

The supplementary material contains the design of the origami (Figure S1), the sequences of the cassettes (Figures S2-S4), the design and sequences of the walker and the schematic drawings of the walking (Figure S5), non-denaturing gels of the cassettes and the walker (Figure S6), agarose gels of the Au-DNA conjugations (Figure S7), the movement of the walker on the origami (Figure S8), images of the dynamic switching for each cassette on the origami (Figure S9), zoomed TEM images for statistical analysis (S10-S11), distance distribution between 5 nm and 10 nm Au on the walker (S12) and the statistical analysis for different additions (Table S1).

Sequences of Origami Helper Strands

Referring to Figure S1, there are totally 202 helper strands, labeled from 1 to 202. The color coding follows the conventions of Figure S1.

5'→3'

- 1: TGAAATTGTTATCCGCATAGCTGT
- 2: TTCCTGTGTTTTTCGGCCAGTGCCAAGCTCGACGTTG
- 3: TAAAACGATTTTCTTCGCTATTACGCCACGATCGGT
- 4: GCGGGCCTTTTTAGCTTTCCGGCACCGCAGATCGCA
- 5: CTCCAGCCTTTTAGGTTACGTTGGTGTATTGACCGT
- 6: AATGGGATTTTTCTTCCTGTAGCCAGCTATAATTTCG
- 7: CGTCTGGCTTTTAGCTGCTCATTTCAGTGCAAATCAACGTAACAA
- 8: GACAAGAACCGGATATTCATTACCAATAAGGC
- 9: TTGCCCTGAGGAACGCCATCAAAATTCATCAA
- 10: CATTAAATGGGAACAAACGGCGGAGATGGGCG
- 11: CATCGTAACGTATCGGCCTCAGGATTCTGGTG
- 12: CCGGAAACCAACTGTTGGGAAGGGGCTGGCGA
- 13: AAGGGGGAAGGGTTTTCCAGTCATGCATGCC
- 14: TGCAGGTCATTCGTAATCATGGTCTCACAATT

15: CCACACAACATACGAGCCGGAAGCATAAAGTG
16: AGTTGATTTCTGGAAGTTTCATTCCGGGTACC
17: GAGCTCGAGACTCTAGAGGATCCCCATGTAAC
18: TACAGGCAGTAGCATTAAACATCCATAAGTTGG
19: GTAACGCCTGTGCTGCAAGGCGATATAAATCA
20: ATGCAATGTTTTAGAACCCCTCATACGCCATTC
21: AGGCTGCGCAGGCAAAGCGCCATTTATTTTAA
22: GCCTGAGAATCTACAAAGGCTATCTTGAGGGG
23: ACGACGACCCGTGCATCTGCCAGTAGGTCATT
24: CGATGCACCCGTGCGATTCTCCGTGTGAGCGAGTAACAACAGTCGCTT
25: AATTGGGCTTTTTGTTAAATCAGCTCATTTTT
26: TAACCAATACGAGAACACCAGAACGAGTAGTA
27: AGGCTGGCTGACCTTCATCAAGAGTAATCTTC
28: GGACAGATGAACGGTGTACAGACCTCAACTTT
29: GCGTTAAATTGAGATGGTTTAATTAGGCGCAT
30: AATCATTGAACGTTAATATTTTGTTAAAATTC
31: TTTGAGAGGTCTGGAGCAAACAAGAGAATCGA
32: TGAACGGTTTAATGCCGGAGAGGGGTAAAGAT
33: ATAAAAATCCTGAGTAATGTGTAGTAGCTATT
34: TCAAAAGGAGAAGCCTTTATTTCAAATAAG
35: TAATAGTAAGGCAAAGAATTAGCAACGCAAGG
36: CAATAAAGCTGAAAAGGTGGCATCAGTAGATT
37: GCTGTA CTTTGGTTACTCGTACAACGTACGGTGCCCAATTCTGCGAACGAATTCTAC
38: TAGTTTGAACATGTTTTAAATATGGAGCTAAC
39: TAAAGCCTGGGGTGCCTAATGAGTCAACTAAA GTTGTACG
40: TCACATTAATTGCGTTGCGCTCACCTGGTGCT
41: GTAGCTCACCATTAGATACATTTCTCATTTGGGCTACGTC

42: ACTTGTATGGGACGTAGCGGCGCGAGCCTCAGAGCATAAAGCGTAATACT
43: TTTGCGGGGTGAGAAAGGCCGGAGCGTTCTAG
44: CTGATAAAAATCGTAAAACCTAGCATGTCAATC
45: AAATTGTATGAATTACCTTATGCGTTGAAAGA
46: ATAAGGGAACCGAACTGACCAACTATTTAAG
47: AACTGGCTCATTATACCAGTCAGGGGTCAATC
48: CAGTTGAGGGAAGATTGTATAAGCAAATATTT
49: CGTGCCTA AAAAGCCCCAAAACAATTTAGGAATACCACAAGATTCAT
50: GTTTACCACCCGGTTGATAATCAGGGTGGCTA
51: ATATGTACGACGACGATAAAAACCTAACCCTC
52: GTCCAATACAATATGATATTCAACACAGTCAA
53: ATCACCATCTGCGGAATCGTCATATGGATAGC
54: GTCTTTACAAAACATTATGACCCTTAAATCGG
55: TTGTACCACCTGACTATTATAGTCAAATCAG
56: AGCTTCAACTGTTTAGCTATATTTGCAAATGG
57: TCAATAACAGCGAACCAGACCGGATTAATTCG
58: TCCACATCGGACTCTGTGCTAGCATTGGCTTAGAGCTTAATTGCTGAATTGCCCGCT
59: TTCCAGTCGGGAAACCTGTCGTGCTTTGCGGAATGCTAGC
60: CCAACAGGTTGATAAGAGGTCATTCAGCTGCA
61: AGCGGATTCTTCAAATATCGCGTTAGCAAACCT
62: TTGAATCCGAATGACCATAAATCAAGAAGCAA
63: GAGAGGCTAGTAAAATGTTTAGACAATATTCA
64: ATGCAGATAAGAGCAACACTATCAAAAATAGC
65: AAGAAAAAATTATTACAGGTAGAATTCAACTA
66: ACTTAGCCGGAACGAGGCGCAGACACGTTGGG
67: ATTGTGTCGAAATCCGCGACCTGCTCCATGTT
68: GGAACAACCTCTACGTTAATAAAAACCTAATAATT

69: TTTTCACGCTAAAGGAATTGCGAAGAATAAC
70: GCTAACTGAATTACGAGGCATAGTACATAACGCCAAAAGGGGTATGCG
71: GGGGTAATTTTGCAAAGAAGTTTAGTTTCGT
72: CACCAGTAATGTACCGTAACACTGTGCCAGAG
73: GAAAACGACCCTCAAATGCTTTAAGGTTGATA
74: TAAGTATATAAGTGCCGTCGAGAGACAGTTCA
75: CCGAAAGAGCATCAAAAAGATTAACCTTGAGT
76: AACAGTGCTTTAACGGGGTCAGTGGAGGAAGC
77: TGCTCCTTTCAGGATTAGAGAGTAAGGCAGGT
78: CAGACGATCATTGACAGGAGGTTGCCTTTAAT
79: TTAATGAATCGGCCAACGCGCGGGGAGAGGCG
80: GTTTGCGTATTGGGCGCCAGGGTGCCAGAGCCAGCGTAAG
81: CTACACGTACCGACATCCTTACGCTGCCGCCAGTGGCCTTGATATTCAGTACTGG
82: TAATAAGTCCGTATAAACAGTTAACTCAGTAC
83: CAGGCGGAGCCCGGAATAGGTGTAAGCCCAAT
84: AGGAACCCCAAACACTACAACGCCTGTAGCATT
85: AGGAACAATTGAAAATCTCCAAAACCTGATAA
86: GTACAACGGAGATTTGTATCATCGAAAAGGCT
87: CAAAAGGAGTTTCAGCGGAGTGAGAATAGAA
88: CACAGACAATTTTCAGGGATAGCATCACCGTA
89: CTCAGGAGGATTAGCGGGGTTTTGTGCCCCCT
90: GCCTATTTTTTTGATGATACAGGAGAAACGAAT
91: GGATCTTCGCCACCAGAACCACAGTTTTTCT
92: TTTCACCAGTGAGACGGGCAACAGCACCAACC
93: TCAGAGCCATTAAAGCCAGAATGGAGCGTCAT
94: TCATGGCTCGGAACCTATTATTCTTCAAGAGA
95: AGGATTAGGTTTAGTACCGCCACCCAGAGCCA

96: CCACCCTCGCCCTCATAGTTAGCGTAACGATC
97: CTTTCAACAGCCTTTAATTGTATCGAAACAAA
98: ACCCCCAGCGATTATACCAAGCGCGGTTTATC
99: AGCTTGCTTTCGAGGTGAATTTCTCATCTTTG
100: GTATGTTATCTGTATGGGATTTTGCTAAACAA
101: **CCAGACGG**GTTAGTAAATGAATTTGCAAACGTAGAAAATAATTACGCA
102: TAGAAAATTGTCGTCTTTCCAGAC**CACTAGTG**
103: TAAAGTTTTCATATGGTTTACCAGACAATCAA
104: AAGGTGAATCAGAACCGCCACCCTCTCAGAAC
105: CGCCACCCTTATCACCGTCACCGAATTCATTA
106: CACCAATGAAGAGGCTGAGACTCCGAAACATG**GTGACGAA**
107: **CTTCATGGCATTTCGTAC**AAAGTATTA AACCATCGATAGCAGGGAAACGT
108: CGGTCATAATTTACCGTTCAGTAAAAGCGCA
109: GTCTCTGAGCCCCCTTATTAGCGTGGCATT
110: **CCGCTATGCACTCGATGCCAGTGAT**CCTCAGAACCGCCACCCTCAGAGCCTGATTGC
111: CCTTCACCGCCTGGCCCTGAGAGAGCCGCCAC**ATCACTGG**
112: TTTTCATAAACCGCCTCCCTCAGAGTTGCAGC
113: TCAGTAGCGTAGCGGTTTTTCATCTTGCCATC
114: ATTTGGGATACCATTAGCAAGGCCACCGTAA
115: ACAAAGGAATATTGACGGAAATTCTTGAGCC
116: AAGGTGGCATAAGTTTATTTTGTCCGCAAAG
117: TTGATACCATGATTAAGACTCCTTCATACATA
118: GGCGAAAGAATACTAAAACACTTAAACAGC
119: CCACTACGAAGGCACCAACCTAAAACGAAAGA
120: GAACTGGCGATAGTTGCGCCGACAATGACAAC
121: AACCATCGGCAATAATAACGGAATACCCAAAA
122: **TCAACGTC**GCAAAGACACCACGGAAACATATAAAAGAAAC**CCACCGAT**

