Integrated Photosynthetic and Metabolic Constitutional Dynamic Networks—An “Artificial Leaf”

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Article

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Abstract

Integration of a photosynthetic network with an assimilation, metabolic network is the fundamental prerequisite to construct an “artificial leaf”. Nucleic acid-based constitutional dynamic networks provide the building modules to construct integrated, intercommunicated networks mimicking photosynthesis. Two constitutional dynamic networks composed each of four constituents provide the photosynthetic and metabolic networks. In the photosynthetic network, photoinduced electron transfer from the Zn(II)-protoporphyrin photosensitizer to a bipyridinium electron acceptor is activated, followed by the biocatalytic reduction of NADP^+ to NADPH, in analogy to photosystem I in native photosynthesis. In the metabolic network, the biocatalyzed-oxidation of lactate to pyruvate proceeds, followed by the metabolic transformation of pyruvate to L-alanine. The guided dynamic feedback-driven intercommunication of the networks is accomplished, leading to the function as an “artificial leaf”.

Main Text

The conversion of solar light energy into fuels or valuable chemicals by artificial photosynthetic systems is one of the holy grail scientific efforts^{1,2}. Different approaches to model the natural photosynthetic apparatus were reported, and these included the design of chemical and supramolecular systems^{3,4}, the assembly of photoelectrochemical photosynthetic models^{5–7}, interfacing nature’s photosynthetic machinery with synthetic materials⁸, and the application of nanomaterials for scalable artificial photosynthesis⁹. These efforts involved the design of photocatalysts mimicking the native chlorophylls¹⁰, the synthesis of supramolecular photosensitizer-electron relay systems for effective electron transfer quenching and charge separation¹¹, the development of catalysts and biocatalysts for H_2 -evolution^{12,13}, CO_2 fixation^{14–16} and photolysis of water^{17–19}. The photosynthetic network is not, however, a self-operating system, and it is tightly coupled to photorespiration and assimilation networks²⁰, and to the overall plant metabolic machinery^{21,22}. The inter-relationships between the photosynthetic apparatus and the coupled dark networks reveal complex intercommunication patterns²³. For example, increased photorespiration and assimilation were reported to enhance operation of the photosynthetic network under appropriate conditions²⁴, yet under different conditions respiration-inhibited photosynthesis was demonstrated²⁵. In addition, mitochondrial metabolism-guided enhanced photosynthesis was demonstrated²⁶. Beyond the fundamental interest to understand the links between the light-harvesting network and the coupled dark networks, the topic has practical significance for improving crop productivity and enhanced growth of bioenergy plants. Within the general topic of artificial photosynthesis, the development of synthetic interlinked networks coupling a photosynthetic cycle with a metabolic cycle is, to the best of our knowledge, unprecedented.

Nucleic acid-based constitutional dynamic networks (CDNs) have been introduced by us as functional frameworks mimicking native networks²⁷. The simplest [2×2] CDN consists of four dynamically interchangeable and equilibrated constituents AA', AB', BA' and BB'. The triggered stabilization of one of

the constituents, *e.g.*, AA' results in the adaptive dynamic reconfiguration of the CDN into a new equilibrated network, where the content of AA' is enriched on the expense of AB' and BA' sharing components with AA'. The dynamic separation of AB' and BA' leads to the recombination of B and B' and to the concomitant enrichment of BB'. The base sequence comprising nucleic acids provides a rich “toolbox” to control the stabilization/destabilization of nucleic acids by fuel/anti-fuel strands^{28–30}, the formation/dissociation of G-quadruplexes (GQ)³¹, and the reversible stabilization/destabilization of duplexes by photoisomerizable intercalator units³². A variety of CDNs revealing adaptive and hierarchically adaptive reconfiguration properties were demonstrated, using fuel/anti-fuel strand³³, G-quadruplex formation/dissociation³⁴ and light³⁵ as input triggers. CDNs of variable complexities, such as intercommunicating³⁶ and feedback-driven CDNs³⁴, and the assembly of [3×3] and three-dimensional CDNs³⁷ were realized. The applications of CDNs are still scarce, and the use of CDNs to build hydrogels exhibiting switchable stiffness for controlled drug release and self-healing³⁸, and the CDN-guided aggregation of nanoparticles for controlled catalysis and switchable optical properties³⁹ were demonstrated. Recently, enzymes were coupled to CDNs and the switchable operation of biocatalytic cascades and the intercommunication of enzyme networks were highlighted⁴⁰.

