CPPC 2021

First Canadian Peptide and Protein Community Virtual Symposium

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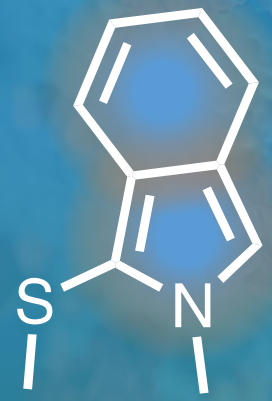
Canadian Peptide Protein Community

biomedicines

MDPI



FlICked ACE2 mimics inhibit SARS-CoV-2 spike protein-ACE2 interaction

Mihajlo Todorovic[1], Antonio A. W. L. Wong[1], Naysilla L. Dayanara, Taylor Navi, David M. Perrin[2]

[1] These presenters contributed equally

[2] Corresponding authors contact: dperrin@chem.ubc.ca | 2036 Main Mall, Vancouver, BC CANADA V6T 1Z1

Coronavirus Disease 2019

COVID-19

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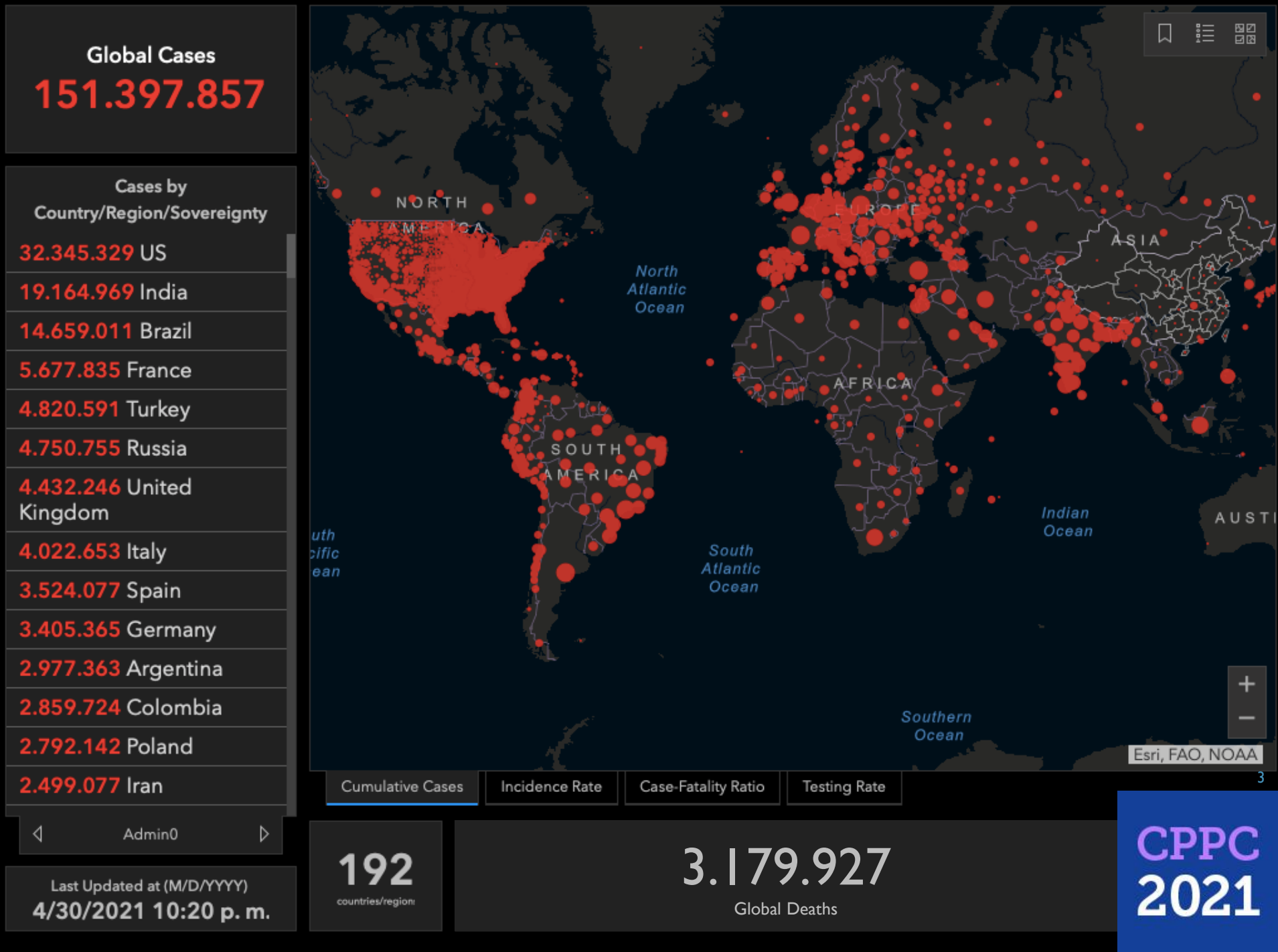
Global Cases
151.397.857
Cases by Country/Region/Sovereignty
32.345.329 US
19.164.969 India
14.659.011 Brazil
5.677.835 France
4.820.591 Turkey
4.750.755 Russia
4.432.246 United Kingdom
4.022.653 Italy
3.524.077 Spain
3.405.365 Germany
2.977.363 Argentina
2.859.724 Colombia
2.792.142 Poland
2.499.077 Iran
Admin0
Last Updated at (M/D/YYYY)
4/30/2021 10:20 p. m.
NORTH AMERICA
EUROPE
ASIA
AFRICA
SOUTH AMERICA
North Atlantic Ocean
South Atlantic Ocean
Indian Ocean
Southern Ocean
Esri, FAO, NOAA
Cumulative Cases
Incidence Rate
Case-Fatality Ratio
Testing Rate
192
countries/region
3.179.927
Global Deaths
CPPC
2021


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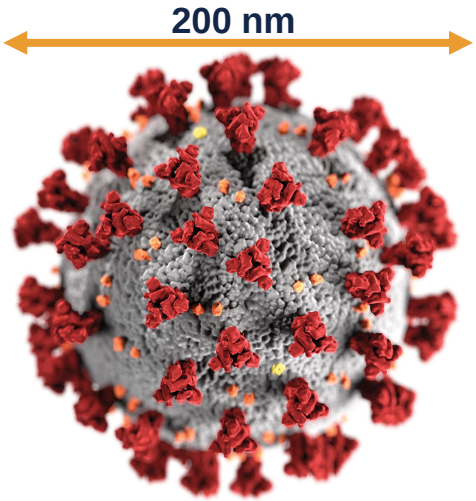

Causative agent of COVID-19

SARS-CoV-2

Severe Acute Respiratory Syndrome Coronavirus 2

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On the surface…

Spike protein homotrimer

Allow penetration and subsequent entry

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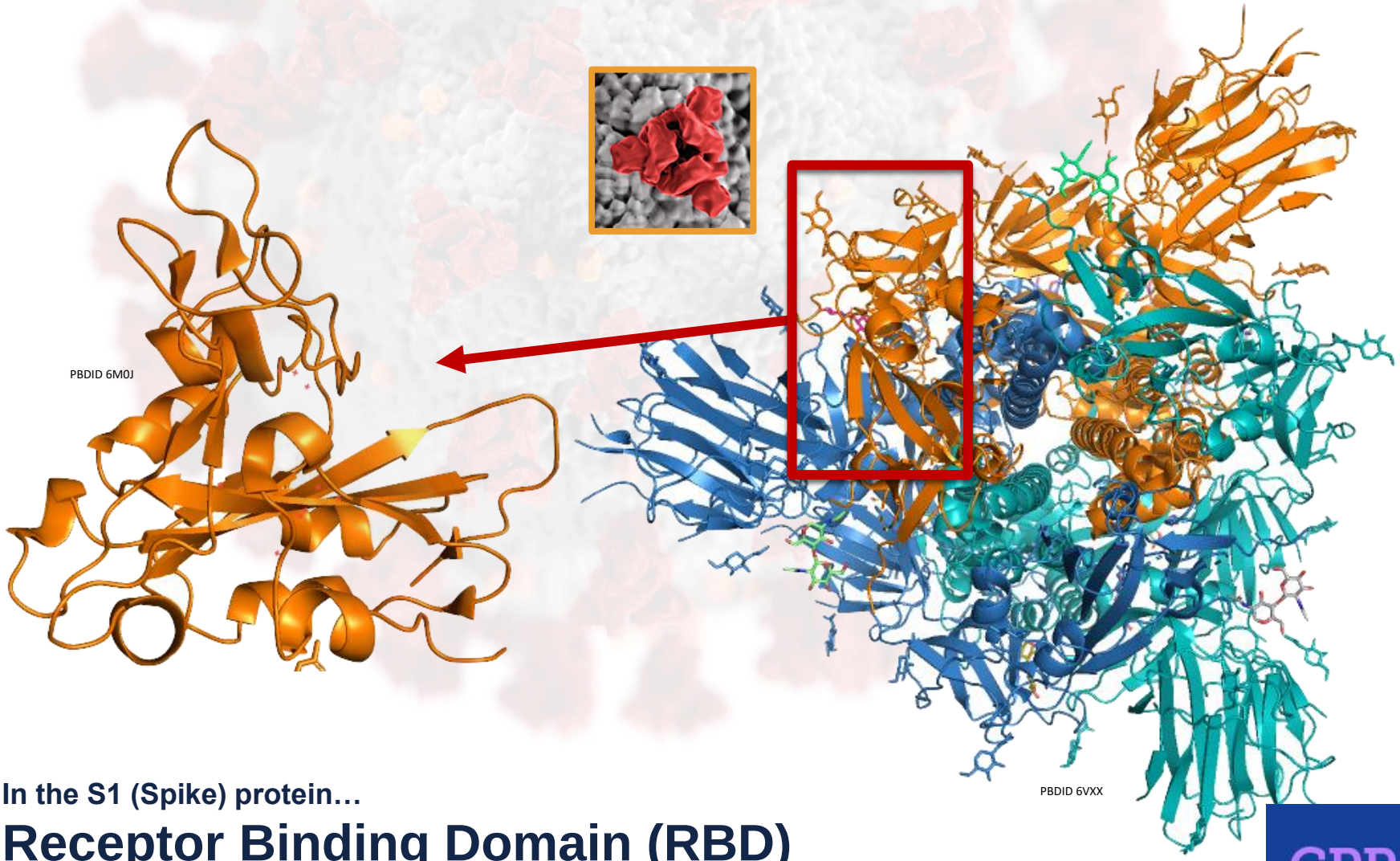

In the S1 (Spike) protein…

Receptor Binding Domain (RBD)

Residue number (resn) 319-541

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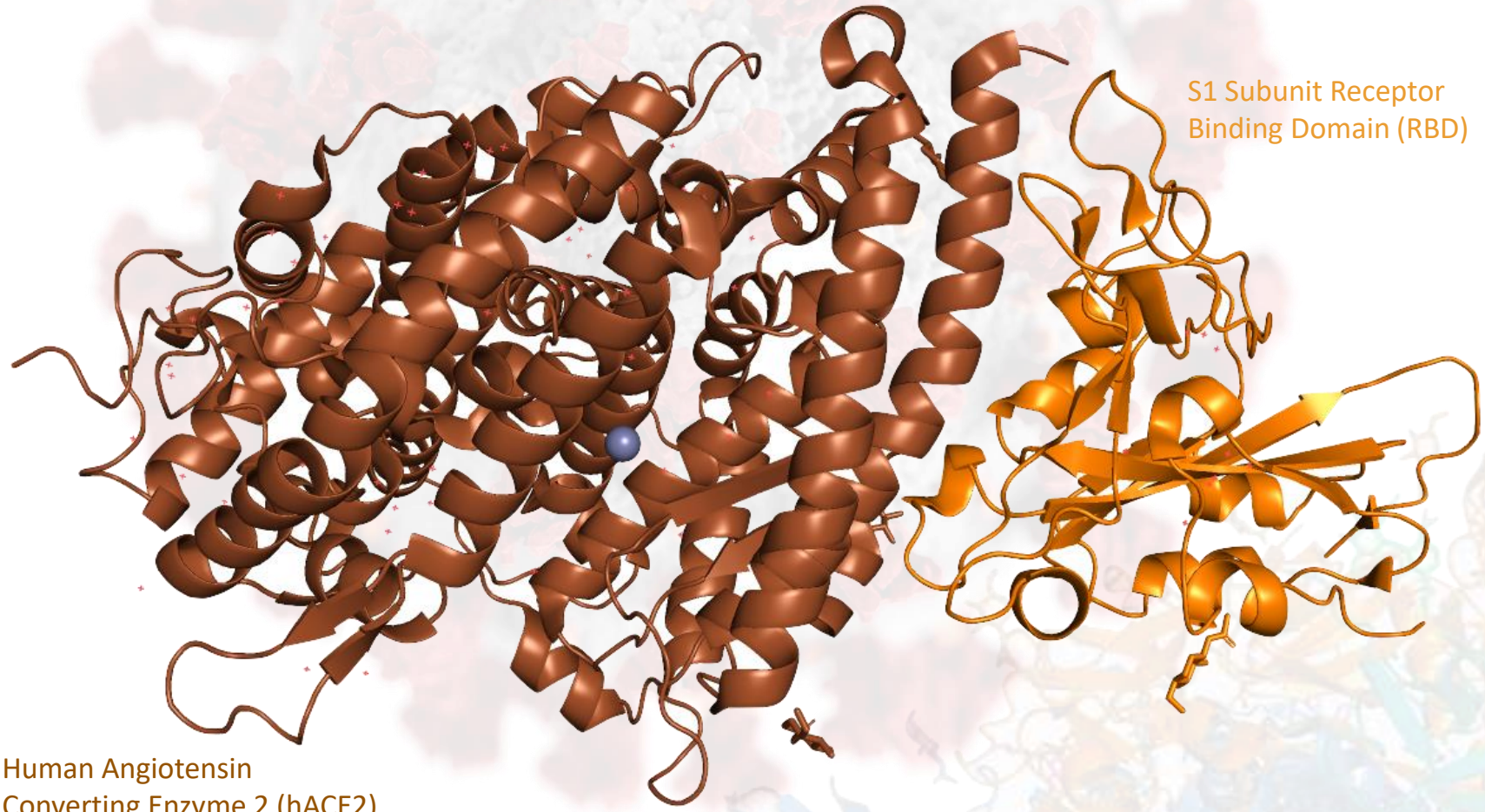

In the S1 (Spike) protein…

Substrate bound X-ray structure

PDBID 6M0J

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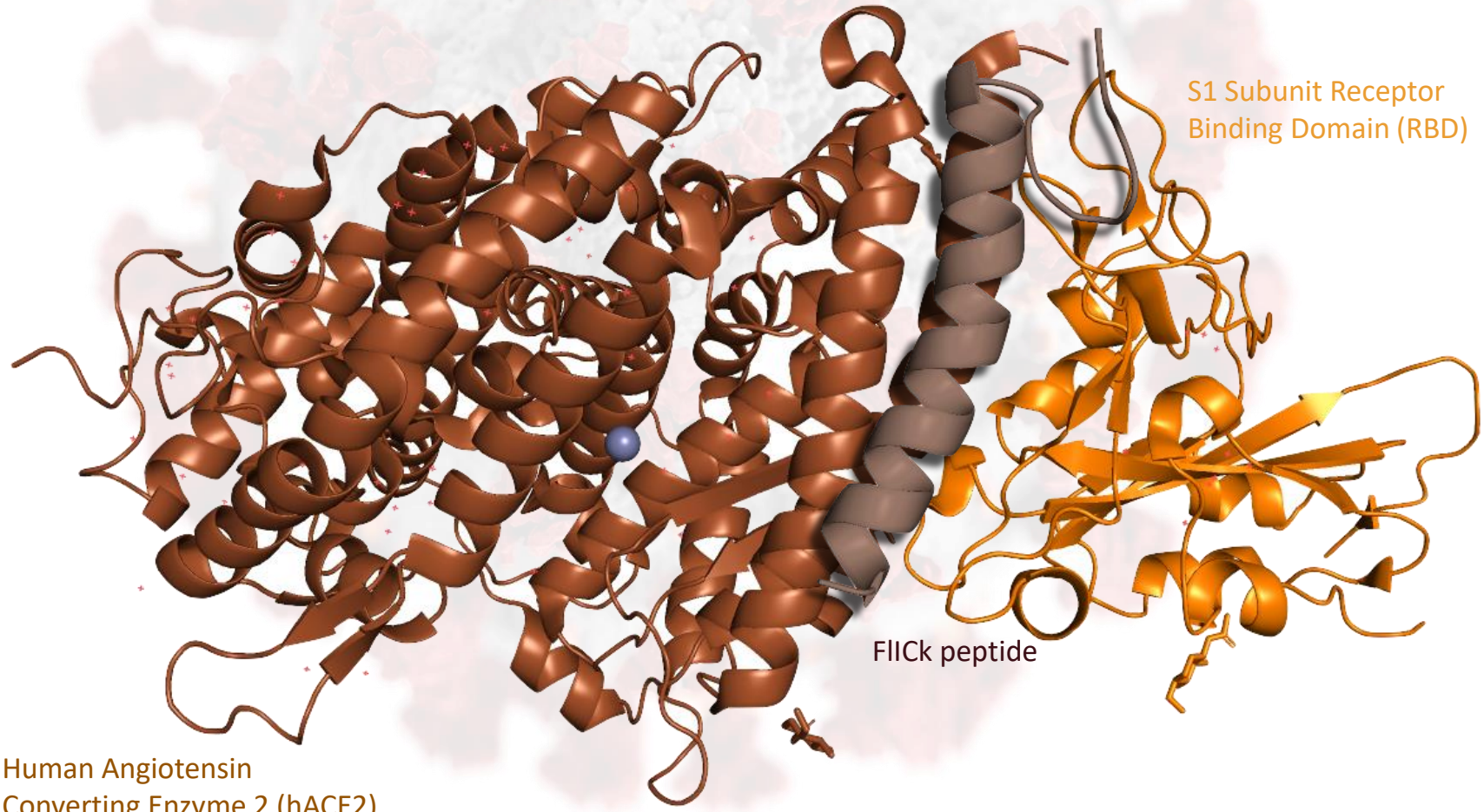

