate = AM-mediated clearance rate + dissolution☼ rate

(☼may be masked due to prolonged retention of bioprocessed particles/ions)

Exposure and Biokinetics of Nanoparticles

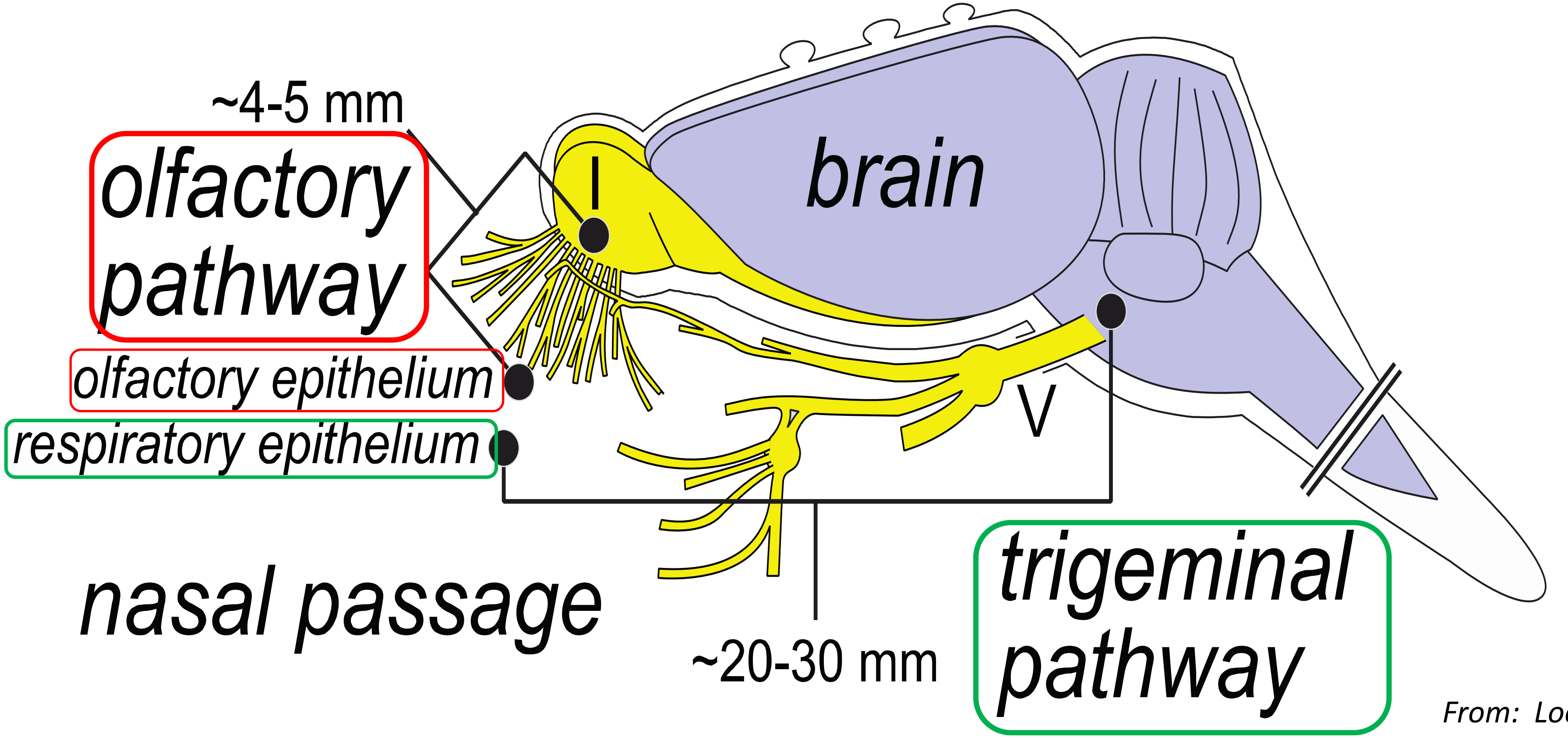


Updated from Oberdörster et al., 2005

Translocation and rates are very low!

Nasal Neuronal Translocation of Inhaled NPs to the Rodent Brain

Axonal transport in sensory neurons of solutes, proteins and solid nano-sized particles has been demonstrated in Rodents

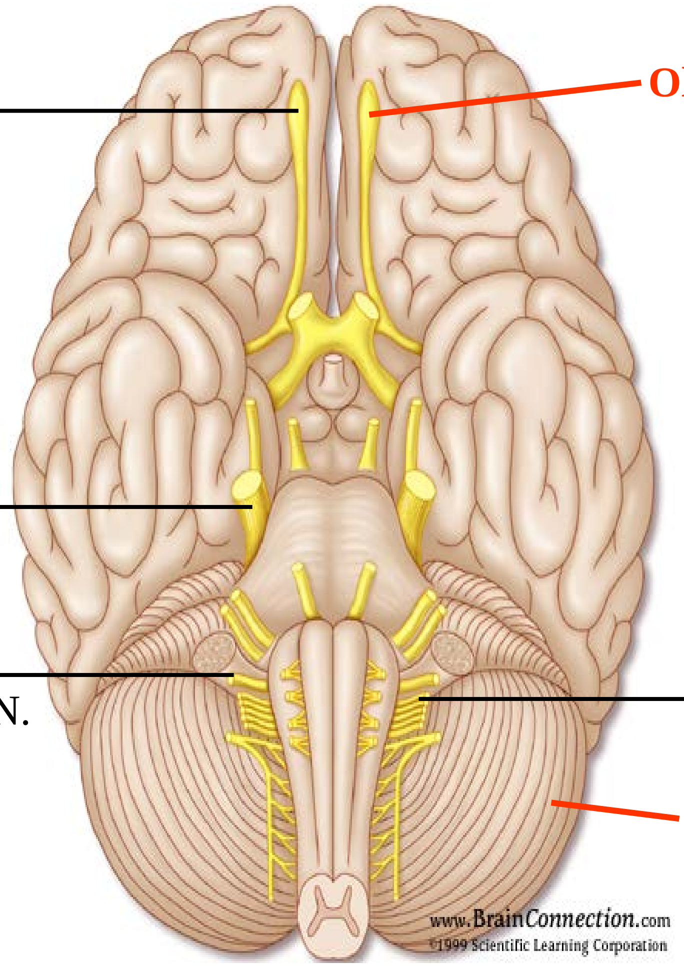


From: Lochhead and Thorne, 2012

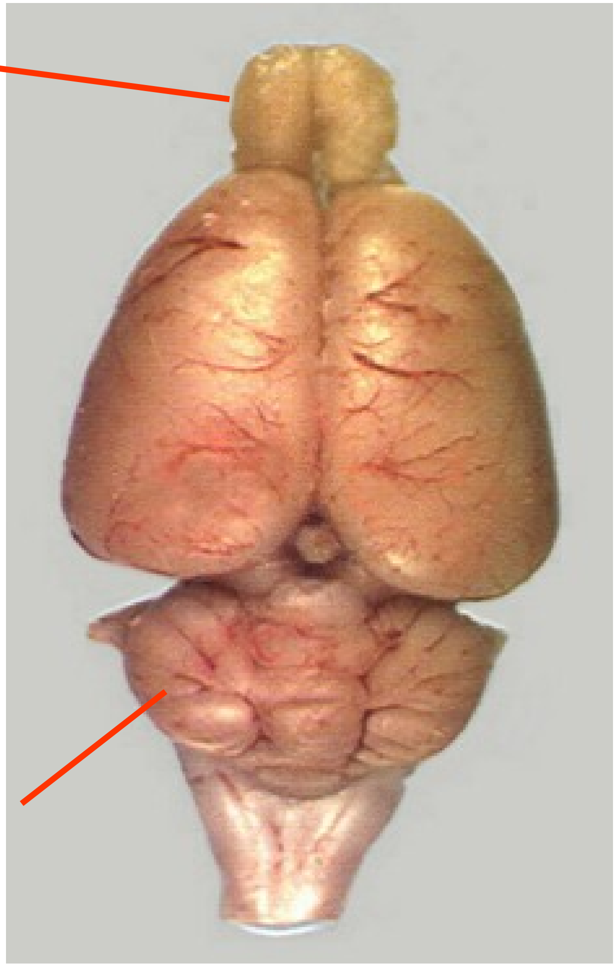
Alzheimer's (AD) and Parkinson (PD) Disease and PM 0.1 uptake?

Translocation Pathways for Inhaled Nanoparticles:

From upper resp. tract via sensory nerves -
From blood circulation via BBB in cerebellum?
Further neuronal translocation within brain?
Extrapolation from rats to humans?

