# Native SDS PAGE

## (OTT ID 1313)

**Inventors: David Petering, Ph.D., Distinguished Professor, Andrew Nowakowski, and William Wobig, The University of Wisconsin-Milwaukee, Department of Chemistry and Biochemistry**

**For further information please contact:**

**Jessica Silvaggi, Ph.D.**

**Senior Licensing Manager**

**1440 East North Ave.**

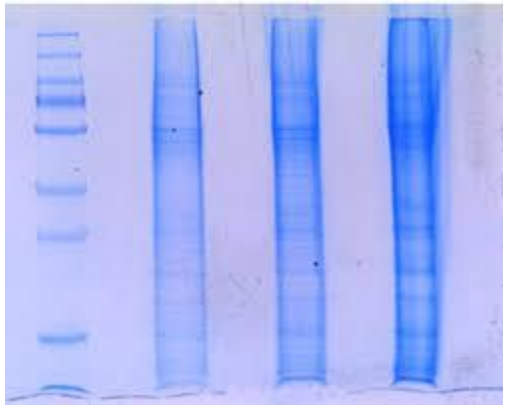
**Milwaukee, WI 53202**

**Tel: 414-906-4654**

**Jessica@uwmrf.org**

# Problems with current PAGE techniques

- In traditional SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) proteins are well separated but are denatured
- Structure and function are no longer adequately maintained for carrying out further functional assays
- Current native PAGE methods lead to poor separation and smearing during electrophoresis



# Our Solution: NSDS-PAGE

- A new method and buffer system named native SDS-PAGE
- New compositions for running buffer and sample buffer
- Low levels of SDS-PAGE enable very good protein separation and clarity on gels and also maintain the native 3-dimensional conformation and functional activity of proteins
- This invention provides inexpensive and quick to market products for companies already selling PAGE gels and buffer systems
- The ability to better resolve proteins in their native state will support numerous research fields including proteomics work, drug discovery, diagnostics, personalized medicine, protein-based therapeutics, and toxicology

# Intellectual Property and Licensing

- U.S. Utility Patent Issued:
  - 9,709,526
- We are seeking partners for licensing and development of the final product
- The buffers can be used with certain pre-cast gels on the market and adapted to other gel types with further testing
- This product is ideal for research laboratories, biotech, and pharma research exploring functional proteins and enzymes and could be used for numerous applications

