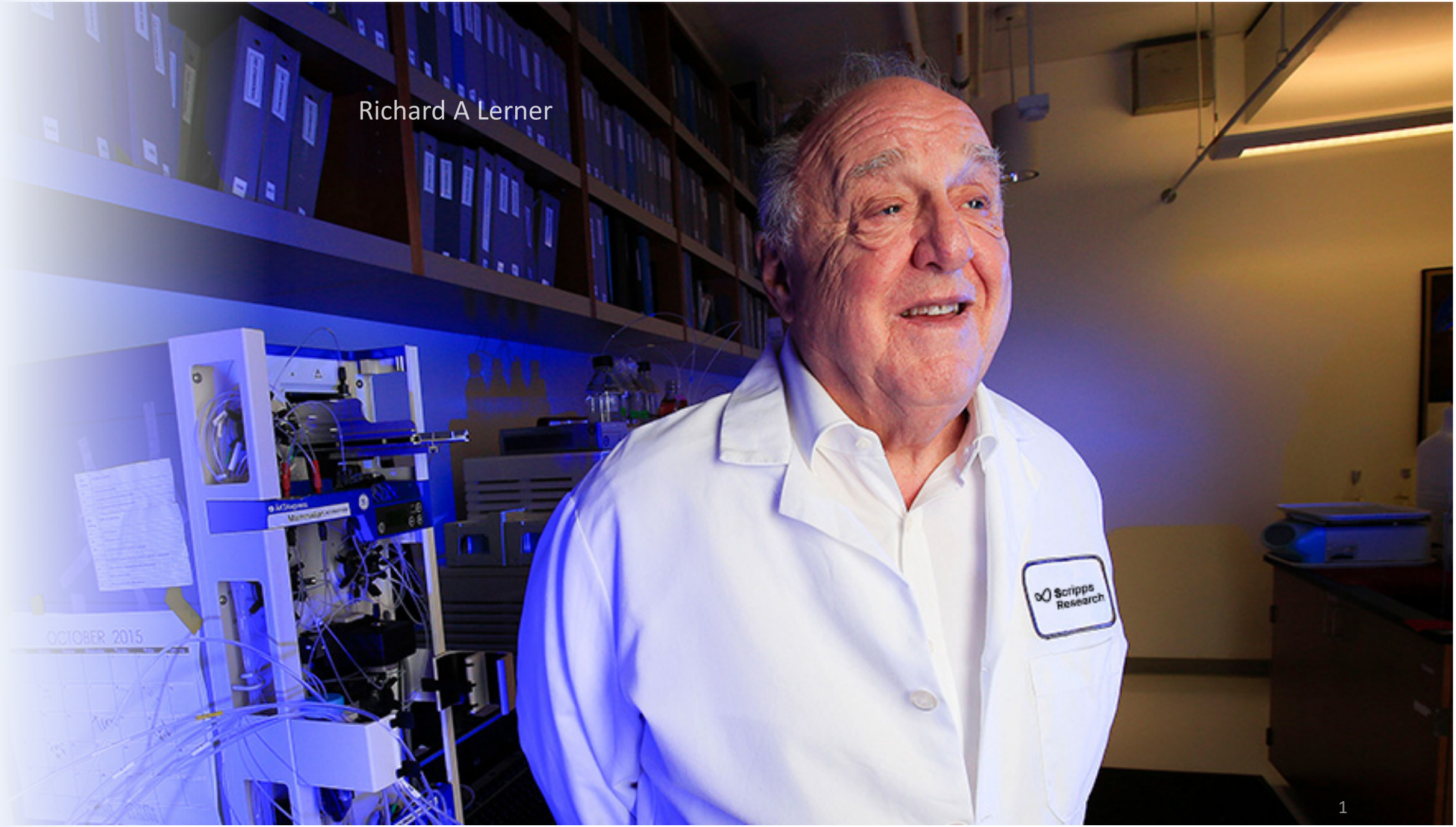

Richard A Lerner
Scripps Research
OCTOBER 2015

Discovery, Expression, and Characterization of Neutralizing Picobodies against SARS-CoV2

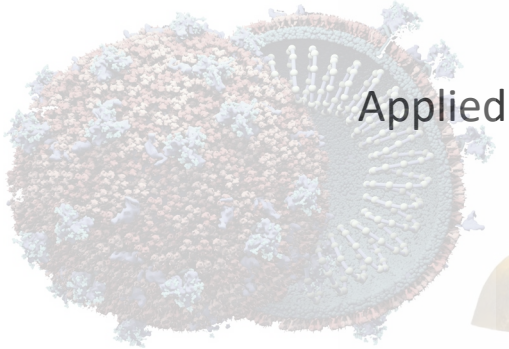
Antibody Engineering and Therapeutics

Dec. 13, 2021

Vaughn Smider, M.D., Ph.D.

