Supporting Information

Origin of the Dendritic Effect in Multivalent Enzyme-Like Catalysts

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1. Mathematical treatment of model $E_n$ (Figure 4)

Starting point is a multivalent catalyst $E_n$, in which $n$ denotes the number of catalytic units. Catalysis occurs either by a single catalytic unit (*single site catalysis*) or by the simultaneous action of two catalytic units (*double site catalysis*), each of them characterized by different binding ($K_{mo}$, $K_{di}$) and rate ($k_{mo}$, $k_{di}$) constants. The maximum number of substrate molecules, $S$, bound to a multivalent catalyst, $E_n$, equals $n$ in case the catalytic site is composed of a single catalytic unit. When the catalytic site is composed of two catalytic units, the maximum number of bound substrate molecules is equal to $n/2$ or $(n-1)/2$ in case $n$ is an odd number. In the model, statistical coefficients affect the association constants between binding site and substrate and therefore the equilibrium constants in this model define association, which is contrary to the Michaelis-Menten parameter, $K_M$, which is generally defined as a dissociation constant.

The model is based on the following assumptions/criteria:

- All binding events are non-cooperative. In other words, binding of a substrate has no effect on the binding constant of the subsequent substrate molecule.
- The binding and rate constants ($K_{mo}$, $k_{mo}$ and $K_{di}$, $k_{di}$) are constant and independent of the size of the multivalent catalyst.
- In this theoretical model statistical coefficients account for *all* the possible modes in which a specific complex between substrate and catalytic site can be formed. No cut-offs in terms of distance between catalytic units are present.

The model bears a strong resemblance to a model recently used by Huskens and Reinhoudt *et al.* to describe the interaction of a multivalent guest with a multivalent monolayer of hosts (Huskens, J.; Mulder A.; Auletta, T.; Nijhuis, C.A.; Ludden, M.J.W.; Reinhoudt, D.N. *J.Am.Chem.Soc.* **2004, 126**, 6784-6797.)
The mass balances for the total concentrations of \( E_n \) and \( S \) are given in equations 1 and 2.

\[
\begin{align*}
[E_n]_{\text{tot}} &= \sum_{p=0}^{m} \sum_{q=0}^{n-2p} \left[ E \cdot (S_{di})_p \cdot (S_{mo})_q \right] \\
[S]_{\text{tot}} &= [S] + \sum_{p=0}^{m} \sum_{q=1}^{n-2p} q \left[ E \cdot (S_{di})_p \cdot (S_{mo})_q \right] + \sum_{p=1}^{m} \sum_{q=0}^{n-2p} \sum_{p=0}^{m} \sum_{q=0}^{n-2p} q \left[ E \cdot (S_{di})_p \cdot (S_{mo})_q \right]
\end{align*}
\]

with \( n \) the valency of the model (\( n=2-8 \)) and \( m \) the maximum number of substrate molecules bound by a catalytic site composed of 2 units (\( m=n/2 \) in case \( n \) is even and \( m=(n-1)/2 \) in case \( n \) is odd).

Binding of the \( q \)th substrate molecule \( S \) to a single catalytic unit of complex \( E\cdot(S_{di})_{p}(S_{mo})_{q-1} \) (mono binding, rows in Figure 4) is described by equation 3.

\[
\begin{align*}
\left[ E \cdot (S_{di})_p \cdot (S_{mo})_q \right] &= \frac{n-2p-q+1}{q} K_{mo} \left[ E \cdot (S_{di})_p \cdot (S_{mo})_{q-1} \right] \times [S]
\end{align*}
\]

with \( 1 \leq q \leq n \) and \( 0 \leq p \leq m-1 \).

Binding of the \( p \)th substrate molecule \( S \) to a double site of complex \( E\cdot(S_{di})_{p-1}(S_{mo})_{q} \) (double binding, columns in Figure 4) is described by equation 4.

\[
\begin{align*}
\sum_{x=1}^{n-2p-q+1} \frac{1}{p} K_{di} \left[ E \cdot (S_{di})_{p-1} \cdot (S_{mo})_{q} \right] \times [S]
\end{align*}
\]

with \( 1 \leq p \leq m \) and \( 0 \leq q \leq n-2 \).

