

**The Challenge of Protein Biomarkers:  
Finding the needle in the haystack and validating it;  
Annexin A3 in prostate cancer**

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**38th Meeting of The International Society of Oncology and  
Biomarkers**

**Munich, September 3-8, 2010**

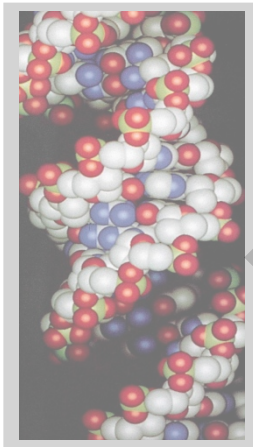
Complexity of proteomes – proteins are first targets of external stimuli:  
Numbers of molecules, time scales and dynamic range

Minutes, hours,  
years

Seconds (?)  
minutes, hours,

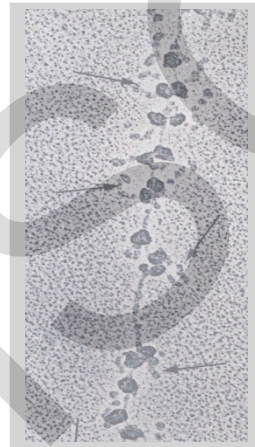
$\mu$ -m-seconds (!)  
minutes, hours, years (!)

Genes (DNA)



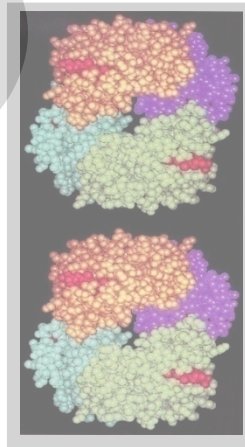
<23,000 in homo s.?

RNA



1 Mio. ?

Proteins



10 Mio. ?

Posttranslational modifications!

- Dynamic protein modification patterns
- Small molecule and protein/protein interactions

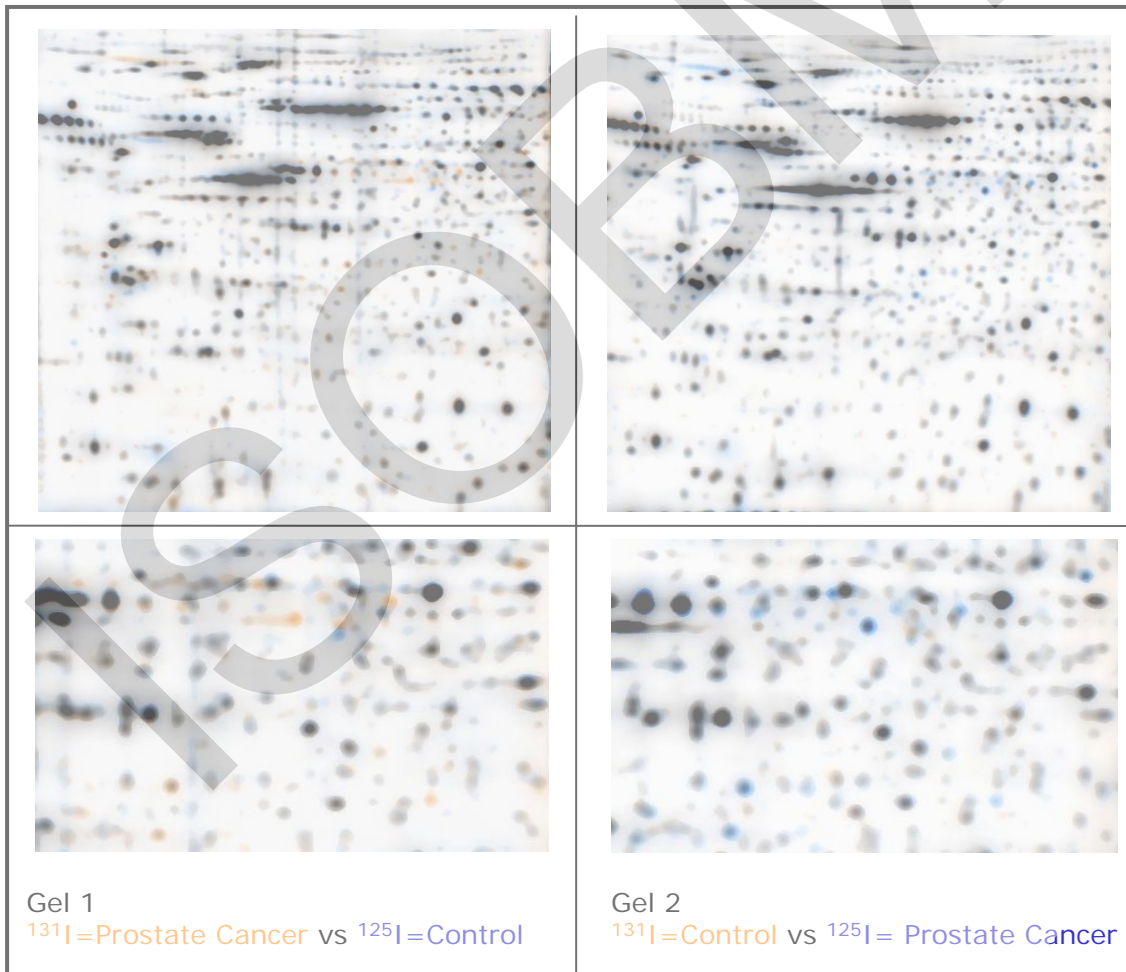
Schrattenholz & Soskic (2008) CMC 15, 1520  
Schrattenholz et al., (2010) Meth.Mol. Biol, in press