In the present study, we introduce CDNs as functional modules to construct an integrated “artificial leaf”. A CDN-guided photosynthetic network that drives the light-induced electron transfer and the photosynthesis of NADPH is coupled to a metabolic CDN assembly that stimulates the biocatalytic oxidation of lactate to pyruvate and the cascaded metabolic amination of pyruvate to L-alanine. We demonstrate the intercommunication between the photosynthetic process and the metabolic assimilation process and highlight the tight inter-relation between the two networks, where the CDN-driven enhanced photosynthesis of NADPH signals the acceleration of the metabolic network as output, and the CDN-driven enhancement of the metabolic network is translated to “information transfer” dictating the acceleration of the photosynthetic network.

Results

A photosynthetic constitutional dynamic network

Fig. 1a introduces the dynamic photocatalytic module mimicking photosynthesis, CDN “X”. The network consists of four constituents where the photosensitizer Zn(II)-protoporphyrin IX (Zn-PPIX) intercalates into the G-quadruplex unit, tethered as photosensitizer to component A of constituent AA', and the N,N'-dialkyl-4,4'-bipyridinium (V^{2+}) electron acceptor is covalently linked to component A' of constituent AA' (Figs. S1-S2). Irradiation of the photocatalytic module results in the effective quenching of the photosensitizer to yield the redox intermediates Zn-PPIX⁺/GQ and MV⁺ (Fig. 1a, panel I). In the presence of 1,4-nicotinamide adenine dinucleotide phosphate (NADP⁺) and ferredoxin-NADP⁺-reductase (FNR), the formation of reduced cofactor NADPH proceeds, in analogy to the photosystem I. Each of the constituents in CDN includes a loop domain, used to shift the CDN equilibrium through the stabilization of a target loop domain, panel II. In addition, each of the constituents is engineered to include a different

Mg²⁺-dependent DNAzyme reporter unit to cleave fluorophore-quencher ribonucleobase-modified substrates for the quantitative evaluation of the concentrations of constituents, panel III.

Figure. 1a and S3 depicts the triggered reconfiguration of CDN “X” in the presence of auxiliary triggers. Subjecting CDN “X” to the strand T₁ leads to the stabilization of the T-A-T triplex structure in the loop domain, resulting in the stabilization of AA' and the reconfiguration of CDN “X” into CDN X_a, where AA' is up-regulated, constituents AB' and BA' are down-regulated and the constituent BB' is up-regulated (Figs. S4-6). The reverse displacement of the trigger T₁ by the counter trigger T₁' regenerates CDN “X”. Similarly, treatment of CDN “X” with the trigger T₂ stabilizes the constituent BA'. BA' and AB' are up-regulated, and AA' and BB' are down-regulated (Fig. S6). The treatment of CDN X_b with the counter trigger T₂' restores CDN “X”. The quantitative contents of the constituents in different CDNs are shown in Fig. 1b and Table S1. The photosensitized electron transfer process proceeding in different CDNs, is stimulated by constituent AA'. The absorption spectra of the photogenerated V^{•+} (Fig. 1c) reveal that the T₁-up-regulated constituent AA' in CDN X_a lead to the enhanced photoinduced electron transfer, whereas the T₂-down-regulation of AA' in CDN X_b inhibited the photoinduced electron transfer. Fig. 1d demonstrates the switchable and reversible control over the photoinduced electron transfer (ET) process guided by the T₁-/T₂-triggered reconfiguration of the CDN modules. The secondary CDN-guided synthesis of NADPH driven by the primary photoinduced V^{•+}, in the presence of FNR, NADP⁺ and mecaptoethanol (electron donor), is presented in Fig. 1e-g. The time-dependent built-up of NADPH (λ = 345 nm) and V^{•+} (λ = 395 nm) is observed. It is evident that the built-up of NADPH by CDN X_a (Fig. 1f) is enhanced as compared to the NADPH generated by CDN “X” (Fig. 1e), whereas the built-up of NADPH by CDN X_b (Fig. 1g) is inhibited as compared to CDN “X”. Fig. 1h shows the time-dependent formation of NADPH at time-intervals of illumination of CDNs X, X_a, and X_b. The efficiency of the photogenerated NADPH is controlled by the efficiency of the primary photoinduced electron transfer process that yields V^{•+}.

