A Kinetic Study of Analyte-Receptor Binding and Dissociation for Biosensor Applications: A Fractal Analysis for Cholera Toxin and Peptide Protein Interactions

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Introduction

- Biosensors monitor analyte-receptor interactions in real-time

- One component (receptor) is immobilized on the biosensor or chip surface

- Receptors can be antibodies, proteins, enzymes, etc.
Molecule (analyte) to be detected is present in the solution.

Analyte can be antigens, receptors, drug molecules, substrate, *etc.*

Analyte-receptor interaction on the biosensor surface generates a signal.
Signal can be a change in the resonance units (SPR biosensors), UV or IR absorption, fluorescence etc.

Signal intensity is directly proportional to the extent and strength of binding
Toxins-Biological Weapons

- Toxins are poisons produced by living organisms and their synthetic equivalents.

- When used for military purposes they are called chemical or biological warfare agents.

- Common biowarfare agents: anthrax, SEB, cholera, bubonic/pneumonic plague etc.
Cholera Toxin

- Toxin secreted by the bacteria *Vibrio cholerae*
- Toxin affects the small intestine
- Causes secretion of chloride ions and blocks absorption of sodium ions
○ Symptoms: Water loss, diarrhea, electrolyte depletion, weakness, hypotension, malaise, headache, vomiting, etc.

○ In severe cases can cause coma and death

○ If toxin contaminates the water supply it becomes a major threat in biological warfare
Mechanism of Action

- Cholera toxin recognizes and binds to pentasaccharide chain of ganglioside GM1

- GM1 present on the membrane of the cells lining the intestinal lumen

- GM1 binding causes clipping of enzymatic portion of toxin and causes cellular entry
- Inside the cell the subunit causes an increase in the cellular cAMP

- Leads to overactivity of the sodium pumps

- Electrolytes in the guts force the water out of the cells leading to diarrhea
Problem Statement

- Biosensor design makes them heterogeneous in nature

- Heterogeneity mainly due to:
  - Ligand immobilization
  - Nature of the surface or the ligand
  - Immobilization chemistry
Biosensor based kinetic assays influenced by:
- Heterogeneity on the surface
- Diffusional (mass transfer) limitations
- Convective effects
- Depletion of analyte
Modeling Analyte-Receptor Binding in Biosensors

- Conventional models:
  - Langmuir model
  - Saturation model, *etc.*
  - Binding and dissociation rate coefficients obtained
  - Surface heterogeneity not considered in the models
What are Fractals?

- Fractals are self-similar disordered systems

- Disorder described by non-integral dimensions

- Many objects in nature better described with a dimension part way between two whole numbers
Part of the fractal object resembles the bigger structure of which it is a part
Leaves on a tree
Tiny bumps on a hill

Heterogeneous distribution of receptors on a biosensor surface could be considered fractal
A fractal structure looks the same over different ranges of scale
Characteristics of fractal kinetics:

- Anomalous reaction orders
- Time dependent rate coefficients

The fractal dimension, $D_f$, - lumped parameter to describe the heterogeneity and other complexities on the biosensor surface
Fractal Theory And Models

*Single-Fractal Association*

\[
(\text{Analyte}.\text{Receptor}) \sim \begin{cases} 
  t^{(3-D_{f,\text{bind}})/2} = t^p & (t < t_c) \\
  t^{1/2} & (t > t_c) 
\end{cases}
\]

\(D_{f,\text{bind}}\) is the fractal dimension during the association phase

\(t_c\) is the cross-over time
Single-Fractal Dissociation

\[(\text{Analyte.Receptor}) \sim -t^{(3-D_{f,diss})/2} \quad (t > t_{diss})\]

\(D_{f,diss}\) represents the fractal dimension of the surface during the dissociation phase

\(t_{diss}\) represents the start of the dissociation step
Single-fractal model does not satisfactorily fit the available data in all cases

We extend the single-fractal model to include two fractal dimensions

The time $t=t_1$ when the surface changes from first to the second fractal dimension is empirical
Dual-Fractal Association

\[(\text{Analyte.Receptor}) \sim \begin{cases} 
  t^{(3-D_{f1,\text{bind}})/2} = t^{p_1} & t < t_1 \\
  t^{(3-D_{f2,\text{bind}})/2} = t^{p_2} & t_1 < t < t_2 = t_c \\
  t^{1/2} & (t > t_c)
\end{cases}\]

Dual-Fractal Dissociation

\[(\text{Analyte.Receptor}) \sim \begin{cases} 
  -t^{(3-D_{f1,\text{diss}})/2} & t_{\text{diss}} < t < t_{d1} \\
  -t^{(3-D_{f2,\text{diss}})/2} & t_{d1} < t < t_{d2}
\end{cases}\]
Results & Discussion

- Fractal analysis applied to analyte-receptor binding data obtained from the literature

- Binding and dissociation rate coefficients and fractal dimensions were obtained

- Analysis provides us with further physical insights into analyte-receptor interactions
Detection of Cholera Toxin (CT) by Flow Cytometry Based Biosensor

- Song et al. (2000) used a biosensor for detecting multivalent proteins (e.g., CT)
- Fluorescence based energy transfer (FERT) is the detection method
- Method detects CT levels of < 10 pM in 30 minutes
Assay Format

- The CT was in the solution in concentrations ranging from 50-2000 pM

- The fluorophore-labeled ganglioside (GM1) was immobilized on the sensor surface

- The rate coefficients and fractal dimensions were obtained by a regression analysis using Corel Quattro Pro 8.0 (1997)
• The binding of CT in the concentration range of 50-500 pM can be described by a single-fractal analysis.

• At the higher concentrations (1000-2000 pM) a dual-fractal analysis is required to model the data.
Single-Fractal Fit for the Binding of 50 pM CT to Immobilized GM1

\[ \left( \frac{I_a}{I_d} \right) / \left( \frac{I_{a0}}{I_{d0}} \right) \]

\[ k = 0.015 \pm 0.001 \]
\[ D_f = 2.081 \pm 0.063 \]
Dual-Fractal Fit for the Binding of 2000 pM CT to Immobilized GM1

\[ k_1 = 0.405 \pm 0.033 \]
\[ k_2 = 1.031 \pm 0.011 \]
\[ D_{f1} = 2.494 \pm 0.091 \]
\[ D_{f2} \approx 3.0 \]
There is apparently a change in the binding mechanism as one goes from the lower to the higher CT concentration.

At the higher concentrations it was noted that an increase in the fractal dimension led to an increase in the binding rate coefficient.
The fractal dimension, $D_f$, or the degree of heterogeneity on the surface decreases as the CT concentration increases in the lower concentration range (50-500 pM).

In the 50-500 pM concentration range, $D_f$ is given by:

$$D_f = (3.923 \pm 0.182) \ [CT]^{-0.1659 \pm 0.0275}$$
- Higher analyte (CT) concentrations may lead to slightly different binding mechanisms (e.g. saturation) leading to a different (higher) degree of heterogeneity on the surface.

- Saturation binding may involve complete filling of the active sites on the surface leading to higher surface heterogeneities.
Conclusions

- A predictive approach using fractals is presented for the binding of CT in solution to GM1 immobilized on a biosensor surface

- Ligand immobilization—surface heterogeneity—fractal dimension

- Both single- and dual-fractal analysis may be used
The treatment is of a general enough nature, and should be applicable to non biosensor applications wherein further physical insights could be obtained.

DNA-hybridization, cell-receptor, antigen-antibody reactions, etc.