

Computational Framework Proposal for Competitive Extracorporeal Binding of Protein-Bound Uremic Toxins

A Conceptual Systems-Level Approach to Enhancing Dialytic Clearance

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Research Line: Bioengineering, Computational Protein Design, Translational Nephrology

Estimated Duration: 36-50 months

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Abstract

Protein-bound uremic toxins (PBUTs), particularly *indoxyl sulfate* (IS) and *p-cresyl sulfate* (pCS), represent a critical limitation of conventional hemodialysis (Vanholder et al., 2014; Niwa, 2010). Although current dialysis membranes effectively remove low-molecular-weight solutes (<500 Da), the clearance of toxins strongly bound to **human serum albumin (HSA)** remains suboptimal.

The high affinity of IS for HSA ($K_d \approx 0.6\text{--}1.2\ \mu\text{M}$) results in >95% of the toxin circulating in a bound state, with albumin acting as an intravascular reservoir during the dialysis session. This restricts the net reduction of IS to approx. 30–40% per session, contributing to chronic inflammation, cardiovascular fibrosis, and increased mortality.

This project outlines a conceptual computational strategy for the de novo design of a synthetic mini-protein (designated **TRAPHOLE-UT**) utilizing structural artificial intelligence tools (**RFdiffusion**, **ProteinMPNN**), pending quantitative and experimental validation.

TRAPHOLE-UT will be:

1. Low molecular weight (<20 kDa).
2. Engineered to bind IS and pCS with sub-micromolar affinity (target $\leq 500\ \text{nM}$).
3. Optimized for extracorporeal immobilization within a biofunctionalized system.

The proposed mechanism involves acting as a **competitive molecular sink**, shifting the HSA-toxin equilibrium through progressive capture of the free fraction, thereby promoting the continuous dissociation of the T-HSA complex.

The strategy prioritizes extracorporeal immobilization, mitigating the risk of systemic immunogenicity. Nevertheless, complement activation and inflammatory response will be evaluated in preclinical studies.

Central Hypothesis:

A de novo designed mini-protein with sub-micromolar affinity, appropriate binding kinetics, and high immobilization density may be capable of competitively displacing albumin-bound toxins under dynamic extracorporeal conditions, thereby increasing total clearance without significant albumin loss.

1. Problem Statement: The Pending Challenge of Modern Hemodialysis

1.1 The Spectrum of Protein-Bound Uremic Toxins

Chronic Kidney Disease (CKD) affects more than 850 million people worldwide, with a rising prevalence of 8–10% in the adult population. When renal function drops below 15%, renal replacement therapy (hemodialysis, peritoneal dialysis, or transplantation) becomes essential for survival. However, despite decades of technological advancements, conventional hemodialysis remains an incomplete therapy.

Uremic toxins are classified into three categories based on their behavior during dialysis:

- **Small water-soluble solutes (<500 Da):** Urea, creatinine, and uric acid. Efficiently removed via diffusion.
- **Middle molecules (500–60,000 Da):** *beta*2-microglobulin, cystatin C. Partially removed by convection in high-flux or hemodiafiltration techniques.
- **Protein-bound uremic toxins (PBUTs):** Indoxyl sulfate (IS), p-cresyl sulfate (pCS), hippuric acid, and 3-carboxy-4-methyl-5-propyl-2-furanopropanoic acid (CMPF). These constitute the unresolved challenge.

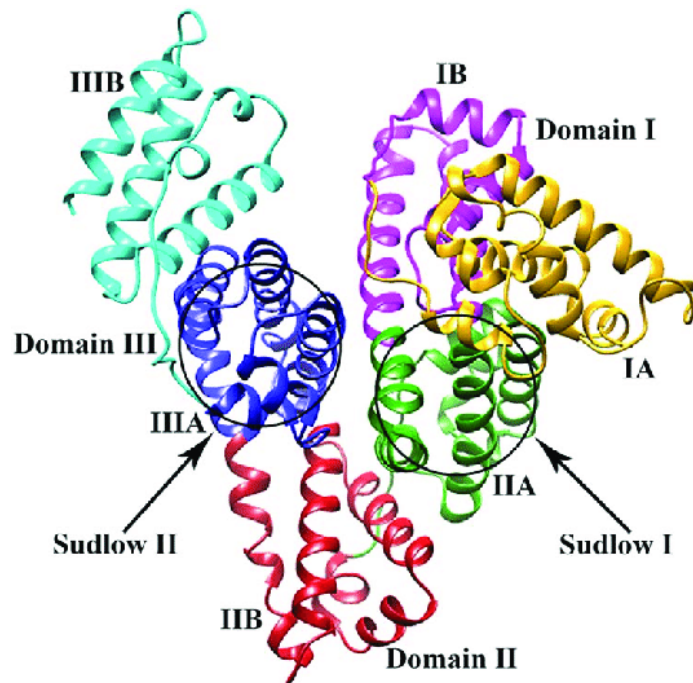
Classification	Characteristics	Examples
Small water-soluble molecules	Molecular weight <500 Da; Easily removed by dialysis	Creatine Creatinine* Guanidine* (ADMA/SDMA) Oxalate Urea* Uric acid Trimethylamine
Middle molecules	Molecular weight >500 Da; Need dialysis membranes with large pores large to be removed; Many are peptides	AGES and AOPP Complement factor D Cystatin C Cytokines Endothelin FGF23 Leptin* β2-Microglobulin*
Protein-bound molecules	Generally of low molecular weight; Difficult to be removed by dialysis	CMPF Hippuric acid Homocysteine Indole-3-acetate Indoxyl sulfate* p-Cresilsulfato* Spermidine/Spermine

