2010 International Conference on Nanotechnology and Biosensors
IPCBEE vol.2 (2011) © (2011) IACSIT Press, Singapore

Deoxyribozyme-based Autonomous Molecular Spiders Controlled by Computing Logic Gates

Renjun Pei, Steven K. Taylor
Department of Medicine, Columbia University
New York, NY, USA
email: rp2164@columbia.edu (R.P.), st2171@columbia.edu (S.K.T.)

***Abstract*—To develop autonomously decision-making molecular walkers, we constructed a set of controllable molecular spiders by incorporating deoxyribozyme-based molecular computing logic gates with deoxyribozyme-based molecular polycatalytic assemblies. The spiders were characterized at the ensemble level using Biacore, a real-time surface plasmon resonance technique. A cargo loaded molecular spider will also be discussed.**

Keywords-deoxyribozyme; logic gates controlled molecular spiders; cargo loaded molecular spider; surface plasmon resonance

I. INTRODUCTION

Engineered DNA walkers that are capable of precisely controlled movement at the molecular or nanometric scale have been reported in the past several years [1-6]. Recently, Seeman's group reported a proximity-based programmable DNA nanoscale assembly line where a DNA walker can collect cargo along a track [7]. Liu's group reported autonomous multistep amine acylation reactions in a single solution without any external intervention by a DNA walker moving along a DNA track [8]. We previously described polycatalytic assemblies that function as walkers and are referred to as molecular spiders. These walkers can diffuse through a substrate-deposited matrix continuously by cleaving the substrates [9]. Later on, we used single-molecular microscopy to confirm that these walkers achieve directional movement by sensing and modifying tracks of substrate laid out on a two-dimensional DNA origami landscape [10]. However, the whole field is still in its infancy. It is very challenging to incorporate these movers with sensors and molecular scale computation [11, 12] to construct autonomous, controllable and decision-making molecular robots to execute a specific action (e.g. cargo delivery). To this purpose, in the current study, the logic gates controlled autonomous molecular spiders and cargo loaded molecular spider were constructed and investigated.

II. MATERIALS AND METHODS

A. Materials and instrumentations

All oligonucleotides were custom-made and purified by Integrated DNA Technologies Inc. (Coralville, USA) or Trilink Biotechnoloies Inc. (San Diego, USA) and used as received. Immunopure avidin and streptavidin were purchased from Pierce (Rockford, USA). Quantum dot 655 streptavidin conjugate (featuring a red-fluorescent quantum dot nanocrystal core within a functional coating averaging 8~10 covalently attached streptavidins) was obtained from Invitrogen (Eugene, USA). We used a Biacore X system (surface plasmon resonance, SPR technique) and commercially available Biacore SA sensor chips, and Biacore C1 sensor chips from GE Healthcare (Piscataway, USA). HBS buffer (10mM HEPES, pH7.4 with 150mM NaCl) was used. HBS-EP (HBS buffer with 3mM EDTA and 0.005% P20 surfactant) and HBS-Zn (HBS buffer with 1mM $ZnCl_2$) were employed as running and sample buffers. For HPLC purification, we used a Shimadzu LC-6AD pump equipped with an SPD-M10A PDA detector. The oligonucleotide sequences used in this study were: the leg for NICK4.2A spider: 5`-biotin-(TEG)-SP18-SP18-CTC TTC TCC GAG CCG GTC GAA ATA GTG AA; the leg for input sensing streptavidin spider: 5`-biotin-(TEG)-SP18-SP18-GCT CGG AGA AGA GAT CCA TAC ATT TAA TAC AGA CTC TTC TCC GAG CCG GTC GAA ATA GTG AAA A; input oligonucleotide i_{1s} : 5`- TCT GTA TTA AAT GTA TGG AT; input $i_{2s}$: 5`- TCT GTA TTA AAT GTA TGG ATA GAG TCC G ; c-input c-i_{2s} (complementary to i_2): 5`-CGG ACT CTA TCC ATA CAT TTA ATA CAG A; two strands for input sensing double-helices DNA spider: 2L-1: 5`-GGC ACA GAG CTA TGG ATC CA-Sp18-GCT CGG AGA AGA GAT CCA TAC ATT TAA TAC AGA CTC TTC TCC GAG CCG GTC GAA ATA GTG AAA A; 2L-2: 5`-TGG ATC CAT AGC TCT GTG CC-Sp18-GCT CGG AGA AGA GAT CCA TAC ATT TAA TAC AGA CTC TTC TCC GAG CCG GTC GAA ATA GTG AAA A; substrate on SPR chips: 5`-biotin-(TEG)-TTT TTT TTC ACT ATrA GGA AGA G; the leg for NOT gate spider: 5`-biotin-(TEG)-SP18-SP18-CTC TTC AGC GAT CCG AGT GAT CTA TCG TAA CTC TCG GCA CCC ATG TTA GTG A; input $i_{NOT}$: 5`-CTT AGT TrAG GAT AGA TCA T; input S_1: 5`-CTT AGT TrAG GAT AGA TCA T; input $S_2$: 5`-TAG TTA GGA TrAG ATC ATC; $YESi_1$: 5`-GGA GAT AGA AAG TTA ACA CCA ATG TCT ATC TCC GAG CCG GTC GAA AAC TAA G; $YESi_2$: 5`-GGA ATC ACC TAC TTA GGA CTA ATG GAT GATT TCC GAG CCG GTC GAA ATC CTA A; i_1: 5`-CAT TGG TGT TAA CTT; $i_2$: 5`-CAT TAG TCC TAA GTA; substrate on bead surface: 5`-biotin-(TEG)-TTT TTT TTC ACT ATrA GGA AGA G-

