DIANA
assay for *in vitro* diagnostics

Overview and collaboration proposal

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

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DNA-linked Inhibitor ANtibody Assay

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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)

DNA-linked Inhibitor Antibody Assay (DIANA) for sensitive and selective enzyme detection and inhibitor screening. Navrátil *et al*. *Nucleic Acids Res* (**2017**); DOI: 10.1093/nar/gkw853

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

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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 develop[1]
Key advantages	• **Ultra-sensitive** : can detect 100 attograms (10^{-16} g) or zeptomoles (10^{-21} mol) of target protein in only 1 µl of clinical sample[2] • **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

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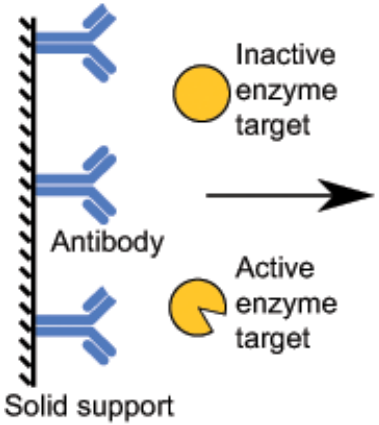
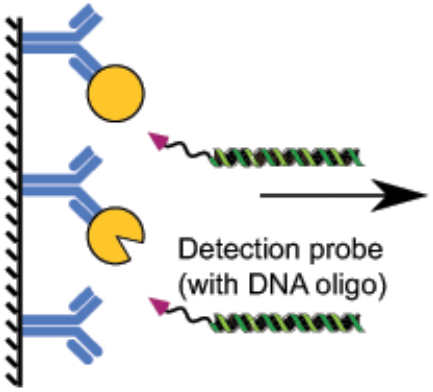
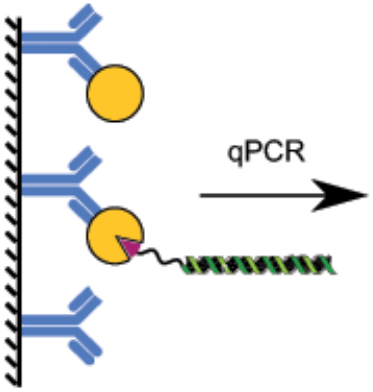
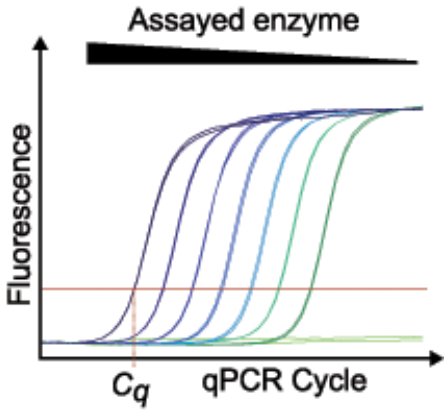
	1 Capturing target from the sample[1]	2 Incubation with detection probe[1]	3 Washing[1]	4 qPCR detection
Protocol				
	• Target protein from the sample (e.g. serum) is captured via antibody	• Detection probe made of known inhibitor / ligand which binds into enzyme active site with attached DNA-oligo is added	• Any probe not bound to the captured target is removed	• Target concentration calculated directly from cycle number difference relative to the calibration
Setup[1]	• Polypropylene qPCR compatible 96- or 384- well plates	• Automated liquid handler / acoustic dispenser (ECHO) • Manual handling possible for small-scale experiments	• Most common washers (BioTek, BlueWasher) • Manual washing possible for small-scale experiments	• All common qPCR systems (Roche, ThermoFisher Scientific, Bio-Rad) • Standard qPCR evaluation software

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

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

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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]	1×10^{-20}	1×10^{-17}	1×10^{-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 ulra-high sensitivity and broad dynamic range