¹Source: Barreto et al., 2017 | doi:10.1159/000479253

1.2 The Albumin Barrier: Molecular Mechanism

Human Serum Albumin (HSA) is the most abundant protein in plasma (35–50 g/L) and serves as a universal carrier for fatty acids, bilirubin, hormones, drugs, and toxins. It possesses two primary binding sites for aromatic ligands (Sudlow, 1975):

- **Site I (Subdomain IIA):** Preference for bulky, heterocyclic ligands.
- **Site II (Subdomain IIIA):** Preference for small aromatic ligands such as IS and pCS.



Source: Ghosh et al., 2017 | doi:10.1016/j.jlumin.2017.07.054

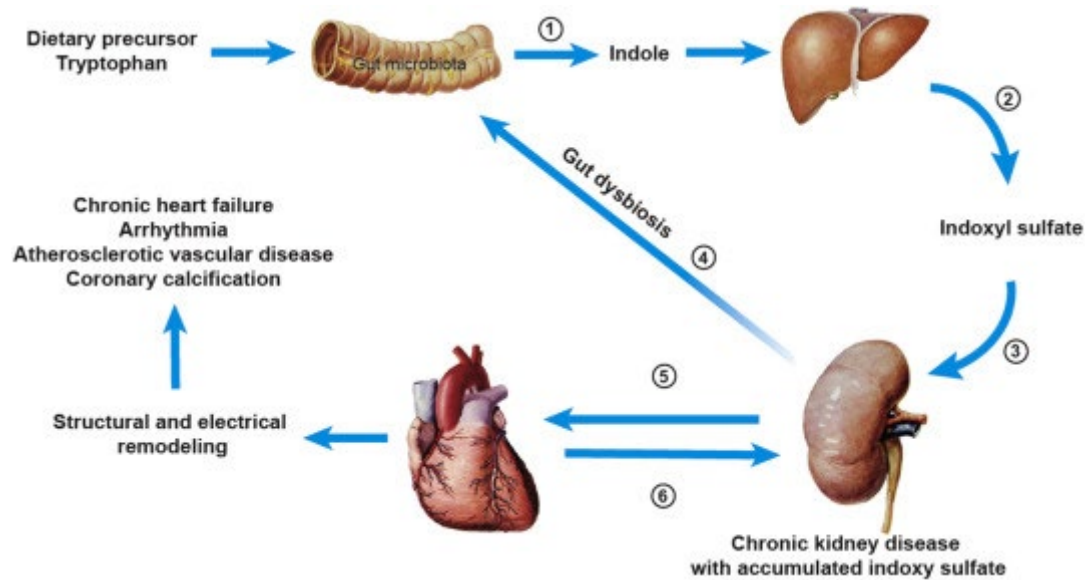
Indoxyl sulfate exhibits significant affinity for HSA ($K_d \approx 0.6\text{--}1.2 \mu\text{M}$), resulting in >95% circulating in a bound state. The efficacy of an alternative binder will not depend solely on surpassing this affinity value, but also on kinetic parameters (k_{off}), surface binding density, and the engineering of the extracorporeal microenvironment.

During a dialysis session, the free fraction is rapidly removed; however, the bound fraction acts as a dynamic reservoir. Effective displacement will require not only competitive affinity but also capture kinetics capable of leveraging the progressive re-equilibration of the system.

1.3 Clinical Consequences of Chronic Accumulation

Accumulated evidence over the last two decades has established IS and pCS as cardiovascular and pro-fibrotic toxins:

- **Oxidative Stress:** Activation of NADPH oxidase in endothelial and renal tubular cells, generating reactive oxygen species (ROS).
- **Inflammation:** Induction of adhesion molecules (ICAM-1, VCAM-1) and pro-inflammatory cytokines (IL-6, TNF-*alpha*).
- **Fibrosis:** Activation of TGF-*beta*/Smad signaling pathways in cardiomyocytes and fibroblasts, promoting myocardial and vascular fibrosis.
- **Erythropoietin Resistance:** Interference with endogenous erythropoietin production and recombinant response.
- **CKD Progression:** Acceleration of residual renal function decline through tubulointerstitial fibrosis.