President

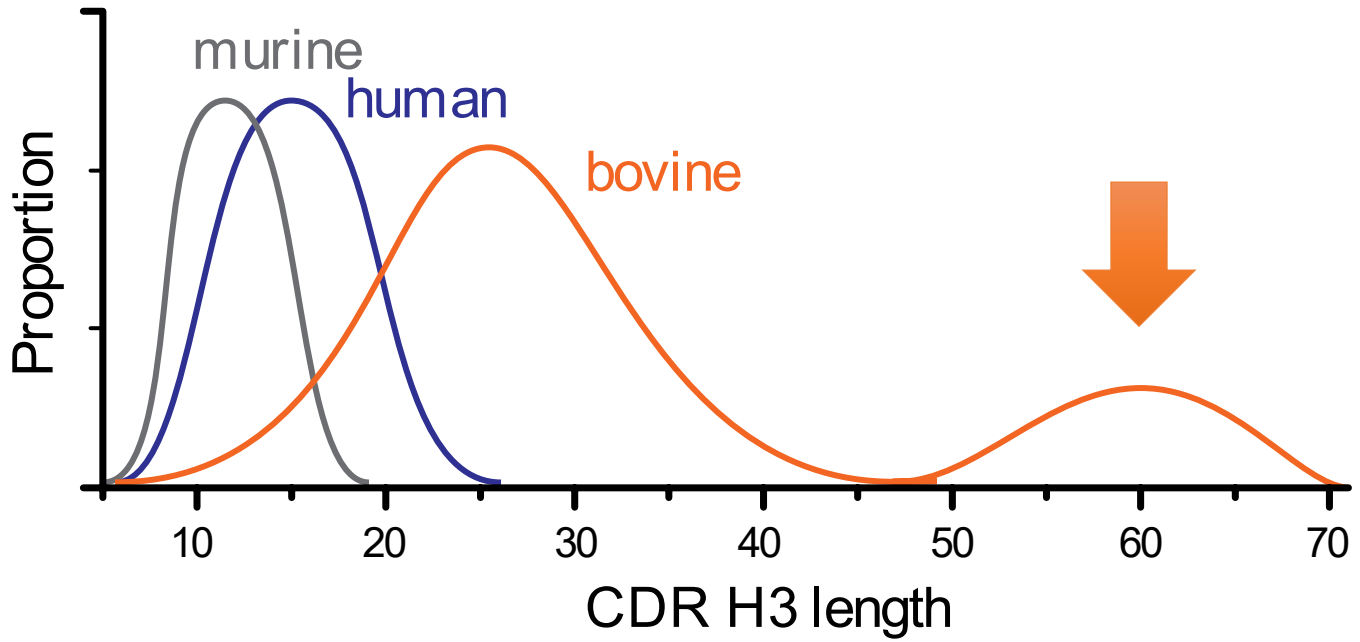
Applied Biomedical Science Institute



Applied
Biomedical
Science Institute

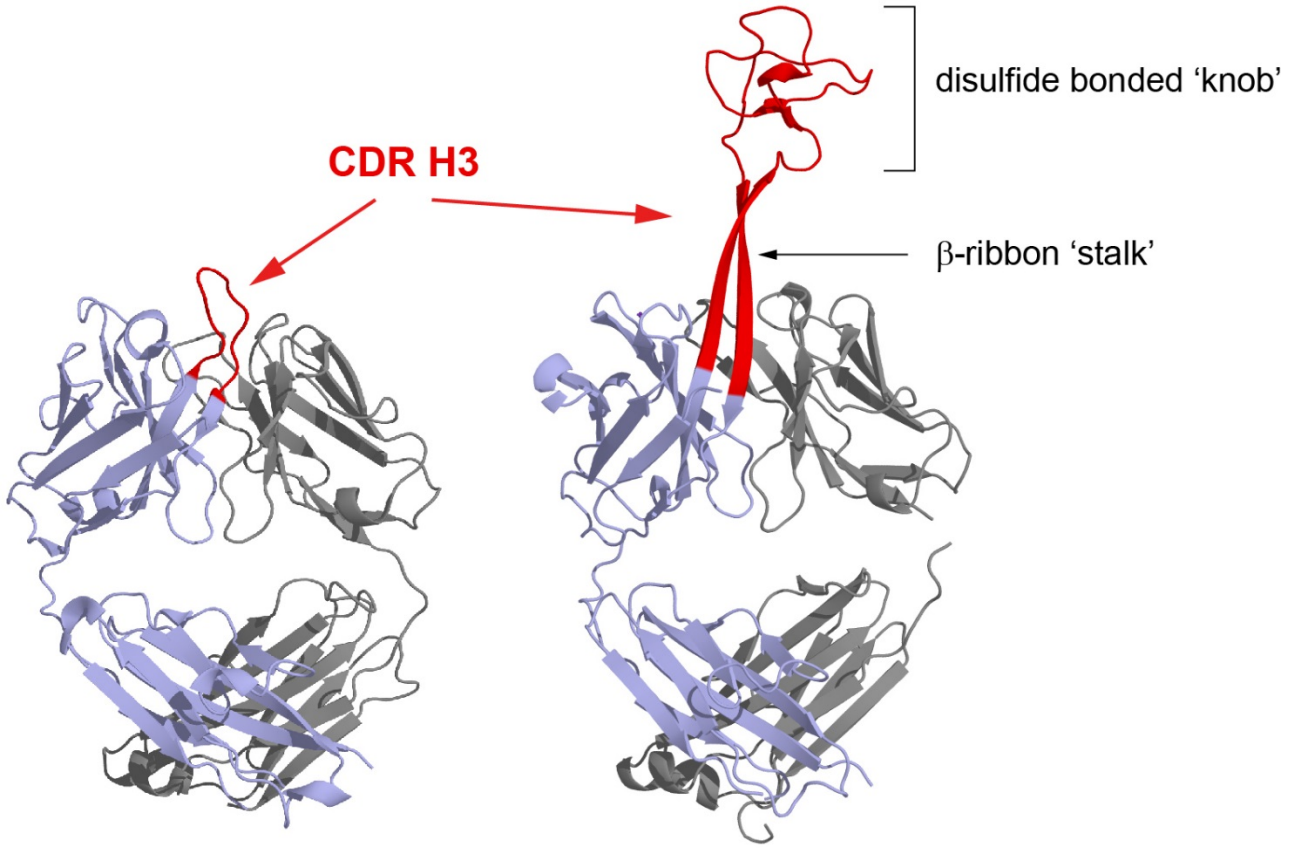
Ligand® OmniTaur™

Cows have long CDR H3s, with an ultralong subset (40-70 amino acids)



Applied
Biomedical
Science Institute

Cow ultralong CDR H3s have protruding ‘stalk’ and ‘knob’ minidomains

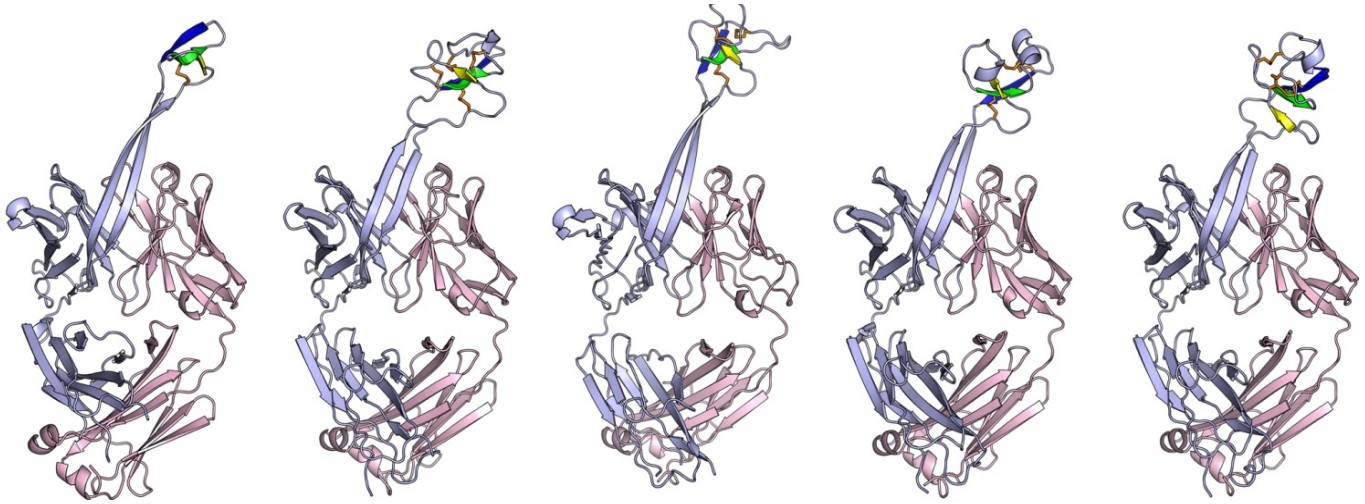


Stanfield, et al. (2017) *Adv Immunol*, 137: 135

Wang et.al. (2013) *Cell* 153: 1379-1393

Applied
Biomedical
Science Institute

Structures reveal conserved and diverse features of ultralong CDR H3s

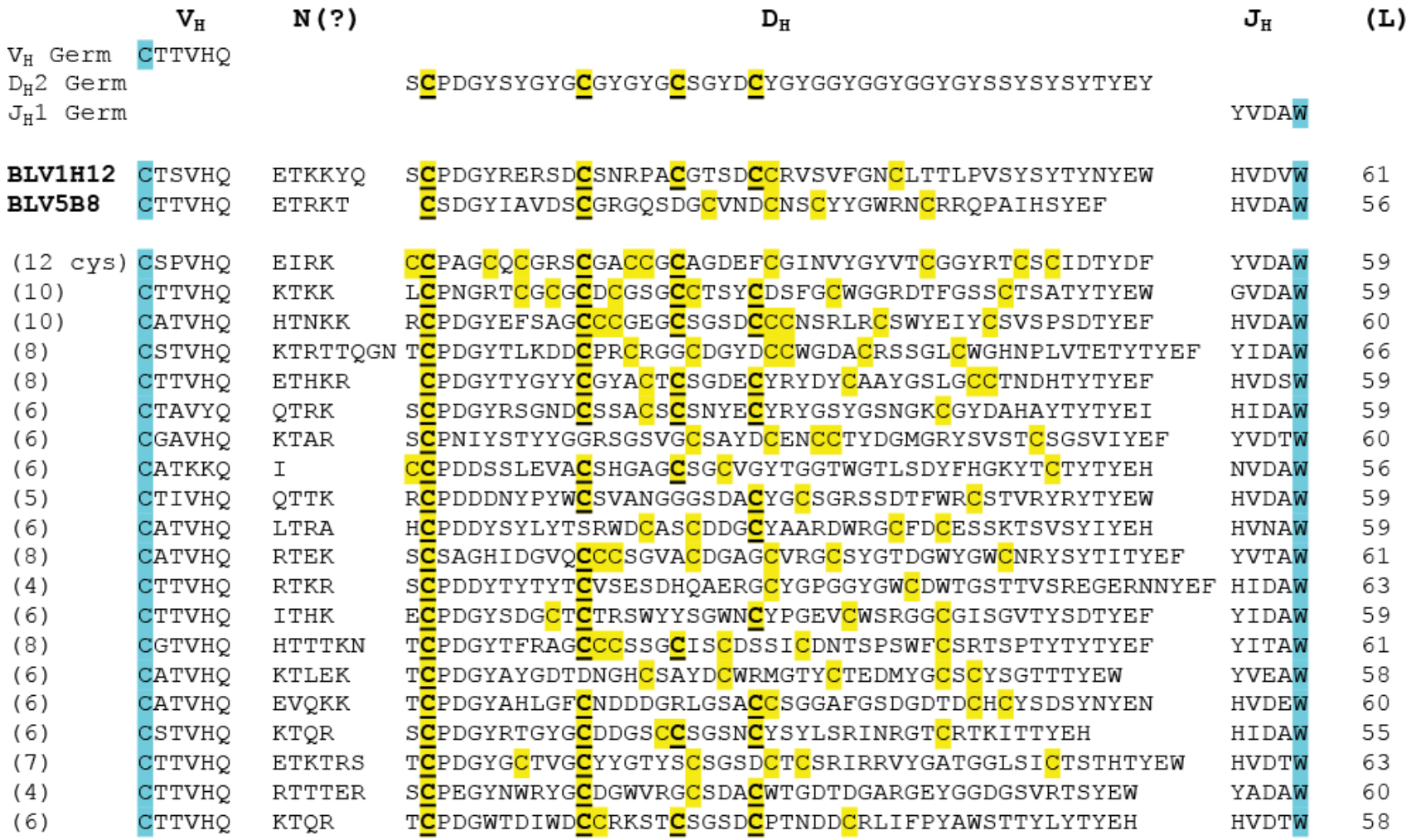


Length:	44	63	61	56	61
Cys:	2	8	6	6	6
Pattern:	1-2	1-4, 2-7, 3-8, 5-6	1-4, 2-6, 3-5	1-3, 2-4, 5-6	1-4, 2-5, 3-6

Stanfield, et.al. (2016) *Science Immunology*: 1(1)