123: GGAAGGTAGCGACATTCAACCGATTATTTATC
124: CCAATCCAAAATAAACAGCCATATTGAGGGAG
125: AGCACCATATTAGAGCCAGCAAAAAAGTACCG
126: ACAAAGGGTAATAAGAGAATATATCACCAGT
127: GTCAGACTGACAGAATCAAGTTTGGTAAATCG
128: TCGCTATTGAATAACCTTGCTTCTCCTTTAGC
129: ACCACCGGATCAAATCACCGGAAATTAATTT
130: TAAAAGTTCCTTTGCCCGAACGTTCCAGAGCC
131: AAGCGGTCCACGCTGGTTTGCCCCAGCAGGCG
132: AAAATCCTGTTTGATGGTGGTTCCACAACCTCGAGTCACAC
133: AACCGTGTCTACGCATCGTGTGACTTATTAATTGAGTAACATTATCATATCAATAT
134: ATGTGAGTAATTAATTTTCCCTTAAGGCATTT
135: TCGAGCCATAAAGTAATTCTGTCCTAATTTGC
136: CAGTTACAAATAAGAAACGATTTTTTGTTTAA
137: GAGGAAACCCACGCATAACCGATACGTAATG
138: AAGTTTCCATTAAACGGGTAAAATATATTCGG
139: TCGCTGAGGAACAAAGTTACCAGAAGGAAACC
140: CGTCAAAGCGTCTTTCCAGAGCCAGACGACG
141: ACAATAAACATGTAATTTAGGCAGGAATCCTT
142: GAAAACATTGGAAACAGTACATAATTTGCGGA
143: ACAAAGAACTTTACAAACAATTCGGAAATCGG
144: CAAAATCCCTTATAAATCAAAGAATTTAGAA
145: GTATTAGAACCACCAGAAGGAGCGGAATTACC
146: TTTTTTAAAGCGATAGCTTAGATTTATTTAACTGTCATAG
147: GGAGTCGGCACTATGACAACGCCAACAACATGTTTCAGCTAATTACCAAC
148: GCTAACGAATGAAAATAGCAGCCTTTACAGAG
149: AGATAGCCGCTTGCAGGGAGTTAATCATGAGG

150: AGGCTTTGAGGACTAAAGACTTTTAGGCCGCT
151: TTTGCGGGATCGTCACCCTCAGCAGGCTACAG
152: TCTTACCGAAGCCCTTTTAAAGAAAAGTAAGC
153: GCAGTTGCCAGAGAGATAACCCACAAGAATTGAGTTAAGCAATAGCTA
154: CAGAGGGTAATTGAGCGCTAATATGCTACGAT
155: AGAATAACATAAAAAACAGGGAAGCAACAAAGT
156: CCAGCTACAATTTTATCCTGAATCTGCAGAAC
157: GCGCCTGTTTATCAACAATAGATATTTTGCAC
158: GTAGGGCTTAATTGAGAATCGCCAAAGACGCT
159: GAGAAGAGTCAATAGTGAATTTATGCTCAACA
160: TAATTACATTTAACAATTTCAATTTGAATTATC
161: ATCATATTCCTGATTATCAGATGAAACAAAAT
162: TAACGACACTTGCATCAGCTATTGACGTCAATAGATAATACATTTGAGGATAGCCCG
163: AGATAGGGTTGAGTGTTGTTCCAGATTAGAGCTCAATAGC
164: CATCAATACACTAACAATAATAGTTTGGAAC
165: TAGGTCTGAAACAAACATCAAGAATGGCAATT
166: ACAAGAAACCAGTATAAAGCCAACCAAAATCA
167: CGGGAGAATCAAGATTAGTTGCTAAGTCCTGA
168: AAGAGCAATTTTTTAACTGAACACCCTGGCATTAGA
169: CAGCATCGTTTTGAAACAATGAAATAGCCCAATAAT
170: GAACGAGGGTAGCAACGCGAAAGA
171: AATTCTTAAATAATATCCCATCCTGTTTTGAAGCCTTAAA
172: AGATGATGAGAGACTACCTTTTTAGTTATACA
173: TTTAGGTGTAATCCTGATTGTTTGGCAAAAGA
174: AAGAGTCCACTATTAAAGAACGTGAAAATATC
175: TTCTGAATTTGAGGAAGGTTATCTGACTCCAA
176: CTTAGGTTCAATTTCAATTACCTGAGATTATAC

177: AGCATGTAGTTTAGTATCATATGCACCTCCGG
178: AGCGAACCTCCCGACTTGCGGGAGAATTTACG
179: TTATCCGGTATTCTAAGAACGCGAGGCGTTTT
180: AAAAGCCTGAAACCAATCAATAATCGGCTGTC
181: TTTCTTAAACACCGGAATCATAATTACTAGA
182: GAATTATTGGGTTATATAACTATATGTAAATG
183: CTGATGCACAAGTTACAAAATCGCGCAGAGGC
184: GAAAGGAATATGGAAGGAATTGAACCAACCAT
185: ATCAAAATTGGTCAGTTGGCAAATCAACAGTA
186: CGTCAAAGGGCGAAAAACCGTCTATCAGGGCG
187: ATGGCCCACTACGTGAACCATCACACCCTCAA
188: TCAATATCTATTAGCACGTAAAACCTGATTGCT
189: TTGAATACAATCCAATCGCAAGACGCGTTAAA
190: TAAGAATATCATTCCAAGAACGGGTAGAAGGC
191: GCCCAATAGCAAGCAAATCAGATATATTAAC
192: CAAGTACCCCGTGTGATAAATAAGAAAGAACG
193: CGAGAAAACAATAACGGATTCGCCAGAAATAA
194: AGAAATTGGAACCTCAAATATCAACCAAATCA
195: AGTTTTTTGGGGTCGAGGTGCCGTAAAGCATC
196: ACCTTGCTCGTAGATTTTCAGGTTTTTACATC
197: GGGAGAACTTTTTCAAATATATTTGGTTTGA
198: AATACCGAGCACTCATCGAGAACAATTACCGC
199: TTTTCATCGTAGGAATCAGCAAGCC
200: GTTTTTATTTTTCTGACCTAAATTTAATTAGTTAA
201: TTTTCATCTTTTTTACAGTAACAGTACCTTAACGTCA
202: GATGAATATTTTAGCAAATGAAAAATCTAAAGCACTAAATCGGA

Figures S1-S5. Molecular Details of the Molecules used in this Work.

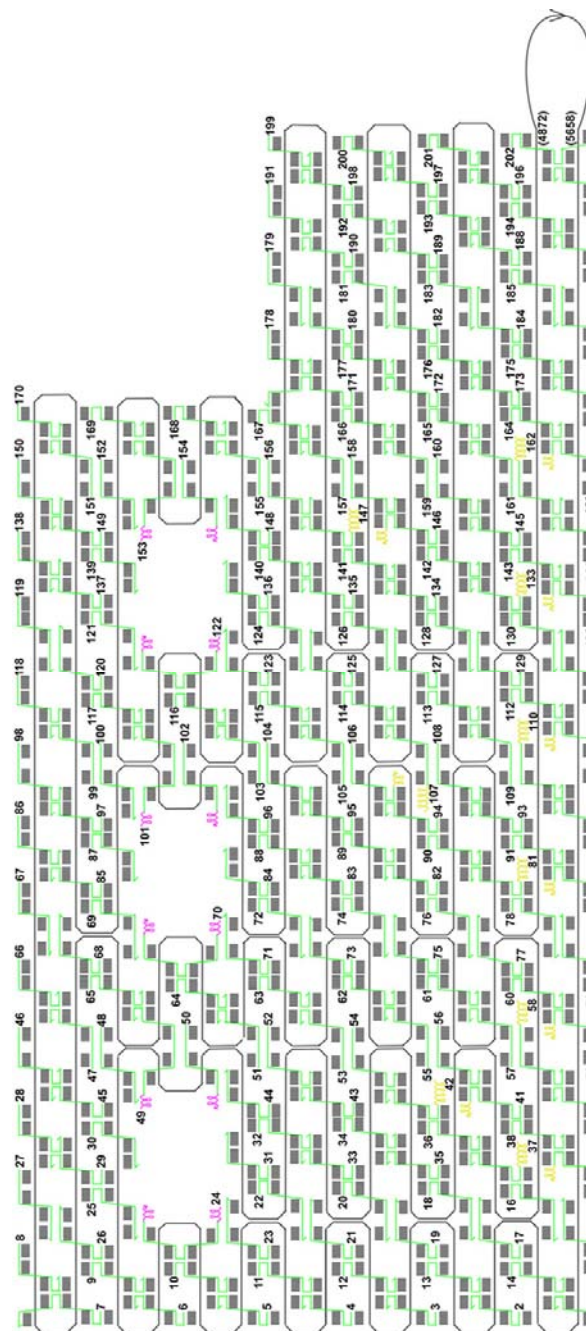


Figure S1. The Strand Structure of the M13 Origami Used in This Work.

202 helper strands were used to form the origami with 3 slots. 9 Single strand extensions are labeled purple around the slots. They were designed to bind with cassettes and position them on the slots. Another 18 single strand extensions are labeled tan for the walker binding. The sequence used for the circular M13 plasmid in this work starts from 5658 and ends at 4872. The colored strands were purified individually by denaturing PAGE.

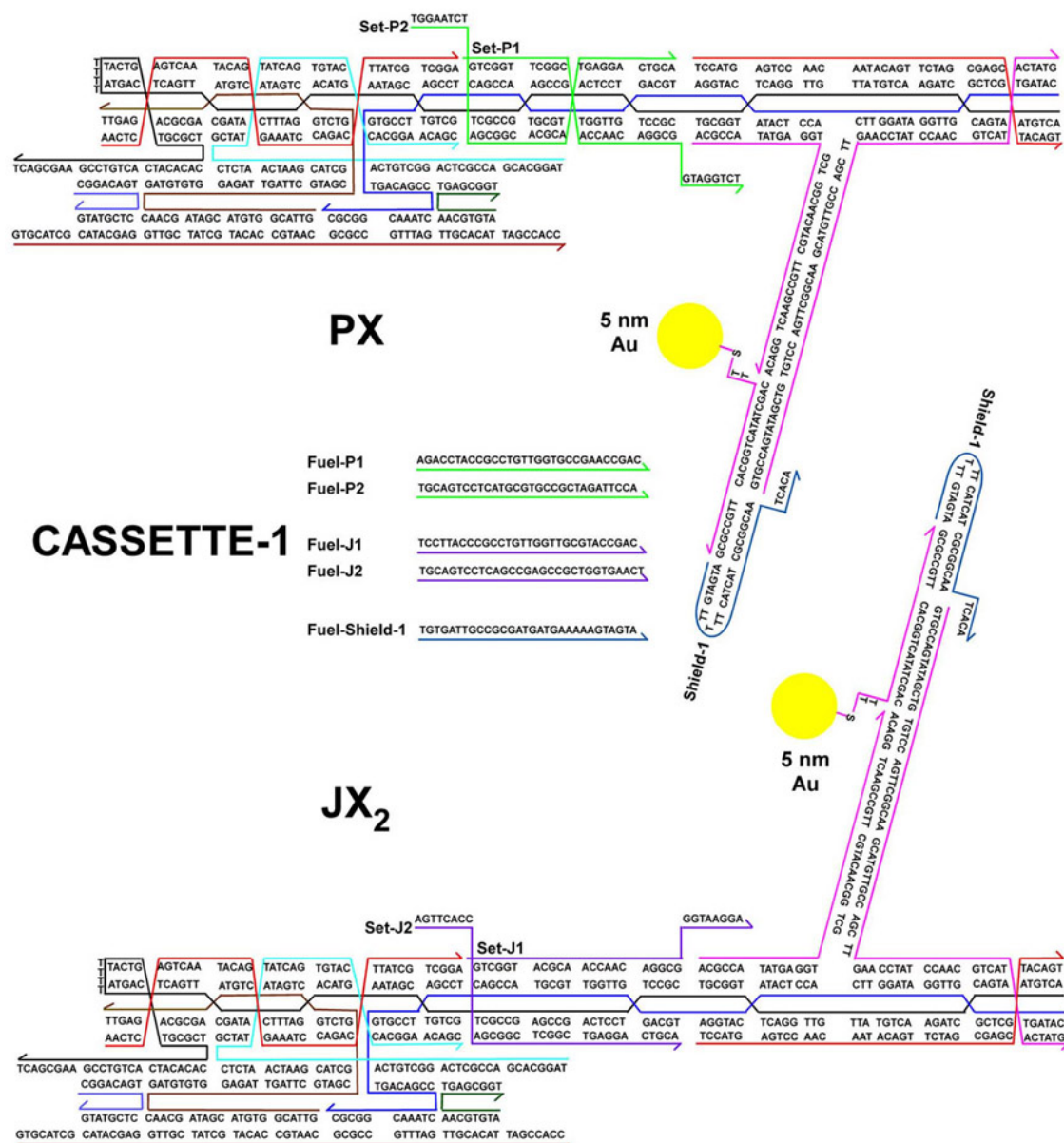


Figure S2. The Sequence of Cassette 1 in the PX and JX₂ States.