Protein biomarkers

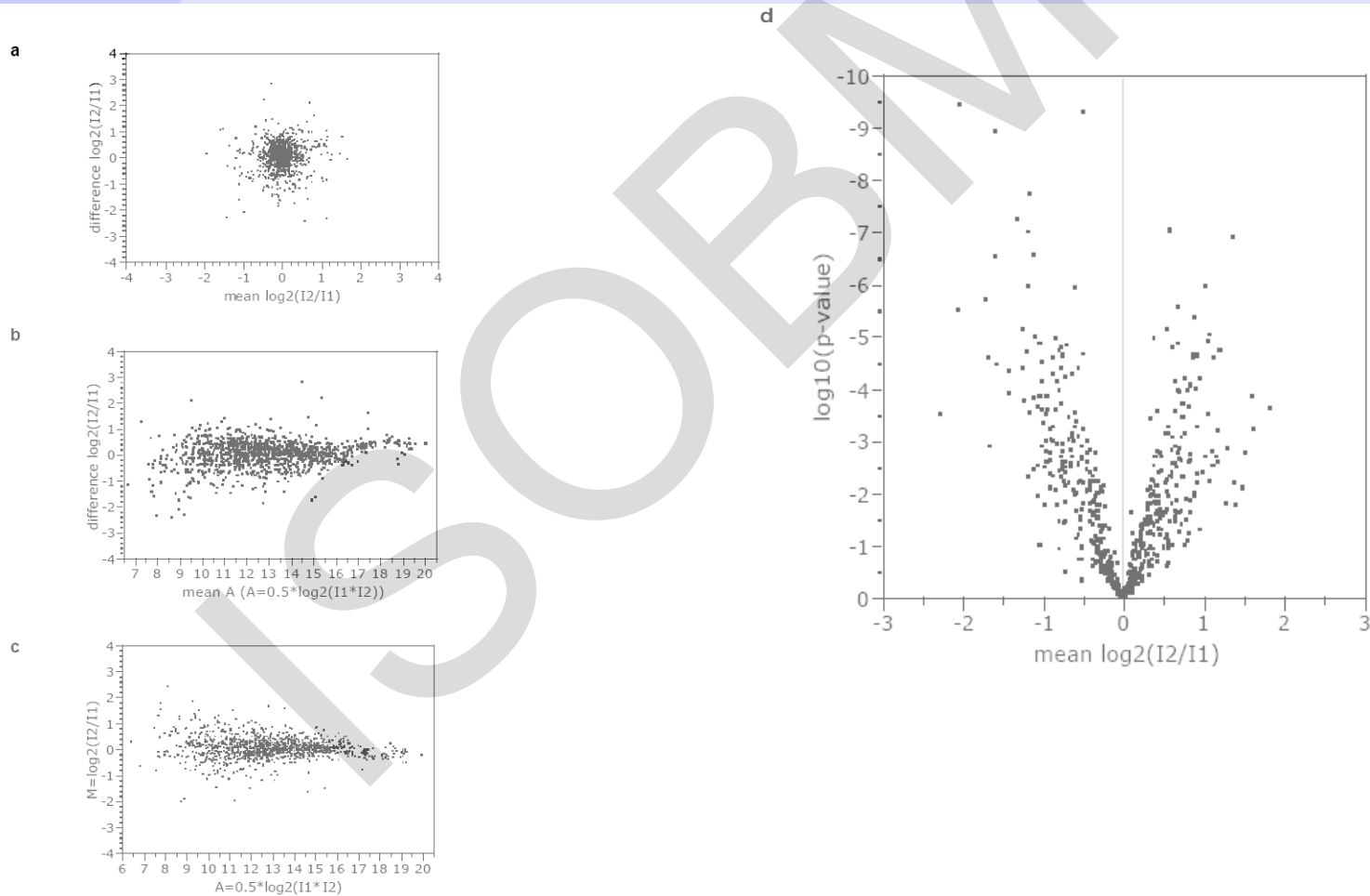
Differential proteomic approach

ISOBM

Quantitative control of differential protein patterns –  
Resolution, normalized primary data and statistical treatments!

