De chemie van Corona-vaccins

Woudschoten Chemie, 6 November 2020

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Coriolis Pharma en

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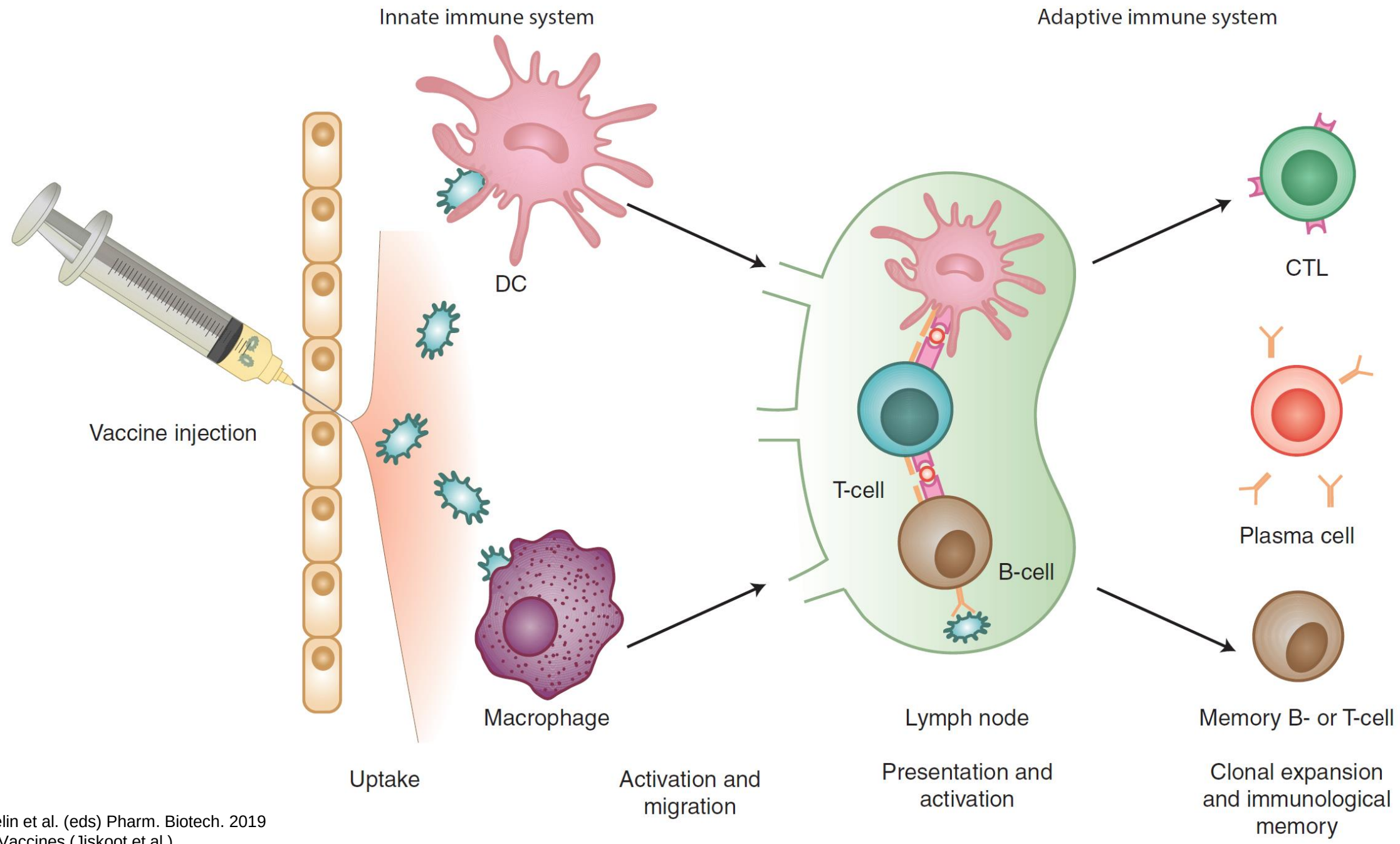
MC Escher, Vlakvulling2

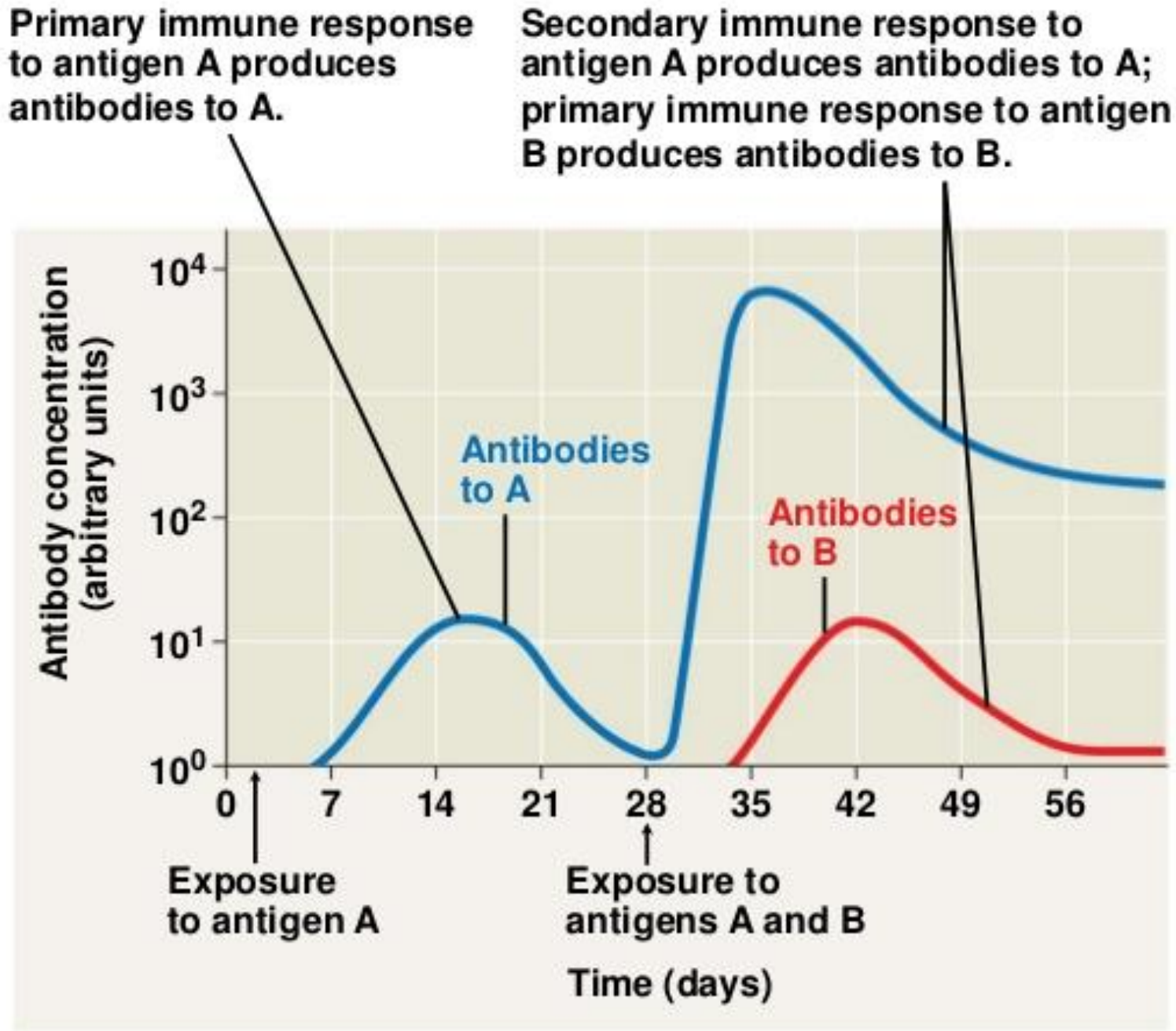
Chemie is cruciaal rol bij vaccinontwikkeling en -productie

- **Biochemie**:
  moleculaire interacties tussen pathogeen/vaccin en immuunsysteem.
- **Analytische chemie**:
  kwantificeren van immuunresponsen en vaccincomponenten.
- **Fysische chemie**:
  colloidchemie van eiwitten en nanodeeltjes.
- **Organische chemie**:
  koppeling suikers – eiwitten, chemische inactivering van virussen
- Anorganische chemie:
  bereiding van minerale nanodeeltjes (o.b.v. Al, Ca of Si)



Crommelin et al. (eds) Pharm. Biotech. 2019
chapter Vaccines (Jiskoot et al.)

Fig. 43-15



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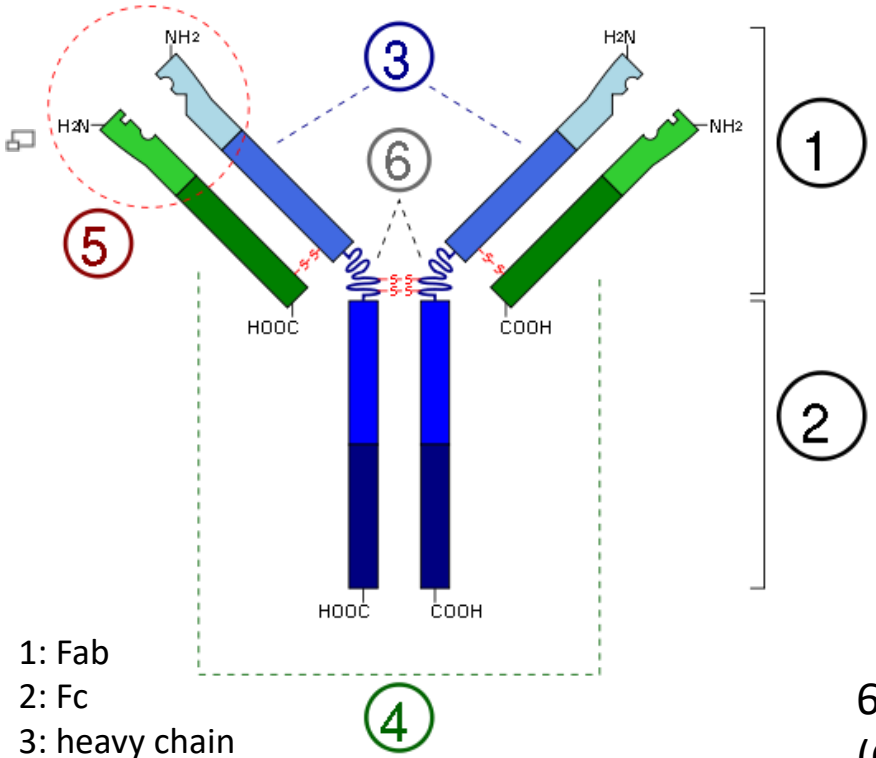
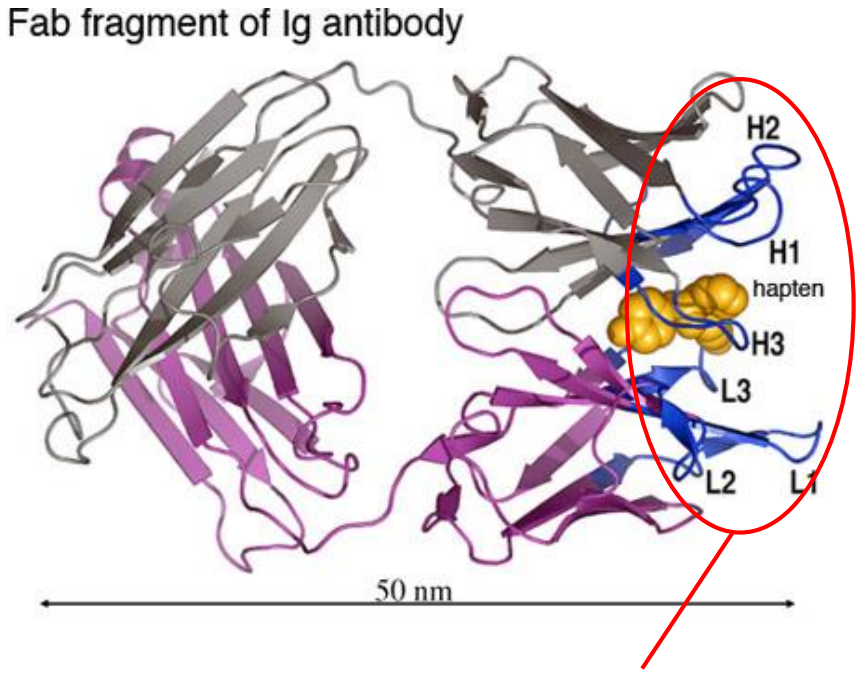
De werkpaarden van het immuunsysteem: antistoffen



