

Introduction

Glucose-6-phosphate dehydrogenase (G6PD) is the rate-limiting enzyme in the pentose phosphate pathway, an important metabolic pathway for cell growth.¹ The enzyme reduces the electron carrier NADP while oxidizing glucose-6-phosphate to 6-phosphogluconate. G6PD deficiency is the most prevalent enzyme deficiency worldwide, accounting for over 400 million cases. Patients with the deficiency present with hemolytic anemia and jaundice due to an X-linked genetic mutation.² Here, we report a study of the wild type enzyme with an inhibitor in as apart of a larger effort to better understand the binding event in the mutated Canton enzyme with the activator.
Keywords: G6PD, NADPH, dehydrogenase, favism, enzyme deficiency

Research

PROTEIN PURIFICATION

G6PD was grown in BL21C40 *E. coli* cells to an optical density (OD) of 0.17. The cells were induced with isopropyl β-D-1-thiogalactopyranoside (IPTG) and arabinose and cells were harvested by centrifugation. The cell pellets were then re-suspended with a lysis buffer containing 50mM pH 8.5 Tris, 1M NaCl, 5% glycerol, and 0.01% Triton X-1000 followed by six rounds of sonication at 70% for 30 seconds. Once lysed, the cells were run through a Ni-NTA column with 50mM pH 8.5 Tris and 300mM NaCl, then eluted with 500mM pH 6.5 imidazole, 50mM pH 8.5 Tris, and 300mM NaCl. Two rounds of dialysis were then performed to further purify the the protein. SDS-PAGE was used to confirm the purification.

CRYSTALLIZATION

Crystals were created with the enzyme bound to the inhibitor. The micro-batch method was used to obtain crystals by pipetting 0.77 ul of the protein with 0.77 ul of the crystallization screen, followed by 16.6 ul of paraffin oil on top of the well. Screening conditions were obtained from Hampton Research. After completing the micro-batch process, the most optimal conditions for crystals were observed and further crystallized using the sitting drop method.

X-RAY CRYSTALLOGRAPHY

Once optimal crystals for G6PD are obtained from the sitting drop method, the enzyme structure with inhibitor can, if diffraction proceeds well, be determined at a synchrotron light source. The plan currently stands to continue monitoring the crystallization plates as some crystals will not form for an extended period.

