A new label-free impedimetric aptasensor for gluten detection.

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Abstract

Celiac disease is a serious autoimmune disorder caused by the ingestion of gluten. Gliadin is the gluten fraction responsible for the triggering of disease. The only cure for the celiac patients, known until now, is a diet with gluten-free foods, classified by the European regulation doesn't exceed 20 ppm in gluten. With the aim to guarantee the food safety for celiac patients in this study the developing and optimization of a fast and reliable label free impedimetric aptasensor for gliadin detection is reported. The aptamer (Gli1) at 0.5 μmol and poly (amidoamine) dendrimer of fourth generation (PAMAM G4) at 2mg/ml were chosen during the developing steps of the sensing platform because the best ones for the detection of low gluten concentrations with the highest sensitivity. The aptasensor showed linearity in the range of 5-50 μg/l and 50-1000μg/l in gliadin, a limit of detection of 5 μg/l corresponding to 5 ppm of gluten, a reproducibility lower than 5% and a storage stability at 4°C of two months. Finally the aptasensor was used to measure gluten, in gluten and gluten-free food products, showing a good agreement with the results obtained with official R5 ELISA method.

Keywords: Aptamer, gluten, gliadin, Electrochemical Impedance Spectroscopy,

Chemical compounds studied in this article

Polyamidoamine dendrimer generation 4 (ethylenediamine core) (PubChem CID:4140276), Sulfuric Acid (PubChem CID:1118), Cysteamine (PubChem CID:6058), Glutaraldehyde, (PubChem CID:3485), Potassium hexacyanoferrate (PubChem CID:26250), Potassium ferrocyanide (PubChem CID:11963580), Sodium phosphate monobasic (PubChem CID:23672064), Sodium phosphate dibasic anhydrous (PubChem CID:23672064), Potassium Chloride (PubChem CID:4873).

1. Introduction

Gluten is a mixture of gliadin, an allergenic protein family responsible for the autoimmune enteropathy generated by Celiac disease, one of the most common chronic digestive disorders defined as a 'small intestinal immune-mediated enteropathy precipitated by exposure to dietary gluten in genetically predisposed individuals' (Ludvigsson et al., 2013). After the diagnosis the only effective safe therapy for celiac disease is based on a rigorous and permanent diet that excludes sources of gluten (wheat, rye, barley) and any foods made with these grains.

Maintaining a true gluten-free diet isn't very simple, because gluten can occurs from cross-contamination by hypothetic gluten free raw material or flavour enhancer, thickener, emulsifier, filler and fortification ingredient (Hüttner & Arendt 2010) posing potential risks for the most sensitive celiac patients.

The main methods for the detection of gluten in foods are based on directly targeting the gliadin (allergenic proteins in the gluten) or its peptide fragments. The detection can occur by isoelectric focusing (IEF), A-PAGE, SDS-PAGE, reversed-phase (RP)-HPLC, size-exclusion HPLC (SE-HPLC), high-performance resolution capillary electrophoresis (HPCE), the combination of HPLC with electrospray ionization (ESI), tandem mass spectrometry detection (LC–MS/MS) and enzyme-

linked immunosorbent assay (ELISA). This latter is the currently accepted method for gluten determination in native and processed foods. However, these gliadin analysis methods are time intensive, expensive and require trained operators

Availability of fast, cheap but sensitive methods for gluten detection are necessary for an effective gluten-free products labelling and thus protecting celiac people from the unaware content of gluten in food higher than the official limit (20 ppm) set by the European regulation.

Electrochemical immunosensors for gluten detection in food products have been developed in the last years exploiting the capability of specific monoclonal antibodies to detect gliadin antigens: a sandwich immunosensor that needs labeling steps followed by enzymatic reaction before measurement, and an amperometric competitive immunosensor based on gliadin immobilization on disposable carbon-nano gold screen-printed electrodes (Manfredi et al., 2016). Recently Chiriacò et al. (2015) proposed a lab on chip platform based on impedimetric immunosensors to detect gluten at 1 ppm.

Nucleic acid aptamers, obtained by the in vitro selection process SELEX represent a new kind of receptors for gliadin detection. The use of aptamers as the biomolecular recognition element for developing gluten sensors is justified by their low cost synthesis and high reproducibility; high affinities comparable to those of monoclonal antibodies but with higher stability due to their nucleic-acid chemical nature and additionally they can be easily combined with different chemical labels/groups that provide flexibility for adaptation to different platforms (Miranda-Castro, de-los-Santos-Álvarez, Miranda-Ordieres, & Lobo-Castañón, 2016).

In the last years aptamers against the 33-mer peptide (amino acid sequence LQLQPFPQPQLPYPQPQLPYPQPQLPYPQPQPF) recognizing the hydrophobic immunodominant fragment of α2-gliadin, have been studied pointing out their capability to bind gliadin from several gluten sources (Pinto et al., 2014; Amaya-González et al., 2014; Amaya-González et al., 2015). Aptamers against the 33-mer peptide, termed Gli1 and Gli4, have been applied in an electrochemical competitive enzyme-linked assay on magnetic particles. The assay

85 based on Gli 4-aptamer was able to achieve a gluten detection of 0.5 ppm but it failed in detecting
86 gluten in heat treated and hydrolyzed food samples contrary to Gli1 who was kinetically favored.
87 Recently Lopez-Lopez et al. (2017) developed a competitive electrochemical enzyme labeled
88 aptasensor for the analysis of gluten in food samples.
89 Electrochemical impedance spectroscopy (EIS) is a powerful informative and non-destructive
90 technique due to the small alternating voltage excitation used during detection, which can be used
91 to study the electrical properties of the sensing device interface and tracing the reactions occurring
92 on it. The application of EIS as a detection analytical technique, based on the direct monitoring of
93 the interaction between the bioreceptor and its target, enables the production of label-free
94 biosensors for food analysis with significant advantages over labeled ones. By avoiding the
95 laborious and expensive labeling steps, which can cause loss of affinity between the labeled
96 receptor and its target, and decrease reproducibility, sensitivity and selectivity of the biosensor, the
97 use of the label-free monitoring reduces biosensor costs and allows analysis in short time (Rhouati
98 et al., 2016). Thanks to EIS transduction technique, food biosensor analysis are performed in real-
99 time by studying the change in electrical properties of the electrode surface which depends only on
100 the binding interaction between the analyte and its receptor (Malvano et al., 2016a).
101 In response to industrial demand that requires simple and fast analytical methods for routine and in
102 situ control of gluten in food processing we propose the first label-free electrochemical aptasensor
103 for the gluten analysis in food products.
104 Poly (amidoamine) dendrimers of fourth generation (PAMAM G4) was used for the
105 biofunctionalization of the gold electrode in order to increase the sensitivity and at the same time
106 reach low detection limit. EIS and cyclic voltammetry (CV) were used to characterize each step of
107 electrode modification and the analytical performances of the aptasensors developed. Finally the
108 aptasensor was used to quantify gluten in raw and processed food samples and the results
109 compared with the official ELISA method based on R5 monoclonal antibody.
110

