


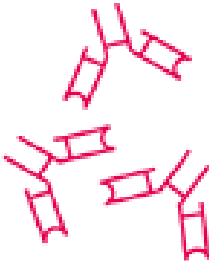
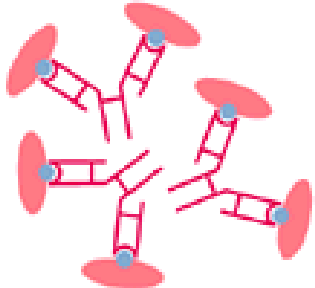

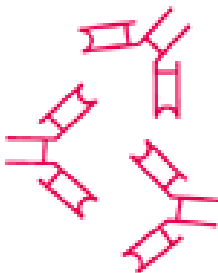
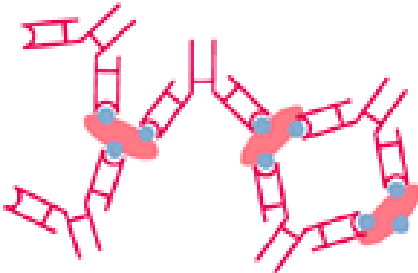
# Utilisation des anticorps *in vitro* principes et techniques

Adrien Six (adrien.six@upmc.fr)

Université Pierre et Marie Curie

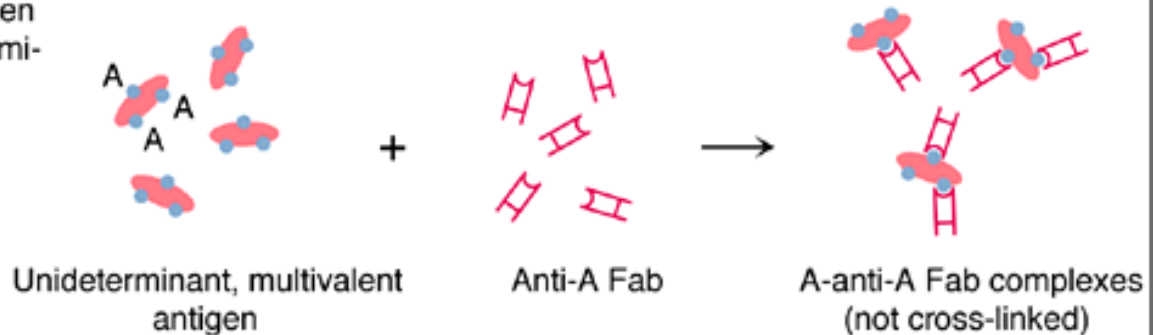
octobre 2004

# Réaction antigène/anticorps

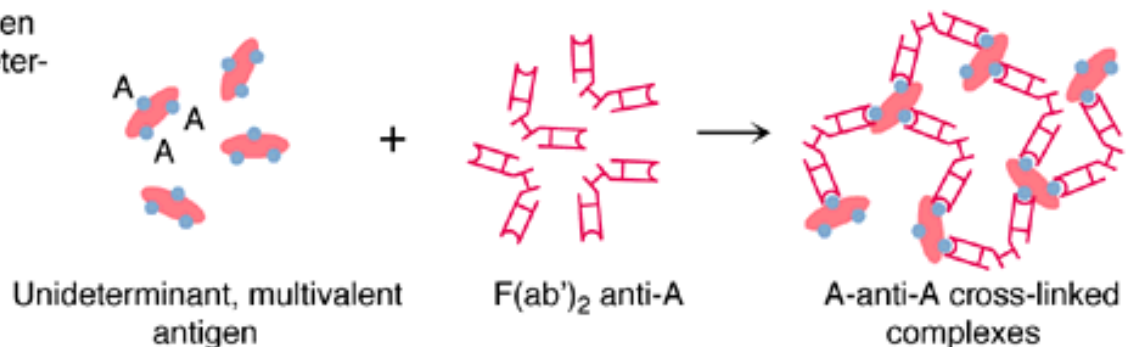
	Antigen/Hapten	Antibody/Fragment	
The reaction between antibody and a hapten.	 Univalent, unideterminant antigen (hapten)	 Anti-A	 A-anti-A complexes (not cross-linked)
The reaction between antibody and a unideterminant, multivalent antigen.	 Unideterminant, multivalent antigen	 Anti-A	 A-anti-A cross-linked complexes

# Réaction antigène/anticorps

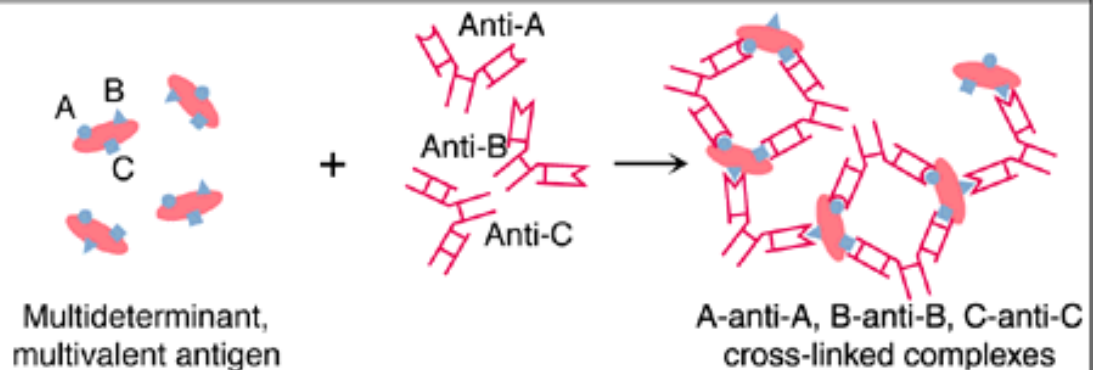
The reaction between Fab and a unideterminant, multivalent antigen.



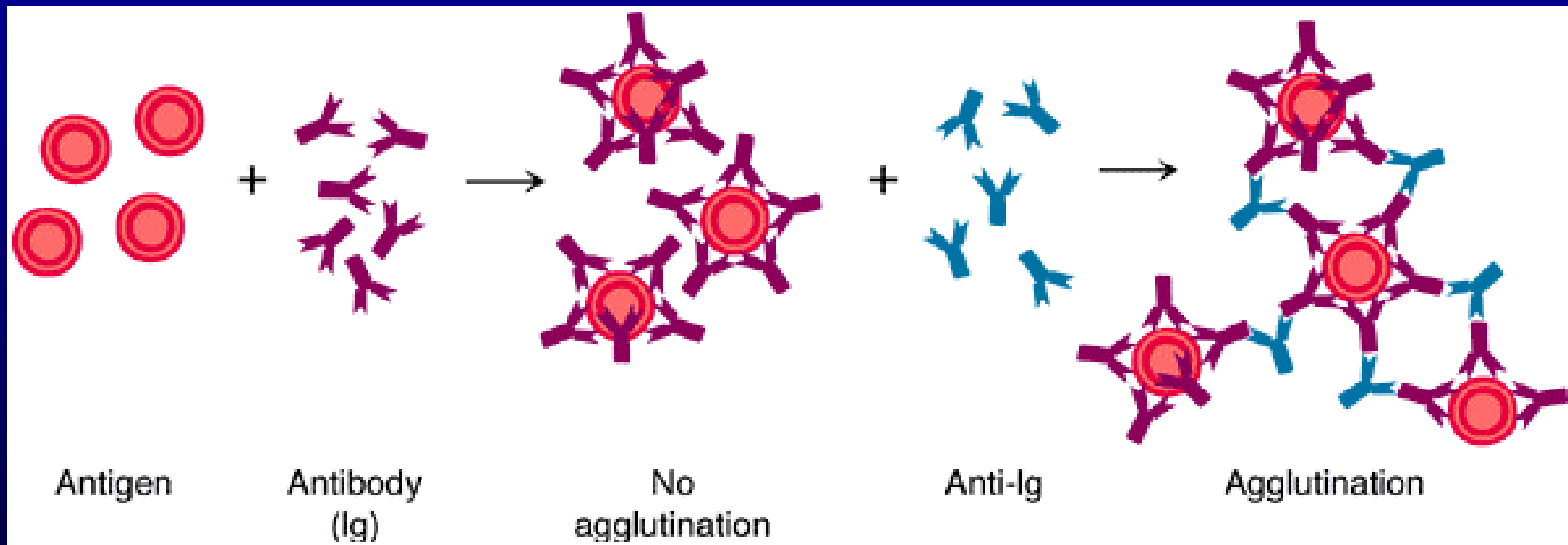
The reaction between  $F(ab')_2$  and a unideterminant, multivalent antigen.



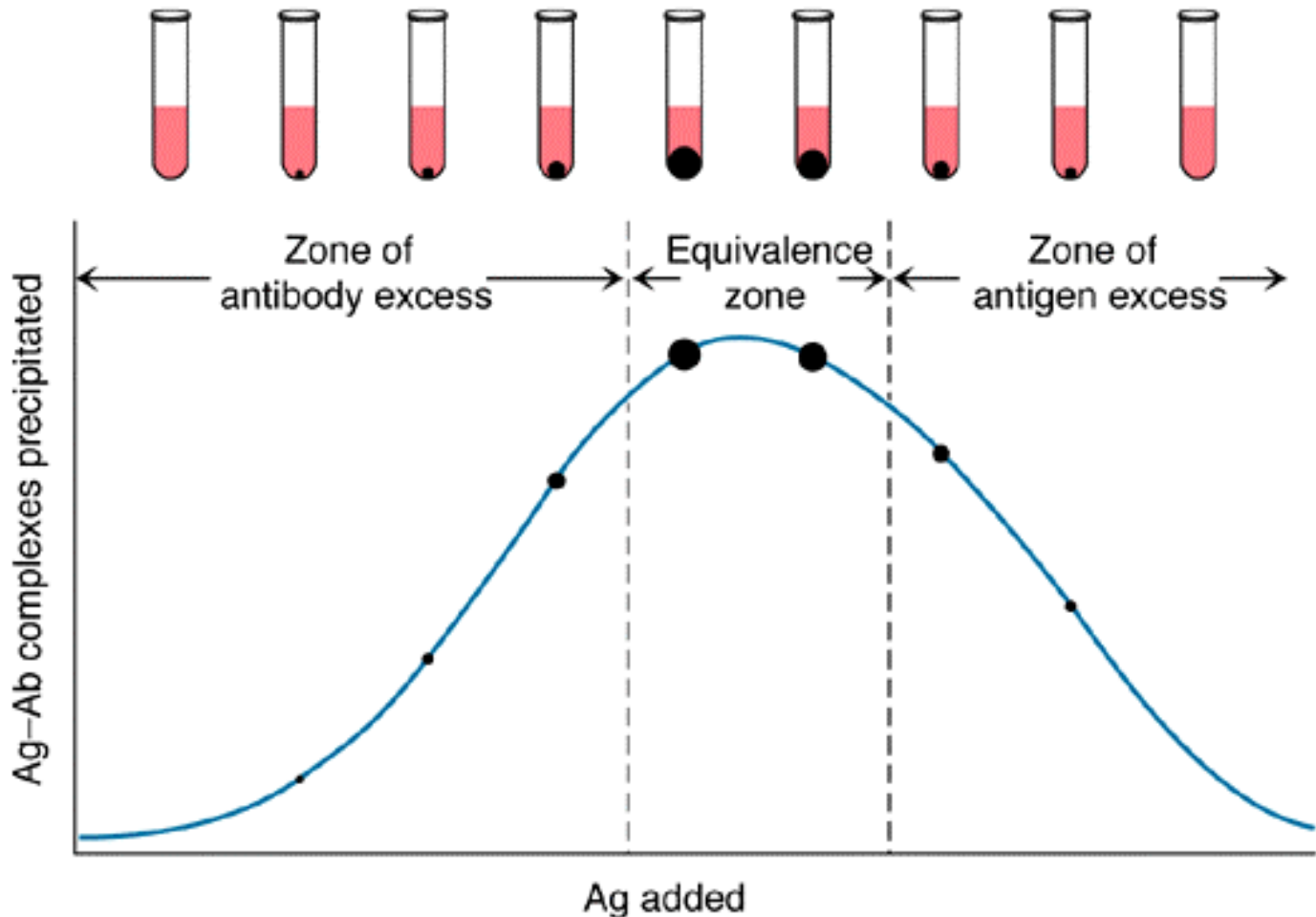
The reaction between antibodies to determinants A, B, and C, and a multivalent, multideterminant antigen with determinants A, B, and C.



