Highly Sensitive Micro-Biosensors for in-situ Monitoring of Mercury (II) Contaminants through Genetic-evolution and Computer modeling of Metal-binding Proteins

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Modeling and computation: Patrick Theofanis, Youyong Li,
Power, Environmental and Energy Research (PEER)
California Institute of Technology

Created in 2000 for Collaborative Research in Energy and Environmental Issues.
Director: Yongchun Tang, co-Director: William A. Goddard III

Mission:
Conduct fundamental research in the science and engineering underlying energy and environmental technologies. Train new scientists and engineers to provide the multidisciplinary knowledge needed to solve these problems.

Scope: PEER spans traditional fields of Chemistry, Geochemistry, Engineering, and Materials, to integrate developments in chemistry, chemical engineering, material sciences, geochemistry, and environmental chemistry to develop affordable energy production technologies while preserving and enhancing the environment.

Key strategy: develop and use computer simulation and modeling (from quantum mechanics to macroscopic) to interpret and guide experiments and to provide new design principles.

Coupled with Materials and Process Simulation Center (MSC) directed by Professor Goddard at Caltech
Peer Industrial Partners

Power, Environmental & Energy Research Center
California Institute of Technology
PEER Projects

Enhanced oil recovery (surfactant flooding)
Gas to Liquids (CH4 to CH3OH)
Heavy Oil Upgrading
Reservoir Simulation
Flow Assurance
Production Chemistry
Gas Isotope Modeling
Thermal Sulfate Reductions
MultiParadigm Strategy enables application of 1st principles to complex systems
Stimulation: industrially supported projects
Always Impossible, forces new theory developments

Chevron Corporation: catalysis CH₄ to CH₃OH, Wax Inhibition in oil pipelines
Dow Corning: Catalysts for Production of Silanes for Silicones
Ford Motor Company: Fuel Cells: degradation of Nafion, Cathode catalysts
Intel Corp: Carbon Nanotube Interconnects, nanoscale patterning
Boehringer-Ingelheim: Structures and Function of GPCRs
Pfizer Corp: Structures and Function of GPCRs
GPC-Rx: Design new pharma for GPCRs
Toshiba: Materials Electronics

Allozyne: non natural AA, Structure GLP-1R and binding to GLP-1
Asahi Glass: Fluorinated Polymers and Ceramics
Asahi Kasei: Ammoxidation Catalysis, polymer properties
Avery-Dennison: Nanocomposites for computer screens Adhesives, Catalysis
Berlex Biopharma: Structures and Function of chemokine GPCRs
BP: Heterogeneous Catalysis (alkanes to chemicals, EO)
Dow Chemical: Microstructure copolymers, Catalysis polymerize polar olefins
Exxon Corporation: Catalysis (Reforming to obtain High cetane diesel fuel)
General Motors - Wear inhibition in Aluminum engines
GM advanced propulsion: Fuel Cells (H2 storage, membranes, cathode)
Hughes Satellites/Raytheon: Carbon Based MEMS
Hughes Research Labs: Hg Compounds for HgCdTe from MOMBE
Kellogg: Carbohydrates/sugars (corn flakes) Structures, water content
3M: Surface Tension and structure of polymers
Nippon Steel: CO + H2 to CH3OH over metal catalysts
Nissan: tribology of diamond like carbon (DLC) films
Owens-Corning: Fiberglas (coupling of matrix to fiber)
Saudi Aramco: Up-Stream additives (Demulsifiers, Asphaltenes)
Seiko-Epson: Dielectric Breakdown, Transient Enhanced Diffusion Implanted B

Completed successfully

Spin-Offs:
Accelrys (public) - software
Schrödinger - software
Eidogen-Sertanty - structures
Allozyne – non-natural AA
Systine (new) – Etching <45nm
Qateomix (new) – CH₄ → liquid
GPC-Rx (new) – pharma GPCRs
Need 1st Principles simulations of macroscale systems so can predict NEW materials never before synthesized and optimize them prior to experiment 1st Principles connect Macro to QM. Strategy use an overlapping hierarchy of methods (paradigms) (fine scale to coarse) Allows Design of new materials and drugs (predict hard to measure properties)

Accurate calculations for bulk phases and molecules (EOS, bond dissociation) Chemical Reactions (P-450 oxidation)

Simulations real devices and full cell (systems biology)

