

Application of biopolymers in immunodetection

- Signal amplification of immunodetection using biopolymers
- High resolution protein localization using biopolymers (PARAPLAY)
- *In situ* detection of NAD⁺ by conversion to biopolymers

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GUIDING GOOD IDEAS

Content

Technology Principle

Application of biopolymers in immunodetection

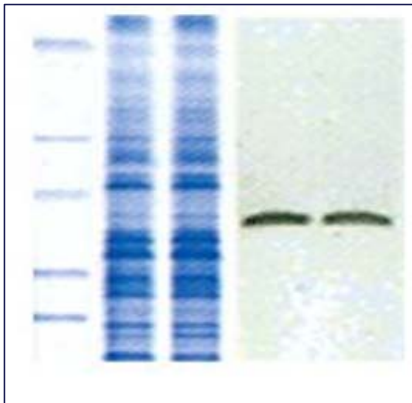
Technology Application

- Signal amplification of immunodetection using biopolymers 
- High resolution protein localization using biopolymers (PARAPLAY) 
- *In situ detection of NAD⁺ by conversion to biopolymers* 

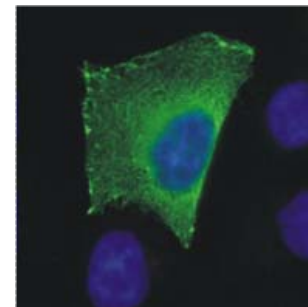
Immunodetection

- Protein detection is essential in research and biomedical diagnostics
- Immunodetection methods provide specificity for the target molecule
- Sensitivity of immunodetection methods is still perfectible

SDS PAGE Western Blot

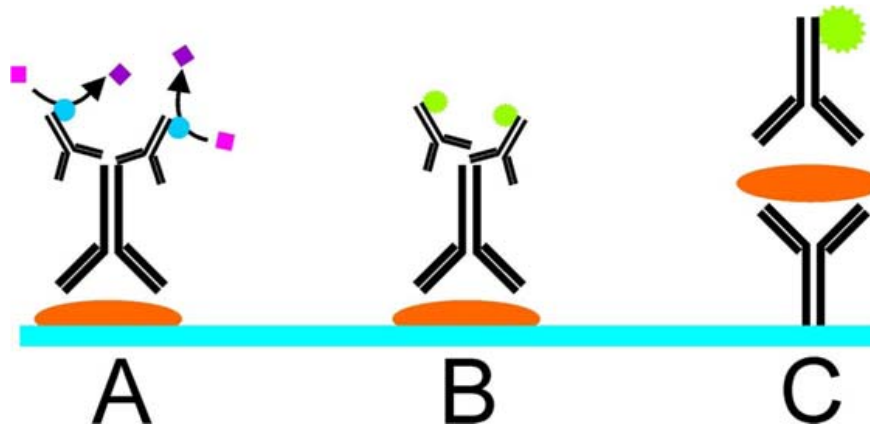


Immunocytochemistry



Principles of Immunodetection

The molecule of interest is detected by a primary antibody, which in turn is recognized by a secondary antibody carrying a reporter signal.



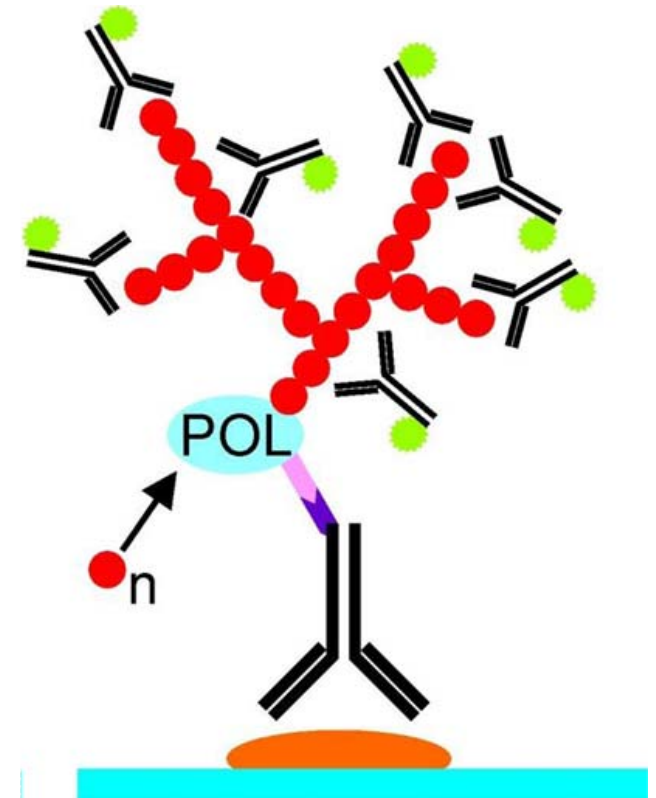
A: secondary antibody-enzyme conjugate, generating a coloured product or light