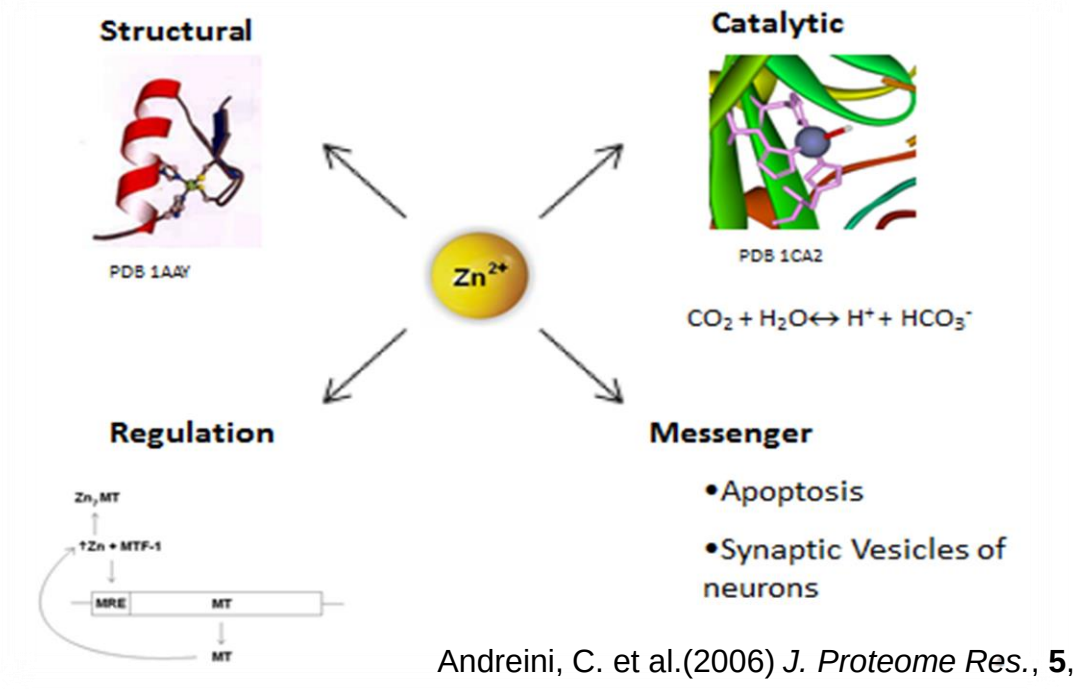
# Market Potential

## Market

- BCC Research reports that the life science tools and reagents market reached $48.2 billion in 2015 and is projected to reach $58 billion by 2020.
- The global proteomics market is predicted to reach $21.87 billion by 2021 according to MarketsandMarkets Research.
- The market for protein therapeutics  is expected to reach $208 billion by 2020 according to RNCOS Industry Research Solutions.

# The Need for Native SDS PAGE

- The Petering laboratory studies metal-binding proteins and are focusing on zinc in biological systems
- Zinc is the 2nd most abundant transition metal (2-3 grams in the body) and it is estimated that there are approximately 2800 Zn binding proteins and 1000 transcription factors containing Zn




Andreini, C. et al.(2006) *J. Proteome Res.*, **5**, 196-201.

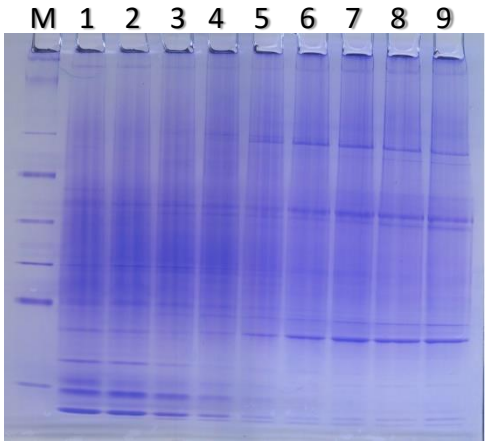
# Current Methods are Limiting

- The ability to isolate, identify, and study the vast array of Zn-proteins is severely limited using available PAGE techniques because SDS-PAGE denatures such proteins, releasing bound Zn

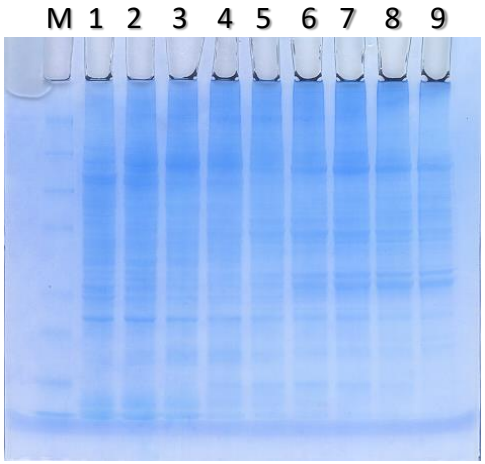
- The inventors developed a new Native SDS-PAGE method to separate proteins of interest and optimized Laser Ablation Inductively Coupled Plasma Mass Spectrometry to visualize Zn associated with proteins on dried PAGE gels

