

DIANA

assay for *in vitro* diagnostics

Overview and collaboration proposal

Institute of Organic Chemistry and Biochemistry AS CR, Prague, Czech Republic



ÚOCHB AV
ČR
IOCB PRAGUE



IOCB TTO
www.iocb-tto.cz

DNA-linked Inhibitor ANTibody Assay

What is DIANA

- A **multi-well plate based assay** (with similar protocol to ELISA)
- **Detection via small-molecule active site ligand** linked to reporter DNA oligo
- **Quantified by qPCR** with high sensitivity and broad dynamic range
- Can be implemented using standard equipment and be fully **automatized**

Two major application markets

1. **DIANA for diagnostics**
Ultra-sensitive detection of active enzymes in range of clinical samples
2. **DIANA for screening**
Screening for target enzyme inhibitors in drug discovery
(covered in detail in a separate document)

DIANA for diagnostics: novel assay suitable for ultra-sensitive target detection

Application

- Suitable for ultra-sensitive detection of active enzymes in range of clinical samples (e.g. human serum or plasma, urine, cell and tissue lysates)
- Validated for multiple targets, incl. Prostate specific membrane antigen (**PSMA**), Carbonic anhydrase 9 (**CA-IX**) - assays for new targets straightforward to develop¹

Key advantages

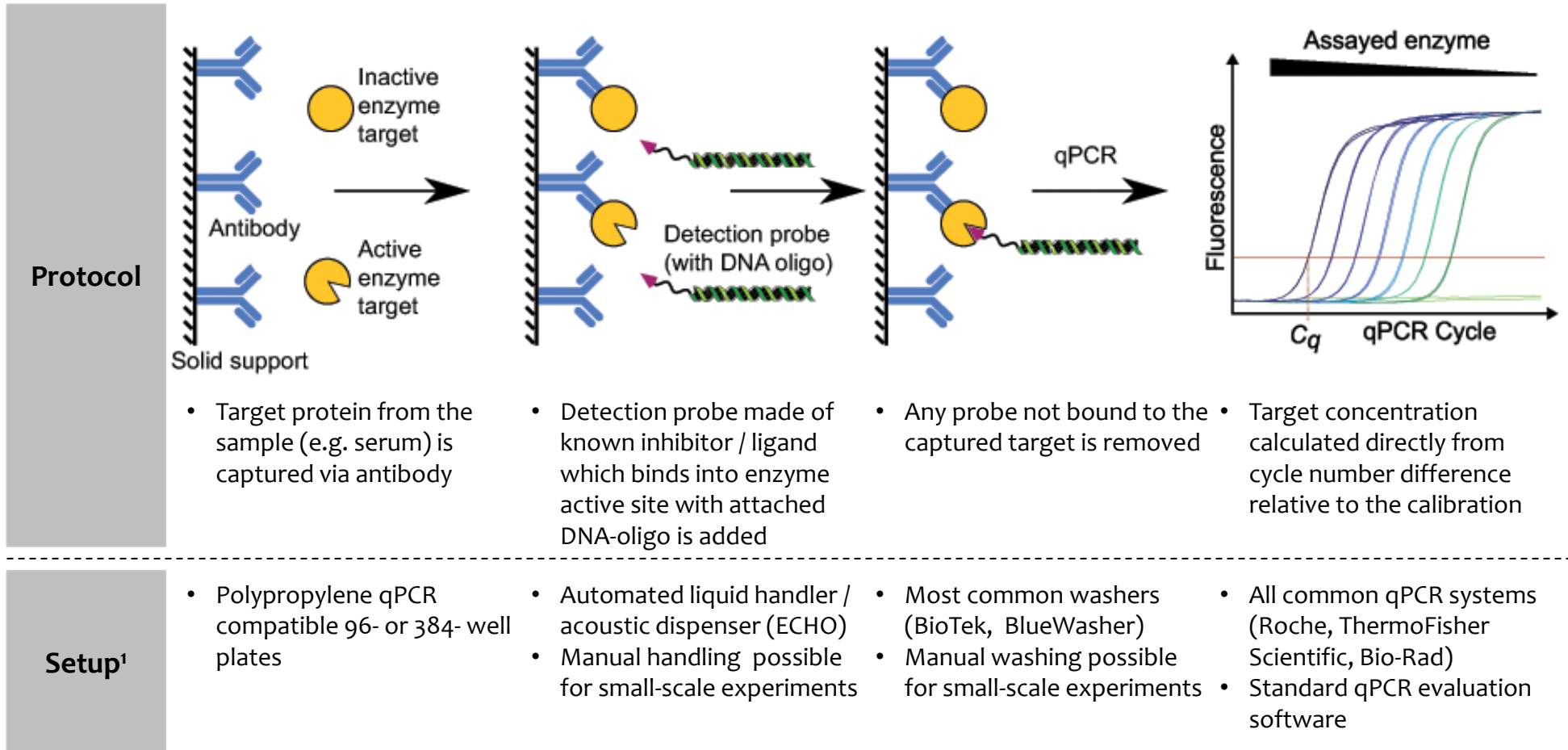
- **Ultra-sensitive** : can detect 100 attograms (10^{-16} g) or zeptomoles (10^{-21} mol) of target protein in only 1 μ l of clinical sample²
- **Selective**: combination of antibody and probe makes it highly selective for the target, detects only proteins with intact active site
- **Broad dynamic range**: precise quantification of concentration over 7-log range
- **Robust**: can be used in range of biological samples, no interfering antibodies, lack of non-specific binding

