

THE MATRIX IS ALWAYS COMPLEX!

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<http://www.ucd.ie/cbni/>

Mapping protein binding sites on the biomolecular corona of nanoparticles

Philip M. Kelly, Christoffer Åberg, Ester Polo, Ann O'Connell, Jennifer Cookman, Jonathan Fallon, Željka Krpetić* and Kenneth A. Dawson*

Nanoparticles in a biological milieu are known to form a sufficiently long-lived and well-organized 'corona' of biomolecules to confer a biological identity to the particle. Because this nanoparticle–biomolecule complex interacts with cells and biological barriers, potentially engaging with different biological pathways, it is important to clarify the presentation of functional biomolecular motifs at its interface. Here, we demonstrate that by using antibody-labelled gold nanoparticles, differential centrifugal sedimentation and various imaging techniques it is possible to identify the spatial location of proteins, their functional motifs and their binding sites. We show that for transferrin-coated polystyrene nanoparticles only a minority of adsorbed proteins exhibit functional motifs and the spatial organization appears random, which is consistent, overall, with a stochastic and irreversible adsorption process. Our methods are applicable to a wide array of nanoparticles and can offer a microscopic molecular description of the biological identity of nanoparticles.

The “Sweet” Side of the Protein Corona: Effects of Glycosylation on Nanoparticle–Cell Interactions

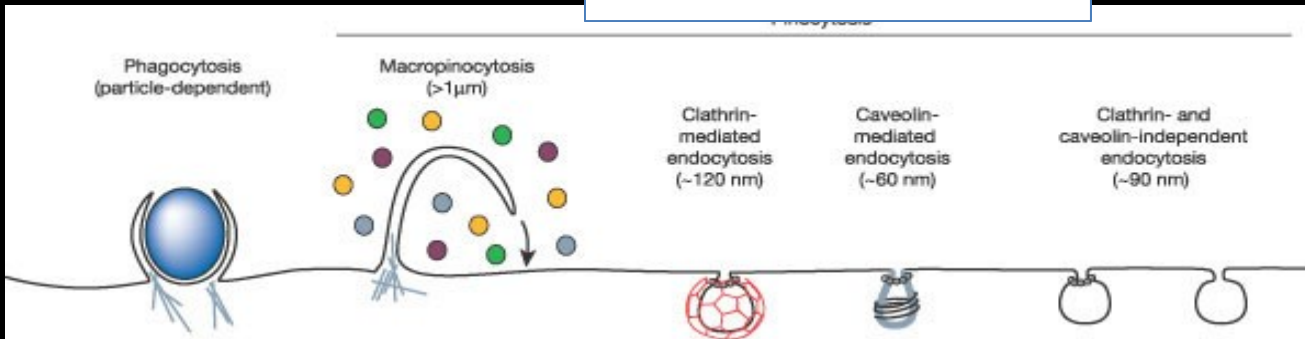
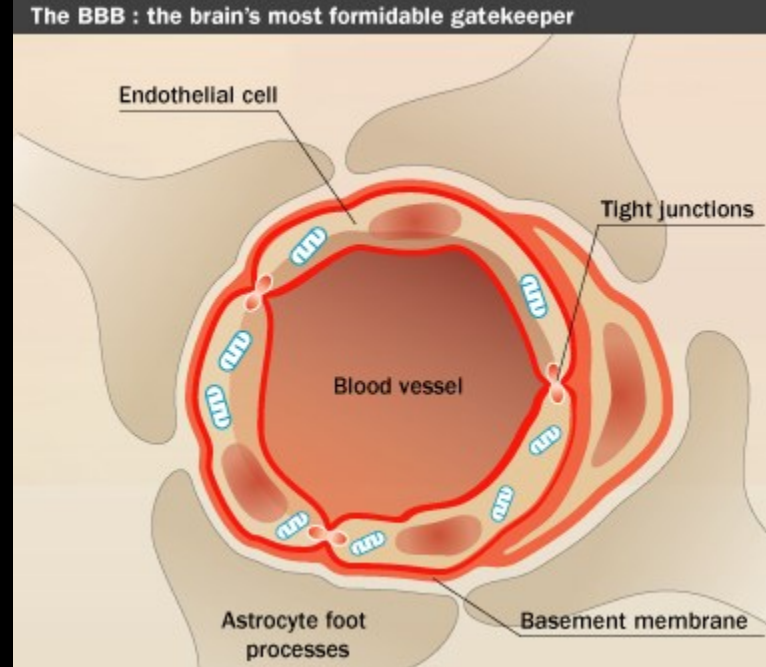
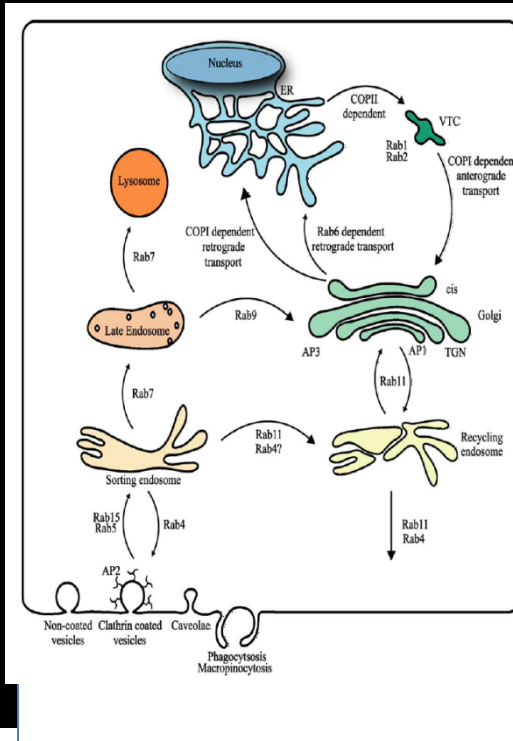
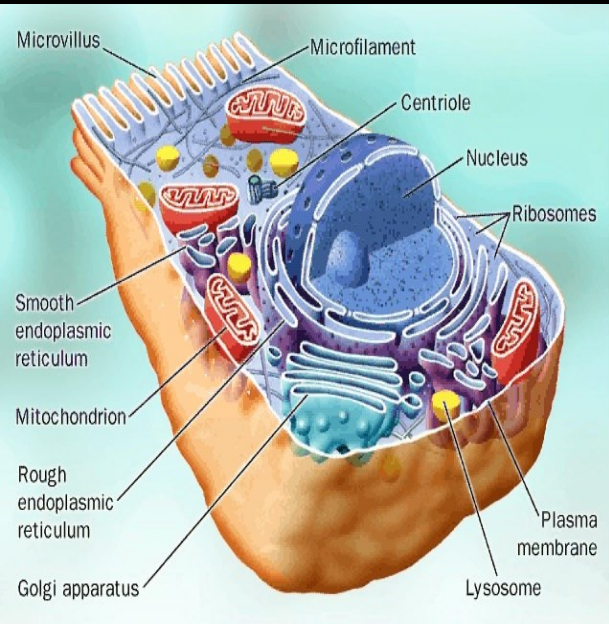
Sha Wan,[†] Philip M. Kelly,[†] Eugene Mahon,[†] Henning Stöckmann,[‡] Pauline M. Rudd,[‡] Frank Caruso,[§] Kenneth A. Dawson,[†] Yan Yan,^{*,§} and Marco P. Monopoli^{*,†}

[†]Centre for BioNano Interactions, School of Chemistry and Chemical Biology, University College Dublin, Dublin 4, Ireland, [‡]NIBRT, GlycoScience Group, NIBRT—The National Institute for Bioprocessing Research and Training, Fosters Avenue, Mount Merrion, Blackrock, Co. Dublin, Ireland, and [§]ARC Centre of Excellence in Convergent Bio-Nano Science and Technology, and the Department of Chemical and Biomolecular Engineering, The University of Melbourne, Parkville, Victoria 3010, Australia

