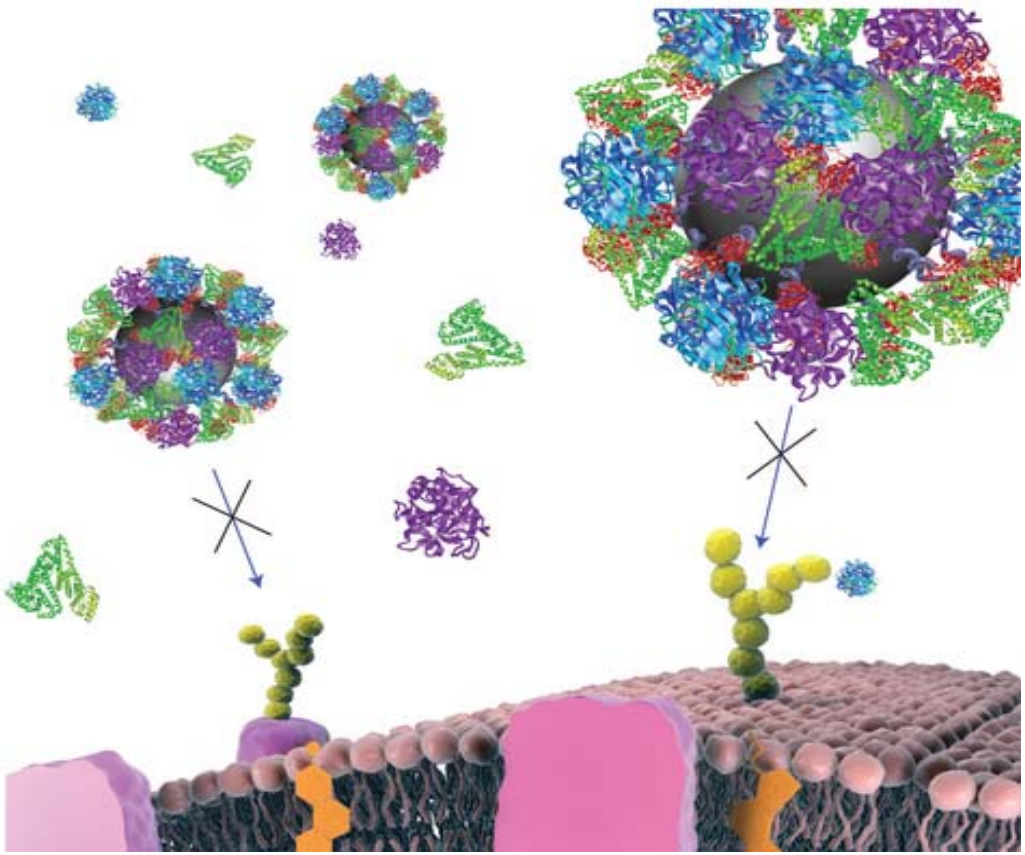
Fundamental nanoscale processes using FCS to examine the formation and kinetics of corona formation

Jason DeRouchey

Department of Chemistry
University of Kentucky

Protein Corona Formation



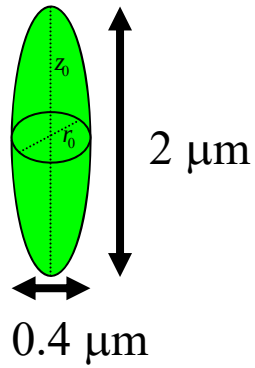
- Due to their high free energy, nanomaterial surfaces in contact with biological media are rapidly covered by biomacromolecules that form a corona.
- This corona is “what the cell sees” directly influencing nanomaterial/cell interactions

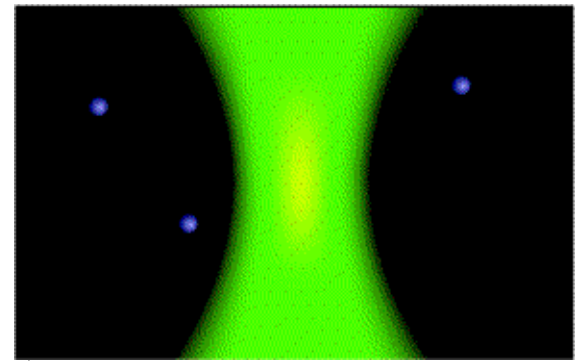
Reprinted from: Salvati, A.; Pitek, AS.; Monopoli, M. P.; Prapainop, K.; Bombelli, F. B.; Hristov, D. R.; Kelly, P. M.; Åberg, C.; Mahon, E.; Dawson, K. A. Transferrin-functionalized nanoparticles lose their targeting capabilities when a biomolecule corona adsorbs on the surface. Nature Nanotechnology. 2013, 8, 137-143.

Fluorescence Correlation Spectroscopy (FCS)