2. Materials and Methods

2.1 Chemicals

Sulfuric Acid (H2SO4, 99.9%), Cysteamine (95%), Glutaraldehyde solution (50% in H2O), Potassium hexacyanoferrate (III) ($[Fe(CN)_6]^{3-}$, >99%), Polyamidoamine (PAMAM) dendrimer generation 4 (ethylenediamine core), were purchased from Sigma-Aldrich (Milano, Italy).

Gli1 aptamer, 5'-tagged with 6-carboxyfluorescein (6FAM), was obtained from Sigma-Aldrich (Milano, Italy) according to the following sequences: 5'(6FAM)CTAGGCGAAATATAGCTACAACTGTCTGAAGGCACCCAAT .

Potassium ferrocyanide ($[Fe(CN)_6]^{4-}$), was obtained from Carlo Erba reagent (Milano, Italy).

Sodium phosphate monobasic (NaH2PO4), Sodium phosphate dibasic anhydrous (Na2HPO4), and Potassium Chloride (KCl) were obtained from Sigma Aldrich (Milano, Italy). The Gliadin standard of Prolamin Working Group (PWG Gliadin) was purchased from R-Biopharm Italia Srl (Melegnano, Italy)

2.2 Apparatus

The electrochemical measurements were carried out with a computer-controlled Autolab PGSTAT 204 Potentiostat (Metrohm), equipped with an Impedance module (FRA32M); the experimental data were analyzed with Nova software (Metrohm). Au thin-film single-electrodes, based on a three-electrode layout (working/auxiliary/reference) were purchased from Micrux Technologies (Oviedo, Spain). The diameter of Au working electrode was 1mm.

2.3 Aptasensor manufacturing

Before modification, gold electrodes were cleaned by applying 13 potential cycles between -1.0 and +1.3 V with 100 mV/s scan rate in 0.05 M sulfuric acid. Cysteamine water solution 20 mM was dropped on the surface of the electrode and a constant potential of 1.2 V vs. Ag/AgCl for 20 min was applied. After the electrode was thoroughly rinsed with water, to remove physically – adsorbed

cysteamine; then 100 μL of glutaraldehyde solution 5% (v/v) were dropped onto the modified working electrode for 1 h and, again, the electrode was rinsed with water.

Before the immobilization, the terminal carboxylic group of Gli1 aptamer was activated in a solution of 75 mM EDC and 15 mM NHS in 100 mM MES buffer (pH 7.4) for 2 h and then the activated aptamer was dropped on Au modified electrode. Afterwards, the immobilization of Gli1 aptamer was carried out in presence and in absence of PAMAM dendrimer. For the immobilization without PAMAM, cysteamine modified electrode was covered with 10 μL of aptamer solution at three different concentrations (0.5 μM, 1 μM 1.5 μM) for 1h at room temperature. The functionalization with PAMAM was carried out by glutaraldehyde deposition on the cysteamine layer, then three different concentrations of PAMAM solution (1 mg/mL, 1.5 mg/mL, 2 mg/mL) were dropped on it and left to react for 3 hours. After that, activated aptamer was incubated on electrode surface for 1 h.

Finally, the electrode was rinsed in PBS (pH 7.5) to remove unbound aptamers. The schematic diagram of gliadin aptasensor fabrication is presented in Fig.1

2.4 Experimental Measurements

Electrochemical Impedance Spectroscopy was used to detect the immobilization processes and the interaction Gli1-gliadin.

For the impedance measurements, a sinusoidal AC potential (10 mV) in the frequency range from 0.1 to 10^4 Hz was superimposed to 0.00 mV (vs. reference electrode) DC potential. The impedance data were plotted in the form of Nyquist plots, where the complex impedance is displayed as the sum of the real and imaginary components (Z^I and Z^{II} respectively) and in the form of Bode diagram, where the total impedance of the system (Z) is plotted versus frequency. All measurements were performed in a solution of 1 mM ferri/ferrocyanide redox couple ($[Fe(CN)_6]^{4/3}$, 1:1) in PBS, pH 7.5, as background electrolyte at room temperature.

Cyclic Voltammetry measurements (CV) were also used to characterize each step of the electrode

modification. The measurements were performed from -0.6 to 0.6 V vs. reference electrode with a scan rate of 0.05 V/s; the redox couple used for the CV was the same as that used for impedance measurements.

PWG gliadin standard solutions and food samples extracts were dropped onto the working area of the aptasensors and incubated for 45 min. Before the impedance measurements, the sensor surface was rinsed thoroughly with copious amounts of PBS.

2.5 Preparation of food samples for gluten measurement detection

The preparation food samples was carried out according to (Méndez, Vela, Immer, & Janssen, 2005)

Five commercial samples were chosen for our tests: beer and gluten-free beer, gluten-free toasted bread, rice and corn flour. 5 mg for grinded solid or 5 ml for liquid samples were mixed with 2.5 mL of "cocktail solution" and incubated for 40 min at 50°C. Cocktail solution contains denaturing and reducing agents, ensuring a very good recovery of gluten proteins also from heat-treated food. After the cooling of the sample at room temperature, 7.5 mL of 80% ethanol were added. After shaking for 2 h at room temperature, the extract was centrifuged at 8000 rpm for 10 min and the supernatant was recovered and diluted with PBS obtaining a final dilution factor of 500.

The results obtained with the aptasensor were compared with those measured with R5 ELISA KIT (R-Biopharm Italy) for gliadin detection.

3. Results and Discussion

3.1 Aptasensor development and optimization of experimental condition

The amount of the bioreceptor is an important factor in the performance of a biosensor as it greatly affects the capability of the biointerface to detect the target in a range of interest.