Source: Edamatsu et al., 2018 | doi:10.1016/j.lfs.2017.07.030

A 2018 meta-analysis (Lin et al.) involving >10,000 patients found that elevated IS levels were associated with a 40% increase in cardiovascular mortality and a 35% increase in all-cause mortality.

1.4 Previous Attempts and Their Limitations

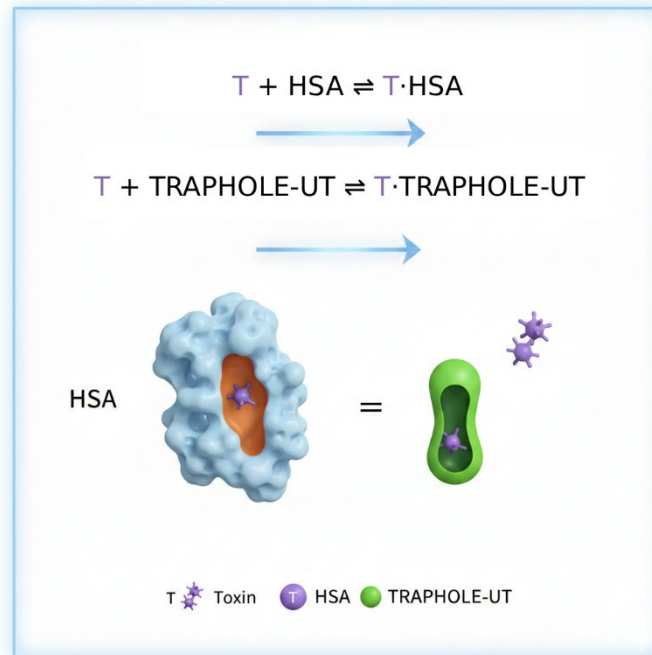
- **Oral Adsorbents (AST-120):** Designed to bind precursors in the gut. Results clinically inconsistent; poor patient compliance.
- **Online Hemodiafiltration (HDF):** Increases convective clearance but fails to remove the albumin-bound fraction.
- **High-Cutoff Dialyzers:** Larger pores allow some albumin passage, but result in undesired loss of essential proteins.
- **Binding Competitors:** Use of drugs (e.g., ibuprofen) to displace toxins. Limited by adverse pharmacological effects and low specificity.

Conclusion: No current technology fully addresses the root of the problem: the high affinity of toxins for albumin and the impossibility of accessing the bound fraction during dialysis. This justifies the exploration of hybrid systems combining passive filtration with specific adsorption through protein engineering.

2. Conceptual Framework and Proposed Mechanism of Action

2.1 Thermodynamic Principle: The Molecular Sink

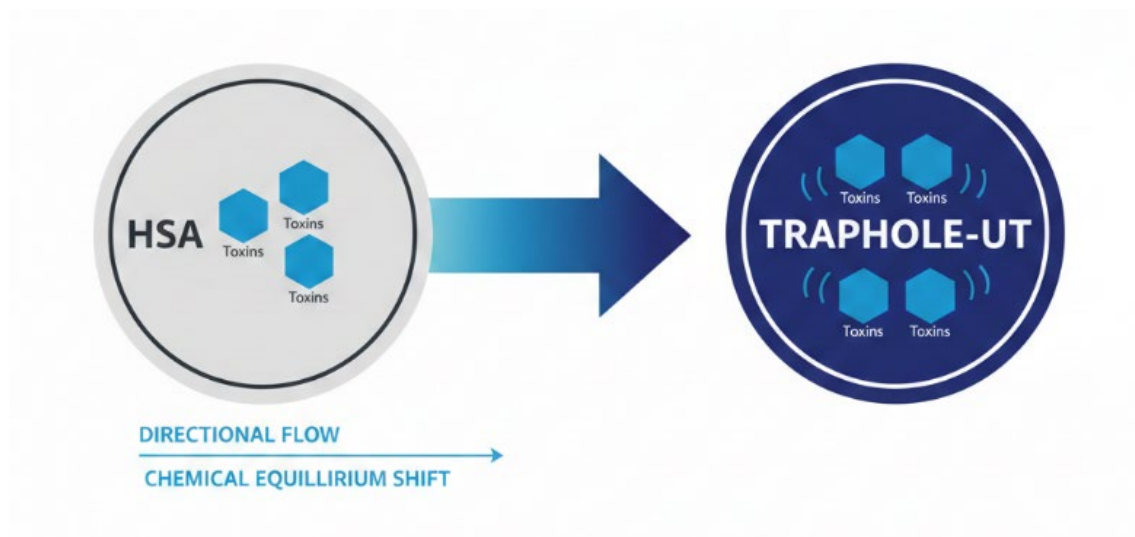
The proposed strategy is based on a simple yet powerful thermodynamic principle: the **Law of Mass Action**. Consider the binding equilibrium between the toxin (T), native albumin (HSA), and the scavenger mini-protein (TRAPHOLE-UT):



The operational principle consists of shifting the equilibrium toward the formation of the **T-TRAPHOLE-UT** complex through thermodynamic competition and control of the extracorporeal environment.

