PROTEINS DO NEARLY EVERY JOB, at every moment, within the body: digesting food, building tissue, transporting oxygen. Over the last century, many drugs have been developed to target and correct malfunctioning proteins: in other words, to cure disease.

Still, the pace of drug discovery has slowed dramatically over the past few years. This slower pace is one of the reasons that the University of Washington and UW Medicine have made the research conducted by the UW Institute for Protein Design (IPD) an institutional priority.

By manipulating molecules and designing proteins, the IPD’s goal is to bring a novel approach to many different areas of medicine — by helping treat diabetes, cancer, flu and malaria, for instance, or in speeding vaccine development.

With this proposal, we request your partnership in two diabetes-related protein design projects being undertaken by researcher David Baker, Ph.D., the director of the Institute for Protein Design. These projects — which have the potential to make ground-breaking discoveries that could affect diabetes treatment — are explained in more detail below.

About Diabetes and the Institute for Protein Design

Type 1 diabetes is an autoimmune disease — a disease in which the body’s immune system goes awry and turns on itself. In the case of type 1 diabetes, T cells attack the beta cells the pancreas uses to produce insulin. And without insulin, the body cannot metabolize blood glucose for energy.

There are approximately 3 million individuals in the U.S. alone with type 1 diabetes, and, unfortunately, it poses many risks to the people who suffer from it. Among the risks are serious complications like blindness, kidney failure and nerve damage. While there are treatments for type 1 diabetes, including lifelong reliance on insulin injections, diet modifications and exercise, there is no cure.

By addressing two different stages in the progression of diabetes, the Institute for Protein Design hopes to provide innovative new protein designs that could lead to new type 1 diabetes therapies. We would welcome your partnership in bringing these two projects to fruition.
DIABETES PROJECT, PART 1: INHIBITING T CELL ACTIVATION

One way to approach a treatment to type 1 diabetes is to address the problem of T cells: the process by which they are activated to destroy the pancreas’ insulin-producing cells, also known as beta cells or islet cells.

Scientists have discovered that type 1 diabetes is preceded by the presence of T cells that react to proteins produced by islet cells, which include insulin and glutamic acid decarboxylase. The T cells incorrectly perceive these proteins as antigens, or substances dangerous to the body.

Researchers also know that the presence of the DQ8 HLA allele is highly correlated with onset of childhood diabetes. What’s more, the DQ8 HLA receptor has been shown to bind insulin antigens with high affinity and specificity. This binding process, receptor to insulin, serves as an alert to the T cells to activate.

Dr. Baker and his colleagues theorize that if they could design a protein — a protein that could bind with even higher affinity and specificity to the DQ8 HLA receptor than insulin does — they could block the receptor from binding with insulin antigens.

The result of designing such a protein? The T cells wouldn’t attack the islet cells, and the development of type 1 diabetes could be thwarted. The project is described in more detail in the section below.

Starting the project: designing the protein

First, an effective binding protein must be designed. To that end, Dr. Baker and his colleagues will insert known DQ8-binding peptides (molecules consisting of two or more amino acids) into small, thermo-stable scaffold proteins. The available crystal structure of a DQ8-peptide complex will guide protein design simulations, allowing for the identification of correctly shaped scaffold proteins and further rational mutagenesis to stabilize the inserted peptide sequence in its DQ8-binding conformation.

Candidate designs will be expressed on yeast and incubated with the target DQ8 protein, after which researchers will use flow cytometry to identify successful designs. After identification of high-affinity binding proteins, yeast display selection methods will be utilized to further increase the proteins' affinity for DQ8.

Final candidates will be screened for binding against other HLA receptors to insure that the binding is very specific to DQ8 receptors, avoiding the possibility of inhibiting normal immune function at non-DQ8 receptors.

After the design stage is completed, in vitro cell testing will commence. Proteins designed to bind to DQ8 will be tested in the tube to see how well they inhibit T-cell activation by insulin peptides.

DIABETES PROJECT, PART 2: DESIGN OF INSULIN-TOLERIZING VACCINES

The second and complementary research project also addresses the topic of immune response and type 1 diabetes, but it addresses a different stage of the disease process. With this project, Dr. Baker and his colleagues are considering the creation of a vaccine that will inhibit B cells in the immune system that target islet cells.
B cells produce antibodies, proteins the body creates in response to antigens. Antibodies “tag” the antigen, a process that notifies T cells to attack the antigen invader. In the case of type 1 diabetes, these antibodies are incorrectly tagging islet cells in the pancreas for T cell destruction.

However, if physicians could use a vaccine to inhibit the B cells’ autoimmune responses, the chain reaction that leads to diabetes — antibody creation, tagging of beta cells, T cell alert, and T cell-initiated destruction of pancreatic cells — could be interrupted. The IPD’s approach to this project is described in more detail in the section below.

**Starting the project: building the nanoparticle**

The IPD has made recent advances in designing protein-based nanoparticles: self-assembling particles constructed of two separate proteins. Mimicking the shape and size of natural viruses, these designed structures will have the potential to serve as potent vaccines by engaging the immune system in a manner similar to a vaccine, without the costly and time-consuming need to grow viruses in a host system.

Unlike standard vaccines that activate the immune system to target a foreign protein, these particles could also be used to create **tolerizing vaccines** — vaccines that inhibit an immune response against a specific protein.

With these two-component nanoparticles, Dr. Baker and his colleagues propose to generate and test a new class of insulin-tolerizing vaccines. On one component, they will deliver insulin; on the other, they will deliver signals designed to suppress B cell signals. In doing so, the vaccine will educate the immune system of diabetics to ignore insulin, thus preventing its degradation and the progression of disease.

With other collaborators at UW Medicine, the IPD will test the initial efficacy of these particles in making B cells insulin-tolerant.

**Note**

Potential designs which block T-cell activation and protein-based vaccines will be tested in animal models by colleagues at at UW Medicine, the Benaroya Research Institute and others. However, this part of the project is outside the scope of this proposal, as it will require additional funding. Such funding will be contingent on the success of initial protein design production and **in vitro** cell tests.
BUDGET

Below is a budget that details the costs related to investigating protein-based solutions to diabetes.

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<th>Inhibiting T Cell Activation (two-year budget)</th>
<th>Purpose</th>
<th>Cost</th>
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OUR REQUEST

David Baker and his colleagues propose nothing less than using a revolutionary new field, protein design, to stop diabetes.

We respectfully request your support of this work. By investing in research into T cell activation and insulin-tolerizing vaccines, you will help the UW Institute for Protein Design pursue promising avenues of investigation — and, potentially, benefit millions of people worldwide.

If you would like more information about this proposal or about the Institute for Protein Design, please contact Katherine Cardinal, MBA, senior director for philanthropy at 206.616.0412 or cardinal@uw.edu. Thank you very much for your consideration.