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Boolean Logic Functions of a Synthetic Peptide Network

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Living cells can process rapidly and simultaneously multiple extracellular input signals through the complex networks of evolutionary selected biomolecular interactions and chemical transformations.[1] Recent approaches to molecular computation have increasingly sought to mimic or exploit various aspects of biology. A number of studies have adapted nucleic acids[2] and proteins[3] to the design of molecular logic gates and computational systems, while other works have affected computation in living cells via biochemical pathway engineering.[4,5] Here we report that de novo designed synthetic peptide networks can also mimic some of the basic logic functions of the more complex biological networks. We show that segments of a small network whose graph structure is composed of five nodes and 15 directed edges (Figure 1) can express OR, NOR, and NOTIF logic.

We described recently the graph structure and experimental analysis of a self-organized synthetic peptide network.[6] The system was designed rationally to operate in neutral aqueous solutions based on the sequence-selective auto- and cross-catalytic template-directed coiled-coil peptide fragment condensation reactions.[7] Given that this relatively simple synthetic network appeared to display some of the basic architectural and dynamic features of the much larger complex systems,[1] we sought to examine whether selected segments of the network[6] (Figure 1) could also carry out basic Boolean logic operations.[1a,3] We have established experimentally that the rates of product formation in isolated reaction mixtures follow closely the relative order of the predicted pathway efficiencies.[6] However, as to be expected, the rates of product formation in the context of the network is significantly different than in isolated reactions since the preponderance of certain nodes depend on the competition between and/or integrated inputs from other node(s). Accordingly, we envisioned that since the formation of a given node within the network can be regulated by more than one template-directed pathway, certain nodes could be evaluated as outputs of logic processes in response to selected system inputs.[1a] Specifically, because of the prominent positions of nodes $\mathbf{T_3}$ and $\mathbf{T_7}$ in the network and their patterns of network interconnectivities (Figure 1), we chose to examine each of these nodes separately as outputs in two input logic operations.

The linear nature of the $\mathbf{T_3} \rightleftarrows \mathbf{T_7} \rightleftarrows \mathbf{T_4}$ sub-network connectivity suggests that $\mathbf{T_7}$ might function as the output of an OR gate with $\mathbf{T_3}$ and $\mathbf{T_4}$ as the inputs. The OR logic function was validated in isolated experiments in which the rate of $\mathbf{T_7}$ production was monitored in reactions mixtures composed of equimolar amounts of $\mathbf{E_7}$ and **N** (100 μM) in the absence or presence of either $\mathbf{T_3}$ (40 μM), $\mathbf{T_4}$ (40 μM), or an equal mixture of $\mathbf{T_3}$ and $\mathbf{T_4}$ (20 μM each). In the absence of added initial amounts of either input, the background autocatalytic rates of $\mathbf{T_7}$ production is low (Figure S1). However, because $\mathbf{T_3}$ and $\mathbf{T_4}$ are both efficient templates for the production of $\mathbf{T_7}$, the presence of either or both inputs in the reaction mixture gives rise to significantly enhanced rates of $\mathbf{T_7}$ production (Figure S1). More significantly, the OR logic function persists even