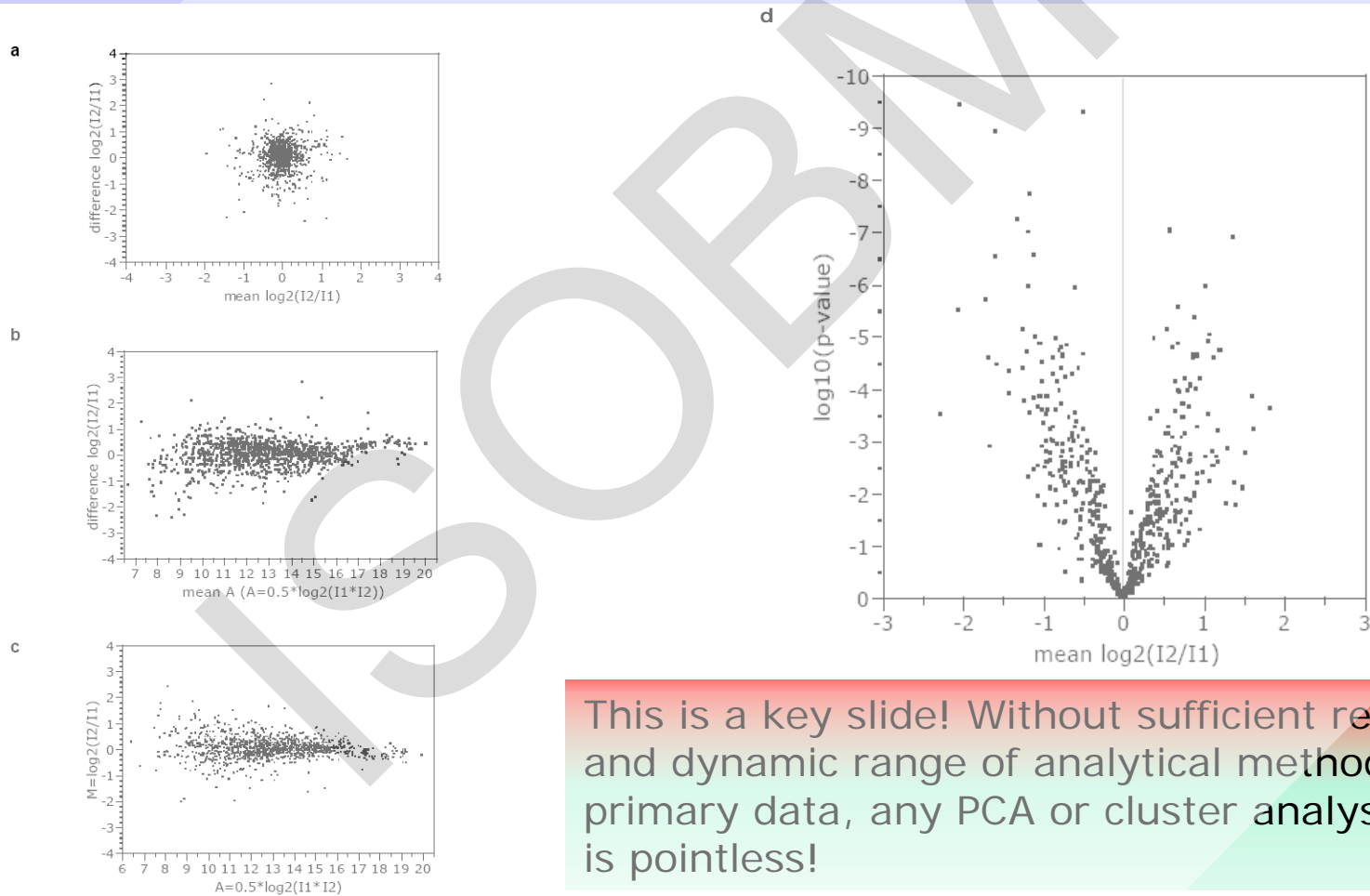


# Quantities, dynamic range of detection and statistics for primary data



Schrattenholz & Groebe (2007) *Electrophoresis*, 28(12), 1970  
 Schrattenholz et al. (2010) *Meth. Molec. Biol.* 2010

## Quantities, dynamic range of detection and statistics for primary data

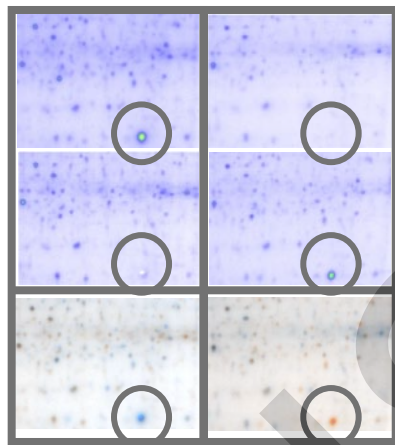


This is a key slide! Without sufficient resolution and dynamic range of analytical method for primary data, any PCA or cluster analysis is pointless!