Applied
Biomedical
Science Institute

Ultralong CDR H3 sequences are enormously diverse



	V_H	N(?)	D_H	J_H	(L)
V_H Germ	CTTVHQ				
D_H2 Germ			SCPDGYSYGYGCGYGYGCSGYDCYGYGGYGGYGGYGYSSYSYSYTYEY		
J_H1 Germ				YVDAW	
BLV1H12	CTSVHQ	ETKKYQ	SCPDGYRERSDCSNRPACGTSDCCRVSVFGNCLTTLPVSYSYTYNYEW	HVDVW	61
BLV5B8	CTTVHQ	ETRKT	CSDGYIAVDSCGRGQSDGCVNDCNSCYYGWRNCRRQPAIHSYEF	HVDAW	56
(12 cys)	CSPVHQ	EIRK	CCPAGCQCGRSCGACCGCAGDEFCGINVYGYVTCGGYRTCSCIDTYDF	YVDAW	59
(10)	CTTVHQ	KTKK	LCPNGRTCGCGCDCGSGCCTSYCDSFGCWGGRDTFGSSCTSATYTYEW	GVDAW	59
(10)	CATVHQ	HTNKK	RCPDGYEFSAGCCCGEGCSGSDCCCNSRLRCSWYEIYCSVSPSDTYEF	HVDAW	60
(8)	CSTVHQ	KTRTTQGN	TCPDGYTLKDDCPRCRGGCDGYDCCWGDACRSSGLCWGHNPLVTETYTYEF	YIDAW	66
(8)	CTTVHQ	ETHKR	CPDGYTYGYYCGYACTCSGDECYRYDYCAAYGSLGCCTNDHTYTYEF	HVDSW	59
(6)	CTAVYQ	QTRK	SCPDGYRSGNDCSSACSCSNYECYRYGSYGSNGKCGYDAHAYTYTYEI	HIDAW	59
(6)	CGAVHQ	KTAR	SCPNIYSTYYGGRSGSVGCSAYDCENCCTYDGMGRYSVSTCSGSVIYEF	YVDTW	60
(6)	CATKKQ	I	CCPDDSSLEVACSHGAGCSGCVGYTGGTWGTLSDYFHGKYTCTYTYEH	NVDAW	56
(5)	CTIVHQ	QTTK	RCPDDDNYPYWCSVANGGGSDACYGCSGRSSDTFWRCSTVRYRYTYEW	HVDAW	59
(6)	CATVHQ	LTRA	HCPDDYSYLYTSRWDCASCDDGCYAARDWRGCFDCESSKTSVSYIYEH	HVNAW	59
(8)	CATVHQ	RTEK	SCSAGHIDGVQCCCSGVACDGAGCVRGCSYGTDGWYGWCNRYSYTITYEF	YVTAW	61
(4)	CTTVHQ	RTKR	SCPDDYTYTYTCVSESDHQAERGCYGPGGYGWCDWTGSTTVSREGERNNYEF	HIDAW	63
(6)	CTTVHQ	ITHK	ECPDGYSDGCTCTRSWYYSGWNCYPGEVCWSRGGCGISGVTYSDTYEF	YIDAW	59
(8)	CGTVHQ	HTTTKN	TCPDGYTFRAGCCCSSGCISCDSSICDNTSPSWFCSRTSPTYTYTYEF	YITAW	61
(6)	CATVHQ	KTLEK	TCPDGYAYGDTDNGHCSAYDCWRMGTYCTEDMYGCSCYSGTTTYEW	YVEAW	58
(6)	CATVHQ	EVQKK	TCPDGYAHLGFCNDDDGRLGSACCSGGAFGSDGDTDCHCYSDSYNYEN	HVDEW	60
(6)	CSTVHQ	KTQR	SCPDGYRTGYGCDDGSCCSGSNCYSYLSRINRGTCRTKITTYEH	HIDAW	55
(7)	CTTVHQ	ETKTRS	TCPDGYGCTVGCYYGTYSCSGSDCTCSRIRRVYGATGGLSICTSTHTYEW	HVDTW	63
(4)	CTTVHQ	RTTTER	SCPEGYNWRYGCDGWVRGCSDACWTGDTDGARGEYGGDGSVRTSYEW	YADAW	60
(6)	CTTVHQ	KTQR	TCPDGWTDIWDCCRKSTCSGSDCPTNDDCRLIFPYAWSTTYLYTYEH	HVDTW	58

Applied
Biomedical
Science Institute

Wang et.al. (2013) *Cell* 153: 1379-1393

Cows are the only species that can rapidly produce broadly neutralizing antibodies against HIV

LETTER

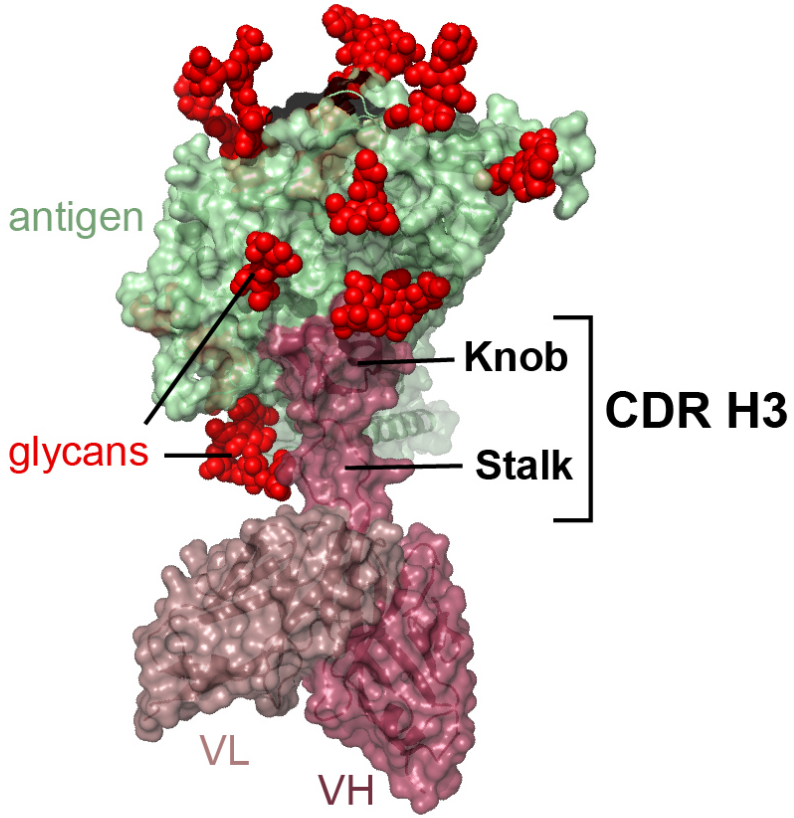
doi:10.1038/nature23301

Rapid elicitation of broadly neutralizing antibodies to HIV by immunization in cows

Devin Sok[1,2,3,4]*, Khoa M. Le[1,2,3,4]*, Melissa L. Vadnais[5], Karen L. Saye-Francisco[1,2,3], Joseph G. Jardine[1,2,3], Jonathan L. Torres[6], Zachary T. Berndsen[6], Leopold Kong[6], Robyn Stanfield[6], Jennifer Ruiz[1,2,3,4], Alejandra Ramos[1,2,3,4], Chi-Hui Liang[1,2,3], Patricia L. Chen[7], Michael F. Criscitiello[7], Waithaka Mwangi[8], Ian A. Wilson[2,3,6], Andrew B. Ward[2,3,6], Vaughn V. Smider[5] & Dennis R. Burton[1,2,3,9]

Sok et.al. (2017) *Nature* 548: 108-111

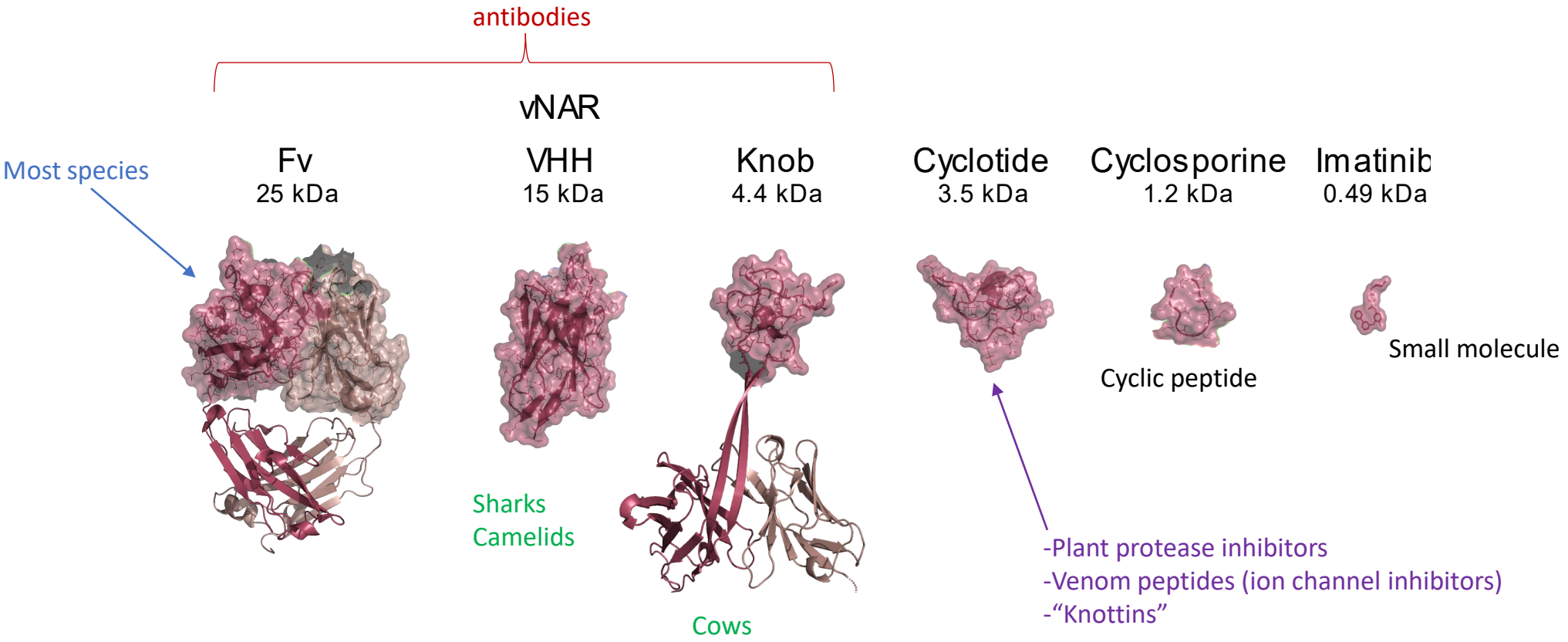
NC-Cow1 Ultralong CDR H3 binds the recessed CD4 epitope on HIV Env



Stanfield, et.al. (2020) *Sci Adv* 6(20): eaba0468.

