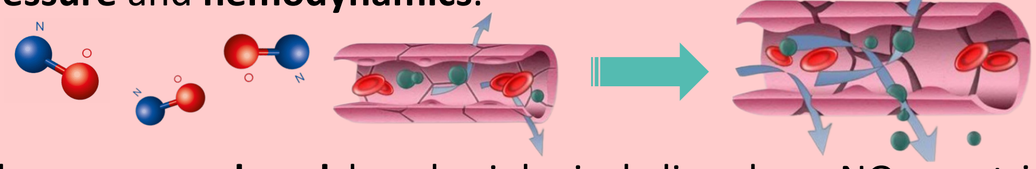
Engineering ssDNA-SWCNT Optical Biosensors for Nitric Oxide Detection

Younes Rhouta*, S. Hashem Sajjadi, Yahya Rabbani, Ardemis A. Boghossian
Laboratory of NanoBiotechnology, École Polytechnique Fédérale de Lausanne (EPFL), 1015 Lausanne, Switzerland

1. Introduction

Nitric oxide (**NO**) is a prolific **vasodilator** that **regulates** blood **pressure** and **hemodynamics**.



When **overproduced** by physiological disorders, NO can trigger severe **inflammatory** and **cardiovascular diseases**. Being a **free radical**, its **short half-life** hinders its detection and quantification.

Single-walled carbon nanotubes (**SWCNTs**) stand-out as cutting-edge **biosensors** given their **nanoscale** precision, **single-molecule** sensitivity and unique **functional properties**.

They emit a **photostable** near-infrared **fluorescence**. It does **not interfere** with **biological tissues** and it **decreases** upon specific **molecule adsorption**. They can be non-covalently **functionalized** with **ssDNA** which enables a **selective** *in-vivo* **monitoring**.

2. Aims

In this study, we demonstrate how the **screening** of a **98-sequence ssDNA** library enables the **engineering**, through machine learning and directed evolution, of an **optimized ssDNA-SWCNT biosensor** with **improved selectivity** and **sensitivity** for **nitric oxide detection**.

3. Materials & Methods

1. Sample preparation

SC-SWCNT + ssDNA → MeOH → [room temp.]