NEW SCIENCE

Engineered Nanoscale written in our biology

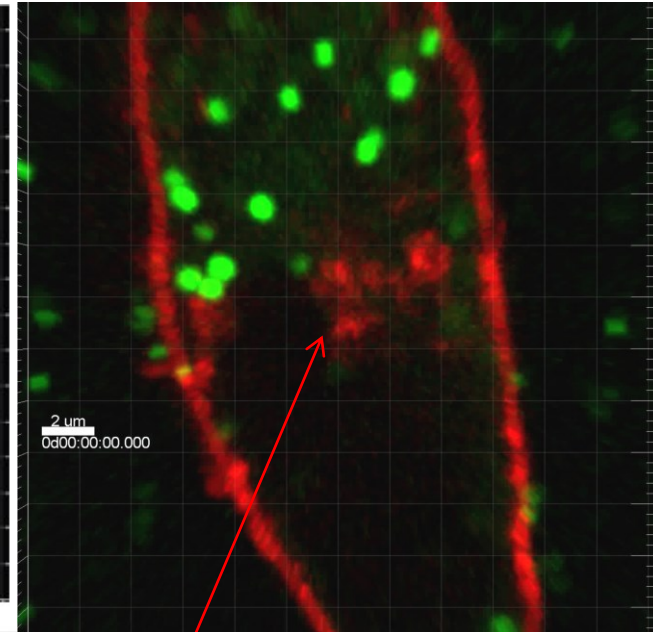
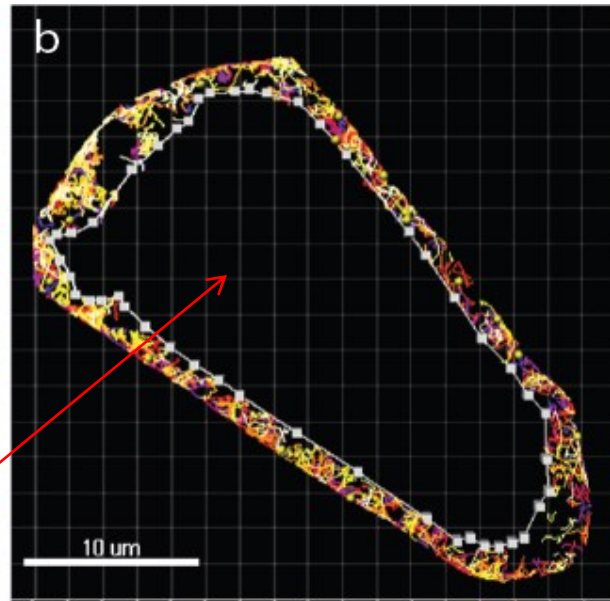
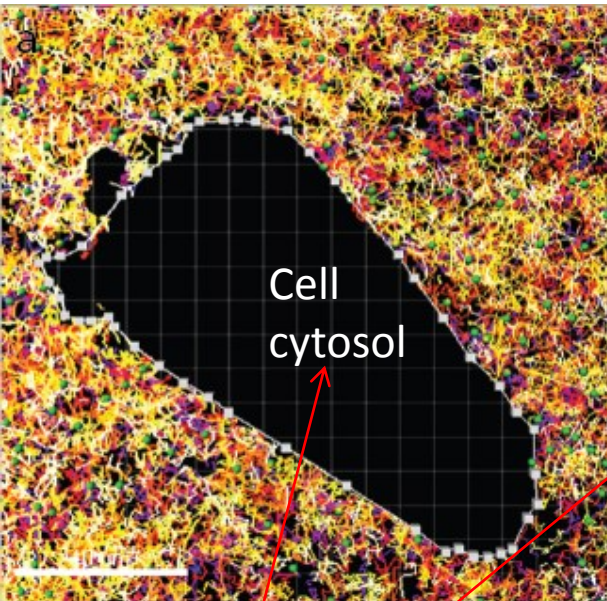
new medicine-new science; ADME Models will not work



Salvati et al.,
Nature Nanotech (2013)
Mahon et al.,
Nanoscale (2014)

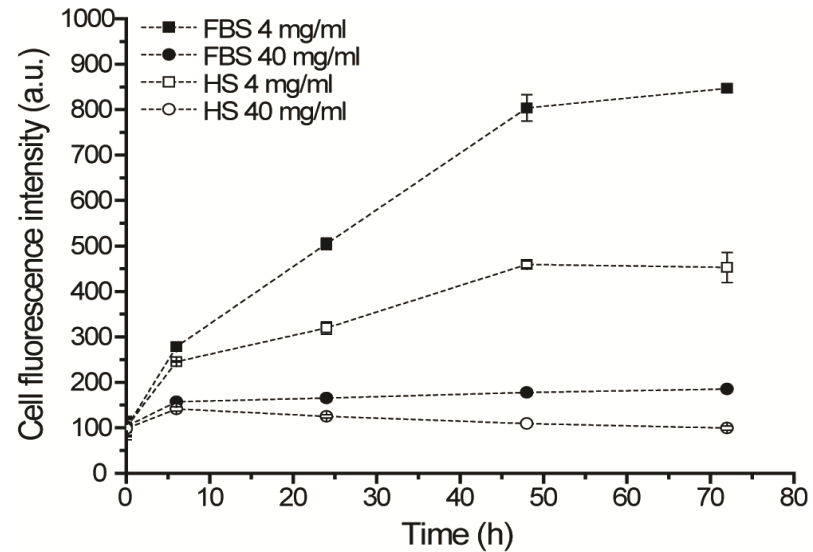
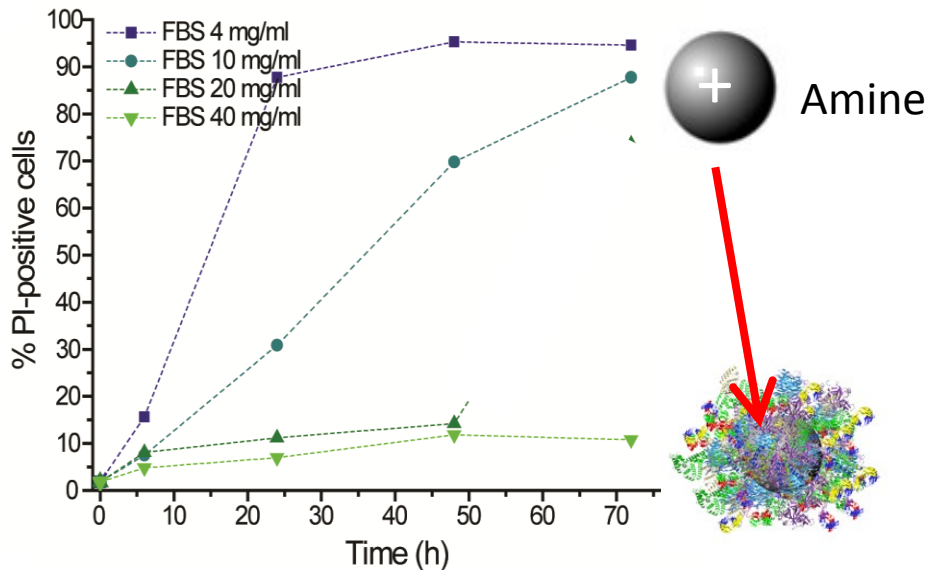
Chemicals Partition but Nanoparticles processed-energy of cell used