Applied
Biomedical
Science Institute

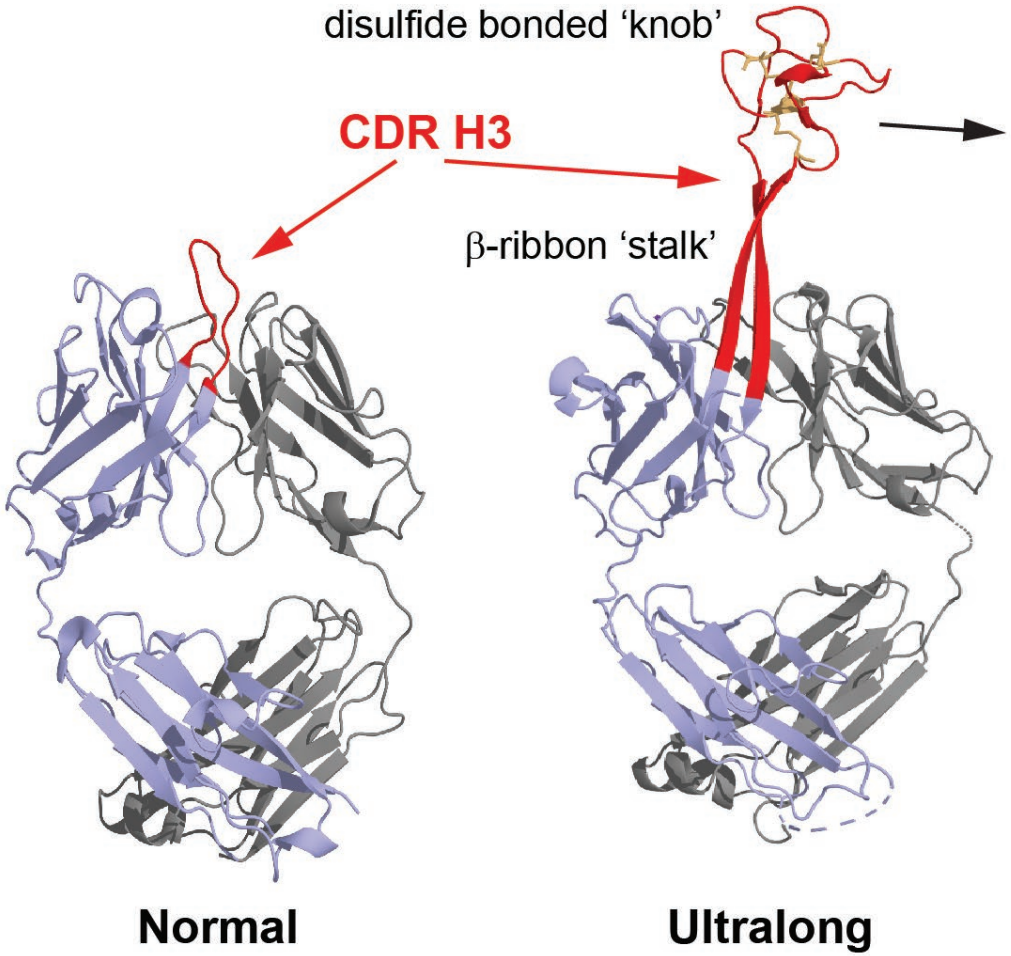
Target binding domains of biologics and small molecules

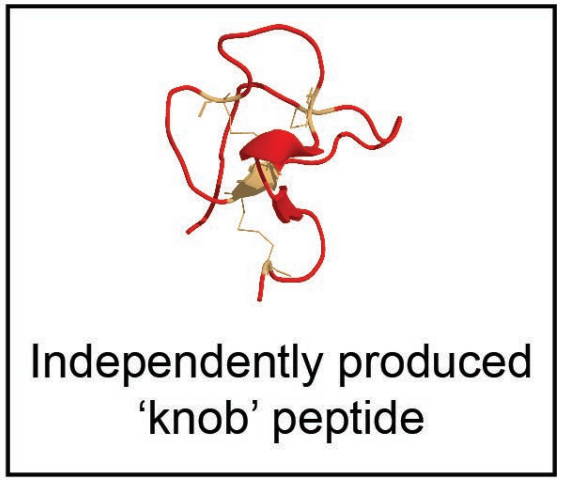


Cow antibody "knob" peptides are the smallest independent antigen binding domain

Applied
Biomedical
Science Institute

Can we produce knobs independently of the antibody?





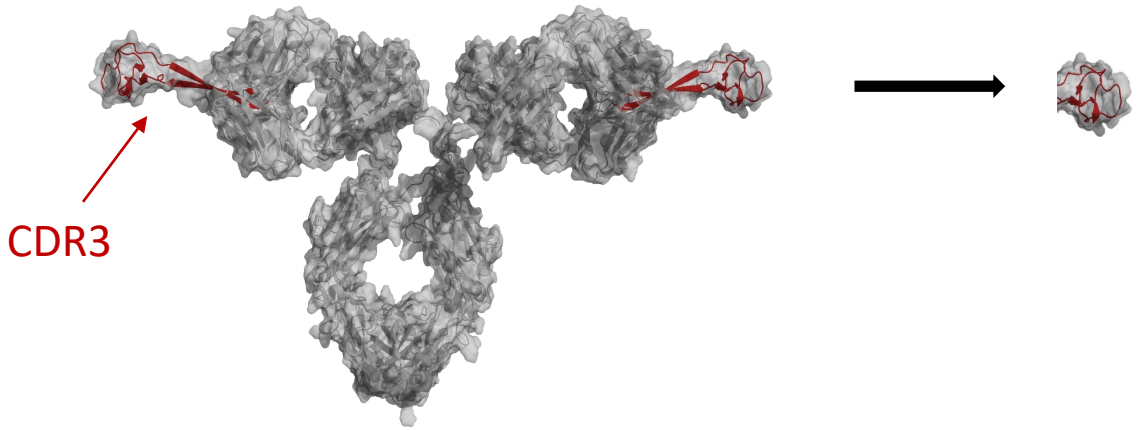
MacPherson A., et.al. *eLife* (2021) 10:e63586

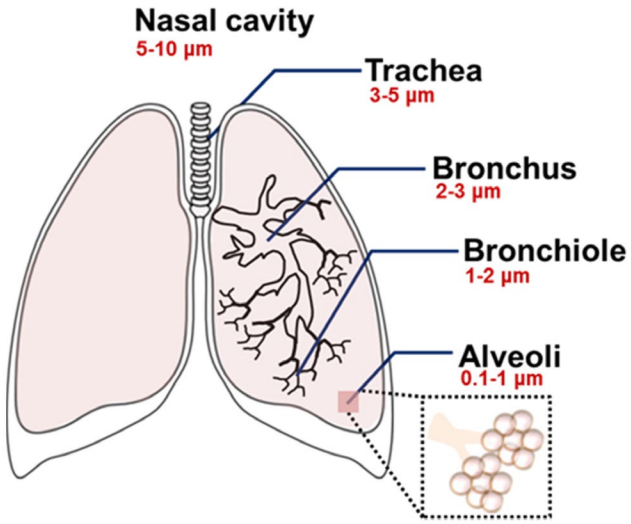
From one immunization, we can potentially discover two classes of therapeutic: IgG, peptide “knob”

Applied
Biomedical
Science Institute

Features of cow antibody-derived peptide (picobody) knobs

Ultralong CDR3 cow antibody **“knob” peptide**

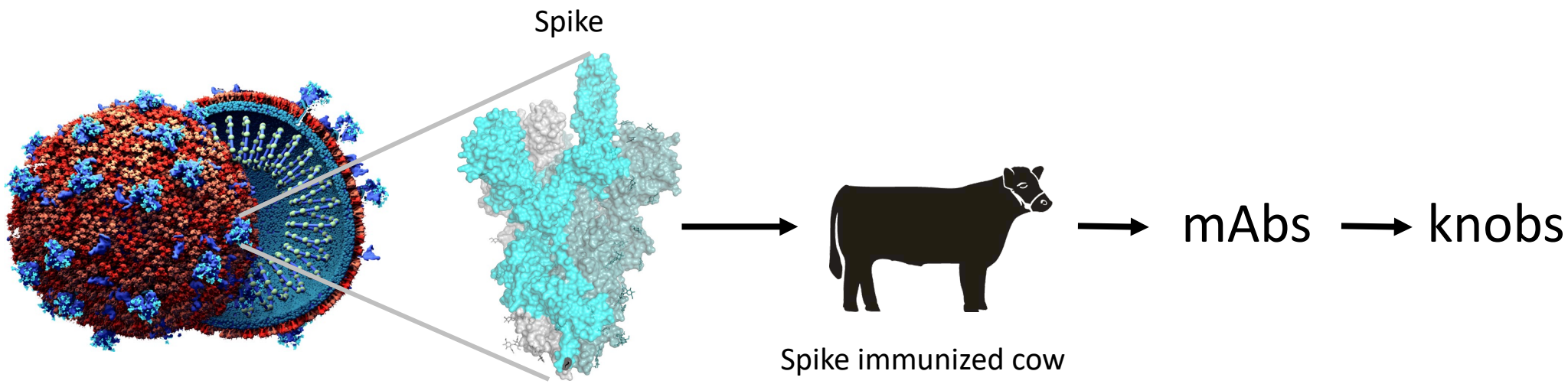




- **Small** - tiny size approaches small molecules
  - Better tissue penetration, alveolar delivery
- **Target binding** - high affinity and epitope coverage, like antibodies
- **Stable and Developable**
  - Rigid disulfide-bonded small domain
  - Avoids aggregates seen in nanobodies and other immunoglobulin domain-based fragments
  - High yield in *E.coli*
- **Simple, immunization-based discovery platform**
  - Peptide structural diversity far greater than any *in vitro* display-based platform
  - Rapid “hit” discovery
  - Each knob has its own novel disulfide-bonded structure

