Biocolloidal Particle Assembly and Bioassays

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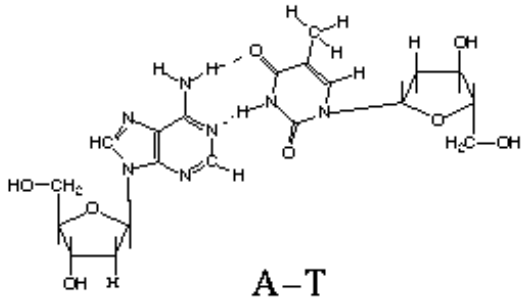
DNA interaction basics

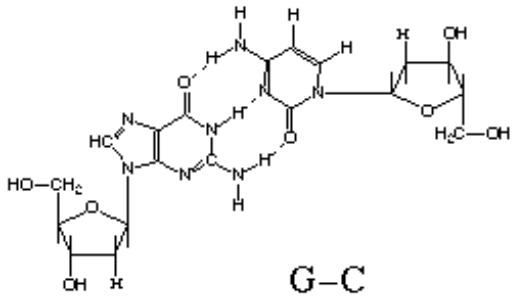
DNA Basepairs



A–T

Adenosine–Thymidine
(Adenine–Thymine)

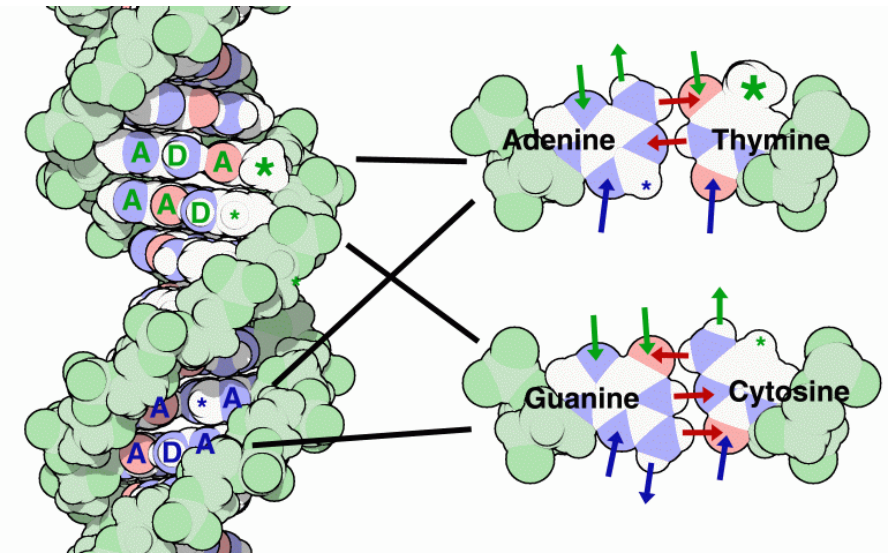
ΔH = 30 kJ mol^{-1} (12 kT)

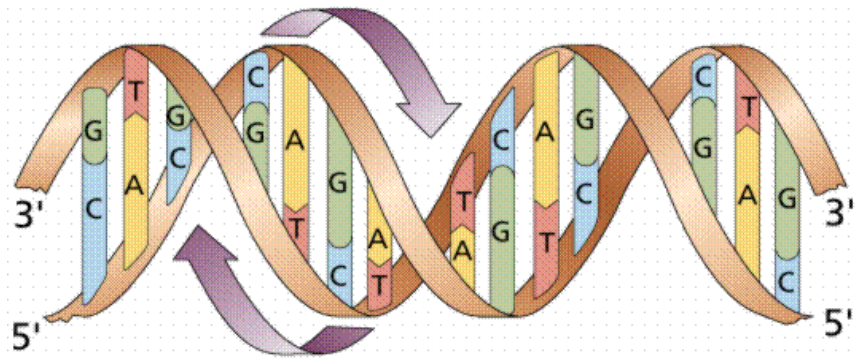


G–C

Guanosine–Cytidine
(Guanine–Cytosine)

ΔH = 48 kJ mol^{-1} (20 kT)

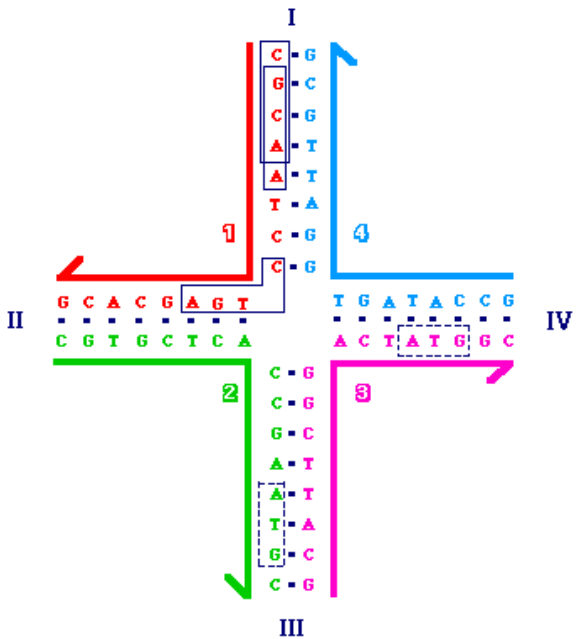




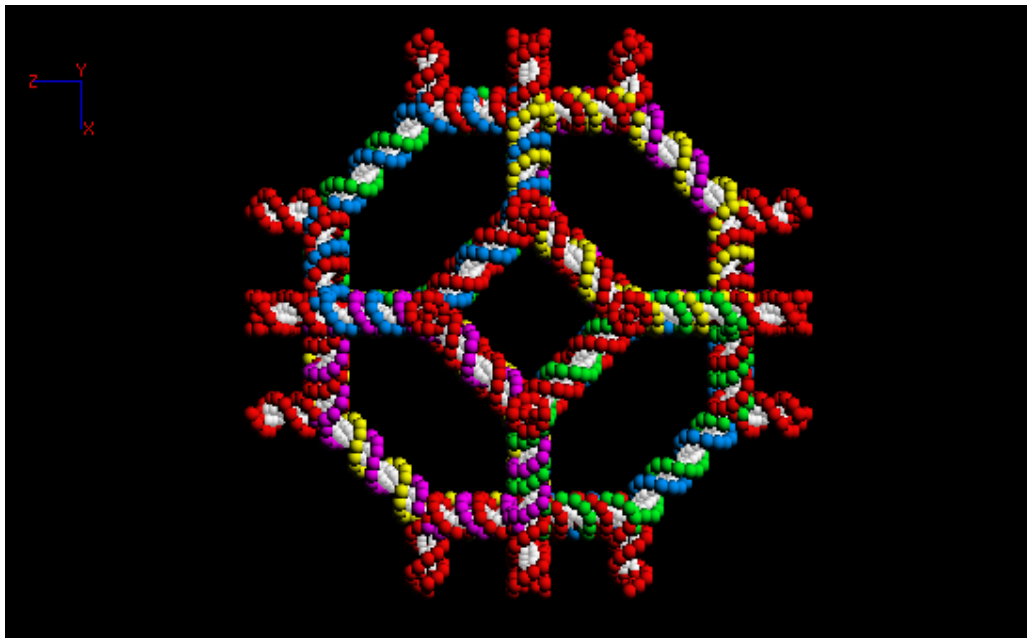
DNA Assembly: 2D crystals and 3D objects

Ned Seeman, New York University

4- way DNA junction.



Octahedron assembly

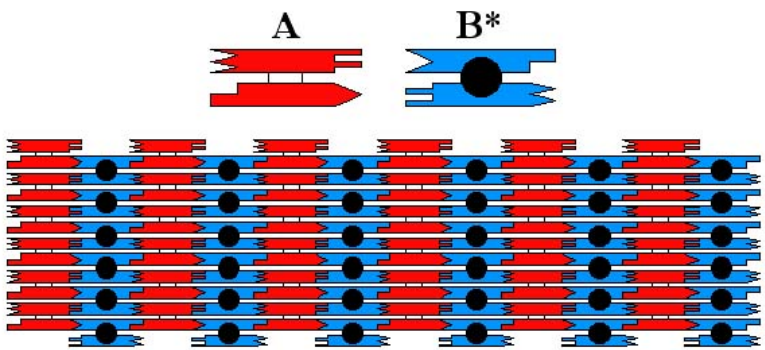


http://seemanlab4.chem.nyu.edu/homepage.html

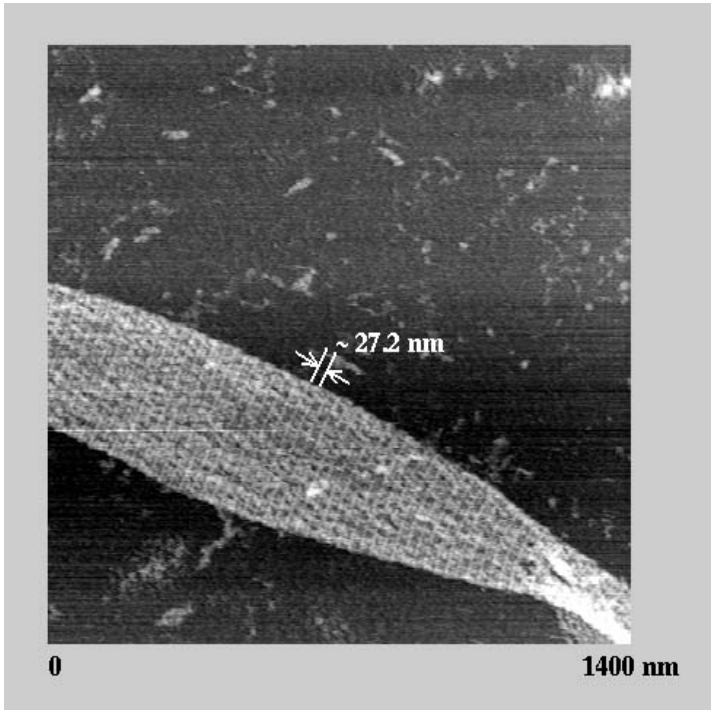
DNA Assembly: 2D crystals and 3D objects

Ned Seeman, New York University

Assembly principle



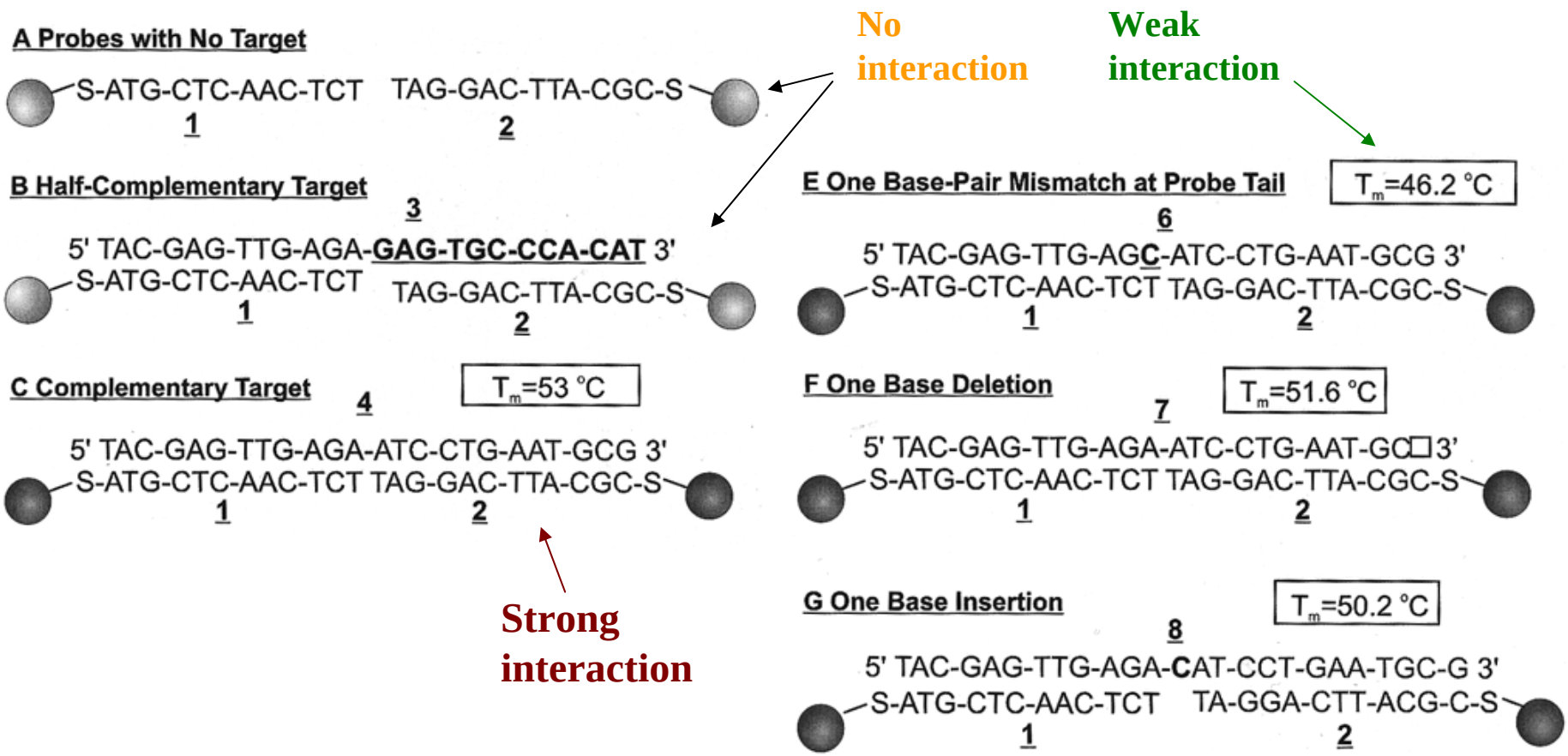
AFM image



Winfree et al., *Nature* **394**, 539 (1998).

