IMPEDIMETRIC LECTIN-BASED BIOSENSORS FOR CANCER-ASSOCIATED O-GLYCANS

M. Luísa S. Silva

Centre of Chemical Research, Autonomous University of Hidalgo State, México

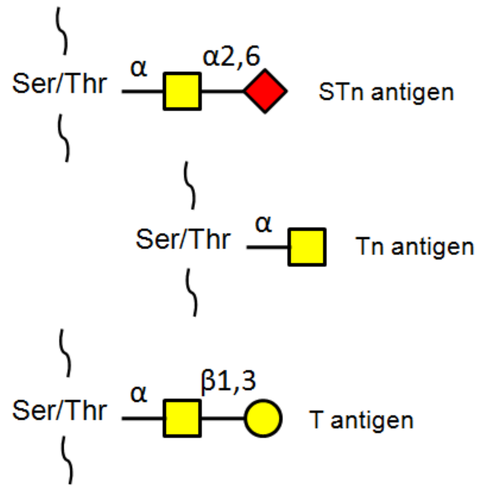
mluisasilva@portugalmail.pt

Introduction

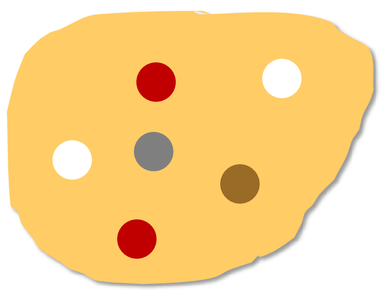
oncogenesis

aberrant protein glycosylation

- **truncation of *O*-glycans**
  - **STn antigen**
  - **Tn antigen**
  - **T antigen**
- **increased fucosylation**
- **increased sialylation**
- **increased β1,6 GlcNAc branching in *N*-glycans**



tumoral cell



secretion

blood

aberrant glycans are cancer biomarkers accessible in blood

Introduction

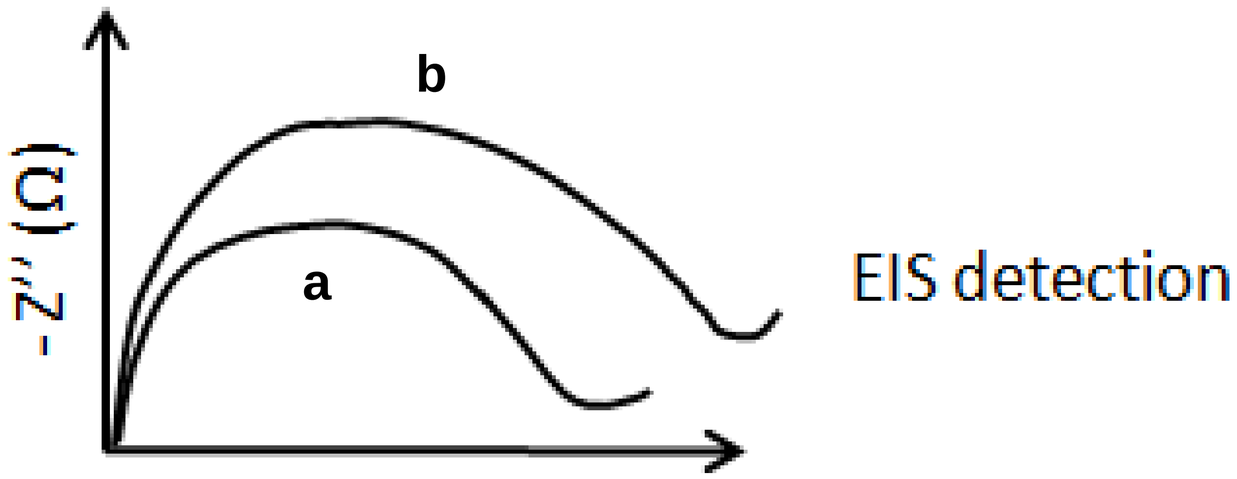
detection of aberrant glycostructures → **lectins**

cancer-associated truncated *O*-glycan	lectin immobilized on the biosensor
STn	***Sambucus nigra* agglutinin (SNA)** specifically recognizes the NeuAc-α2-6GalNAc-α1-O-Ser/Thr structure
Tn	***Vicia villosa* agglutinin (VVA)** specifically recognizes the GalNAc-α1-O-Ser/Thr structure
T	***Arachis hypogeae* agglutinin (PNA)** specifically recognizes the Gal-α1-3GalNAc-α1-O-Ser/Thr structure

Introduction

The binding event between each lectin and the corresponding aberrant *O*-glycan was monitored by electrochemical impedance spectroscopy, measuring the increase in the biosensor's impedance after incubating the samples. The increase in impedance was related to the lectin-glycan complex formation.

