



# ACE2 and SARS-CoV-2-Main Protease Capillary Columns for Affinity Chromatography: Testimony of the Binding of Dexamethasone and its Carbon Nanotube Nanovector

Yves Claude Guillaume<sup>1,2,3</sup> · Claire André<sup>1,2,3</sup>

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## Abstract

In this paper, each of the two following proteins, the angiotensin-converting enzyme 2 (ACE2) and the Main protease (Main pro) of the Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) were grafted for the first time on homemade neutravidin poly(GMA-co-EDMA) capillary columns for the research of their ligands. The effect of the column diameter on the quantity of immobilized biotinylated protein was studied. For a capillary length of 40 mm, when its internal diameter varied from 75 to 25  $\mu\text{m}$ , the grafted quantity of ACE2 decreased by 85% (from 1.50 to 0.24  $\mu\text{g}$ ). Among all the studied ligands, a particular vigilance has been given for dexamethasone, a widely used molecule today for adult patients hospitalized with SARS-CoV-2. Competition experiments were performed with SARS-CoV-2 Receptor Binding Domain used as reference molecule with the ACE2 affinity column to assess the orthosteric binding site of dexamethasone (Dex) on ACE2. This ligand was then immobilized on Multiwall Carbon Nanotubes (Dex/MWCNT). By comparison of the normalized breakthrough curves measured for Dex and Dex/MWCNT on both the ACE2 and Main pro affinity columns, it was showed for the first time that nanovectorisation of Dex with MWCNT enhanced and stabilized its binding to both ACE2 and Main pro. This last result reinforced the use of Dex and the interest of MWCNT for boosting immune health against COVID 19.

**Keywords** Nano affinity chromatography · ACE2 · Main protease · Receptor binding domain · SARS-CoV-2 · Ligands · Dexamethasone · Multi wall carbon nanotubes

## Introduction

Bio-affinity chromatography was employed to study biological interactions, such as protein–protein, drug–protein, enzyme–inhibitor and receptor–ligand interactions [1–4]. In the past few years, miniaturized affinity LC systems [5, 6] were developed. Nano LC columns are generally 75  $\mu\text{m}$  in diameter, because the particle sizes for packed Nano columns were between 3 and 5  $\mu\text{m}$  [1].

The alternative to the particle packed columns were Poly glycidyl methacrylate-co-ethylene dimethacrylate) (Poly (GMA-co-EDMA)) monoliths columns [7–11]. So as to decrease strongly the time required for the immobilization procedure of the receptor in the capillary column, neutravidin (or streptavidin) [12–15] has been first grafted on the organic monolith surface. This neutravidin (or streptavidin) poly(GMA-co-EDMA) monolithic LC capillary column can be stored for at least 3 months at 4 °C and used just before the grafting of the biotinylated receptor which took only a few minutes. A such immobilization process with Nano LC capillary columns has thus been used for human serum albumin (HSA) [12], arginase [13], SARS-CoV-2 Receptor Binding Domain (RBD) [14] and acetylcholinesterase (AChE) [15] for the purpose of the research of their potential ligands.

In this work, homemade nano-organic neutravidin monolithic LC capillary columns were used for the first time for the grafting of (1) the angiotensin-converting enzyme 2 (ACE2) which was the host functional receptor for the SARS CoV2 causing COVID19 [16] and (2) the

✉ Yves Claude Guillaume  
yves.guillaume@univ-fcomte.fr

<sup>1</sup> Univ Franche-Comté, 25000 Besançon, France

<sup>2</sup> EA481 Neurosciences Intégratives et Cliniques/Pôle Chimie Analytique Bioanalytique et Physique (PCABP), 25000 Besançon, France

<sup>3</sup> Pôle Pharmaceutique, CHRU Besançon, 25000 Besançon, France

SARS-CoV-2-3CL-Mpro proteinase which played a critical role in viral protein maturation by cleaning proproteins after their translation into the host cell cytosol [17].

Angiotensin-converting enzyme 2 (ACE2) consists of 805 amino acids and is a type I membrane protein expressed in lung heart kidney and intestine [18]. The full length ACE2 consists of an N terminal peptidase domain (PD) (residues Ser19–Asp615) and a C terminal collectrin-like domain (CLD) that ends with a transmembrane alpha helix and an approximately 40-residue intracellular segment [18].

In the case of SARS-CoV-2, the glycoprotein (S protein) on the virion surface exploits ACE2 for host infection [19]. It was demonstrated that the ectodomain of SARS-CoV-2-S protein binds to the PD of ACE2 with a dissociation constant  $K_d = 15$  nM [20].

The 3CL Mpro proteinase is composed of Domain I (residues 8–101), Domain II (residues 102–184) and Domain III (residues 201–303). Domains I and II are composed of antiparallel beta barrel structures and are the catalytic structures. Domain III is composed of five alpha helices and plays an essential role in the protease function [21].