Under basal conditions, the toxin is distributed between a free fraction (minority) and an HSA-bound fraction (majority). By introducing TRAPHOLE-UT with affinity comparable to or higher than HSA, a competition is established:

1. **TRAPHOLE-UT** captures the free toxin fraction.
2. The reduction of free [T] shifts the HSA-toxin equilibrium toward dissociation (*Le Chatelier's principle*).
3. The toxin released by HSA is immediately captured by TRAPHOLE-UT.
4. The cycle continues until the available toxin is transferred to TRAPHOLE-UT.



The displacement efficacy will depend not only on the relative affinity (K_d) but also on the dissociation kinetics (k_{off}) of the T-HSA complex. This parameter will be estimated through **metadynamics simulations** and residence time analysis to evaluate the dynamic viability of the competitive process.

2.2 Design Requirements for TRAPHOLE-UT

To ensure an effective mechanism, TRAPHOLE-UT must meet rigorous criteria:

- **Affinity superior or equivalent to HSA:** Target $K_d < 500$ nM for IS and < 1 micromolar for pCS.
- **Selectivity:** Preferential binding to IS/pCS over endogenous metabolites (bilirubin, fatty acids, thyroid hormones) to avoid physiological interference.
- **Thermal stability:** $T_m > 65^\circ\text{C}$ to withstand storage conditions and sterilization cycles.
- **Plasma stability:** Resistance to serum proteases for at least 4–6 hours.
- **Molecular mass:** Optimized for high-density immobilization (initial target < 20 kDa).
- **Anchor point:** Surface-exposed cysteine (Cys) residue distal to the active site for oriented covalent immobilization.
- **Low immunogenicity:** Minimization of T-cell epitopes via *in silico* filtering.

2.3 Structural Inspiration: HSA Sudlow Site II

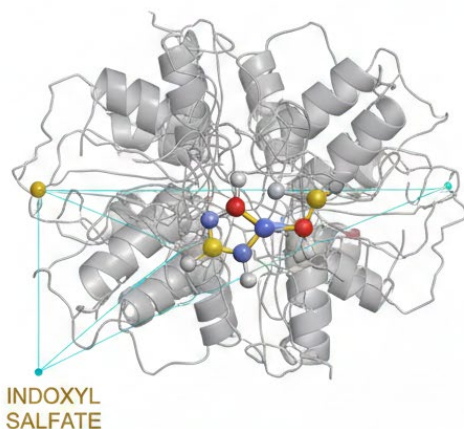
The conceptual starting point is Subdomain IIIA of HSA (residues 384-585), which constitutes **Site II**. Structural analysis of HSA crystallographies in complex with IS (PDB: 2BXH, 4L8U, 4N0F) reveals key molecular determinants:

Critical HSA Site II residues for IS/pCS binding:

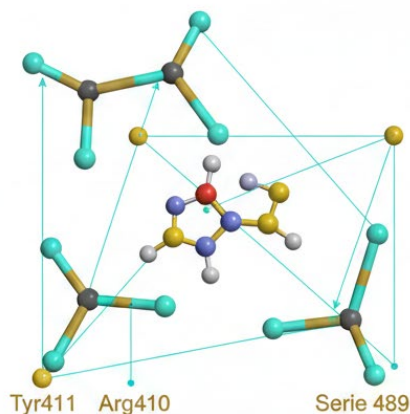
- **Tyr411:** Aromatic; *pi-pi* stacking with the indole ring of IS.
- **Arg410:** Cationic; electrostatic interaction with the sulfate group.
- **Leu407 & Val433:** Hydrophobic; cavity stabilization and van der Waals contacts.
- **Ser489:** Polar; hydrogen bonding with sulfate oxygens.

The design strategy is not to replicate this pocket exactly, but to extract these **functional motifs** and incorporate them into a minimalist, stable protein scaffold.

**HUMAN SERUM ALBUMIN
SUBDOMAIN IIIA
(SUDLOW SITE II)**



**MINIMALIST THEOZYME
(TRAPHOLE-UT)**



2.4 Preliminary Quantitative Feasibility Considerations

While the present framework is primarily conceptual, preliminary quantitative reasoning is essential to evaluate its physical plausibility under realistic dialysis conditions.

