

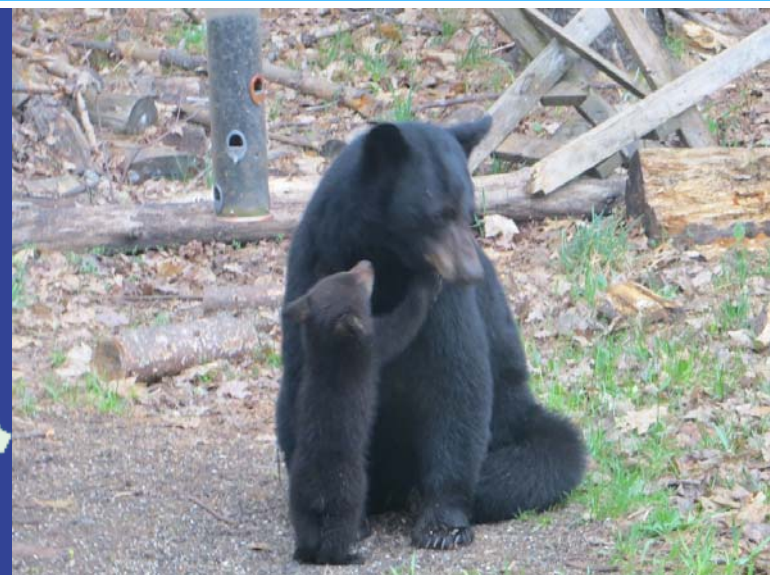
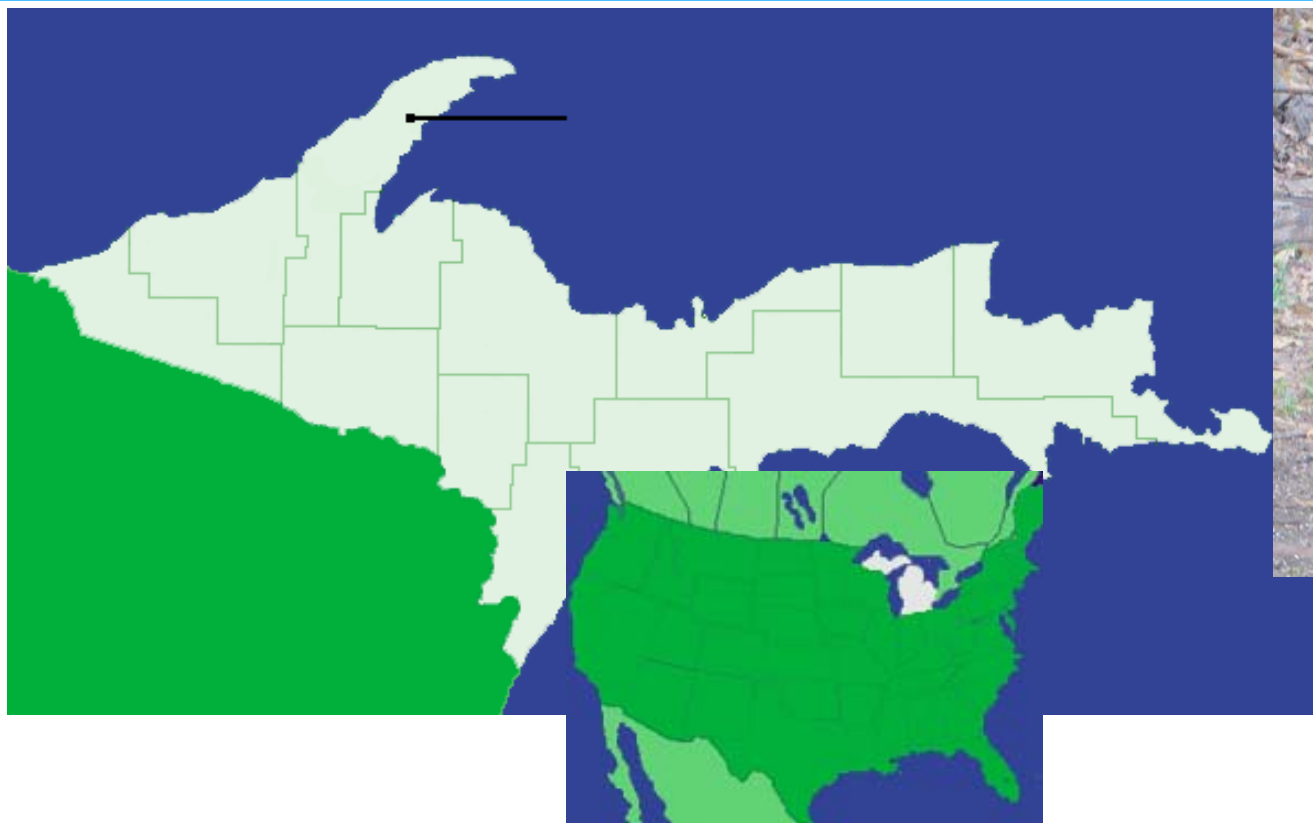
# Method Validation: Outsider View

