Supporting information --- ---

Ordered self-assembly of proteins for computation in mammalian cells

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Experimental Section

B. cereus **strains and culture conditions.**

The wild-type *B. cereus* reference strain for Nhe production NVH 75/95 was designated MHI 1491 in this work (1). The mutant strains producing only parts of the three components of Nhe complex were designated as MHI 1761 (no expression of NheA) and MHI 1672 (no expression of NheC), genetic and expression profiles of these strains have been previously demonstrated by PCR, ELISA, and cell proliferation assay (2). For the production of the intact Nhe toxin or functional active Nhe components, CGY medium plus 1% glucose was used as previously described (3). Briefly, *B. cereus* was inoculated into 20 mL CGY medium plus 1% glucose, and cultured in a water bath for 17 h at 32 °C. Subsequently 0.1 mL of the culture liquid was transferred into 20 mL CGY medium plus 1% glucose and incubated for another 6 h at 32 °C. 200 μL EDTA (1mM) was added at the harvesting time, the supernatants were collected by centrifugation for 20 min at 3 000 g, at 4 °C, then passed through 0.22 μm filter to get the cell-free supernatants for further use. More details of the strains used in this work are listed in Table S2.

Mammalian cell lines and culture conditions.

A549, Caco-2 and IPEC-J2 cell lines were provided by the German Collections of Microorganisms and Cell Cultures (DSMZ). HEp-2 and Vero cell lines were purchased from the European Collections of Cell Cultures. Cells were cultured at 37 °C and 7% CO_2 in cell culture media as recommended by the suppliers (4).

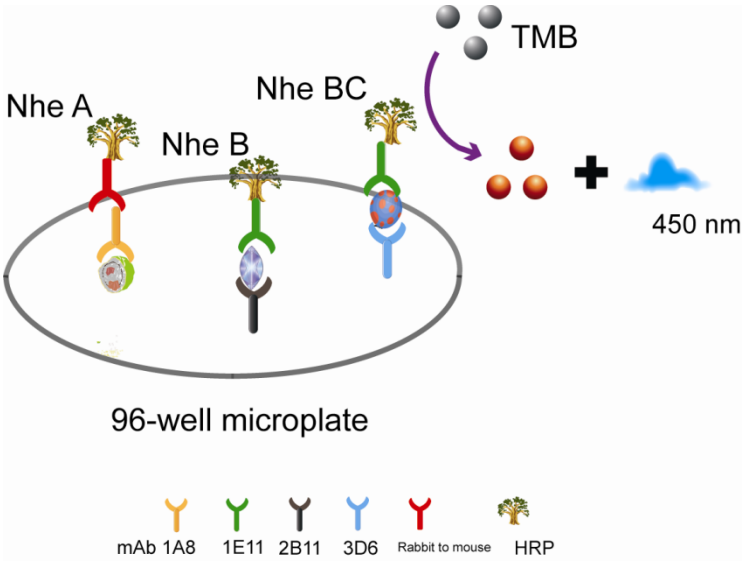
Cell proliferation assay.

Cytotoxic effects of the different components of Nhe complex from *B. cereus*, and the recombinant components of Nhe were tested on diverse cells under simultaneous incubation conditions with the addition of WST-1 (water-soluble tetrozolium salt, Roche Diagnostics) and PI (propidium iodide, Fluka) to the culture media, subsequently the cellular viability was determined as endpoint titer by Tecan photometer and VICTOR™ Multilabel Plate Reader as previously described (2). Briefly, serial dilutions of the Nhe components or the tripartite complex were placed into 96-well microplates (0.1 mL/well) and 0.1 mL cell suspension (A549, Caco-2 and HEp-2, 2×10^4 cells/well; IPEC-J2, 5×10^3 cells/well; and Vero, 1×10^4 cells/well) were added immediately afterwards. The growth media and dilution solutions consisted of MEM or DMEM supplemented with additional nutritional factors as claimed by the suppliers. After 24 h incubation of the test mixture at 37 °C in a 7% CO_2 atmosphere, the morphology of the cells was checked by light microscopy. Moreover, the mitochondrial

