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Supplemental Information

The 2.8 Å Electron Microscopy Structure

of Adeno-Associated Virus-DJ Bound

by a Heparinoid Pentasaccharide

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Figure S1: Controls for the transduction inhibition assay (Figure 2). Transduction in HeLa cells was measured using an eGFP-AAV2 vector, but otherwise as in Figure 2. The histogram shows that the inhibition, specific for fondaparinux, is not shared by equimolar treatments with sucrose. Viability was assayed by trypan blue exclusion, counted by hand.

	10	20	30	40	50	60	70	80	90	100
AAVDJ	MAADGYLPDWLEDI	LSEGIROW	KLKPGPPPPKI	PAERHKDDSR	GLVLPGYKYL	 GPFNGLDKGE	PVNEADAAALI	HDKAYDROL	 DSGDNPYLKY	NHADAEF
AAV2	MAADGYLPDWLEDI	LSEGIRQWW	KLKPGPPPPKI	PAERHKDDSR	GLVLPGYKYL	GPFNGLDKGE	PVNEADAAALI	EHDKAYDRQL	DSGDNPYLKY	NHADAEF
AAV8	MAADGYLPDWLEDN	LSEGIREWW	ALKPGAPKPK	anqqkq dd g r	GLVLPGYKYL	GPFNGLDKGE	PVNAADAAALI	ehdkayd og l	QAGDNPYLRY	NHADAEF
AAV9	MAADGYLPDWLEDN	LSEGIREWW	ALKEGAEŐEK	ANQQHQ U NA R	GLATECAKAT	GP GNGLDKGE	PVNAADAAALI	CHDKAYDQQL	KAGDNPYLKY	(NHADAEF
					AAV2					
	110	120	130	140	150	160	170	180	190	200
		1	1		1		1	1		1
AAVDJ	QERLKEDTSFGGNI	GRAVFQAKK	RLLEPLGLVE	EAAKTAPGKK	RPVEHSPVE-	PDSSSGTGKA	GQQPARKRLNI	GOTGDADSV	PDPQPIGEPP	AAPSGVG
AAV2 AAV8	OERLOEDTSFGGNI	GRAVEQARE.	RVLEPLGLVEI	E PVKTAPGKK EGAKTAPGKK	REVERSEVE REVEPSPORS	PDSSTGTGKA	GOOPARKRINI	GOTGDSESV	PDPQPLGQPP PDPOPLGEPF	AAPSGLG
AAV9	QERL KEDTSFGGNI	GRAVFQAKK	RLLEPLGLVE	EAAKTAPGKK	RPVEQSPQE-:	PDSS <mark>AG IGKS</mark> (GÃ ÕPAKKRLNI	GÕTGDTESV	PDPÕPIGEPP	AAPSGVG
		←	AAV9	→		<u> </u>	AV2 -	 →	· 🔶 /	4AV9
	-									
	210	220	230	240	250	260	270	280	290	300
AAVDJ	SLTMAAGGGAPMAD	NNEGADGVG	NSSGNWHCDS	TWMGDRVITT	STRTWALPTY	NNHLYKQISN	STSGGSSNDN	YFGYSTPWG	YFDFNRFHCH	IFSPRDWQ
AAV2	TNTMATG SGAPMAD	NNEGADGVG	NSSGNWHCDS	TWMGDRVITI	STRTWALPTY	NNHLYKQIS <mark>S</mark>	QSGASNDN	YFGYSTPWG	YFDFNRFHCH	IFSPRDWQ
AAV8 AAV9	PNTMAAGGGAPMAD	NNEGADGVG	SSSGNWHCDS	TWLGDRVITT ONIGDRVITT	STRTWALPTY	NNHLYKQISN NNHLYKOISN	GTSGGATNDN'	YFGYSTPWG	YFDFNRFHCH	IFSPRDWQ
7010 5			0000000000	×"BODK"III	SIRIMAL II.		515005511511			
		+ +		AAV2	> <		AAV9	→	. 🔶	
						-				
	310	320	330	340	350	360	370	380	390	400
AAVDJ	RT.TNNNWGFRPKRT	SFKLENTOV	KEVTONEGTK'	 TANNLTSTT	OVETENSE YOL	PYVLGSAHOGI	T.PPFPADVF	ITPOYGYT.TT.	NNGSOAVGRS	SEYCLEY
AAV2	RLINNNWGFRPKRI	NFKLFNIQV	KEVTONDGTT	TIANNLTSTV	QVFTDSEYQL	PYVLGSAHQG	CLPPFPADVFI	IVPQYGYLTL	NNGSQAVGRS	SFYCLEY
AAV8	RLINNNWGFRPKRI	SFKLFNIQV	KEVTQNEGTK'	TIANNLTSTI	QVFTDSEYQL	PYVLGSAHQG	CLPPFPADVFI	1IPQYGYLTL	NNGSQAVGRS	SFYCLEY
AAV9	RLINNNWGFRPKRI	NFKLFNIQV	KEVT <mark>DNN</mark> GVK'	TIANNLTSTV	QVFTDS DYQL	PYVLGSAHEG	CLPPFPADVFI	1IPQYGYLTL	NDGSQAVGRS	SFYCLEY
					A4V8		-			
	410	420	430	440	450	460	470	480	490	500
	I	1	1	1	I.	I	1	1	1	1
AAVDJ DDV2	FPSQMLRTGNNFQF	'TYTFEDVPF'	HSSYAHSQSLI	DRLMNPLIDQ	YLYYLSRTOT	TGGTTNTQTL	GFSQGGPNTMU	NOAKNWLPG	PCYRQQRVSK	TS ADNNN
AAV8	FPSQMLRTGNNFQF	TYTEDVER	HSSYAHSQSLI	DRLMNPLIDQ	YLYYLSRTQT	TGGTANTQTL	GFSQGGPNTM	NQAKNWLPG	PCYRQQRVST	TTGQNNN
AAV9	FPSQMLRTGNNFQF	SYEFENVPF	HSSYAHSQSLI	DRLMNPLIDQ	YLYYLSKTIN	GSGQ-NQQTL	KFSVAGP SNMU	AVQGRNYIPG:	P SYRQQRVS I	TVTQNNN
						→ ←		/8		
										•
	510	520	530	540	550	560	570	580	590	600
aavn.t	SEVSMIC DUKVHI.N		 ופתתאנצמאנים	EKEEPOSGUL	TECKOCSEKT	NVDTEKVMT7	 NEERTRINE	72/07/2575		 תיאוטנרו ביתי ב
AAV2	SEYSWIGATKYHLN	GRDSLVNPG	PAMASHKDDEI	EKFFPQSGVL	IFGKQGSEKT	NVDIEKVMIT	DEEEIRTINP	ATEQYGSVS	TNLQRGNRQA	ATADVNT
AAV8	SNFAWTAGTKYHLN	IGR <mark>N</mark> SL ANP G	IAMA THKDDEI	ERFFP SNG IL	IFGKQNAARD	NADYSDVMLT	SEEEIKTINP	ATEEYG IVA	D NLQ QQ NT AF	QIGTVNS
AAV9	SEFAWPGASSWALN	IGRNSLMNPG	PAMASHKEGE	DRFFPLSGSL	IFGKQ <mark>G</mark> TGRD	NVDADKVMIT	NEEEIKTINP	/ATESYGQVA	IN HOSAQAQA	NGLORIN
					A AU2					
				_	AAVZ					
	610	620	630	640	650	660	670	680	690	700
AAVDJ	OGVLPGMVWODRDV	YLOGP IWAK	IPHTDGHFHP:	SPLMGGFGLK	HPPPOILIKN	TP VP ADP PTT	FNOSKLNSFI	POYSTGOVSV	EIEWELOKEN	ISKRWNPE
AAV2	QGVLPGMVWQDRDV	YLQGP IWAK	IPHTDGHFHP	SPLMGGFGLK	HPPPQILIKN	TPVPANPSTT	SAAKFASFI	QYSTGQVSV	EIEWELÕKEN	SKRWNPE
AAV8	QGALPGMVWQNRDV	YLQGP IWAK	IPHTDG NFHP:	SPLMGGFGLK	HPPPQILIKN	TPVPADPPTT	FNQSKLNSFI	rqystgqvsv	EIEWELQKEN	ISKRWNPE
AAV9	QG LLPGMVWQDRDV	YLQGPIWAK	IPHTDG NFHP:	SPLMGGFGMK	HEBEÖIFIKN	TPVPADPPTA	FNKDKLNSFI	IQYSTGQVSV.	SIEWELQKEN	ISKRWNPE
				_			AA	vs —		
	710	720	730							
AAVDJ	IQYTSNYYKSTSVE	FAVNTEGVY	SEPRP IGTRY	LTRNL						
AAV2	IQYTSNYNKSVNVE	FTVDTNGVY	SEPRPIGTRY	LTRNL						
AAV8	IQYTSNYYKSTSVE	FAVNTEGVY	SEPRPIGTRY	LTRNL						
AAV 2	TÄLLENIIKENNAF	e ave tegv y	995 KE TOLKAI	UTKNU						
	←──	AAV8		→						