Investigations proved that low doses of dexamethasone could reduce the mortality in patients with severe COVID-19. The binding mechanism of the dexamethasone ligand to these two receptors has been particularly scrutinized. Carbon nanotubes were promising agents to increase the binding of some ligands to their molecular targets [22]. Thus, the difference on the binding to ACE2 or Mainpro of Dexametason and its nanovector (i.e., Dex/MWCNT) was studied in this paper for the first time.

## Materials and Methods

### Equipment

The ThermoScientific Ultimate 3000 RSLCNano system with a UV detection was used.

The poly(GMA-co-EDMA) monoliths were prepared as explained in our previous work [23]. This monolith had a specific area of  $4 \text{ m}^2 \text{ g}^{-1}$  and a permeability  $K = 6.10^{-14} \text{ m}^2$ . The medium size for macropores was approximately  $2.0 \text{ }\mu\text{m}$  and the skeleton size was approximately  $1.0 \text{ }\mu\text{m}$ . The grafting process of neutravidin onto the surface of these monoliths was previously described by the use of the Schiff base method [12–15]. These neutravidin poly(GMA-co-EDMA) monolith capillary columns ( $75 \text{ }\mu\text{m}$  or  $25 \text{ }\mu\text{m}$  i.d.) were stable at least 3 months at  $4 \text{ }^\circ\text{C}$  and can be used just before the fast immobilization process of the biotinylated receptor (ACE2 or Main pro).

### Reagents

Dexamethasone, chloroquine, hydroxychloroquine, isorhamnetin, quercetin, phenylalanine, chlorogenic acid, piceatanol, kaempferol, rutin and Polyethylene Glycol—4000 (PEG 4000) were obtained from Sigma Aldrich. The recombinant angiotensin-converting enzyme 2 (ACE2), the SARS-CoV-2-Main protease (Main pro) and the SARS-CoV-2-Receptor Binding Domain (RBD) were purchased from Acrobiosystem. Short length multiwall carbon nanotubes (median length  $600 \text{ nm}$ ) were obtained from Nanothinx (Greece). All the other chemical products were of analytical grade and all the buffer solutions were filtered through a  $0.45 \text{ }\mu\text{m}$  membrane filter and degassed before their use for HPLC.

The receptor (ACE2 or Main pro) was biotinylated with a molar ratio RBD/biotin = 2 with the EZ-link NHS-Biotin Reagent (Thermoscientific) following the manufacturer's protocol.

### Preparation of Dex/MWCNT Nanovector

The Dex/MWCNT nanovector was prepared as explained in a previous work [24]. MWCNTs were first suspended in a 3:1 mixture of concentrated sulfuric and nitric acids (96% and 69%, respectively) for 4 h. The resulting oxidized nano MWCNT (oMWCNTs) were collected through a  $0.2 \text{ }\mu\text{m}$  PTFE membrane, and washed with water until reaching neutral pH and dried under vacuum for 2 h. Then, 6 mg of oMWCNTs and 6 mg of PEG 4000 were mixed and dispersed in 100 mL of deionized water. The mixture was sonicated in a water bath and stirred at room temperature for 60 min. 100 mL of Dex at  $30 \text{ }\mu\text{M}$  was thus added in this solution for 2 h. The Dex/MWCNT particles were collected through a  $0.45 \text{ }\mu\text{m}$  Millipore filter rinsed with water and dried under vacuum for 4 h. The mass fraction of Dex (0.096 (m/m)) in the Dex/MWCNT nanovector was inferred from the remaining free Dex contained in the solution and calculated using UV absorbance of Dex at 238 nm ( $E_{\text{Dex}} = 0.039 \text{ L mg}^{-1} \text{ cm}^{-1}$ ).

### Frontal Analysis Experiments

Different concentrations of potential ligands (L) were prepared in the 50 mM PBS pH 7.4 and were flushed at a fixed flow-rate through our prepared miniaturized affinity capillary columns (ACE2 or Main pro affinity column). All the experiments were repeated three times.

Sigmoidal breakthrough curves were obtained and studied by the use of the frontal analysis equation [25]:

$$1/Q = K_d/(m_L * [L]) + 1/m_L.$$

In this equation,  $Q$  was the number of moles of ligands (L) needed to reach the midpoint of the sigmoidal curve,  $K_d$  was the dissociation constant of the ligand to its studied receptor and  $m_L$  was the total moles of bound receptors on the affinity column.

The dead time  $t_0$  of the affinity column was determined by the injection of DMSO in the chromatographic system.

For some experiments the breakthrough curves were given in the Normalized format.

In this format the  $y$ -axis described the ratio of the UV absorbance of the ligand at the column outlet to that of the UV absorbance of the ligand at the column inlet (Abs. Normalized) with values between 0 and 1. In the breakthrough curve, the stable plateau formed at saturation time  $t_p$  was characterized by an Abs. Normalized = 1.