concentration fluctuations in a small fixed confocal volume

volume	:	1 μm^3 (~1 femtoliter)
conc.	:	~ 1 nM
# of particles		~ 1





w_0 : width

z_0 : height of illumination volume

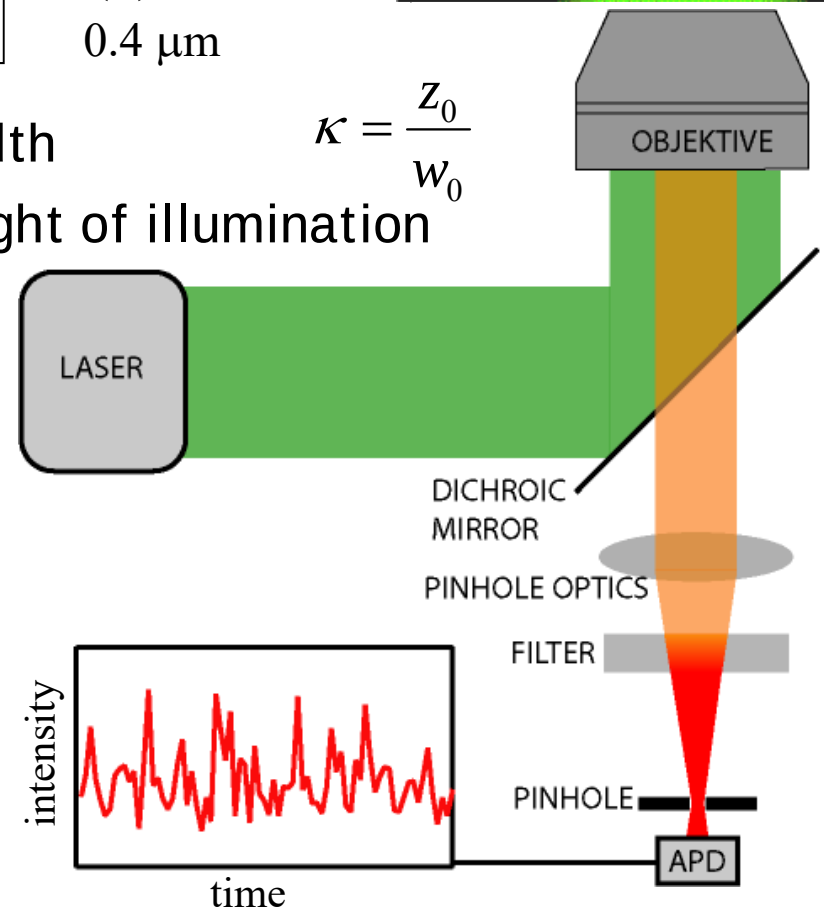
$$\kappa = \frac{z_0}{w_0}$$

fluorescence intensity

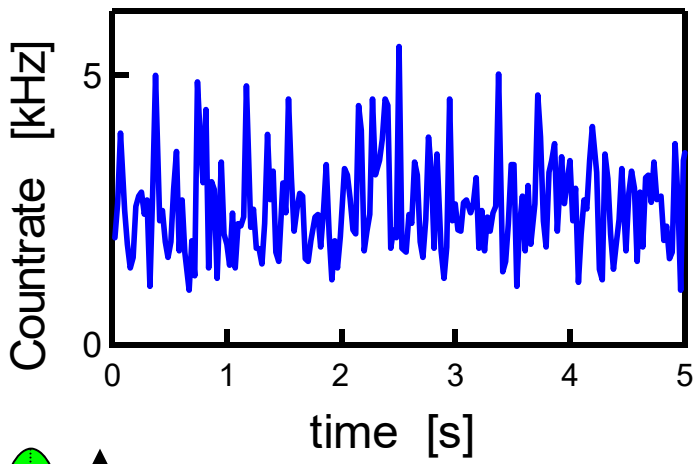
$$I(t) = \int W(\mathbf{r}) \cdot C(\mathbf{r},t) \; d^3\mathbf{r}$$

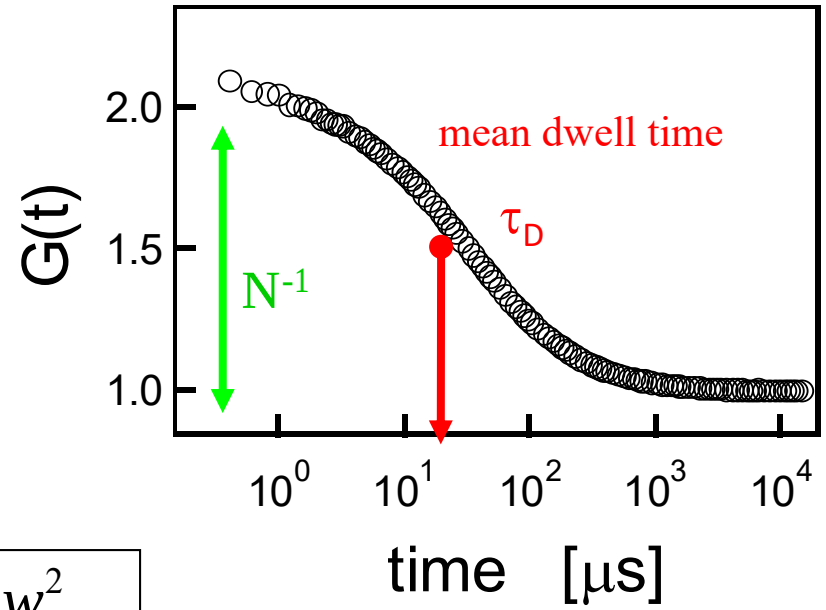
Spatial filter function (approx. Gaussian)

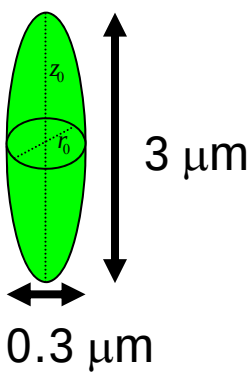
Particle concentration



FCS Auto-Correlation Function of Diffusing Particles





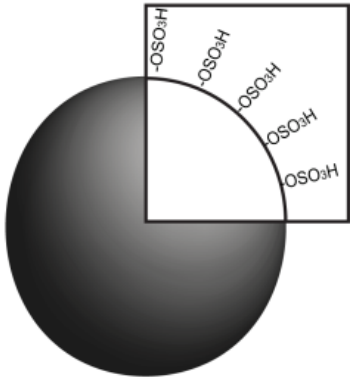


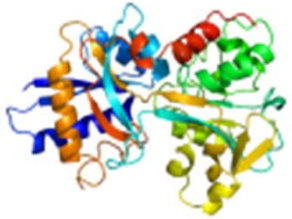
diffusion time : $\tau_D = \dfrac{w_0^2}{4D}$

$$G(t) = \frac{1}{cV_{eff}}\left(1+\frac{t}{\tau_D}\right)^{-1}\left(1+\frac{t}{\tau_D\,\kappa^2}\right)^{-1/2}+1$$

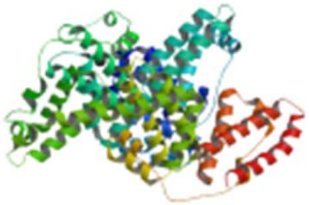
* concentration
* diffusion coefficient
* reaction kinetics