Micromechanical modeling Protein clusters

Deformation and Failure Protein Structure and Function

Big breakthrough making FC simulations practical: reactive force fields based on QM Describes: chemistry, charge transfer, etc. For metals, oxides, organics.
Materials Design Requires Improvements in Methods for Maximum Accuracy. MSC Focus:

1: Quantum Mechanics
Challenge: increased accuracy
• New Functionals DFT (dispersion)
• Quantum Monte Carlo methods
• Tunneling thru molecules (I/V)

2: Force Fields
Challenge: chemical reactions
• ReaxFF - Describe Chemical Reaction processes, Phase Transitions, for Mixed Metal, Ceramic, Polymer systems
• Electron Force Field (eFF) describe plasma processing

3: Molecular Dynamics
Challenge: Extract properties essential to materials design
• Non-Equilibrium Dynamics
  – Viscosity, rheology
  – Thermal Conductivity
• Solvation Forces (continuum Solv)
  – surface tension, contact angles
• Hybrid QM/MD
• Plasticity, Dislocations, Crack
• Interfacial Energies
• Reaction Kinetics
• Entropies, Free energies

4: Biological Predictions
1st principles structures GPCRs
1st principles Ligand Binding

5: MesoScale Dynamics
Coarse Grained FF
Hybrid MD and Meso Dynamics

6: Integration: Computational Materials Design Facility (CMDF)
• Seamless across the hierarchies of simulations using Python-based scripts
DOE environmental cleanup operations face enormous challenges to remediate over 1.7 trillion gallons of contaminated ground water, 40 million cubic meters of contaminated soil and debris, and 3 million cubic meters of waste buried in landfills, trenches and spill areas.

- > 50% of the facilities and 35% waste sites have metal and radionuclide contamination in ground water, soils and sediments.

- Hg$^{II}$ and RHg are major hazardous contaminants at most DOE facilities, especially in the ground water plumes.

- Current detection and monitoring methods for Hg(II) and RHg are slow and expensive (AFS, CVAAS, CVAFS, ICP-MS).

- Need new tools for field detection and monitoring of Hg(II) and RHg contaminants, both before and after remediation efforts.
Current Hg(II) Detection Methods

<table>
<thead>
<tr>
<th>Methods</th>
<th>Apparatus</th>
<th>Detection Limits</th>
<th>Applicable</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPA 245.1</td>
<td>Cold Vapor Atomic Absorption - Manual</td>
<td>1nM (0.2 ppb)</td>
<td>Drinking &amp; surface water, domestic &amp; indus. Waste</td>
</tr>
<tr>
<td>EPA 245.2</td>
<td>Cold Vapor Atomic Absorption - Automatic</td>
<td>1nM (0.2 ppb)</td>
<td>Surface Water</td>
</tr>
<tr>
<td>EPA 245.3</td>
<td>Cold Vapor Atomic Absorption - Manual</td>
<td>1nM (0.2 ppb)</td>
<td>Soils, sediments, bottom deposits and sludge</td>
</tr>
<tr>
<td>EPA 245.7 (Mar. 2007)</td>
<td>Cold Vapor Atomic Fluorescence Spectrometry (CVAFS)</td>
<td>0.025nM (5 ppt)</td>
<td>Water</td>
</tr>
</tbody>
</table>

- Requires Expensive Equipment – CVAAS or CVAFS
- Requires Well-trained Technician
- Works by convert Hg\(^{ll}\) to Hg(0) vapor
- Cannot detect bio-available Hg

EPA drinking water standard: 10nM = 2 ppb
Biosensors use biological reactions to detect target analytes. They couple a biological recognition element (interacting with the target analyte) with a physical transducer that translates the biorecognition event into a useful electrical signal.

Advantages:
- Specificity,
- Sensitivity,
- Stability (longer lifetime),
- Simplicity,
General mechanism of MerR regulator for transcriptional activation.

Without Hg, MerR binds with polymerase to form inactive complex

With Hg, MerR twists the DNA to align with the promoter elements

(Lund et al., 1986; Hobman, 2007)
Background RFU of various fluorescence protein-producing live cells

mCherry was chosen for our applications because it showed the lowest background RFU
Two whole-cell mercury biosensor designs

(A) Construct A to detect inorganic form of Hg(II)

(B) Construct B to detect both inorganic Hg(II) and Methyl-Hg(II)

HgCl₂ (nM)  CH₃HgCl (nM)

Wild type
How improve the sensitivity?