The  $x$ -axis described the ratio of the saturation  $t_p$  of the plateau to the dead time of the affinity column (Normalized. Time;  $t_p/t_0$ ).

When the ligand displayed no interactions with the affinity column, the Normalized breakthrough time  $t_p/t_0 \rightarrow 1$  [26].

### Biotinylated-Receptor Immobilization on the Neutravidin Poly(GMA-co-EDMA) Monolithic LC Capillary Columns

For the receptor ACE2, a biotinylated—ACE2 solution with a concentration of 10  $\mu\text{M}$  flushed through our neutravidin column (40 mm length, 75  $\mu\text{m}$  internal diameter) in a 50 mM PBS pH 7.4 at 300  $\text{nL min}^{-1}$  and 280 nm produced a sigmoidal curve with a breakthrough time equal to  $t_p = 6.36$  min due to the strong and rapid noncovalent biotin—neutravidin interaction ( $K_d = 10^{-15}$  M). The column dead time  $t_0$  at the same chromatographic conditions measured by the breakthrough time of DMSO was 0.45 min. The corresponding reduced breakthrough time ( $t_r = t_p - t_0$ ) was thus equal to 5.91 min. These values corresponded to a total mole of bound receptors on the ACE2 column equal to  $m_{\text{ACE2}} = 30 \cdot 0.10^{-9} \cdot 5.91 \cdot 10 \cdot 10^{-6} = 17.7 \times 10^{-12}$  mol = 17.7 pmol. For a set of 6 columns, around 18.00 pmol of RBD were thus immobilized ( $18.00 \pm 0.31$  pmol,  $n = 6$ ) which corresponded to 1.50  $\mu\text{g}$  of ACE2 (MW 85 kDa) for a column length of 40 mm with a 75  $\mu\text{m}$  i.d.

The same procedure was used for the immobilisation of biotinylated-ACE2 onto the neutravidin column with the same length (40 mm) but with an internal diameter of 25  $\mu\text{m}$ . The concentration of the flushed biotinylated-ACE2 solution was 15  $\mu\text{M}$  in the 50 mM PBS pH 7.4 and the flow rate was fixed at 30  $\text{nL min}^{-1}$ . The corresponding reduced breakthrough time was 6.24 min. These values corresponded now to a  $m_{\text{ACE2}}$  value equal to  $30 \cdot 10^{-9} \cdot 6.24 \cdot 15 \cdot 10^{-6} = 2.80 \times 10^{-12}$  mol = 2.80 pmol.

For a set of 6 columns, around 2.80 pmol of ACE2 were thus immobilized ( $2.80 \pm 0.09$  pmol,  $n = 6$ ) which

corresponded to 0.24  $\mu\text{g}$  of ACE2 (MW 85 kDa) for a column length of 40 mm with a 25  $\mu\text{m}$  i.d.

The use of a column with an internal diameter of 25  $\mu\text{m}$  allowed to decrease strongly the quantity of grafted protein on the chromatographic support (86%). Therefore, the Main pro receptor was grafted onto the neutravidin poly(GMA-co-EDMA) monolith inside a capillary column with a 25  $\mu\text{m}$  internal diameter. The concentration of the flushed biotinylated-main pro solution was 10  $\mu\text{M}$  in the 50 mM PBS pH 7.4 and the flow rate was fixed at 30  $\text{nL min}^{-1}$ . The corresponding reduced breakthrough time was 5.33 min. These values corresponded now to a  $m_{\text{Main pro}}$  value equal to  $30 \cdot 10^{-9} \cdot 5.33 \cdot 10 \cdot 10^{-6} = 1.60 \times 10^{-12}$  mol = 1.60 pmol. The quantity of immobilized Main pro was ( $1.60 \pm 0.05$  pmol,  $n = 6$ ).