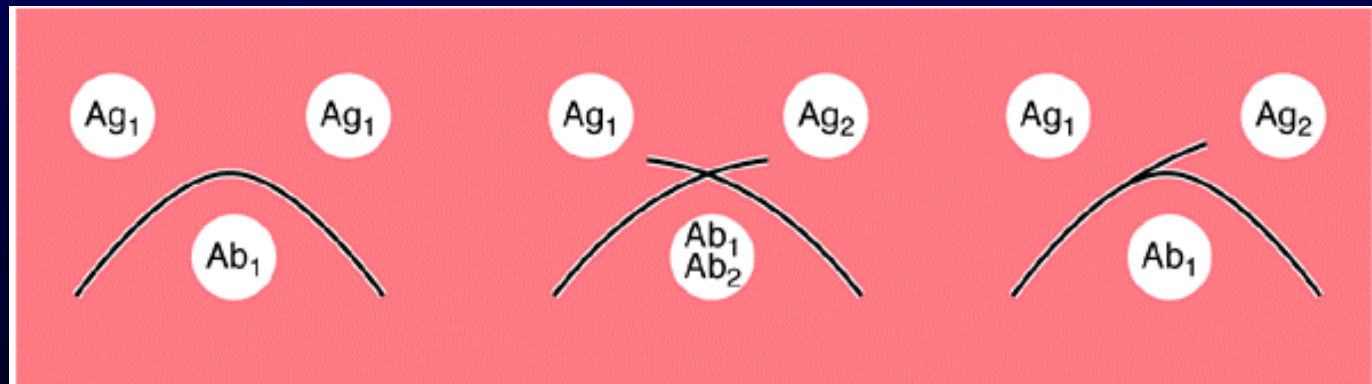
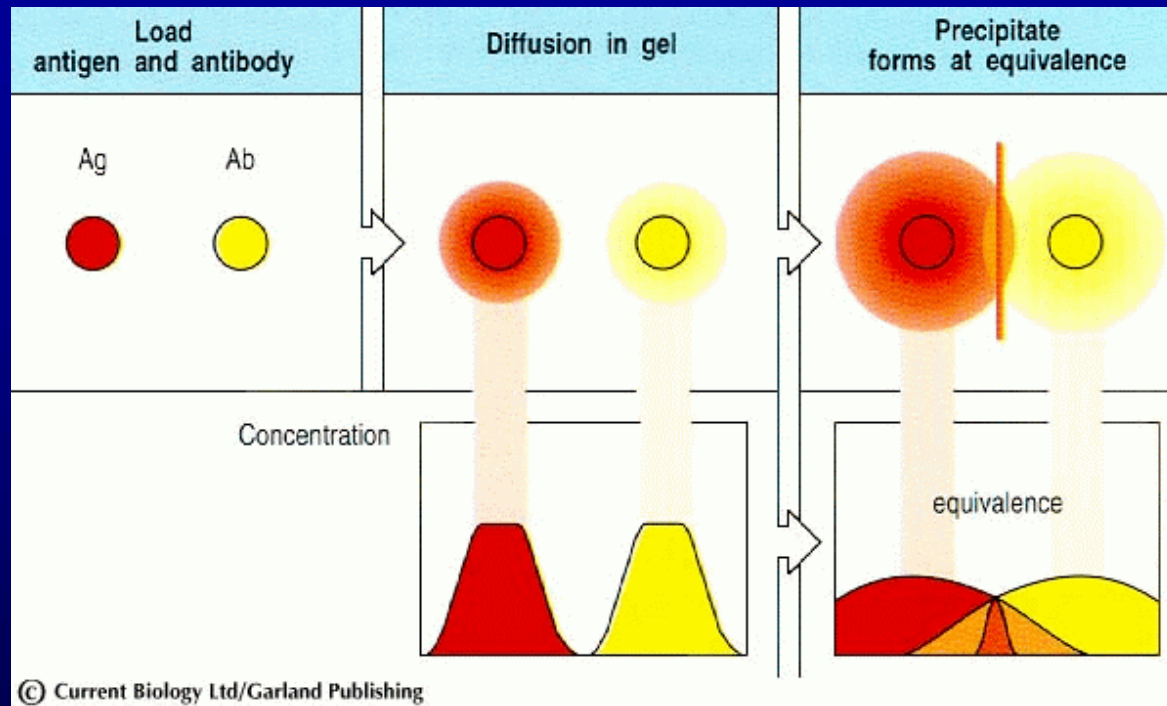
# Réaction d'agglutination