In the S1 (Spike) protein…

Substrate bound X-ray structure

PDBID 6M0J

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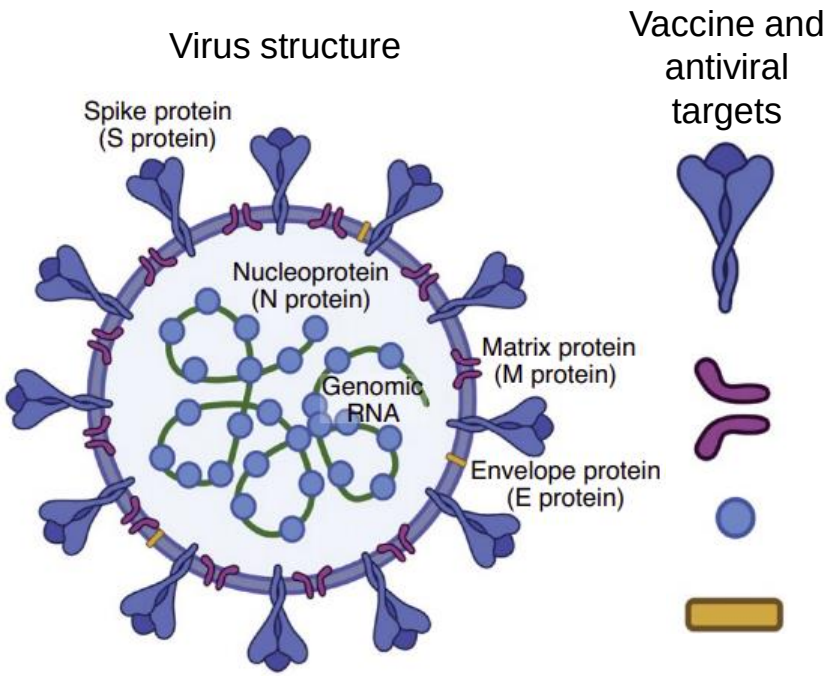
(Peptide Introduction): Challenges associated with mass vaccination

- Production and distribution of vaccines
- Variable vaccine acceptance
- Imperfect vaccine efficacy
- Development of vaccination-resistant strains



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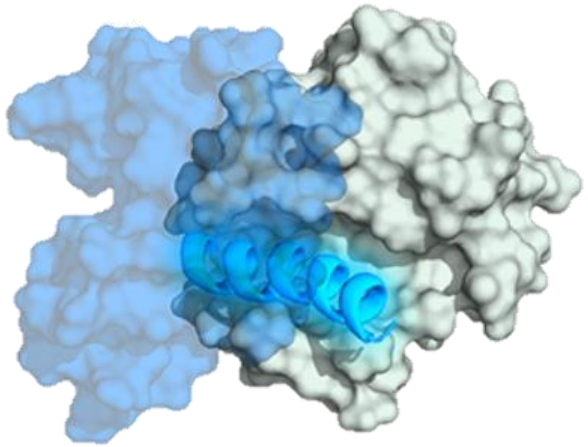
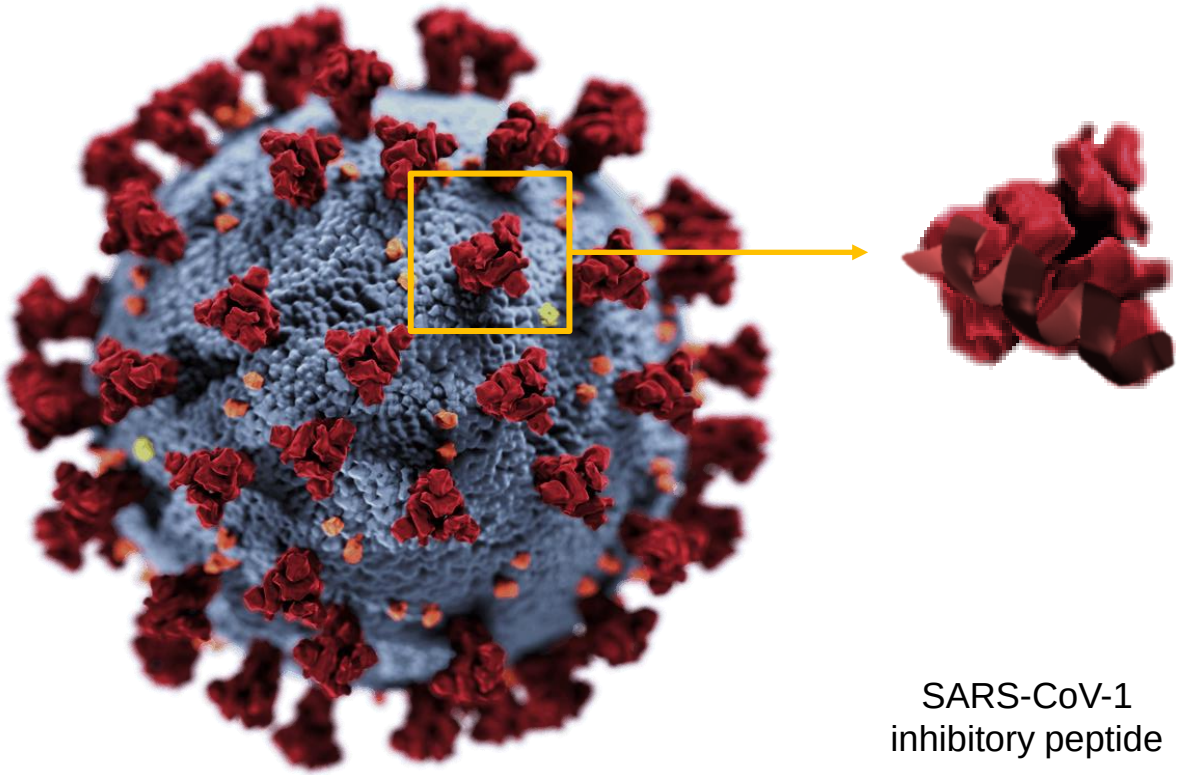
(Peptide Introduction): Peptides as drugs

- Better at targeting protein-protein interactions
- Effective for extracellular targets
- Less prone to resistance
- Stapling increases protease resistance



(Peptide Introduction): SARS-CoV-1 inhibitory peptides



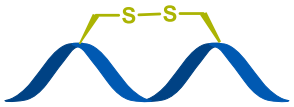
SARS-CoV-1
inhibitory peptide

Ho, T. Y.; Wu, S. L.; Chen, J. C.; Wei, Y. C.; Cheng, S. E.; Chang, Y. H.; Liu, H. J.; Hsiang, C. Y. *Antiviral Res.* **2006**, *69* (2), 70–76. https://doi.org/10.1016/j.antiviral.2005.10.005.

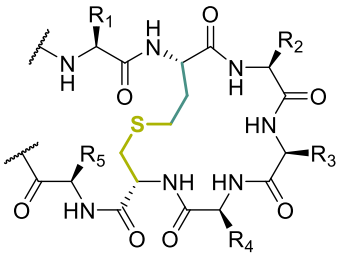
(Peptide Introduction): Peptide Stapling

Natural Peptide Crosslinks

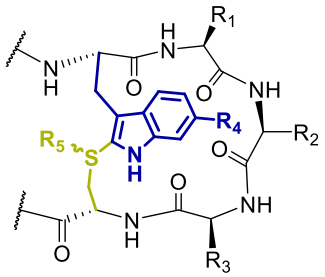
Disulfide Linkage



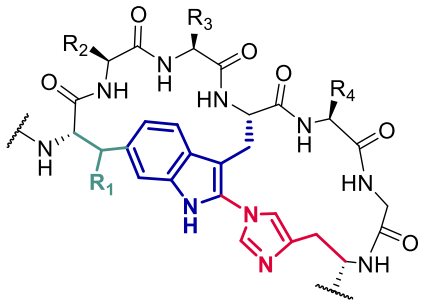
Thioether Bridge



Tryptathionine Bridge



Tryptophan-Histidine Crosslink

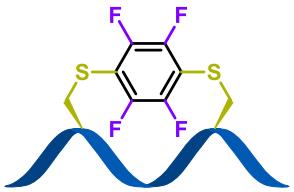


Synthetic Peptide Crosslinks

Olefin Metathesis

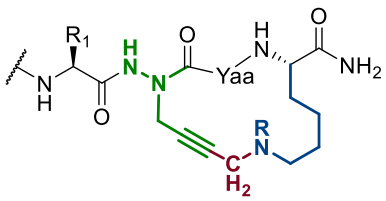
Blackwell, Helen E., & Grubbs, Robert H. (1998). *Angewandte Chemie International Edition*, *37*(23), 3281–3284.

Perfluoroaryl Stapling



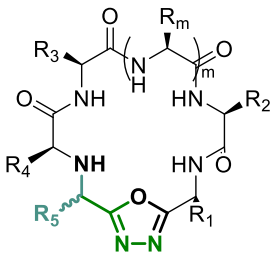
Spokoyny, A. M., Zou, Y., Ling, J. J., Yu, H.,
Lin, Y. S., & Pentelute, B. L. (2013). *Journal of the American Chemical Society*, *135*(16), 5946–5949.

A^3 Cyclization



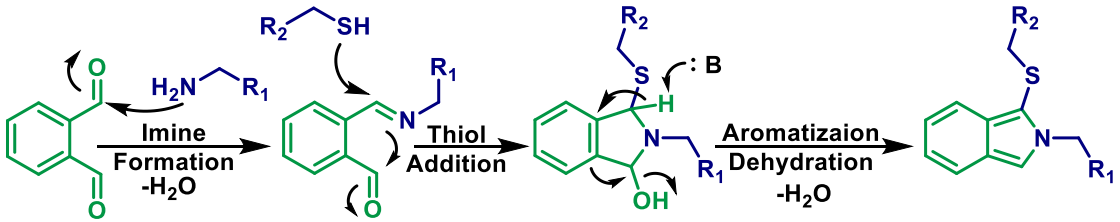
Zhang, J., Mulumba, M., Ong, H., & Lubell, W. D. (2017). *Angewandte Chemie – International Edition*, *56*(22), 6284–6288.

Oxadiazole Grafting



Frost, J. R., Scully, C. C. G., & Yudin, A. K.
(2016). *Nature Chemistry*, *8*(12), 1105 1111.
https://doi.org/10.1038/nchem.2636

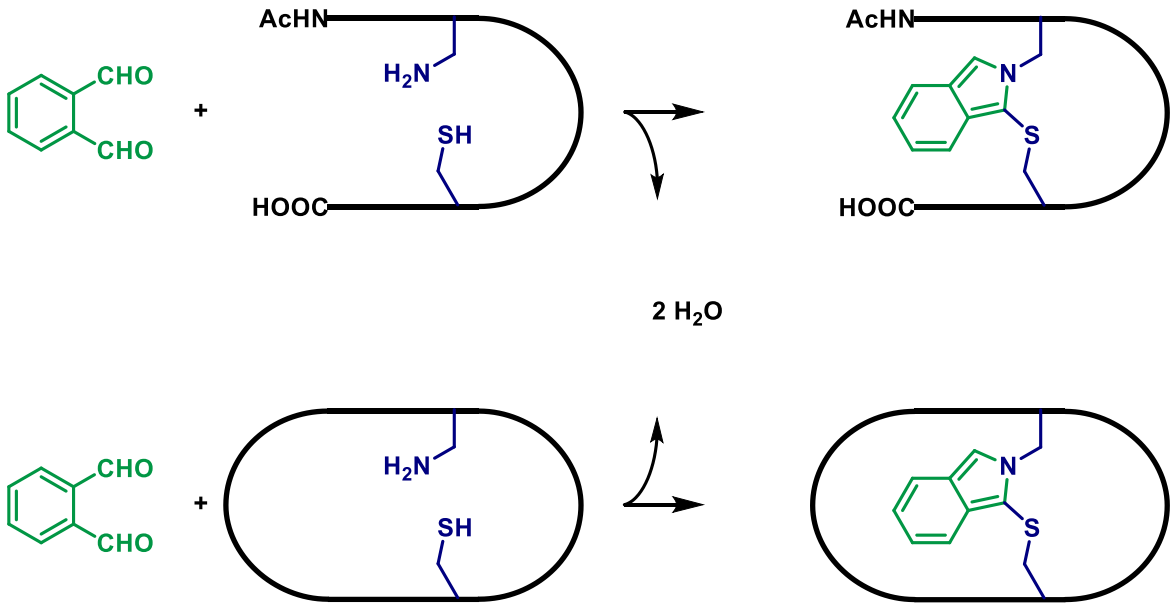
(FIICk): Isoindole Formation



- **Isoindole Formation** is a classic analytical reaction
- Condensation of ortho-phthalaldehyde with an amine and a thiol results in a fluorescent heterocycle
- Used to quantify amino acids and peptides and as an active site titrant

Todorovic, M.; Schwab, K. D.; Zeisler, J.; Zhang, C.; Bénard, F.; Perrin, D. M. *Angew. Chemie - Int. Ed.* **2019**, *58* (40), 14120–14124. https://doi.org/10.1002/anie.201906514.

(FIICk): Monocycles and Bicycles



Todorovic, M.; Schwab, K. D.; Zeisler, J.; Zhang, C.; Bénard, F.; Perrin, D. M. *Angew. Chemie - Int. Ed.* **2019**, *58* (40), 14120–14124. https://doi.org/10.1002/anie.201906514.