Colloidal aspects: DNA interaction on nanoparticles

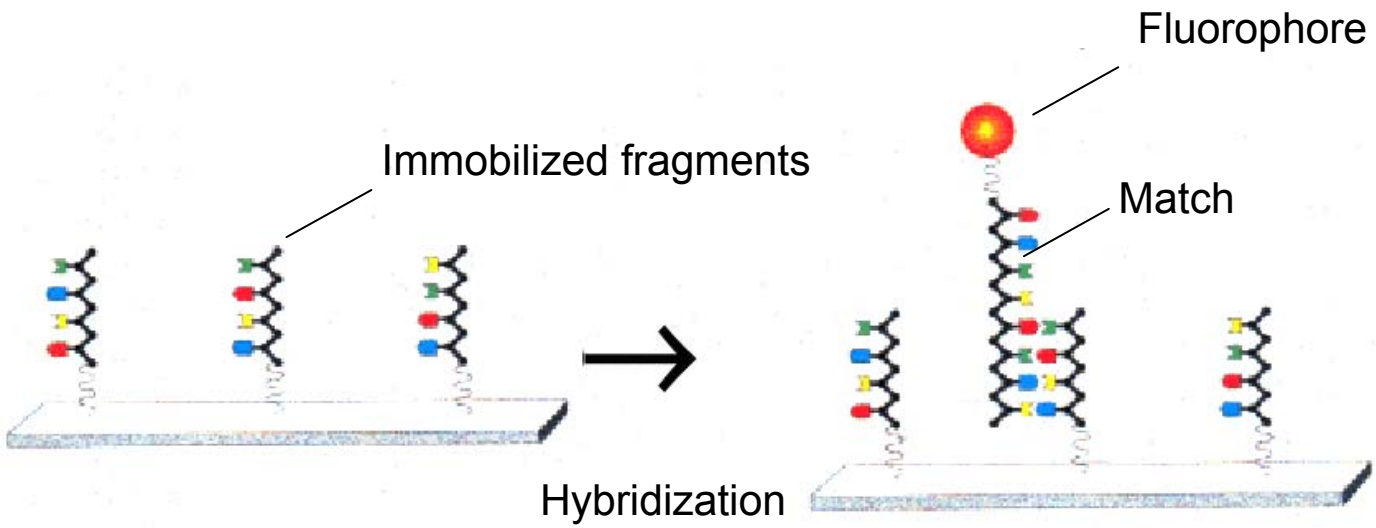
Particle linking: the strength of the interaction (measured by the "melting" temperature) depends on the DNA complementarity



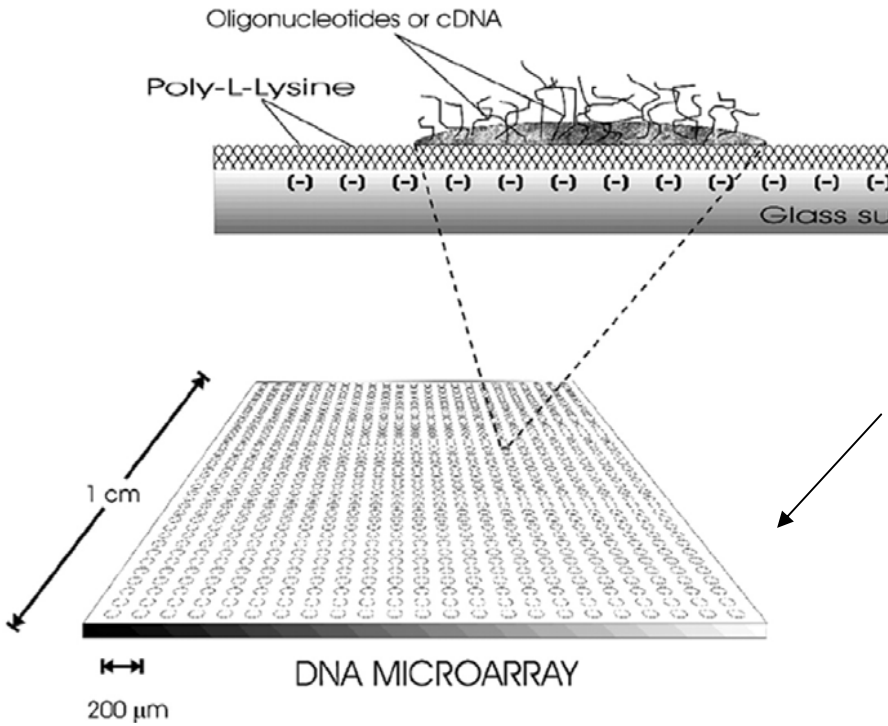
(Mirkin et al., JACS, 120:12674, 1998)

DNA Array Chips – Basic Principles

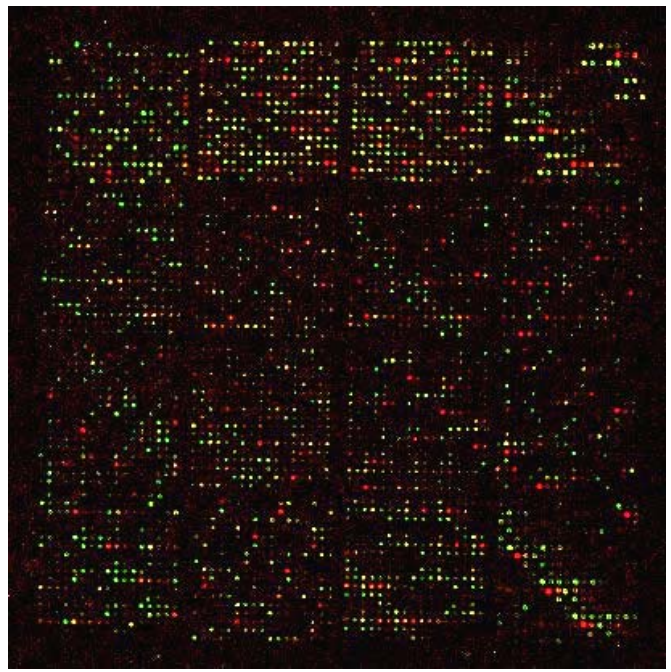
- Human genome contains ~ 30000 genes which encode more than 90000 RNA species and basic proteins. The possible mutations increase this number multiple fold.
- Many genes work in combination with others, so understanding and using their function requires characterization of multiple genes.
- Massively parallel detection and analysis is required.
- The amount of reagents and samples is small and they are very expensive so it all needs to be done on a miniature scale.



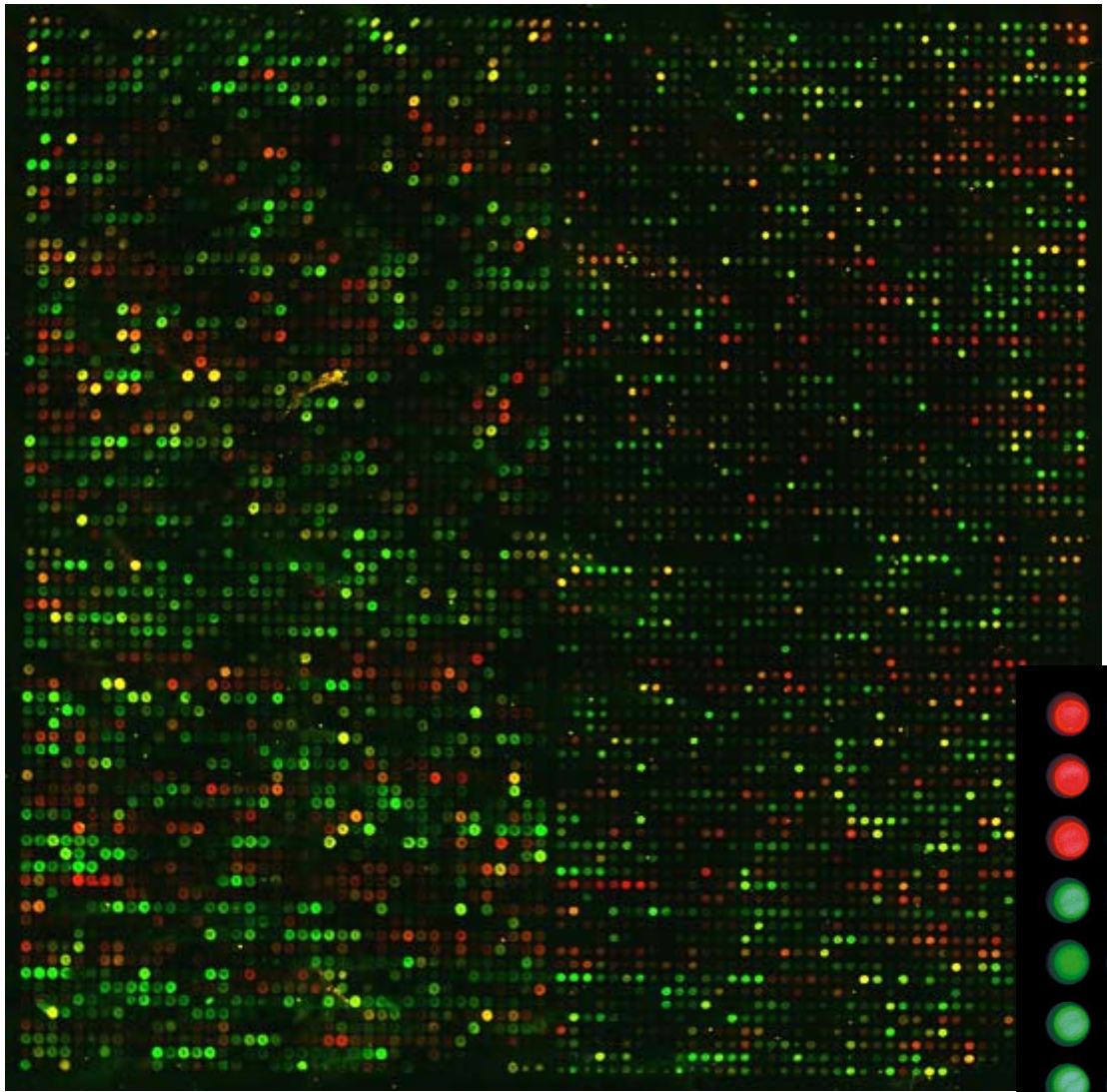
DNA Array Chips – Basics

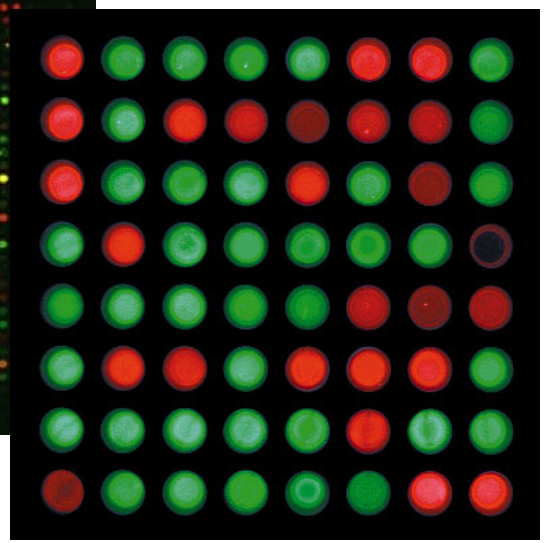


Basics of what's on the surface of a DNA chip



Bioarrays: The future of bioresearch and medicine

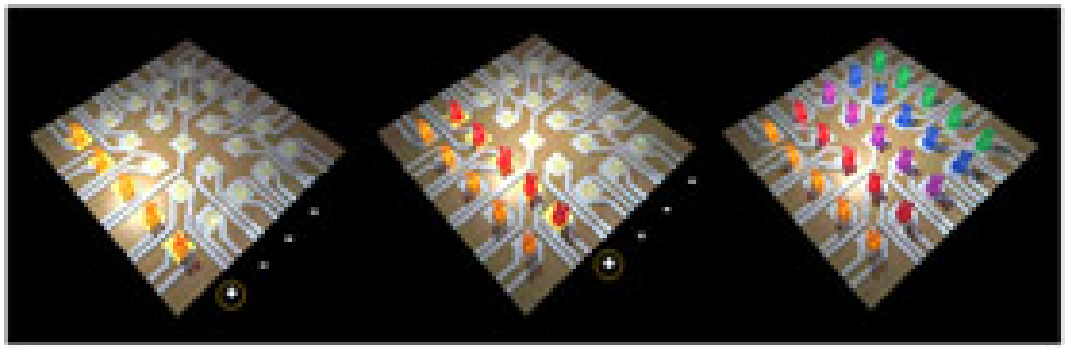




Thousands of genes checked on chip

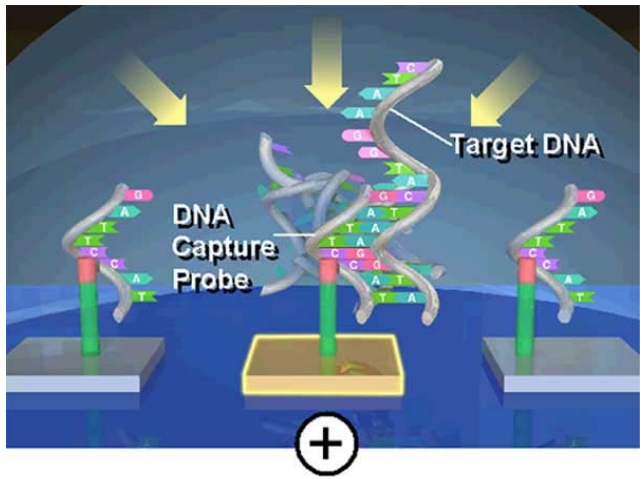
Clinical diagnostics
Genetic fingerprinting
Drug screening
Genetic research
Cell research

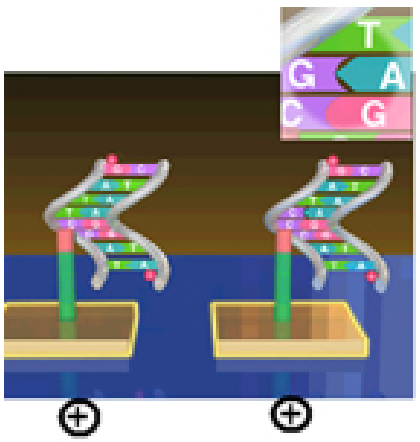
Moving the DNA molecules around: Nanogen's electrophoretic approach

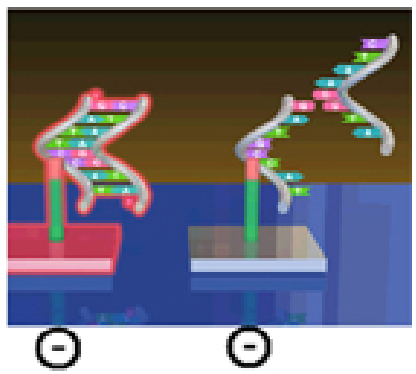


The DNA patches are situated on electrode arrays …

… that allow to attract and move the DNA sample, so masstransfer and binding are quick …



