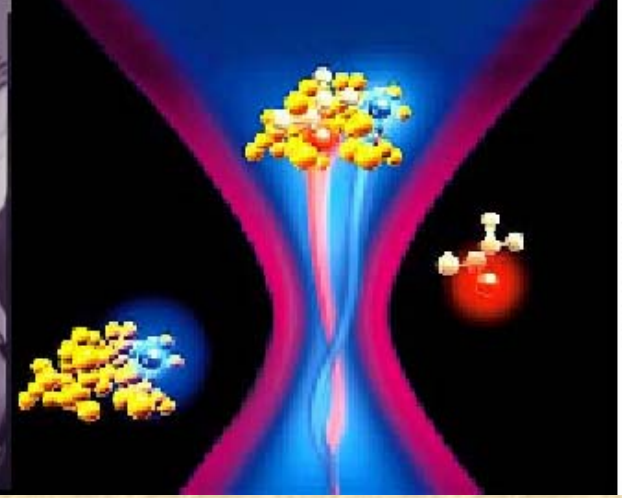
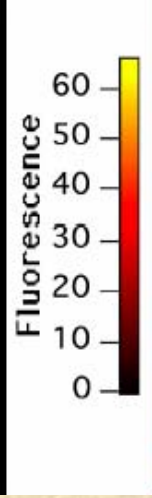
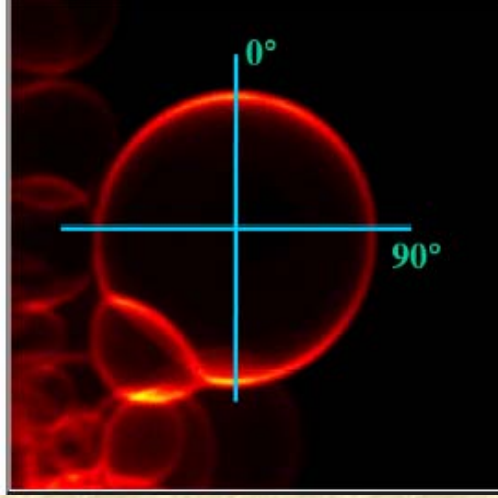






Fluorescence Workshop
UMN Physics
June 8-10, 2006

Introduction to Fluorescence Essays
Joachim Mueller

Where is Fluorescence Used?

Microscopy

Drug Discovery

Chemical Analysis

Enzymatic Assays

Contamination Detection

Environmental Monitoring

Molecular Organization

Why is Fluorescence Used?
Advantages of Fluorescence Based Analysis Systems

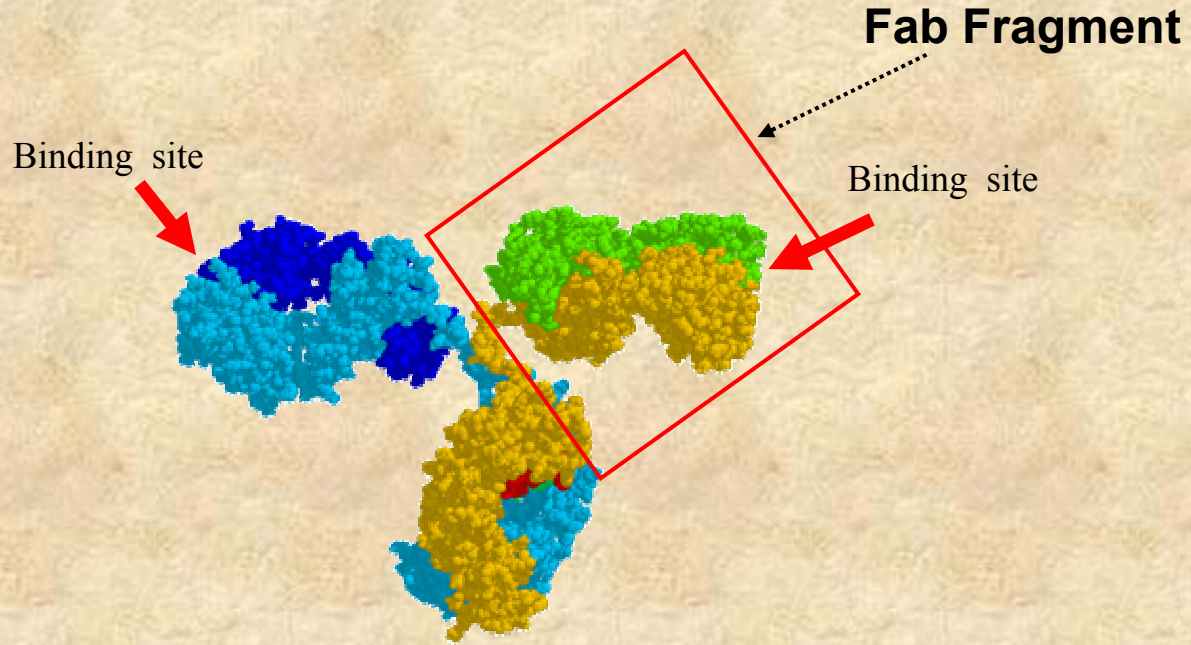
1. Sensitive
   - High signal to noise ratio
   - Single molecule sensitivity
2. Flexible
   - Broad array of possible parameters to measure (Intensity, Spectra Shifts, Anisotropy, Decay parameters, FRET, …)
3. Information can be multidimensional
   - Use of multiple probes or probes that report a variety of environmental parameters.
4. Real-time data acquisition
   - System dynamics
   - Kinetic events
5. Monitoring can be distant
   - Chambers for harsh environmental conditions

