In situ Measurements of Surface Chemistry Biologically and Environmentally Relevant Ligands on Metal Oxide Nanoparticles

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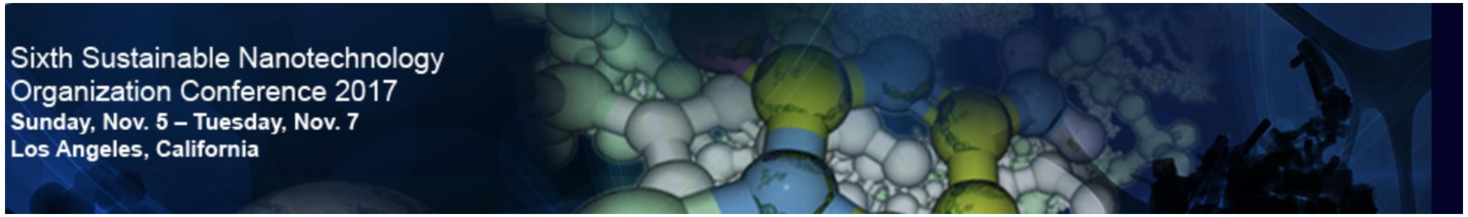


Special Tribute To Professor Pedro Alvarez, Rice University

Former Colleague

Academic Appointments

2015-present	Director, NEWT ERC	Rice University, Houston, TX
2004-present	G.R. Brown Professor	Rice University, Houston, TX
2004-present	CEE Dept. Chair	Rice University, Houston, TX
2001-2003	Professor	The University of Iowa, Iowa City
1999	Visiting Professor	EAWAG, Switzerland
1998-2003	Associate Director	Center for Biocatalysis & Bioprocessing
1997-2001	Associate Professor	The University of Iowa, Iowa City
1993-1997	Assistant Professor	The University of Iowa, Iowa City
1985-1988	Environ. Engineer	Tetratech Inc., San Bernardino, CA

Special Tribute To Professor Pedro Alvarez, Rice University

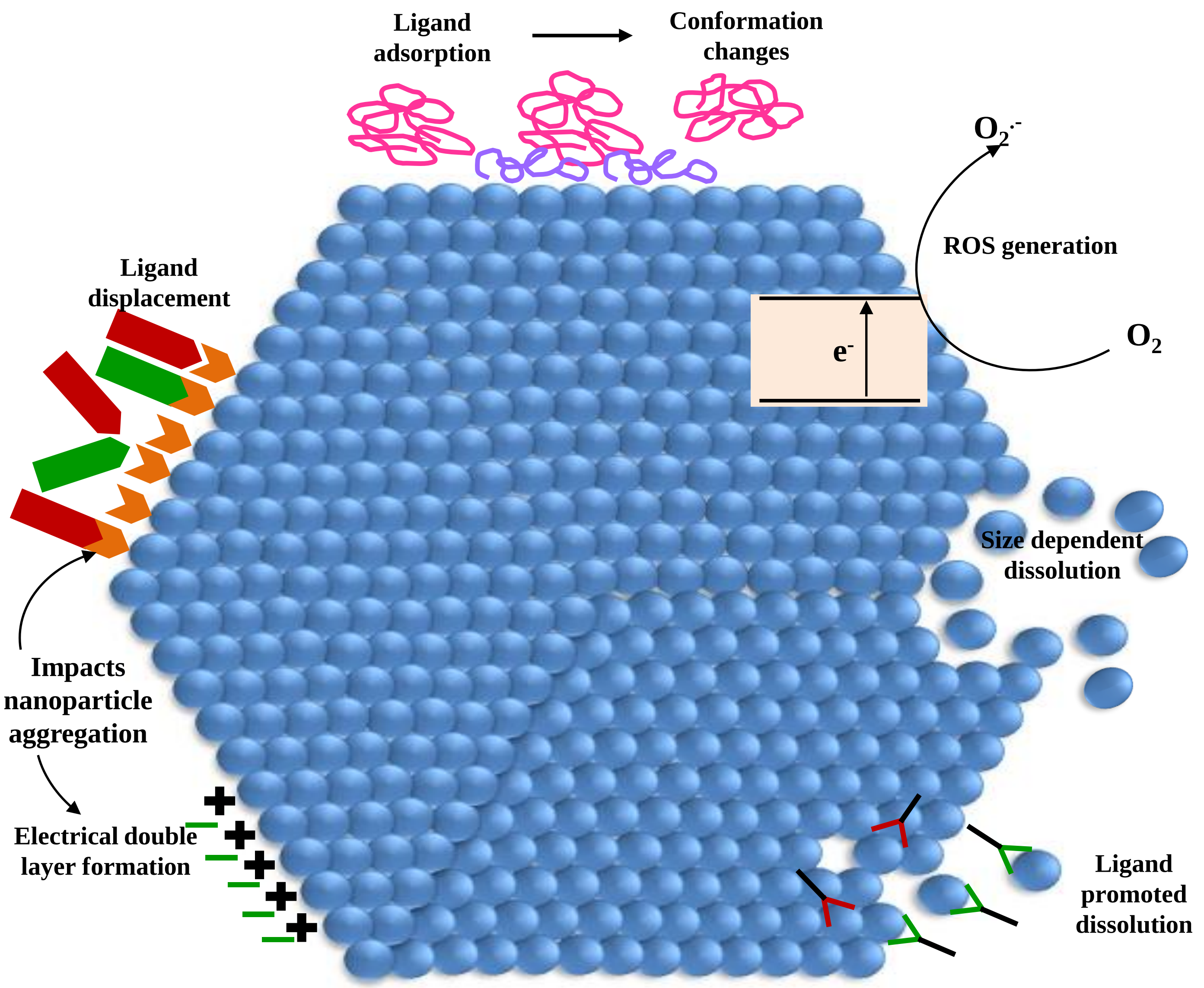
- Leader in the Field of Environmental Engineering

- Leader in the Fate and Transport of Nanomaterials in the Environment

- Leader in the Applications of Nanomaterials in Water Quality

Nanoparticle Surfaces – A Closer Look

Complex Interactions at the Aqueous-Nanoparticle Interface



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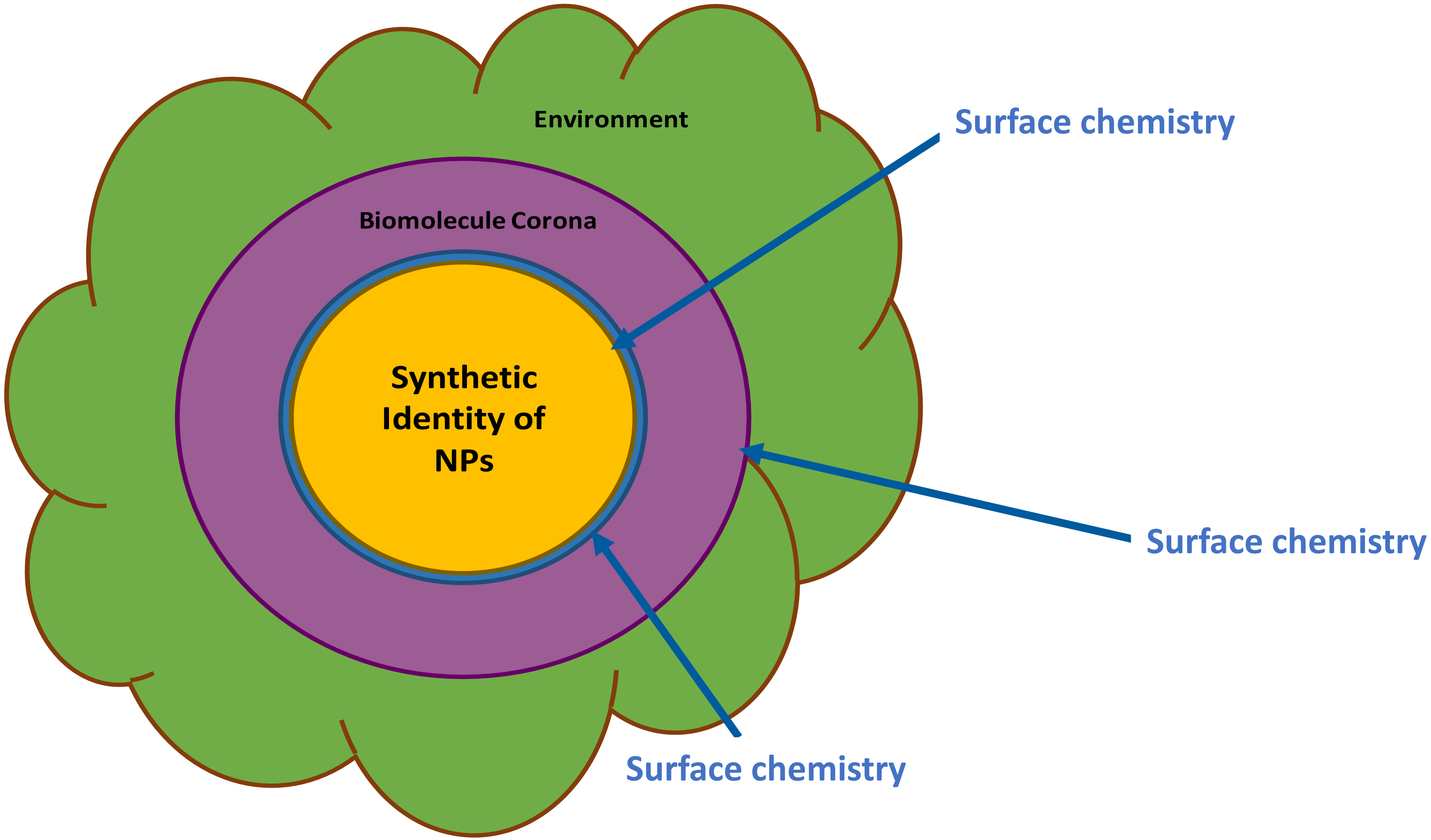
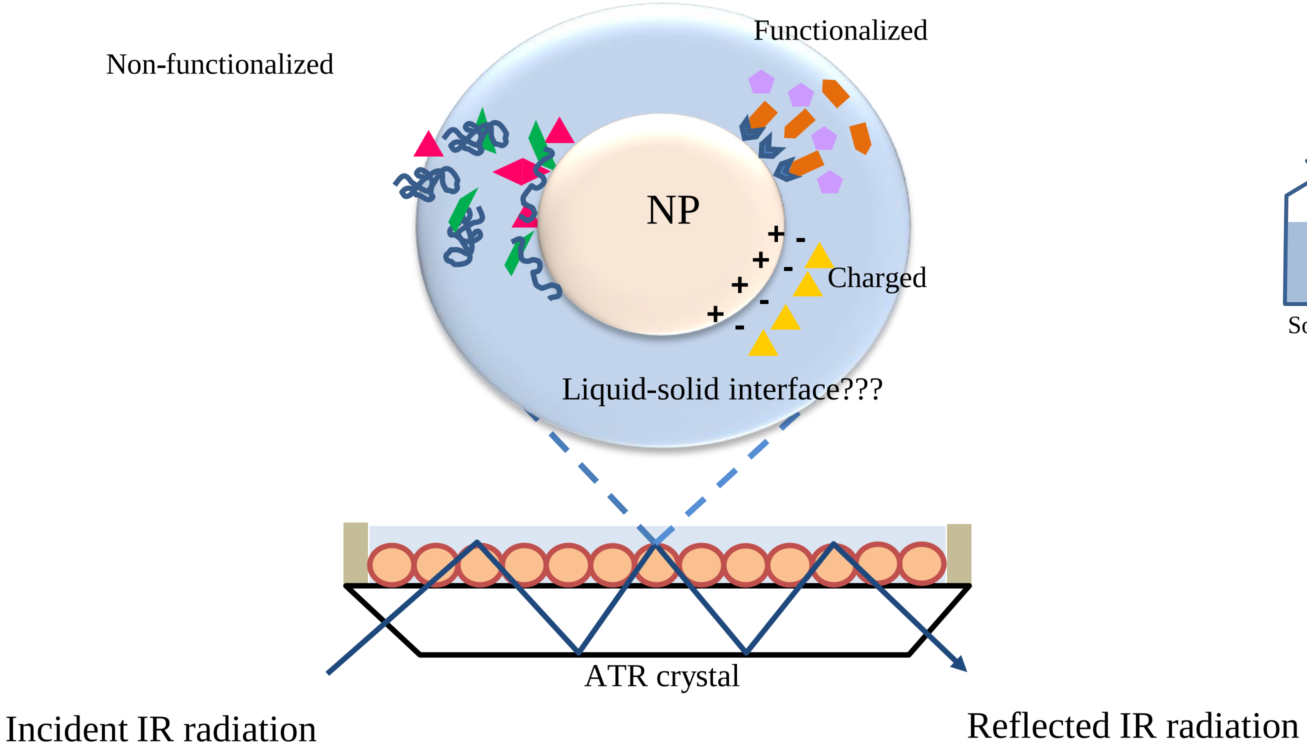
Nanoparticle Surfaces – A Closer Look

