

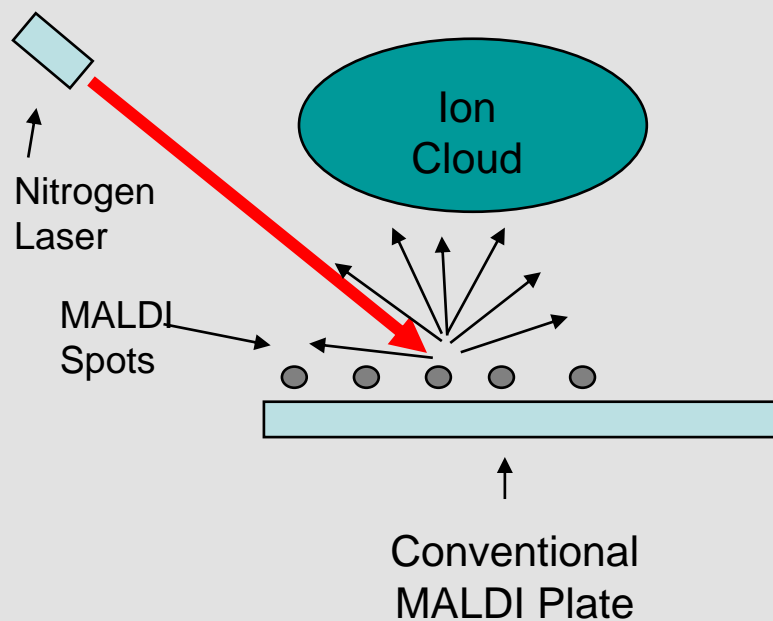
# Introduction

- Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry (MALDI-TOFMS) has become one of the most popular analytical techniques for analysis in the pharmaceutical and biological research areas.
- In a traditional MALDI experiment, the analyte is mixed in solution with a matrix material and deposited in small spots on a stainless steel plate. The identity of the spots is maintained by their location on the target plate. The spots are then dried and the entire plate is loaded into the vacuum system of the TOFMS.
- A laser pulse (typically from a N<sub>2</sub> Laser) is allowed to impinge on the spot. The laser energy is absorbed by the matrix material, causing a supersonic expansion which results in both the desorption and ionization of the analyte material. (Figure 1A)
- The resultant ions are allowed to stabilize for a short while (delayed extraction conditions) before they are ejected into the flight tube by the application of a high voltage pulse to the sample plate. Once in the drift tube, the ions separate by mass as they move toward the microchannel plate ion detector.
- The arrival of each ion is precisely recorded by the microchannel plate detector and high speed digitizer.

# Discussion

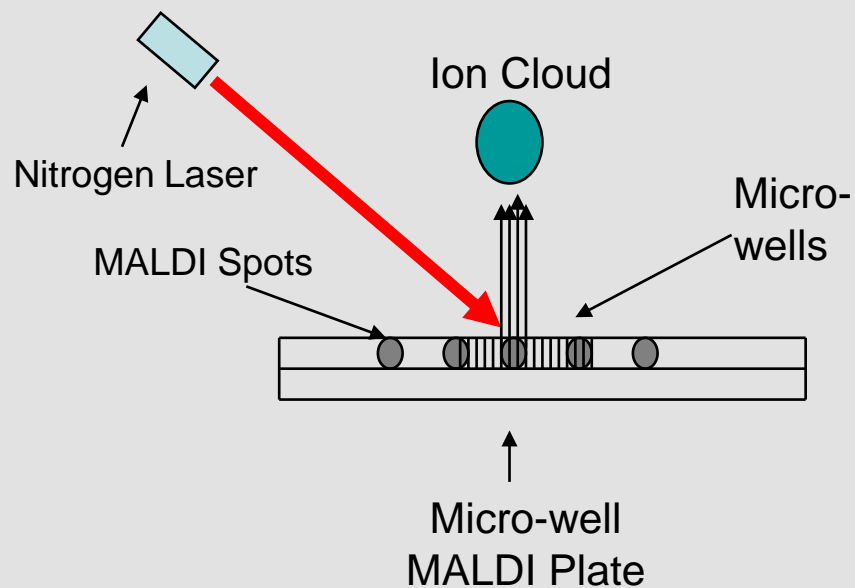
- It was believed that the performance of a MALDI TOFMS could be improved through the development of improved MALDI target plates.
- The supersonic expansion triggered by the laser produces ions that leave the surface with a large range of angles, producing a rather large cloud of ions. Ions of the same mass are generally not located in the same spatial position when the ion extraction pulse is applied. Ions of the same mass which exit the source at different times do not arrive at the detector at the same time. This time jitter can lead to degraded mass resolution and accuracy. Containing the spots within micro-wells could limit the ion cloud spatial distribution and provide directionality to the ion plume desorbing from the plate surface. (Figure 1B)
- Samples spotted on conventional MALDI targets can run or smear causing samples to mix and lose integrity.
- During drying, the matrix material often agglomerates and does not remain homogeneously distributed in the sample spot. Forcing the sample to dry in a series of cells would help ensure the solid sample would be evenly distributed.

Illustration of an Ion Cloud Resulting from the Laser Excitation of the Matrix Material



A.)

Potential Ion Cloud produced from a Micro-Well Target



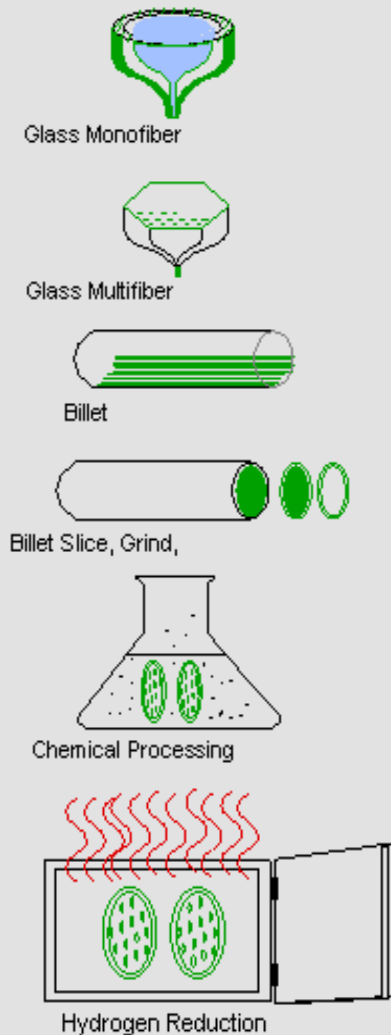
B.)

