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WILLAMETTE UNIVERSITY

Interactions of 17β-Estradiol with Fe(II)-Activated Nontronite

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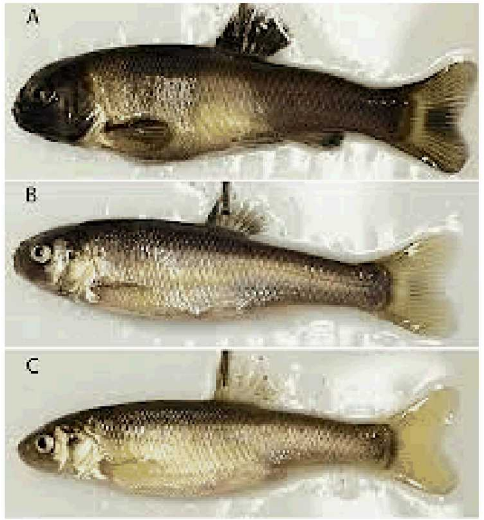
Implications of Estrogen Contamination



Fathead Minnows
A: normal male
B: feminized male
C: normal female
Figure from Keyeles, EPA, 2014

NATIONAL GEOGRAPHIC

About 85 percent of male smallmouth bass collected in national wildlife refuges in the Northeastern U.S. had eggs were growing in their testes. Pollutants that mimic sex hormones are the suspected culprit.

PHOTOGRAPH BY JOEL SARTORE, NATIONAL GEOGRAPHIC PHOTO ARK

Why Are These Male Fish Growing Eggs?

Fish in wildlife refuges are feminized, probably by hormone-skewing pollution. What does this portend for the health of all creatures—and people?

7 MINUTE READ

BY LINDSEY KONKEL

Male fish mutating into females because of waste chemicals, expert warns

Expert calls for stronger stance on chemicals and drugs that are likely to have 'sub-lethal' effects on wildlife

Ian Johnston Environment Correspondent | @montaukian |
Monday 3 July 2017 13:00 | 2.3K shares |

Like Click to follow The Independent

Birth-control pills are partly to blame for the changes in fish anatomy (Getty/iStockphoto)

PNAS

Collapse of a fish population after exposure to a synthetic estrogen

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Edited by Deborah Swackhamer, University of Minnesota, Minneapolis, MN, and accepted by the Editorial Board March 29, 2007 (received for review October 27, 2006)

Science News *from research organizations*

Estrogen in birth control pills has a negative impact on fish

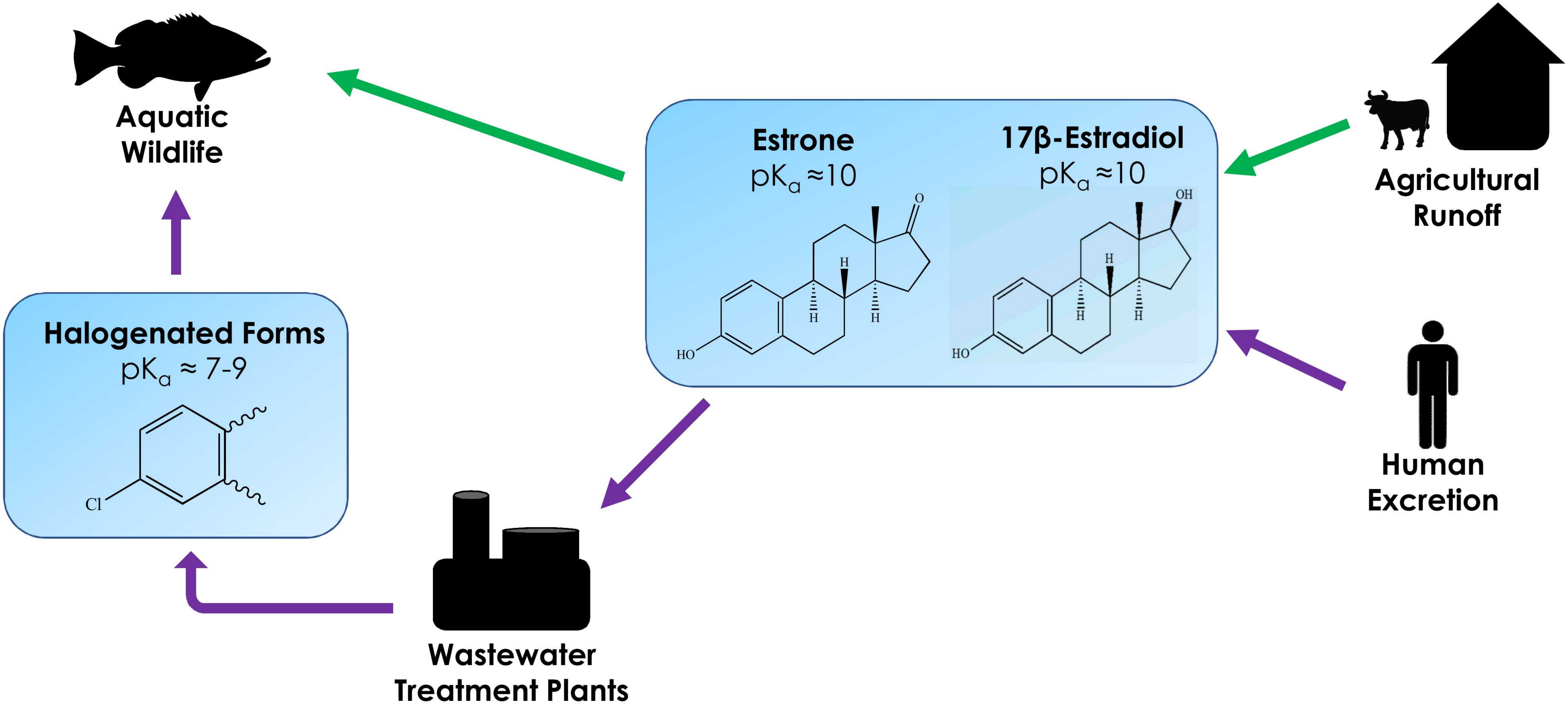
Date: March 4, 2016

Source: Lund University

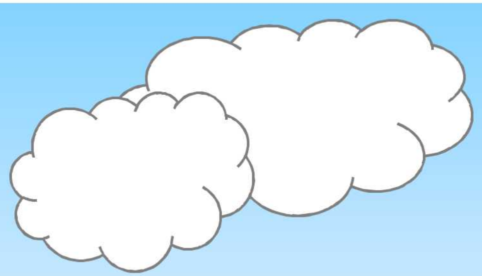
Summary: New research shows that hormones found in birth control pills alter the genes in fish, which can cause changes in their behavior.