EARLY LIFE
DETERMINED
BY MILEU



Many particle trajectories
Most unsuccessful in
entering cell

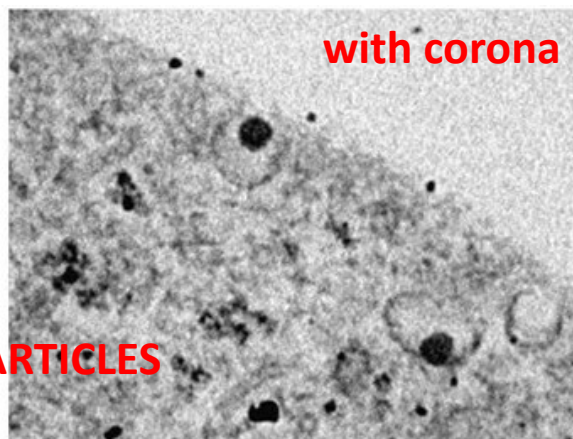
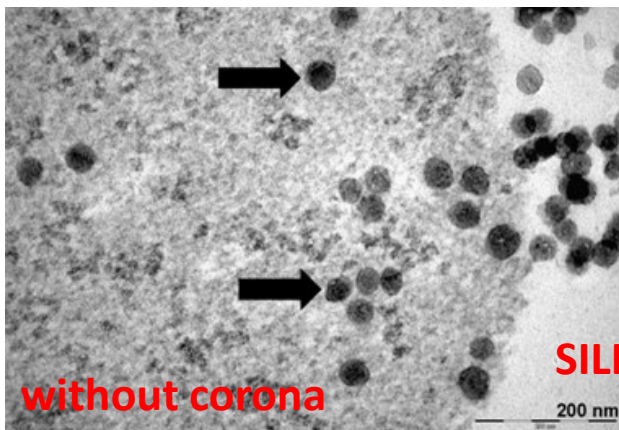
There are few that enter
And they do so by regulated pathways
(later)



In vitro conditions: massive cell death

In vivo conditions: completely benign

EM confirms higher uptake and some NPs free in the cytosol in absence of serum

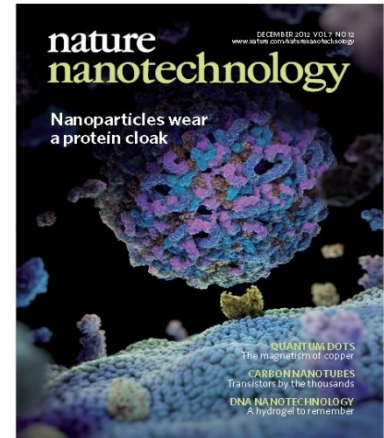
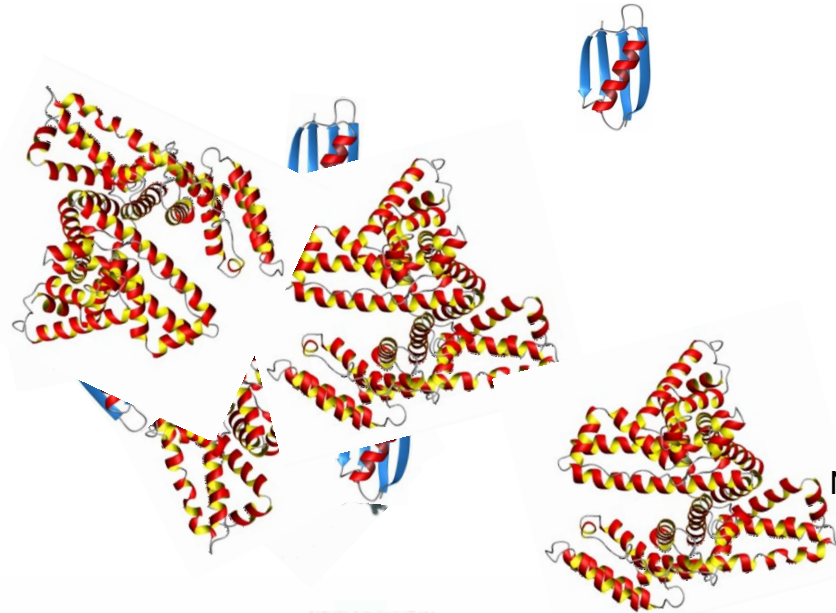


REASON FOR MUCH CONTROVERSY
Wrong conditions
Meaningless outcome?

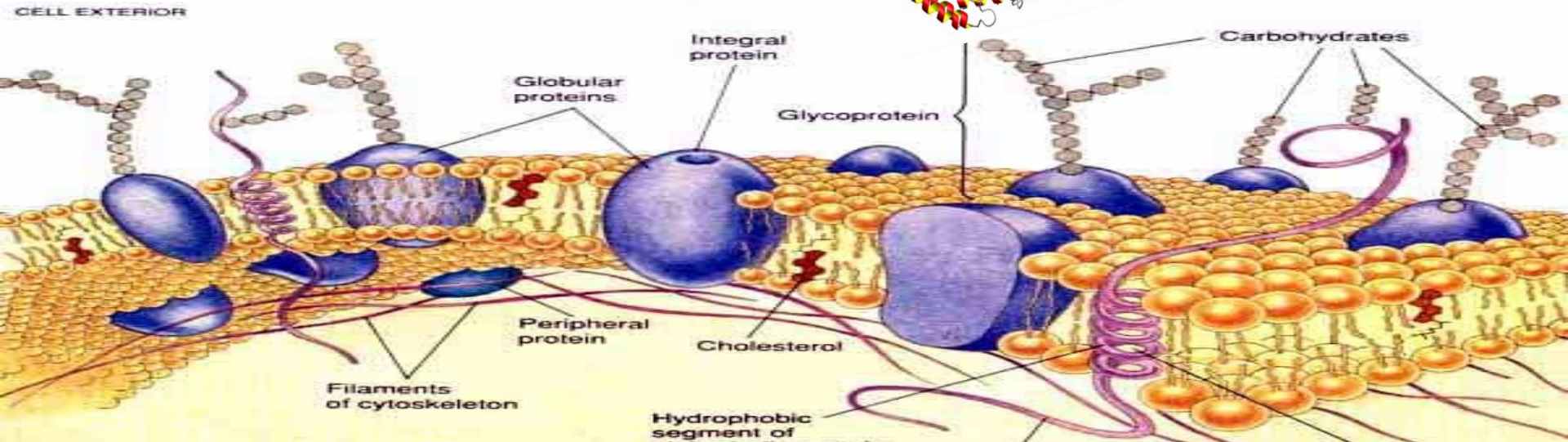
RECOGNITION
IS THE
NANOSCALE
PARADIGM

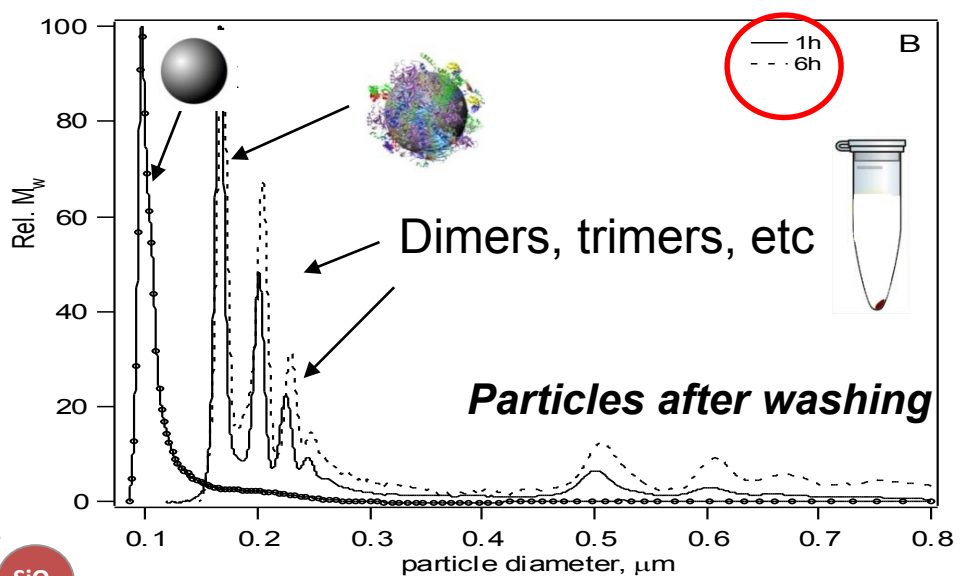
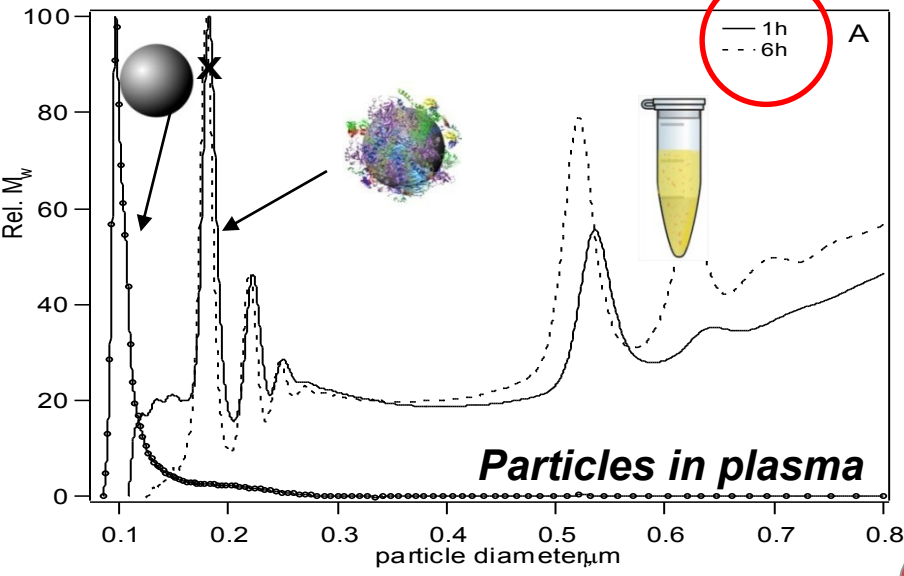
PNAS, 2007, 104,
2050-2055 (2007)