Estimated Toxin Load per Session

Reported plasma concentrations of indoxyl sulfate (IS) in end-stage renal disease patients range between approximately 20–80 μM . Considering an effective processed plasma volume of approximately 60–90 L during a standard 4-hour hemodialysis session (accounting for continuous recirculation), the cumulative toxin exposure to the extracorporeal circuit may reach the order of 1–5 millimoles when integrated over time.

Even assuming partial redistribution and incomplete equilibration, achieving a 30–50% reduction in total circulating IS would require competitive binding capacity in the range of hundreds of micromoles per session.

This estimation highlights that binding affinity alone is insufficient; total accessible binding capacity and surface density are critical determinants of system performance.

Competitive Context Against Albumin

Human serum albumin circulates at approximately 600 μM concentration. Any synthetic competitive binder must therefore operate in the presence of a vast endogenous binding reservoir.

System effectiveness will depend on:

- Relative binding affinity (K_d)
- Association and dissociation kinetics (k_{on} , k_{off})
- Density of immobilized active sites
- Residence time under extracorporeal flow
- Total accessible binding capacity

This defines a multi-parameter optimization challenge rather than a single affinity target.

Scaling Considerations

Assuming one functional binding site per mini-protein (<20 kDa), the molar quantity required to significantly influence equilibrium may correspond to gram-scale immobilized protein depending on achievable surface density and orientation efficiency.

Further computational modeling and mass-balance simulations are required to refine these estimations. However, the order-of-magnitude analysis suggests that the approach remains within physically plausible engineering limits.

3. Computational Design Methodology: High-Throughput Pipeline

3.1 Computational Infrastructure

The pipeline will be executed on a dedicated workstation featuring:

- **Processor:** 2x AMD EPYC 9554 (64 cores, 128 threads).
- **GPUs:** 4x NVIDIA RTX 5090 (32 GB VRAM each, Blackwell architecture).
- **RAM:** 256 GB DDR5.
- **Storage:** 4 TB NVMe SSD (RAID 5) for active data + 16 TB HDD for archiving.
- **OS:** Rocky Linux 9 LTS.
- **Software:** CUDA 12.4, GROMACS 2024 (GPU-supported), RFdiffusion, ProteinMPNN, PyRosetta, AlphaFold3.

3.2 Stage 1: Structural Motif Extraction and Theozyme Definition

Objective: Identify the optimal spatial arrangement of functional groups for binding IS/pCS.

Procedure:

1. Download HSA-ligand crystallographic structures (PDB: 2BXH, 4L8U, 4N0F, 1E78, 1HA2).
2. Structural alignment and binding site superposition.
3. **Protein Pharmacophore Identification:** Distance and orientation between the aromatic ring (*pi-stacking* acceptor) and the cationic group (sulfate interaction).
4. Define target coordinates (*theozyme*) for structural generation.

3.3 Stage 2: Backbone Generation with RFdiffusion

Objective: Generate >10,000 compact protein scaffolds incorporating the functional residues.

- **Theoretical Basis:** RFdiffusion (Watson et al., 2023) utilizes denoising diffusion probabilistic models (DDPM).
- **Parameters:** Length 50–120 amino acids; 10,000 designs; Sampling temperature 0.1–0.3.
- **Quality Metrics:** pLDDT > 80, pAE < 5.

3.4 Stage 3: Sequence Optimization with ProteinMPNN

Objective: Assign amino acid sequences that maximize stability and toxin affinity.

- **Procedure:** Execute ProteinMPNN (temp 0.1) generating 8 sequences per backbone.
- **Design Options:** Fix functional residues; allow conservative mutations; introduce surface Cys[k] for anchoring.
- **Metrics:** ProteinMPNN score (log-likelihood); predicted stability (T_m via FoldX/Rosetta); immunogenicity (NetMHCIIpan 4.0).
- **Target:** $T_m \geq 65^\circ\text{C}$.

3.5 Stage 4: Molecular Dynamics Validation (GROMACS)

Objective: Evaluate structural stability and binding free energy in physiological conditions.