activity of viable cells was detected by colorimetric measurement at 450 nm after the addition of WST-1, and the fluorescence of PI bound to DNA was measured by setting excitation and emission wavelength to 535 nm and 617 nm, respectively. The dose dependent curves were used to evaluate the 50% inhibitory concentrations of the tested targets of interest by linear interpolation. The highest dilution of reference strain MHI1491 that resulted in 50% dead cells was defined as 100% cytotoxicity.

PCR and ELISA.

The *nhe* genes of the three components in each strain were detected according to the previous report (2). On the protein level, detection of the Nhe component was performed using different formats of ELISA, as shown in Scheme S1. NheA was detected by an indirect ELISA using monoclonal antibody (mAb 1A8) as the primary antibody, meanwhile, two mAbs 1E11 and 2B11, which recognize different epitopes of NheB, were employed for the sandwich ELISA (5). No free NheC can be detected in solution, because it forms a stable complex with NheB (6). Therefore, a complex ELISA was established to detect this toxin component. In this ELISA, the NheC specific mAb 3D6 was used as the capture antibody, and the horseradish peroxidase (HRP) labeled mAb 1E11 against NheB was used as detection antibody.



Scheme S1. Schematic representation of different formats of ELISA used for the detection of Nhe components produced by *B. cereus*. NheA, indirect ELISA (mAb 1A8), NheB, sandwich ELISA (mAbs 1E11 and 2B11) and NheBC, complex ELISA (mAb 3D6 and 1E11), respectively.

Tables

Strain (MHI)		*nhe* gene	Nhe proteins A	B	C			
1	1477	+	+	+	+	1		
2	1489	+	+	+	+	1		
3	1493	+	+	+	+	1		
4	1496	+	+	+	+	1		
5	1503	+	+	+	+	1		
6	1504	+	+	+	-	0	0	1
7	1507	+	+	+	+	1		
8	1522	+	+	+	+	1		
9	1527	+	+	+	+	1		
10	1541	+	+	+	+	1		
11	1543	+	+	+	+	1		
12	1556	+	-	-	-	0	0	0
13	1647	+	+	-	-	0	0	1
15	1668	+	+	+	+	1		
16	1670	+	-	+	+	0	1	0
17	1692	+	+	+	+	1		
18	1698	+	-	-	-	0	0	0
19	1699	+	-	-	-	0	0	0
20	1700	+	-	-	-	0	0	0
21	2963	+	+	+	+	1		
22	2965	+	+	+	+	1		
23	2967	+	+	+	+	1		
24	2968	+	-	+	+	0	1	0
28	2970	+	+	+	+	1		
29	3038	+	+	+	+	1		
30	3086	+	+	+	+	1		
	Total	**30**	**24**	**25**	**24**	**22**	**2**	**2**

Table S1. Comparison of the actual toxicity of the Nhe complex, produced by diverse *B. cereus* strains (= input X1), with their gene and protein profile; *nhe* genes were detected by PCR; proteins NheA, B, and C were detected by ELISA based on monoclonal antibodies; toxicity is presented as 0/1 result of the Buffer gate; 0/1 result of the AND gates provides additional information about the toxin profile.

	Strains	Nhe components			References
B. cereus	MHI 1672[a]		B		*2, 7*
	MHI 1672	A	B		*7*
	MHI 1761		B	C	*7*
	MHI 1491	A	B	C	*1,2,7*
E. coli	LMG 194	rNhe A			*8*
	LMG 194			rNhe C	*5, 8*

Table S2. Strains of *B. cereus* and *E. coli* and the associated Nhe-components used in this study.

Note: The *B. cereus* reference strain for Nhe complex NVH 75/95 was designated MHI 1491.

