



Characterizing Heteroaggregation between Carboxylated Carbon Nanotubes and Kaolinite Using Flow Cytometry

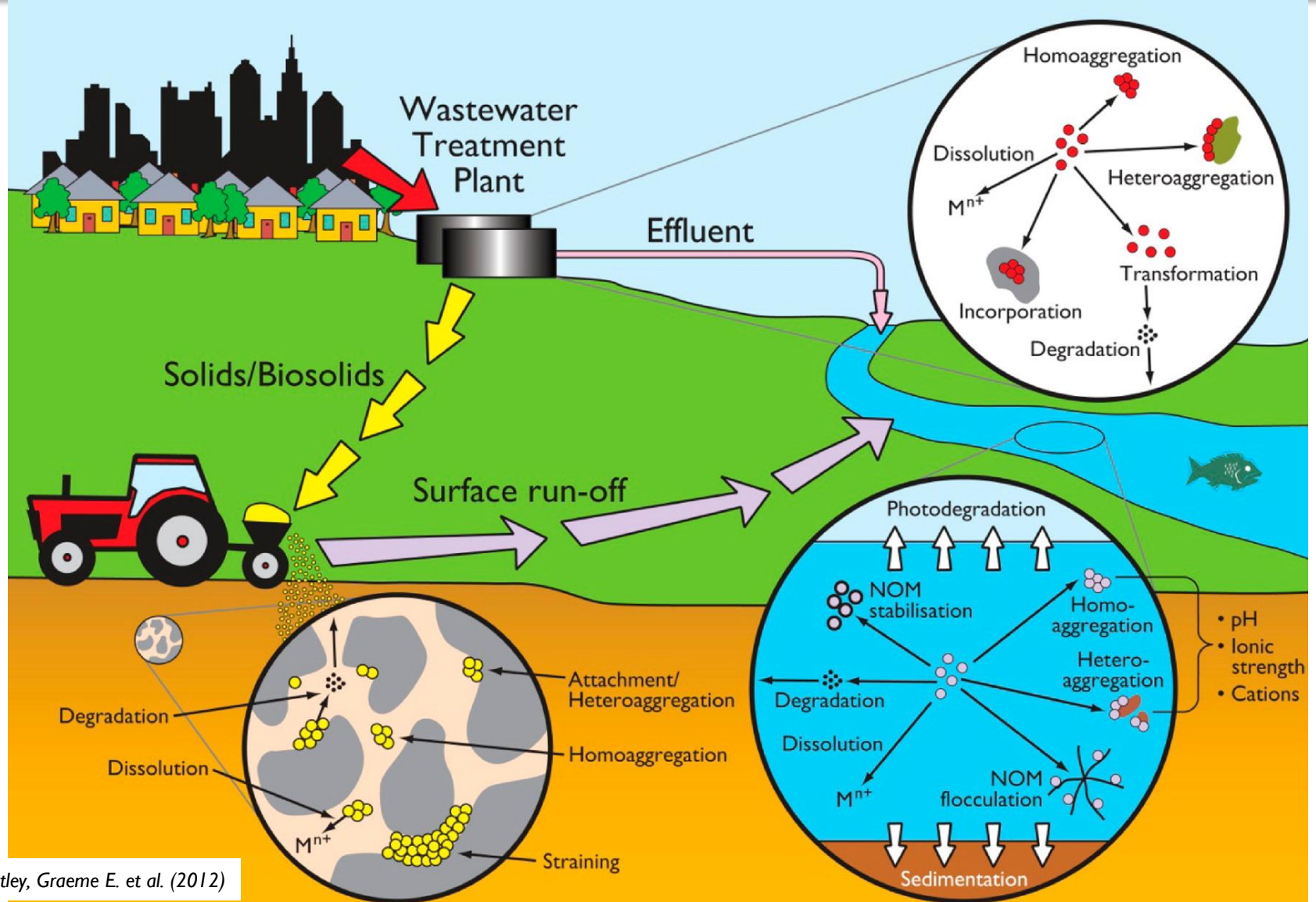
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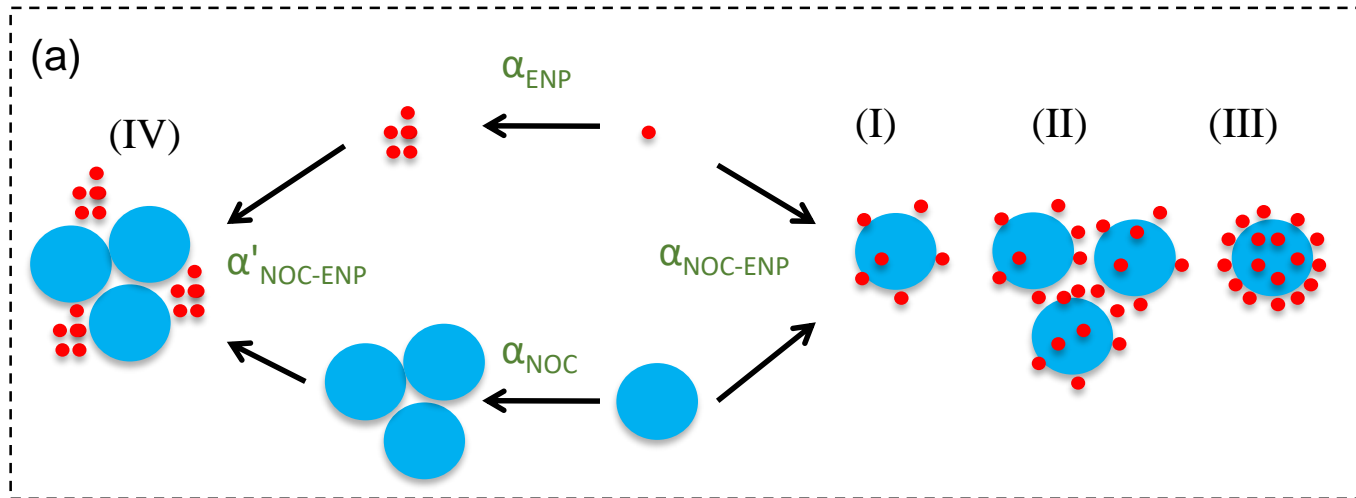
November 5, 2017

Engineered Nanomaterials (ENMs) and Naturally Occurring Colloids (NOCs)

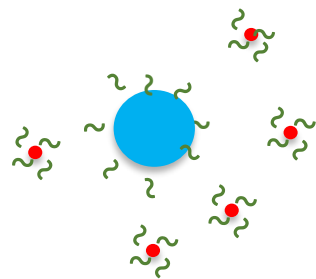




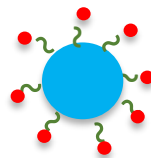
Complex Interactions among ENMs, NOCs and NOM



(b)



(c)



NOC

• ENM

~ NOM

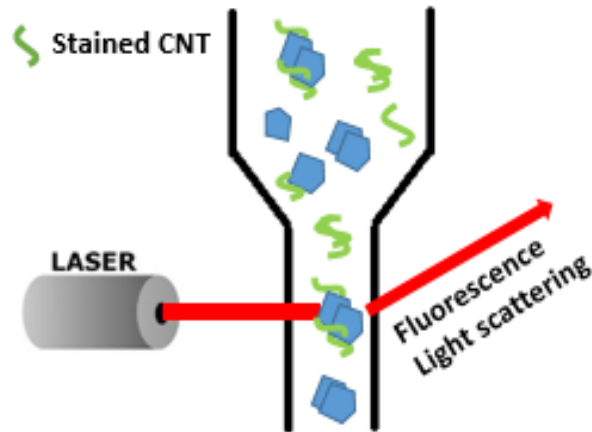


Challenges in Studying Heteroaggregation

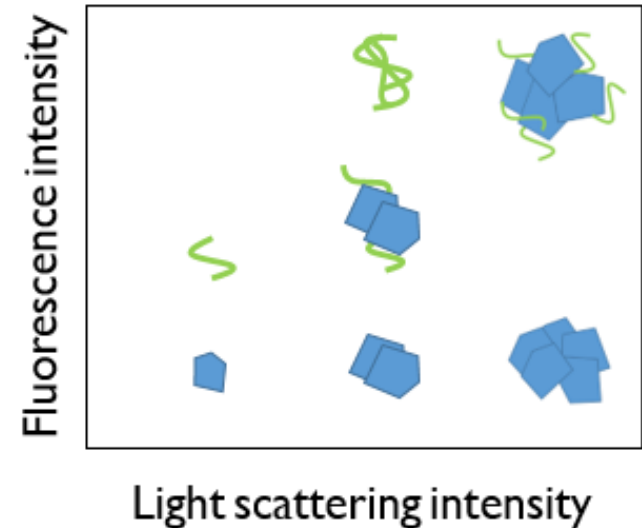
- Conventional methods
 - DLS
 - Not able to distinguish hetero- vs. homo- aggregation
 - No structural/compositional information
 - Relatively high particle concentration
 - Cryo TEM
 - expensive
 - small number of particles
 - unable to analyze samples in situ or real time
- Flow cytometry



Characterizing Small Particles Using Flow Cytometry



Plot of a mixture sample



Advantages:

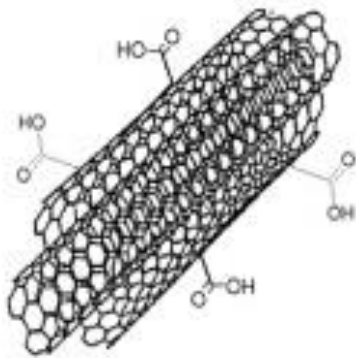
- ❑ Single particle detection
- ❑ Rapid, non-destructive measurement
- ❑ Real-time, simultaneous measurement of size and fluorescence
- ❑ Measurement at very low particle concentrations



Research Objectives

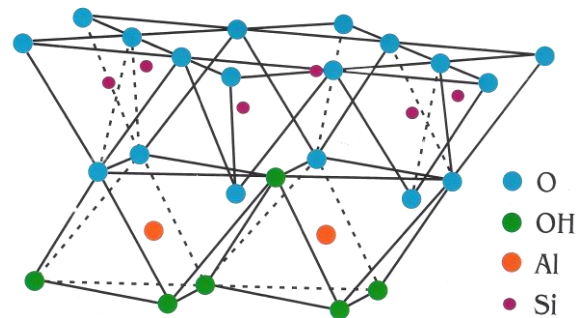
- ❑ Characterize heteroaggregation of COOH-CNTs and kaolinite using flow cytometry
- ❑ Study the effect of solution chemistry (pH and IS) on heteroaggregation.