The initial rates originate from single site catalysis (\( \nu_{\text{init,mo}} \)) and double site catalysis (\( \nu_{\text{init,di}} \)) according to equations 5 and 6, respectively.

\[
\begin{align*}
\nu_{\text{init,mo}} &= k_{mo} \sum_{p=0}^{m} \sum_{q=0}^{n-2p} \left[ E \cdot (S_{di})_p \cdot (S_{mo})_q \right] \\
\nu_{\text{init,di}} &= k_{di} \sum_{p=1}^{m} \sum_{q=0}^{n-2p} \sum_{p=0}^{m} \sum_{q=0}^{n-2p} \left[ E \cdot (S_{di})_p \cdot (S_{mo})_q \right]
\end{align*}
\]

with \( k_{mo} \) and \( k_{di} (s^{-1}) \) the rate constants for both pathways, respectively.

The calculated initial rate \( \nu_{\text{init}} \) is the sum of \( \nu_{\text{init,mo}} \) and \( \nu_{\text{init,di}} \).
2. Implementation of the models in Scientist

In Figure 4 of the manuscript the model is described using sequential binding of substrate molecules S to 1 or 2 catalytic units using the equations described in the first section of the Supporting Information. For implementation of the models in the software used for simulation (MicroMath® Scientist® for Windows™, version 2.01) we preferred to describe the concentration of each separate complex as an equilibrium with the free components $E_n$ and $S$ according to the general equation 7.

$$[E_n] + p[S] + q[S] \xleftrightarrow{\pm K_{di}^p K_{mo}^q} E_{n-2p-q} \cdot S_{di,p} \cdot S_{mo,q}$$

with $n$ the number of catalytic sites in the multivalent system ($2 \leq n \leq 8$), $p$ the number of substrate molecules bound to two catalytic units ($0 \leq p \leq m$, with $m=n/2$ in case $n$ is even and $m=(n-1)/2$ in case $n$ is odd), $q$ the number of substrate molecules bound to a single catalytic unit ($0 \leq q \leq n$), with the general condition that $2p+q \leq n$. Binding to a double or single catalytic unit is defined by the association constants $K_{di}$ or $K_{mo}$, respectively. All statistical coefficients for the formation of the specific complex $E_{n-2p-q}S_{di,p}S_{mo,q}$ are compiled in a single factor $x$. The statistical factors $x$ for each given complex of Figure 4 are calculated and given in Table SI-1. The complexes of Figure 4 are treated row-wise. The general indication $ESD_{p}SM_{q}$ indicates that $p$ substrate molecules are bound to two catalytic units and $q$ substrate molecule to a single catalytic unit. In Table SI-2 the reduced statistical coefficients are given in which based on the assumption that starting from dendrimer $E_3$ each subsequent generation loses 10% of the potential binding sites composed of two catalytic units for binding of the first (and also subsequent) substrate molecules.
Table SI-1. Statistical coefficients $x$ as used in the Scientist models E2-E8

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</table>

SI -6-
3. Scientist files for models E2-E8 (using the statistical coefficients of Table SI-1)

// MicroMath Scientist Model File E2
IndVars: S0
DepVars: vinit, vinitm, vinitd, S, E, ESM1, ESM2, ESD1, STOT, ETOT
Params: E0, kcatm, Km, kcatd, Kd
ESM1=2*Km*E*S
ESM2=Km^2*E*S^2
ESD1=Kd*E*S
S=S0-ESM1-2*ESM2-ESD1
E=E0-ESM1-ESM2-ESD1
vinitm=kcatm*(ESM1+2*ESM2)
vinitd=kcatd*ESD1
vinit=vinitm+vinitd
STOT=S+ESM1+2*ESM2+ESD1
ETOT=E+ESM1+ESM2+ESD1
//boundaries
0<E<E0
0<S<S0
0<ESM1<E0
0<ESM2<E0
0<ESD1<E0
***

