

THE MATRIX IS ALWAYS COMPLEX!

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

Mapping protein binding sites on the biomolecular corona of nanoparticles

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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

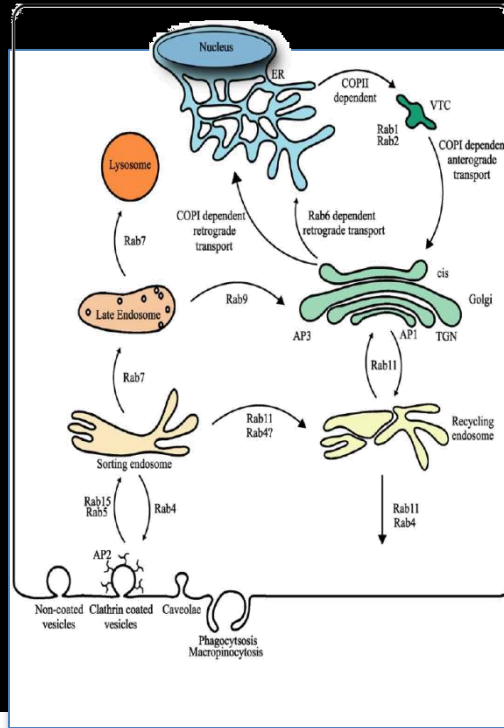
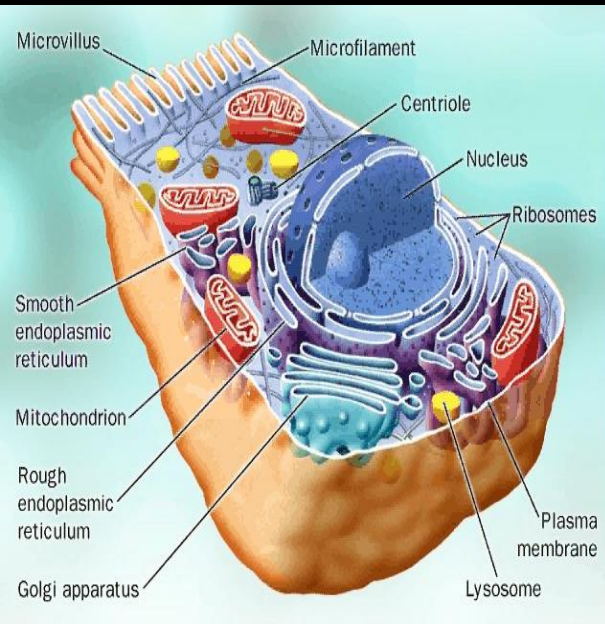
Sha Wan,[†] Philip M. Kelly,[†] Eugene Mahon,[†] Henning Stöckmann,[‡] Pauline M. Rudd,[‡] Frank Caruso,[§] Kenneth A. Dawson,[†] Yan Yan,^{*,§} and Marco P. Monopoli^{*,†}

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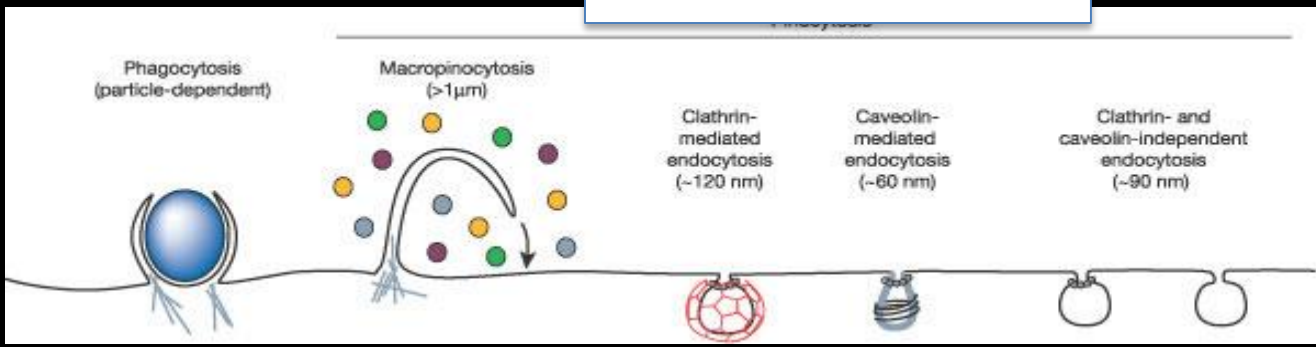
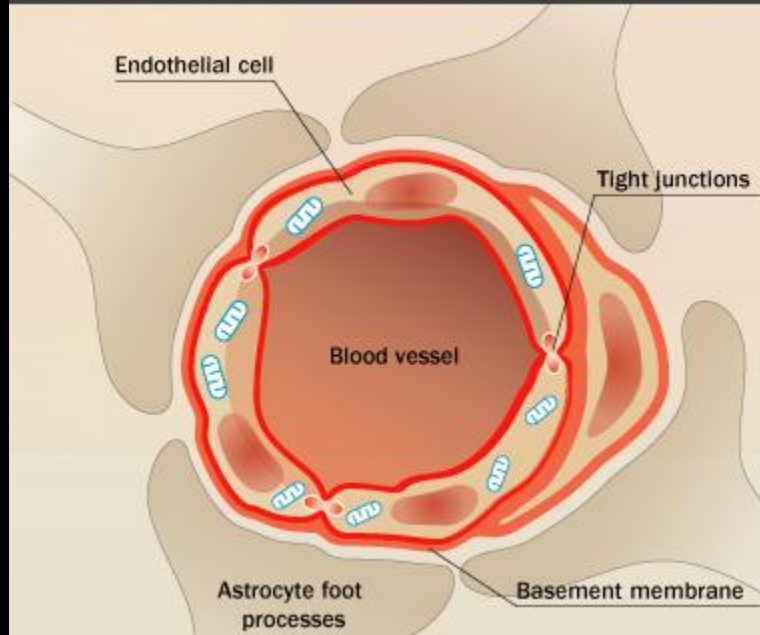
NEW SCIENCE