I-D ENM



COOH-MWCNT

Model NOC

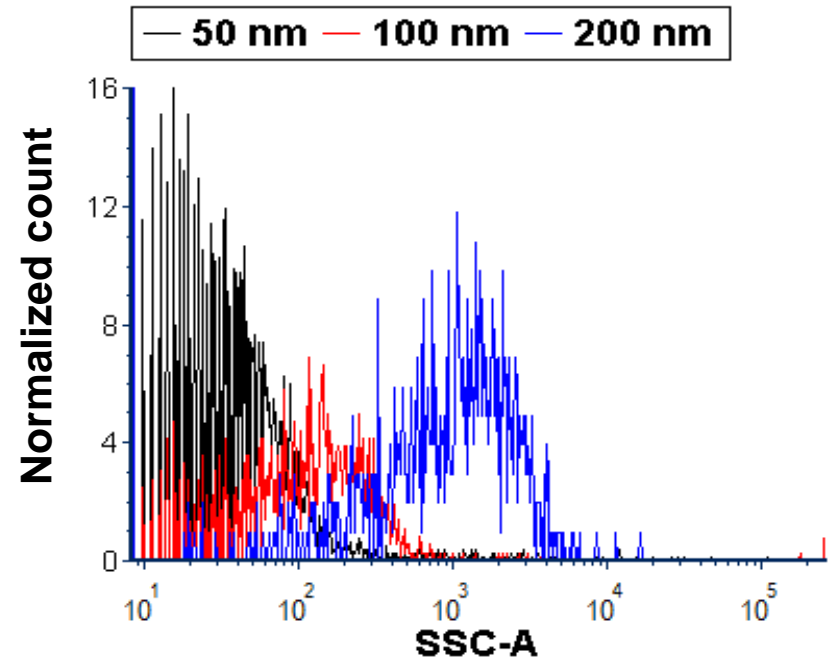
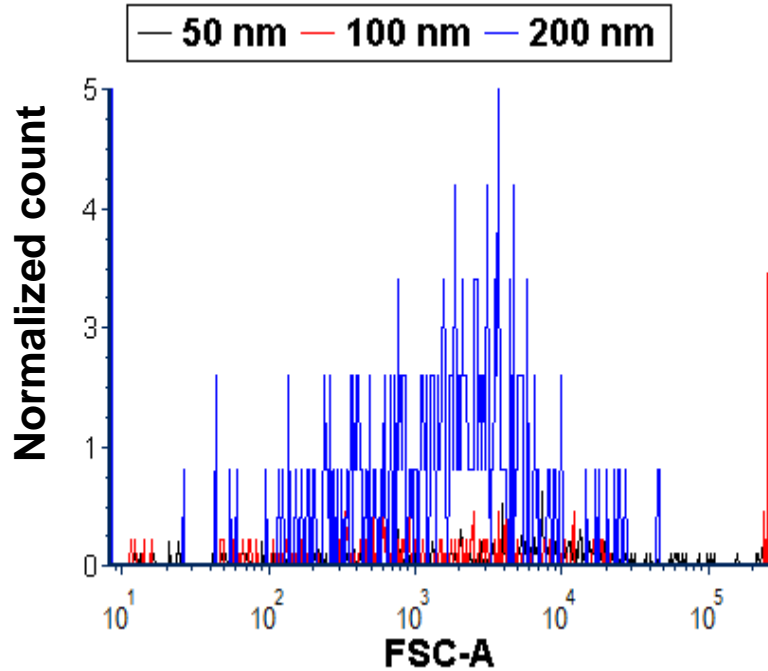