B: secondary antibody-fluorophor conjugate, fluorescent reporter signal

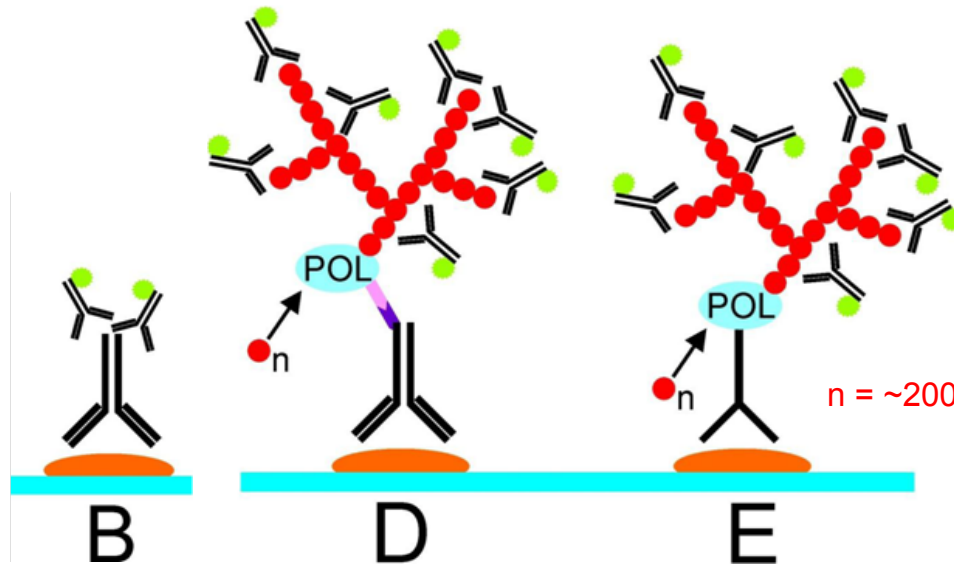
C: ELISA principle, immobilized antibody captures the antigen, which is recognized by a antibody fluorophor conjugate

Improvement of Immunodetection Sensitivity

- The target molecule is decorated with an immunodetectable biopolymer
- The biopolymer provides numerous binding sites for a secondary antibody
- The biopolymer is detected by an antibody carrying the reporter signal
- The multiplication of epitopes, i.e. binding sites for the antibody carrying the reporter signal, results in signal amplification



Improvement of Immunodetection Sensitivity



The same amount of antigen is able to bind a much higher number of antibodies carrying the reporter signal when a polymer decoration step is included.

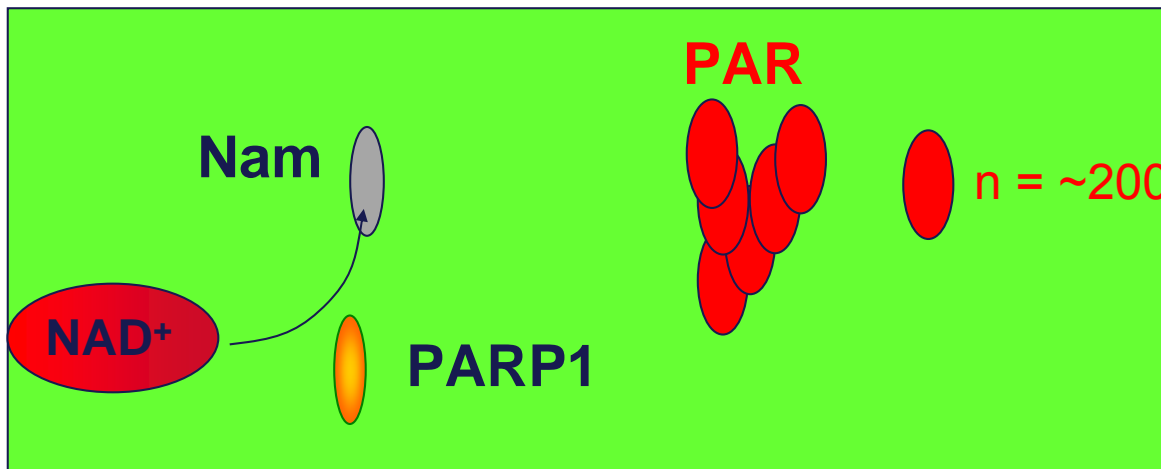
B: Conventional Immunodetection

D: Decoration with a biopolymer generated by a polymerase bound to the primary antibody, for example via a biotin streptavidin interaction, results in signal amplification

E: Immunodetection of a biopolymer generated by a polymerase as part of a chimeric fusion protein, consisting of the polymerase activity and a single polypeptide chain antibody (for example from llama)

Improvement of Immunodetection Sensitivity

The catalytic domain of PARP1 generates immunodetectable biopolymers from NAD⁺



A signal amplification of more than ~10fold is achievable

Comparison old and new

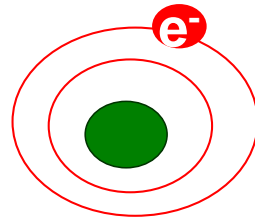
- Basic principles of immunodetection are applied
→ same laboratory procedures as for current immunodetection
- Similar possibilities of fluorophors and/or reporter signals
- Enhanced signal intensity
- Increased sensitivity through biopolymer-based amplification
- $> \sim 10x$ signal amplification is achieved