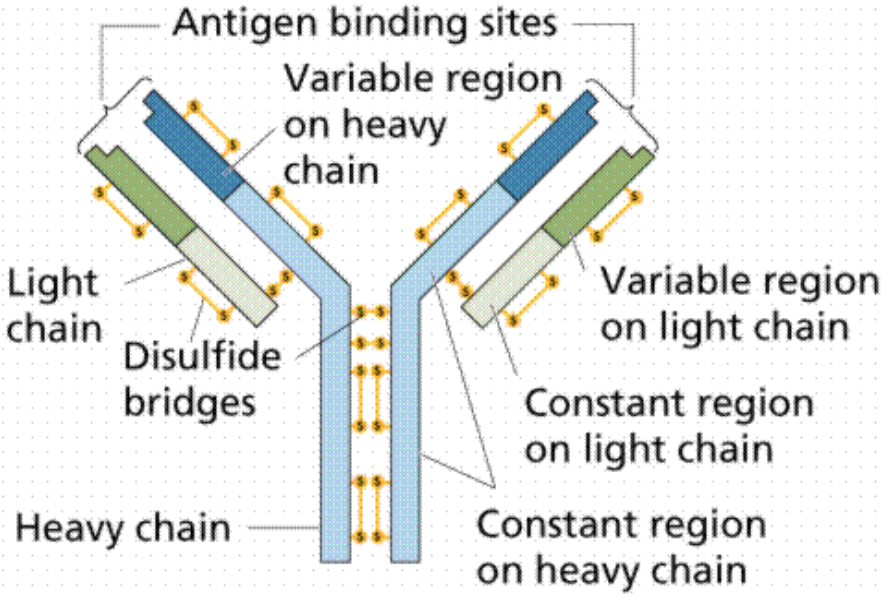


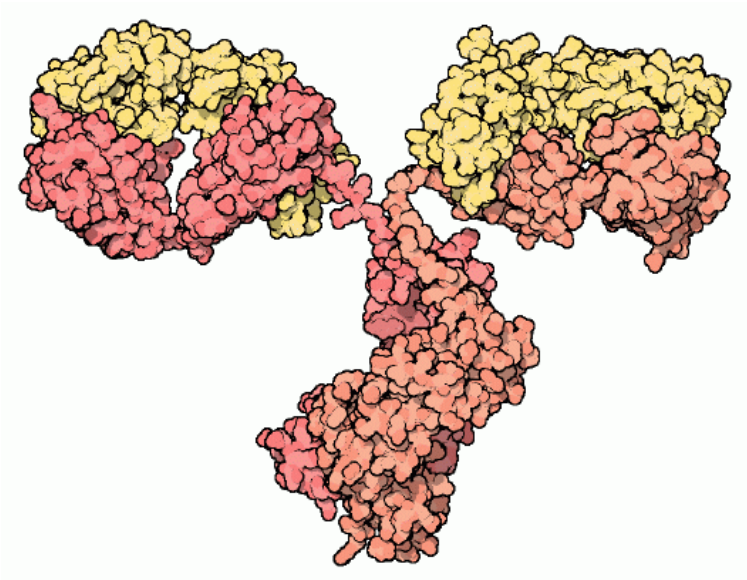


… and by reversing the charge remove the (weakly bound) molecules with no full complementarity

Immunological Antibody-Antigen Interactions

Immunoglobulin (IgG)-Antibody

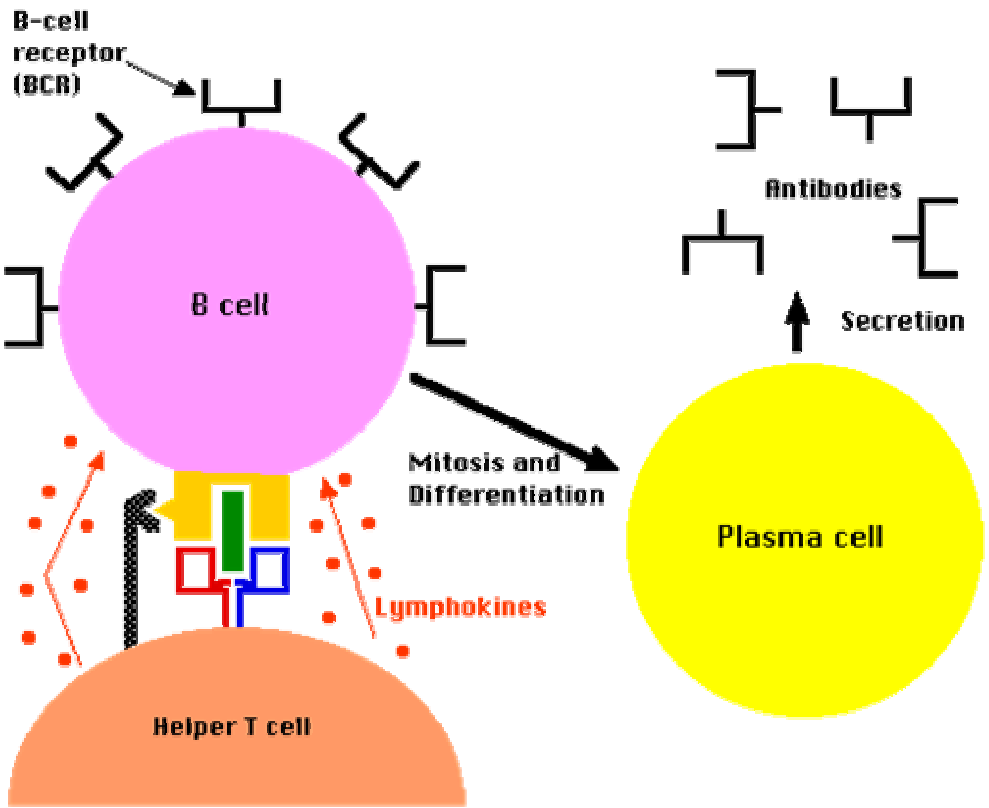




$K_d < 10^{-5} – 10^{-7}$

Immunoglobulins are part of the immune defense system

Antigen:
protein,
polysaccharide,
toxin,
DNA/RNA, etc



Tag and remove antigens

"Lock-and-key" Protein-Ligand Interactions

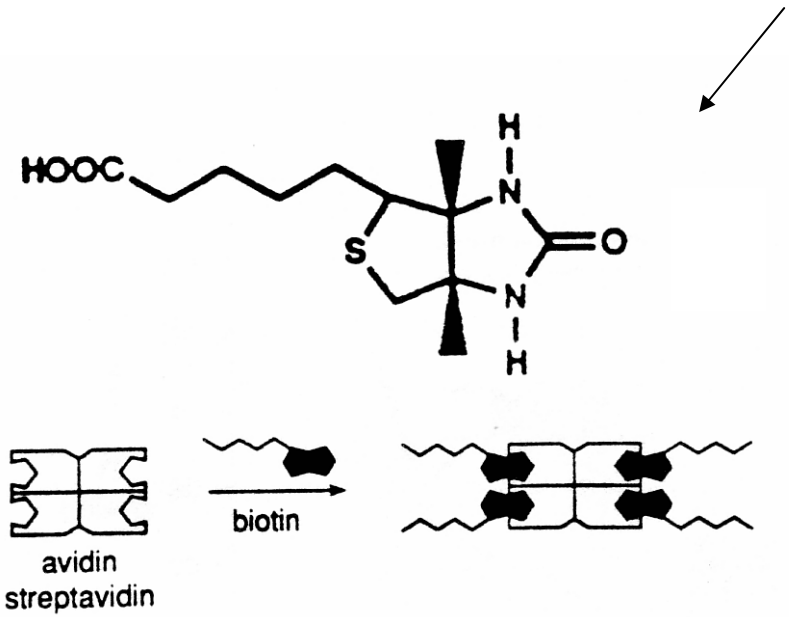
$$\mathbf{P + A \leftrightarrow PA}$$

- Strong
- Irreversible
- Measured by K_d

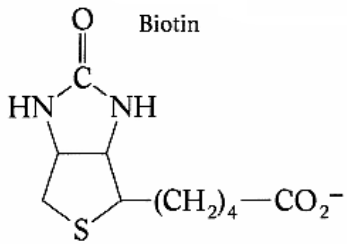
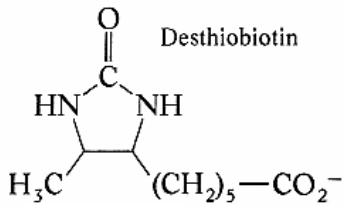
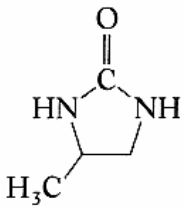
$$K_d = \frac{[P][A]}{[PA]}$$

Basic example:

Avidin-Biotin or Streptdavidin-Biotin



Binding of Biotin Derivatives to Avidin

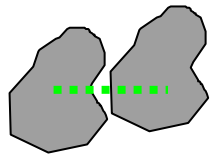
Derivative	Dissociation constant (M)	Free-energy contribution to binding (kcal/mol)
Biotin 	1.3×10^{-15}	
Desthiobiotin 	5×10^{-13}	
	3.4×10^{-5}	−13.3
$CH_3-(CH_2)_4-CO_2^-$	3×10^{-3}	−10.7

Data from N. M. Green, *Adv. Protein Chem.* 29:85 (1975).

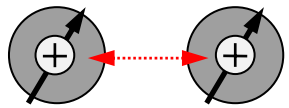
Protein - protein interactions: Calculating the potential W(r)

$$W(r) = W_{disp}(r) + W_{q-q}(r) + W_{q-\mu}(r) + W_{\mu-\mu}(r) + W_{HS}(r) + W_{OH}(r)$$

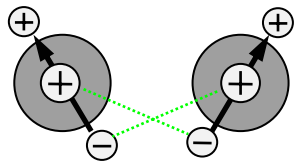
W_{disp} - van der Waals attraction



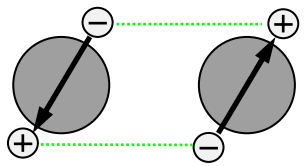
$W_{q\text{-}q}$ - charge - charge electrostatic repulsion



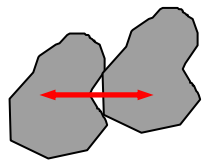
$W_{q\text{-}\mu}$ - charge - dipole electrostatic attraction



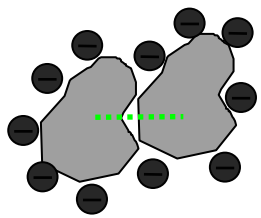
$W_{\mu\text{-}\mu}$ - dipole - dipole electrostatic attraction



W_{HS} - hard-sphere repulsion

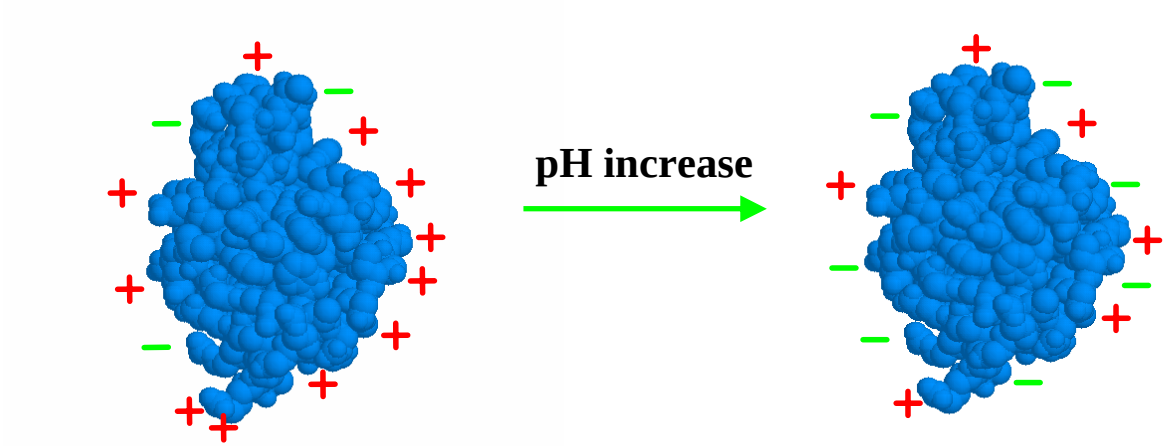


W_{OH} - short-ranged hydrophylic or hydrophobic attraction

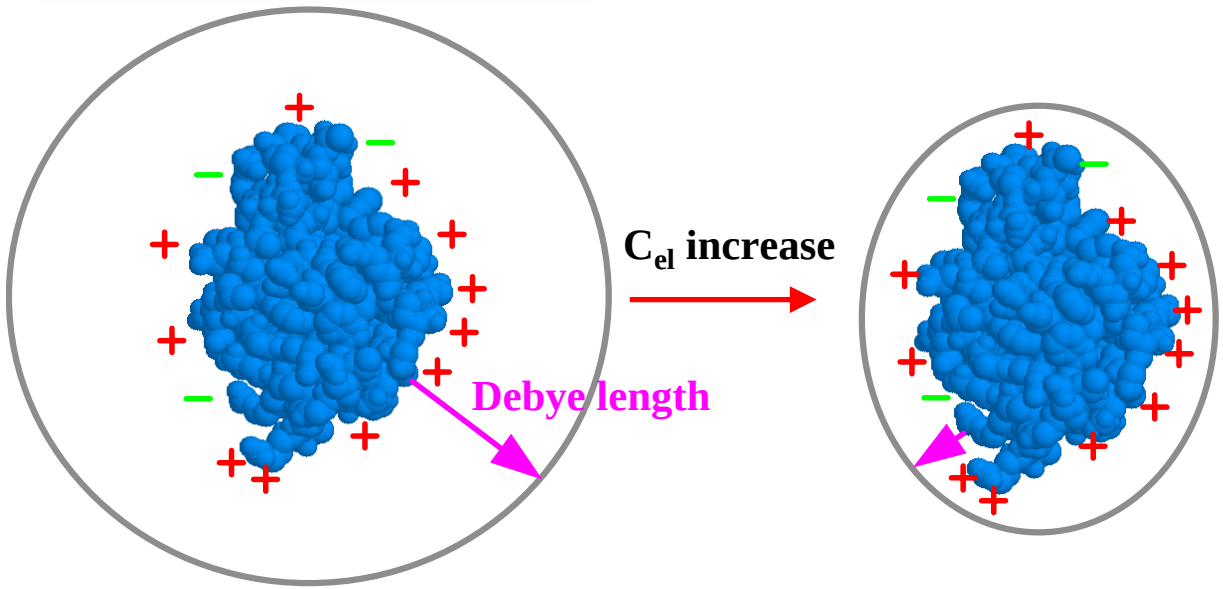


The two basic parameters affecting protein interactions

pH



Electrolyte



Correlating protein interactions to macroscopic properties of protein solutions via the second virial coefficient

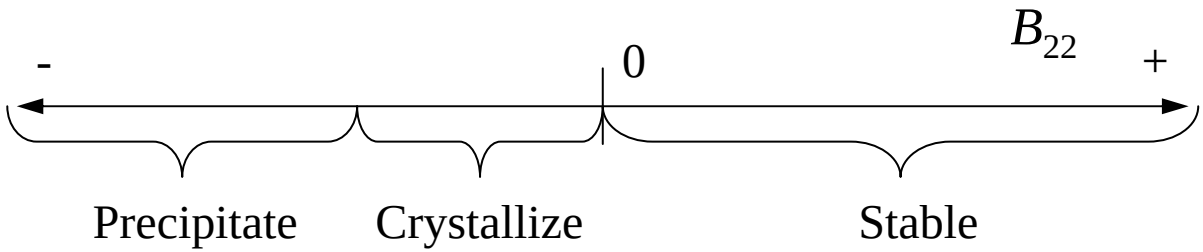
- Theoretically, the second virial coefficient, B_{22}, characterizes two-body interactions between protein molecules in dilute solution
- It can be calculated from the energy of interaction

$$B_{22} = -\int_{\Omega}\int_{r}\left(e^{-\Delta w(r,\Omega)/kT} - 1\right) r^2\, dr\, d\Omega$$

- It can be measured experimentally via the osmotic pressure, π, or by light scattering