System Studied

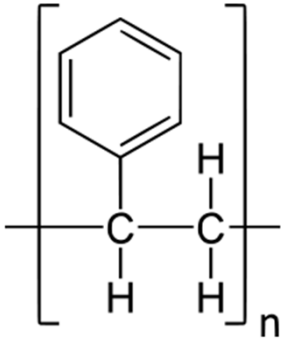




3.7 nm



3.5 nm



PS

<u>Bovine Serum Albumin (BSA)</u>

Most abundant protein in blood plasma

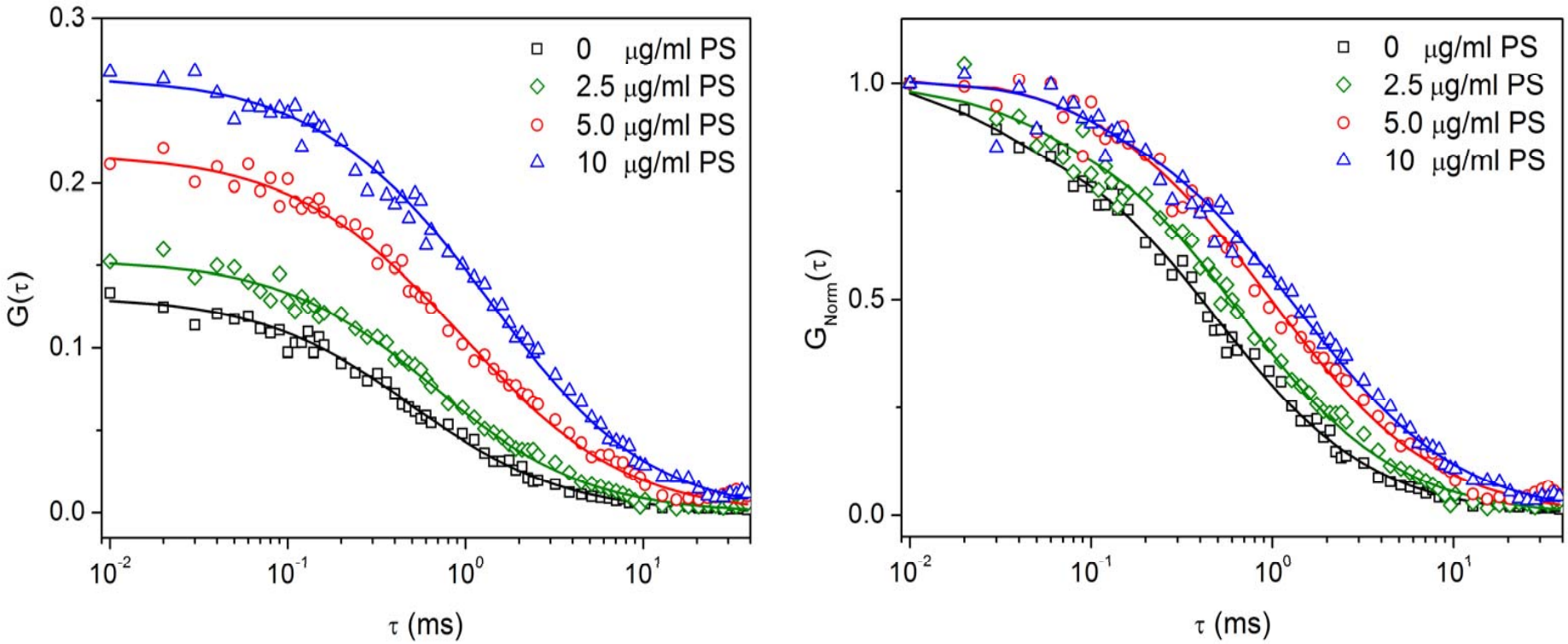
High hydrophobicity

<u>Human transferrin (Tf)</u>

Abundant human plasma glycoprotein

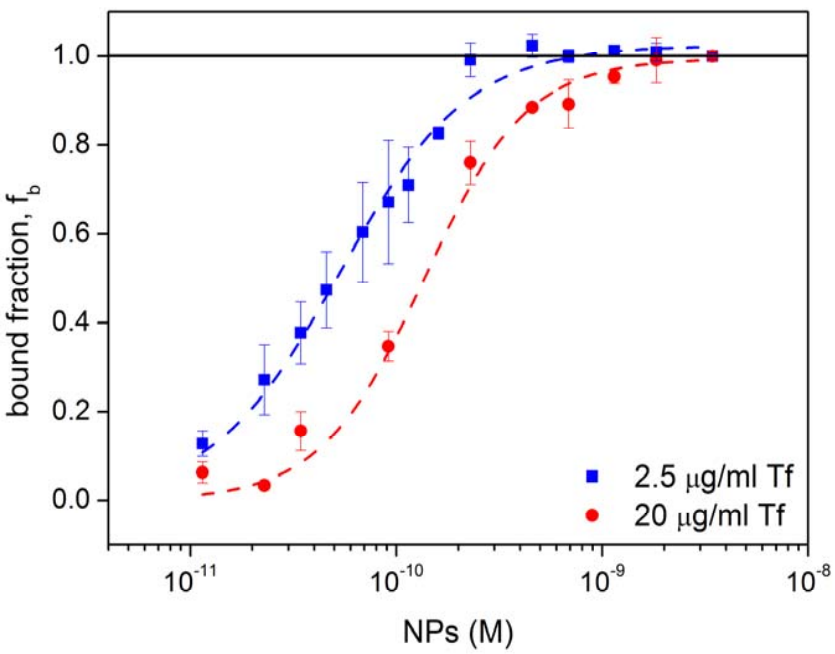
Widely used for site-specific drug delivery and cancer treatment

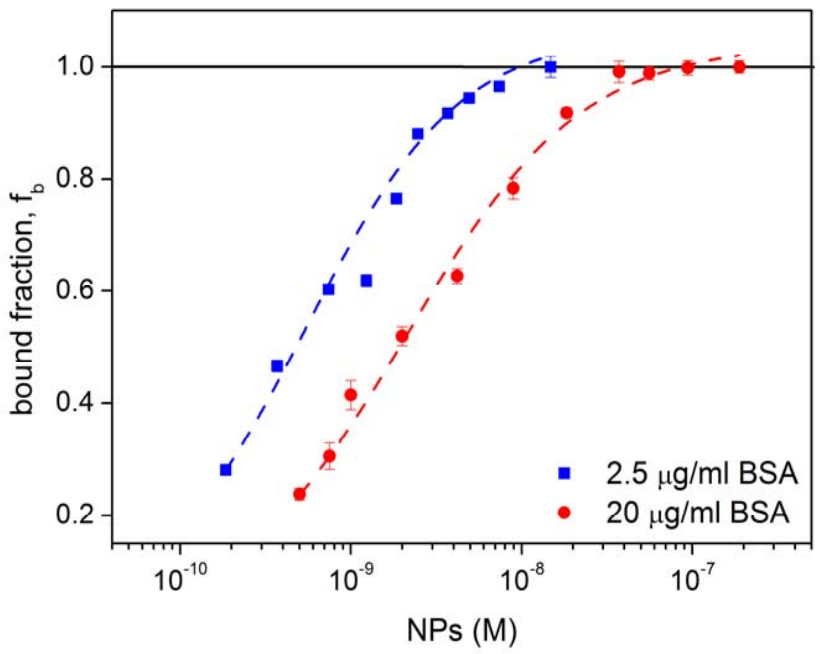
More bound proteins observed by FCS with increased NP concentration



