

*Immunochemical Characterization  
of Antigens and the Prediction  
of Efficacy*

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# Historical Immunochemical Characterization:

- **Potency by Direct Antigen Quantitation**
  - **Direct count of attenuated organisms**
  - **Sub-units/recombinants – ELISA**
  - **Complex antigens - RelPot**

**[dependent on precise determination of MID]**
- **Potency by Indirect Antigen Quantitation**
  - **Toxin/antitoxin relationships (tetanus)**
  - **Protection Models (*Pasteurella multocida*,  
*Leptospira* serovars, rabies)**

**[more qualitative?]**

# **Problems – contributing to variability:**

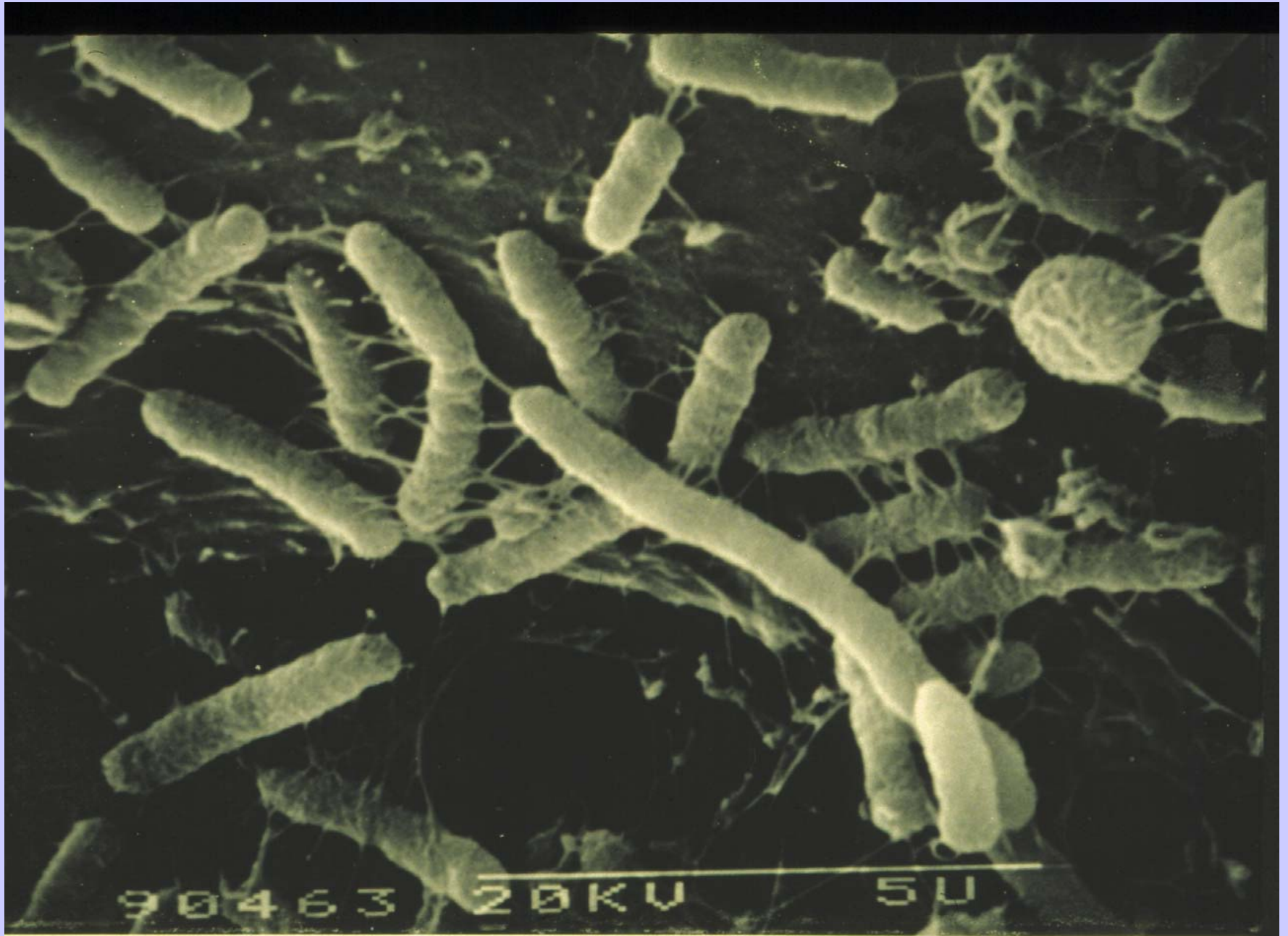
- 1. Complex, inactivated antigens**
  - Failure to account for multiple protective antigens**
  - Stability of multiple components**
- 2. Inconsistent challenge models**
- 3. Complexities of CMI – immunogenetics**
- 4. Strain, serovars – variable efficacy**
- 5. Species differences (mouse/cow – mouse/pig)**
- 6. Complex pathogenesis**
- 7. Biological complexity of the immune response**