Kaolinite



Size Measurement by Side Light Scattering

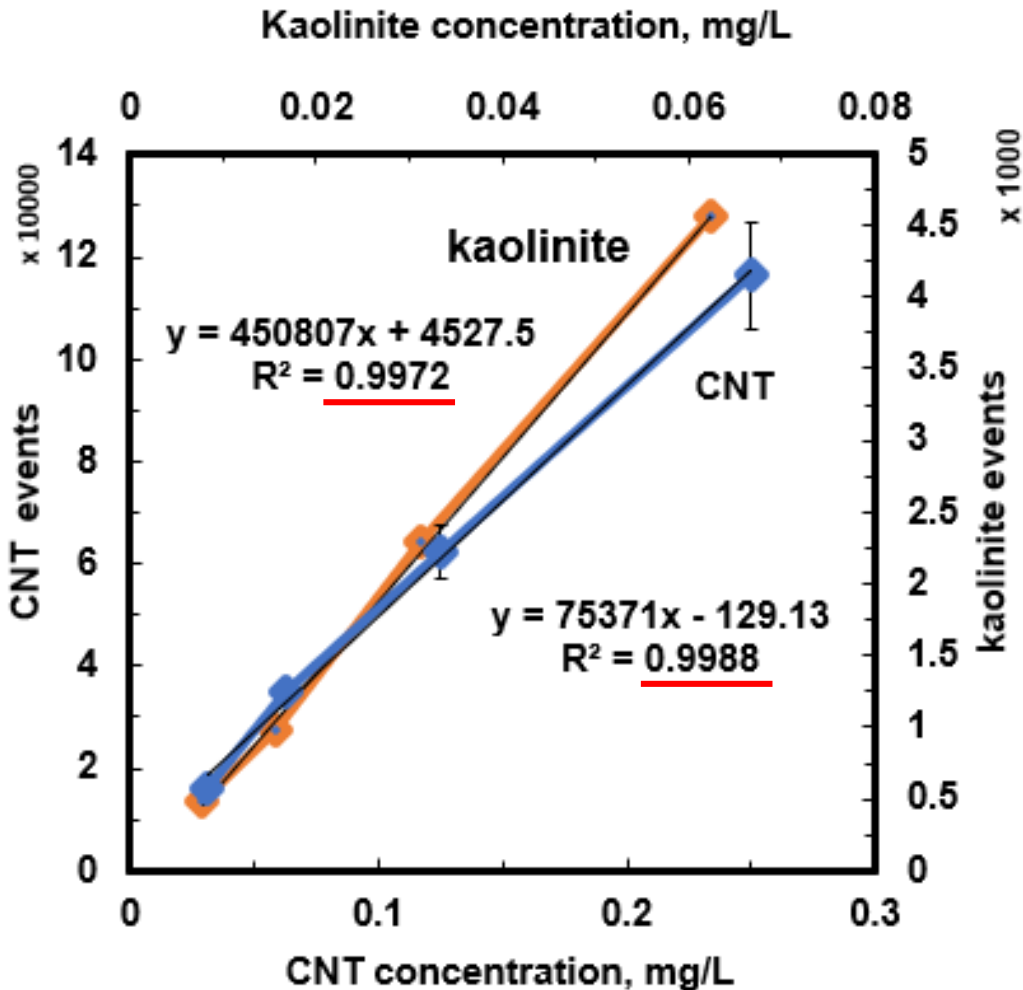
Spherical polystyrene particle



Size	FSC-A mean	SSC-A mean
50 nm	478	67
100 nm	646	213
200 nm	694	571



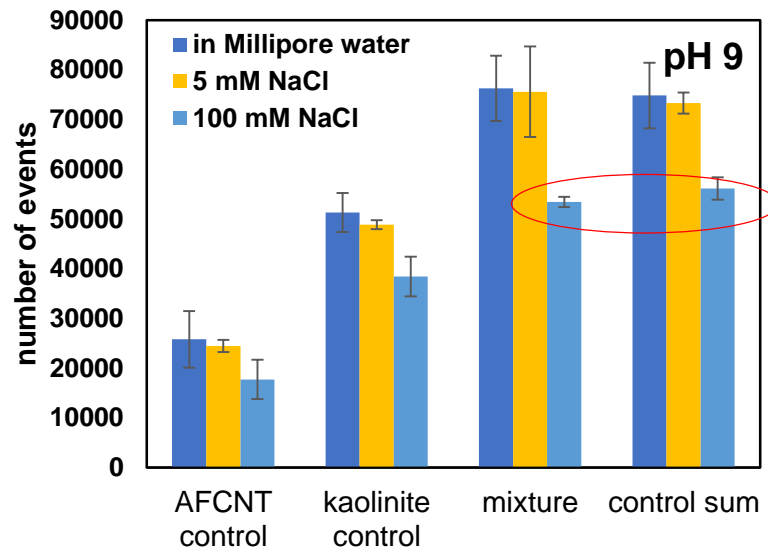
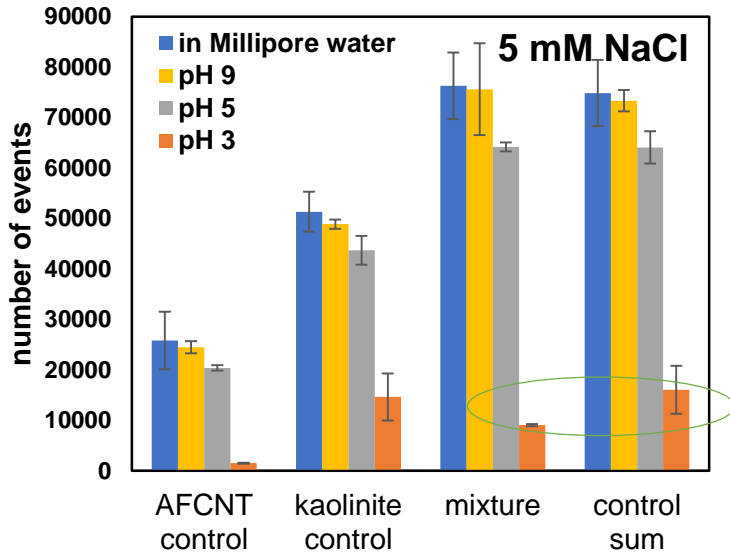
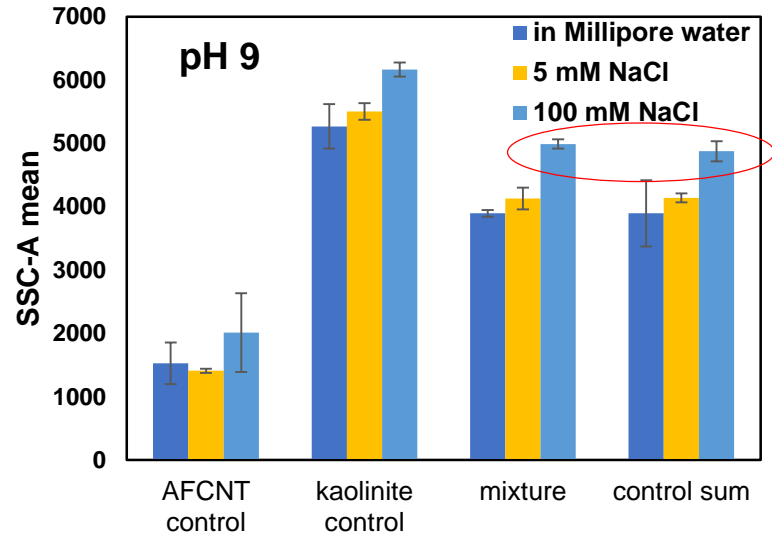
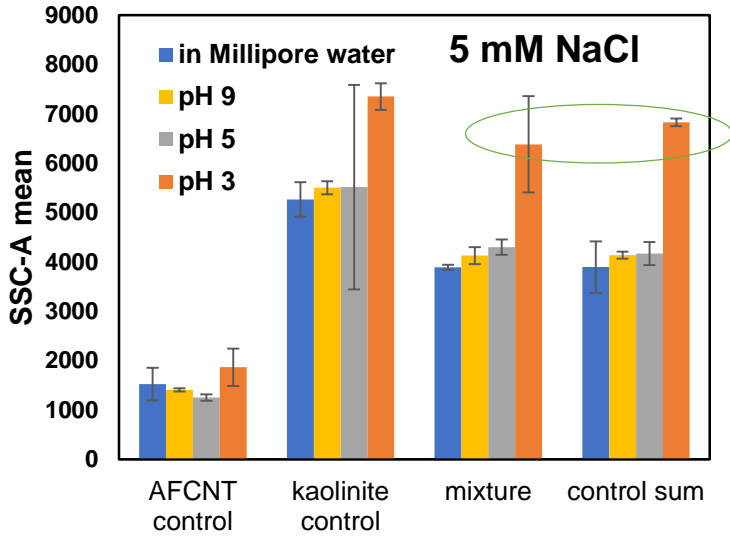
Swarm Effect negligible



- ❖ When particle sizes are small, an event can be generated by multiple particles.
- ❖ Swarm effect is negligible for both CNT and kaolinite in this study.



Characterization Using Side-Scattering Alone





Characterizing Heteroaggregation Using Fluorescently Labeled CNTs

Alexa Fluor 633 hydrazide (dye)
& EDC



Shake 16 hrs
in dark



Centrifuge > 3 times
(washing)

COOH-MWCNT
in MES buffer



1 hr 400k rpm

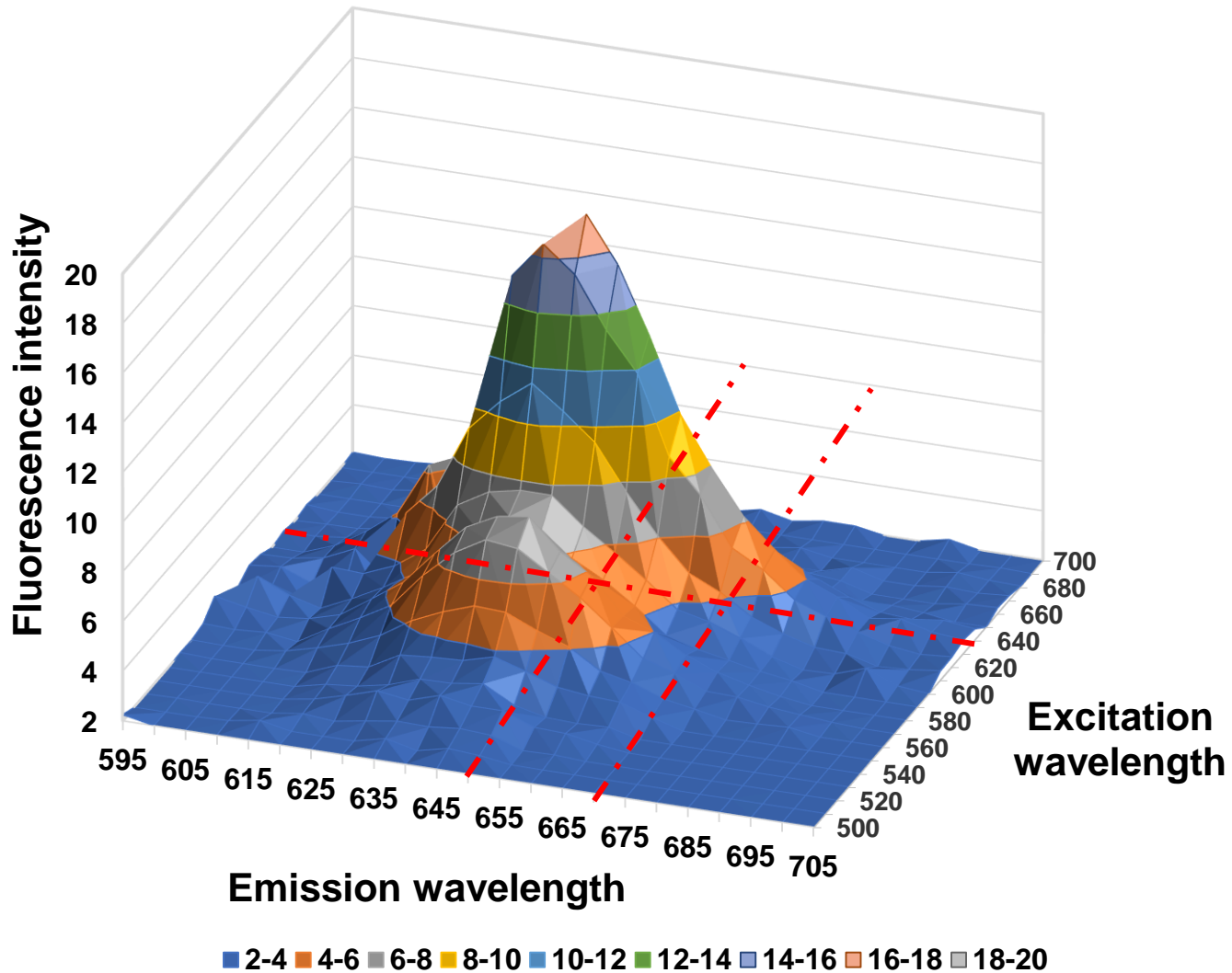


0.0625 ppm AF-CNT
0.625 ppm kaolinite
0.0625 ppm AF-CNT & 0.625 ppm kaolinite mixture
(AF-CNT: kaolinite ratio 0.1)

