Characterisation of fragments binding to the translation initiation factor eIF4E.

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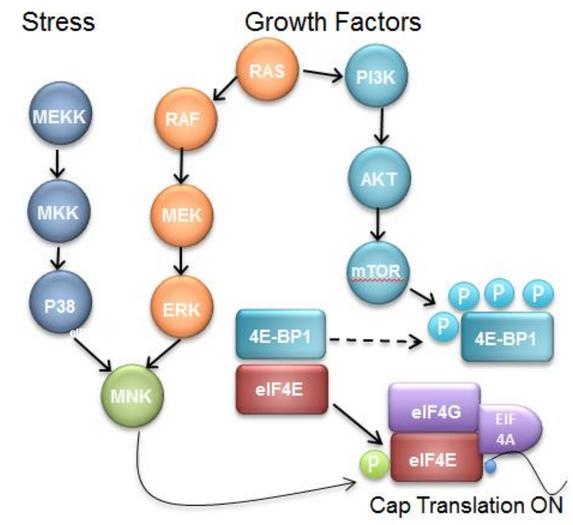
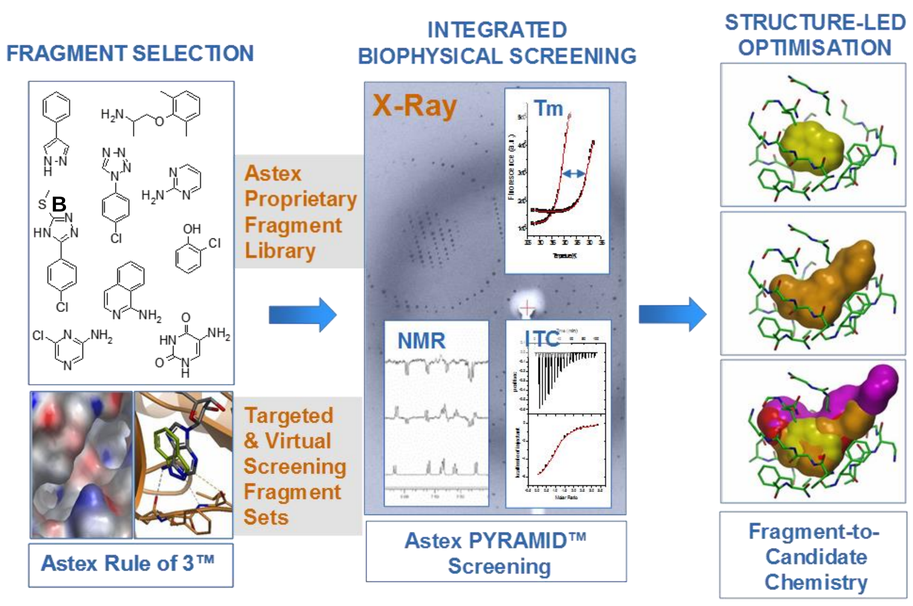
Introduction

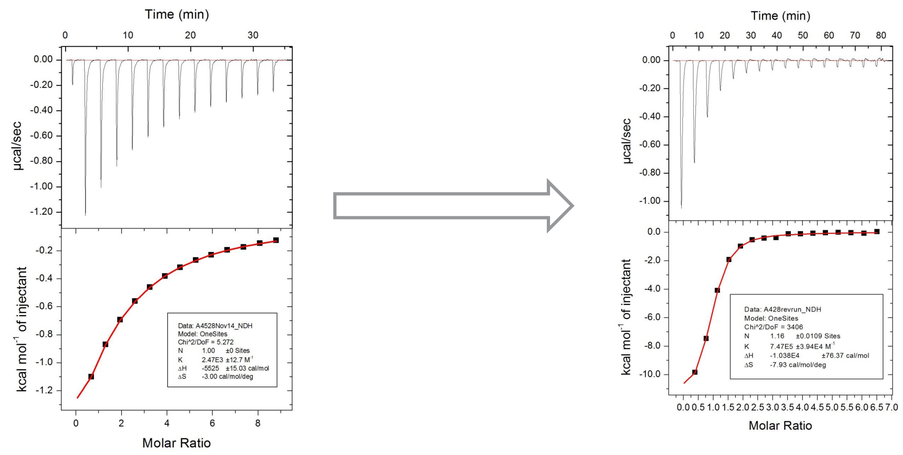
- Eukaryotic translation initiation factor 4E (eIF-4E) is a key component of the m7G-cap-binding protein complex eIF-4F and is required for cap-dependent translation initiation. Activity of the eIF4F complex is tightly controlled by both the PI3K/Akt/mTOR and Raf/Mek/ERK pathways, via mTOR phosphorylation of the eIF4E sequestering proteins 4E-BP1-3 and phosphorylation of eIF4E by MNK1/2, downstream of ERK. EIF4E is therefore a key node downstream of pathways that are frequently dysregulated in cancer.
- Formation of the eIF4F complex leads to translation of 'weak' mRNAs, encoding key cell growth and survival proteins such as cyclin D1, c-MYC and Mcl1, supporting cancer cell proliferation, and has been associated with resistance to MAPK and PI3K inhibitors[1,2]. Identification of an inhibitor of eIF4E would therefore be of therapeutic value.
- The Astex fragment screening platform was used to identify fragment hits binding to an unprecedented binding site on eIF4E. These weak hits were optimised using structure guided design into <100nM leads and profiled in a range of biophysical and biological assays to show functional effects on cap dependent translation by inhibiting the formation of eIF4F translation initiation complex.