Figure 1

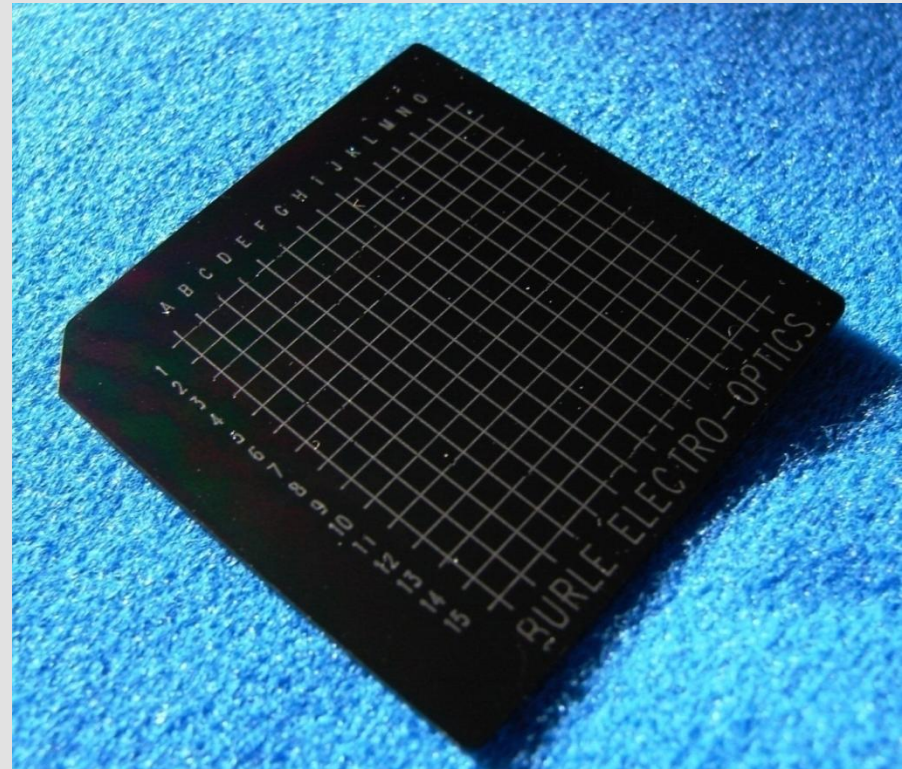
# Experimental Design I

- Wafers were prepared using Microchannel Plate manufacturing methods. (Figure 3A)
- Prototype targets were produced in various sizes and shapes to accommodate various carriers. Figure 3B illustrates a typical prototype target.
- Etching experiments were performed in order to determine the etch rate of the core material removal. Once established, the depth of the Micro-wells could be precisely controlled. (Figure 4)
- The resultant Micro-well structure was then hydrogen fired in order to render the device electrically conductive to prevent charging effects.
- The resultant Micro-well targets of various pore depths were then spotted with peptide samples and their performance compared to that produced using the standard stainless steel targets. A Bruker Reflex® III MALDI TOFMS was used for this work.

# Fabrication Sequence for Micro-well MALDI Targets



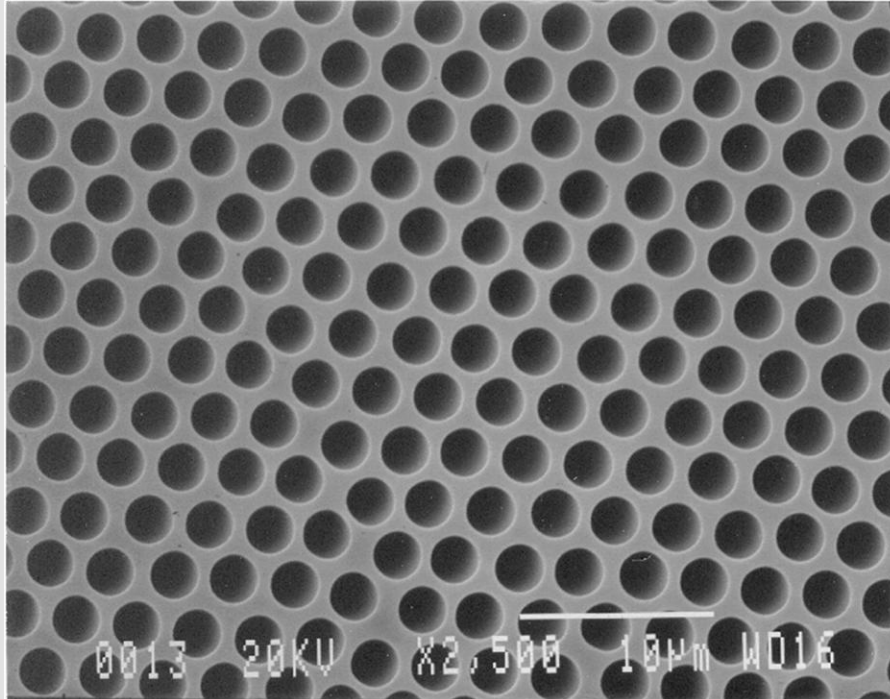
# Prototype Micro-well MALDI Target



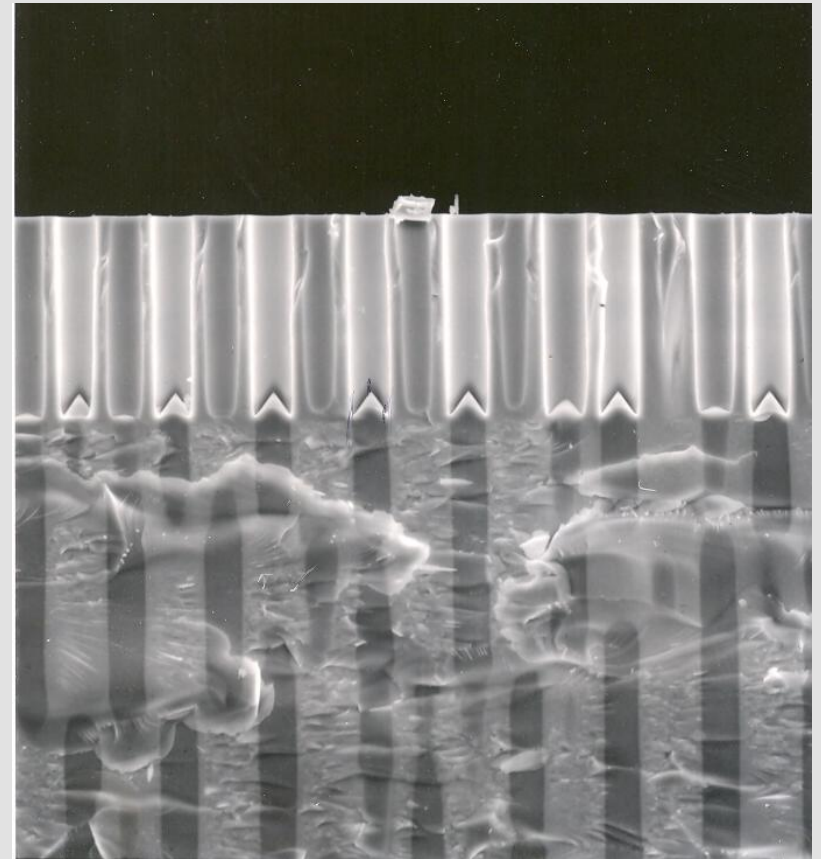
B.)