1: Fab
2: Fc
3: heavy chain
4: light chain
5: paratope
6: hinge regions



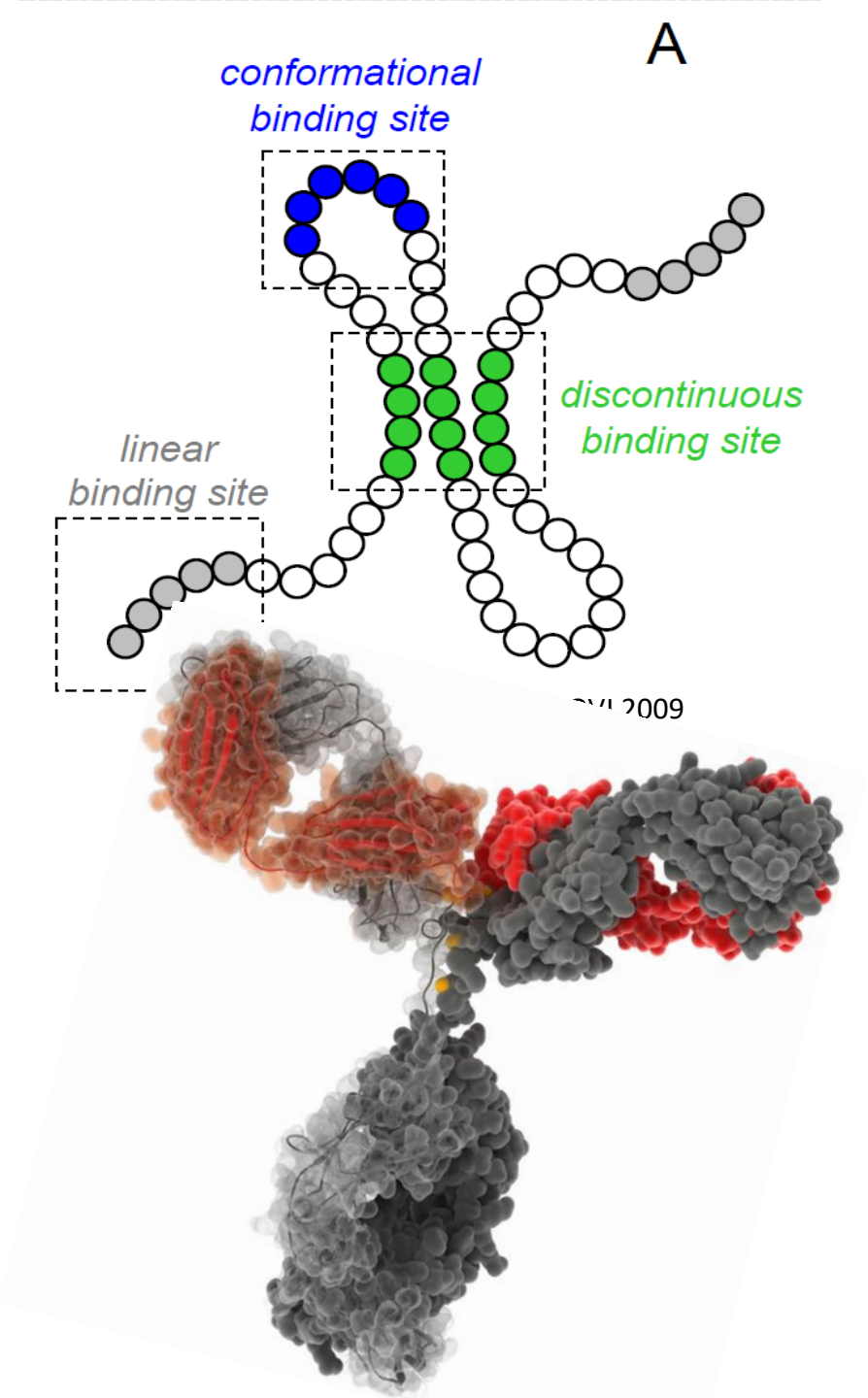
6 Complementarity Determining Regions (CDR) bind antigen

3 loops on the heavy chain
3 loops on the light chain

Theoretisch geschat humaan antilichaamrepertoire: 10 miljard

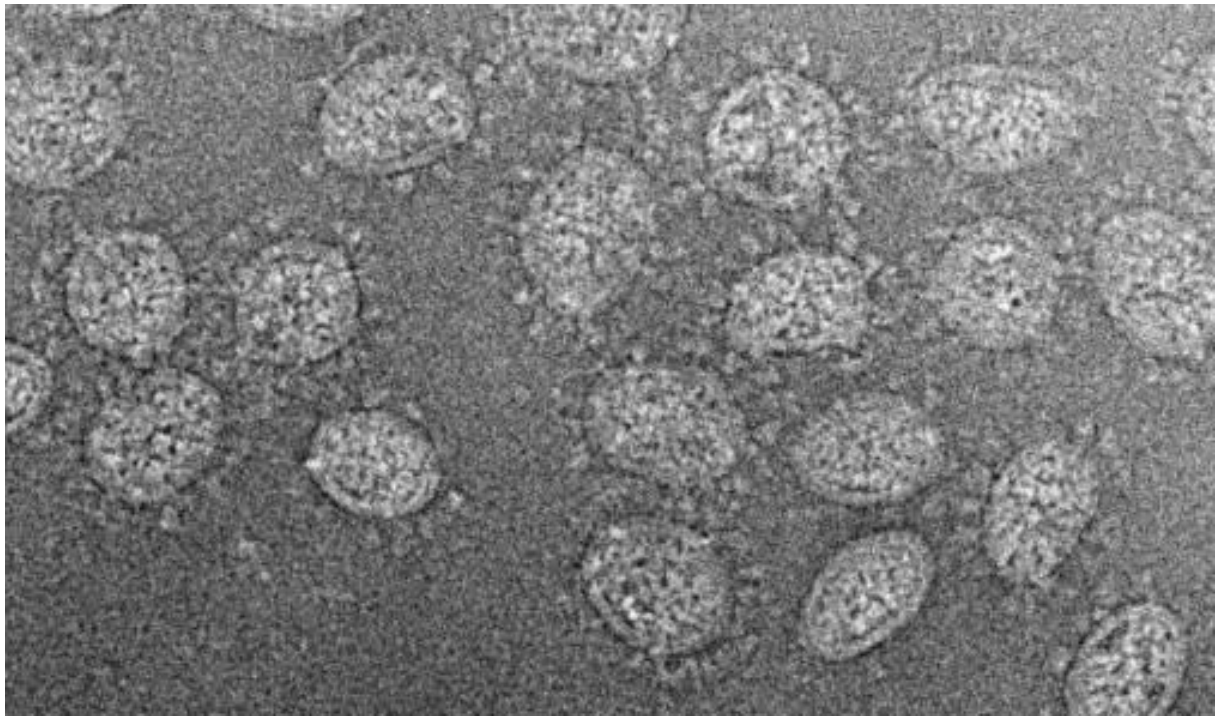
Antistoffen binden aan epitopen

- 15-22 aminozuren
- 5-6 aminozuren dragen meeste bij aan bindingsenergie
- Grootte: 6,5 – 9 nm^2
- 75 – 120 waterstofbruggen
- Andere electrostatische interacties en hydrofobe interacties
- Conformatie vaak cruciaal