Approaches we tried:

1) MerT Approach -- increase the amount of mercury ions, especially inside cell;

2) MerR Quantity Approach -- Increase the amount of MerR;

3) MerR Quality Approach -- Change the performance or quality of MerR.
1. MerT (mercuric transporter) Strategy

MerT is a Hg\textsuperscript{II} transport integral membrane protein responsible for transport of the Hg\textsuperscript{2+} ion.

We introduced MerT to facilitate transport of Hg\textsuperscript{II} into the cell.
MerT ➔ ONLY MARGINAL increase in sensitivity

The RFU was measured with 50nM HgCl₂ at 37°C
2. MerR Quantity Approach-case I

Engineered *cl*-mediated genetic circuit amplifier to increase MerR expression

\[ P_{RM} \] promoter is overlapped by the right operator region, which contains three sites: *Or1*, *Or2* and *Or3*.

When *Or3* was mutated, repressor cl cannot effectively bind to *Or3*, so cl keeps a high transcription.

So does the clustered MerR.

This results in high-level expression of MerR to facilitate the expression of reporter gene mCherry even at the low concentration of mercury.
Results of Engineering *cl*-mediated genetic circuit amplifier

Sensitivity increased only a factor of 2 (40 to 80 RFU) – not enough

Two Or3 mutants (Red open or filled square) show higher sensitivity than wild type strains (Pink open or filled square) with or without *cl*-mediated genetic circuit.

The sensitivity increases only a factor of 2.
2. MerR Quantity Approach – case II

Engineering the $P_m$-XylS2 gene cascade system

The cascade expression system has two salicylate-response transcriptional activator proteins, NahR and XylS2. The NahR protein induces expression of XylS2 from the Psal promoter in the presence of salicylate.

Salicylate also activates XylS2, which induces high-level expression of the gene from the $P_m$ promoter.

Using these two transcriptional regulators in a sequential cascade amplifies expression levels nearly 20-fold compared to expression from either promoter individually (A).

We replaced NahR by MerR, which is activated in the presence of mercury (II), Also the expression of mCherry was enhanced (B).
Results of engineering *Pm-XylS2* gene cascade system

Increased sensitivity 2.5 fold – not enough

Pink: without *Pm-XylS2* system, Red: with *Pm-XylS2* system.

The concentration of HgCl₂ is 50nM, and Salicylate is 2mM.
Wild type not sufficiently Sensitive
To improve Performance of MerR use Directed evolution of MerR

NEXT GENERATION

Parental gene(s)

Random mutagenesis (Mutagenic PCR)

DIVERSITY (Library)

SUPER MUTANT

Screening

Selection

Transformation

Cloning

Transformation
Rapid Screening: Color-based plate assay plus Microplate Fluorescence Screening

1. Cell containing basic construct (MerR-mCherry) develops visible color in the M9 plate containing mercuric ion. (1000nM Hg(II) the optimal concentration for screening, 5000nM Hg(II) inhibits the cell growth)

2. Grow and then incubate with 1-10 nM Hg\textsuperscript{II}

3. Microplate fluorescence screening to find best mutants
Results of Screening of 50,000 mutants

> 22 single-mutations found with higher sensitivity than Wild Type (WT)
Tried 4 different growth environments, with little effect on bio-sensors (M9: minimal salt media; M9CA: M9 with 0.5% casamino acids; M9CAG: M9 with 0.5% casamino acids and 0.4% glucose; LB: Luria-Bertani Media, rich media.)
The location of mutations in MerR

helix-turn-helix DNA binding domain from I10 to R29.

“coupling” domain from K30 to H81 which may convey the status of the Hg(II) binding site to the DNA binding site.