Astex fragment screening platform



Biophysical screening for fragment hit optimisation



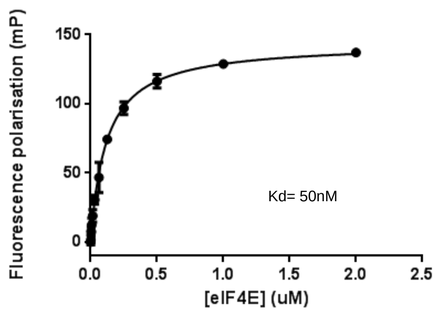
Fragment Hit
Kd = 400µM (LE = 0.36)

Chemical Probe
Kd = 1.1µM (LE = 0..34)

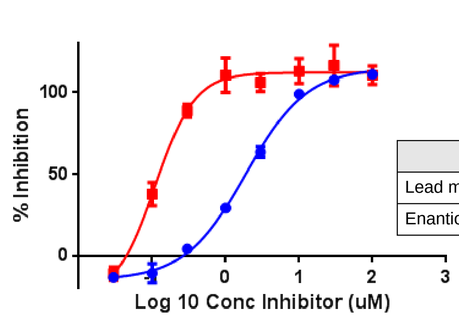
- Fragment hits were optimised using a combination of protein-ligand x-ray crystallography and biophysical methods (NMR, ITC and SPR). One of these optimised fragment hits was used to generate a chemical probe for fluorescence polarisation bioassay development

Fluorescence Polarisation Assay for hit optimisation

A. Kd determination of FAM labelled probe molecule for FP



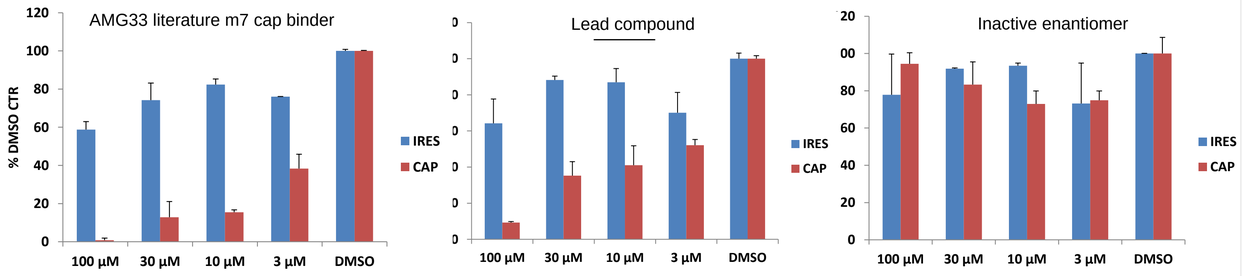
B. IC50 determination of lead molecule and its less active enantiomer



	IC_{50} (uM)
Lead molecule	0.1
Enantiomer	1.9

- The probe molecule was fluorescently labelled with fluorescein to generate a probe for fluorescence polarisation assay development. This assay was then used to support structure based optimisation of compounds to generate leads with ~100nM binding affinity to eIF4E. Less active enantiomers of the lead molecules were also synthesised and used as negative control tool compounds.