The PX state is shown on the top and the JX₂ state is on the bottom. The cassette consists of four DNA duplex domains. The bottom two duplexes form the DX motif, which can be inserted into the left slot of the origami by double cohesion. The top two duplex domains form the PX/JX₂ device motif. The strands which set the cassette to the PX or JX₂ state were labeled as Set-P1/2 or Set-J1/2, respectively. The strands that are completely complementary to the set strands were labeled as Fuel-P1/2 or Fuel-J1/2, respectively. The same convention applies for Figure S3 and S4. The cargo strand for carrying a 5 nm Au nano-particle on the duplex arm was modified with a -SH group. The overhang part of the cargo strand was protected by the Shield-1 strand, which could be removed by its completely complementary strand Fuel-Shield-1.

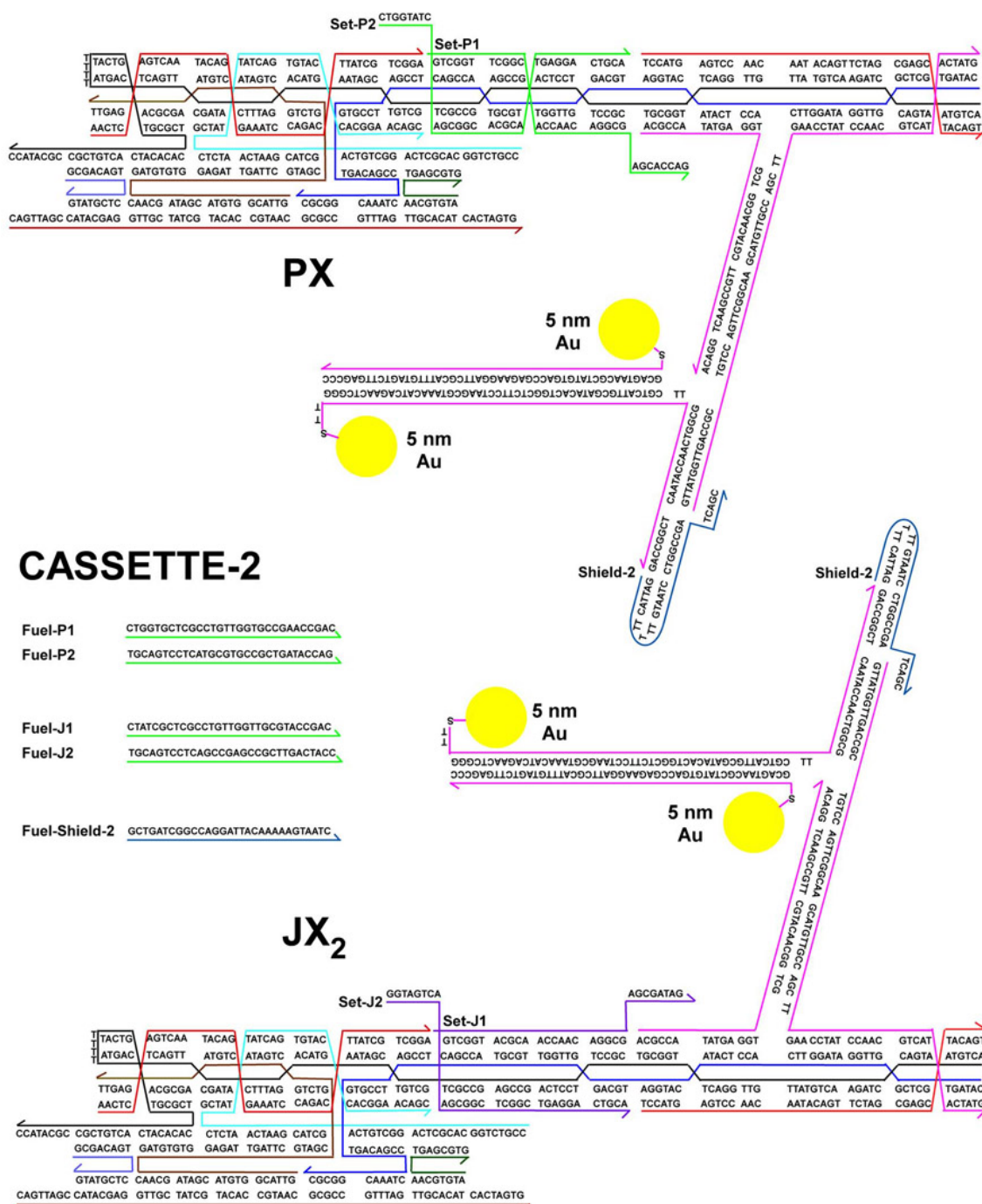


Figure S3. The Sequence of Cassette 2 in the PX and JX₂ States.

The same conventions apply as in Figure S2. The cargo strands for carrying double 5 nm Au nanoparticles on the duplex arm were modified with a -SH group. The overhang part of the longer cargo strand was protected by the Shield-2 strand, which could be removed by its completely complementary strand Fuel-Shield-2.

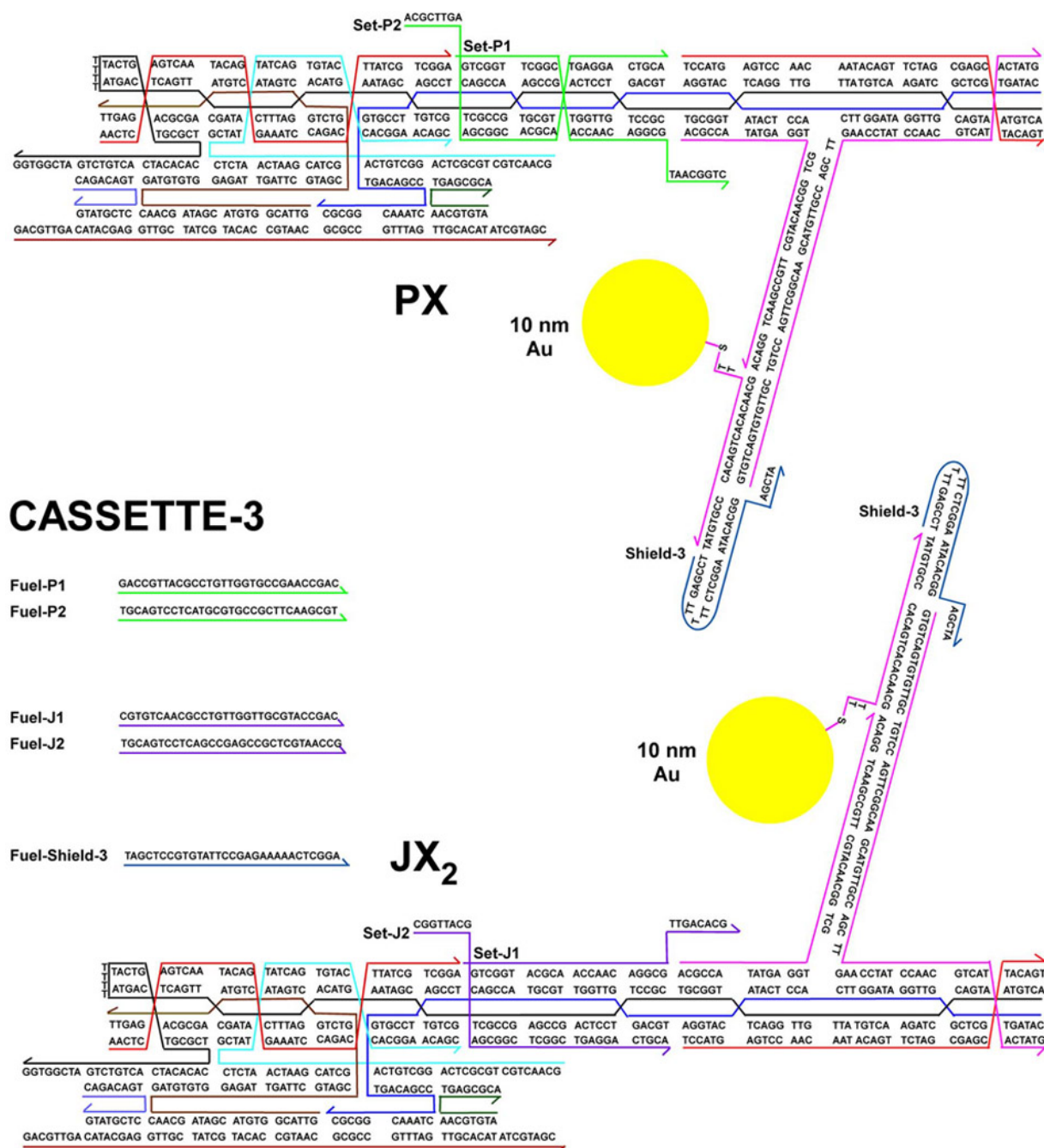


Figure S4. The Sequence of Cassette 3 in the PX and JX₂ States.

The same conventions apply as in Figure S2. The cargo strand for carrying a 10 nm Au nano-particle on the duplex arm was modified with a -SH group. The overhang part of the cargo strand was protected by the Shield-3 strand, which could be removed by its completely complementary strand Fuel-Shield-3.