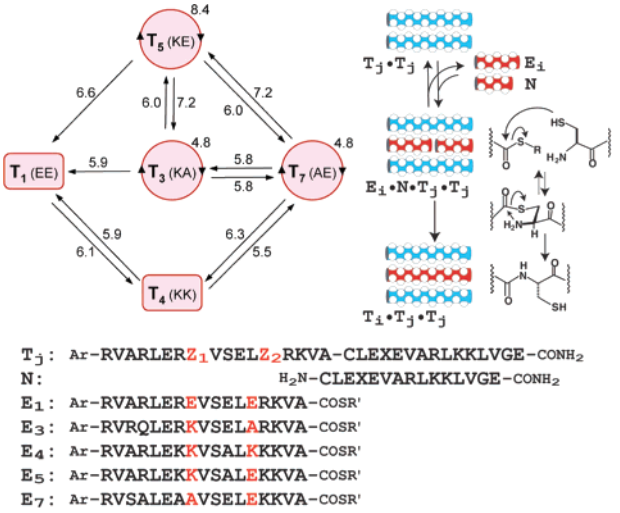

Figure 1. Synthetic network (top left), template-directed peptide fragment ligation (top right), and the peptide sequences employed in this study (bottom). Each node in the graph represents a distinct peptide template or product, with the edges (arrows) designating the experimentally observed template-assisted ligation pathways (condensation of the electrophilic $\mathbf{E_i}$ and nucleophilic **N** fragments by $\mathbf{T_j}$ to produce $\mathbf{T_i}$) pointing from the template sequence to the product. The circular nodes depict the peptide sequences with inherent autocatalytic activities.[6] A reaction is autocatalytic when $\mathbf{T_i} = \mathbf{T_j}$ and cross-catalytic when $\mathbf{T_i} \neq \mathbf{T_j}$. The primary sequence of a given node $\mathbf{T_j}$ is labeled on the graph according to the amino acids Z_1 and Z_2 (parentheses) employed at the corresponding positions on its constituent electrophilic fragment $\mathbf{E_i}$. Peptide sequence numbering was intentionally kept as that in ref 6 to allow facile comparisons. Numerical values represent theoretical estimation of the relative efficiency (edge weight) of depicted pathways. These values were estimated on the basis of the calculated difference in the stability of the trimeric product species as described previously.[6] Ar = 4-acetamidobenzoic acid (ABA), X = Lys-ABA, R′ = ethanesulfonic acid.

in the more complex reaction mixture composed of **N** (200 μM) and equimolar amounts of (~80 μM each) $\mathbf{E_3}$, $\mathbf{E_4}$, and $\mathbf{E_7}$ (Figure 2a). It is important to note that although $\mathbf{T_3}$ possesses modest inherent autocatalytic activity (Figure S2), in the context of the network this activity is practically lost because of its greater efficiency as template for the cross-catalytic production of $\mathbf{T_7}$ as also reflected in the relative efficiency scores calculated for these processes (4.8 vs 5.8, respectively). Therefore, the presence of either or both $\mathbf{T_3}$ and $\mathbf{T_4}$ as inputs leads to the enhanced production of the $\mathbf{T_7}$ output signal even though the substrates for the competing pathways are present in the reaction mixture.

Reaction pathways can be negatively regulated when substrates compete for binding to a given catalyst to prevent its normal function and/or redirect its participation into an alternative pathway. The simplest negative regulation is the inverter NOT function. As an example, the autocatalytic production of $\mathbf{T_3}$ in a reaction mixture composed of **N**, $\mathbf{E_3}$, and $\mathbf{T_3}$ can be negatively affected by the addition of $\mathbf{E_5}$ that sequesters **N** and $\mathbf{T_3}$ for the production of $\mathbf{T_5}$

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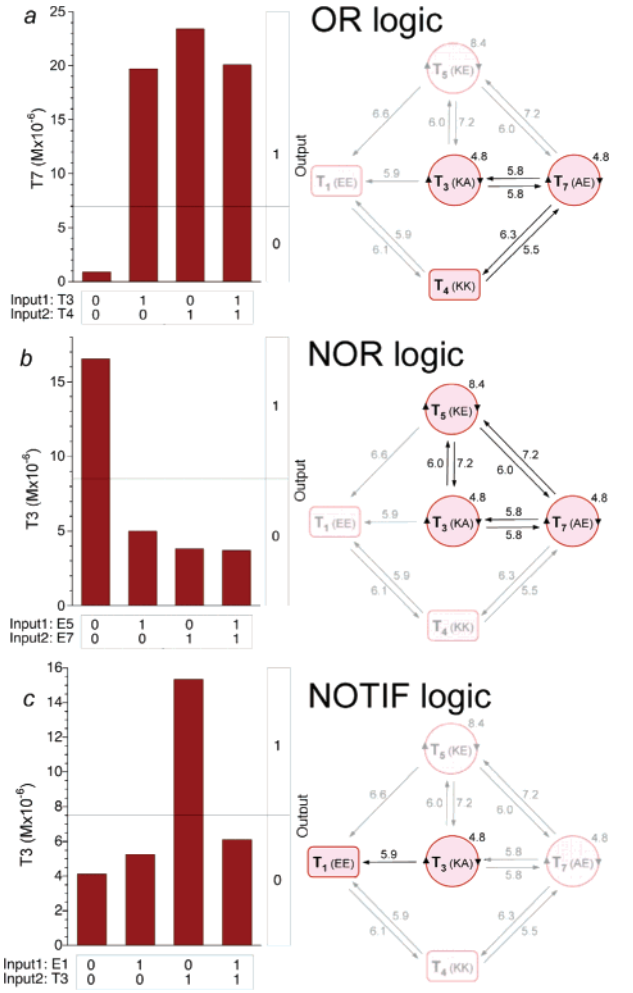

Figure 2. Logic gate operations expressed by selected peptide sub-networks. In each panel (a−c), the relevant network is illustrated on the right and the amounts of product formed (output), in the absence (0) or presence (1) of different combinations of input peptides, are depicted on the left. Results shown are the amounts of products formed after 30 (a) or 60 min (b and c). Reactions were performed in 100 mM MOPS, pH 7.2, at 22 °C in the presence of 5 mM tris(2-carboxyethyl)phosphine (TCEP) as the reducing agent. The rates of product formation were monitored by RP-HPLC, and products were identified by mass spectrometry and by comparisons with authentic samples. See Supporting Information for complete graphs of product formation in time.