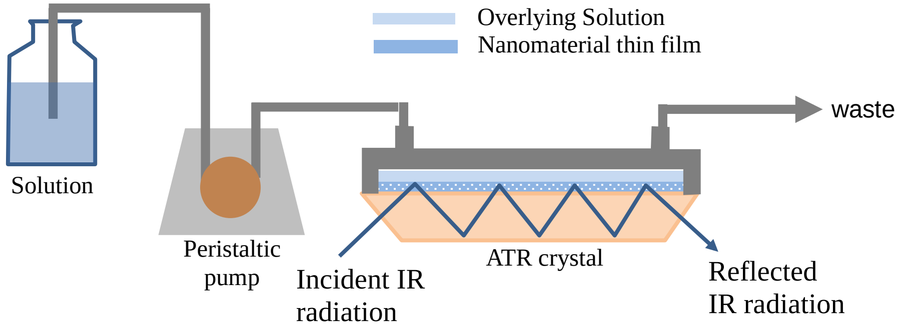


Dr. Natalia Gonzalez Pech

Monitoring Surface Composition and Surface Chemistry

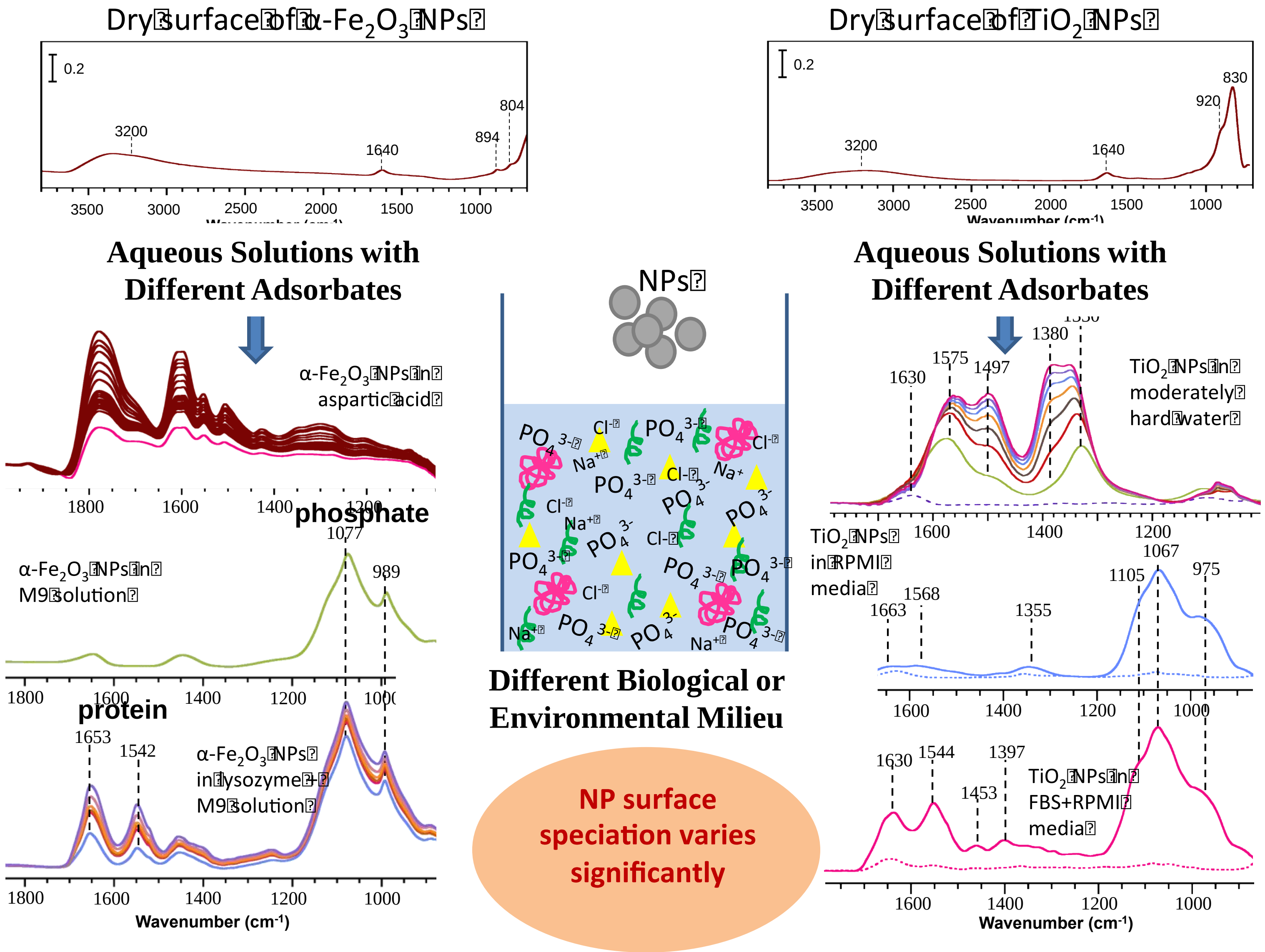
ATR-FTIR Spectroscopy to Probe Nanoparticle Surfaces





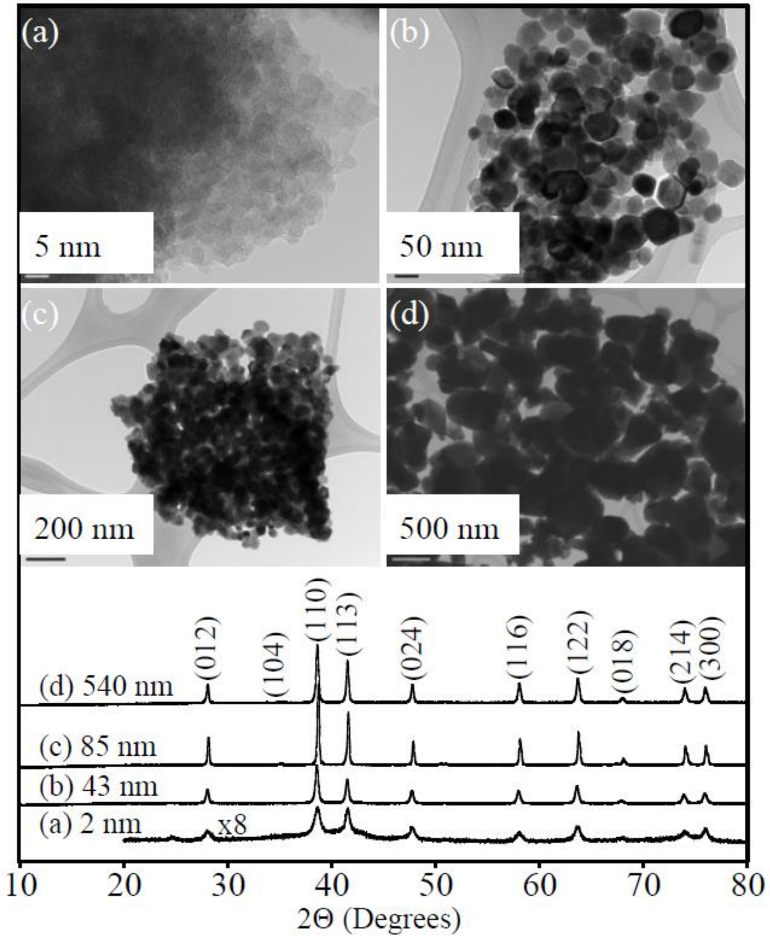
Mudunkotuwa, Al Minshid, and Grassian Analyst 2014, 139, 870-881.