MHI 1672[a], Nhe B was purified by immunoaffinity chromatography (IAC) from the supernatant of MHI 1672. *E. coli* was used as the vector to produce the recombinant NheA and C, respectively.

Figures

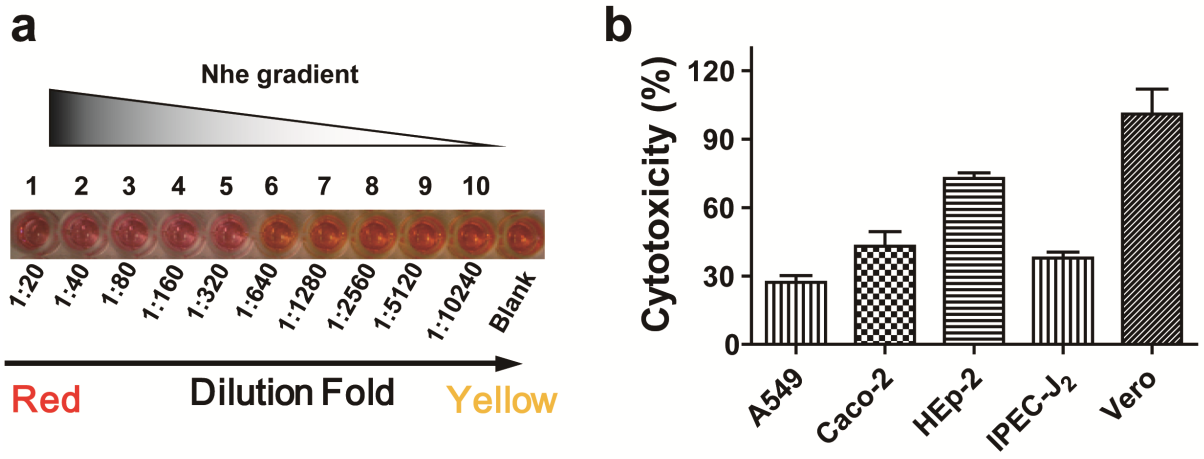


Fig. S1 (a) Photograph of Nhe complex-treated cells with WST-1. Well 1-11, 1×10^4 Vero cells were inoculated into each well; Wells 1-10, Nhe was added in a two-fold serial dilution, starting at 1:20. Well 11, blank control without Nhe complex. (b) Susceptibility of several cell lines to Nhe complex. Five different cell lines, A549, Caco-2, HEp-2, IPEC-J_2 and Vero cells were treated with Nhe complex to trigger full cytotoxicity.