A metabolic constitutional dynamic network

The metabolic CDN module is introduced in Fig. 2 and S7. CDN “Y” is composed of the constituents CC', DC', CD' and DD', where the components of DD' are modified with lactate dehydrogenase (LDH) and nicotinamide adenine dinucleotide (NAD⁺), Figs. S2, S8. The metabolic biocatalytic transformation proceeding in CDN “Y” involves the LDH-biocatalyzed reduction of NAD⁺ to NADH by lactic acid, and the concomitant formation of pyruvic acid. The biocatalyzed formation of NADH is coupled to the secondary reduction of methylene blue (MB⁺) to colorless MBH, a process that allows the spectroscopy readout of the time-dependent formation of NADH (Fig. 2a, panel I). In addition, the biocatalyzed formation of NADH and pyruvic acid is coupled to the secondary reductive amination of pyruvic acid, in the presence of NH₄⁺ and alanine dehydrogenase (AlaDH), to form L-alanine as metabolic product.

Subjecting CDN “Y” to trigger T₃ stabilizes constituent DD', resulting in the reconfiguration of CDN “Y” to CDN Y_a, where DD' is up-regulated, DC' and CD' are down-regulated and CC' is up-regulated (Figs. S9-11).

The reverse treatment of CDN Y_a with T_3' displaces T_3 from DD' and resulting in the regeneration of CDN “Y”. In addition, treatment of CDN “Y” with T_4 stabilizes the constituent CD' , leading to the reconfiguration of CDN “Y” to CDN Y_b , where CD' and DC' are up-regulated and the constituents CC' and DD' are down-regulated. Fig. 2b and Table S2 show the concentrations of the constituents. The CDNs-guided time-dependent operation of the biocatalytic cascade corresponding to LDH-catalyzed reduction of NAD^+ by lactic acid to NADH, and the subsequent reduction of MB^+ ($\lambda = 630\text{ nm}$) are presented in Fig. 2c and S12-13. The time-dependent depletion of MB^+ to MBH is enhanced, in the presence of the T_3 -triggered reconfigured CDN Y_a , and retreated in the presence of the T_4 -reconfigured CDN Y_b , consistent with the up-regulation of the constituent DD' in CDN Y_a and the down-regulation of DD' in CDN Y_b , respectively. In addition, Fig. 2d presents the time-dependent CDNs-driven metabolic cascade, where the LDH-biocatalyzed reduction of NAD^+ to NADH by lactate is followed by the AlaDH-catalyzed amination of the generated pyruvic acid to yield L-alanine (Fig. S14, Table S3-S5). The rate of formation of L-alanine metabolite is enhanced in the presence of CDN Y_a and dampened by CDN Y_b , respectively.