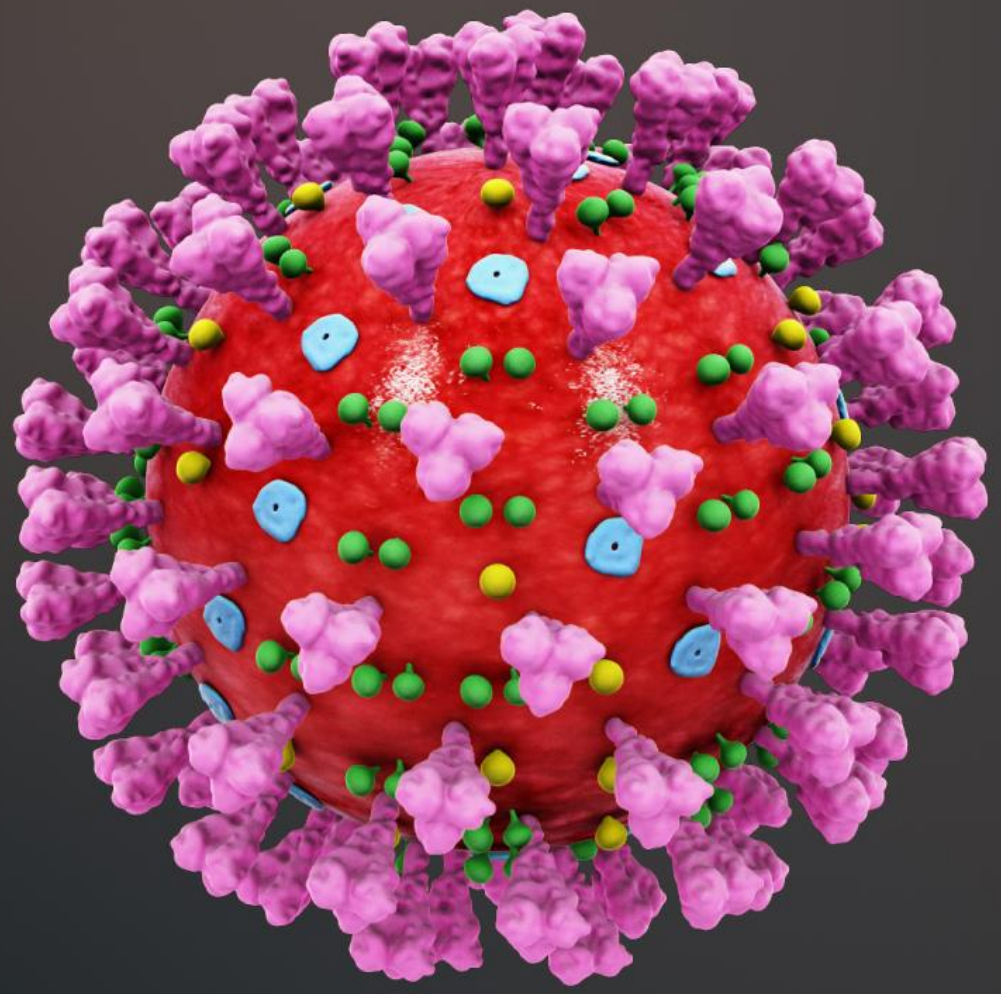
Coronavirus

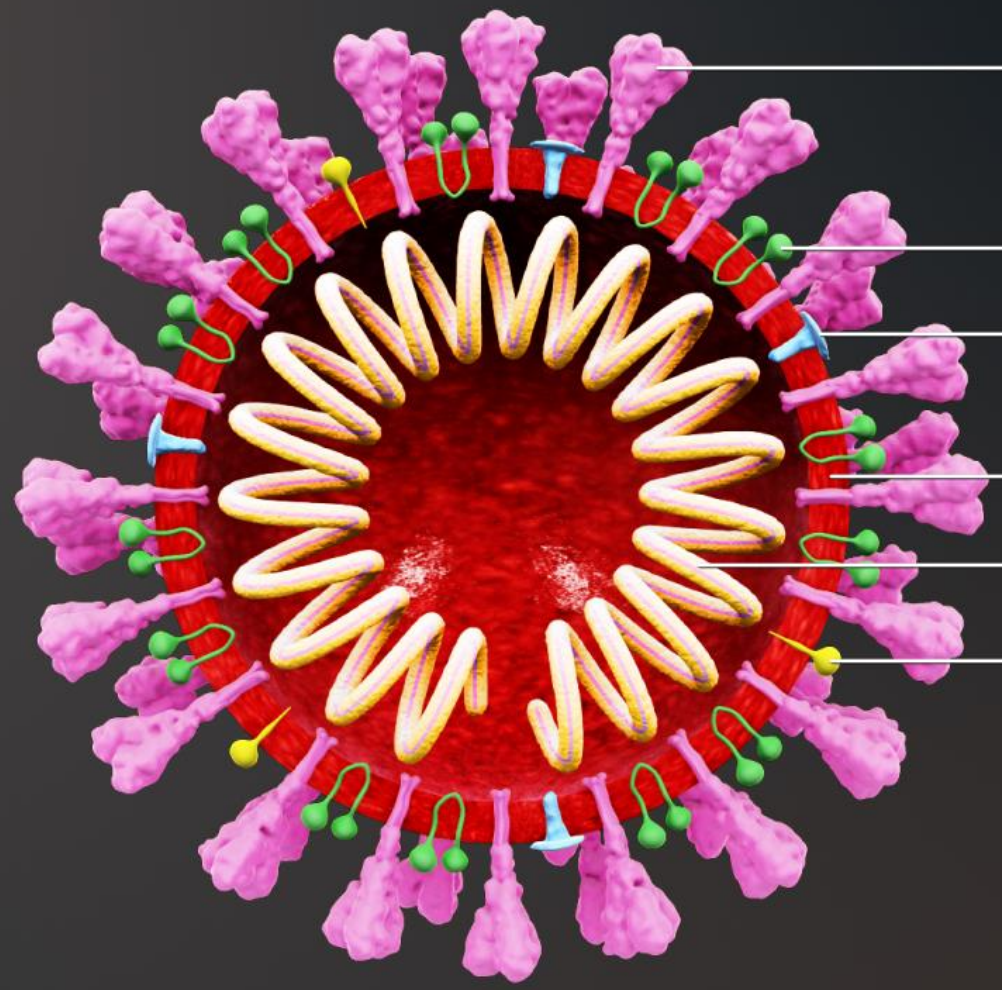
- Vier ‘oude’ humane stammen veroorzaken verkoudheid
- Drie niet-humane, zoonotische stammen veroorzaken ernstige ziekte:
  - SARS-CoV
  - MERS-CoV
  - SARS-CoV-2
- RNA virussen
- Lipide envelop
- 50-200 nm



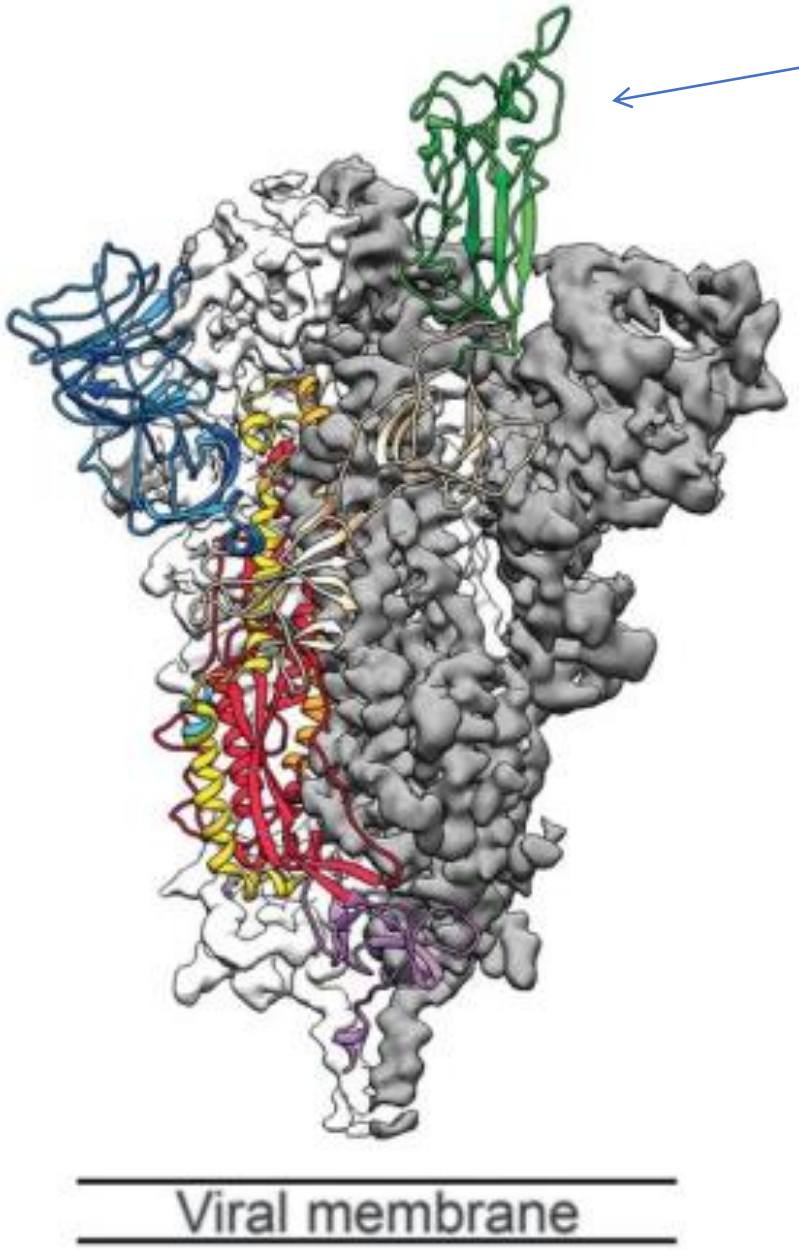
SARS, cryo-EM

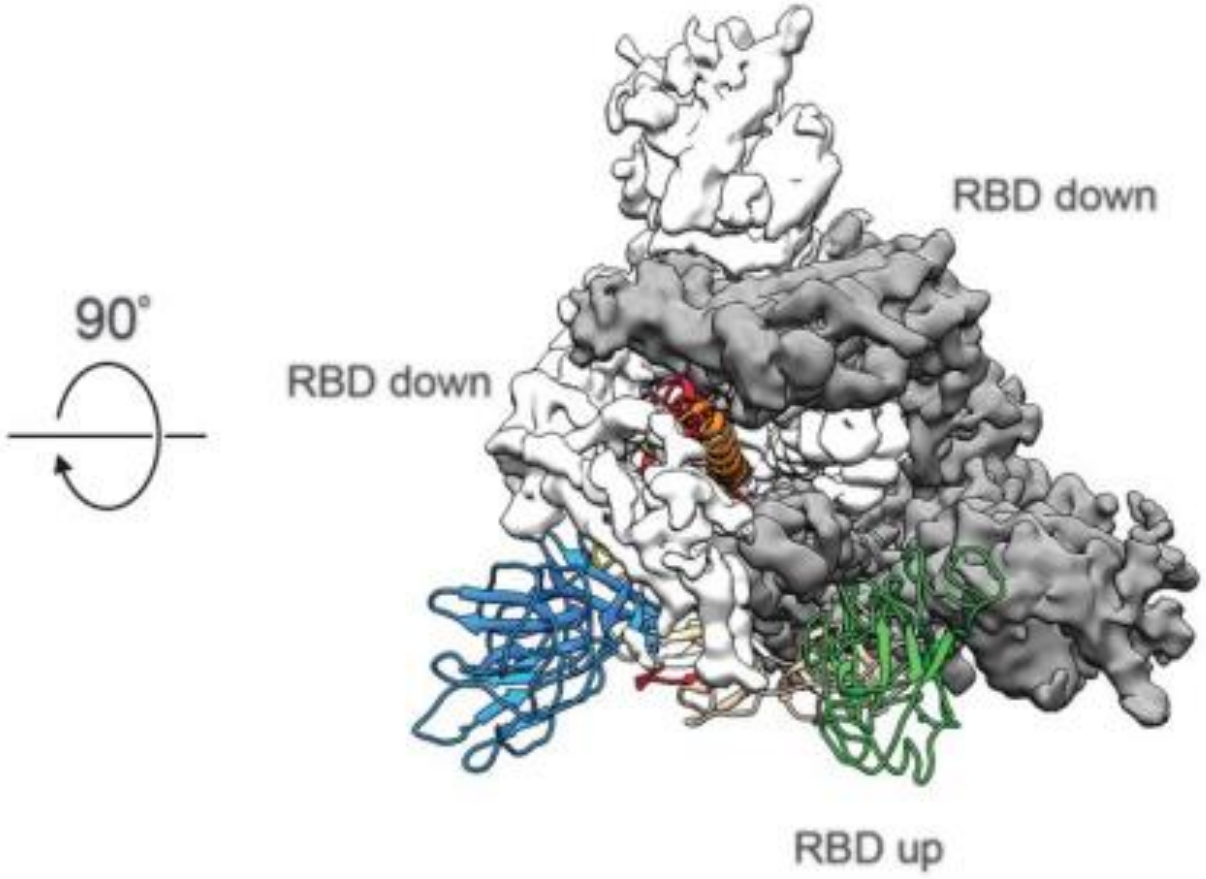
Neumann et al J Virol 2006



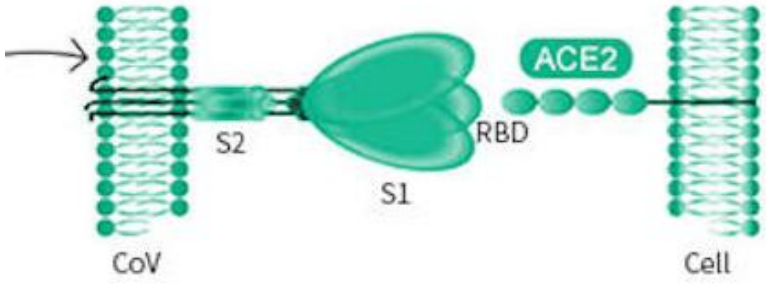

Spike Glycoprotein (S)
M-Protein
Hemagglutinin-esterase dimer (HE)
Envelope
RNA and N protein
E-Protein