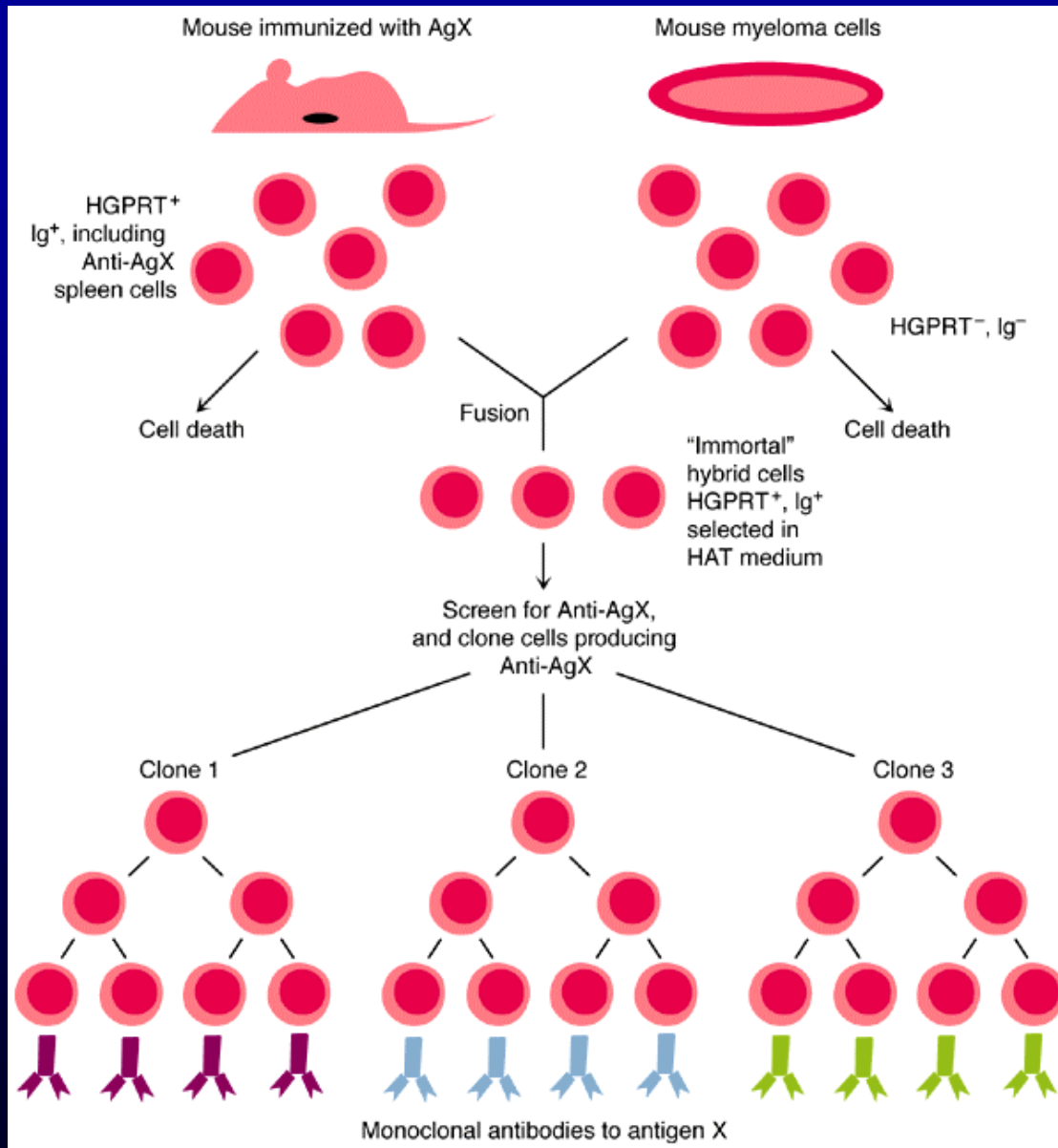
# Précipitation en milieu liquide



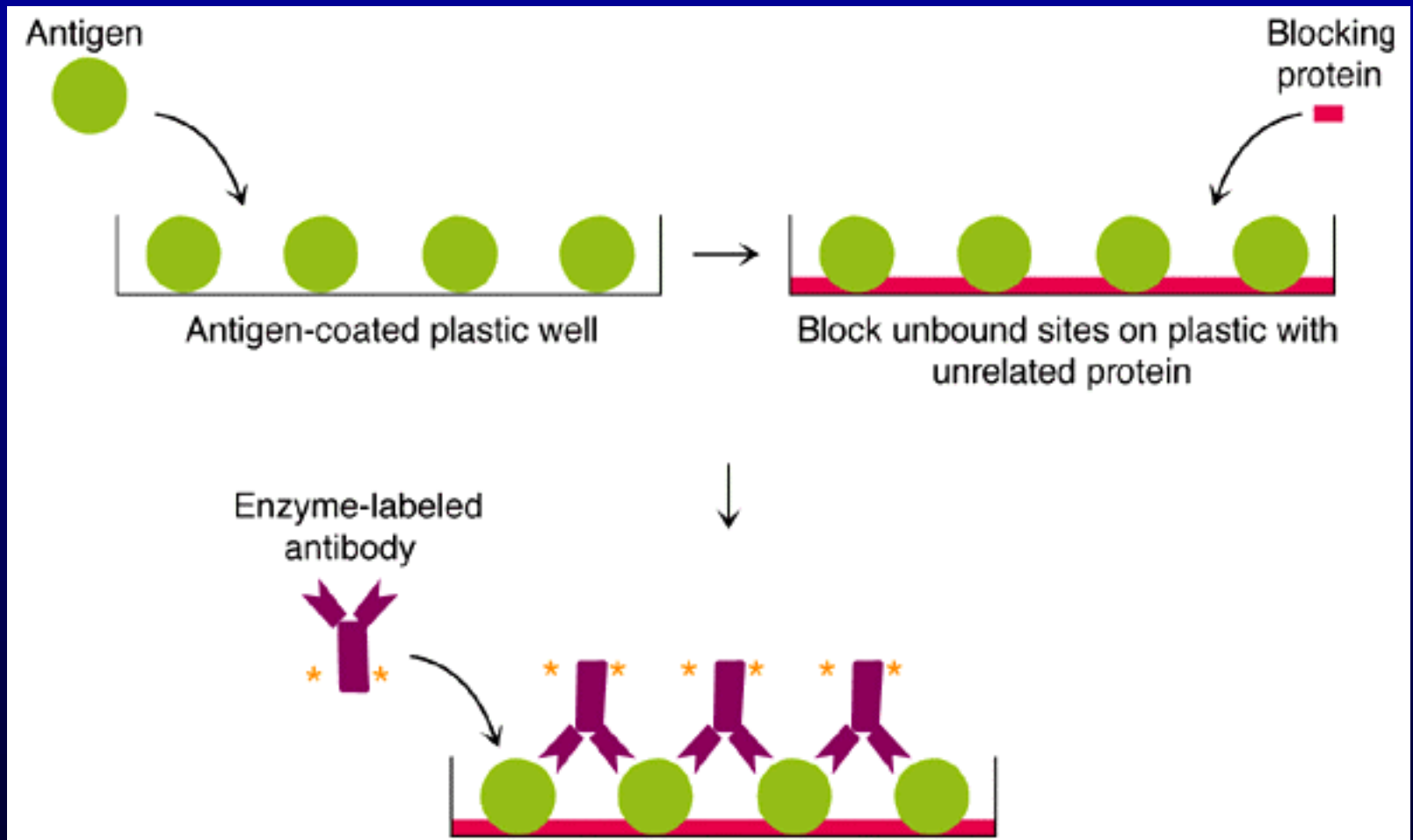
# Précipitation en milieu solide



# Production d'anticorps monoclonaux



# RIA/ELISA

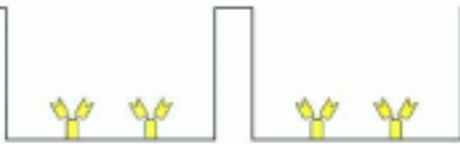


RadiolImmunoAssay/Enzyme-linked ImmunoSorbentAssay

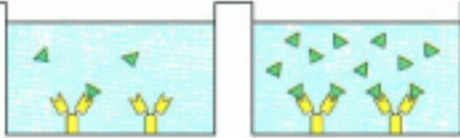


# Sandwich/Compétition

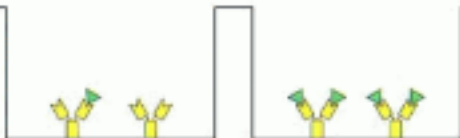
Coat plastic wells with antibody to IL-2



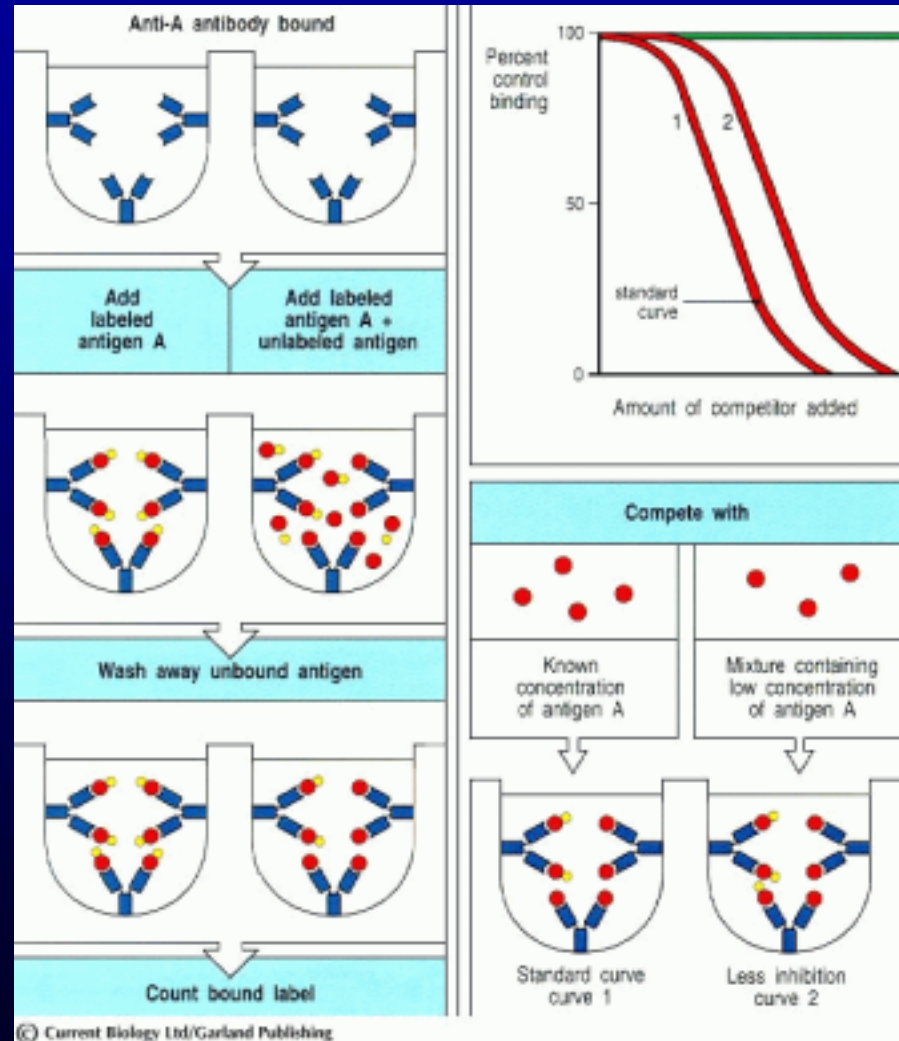
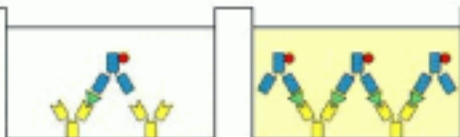
Incubate wells with solutions containing different concentrations of IL-2