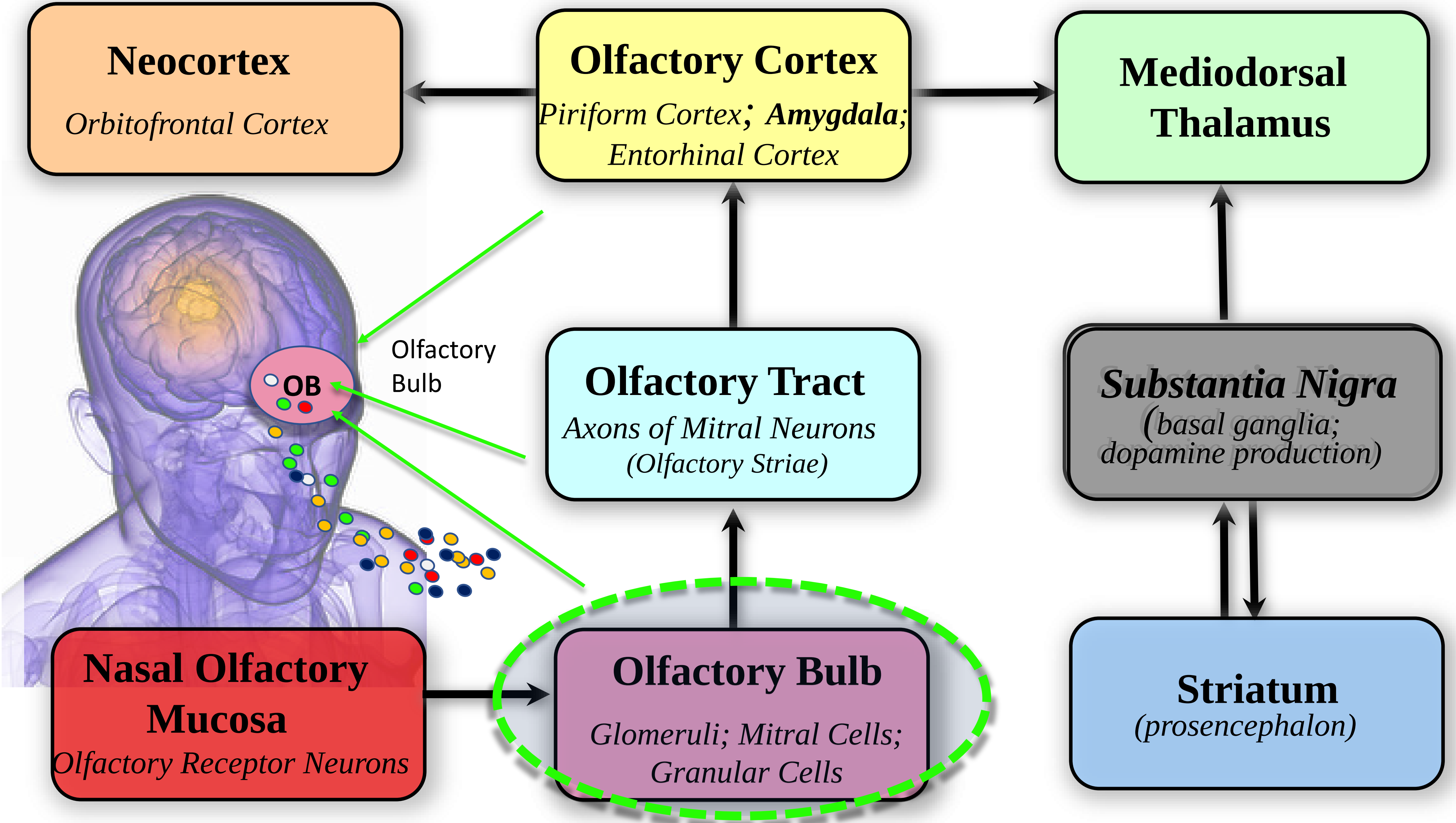


Human brain, ventral view

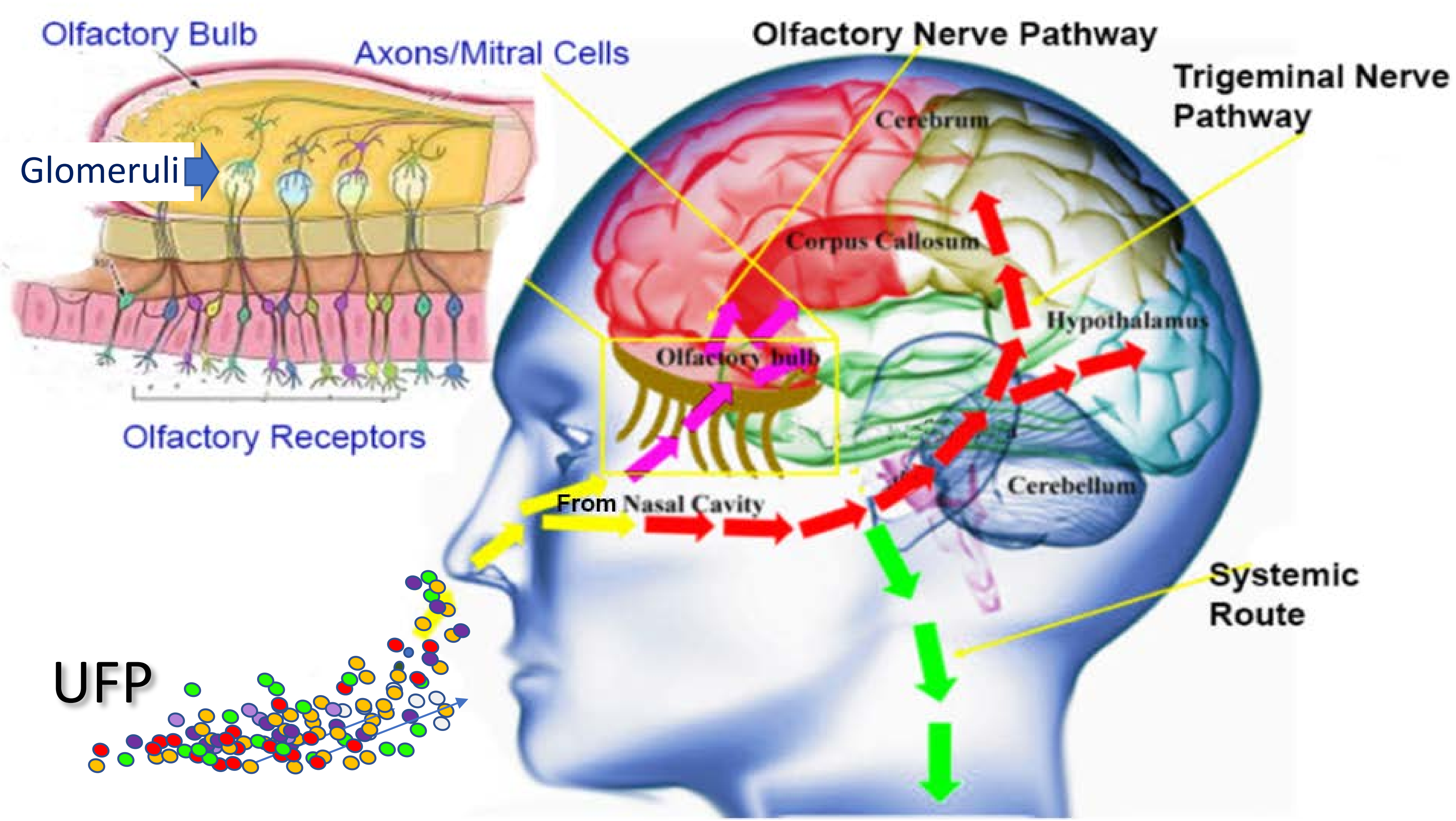


Rat brain, dorsal view

From the Nose to the Brain, Neuronal Pathways for Nanoparticles?



Nanoparticles have been shown to enter the brain via olfactory and trigeminal neuronal pathways and, when blood-bound, have the potential to cross the BBB, potentially causing neurotoxicity, neuroinflammation and neurodegeneration of the central nervous system.



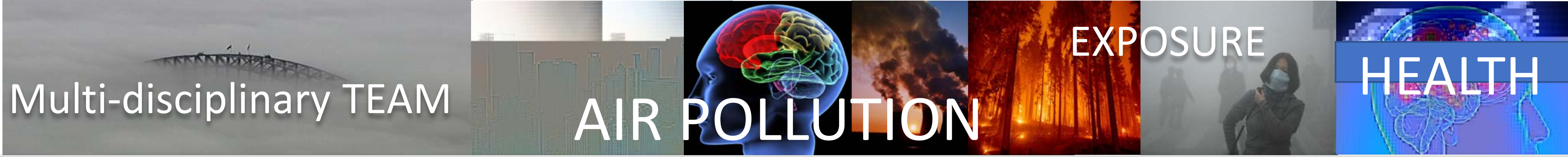
Chemical Research in Toxicology

2020 33 (5), 1145 - 1162

Tissue Specific Fate of Nanomaterials by Advanced Analytical Imaging Techniques - A Review

Uschi M. Graham,* Alan K. Dozier, Günter Oberdörster, Robert A. Yokel, Ramon Molina, Joseph D. Brain, Jayant M. Pinto, Jennifer Weuve, and David A. Bennett

***Objective*: Hi-Res imaging and phys-chem characterization for comparing ambient environmental particles and particles discovered in autopsied human olfactory bulbs, focusing on particle transformation and their association with pathology**

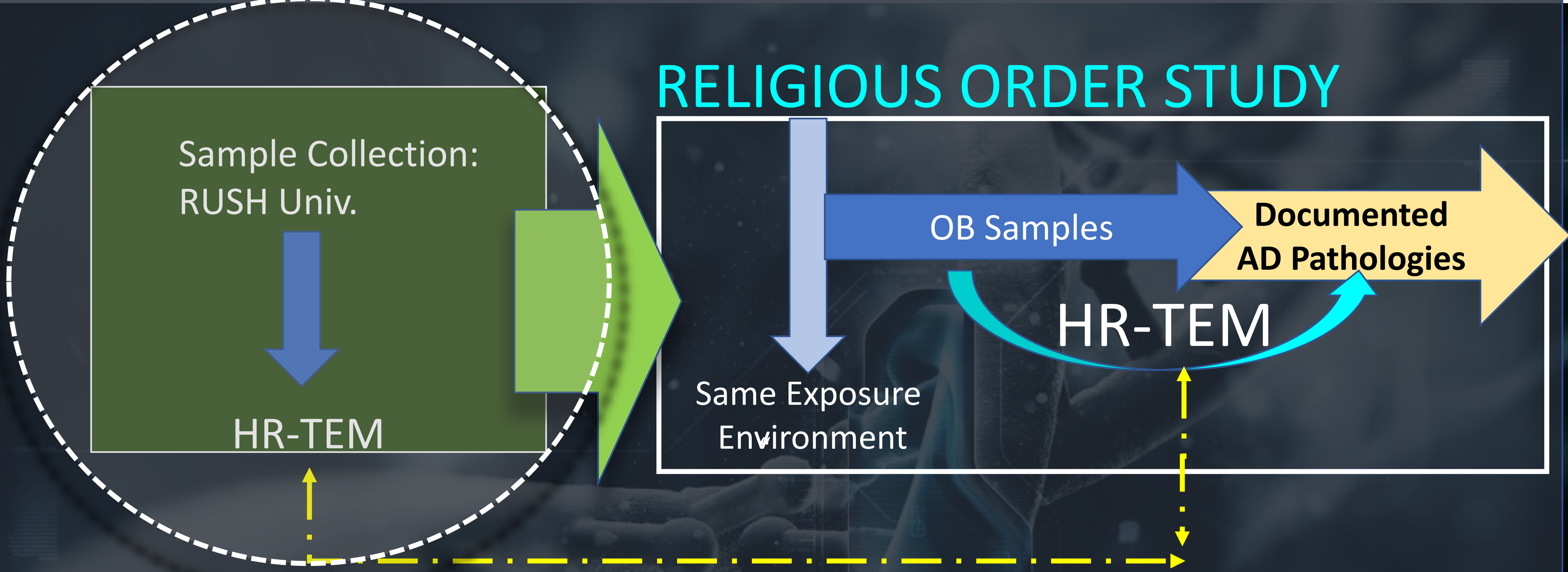


Funding:
1R01AG067497-01

Air Pollution and Alzheimer's Dementia: Neuropathologic and Olfactory Mechanisms in Multi-Ethnic Longitudinal Cohorts

Epidemiology	Surgery	Neurology	Pathology	Nanotoxicology	Nanotechnology
J Weuve	**JJ Pinto**	**DA Bennett**	**J Schneider**	**G Oberdörster**	**UM Graham**

"Nanoscale Analyses of Particulate Matter Air Pollution in the Human Olfactory Bulb"

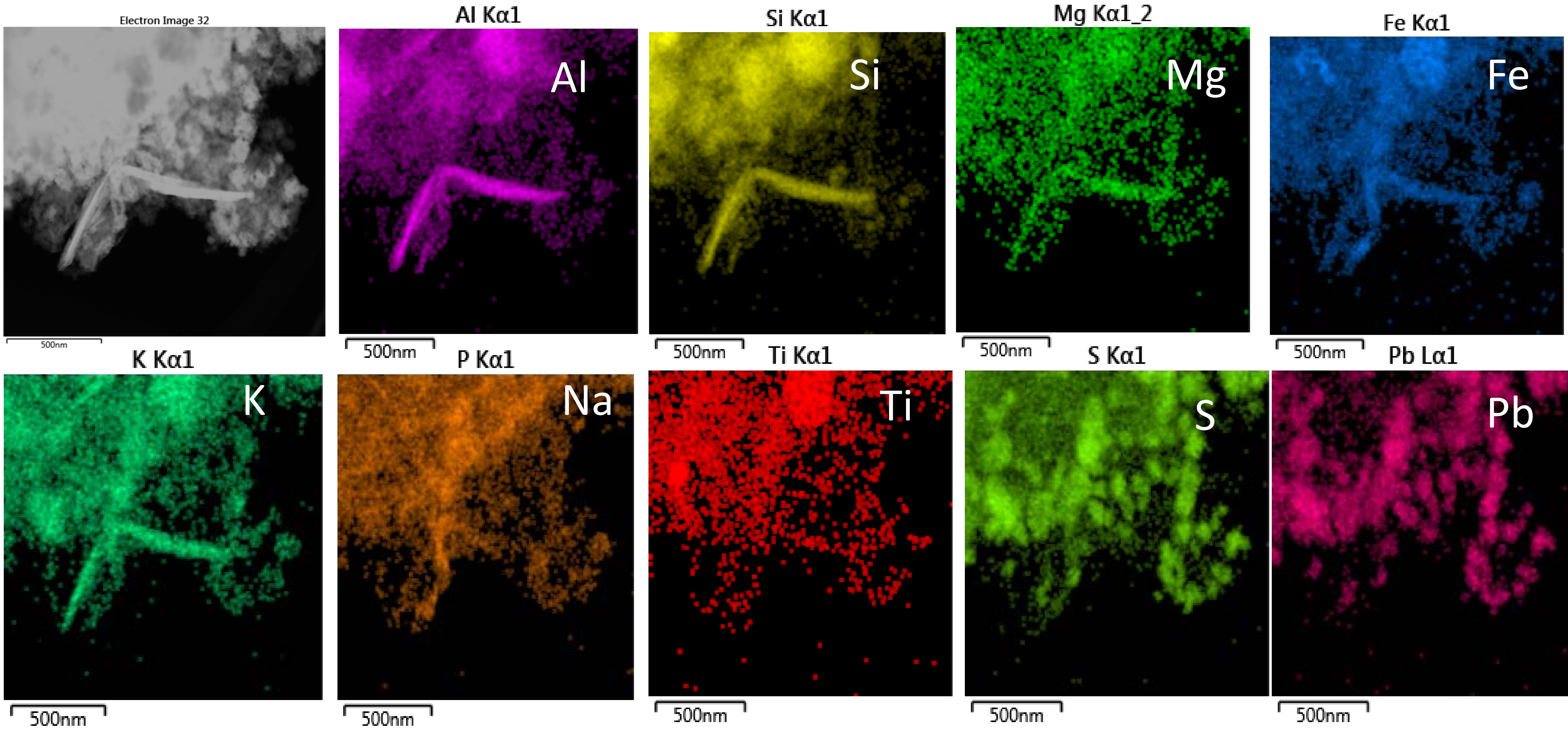


Environmental PM in OB linked to AD?

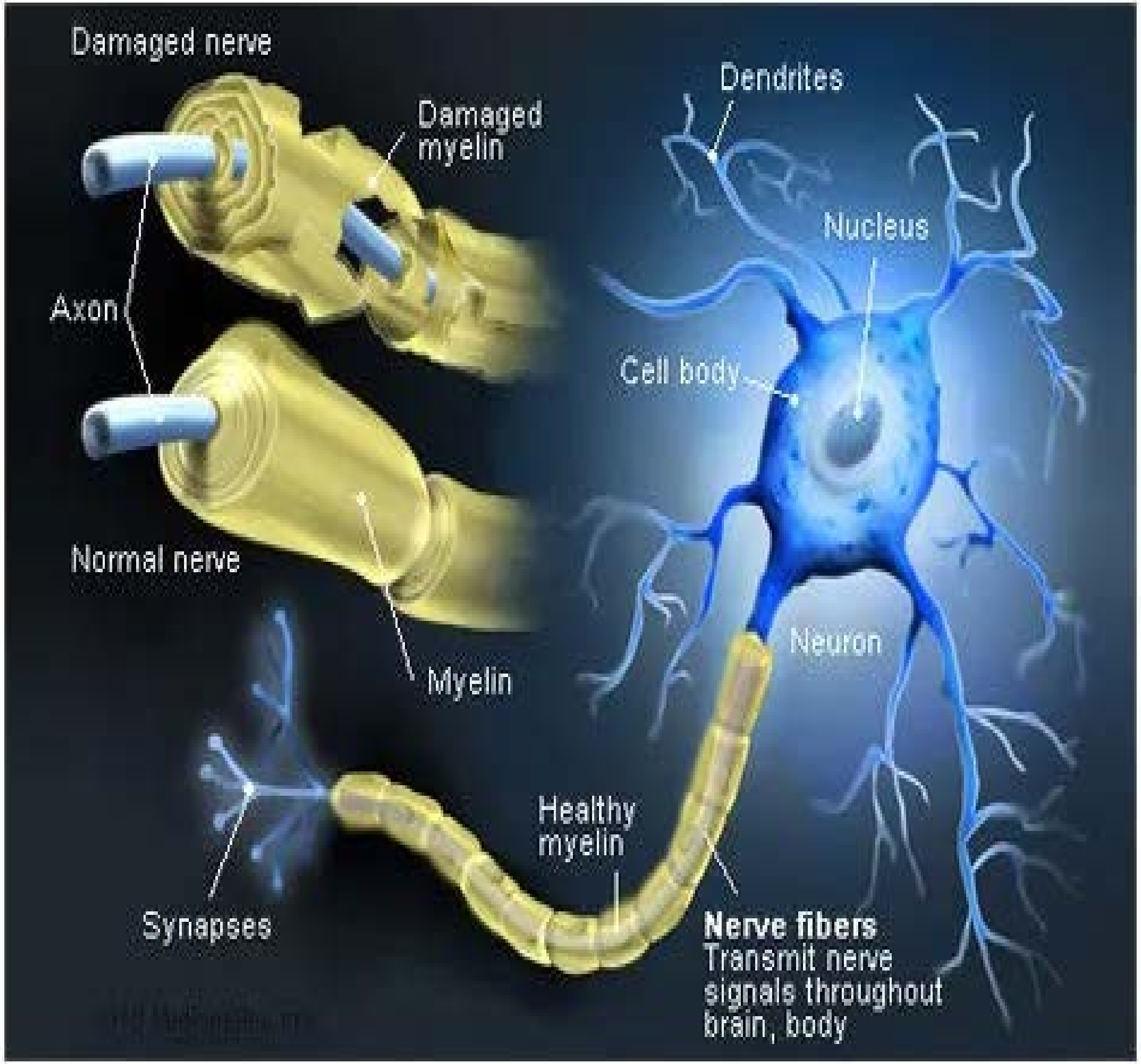
Religious Order Study and Rush Memory and Aging Project

