

1
2 **SUPPLEMENTAL INFORMATION**

3 **Slow leakage of Ca-dipicolinic acid from individual *Bacillus***
4 **spores during initiation of spore germination**

5 Shiwei Wang¹, Peter Setlow², and Yong-qing Li^{1*}

6 ¹*Department of Physics, East Carolina University, Greenville, North Carolina 27858-4353, and*

7 ²*Department of Molecular Biology and Biophysics, University of Connecticut Health Center,*

8 *Farmington, Connecticut 06030-3305*

9 Key words: CaDPA release; *Bacillus*; spores; germination; commitment

10 Running title: Slow DPA leakage during initiation of spore germination

11 *Corresponding author

12 Address: Department of Physics, East Carolina University, Greenville, North Carolina 27858-
13 4353

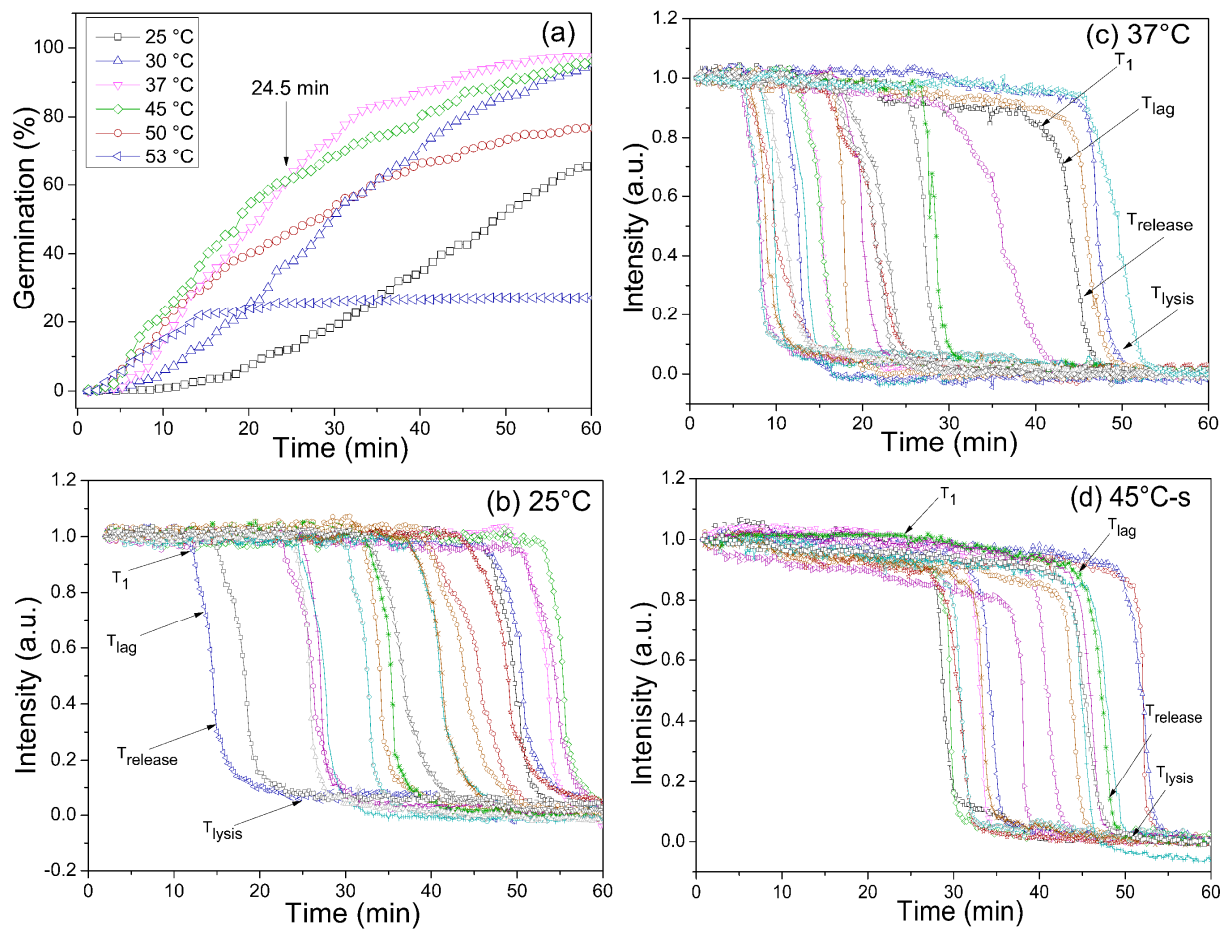
14 Phone: 252-328-1858

15 Fax: 252-328-6314

16 Email: liy@ecu.edu

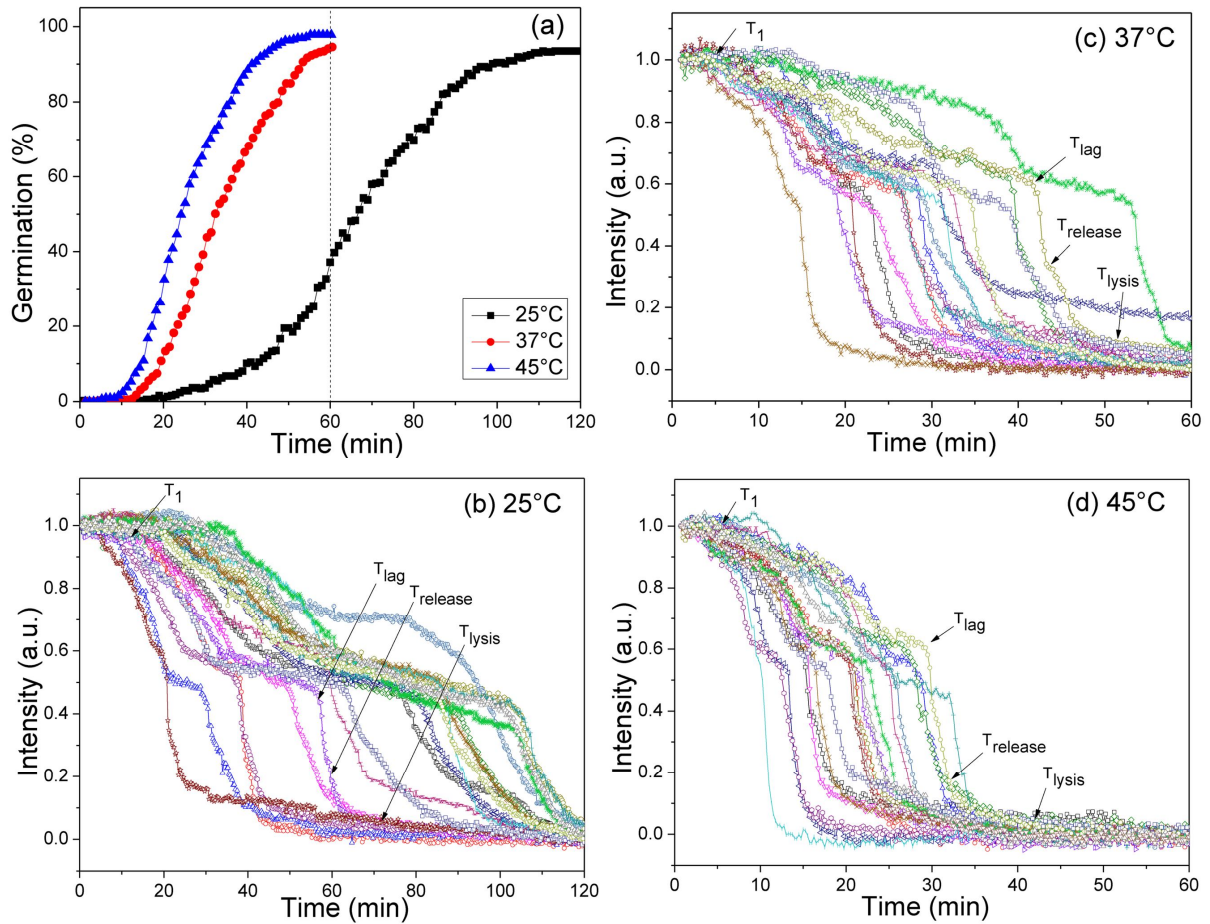
17

*Address correspondence to Yong-qing Li, East Carolina University, Department of Physics, Greenville, North Carolina 27858-4353 United States of America; Tel: 252-328-1858; Fax: 252-328-6314; E-mail: liy@ecu.edu