High resolution protein localization using biopolymers (PARAPLAY)

- Precise subcellular location of a protein is vital to understand its function
- Challenge of established technologies:
Discrimination between an external association to an organelle and luminal localization is achieved only with enormous experimental efforts

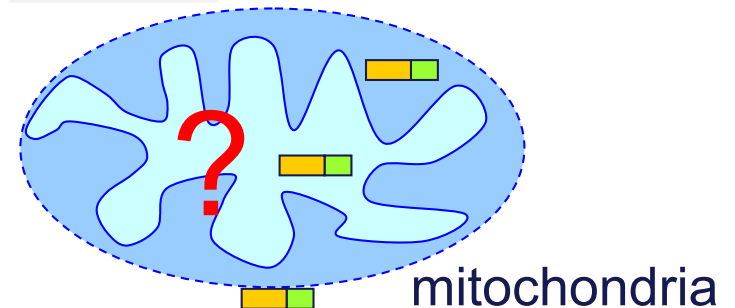
Methods to study subcellular distribution of proteins harbor obstacles

- Subcellular fractionation by differential centrifugation
 - false positive results (contamination)
 - false negative results (loss of material)
- Electron microscopy
 - High technical efforts
- Immunocytochemistry and fluorescence microscopy
 - Insufficient suborganellar resolution

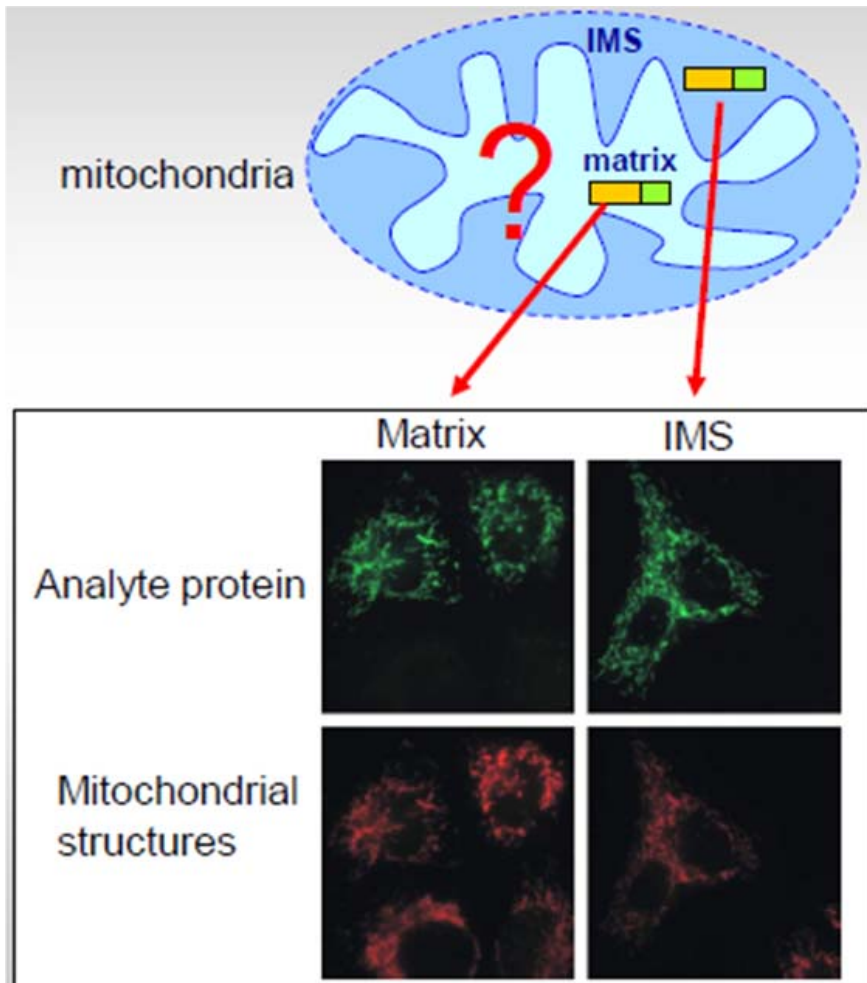


Prior art: immunocytochemistry and fluorescence microscopy

- Overexpression of the target protein in fusion with fluorescent proteins (e.g., GFP)
- Immunodetection of overexpressed proteins with an epitope-tag (FLAG, myc, etc.)
- Immunodetection of endogenous proteins
- **Drawback:** Only association with an organelle can be established, no suborganellar resolution



Current light microscopy techniques fail to distinguish between suborganelle locations