Figure 3

# Photomicrographs of Micro-well Structures



Top View



Cross Section

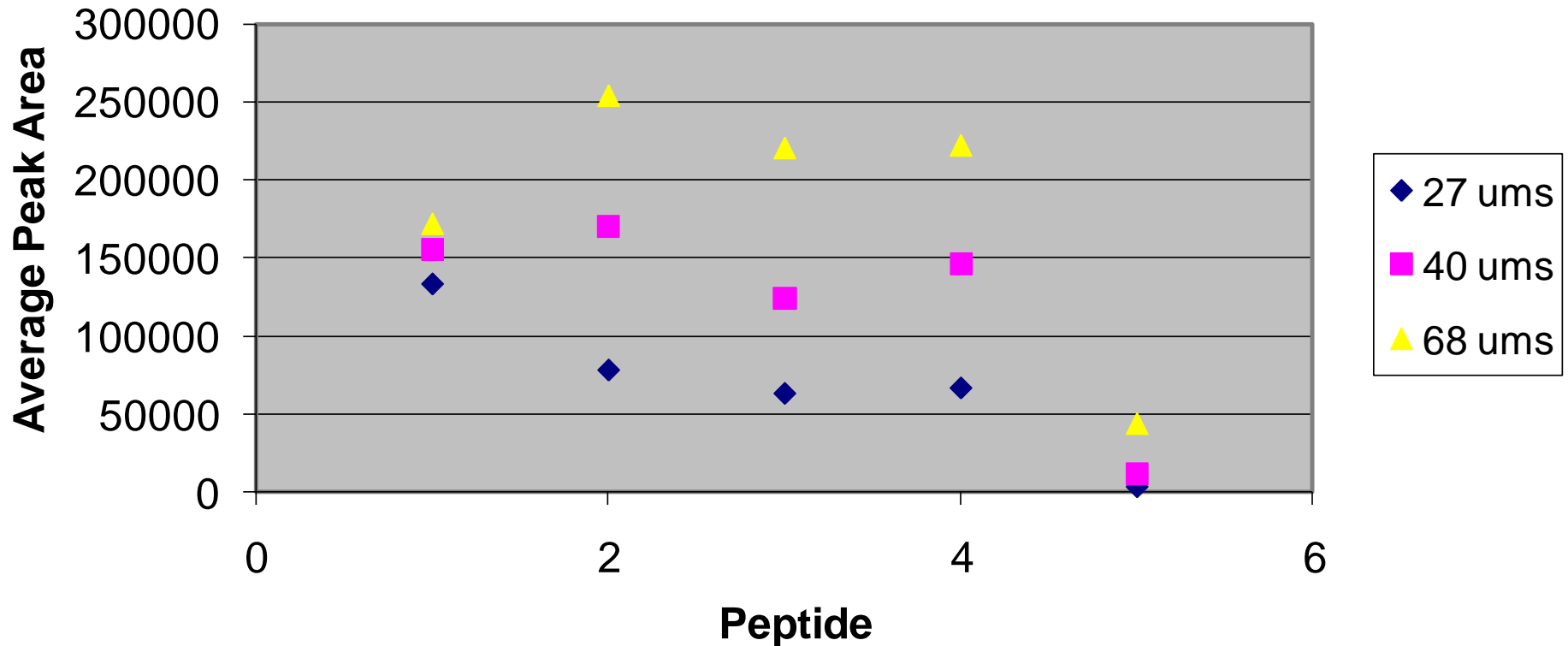
Figure 4

# Preliminary Samples

Mixture	A2 pmole/uL	A2/2 pmole/uL	A2/5 pmole/uL	A2/10 pmole/uL
Ang I (1046)	2.28	1.14	0.46	0.23
Bradykinin (1060)	6.74	3.37	1.35	0.67
Ang II (1296)	3.68	1.84	0.74	0.37
Neurotensin (1672)	5.69	2.85	1.14	0.57
Glucagon (3481)	20.52	10.26	4.10	2.05
Vol pep mix	5	5	5	5
Vol CHCA	47.5	47.5	47.5	47.5
M/A	2282	4564	11409	22818