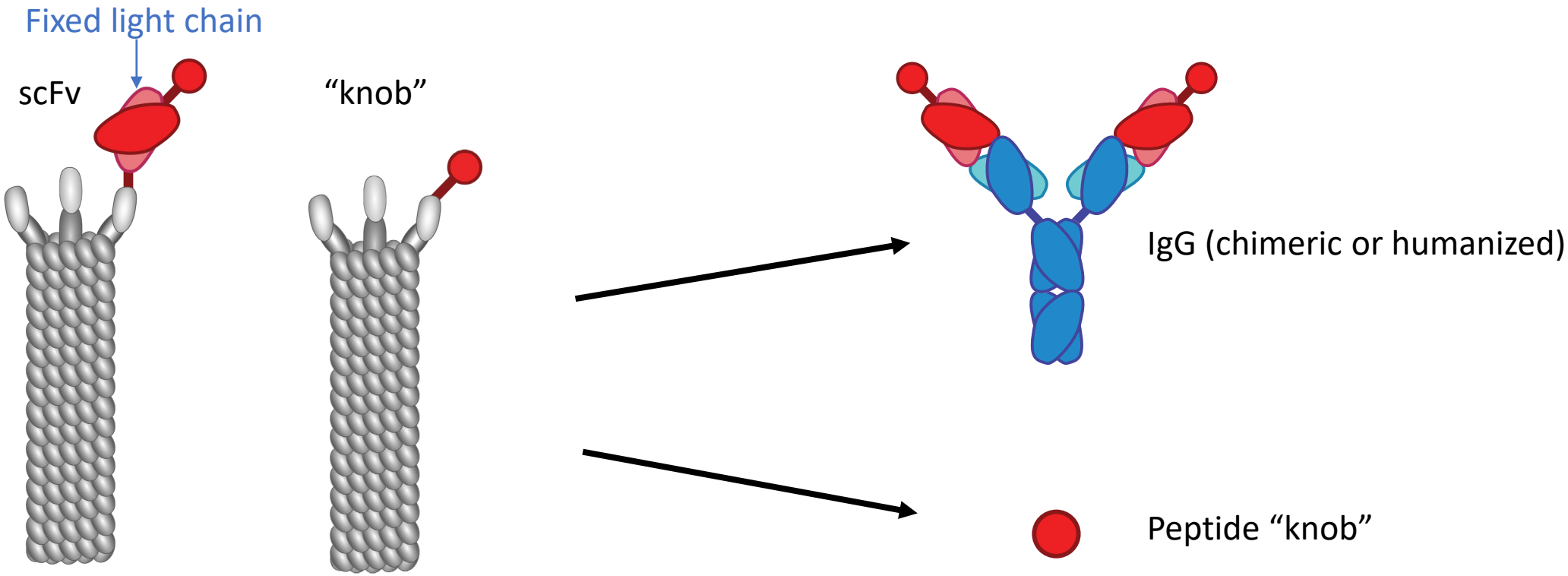
Applied
Biomedical
Science Institute

Discovery of cow anti-coronavirus antibodies



Applied
Biomedical
Science Institute

A novel phage display system to discover antibodies and knobs



Phage ELISA ➡ IgG ELISA (titration) ➡ Pseudovirus neutralization ➡ Live virus PRNT

Applied
Biomedical
Science Institute

Ultralong CDR H3s bind SARS-CoV-2 variants

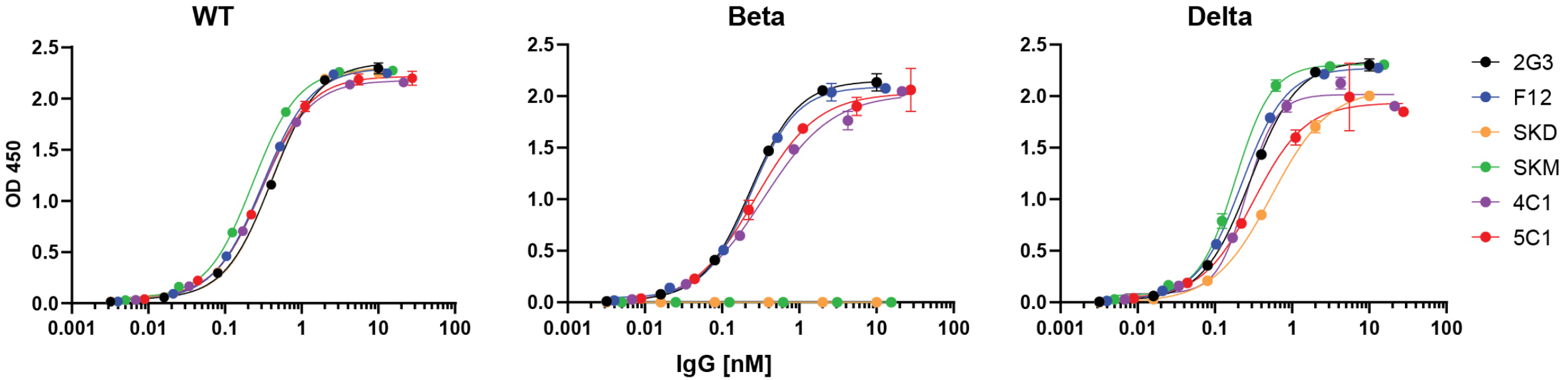
	V	N	D	J	L	#Cys	Epitope
Germ	CTTVHQ		SCPDGYSYGYGCGYGYGCSGYDCYGYGGYGGYGGYGYSSYSYSYTYEY	YVDAW			
SKD	CTTVHQ	RTSEKR	SCPGGSSRRYPSGASCDVSGGACACYVSNCRGVLCPTLNEIVAYTYEW	HVDAW	61	6	RBD
SKM	CTTVHQ	ETLR	SCPDGYIDNSGCTADWGCAALDCWRRRFGYHSTDPSHYTGATYIYTYSL	HIDAW	60	4	RBD
4C1	CATVHQ	KTRKEK	NCPDGYIYSSNTASGYDCGVWICRRVGSAFCSRTGDYTSPSEFDIYEF	YVEGW	61	4	spike
5C1	CATVHQ	KTRKEK	SCPDGYLYSSNTGRGYDCGVWTCRRVGGEFCSATGDWTSPSEEDFYEF	YVDTW	61	4	spike
3A3	CATVHQ	KTRKEK	NCPDGYIYSSNITSGFDCGVWICRRVGSAFCSRTGDYTSPTELDIYEF	YVEGW	61	4	spike
F12	CATVDQ	KTKN	ACPDDFDYRCSCIGGCGCARKGCVGPLCCRSDLGGYLTDSPAYIYEW	YIDLW	58	8	RBD
2G3	CATVHQ	KTAEGDK	TCPDGYEHTCGCIGGCGCKRSACIGALCCQASLGGWLSDGETYTYEF	HVDTW	61	8	RBD
2D9	CAIVQQ	ITHK	TCPNGYNWFDRCCSWDGTCGDGCCSNRAWPSGNGRADSSIGETYGYEF	HVAAW	59	6	RBD

Selected on Beta (later)

Applied
Biomedical
Science Institute

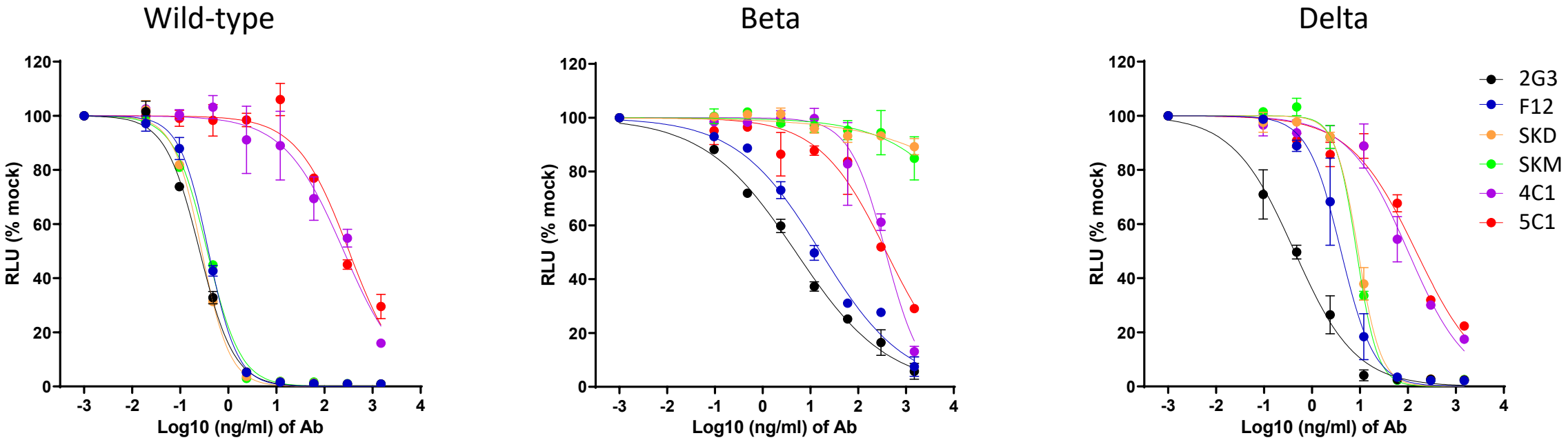
Most antibodies cross-react with SARS-CoV-2 variant strains

SKD and SKM completely lose binding against beta



Applied
Biomedical
Science Institute

Ultralong CDR H3 antibodies maintain neutralization activity against SARS-CoV-2 variant strains