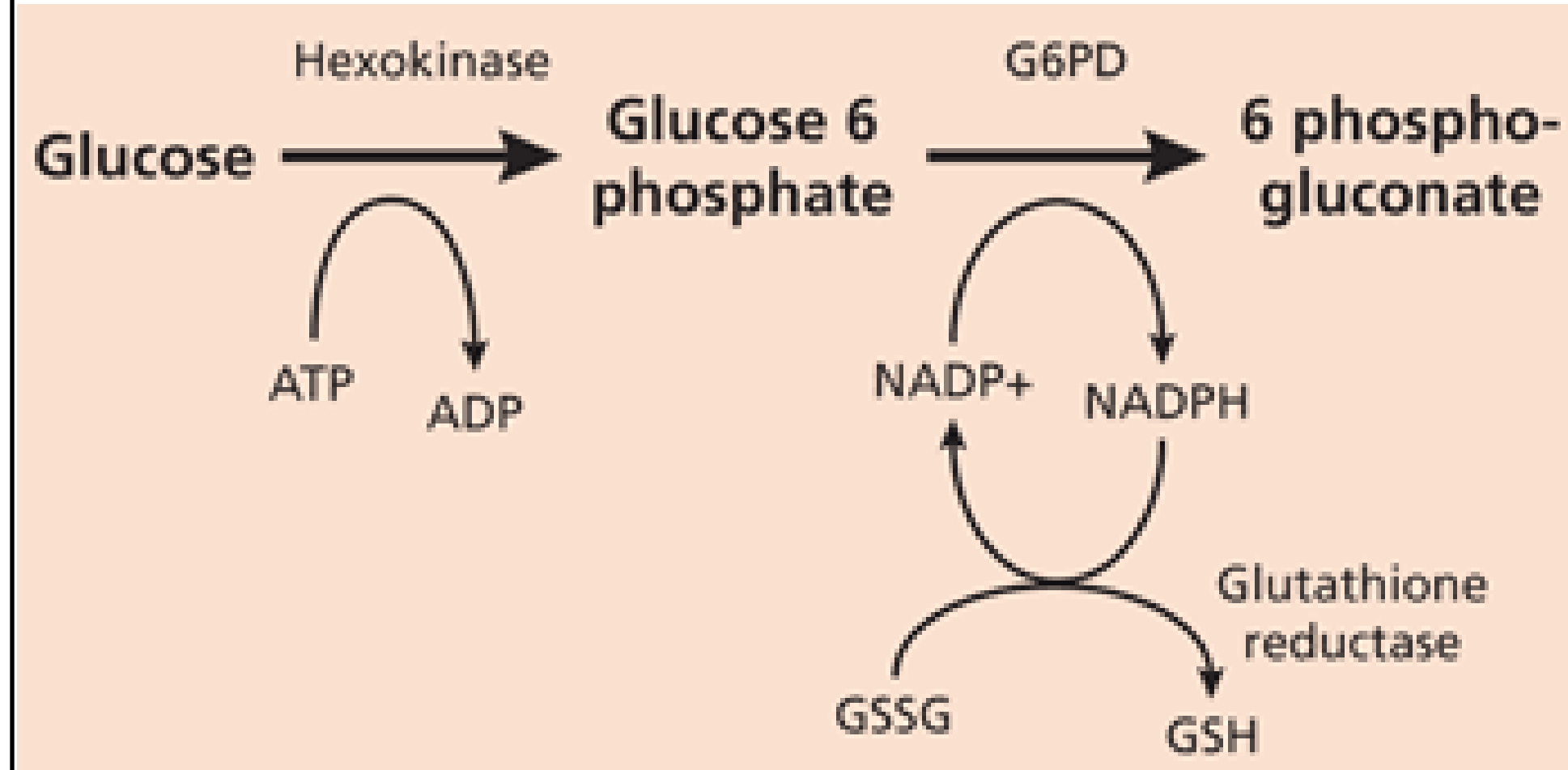


Figure 1. Role of G6PD in the Pentose Phosphate Pathway. G6PD allows for reduction of oxidants by catalyzing the reduction of NADP+ to NADPH. Frank et. Al 2005.