With the aim to develop an aptasensor able to detect gluten in a wide range of raw and processed foods Gli1 was selected, among the aptamers against 33-mer (Amaya-Gonzalez et al., 2014),

because of its high affinity not only to intact gliadin but also to its peptide fraction caused by enzyme reaction in fermentation process. (e.g. the beer). Three different amount of Gli1 aptamers were directly immobilized on the surface of cysteamine modified gold electrodes.

The immobilization steps were monitored by EIS and CV (Fig. 2).

The voltammogramm of the Au electrode display well defined anodic and cathodic peaks due to the reversible interconversion of $Fe(CN)_6^{3-/4-}$ (Fig.2a). The formation of the cysteamine layers and the aptamer binding, causes the decrease of both peaks due to hindering effects of the layers on the electron transfer rate. Nyquist plots showed an increase of impedance during the immobilization steps due to the blocking layer coating on electrode surface, which became thicker with the assembly procedure. Moreover, impedance increases with the increasing of aptamer concentration pointing out a greater immobilization of aptamer on the surface of the electrode.

When the aptasensor reacts with increasing concentration of PWG gliadin standard an increase of semicircle diameters of Nyquist plots was observed (Fig. S1) corresponding to the charge-transfer resistance R_{ct} of Randle circuit used for data fitting. This parameter versus the concentration of PWG gliadin was then used to calibrate the three aptasensors fabricated (Fig.3).

Even if higher amounts of Gli1 result in higher signals, the aptamer at 0.5 μmol showed the lowest limit of detection (LOD) equal to (50 μg/l) of gliadin, calculated using the sum of average blank solution and three times the standard deviation.

The process related to gliadin coupling with Gli1 aptamer was monitored by single frequency impedance (SFI) that is able to monitor total impedance in a single frequency versus time. In our study SFI tests were carried out a 0.1 Hz chosen on the basis of the maximum differences among the Bode plots corresponding to different gliadin concentration (Fig. S2). A significant change in impedance was observed for an incubation time of 40 min, then no change was registered (Fig. S3). These results justified the incubation times of 45 min used in this study for Gli1 PWG gliadin coupling.

Taking into consideration that gluten is a *50/50* blend of gliadin and glutenin and in a typical

analysis of gluten in food matrix a dilution 1:500 is required, the LOD of the aptasensor was calculated equal to 50 ppm of gluten. With the aim to improve the analytical performance of the aptasensor to detect the official limit in gluten (20 ppm) imposed by the European Regulation for the labelling of gluten-free foods a PAMAM dendrimer was used as linker for Gli1 aptamer (Fig. 1). Three different concentration of PAMAM (1-1.5 and 2 mg/ml), were used for the immobilization of Gli1 at 0.5 μmol. Also in this case the immobilization of different layers was investigated by EIS showing the increase of total impedance in each immobilization step used during the fabrication of the aptasensors (Fig. S4). When PAMAM was loaded on cysteamine modified electrode, R_{ct} progressively increased with PAMAM concentration (42 K Ω for 1 mg/ml; 53 K Ω for 1.5 mg/ml 70K Ω for 2 mg/ml) suggesting that higher amount of dendrimer were immobilized on the electrode surface.

The three aptasensors constructed with PAMAM were able to detect lower PWG gliadin amount (equal to 5 μg/l) than those registered without the dendrimer. This data is consistent with Lee et al. (2009) and Mori et al. (2009) who verified the advantages of PAMAM dendrimer, over self-assembled monolayer surface coatings (SAMs), to increase the bio-availability and sensor sensitivity. Moreover PAMAM maintains flexibility of the branches after fixation to the solid surface exposing bioactive moieties in a more effective way than in monolayer linkers (Katzur et al., 2012)

For all three aptasensors the linearity was in separated concentration ranges of 5-50 μg/l and 50-1000 μg/l in gliadin with a LOD of 5 μg/l corresponding, as stated above, to 5 ppm of gluten in food products (Fig. 4). This result was consistent with Amaya-Gonzales et al. (2015) who verified the ability of Gli1 aptamer in gluten detection by a competitive electrochemical aptamer based assay.

The highest sensitivity was found for the aptasensor fabricated with 2mg/ml of PAMAM.

This result could be due to the spherical shape of the dendrimers and the high density of reactive groups on their surface that raise the specific surface areas of the probe, thereby increasing the

242 amount of immobilized aptamer (Benters, Niemeyer, and Wöhrle 2001; Degenhart et al., 2004;
243 Ajikumar et al., 2007).
244 This latter was confirmed by the analysis of EI spectra registered during the construction of the
245 sensor with dendrimer who highlighted the increase in ΔR_{ct}, (56 KΩ, 58KΩ and 95KΩ for 1, 1.5
246 and 2 mg/ml of PAMAM respectively) calculated before and after the binding of the aptamer at 0.5
247 μmol. Moreover as previous reported (Malvano, et al. 2016b) higher level of biomolecule
248 immobilization raised the sensitivity of the sensor increasing the coupling capacity between
249 bioreceptor and target.
250
251 *3.2 Analytical performance of aptasensor*
252 The reproducibility calculated on five different aptasensors, at 100 μg/l PWG standard, showed a
253 good relative standard deviation (RSD) for all aptasensors developed with PAMAM: 4.56% , 5.12%
254 and 4.25% for 1, 1.5 and 2 mg/ml PAMAM respectively.
255 The storage stability of aptasensors fabricated with 2mg/ml PAMAM was also determined. For this
256 purpose different aptasensors were stored for two months at 4°C without chemical preservatives
257 and characterized at regular interval times. After the investigated storage period the aptasensors
258 showed a negligible loss of activity.
259 In table 1 a performance comparison of aptamer-based electrochemical assays, previously
260 developed, for gliadin detection is reported. Gli 4 showed highest affinity for gliadin even if it fails
261 in detecting the peptide fraction in solution (Amaya-González et al., 2015). Anyway a good
262 agreement with literature was observed for Gli1. It is worth noting that our aptasensor is the first
263 one based on the immobilization of aptamer on the modified electrode and all the others were based
264 on competitive assays. This condition makes the analysis of gliadin very fast and easier than
265 aptamer competitive assays that required addition of an enzyme labelled aptamer to the food sample
266 and then the enzymatic substrate.
267 The analysis of the impedimetric data registered during the interaction tests between the

aptasensors, with and without dendrimer, and increasing PWG gliadin concentration, also provides an estimation of dissociation constant (K_d) for aptamer PWG gliadin interaction.