Au/SPE low temperature cure ink gold electrode; E_w (4 mm diameter)



Nyquist plots obtained (a) before and (b) after sample incubation

Biosensor construction

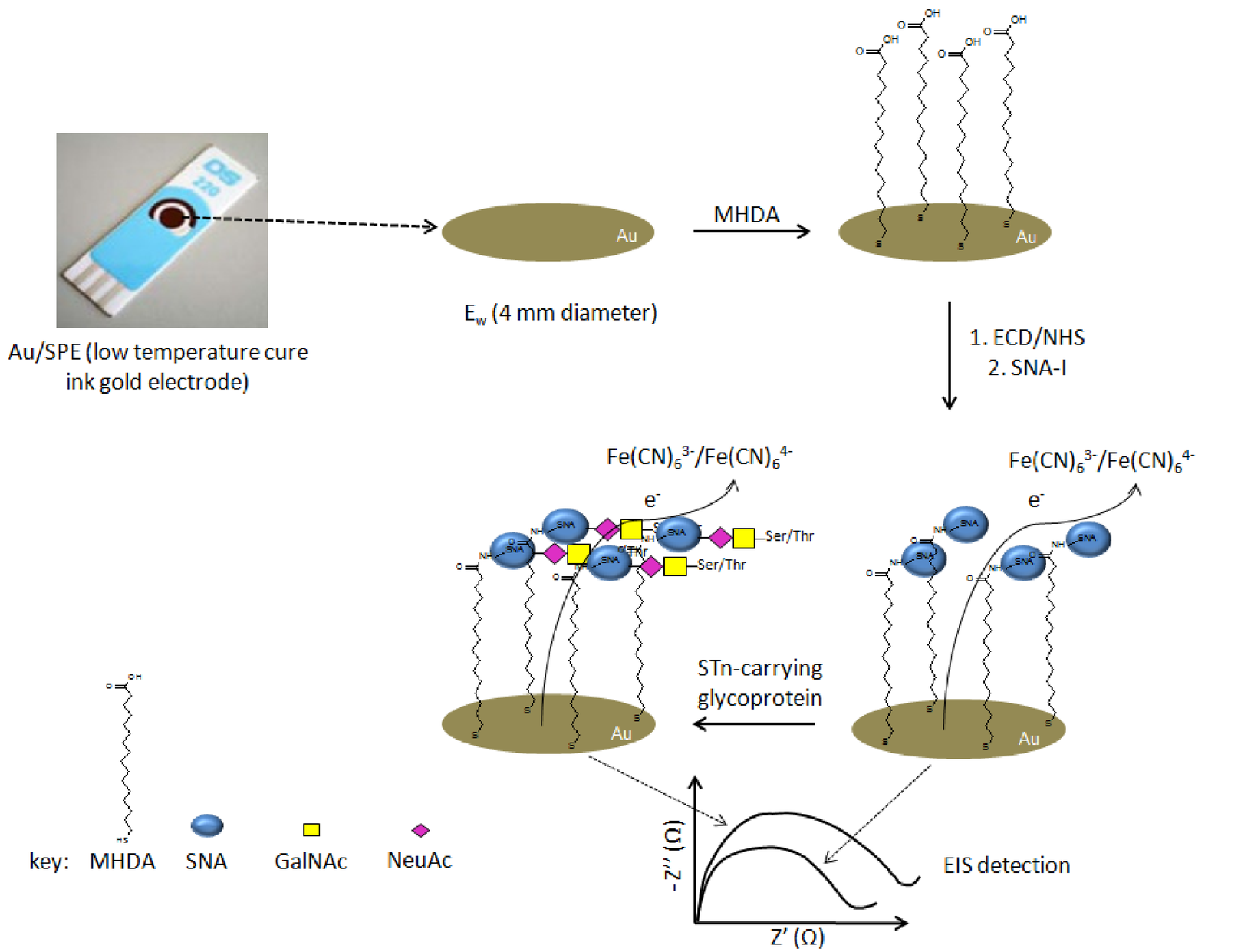
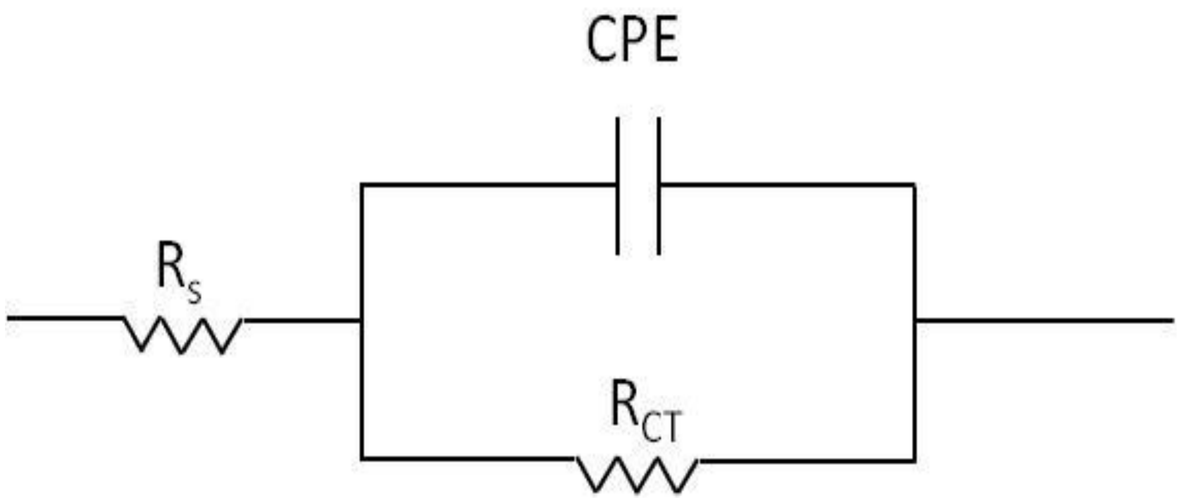