Intercommunicated photosynthetic and metabolic dynamic networks

In the next step, efforts to couple photosynthetic module and the metabolic assimilation module were undertaken, similarly to the interlinked processes in plants. The principle to intercommunicate between the two modules is displayed in Fig. 3a. The constituents BB' and CC' in CDNs “X” and “Y” were pre-engineered to include each extra Mg^{2+} -dependent DNAzyme units. These units are termed “activators”, integrated into the composite in order to intercommunicate between the networks. To intercommunicate between the networks, we added two hairpins, H_a or H_d into the mixture of two CDNs. The H_a is designed to be cleaved by the activator associated with constituent CC' to yield the fragmented strand H_{a-1} that interacts with constituent AA' by the stabilization of the triplex T-A·T in the loop domain of AA' (Fig. 3a route I and Fig. S15). This results in the up-regulation of AA' and BB' and down-regulation of AB' and BA' (Figs. S16-17). That is, the cleavage of H_a by the metabolic module is anticipated to enhance the performance of the photosynthetic module by up-regulating AA' . On the other hand, the cleavage of hairpin H_d by the activator of constituent BB' yields the fragmented strand H_{d-1} that provides an information strand to control the activity of CDN “Y” (Fig. 3a route II and Fig. S18). The binding of H_{d-1} to the loop domain of DD' , and the formation of the T-A·T triplex lead to the stabilization of DD' , the up-regulation of DD' and CC' and the down-regulation of CD' and DC' (Figs. S19-20). The resulting time-dependent up-regulation of DD' leads, then, to a time-dependent increase in the metabolic performance of CDN “Y”.

In the first step, the unidirectional intercommunication between the networks using hairpin H_a or H_d was evaluated. Fig. 3b and S21 show the formation of NADPH by the photosynthetic module upon exposure to the CDN “Y”-synthesized H_{a-1} at different time-intervals. As the time-interval is prolonged, the photosensitized generation of NADPH by the photosynthetic CDN “X”/FNR is enhanced, consistent with the continuous enrichment of the constituent AA' by H_{a-1} . Fig. 3c depicts the metabolic performance of

CDN “Y” before and after the strand H_{a-1} was supplied to CDN “X”, by following the reduction of MB^+ to MBH. As expected, the metabolic module is unaffected upon supplying H_{a-1} as a trigger to CDN “X”. Fig. 3d shows the rate of synthesis of L-alanine by CDN “Y” upon feeding the two CDNs with H_{d-1} generated at different time-intervals (Fig. S22, Tables S6-S9). As the time interval of the generation of H_{d-1} is prolonged, the synthesis of L-alanine is enhanced, consistent with the stabilization and time-dependent overexpression of DD', in the presence of H_{d-1} . Fig. 3e shows the spectra of NADPH generated by the photosynthetic module (1 h-irradiation) before and after the generation of H_{d-1} , implying CDN “X” is unaffected upon the transfer of the information strand (H_{d-1}) to CDN “Y”. Noted that the discussion introduced the positive intercommunication “dialog” between the CDNs. One may envisage, however, the negative intercommunication between the CDNs. For example, subjecting coupled CDNs to hairpin H_n (cleaved by the activator of BB') leads to the generation of fragmented product H_{n-1} that stabilizes CD', the down-regulation of DD' proceeds, resulting in the inhibition of the metabolic module (Figs. S23-26).