Schrattenholz et al. (2010) Meth. Molec. Biol., in press

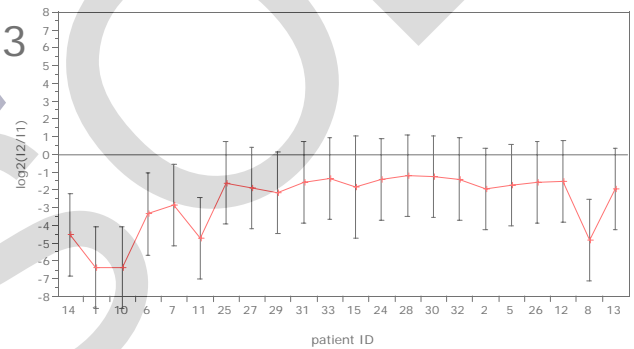
# The start: Proteomics – cancer versus benign

**Inverse replicate for each patient.  
2D-PAGE pH 4-7**

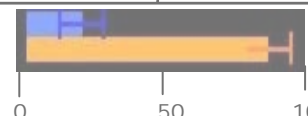


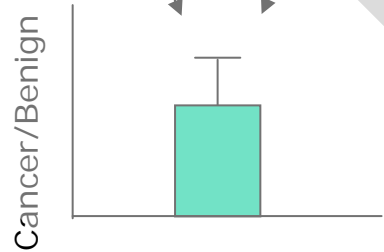
x 33

**Average spot differentials with error and significance for all 33 patients**



**Mean average differentials with error of means for all patients**

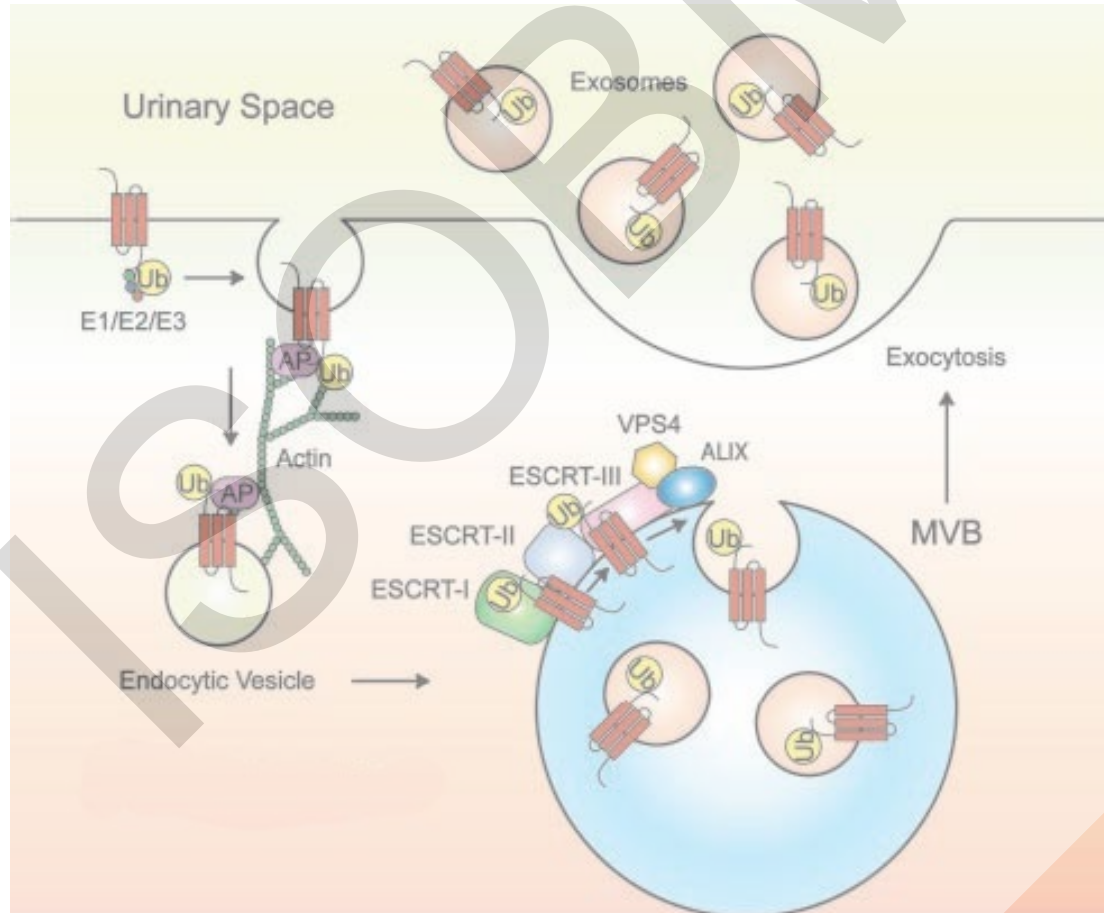
ANXA3		
Protein Name	PMF score	P-value
Annexin A3	160	>0.00001
		Benign Cancer