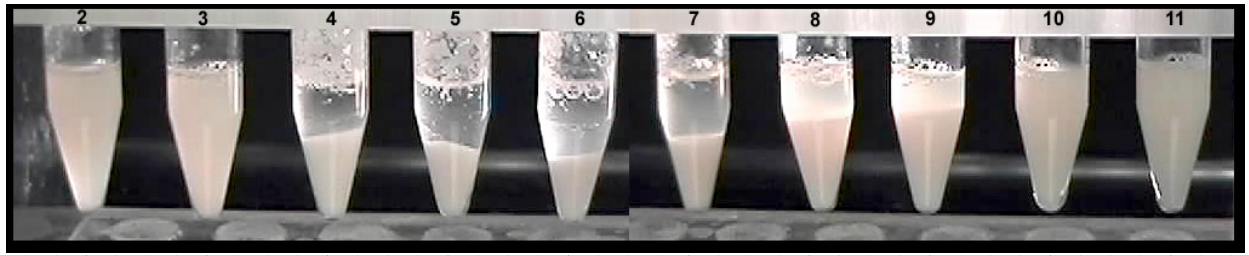
$$\pi = R\,T\,C_p\,(1 + B_{22}\,C_p + \ldots)$$

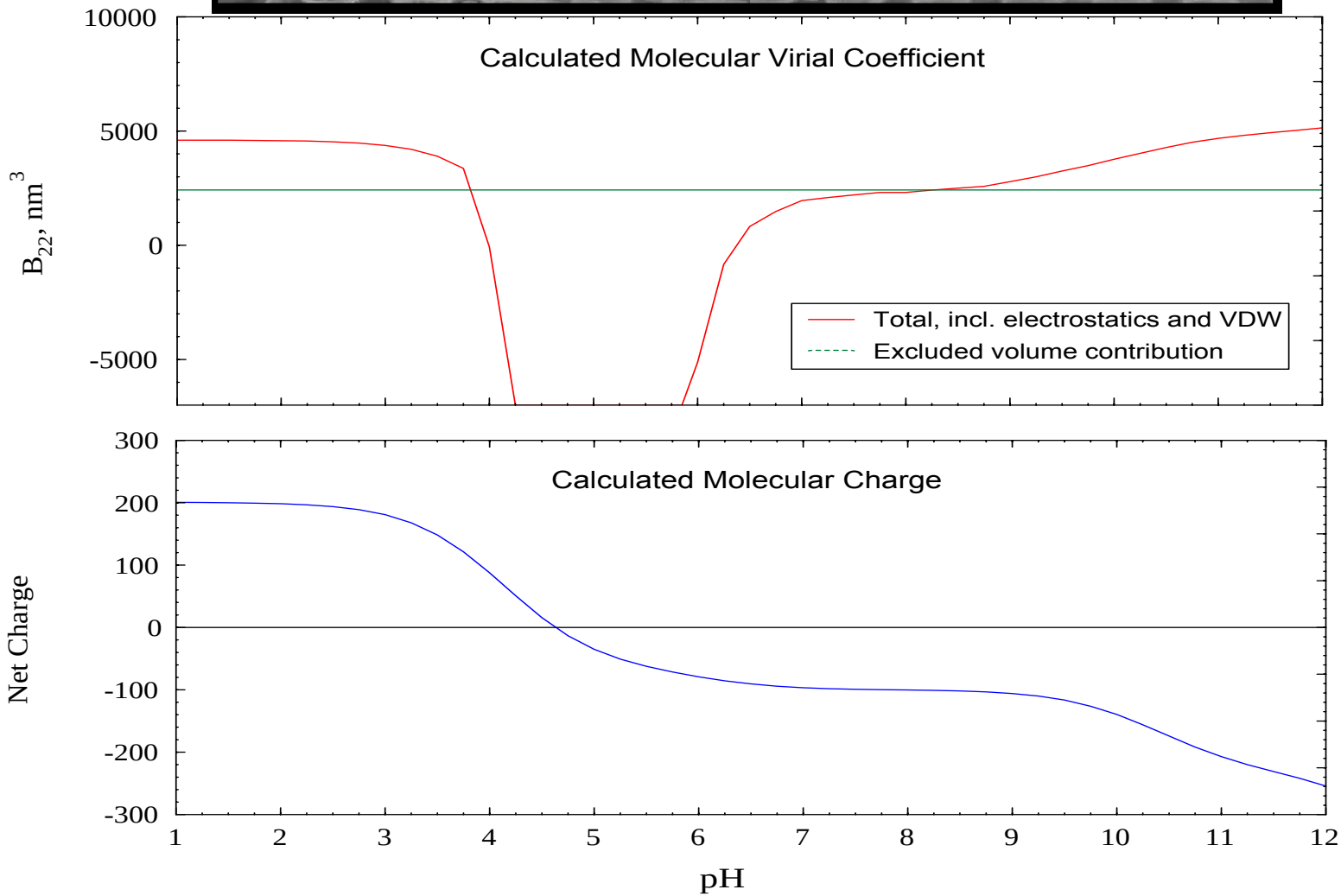
- B_{22} is a major predictor for the properties and separation processes in protein solutions



Correspondence between Charge and Precipitation Equilibria - Soy Protein

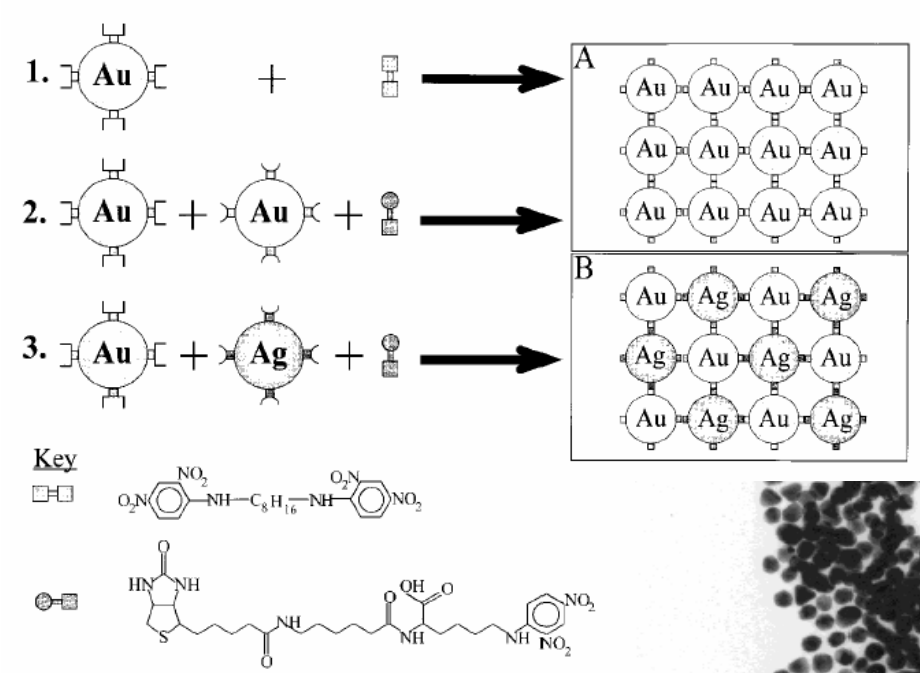
Hinderliter
and Velev





Assembling particles via Lock-and-Key interactions

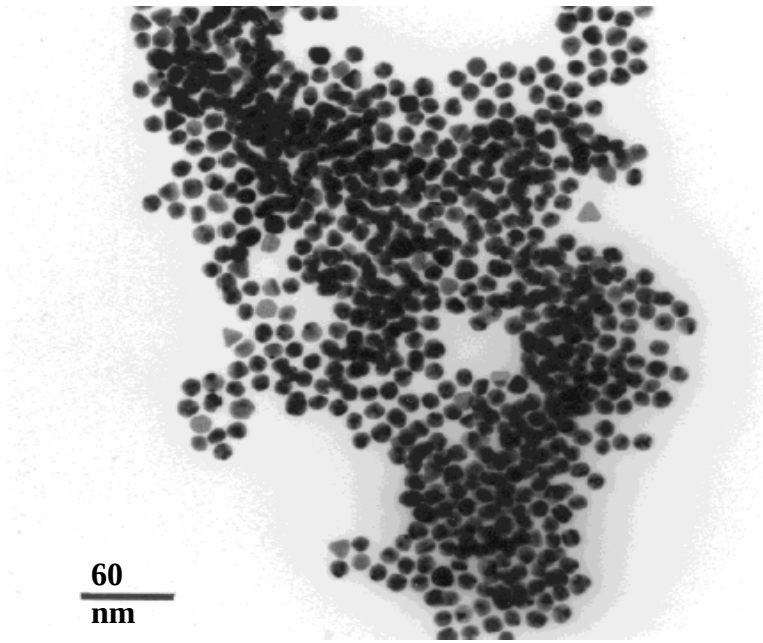
Metallic nanoparticles: Mann et al., Adv. Mater., 1999, 11:449.



Schematic representation showing possible approaches to the directed self-assembly of metallic (routes 1 and 2), and bimetallic (route 3) macroscopic materials using antibody–antigen cross-linking of inorganic nanoparticles. The structures shown are idealized; in reality the materials are highly disordered. 1) Au nanoparticles with surface-attached anti-DNP IgE antibodies and homo-Janus DNP–DNP antigen connector. 2) Au nanoparticles with either surface-attached anti-DNP IgE or anti-biotin IgG antibodies and hetero-Janus DNP–biotin antigen. 3) 1:1 mixture of Au/anti-DNP IgE and Ag/anti-biotin IgG nanoparticles in association with DNP–biotin bivalent antigen.

Principle

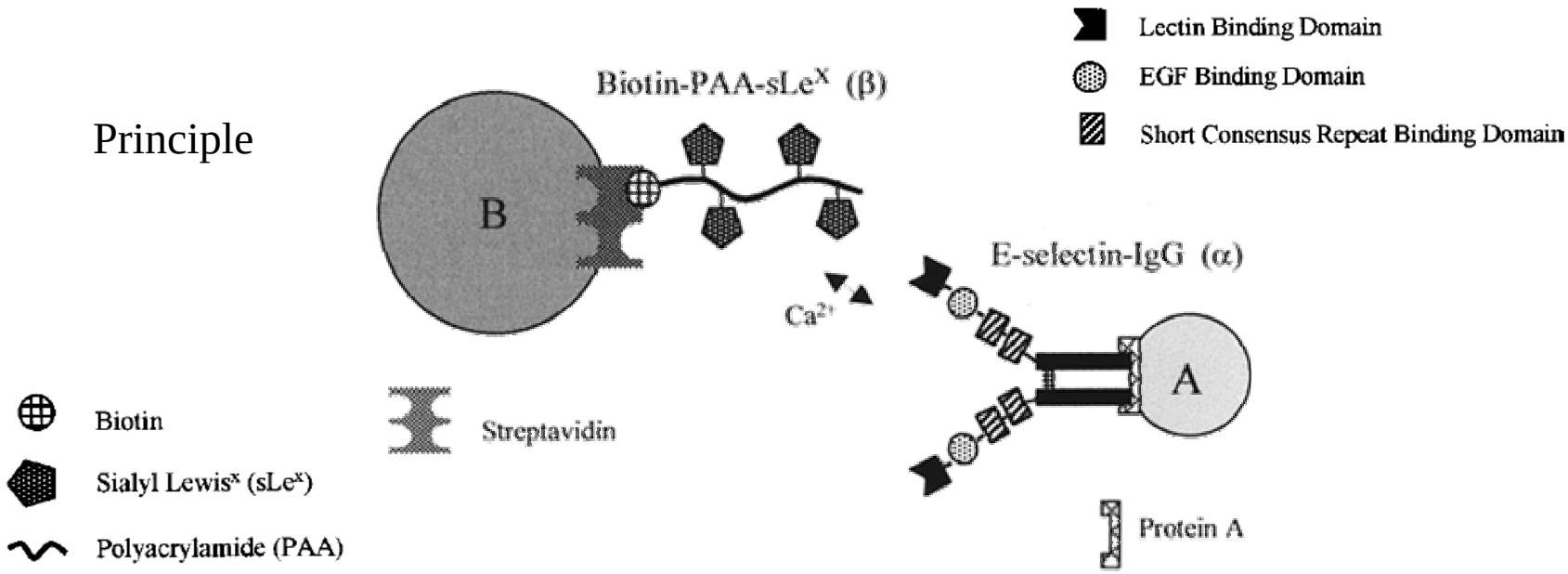
TEM image



Assembling particles via Lock-and-Key interactions 2

Amy Hiddessen et al., Langmuir, 2000, 16:9744.

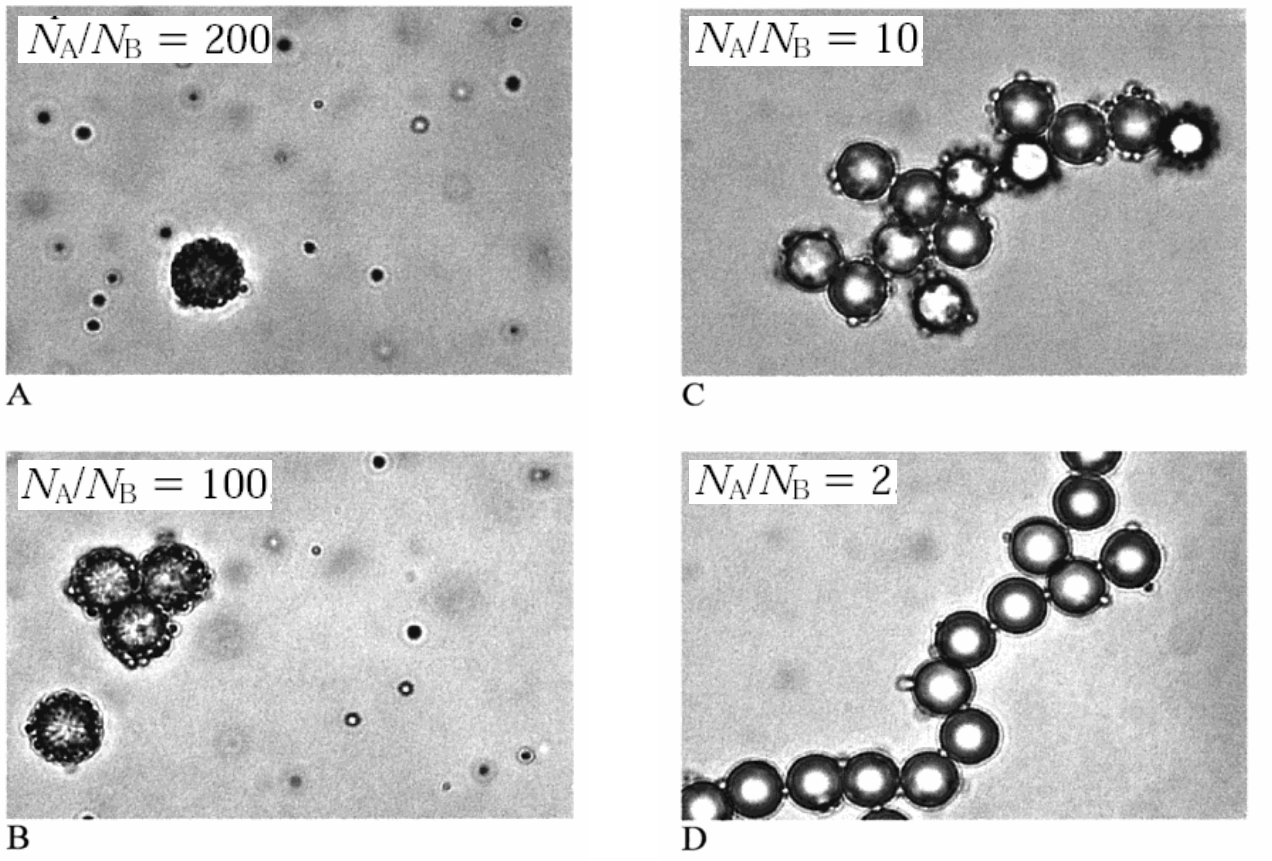
Principle



- A particles: 0.94-μm protein A modified polystyrene particles coated with E-selectin-IgG.
- B particles: 5.5-m streptavidin modified B polystyrene particles coated with sLe^X
- In the E-selectin/sLe^X interaction, sLe^X binds to the lectin-binding domain of E-selectin (in the presence of calcium ions).

