

Supporting information

Discovery and evaluation of peptide ligands for selective adsorption and release of Cas9 nuclease on solid substrates

Kevin Day^{†,§}, Raphael Prodromou^{†,§}, Sahand Saberi Bosari[†], Ashton Lavoie[†], Mohammad Omary[†], Connor Market[†], Adriana San Miguel[†], and Stefano Menegatti^{†,‡,*}

[†] Department of Chemical and Biomolecular Engineering, North Carolina State University, 911 Partners Way, Raleigh 27695, USA

[‡] Biomanufacturing Training and Education Center (BTEC), North Carolina State University, 850 Oval Dr, Raleigh, NC 27606, USA

Corresponding author: smenega@ncsu.edu; (919) 515 6398; Engineering Building 1, 911 Partners way, Raleigh, NC 27695-7905.

§ denotes equal contribution.

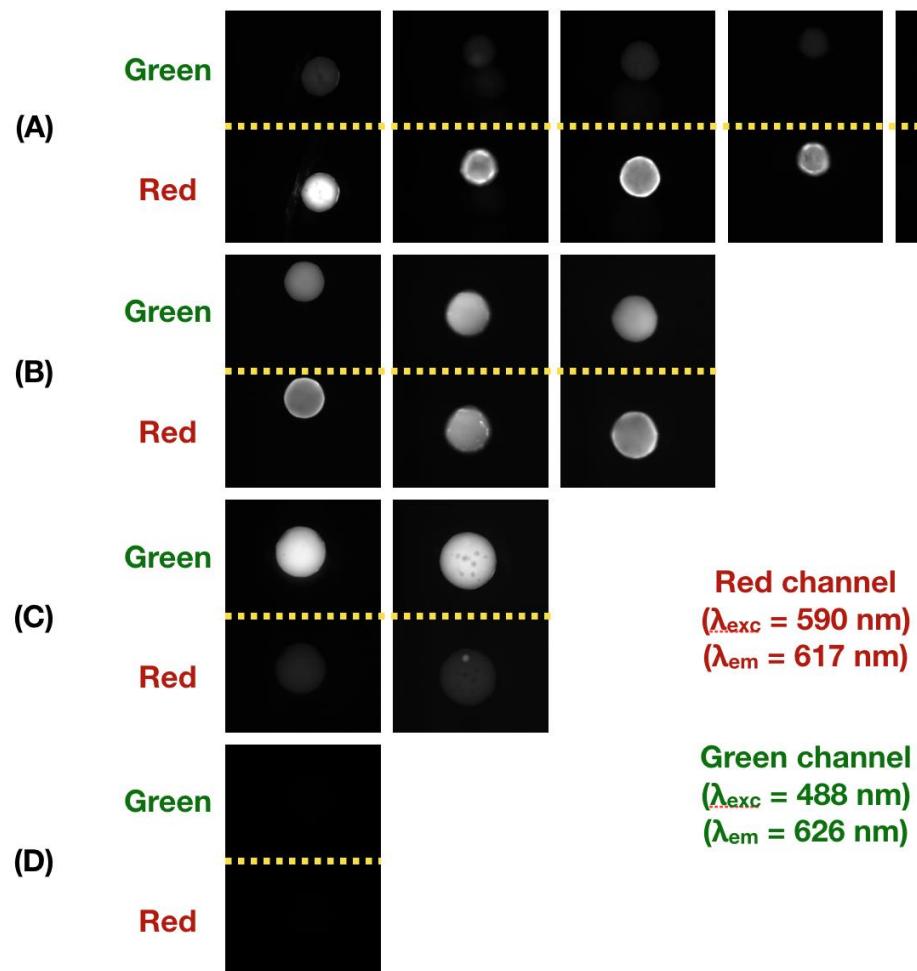
Table S1. Candidate Cas9-binding peptide sequences and corresponding values of Cas9 binding-and yield.

Sequence	Cas9 yield (mg eluted / mg bound)	Sequence	Cas9 yield (mg eluted / mg bound)
SYRQLYR-GSG	49.5%	SGAYLSYQ-GSG	35.1%
SYAYARSG-GSG	49.1%	SRRALLDY-GSG	47.2%
EHLRRQRR-GSG	43.3%	LSAYLSLR-GSG	54.9%
EGQRLLYG-GSG	34.4%	SGYRAAYL-GSG	47.5%
GRRYLDRS-GSG	48.8%	EQYGRRLL-GSG	47.4%
SYRHYYRE-GSG	54.7%	SEYSREGG-GSG	34.8%
SEGYYRRG-GSG	49.6%	GALLRYGQ-GSG	44.7%
SYAYARSG-GSG	49.8%	GYYRYSEYG-SG	56.0%
DYGLYGGR-GSG	48.6%	YQRGGYGA-GSG	50.2%
YYHRHGLQ-GSG	53.8%	SRSGRLGQ-GSG	40.8%
YYRSSEGS-GSG	45.5%	GYGHYQRA-GSG	53.6%
YGRRRDYD-GSG	49.3%	SSEEERLE-GSG	55.8%
YQRYGAGG-GSG	44.8%	DDSADGRY-GSG	3.06%
SYRGSLL-GSG	45.9%	ALHLLRRE-GSG	44.8%
SYLYAQLE-GSG	37.6%	SYRYLRRGG-GSG	50.6%

20 **Table S2.** Free binding energy (ΔG) of the Cas9:peptide complexes obtained by *in silico* peptide docking
 21 on Cas9 (PDB ID: 4008).