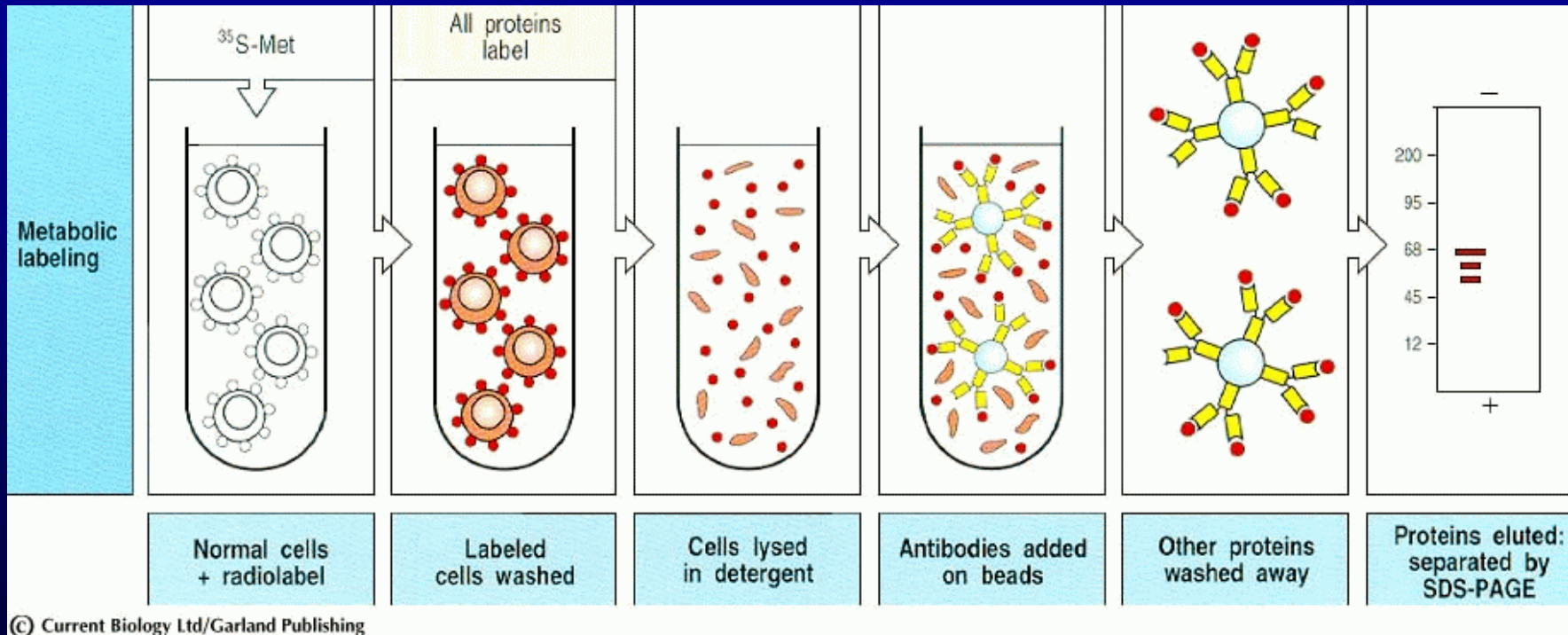
Wash away unbound IL-2



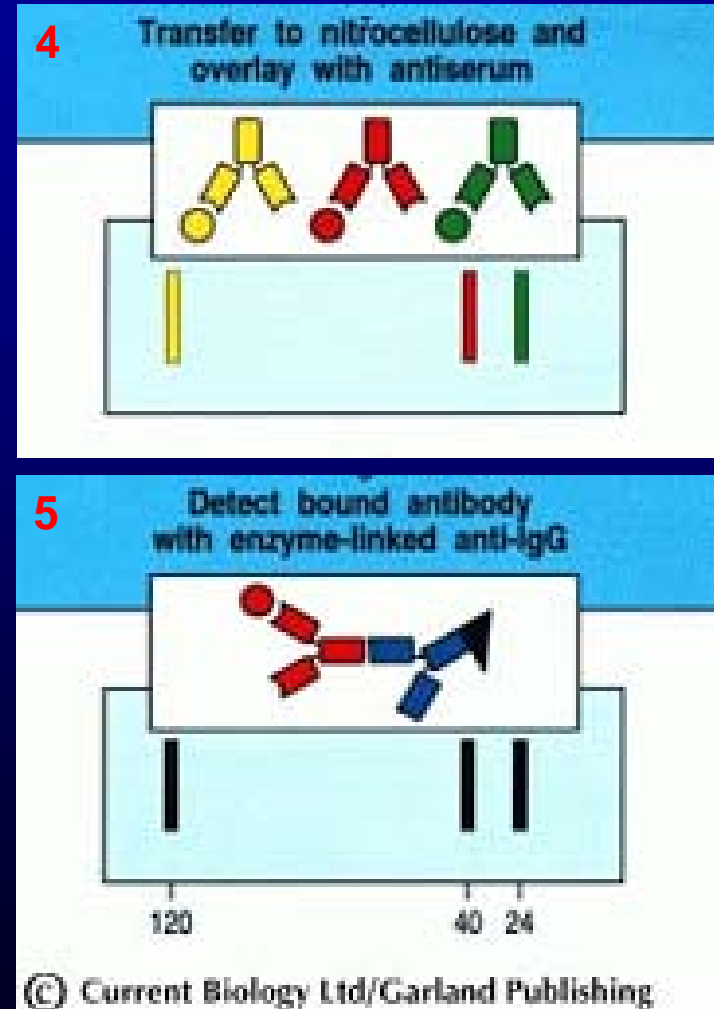
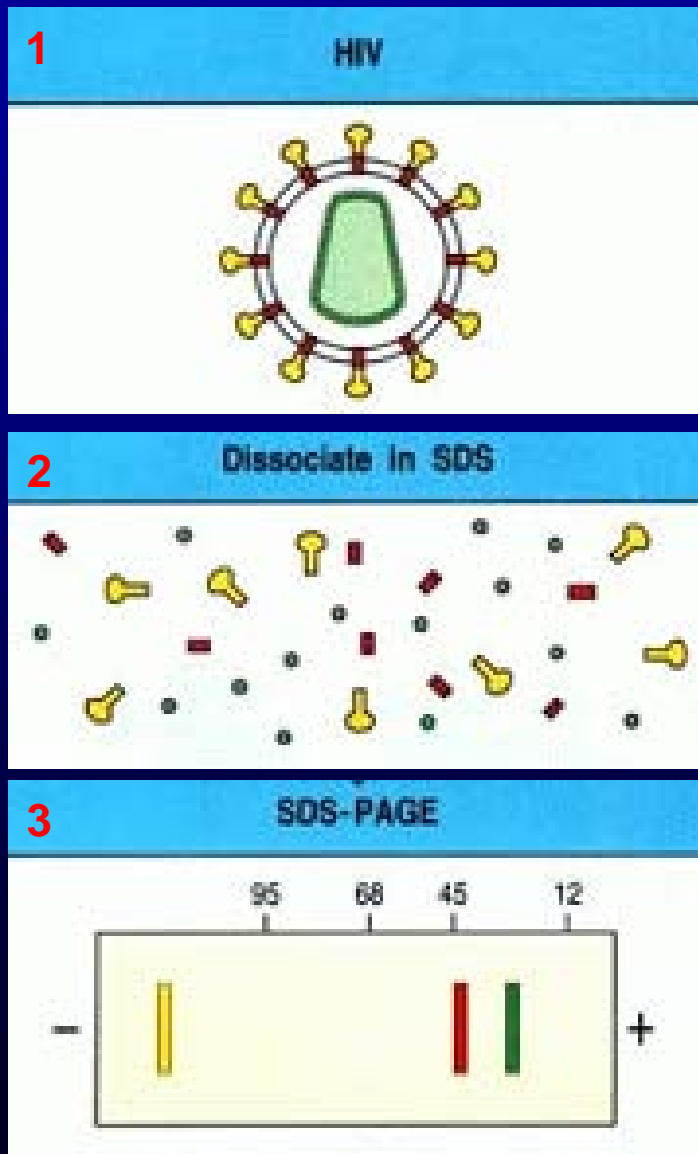
Monitor amount of bound IL-2 using second IL-2-specific antibody coupled to an enzyme. The amount of enzyme bound correlates with the concentration of IL-2 added to each well