***EXAMPLES:***

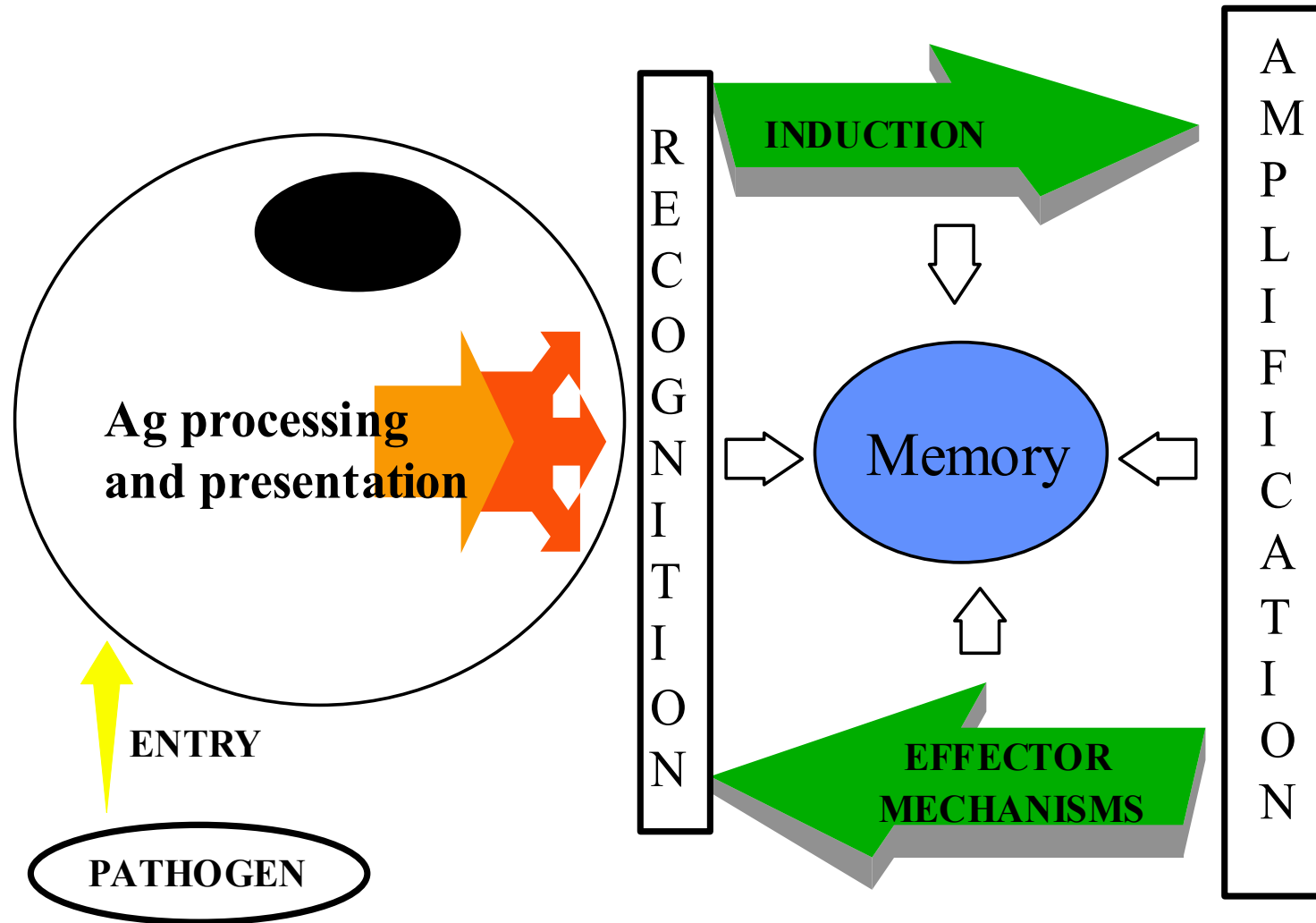
*Salmonella enterica* s. Typhimurium



*Salmonella enterica* s. Typhimurium

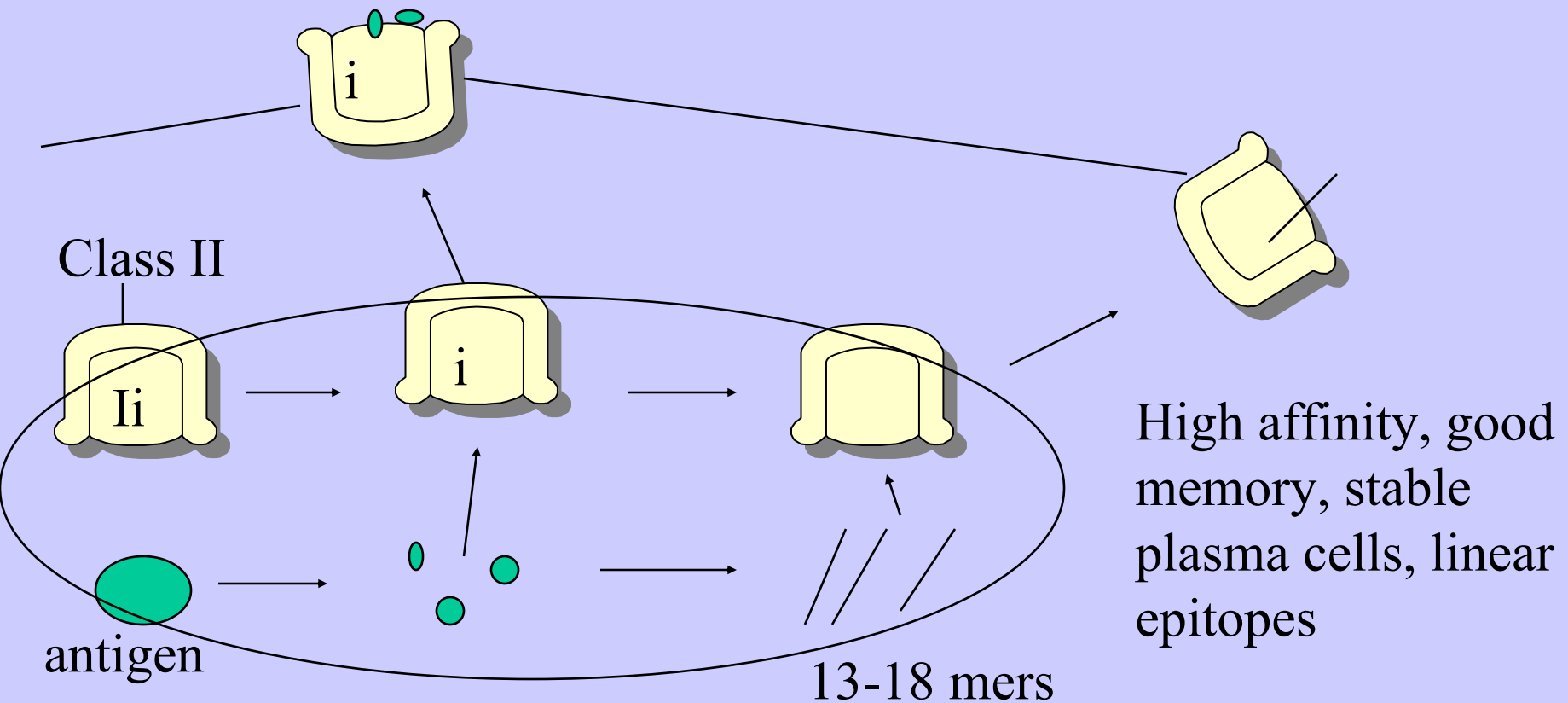


# Phases in the Development of Specific Immunity:



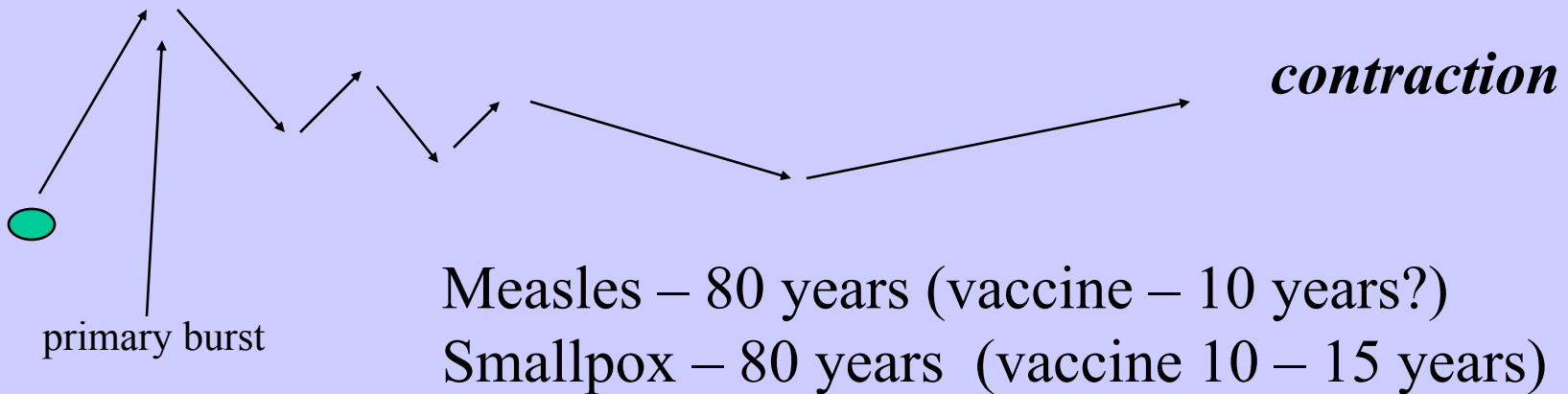
# APC - Phagolysosome/RER progression

Early, inefficient presentation, conformational epitopes, low affinity



# CD8 cellular expansion:

1. Naive cells - expand 1000 – 5000 fold (8 mitotic divisions) – *primary burst*
2. Only 10% survive in memory pools.
3. Cycles of maintenance



# **lymphocyte cellular contraction:**

- 1. Contraction is programmed for immune responses to both bacterial and viral pathogens**
- 2. Contraction kinetics are independent of:**
  - a. the magnitude and duration of exposure (infection)**
  - b. amount of antigen displayed by dendritic cells**

# CD4-mediated regulation is critical :

1. With some peptide antigens, CD8+ cells will expand but not differentiate to mature active forms (binding avidity and cytolytic activity)
2. Anatomic “spread” may be restricted
3. Maturation of CD4 help may regulate physiological contraction

## ***CRITICAL:***

***Priming – 1. magnitude of primary burst***

***2. onset of appropriate CD4 function***

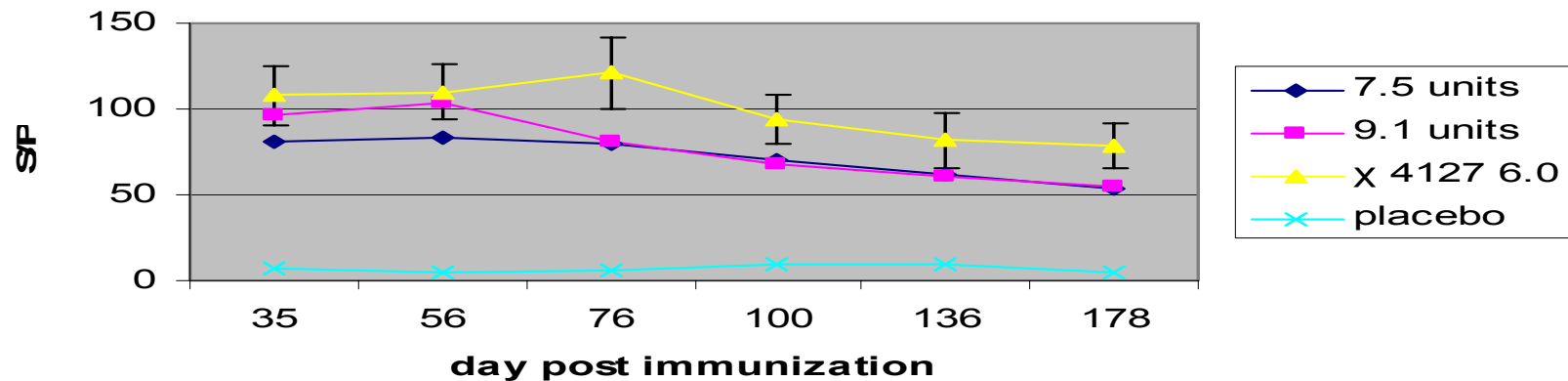
***Boost – delivery of antigen to key dendritic cell populations within functional memory period***