Surface Speciation Can Be Followed in Different Aqueous Environments



Mudunkotuwa and Grassian ES:Nano, 2015, 2, 429-439

Iron Oxide Nanoparticles Inhibition of Anti-Microbial Peptides



Environmental Science Nano

ROYAL SOCIETY OF CHEMISTRY

PAPER

Cite this: DOI: 10.1039/c3en00029j

Iron oxide nanoparticles induce *Pseudomonas aeruginosa* growth, induce biofilm formation, and inhibit antimicrobial peptide function†

Jennifer Borcherding,[a] Jonas Baltrusaitis,[b] Haihan Chen,[b] Larissa Stebounova,[b] Chia-Ming Wu,[b] Gayan Rubasinghege,[b] Imali A. Mudunkotuwa,[b] Juan Carlos Caraballo,[a] Joseph Zabner,[a] Vicki H. Grassian*[b] and Alejandro P. Comellas*[a]

***Environmental Science: Nano* 2014, *1*, 123 - 132.**

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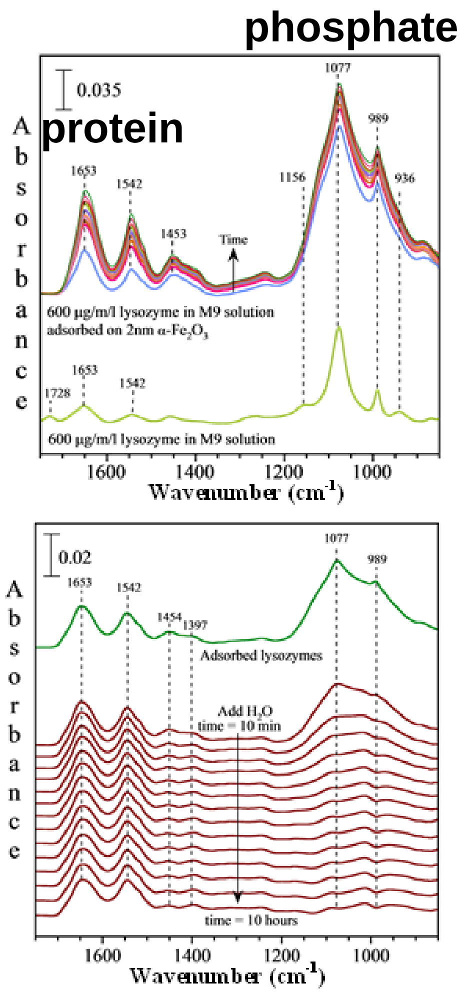
AMPs Adsorb to Nanoparticle Surfaces

ATR-FTIR Spectroscopy Shows that AMPs Adsorb to Nanoparticle Surface in an an Irreversible Manner



AMP Less Effective Due in Part to Irreversible Adsorption on Nanoparticle Surface and Loss of Activity

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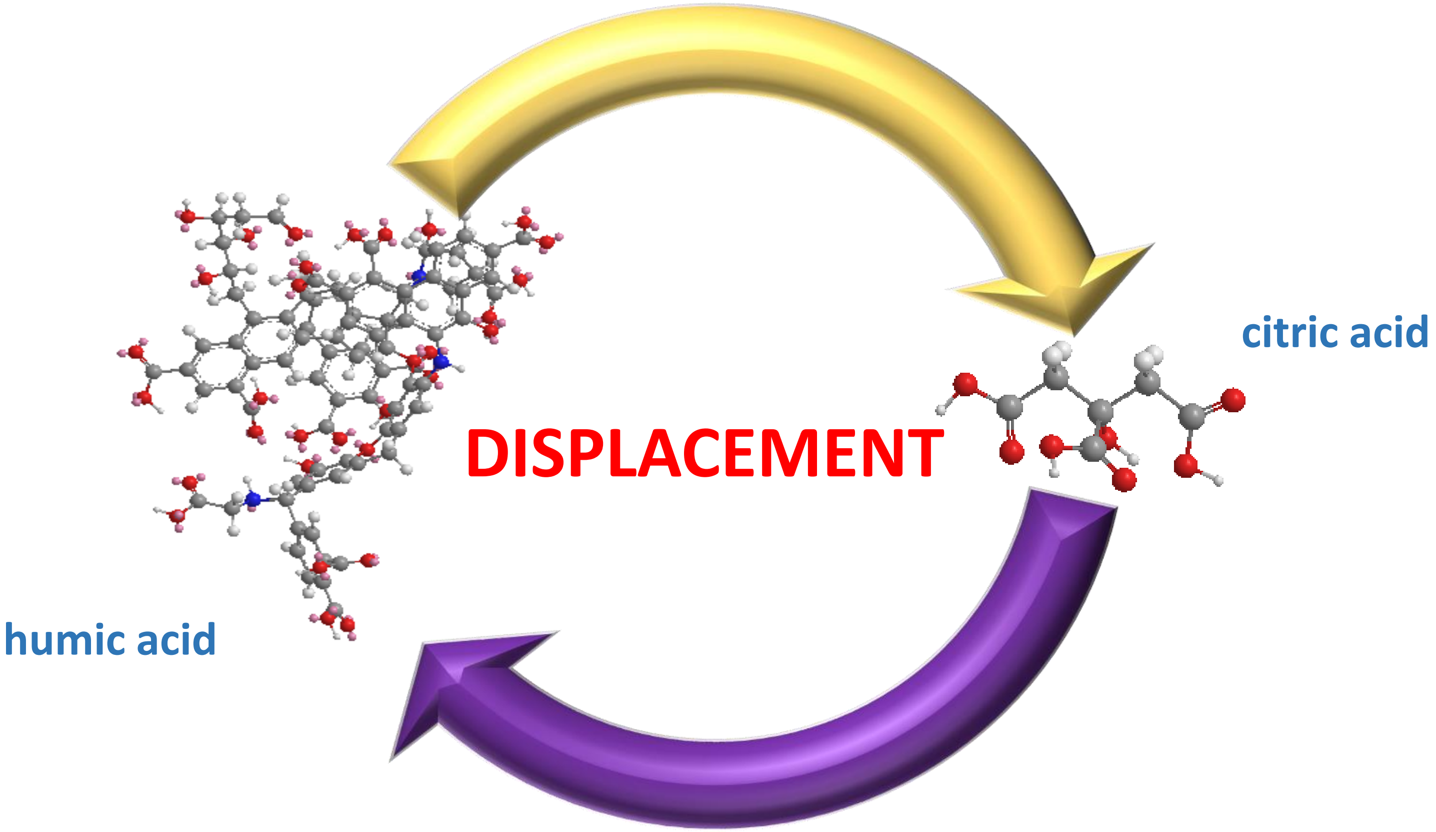
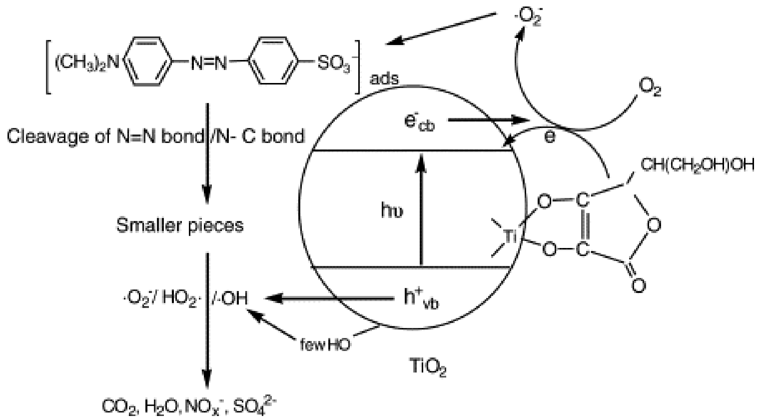
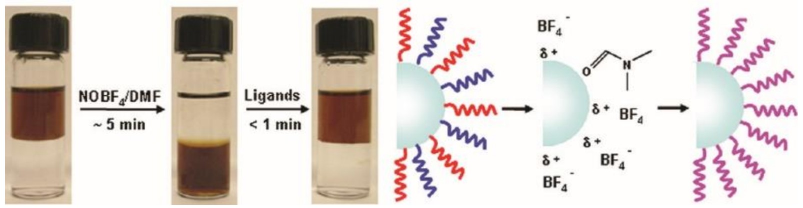
Surface Composition Can Be Dynamic



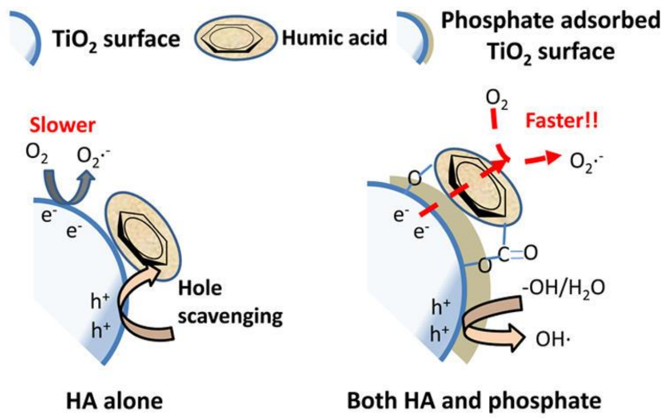
Displacement Reactions Can Impact A Range of Behaviors



Surface ligands affecting photocatalytic process on the surface[1]



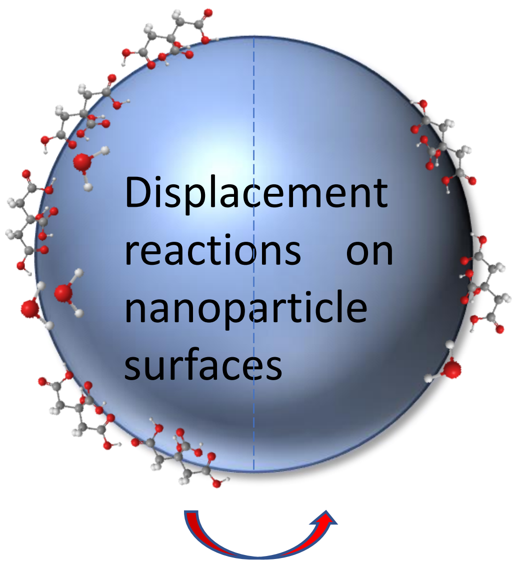
Ligands exchange affecting solubility in different polar solvents[2]



Strong Inhibitory Effect!