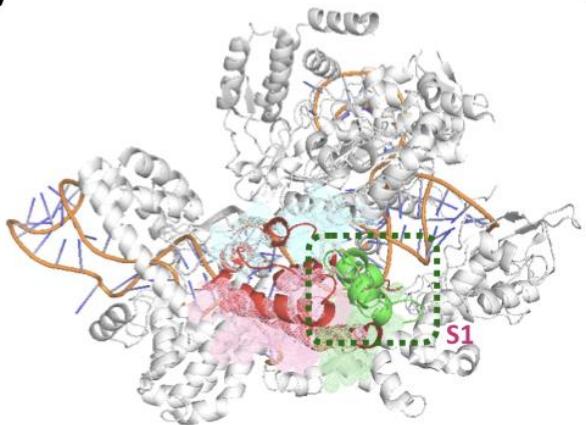
Sequence	Site 1 ΔG (kcal/mol)	Site 2 ΔG (kcal/mol)	Site 3 ΔG (kcal/mol)	Site 4 ΔG (kcal/mol)	Site 5 ΔG (kcal/mol)	Site 6 ΔG (kcal/mol)
SYRYLRGG-GSG	-7.963	-6.499	-5.473	-4.976	--	--
SYRHYYRE-GSG	-6.042	-7.360	-5.864	--	--	--
SYRYQLYR-GSG	-7.530	-6.809	-6.375	--	--	--
DYGLYGGR-GSG	-6.218	-5.521	-5.636	5.450	--	--
GYYRYSEY-GSG	-7.346	-6.969	-5.617	-6.651	-5.511	-5.389
YYHRHGLQ-GSG	-9.724	-5.466	-4.782	--	--	--

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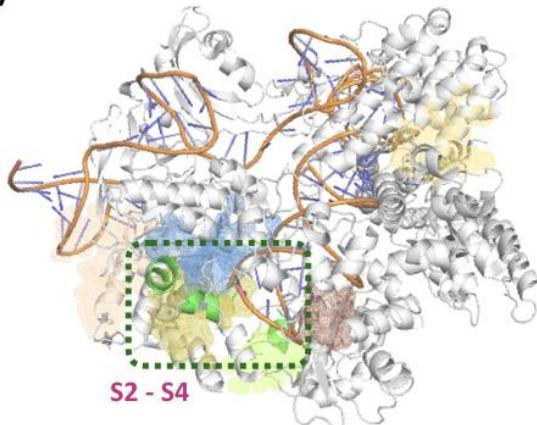


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 25 **Figure S1.** **(A)** Positive beads (red fluorescence only) carrying sequences SYRHYYRE-GSG, SYRYQLYR-GSG,
 26 DYGLYGGR-GSG, GYYRYSEY-GSG, and YYHRHGLQ-GSG; **(B)** Negative beads (red and green fluorescence); **(C)**
 27 Negative beads (green fluorescence only); **(D)** Negative beads (no fluorescence).

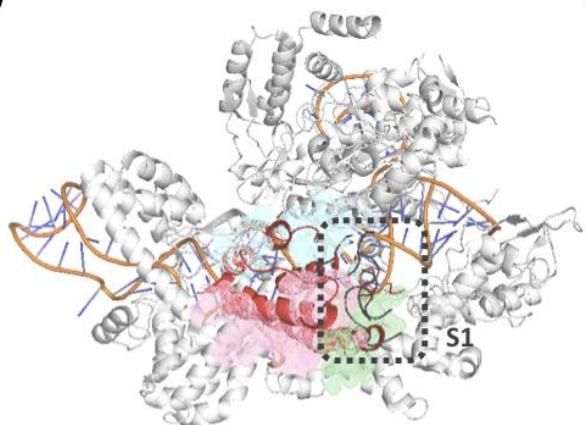
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29 (A)



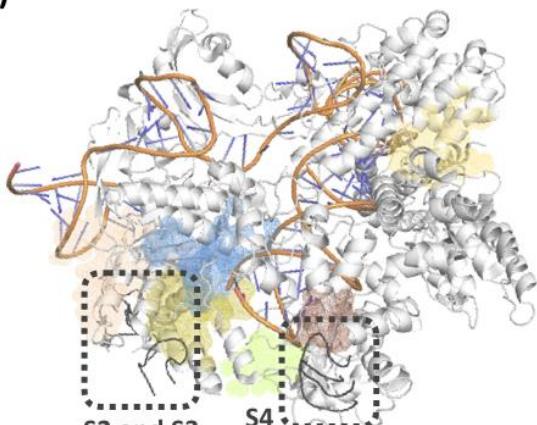
30 (B)



31 (C)



32 (D)



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30 **Figure S2.** Docking of SYRYLRGG-GSG on (A) front and (B) back sides of Cas9 (PDB ID: 4OO8); docking of
31 DYGLYGGR-GSG on (C) front and (D) back sides of Cas9 (PDB ID: 4OO8). The putative binding sites are highlighted
32 as reported in **Figure 1**. Peptide SYRYLRGG is in green, peptide DYGLYGGR is in black, Cas9 is in grey, and the DNA
and guide RNA are in orange/blue.