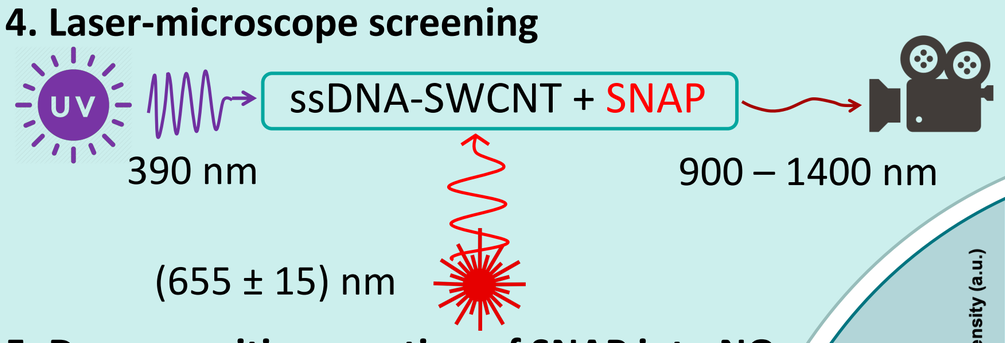
2. Sample purification

NaCl → SC + MeOH → 100% EtOH [-20°C] → [vortex & centrifuge] → 70% EtOH

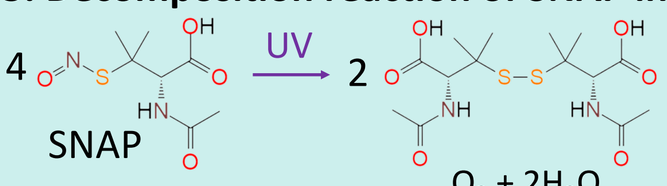
3. Sample resuspension

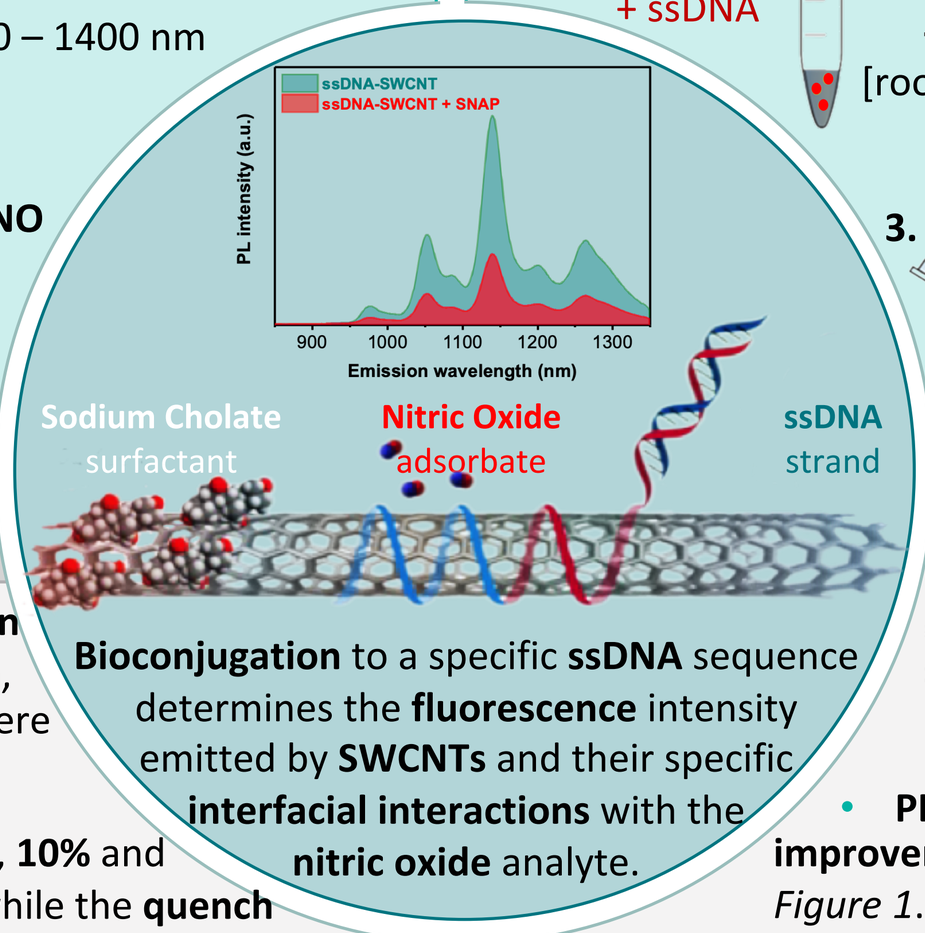
supernatant removal → DI water + PBS 10x