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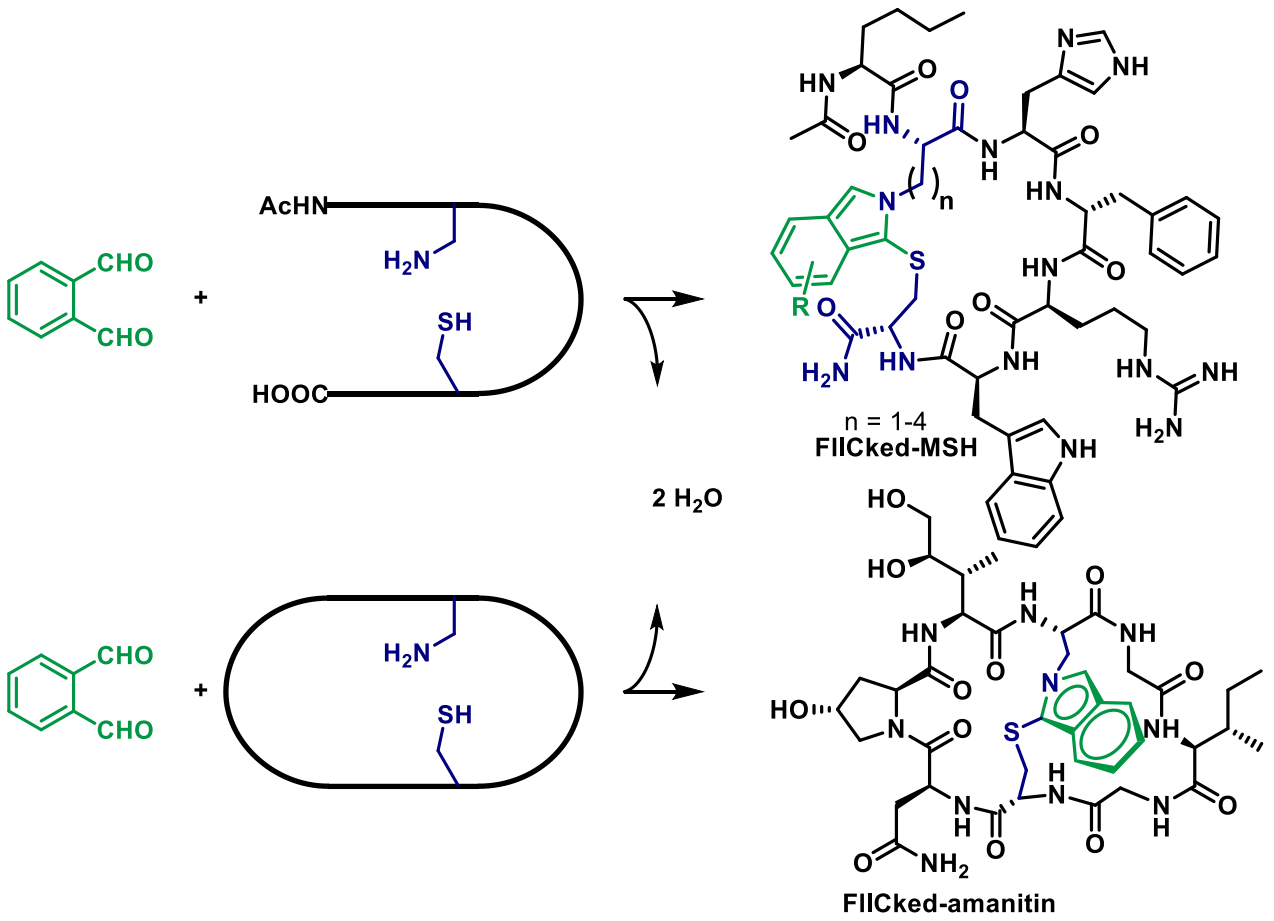
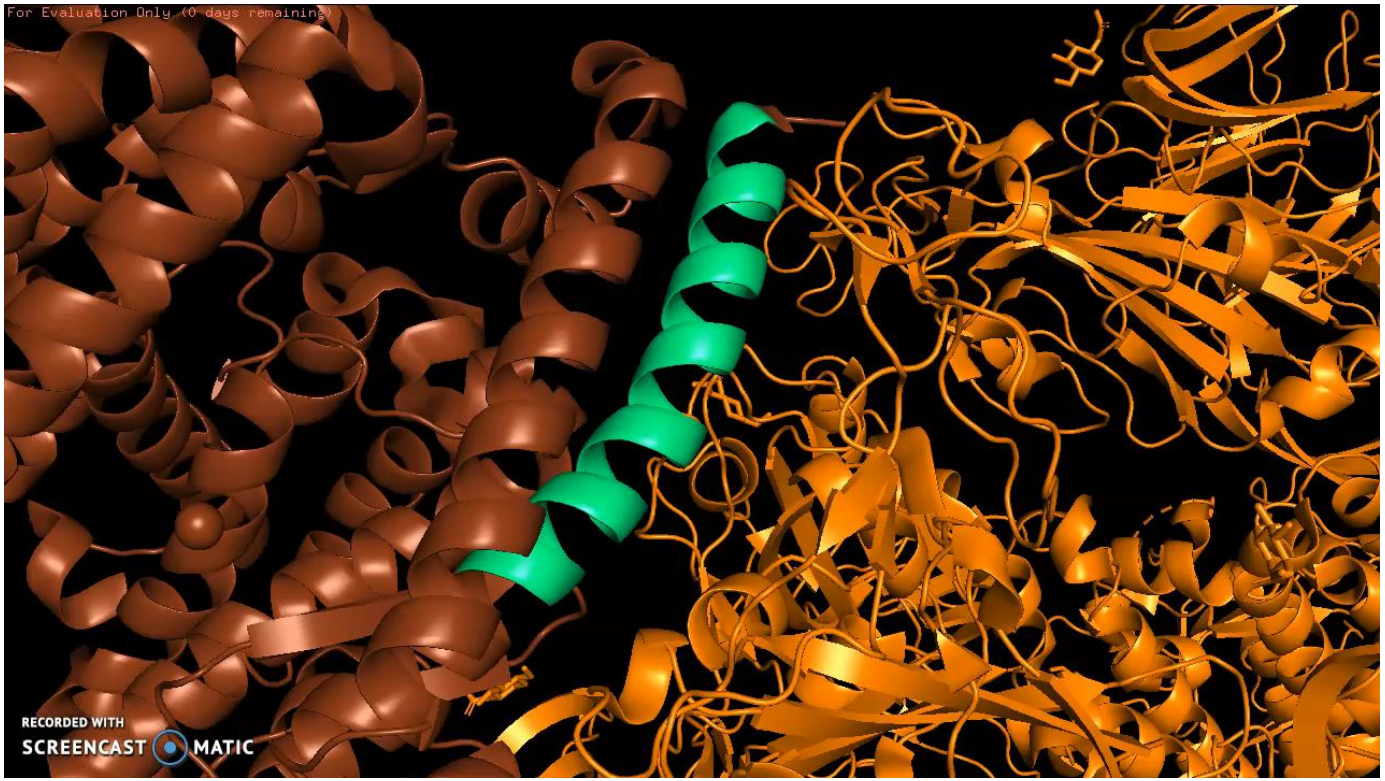
(FIICk): Proof of Concept Peptides



Todorovic, M.; Schwab, K. D.; Zeisler, J.; Zhang, C.; Bénard, F.; Perrin, D. M. *Angew. Chemie - Int. Ed.* **2019**, *58* (40), 14120–14124. https://doi.org/10.1002/anie.201906514.

(FIICk): ACE2 Mimicking Peptide Literature Precedence

- Direct cut-outs of N-terminal fragment of hACE2 were made by Pentelute[1] and Altseens[2]
- Binding to Spike protein tested by Biolayer Interferometry (BLI) or Atomic Force Microscopy
- Little binding in the high micromolar range was reported and no inhibition of the ACE2-Spike protein interaction was observed in our hands
- Doubled stapled (olefin metathesis) versions of ACE2 helix made by Debnath[3]
- Although binding measured by BLI, activity in in-vitro antiviral show



1) Zhang, G.; Pomplun, S.; Loftis, A. R.; Loas, A.; Pentelute, B. L. *bioRxiv* **2020**, 2020.03.19.999318. https://doi.org/10.1101/2020.03.19.999318.
2) Yang, J.; Petitjean, S. J. L.; Koehler, M.; Zhang, Q.; Dumitru, A. C.; Chen, W.; Derclaye, S.; Vincent, S. P.; Soumillion, P.; Alsteens, D. *Nat. Commun.* **2020**, *11*https://doi.org/10.1038/s41467-020-18319-6.
3) Curreli, F.; Victor, S. M. B.; Ahmed, S.; Drelich, A.; Tong, X.; Tseng, C.-T. K.; Hillyer, C. D.; Debnath, A. K. *MBio* **2020**, *11* (6), e02451-20.

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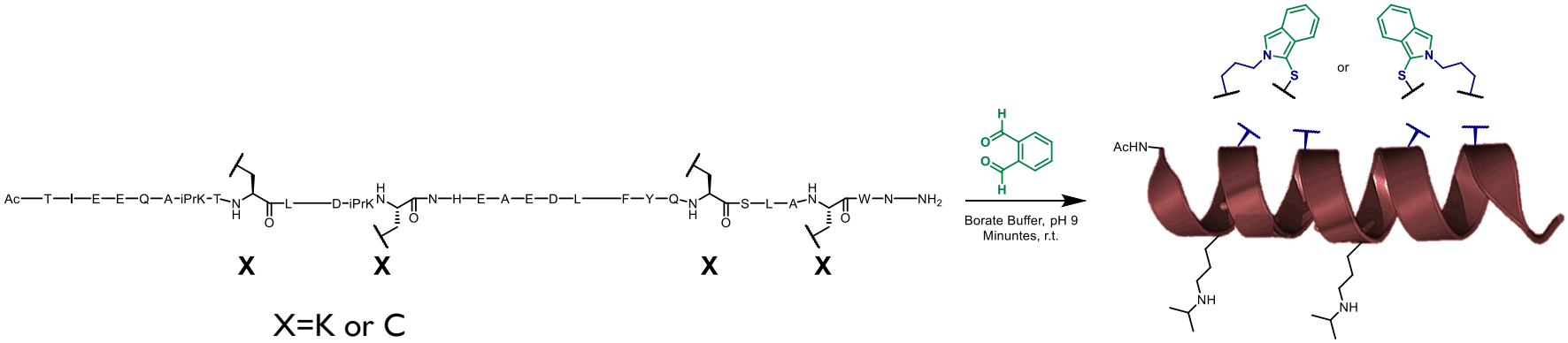
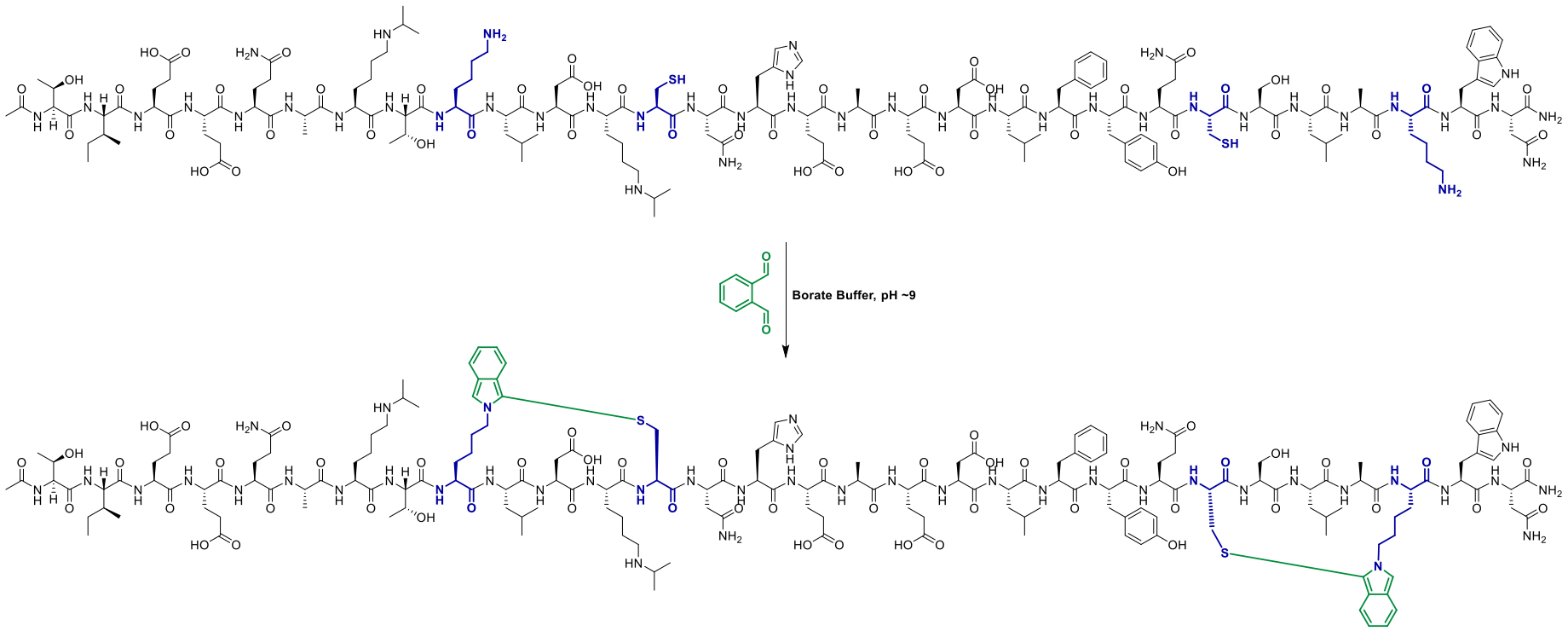
(FlICk): Double FlICked ACE2 Peptides



X=K or C

Peptide	Sequence
1	CKCK
2	CKKC
3	KCCK
4	KCKC

(FlICk): Peptide 3 Double Flicking



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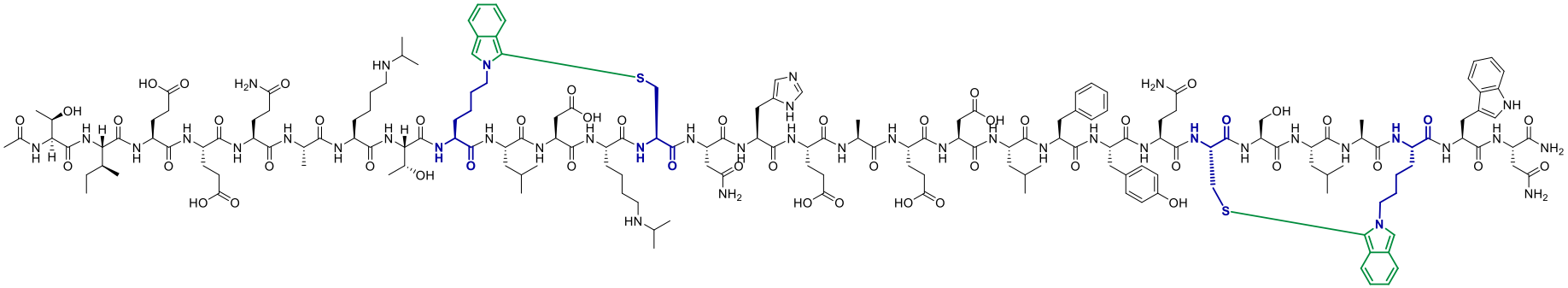
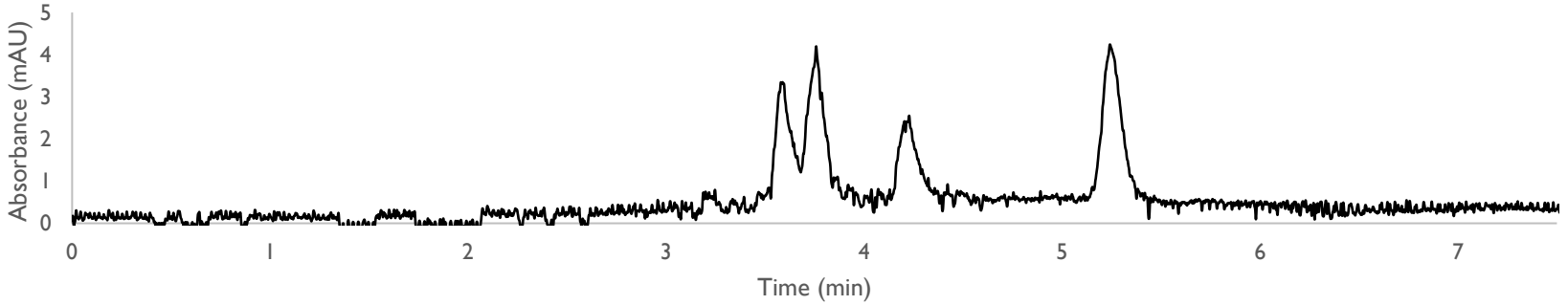
(FIICk): Peptide 3 Double Flicking