Subjecting the mixture of the two CDNs to the two hairpin has, however, a significant effect on the intercommunication between the two CDNs (Fig. 4a). The cleavage of the hairpin H_a yields the strand H_{a-1} that provides the information to up-regulate AA' in the photosynthetic module, thus enhancing the photosynthetic module leading to the time-dependent increase in the photosensitized electron transfer process and the FNR-catalyzed synthesis of NADPH. The up-regulation of AA' is accompanied by the up-regulation of BB' that leads to the time-dependent enhancement of the cleavage of H_d to form H_{d-1} . The latter product provides the information strand to enhance the metabolic module synthesizing L-alanine. The stabilization and up-regulation of DD' is accompanied by the up-regulation of CC' and, thus, the further enhancement of the cleavage of H_a and the enhancement of the photosynthetic module. In the presence of the hairpin H_a and H_b , a positive feedback mechanism intercommunicating the CDNs is established (Figs. S27-29). The time-dependent increase in the performance of the photosynthetic model is reflected by an information transfer to the metabolic CDN module to enhance its activity and *visa versa*. The control over the concentrations of the constituents by the two hairpins is reflected in the photosynthetic and metabolic processes occurring in CDNs “X” and “Y”. Fig. 4b and S30 show the photosensitized-NADPH generated at time-intervals of the feedback-driven intercommunication of the two networks, indicating the generation of NADPH by the photosynthetic module is enhanced. Fig. 4c depicts the rates of the metabolism (lactate/LDH/AlaDH/ MB^+ cascade) at time-intervals of the intercommunication between the networks. As the feedback process enriches AA' and DD', the concentration of NADPH is higher and the biocatalytic cascade is enhanced. The results demonstrate a tight relation between the photosynthetic module and the assimilation, metabolic module. The enhancement of the photosynthetic process channels the information to enhance the metabolic path, and the enhanced metabolic reactions are translated into transfer of information for enhanced photosynthesis.

Conclusions

The study introduces two complementary dynamic networks that mimic the functions of plants- an “artificial leaf”. One network introduces photosynthetic path, where the control over the light-induced electron transfer and the subsequent catalyzed synthesis of NADPH proceeds, in analogy to the transformation driven by photosystem I. A second dynamic network demonstrates a metabolic path mimicking plant assimilation and mitochondrial metabolism by the input-driven oxidation of lactate and its metabolic transformation to L-alanine. The two networks are intercommunicated by demonstrating the guided activation of the metabolic network by the photosynthetic network and the counter control over the photosynthetic network by means of the metabolic network. Finally, the integrated feedback-driven operation of the photosynthetic network and the metabolic network is established by introducing the coupled “leaf-like” operation of the two networks, where “information transfer” between the two networks exists. The photosynthetic network enhances the activity of the metabolic network, and the activity of the metabolic network guides transfer of information for enhancing the photosynthetic network. Beyond highlighting the integration of the photosynthetic and metabolic networks in leaf-mimicking module, the study introduces an important path to guide the synthesis of useful materials (L-alanine) by the metabolic network⁴¹.

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Methods

Modification of strand A' with V²⁺

60 μL of 0.05 M V^{2+} (10 eq) and 30 μL of 0.01 M sulfo-EMCS (1 eq) were mixed in PBS buffer (20 mM, pH = 7.24) and incubated at room temperature for 1 hour. Then 30 μL of 1 mM strand A' (0.1 eq) was added and incubated for another 2 hours. Excess reactants were removed using Amicon 10 kD cutoff filters. The synthesis route was shown in Fig. S2a.

Modification of strand D with LDH

10 μM of LDH and 1.2 mM SPDP in HEPES buffer (10 mM, pH = 8) were incubated for 1 hour. Excess SPDP was removed using Amicon 30 kD cutoff filters. Before modification of strand D with LDH, strand D was treated with TCEP (100-fold excess) for 2 hours and washed by using Amicon 3 kD cutoff filters. Next, SPDP-modified LDH was conjugated to strand D (8-fold excess) through a disulfide bond exchange of the activated pyridyldithiol group (see synthetic scheme in Fig. S2b). The reaction was performed in HEPES buffer (10 mM, pH = 8) for 2 hours. The coupling efficiency was evaluated by monitoring the increase in absorbance at 343 nm due to the release of pyridine-2-thione (Fig. S10a-b, extinction coefficient: $8,080 \text{ M}^{-1} \text{ cm}^{-1}$). Excess DNA was removed using Amicon 30 kD cutoff filters. The enzymatic activity of DNA-modified LDH was $\sim 75\%$ of the activity of the native enzyme (Fig. S10c).

