Dendrimers

Comparison to micelles and DNA

Electron transfer on dendrimers and DNA

Toward measuring thoughts one mRNA molecule at a time with Molecular Beacons
Examples of supramolecular hosts

G = guest

C₆₀  Micelle  Zeolite1  Zeolite2  DNA
isotope labeling

simultaneous observation of superexchange between incarcerated paramagnetic nitroxide and nitroxide in the external aqueous phase in water
isotope labeling

\[ ^{14}\text{T}^+ \]

\[ \text{BP}^{15}\text{T} \]

in water
Electron spin transfer in solution at a fixed time after excitation in solution
Soluble in water above pH ~9.

Encapsulation of hydrophobic guest molecules accomplishes the formation of capsular assembly in aqueous medium.

$^{14}$NO spin acceptor outside capsule, $^{15}$NO spin donor inside capsule.
paramagnetic multi-guest system

$^{14}\text{T}^+$

$\text{BP}^{-15}\text{T}@(\text{OA})_2$

$\text{BP}^{-15}\text{T}@(\text{OA})_2 + ^{14}\text{T}^+$
Overall electron spin polarization transfer mechanism

Spin polarization transfer from BP-$^{15}$T to $^{14}$T$^\oplus$

Energy levels:
- $S_0$
- $S_1$

Energy differences:
- $E = 10^6$ kcal/mol
- $10^{10}$

Excitation at 266 nm
Experimental demonstrating of spin polarization transfer from $\text{BP}^{-15}\text{T@capsule}$ to $^{14}\text{T}$

Gauss

$3450 \rightarrow 3460 \rightarrow 3470 \rightarrow 3480 \rightarrow 3490 \rightarrow 3500 \rightarrow 3510$

$\text{CW}$

$\text{TR-CW}$
Examples of supramolecular guest@host systems

G = guest

C_{60}  Micelle  Zeolite  Dendrimer  DNA
Dendrimers: covalent micelles

A dendrimer: a hyperbranched polymer

Generation increasing ➔
Polyamidoamine (PAMAM) Dendrimers

Potential Applications
- drug delivery
- gene transfection
- water purification

repeating unit: \((\text{CH}_2 - \text{CH}_2 - \text{CO} - \text{NH} - \text{CH}_2 - \text{CH}_2 - \text{N})\)

<table>
<thead>
<tr>
<th>Dendrimer</th>
<th># amines</th>
<th>pKa</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-SBD</td>
<td>14/16</td>
<td>3-6/7-9</td>
</tr>
<tr>
<td>4-SBD</td>
<td>62/64</td>
<td></td>
</tr>
<tr>
<td>6-SBD</td>
<td>254/256</td>
<td></td>
</tr>
</tbody>
</table>

G-2-SBD 29 Å
G-4-SBD 45 Å
G-6-SBD 67 Å
**Synthesis: Divergent Method**

‘From-Core-to-Periphery’ Strategy
High generation dendrimer can be prepared.
Reaction should be highly selective.
Defect can not be avoided in high generation dendrimer.

**Examples**
(side or incomplete reaction)

**One Generation**

Examples:
- **PAMAM**
  - Poly (propyleneimine)
- **Poly (propyleneimine)**

**Drug Delivery System**

Topological Entrapment

- Addition of guest

Poly (propyleneimine)
Dendrimer with 64 amines

Guest molecules are physically encapsulated in Dendritic Box.