The dissociation reaction for PWG gliadin – Gli1 complex could be expressed for the dissociation constant, K_d as following:

$AG \rightarrow A+G$

In which A is the aptamer, G is the target, and AG is the aptamer-target complex.

The equilibrium can be described using dissociation constant K_d:

$K_d=[A][G]/[AG]$

Assuming the surface coverage of PWG gliadin – aptamer complex is α, the surface coverage of unbound aptamer will be 1-□α:

$$K_d = \left(\frac{1-\alpha}{\alpha}\right)[G]$$

K_d can be obtained experimentally by measure of fraction occupied sites by:

$f = R_{cteq}-R_{ct0}/ R_{ct0}$

Where R_{cteq} is R_{ct} at equilibrium and R_{ct0} is the initial R_{ct} (Jing and Bowser, 2011; Fan, Zhao, Shi, Liu, & Li, 2013).

According to Langmuir adsorption isotherm, this fraction f can be directly related to the surface coverage of the gliadin–aptamer complex:

$$f = \alpha * f_{sat}$$

where with α =1, the standard signal reaches the maximum value f_{sat}.

The use of dimensionless *f* data allows transformation of the adsorption isotherm in to the Hanes–Woolf form, where overweighting of the low concentration results is avoided (Schuler and Kargi, 2002)

293

294 $$f = \frac{f_{sat} \cdot [G]}{K_d + [G]}$$

295

296 A plot of [G]/f versus [G] for the aptasensors with and without PAMAM was shown in Fig. 5.

297 The slope of linear regressions and the y-intercept for each aptasensors let the calculation of K_d as

298 reported in table 2.

299 Highest affinity was calculated when PAMAM was used for the aptamer anchorage on the electrode

300 confirming the advantages of dendrimer to preserve the native biomolecule conformation.

301 For all aptasensor developed Gli1 showed higher affinity to PWG gliadin and the value estimated

302 were consistent with other authors (Amaya-Gonzalez et al., 2015) who calculated a K_d value of 58

303 nM for Gli1-PWG gliadin interaction. It is worth noting that K_d value is influenced by the analytical

304 technique used for the calculation, the experimental procedure (aptamer immobilized or free in

305 solution), if it is native or 5-tagged (for its binding on solid surface), and by the type of moiety used

306 for the tagging (Jing & Bowser, 2011).

307

308 *3.3 Analysis of gluten in food samples*

309 In order to highlight the gluten detection capability of the aptasensor (with PAMAM at 2 mg/ml)

310 processed and unprocessed food labeled as "gluten free" were chosen as samples. The aptasensor

311 was also tested with corn flour and rice to evaluate the reactivity of Gli1 versus other prolamines

312 non celiac disease triggering. The extraction of gluten was carried out by cocktail solution®

313 (Méndez et al., 2005) according to extraction protocol of official method based on R5

314 immunoassay. R5 ELISA was used as comparison method. The amount of gluten detected in food

315 samples by both methods was reported in table 3. Both methods gave concordant results for beer

316 and bread "gluten free" labelled confirming their safety in celiac patients. Contrasting gluten

317 concentrations were measured by aptasensor and ELISA kit. The capability of Gli1 aptamer to

318 recognize also hydrolysed gliadin in contrast to R5 sandwich ELISA could be an explanation of

different gluten amount detected in lager beer samples. Finally corn flour contains gluten near the threshold established by European regulation. R5 recognizes only the amino acid sequence QQPFP and similar sequences present in prolamins from wheat (gliadin), rye (secalin), and barley (hordein) (Valdés, García, Llorente, & Méndez, 2003) thus the amount of about 20 ppm measured by ELISA kit and aptasensor could be due to a cross-contamination during the manufacturing process of corn flour.

4. Conclusion

A new label-free impedimetric aptasensor for gluten detection, based on the immobilization of aptamer (Gli1) on the gold electrode modified with PAMAM, was reported. PAMAM has been proven to increase the sensitivity of the aptasensor with a high binding affinity to PWG gliadin. The aptasensors developed were very sensitive to gluten with a detection limit of 5 ppm. Moreover, a good agreement between R5 ELISA official method and aptasensor was obtained in gluten content analyzed in food samples. The analysis of gluten does not require the use of other reagents but only the gliadin extract, thus it makes the impedimetric aptasensor a fast and simple method for the control of food safety in food products addressed to celiac patients diet.

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List of Figures and Tables

441 Table 3: Gluten results obtained by impedimetric aptasensor and R5 ELISA kit.

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466 **Table 1**

467

	Assay type	Transduction technique	LOD [gliadin ug/L]	LOD [gluten ppm]	References
33-mer onto magnetic beads + biotin-Gli1+gliadin+streptavidin-peroxidase	competitive	amperometry	4.9	4.9	[Amaya Gonzalez-et al., 2015]
33-mer onto magnetic beads + biotin-Gli4+gliadin+streptavidin-peroxidase	competitive	amperometry	0.5	0.5	[Amaya Gonzalez-et al., 2015]
SPCE-33mer + biotin-Gli4+gliadin+streptavidin-peroxidase	competitive	amperometry	0.11	0.11	[Lopez-Lopez et al., 2017]
GE-Cys-PAMAM-Gli1	direct	Impedance	5.0	5.0	This work

468 GE gold electrode^{-}: SPCE Screen-Printed Carbon Electrode

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472 Table 2.

aptamer Gli1	without PAMAM	PAMAM 1mg/ml	PAMAM 1.5 mg/ml	PAMAM 2 mg/ml
k_d/nM	73,63	6,66	9,03	9,86

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477 Table 3:

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Sample	Aptasensor ppm	R5 ELISA kit ppm
lager beer	34.29±0,20	21.67±6.13
beer gluten free	6.03±0,04	6.59±0.27
bread gluten free	7.97±0,40	8.08±0.29
rice	nd	nd
corn flour	21.02±0.65	18.19±0.88

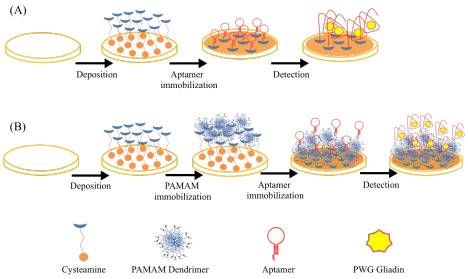

Fig. 1. Schematic diagram of the aptasensor with and without PAMAM.

