Supplementary Information

Multivalent bicyclic peptides are an effective antiviral modality that can potently inhibit SARS-CoV-2

Katherine U Gaynor, Marina Vaysburd, Maximilian A J Harman, Anna Albecka, Phillip Jeffrey, Paul Beswick, Guido Papa, Liuhong Chen, Donna Mallery, Brian McGuinness, Katerine Van Rietschoten, Steven Stanway, Paul Brear, Aleksei Lulla, Katarzyna Ciazynska, Veronica T Chang, Jo Sharp, Megan Neary, Helen Box, Jo Herriott, Edyta Kijak, Lee Tatham, Eleanor G Bentley, Parul Sharma, Adam Kirby, Ximeng Han, James P Stewart, Andrew Owen, John A. G. Briggs, Marko Hyvönen, Michael J Skynner & Leo C James

Contents

Supplementary Table 1: Aligned sequences from phage sanger sequencing.

Peptide sequences from selection against Spike, organized by epitope group.

Epitope	Scaffold	Sequence
1*	TATA	A C **E** **Y** V **G** **P** M C Y **R** **L** **Y** C A
1	TATA	A C **E** **Y** N **G** **P** Y C Y **R** **L** **Y** C A
1	TATA	A C **E** **Y** Q **G** **P** H C Y **R** **L** **Y** C A
2*	TCMT	A C **P** Y V A **G** R **G** T C **L** **L** C A
2	TCMT	A C **P** F K P **G** V **G** T C **L** **L** C A
2	TCMT	A C **P** F P P **G** M **G** T C **L** **L** C A
2	TCMT	A C **P** H M P **G** S **G** T C **L** **L** C A
2	TCMT	A C **P** H P P **G** R **G** T C **L** **L** C A
2	TCMT	A C **P** H Q P **G** F **G** T C **L** **L** C A
2	TCMT	A C **P** W E A **G** K **G** T C **L** **L** C A
2	TCMT	A C **P** Y A P **G** M **G** T C **L** **L** C A
2	TCMT	A C **P** Y A P **G** N **G** T C **L** **L** C A
2	TCMT	A C **P** Y L A **G** T **G** T C **L** **L** C A
2	TCMT	A C **P** Y N A **G** T **G** T C **L** **L** C A
2	TCMT	A C **P** Y N K **G** E **G** T C **L** **L** C A
2	TCMT	A C **P** Y Q P **G** S **G** T C **L** **L** C A
2	TCMT	A C **P** Y R E **G** T **G** T C **L** **L** C A
2	TCMT	A C **P** Y S P **G** Q **G** T C **L** **L** C A
2	TCMT	A C **P** Y S P **G** S G T C **L** **L** C A
2	TCMT	A C L Y P P **G** K G T C **L** **L** C A
2	TCMT	A C **P** S P A **G** R G T C **L** **L** C A
2b*	TATA	A C **M** F V **P** C A V **R** H A **L** **G** **L** C A
2b	TATA	A C **M** F T **P** C H V **R** E I **L** **G** **L** C A
2b	TATA	A C **M** F V **P** C A A **R** H E **L** **G** **L** C A
2b	TATA	A C **M** F V **P** C A A **R** V E **L** **G** **L** C A
2b	TATA	A C **M** F V **P** C A I **R** Q T **L** **G** **L** C A
2b	TATA	A C **M** F V **P** C A T **R** H E **L** **G** **L** C A
2b	TATA	A C **M** F V **P** C A T **R** H Q **L** **G** **L** C A
2b	TATA	A C **M** F V **P** C A T **R** H S **L** **G** **L** C A
2b	TATA	A C **M** F V **P** C A T **R** L A **L** **G** **L** C A
2b	TATA	A C **M** F V **P** C A T **R** L Q **L** **G** **L** C A
2b	TATA	A C **M** F V **P** C A T **R** Q E **L** **G** **L** C A
2b	TATA	A C **M** F V **P** C A T **R** Q M **L** **G** **L** C A
2b	TATA	A C **M** F V **P** C A T **R** V A **L** **G** **L** C A
2b	TATA	A C **M** F V **P** C A V **R** E E **L** **G** **L** C A
2b	TATA	A C **M** F V **P** C A V **R** E I **L** **G** **L** C A
2b	TATA	A C **M** F V **P** C A V **R** H S **L** **G** **L** C A
2b	TATA	A C **M** F V **P** C A V **R** K D **L** **G** **L** C A
2b	TATA	A C **M** F V **P** C A V **R** Q T **L** **G** **L** C A
2b	TATA	A C **M** G V **P** C K V **R** E I **L** **G** **L** C A
3*	TATA	A C E D N D **W** V **Y** C **S** **T** C A
3	TATA	A C E D H D **W** V **Y** C **S** **T** C A
3	TATA	A C E S N D **W** V **Y** C **S** **T** C A
3	TATA	A C L D E T **W** I **Y** C **S** **T** C A
3	TATA	A C P D E T **W** V **Y** C **S** **T** C A