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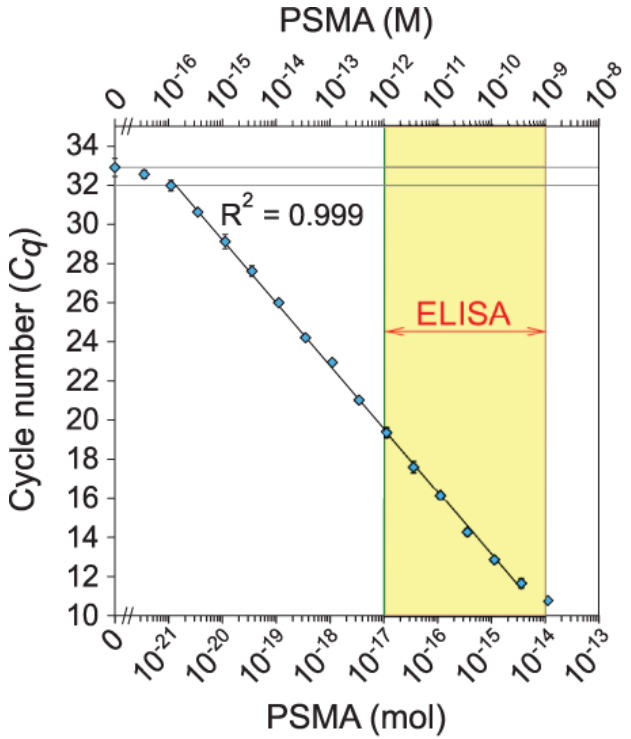
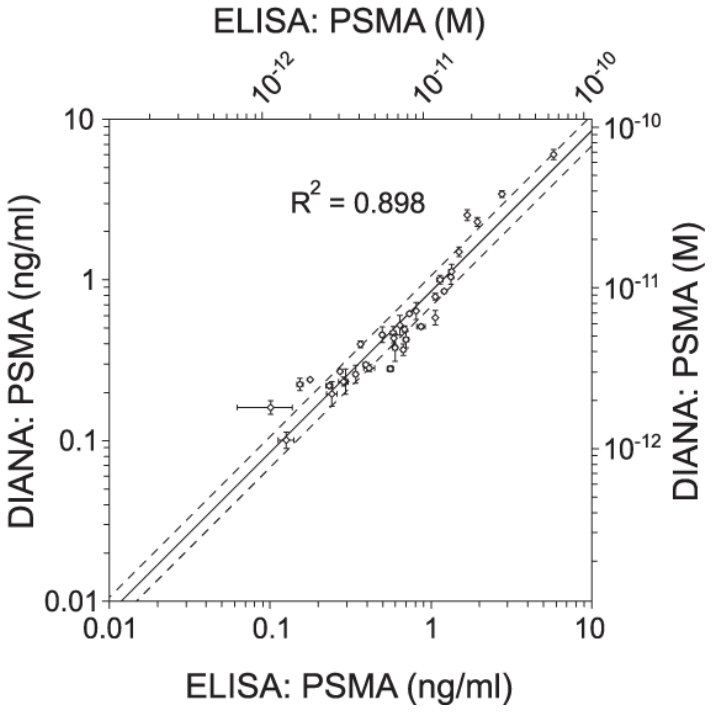
Sensitive detection …

- Limit of detection of 100 ag of recombinant protein (1×10^{-21} moles) or ~ 0.001 ng/ml PSMA in a 1 µl 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

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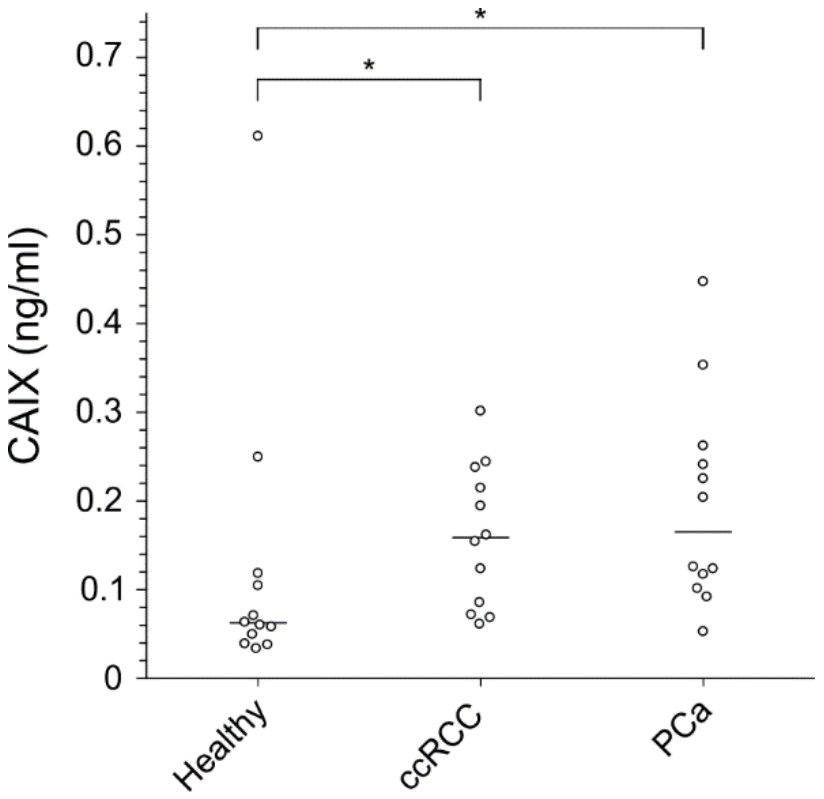
DIANA used to measure CA-IX levels in blood

- 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 µl 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

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	Target	Medical relevance	Development status
Existing detection assays	**Prostate specific membrane antigen (PSMA)**	• Oncology (Prostate cancer) • Inflammation (IBD) • Neurology (CNS)	• Validated detection from blood serum (possible in 10nl), urine, cells and tissues
	Carbonic anhydrase 9 (CA-IX)	• Oncology	• Validated detection from blood serum (possible in 1µl), cells and tissues • Serum levels demonstrated to be upregulated in cancer patients
	Fibroblast activating protein (FAP)	• Oncology • Metabolic disorders	• Validated detection from blood serum (possible in 10nl)
On demand targets	• **Assay can be promptly developed on demand to majority of relevant protein targets[1]** • **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

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	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

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Contacts

Václav Navrátil – Head of R&D

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

Jaromír Zahrádka & Martin Dienstbier – TTO

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- 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=hrh82euICfU

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

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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