Fragment Based Design to Disrupt the Oligomeric Interface of *Plasmodium Falciparum* Malate Dehydrogenase

Atilio Reyes Romero [(1)] , Grzegorz Popowicz [(2)], Matthew Groves [(1)], Michael Sattler[(2)], Alexander Dömling [(1)]

(1) Drug Design Group, Groningen Research Institute of Pharmacy, University of Groningen, A. Deusinglaan 1, 9713 AV, Groningen, the Netherlands.

(2) Institute of Structural Biology, Helmholtz Zentrum München, Ingolstädter Landstr. 1 85764 Neuherberg Germany

Introduction

- Although there are still effective treatments against the spread and pathogenesis of malaria in the market, new protein targets for the development of new drug classes have emerged recently.[1]
- Malate dehydrogenase (*Pf*MDH) catalyses the reversible NAD(P)+-dependent oxidation of oxaloacetate to malate in cytosol and transports malate into the mitochondrion via a potential malate/aspartate shuttle to feed the respiratory chain.[2]
- In order to facilitate the discovery of further new targets we proposed a method based on the introduction of point mutations of amino acid residues that are part of the oligomeric interface.[3]

Objectives

1. Disclose the oligomeric interface
2. High throughput screening of large and chemical diverse fragments database
3. Selection of the top five candidates
4. Co – crystallization and hit optimization

Matherial and Methods

1. Mutants generation:
   - Phusion Site-Directed Mutagenesis Kit
   - Sequencing
2. Static light scattering to characterize the new oligomeric assembly (SLS)
3. Saturation-Transfer Difference (STD) NMR for ligand screening
   - 1500 fragments from the Maybridge Ro3 diversity library in deuteraded DMSO – PAINS free
   - 1:100 ratio protein:compounds
   - 600 MHz NMR. Cryoprobe
   - 10 uM protein in 100 mM sodium phosphate buffer, 400 mM NaCl pH 7.4

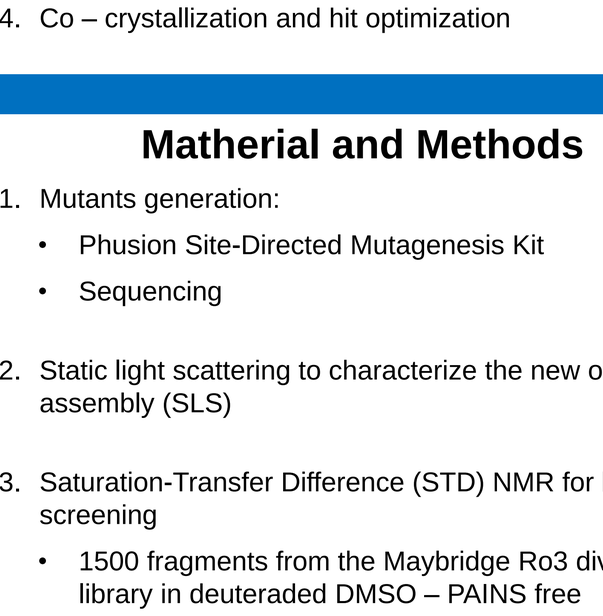


Fig 1: schematic representation of saturation transfer difference (SDT) applied in fragment based drug design.. The difference spectrum (lower row) only contains the ligand signals perturbed upon binding, whose intensities reflect the proximity of each proton to the protein surface. (picture taken from https://glycopedia.eu/Saturation-Transfer-Difference)