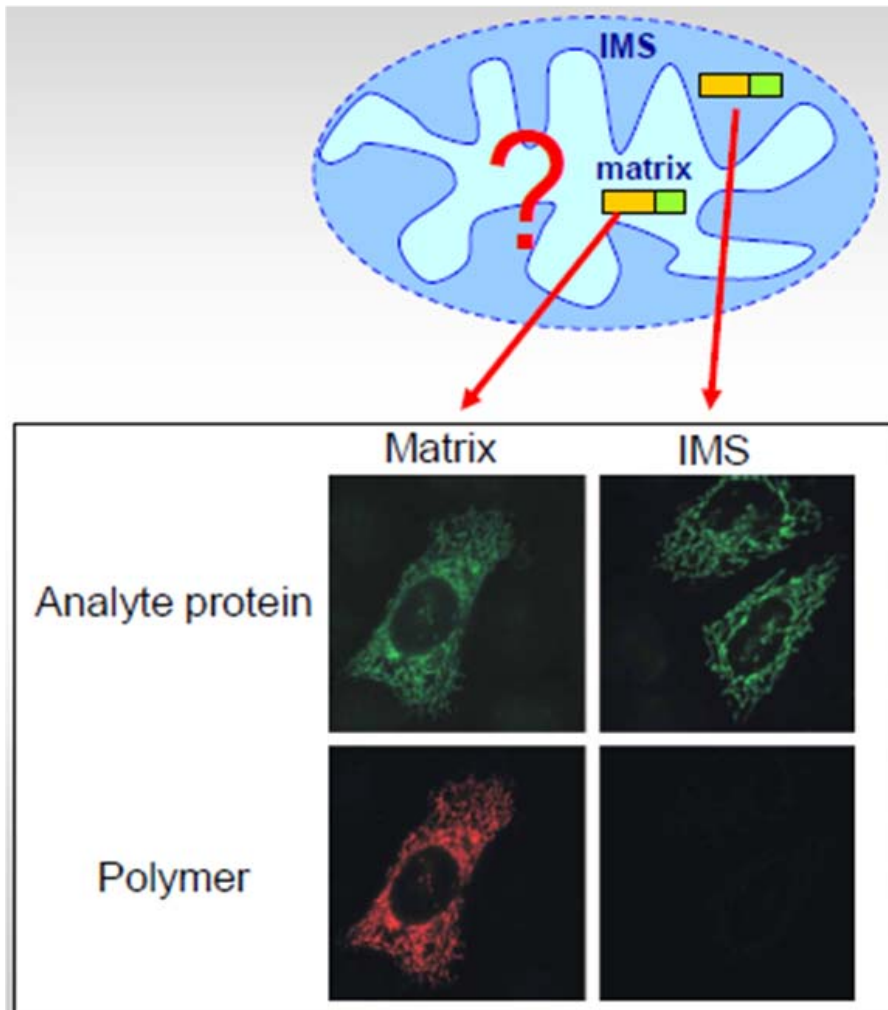


PROBLEM:

Both proteins apparently colocalize even though they reside in different compartments

adapted from Dölle *et al.*, 2010

Poly-ADP-Ribose Assisted Protein Localization Assay (PARAPLAY) resolves suborganellar distribution



Polymer formation is only achieved in the organellar lumen (matrix)

ADVANTAGE:

Different suborganellar locations can be distinguished

adapted from Dölle *et al.*, 2010

Advantages of PARAPLAY

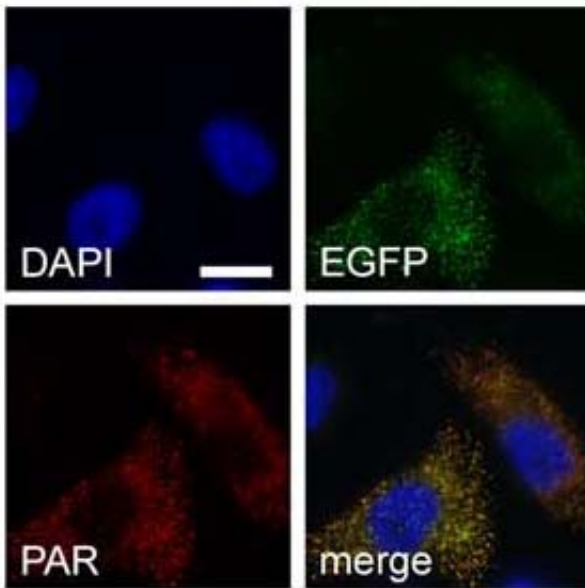
- Superior resolution compared to GFP fusion or conventional immunodetection
- Applicable for all light microscopy techniques
- Suborganellar protein localization is revealed
- No sample disruption necessary
- No additional equipment or skills required
- Based on well established molecular biological techniques