Share:

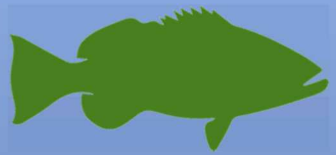
Sources of 17β-Estradiol and Estrone



Environmental Fate of 17β-Estradiol and Estrone



Photodegradation

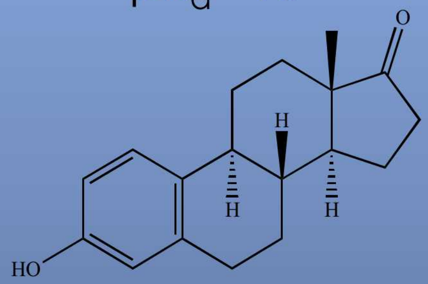


Bioaccumulation

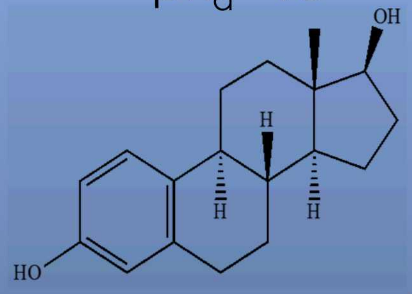
- Toxicity observed at 100 ng/L for E1 and E2 alone
- Together toxicity observed at 50 ng/L

Routledge et al. 1998, Environ. Sci. Tech

Estrone
$pK_a \approx 10$



17β-Estradiol
$pK_a \approx 10$

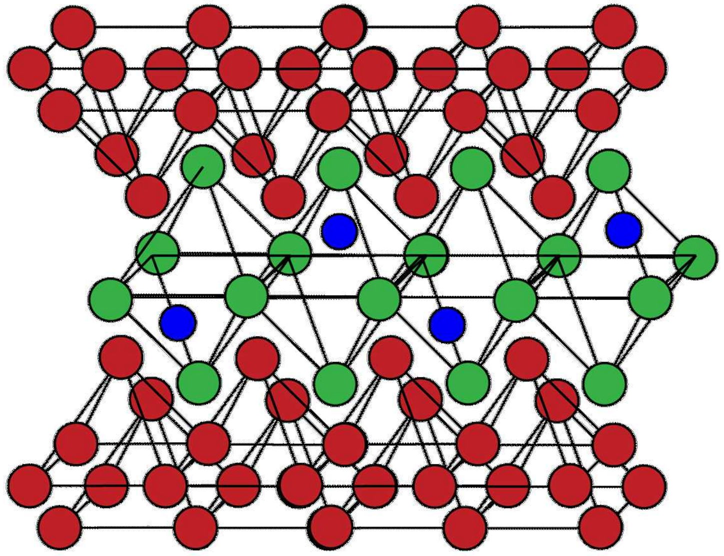


Biodegradation

Sedimentation

Redox Reactions on Mineral Surfaces

- Removal and Degradation of E2 by mineral surfaces
  - Manganese oxides: 80% oxidation and 95% sorption[1,2]
  - Montmorillonite: 100% oligmerization[3]
- Nontronite (NAu-1): 2-1 clay mineral
  - Two tetrahedral sheets and one octahedral sheet
  - Structural iron in octahedral sheet is redox-active. [4-9]
  - Activated NAu-1 oxidizes/reduces inorganic contaminants and organic contaminants.[6-8]



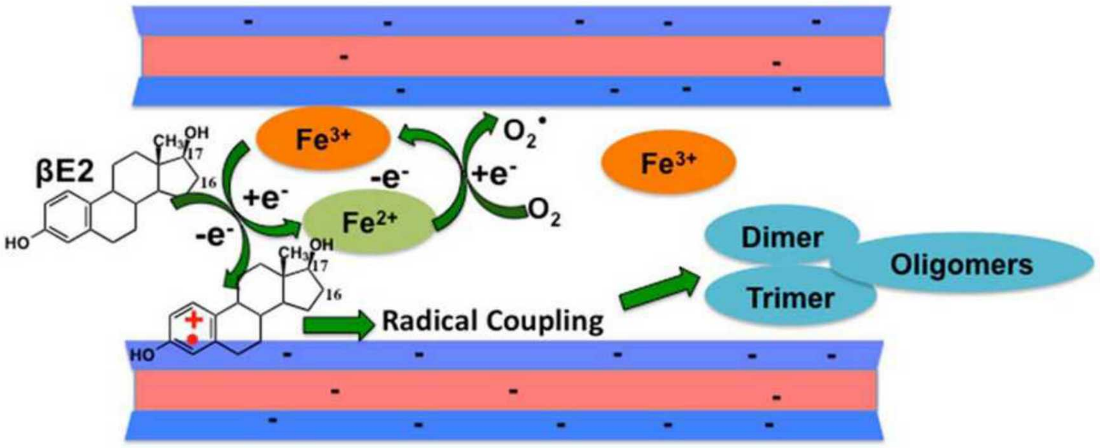


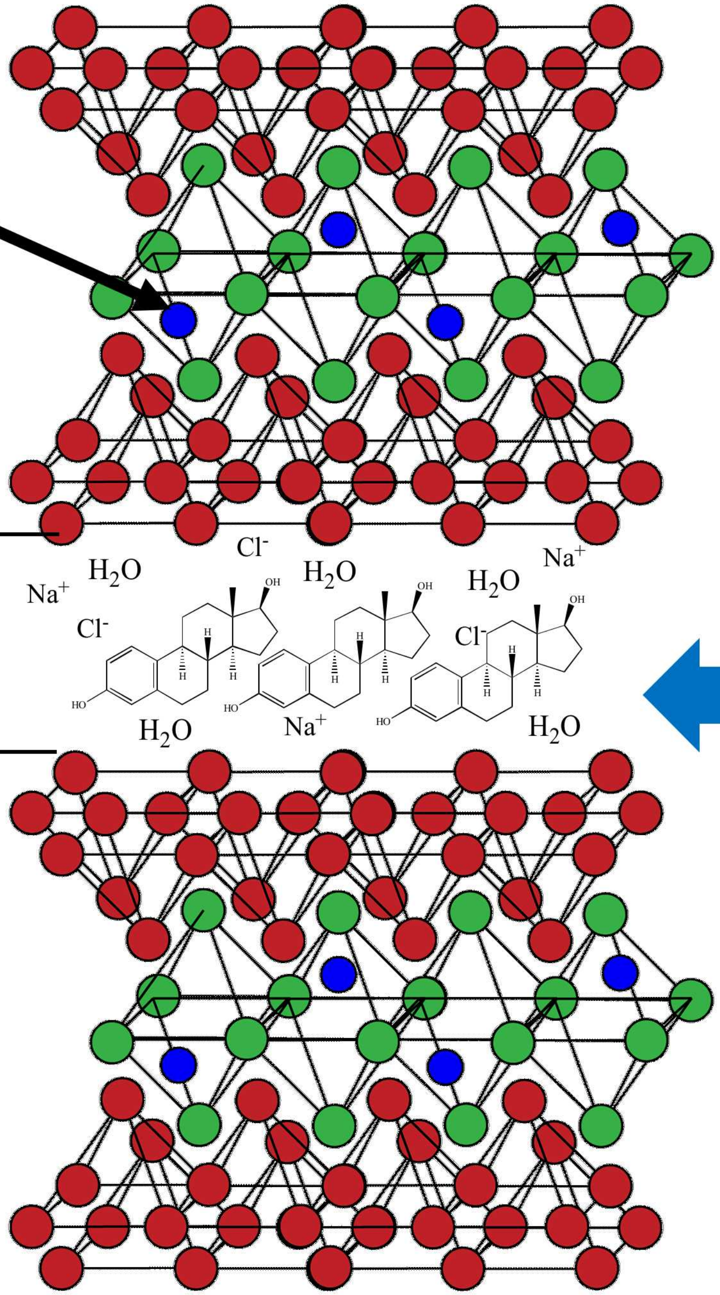
Figure from Qin et al., Envr Sci Tech, 2014