Figure 2 – Schematic diagram describing the construction of each lectin biosensor and detection of aberrant *O*-glycans by EIS: (a) alkanethiol/mixed alkanethiols self-assembled monolayer is formed via incubation of screen-printed electrodes for 24 h; (b) the carboxylic acid end of the alkanethiols are activated with ECD and NHS to allow covalent binding with the lectin; (c) the truncated *O*-glycan present in glycoproteins is captured based on the affinity of the lectin to the referred structure; (d) the formation of the complex lectin-truncated *O*-glycan is monitored by the increase in the electrode impedance (by electrochemical impedance spectroscopy).

Biosensor construction

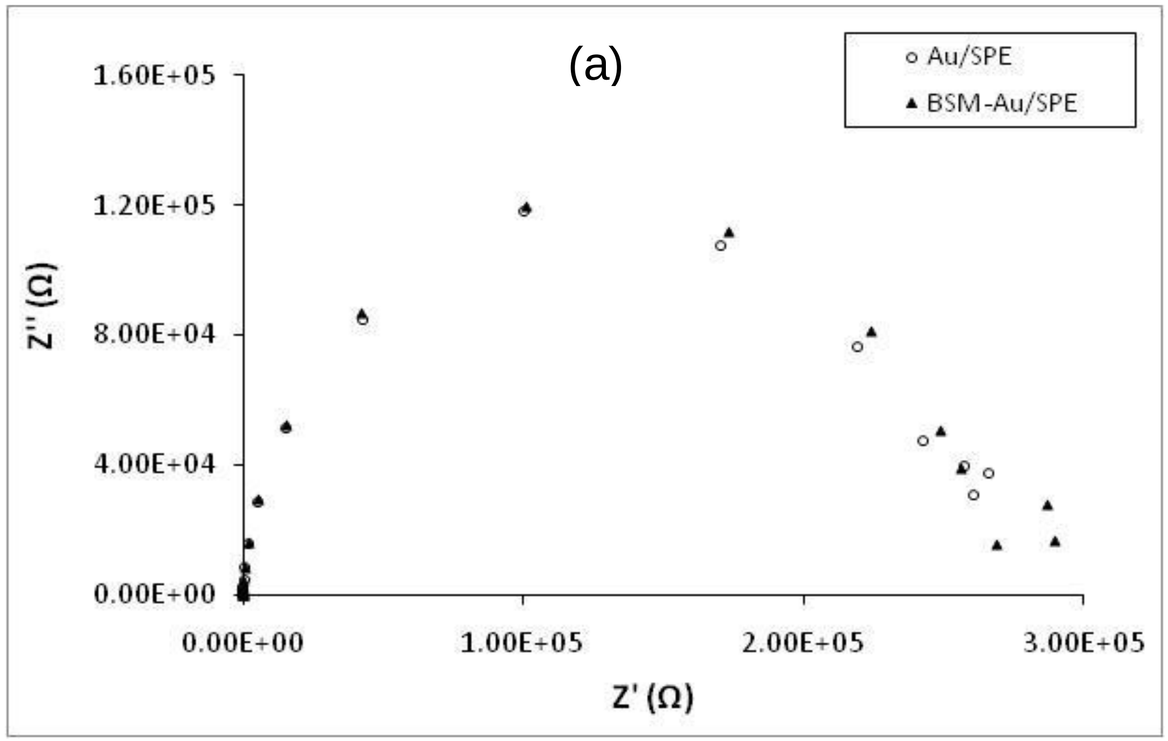
cancer-associated truncated *O*-glycan	lectin immobilized on the biosensor	model glycoprotein used to monitor complex formation during optimization
STn	**SNA**	bovine submaxillary mucin; human transferrin
Tn	**VVA**	asialofetuin; asialo-bovine submaxillary mucin
T	**PNA**	asialofetuin