Cozzarelli
Prize NAS
2008

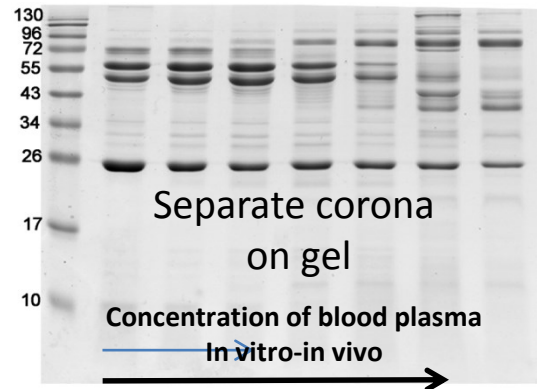


Nature Nanotech 7, 779–786 (2012)





SiO_2



Map out protein composition
Quantitatively mass spec.

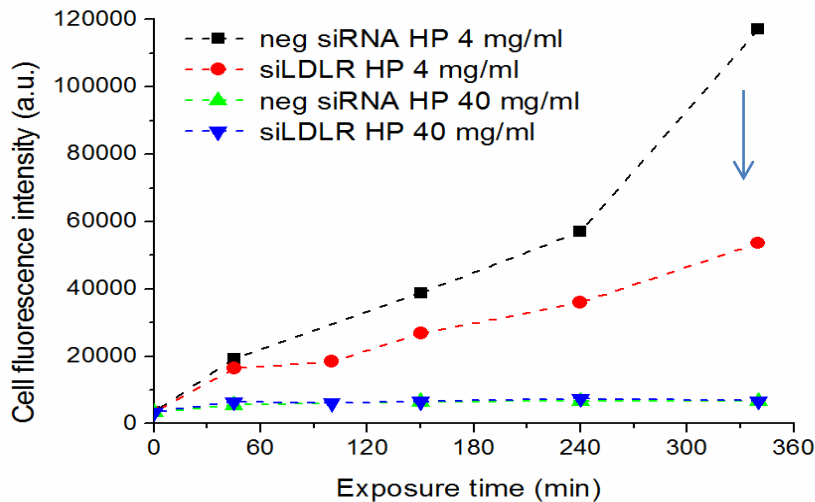


$$\left[(\rho_c - \rho_f) D^2 \right] \cdot t = \frac{18\eta}{\omega^2} \ln \left(\frac{R_f}{R_0} \right)$$

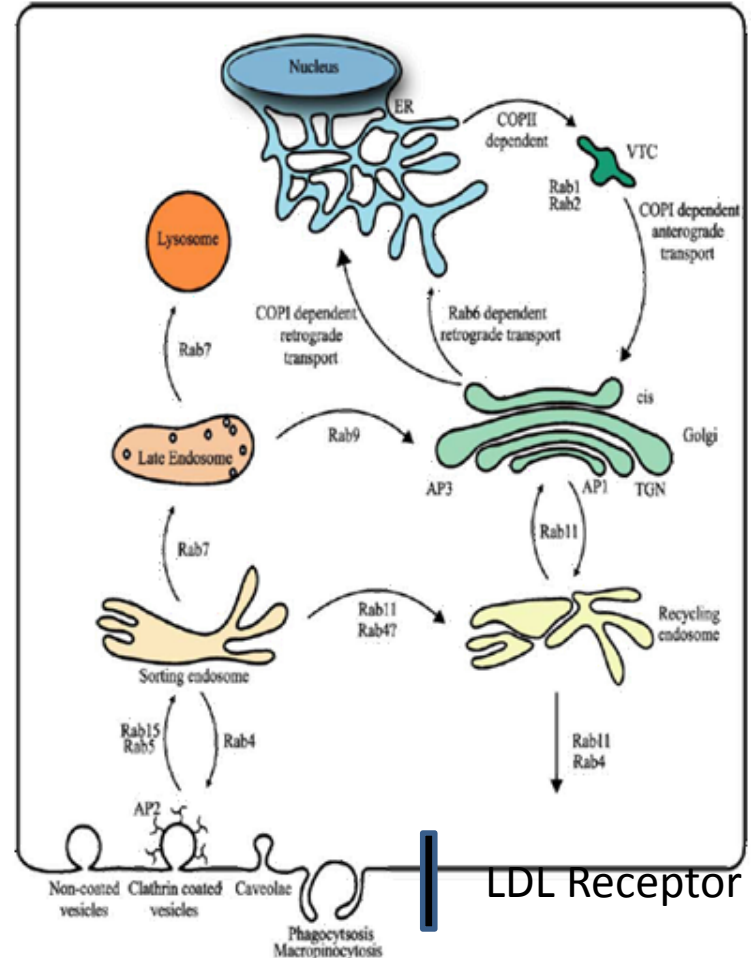
M. P. Monopoli, *Journal of the American Chemical Society*, 2011, **133**, 2525-2534.
 D. Walczyk, *Journal of the American Chemical Society*, 2010, **132**, 5761-5768.

The Details of Recognition are dependent
On the Concentration of Serum
(and of course serum type-match species)

siLDLR
(10% human plasma)