Peptide 3 Double Flicked Crude (335nm) 2eq OPA



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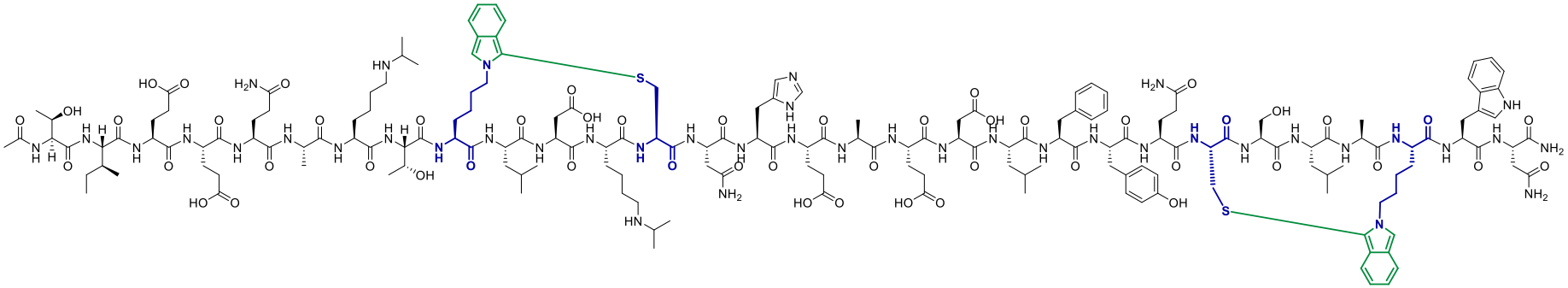
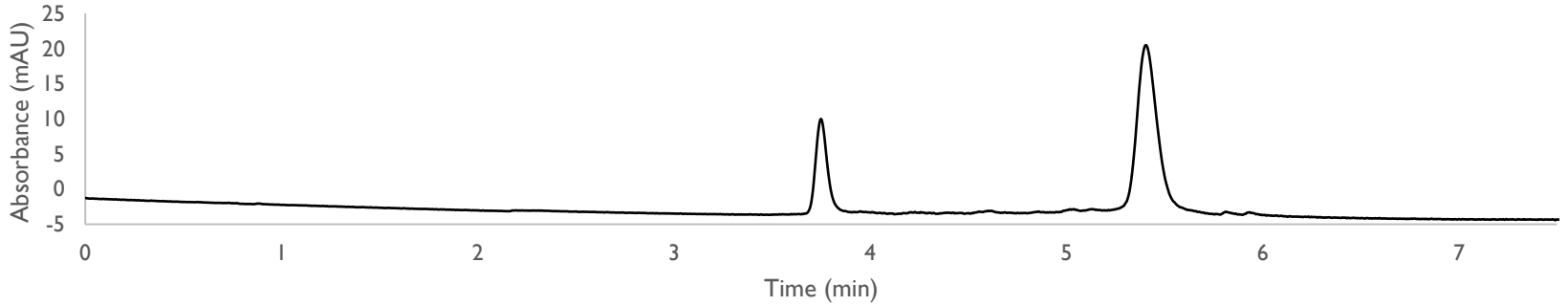
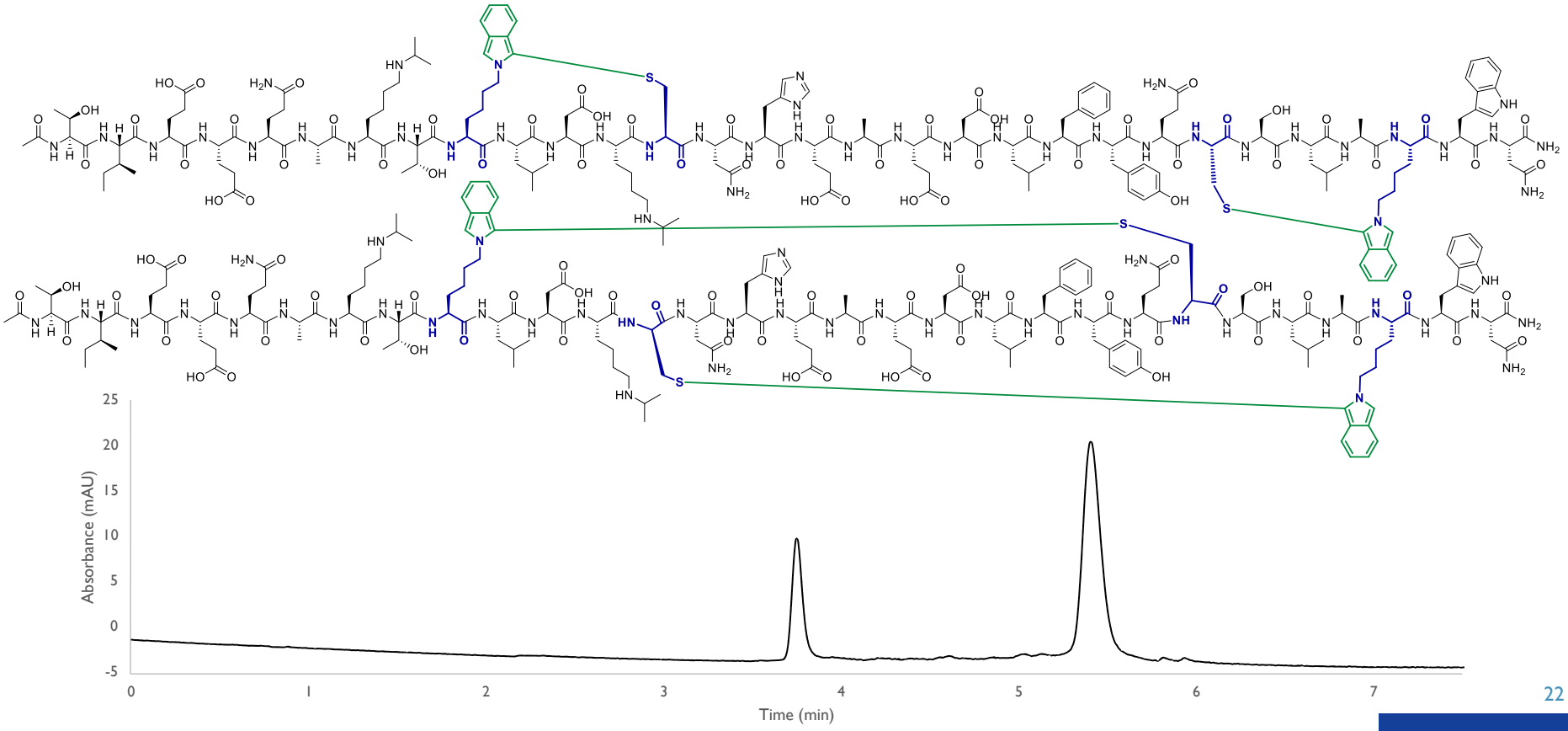
(FIICk): Peptide 3 Double Flicking





(FIICk): Peptide 3 Double Flicking



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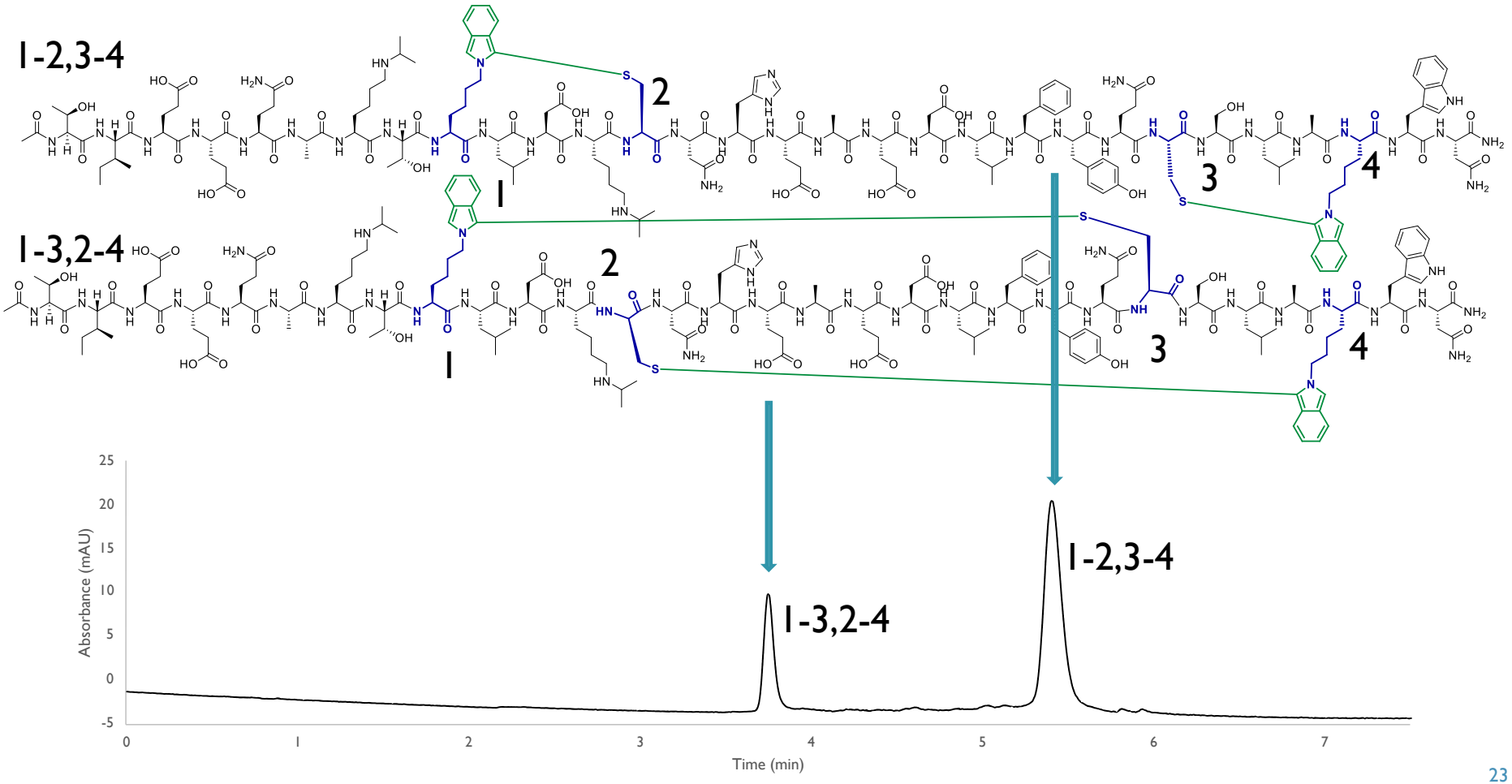
(FIICk): Peptide 3 Double Flicking



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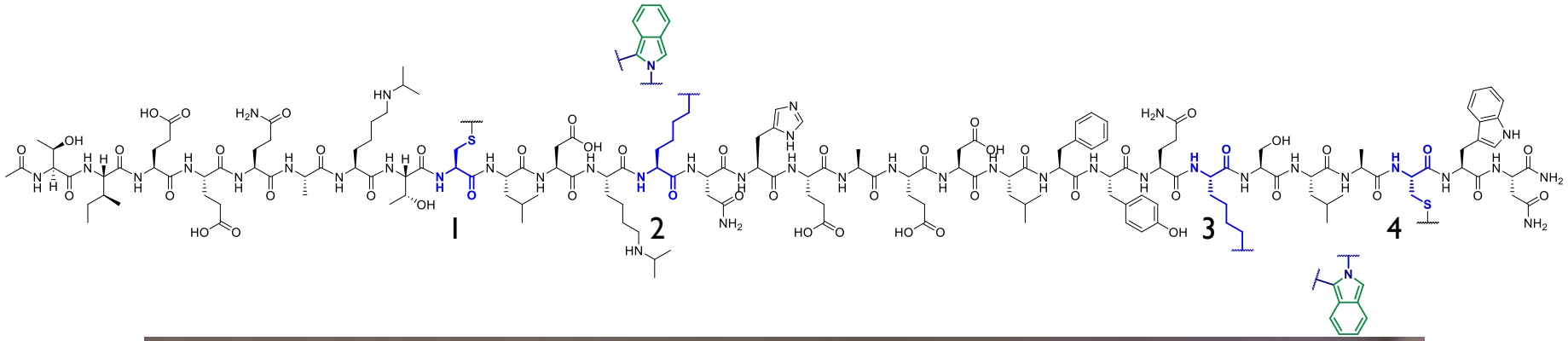
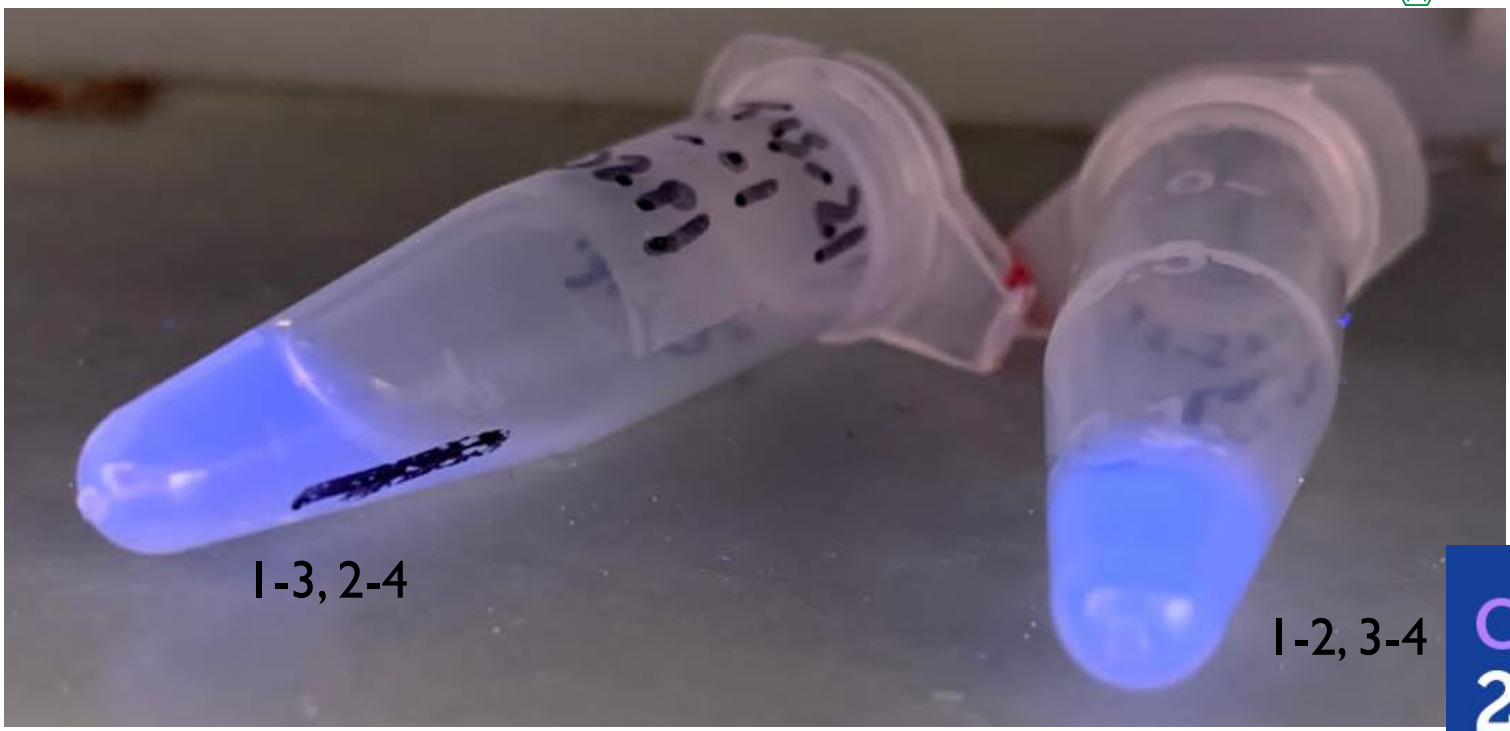
(FlICk): Double Flicked Peptide Fluorescence





