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Nucleic Acid Aptamers for Sensing and Imaging

NOVEL DIAGNOSTICS FOR INFECTIOUS DISEASES
25-26 March, 2024; Royal Society, London

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Aptamers-Strengths as Sensing Molecules

- Well-defined at the molecular level
- Available in high quantity and quality via chemical synthesis
- Low Cost (<1% that of antibodies per mole at a research scale)
- Precision chemical modification either during, or post-synthesis
- High stability (with suitable modification)
- Sraightforward Rules for Intramolecular Interactions

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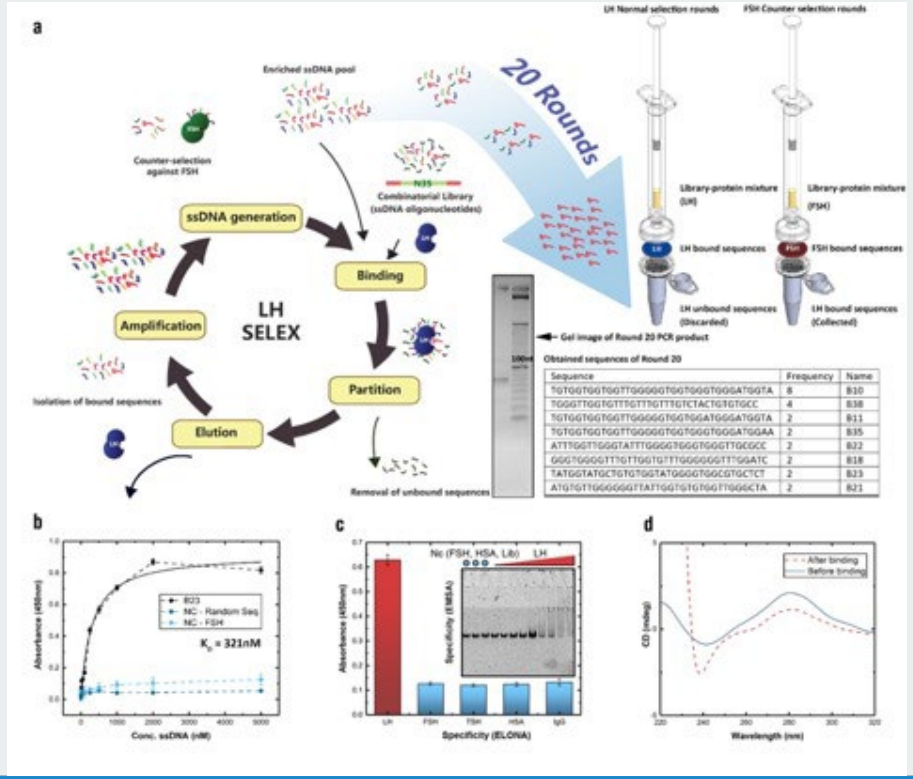
Aptamer Selection via SELEX



Other Flavours of SELEX:

1. Capture SELEX
2. Mag Bead SELEX
3. Microfluidic SELEX
4. CE-SELEX
5. Proximity Ligation SELEX

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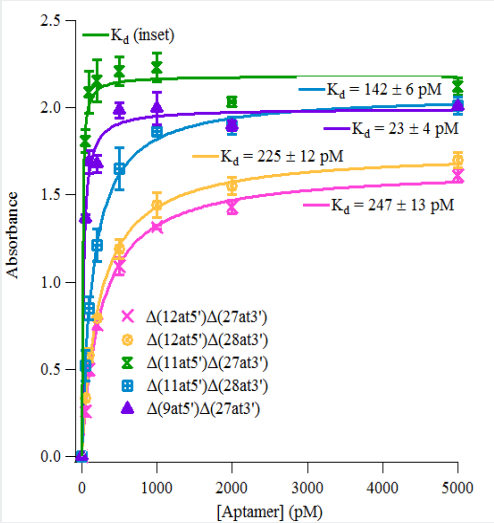
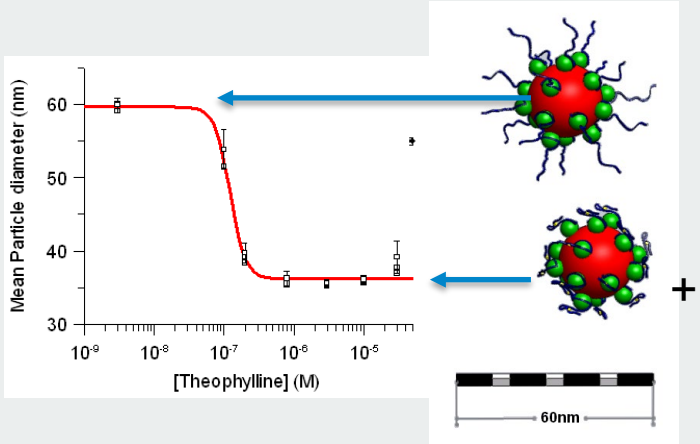
Post-SELEX Modification

