SELECTION, SYNTHESIS AND INTERACTION OF hACE2 PEPTIDE FRAGMENTS TOWARDS SPIKE PROTEIN OF THE SARS-COV-2 VIRUS

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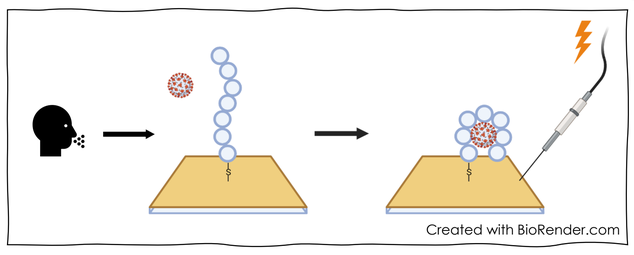
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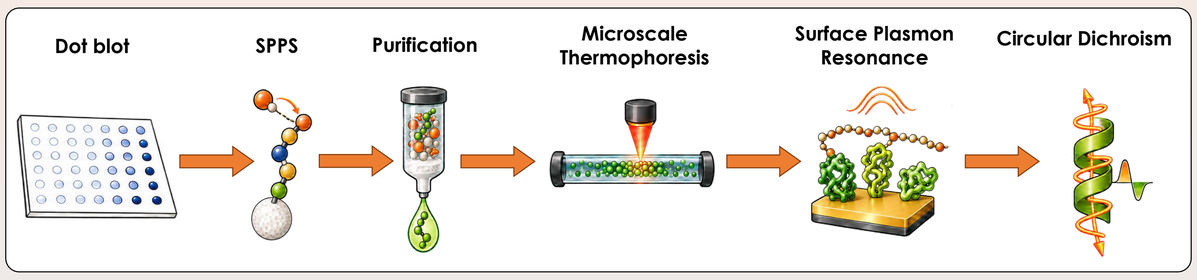
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AIM OF THE STUDY

To identify hACE2 peptide fragments capable of recognising the SARS-CoV-2 spike protein and to evaluate their potential as biorecognition elements for detecting virus in exhaled air and indor environmental samples

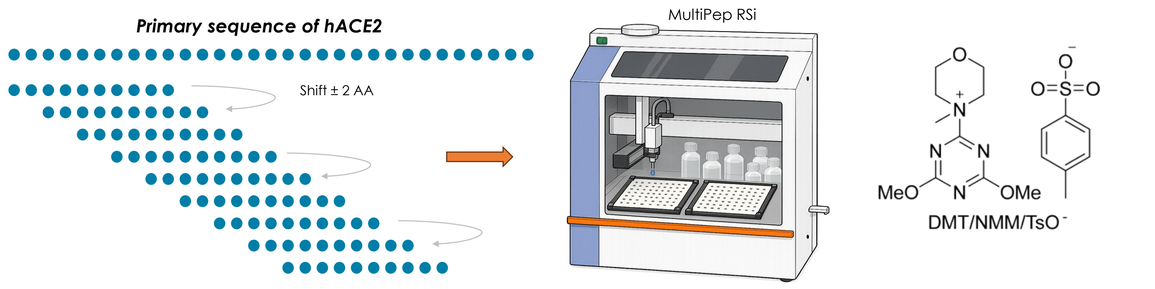


METHODS



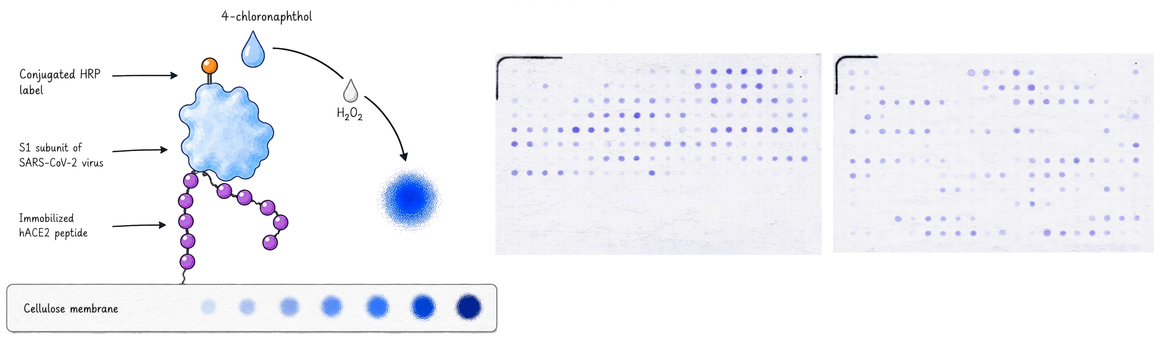
RESULTS

SPOT SYNTHESIS



Frączyk J., Walczak M., Kamiński Z.J. J. Pept. Sci. 2018, 24, e3063.

