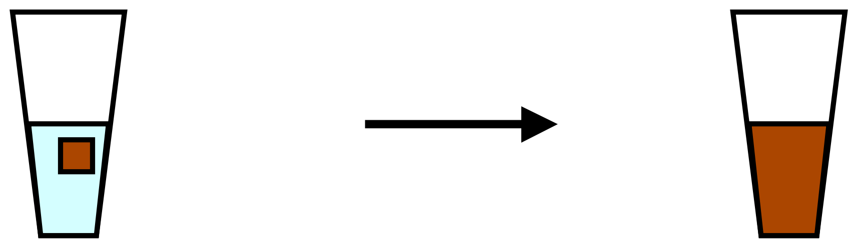
Extreme Nucleic Acid Biosensing



1 μL

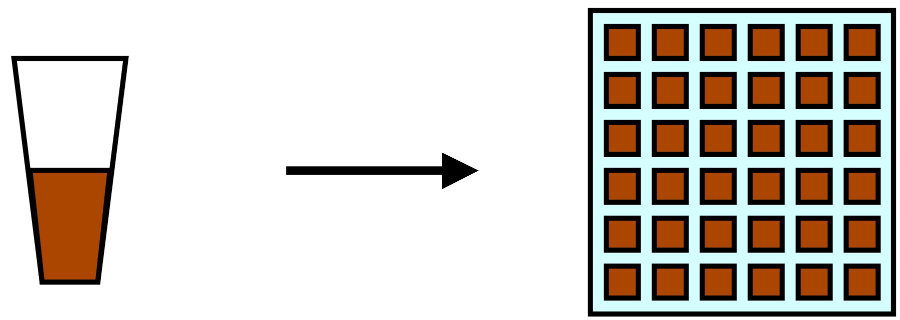
One 100 MBase DNA molecule

1 μL

One million DNA 100mer molecules

One genomic DNA molecule, enzymatically cut up into one million different 100mers.

Extreme Nucleic Acid Biosensing



Is it possible?

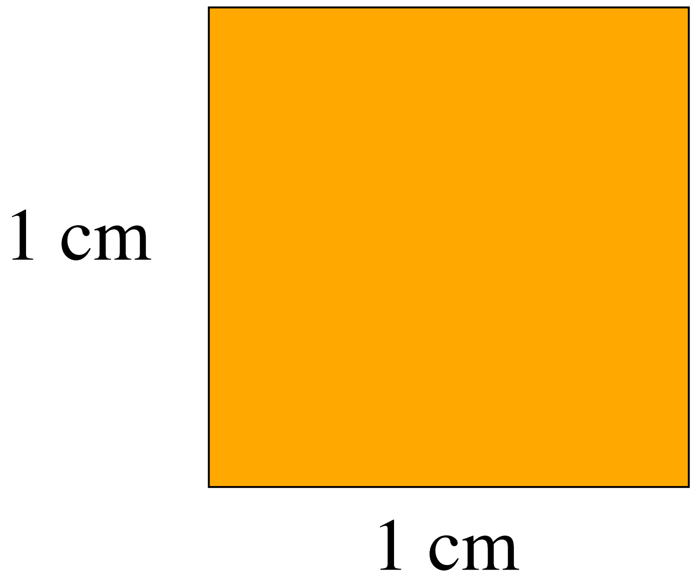
1 μL

One million
DNA 100mers

1 Megapixel
DNA array

One million simultaneous true single molecule detection and identification events by surface bioaffinity adsorption.

1. Stoichiometry, areas and concentrations



Area = A = 1 cm^2

Surface site density $\Gamma = 10^{12}$ probe molecules cm^{-2}

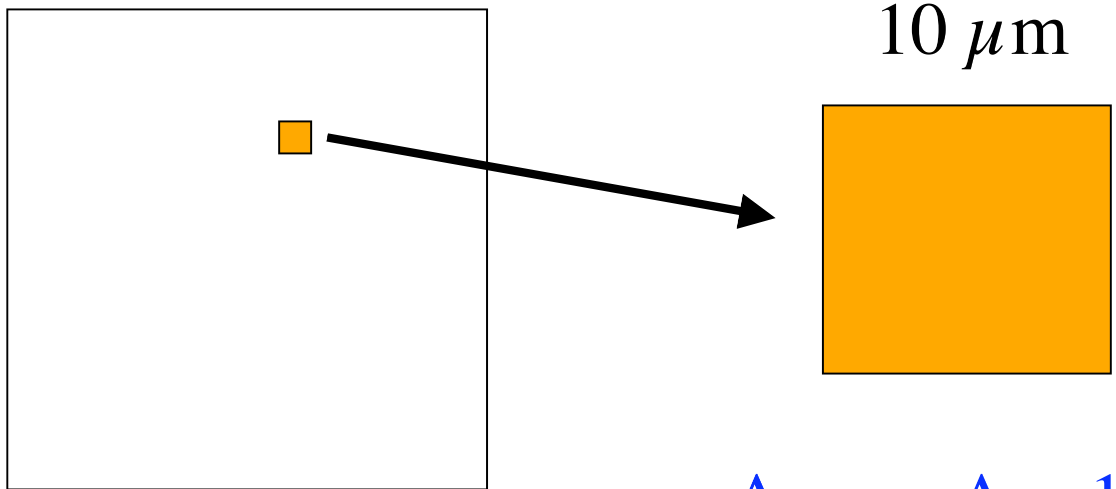
molecules = $\Gamma A = 10^{12}$ molecules = 2 picomoles