Biochemical & Chemical Assays for Detection

The Ideal Assay

1. Appropriate sensitivity for real-world samples
2. Recognition only to the target molecule
3. High signal/noise ratio
4. Low volume requirements
5. Fast reaction completion
6. Fast sampling

A Robust General System: Immunoassays

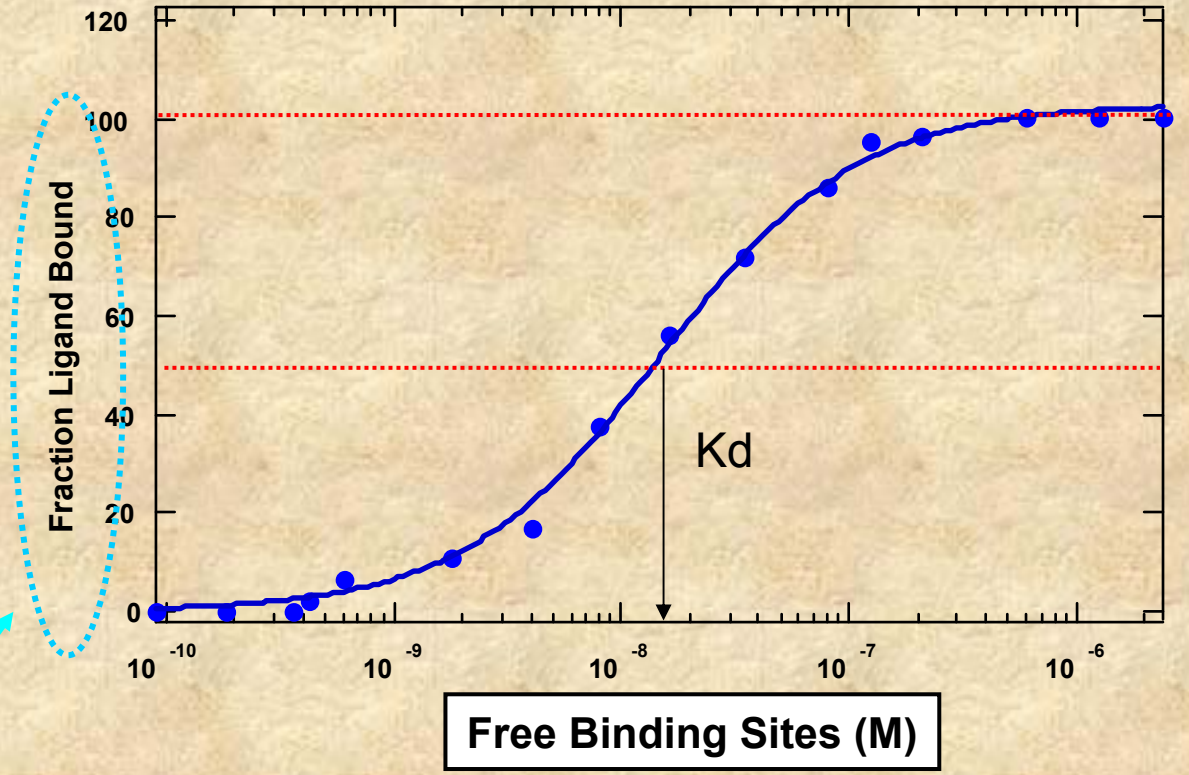


Mouse IgG: The two heavy chains are shown in yellow and light blue. The two light chains are shown in green and dark blue..