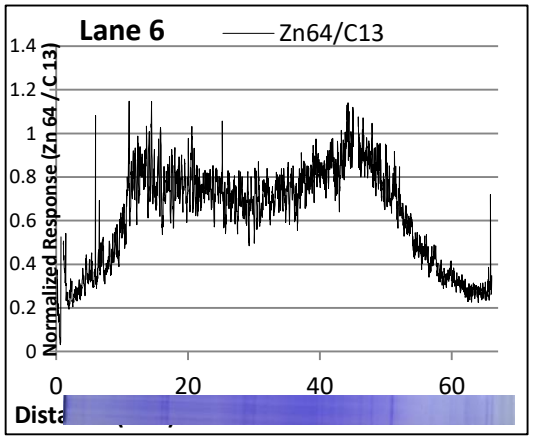
# Native PAGE and SDS-PAGE Problems

**BN-PAGE "Native"**

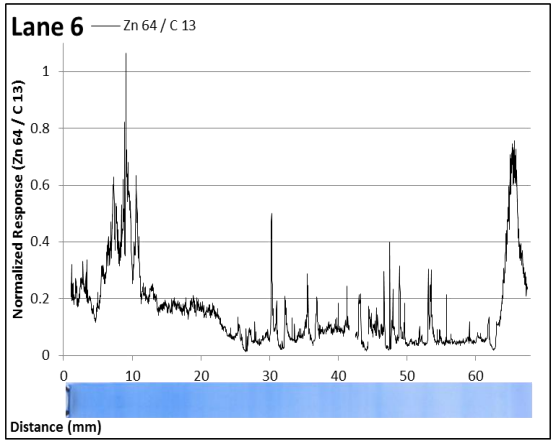



**SDS-PAGE "Denaturing"**




Poor Resolution




Superior Resolution

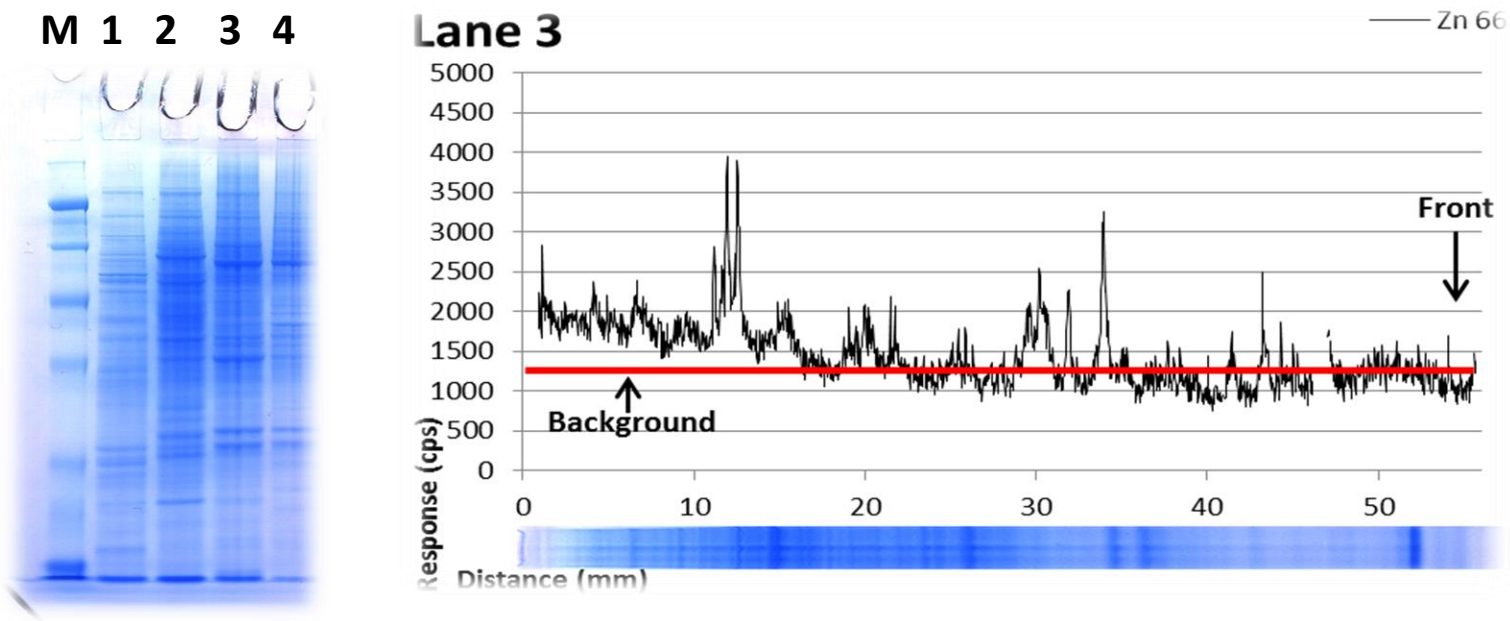
## Native PAGE

- Proteins remain native as evident from presence of Coomassie staining associated with Zn
- Resolution is poor such that no single protein band can be identified as a Zn-protein

## SDS-PAGE

- Denatured Zn proteins as evident from large loss of Zn which migrates at the dye front
- Some proteins retain Zn in specific protein peaks