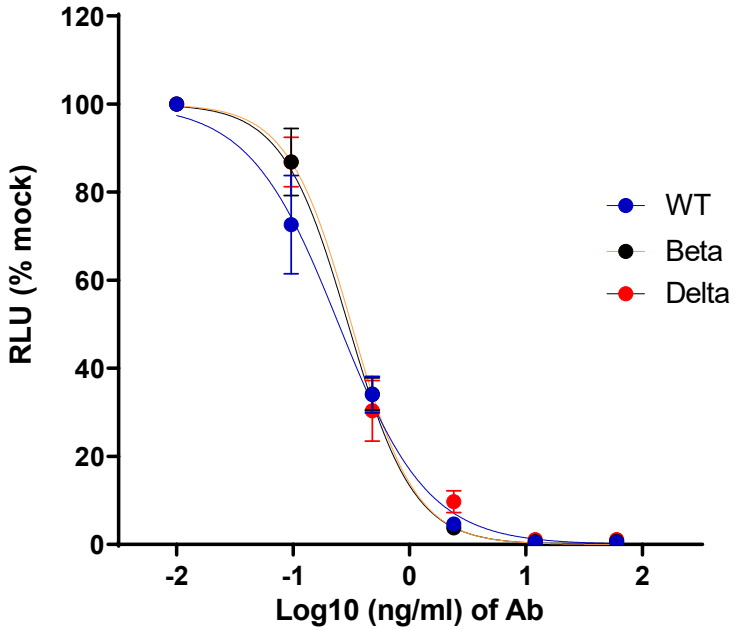


pM potency

K.C. Cheong lab, KSU

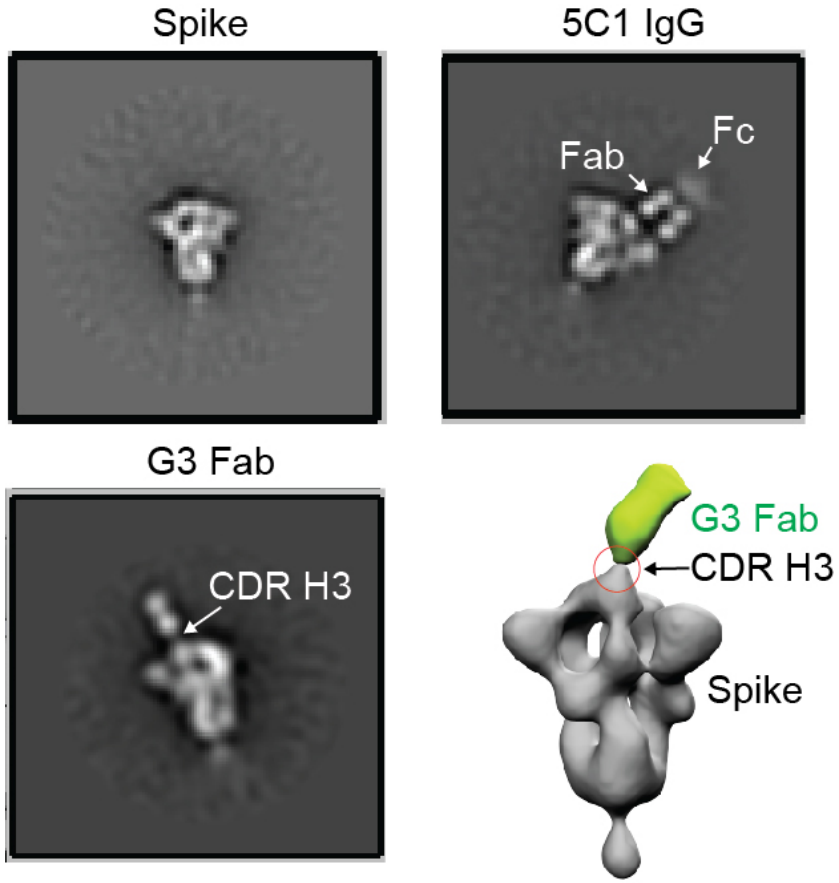
Applied Biomedical Science Institute

2D9 (selected on beta) is equally active against wt, beta, and delta variants



Applied
Biomedical
Science Institute

Preliminary structural biology – EM

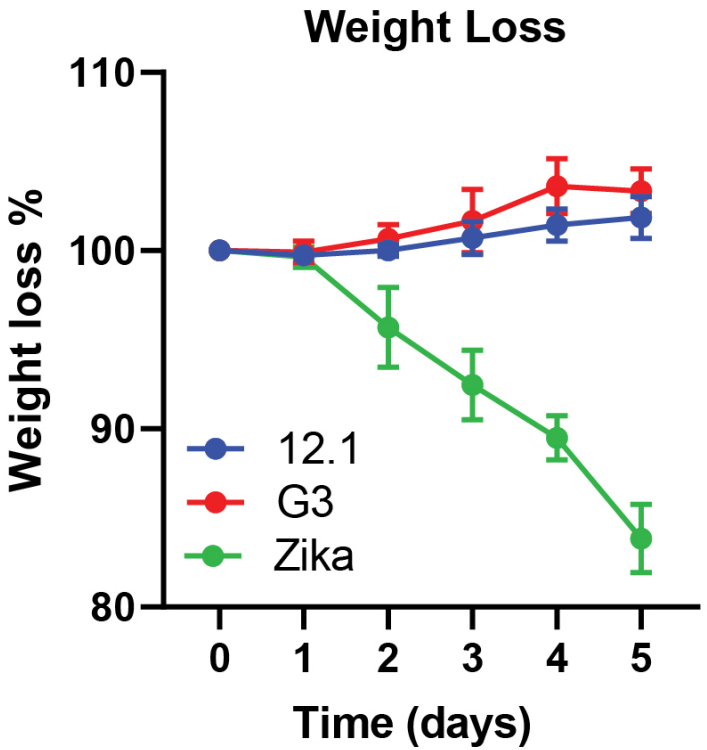


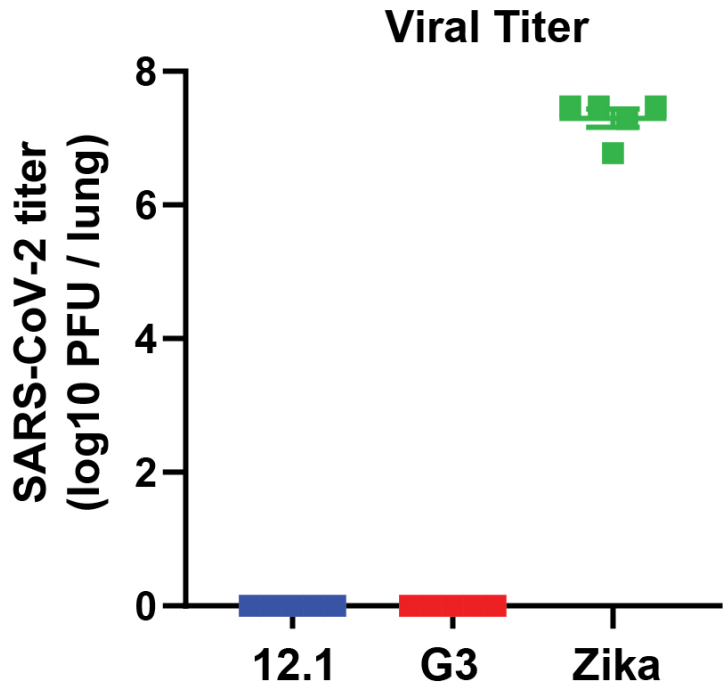
Andrew Ward lab, Scripps Research

Applied
Biomedical
Science Institute

2G3 prevents SARS-CoV-2 infection *in vivo*

single IP dose before infection, K18 ACE2 Tg model

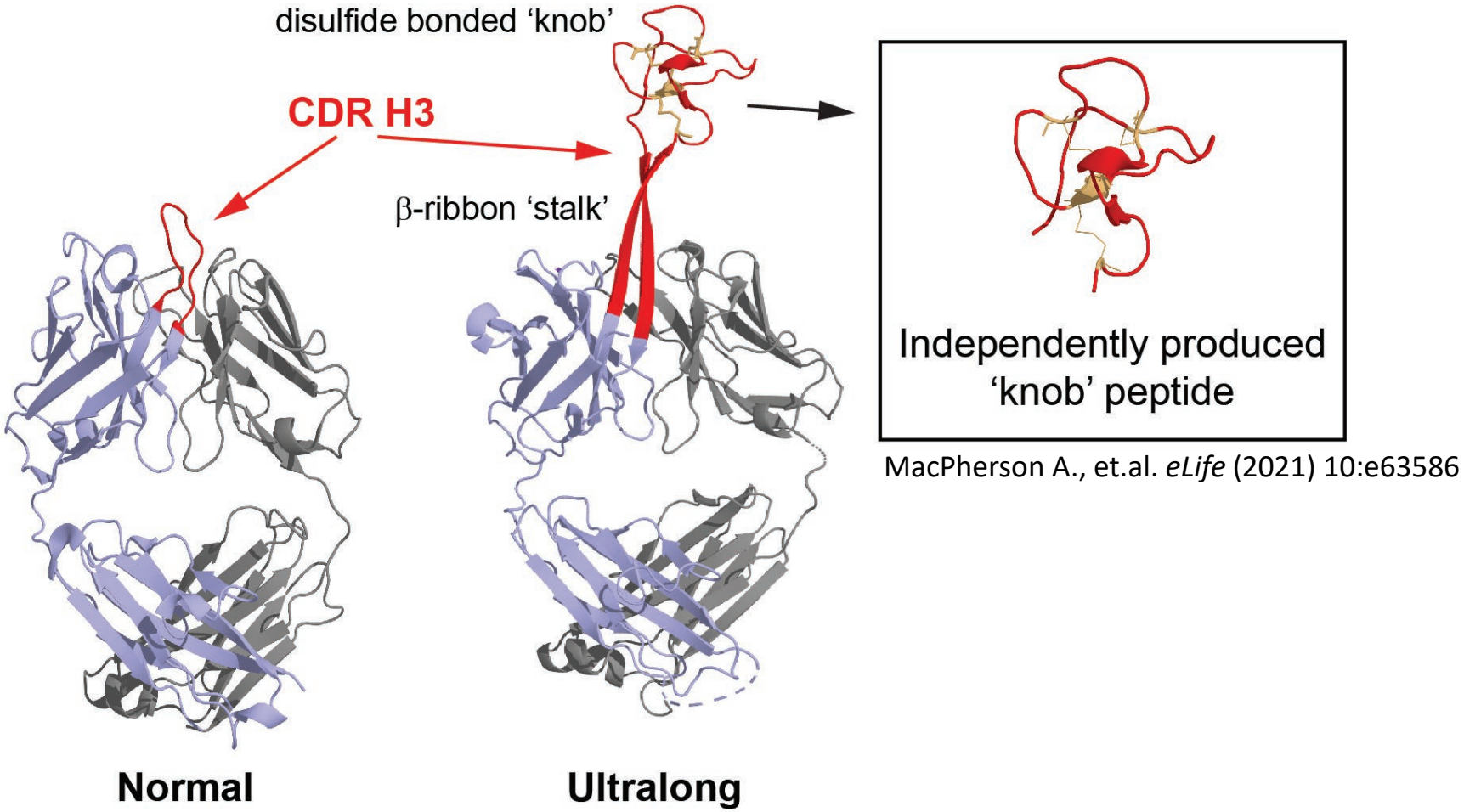