3	TATA	A	C	P	D	V	S	**W**	I	**Y**	C	**S**	**T**	C	A			
3	TATA	A	C	E	Q	N	G	**W**	I	**Y**	C	**S**	**T**	C	A			
3	TATA	A	C	P	N	I	S	**W**	I	**Y**	C	**S**	**T**	C	A			
3	TATA	A	C	T	D	R	S	**W**	I	F	C	**S**	**T**	C	A			
3	TATA	A	C	A	P	T	S	G	**W**	I	**Y**	C	**S**	**T**	C	A		
3	TATA	A	C	G	R	D	S	S	**W**	I	**Y**	C	**S**	**T**	C	A		
3	TATA	A	C	P	E	A	N	S	**W**	V	**Y**	C	**S**	**T**	C	A		
3	TATA	A	C	R	G	T	P	A	**W**	K	A	C	A	I	C	A		
4*	TATB	A	C	I	P	L	**D**	**W**	**T**	C	M	I	A	C	A			
4	TATA	A	C	K	I	H	**D**	**W**	**T**	C	L	L	R	C	A			
4	TATB	A	C	**D**	**W**	**T**	C	Y	F	R	P	L	P	C	A			
4	TATB	A	C	**D**	**W**	**T**	C	Y	L	R	P	L	P	C	A			
4	TATB	A	C	**D**	**W**	**T**	C	Y	L	T	M	M	P	C	A			
4	TATB	A	C	**D**	**W**	**T**	C	Y	M	S	M	K	P	C	A			
4	TATB	A	C	**D**	**W**	**T**	C	Y	I	S	P	M	F	D	C	A		
4	TATB	A	C	**D**	**W**	**T**	C	Y	L	N	I	Y	H	E	C	A		
4	TATB	A	C	**D**	**W**	**T**	C	Y	L	R	I	H	E	A	C	A		
4	TATB	A	C	**D**	**W**	**T**	C	Y	M	D	Y	L	S	N	C	A		
4	TATB	A	C	**D**	**W**	**T**	C	Y	M	R	I	N	D	A	C	A		
4	TCMT	A	C	**D**	**W**	**T**	C	Y	I	N	I	Y	N	T	C	A		
4	TCMT	A	C	F	D	**D**	**W**	**T**	C	Y	I	Q	M	C	A			
5*	TATB	A	C	A	N	P	D	**N**	**P**	**V**	C	**R**	**F**	**Y**	C	A		
5	TATB	A	C	A	N	H	D	**N**	**P**	**V**	C	**R**	**F**	**Y**	C	A		
5	TATB	A	C	A	S	P	D	**N**	**P**	**V**	C	**R**	**F**	**Y**	C	A		
5	TATB	A	C	E	N	M	D	**N**	**P**	**V**	C	**R**	**F**	**Y**	C	A		
5	TATB	A	C	F	N	I	D	**N**	**P**	**V**	C	**R**	**F**	**Y**	C	A		
5	TATB	A	C	H	N	L	E	**N**	**P**	**V**	C	**R**	**F**	**Y**	C	A		
5	TATB	A	C	H	N	P	S	**N**	**P**	**V**	C	**R**	**F**	**Y**	C	A		
5	TATB	A	C	K	N	Y	E	**N**	**P**	**V**	C	**R**	**F**	**Y**	C	A		
5	TATB	A	C	L	N	A	E	**N**	**P**	**V**	C	**R**	**F**	**Y**	C	A		
5	TATB	A	C	L	N	K	H	**N**	**P**	**V**	C	**R**	**F**	**Y**	C	A		
5	TATB	A	C	L	N	P	E	**N**	**P**	**V**	C	**R**	**F**	**Y**	C	A		
5	TATB	A	C	L	N	V	E	**N**	**P**	**V**	C	**R**	**F**	**Y**	C	A		
5	TATB	A	C	M	N	A	A	**N**	**P**	**V**	C	**R**	**F**	**Y**	C	A		
5	TATB	A	C	M	N	E	D	**N**	**P**	**V**	C	**R**	**F**	**Y**	C	A		
5	TATB	A	C	M	N	P	D	**N**	**P**	**V**	C	**R**	**F**	**Y**	C	A		
5	TATB	A	C	M	N	T	D	**N**	**P**	**V**	C	**R**	**F**	**Y**	C	A		
5	TATB	A	C	N	N	P	A	**N**	**P**	**V**	C	**R**	**F**	**Y**	C	A		
5	TATB	A	C	Q	N	P	G	**N**	**P**	**V**	C	**R**	**F**	**Y**	C	A		
5	TATB	A	C	R	N	P	E	**N**	**P**	**V**	C	**R**	**F**	**Y**	C	A		
5	TATB	A	C	S	N	P	E	**N**	**P**	**V**	C	**R**	**F**	**Y**	C	A		
5	TATB	A	C	Y	N	Q	E	**N**	**P**	**V**	C	**R**	**F**	**Y**	C	A		
6*	TATB	A	C	D	H	Y	H	C	P	W	L	A	L	G	G	S	C	A
7*	TATB	A	C	I	N	P	Y	C	E	H	H	I	Y	L	E	H	C	A
9*	TATB	A	C	**M**	**N**	**P**	**F**	F	Y	D	C	E	T	V	C	A		
9	TATB	A	C	**M**	**N**	**P**	**F**	F	Y	D	C	D	H	I	C	A		
9	TATB	A	C	**M**	**N**	**P**	**F**	F	Y	D	C	E	D	R	C	A		