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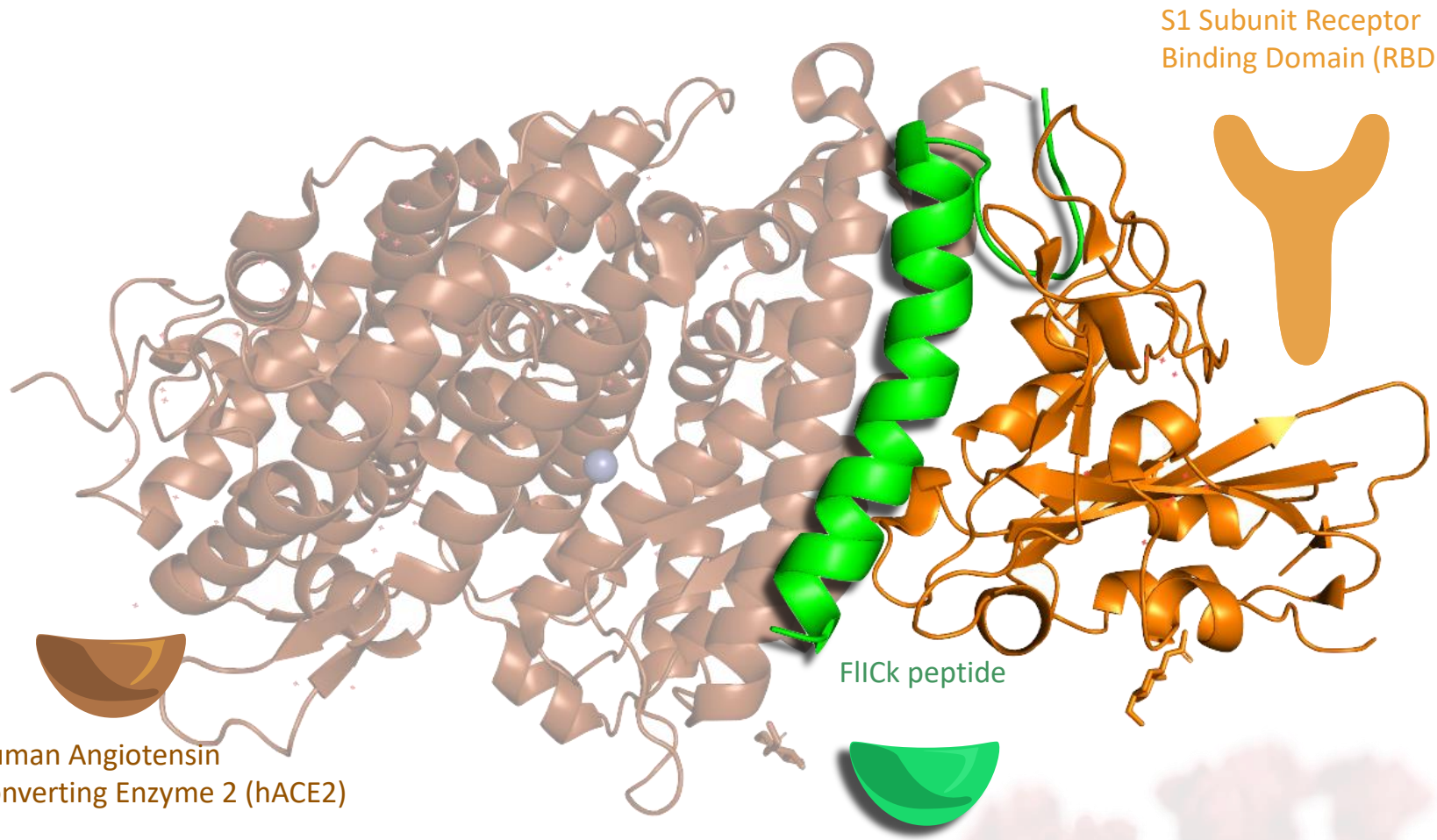


Enzyme ImmunoSorbent Assay

ELISA

ELISA: Enzyme ImmunoSorbent Assay

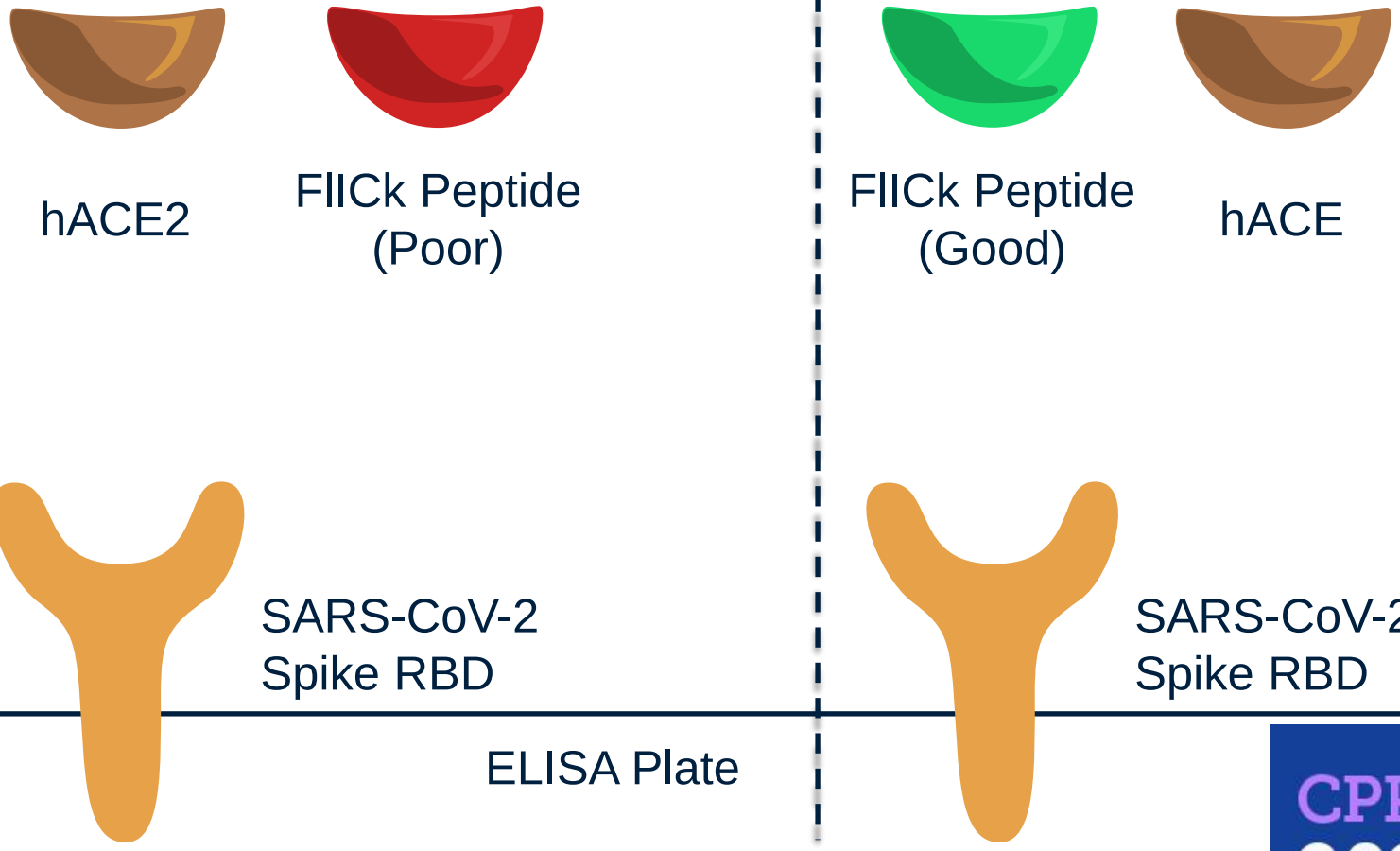
Negative results/ poor binder

Positive results/ good binder

ELISA: Enzyme ImmunoSorbent Assay

Negative results/ poor binder

Positive results/ good binder



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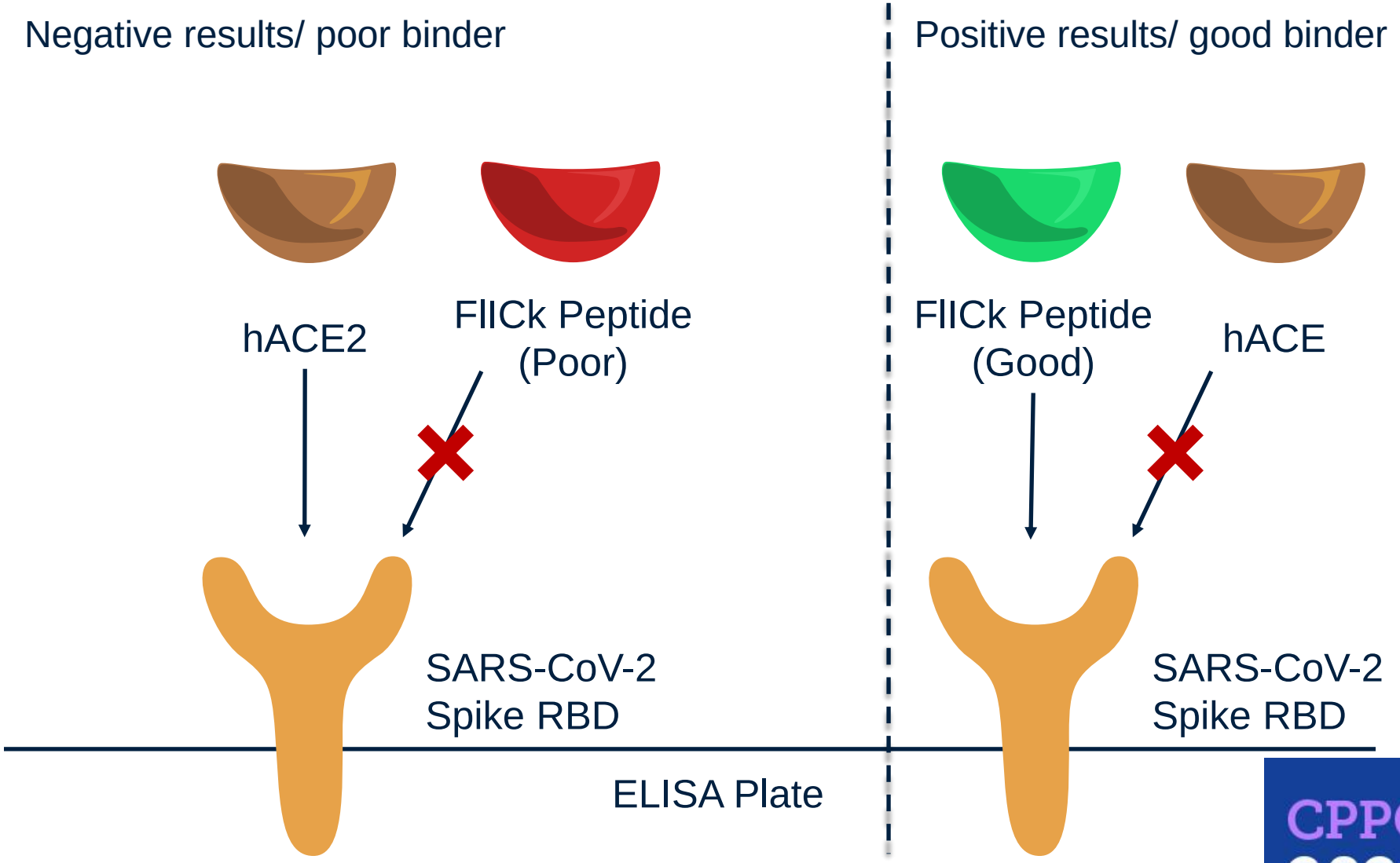
ELISA: Enzyme ImmunoSorbent Assay



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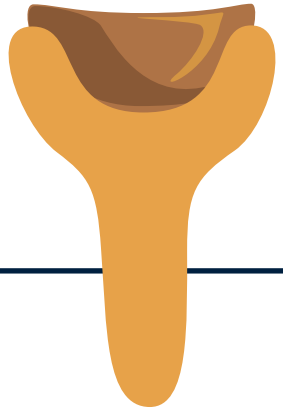
ELISA: Enzyme ImmunoSorbent Assay

Negative results/ poor binder

Positive results/ good binder

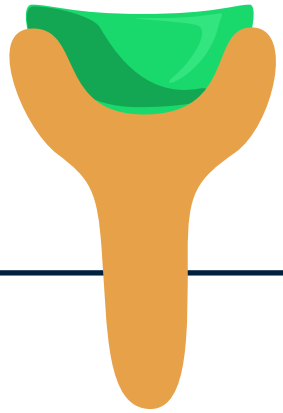
FlICk Peptide
(Poor)

hACE



hACE2

SARS-CoV-2
Spike RBD



FlICk Peptide
(Good)

SARS-CoV-2
Spike RBD

ELISA Plate

ELISA: Enzyme ImmunoSorbent Assay

Negative results/ poor binder

Positive results/ good binder

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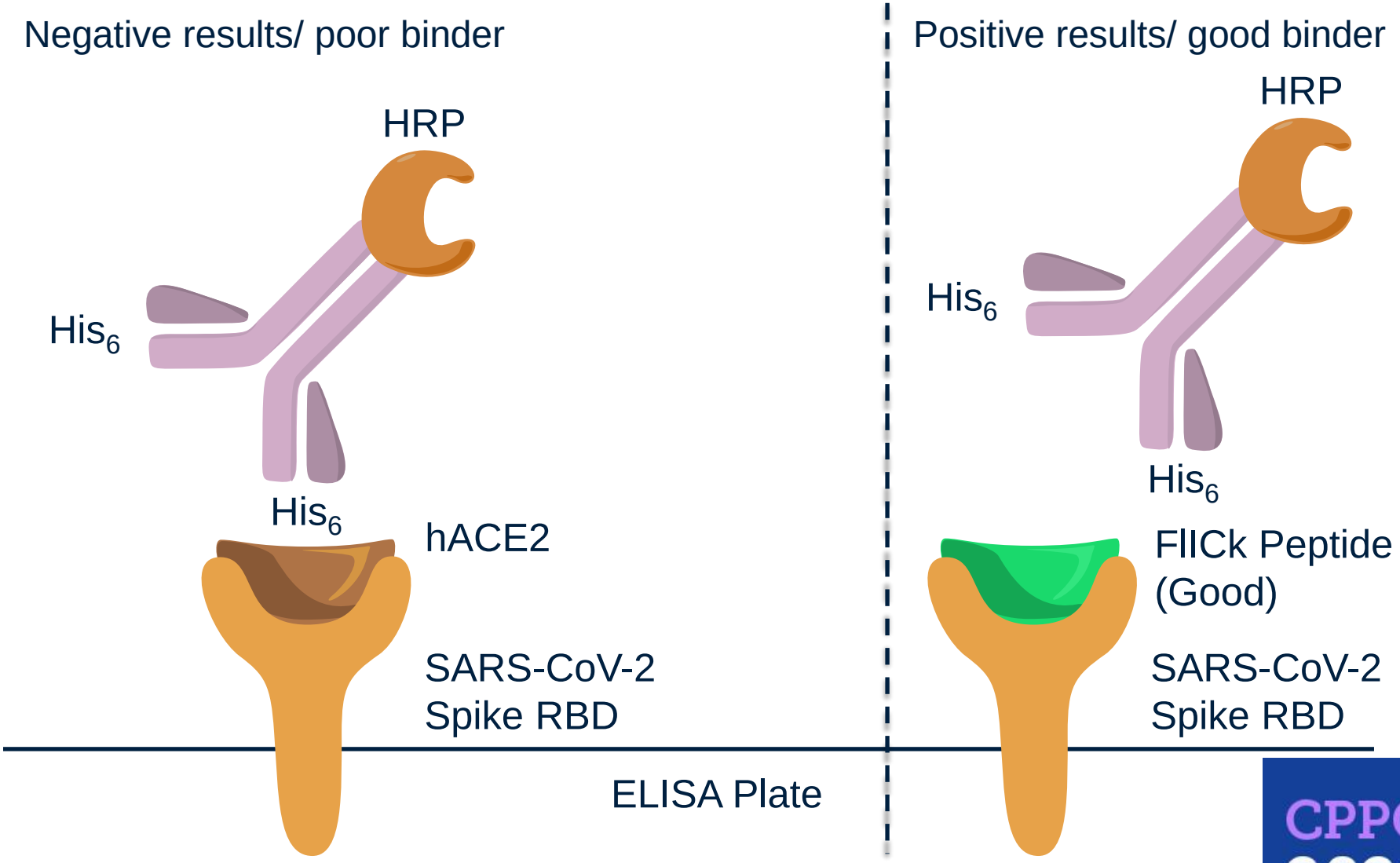
ELISA: Enzyme ImmunoSorbent Assay