fluorescein; the single strand attached to the maleimide functionalized BSA: 5`-(ThioMC6-D)-GAT AGA TGA CAC GAG AGA TAT GAC TGA GTG; the single strand attached to streptavidin for 3+1 spider: 5`-biotin-(TEG)-CAC TCA GTC ATA TCT CTC GTG TCA TCT ATC. TEG is a tetraethylene glycol spacer, SP18 is an 18-atom hexaethylene glycol spacer, "r" precedes a ribonucleotide, ThioMC6-D is a thiol group with C6 spacer (in disulfide form).

B. Systhesis of spiders

The synthesis of input sensing molecular spiders, logic gates controlled molecular spiders and 3+1 spider, with purification by IE-HPLC and characterization by PAGE, are similar to standard NICK spiders, see ref. [9] and ref. [10]. The synthesis of bovine serum albumin (BSA) loaded spider is illustrated in Figure 3. BSA was reacted with 10 equivalents of N-ethyl-maleimide (NEM, Pierce) to block the free sulfhydryl group according to the accompanying Pierce protocol. The BSA-NEM product was then reacted with three equivalents of the heterobifunctional crosslinking reagent N-e-Maleimidocaproyloxy]sulfosuccinimide ester (Sulfo-EMCS, Pierce) according to the protocol of Kukolka et al. [13]. 0.1 equivalents of sulfhydryl functionalized oligonucleotide was added to the maleimide functionalized BSA [14]. The BSA-oligonucleotide product was hybridized with complementary 3+1 spider [10].

C. Measurements

Depositing substrates on dextran-matrix surfaces and 2D monolayer surfaces of SPR chips are similar to ref. [9] and [10]. For 2D monolayer surface, the average intersubstrate distance is 5.7 nm. Spiders (0.05nM~10nM for 3~5min) were loaded only on channel 2, with channel 1 used as a negative control. The amount of spider applied was controlled by adjusting concentration and the reaction time of spiders. Monitoring the cleavage of the substrate was initiated by switching to HBS-Zn buffer with the Biacore X system 'Working Tools Wash'. For input sensing molecular spiders, input or c-input (10μM) were added and incubated with chip for 20 min. All cleavage reactions were monitored with a flow rate of 20 μL/min. For logic gates controlled molecular spiders on beads: substrates were attached to streptavidin-functionalized-agarose microspheres (Pierce). The experiments were run in HEPES (10mM) buffer at pH 7.4 with 1M NaCl and 1 mM $ZnCl_2$. At each time point, the beads were spun down and an aliquot withdrawn for fluorescence measurement and then returned to the bulk reaction mixture.