*J.Harris, S.B.Larson, K.W.Hasel, A.McPherson, "Refined structure of an intact IgG2a monoclonal antibody", Biochemistry 36: 1581, (1997).*

Many Recognition Assays Rely on an Equilibrium Condition

$$K_d = \frac{[Sites_{free}][Ligand_{free}]}{[Sites \bullet Ligand]}$$

$$F_b = \frac{m \cdot S_{free}}{K_d + S_{free}} + c$$



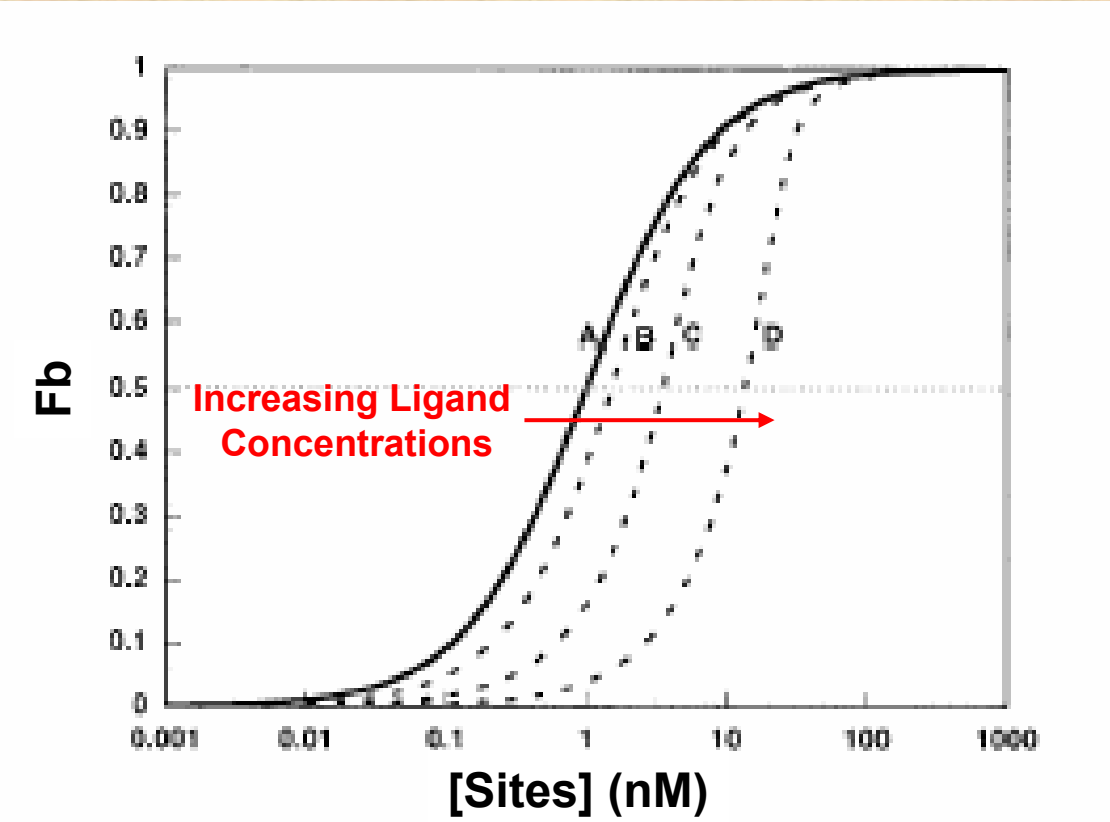
Critical Point

What should you titrate?