**We found ANXA3 average 4-fold more abundant in tumor biopsies.**

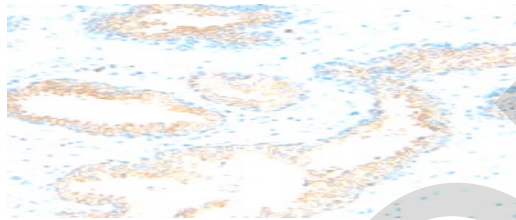
Wozny et al. (2007) Proteomics 7, 313

The initial observation- ANXA3 in urine: identification and proteomic profiling of exosomes in human urine

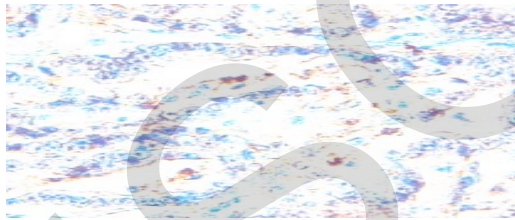
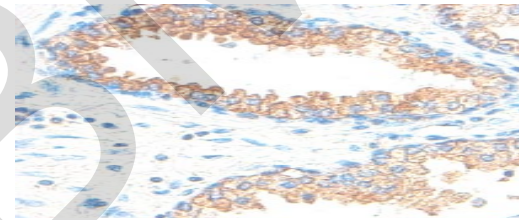


Pisitkun et al. 2001

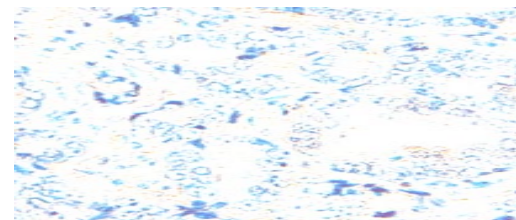
The evidence: ANXA3 localization and abundance is cancer dependent