CHCA matrix solution was made by dissolving 18.56mg of matrix into 1ml of MeOH

# Well Depth Optimization



Angiotensin II – Peptide 1

Bradykinin – Peptide 2

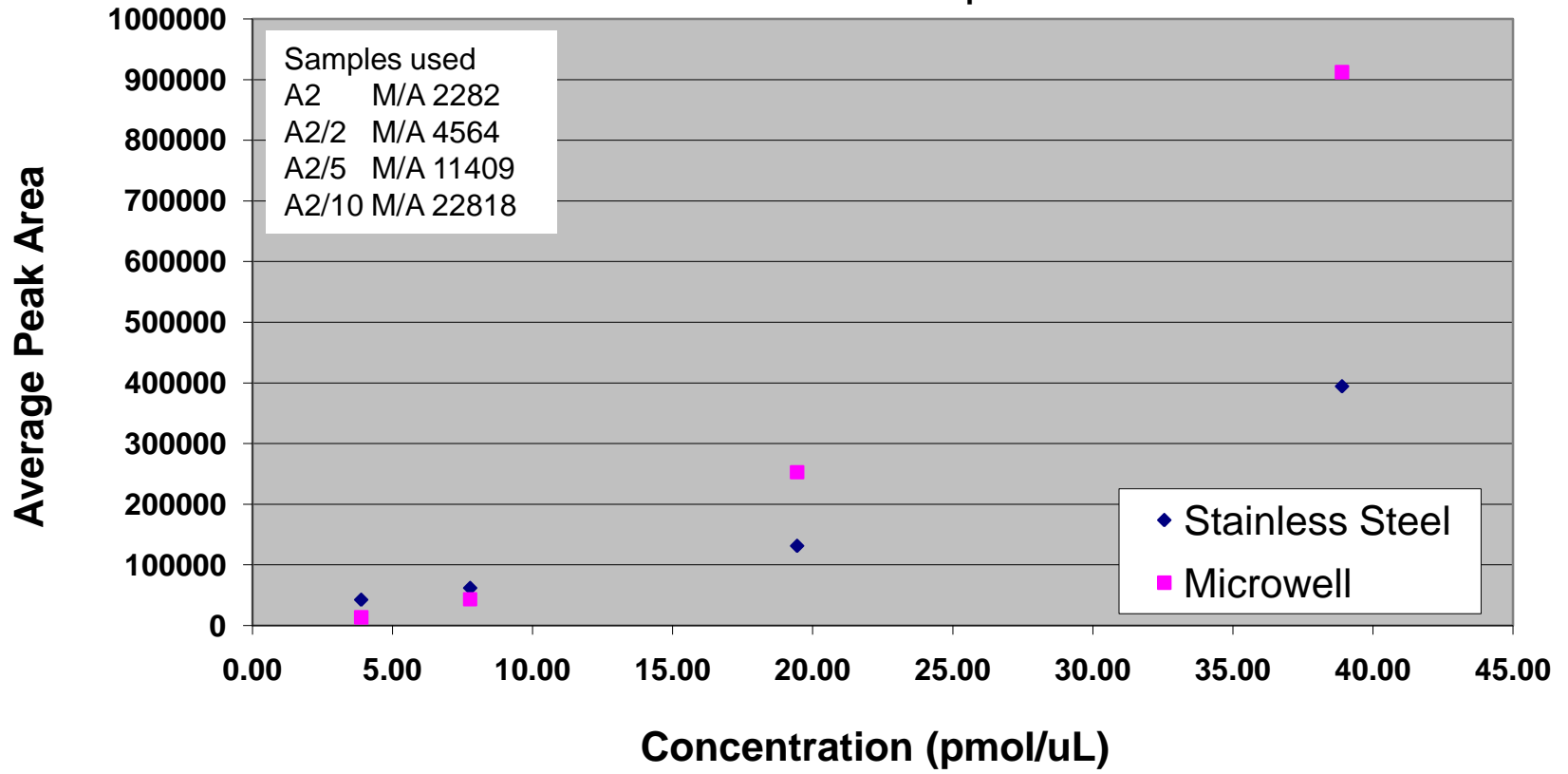
Angiotensin I – Peptide 3

Neurotensin – Peptide 4

Glucagon – Peptide 5

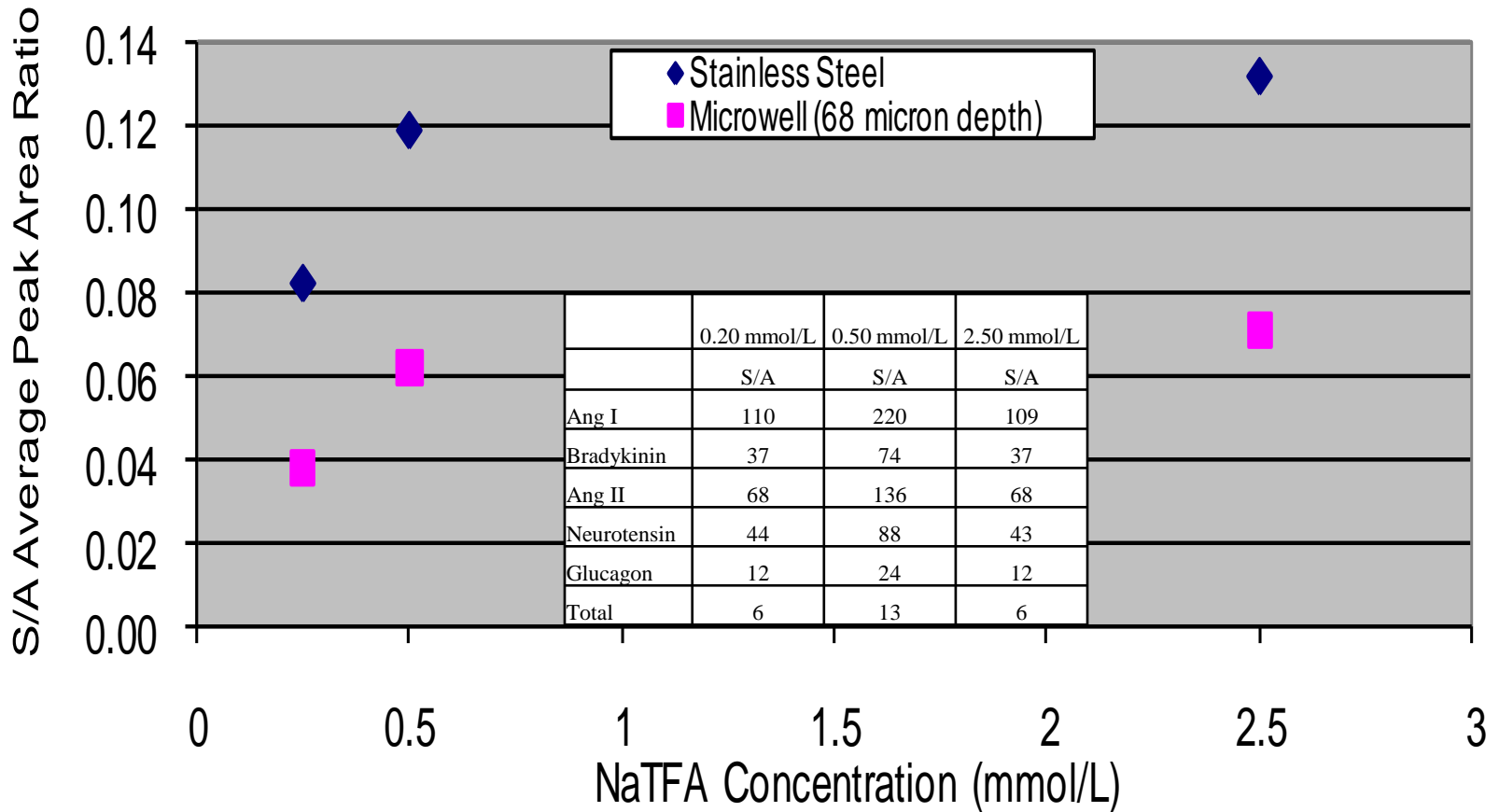
# Sensitivity

**Total Peak Area**  
Sum of All Samples

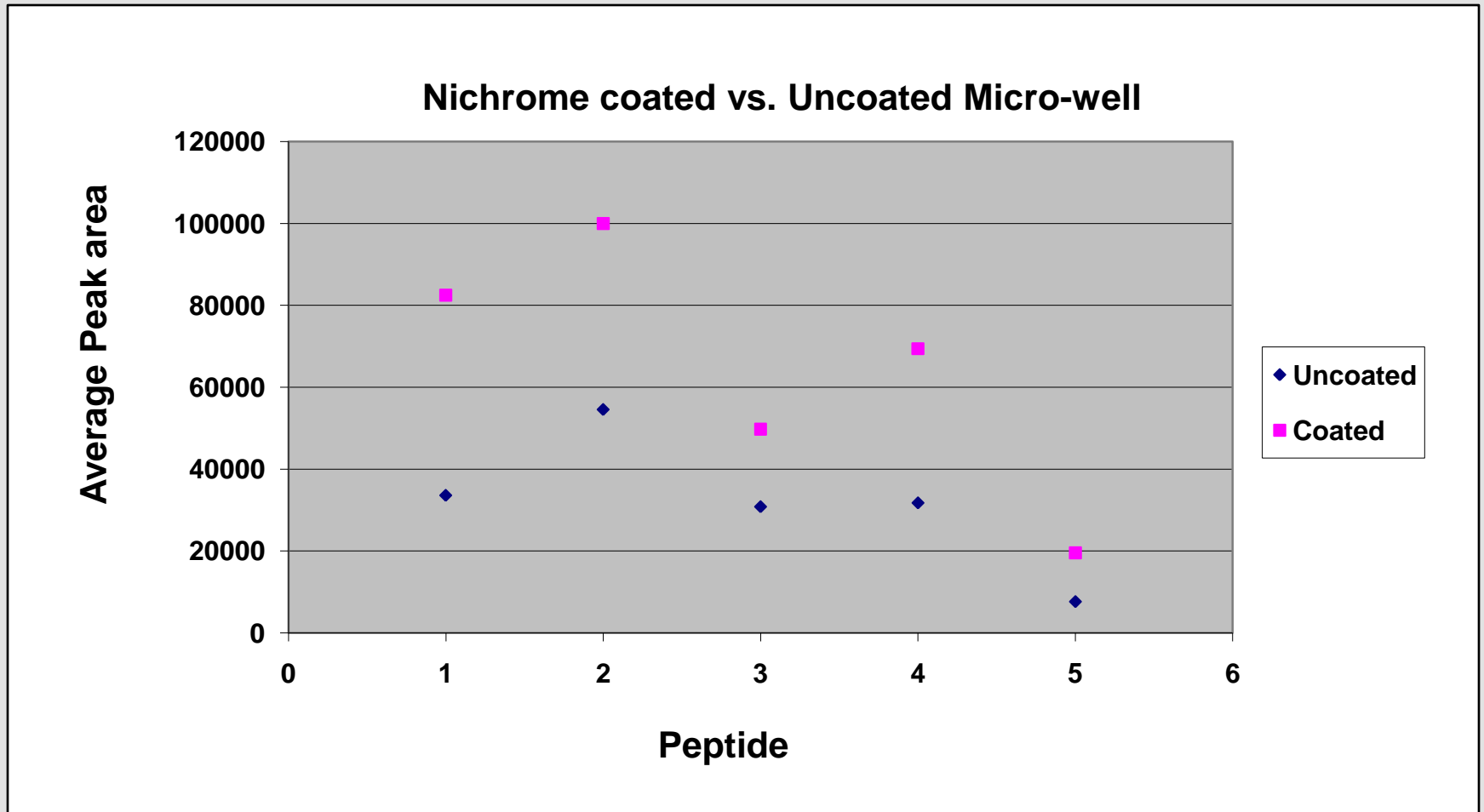


# Alkali Scrubbing Effect

Total Peak Area Ratio – Sum of All Samples



# Sensitivity with Metal Coating



27µm well depth plates used  
 A2 M/A 2282 sample used

# Experimental Design II

- Further experiments were run to investigate the improvement seen in the Nichrome coated samples. Micro-well plates were coated with Nichrome and gold and compared to uncoated Micro-well and standard stainless steel plates
  - Concentration Effect
    - 2, 5, 10, 50 and 100 fold dilutions on all 4 surfaces
  - Mass Accuracy
    - 3 and 4 point calibrations on all 4 surfaces
  - Micro-well sample plate lifetime
    - Non-coated plates from May 2007 and Feb 2008
  - Mass Resolution
    - 4 peptide mixture on all 4 surfaces

# Samples – Effect of Concentration

	Vol(uL)	A2 pmole/uL	A2/2 pmole/uL	A2/5 pmole/uL	A2/10 pmole/uL
Bradykinin	2	17.96	8.98	3.59	1.80
Ang I	4	29.38	14.69	5.88	2.94
Neurotensin	3.5	19.93	9.96	3.99	1.99
water	0	0.00	0.00	0.00	0.00
Ang II	0.5	4.55	4.55	4.55	4.55
	M/A	1260	2370	5026	8023