Adapted from S. Tetin, K. Swift, & , E, Matayoshi

Common Errors in Binding Studies

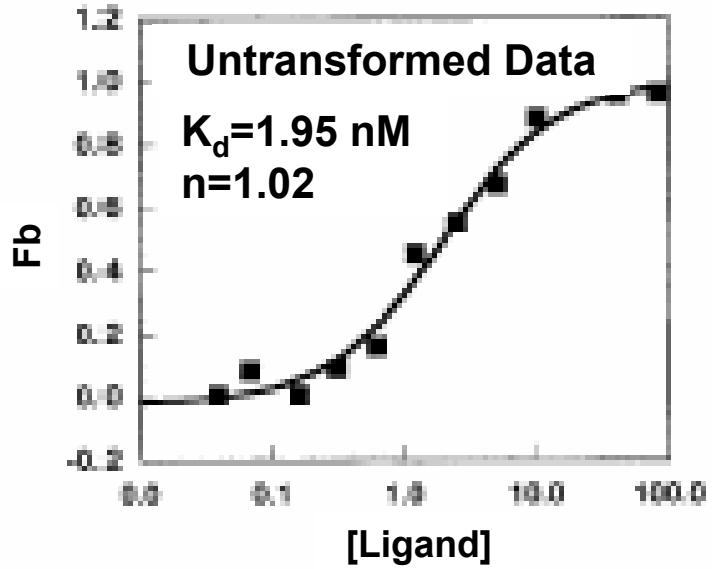
Concentrations Too High !

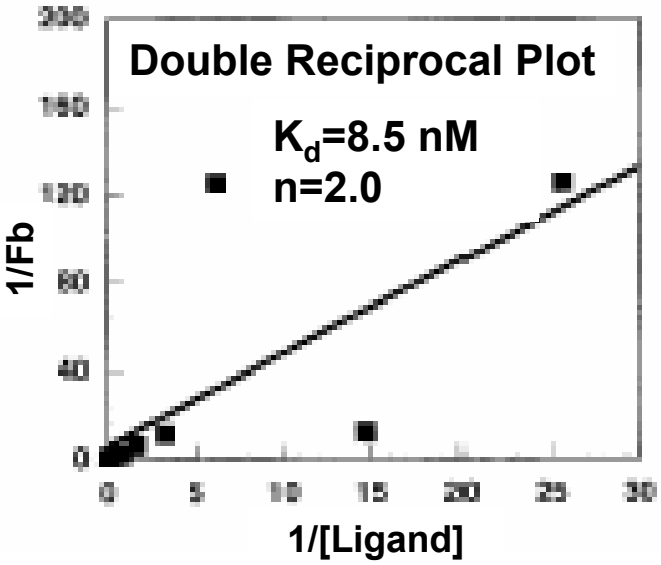


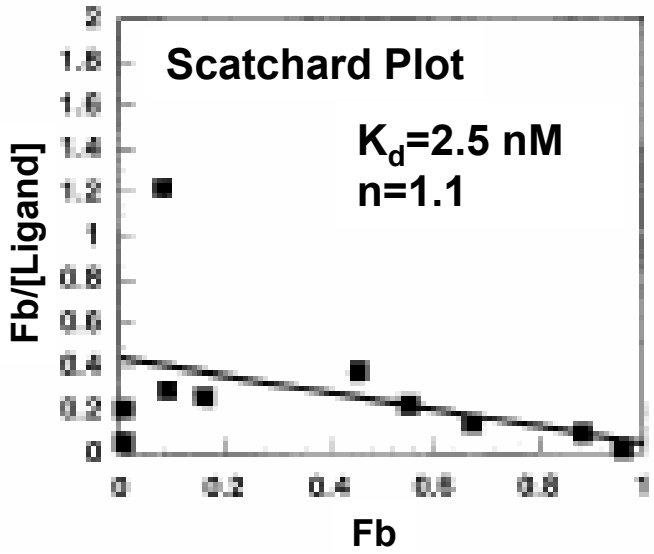
Tetin & Hazlett (2000) Methods 20, 341-361