FACS Canto II
Flow cytometer measurement

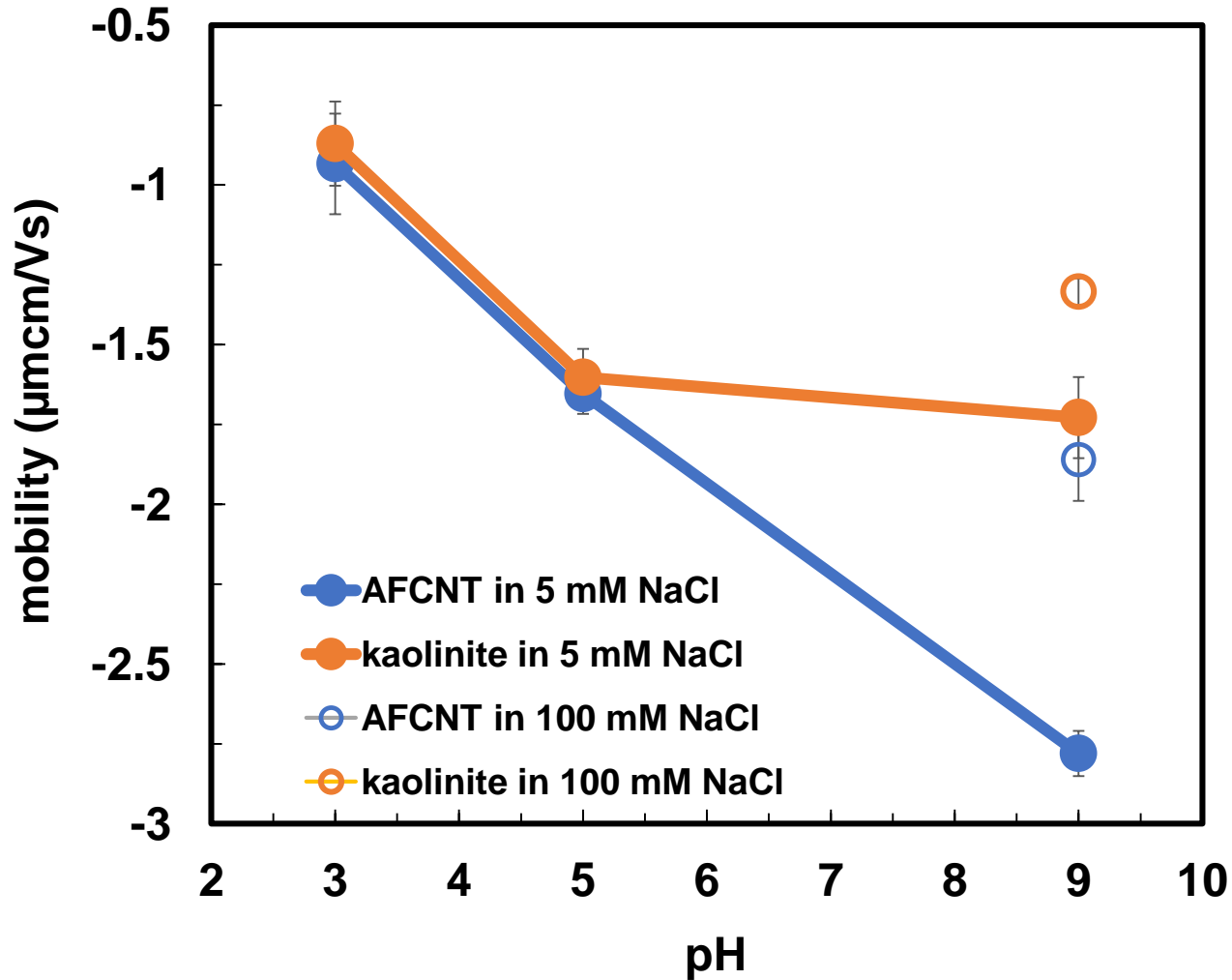


Fluorescence Detection of CNTs



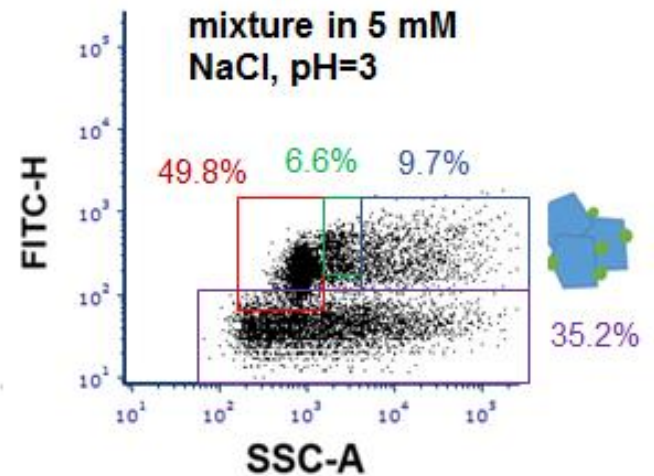
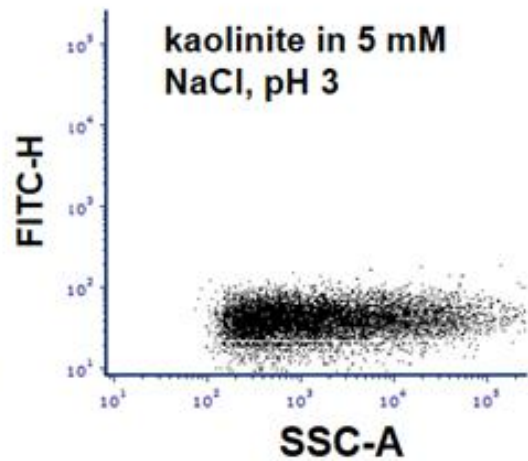
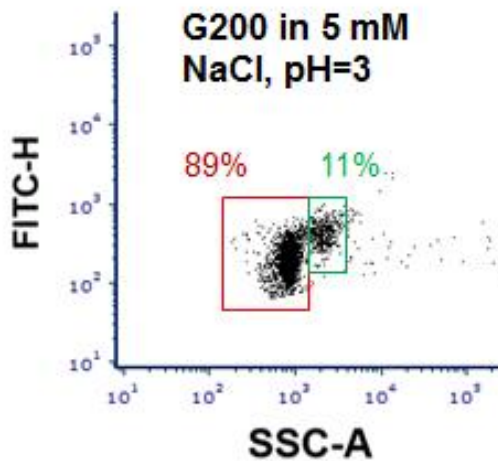
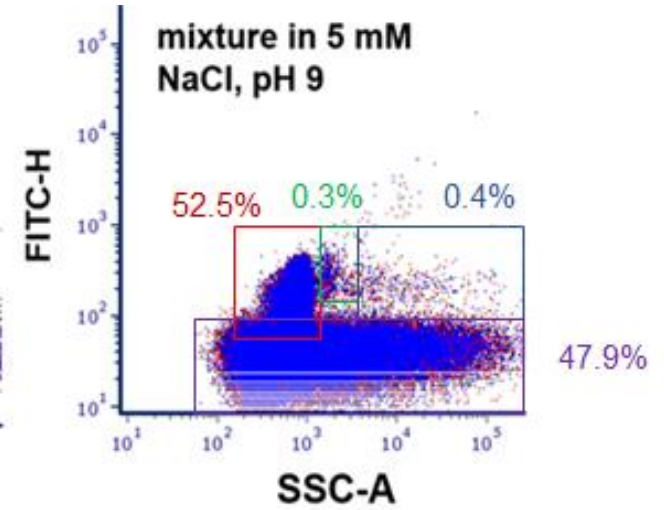
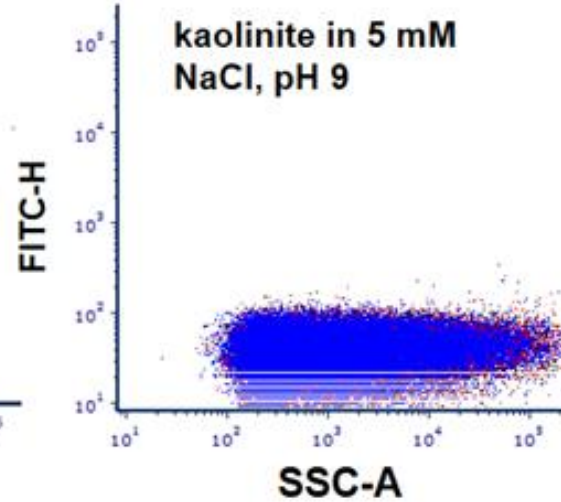
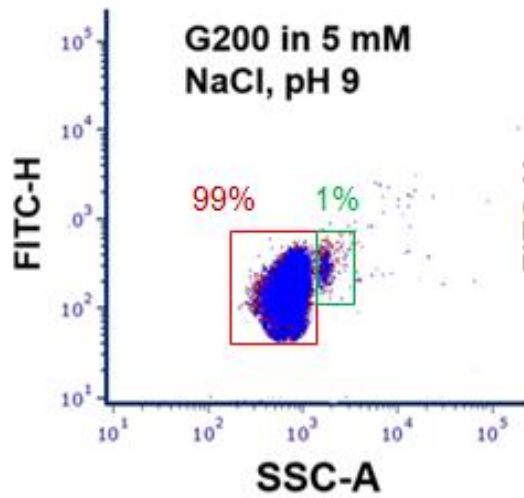