\*internal calibrant

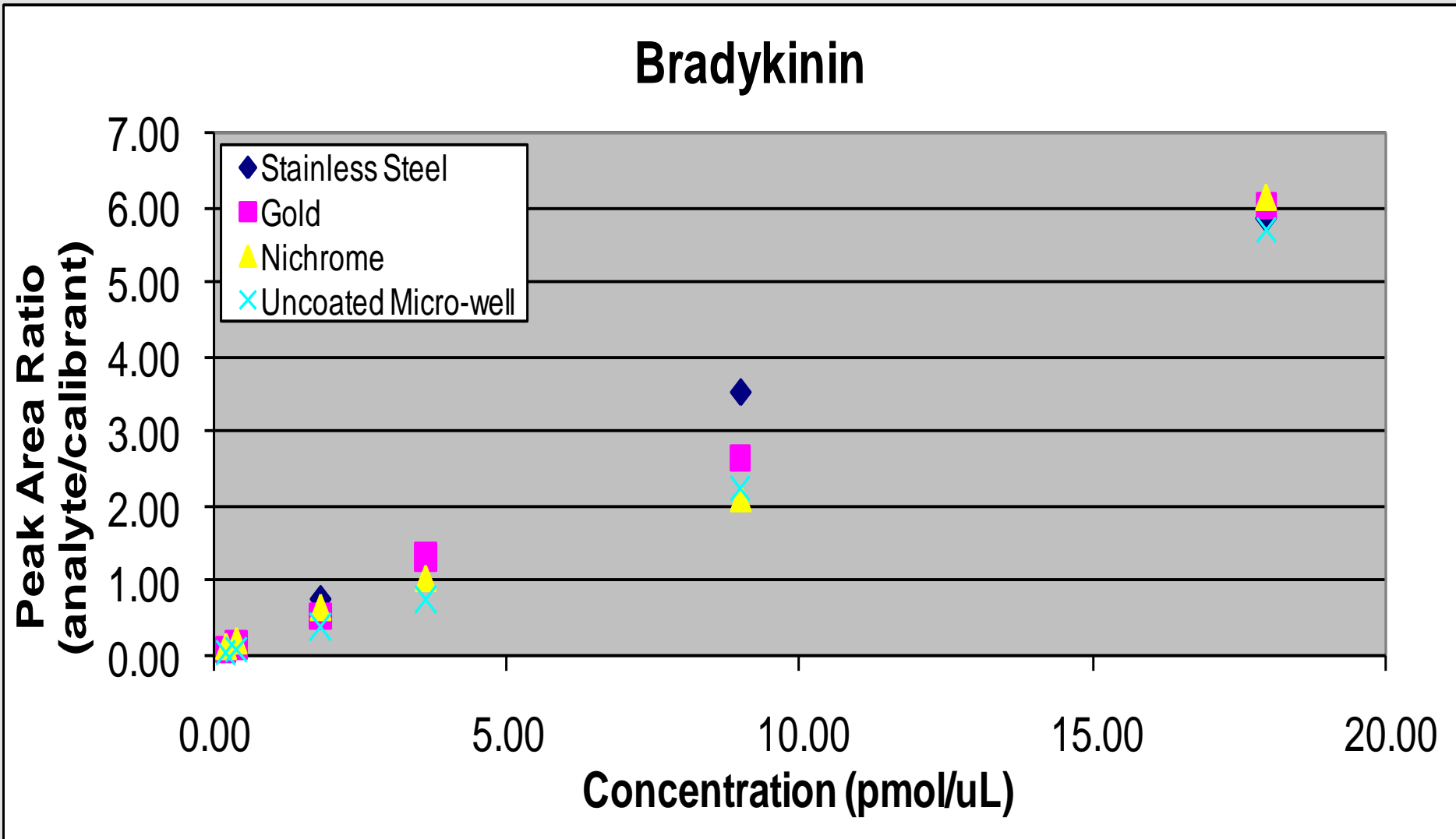
CHCA matrix solution was made by dissolving 18.89mg of matrix into 1ml of MeOH

	Vol(uL)	A2/10 pmole/uL	A2/50 pmole/uL	A2/100 pmole/uL
Bradykinin	2	1.80	0.36	0.18
Ang I	4	2.94	0.59	0.29
Neurotensin	3.5	1.99	0.40	0.20
water	90	0.00	0.00	0.00
Ang II	0.5	0.46	0.46	0.46
	M/A	1260	5026	8023

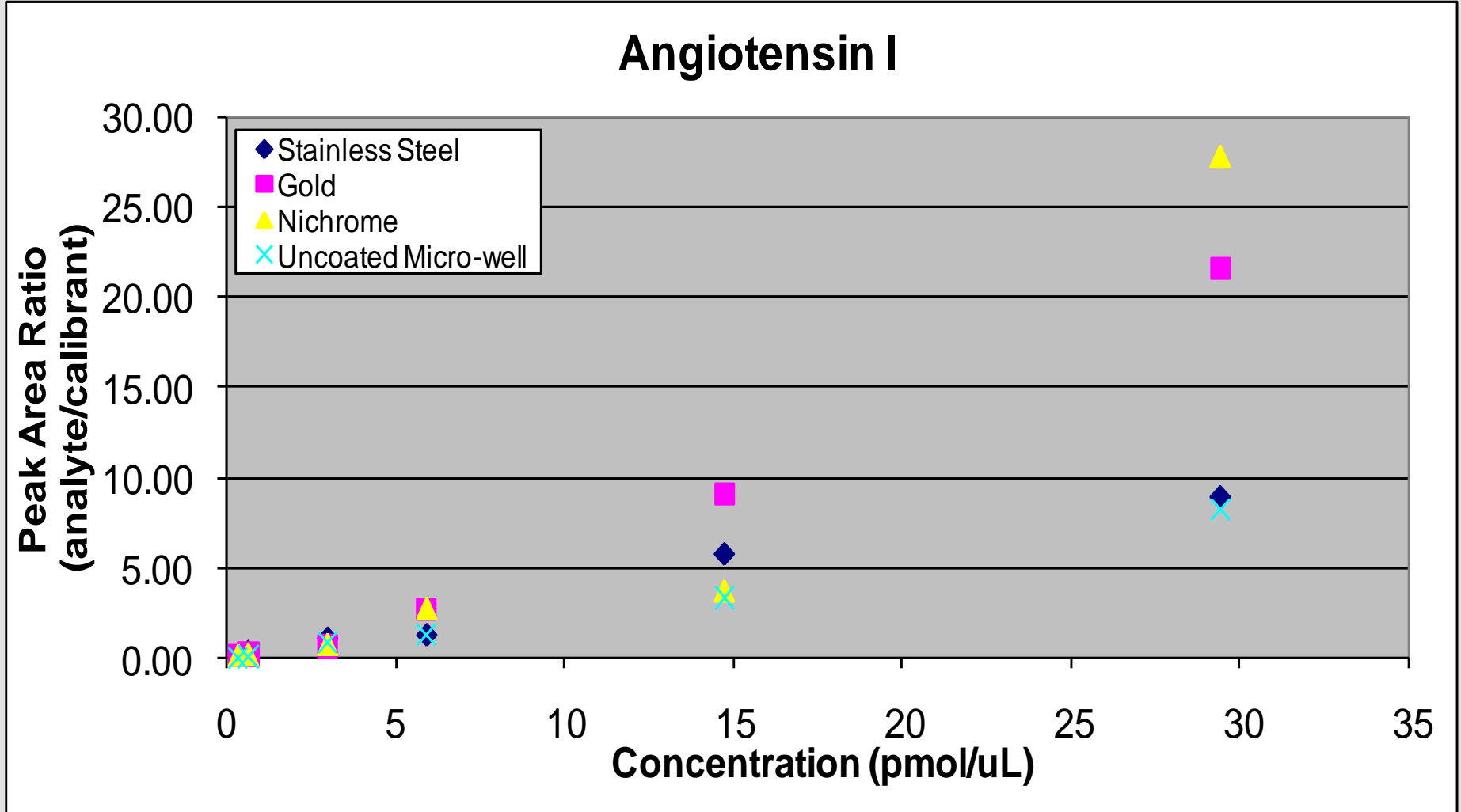
\* internal calibrant

CHCA matrix solution was made by dissolving 1.90mg of matrix into 1ml of MeOH  
1uL of a 47.5 uL matrix/5uL analyte mixture was spotted on each plate