9	TATB	A	C	**M**	**N**	**P**	**F**	F	Y	D	C	E	E	I	C	A	
9	TATB	A	C	**M**	**N**	**P**	**F**	F	Y	D	C	E	N	P	C	A	
9	TATB	A	C	**M**	**N**	**P**	**F**	F	Y	D	C	E	R	T	C	A	
9	TATB	A	C	**M**	**N**	**P**	**F**	F	Y	D	C	E	Y	V	C	A	
9	TATB	A	C	**M**	**N**	**P**	**F**	F	Y	D	C	H	E	Q	C	A	
9	TATB	A	C	**M**	**N**	**P**	**F**	F	Y	D	C	K	V	V	C	A	
9	TATB	A	C	**M**	**N**	**P**	**F**	Y	Y	D	C	E	E	V	C	A	
11*	TCMT	A	C	**F**	**P**	E	**P**	**W**	**L**	**G**	**L**	C	**T**	**P**	C	A	
11	TCMT	A	C	**F**	**P**	A	**P**	**W**	**L**	**G**	**L**	C	**T**	**P**	C	A	
12*	TATA	A	C	**S**	S	K	**F**	C	D	**A**	**W**	W	N	**F**	**N**	R	C	A
12	TATA	A	C	**S**	D	A	**F**	C	S	**A**	**W**	W	G	**F**	**N**	Q	C	A
12	TATA	A	C	**S**	D	D	**F**	C	S	**A**	**W**	W	G	**F**	**N**	H	C	A
12	TATA	A	C	**S**	D	E	**F**	C	S	**A**	**W**	W	G	**F**	**N**	E	C	A
12	TATA	A	C	**S**	N	K	**F**	C	D	**A**	**W**	W	N	**F**	**N**	R	C	A

* denotes the lead sequence as described in Table 1. Note for Epitope 6 and 7, these sequences appeared as "singletons" with no similar motifs appearing in outputs.

text in bold denotes conserved residues

Supplementary Table 2: Domain mapping of *Bicycles* against Spike by SPR. *Bicycle* representatives of each epitope were screened against six protein constructs; Spike Trimer, S1, S1-RBD, S1-NTD, S2 and ACE2. The equilibrium dissociation constant (K_D) is displayed as a geometric mean (geomean) value (N=3) with upper (UCI) and lower (LCI) confidence intervals.