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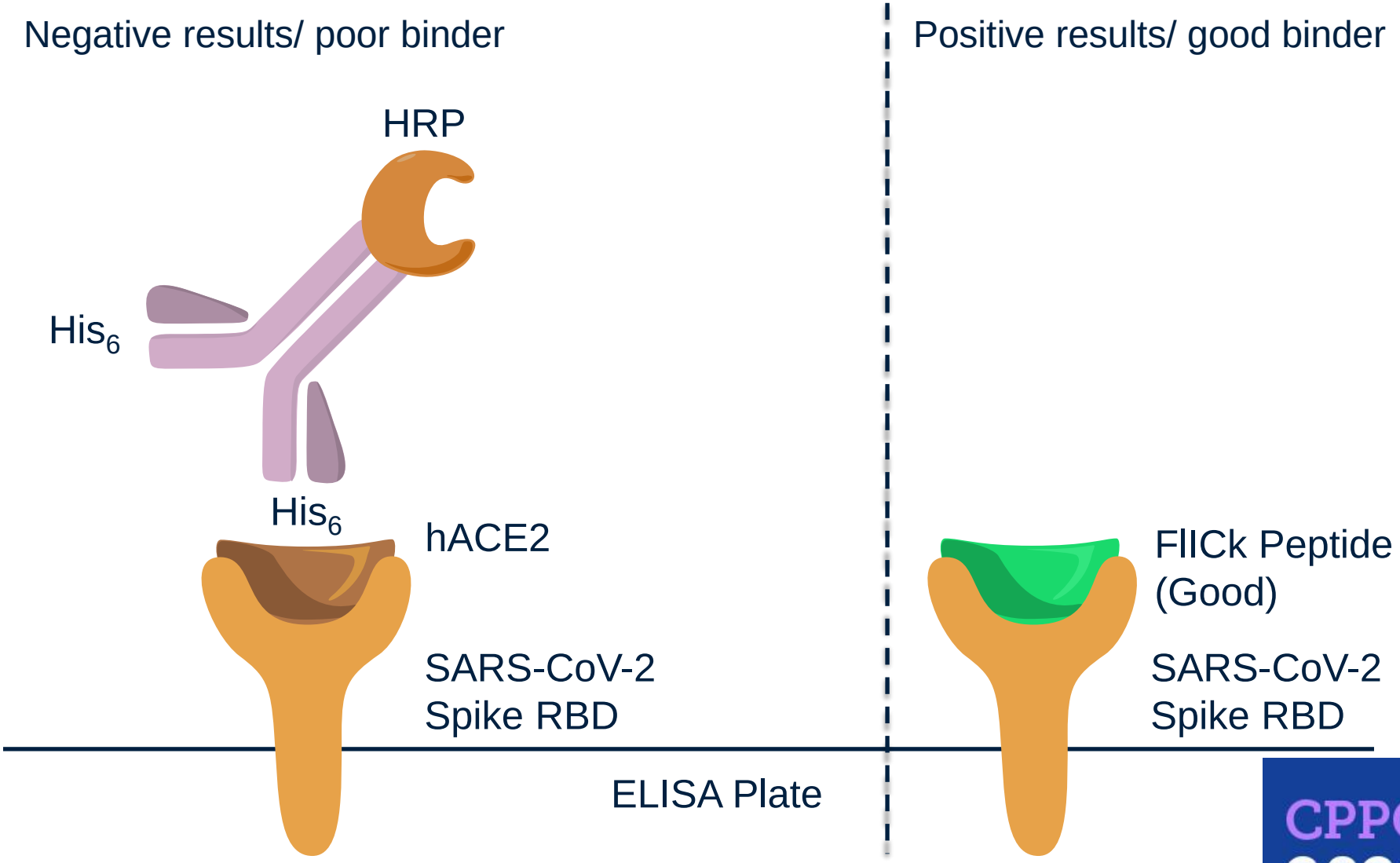
ELISA: Enzyme ImmunoSorbent Assay



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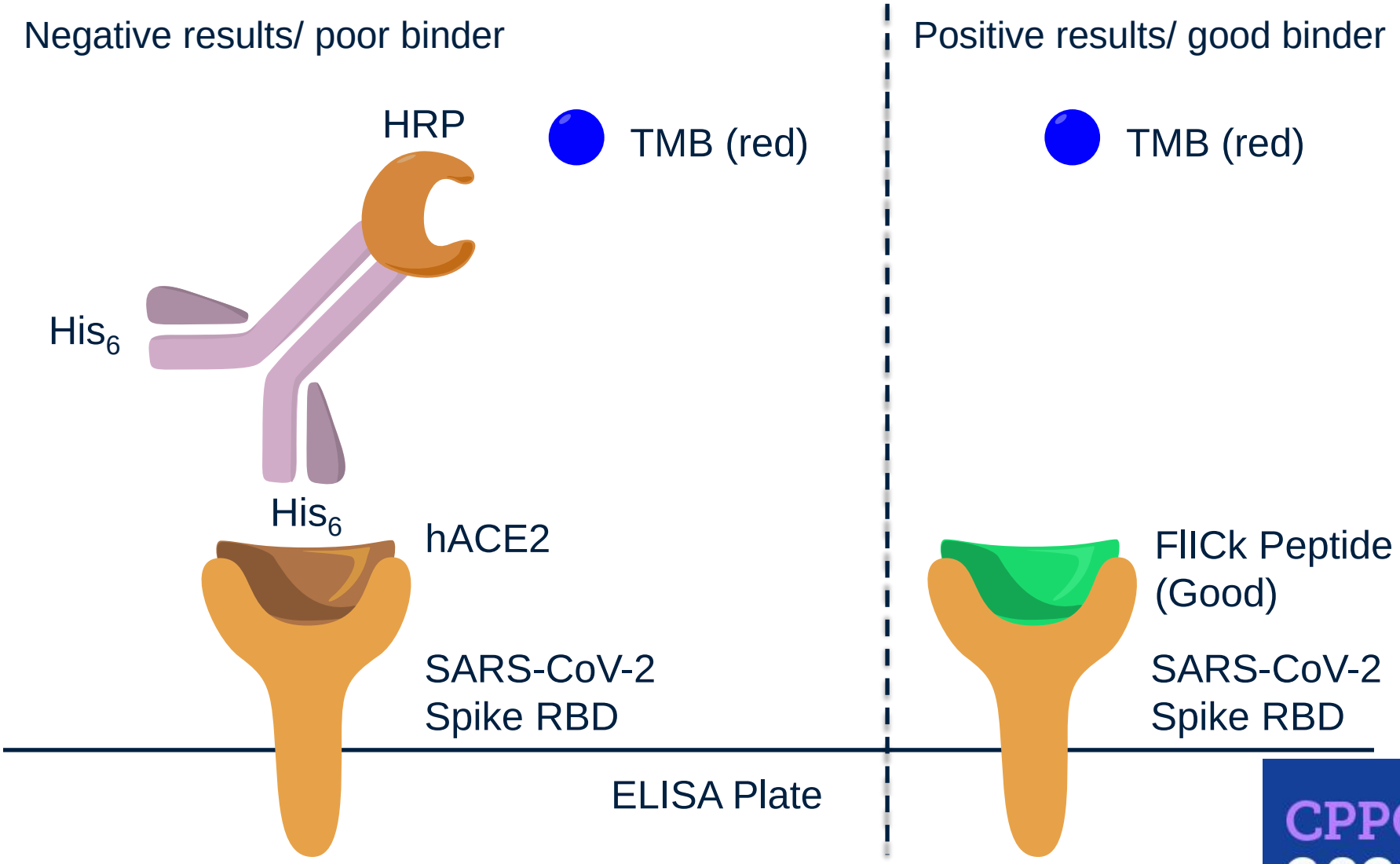
ELISA: Enzyme ImmunoSorbent Assay



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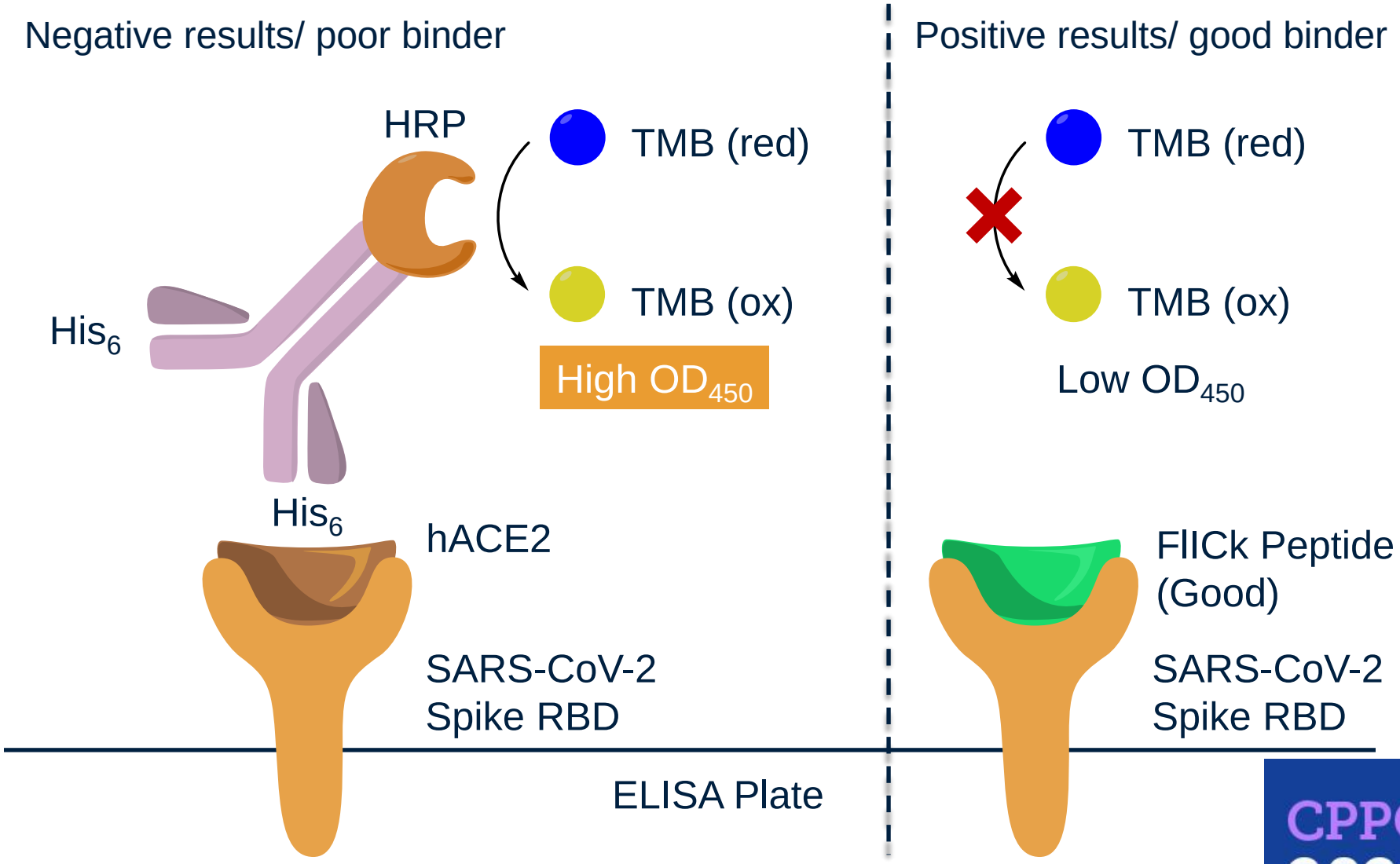
ELISA: Enzyme ImmunoSorbent Assay



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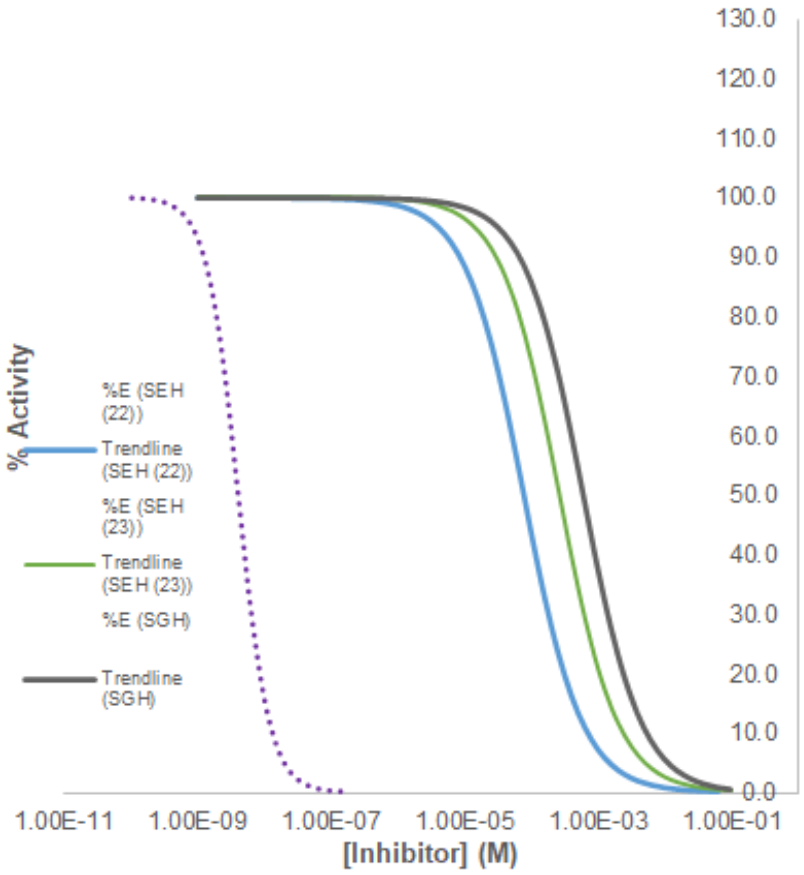
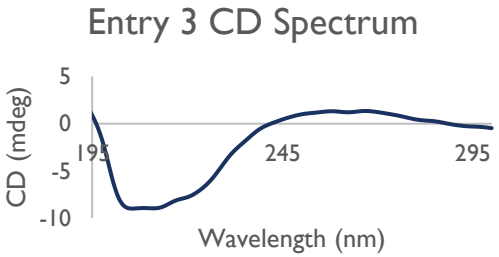
(FIICk): Double Flicked Peptide IC50s

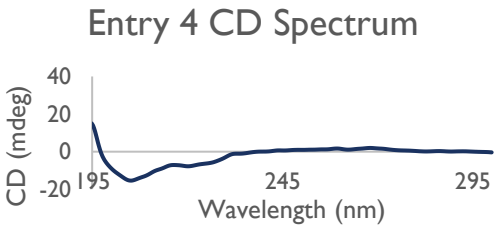


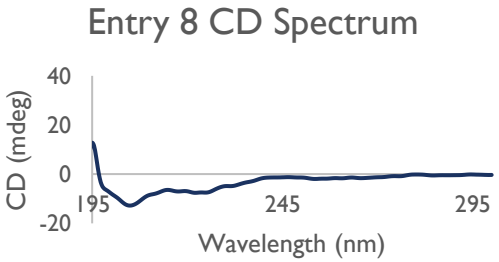
Entry	1234	Topology	R.T. (min)	IC50(μM)
1	CKCK	1-4,2-3	4.71	Not active (>900)
2	CKCK	1-2,3-4	5.21	Not active (>900)
3	CKKC	1-3,2-4	3.57	77
4	CKKC	1-2,3-4	5.22	260
5	KCCK	1-3,2-4	3.75	Not active (>500)
6	KCCK	1-2,3-4	5.39	Not active (>900)
7	KCKC	1-4,2-3	5.00	Not active (>900)
8	KCKC	1-2,3-4	5.36	620

(FIICk): Double Flicked Peptide IC50s

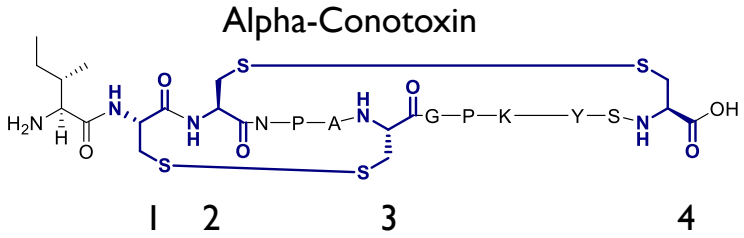
Entry	XXXX 1234	Topology	R.T. (min)	IC50(μM)
1	CKCK	1-4,2-3	4.71	Not active (>900)
2	CKCK	1-2,3-4	5.21	Not active (>900)
3	CKKC	1-3,2-4	3.57	77
4	CKKC	1-2,3-4	5.22	260
5	KCCK	1-3,2-4	3.75	Not active (>500)
6	KCCK	1-2,3-4	5.39	Not active (>900)
7	KCKC	1-4,2-3	5.00	Not active (>900)
8	KCKC	1-2,3-4	5.36	620