Target voor antistoffen:
Receptor Binding Domain (RBD)

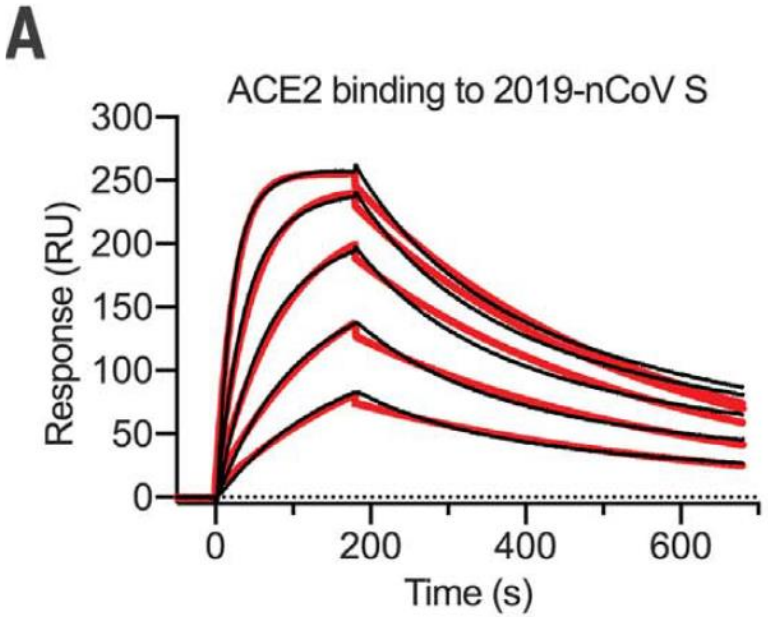




S-protein (Wrapp et al 2020)



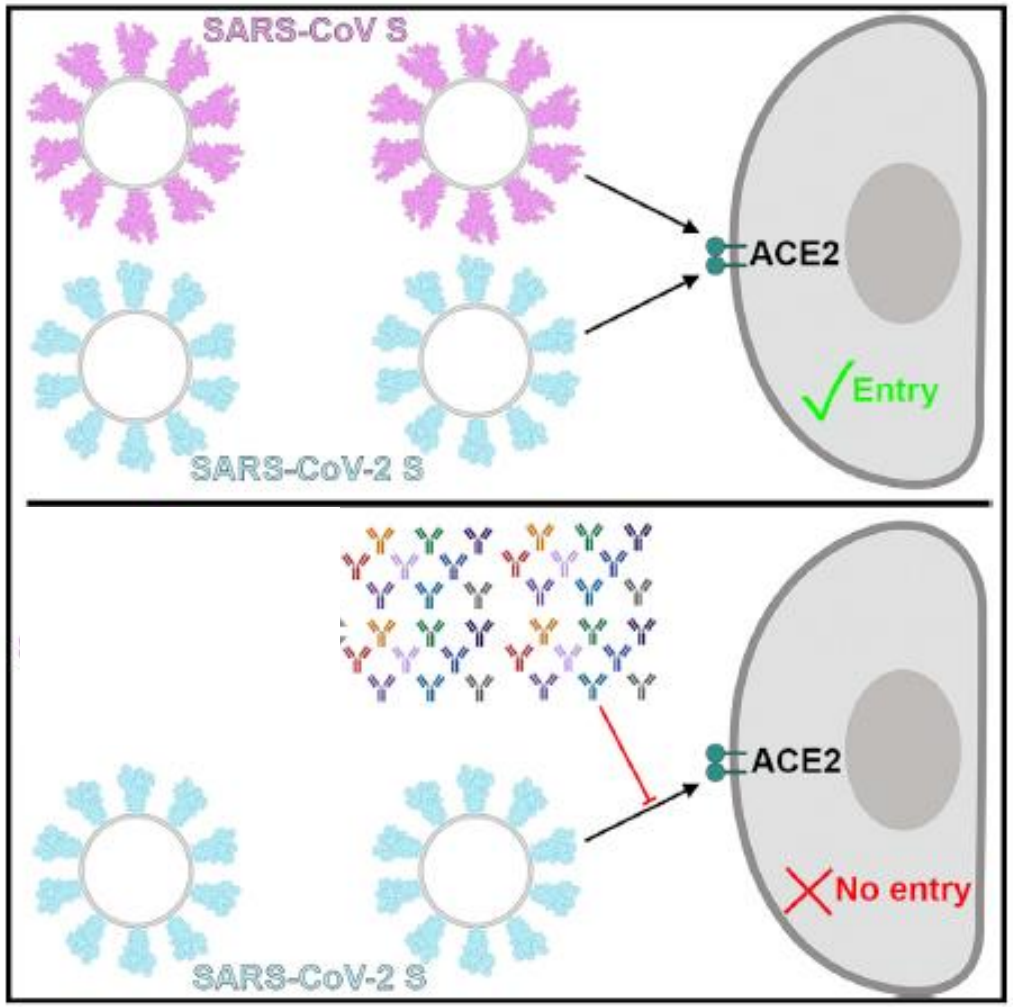
Rapid microbiology



K_D = 14.7 nM

k_a = 1.88 x10^5 $M^{-1}s^{-1}$

k_d = 2.76 x 10^{-3} s^{-1}



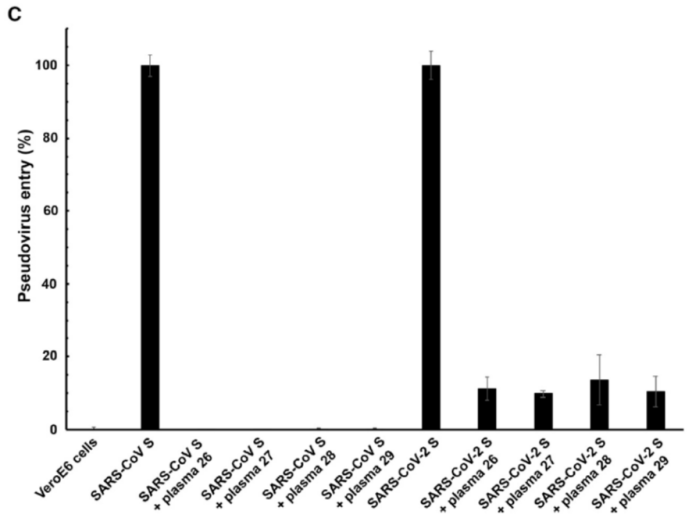
Antistoffen: K_D: 10^{-6} – 10^{-10} M

Walls et al. 2020

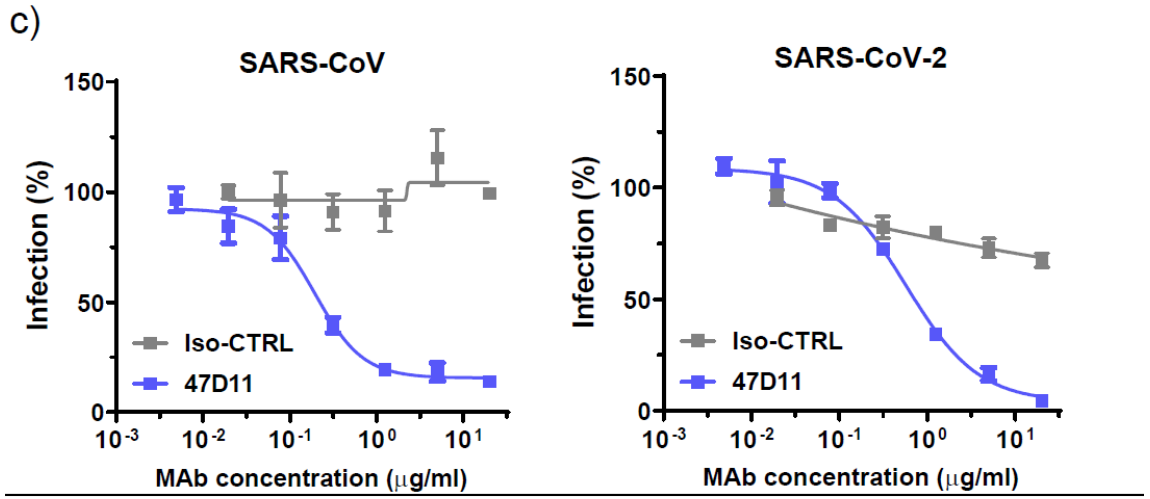
Wrapp et al Science 2020

Aanwijzingen dat antistoffen werken.

- Antistoffen tegen SARS-CoV neutraliseren SARS-CoV-2 (muizen).
- Sommige herstelde patienten hebben hoge titers virusneutraliserende antistoffen.
- Sommige monoclonale antistoffen neutraliseren SARS-CoV-2.