Mitigated Inhibitory Effect!

Co-existed phosphate molecules changing Humic acid (HA) binding resulting in different electron transfer pathways[3]



- Natural water system has a wide range of organic and inorganic ligands (e.g. phosphate, acetate, citrate, humic acid and fulvic acid).
- Citrate is also widely used as ligands for many nanomaterials.

1. Ou *et. al. J. Mol. Catal. A: Chem.*, **2005**, *241*, 59;
2. Dong *et. al. J. Am. Chem. Soc.* **2011**, *133*, 998;
3. Long *et. al. Environ. Sci. Technol.*, **2017**, *51,* 514.

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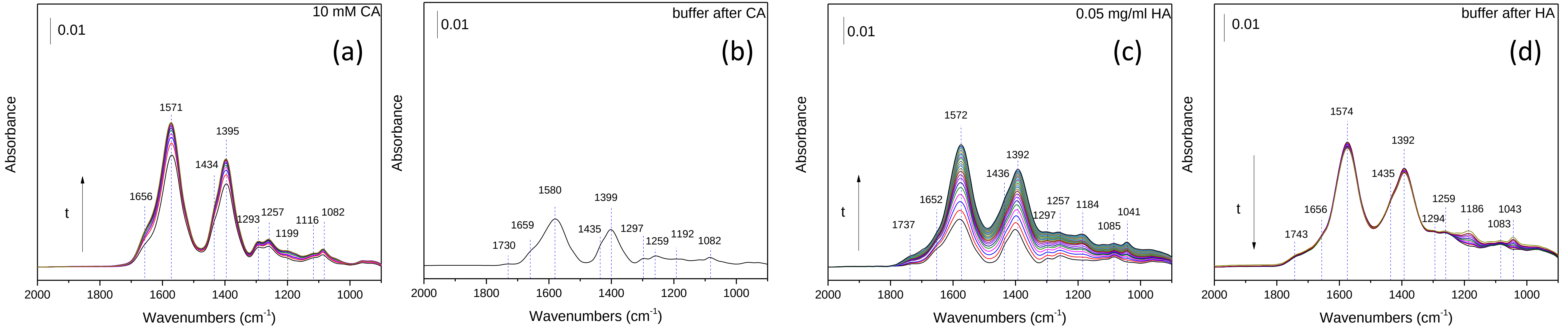
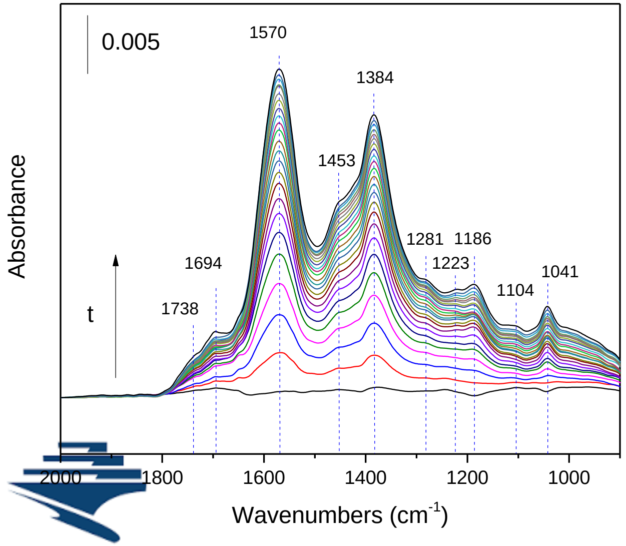
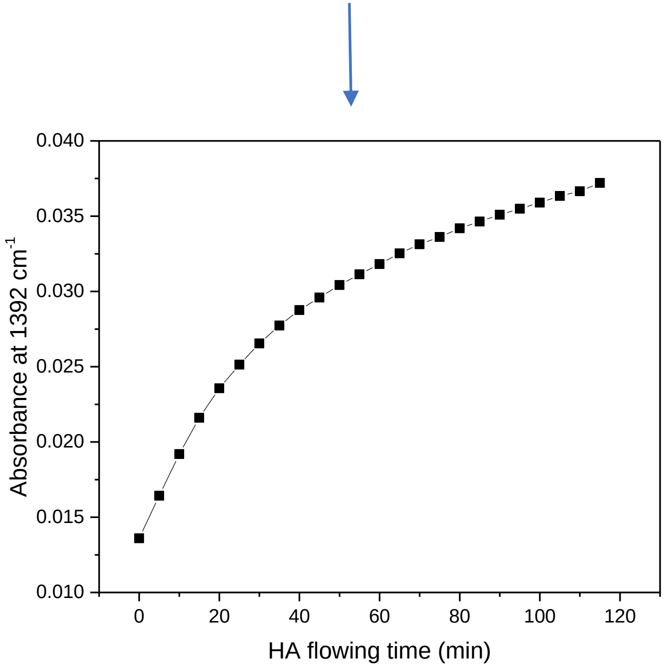
Citric Acid-Humic Acid: A Changing Surface



Subtraction b from c

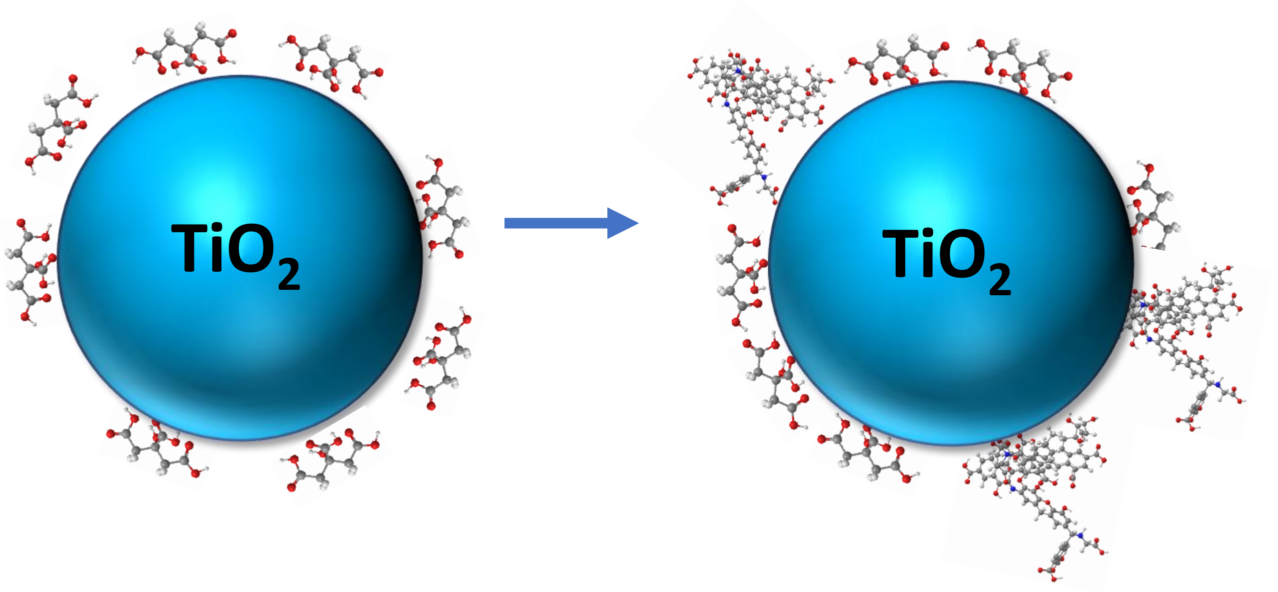


The symmetrical –COO$^-$ of HA on the TiO_2 film is around 1380 cm-^{1}. The difference show the peaks shift from 1399 cm^{-1} to 1384 cm^{-1}.