// MicroMath Scientist Model File E3
IndVars: S0
DepVars: vinit, vinitm, vinitd, S, E, ESM1, ESM2, ESM3, ESD1, ESD1M1, STOT, ETOT
Params: E0, kcatm, Km, kcatd, Kd
ESM1=3*Km*E*S
ESM2=3*Km^2*E*S^2
ESM3=Km^3*E*S^3
ESD1=3*Kd*E*S
ESD1M1=3*Kd*Km*E*S^2
S=S0-ESM1-2*ESM2-3*ESM3-ESD1-2*ESD1M1
E=E0-ESM1-ESM2-ESM3-ESD1-ESD1M1
vinitm=kcatm*(ESM1+2*ESM2+3*ESM3+ESD1M1)
vinitd=kcatd*(ESD1+ESD1M1)
vinit=vinitm+vinitd
STOT=S+ESM1+2*ESM2+3*ESM3+ESD1+2*ESD1M1
ETOT=E+ESM1+ESM2+ESM3+ESD1+ESD1M1
//boundaries
0<E<E0
0<S<S0
0<ESM1<E0
0<ESM2<E0
0<ESM3<E0
0<ESD1<E0
0<ESD1M1<E0
***
// MicroMath Scientist Model File E4

IndVars: S0
DepVars: vinit, vinitm, vinitd, S, E, ESM1, ESM2, ESM3, ESM4, ESD1, ESD1M1, ESD1M2, ESD2, STOT, ETOT
Params: E0, kcatm, Km, kcatd, Kd

ESM1=4*Km*E*S
ESM2=6*Km^2*E*S^2
ESM3=4*Km^3*E*S^3
ESM4=Km^4*E*S^4
ESD1=6*Kd*E*S
ESD1M1=12*Kd*Km*E*S^2
ESD1M2=6*Kd*Km^2*E*S^3
ESD2=3*Kd^2*E*S^2
S=S0-ESM1-2*ESM2-3*ESM3-4*ESM4-ESD1-2*ESD1M1-3*ESD1M2-2*ESD2
E=E0-ESM1-ESM2-ESM3-ESM4-ESD1-ESD1M1-ESD1M2-ESD2
vinitm=kcatm*(ESM1+2*ESM2+3*ESM3+4*ESM4+ESD1M1+2*ESD1M2)
vinitd=kcatd*(ESD1+ESD1M1+ESD1M2+2*ESD2)
vinit=vinitm+vinitd
STOT=S+ESM1+2*ESM2+3*ESM3+4*ESM4+ESD1+2*ESD1M1+3*ESD1M2+2*ESD2
ETOT=E+ESM1+ESM2+ESM3+ESM4+ESD1+ESD1M1+ESD1M2+ESD2

//boundaries
0<E<E0
0<S<S0
0<ESM1<E0
0<ESM2<E0
0<ESM3<E0
0<ESM4<E0
0<ESD1<E0
0<ESD1M1<E0
0<ESD1M2<E0
0<ESD2<E0
***
// MicroMath Scientist Model File E5

IndVars: S0
DepVars: vinit, vinitm, vinitd, S, E, ESM1, ESM2, ESM3, ESM4, ESM5, ESD1, ESD1M1, ESD1M2, ESD1M3, ESD2, ESD2M1, STOT, ETOT

Params: E0, kcatm, Km, kcatd, Kd

ESM1=5*Km*E*S
ESM2=10*Km^2*E*S^2
ESM3=10*Km^3*E*S^3
ESM4=5*Km^4*E*S^4
ESM5=Km^5*E*S^5
ESD1=10*Kd*E*S
ESD1M1=30*Kd*Km*E*S^2
ESD1M2=30*Kd*Km^2*E*S^3
ESD1M3=10*Kd*Km^3*E*S^4
ESD2=15*Kd^2*E*S^2
ESD2M1=15*Kd^2*Km*E*S^3

S=S0-ESM1-2*ESM2-3*ESM3-4*ESM4-5*ESM5-ESD1M1-2*ESD1M2-ESD1M3-3*ESD1M4-4*ESD1M5-5*ESD1M6-6*ESD1M7
E=E0-ESM1-ESM2-ESM3-ESM4-ESM5-ESD1-ESD1M1-ESD1M2-ESD1M3-ESD1M4-ESD1M5-ESD1M6-ESD1M7

vinitm=kcatm*(ESM1+2*ESM2+3*ESM3+4*ESM4+5*ESM5+ESD1M1+2*ESD1M2+3*ESD1M3+ESD2M1)
vinitd=kcatd*(ESD1+ESD1M1+ESD1M2+ESD1M3+2*ESD1M4+2*ESD1M5)
vinit=vinitm+vinitd