Potential customers

- **Clinical laboratories**: *in vitro* diagnostics – detection of disease-relevant markers
- **R&D laboratories**: sensitive quantification of hard-to-detect targets in experimental samples

1. Assuming at least one small-molecule inhibitor (even non-specific) exists and have suitable chemistry for modification, one antibody to complement the assay is also needed.
2. Up to 4 orders of magnitude more sensitive than Western blot, ELISA or immuno-PCR

Experimental protocol: 4 steps easy to implement in most laboratories



1. No temperature sensitive incubations. Flexibility in incubation times. Suitable for automation.

DIANA advantages: superior to standard ELISA or immuno-PCR assays

DIANA assay unique properties ...

- Ultra-high **sensitivity** and very broad **dynamic range**
- **Highly selective** for the target (validation by titration with free inhibitor possible)
- **Minimal** sample and probe **consumption**
- **Robust** – capable to work with range of biological samples (incl. blood serum)
- Lack of antibody interference and non-specific binding – very **low background**
- DNA Probe superior to antibody of similar affinity
- Easy protocol **suitable for detection kit format** (manual or automated protocol)
- High level of reproducibility, **suitable for CE IVD**

... providing clear advantage over other diagnostics assays

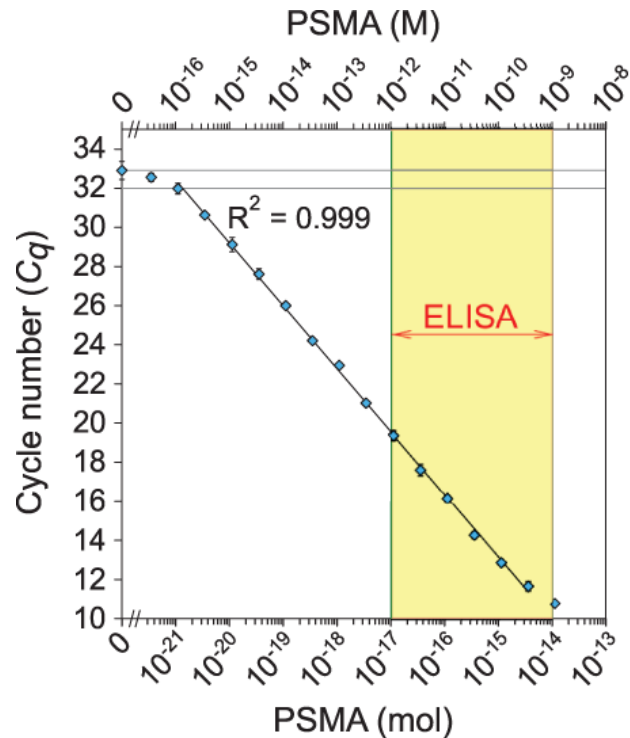
	DIANA	ELISA	Immuno-PCR
Biological matrices	✓	✓	✓
No interfering antibodies	✓	x	x
Low background	✓	x	x
Detects only active enzyme	✓	x	x
Sample requirement	≤ 1 µl	100 µl	≤ 1 µl
Dynamic range	7-logs	3-logs	5-7-logs
Sensitivity (mol) ¹	1 x 10 ⁻²⁰	1 x 10 ⁻¹⁷	1 x 10 ⁻¹⁹