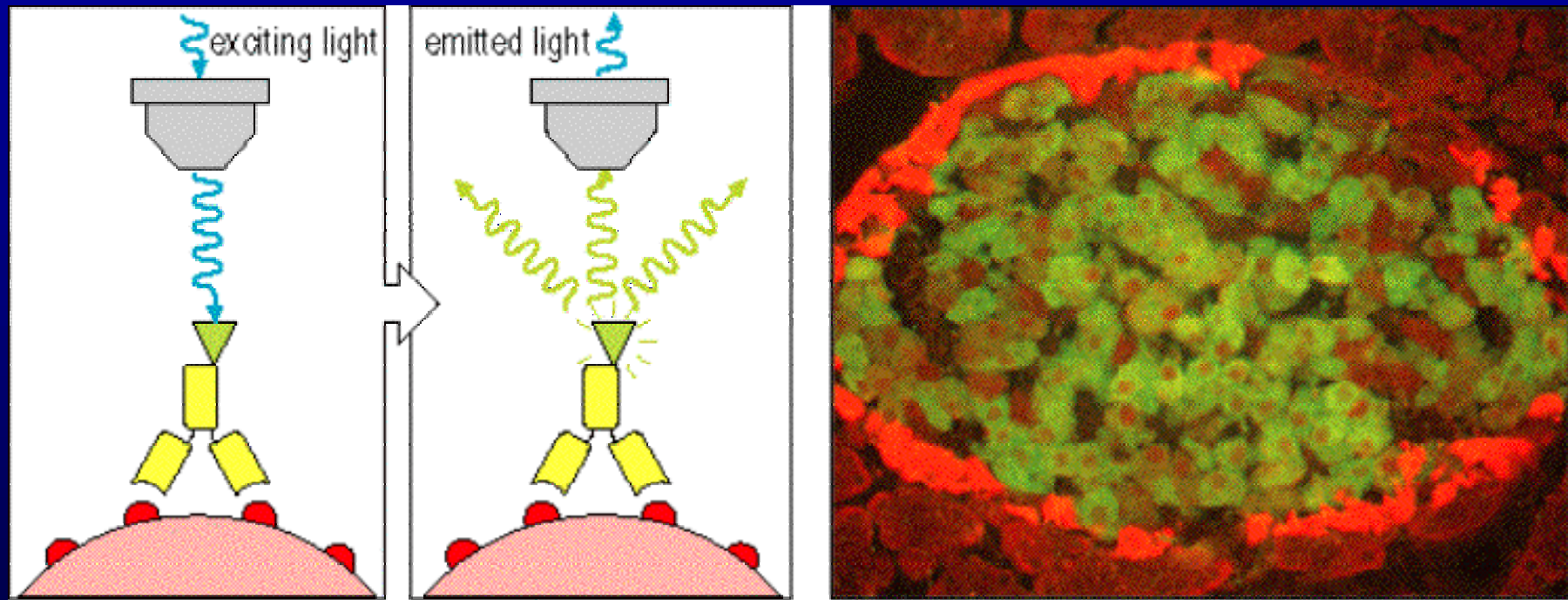
# Immunoprécipitation



# Western blot



# Immunofluorescence

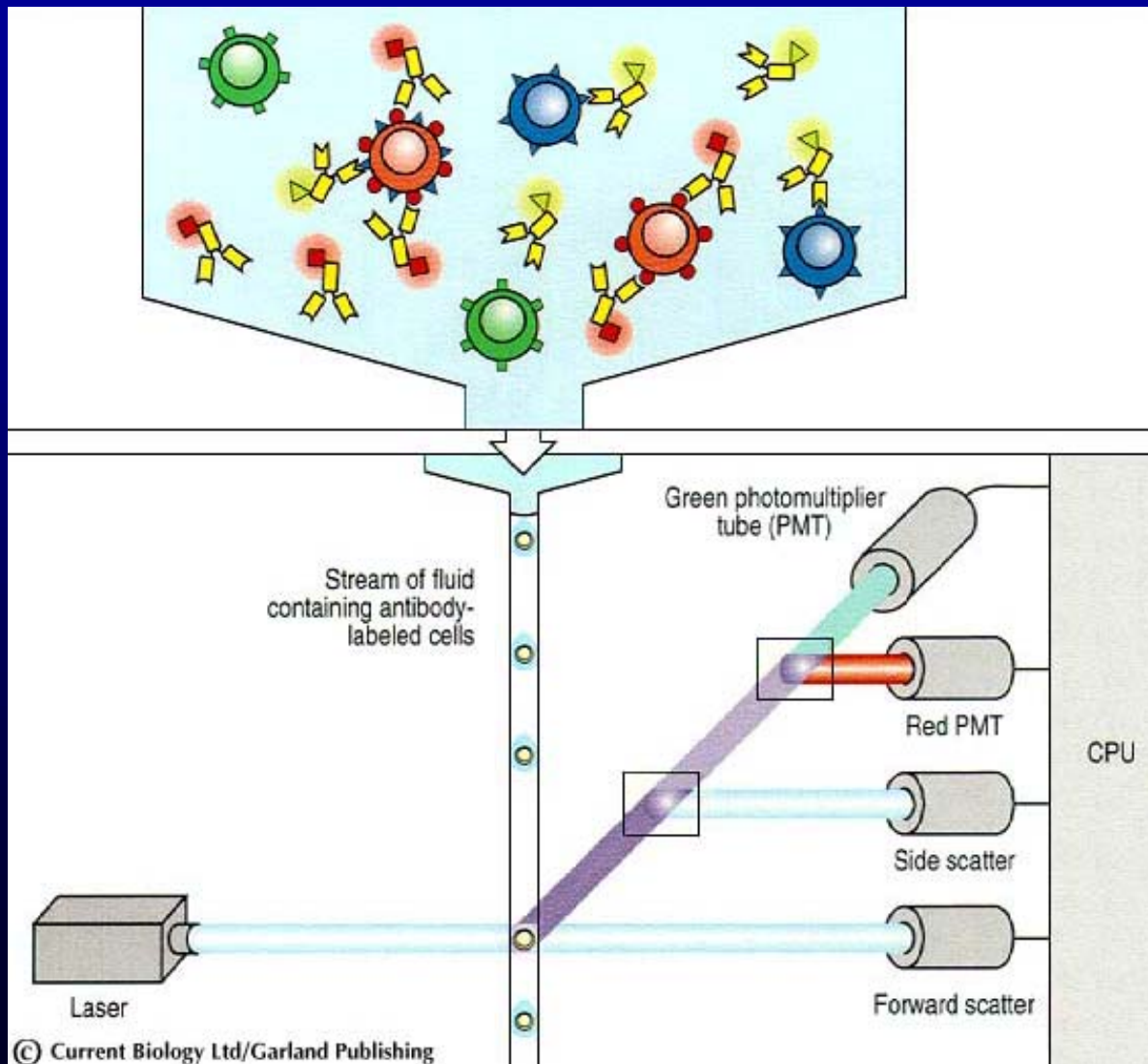


## Coupe de pancréas:

Anticorps « vert » dirigé contre GAD (*glutamic acid decarboxylase*)  
marque les cellules  $\beta$  des îlots de Langerhans

Anticorps « orange » dirigé contre l'hormone glucagon marque les  
cellules  $\alpha$

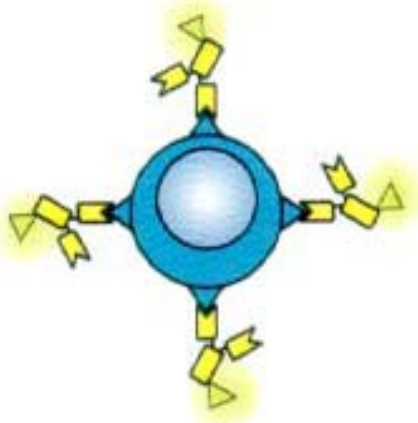
# Cytométrie de flux (1)



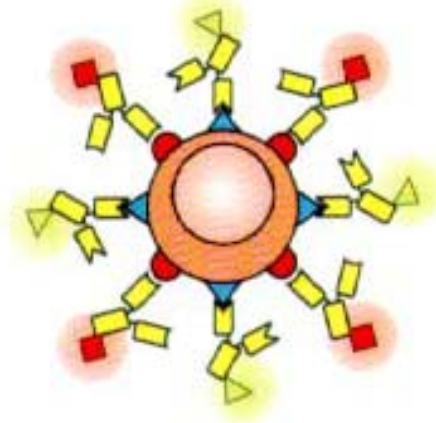


# Cytométrie de flux (2)

One antibody

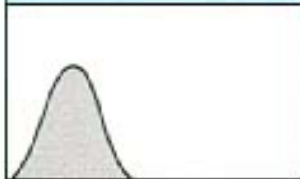


Two antibodies

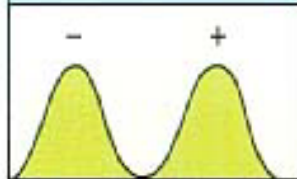


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Control



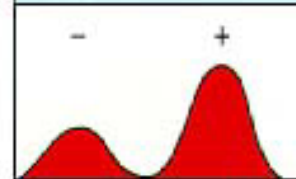
Antibody



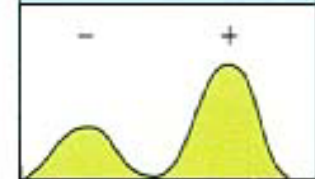
Fluorescence intensity

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Red antibody

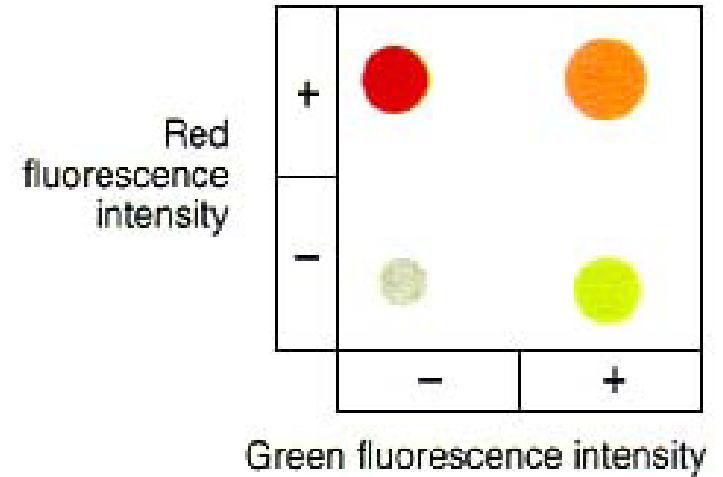
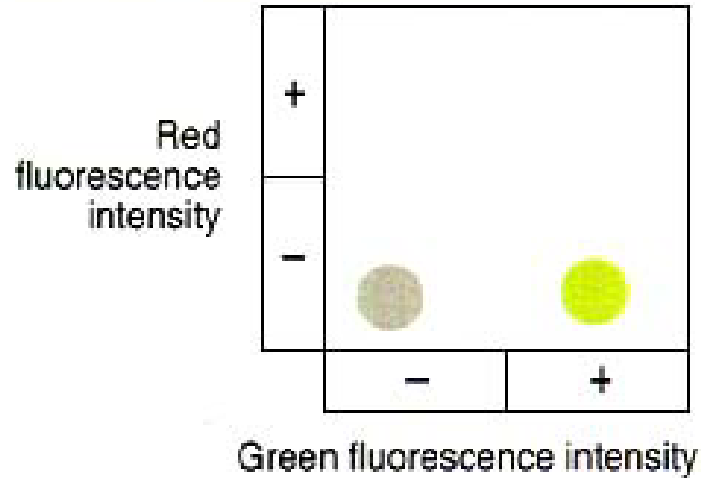


Green antibody



Fluorescence intensity

# Cytométrie de flux (3)



# Tri cellulaire par « panning »

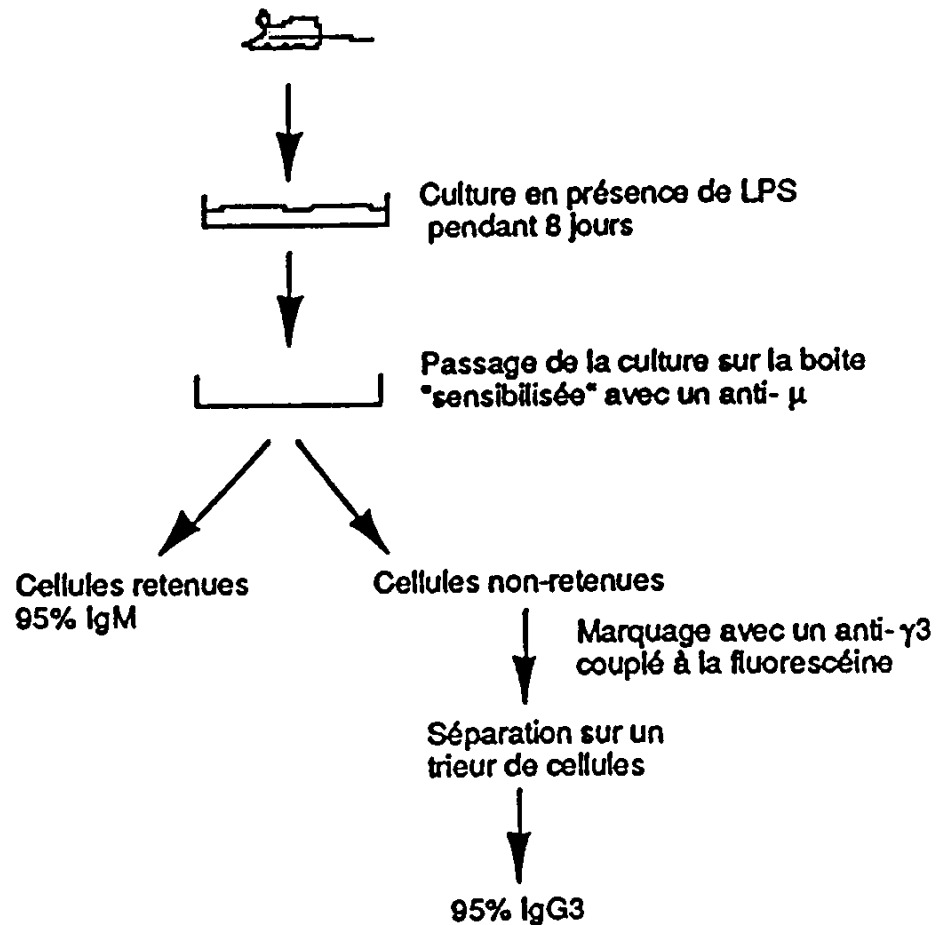
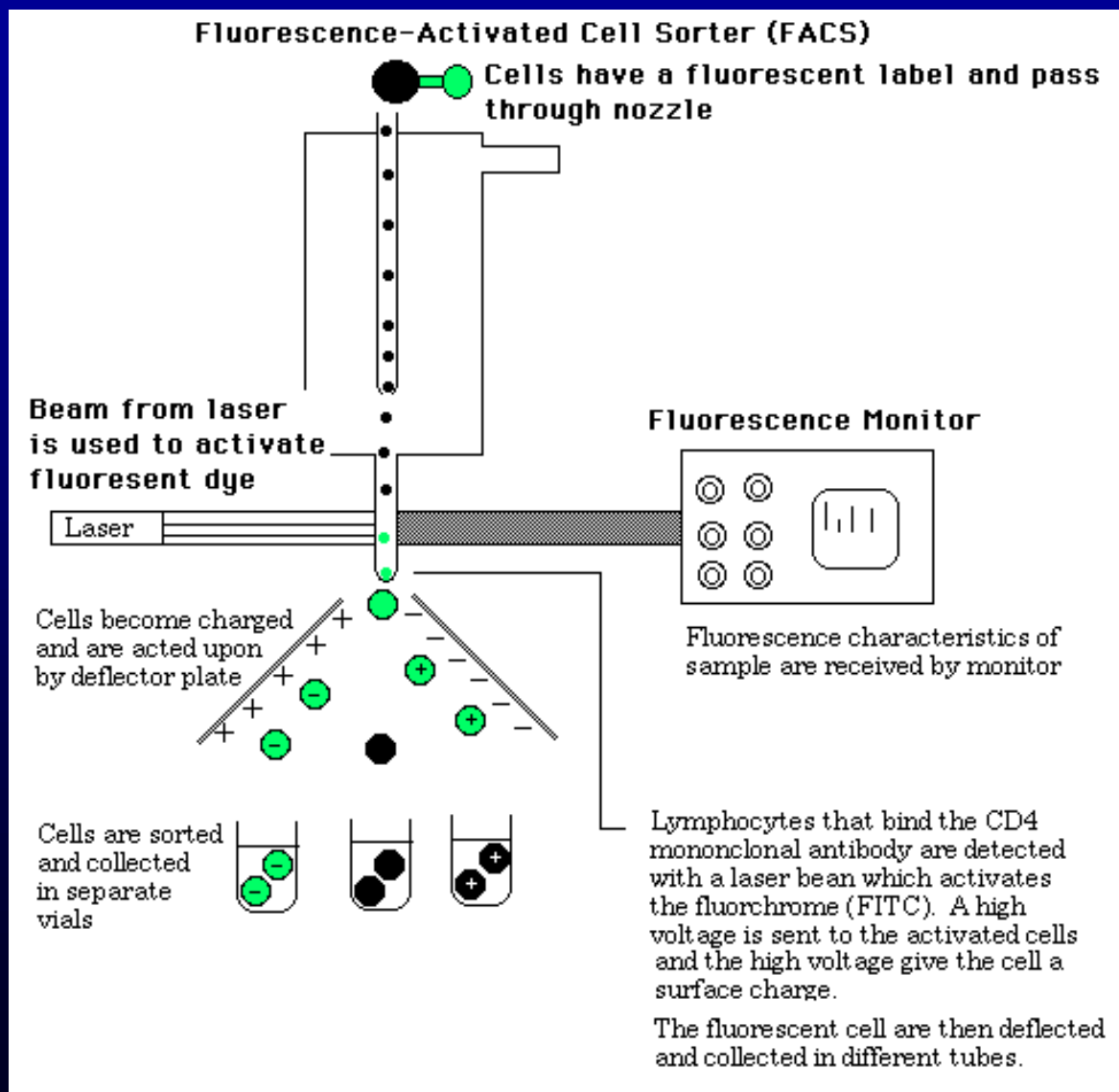