STOT=S+ESM1+2*ESM2+3*ESM3+4*ESM4+5*ESM5+ESD1+2*ESD1M1+3*ESD1M2+4*ESD1M3+2*ESD2+3*ESD2M1
ETOT=E+ESM1+ESM2+ESM3+ESM4+ESM5+ESD1+ESD1M1+ESD1M2+ESD1M3+ESD2+ESD2M1

//boundaries
0<E<E0
0<S<S0
0<ESM1<E0
0<ESM2<E0
0<ESM3<E0
0<ESM4<E0
0<ESM5<E0
0<ESD1<E0
0<ESD1M1<E0
0<ESD1M2<E0
0<ESD1M3<E0
0<ESD2<E0
0<ESD2M1<E0

***
// MicroMath Scientist Model File E6

IndVars: S0
DepVars: vinit, vinitm, vinitd, S, E, ESM1, ESM2, ESM3, ESM4, ESM5, ESM6, ESD1, ESD1M1, ESD1M2, ESD1M3, ESD1M4, ESD2, ESD2M1, ESD2M2, ESD3, STOT, ETOT
Params: E0, kcatm, Km, kcatd, Kd

ESM1=6*Km*E*S
ESM2=15*Km^2*E*S^2
ESM3=20*Km^3*E*S^3
ESM4=15*Km^4*E*S^4
ESM5=6*Km^5*E*S^5
ESM6=Km^6*E*S^6
ESD1=15*Kd*E*S
ESD1M1=60*Kd*Km*E*S^2
ESD1M2=90*Kd*Km^2*E*S^3
ESD1M3=60*Kd*Km^3*E*S^4
ESD1M4=15*Kd*Km^4*E*S^5
ESD2=45*Kd^2*E*S^2
ESD2M1=90*Kd^2*Km*E*S^3
ESD2M2=45*Kd^2*Km^2*E*S^4
ESD3=15*Kd^3*E*S^3

S=S0-ESM1-2*ESM2-3*ESM3-4*ESM4-5*ESM5-6*ESM6-ESD1-2*ESD1M1-3*ESD1M2-4*ESD1M3-5*ESD1M4-2*ESD2-3*ESD2M1-4*ESD2M2-3*ESD3
E=E0-ESM1-ESM2-ESM3-ESM4-ESM5-ESM6-ESD1-ESD1M1-ESD1M2-ESD1M3-ESD1M4-ESD2-ESD2M1-ESD2M2-ESD3

vinitm=kcatm*(ESM1+2*ESM2+3*ESM3+4*ESM4+5*ESM5+6*ESM6+ESD1M1+2*ESD1M2+3*ESD1M3+4*ESD1M4+2*ESD2M1+2*ESD2M2+3*ESD3)
vinitd=kcatd*(ESD1+ESD1M1+ESD1M2+ESD1M3+ESD1M4+2*ESD2+2*ESD2M1+2*ESD2M2+3*ESD3)
vinit=vinitm+vinitd

STOT=S+ESM1+2*ESM2+3*ESM3+4*ESM4+5*ESM5+6*ESM6+ESD1+2*ESD1M1+3*ESD1M2+4*ESD1M3+5*ESD1M4+2*ESD2+3*ESD2M1+4*ESD2M2+3*ESD3
ETOT=E+ESM1+ESM2+ESM3+ESM4+ESM5+ESM6+ESD1+ESD1M1+ESD1M2+ESD1M3+ESD1M4+ESD2+ESD2M1+ESD2M2+ESD3

//boundaries
0<E<E0
0<S<S0
0<ESM1<E0
0<ESM2<E0
0<ESM3<E0
0<ESM4<E0
0<ESM5<E0
0<ESM6<E0
0<ESD1<E0
0<ESD1M1<E0
0<ESD1M2<E0
0<ESD1M3<E0
0<ESD1M4<E0
0<ESD2<E0
0<ESD2M1<E0
0<ESD2M2<E0
0<ESD3<E0
***
// MicroMath Scientist Model File E7

IndVars: S0
DepVars: vinit, vinitm, vinitd, S, E, ESM1, ESM2, ESM3, ESM4, ESM5, ESM6, ESM7, ESD1, ESD1M1, ESD1M2, ESD1M3, ESD1M4, ESD1M5, ESD2, ESD2M1, ESD2M2, ESD2M3, ESD3, ESD3M1, STOT, ETOT