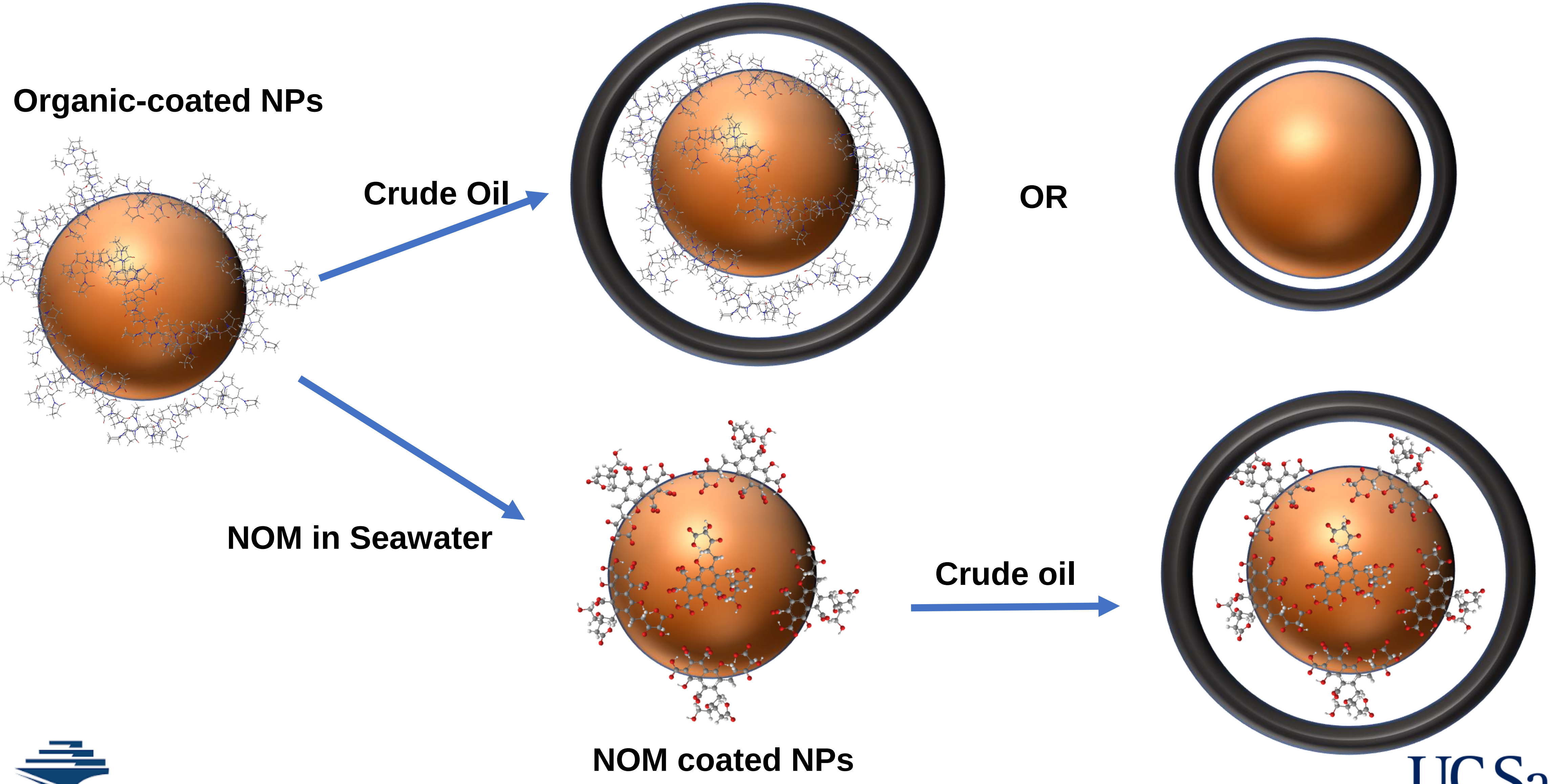
Haibin Wu

Co-Adsorption of Citric and Humic Acid



NP Surfaces During A Simulated Environmental Application

Oil Spill Cleanup



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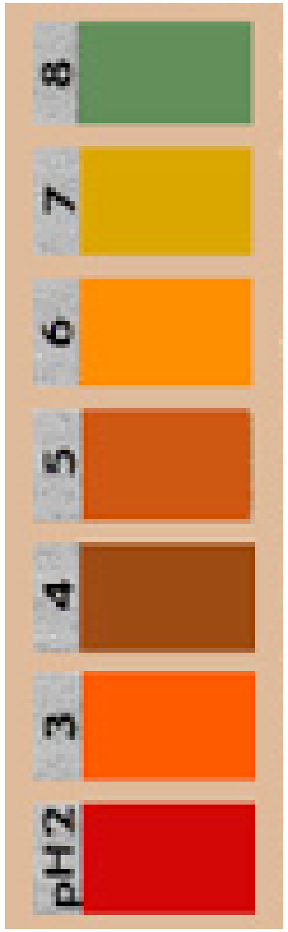
Environmental and Biological Media – Different pH Ranges



pH	Mileu (Select Examples)
8.0	Small Intestine
7.4	Blood / Rivers
7.2	Interstitial Fluid in Lung
4.7	Saliva / Rain
4.2	Alveolar Macrophage
3.7	Small Intestine
3.0	Stomach
2.0	Acid Rain

Role of pH in Nanoparticle-Biological/Environmental Interactions

- Wide range of pH in different environmental/biological media
- Chemical speciation of solution phase molecules vary as a f(pH)
- Biomolecule secondary and tertiary structures changes as a f(pH)
- Surface functionality varies as a f(pH)
- Dissolution depends on pH (implications for nanotoxicity)

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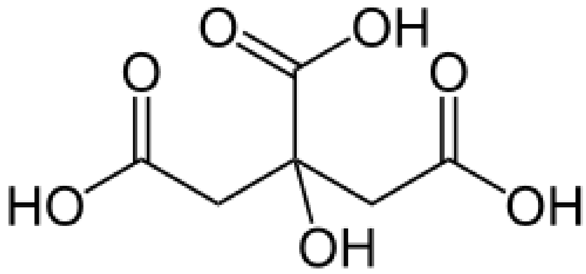
Speciation as a f(pH)

Aqueous Phase Speciation of Citric Acid



Citric Acid

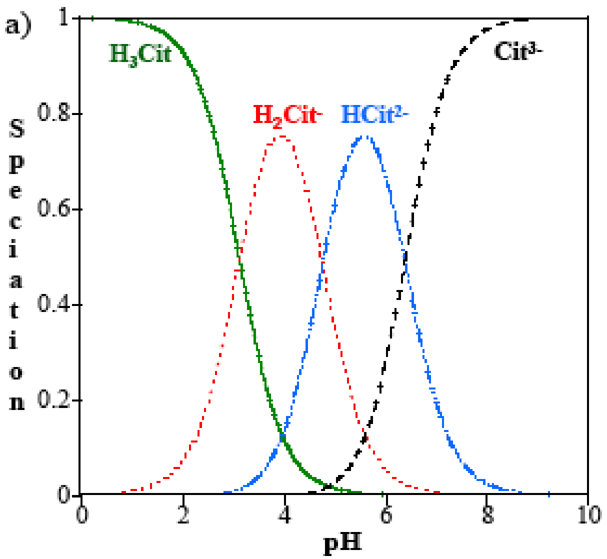
$pK_{a1} = 3.13$

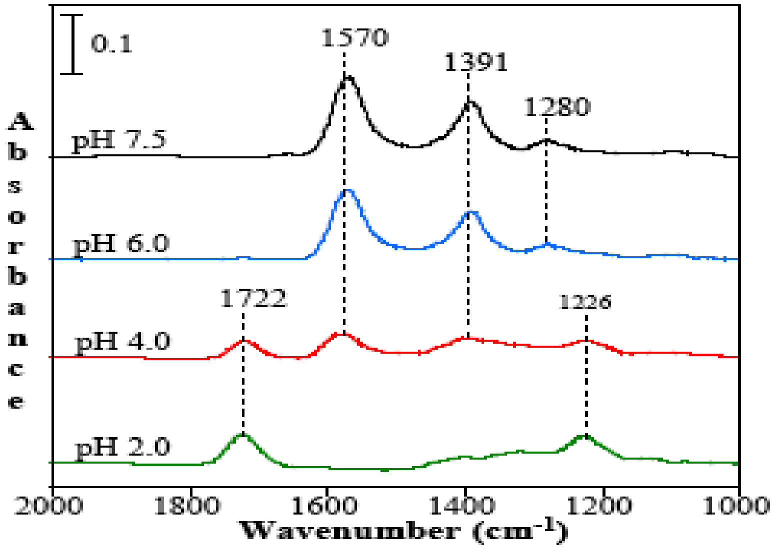
$pK_{a2} = 4.76$

$pK_{a3} = 6.40$

Henderson–Hasselbalch equation

$$pH = pK_a + \log_{10}\left(\frac{[A^-]}{[HA]}\right)$$





pH Dependent Changes in Molecular Structure Are Reflected in ATR-FTIR Spectra

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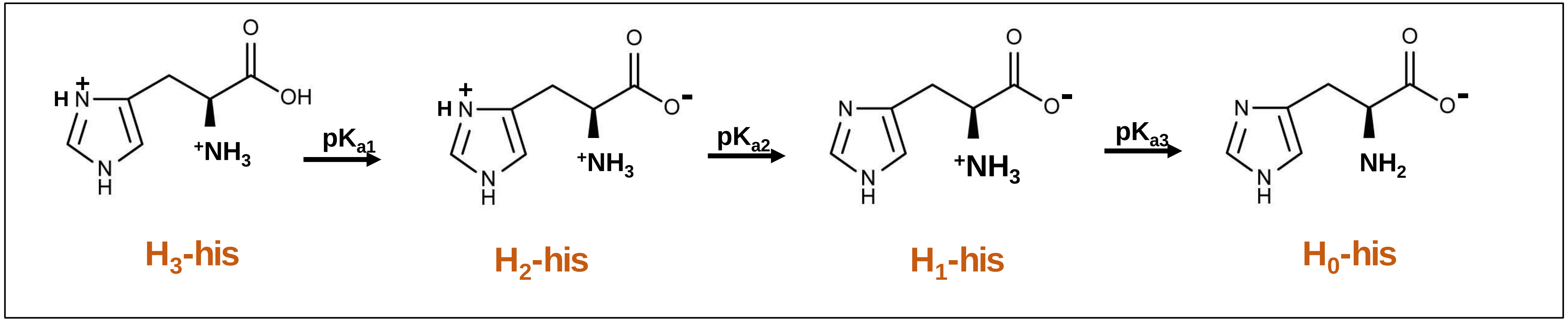
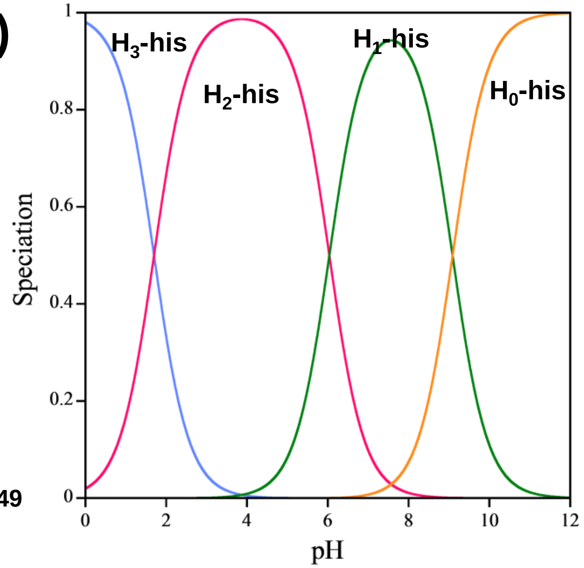
Biological Molecules Including Amino Acids and Proteins