## Results and Discussion

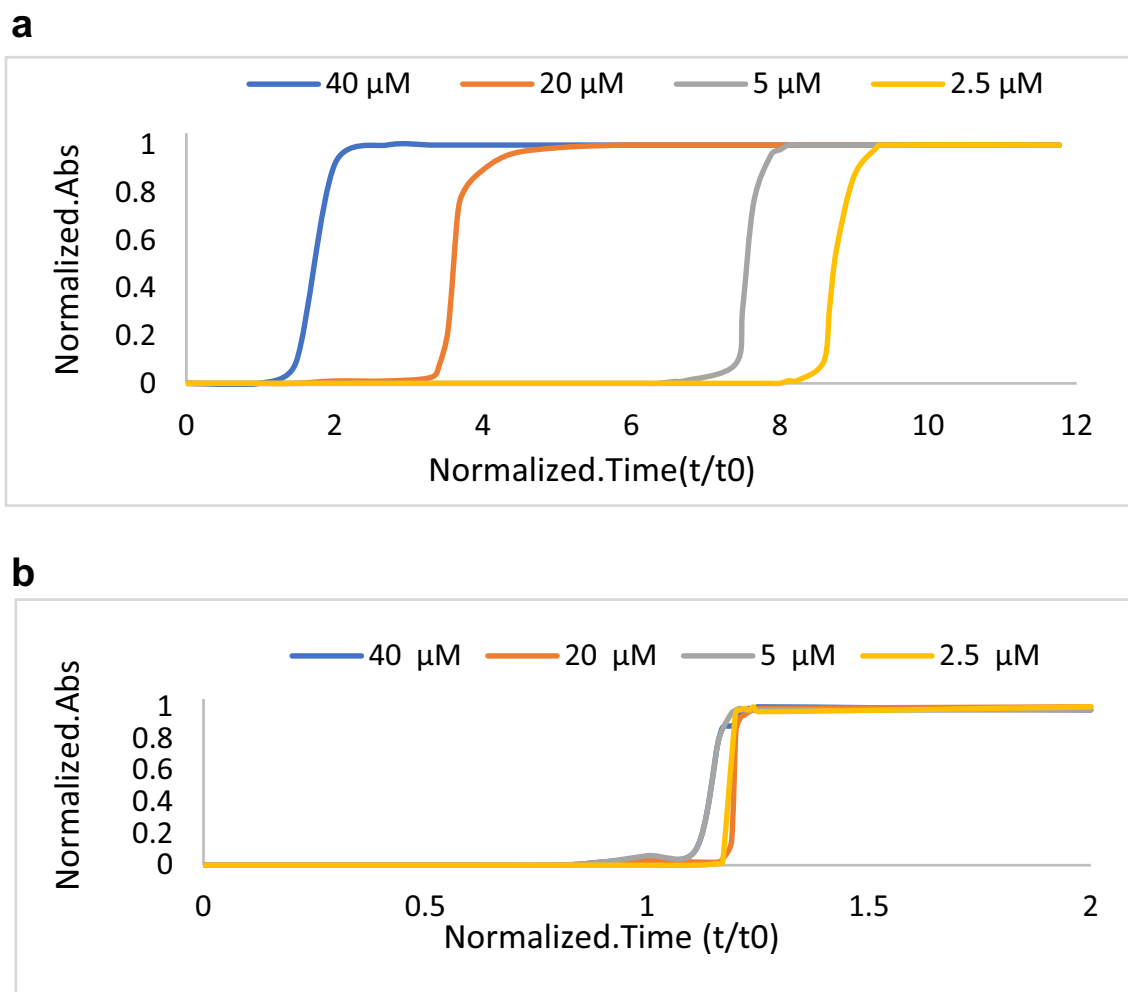
### Research of Ligand Targeting ACE2

Typical breakthrough curves were obtained for a series of potential ligands of ACE2 (including dexamethasone) by the use of the two capillary columns with 25  $\mu\text{m}$  and 75  $\mu\text{m}$  internal diameter.

A solution of the ligand at a given concentration (in the range 2–50  $\mu\text{M}$ ) was flushed in a 50 mM PBS pH 7.4 through the miniaturized column. The column temperature was 25  $^{\circ}\text{C}$  and the detection wavelength was 254 nm for all the studied ligands (except for dexamethasone  $\lambda = 238$  nm). The column length was 40 mm. The flow rate was equal to 200  $\text{nL min}^{-1}$  and 30  $\text{nL min}^{-1}$  for a column diameter, respectively, equal to 75  $\mu\text{m}$  and 25  $\mu\text{m}$ .

An example is given in Fig. 1A. The curves  $1/Q$  versus  $1/[L]$  were drawn for each ligand and were linear ( $r^2 > 0.997$ ). For example, the linear equation obtained for quercetin with the column of 75  $\mu\text{m}$  i.d. was  $1/Q = 2.876 \cdot 10^5 / [\text{quercetin}] + 5.639 \cdot 10^{10}$  ( $r^2 = 0.9981$ ). From their slopes and intercepts, the corresponding values of  $m_L$  and  $K_d$  for each ligand were calculated (Table. 1). For each ligand, the  $K_d$  values obtained on the two columns were similar and chloroquine and dexamethasone described similar  $K_d$  values as those obtained by cell chromatography ( $K_d = 1.30 \mu\text{M}$ ) for chloroquine [27] or by surface plasmon resonance ( $K_d = 9.0 \mu\text{M}$ ) [28] for dexamethasone. These results confirmed the excellent efficiency of our immobilization protocol of the ACE2 receptor onto the organic monolith.