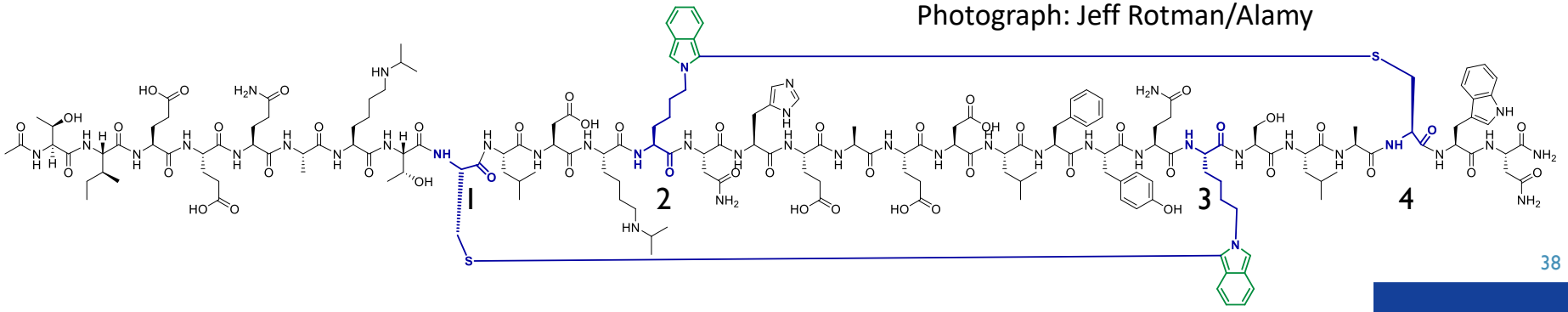




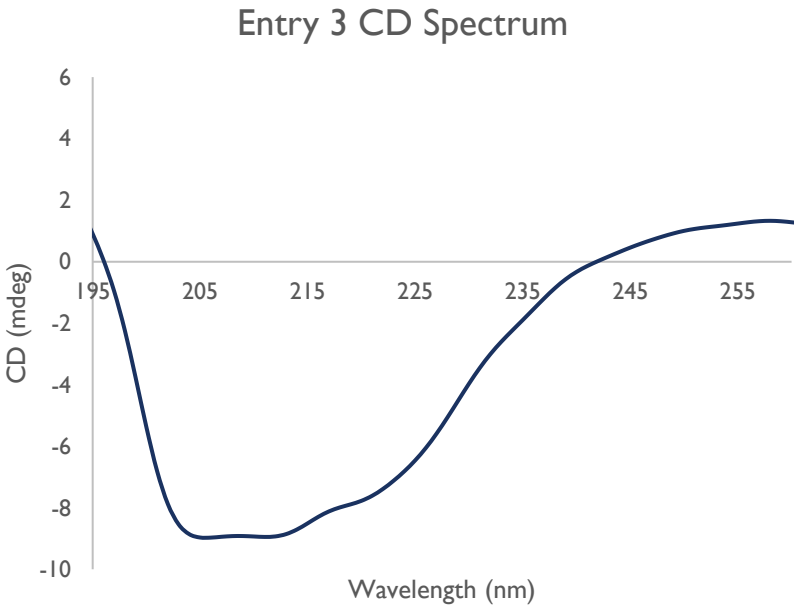
(FIICk): Alpha Conotoxin Topology

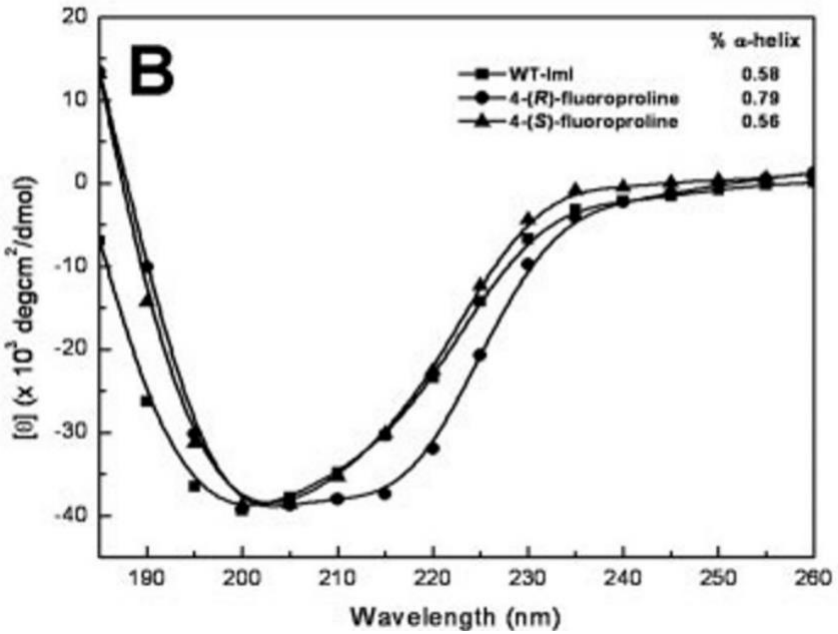


Photograph: Jeff Rotman/Alamy



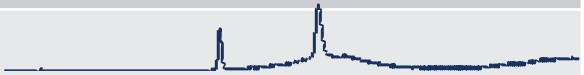
(FIICk): Alpha Conotoxin Topology



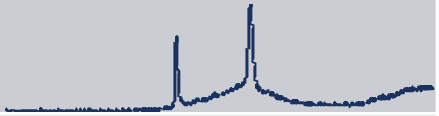
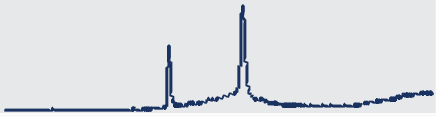
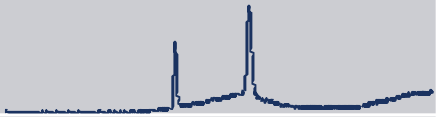
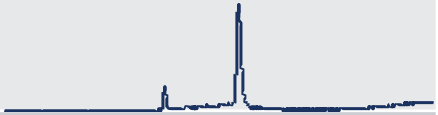
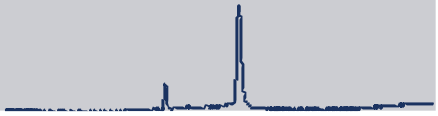


Armishaw, C.; Jensen, A. A.; Balle, T.; Clark, R. J.; Harpsøe, K.; Skonberg, C.; Liljefors, T.; Strømgaard, K. *J. Biol. Chem.* **2009**, *284* (14), 9498–9512. https://doi.org/10.1074/jbc.M806136200.

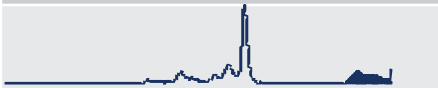
(FIICk): Effect of chaotropes and salts on Peptide 3 crosslinking selectivity

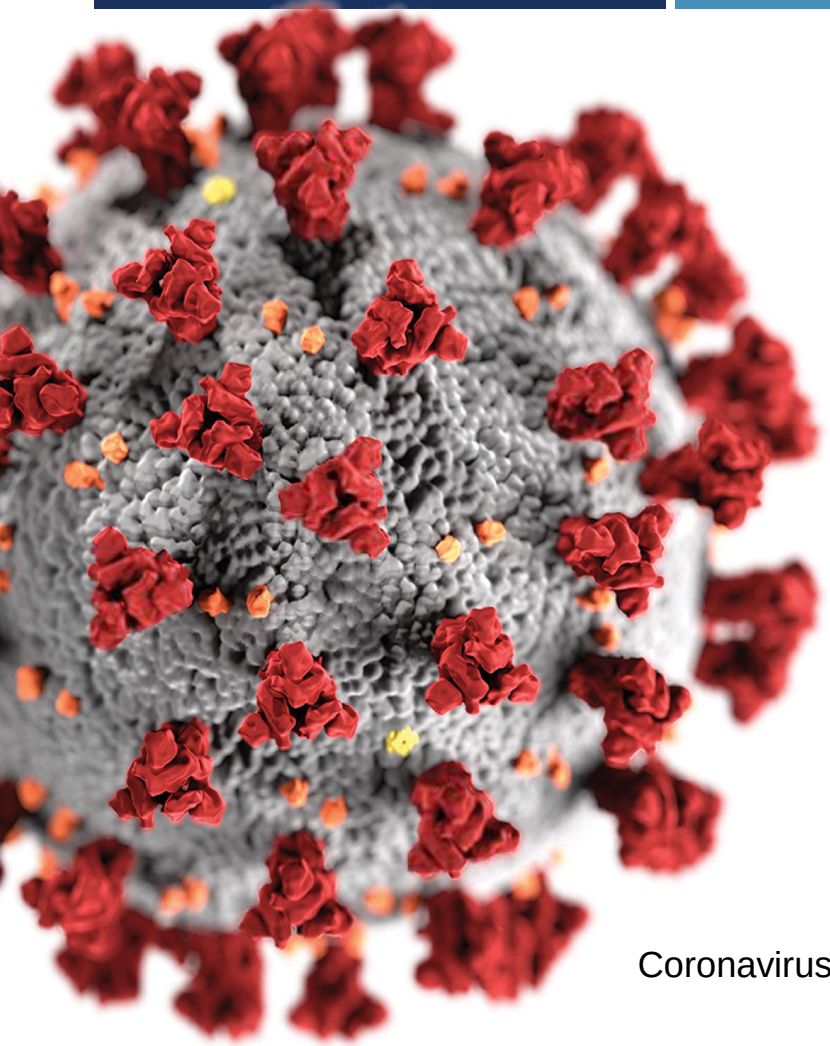
Chaotrope/salt (concentration)	1-2,3-4 : 1-3,2-4 ratio	HPLC chromatogram
none	2.5	
Guanidinium chloride (2% w/w)	2.5	
Urea (2% w/w)	2.4	
Polysorbate 20 (2% w/w)	3.2	
Polysorbate 80 (2% w/w)	2.7	
Triton X-100 (2% w/w)	3.5	
Mr. Clean® dish detergent (2% w/w)	2.9	
Magnesium chloride (450 mM)	2.3	

(FIICk): Effect of temperature and solvent on Peptide 3 crosslinking selectivity

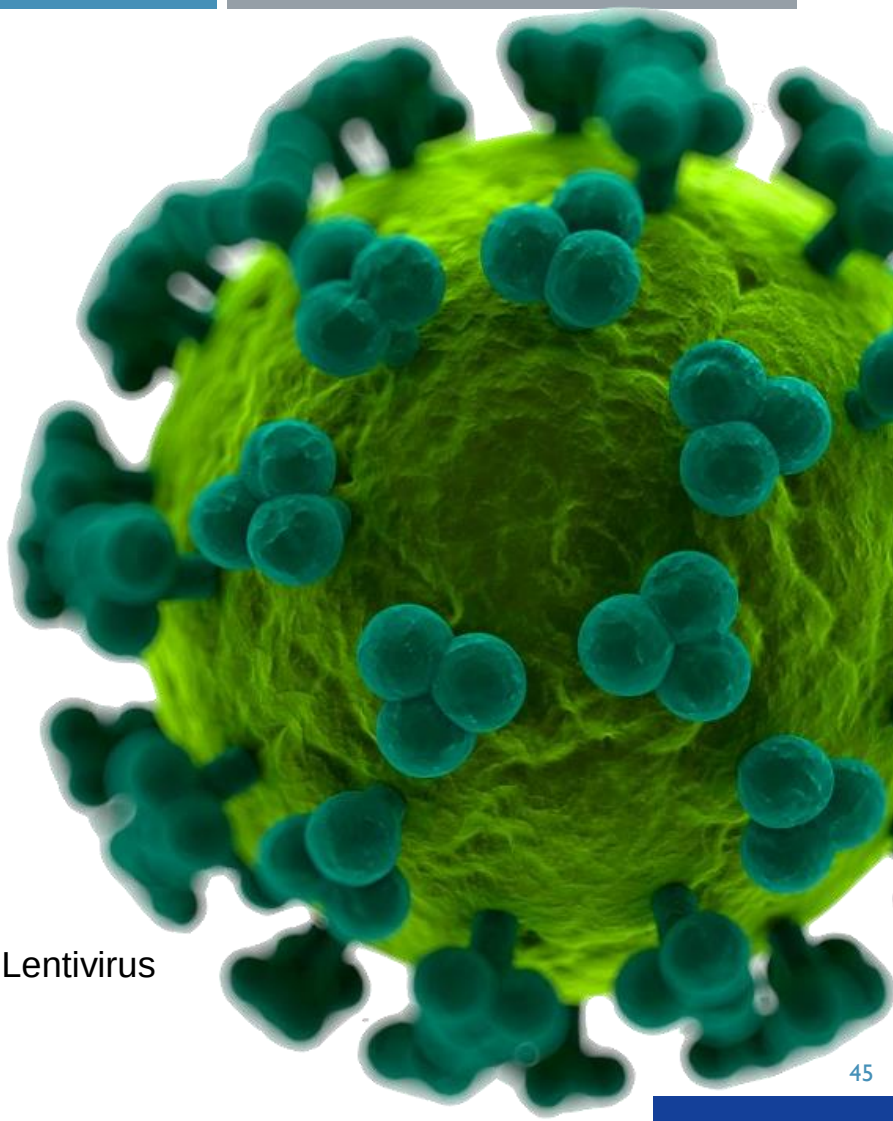
Solvent	Temperature (°C)	1-2,3-4 : 1-3,2-4 ratio	HPLC chromatogram
Sodium borate buffer, pH 9	4	1.90	
Sodium borate buffer, pH 9	20	2.52	
Sodium borate buffer, pH 9	37	2.34	
Sodium borate buffer, pH 9/EtOH (1:1)	-20	7.78	
Sodium borate buffer, pH 9/EtOH (1:1)	20	7.46	

(FIICk): Effect of solvent on Peptide 3 crosslinking selectivity

Peptide	Solvent	1-2,3-4 : 1-3,2-4 ratio	HPLC chromatogram
3	Sodium borate buffer, pH 9	2.52	
3	Sodium borate buffer, pH 9/EtOH (1:1)	7.46	
4	Sodium borate buffer, pH 9	2.26	
4	Sodium borate buffer, pH 9/DMF (1:1)	3.95	
4	Sodium borate buffer, pH 9/DMA (1:1)	3.39	
4	Sodium borate buffer, pH 9/MeCN (1:1)	2.46	
4	Sodium borate buffer, pH 9/THF (1:1)	6.98	