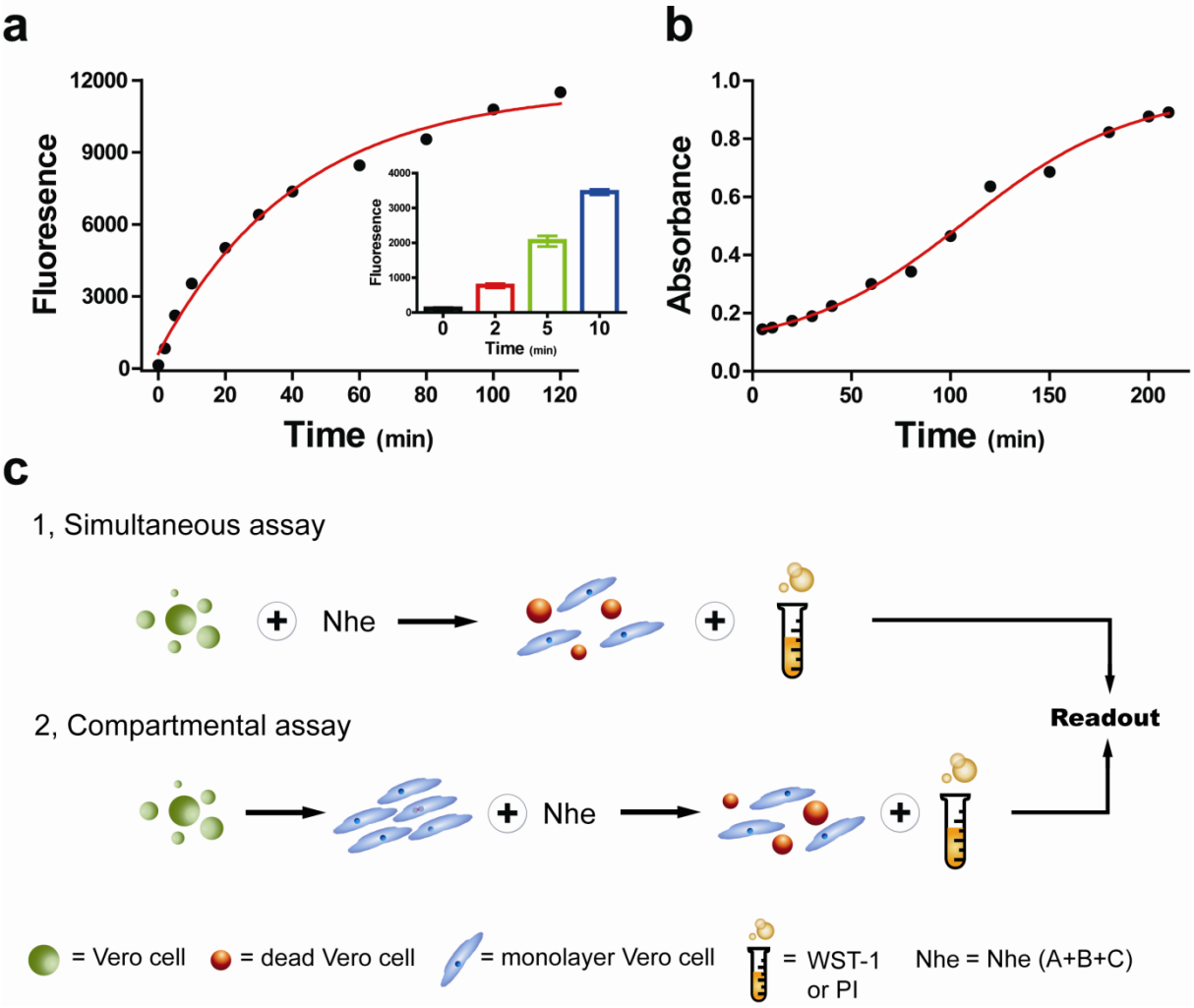


Fig. S2 Time-dependent cytotoxicity of the Nhe complex to Vero cells. A monolayer of cells was treated with Nhe complex for different time periods ranging from 0 min to 120 min (PI measurement, a) and from 0 min to 200 min (WST-1 measurement, b). The inset shows the PI response during the first 10 min. (c) Scheme of simultaneous and compartmental assays used in this work.