III. Results And Discussion

A. Input sensing molecular spiders

We previously described the behavior of polycatalytic assemblies (molecular spiders) in a substrate-displaying matrix [9]. The standard molecular spiders comprise one streptavidin as an inert body and four identical catalytic 8-17 deoxyribozymes [15] as legs (Fig. 1a,b, NICK4.2A). The legs bind and cleave oligonucleotide substrates into two shorter products. Each leg moves independently from one site to an accessible neighbor site and will stay longer on average on a substrate site than a product site [16]. After cleaving, each leg will explore neighbor sites until it finds another substrate to bind and cleave. Multivalent binding allows molecular spiders to stick on the surface very tightly and hinders complete dissociation. This ensures that the molecular spiders stay in the matrix to diffuse by moving continuously towards the substrate region [9, 10]. In this study, we integrated our computing logic gates with the molecular spiders to control the behavior of the spiders. Input sensing gates [11] were chosen as legs for the molecular spiders and the cleavage sensorgrams were monitored in the real time by SPR technique (Fig.1c,d,e). We also constructed spiders using solely DNA with double-helices, three- and four-way junctions as inert body in place of streptavidin. The advantage of DNA spiders is that the lengths of their inert bodies can be finely tuned, the bodies can be easily labeled by fluorophores, and the synthesis is much easier with the potential to get any number of legs. The input sensing double-helices DNA molecular spiders were assembled using input sensing gates. Gates were switched on in the presence of input (Fig.1f).

B. Logic gates controlled molecular spiders

We used a NOT gate [11] (adapted from deoxyribozyme E6 [17]) to assemble a NOT gate molecular spider. This spider cleaved fluorescein labeled substrates, tethered to beads, in the absence of input oligonucleotide (Fig2a, b). By using two inputs (which are substrates for two independent YES gates) for the NOT gate spider, an AND gate was built for external computational control of molecular spider (Fig2c, d).

C. Cargo loaded molecular spider

Our long-term goal is to integrate molecular spiders with molecular scale computation to construct autonomous, controllable and decision-making molecular robots to execute a specific action (e.g. cargo delivery). These molecular robots will find a broad diagnosis and therapeutic applications. To this goal, first, we need to know how the loaded cargo affects the movement of the molecular spiders. We used BSA as a model cargo molecule. 3+1 spider (three identical 8-17 deoxyribozyme legs and one capturing oligonucleotide) was synthesized as before [10]. The capturing oligonucleotide formed a double helix with its complementary oligonucleotide which was coupled with BSA in a 1:1 ratio. The BSA loaded spider had nearly the same rate as the control spider. We also studied the cleavage of BSA loaded spider on 2D monolayer surface (Fig.4).

Acknowledgment

The authors are grateful to Dr. Milan N. Stojanovic for support of this work. This work was supported by the US National Science Foundation (NSF).

References

[1] J. S. Shin and N. A. Pierce, "A synthetic DNA walker for molecular transport," J. Am. Chem. Soc., vol. 126, Sep. 2004, pp. 10834-10835, doi: 10.1021/ja047543j.

[2] W. B. Sherman and N. C. Seeman, "A precisely controlled DNA bipedal walking device," Nano Lett., vol. 4, Jul. 2004, pp. 1203-1207, doi: 10.1021/nl049527q.

[3] P. Yin, H. Yan, X. G. Daniell, A. J. Turberfield, and J. H. Reif, "A unidirectional DNA walker that moves autonomously along a track," Angew. Chem. Int. Ed., vol. 43, Sep. 2004, pp. 4906-4911, doi: 10.1002/anie.200460522.

[4] Y. Tian, Y. He, Y. Chen, P. Yin. And C. D. Mao, "A DNAzyme that walks processively and autonomously along a one-dimensional track," Angew. Chem. Int. Ed., vol. 44, Jul. 2005, pp. 4355-4358, doi: 10.1002/anie.200500703.

[5] J. Bath, S. J. Green, K. E. Allen, and A. J. Turberfield, "Mechanism for a directional, precessive, and reversible DNA motor," Small, vol. 5, Jul. 2009, pp. 1513-1516, doi: 10.1002/smll.200900078.

[6] T. Omabegho, R. Sha, and N. C. Seeman, "A bipedal DNA brownian motor with coordinated legs," Science, vol. 324, Apr. 2009, pp. 67-71, doi: 10.1126/science.1170336.