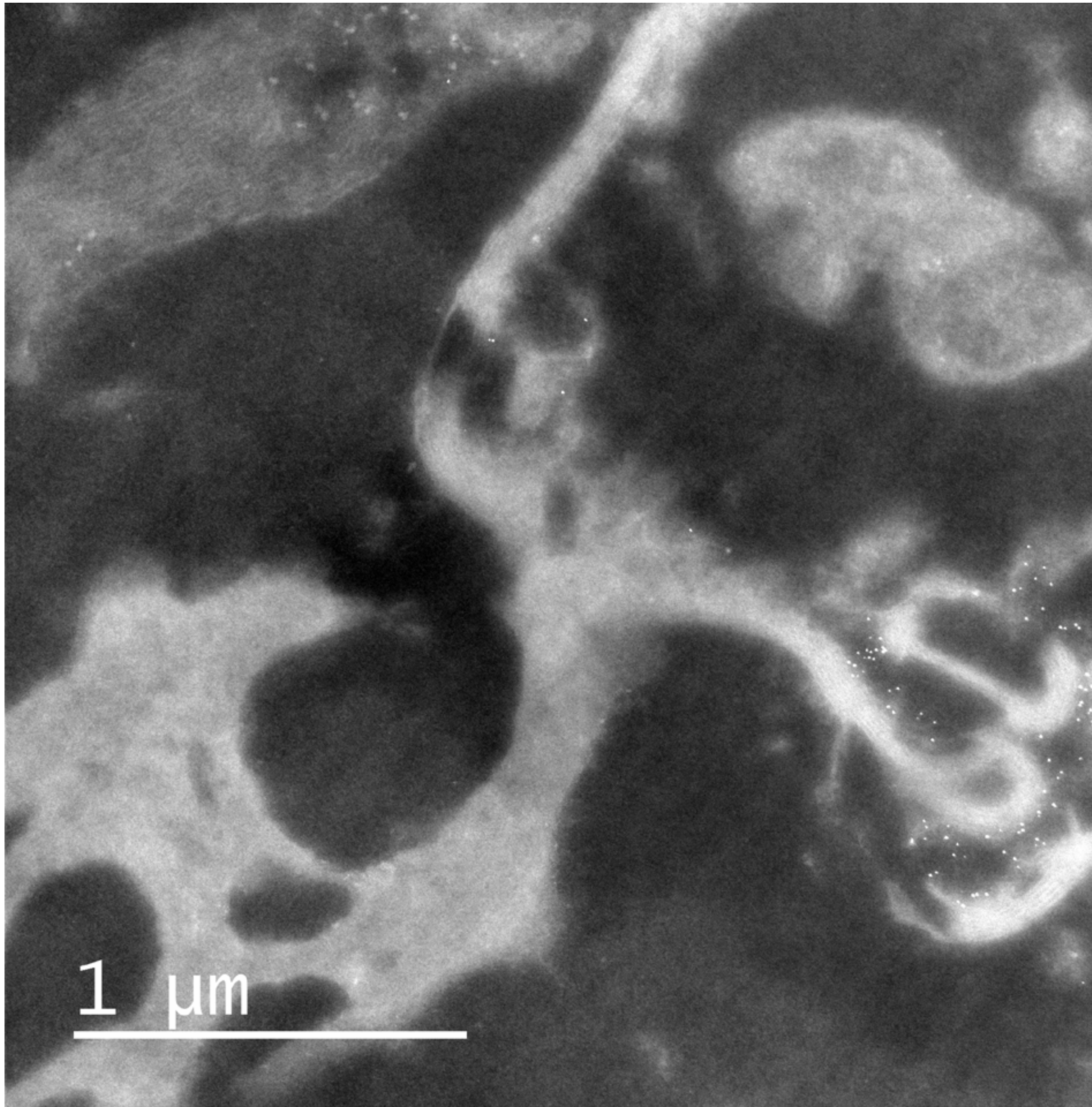
Subjects with different progression of AD.

Environmental Sample:



Nanoparticle Uptake to the Olfactory Tract: Observed damaged myelin sheets!





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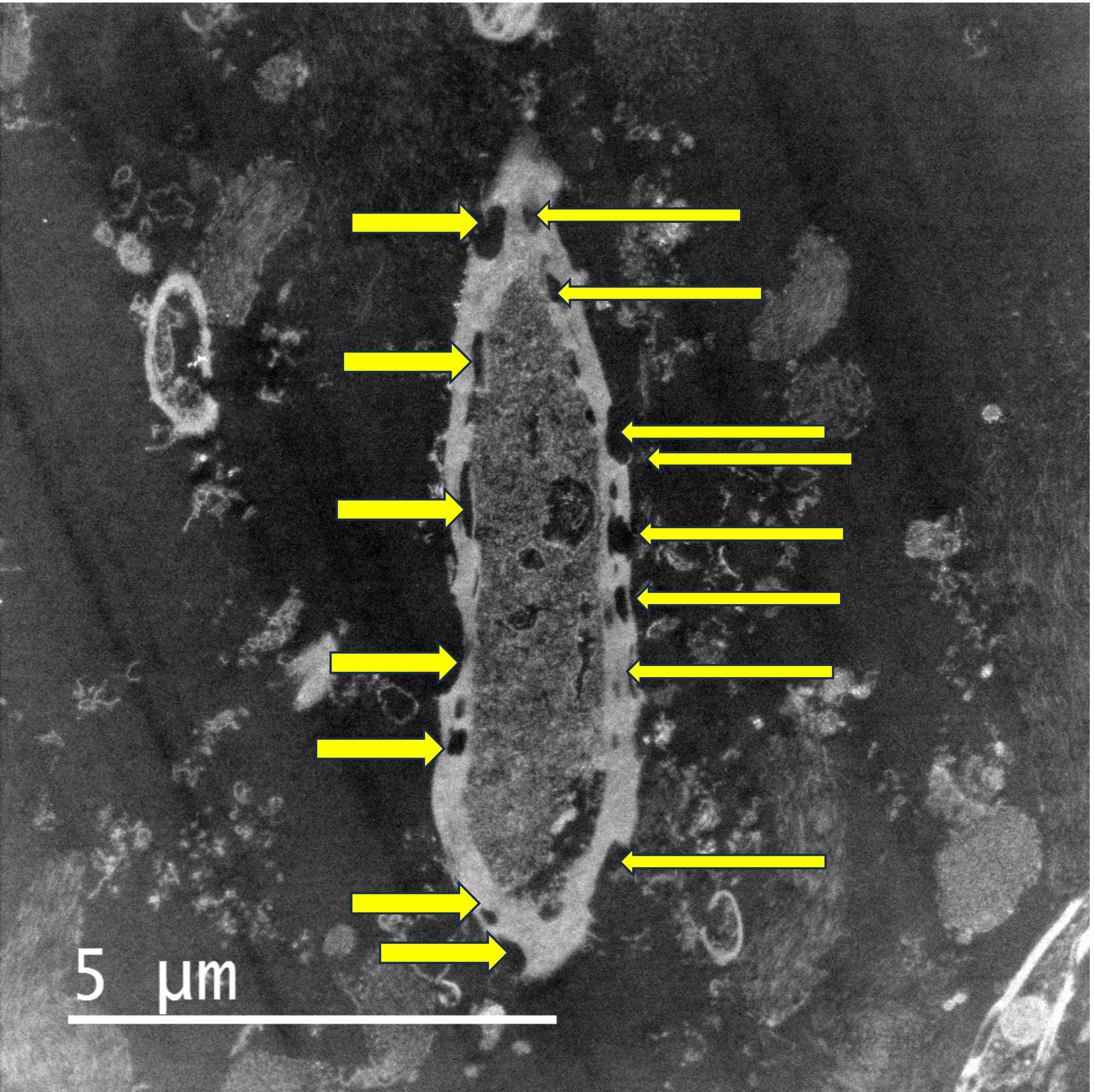
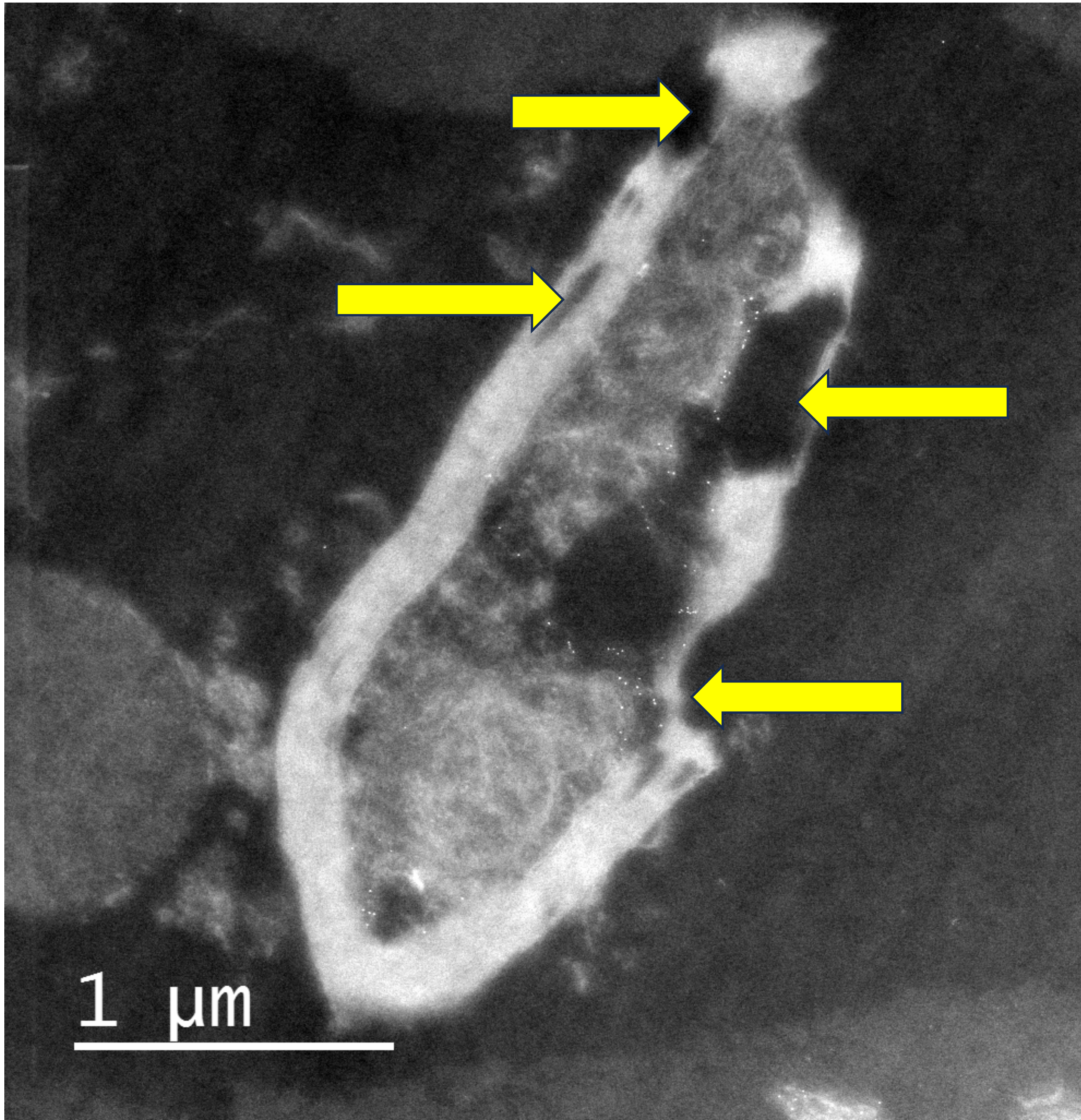
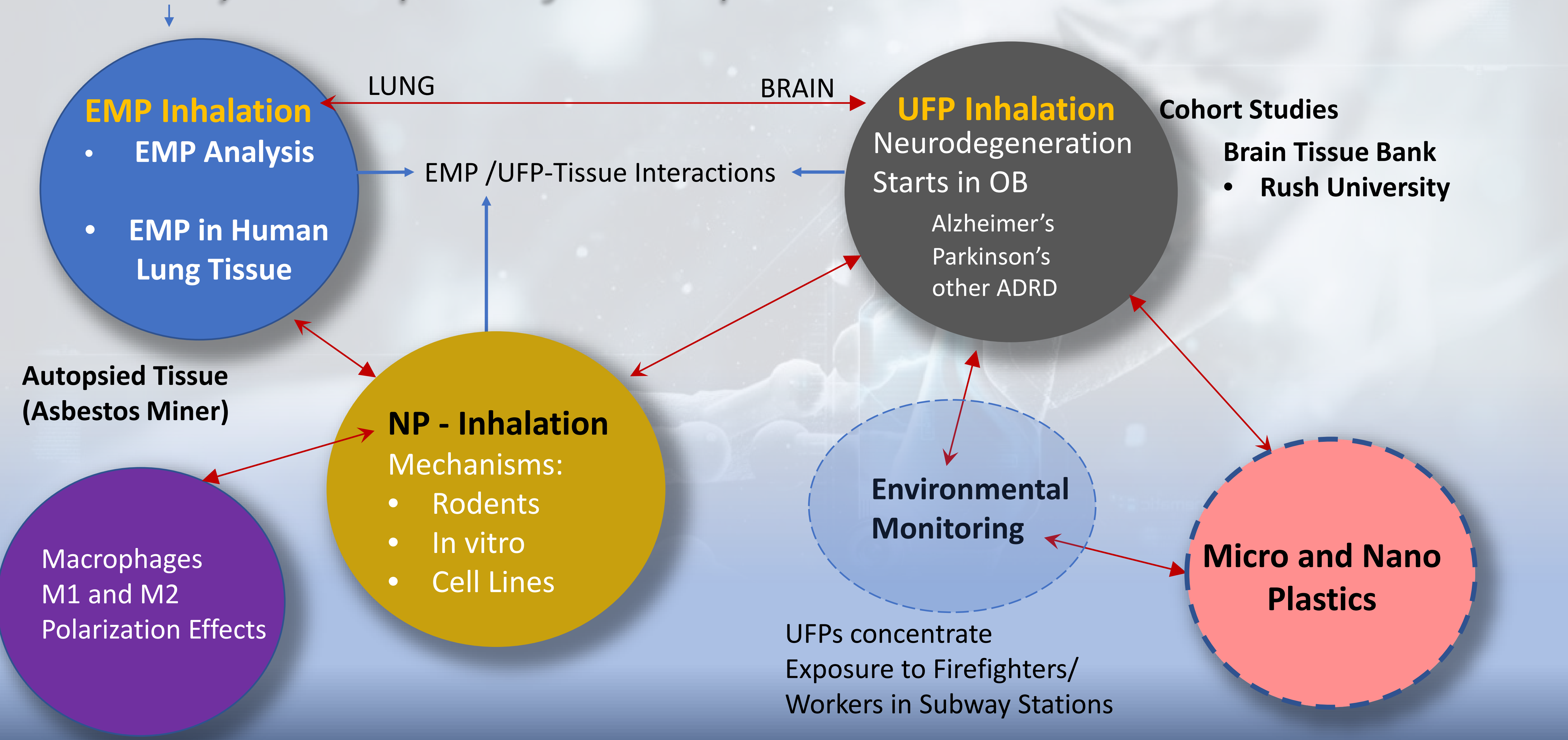