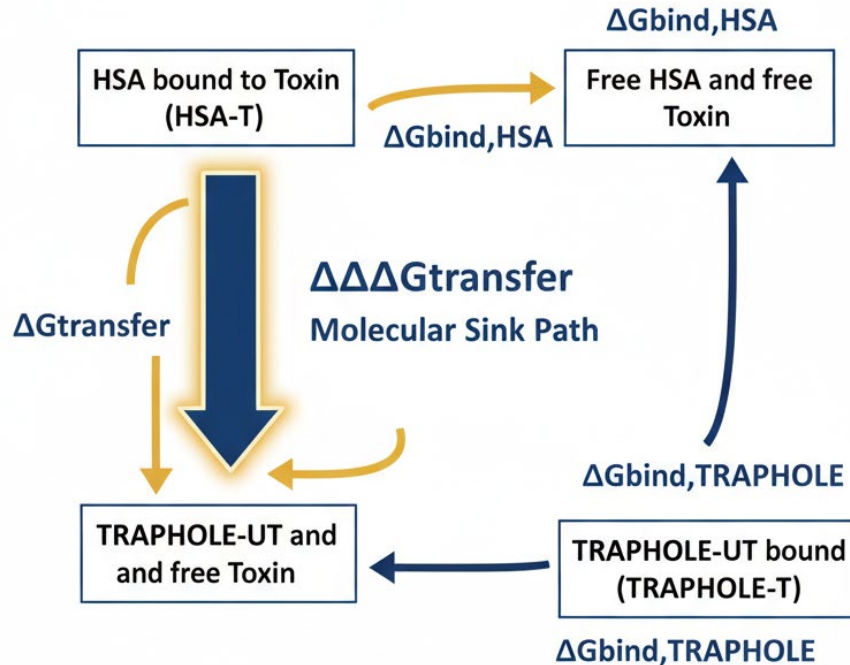
- **Protocol:** Modeling with AlphaFold3; protonation at pH 7.4; explicit water solvation (TIP3P).
- **Simulation:** Force field CHARMM36m; 500 ns production per replica (3 replicas).
- **Analysis:** RMSD, RMSF, Radius of Gyration; Binding free energy via MM/PBSA.

3.6 Stage 5: Competitive Validation Against HSA

Objective: Demonstrate *in silico* that TRAPHOLE-UT can effectively compete with HSA.

- **Procedure:** Construct ternary system (HSA + toxin + TRAPHOLE-UT).

- **Analysis:** Relative Binding Free Energy (RBFE) via FEP (Free Energy Perturbation) or TI (Thermodynamic Integration).
- **Success Criteria:** Delta-Delta G consistently favoring TRAPHOLE-UT; sustained complex stability.



4. Clinical Implementation Strategy: Biofunctionalized Device

4.1 Fundamentals of the Extracorporeal Strategy

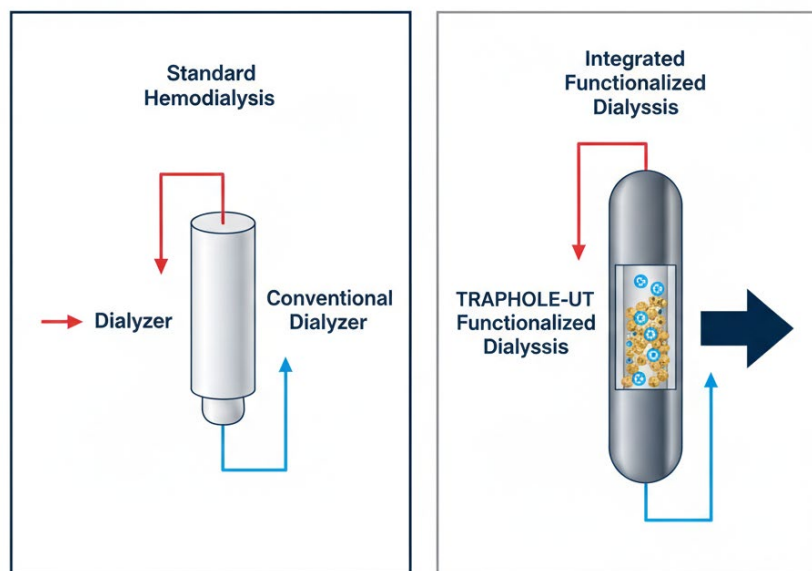
The decision to immobilize **TRAPHOLE-UT** within an extracorporeal device rather than administering it as a systemic drug is based on regulatory, safety, and pharmacokinetic considerations:

- **Circulating Protein (Drug):** High systemic exposure; requires exhaustive toxicology; high risk of neutralizing antibodies; regulation as a biologic (*BLA*); high development cost (Phase I, II, III).
- **Immobilized Protein (Proposed):** Zero systemic exposure; only extracorporeal contact; reduced immunogenicity risk. Requires evaluation of complement activation and hemocompatibility; regulation as a functionalized medical device (FDA 510(k) or CE marking pathway); moderate development cost.

4.2 Device Architecture

Two potential configurations are under consideration:

- **Configuration A: Functionalized Dialyzer.** High-flux polysulfone membrane with TRAPHOLE-UT covalently immobilized on the inner surface of the hollow fibers (blood side). Toxins transfer to the scavenger during blood transit.
- **Configuration B: Post-dialyzer Adsorbent Cartridge.** Independent cartridge placed in series after the dialyzer. Contains porous particles functionalized with TRAPHOLE-UT.
- **Advantage:** Does not modify the approved dialyzer; independent development.