[7] H. Gu, J. Chao, S-J. Xiao, and N. C. Seeman, "A proximity-based programmable DNA nanoscale assembly line," Nature, vol. 465, May 2010, pp. 202-205, doi: 10.1038/nature09026.

[8] Y. He and D. R. Liu, "Autonomous multistep organic synthesis in a single isothermal solution mediated by a DNA walker," Nature Nanotechnology, vol.5, Nov. 2010, pp. 778-782, doi: 10.1038/NNANO.2010.190.

[9] R. Pei et al., "Behavior of polycatalytic assemblies in a substrate-displaying matrix," J. Am. Chem. Soc., vol. 128, Sep. 2006, pp. 12693-12699, doi: 10.1021/ja058394n.

[10] K. Lund et al., "Molecular robots guided by prescriptive landscapes," Nature, vol. 465, May 2010, pp. 206-210, doi: 10.1038/nature09012.

[11] M. N. Stojanovic, T. E. Mitchell, and D. Stefanovic, "Deoxyribozyme-based logic gates," J. Am. Chem. Soc., vol. 124, Apr. 2002, pp. 3555-3561, doi: 10.1021/ja016756v.

[12] R. Pei, E. Matamoros, M. Liu, D. Stefanovic, and M. N. Stojanovic, "Training a molecular automaton to play a game," Nature Nanotechnology, vol. 5, Nov. 2010, pp. 773-777, doi: 10.1038/NNANO.2010.194.

[13] F. Kukolka, M. Lovrinovic, R. Wacker, and C. M. Niemeyer, "Bioconjugation Protocols: Strategies and Methods," in Methods in Molecular Biology, vol. 283, C. M. Niemeyer, Eds. New Jersey: Humana Pree Inc., 2004, pp. 181-196.

[14] S. K. Taylor, R. Pei, B. C. Moon, S. Damera, A. Shen, and M. N. Stojanovic, "Triggered release of an active peptide conjugate from a DNA device by an orally administrable small molecule," Angew. Chem. Int. Ed., vol. 48, Jun. 2009, pp. 4394-4397, doi: 10.1002/anie.200900499.

[15] S. W. Santoro and G. F. Joyce, "A general purpose RNA-cleaving DNA enzyme," Proc. Natl. Acad. Sci. USA, vol. 94, Apr. 1997, pp. 4262-4266.

[16] T. Antal and P. L. Krapivsky, "Molecualr spiders with memory," Phys. Rev. E, vol. 76, Aug. 2007, no. 021121, doi: 10.1103/PhysRevE.76.021121.

[17] R. R. Breaker and G. F. Joyce, "A DNA enzyme with Mg^{2+}-dependent RNA phosphoesterase activity," Chem. Biol., vol. 2, Oct. 1995, pp. 655-660.