Kit content

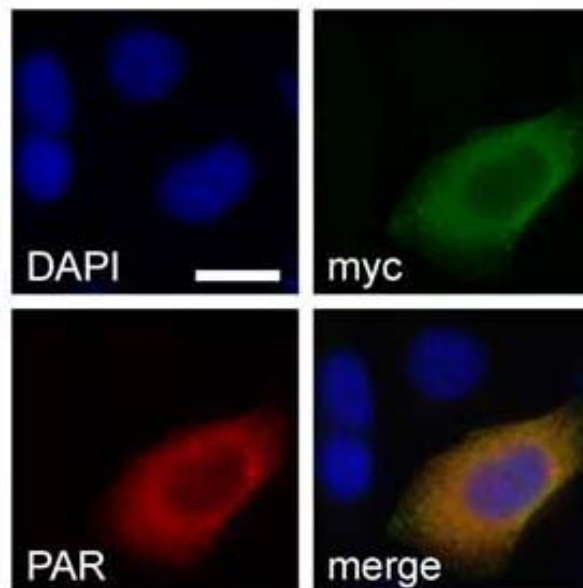
- Eukaryotic PARAPLAY expression vectors to express the protein of interest
- PARAPLAY antibodies
- Control expression vectors of organellar markers positive in PARAPLAY

General applicability of the PARAPLAY assay for cellular organelles

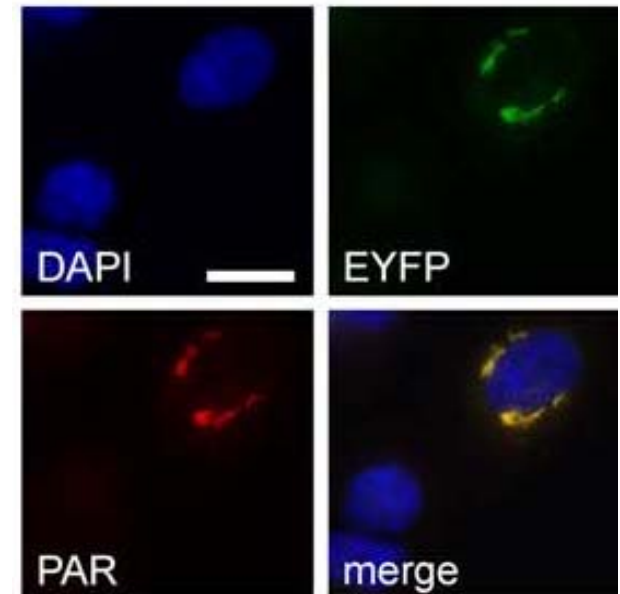
Peroxisomes



Endoplasmic reticulum



Golgi apparatus



Our Novel Technology

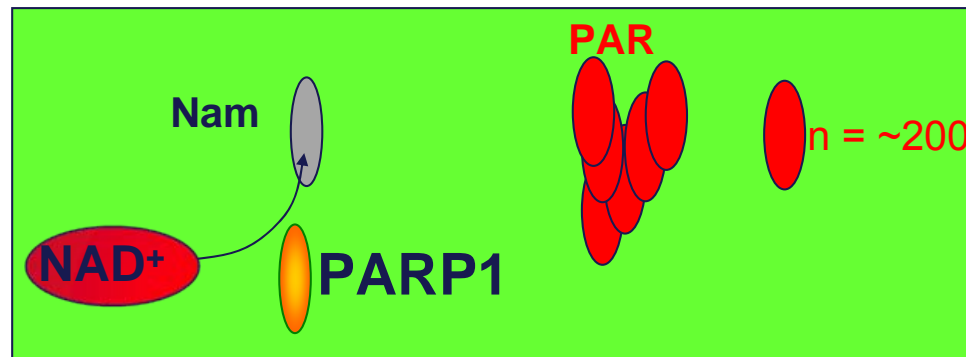
Basic principle

The protein of interest is fused to the catalytic domain of PARP1, which generates large homo-polymers (poly-ADP-ribose) from a small biomolecule (NAD⁺).

Fusion protein:



Polymer Generation:

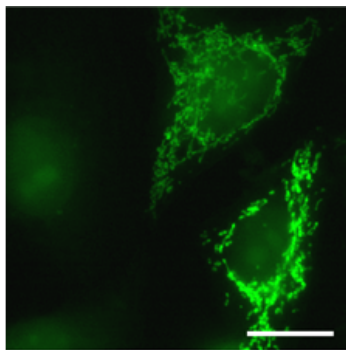


Antibodies specifically recognizing these polymers are used for highly efficient immunochemical detection.

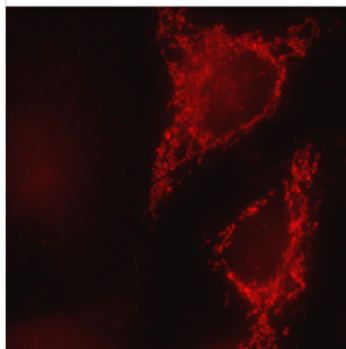
The fusion protein harbouring the catalytic domain of PARP1 targeted to the mitochondrial matrix generates polymers (PAR)

