Simplify complex pathway dissection by combining the power of HTRF® cellular phospho-assays and the flexibility of the CyBi®-FeliX liquid handling system

F. Charrier-Savournin[1], I. Pribilla[1], B. Roux[1], A. Jean[1], K. Undisz[2], H. Pruefer[2], X. Scerri[3], M. Beil-Peter[2], S. Oberleitner[1]
[1]Cisbio Bioassays, Codolet, France - [2]CyBio AG, Jena, Germany - [3]CyBio France, Savigny-le-Temple, France

Concept

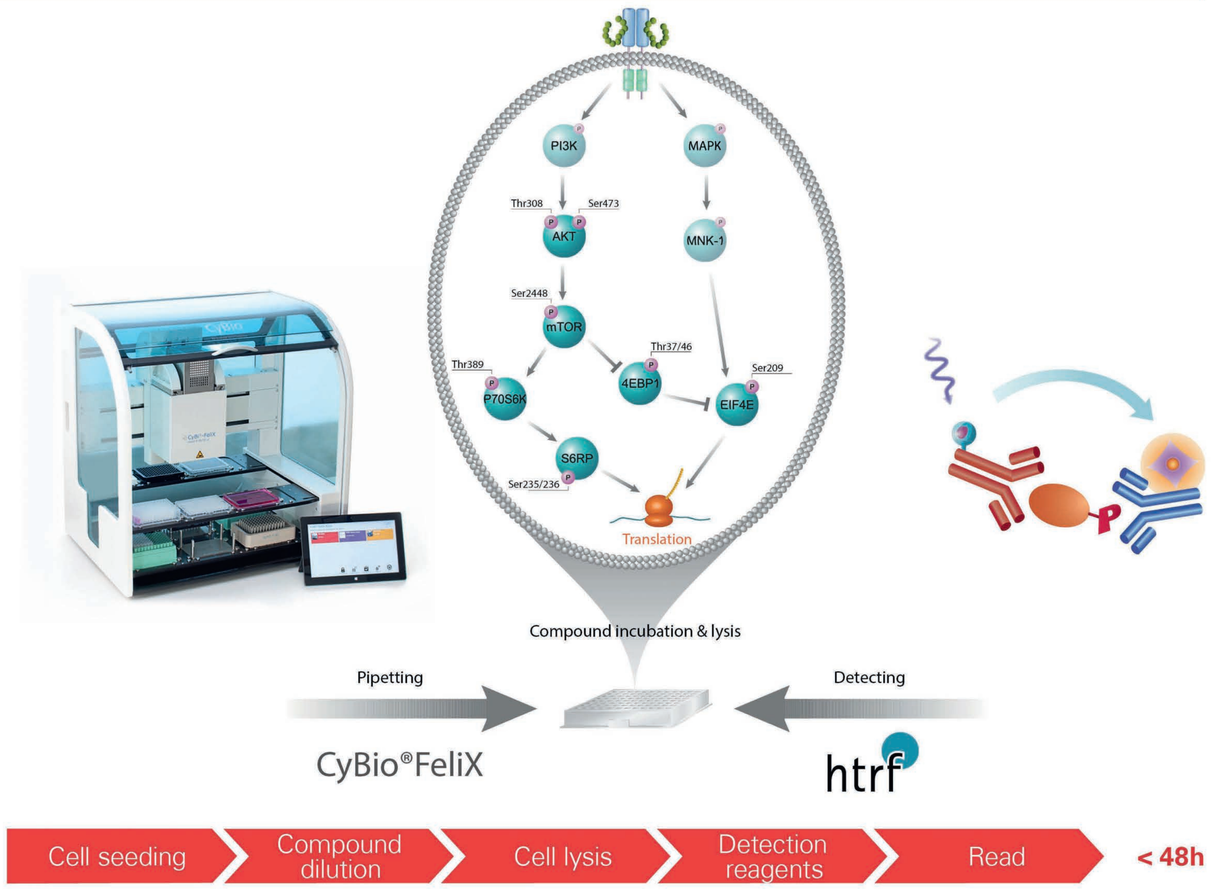
What if you had a proliferation inhibitor and you don't know the molecular target or its mechanism in the cell? Exploring the regulatory networks within the cell is the basis of understanding drug actions in disease and for finding appropriate therapies. Disease biology is complex, as are the signaling pathways that drive the biological responses. Smart tools that facilitate pathway profiling and target deconvolution are the key to success in phenotypic and target-based approaches in drug discovery.