Walls et al. Cell 2020



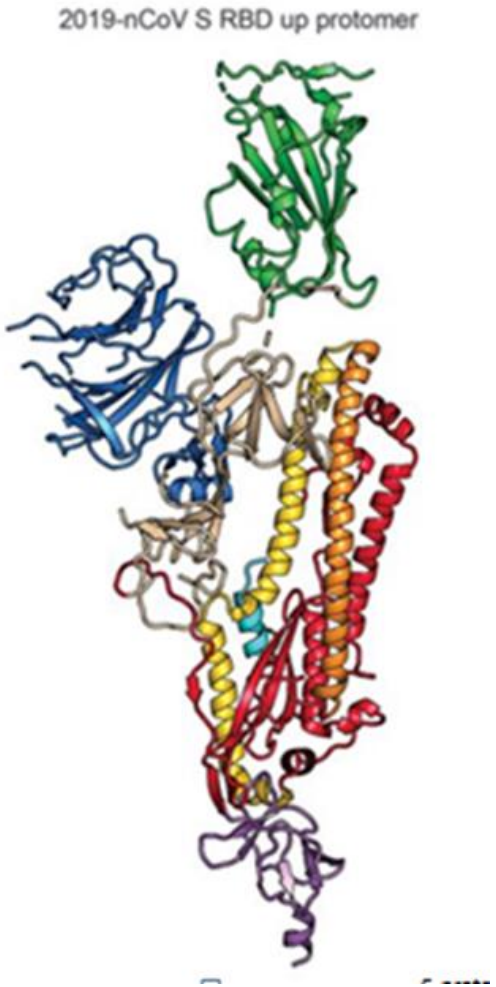
Wang et al. ArXiv

Onzekerheden

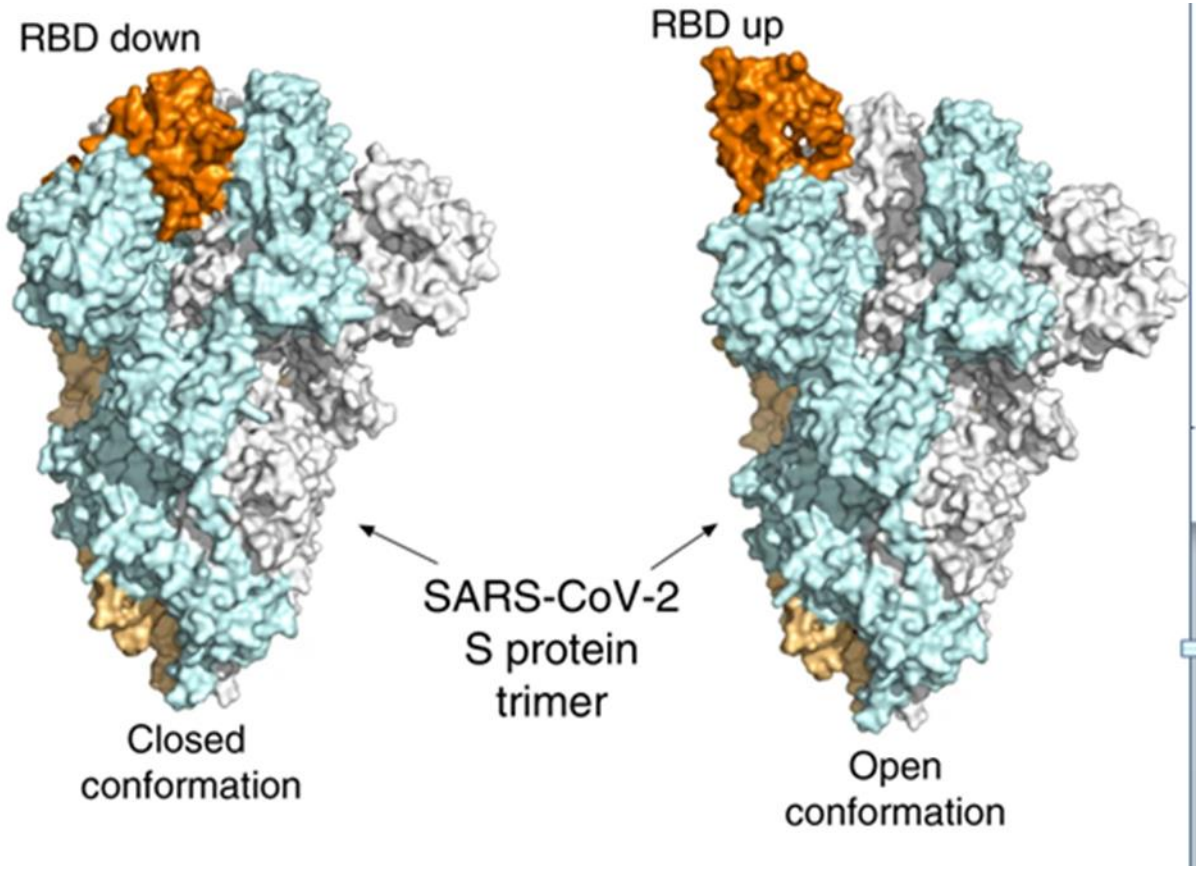
- Dynamiek van het S-eiwit:
  - **‘Dicht/open’ conformatie**
  - **Pre- en post-fusie conformatie**
  - Huidige modellen S-eiwit gebaseerd op statische methoden zoals rontgendiffractie en cryo-EM
  - Nieuwe inzichten door *in silico* modellering eiwitdynamiek (zie Folding@home)
- **Glycosylering S-eiwit**
- Is de ACE2 route de enige weg om binnen te komen? (rol siaalzuur op celoppervlak?)
- Kunnen zwak bindende antistoffen infectie verergeren?
- Is S-eiwit geconserveerd?

RBD ‘ingeklapt’ afgeschermd voor antistoffen(?)