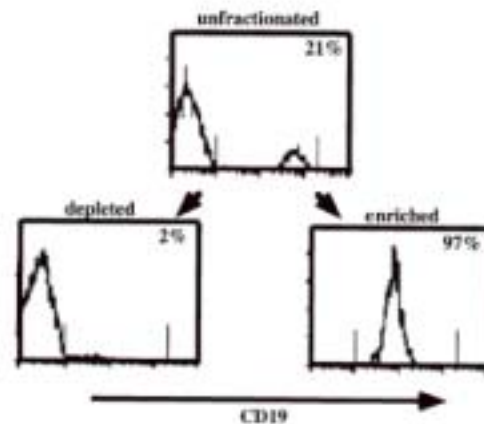
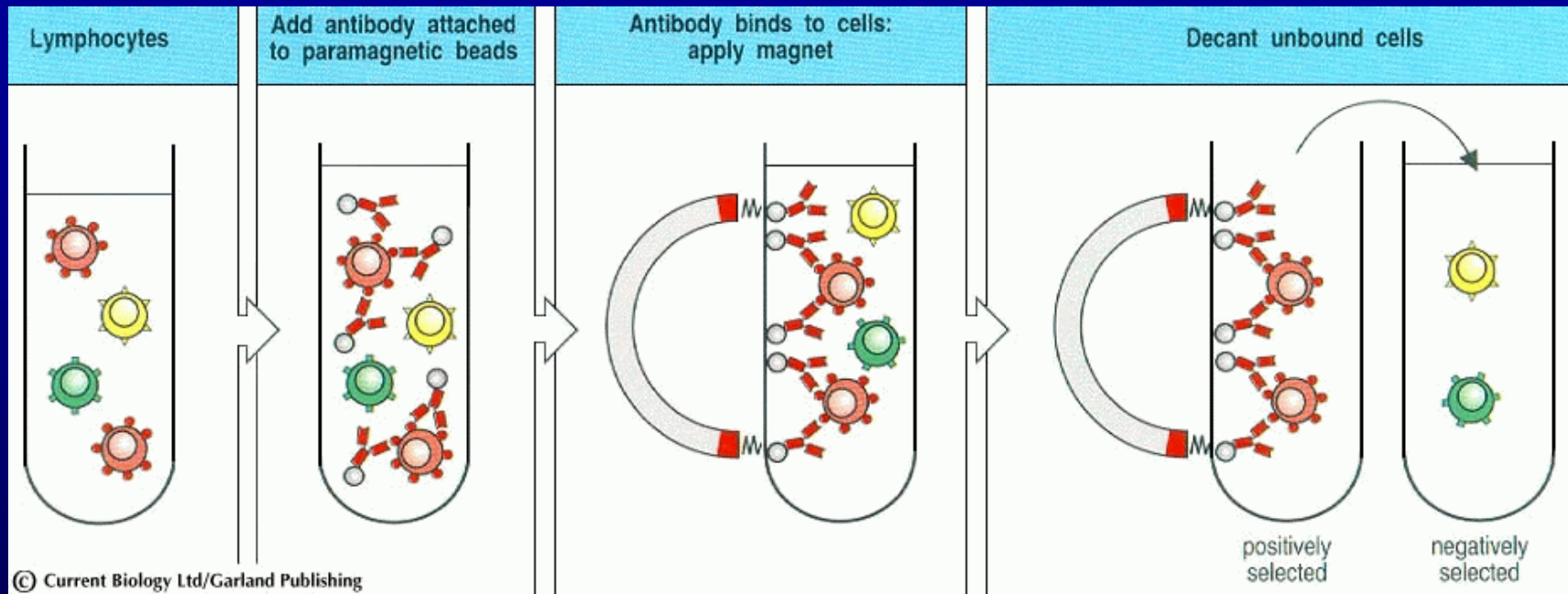
Figure 1



# Tri cellulaire par cytométrie



# Tri cellulaire magnétique

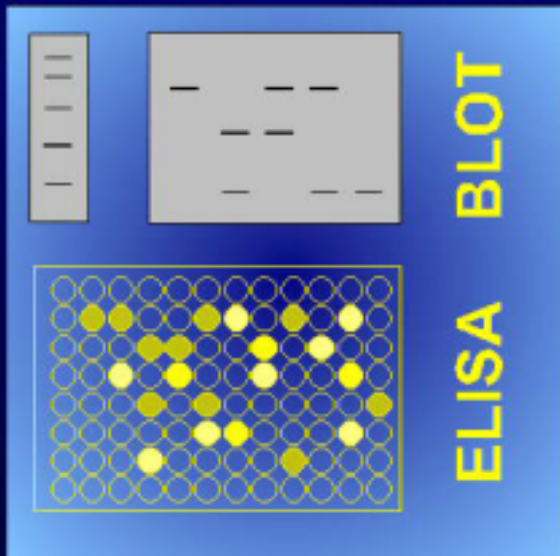


# Cartographie épitopique (1)

## "Classical" epitope mapping

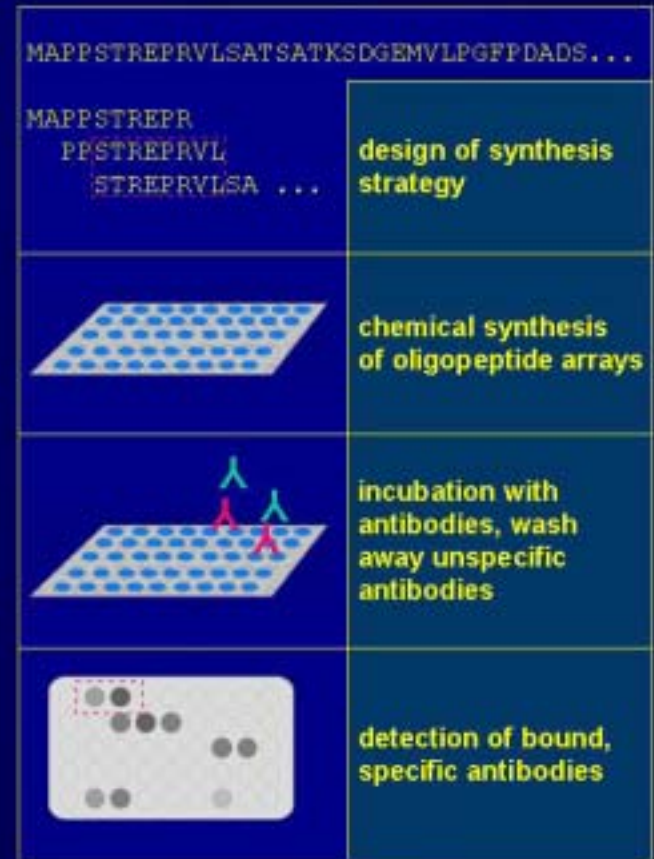
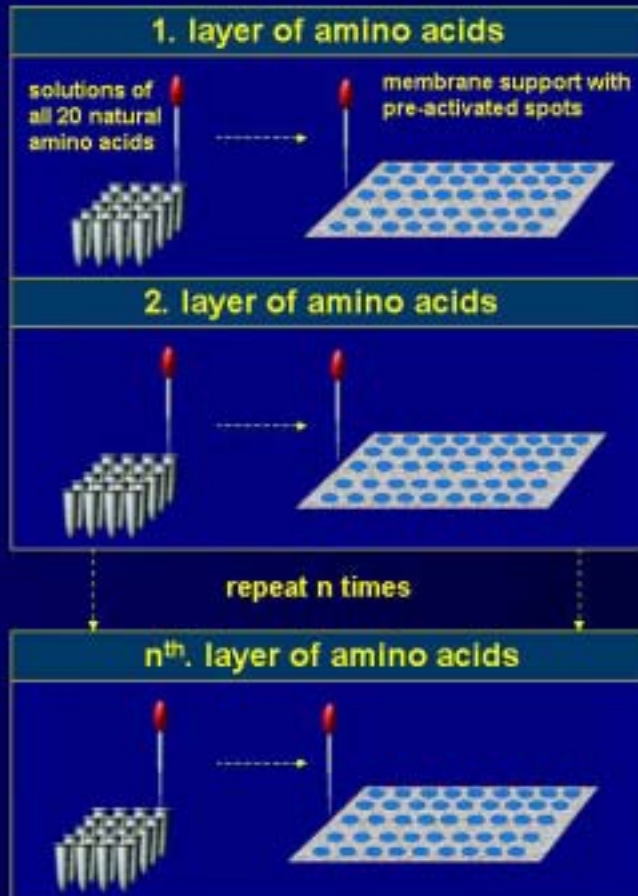