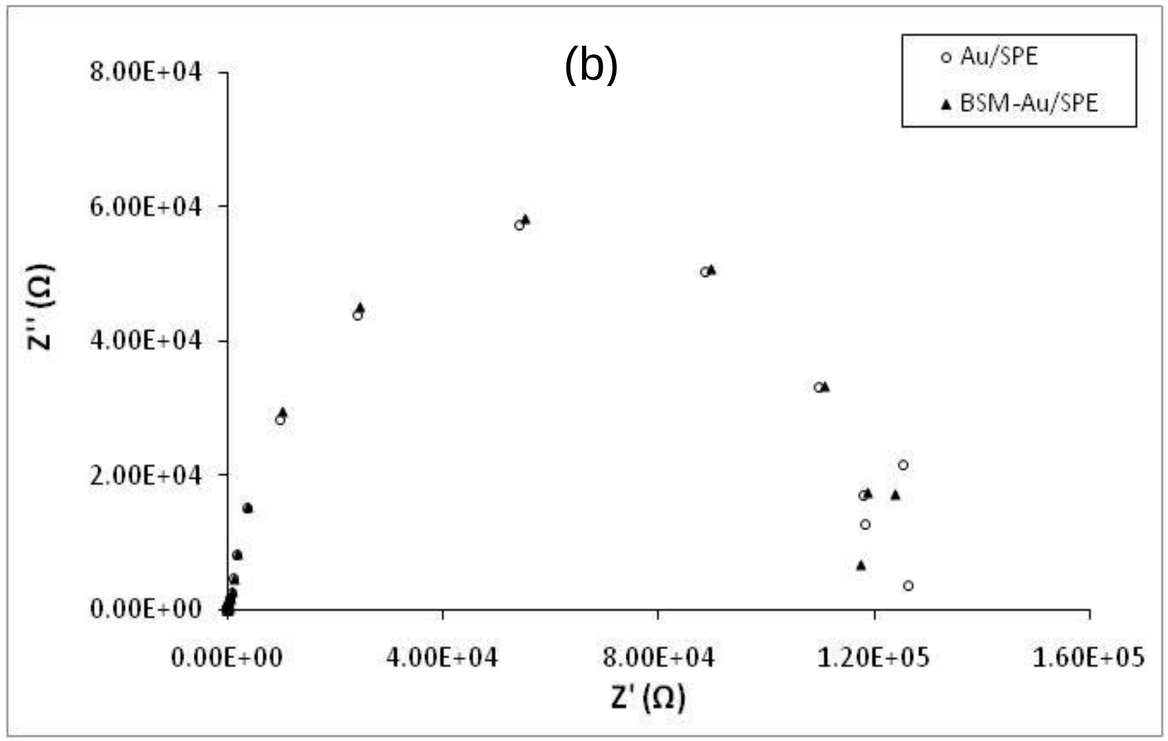


Randles equivalent circuit for the developed biosensors. R_s – resistance of the electrolyte solution; CPE – constant phase element; R_{CT} – charge transfer resistance.