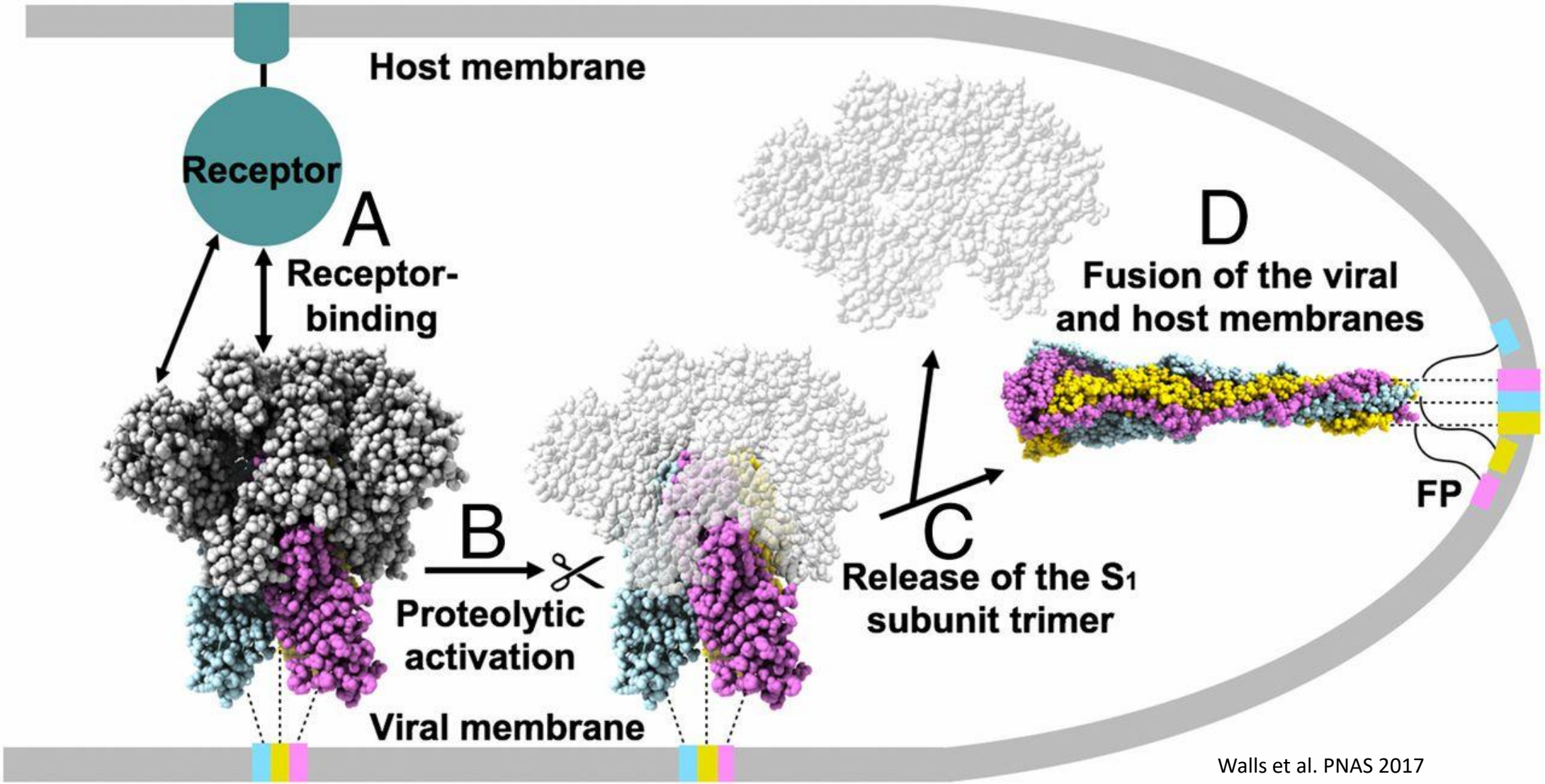
In/down conformatie



Uit/up conformatie

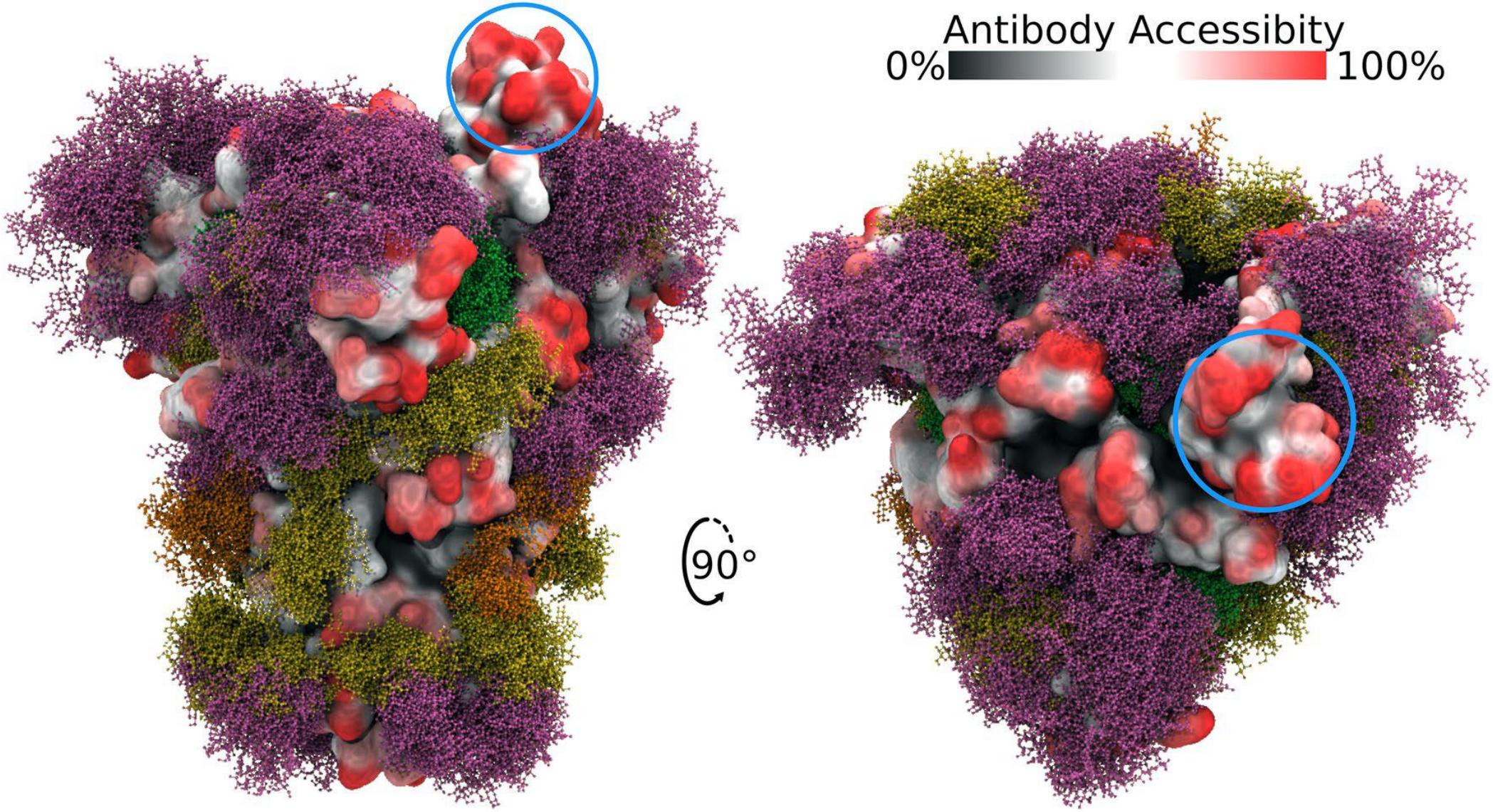


Pre-/post fusie conformatie
Na binding van virus: membraanfusie



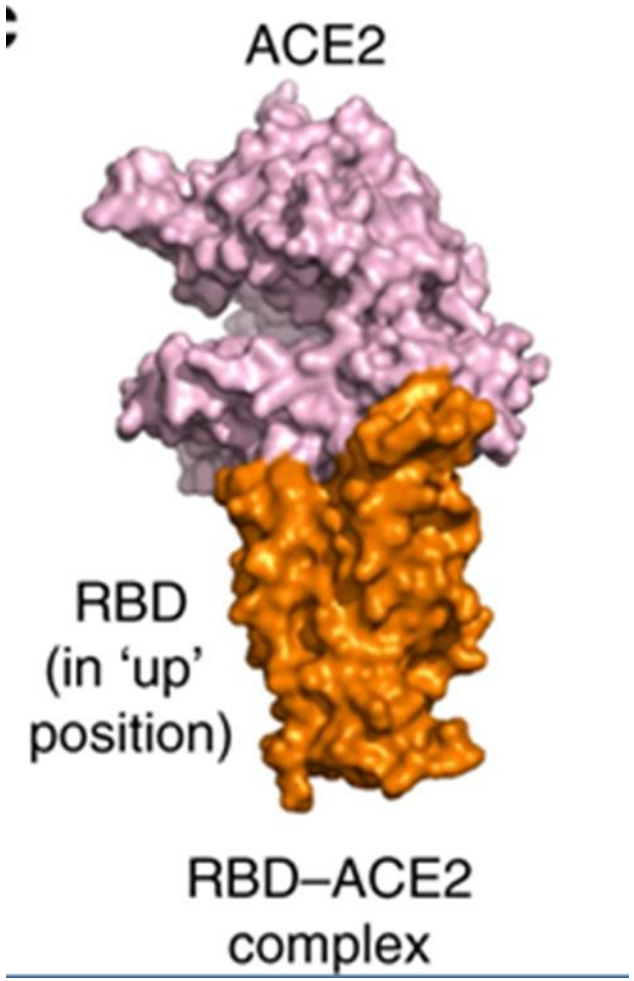
Walls et al. PNAS 2017

S-eiwit heeft 22 (66) glycosyleringsplaatsen

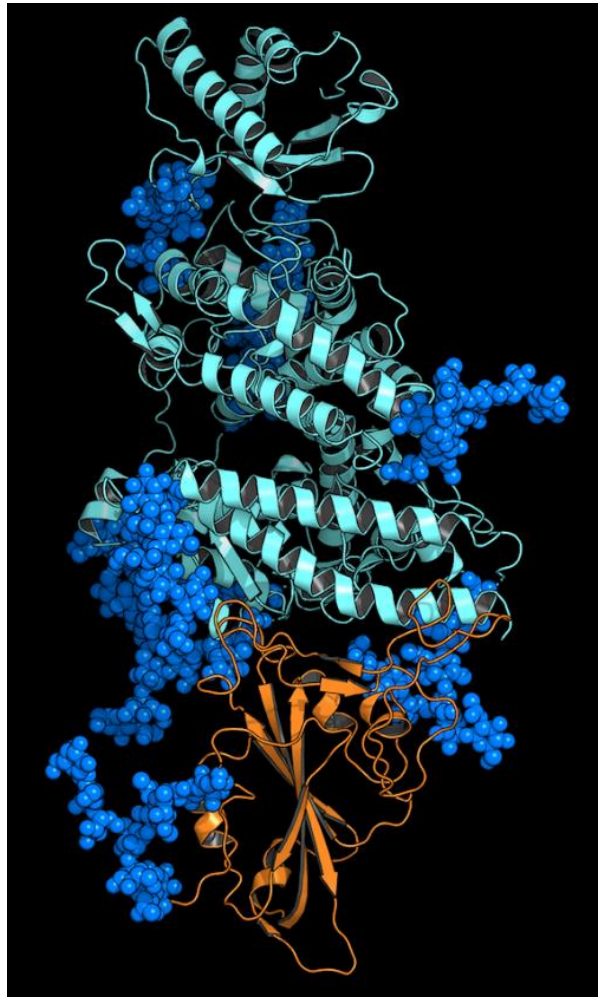


Grant et al Sci Rep 2020

Gebruik alleen wat je nodig hebt?
Voor vaccin is RBD wellicht voldoende of optimaal.



Zhang et al. Nature Com 2020



Folding@home

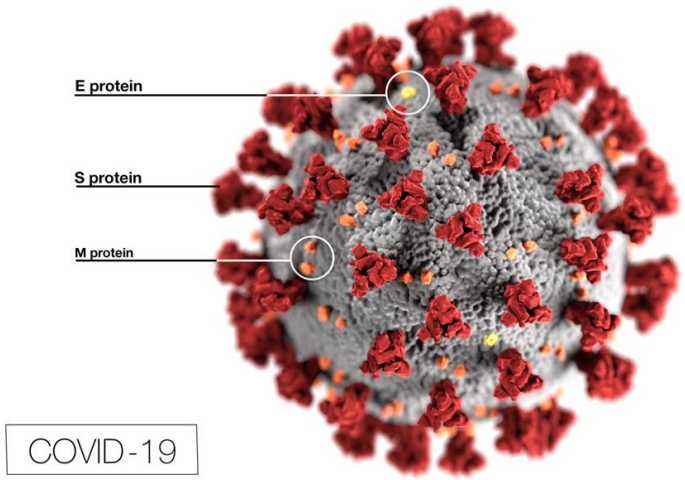
Veel vaccin-families

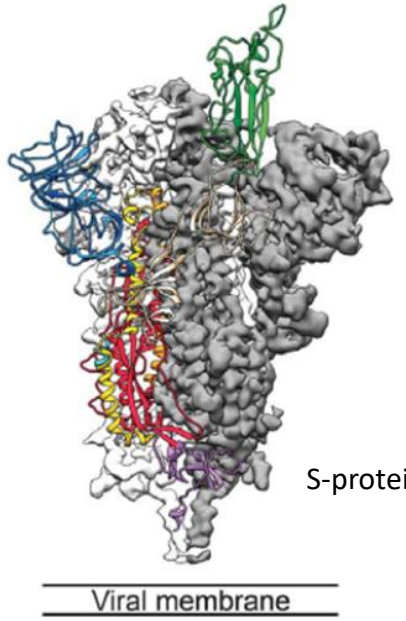
Op de markt:

- Levend verzwakt
- **Geinactiveerd**
- **Subunit** (gezuiverde eiwitten, polysacchariden, virus-like particles)

Experimenteel:

- Recombinant vector vaccins
- **DNA/RNA**
- Synthetische peptides

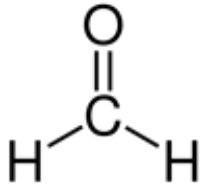




S-protein (Wrapp et al 2020)

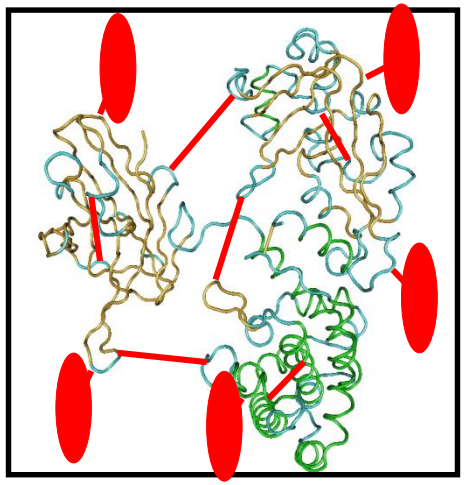
Geinactiveerd vaccins

- Chemische inactivering met formaldehyde
- Covalente veranderingen aan eiwit (en genetisch materiaal)