Histidine



Speciation of histidine as a f(pH)



- Histidine found in many of the active sites in proteins (e.g. hemoglobin, carbonic anhydrase, histidine kinase)

- Three pK_a values:

  pK_{a1}= 1.70 pK_{a2}= 6.04 pK_{a3}= 9.09

Özcan, A. A.; Say, R. d.; Denizli, A.; Ersöz, A. *Analytical Chemistry* 2006, *78*, 7253-7258
Heyda, J.; Mason, P. E.; Jungwirth, P. *The Journal of Physical Chemistry B* 2010, *114*, 8744-8749

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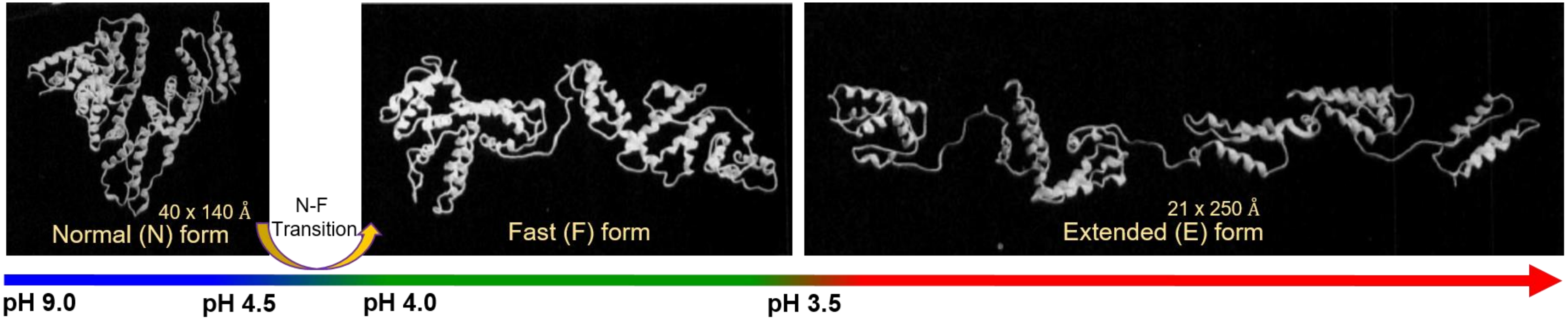
Protein Structure Depends on pH: Bovine Serum Albumin (BSA)

BSA form and dimensional changes as a function of pH



– Common blood protein with high abundance

– BSA has similar properties with its human variant (shares 98% percent amino acid sequence)

– Molecular weight: 66,463 Da (= 66.5 kDa)

Carter, D. C.; Ho, J. X. Adv. Protein Chem. 1994, 45 (45),153–203

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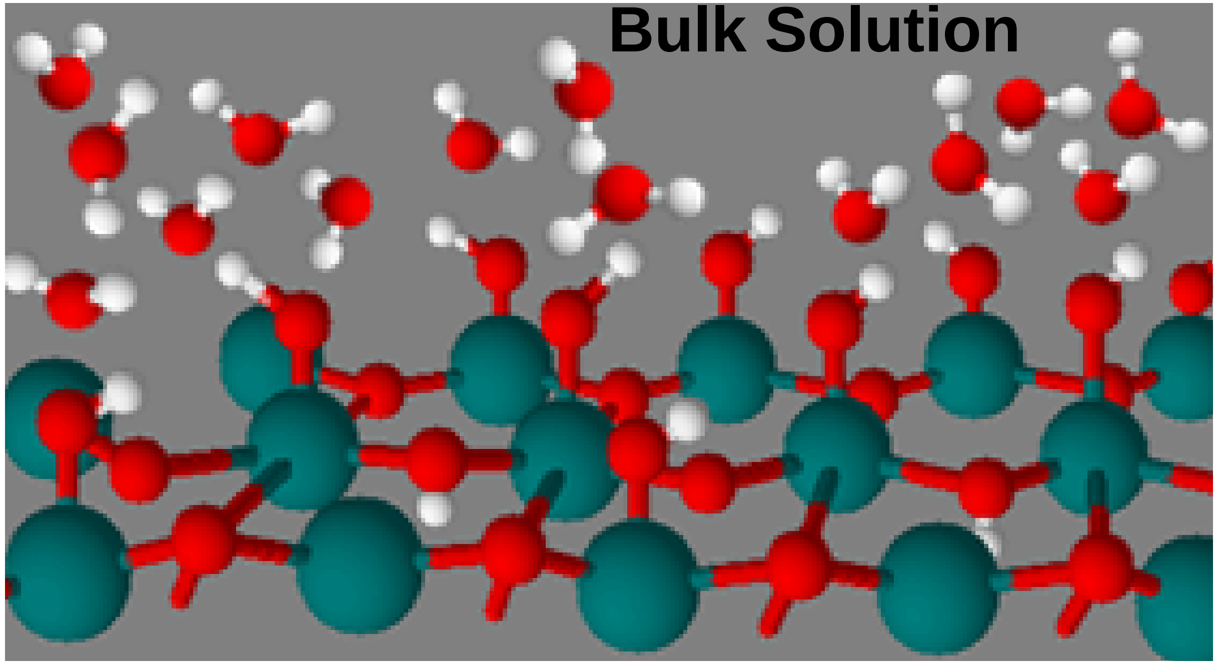
Nanoparticle Surface pH Dependence

Nanoparticle Surface pH Dependence: Hydroxylated and Surface Functionalized



$–OH_2^+$ $–OH$ $–O^-$

→

Low pH High pH

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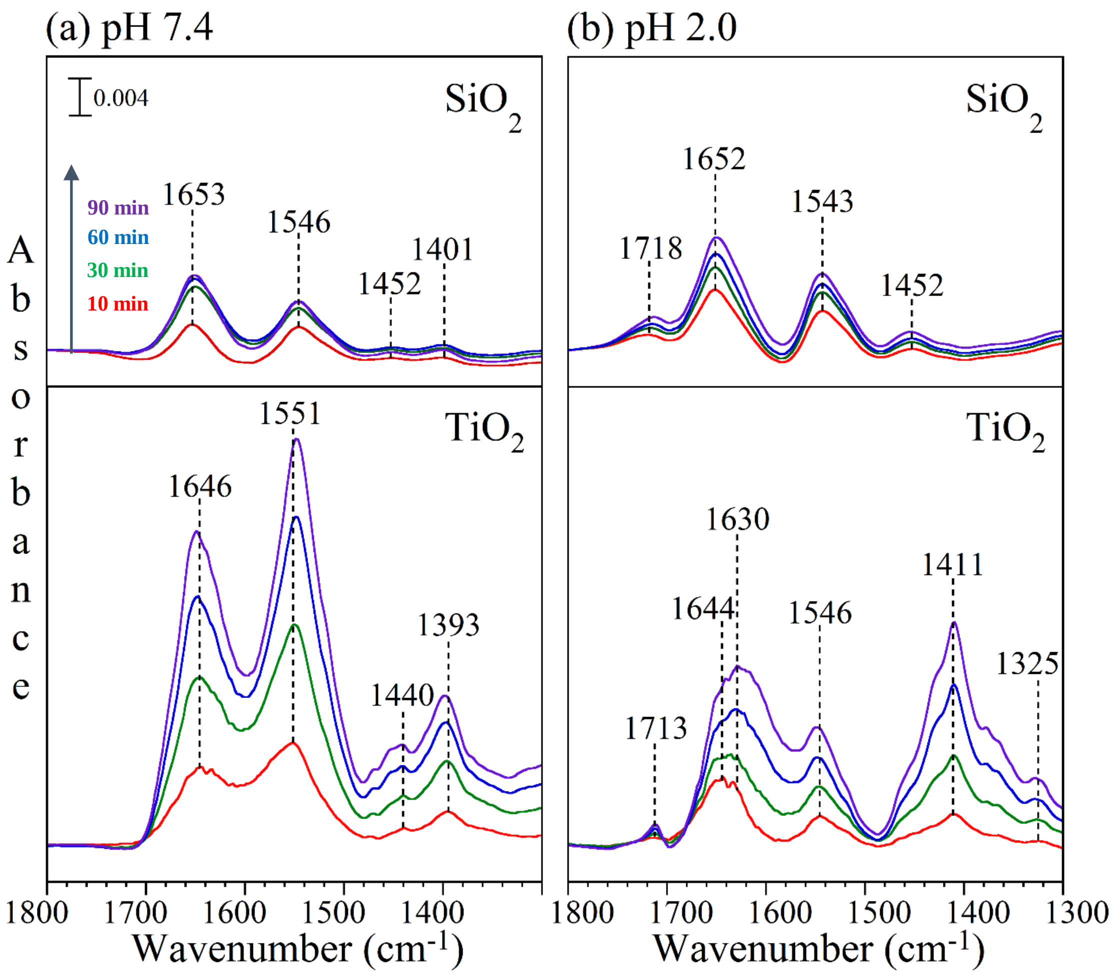
BSA Adsorption on 20 nm SiO_2 and TiO_2 Surfaces

A Tale of Two Nano-Bio Surface Interactions



- Bands intensity increases over time
- Adsorption depends on substrate
- Adsorption depends on pH

> ➢ Amide I (ca. 1650) and Amide II (ca. 1550) bands observed in the spectra
>
> ➢ pH effect especially obvious for BSA on TiO_2 at pH 2.0
>
> ➢ Greater intensity and larger peak shifts (relative to solution) for BSA on TiO_2

Givens, B.; Xu, Zhenzhu; Fiegel, J.; Grassian, V.H. Journal of Colloid and Interface Science 2017, 493, 334-341.