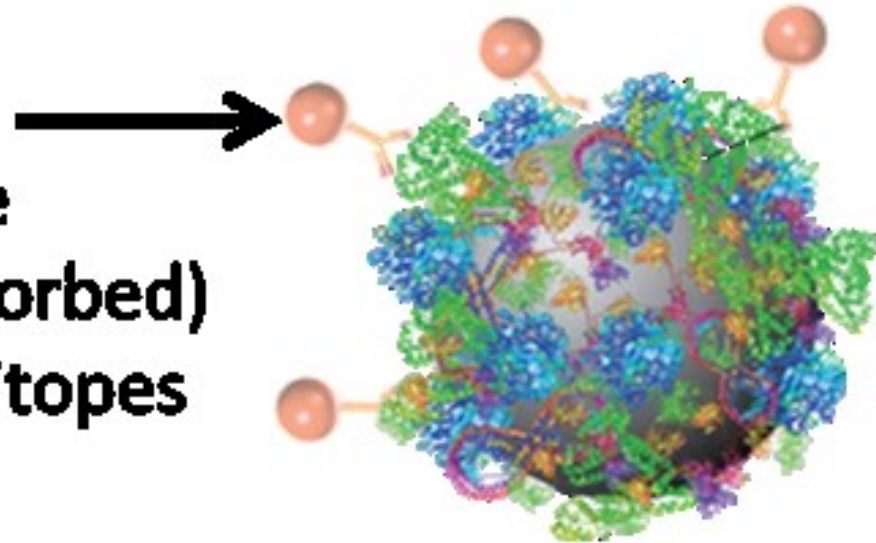


50 nm silica 125 ug/mL



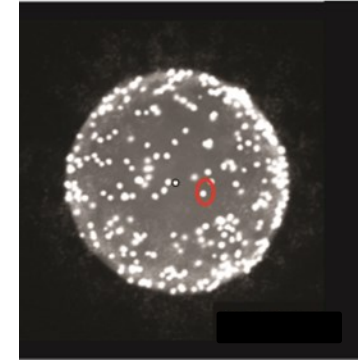
PREDICTING
THE
INTERACTION OF PARTICLES
WITH CELLS

**Immunogold
(or other) probe
for specific (adsorbed)
biomolecule epitopes**

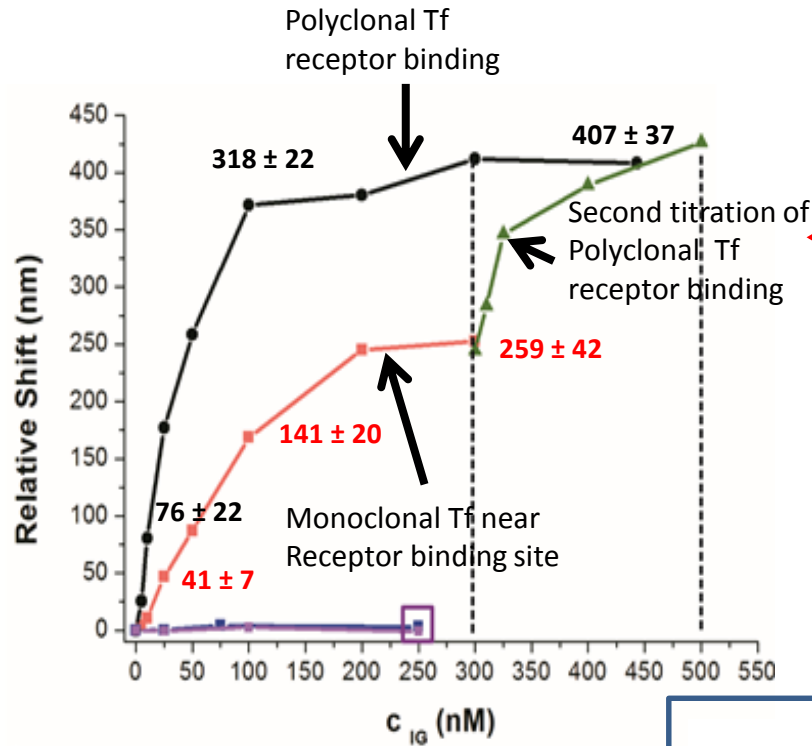


EACH SPECIFIC FUNCTIONAL ELEMENT OF EACH PROTEIN ON THE CORONA
CAN NOW BE MAPPED OUTPROVIDING A PROPOSAL FOR THE
LIKELY INTERACTIONS OF NANOPARTICLES IN THAT EXPOSURE MEDIUM WITH
THOSE CELLS

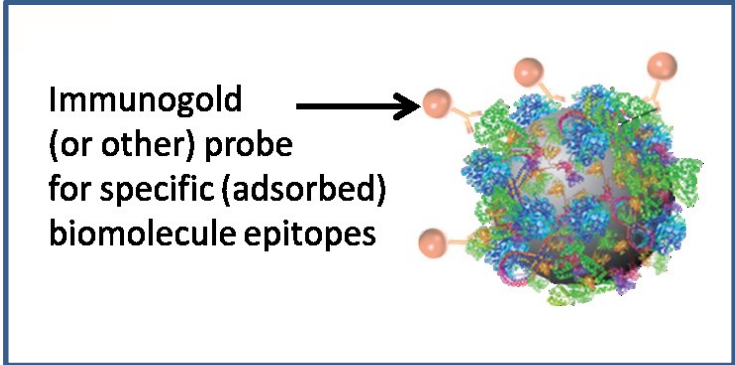
Polystyrene and adsorbed
Transferrin monolayer
mapped with ImmunoGold



Progressive binding and
epitope SATURATION
monitored using DCS
and electron microscopy
- counting of epitopes

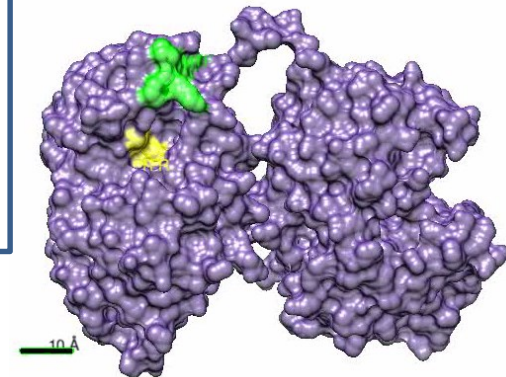


'Distributions' replace
concept of fixed structures
- averaged numbers of
IG bound from EM
*In this example
most particles similar*

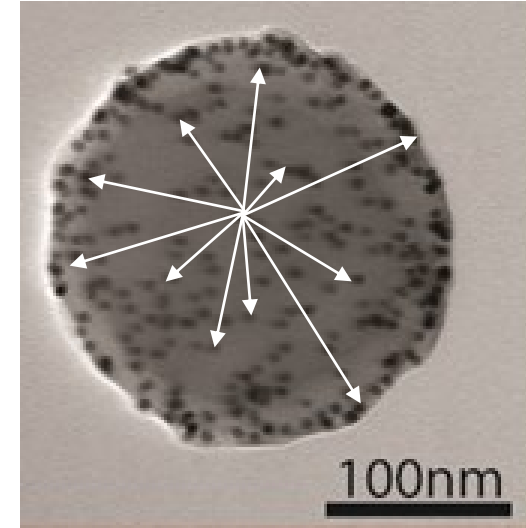
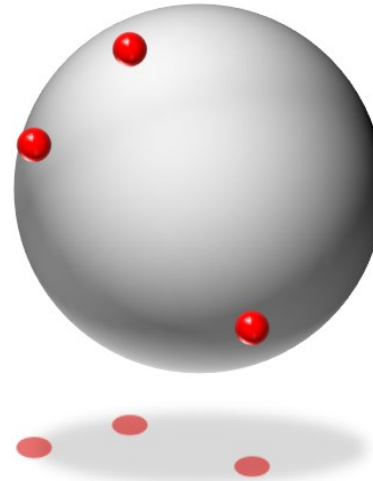
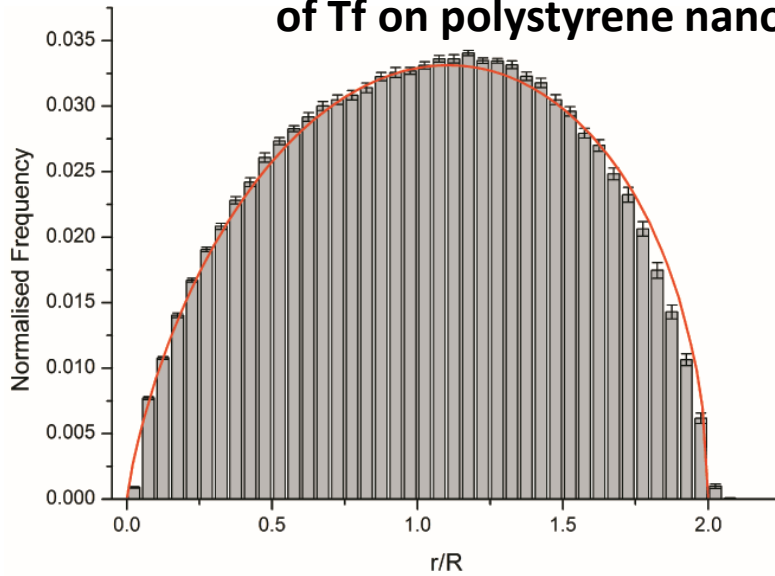


Transferrin Epitopes:

- TfR - **Yellow** binding
- Monoclonal **Green**
(aa. 142-145)



Distribution of projected distances between epitopes of Tf on polystyrene nanoparticles