# Native SDS-PAGE = Excellent Resolution

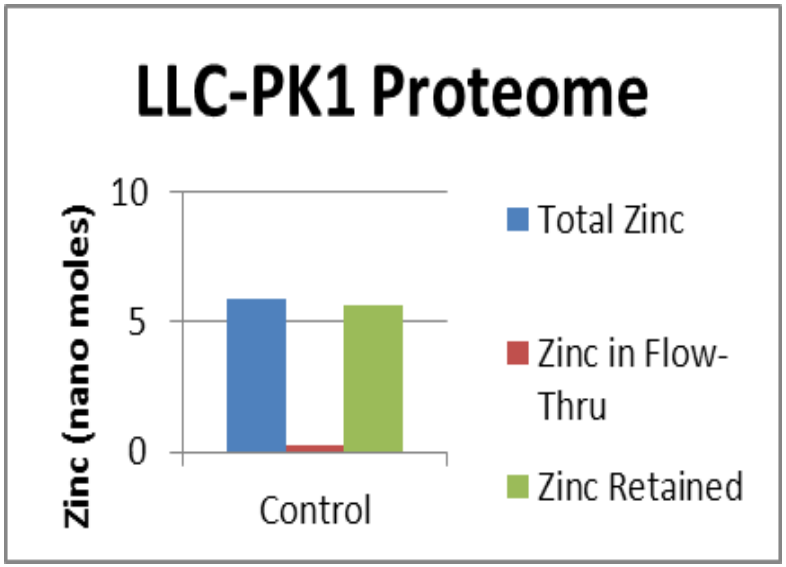



- Zn proteins remain native; Zinc remains associated with specific protein bands and not dissociated at the dye front
- The gel shows high resolution of bands

# PAGE Methods Retention of Zinc

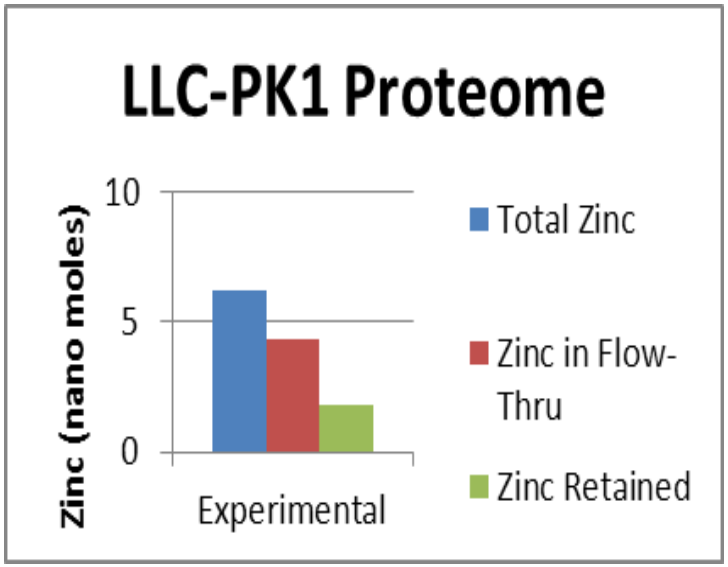
**Stability of LL-CPK$_1$ proteomic $Zn^{2+}$**

One hour incubation before centrifugal filtration.




**NSDS-PAGE**

Almost all Zn retained in proteome fraction after incubation with NSDS-PAGE run buffer and filtration




**SDS-PAGE**

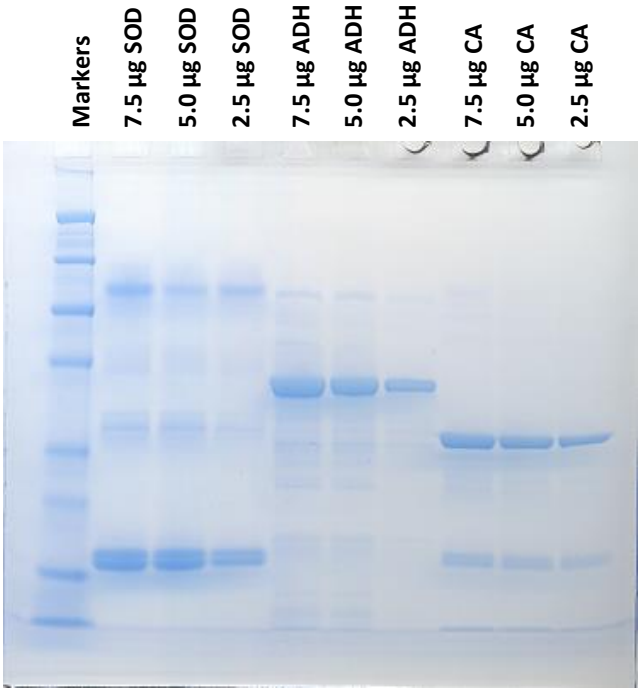
Large majority of Zn is released from Zn-proteins after incubation with SDS-PAGE buffer and filtration