[1] Daniel Sheng et. al, 2009
[2] Jiang et. al, 2009
[3] Qin et. al, 2015
[4] Hofstetter et.al, 2003
[5] Stucki, 2006
[6] Ilgen et al., 2012
[7] Ilgen et al., 2017
[8]Hofstetter et al., 2006
[9] Neumann et al., 2013

Predicted Sorption of Estradiol on Non-Activated NAu-1

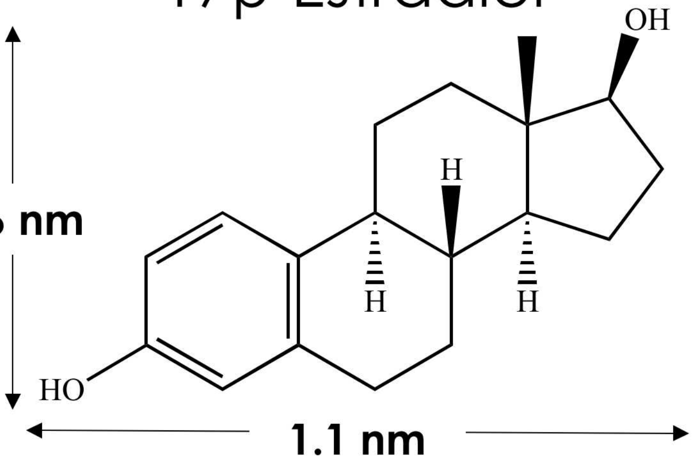
Structural Fe(III)

Interlayer with Exchangeable Cations ≈3 nm

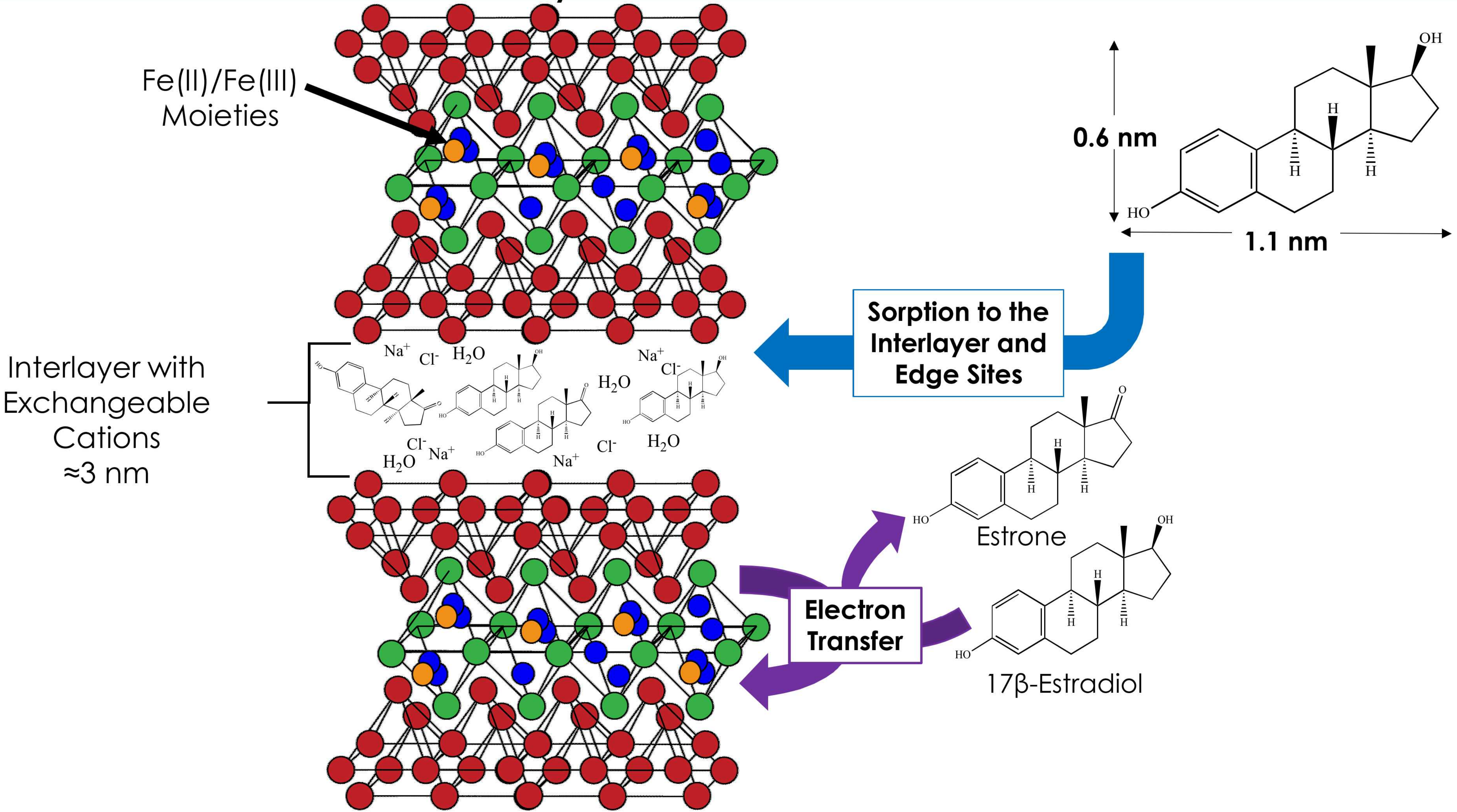


Sorption to the Interlayer and Edge Sites

17β-Estradiol



Predicted Reactivity of Estradiol on Activated NAu-1



Kinetic Experiments to Analyze Interactions of E2 on NAu-1

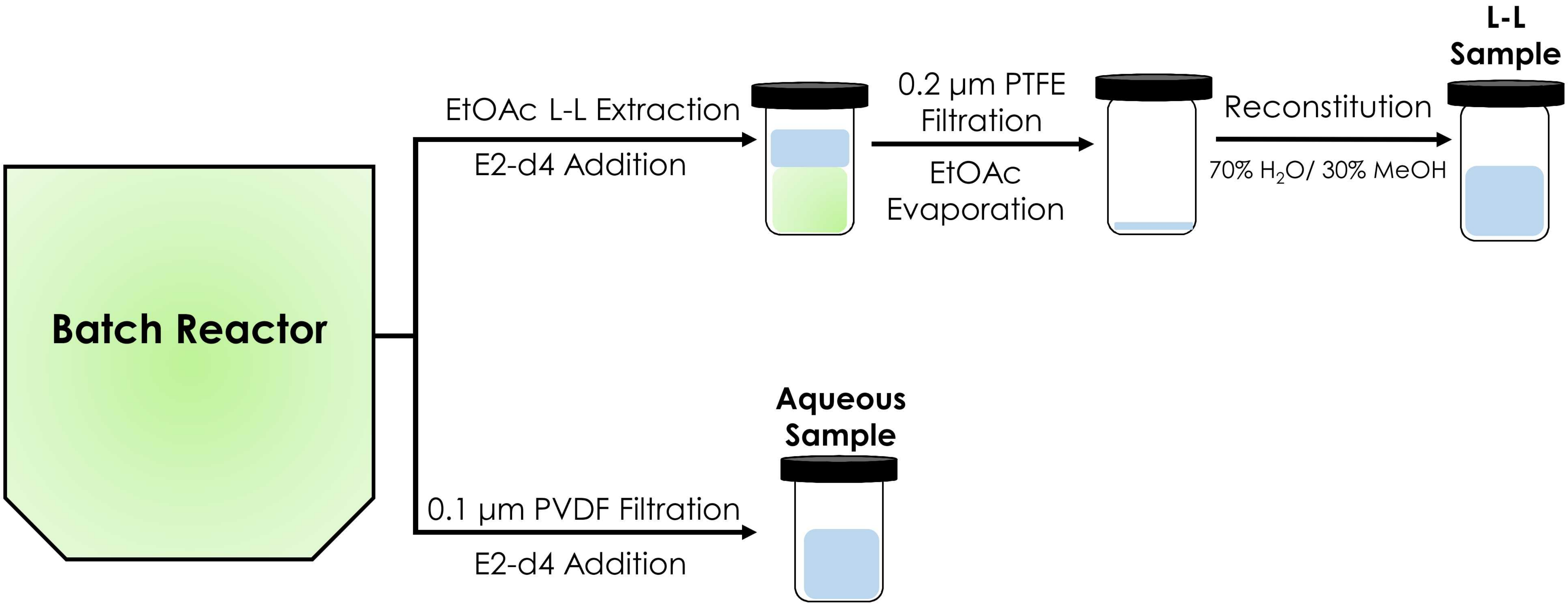
- Is there sorption of E2 onto NAu-1?
- Will there be redox reactivity between E2 and activated NAu-1?