John Teijaro lab, Scripps Research

Applied
Biomedical
Science Institute

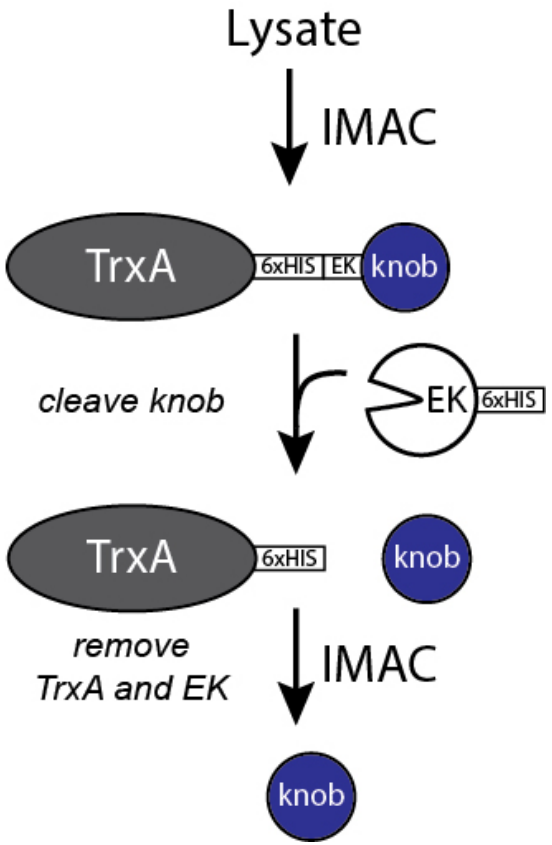
Can we produce knobs independently of the antibody?

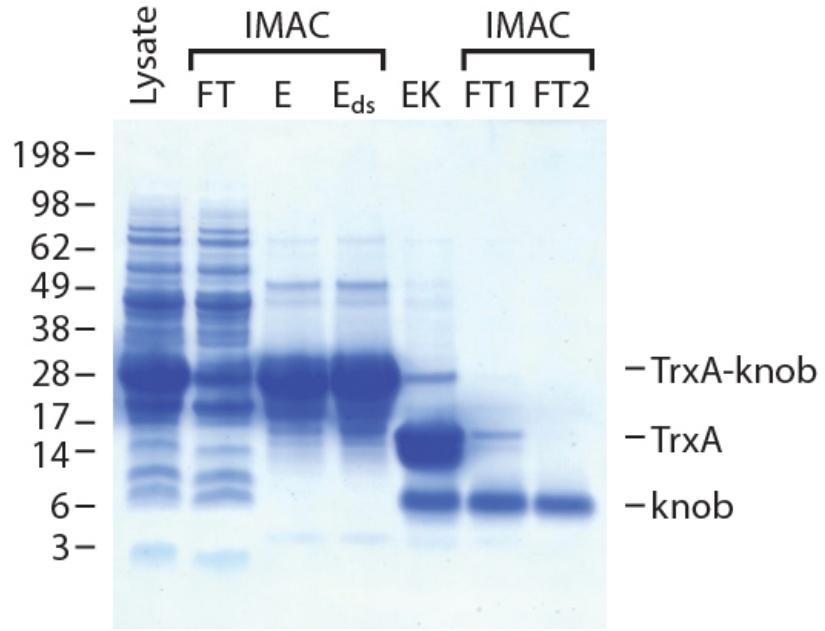


MacPherson A., et.al. *eLife* (2021) 10:e63586

Applied
Biomedical
Science Institute

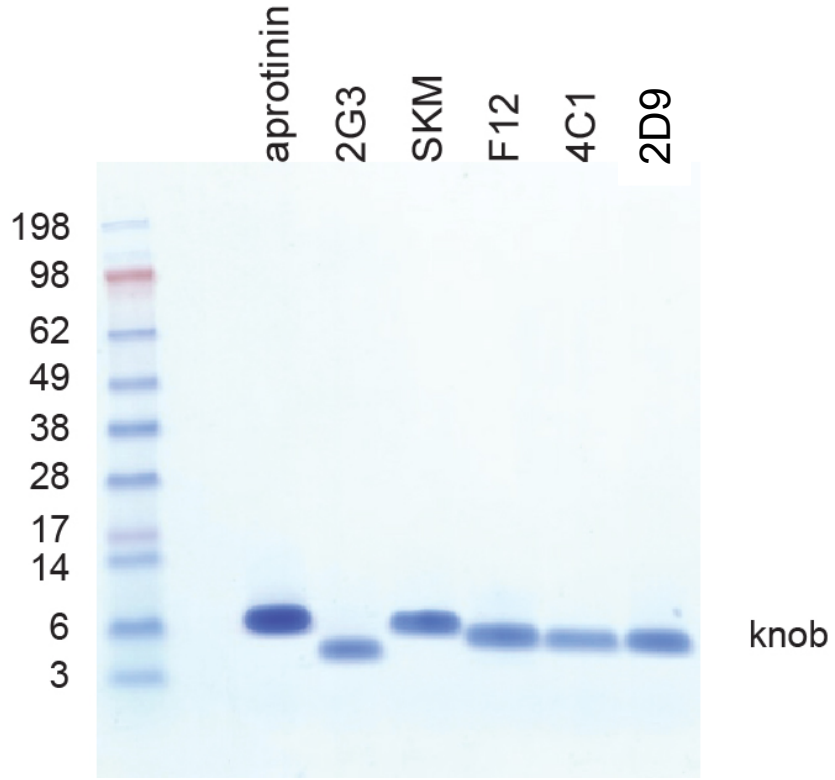
A straightforward, scalable, knob production process in *E.coli*



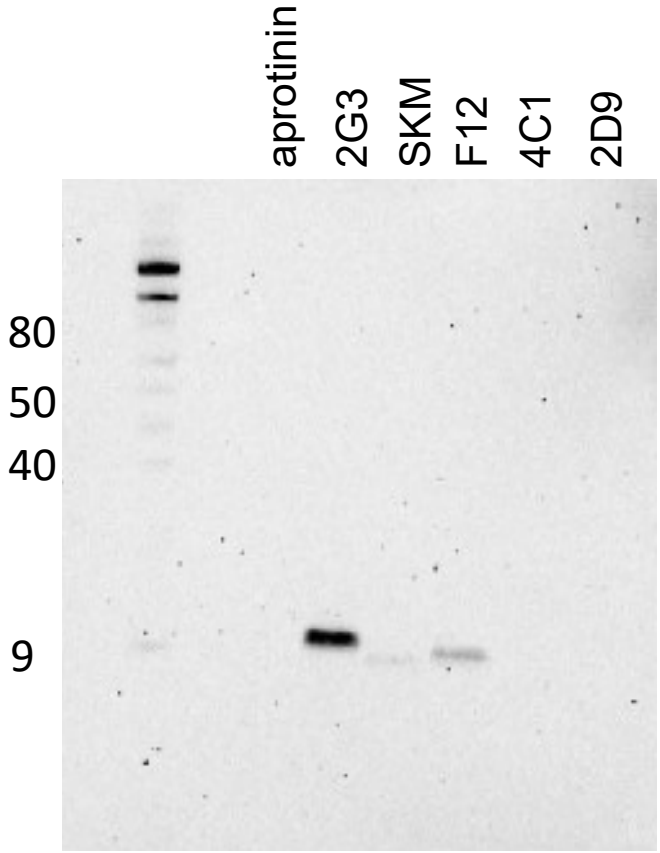


Applied
Biomedical
Science Institute

Purified knobs- some knobs are stable to heat and SDS



SDS-PAGE Coomassie (+DTT)