1. Stoichiometry, areas and concentrations

1 Megapixel array
$10^6 = (1000 \times 1000)$

1 pixel

10 μm



1 cm

10 μm

1 cm

Area = A = 10^{-6} cm^2

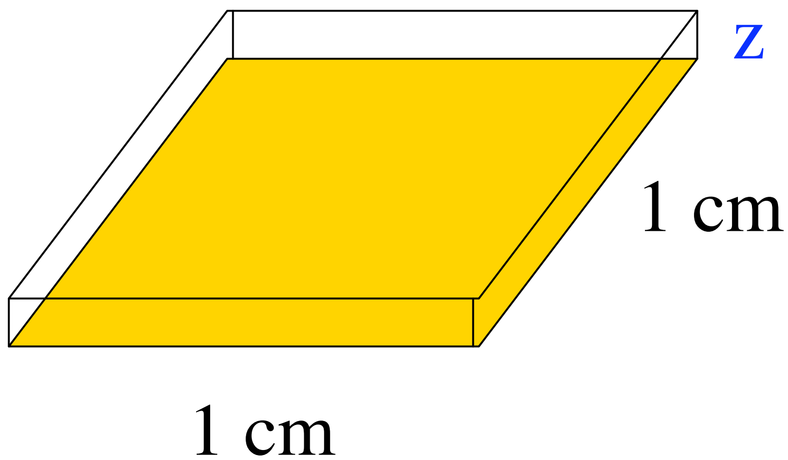
Surface site density $\Gamma = 10^{12}$ probe molecules cm^{-2}