As well, the normalized breakthrough times obtained on our 75  $\mu\text{m}$  and 25  $\mu\text{m}$  neutravidin poly(GMA-co-EDMA) monolithic LC capillary columns (i.e., with no immobilized ACE2) was the same upon ligand concentration with a  $t_{\text{plateau}}/t_0 \rightarrow 1$  (1.20) (Fig. 1B) showing that non-specific



**Fig. 1** Normalized breakthrough curve of quercetin solution at four concentration on the ACE2 affinity column (A) and the corresponding Normalized breakthrough curve on the control column (with no ACE2 immobilized) (B). The column diameter and length were

75  $\mu\text{m}$  and 40 mm, respectively. Mobile phase: 50 mM PBS pH 7.4. Flow-rate: 200 nL/min. Column temperature: 25  $^{\circ}\text{C}$ . Detection wavelength: 254 nm

interaction during the binding process remained negligible [29].

For fragment-based drug discovery (FBDD), competition experiments are very useful to confirm the receptor binding site of a fragment. So as to demonstrate that our homemade miniaturized columns can be used for a such purpose, normalized breakthrough curves were compared in the absence and presence of the competition agent in the percolated solution inside the miniaturized capillary column (25  $\mu\text{m}$ , id 40 mm length).

This was highlighted for dexamethasone in the presence of the competitive agent SARS-CoV-2 RBD which bind to ACE2 with an equilibrium dissociation constant in the nanomolar range [30]. A solution of dexamethasone (5  $\mu\text{M}$ ) was flushed in a 50 mM PBS pH 7.4 through successively (1) the neutravidin column (i.e., control column with no ACE2), (2) the ACE2 column in the presence of

5  $\mu\text{M}$  of the SARS-CoV-2 RBD competition agent and (3) the ACE2 column without competition agent. The flow-rate was 30 nL  $\text{min}^{-1}$ , the column temperature was 25  $^{\circ}\text{C}$  and the detection wavelength was 238 nm. The corresponding normalized breakthrough curves are given in Fig. 2.

The normalized breakthrough time of Dex decreased strongly when SARS-CoV-2 RBD was added in the percolated solution and close to the value obtained on the Neutravidin column (control column with no ACE2) ( $t_p/t_0 \rightarrow 1$ ). This result confirmed that dexamethasone bound on the orthosteric site of ACE2 with specific interaction.

### Research of Ligand Targeting the Main Pro of SARS-CoV-2

The inhibition of viral proteases can reduce the assembly of mature viral particles [13] and can thus reduce the COVID19

**Table 1** Total moles of bound receptors on the ACE2 affinity column ( $m_L$ ) and the  $K_d$  ( $\mu\text{M}$ ) values for five ligands with a mobile phase 50 mM PBS pH 7.4 and a column temperature of 25 °C

Quercetin	75 ( $\mu\text{M}$ )	25 ( $\mu\text{M}$ )
$m_L$ (pmol)	17.73	2.65
$K_d$ ( $\mu\text{M}$ )	5.12	5.67
Dexamethasone	75 ( $\mu\text{M}$ )	25 ( $\mu\text{M}$ )
$m_L$ (pmol)	16.78	2.50
$K_d$ ( $\mu\text{M}$ )	10.6	12.0
Chloroquine	75 ( $\mu\text{M}$ )	25 ( $\mu\text{M}$ )
$m_L$ (pmol)	17.89	2.56
$K_d$ ( $\mu\text{M}$ )	1.32	1.43
Hydroxychloroquine	75 ( $\mu\text{M}$ )	25 ( $\mu\text{M}$ )
$m_L$ (pmol)	16.60	2.43
$K_d$ ( $\mu\text{M}$ )	1.76	1.92
Isoharmetin	75 ( $\mu\text{M}$ )	25 ( $\mu\text{M}$ )
$m_L$ (pmol)	17.54	2.58
$K_d$ ( $\mu\text{M}$ )	3.10	3.56

The column length was 40 mm. The mobile phase flow-rate was 200 nL/min and 30 nL/min for an i.d. column, respectively, equal to 75  $\mu\text{m}$  and 25  $\mu\text{m}$ . Detection wavelength: 254 nm (except for dexamethasone  $\lambda = 238$  nm)

infection. Works remained to be done on developing rapid analytical method in searching for drugs inhibiting the Main protease (Main pro) of SARS-CoV-2. For this purpose, normalized breakthrough times were compared for two extreme drug concentrations. Indeed, if a molecule was a ligand of Main pro, at a very high concentration of this molecule, the total binding sites onto the Main pro capillary column are thus occupied and the breakthrough time of this molecule measured on the Main pro immobilized column was obviously close to the one measured on the Neutravidin control column (with no immobilized Main pro). This made it

possible to avoid changing the column during experiments (time saving) and potential uncertainties of the measures on the control column. This strategy was used for the first time for the study of the binding of 5 potential ligands to Main pro.