NAu1-Fe(II)-E2
Activated NAu-1
367 μM E2
0.1 M NaCl
1.1 M $FeCl_2$

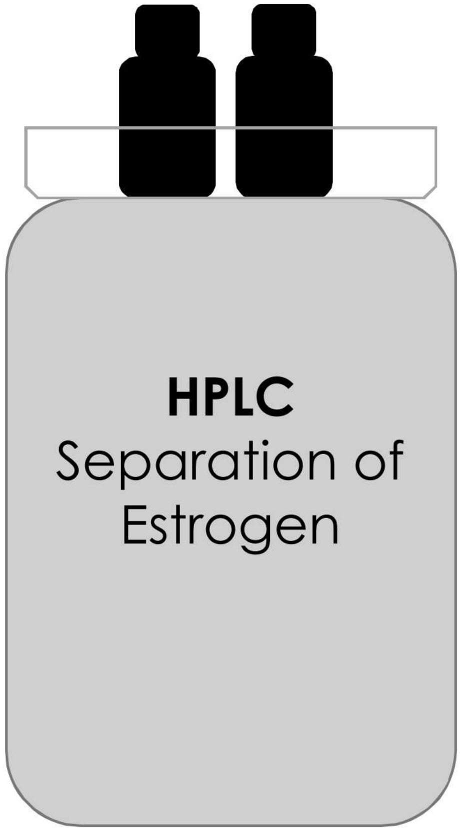
NAu1-E2
Non-activated NAu-1
367 μM E2
0.1 M NaCl

Fe(II)-E2
367 μM E2
0.1 M NaCl
1.1 M $FeCl_2$

Kinetics Experiments



High-Performance Liquid Chromatography



Mass Spectrometer

Electrospray Ionization
Fragmentation of Estrogens in Negative Mode

Tandem Mass Analyzer
Quantification of Estrogens

UV Detector
Quantification of E1 and E2

<u>Two Methods of HPLC</u>

- HPLC coupled to a UV-detector cannot separate E2-d4 from E2
- E2-d4 was quantified using HPLC coupled to tandem mass spectrometry

HPLC Data Analysis

- Peak areas of both HPLC methods

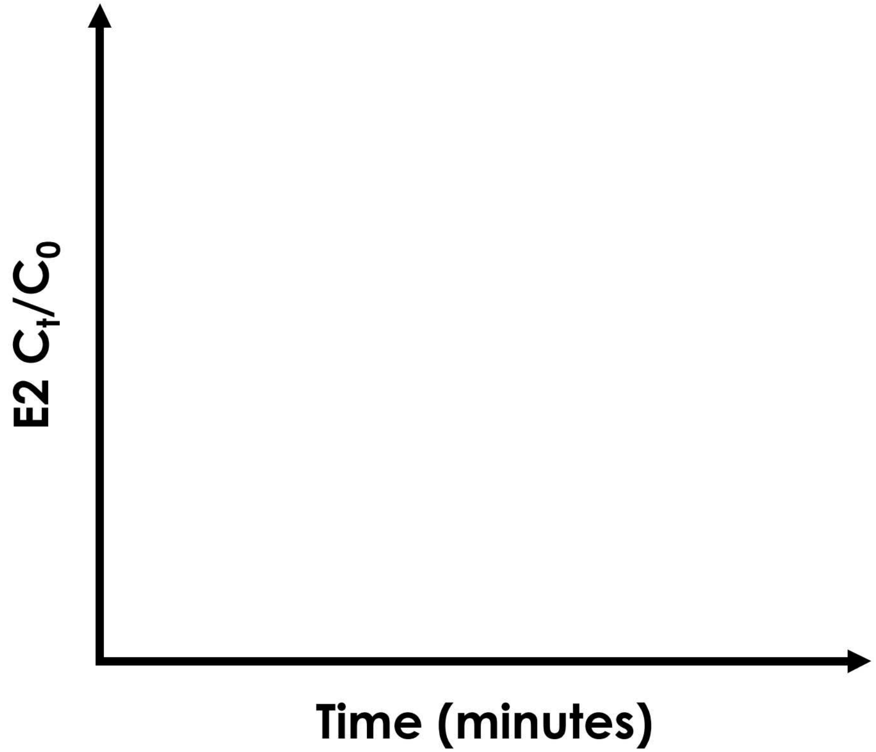
  L-L Peak Area – Aqueous Peak Area = Solid Peak Area