mitoPARP

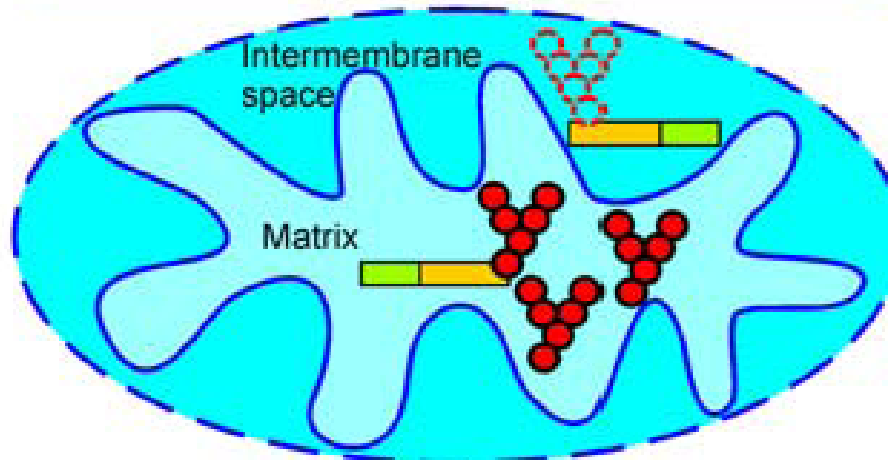
EGFP



PAR



PARP1cd fusion construct



Niere *et al.*, 2008
Dölle *et al.*, 2010

***In situ* detection of NAD⁺ by conversion to biopolymers**

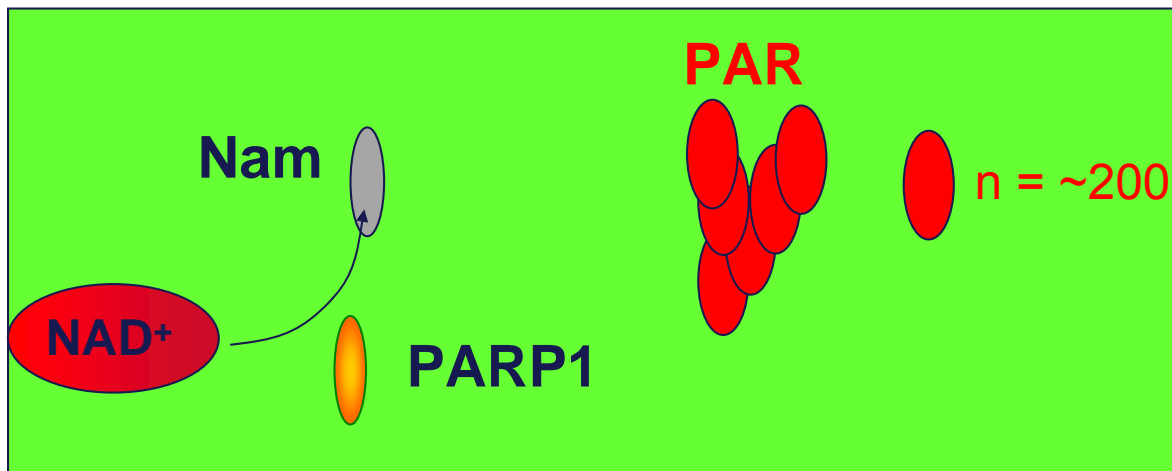
Nicotinamide adenine dinucleotide (NAD)

- Small essential molecule in all cells
 - Major electron carrier in metabolism
 - (Conversion $\text{NAD}^+ \leftrightarrow \text{NADH}$)
 - NAD^+ is substrate for signalling reactions controlling gene expression, lifespan, calcium flux, metabolism
- Determination of the NAD content of subcellular organelles and monitoring specific changes is important to understand these processes.

Prior art: Detection of NAD⁺

- Undetectable by immune assays and in intact cells
- Tissue or cell homogenization, acidic extraction
- Chromatographic separation and detection (HPLC, Mass spectrometry)
- **Disadvantages:**
 - Loss of material during preparation
 - change of redox state
 - no subcellular pools distinguishable

Our novel technology: Conversion of NAD⁺ to the immunodetectable biopolymer, PAR

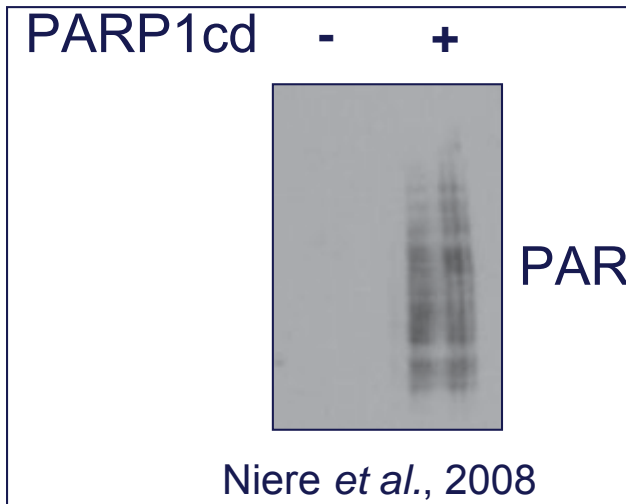


- The enzymatic activity of Poly-ADP-Ribose Polymerase 1 (PARP1) generates poly-ADP-ribose (PAR)
 - PAR is immunodetectable using PAR-specific antibodies
- Conversion of NAD⁺ to PAR makes it accessible for immunodetection