# Effect of Concentration

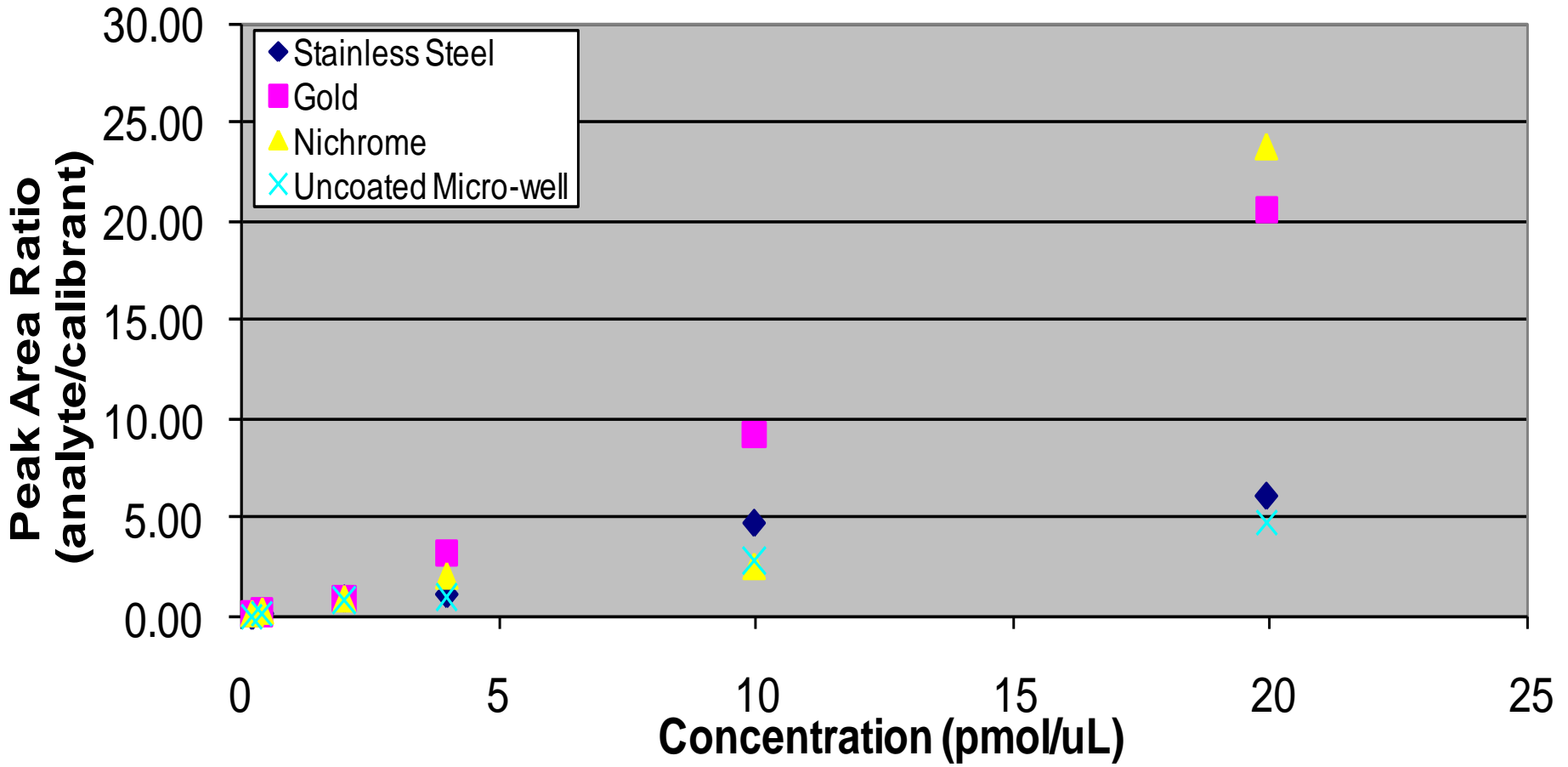


# Effect of Concentration



# Effect of Concentration

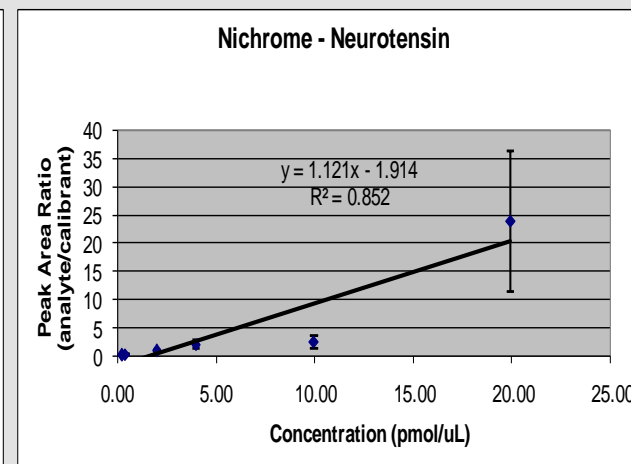
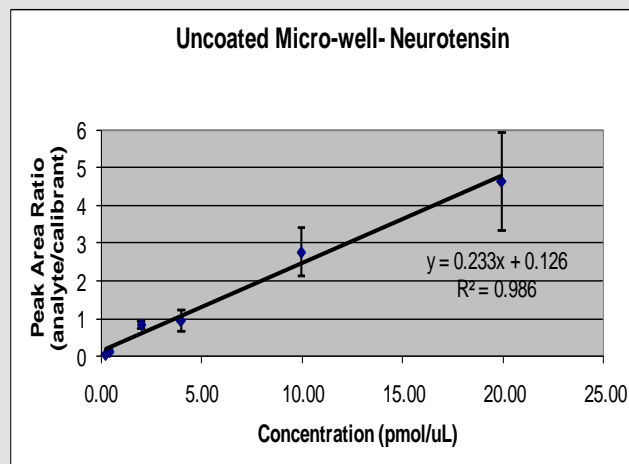
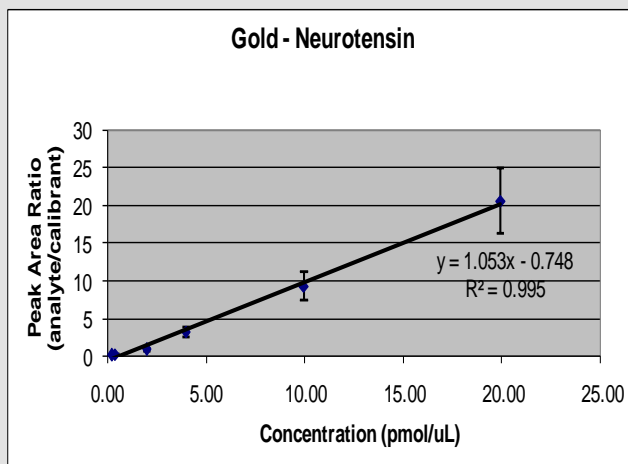
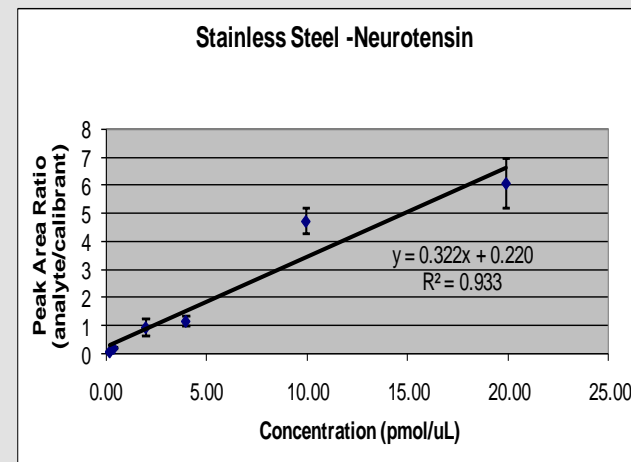
## Neurotensin



# Concentration Effect Results

Slope	Stainless Steel	Gold	Nichrome	Uncoated Micro-well
Bradykinin	0.3294	0.3299	0.3277	0.3145
Angiotensin I	0.3136	0.7471	0.8974	0.2732
Neurotensin	0.3223	1.0533	1.1213	0.2333

The slope of the linear fits of the concentration curves indicate the Nichrome and Gold coated Micro-well plates have higher sensitivity for certain analytes.