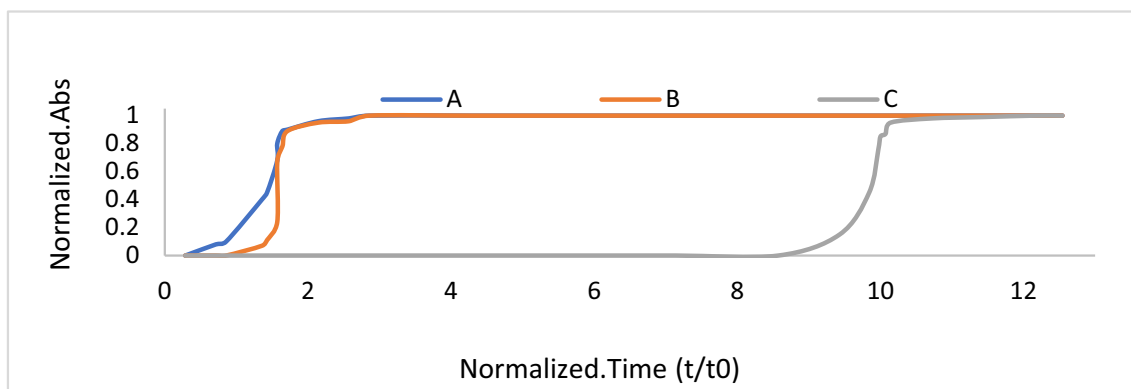
A solution of the drug at a low concentration (10  $\mu\text{M}$ ) and a high concentration (1400  $\mu\text{M}$ ) was thus successively flushed through our Main pro column (40 mm length, 25  $\mu\text{m}$  internal diameter) in a 50 mM PBS pH 7.4 at 30  $\text{nL min}^{-1}$ , at a column temperature equal to 25 °C with a detection wavelength equal to 254 nm.

Phenylalanine described identical breakthrough times at a low (5  $\mu\text{M}$ ) and a very high concentration (1400  $\mu\text{M}$ ) and were thus non-ligands for Main pro (Fig. 3). On the other hand, significant differences between breakthrough times were observed at these two extreme concentrations for four molecules (Fig. 3). When the normalized breakthrough time increased the affinity of the ligand to the receptor increased. These compounds have thus an affinity for Main pro in the sequence chlorogenic acid < piceatannol < kaempferol < rutin (Fig. 3). This result was in good agreement with a previous result demonstrating that rutin was a ligand of Main Pro [31].

### Role of Carbon Nanotube on the Dexamethasone/ Receptor Binding

Carbon nanotubes have been recognized as promising nano-carriers for an extended range of molecules [22]. For the moment, the corticosteroid dexamethasone is a widely used molecule for adult patients hospitalized with COVID-19 [32] due to its binding to both ACE2 and Main pro receptors of SARS CoV-2.

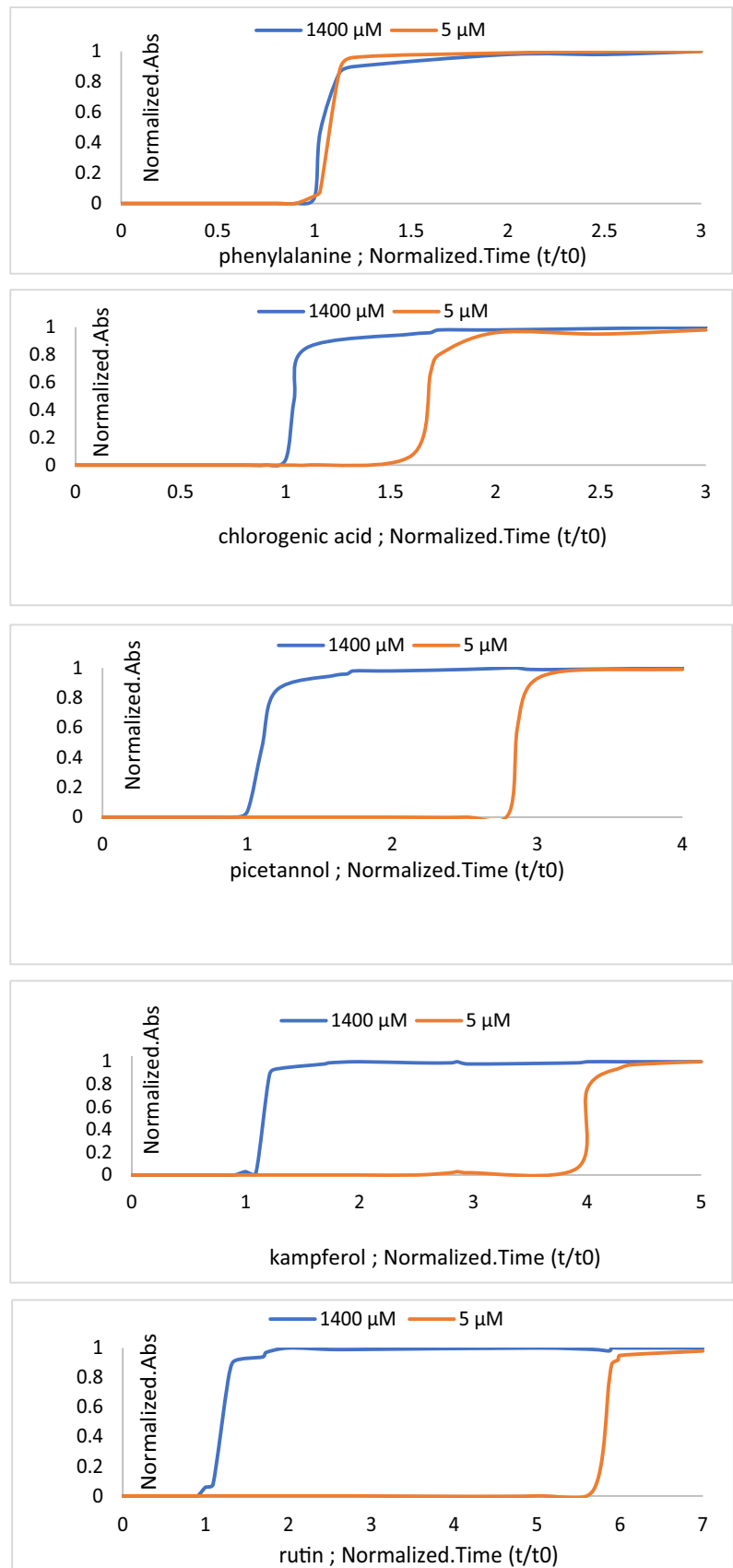
A solution of dexamethasone (5  $\mu\text{M}$ ) and a solution of Dex/MWCNT (5  $\mu\text{M}$ ) were successively flushed at a flow