NEMC2018



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# Literally Outsiders: Upper Peninsula of MI



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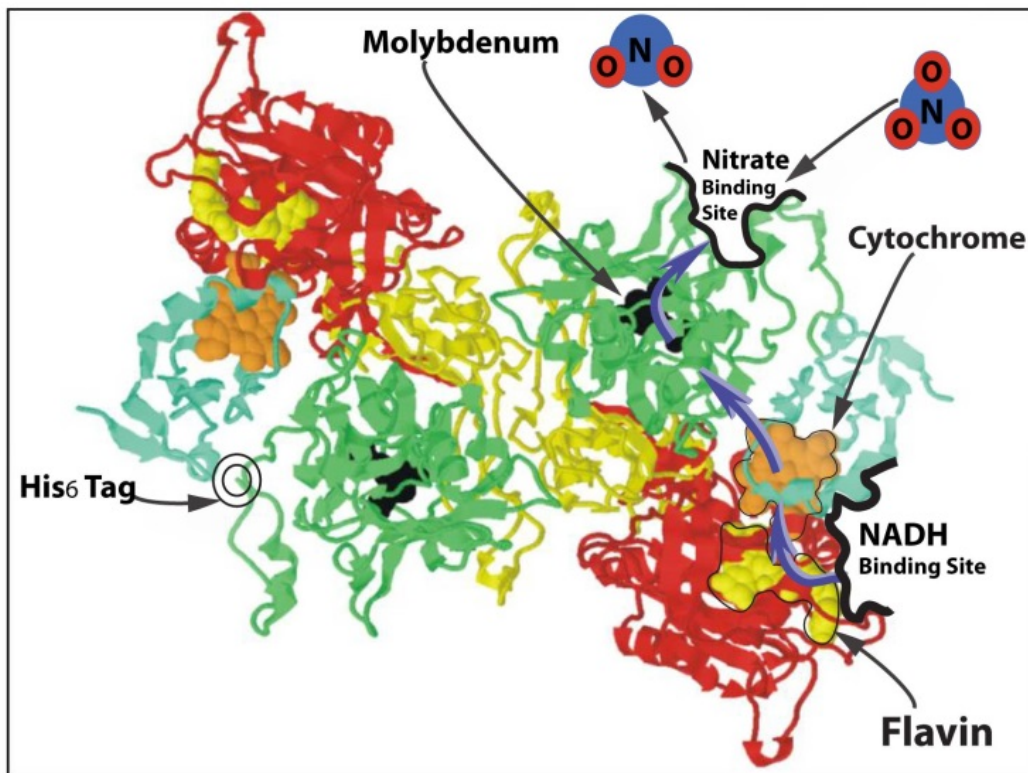
# Initiation: Pharmacology Research

- Internship and 1<sup>st</sup> lab experience
- Project: How do diuretics work at the molecular level in the kidney?
- Expertise and skills required: Cellular structure, organic synthesis, small animal surgery, analytical chemistry
- Diuretics work by inhibiting carbonic anhydrase in the cell walls of the nephrons of the kidney
- Needed derivatives of 3 sizes
- Design, synthesize, identify derivatives, develop relevant HPLC (UV & Fluorescence) assay
- Experiments



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# Next stage: Nitrate Reductase in Academia



