

Accelerated RNA detection using tandem CRISPR nucleases

Tina Y. Liu^{1,2}, Gavin J. Knott¹⁻³, Dylan C. J. Smock^{1,2}, John J. Desmarais¹, Sungmin Son⁴, Abdul Bhuiya^{4,5}, Shrutee Jakhanwal^{1,2}, Noam Prywes², Shreeya Agrawal^{2,4}, María Díaz de León Derby^{4,5}, Neil A. Switz⁷, Maxim Armstrong^{4,8}, Andrew R. Harris⁴, Emeric J. Charles¹, Brittney W. Thornton¹, Parinaz Fozouni⁹⁻¹², Jeffrey Shu^{9,12}, Stephanie I. Stephens^{9,12}, G. Renuka Kumar^{9,12}, Chunyu Zhao^{9,13}, Amanda Mok¹⁴, Anthony T. Iavarone¹⁵, Arturo M. Escajeda¹⁶, Roger McIntosh¹⁶, Shin E. Kim^{1,2}, Eli J. Dugan^{1,2}, IGI Testing Consortium², Katherine S. Pollard^{9,13,17}, Ming X. Tan¹⁶, Melanie Ott^{9,12}, Daniel A. Fletcher^{4,5,13}, Liana F. Lareau^{2,4}, Patrick D. Hsu^{2,4}, David F. Savage^{2,4}, Jennifer A. Doudna^{1,2,8,9,18-21}

¹Department of Molecular and Cell Biology, University of California, Berkeley, CA, USA

²Innovative Genomics Institute, University of California, Berkeley, Berkeley, CA, USA

³Monash Biomedicine Discovery Institute, Department of Biochemistry & Molecular Biology, Monash University, VIC 3800, Australia

⁴Department of Bioengineering, University of California, Berkeley, CA, USA

⁵UC Berkeley-UC San Francisco Graduate Program in Bioengineering, University of California, Berkeley, Berkeley, CA, USA

⁶Division of Biological Systems and Engineering, Lawrence Berkeley National Laboratory, Berkeley, CA, USA

⁷Department of Physics and Astronomy, San José State University, San José, CA, USA

⁸Molecular Biophysics and Integrated Bioimaging Division, Lawrence Berkeley National Laboratory, Berkeley, CA, USA

⁹Gladstone Institute of Virology, Gladstone Institutes, San Francisco, CA, USA.

¹⁰Medical Scientist Training Program, University of California, San Francisco, San Francisco, CA 94143, USA

¹¹Biomedical Sciences Graduate Program, University of California, San Francisco, San Francisco, CA 94143, USA

¹²Department of Medicine, University of California, San Francisco, San Francisco, CA 94143, USA

¹³Chan-Zuckerberg Biohub, San Francisco, CA, USA

¹⁴Center for Computational Biology, University of California, Berkeley, Berkeley, CA, USA;

¹⁵QB3/Chemistry Mass Spectrometry Facility, University of California, Berkeley, Berkeley, CA, USA

¹⁶Wainamics Inc., Pleasanton, CA, USA

¹⁷Department of Epidemiology & Biostatistics, University of California, San Francisco, CA, USA

¹⁸Howard Hughes Medical Institute, University of California, Berkeley, Berkeley, CA, USA

¹⁹Department of Chemistry, University of California, Berkeley, Berkeley, CA, USA

²⁰Gladstone Institute of Data Science and Biotechnology, Gladstone Institutes, San Francisco, CA, USA

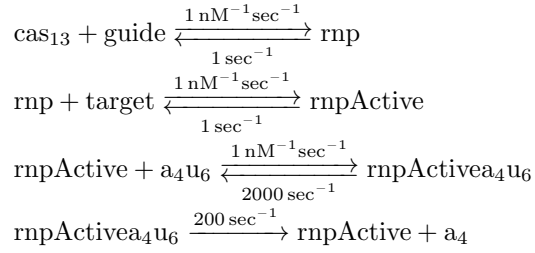
²¹California Institute for Quantitative Biosciences (QB3), University of California, Berkeley, Berkeley, CA, USA.