Bicycle (Epitope)	Protein Construct	Geomean K_D (nM)	Lower 95% CI (nM)	Upper 95% CI (nM)	N
BCY17548 (E1)	Spike Trimer	6200	8200	4600	3
	S1	5100	15000	1800	3
	S1-RBD	3600	5000	2600	3
	S1-NTD	>	-	-	3
	S2	>	-	-	3
	ACE2	>	-	-	3
BCY16591 (E2)	Spike Trimer	730	1100	490	3
	S1	830	1100	620	3
	S1-RBD	650	1300	310	3
	S1-NTD	>	-	-	3
	S2	>	-	-	3
	ACE2	>	-	-	3
BCY17543 (E3)	Spike Trimer	5100	15000	1700	3
	S1	2500	7000	910	3
	S1-RBD	2300	4500	1200	3
	S1-NTD	>	-	-	3
	S2	>	-	-	3
	ACE2	>	-	-	3
BCY18150 (E9)	Spike Trimer	3100	3300	2800	3
	S1	3800	6000	2400	3
	S1-RBD	4100	7300	2300	3
	S1-NTD	>	-	-	3
	S2	>	-	-	3
	ACE2	>	-	-	3
BCY15466 (E4)	Spike Trimer	110	290	42	3
	S1	2900	28000	300	3
	S1-RBD	>	-	-	3
	S1-NTD	440	1000	190	3
	S2	>	-	-	3
	ACE2	>	-	-	3
BCY16107 (E6)	Spike Trimer	970	3800	250	3
	S1	>	-	-	3
	S1-RBD	>	-	-	3
	S1-NTD	3900	6100	2500	3
	S2	>	-	-	3
	ACE2	>	-	-	3
BCY16115 (E7)	Spike Trimer	3200	3800	2700	3
	S1	>	-	-	3
	S1-RBD	>	-	-	3
	S1-NTD	4000	6400	2500	3
	S2	>	-	-	3
	ACE2	>	-	-	3
BCY16903 (E5)	Spike Trimer	15	150	1.6	2
	S1	24	240	2.4	2
	S1-RBD	>	-	-	3
	S1-NTD	>	-	-	3
	S2	>	-	-	3
	ACE2	>	-	-	3
BCY19905 (E11)	Spike Trimer	450	1000	190	3
	S1	>	-	-	3
	S1-RBD	>	-	-	3
	S1-NTD	>	-	-	3
	S2	530	780	360	3
	ACE2	>	-	-	3
BCY19901 (E12)	Spike Trimer	610	700	530	3
	S1	>	-	-	3
	S1-RBD	>	-	-	3
	S1-NTD	>	-	-	3
	S2	810	1200	530	3
	ACE2	>	-	-	3

Supplementary Table 3: AlphaScreen competition binding to determine which *Bicycles* target overlapping epitopes. Unmodified *Bicycle* representatives of each epitope were competed against representative biotinylated *Bicycles* of each epitope binding a certain Spike protein construct dependent on the domain mapping profile. The half maximal inhibitory concentration (IC_{50}) is displayed as the arithmetic mean average of two technical replicates.

Bicycle ID (Epitope)	Biotinylated Bicycle (Epitope)	Spike Domain	IC_{50} (μM)	n
BCY15354 (E1)	BCY15762 (E1)	S1	0.36	2
BCY16591 (E2)			0.11	2
BCY15231 (E3)			0.31	2
BCY16207 (E9)			-	2
BCY15354 (E1)	BCY15760 (E2)	S1	1.6	2
BCY16591 (E2)			0.35	2
BCY15231 (E3)			-	2
BCY16207 (E9)			-	2
BCY15354 (E1)	BCY15763 (E3)	S1	0.55	2
BCY16591 (E2)			-	2
BCY15231 (E3)			0.40	2
BCY16207 (E9)			1.1	2
BCY16107 (E6)	BCY16257 (E6)	S-Trimer	0.20	2
BCY16115 (E7)			-	2
BCY16107 (E6)	BCY16274 (E7)	S-Trimer	-	2
BCY16115 (E7)			1.0	2
BCY19905 (E11)	BCY19320 (E11)	S2	0.064	2
BCY19901 (E12)			-	2
BCY19905 (E11)	BCY19908 (E12)	S2	-	2
BCY19901 (E12)			0.22	2

Supplementary Table 4: AlphaScreen competition assay to determine ability of *Bicycles* to inhibit ACE2 binding. Unmodified monomeric *Bicycle* representatives of each epitope were competed against Spike Trimer:ACE2 binding interaction. The half maximal inhibitory concentration (IC_{50}) is displayed as a geometric mean (geomean) value (minimum N=2) with upper (UCI) and lower (LCI) confidence intervals. A non-Spike binding Bicycle was used as a negative control.