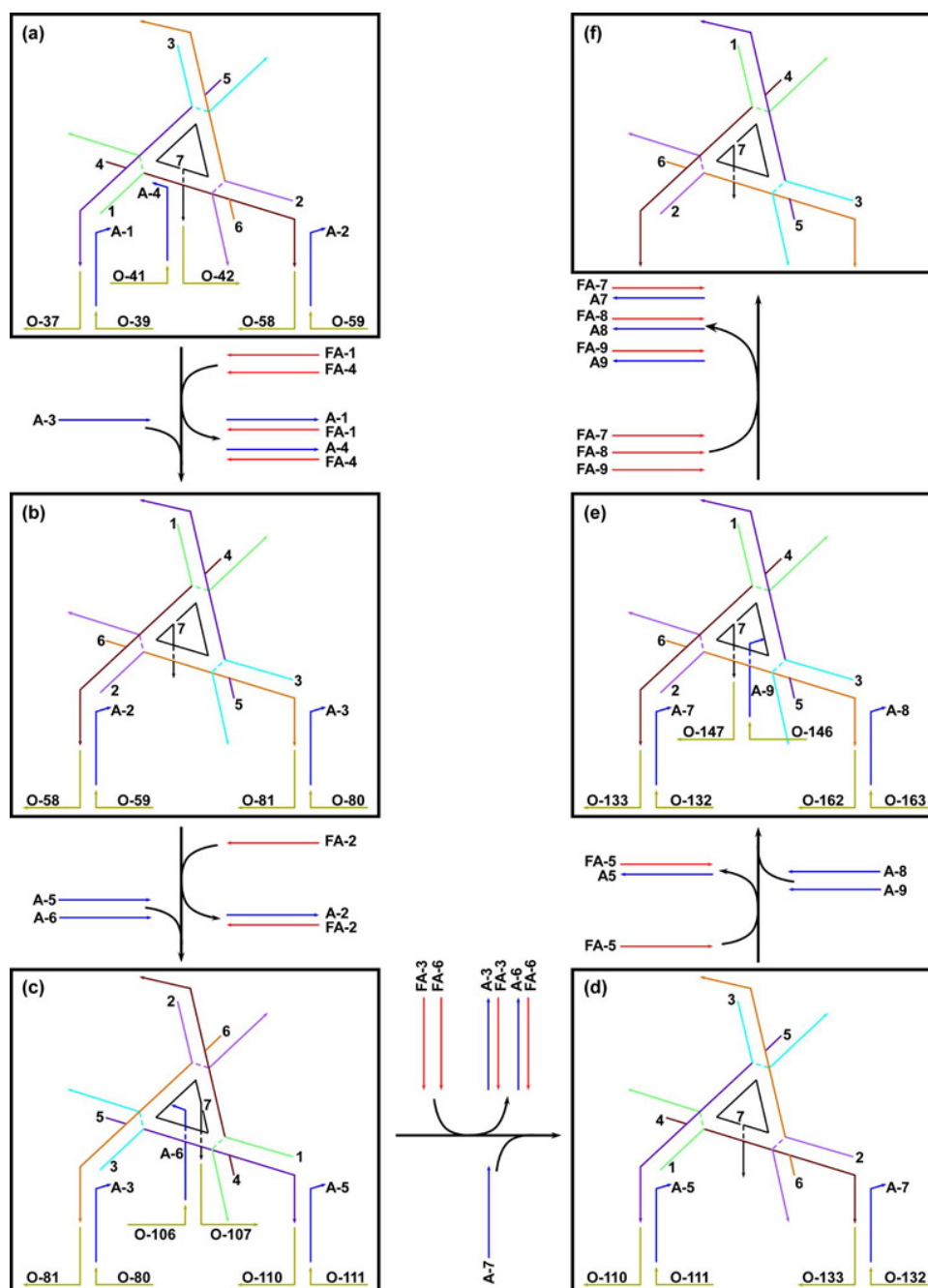


Figure S5. Schematic Drawings of the Whole Walking Process and the Sequence of the Walker.

The walker consists of 7 strands, which were labeled as 1 to 7. Single strand extensions from strand 4, 5, 6 and 7 act as feet of the walker. The feet could bind with the origami helper strands (labeled as O-*) by anchor strands (labeled as A-*). The anchor strands could be removed by the completely complementary fuel strands (labeled as FA-). Totally there are 9 anchor strands and 9 fuel strands.

* Some labels refer to origami strands in Figure S1, different from those in the main text. To clarify, the correspondence between the notation in Figure S5 and in Figure 2 is shown in the table below.

O37 ↔ 1a	A1 (in part) ↔ the complement to 1a
O42 ↔ 4a	A2 (in part) ↔ the complement to 2a
O58 ↔ 2a	A3 (in part) ↔ the complement to 3a
O81 ↔ 3a	A4 (in part) ↔ the complement to 4a
O107 ↔ 4b	A5 (in part) ↔ the complement to 1b
O110 ↔ 1b	A6 (in part) ↔ the complement to 4b
O133 ↔ 2b	A7 (in part) ↔ the complement to 2b
O147 ↔ 4c	A8 (in part) ↔ the complement to 3b
O162 ↔ 3b	A9 (in part) ↔ the complement to 4c

Sequences of Walker, Anchor, and Fuel Strands:

5'→3'

Walker-1: GCATCGTATGTAGCGCGTAGTGGTCGCACAATACACGGGTGTCAGTGTGTTGC

Walker-2: TACGTGATGACGTAAGTACTGATGTGGTAGGCTGCTGGCCGAGTTATGGTTGACCGC

Walker-3: GATCATGCAGTGGCTAGTCGTGGTATCGCTCGCGGCAAGTGCCAGTATAGCTG

Walker-4:

TGTGCGACCTGCTCTCGTGCTGATATGCCTTGATCGGACATCAGTACGTCATCACGTATTT
TATCTGTTAGCA

Walker-5:

AGCGATACCTGATAATAGACTGTGAATAGTTGTACGGACTACGCGCTACATACGATGCTTT
TGTGACGGCGTC

Walker-6:

CAGCCTACCTGGTGAGTAACGTCCATGTTCGATTACAGGACGACTAGCCACTGCATGATCTTT
TGCGCCAATGGC

Walker-7:

TCAGCACGAGAGCACCGTACAACCTATTCACAGTCTATTATCACCTGAATCGACATGGACG
TTACTCACCACCGATCAAGGCATATTTTGATAGGATCT

A-1: AGTAACCAAAGTACAGCACTGCGACGCCGTCACCAATAC

A-2: ACAGAGTCCGATGTGGATGTCATGCTAACAGATATCAGT

A-3: GATGTCGGTACGTGTAGCCACGGCCATTGGCGCTGCACG

A-4: CCATACAAGTATGTAGATCCTATCCATGAC

A-5: CATCGAGTGCATAGCGGTCGAGGACGCCGTCACGTAGTC

A-6: TGCCATGAAGTTGAAGATCCTATCGAACAT

A-7: GATGCGTAGACACGGTTAGCAGTGCTAACAGATACGGCA

A-8: TGATGCAAGTGTCGTTATGCGAGCCATTGGCGCTATACG

A-9: TGCCGACTCCAATGAGATCCTATCTGGACA

FA-1: GTATTGGTGACGGCGTCGCAGTGCTGTACTTTGGTTACT

FA-2: ACTGATATCTGTTAGCATGACATCCACATCGGACTCTGT

FA-3: CGTGCAGCGCCAATGGCCGTGGCTACACGTACCGACATC

FA-4: GTCATGGATAGGATCTACATACTTGTATGG

FA-5: GACTACGTGACGGCGTCCTCGACCGCTATGCACTCGATG

FA-6: ATGTTGATAGGATCTTCAACTTCATGGCA

FA-7: TGCCGTATCTGTTAGCACTGCTAACCGTGTCTACGCATC

FA-8: CGTATAGCGCCAATGGCTCGCATAACGACACTTGCATCA

FA-9: TGTCCAGATAGGATCTCATTGGAGTCGGCA

A-Biotin-Walker-7 is a special biotin modified strand that can anchor the walker to the streptavidin bead by binding with the foot on Walker-7. FA-Biotin-Walker-7 is A-Biotin-Walker-7's complementary strand.

A-Biotin-Walker-7: TT-Biotin-TT-Biotin-TTTTTTTTTTTAGATCCTATC

FA-Biotin-Walker-7: GATAGGATCTAAAAAAAAA

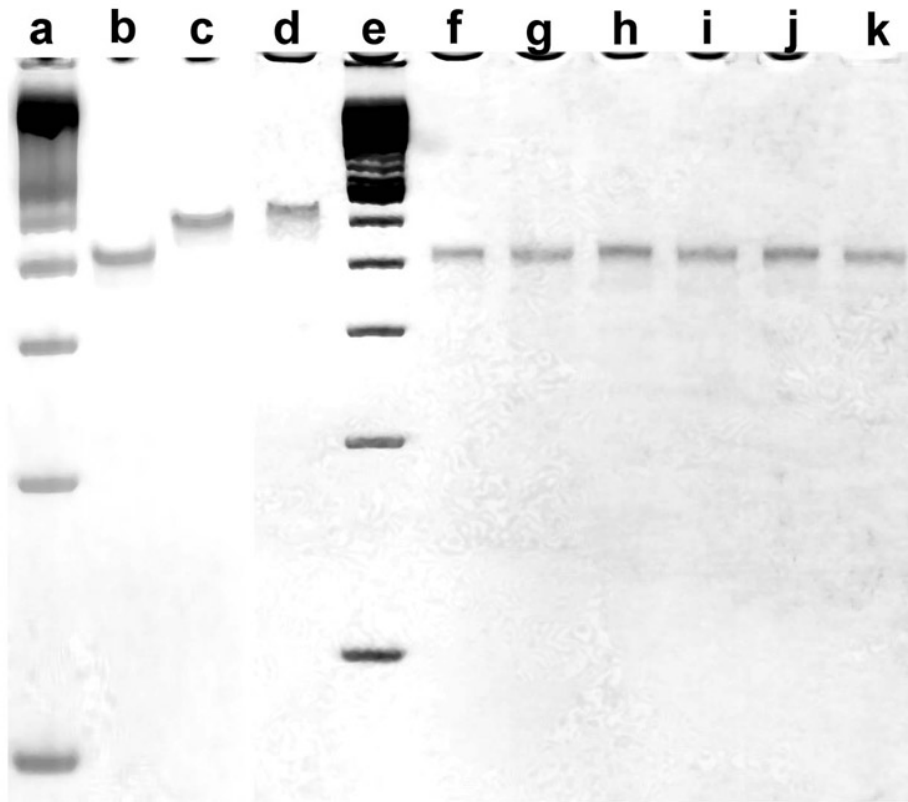


Figure S6. Non-Denaturing Gels of Components of the System.

The images are 5% non-denaturing gels run at 25 °C. Lane a contains a 100 nucleotide pair marker; lane b contains the walker without the foot and hand extensions; lane c contains the walker with the foot extensions, but without the hand extensions; lane d contains the walker with the foot and the hand extensions; lane e contains another 100 nucleotide pair marker; lanes f and g contain cassette 1 in the JX₂ and the PX states, respectively; lanes h and i contain cassette 2 in the JX₂ and the PX states, respectively; lanes j and k contain cassette 3 in the JX₂ and the PX states, respectively. For lanes b to d and f to k, 20 μ L of each molecule at a concentration of 50 nM were loaded into the well.

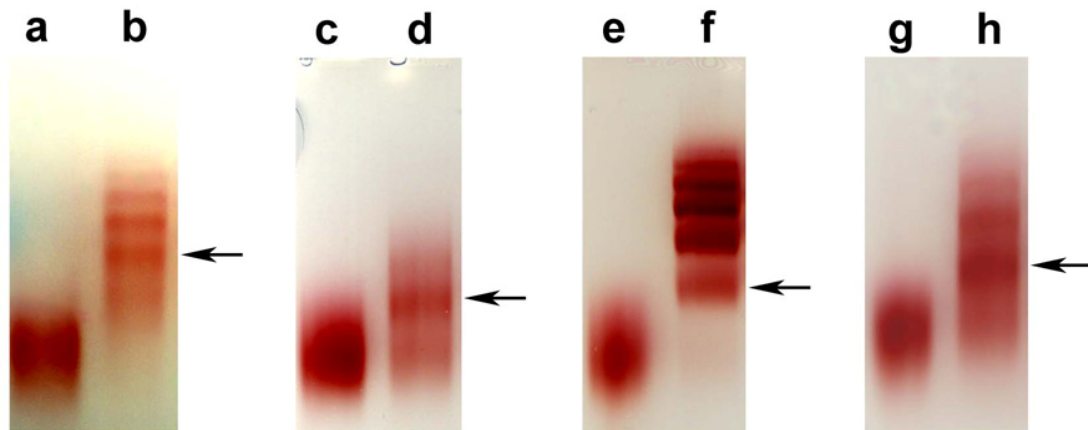


Figure S7. 3% Agarose Gels showing the Au-DNA Conjugates.