# Typical Vaccine Development :

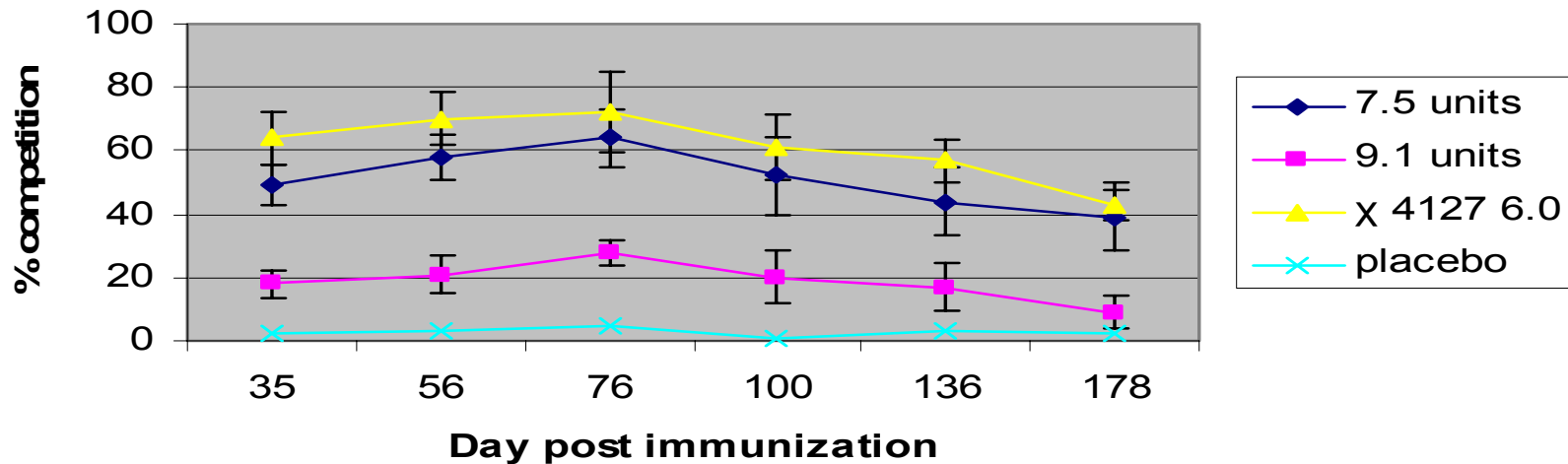
- 1. Formulation selection – minimum immunogenicity dose**  
**Often serologically based - cost of goods and manufacturing robustness is an issue.**
- 2. Efficacy challenge – onset of immunity and doi**
- 3. Safety – production ranges?**

<b>Vaccine</b>	<b>Dose/Potency</b>	<b>Serum IgG S/P</b>	<b>Competition %</b>	<b>Survival Day 7</b>	<b>Survival Day 21</b>
$\chi$ 4127	4.0 units	0.61 $\pm$ 0.14	53%	15%	15%
$\chi$ 4127	6.0 units	1.08 $\pm$ 0.17	64%	90%	85%
$\chi$ 4127	8.0 units <i>K9!!</i>	0.97 $\pm$ 0.26	51%	100%	100%
wt – inactivated, adjuvant	5.0 units	0.32 $\pm$ 0.15	24%	5%	0%
wt – inactivated, adjuvant	7.5 units <i>K9!!</i>	0.81 $\pm$ 0.21	49%	75%	65%
wt – inactivated, adjuvant	9.1 units	0.97 $\pm$ 0.41	18%	65%	50%
saline placebo	0	0.05 – 0.09	2%	0 %	-

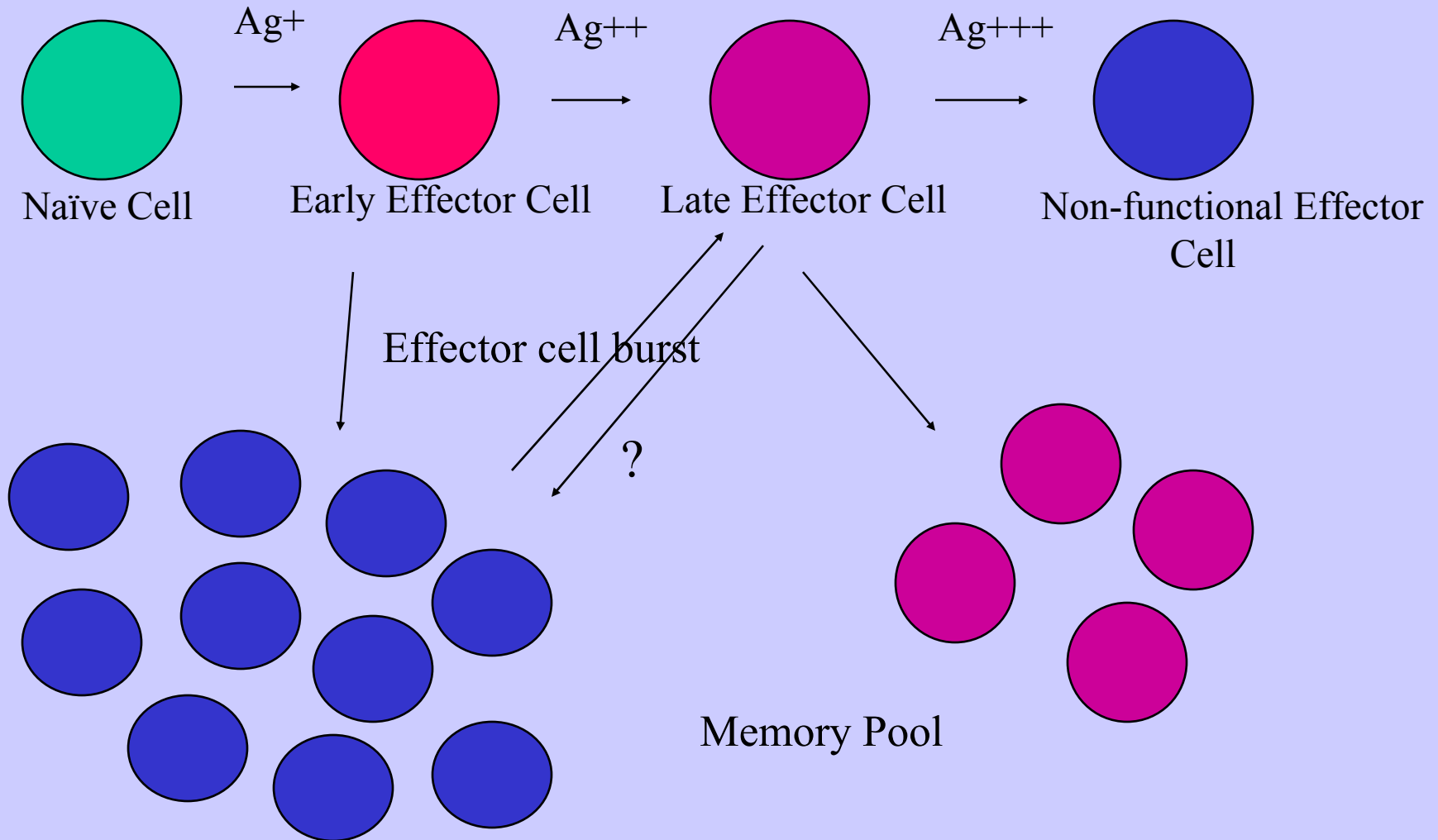
### Serum IgG S/P



### Serum IgG Comp

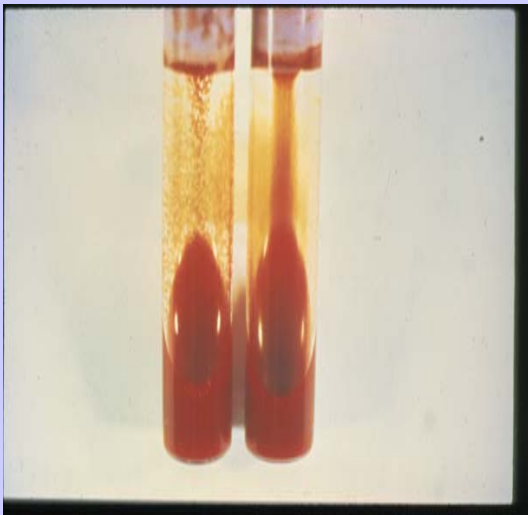


# Limited-Potential Hypothesis



By-stander stimulation  
of B-cells, general  
increase in IgG  
production, auto-reactive  
clones!