Engineered Nanoscale written in our biology

new medicine-new science; ADME Models will not work



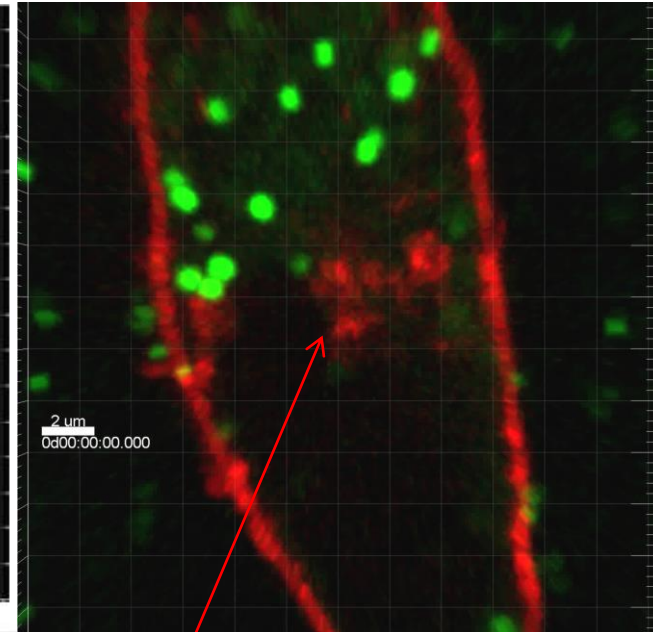
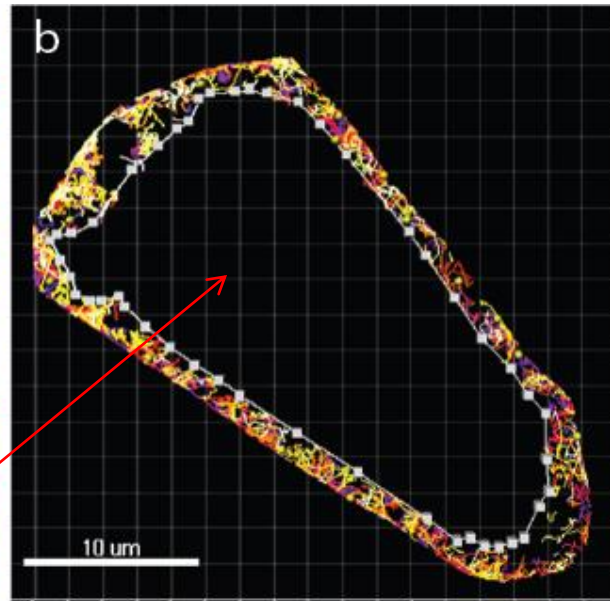
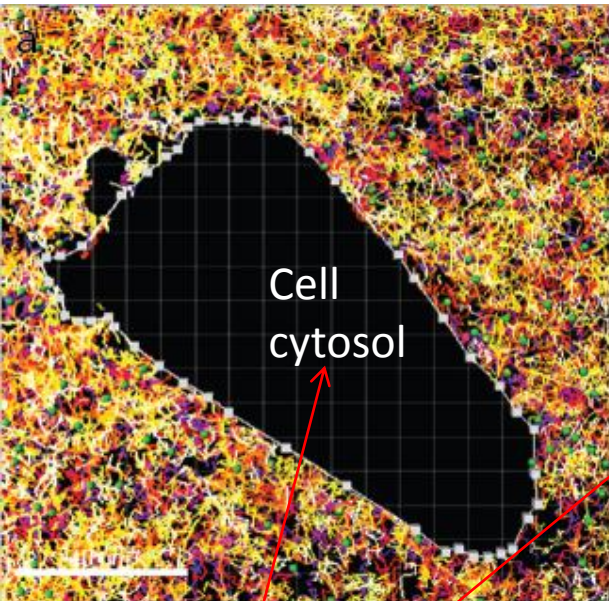
The BBB : the brain's most formidable gatekeeper



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

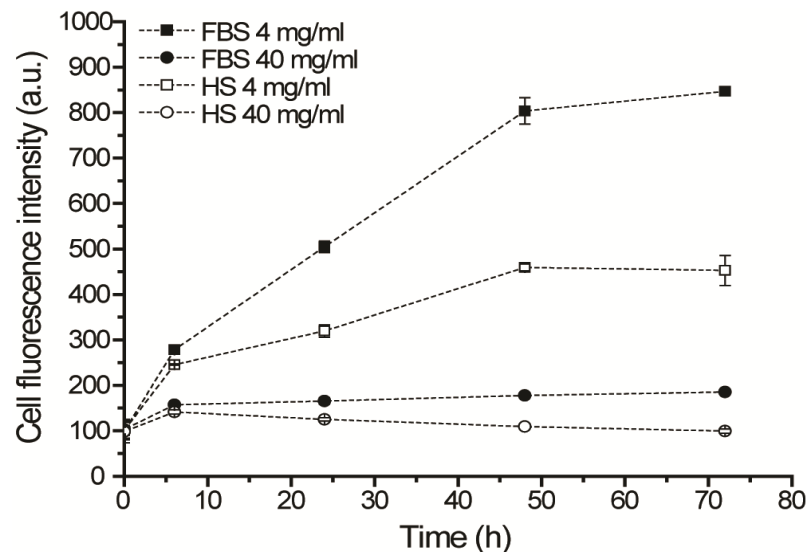
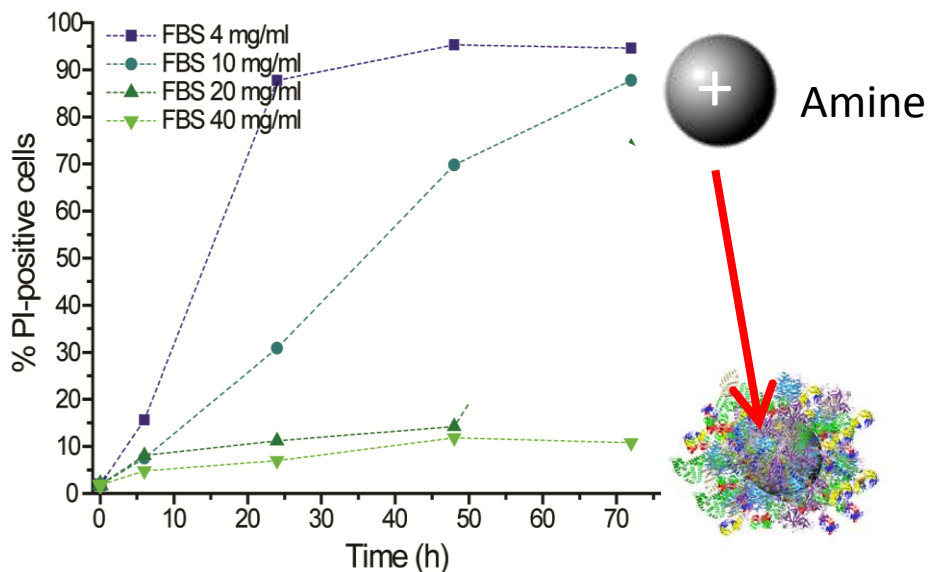
Chemicals Partition but Nanoparticles processed-energy of cell used

EARLY LIFE
DETERMINED
BY MILEU



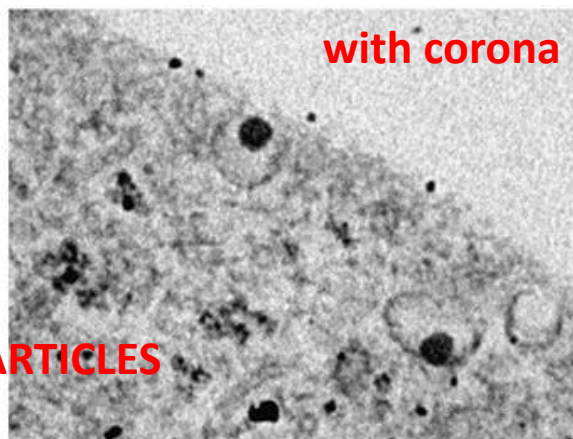
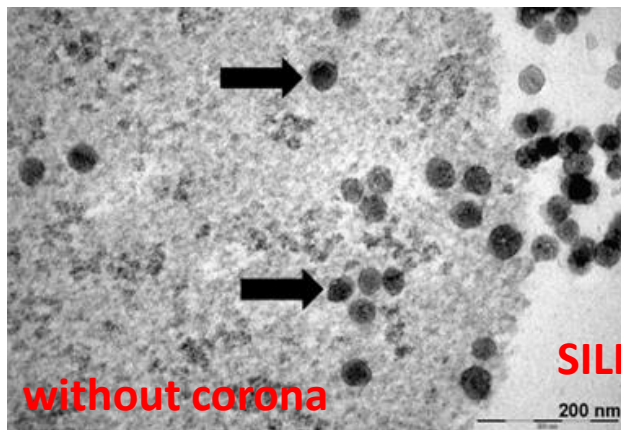
Many particle trajectories
Most unsuccessful in
entering cell