Hg(II)-binding domain, long helical region from C82 to C117 which constitutes both the dimmer interface and, with the loop containing C126.
Mutated Hg(II) Biosensors Show HIGH Specificity

No metal ions tested (at 1000nM concentration) produced response signal except Hg (II)
Characterization of Mutated biosensors I

- **0.1 nM = 0.02 ppb = 0.02 μg/L**
- **good response after 2-hour incubation**
- K15E had fastest response time; V109E had highest sensitivity

**Graph:**
- RFU vs. HgCl₂ (nM)
- WT, K15E, V109E, S129P, L74Q, K99N

**Legend:**
- 0.1 nM = 0.02 ppb
- 1 nM = 0.2 ppb = 0.2 μg/L

**Biosensor sensitivity 0.1 nM = 0.02 ppb = 0.02 μg/L**

**Note:**
- good response after 2-hour incubation
- K15E had fastest response time; V109E had highest sensitivity
Biosensor sensitivity 0.1 nM = 0.02 ppb = 0.02 μg/L
excellent response after 4-hour incubation
K15E had fastest response time; V109E had highest sensitivity
Portable PEER-Caltech Hg Sensor Ver. 1.2

Box dimension 9”x6”x4”

180μl Water sample mixed with 20μl (O.D 1.0) of cells

Green laser pointer

Digital Readout
Summary

- Successfully built whole-cell based biosensors to detect both inorganic Hg(II) and Methyl-Hg(II).
- Used directed evolution of MerR with subsequent high throughput microplate screening to increase detection sensitivity from 10 nM for WT to 0.1 nM for single mutants.
- Hg(II) biosensor with mutant MerR can detect Hg(II) at concentrations of 0.1 nM (20 ppt = 0.02 ppb = 0.02 μg/L).
- Hg(II) biosensors have high specificity only to Hg(II) ions.
- Whole-cell Hg(II) biosensors stable up to 7 days.
- Prototype hand held detector; portable and inexpensive.
Next step, ongoing

Use theory, modeling, and simulation to build upon and interpret results from directed evolution.

Use this information for rational redesign of the active site to enhance Hg binding and the effect of Hg binding on transcription rates of MerR and MerB.

Expect to finish by June 2009.
Dimer Has Two Helix-Turn-Helix DNA Binding Domains

A

MerR regulator homodimer

B

Hg bound to MerR regulator homodimer
MerR Homodimer With the Best Mutants

- DNA Binding Domain
- Mercury Binding Domain
- Coupling Domain

F56L
V109E
M106T
V124D

a realistic interpretation of the various domains
1. Hg\(^{2+}\) binding improves binding constant of helix-turn-helix DNA binding domain in MerR

2. Hg\(^{2+}\) binding signal is relayed across MerR and coupling region fidelity improves MerR’s ability to activate second DNA promoter site
1. Mutants Improve Binding Constant

- Mercury binding activates helix-turn-helix DNA binding domain here
- $\text{Hg}^{2+}$ binds here
- Mutations here improve geometry of helix-turn-helix binding domain and improve binding constant
Angle between helices affects DNA binding constant

Must do average angle over all MD timepoints

How does the angle affect the binding constant?

Performance: V109E > M106T > V124D > F56L > WT
2. Strength of Coupling Domain Dictates Effectiveness of Promotion

Coupling domains help push 2\textsuperscript{nd} promoter region towards promoter site

- One helix-turn-helix binding domain is always bound to the DNA strand
- Upon binding Hg\textsuperscript{2+}, a structural change causes MerR to bend or unbend the DNA strand so that the second DNA promoter element finds its polymerase binding site
- We hypothesize that the second MerR h-t-h binding site is activated on Hg\textsuperscript{2+} binding and acts to push the DNA strand towards the second polymerase site
- The coupling regions’ structural fidelity is crucial to relaying the Hg\textsuperscript{2+} binding signal: stronger arms are more capable of torqueing the DNA towards the polymerase
All mutants exhibit greater coupling domain helicity!

Performance: V109E > M106T > V124D > F56L > WT
Ramachandran Plots of Coupling Domain Helices for all MD points

All good mutants much closer to standard alpha-helix than WT

Performance: V109E > M106T > V124D > F56L > WT
**Quantify Difference in Coupling Region Helicity**

Standard Deviation from Perfect \( \alpha \)-helix

<table>
<thead>
<tr>
<th></th>
<th>Protein</th>
<th>( \sigma )</th>
<th>( \sigma_\phi )</th>
</tr>
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<tbody>
<tr>
<td>baaad</td>
<td>WT</td>
<td>30.5</td>
<td>42.7</td>
</tr>
<tr>
<td>2(^{nd})</td>
<td>F56L</td>
<td>16.6</td>
<td>25.1</td>
</tr>
<tr>
<td>4(^{th})</td>
<td>M106T</td>
<td>22.2</td>
<td>32.2</td>
</tr>
<tr>
<td>5(^{th}) (best)</td>
<td>V109E</td>
<td>24.2</td>
<td>38.1</td>
</tr>
<tr>
<td>3(^{rd})</td>
<td>V124D</td>
<td>28.4</td>
<td>36.8</td>
</tr>
</tbody>
</table>