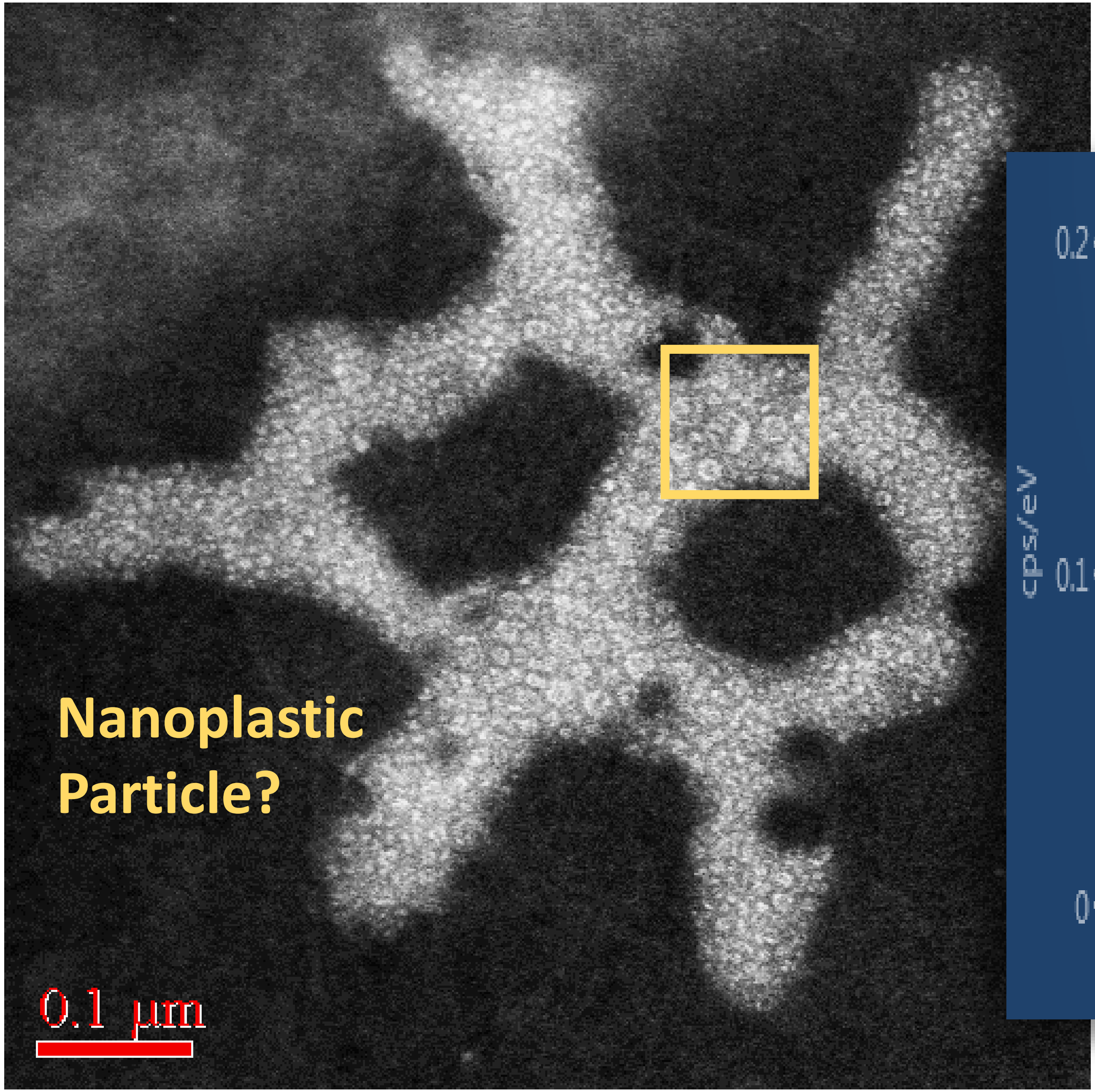


Study Examples of Nanoparticle Tissue Interactions

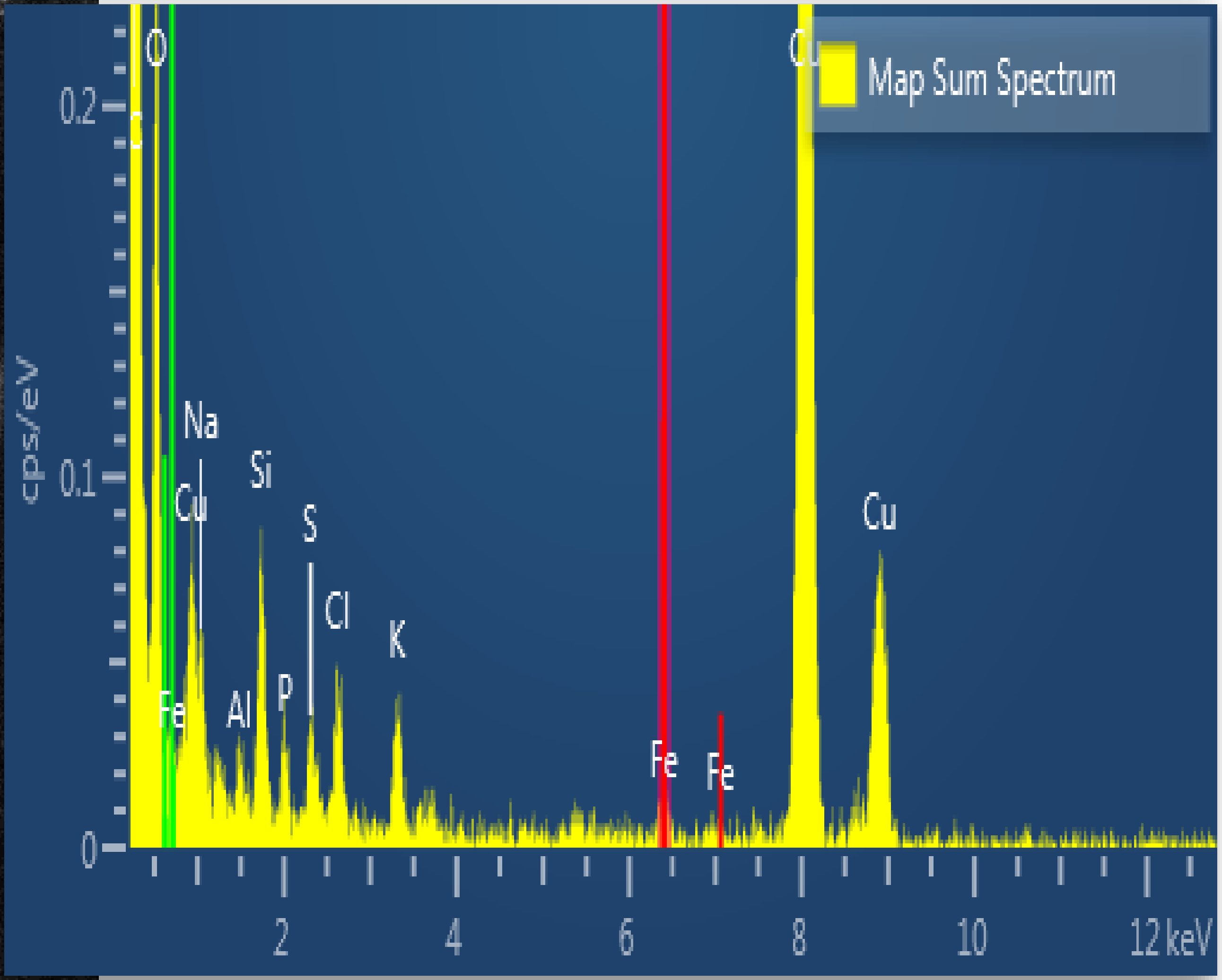


Analytical High-Resolution Imaging of Tissues performed at NIOSH (Cincinnati)

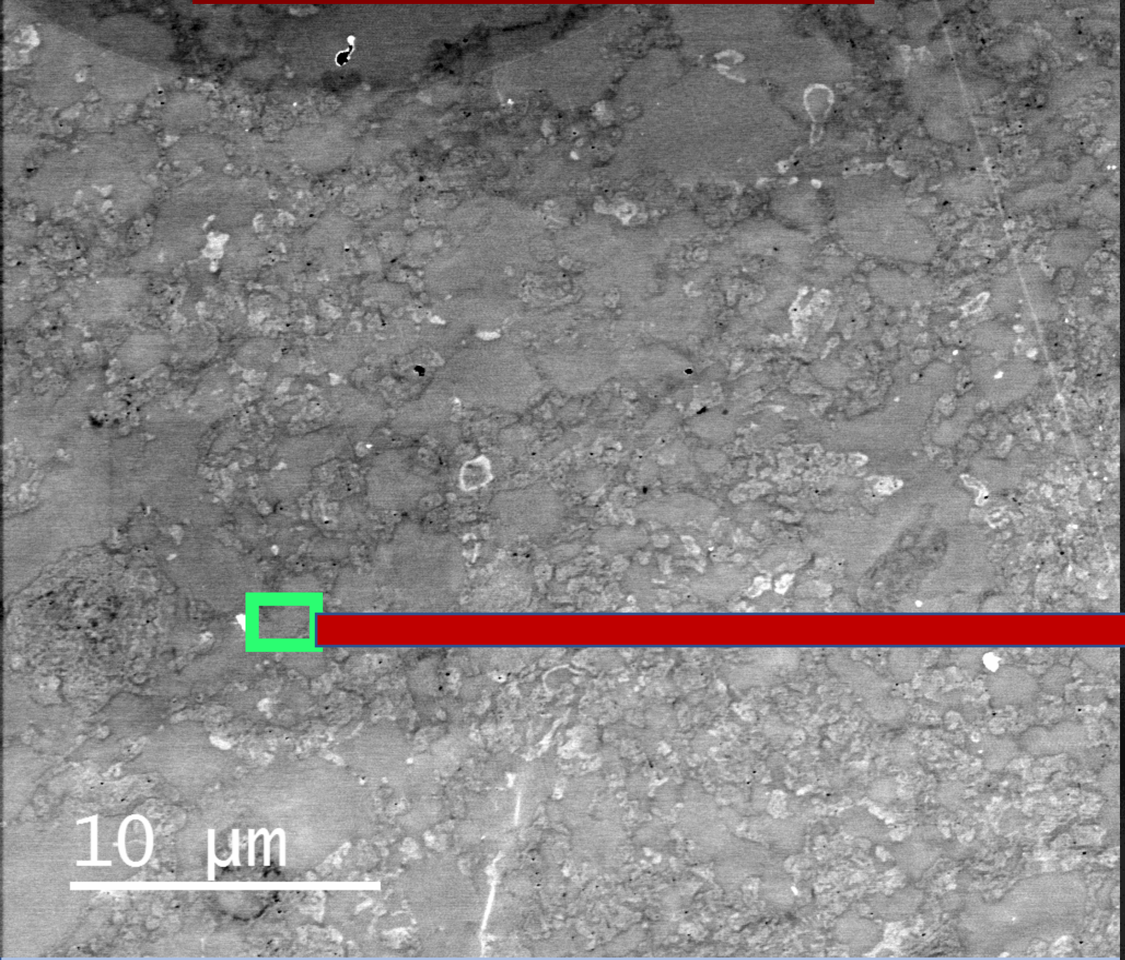
OB Sample: 10459674 H1



Trojan Horse –Mechanism:

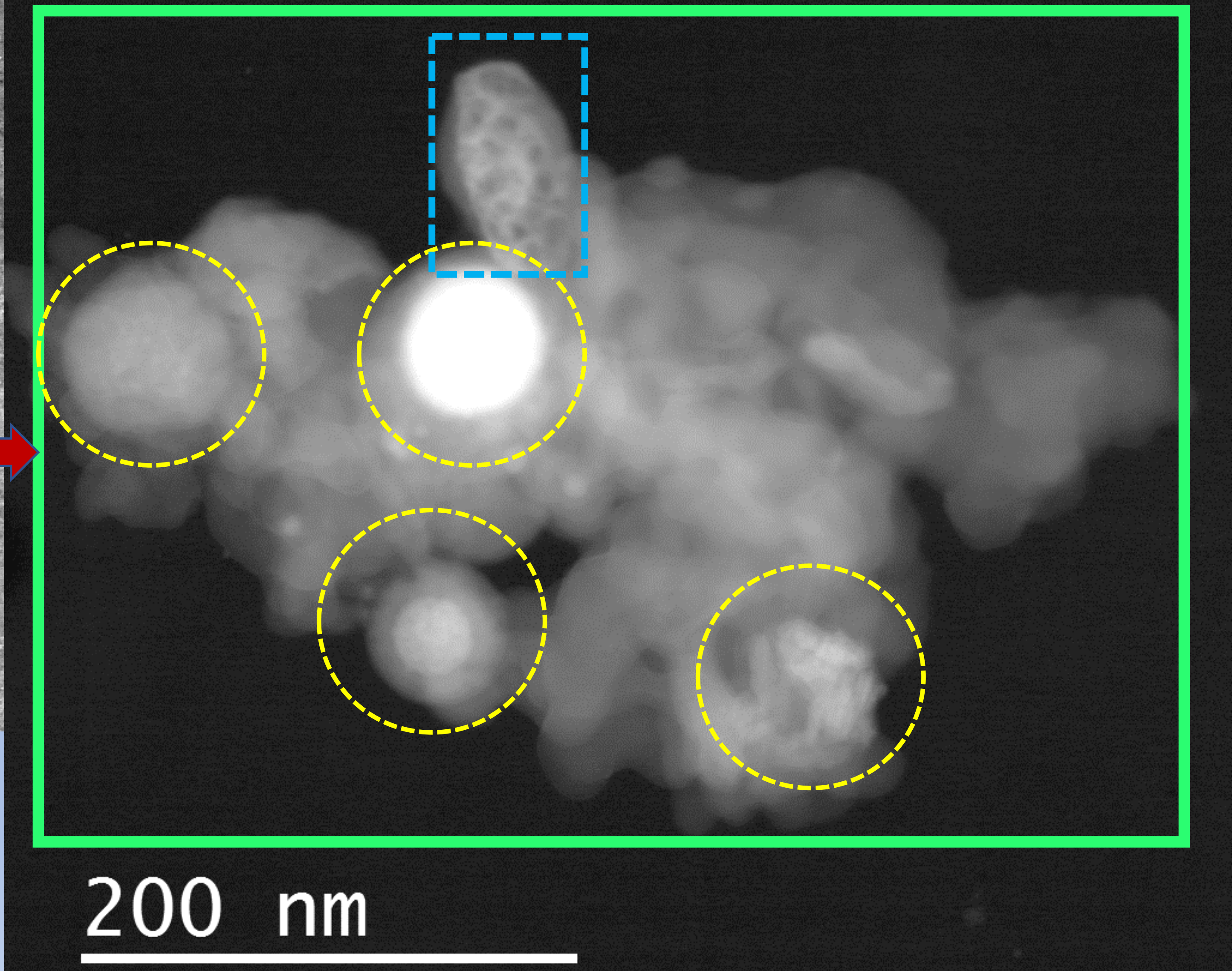


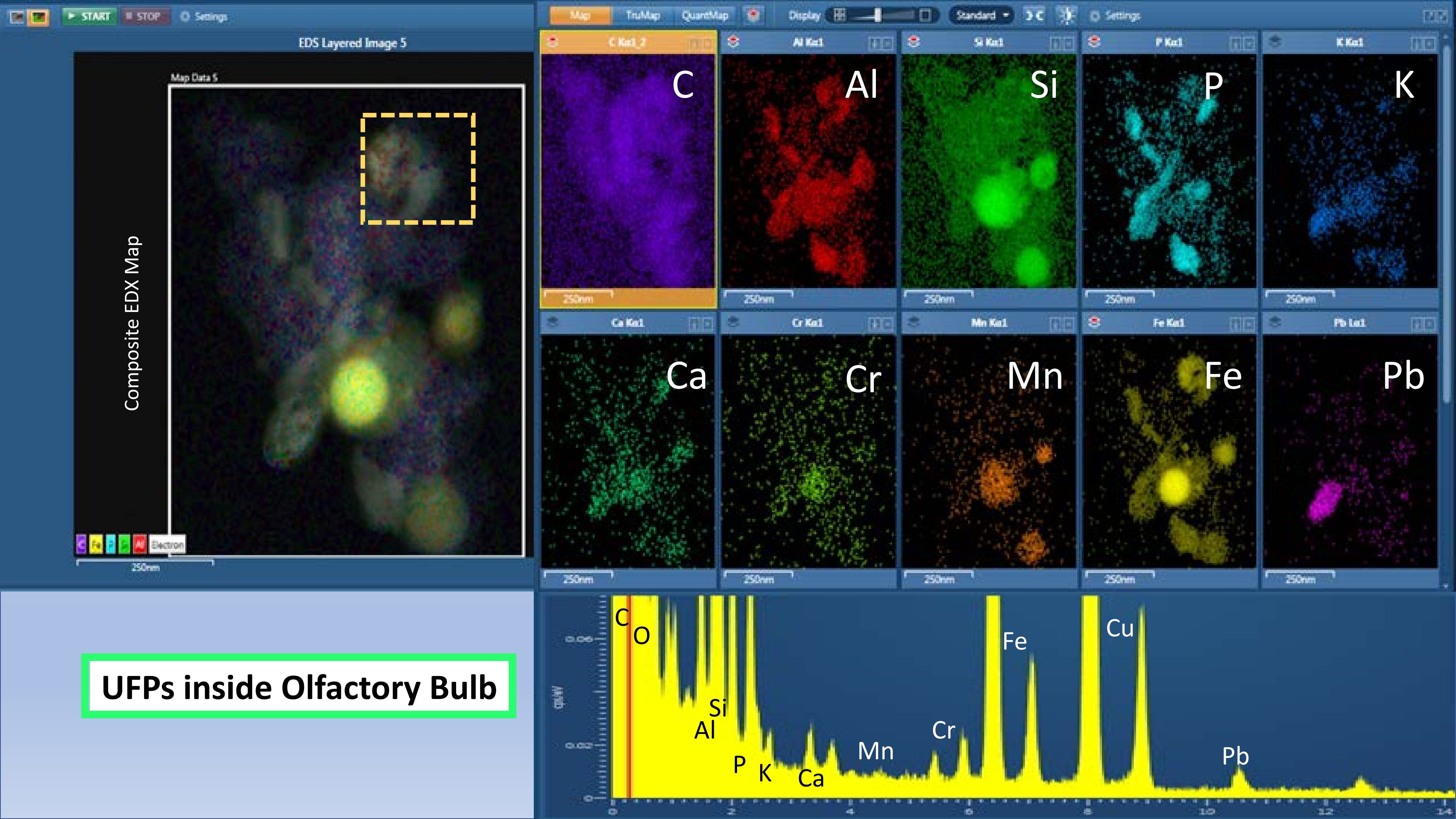
OLFACTORY BULB



UFPs inside Olfactory Bulb

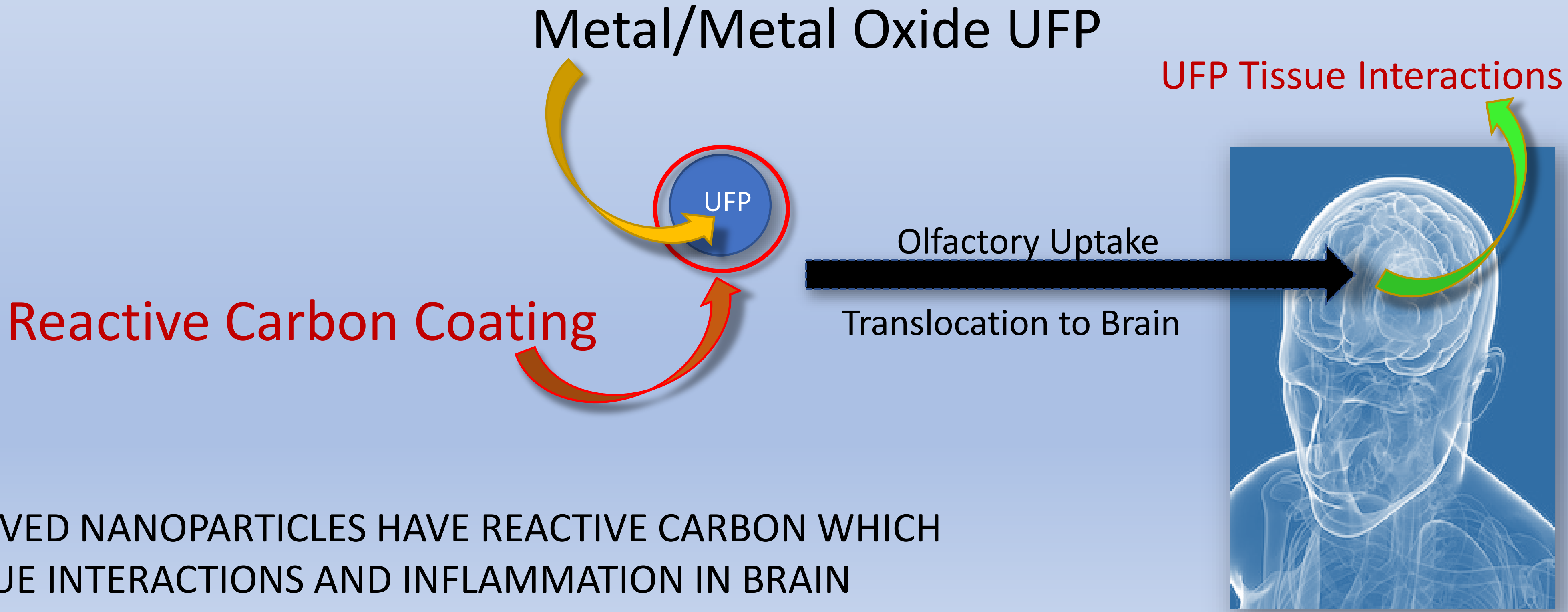
Combustion Particles "Spheres" with carbon Coatings