This study illustrates the parallel implementation of several HTRF® cellular phospho-protein assays on the CyBi®-FeliX liquid handling platform. A homogeneous and robust phospho-protein assay platform based on TR-FRET has been developed for the detection of endogenous phosphorylation, to help dissect cellular signaling cascades. The CyBi®-FeliX, with its 12 deck positions on two levels, was used as a standalone fully automated system to perform all HTRF®-assay specific liquid handling tasks with one pipetting head.

The pharmacological response of the cell to several well-known kinase inhibitors was tested on significant nodes in the PI3K/Akt/mTOR translational control pathway. The IC50s were determined for each pathway step and converted into a "signature of inhibitor action". Displayed as a heat map, the signatures of reference inhibitors enable the assessment of the cellular mechanism of action of new inhibitors.

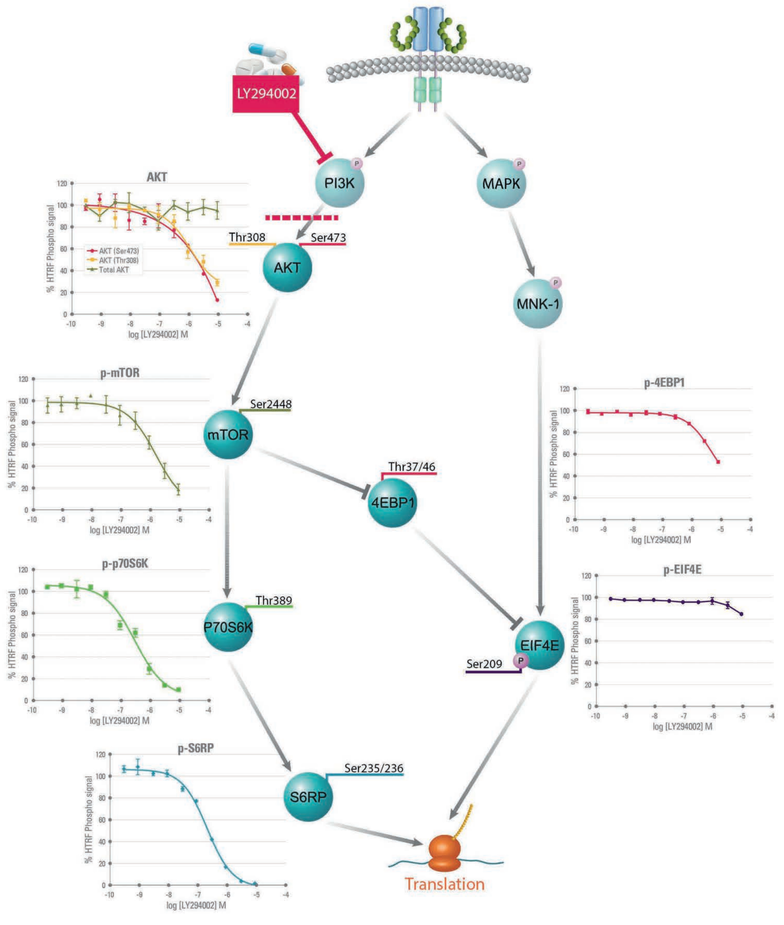
Assay Workflow

Seven different reference compounds, as indicated, were diluted in parallel over 5 orders of magnitude in cell medium with 10% DMSO in a 96-well V-bottom plate. The entire compound plate was replicated and diluted 1:10 with cell medium to reduce the DMSO concentration to 1%. Afterwards, 4 x 50µl from each well were transferred into 4 identical 96-well plates, which had been seeded with 50µl HEK293 cells (at 50k cells/well) the day before. After 3h incubation at 37°C the supernatant was discarded and 60µl supplemented lysis buffer was added. After 30 min at RT on a microplate shaker either 8µl or 16µl of the cell lysates were transferred in duplicates into white 384-well small volume assay plates. Lysis buffer was added to give a final volume of 16µl before the addition of 4µl of HTRF detection reagents. Plates were read after 16h incubation at RT in a BMG PHERAstar FS reader. All microplates used were from Greiner Bio One.