# Mass Accuracy

## Samples

Mixture	A2 pmole/uL
Angiotensin II (1046)	20.22
Bradykinin (1060)	19.96
Angiotensin I (1296)	16.32
Neurotensin (1672)	18.98
Vol pep mix	5
Vol CHCA	47.5
M/A	1197

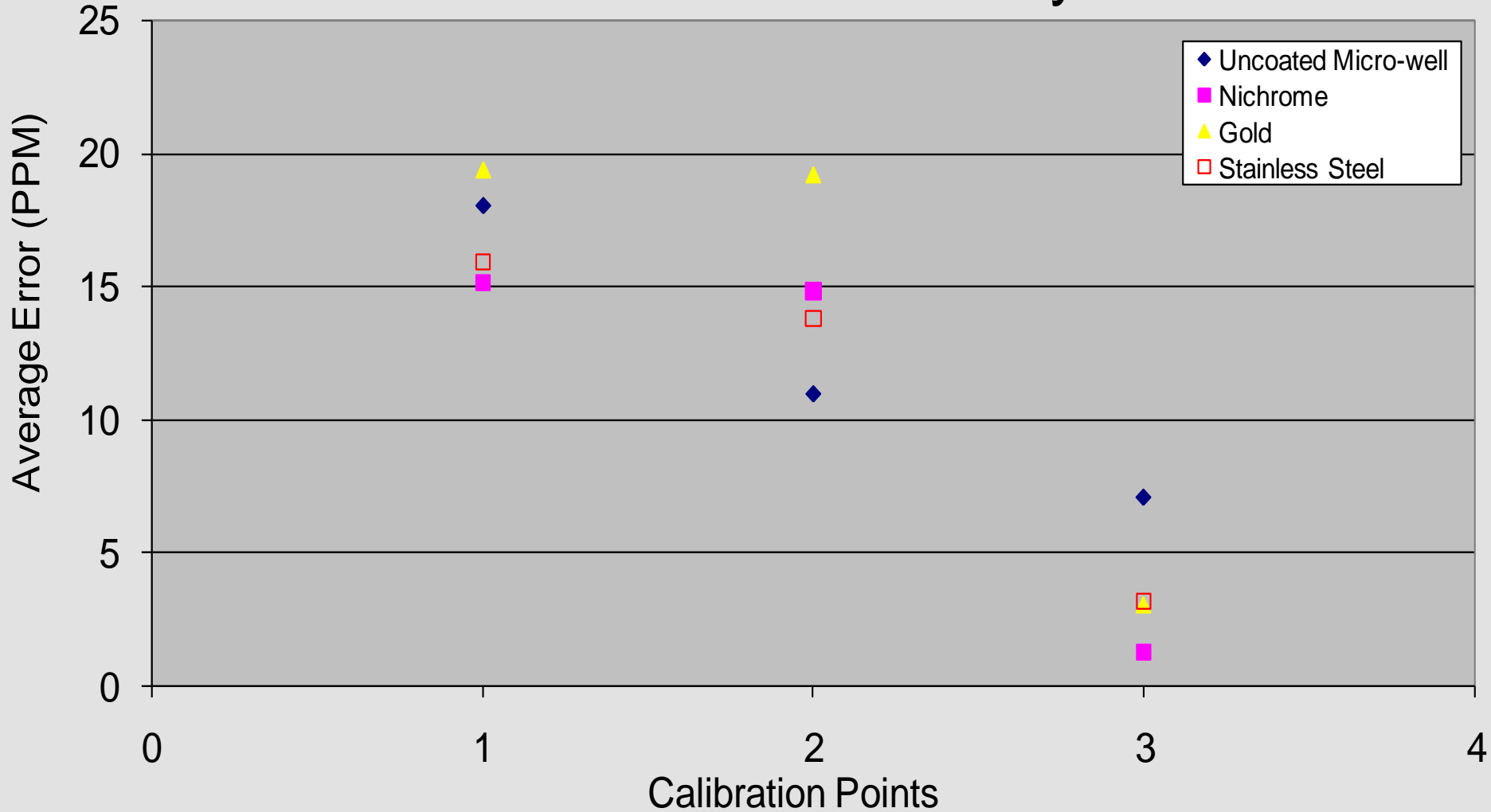
CHCA matrix solution was made by dissolving 60.05mg of matrix into 2ml of MeOH

Stage 3 Micro-well (40 $\mu$ m deep) Micro-well sample plates with no coating, nichrome coating and gold coating from Feb 2008 were used

## Instrument Calibration

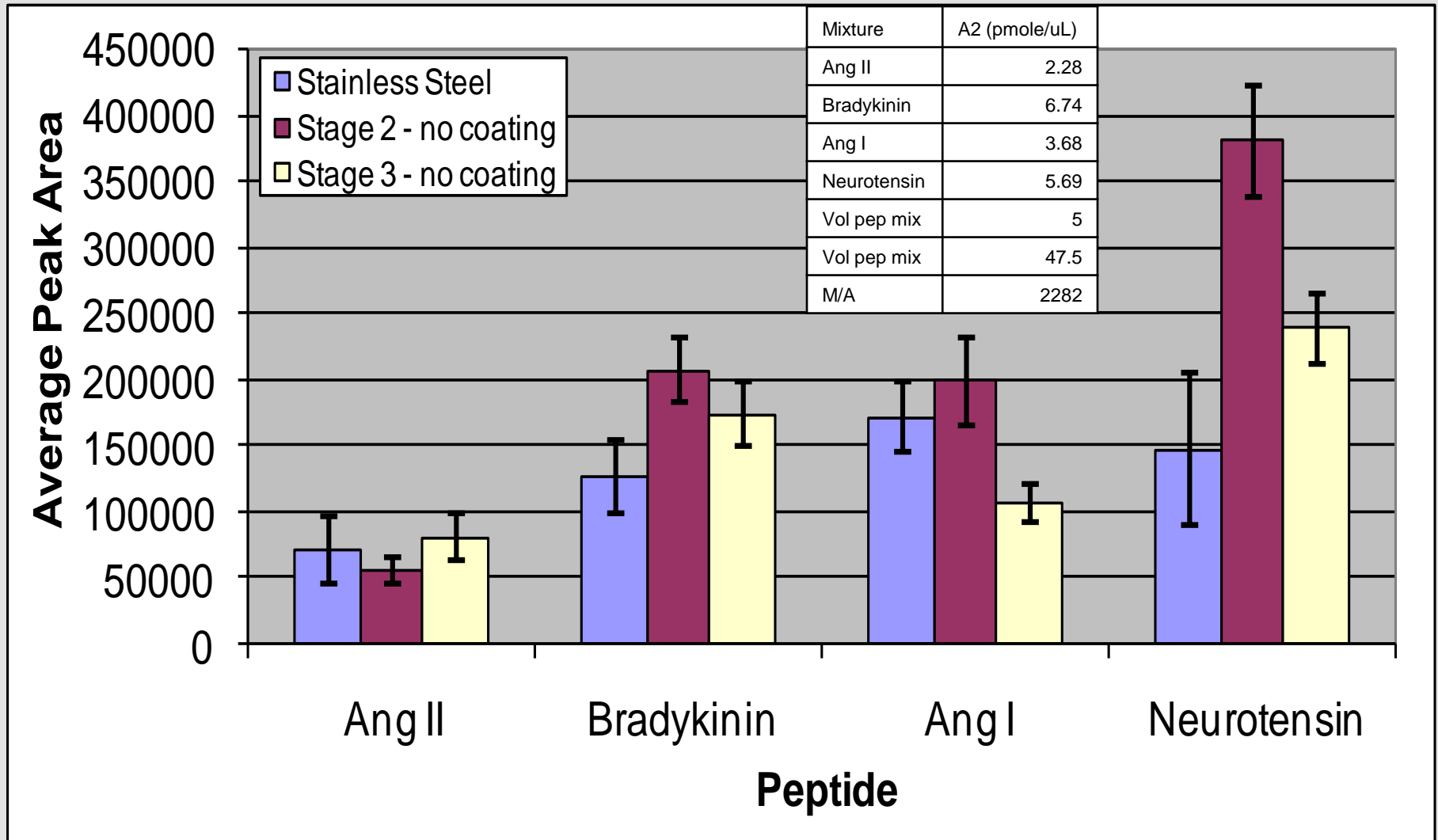
- The mass scale of the instrument was calibrated using the calibration routine which is part of the Bruker XTOF version 5.0.1 software.
- The 4 peptides were selected from a list which contained the exact masses of each peptide peak.
- Once all peaks were selected a linear fit was calculated.
- This procedure was then repeated twice for 3 peptides at a time in which Bradykinin, and Angiotensin I were excluded one at a time.