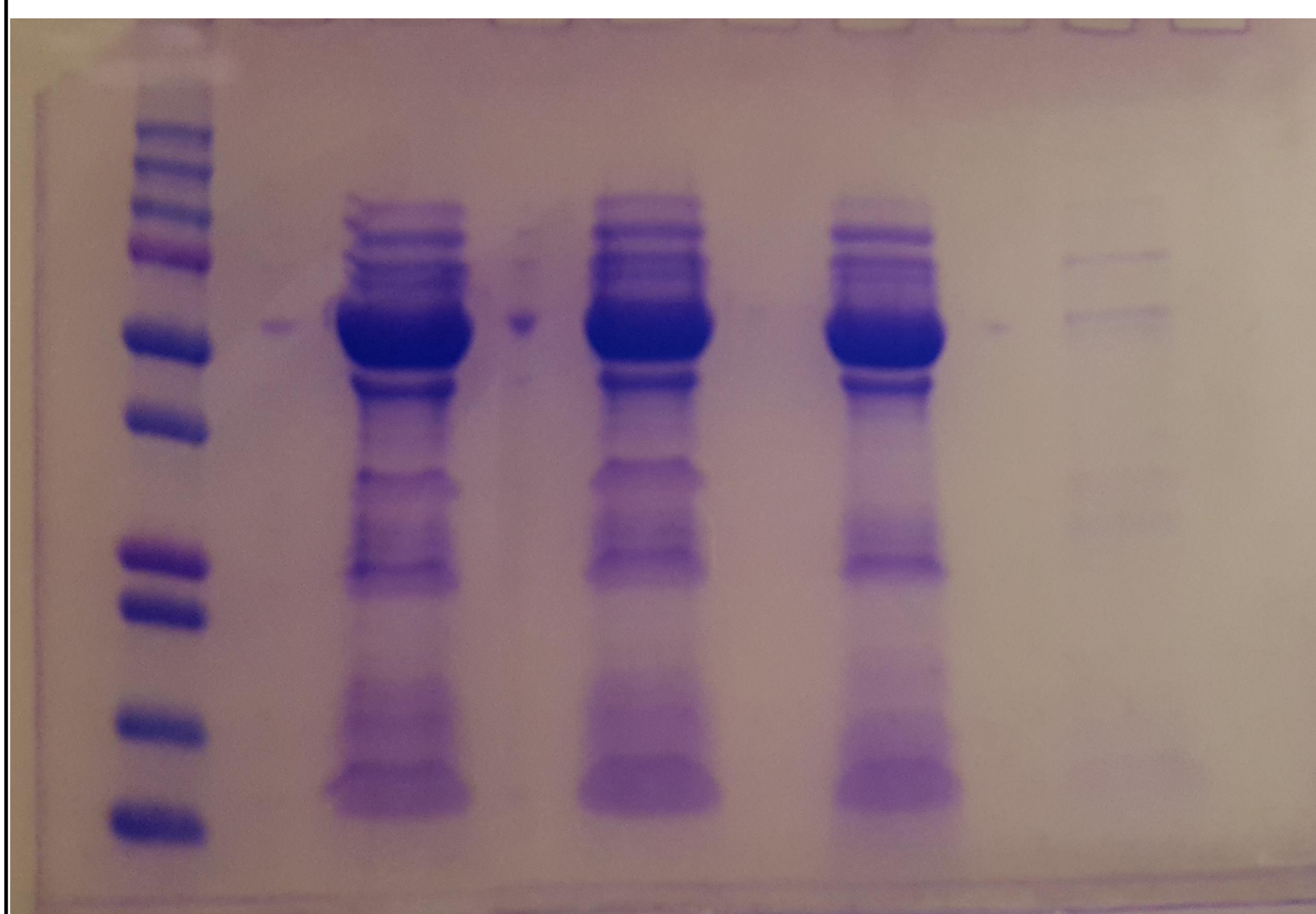


Figure 2. SDS-PAGE of G6PD bound to inhibitor, G6P, and NADP. Lane 1 is the ladder, lane 2 is G6PD bound to 1 mm of inhibitor, lane 3 is G6PD bound to inhibitor & NADP+, lane 4 is G6PD bound to inhibitor, NADP+, and G6P. Lane 5 is the precipant from adding NADP+ and G6P.