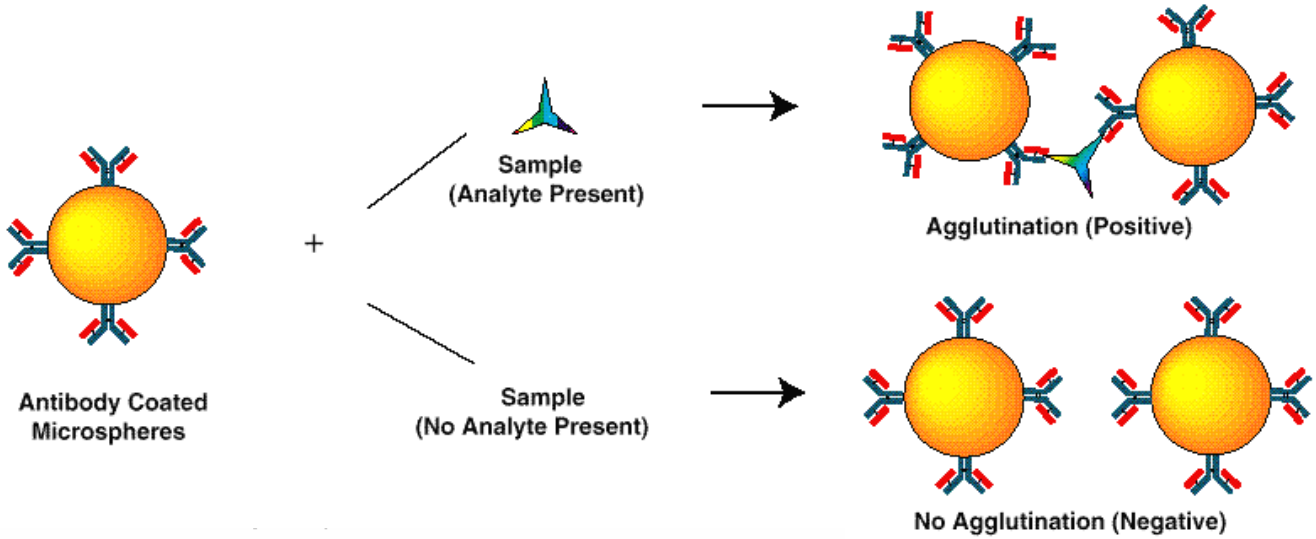
Assembly via Lock-and-Key interactions contd.

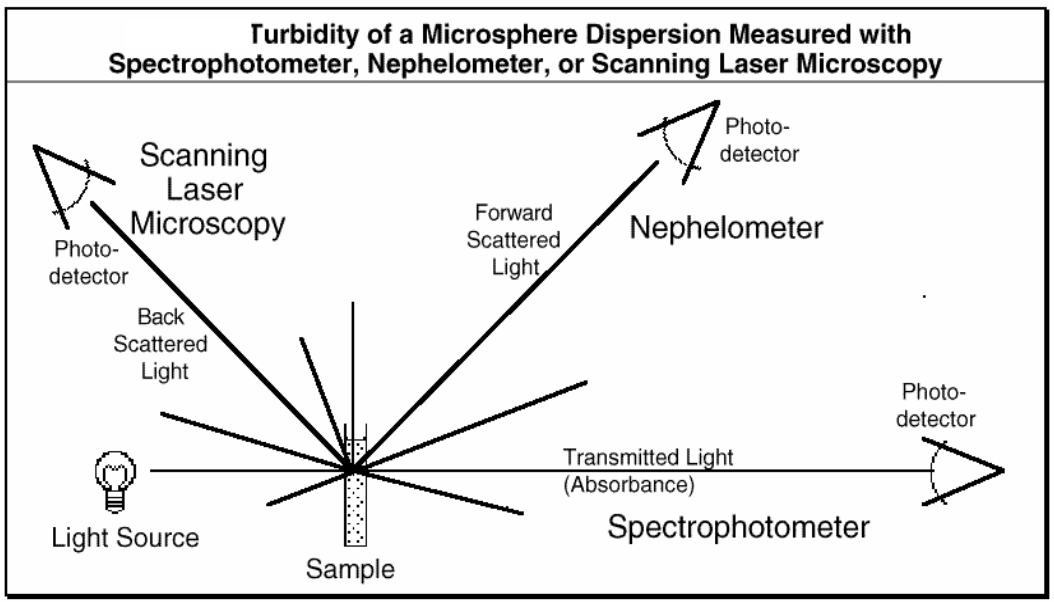
Effect of small/large particles ratio



Amy Hiddessen et al., Langmuir, 2000, 16:9744.

Immunological Antibody-Antigen Interactions: Latex Agglutination Assays

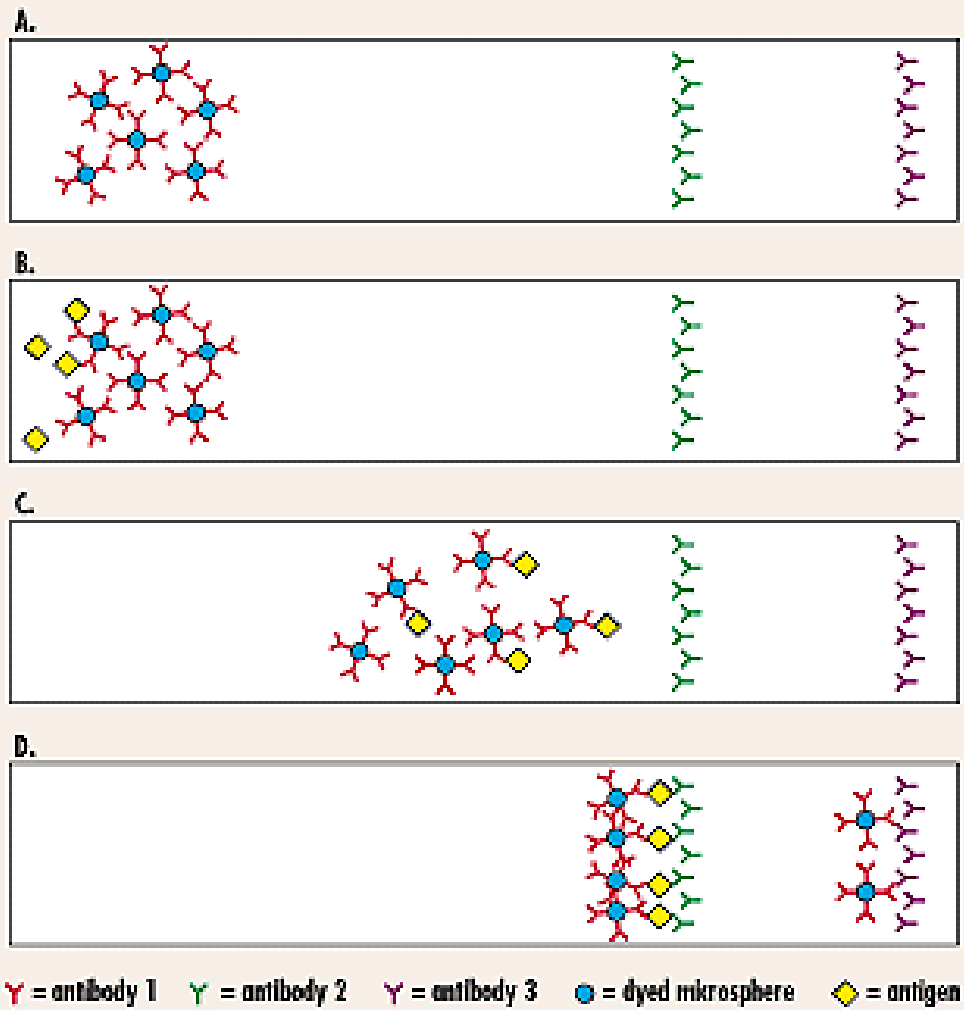




Figures from L. B. Bangs, Tech. Notes #39 and #40, Bangs Laboratories Inc.

Basic “chromatographic strip test”

Principle



A. Dry strip.

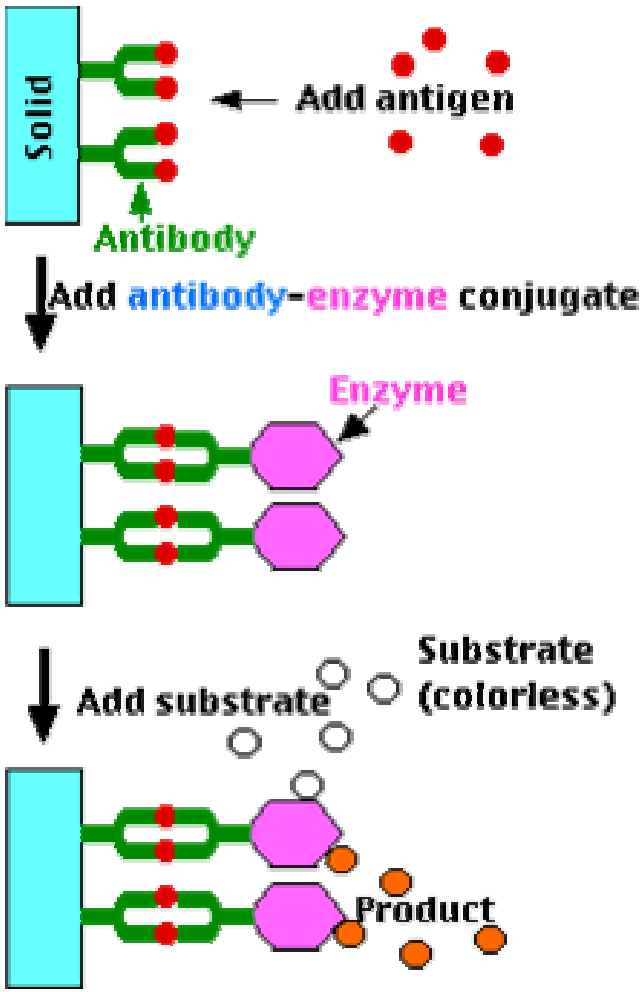
B. Sample (with antigen) added.

C. Sample flow moves microspheres; antigen forms sandwich.

D. Dyed microspheres form colored lines for positive test and control.

Enzyme-Linked Immunosorbent Assay (ELISA)

Principle

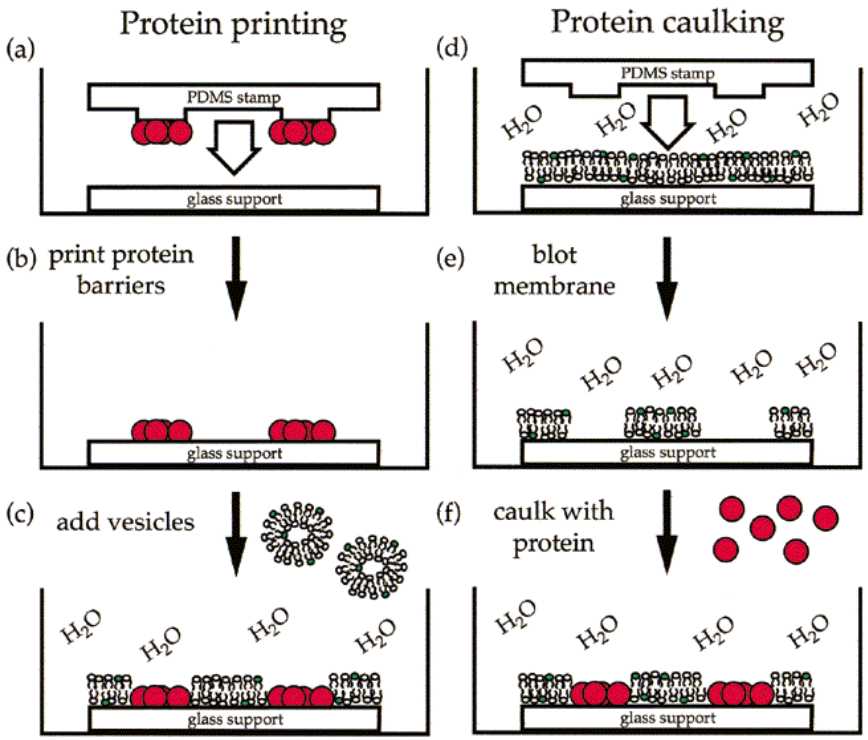


ELISA is a widely-used method for quantitatively measuring the concentration in a fluid such as serum or urine of :

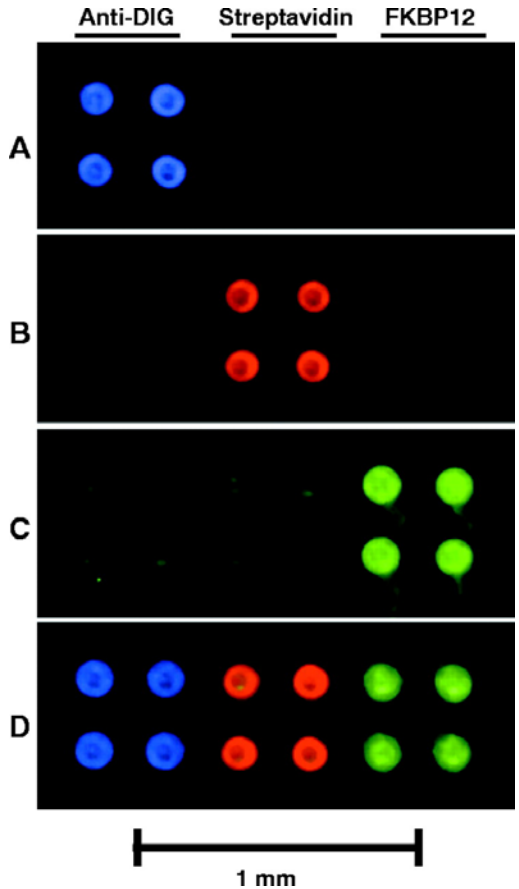
- Hormone levels (pregnancy, anabolic steroids, HGH) Infections diseases
- Allergens in food and house dust
- Autoantibodies (e.g. "rheumatoid factors")
- Toxins, illicit drugs

Moving towards the next frontier: Proteomics

Fabrication of protein arrays by printing
Boxer et al., *Langmuir*, **16**, 6773 (2000)