**Strong expression of ANXA3 in prostatic epithelium of typical BPH**

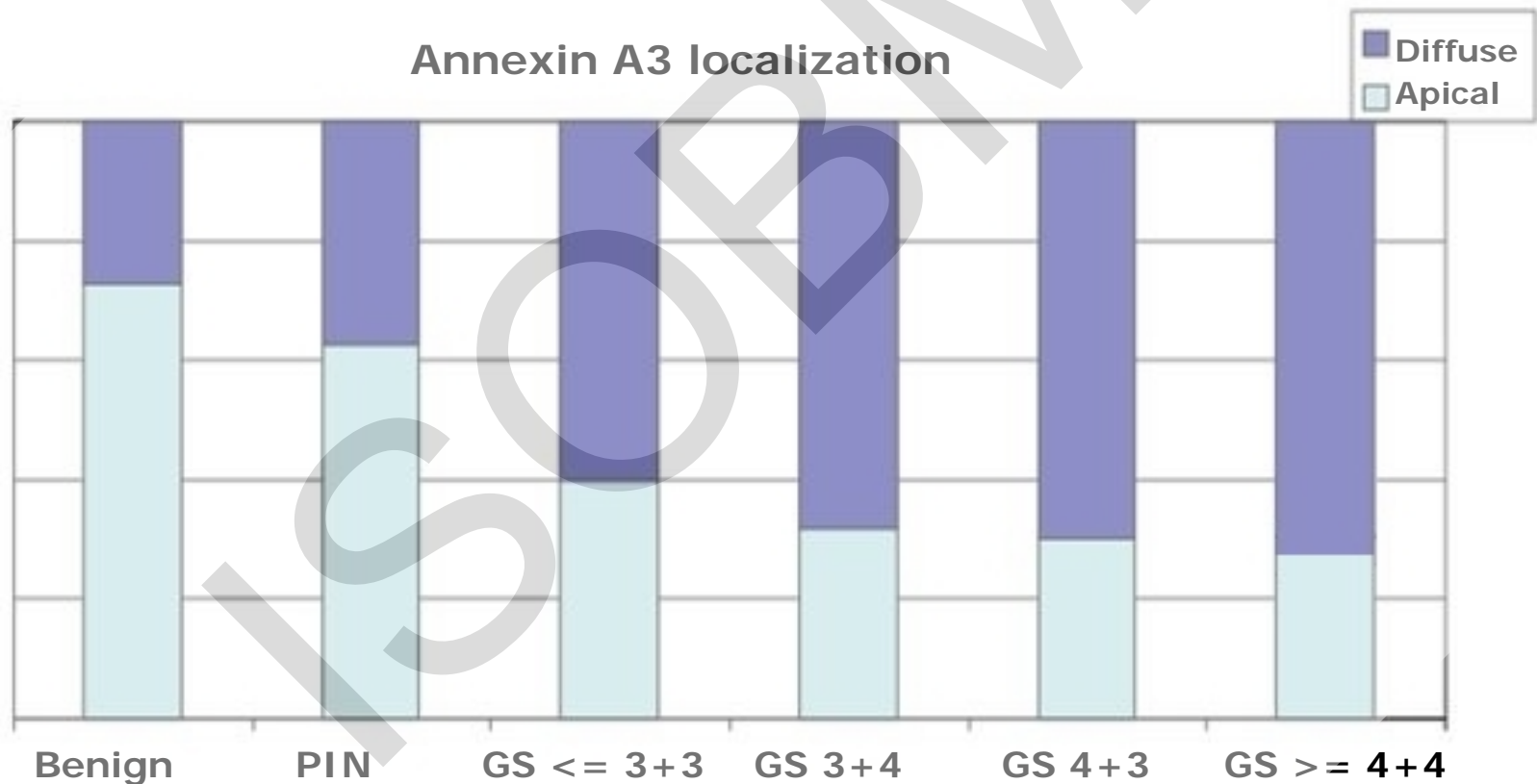


**Weaker expression of ANXA3 in prostatic epithelium of typical Pca**



**Weak expression of ANXA3 in advanced Pca**

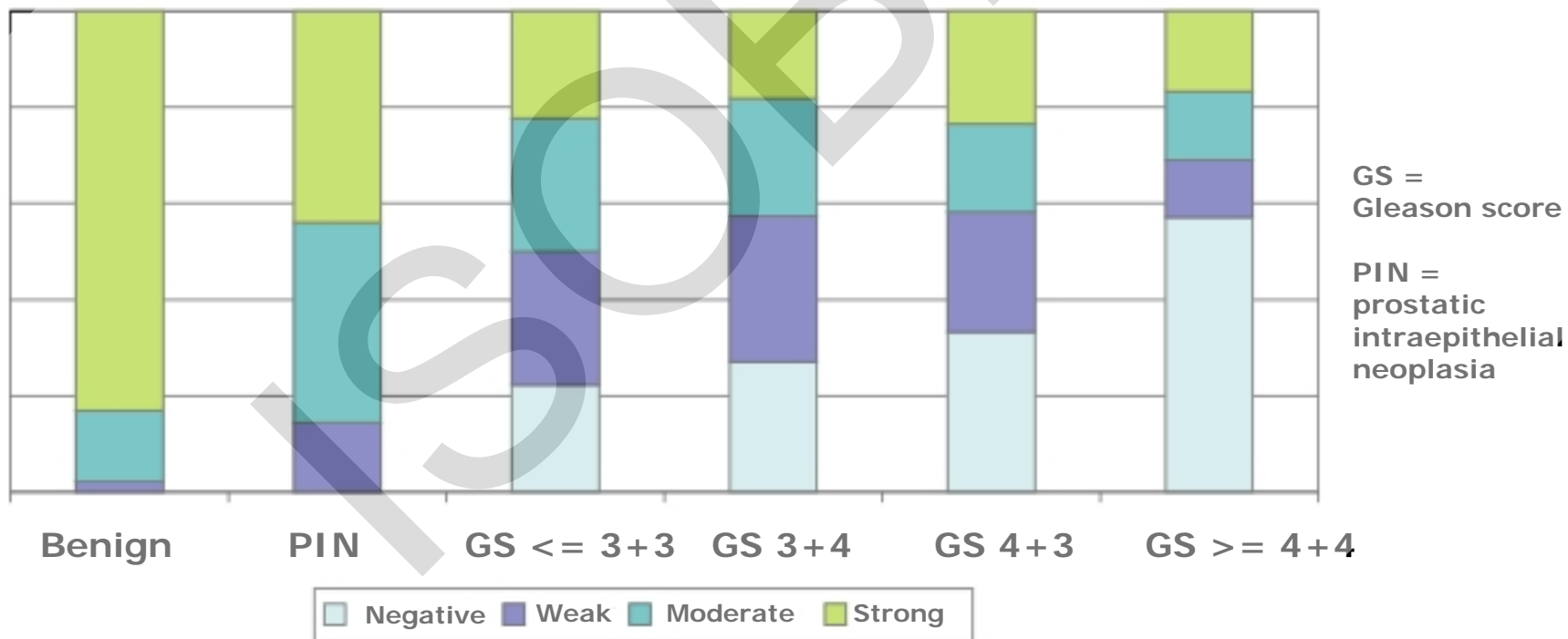
Also ANXA3 localization is correlated to progression of the prostatic transformation