- Normalization of peak areas to internal standard

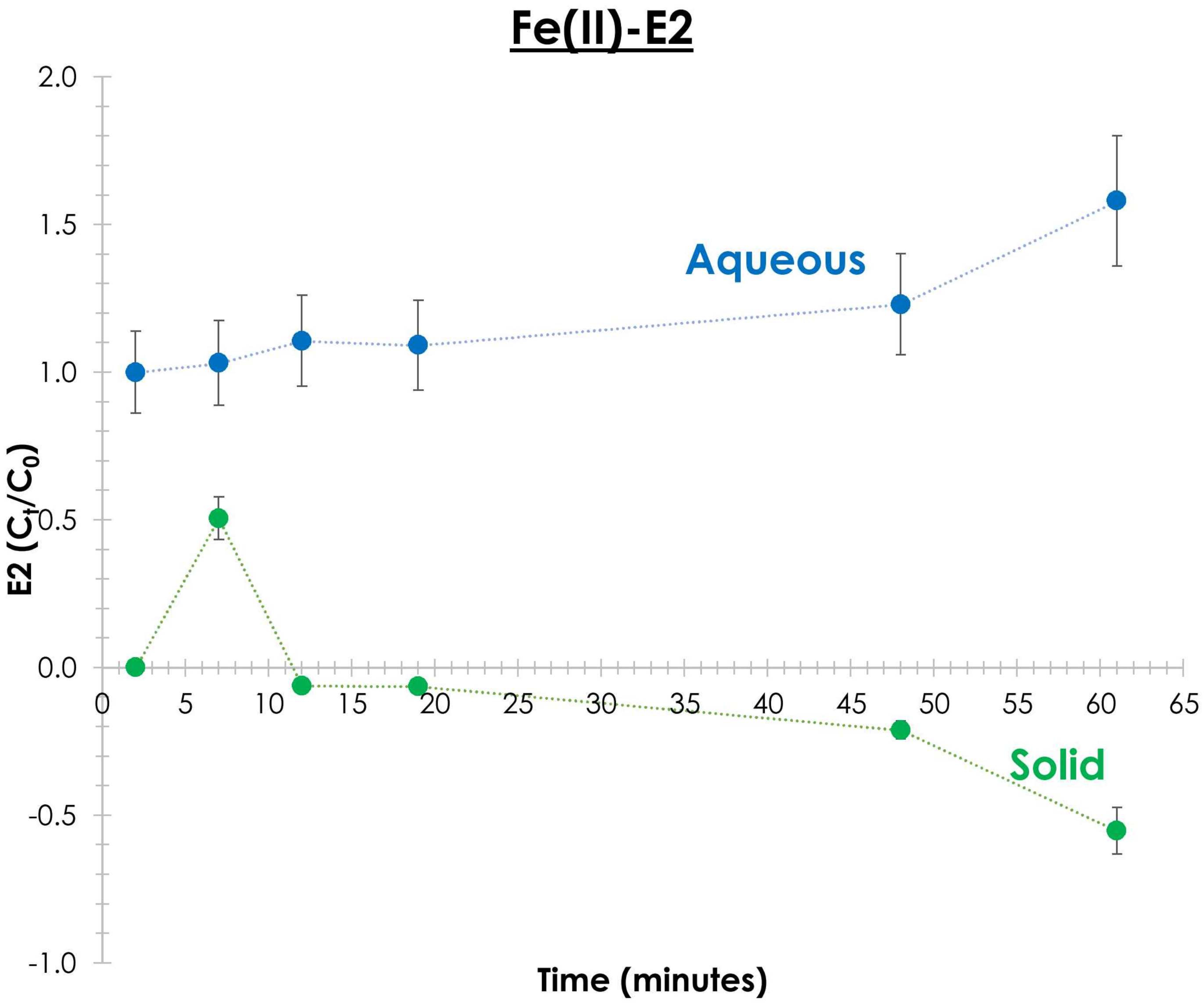
  $$\text{E2 Peak Area} \,/\, \text{E2-d4 Peak Area}$$

- Each time point normalized to first time point

  $$\frac{\text{E2 Peak Area}_{\text{time=T}} \,/\, \text{E2-d4 Peak Area}_{\text{time = T}}}{\text{E2 Peak Area}_{\text{time=0}} \,/\, \text{E2-d4 Peak Area}_{\text{time = 0}}}$$