1. Sensitivities in biological matrices are listed. The value for each target depends also on affinity of the probe / antibody. However small-molecule DIANA probes have superior performance when compared to antibodies of similar affinity (ELISA, immuno-PCR)

PSMA detection: validated DIANA assay with ultra-high sensitivity and broad dynamic range

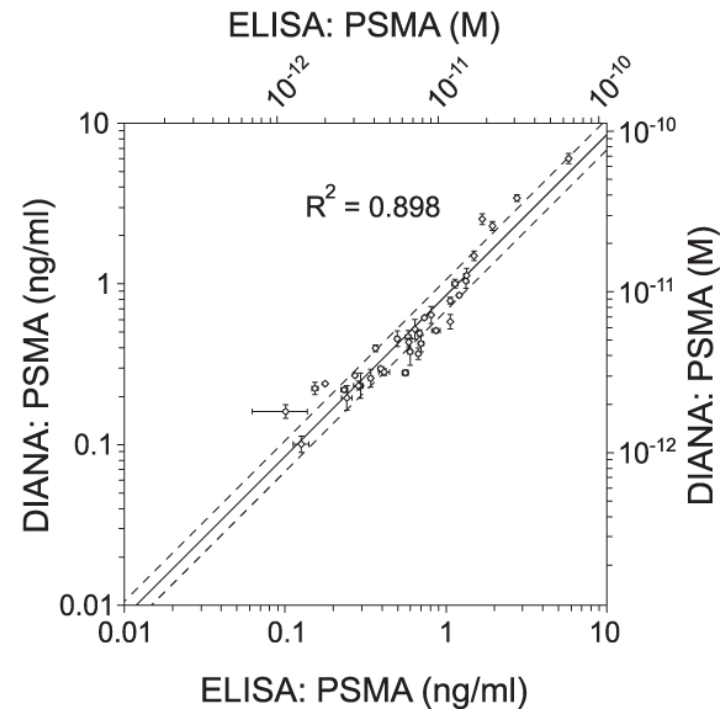
Sensitive detection ...

- Limit of detection of 100 ag of recombinant protein (1×10^{-21} moles) or ~ 0.001 ng/ml PSMA in a $1 \mu\text{l}$ human serum sample (about 4 orders of magnitude more sensitive than our ELISA assay)



... validated using ELISA assay

- High correlation between PSMA quantification using DIANA and our ELISA assay (no commercial ELISA available) demonstrated

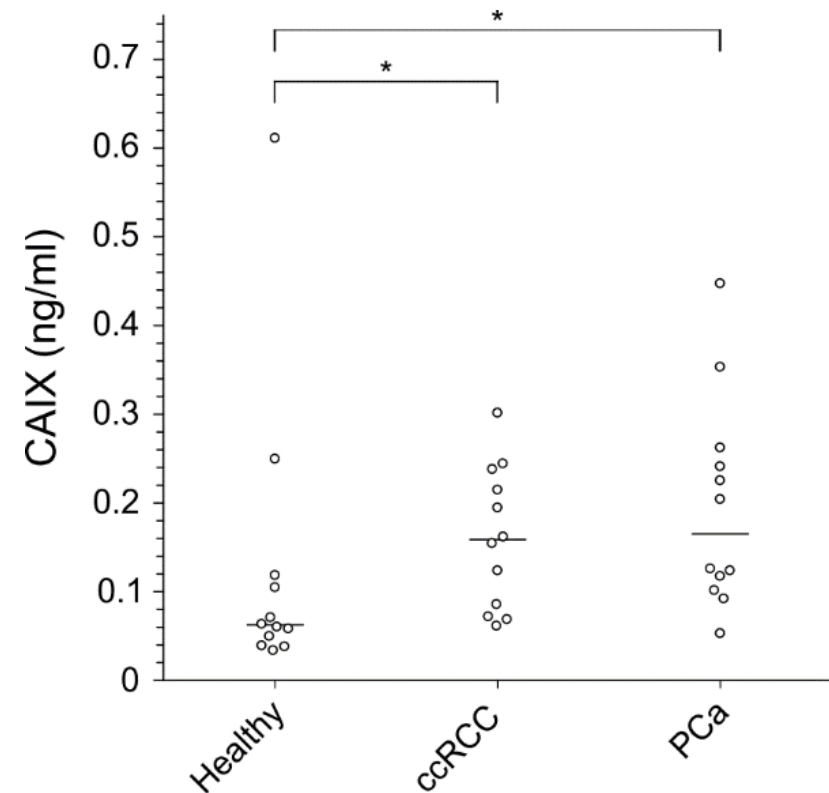


DIANA validated on clinical samples: Carbonic anhydrase 9 indicated as potential cancer marker