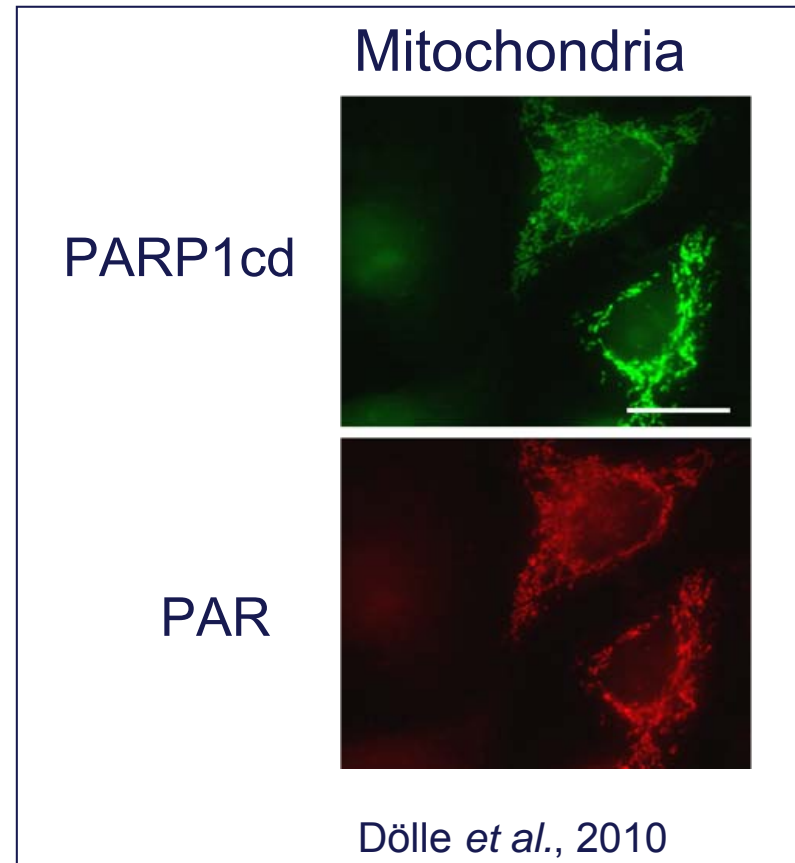
Advantages of the new approach

- *In situ detection of NAD⁺ in subcellular organelles*
- Monitoring changes in subcellular pools of NAD⁺ in response to external stimuli.
- Easy detection by **immunochemistry**.
- Signal reflects organellar NAD⁺ content.
- Based on **standard cell culture techniques**.
- No fractionation or extraction steps required
- Superior precision and resolution.