SELECTION

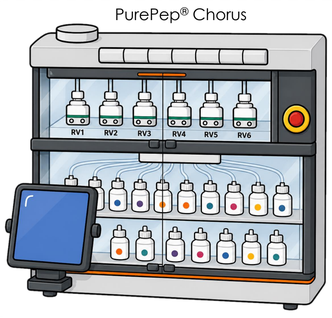


Fragments strongly interacting with the S1 subunit of the SARS-CoV-2 virus

No.	Locant	AA	Peptide fragment
1.	505-526	22	H-HVSNDYSFIRYYTRTLYQFQFQ-OH
2.	667-692	26	H-EEDVRVANLKPRISFNFFVTAPKNVS-OH
3.	545-562	18	H-SNSTEAGQKLFNMLRLGK-OH
4.	641-666	26	H-YLFRSSVAYAMRQYFLKVKNQMILFG-OH
5.	611-632	22	H-SPYADQSIKVRISLKSALGDKA-OH
6.	587-606	20	H-YFEPLFTWLKDQNKNSFVGW-OH
7.	755-776	22	H-VGIVILIFTGIRDRKKKNKARS-OH
8.	699-718	20	H-EVEKAIRMSRSRINDAFRLN-OH
9.	265-282	18	H-HLLGDMWGRFWTNLYSLT-OH
10.	103-122	20	H-NGSSVLSEDKSKRLNTILNT-OH

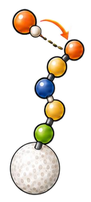
Kolesińska B. et al. *Engineering of Biomaterials* **2018**,*148*, 41.