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

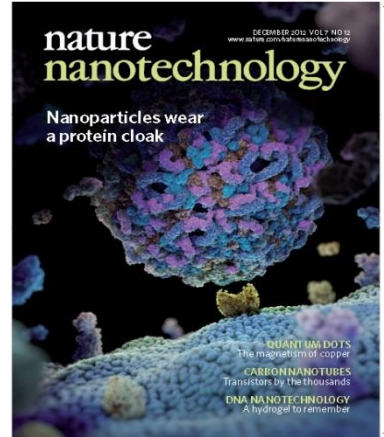
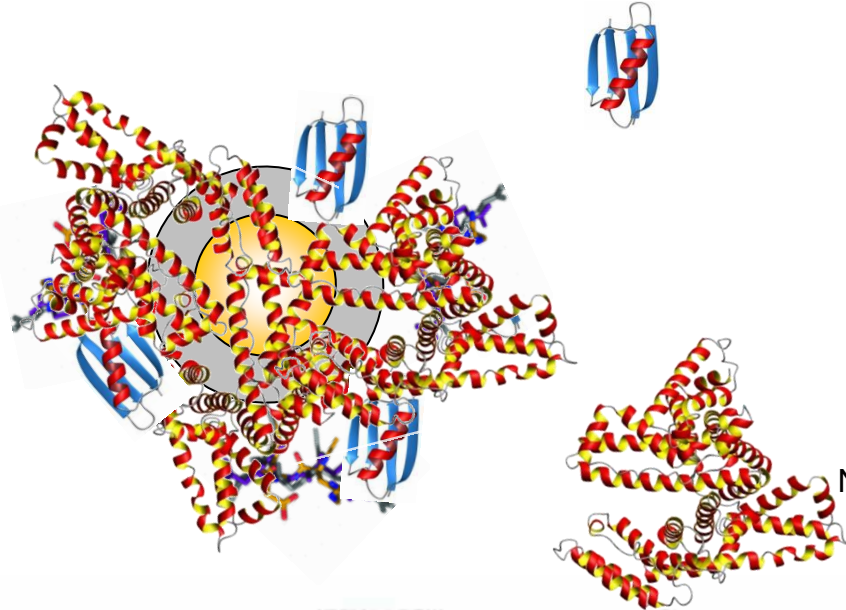


REASON FOR MUCH CONTROVERSY
Wrong conditions
Meaningless outcome?

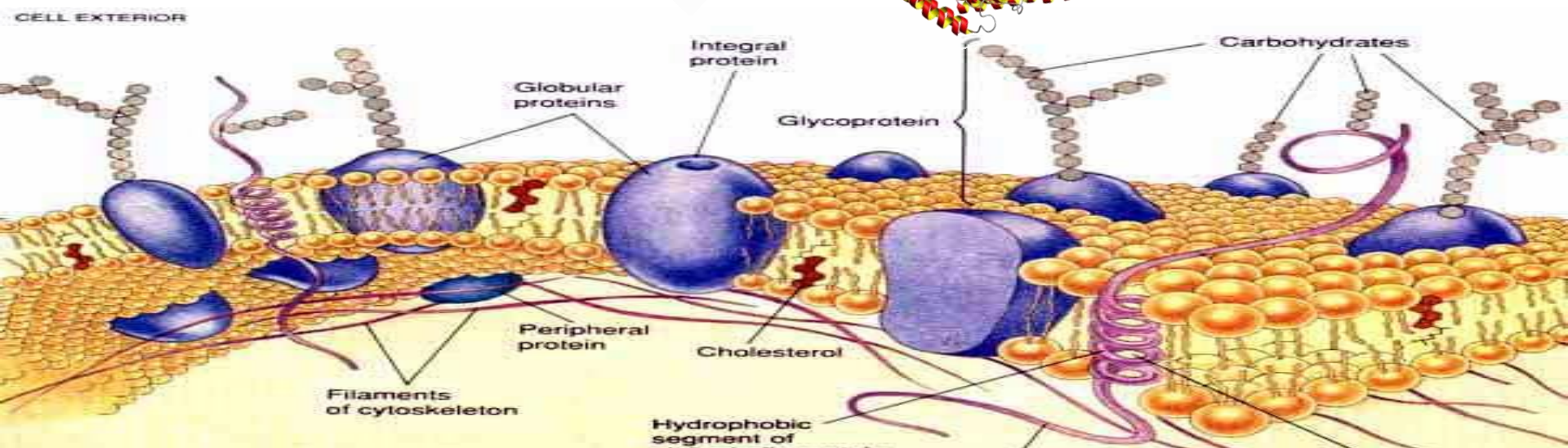
RECOGNITION
IS THE
NANOSCALE
PARADIGM

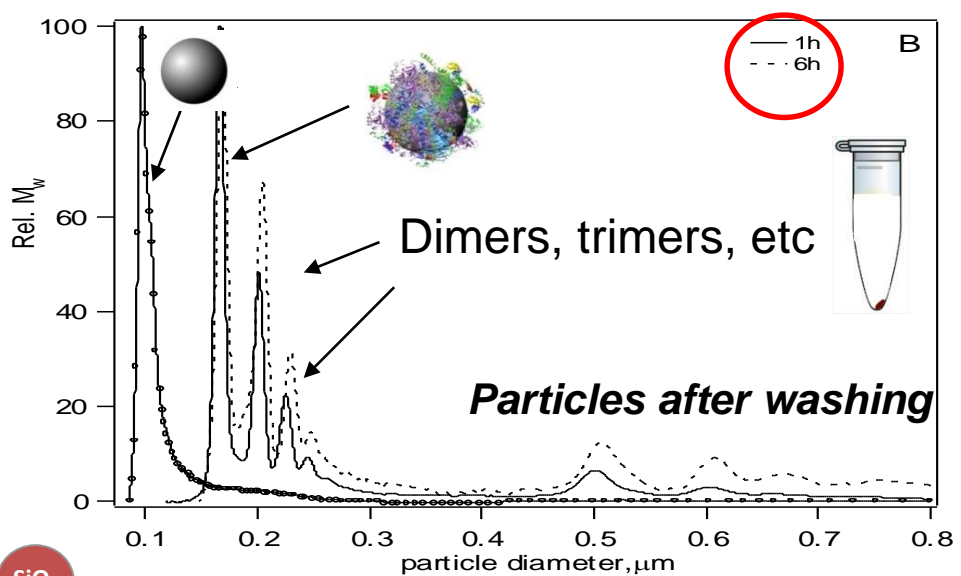
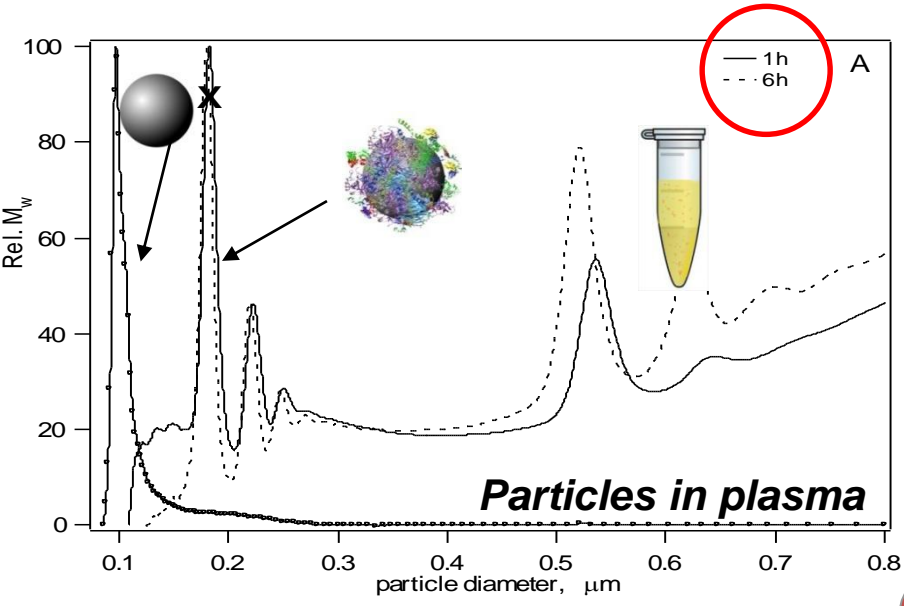
PNAS, 2007, 104,
2050-2055 (2007)

Cozzarelli
Prize NAS
2008

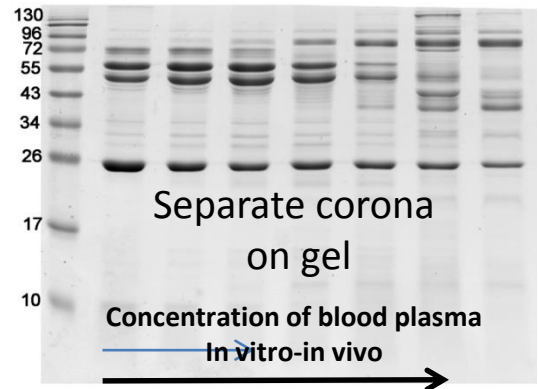


Nature Nanotech 7, 779–786 (2012)





SiO₂



Map out protein composition
Quantitatively mass spec.