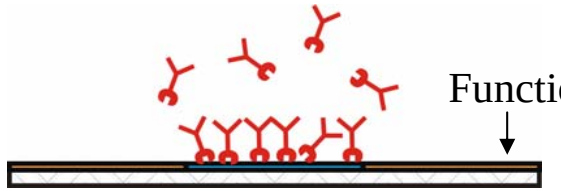
Detecting targets of small molecules by proteins immobilized on glass slides
MacBeath and Schreiber, *Science*, 289, 1760 (2000)



Nanoparticle immunoassay schematics

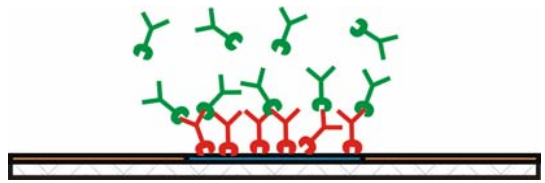
S. Gupta, P. Kilpatrick and O. Velev

Base IgG attachment
(5 mins)



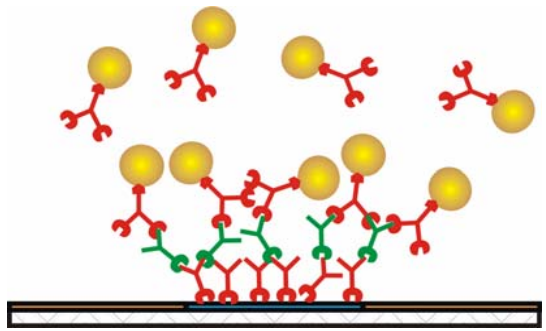
Receptor antibody

Analyte detection
(20 mins)



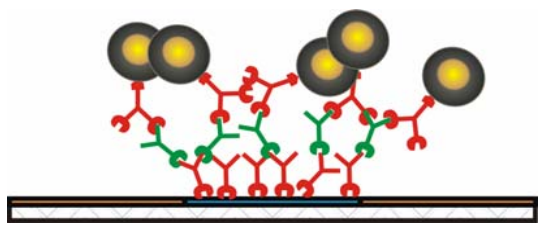
Antigen

Gold tagging
(45 mins)



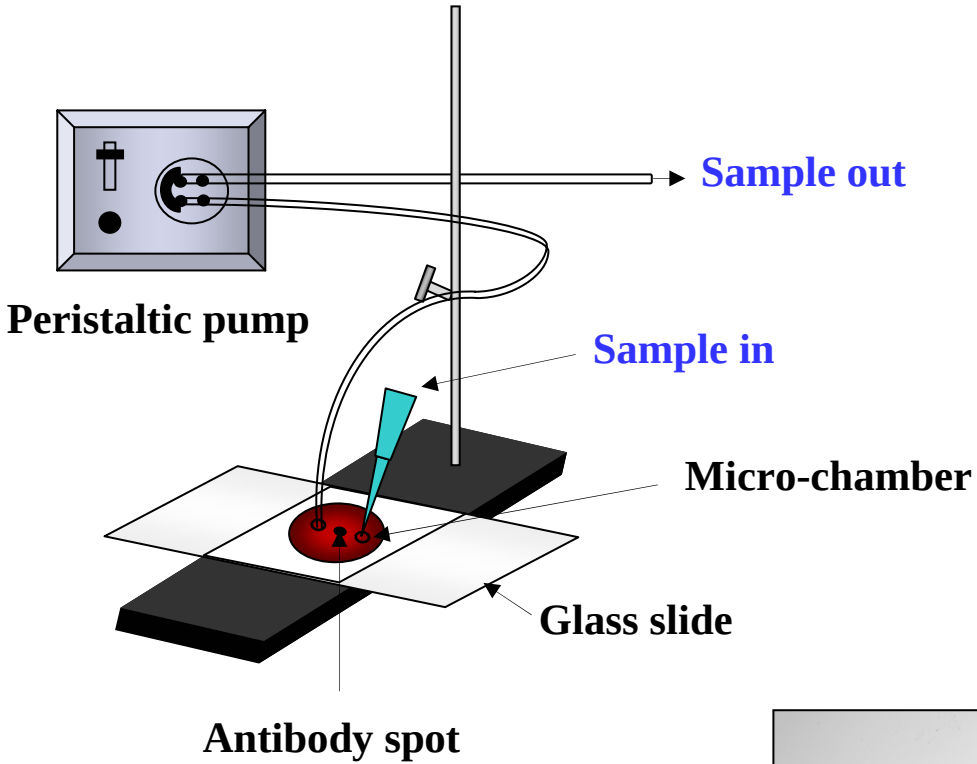
Gold-conjugated antibody

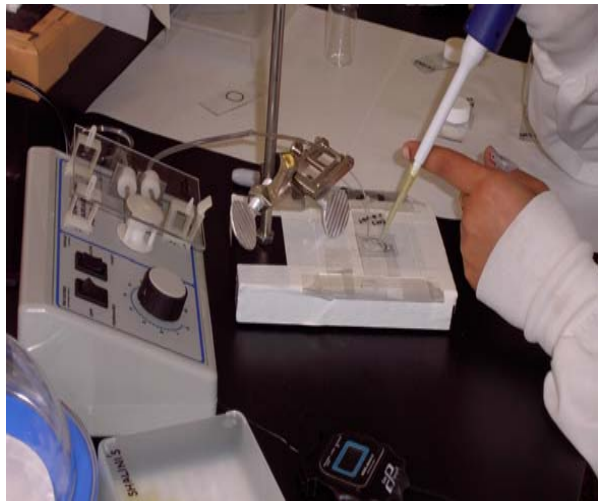
Silver enhancement
(10 mins)



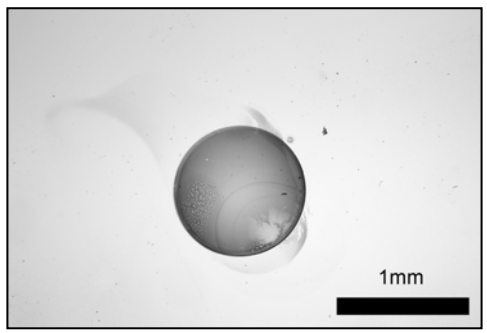
Silver enhanced gold

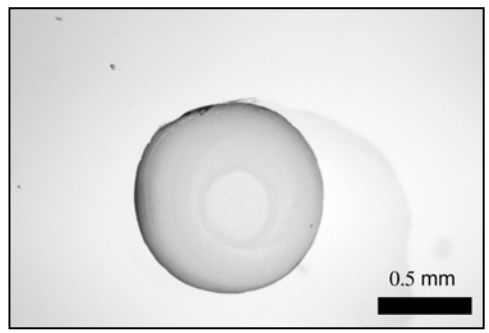
Set-up for preparing sandwich assays





Silver-enhanced spots





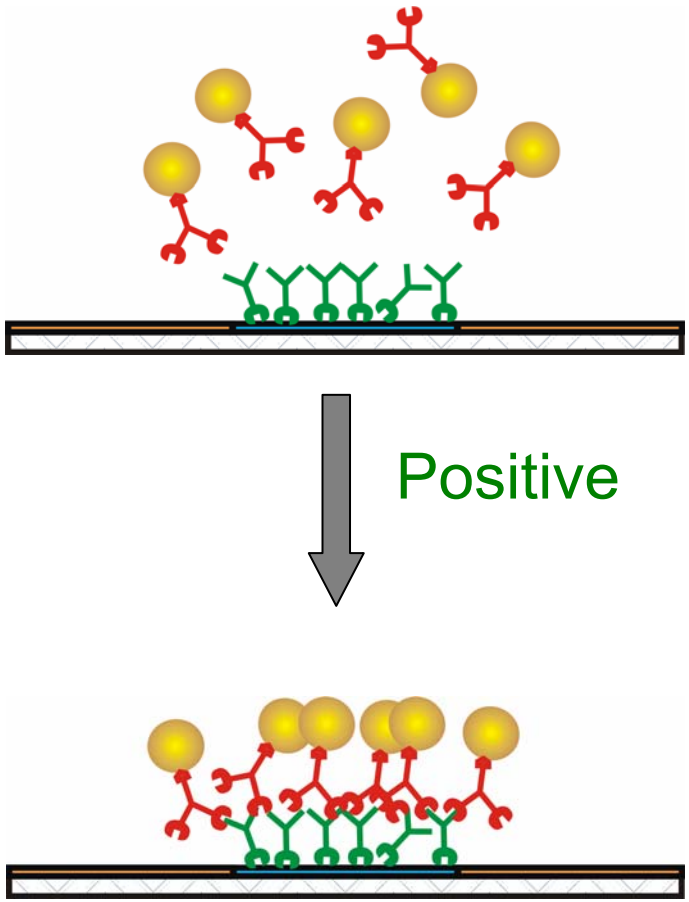
Micro-chamber

- diameter = 13 mm
- depth = 0.25 mm
- volume = 30 µL

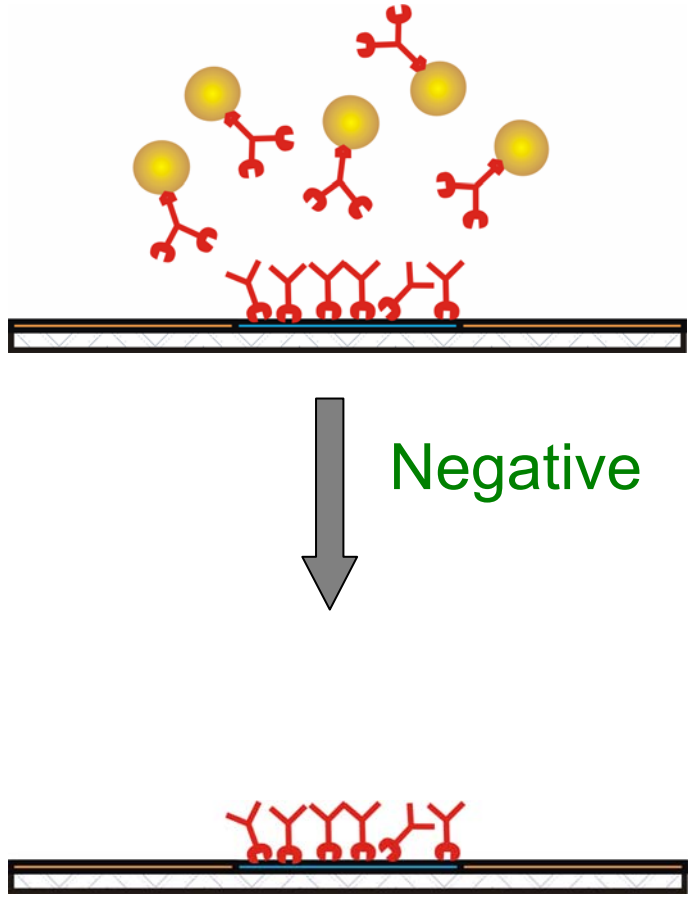
[Mouse IgG] →	**30 µg/mL**	**1 µg/mL**
Gold incubation →	**30 mins**	**45 mins**

Expected selectivity for direct assays

Complementary Antibodies

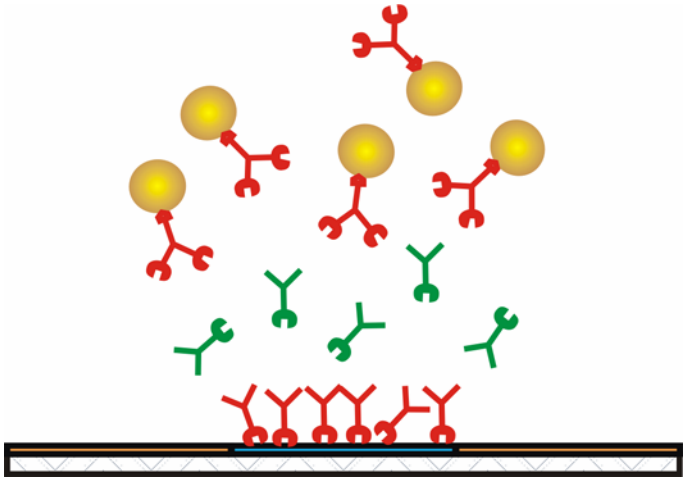


Non-complementary Antibodies

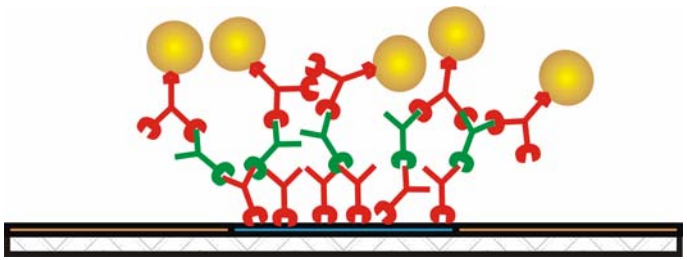


Expected selectivity for sandwich assays

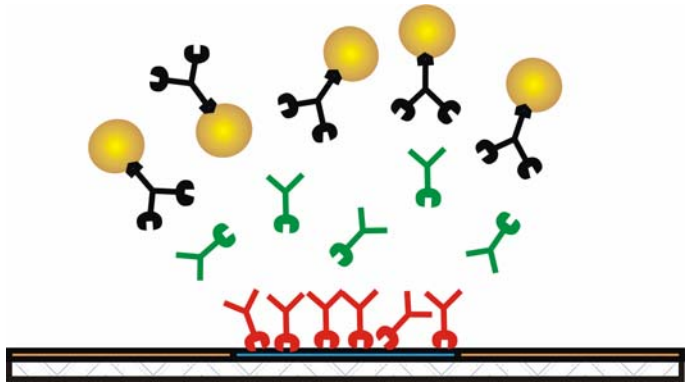
Complementary Antibodies



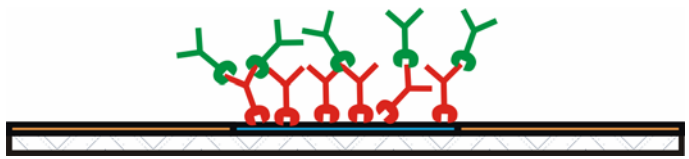
Positive



Non-complementary Antibodies



Negative



Selectivity table

Direct assays

Sandwich assays

	GAMg	GARg	M-GAMg	M-GARg	R-GAMg	R-GARg
M	√, √	X, √	√, √	√, √	√, √	√, √
R	X, X	√, √	X, √	√, √	√, X	√, √
GAR	X, X	X, X	X, X	X, X	X, X	√, √
GAM	X, X	X, X	√, √	X, X	X, X	X, X