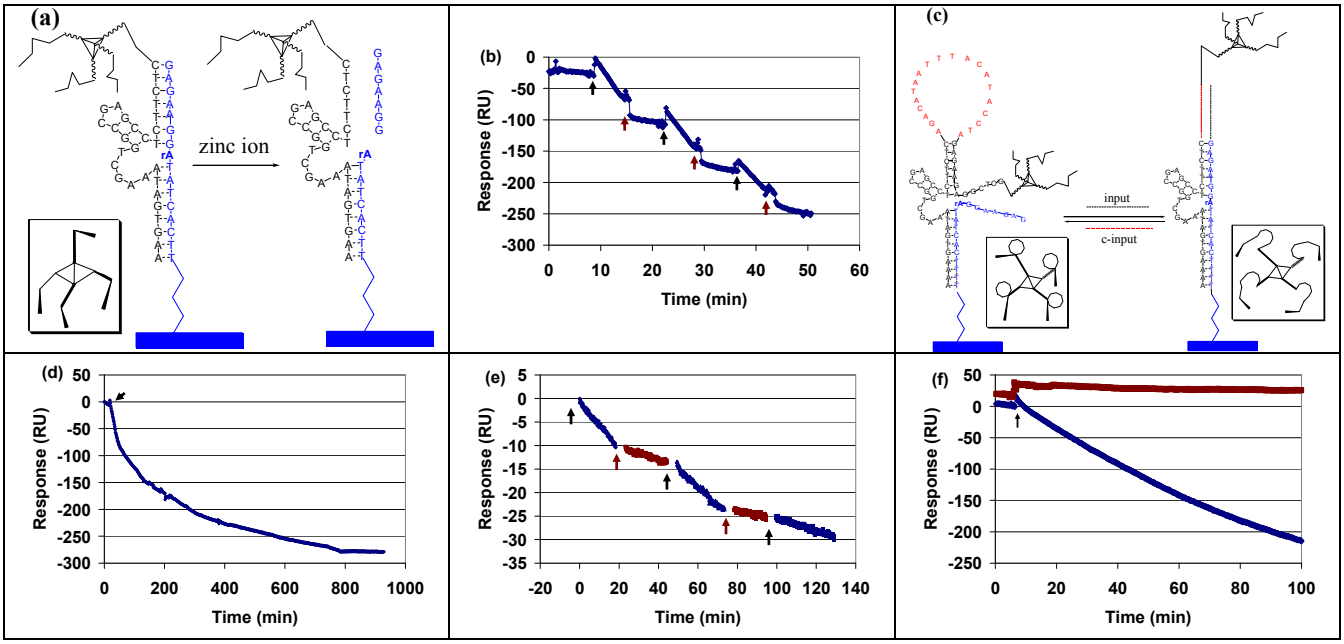

Figure 1. Input sensing molecular spiders. (a) The standard streptavidin molecular spider, NICK4.2A. The spider consists of a streptavidin core, with four identical 8-17 deoxyribozyme legs. The legs cleave the substrates deposited on the dextran-matrix surface of SPR chip, release the product and bind the next substrate. (b) Switch on/off by alternatively switching running buffers of HBS-Zn (black arrows) and HBS-EP (red arrows) (1:300 ratio of spider to substrate). (c) Input sensing streptavidin spider. The spider consists of a streptavidin core, with four identical input sensing gates as legs. The gates are activated by the input oligonucleotide. (d) The sensorgram shows the time course of substrate cleavage by input sensing streptavidin spider at a 1:550 ratio of spider to substrate. Cleavage is initiated by the adding of input oligonucleotide (i1s, black arrow) and is visualized by a decrease in mass on the chip due to the release of the product. (e) Switch on/off of input sensing streptavidin spider by alternatively adding of input (i2s, black arrow) and c-input (c-i2s, red

arrow) (1: 1340 ratio of spider to substrate, the reaction phase for input and c-input was omitted for clarification). (f) The time course of substrate cleavage by input sensing double-helices DNA spider (1:300 ratio of spider to substrate, the blue line for spider with input oligonucleotide i1s and the red line for spider without input oligonucleotide). The spider consists of a double-helices core, with two identical input sensing gates as legs. Cleavage is initiated by switching the running buffer to HBS-Zn (black arrow) from HBS-EP.