Results

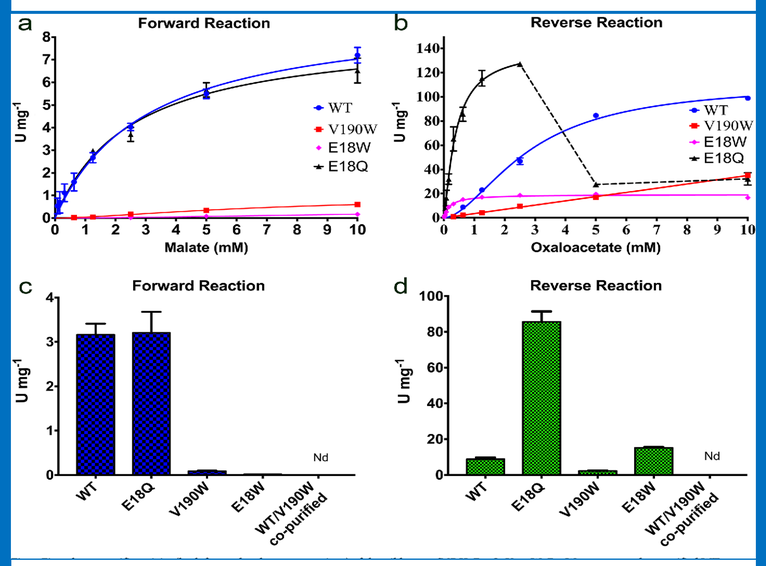


Fig 2: :U =µmol of NADH oxidized or (NAD+) reduced per minute (a &b). At1.6 mM malate dimeric PfMDH mutants had significantly reduced specific activity. At 0.625 mM oxaloacetate V190W mutant showed little or no measu rable activity.

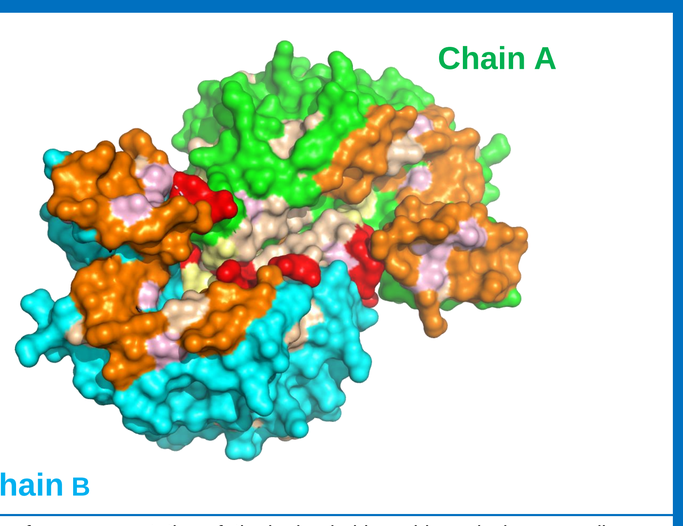
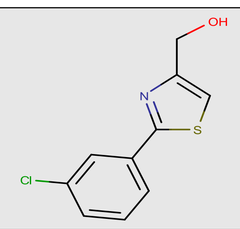
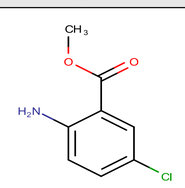
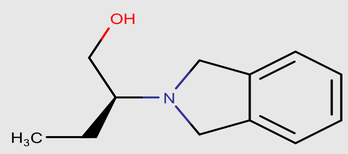
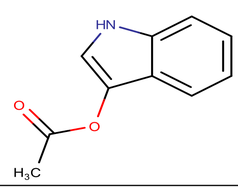
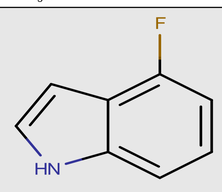


Fig 3: surface representation of the hydrophobic residues (pale rose, yellow and grey) calculated with PISA (**http://www.ebi.ac.uk/pdbe/pisa/).** The picture is rendered in Pymol.

ID	structure
HMGU - 912	
HMGU - 551	
HMGU - 1468	
HMGU - 1433	
HMGU - 1469	

Tab 1: Chemical structure of the fragment candidates after inspection of the binders of v190w and wtMDH. Only those compounds that were found in the wild type and not in the mutant ones were selected for co-crystallization and lead optimization.

Discussion

- Cluster analysis revealed that most of the fragment candidate contain an indole ring
- Indole ring of tryptophan 190 introduces steric clashed and prevents the assembly into an active conformation.
- The activity is no longer retained for oxidation from malate to oxaloacetate, but doesn't significantly change for the reverse reaction.
- STD is useful for epitope mapping and assess the ligand orientation in the binding pocket.
- Selective ^{13}C labeling of hydrophobic side chains of the oligomeric interface (e.g. isoleucine, valine and leucine) to monitor the interaction at the interface.

Conclusions

1. Properly formed oligomeric assembly is required for correct catalytic function of *Pf*MDH.
2. Oligomeric interference approach could be used in the future in order to assess druggability of other challenging human pathogen drug targets.
3. Fragment based technique such as NMR provides robust results for chemical optimization of compounds with high ligand efficiency *per se*.

References

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Prof. Michael Sattler, Dr. Gregorz Popowicz

Contacts

e-mail address: a.reyes.romero@rug.nl

Tel: +31 50 363 9107 (office)

Tel. : +31 61 7490 366 (mobile)

AEGIS website: **http://www.aegis-itn.eu/fellows/atilio-reyes-romero/**