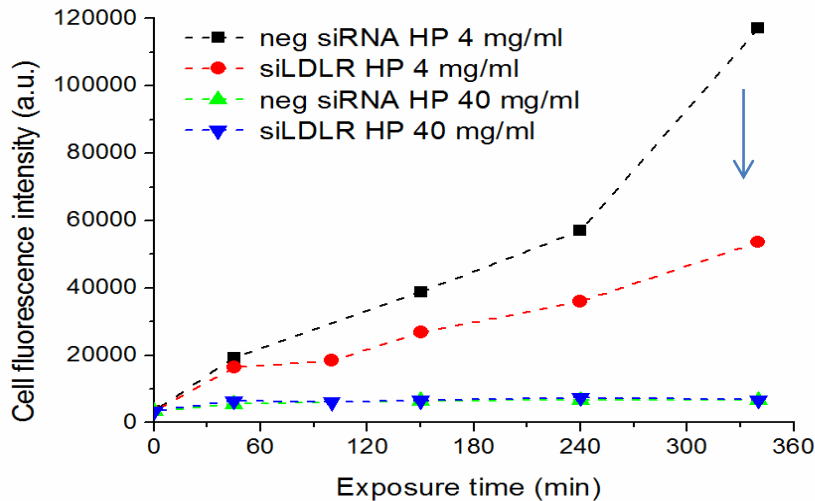
Gel band Mw	Acc number	Protein Identity	NSpC	NSpC
			[10% plasma]	[55% plasma]
500kDa	P04114	Apolipoprotein B 100	0.96	0.91
120kDa	P07996	Thrombospondin-1	0.01	1.37
90 kDa	P04196	Histidine-rich glycoprotein	4.02	13.93
90 kDa	P00747	Plasminogen	0.87	3.27
90 kDa	P02787	Transferrin	0.02	0.52
72 kDa	P06396	Gelsolin	-	0.63
90 kDa	P02671	Fibrinogen alpha chain	15.43	4.88
72 kDa	P02768	Serum albumin	1.80	9.67
72 kDa	P01042	Kinogen-1	1.54	2.22
60 kDa	P02675	Fibrinogen beta chain	23.92	7.99
50 kDa	P02679	Fibrinogen gamma chain	18.40	6.52
50 kDa	P00748	Coagulation factor XII	1.05	4.15
43 kDa	P49908	Selenoprotein P	0.16	0.87
40 kDa	P02765	Alpha-2-HS-glycoprotein	-	0.16
28 kDa	P02749	Beta-2-glycoprotein	-	0.74
30 kDa	P02649	Apolipoprotein E	3.13	3.87
		Complement C1q subcomponent		
30 kDa	P02746	Beta	2.28	0.58
26 kDa	P02647	Apolipoprotein A-I	9.45	14.83
12 kDa	P01834	Ig kappa chain C region	3.26	5.13

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

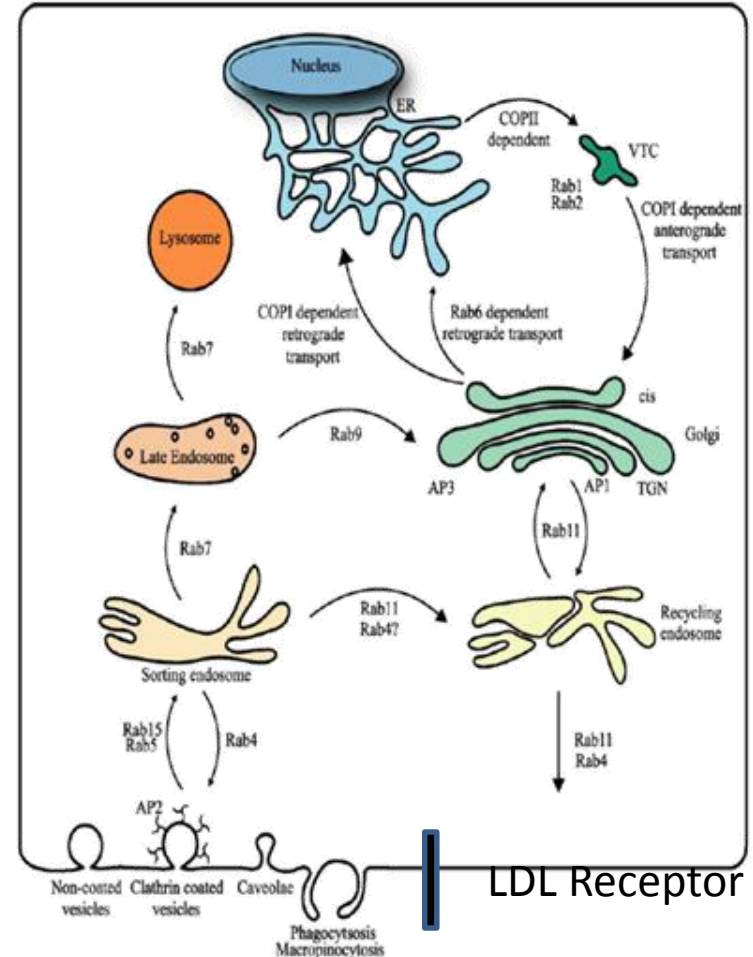
M. P. Monopoli, *Journal of the American Chemical Society*, 2011, **133**, 2525-2534.
 D. Walczyk, *Journal of the American Chemical Society*, 2010, **132**, 5761-5768.

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

siLDLR
(10% human plasma)