Bicycle ID	Epitope	Multimeric State	Geomean IC_{50} (nM)	Lower 95% CI (nM)	Upper 95% CI (nM)	N
-ve Control	Non-binder	Monomer	>21000	-	-	6
BCY17548	E1	Monomer	>20000	-	-	2
BCY16591	E2	Monomer	520	360	750	4
BCY17543	E3	Monomer	>20000	-	-	2
BCY15446	E4	Monomer	>16000	-	-	2
BCY16903	E5	Monomer	>20000	-	-	2
BCY16107	E6	Monomer	>20000	-	-	2
BCY16115	E7	Monomer	>14000	-	-	2
BCY18150	E9	Monomer	>20000	-	-	2
BCY19905	E11	Monomer	>20000	-	-	2
BCY19901	E12	Monomer	>20000	-	-	2

Supplementary Table 5: Data collection and refinement statistics. RBD complexes with *Bicycle* ligands. Statistics for both data integration and model refinement are given (values for the highest resolution shell are given in parentheses).

	sRBD:E2	sRBD:E2b
PDB code	7Z8O	8AAA
Data collection		
Wavelength (Å)	0.8	0.98
Temperature (K)	100	100
Beamline	Diamond Light Source I04	Diamond Light Source I04
Detector	Eiger2 XE 16M	Eiger2 XE 16M
Rotation per image (°)	0.1	0.05
Total rotation range (°)	300	360
Exposure time per image (s)	0.05	0.02
Space group	$P2_12_12_1$	P32 21
a, b, c (A)	44.2 55.7 82.7	112.2 112.2 35.5
α, β, γ (°)	90, 90, 90	90, 90, 120
Resolution range	46.2 - 0.96 (0.98 - 0.96)	56.1 - 1.9 (1.97 - 1.90)
Total No of reflections	2313853 (34008)	266723 (10644)
No of unique reflections	123986 (5517)	13749 (686)
Completeness	99.2 (90.4)	91.5 (65.2)
Multiplicity	18.7 (6.2)	19.4 (15.5)
<I>/<σ(I)>	19.6 (0.9)	9.3 (1.9)
R_{merge}	0.06 (1.51)	0.25 (1.68)
$CC_{1/2}$	1.00 (0.37)	0.99 (0.86)
Refinement		
Rfactor	0.1191 (0.342)	0.181 (0.394)
R-free	0.1334 (0.301)	0.2276 (0.3432)
R.m.s. deviations (angle, °)	1.99	0.94
R.m.s. deviations (length, Å)	0.017	0.008
Ramachandran plot		
Ramachandran favored (%)	97.47	95.63
Ramachandran allowed (%)	2.53	4.37
Ramachandran outliers (%)	0	0
Average B-factor	16.4	35.8
macromolecules	14.2	35.8
ligands	15.3	35.1
solvent	28	35.5

Supplementary Table 6: AlphaScreen competition assay to determine ability of multimerized E2 *Bicycles* to inhibit ACE2 binding. E2 *Bicycle* of constant sequence and variable multimeric state were competed against Spike Trimer:ACE2 binding interaction. The half maximal inhibitory concentration (IC_{50}) is displayed as a geometric mean (geomean) value (minimum N=2) with upper (UCI) and lower (LCI) confidence intervals. A non-Spike binding Bicycle was used as a negative control. The lower limit of quantification (LoQ) was defined as [Spike Protein]/2, in this case equal to 0.1 nM.

Bicycle ID	Epitope	Multimeric State	Geomean IC_{50} (nM)	Lower 95% CI (nM)	Upper 95% CI (nM)	N
-ve Control	Non-binder	Monomer	>21000	-	-	6
BCY16591	E2	Monomer	520	360	750	4
BCY17023	E2	Homodimer	0.74	0.047	12	2
BCY17021	E2	Homotrimer	0.075*	0.014	0.42	2
BCY17022	E2	Homotetramer	0.096*	0.094	0.097	2