Electrophoretic mobilities of AFCNT and kaolinite



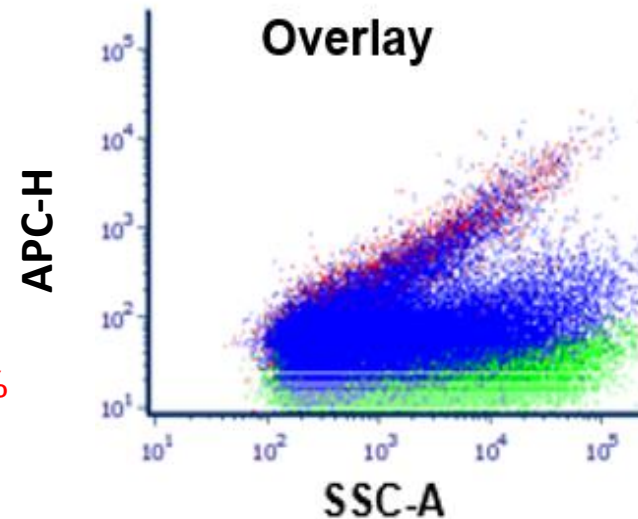
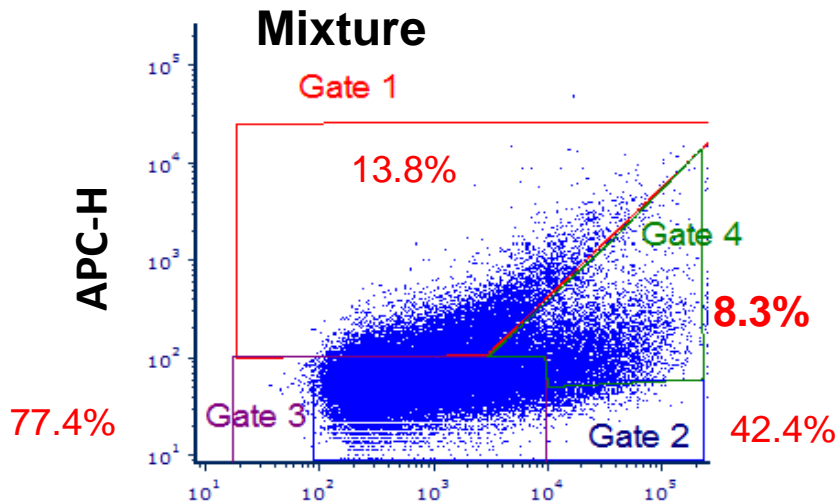
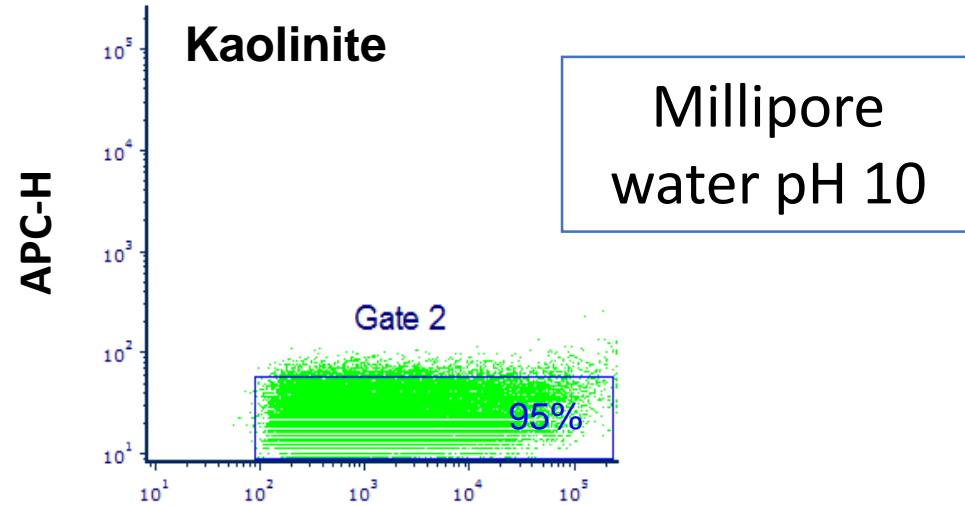
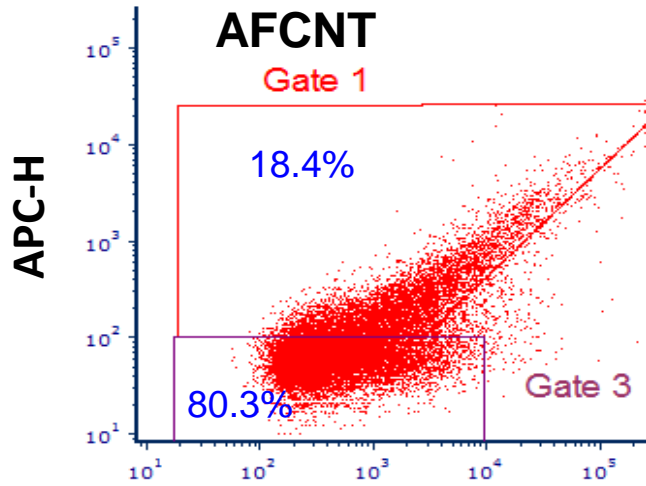


Validation Using Standard Nanoparticles



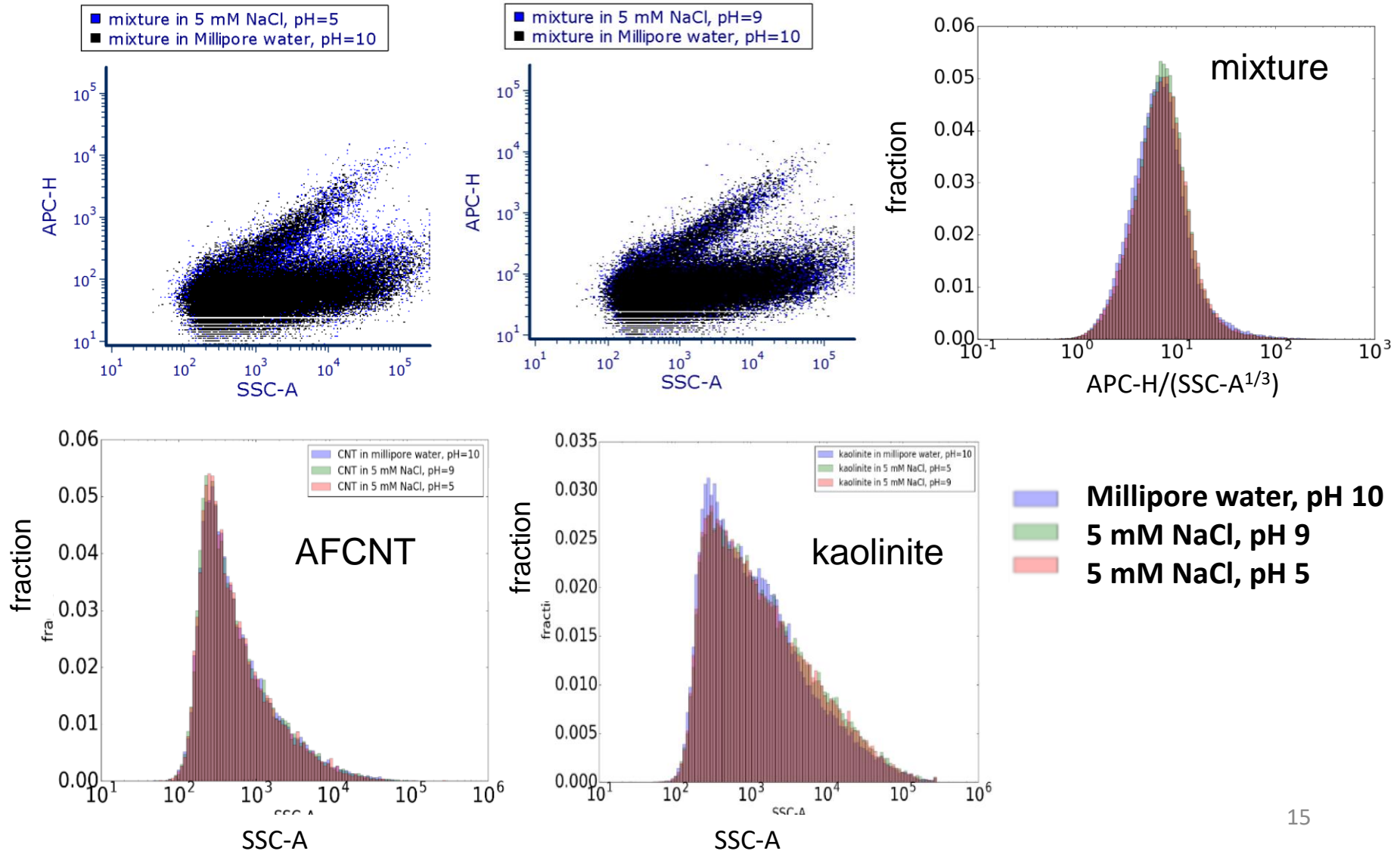


CNT-Kaolinite Interaction at Low IS and High pH



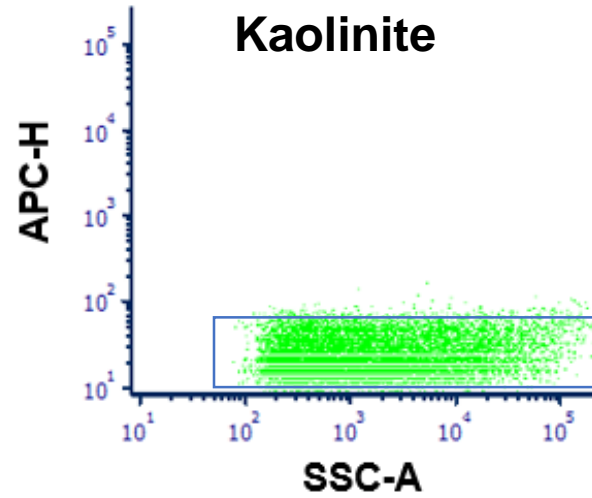
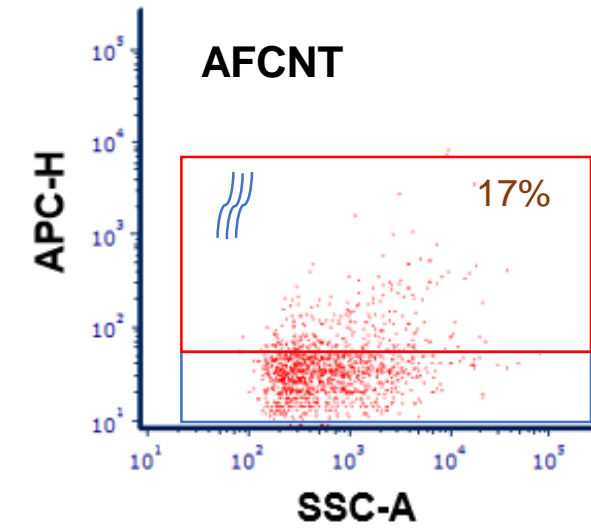