NAD⁺ detection within subcellular organelles

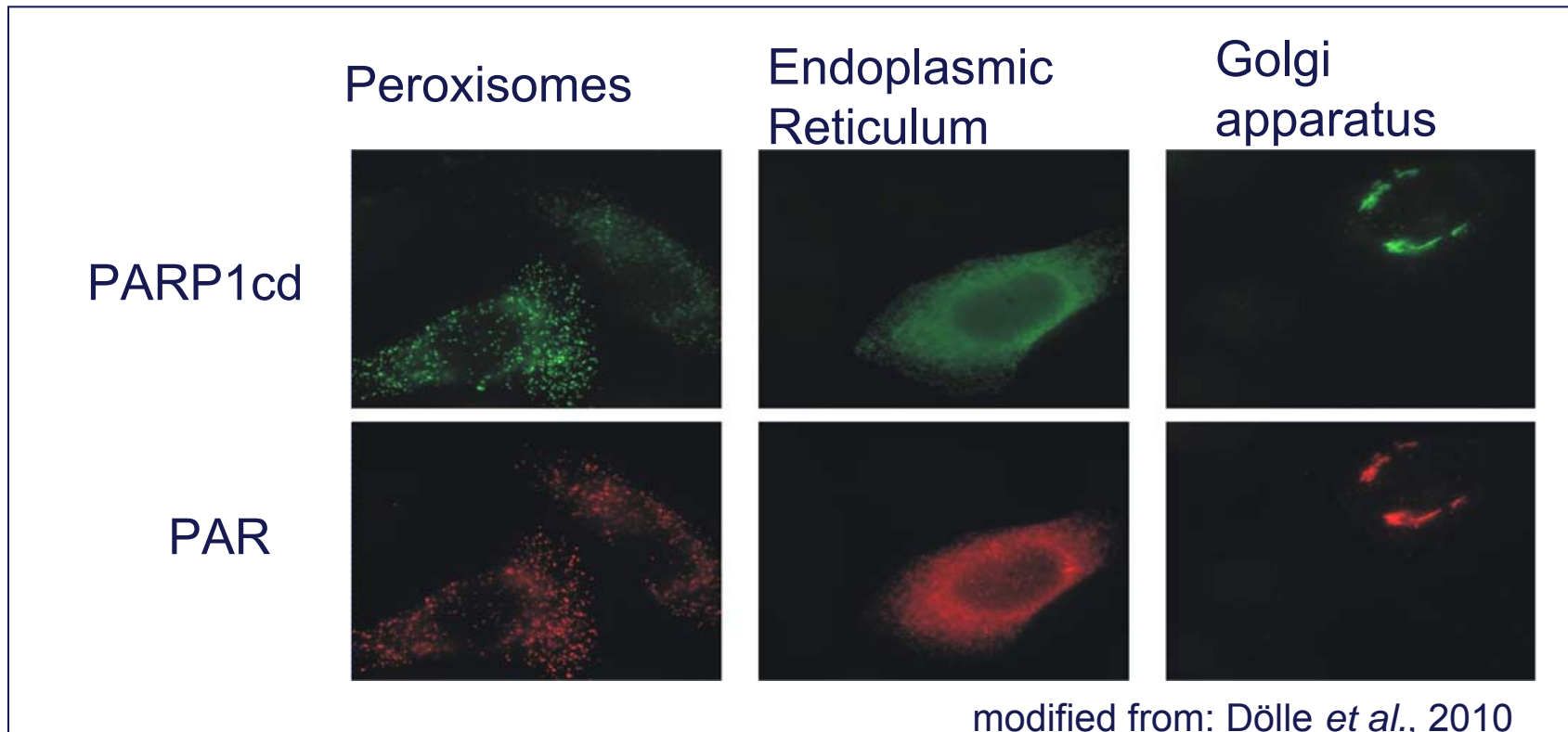


The polymerase generates immunodetectable PAR when targeted to an organelle



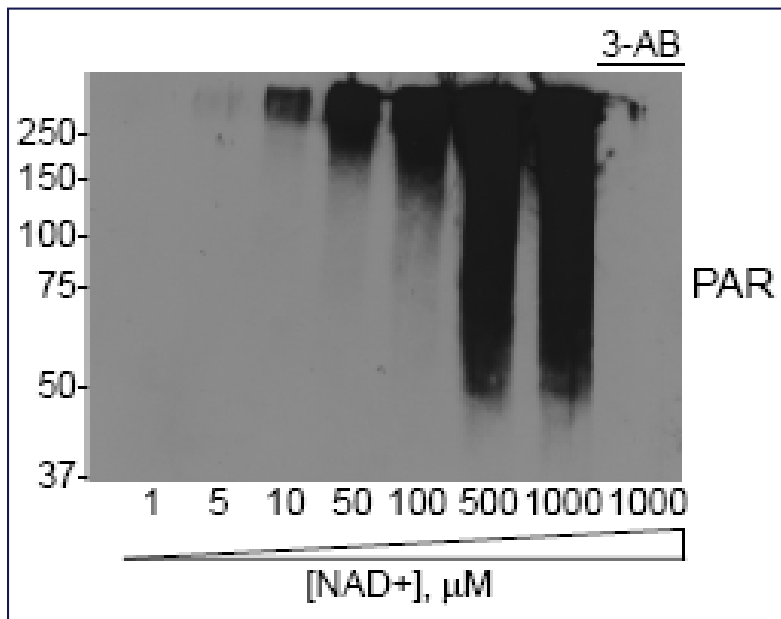
Organelle targeted PARP1cd only converts organelle specific NAD⁺ pool

NAD⁺ detection is possible in a variety of subcellular organelles

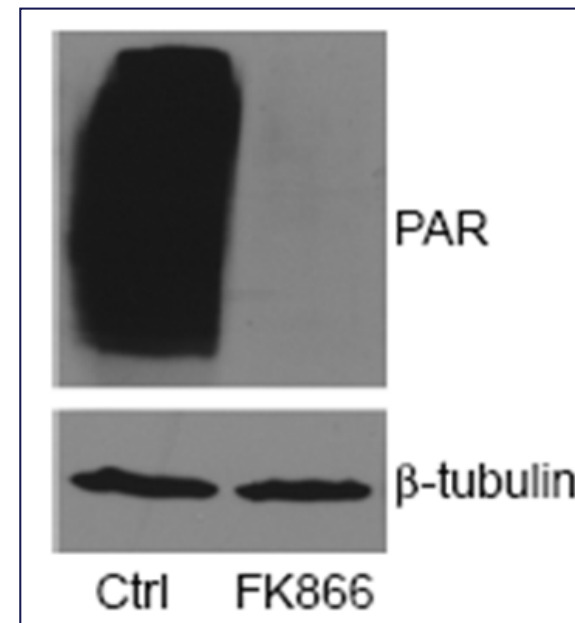


Targeted expression of PARP1cd generates PAR and monitors NAD⁺ content in several different organelles

NAD⁺ detection is sensitive to the NAD content



PAR formation by PARP1cd depends on NAD⁺ concentration



PAR signal is abolished if NAD⁺ biosynthesis is inhibited (FK866)

Even though whole cell lysates are examined, only the NAD pool of organelles where PARP was expressed is revealed by PAR

Kit content

- Vectors for expression of organellar targeted polymerase
- Antibodies for polymer detection
- Expression vectors are based on commercial vector systems, but can be adapted/ modified to suit other systems



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