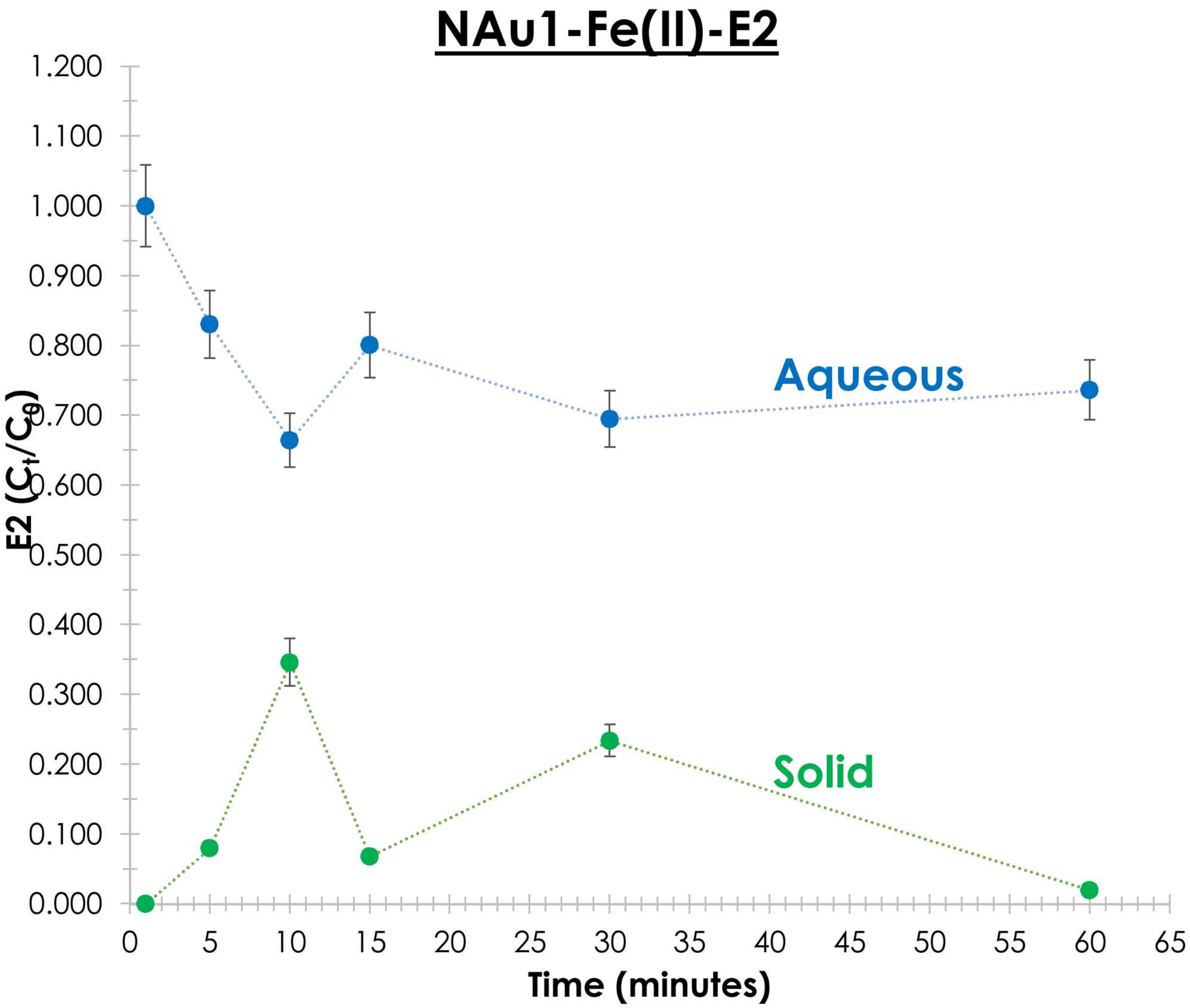


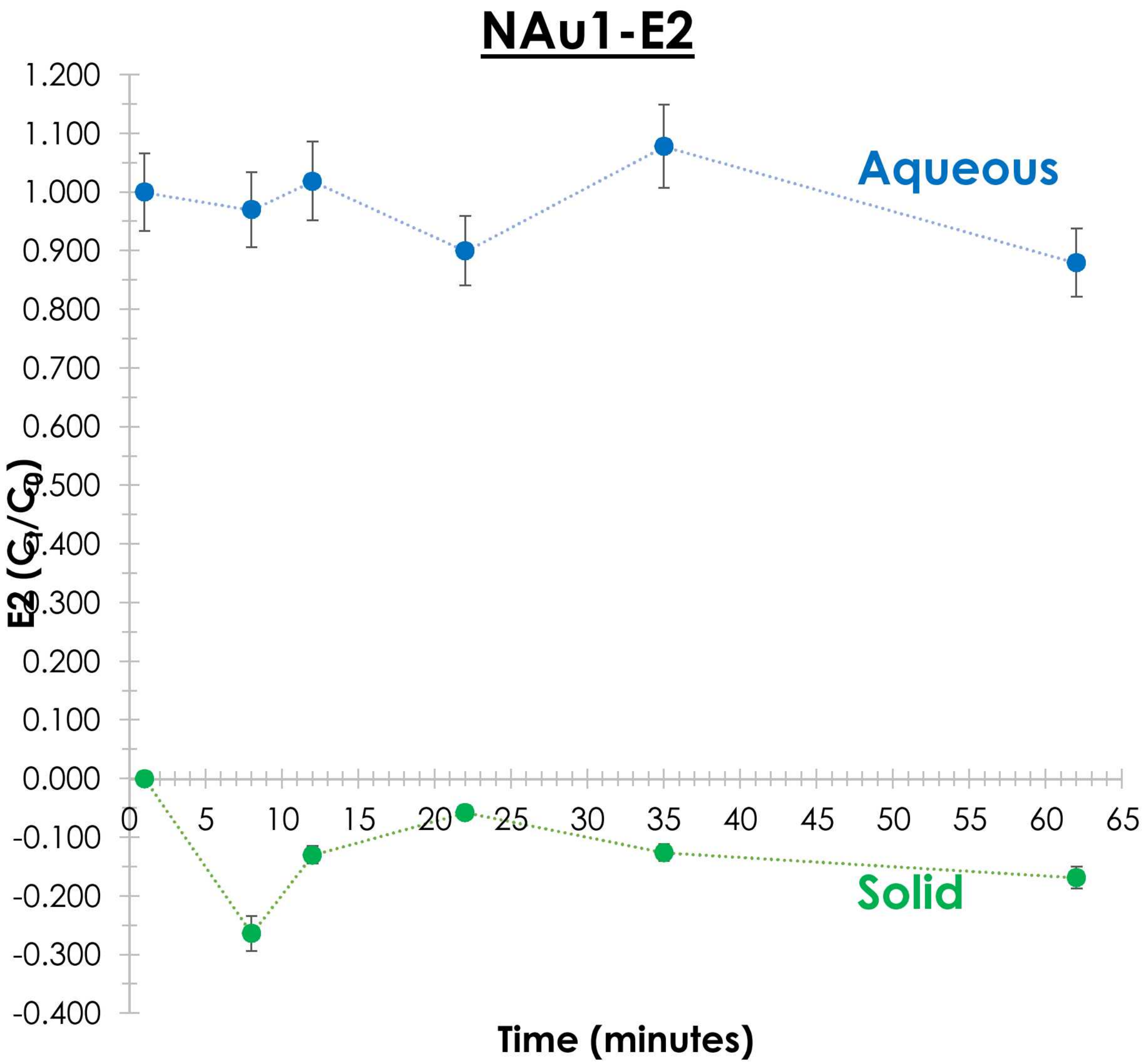
HPLC Kinetics Data



- Lack of redox reactivity or sorption
- Mass-balance obtained

HPLC Kinetics Data





Quantification of Fe(II)/Fe(III) by Diffuse Reflectance

Research Question:

- How does the Fe^{2+}/Fe^{3+} ratio change as NAu-1 is reacted with E2?

Instrumentation: Diffuse reflectance

- Quick analysis
- Hard quantitative analysis- stray light and spectral noise

Experiments:

- Measure diffuse reflectance response after E2 has reacted with NAu-1 or activated NAu-1 for 30 minutes
- Integrated area between 675-775 nm to quantify Fe(II)-Fe(III) intervalence electron transfer band at ≈750 nm

