Surface functionalization strategies for the design and fabrication of thermoplastic microfluidic devices and the development of new analytical tools


fanny.dorlye@chimie-paristech.fr
Pharmaceuticals
Endocrinal disruptors
Emerging contaminants*
Unknown impact on aquatic environment and human health

* sub μg.L⁻¹

Diagnostic for emerging water contaminants

12 August 2013
Watch list with substances that have to be monitored in the EU (2013/39/U), among which 3 pharmaceuticals:
diclofenac, 17-beta-estradiol and 17-alpha-ethinylestradiol

DICLOFENAC: Painkiller
Analytical microsystem

Integrated approach
Reduced cost
Transportable, suitable for field analysis
Fast analysis with reduced sample and solvent consumption

Conservation of selectivity and sensitivity
Adsorption on channel walls
Fluid flow control
Microsystem design and interfacing

- Sample pretreatment
- Separation
- On-line Detection

Sample reservoir
Electrolyte reservoir

Ligand confinement zone

Target analyte binding to immobilized specific ligands:
- Analyte concentration
- Matrix elimination

Hydrodynamic continuous injection of sample

Specific and sensitive detection

Electrochemical

Fluorescence

Working electrode
Auxiliary electrode
Reference electrode

Hyphenated Detection
Mass spectrometer

NEXTLAB 2014, Rueil-Malmaison (France)
2-4 April 2014
Methodological and technological issues

**Issue 1:**
1. Substrate material
2. Surface treatment/functionalization

**Issue 2:**
1. Selective concentration
2. Efficient separation

**Issue 3:**
On-line detection for trace level quantitation
Selection of microsystem substrate materials

Thermoplastic polymers
COC (cyclic olefin copolymers)
Dyneon (fluorinated polymers)

Structure of COC

Structures of Dyneon monomers

Transparent in UV-visible from 270 nm
Chemical inertness
Semi-clean room microfabrication
Easy to mass produce

Very low surface reactivity

Deposition of a brominated polymeric thin film before further functionalization

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What are aptamers?

• « aptus » + « -mer » = « polymer which is adapted to a function »

• Developed in the 90’s [1-5]

• Short oligonucleotides (ssDNA or RNA) presenting a high affinity to a wide variety of TARGETS
  – Small molecules
  – Nucleic acids
  – Proteins
  – Complex targets :
    • Viruses
    • Cells

• High affinity :
  – Proteins : $10^{-10} < K_d < 10^{-8}$ M
  – Small molecules : $10^{-7} < K_d < 10^{-6}$ M


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Aptamer-based molecular capture

Selected by SELEX* from an oligonucleotide database

Easy to produce once selected (chemical synthesis)

On demand modification / labelling

**Stability** for long-term storage and easy regeneration after denaturation

High affinity interaction in various conditions $(K_D = 50 \text{ nM for diclofenac})$

Use of aptamer to extract the target from the matrix and preconcentrate

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Channel functionalization strategy

3-step strategy

**Step 1** Bromination

Substrate → Br

**Step 2** Substitution

Substrate → NaN₃

**Step 3** Click chemistry

Substrate → R, Cu(I)

Aptamers

COC, Dyneon

Photopolymerisation → Plasma deposition

Brominated substrate

Substitution Br → N₃

Azido substrate

Localized click reaction → Batch click reaction

Selective and specific ligand for extraction and concentration of the target compound

Polymers with various properties (charge, hydrophobicity) to prevent adsorption and control fluid flow

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### Characterization methods

**Step 1: Bromination**

<table>
<thead>
<tr>
<th>Method</th>
<th>Measured signal</th>
<th>Information obtained</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contact angle</td>
<td>Wettability</td>
<td>Surface modification</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Stability</td>
</tr>
<tr>
<td>XPS</td>
<td>Elemental composition in the measured zone</td>
<td>% of brome in the measured zone (quantitative)</td>
</tr>
<tr>
<td>Ellipsometry</td>
<td>Light polarization state</td>
<td>Thickness of the deposited layer</td>
</tr>
<tr>
<td>Infrared spectroscopy (ATR)</td>
<td>Molecular bonds and functional groups in the measured zone</td>
<td>Presence of azide groups or ligands</td>
</tr>
<tr>
<td>Fluorescence</td>
<td>Light intensity</td>
<td>Validation of the overall functionalization strategy</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Homogeneity of the functionalization</td>
</tr>
</tbody>
</table>

**AF** = fluorescent ligand

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XPS: X-ray photoelectron spectroscopy