Modification of strand D' with NAD^+

The preparation of D'- NAD^+ (see synthetic scheme in Fig. S2c) was followed our previous paper⁴⁰ and characterized by mass spectrum.

Preparation of CDNs

A sample of 1 mL of CDN (each component 2 μM) was taken as an example to explain the procedure of the preparation of CDNs:

CDN X, including the constituents AA', BB', AB', BA', was prepared as follows: A (20 μL , 100 μM), A' (20 μL , 100 μM), B (20 μL , 100 μM), B' (20 μL , 100 μM) and PPIX (2 μL , 1 mM) were mixed in Tris buffer (10 mM, pH = 7.29) that includes 20 mM MgCl_2 and 100 mM K^+ . The mixture was annealed at 37 $^\circ\text{C}$, cooled down to 25 $^\circ\text{C}$ at a rate of 0.33 $^\circ\text{C}/\text{min}$ and equilibrated at 25 $^\circ\text{C}$ for 12 h. CDN Y, including the constituents LDH/ NAD^+ -DD', LDH-DC', CD'- NAD^+ , CC' was prepared as follows: LDH-D (20 μL , 100 μM), D'- NAD^+ (20 μL , 100 μM), C (20 μL , 100 μM), C' (20 μL , 100 μM) were mixed in Tris buffer (10 mM, pH = 7.29) that includes 20 mM MgCl_2 and 100 mM K^+ . The mixture was annealed at 37 $^\circ\text{C}$ for 1 hour, cooled down to 25 $^\circ\text{C}$ at a rate of 0.33 $^\circ\text{C}/\text{min}$, and equilibrated at 25 $^\circ\text{C}$ for 12 h.

For the triggered transition of CDN X, triggers T_1 , T_1' or T_2 , T_2' are 1.67-fold excess than each component of CDN. After adding triggers into initial CDN, the final concentration of each component of CDN was 1 μM and the final concentration of trigger was 1.67 μM . The solution was incubated at 28°C overnight to equilibrate. For the triggered transition of CDN Y, triggers T_3 , T_3' or T_4 , T_4' are 2.5-fold excess than each component of CDN. After adding triggers into initial CDN, the final concentration of each component of CDN was 1 μM and the final concentration of trigger was 2.5 μM . The solution was incubated at 28°C overnight to equilibrate. After equilibration, the equilibrated CDN (each component 1 μM) was treated with one substrate (5 μM) (sub 1 for AA', sub 2 for BB', sub 3 for BA', sub 4 for AB', sub 5 for DC', sub 6 for CD', sub 7 for CC' and sub 8 for DD'). The time-dependent fluorescence changes generated by the cleavage of the different substrates by DNAzyme reporter units were measured. By following the rate of formation of the fluorophore-labeled fragment and using appropriate calibration curves of the intact constituent (Figs. S4-S5 and S9-S10), the quantitative evaluation of the concentrations of constituents is achieved.

Declarations

Acknowledgments

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Author Contributions

C.W. performed the experiments, analyzed the results and participated in writing the paper. E. N and R. N participated in the synthesis of FNR and the analysis of results. I.W. supervised the project and wrote the paper.

Competing interests

The authors declare no competing interests.

Data availability

All data are available in the main text or the supplementary materials.

Supplementary materials

Materials and methods

Figs. S1-S30

Tables S1-S9

Figures

Figure 1

Assembly and operation of the photosynthetic constitutional dynamic network (CDN). A) Schematic composition of the 2×2 photosynthetic CDN X , its reversible triggered transition into CDN X_a or CDN X_b and the light-induced photosynthesis of NADPH by the CDNs. Panel I-The light-induced electron transfer activated by the photosynthetic network, followed by the biocatalyzed synthesis of NADPH. Panel II-Schematic stabilization of a constituent through the T_i -triggered formation of T-A-T triplex and its