√ → Enhancement

X → No enhancement

Experiments → 1 2

False positives

Summary: assay selectivity

Conclusions

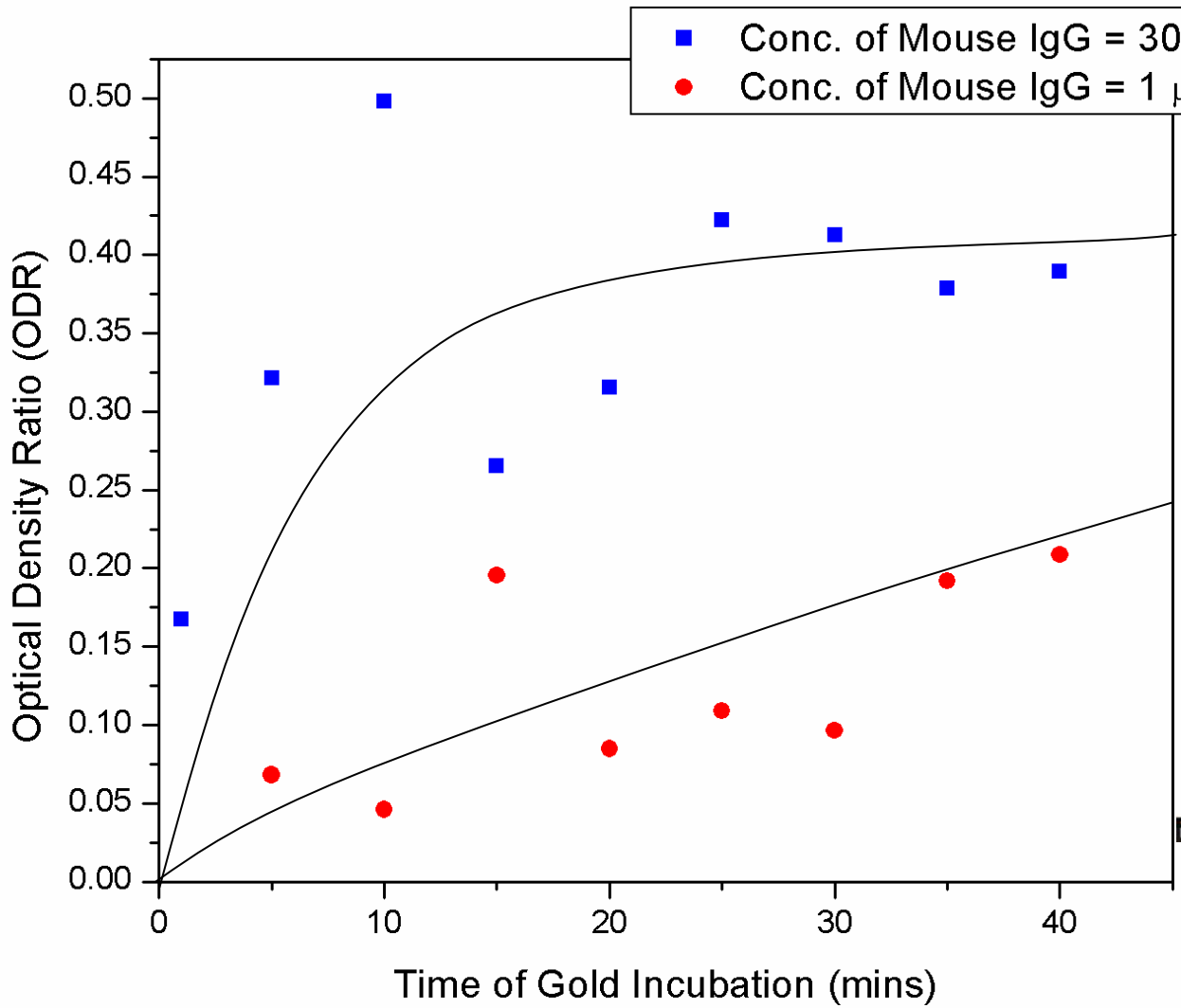
- Sandwich assays possess high level of selectivity when **antibody** (GAM & GAR IgG) is immobilized on the surface.
- False positives can occur in direct and sandwich assays when **antigen** (M & R IgG) is immobilized on the surface.

Explanation

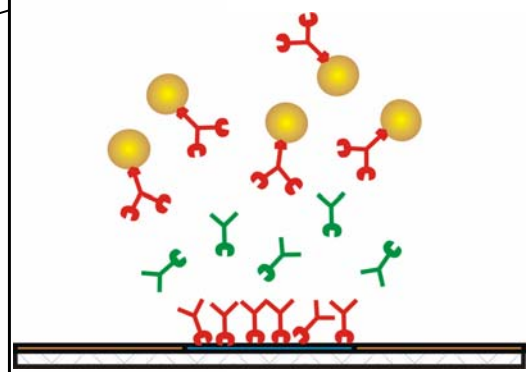
Polyclonal antigen-

I. On **surface**: can interact **non-specifically** with antibodies in solution. Hence, false positives.
II. In **solution**: needs double cross-reactivity for enhancement. No false positives.

Effect of gold incubation time on spot enhancement in GAM-M-GAMg immunoassay

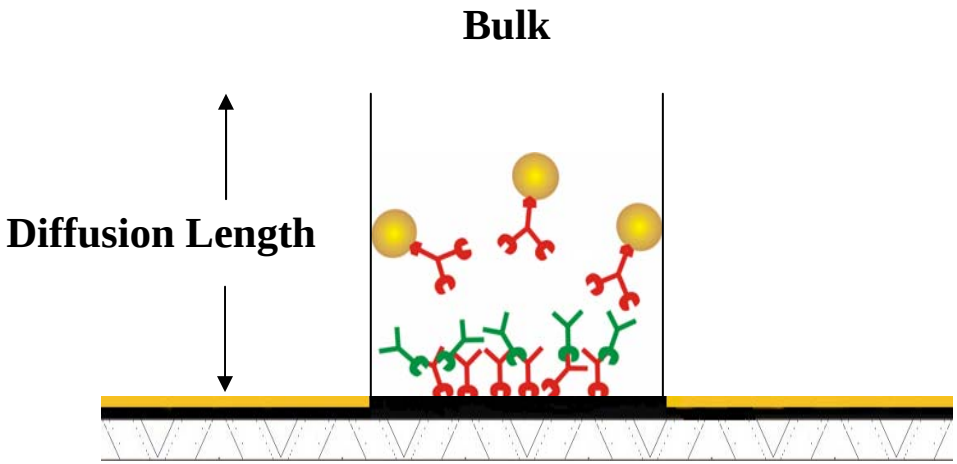


Vary time of gold incubation



Curves fitted to guide the eye

Diffusion limited assay binding



$$\frac{\partial C(x,t)}{\partial t} = D\frac{\partial^2 C(x,t)}{\partial x^2}$$

$$D\frac{\partial C(0,t)}{\partial x} = \frac{d\Gamma(t)}{dt}$$

Boundary conditions

1. $C(x,0) = C_0$
2. $C(l,t) = C_0$
3. $\Gamma(0) = 0$
4. $C(0,t) = f(t)$

$C_0 \rightarrow Conc.\ of\ \ gold\ \ in\ the\ bulk\ (kg/m^3)$

$C \rightarrow Conc.\ of\ \ gold\ \ in\ the\ diffusion\ layer\ (kg/m^3)$

$t \rightarrow Time\ (s)$

$D \rightarrow Diffusivity\ of\ \ gold\ \ conjugated\ \ IgG\ (m/s^2)$

$l \rightarrow Diffusion\ length\ (m)$

$\Gamma \rightarrow Surface\ concentration\ (kg/m^2)$

Model solution

$$\Gamma(t) = 2\frac{D}{\pi}\left[C_0\sqrt{t} - \frac{1}{2}\int_0^t \frac{f(z)}{(t-z)^{1/2}}dz \right]$$

(Ward and Tordai)

For,

$$f(t) = C_o\left(1 - \exp(-\phi\theta)\right)$$

$$\Gamma_{eq} = \frac{C_0 l}{\phi}$$

$$\Gamma = C_0 l\left(\frac{1}{\phi} - \frac{\cot\left(\sqrt{\phi}\right)}{\sqrt{\phi}}\exp(-\phi\theta) - 2\sum_{n=1}^{\infty}\frac{\exp\left(-n^2\pi^2\theta\right)}{\left(n^2\pi^2\phi\right)} \right)$$

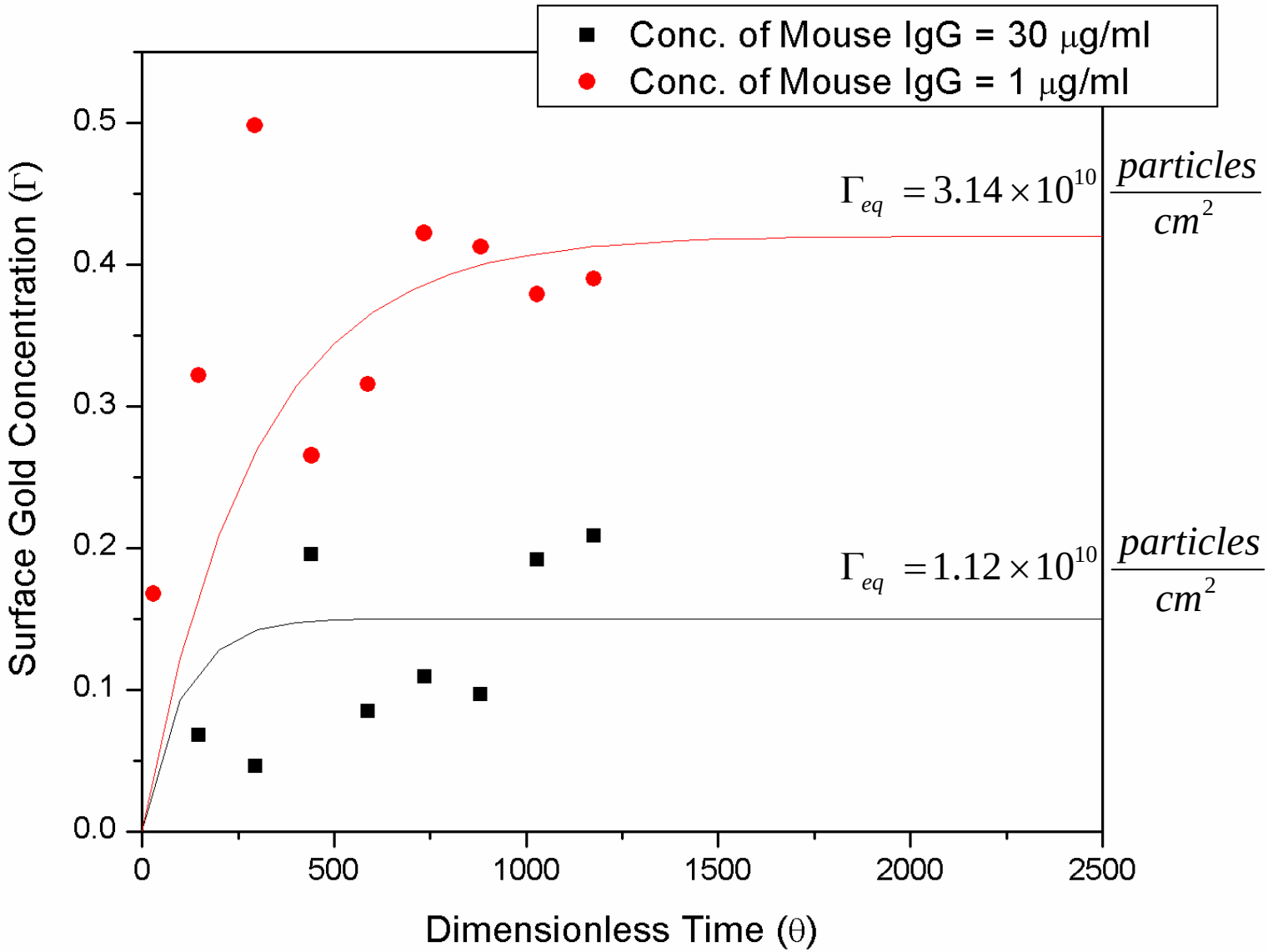
$$\theta = \frac{Dt}{l^2}\left(Time\right)$$

$$\phi = \frac{C_0 l}{\Gamma_{eq}}\ \left(Fitted\ \ parameter\right)$$

Dimensionless parameters

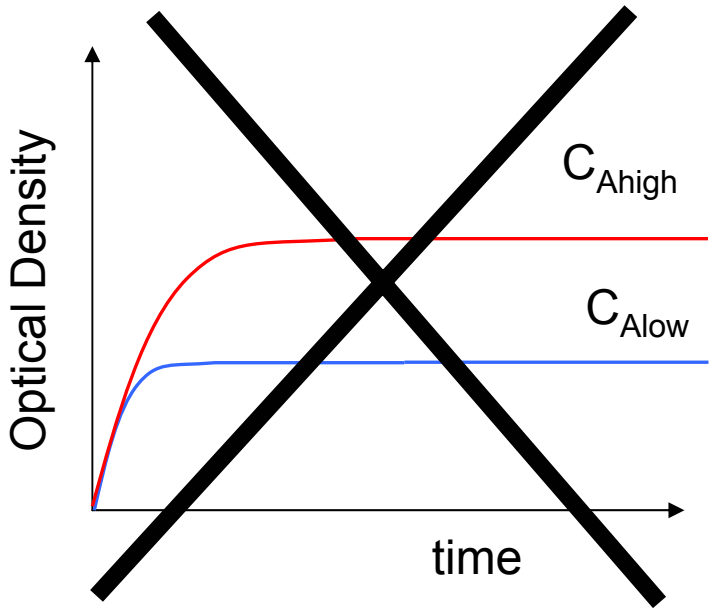
Johannsen *et al.*, Colloids and Surfaces 1991

Model fitting

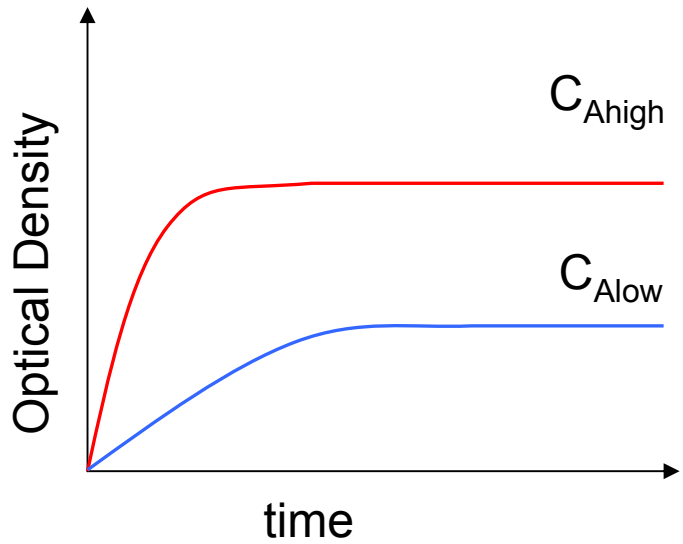


Saturation rate analysis

Model



Experimental



We have to consider additional effects at lower concentrations such as lateral surface diffusion, desorption, rotational reorientation etc.

Summary on nanoparticle-immunoassays

- Developed and characterized silver-enhanced nanoparticle based biosensors which have-

  Low-cost: Simple, low volume consuming equipment.