Errors in analysis: data transformation

Theophylline & anti-Theophylline IgG. K_d = 2 nM & n=1





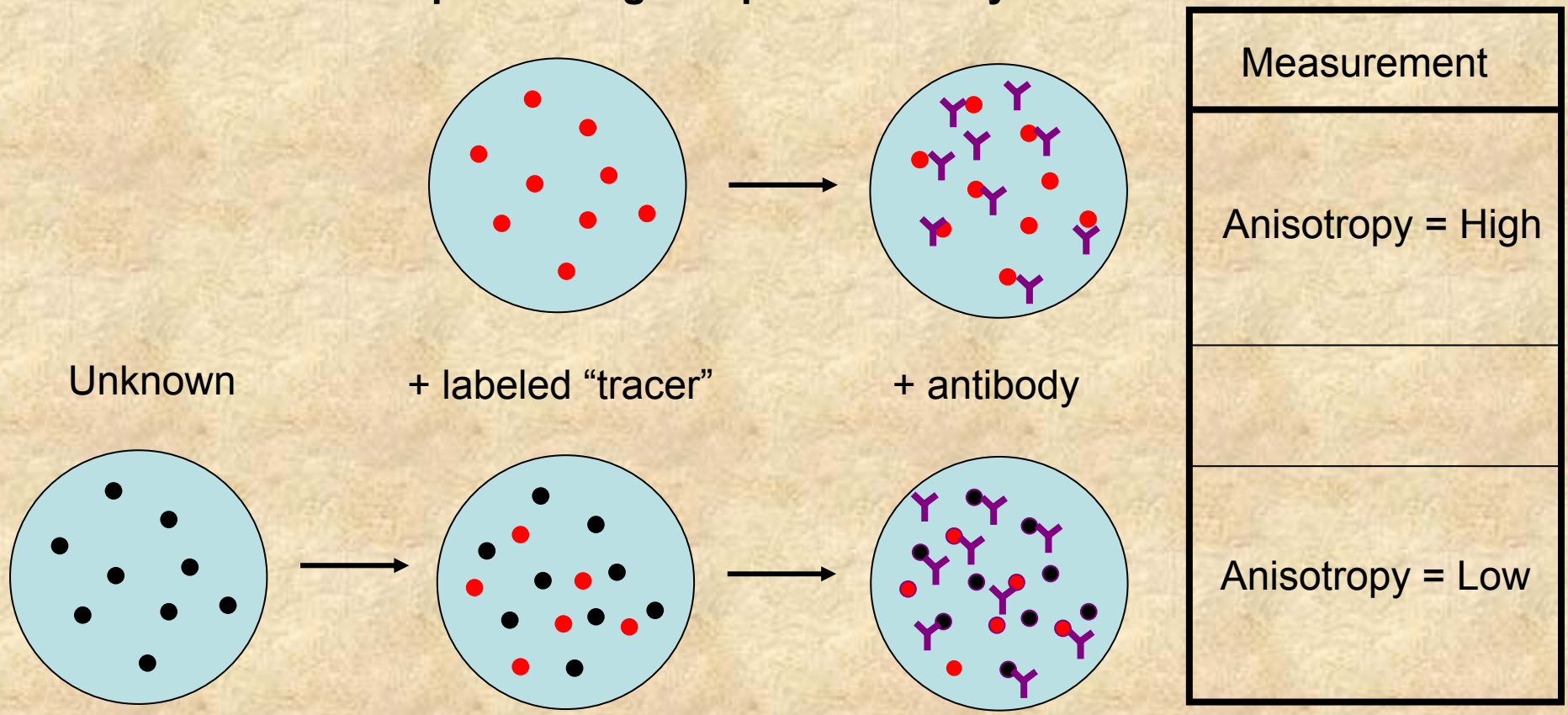


Tetin & Hazlett (2000) Methods 20, 341-361

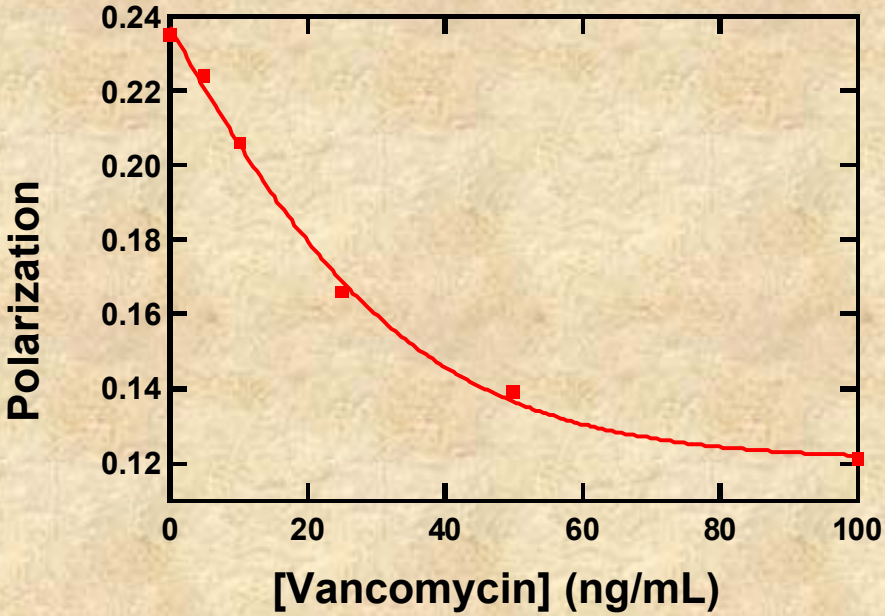
How do we create an assay?

The test samples do not have labeled material, do they?

1. The simple binding competition assay:



Abbott Laboratory Polarization Assay



Vancomycin (ng/mL)	Polarization	[Tracer] bound (%)
0	0.234	100