CNT-Kaolinite Interaction at Low IS (pH 5 and 9)

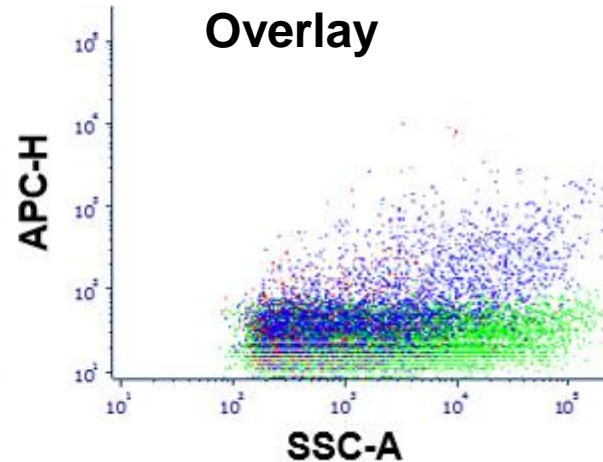
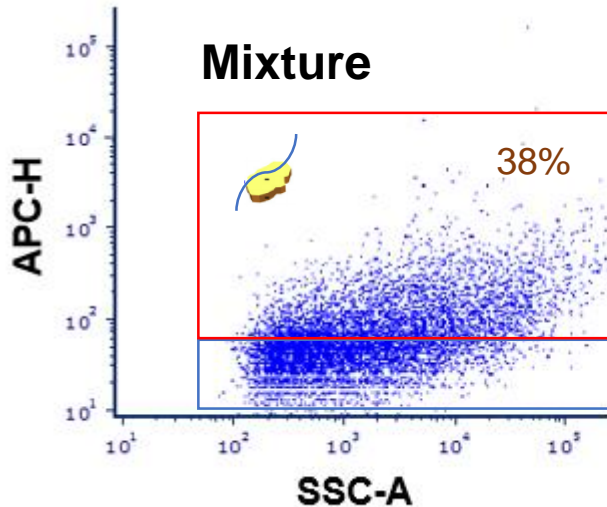




CNT-Kaolinite Interaction at Low IS and Low pH

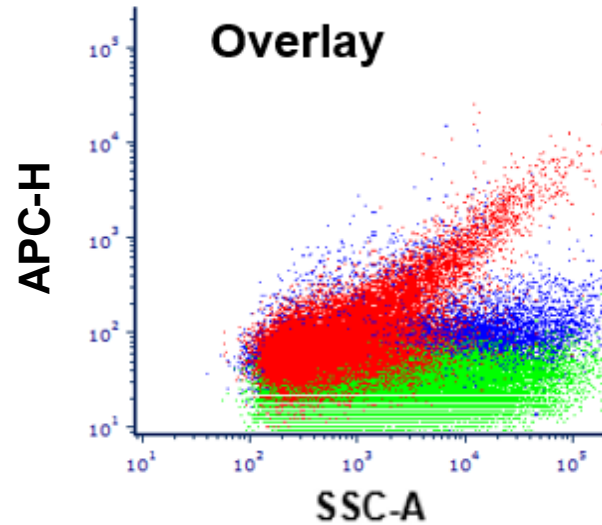
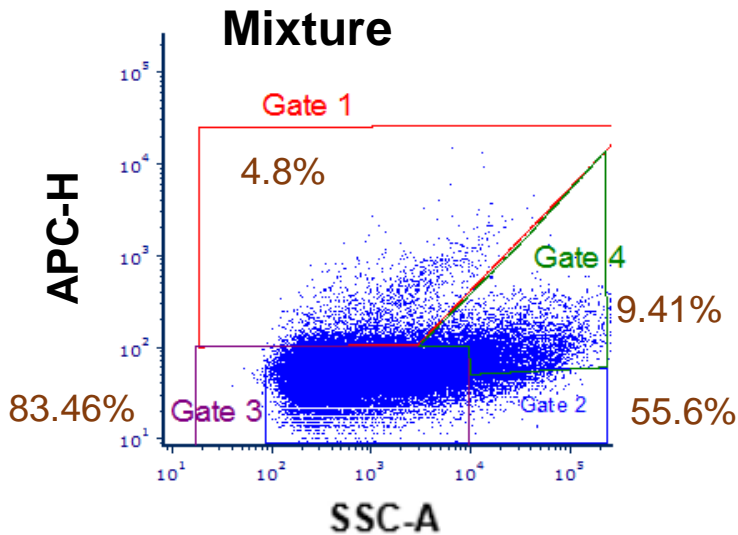
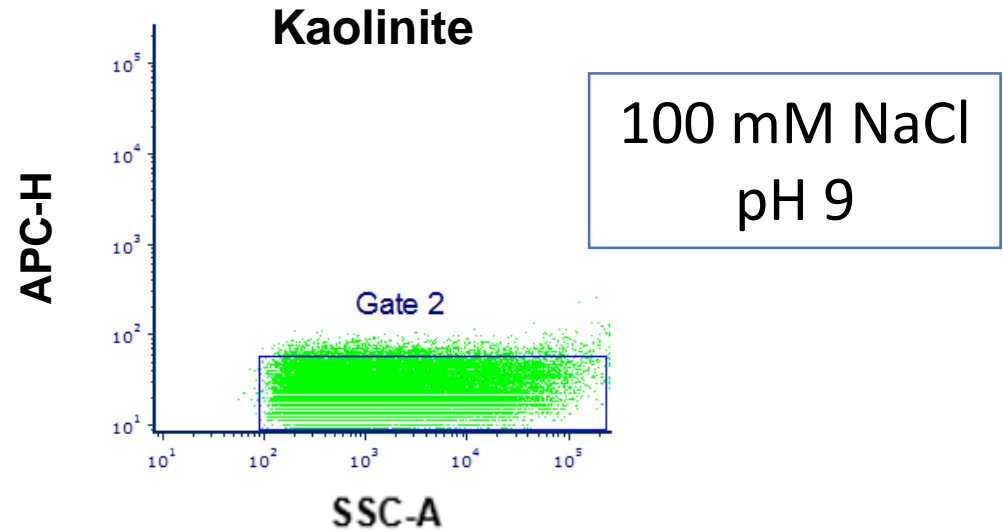
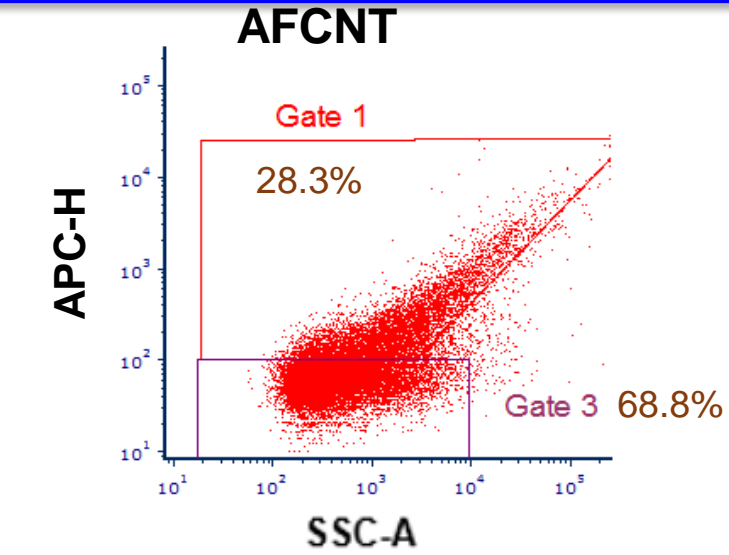


5 mM NaCl
pH 3





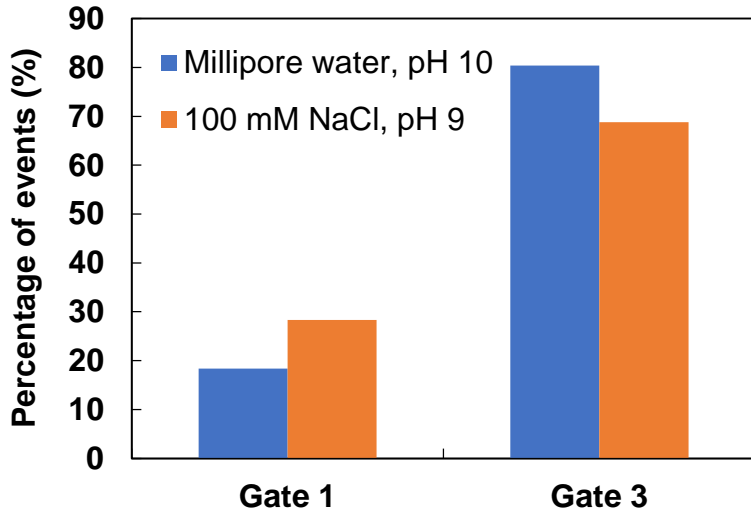
CNT-Kaolinite Interaction at High IS and High pH