4. Laser-microscope screening



5. Decomposition reaction of SNAP into NO

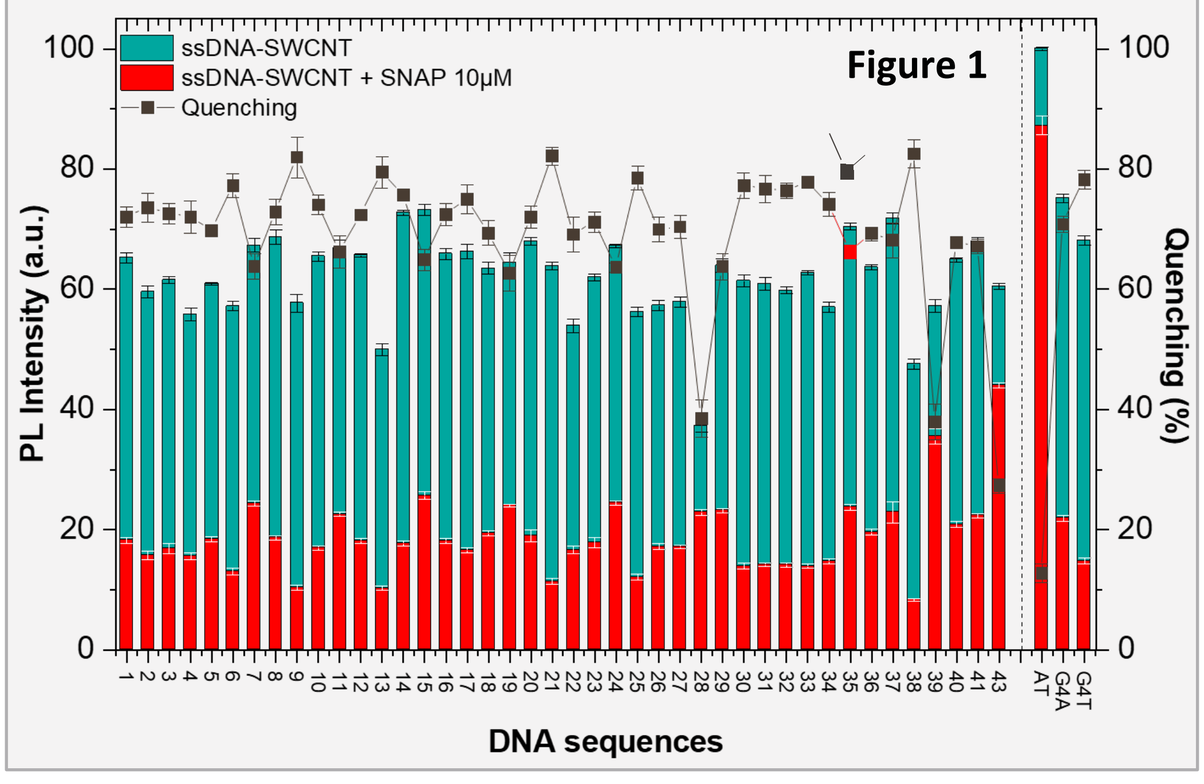




4. Results & Discussion

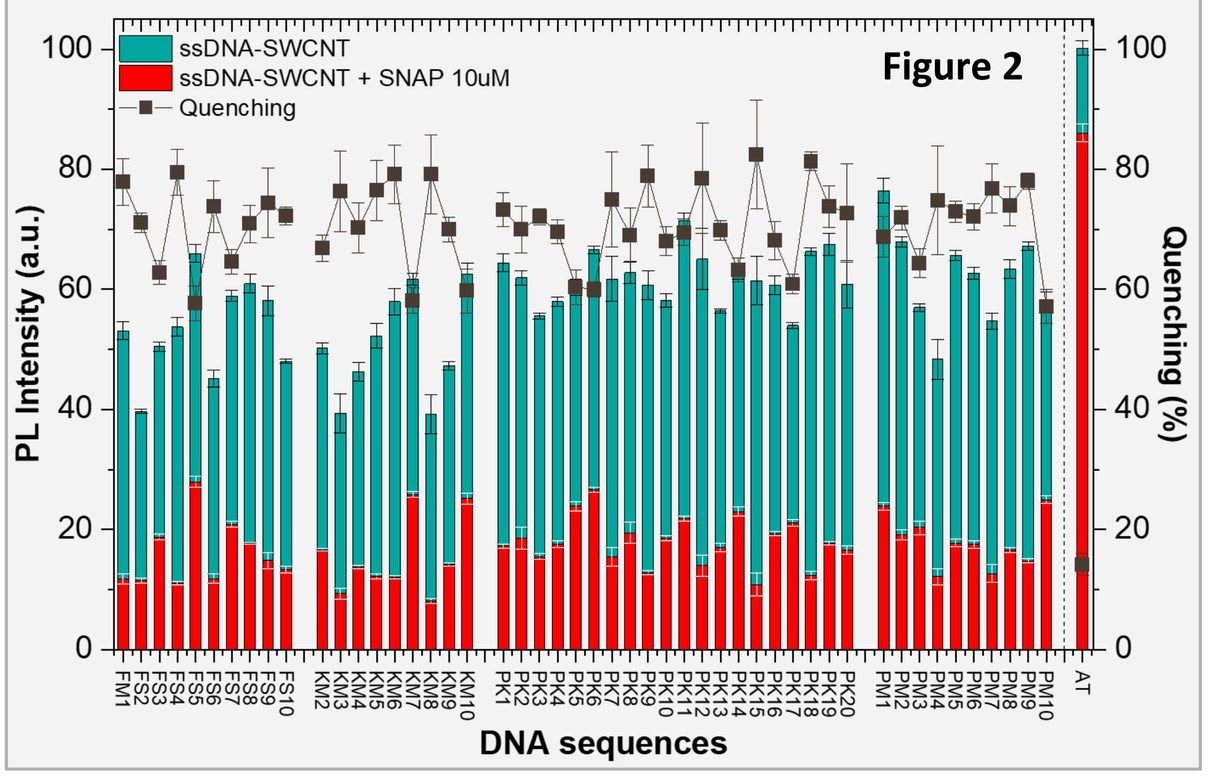
- ML algorithms generated **1 million random** ssDNA sequences. From them, **42 dissimilar** ones (*Figure 1*) were selected using clustering methods.
- **PL intensities** range from 36 to 72 a.u., **10%** and **8% lower** than the range in *Figure 2*; while the **quench** ranges from 36 to 82%, **1.6 times lower** than that in *Figure 2*.
- Sample no. **14** provides an **initial PL** of (74 ± 0.44) a.u. and a **quenching** of (76 ± 0.98)%, outperforming the **G4T** control.

Figure 1



- The ssDNA sequences in *Figure 2*, generated via ML, combine **recurrently shared DNA** from sequences with **high brightness** and **NO sensitivity**.
- **PL** values range from 40 to 78 a.u., a **5 a.u. improvement** at both extremes compared to *Figure 1*. The **quenching** response, 59-83%, spans a 24% spread, **2 times narrower** than *Figure 1*.
- **PM** and **PK** exhibit the **highest initial fluorescence**, averaging 62 a.u., **1.2 times more** than the **F** and **KM** sets. Within each class, the **quenching** averages 71%.

Figure 2



5. Conclusions

This study highlighted how incorporating **directed evolution** insights in **biosensor design** results in **enhanced fluorescence** and **NO responsiveness**. We achieved a **10% quenching** range **improvement** compared to randomly generated sequences.

This dataset serves as a **valuable framework** for **algorithm training**. While **directed evolution** is effective, **assisting** it with **machine learning** allows **pattern recognition**, precise **nucleotide** selection and **rational** ssDNA sequence **engineering**.

*younes.rhouta@epfl.ch

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