4.3 Immobilization Chemistry

To ensure uniform orientation and maximum binding activity:

1. **Anchor Cysteine Introduction:** Design TRAPHOLE-UT with a unique surface Cys residue distal to the active site (N or C-terminal).
2. **Surface Activation:** Polysulfone membranes treated with oxygen plasma followed by silanization with *GPTMS* to introduce epoxy groups.
3. **Coupling:** Thiol-specific reaction between the Cys residue and surface epoxy/maleimide groups (pH 7.0–8.0).
4. **Characterization:** Quantification via modified Bradford assay and ELISA; residual binding activity under flow.

4.4 Ex Vivo Validation in Human Plasma

Objective: Evaluate capture capacity under dynamic conditions simulating a real hemodialysis session.

Experimental Protocol:

1. Obtain plasma from hemodialysis patients (Ethics Committee approval required).
2. Recirculate plasma through a prototype mini-column at 37°C.
3. Serial sampling at $t = 0, 15, 30, 60, 120$, and 240 minutes.
4. Quantification of total and free IS/pCS via **UPLC-MS/MS**.

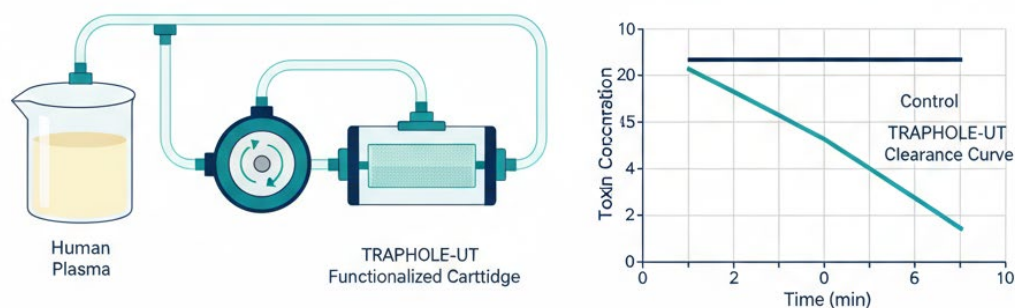
Performance Metrics:

- Toxin reduction percentage.
- Maximum adsorption capacity (mg toxin / g TRAPHOLE-UT).
- Capture kinetics (*Langmuir* or pseudo-first order models).
- Selectivity (Albumin retention).

Success Criteria:

- Target reduction benchmark: >50% total IS reduction in 4 hours (to be experimentally validated).

- Total pCS reduction >40% in 4 hours.
- Albumin loss <5%.
- No evidence of scavenger leaching or degradation.



5. Computational Infrastructure and Resources

5.1 Hardware Platform

- See **Section 3.1** for detailed infrastructure description

5.2 Scientific Software Suite

- **RFdiffusion**: De novo structural design and scaffolding.
- **ProteinMPNN**: Sequence optimization and recovery.
- **AlphaFold3 / ESMFold**: High-accuracy structural prediction.
- **AutoDock Vina**: Initial ligand-protein docking.
- **GROMACS 2024**: GPU-accelerated molecular dynamics simulations.
- **FoldX / Rosetta**: Protein stability and Delta-Delta G calculations.
- **NetMHCIIpan 4.0**: In silico immunogenicity and T-cell epitope prediction.

5.3 Licensing and Accessibility

The pipeline primarily utilizes *open-source* or *academic license* tools. Commercial licensing costs (where applicable for proprietary force fields or specialized software) are estimated as part of the operational budget to ensure full compliance and professional support.

6. Transition to Validation Phase and Funding Strategy

6.1 Current Project Status

This document consolidates the conceptual and preliminary computational design phase, establishing:

- Theoretical framework and mechanism of action.
- Detailed computational pipeline.
- Immobilization strategy and infrastructure.
- Success and validation criteria.

The project is currently at **TRL 1-2** (Basic concept/technology formulation). The next phase aims to reach **TRL 3-4** (Experimental proof of concept).

6.2 Development Plan by Phases

- **Phase I: Advanced Computational Validation** (6–8 months). Activities: 10,000 designs, 500 ns simulations. TRL 3. Estimated Budget: \$50,000.
- **Phase II: In Vitro Synthesis and Characterization** (8–10 months). Activities: *E. coli* expression, purification, affinity measurement (*SPR/ITC*). TRL 4. Estimated Budget: \$150,000.
- **Phase III: Ex Vivo Validation** (4–6 months). Activities: Patient plasma testing, small-scale columns. TRL 5. Estimated Budget: \$100,000.
- **Phase IV: Functional Prototype** (6–8 months). Activities: Cartridge integration, flow testing, sterilization. TRL 6. Estimated Budget: \$200,000.
- **Phase V: Preclinical Studies** (12–18 months). Activities: Large animal models (porcine/ovine), safety and efficacy. TRL 7. Estimated Budget: \$500,000.

Total Estimated Budget: ~\$1,000,000 USD | **Total Timeline:** 36–50 months.