Both proteins, Tf and BSA readily bind to sulfonated PS beads

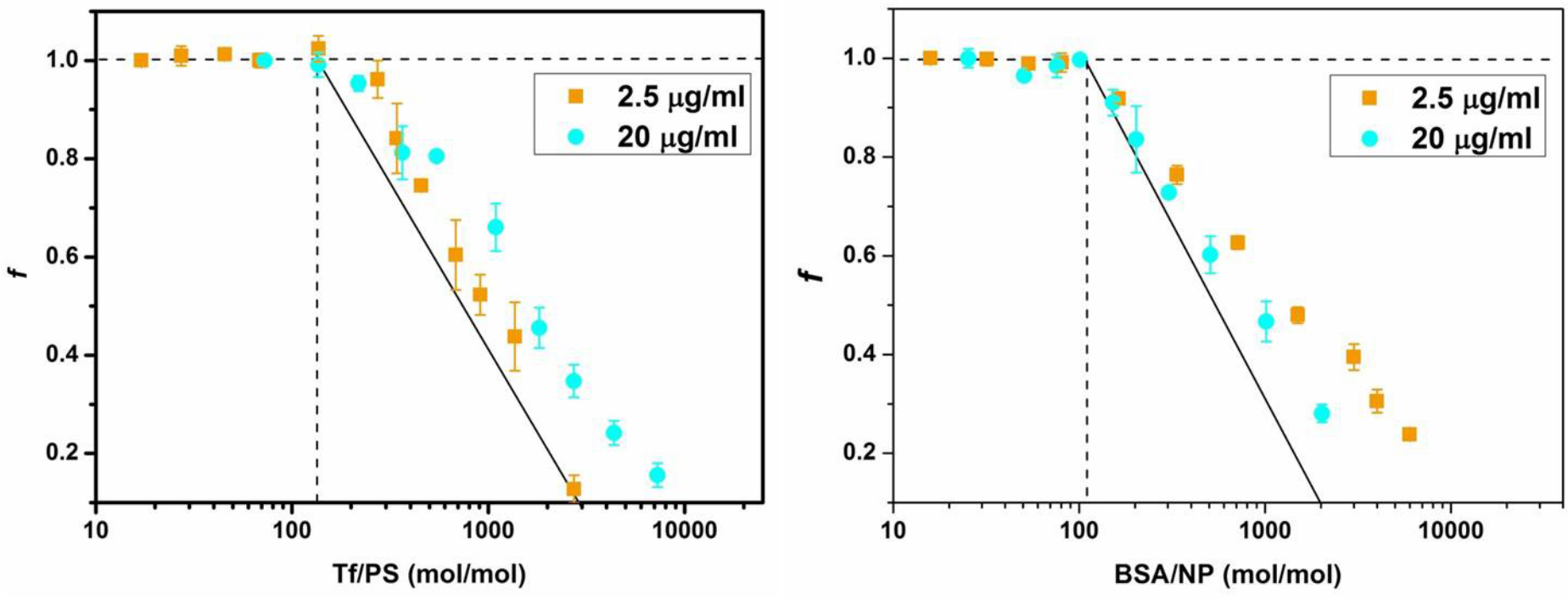
Protein adsorption consistent with Langmuir adsorption isotherms





Experiments performed at fixed concentrations
Fraction bound increases with nanoparticle concentration.
Dashed lines are Langmuir adsorption curves.

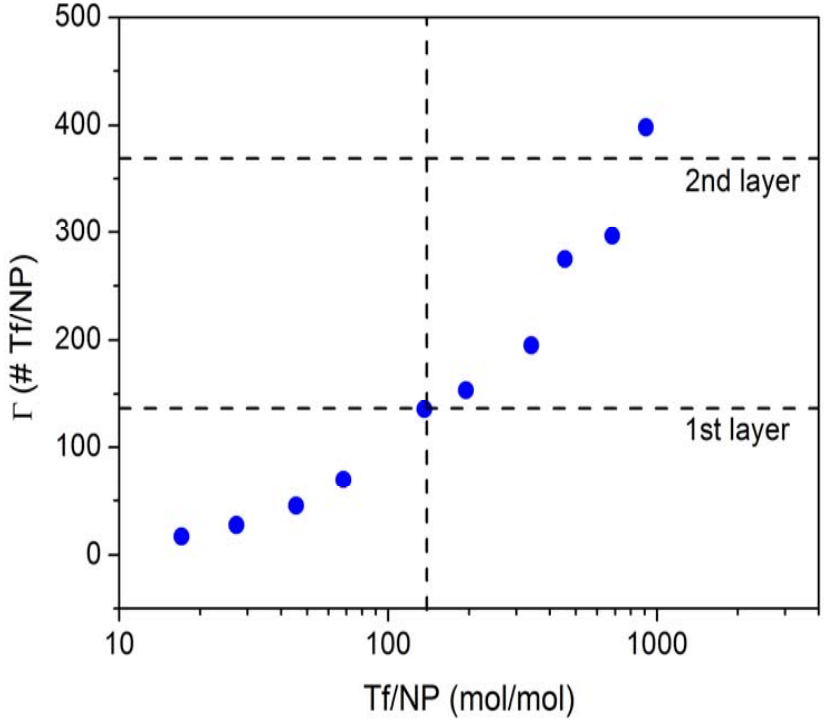
Fraction bound vs the ratio of protein to NPs

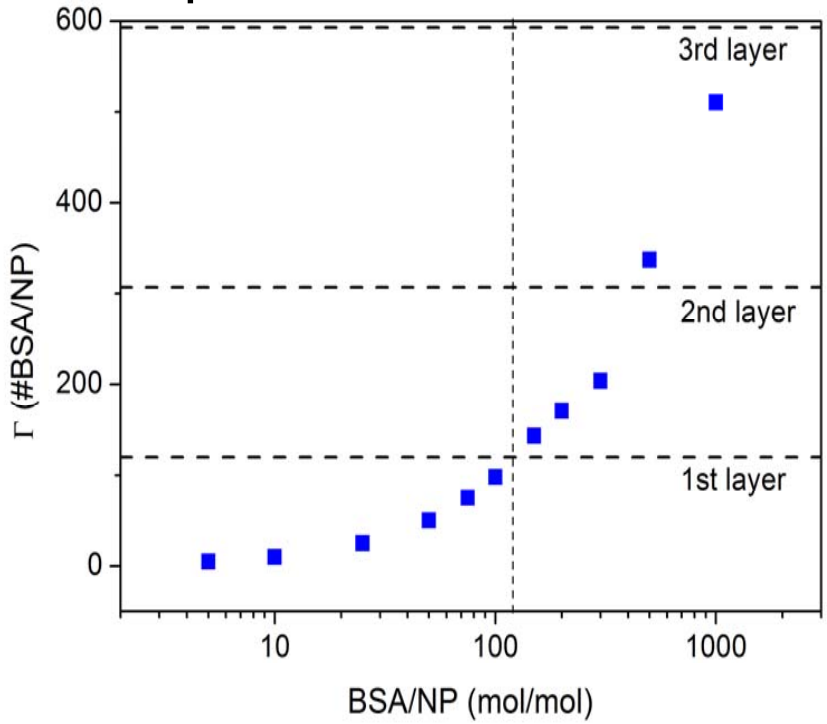


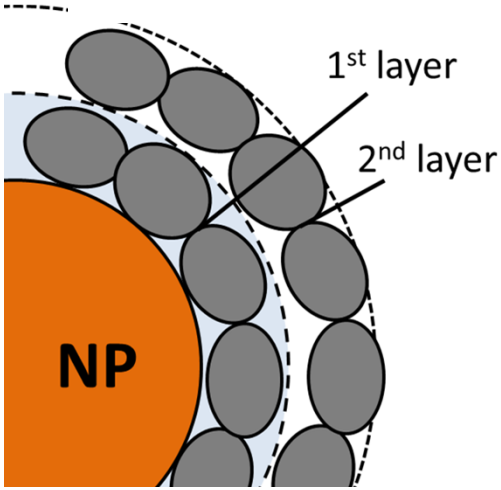
Molar ratio ~130 Tf / 110 BSA is reasonable for calculated surface area of 50 nm nanoparticle

The bound fraction does not decrease as rapidly as predicted for a simple strong binding model indicating that at higher protein/NP concentrations beyond a single layer of protein is binding to the nanoparticles.