# Native SDS-PAGE Excels

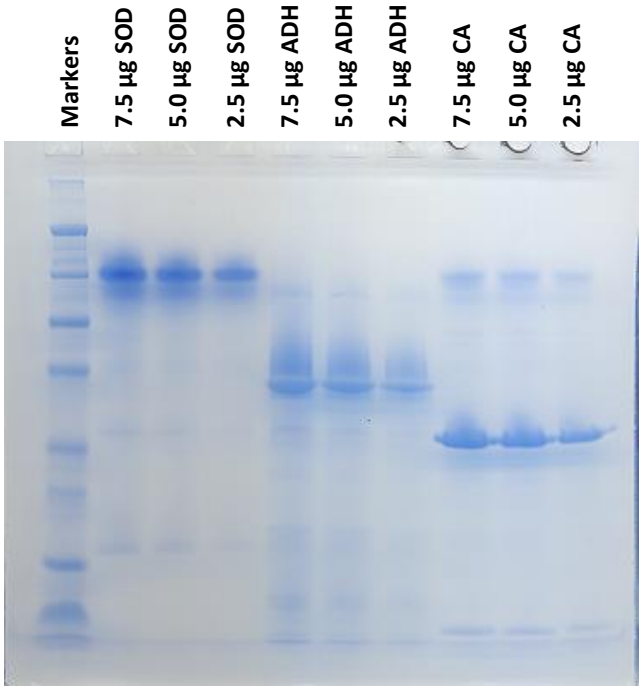
The Native SDS-PAGE buffers and protocol provide excellent resolution while maintaining protein function and structure

SOD = Superoxide dismutase (**Cu,Zn enzyme**)
ADH = Alcohol dehydrogenase (**Zn enzyme**)
CA = Carbonic anhydrase

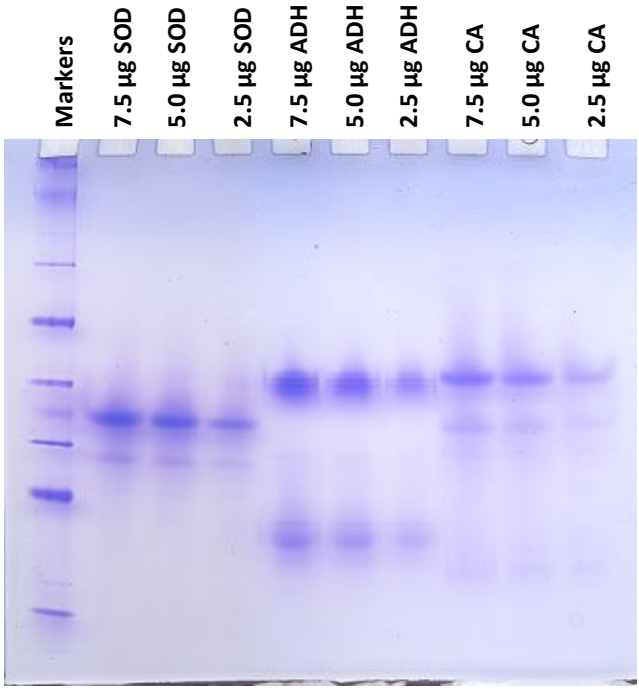
**SDS PAGE gel per Invitrogen protocol and with Invitrogen Chemicals**