Effects on other responses?

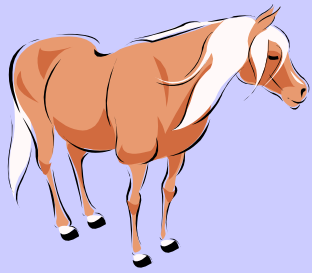


Vaccine	Rabies Titers	RF Test +	NPssDNA
7.5 units	↓ 10% – 16 %	3/20	0/20
9.1 units	↓ 18% - 42%	12/20	14/20
χ4127	↓ 8% – 17 %	1/20	1/20
placebo	~ 0%	0/20	0/20

# Equine Log 2 SN Titers



<b>Vaccine</b>	<b>Day 0</b>	<b>Day 28</b>	<b>Day 365</b>	<b>Re-Call 1 yr</b>	<b>Day 1100</b>	<b>Re- Call 3 yrs</b>
A1	< 0.2	$6.7 \pm 0.2$	$3.2 \pm 0.8$	$5.8 \pm 0.7$	$2.0 \pm 0.7$	$4.3 \pm 0.6$
A2	< 0.2	$6.9 \pm 0.4$	$2.9 \pm 0.7$	$5.0 \pm 0.4$	$1.8 \pm 1.1$	$4.0 \pm 0.3$
B1	< 0.2	$3.4 \pm 0.8$	$2.5 \pm 0.3$	$4.3 \pm 0.5$	$2.3 \pm 0.8$	$4.6 \pm 0.4$
B2	< 0.2	$3.8 \pm 0.2$	$2.7 \pm 0.4$	$4.7 \pm 0.1$	$2.1 \pm 0.4$	$4.4 \pm 0.7$
C	< 0.2	$6.0 \pm 0.5$	$2.4 \pm 0.6$	$3.7 \pm 0.4$	$1.6 \pm 0.9$	$3.1 \pm 0.8$



## Testing of multiple serials!

Vaccine	Day 0	Day 28
A1	$< 0.2$	$6.7 \pm 0.2$
A2	$< 0.2$	$6.9 \pm 0.4$
A3	$< 0.2$	$4.0 \pm 0.2$
A4	$< 0.2$	$3.2 \pm 1.2$
A5	$< 0.2$	$7.3 \pm 0.7$

## *The Key Questions:*

- 1. Considering the complexity of infectious disease and immune responses, can we develop characterizing assays that robustly predict the quality (efficacy/effectiveness) of immune responses? (true immunological strength)**
- 2. Can we reduce the time required to generate data and reduce the amount of in vivo testing?**

## *Possible Approaches:*

### **1. Antigen Presentation models**

- **In vitro models of macrophages and dendritic cells**
- **Antigen degradation – traffic flow**
- **Co-stimulatory molecule expression**
- **Cytokine responses**
- **Ability to stimulate B-cell and T-cell clones**
- **Species-specific secondary lymphoid tissue models**

**Characterize “reference” serials in such assays  
– use for re-qualification, stability**

## *Possible Approaches:*

### **2. Serological Evaluation (Effector T-cells)**

- **Affinity (binding avidity), maturation and memory**
- **Clonal selection and balance**
- **Primary burst kinetics**
- **Stable plasma cells (CTL's)**
- **Functional characterization – appropriate specificities**

**Highest titer not always the best titer!**

## *Possible Approaches:*

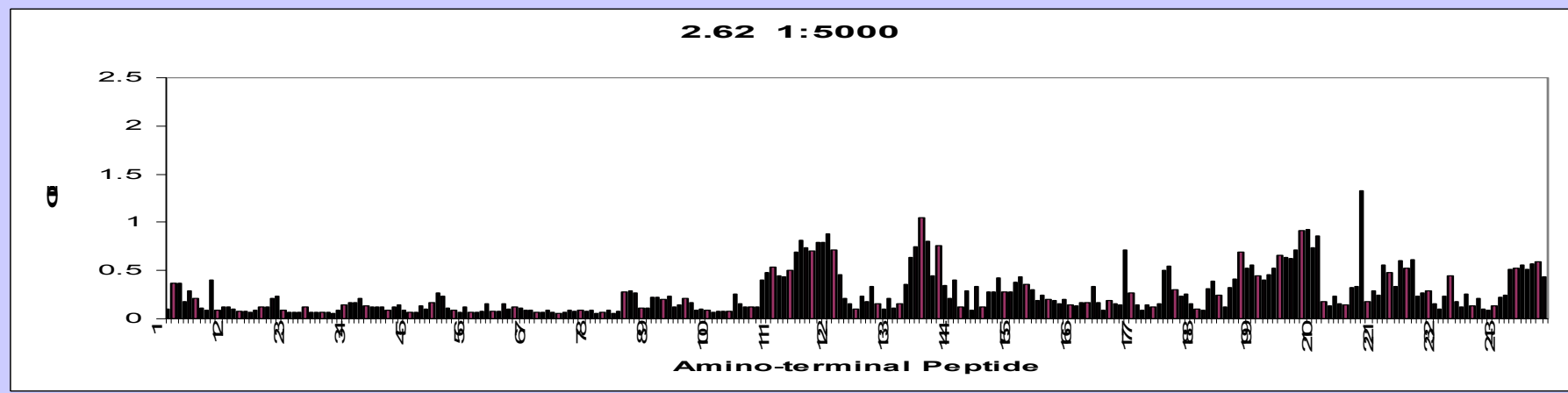
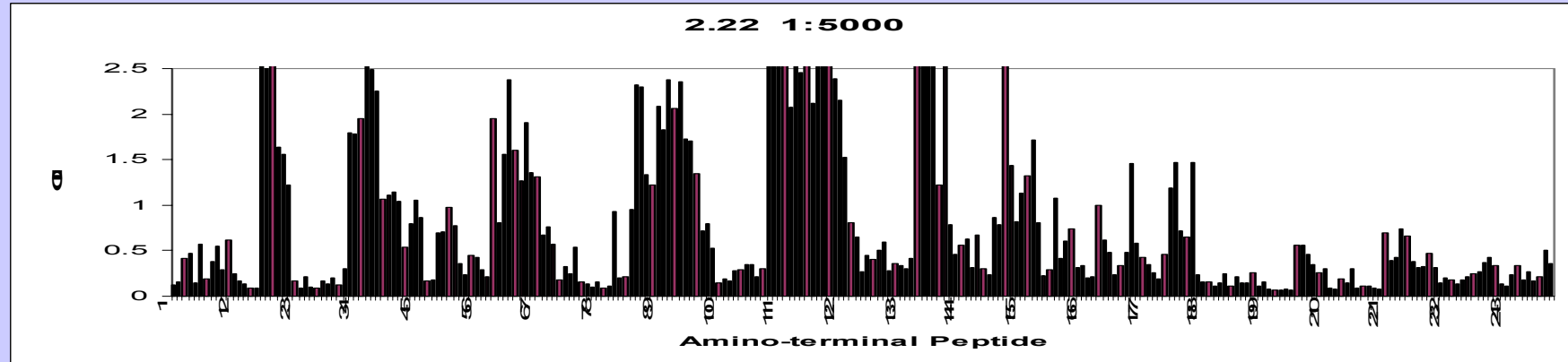
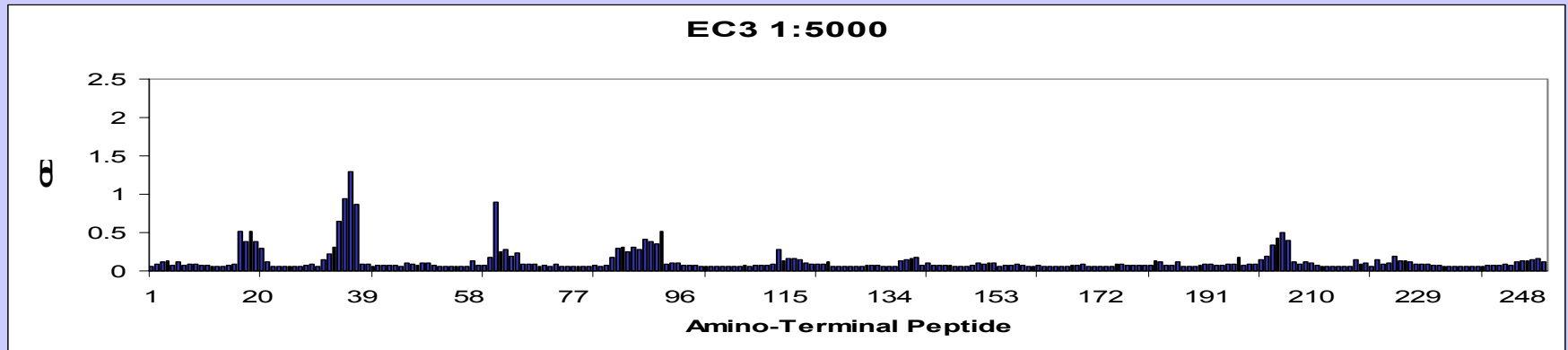
### **3. Physical/Chemical Characterization**