Surface coverage (number of protein molecules bound per NP)



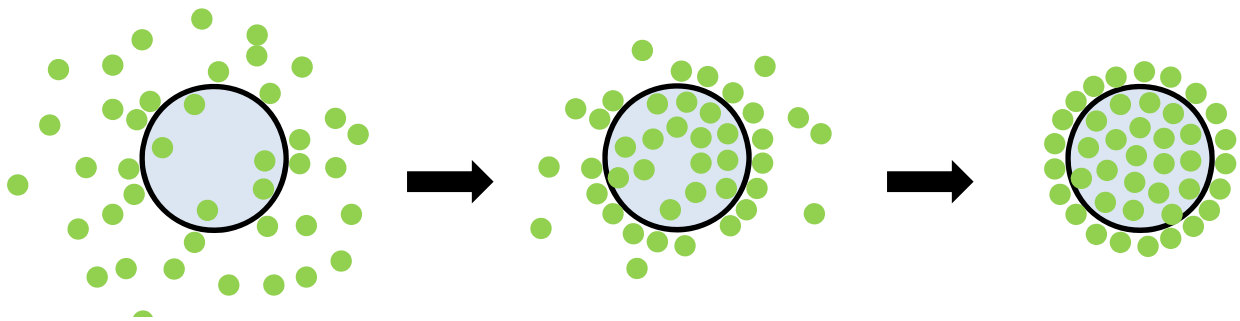


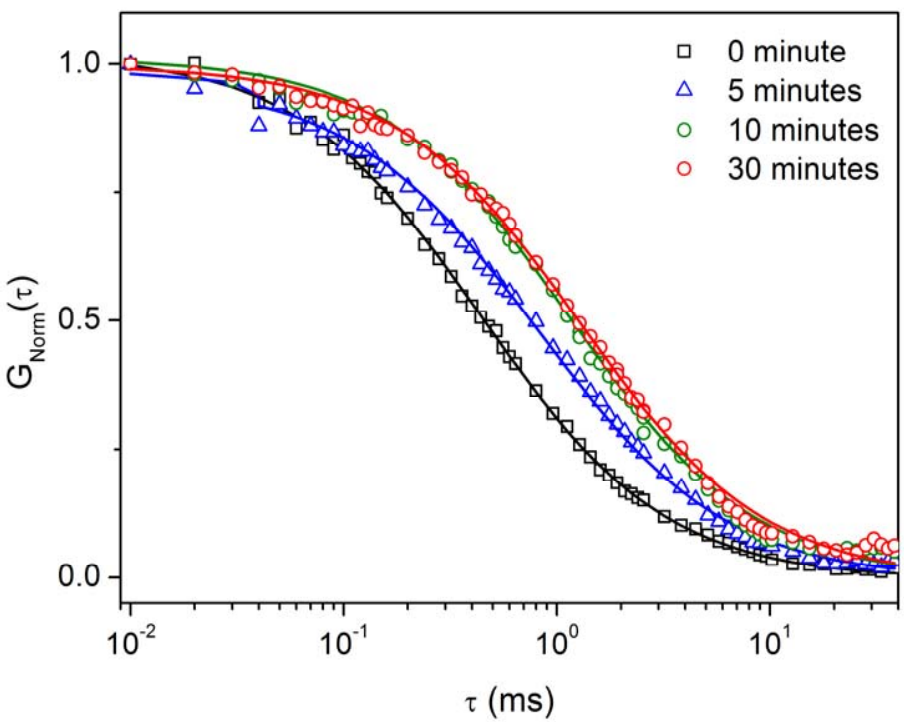


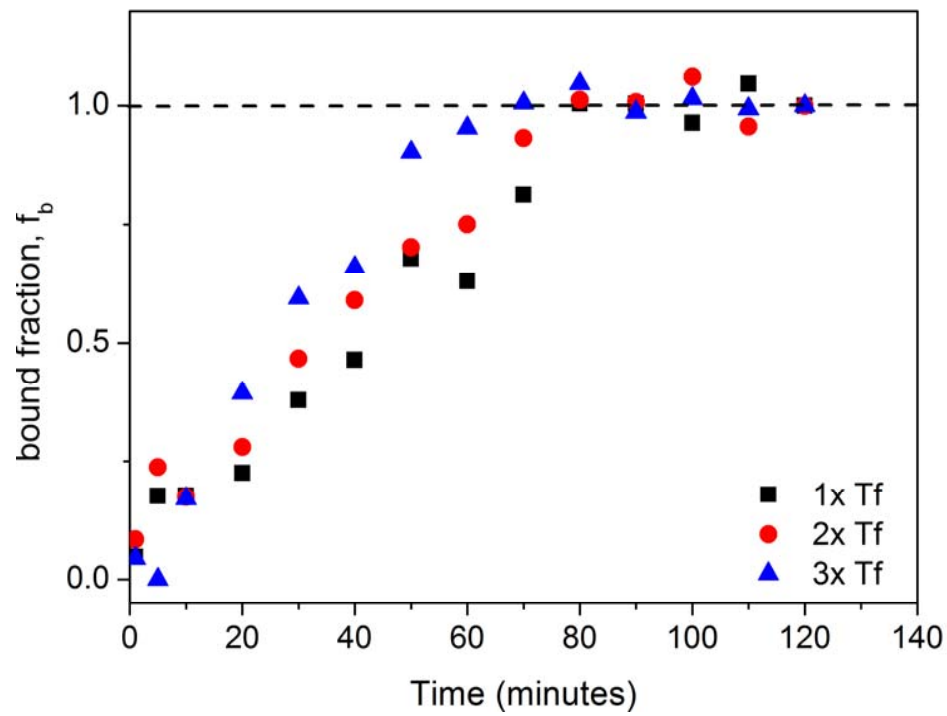
* Model assumes strong interaction of protein with uncovered NP surface and weak interaction of protein with 1st layer

* The horizontal dash lines indicate the calculated maximum numbers of protein per layer based on surface area per protein.