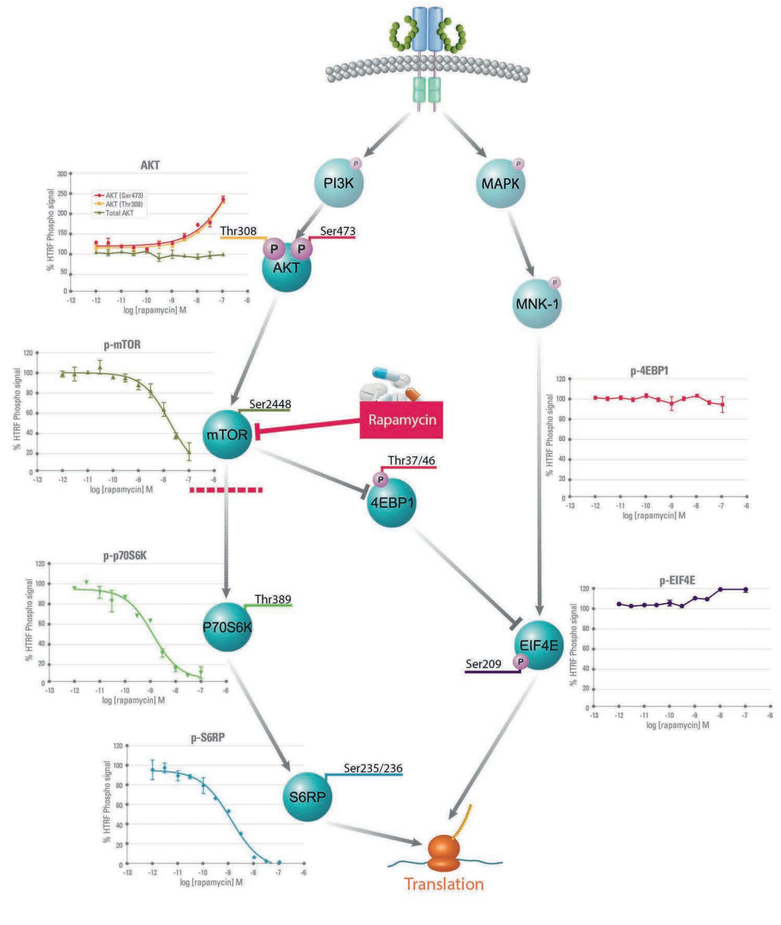


Phosphorylation signatures of inhibitors on the translational control pathway

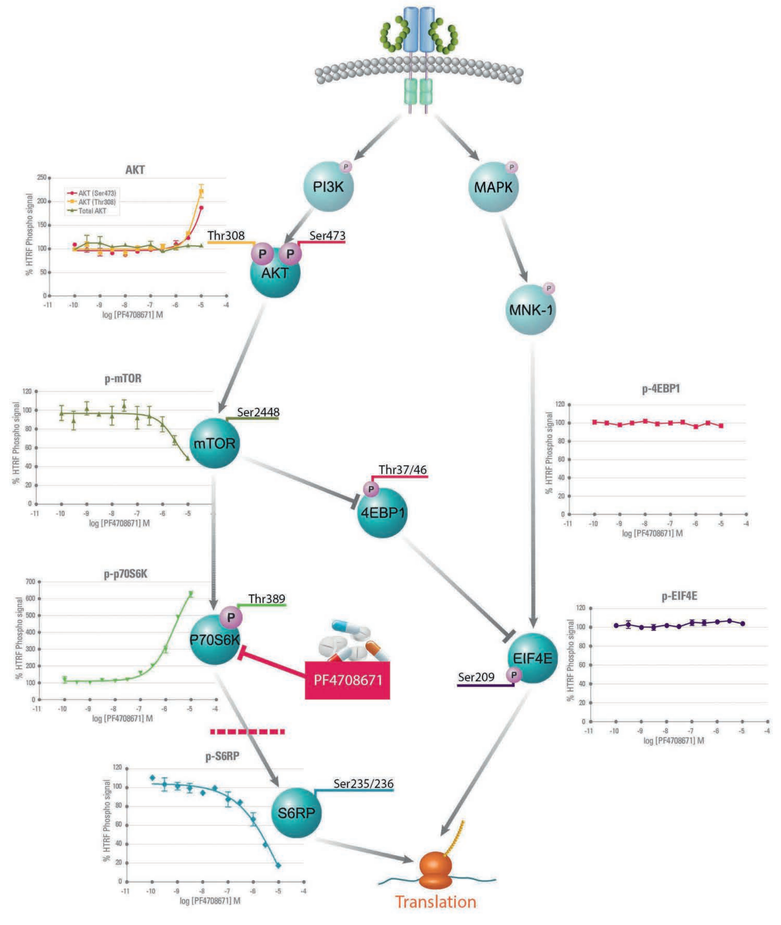
1. LY294002 dose responses



2. Rapamycin dose responses



3. PF4708671 dose responses



Heat map of phosphorylation signatures of inhibitors on the translational control pathway: IC50 values [nM]

Inhibitor	LY294002	Wortmannin	PP242	Rapamycin	PF-4708671	CGP57380	Cerco sporamide
Kinase target / *Assay*	PI3K		PI3K + mTOR	mTOR	p70S6K	Mnk1	
AKT Total							
p-AKT (Ser473)	2,500	500	50	100	>10,000		
p-AKT (Thr308)	3,000	500	19	100	>10,000		
p-mTor (Ser2448)	2,000	2,000	40	18	4,000		
p-P70S6K (Thr389)	330	1,000	15	1	2,300		> 10,000
p-S6RP (Ser235/236)	250	500	5	1	3,000	500	
p-4EBP1 (Thr37/46)	~15,000	~10,000	250				
p-EIF4E (Ser209)			190			2,000	430
Reference IC50 values (nM)*	500 - 6,000	2 - 400	8-200	0.1-10	200	2,000	10-150

partial inhibition | *activation*
full inhibition | *no effect*

Conclusion

This study shows how easy it is to analyze the mechanisms of actions of drugs with HTRF® cellular phospho-assays implemented on the compact and flexible CyBi®-FeliX pipettor. Here we dissected the complex PI3K/AKT/mTor translational control pathway into individual measurable steps. The results obtained correlate well with the known pharmacology of the reference inhibitors published in the literature*. The heat map of phosphorylation signatures obtained for reference inhibitors allow clustering of your leads according to their cellular mechanism of action and assessment of their molecular targets.