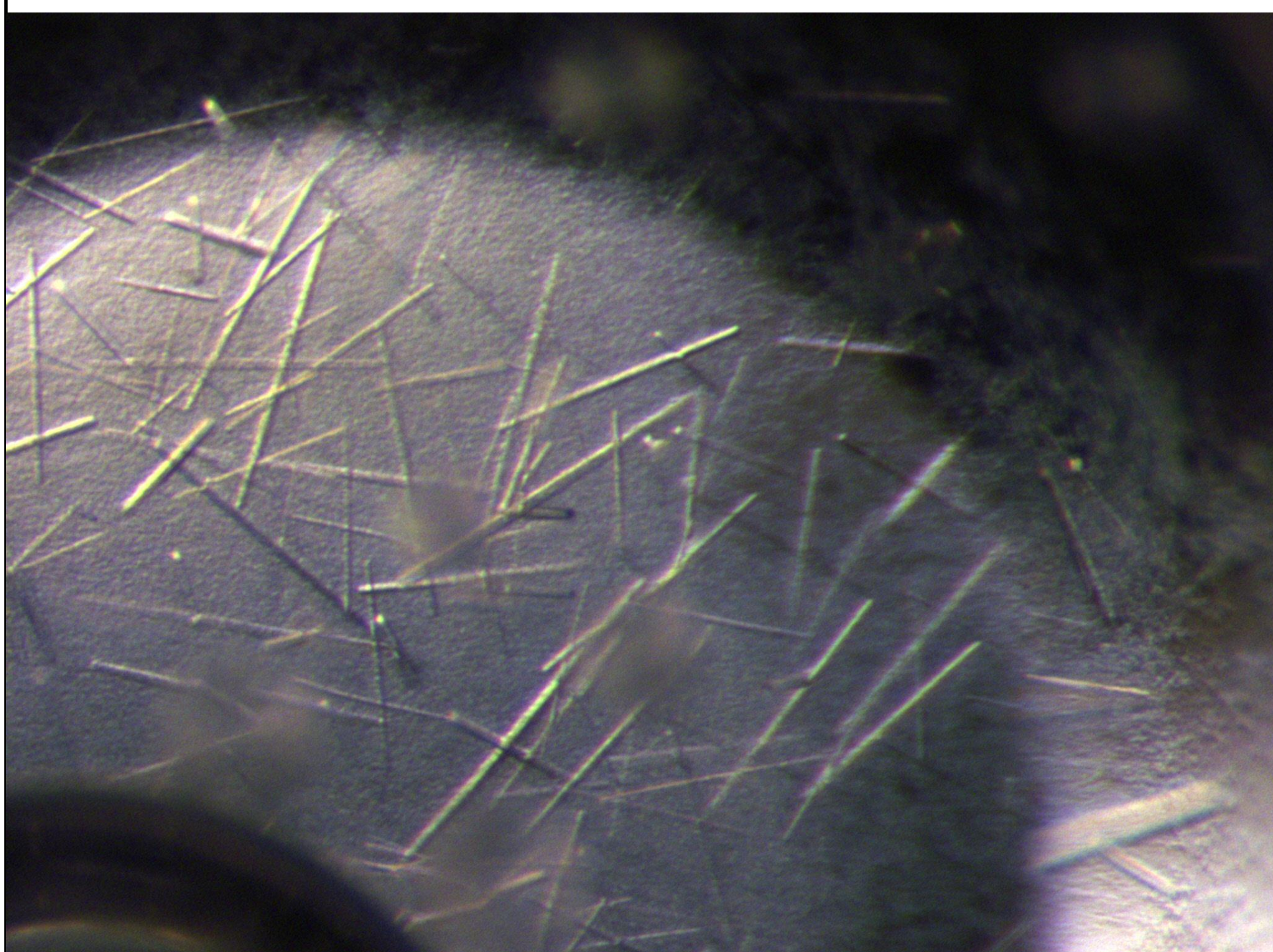


Figure 3. Crystals of G6PD bound to inhibitor. Crystal screen condition used was Wizard 1 #8 from Hampton Research containing 2 M ammonium sulfate, 100 mM sodium citrate/ citric acid pH 5.5