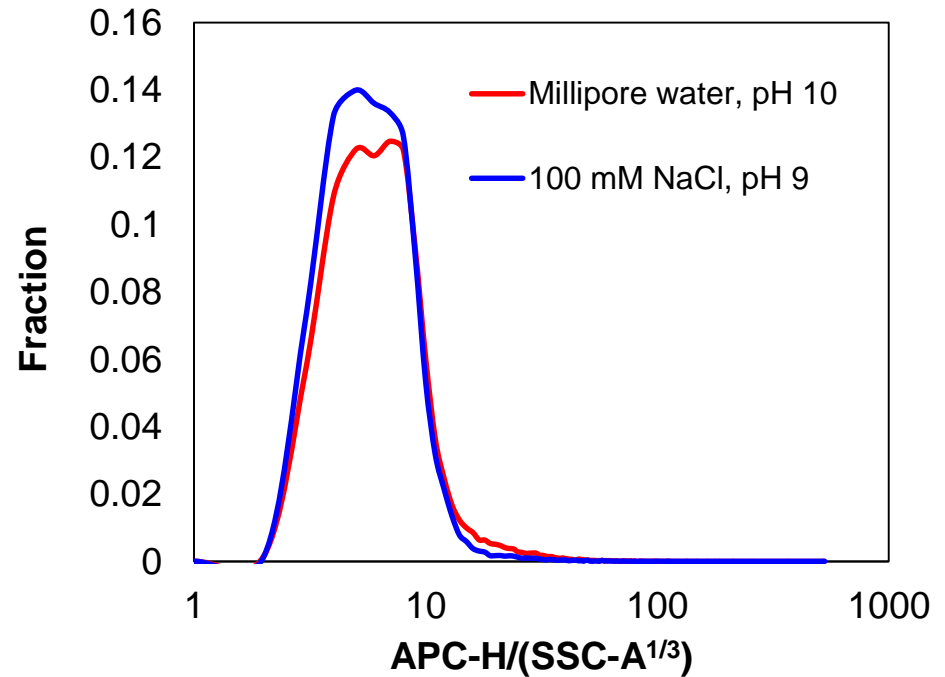
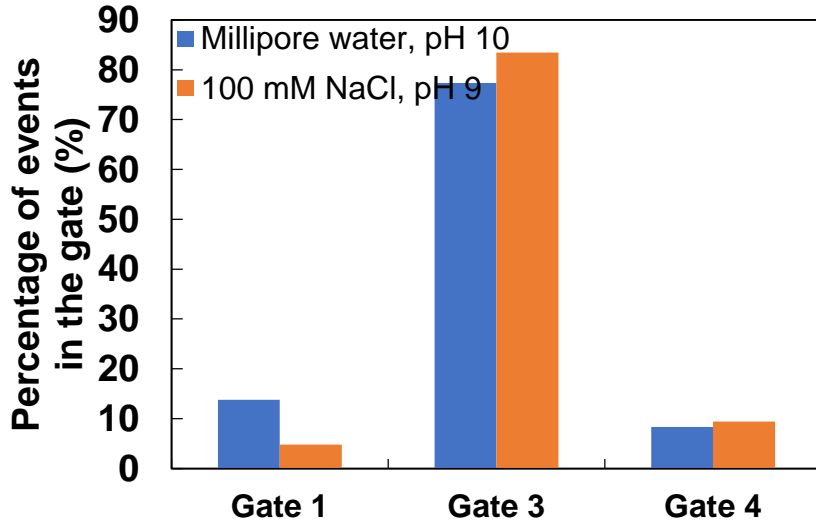


Quantification of Aggregation

AFCNT

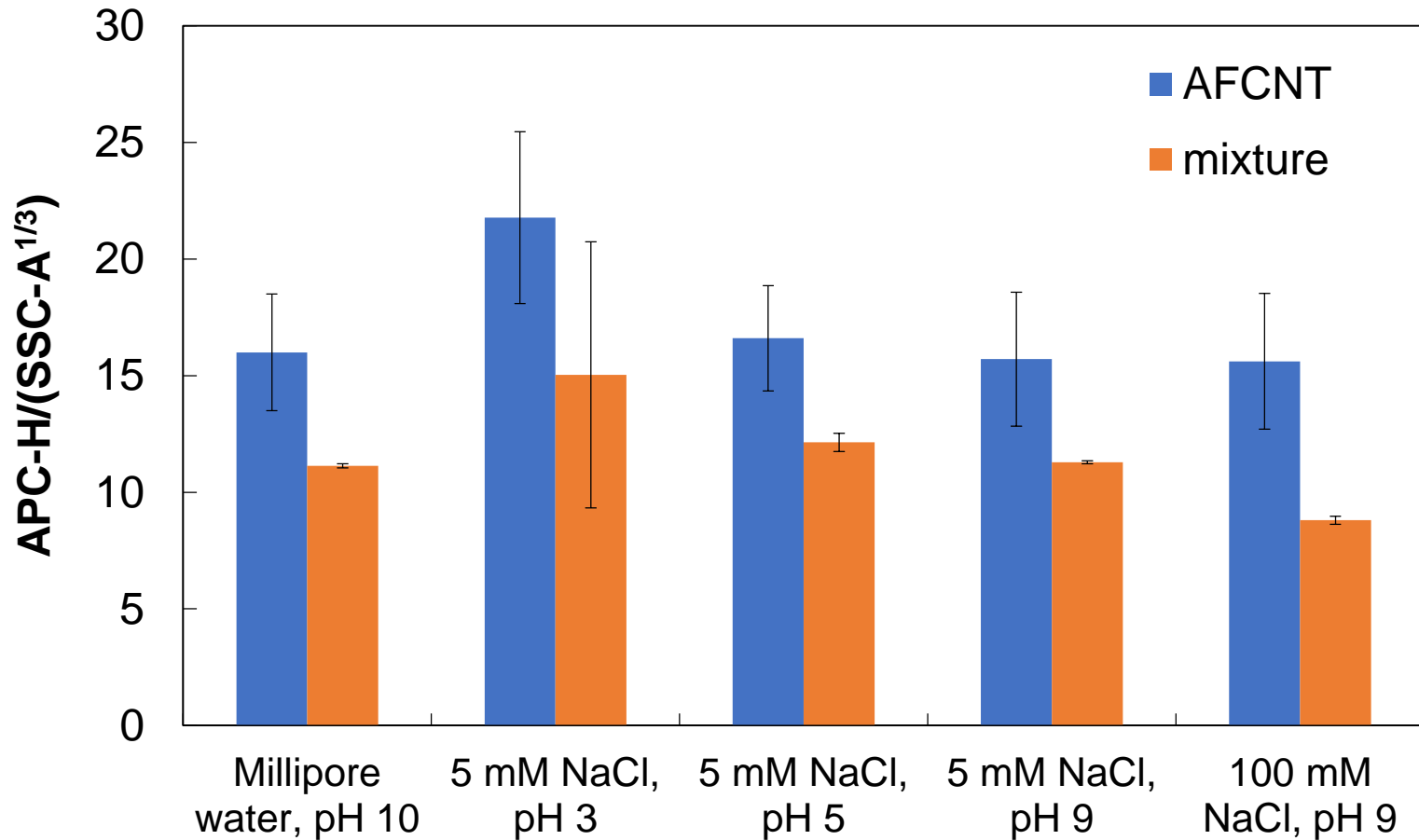


Mixture





Quantitative Indicator for Heteroaggregation





Conclusions

- ❑ Simultaneous light scattering and fluorescence measurement by flow cytometry can detect heteroaggregation events of submicron particles in both stable and unstable conditions
- ❑ Heteroaggregation may reduce homoaggregation under unstable conditions
- ❑ Single particle fluorescent intensity may be a reasonable indicator of heteroaggregation



Acknowledgement

Effect of Kaolinite on the Fate and Transport of Carboxylated Multi-walled Carbon Nanotubes