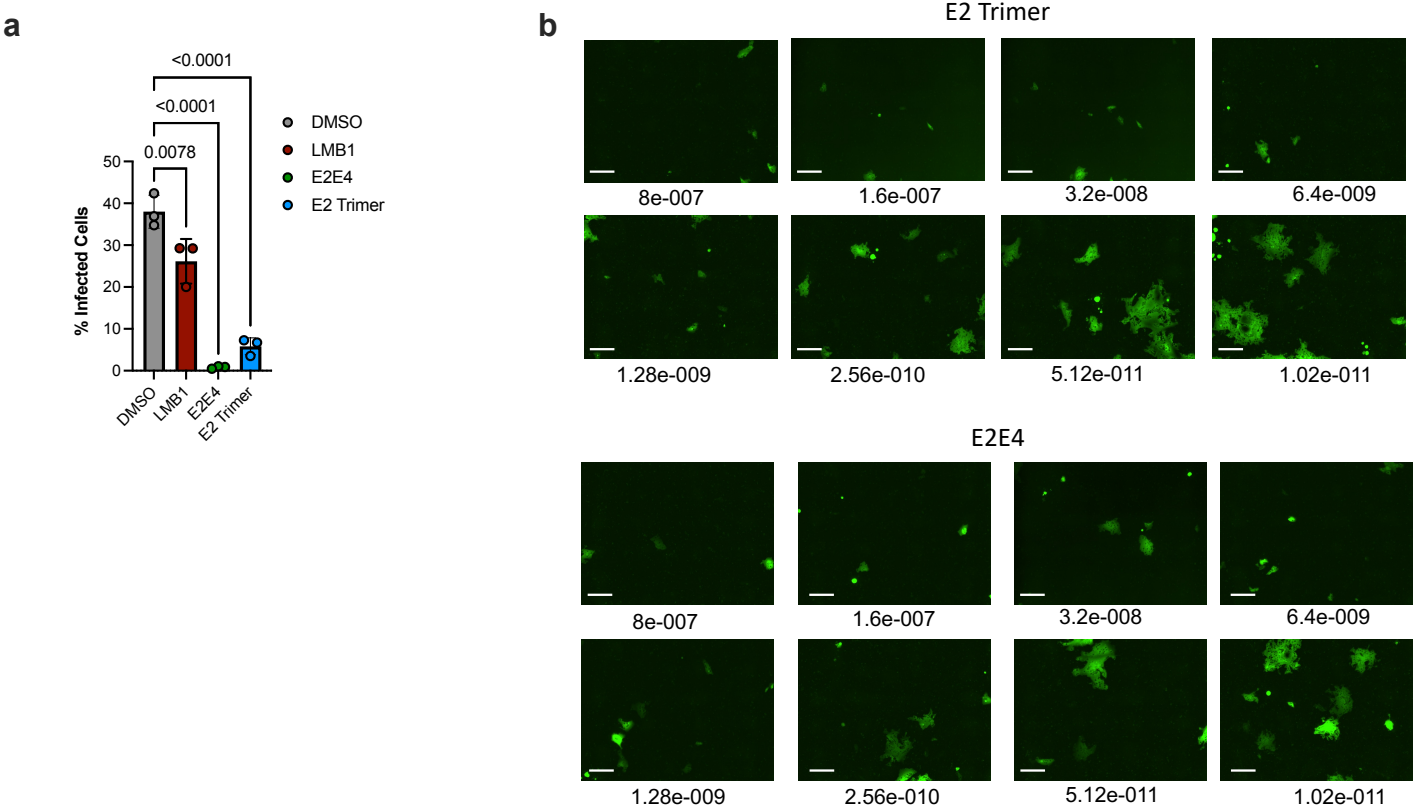
*LoQ = 0.1 nM

Supplementary Table 7: List of *Bicycle* IC90s. IC90 values are given by figure panel for each Bicycle as calculated from each dose-response fit.