Truncation



5' Truncations

Fusions (Chimaeras)



+ Theophylline

Streptavidin~Theophylline Binding RNA Aptamers

Labelling

Redox active groups
Fluorophores
Biotin
Nanoparticles

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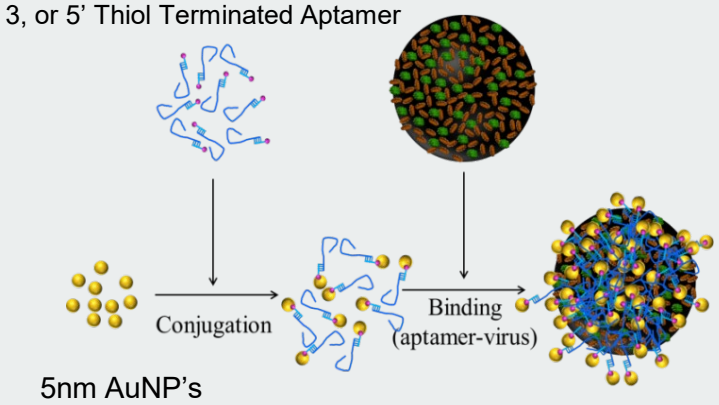
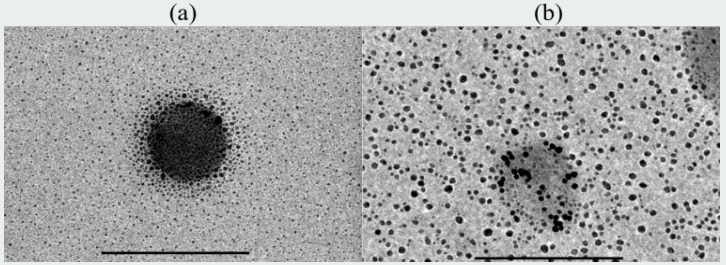
Aptamers in Virus Detection



Assay Design



Virus Specific Aptamer Random DNA Sequence

After George Whitesides

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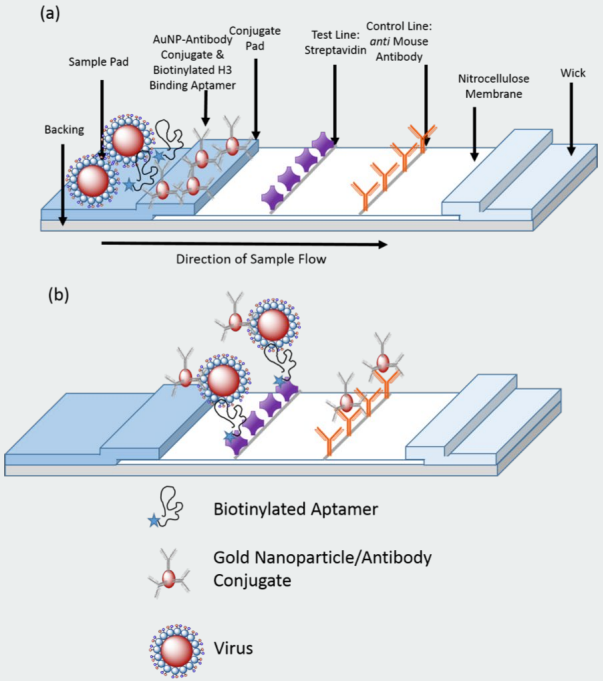
DRELFA: Dual Recognition Element Lateral Flow Assays



Standard Lateral Flow Format

1. Biotinylated aptamer for specificity
2. Streptavidin Capture Line
3. AuNP labelled antibody for detection
4. *anti* mouse antibody for QA
5. Avoids immobilised aptamer ‘lines’

Le T. et al. *Anal. Chem.* **2017**, *89* (12), pp 6781–6786

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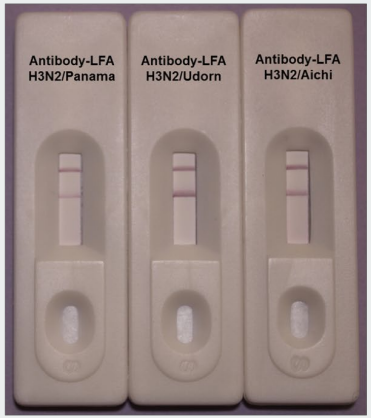
DRELFA Performance