cDNA fragments  
are expressed as  
recombinant proteins

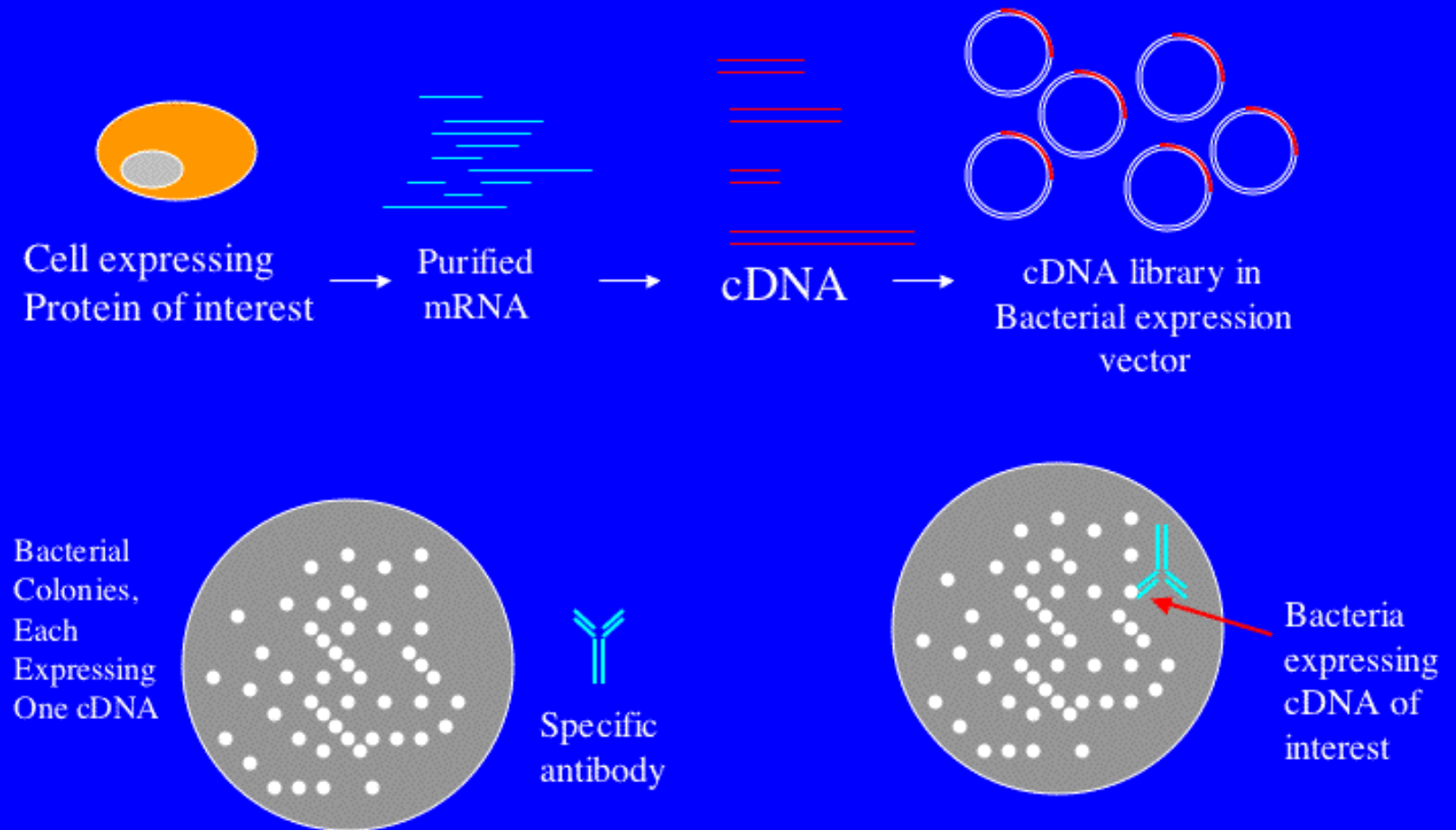


Identification of  
immunoreactive  
recombinant fragments  
by ELISA or Westernblot

# Cartographie épitopique (2)

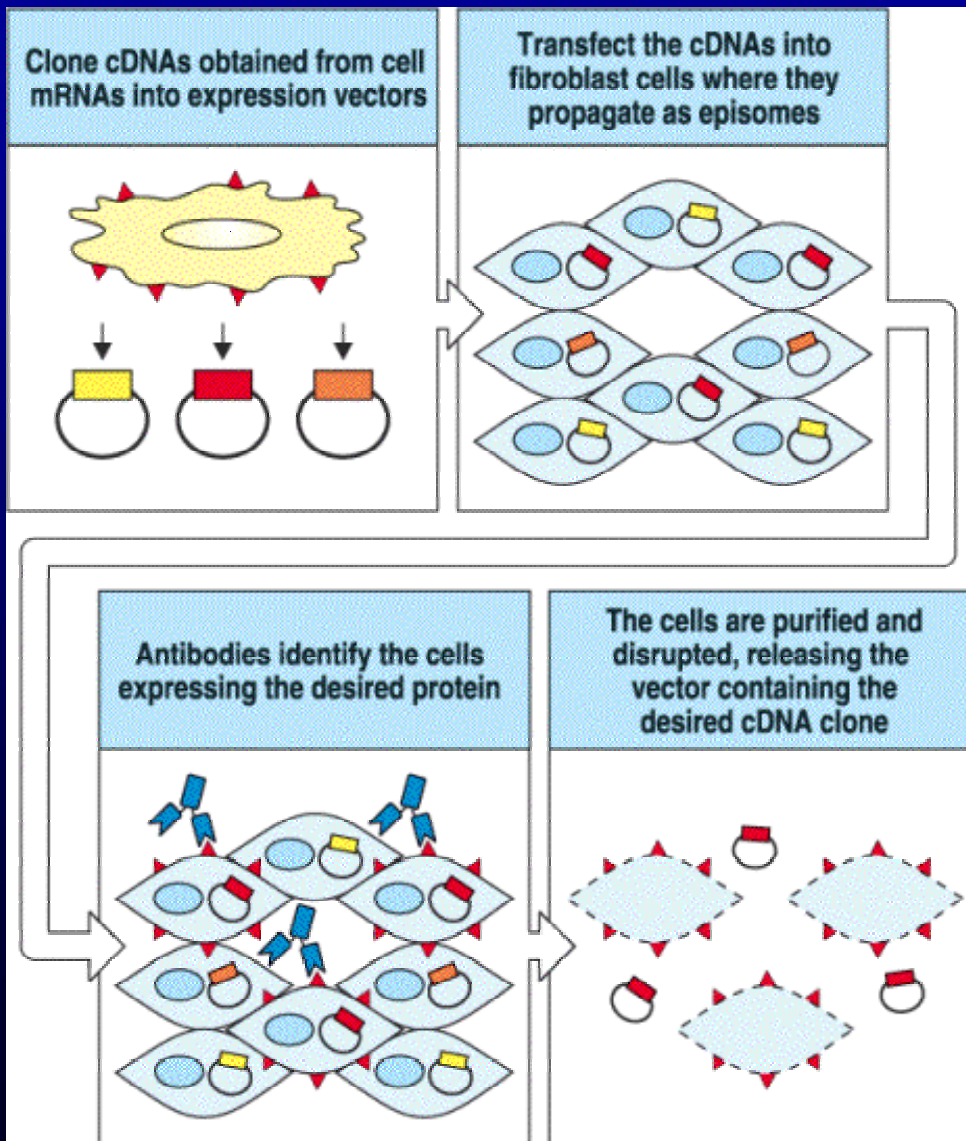


# Clonage et criblage (1)



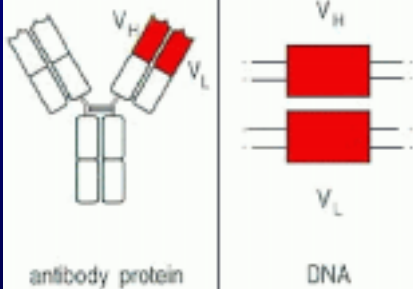


# Clonage et criblage (2)



# Clonage et criblage (3)

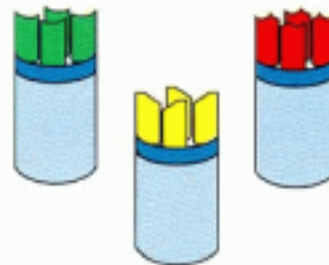
Isolate population of genes encoding antibody variable regions



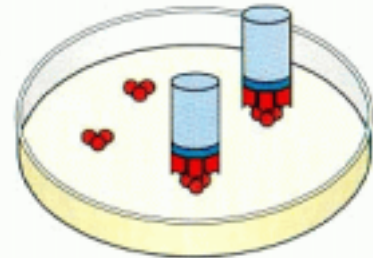
Construct fusion protein of V region with a bacteriophage coat protein