Params: E0, kcatm, Km, kcatd, Kd

ESM1=7*Km*E*S
ESM2=21*Km^2*E*S^2
ESM3=35*Km^3*E*S^3
ESM4=35*Km^4*E*S^4
ESM5=21*Km^5*E*S^5
ESM6=7*Km^6*E*S^6
ESM7=Km^7*E*S^7
ESD1=21*Kd*E*S
ESD1M1=105*Kd*Km*E*S^2
ESD1M2=210*Kd*Km^2*E*S^3
ESD1M3=210*Kd*Km^3*E*S^4
ESD1M4=105*Kd*Km^4*E*S^5
ESD1M5=21*Kd*Km^5*E*S^6
ESD2=105*Kd^2*E*S^2
ESD2M1=315*Kd^2*Km*E*S^3
ESD2M2=315*Kd^2*Km^2*E*S^4
ESD2M3=105*Kd^2*Km^3*E*S^5
ESD3=105*Kd^3*E*S^3
ESD3M1=105*Kd^3*Km*E*S^3

S=S0-ESM1-2*ESM2-3*ESM3-4*ESM4-5*ESM5-6*ESM6-7*ESM7-ESD1M1-2*ESD1M2-3*ESD1M3-4*ESD1M4-5*ESD1M5-2*ESD2-3*ESD2M1-4*ESD2M2-5*ESD2M3-3*ESD3-4*ESD3M1
E=E0-ESM1-ESM2-ESM3-ESM4-ESM5-ESM6-ESM7-ESD1-ESD1M1-ESD1M2-ESD1M3-ESD1M4-ESD1M5-ESD2-ESD2M1-ESD2M2-ESD2M3-ESD3-ESD3M1
vinitm=kcatm*(ESM1+2*ESM2+3*ESM3+4*ESM4+5*ESM5+6*ESM6+7*ESM7+ESD1+2*ESD1M1+3*ESD1M2+4*ESD1M3+5*ESD1M4+6*ESD1M5+2*ESD2+3*ESD2M1+4*ESD2M2+5*ESD2M3+3*ESD3+4*ESD3M1)
vinitd=kcatd*(ESD1+ESD1M1+ESD1M2+ESD1M3+ESD1M4+ESD1M5+2*ESD2+2*ESD2M1+2*ESD2M2+2*ESD2M3+3*ESD3+3*ESD3M1)
vinit=vinitm+vinitd
STOT=S+ESM1+2*ESM2+3*ESM3+4*ESM4+5*ESM5+6*ESM6+7*ESM7+ESD1+2*ESD1M1+3*ESD1M2+4*ESD1M3+5*ESD1M4+6*ESD1M5+2*ESD2+3*ESD2M1+4*ESD2M2+5*ESD2M3+3*ESD3+4*ESD3M1
ETOT=E+ESM1+ESM2+ESM3+ESM4+ESM5+ESM6+ESM7+ESD1+ESD1M1+ESD1M2+ESD1M3+ESD1M4+ESD1M5+ESD2+ESD2M1+ESD2M2+ESD2M3+ESD3+ESD3M1

//boundaries
0<E<E0
0<S<S0
0<ESM1<E0
0<ESM2<E0
0<ESM3<E0
0<ESM4<E0
0<ESM5<E0
0<ESM6<E0
0<ESM7<E0
0<ESD1<E0
0<ESD1M1<E0
0<ESD1M2<E0
0<ESD1M3<E0
0<ESD1M4<E0
0<ESD1M5<E0
0<ESD2<E0
0<ESD2M1<E0
0<ESD2M2<E0
0<ESD2M3<E0
0<ESD3<E0
0<ESD3M1<E0***
// MicroMath Scientist Model File E8

IndVars: S0
DepVars: vinit, vinitm, vinitd, S, E, ESM1, ESM2, ESM3, ESM4, ESM5, ESM6, ESM7, ESM8, ESD1, ESD1M1, ESD1M2, ESD1M3, ESD1M4, ESD1M5, ESD1M6, ESD2, ESD2M1, ESD2M2, ESD2M3, ESD2M4, ESD3, ESD3M1, ESD3M2, ESD4, STOT, ETOT