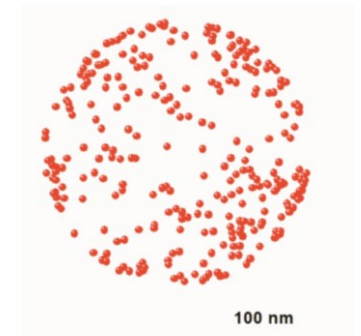
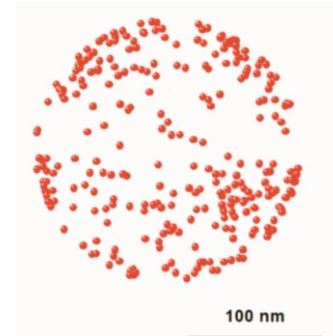
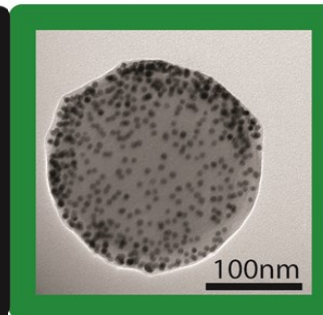
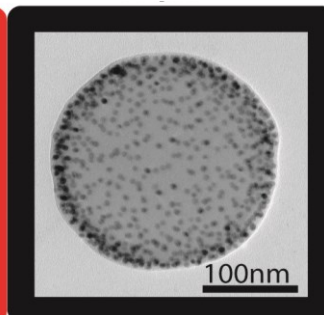
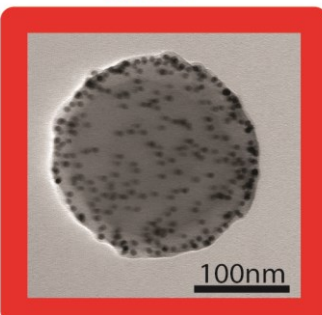


THE CONCEPT OF 'GEOMETRY' IN MOLECULAR SCIENCES WILL BE REPLACED BY DISTRIBUTIONS OF DISTANCES BETWEEN FUNCTIONAL EPITOPES OF NANOPARTICLES - ULTIMATELY THIS COMPLETELY DEFINES RELEVANT PROPERTIES OF ENSEMBLE OF NANOPARTICLES

aa. 142-145

N-Terminal

Double



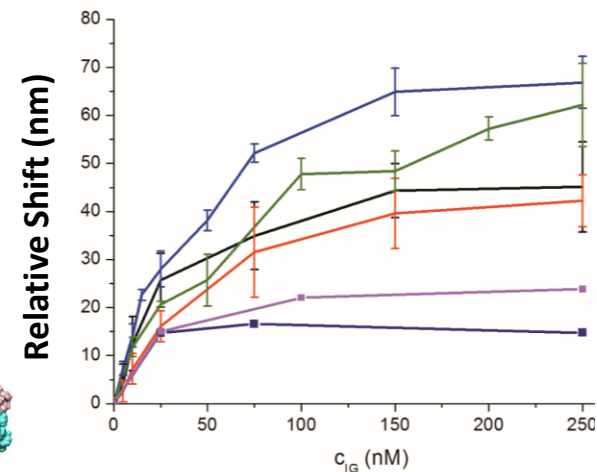
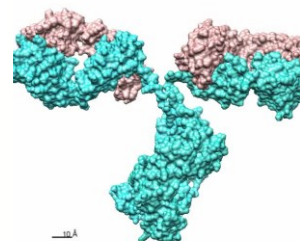
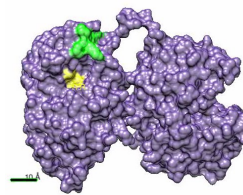
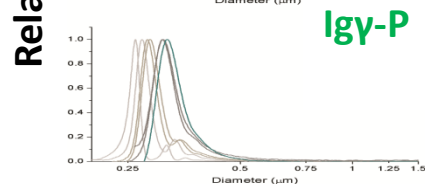
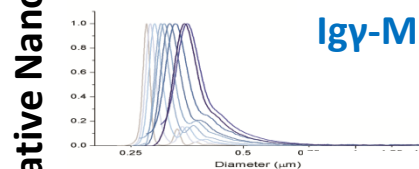
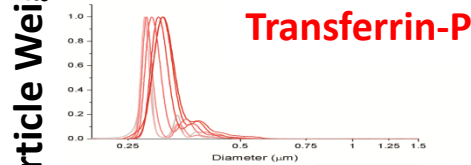
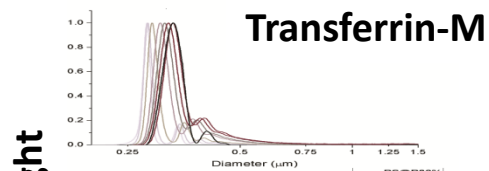
Population analysis yields the same result as mass spec.

Ratio of Tf to IGG

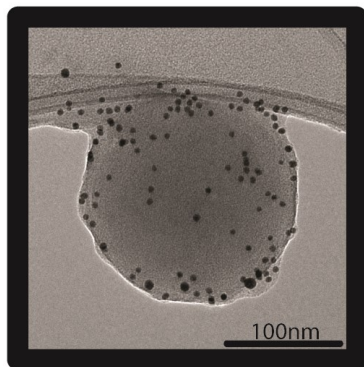
Mass Spec = 93 %

DCS = 89 %

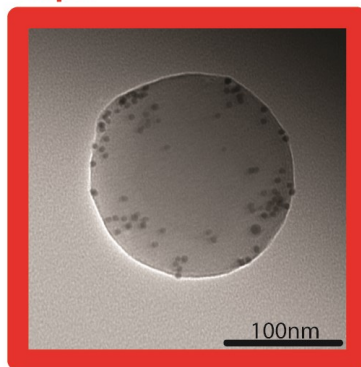
Single particle analysis shows the individual biological Identity