Guest molecules include:
- 3-carboxyl-PROXYL
- Rose Bengal
- Erichrome Black T
Photoinduced electron transfer between cationic species on the bound to the external surface of anionic hosts

\[ *\text{Ru(II)} + \text{EA}^+n \rightarrow \text{Ru(III)} + \text{EA}^{+(n-1)} \]

\[ *\text{RuL}_3^{2+} + \text{MV}^{2+} \rightarrow \text{RuL}_3^{3+} + \text{MV}^+ \quad (1) \]

\[ *\text{RuL}_3^{2+} + \text{CoL}_3^{3+} \rightarrow \text{RuL}_3^{3+} + \text{CoL}_3^{2+} \quad (2) \]

\[ *\text{RuL}_3^{2+} + \text{Fe(CN)}_6^{4-} \rightarrow \text{RuL}_3^{3+} + \text{Fe(CN)}_6^{3-} \quad (3) \]
Metal complexes involved in photoinduced electron transfer to methyl viologen (MV$^{2+}$)

Modify guest ligands

Chiral complexes

Organic electron acceptor
Photoinduced electron transfer on the surface of dendrimers as a function of generation

Table I.
Quenching Rate Constants for Luminescence Quenching of Ru(phen)$_3^{2+}$ by MV$^{2+}$ in Various Starburst Dendrimers

<table>
<thead>
<tr>
<th>starburst</th>
<th>$k_3^a$</th>
<th>$R,^b$ Å</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>$5.0 \times 10^9$ M$^{-1}$ s$^{-1}$</td>
<td>14</td>
</tr>
<tr>
<td>0.5 G</td>
<td>$5.6 \times 10^9$ M$^{-1}$ s$^{-1}$</td>
<td>18</td>
</tr>
<tr>
<td>1.5 G</td>
<td>$4.1 \times 10^9$ M$^{-1}$ s$^{-1}$</td>
<td>24</td>
</tr>
<tr>
<td>2.5 G</td>
<td>$4.9 \times 10^9$ M$^{-1}$ s$^{-1}$</td>
<td>33</td>
</tr>
<tr>
<td>3.5 G</td>
<td>$1.2 \pm 0.6 \times 10^7$ s$^{-1}$</td>
<td>44</td>
</tr>
<tr>
<td>4.5 G</td>
<td>$7.4 \pm 0.5 \times 10^6$ s$^{-1}$</td>
<td>56</td>
</tr>
<tr>
<td>5.5 G</td>
<td>$5.1 \pm 0.4 \times 10^6$ s$^{-1}$</td>
<td>63</td>
</tr>
<tr>
<td>6.5 G</td>
<td>$2.3 \pm 0.3 \times 10^6$ s$^{-1}$</td>
<td>74</td>
</tr>
<tr>
<td>7.5 G</td>
<td>$1.8 \pm 0.3 \times 10^6$ s$^{-1}$</td>
<td>87</td>
</tr>
<tr>
<td>8.5 G</td>
<td>$7.7 \pm 0.4 \times 10^5$ s$^{-1}$</td>
<td>105</td>
</tr>
<tr>
<td>9.5 G</td>
<td>$6.4 \pm 0.4 \times 10^5$ s$^{-1}$</td>
<td></td>
</tr>
</tbody>
</table>
External surface “closes”

Similar to a “cmc” for micelles
Examples of supramolecular guest@host systems
Comparison of micelles, dendrimers and DNA
Metal complexes involved in photoinduced electron transfer to methyl viologen (MV$^{2+}$)

Modify guest ligands

Chiral complexes

Organic electron acceptor
Binding sites for metal complexes near an ionic interface

AQUEOUS PHASE (a)

ATMOSPHERIC LAYER (b)

COMPACT DOUBLE LAYER (c)

CORE PHASE

UNBOUND TERRITORIAL BINDING IONIC SITE BINDING SURFACE BINDING INTERCALATIVE BINDING

Chiral grooves
A molecular “light switch”: Off in water, On when bound to DNA
Chiral separation of complexes by chiral DNA
DNA possesses chiral grooves on its surface that allow enantioselective adsorption of chiral metal complexes.
Molecular beacons for recognition of DNA and mRNA

Top: Beacon attached to target (ON)

Bottom: Beacon closed (Off)
Electronic energy transfer: $^*D + A \rightarrow D + ^*A$

FRET: Fluorescence resonance energy transfer

![Diagram of energy transfer]
Classical Molecular Beacon System

(A) Stem-Loop Molecular Beacon

Fluorescent switch → Loop

Stem

No Fluorescence from Cy
Fluorescence of *Cy is Quenched completely by Q

MB “Off”