- **Emulsion/adjuvant/antigen stability**
- **Antigen conformation**
- **Degree of “degradability”**
- **Red/Ox properties**
- **Preservative interactions**
- **Cellular toxicity models**
- **Many others**

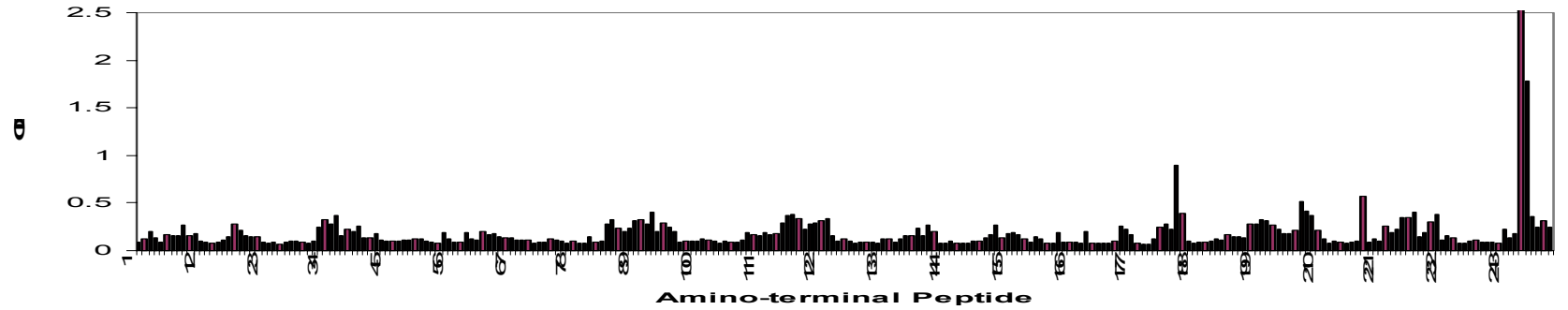
***CONSIDER: Consider a period of post-registration characterization of responses to refine long-term potency/stability plans.***

## *Example:*

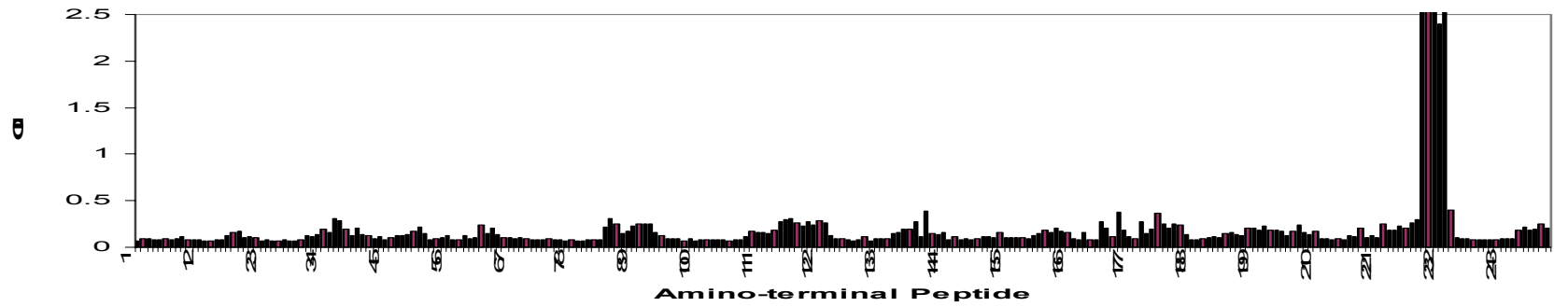
- **Epitope mapping of recombinant antigen to define the most appropriate and formulation-specific epitopes for classic in vitro potency**
- **Relevance to “neutralization”**
- **Mice vs. cattle vs. goats**
- **Molecular surprises!**