**The ANXA3 localization is the basis for the pathogenic mechanism and the stability of yields in exprimate urine.**

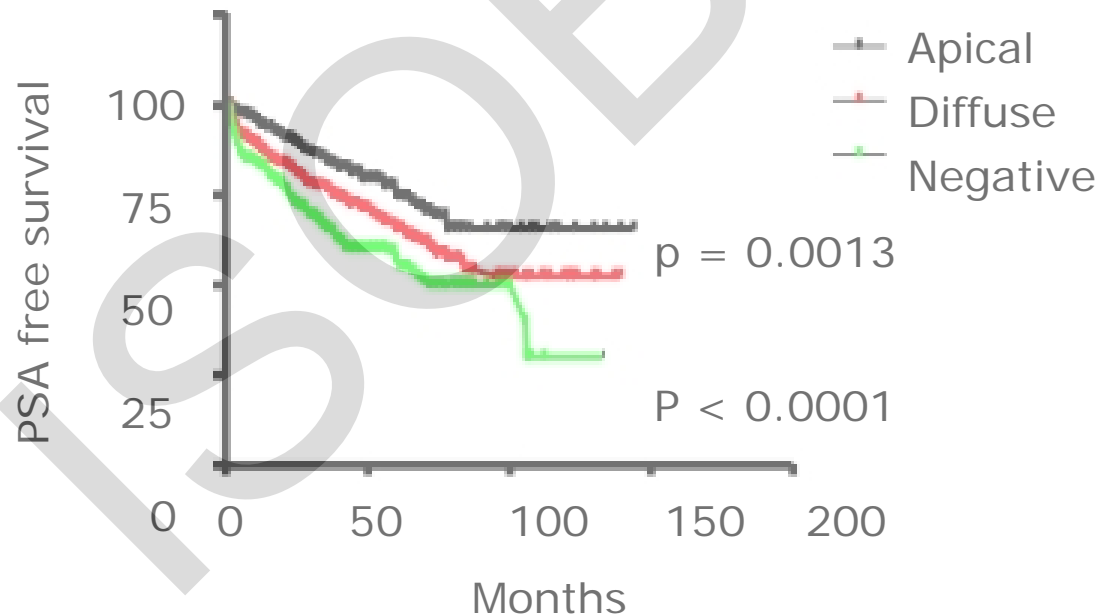
ANXA3 abundance is perfectly correlated with the progression of prostatic transformation

### Annexin A3 staining



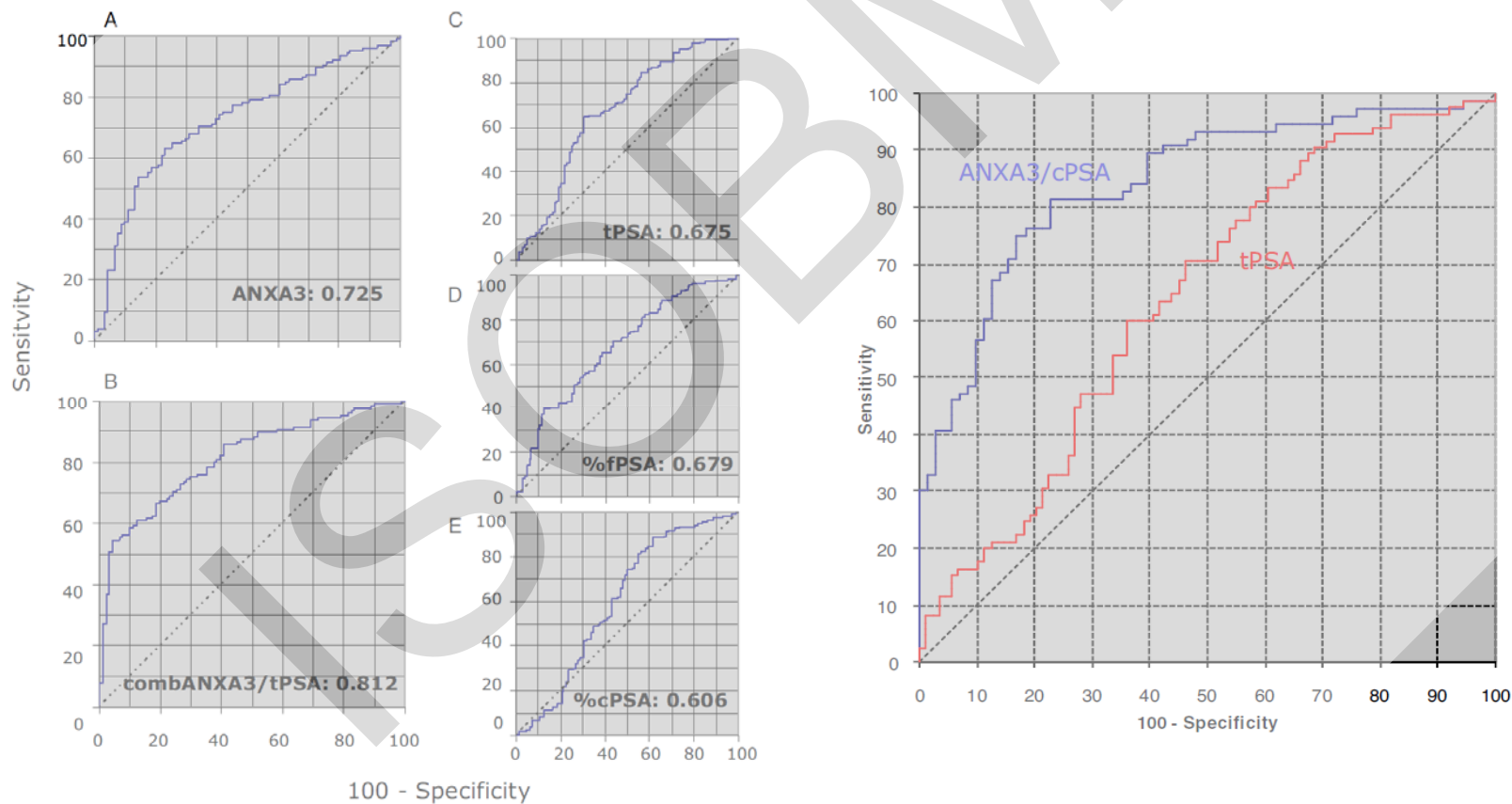
Köllermann et al. (2008) Eur. Urol. 54, 1314