All good mutants closer to standard alpha-helix than WT but the correlation is not perfect

Performance: **V109E > M106T > V124D > F56L > WT**
Summary for MD Simulations

• The helix-turn-helix angle correlates with performance: smaller angles correspond to better DNA binding

• Coupling region helicity, or strength, has some correlation with improved Hg²⁺ binding signal translation and this promotes mCherry expression

• Do not yet have clear-cut mechanism to explain why these particular single mutations confer preferable structural changes relative to the WT MerR

• Mutations that reduce the helix-turn-helix angle, force coupling region helicity, which increases monomer coordination. This should improve fluorescence yields

• Need longer more complete MD simulations to determine the mechanism which is essential for rational design of improve mutants
### Biosensor advantages: portability, sensitivity, low Costs
(Both Initial investment and operational costs)

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Biosensor investment costs</strong> (% less than conventional analysis)</td>
<td>50 - 95 % less than CVAAS or CVAFS</td>
</tr>
<tr>
<td><strong>Biosensor sample costs</strong> (% less than conventional analysis)</td>
<td>50 - 90 % less than CVAAS or CVAFS</td>
</tr>
<tr>
<td>Cost of each sample analysis by conventional methods</td>
<td>&gt; $100 for CVAAS or CVAFS</td>
</tr>
<tr>
<td>Cost of each sample analysis by Biosensor</td>
<td>&lt; $10 for Biosensor</td>
</tr>
<tr>
<td>Cost of Conventional Equipment</td>
<td>$50,000 to $500,000 CVAAS, CVAFS</td>
</tr>
<tr>
<td>Cost of PEER-Caltech Biosensor</td>
<td>&lt; $1000 biosensor (~$100 if mass produced)</td>
</tr>
</tbody>
</table>

Equally important: hand held biosensor can be used on site, no need to Transport to analysis facility

CVAAS = Cold Vapor Atomic Absorption – Manual
CVAFS = Cold Vapor Atomic Fluorescence Spectrometry
1. Task 2.2 Second round mutation studies via in vitro DNA shuffling
2. Task 3.2 Build and improve micro-biosensors for detection of Hg(II)
3. Task 3.3 Evaluate the technology for Cr$^{VI}$ and future applications

Budget reduced by $300K from $889K to $590K
Future Directions (2-year continuation research program)

- Use predictions from Computer models to predict better mutations to further improve sensitivity and response time.
- Do 2nd round of directed evolution of MerR to improve sensitivity (by factor of 10 to 2ppt, ~ EPA245.7), improve fluorescent signal stability, and decrease response time of biosensors.
- Improve immobilization techniques to increase the cell stability and lifetime of the biosensors.
- Fabrication 2nd generation of portable red-fluorescence detector.
- Computer design active site to selectively recognize other metal binding proteins.
- Build biosensor for commercial & consumer use.

Estimated funding required -- $700,000
## Metal biosensors opportunities that could be developed using our technology