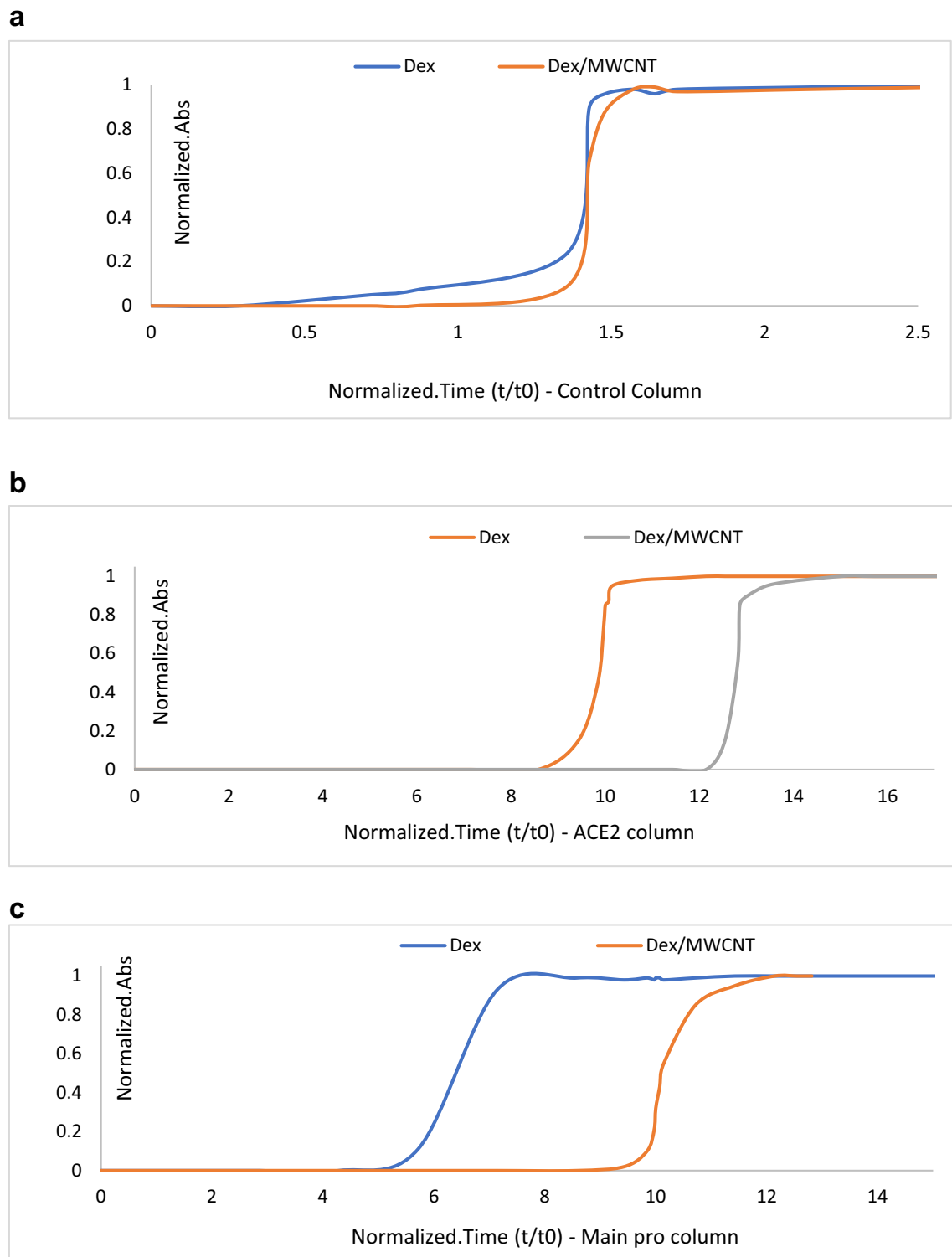


**Fig. 2** Normalized breakthrough curves of a dexamethasone solution (5  $\mu\text{M}$ ) on the: **A** Neutravidin column (i.e., control column with no ACE2). **B** ACE2 affinity column with the SARS-CoV-2 RBD competition agent of high affinity. **C** ACE2 affinity column without compe-

tion agent. The column diameter and length were 25  $\mu\text{m}$  and 40 mm, respectively. Mobile phase: 50 mM PBS pH 7.4. Flow-rate: 30 nL/min. Column temperature: 25 °C. Detection wavelength: 238 nm

**Fig. 3** Normalized breakthrough curve of 5 compounds at a low and a high-level concentration in PBS on the homemade Main pro column. The column diameter and length were 25  $\mu\text{m}$  and 40 mm, respectively. Mobile phase: 50 mM PBS pH 7.4. Flow rate: 30 nL/min. Column temperature: 25  $^{\circ}\text{C}$ . Detection wavelength: 254 nm





**Fig. 4** Normalized breakthrough curves of Dex and Dex/MWCNT solutions (5  $\mu$ M) on the **A** Neutravidin column (control column), **B** ACE2 affinity column and **C** main Pro affinity column. The column

diameter and length were 25  $\mu$ m and 40 mm, respectively. Mobile phase: 50 mM PBS pH 7.4. Flow-rate: 30 nL/min. Column temperature: 25  $^{\circ}$ C. Detection wavelength: 238 nm

rate of  $30 \text{ nL min}^{-1}$  and  $25 \text{ }^\circ\text{C}$  in a  $50 \text{ mM PBS pH } 7.4$  through (1) the Neutravidin column (i.e., control column with no ACE2), (2) the ACE2 column, and (3) the Main Pro column. The column diameter and length were, respectively, equal to  $25 \text{ }\mu\text{m}$  and  $40 \text{ mm}$  and the detection wavelength was  $238 \text{ nm}$ . The corresponding normalized breakthrough curves are given in Fig. 4.

Dex and Dex/MWCNT described identical breakthrough times on the neutravidin poly(GMA-co-EDMA) capillary column with ( $t/t_0 \rightarrow 1$ ) (Fig. 4A) showing very weak non-specific interactions with the chromatographic support on absence of ACE2 and Main pro.

In opposition, the normalized breakthrough curves for Dex and MWCNT/Dex obtained on both the ACE2 column (Fig. 4B) and on the Main pro column (Fig. 4C) described normalized breakthrough times values well above 1 and always the highest for the nanovector.