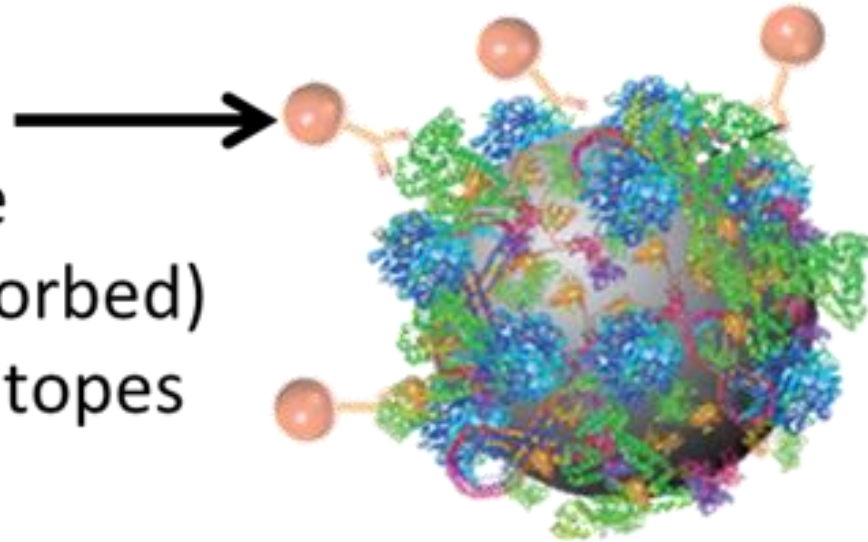


50 nm silica 125 ug/mL



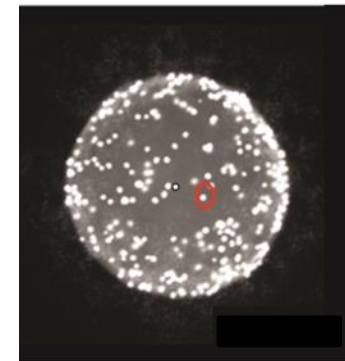
PREDICTING
THE
INTERACTION OF PARTICLES
WITH CELLS

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

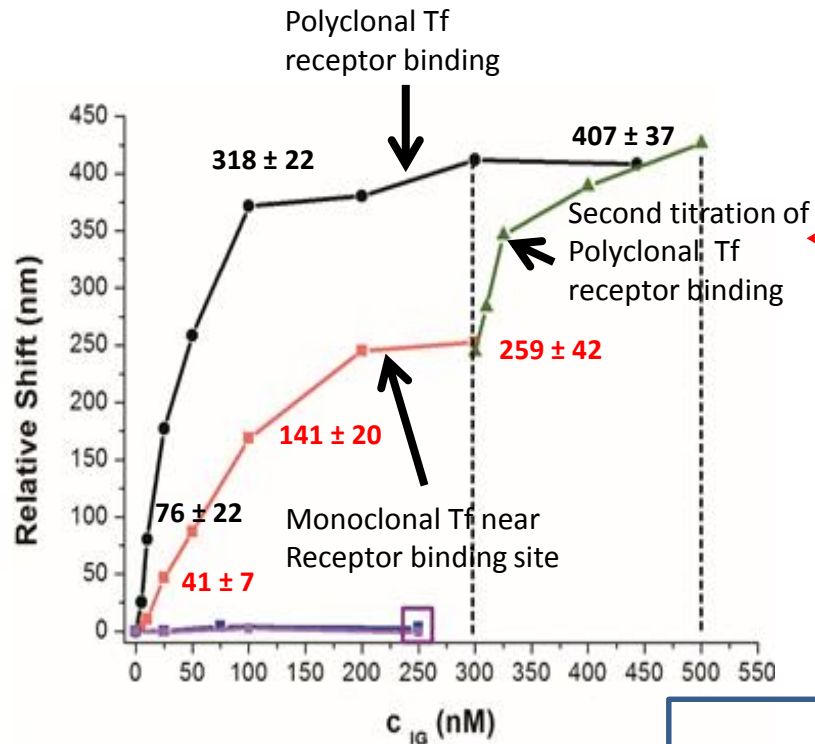


EACH SPECIFIC FUNCTIONAL ELEMENT OF EACH PROTEIN ON THE CORONA
CAN NOW BE MAPPED OUTPROVIDING A PROPOSAL FOR THE
LIKELY INTERACTIONS OF NANOPARTICLES IN THAT EXPOSURE MEDIUM WITH
THOSE CELLS

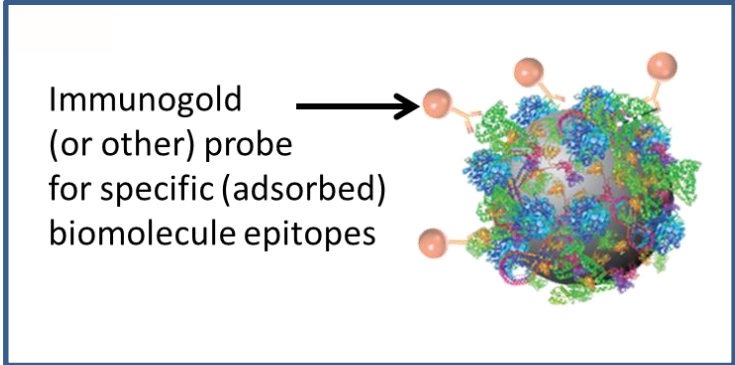
Polystyrene and adsorbed
Transferrin monolayer
mapped with ImmunoGold



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

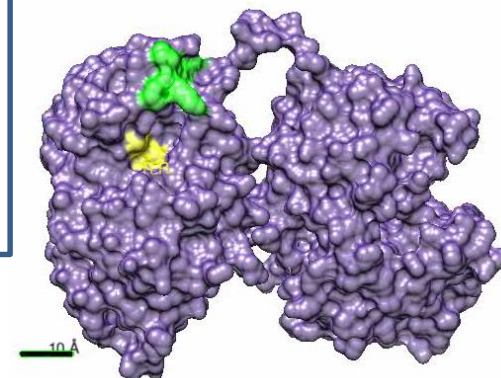


'Distributions' replace
concept of fixed structures
- averaged numbers of
IG bound from EM
***In this example
most particles similar***

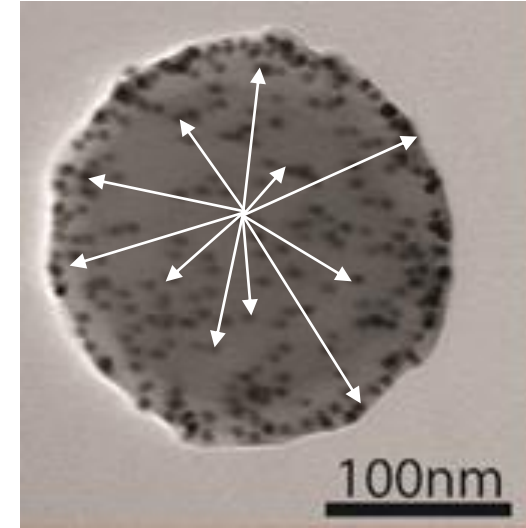
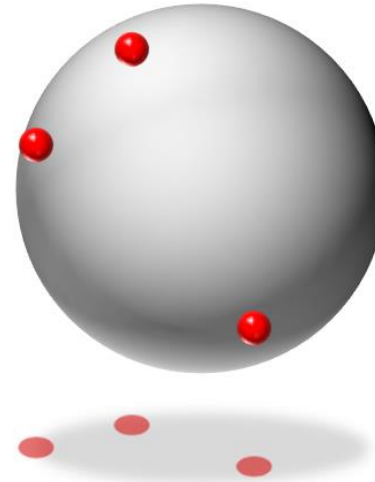
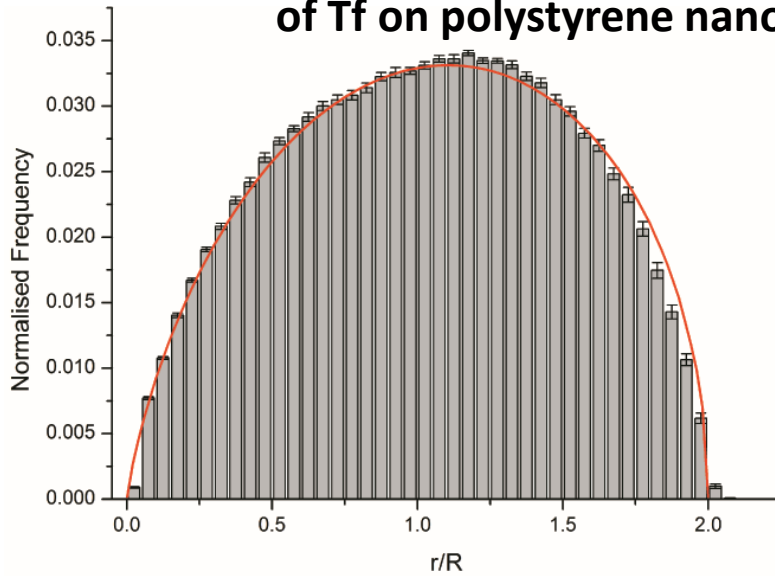


Transferrin Epitopes:

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



Distribution of projected distances between epitopes of Tf on polystyrene nanoparticles