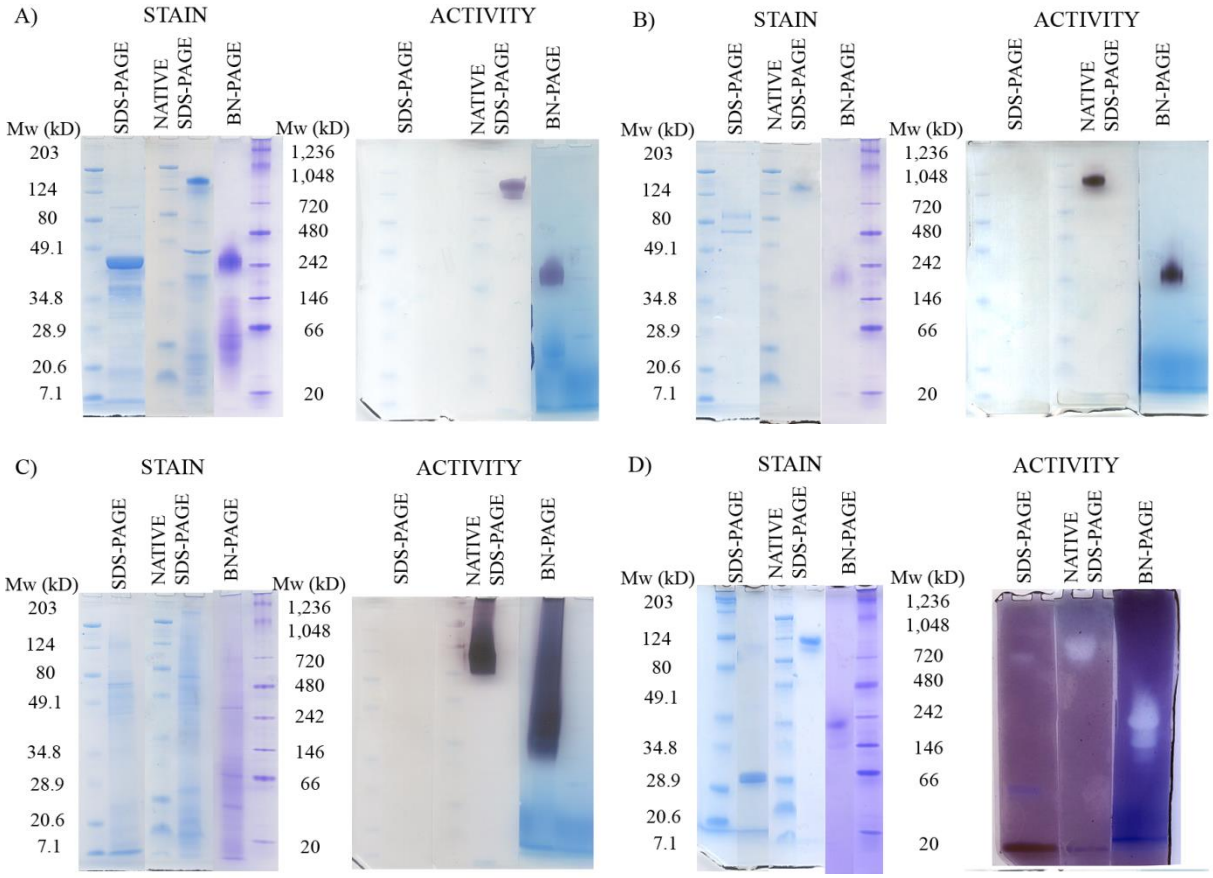

**SDS PAGE gel per our modified "Native" protocol**




**BN PAGE gel per Invitrogen protocol and with Invitrogen Chemicals**



# Enzymatic Activity Maintained in NSDS-PAGE

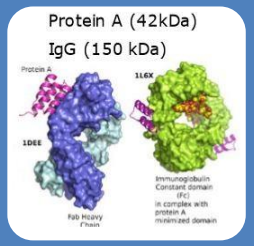



(A) Alcohol dehydrogenase
(B) β-Galactosidase
(C) Alkaline phosphatase (Zn enzyme)
(D) Superoxide dismutase

- SDS-PAGE, NSDS-PAGE, and BN-PAGE were compared for residual enzymatic activity
- SDS-PAGE only showed slight activity for SOD; NSDS- and BN-PAGE retain activity

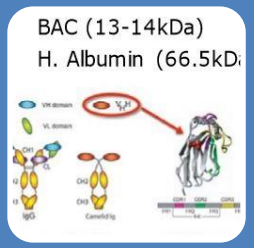
# Protein Complex Preparation

- 2 protein models were developed to explore migration as:
  - "Complexed" in the Native gel (BN) and in the NSDS system.
  - 2 separated proteins under denaturing conditions.