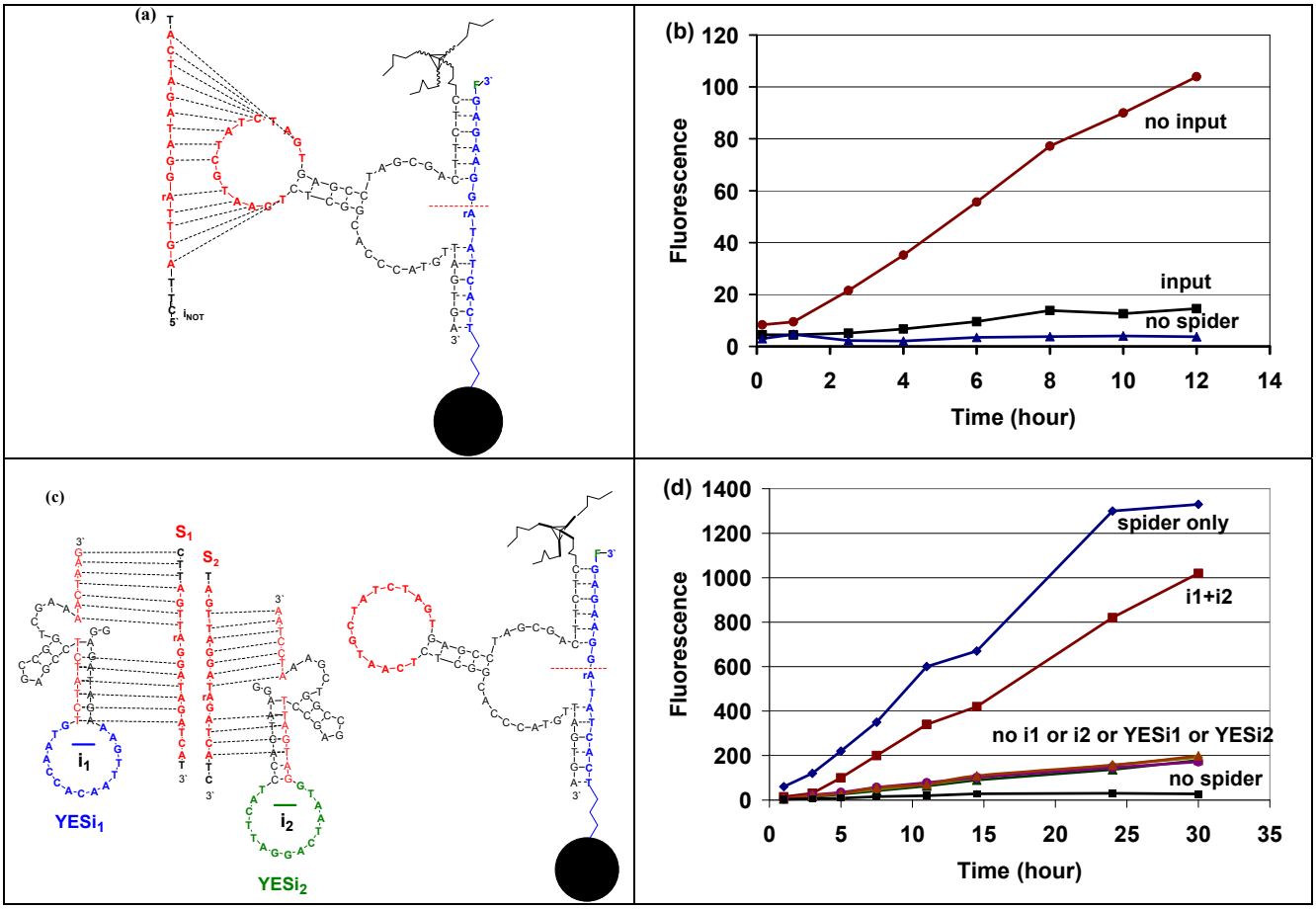

Figure 2. Logic gates controlled molecular spiders. (a) NOT gate molecular spider. The spider consists of a streptavidin core, with four identical NOT gate legs. (b) Fluorescence response of NOT gate spider by cleaving fluorescein labeled substrates on bead surface. (c) AND gate external control of spider. Two inputs (S1 and S2) of NOT gate are also substrates for two independent YES gates (YESi1 and YESi2). Only in the presence of both i1 and i2, the activation of YESi1 and YESi2 leads the cleavage of S1 and S2 to restore the activity of NOT gate spider. (d) Fluorescence response of AND gate external control of spider (NOT gate spider in a spider to substrate ratio of 1:1500, 2μM S1, 2μM S2, 100nM YESi1, 100nM YESi2, 1μM i1, 1μM i2).