Real-time tracking of protein corona formation

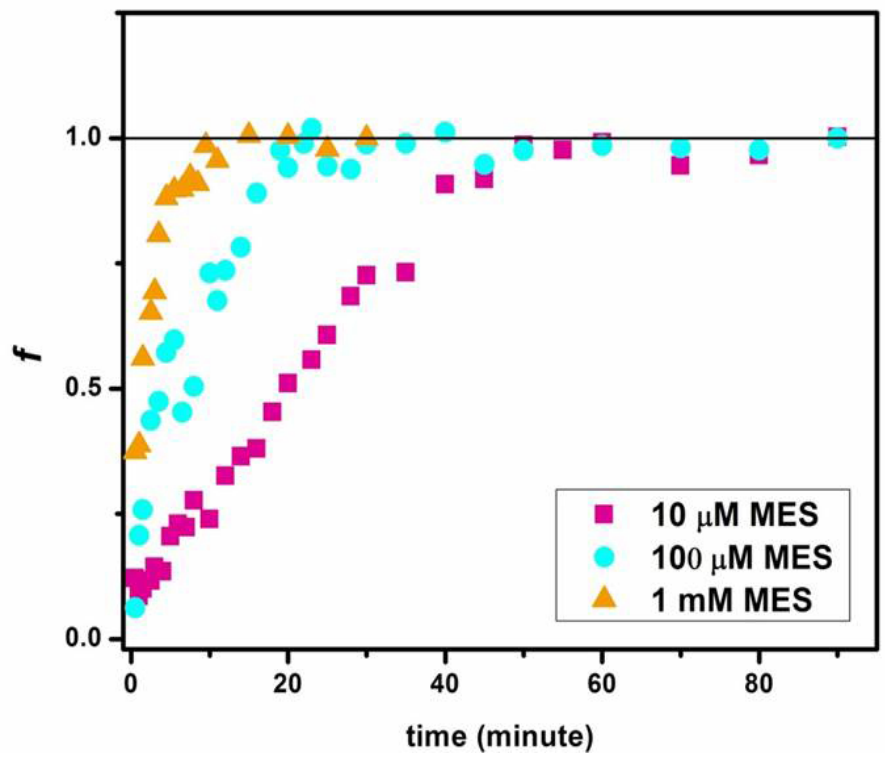




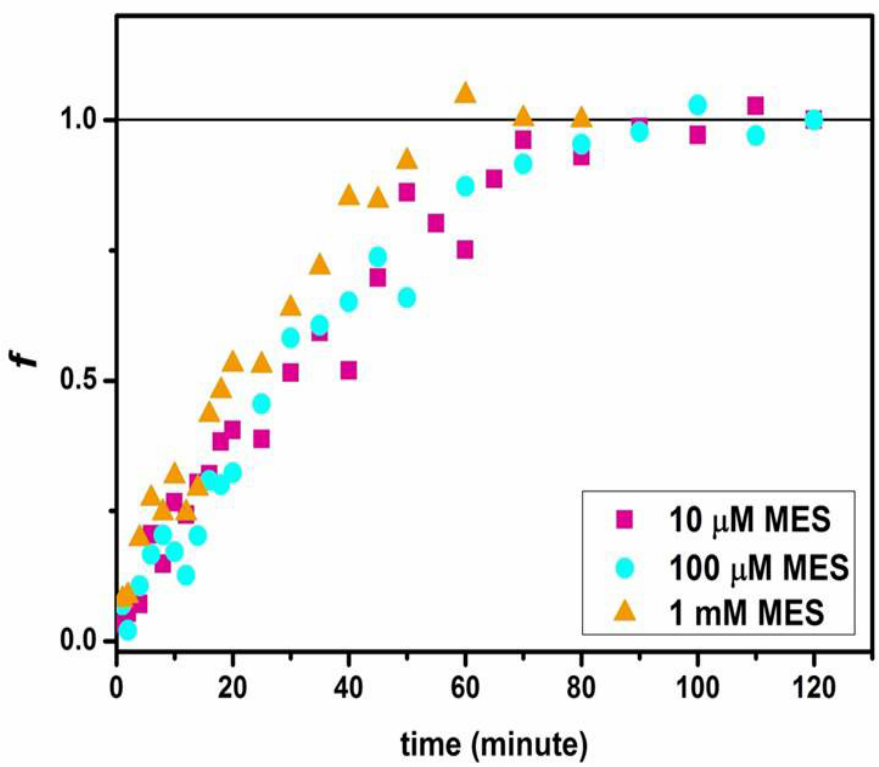


Buffer concentration effect on protein binding rate

Tf-NPs

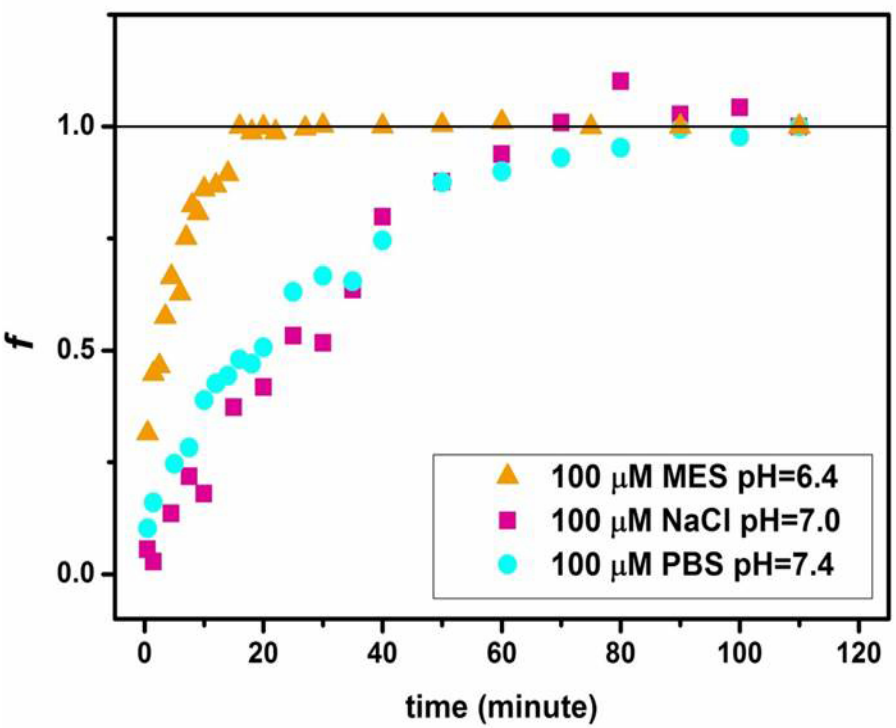


BSA-NPs

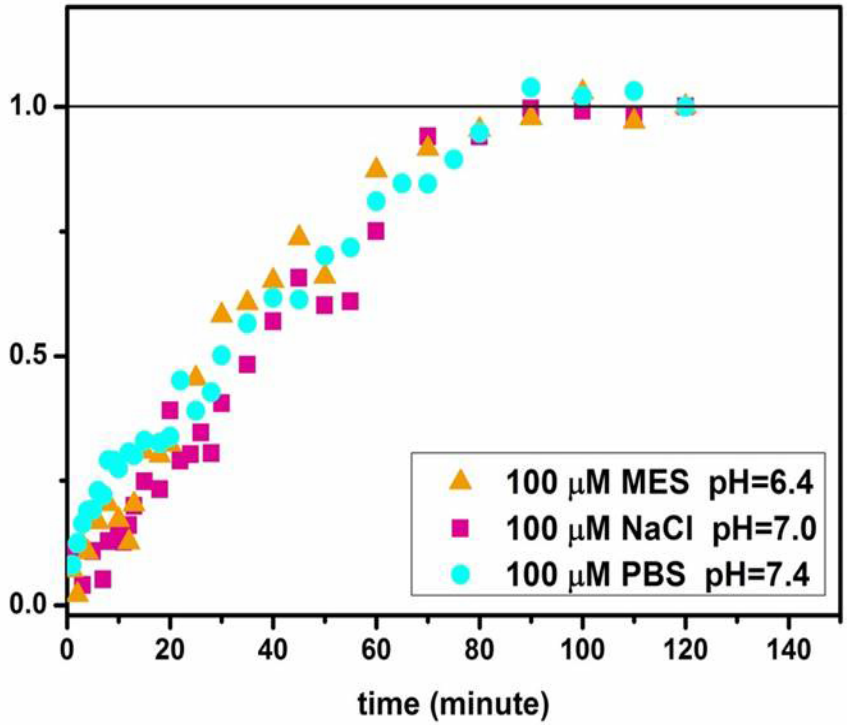


Lower pH leads to a significantly faster binding rate for Tf

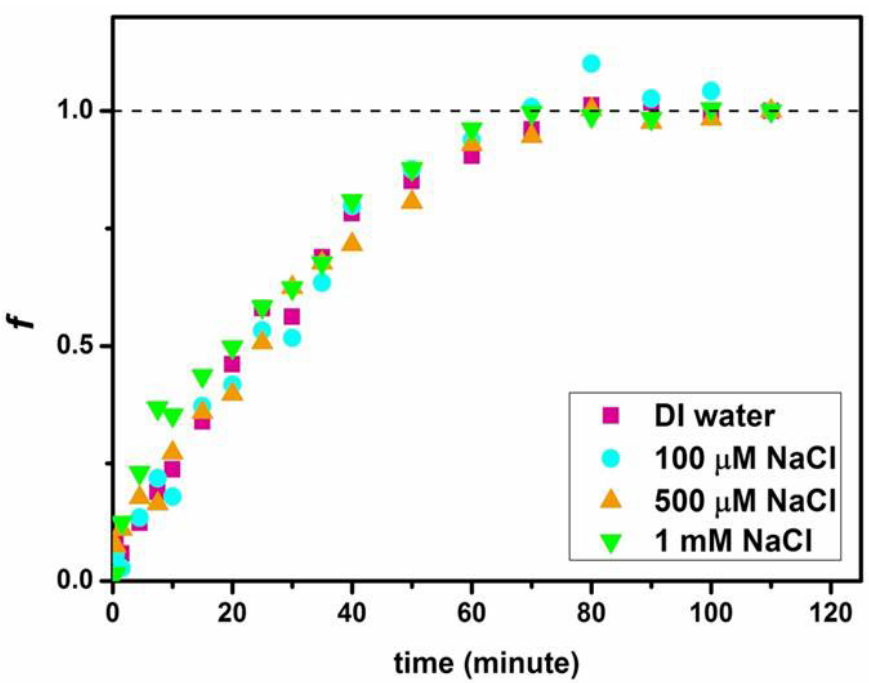
Tf-NPs

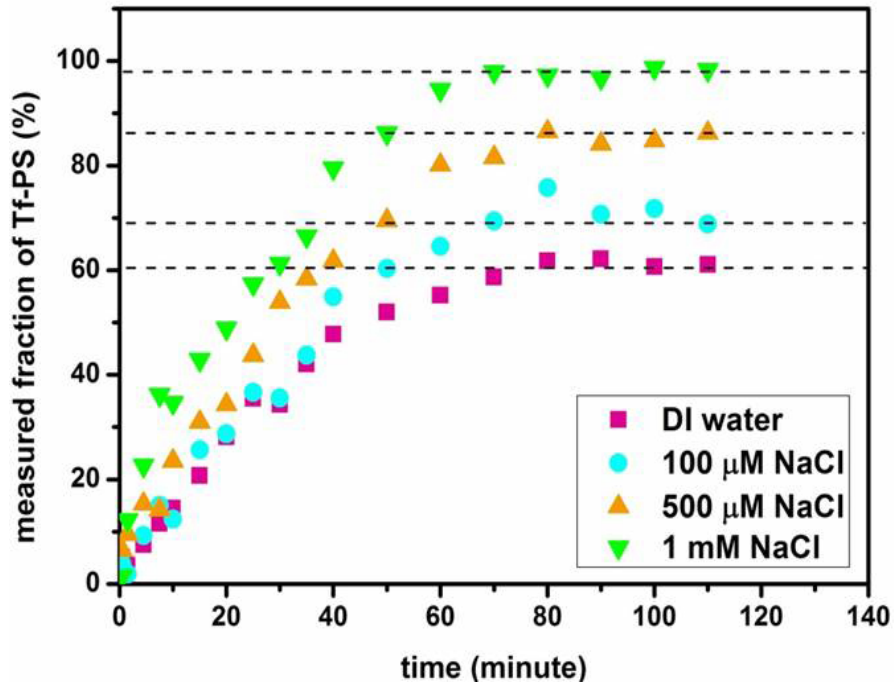


BSA-NPs

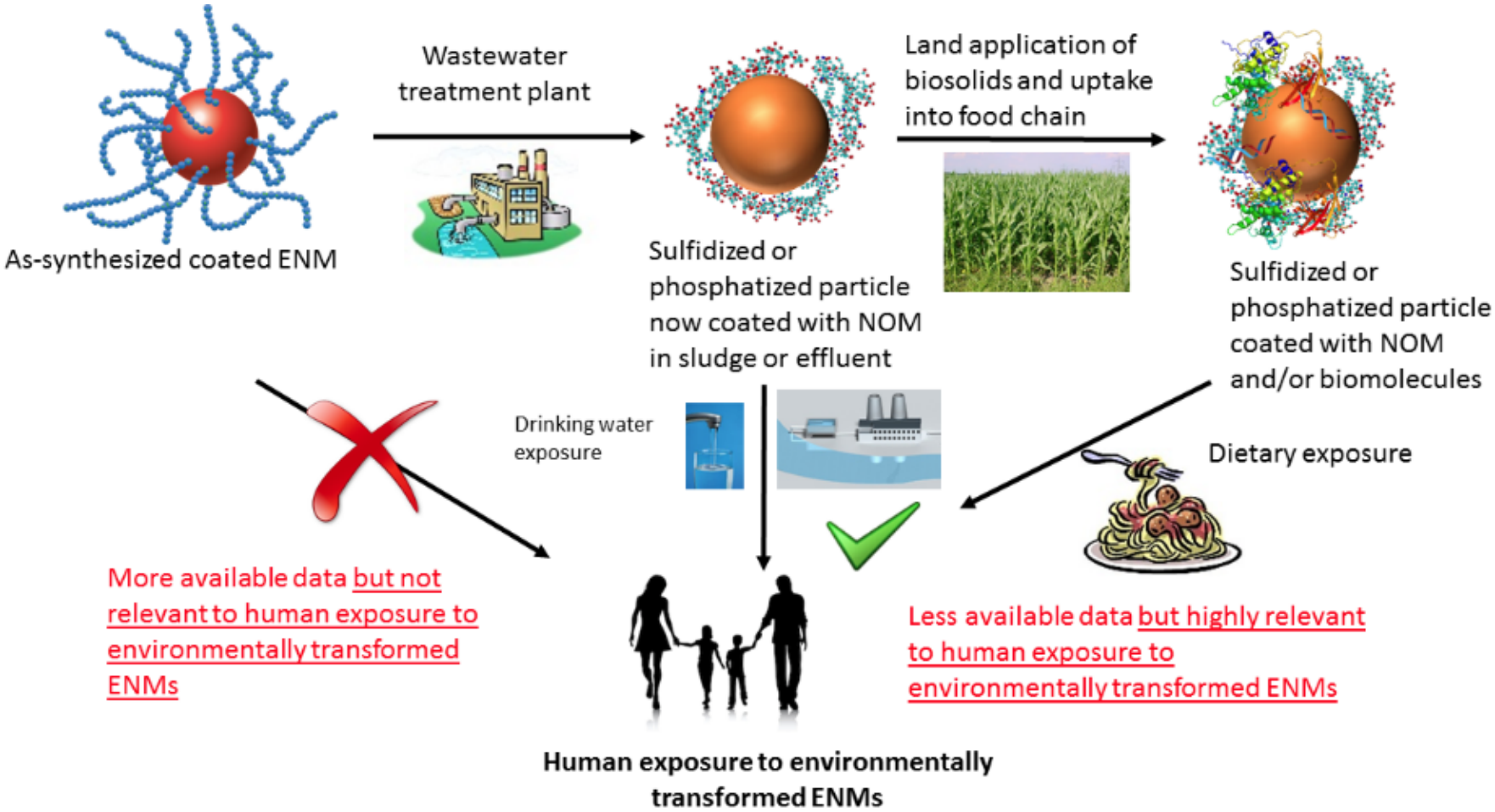