Params: E0, kcatm, Km, kcatd, Kd

ESM1=8*Km*E*S
ESM2=28*Km*2*E*S^2
ESM3=56*Km*3*E*S^3
ESM4=70*Km*4*E*S^4
ESM5=56*Km*5*E*S^5
ESM6=28*Km*6*E*S^6
ESM7=8*Km*7*E*S^7
ESM8=Km*8*E*S^8
ESD1=28*Kd*E*S
ESD1M1=168*Kd*Km*E*S^2
ESD1M2=420*Kd*Km*3*E*S^3
ESD1M3=560*Kd*Km*4*E*S^4
ESD1M5=168*Kd*Km*5*E*S^5
ESD1M6=28*Kd*Km*6*E*S^6
ESD2=210*Kd*2*E*S^2
ESD2M1=840*Kd*Km*E*S^3
ESD2M2=1260*Kd*2*Km*2*E*S^4
ESD2M3=840*Kd*2*Km*3*E*S^5
ESD2M4=210*Kd*2*Km*4*E*S^6
ESD3=420*Kd*3*E*S^3
ESD3M1=840*Kd*3*Km*E*S^4
ESD3M2=420*Kd*3*Km*2*E*S^5
ESD4=105*Kd*4*E*S^4

S=S0-ESM1-2*ESM2-3*ESM3-4*ESM4-5*ESM5-6*ESM6-7*ESM7-8*ESM8-ESD1-2*ESD1M1-3*ESD1M2-4*ESD1M3-5*ESD1M4-6*ESD1M5-7*ESD1M6-2*ESD2-3*ESD2M1-4*ESD2M2-5*ESD2M3-6*ESD2M4-3*ESD3-4*ESD3M1-5*ESD3M2-4*ESD4
E=E0-ESM1-ESM2-ESM3-ESM4-ESM5-ESM6-ESM7-ESM8-ESD1-ESD1M1-ESD1M2-ESD1M3-ESD1M4-ESD1M5-ESD1M6-ESD2-ESD2M1-ESD2M2-ESD2M3-ESD2M4-ESD3-ESD3M1-ESD3M2-4*ESD4

vinitm=kcatm*(ESM1+2*ESM2+3*ESM3+4*ESM4+5*ESM5+6*ESM6+7*ESM7+8*ESM8+ESD1M1+2*ESD1M2+3*ESD1M3+4*ESD1M4+5*ESD1M5+6*ESD1M6+ESD2+2*ESD2M1+3*ESD2M2+3*ESD2M3+4*ESD2M4+ESD3+3*ESD3M1+3*ESD3M2+4*ESD4)
vinitd=kcatd*(ESD1+ESD1M1+ESD1M2+ESD1M3+ESD1M4+ESD1M5+ESD1M6+2*ESD2+2*ESD2M1+2*ESD2M2+2*ESD2M3+2*ESD2M4+3*ESD3+3*ESD3M1+3*ESD3M2+4*ESD4)

vinit=vinitm+vinitd
STOT=S+ESM1+2*ESM2+3*ESM3+4*ESM4+5*ESM5+6*ESM6+7*ESM7+8*ESM8+ESD1+2*ESD1M1+3*ESD1M2+4*ESD1M3+5*ESD1M4+6*ESD1M5+7*ESD1M6+2*ESD2+3*ESD2M1+4*ESD2M2+5*ESD2M3+6*ESD2M4+3*ESD3+4*ESD3M1+5*ESD3M2+4*ESD4
ETOT=E+ESM1+ESM2+ESM3+ESM4+ESM5+ESM6+ESM7+ESM8+ESD1+ESD1M1+ESD1M2+ESD1M3+ESD1M4+ESD1M5+ESD1M6+ESD2+ESD2M1+ESD2M2+ESD2M3+ESD2M4+ESD3+ESD3M1+ESD3M2+ESD4

//boundaries
0<E<E0
0<S<S0
0<ESM1<E0
0<ESM2<E0
0<ESM3<E0
0<ESM4<E0
0<ESM5<E0
0<ESM6<E0
0<ESM7<E0
0<ESM8<E0
0<ESD1<E0
0<ESD1M1<E0
0<ESD1M2<E0
0<ESD1M3<E0
0<ESD1M4<E0
0<ESD1M5<E0
0<ESD1M6<E0
0<ESD2<E0
0<ESD2M1<E0
0<ESD2M2<E0
0<ESD2M3<E0
0<ESD2M4<E0
0<ESD3<E0
0<ESD3M1<E0
0<ESD3M2<E0
0<ESD4<E0
***
4. Saturation profile for TACN-Zn\(^{II}\)