through the more efficient $\mathbf{T_3} \rightarrow \mathbf{T_5}$ pathway (Figure 2b). The NOT function is part of the more interesting two-input NOR and NOTIF logic operations.[8] The NOR logic was explored in the context of the circular subnetwork of $\mathbf{T_3}$, $\mathbf{T_5}$, and $\mathbf{T_7}$. The rate of $\mathbf{T_3}$ production (output) was monitored in reactions mixtures composed of **N** (200 μM), $\mathbf{E_3}$ (80 μM), and $\mathbf{T_3}$ (40 μM) fragments in the absence or presence of the inputs: either $\mathbf{E_5}$ (80 μM), $\mathbf{E_7}$ (80 μM), or an equal mixture of $\mathbf{E_5}$ and $\mathbf{E_7}$ (80 μM each). In the absence of any input, $\mathbf{T_3}$ production proceeds efficiently through its autocatalytic cycle. However, in the presence of the inputs $\mathbf{E_5}$ and/or $\mathbf{E_7}$, the autocatalytic rate of $\mathbf{T_3}$ production is diminished because of its involvement in the more efficient $\mathbf{T_3} \rightarrow \mathbf{T_5}$ and/or $\mathbf{T_3} \rightarrow \mathbf{T_7}$ pathways, respectively (Figure 2b).

The NOTIF logic function was studied in the context of the $\mathbf{T_3} \rightarrow \mathbf{T_1}$ pathway. The autocatalytic rates of $\mathbf{T_3}$ production as the output were monitored in reaction mixtures composed of **N** (200 μM) and $\mathbf{E_3}$ (80 μM) in the presence of either inputs $\mathbf{E_1}$ (80 μM) or $\mathbf{T_3}$ (40 μM) or a mixture of $\mathbf{E_1}$ (80 μM) and $\mathbf{T_3}$ (40 μM). A strong output signal is observed in the presence of $\mathbf{T_3}$ only when $\mathbf{E_1}$ is absent (Figure 2c). The observed NOTIF function is consistent with the inhibition of $\mathbf{T_3}$ autocatalysis in the presence of competing substrate $\mathbf{E_1}$ that redirects $\mathbf{T_3}$ for the production of $\mathbf{T_1}$, which is neither an autocatalyst nor can back-catalyze the formation of $\mathbf{T_3}$. Two other NOTIF logic operations are also present in the context of the $\mathbf{T_3} \rightleftarrows \mathbf{T_5}$ sub-network (see Figure S6).

In summary, we have demonstrated that simple synthetic chemical systems can be designed to express network-dependent logic operations in response to external stimuli. We suggest that the ability to rationally construct predictable chemical circuitry might be useful in advancing the modeling and better understanding of some of the basic dynamic information processing characteristics of the more complex cellular networks.

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Supporting Information Available: Experimental procedures, reaction conditions, and the kinetic profiles. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Bray, D. *Nature* **1995**, *376*, 307−312. (b) Arkin, A.; Ross, J. *Biophys. J.* **1994**, *67*, 560−578. (c) Bhalla, U. S.; Iyengar, R. *Science* **1998**, *283*, 381−387. (d) Vohradsky, J. *FASEB J.* **2001**, *15*, 846−854. (e) Lee, T. I.; Rinaldi, N. J.; Robert, F.; Odom, D. T.; Bar-Joseph, Z.; Gerber, G. K.; Hannett, N. M.; Harbison, C. T.; Thompson, C. M.; Simon, I.; Zeitlinger, J.; Jennings, E. G.; Murray, H. L.; Gordon, D. B.; Ren, B.; Wyrick, J. J.; Tagne, J.-B.; Volkert, T. L.; Fraenkel, E.; Gifford, D. K.; Young, R. A. *Science* **2002**, *298*, 799−804. (f) Ravasz, E.; Somera, A. L.; Mongru, D. A.; Oltvai, Z. N.; Barabasi, A. L. *Science* **2002**, *297*, 1551−1555. (g) Pawson, T.; Nash, P. *Science* **2003**, *300*, 445−452. (h) McAdams, H. H.; Shapiro, L. *Science* **2003**, *301*, 1874−1877. (i) Mangan, S.; Alon, U. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 11980−11985.