For each gel, the left lane contains Au nano-particles (AuNp) as a marker. The right lane contains the Au-DNA conjugates. From bottom to top, each band corresponds to particles bearing an increased number of DNA strands. The first conjugate was collected (arrow head) to form the cassettes. Lane a contains 5 nm AuNp; Lane b contains 5 nm AuNp and DNA duplex (arm of cassette 1) conjugates; Lane c contains 5 nm AuNp; Lane d contains 5 nm AuNp and the shorter DNA strand (arm of cassette 2) conjugates; Lane e contains 5 nm AuNp; Lane f contains 5 nm AuNp and the longer DNA strand (arm of cassette 2) conjugates; Lane g contains 10 nm AuNp; Lane h contains 10 nm AuNp and DNA duplex (arm of cassette 3) conjugates.

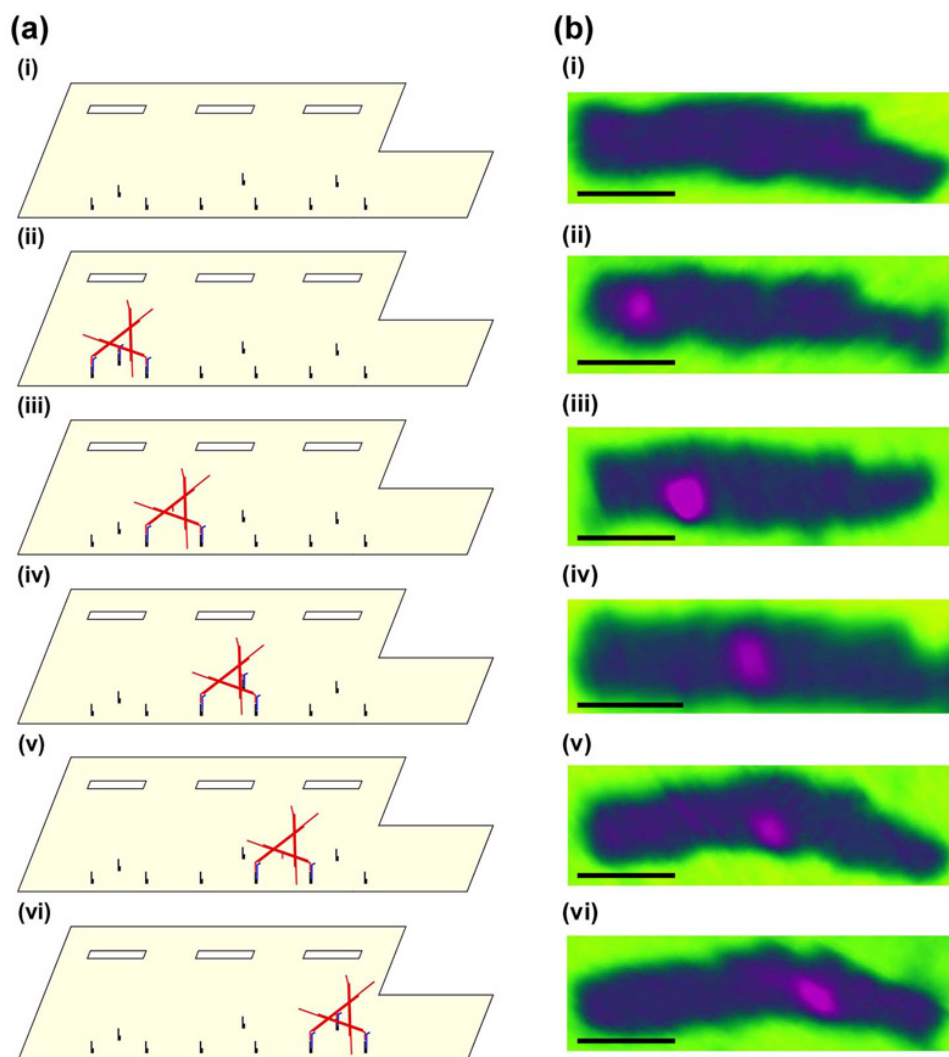


Figure S8. Schematics (a) and Atomic Force Micrographs (b) of the Walking on the Origami.

The origami with three slots is shown in light yellow in (a). Nine single strand extensions from the origami helper strands are shown as black stems. They are shown below the slots, and they act as stations for the walker. The walker is shown in red. AFM was performed in tapping mode in air in panel (b). Panel i of (a) illustrates the origami array containing slots for the cassettes, helper strand extensions for the walker and a notch to enable recognition of orientation; only the notch is visible in the AFM in (b), dehydration in air shrinks the origami, making the slots invisible. Panel (ii) of (a) illustrates the binding of the walker at the first transfer station shown below the left slot of the origami. The presence of the walker is evident in the AFM image in (b). Panels (iii) show the first step walking forward along the origami in the schematic (a) and in the AFM image (b). Panels (iv) illustrate the second step walking forward (a), bringing the walker to the second transfer station shown below the middle slot of the origami, which is evident in the AFM image (b). Panels (v) show the third step walking forward, both schematically (a) and in the AFM image (b). Panels (vi) illustrate the fourth step walking forward, positioning the walker at the third transfer station, shown below the right slot of the origami (a); it is visible in the AFM image (b). All scale bars are 50 nm.

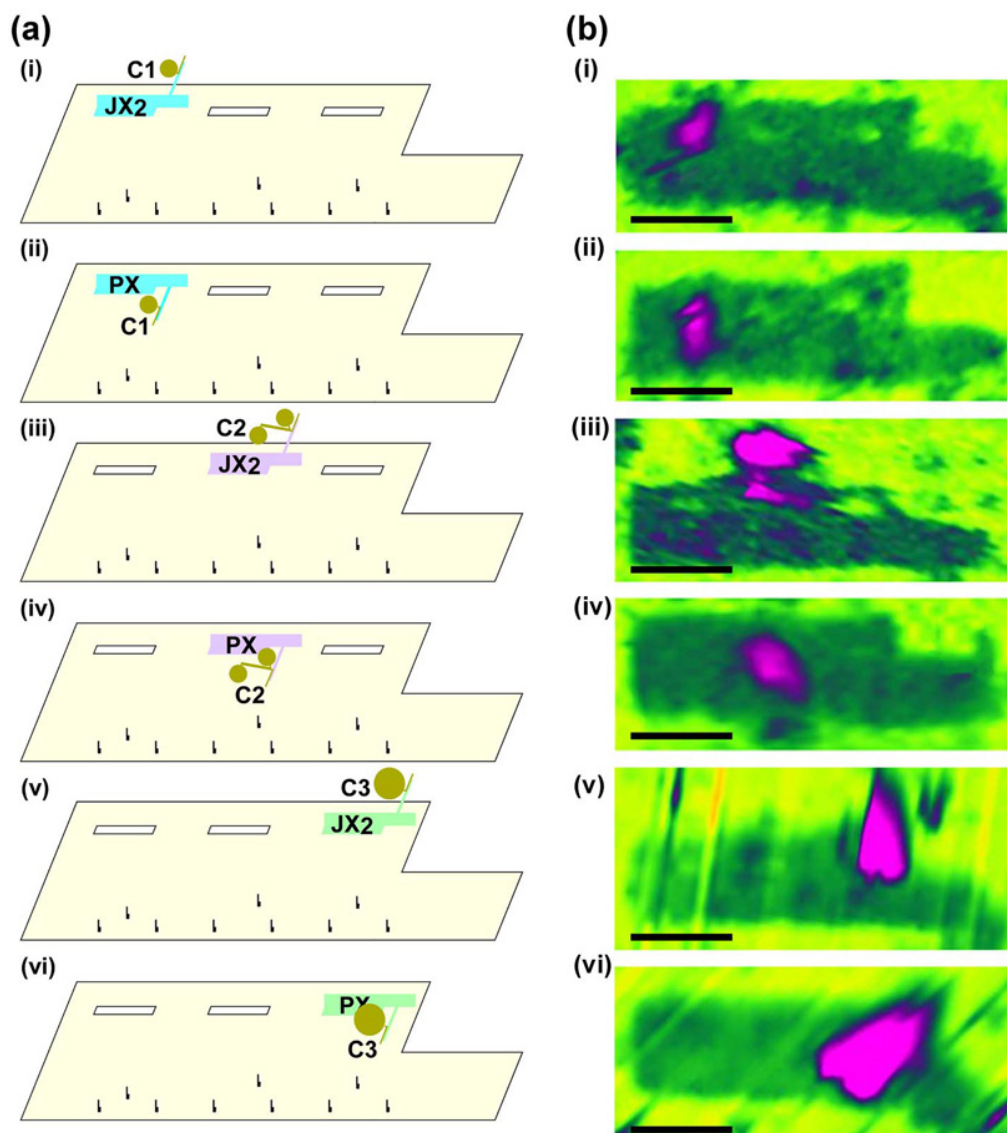
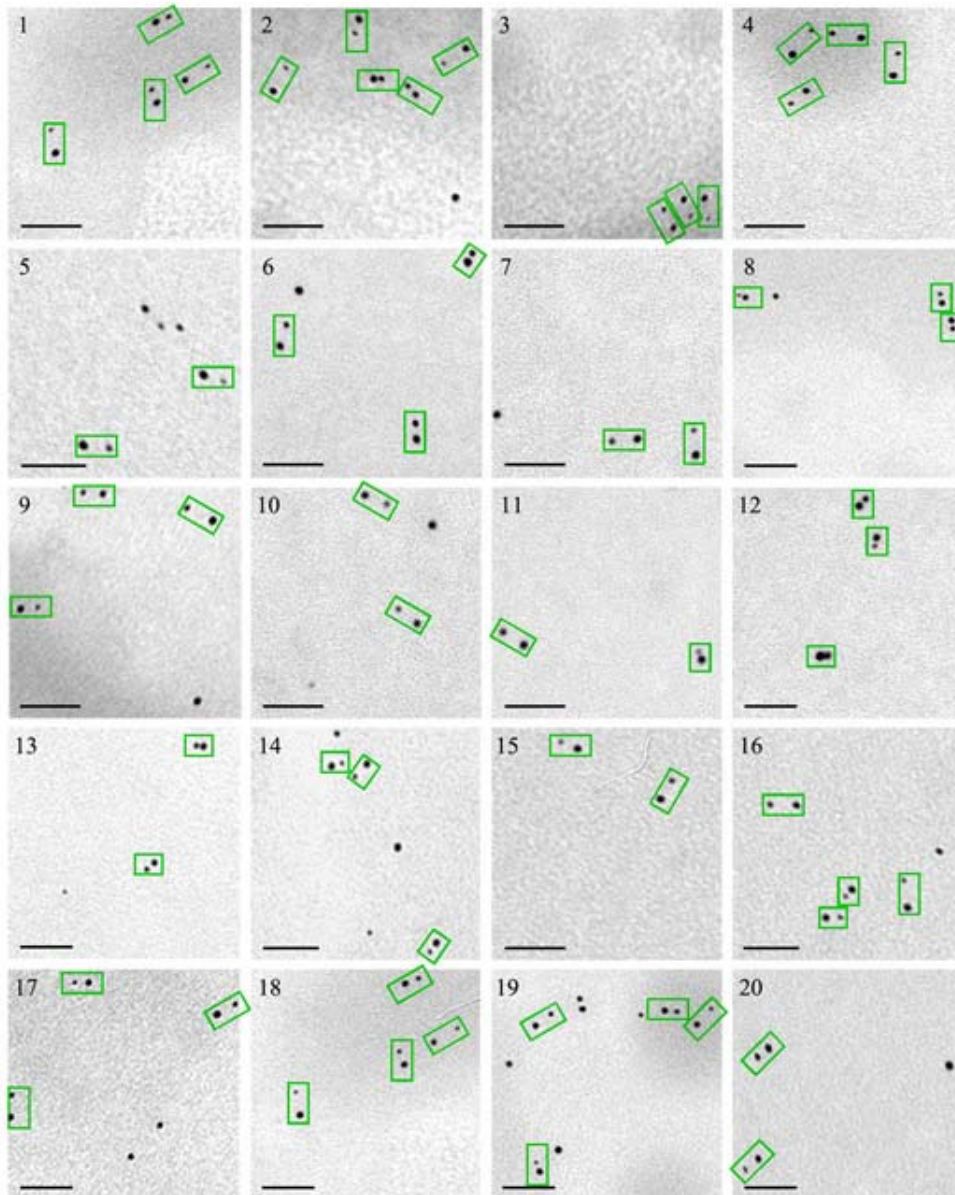
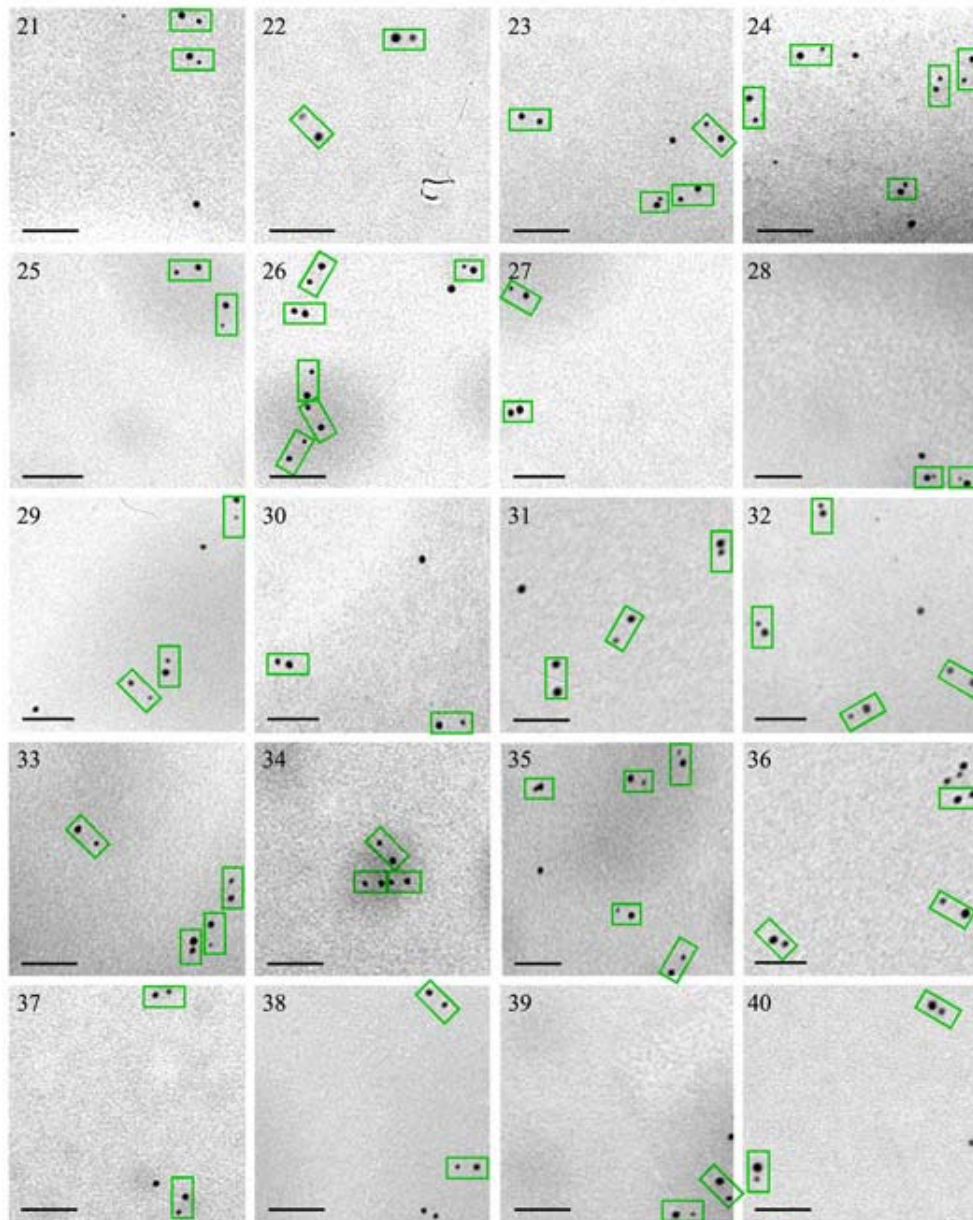