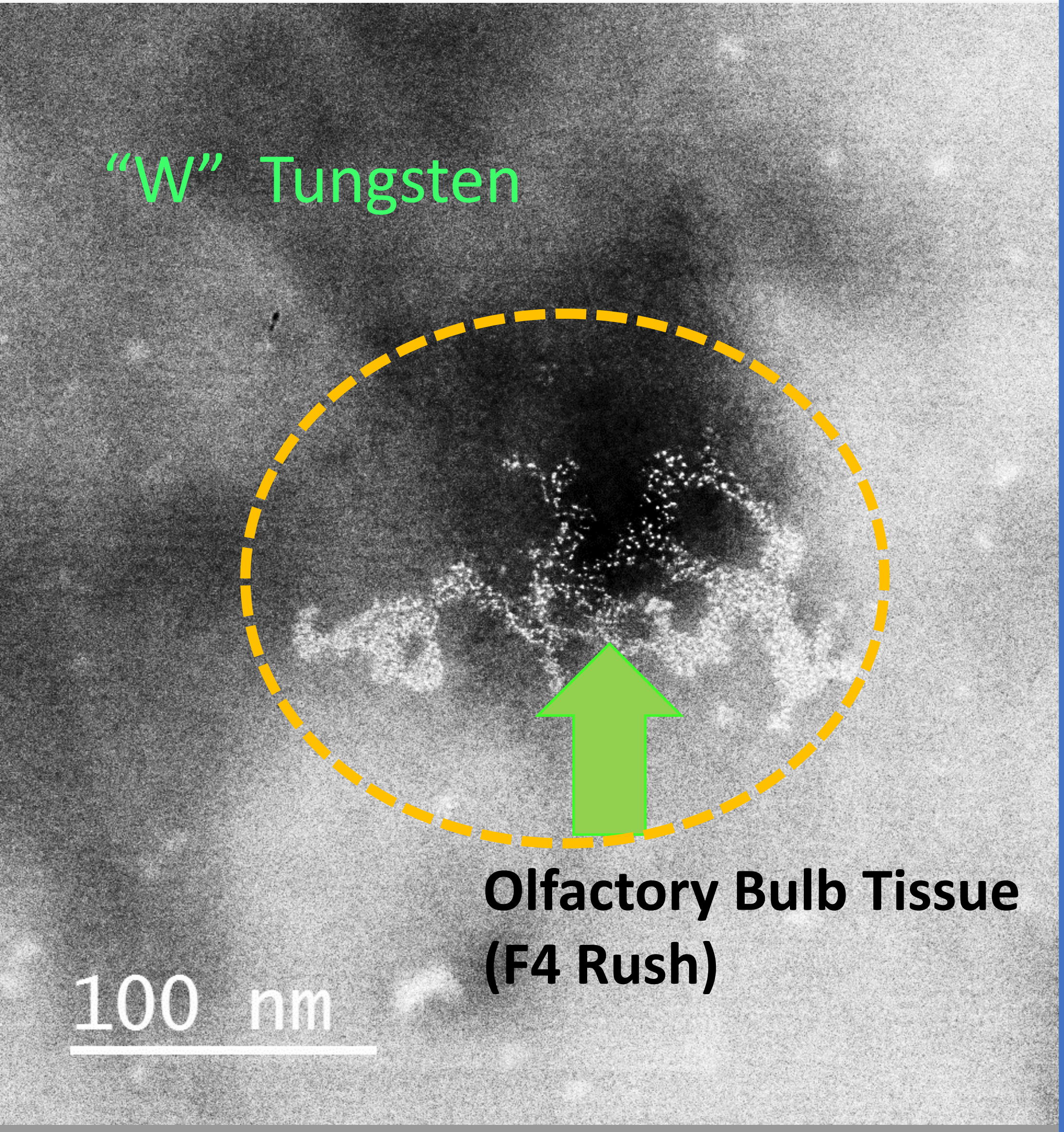

START
STOP
Settings
EDS Layered Image 5
Map Data 5
Composite EDX Map
Electron
250nm
Map
TruMap
QuantMap
Display
Standard
Settings
C Kα1_2
Al Kα1
Si Kα1
P Kα1
K Kα1
Ca Kα1
Cr Kα1
Mn Kα1
Fe Kα1
Pb Lα1
C
Al
Si
P
K
Ca
Cr
Mn
Fe
Pb
UFPs inside Olfactory Bulb
C
O
Al
Si
P
K
Ca
Mn
Cr
Fe
Cu
Pb

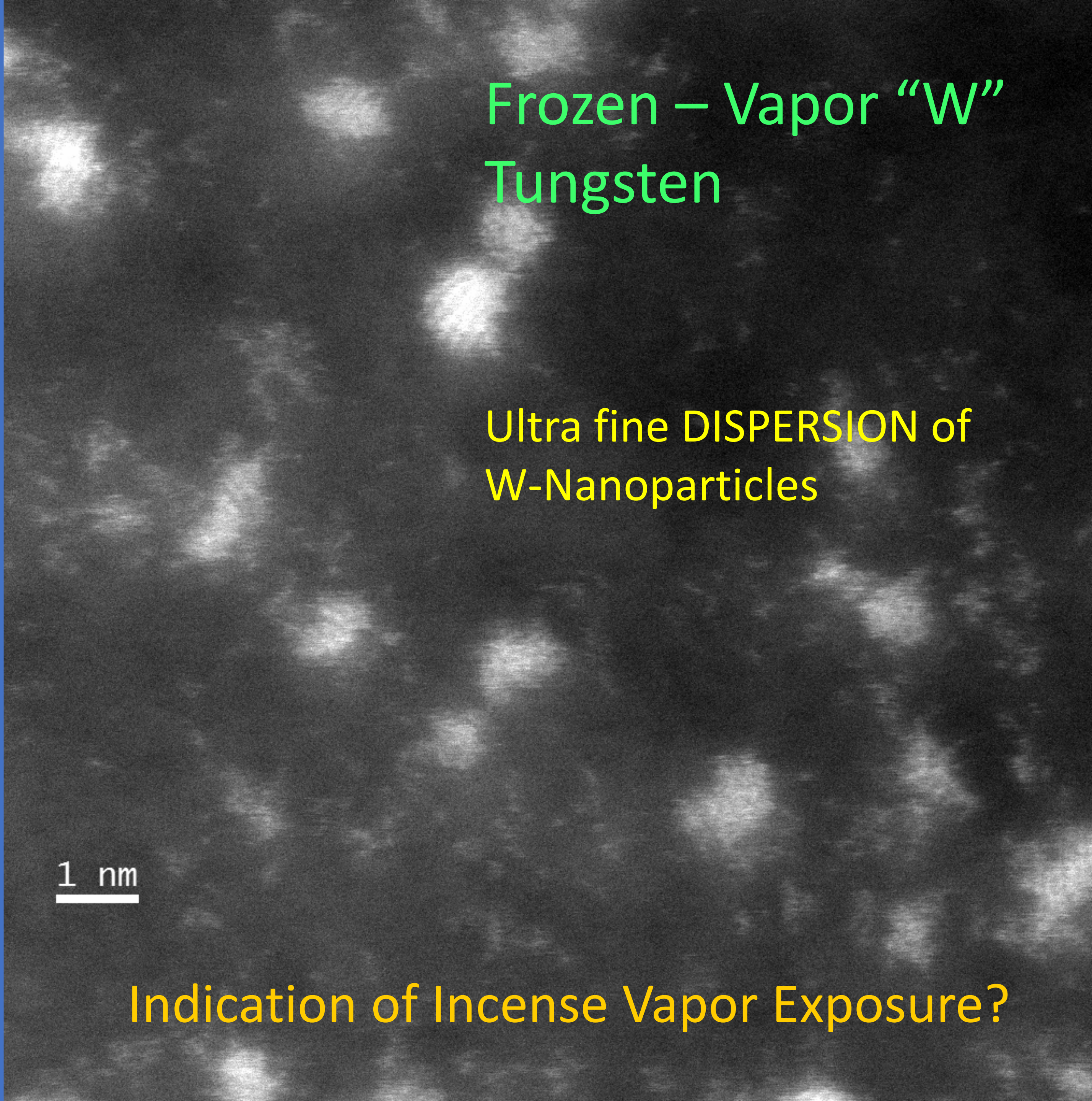
NEW DISCOVERY

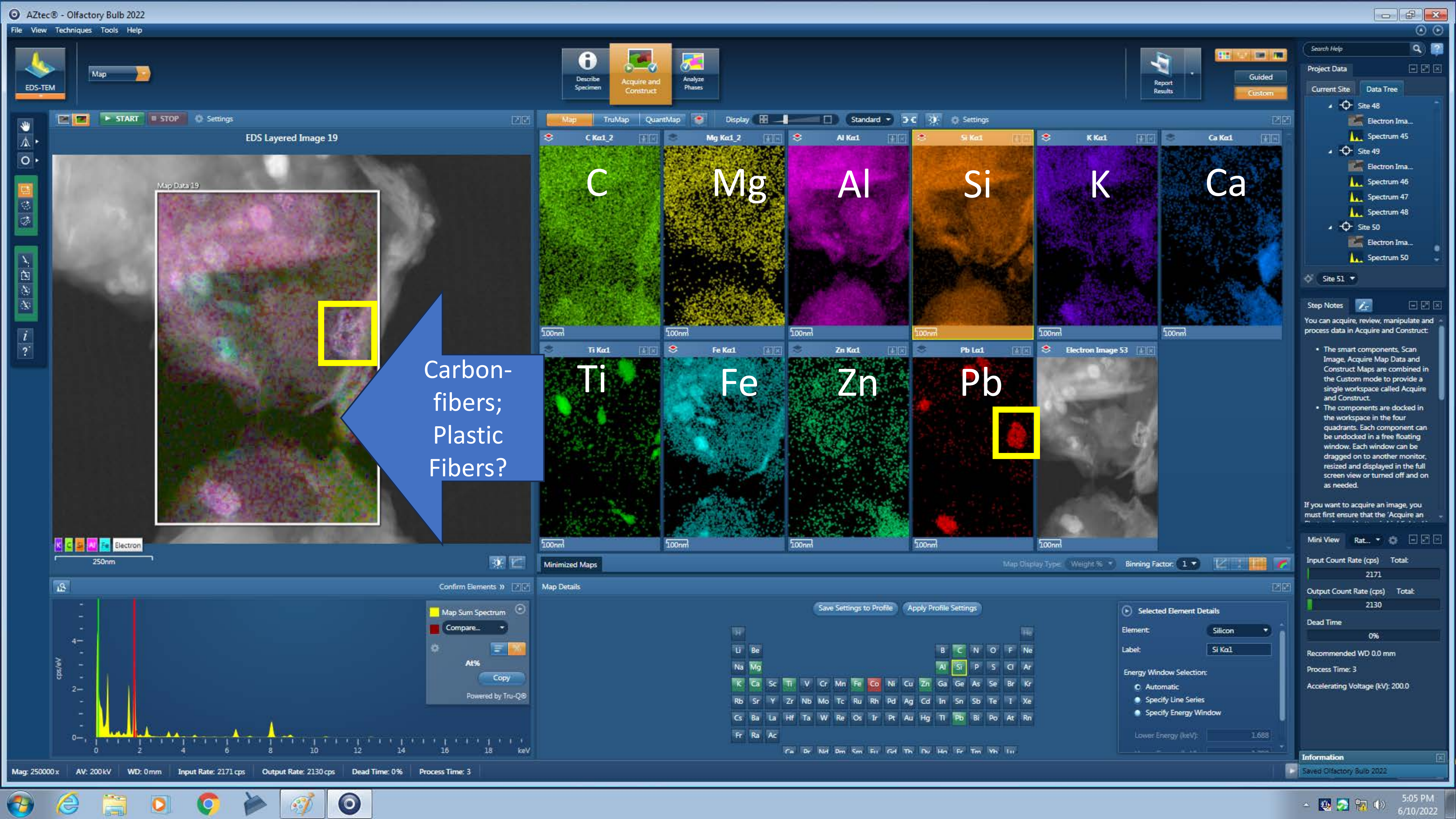
We show for the first time the presence of carbon coatings on the surfaces of UFPs that translocated to the Human Olfactory Bulb (Cohort Subject with Neurodegeneration).