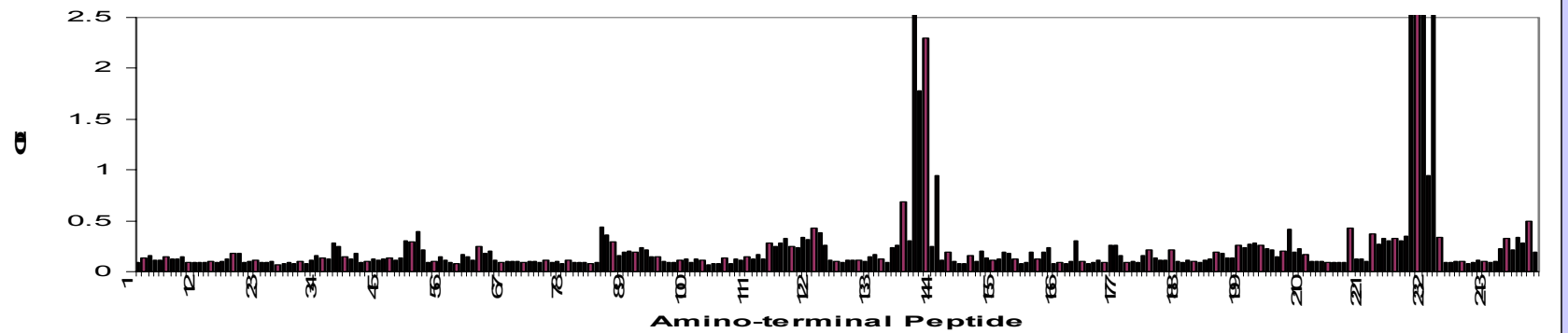
1.89 1:5000



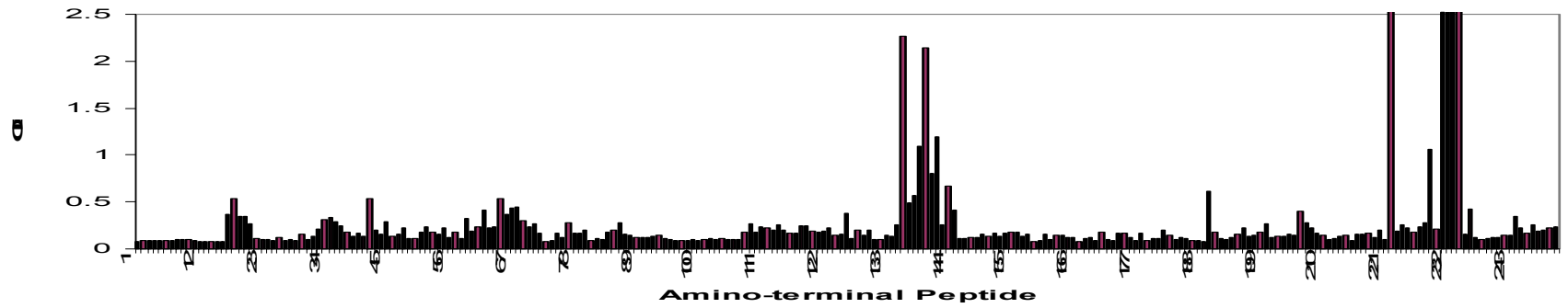
1.85. 1:5000



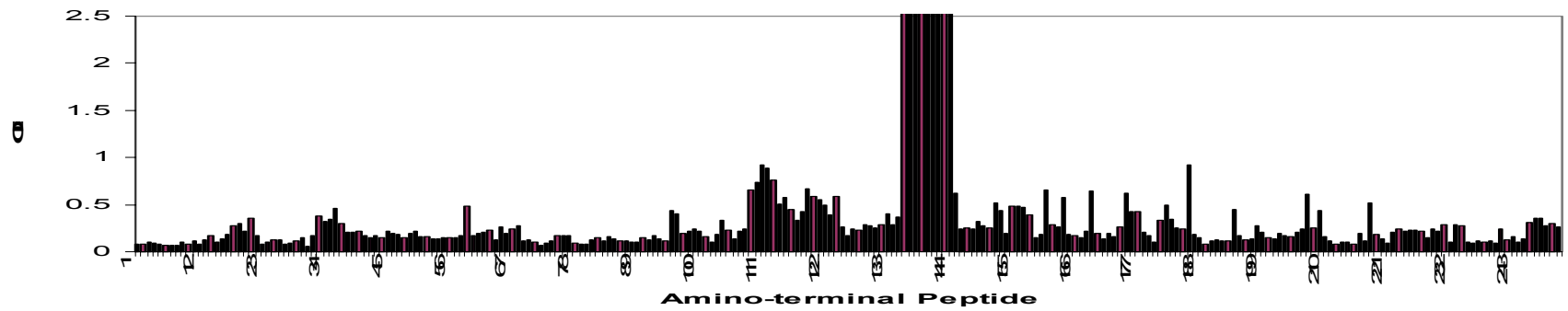
2.14 1:5000



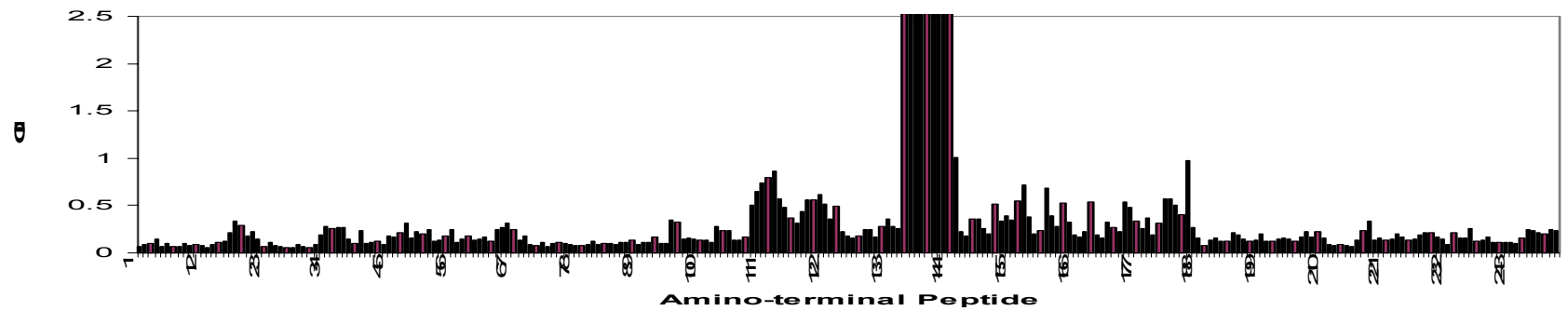
2.32 1:5000



2.18 1:5000



2.46 1:5000



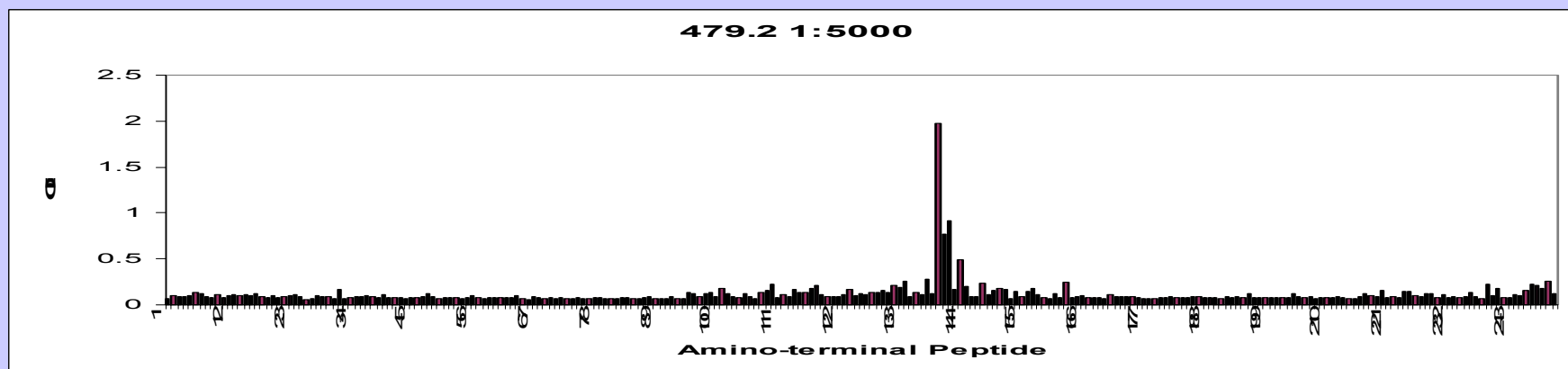
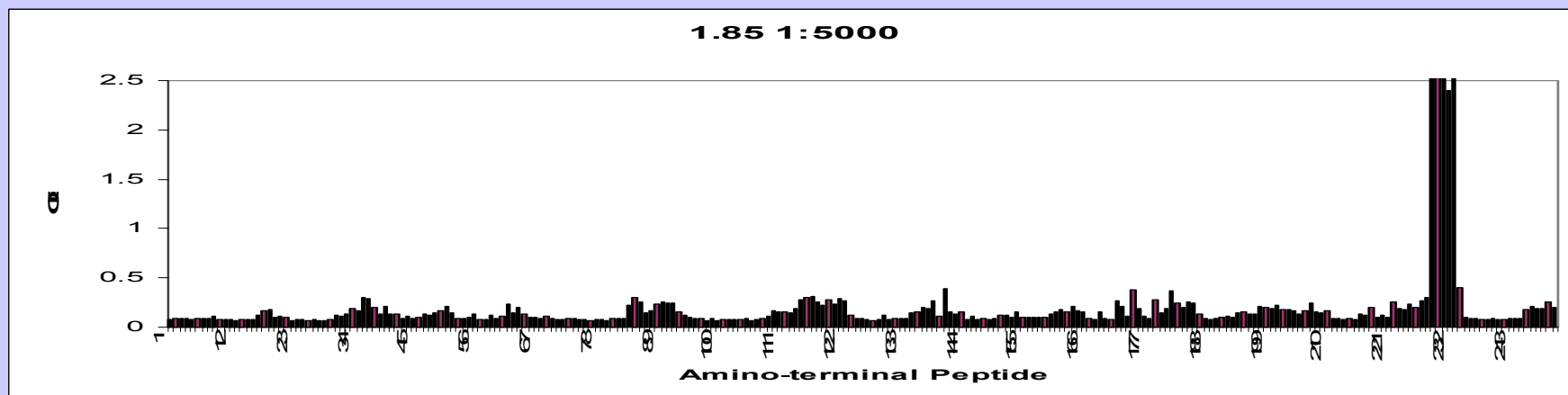
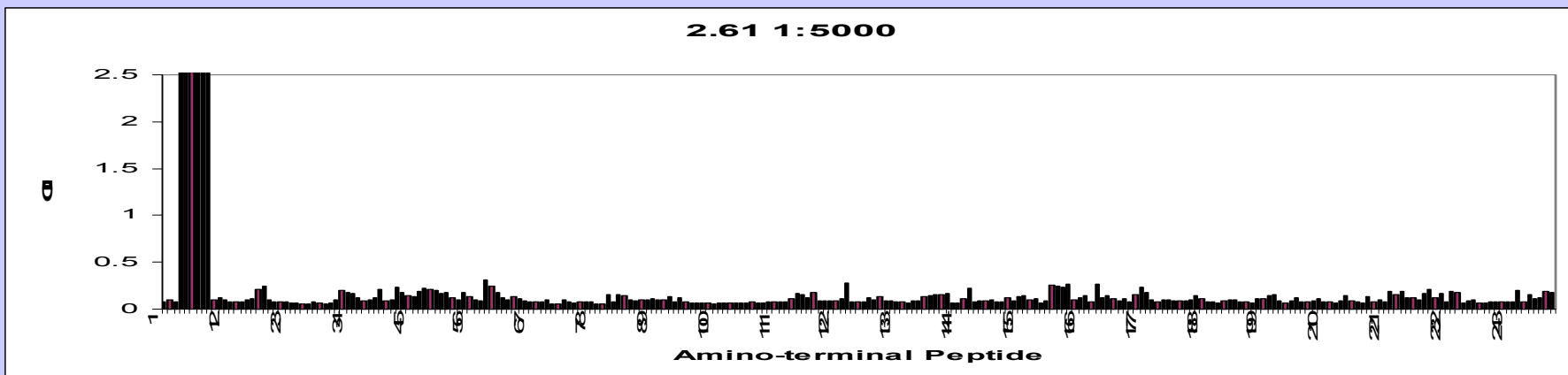