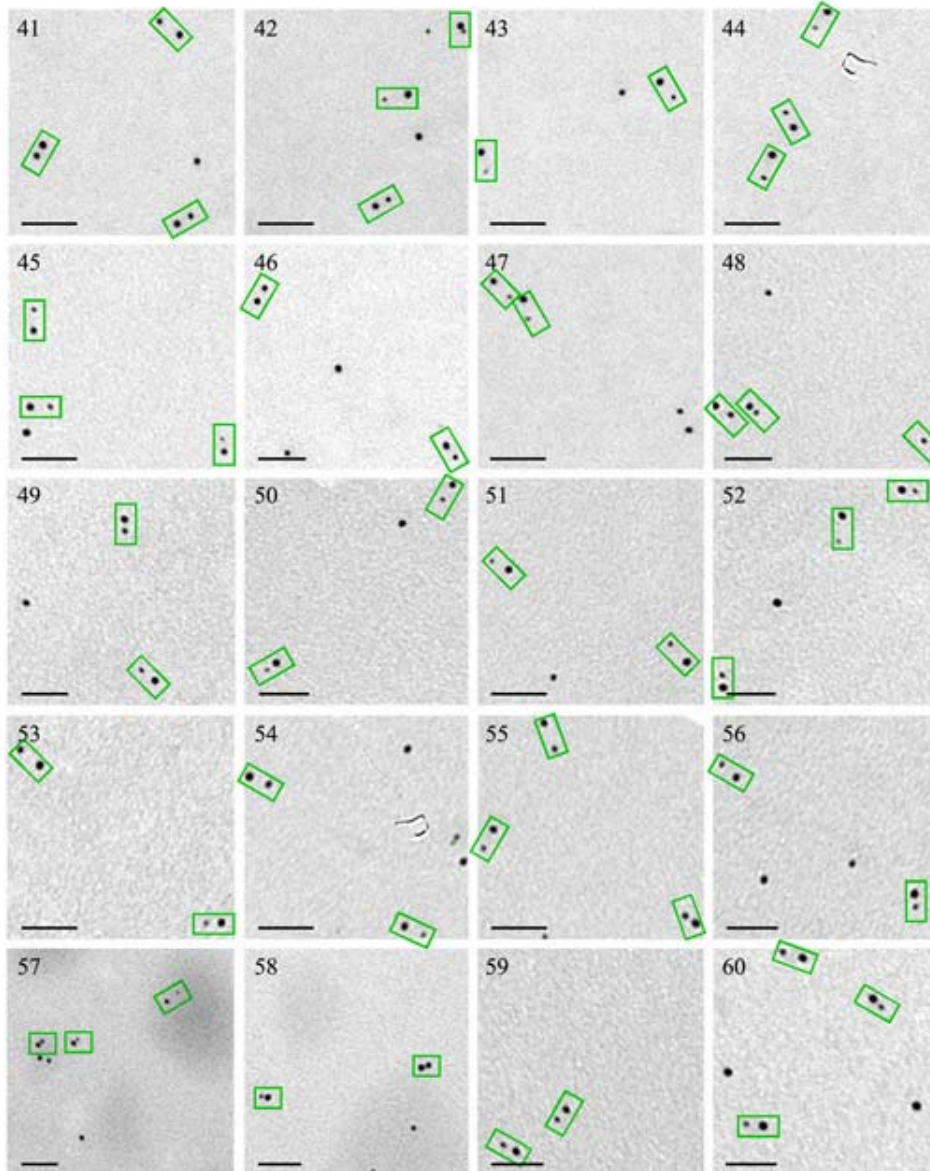
Figure S9. Schematics (a) and Atomic Force Micrographs (b) of the JX_2 to PX Switching for Each Cassette.

AFM was done in tapping mode in buffer. Panel (i) of (a) shows cassette 1 with cargo-1 (5 nm Au) in the JX_2 state was placed into the left slot; it is visible in the AFM (b). Panel (ii) of (a) illustrates the state of cassette 1 dynamically switching from JX_2 to PX, bringing the 5 nm Au toward the walker pathway, which is shown clearly in the AFM (b). Panels (iii) to (iv) of (a) schematically show the result of insertion into the middle slot, cassette 2 with cargo-2 (double 5 nm Au) switching from the JX_2 state to the PX state; the AFM image (b) clearly shows the change of Au position. Panels (v) to (vi) of (a) illustrate insertion into the right slot, cassette 3 with cargo-3 (10 nm Au) programmed from JX_2 to PX state in the schematics; the reprogramming switched Au from up to down, which is evident in the AFM images (b).

Figure S10. Additional Zoomed TEM Images of the Walker with Cargo-1 and Cargo-3 for Statistical Analysis.







