A. Specific Aims

Guanidinoacetate methyltransferase (GAMT) deficiency is one of the three inborn errors of metabolism resulting in cerebral creatine deficiency. GAMT deficiency causes a combination of intellectual disability, autistic behavior, seizures, moderate to severe speech delay, hypotonia, and, in some cases, movement disorders. The two other inborn errors of cerebral creatine deficiency, arginine:glycine amidinotransferase (AGAT) deficiency and creatine transporter defect (CRTR), present Similarly, but respond better to standard anticonvulsivant or other therapies. Treatment of GAMT deficiency aims for both repletion of cerebral creatine and reduction of guanidinoacetate concentrations. Creatine is supplemented orally at doses between 400 and 800 mg/kg/day. Cerebral creatine levels may take months or years to be restored. Guanidinoacetate is synthesized from arginine and glycine. High-dose ornithine supplementation (400 to 800 mg/kg/day), an arginine-restricted diet with or without medical food, and sodium benzoate (100 mg/kg/day: benzoate binds to and reduces the concentration of glycine) are also used alone or in combination to reduce cerebral guanidinoacetate concentrations. Even with the best available therapy, the concentration of guanidinoacetate remains elevated several folds above normal. The toxicity of guanidinoacetate is the likely cause of the incomplete response of GAMT deficiency to existing therapies and the poor response of seizures to anticonvulsivants.

The objective of this proposal is to discover inhibitor of the synthesis of guanidinoacetate capable of normalizing guanidinoacetate concentration in patients with GAMT deficiency. These novels drugs, given with creatine supplements, should normalize the brain chemistry of patients with GAMT deficiency, reducing the frequency of seizures and facilitating intellectual development. To accomplish this objective the following specific aims will be accomplished:

Aim 1. To discover small molecule inhibitors of the human AGAT enzyme.

In silico virtual screening performed by Atomwise has identified about 100 compounds capable of binding and complexing to the AGAT enzyme. We propose to test *in vitro* these molecules for their capacity to inhibit the AGAT enzyme. To achieve this objective, we have established methods to purify sufficient amount of active AGAT. We are developing a robust tandem mass spectrometry (MS/MS) approach to the measurement of the products of the reaction (ornithine and guanidinoacetate) that can be used to identify suitable inhibitors. Small molecule compounds that inhibit the human AGAT activity (i.e., positives) will be re-tested and those confirmed will be characterized in Aim 2

Aim 2. To prioritize the positives identified from the primary screen.

Given that our goal is to identify potent and selective AGAT inhibitors, the confirmed positives from Aim 1 will be further characterized by: **(a)** Determination of their IC_{50}'s. **(b)** Assessment of their selectivity towards AGAT. **(c)** Assay for their inherent toxicity in cell culture experiments. **(d)** Evaluation of their efficacy to prevent guanidinoacetate accumulation in cells derived from patients with GAMT deficiency. These experiments **(a-d)** will help rank the confirmed positives based on potency, selectivity, toxicity, efficacy, and identify future chemical optimization schemes. Although it is outside the scope of the current application, we plan to investigate the mechanisms of action of the priority AGAT inhibitors by enzyme kinetics and possibly X-ray crystallography in the future.

Identification of potent, selective inhibitors of the AGAT enzyme can provide a novel therapeutic avenue for patients with GAMT deficiency, with the potential of reducing seizures and improving intellectual development.