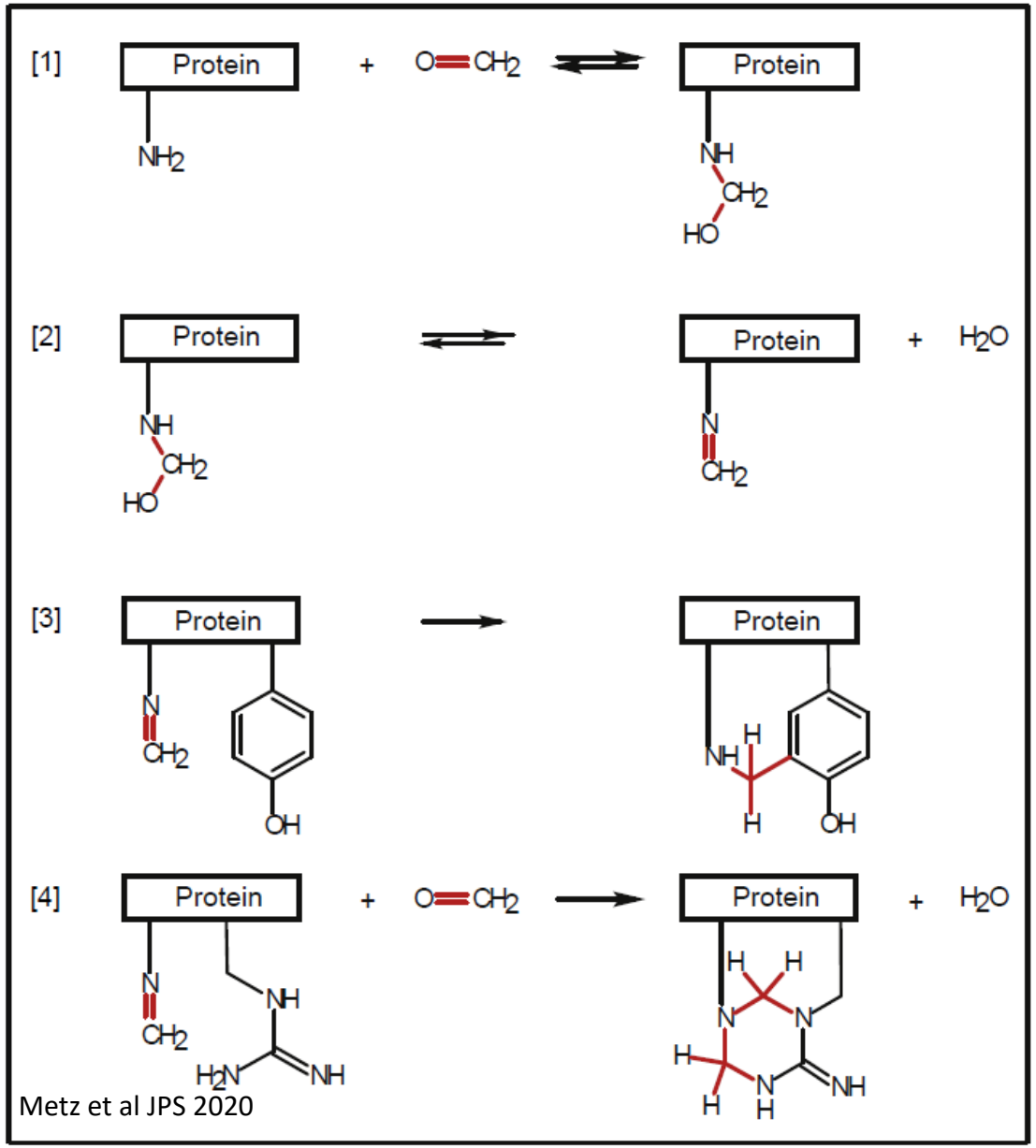


Formaldehyde

Amino acids



Formaldehyde – eiwit crosslinks

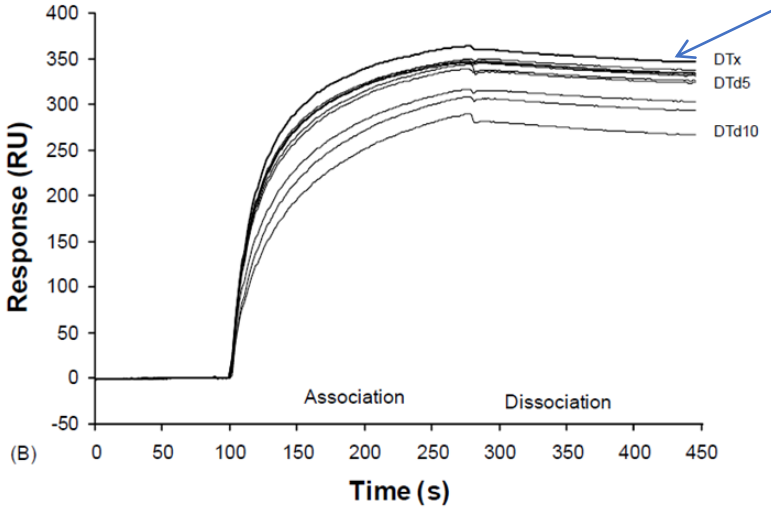


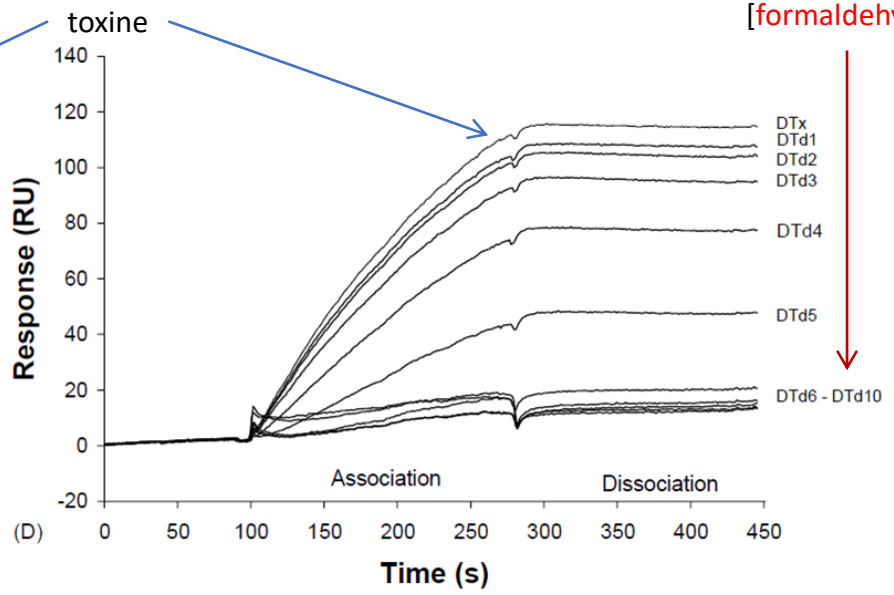
Metz et al JPS 2020

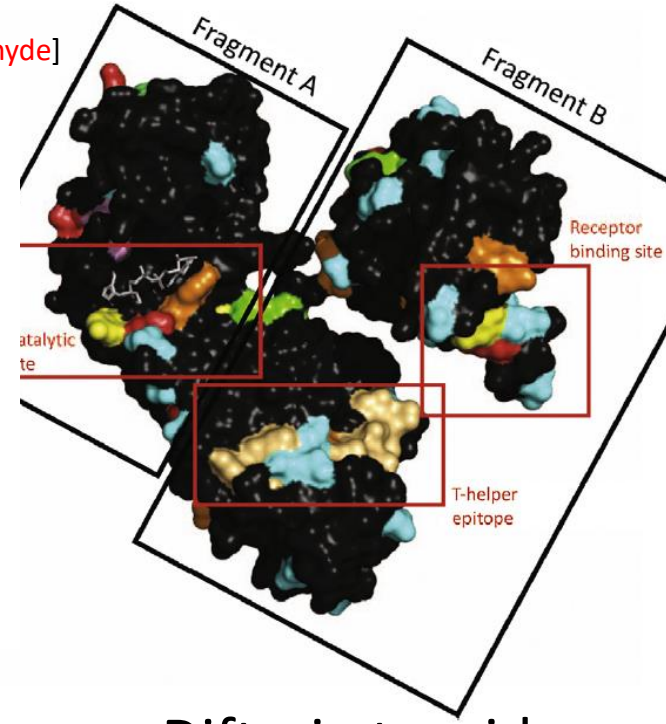
Example structure	Reactive residues
N Protein	K, Q, N, W, H
O X N Protein HN	Free *N* termini
Protein N H Protein HO	K, Y
N H Protein	K
Protein N N Protein H_2N N	K, R
HN Protein HO	K, Q, N, W, H
HO O N H Protein HO	H, W, Q, N
	Y
Protein N N O OH H_2N N	R

Michiels et al Sci Rep 2020

Formaldehyde kan delen van het eiwit modificeren die van belang zijn voor herkenning door het immuunsysteem







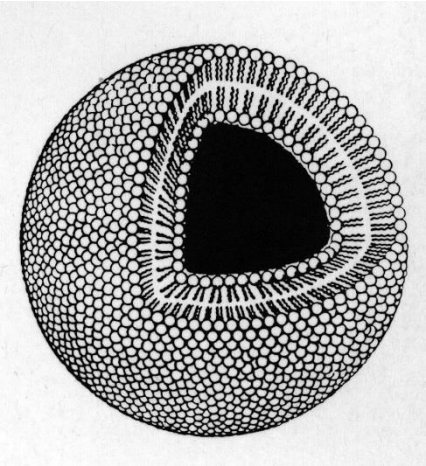
Binding antistof 1 aan toxine en toxoid
Formaldehyde reageert niet met epitoop 1

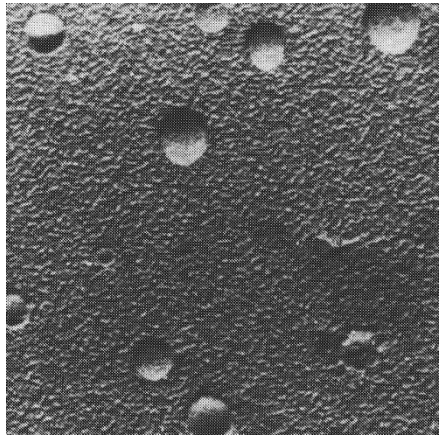
Binding antistof 2 aan toxine en toxoid
Formaldehyde modificeert epitoop 2

Difterie toxoid met formaldehyde modificaties

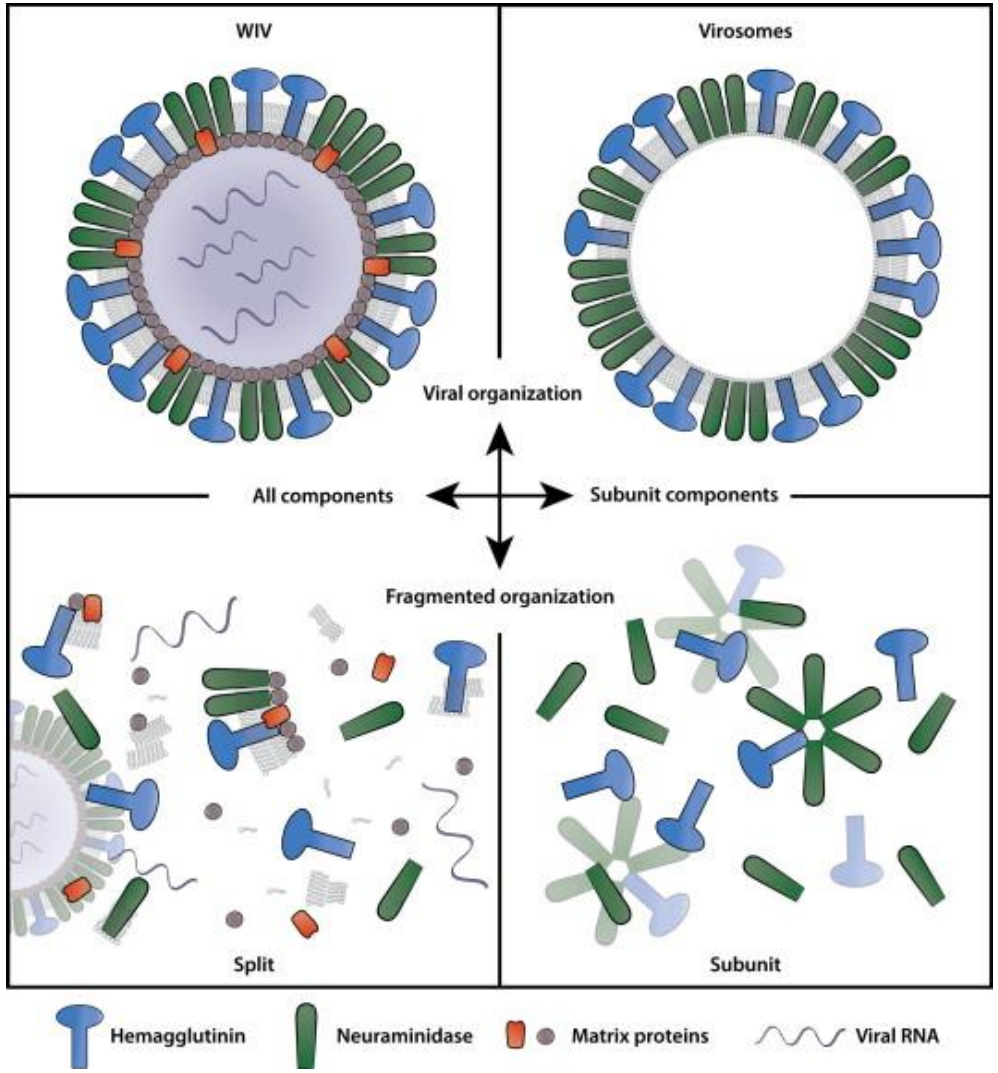
Metz et al. Vaccine 2003
Metz et al. JPS 2020