Fig 2

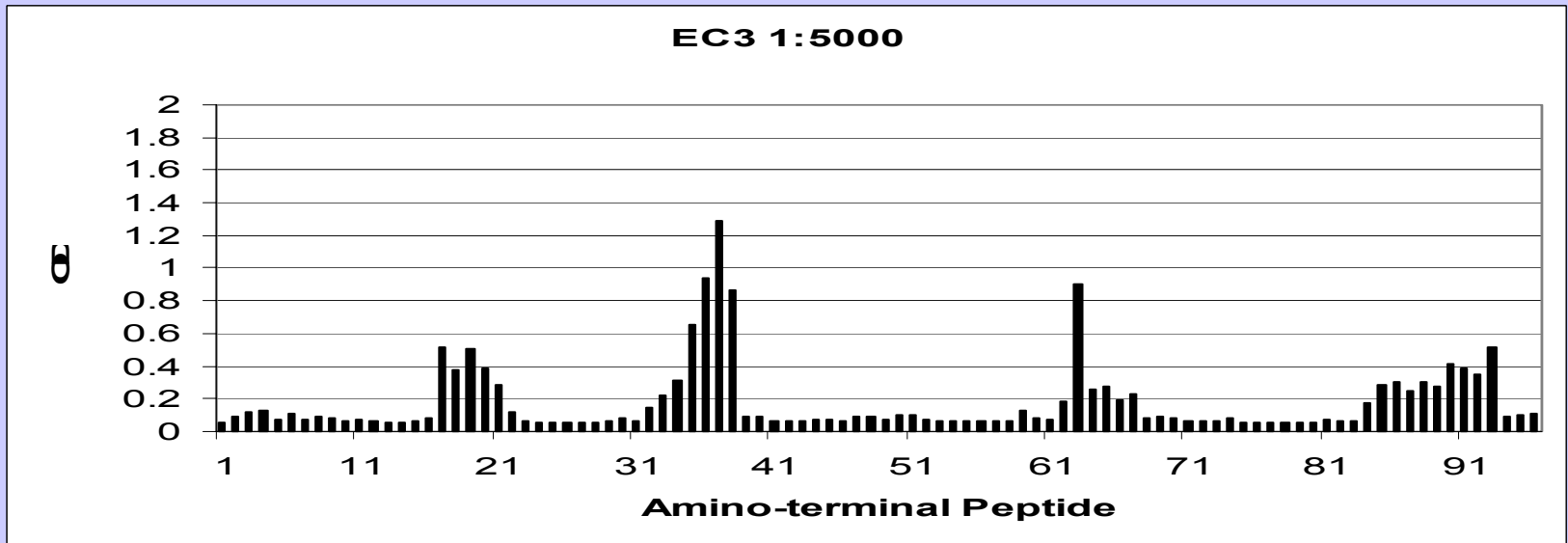
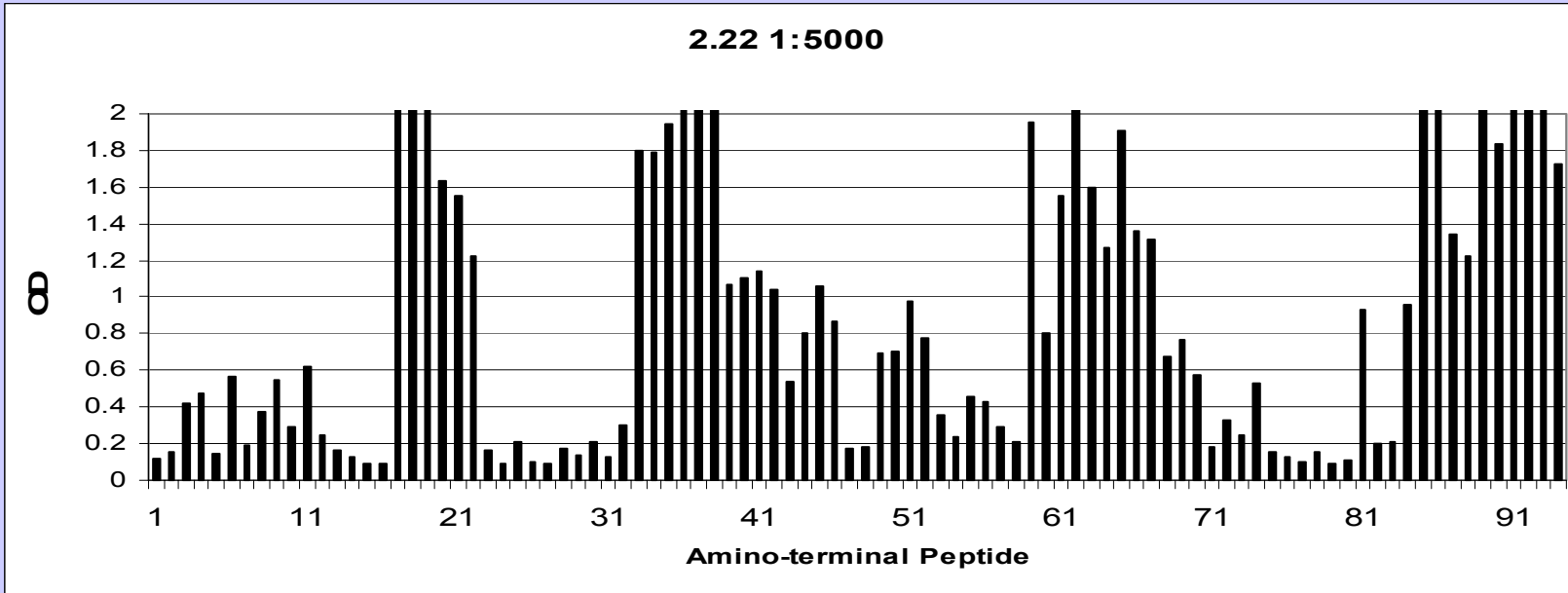
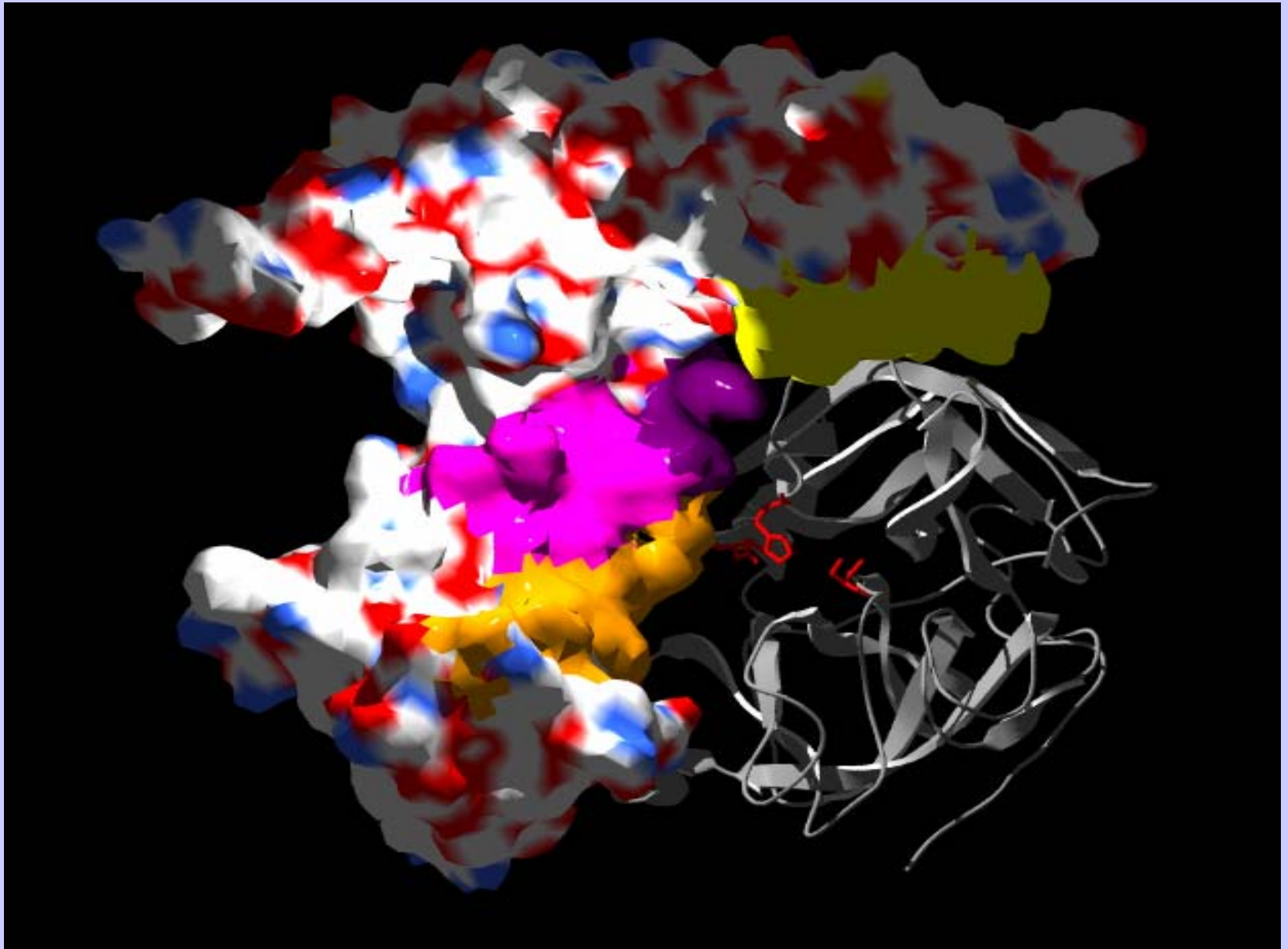