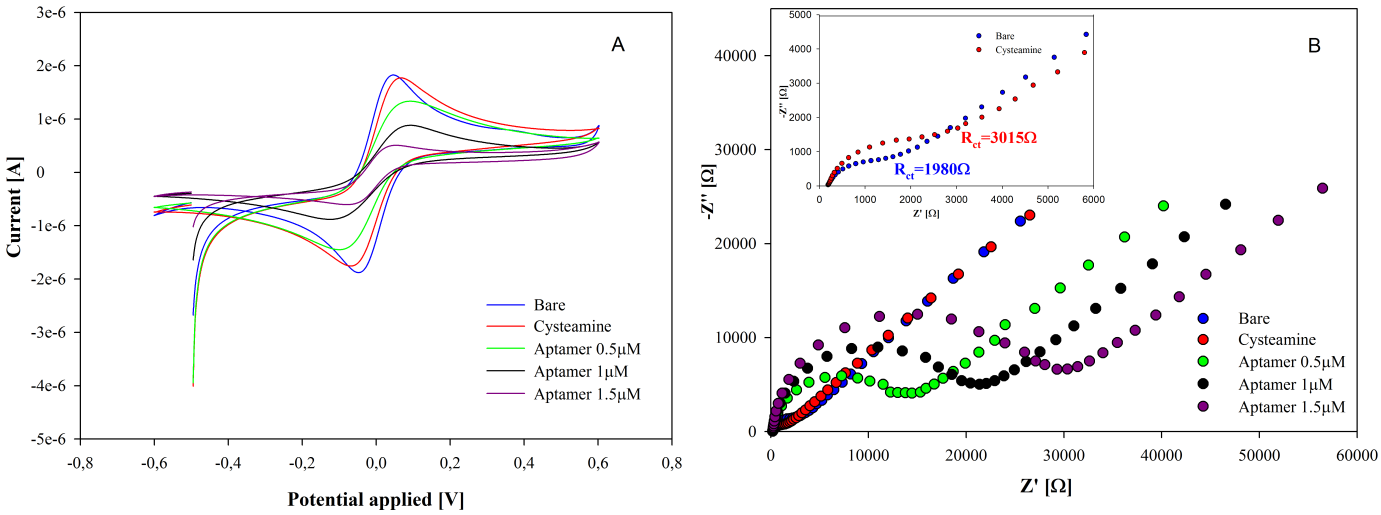

Fig. 2. (A) Cyclic voltammograms in 1 mM ([Fe(CN)6]4/3 and (B) EIS responses after each step of aptasensor construction with different amount of Gli1. The inset corresponds to the impedance spectra for bare and cysteamine modified electrodes.