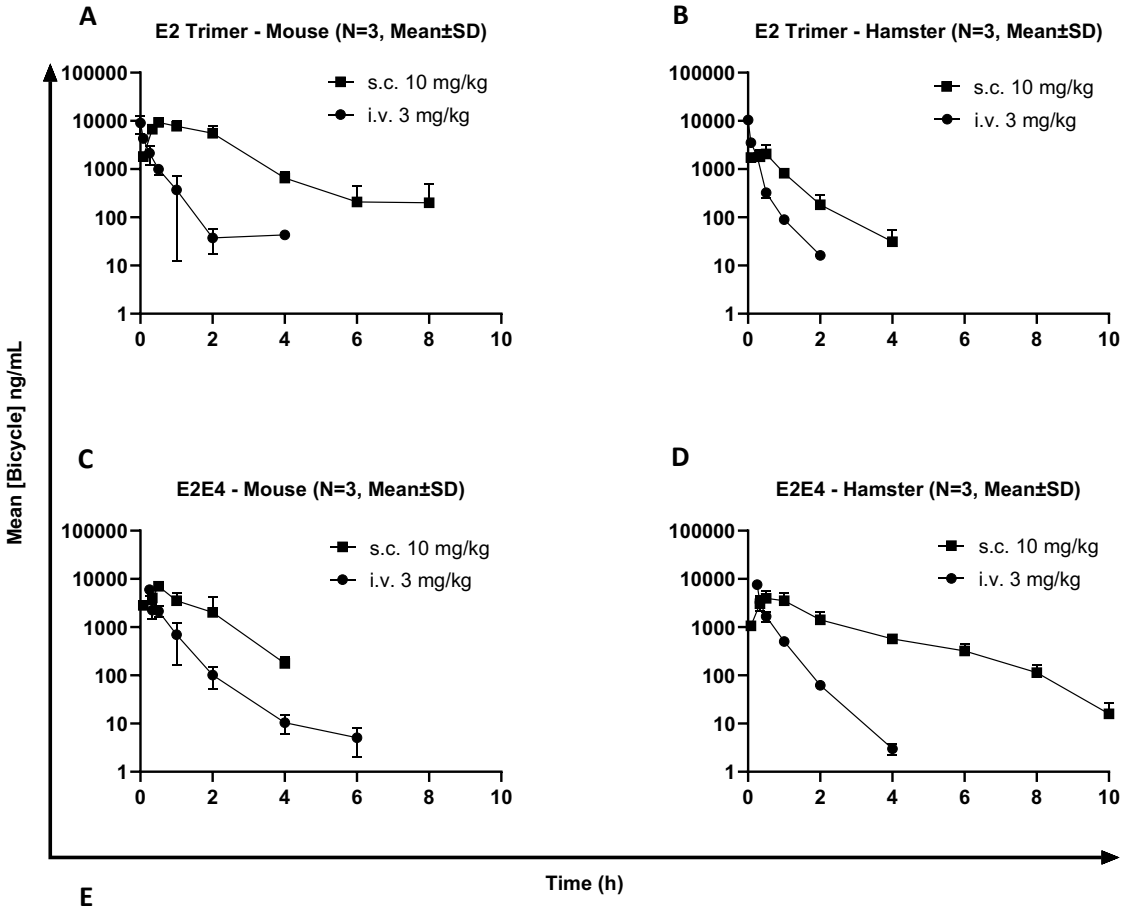
Figure 2B	
Bicycle	**IC90 (M)**
E2 Monomer	1.6×10^{-2}
E2 Dimer	7.3×10^{-5}
E2 Trimer	7.2×10^{-7}
E2 Tetramer	4.3×10^{-7}
Figure 2C	
Bicycle	**IC90 (M)**
PEG1	2.6×10^{-8}
PEG5	5.3×10^{-8}
PEG10	4.9×10^{-8}
PEG23	1.3×10^{-7}
Figure 2D	
Bicycle	**IC90 (M)**
E3 Trimer	8.3×10^{-6}
E5 Trimer	2.2×10^{-6}
Figure 2E	
Bicycle	**IC90 (M)**
E1E2	7.6×10^{-4}
E2E3	1.6×10^{-6}
E1E5	8.3×10^{-6}
E2E5	1.8×10^{-7}
E3E5	1.1×10^{-6}
Figure 2G	
Bicycle	**IC90 (M)**
E1E4	6.3×10^{-6}
E2E4	8.3×10^{-8}
E3E4	1.9×10^{-6}
E4E5	2.4×10^{-6}
Figure 3A	
Bicycle	**IC90 (M)**
E2 Trimer	3.9×10^{-9}
E2E4	8.1×10^{-9}
Figure 3B	
Bicycle	**IC90 (M)**
E2	2.1×10^{-7}
E2E4	8.9×10^{-7}
Figure 3C	
Bicycle	**IC90 (M)**
E2	2.56×10^{-8}
E2E4	2.17×10^{-8}
Figure 4B	