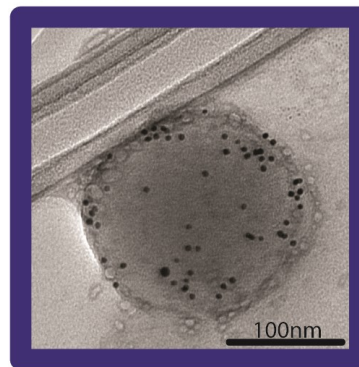
IG-mTf



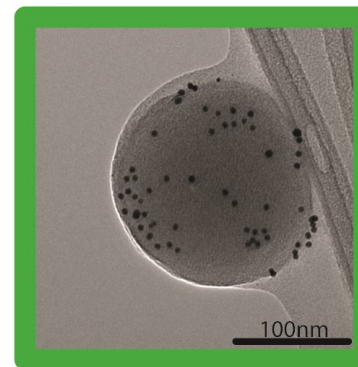
IG-pTf



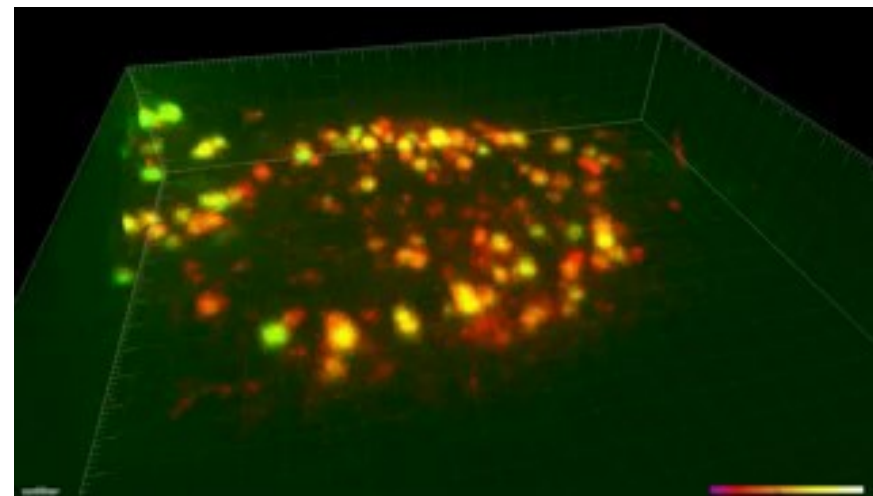
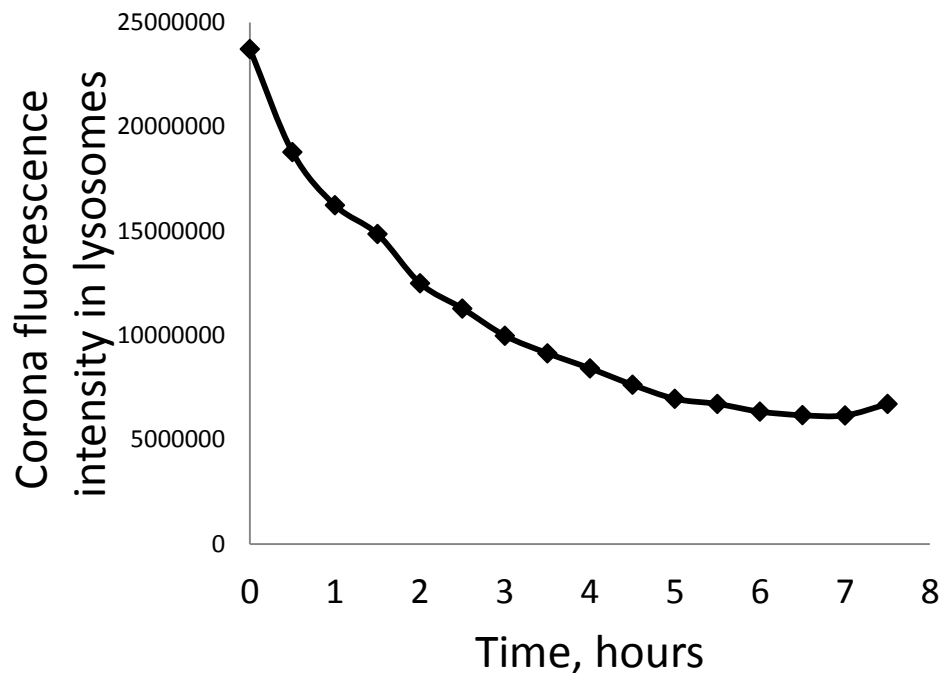
IG-mIGG



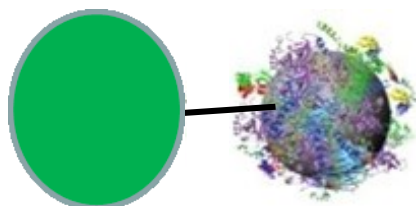
IG-pIGG



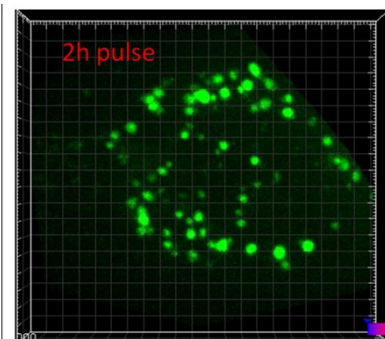
INSIDE THE CELL



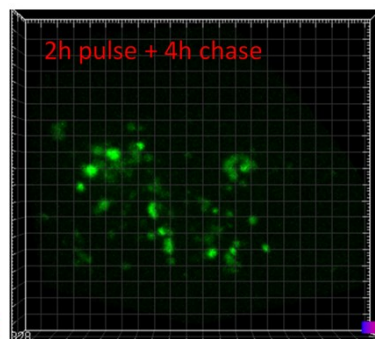
Particles surrounded by corona (green) in lysosomes (red)-Corona degraded after 3-5 hrs



Serum labelled green



2h pulse

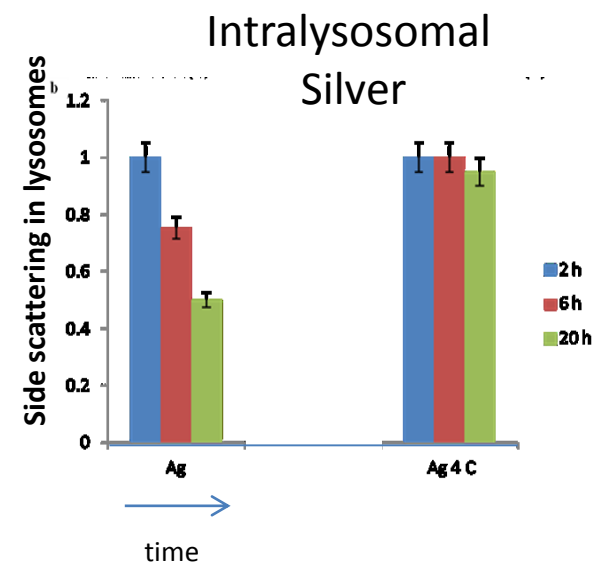
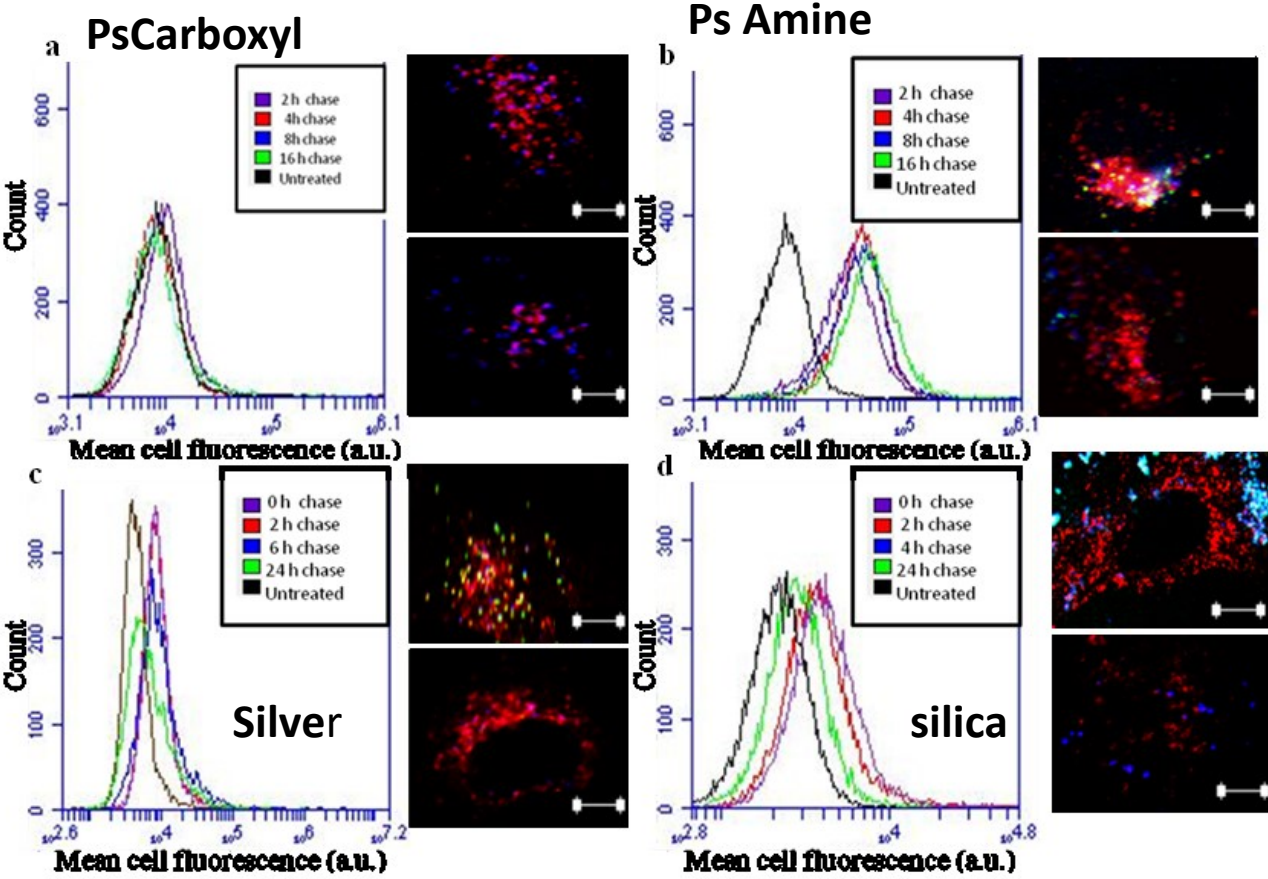