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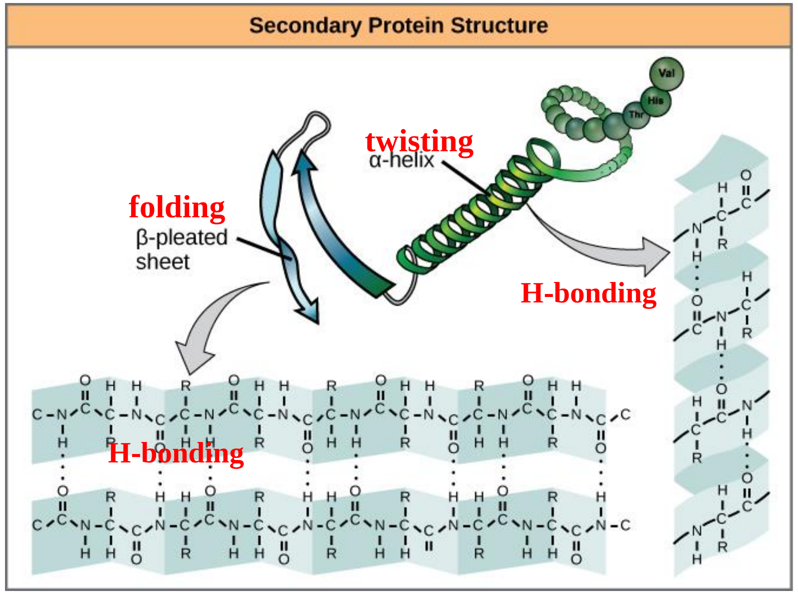
Amide I Band Used for Structure Analysis

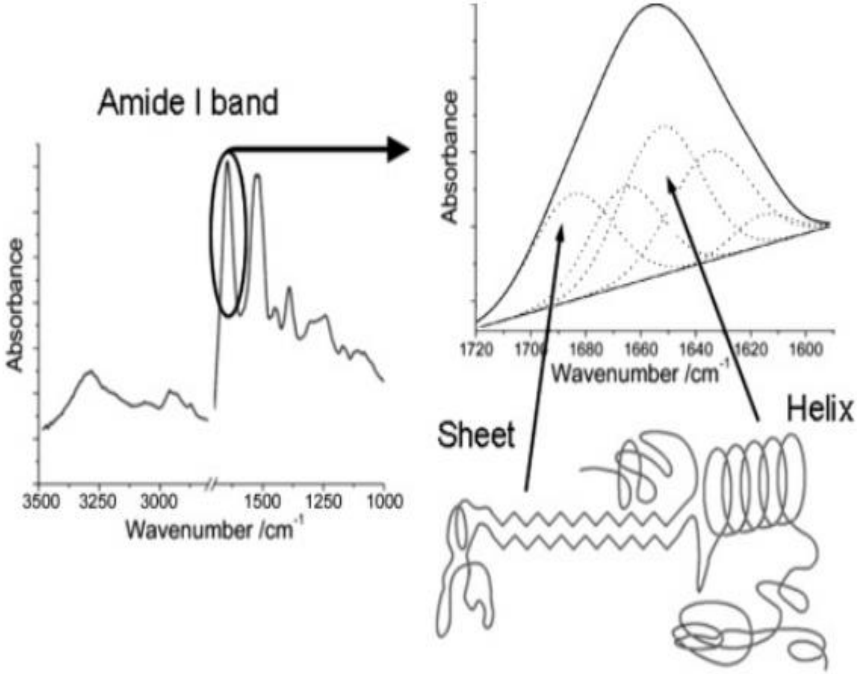
Secondary structures of protein



https://www.boundless.com/biology/textbooks/boundless-biology-textbook/biological-macromolecules-3/proteins-56/protein-structure-304-11437/

Secondary structural analysis

➢ Curve fitting



Roach, Paul, David Farrar, and Carole C. Perry. "Surface tailoring for controlled protein adsorption: effect of topography at the nanometer scale and chemistry." Journal of the American Chemical Society 128.12 (2006): 3939-3945.

Secondary structure	Vibrational frequency (cm^{-1})
β-sheets/turns	1685-1663
α-helices	1655-1650
Random chains	1648-1644
Extended chains/β-sheets	1639-1621
Side chain moieties	1616-1600

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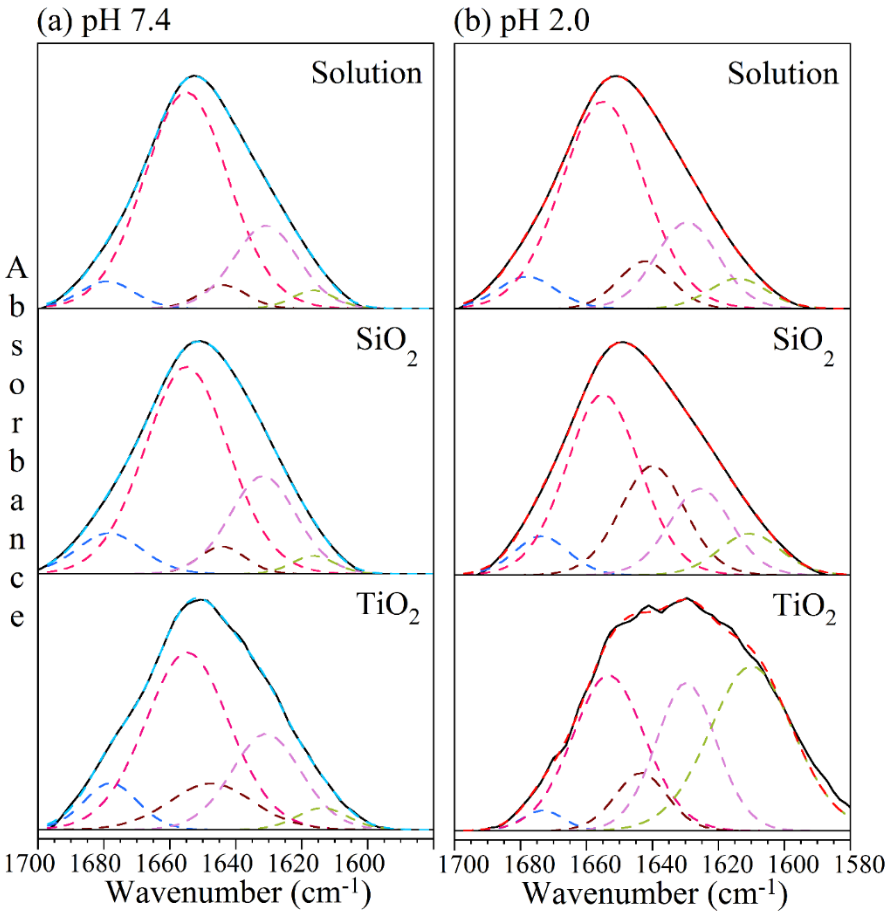
Secondary Protein Structural Analyses of BSA

Solution phase compared to adsorbed
TiO_2 compared to SiO_2
pH 7.4 compared to 2.0

Normalized BSA amide I band for secondary structural analysis through curve fitting



Secondary structure	Vibrational frequency (cm^{-1})
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Extended chains/β-sheets	1639-1621
Side chain moieties	1616-1600

The secondary structure content (%) in BSA determined via curve fitting for BSA in solution and after adsorption on to the nanoparticle surfaces; SiO_2 and TiO_2

pH	Secondary structure	Solution phase BSA	Adsorbed BSA on SiO_2 (Δ from solution)[a]	Adsorbed BSA on TiO_2 (Δ from solution)[a]
7.4	β-sheets/turns	5	9 (+4)	9 (+4)
	α-helices	68	59 (-9)	51 (-17)
	Random chains	4	15 (+11)	13 (+9)
	Extended chains/β-sheets	20	12 (-8)	23 (+3)
	Side chain moieties	3	5 (+2)	4 (+1)
2.0	β-sheets/turns	6	5 (-1)	2 (-4)
	α-helices	60	48 (-12)	30 (-30)
	Random chains	9	15 (+6)	8 (-1)
	Extended chains/β-sheets	19	25 (+16)	24 (+15)
	Side chain moieties	6	7 (+1)	36 (+30)