INDUCTION-HEATED SPPS



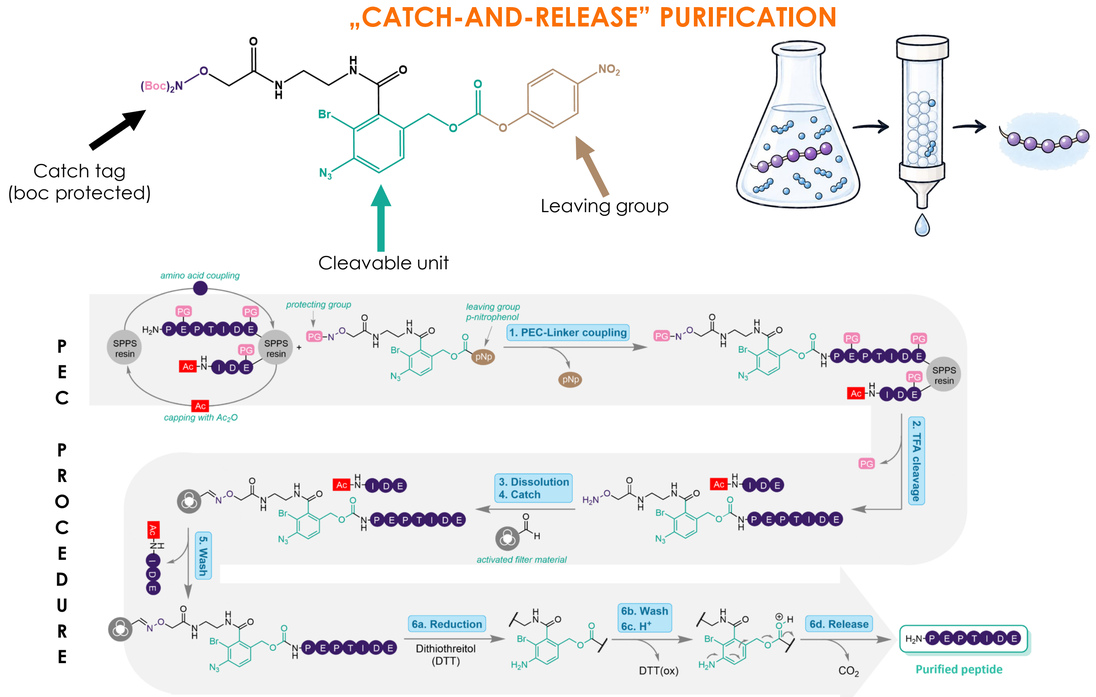
IH-SPPS conditions

- Fmoc/tBu strategy
- 50-200 µmol scale
- Preloaded Wang or TCP resin
- HCTU/DIPEA or DIC/OxymaPure coupling reagents
- RT, 60 or 90 °C



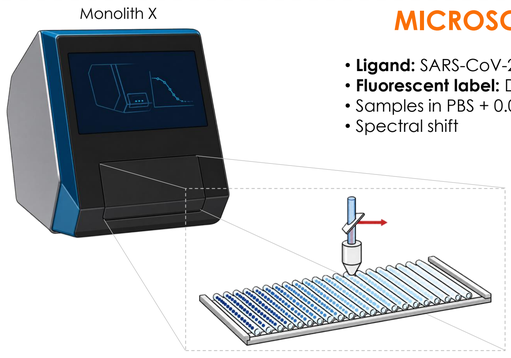
The synthesis conditions were adjusted individually according to peptide length, hydrophobicity and aggregation tendency

„CATCH-AND-RELEASE" PURIFICATION



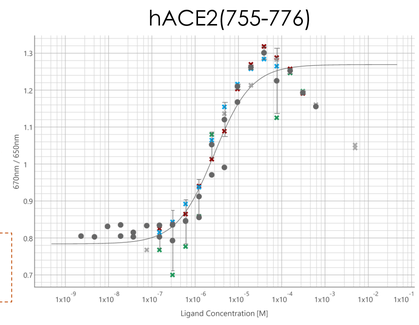
Zitterbart R., Berger N., Reimann O., Noble G.T., Lüdtke S., Sarma D., Seitz O. Chem. Sci. **2021**, 12, 2389.

MICROSCALE THERMOPHORESIS

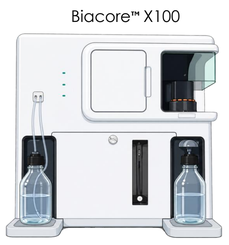


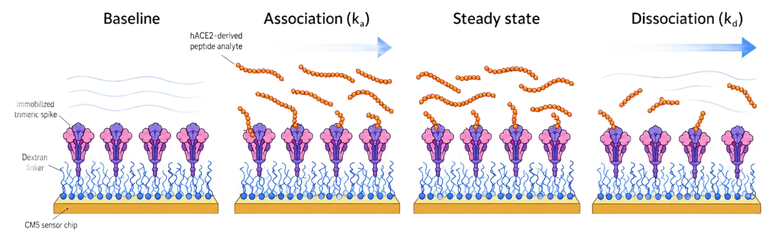
- **Ligand:** SARS-CoV-2 hFc with His tag
- **Fluorescent label:** Dye 2nd Gen (MST)
- Samples in PBS + 0.05% Tween-20
- Spectral shift

$K_D \approx 2.74$ µM (confidence interval 1.86-4.02 µM)



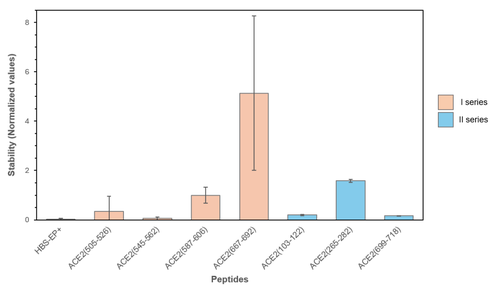
SURFACE PLASMON RESONANCE





- **Ligand:** trimeric SARS-CoV-2 spike protein
- **Analyte:** selected hACE2 peptides
- **Sensor:** CM5
- **Buffer**: HBS-EP+

Ligand was immobilised on a sensor chip by amine coupling. Selected hACE2 peptides were injected as analytes and their binding responses were monitored in real time