molecules = $\Gamma A = 10^6$ molecules = 2 attomoles

1. Stoichiometry, areas and concentrations

1 target molecule in 1 microliter = 1.7e-18 M = 2 aM

1 MPixel array



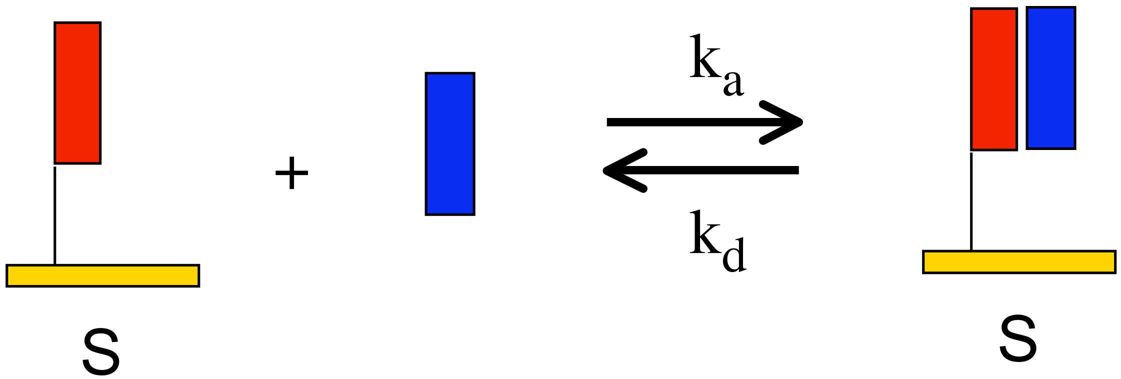
$1\ \mu L = 10^{-3}\ cm^3$

For $1\ \mu L$,

$z = 10\ \mu m$

1 fM = 600 molecules/microliter

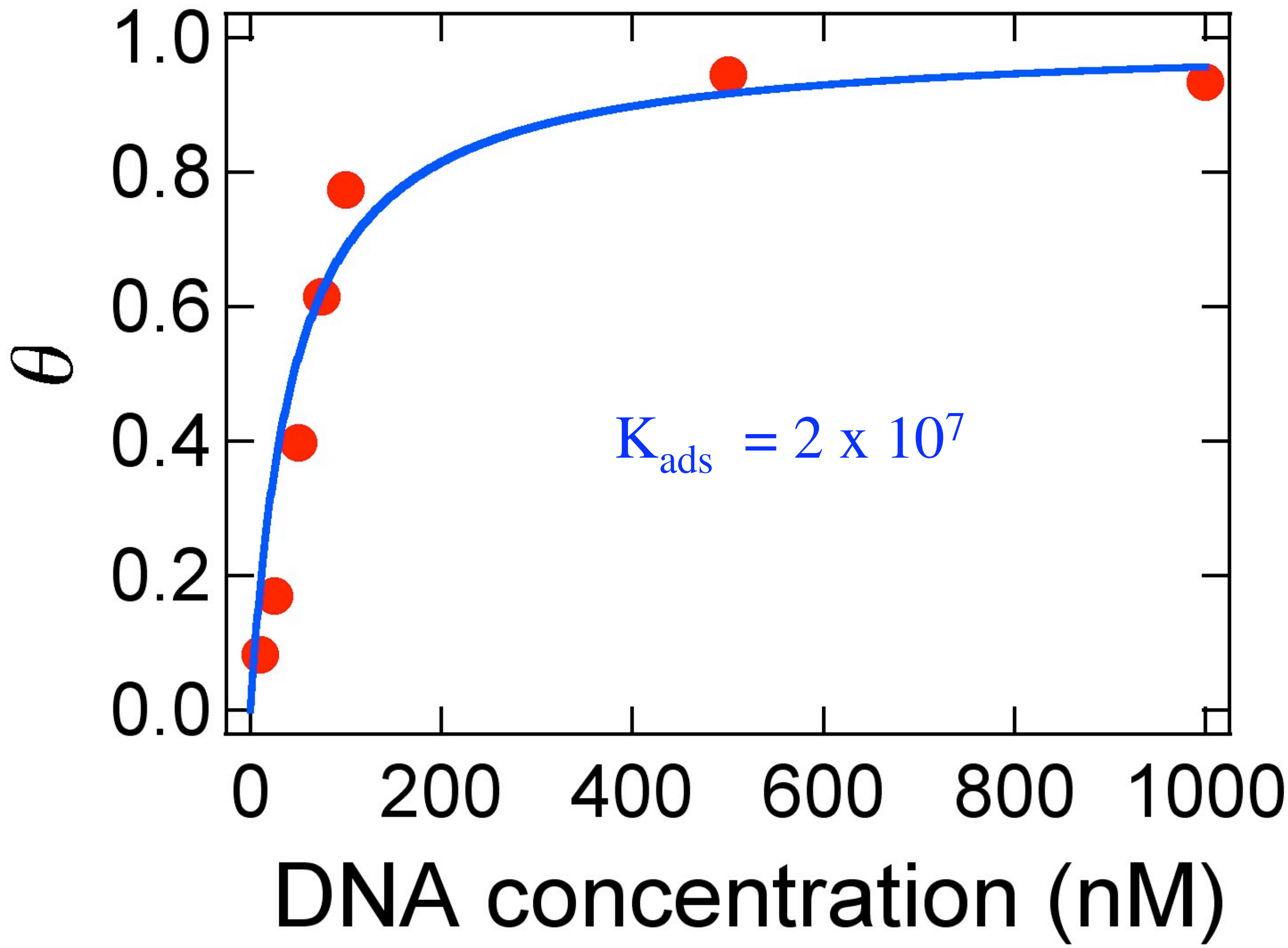
2. The tyranny of the Langmuir isotherm



$$\theta = \frac{\Gamma}{\Gamma_{\max}} = \frac{K_{ads}C}{1 + K_{ads}C} \approx K_{ads}C$$ Low coverage limit

θ = relative surface coverage

Langmuir Isotherm for DNA Hybridization Adsorption



2. The tyranny of the Langmuir isotherm

Low coverage limit:

$\theta = K_{ads}C$ for $\theta << 1$

$\theta = (10^{+9}\ M^{-1}) \times (2 \times 10^{-18}\ M) = 2 \times 10^{-9}$ 2 aM

$\theta = (10^{+9}\ M^{-1}) \times (10^{-15}\ M) = 10^{-6}$ 1 fM

2. The tyranny of the Langmuir isotherm

$\Gamma_{max} = 10^{12}$ molecules cm^{-2}

$\Gamma = \theta\ \Gamma_{max} = 2 \times 10^{-9} \times 10^{12}$ molecules cm^{-2}