Results – selectivity

SNA biosensor

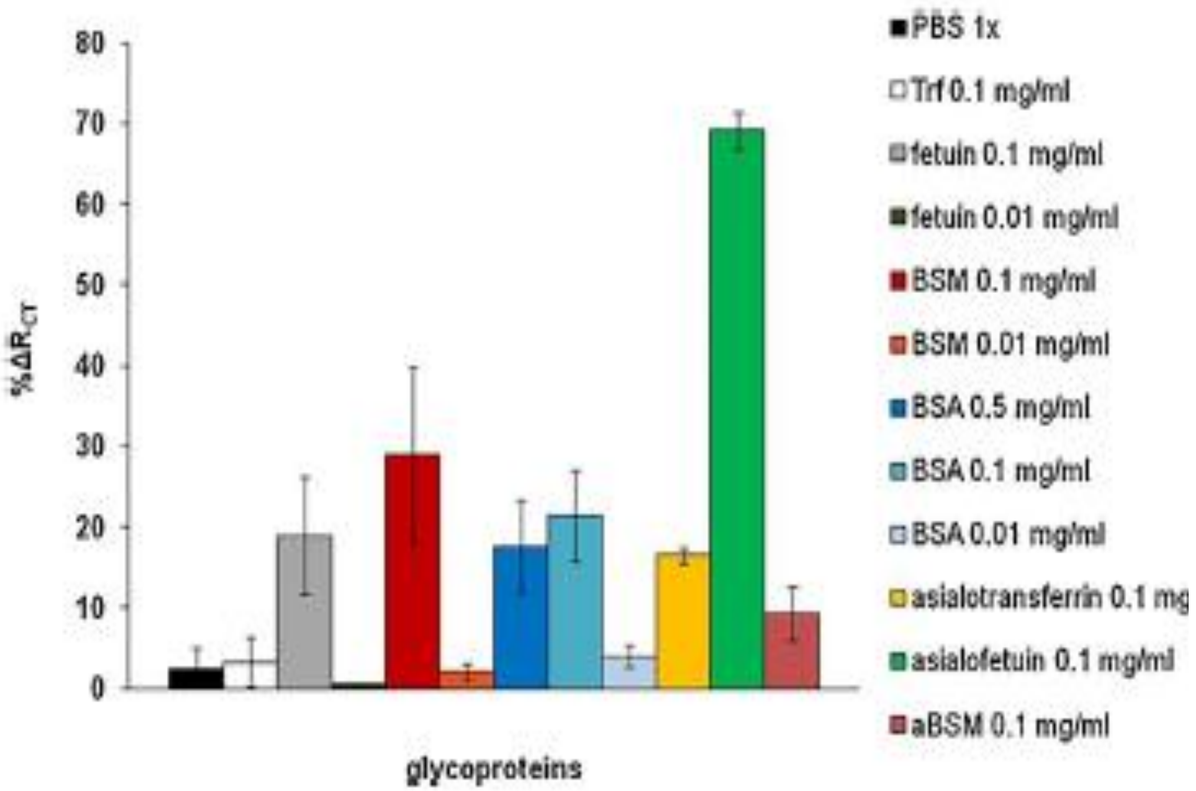




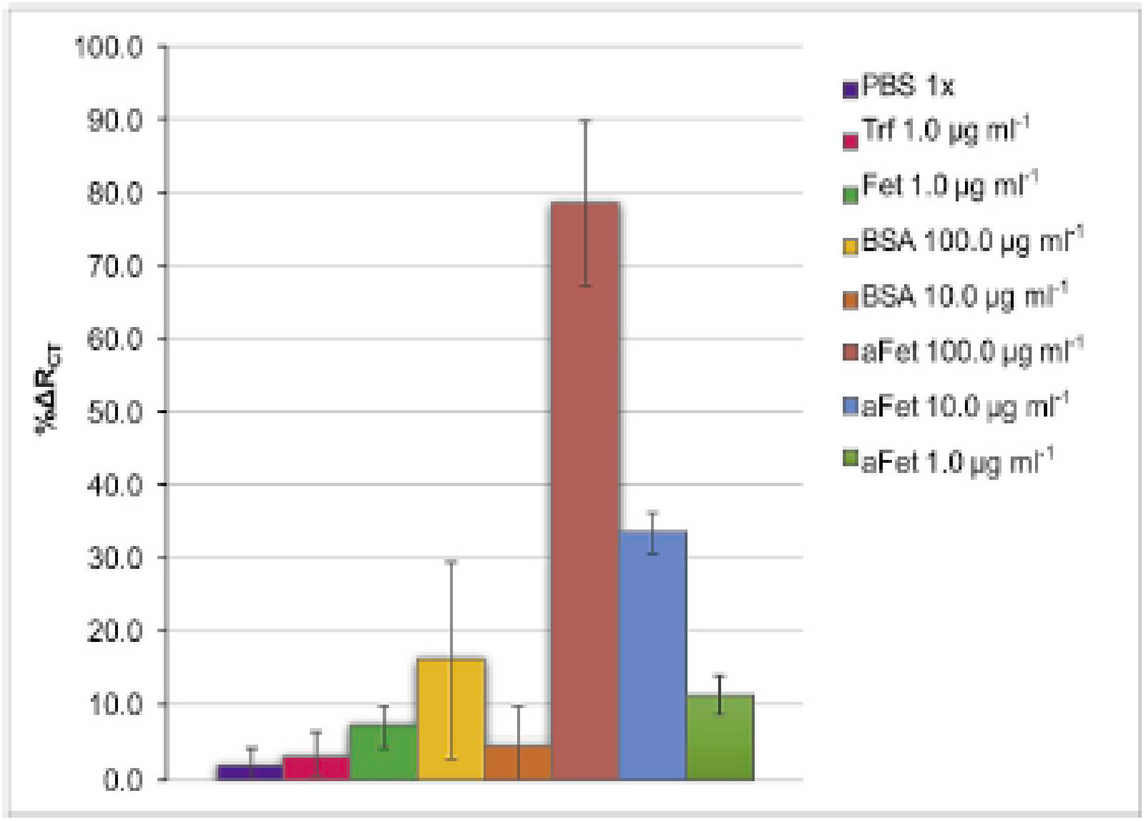
Nyquist plots obtained before and after incubating the blank biosensor (with no lectin) with BSM solutions (a) 0.01 µg ml^{-1} and (b) 1.0 µg ml^{-1}, for 5 min at room temperature.