[a] – difference between adsorbed and solution phase structure content

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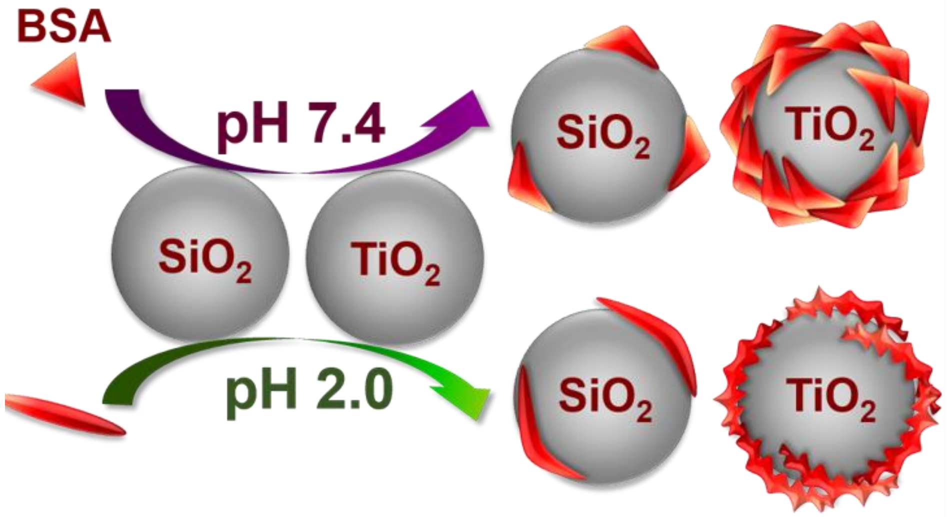
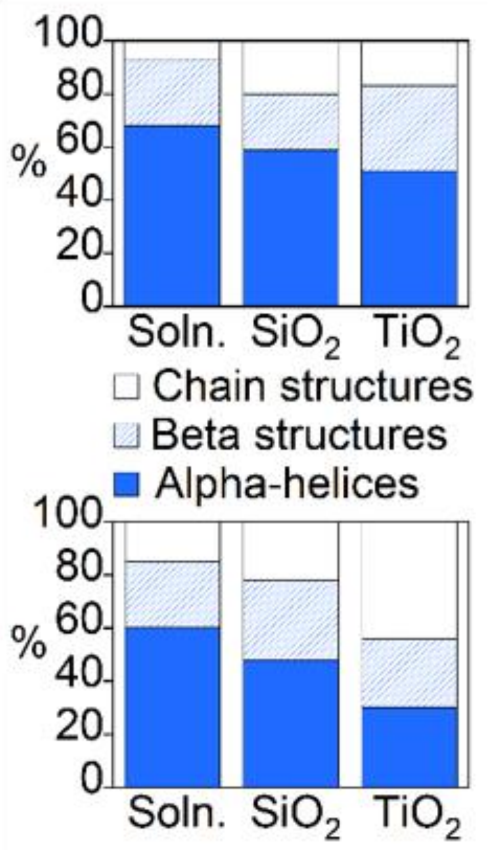
Summary of Results: BSA at Circumnetural and Acidic pH





-Protein conformation changes as a function of pH

- Solution phase BSA
- Adsorbed BSA

-Protein conformation upon adsorption changes and this differs on the two oxide surface

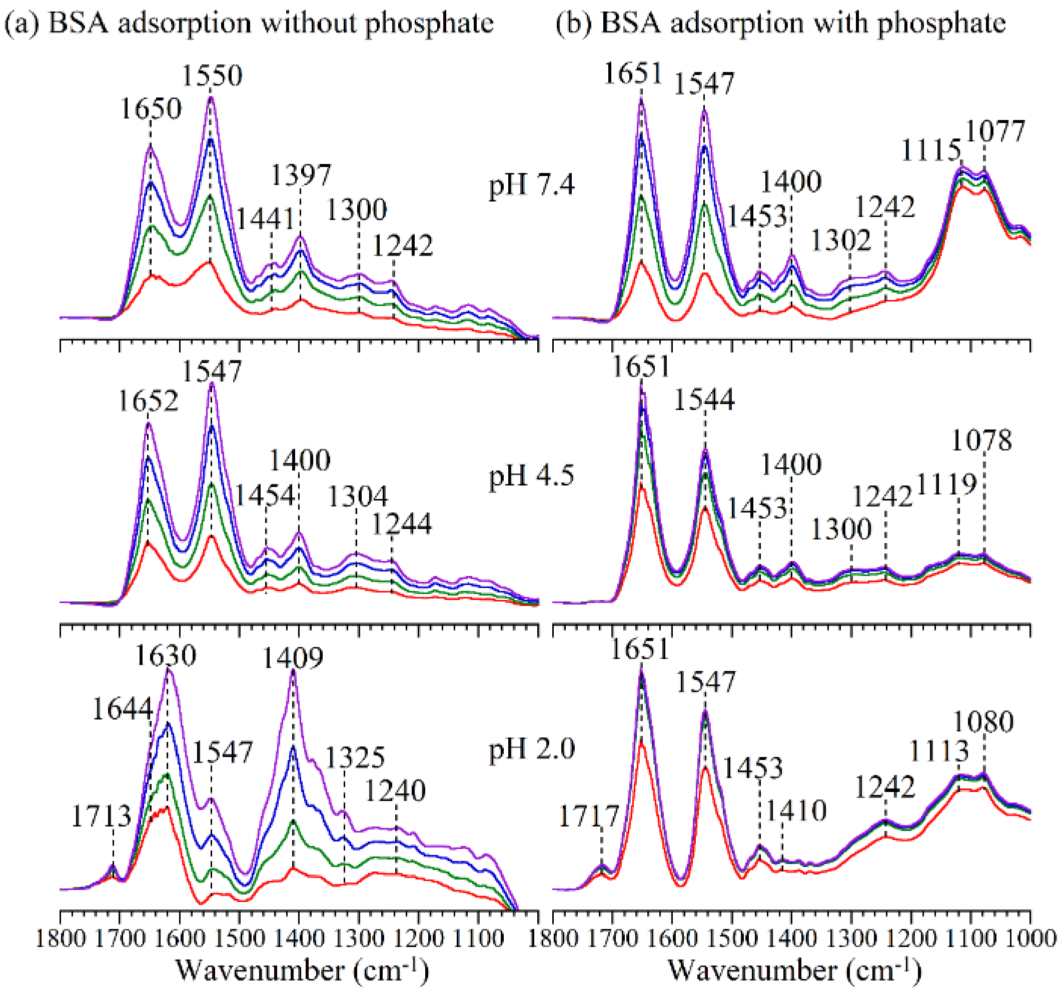
-Protein interaction is strongest with the TiO_2 NP surface

- Higher surface coverage
- Larger change in protein conformation
- Interaction with surface OH groups (possibly as OH_2^+) may be important for this interaction and that is greatest on TiO_2

Impact of Phosphate Adsorption

Effects of co-adsorption of phosphate on protein-surface interaction and protein structure

BSA adsorption on TiO2 (22 nm) in the presence/absence of phosphate



- Phosphate bands (ca. 1117 and ca. 1077) observed in the spectra.
- Growth of both protein bands and phosphate indicates co-adsorption of phosphate.
- Phosphate inhibits BSA from denaturation on TiO_2 at acidic pH.

Xu, Zhenzhu; Grassian, V.H.
Journal of Physical Chemistry C
2017, 121, 21763-21771.

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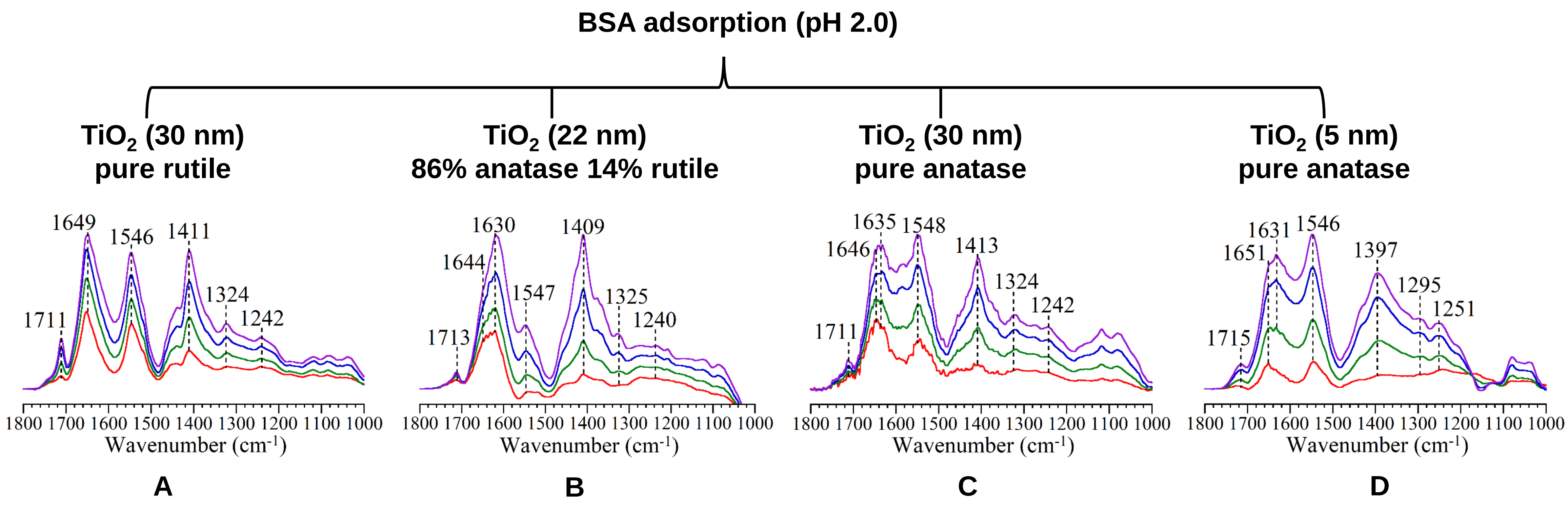
Impact of Nanoparticle Crystalline Phase



- Protein adsorption highly depends on the crystalline phase of TiO_2 nanoparticles
- Anatase vs rutile: Only anatase can cause complete BSA denaturation upon adsorption at very acidic pH.

Zhenzhu Xu

Conclusions

1 Molecular Probes of Nanoparticle Surfaces Provide Important Insights

2 pH of Aqueous Phase Impacts Nano-Bio Interactions

3 Protein Structure Impacted Nanoparticle Composition, Surface Structure and the Presence of Co-Adsorbates

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