(B) Stem-Loop Molecular Beacon Hybridized with Target mRNA

mRNA Target

Fluorescence from Cy

MB “On”
Experimental example of a molecular beacon in vitro
Beacon based on pyrene excimer

The pyrene excimer paradigm
Pyrene binary molecular beacons

Probe 1

Probe 2

Target hybridized with probes

Probe 1

Probe 2

+ Target
A pyrene MB that binds both proteins and ssDNA

Binding to the target brings two pyrenes together
Beacon open: Monomer fluorescence.
Beacon closed: Excimer fluorescence

\[ [\text{MB}] = 200 \text{ nM} \]
20 mM Tris buffer
25 °C
\[ \lambda_{ex} = 337 \text{ nm} \]
ss-DNA displaces the protein from the MB/protein complex

Target DNA completely displaces the bound protein!
The protein cannot displace the complex of ss-DNA and MB

Target DNA bound to MB is not displaced by protein!
Combinatorial Fluorescence Energy Transfer Tags (CFET) for Biological Imaging

Distance dependent energy transfer

Many fluorescence signatures with a few dyes: Bar code metaphor
Coupling of CFET and MB ideas

- Molecular beacon
- CFET Tag

Target mRNA

CFET-MB Target mRNA Hybrid
CFET Molecular Beacon System (Many Variations Possible with a small starting library of dyes)
$\lambda_{\text{ex}} = 488 \text{ nm}$

$\lambda_{\text{em}} = 525 \text{ nm}$

$\lambda_{\text{em}} = 585 \text{ nm}$

$\lambda_{\text{em}} = 670 \text{ nm}$

A real system!
The challenge: Detect mRNA in living neurons using imaging agents
Molecular Beacon Design

Select candidate genes from *Aplysia* cDNA database

Predict possible mRNA secondary structures

Take most probable structures (~30) and find single-stranded regions as targets

Add GC-rich stems (5-6 bps) to beacon loops (14-24 bps complementary to targets)

Sensorin mRNA

Molecular beacon

mRNA – specific loop

Fluorophore

Quencher

Synthesize, test *in vitro*

Select dyes and quenchers

Predict secondary structures and self-dimers for each stem+loop

Calculate Tm for each loop+target and stem+loop combination

Select candidate genes from *Aplysia* cDNA database

Predict possible mRNA secondary structures

Take most probable structures (~30) and find single-stranded regions as targets

Add GC-rich stems (5-6 bps) to beacon loops (14-24 bps complementary to targets)

Sensorin mRNA

Molecular beacon

mRNA – specific loop

Fluorophore

Quencher

Synthesize, test *in vitro*

Select dyes and quenchers

Predict secondary structures and self-dimers for each stem+loop

Calculate Tm for each loop+target and stem+loop combination
1. ca. 650 bases
2. Sequence available
3. Several positions are partially opened.

Proposed positions for binding of mRNA probes.
Problems with “classical” molecular beacons: false positive signals and auto-fluorescence of cells

Injection of

Target mRNA
Specific MB opening

The fluorescent signal could be the same even in the absence of target.

No target mRNA
Non-Specific MB opening
False positive signal!

auto-fluorescence
Solution to false positive signals: binary probes

Binary probe (BP)

Very dilute: No FRET

Special pair: strong FRET

Binary probes are “open” in the absence of target and “closed” in the presence of target
Solution to auto-fluorescence challenge: time resolution

Auto-fluorescence of cells decays in nanoseconds

Delay the fluorescence of the acceptor fluorescence (somehow)
Binary probes for triplet-singlet SF-RET

Probes separated in solution: No SF-RET

Triplet energy donor
Singlet energy acceptor
Results in a medium that mimics cell auto-fluorescence

Steady state SF-RET

Time resolved SF-RET

S/B = 2.5

S/B = 10
Binary probes work in living cells