In this work we decided to concentrate our (theoretical) analysis on double site catalysis only (ignoring contributions from single site catalysis). This choice could be confirmed by extending the Michaelis-Menten curves of Figures 2a and b (manuscript) to higher substrate concentrations (as done in the simulations) resulting in constant \(V_{\text{max}}\) values. Suffering from relatively high \(K_M\) values (weak binding) the analysis of our dendrimers should undoubtedly benefit from this. However, we experienced experimental difficulties in increasing the substrate concentration beyond 2.0 mM because at these concentrations the buffering conditions (HEPES = 10 mM) of the solution became insufficient. Attempts to work at higher buffer concentrations gave unsatisfactory results as we found that under these conditions substrate binding is weaker. Therefore, regretfully, we are experimentally not able to reach higher saturation levels for the dendrimers reported here.

In order to add experimental evidence in support of our choice to concentrate on double site catalysis we therefore performed a study on the saturation behavior of the single TACN-Zn\(^{II}\) complex under the same experimental conditions as for the dendrimers. (Figure SI-1; [TACN]=2x10\(^{-5}\) M, [Zn\(^{II}\)] = 2x10\(^{-5}\) M, [HEPES]=1x10\(^{-2}\) M, pH = 7.5, T = 40°C, H\(_2\)O:CH\(_3\)CN=7:3). Importantly, a strictly linear behavior is observed between \(v_{\text{init}}\) and [HPNPP], indicating indeed a very low binding constant between the single TACN-Zn\(^{II}\) complex and HPNPP. These data justify our assumption to ignore single site catalysis.

**Figure SI-1.** Observed initial rate as a function of [HPNPP] for the TACN-Zn\(^{II}\) complex. ([TACN]=2x10\(^{-5}\) M, [Zn\(^{II}\)] = 2x10\(^{-5}\) M, [HEPES]=1x10\(^{-2}\) M, pH = 7.5, T = 40°C, H\(_2\)O:CH\(_3\)CN=7:3)
5. ‘Retro-fitting’ in case of both single- and double site catalysis (Figure 5b)

The simulated curves of Figure 5b (manuscript) are ‘retro-fitted’ using only the initial part of the curves (up till the maximum) as input values for the Michaelis-Menten equation similar as described in the manuscript for Figures 5c and d. The resulting values for the Michaelis-Menten parameters are shown in Figure SI-2. Also in this case, the system is characterized by a linear increase in $k_{cat,den}$ (Fig. SI-2a) and a continuous decrease in $K_M$ (Fig. SI-2b) as a function of the number of catalytic units present. The positive dendritic effect is evident from the plot of $k_{cat,den}/K_M$ against the number of catalytic units (Fig. SI-2c).

![Figure SI-2](image_url)

**Figure SI-2.** Michaelis-Menten parameters (a) $k_{cat,den}$, (b) $K_M$, and (c) $k_{cat,den}/K_M$ for models E2-E8 obtained by fitting the calculated saturation curves from Figure 5b (manuscript). (■: ‘even’ models, □: ‘odd’ models)
It is evident, though, that the occurrence of single site catalysis causes the dendritic effect to be weaker (Fig. SI-2c is ‘less’ exponential with respect to Figure 6c of the manuscript). The higher the relative contribution from single site catalysis, the more the observed effect shifts towards the situation of Figure 5a (manuscript). Here, catalysis only occurs by single site catalysis and no dendritic effect is observed at all.

A second consequence is that the ‘odd/even’ effect has disappeared almost completely. It is remembered that the ‘odd/even’ effect resulted from the fact that ‘odd’ and ‘even’ models were not saturated to the same level in the double site analysis. Allowing a single-site contribution to catalysis this difference in saturation level has (almost) completely disappeared. It is important to point out that we have observed the ‘odd/even’ effect experimentally (dendrons vs dendrimers), which is another strong indication that the contribution of a single TACN•Zn^{II} unit to catalysis in our system can be neglected.