ANXA3 localization is significantly correlated with PSA free survival of patients after prostatectomy



Köllermann et al. (2008) Eur. Urol. 54, 1314  
 Schlomm et al., (2010) Meth Molec. Biol., 664, 177

First clinical study: Schostak et al. (2008) J.Urol.181, 343  
 Another big study (1000 patients) under way



PSA range 2-6

PSA range 2-6; DRE negative patients

## Annexin A3– early detection of prostatic diseases

**ANXA3 follows a completely new principle, based on a novel mechanistic discovery potentially related to autoimmunity in prostate cancer.**

→ vs. other isoforms of PSA

**ANXA3 combines a proven and successful diagnostic marker (PSA) with a new marker in order to eliminate the key weaknesses of the PSA system.**

→ complementarity with PSA

**ANXA3 uses a technology platform (ELISA) which is stable proven and successful.**

→ vs. RNA based diagnostics

**Moreover:**

**ANXA3 is a novel tissue biomarker which delivers a full representation of the entire prostatic transformation and for the first time enables prognostic risk assessment after biopsy using a single marker.**

## ANXA3 and biology of prostatic disease

- BPH releases large quantities of prostasomes/exosomes containing ANXA3 into extracellular space (measured as u.anx.)
  - Apical ANXA3 staining of vesicular structures
  - Role of exosomes in regulation of innate immune system
  - This provides protection against progression of cancer (role of ANXA3 in angiogenesis: Park et al., 2005, BBRC 337,1283)
- PCA down-regulates ANXA3 and in particular apical/exosomal expression of ANXA3
  - Pca induces autoimmune antibodies against prostasomes (Larsson et al., 2006, Urol Oncol. 24, 195) and ANXA3 (our study)
  - T-cells (regulatory) in advanced PCA-tissue
- Latest publications confirm these lines of thinking:
  - Chen YW et al. (2010) TMPRSS2, a serine protease expressed in the prostate on the apical surface of luminal epithelial cells and released into semen in prostasomes, is misregulated in prostate cancer cells. Am J Pathol. 176(6):2986-96. Epub 2010 Apr 9. PMID: 20382709 [PubMed - in process]
  - Ronquist KG et al. (2010) Proteomic analysis of prostate cancer metastasis-derived prostasomes. Anticancer Res. 30(2):285-90. PMID: 20332430 [PubMed - indexed for MEDLINE]
  - Babiker AA et al. (2010) Mapping pro- and antiangiogenic factors on the surface of prostasomes of normal and malignant cell origin; Prostate. 70(8):834-47. PMID: 20127731
  - Schlomm T et al. (2010) From Gene to Clinic: TMA-Based Clinical Validation of Molecular Markers in Prostate Cancer; Methods Molec. Biol. 664: 177-89. [PubMed - indexed for MEDLINE]