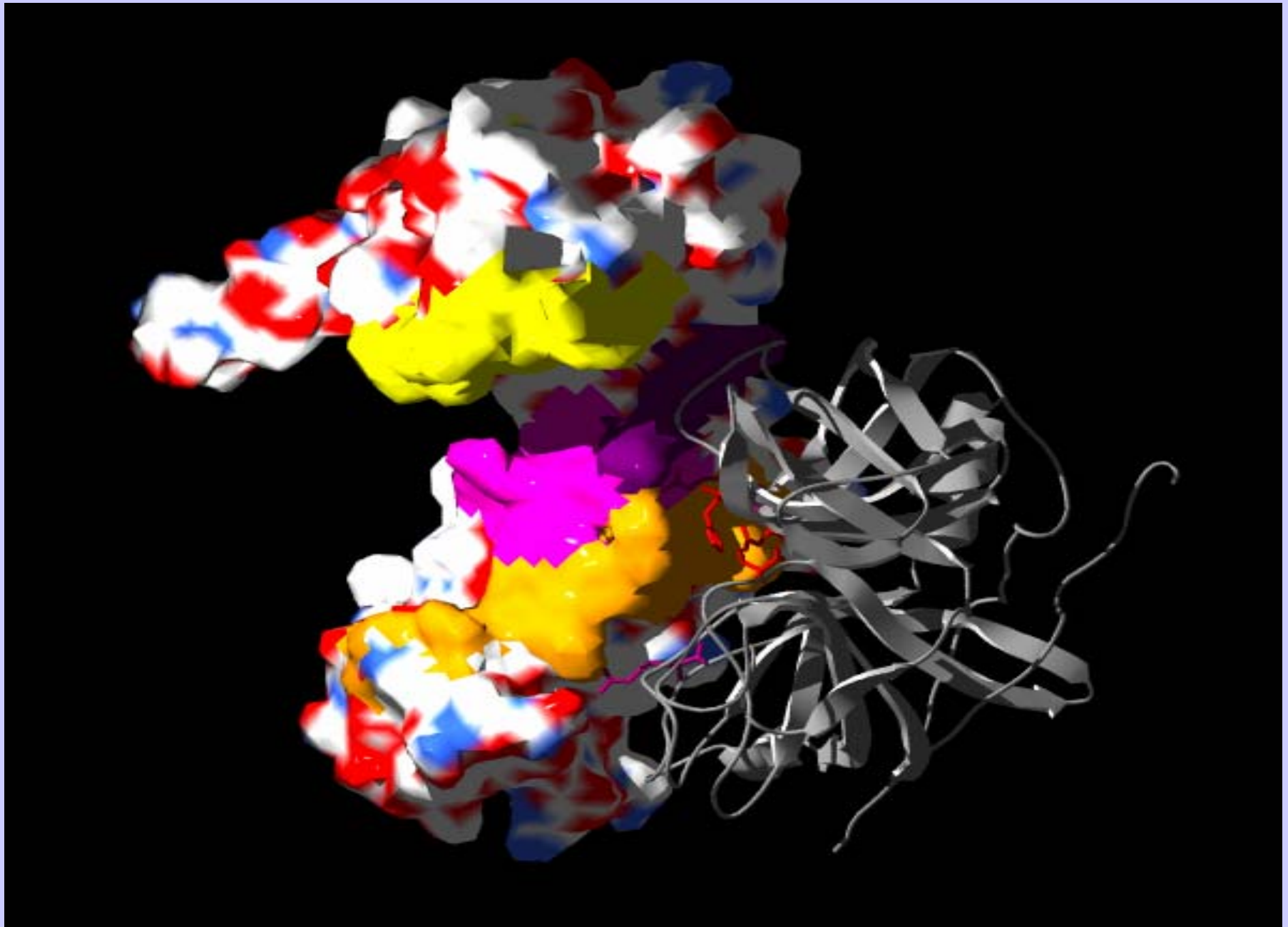
Fig 3

37 -- I L I R G I H H V L T ---  
                  +           + +  
                  -           + +  
59 -- - L L D A I R H Q M V ---

Front:



Side:



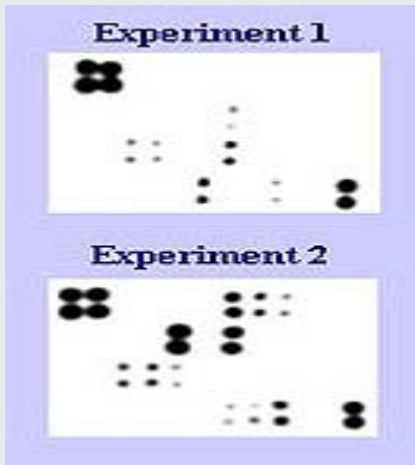
- TABLE 4. Inhibition of EC3 or 2.22 Binding to recombinant protein
- Inhibition of binding of conjugated mAb1:

Blocking mAb	2.22	EC3	Goat	Bovine
• 2.22	99%	98%	+++	+++
• EC3	67%	96%	++	+++
• 2.62	22%	35%	-	-
• 1.89	53%	9%	+	+
• 1.85	34%	8%	++	++
• 2.14	12%	21%	-	+/-
• 479.2	30%	12%		
• 57.1	26%	28%		
• 2.32	14%	31%		
• 2.18	6%	4%		
• 2.46	5%	8%		
• 2.61	4%	17%	+	-

# ***CONCLUSIONS:***

- 1. Potency testing dependent on antigen quantitation is useful and has been successful. Refinement of serological tools will improve the power of such assays.**
- 2. There is a need to mechanistically characterize immunogenic formulations and the technology is becoming more available . (Quality – not just quantity)**
- 3. This would be particularly useful for stability testing, reference re-qualifications, necessary production process changes (i.e. – reduction and removal of animal origin components), and addition of new antigens.**
- 4. Consider post-registration assay development to sustain success!**

# Technology:



@Biacore

RayBio™ Inflammation Antibody Array I and 1.1

**RayBiotech, Inc.**  
The Protein Array Pioneer Company