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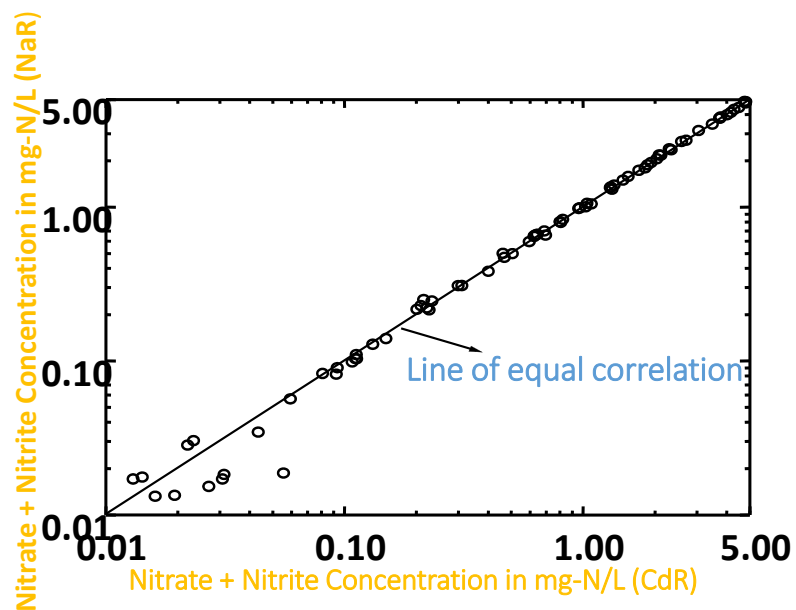
# What we had to do to Understand NaR

- Purify 1.0 mg to make antibodies
- Produce MAbs as protein purification tools
- 100s of mg required for Structure info
- Develop nitrate assay to correlate RNA messages with protein expression
- Blue Sepharose affinity LPC
- Set and run mammalian cell culture facility
- MAbs increased production to 25 – 100 mg per run
  - Samples of all of these proteins and MoBio tools distributed to researchers worldwide



# So we started a company!

- First products: Nitrate Reductase purified from Corn Seedlings by immobilized monoclonal antibody affinity chromatography
- USGS publication in *ES&T* used this form of NaR



# Everyone measures Nitrate!



**Agriculture +  
Farmers**



**Environmental  
+ Water Quality**



**Home Owners  
Wells**



**Education +  
Citizen Monitoring**



**Utilities, Industrial and  
+ Commercial Labs**



**Researchers +  
Clinical**



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# Validation experiences

- First customers: Biomedical research companies for Nitric Oxide Synthase assay kits
  - This was simple: Microplate format, picomoles in biological and tissue culture fluids. The customers performed the validations.
- Next: Agricultural Samples
  - This was unsatisfactory. No standard samples, commercial labs use ISE for plant extracts and soil or ineffective/nonreproducible sample prep, same sample to multiple labs returned wildly varied results.
- Water: BARRIERS
  - Habit, resistance to change, differences in style.
  - Conflicting demands by regulator, agency, water type.



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# A Few Suggestions

- **Qualified source of affordable and fully characterized standard samples**
- **SOPs for validation studies**
- **Concordance across disciplines on what qualifies as a reliable assay**
- **Rational accuracy definitions and demands**
- **Faster adoption of innovative methods**
- **What else?**



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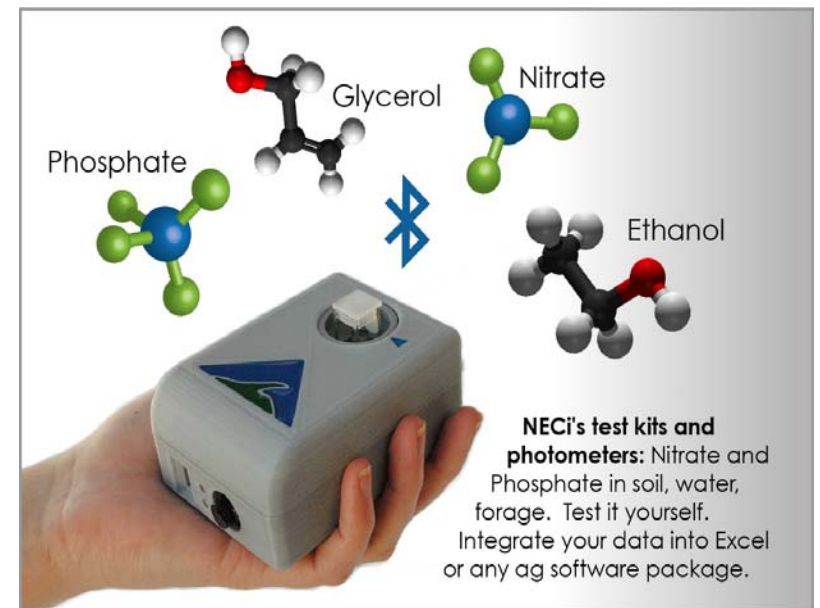
# Thanks to all our Collaborations and to the grant agencies that have funded our R&D

NIH's SBIR program funded expression of recombinant of YNaR and AtNaR.

USDA's SBIR program funded applications, from test kits to reagents for Discrete Analyzers.

NSF's STTR program helped fund our handheld photometer.

NECi: Biotechnology Chemists can Use



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