5	0.223	91
10	0.207	76
25	0.167	41
50	0.139	16
100	0.119	0

Calibration curve for a vancomycin immunoassay. Vancomycin tracer and varying concentrations unlabeled vancomycin were added to sera and polarization values were collected using the TDx assay instrumentation. Curve was fit to a 4-parameter logistic function (empirical fit).

Considerations

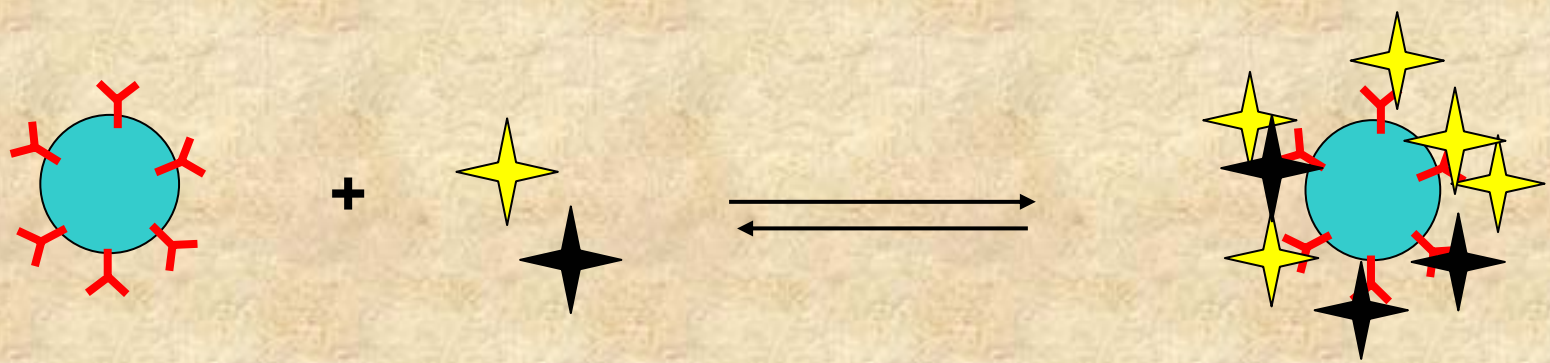
Speed
Accuracy
Background contributions
Measuring errors

