Profiling of cyclin-dependent kinase (CDK) inhibitors: use case of a novel DIANA method for efficient kinase inhibitor discovery

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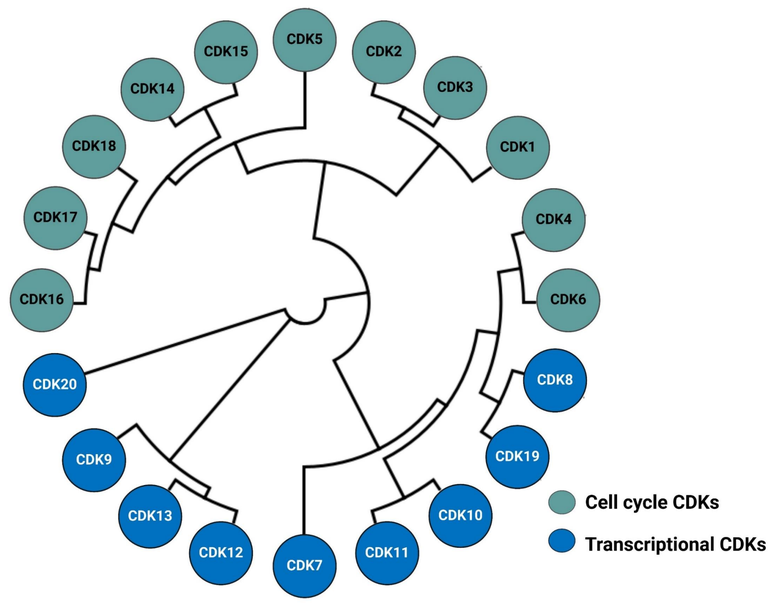
Overview

- A novel **DIANA** (**D**NA-linked **I**nhibitor **AN**tibody **A**ssay) technology enabling efficient inhibitor profiling and HTS (1).
- Convenient determination of enzyme inhibition constants (K_i) in a 384-well plate format.
- Reliable determination of K_i from a single well.
- Selectivity profiling of target compounds against the family of CDKs.