10 μm pixel: $A = 10^{-6}\ cm^2$

$\Gamma A = 2 \times 10^{-3}$ molecules 2 aM

2. The tyranny of the Langmuir isotherm

$\Gamma_{max} = 10^{12}$ molecules cm^{-2}

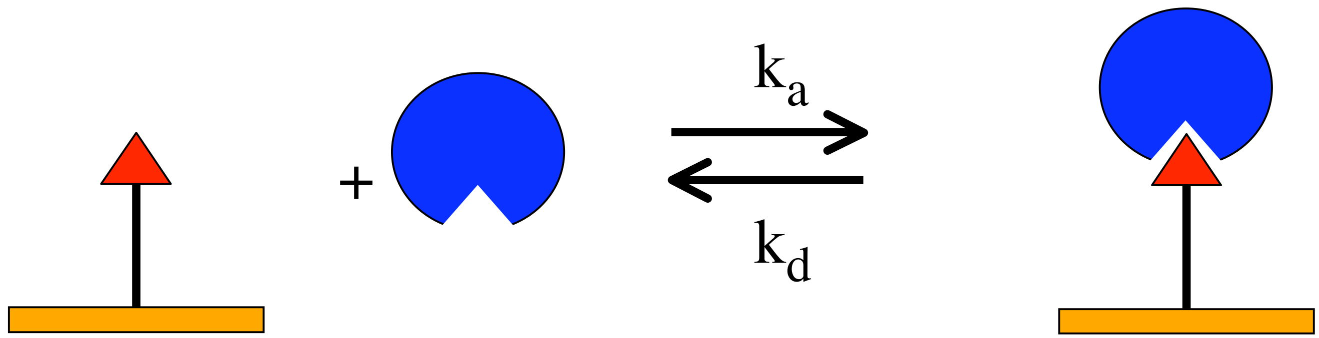
$\Gamma = \theta\ \Gamma_{max} = 10^{-6} \times 10^{12}$ molecules cm^{-2}

10 μm pixel: $A = 10^{-6}$ cm^2

$\Gamma A = 1$ molecule

1 fM

2. Langmuir surface adsorption kinetics



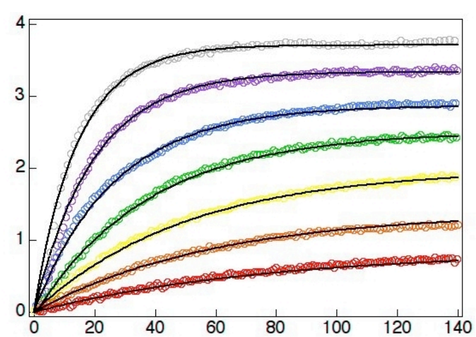
S protein - S peptide

$$\theta(t) = \theta^{eq}\left(1 - \exp(-(k_a C + k_d)t)\right)$$

$$\theta(t) = \theta^{eq} \exp(-k_d t)$$

$$K_{ads} = \frac{k_a}{k_d} = \frac{\theta^{eq}}{C(1-\theta^{eq})}$$

$$\theta = \Gamma / \Gamma_{tot}$$



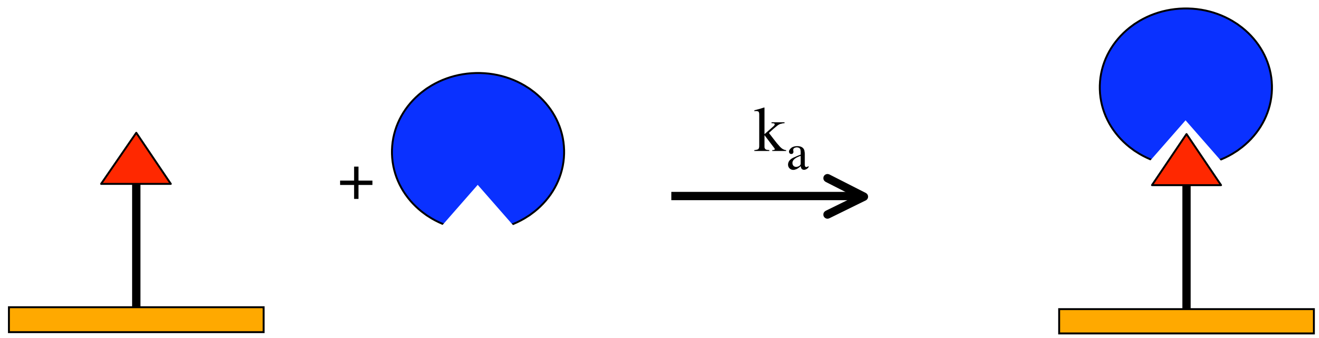
$k_a = 1.9 \text{ x } 10^5 \text{ M}^{-1} \text{ s}^{-1}$

$k_d = 1.1 \text{ x} 10^{-2} \text{ s}^{-1}$

Dr. Greta Wegner

G. J. Wegner et al. Analytical Chem., 76 5667-5684 (2004).

2. Surface adsorption kinetics



Irreversible adsorption

$$\theta(t) = \theta^{eq}\left(1 - \exp(-k_a C t)\right)$$

1 fM

$1/k_aC = 1/(1e06\ M^{-1}\ s^{-1} \times 1e{-15}\ M) = 1e09$ seconds!!!

(30 years)