## 1. Protein A and Mouse IgG

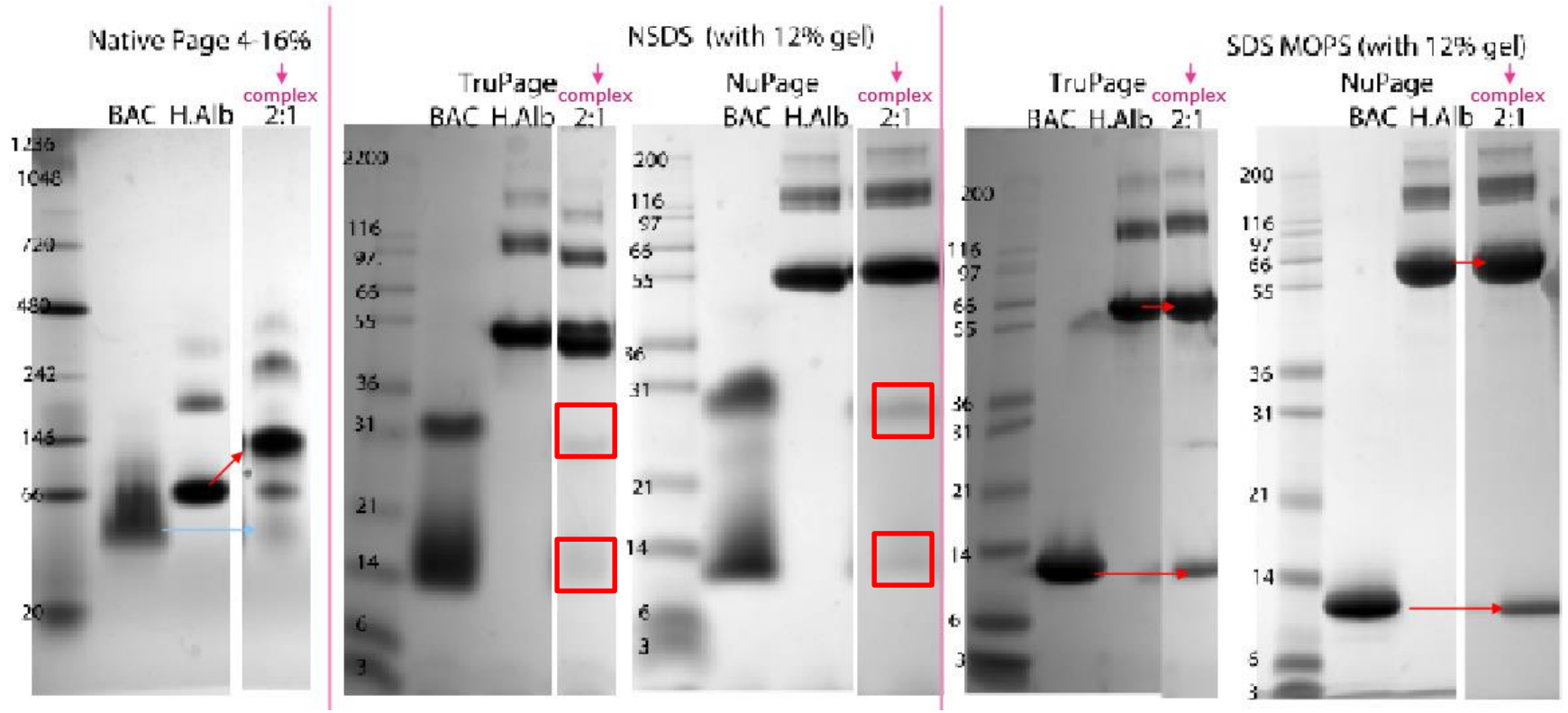
- Protein A (1.47mM) and Ms IgG (16uM) were diluted in PBS to 8uM. Solution was mixed to different micromolar ratios – from 5:1 to 2:1 of IgG /Protein A