(2) (a) Adleman, L. M. *Science* **1994**, *266*, 1021−1024. (b) Lipton, R. J. *Science* **1995**, *268*, 542−545. (c) Guarnieri, F.; Fliss, M.; Bancroft, C. *Science* **1996**, *273*, 220−223. (d) Ouyang, Q.; Kaplan, P. D.; Liu, S.; Libchaber, A. *Science* **1997**, *278*, 446−449. (e) Faulhammer, D.; Cukras, A. R.; Lipton, R. J.; Landweber, L. F. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 1385−1389. (f) Liu, Q.; Wang, L.; Frutos, A. G.; Condon, A. E.; Corn, R. M.; Smith, L. M. *Nature* **2000**, *403*, 175−179. (g) Mao, C.; LaBean, T. H.; Reif, J. H.; Seeman, N. C. *Nature* **2000**, *407*, 493−496. (h) Benenson, Y.; Paz-Elizur, T.; Adar, R.; Keinan, E.; Livneh, Z.; Shapiro, E. *Nature* **2001**, *414*, 430−434. (i) Carbone, A.; Seeman, N. C. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 12577−12582. (j) Stojanovic, M. N.; Stefanovic, D. *J. Am. Chem. Soc.* **2003**, *125*, 6673−6676. (k) Saghatelian, A.; Voelcker, N. H.; Guckian, K. M.; Lin, V. S. Y.; Ghadiri, M. R. *J. Am. Chem. Soc.* **2003**, *125*, 346−347. (l) Stojanovic, M. N.; Stefanovic, D. *Nat. Biotechnol.* **2003**, *21*, 1069−1074. (m) Yan, H.; Feng, L.; LaBean, T. H.; Reif, J. H. *J. Am. Chem. Soc.* **2003**, *125*, 14246−14247.

(3) (a) Hjelmfelt, A.; Weinberger, E. D.; Ross, J. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 10983−10987. (b) Hjelmfelt, A.; Weinberger, E. D.; Ross, J. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 383−387. (c) Dueber, J. E.; Yeh, B. J.; Chak, K.; Lim, W. A. *Science* **2003**, *301*, 1904−1908. (d) Sivan, S.; Tuchman, S.; Lotan, N. *BioSystems* **2003**, *70*, 21−33.

(4) (a) Elowitz, M.; Leibler, S. *Nature* **2000**, *403*, 335−338. (b) Gardner, T.; Cantor, C.; Collins, J. *Nature* **2000**, *403*, 339−342. (c) Guet, C.; Elowitz, M.; Hsing, W.; Leibler, S. *Science* **2002**, *296*, 1466−1470. (d) Hasty, J.; McMillen, D.; Collins, J. J. *Nature* **2002**, *420*, 224−230. (e) Yokobayashi, Y.; Weiss, R.; Arnold, F. H. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 16587−16591. (f) Atkinson Mariette, R.; Savageau Michael, A.; Myers Jesse, T.; Ninfa Alexander, J. *Cell* **2003**, *113*, 597−607.

(5) For a recent reviews of synthetic molecular logic gates, see: (a) Raymo, F. M. *Adv. Mater.* **2002**, *14*, 401−414. (b) de Silva, A. P.; McClenaghan, N. D. *Chem.−Eur. J.* **2004**, *10*, 574−586. (c) *Molecular Computing*; Sienko, T., Adamatzky, A., Rambidi, N. G., Conrad, M., Eds.; MIT Press: Cambridge, MA, 2003.

(6) Ashkenasy, G.; Jagasia, R.; Yadav, M.; Ghadiri, M. R. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 10872−10877.

(7) (a) Lee, D. H.; Granja, J. R.; Martinez, J. A.; Severin, K.; Ghadiri, M. R. *Nature* **1996**, *382*, 525−528. (b) Severin, K.; Lee, D. H.; Kennan, A. J.; Ghadiri, M. R. *Nature* **1997**, *389*, 706−709. (c) Lee, D. H.; Severin, K.; Yokobayashi, Y.; Ghadiri, M. R. *Nature* **1997**, *390*, 591−594. (d) Severin, K.; Lee, D. H.; Martinez, J. A.; Vieth, M.; Ghadiri, M. R. *Angew. Chem., Int. Ed.* **1998**, *37*, 126−128. (e) Yao, S.; Ghosh, I.; Zutshi, R.; Chmielewski, J. *Nature* **1998**, *396*, 447−450. (f) Kennan, A. J.; Haridas, V.; Severin, K.; Lee, D. H.; Ghadiri, M. R. *J. Am. Chem. Soc.* **2001**, *123*, 1797−1803.

(8) (a) Mendelson, E. *Boolean Algebra and Switching Circuits*; McGraw-Hill: 1970. (b) http://edda.csie.dyu.edu.tw/course/verilog/ver_chap5_2up.pdf.

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