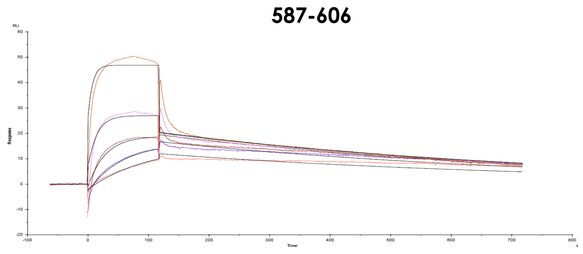


Key observations

- hACE2(587–606) produced a reproducible binding response.
- hACE2(265–282) also showed reproducible interaction with the immobilised spike protein.
- Responses obtained for hACE2(641–666) and hACE2(667–692) were not fully reproducible.
- hACE2(755–776) produced a complex response that could not be reliably interpreted using the applied SPR conditions.

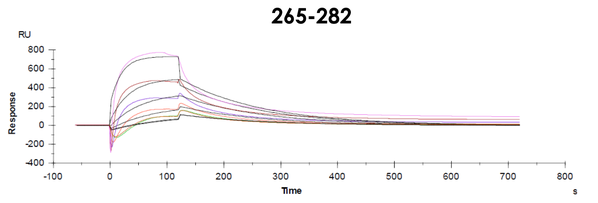
Kinetic studies

587-606



Model	k_{a1} [M^{-1} s^{-1}]	k_{d1} [s^{-1}]	Apparent K_D [µM]	R_{max} [RU]	Chi^2 [RU^2]	U-value
1:1	2669	0.001506	0.564	20.77	2.77	5

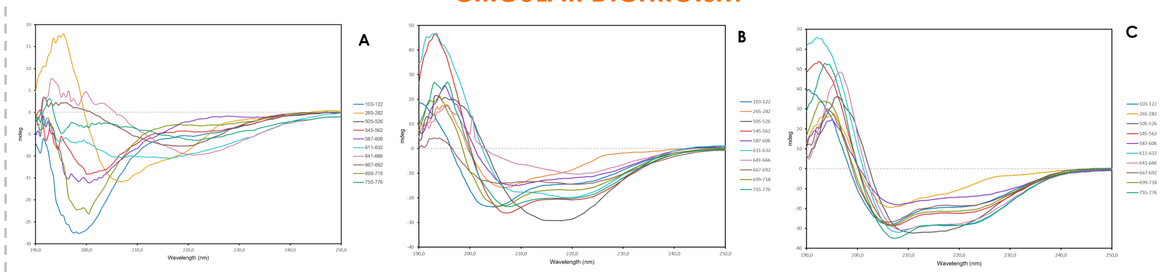
265-282



Model	k_{a1} [M^{-1} s^{-1}]	k_{d1} [s^{-1}]	Apparent K_D [µM]	R_{max} [RU]	Chi^2 [RU^2]	U-value
1:1	454.4	0.006918	15.2	584.3	1520	5

M. Quagliata et al. *ACS Omega* **2023**, *8*, 25, 22665-22672.

CIRCULAR DICHROISM



A – phosphate buffer (PB); **B** – 25% TFE in PB; C – 50% TFE in PB

TFE promoted a-helical structure formation in the selected hACE2 fragments, revealing their intrinsic conformational propensity. **The highest a-helical content was observed for hACE2(755–776).**

TAKE-HOME MESSAGE

- Dot-blot screening enabled the selection of ten hACE2-derived sequences for further investigation.
- The selected peptides were successfully produced using individually optimised SPPS and purification strategies.
- MST and SPR identified hACE2 fragments capable of interacting with SARS-CoV-2 spike constructs, although the observed behaviour depended on the applied method.
- CD analysis revealed pronounced environment-dependent conformational changes, with hACE2(755–776) showing the highest a-helical propensity
- **Combining complementary biophysical methods is essential for the reliable evaluation of peptide–spike interactions.**

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Peptide synthesis and purification, surface plasmon resonance measurements, and circular dichroism spectroscopy were carried out in collaboration with PeptLab, University of Florence, Italy

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LET'S CONNECT!



ALEKSANDRA CZERCHAWY, MSc.
PhD candidate in Chemical Sciences
Peptide Chemistry • Interaction studies

LET'S TALK ABOUT

- Peptide-based biorecognition
- Spike-peptide interactions
- Detection of viral markers

Open to scientific collaborations in peptide chemistry, biochemistry and related research areas

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