Bicycle	IC90 (M)
Beta	2.7×10^{-4}
Beta 484	7.3×10^{-8}
Delta	1.4×10^{-4}
Delta 452	5.1×10^{-9}
Figure 4C	
Bicycle	**IC90 (M)**
E3E5	1.7×10^{-7}
E4E5	5.5×10^{-8}
E2c Trimer	2.0×10^{-7}
E2E4	1.3×10^{-7}
Figure 4D	
Bicycle	**IC90 (M)**
E3E5	1.2×10^{-6}
E4E5	6.0×10^{-7}
E2c Trimer	1.3×10^{-6}
E2E4	1.8×10^{-6}
Figure 4E	
Bicycle	**IC90 (M)**
E3E5	4.9×10^{-6}
E4E5	2.0×10^{-4}
E2c Trimer	5.9×10^{-6}
E2E4	9.7×10^{-5}
Figure 4F	
Bicycle	**IC90 (M)**
E2E4	2.1×10^{-7}
Figure 4G	
Bicycle	**IC90 (M)**
E3E5	3.3×10^{-5}
E2c Trimer	1.6×10^{-5}

Supplementary Figure 1: Immunofluorescence data of *Bicycles* inhibition of SARS-CoV-2 infection and cell-cell fusion. (a) Quantification of NP expression in Vero TMPRSS2/ACE2 cells 18 hours post-infection with SARS-CoV-2 (Wuhan-Hu-1 strain) in the presence of homotrimeric E2 or biparatopic E2E4. Cells were fixed and stained with anti-NP antibody (CAT). A representative image of at least 3 biological replicates is shown. Data are presented as mean values +/- SEM. Ordinary one-way ANOVA was used for statistical analysis and significance indicated with respect to DMSO condition. **(b)** Immunofluorescence images of GFP positive syncytia 12 hours post-Spike transfection in the presence of E2E4 and E2 trimer *Bicycles* at a range of concentrations. Quantification of this data is shown in Figure 3E. Scale bar is 200 µM.



Supplementary Figure 2: Summary of intravenous (i.v.) and sub-cutaneous (s.c.) pharmacokinetics for E2 trimer and E2E4 biparatopic. (A,B,C,D) *Bicycle* multimers were administered to both mice and hamsters (N=6, alternate composite sampling resulting in N=3 per timepoint) via i.v. and s.c. routes of administration. *Bicycle* plasma concentration over time data displayed as mean +/- standard deviation (SD) for N=3 biological replicates. **(E)** Pharmacokinetic parameters were subsequently evaluated.



E

Compound	BCY17021				BCY18656			
Species	Mouse		Hamster		Mouse		Hamster	
Route of Administration	i.v.	s.c.	i.v.	s.c.	i.v.	s.c.	i.v.	s.c.
Nominal Dose (mg/kg)	3.0	10	3.0	10	3.0	10	3.0	10
C_{max} (ng/mL)	9000	9300	10000	2100	6000	7000	7600	4300
T_{max} (h)	0.0	0.5	0.0	0.5	0.25	0.5	0.25	0.6
$T_{1/2}$ (h)	0.6	1.2	0.4	0.7	0.6	0.7	0.4	1.1
Vd_{ss} (L/kg)	0.45	-	0.27	-	0.64	-	0.46	-
Cl (mL/min/kg)	16	-	19	-	20	-	20	-
$AUC_{0\text{-}inf}$ (ng.h/mL)	3100	19000	2600	2100	2500	8900	2500	8600
F (%)	-	NR*	-	16	-	100	-	100

*inconsistent plasma concentration-time profile, therefore bioavailability not reported (NR).

Supplementary Figure 3: Pharmacokinetic-pharmacodynamic (PKPD) models. PKPD models were used to select an appropriate in vivo dosing regimen that would sustain a minimum of 90% target coverage at trough for both the E2 Trimer and E2E4 biparatopic. Using the appropriate data the target coverage predictions are; **(A)** E2 Trimer mouse s.c. administration **(B)** E2 Trimer hamster s.c. administration, **(C)** E2E4 biparatopic mouse s.c. administration **(D)** E2E4 biparatopic hamster s.c. administration. Each panel is expressed as % Bicycle Target Coverage over time where black dotted lines reference 90% coverage at 8 hours, indicating 90% target coverage at trough based on a t.i.d. dosing regimen.