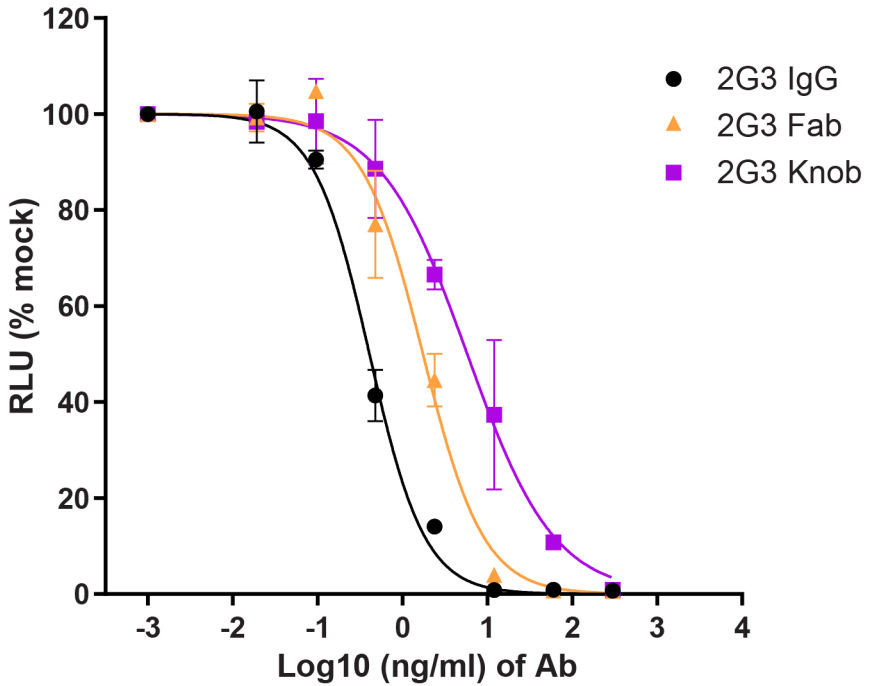


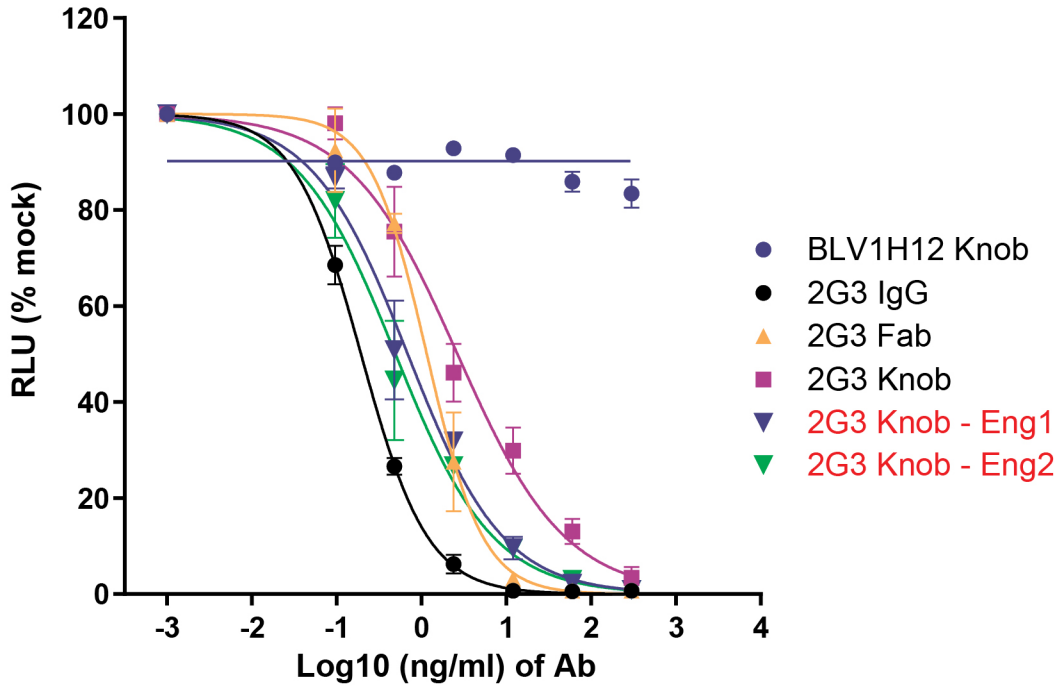
Modified western detected with biotinylated RBD
(-DTT)

Applied
Biomedical
Science Institute

Independently expressed “knobs” neutralize SARS-CoV-2

Knob molecular evolution





Applied
Biomedical
Science Institute

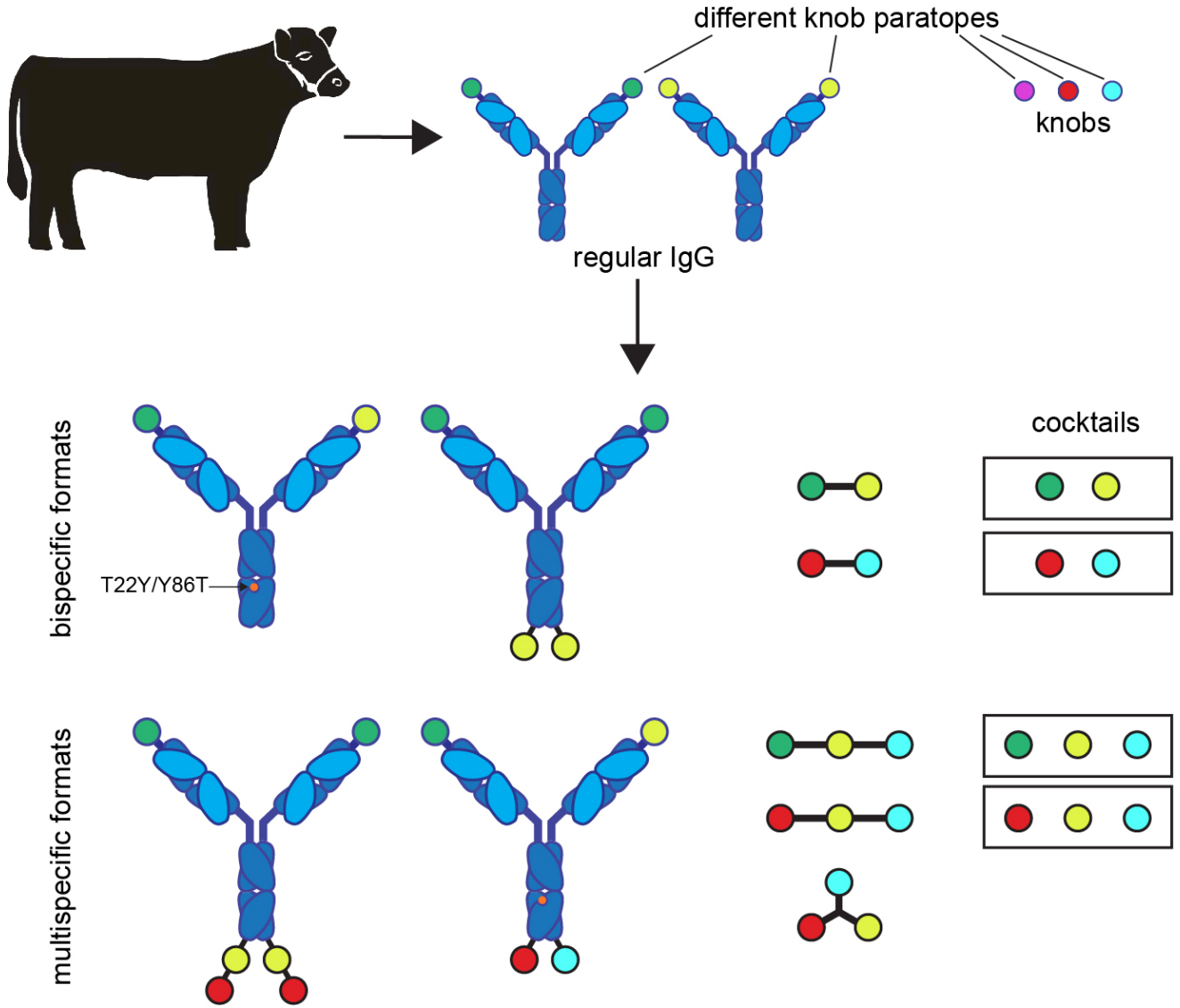
Next steps

- Define start and end of knobs (Gabrielle Warner poster)
- Stability studies (serum, proteases, pH, etc.), oral availability?
- Intranasal/inhaled animal model(s)
- PK, PD, Production process, scale (Dianne Retallack – next!)
- More knobs against emerging strains, cross coronavirus neutralization
- Novel bispecific construction

- *See Ruiqi Huang's poster too!*

Applied
Biomedical
Science Institute

Picobodies as building blocks for novel therapeutics



Applied
Biomedical
Science Institute

Conclusions

- We have discovered extremely potent (pM) antibodies targeting coronavirus SARS-CoV-2
  - 2G3, F12, 2D9 (very potent) cross-react with SA strains
  - 4C1, 5C1 (less potent, still ~nM) cross-react with SARS-CoV-1
- Most potent mAbs have “ultralong” CDR H3 regions (>60 amino acids)
- “Knob” regions of CDR H3s can be produced as independent peptides (“picobodies”)
  - Expression in *E.coli* - rapid and scalable process
  - Retain binding and activity
- Standard discovery system applicable to any target

Applied
Biomedical
Science Institute

Acknowledgements

Smider Lab / ABS Institute

Applied
Biomedical
Science Institute

Riqui Huang

Gabrielle Warner

Duncan McGregor

Abigail Kelley

Alexandra Stambaugh

Jeremy Haakenson

Scripps Research

Scripps

Dennis Burton

Devin Sok

Fangzhu Zhao

Ian Wilson

Robyn Stanfield

Andrew Ward

Jonathan Torres

Abigail Jackson

John Teijaro

K-STATE

Kansas State University

Waithaka Mwangi

K.C. Cheong

Yunjeong Kim

Ligand

Bill Harriman

Philip Leighton

Sam Zeng

Funding: NIH, USDA

Applied
Biomedical
Science Institute

Thank You!

Ligand®

OmniTaur™

https://www.omniab.com/

Applied
Biomedical
Science Institute

https://absinstitute.org/