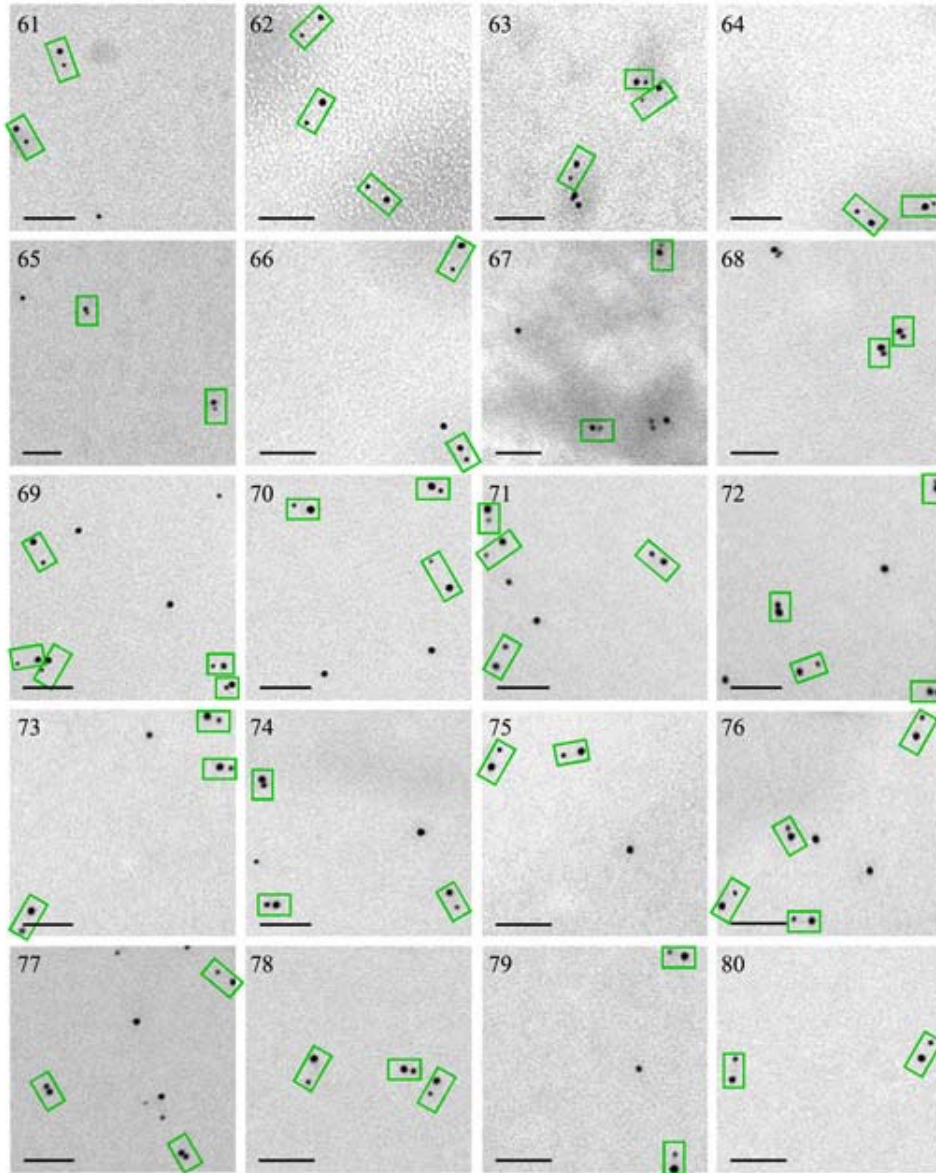
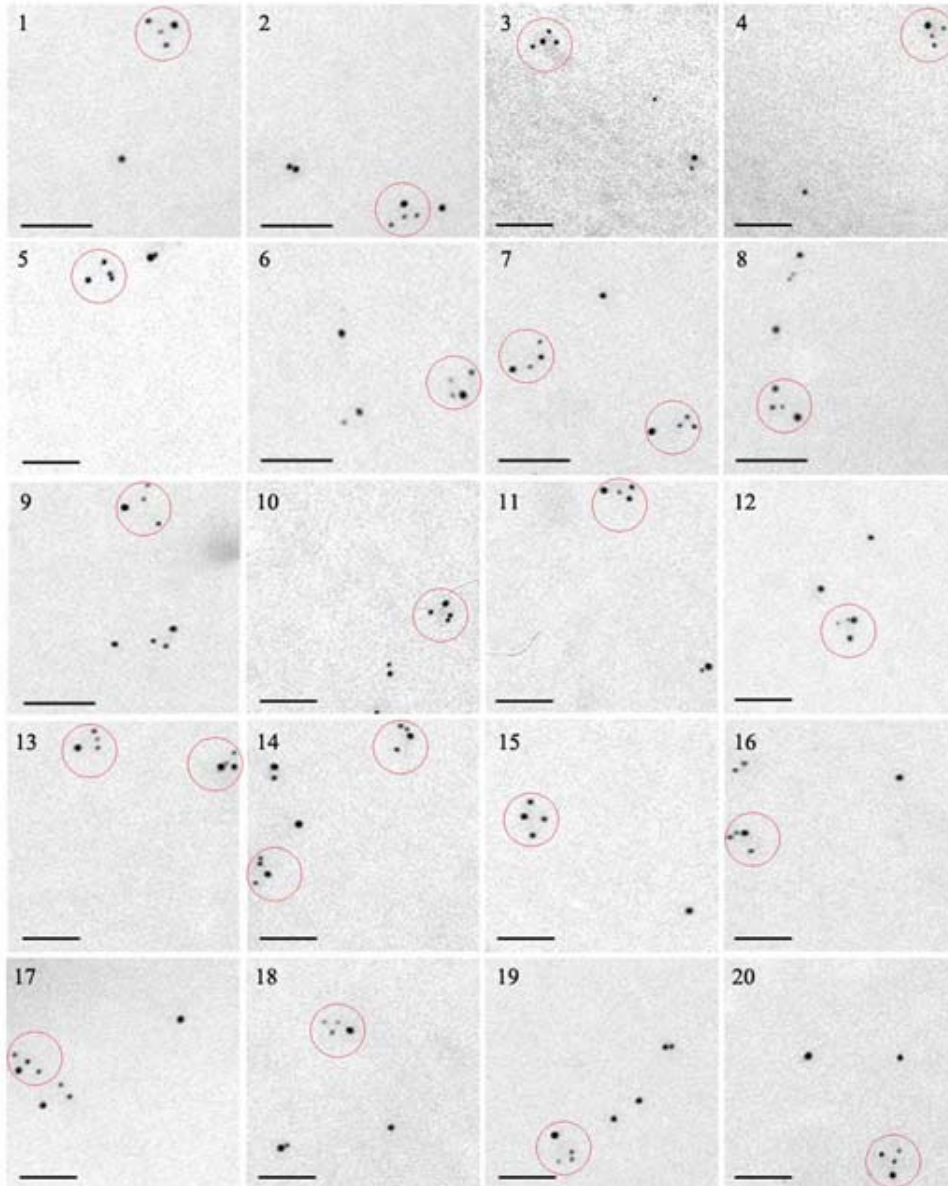
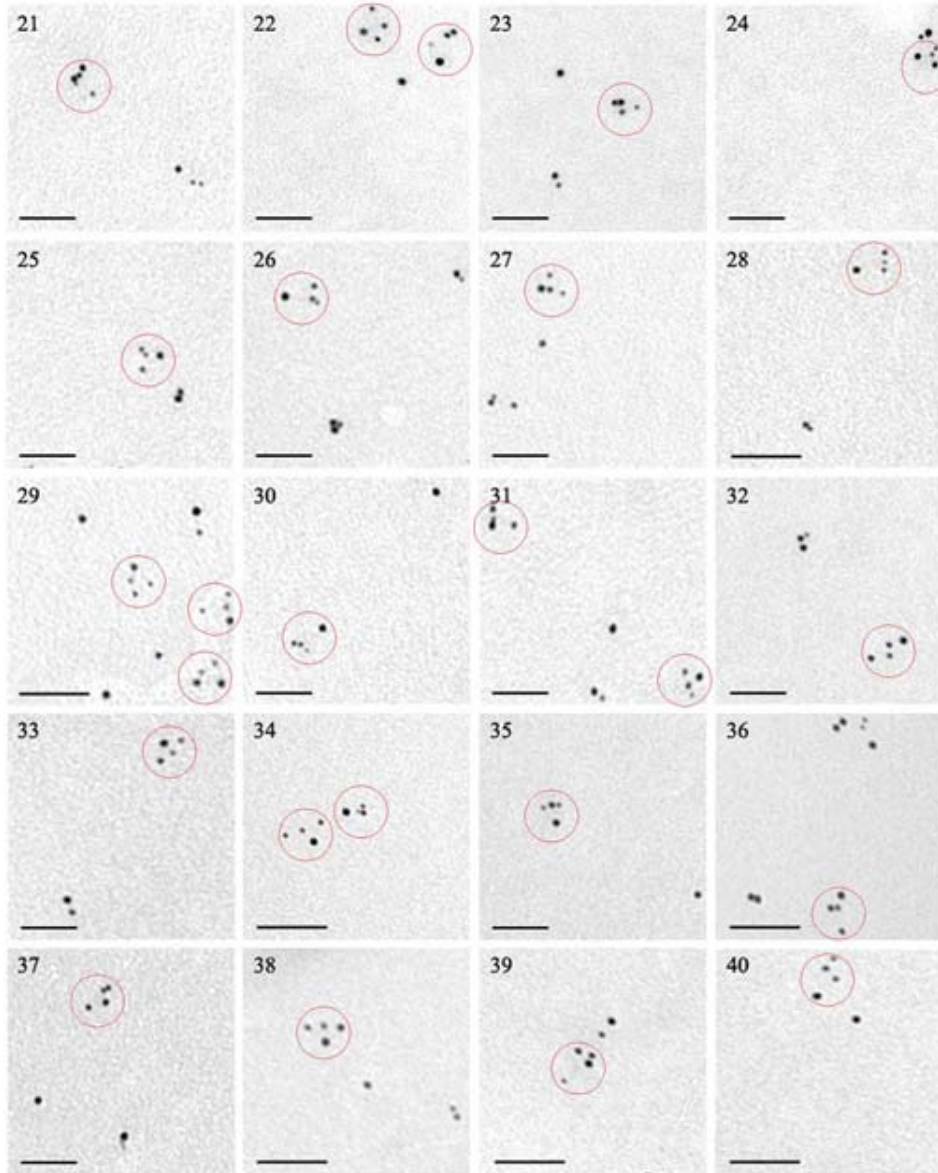
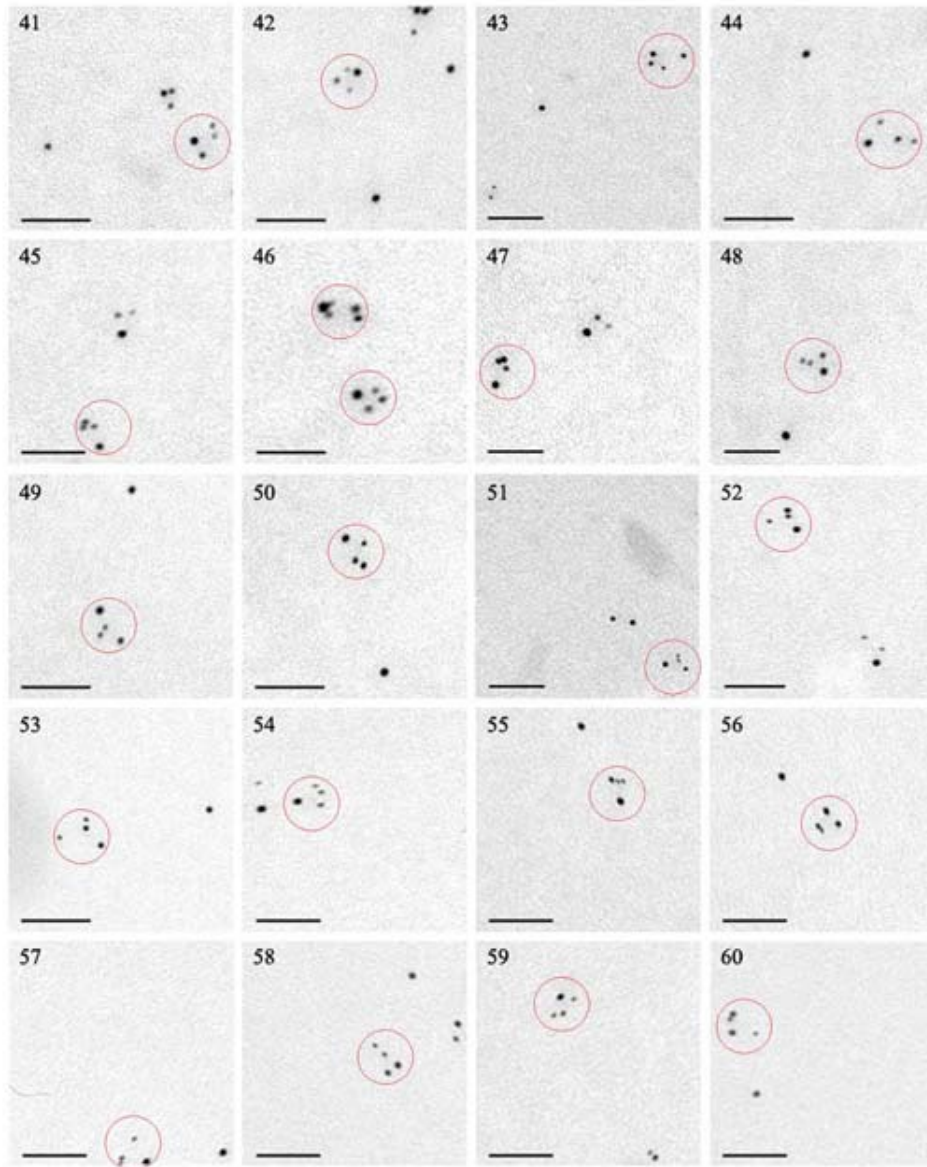
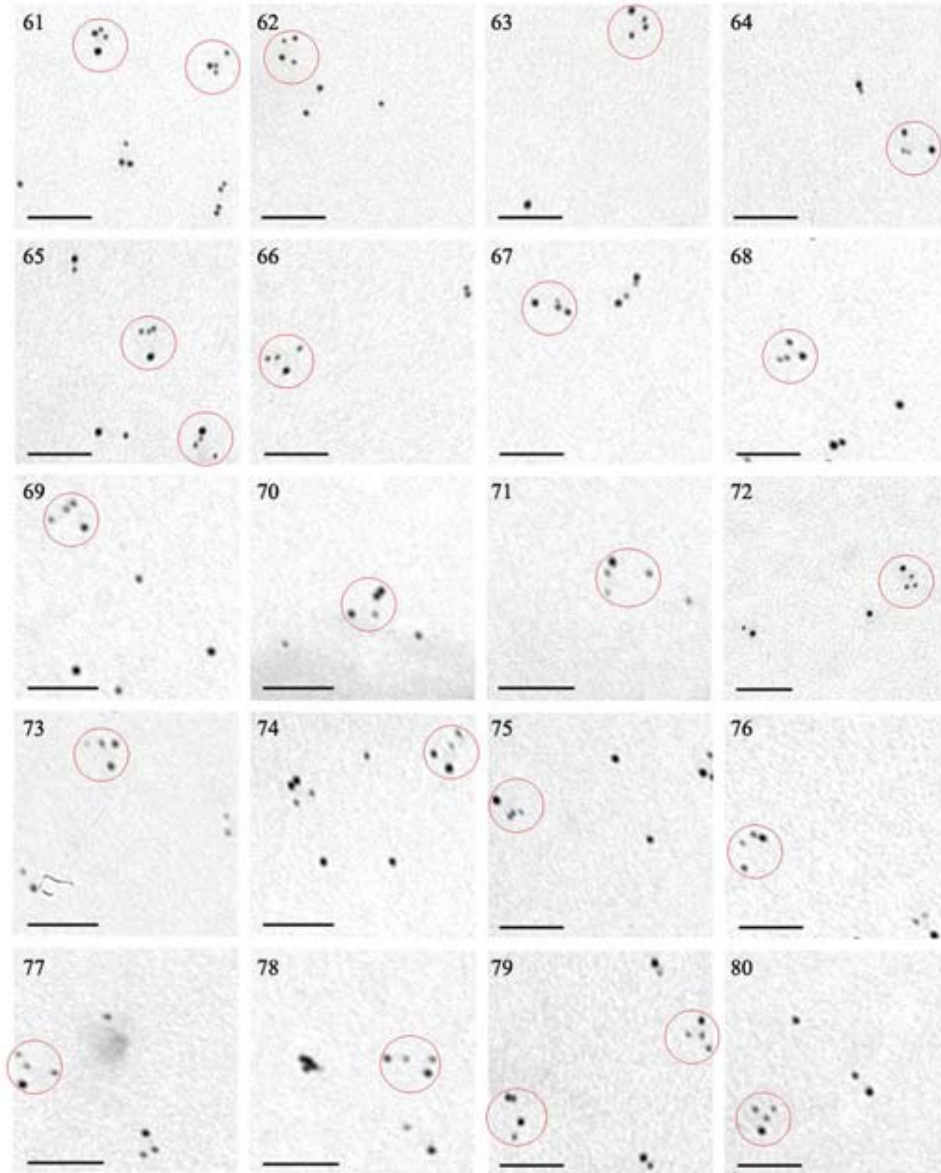