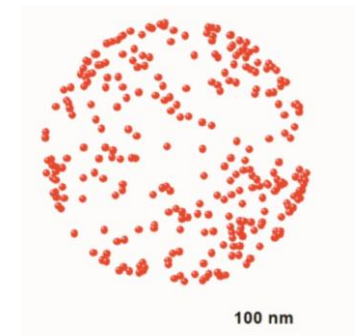
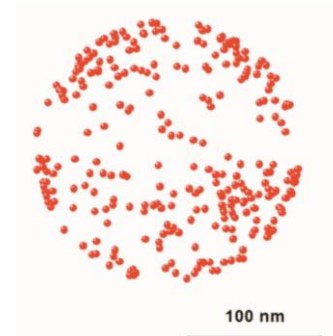
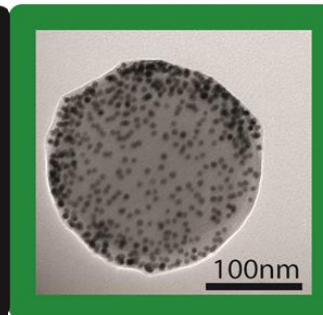
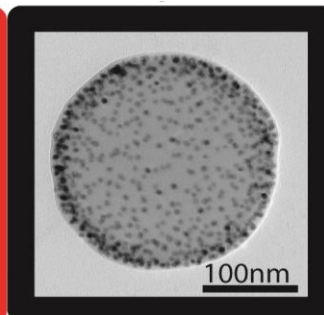
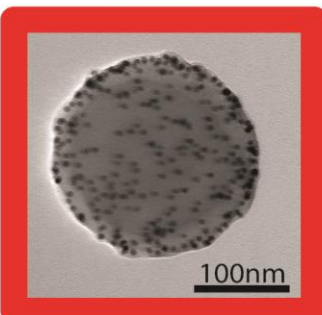


THE CONCEPT OF 'GEOMETRY' IN MOLECULAR SCIENCES WILL BE REPLACED BY DISTRIBUTIONS OF DISTANCES BETWEEN FUNCTIONAL EPITOPES OF NANOPARTICLES - ULTIMATELY THIS COMPLETELY DEFINES RELEVANT PROPERTIES OF ENSEMBLE OF NANOPARTICLES

aa. 142-145

N-Terminal

Double



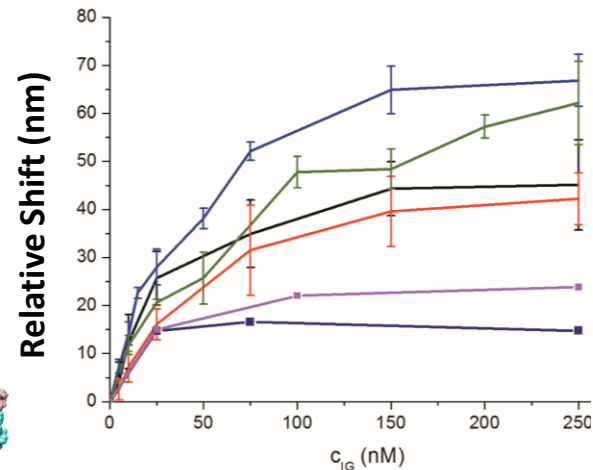
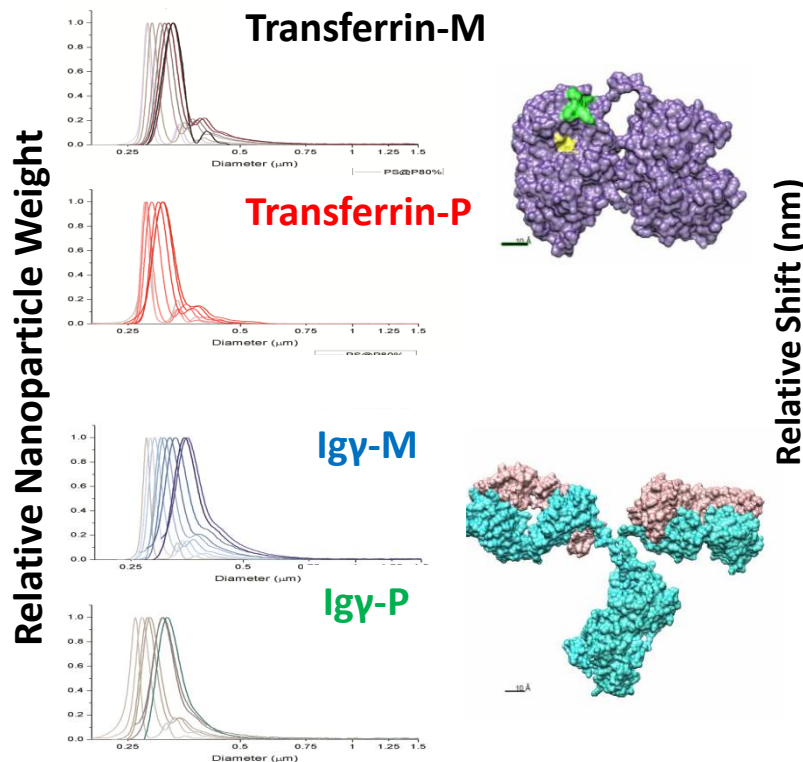
Population analysis yields the same result as mass spec.

Ratio of Tf to IGG

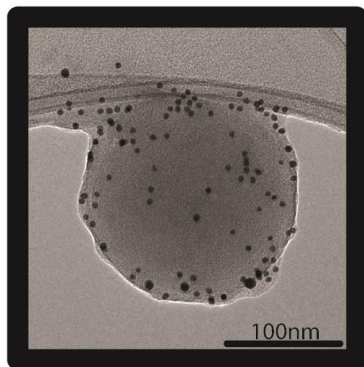
Mass Spec = 93 %

DCS = 89 %

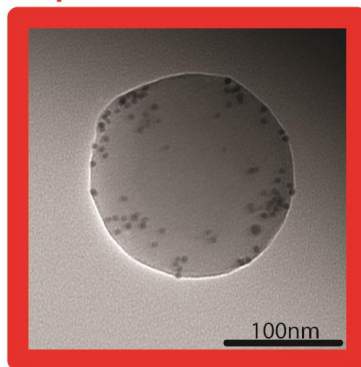
Single particle analysis shows the individual biological Identity