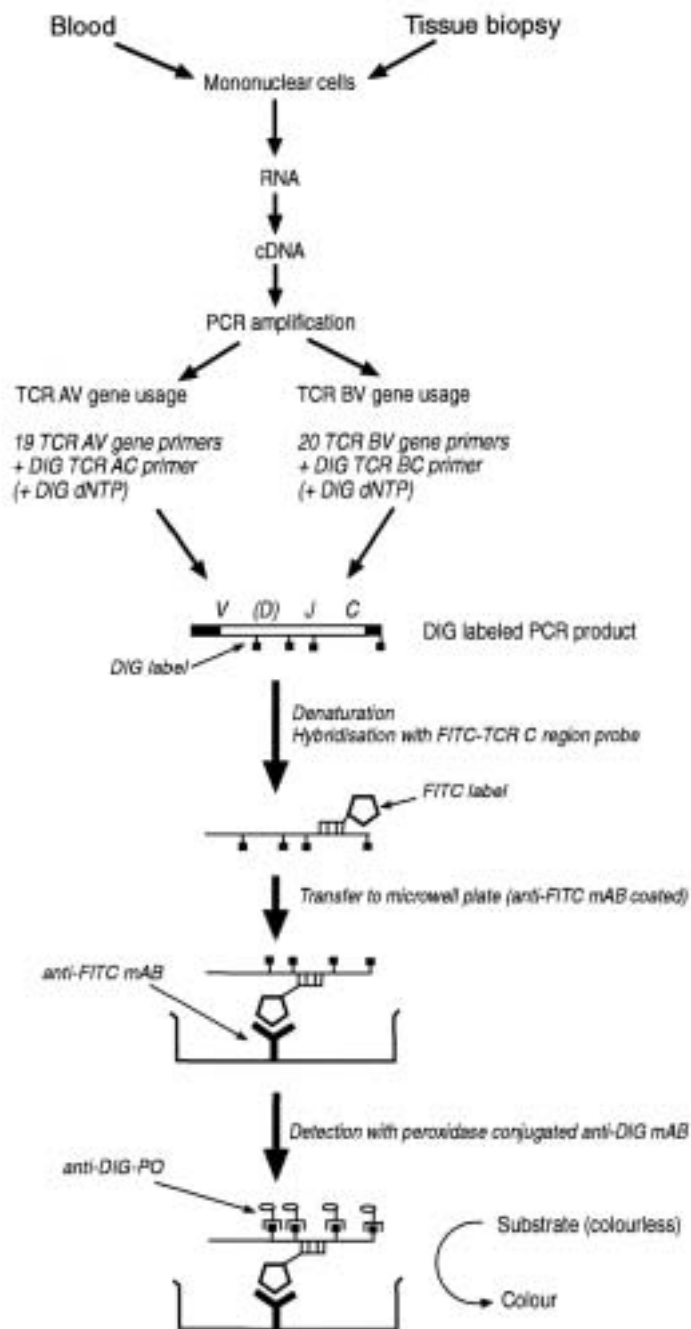


Cloning a random population of variable regions gives rise to a mixture of bacteriophage — a phage display library



Select phage with desired V regions by specific binding to antigen



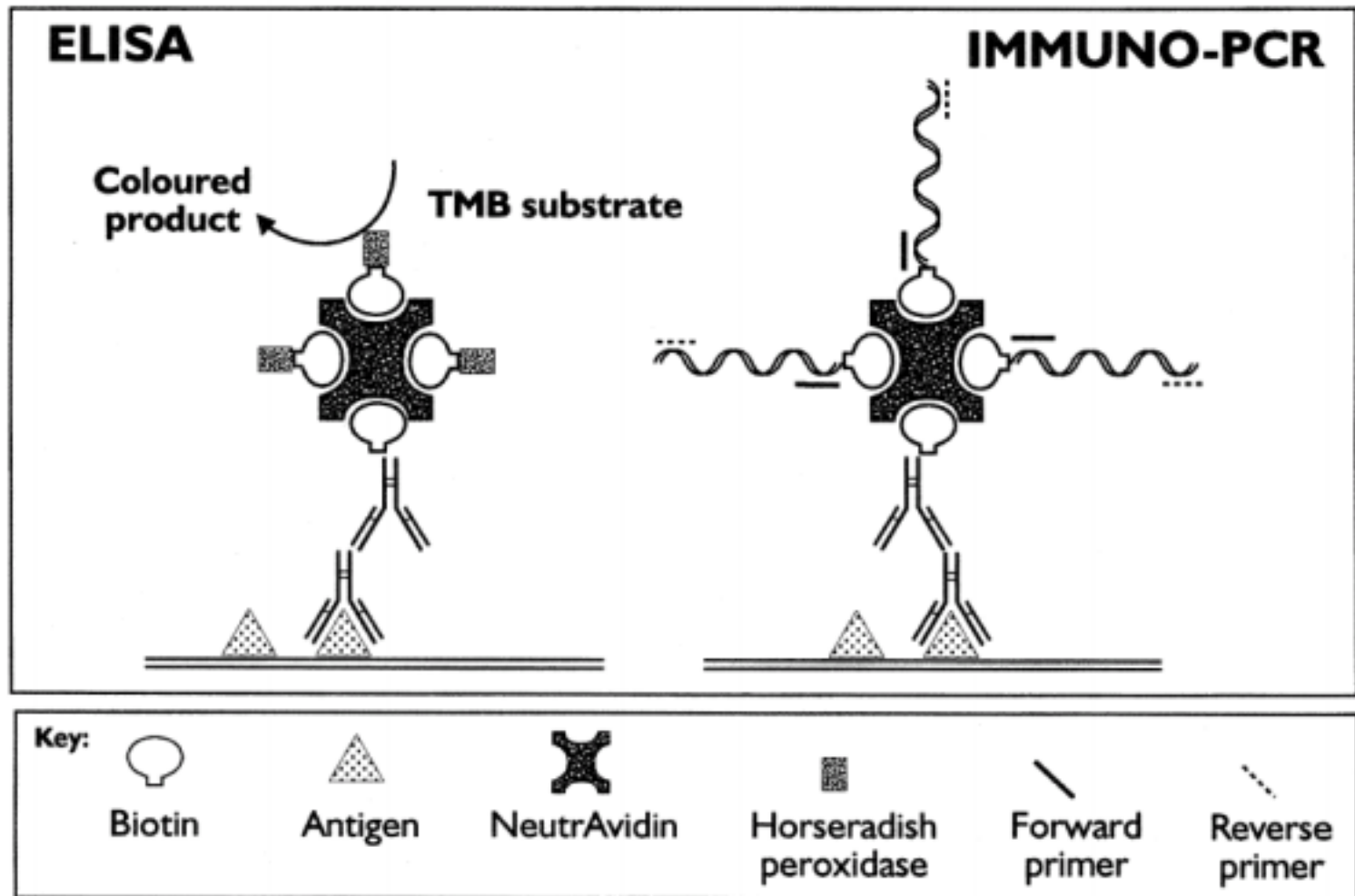


# PCR-ELISA

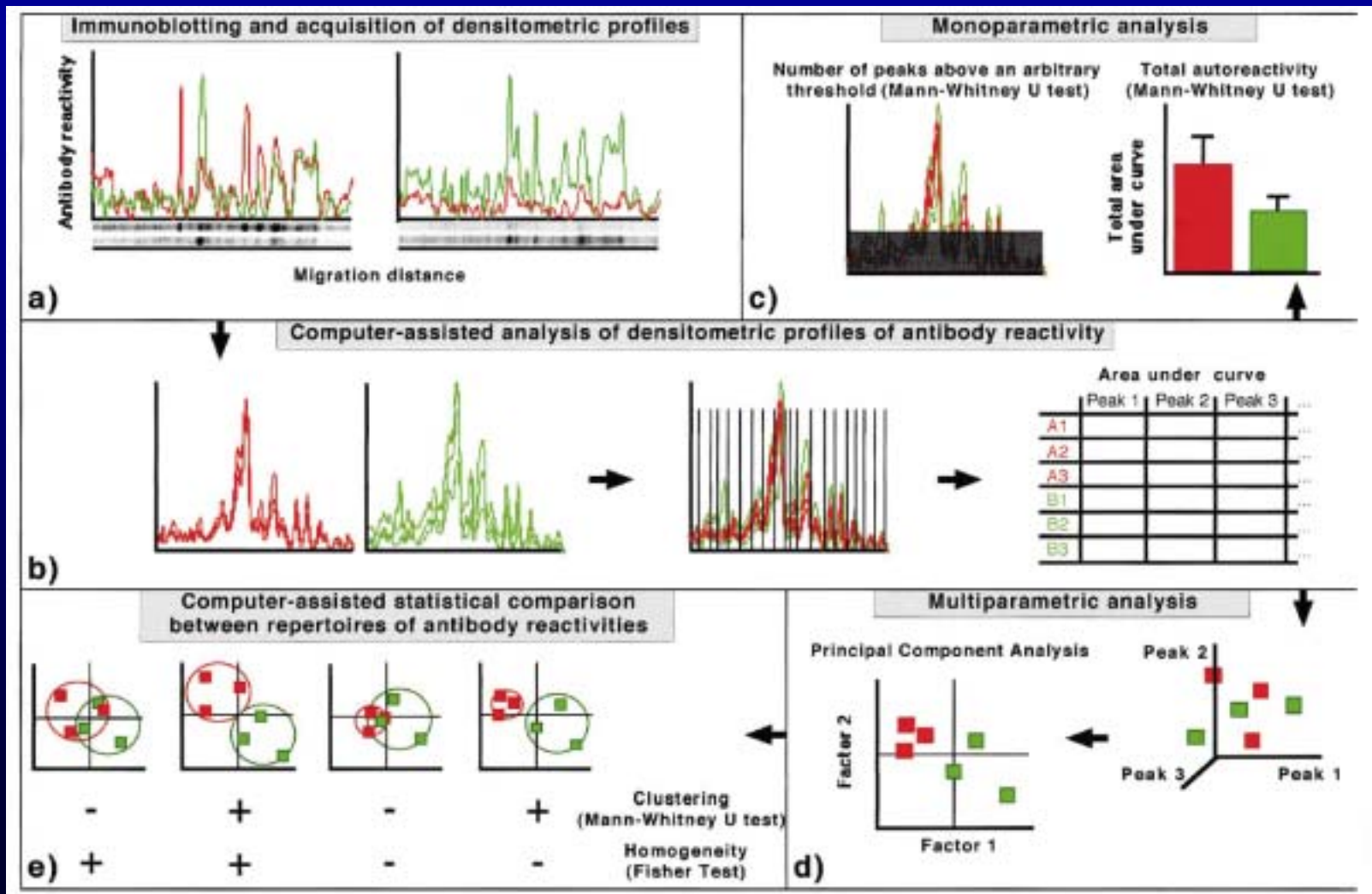
A. VanderBorgh et al. (1999)  
J.Immunol.Methods 223:47.



# Immuno-PCR



# Panama-blot



# Immunoproteomics

Francisco J. Quintana, Peter H.  
Hagedorn, Gad Elizur, Yifat Merbl, Eytan  
Domany, and Irun R. Cohen.

Functional immunomics: Microarray  
analysis of IgG autoantibody repertoires  
predicts the future response of mice to  
induced diabetes.

PNAS 101:14615-14621, 2004.