S.Y. Tetin et. al. Analytical Biochemistry 307 (2002) 84–91

Solid Support Immunoassays

Nanoparticle Immunoassays employing fluorescence correlation spectroscopy

Competitive Immunoassay



Particle with IgG attached

Fluorescent Antigen & Antigen

Complex Slower Diffusion & Bright

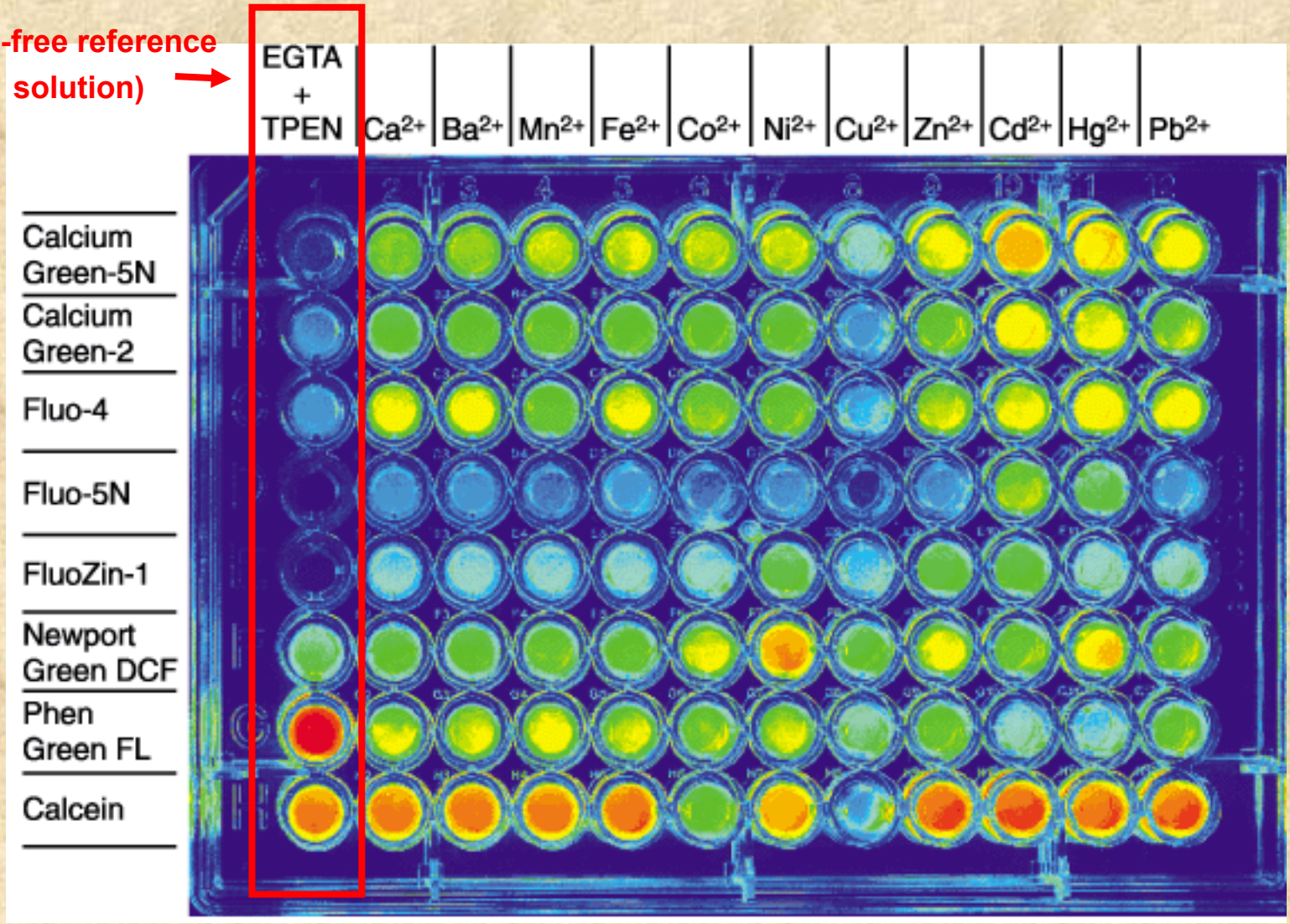
Potential Advantages

Fast separation of soluble, unattached fluorescent antigen

Large, dilute volumes can be assayed (depends on K_d)