Crystallization Setup

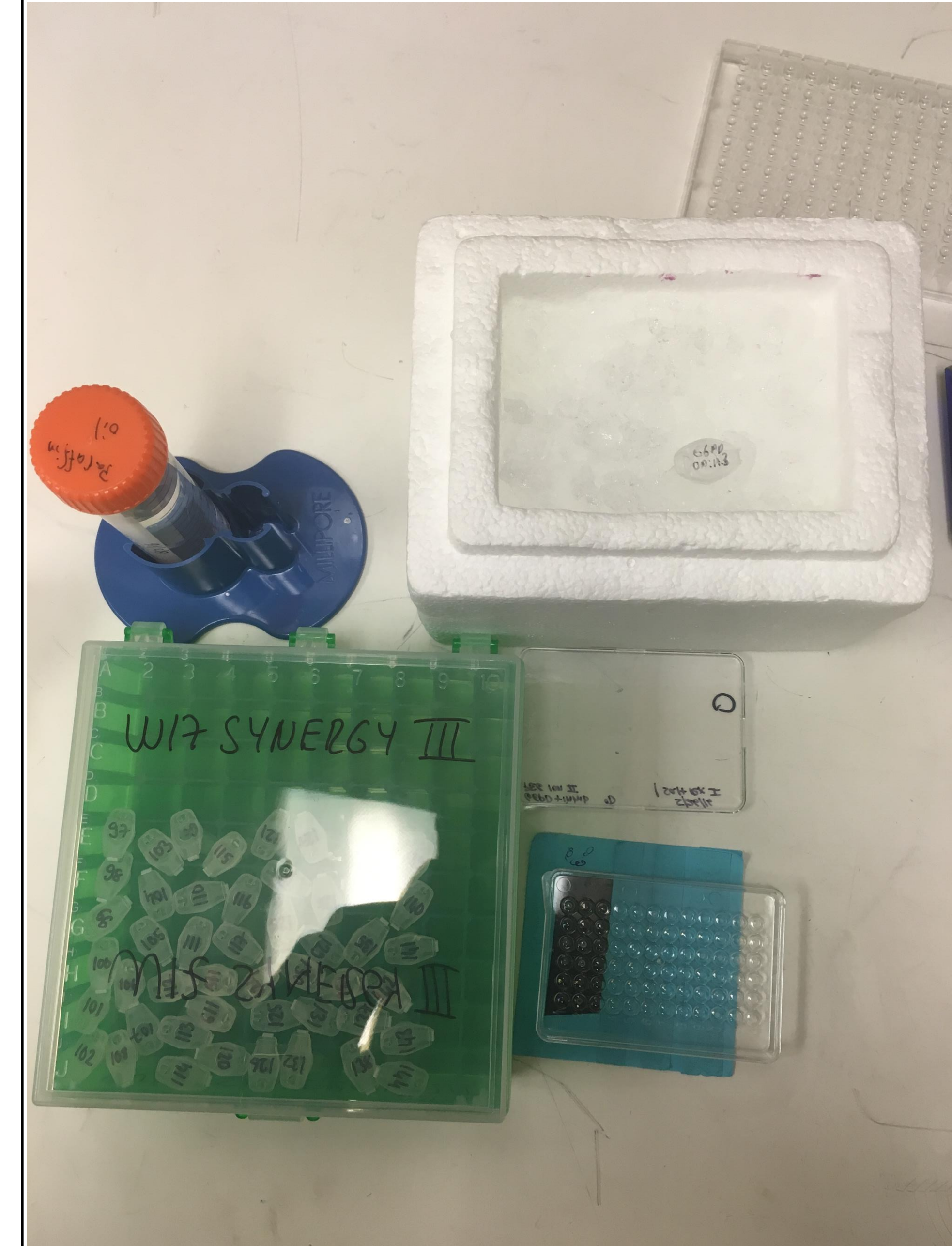


Figure 4. Illustration of microbatch crystallization procedure for G6PD. Pipettes not shown. Protein is kept on ice (top right), Paraffin oil (top left), Crystallization conditions (bottom left), microbatch plate (bottom right)

Future Work

The project is currently halted at the crystallization stage. Recently, wild type G6PD was tested a SSRL but did not diffract to a high resolution. The plan stands to begin growing more BL21C40 *E. coli* cells expressing G6PD for future attempts at crystallography.

References

- Cappellini, Maria Domenica, and G. Fiorelli. "Glucose-6-phosphate dehydrogenase deficiency." *The lancet* 371.9606 (2008): 64-74.
- Tian, W., Braunstein, L., Pang, J., Stuhlmeier, K., Xi, Q., Tian, X., Stanton, R. (1998) *J.Biol. Chem.* 273, 10609-10617.

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References

Tian, W., Braunstein, L., Pang, J., Stuhlmeier, K., Xi, Q., Tian, X., Stanton, R. (1998) *J.Biol. Chem.* 273, 10609-10617.
Cappellini, Maria Domenica, and G. Fiorelli. "Glucose-6-phosphate dehydrogenase deficiency." *The lancet* 371.9606 (2008): 64-74.