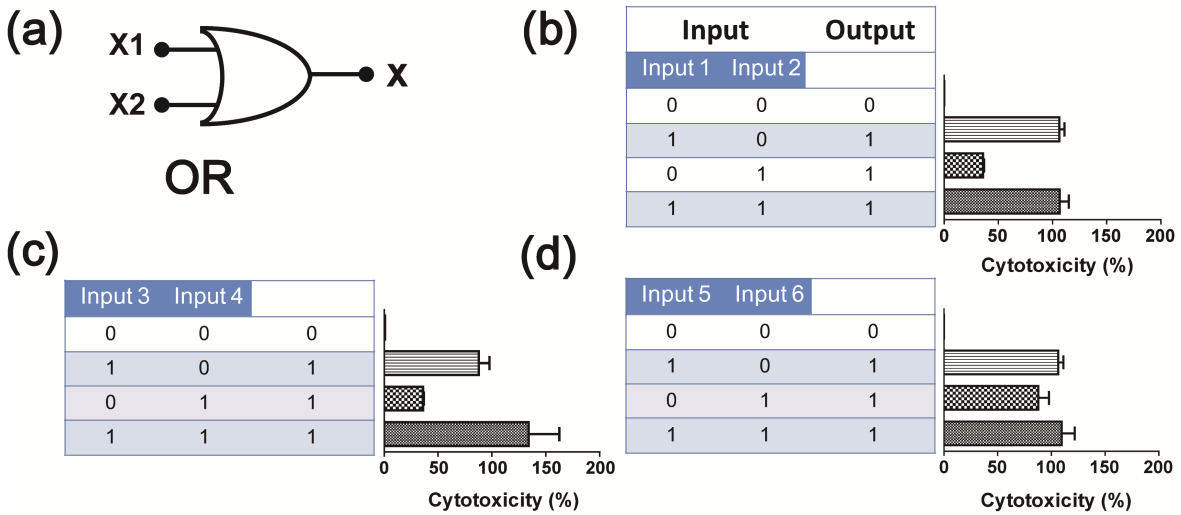


Fig. S3 Nhe-based cellular OR gates. Equivalent circuit (a) and truth tables of the cellular OR gates and the associated cytotoxicity. b) OR gate of NheA, NheA (input 1) and rNheA (input 2), c) OR gate of NheB, NheB of MHI 1672 (input 3) and NheB of MHI 1761 (input 4), and (d) OR gate of NheC, NheC (input 5) and rNheC (input 6). Each gate contained the complementary Nhe components to trigger full cytotoxicity.

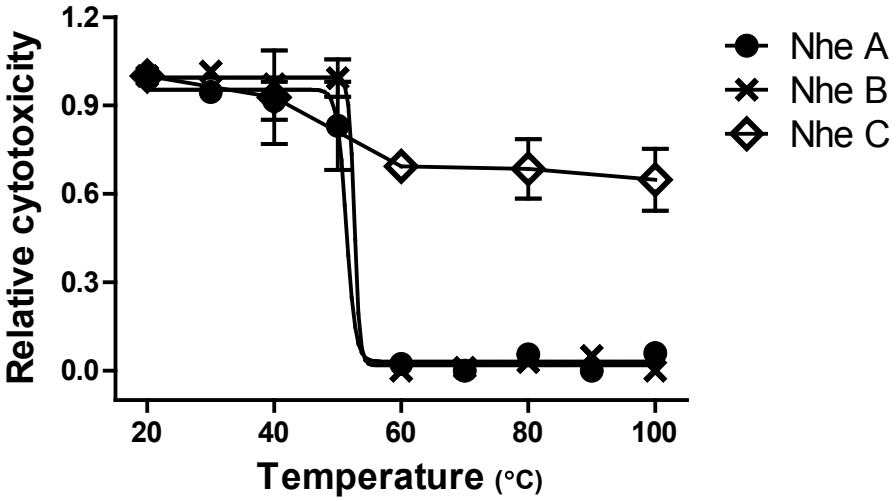


Fig. S4 Thermostability of each component in the tripartite Nhe complex. The Nhe complex was heated for 10 min from 20 °C to 100 °C. The relative cytotoxicity was defined as the ratio of the cytotoxic titer of the heated components to that of the control.