2h pulse + 4h chase

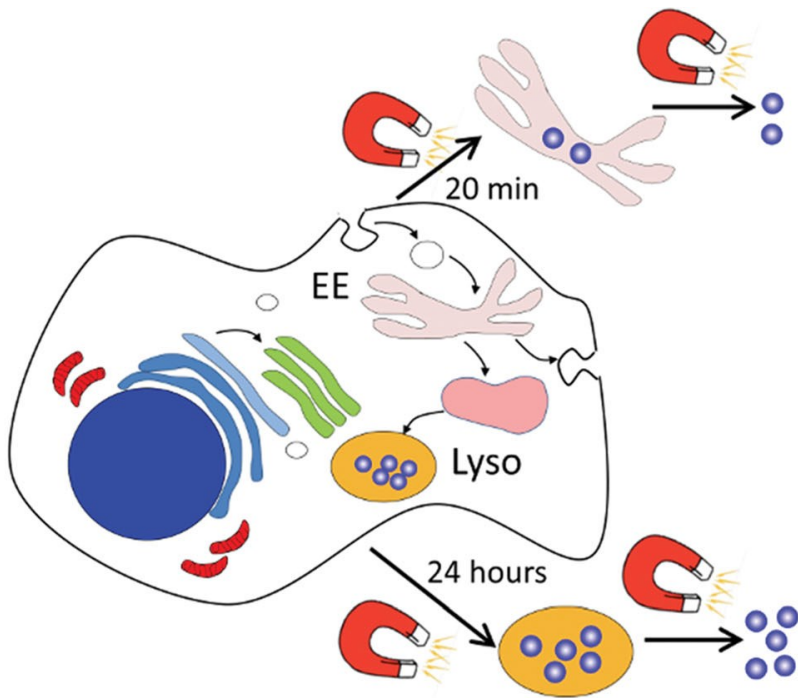


2h pulse + 7h chase

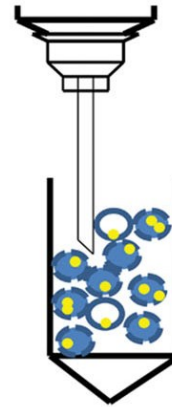
MOST PARTICLES TAKE IN CORONA WITH THEM



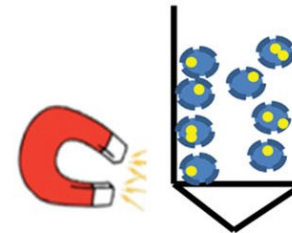
THIS MATTERS
MANY DETAILED
PREDICTIONS OF INTRACELLULAR
CELLULAR SIGNALLING
(“SYSTEMS BIOLOGY”)
DEPEND ON HOW THE
CORONA WAS CARRIED INTO CELL

Bertoli *et al.* Small (2014)

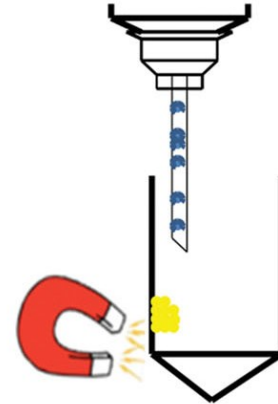
Cell lysis



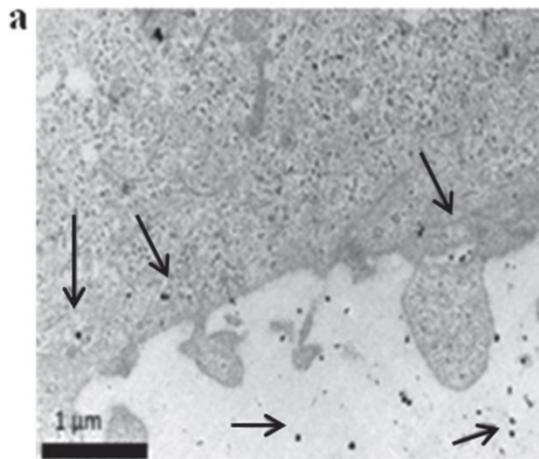
Magnetic separation



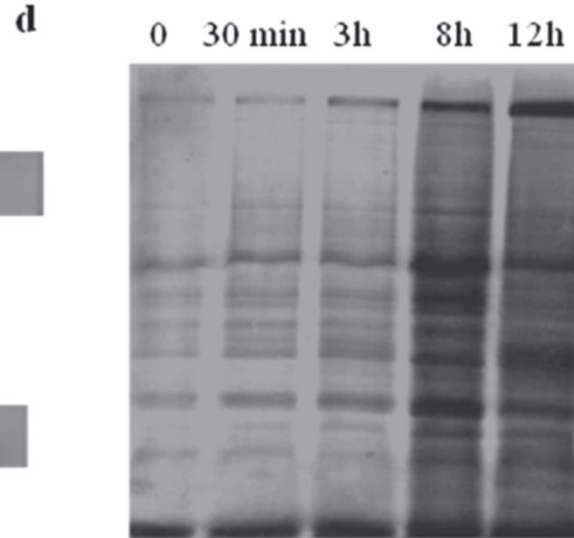
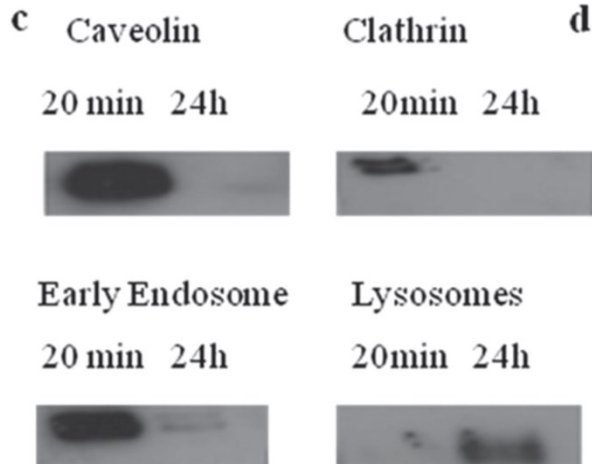
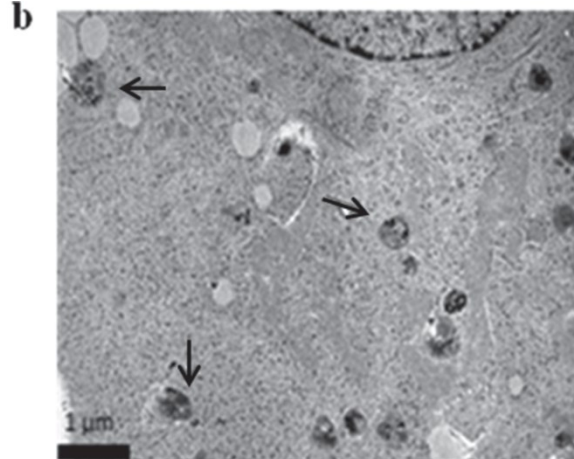
Pellet isolation



After 20 minutes:



After 24 hr:



The Accumulation
Of Corona Proteins
Inside Lysosomes

A549 cells incubated with 250 ug/mL silica coated magnetite
20 min pulse with varying a) 20 min and b) 24hr chase

Bertoli *et al.* Small (2014)

WE WERE BUILT TO PROCESS NANOPARTICLES

PROTEINS MATTER AND SO DO THE SUGARS AT THE
INTERFACE

THERE ARE WELL DEFINED LAWS GOVERNG THIS FIELD,
DIFFERENT FROM THOSE WITH CHEMICALS, AND WE ARE
PROGRESSIVELY MASTERING THEM

WE WILL, IF WE ARE DRIVEN TO DO SO, ONE DAY
UNDERSTAND THESE MECHANISMS AND PROCESSES
UNDERLYING NANOPARTICLES AND LIVING ORGANISMS
BETTER THAN THOSE WITH CHEMICALS

IT IS FOR US TO CHOOSE WHAT WE WILL BECOME