Added salt enhances binding affinity but does not affect binding rates





Future Directions



In collaboration with Jason Unrine, Robert Yokel, and Eric Grulke (UK)

Exposure of *C. elegans* to artificially sulfidized Ag NPs

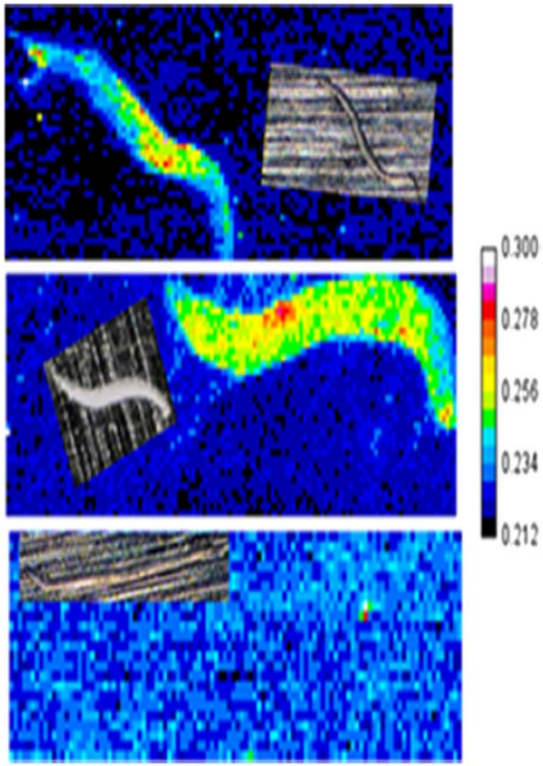
X-ray fluorescence microscopy

Ag accumulation at LC_{30}

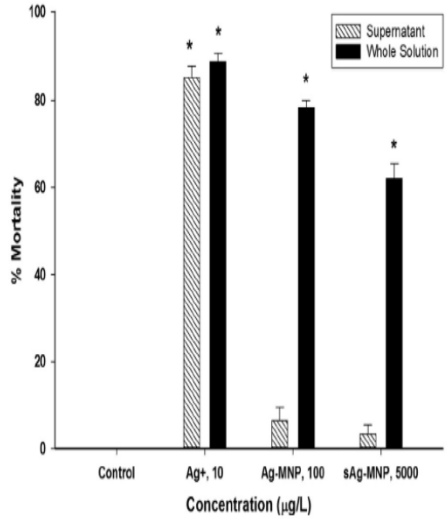
$AgNO_3$

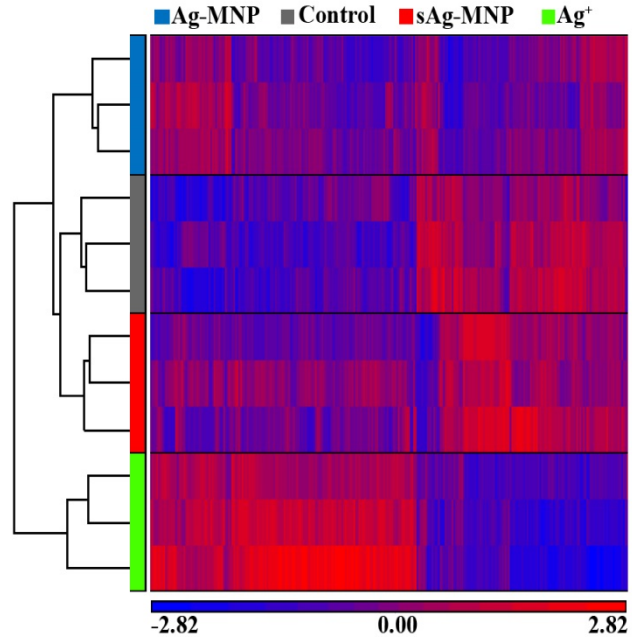
PVP-AgNP

Ag_2S-NP

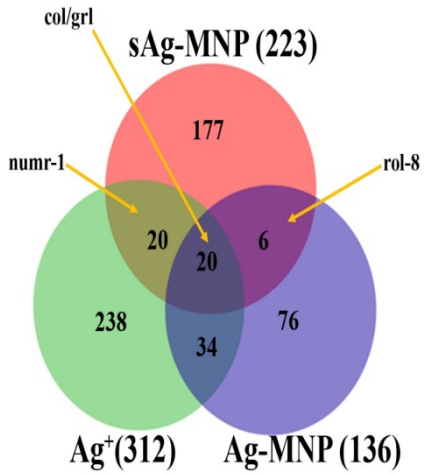


Supernatant exposures





Whole genome transcriptome for *C. elegans* exposed at the EC30 for reproduction



Venn diagram of differentially expressed genes

Work of Jason Unrine (UK)

Environmental Pollution 196 (2015) 239e246

Conclusions

Apologizes to TED.com

1) FCS is a useful tool for examining corona self-assembly, size and time scales

2) Significant differences are seen in protein adsorption to PS; most likely due to favorable hydrophobic interactions

3) We are currently leveraging this capability to a wide-variety of nanosystems to look at effects of aging

UK UNIVERSITY OF KENTUCKY

NSF