18

19 Fig. S1 (a-d) Germination extents and phase contrast image intensities of individual wild-type *B.*
 20 *subtilis* spores germinating with AGFK at various temperatures. PS533 spores (wild-type) were
 21 germinated with 25 mM AGFK at various temperatures and spore germination was monitored by
 22 phase contrast image intensity changes as described in Methods. The phase contrast image
 23 intensities (a.u.) were normalized to 1 based on the respective values at the first time of
 24 measurement, and phase contrast image intensities at the end of the experiment were set at 0. The
 25 black arrows indicate the times of T_1 , T_{lag} , $T_{release}$ and T_{lysis} for a single spore. In a, the
 26 germination curves at the different temperatures are from data with > 427 spores each, and a
 27 germinated spore was defined as one that had reached $T_{release}$. In panel (d), s means spores
 28 germinating slowly at 45 °C ($T_{release} > 24.5$ min in panel (a)).



29

30 Fig. S2 (a-d) Germination extents (a) and rates (b-d) of multiple individual *B. subtilis* FB111
 31 (*cwlJ*) spores germinating with L-valine at various temperatures. FB111 spores were germinated
 32 with 10 mM L-valine at various temperatures and spore germination was monitored by PC image
 33 intensity changes as described in Methods. The PC image intensities (a.u.) were normalized to 1
 34 based on the respective values at the first time of measurement, and PC image intensities at the
 35 end of the experiment were set at 0. The black arrows indicate the times of T_1 , T_{lag} , $T_{release}$ and
 36 T_{lysis} for a single spore. Note that in FB111 (*cwlJ*) spores T_{lag} is much larger and I_{lag} is much
 37 smaller than in germinating wild-type PS533 (Table 5). In panel (a), the germination curves at

38 the different temperatures are from data with > 323 spores each, and a germinated spore was
39 defined as one that had reached T_{release} .

40

Table S1

41 Mean values and standard deviations of germination parameters of multiple individual wild-type
 42 *B. subtilis* spores germinating with AGFK at different temperatures*

Germination temperature	T ₁ (min)	T _{lag} (min)	ΔT _{leakage} (min)	T _{release} (min)	ΔT _{release} (min)	I _{lag}	No. of germinated spores (% germination)
23°C	40.3±16.0	42.4±16.4	2.1±1.1	45.0±16.7	2.7±0.8	0.84±0.06	67.2%(287)
30°C	24.4±12.6	25.9±13.1	1.5±1.1	28.6±13.4	2.7±0.8	0.87±0.08	95.3%(465)
37°C	15.2±8.5	18.2±10.2	2.9±3.8	21.1±10.6	2.9±0.9	0.86±0.10	97.2%(418)
45°C	14.7±9.8	22.9±14.9	8.2±9.1	25.7±15.5	2.7±2.3	0.84±0.09	95.3%(444)
50°C	12.5±10.6	21.1±15.0	8.5±8.7	23.7±15.3	2.6±1.0	0.85±0.09	76.7%(419)

43

44 *As described in the legend to Fig. S1, PS533 (wild-type) spores were germinated with 25 mM
 45 AGFK at various temperatures, and spore germination parameters were determined as described
 46 in Method.

47

48

Table S2

49 Mean values and standard deviations of germination parameters of *B. subtilis* spores germinating
 50 with CaDPA or dodecylamine and *B. cereus* and *B. megaterium* spores germinating with
 51 nutrients*

Spores	Germinant	T ₁ (min)	T _{lag} (min)	ΔT _{leakage} (min)	T _{release} (min)	ΔT _{release} (min)	I _{lag}
PS533	50 mM CaDPA	11.2±5.4	34.1±12.0	22.9±10.5	35.8±11.9	1.7±0.9	0.72±0.01
PS832	0.5 mM Dodecylamine	17.5±16.9	35.1±15.5	17.6±12.2	36.9±15.5	1.7±0.8	0.80±0.08
<i>B. cereus</i>	2 mM L- alanine	4.6±2.5	11.9±5.3	7.3±4.8	13.0±5.5	1.1±0.4	0.92±0.05
<i>B. megaterium</i>	10 mM Glucose	15.2±8.8	20.1±9.9	4.8±2.4	22.7±10.3	2.6±0.9	0.81±0.06

52

53 *As described in the legend to Fig. 6, spores were germinated with various agents, and
 54 germination parameters were determined as described in Methods.

55