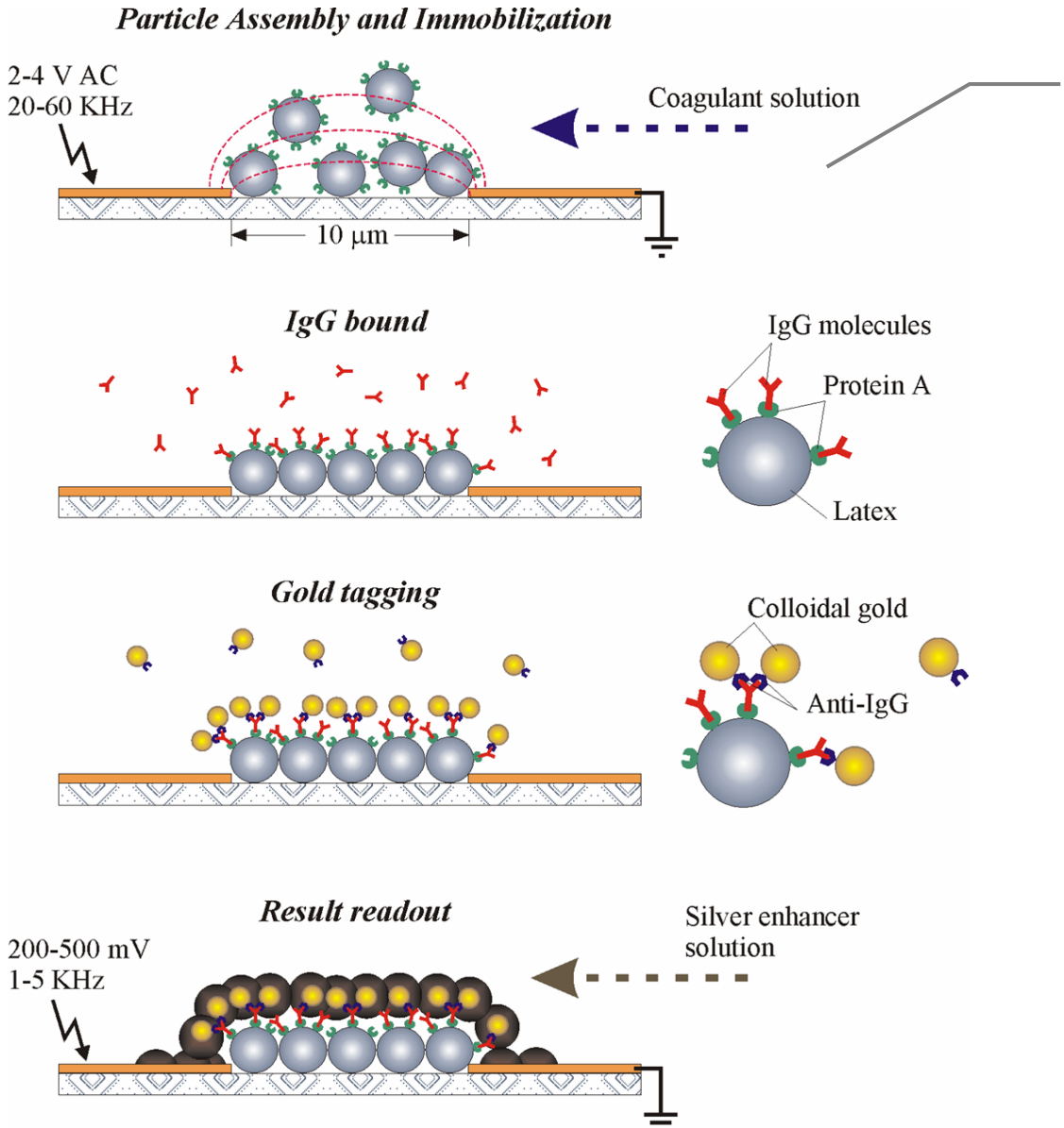
  Speed: Naked-eye evaluation (qualitative), 2 hrs for 4-6 tests (quantitative)

  Sensitivity: 0.1 μg/mL

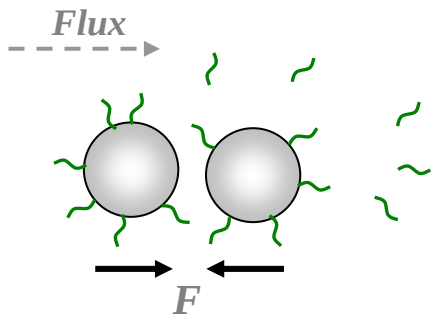
  Selectivity: No false positives for sandwich assays with surface bound antigen.

- Optimized bioasays could be made by modeling theoretical behavior of assays using mass-transfer fundamentals.

Protein arrays by dielectrophoretic assembly of latex microspheres - principle

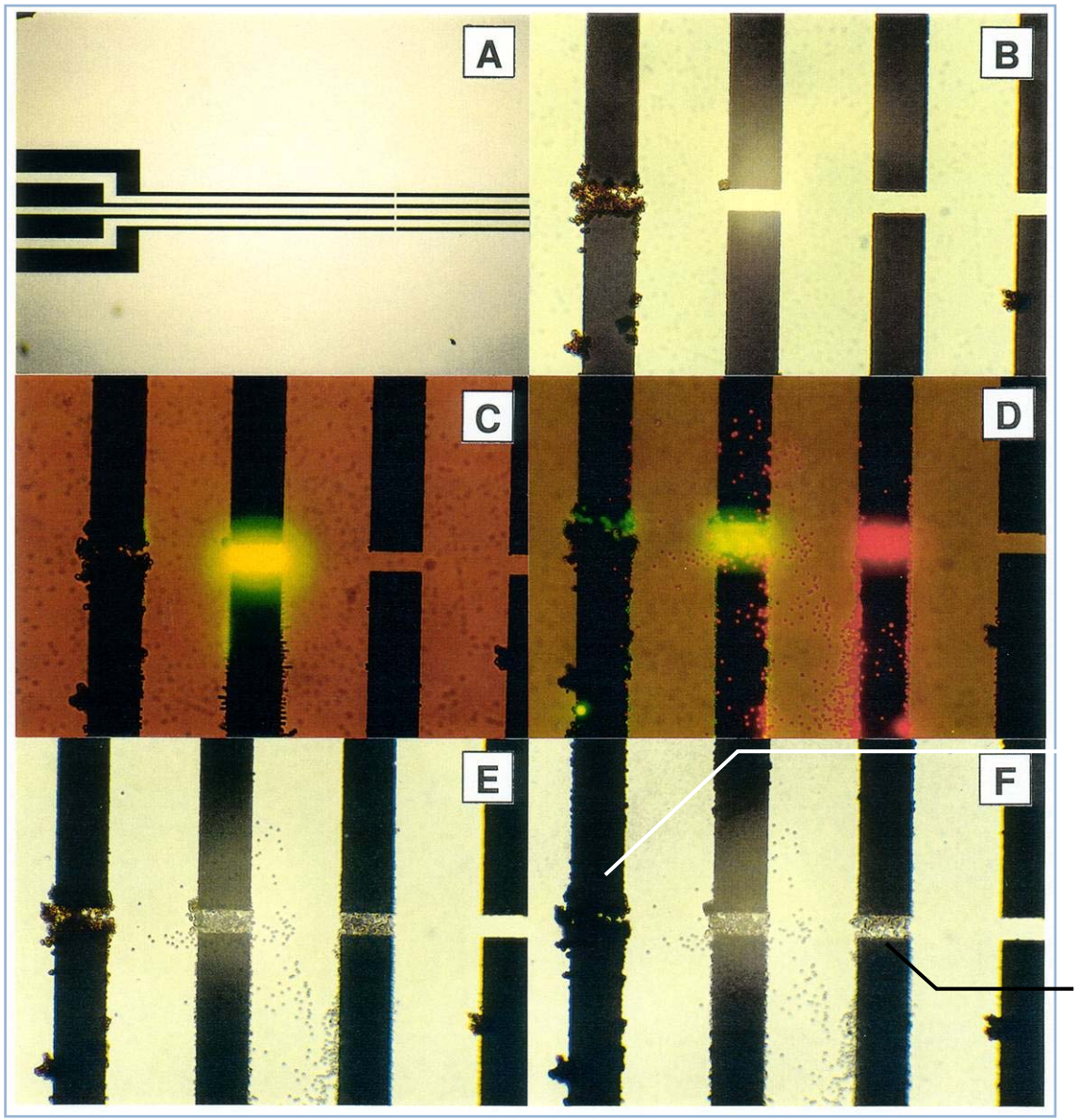


Requires particle coagulation via agent in the flux



Velev and Kaler, *Langmuir*, 15, 3693 (1999).

Optical micrographs of the sensor area

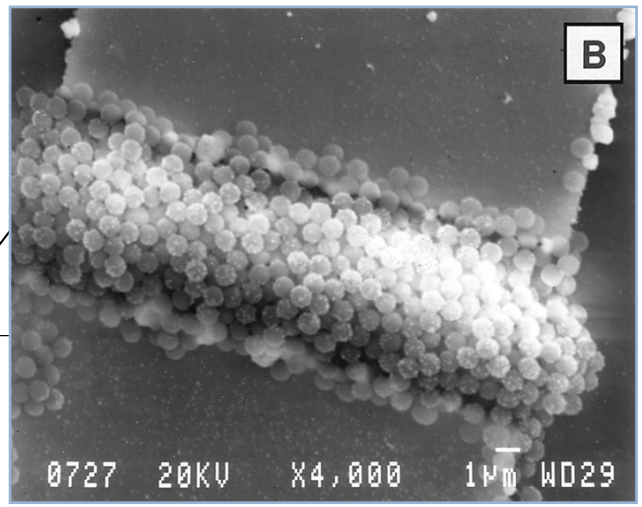


Positive result:
see SEM frame (A)

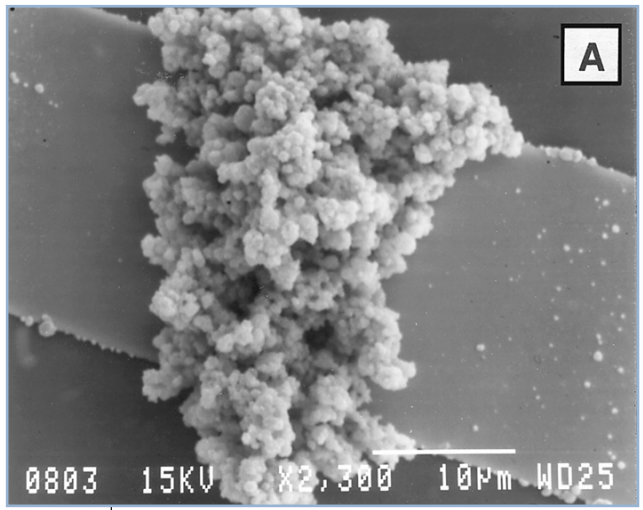
Negative control:
see SEM frame (B)

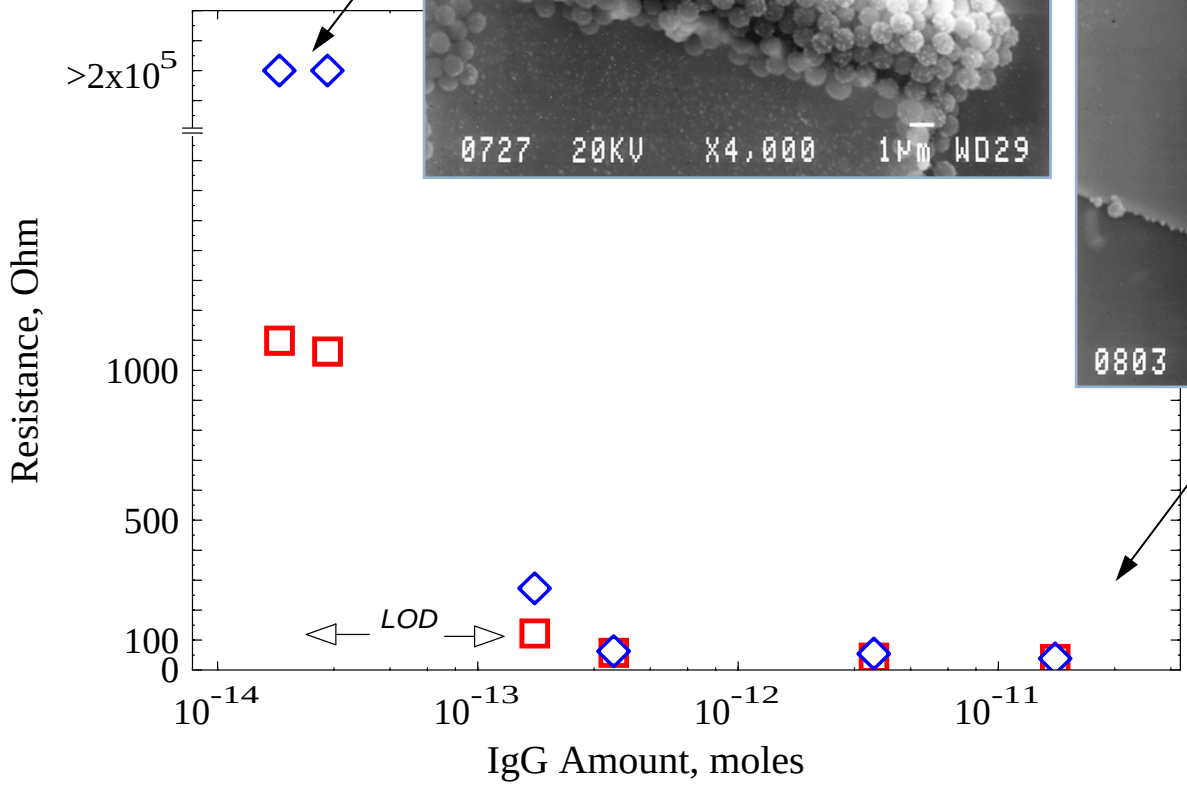
Protein arrays by dielectrophoretic assembly of latex microspheres – direct electric detection

Non-functionalized particles: negative

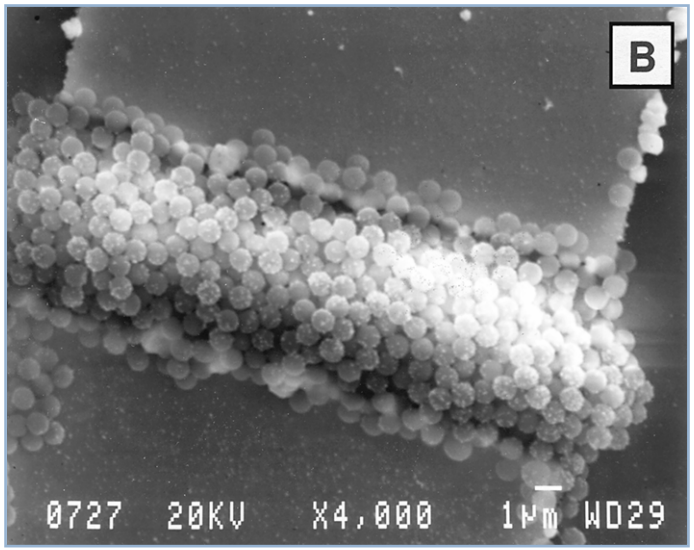


Positive result: electrodes short-circuited





On-chip electric sensor assembly: Summary



- Microscopic and highly sensitive
- First demonstration of direct electric readout
- Multiple sensors can be assembled on the same chip

Velev and Kaler, *Langmuir*, 15, 3693 (1999).

Acknowledgements

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Ketan Bhatt

Velev Group

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