## 2. BAC ligand and Human Albumin

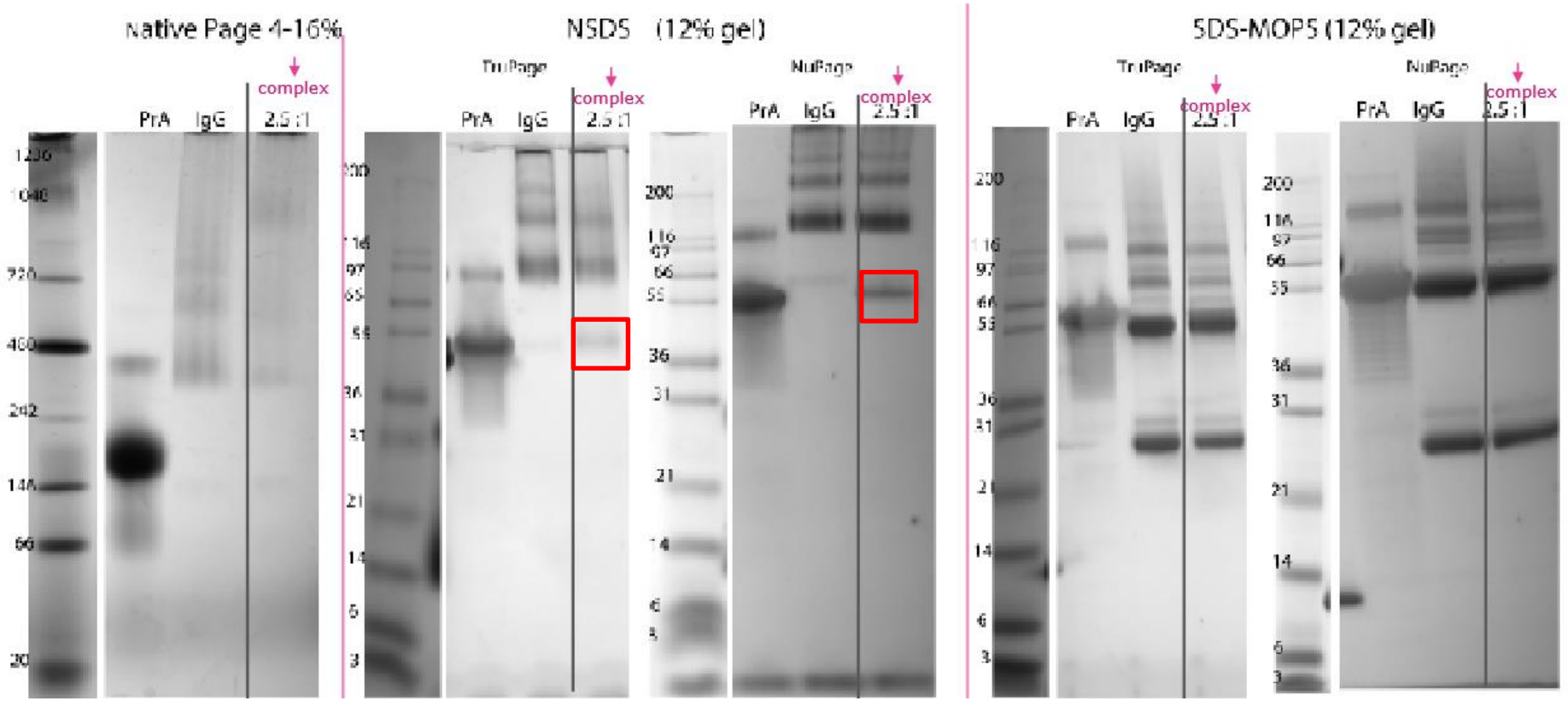
- VHH antibody fragment (0.8mM) and Human Albumin (0.8mM solution) were diluted in PBS and mixed to different micromolar ratios – from 0.75:1 to 3:1 Albumin BAC ligand

# Data for BAC-Albumin Protein complex




- Native and NSDS maintain the complex, while SDS PAGE shows separate proteins with the same band migration
- In the complex lane, there is a lack of BAC as it is in complex with Albumin

# Data for Protein A-IgG Protein complex




- Native and NSDS maintain the complex, while SDS PAGE shows separate proteins with the same band migration
- In the complex lane, there is less of Protein A alone as it is bound to IgG

# Discussion and Conclusions

**Advantages:**

- Can be performed using commercial pre-casted gels
- Simple technique and requires only one buffer system
- Can retain the functional activity of proteins tested
- Can retain binding of protein complexes
- The neutral pH of the NSDS buffer system has an advantage over the high pH Tris-glycine system for separation of the pH sensitive proteins

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