Results – selectivity

VVA biosensor



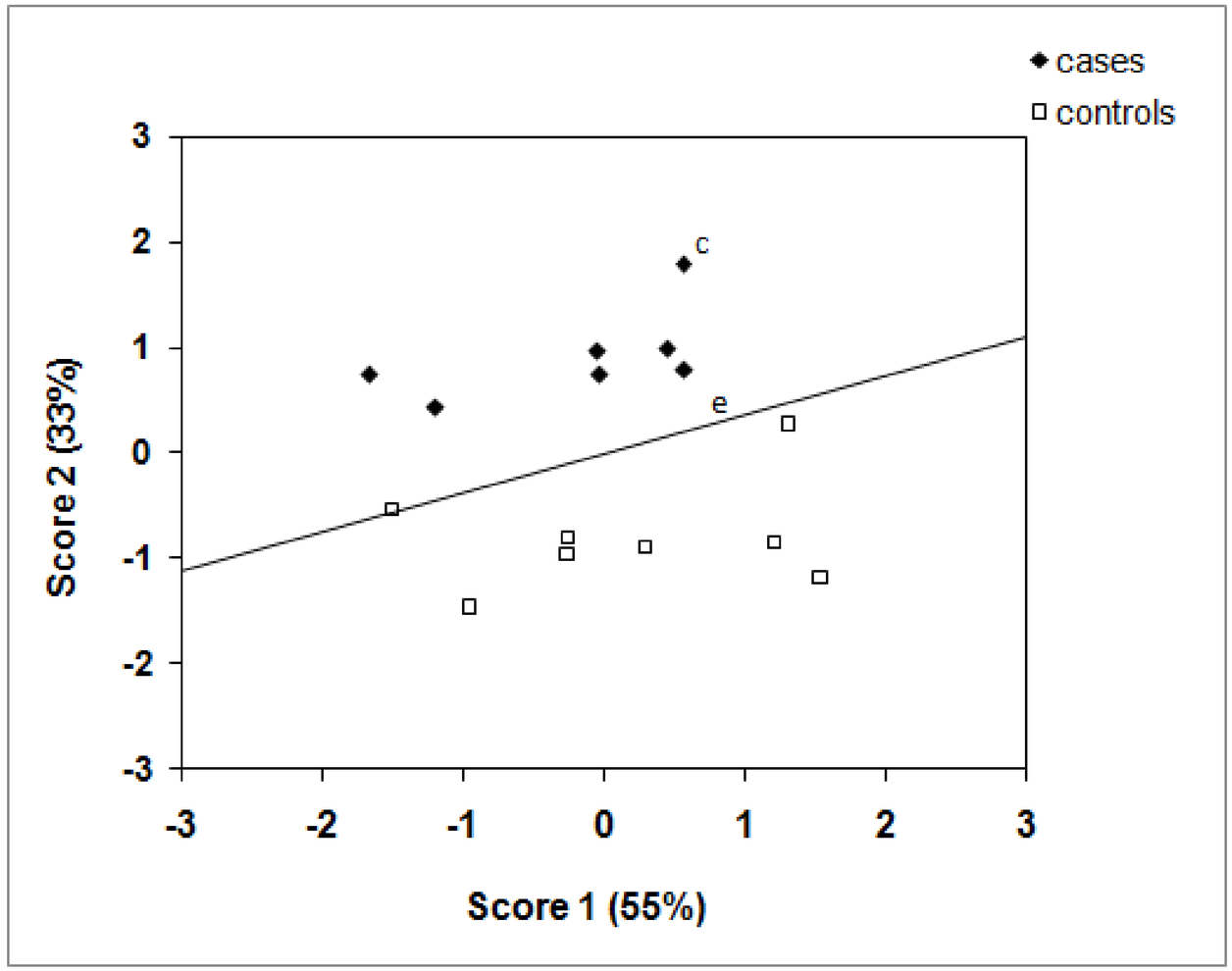
PNA biosensor



Response for several glycoprotein solutions, incubated for 10 min. Error bars indicate standard deviations of duplicate measurements with two independent biosensors for each solution.