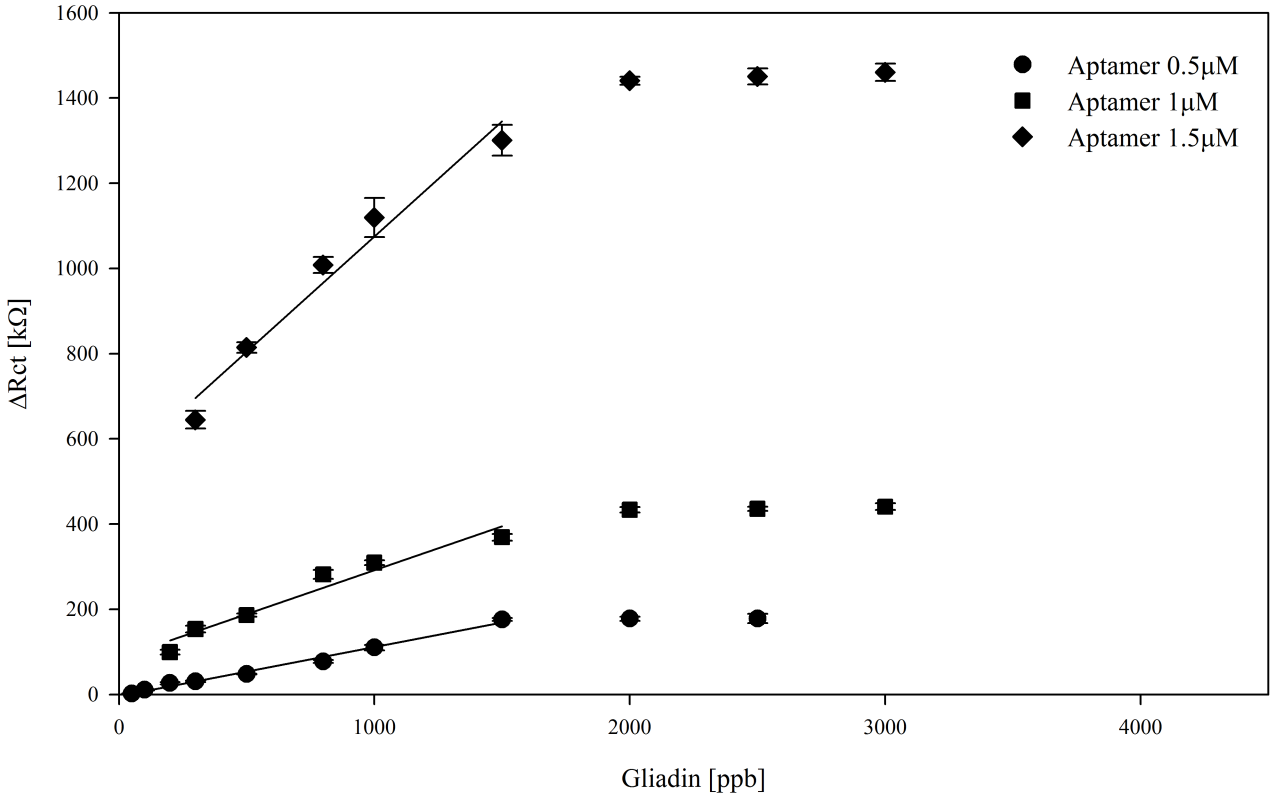

Fig. 3. Calibration curves for PWG gliadin with different aptamer Gli1 loading

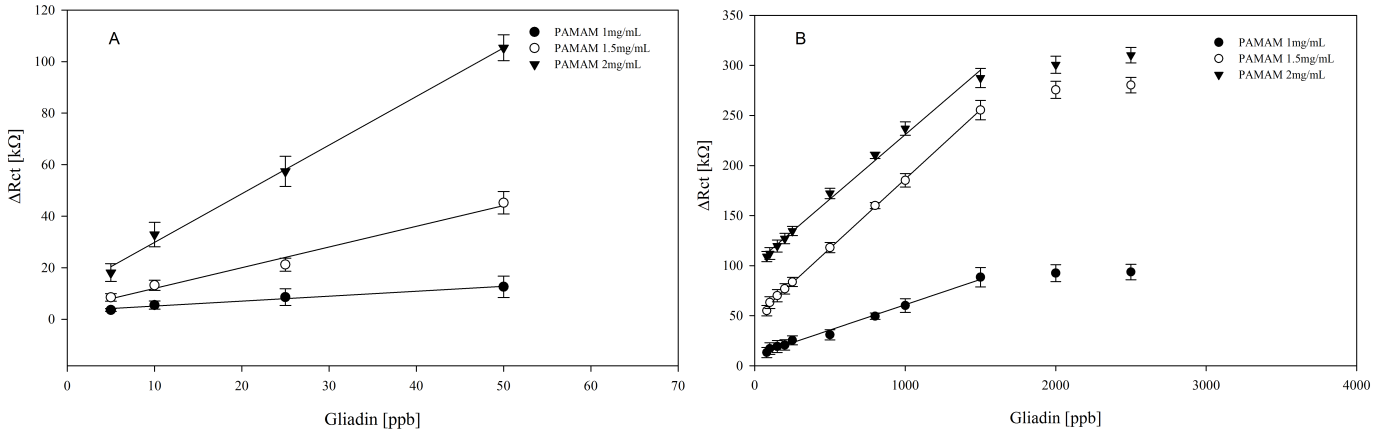

Fig. 4. Calibration curves for PWG gliadin for aptasensor with 0,5umol Gli1 and PAMAM at 1, 1,5 and 2 mg/ml. (A) Linear range PWG gliadin 5e50 mg/l; (B) Linear range PWG gliadin 50e1000 mg/l.

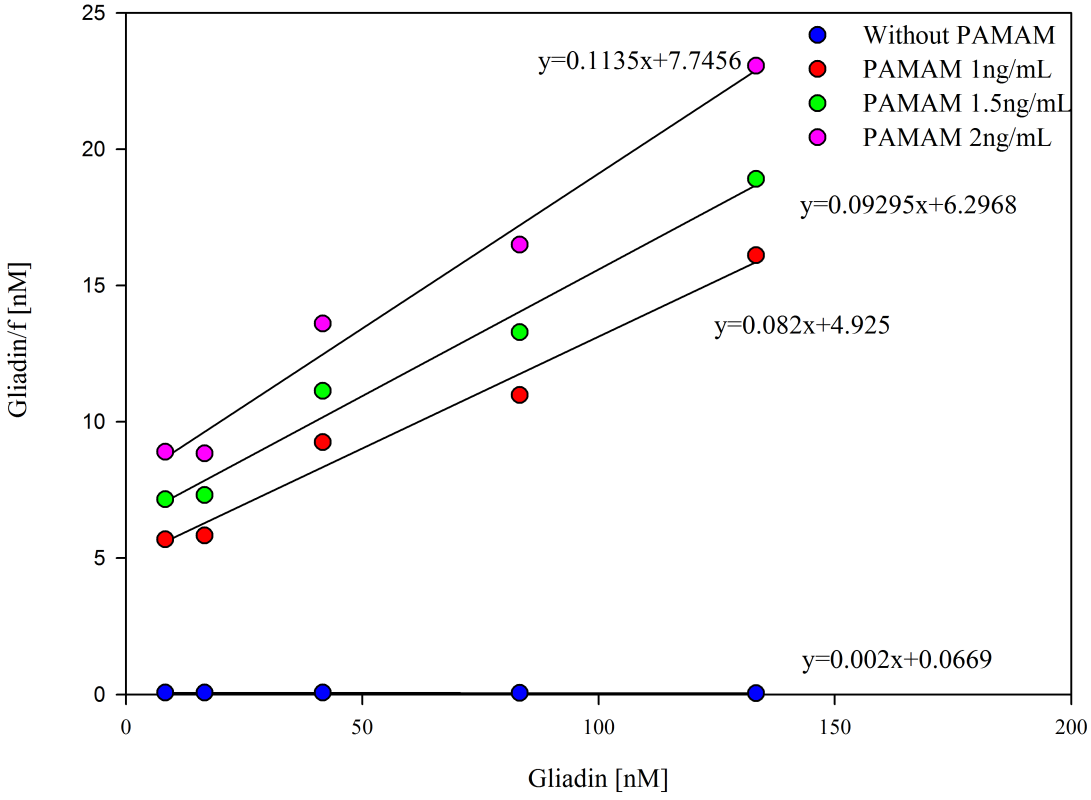

Fig. 5. HaneseWoolf plots for determining the dissociation constant.