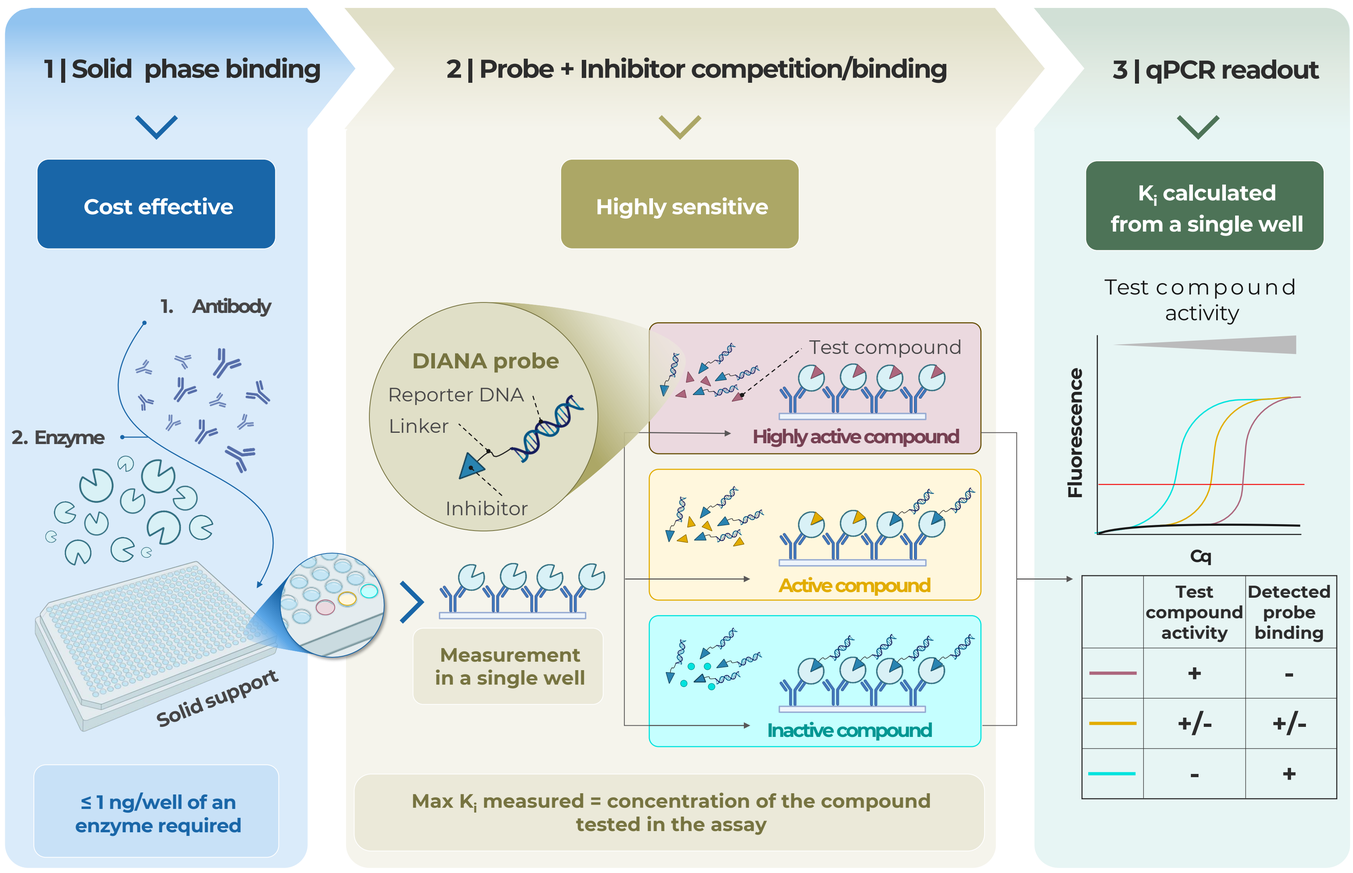


Introduction

Cyclin Dependent Kinases (CDKs) are serine/threonine kinases, members oft he CMGC kinase family, whose activity is regulated by their binding partners, cyclins (2). To this day, 20 CDKs have been identified (3). They play a role in essential cellular processes such as regulation of the cell cycle, transcription, and pre-mRNA splicing.

Here, we present DIANA CDK panel – a set of microplate-based binding assays for CDKs based on the patented DIANA (DNA-linked inhibitor antibody assay) technology. These assays can be used for convenient measurement of inhibitors' K_i and selectivity profiling of target compounds against the whole family of CDKs. Alternatively, DIANA CDK assays can be applied as a robust HTS (high-throughput screening) method to search for new active compounds against CDKs.

Methods

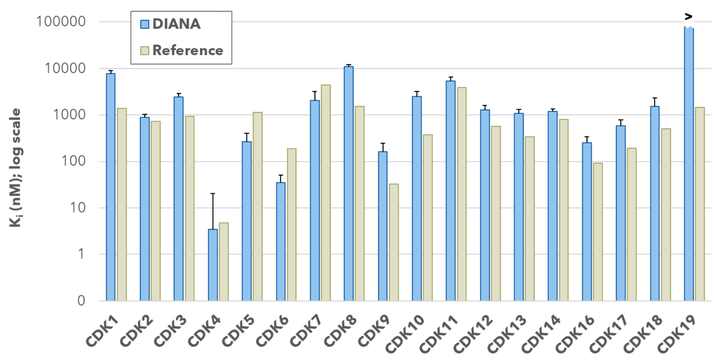


Features of DIANA CDK panel

- Based on the proprietary patented DIANA technology.
- 384-well plate format → **high-throughput.**
- Low consumption of reagents → **cost-effective.**
- Quantitative PCR-based readout → **K_i from single well.**
- Any CDK included in the DIANA CDK inhibitor profiling panel can be used in **HTS campaigns (Z' > 0.7) using our in-house compound libraries.**