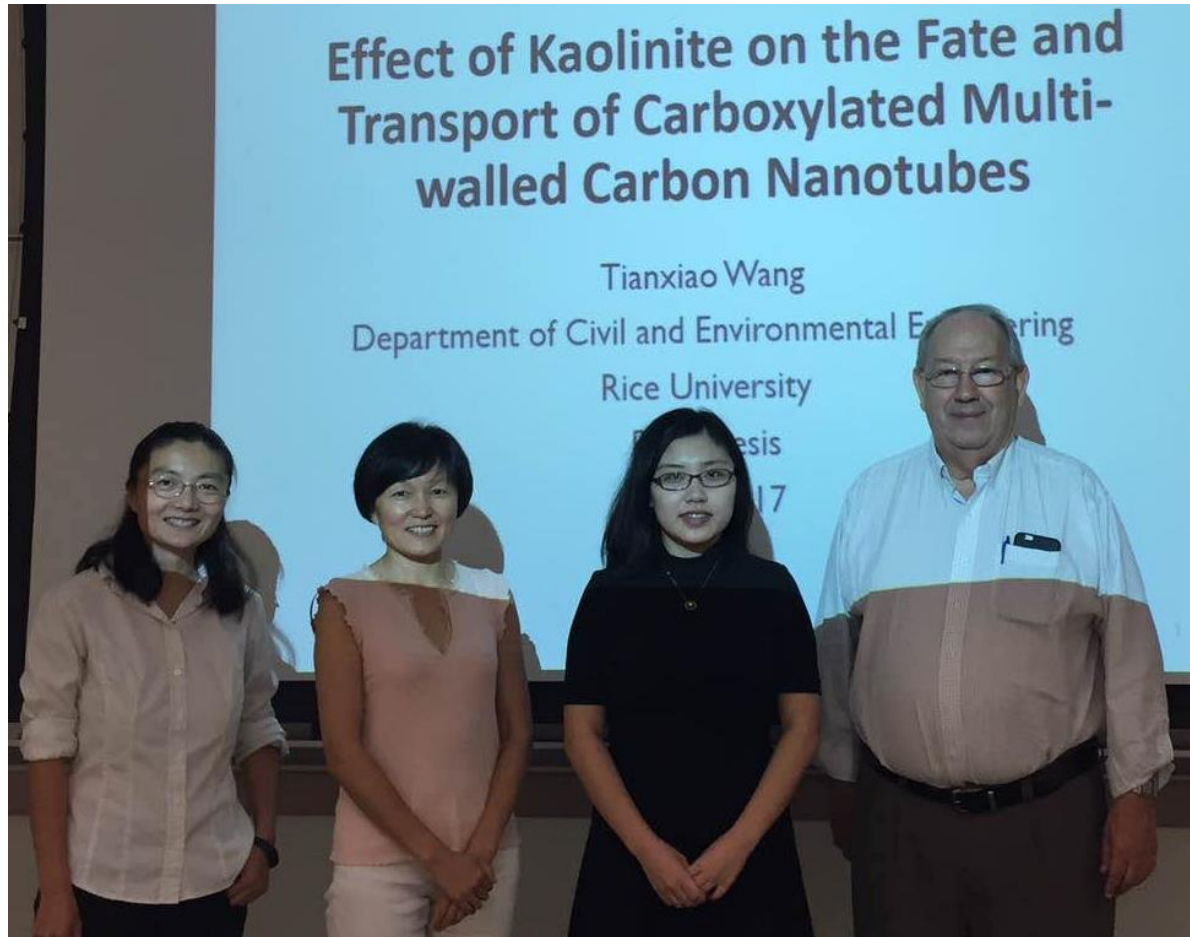
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Thesis

2017

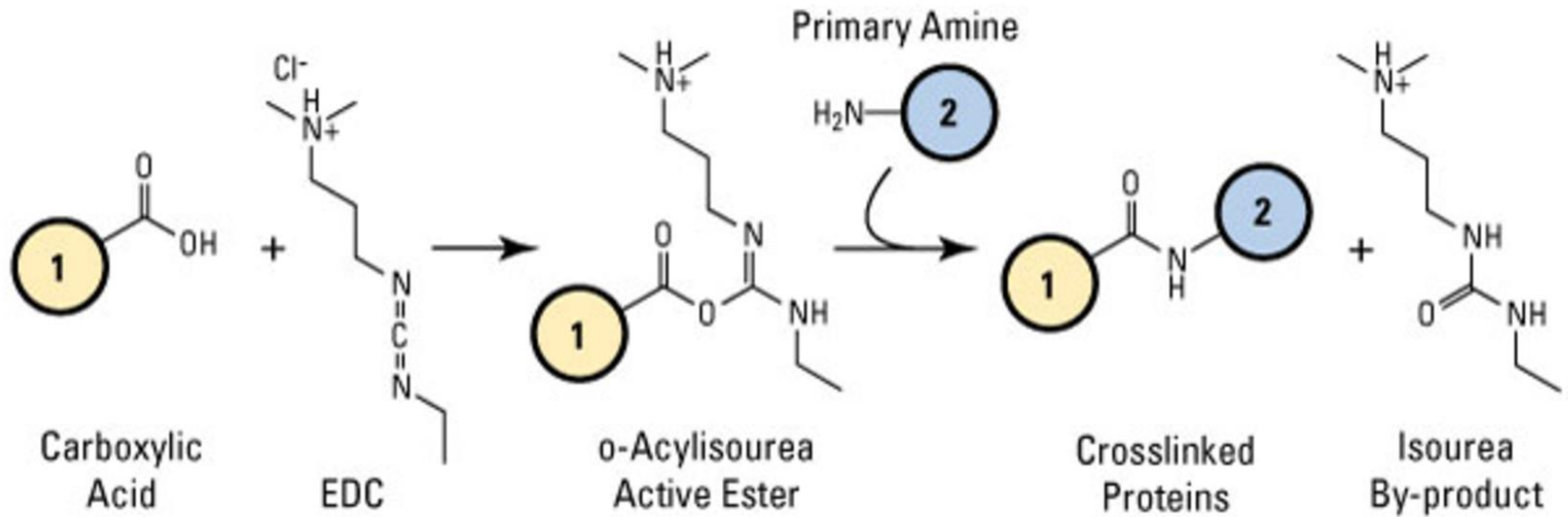


- ❑ China Scholarship Council
- ❑ Rice E2I
- ❑ Dr. Prateek Adora, Indian Institute of Science, Education and Research
- ❑ Dr. Angelo Benedetto, Rice University

Backup slides



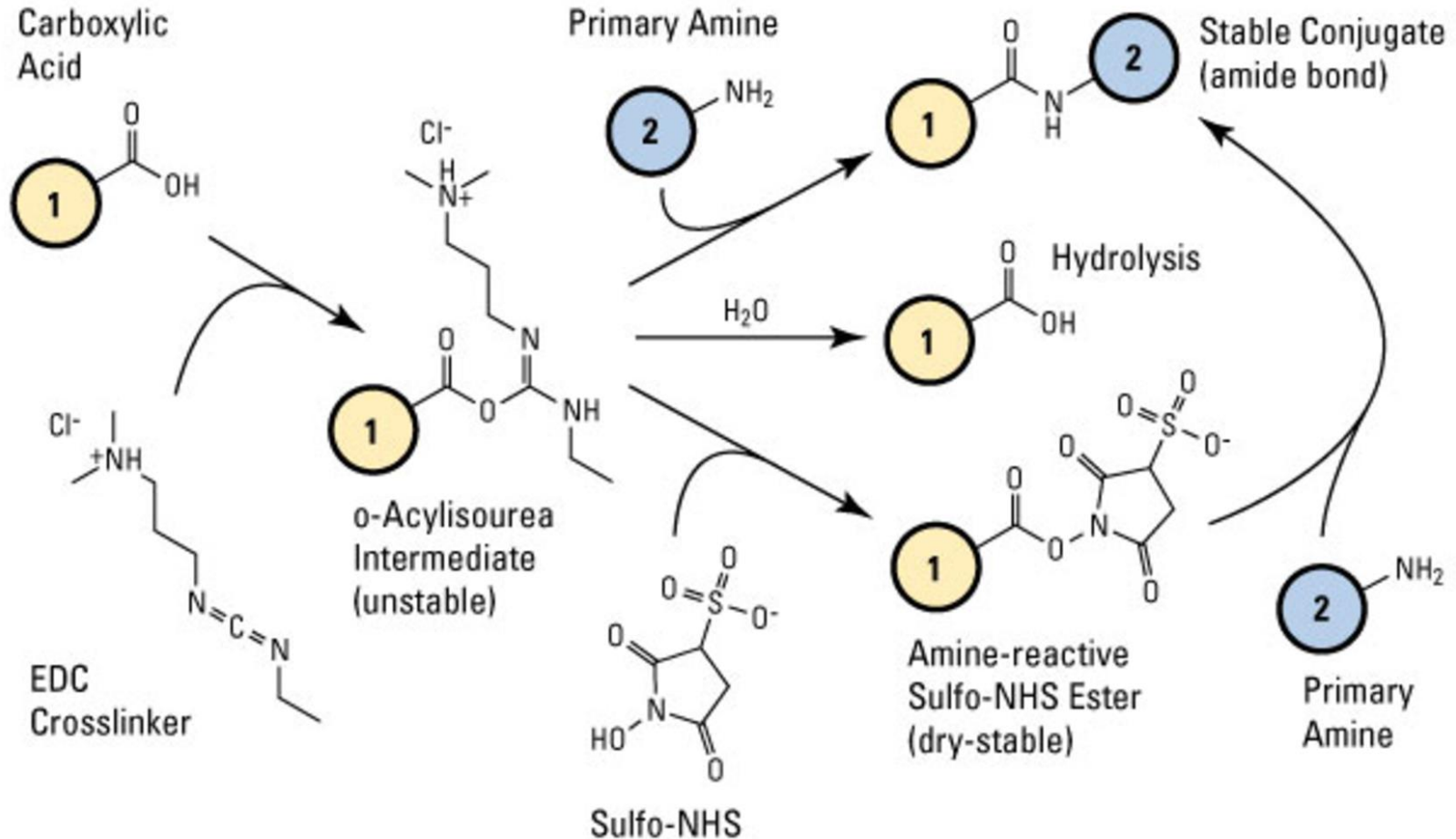
EDC (carbodiimide) crosslinking reaction



Source: <https://www.thermofisher.com/us/en/home/life-science/protein-biology/protein-biology-learning-center/protein-biology-resource-library/pierce-protein-methods/carbodiimide-crosslinker-chemistry.html>



Sulfo-NHS plus EDC crosslinking reaction





Experimental Methods: Data Analysis

Fluorescence channel height: APC-H

Side light scattering channel area: SSC-A

- Fluorescence Vs. Size** → **Dot plots of APC-H Vs. SSC-A**
- Size distribution** → **Relative frequency histograms of SSC-A**
- Aggregation status** (fluorescence over surface area) → **Relative frequency histograms of $APC-H / (SSC-A)^{1/3}$**