# Mass Accuracy



[1: 4 peptide calibration, 2: 3 peptide (excluding Bradykinin), 3: 3 peptide (excluding Ang I)]

# Micro-well Lifetime Comparison



CHCA matrix solution was made by dissolving 60.05mg of matrix into 2ml of MeOH  
 Stage 2 non-coated Micro-well (40um deep) MALDI sample plates were from May 2007  
 Stage 3 non-coated Micro-well (40um deep) MALDI sample plates were from Feb 2008

# Samples – Mass Resolution

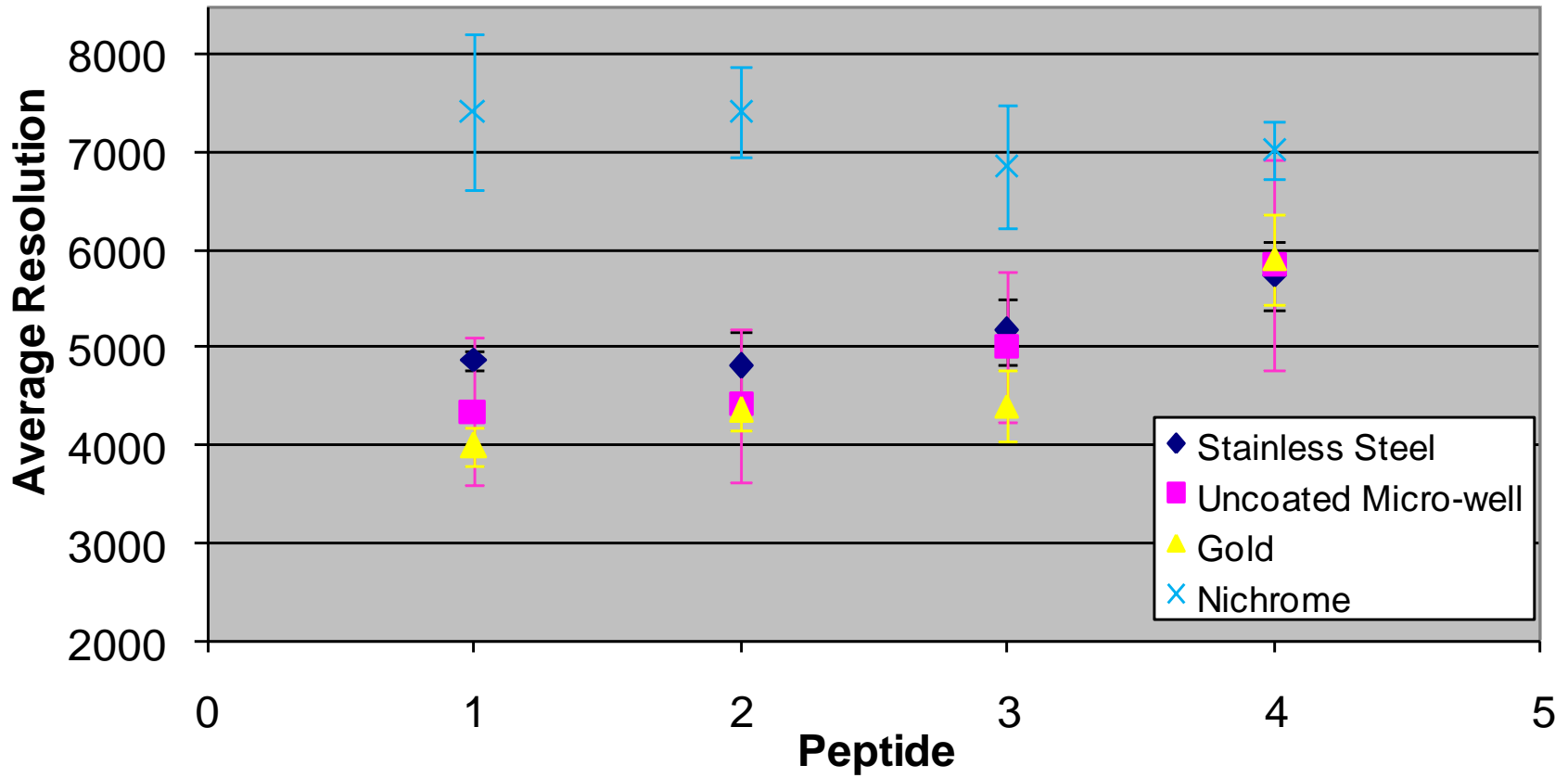
Peptide	A2 pmole/uL
Angiotensin II (1046)	18.20
Bradykinin (1060)	17.96
Angiotensin I (1296)	29.38
Neurotensin (1672)	22.77
Vol pep mix	5
Vol CHCA	47.5
M/A	1023

CHCA matrix solution was made by dissolving 18.89mg of matrix into 1ml of MeOH

Stage 3 Micro-well (40 $\mu$ m deep) sample plates with no coating, nichrome coating and gold coating from Feb 2008 were used

Instrument parameters were optimized for each plate.

## Resolution



# Conclusions- Experimental Design I

- Sample spots are very well contained within the Micro-well structure.
- Little peptide signal is observed without matrix. With matrix, good signal is observed.
- Higher mass resolution was observed for samples desorbed from the Micro-well sample plates when compared to the standard stainless steel plates.
- For the angle of incidence of the laser in the Bruker Reflex® III MALDI TOFMS instrument, the 68  $\mu\text{m}$  deep wells consistently produced the highest signal levels.
- Sensitivity enhancements of between a factor of 2-5 were observed on 5 selected analytes when compared to the standard targets.
- “Alkali scrubbing” by the Micro-well plate surface resulted in a 40-75% reduction in the area of the cationized analyte peaks observed.
- Adding a reflective coating to the Micro-well walls and bottom nearly doubles the sensitivity.

# Conclusions- Experimental Design II

- Concentration Effect
  - In general, Micro-well plates produced stronger signals than stainless steel plates at higher analyte concentrations
  - Micro-well plates, regardless of coating, were comparable to stainless steel at low concentrations
  - Slopes of the calibration curves indicate that the Nichrome and Gold coated Micro-well plates have greater sensitivity for certain compounds
- Mass Accuracy
  - Mass accuracy varied depending on the calibrants selected, but is comparable between Micro-well and stainless steel plates
  - In all cases the error is well below the specified mass accuracy of the instrument used
- Micro-well Sample Plate Lifetime
  - Micro-well plates continue to perform comparably to or better than stainless steel plates even after 1+ year of storage
  - Variations in Micro-well plate results over time may be related to processing parameters (i.e., conductivity of the Micro-well plates)
- Resolution
  - Nichrome coated Micro-well plates were consistently better than stainless steel
  - Gold coated and uncoated Micro-well plates are comparable to stainless steel plates