destabilization by a Ti^{+} -induced strand displacement process. Panel III-Schematic cleavage of the fluorophore/quencher-functionalized substrate by Mg^{2+} -dependent DNAzyme reporter units associated with the constituents. B) Composition of the constituents in the different reconfigured CDNs. C) Absorbance spectra corresponding to the bipyridinium radical cation (V^{+}) generated by the photosynthetic network of: (i) CDN X, (ii) CDN Xa, (iii) CDN Xb (irradiation time-interval 30 min). D) Switchable and reversible formation of the V^{+} , upon the transition $CDN\ X \rightarrow Xa \rightarrow X$ and $X \rightarrow Xb \rightarrow X$. E-G) Time-dependent absorbance spectra corresponding to the FNR-biocatalyzed photosynthesis of NADPH by CDN X, Xa and Xb, respectively (spectra recorded at time intervals of 5 min for 30 min). H) Time-dependent photoinduced generation of NADPH by CDNs X, Xa and Xb.

Figure 2

Assembly and triggered reconfiguration of the metabolic network. A) Schematic composition of a 2x2 CDN Y, its triggered reversible reconfiguration across CDNs Y, Ya and Yb, and the triggered control over metabolic paths. Panel I-Lactate dehydrogenase (LDH)-catalyzed oxidation of lactate to pyruvate and the subsequent reduction of methylene blue (MB+) to MBH as metabolic path (1), and the subsequent alanine dehydrogenase (AlaDH)-metabolic amination of pyruvate to L-alanine, path (2). Panel II-Schematic triggered reversible formation/dissociation of T-A-T triplex in the loop domain of a constituent. Panel III-Schematic cleavage of the fluorophore/quencher-functionalized substrate by Mg²⁺-dependent DNAzyme reporter units associated with the constituents. B) Contents of the constituents in CDNs Y, Ya and Yb. C) Time-dependent absorbance changes corresponding to the reduction of MB+ to MBH by the metabolic path (1), in the presence of different CDNs Y, Ya and Yb. D) Time-dependent formation of L-alanine by the metabolic path (2) using the different CDNs Y, Ya and Yb.

Figure 3

Intercommunication between the photosynthetic network (X) and the metabolic network (Y) using hairpin stimulants. A) The mixture M consisting of CDNs X and Y subjected to the stimulant Ha leads to CDN Y-driven transition of the mixture into state Ma that includes CDN Xa and unaffected CDN Y. The cleavage of Ha by CC' of CDN Y leads to a fragmented strand that stabilizes AA', resulting in the reconfiguration of CDN X into Xa (route I). Subjecting mixture M to Hd leads to the photosynthetic network-guided transition of M into Mb through the cleavage of Hd by BB' and the generation of a fragmented strand that stabilizes DD', while CDN X is unaffected (route II). B) Time-dependent formation of NADPH by the photosynthetic network at time-intervals of treatment of mixture M with the stimulant Ha. C) Time-dependent reduction of methylene blue by the metabolic network: (i) before and (ii) after treatment of mixture M with the stimulant Ha. D) Time-dependent formation of L-alanine by CDN Y at time-intervals of treatment of mixture M with the stimulant Hd. E) Absorbance spectra of photosynthesized NADPH by CDN X: (i) before and (ii) after treatment of mixture M with the stimulant Hd.

Figure 4

Integrated feedback-driven intercommunicating networks X and Y using the mixture of stimulants Ha and Hd. A) Subjecting the mixture M to the two stimulants Ha and Hd results in the Ha-triggered time-

dependent metabolic network (Y)-driven transition of X into Xa and the concomitant Hd-triggered time-dependent photosynthetic network (X)-guided transition of CDN Y into Ya. The intercommunicating photosynthetic and metabolic network act as an artificial leaf. B) Time-dependent formation of NADPH by the photosynthetic network X at time-intervals of activation by stimulant Ha-triggered operation of the metabolic network Y. C) Time-dependent operation of metabolic network (followed by the reduction of MB⁺ to MBH) at time-intervals of Hd-triggered operation of the photosynthetic network.

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