Results – sample analysis

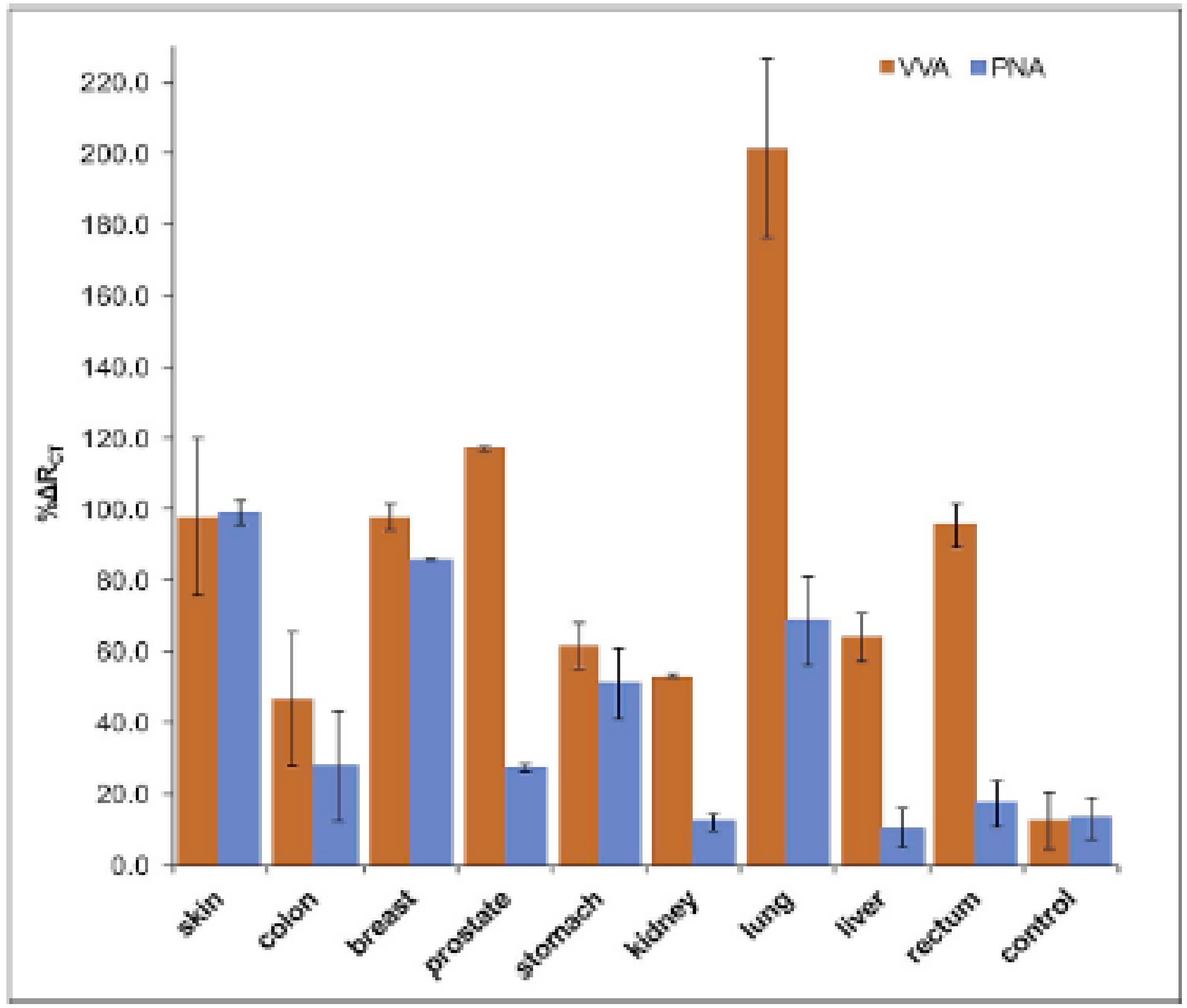
SNA biosensor



Graphical representation of the first two scores of a PCA performed on the impedimetric data from sample analysis using the SNA biosensor. Each point represents an individual analysis of a sample; (a) – breast carcinoma, (b) – retroperitoneal located malignant tumour, (c and e) – pools with 25 different cancer samples, (d) – cervical-uterine carcinoma.

Results – sample analysis

VVA and PNA biosensors



Results obtained in sample analysis for VVA and PNA biosensors. Each sample pool refers to a type of carcinoma. Ctrl represents a pool of samples from healthy donors. Error bars indicate standard deviations of duplicate measurements with two independent

Conclusions

1. **The developed biosensors showed high selectivity and high discrimination capacity between controls and cases.**

2. **For all the developed biosensors, in the optimized conditions, the assays were fast (around 20 min).**

3. **The EIS-based label-free detection simplified the construction and detection procedure.**

4. **The construction process was highly flexible and, with small, changes, could be applied to all lectin-based biosensors.**

5. **By using all biosensors for the analysis of the same cancer type, different glycosylation patterns could be observed, according to lectin specificity.**

References

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2. M. Luísa S. Silva, María G. H. Rangel. Sens. Actuators B 252 (2017) 777-784.
3. María. G. H. Rangel, M. Luísa S. Silva. Biosens. Bioelectron. 141 (2019) 111401.

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