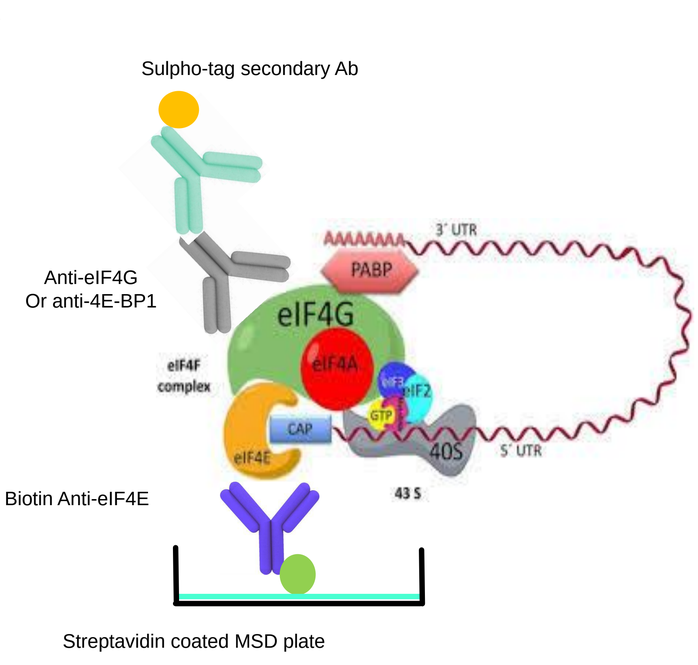
Lead compounds inhibit Cap-dependent translation



- A Dual luciferase reporter vector was used to measure cap and IRES driven translation in HeLa cell lysates (HeLa *In vitro* translation assay kit, Thermo life sciences)
- Lead compounds inhibit cap-dependent , but not IRES driven translation in this *invitro* translation HeLa cell lysate assay. The assay was validated using a published inhibitor that binds to the m7-Cap site (AMG33)[3]

Lead compounds disrupt eIF4F complex formation

A



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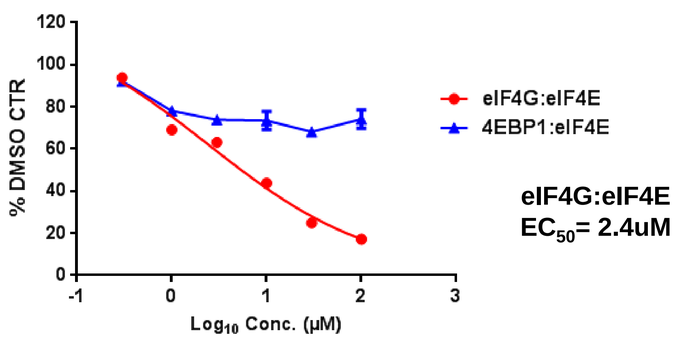

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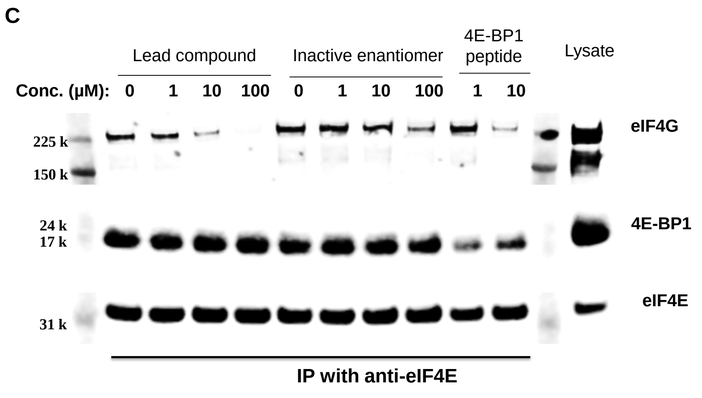

- A. Meso-scale discovery (MSD) assay format to measure the interaction of eIF4E with 4E-BP1 or eIF4G in SW620 lysates
- B. Lead compound activity in eIF4E MSD interaction assay. The lead compound inhibits binding of eIF4E to eIF4G but not to 4E- BP1 in SW620 lysates
- C. Immunoprecipitation assay confirming MSD assay data. The lead compound inhibits the interaction between eIF4G but not 4E-BP1. The less active enantiomer of the lead compound does not affect the PPI to the same extent.

Summary and Conclusions

- Upregulation of translation initiation complex eIF4F has been shown to occur in a variety of cancer types, and may be associated with resistance to vemurafenib and mTOR inhibitors.[1,2]
- Using a fragment based drug discovery approach we generated inhibitors of the eIF4F translation initiation complex that potently bind to a novel binding site on eIF4E, prevent eIF4F complex formation and inhibit cap-dependent translation.
- The data demonstrates that a fragment based discovery approach can be utilised for this challenging but promising cancer drug target

References

1. Boussemart et al (2014) Nature; 513, 105-110
2. Cope et al (2014) J. Cell Science; 127, 788-800
3. Chen et al (2012) J Med Chem; 55, 3837-3857

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