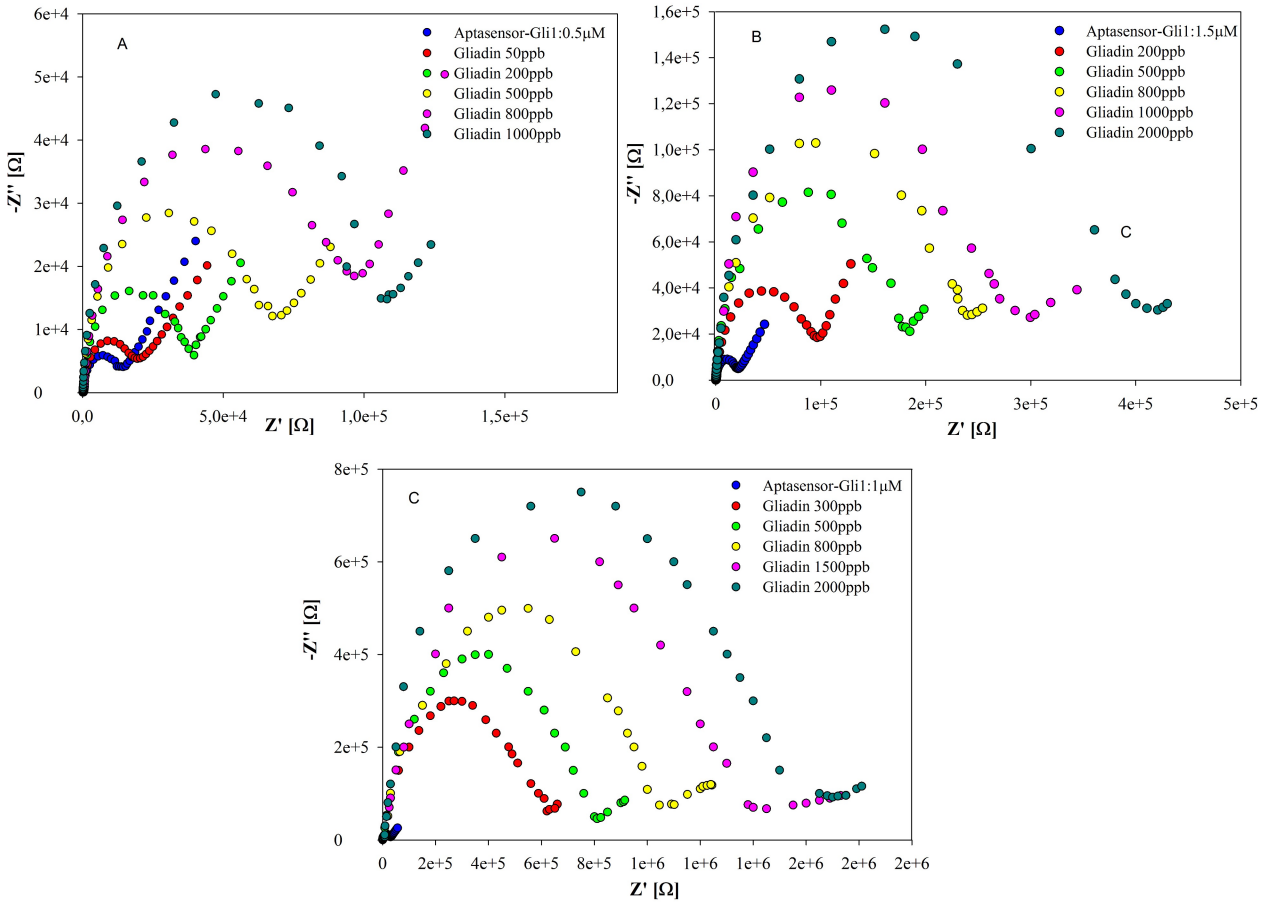

Figure S1: Nyquist plots of aptasensors after incubation of different PWG gliadin concentrations: (A: Gli1 = 0,5 [?]M; B: Gli1= 1 [?]M; C: Gli1=1,5[?]M)

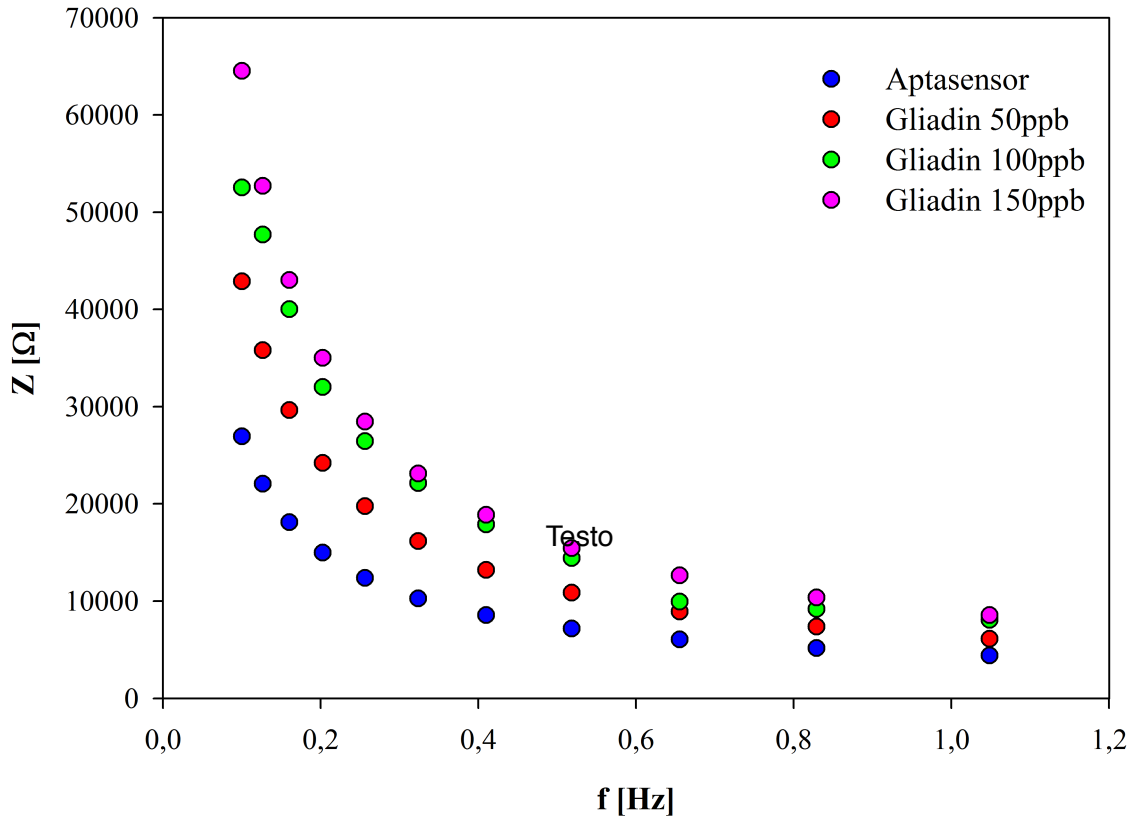

Figure S2 Bode plot in impedance measurements for aptasensor with Gli 1 after incubation of different PWG gliadin concentrations steps in the frequency range 0.1–1 Hz.