ATR: Attenuated total reflectance
Global functionalization
Channel global functionalization strategy

3-step strategy

**Step 1** Bromination

Bromination of substrate

**Step 2** Substitution

Substitution of Br with NaN₃ to form azido substrate

**Step 3** Click chemistry

Localized click reaction

Batch click reaction

Selective and specific ligand for extraction and concentration of the target compound

Polymers with various properties (charge, hydrophobicity) to prevent adsorption and control fluid flow

COC, Dyneon

Photopolymerisation

Plasma deposition

Brominated substrate

Substitution Br => N₃

Azido substrate

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Step 1 – plasma deposition

Plasma = ionized gas

Monomer

Precursor : $C_3H_7Br$

RF ELECTRODE

COC

RF ELECTRODE

Plasma = ionized gas

Precursor :

$C_3H_7Br$

\[ \text{Plasma} \rightarrow \text{RF ELECTRODE} \rightarrow \text{COC} \rightarrow \text{RF ELECTRODE} \rightarrow \text{pump} \]

Brominated polymeric thin film deposition

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Step 1 – Plasma bromination COC substrate*

Characterization of COC surface by XPS before and after plasma deposition (Step 1)

**Sample** | C (%) | O (%) | Br (%)  
--- | --- | --- | --- |
Bare COC surface | 86.7 | 11.4 | – |
Br-modified COC surface | 75.4 | 15.4 | 8.7 |

Step 2 – Substitution COC substrate*

COC 

Step 1
Plasma bromination

Step 2
Substitution

Step 3
Click Chemistry

AF = ligand fluorescent

Infrared (FTIR-ATR) characterization of COC surface after step 2


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**Step 3 – Click chemistry**

**COC substrate**

AF = fluorescent ligand

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**Infrared (FTIR-ATR) characterization of COC surface after step 2 and step 3**

**Fluorescence characterization of COC surface after step 3**

Fluorescence gain: 

\[
G = \frac{I_f - I_n}{I_n} \times 100
\]

**Fluorescence gain:** $G = 20\%$

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**Step 3 – Click chemistry**

**Dyneon substrate**

**Infrared (FTIR-ATR) characterization of THV surface after step 2 and step 3**

**Fluorescence gain:**

\[ G = \frac{I_f - I_n}{I_n} \times 100 \]


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Local functionalization
Photopolymerisation
Substitution $Br \Rightarrow N_3$
Localized click reaction
Plasma deposition
Brominated substrate
Aptamers
Selectively and specific ligand for extraction and concentration of the target
Polymers with various properties (charge, hydrophobicity) to prevent adsorption and control fluid flow
Localized click chemistry on ITO (Indium tin oxide) platelets

Platinum electrode, diameter 25 µm

-0.3V during CuII for 30 min

Glass slide covered with ITO

* Scanning electrochemical microscopy

*SECM imaging

Substrate potential: 0.25 V vs Ag/AgCl

Redox mediator: ferrocyanide; Probe potential: -0.1 V vs Ag/AgCl

Spot ≈ 80 µm

*Fluorescence imaging

Spot ≈ 200 µm

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Localized click chemistry
Inside Dyneon microchannels

Size control (10-100 µm size-range)

Patent pending
Perspectives
Functionalized aptamers as selective agents?

Binding affinity

Microchip electrophoresis in frontal mode


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Functionalized aptamers as selective agents?

Binding affinity

\[ k_d = 7.6 \pm 1.8 \text{ nM} \]
\[ n = 0.05 \pm 0.01 \]

\[ k_d = 16.0 \pm 1.6 \text{ nM} \]
\[ n = 0.06 \pm 0.01 \]

M. Girardot, F. d'Orlye, Fanny et al., ANALYTICAL AND BIOANALYTICAL CHEMISTRY, 406 (2014) 1089-1098
M. Girardot, F. d'Orlye, Fanny et al., ANALYTICAL BIOCHEMISTRY, 435 (2013) 150-152
Target preconcentration and separation

Confinement

Release and separation

Chemical/Thermal control
Electrochemical detection integration

Injection

Concentration

Détection

Sample reservoir

Ligand confinement zone

Electrolyte reservoir

Fluorescence

Electrochemical Working electrode

Analyse du DCF 1mM, v= 100mV/s

Blanc

Direct or indirect detection

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Conclusion

Simple lab-on-a-chip
New materials

Global / local functionalization

Sensitive detection

An original aptamer-based molecular capture
Thank you for your attention