Figure S11. Additional Zoomed TEM Images of the Walker with Cargo-1, Cargo-2, and Cargo-3 for Statistical Analysis.









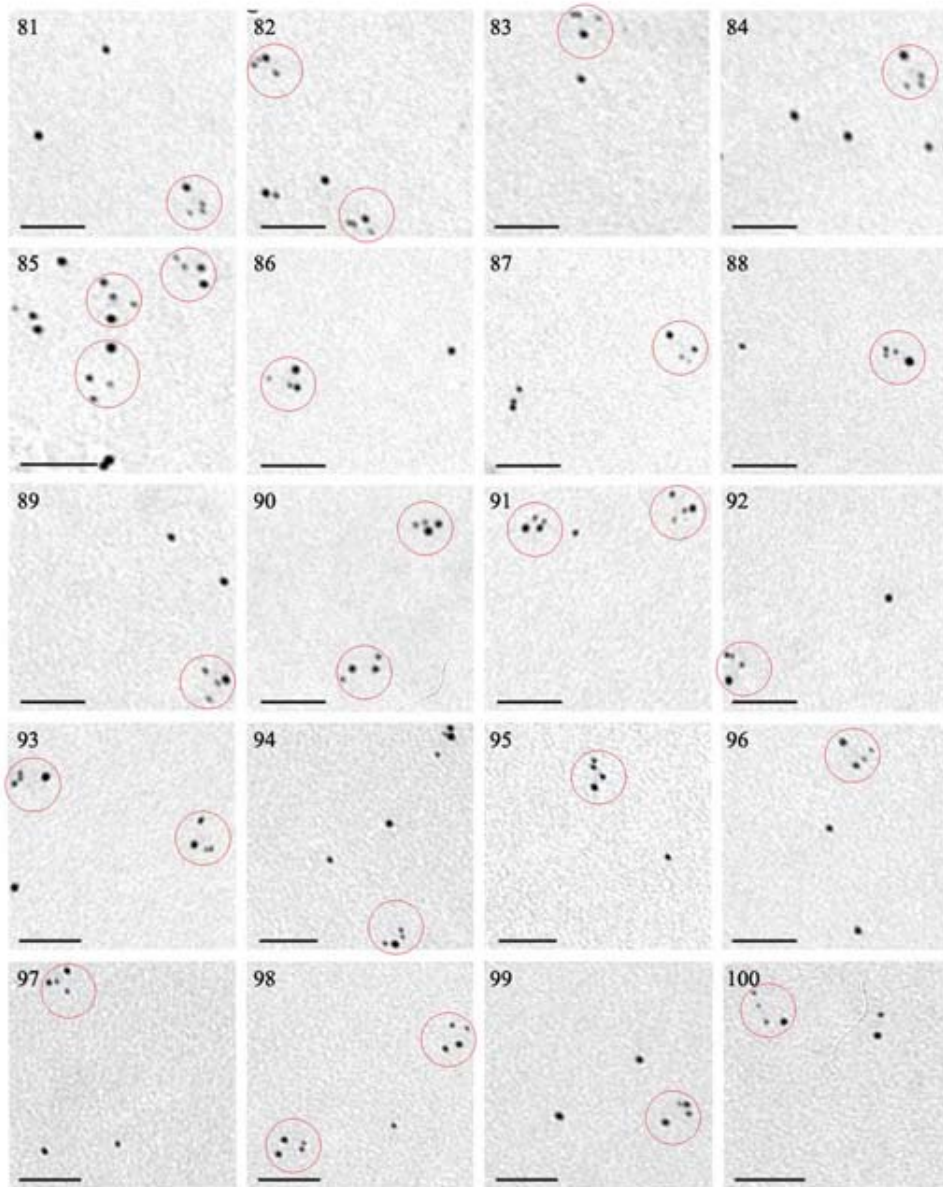


Figure S12. Distance Distribution between the 5 nm Au nanoparticle and the 10 nm Au nanoparticle on the Walker.

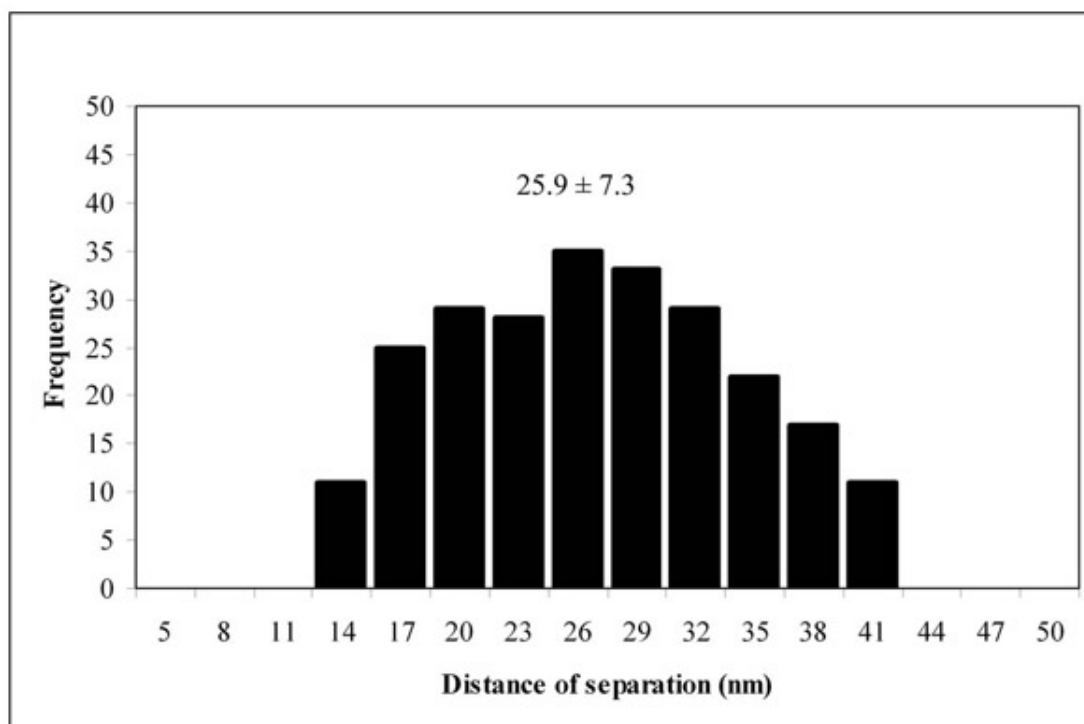


Table S1. Statistical Analysis for Different Additions.

When the 3 kinds of gold are all set to be transferred to and carried by the walker, the yield is around 43%.			
	Frequency	Yield	
(5)/(5-5)/(10)	119	43%	Target products
(5)/(5-5) or (5)/(10) or (5-5)/(10)	54	20%	Partly formed products
(5) or (5-5) or (10)	102	37%	Partly formed products
The numbers are counted from 100 zoom-in TEM pictures.			
When any 2 out of the 3 kinds of gold are set to be transferred to and carried by the walker, the yield is around 70%.			
Take (5)/(10) as an example:			
	Frequency	Yield	
(5)/(10)	225	72%	Target products
(5)/(5-5) or (5-5)/(10) or (5-5) or (5)/(5-5)/(10)	7	2%	Mistaken products
(5) or (10)	82	26%	Partly formed products
The numbers are counted from 80 zoom-in TEM pictures.			
When any 1 out of the 3 kinds of gold are set to be transferred to and carried by the walker, the yield is above 90%.			