6.3 Proposed Team Structure

- **Principal Investigator (PI) - Orlando Samayoa:** General coordination, computational design, and funding relations.
- **Structural Bioinformatics:** Execution of RFdiffusion/ProteinMPNN pipeline and MD simulations.
- **Molecular Biology & Biochemistry:** Protein expression, purification, and biophysical characterization.
- **Biomaterials Engineering:** Surface functionalization and cartridge prototype design.
- **Clinical & Regulatory Advisory:** Access to patient samples and FDA/EMA regulatory strategy.

6.4 Identified Funding Sources

- **International:** NIH (R21/R01), ERC Starting Grant, Wellcome Trust, Bill & Melinda Gates Foundation.
- **Renal Foundations:** National Kidney Foundation (NKF), American Society of Nephrology (ASN).
- **Industry:** Fresenius Medical Care, Baxter, B. Braun (Innovation Funds).
- **Regional (Mexico):** CONAHCYT (Ciencia de Frontera).

6.5 Intellectual Property (IP)

The IP strategy includes:

1. **Composition of Matter Patent:** TRAPHOLE-UT sequences and variants.
2. **Method of Use Patent:** Extracorporeal system for PBUT removal.
3. **Know-how:** Specific immobilization protocols and operational conditions.

Timeline: Provisional application prior to Phase I results publication.

IP actions will be aligned with publication strategy and collaborative agreements to ensure transparency while protecting translational potential.

6.6 Risk Analysis and Mitigation

- **Insufficient Affinity:** Iterative computational redesign.
- **Slow Dissociation from HSA:** Metadynamics evaluation and surface density optimization.
- **Regulatory Risk:** Early consultation with FDA/EMA advisors.
- **Hemocompatibility:** ISO 10993 compliant testing and C3a/C5a activation assays.

6.7 Limitations and Unresolved Variables

Despite the structured computational and translational framework presented, several critical uncertainties remain:

1. **Kinetic Constraints**
The dissociation rate (k_{off}) of IS and pCS from HSA under dynamic extracorporeal flow may limit real-time displacement efficiency beyond equilibrium predictions.
2. **Mass Transport Limitations**
Diffusion gradients and boundary layer effects within hollow fiber systems may reduce effective contact between toxins and immobilized TRAPHOLE-UT.
3. **Competitive Endogenous Ligands**
Fatty acids, bilirubin, and protein-bound drugs may compete for similar aromatic or cationic binding motifs, potentially reducing selectivity.
4. **Surface Fouling and Protein Corona Formation**
Adsorption of plasma proteins onto functionalized surfaces may reduce active site accessibility during prolonged dialysis sessions.
5. **Orientation and Density Effects**
Immobilization efficiency and spatial orientation of TRAPHOLE-UT molecules will critically determine functional binding capacity.
6. **Scaling from In Vitro to Clinical Conditions**
Performance observed in plasma recirculation systems may not directly extrapolate to full clinical dialysis environments.

Addressing these variables will require integrated computational modeling, in vitro experimentation, and fluid dynamic simulations prior to translational advancement.

7. Expected Impact and Contributions

7.1 Scientific Impact

- Proposal of a de novo mini-protein design framework specifically targeting protein-bound uremic toxins.
- Validation of a high-throughput computational pipeline for clinically relevant protein-ligand problems.
- Contribution to the emerging field of **biofunctionalized medical devices** and smart biomaterials.

7.2 Clinical Impact

- Potential long-term improvement in dialysis efficiency and quality of life, pending successful validation.
- Potential contribution to reduction of cardiovascular morbidity and mortality associated with chronic PBUT accumulation, pending long-term validation.
- Possibility of extending dialysis intervals or significantly increasing the efficiency of standard 4-hour sessions.

7.3 Commercial Impact

- **Market Size:** The global renal replacement therapy market is valued at tens of billions of dollars annually.
- **Adsorbent Niche:** The specific adsorbent market is growing at a CAGR of 8–10%.

- **Licensing Potential:** High attractiveness for major medical device manufacturers (Fresenius Medical Care, Baxter, Nikkiso, Toray).

7.4 Exit Strategy and Technology Transfer

The project contemplates several paths for scalability:

1. **Early-stage Licensing:** Transferring IP after successful Phase II (In vitro) validation.
2. **Biotech Spin-off:** Creating a dedicated entity to drive clinical trials and regulatory approval.
3. **Co-development:** Partnering with an established dialyzer manufacturer to integrate TRAPHOLE-UT into existing product lines.
4. **Joint Venture:** Collaboration with global renal care providers for large-scale implementation.

Author Positioning Statement

This document represents an independent interdisciplinary research proposal. The author does not hold formal clinical specialization in nephrology and approaches the problem from a systems-level perspective grounded in computational modeling and bioengineering. The intent of this work is to stimulate collaboration with domain experts for rigorous validation and translational development.

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