A New Toolkit in Drug Target Validation: the Protein Interference Assay

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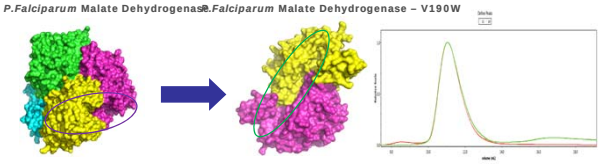
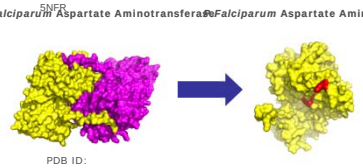
Introduction

- 60% of non-redundant protein structures available in the Protein Data Bank (PDB) represent dimerization or higher oligomerisation order (Hashimoto *et al.*, 2011)
- Large surface area of the intraoligomeric interfaces and evolutional diversity allow oligomeric partners selectively bind to each other with no cross-reactivity in the system
- Direct interference with protein self-assembly would provide an opportunity for a highly selective modulation of protein activity or function both in vitro and in vivo.

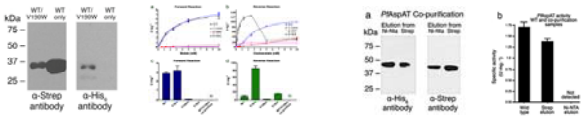
Results

- Insertion of tryptophan splits the native tetramer apart in a couple of dimers, as measured by static light scattering (SLS)

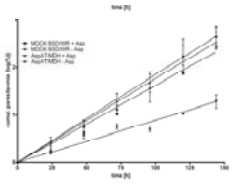




- Western Blot analysis demonstrates the ability of His_6-tagged *Pf*MDH-V190W and *Pf*AspAt mutants to incorporate into pre-formed native Strep-tagged oligomeric assembly post-expression

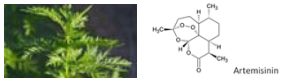


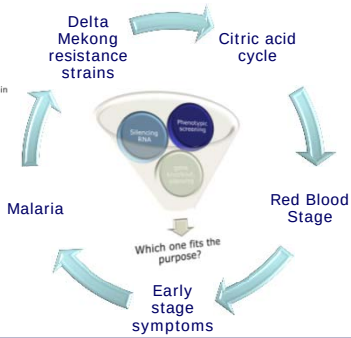
- Proliferation curves shows While no effect of double transfection with PfMDH-V190W and PfAspAT-Y68A/R257A was observed in aspartate-rich media, the parasite's viability was significantly hampered in the aspartate-limited culture



Hunting for New Antimalaria Targets

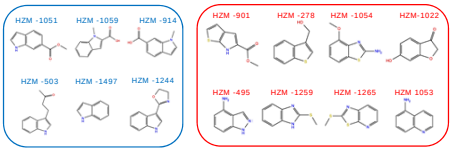
- TCA metabolism does occur in asexual Plasmodium, but at low turnover and the exact function is still a subject of debate, as it does not seem to function like a conventional TCA cycle





Rational Fragment Based Drug Design

Step 1 : Screening

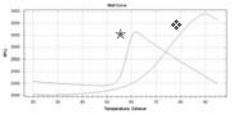


Saturation transfer difference (STD)

April 2017 - Helmholtz Zentrum library (1550 fragments)

★ = PfAspAt =

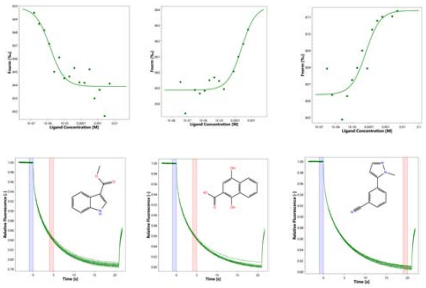
❖ = *Pf*AspAt + 5 mM fragment



Thermal Shift Assay (TSA)

January 2017 – RUG library (700 fragments)

Step 2: Hit Validation



Microscale Thermophoresis

Compound	K_d
HZM - 7	1.32 uM
HZM - 408	186 uM
HZM - 434	1.18 mM
HZM - 433	35 uM
Alex library – 2G7	247 uM
HZM - 242	235 uM
HZM – 28	2.81 uM

Future prospective: how far can we go?

- PIA will allow re-evaluation of the previously studied promising targets where conventional validation approaches have failed (in vivo)
- Future drug targets to treat malarial infection may be found within downstream components of the aspartate metabolism pathway

Acknowledgments

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