The shape of the breakthrough curve was less steep on the Main pro column than on the ACE2 column. This can be explained by the fact that linear chromatographic conditions were favored on the Main Pro column [33]. The equivalent in zonal chromatography would be a more symmetrical peak obtained on the Main Pro column. In addition, these results suggested that the  $K_d$  dissociation constant values for Dex and its nanovector for Main pro became appreciable relative to the concentration of the ligand at the column inlet (i.e.,  $K_d > > 5 \text{ }\mu\text{M}$ ) [33].

These results demonstrated that the dexamethasone ligand and its nanovector were binders for both ACE2 and Main Pro with specific interactions. As well, it was showed that the nanovectorisation of dexamethasone with MWCNT enhanced and stabilized its recognition mechanism with ACE2 and Main pro and may reinforced its efficiency in clinical trial against COVID19.

## Conclusions

Here, we have showed for the first time, that our homemade neutravidin poly(GMA-co-EDMA) capillary columns, can be used for distinguish ligand or non-ligands of the ACE2 and the Main pro of the Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2). Competition experiments were carried out to confirm the binding of dexamethasone to the orthosteric site of ACE2 by the use of SARS-CoV-2 RBD reference molecule. As well, and for the first time, by comparison of the normalized breakthrough curves of dexamethasone and its nanovector obtained on the ACE2 and Main pro affinity columns, it was demonstrated that the nanovector stabilized and reinforced the dexamethasone binding to ACE2 and Main pro. This nanovector could be thus a useful tool to increase the therapeutic efficiency of dexamethasone against COVID19.

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## Declarations

**Conflict of interest** Yves Claude Guillaume has no conflict of interest regarding the publication of this article. Claire André has no conflict of interest regarding the publication of this article.

**Ethical approval** This article does not contain any studies with human participants or animals performed by any of the authors.

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