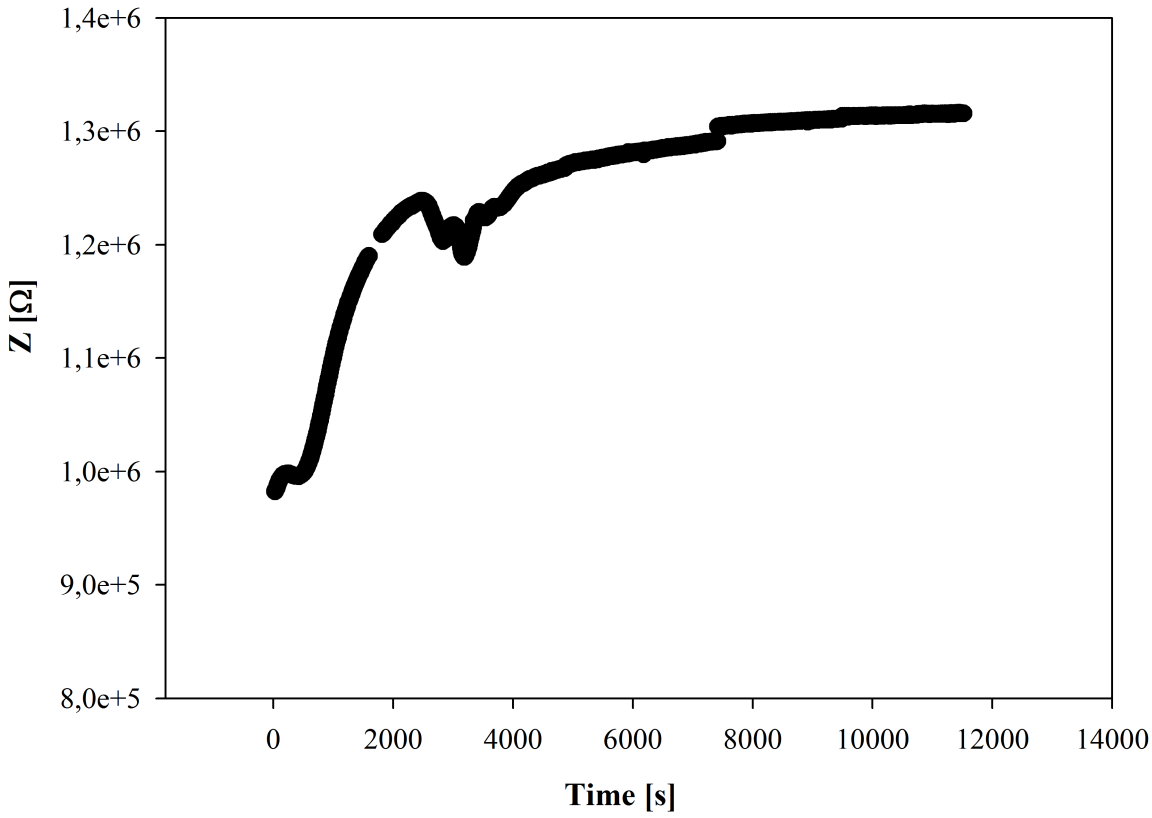

Figure S3: PWG Gliadin coupling with Gli1 aptamer monitored by SFI at 0.1 Hz

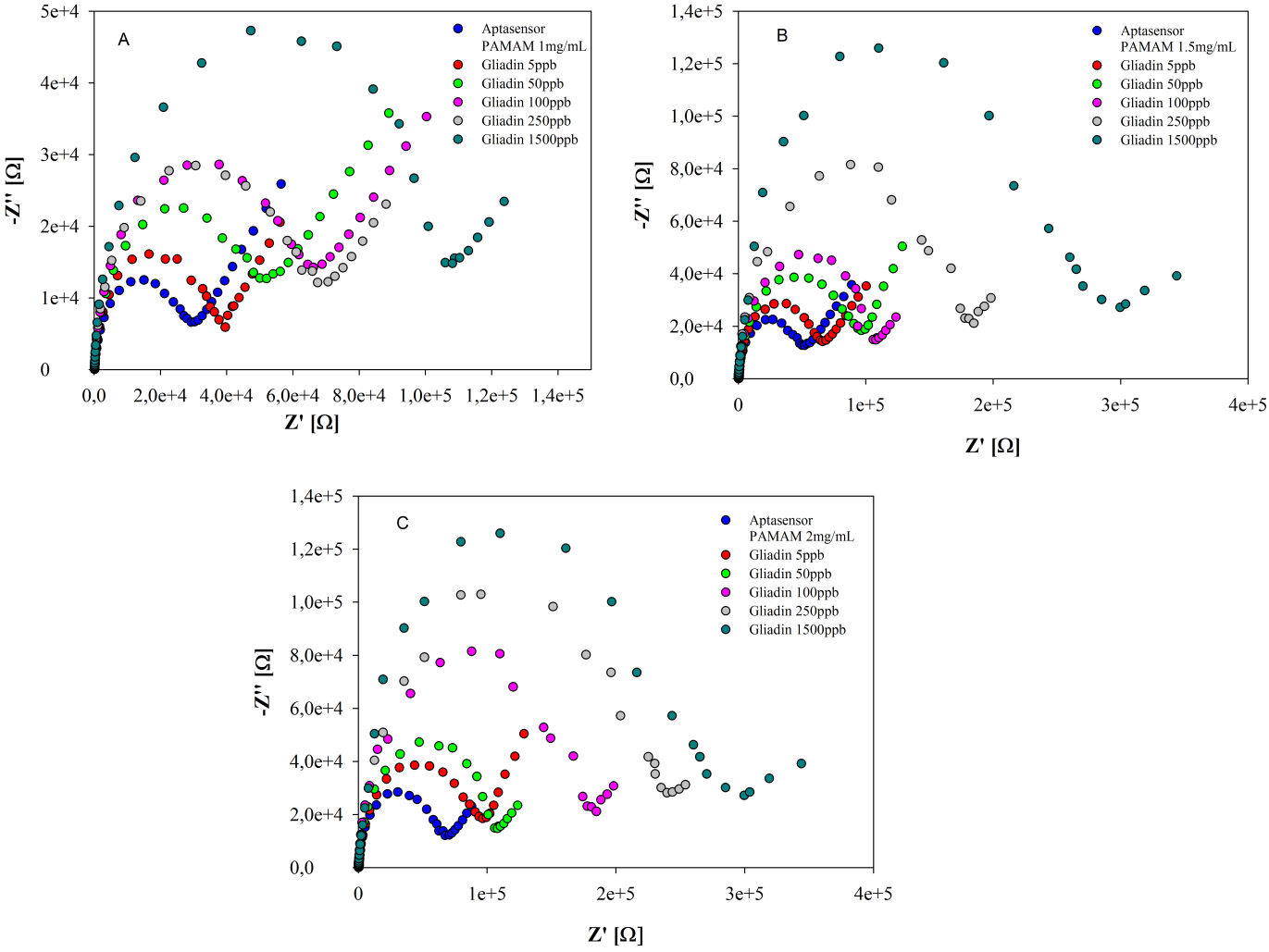

Figure S4: Nyquist plots of aptasensor with Gli1 = 0.5 µmol and different amount of PAMAM