

Rapid assessment of changes in phage bioactivity using dynamic light scattering

Tejas Dharmaraj^{1,2}, Michael J. Kratochvil³, Julie D. Pourtois⁴, Qingquan Chen¹, Maryam Hajfathalian¹, Aviv Hargil¹, Yung-Hao Lin⁵, Zoe Evans¹, Agnès Oromí-Bosch⁶, Joel D. Berry⁶, Robert McBride⁶, Naomi L. Haddock¹, Derek R. Holman⁷, Jonas D. van Belleghem¹, Tony H. Chang¹, Jeremy J. Barr⁸, Rob Lavigne⁹, Sarah C. Heilshorn³, Francis G. Blankenberg¹⁰, Paul L. Bollyky¹.

¹Division of Infectious Diseases and Geographic Medicine, Department of Medicine, Stanford University School of Medicine, Stanford, CA 94305, USA.

²Sarafan ChEM-H, Stanford University, Stanford, CA 94305, USA.

³Department of Materials Science and Engineering, Stanford University, Stanford, CA 94305

⁴Hopkins Marine Station, Department of Biology, Stanford University, Pacific Grove, CA 93950, USA.

⁵Department of Chemical Engineering, Stanford University, Stanford, CA 94305, USA.

⁶Felix Biotechnology, South San Francisco, CA, 94080.

⁷Division of Gastroenterology and Hepatology, Department of Medicine, Stanford University School of Medicine, Stanford, CA 94305, USA.

⁸School of Biological Sciences, Monash University, Clayton, 3800, VIC, Australia

⁹Department of Biosystems, KU Leuven, Leuven, 3001, Belgium

¹⁰Division of Pediatric Radiology and Nuclear Medicine, Department of Radiology, Lucile Packard Children's Hospital, Stanford, CA 94305, USA.

Corresponding Author: Paul L. Bollyky, MD, PhD, Division of Infectious Diseases and Geographic Medicine, Department of Medicine, Stanford University School of Medicine, Beckman Center for Molecular and Genetic Medicine, 279 Campus Drive, Stanford, CA 94305, USA. pbollyky@stanford.edu.

Email: pbollyky@stanford.edu

This PDF file includes:

Figures S1 to S2

Tables S1 to S2

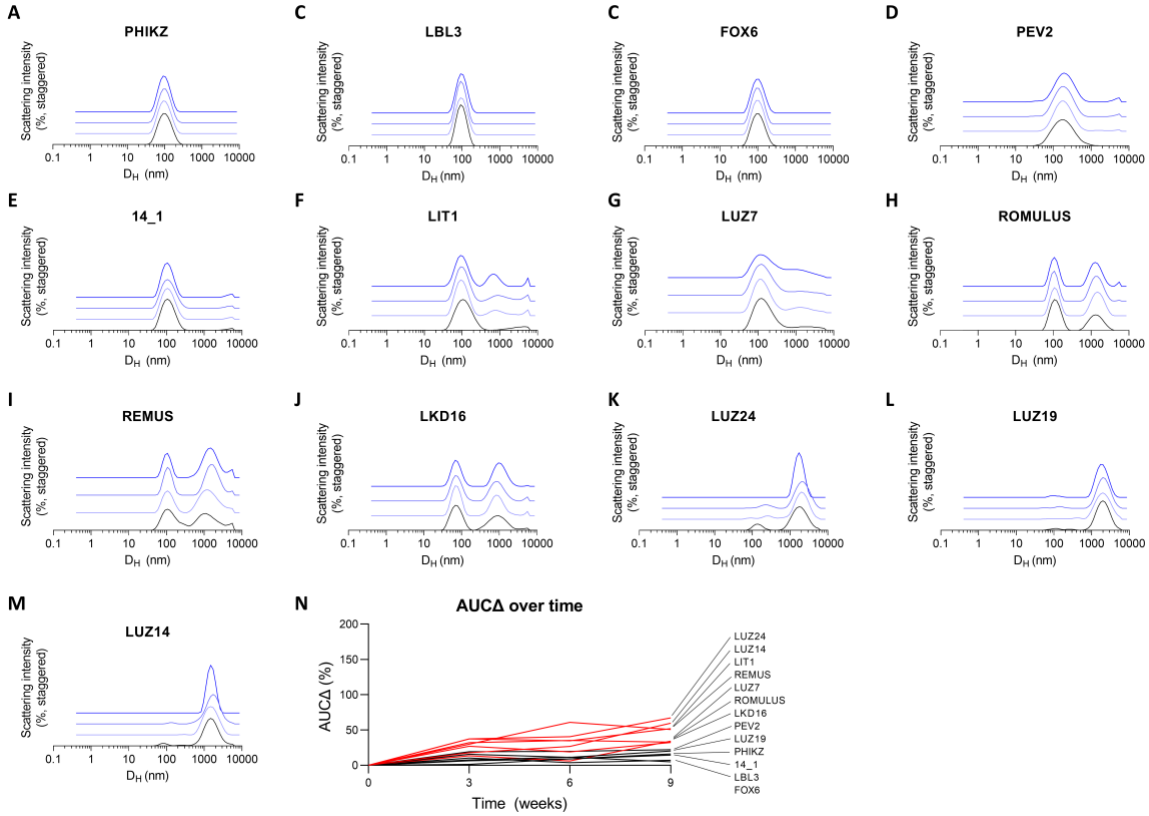


Fig. S1. Phages spontaneously fragment and aggregate in refrigerated storage over time (Belgian cohort).

(A through M) DLS spectra of phages from the Belgian cohort (BC) over a monitoring period of two months. Shown are averages of $n=3$ DLS measurements per phage. $n=13$ phages were assessed. Darker colors and vertical staggering are used to show progression in time. Few phages remained largely intact (A through C). Most phages aggregated over the monitoring period (D through M). (N) AUC Δ identifies the most-changed phage over the monitoring period and is consistent with our qualitative assessment of the DLS spectra.

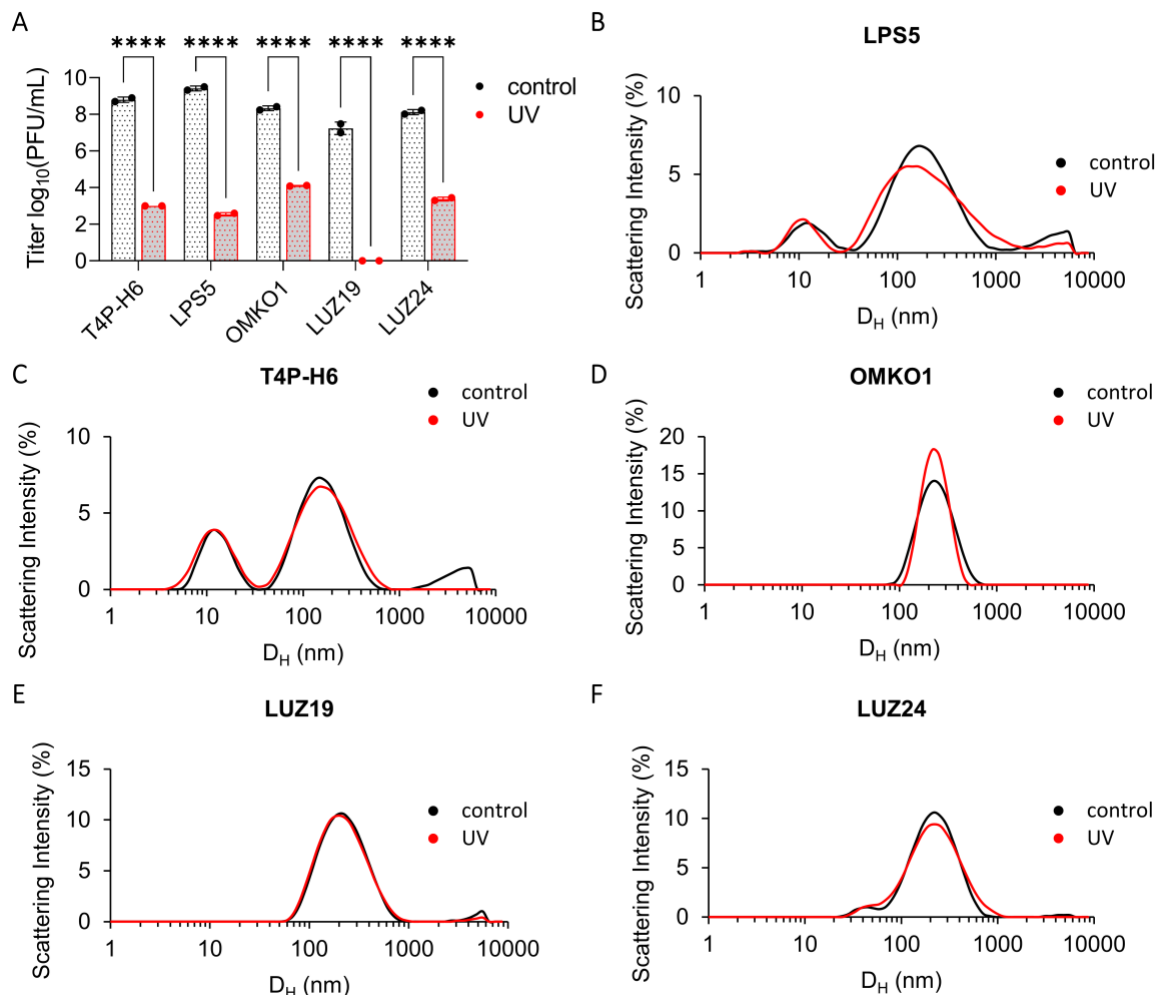


Fig. S2: DLS captures changes in phage size, but it does not capture genomic damage.

