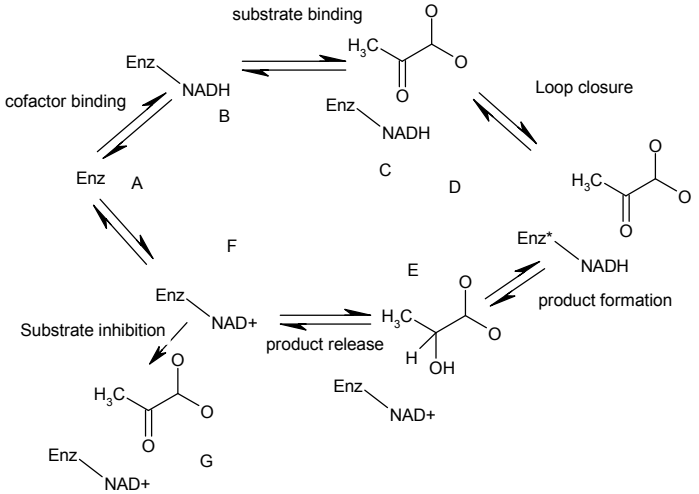
Catalytic Cycle of Plasmodium Falciparum Lactate Dehydrogenase, Andrea Hadfield,* Claire Limpkin, Jon Read, Camille Shammas, Debbie Shoemark, Rebecca Tranter and Leo Brady, *Department of Biochemistry, University of Bristol, School of Medical Sciences, Bristol, BS8 1TD, UK*. E-mail: a.t.hadfield@bris.ac.uk

Keywords: time-resolved; dehydrogenase; protein crystallography

Catalytic Cycle of *Plasmodium Falciparum* Lactate Dehydrogenase

Malaria is one of the major diseases of mankind, claiming 3 million lives worldwide annually. Resistance to existing antimalarial drugs is a large and increasing problem. The first high-resolution structure of an enzyme from the Plasmodium falciparum parasite, the causative agent of malaria was that of the essential glycolytic enzyme *P. falciparum* lactate dehydrogenase (*pf*LDH) (*1*). The deduction and confirmation of the precise molecular changes that accompany catalysis in this enzyme would provide an invaluable basis for the design of transition-state based inhibitors. The kinetic mechanism of action of lactate dehydrogenase has been studied at in great detail in a number of species of the enzyme (reviewed in (*2*)). This poster describes our progress towards elucidating the structural changes around the catalytic cycle (Figure 1) within crystals of pfLDH.



We have determined structures representing each stage in the cycle shown in figure 1, at resolutions varying from 1.2 Å (B, D, G) to 1.8 Å. Binary crystals (pfLDH + NADH) show disorder of the substrate binding loop. After soaking these crystals with substrate for 1 hour, the loop becomes ordered in the crystal (structure D) in a closed conformation. A combination of a slow turnover mutant and flash freezing has been used to capture states which we believe represent C and E with the substrate/product visible in the active site and the binding loop partially ordered. This work is being repeated using the wild-type enzyme. Structure G confirms the addition of pyruvate to NAD^+ at the C4 position in the nicotinamide ring to form a covalent adduct that has a characteristic yellow absorption.

[1] Dunn, C. R., Banfield, M. J., Barker, J. J., Higham, C. W., Moreton, K. M., TurgutBalik, D., Brady, R. L., and Holbrook, J. J. (1996) *Nature Structural Biology 3*, 912-915.

[2] Clarke, A. R., and Dafforn, T. (1998) in *Comprehensive Biological Catalysis* pp 1-80