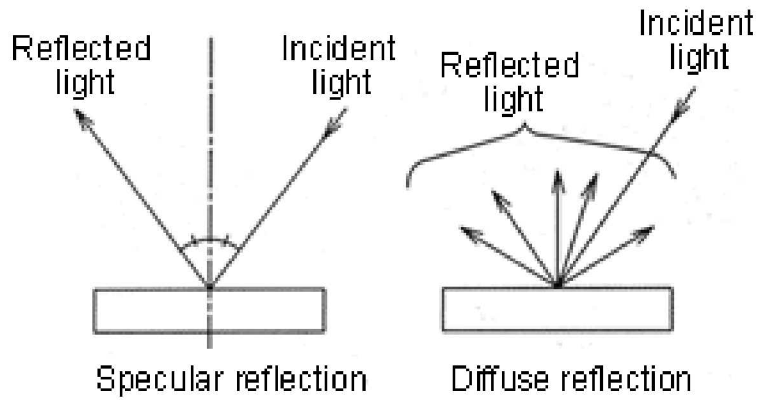
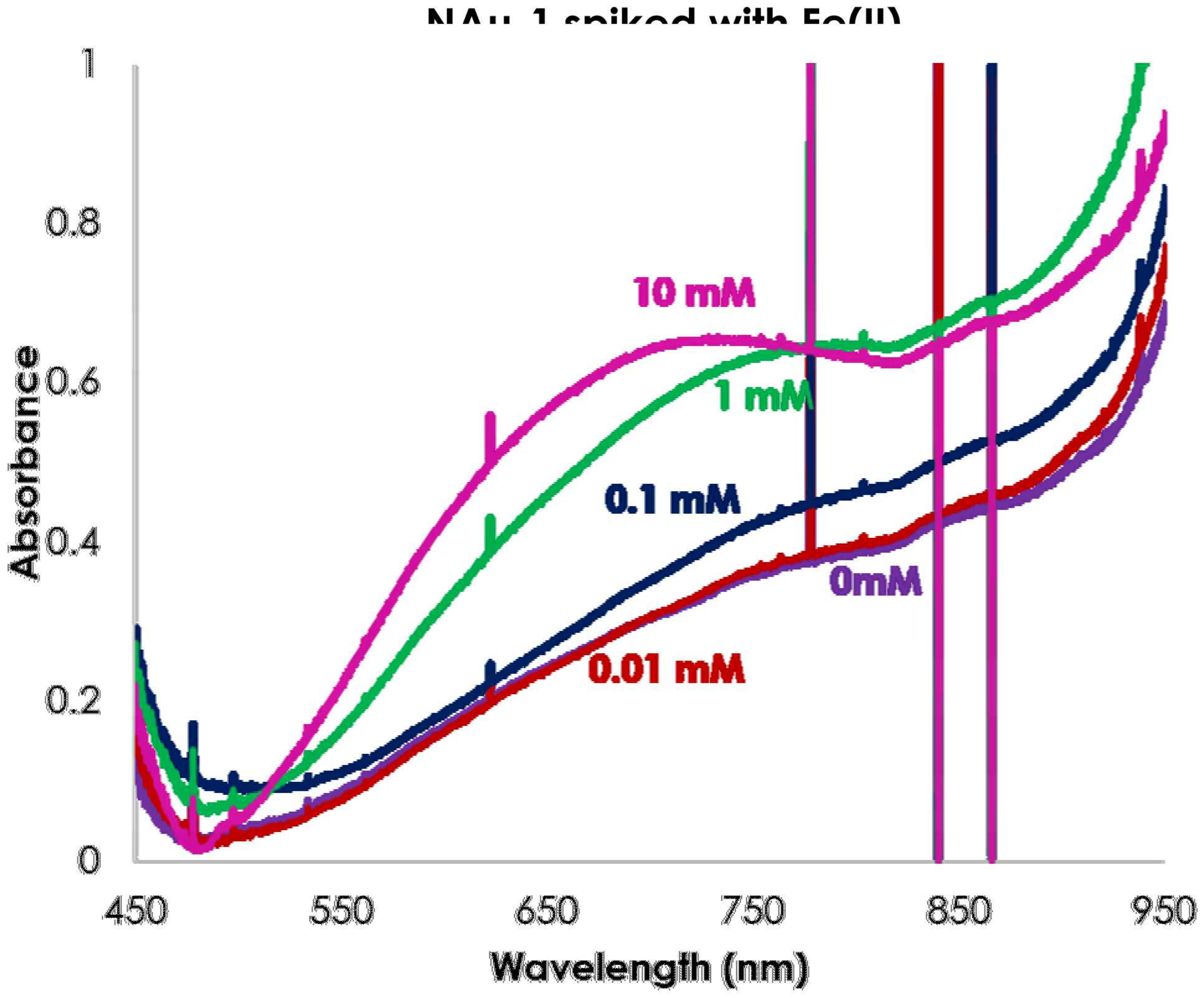
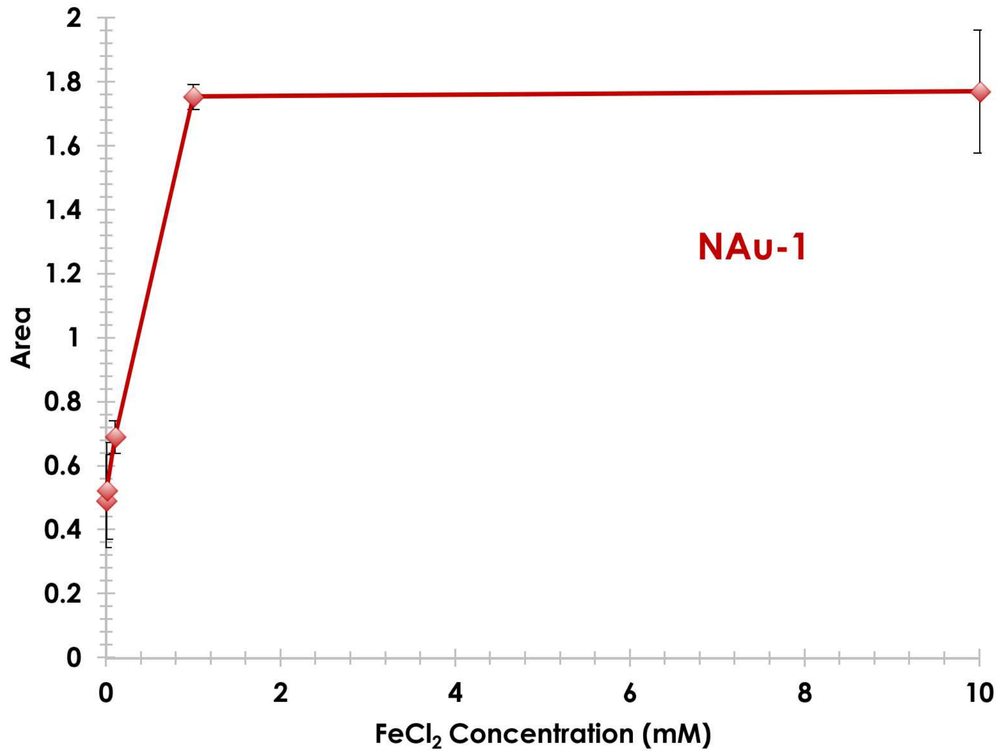


Figure from Shamadzu website, accessed 2018

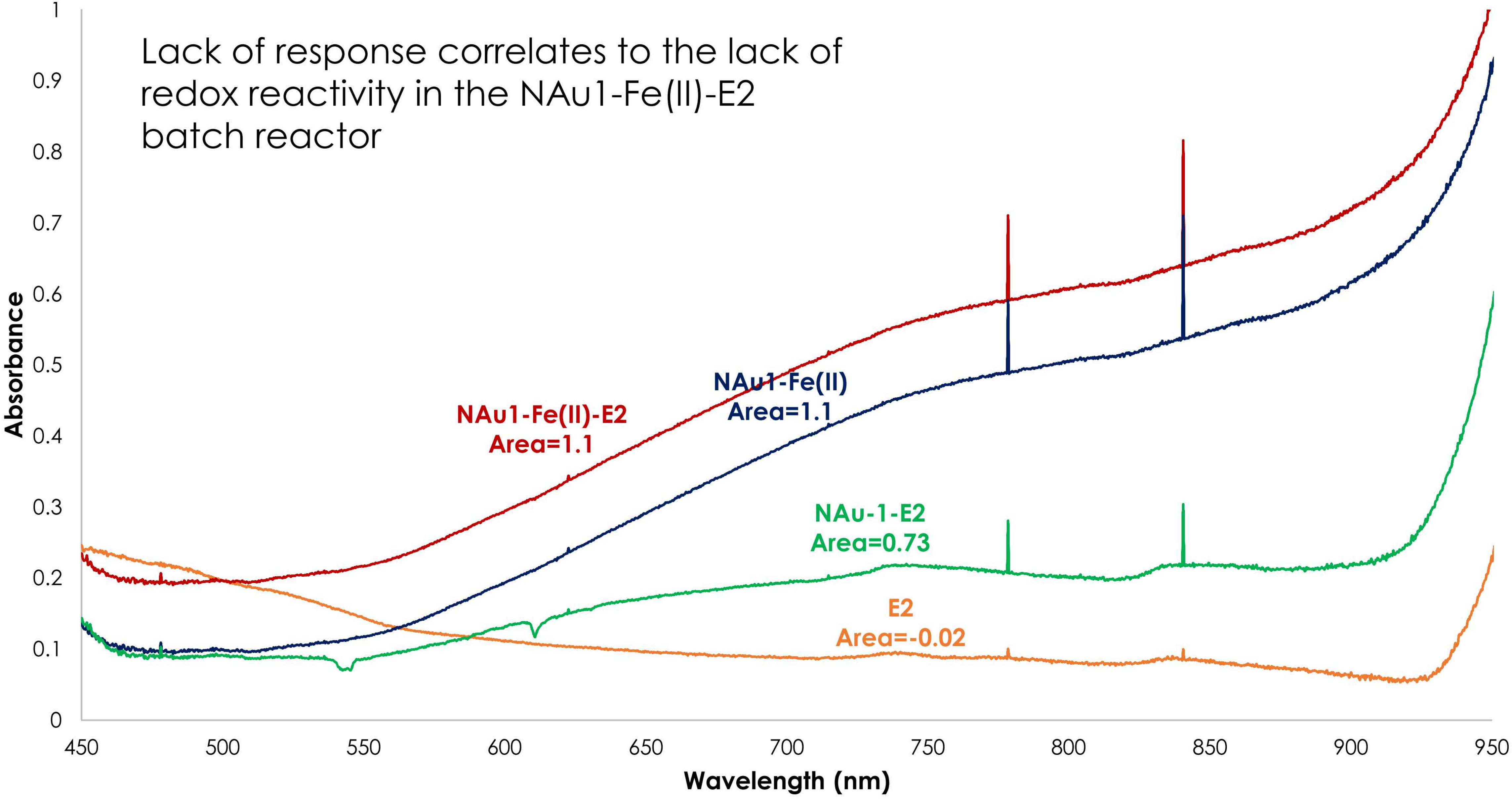
Diffuse Reflectance Response to Fe(II)/Fe(III) Moieties