Figure S2: Sequence and structural alignment of AAV-DJ with its parental strains. Residues differing in amino acid type from AAV-DJ are shown in red. In places, the absence of sequence

differences uniquely defines the parental origin, but in other regions of higher serotype sequence identity, the designation (shown with arrows under the sequences) is ambiguous, so a parsimonious choice has been made that minimizes the number of sequence cross-overs, and gives have closest C_{α} alignment, in the 3D structures.

Supplemental Table

Serotype	Antibody	Neutralizing	Mapping	Resolution	Citation
AAV-1	ADK1a	Yes	Cryo-EM	11 Å	(1)
	ADK1b	Yes	Cryo-EM	11 Å	(1)
	4E4	Yes	Cryo-EM	12 Å	(2)
	5H7	Yes	Cryo-EM	23 Å	(2)
AAV-2	A20	Yes	Cryo-EM	8.5 Å	(3)
			Site-directed mutagenesis		(4)
			Peptide insertion		(5)
			Peptide scanning		(6)
	C37B	Yes	Cryo-EM	11 Å	(2)
			Peptide insertion		(5)
	D3	No	Peptide scanning		(6)
	C24B	Yes	Peptide scanning		(6)
AAV-5	ADK5a	No	Cryo-EM	11 Å	(1)
	ADK5b	Yes	Cryo-EM	12 Å	(1)
	3C5(B)	No	Cryo-EM	15 Å	(2)
AAV-6	5H7	Yes	Cryo-EM	15 Å	(2)
AAV-8	ADK8	Yes	Cryo-EM	19 Å	(7)
			Site-directed mutagenesis		(7)
			Peptide insertion		(7)

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