<table>
<thead>
<tr>
<th>Metal</th>
<th>Protein</th>
<th>Family (species)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hg(II)</td>
<td>MerR</td>
<td>MerR (<em>P. aeruginosa</em>)</td>
</tr>
<tr>
<td>Mn(II)</td>
<td>MntR</td>
<td>DtxR (<em>B. subtilis</em>)</td>
</tr>
<tr>
<td>Fe(II)</td>
<td>Aft1p/Aft2p</td>
<td>DtxR (<em>S. cerevisiae</em>)</td>
</tr>
<tr>
<td></td>
<td>DtxR</td>
<td><em>DtxR</em>(Corynebacterium diphtheriae)*</td>
</tr>
<tr>
<td>Co(II)</td>
<td>CoaR</td>
<td>MerR(<em>Synechocystis</em> sp. PCC 6803)</td>
</tr>
<tr>
<td>Ni(II)</td>
<td>NikR</td>
<td>NikR (<em>E. coli</em>)</td>
</tr>
<tr>
<td>Cu(I)</td>
<td>CueR</td>
<td>MerR (<em>E. coli</em>)</td>
</tr>
<tr>
<td></td>
<td>CsoR</td>
<td>DUF156 (<em>M. tuberculosis</em>)</td>
</tr>
<tr>
<td></td>
<td>MacI</td>
<td>(<em>S. cerevisiae</em>)</td>
</tr>
<tr>
<td></td>
<td>Ace</td>
<td>(<em>S. cerevisiae</em>)</td>
</tr>
<tr>
<td></td>
<td>Amt1</td>
<td>(<em>C. glabrata</em>)</td>
</tr>
<tr>
<td>Zn(II)</td>
<td>ZntR</td>
<td>MerR (<em>E. coli</em>)</td>
</tr>
<tr>
<td></td>
<td>Zap1</td>
<td>(<em>S. cerevisiae</em>)</td>
</tr>
<tr>
<td>As(III)</td>
<td>ArsR</td>
<td>ArsR/SmtB (<em>E. coli</em>)</td>
</tr>
<tr>
<td>Ag(I)</td>
<td>SilE</td>
<td>(<em>Salmonella</em>)</td>
</tr>
<tr>
<td></td>
<td>CueR/CsoR</td>
<td>MerR (<em>E. coli</em>)/DUF-156 (<em>M. tuberculosis</em>)</td>
</tr>
<tr>
<td>Cd(II)</td>
<td>CadR</td>
<td>MerR (<em>P. putida</em>)</td>
</tr>
<tr>
<td>Au(I)</td>
<td>GolS</td>
<td>MerR (<em>Salmonella</em> and <em>R. metallidurans</em> CH34)</td>
</tr>
<tr>
<td>Pb(II)</td>
<td>PbrR</td>
<td>MerR (<em>R. metalliduran</em> CH34)</td>
</tr>
</tbody>
</table>
Pu$^\text{V}$, Tc$^\text{VII}$, U$^\text{VI}$, $^{91}\text{Sr}$, $^{137}\text{Cs}$, $^{237}\text{Np}$

No known biological system to build upon

Instead use theory and simulation to redesign known systems: MerR, MerT, etc

to selectively recognize Pu$^\text{V}$, Tc$^\text{VII}$, U$^\text{VI}$,
Strategy for selective binding of Methylmercury

- Screen and design Methylmercury binding domain;
- Insert binding domain into Fluorescence protein (FP) to produce hybrid protein, which changes fluorescence signal only in the presence of methylmercury;
- Use cell surface display of hybrid methylmercury-binding FP for methylmercury detection;
- Improve the performance of hybrid methylmercury-binding FP by directed protein evolution (improving binding specificity, affinity, and stability).
New concept remediation of Toxic Metals

Solution: Selective Encapsulation and Release/Destruction

Low-cost dendrimer-like macromolecules with tunable contaminant binding sites
Allows for low pressure membrane (MF/UF) separation
Scalable – for small or large scale applic.
Can treat:
- Cations
- Anions
- Organic compounds
- Water-borne bacteria and viruses
- Catalysts for contaminants

Solution: Water Treatment using nanotechnology: Dendrimer Enhanced Filtration

Mamadou S. Diallo and wag Caltech
Jean Frechet (UC Berkeley)
What is special about dendrimers?

Can design in special chemical character inside or outside

Generation 4
64 primary amines on outside
plus 62 tertiary amines on inside
Plus 62 amides on inside
At pH > 10 the whole dendrimer is neutral
At pH ~ 7-8 get 64 protonated primary amines
At pH < 6 get also 62 protonated tertiary amines for a total charge of 126 on one molecule!

Can tune to bind metals (Cu, Fe, Cr, Hg, U, Pt, Tc) at one pH and the recover dendrimer by rejecting ions at another pH
Radial density distribution of G4-NH₂ PAMAM dendrimer at various pH values.

Snapshots from MD simulations shown in insert.

Very different internal structure despite very similar size

Differentially binds anions, cations while retaining size

Excellent agreement. Thus trust MD

Size independent of pH, despite
0 H⁺ for high pH (>10)
64 H⁺ for neutral pH (~7)
126 H⁺ for low pH (<5)
Detailed local structure determines which contaminants will be bound or rejected.