DIANA used to measure CA-IX levels in blood

- Analyzed **blood serum samples** drawn from patients with histologically proven **prostate carcinoma (PCa)**, **clear cell renal carcinoma (ccRCC)** or healthy volunteers
- DIANA assay used to **quantify CA-IX and PSMA** levels - successful quantification **using only 1 μ l of blood sample**
- While there was no correlation found for PSMA, **CA-IX levels were significantly upregulated in samples from cancer patients** (compared to healthy volunteers)

CA-IX found upregulated in cancer samples



Catalogue of targets: expanding range of assays for medically relevant targets

	Target	Medical relevance	Development status
Existing detection assays	Prostate specific membrane antigen (PSMA)	<ul style="list-style-type: none"> • Oncology (Prostate cancer) • Inflammation (IBD) • Neurology (CNS) 	<ul style="list-style-type: none"> • Validated detection from blood serum (possible in 10nl), urine, cells and tissues
	Carbonic anhydrase 9 (CA-IX)	<ul style="list-style-type: none"> • Oncology 	<ul style="list-style-type: none"> • Validated detection from blood serum (possible in 1µl), cells and tissues • Serum levels demonstrated to be upregulated in cancer patients
	Fibroblast activating protein (FAP)	<ul style="list-style-type: none"> • Oncology • Metabolic disorders 	<ul style="list-style-type: none"> • Validated detection from blood serum (possible in 10nl)
On demand targets	<ul style="list-style-type: none"> • Assay can be promptly developed on demand to majority of relevant protein targets¹ • CE IVD certification possible to allow use in clinical diagnostics 		

1. Assuming at least one small-molecule inhibitor (even non-specific) exists and have suitable chemistry for modification, one antibody to complement the assay is also needed.

Our proposition: looking for partnerships to introduce DIANA to *in vitro* diagnostics market

Our proposition

Potential partners

Assay development and application

- We can **develop DIANA based detection assays for any new target** of partner's interest
- We **support implementation** of DIANA detection assay **at partner's site** and its adjustment to specific project needs
- We **synthesize the detection probe** and provide other reagents required

- Academic research groups
- Clinical diagnostic facilities

Distribution of detection kits

- We **co-develop ready-made detection kits** and optimized instrumental setup – *see details in Appendix*
- We collaborate on **studies required for CE IVD** for relevant targets
- We **distribute the kits via partner's product catalogue** to research and diagnostic laboratories

- *In vitro* diagnostics assay manufacturer
- Clinical diagnostic facilities
- Lab technology / chemical distributor

Contacts for enquiries & other resources

Contacts

Václav Navrátil – Head of R&D

- vaclav.navratil@uochb.cas.cz
- Methodology questions, assay development requirements

Jaromír Zahrádka & Martin Dienstbier – TTO

- zahradka@iocb-tto.cz, dienstbier@iocb-tto.cz
- Business questions, collaboration deals

Other contacts

- Martin Fusek, Jan Konvalinka, Pavel Šácha

Further resources

Research paper

- Navratil et al. (2016): DIANA for sensitive and selective enzyme detection and inhibitor screening. *Nucleic Acids Research*
- Screening methodology & Clinical diagnostics papers. *In preparation*

Introductory video

- www.youtube.com/watch?v=hrh82eulCfU

Institute of Organic Chemistry and Biochemistry, Academy of Science.
Flemingovo namesti 2, 16610, Prague 6, Czech republic



ÚOCHB ^{AV}_{ČR}
IOCB PRAGUE



IOCB TTO
www.iocb-tto.cz

Appendix: DIANA-based detection kits can be developed for range of targets

Application

- **Ultrasensitive quantification of target proteins** in biological matrixes
- **IVD certification** to enable use in clinical diagnostics

Kit content

- **Antibody coated 96- or 384-well plate**
- Target specific **detection probe**
- Target protein **standard**
- Reconstitution buffers (for protein and probe)
- 10x assay buffers (protein and probe dilution, wash)
- *Optional:* qPCR reagents

Protocol and equipment required

- **Simple 4-step protocol:** sample incubation, (wash), incubation with probe, washing, qPCR detection (about 3 hours, all incubations at room temperature)
- Requires **standard lab equipment** and **multiwell-plate qPCR system**
- Both **manual** and **automated** settings possible