(A) Titer of phages after irradiation for 20 minutes with germicidal UV-C light. Results are from one experiment. Titer was measured with $n=2$ plaque assays per phage per condition. Two-way ANOVA with Tukey correction. **** = $p < 0.0001$. (B through F) DLS spectra of phages before and after irradiation. Shown are averages of $n=3$ DLS measurements per phage per condition.

Table S1. Physical and biological characteristics of CYPHY phages.

Phage	T4P-H6	LPS5	OMKO1
Plaque characteristics	Clear, well-circumscribed Size: 0.3 – 1 mm	Clear, well-circumscribed Size: 1.5 – 2.0 mm	Clear, well-circumscribed Size: 0.5 – 1.5 mm
Dimensions	Tail length: 190 ± 5 nm Tail width: 16 ± 2 nm Head length: 74 ± 2 nm Head shape: isometric	Tail length: 140 ± 2 nm Tail width: 20 ± 1 nm Head length: 70 ± 3 nm Head shape: isometric	Tail length: 212 ± 2 nm Tail width: 28 ± 1 nm Head length: 134 ± 4 nm Head shape: isometric
Morphology	<i>Siphoviridae</i>	<i>Myoviridae</i>	<i>Myoviridae</i>

Table S2. Phages used in this study.

Phage	Taxonomy	Bacterial Host	Morphology	genome size (kb)	dim. (nm) capsid / tail
LUZ14	<i>Autographiviridae</i>	<i>P. aeruginosa</i> C1	podovirus	~43	62/12
LUZ19	<i>Autographiviridae</i> , <i>Phikmvvirus</i>	<i>P. aeruginosa</i> PA01 K	podovirus	43.5	65/12
LUZ24	<i>Bruynoghevirus</i>	<i>P. aeruginosa</i> Li010	podovirus	45.6	63/12
LKD16	<i>Autographiviridae</i> , <i>Phikmvvirus</i>	<i>P. aeruginosa</i> GHB15	podovirus	43.2	65/12
LUZ7	<i>Schitoviridae</i> , <i>Luzseptimavirus</i>	<i>P. aeruginosa</i> Br257	podovirus	74.9	76/30
PEV2	<i>Schitoviridae</i> , <i>Litunavirus</i>	<i>P. aeruginosa</i> PA01 K	podovirus	72.7	70/30
LIT1	<i>Schitoviridae</i> , <i>Litunavirus</i>	<i>P. aeruginosa</i> US449	myovirus	72.5	74/30
LBL3	<i>Pbunavirus</i>	<i>P. aeruginosa</i> C1	myovirus	64.4	73/148
14_1	<i>Pbunavirus</i>	<i>P. aeruginosa</i> Li010	myovirus	66.2	73/148
PhiKZ	<i>Phikzvirus</i>	<i>P. aeruginosa</i> Aa245	myovirus	280.3	145/200
Romulus	<i>Herelleviridae</i> , <i>Silviavirus</i>	<i>S. aureus</i> (broad host range)	myovirus	131.3	90/204
Remus	<i>Herelleviridae</i> , <i>Silviavirus</i>	<i>S. aureus</i> (broad host range)	myovirus	134.6	90/204
Fox6	<i>Carmasinavirus</i>	<i>X. campestris</i> pv. <i>campestris</i> I11008	myovirus	61.1	78/156
OMKO1	<i>Phikzvirus</i>	<i>P. aeruginosa</i> PAO1	myovirus	281.8	134/212
LPS5	<i>Pakpunavirus</i>	<i>P. aeruginosa</i> PAO1	myovirus	93.1	70/140
T4P-H6	<i>Nipunavirus</i>	<i>P. aeruginosa</i> PA14	siphovirus	57.4	74/190
T2	<i>Straboviridae</i> , <i>Tequatrovirus</i>	<i>E. coli</i> B	myovirus	163.8	111/78
T3	<i>Autographiviridae</i> , <i>Teetrevirus</i>	<i>E. coli</i> B	podovirus	38.3	60/30
T4	<i>Straboviridae</i> , <i>Tequatrovirus</i>	<i>E. coli</i> B	myovirus	168.9	111/78
T6	<i>Straboviridae</i> , <i>Tequatrovirus</i>	<i>E. coli</i> B	myovirus	170	120/86
T7	<i>Autographiviridae</i> , <i>Tespetimavirus</i>	<i>E. coli</i> B	podovirus	39.9	55/29