Diffuse reflectance response due to an increase of Fe(II)/Fe(III) moieties as Fe(II) is added to NAu-1 surface

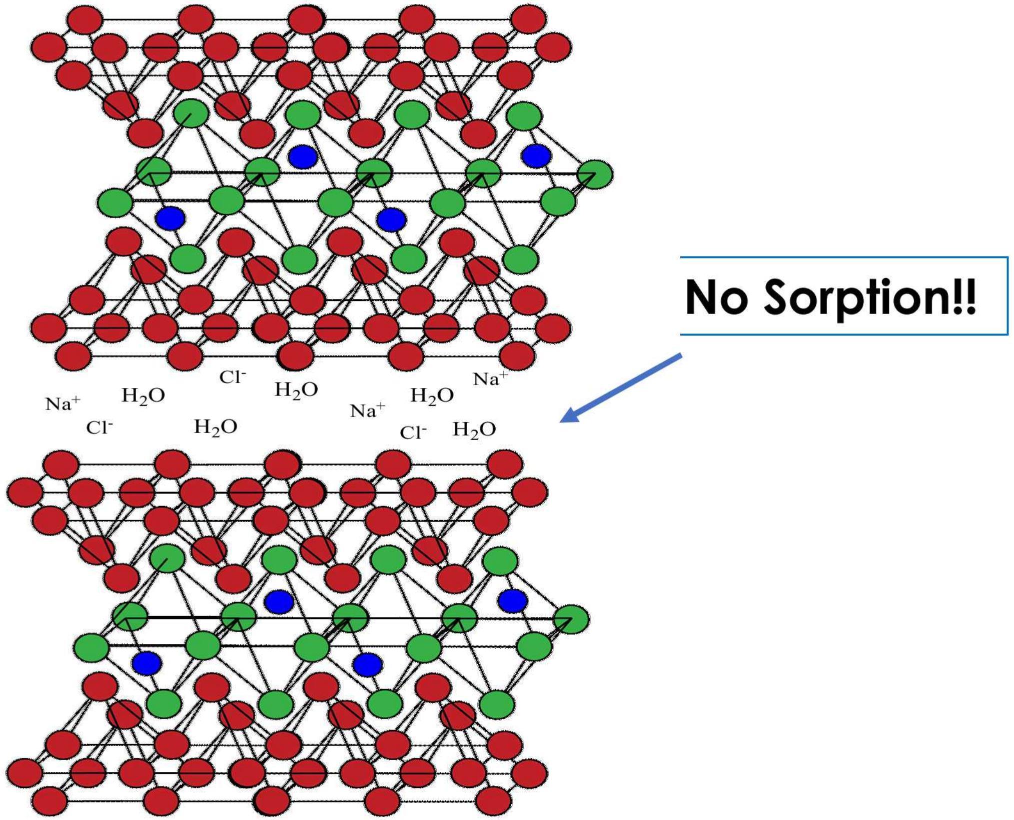


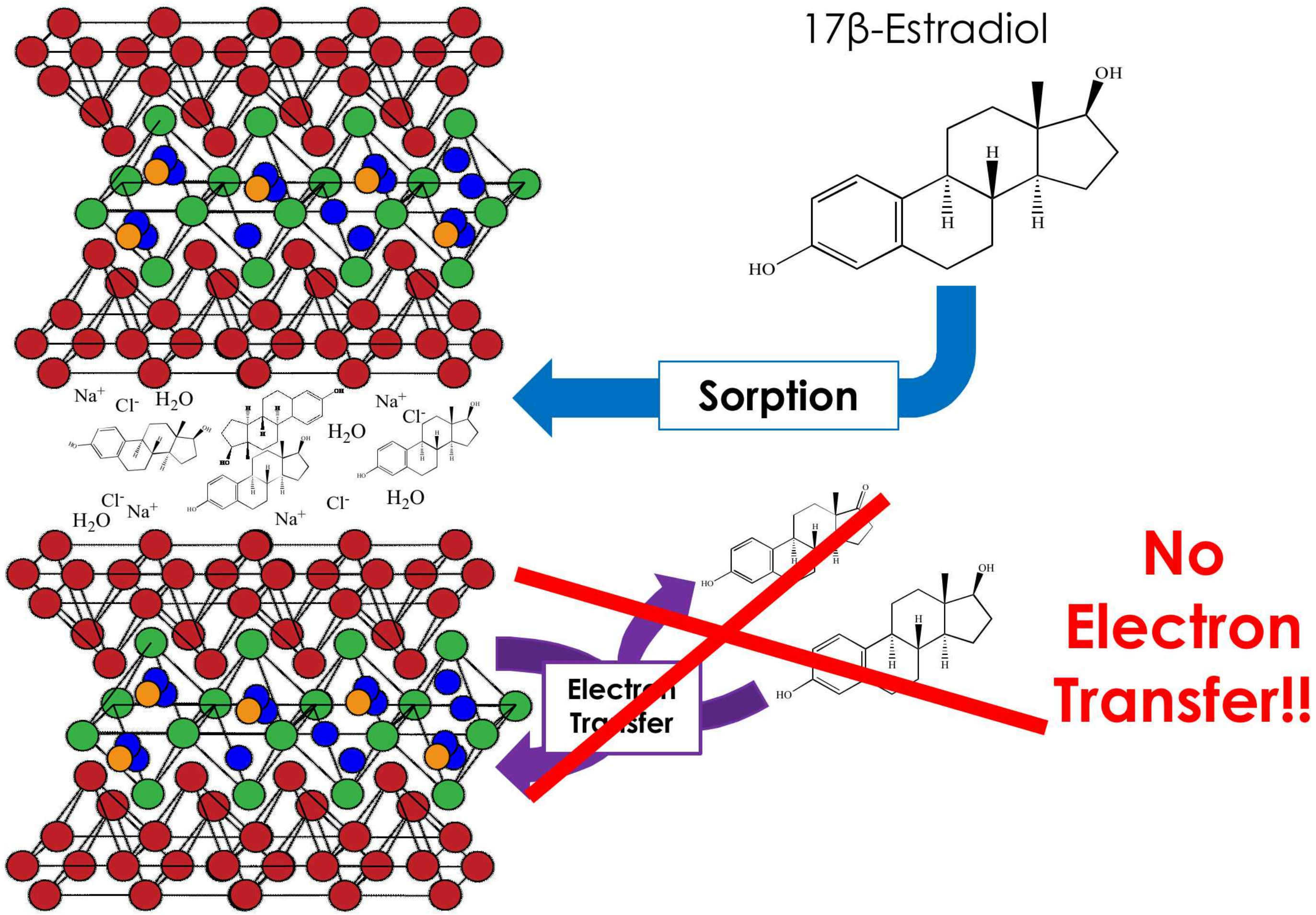
Diffuse Reflectance Spectra



Conclusions

Results





Future Work

- Longer kinetics experiments
- Sorption experiments