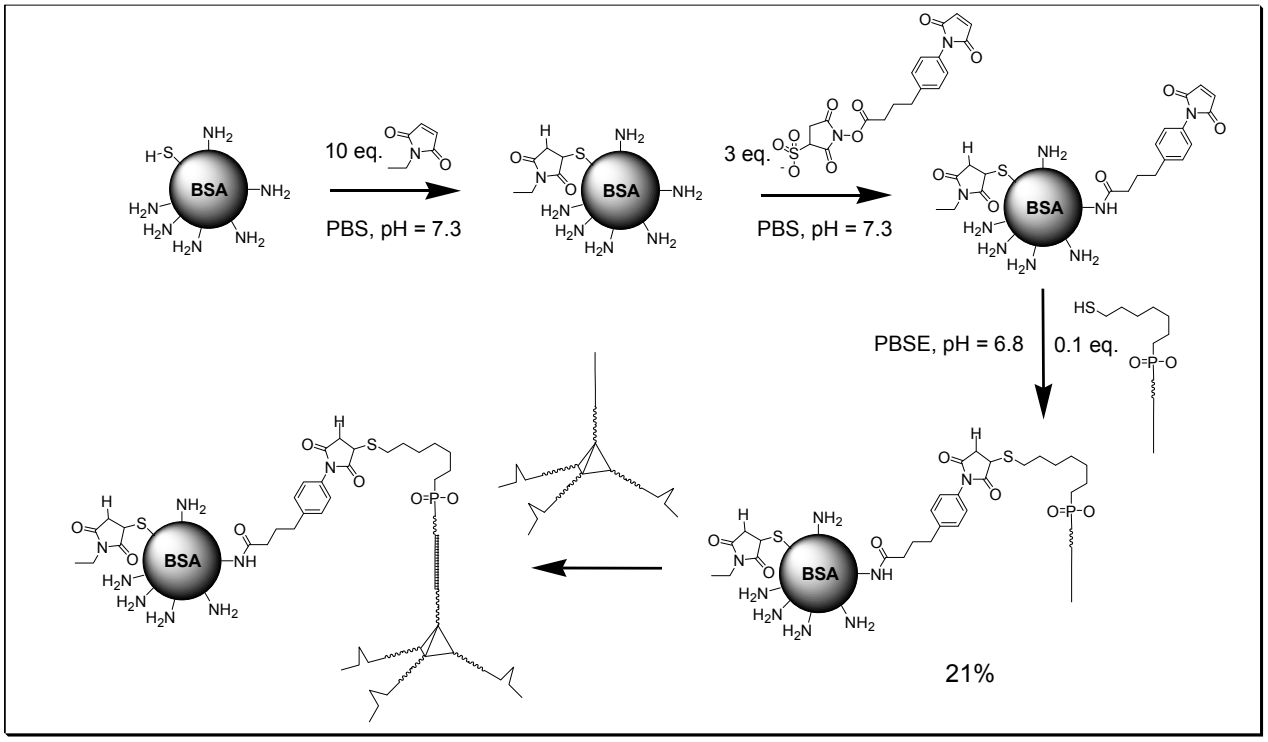

Figure 3. The synthesis of BSA loaded molecular spider.

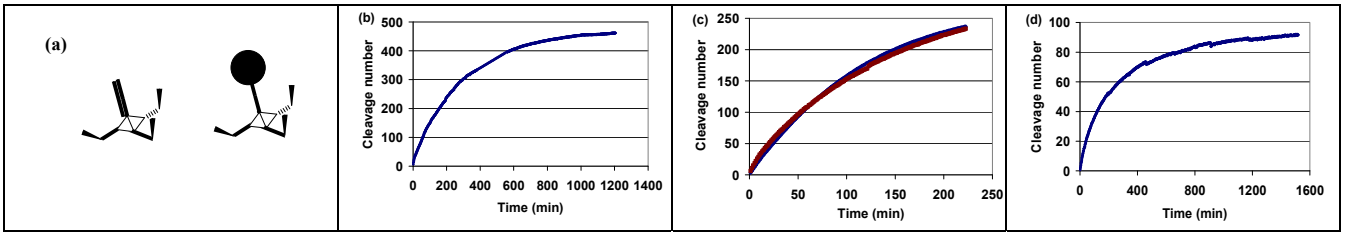

Figure 4. BSA loaded molecular spider. (a) 3-legged spider with a double-helices as a tag (the control spider) and BSA loaded spider. (b) The cleavage (per spider) sensorgram of BSA loaded spider on the dextran-matrix surface of SPR chip at a 1:560 ratio of spider to substrate. (c) Comparison of the cleavage sensorgrams of BSA loaded spider (1:350 ratio of spider to substrate, blue line) and the control spider (1:340 ratio of spider to substrate, red line) on the dextran-matrix surface. (d) The cleavage sensorgram of BSA loaded spider on 2D monolayer flat surface at a 1:120 ratio of spider to substrate.