Speed up the time from phenotype to target by taking advantage of HTRF® cellular phospho-assays implemented on the CyBi®-FeliX liquid handling system to dissect the translational control pathway. Extend the approach to many other pathways and other cell types with Cisbio's broad cellular HTRF® phospho-assays portfolio and our custom assay development services.

Make sure that your leads show the desired Mechanism of Action in signal transduction and control the cell signaling events associated with the disease state in a pathological cellular background.

* References

Chaussade et al. Biochem. J. (2007) 404: 449-458
Yano et al. J. Biol. Chem. (1993) 268: 25846-25856
Apsel et al. Nat Chem Biol. (2008) 4(11): 691-9
Edwards et al. J. Biol. Chem. (2007) 282: 13395-13401
Pearce et al. Biochem J. (2010) 431(2): 245-55
Konicek et al. Cancer Res. (2011) 71(5): 1849-57
Knauf et al. Mol. Cell. Biol. (2001) 21(16): 5500-5511

Cisbio Bioassays

Headquarters & Europe
Cisbio Bioassays
Phone : +33 (0)4 66 79 67 05
Fax : +33 (0)4 66 79 19 20
E-mail : bioassays@cisbio.com

USA
Cisbio US, inc.
Phone : 888-963-4567
Fax : 781-687-1500
E-mail : htrfinfo@cisbio.us

Analytik Jena AG
Phone +49 3641 77 70
Fax +49 3641 77 9279
info@analytik-jena.com
www.analytik-jena.com

www.cisbio.com
www.analytik-jena.com