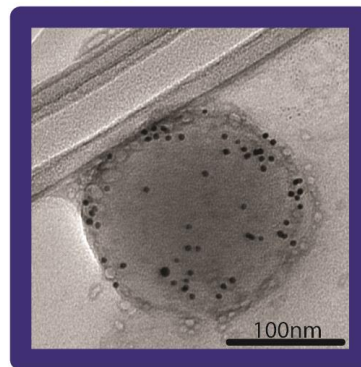
IG-mTf



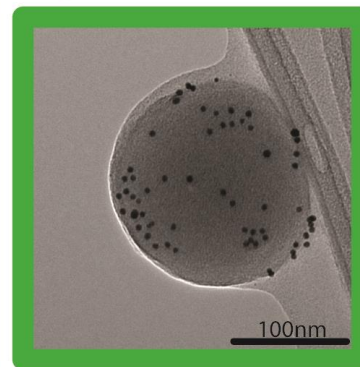
IG-pTf



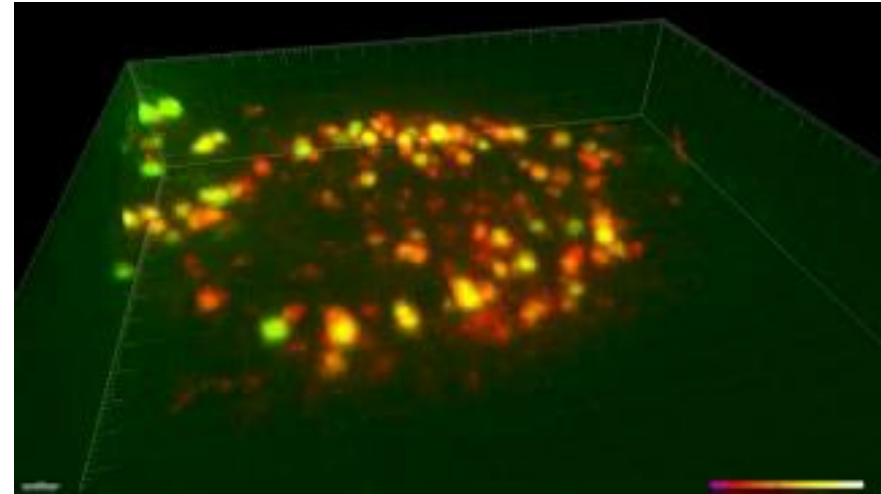
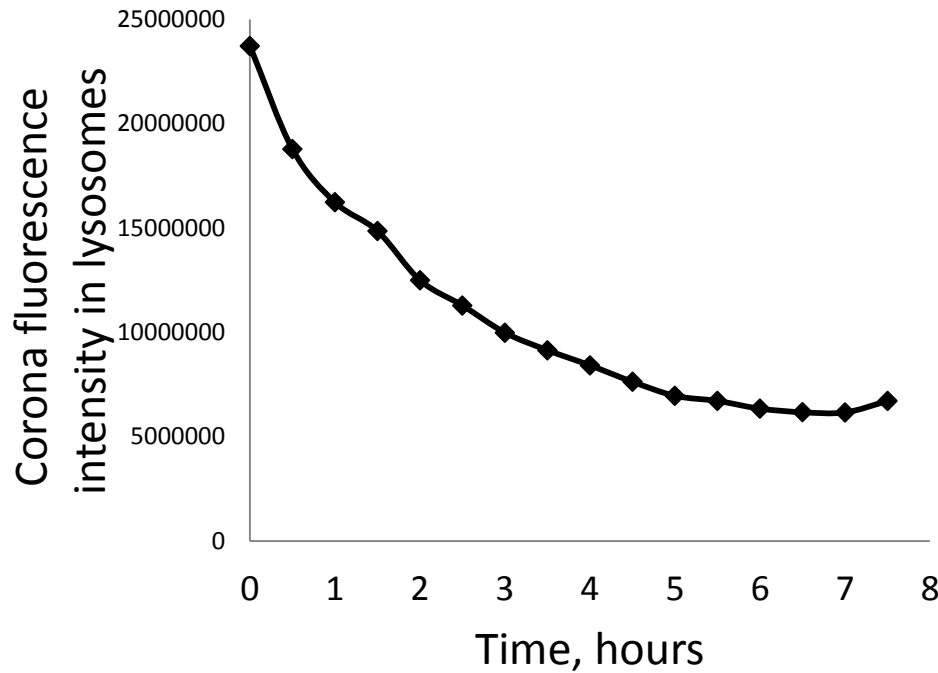
IG-mIGG



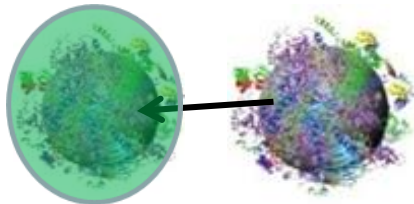
IG-pIGG



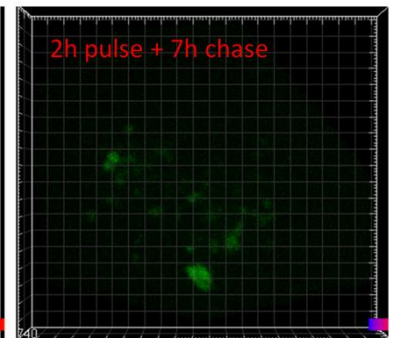
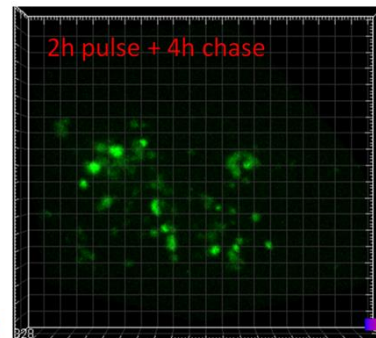
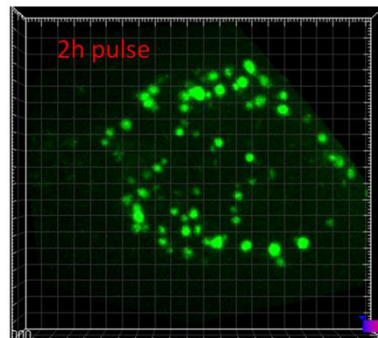
INSIDE THE CELL



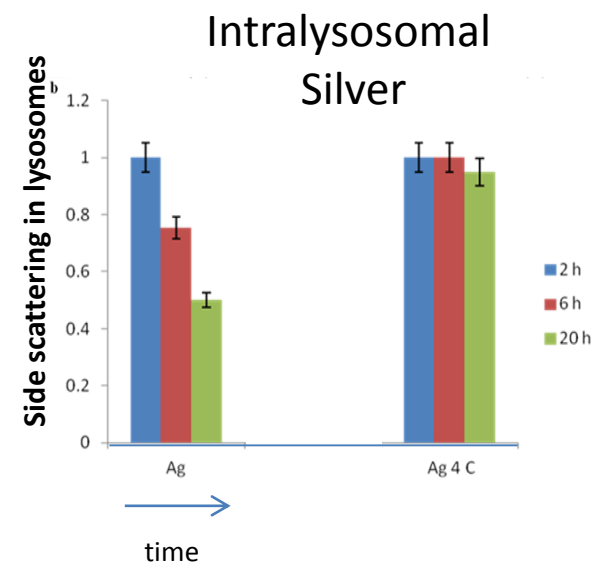
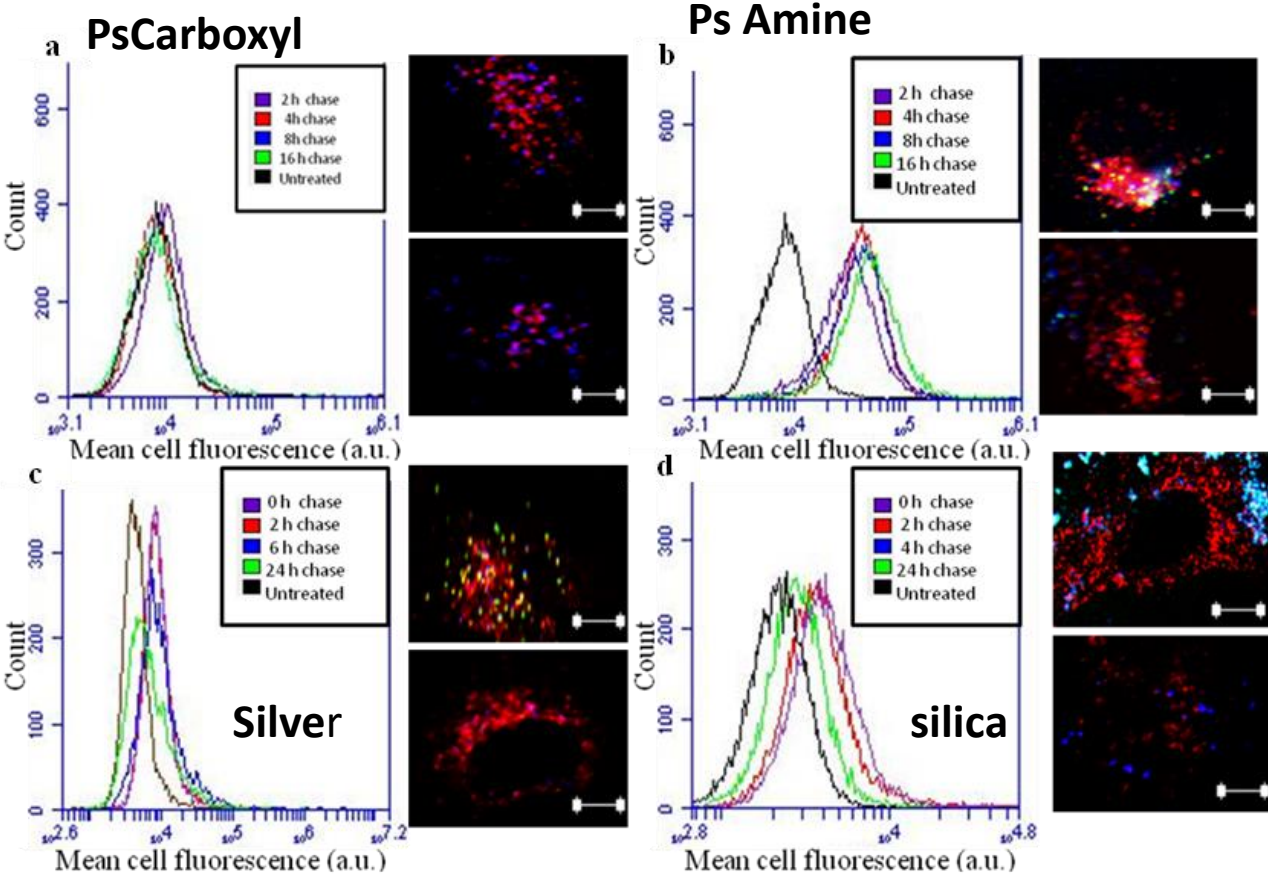
Particles surrounded by corona (green) in lysosomes (red)-Corona degraded after 3-5 hrs