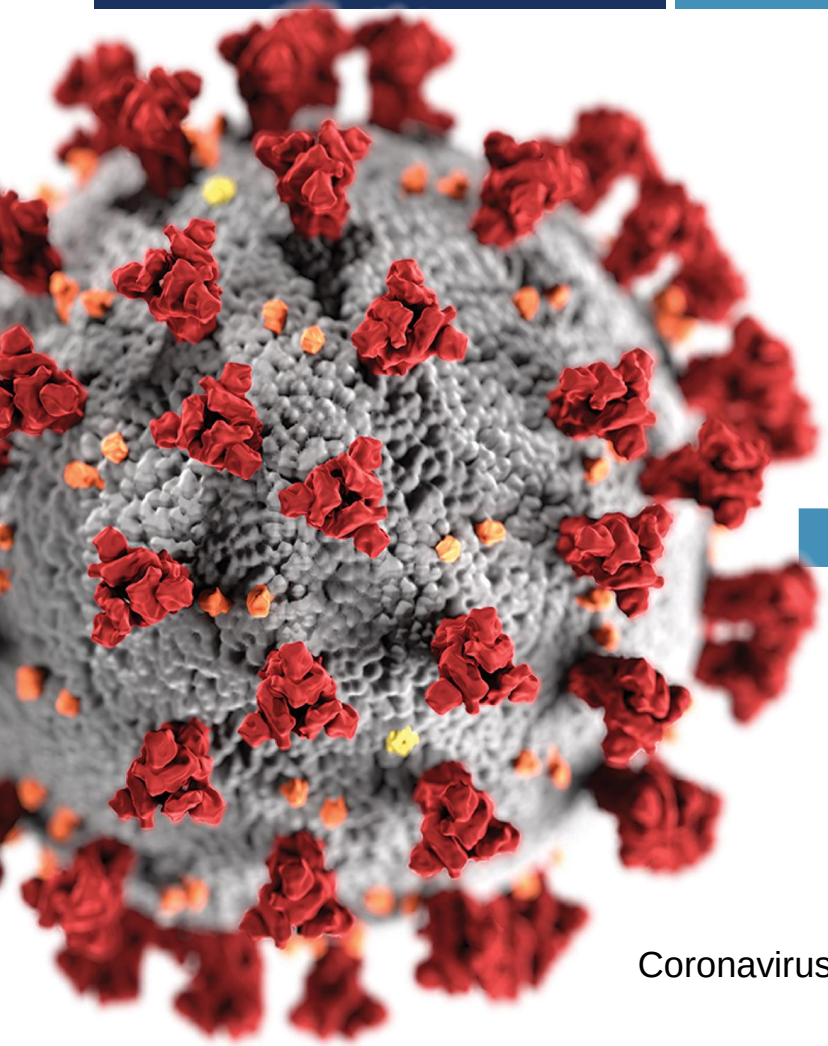
Coronavirus



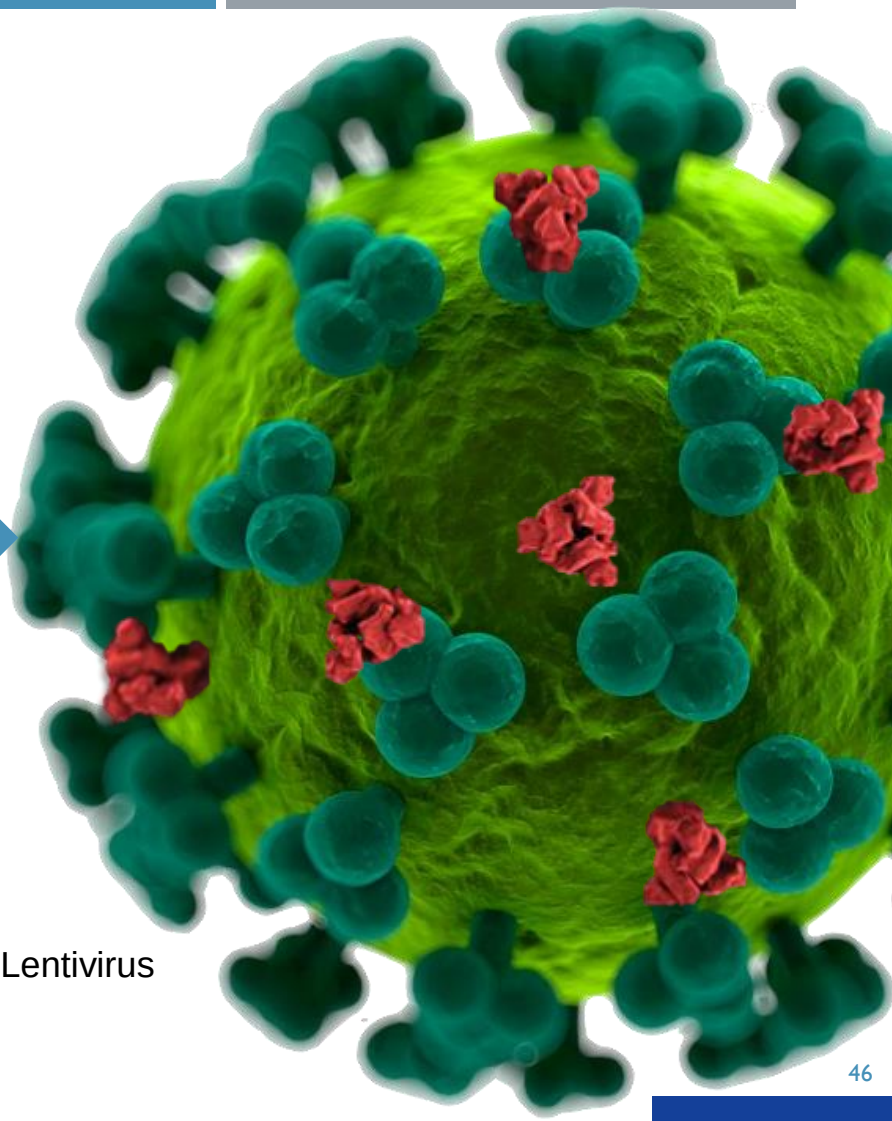
Lentivirus

Future direction

Psuedovirus Neutralization Assay (PNA)



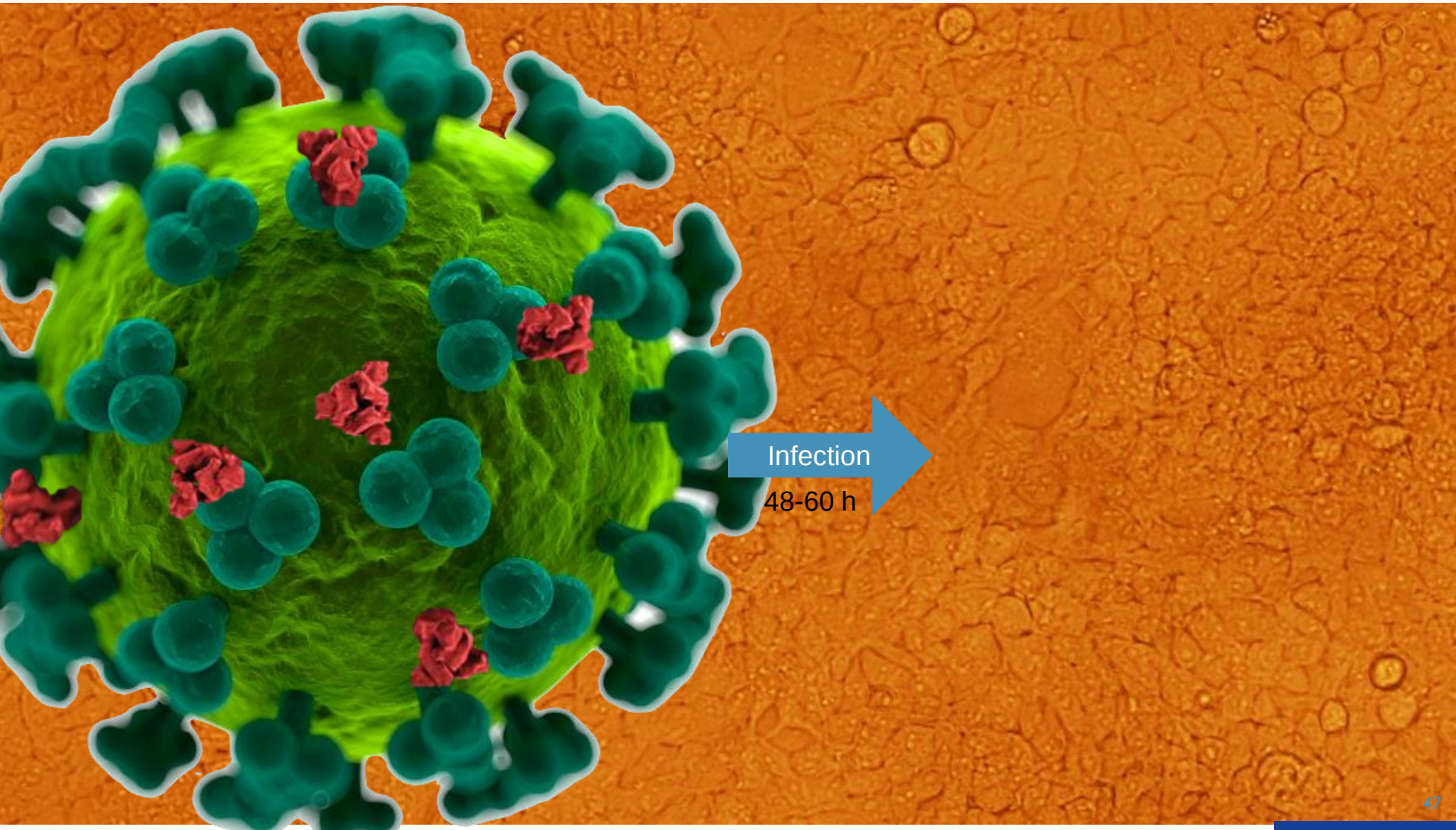
Coronavirus



Lentivirus

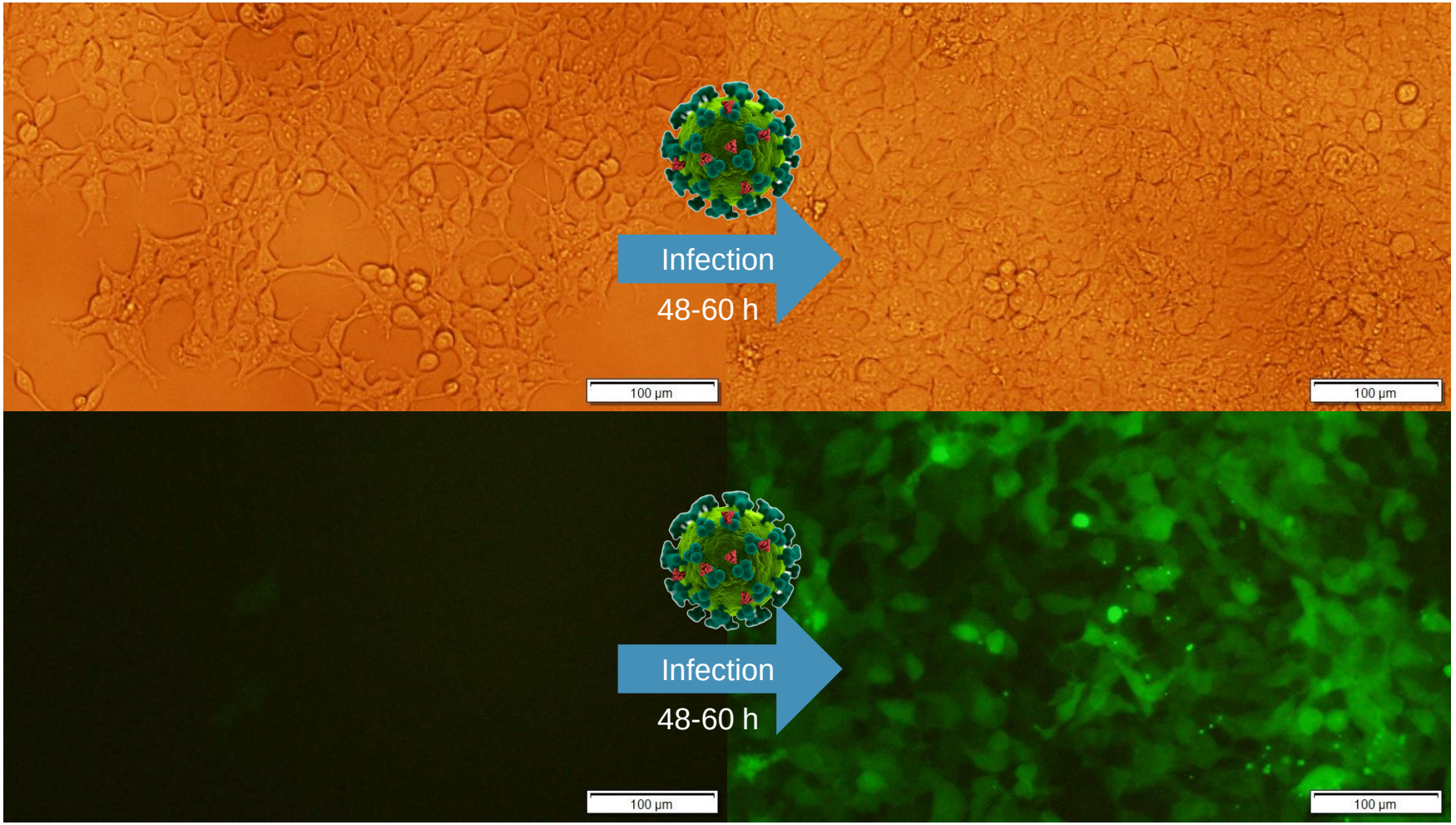
Future direction

Psuedovirus Neutralization Assay (PNA)



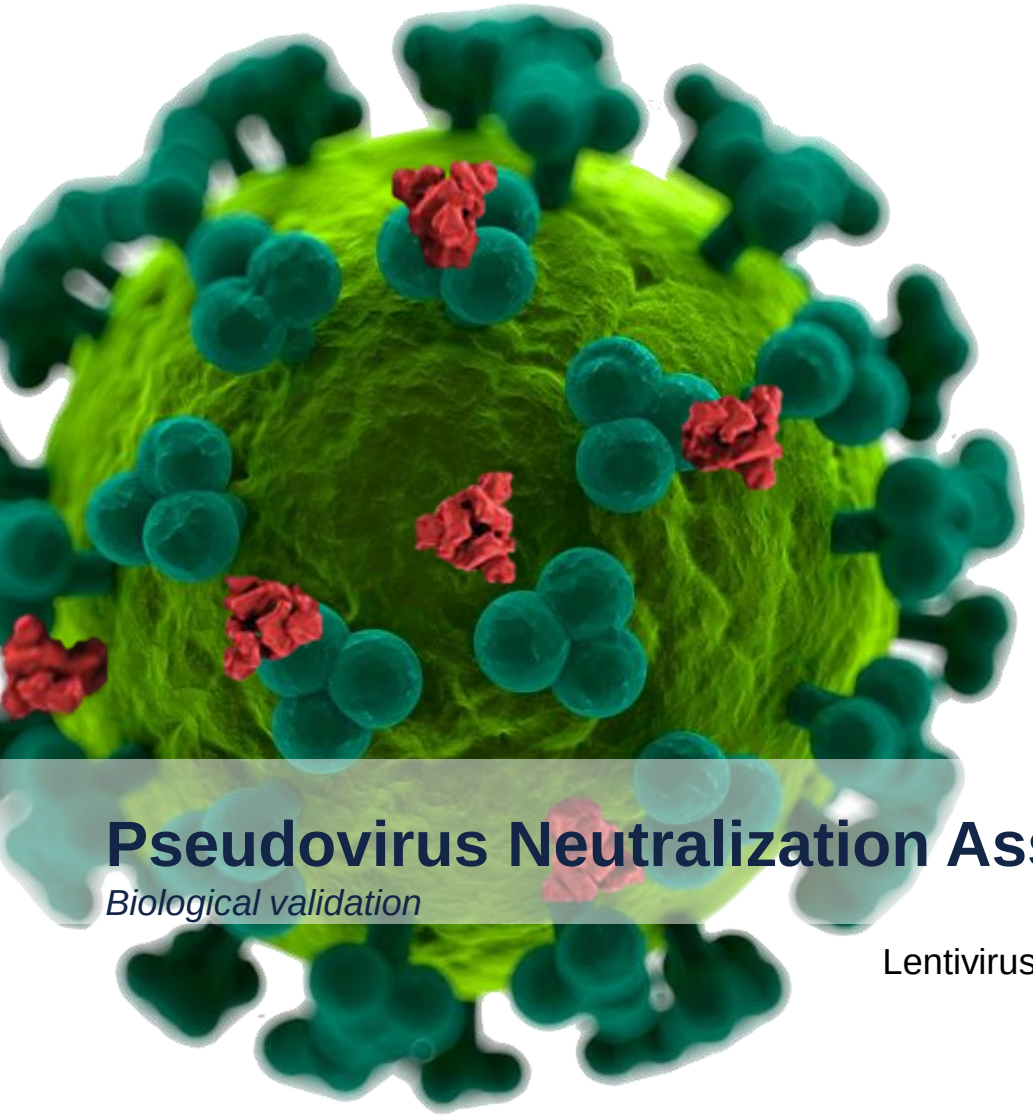
Future direction

Psuedovirus Neutralization Assay (PNA)



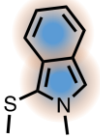
Future direction

Psuedovirus Neutralization Assay (PNA)



+ ACE2⁺ cells

Infection



+ **inhibitor**

+ ACE2⁺ cells

Infection

Pseudovirus Neutralization Assay

Biological validation

Lentivirus

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Faculty of Science

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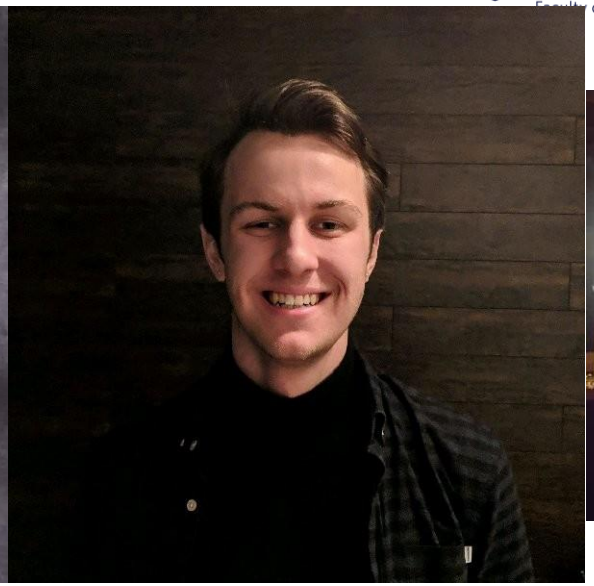
Naysilla Dayanara



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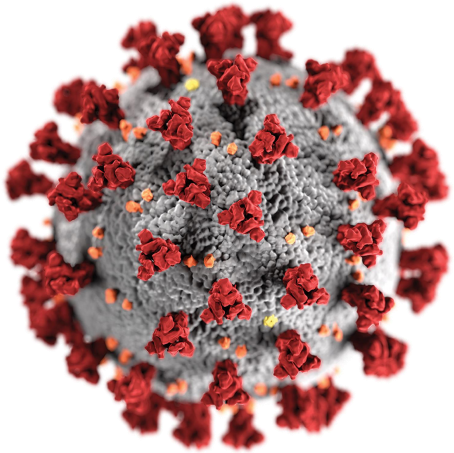

Questions?

CPPC 2021