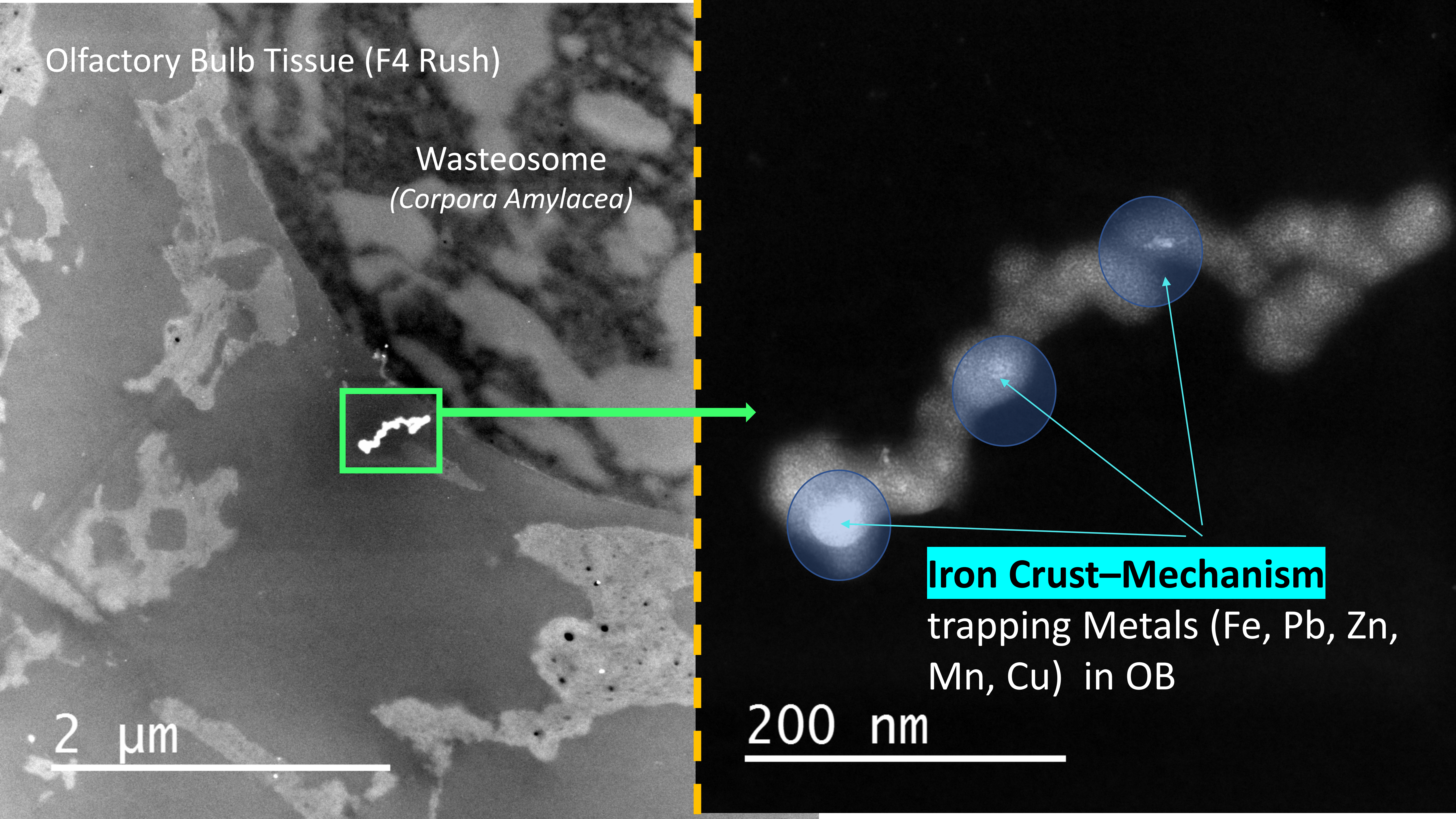


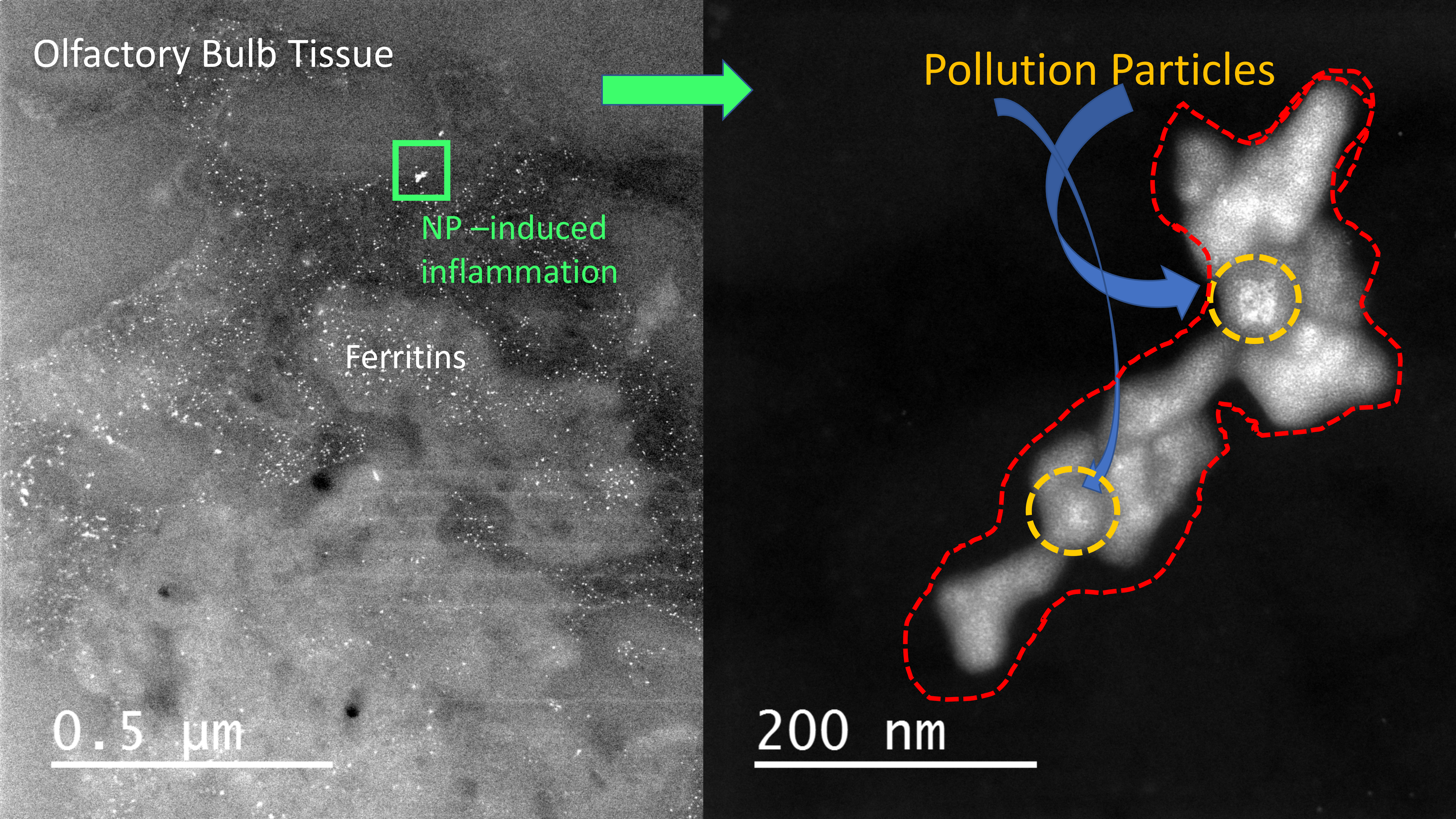
COMBUSTION DERIVED NANOPARTICLES HAVE REACTIVE CARBON WHICH LIKELY AFFECT TISSUE INTERACTIONS AND INFLAMMATION IN BRAIN


"W" Tungsten
Olfactory Bulb Tissue
(F4 Rush)
100 nm

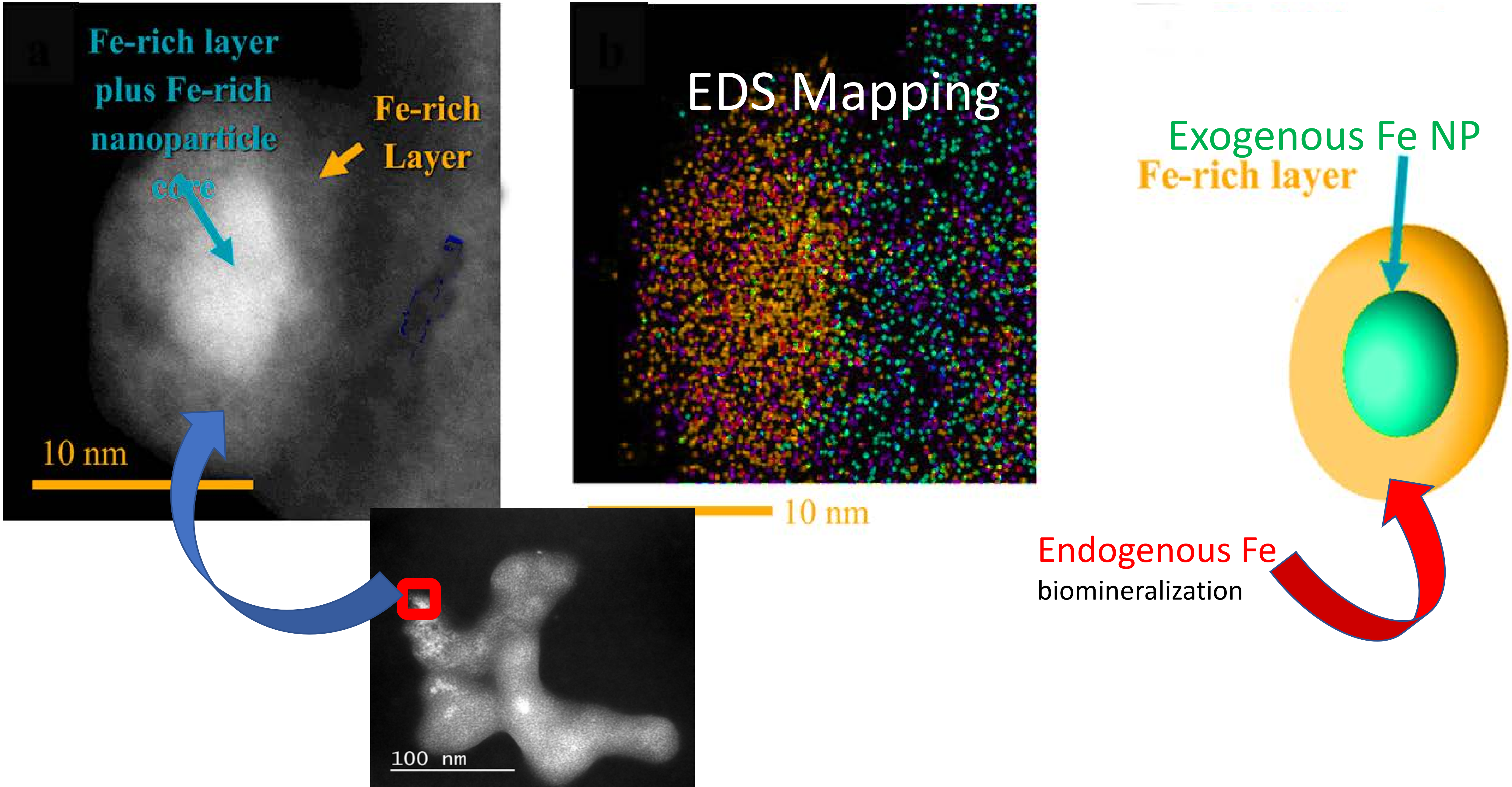

Frozen – Vapor "W"
Tungsten
Ultra fine DISPERSION of
W-Nanoparticles
1 nm
Indication of Incense Vapor Exposure?

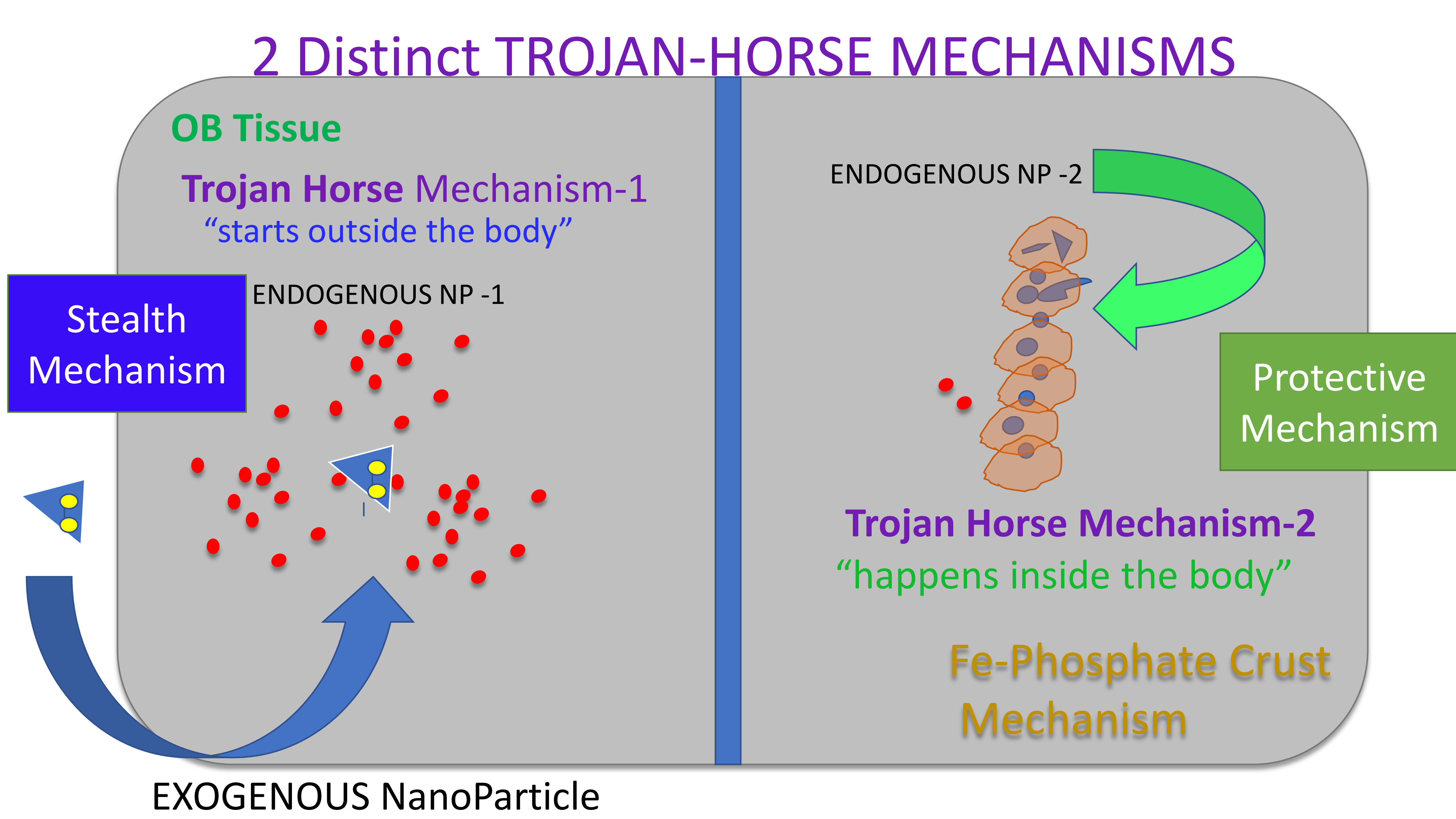

AZtec® - Olfactory Bulb 2022
File View Techniques Tools Help
EDS-TEM
Map
Describe Specimen
Acquire and Construct
Analyze Phases
Report Results
Guided
Custom
Project Data
Current Site
Data Tree
START
STOP
Settings
EDS Layered Image 19
Map Data 19
Carbon-fibers; Plastic Fibers?
250nm
Map
TruMap
QuantMap
Display
Standard
C Kα1_2
Mg Kα1_2
Al Kα1
Si Kα1
K Kα1
Ca Kα1
C
Mg
Al
Si
K
Ca
100nm
Ti Kα1
Fe Kα1
Zn Kα1
Pb Lα1
Electron Image 53
Ti
Fe
Zn
Pb
Minimized Maps
Binning Factor: 1
Confirm Elements
Map Details
Map Sum Spectrum
Compare...
At%
Copy
Powered by Tru-Q®
Save Settings to Profile
Apply Profile Settings
Selected Element Details
Element: Silicon
Label: Si Kα1
Energy Window Selection:
Automatic
Specify Line Series
Specify Energy Window
Lower Energy (keV): 1.688
keV
Step Notes
You can acquire, review, manipulate and process data in Acquire and Construct.
The smart components, Scan Image, Acquire Map Data and Construct Maps are combined in the Custom mode to provide a single workspace called Acquire and Construct.
The components are docked in the workspace in the four quadrants. Each component can be undocked in a free floating window. Each window can be dragged on to another monitor, resized and displayed in the full screen view or turned off and on as needed.
If you want to acquire an image, you must first ensure that the 'Acquire an
Mini View
Input Count Rate (cps) Total: 2171
Output Count Rate (cps) Total: 2130
Dead Time 0%
Recommended WD 0.0 mm
Process Time: 3
Accelerating Voltage (kV): 200.0
Information
Saved Olfactory Bulb 2022
Mag: 250000 x AV: 200 kV WD: 0 mm Input Rate: 2171 cps Output Rate: 2130 cps Dead Time: 0% Process Time: 3
5:05 PM
6/10/2022