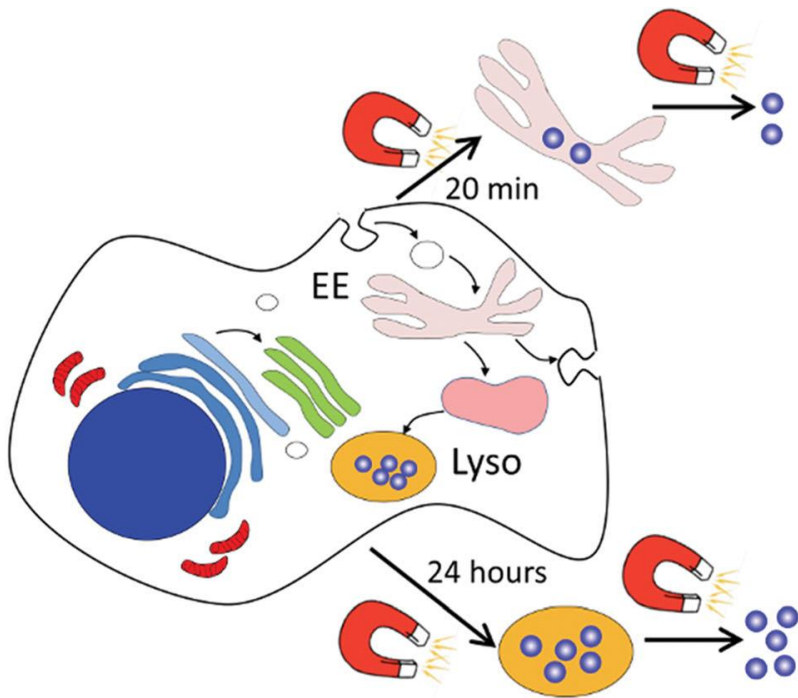
Serum labelled green



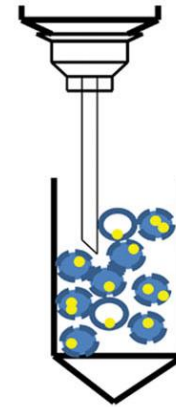
MOST PARTICLES TAKE IN CORONA WITH THEM



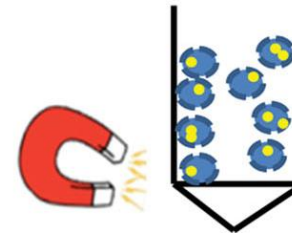
THIS MATTERS
MANY DETAILED
PREDICTIONS OF INTRACELLULAR
CELLULAR SIGNALLING
(“SYSTEMS BIOLOGY”)
DEPEND ON HOW THE
CORONA WAS CARRIED INTO CELL



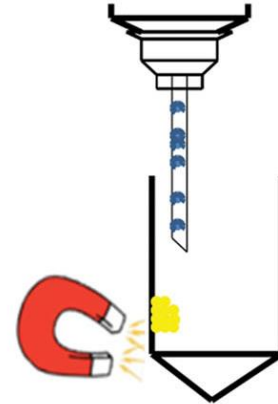
Cell lysis



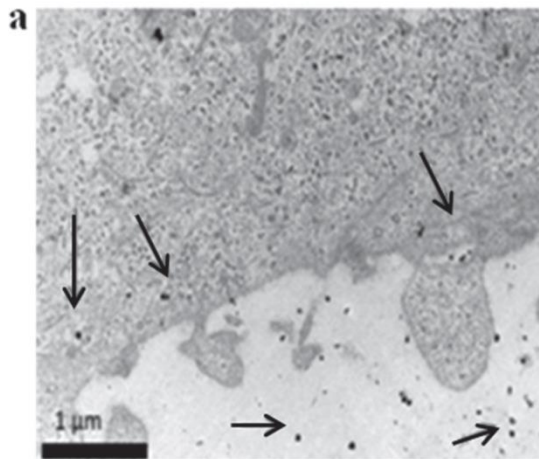
Magnetic separation



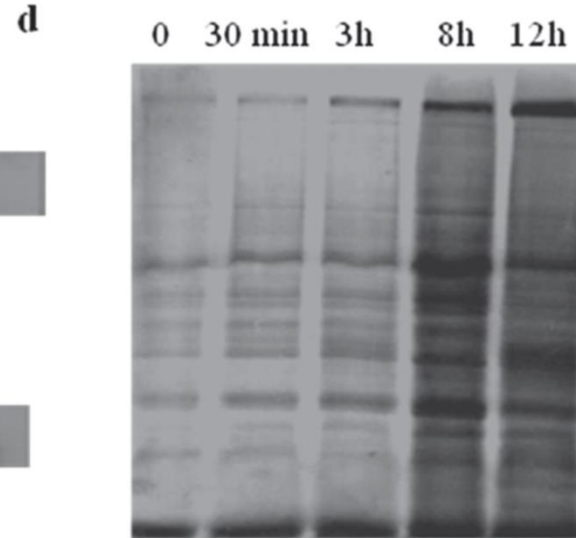
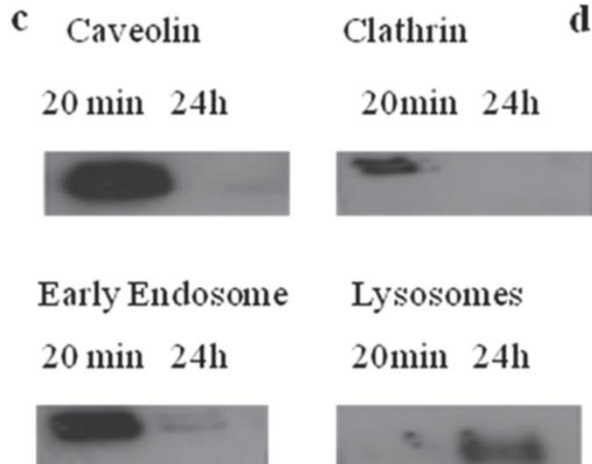
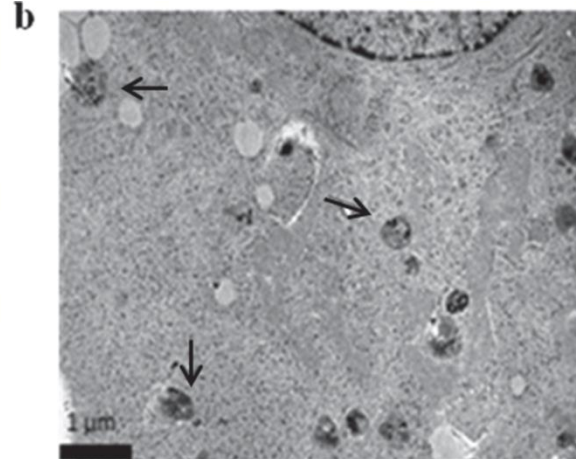
Pellet isolation



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