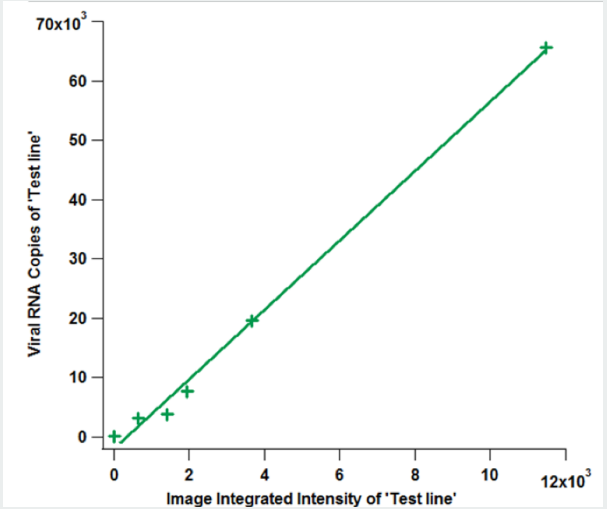
(a)

Conventional 2-antibody



(b)

DRELFA



Aptamer *anti* H3N2/Panama
Antibody *anti* H3

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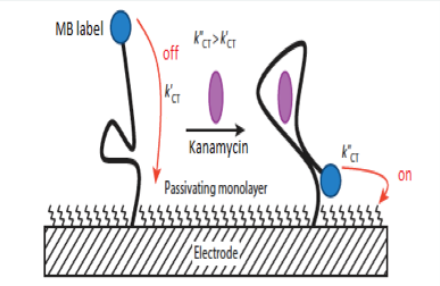
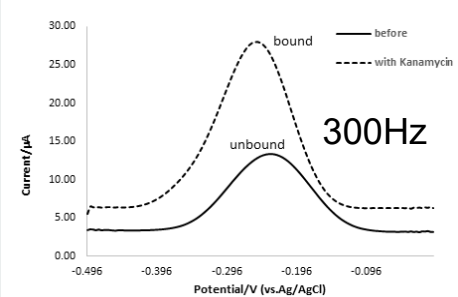
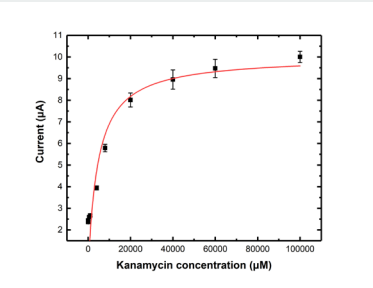
Conformational Switching. Electrochemical Aptasensors







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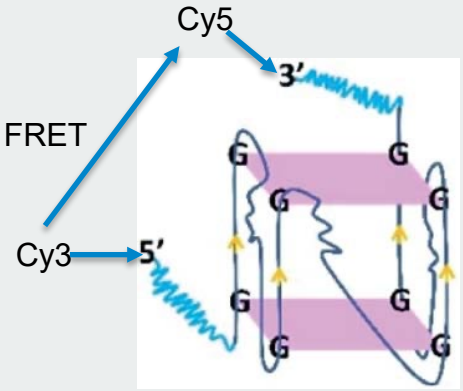
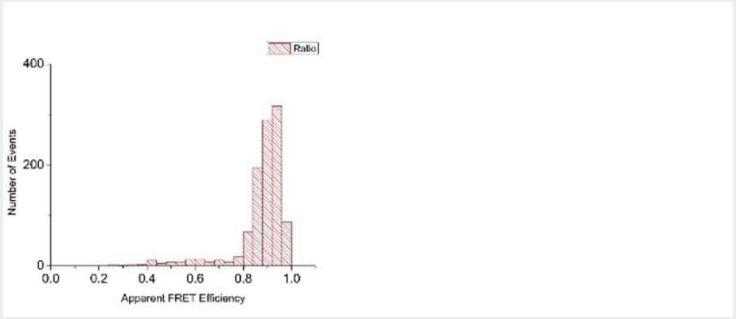
Aptamer Stability

G-Quadruplexes



G-quadruplex that binds Retinoblastoma protein



Acceptor Photobleaching FRET

→ Intracellular Stability

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Aptamer ‘Specificity’ A Cautionary Tale

Nucleic Acid Aptamers are often claimed to be ‘Highly Specific”

Some Counter Examples

“Cocaine Binding Aptamer”	Binds quinine and cholesterol
Nucleolin Binding Aptamer	A ‘control’ aptamer with a randomized sequence (AS1411) has a higher affinity
Arsenite/arsenate Binding Aptamer	Binds neither, flawed assay

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Acknowledgements

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