Olfactory Bulb Tissue (F4 Rush)
Wasteosome
(Corpora Amylacea)
2 µm
Iron Crust–Mechanism
trapping Metals (Fe, Pb, Zn, Mn, Cu) in OB
200 nm

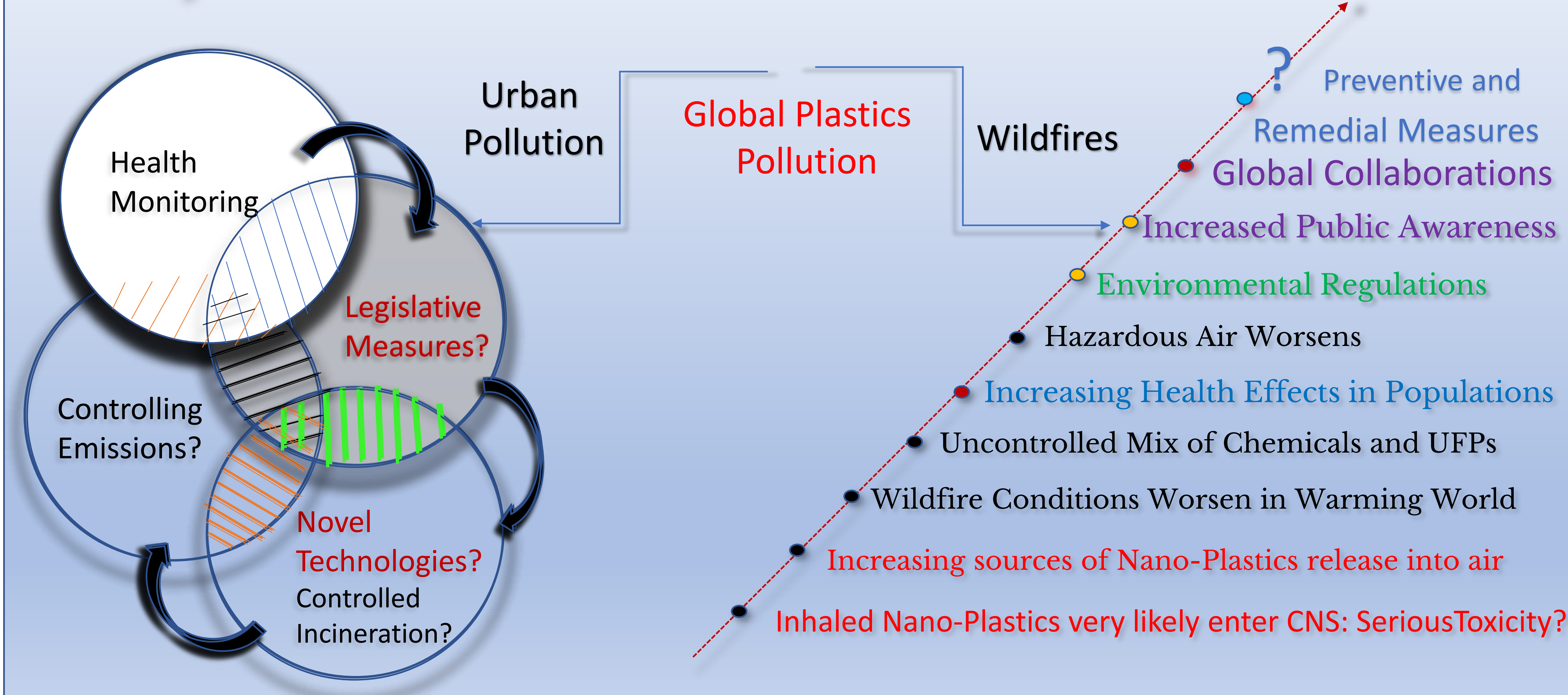

Olfactory Bulb Tissue
NP –induced
inflammation
Ferritins
0.5 μm
Pollution Particles
200 nm

Fe- Phosphate “crusts” around Fe-rich NP in Olfactory Bulb

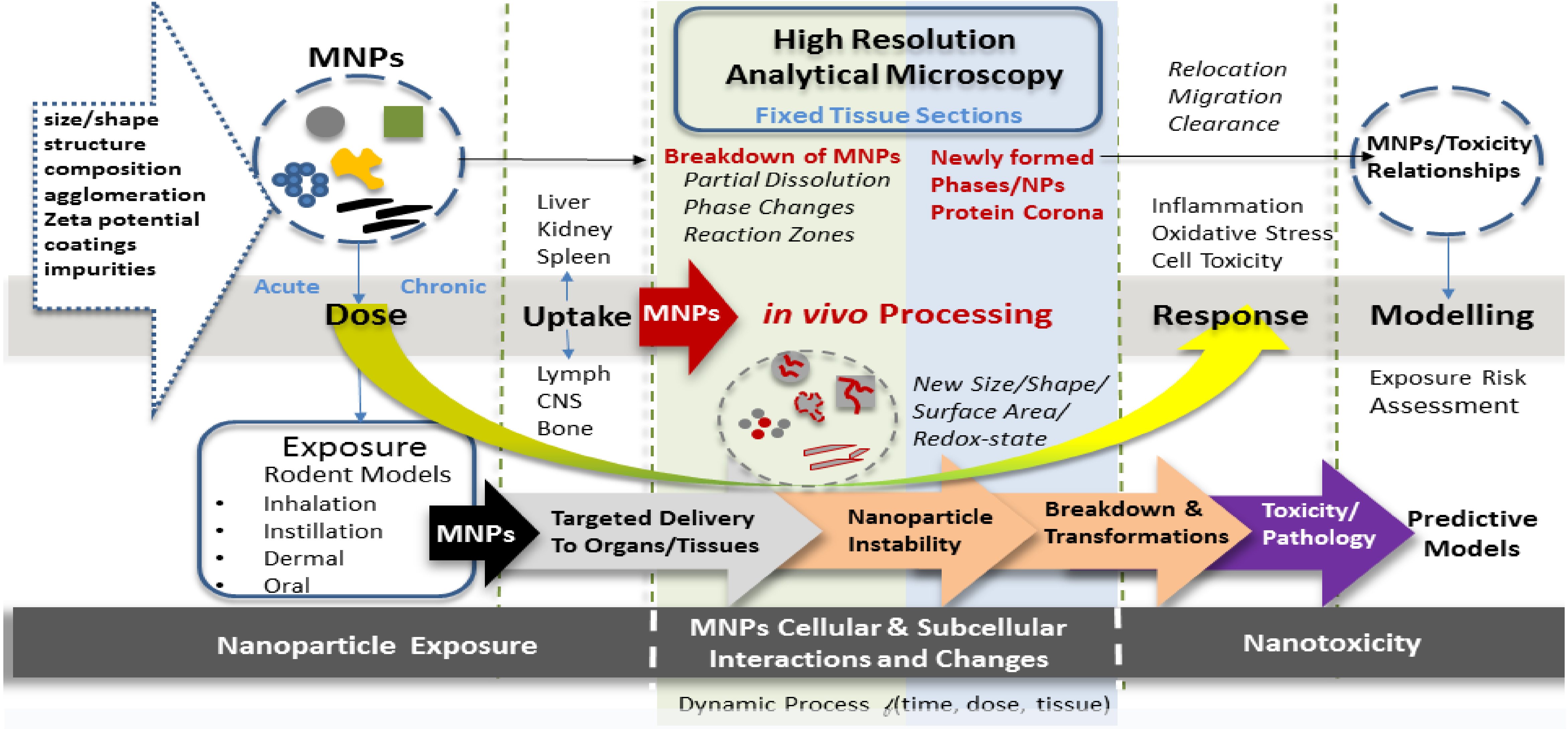



2 Distinct TROJAN-HORSE MECHANISMS
OB Tissue
Trojan Horse Mechanism-1
“starts outside the body”
Stealth Mechanism
ENDOGENOUS NP -1
EXOGENOUS NanoParticle
ENDOGENOUS NP -2
Protective Mechanism
Trojan Horse Mechanism-2
“happens inside the body”
Fe-Phosphate Crust Mechanism

Inhaled particles will deposit throughout the respiratory tract as portal of entry for distributing to secondary organs, including the brain if particles are nanosized



Reserve Figures



HYPOTHESIS:

Nanoparticle –induced inflammation will trigger a defense response involving in vivo FERRITIN NPS

SUMMARY: Nanoscale Analyses of Particulate Matter Air Pollution in the Human Olfactory Bulb

- In vivo processing of translocated UFP (and NANO PLASTICS) involves Fe-phosphate crust formation.
- Fe-phosphate crusts are amorphous and form at the exterior of UFP (including Nano Plastics) utilizing endogenous Fe source.

- Fe-phosphate crust formation mechanism is same for UFP of different composition.
- **DOES ASSOCIATION MEAN CAUSALITY?** Abundance of Ferritin NP (*acute phase protein*) and Fe-phosphate "crusts" due to Fe- biomineralization are indicators of inflammation in OB.

•Need for studying UFP (including NANO PLASTICS) in OB – and enrichment of Fe and P in context with histopathology and molecular biology mechanisms.

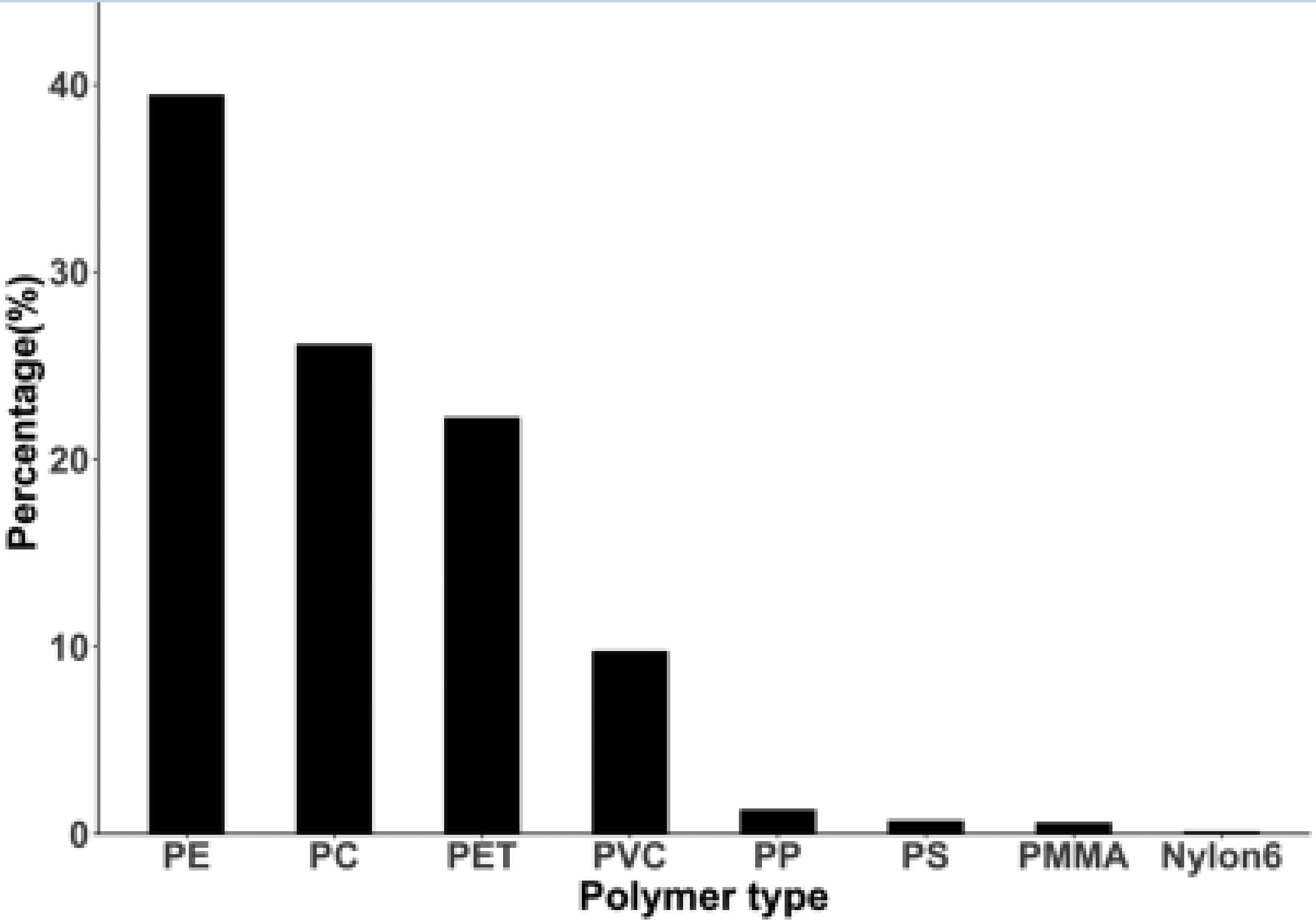


Figure 7. Polymer types observed in atmospheric MP samples displayed as the percentage of total polymer mass detected.

Fan et al, 2022