Adapted from Meyers-Almes (2001), in Fluorescence Correlation Spectroscopy, Rigler & Elson, Eds.

Ion Probes Specificity

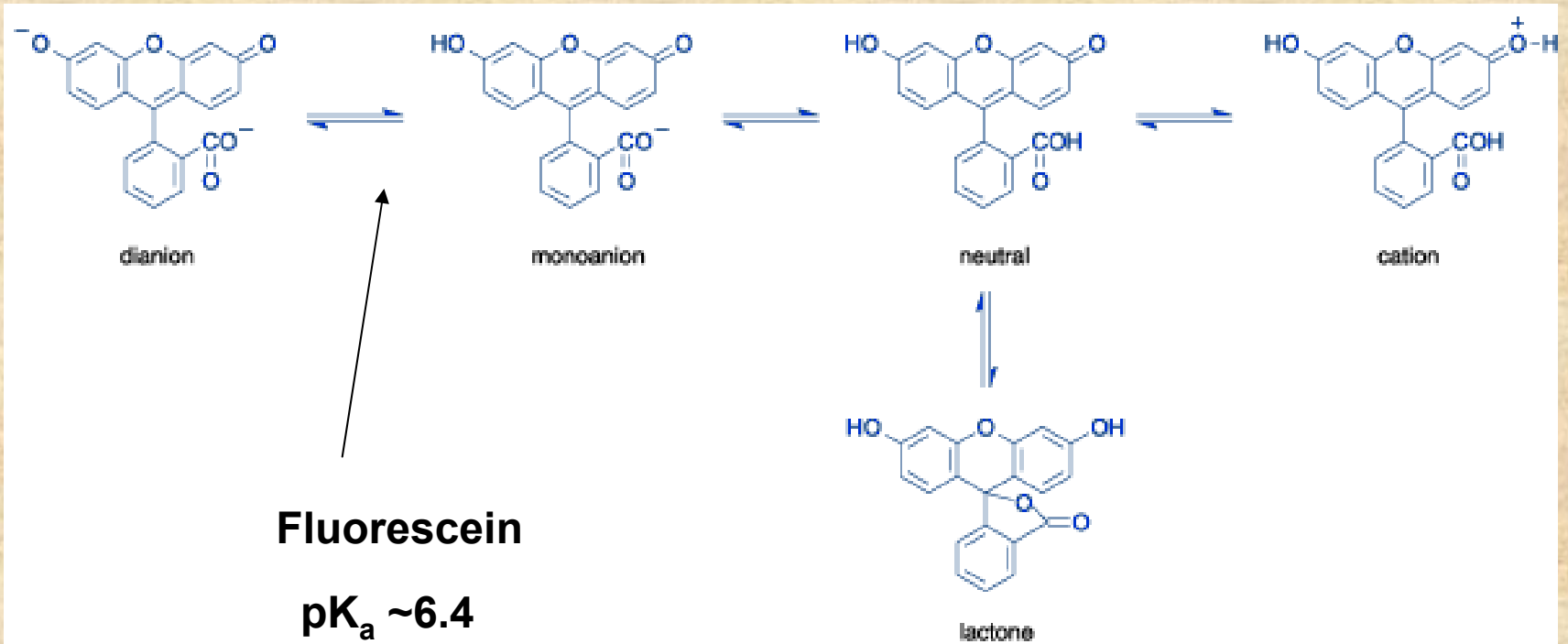


(Fluorescence: high = red > orange > yellow > green > blue = low).

Molecular probes, www.probes.com

Measuring H^+

Probes with titratable groups can have pH dependent spectra



Fluorescein Data