Delivery systeem voor subunit vaccin: virus-like particles (virosomen)



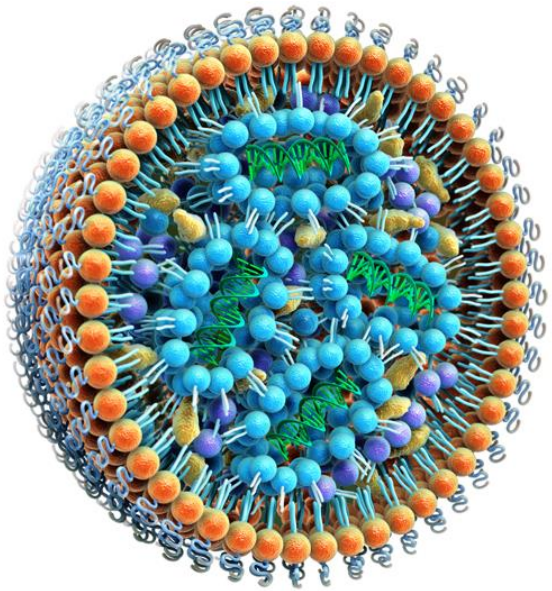


liposoom



Soema et al. 2015

Delivery systeem voor RNA vaccins: lipid based



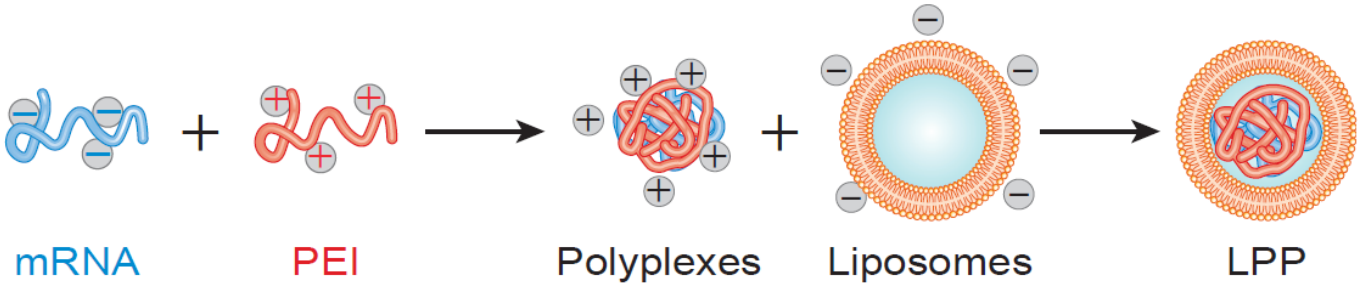
Precision Nanosystems

Lipid nanoparticles (LNP):

- Geladen lipiden (lichtblauw) -> interactie met RNA
- Fosfolipiden (paars)
- Cholesterol (geel)
- PEG-Lipide conjugaten (oranje) -> camouflage

Lipopolyplexes (LPP)

- Polyethyleenimine
- Fosfolipiden
- Cholesterol
- Geladen lipiden

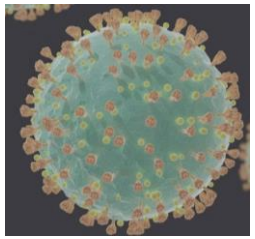


Perche et al Mol Ther Nucl Acids 2019

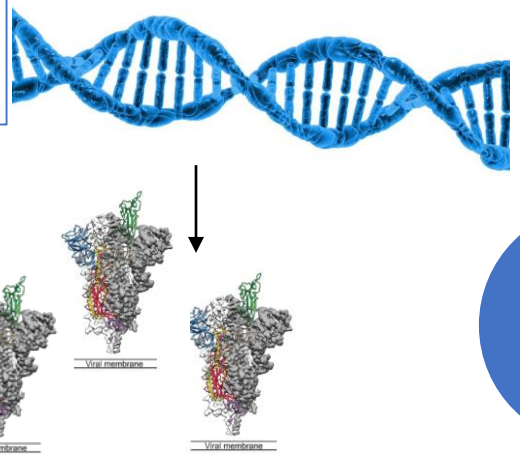
Covid-19 vaccin concepten

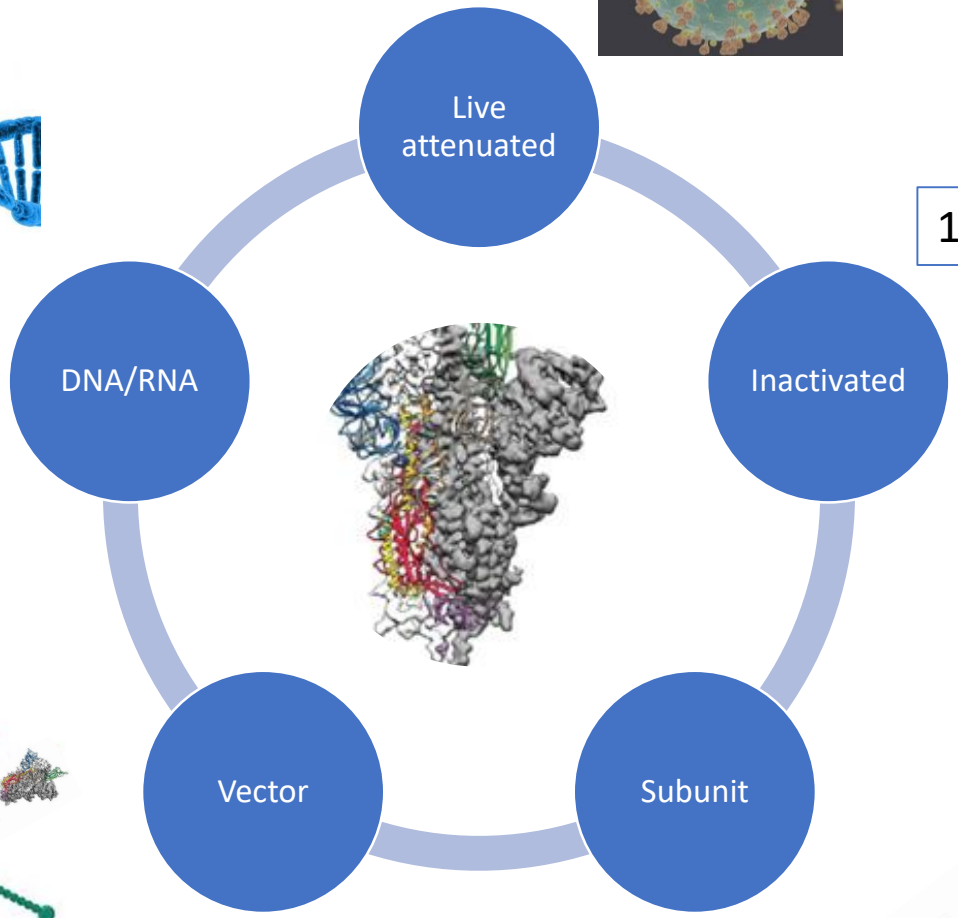
Aantal initiatieven per 19 October (bron: WHO)

3



18 DNA
25 RNA





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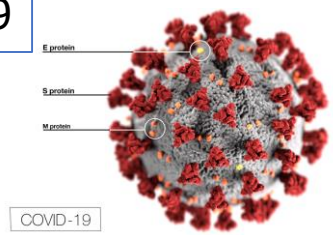

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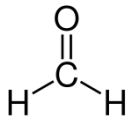
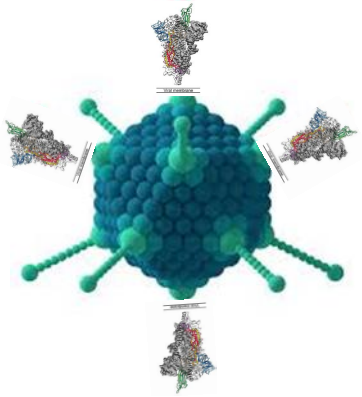
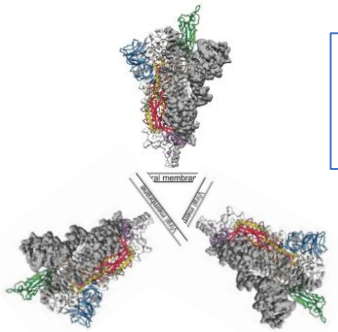


27 replicating
22 non-replicating





69 subunit
15 virus-like particle

Klinische ontwikkeling van Covid-19 vaccins

- Fase 3:
  - 10 vaccins (4 vector vaccins*), 3 geinactiveerd*), 2 RNA, 1 subunit)
- Fase 2:
  - 2 vaccins (1 RNA, 1 subunit)
- Fase 1/2:
  - 11 vaccins (3 geinactiveerd, 4 DNA, 1 RNA, 2 subunit, 1 VLP)
- Fase 1:
  - 21 vaccins (1 geinactiveerd, 8 vector, 9 subunit, 2 RNA, 1 VLP)

*)Sinovac en Gamaleya Research Institute : fase 4(?)

Status 19-oct-2020

Tot slot

Immunologie, vaccinologie, bioprocestechnologie, moleculaire biologie

vormen de basis voor succesvolle SARS-CoV2 vaccinontwikkeling en – productie maar uiteindelijk zijn dit toepassingen van

chemie, natuurkunde, wiskunde

Vlakvulling II,
MC Escher

Dank voor Uw aandacht