Protein Interference Assay (PIA) in Drug Target Validation.

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In order to assess a gene's product role one must possess a set of tools to modulate the target function *in vivo* with sufficient specificity. However, in many pathogenic systems, including human malaria, conventional genetic manipulation techniques or small molecule inhibitor approaches do not always provide the desired efficacy (Meissner *et al.*, 2016). A number of classic techniques such as silencing RNA (Agrawal *et al.*, 2003, Hammond, 2005) have already been reported to be non-effective in certain cases (Barnes *et al.*, 2012, Mueller *et al.*, 2014, Kolev *et al.*, 2011, Baum *et al.*, 2009).

In addition, the use of small-molecule inhibitor approaches *in vivo* is associated with high costs and is often limited due to the variety of host-specific reasons that are difficult to predict, such as rapid metabolism, poor membrane transport or localisation. Thus, potential drug targets may remain unexplored due to the inability to use the existing validation tool set.

We have recently proposed a novel promising drug-target validation approach that relies on common feature of all biological systems - oligomerisation (Meissner *et al.*, 2016). Oligomerisation is a self-assembly of two or more copies of one protein molecule (or different molecules) into one object. Large surface area of the intraoligomeric interfaces and evolutional diversity allow oligomeric partners to selectively bind to each other with very limited cross-reactivity in the system. Unlike the active sites and cofactor binding sites where evolutionary constraints restrict the sequence diversity to retain the function, oligomeric interfaces are significantly less conserved amongst homologous proteins (Caffrey *et al.*, 2004, Valdar & Thornton, 2001). Thus, 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*.

Protein Interference Assay (PIA; Meissner *et al.*, 2016) involves the utilisation of structural knowledge and mutagenic modifications of one (or more) partner proteins in the oligomeric assembly. These modifications may affect the binding site for a cofactor, catalytic activity or disrupt the oligomeric interface of the target protein. Introduction of such mutants in the native assembly (e.g. via co-expression or transfection) allows the formation of the complex with modified activity both *in vitro* and *in vivo,* and thus, making possible the quantitative analysis and highly selective tuning of the function of the target protein, that are essential for its validation as drug-target. Despite the obvious limitation of PIA

approach to oligomeric proteins, this assay would still allow partial assessment of the system of interest, as many of the studied pathways are likely to involve at least one oligomeric assembly. We suggest that PIA would also allow re-evaluation of the previously studied promising targets where conventional validation approaches have failed (Ke *et al.*, 2015).

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