*Correspondence to: doudna@berkeley.edu

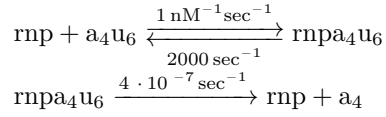
Supplementary Information

1 Chemical equations for cas13 to csm6 transduction model

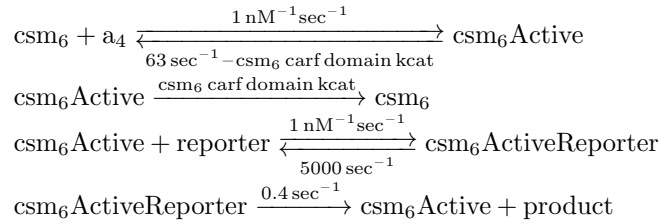
1.1 cas13 on target activity



1.2 cas13 background activity



1.3 csm6 activity



2 cas13 to csm6 transduction model

2.1 Ordinary differential equations for component concentrations

$$\begin{aligned} c'_{a4}(t) &= -c_{a4}(t)c_{csm6}(t) + (63 - csm6 \text{ Carf domain kcat}) \\ &\quad c_{csm6Active}(t) + \frac{c_{rnpa4u6}(t)}{2500000} + 200c_{rnpActivea4u6}(t), \end{aligned}$$

$$\begin{aligned} c'_{a4u6}(t) &= -c_{a4u6}(t)c_{rnp}(t) - c_{a4u6}(t)c_{rnpActive}(t) + \\ &\quad 2000c_{rnpa4u6}(t) + 2000c_{rnpActivea4u6}(t), \end{aligned}$$

$$c'_{cas13}(t) = c_{rnp}(t) - c_{cas13}(t)c_{guide}(t),$$

$$\begin{aligned} c'_{csm6}(t) &= -c_{a4}(t)c_{csm6}(t) + \\ &\quad (63 - csm6 \text{ Carf domain kcat})c_{csm6Active}(t) + \\ &\quad csm6 \text{ Carf domain kcat}c_{csm6Active}(t), \end{aligned}$$

$$\begin{aligned} c'_{csm6Active}(t) &= c_{a4}(t)c_{csm6}(t) - (63 - csm6 \text{ Carf domain kcat})c_{csm6Active}(t) - \\ &\quad csm6 \text{ Carf domain kcat}c_{csm6Active}(t) - c_{csm6Active}(t) \\ &\quad c_{reporter}(t) + 5000.4c_{csm6ActiveReporter}(t), \end{aligned}$$

$$c'_{csm6ActiveReporter}(t) = c_{csm6Active}(t)c_{reporter}(t) - 5000.4c_{csm6ActiveReporter}(t),$$

$$c'_{guide}(t) = c_{rnp}(t) - c_{cas13}(t)c_{guide}(t),$$

$$c'_{product}(t) = 0.4c_{csm6ActiveReporter}(t),$$

$$c'_{reporter}(t) = 5000c_{csm6ActiveReporter}(t) - c_{csm6Active}(t)c_{reporter}(t),$$

$$\begin{aligned} c'_{rnp}(t) &= -c_{a4u6}(t)c_{rnp}(t) + c_{cas13}(t)c_{guide}(t) - c_{rnp}(t)c_{target}(t) - \\ &\quad c_{rnp}(t) + \frac{5000000001c_{rnpa4u6}(t)}{2500000} + c_{rnpActive}(t), \end{aligned}$$

$$c'_{rnpa4u6}(t) = c_{a4u6}(t)c_{rnp}(t) - \frac{5000000001c_{rnpa4u6}(t)}{2500000},$$

$$c'_{rnpActive}(t) = -c_{a4u6}(t)c_{rnpActive}(t) + c_{rnp}(t)c_{target}(t) - c_{rnpActive}(t) + 2200c_{rnpActivea4u6}(t),$$

$$c'_{rnpActivea4u6}(t) = c_{a4u6}(t)c_{rnpActive}(t) - 2200c_{rnpActivea4u6}(t),$$

$$c'_{target}(t) = c_{rnpActive}(t) - c_{rnp}(t)c_{target}(t),$$

2.2 Starting concentrations of components

$$\begin{array}{lll} c_{a4}(0) = 0nM & c_{csm6}(0) = 100nM & c_{guide}(0) = 13.5078nM \\ c_{a4u6}(0) = 2000nM & c_{csm6Active}(0) = 0nM & c_{product}(0) = 0nM \\ c_{cas13}(0) = 13.5078nM & c_{csm6ActiveReporter}(0) = 0nM & c_{rnp}(0) = 36.4922nM \\ c_{rnpa4u6}(0) = 0nM & c_{rnpActive}(0) = 0nM & c_{rnpActivea4u6}(0) = 0nM \\ c_{target}(0) = target & & \end{array}$$