Can tune terminal groups and internal groups to selectively bind specific metals (Cu, Fe, Cr, Hg, U, Pt, Tc) at one pH and the recover dendrimer by rejecting ions at another pH.
AquaNano LLC (Pasadena CA) established to commercial this technology, looking for A round
Selective Encapsulation and Release of Anions from Water

Already have dendritic materials selective for

- Nitrates (NO$_3^-$)
- Phosphates (HPO$_4^{2-}$)
- Perchlorate ((TcO$_4$)$^-$)
- Bromate (BrO$_3^-$)
- Copper (II)

Maybe could use for

- (TcO$_4$)$^-$
- (UO$_2$)$^{2+}$
Stop already
## Tasks

### Task One

1. **Literature Review, Data Organization and Evaluation**
   - **1.1** PCR clone the MerR gene from tn501 operon
   - **1.2** Engineer recombinant E. Coli bacteria cells
   - **1.3** Build whole-cells biosensors with native MerR

**Milestone 1:** model E. Coli whole-cell based biosensors that will detect Hg(II)

### Task Two

1. **Conduct the first round mutagenesis of MerR gene via Error-prone PCR.**
2. **Second round mutation studies via in vitro DNA shuffling**

**Milestone 2:** mutant MerR genes with improved functionalities towards building desired biosensors

**Milestone 3:** much improved model E. Coli whole-cell based biosensors that could be used for in situ applications

### Task Three

1. **computer-assisted protein modeling/design**
2. **Evaluate the technology for future applications**

**Milestone 4:** biosensors in microbial strains that are suitable for subsurface applications

<table>
<thead>
<tr>
<th>0 mo.</th>
<th>9 mo.</th>
<th>18 mo.</th>
<th>27 mo.</th>
<th>36 mo.</th>
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<tr>
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<th>Year One</th>
<th>Year Two</th>
<th>Year Three</th>
<th>Year One</th>
<th>Year Two</th>
<th>Year Three</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6/1/07 - 12/31/07</td>
<td>1/1/08 - 12/31/08</td>
<td>1/1/09 - 12/31/09</td>
<td>1/1/07 - 12/31/07</td>
<td>1/1/08 - 12/31/08</td>
<td>1/1/09 - 12/31/09</td>
</tr>
<tr>
<td><strong>Caltech PEER (Off-site Facility) Budget</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Total Direct Costs (PEER)</td>
<td>$120,584</td>
<td>$124,301</td>
<td>$124,298</td>
<td>$217,243</td>
<td>$180,010</td>
<td>$162,860</td>
</tr>
<tr>
<td>Overhead (26%, excl equip, PEER)</td>
<td>$22,693</td>
<td>$28,728</td>
<td>$32,318</td>
<td>$40,883</td>
<td>$41,603</td>
<td>$42,344</td>
</tr>
<tr>
<td><strong>Total Off-site Budget</strong></td>
<td>$143,277</td>
<td>$153,028</td>
<td>$156,616</td>
<td>$258,126</td>
<td>$221,612</td>
<td>$205,204</td>
</tr>
<tr>
<td><strong>Caltech Campus Budget</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Direct Costs (Campus)</td>
<td>$22,668</td>
<td>$28,995</td>
<td>$32,953</td>
<td>$41,531</td>
<td>$42,701</td>
<td>$43,908</td>
</tr>
<tr>
<td>Overhead (59.3%, excl equip, tuition rem.) (Campus)</td>
<td>$14,054</td>
<td>$17,977</td>
<td>$20,431</td>
<td>$24,628</td>
<td>$25,322</td>
<td>$26,037</td>
</tr>
<tr>
<td><strong>Total Campus Budget</strong></td>
<td>$36,723</td>
<td>$46,972</td>
<td>$53,384</td>
<td>$66,159</td>
<td>$68,023</td>
<td>$69,945</td>
</tr>
<tr>
<td><strong>Grand Total, Caltech</strong></td>
<td>$180,000</td>
<td>$200,000</td>
<td>$210,000</td>
<td>$324,284</td>
<td>$289,636</td>
<td>$275,149</td>
</tr>
<tr>
<td>Total approved</td>
<td>590,000</td>
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<tr>
<td>Total proposed</td>
<td>889,069</td>
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<tr>
<td><strong>Total Budget Cut</strong></td>
<td><strong>$299,068</strong></td>
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</tr>
</tbody>
</table>
Characterization of Mutated biosensors II

0.5 nM HgCl₂

1.0 nM HgCl₂