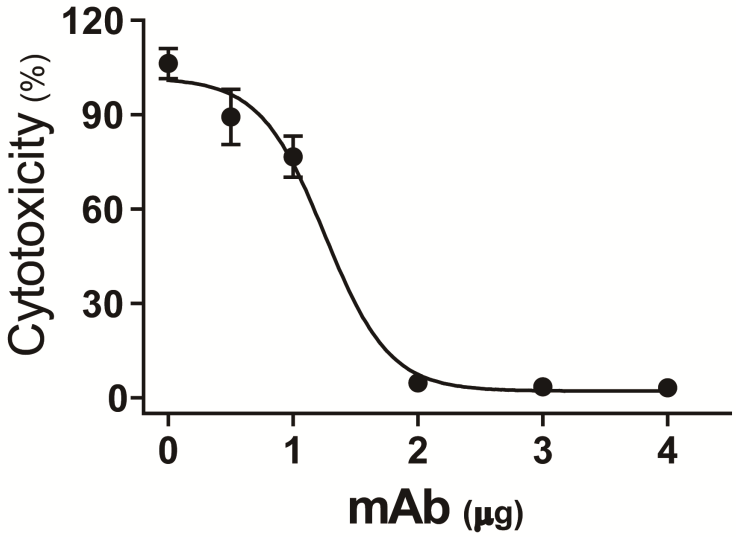


Fig. S5 Inhibition of cytotoxicity by mAb (1E11) against NheB; the antibody was used to construct an INHIBIT logic gate.

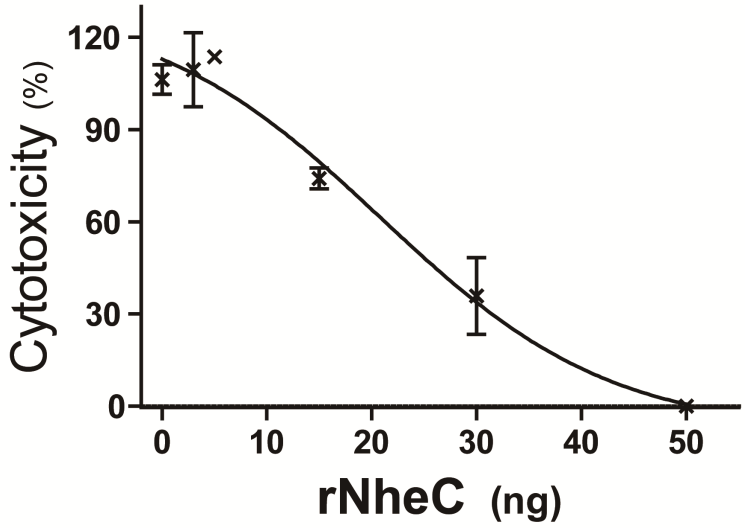
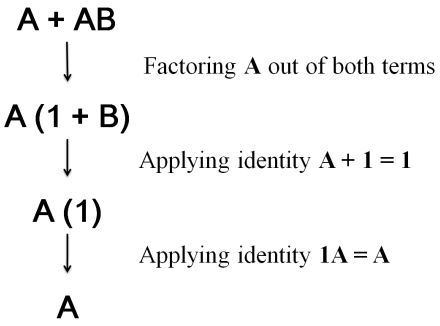


Fig. S6 Inhibition of cytotoxicity by excess rNheC; an excess amount of rNheC was used to construct an INHIBIT logic gate.

a



b

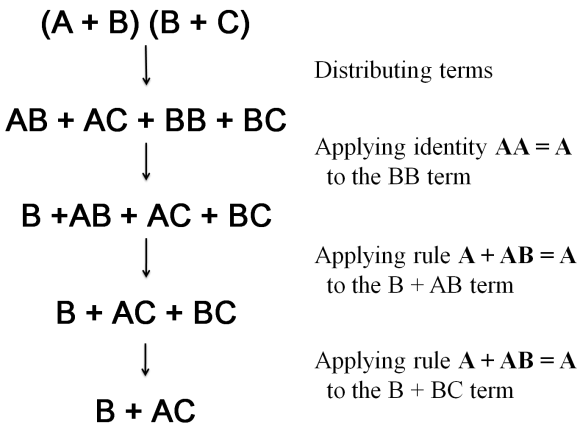


Fig. S7 The proofs of the two Boolean rules for simplification in the Nhe-based cellular logic system.

References

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