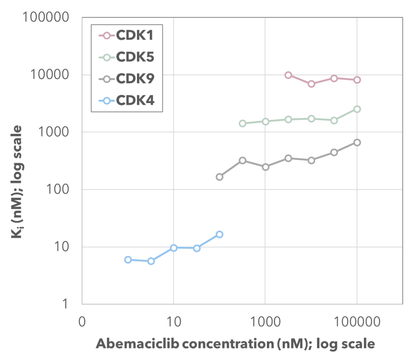
Results

Comparison of Abemaciclib inhibitor profile with a reference method



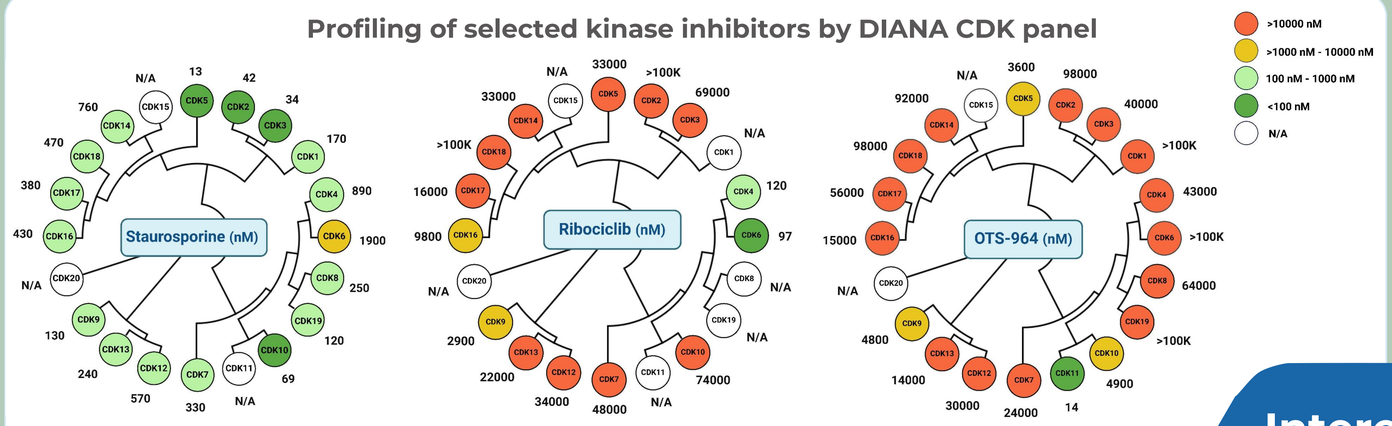
K_i values measured with the DIANA CDK panel are comparable with reference data; **>** indicates K_i above assay sensitivity.

K_i values calculated from different concentrations of Abemaciclib



A single concentration of compound within the assay dynamic range is used for precise K_i determination.

Profiling of selected kinase inhibitors by DIANA CDK panel



Pan-specific inhibitor | CDK4/6-specific inhibitor | CDK11-specific inhibitor

Parameters of DIANA CDK panel

CDK	Dynamic range (fold change)	MIN K_i (nM; 1 µM cpd used)	MAX K_i (nM; 1 µM cpd used)
CDK1/cycB1	15	110	1700
CDK2/cycA2	2100	1.0	2200
CDK3/cycE1	1600	1.4	2300
CDK4/cycD1	7.7	210	1600
CDK4/cycD2	13	130	1600
CDK4/cycD3	45	44	1900
CDK5/p25	550	3.4	1900
CDK5/p35	700	2.6	1800
CDK6/cycD1	17	77	1300
CDK6/cycD2	5.4	330	1700
CDK6/cycD3	26	77	2000
CDK7/ cyc H1	15	100	1600
CDK7/ cyc H1/ MNAT1	1500	0.90	1300
CDK8/cycC	13	93	1200
CDK9/cycT1	320	2.8	910
CDK9/cycT2	290	5.3	1500
CDK10/cycQ	75	26.0	1900
CDK11A	49	25	1200
CDK12/cycK	62	35	2200
CDK13/cycK	61	27	1700
CDK14/cycY	330	5.7	1900
CDK16/cycY	35	58	2000
CDK17/cycY	57	39	2200
CDK18/cycY	7.5	260	1900
CDK19/cycC	160	12	1900

Cell cycle CDKs Transcriptional CDKs

Conclusions

- DIANA method enables robust, precise, and cost-effective inhibitor profiling.
- DIANA CDK panel comprises 25 CDK/cyclin complexes.
- Results of the DIANA CDK panel were validated with two reference methods.
- K_i determination from a single well.

Interested in using the DIANA CDK panel in your drug discovery pipeline?

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DIANA Biotechnologies

References

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2. Malumbres et al., Genome Biology 2014
3. Lukasik et al., Int. J.Mol. Sci. 2021